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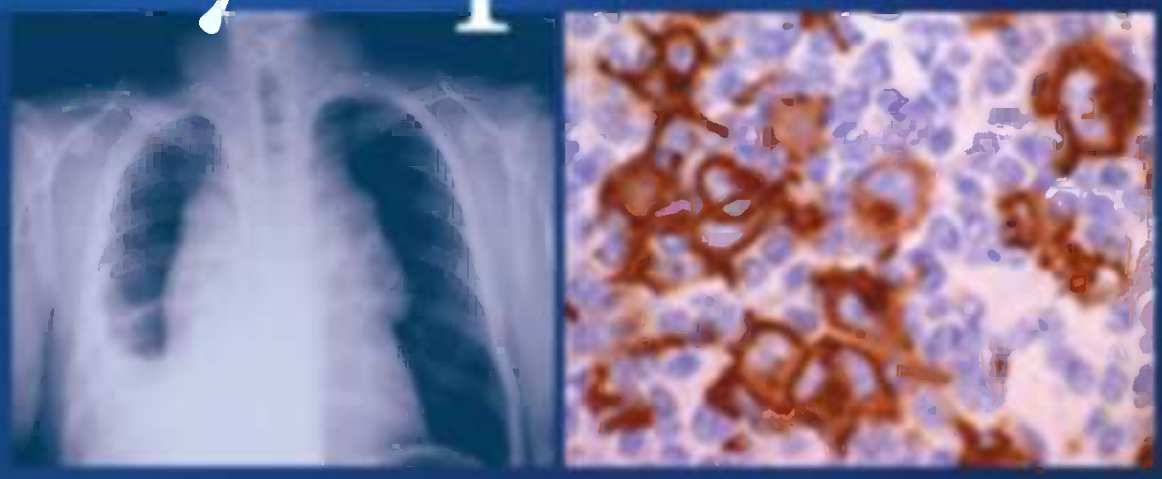
Editors

PEDIATRIC ONCOLOGY

Pediatric Lymphomas



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Howard J. Weinstein
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Preface

This is the first edition of *Pediatric Lymphomas*. The editors have been colleagues for more than 25 years and have been involved in the design and coordination of clinical trials and multimodality approaches for children with Hodgkin and non-Hodgkin lymphomas. Progress in elucidating the pathogenesis and in the diagnosis and treatment of lymphomas in children has been one of the great success stories in pediatric oncology. Prior to 1970, fewer than 20% of children with malignant lymphomas survived. Today, more than 90% of children diagnosed with Hodgkin lymphoma survive and more than 80% of children with non-Hodgkin lymphoma are considered cured. Continued improvement in survival has occurred as the result of combination chemotherapy, multidisciplinary care, supportive care and new insights into lymphoma biology. In addition, the rarity of childhood lymphoma has fostered national and international collaborations to test new therapies and to better understand the molecular biology of lymphomas that occur in children.

Our first edition of *Pediatric Lymphomas* provides comprehensive chapters on the diagnosis and treatment of both Hodgkin and non-Hodgkin lymphomas, and lymphoproliferative disorders associated with immunodeficiency. In addition, three chapters focus on the pathology, molecular biology, and genetics of Hodgkin and non-Hodgkin lymphoma including the rare cutaneous lymphomas. We hope that Pediatric *Lymphomas* will be a useful resource for practitioners from the many different disciplines involved in the comprehensive care of children with lymphomas.

The authors are all leading experts in the area of childhood lymphomas. We wish to thank them for all of the time and effort that went into their contributions. If this first edition is helpful to our diverse readership, it is because of the authors. We also want to acknowledge our desk editor, Meike Stoeck, and our partnership with Springer.

As pediatric oncologists, we are dedicated to improving the lives of children facing malignancy. We hope this book contributes to the field of pediatric oncology and to the benefit of our patients and their families.

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Introduction and Historical Background: Pediatric Hodgkin Lymphoma

S.S. Donaldson

Contents

1.1 The Way It Was

Prior to 1960, Hodgkin lymphoma was considered a uniformly fatal cancer. Hodgkin described the disease in 1832, but most attributed its pathophysiology to an inflammatory condition. Sternberg and Reed are credited for the first definitive description of the histopathology (Reed 1902; Sternberg 1898). Fox later refined the definition, and noted the occurrence of this entity in young patients (Fox 1926). However, little was known about its etiology and/or epidemiology. There was neither a uniform histopathologic classification, nor consistently used workup or staging system. The clinical assessment, largely by observation and palpation, was gradually supplemented by laboratory studies that characterized specific abnormalities. Imaging studies lacked precision, and staging was not precise. Therapy was largely symptomatic.

Early after Roentgen's discovery of the X-ray in 1896, Pusey first used X-rays to treat a young man with a presumed lymphoma; he noted dramatic shrinkage of enlarged neck adenopathy after 21 exposures to the X-rays (Pusey 1902). He soon used the X-ray treatment on a 4-year-old boy with bilateral cervical swelling from Hodgkin lymphoma and reported the swollen glands were "reduced to the size of an almond" within 2 months. During the time period from 1920 to 1940, there was some interest in the use of radical surgery followed by postoperative radiotherapy for the treatment of localized lymphoma. The good results from the X-ray treatment, and the cosmetic disfigurement from the extensive surgery, led most surgeons to consider that radical surgery was not indicated in the treatment of this disease.

As a byproduct of the World War II development of mustard gases, investigators observed nitrogen mus-

tard's lympholytic effect on lymphoid tissues. This observation initiated drug development, specifically the use of single agent chemotherapeutic agents in the treatment of leukemia and lymphoma. Multiple single agents were tried. But the introduction of the highly effective four-drug "MOPP" regimen (Devita et al. 1970) is credited for the 10- year and greater relapse-free survival for patients with advanced Hodgkin's disease.

While several investigators used low energy X-rays successfully in the treatment of Hodgkin's disease, the introduction of megavoltage radiotherapy, ushered in during the late 1950s and early 1960s, permitted treatment of large areas of the body with high radiation doses, thus providing potentially curative treatment to patients with lymphadenopathy above and below the diaphragm. Kaplan and investigators at Stanford are credited with the definition of standard treatment fields of mantle, inverted Y, total nodal and total lymphoid radiation (Kaplan 1970). The term involved field was employed for localized disease limited to involved lymph node chains, while prophylactic, complementary, or extended field radiotherapy was used for the treatment of apparently uninvolved lymphatic regions.

With increasingly effective treatment, the search for the optimal therapy began with a series of prospectively randomized trials investigating treatment approaches. The first randomized clinical trial undertaken in patients with Hodgkin lymphoma was initiated at Stanford University (Kaplan and Rosenberg 1966, 1973). Soon thereafter a large-scale randomized national cooperative group clinical trial was developed (Nickson 1966; Nickson et al. 1976). These studies were not age dependent; children, adolescents and adults participated in these investigations. However, the clinical trials required pathology and staging definitions for eligibility.

The Jackson and Parker classification (Jackson and Parker 1944), which designated three histologic subcategories (paragranuloma, granuloma, and sarcoma), was modified by Lukes, Butler and Hicks (Lukes et al. 1966a) to comprise six categories: lymphocytic/histiocytic nodular; lymphoctic/histiocytic diffuse; nodular sclerosis; mixed; diffuse fibrosis; and reticular. This was later modified at the Rye conference into a four-subcategory classification (lymphocytic predominance, nodular sclerosis, mixed cellularity, lymphocytic depletion) (Lukes et al. 1966b). The Rye classification was successfully used for over 25 years, and only recently has been modified slightly into a World Health Organization (WHO) classification (Stein et al. 2001). This system recognizes the disease as a lymphoma and designates Hodgkin lymphoma to be used synonymously with Hodgkin's disease. In addition, nodular lymphocyte predominant Hodgkin lymphoma (with or without diffuse areas) is clearly separate from other types of classical Hodgkin lymphoma, in view of its distinct biologic, histologic, and clinical features. Classical Hodgkin lymphoma is subdivided as: nodular sclerosis, lymphocyte-rich, mixed cellularity, and lymphocyte depletion.

An anatomic staging system evolved with greater specificity and clarity than the generalized terms of localized and regional and was ratified at the Paris 1965 (Tubiana 1996) and Rye New York meetings (Rosenberg 1966). Further modifications were adopted as the Ann Arbor staging classification in 1971 (Rosenberg et al. 1971). This system refined stages as I-IV, with or without systemic symptoms (A or B), with or without extranodal organ or site involvement (E), and differentiated clinical stage (CS) and pathologic stage (PS). The Ann Arbor classification for Hodgkin lymphoma was adopted by the Union Internationale Centre le Cancer (UICC) staging committee as the official staging system (Sobin and Wittekind 1997). This system was associated with recommendations for diagnostic evaluation, and impacted therapeutic options and management. New diagnostic tests evolved which became widely used, such as computed tomographic (CT) imaging. The concept of prognosis related to stage and the importance of bulky disease became apparent. To update the Ann Arbor staging classification and justify the use of imaging techniques such as CT imaging, a meeting in the Cotswolds, UK, in 1988 was held which recommended minor revisions to the Ann Arbor staging system (Lister et al. 1989). Following this, there has been a gradual evolution of required and recommended studies for the initial evaluation of patients with Hodgkin lymphoma.

With new tools to aid in establishing the diagnosis, and to define the extent of disease, came changes in

management and treatment. The large clinical trials gradually defined appropriate treatment options as a function of stage and prognostic factors. Initially, these studies tested radiation alone, chemotherapy alone, and various combinations of combined modality therapy for differing clinical and pathologic situations, accounting for favorable vs. unfavorable prognostic factors. One of the first observations from this new curative therapy was the observation of sequelae, which were first apparent in the youngest children to be cured of the disease.

1.2 Lessons from the Children

The standard approach for Hodgkin lymphoma evolved to become surgical staging with laparotomy and splenectomy, followed by standard dose (36–44 Gy) extended field radiation, with or without combination chemotherapy. Survival and disease-free survival rates dramatically rose. Associated with this success came the unanticipated observation of substantial sequelae in children who survived their disease. The first observation was one of impairment of growth and development in youngsters who had received high-dose, large-field radiation. Based upon a prior observation of disproportionate inhibition of axial skeletal growth among a few pediatric survivors of medulloblastoma treated with cranial spinal radiotherapy (Probert et al. 1973), the first protocol using low-dose radiotherapy in combination with combination chemotherapy was initiated at Stanford in the spring of 1970. Two boys, aged 21 and 50 months, with advanced stage disease were treated with low-dose (15 and 20 Gy) total lymphoid radiation and six cycles of MOPP chemotherapy. Their disease disappeared, and they remained disease-free and grew within the normal range for their ages. A formal protocol evolved using radiation dose as a function of bone age: 15 Gy for those less than age 6; 20 Gy for those aged 6–10; and 25 Gy for those aged 11–14; and volume as an involved field defined by pathologic staging. All patients received six cycles of MOPP chemotherapy (Donaldson 1980). This approach of combined modality therapy, using less than standard doses of radiation with chemotherapy, emerged as a major advance in the curative treatment of children with Hodgkin's disease. The 10-year update of the Stanford protocol confirmed the unprecedented and unexpected finding of overall survival of 89%, freedom from relapse of 90% (Donaldson and Link 1987). While growth and development have not proven to be serious problems using this approach, other unexpected findings did occur.

1.3 The Hidden Secrets – The Discovery of Late Effects

With curative therapy resulting in an increasing cohort of survivors, new problems became apparent, the most significant of which was the occurrence of another malignancy. The incidence of secondary leukemia and/or myelodysplastic syndrome became apparent with the increased use of alkylating agent chemotherapy, first noted with the MOPP combination. The 5-year survival from those affected was less than 5% (Henry-Amar 1992). The development of non-Hodgkin lymphoma after the successful treatment of Hodgkin's disease also appeared and was correlated with the lymphocyte predominant histology, extensive treatment, and immunosuppression. The risk of developing a secondary solid tumor occurred later, more than 10–15 years after the initial therapy. While many tumor types were observed, lung, skin, gastrointestinal, and breast cancers remain the most commonly reported. Among young girls, the breast cancer risk is thought to be a reflection of greater sensitivity of the developing breast tissue to ionizing radiation, especially among those under age 30 when irradiated with high doses (Hancock et al. 1993b).

Impairment of growth and development was first noted in children who received high-dose, extended-field radiation (Willman et al. 1994). These children exhibited a measurable decrease in sitting height as compared to standing height. Soft-tissue abnormalities, including atrophy, most commonly seen in the neck, were observed later.

Cardiovascular disease then emerged, contributing 10–15% of late morbidity. A wide spectrum of cardiac complications has been observed, but the most common fatal complication is acute myocardial infarction secondary to coronary artery disease (Hancock et al.

1993a). The risk of cardiac death has been shown to be associated with mediastinal irradiation. The long-term toxicity of cardiotoxic chemotherapeutic agents remains unknown but is also of concern, especially in children.

Reported pulmonary toxicity has ranged from acute interstitial pneumonitis to chronic lung fibrosis and recurrent pleural effusions. This has been associated with radiation dose, volume, and technique, as well as the use of specific chemotherapeutic agents such as bleomycin (Marina et al. 1995). Judicious use of these modalities in contemporary regimens has reduced the incidence of these complications.

Infectious complications including overwhelming bacteremia was first associated with splenectomy and aggressive immunosuppressive therapy (Donaldson et al. 1978). Administration of appropriate immunizations and prophylactic antimicrobial therapy can be life-saving in at-risk survivors. Viral, fungal, and opportunistic infections have also been observed, but are less likely to be life threatening.

Sterility is observed in children requiring high-dose pelvic radiotherapy, without appropriate gonadal shielding, and in males receiving alkylating agent chemotherapy. Other late effects that require attention are endocrine dysfunction, particularly hypothyroidism. Psychosocial issues, including mood disturbances and chronic fatigue, have also been observed.

1.4 Current Optimal Management

The lessons learned over the past 50 years have brought us to a new era in the management of children with Hodgkin lymphoma, where the goal of therapy is cure, freedom from late effects, and optimal quality of life. Today, appropriate management begins with careful clinical staging, without routine surgical staging and splenectomy. Histopathologic material must be confirmed by a hematopathologist with expertise in the malignant lymphomas. Workup and staging evaluation are undertaken with careful physical examination by pediatric and radiation oncologists at the time of diagnosis. Recommended imaging studies include a chest radiograph, CT imaging of the neck, chest, abdomen, and pelvis. No longer are tomograms and lymphograms required. Magnetic resonance imaging, ultrasonography and radioisotope bone scanning are useful in only select cases. Positron emission tomography with 18-fluoro-2-deoxyglucose is being investigated and increasingly utilized in place of gallium citrate 67 scanning. Bone marrow biopsy is reserved for the child presenting with systemic symptoms, who has clinically apparent disease on both sides of the diaphragm.

Optimal therapy has evolved to risk-adapted treatment, with risk groups most commonly defined as low, intermediate, and high. This involves combined modality therapy using low-dose, involved-field radiation and multiagent chemotherapy in the majority of children (Nachman et al. 2002; Ruhl et al. 2004). The details regarding specific drug dose and duration are dictated by individual protocol. The overriding goal of therapy today is to define the least amount of therapy which affords the highest event-free and overall survival for all. It is highly likely that continual refinement of therapy will show this optimal therapy to be a limited number of cycles of non-toxic chemotherapy, with low-dose, conformal radiation, in clinically staged children managed in pediatric centers where there is expertise in the diagnosis, staging, and treatment of children with malignant disease.

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Biology and Pathology of Hodgkin's Disease

R.E. Hutchison and A. Uner

Contents

2.1 History of Hodgkin's Disease Pathologic Classification

Hodgkin's disease, now referred to as Hodgkin lymphoma (HL) in current pathology nomenclature (Stein et al. 2001a), has long been grossly and microscopically characterized, but only recently has its biology been more than hypothetical. While the history of this disease is addressed in other chapters of this book, some historical notes related to its pathology may be of particular interest, since the histopathologic descriptions have changed little in over 100 years, and some commonly used nomenclature is derived from old classifications.

This disease was grossly described in 1832 by Thomas Hodgkin, MD, curator of the Museum at Guy's Hospital, London (Hodgkin 1832). He read his paper to the Medical and Chirurgical Society of London with watercolor illustrations of the disease by his friend, Sir Robert Carswell, on display. Some of these can be seen in our medical libraries (Dawson 1968). What he described was "peculiar enlargement" and "affection" of the absorbent glands (lymph nodes) of the neck and other parts of the body accompanied by enlargement and deposits in the spleen and sometimes liver of firm tubercle-like nodules, some the shape of eggs of different sizes. He detailed the gross pathologic findings of six cases from the museum, the first two of which were boys age 9 and 10 years, and a seventh case seen by Dr. Carswell. Only two of the cases, including the 10-year-old boy, were later confirmed to be what we now call HL (Jackson and Parker 1947). He made several related observations and then described clinical and pathologic findings of seven more cases from his clinical experience. One of these observations was that the splenic nodules, resembling glandular inflam-

mation, were likely arising in pre-existing structures which Malpighi had considered "glands" (Hodgkin 1832), thus stating that the spleen contains portions of the same tissue as lymph nodes.

Samuel Wilks, in 1856, described similar cases of what he considered unusual forms of "lardaceous affections" (resembling bacon rind or suet) involving at the same time cervical and other "glands" and spleen (Wilks 1856). These disorders likely included tuberculosis and perhaps other infections, but the cases he described were distinct. This report was apparently independent of knowledge of Hodgkin's prior work, but in 1865, Wilks acknowledged that Hodgkin had been the first to describe a distinct disease and he called it "Hodgkin's Disease" (Wilks 1865). He separated it from the "lardaceous affections" and noted its "likeness to cancer", stating: "A new growth, it may be observed, which thus destroys surrounding parts, is usually styled malignant."

Other 19th-century pathologists and medical scientists, including Wunderlich, Virchow, Cohnheim, Rousseau, Pel, Ebstein and Billroth, made subsequent observations on the pathology and clinical findings of Hodgkin's disease and assigned to it a variety of names (Reed 1902). The discovery and description of the malignant cell are usually credited to both Carl Sternberg and Dorothy Reed (Sternberg 1898; Reed 1902). Sternberg described it first, and Reed did so in more detail (Jackson and Parker 1947).

At age 26, eight years after beginning his medical studies, Sternberg described the cellular elements of HL. He noted: "In the middle between the lymphatic elements we find a fairly abundant quantity of large cells, each rich in protoplasm, with a large nucleus which in general is rather strongly stained with Hämalaun (Meyers hematoxylin). The majority of these cells have a mostly round, but often oval or lobed, rather large nucleus, in which nuclear corpuscles are often recognizable. Very frequently these cells have multiple nuclei. They lie in the middle among the lymphocytes, but often are plainly seen to hang together with the flow" (Sternberg 1898; Schmidt 1992).

Reed credited Sternberg's extensive research and influence, but argued that his conclusion that HL is a form of tuberculosis, based on finding bacilli in eight of 13 cases, was inaccurate. Her description of HL was highly insightful and remains instructive today. She described the presentation, most frequently in young people and more often in boys, as progressive painless enlargement of lymphatic glands almost always arising in the cervical region, at first unilateral, with extension to adjacent glands and leading to massive enlargement without skin involvement. Anemia without leukocytosis and cachexia eventually developed. Gland enlargement extended to, though usually not beyond, the abdomen, with the inguinal region usually not involved. "The spleen is almost always enlarged and may be enormous", and the liver was only occasionally involved. Smaller and younger tumors were soft, and larger, later ones firm and hard. Secondary infections occurred eventually, tuberculosis was the most common cause of death after one to four years, and the mediastinum was involved at autopsy.

Microscopically, the process showed increasing "endothelium" with decreasing germinal centres, abundant lymphocytes, plasma cells and eosinophils (none of which was always present). There were large cells free in the tissue with giant cells attached to "endothelium" (apparently referring to mesenchymal tissue in general) and present in sinuses. Nodules of connective tissue formed, and there was often necrosis within them, and mild capsular fibrosis. Giant cells varied from the width of two to twenty times the size of a red blood cell, with one or more nuclei (up to eight to twenty) which were round or bean shaped and contained one or two large acidophilic nucleoli. The cells showed homogenous protoplasm. The particular giant cells were felt to be peculiar to this disease, and Reed hypothesized them to be derived from endothelium, "though not the endothelium of the blood vessels". The large mononuclear cells free in the tissue were considered to be derived from the "mother cells of the germinal centres which also give rise to lymphocytes and plasma cells" (perhaps presaging the current belief). Langhans's giant cells were also present, and related to secondary infection. All but the characteristic large cells were considered inflammatory.

Very little technical detail was given, but paraffin sectioning and differential staining were well developed by that time. The fixatives alcohol and Zenker's fluid were utilized for at least some cases, and polychrome methlyene blue staining described several

times, likely for special effect, and sometimes showing mast cells.

Gall and Mallory described over 600 cases of HL in 1942 (Gall and Mallory 1942) and Jackson and Parker proposed a classification for HL in 1947 that included three types: paragranuloma, granuloma, and sarcoma (Jackson and Parker 1947). Lukes, Lennert, and others characterized the histopathology of the subtypes including the host response and relationships to stage and disease progression (Lennert 1953; Lukes 1963). The Jackson and Parker classification was utilized through publication of Rappaport's highly influential "Tumors of the Hematopoietic System", which is mostly known for his classification of non-Hodgkin's lymphoma, originally submitted in 1959 but published in 1966 (Rappaport 1966). Rappaport described that "Sternberg-Reed" cells were malignant histiocytes and that the mononuclear variants were less diagnostically specific than multinucleated forms.

Paragranuloma (corresponding to lymphocyte predominant HL) was an indolent, often nodular lymphoma with few neoplastic cells, eosinophils, or plasma cells and excellent survival. It was often confused with nodular "lymphosarcoma" (which Rappaport renamed poorly differentiated lymphocytic lymphoma). Granuloma type (now classical HL) contained more classical Sternberg-Reed cells, eosinophils, plasma cells, neutrophils, fibroblasts, and benign histiocytes, occasionally necrosis, and was more aggressive with poor survival. Sarcoma type, much less common, exhibited a predominant population of atypical and bizarre cells with little inflammatory component (Rappaport 1966).

The current histopathologic classification of HL is closely derived from Lukes's and Butler's proposal, presented and slightly modified in 1966 at a conference in Rye, New York (Lukes and Butler 1966; Lukes et al. 1966). The proposal was for six types: lymphocytic and/or histiocytic (from which the term "L&H", or LH cell is derived); diffuse and nodular; nodular; mixed; diffuse fibrosis; and reticular. The nomenclature committee of the Rye conference simplified the proposal into four types: lymphocyte predominance (LP), nodular sclerosis (NS), mixed cellularity (MC), and lymphocyte depletion (LD). Used in conjunction with the Ann Arbor clinical staging system (Carbone et al. 1971), this has remained the basis for pathologic diagnosis and classification.

The stability of HL pathologic classification over the last four decades is in contrast with the cacophany of non-Hodgkin lymphoma classifications during that same time. All hematopoietic malignancies are now included in a unified classification sponsored by the World Health Organization (WHO), Pathology and Genetics, Tumours of Haematopoietic and Lymphoid Tissues (Jaffe et al. 2001). This provides researchers and practitioners with an unified nomenclature for investigation and treatment.

The recommended term "Hodgkin lymphoma (HL)" encompasses two basic diseases, a relatively common form now referred to as classical Hodgkin lymphoma (CHL) and the very uncommon disease of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) (Stein et al. 2001a). Classical HL is a malignant tumor that may be subclassified into histological groups sharing biologically and morphologically similar neoplastic cells, Hodgkin Reed-Sternberg (HRS) cells. NLPHL is in contrast viewed as an indolent tumor sharing features with some B-cell non-Hodgkin lymphomas. The current concept in classification, two basic diseases, remains very close to that of Jackson and Parker while the terminology is similar to Lukes and Butler.

2.2 Lineage of Hodgkin Reed-Sternberg cells; B-cell phenotype

In CHL, HRS cells have an unusual immunophenotype (Thomas et al. 2004). They usually don't express leukocyte common antigen (LCA; CD45), and B-cell surface markers are either not expressed or expressed on only a small proportion of malignant cells. B-cell receptor (BCR; surface immunoglobulin) is absent, and a majority of immunoglobulin transcription factors are downregulated. Some markers related to dendritic cells, cytotoxic T/NK cells, and myeloid lineages are expressed. This unusual phenotype has been confusing, and derivation from precursors of different lineages has been proposed at different times. The paucity of neoplastic cells in the tumor has inhibited accumulation of necessary data to establish the origins of HRS cells.

Evidence emerged in the early 1990s that HRS cells are monoclonal and derived from germinal center B cells (Ingharami et al. 1994; Tamaru et al. 1994). This was confirmed by the group of Rajewsky, Kuppers and others using individually isolated HRS cells (Kuppers et al. 1994). They were able to amplify the immunoglobulin (Ig) heavy (IgH) and light chain genes and show that they carry monoclonal somatically mutated immunoglobulin variable region genes. Their findings suggested that the majority of HL are derived from germinal center B cells or their progeny, though some appeared polyclonal in at least one study (Hummel et al. 1995). It was initially thought that HRS cells contained crippling somatic mutations with stop codons limiting antigenic selection (Kanzler et al. 1996). Subsequent work has suggested that HRS cells in CHL are uniformly clonal and generally lack crippling mutations but have lost their Ig gene translation ability due to functional defects in regulatory elements (Marafioti et al. 2000). One proposed mechanism is inactivation of the transcriptional machinery (Thomas et al. 2004), and indeed, HRS cells lack Ig transcription factors (Stein et al. 2001c). In rare cases of CHL, T-cell derivation has been established (Seitz et al. 2000). It is interesting that other rare cases of composite CHL and B-cell non-Hodgkin lymphoma have been shown to be clonally related to one another (Brauninger et al. 1999; Marafioti et al. 1999; Bellan et al. 2002).

LH cells in nodular lymphocyte predominant Hodgkin lymphoma are also clonal B cells. These show ongoing somatic mutations with interclonal diversity suggesting that they are derived from selected germinal center B cells (Braeuninger et al. 1997). The LH cells in NLPHL consistently express B-lineage associated markers such as CD19, CD20, CD22, CD79a, and Ig J-chain, consistent with derivation from B-lineage.

Single-cell studies including immunohistochemistry and IgH sequencing of micromanipulated HRS cells have also been performed in lymphocyte-rich classical Hodgkin lymphoma (LRCHL), a form of CHL which resembles NLPHL. Those results show similarities to CHL rather than NLPHL (Brauninger et al. 2003).

2.3 Some Evidence of an Antigen-Presenting Function

HRS cells have some features of antigen-presenting cells, including expression of MHC class II molecules. Class II-associated invariant chain peptides (CLIP) are associated with many of these molecules (Bosshart and Jarrett 1998). CLIP is a probable target of autologous graft versus host disease (Bosshart 1999; Hess et al. 1997).

Cytoplasmic linker protein (CLIP)-170/restin (*Re*ed-*St*ernberg *in*termediate filament-associated protein) is a different molecule involved in the antigen uptake process and is highly expressed in HRS cells. Restin and CLIP-170 are produced as splice variants of the same gene and are thought to play a role in macropinocytosis (Sahin et al. 2002; Bilbe et al. 1992; Delabie et al. 1992) and the binding of endocytic vesicles to the cytoskeleton (Rickard and Kreis 1991). Expression of this gene is normally high in dendritic cells, and it is also expressed in activated B cells. Its presence, along with dendritic cell specific molecules such as the actin bundling protein fascin, suggests that HRS cells may function as antigen-presenting cells even though their lineage is B cell (Sahin et al. 2002).

2.4 Apoptosis

Apoptosis is programmed cell death with characteristic morphologic changes and DNA degradation without necrotic response. It is induced through one of two pathways. Death receptor (DR) mediated, or extrinsic pathway, apoptosis is induced by the engagement of TNFR family members, including Fas/CD95, with their ligands, recruitment of adaptor molecule Fas–associated death domain (FADD), and translocation of caspase-8 towards the plasma membrane to form the death-inducing signaling complex (DISC). This activates effector caspases-3 and -7 and other proteases including granzymes, cathepsins, and calpains to degrade structural and regulatory proteins (Eldering and Vanlier 2005). Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) activates this pathway for selective killing of cancer cells.

Apoptosis following DNA damage or stress is induced through an intrinsic (mitochondrial) pathway

involving permeabilization of the mitochondrial outer membrane (MOM) and modulated by pro- and anti-apoptotic bcl-2 family proteins. Intrinsic pathway activation by p53 or pro-apoptotic Bcl-2 family members such as Bax and Bad induces permeability of the MOM with release of apoptogenic proteins including cytochrome c. Cytochrome c binds to apoptotic protease-activating factor (APAF)-1 and procaspase-9, leading to apoptosis (Delhalle et al. 2004). Regulation of caspase-9 activation involves inhibitors of apoptosis (IAP) family members such as XIAP, the released mitochondrial protein XMAC/DIABLO, mitochondrial HtrA2 and inhibitor of XIAP (Eldering and Vanlier 2005).

Immature B cells undergo apoptosis when an antigen binds to their surface Ig (BCR), while mature germinal center B cells are activated under some circumstances. Immature B cells also undergo growth arrest and apoptosis when their BCR is cross-linked by antibody against it (Bras and Ruiz-Vela 1999). This apoptosis is inhibited by Bcl-2 and by CD40 ligation. The mechanism of B-cell apoptosis following BCR engagement is, itself, still obscure. The apoptosis appears to be of the intrinsic type and involves caspase-2 and Bcl-2 family proteins Bad, Bim, and Bid (Eldering and Vanlier 2005). These are members of the pro-apoptotic BH3-only branch of the Bcl-2 family, which share structural homology to Bcl-2 only at the BH3 domain (Petros et al. 2004).

In normal germinal center B-cell development, positive selection is dependent on high-affinity BCR, and B cells without a functional BCR, such as HRS cells, would be expected to undergo apoptosis. The mechanisms which save HRS cells from apoptotic cell death have not been fully described, but include a FAS-resistant phenotype, constitutive expression of c-FLIP, and perhaps lineage infidelity (Thomas et al. 2004). Activation of the nuclear factor kappa B (NF-κB) pathway likely plays a role in many if not all cases.

HRS cells express Fas/CD95, which is required for FAS-ligand (CD95-L) induced apoptosis. They also, however, constitutively express caspase-8/FADD-like-IL-1ß-converting enzyme inhibitory protein (c-FLIP), a potent anti-apoptotic mediator normally expressed on B cells with high affinity BCR (Re et al. 2000; Thomas et al. 2004). C-FLIP is an inactive caspase analogue which competitively inhibits caspase-8 and -10 (Irmler et al. 1997). This may provide one mechanism to inhibit apoptosis in the absence of BCR signaling. Increased expression of anti-apoptotic genes encoding regulators including Bcl-2, Bcl-xL, survivin, and NF-κB, as well as downregulation of pro-apoptotic regulators such as Bax, is also thought to play a role in the resistance of HRS cells to apoptosis (Garcia et al. 2003).

2.5 NF-kappaB

NF-κB, a ubiquitous mediator of inducible gene expression in response to inflammatory cytokines, is a dimeric complex of various members of the Rel family; p105/50, p100/52, p65 (RelA), RelB, and c-Rel (Delhalle et al. 2004). These have in common a Rel homology domain (RHD) which allows DNA binding, dimerization, and nuclear localization. NF-κB proteins are normally sequestered in the cytoplasm by inhibitors of kB (IkB). These inhibitors are phosphorylated following cytokine or other stimulation, which leads to ubiquitinylation and degradation by the 26S proteosome. NF-κB then enters the nucleus and activates transcription of target genes. IkB degradation is dependant on activation of the IkB kinase (IKK) complex. IKK is made up of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, IKKγ, also known as NF-κB essential modulator (NEMO). The latter is a regular of NF-κB activation. Bcl-10 promotes activation of NF-κB transcription factors through ubiquitinylation of NEMO (Zhou et al. 2004).

NF-κB is constitutively activated in CHL (Bargou et al. 1996). In turn, it activates a variety of anti-apoptotic gene products including IAP, c-FLIP, and Bcl-2 family proteins (which have competing regulatory effects), and also represses some other pro-apoptotic factors (Bargou et al. 1997). In some cases, mutations of NF-κB inhibitors in Epstein-Barr (EBV)-negative CHL are considered central transforming events (Jungnickel et al. 2000; Cabannas et al. 1999; Emmerich et al. 1999, 2003). In others, amplification of the NF-κB/Rel locus at 2p13-16 causes dysregulation. In EBV-associated HL, activation results from activity of LMP1 and LMP2a (Thomas et al. 2004).

Additionally, alterations in pathways mediated through members of the tumor necrosis factor recep-

tor (TNFR) family, such as CD30, CD40, and receptor activator of NF-κB (RANK), can lead to overexpression of NF-κB. This overexpression results in promotion of a number of signal transducers, cytokines and chemokines associated with CHL, including STAT5a, interleukin-13, and CC chemokine receptor 7 (CCR7) (Hinz et al. 2002).

2.6 Jak/STAT Pathways

Cell survival depends on multiple factors, and in each particular neoplasm different mechanisms leading to survival advantage may be in play. Although the NF-κB pathway appears to play a dominant role in HL, other transcription factors such as AP-1 or signal transducer and activator of transcription (STAT) are also involved. Cytokine interaction with surface receptors leads to intracellular cascades mediated through members of the Janus kinase (Jak) family, which phosphorylate cytoplasmic substrates. Principal substrates include members of the STAT family of proteins, which are central to cytokine signaling. STAT5 is mentioned above. Constitutive activation of STAT6 in HRS cells has been demonstrated in a majority of CHL and is associated with IL-13 signaling (Skinnider et al. 2002). STAT3 is also constitutively phosphorylated (activated) in a majority of cases. It is activated by a wide range of signals including LMP1, CD40, and a number of cytokines and contributes to cell growth, but is not specific to HL (Skinnider and Mak 2002). STAT1 and STAT3 overexpression is characteristic of EBV-associated CHL (Garcia et al. 2003). STAT5a appears to be an important downstream effector of NF-κB and is constitutively upregulated in many cases (Hinz et al. 2002).

2.7 Tumor Necrosis Factor Receptor (TNFR) Family

Tumor necrosis factor receptor (TNFR) and ligand families also play a very large role in CHL. These have broad roles in inflammation and the immune response. TNFRs include TNFRI, TNFRII, CD40, CD30, CD27, OX40, and receptor activator of NF-κB (RANK) (Skinnider and Mak 2002). Ligands include TNF-α, lymphotoxin α (LT-α), and FAS. TNF-α is a mediator of macrophage activity and present in both HRS cells and associated infiltrates, as is LT-α, which is partly homologous to TNF-α.

CD40/CD40L interaction stimulates the T-cell-dependent humoral response. Activation of CD40 requires CD40L on adjacent cells. Activated CD40 is found on HRS cells while CD40L is found in the reactive infiltrates. Their interaction activates NF-κB, mediated by tumor necrosis factor receptor-associated factor (TRAF)-3 proteolysis (Annunziata et al. 2000). Elevated levels of NF-κB in turn maintain c-CLIP, CD40, and CD86 (Hinz et al. 2001). Associated signaling pathways can be activated by EBV LMP1 (Skinnider and Mak 2002).

CD30 is ubiquitously over-expressed in CHL and is likely a primary factor in both diminished apoptosis and proliferation of the disease. Over-expressed CD30 self-aggregates, recruits TRAF2 and TRAF5, and activates NF-kappaB independent of CD30 ligand (Horie et al. 2002b, 2003). Ligation of CD30 promotes proliferation of HL cells in culture, in contrast to an opposite death-inducing effect in anaplastic large cell lymphoma cell lines (Smith et al. 1993).

The mitogen-activated protein kinase (MAPK) / extracellular signal-regulated kinase (ERK) pathway is implicated in the growth and proliferation of several tumors, and appears to be involved in CHL as well. Ligand activation of CD30, CD40, and RANK receptors increases ERK phosphorylation and promotes HRS cell survival (Zheng et al. 2003).

2.8 Tumor Necrosis Factor Receptor-Associated Factors (TRAFs)

Tumor necrosis factor receptor-associated factors (TRAF) refer to a family of proteins involved in the intracellular transduction of members of the TNFR superfamily that promote cell survival and activation of NF-κB. TRAF1 is normally dependent on EBV LMP1 signaling, but TRAF1 and -2 are constitutively activated in CHL, with moderate expression of TRAF4 and 6 (Izban et al. 2000; Siegler et al. 2003). TRAF3 proteolysis is involved in NF-κB activation, as above.

TRAF2 and TRAF5 cytoplasmic aggregation occurs in response to CD30 signaling (Horie et al. 2002a).

2.9 Cytokines and Chemokines

It has been recognized for a long time that patients with HL have impaired cell-mediated immunity. This has been attributed in the past to lymphocytopenia, decrease in the number of CD4+ cells in the peripheral blood, and defects in T lymphocyte function (Hillinger and Herzig 1978).

Recent studies have shown that HRS cells produce numerous cytokines and chemokines which may account for the characteristic background inflammatory cells. In fact, in Hodgkin lymphoma the majority of the tumor mass is due to inflammatory cells which accompany the neoplastic cells. Despite this, an effective immune response against genotypically and immunophenotypically altered neoplastic cells is not rendered. The cause of this failure is one of the central questions in understanding the biology of Hodgkin lymphoma.

Cytokines are potent low-molecular-weight regulatory proteins, and chemokines are cytokines with chemo-attractant properties. These are produced by T-helper cells, macrophages, and other cells. Many, including interleukin (IL)-1, IL-6, IL-7, IL-9, IL-13, and IL-14, have been implicated as growth factors in CHL, while others induce the inflammatory background (i.e. IL-5) or act as immunosuppressive agents [IL-10 and transforming growth factor (TGF)-ß] (Maggio et al. 2002).

Th2 T cells appear to be the dominant T cells in HL, but Th1 cells also play a role. T-helper cells expressing CD4 and producing cytokines are of two primary classes, Th1 and Th2. Th1 cells facilitate cell-mediated immunity and B-cell production of complement-fixing and opsonizing antibodies and require IL-12 for differentiation. Th1 cells produce IL-2, IL-12, interferon (IF)-γ, and other cytokines. IF-γ is also produced by some HRS cells. Th2 cells provide B-cell help for production of non-complement-fixing antibodies, require IL-4, and produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 (Skinnider and Mak 2002).

IL-13 with IL-13-specific receptor chain (IL-13R alpha1) acts as an autocrine growth factor in CHL and activates STAT-6 (Skinnider et al. 2002). IL-13 and IL-4 enhance production of macrophage derived chemokine (MDC), also produced in HRS cells, and lead to attraction of Th2 T-cells which, along with HRS cells, produce more IL-13 and IL-4 in an amplification loop. IL-5 is essential for the growth of eosinophils and is expressed by HRS cells in cases of CHL with tissue eosinophilia. IL-6 induces plasma cell differentiation and possibly acts as an autocrine growth factor. IL-9, a T-cell and mast cell growth factor, is also frequently expressed.

IL-7, produced by stromal cells (Hofmeister et al. 1999), stimulates T-cells in CHL. IL-17, produced in a broad spectrum of T cells, neutrophils, and eosinophils (Kawaguchi et al. 2004), induces proliferation and inhibits apoptosis by activating NF-κB (Maggio et al. 2002). TGF-ß, produced by T cells and HRS cells, is involved in the fibrosis of NSHL. IL-10 inhibits inflammation through IL-2 and IFN-γ suppression and is a B-cell growth factor. It shows a negative influence on response to therapy (Maggio et al. 2002), is produced by EBV (vIL-10) as well as in human cells, and is increased in cases involved with EBV, though most IL-10 in those cases is of human origin (Skinnider and Mak 2002; Maggio et al. 2002). Thymus and activated related chemokine (TARC), normally produced by antigen-presenting cells, is also produced in HRS cells and attracts Th2 T cells which express its receptor, chemokine receptor (CCR)4, thus augmenting the effect of another chemokine, MDC. The chemokine eotaxin, along with IL-5, is involved in eosinophilia in HL by interaction with CCR3 on eosinophils. It is not clear if IL-5 is produced by HRS cells, but it is more likely produced by fibroblasts stimulated by TNF-α, which is produced by HRS cells (Skinnider and Mak 2002).

Protein regulated on activation, normal T-cell expressed and secreted (RANTES), a chemokine produced by Th1 T-cells and also HRS cells, attracts monocytes, T-cells, eosinophils, and mast cells, and interacts with CCR3 and CCR5. It is likely responsible for mast cell increases in some cases (Fischer et al. 2003). Other Th1-associated cytokines increased in CHL include IP-10, Mig-1, MIP-1α, and MIP-1β. The receptor for the latter two, CCR5, is present on lymphocytes in the reactive infiltrates. IL-8, a neutrophil-attractant chemokine, is also expressed by the inflammatory infiltrate.

The cytokine expression profile of HL cell lines themselves, with high levels of IL-10 and TGF-β, resembles that of a subset of CD4+ regulatory T cells distinct from Th1 or Th2 cells (Groux et al. 1997; Malec et al. 2004; Skinnider and Mak 2002; Hsu et al. 1993; Rao et al. 2005). Both of these cytokines can suppress T-cell responses and may play a role in the lack of an effective anti-tumor immune response.

Tumor infiltrating lymphocytes (TIL) in HL are usually hyporesponsive to various stimuli and exhibit impaired ability to mount Th-1 type immune responses (Marshall et al. 2004; Poppema and van den Berg 2000). Furthermore, the presence of regulatory T-cell populations with the ability to suppress the functions of effector T cells through mechanisms such as IL-10 production, cell-cell contact, and CTLA-4 engagement have been demonstrated among TILs (Marshall et al. 2004).

2.10 Cytogenetics

Cytogenetic studies in HL are characterized by genetic instability. Structural and numerical chromosomal abnormalities are observed in HRS cells using karyotyping and in situ hybridization procedures. Aneuploidy and hyperdiploidy are often observed, and the karyotypic abnormalities are often complex. Fluorescent in-situ hybridization combined with immunohistochemical studies (FICTION) have shown that chromosomal abnormalities could be identified in all CD30-positive HRS cells (Weber-Matthieson et al. 1995). Structural abnormalities include alterations of 2p, 3q, 6q, 7q, 9p, 13p, 14p, and 17p. Non-random breakpoints include 3q27, 6q15, 7q22, 11q23, and 14q32 (Re et al. 2002). Recurrent gains of 2p, 12q, and 9p have been noted, and amplifications of 4q16, 4q23-24, 9p23-p24 (involving the JAK2 gene), and 12q14 (MDM2 gene locus) (Re et al. 2002). In addition, in one comparative genomic hybridization (CGH) study, gains or losses of 1p, 19p, 19q, 4q, and 12q were increased (Ohshima et al. 1999). Abnormalities of 2p at the REL locus, in particular, may be involved in Nfkappa-B activation through overexpression of c-REL protein in CHL (Barth et al. 2003). Gains of 2q, 4q, 5q, 6q, and 11q have been noted in NLPHD (Re et al. 2002).

2.11 Gene Profile

Gene expression profiling studies performed on Hodgkin lymphoma cell lines have revealed that HRS cells represent a distinct entity and show an activated germinal center B-lymphocyte phenotype (Kuppers et al. 2003). Specifically upregulated genes include transcription factors such as GATA-3, ABF1, Nrf3, and EAR3, actin-bundling protein Fascin, and the chemokine TARC. Downregulated genes include many B-lineage specific genes encoding for cell surface molecules such as CD19 and CD20, CD52, tyrosine kinases involved in BCR signaling (i.e. Syk, Lyn, Blk), and transcription factors such as Spi-B, Lyl-1, and A-myb (Kuppers et al. 2003). Frequent expression of T-cell transcription factors including GATA-3, T-bet, and c-maf and their target cytokines have been shown by quantitative PCR and immunohistochemical analysis (Atayar et al. 2005). These findings may in part explain the production of cytokines by HRS cells. cDNA libraries from micromanipulated live single HRS cells also show an activated germinal center B-lymphocyte phenotype which resists apoptosis through CD40 and NF-κB signaling (Cossman 2001). They do not show a dendritic cell expression pattern (Cossman et al. 1999).

Increased expression of several cyclins and cyclin-dependent kinases (CDKs) has been demonstrated as well as inactivation of one or more of tumor suppressor pathways including $p14^{ARF}$-p53-$p21^{WAF}$, $p16^{INK4a}$-RB, and $p27^{Kip1}$ (Garcia et al. 2003).

2.12 Association with EBV

Epstein-Barr Virus (EBV) has been widely implicated as a possible etiologic agent in CHL. The idea that HL could be associated with an infectious agent was first proposed by MacMahon (MacMahon 1966). Later, Levine et al. (Levine et al. 1971) demonstrated elevated antibody titres to EBV antigen in HL patients. While epidemiologic and serologic studies provided circumstantial evidence, later molecular studies showed monoclonal EBV sequences in CHL by Southern blot analysis suggesting that the EBV infection occurred prior to the malignant transformation in HRS cells

(Anagnostopoulos et al. 1989). In-situ hybridization studies unequivocally showed EBV sequences associated with HRS cells in approximately one-half of cases of CHL in developed nations, the United States and Japan, and only rarely in LPHL (Uhara et al. 1990; Weiss et al. 1991). Soon after, it was shown that EBV was present in HRS cells of CHL in more than 90% of cases in a developing nation, Peru, somewhat analogous to the geographically variable association of EBV with Burkitt lymphoma (Chang et al. 1993). Geographic variability of EBV expression has been confirmed by many subsequent studies (Weinreb et al. 1996; Thompson and Kurzrock 2004). The relationship of EBV to HL is thought to be causal in a proportion of cases (Jarrett 2003).

Epidemiological studies indicate that EBV is mostly associated with mixed cellularity type HL, shows a male predominance, is more frequent among children under the age of 10 and older patients, compared to young adults, and is also more frequent with lower education level or socioeconomic status (Jarrett 2003; Gandhi et al. 2004). There is accumulating evidence to support an association between EBV-positive HL and infectious mononucleosis (IM), particularly with late first exposure (Hjalgrim et al. 2003; Alexander et al. 2003). There is not, however, a similar association between IM and EBV-negative HL, which constitute about half of cases. EBV expression does not have a proven effect on outcome but may be beneficial in some groups, particularly young adults (Flavell et al. 2003; Gandhi et al. 2004; Krugman et al. 2003).

The transforming ability of EBV was first confirmed in a series of studies in the late 1960s and early 1970s (Nilsson et al. 1971; Miller and Lipman 1973). These studies demonstrated that EBV could transform resting B cells and form clonal populations. Today, evidence on the pathogenetic mechanism of this transformation process is accumulating rapidly (Thompson and Kurzrock 2004; Gandhi et al. 2004; Hammerschmidt and Sugden 2004; Thomas et al. 2002).

EBV infects B cells through binding of its BLLF-1 glycoprotein (gp350/220) to the CD21 molecule which happens to be the C3d receptor. Infected B cells are transformed/immortalized to produce "lymphoblastoid" cell lines (LCL) (Fingeroth et al. 1984; Tanner et al. 1987). The EBV-encoded latent genes are primarily involved in this transformation process (Young et al. 1989). The associated pattern of gene transcription is different from that observed during the lytic cycle of the virus. The lytic cycle includes expression of Epstein-Barr nuclear antigens (EBNAs 1, 2, 3A, 3B, and EBNA-LP), the three latent membrane proteins (LMPs 1, 2A, and 2B), and small polyadenylated RNAs (EBERs 1 and 2). EBV-associated large B-cell lymphomas also show expression of all these latency genes in a pattern called latency type III. The pattern of transcription observed in HL is restricted to expression of EBNA1, LMP1, 2A, and 2B, and the EBER RNAs. It is called latency type II and is the pattern also seen in nasopharyngeal carcinomas and T-cell lymphomas (Deacon et al. 1993).

EBNA 1 is expressed in all EBV-infected cells and is therefore found in all EBV-associated malignancies as well as chronic active infections. This is because EBNA1 is crucial for maintenance of the viral episome, which it tethers to the host chromatin, enabling its transmission to daughter cells and coordinating its replication with cellular DNA. The oncogenic activity of EBNA 1 is controversial, with no direct evidence as of yet, although transgenic mice expressing EBNA1 have been shown to develop follicular lymphomas (Wilson et al. 1996). LMP1, on the other hand, is thought to be the major protein responsible for the transforming effects of EBV. It functions as a constitutively activated tumor necrosis factor receptor (TNFR) and resembles CD40, but acts in a ligand independent manner (Eliopoulos et al. 1997). It regulates cell growth and differentiation and, most importantly, prevents apoptotic death through its interactions with TRAFs (Devergne et al. 1996; Kaye et al. 1996; Izumi et al. 1997).

LMP1 is involved in the upregulation of antiapoptotic proteins, cytokine production, and downregulation of CD99 (Kim et al. 1998, 2000). Its principal transforming activities are due to NF-κB which it activates via the phosphorylation and degradation of IkBα (Herrero et al. 1995; Sylla et al. 1998).

LMP2a, although not crucial for the transformation of B cells, is thought to substitute for BCR signaling and thus function to prevent apoptosis and allow for survival of B cells lacking immunoglobulin (Caldwell et al. 1998; Casola et al. 2004). Furthermore, it is also thought

to have a role in repression of the lytic cycle and has been shown to interfere with normal B-cell gene expression by interfering with global transcription factor regulation in B-cell development, downregulating transcription factors such as TF-E2A, EBF, and Pax-5 and increasing the expression of genes associated with cell cycle induction and inhibition of apoptosis (Portis et al. 2003; Portis and Longnecker 2003). LMP2B, on the other hand, is thought to function as a negative regulator of LMP2A (Longnecker and Miller 1996).

The role of EBERs in EBV-associated HL is still unresolved. It is suggested that they may be involved in IL-10 expression and hence immune evasion (Kitagawa et al. 2000). Transformation induced by EBV in CHL likely rescues HRS cells from apoptotic death, providing just one mechanism of oncogenesis in HL.

2.13 Pathology of Classical Hodgkin Lymphoma

Classical HL is histologically defined as a monoclonal lymphoid neoplasm composed of mononuclear Hodgkin and multinucleated Reed-Sternberg cells, collectively termed Hodgkin Reed-Sternberg cells (HRS), present in variable numbers within an immunoreactive background (lymphocytes, eosinophils, neutrophils, histiocytes, plasma cells, fibroblasts, and collagen) (Stein et al. 2001a). HRS are felt to be the malignant cells even though they are usually in the minority, with the reactive milieu present in response to cytokines produced by the tumor. The Reed-Sternberg (RS) cell is a large cell with abundant cytoplasm and a bi- or multilobated nucleus containing a prominent inclusion-like eosinophilic nucleolus within each lobe. Frequently in clinical practice, there is a tendency to focus on cells with a bilobed nucleus of which the nucleoli impart an owl-eye appearance, but multilobated forms are often more frequent, particularly in the nodular sclerosis subtype, and are of equal diagnostic importance (Figs. 2.1–2.6). When only mononuclear neoplastic cells are seen, it is more difficult to distinguish the disease from NHL (Fig. 2.7). "Mummified" cells are degenerating or apoptotic HRS cells which appear darkly stained and contracted, though otherwise often retain the morphology of HRS (Fig. 2.8). Cells similar to Hodgkin cells but without the inclusion-like nucleoli are frequent in NSHD, often in clusters also containing typical HRS cells, and are referred to as "lacunar" cells (Fig. 2.9). This term comes from cytoplasmic retraction in formalin-fixed tissue which gives the appearance of lacunae. It is interesting that Dorothy Reed did not describe these. (She appears to have utilized alcohol and mercuric Zenker's fixative, the latter of which along with B5 fixative and formalin compounds containing zinc provides better nuclear detail than formalin and does not produce "lacunae". Zenker's came into common use again for a time in the 1970s when NHL classifications required improved fixation, but has been largely supplanted.)

The immunoreactive background of CHL consists predominantly of small lymphocytes with round to slightly irregular nuclei which are mostly reactive T cells. Scattered immunoblasts are sometimes seen, particularly when a lymph node is only partially involved by tumor, in which case residual normal nodal

Table 2.1 Immunohistochemical findings in histologic types of Hodgkin lymphoma

	CHL	LRCHL	NLPHL
CD30	++	++	–
CD15	+/–	+/–	–
CD20	–/+	–/+	++
CD79a	–/+	–/+	++
J-chain	–	–	++
BSAP	+/–	+/–	++
BOB.1	–/+	–/+	++
OCT.2	–/+	–/+	++
MUM.1	++	++	++
BCL-6	–	++	++

Abbreviations: CHL = classical Hodgkin lymphoma; NLRCHL = nodular lymphocyte rich classical Hodgkin lymphoma; J-chain=immunoglobulin joining chain; BSAP=B-cell specific activator protein; BOB.1= B-cell Oct-binding protein 1; OCT.2=octomer protein 2; MUM.1=multiple myeloma-1

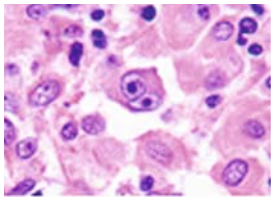

Figure 2.1
A classic Reed-Sternberg cell with bilobed nucleus

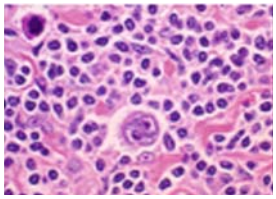

Figure 2.2
A Reed-Sternberg cell with slightly different morphology

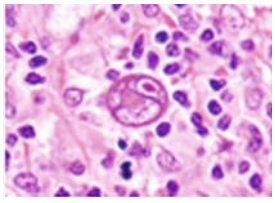

Figure 2.3
Slight degeneration in a Reed-Sternberg cell

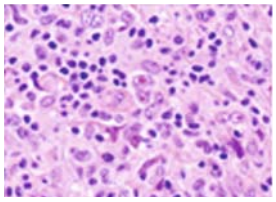

Figure 2.4
A small Reed-Sternberg cell

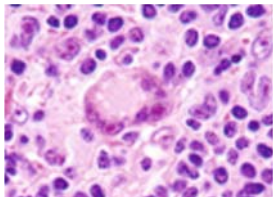

Figure 2.5
A multinucleated Reed-Sternberg cell with wreath-like nucleus

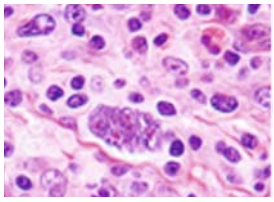

Figure 2.6
A highly lobated Reed-Sternberg cell nucleus

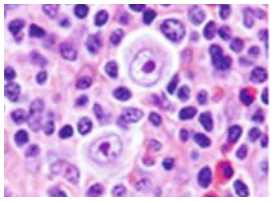

Figure 2.7
Mononuclear Reed-Sternberg cell variants

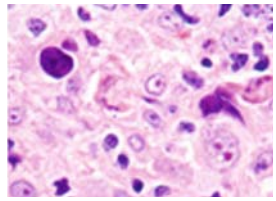

Figure 2.8
Two mummified cells as well as a Reed-Sternberg cell

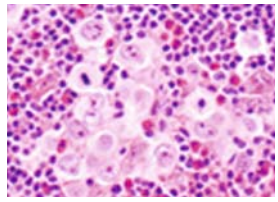

Figure 2.9
A cluster of lacunar cells in NSHL

structure is present. Plasma cells and eosinophils are usually present in varying numbers in the reactive background. They are, however, in no way specific for HL and are not always present.

2.14 Immunophenotype of Classical HRS Cells

The immunophenotype of HRS cells in paraffin sections is somewhat puzzling in terms of the putative cell of origin, but it is generally characteristic and diagnostically helpful (Table 2.1). It is especially helpful when morphologic features overlap with NHL such as anaplastic large cell lymphoma (ALCL) or large B-cell lymphoma. The most common phenotype of HRS cells is expression of CD30, CD15, and fascin with absence of CD45 (Kurtin and Pinkus 1985) and T-cell markers. B-cell associated markers CD20 (20%) and CD79a (10%) are expressed in a minority of cases (Korkolopoulou et al. 1994; Tzankov et al. 2003) and are usually focal and weak. Occasionally, strong surface CD20 expression may be noted. CD20 expression may be an adverse feature (Portlock et al. 2004). B-cell transcription factors Oct.2 and BOB.1 are weak or absent (Stein et al. 2001c), as are immunoglobulin J chain, CD75 (Stein et al. 2001a), and bcl-6 (Brauninger et al. 2003). B-cell specific activator protein (BSAP) is, however, expressed in 90% of cases (Foss et al. 1999). Light chain immunoglobulin antibodies may show generalized cytoplasmic labeling which appears polyclonal and nonspecific.

CD30 is variably expressed in the Golgi and cytoplasm and on the surface of almost all cases of classical HL (Fig. 2.10) (Stein et al. 1981, 1985, 2001a). It is not, however, specific and is also expressed by anaplastic large cell lymphoma and by lymphoid tissue stimulated by EBV, HTLV-1, and *Staphylococcus aureus* (Stein et al. 1985). CD15 is a less sensitive marker of CHL but is very helpful when found in conjunction with CD30 (Norton and Isaacson 1985). Approximately 80% of HRS cells in CHL express CD15 (Hall and D'Ardenne 1987), but it may be weak and only present in a minority of cells (Stein et al. 2001b), usually in the Golgi (Fig. 2.11). CD45 is usually negative (Kurtin and Pinkus 1985). ALK1 is consistently not expressed, nor are histiocyte-associated antibodies including CD68, nor epithelial membrane antigen (EMA). Fascin is expressed in virtually all cases of CHL, but is also present in ALCL and expressed by dendritic cells, activated B-cells, and others (Pinkus et al. 1997; Fan et al. 2003). Fascin staining is useful to highlight HRS cells and, when negative, argues for another diagnosis.

Expression of EBV-encoded LMP1 is detected in EBV-infected HRS cells in 10–40% of NSHL and 75% of MCHL (Stein et al. 2001b). CHL shows limited expression of EBV nuclear antigens, consistent with multistep pathogenesis, with expression of EBNA-1 accompanied by LMP1, LMP2A, and LMP2B (Deacon et al. 1993). In situ hybridization (ISH) for small EBV-encoded ribonucleotides (EBER-1 and -2) is detectable in similar numbers of cases (Fig. 2.12) (Herbst et al. 1992). Detection of EBV in HRS cells tends to support a diagnosis of CHL, but EBV may also be present in large B-cell NHL, particularly in immune-suppressed patients, and EBV mononucleosis itself often mimics HL.

The number of antigens and other targets utilized in diagnostic studies has been largely restricted in the past to those considered to be highly specific for a diagnosis. Immunophenotyping combined with ISH may also provide insight into apoptotic and proliferative pathways by using antibodies to semiquantitatively measure and correlate the expression of various molecules with findings from genetic studies and direct protein analyses. Tissue array techniques allow comparison of immunohistochemically detectable antigen expressions across large numbers of cases and may minimize technical cost and labor. Their use, however, requires a great deal of organization to collect appropriate cases, such as from a clinical trial. They do not eliminate all variables associated with immnohistochemical studies, such as those associated with fixation.

Bcl-2 family expression has been analyzed in 26 cases of CHL with more than 50% showing expression of Bax, Bcl-xL, and Bcl-x, and 44% with immunohistochemically detectable Bcl-2 expression. Results suggested that pro-apoptotic Bax was overwhelmed by Bcl-xL or Bcl-2 (Kim et al. 2004). Another study compared expression in 62 cases of CHL of Bcl-2, p53, retinoblastoma gene (Rb), p21, Ki67 (MIB 1), and topoisomerase IIalpha (TopoIIalpha), along with EBV status and apoptosis (Wang and Taylor 2003). Aggressive disease was associated with increased Ki-67 and TopoIIalpha, and loss of Rb and p21. Another study of 288 cases utilized IHC and tissue arrays to analyze expression of 29 genes. Results showed marked overexpression of cyclin E, CDK2, CDK6, STAT3, Hdm2, Bcl-2, Bcl-X_L, survivin, and NF-κB proteins, with alterations in both proliferative checkpoints and apoptosis regulators (Garcia et al. 2003). It is highly likely that such studies will dramatically change our view of the role of immunohistology in delineating the biology as well as diagnostic features of this disease.

2.15 Flow Cytometry

Flow cytometry is not diagnostic in HL, as it characterizes the inflammatory and background milieu rather than the malignant cells. HRS cells constitute a small minority of cells in most cases and are mostly too large for analysis by standard hematologic flow cytometric methods. These studies are often performed, however, on initial biopsies for which the differential diagnosis includes non-Hodgkin lymphomas, and the results may be confusing.

Most often flow cytometry shows a predominance of reactive T cells with CD4 predominance and an elevated CD4:CD8 ratio (Pituch-Noworolska et al. 2004), occasionally as high as 12:1 or more, in our experience, and 28:1 in the literature (Gorczyca et al. 2002). This may suggest peripheral T-cell lymphoma,

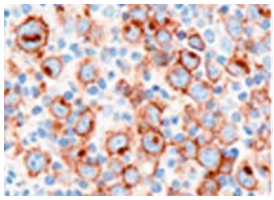

Figure 2.10
CD30 labeling of surface membrane and Golgi region

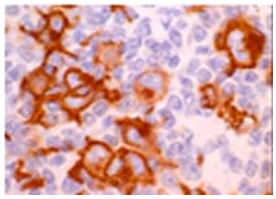

Figure 2.11
CD15 labeling of HRS cells

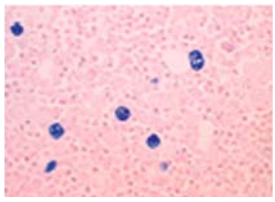

Figure 2.12
EBER-1 nuclear labeling of HRS cells in MCHL

but there is no loss of pan T-cell markers, no clonal T-cell receptor rearrangement, and malignant cells do not label with T-cell markers by immunocytochemistry or immunohistochemistry. When HL involves the thymus, flow cytometry may show immature thymic T cells and suggest lymphoblastic lymphoma, but attention to patterns of surface CD3 and light scatter may minimize this problem (Gorczyca et al. 2004). Morphologic examination is, however, important.

Southern blot and routine polymerase chain reaction (PCR) molecular analysis of immunoglobulin (Ig) and T-cell receptor (TCR) genes usually show polyclonal populations. While most HRS cells within a tumor (or nodule) share a clonal Ig heavy chain rearrangement as detected from microdissected cells (Marafioti et al. 2000), the number of clonal cells is typically below the sensitivity of assays performed on homogenized tissue sections. These assays are typically designed to detect only substantial clones with limited false positivity rates. Approximately one-third of cases will show IgH rearrangement by clinical PCR (Manzanal et al. 1997). A very small proportion (1-2%) of CHL are considered to be of T-cell origin (Seitz et al. 2000), and clonal TCR rearrangement is only rarely seen, though sometimes in HL associated with cutaneous T-cell lymphoma (Kadin et al. 2001). T-cell receptor clonality suggests an alternate diagnosis of peripheral T-cell lymphoma, or concurrent separate lymphoma (Brown et al. 2004).

2.16 Histologic Classification of CHL

2.16.1 Nodular Sclerosis HL

Nodular sclerosis is the most common type of HL in young people. It is most frequent in adolescents and young adults and is the only HL subtype with a slight female predominance, which is also unusual for all lymphomas in young people. Most cases (80%) involve the mediastinum, and there is evidence in some cases of derivation from a thymic B cell, similar to mediastinal large B-cell lymphoma (Copie-Bergman 2002;). Fibrosis in NSHL is speculated to be a recapitulation of thymic septae. Application of anticytokeratin antibodies in mediastinal NSHD often reveals entrapped thymic epithelium, which simply implies involvement of usually involuted thymus (Policarpio-Nicolas and Hutchison 2002). Many cases diagnosed as mixed cellularity HL from fragmented small mediastinal biopsies are actually samples of NSHL in which the tumor fragments along fibrous septae and the fibrosis itself is not well visualized.

A defining histologic feature of NSHL, regardless of location, is the formation of clusters of HRS and lacunar cells (mononuclear Hodgkin cells with only moderately prominent nucleoli) (Fig. 2.13). The number and contiguous arrangement of these cells vary, and their formation often precedes fibrosis. Lymph nodes involved by spread of NSHL first show scattered loose clusters of tumor cells, which increase in prominence while there is lymph node capsular fibrosis. Fibrous bands extend from the capsule to eventually surround clusters and form nodules.

The histology of early involvement, presenting in about 1/3 of pediatric cases, is often called the "cellular phase" of NSHL and resembles mixed cellularity type except for the clustering of tumor cells and usually some capsular fibrosis (Norris et al. 1975). In well developed histology, fibrous bands surround variable numbers of tumor cell clusters (Fig. 2.14), which may contain sheets of HRS and lacunar cells and sometimes

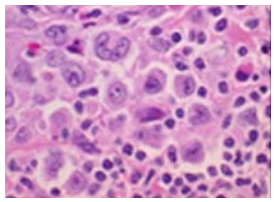

Figure 2.13
A cluster of HRS cells and lacunar cells in NSHL

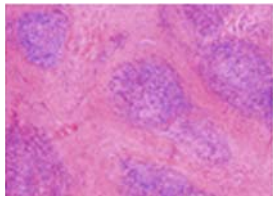

Figure 2.14
Characteristic fibrosis in NSHL

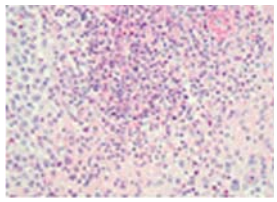

Figure 2.15
An area of necrosis in NSHL

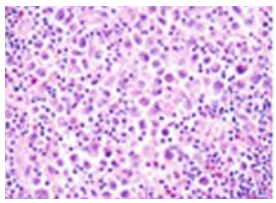

Figure 2.16
A sheet of pleomorphic HRS cells in NSHL, type II

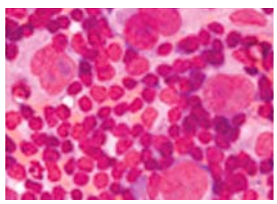

Figure 2.17
Wright Giemsa-stained touch imprint of classical HL

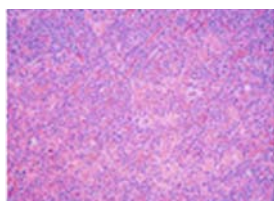

Figure 2.18
Low power view of MCHL

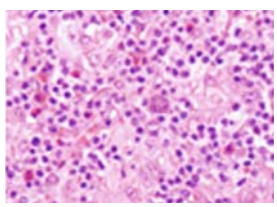

Figure 2.19
Higher power view of MCHL with HRS cells, lymphocytes, histiocytes, and occasional plasma cells and eosinophils

undergo central necrosis (Fig. 2.15). Occasionally, necrotic nodules are surrounded by palisaded histiocytes similar to benign granulomatous disease (such as cat-scratch or lymphogranuloma venereum), and only rare neoplastic cells are notable among the histiocytes.

Cases of NSHL with nodules containing sheets of tumor cells with or without necrosis have been called "syncytial variant" or lymphocyte depleted phase (Strickler et al. 1986). In a grading system proposed by the British National Lymphoma Investigation (BNLI), cases are designated grade II when >25% of nodules contain reticular or pleomorphic lymphocyte depletion, if >80% show fibrohistiocytic lymphocyte depletion, or if > 25% or nodules contain bizarre pleomorphic HRS cells without lymphocyte depletion (MacLennan et al. 1989) (Fig. 2.16). Other cases are designated grade I. This has had prognostic significance in some studies but is not a generally accepted prognostic indicator. A more recent proposal designates high risk based on presence of tissue eosinophilia (>5% of cells in nodule), lymphocyte depletion (<33% lymphocytes in the whole section), or > 25% bizarre/anaplastic HRS cells (von Wasielewski et al. 2003).

Cases with advanced involvement may show fibrous obliteration of nodules. Occasionally, a case of apparently obvious NSHD will exhibit only rare HRS cells even on examination of multiple sections. Sometimes HRS cells can be seen on touch imprints in these cases (Fig. 2.17).

2.16.2 Mixed Cellularity HL

Mixed cellularity HL is less common than NS and comprises about 25% of CHL with a male predominance (70%). It usually presents in the peripheral lymph nodes of the upper body and is often at an advanced stage (III or IV). It is histologically characterized by diffuse node involvement by a polymorphous infiltrate of lymphocytes with variable numbers of histiocytes, eosinophils, and plasma cells and with HRS cells evenly scattered throughout (Fig. 2.18). Bilobed HRS cells are usually not difficult to find and generally outnumber multinucleated variants (Fig. 2.19). Lacunar cells are typically not present, but some cells resembling them may be seen. Clustering of epitheloid histiocytes is common, may include Langhans's giant

cells, and may resemble infectious granulomas. Granulomatous inflammation, as noted in the 19th century, may occur in patients with HL due to impaired cellular immunity, so the presence of granulomas following therapy is not diagnostic of recurrent HL.

Partial involvement of a lymph node by MCHL often involves the paracortex or T-zone of the lymph node with sparing of germinal centers. This is referred to as "interfollicular" HL. Formerly, all cases of interfollicular HL were categorized as MC (Lukes and Butler 1966), but this is no longer the case, and NSHL may also partially involve the lymph node. Interfollicular MCHL can be difficult to differentiate from viral lymphadenopathy. Lymph nodes that show discrete zones of pale eosinophilic coloration at low power should be scrutinized carefully for HRS cells hiding in the background cellularity. The immunophenotype of MCHL is that of other classic HL. EBV is more frequently found in MC (~75%) than in NS.

2.16.3 Nodular Lymphocyte Rich CHL

NLRCHL is an uncommon form of HL that was most likely included with lymphocyte predominance HL prior to immunophenotyping. It became noticed when a very large series of cases of HL diagnosed as lymphocyte predominant were examined and found by immunophenotyping to contain many cases of classical HL (Diehl et al. 1999). It consists of nodules or a diffuse background of small lymphocytes with few or no neutrophils or eosinophils, and only occasional scattered HRS cells (Fig. 2.20). Nodular forms show large, closely spaced nodules with little intervening paracortex. Small germinal centers are eccentrically located within expanded mantle zones and best seen by labeling the dendritic meshwork with CD21. HRS cells may be found within the mantles as well as elsewhere in the nodules (Figs. 2.21 and 2.22). Diffuse cases are distinctly uncommon, and the background consists of T cells. HRS label similarly to those in other CHL. The disease is usually localized at diagnosis (stage I or II), and the prognosis may be similar to lymphocyte predominant HL. Bcl-6 has been reported to be positive in neoplastic cells (Kraus and Haley 2000). This type of CHL appears to be uncommon in pediatric populations.

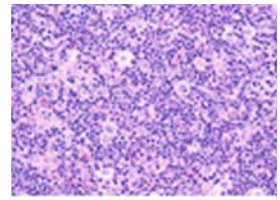

Figure 2.20
NLRCHL resembling NLPHL, but showing the phenotype of classical HL

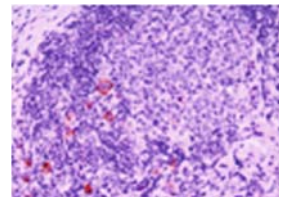

Figure 2.21
NLRCHL with CD30+ cells in the mantle of a reactive follicle

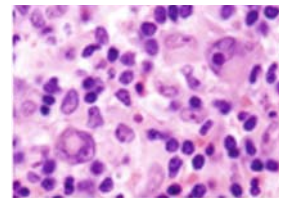

Figure 2.22
HRS variant cells in other areas of the case seen in Fig. 2.21

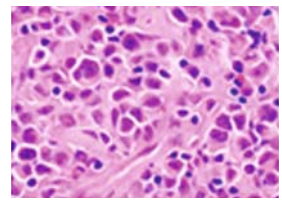

Figure 2.23
Sheets of tumor cells in LDHL

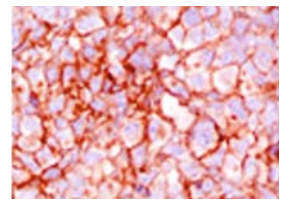

Figure 2.24
CD30 in LDHL

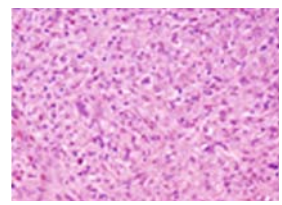

Figure 2.25
An area of diffuse fibrosis and lymphocyte depletion in LDHL

2.16.4 Lymphocyte Depleted HL

LDHL is rare but occasionally occurs in children. It typically presents with advanced disease and often involves the bone marrow, retroperitoneal lymph nodes, and/or abdominal organs, with frequent constitutional symptoms. HRS are abundant (Figs. 2.23 and 2.24), small lymphocytes are few, and fibrosis is often diffusely present. A sarcomatoid appearance is not uncommon (Fig. 2.25). The WHO classification does not provide a threshold number of cells to diagnosis LDHL. These tumors are difficult to distinguish from T or B NHL or other tumors and overlap with both MC and advanced NSHL with grade 2 histology and areas of obliterative fibrosis. The HRS cells label similarly to other CHL.

2.17 Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL)

NLPHL is a monoclonal B-cell neoplasm (Stein et al. 2001c). The histology shows effacement of lymph node architecture by a nodular infiltrate of small B cells with an associated follicular dendritic network and scattered or clustered large cells referred to as lymphocytic and histiocytic (L&H or LH) cells (Figs. 2.26 and 2.27). Nodularity may be vague. L&H cells are variable in cytology but are most often described as exhibiting large nuclei with vesicular chromatin, moderately prominent nucleoli, and convoluted nuclei resembling popcorn ("popcorn cells") (Fig. 2.27). Cytoplasm is moderate in amount and clear, retracted, or slightly eosinophilic. Frequently, the neoplastic cells show round to oval nuclei with distinct eosinophilic or basophilic nucleoli, but much less prominent than the inclusion-like nuclei in HRS cells of CHL (Fig. 2.28). Occasional cells resembling HRS may be seen, but rarely are numerous. The phenotype of these cells is different than classical HRS cells. L&H cells are loosely aggregated in nodules as well as scattered about. In some cases, they coalesce, form sheets, and the disease may progress to diffuse large B-cell lymphoma.

L&H cells are mature B cells, expressing CD45, CD20, CD22, CD79a, bcl-6, BOB.1, Oct-2, CD75, and usually J-chain (Fig. 2.29). CD30 and CD15 are usally absent, and EMA is frequently expressed. The nodules are composed predominantly of B cells with admixed CD3/CD57-positive T-cells which often form rosettes around L&H cells (Fig. 2.30). These are not always present but are diagnostically helpful when they are. Nodules contain a follicular dendritic network, highlighted by staining with CD21, CD35, or CD23 antibodies (Pileri et al. 2002). Cytoplasmic Ig is present but does not appear clonal even though they have been proven to be monoclonal B cells by single cell PCR (Stein et al. 2001b). Southern blot assays for clonal IgH gene rearrangement are negative, and clinical PCR for IgH is similarly negative in the majority of cases due to sensitivity of the assays, which are designed for detecting clonality in NHL.

Less is known about NLPHL than other forms of the disease. This is in part because of its rarity, constituting less than 5% of HL, its usual indolence, and lack of associated cell lines. The diagnosis is important, however, due to differences in behavior and outcome compared with other similar appearing tumors, particularly T-cell and histiocyte rich large B-cell lymphoma, and also NLRCHL. L&H cells are of germinal center or postgerminal center derivation with somatic mutations of Ig genes which are ongoing in about half of studied cases (Braeuninger et al. 1997; Pileri et al. 2002). Progressive transformation of lymph node germinal centers (PTGC) has been described as a precursor lesion to NLPHL, but there is no proven relationship. Most cases of PTGC do not lead to lymphoma (Ferry et al. 1992; Osborne et al. 1992).

With some exceptions, L&H cells retain most normal markers of B-cell differentiation, in contrast to HRS of CHL. Lyn kinase, a B-cell switch molecule which is overexpressed in chronic lymphocytic leukemia (Contri et al. 2005) is diminished in NLPHL (Marafioti et al. 2004).

While NLPHL is considered to be an indolent or even benign disease, a large clinical study of more than 400 cases (mostly adults) diagnosed as lymphocyte predominant Hodgkin's disease (LPHD) in the Revised European-American Lymphoma (REAL) classification provides some interesting lessons (Diehl et al. 1999). Almost 50% of cases were misclassified and actually represented CHL, mostly NLRCHL (lymphocyte rich classical Hodgkin disease, LRCHD, in the REAL).

LPHD could not be classified correctly without immunophenotyping. When adjusted for stage, 20% of cases of LPHD were stage III or IV, and those did no better than CHL. Furthermore, LPHD showed a higher frequency of multiple relapses. The favorable outcome of this rare disease (3.3% of 6000 cases in the study by Diehl et al.) may be due to usually limited stage and young age.

2.18 Hodgkin Lymphoma in the Setting of HIV Infection

The incidence of Hodgkin lymphoma is increased in patients with HIV, though it is not as frequent as non-Hodgkin lymphoma and is not an AIDS-defining illness. A majority are in young men with advanced HIV infection, low CD4 counts, history of intravenous drug abuse, and advanced disease at diagnosis (Tirelli et al. 1992; Thompson et al. 2004). The morphology and immunophenotype are similar to other CHL. Cases with CNS involvement, with and without EBV association, have been described (Massarweh et al. 2003). They also have a higher incidence of adverse histologic types, aggressive course, and poor response to therapy compared with others (Spina et al. 2000). There is greater association with EBV and poor survival, particularly in young adults, but the outcome appears to be improving with highly active antiretroviral therapy (Glaser et al. 2003). EBV association is present in 80–100% and shows high levels of LMP1 expression (Dolcetti et al. 2001).

HIV-associated HL appears to be rare in children, limited mostly to case reports, but HL is reported to be increased in children with HIV (Biggar et al. 2000). In one case, an 8-year-old boy with HIV and EBV-associated HL, fatal hemophagocytic syndrome occurred (Preciado et al. 2001).

2.19 Bone Marrow Involvement and Histologic Staging

Staging of HL by the Ann Arbor system (Carbone et al. 1971) or more recent modifications requires knowledge of extent of involvement of lymph nodes and involvement of liver, spleen, and bone marrow. While imaging studies now provide much of this information, bone marrow involvement is still evaluated in most cases by direct pathologic examination of the marrow. Bone marrow examination is usually performed by aspiration and/or trephine biopsy, but the trephine biopsy is required for detection of most cases of HL (Howell et al. 2002). Bilateral biopsy is currently recommended for patients with intermediate to advanced stage disease and for localized disease with B symptoms or adverse risk factors (Cavalieri et al. 2005).

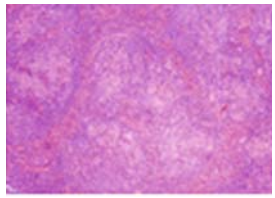

Figure 2.26
Low-power view of nodular LPHL

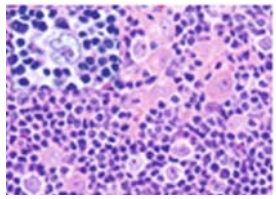

Figure 2.27
NLPHL with L&H cells. Insert shows a "popcorn" L&H cell

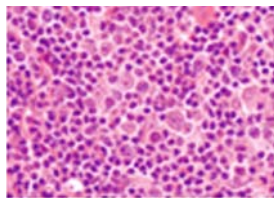

Figure 2.28
Another case of NLPHL with L&H cells

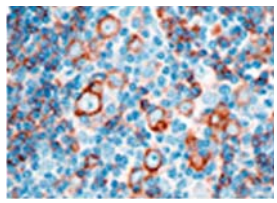

Figure 2.29
CD20-positive L&H cells in NLPHL

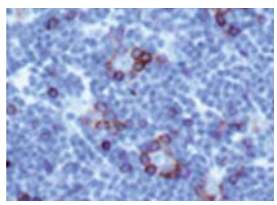

Figure 2.30
CD3+ T cells resetting around neoplastic B cells in NLPHL

Marrow involvement is present at initial diagnosis in approximately 10-15% of patients and is more common in older males with constitutional symptoms (Brunning and McKenna 1994). The frequency varies with histologic type from 5% for NS, to 10% for MC, and >50% for LD (Kurtin 2001). The presence of anemia, leucopenia, and/or thrombocytopenia unrelated to therapy may be associated with marrow involvement (Weiss et al. 1975), although this is not well substantiated. Neutrophilia, eosinophilia, thrombocytosis, and anemia are common and are likely associated with cytokine stimuli.

The marrow involvement in CHL, as in lymph nodes, consists primarily of variable numbers of inflammatory cells, fibrosis, and often very few HRS. When present, the involvement is often diffuse but may be focal, and it may show areas of diffuse and focal involvement even in the same biopsy. The appearance is often that of a granulomatous infiltrate (Fig. 2.31). Frequently, in addition, patients with HL have secondary inflammatory infiltrates due to cytokines and stimulation and/or secondary infection. Thus, the presence of neoplastic cells must be confirmed to diagnose marrow involvement. This may be done by morphology, in which the accepted norm is the presence of at least one HRS cell, either mononuclear, bilobed, or multinuclear variant (Brunning and McKenna 1994) (Fig. 2.32). The use of immunohistochemistry is helpful to find neoplastic cells which are morphologically difficult to detect, and to confirm their presence, but does not replace morphology.

Marrow involvement occurs in less than 3% of cases of NLPHL, but its presence is an adverse finding. Most of these patients have other evidence of advanced disease, and it is possible that this represents transformation to large B-cell lymphoma. It is difficult by any criteria to distinguish this from T-cell rich large B-cell lymphoma (Khoury et al. 2004).

Although staging laparotomy is no longer routinely performed, when it is done, careful attention to gross and microscopic findings are required to accurately assess the extent of the disease (Kurtin 2001). Specimens examined include intra-abdominal lymph nodes, spleen, and liver as well as bone marrow samples. The anatomic location of lymph nodes must be carefully recorded, and particular attention must be paid to gross examination of the spleen, with sectioning at 2 mm intervals. The number of nodules should be noted, since the presence of six or more confers a greater risk of recurrence. The diagnosis is based on finding HRS cells in the characteristic inflammatory background. The earliest involvement of the lymph nodes is seen at the junctions of the follicles and paracortex and in the spleen in the T-zones of the white pulp. The liver is rarely involved without the spleen.

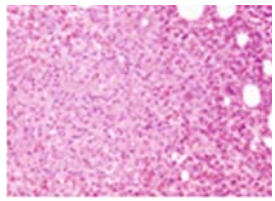

Figure 2.31
A vague granulomatous area in the bone marrow, with a single HRS cell

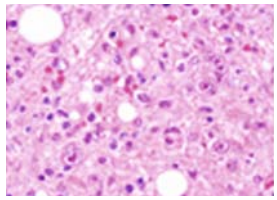

Figure 2.32
Several HRS cells in another area of the marrow in Fig. 2.31

2.20 Pathology of Relapse and Second Malignancy

With modern therapy, a successful long-term outcome is achieved for most patients, but relapse does occur in a minority. In most instances when a relapse occurs at an untreated site, the original histologic appearance is maintained. The morphologic appearance, however, becomes more pleomorphic and bizarre when a relapse occurs at a previously irradiated site. Involvement of extranodal sites is common in late relapses (Grogan et al. 1982).

The risk of developing non-Hodgkin lymphoma (NHL) following HL is increased compared with the general population, with a cumulative risk of 4.1% at 20 years (van Leeuwen et al. 1994). Synchronous or metachronous occurrence of HL and NHL occurs but is rare. When it does, the NHL is usually derived from B cells, though some show T-cell phenotype (Casey et al. 1990; Tinguely et al. 2003; Rosenquist et al. 2004; Brown et al. 2004). BCR and TCR gene rearrangement studies performed on paired specimens have demon-

strated the presence of the same rearranged clone in both tumor specimens in a subset of these cases. This finding suggests that at least a subset of these lymphomas are clonally related and that they either share a common precursor which shows genomic instability or that one arose by transformation of the other (Brown et al. 2004).

In children surviving HL, there is an increase in acute leukemia, primarily in the years immediately following therapy. (Kaldor et al. 1990; Metayer et al. 2000). This effect subsides by 20 years, but thyroid, respiratory tract, digestive tract, and female breast cancers are also increased for HL survivors treated in childhood or as adults (Dores et al. 2002).

2.21 Differential Diagnosis of HL

The pathologic differential diagnosis of HL varies with patient age, histologic type, and anatomic location. Mediastinal tumors are often the most problematic due to the frequency of small biopsies as well as a variety of disparate lesions which may resemble each other in small samples. Lesions which mimic HL include reactive conditions and benign tumorous growths as well as other malignancies. Many lymphoid neoplasms may contain cells resembling HRS cells, though characteristics of the primary disorder usually dominate.

Infectious mononucleosis is a common mimic of CHL. These infectious lymphadenopathies are most often due to EBV, but other viruses, particularly cytomegalovirus, are also implicated. The clinical findings show cervical (often posterior cervical) lymphadenopathy with pharyngitis, malaise, low-grade fever, and often splenomegaly. The monospot test is positive, and there are rising titers of IgG and IgM viral capsid antibodies (Hess 2004). Histology shows reactive features with nonuniform expansion of interfollicular areas with sheets and nodules of immunoblasts showing frequent mitoses and variable Hodgkin-Reed-Sternberg-like cells, plasma cells, histiocytes, abundance of post-capillary venules, and necrosis (Strickler et al. 1993; Childs et al. 1987). Immunoblasts and HRS-like cells are often CD30-positive, predominantly B-cell phenotype with variable T cells, but lacking CD15 expression (Segal et al. 1994; Anagnostopoulos et al. 1995; Reynolds et al. 1995). Other viral and bacterial infections may produce abundant immunoblasts and mimic HL.

Hypersensitivity reactions, such as due to phenytoin (Dilantin) therapy, also mimic HL. Patient symptoms include fever, rash, lymphadenopathy, hepatitis, and eosinophilia (Powers and Carson 1987). The lymph node findings may also mimic infectious mononucleosis and include HRS-like cells (Dorfman and Warnke 1974).

Anaplastic large cell lymphoma (ALCL) frequently resembles CHL, particularly type II NSHL and LDHL. This is less of a diagnostic dilemma now that ALCL is well recognized, but the morphologic features often overlap a great deal (Delsol et al. 2001). ALCL comprises 10–15% of pediatric lymphomas, while HL is more frequent and comprises slightly more than half of cases. Many ALCL contain HRS-like cells, and morphologic variants of ALCL show distinct Hodgkin-like features. Sclerosis with nodule formation occurs in formation occurs in some cases (Benharroch et al. 1998). While lymph node sinus involvement is frequent in ALCL, it is not constant and also occurs in CHL, as first described by Dorothy Reed. Both diseases are characterized by strong CD30 expression, but there are substantial differences. CHL is a disease of B cells while ALCL is usually demonstrated to have T-cell lineage, though up to 30% do not show T-cell markers. Demonstration of T-cell markers or T-cell gene rearrangement is strong evidence of ALCL rather than CHL. ALCL is also sometimes CD45-positive while HL rarely is. In children, adolescents, and young adults, most cases of ALCL are associated with ALK translocations and show reactivity with anti-ALK antibodies. ALCL in young people typically shows a phenotype of CD30+/ALK+ with negative CD45 and one or more T-cell markers or T-cell gene rearrangement. CHL shows CD30+/CD15+ with negative T-cell markers and negative T-cell receptor gene rearrangement and variable, often negative, B-cell markers/gene rearrangement. Clonal Ig gene rearrangement is consistent with HL when neoplastic cells are abundant. ALCL only occasionally shows the inclusion-like nucleoli characteristic of CHL.

Primary cutaneous CD30-positive T-cell lymphoproliferative disorder includes primary cutaneous ALCL (formerly known as atypical regressing histiocytosis)

and lymphomatoid papulosis (Ralfkiaer et al. 2001). These cutaneous disorders have relapsing/remitting courses and morphologically contain pleomorphic cells which often resemble HRS cells. They express CD30 but not ALK or CD15. T-cell markers and/or gene rearrangements are usually present. Although CHL only rarely involves the skin, there may be an association between LP and HL (Beljaards and Willemze 1992; Kadin 1991).

Peripheral T-cell lymphomas (PTCL) may occasionally contain HRS-like cells and show a CD30+/CD15+ phenotype (Barry et al. 2003). Attention to lineage-specific markers and genotype may be required to make the correct diagnosis. Cases have been described of peripheral T-cell lymphoma with Reed-Sternberg cells of B-cell phenotype/genotype associated with EBV infection (Quintanilla-Martinez et al. 1999). This is postulated to be a possible precursor to HL following PTCL.

Undifferentiated carcinoma of the nasopharyngeal type (UDNT; nasopharyngeal carcinoma) frequently mimics CHL in adults. Some cases show eotaxin expression with eosinophilia and share some molecular features with HL (Charafe-Jauffret et al. 2003). Immunophenotyping with anticytokeratin antibodies should be diagnostic.

Plasma cell type of Castleman's disease, with lymph node hyperplasia and sheets of plasma cells, may superficially resemble CHL. Cases have also been described with concurrence of the two disorders (Frank et al. 2001; Saletti et al. 1999).

Mediastinal tumors which enter into the differential diagnosis of HL include non-Hodgkin lymphoma, carcinomas, either of thymic or metastatic origin, thymomas, and germ cell tumors including benign and malignant teratomas, seminoma, and malignant nonseminomatous germ cell tumors (Wakely 2002; Dulmet et al. 1993). For most of these, careful attention to morphology, clinical and radiologic findings, and immunophenotype is essential to avoid incorrect diagnoses.

Thymomas are difficult to evaluate versus HL because HL often involves the thymus, and small biopsies or aspirations will contain thymic material which may or may not be part of the tumor. Phenotyping will reveal thymocytes, and it is possible to make a mistaken diagnosis of lymphoblastic lymphoma or to miss a HL. Imaging of thymomas often shows an encapsulated and/or pedunculated tumor with a clinical history of slow growth.

T-lymphoblastic lymphoma (LBL) of the mediastinum enters into the clinical diagnosis of HL. Flow cytometry of thymic material within which unseen HRS cells reside can be mistaken for LBL. Cytologic preparations of thymic material often contain blast-like immature thymocytes.

Mediastinal (thymic) large B-cell lymphoma (MLBCL) is an interesting mimic of CHL. The presence of a fibrous stroma and occasionally HRS-like cells in a mediastinal location may resemble CHL, but there are usually sheets of tumor cells that label strongly with B-cell markers including CD20. CD30 may be positive but is usually not as strong as in CHL, and CD15 is usually absent. Immunoglobulin gene rearrangement assays are usually positive, while in CHL they are often negative. Gene array studies show that MLBCL shares a gene expression profile with NSHL (Savage et al. 2003; Rosenwald et al. 2003). Grey-zone lymphomas with features intermediate between NSHL and MLBCL have been described (Calvo et al. 2004).

T-cell/histiocyte rich large B-cell lymphoma (TCHRLBCL) is often virtually identical to NLPHL in terms of histomorphology and immunophenotype of neoplastic cells (CD45/CD20/CD79a/J-chain/Oct-2/EMA). The background lymphocytes in TCHRLBCL are mostly CD8+ cytotoxic T cells and histiocytes while a majority of those in NLPHL are B cells with CD3/CD57-positive T cells seen rosetting around the neoplastic large cells and with follicular dendritic cells forming expanded meshworks. NLPHL is usually localized while TCHRLBCL is usually advanced at diagnosis. The fact that NLPHL may behave aggressively when in an advanced stage raises the question of whether the two diseases are truly distinct, and molecular studies may someday provide that answer (Boudova et al. 2003).

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Treatment of Pediatric Hodgkin Lymphoma

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Contents

3.0 Introduction

Treatment for children and adolescents with Hodgkin lymphoma (HL) aims to achieve cure without long-term morbidity. Earlier treatment approaches did not consider the developmental issues in pediatric patients that resulted in unacceptable musculoskeletal hypoplasia, cardiovascular and pulmonary dysfunction, and the development of subsequent primary cancers. Recognition of these life-altering and life-threatening late treatment effects motivated the development of combined-modality therapy regimens in which cycles of chemotherapy replaced a portion of the radiation therapy in laparotomy-staged children. Demonstration of the effectiveness of combined-modality therapy in children and progress in diagnostic imaging technology eventually permitted the abandonment of surgical staging. Over time, investigators undertook further modifications that decreased the number of chemotherapy cycles, restricted or eliminated specific agents that predisposed to greater treatment toxicity, and reduced radiation treatment fields and doses. Most contemporary trials for children, adolescents, and young adults with HL involve a risk-adapted approach that considers disease-related factors like presence of B symptoms, stage, the number of involved nodal regions, and the presence of tumor bulk in treatment recommendations. In general, patients with favorable disease presentations receive fewer cycles of multiagent chemotherapy alone or combined with low-dose, involved-field radiation than those with advanced and unfavorable clinical presentations. Because of specific treatment toxicities that are unique to age and gender, these factors may also influence treatment decisions. Therefore, no single treatment approach is uniformly appropriate for all patients. Instead, therapy duration

and intensity are selected to optimize opportunities to achieve and maintain long-term remission with minimal treatment-related morbidity.

3.1 Clinical Presentation

Clinical presentations of HL range from the coincidental discovery of mediastinal lymphadenopathy during an evaluation for an orthopedic injury, to a chronic cough or pruritus, to life-threatening airway obstruction or spinal cord compression. In most, the presentation is asymptomatic or less dramatic because of the indolent onset of symptoms. Persistent painless cervical or supraclavicular lymphadenopathy represents the most common presentation of pediatric HL. Involved lymph nodes are typically rubbery or firm and non-tender, although they may be sensitive to palpation if they have grown rapidly. With disease progression, abnormal nodes form large aggregate nodal masses that may become fixed to underlying tissues. Because reactive lymphadenopathy is quite common in the pediatric age group, often several courses of antimicrobial therapy have been administered before referral for biopsy. The presence of supraclavicular lymphadenopathy should prompt earlier consideration of a malignant pathogenesis, as opposed to cervical nodal abnormalities, which are commonly enlarged in association with pediatric infectious and inflammatory conditions. Cervical nodal disease is accompanied by mediastinal involvement in two-thirds or more of children and adolescents. Intrathoracic HL is often asymptomatic, but may be associated with a nonproductive cough, dyspnea, chest pain, or superior vena cava syndrome. When lymphoma is considered in the differential diagnosis, posteroanterior and lateral thoracic radiographs should be performed to evaluate for mediastinal lymphadenopathy and airway patency, particularly if sedation or general anesthesia is planned for diagnostic procedures. Occasionally, mediastinal lymphadenopathy may be difficult to differentiate from a large, normal thymus in younger children. Uncommonly, patients present with axillary or inguinal lymphadenopathy. HL limited to infradiaphragmatic sites develops in less than 5% of pediatric cases (Krikorian et al. 1986). These patients usually have lymphoma involving the peripheral inguinal, femoral, or superficial iliac lymph nodes.

Table 3.1 Demographic and clinical characteristics at presentation of pediatric Hodgkin lymphoma

	Children [a, b] (%)	Adults[b] (%)
Number of patients	1985	1912
<10 yrs of age	360 (18.1)	
≥10 yrs of age	1615 (81.4)	
>17 yrs of age	10 (0.5)	1912 (100)
Gender		
Male	1100 (55.4)	1147 (60.0)
Female	885 (44.6)	765 (40.0)
Histology		
Lymphocyte predominant	192 (9.7)	96 (5.0)
Mixed cellularity	307 (15.5)	325 (17.0)
Nodular sclerosis	1431 (72.1)	1377 (72.0)
Not classified and lymphocyte depleted	55 (2.8)	115 (6.0)
Stage[c]		
I	229 (11.5)	210 (11.0)
II	1078 (54.3)	899 (47.0)
III	391 (19.7)	593 (31.0)
IV	287 (14.5)	210 (11.0)
B symptoms		
present	564 (28.4)	612 (32.0)
absent	1421 (71.6)	1300 (68.0)

[a] Data taken from Ruhl et al. 2001 and Nachman et al. 2002.
[b] Data taken from Cleary et al. 1994.
[c] Data derived from both pathologically and clinically staged patients.

The histologic subtypes of HL (reviewed in Chapter 2) have unique characteristics that are reflected in their clinical presentations. Nodular lymphocyte predominant HL usually presents as clinically localized disease involving the cervical, axillary, or inguinal-femoral

nodal regions. This subtype is more common in male and younger patients and may be preceded by or coexist with progressive transformation of germinal centers, a pattern of benign lymphoid hyperplasia. Nodular sclerosis HL more frequently involves the cervical, supraclavicular, and mediastinal lymph nodes. The abundant collagen characteristic of the nodes involved by this subtype contributes to the development of bulky aggregate nodal masses that may not completely regress after completion of therapy. Nodular sclerosis HL is the most common subtype observed in adolescents. Mixed cellularity HL frequently presents as advanced disease with extranodal involvement and has the strongest association with Epstein-Barr virus. This subtype may exhibit an unusual "skip" pattern of clinical staging with radiographically uninvolved lymph node regions in the thoracic cavity contiguous to involved nodal regions in the neck and abdomen. Mixed cellularity HL more commonly occurs in children younger than 10 years of age. Lymphocyte depleted HL is very rare in the pediatric age range but may develop in the setting of acquired immunodeficiency, e. g., human immunodeficiency virus infection or chronic immunosuppression following solid organ transplantation. This subtype is characterized by widespread disease involving the bones and bone marrow. The relationship of age at presentation, stage, gender, and histologic subtype is illustrated in Table 3.1.

3.1.1 Systemic Symptoms

Cytokine production by Hodgkin and Reed-Sternberg cells is felt to be responsible for many of the clinical features of HL (Table 3.2) (Kadin and Liebowitz 1999). Nonspecific systemic symptoms commonly observed at diagnosis include fatigue, anorexia, and mild weight loss. Approximately 30% of pediatric patients present with any one of three specific constitutional or B symptoms that have been correlated with prognosis: unexplained fever with temperatures above 38.0°C orally, unexplained weight loss of 10% within 6 months preceding diagnosis, and drenching night sweats. The Pel-Epstein fever associated with HL is characteristically intermittent, recurrent over variable intervals of days to weeks, more noticeable in the evening, and becomes more severe and continuous over time. In some studies, night sweats were not as prognostically significant as fever and weight loss (Gobbi et al. 1985; Crnkovich et al. 1986).

Other symptoms observed in patients with HL that are not defined as B symptoms for staging include pruritus and alcohol-induced pain. Severe pruritus may be an important marker of disease activity in some patients with HL (Gobbi et al. 1985). Pruritus, which may present months or even a year before lymphadenopathy is discovered, is usually generalized and may be associated with extensive excoriations from excessive scratching. Pruritus is more common in women and in patients with advanced disease. Hodgkin-induced pruritus has been speculated to result from cytokine production following tumor lysis (Newbold 1970). In older patients, alcohol ingestion may produce severe pain in sites of involved nodes or bony metastases, and radiate to the extremities or back. The mechanism for this unusual symptom is unknown. Pruritus and alcohol-induced pain uniformly resolve when HL responds to therapy.

3.1.2 Laboratory Evaluation

Laboratory evaluation in the patient with HL is undertaken to identify aberrations in hematologic and chemical blood parameters that may correlate with disease extent and confirm satisfactory renal and hepatic function before initiating therapy. In children, bone marrow involvement is usually focal, so the presence of extranodal disease in the marrow cannot be reliably assessed by blood counts. Nonspecific hematologic abnormalities observed in HL may include neutrophilic leukocytosis, lymphopenia, eosinophilia, and monocytosis. Lymphopenia is more commonly observed in patients with extensive disease (Tan et al. 1982). Other hematologic manifestations of HL include a normochromic normocytic anemia that is typically associated with advanced disease (Ratkin et al. 1974) and rarely, a Coombs' positive hemolytic anemia (Cline and Berlin 1963).

Elevations of acute phase reactants like the erythrocyte sedimentation rate (ESR), serum copper, ferritin, and C-reactive protein (CRP) are commonly observed at presentation of HL. Of these, the ESR and more recently, CRP, have been used at diagnosis as prognostic

Table 3.2 Clinical features of Hodgkin lymphoma related to cytokine production

Clinical features of Hodgkin's disease	Cytokines
Constitutional (B) symptoms	TNF, LT-α, IL-1, IL-6
Polykaryon formation	Interferon-γ , IL-4
Sclerosis	TGF-β, LIF, PDGF, IL-1, TNF
Acute phase reactions	IL-1, IL-6, IL-11, LIF
Eosinophilia	IL-5, granulocyte M-CSF, IL-2, IL-3
Plasmacytosis	IL-6, IL-11
Mild thrombocytosis	IL-6, IL-11, LIF
T-cell and Hodgkin and Reed-Sternberg cell interaction	IL-1, IL-2, IL-6, IL-7, IL-9, TNF, LT-α, CD30L, CD40L, B7 ligands (CD80 and CD86)
Immune deficiency	TGF-β, IL-10
Autocrine growth factors (?)	IL-6, IL-9, TNF, LT-α, CD30L, M-CSF
Increased alkaline phosphatase	M-CSF
Neutrophil accumulation/activation	IL-8, TNF, TGF-β

Abbreviations: IL, interleukin; LIF, leukemia inhibitory factor; LT, lymphotoxin; M-CSF, macrophage colony-stimulating factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor
Adapted from Kadin and Liebowitz 1999:139

factors and during therapy to nonspecifically monitor response (Wieland et al. 2003). Alkaline phosphatase may correlate with the presence of bony metastatic disease; elevations beyond what is appropriate for age should prompt further investigation for skeletal sites of extranodal disease. Other chemical parameters that have been used as prognostic factors include hypoalbuminemia and elevations of lactate dehydrogenase.

Autoimmune disorders including nephrotic syndrome, autoimmune hemolytic anemia, autoimmune neutropenia, and immune thrombocytopenia (ITP) have been observed as paraneoplastic phenomena in patients with HL. Of these, ITP is the most commonly reported, occurring in 1–2% of HL cases (Sonnenblick et al. 1986; Xiros et al. 1988; Bradley et al. 1993). Thrombocytopenia may develop before, at the same time, or after the diagnosis of HL. ITP frequently occurs in patients in remission after completion of therapy for HL and is not usually associated with relapse. The treatment approach and response for ITP in patients with HL is similar to that in patients without malignancy (Sonnenblick et al. 1986). ITP may also develop in association with autoimmune hemolytic anemia (Xiros et al. 1988).

3.1.3 Immunologic Status

Active HL is characterized by generalized cellular immune deficiency and ineffective host antitumor response (Slivnick et al. 1990). The universal anergy associated with HL is considered a primary attribute of the disease. Mechanisms hypothesized to be responsible for impaired cellular immunity include abnormal T-cell subset populations, prostaglandin E_2 mediated suppression, enhanced sensitivity to suppressor monocytes and suppressor T cells, inherent T-lymphocyte defect, reduced interleukin-2 production and transforming growth factor β (TGF-β) secretion by HRS cells (Slivnick et al. 1990). T-cell immune deficits may persist in long-term disease-free survivors. Consequent to their cellular immunodeficiency, patients with HL exhibit an increased risk of infection with op-

portunistic pathogens including fungi, viruses, and tuberculosis (Casazza et al. 1966). Even with contemporary antimicrobial agents, varicella zoster and human papilloma infections commonly occur during and after completion of therapy.

Natural killer cell cytotoxicity is also depressed in newly diagnosed patients with HL, with more pronounced deficits in patients with advanced and symptomatic disease (Ruco et al. 1982). NK function typically normalizes after treatment in patients who attain a complete remission (Liberati et al. 1987). The etiology of depressed NK cell cytotoxicity associated with HL is unknown.

Humoral immunity is usually intact at diagnosis, but may become transiently depressed following therapy. In a study evaluating antibody production following *Haemophilus influenzae* type B in adults who had completed therapy for HL, patients treated with combination chemotherapy, particularly in association with total nodal radiation, exhibited significantly reduced antibody production following immunization (Weitzman et al. 1977). Humoral immune deficits typically recover with increasing time from therapy (Minor et al. 1979). *In vitro* and *in vivo* studies have provided insights regarding the mechanism of immune dysregulation in HL. Chemokine and cytokine production (discussed further in Chapter 2) appear to contribute to the development of an environment in which Hodgkin and Reed-Sternberg cells can proliferate, escape apoptosis, and evade host immune surveillance. Table 3.3 summarizes the characteristic immune profiles observed in patients with HL (Slivnick et al. 1990).

Table 3.3 Immune profiles in Hodgkin lymphoma

Activity	Untreated active disease	Disease-free survivors
Antigen-induced antibody production	Normal	Transiently depressed
Polymorphonuclear function:		
Chemotaxis	Decreased	Decreased
Metabolic reactivity	Decreased	Decreased
Delayed-hypersensitivity skin tests:		
Recall antigens	Anergic	Reactive
Neoantigens	Anergic	Anergic
E rosette formation	Decreased	Decreased
Mitogen-induced T-cell proliferation	Decreased	Decreased
Mixed lymphocyte-induced proliferation:		
Autologous	Decreased	Decreased
Allogeneic	Slightly decreased	Slightly decreased
Sensitivity to suppressor monocytes	Enhanced	Enhanced
Sensitivity to suppressor T cells	Enhanced	Enhanced
CD4:CD8 ratio	Slightly decreased	Decreased

From Slivnick et al. 1990;17:673, with permission.

3.2 Differential Diagnosis

The differential diagnosis for HL includes other infectious, inflammatory, and neoplastic conditions presenting with lymphadenopathy (Green 1998). A variety of bacterial, viral, and fungal organisms prominently feature lymphadenopathy in their presentation. The infectious agents most commonly considered in the differential diagnosis of HL are those that present with an indolent course (e. g., atypical mycobacterium, *Bartonella henselae*, histoplasma, and toxoplasmosis). Pyogenic bacterial lymphadenitis is typically unilateral, and associated with erythema, pain, and tenderness. Bilateral acute cervical lymphadenitis is more likely to be viral in etiology. Individuals with

chronic localized lymphadenopathy should be evaluated for signs of a persistent regional infection. Tuberculosis typically presents with localized lymphadenopathy in the mediastinal, mesenteric, anterior cervical nodes, but generalized lymphadenopathy may be observed with hematogenous spread of the organism. A positive tuberculin skin test is helpful in confirming the diagnosis. Nontuberculous mycobacteria may also cause cervical or submandibular lymphadenitis that is typically unilateral. Cat-scratch is a common cause of localized, usually painful, lymphadenopathy that may persist for weeks to months. A positive exposure history and *Bartonella henselae* titer should facilitate making this diagnosis.

Infectious mononucleosis is most often accompanied by symmetrical cervical lymphadenopathy in association with pharyngitis. Generalized lymphadenopathy may also occur, and hepatosplenomegaly is common. A positive Epstein-Barr viral titer (IgM fraction) establishes the diagnosis of acute infection, but it should be noted that HL may also present in the setting of an acute EBV infection. Therefore, further investigation should be undertaken if abnormal lymphadenopathy persists or progresses after resolution of infectious symptoms. Several microbes may simulate infectious mononucleosis in their presentation. Cytomegalovirus infection is associated with generalized lymphadenopathy, hepatosplenomegaly, and fever. Toxoplasmosis usually causes cervical, suboccipital, supraclavicular, or generalized lymphadenopathy and fever. In healthy hosts, these infections are usually self-limited.

Pulmonary histoplasmosis produces mediastinal and hilar lymphadenopathy that may be difficult to clinically distinguish from lymphoma in an asymptomatic patient. A history of a recent respiratory infection, exposure to a high-risk area of fungal spore contamination, and positive histoplasma serology are helpful in avoiding invasive diagnostic procedures. Hilar adenopathy is also a prominent feature of sarcoidosis, a multisystem disorder that may be accompanied by nonspecific constitutional symptoms (fatigue, malaise, anorexia, or weight loss) and characteristic lung, eye, and skin findings. The diagnosis may be suspected based on clinical presentation, but requires biopsy for definitive confirmation.

Lymphadenopathy is also frequently observed in immunodeficiency syndromes and phagocytic dysfunction. Suppurative lymphadenitis is a common complication in children with chronic granulomatous disease and hyper-IgE syndrome. Acquired immunodeficiency syndrome associated with chronic human immunodeficiency virus (HIV) is characterized by generalized lymphadenopathy, hepatosplenomegaly, fever, and failure to thrive. Chronic immunosuppression associated with HIV increases the risk of a variety of malignancies, including HL and NHL. Therefore, children with chronic HIV infection should undergo prompt evaluation of lymph node changes suggesting the development of a malignant process.

Non-Hodgkin lymphoma (NHL) is the most common pediatric malignancy that should be considered in the differential diagnosis of HL. Typically, the growth rate of the affected lymph nodes in NHL is more rapid than in HL and more frequently associated with abnormal chemical parameters such as elevated levels of uric acid or lactic dehydrogenase. Cervical lymphadenopathy is observed in the presentation of other pediatric malignancies including nasopharyngeal carcinoma, rhabdomyosarcoma, neuroblastoma, and thyroid carcinoma. For further discussion regarding pathologic features to consider in the differential diagnosis of HL, see Chapters 8–11.

3.3 Diagnostic Evaluation and Staging

Table 3.4 summarizes the recommended steps in the diagnostic work-up for pediatric HL. Biopsy is required to establish the diagnosis. Lymph node excision is preferred because the malignant Hodgkin and Reed-Sternberg cells can be evaluated within the context of the nodal architecture unique to individual histologic subtypes. If a noninvasive needle biopsy is planned, multiple tissue passes should be performed to facilitate the assessment of the nodal architecture. Physical examination should include an evaluation of all node-bearing areas, including Waldeyer's ring, and measurement of enlarged nodes that can be monitored for response to therapy.

The goal of the staging evaluation is to identify sites and characteristics of disease to permit as accurate a

risk assessment as possible for treatment planning. Historically, cervical lymph nodes have been evaluated exclusively by physical examination. Because ongoing risk-adapted treatment protocols use response as a parameter to escalate or truncate therapy, computed tomography (CT) evaluation of Waldeyer's ring and cervical soft tissues is recommended to permit more accurate assessment of the nodal response.

A chest radiograph provides preliminary information about mediastinal involvement and intrathoracic structures. Mediastinal lymphadenopathy measuring 33% or more of the maximum intrathoracic cavity at the dome of the diaphragm on an upright chest radiograph is designated "bulky" in the risk assessment (Fig. 3.1). CT of the chest provides more detailed information regarding involvement of the intrathoracic lymph nodes, pulmonary parenchyma, chest wall, pleura, and pericardium that may not be apparent on radiographs (Rostock et al. 1982). Although magnetic resonance imaging (MRI) is an effective tool for evaluating intrathoracic structures, thoracic CT is superior to MRI in the evaluation of the pulmonary parenchyma.

Since the abandonment of staging laparotomy and lymphography, CT is most often used to evaluate sites of infradiaphragmatic disease. Oral and intravenous contrast administration is required to accurately delineate abdominal/pelvic nodes from other infradiaphragmatic structures and organ involvement. Suboptimal bowel contrast and the lack of retroperitoneal fat in some patients may limit the sensitivity of CT in detecting abdominal adenopathy (Baker et al. 1990). In these cases, MRI may provide better evaluation of fat-encased retroperitoneal lymph nodes (Hanna et al. 1993). The size of abdominal and pelvic nodes is used to estimate lymphomatous involvement. Abdominal nodes smaller than 1–1.5 cm and pelvic nodes smaller than 2–25 cm are usually considered normal. Functional imaging with positron emission tomography (PET) now enables identification of disease in smaller nodes.

Splenic involvement occurs in 30–40% of patients with HL, whereas hepatic involvement is rare in the pediatric age group. Abnormal densities on CT or MRI suggest lymphomatous involvement of the liver and spleen. Because tumor deposits in these organs may be less than 1 cm in diameter, the disease status cannot be dependably assessed by organ size alone. Liver function studies are also unreliable indicators of hepatic disease. Previous studies in children undergoing staging laparotomy demonstrate that CT findings of intrinsic spleen lesions and porta hepatic and celiac lymphadenopathy were infrequent but highly predictive of lymphomatous involvement (Mendenhall et al. 1993). Definitive evaluation of disease involving the liver and spleen requires histologic assessment, which is no longer required for contemporary treatment planning.

Functional nuclear imaging is an important diagnostic and monitoring modality in patients with HL. Earlier investigations established the sensitivity and predictive value of gallium-67 (^{67}Ga) avidity, particularly in the evaluation of supradiaphragmatic disease (Weiner et al. 1991). PET has largely replaced ^{67}Ga imaging because of the latter modality's low resolution and its suboptimal physiological biodistribution, which limits evaluation of the abdominal and pelvic lymph nodes (Hueltenschmidt et al. 2001; Bar-Shalom et al. 2003; Hudson et al. 2004). PET provides an assessment of proliferative activity in tumors undergoing anaerobic glycolysis through uptake of the radioactive glucose analogue, 18-fluoro-2-deoxyglucose (FDG). PET-CT is both an accurate and cost-effective imaging modality that integrates functional and anatomic tumor characteristics (Jerusalem et al. 1999). Moreover, PET or PET-CT imaging can be completed in a single day, has a higher resolution, better dosimetry, less intestinal activity than ^{67}Ga imaging. Like ^{67}Ga, FDG avidity persisting after therapy is prognostic of outcome and helpful in determining the need for additional therapy (Jerusalem et al. 1999; Spaepen et al. 2001; Friedberg et al. 2004).

Clinicians should be aware of the limitations of FDG-PET in the pediatric setting. Patient cooperation or sedation is critical to minimize FDG uptake that is unrelated to tumor activity. Interpretation of lymphomatous involvement may be confounded by FDG avidity in normal tissues, e. g., brown fat of cervical musculature. Also, a variety of nonmalignant conditions including thymic rebound commonly observed after completion of lymphoma therapy exhibit FDG avidity (Kaste et al. 2005). Lastly, tumor activity cannot be assessed in diabetic patients with poorly controlled blood glucose. Prospective trials evaluating FDG-PET in pediatric HL are ongoing (Korholz et al. 2003).

Bone marrow involvement at initial presentation of pediatric HL is uncommon and rarely occurs as an isolated site of extranodal disease. Lymphomatous marrow infiltration may be diffuse or focal and is frequently accompanied by reversible marrow fibrosis. Bone marrow aspiration is an inadequate method of assessment for disease. Bone marrow biopsy should be performed in any patient with advanced (clinical stage III to IV) or symptomatic (B symptoms) disease, or during restaging of patients with recurrent disease. Bone marrow involvement in newly diagnosed stage I to IIA disease is very rare. Consequently, the low yield from bone marrow biopsy in these patients does not support routine marrow evaluation for this favorable risk group.

Historically, evaluations undertaken for the assessment of sites of skeletal metastases included technetium-99 nuclear imaging and radiography-identified sites of abnormalities. Because of the rarity of bone involvement in pediatric HL, these studies were reserved for children with bone pain, elevations of serum alkaline phosphatase beyond that expected for age, or other sites of extranodal disease. PET-CT, which assesses both cortical bone and bone marrow, may ultimately replace these previously used modalities.

The currently used Ann Arbor staging system, adopted in 1971, is based on the observation that Hodgkin lymphoma appears to spread along contiguous lymph nodes until late in the course of disease (Table 3.5) (Carbone et al. 1971). The substage classifications A, B, and E amend each stage based on defined clinical features. Substage A indicates "asymptomatic" disease. B symptoms include fever exceeding 38°C for 3 consecutive days, drenching night sweats, and an unexplained loss of at least 10% of body weight over 6 months. Substage E denotes extranodal involvement arising from extension of disease from contiguous nodal regions. Substage S denotes involvement of the spleen.

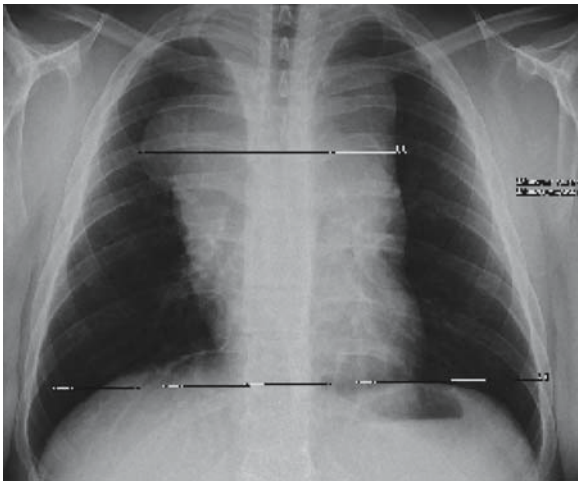

Figure 3.1

Mediastinal bulk is determined by calculating the ratio of maximum diameter of the mediastinal mass to the maximum diameter of the intrathoracic cavity measured at the dome of the diaphragm on an upright chest radiograph. A measurement of 33% or more is designated "bulky" in the risk assessment

Table 3.4 Diagnostic evaluation for children with Hodgkin lymphoma

Physical examination with measurement of lymph nodes
Complete blood cell count with differential, erythrocyte sedimentation rate or C-reactive protein, renal and hepatic function tests, alkaline phosphatase level
Lymph node biopsy
Chest radiograph with measurement of ratio of mediastinal mass to maximum intrathoracic cavity at the dome of the diaphragm
Computed tomography of neck and chest
Computed tomography or magnetic resonance imaging of abdomen and pelvis
Bone marrow biopsy[a]
Bone scan[b]
Gallium or positron emission tomography (PET) scan

[a] Recommended for all children except those with stages IA/IIA.

[b] Recommended for children with bone pain and elevated alkaline phosphatase.

Table 3.5 Ann Arbor staging classification for Hodgkin lymphoma

Stage	Description
I	Involvement of a single lymph node region or lymphoid structure, e. g., spleen, thymus, Waldeyer's ring, or single extralymphatic site (IE)
II	Involvement of two or more lymph node regions on the same side of the diaphragm, or localized contiguous involvement of only one extranodal organ/site and lymph node region on the same side of the diaphragm (IIE)
III	Involvement of lymph node regions on both sides of the diaphragm (III), which may be accompanied by involvement of the spleen (III_S) or by localized contiguous involvement of only one extranodal organ site (III_E) or both (III_{SE})
III1	With or without involvement of splenic hilar, celiac, or mesenteric nodes
III2	With involvement of para-aortic, iliac, or mesenteric nodes
IV	Diffuse or disseminated involvement of one or more extranodal organs or tissues, with or without associated lymph node involvement
Designations applicable to any stage	
A	No symptoms
B	Fever (temperature > 38°C), drenching night sweats, unexplained loss of > 10% of body weight within the preceding 6 months
E	Involvement of a single extranodal site that is contiguous or proximal to the known nodal site

3.4 Prognostic Factors

Advances in the treatment of HL have diminished the importance of prognostic factors. As a corollary, prognostic factors change as therapy changes and improves. Yet, they remain useful as tools for predicting outcome, defining risk groups for patient stratification, and providing insight into the disease process (e. g., natural history, biology). Prognostic factors in HL can be divided into those that are patient-related (e. g. age, gender) and tumor-related (e. g. pathologic subtype, disease extent). They can also be grouped according to the time point at which they were recorded: at diagnosis or during therapy. The degree to which prognostic factors are interrelated (e. g., disease stage, bulk, biologic aggressiveness) and therapy-dependent will determine their general applicability. Definitions of the prognostic factors will, of course, affect their interpretability (e. g., bulk disease, sites of involvement, age cutoffs, relevant ranges of laboratory values) (Specht 1996). Finally, some factors may be prognostic for certain therapies or for certain stages.

Prognostic factors used in various current clinical trials for children are as follows:

- CS I/II disease treated with combined modality therapy: disease bulk (peripheral and mediastinal), number of disease sites, B symptoms, erythrocyte sedimentation rate, histologic subtype, gender.
- CS III/IV disease but also early stage with B symptoms: disease bulk, B symptoms, histologic subtype, extranodal extension and organ involvement, laboratory values including anemia, leukocytosis, lymphopenia, hypoalbuminemia.
- Retrieval therapy with or without high-dose therapy and hematopoietic cell rescue: intensity of initial chemotherapy, treatment with radiation, response to initial therapy, duration of initial remission, response to salvage therapy, disease stage at relapse, disease bulk at relapse, extranodal relapse, B symptoms at relapse.

Disease extent and biology have been correlated with the following prognostic factors currently in use or under investigation. The stage of disease persists as the most important prognostic variable. The Ann Arbor staging classification was demonstrated to be prognostic for more than 14,000 patients in the International Database on HL (Henry-Amar et al. 1990), and its utility has persisted despite obvious limitations in its precision. These include its failure to consider other factors such as disease burden and biology. Yet, it is used in most trials for patient stratification. Patients with advanced stage disease, especially stage IV, have an inferior outlook compared with patients with early stage disease (Henry-Amar et al. 1990; Bader et al. 1993; Nachman et al. 2002; Smith et al. 2003).

Disease burden encompasses the bulk of disease as reflected by the disease stage, but more specifically by the volume of distinct areas of involvement and the number of disease sites. Large mediastinal adenopathy places a patient at a greater risk for disease recurrence when treated with radiation therapy alone, and also in trials using combined modality therapy. A slightly inferior survival rate is also apparent in some studies despite the effectiveness of salvage therapy (Gobbi et al. 1985; Mauch et al. 1988; Maity et al. 1992; Specht 1996; Nachman et al. 2002; Smith et al. 2003). Of interest, however, is the recent DAL-HD-90 trial, in which bulk disease did not influence the outcome (Dieckmann et al. 2003). Escalating radiation doses for patients with bulk or residual postchemotherapy disease may have obviated the significance of bulk in this trial. Patients (at least those staged only clinically) with several sites of involvement, generally defined as 4 or more, fare less well (Mauch et al. 1988; Maity et al. 1992; Specht 1996). Patients with stage IV disease who have multiple organs involved fare especially poorly.

Systemic symptoms, which presumably result from cytokine secretion, reflect biologic aggressiveness and confer a worse prognosis (Vecchi et al. 1993; Schellong 1996; Landman-Parker et al. 2000; Nachman et al. 2002; Smith et al. 2003). The constellation of symptoms appears to be relevant to this observation. That is, patients with night sweats only (at least among patients with PS I and II disease) appear to fare as well as PS I to IIA patients, while those with both fevers and weight loss have the worst prognosis (Crnkovich et al. 1986).

Laboratory studies, including the erythrocyte sedimentation rate, serum ferritin, hemoglobin level, serum albumin, and serum CD8 antigen levels, have been reported to predict a worse outcome (Pui et al. 1989; Specht 1996; Landman-Parker et al. 2000; Smith et al. 2003; Montalban et al. 2004). This could reflect disease biology or bulk. Other investigational serum markers associated with an adverse outcome include soluble vascular cell adhesion molecule-1 (Christiansen et al. 1998), tumor necrosis factor (Warzocha et al. 1998), soluble CD30 (Nadali et al. 1998) and CD 20 (Tzankov et al. 2003), beta-2-microglobulin (Chronowski et al. 2002), transferrin and serum IL-10 (Bohlen et al. 2000), bcl-2 expression (Sup et al. 2005). High levels of caspase 3 in Hodgkin and Reed-Sternberg cells has been correlated with a favorable outcome (Dukers et al. 2002). A recent study of children suggests that EBV infection may be predictive of an inferior outcome in those with advanced stage disease or NSHL subtype (Claviez et al. 2005).

Histologic subtype is relevant, at least among adults. Patients with clinical stage I to II MCHL have an increased frequency of subdiaphragmatic relapse, and disease subtype independently influences survival in some reports (Mauch et al. 1988). Grade 2 NSHL histology has conferred poor outcome in some, but not all studies (Schellong 1996; von Wasielewski et al. 2003). Patients with LDHL fare poorly. However, a recent report from the United Kingdom Children's Cancer Study Group assessing the relevance of histology in 331 children is revealing. Less than 1% had LDHL, obviating any meaningful assessment of its prognostic significance. For patients with other histologies treated with combined therapy, no difference in outcome was observed (Shankar et al. 1997). As previously discussed, patients with nLPHL have distinctive differences in disease-free and overall survival (Sandoval et al. 2002).

Age is a significant prognostic factor is some studies. Survival rates for children with HL approach 85–95%. In a report from Stanford, the 5- and 10-year survival for children with HL less than or equal to 10 years of age is 94% and 92%, respectively, compared with 93% and 86% for adolescents (aged 11 to 16 years old) and 84% and 73% for adults (Cleary et al. 1994). Several features of the youngest patient group may in-

Table 3.6 Prognostic factors identified in pediatric Hodgkin lymphoma trials

Center	Patient no. (study period)	Treatment outcome	Prognostic factors
Associazione Italiana di Ematologia ed Oncologia Pediatrica-MH-83 (AIEOP) (Vecchi 1993)	215 (1983–1989)	7-yr FFP: 86%	B symptoms Mediastinal bulk[a] Histology
German multicenter DAL 90 (Schellong 1996)	578 (1990–1995)	5-yr EFS: 91%	Histology: Nodular sclerosis, grade 2 B symptoms
Stanford, Dana Farber & St. Jude Consortium (Smith 2003)	328 (1990–2000)	5-yr DFS: 83%	Male sex Stage IIB, IIIB, IV Mediastinal bulk[a] WBC > $13.5 \times 10^3/mm^3$ Hemoglobin < 11.5 gm/dL

[a] Defined as mediastinal mass/thoracic cavity ratio > 0.33 on upright chest radiograph
Abbreviations: DFS, disease-free survival; EFS, event-free survival; FFP, freedom from progression

fluence their improved prognosis, including higher frequency of LP and MC subtypes and of stage I disease, a lower frequency of systemic symptoms, and the more common use of combined modality therapy. Multivariate analysis of these data showed that age, stage, histology, and treatment modality (combined radiation and chemotherapy versus radiation alone) were all independent prognostic variables for survival (Cleary et al. 1994). Although children less than 4 years of age with HL are uncommon, even these children would appear to have an excellent prognosis (Kung 1991).

The rapidity of response to initial therapy is an important prognostic variable in many forms of cancer, including HL. In some trials, the rapidity of response to chemotherapy is used to determine subsequent therapy (Bierman et al. 2002; Carde et al. 2002; Lieskovsky et al. 2004). Early response to therapy as measured by FDG-PET imaging is under investigation as a possible marker of prognosis (Korholz et al. 2003).

Table 3.6 provides examples of factors significant on multivariate analysis in recently published studies with more than 200 patients. Although prognostic factors will continue to be influenced by choice of therapy, parameters such as disease, bulk, number of involved sites, and systemic symptomatology are likely to remain relevant to the outcome. Nonetheless, as therapy both improves and becomes increasingly tailored to prognostic factors and therapeutic response, the overall outcome should become less affected by those parameters.

3.5 Combination Chemotherapy

Its contiguous nodal pattern of disease dissemination permitted HL to be one of the few tumors curable with radiation alone. However, cure was limited to those whose entire extent of disease could be detected by available staging studies and included within radiation fields. The introduction of effective chemotherapy for HL made cure possible for patients with more extensive disease. In addition to treating sites of unrecognized metastasis, chemotherapy permitted the reduction of radiation fields and total dose, thus reducing the risk of hypoplasia in growing children. Table 3.7 summarizes the most common regimens used today in the treatment of pediatric Hodgkin lymphoma.

Although single agents induced a response, combination chemotherapy resulted in sustained disease control that impacted survival rates. MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone; DeVita et al. 1980) and ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine; Santoro et al. 1982)

were developed by combining active agents with different mechanisms of action and minimal overlap of toxicity. Similar therapeutic responsiveness to chemotherapy made these combinations valuable for the treatment of both adults and children. When combined with radiation, cure rates in the range of 85–90% are achievable. Unfortunately, many patients suffer from adverse long-term effects of the chemotherapy. The increased vulnerability of children to late treatment complications (Donaldson and Kaplan 1982; Mauch et al. 1983) and their expected long-term survival have motivated the goals of pediatric trials to mitigate these effects.

A review of the modern treatment of pediatric HL (Thomson and Wallace 2002) is an overview of attempts to select optimal combinations of therapy that might offer maximal efficacy with minimal toxicity. MOPP and ABVD have been used alone, in alternating fashion, and as a hybrid cycle in children. In adult trials, regimens including doxorubicin appear to enhance the outcome (Canellos et al. 1992; Duggan et al. 2003). Although ABVD does not have the significant risk of sterility or secondary malignancy associated with MOPP or COPP (cyclophosphamide, vincristine, procarbazine, prednisone; da Cunha et al. 1984; Bramswig et al. 1990; Lipton et al. 1996; Schellong et al. 1997, 1999), the combination does carry the risk of anthracycline-induced cardiotoxicity and bleomycin-induced pulmonary toxicity. Hybrid and alternating regimens have the advantage of administering restricted cumulative doses of each effective agent. The Pediatric Oncology Group (POG) achieved excellent results using MOPP alternating with ABVD with low-dose radiation as the backbone of therapy for children, limiting cumulative doses of therapy even for those with the most advanced disease (Weiner et al. 1997). In a more recent study, Nachman et al. relied on the hybrid regimen COPP/ABV (Nachman et al. 2002). An alternative approach was pioneered in the German Austrian investigators who attempted to minimize the use of alkylating agents in an effort to reduce the incidence of sterility and secondary malignancy (Schellong et al. 1999). Using the regimen known as OPPA (vincristine, procarbazine, prednisone, doxorubicin)/COPP, this group replaced nitrogen mustard with doxorubicin in one cycle (OPPA) and with cyclophosphamide in the other (COPP). When the subsequent elimination of procarbazine resulted in a significant increase in therapeutic failure in higher risk patients, etoposide was used in lieu of procarbazine (OEPA) in boys in an effort to preserve testicular function. With this change, the gender-based approach produced less gonadal toxicity while maintaining efficacy (Bramswig et al. 1990; Ruhl et al. 2001).

Other chemotherapeutic agents may have fewer or non-overlapping toxicities. Cytosine arabinoside and etoposide have been incorporated into several regimens in an effort to reduce gonadal toxicity and enhance antitumor activity (Ekert et al. 1993; Nachman et al. 2002). Non-cross-resistant agents with fewer or different effects have also been studied. Investigators at Stanford, St. Jude, Boston consortium combined methotrexate with vinblastine, doxorubicin, and prednisone to establish VAMP, an effective regimen in low-stage disease that does not include alkylating agents (Donaldson et al. 2002). Other combinations including DBVE (doxorubicin, bleomycin, vincristine, etoposide), VBVP (vinblastine, bleomycin, etoposide, prednisone), and OEPA (vincristine, etoposide, prednisone, doxorubicin) have also successfully eliminated the use of alkylating agents (Landman-Parker et al. 2000; Schwartz et al. 2002; Dorffel et al. 2003). VAMP has the advantage of not including etoposide and its potential leukemogenic effect, while EBVP avoids the need for anthracycline. OEPA is the only regimen without an alkylating agent that has been shown to be effective for low-stage disease without radiation, producing 97% event-free survival (EFS) in IA/IIA patients (Dorffel et al. 2003). ABVD derivative regimens including etoposide have not been effectively used in high-stage disease. VEPA (vinblastine, etoposide, prednisone, doxorubicin) combined with response-based (15–25.5 cGy) involved-field radiation resulted in only a 70% and 49% 5-year EFS for stages III and IV HL, respectively (Friedmann et al. 2002). Results with VEEP (vincristine, etoposide, epirubicin, prednisolone) and AOPE (doxorubicin, vincristine, prednisone, etoposide) and involved-field radiation ($\geq$30 Gy) were also suboptimal for patients with advanced and unfavorable disease, suggesting that outcomes are compromised for some high-risk patients with the elimination of alkylating agents (Sackmann-Muriel et al. 1997; Shankar et al. 1998).

Table 3.7 Chemotherapy regimens for Hodgkin lymphoma (repeat cycle every 28 days)

Name	Drugs	Dosage	Route	Days
MOPP & derivatives:				
MOPP (Donaldson 1987)	Mechlorethamine	6.0 mg/m^2	IV	1, 8
	Vincristine	1.4 mg/m^2	IV	1, 8
	Procarbazine	100 mg/m^2	PO	1–15
	Prednisone	40 mg/m^2	PO	1–15
COPP (Baez 1997)	Cyclophosphamide	600 mg/m^2	IV	1, 8
	Vincristine	1.4 mg/m^2	IV	1, 8
	Procarbazine	100 mg/m^2	PO	1–15
	Prednisone	40 mg/m^2	PO	1–15
OPPA (Schellong 1996)	Vincristine	1.5 mg/m^2	IV	1, 8, 15
	Procarbazine	100 mg/m^2	PO	1–15
	Prednisone	60 mg/m^2	PO	1–15
	Doxorubicin	40 mg/m^2	IV	1, 15
ChlVPP (Shankar 1997)	Chlorambucil	6 mg/m^2	PO	1–14
	Vinblastine	6 mg/m^2	PO	1, 8
	Procarbazine	100 mg/m^2	PO	1–14
	Prednisone	40 mg/m^2	PO	1–14
ABVD & derivatives:				
ABVD (Santoro 1982)	Doxorubicin	25 mg/m^2	IV	1, 15
	Bleomycin	10 U/m2	IV	1, 15
	Vinblastine	6 mg/m^2	IV	1, 15
	Dacarbazine	375 mg/m^2	IV	1, 15
OEPA (Schellong 1996)	Vincristine	1.5 mg/m^2	IV	1, 8, 15
	Etoposide	125 mg/m^2	IV	3–6
	Prednisone	60 mg/m^2	PO	1–15
	Doxorubicin	40 mg/m^2	IV	1, 15
VAMP (Donaldson 2002)	Vinblastine	6 mg/m^2	IV	1, 15
	Doxorubicin	25 mg/m^2	IV	1, 15
	Methotrexate	20 mg/m^2	IV	1, 15
	Prednisone	40 mg/m^2	PO	1–14
VBVP (Landman-Parker 2000)	Vinblastine	6 mg/m^2	IV	1, 8
	Bleomycin	10 U/m^2	IV	1
	Etoposide	100 mg/m^2	IV	1–5
	Prednisone	40 mg/m^2	PO	1–8
DBVE (Schwartz 2002)	Doxorubicin	25 mg/m^2	IV	1, 15
	Bleomycin	10 U/m^2	IV	1, 15
	Vincristine	1.5 mg/m^2 (2 mg max)	IV	1, 15
	Etoposide	100 mg/m^2	IV	1–5

Table 3.7 Chemotherapy regimens for Hodgkin lymphoma (repeat cycle every 28 days)

Name	Drugs	Dosage	Route	Days
VEPA (Friedmann 2002)	Vinblastine	6 mg/m^2	IV	1, 15
	Etoposide	200 mg/m^2	IV	1, 15
	Prednisone	40 mg/m^2	PO	1–14
	Doxorubicin	25 mg/m^2	IV	1, 15
Dose-intensive MOPP/ABVD combination derivatives:				
COPP/ABV (Nachman 2002)	Cyclophosphamide	600 mg/m^2	IV	1
	Vincristine	1.4 mg/m^2	IV	1
	Procarbazine	100 mg/m^2	PO	1–7
	Prednisone	40 mg/m^2	PO	1–14
	Doxorubicin	35 mg/m^2	IV	8
	Bleomycin	10 U/m^2	IV	8
	Vinblastine	6 mg/m^2	IV	8
DBVE-PC (Schwartz 2002)	Doxorubicin	25 mg/m^2	IV	1, 2
	Bleomycin	5 U/m^2 10 U/m^2	IV	1 8
	Vincristine	1.4 mg/m^2 (2.8 mg max)	IV	1, 8 7
	Etoposide	125 mg/m^2	IV	1–3
	Prednisone	40 mg/m^2	PO	1–7
	Cyclophosphamide	800 mg/m^2	IV	1
BEACOPP (Kelly 2002)	Bleomycin	10 U/m^2	IV	8
	Etoposide	200 mg/m^2	IV	1–3
	Doxorubicin	35 mg/m^2	IV	1
	Cyclophosphamide	1200 mg/m^2	IV	1
	Vincristine	2 mg/m^2 (2 mg max)	IV	8
	Procarbazine	100 mg/m^2	PO	1–7
	Prednisone	40 mg/m^2	PO	1–14
Stanford V (Horning 2002)	Mechlorethamine	6 mg/m^2	IV	1, 15
	Vinblastine	6 mg/m^2	IV	1, 15
	Doxorubicin	25 mg/m^2	IV	1
	Etoposide	60 mg/m^2	IV	15, 16
	Vincristine	1.4 mg/m^2 (2 mg max)	IV	8, 22
	Bleomycin	5 U/m^2	IV	8, 22
	Prednisone	40 mg/m^2	PO	Every other day

Dose-intensive regimens designed to improve efficacy have been used to increase the antitumor effect and limit cumulative doses of potentially toxic agents. Adult groups have explored the concept of dose/time intensification in advanced stage disease. Stanford V limited the duration and total dose of chemotherapy, achieving a 3-year EFS of 87% and a 5-year progression-free survival of 89% in adults with advanced and unfavorable HL (Horning et al. 2002). BEACOPP and escalated BEACOPP are dose-intensive regimens with improved efficacy compared to COPP/ABVE (Diehl et al. 2003). Children with advanced-stage disease have received escalated BEACOPP followed by response-directed, gender-specific therapy with excellent early outcomes (Kelly et al. 2002). Dose-intensive regimens of short duration can potentially minimize cumulative doses and thus long-term toxicity, although acute toxicity associated with myelosuppressive and neuropathic side-effects may be greater than that observed following conventional chemotherapy administered on a twice-monthly schedule. Instead of further cumulative dose escalation, ongoing pediatric trials utilize dose-intensive delivery to limit cumulative cytotoxic therapy. The current Children's Oncology Group's intermediate risk trial features a dose/time-intensive approach with ABVE-PC as the backbone therapy that eliminates procarbazine and restricts doxorubicin and etoposide dose. Similarly, pediatric Hodgkin's consortium investigators from St. Jude, Stanford, and Boston are testing Stanford V and response-based (15–25.5 Gy), involved-field radiation therapy for children and adolescents with advanced and unfavorable disease. Longer follow-up is required to determine if outcome will be improved with this approach.

Overall 5-year survival for pediatric HL approaches 90% for most patients, but with prolonged follow-up beyond 10 years from diagnosis, the risk of death due to disease is almost equaled by the risk of death due to other causes, particularly the long-term consequences of therapy (Hudson et al. 1998; Mertens et al. 2001). Chemotherapy with lower dose (15–25 Gy) and limited field radiation provides excellent disease control without cosmetically significant hypoplasia (Landman-Parker et al. 2000; Donaldson et al. 2002; Dorffel et al. 2003). Recognition of the risks of breast cancer (Bhatia et al. 2003; Travis et al. 2003; van Leeuwen et al. 2003; Kenney et al. 2004; Guibout et al. 2005) and atherosclerotic heart disease (Hancock et al. 1993; Adams et al. 2003) occurring 10 to 20 years after full-dose radiation has led to the use of combined modality therapy with low-dose radiotherapy rather than full-dose radiotherapy even for postpubertal adolescents. With rare exceptions, chemotherapy is now recommended for all children and adolescents (Hudson 2002; Hudson and Constine 2004). Chemotherapy also has significant long-term effects on children and adolescents including sterility/infertility (alkylating agents; Horning et al. 1981; Bramswig et al. 1990; Ortin et al. 1990; Byrne et al. 1992; Hobbie et al. 2005), secondary leukemia (alkylating agents, etoposide; van Leeuwen et al. 2000; Bhatia et al. 2003; Lin and Teitell 2005), pulmonary fibrosis (bleomycin; Marina et al. 1995; Polliack 1995; Bossi et al. 1997), and cardiomyopathy (anthracyclines; Kadota et al. 1988; Hancock et al. 1993; Adams et al. 2004). To avoid these late toxicities, treatment regimens evaluated in children have focused on the determination of minimal therapies necessary to effect cure.

3.6 Chemotherapy Alone Versus Combined Modality Therapy

Treatment with combination chemotherapy alone is effective for pediatric HL and avoids the potential long-term complications associated with radiation therapy (Behrendt et al. 1987; Ekert et al. 1988, 1993; Lobo-Sanahuja et al. 1994; Baez et al. 1997; Sackmann-Muriel et al. 1997; van den Berg et al. 1997; Hutchinson et al. 1998; Atra et al. 2002). This treatment approach is preferred in centers that do not have access to the radiation facilities, trained personnel, and diagnostic imaging modalities needed for clinical staging. Earlier chemotherapy-alone trials that prescribed MOPP and MOPP-derivative combinations supported the therapeutic efficacy of the combination but did not provide information about long-term toxicity (Behrendt et al. 1987; Ekert et al. 1988; Atra et al. 2002). In an effort to avoid gonadal and neoplastic complications associated with alkylating agent combinations, subsequent trials of chemotherapy alone tested ABVD or derivative combinations in alternation with MOPP-

derivative therapy (Lobo-Sanahuja et al. 1994; Sripada et al. 1995; Baez et al. 1997; Hutchinson et al. 1998). Outcomes observed after these treatments were comparable to those achieved with combined modality regimens, particularly in patients with localized disease presentations. Attempts to reduce toxicity by eliminating alkylating agents in regimens by using ABVD or derivative therapy produced suboptimal results in patients with advanced stage disease (Ekert et al. 1993; Behrendt et al. 1996; Shankar et al. 1998). The alternative approach to chemotherapy-alone treatment in pediatric patients utilizes combination chemotherapy with low-dose radiation delivered to involved sites of disease. The superiority of treatment with combined modality versus chemotherapy alone continues to be intensely debated by investigators because of individual biases regarding chemotherapy- and radiation-related toxicities. To date, only a few longitudinal, controlled, randomized trials have been undertaken (Sackmann-Muriel et al. 1981, 1997; Weiner et al. 1997; Hutchinson et al. 1998; Nachman et al. 2002). Early studies failed to demonstrate an event-free survival advantage with the addition of radiation to combination chemotherapy. However, these results are limited in their clinical relevance because some trials required staging laparotomy with splenectomy for treatment assignment, administered radiation to extended treatment volumes in combined modality regimens, prescribed an excessive (by contemporary standards) number of chemotherapy cycles, or utilized the more leukemogenic MOPP combination chemotherapy.

A recent, more clinically pertinent study undertaken by the Children's Cancer Group investigators prospectively evaluated the benefit of adding low-dose, involved-field radiation to hybrid COPP/ABV combination chemotherapy (Nachman et al. 2002). The trial featured a risk-adapted treatment assignment based on the presence of B symptoms, hilar adenopathy, mediastinal and peripheral lymph node bulk, and the number of involved nodal regions. Patients who achieved a complete response to COPP/ABV hybrid chemotherapy were eligible for randomization to receive low-dose, involved-field radiation or no further therapy. A significantly higher number of relapses among patients treated with chemotherapy alone prompted early closure of the study. Radiation was associated with a 12% EFS differential in those with a good response after 4–6 cycles of COPP/ABV. Surprisingly, this effect was apparent in all stages of the disease, although radiation was most important for those with stage IV disease and those with B symptoms or "bulky" disease (Nachman et al. 2002). Due to successful salvage therapy after relapse, the overall survival estimates did not differ between the randomized groups, but the follow-up of the cohort is still too short.

In another prospective trial, German-Austrian pediatric Hodgkin's disease investigators assigned radiation on the basis of the clinical response at the end of therapy (Dorffel et al. 2003). Radiation was omitted for patients responding completely to risk- and gender-based OEPA or OPPA/COPP chemotherapy. Intermediate-risk and high-risk patients with complete response to chemotherapy had an inferior outcome compared with those with >75% response but not CR (81% vs. 92% EFS; p=0.01), a finding attributable to the radiation given to those with residual disease (Dorffel et al. 2003). Relapses included involved nodal areas in all of the non-radiated group and in 65% of those receiving radiation. There was no difference in DFS among irradiated and nonirradiated patients assigned to the favorable-risk group. Due to the effectiveness of retrieval therapy, overall survival was not significantly reduced.

Radiation clearly plays an important role in treating HL, yet with risks of breast cancer and myocardial infarction, identification of the group who can safely be treated with chemotherapy alone would be beneficial. One hypothesis is that patients with the most chemotherapy-sensitive disease do not need an additional modality of therapy. Studies that eliminate radiation for a subset of patients have routinely defined the subset as those with complete response at the end of therapy. The results of recent studies indicate that early response to chemotherapy may appropriately predict those who can be treated with reduced or single modality therapy. Patients in the Pediatric Oncology Group (POG) study 8725 with complete response (CR) after 3 cycles of MOPP/ABVD had improved outcome, while CR after 6 cycles was not predictive of outcome (Weiner et al. 1991). Donaldson et al. (2002) used re-

sponse after 2 cycles of VAMP to determine radiation dose (15 Gy vs. 25.5 Gy). Preliminary results of recently concluded POG studies showed that an early response could be used to determine an appropriate duration of chemotherapy (Schwartz et al. 2002). This approach is under investigation in several ongoing trials for pediatric HL.

Radiation therapy with its long-term risks (hypoplasia, hypothyroidism, cardiopulmonary fibrosis, breast cancer, myocardial ischemia) remains a major therapeutic modality for the treatment of HL. The challenge is to identify patients for whom radiation represents a less toxic modality than the chemotherapy necessary for an equivalent outcome or for whom radiation is a unique modality necessary for cure. Optimistically, the long-term risks previously noted will be less substantial in the modern era of lower dose radiotherapy to tailored fields.

3.7 Risk-Adapted Therapy

To optimize the opportunity for long-term disease control and minimize cancer-related morbidity, contemporary treatment for pediatric HL utilizes a risk-adapted approach that considers host- and cancer-related factors at diagnosis. Therapy type and intensity are largely determined by cancer-related factors, but host-specific risks for treatment toxicity may be taken into account when equally effective alternative modalities are available. Host-related factors that are most often considered in the context of risk for specific treatment-related toxicities are age and gender. Younger age at diagnosis increases the risk of musculoskeletal and soft-tissue deformity after radiation therapy and cardiovascular dysfunction after mediastinal radiation and anthracycline therapy (Donaldson and Link 1991; Adams et al. 2003, 2004). At equivalent doses of alkylating agent chemotherapy, boys exhibit a higher frequency of gonadal dysfunction than girls (Horning et al. 1981; Bramswig et al. 1990; Ortin et al. 1990; Hobbie et al. 2005). In contrast, teenage girls have a substantially increased risk of breast cancer following thoracic radiation, which is not observed in boys (Bhatia et al. 2003). At equivalent doses of anthracycline chemotherapy, females are at a higher risk for anthracycline-induced cardiomyopathy than males (Lipshultz et al. 1991). The desire to avoid a particular treatment toxicity may influence an investigator's preferred treatment approach in a given patient. Overall, however, most frontline treatment protocols for pediatric HL limit exposure to agents and modalities, taking into account risks for treatment side-effects that are unique to age and gender.

Cancer-related factors considered in the risk assessment at diagnosis include the presence of B symptoms, Ann Arbor stage, number of involved nodal regions, lymph node bulk, and extranodal extension of disease to contiguous structures. Histological subtype has anecdotally been used to direct therapy in patients with localized, completely resected, nodular lymphocyte predominant HL (Murphy et al. 2003; Pellegrino et al. 2003). This treatment approach will be prospectively studied in an ongoing Children's Oncology Group trial. Risk designations using these cancer-related parameters have varied among pediatric investigators, but typically define characteristics of low-, intermediate-, and high-risk disease presentations.

3.7.1 Treatment of Low-Risk Disease

A low-risk clinical presentation is uniformly characterized by localized (stage I/II) nodal involvement in the absence of B symptoms and lymph node bulk. Bulky mediastinal lymphadenopathy is designated when the ratio of the maximum measurement of the mediastinal lymph nodes to the intrathoracic cavity on an upright chest radiograph is 33% or more. Some studies also consider lymph node bulk outside the mediastinum in the risk assessment; this designation has ranged across studies from 4 cm to 10 cm. Likewise, the number of nodal sites considered as low risk has been variable, but generally is defined as fewer than 3 to 4 involved nodal regions.

Numerous investigations have demonstrated that children and adolescents with low-risk presentations of HL are excellent candidates for reduced therapy. The standard treatment approach for low-risk patients involves 2 to 4 cycles of chemotherapy with low-dose, involved-field radiation. The most popular multiagent regimens used for low-risk patients are characterized by little or no alkylating agent chemotherapy (see Ta-

Table 3.8 Treatment results for selected low-risk pediatric Hodgkin lymphoma

Group or institution	Number of patients	Stage	Chemotherapy	Radiation (Gy), field	Survival (%)		Follow-up interval (years)
					DFS, EFS, or RFS	Overall	
Combined modality trials:							
Stanford/St. Jude/ Boston Consortium (Hudson 2004)	15 62	I II	3 VAMP/ 3 COP 3 VAMP/ 3 COP	15–25.5, IF 15–25.5, IF	100 78	NA NA	5.8
German Multicenter (Dorffel 2003)	281 212	I-IIA II_EA, IIB, IIIA	2 OPPA/OEPA 2 OPPA/OEPA + 2 COPP	20–35, IF	94 92	NA	5
Stanford/St. Jude/ Boston Consortium (Donaldson 2002)	110	I/II (without lymph node bulk)[a]	4 VAMP	15–25.5, IF	93	99	5
U.S. Children's Cancer Group (Nachman 2002)	294	IA/B, IIA (without adverse features)[b]	4 COPP/ABV	21, IF	97	100	3
French Society of Pediatric Oncology (Landman-Parker 2000)	171 27	I-II I-II	4 VBVP, good responders 4VBVP + 1-2 OPPA, poor responders	20, IF 20, IF	91 78	97.5	5 5
Royal Marsden (Shankar 1998)	46	I-III	8 VEEP	30–35, IF	82	93	5
Royal Marsden (Shankar 1997)	125	II	6–10 ChlVPP	35, IF	85	92	10
Stanford (Hunger 1994)	44	I-III (some PS)	3 MOPP/ 3 ABVD	15–25.5, IF	100	100	10
St. Jude (Hudson 1993)	58	II/III	4–5 COP(P)/ 3–4 ABVD	20, IF	96/97	96/100	5
French Society of Pediatric Oncology (Oberlin 1992)	79 67 31	I-IIA I-IIA IB-IIB	4 ABVD 2 MOPP/ 2 ABVD 3 MOPP/ 3 ABVD	20–40, IF 20–40, IF 20–40, EF	90 87	 92	6 6 6
Stanford (Donaldson 1987)	27	PS I, II	6 MOPP	15–25, IF	96	100	5
Chemotherapy-alone trials:							
German Multicenter (Dorffel 2003)	113 52	I-IIA II_EA, IIB, IIIA	2 OPPA/OEPA 2 OPPA/OEPA + 2 COPP	None	97 78	NA	5
U.S. Children's Cancer Group (Nachman 2002)	106	IA/B, IIA (without adverse features)[b]	4 COPP/ABV	None	91	100	3
Nicaragua (Baez 1997)	14	I, IIA	6 COPP	None	100	100	3
Argentina (Sackmann-Muriel 1997)	10 16	IA, IIA IB, IIB	3 CVPP 6 CVPP	None None	86 87	NA	6.7 6.7
Costa Rica (Lobo-Sanahuja 1994)	52	IA-IIIA	6 CVPP	None	90	100	5

Abbreviations: **DFS**, disease-free survival; **EF**, extended field; **EFS**, event-free survival; **IF**, involved field; **PS**, pathologic stage; **RFS**, relapse-free survival.
ABVD, Adriamycin, bleomycin, vinblastine and dacarbazine; **ChlVPP**, chlorambucil, vinblastine, procarbazine, and prednisolone; **CVPP**, cyclophosphamide, vinblastine, procarbazine, prednisone; **COP(P)**, cyclophosphamide, Oncovin, prednisone, and procarbazine; **COPP/ABV**, cyclophosphamide, Oncovin, procarbazine, prednisone/Adriamycin, bleomycin, vinblastine; **MOPP**, nitrogen mustard, Oncovin, procarbazine, and prednisone; **OEPA**, Oncovin, etoposide, prednisone, Adriamycin; **OPPA**, Oncovin, procarbazine, prednisolone, and Adriamycin: **VAMP**, vinblastine, doxorubicin, methotrexate, and prednisone; **VBVP**, vinblastine, bleomycin, etoposide, and prednisone; **VEEP**, vincristine, etoposide, epirubicin, prednisolone.

[a] Tumor bulkdefined as mediastinal mass to thoracic cavity ratio of $\geq$ 33% or node or nodal aggregate $\geq$ 6 cm.

[b] Adverse features defined as hilar adenopathy, involvement of more than 4 nodal regions, mediastinal tumor $\geq$ 33% of chest diameter, node or nodal aggregate > 10 cm.

ble 3.8) (Schellong 1996; Landman-Parker et al. 2000; Donaldson et al. 2002; Nachman et al. 2002). Pediatric investigators from Stanford, Dana Farber, and St. Jude reported excellent outcomes using 4 cycles of vinblastine, doxorubicin, methotrexate, and prednisone (VAMP) and low-dose, involved-field radiation therapy (Donaldson et al. 2002). Five-year event-free survival and overall survival for low-risk patients treated with combined modality including the VAMP regimen was 93% and 99%, respectively (Donaldson et al. 2002). Other groups have demonstrated comparable results using regimens prescribing lower doses of anthracyclines. These trials feature regimens that use etoposide in lieu of anthracyclines or alkylators (Schellong 1996; Landman-Parker et al. 2000; Schwartz et al. 2002; Dorffel et al. 2003), which has been controversial among some investigators because of the agent's association with secondary acute myeloid leukemia (s-AML; Smith et al. 1999). French investigators observed a 5-year EFS of 91.5% in favorable-risk patients who achieved a good response following 4 cycles of vinblastine, bleomycin, etoposide, and prednisone (VBVP), a regimen including neither alkylating agent nor anthracycline chemotherapy, followed by 20 Gy to involved fields (Landman-Parker et al. 2000). Similarly, German-Austrian trials demonstrated that DFS could be maintained with a potential for less gonadal toxicity in male patients by substituting etoposide for procarbazine in the vincristine, prednisone, procarbazine, and doxorubicin (OPPA) regimen (Schellong 1996; Dorffel et al. 2003). To date, the cumulative dose of etoposide in these trials has been associated with only a rare case of s-AML (Landman-Parker et al. 2000). Therefore, the benefits of reduced gonadal and cardiac toxicity far outweigh the risk of leukemogenesis with the restricted use of etoposide in low-risk HL.

In addition to efforts at reducing chemotherapy-associated toxicity, several recent trials have aimed to eliminate radiation therapy for low-risk patients with favorable responses to chemotherapy. St. Jude investigators demonstrated that local control was not compromised by reducing involved-field radiation dose to 15 Gy in patients who achieved an early complete response to VAMP chemotherapy (Krasin et al. 2005). Their ongoing consortium trial is investigating whether radiation can be eliminated for this favorable group. In a prospective study that randomized low-risk patients who achieved a complete response to 4 cycles of COPP/ABV hybrid chemotherapy, North American investigators observed a significantly higher 3-year EFS in patients who received 21 Gy involved-field radiation consolidation (97%) compared with those who were treated with 4 cycles of COPP/ABV chemotherapy alone (91%; Nachman et al. 2002). Despite this significant difference, the still very good early treatment results in the group randomized to chemotherapy alone suggests that many patients with low-risk disease can be cured using this approach. This is supported by the results of the most recent GPOH-HD trial, which established that the outcome was not compromised by omitting involved-field radiation in low-risk patients who achieved a complete response following treatment with 2 cycles of OPPA or OEPA chemotherapy (Dorffel et al. 2003).

3.7.2 Treatment of Intermediate- and High-Risk Disease

The intermediate-risk group most often includes patients with stage I/II disease who have one or more unfavorable features, and sometimes patients with stage IIIA disease. Patients with localized disease with unfavorable features have been treated similarly to those with advanced stage (III/IV) disease in some risk-adapted pediatric trials or assigned a therapy that is intermediate in intensity in others. The criterion for unfavorable disease features has not been consistent across trials and may include the presence of B symptoms, lymph node bulk, hilar lymph involvement, involvement of 3–4 or more lymph node regions, and extranodal extension to contiguous structures. A high-risk presentation includes patients with advanced stage IIIB or IVA/B. This risk designation earns patients the most dose-intensive chemotherapy assignment therapy, which in most cases includes low-dose, involved-field radiation consolidation.

Chemotherapy used for intermediate- and high-risk HL generally includes derivative combinations of MOPP and ABVD. Because cyclophosphamide is less myelosuppressive and leukemogenic than mechlorethamine, COPP has largely replaced MOPP in pediatric trials (Schellong et al. 1997). Similar to combina-

tions used in studies of low-risk HL, etoposide has been incorporated in regimens for intermediate- and high-risk HL with the goal of improving antitumor activity and reducing cumulative doses of alkylating and anthracycline chemotherapy. The standard treatment approach prescribes a noncross-resistant chemotherapy combination on a twice-monthly schedule for a total of 6 months. Low-dose (15.0 to 25.5 Gy), involved-field radiation therapy may be delivered between treatment cycles or, more commonly, following completion of chemotherapy to consolidate remission. An alternative approach utilizes dose-intensive multiagent chemotherapy administered at weekly intervals for a period of 3 to 5 months during which myelosuppressive agents are alternated with nonmyelosuppressive agents. The abbreviated therapy duration provides the advantage of increased dose intensity, reduced therapy duration, decreased cumulative chemotherapy doses, which should theoretically reduce the potential for the development of chemotherapy resistance and treatment toxicity. Most pediatric trials consolidate with low-dose, involved-field radiation to sites of bulky or residual disease.

Treatment results for selected intermediate- and high-risk pediatric HL are summarized in Table 3.9 and are notable for the following observations: Patients at intermediate risk, i.e., those with clinically localized disease (stages I–IIIA) with unfavorable features like lymph node bulk, extranodal extension, etc., have excellent treatment outcomes following therapy reduction to 4 chemotherapy cycles (Schellong 1996; Ruhl et al. 2001; Dorffel et al. 2003). Trials in high-risk patients evaluating the substitution of nonalkylating agent chemotherapy like methotrexate or etoposide as an alternative to alkylating agent chemotherapy observed inferior EFS among patients with high-risk clinical presentations (Schellong et al. 1992; Shankar et al. 1998; Friedmann et al. 2002; Hudson et al. 2004). Preliminary results of the North American pediatric cooperative groups support the feasibility of using a compacted, dose-intensive therapy approach combined with low-dose radiation therapy (Kelly et al. 2002). However, long-term follow-up is not yet available to evaluate the efficacy and treatment sequelae. Therapy intensification for high-risk pediatric HL using hematopoietic stem cell transplantation (HSCT) as a frontline therapy has not been pursued because the EFS of these patients is well in excess of 50%. Ongoing studies evaluating the relationship of metabolic tumor activity by PET to early chemotherapy response and treatment outcomes may help identify patients who may benefit from intensification of therapy with HSCT. To date, consensus has not been established among investigators regarding prognostic features that justify the risks of this aggressive approach. Until these issues are further clarified, HSCT should be reserved for patients after relapse or for those who are refractory to primary conventional therapy, including alkylating agents.

3.8 Principles of Radiation Therapy

The complexity of current treatment strategies and the vulnerability of the developing child to both radiation and chemotherapy require a comprehensive understanding of both modalities. Newly diagnosed children will be treated with risk-adapted chemotherapy alone or combined-modality therapy including low-dose, involved-field radiation. The use of radiation alone, even for fully grown adolescents with early stage disease, has been largely abandoned. This shift derives from recognition of the cardiopulmonary and musculoskeletal morbidities of full-dose radiation and the occurrence of secondary malignant neoplasms (Donaldson and Kaplan 1982; Mauch et al. 1983; Donaldson and Link 1991). However, most mature reports demonstrate an advantage of involved-field radiation in combination with chemotherapy, and particularly in children with advanced stages of disease (Ruhl et al. 2001; Nachman et al. 2002). The principles of radiotherapeutic management are generally consistent across institutions (e. g., high-energy machines, immobilization and simulation of patients), but the nuances of volume and dose vary and will depend on the overall treatment regimen. The radiation oncologist ideally will participate in the initial evaluation and ultimate determination of therapy. Most children will be entered into institutional or group studies, and understanding (as well as agreeing with) protocol requirements is necessary. In the environment of a formal study, a central review of radiation compliance can enhance the quality of therapy. A recent review of

Table 3.9 Treatment results for selected intermediate- and high-risk pediatric Hodgkin lymphoma

Group or institution	Number of patients	Stage	Chemotherapy	Radiation (Gy), field	Survival (%)		Follow-up interval (years)
					DFS, EFS, or RFS	Overall	
Combined modality trials:							
German Multicenter (Schellong 1996)	179	II_EB, III_EA/B, IIIB, IVA/B	2 OEPA/OPPA + 4 COPP	20, IF	83/91	98/89	5
German Multicenter (Dorffel 2003)	265	II_EA, III_EA/B, IIIB, IVA/B	2 OPPA/OEPA + 4 COPP	20–35, IF	90	90	5
St. Jude (Hudson 1993)	27	IV	4–5 COP(P)/ 3–4 ABVD	20, IF	85	86	5
Stanford (Donaldson 1987)	28	III-IV	6 MOPP	15–25.5, IF	84	78	7.5
Stanford (Hunger 1994)	13	III-IV	3 MOPP/ 3 ABVD	15–25.5, IF	69	85	10
Stanford/St. Jude/Boston Consortium (Friedmann 2002)	56	I/II[a] (n=26) III/IV (n=30)	6 VEPA	15–25.5, IF	67.8	81.9	5
Stanford/St. Jude/Boston Consortium (Hudson 2004)	82	III IV	3 VAMP/ 3 COP	15–25.5, IF	68.9 68.5	92.7 (all)	5.8
Toronto (Jenkin 1990)	57	IIA-IV	6 MOPP	25–30, EF	80	85	10
U.S. Children's Cancer Group (Fryer 1990)	54	PS III/IV	6 ABVD	21, EF	87	90	4
Pediatric Oncology Group (Weiner 1997)	80	IIB, $IIIA_2$, IIIB, IV (some PS)	4 MOPP/4 ABVD	21, EF	80	87	5
U.S. Children's Cancer Group (Nachman 2002)	394	I/II[b], IIB, III	6 COPP/ABV	21, IF	87	95	3
U.S. Children's Cancer Group (Nachman 2002)	141	CS IV	COPP/ABV + CHOP + Ara-C/ VP-16	21, IF	90	100	3
Chemotherapy-alone trials							
German Multicenter (Dorffel 2003)	57	II_EA, III_EA/B, IIIB, IVA/B	2 OPPA/OEPA + 4 COPP	None	80	90	5
U.K. Children's Cancer Study Group (Atra 2002)	67	CS IV	6–8 ChlVPP	None	55.2	80.8	5
U.S. Children's Cancer Group (Nachman 2002)	394	CS I/II[b], CS IIB, CS III	6 COPP/ABV	None	83	100	3
U.S. Children's Cancer Group (Nachman 2002)	141	IV	COPP/ABV + CHOP + Ara-C/VP-16	None	81	94	3
Australia/New Zealand (Ekert 1999)	53	I-IV	5–6 VEEP	None	78	92	5
U.S. Children's Cancer Group (Hutchinson 1998)	57	PS III/IV	6 MOPP/ 6 ABVD	None	77	84	4
Nicaragua (Baez 1997)	23	IIIB, IV	8–10 COPP-ABV	None	75		3
The Netherlands (van den Berg 1997)	21 17 21	I-IV (< 4 cm node) I-IV	6 MOPP 6 ABVD 3 MOPP/ 3 ABVD	None	91 70 91	100 94 91	5
Pediatric Oncology Group (Weiner 1997)	81	IIB, III_2A, IIIB, IV	4 MOPP/ 4 ABVD	None	79	96	5
Costa Rica (Lobo-Sanahuja 1994)	24	IIIB, IV	6 CVPP/ 6 EBO	None	60	81	5
Madras, India (Sripada 1995)	43	IIB-IVB	6 COPP/ABV	None	90		5

Abbreviations: **FS**, disease-free survival; **EF**, extended field; **EFS**, event-free survival; **IF**, involved field; **PS**, pathologic stage; **RFS**, relapse-free survival; **ABVD**, Adriamycin, bleomycin, vinblastine, and dacarbazine; **COPP**, **CCNU**, vincristine, procarbazine, prednisone; **ChlVPP**, chlorambucil, vinblastine, procarbazine, and prednisolone; **CHOP**, cyclophosphamide, Adriamycin, Oncovin, prednisone; **COP(P)**, cyclophosphamide, Oncovin, prednisone, and procarbazine; **COPP/ABV**, cyclophosphamide, Oncovin, procarbazine, prednisone/Adriamycin, bleomycin, vinblastine; **CVPP**, cyclophosphamide, vinblastine, procarbazine, prednisone; **EBO**, epirubicin, bleomycin, vincristine; **MOPP**, nitrogen mustard, Oncovin, procarbazine and prednisone; **OEPA**, Oncovin, etoposide, prednisone, Adriamycin; **OPA**, Oncovin, prednisone, Adriamcyin; **OPPA**, Oncovin, procarbazine, prednisolone, and Adriamycin; **VBVP**, vinblastine, bleomycin, etoposide, and prednisone; **VEEP**, vincristine, etoposide, epirubicin, prednisolone; **VEPA**, vinblastine, etoposide, prednisone, Adriamycin.

[a] With tumor bulk defined as mediastinal mass to thoracic cavity ratio of ≥ 33% or node or nodal aggregate ≥ 6 cm.

[b] With adverse features defined as hilar adenopathy, involvement of more than 4 nodal regions, mediastinal tumor ≥ 33% of chest diameter, node or nodal aggregate > 10 cm.

the DAL-HD-90 trial (German-Austrian pediatric multicenter trial) showed that up-front centralized review of patients entered into the study altered the treatment approach in a large number of children (Dieckmann et al. 2002). Unpublished data from the POG also support the superior outcome of children treated with appropriate radiation fields and doses, in contrast to those in whom protocol violations occurred.

3.8.1 Volume Considerations

The tandem development of eloquent diagnostic imaging and effective combination chemotherapy has provoked redefinition of the treatment volume appropriate for children treated with multimodality therapy. Future studies on patterns of disease recurrence in this setting will augment our understanding of the necessary radiation volumes. Nevertheless, knowledge, judgment, and skill are required to irradiate children appropriately. Table 3.10 provides an example of involved-field definitions for children. These definitions depend on the anatomy of the region in terms of lymph node distribution, patterns of disease extension into adjacent areas, and consideration for match line problems should disease recur. In fact, these definitions are not static, since the uncertainty regarding the presence of subclinical disease has diminished with advanced imaging techniques, including PET. However, a conservative approach remains appropriate for patients not treated on protocols. Thus, involved fields typically still include not just the identifiably abnormal lymph nodes, but the entire lymph node chain containing the involved node(s). For example, the cervical and supraclavicular lymph nodes are generally treated when abnormal nodes are located anywhere within this area; this is consistent with the anatomic definition of lymph node regions used for staging purposes (Kaplan and Rosenberg 1966). However, confining the volume to include either the cervical or the supraclavicular region with an appropriate margin (e. g. 2 cm) may be equally effective with less normal tissue exposure. A conservative approach adopts the former strategy, but some protocols specify the latter. The traditional definitions of lymph node regions can be helpful, but are not necessarily sufficient. That is, confining the radiation volume to the involved region may not fulfill the criteria of a judgmental approach. For example, the hila are generally irradiated when the mediastinum is involved, despite the fact that the hila and mediastinum are separate lymph node regions. Similarly, the SCV is often treated when the axilla or the mediastinum is involved, and the ipsilateral external iliac nodes are often treated when the inguinal nodes are involved. However, in both these situations care must be taken to shield relevant normal tissues to the degree possible, such as the breast in the former situation, and ovaries in the latter. Moreover, the decision to treat the axilla or mediastinum without the SCV, and the inguinal nodes without the iliacs, might be appropriate depending on the size and distribution of involved nodes at presentation. In a very young child, consideration may be given to treating bilateral areas (e. g., both sides of the neck) to avoid growth asymmetry. However, this is less of a concern with low radiation doses, and thus

unilateral fields are usually appropriate if the disease is unilateral. Maneuvers to exclude vulnerable normal tissues (e. g. breast, ovaries, heart) are always a component of the planning process.

From the above discussion, it is clear that treating an involved supradiaphragmatic or mantle field requires precision because of the distribution of lymph nodes and the critical adjacent normal tissues. These fields can be simulated with the arms up over the head, or down with hands on the hips. The former pulls the axillary lymph nodes away from the lungs, allowing greater lung shielding. However, the axillary lymph nodes then move into the vicinity of the humeral heads, which should be blocked in growing children. Thus, the position chosen involves weighing concerns regarding lymph nodes, lung, and humeral heads. Efforts should be made to exclude breast tissue altogether or to position it under the lung/axillary blocking. Equally weighted anterior and posterior fields are treated daily. Anteriorly weighted mediastinal fields excessively irradiate the anterior heart with associated cardiac morbidity (Gottdiener et al. 1983). Dose calculations should be based on the patient separation at the central axis. Nodes in the neck and axilla may receive a higher dose because of the decreased patient thickness compared with the midthorax. Therefore, separate axillary, neck, and low mediastinal dosimetry should be performed, and compensating filters or other modifications should be used to minimize inhomogeneity. Extended source-to-skin distances decrease dose inhomogeneity in these different areas. An anterior laryngeal and a posterior occipital block is often used throughout treatment if the disease is not thereby shielded. A posterior cervical spine block might also be appropriate to limit this structure to a chosen dose, depending on disease location (e. g., involved cervical nodes are usually not midline) and the total dose used. Blocking the thoracic cord is not recommended because it risks underdosing the mediastinal nodes (Prosnitz et al. 1997). Lung blocks should allow adequate (1 to 2 cm) margins around the mediastinal disease. The width of the mediastinal/hilar field is generally based on the postchemotherapy residual disease, whereas the cephalad-caudad dimension respects the original disease extent. Humeral head blocks are used unless bulky axillary adenopathy would thereby be shielded. Depending on the response of the disease to chemotherapy and the dose administered, field reductions may be possible. Because 10- to 15-Gy doses can cytoreduce HL, increasing the size of the lung or cardiac blocks is often possible during the course of therapy; however, it is uncommon to use RT in a setting where large disease bulk has not already been cytoreduced by chemotherapy. The entire heart or lungs are rarely treated above doses of 10 to 16 Gy, depending on the distribution of disease and chemotherapy used. More specifically, the indications for whole-heart irradiation include pericardial involvement as suggested by a large pericardial effusion or frank pericardial invasion with tumor; such patients will generally receive combined modality therapy and 10 to 15 Gy to the entire heart. Whole-lung irradiation with partial transmission blocks is a consideration in the setting of overt pulmonary nodules. Again, this is protocol-dependent since some children treated for advanced stage HL will receive RT only to areas of initial bulk disease or postchemotherapy residual disease. However, this approach remains investigational, and involved field radiation therapy is usually the appropriate treatment approach, as demonstrated by recent reports (Ruhl et al. 2001; Nachman et al. 2002). Thus, for children with pulmonary nodules at diagnosis, whole-lung irradiation to 10 to 15 Gy is a consideration. A gap should be calculated when matching the paraaortic field.

Radiation therapy to a subdiaphragmatic region requires the same types of considerations and, of course, is dependent on the distribution of involved sites. The spleen or pedicle is included in patients who have splenic involvement, while minimizing the radiation dose to the kidneys. Whether the spleen (or pedicle) should be routinely treated in the setting of paraaortic but not overt splenic involvement is controversial. In the absence of a study, elective splenic irradiation is generally advised. Usually the upper pole of the left kidney is within the irradiated volume. A treatment planning CT or diagnostic information obtained from the CT or MRI is helpful in determining the blocks. When treating the pelvis, special attention must be given to the ovaries and testes. The ovaries should be relocated, and marked with surgical clips, laterally along the iliac wings, or centrally behind the uterus. In this manner appropriate shielding may be used in or-

Table 3.10 Involved field radiation guidelines

Involved node(s)	Radiation field
Cervical	Neck and infraclavicular/ supraclaviculara
Supraclavicular	Neck and infraclavicular/ supraclavicular +/– axilla
Axilla	Axilla +/– infraclavicular/ supraclavicular
Mediastinum	Mediastinum, hila, infraclavicular/ supraclavicular[a,b]
Hila	Hila, mediastinum
Spleen	Spleen +/– para-aortics
Para-aortics	Para-aortics +/– spleen
Iliac	Iliacs, inguinal, femoral
Inguinal	External iliac, inguinal, femoral
Femoral	External iliac, inguinal, femoral

[a] Upper cervical region not treated if supraclavicular involvement is extension of the mediastinal disease.
[b] Prechemotherapy volume is treated except for lateral borders of the mediastinal field, which is postchemotherapy.

der to administer as little irradiation as possible, and certainly less than 6 Gy. The testes receive 5–10% of the administered pelvic dose, which is sufficient to cause transient or permanent azoospermia, depending on the total pelvic dose. The greatest shielding can be afforded to the testes if the patient is placed in a frog-legged position with an individually constructed testes shield. If multileaf collimation is available, the multileaf can be placed over the testes, additionally decreasing the transmitted dose. As previously stated, radiation therapy for unfavorable and advanced HL is variable and protocol-dependent. Although IFRT remains the standard when patients are treated with combined modality therapy, restricting RT to areas of initial bulk disease (generally defined as 5 cm or more at the time of disease presentation) or postchemotherapy residual disease (generally defined as 2 cm or more, or residual PET avidity) is under investigation.

3.8.2 Dose Considerations

In the setting of combined modality therapy, the radiation dose will depend on the overall treatment regimen and the specific chemotherapy utilized. Most data describing the radiation dose response of HL are based on studies in adults, including the Patterns of Care reports (Coia and Hanks 1988; Schewe et al. 1988; Sears et al. 1997; Nadali et al. 1998). In the absence of chemotherapy, subclinical disease is reliably (95%) controlled with 25 to 30 Gy, small bulk disease (variously defined but less than 5 cm in most reports) requires 30 to 35 Gy, and large bulk disease requires an additional 5 to 10 Gy. The doses per fraction should be 1.5 to 1.8 Gy daily, five times a week. Patients treated with large volumes may only tolerate 1.5 Gy fractions. Studies randomizing children to different radiation doses, in order to determine the "correct" dose, are lacking. In the setting of combined therapy, the intensity of the chemotherapy must be understood in order to determine the radiation dose and volume. In general, doses of 15 to 25 Gy are used with shrinking fields and individualized boosts. When the decision is made to include some or all of a critical organ (liver, kidney, or heart) in the radiation field, then normal tissue constraints will relate to the chemotherapy used and patient's age. In the tables summarizing recent clinical trials for early and advanced stage HL, the radiation doses selected to complement the chemotherapy regimen are provided. In general, doses of greater than 25 Gy are uncommon in the pediatric setting. Caution must be used in applying the results from published reports. For example, in the recently analyzed DAL-HD-90 trial, doses of 20–25 Gy were administered in combination with OEPA or OPPA, with or without COPP (Dieckmann et al. 2002). However, a local boost of 5–10 Gy was delivered for insufficient remission following chemotherapy. Tumor burden, indicated by bulky disease or number of involved nodes, proved not to be prognostically significant in this report, perhaps due to the boost doses. Despite the excellent tumor control in this study, radiation doses of 25–35 Gy are rarely recommended in most current investigations. Also of interest are recent data from a randomized trial by the German Hodgkin Lymphoma Group in which patients (adults) with stage I to IIIA disease received 20, 30, or 40 Gy to non-

bulky or uninvolved sites following 4 months of chemotherapy. Bulk (greater than 7.5 cm) disease always received 40 Gy. With this constraint, no difference was observed for the various doses (Loeffler et al. 1997).

3.8.3 Energy

Megavoltage energies are necessary. A 4- to 6-MV linear accelerator should be used for supradiaphragmatic fields, thereby ensuring adequate doses to the superficial nodes in the build-up region as well as to deep nodal areas such as the mediastinum. Higher energy machines (8 to 15 MV) may be appropriate for treating paraaortic nodes. If high-energy machines must be used for treatment of the mantle field, some therapists introduce a beam spoiler or bolus on the neck and supraclavicular regions. Cobalt 60 units can underdose the field edge, and orthovoltage units are absolutely inappropriate. Distances of less than 80 cm are also contraindicated because of suboptimal depth-dose characteristics.

3.9 Summary Recommendations for Primary Disease/Selection of Therapy

Multidisciplinary collaboration facilitates optimal treatment planning. Ideally, the pediatric and radiation oncologist should meet at diagnosis and response evaluations to review diagnostic imaging studies following examination of the patient. The treatment approach should consider host factors that may enhance the risk for specific treatment toxicities, as well as disease factors that may permit therapy reduction or require dose intensification. Since patients with low-risk disease presentations can achieve long-term DFS using regimens that do not contain alkylators, ABVD or derivative chemotherapy is preferred for this group. An alternative strategy is to add alkylating agents or etoposide to the regimen, which may preserve cardiac function by reducing anthracycline cumulative dosage without compromising disease control. Combined modality treatment regimens using limited cycles of combination chemotherapy and low-dose, involved-field radiation therapy have produced excellent results in pediatric patients with low-risk disease. Combinations derived from both ABVD and MOPP still provide the most effective chemotherapy strategies for children and adolescents with intermediate- or high-risk disease presentations. Etoposide is often added to these regimens to increase the antitumor activity and reduce the gonadal toxicity associated with alkylating agent chemotherapy. Attempts to restrict or completely omit alkylating agents in these high-risk groups have resulted in unsatisfactory outcomes, as have protocols prescribing ABVD or derivative chemotherapy alone (Shankar et al. 1998; Ekert et al. 1999; Friedmann et al. 2002; Hudson et al. 2004). Regimens reporting the best long-term outcomes prescribe low-dose radiation therapy to nodal regions involved at diagnosis or a radiation boost to areas with suboptimal response to chemotherapy (Nachman et al. 2002; Dorffel et al. 2003). A summary of recommended treatment approaches for low-, intermediate-, and high-risk disease presentations is outlined in Table 3.11.

3.10 Acute Effects of Therapy

3.10.1 Chemotherapy Side-Effects

The nausea and vomiting associated with chemotherapy administration for pediatric HL is generally responsive to serotonin receptor antagonist antiemetics, such as ondansetron. Premedication with benzodiazepines is usually effective in controlling anticipatory nausea that may develop later as therapy progresses. Most treatment regimens will cause some degree of reversible alopecia. Myelosuppression remains the most common dose-limiting acute toxicity of contemporary multiagent chemotherapy. Administration of granulocyte colony stimulating factor support prevents treatment delays and facilitates maintenance of chemotherapy dose intensity. Hospitalization for antimicrobial therapy for febrile neutropenia and blood product transfusion is occasionally required. *Varicella zoster* infections are quite common during and after therapy for HL, with a frequency that has been directly related to the intensity of treatment (Reboul et al. 1978).

Several agents, including nitrogen mustard, vincristine, vinblastine, and doxorubicin, may result in local tissue damage if subcutaneous tissue extravasation oc-

Table 3.11 Recommended treatment approaches in pediatric Hodgkin lymphoma

Clinical presentation	Recommended treatment approach
Low risk: Localized disease (stage I or II) < 3–4 involved nodal regions without B symptoms, lymph node bulk[a] or extranodal extension of disease from contiguous lymph node structures	Recommended therapy: 2–4 cycles non-cross-resistant chemotherapy without alkylators plus low-dose, involved-field radiation (15–25 Gy) Other considerations: 6 cycles non-cross-resistant chemotherapy alone In clinical trial setting only: 4 cycles of chemotherapy alone
Intermediate risk: Localized disease (stage I or II) with ≥ 3–4 involved nodal regions, lymph node bulk[a], extranodal extension of disease from contiguous lymph node structures Some stage IIB[b] disease (e. g., sweats only) Stage IIIA disease	Recommended therapy: 4–6 cycles non-cross-resistant chemotherapy plus low-dose, involved-field radiation (15–25 Gy) Other considerations: 6–8 cycles non-cross-resistant chemotherapy alone
High risk: Some stage IIB[b] disease (e. g., fever or weight loss) Stage IIIB Stage IVA/B	Recommended therapy: 6–8 cycles of non-cross-resistant chemotherapy plus low-dose, involved-field radiation (15–25 Gy) Other considerations: 8 cycles non-cross-resistant chemotherapy alone

[a] Mediastinal bulk defined as ratio of measurement of diameter of mediastinal mass to maximum intrathoracic cavity at the dome of the diaphragm of ≥ 33%; other lymph node defined as lymph node mass ≥ 6–10 cm.

[b] Stage IIB patients have been variably treated as intermediate or unfavorable risk. Some studies use associated factors, e. g., weight loss, bulk disease, extranodal extension, for further risk stratification.

curs during administration. Vinca alkaloids (vincristine, vinblastine) comprise an important component of contemporary, dose-intensive, multiagent chemotherapy programs. Peripheral neuropathy commonly develops when these agents are given on a weekly schedule for an extended period of time. Gabapentin may be helpful in managing severe or refractory pain or paresthesias associated with sensory neuropathy. Motor neuropathy universally results in temporary loss of deep tendon reflexes. In cases with progressive motor dysfunction, e. g., foot drop or hoarseness associated with vocal cord paralysis, vinca alkaloid therapy should be withheld until there is an improvement in the neurologic function, at which time administration of a reduced dose should be considered. Autonomic neuropathy associated with vinca alkaloid therapy may cause severe constipation/obstipation; aggressive supportive care is essential to prevent this complication.

Bleomycin administration causes acute, largely a symptomatic, pulmonary toxicity that can be detected on formal pulmonary function testing. Bleomycin can be withheld following acute declines of 20% or more in spirometry or diffusion without compromising outcome (Hudson et al. 1993). A substantial proportion of these abnormalities will improve or resolve during follow-up after completion of therapy (Marina et al. 1995). Acute cardiac toxicity associated with anthracycline agents like doxorubicin rarely occurs with the restricted doses of these agents prescribed in contemporary regimens. Periodic screening of cardiac function is recommended after completion of therapy because subclinical cardiac injury may become more clinically significant in aging survivors (see Chapter 12).

3.10.2 Radiation Side-Effects

The radiation doses used in contemporary combined modality treatment protocols are generally well tolerated. Premedication with antiemetics controls nausea and vomiting and permits the patient an excellent quality of life during treatment. Transient skin effects may include mild erythema or hyperpigmentation. Depending on the extent of the cervical treatment fields, patients may experience a transient occipital alopecia, mild dysphagia, xerostomia, and taste alterations. Because treatment fields are limited to involved nodal regions, declines in blood counts are relatively uncommon unless the disease extent requires total nodal irradiation. Even in those cases, anemia, granulocytopenia, and thrombocytopenia most often reflect bone marrow suppression from prior chemotherapy and uncommonly require interventions like transfusion or colony-stimulating growth factor support. A rare subacute effect of mantle radiation is a transient myelopathy (Lhermitte's syndrome) that may produce a sensation of an electric shock radiating down the back and into the extremities on flexion of the neck. This condition, which is uncommon following radiation doses below 30 to 35 Gy, is self-limited and resolves without neurologic sequelae. In general, acute radiation effects are mild and reversible.

3.11 Future Directions

HL has been curable for many decades. The long history of successful treatments have led to many survivors. From them we have learned the toxicity of our therapies. In this next millennium, we hope to learn to cure with minimal toxicity. Better paradigms of care will be our initial steps toward this goal, but ultimately it will be the biologic understanding of HL that will allow for the development of more efficacious and less toxic therapies. Understanding aberrant pathways of cell death, particularly abnormal apoptotic pathways attributable to constitutive activation of NF-κB, may allow targeted therapies that may enhance the response (Bargou et al. 1996). Early trials using proteasome inhibitors have been initiated (Adams 2001). Immunomodulatory approaches designed to enhance the T-cell response to EBV (Rooney et al. 1995) or to the tumor are also being considered. With such approaches, it may be possible to achieve the goal of optimal response without enhanced toxicity.

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Treatment of Relapsed/Refractory Hodgkin Lymphoma

T.M. Trippett • A. Chen

Contents

4.1 Introduction

In the past 10–20 years, the treatment outcome for patients with pediatric Hodgkin disease (HL) has improved remarkably; however, 10–20% of the patients still relapse. Historically, retrieval approaches for patients with recurrent HL have utilized regimens that were previously used in frontline therapy. Generally, the degree of response to these regimens predicted the ability to rescue patients. In children and adolescents, the tolerance of salvage therapy has been exceptional, permitting the evaluation of novel therapeutic strategies. Given this fact, the introduction of new chemotherapeutic, immunologic and biologic agents is necessary to improve the response rate of pediatric patients with recurrent/refractory HL. Because of the significant risk of treatment-related secondary malignancies in pediatric patients associated with the use of alkylating agents and epipodophyllotoxin chemotherapy, agents frequently used in the treatment of HL (Krishnan et al. 2000; Pedersen-Bjergaard et al. 1997; Wheeler et al. 2001), alternative therapeutic approaches for retrieval that are both efficacious and safe must be considered for pediatric patients with relapsed/refractory HL.

4.2 Strategies for Re-induction

Combined modality chemotherapy and radiotherapy have resulted in the cure of 80–90% of pediatric patients with HL. Approximately 10–20% of patients with advanced stage HL relapse after front-line treatment. Historically, a failure to respond to treatment with standard-dose conventional chemotherapy has resulted in low complete remission rates and minimal

survival benefit. Longo et al. reported a median survival of 16 months in patients who never attained a CR in a series of 51 patients treated with Methotrexate, Oncovin, Procarbazine and Prednisone (MOPP) (Longo et al. 1992). Likewise, Bonfante et al. reported similar results in patients who failed MOPP or MOPP/ABV hybrid or alternating regimens with a long-term event-free survival (EFS) of 8% (Bonfante et al. 1997). Failure to respond or relapse is directly related to the duration of the initial response (Longo et al. 1992). Progression during induction therapy or within 12 months of completion of treatment resulted in a dismal prognosis with 5-year disease-free survival rates of 0% and 20%, respectively (Longo et al. 1992). Relapses occurring 12 months or later were amenable to salvage chemotherapy, but overall survival rates were 20–50% with conventional chemotherapy (Fisher et al. 1979; Longo et al. 1992; Viviani et al. 1990).

4.2.1 Role of Re-induction Chemotherapy

Response to cytoreductive (re-induction) chemotherapy prior to high-dose therapy in patients with relapsed/refractory HL predicts overall survival (OS) regardless of the type of salvage therapy. Yuen et al. reported that sensitivity of disease reflected by response to cytoreductive therapy prior to high-dose therapy in patients with relapsed/refractory HL was a significant predictor of OS regardless of the type of salvage therapy (Yuen et al. 1997). Likewise, Rapoport et al. demonstrated that high-dose therapy was most effective for low-risk patients who enter with minimal or sensitive disease (Rapoport et al. 1993). In this series, minimal disease status at the time of transplant was the major predictor of improved EFS for patients with HL and non-Hodgkin's lymphoma (NHL). Moskowitz et al. reported on 65 relapsed/refractory HD patients treated at Memorial Sloan-Kettering Cancer Center who underwent induction chemotherapy with ICE prior to high-dose therapy; there was a response rate to ICE of 88% and an EFS of 68% for patients (median follow-up 43 months) who underwent transplantation (Moskowitz et al. 2001). The EFS among HL patients with a positive response to salvage was 58% vs 35% in those who did not respond (p=0.12). Thus, the advantages of induction chemotherapy may be to decrease tumor burden before high-dose therapy and to select appropriate candidates for high-dose treatment. Further well-designed prospective studies are needed to test and substantiate this hypothesis.

4.2.2 Standard Re-induction with ICE

As single agents or in combination, the chemotherapeutic agents ifosfamide, carboplatin, and etoposide have been effective in the treatment of adult (Moskowitz et al. 1999, 2001) and pediatric patients with HL and NHL. Consequently, the combination is a commonly used re-induction regimen in patients with relapsed/refractory disease. Kung et al. reported a response rate of 80% in a phase II trial in pediatric patients with recurrent non-Hodgkin lymphoma treated with ICE (Kung et al. 1995). Limited data, however, are available from pediatric phase I/II studies regarding response in HL to ICE. In the one available study, Moskowitz et al. at Memorial Sloan-Kettering Cancer Center reported an 88% response rate with ICE in a combined trial with adult and pediatric patients with relapsed/refractory HL (Moskowitz et al. 2001).

Observation of an increased incidence of treatment-related secondary malignancies associated with the use of alkylating agents and the epipodophyllotoxins (etoposide and teniposide) mandates consideration of alternative therapeutic approaches for re-induction that incorporate novel, effective, and less toxic agents. Etoposide, which has been shown to be a highly active agent in the treatment of HL and other pediatric tumors, has been associated with the development of myelodysplastic syndrome and secondary acute myelogenous leukemia. Given this finding, alternative re-induction approaches must be explored utilizing combinations of agents with non-overlapping mechanisms of action and toxicity as well as acceptable short- and long-term safety profiles. Two re-induction regimens incorporating novel agents with acceptable toxicity profiles are being evaluated in Children's Oncology Group (COG) phase II trials combining the chemotherapeutic agents, ifosfamide and vinorelbine (AHOD00P1) and gemcitabine and vinorelbine (AHOD0321).

4.2.3 Re-induction Therapy with Ifosfamide/ Vinorelbine (IV)

Vinorelbine (Navelbine, VRB), a semisynthetic alkaloid, exhibits marked clinical activity in HL and NHL (Borchmann et al. 1998; Devizzi et al. 1994, 1996). Similar to other vinca alkaloids, the mechanism of action of VRB is inhibition of microtubule formation (Toh et al. 1998). Vinorelbine, however, demonstrates more selective inhibition of mitotic microtubule formation as opposed to the inhibition of neural axonal formation observed with vinca alkaloids, thereby diminishing the likelihood of neurotoxicity. Preclinical studies indicated broad-spectrum antitumor activity in in vitro and in vivo model systems in a variety of murine cell lines, L1210 leukemia, P388 leukemia, B16 melanoma, and human tumor cell lines (leukemia, colorectal carcinoma, central nervous system, breast carcinoma, non-small-cell and small-cell lung carcinoma) (Toh et al. 1998).

Vinorelbine has been studied in adult and pediatric phase I clinical trials. The adult maximum tolerated dose (MTD) ranged from 30 to 35 mg/m^2/week. Adult phase I studies evaluated the toxicity profile of VRB administered on a weekly intravenous bolus dose schedule. Extensive experience in these studies has demonstrated that VRB has limited severe toxicities. The dose-limiting toxicity (DLT) was granulocytopenia, noted in 60% of patients. The predominant nonhematologic toxicities include transient elevation in hepatic transaminases, alkaline phosphatase, and bilirubin. Reversible peripheral neuropathy was observed in 20% of patients. Asthenia, injection site reactions (phlebitis < 5%) nausea, vomiting, and constipation were uncommon. In the phase II studies, response rates as high as 50% have been reported when VRB is given weekly as a single agent to heavily pretreated patients with relapsed or refractory HL (Rule et al. 1998; Devizzi et al. 1996), with some complete responses (CR) seen. Grade 3–4 granulocytopenia was reported in ~ 50% of patients. Local injection site reactions and constipation were uncommon. In a pediatric phase I clinical trial in patients with recurrent or refractory pediatric malignancies, the MTD was established at 33.75 mg/m^2/dose. In a phase II study conducted by the Children's Cancer Group, A09705, VRB was administered on a weekly schedule for 6 weeks in 50 children with recurrent or refractory pediatric malignancies. Due to significant neutropenia resulting in frequent treatment delays, the dose of VRB was reduced from 33.75 mg/m^2/dose to 30 mg/m^2/dose. Nonhematologic toxicity at either dose seemed to be less frequent than that reported in adult trials.

The combination of ifosfamide and vinorelbine (IV) has been evaluated in a phase II trial in adult patients with refractory/recurrent HL (Bonfante et al. 1997). An overall response rate of 80% (40% CR and 40% PR) was achieved with a median of two cycles of IV in 20 patients (Bonfante et al. 1997). The results were particularly encouraging in patients with extranodal disease who had a response rate of 89%. This combination was well-tolerated with no apparent cumulative toxicity after as many as ten consecutive cycles. The toxicity profile of this combination was limited to grade 3–4 neutropenia in only 50% of the cycles with a median duration of 4 days. Fanconi's tubular dysfunction was not observed after IV as with ICE (Ho 1995). In the pediatric setting, a phase II COG pilot study (AHOD00P1) was conducted to evaluate IV as a novel re-induction regimen for patients with relapsed/refractory HL prior to stem cell transplantation (Trippett et al. 2004). The schedule of administration comprised a 21-day treatment cycle consisting of ifosfamide 3000 mg/m^2/day administered by continuous intravenous infusion for 4 consecutive days and VRB 25 mg/m^2/dose administered by intravenous bolus on days 1 and 5. The treatment schema is shown in Fig. 4.1. The primary objectives of this study were to assess the toxicity, capability to mobilize hematologic stem cells, and response rate of this novel re-induction regimen. An acceptable toxicity profile was demonstrated with the predominant toxicity being reversible myelosuppression. The major grade 3 toxicities included neutropenia (81%), thrombocytopenia 44%, and anemia 69%. The incidence of nephrotoxicity and neurotoxicity was negligible, 3% and < 1%, respectively. Acceptable stem cell mobilization rates were noted as well. Response data in pediatric patients with heavily pretreated relapsed/refractory HL demonstrated an objective response rate (ORR; complete/partial response: CR/PR) of 78%. Successful mobilization of peripheral blood stem cells was accomplished in the majority of patients

(Trippett et al. 2004). These data substantiate IV as an acceptable re-induction regimen for pediatric patients with relapsed/refractory HL.

4.3 High-Dose Therapy

Since its introduction 20 years ago, high-dose therapy with autologous stem cell rescue has become the treatment of choice for patients with relapsed or refractory HL. The increase in the use of high-dose therapy is due largely to the marked reduction in early transplant-related mortality, improved disease free-survival, and widespread availability of this approach. In multiple series, sustained remissions have been seen after high-dose therapy and autologous bone marrow rescue, and more recently, peripheral stem cell transplantation with response rates reaching 50% in most studies and early transplant-related mortality rates <10%. A variety of high-dose therapy regimens including cyclophosphamide, busulfan, etoposide (CBV), busulfan, etoposide, cytarabine, melphalan (BEAM), or high-dose melphalan with or without total body irradiation (TBI) have been used; however, none of these regimens have been shown to be superior (Chopra et al. 1993; Jagannath et al. 1989; Kessinger et al. 1991; Reece et al. 1994; Schmitz et al. 2002). Despite maximal intensification of therapy with autologous stem cell rescue, only 40–50% of patients are salvaged. The predominant reason for failure in patients undergoing salvage therapy is relapse. Therefore, the need for effective novel retrieval strategies as an adjunct to high-dose therapy is paramount.

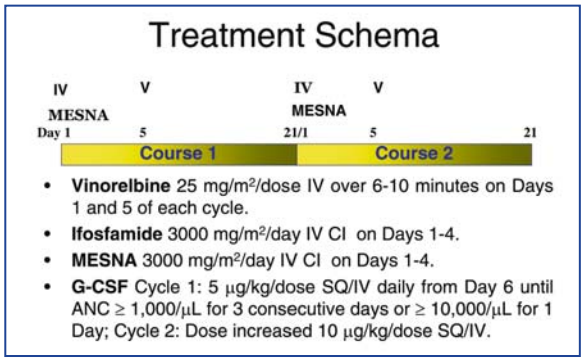

Figure 4.1

Treatment schema for AHOD00P1, Phase II Study of Ifosfamide/Vinorelbine

In adult patients, high-dose therapy with autologous hematopoietic stem cell rescue (ASCR) has become the standard option for salvage therapy over conventional allogeneic stem cell transplantation in patients with relapsed or refractory HL, with reported survival rates ranging between 30 and 50%. The role of conventional allogeneic stem cell transplantation has been limited to younger patients because of high non-relapse mortality rates (43–61%) and graft-versus-host disease (GVHD) (Akpek et al. 2001; Anderson et al. 1993; Milipied et al. 1996; Gajewski et al. 1996). Retrospective data in children undergoing ASCT, although limited, have demonstrated similar 5-year OS, EFS, and PFS rates to that of adult patients, with a survival advantage for patients with refractory disease or relapse within 12 months of completion of front-line therapy (Baker et al. 1999; Lieskovsky et al. 2004). The therapeutic benefit of high-dose therapy in patients who relapse more than 1 year after completion of front-line therapy remains controversial. In this regard, Ardeshna et al. reported a survival advantage in this group of patients over salvage with conventional chemotherapy (Ardeshna et al. 2005). Conversely, the United Kingdom Children's Cancer Study Group concluded that overall survival in patients treated with ASCT did not differ significantly from that of those treated with conventional salvage therapy following a retrospective study in 51 pediatric patients with relapsed or refractory HL (hazard ratio = 1.5; 95% confidence interval= 0.9–8.2; p= 0.4) (Schmitz et al. 2002). Moreover, survival data did not differ among the patients who underwent ASCT or conventional chemotherapy if the duration of first remission was less than or greater than 1 year (p = 0.5; stratified log-rank). Despite these conflicting results, the general consensus is that ASCT enhances the potential for long-term cures and should be considered in children and adolescents with relapsed/refractory HL.

Although high-dose therapy appears to be an effective therapeutic modality for the treatment of recurrent or refractory HL, approximately 40–50% of children and adolescents will experience a subsequent relapse of their disease. The patterns of relapse follow-

ing high-dose chemotherapy occur in the majority of the cases (81%) in the sites of prior disease. However, first-time presentation of intrapulmonary disease has been demonstrated in 53% of the cases (Stoneham et al. 2004). Several prognostic factors have been identified in the adult literature that determine the outcome after transplantation including bulk of disease at transplantation, systemic symptoms at relapse, extranodal disease at relapse, number of prior treatment regimens, duration of initial remission, performance status, and relapse within a prior radiation field (Bonfante et al. 1997; Burns et al. 1995; Chopra et al. 1993; Crump et al. 1993; Jagannath et al. 1989; Moskowitz et al. 2001; Rapoport et al. 1993). In the pediatric literature, additional prognostic factors include female sex, interval from diagnosis to ASCT <15 months, elevation of LDH levels, and disease sensitivity at the time of ASCT (Baker et al. 1999; Lieskovsky et al. 2004). The most significant factors in children that are predictive for poor OS, EFS, and PFS after ASCT as in adults were extranodal disease at the time of relapse and bulky mediastinal mass at the time of transplantation. Based upon these findings, additional therapeutic approaches must be explored to augment the response to high-dose chemotherapy.

4.3.1 Immunomodulation as a Therapeutic Strategy to Augment High-Dose Therapy

The observation that immunologic effector mechanisms are not cross-resistant with chemotherapy and radiation therapy suggests a potentially beneficial role of immunotherapy (Fuchs et al. 1995; Kontny et al. 1998). Further support includes data demonstrating an allogeneic effect after bone marrow transplantation in HL, albeit offset by transplant-related morbidity (Anderson et al. 1993; Jones et al. 1991). Infusion of donor lymphocytes has been reported to produce a response in recurrent HL after bone marrow transplantation (Russell et al. 1996). Immunomodulation with interferon-γ and interleukin-2 (IL-2) following autologous stem cell rescue has also been demonstrated to reduce the rate of relapse and to improve survival compared to historical controls (Nagler et al. 1997). Based on these data, investigators in the COG are conducting a phase II clinical trial, ADHOD0121, evaluating the feasibility and efficacy of a novel therapeutic approach that combines high-dose therapy with immunotherapy with cyclosporine, interferon-γ, and IL-2 to stimulate autologous GVHD during recovery following ASCR which may result in an antitumor effect (Chen et al. 2005). The primary aims of the study are to improve survival in patients with recurrent or refractory HL and to provide proof of principle for immunotherapy after autologous stem cell rescue (ASCR). The initial or feasibility phase of the study has been completed. Patients with biopsy-proven recurrent or refractory HL were enrolled in the study and received immunomodulation with cyclosporine, interferon-γ, and IL-2 following high-dose BEAM as a preparative regimen and ASCR. Expected reversible complications including febrile neutropenia, pancytopenia, nausea, vomiting, anorexia, mucositis, and electrolyte disturbances were observed. Two patients developed pneumonitis after receiving immunotherapy, one of whom died of respiratory failure 6 weeks after study entry. The latter patient received only 2 doses of IL-2 before developing pneumonia. Bronchoalveolar lavage failed to demonstrate an etiology for the pneumonia; however, a culture obtained from an open lung biopsy was positive for *Staphylococcus epidermidis*. One patient developed a rash, and one patient developed liver abnormalities during immunotherapy. Peripheral blood samples were obtained at weekly intervals during immunotherapy to test for autoreactivity in mixed lymphocyte cultures and by cytokine assays with autologous stimulator cells. In 11 of 14 evaluable patients, there was significant in vitro lymphocyte autoreactivity. Based upon these findings, the immunotherapy regimen was found to have acceptable tolerance and induced autoreactivity in a sufficient proportion of patients to warrant proceeding with the second phase of the study, testing the efficacy of the regimen by randomization of patients with chemosensitive recurrent/refractory HL to receive immunotherapy or not following conditioning with BEAM and ASCR. Patients with refractory HL will undergo nonrandom assignment to receive immunotherapy.

4.3.2 Reduced-Intensity/Non-myeloablative Allogeneic Stem Cell Transplantation

The role of reduced-intensity allogenic or non-myeloablative stem cell transplantation (NST) as a salvage approach in HL remains controversial. The incorporation of reduced-intensity conditioning utilizing fludarabine-containing regimens with or without early cyclosporine withdrawal and donor lymphocyte infusions provides potential advantages including sufficient immunosuppression for allogeneic engraftment, decreased toxicity in comparison to standard high-dose conditioning regimens, reduction in nonrelapse-related mortality rates, as well as the potential induction of a graft-versus-lymphoma (GVL) effect to improve efficacy. Recent reports of favorable outcomes with NST in small cohorts of patients with recurrent/refractory HL have resulted in renewed interest in allografting in HL (Carella et al. 2001; Peggs et al. 2005; Phillips et al. 1989). Peggs et al. reported a response rate of 56% (8 CR, 1 PR) in a series of 49 patients with multiply relapsed HL who had progression of disease after prior autologous transplantation with a nonrelapse-related mortality rate of 16.3% at 730 days (7.3% for patients with related donors and 34.1% for those with unrelated donors). Despite these intriguing results, the efficacy of transplantation after reduced-intensity conditioning remains controversial. In several multicenter studies, 2-year progression-free survival rates have been reported ranging from 16 to 26%. A major factor in determining outcome related to NST was disease status prior to transplantation (Robinson et al. 2004). The disease status prior to NST was the only predictive factor for a high relapse rate. Chemoresistant patients demonstrated a significantly worse PFS rate.

Currently, NST has been utilized in patients with refractory HL as an adjunct to high-dose therapy with ASCR or as an alternative salvage approach in patients with multiply relapsed HL after failure of ASCT. To date, NST has been restricted to use in high-risk patients with chemosensitive disease, decreased tumor burden prior to allografting, and as a treatment of choice in patients where the toxicity of standard ablative therapy is considered unacceptable, i.e., patients with organ dysfunction or comorbidities. Patients with unresponsive or bulky residual disease have been considered poor candidates for treatment with this modality.

Confounding variables which restrict the ability to assess the impact of NST include the small numbers of patients treated, patient selection, and the inability to confirm a GVL effect in patients undergoing this procedure. The value of NST will ultimately require validation of the efficacy of this modality in randomized clinical trials. Future considerations to improve the outcome after NST include optimization of preparatory regimens and the development of techniques to selectively eliminate alloreactive T cells responsible for GVHD from T cells associated with GVL and infection control potential.

4.4 Salvage Strategies Following Transplantation

Historically, salvage approaches for patients who fail second-line therapy have consisted of either sequential single-agent chemotherapy or multiagent chemotherapy. In patients who receive further treatment after failure of high-dose therapy and demonstrate continued chemosensitivity, a survival advantage has been reported (13 vs 4 months median, p= 0.0001) (Schmitz et al. 2002). A trend toward longer survival was observed in patients whose disease recurred later than 6 months following high-dose chemotherapy particularly in those who received combination chemotherapy. Thus, administration of additional therapy in patients who experience treatment failure following high-dose therapy should be considered. Further understanding of the biology of HL may broaden the spectrum of options of therapeutic strategies by the development of targeted therapy.

The introduction of novel therapeutic approaches incorporating new single agents or combination chemotherapeutic regimens and/or targeted biologic or immunologic agents is needed to overcome resistance, to provide a potential benefit to patients who fail treatment with first- and second-line therapy, and to minimize the short- and long-term toxicity in heavily pretreated patients. The following sections provide a summary of the variety of novel therapeutic approaches that are currently being investigated.

4.4.1 Combination Chemotherapy with Gemcitabine/Vinorelbine (GEM/VRB)

Gemcitabine, 2',2'–difluorodeoxycytosine (GEM), a deoxycytidine analog which inhibits DNA synthesis and repair (Plunkett et al. 1995), has demonstrated significant single-agent activity in patients with relapsed or refractory HD. Like cytosine arabinoside, GEM is a prodrug which requires intracellular phosphorylation by deoxycytidine kinase to the active diphosphate and triphosphate forms. In vitro, GEM has a higher affinity for deoxycytidine kinase than cytarabine, as well as a longer intracellular retention (Heinemann et al. 1988). In adults, the maximally tolerated dose (MTD) of GEM varied significantly depending on both the schedule of administration (frequency and duration of infusion) and patient factors (e. g., prior chemotherapy). Adult MTDs ranged from 800 mg/m^2 in heavily pretreated patients to 4800 mg/m^2 when given as a prolonged infusion over 480 min to less heavily pretreated patients (Grunewald et al. 1992). The MTD in the pediatric phase I in children with refractory hematologic malignancies (leukemia/NHL) was 3600 mg/m^2/week (10 mg/m^2/min for 360 min) when administered weekly for three consecutive weeks (Steinherz et al. 2002). The DLT was hepatotoxicity. Some 30–50% of patients exhibited allergic-type symptoms including fever, rash, or myalgia. In phase II trials of GEM as a single agent in the treatment of patients with relapsed or refractory lymphomas, a range of dosing and schedules has been used (Bernell and Ohm 1998; Dumontet et al. 2001; Fossa et al. 1999; Lucas et al. 1999; Santoro et al. 2000; Savage et al. 2000; Venkatesh et al. 2004; Zinzani et al. 2000). Overall response rates in HL have been as high as 39%–43% (Santoro et al. 2000; Zinzani et al. 2000).

As a single agent, GEM has a favorable safety profile with a similar spectrum of toxicities in adults and children (Green 1996). The major toxicity was myelosuppression. Sporadic grade 4 lymphopenia, grade 3 transaminase elevation, abnormal clotting studies, myalgias, fainting, grade 3 proteinuria, grade 3 constipation, and hypotension with fever were also reported. The incidence of noncardiogenic pulmonary edema (NCPE) in adults was low (<2%). NCPE is a potentially fatal complication of therapy with GEM characterized by the simultaneous presence of grade 3 or 4 hypoxia and bilateral alveolar infiltrates noted on chest radiograph persisting for at least 3 days without evidence of other etiologies, i.e., congestive heart failure, infection, left atrial hypertension, metabolic abnormalities, or cancer-related causes (e. g., malignant pericarditis). No cases of NCPE were reported in children in 115 administered courses of gemcitabine (Reid et al. 2004).

VRB as previously described has significant single-agent activity (50%) in adult and pediatric patients with relapsed or refractory HL with a limited toxicity profile. Preclinical models have demonstrated additive activity when GEM is combined with VRB with little increased toxicity over a wide range of doses (Herbst et al. 2001). Published data regarding the use of this combination in adult patients with relapsed/refractory HL has demonstrated significant antitumor activity particularly in patients with a second recurrence after high-dose therapy. In one series, six of eight treated patients with HL had disease stabilization or response following treatment with GEM 1000 mg/m^2 and VRB 25 mg/m^2 on days 1 and 8, followed by G-CSF support until neutrophil recovery (Spencer et al. 2002). In the Memorial Sloan-Kettering Cancer Center experience, a larger series of 13 adult patients with relapsed or refractory HL following autologous stem cell transplantation received GEM 1275 mg/m^2 and VRB 30 mg/m^2 on a biweekly schedule (Hamlin et al. 2002). Of 178 treatments administered, 172 were given at the intended dose level. The ORR was 62% (6 PRs and 2 CRs). The median time to maximum response was six cycles (range 5–26). In contrast to the high rates of pulmonary toxicity observed following front-line pilot studies incorporating bleomycin with GEM, neither series reported NCPE (Bredenfeld et al. 2004; Friedberg et al. 2003).

Anecdotal cases have been reported in the literature using the GEM/VRB combination as a salvage regimen after ASCT in the pediatric setting (Ozkaynak and Jayabose 2004). A COG phase II study, AHOD0321, is currently underway in an effort to introduce novel and hopefully nontoxic agents to the therapeutic approach for patients with relapsed/refractory HL. This study will evaluate the efficacy and toxicity of the combination gemcitabine/vinorelbine in a large series of

pediatric patients in second or greater relapse or refractory HL. The schedule of administration will comprise a 21-day treatment cycle consisting of two weekly doses of gemcitabine administered at 1000 mg/m^2/dose and vinorelbine 25 mg/m^2/dose. The schema of the therapeutic regimen is shown in Fig. 4.2. It is hoped that the combination GEM/VRB may show promise as a novel salvage approach for children and adolescents with relapsed or refractory HL.

4.4.2 Molecular Targeting of the NF-κB Pathway

Better understanding of the mechanism of malignant transformation of HL and the role of nuclear factor-kappa B (NF-κB) in this process affords the opportunity to develop biologically based therapy for HL (Krappmann et al. 1999; Stein and Hummel 1999). Recent studies have evaluated the origin of the Hodgkin and Reed Sternberg (H/RS) cells (Kornacker et al. 1999). Studies of single-cell DNA amplification have also documented the importance of signaling through NF-κB transcription factor both in the proliferation of H/RS cells and in the suppression of apoptosis (Bargou et al. 1996; Krappmann et al. 1999). More importantly, inhibition of this pathway also inhibits cell proliferation, induces apoptosis, and renders H/RS cells less able to form tumors when transplanted into nude mice (Bargou et al. 1997).

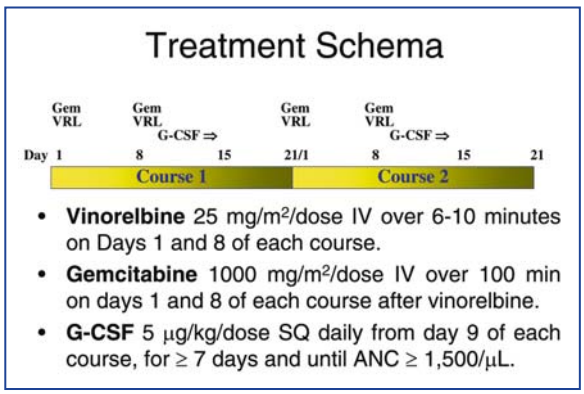

Figure 4.2

Treatment schema for AHOD321, Phase II Study of Gemcitabine/Vinorelbine

4.4.2.1 Activation of NF-κB

NF-κB, a nuclear transcription factor, is constitutively activated in HL. Extensive research has demonstrated that NF-κB regulates the expression of a variety of genes that play a crucial role in viral replication, tumorigenesis, apoptosis, various autoimmune diseases, and inflammation (Younes et al. 2003). NF-κB under normal conditions is found in the cytoplasm in an inactive state as a heterotrimer consisting of p50, p65, and IκBα subunits (Younes et al. 2003). In nonproliferative cells, the inhibitor protein IκB sequesters NF-κB in the cytoplasm. Cellular stress results in ubiquitination and the subsequent degradation of IκBα. When IκBα is degraded, nuclear localization signals are exposed on the p50-p65 heterodimer, resulting in nuclear translocation of free NF-κB, phosphorylation, and binding to a specific DNA sequence that results in DNA transcription (Younes et al. 2003) (Figs. 4.3–4.5). Subsequently, the promoter regions of numerous genes are activated, including genes encoding for several antiapoptotic proteins such as bcl-2, X-linked inhibitor of apoptosis protein (XIAP), and c-Jun N-terminal kinase (JNK) (Karin et al. 2002; Li and Stark 2002).

Constitutive activation of NF-κB in HD can occur through a variety of mechanisms, including NF-κB gene amplification, NF-κB chromosomal rearrangements, IκB mutations, induction of IκB kinases (IKK), and the induction of upstream regulators of NF-κB (Younes et al. 2003). Mechanisms of NF-κB activation found in H/RS cells include amplification of IκB kinase activity (Krappmann et al. 1999), C-terminal IκB mutations (Emmerich et al. 1999), EBV-mediated LMP-1 expression (McFarland et al. 1999), CD30 overexpression (Horie et al. 2002), c-Jun overexpression (Matthas et al. 2002), and increased expression of soluble RANKL (Fiumara et al. 2001). Because NF-κB activation can enhance the expression of several proteins implicated in protection from apoptosis in H/RS cells (Hinz et al. 2002), NF-κB inhibition is postulated to sensitize malignant cells to chemotherapy and radiation (Turco et al. 2004; Jeremias et al. 1998; Wang et al. 1999). Several in vitro studies support this hypothesis. Pajonk et al. demonstrated that NF-κB inhibition enhanced H/RS cell sensitivity to both radiotherapy and chemotherapeutic agents (Pajonk et al. 2000).

Thus, inhibition of NF-κB would be an attractive biologic or molecular targeted strategy in the treatment of relapsed/refractory HL.

4.4.2.2 Inhibition of NF-κB Through Proteasome Inhibition

The focus of future targeted studies in pediatric patients with HL will incorporate novel agents (chemotherapeutic and biologic agents) and therapeutic strategies which act to perturb the NF-κB pathway through inhibition of NF-κB. One strategy to inhibit NF-κB would be through proteasome inhibition which results in the stabilization of IκBα. Current novel therapeutic strategies incorporating proteasome inhibition are underway in a variety of cancers.

Bortezomib (Velcade, PS341), a dipeptidyl boronic acid, is a selective inhibitor of NF-κB activation and of the ubiquitin proteasome pathway (UPP), which is essential for the degradation of most short-lived and many long-lived intracellular proteins in eukaryotic cells (Adams et al. 1999) (Fig. 4.6). Important regula-

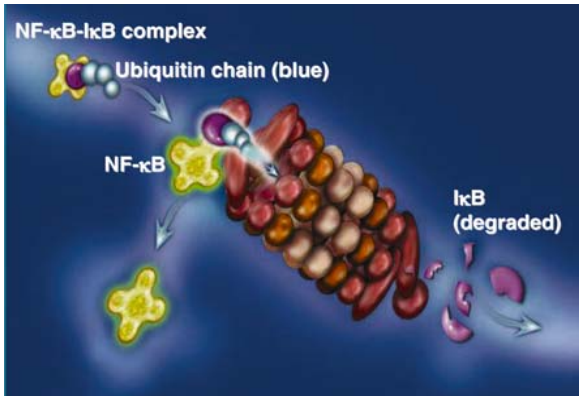

Figure 4.3

Sequential activation of NF-κB after IκB degradation by the proteasome (courtesy of Millenium Pharmaceuticals, Inc.)

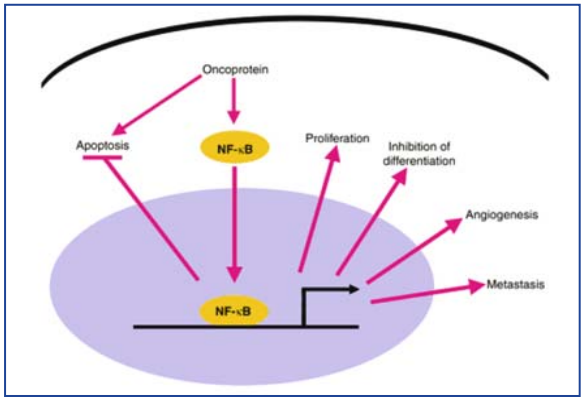

Figure 4.5

Transcriptional activation of NF-κB (courtesy of Millenium Pharmaceuticals, Inc.)

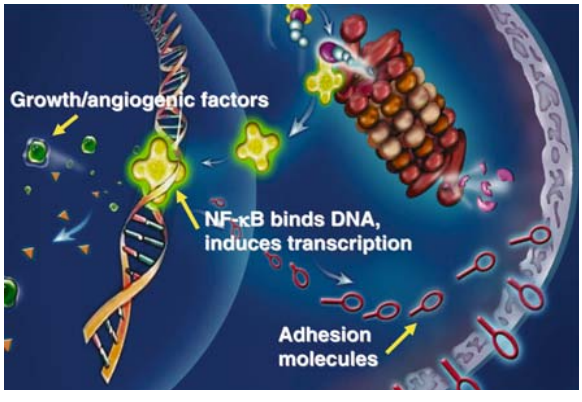

Figure 4.4

Effects of NF-κB activation (courtesy of Millenium Pharmaceuticals, Inc.)

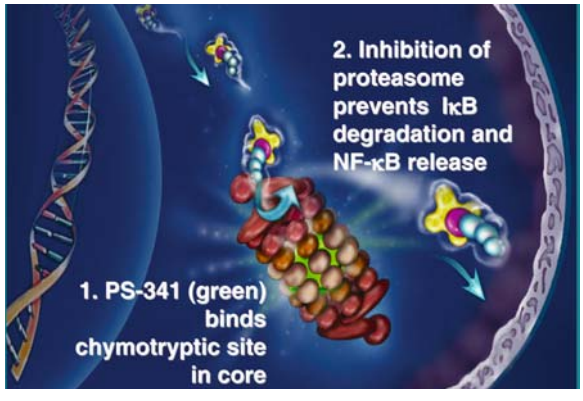

Figure 4.6

Proteasome inhibition by Bortezomib (PS341) (Courtesy of Millenium Pharmaceuticals, Inc.)

tory proteins affected by inhibition of the UPP system include NF-κB, p53, bcl-2, and other cell cycle regulatory proteins such as the cyclin-dependent kinase inhibitors p21 and p27 (Hochstrasser 1995). Proteasome inhibition stabilizes many cell cycle regulatory proteins and appears to sensitize malignant cells to apoptosis. Proteasome inhibition can also change the balance of pro- and anti-apoptotic proteins in the mitochondrial membrane and may block anti-apoptotic responses to chemotherapy (Adams et al. 2000).

Bortezomib specifically inhibits the 26S proteasome, an ATP-dependent multi-subunit protein that degrades proteins involved in multiple cellular processes, including cell cycle regulation, transcription factor activation, apoptosis, and cell trafficking (Tiecher et al. 1999). Bortezomib has been shown in vitro to be cytotoxic in leukemia and cancer cell lines due to induction of apoptosis (Zheng et al. 2004; Schenkein 2002). Cell death is believed to be preceded by p21WAF1/CIP1 accumulation (an alternative marker of proteasome inhibition) and by cleavage of PARP and Rb proteins and nuclear fragmentation. Apoptosis following proteasome inhibition is seen in malignant HL cell lines (Zheng et al. 2004; Schenkein 2002) as well as in primary HL cells (Pajonk et al. 2000), but not in normal hematopoietic progenitors (Masdehors et al. 2000). Although much preclinical work has focused on the inhibition of NF-κB following proteasome inhibition, the precise mechanism of bortezomib cytotoxicity is not clear. 26S-proteasome inhibition results in rapid cytochrome c release (3–6 h) from the mitochondrial membrane, followed by activation of caspases 8 and 9 (12 h) and caspases 3 and 7 (24 h) (Marhansky et al. 2001; Ling et al. 2002). Bortezomib enhanced in vitro H/RS sensitivity to gemcitabine (Schenkein 2002, 2005), TNF-related apoptosis-inducing ligand (TRAIL) (Zheng et al. 2004) and dexamethasone (An et al. 2004). Bortezomib has also enhanced solid tumor sensitivity to a variety of chemotherapy agents, including cyclophosphamide, in xenograft models (Teicher et al. 1999). Recent data demonstrated that the action of bortezomib in Hodgkin-derived cell lines may be enhanced in vitro and in vivo when preceded by anti-CD30 antibody activation of NF-κB (Boll et al. 2005). Boll et al. demonstrated that CD30 stimulation via 5F11, a fully humanized monoclonal antibody directed against CD30, activates NF-κB and its target cellular Fas-associating protein with death domain-like interleukin-1B-converting enzyme (FLICE) inhibitory protein (c-flip), which can also be inhibited by bortezomib. Cytotoxic synergy in vitro and in vivo was seen with the combination of 5F11 and bortezomib.

4.4.2.3 Adult Clinical Trials

Bortezomib has been evaluated as a single agent in multiple myeloma and NHL in adults. The MTD in adults varied with dosing schedule and ranged from 1.04 mg/m^2 (twice weekly for 4 weeks every 6 weeks) to 1.6 mg/m^2 (weekly for 2 weeks every 3 weeks) (Orlowski et al. 2002; Richardson et al. 2004). The most frequently reported adverse events (≥ 10%) among the 123 patients with advanced malignancies treated in phase 1 studies with bortezomib included fatigue (58%), anemia (47%), nausea (45%), constipation (43%), diarrhea (41%), vomiting (33%), headache (26%), pyrexia (24%), dyspnea (22%), abdominal pain (20%), and thrombocytopenia (19%). Grade 3 or grade 4 AE reported included thrombocytopenia (9%), anemia (6%), diarrhea (9%), and fatigue (4%) (Orlowski et al. 2002; Papandreou et al. 2004).

Two published studies have examined the efficacy of bortezomib as a single agent in phase II trials in adults with relapsed/refractory NHL (O'Connor et al. 2005; Goy et al. 2005). O'Connor et al. conducted a phase II clinical trial in indolent NHL and mantle cell lymphomas (O'Connor et al. 2005). The ORR (3 CR, 8 PR) was 50% in 24 evaluable patients. The toxicity profile was acceptable, consisting of one episode of grade 4 hyponatremia and grade 3 lymphopenia (58%) and thrombocytopenia (41%). Goy et al. reported an ORR for patients with mantle cell lymphoma of 41% (6 CR, 6 PR) with a median follow-up time of 9.3 months (range 1.7–24 months) and an ORR of 19% (2 CR, 2 PR) in patients with other B-cell lymphomas (small lymphocytic lymphoma, diffuse large B-cell lymphoma, and Waldenstrom's macrogloblinemia). The toxicity profile demonstrated grade 3 thrombocytopenia (47%), gastrointestinal disturbances (20%), fatigue (13%), neutropenia (10%), and peripheral neuropathy (5%). Grade 4 toxicity occurred in 9 patients (15%),

and 3 deaths were reported from disease progression within 3 days of withdrawal from the study. Two additional studies are in progress to evaluate bortezomib as a single agent in HL and in combination with a conventional chemotherapeutic regimen with etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (EPOCH).

4.4.2.4 Pediatric Clinical Trials

A phase I study evaluating bortezomib in pediatric patients with relapsed/refractory solid tumors has been conducted (Blaney et al. 2004). The dosing schedule consisted of twice weekly bolus dosing of bortezomib administered for 2 consecutive weeks at either 1.2 mg/m^2 or 1.6 mg/m^2 followed by a 10-day rest period. Fifteen patients were enrolled in the study. Thrombocytopenia was the DLT in the 12 patients evaluable for toxicity. Grade 3 or 4 toxicities included neutropenia (3), anemia (2), thrombocytopenia (3), and transient elevation in ALT (1). Inhibition of 20S proteasome activity in children appeared to be dose-dependent, with an average inhibition 1 h after drug administration on day 1 of 67% + 7% at 1.2 mg/m^2 and 77% + 3% at 1.6 mg/m^2. A phase I study of bortezomib in relapsed/refractory pediatric leukemia is currently underway.

4.4.2.5 Novel Retrieval Strategies Incorporating Proteasome Inhibition with Bortezomib

By exploiting the potential targeted activity of bortezomib in relapsed/refractory HL through inhibition of NF-κB, a variety of therapeutic strategies is being explored. The German Hodgkin Study Group is currently evaluating a combination of bortezomib and dexamethasone in a relapsed setting. A phase II pilot study will be conducted by the COG, AHOD0521, evaluating the safety and efficacy of a novel re-induction regimen consisting of bortezomib in combination with the re-induction regimen IV. Based on the data reported by Boll et al. (2005), molecularly targeted strategies incorporating bortezomib with immunologic agents such as CD30 monoclonal antibodies may serve as a novel method to potentiate its efficacy in the clinical setting.

4.4.3 Targeted Immunotherapy Strategies

4.4.3.1 Epstein-Barr Virus Directed Therapy

Approximately 40–50% of cases of HL are associated with expression of Epstein-Barr virus (EBV) derived antigens in malignant H-RS cells and their variants. As a result, targeted immunotherapeutic approaches in EBV-specific malignancies including HL have been developed which incorporate adoptive transfer of EBV-specific cytotoxic T lymphocytes (CTL). In contrast to EBV-lymphoproliferative disorders (EBV-LPD), EBV-positive HL demonstrate type II latency characterized by the expression of a limited number of EBV-derived antigens, EBNA-1, LMP1, and LMP2, EBERs and BARTs which provide valid targets for immunotherapy. These antigens, however, are weakly immunogenic.

There is limited clinical experience using EBV-specific CTL in patients with recurrent/refractory HL. Autologous as well as allogeneic EBV-specific T cells have been developed and evaluated in patients with recurrent/refractory HL (Bollard et al. 2004; Gottschalk et al. 2005; Lucas et al. 2004). Autologous EBV-specific CTL generally have been shown to be well tolerated, persist for up to 12 months after infusion, exhibit a homing mechanism directed to the sites of tumor involvement and enhance EBV-specific immunity by expanding several logs in vivo after infusion and contributing to the memory pool (Bollard et al. 2004; Gottschalk et al. 2005). Biologic and antitumor activity was demonstrated. Reduction in viral load was observed suggesting biologic activity. Bollard et al. reported five patients with CR, one PR, and five SD in a series of 14 patients. Lucas et al. reported a series of six patients with matched or partially matched allogeneic EBV-specific CTL. Three patients were treated with CTL only (5×10^6 cells/kg) and experienced a partial response to therapy, with durable responses in two patients who were alive 6 and 22 months after infusion. Three patients were treated with fludarabine 30 mg/m^2 for 3 days followed by 1.5×10^7 cells/kg. Two of the three patients demonstrated partial responses, but it was unclear whether the response was due to fludarabine or CTL infusion. Persistence of donor CTL, however, was not demonstrated.

Overall, the results thus far indicate that the use of adoptive immunotherapy, although promising, is less effective in EBV-positive HL than in EBV-LPD. The lack of efficacy may be attributed to immunosuppressive factors secreted by H-RS cells or to the limitations of current methods utilized for the generation of EBV-specific CTL which may result in CTL lines that are dominated by clones reactive to viral proteins not expressed in HL. Thus, novel methods are being developed to enhance the potency of EBV-specific CTL by targeting CTL to subdominant EBV proteins (e. g., LMP1-specific, LMP2-specific) and by genetically modifying the expanded CTL to render them resistant to inhibitory cytokines or immunosuppressive medications.

4.4.3.2 Monoclonal Antibodies Targeting Receptors Expressed in HL

H/RS cells express several receptors that belong to the tumor necrosis factor (TNF) receptor family including CD30, CD40, and RANK. The CD30 receptor is selectively overexpressed in HL and thus is an excellent target for antibody-based immunotherapy. In a small subgroup of HL, CD20 is overexpressed at a high density over the surface of H/RS cells, rendering the antigen an excellent target for these patients. With the advent of a newer generation of chimeric and human monoclonal antibodies, the role of these agents in selective immunotherapy may be enhanced.

With the advent of chimeric human/mouse monoclonal antibodies directed toward the CD20 antigen (rituximab), successful salvage strategies have been developed for patients with recurrent lymphocyte predominant HL and other subtypes of CD20-positive HL either as monotherapy (Ekstrand et al. 2003; Rewald et al. 2003) or in combination with radiation therapy (Ibom et al. 2003; DeVita 2003). Rituximab has also been shown to sensitize lymphoma cell lines to cytotoxic agents. Recently, the use of rituximab for salvage therapy has been evaluated in a broader context in patients with recurrent classical HL, where CD20 is expressed in 20% of H/RS cells, to eradicate normal infiltrating B cells in an effort to deprive H/RS cells of important growth factors. Younes et al. postulated that eliminating $CD20^+$ bystander B cells might abort cytokine-mediated stimulation of H/RS cells (Younes et al. 2003). Benign infiltrating B cells in HL lesions can express CD40 ligand and CD30 ligand which may contribute to the survival of H/RS cells in vivo and may be involved in regulating cytokine and chemokine expression (Clodi et al. 2002; Gattei et al. 1997; Younes et al. 1996). A pilot study of six weekly doses of 375 mg/m^2 rituximab was conducted at MD Anderson Cancer Center in patients with classical HL irregardless of their CD20 expression in H/RS cells in order to selectively eliminate infiltrating B cells (Younes et al. 2003). Twenty-two patients were evaluable for response. Five patients (22%) achieved either a CR or PR with a median duration of response of 7.8 months (range 3.3–14.9 months). Responses were limited to nodal or splenic sites only and were associated with a decline in IL-6 cytokine levels in two patients with a PR. B symptoms resolved in six out of seven patients after therapy. Therefore, rituximab may have a potential therapeutic role in the treatment of patients with recurrent, classic HL limited to nodal sites and/or the spleen. Further studies are underway based upon these findings.

CD30 monoclonal antibodies have been evaluated extensively as a salvage approach in patients with recurrent/refractory HL; however, their efficacy has not been as promising as the results with rituximab. This has been largely due to the lack of efficacy demonstrated in HL patients with bulk disease at relapse. Recently, two monoclonal antibodies, the human 5F11 (Borchmann et al. 2003) and the humanized SGN-30 (Wahl et al. 2002), have exhibited in vitro cytotoxicity against HL-derived cell lines; however, limitations in sensitivity in clinical trials have been observed. Preclinical data suggest that limited sensitivity to CD30 monoclonal antibodies may be due to growth stimulation in $CD30^+$ HL through activation of NF-κB, an important antiapoptotic factor in HL, resulting in resistance to apoptosis after CD30 signaling. (Boll et al. 2005). The development of bispecific molecules such as anti-CD30/anti-CD64 reagent H22xKi-4 or the Ki-4 J 131 radioimmunoconjugate may abrogate this problem and warrant further investigation. Additionally, strategies incorporating anti-CD30 monoclonal antibodies in combination with targeted agents that suppress NF-κB activation, such as bortezomib, may also lead to more effective strategies to eradicate relapsed/refractory disease.

4.4.3.3 Radiolabeled Immunoglobulin Therapy in HL

Salvage approaches for HL have resulted in promising results with the introduction of radiolabeled immunoglobulin therapy RIT (Order 1988; Vriesendorp et al. 1991; Vriesendorp and Quadri 2000). The tumor-associated antigen used for RIT in HL has been ferritin, a high-molecular-weight protein present in the interstitium and cytoplasm (Eshbar et al.1974). Radiolabeled antiferritin targets tumor interstitium and shrinks tumors by radiation effects, not immunologic effects. Currently available radiolabeled antibody treatment has significant advantages over other systemic modalities of therapy for recurrent/relapsed HL. The advantages of radiolabeled antiferritin include: a higher therapeutic ratio than that observed in most phase I trials of chemotherapeutic agents because significant increases in tumor dose can be obtained without an increase in normal tissue toxicity; rare incidence of anti-antibody formation; lower cost; and absence of immunologic, pharmacologic or microbiologic complications in vivo. The predominant toxicity is bone marrow depression, particularly thrombocytopenia. More importantly, significant dose-response relationships to tumor remission have been reported with radioconjugates including ^{131}I antiferritin (40% PR) and ^{90}Y-labeled antiferritin (CR rates 50%). New developments in the stabilization of antibody fragments and design of labile linker chelates are expected to increase the radioisotope delivery to the tumor by monoclonal radioimmunoconjugates without jeopardizing the therapeutic ratio. Responses were more commonly reported in patients with smaller tumor volumes (<30 cm^3) and in patients with longer disease histories. In addition, a higher response rate was noted in patients who received dose 0.4 mCi ^{90}Y-labeled antiferritin/kg body weight. To date, limited data are available in children, and the safety must be established.

4.5 Future Considerations

As a better understanding is gleaned of the biology of HL, more effective approaches to the eradication of recurrent or refractory disease will be determined. Given that the Hodgkin/Reed Sternberg cells of HL aberrantly express the activated p50/p65 (Rel A) heterodimer for NF-κB, molecular targeting and inhibition of this pathway may prove to be valuable in the treatment of these patients. The focus of future retrieval approaches in pediatric patients with relapsed/refractory HL will incorporate novel agents (chemotherapeutic, biologic, and immunologic agents) and therapeutic strategies which act to perturb the NF-κB pathway through direct or indirect inhibition of NF-κB. Future challenges include the development of strategies to overcome resistance, minimization of short- and long-term toxicity, and the design of immunotherapy approaches to augment the immune response in an effort to improve the overall efficacy of these therapeutic strategies.

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Non-Hodgkin's Lymphoma
Introduction and Historical Background

Sharon B. Murphy

Contents

5.1 Introduction

The history of advances in our understanding and management of non-Hodgkin's lymphoma (NHL) in the last half of the twentieth century is arguably one of the modern success stories of pediatric oncology. Advances in classification, staging, treatment, and supportive care have transformed a once invariably fatal disease into one of the most curable of pediatric cancers.

Progress in the field coincided with the introduction in the 1960s of combination chemotherapy and combined modality treatments and paralleled advances in the management of childhood acute lymphoblastic leukemia (ALL) and Hodgkin lymphoma. The pathologic classification has shifted in the meantime from mere morphologic observations and vague terminology (lymphosarcoma or reticulum cell sarcoma) to a sophisticated understanding of tumor origins, stratified according to T- and B-cell lineage, recognizing a number of distinctive lymphoid neoplasms, associated with characteristic immunophenotypic, genetic, and clinical features.

Particularly gratifying has been the unprecedented level of cooperation and communication between investigators from around the world which has facilitated the extraordinary progress achieved. This historical overview of progress in the field of pediatric NHL will attempt to provide a chronology of the advances. Burkitt's lymphoma, a discrete clinical and geographic entity common among children in both endemic and nonendemic areas, was first described in 1958 and occupies a very important place in the history of lymphoma, but is discussed in detail elsewhere in this book and will not be covered in this chapter.

5.2 The Early Era, Pre-1970

Writing in 1960 in the first really comprehensive pediatric oncology textbook, Harold Dargeon, then Chief of the Pediatric Service at Memorial Hospital in New York City, lamented the lack of a satisfactory classification of lymphoid tumors and noted the frequent confusion with and/or evolution to leukemia. He recognized that separation of patients with lymphosarcoma in whom leukemia subsequently developed, «aka» leukosarcoma, from those in whom it did not may be challenged as arbitrary since, had the latter cases simply lived longer, leukemia might have developed (Dargeon 1960). This nosologic confusion was perpetuated by many investigators, resulting in continuing confusion regarding "leukemic transformation of lymphosarcoma" and the even more puzzling "lymphoma-leukemia syndrome" or "lymphomatous ALL". Dargeon presciently observed that "great opportunities for confusion among clinicians are afforded", noting the terms used to designate the disease are of more than academic interest but would impact estimates of incidence, management, and prognosis. However, in those early days, it hardly mattered, since the results were consistently discouraging, with most children dying within weeks to months. Occasional long-term survivors who presented with localized disease were noted, however, following surgery, irradiation, and treatment with the single agents then available, such as Coley's toxin or nitrogen mustard. Reporting the results of 69 children with lymphosarcoma seen at Memorial from 1928 to 1953, Dargeon and his co-authors (Rosenberg et al. 1958) observed that 17.4% survived five years or more and noted that this "not infrequent long-term survival should plant seeds of optimism in the physicians responsible for the treatment of these children".

Contemporary experiences of many other pioneering pediatric oncologists echoed the same general experience with lymphosarcoma in childhood, painting a picture of a generally fatal disease, with a high incidence (around 30%) of leukemic transformation, and a small number (about 10%) of long-term survivors, most of whom presented with localized disease. Jones and Klingberg (1963) summarized their experience with 43 cases of lymphosarcoma seen at St. Louis Children's Hospital between 1945 and 1960 and reviewed data from several similar series of cases reported from other institutions, noting that only 50% of cases were alive at the end of six months, and only 9% survived five years. Jenkin and Sonley (1969) summarized their experience with 121 NHL cases seen at the Hospital for Sick Children in Toronto from 1930 to 1965. The end result was that 14/121 (12%) were cured, and 12 of these 14 long-term survivors had grossly complete surgical excision of a primary gastrointestinal tumor followed by wide-field whole abdominal irradiation, highlighting the curability of this favorable subgroup of abdominal primaries. This favorable outcome was in marked contrast to other NHL cases treated with irradiation in whom responses were limited to three or four weeks because of disease appearing at other sites.

By the end of the 1960s, irradiation was firmly established as a modality essential for the cure of Hodgkin's disease. Glatstein, Donaldson, Kaplan, and colleagues at Stanford attempted to directly apply their approach so successful in pediatric Hodgkin's disease to the treatment of NHL in children (Glatstein et al. 1974). They reported the outcome of a series of children treated from 1961 to 1971, attempting to eradicate all known disease with high-dose radiation therapy (minimum 3500 rad) to all involved or extended fields. In a series of 25 confirmed pediatric NHL cases who presented with Ann Arbor stage I, II, or III disease (excluding stage IV patients), they reported ten of the 25 survived more than two years, half of whom had stage I disease, with the majority who failed relapsing outside the irradiated field at extra nodal sites, particularly bone marrow. They concluded that chemotherapy as well as irradiation was essential for the management of children with NHL. Investigators at St. Jude Children Research Hospital, led by Donald Pinkel, also recommended that local radiotherapy be combined with multiple-agent chemotherapy, based on their favorable experience with apparently curing six out of eight children with localized lymphosarcoma with this approach (Aur et al. 1971).

5.3 The Latter Era, Post-1970s

The experience of the Toronto, Stanford, and St. Jude groups in apparently curing half or more of favorable localized cases of pediatric NHL with irradiation led to

its firm establishment as a modality considered essential in the treatment armamentarium for NHL. Results of controlled trials which assessed the contribution of irradiation to the overall success of combined modality treatment regimens for NHL were much later in coming, the result being that, for the next two to three decades, children with NHL routinely received both radiation and multidrug chemotherapy. Such approaches resulted in dramatic improvements in end results of therapy, compared to historical controls treated less aggressively, but produced significant acute toxicity and serious sequelae among survivors. In the first controlled trial of the role of radiotherapy in childhood NHL in 1975–1978, I conducted a stage-stratified and randomized trial of the efficacy of adding involved field radiotherapy to multiple drugs during induction for patients with a poor prognosis (stage III–IV) and found no benefit of radiation whatsoever (Murphy and Hustu 1980). This finding led to a series of controlled trials by the Pediatric Oncology Group (POG), conducted over more than a decade, randomizing radiotherapy while treating children with localized (stage I–II) disease with a chemotherapy regimen of reduced intensity and short duration (Link et al. 1990). Results conclusively demonstrated that radiotherapy could safely be omitted. Present-day regimens for childhood NHL no longer include radiotherapy.

The most striking improvements in end-results of treatment for all stages of NHL in children came about in the late 1970s and early 1980s from the systematic and widespread application of successful multidrug antileukemia regimens for the treatment of children with lymphoma, generally (and in hindsight unfortunately) combined with irradiation to involved fields or sites of bulk disease.

In addition to the pioneering efforts at St. Jude in adapting Total Therapy leukemia regimens for lymphoma treatment, Norma Wollner and her colleagues at Memorial produced great excitement in the field by their reports of striking success with adaptation of the L_2 leukemia regimen for lymphomas, dubbing the protocol LSA-L_2. The LSA-L_2 protocol was developed in 1971 and was essentially identical to the L_2 regimen used at Memorial for the treatment of ALL, with the addition of a single large dose of cyclophosphamide at the onset of therapy plus individualized radiation treatment in a substantial proportion of patients to areas of bulky tumor. In her initial report, Wollner reported results significantly superior to historical controls, i.e., 76% two-year disease-free survival in a series of 43 patients, almost half of whom had stage IV disease, many of whom would have conventionally been considered to have leukemia (Wollner et al. 1976). This experience from Memorial provided the first convincing demonstration that intensive combination chemotherapy had substantially altered the previously poor prognosis for childhood NHL.

This reported success led directly to a large randomized trial by the members of the Children's Cancer Study Group, conducted from 1977 to 1979, comparing the ten-drug LSA-L_2 regimen to a four-drug regimen (COMP), adapted from the earlier work of Ziegler, combining high-dose cyclophosphamide with a variation of moderate-dose methotrexate, originally pioneered by Djerassi and piloted by Meadows (Anderson et al. 1983). Both regimens incorporated irradiation to sites of bulk disease and prophylactic treatment of the central nervous system with repeated intrathecal injections of methotrexate. The results of this large trial were important in confirming the powerful prognostic importance of stage and in highlighting the influence of histologic subtype of NHL on the likelihood of success for patients with nonlocalized presentations of disease. This trial provided the first clear evidence that chemotherapy strategies for different histologic subsets of NHL must be selective. While both treatments were effective in curing over 80% of children with localized disease, the LSA_2-L_2 regimen was more effective for nonlocalized lymphoblastic lymphomas than COMP (76% vs 26%, respectively). The outcome of patients presenting with nonlocalized nonlymphoblastic disease was superior when treated with COMP (57% vs 28%), though neither regimen was particularly effective when there was initial involvement of the marrow or central nervous system. (Anderson et al. 1983).

Concurrently from 1976 to 1979, members of the Pediatric Division of the Southwest Oncology Group (which later became the Pediatric Oncology Group) conducted a nonrandomized trial of modified LSA_2-L_2 therapy for childhood NHL, excluding Burkitt's, and confirmed the effectiveness of the regimen in curing the majority of patients (Sullivan et al. 1985). Pediatric

Oncology Group investigators subsequently conducted a randomized study (POG 7905) comparing LSA_2L_2 with the $ACOP^+$ regimen, but discontinued the randomization due to the emerging evidence of inferiority of the LSA_2-L_2 regimen for nonlymphoblastic histology. The results with the $ACOP^+$ regimen confirmed the previously reported importance of stage and provided important evidence of a difference in outcome between small noncleaved cell and large cell types of nonlymphoblastic lymphomas (Hvizdala et al. 1991). Results of this pivotal trial for POG cemented a stage- and histology/phenotype-specific stratification strategy for the subsequent generation of POG NHL trials.

Meantime, national clinical trials groups in Germany (BFM) and France (SFOP) were developing the early trials which laid the groundwork for their highly successful modern protocols for childhood NHL. The results of BFM Study 1975/81 for NHL, conducted in parallel and with treatments similar to their ALL study, confirmed the prognostic importance of stage and histology/immunophenotype (Müller-Weihrich et al. 1982) and led to the design of subsequent stratified trials, separating "B" from "non-B" types of disease. In the successor trial, NHL-BFM81, a new treatment regimen was introduced for patients with B-cell lymphomas (small noncleaved and diffuse large-cell), while non-B NHL (mainly T-cell lymphoblastic) were treated as ALL, and treatment duration was adapted to risk, i.e., stage. This strategy was further refined in NHL-BFM-83 and -86, leading to outstanding overall event-free survival (80%) for all NHL subtypes (Reiter et al. 1995). French investigators, meanwhile, led by Catherine Patte of the Institut Gustave-Roussy in Villejuif, built upon a pulsed COPAD protocol, adding high doses of methotrexate and cytosine arabrinoside plus a "mini"-BACT regimen which had proved useful to salvage relapsed patients, thus devising LMB 0281, a protocol specifically designed for B-cell NHL and B-ALL (Patte et al. 1986). Thus was launched the highly successful series of LMB protocols, culminating in the stunning achievement of 91% failure-free survival reported for LMB-89 (Patte et al. 1997). This success triggered the international randomized FAB (French American-British) Cooperative Study of B-NHL and B-ALL.

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Biology and Pathology of Pediatric Non-Hodgkin Lymphoma

S.L. Perkins • S.W. Morris

Contents

6.1 Introduction

The pediatric non-Hodgkin lymphomas (NHLs) are a diverse collection of lymphoid malignancies with varied pathologies, cells of origin, natural history, and response to treatment. The histologic diagnosis of NHL is among the most difficult tasks that surgical pathologists are asked to undertake. A general diagnosis of NHL is accurate in roughly 90% of cases; however, consensus among pathologists regarding the identification of specific histologic subtypes occurs only slightly more than half of the time in past studies using morphology only [35, 108, 179, 386]. Integration of morphologic, immunophenotypic, and genetic information into classifications of NHL has been invaluable in providing the basis for our current approaches to the diagnosis and therapeutic management of these malignancies by providing a framework to subdivide the disease into clinically meaningful and diagnostically reproducible subgroups. Refinements in these classification schemes are constantly ongoing as additional parameters are identified and shown to permit the detection of subgroups that possess unique biologic features and/or that have either a specific response to traditional therapies or are amenable to more directed forms of treatment.

6.2 Non-Hodgkin Lymphoma

Non-Hodgkin lymphomas (NHL) comprise approximately 10% of all childhood cancers and are a diverse collection of malignant neoplasms of lymphoreticular cells [54]. Pediatric NHL includes a varied group of neoplasms that derive from both mature and immature (blastic) cells of both B-cell and T-cell origin.

Table 6.1 Morphologic, immunologic, molecular, and cytogenetic findings in pediatric NHL

–	LBL	BL	DLBCL	ALCL
Morphology	–	–	–	-
Cell size	Small to intermediate	Intermediate	Large	Variable, small to large, multinucleated
Nuclear chromatin	Fine, blastic	Coarse	Clumped, vesicular	Clumped, vesicular
Nucleoli	Absent	Variable, multiple inconspicuous	Variable, single prominent or inconspicuous	Variable
Cytoplasm	Scanty	Moderate to scanty with prominent vacuoles	Moderate to abundant	Moderate to abundant
Nodal pattern	Diffuse, starry-sky pattern may be seen	Diffuse, starry-sky pattern	Diffuse	Sinusoidal or diffuse
Immunophenotype	Precursor T cell (80%), Precursor B cell (20%)	Mature B cell	Mature B cell	T cell, null cell, CD30+
Molecular genetics	TCR or Ig gene rearrangements	Ig gene rearrangements	Ig gene rearrangements	TCR rearrangements
Cytogenetics	T cell; t(7;14), t(1;14), t(14;14), t(8;14), t(11;13), TAL-1 or NOTCH rearrangements. B cell; hyperdiploid, additional 21q material	t(8;14), t(8;22), t(2;8) Translocations of C-MYC	Complex abnormalities including structural and numerical abnormalities	t(2;5), ALK translocation with chromosomes 1, 2, 3, and 17

LBL = lymphoblastic lymphoma; **BL** = Burkitt lymphoma; **DLBCL** = diffuse large B-cell lymphoma; **ALCL** = anaplastic large-cell lymphoma; **TCR** = T-cell receptor; **Ig** = immunoglobulin.

These neoplasms in children are typically intermediate to high grade (clinically aggressive) tumors. This is in direct contrast to NHL in adults, in which more than two-thirds of the tumors are indolent, low-grade malignancies [259, 289, 290, 338]. Pediatric NHL also appears very different from adult lymphomas in that all of the tumors are diffuse neoplasms, and follicular (nodular) lymphomas are exceedingly rare [2, 338, 371]. Similarly, the immunophenotypic subclassification of pediatric NHL shows marked differences from adult lymphomas. Pediatric NHLs are almost evenly split between B- and T-cell neoplasms, whereas T-cell neoplasms make up less than 10% of adult NHL. Pediatric NHL also has many more lesions that are derived from blast-like or precursor B or T cells than are seen in adults [2, 289, 290, 338]. There are four major pathologic subtypes of pediatric NHL that will be discussed in further detail below: Burkitt lymphomas (BL), diffuse large B-cell lymphoma (DLBCL), anaplastic large cell lymphoma (ALCL) and lymphoblastic lymphomas (LBL) (see Table 6.1, Fig. 6.1).

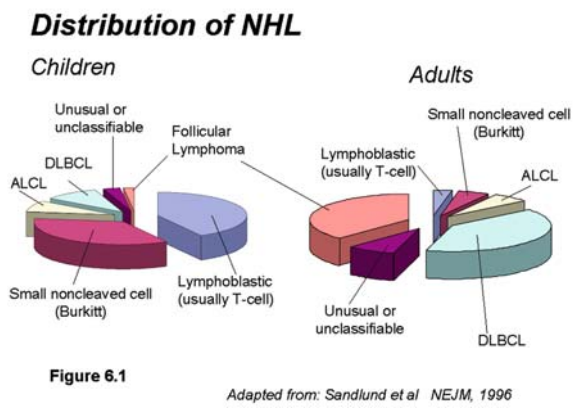


Figure 6.1

Schematic representation demonstrating the difference in pediatric NHL compared with adult NHL highlighting the higher incidence of more aggressive lymphomas in children. Adapted from [338]

Table 6.2 Pathologic work-up of pediatric non-Hodgkin lymphoma

Tissue should be allocated for following studies (as required):
Morphology: fixed tissue optimal, frozen tissue
Immunophenotyping:
Immunoperoxidase stains: fixed or frozen tissue
Flow cytometry: fresh tissue
Cytogenetics: fresh tissue (good viability that contains tumor)
Molecular testing
Reverse transcriptase polymerase chain reaction (RT-PCR) analysis: fresh, frozen or fixed tissue
Southern blot: fresh or frozen tissue
Fluorescent *in situ* hybridization (FISH): fresh, frozen or fixed tissue (probe dependent)

6.3 Pathologic Analysis

The diagnosis of pediatric NHL requires an extensive workup both to ensure proper classification as well as to obtain important prognostic information (see Table 6.2). A diagnosis of NHL may require multiple ancillary studies such as immunophenotyping by flow cytometry or immunohistochemistry [380, 394], genetic or molecular analysis [15, 129]. In addition, many prognostic factors can be identified by either immunophenotyping or molecular/cytogenetic studies [15, 27, 44, 128, 129, 140, 151]. An important role for the pathologist in the workup of a suspected NHL is a collection of appropriate and adequate tissue for ancillary testing.

Most pediatric biopsies come to the pathologist in a fresh state to allow for tissue allocation for ancillary testing. A suspected lymphoma diagnosis may be confirmed by performance of a frozen section of the tissue submitted to confirm that a neoplasm is present. Although frozen section is usually not sufficient to make a definitive diagnosis, it will help to direct the handling of the tissue to ensure that the appropriate testing can be undertaken [361].

6.3.1 Morphology

Morphology provides the keystone for the diagnosis of NHL. This requires that adequate tumor tissue be sampled [289]. In pediatric NHL, most diagnoses will require tissue sampling either by excisional or incisional biopsies or needle core biopsy. However, some diagnoses may be possible on the basis of cytologic analysis using fine needle aspiration [141, 163, 196, 214]. It should be emphasized that often cytologic analysis may not be definitive, and subsequent tissue biopsy may be necessary to confirm a suspected diagnosis. Because of the extensive ancillary testing that may be required in lymphoma diagnosis, the pathologist and the surgeon or other personnel performing the biopsy must work together to ensure that adequate tissue is collected [289]. If limited tissue is available for analysis, the pathologist must make sure that sufficient tissue is submitted for diagnostic morphologic evaluation [289]. Bone marrow evaluations for metastatic disease are also an important part of the staging of NHL. To ensure that sufficient bone marrow is collected for adequate staging, sampling of the marrow cavity below the subcortical bone and/or bilateral biopsies will be required [73, 86, 91, 203, 322].

Often additional studies, including immunophenotypic analysis by immunohistochemistry or molecular testing using methods such as reverse transcriptase-polymerase chain reaction (RT-PCR) may be performed on fixed tissues following morphologic exami-

nation, although the use of fresh tissue in the latter usually yields superior results. Other testing, such as flow cytometric analysis [394] and conventional cytogenetics, absolutely requires fresh tissue. Certain types of molecular testing, including some fluorescence in situ hybridization (FISH) for specific cytogenetic abnormalities, Southern blot analysis, or microarray analyses may require unfixed (fresh or frozen) tissue for optimal results [61, 130]. The pathologist must allocate tissue based on the size and quality of the specimen provided.

Because of the important role of morphology in a diagnosis, proper tissue fixation and preparation of slides are essential for optimal evaluation. Most tissues will be fixed in a fixative such as formalin, which allows for good morphology as well as optimal immunohistochemical and molecular testing [249, 366, 396]. In NHL, nuclear and cytoplasmic morphologic features may be better preserved in specific fixatives. Commonly utilized fixatives include the mercury-based B5 fixative and zinc formalin. These are both coagulative-type fixatives that ensure excellent retention of morphologic nuclear detail, but degrade nucleic acids so as to make them suboptimal for molecular testing [366, 396]. In addition, B5 fixative is less commonly used due to hazardous waste issues concerning the disposal of the mercury compounds. Other fixatives that may be used include Bouin's fixative, Zenker's fixative, and some alcohol-based fixatives. However, these other fixatives do not provide the overall usability and good morphologic preservation seen with formalin. Therefore, when limited material is available, it is suggested that formalin be the primary fixative, with additional fixatives, such as B5 or zinc formalin being utilized to enhance morphologic details only if sufficient tissue is available. Fixation of tissues in alcohol to optimally preserve protein antigen epitopes may also be considered, as this may provide excellent samples for immunophenotypic analysis by immunohistochemical staining.

Following tissue fixation, microscopic slides will be prepared. Optimal morphology requires that the tissue be fixed adequately. It should be noted that most fixatives penetrate tissue at a rate of 1–2 mm per hour. Therefore, fixation of large pieces of tissue for short periods of time will markedly impact upon the preservation of morphologic features. The pathologist must cut thin sections from the specimen to allow adequate impregnation by the fixative, and if inadequate time is available, may wish to process a small section of the submitted material and allow other materials to fix overnight or for a longer period to allow tissue preservation fixation. Once the tissue is fixed, it will be processed. Processing usually involves sequential dehydration of the tissue with its placement in alcohol baths of increasing concentrations in an automated processor. The time of tissue processing ranges from 4 to 6 hours, depending on the technology used. More rapid processing of small biopsies or needle cores may be performed using microwave technology; however, this may negatively impact on morphologic quality. Following processing and dehydration, the tissue will be embedded in paraffin wax prior to sectioning with a microtome. The thickness of the microtome sections will affect the morphologic quality and the ability to interpret morphologic features. Good sections, ranging from 4 to 6 microns thick, with minimal folding or breakage are optimal for analysis. Poor fixation or improper processing will adversely impact on the ability to cut good histologic sections. Sections are placed on glass slides and stained. Most tissue sections will be stained with a hematoxylin and eosin stain for initial analysis. If there is very limited material available, sections for additional studies such as immunohistochemistry or other staining may be cut at the same time as the initial histologic sections to ensure conservation of tissue. Following staining, the slides will be cover-slipped to protect the tissue.

6.3.2 Immunophenotypic Analysis

When the pathologist examines the histologic section, a differential diagnosis will be made based on specific morphologic features within the specimen. This, in combination with clinical features, may be sufficient to make a diagnosis. However, most pediatric NHLs require additional studies to allow for diagnosis [243]. Often the first approach is immunophenotypic analysis. Each type of NHL will have specific antigens that may be more or less specific for that type [3, 187, 288], allowing a definite diagnosis to be made. In cases with fixed tissue, this analysis will be done by immunohis-

tochemical staining. Immunohistochemical staining involves the use of a specific antibody that will recognize a given antigenic epitope expressed on a tumor cell (Table 6.3). The use of panels of antibodies and observation of the staining patterns generated by those antibodies will help with the diagnosis (see below for specific diagnostic entities). The choice of antibodies to be used in staining will be influenced by the morphologic features as well as the clinical impression [288, 395].

Immunohistochemical staining may be done by hand, or more commonly, using automated immunohistochemical stainers. A slide will often be pretreated either with heat or enzymatic digestion to expose antigenic epitopes that may have been cross-linked during tissue fixation and processing (a method known as antigen retrieval) [132]. This is most often done by heat methods, using pressure cookers or microwaves as heat sources. The slide will then be exposed to a specific antibody, which will bind to the appropriate epitope if it is present on the tissue. The staining is then detected by use of a chromagen detection system, often diamino-benzene (DAB), which will form a brown-colored precipitate when staining is present (Fig. 6.2). Interpretation of immunostains requires knowledge of the expected staining pattern, as well as cross-reactivities for each antibody and tumor type. It is not recommended that a diagnosis be made on the basis of a single antibody stain, but rather integration of positive and negative staining results using a panel of antibodies will be required [288]. As noted above, the differential diagnosis based on morphologic and clinical impressions will direct the choice of antibodies to be utilized.

Immunophenotyping may also be done on fresh tissues using flow cytometry [14, 394, 395]. If NHL is suspected, a small portion of the fresh, unfixed, submitted tissue, blood, or bone marrow may be sent to the flow cytometry lab for analysis. Using this approach, a specific fluorescently labeled antibody will bind to the cell if a specific antigen epitope is present. The cells are then passed through a flow cytometric instrument and analyzed by a laser light source, which can generate data on a variety of different cell parameters including cell size and complexity (for example, granularity), as well as the expression of fluorescence

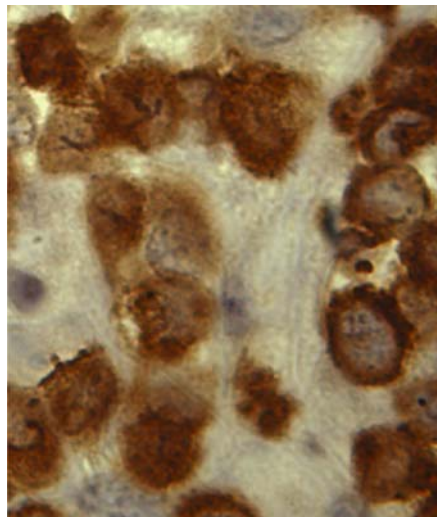


Figure 6.2

Immunohistochemical staining with antibodies against CD20 in a pediatric diffuse large-cell lymphoma (×1000, CD20 immunohistochemical stain)

imparted by the binding of the antibody. Flow cytometers that can analyze up to four or six different fluorochrome stains allow for integration of multiple antigenic signals from a single cell simultaneously [40, 45, 197, 237, 242, 282]. As with tissue-based immunohistochemical staining, flow cytometry requires integration of the patterns of antigen expression by the neoplastic cells in order to make a diagnosis and requires that panels of antibodies be used, based on the suspected diagnosis [395].

6.3.3 Molecular and Cytogenetic Analysis

Another feature incorporated into NHL classification systems concerns cytogenetic or molecular genetic lesions that are of pathogenic importance in selected forms of NHL. Progress in this area over the past 20 years has defined structural chromosomal abnormalities, including translocations, deletions, inversions, amplifications, and base pair mutations, which alter critical genes that normally regulate growth and/or differentiation (oncogenes and tumor suppressor genes, or anti-oncogenes) that are associated with the development of NHL [57, 98, 129, 181, 358, 367] (Ta-

Table 6.3 Selected antibodies useful in immunophenotypic analysis of pediatric non-Hodgkin lymphomas

Antibody	Reactivity
CD1	T lymphoblasts
CD2	T cells and natural killer cells
CD3	T cells
CD4	Helper and inducer T cells
CD5	T cells
CD7	T cells and natural killer cells
CD8	T-cytotoxic and suppressor cells and natural killer cells
CD10 (CALLA)	Progenitor B and T lymphoblasts, some diffuse large B-cell lymphoma
CD15 (Leu M1)	Reed-Sternberg cells
CD20	B cells
CD22	B-cell subset
CD30 (Ki-1)	Reed-Sternberg cells, anaplastic large-cell lymphoma, activated T and B cells
CD43	T cells, B-cell subset, granulocytes, and monocytes and macrophages
CD45	Leukocytes
CD45RA	B cells, T-cell subset, granulocytes, and monocytes
CD45RO	T cells, B-cell subset, granulocytes, and monocytes and macrophages
CD56	Natural killer cells and T-cell subset
CD79a	B cells
IgG, A, M, D, and E	Immunoglobulin heavy chains
κ, λ	Immunoglobulin light chains
Anti-TCR αβ, F1β	αβ T cells
Anti-TCR γδ	γδ T cells
Anti-TdT	Lymphoblasts and some myeloblasts
ALK-1	ALK protein overexpression in anaplastic large-cell lymphoma
EMA	Anaplastic large-cell lymphoma
Ki-67, PCNA, MIB-1	Nuclear proliferation antigens
Anti-perforin; anti-granzyme A,B, and C; and anti-TIA-1	Cytolytic granule-associated proteins in natural killer cells and cytotoxic T cells

CD=cluster designation; CALLA=common lymphoblastic leukemia antigen; Ig=immunoglobulin; TCR=T-cell receptor; TdT=terminal deoxytidyl transferase; ALK=anaplastic lymphoma kinase; EMA=epithelial membrane antigen; TIA=T-cell intracytoplasmic antigen

ble 6.4, Fig. 6.3). With the currently available molecular methods, genetic abnormalities of various types can be identified in essentially all individual cases of NHL. It is now known that characteristic chromosomal translocations or rearrangements act to alter the function of a particular gene or subset of genes, contributing to the development of neoplasia. For example, Burkitt lymphoma was the first human tumor in which a chromosomal translocation was demonstrated by molecular analysis to be involved in its pathogenesis [76, 234, 377, 416]. Although the possibility of therapies aimed at malignancy-specific ge-

Table 6.4 Chromosomal translocations involved in oncogenesis of pediatric NHL

Cytogenetic abnormality[a]	Disease (predominant subtypes)	Involved/presumed target gene(s) (aliases)	Presumed function of target gene(s)
B cell	–	–	–
t(1;14)(q21;q32)	B-ALL, B-NHL	*BCL9*	Required for Wnt signal transduction at the level of nuclear beta-catenin
t(1;14)(q21;q32)	BL with dup (1q)	*IRTA1/2*	Immunoglobulin receptor superfamily
t(1;14)(q21;q32)	DLBCL	*MUC1 (EMA, CD227, PEM, PEMT, H23AG, PUM)*	Mucin (episialin); cell-surface transmembrane glycoprotein
		MDC15 (ADAM15)	Metalloproteinase-like disintegrin-like and cysteine-rich protein
t(2;14)(p13;q32)	DLBL	*BCL11A (EV19)*	Zinc finger transcriptional repressor
t(3;14)(q27;q32)	DLBCL and others	*BCL6 (BCL5, LAZ3)*	Zinc finger transcriptional repressor
t(5;14)(q31;q32)	B-ALL	*IL3 (MCGF)*	Interleukin-3 (colony-stimulating factor); hematopoietic growth factor
t(6;14)(p21;q32)	DLBCL	*CCND3*	Cyclin D3, essential for control of the cell cycle at the G1/S (start) transition; interacts with the CDC2 protein kinase
t(8;14)(q24;q32)	BL, DLBCL	*cMYC*	BHLH/zip transcription factor; activates/represses expression of multiple target genes
t(10;14)(q24;q32)	DLBCL	*NFKB2 (LYT10, H2TF1)*	49-kDa DNA-binding subunit (p52;p100) of heterodimeric NFκB transcription factor; complex regulates the expression of inflammatory and immune genes
t(11;14)(q23;q32)	Mediastinal B-NHL	*PAFAHα2*	Platelet-activation factor acetylhydrolase
t(11;14)(q23;q32)	DLBCL	*RCK (HLR2, p54, DEAD/H BOX 6)*	DEAD/H box ATP-dependent RNA helicase
t(12;14)(a23;q32)	DLBCL	*C4ST-1*	Chrondroitin-4-O-sulfotransferase 1
t(12;14)(q24;q32)	BL	*BCL7A*	Unknown; shares homology with actin-binding protein caldesmon
t(12;15)(q32;q11-13)	DLBCL	*BCL8 (BCL8A)*	Unknown; related BCL8B protein shares extensive homology to the RG Drosophila PKA anchoring protein.
t(14;18)(q32;q21)	DLBCL	*BCL2*	Apoptosis inhibitor
T cell	–	–	–
t(1;7)(p34;q34)	T-LBL	*LCK (p56-LCK, LSK)*	Cytoplasmic tyrosine kinase; participates in antigen-induced T-cell activation; bound to cytoplasmic domains of either CD4 or CD8
t(1;14)(p32;q11)	T-LBL	*TAL1 (SCL, TCL5)*	bHLH DNA-binding transcription factor
t(2;5)(p23;q35)	Anaplastic large cell lymphoma	*NPM-ALK*	Anaplastic lymphoma kinase; receptor tyrosine kinase that binds the ligands pleiotrophin and midkine

t(5;14)(q35;q32)	T-LBL	*HOX11L2 (RNX, TLX-3)*	Homeodomain transcription factor
t(7;9)(q34;q34.3)	T-LBL	*NOTCH1 (TAN1)*	Transmembrane protein that functions as receptor for membrane-bound ligands Jagged 1, Jagged2 and Delta1; intracellular domain is proteolytically released upon ligand binding, translocates to nucleus, and acts as a transcriptional activator
t(7;9)(q35;q34)	T-LBL	*TAL2*	bHLH DNA-binding transcription factor
t(7;19)(q35;p13)	T-LBL	*LYL1*	bHLH DNA-binding transcription factor
t(8;13)(p11;q12)	T-LBL	*FGFR1 (bFGFR, CEK, GLT2)*	Fibroblast growth factor receptor 1; receptor tyrosine kinase that binds basic FGF
t(8;14)(q24;q11)	T-LBL	*cMYC*	bHLH/zip transcription factor; activates/represses expression of multiple target genes
t(10;14)(q24;q11)	T-LBL	*HOX11 (TCL3)*	Homeodomain transcription factor
t(11;14)(p13;q11)	T-LBL	*LMO2 (RBTN2, TTG2)*	LIM-only protein 2; cysteine-rich LIM domain transcriptional regulator
t(11;14)(p15;q11)	T-LBL	*LMO1 (RBTN1, TTG1)*	LIM-only protein 1; cysteine-rich LIM domain transcriptional regulator

The information displayed in this table focuses on genes involved in the pathogenesis of the non-Hodgkin lymphomas; however, genes that are recurrently involved in the genesis of selected other B- or T-cell lymphoid malignancies are also shown.

[a] Variant translocations affecting other *Ig* or *TCR*, or non-*Ig* or non-*TCR*, loci than the one shown do occur less commonly for many of the translocations.

Functional data were obtained from the S.O.U.R.C.E. database (http://genome-www4.stanford.edu/cgi-bin/SMD/source/sourceSearch), as available.

B-ALL=B precursor acute lymphoblastic leukemia; BL=Burkitt lymphoma; B-NHL=B-cell non-Hodgkin lymphoma; DLBCL=diffuse large B-cell lymphoma; T-LBL=T-lymphoblastic lymphoma

netic lesions in NHL remains to be realized in the clinical setting, this promises to change dramatically over the next decade with the current emphasis on the development of targeted therapeutic approaches for cancer [113, 379]. The identification of genes involved in the pathogenesis of NHL has already had the practical benefit of allowing the development of highly specific and sensitive molecular assays to detect these genetic changes. These assays can often resolve diagnostic ambiguities brought about by the more traditional classification methods and can be used to follow therapeutic responses, as well as for the early detection of recurrence [111, 329]. In some instances, as described later in this chapter, the presence of specific genetic abnormalities may be of prognostic importance also [14, 15, 27, 44, 140, 151, 199, 258, 369, 370, 413].

Cytogenetic analysis requires fresh samples that contain malignant cells. Bone marrows (usually 1–2 ml), blood (approximately 10 ml) or a tissue sample (usually 0.2–0.5 mm^3) may be studied. Large amounts of necrosis or normal (non-neoplastic) tissue may impact the ability to do adequate cytogenetic analysis of the neoplastic cell population. The samples should be processed as soon as possible to ensure optimal cell viability. Analysis may be performed on either cultured or uncultured cells. Often the tissue will be incubated with a mitogen to stimulate proliferation of the neoplastic cells. Cells will have colchicine added to arrest cell division, and a metaphase chromosome spread will be prepared (see Fig. 6.4). Usually, the chromosome spread will be stained to identify internal chromosomal structures, such as chromosomal bands. This allows for the specific identification of each chromosome as well as structural abnormalities. Many of the abnormalities identified by cytogenetic analysis may be subtle, requiring an experienced cytogeneticist or additional staining. Conventional cytogenetic approaches are useful in that they provide the ability to view the entire chromosomal component [369, 370].

This allows identification of most abnormalities and is particularly useful in demonstrating complex cytogenetic abnormalities.

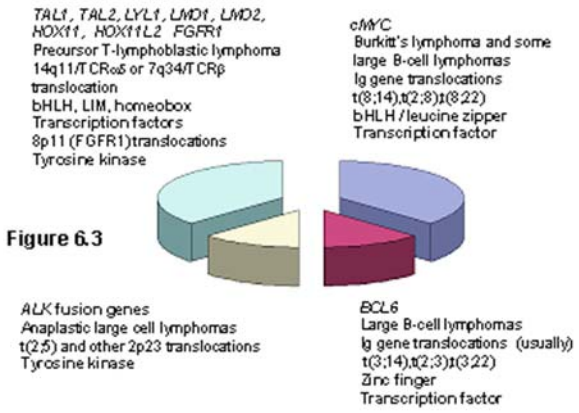


Figure 6.3

Figure 6.3

Molecular genetic aspects of non-Hodgkin lymphoma associated with translocations. Genes altered by commonly occurring chromosomal translocations in pediatric NHL are shown in italics along with the associated lymphoma subtype in which they have pathologic significance. The functional family for each gene is indicated in bold. The genes shown in this figure occur in the significant portion (but not all) cases of pediatric NHL of the indicated subtypes

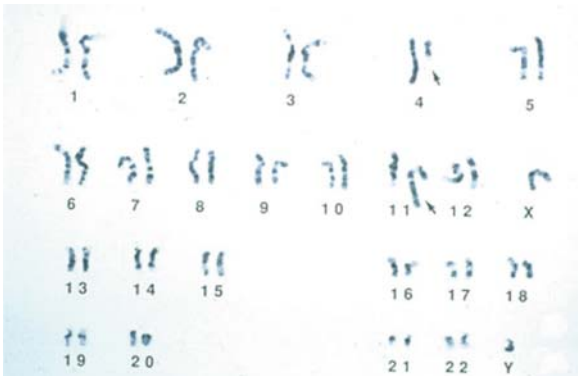


Figure 6.4

Classic cytogenetic metaphase spread. Demonstration of a metaphase spread from a 10-year-old boy with diffuse large B-cell lymphoma indicating abnormalities in chromosome 11

Genetic testing using fluorescence in situ hybridization (FISH) is another cytogenetic approach that is often utilized in pediatric NHL [128, 146, 187, 317]. FISH technology uses specific, fluorescently labeled DNA probes to identify specific chromosomal abnormalities. This approach is useful because it is much more rapid, requiring only 24–48 h compared with 5–7 days for conventional cytogenetics. However, FISH technology will identify only those abnormalities for the specific probe(s) employed [112, 373]. Therefore, this testing has a limitation in not being able to pick up additional cytogenetic abnormalities. However, when a specific diagnosis is suspected, such as anaplastic large-cell lymphoma, FISH technology provides a rapid means to confirm the diagnosis as it can target the tumor-specific *ALK* (anaplastic lymphoma kinase) gene locus to identify translocations (Fig. 6.5). Likewise, FISH using *cMYC* gene probes can identify rearrangements of this gene that are characteristic of Burkitt lymphomas, and many other examples exist as well for identifying particular gene abnormalities associated with NHL pathogenesis. FISH technology is also useful in following minimal residual disease when a specific abnormality is known to be present in a tumor [300, 347].

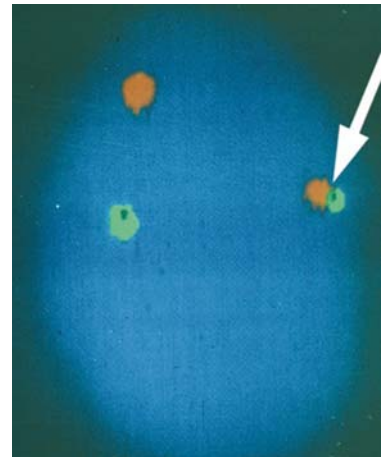
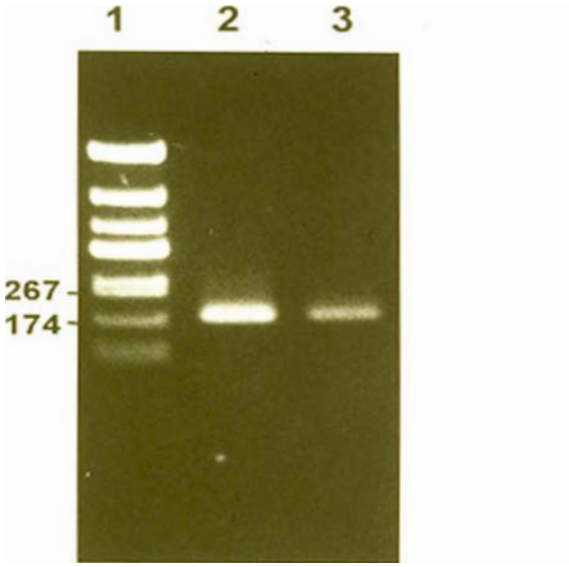


Figure 6.5

Fluorescent in situ hybridization (FISH) demonstrating a translocation as indicated by the fusion signal indicated by the *arrow*. The cell also demonstrates a normal chromosomal pair as indicated by the two separate (red and green) signals



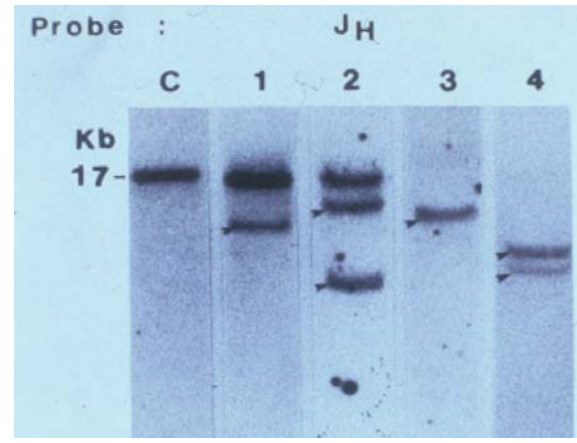


Figure 6.6

Molecular testing useful in characterization of non-Hodgkin lymphoma. Panel A demonstrates a Southern blot requiring fresh tissue to demonstrate immunoglobulin gene rearrangements. Panel B demonstrates RT-PCR performed on paraffin-embedded tissues also demonstrating a monoclonal immunoglobulin gene rearrangement

Molecular testing for specific molecular and cytogenetic abnormalities is also becoming much more common in pediatric NHL [129, 236, 345]. Several molecular tests have been developed using either Southern blot analysis (requiring fresh or frozen tissue) or RT-PCR (which can use fresh, frozen or fixed tissue) (Fig. 6.6). The sensitivity of molecular testing is very high, and it therefore has the ability to detect very small numbers of tumor cells harboring a specific defect [129]. This is particularly useful in the monitoring of minimal residual disease following therapy [300, 347]. As with the FISH analysis described above, molecular testing will only test for a specific abnormality. Therefore, it is often not as useful as conventional cytogenetics at initial diagnosis. Molecular testing often requires 24–48 h as compared to the longer turnaround time for conventional cytogenetics. In addition, many molecular tests may be more sensitive than conventional cytogenetics in identifying some abnormalities particularly when there is a low level of disease present [129, 345]. Because it is possible to use RT-PCR on fixed tissues, it may also provide the ability to observe specific defects that may not have been otherwise identifiable due to a lack of fresh or frozen tissue. As noted previously, the type of fixative used may impact the sensitivity of molecular testing [136, 366, 396]. Formalin fixation is considered optimal for subsequent PCR testing as it produces the least amount of nucleic acid coagulative change and provides the best preservation of nucleic acids for molecular testing, whereas B5 and zinc formalin fixatives are considered less favorable [366].

6.3.4 WHO Classification of Hematolymphoid Tumors

Increased understanding and insights into biologic mechanisms underlying hematologic neoplasms have led to enhanced knowledge of the pathogenesis of many hematolymphoid tumors, including NHL. Recent classification systems, such as the REAL [144] or the WHO Classifications [3] incorporate morphologic, immunophenotypic, and molecular characteristics into the definition of each disease entity. This is in direct contrast with earlier classification systems [1, 318] that made use only of morphologic or cytochemical staining characteristics. It is anticipated that the use of defined immunophenotypic and/or molecular/cytogenetic data in the diagnosis of specific entities will help lead to more objective diagnostic criteria and foster the creation of more uniform dis-

ease categories and diagnoses. Specific criteria for diagnosis will be further outlined for each NHL category below.

6.4 Molecular Pathogenesis of Pediatric NHL

Two distinct consequences have been found to occur as a result of malignancy-associated chromosomal translocations in pediatric NHL. In one common scenario, translocations fuse sequences from one chromosome (often encoding either a transcription factor, or a receptor or cytoplasmic tyrosine kinase) to those of an unrelated gene present on the other chromosome, producing a chimeric gene and protein that possesses oncogenic capabilities. The other common mechanism whereby translocations deregulate gene function is by the relocation of a gene to the vicinity of highly active promoters or enhancers from other chromosomes (in the lymphoid malignancies, often within either the immunoglobulin or T-cell receptor gene loci) that drive the expression of an otherwise unaltered gene product. Because the chimeric genes are unique to the cancer cells that contain them, diagnostic assays such as FISH or RT-PCR can specifically detect cases of a given translocation-containing tumor type [145, 328, 346]. The overexpression of an otherwise unaltered gene leading to increased gene transcripts or encoded protein expression (which may be detectable by immunohistochemical methods) within cells that do not normally express the gene is often also helpful in making or confirming a diagnosis [111].

A major advance in the characterization of the genetic abnormalities important in lymphomagenesis was the realization that the immunoglobulin (*Ig*) and T-cell receptor genes (*TCR*) are common sites of illegitimate interchromosomal translocation. For example, the immunoglobulin heavy chain (*IgH*) gene locus at human chromosome 14, band q32, is frequently interrupted in t(8;14)(q24;q32) translocations involving the *cMYC* gene found on chromosome 8q24 in Burkitt lymphomas, resulting in aberrant overexpression of the cell proliferation-enhancing *cMYC* protein in Burkitt tumor cells. A number of other examples of *IgH* involvement in lymphoid tumor-associated chromosomal rearrangements exist; for instance, another prototypical *IgH* rearrangement is the t(14;18)(q32;q21) found mainly in the adult B-lymphoid malignancy follicular center cell lymphoma, in which overexpression of the anti-apoptotic *BCL2* gene at human chromosome 18q21 occurs, contributing to lymphomagenesis. Illegitimate recombination of the kappa light-chain gene on chromosome 2p12 or lambda light-chain gene on chromosome 22q11 with oncogenic genes is also a molecular mechanism associated with lymphomagenesis, as in the Burkitt lymphomas that contain either the t(2;8)(p12;q24) or the t(8;22)(q24;q11) that activates *cMYC* and the large-cell lymphomas containing either the t(2;3)(p12;q27) or t(3;22)(q27;q11) that results in overexpression of the *BCL6* protein.

Like the *Ig* gene loci, the genes encoding the α, β, γ, and δ *TCR* chains have been cloned and characterized [359]. These genes are evolutionarily related but distinct from the *Ig* genes, and they likewise undergo molecular rearrangement to assemble the mature TCRs expressed on the T-cell surface that possess antigen-specific recognition capabilities. The β and γ *TCR* genes are located on chromosomes 7q34 and 7p15, respectively, whereas the δ *TCR* gene is located within the α *TCR* locus on chromosome 14q11. As with the *Ig* loci, illegitimate recombination of the *TCR* gene loci with genes normally important for the control of cellular growth and/or development can lead to transformation. This mechanism of oncogene activation is exemplified in the case of the multiple *TCR* gene translocations characteristically found in the T-lineage lymphoblastic lymphomas.

6.5 Burkitt Lymphomas

6.5.1 Pathology

The Burkitt lymphomas (BL) were first described as a distinct clinical entity by Denis Burkitt while studying children from equatorial Africa, where this tumor is endemic [51]. Following this discovery, it was found that BL occurs in a worldwide distribution, although there are clinicopathologic differences observed between the endemic (African) and the sporadic forms [90]. BL accounts for 40–50% of pediatric NHL in nonendemic areas. Cases occurring outside of Africa

are morphologically indistinguishable from those occurring in the endemic areas. However, endemic BL tends to have a high propensity for involvement of the bones of the face (particularly the jaw and maxilla) and occurs in younger children. The nonendemic or sporadic form of BL also tends to involve extranodal sites, but is more common in the GI tract, particularly the ileocecal area, as well as in the kidneys and ovaries; by contrast, involvement of the bones of the face is unusual [90, 228]. Extensive involvement of the bone marrow is not frequently seen at diagnosis, but may be prominent in late-stage progressive disease [55, 56]. A small percentage, 1–2% of patients, may present with disseminated disease including extensive peripheral blood and bone marrow involvement. Previously termed ALL-L3 (by the French-American-British or FAB Classification) [34], it is not an acute leukemia but rather the leukemic phase of BL [144]. CNS involvement may also be seen in BL, especially with disseminated disease [56]. Burkitt lymphoma is also common in HIV-infected or other immunocompromised individuals [153, 194, 384].

Morphologically, BL are characterized by intermediate-sized homogeneous cells with round to oval nuclei containing multiple, variably prominent, basophilic nucleoli (Fig. 6.7A). The cells have a modest amount of somewhat basophilic cytoplasm, which will appear vacuolated, due to lipid droplets, on cytologic preparations (Fig. 6.7B). These tumors have very high mitotic activity, and tissue sections will often show a "starry sky" appearance that results from reactive macrophages scattered among the malignant lymphoid cells that are engulfing apoptotic debris from the rapidly dividing tumor cells (Fig. 6.7C) [3]. It should be noted that the "starry sky" appearance is not specific for BL, but can be seen in any rapidly dividing NHL [289]. Mitoses are numerous, and the neoplastic cells often appear to form syncytial masses with molding of the cell membranes. In some areas, the tumor cells may preferentially invade germinal centers [228, 233].

The Burkitt lymphomas also include a related histologic subtype that has previously been termed non-Burkitt, or Burkitt-like, lymphoma [1]. These tumors have also been called high-grade mature B-cell lymphomas in the REAL classification [144] or atypical Burkitt lymphoma in the most current WHO classification [3]. The atypical Burkitt or Burkitt-like lymphomas have many features that are similar to the BLs, but are characterized by more cellular pleomorphism, variable nuclear irregularities, and variable numbers of nucleoli that may be more prominent than are typically seen in BL (Fig. 6.8). In tissue sections, the atypical Burkitt lymphomas may not have the typical "starry sky" pattern imparted by reactive macrophages, and may show a slightly less elevated mitotic rate [3, 190]. The ability to reproducibly distinguish Burkitt from Burkitt-like or atypical Burkitt lymphoma strictly on the basis of morphology is very unreliable, with poor intraobserver consensus shown in numerous studies between pathologists [216, 401], and requires optimally fixed and prepared tissue sections. However, it should be noted that these two entities are more clinically alike than different, and the value of making this distinction in pediatric NHL, other than for descriptive purposes, has not been conclusively demonstrated.

Immunophenotypic features of both the Burkitt and atypical Burkitt lymphomas are nearly identical (Fig. 6.9). Both are composed of mature B cells that express cell surface CD19, CD20, CD22, CD10, and cell-surface immunoglobulin. Usually the immunoglobulin is IgM heavy chain with light-chain restriction [3, 228, 289]. The atypical Burkitt or Burkitt-like tumors tend to have more variability in cell surface antigen expression, with variable CD10 or expression of cell surface IgG [365]. The expression profile of a mature B cell (cell surface CD20 and immunoglobulin expression) phenotype with coexpression of CD10 suggests that the BLs are derived from a follicular center cell [233]. It has been shown in some studies that Burkitt-like or atypical Burkitt lymphomas may have a higher level of protein expression of the germinal center marker BCL6 than BL [169]. Neither BL nor atypical Burkitt lymphomas express significant levels of the antiapoptotic protein BCL2. This can be very helpful in distinguishing BL from DLBCL, where BCL2 expression is more commonly seen [122]. Immunohistochemical staining for *cMYC* protein is positive in BL[122, 169], but may also be seen in DLBCL [122].

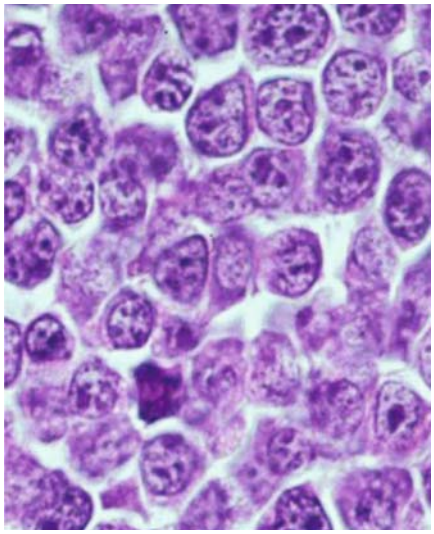
BL is one of the most rapidly proliferating human tumors, with a doubling time of approximately 12–24 h [172]. Immunohistochemical stains with proliferation markers, such as Ki-67 or MIB-1, will show stain-

ing in excess of 99% of the tumor cells [122, 401]. The atypical Burkitt or Burkitt-like lymphomas also demonstrate a very high proliferation rate, but may be slightly more variable (in the range of 90–99% of tumor cells) [175].

Cytogenetic analysis of BL will demonstrate characteristic translocations involving the *cMYC* oncogene locus on chromosome 8q24 in most cases (Table 6.5) [226, 339], and the WHO Classification requires demonstration of a *cMYC* translocation in order to make a definitive diagnosis of BL. Roughly 80% of BLs contain

Table 6.5 Cytogenetic findings in Burkitt lymphoma

C-MYC translocations
t(8;14)(q24;q32) – 80% of cases
t(8;22)(q24;q11)
t(2;8)(p12;q24)
Other abnormalities – seen in 50–60% of cases
deletion 13q
duplication 1q
deletion 6q



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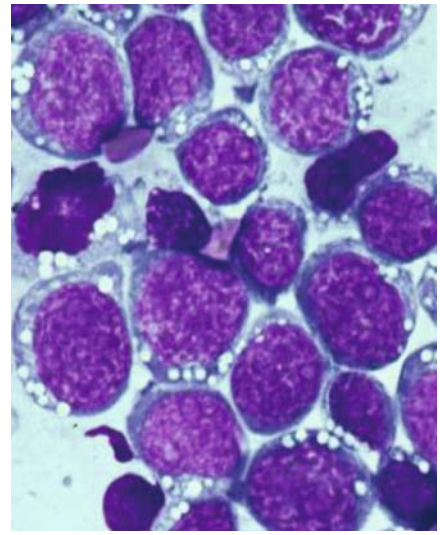

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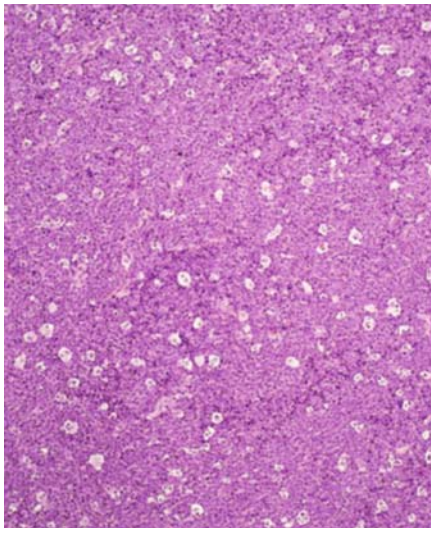


C

Figure 6.7

Burkitt's lymphoma. Panel A shows a high-power view demonstrating the vesicular chromatin, relatively monomorphic appearance and scant cytoplasm characteristic of Burkitt lymphoma (×1000, H&E stain). Panel B demonstrates a cytologic preparation with characteristic basophilic cytoplasm with lipid vacuoles, round vesicular nuclei with no prominent nucleoli identified (×1000, Wright stain). Panel C shows a low-power view of a lymph node involved by Burkitt's lymphoma demonstrating numerous tingible body macrophages giving rise to the starry sky appearance (×100, H&E stain)

a t(8;14)(q24;q32) rearrangement in which translocation of one allele of the prototypic bHLH/leucine zipper transcription factor gene *cMYC*, normally on chromosome 8, occurs to the immunoglobulin heavy-chain gene locus on chromosome 14 [5, 76, 153, 235, 376]. The remaining cases have either a t(2;8)(p12;q24) (found in 15% of cases) or a t(8;22)(q24;q11) (5% of cases) involving *cMYC* and either the kappa or lambda immunoglobulin light-chain gene loci on chromosomes 2 or 22, respectively [75, 104, 106, 162, 312, 319, 376]. In these two rearrangements, the *cMYC* gene remains on chromosome 8, and the involved light-chain gene is translocated downstream of the *cMYC* locus. In spite of the large variation in their positions, the breakpoints on chromosome 8 in these translocations never disrupt the coding regions of the *cMYC* protein. Differences in the chromosome 8 breakpoint location have been noticed between endemic and sporadic Burkitt tumors [227, 285]. The majority of endemic tumors possess breakpoints far upstream (with a smaller number far downstream) of *cMYC*, whereas sporadic tumors almost always have breakpoints within or close to the *cMYC* locus. In all translocations, *cMYC* transcription is deregulated as a result of the juxtaposition of the gene with strong immunoglobulin gene enhancers, resulting in constitutive high-level overexpression of the *cMYC* protein. Because the positions of the chromosomal breakpoints in *cMYC* and the various *Ig* genes are widely dispersed, it has been difficult to design PCR-based tests to detect *cMYC/Ig* fusion genes. As a result, these *cMYC* translocations are most reliably identified primarily by karyotyping of metaphase chromosomes or FISH on either metaphase chromosomes or interphase nuclei [84].

In addition to *cMYC* translocations, more than half of children with BL will have additional cytogenetic abnormalities (Table 6.5). The most commonly seen additional chromosomal changes include deletion 13q, duplication 1q, and deletion 6q that may impact upon the prognosis [125, 218, 226, 339]. Study of the translocations seen in Burkitt-like lymphomas in adults shows much more heterogeneity and reveals both *cMYC* translocations or translocations involving the *BCL2* locus, which are more commonly seen in follicular lymphomas or DLCBL [226]. In children, the *cMYC* translocation in the atypical Burkitt category appears to be much more common, and is seen in 85% of cases [175, 218]. The remaining cases often display complex cytogenetic abnormalities, and those cases with *cMYC* translocation invariably have additional cytogenetic aberrations, similar to those seen in BL. Translocations involving the *BCL2* locus are distinctly uncommon in children [218, 339]. These findings further support the concept that in the pediatric population, Burkitt and the Burkitt-like or atypical Burkitt lymphomas are very closely related and may represent different neoplasms than the Burkitt-like lymphomas described in adults.

6.5.2 Biology and Molecular Mechanisms

cMYC is a transcription factor that promotes cell cycle progression and cell transformation, as well as inhibiting differentiation [79, 80, 126, 281]. Overexpression of *cMYC* in growth factor-deprived cells is sufficient to overcome cell cycle arrest and push cells into S phase

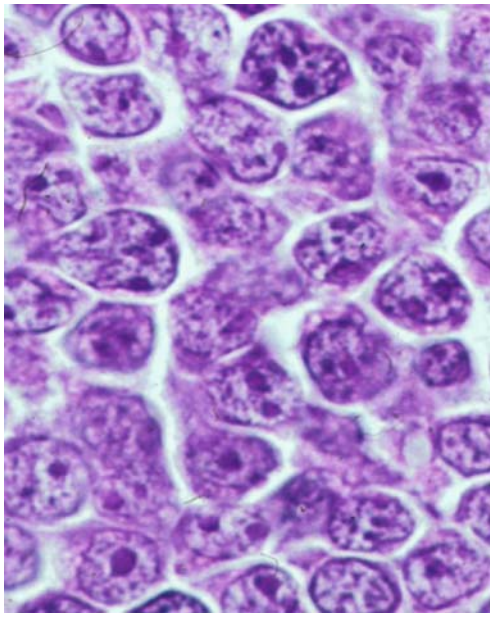
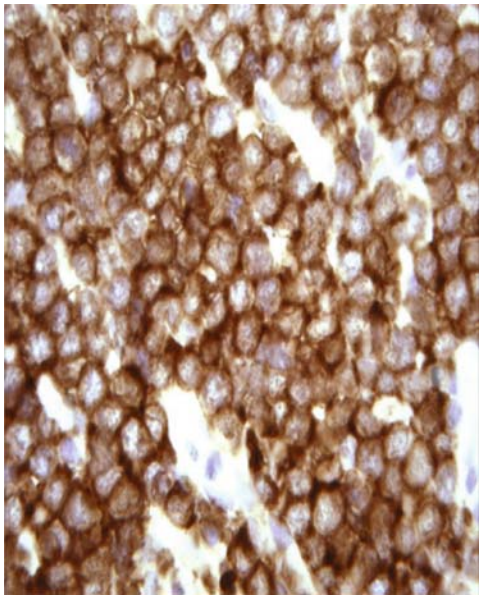


Figure 6.8

Atypical Burkitt lymphoma demonstrating increased heterogeneity of malignant cells with variation in cell size, amount of cytoplasm, and variably prominent nucleoli (×1000, H&E stain)



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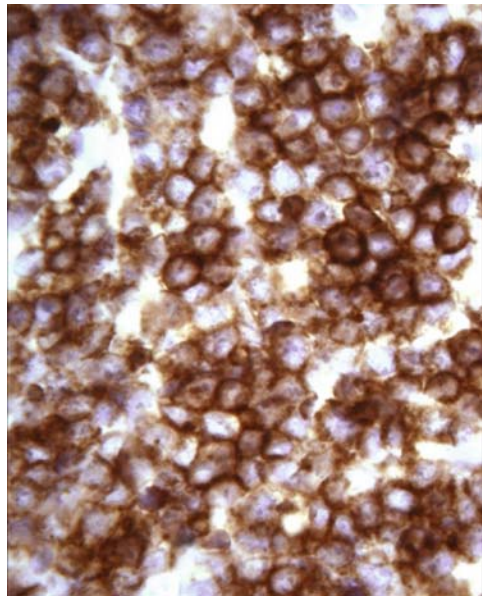

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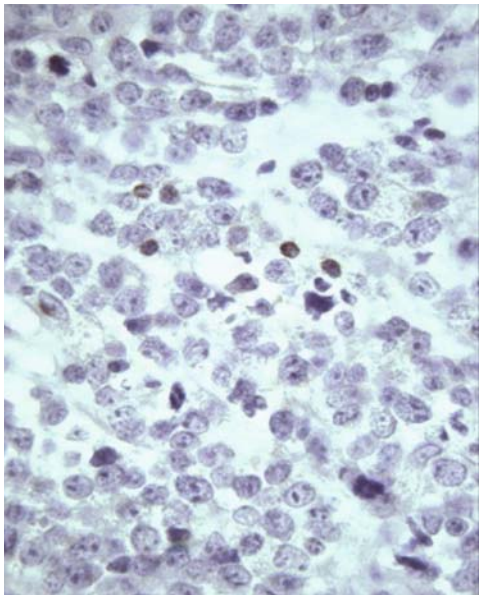

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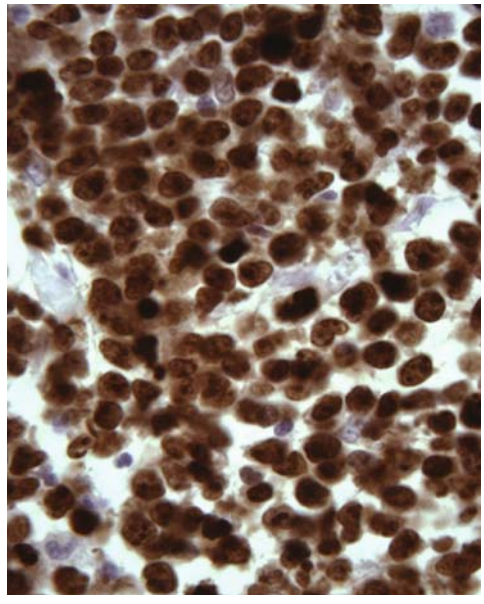


D

Figure 6.9

Immunoperoxidase staining profile for Burkitt lymphoma. Panel A shows strong membrane in cytoplasmic staining with antibodies against CD20 (×1000, anti-CD20 immunoperoxidase staining). Panel B shows strong membrane and cytoplasmic staining with CD10 (×1000, anti-CD10 immunoperoxidase stain). Panel C shows negative staining with BCL2 in the tumor cells. Note rare reactive T cells that stain positively with the antibody. (×1000, anti-BCL2 immunoperoxidase stain). Panel D demonstrates very high proliferative rate by staining with MIB1, a proliferation marker, with <99% of the tumor cells staining positively (×1000, MIB1 immunoperoxidase stain)

[18, 100, 107]. Ablation of *cMYC* in cells using either antisense oligonucleotides or expression constructs blocks entry into S phase [156, 303, 350]. In vitro, the pathogenesis of BL has been experimentally reproduced by demonstrating the tumorigenic conversion of EBV-infected B lymphocytes upon the introduction of an activated *cMYC* gene into the cells [215]. Transgenic mice engineered to overexpress *cMYC* in their lymphoid cell compartments initially develop a polyclonal pre-B-cell hyperplasia that progresses within a short period of time to a monoclonal malignancy. The time course of tumor development suggests that *cMYC* overexpression alone is insufficient to produce transformation, requiring cooperating genetic abnormalities for the development of a full-fledged malignancy [6, 204]. A number of genes that cooperate with *cMYC* to enhance tumorigenesis have been identified, including *BCL2, PIM-1, RAS, ABL, BMI-1*, and *RAF-1* [153, 281].

The exact mechanism by which *cMYC* acts to promote cellular transformation is not completely clear, but is thought to involve the transcriptional regulation of relevant target genes. It is known that *cMYC* exists in cells as a heterodimer with another bHLH/leucine zipper protein called MAX. cMYC/MAX heterodimers exhibit efficient sequence-specific DNA binding and tend to activate gene transcription [39, 133, 302]. However, MAX can homodimerize and also heterodimerize with a number of other related bHLH/leucine zipper proteins including MAD[20], MXI-1 (MAD2) [121, 343, 417] and MNT [168] to produce dimeric protein complexes that repress transcription while retaining the ability to bind to the DNA sequences also bound by cMYC/MAX heterodimers. Thus, it is generally accepted that cells contain cMYC/MAX heterodimers, MAX/MAX homodimers, as well as MAX/MAD, MAX/MXI-1, and MAX/MNT heterodimers that compete for binding to similar gene target sequences and either activate or inhibit transcription [22, 39, 302, 343]. Because the cMYC mRNA and protein are short-lived and MAX is relatively stable and abundant, the level of cMYC/MAX heterodimers largely depends upon the cMYC concentration in the cell. Factors that result in the increased production of cMYC (like the chromosomal translocations characteristic of BL) are thought to favor the formation of cMYC/MAX complexes resulting in the transactivation of a set of genes essential for progression through the G_1 phase of the cell cycle [11]. Overexpression of cMYC also effectively decreases the heterodimerization of MAX with its other dimerization partners such as MAD and MXI1, preventing the formation of MAX complexes with the latter proteins that are associated with cellular differentiation [20, 21, 206, 417].

Transcriptional activation of genes by cMYC/MAX heterodimers involves changes in the balance of histone acetylation and deacetylation, thereby altering the chromatin structure [85, 209, 321]. For example, MAD, MXI-1, and MNT all interact with a transcriptional repressor complex that possesses histone deacetylase activity. Increased recruitment of this complex to bind with promoter elements correlates with histone deacetylase-dependent silencing of transcription and cellular growth arrest plus enhanced differentiation [9, 143, 157, 168, 343, 362]. By contrast, cMYC interacts with the transactivation/transformation domain-associated protein (TRRAP), which recruits GCN5, a known histone acetyltransferase [134, 246, 247, 334]. The binding of TRRAP correlates with the oncogenic ability of cMYC, pointing out the critical importance of histone acetylase activity and alterations of chromatin structure in the cellular transformation process. Additional modulation of transcriptional control by cMYC occurs through interactions with multiple other proteins. For example, p107, Bin1, MM1, PAM, and AMY1 interact with N-terminal cMYC residues including the transcriptional activation domain [80, 333]. Likewise, the C-terminal portion of cMYC interacts with a host of proteins implicated in the regulation of transcription such as YY1, AP2, BRCA1, TFII-I, and MIZ1 [80, 333]. The significance of these cMYC-interacting proteins in cellular transformation is a matter of ongoing study.

cMYC expression and deregulation influence multiple cellular processes including cell cycle progression, differentiation, metabolism, apoptosis, immortalization, and adhesion. Because terminal cellular differentiation requires exit from the cell cycle and given that cMYC promotes constant cycling, it follows that overexpression of cMYC interferes with cellular maturation. Recent studies of mice expressing a regulated *cMYC* gene (allowing the gene to be turned off by ad-

ministration of doxycycline) has shown that the majority of cMYC-induced tumors will undergo cell cycle arrest, terminal differentiation, and regression upon downregulation of the gene [114, 176], implying that continued expression of cMYC is necessary for persistence of the tumor.

A number of metabolic pathways are also affected by cMYC due to its regulation of certain target genes involved in nucleotide synthesis, protein synthesis, and iron metabolism [153]. In addition, lactate dehydrogenase A (LDH-A) transcription is activated by cMYC [351] and correlates with the ability of cells to participate in aerobic glycolysis and grow under hypoxic conditions. cMYC may also be able to maintain the expression of telomerase, an enzyme that contributes to the immortalization of cells by permitting the indefinite maintenance of the chromosomal ends (i.e., the telomeres), which normally shorten as aging occurs. This shortening process is thought to contribute to cellular senescence. cMYC can directly induce transcription of the catalytic subunit of telomerase, known as telomerase reverse transcriptase (TERT) [392, 406]. Enforced expression of TERT has been demonstrated to immortalize rodent cells experimentally and to make cells susceptible to oncogenic transformation [400, 406].

In endemic African BL, approximately 95% of tumors contain clonal Epstein-Barr virus (EBV) DNA, whereas only some 20% of sporadic tumors in the USA and Europe are EBV-associated [153, 213, 221, 229, 309, 414]. Likewise, only a subset (approximately 40%) of human immunodeficiency virus (HIV)-associated Burkitt lymphomas are EBV-associated [153, 194]. The exact contribution of the EBV genome to the development of BL is not yet clear, but it is well known that EBV is capable of immortalizing B lymphocytes, forming cell lines that will proliferate indefinitely in vitro [227]. Clonal EBV genomes in BL indicate that EBV cell infection and immortalization are early events, occurring before the development of frank malignant transformation [264]. Presumably, the activity of several specific EBV genome-encoded proteins expressed during acute infection drives polyclonal lymphoproliferation, which is followed by the establishment of a monoclonal tumor population once the *cMYC* gene is activated [153, 227]. The latent pattern of EBV-associated gene expression observed in BL is more restricted than the expression pattern observed in acute infections and is accompanied by the downregulation of adhesion molecules such as LFA-3 and intercellular adhesion molecule 1, HLA class I molecules, and immunogenic EBV proteins (e. g., EBNA2 and latent membrane protein 1), suggesting that EBV infection may facilitate escape of the lymphomas from normal immune surveillance mechanisms [221]. Epstein-Barr nuclear antigen 1 (EBNA1), one of the few EBV genes that continues to be expressed in Burkitt tumors, has been shown to induce B-cell lymphomas in transgenic mice [402], and the latently expressed EBV-encoded RNA (*EBER*)-1 and *EBER*-2 transcripts exhibit oncogenic potential in cell lines [195]. Thus, an integrated model (yet to be unequivocally proven) of the role of EBV in the pathogenesis of BL hypothesizes that the acute pattern of EBV gene expression initially drives a polyclonal B-cell expansion. Upon acquisition of *cMYC* rearrangement and overexpression, a tumor cell clone converts to the latent EBV gene expression pattern that may enable tumor escape from immune surveillance, helping to sustain the fully transformed state.

6.6 Diffuse Large B-cell Lymphoma

6.6.1 Pathology

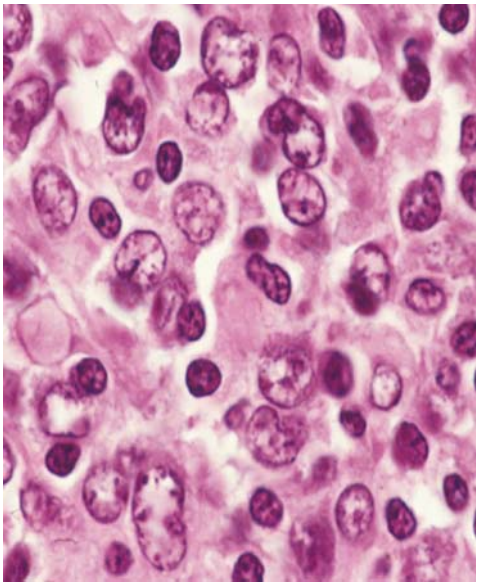
Diffuse large B-cell lymphomas (DLBCL) make up approximately 20% of pediatric NHL [54, 338]. DLBCL tends to occur in slightly older age groups and is the most common histology of NHL seen in children older than 5 years of age and teenagers. DLBCL frequently occurs at a single site, with mediastinal and abdominal disease being most common [53, 54, 338]. Nodal disease is also common, in contrast to BL. Diffuse large B-cell histology is also strongly associated with immunodeficiency states (inherited or iatrogenic) and is the most common subtype of immunodeficiency-associated lymphoma seen in childhood [54, 74, 286, 301, 384].

DLBCL may display a variety of morphologic appearances including tumors composed of large noncleaved cells (Figure 6.10A), large cleaved cells, polylobated cells, and immunoblastic cells (exhibiting a

single prominent eosinophilic nucleolus) (Fig. 6.10B) [289, 294, 349]. In early morphology-based classifications, such as the Working Formulation, these histologic subtypes were thought to have clinical significance [1]. However, in more recent classifications, such as the WHO and REAL Classifications, all DLBCL are considered to be of similar clinical aggressiveness, without a significant clinical impact imparted by histologic subtype [3, 144]. The neoplastic lymphoid cells by definition are large and have a nucleus that is at least the size of a tissue histiocyte or twice the size of a small, reactive lymphocyte. The cytoplasm in DLBCL is variable and may range from pale to plasmacytoid in appearance. The cytoplasm may also vary in abundance, but is always significantly more abundant than that seen in BL or lymphoblastic lymphoma. The overall growth pattern is diffuse [3, 144, 289].

Several morphologic subtypes of DLBCL have been described in the pediatric population including multilobated DLBCL (Fig. 6.11) [268]. T-cell-rich diffuse large B-cell lymphomas (TCR DLBCL) have also been reported in pediatric populations and consist of tumors with relatively small numbers of neoplastic large B cells and an abundant infiltrate of small reactive T cells that may obscure the malignant large B lymphocytes (Fig. 6.12) [217]. Primary mediastinal (thymic) large B-cell lymphomas are also seen in the pediatric population, predominantly in older adolescents. These tumors arise in the mediastinum from thymic B cells and show a diffuse large-cell proliferation with a variable amount of sclerosis that compartmentalizes groups of cells (Fig. 6.13) [59, 217, 293, 344, 404]. This type of lymphoma may be difficult to distinguish clinically and morphologically from Hodgkin lymphoma in small mediastinal biopsies because of extensive sclerosis and necrosis, although immunophenotypic analysis will reliably separate these entities [59].

Immunophenotypic characterization of DLBCL demonstrates a mature B-cell phenotype with expression of cell surface immunoglobulin and B-cell-spe-



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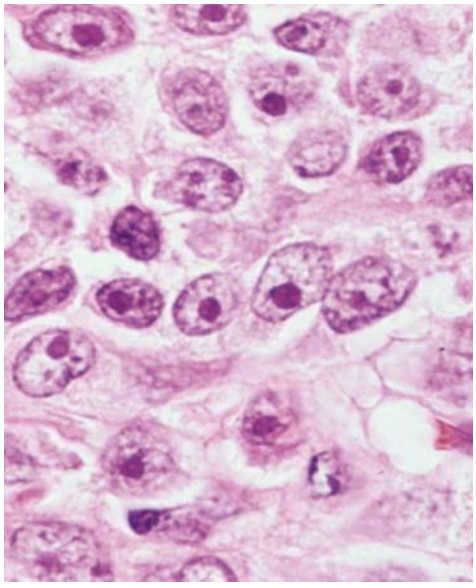


B

Figure 6.10

Panel A demonstrates a centroblastic appearance of diffuse large B-cell lymphoma with large neoplastic cells that vary between 3 and 5 times the size of a small reactive lymphocyte with abundant cytoplasm. The nuclei are vesicular without prominent nucleoli (×1000, H&E). Panel B shows diffuse large B-cell lymphoma demonstrating immunoblastic morphology with prominent eosinophilic nucleoli and abundant eosinophilic cytoplasm. The tumor cells are variable, but range from 3 to 6 times the size of a small reactive lymphocyte (×1000, H&E)

cific lineage markers such as CD19, CD20, CD22, and CD79a [3, 288, 289]. A subset of cases arising from thymic B cells seen in most primary mediastinal large B-cell lymphomas may lack cell surface immunoglobulin expression, but will display cytoplasmic immunoglobulins [59, 294]. Expression of CD10 is variable, but can be seen in approximately half of the cases of pediatric DLBCL [122]. CD5 expression is not commonly seen. The activation marker, CD30, may be seen in some cases of DLBCL, and is most commonly expressed in primary mediastinal diffuse large B-cell lymphomas [160].

There are no specific, highly recurrent characteristic cytogenetic abnormalities associated with DLBCL in children and adolescents [154]. Unlike adults, where 20–30% of cases will demonstrate a *BCL2* translocation such as the t(14;18)(q32;q21) that is also associated with follicular lymphoma, *BCL2* translocation is rarely seen in children [154]. It should also be noted that translocations associated with the *cMYC* oncogene, such as t(8;14), are much more frequently seen in pediatric DLBCL, suggesting a possible relationship with BL [154]. Despite the lack of highly recurrent abnormalities, nearly all pediatric DLBCL will contain cytogenetic aberrations of some sort. DLBCL usually displays complex cytogenetic findings that include structural abnormalities in nearly all cases and numerical abnormalities in over half of the cases studied. Most cases will contain three or more cytogenetic aberrations [154, 263].

6.6.2 Biology and Molecular Mechanisms

Analysis of DLBCL has identified cytogenetically detectable chromosomal abnormalities affecting band 3q27 in 10–12% of adult cases [30, 266], usually reciprocal translocations between 3q27 and a number of chromosomal loci (>10), but most commonly the immunoglobulin heavy (14q32) or light (2p12, 22q11) chain gene loci. The gene on 3q27 altered by these

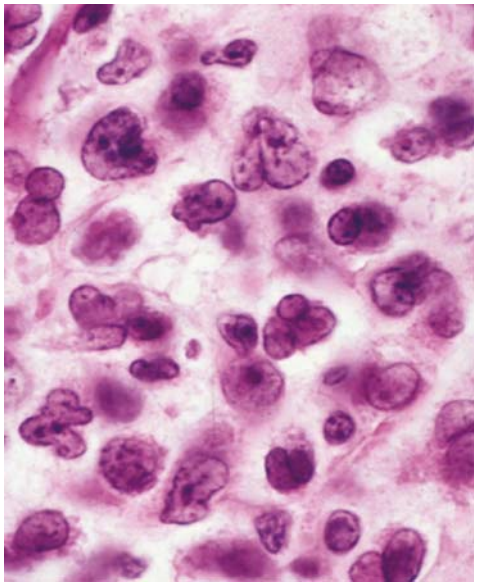


Figure 6.11

Diffuse large B-cell lymphoma demonstrating a multilobated appearance. The large neoplastic cells show numerous nuclear enfolding giving a polylobated appearance (×1000, H&E)

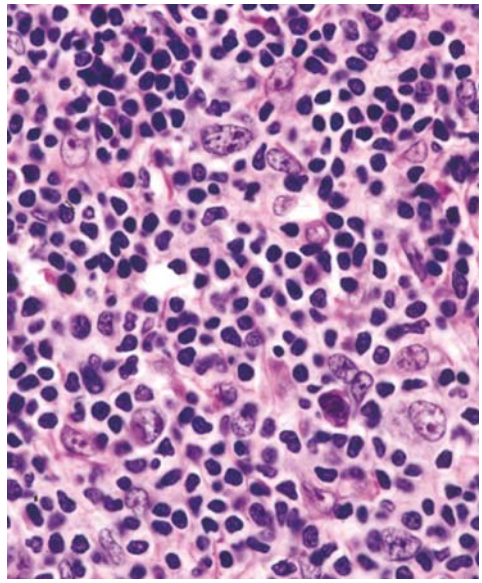


Figure 6.12

T-cell-rich large B-cell lymphoma. The neoplastic large cells are admixed with a large number of small reactive T lymphocytes. The neoplastic B cells make up a minority of the lymphoid cells seen in the section (×400, H&E)

translocations encodes a transcription factor, BCL6 (formerly also called LAZ3 or BCL5)[251, 412]. The aminoterminal portion of BCL6 contains a POZ domain, a protein-protein interaction motif found in some zinc-finger transcription factors. A specific BCL6 DNA-binding sequence has been identified, and BCL6 binding to these sequences decreases gene transcription, suggesting that the normal function of protein is to inhibit the expression of its target genes [26, 62, 186]. Normal BCL6 expression is tightly regulated during B-cell development, being expressed in mature B cells but not in their precursors or their more mature progeny (i.e., immunoblasts and plasma cells) [58, 110, 119, 120, 276]. Within the lymph nodes, BCL6 expression is present only within the germinal centers [225] and is thought to be important for germinal center-associated functions [10].

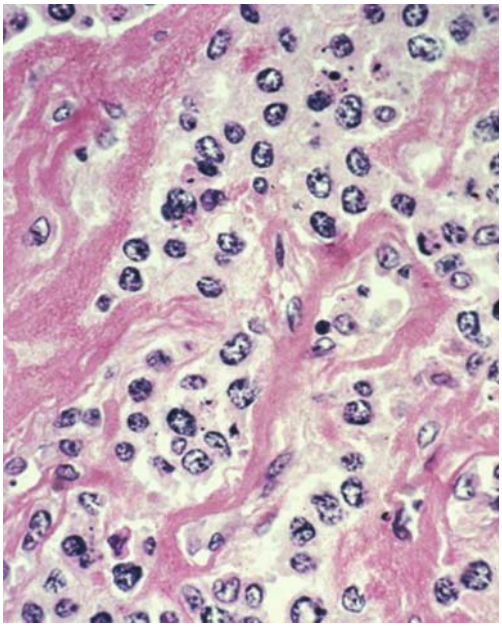


Figure 6.13

Diffuse large B-cell lymphoma with sclerosis or primary mediastinal large B-cell lymphoma. This biopsy of a mediastinal mass shows a neoplastic infiltrate of large B cells with extensive, compartmentalizing sclerosis. The tumor cells demonstrate characteristic abundant, lightly eosinophilic cytoplasm (×1000, H&E)

The frequency of *BCL6* gene rearrangement detected by molecular analysis significantly exceeds the 10–12% predicted by the cytogenetic study of 3q27 abnormalities in adult DLBCL, indicating that rearrangements also occur as a consequence of submicroscopic chromosomal abnormalities. Approximately one-third of adult DLBCL have molecular *BCL6* gene rearrangement identified by Southern hybridization analysis [29, 267, 269, 280]. *BCL6* gene rearrangement is not uniquely restricted to DLBCL and is also identified in 5–14% of follicular lymphomas in adults [29, 269, 280, 412]. *BCL6* rearrangements tend to occur exclusive of rearrangement of *BCL2*, suggesting that *BCL6* is probably specifically involved in the pathogenesis of de novo large-cell lymphoma as opposed to large-cell tumors that result from the transformation of follicular lymphoma [77]. Rearrangement of *BCL6* has been associated with primary extranodal DLBCL and tumors that lack bone marrow involvement [77]. It has also been suggested that *BCL6* alterations may be associated with lymphomas that have a favorable prognosis after chemotherapy [267], although this correlation has not been readily evident in all studies [29].

All *BCL6* breakpoints occur in or around the nontranslated first exon and leave the coding region of the gene intact. The *BCL6* translocations that have been fully characterized have been shown to result in the deregulated expression of a normal *BCL6* protein because of promoter substitution [78, 124, 411]. The ultimate consequence of these translocations appears to be to prevent the physiologic downregulation of *BCL6* that occurs during B-cell differentiation into plasma cells [10, 58, 120, 276].

In addition to chromosome translocations, involvement of *BCL6* in DLBCL can occur as a result of somatic mutations that cluster in the same 5'-noncoding region of the gene in which the 3q27 breakpoints occur [250]. These mutations are often multiple and biallelic, and are present in cases containing either normal or rearranged *BCL6* alleles and therefore occur independently of mechanisms involved in chromosomal translocation [178, 285, 314, 374]. Mutations of *BCL6* were observed in almost three-fourths of adult DLBCL, indicating that this is a very common form of genetic instability that may implicate *BCL6* in the pathogenesis of these tumors. The incidence of *BCL6*

translocations and mutations in pediatric DLBCL is unknown, but karyotypic abnormalities involving 3q27 have been reported in <10% of cases [154]. Molecular analysis for nontranslocation-mediated *BCL6* mutations in pediatric DLBCL has not well described. Thus, it appears that other molecular mechanisms may be more important in the development of DLBCL in the pediatric population. However, there is currently little information as to what molecular events lead to the development of DLBCL in children, and this is an important area for future investigation. "It is worthy to note that lymphomas in which aberrant *BCL6* expression is of pathogenic importance could be a target in the future for therapy directed at the transcription factor, given that experimental peptide-based blockade of the interaction of *BCL6* with co-repressor complexes has been shown to cause apoptosis and cell cycle arrest of *BCL6*-expressing lymphoma cells" [296].

Three major gene expression profiling (microarray) studies have addressed the biological and clinical heterogeneity of adult DLBCL [8, 327, 356]. In the initial DLBCL gene expression profiling study, lymph node biopsy samples from previously untreated adult patients were analyzed [8]. Genes that define the germinal center stage of B-cell differentiation were used to define two prominent DLBCL subgroups. The "germinal center B-cell-like" DLBCL subgroup (GCB DLBCL) expressed genes characteristic of normal germinal center B cells (e. g., *CD10, BCL6, A-myb*), whereas the "activated B cell-like" DLBCL subgroup (ABC DLBCL) expressed genes that are induced during mitogenic activation of peripheral blood B cells (e. g., *BCL2, IRF-4, cyclin D2*) (Fig. 6.14). A larger gene expression profiling study of DLBCL cases confirmed the existence of these two DLBCL subgroups, but also identified another set of cases, termed "type 3" DLBCLs, that do not resemble GCB or ABC DLBCL and may represent an additional molecular subgroup of DLBCL [327].

These observations raised the possibility that the DLBCL subgroups represent pathogenetically distinct entities that are derived from cells at different stages of B-lymphoid differentiation. Support for this hypothesis has come from the analysis of immunoglobulin gene mutations and oncogenic abnormalities in the DLBCL subgroups. Although DLBCLs in both the GCB and ABC subgroups were found to have mutated immunoglobulin genes, only GCB DLBCLs had ongoing somatic hypermutation of the immunoglobulin locus within the tumor clone [219]. Since somatic hypermutation of immunoglobulin genes is a hallmark of germinal center B cells, this finding suggests that the GCB DLBCL tumors retain some of the biological characteristics of normal B cells at this stage of differentiation.

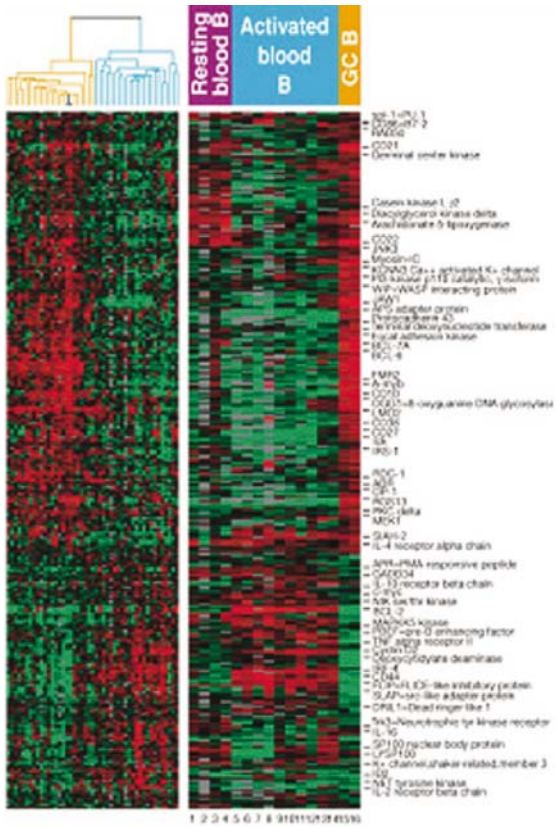


Figure 6.14

Gene microarray analysis of diffuse large B-cell lymphoma demonstrating hierarchical clustering that separates tumors of germinal center B-cell derivation from activated B-cell derivation. Shades of red indicate higher expression, while shades of green indicate lower expression, and black indicates median expression. Each *column* indicates one tumor sample, and each *row* represents a single gene. (Modified from [8])

Two recurrent chromosomal alterations in DLBCL were detected exclusively in the GCB DLBCL subgroup [167, 327]. The t(14;18)(q32;q21) translocation involving the antiapoptosis *BCL2* gene was detected in 23–35% of GCB DLBCL cases but never in ABC or type 3 DLBCL cases. Similarly, amplification of the *cREL* locus on chromosome 2p was detected in 15% of GCB DLBCLs but not in the other subgroups [327]. By contrast, ABC DLBCLs were found to have constitutive activity of the NF-κB pathway, which is not a feature of GCB DLBCLs [83]. These findings suggest that the DLBCL subgroups utilize distinct oncogenic mechanisms, which may have implications for targeted therapy in the future. Indeed, interference with the NF-κB pathway was found to kill ABC DLBCL cells in vitro, suggesting that pharmacological targeting of this pathway may be beneficial for patients with this type of DLBCL [83].

The DLBCL gene expression subgroups were found to have distinct overall survival rates following anthracycline-based multiagent chemotherapy (e. g., the CHOP regimen) [8, 327]. The 5-year survival rates for the GCB, ABC, and type 3 DLBCL subgroups were 60%, 35%, and 39%, respectively [327]. However, the DLBCL subgroup distinction did not fully account for the variable response of these patients to chemotherapy, suggesting that further molecular differences among the lymphomas influence their response to chemotherapy. Unfortunately, no studies of pediatric DLBCL are currently available to know if DLBCL in this age group is similarly heterogeneous or instead demonstrates more homogeneous gene expression profiles.

6.7 Anaplastic Large-Cell Lymphoma

6.7.1 Pathology

Primary systemic ALCL is the most common mature T-cell lymphoma seen in children and makes up approximately 10% of all pediatric NHL and approximately 30–40% of the large-cell lymphomas seen in the pediatric population [54, 289, 338]. ALCL is often associated with lymphadenopathy in combination with extranodal disease, most commonly involving the skin, bone, and soft tissues [7, 47, 193]. CNS involvement is more commonly seen in children than adults [193, 262]. Primary cutaneous ALCL (limited to the skin only) is not commonly seen in the pediatric population, but when present typically occurs in older adolescents [193]. Primary systemic ALCL patients are most commonly male and older than 10 years of age. Most patients will present with advanced (stage III or IV) disease [7, 239].

Morphologically, primary systemic ALCL can be quite variable [180]. The nodal architecture is usually effaced by a sinusoidal, paracortical, or diffuse infiltrate of cohesive-appearing medium-to-large sized tumor cells. There have been several morphologic subtypes of ALCL described. Classic ALCL has a predominance of tumor cells that are large, pleomorphic, and often multinucleated. Often these cells contain eccentric horseshoe-shaped nuclei with abundant clear-to-basophilic cytoplasm with an area of eosinophilia near the nucleus (termed "hallmark cells") (see Fig. 6.15A). These hallmark cells may resemble Reed-Sternberg cells, although they typically do not have as prominent nucleoli. A small-cell variant of ALCL has also been described where the cells are smaller in size, more monomorphic, and show minimal cytologic variation (see Fig. 6.15B). The tumor cells tend to be round-to-oval with slight nuclear irregularities and dispersed chromatin. Nucleoli are variably prominent, and the cytoplasm is moderately abundant, imparting a pale appearance to the tumor infiltrates. Rare or scattered multinucleated large cells may be seen. The small-cell variant tends to invade small blood vessels and may most easily be identified in a perivascular location [192, 193]. In another type of ALCL histology, there may be a prominent histiocytic component accompanying the tumor, and this has been termed the lymphohistiocytic variant of ALCL [180, 193]. The classical histology makes up approximately 75% of pediatric ALCL, whereas the lymphohistiocytic and small-cell variants comprise approximately 10% each. Other, less well described and relatively rare morphologic variants include the sarcomatoid, signet ring, neutrophil-rich, and giant-cell types [3]. Because of the tendency of the tumor cells of ALCL to infiltrate in a sinusoidal or perivascular pattern, the neoplastic infiltrate may be subtle and easily missed or confused

with poorly differentiated metastatic cancers. The small-cell, lymphohistiocytic, and neutrophil-rich variants may be confused with reactive processes. In extranodal sites or skin lesions, the neoplastic infiltrate is also variable and may show a perivascular distribution or a sheet-like pattern of growth with few interspersed inflammatory cells and a relatively high mitotic rate [180, 193].

ALCL is defined as having tumor cells which express the CD30 (Ki-1) antigen in virtually all cases (see Figure 6.16A). The majority of tumors will have a T-cell phenotype when tested for a sufficient number of T-cell antigens (Table 6.3) [3]. The use of antibodies to detect a wide range of T-cell antigens on paraffin-embedded tissues (including CD2, CD3, CD4, CD5, CD7, CD8) helps to define most of these tumors as T-cell neoplasms, which can often be confirmed by molecular studies [67, 129, 265]. Previous studies, using much more limited T-cell antigen panels, reported 20–30% of ALCL as being "null cell" (expressing neither B- nor T-cell specific antigens) [144, 290]. With an expanded T-cell panel, >80% of cases will stain for a T-cell phenotype, however. Antigen deletion of T-cell markers, especially CD3, CD5, and/or CD7, is common. Most tumors will express CD4, and the less specific T-cell associated markers CD43 and CD45RO are also often expressed[3, 289]. The WHO Classification recognizes ALCL as a T-cell disorder only[3]. Nearly all systemic and cutaneous ALCL will demonstrate T-cell receptor gene rearrangement even if immunophenotypic analysis fails to demonstrate definitive T-cell antigen protein expression [129]. A CD30-positive B-cell lymphoma has been described, but these tumors are more appropriately classified as a DLBCL rather than ALCL, and they will lack expression of the ALK (anaplastic lymphoma kinase) protein as detected by ALK-1 or other anti-ALK antibodies (see below) [160, 239, 349]. Epithelial membrane antigen (EMA) and the cytolytic granule protein TIA-1 are very frequently seen in both the T-cell and null-cell types of ALCL. CD45 expression may vary from strong to weak or absent and may be focally expressed. Weak cytokeratin expression has been described in some ALCL, which may complicate resolution of a differential diagnosis that includes metastatic tumor [193]. Particularly in pediatric ALCL, expression of the ALK protein by immunohistochemistry is extremely common (see below and Fig. 6.16B). ALK staining has been strongly associated with systemic disease and is characteristically absent in primary cutaneous ALCL [47, 265, 306].

Cytogenetic and molecular analyses very often demonstrate characteristic genetic alterations involving the *ALK* gene locus on chromosome 2 (see Fig. 6.17A) [94, 201]. Classically, this has been manifested as a t(2;5)(p23;q35) translocation. The molecular cloning of the t(2;5) in 1994 revealed that this chromosomal rearrangement produces a fusion gene, *NPM-ALK*, encoding the aminoterminal portion of nucleophosmin (NPM), a nucleolar phosphoprotein encoded on chromosome 5, that is linked to the cytoplasmic part of ALK, a receptor tyrosine kinase of the insulin receptor superfamily encoded on chromosome 2 [97, 254, 305].

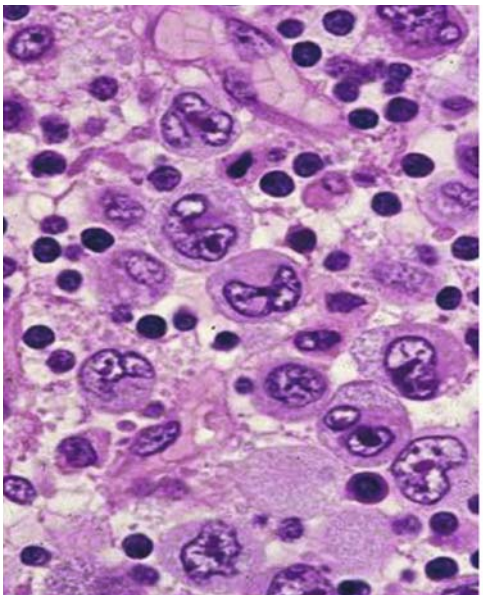
The cloning of the t(2;5)-associated *NPM-ALK* fusion gene permitted the development of RT-PCR, FISH, and Southern blot hybridization assays for the diagnosis of NHL containing this abnormality [50, 93, 102, 240, 340, 391, 393, 397]. In addition, because ALK is not normally expressed at significant levels in lymphoid cells, immunohistochemical staining of biopsy samples with anti-ALK antibodies can be used to reliably detect overexpression of NPM-ALK and other ALK chimeric proteins [111, 159, 202, 306, 354, 355].

Anti-ALK immunohistochemical staining of NPM-ALK-expressing ALCL cases shows a typical staining pattern, in which the chimeric protein is present in both the cytoplasm and the nucleus of the tumor cells [33]. The dual cytoplasmic and nuclear localization of NPM-ALK in tumor cells is due to its hetero-oligomerization with normal NPM, which functions in bidirectional shuttling of proteins between the cytoplasm and nucleus [38, 238]. However, approximately 20–25% of ALCLs exhibit anti-ALK staining in the cytoplasm of the tumor cells only, and these cases have been shown to possess variant chromosomal translocations involving the *ALK* gene locus at chromosome 2p23 but not the *NPM* gene from chromosome 5. These other, less common, translocations include rearrangements of *ALK* to partner genes on chromosomes 1, 2, 3, 17, 19, 22, and X (see Fig. 6.17B). Over the past 5 years, these variant *ALK* rearrangements have been molecularly cloned, and the *ALK* fusion partners identified. Included among these variant *ALK* fusion pro-

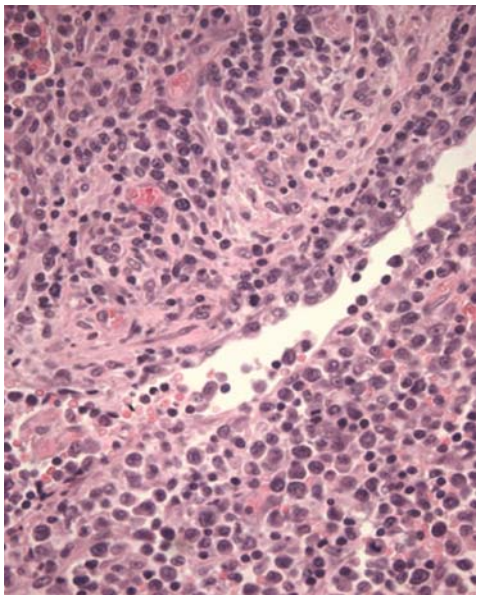
teins are TPM3 (non-muscle tropomyosin-3) ALK, ATIC (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase) ALK, CLTC (clathrin heavy chain) ALK, TFG (TRK-fused gene) ALK, and MSN (moesin)/ALK [97, 109, 256, 305, 308]. Interestingly, while ALK fusion proteins are found mainly in T- and null-cell ALCL, very recent studies suggest that occasional (probably <1%) diffuse large B-cell lymphomas in both children and adults can also be ALK-positive, with CLTC-ALK and NPM-ALK fusions reported in such cases [127, 272-274]. All of these translocations result in upregulation of ALK expression and constitutive activation of its kinase function.

ALK translocations may be detected by conventional cytogenetics, RT-PCR, or FISH using *ALK*-specific probes (see Fig. 6.18) [94, 129]. The result of all of the various *ALK* translocations is overexpression of a truncated *ALK* protein that can be detected by immunohistochemical methods using anti-ALK antibodies including the ALK-1 monoclonal antibody and others. The pattern of ALK antibody staining is nuclear with or without cytoplasmic staining in the most common t(2;5)-containing cases, and staining of the cytoplasm only for most of the alternative translocations (see Fig. 6.16C) [265, 349]. It is important to note that *ALK* translocations are thought to occur rarely, if ever, in primary cutaneous ALCL and are seen with much lower frequency in adult cases of primary systemic ALCL as compared to cases in children and adolescents.

ALK-positive ALCLs occur predominately in children and young adults. For example, Pulford and colleagues [306] identified ALK expression in 88.5% of 26 children, but in only 34% of 47 adults with CD30-positive ALCL. Similarly, Falini [109] examined 96 CD30-positive ALCLs (58 ALK-positive and 38 ALK-negative) and found the mean age of the ALK-positive cases to be 22.0 years (range 3–52), whereas ALK-negative cases occurred at a mean age of 43.3 years. In pediatric populations, an ALK translocation is present in >90% of cases when a combination of ALK immunostaining, cytogenetics, and FISH is employed [54]. Most patients with



A

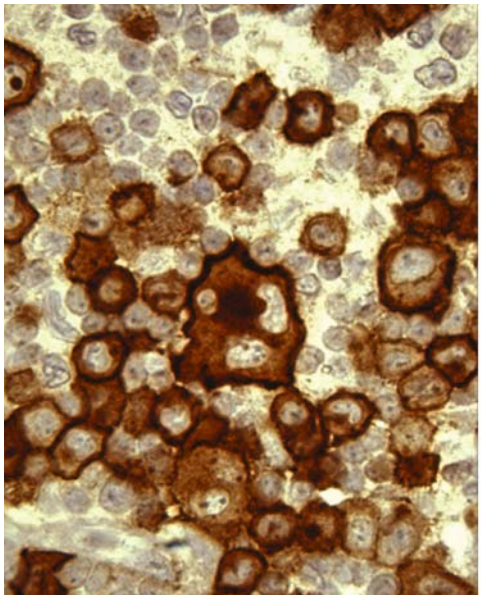


B

Figure 6.15

Anaplastic large-cell lymphoma. Panel A demonstrates the classic variant of anaplastic large-cell lymphoma with numerous large tumor cells including multinucleated and wreath-like forms (×1000, H&E). Panel B demonstrates a small-cell variant with smaller neoplastic cells with characteristic vascular invasion (×1000, H&E)

ALK-positive ALCL present with systemic nodal (with or without extranodal) disease. Although carefully randomized trials of uniformly treated and age-matched patients comparing the prognosis of ALK-positive with ALK-negative ALCL have yet to be performed, in almost all studies ALK-positive ALCL patients have had a significantly better outcome following conventional combination chemotherapy, with overall 5-year survival rates of 79–88% compared to the 28–40% seen for ALK-negative ALCL cases. It appears the superior prognosis of ALK-positive ALCL occurs irrespective of the specific ALK fusion partner, with the variant fusions being associated with an equally good outcome to that observed in the *NPM-ALK*-positive cases [109].



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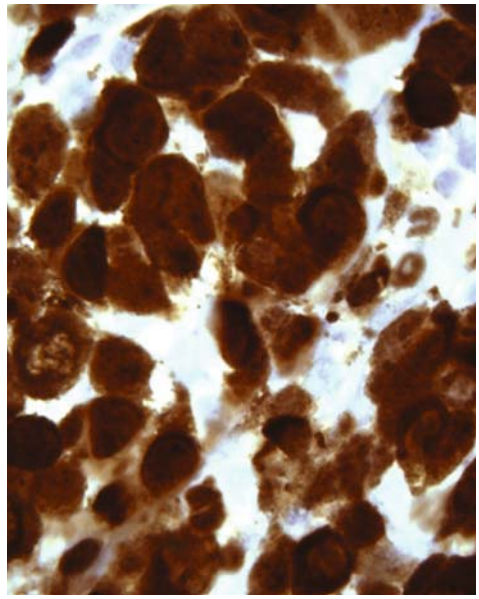
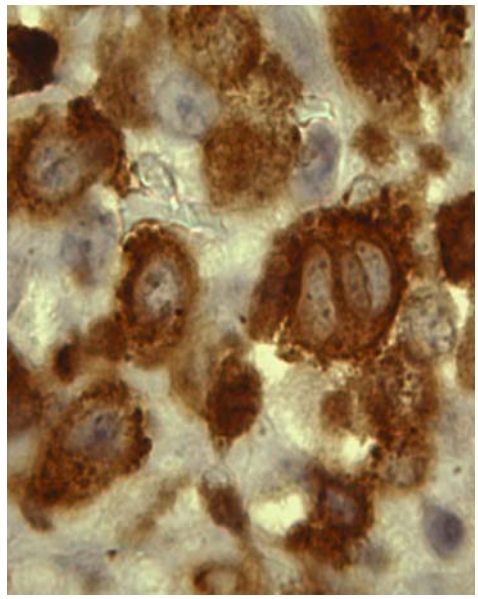


B

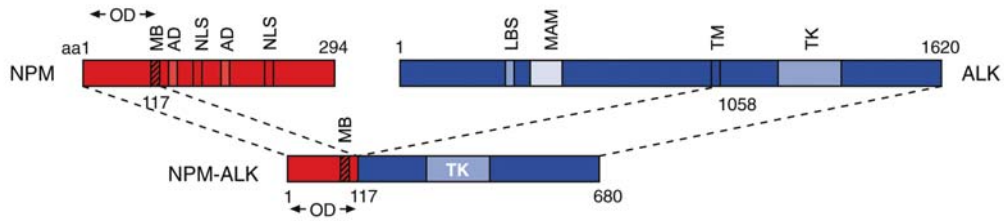


C

Figure 6.16

Immunohistochemical staining of anaplastic large-cell lymphoma. Panel A demonstrates the strong characteristic staining with anti-CD30 antibody in a membrane and gold g pattern of the neoplastic cells (×1000, anti-CD30 immunohistochemical stain). Panel B shows the classic pattern of ALK staining of an anaplastic large-cell lymphoma with a t(2;5) containing tumor with nuclear and cytoplasmic staining (×1000, anti-ALK immunohistochemical stain). Panel C demonstrates an anaplastic large-cell lymphoma with an alterative translocation demonstrating lack of nuclear staining and prominent cytoplasmic staining (×1000, anti-ALK immunohistochemical stain)

A



B

Translocation	Frequency in ALK + Lymphoma	Occurrence in IMT	Localization
t(2;5)(p23;q35)	~75%	–	N/C
t(1;2)(q25;p23)	~18%	+	C
inv(2)(p23q35)	~2%	–	C
t(2;17)(p23;q23)	~2%	+	C
t(2;3)(p23;q21)	~1%	–	C
t(2;3)(p23;q21)	~1%	–	C
t(2;19)(p23;p13.1)	<1%	+	C
t(2;X)(p23;q11-12)	<1%	–	CM
t(2;2)(p23;q11-13)? or inv(2)(p23q11-13)?	—	+	NM

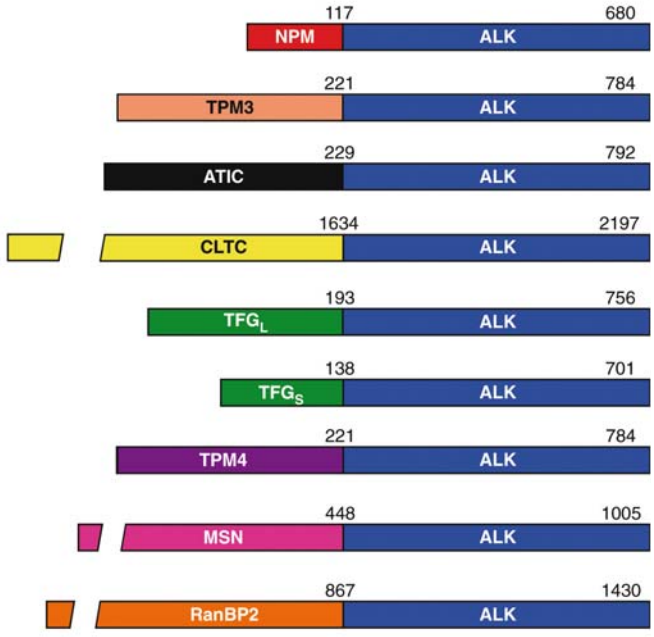


Figure 6.17

Panel A: Schematic of the ALK receptor tyrosine kinase and the NPM-ALK fusion protein resulting from the t(2;5). Fusion of the chromosome 5 gene encoding nucleophosmin (NPM) to the chromosome 2 gene encoding anaplastic lymphoma kinase (ALK) generates the chimeric tyrosine kinase, NPM-ALK. NPM contains an oligomerization domain (OD; residues 1–117) a putative metal-binding domain (MB; residues 104–115), two acidic amino acid clusters (AD; Asp/Glu-rich acidic domain; residues 120–132 and 161–188) that function as acceptor regions for nucleolar targeting signals, and two nuclear localization signals (NLS: residues 152–157 and 191–197). ALK contains a single MAM (Meprin/A5/protein tyrosine phosphatase Mu) domain, a region of about 170 aa present in the extracellular portions of a number of functionally diverse proteins that may have an adhesive function (residues 480–635). The ligand-binding site (LBS) for pleiotrophin and midkine (ALK residues 391–401) is indicated. TM=transmembrane domain; TK=tyrosine kinase catalytic domain. Panel B: ALK fusion proteins, the chromosomal rearrangements that generate them, their occurrence in ALK-positive lymphomas and inflammatory myofibroblastic tumors (IMT), and their subcellular localizations. The frequency of the various ALK fusions expressed in IMT has not yet been determined. 7.17C=cytosolic; N=nuclear; CM=cell membrane; NM=nuclear membrane; TPM3=non-muscle tropomyosin-3; ATIC=5-aminoimidazole-4-carboxmide ribonucleotide formyltransferase/IMP cyclohydrolase; CLTC=clathrin heavy chain; TFG=TRK-fused gene; TPM4=non-muscle tropomyosin-4; MSN=moesin; RanBP2=Ran-binding protein2

Primary cutaneous ALCL typically occurs in older patients, with a median age of 60 years, and approximately 25% of these cases spontaneously undergo either partial or complete regression without treatment, in contrast to the aggressive clinical course typical of untreated primary systemic ALCL [32, 89, 185, 224]. Except for those patients with generalized skin involvement, who appear to be at greater risk of developing extracutaneous involvement and may benefit from multiagent systemic chemotherapy, primary cutaneous ALCL patients can be treated by excision of their lesions, with or without radiation. Thus, it is important if possible to discriminate between primary systemic ALCL with cutaneous involvement and primary cutaneous ALCL, which may appear essentially identical histologically. ALK staining is rarely, if ever, seen in primary cutaneous ALCL. Thus, although the detection of ALK cannot be used to unequivocally distinguish between these two entities, ALK expression is highly associated with a diagnosis of primary systemic ALCL.

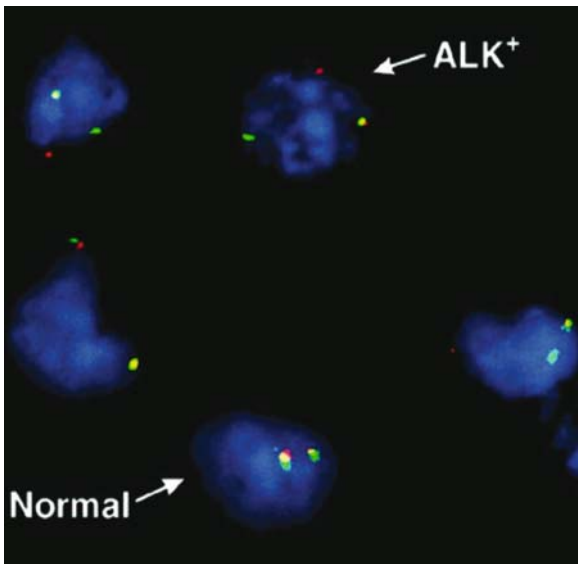


Figure 6.18

Fluorescent in situ hybridization (FISH) in a case of anaplastic large-cell lymphoma using a ALK break apart probe. A normal cell will show a yellow fusion signal with an intact ALK gene. A translocation results in splitting apart of the red and green signals and indicates that a ALK translocation is present (×1000)

Other lymphoproliferative diseases that can mimic primary systemic ALCL (such as classical Hodgkin lymphoma, lymphomatoid papulosis, and mycosis fungoides) appear to be uniformly ALK-negative [256]. As ALCL and classical Hodgkin lymphoma share a number of features in common, including CD30 expression in large tumor cells, the presence of Reed-Sternberg-like cells in some cases of ALCL, and a similar bimodal age distribution, it is important to distinguish ALCL from Hodgkin lymphoma since the therapeutic approaches are different [210]. Lymphomatoid papulosis (LyP) is a clonal cutaneous CD30-positive T-cell lymphoproliferative disorder that occurs mainly, but not exclusively, in adults in which recurrent crops of ulcerating papules regress with scar formation in a few weeks. In 5–20% of cases, LyP progresses to overt lymphomas such as Hodgkin's disease, ALK-negative ALCL, or mycosis fungoides. This observation, together with the presence of lesions with similar histological features shared between LyP and ALCL, suggests that LyP and primary cutaneous ALCL represent a histopathological continuum of CD30-positive lymphoproliferative disorders of the skin. A large number of studies have examined *ALK* expression in LyP and found no evidence for involvement of the gene in the genesis of the disorder [256].

EBV is seen in a small proportion of cases of ALCL, and expression of EBV-associated genes has varied between 5 and 40% in a variety of studies [3, 193, 262]. In most pediatric cases, EBV expression appears to be infrequent or absent [262].

6.7.2 Biology and Molecular Mechanisms

NPM is a highly conserved and ubiquitously expressed 38-kDa nonribosomal RNA-binding protein that shuttles ribosomal ribonucleoproteins between the nucleolus and the cytoplasm and is involved in the late stages of preribosomal particle assembly [43, 60, 117, 341, 342, 372]. NPM has also recently been reported to control centrosome duplication initiated by CDK2/cyclin E-mediated phosphorylation of the protein and to therefore be critical for the normal progression through mitosis [270]. Recent work has indicated that NPM also functions normally to enhance p53 activity, in part by preventing the proteolytic degradation of p53

[69, 200]. It is presently unclear whether these normal functions of NPM (or alterations of them) play a significant role in the development of ALCL. ALK is normally expressed mainly in the central and peripheral nervous systems [173, 255, 305, 308], but understanding of the normal functions of this receptor tyrosine kinase remain incomplete, and mice engineered to lack expression of the protein have a normal lifespan and no grossly evident abnormalities [97, 305, 308].

As a result of the t(2;5), the transcription of the ALK kinase domain is driven by the strong *NPM* gene promoter, leading to its inappropriate expression in lymphoid cells (in which little or no ALK is normally expressed) [254, 353]. In addition, due to NPM-ALK homo-oligomerization mediated by the NPM portion of the fusion (which contains a self-association domain), the ALK kinase catalytic function is constitutively activated and can engage and activate mitogenic signaling substrate proteins by their phosphorylation, therefore leading to oncogenic transformation of cells (Figs. 6.17–6.19) [38, 97, 123, 305, 308]. Lethally irradiated mice rescued by transplantion with bone marrow that expresses NPM-ALK have been demonstrated to develop lymphomas after a 3- to 4-month latency period [198], and transgenic mice engineered to express NPM-ALK in their lymphoid cells develop both T- and B-cell malignancies that are rapidly fatal [66].

The same basic cellular transforming mechanism appears to be operative for both NPM-ALK and the variant ALK fusions. Specifically, the N-terminal portion of the particular ALK partner protein contains some form of oligomerization domain that mediates constitutive self-association of the chimeric ALK protein, which in turn initiates aggregation of the fusion protein and ALK kinase catalytic domain activation. Because these fusion proteins are constantly kinase-active, they transmit unremitting mitogenic signals, resulting in uncontrolled cellular proliferation (Figs. 6.17–6.19). The ability of ALK fusion proteins to oncogenically transform cells is not limited to lymphocytes during lymphomagenesis. ALK has recently been shown to participate in the genesis of a nonhematopoietic malignancy, inflammatory myofibroblastic tumor (IMT), with some of the same ALK fusion proteins that occur in ALCL also contributing to the development of IMT. A recent study of IMTs has shown 44 of 73 cases (60%) to aberrantly express ALK proteins [71]. Thus, while the histological appearance of IMT is not likely to be confused by an experienced pathologist, it is nonetheless important to remember that the expression of an ALK fusion protein in a malignant tumor does not invariably equate with a diagnosis of ALCL.

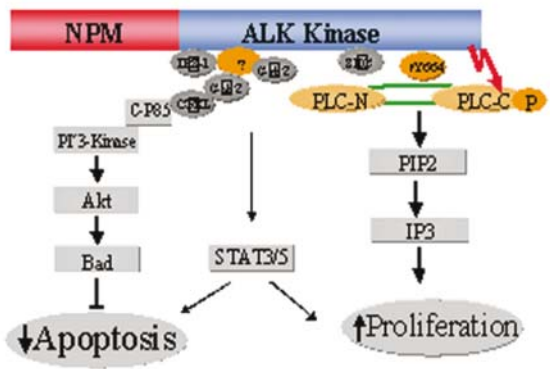


Figure 6.19

Schematic representation of the signaling cascades know to be activated by NPM-ALK and other ALK fusion proteins

All of the described histological variants of ALCL have been reported to express ALK fusions, but with varying frequencies. For example, the monomorphic subtype of ALCL is ALK-positive in greater than 80% of cases, while less than 15% of the so-called Hodgkin's-like ALCL cases express ALK fusions. Intriguingly, although they are histologically indistinguishable from ALK-positive ALCLs, the genetic abnormalities underlying the development of ALCLs that lack expression of ALK fusions (ALK-negative ALCLs) do not appear to involve deregulated kinase activity, and the mechanism of lymphomagenesis in these ALK-negative cases remains unknown at this time.

Approximately 30% of ALK-positive ALCL patients fail to respond favorably to conventional multiagent chemotherapy. ALK-specific targeted therapies including ATP-competitive small molecule inhibitors analogous to Gleevec/Glivec, which inhibits the BCR-ABL fusion kinase associated with chronic myeloid leukemia, are under development, and are likely to be beneficial in the future management of patients refractory to currently available treatments.

Table 6.6 Common antigen expression patterns of the precursor B- and T-lymphoblastic lymphomas

B cell										
-			CD19	CD20	CD22	CD24	CD10	CD34	Tdt	HLA-DR	SIg
B-Precursor ALL			+	–/+	–	+	+/–	+	+	+	–
Pre-B ALL			+	+/–	–/+	+	+	+	+/–	+	–
B-ALL			+	+	+	+	+/–	–	–	+	+
T cell											
-	CD1	CD2	CD3	CD4	CD5	CD7	CD8	CD10	CD34	Tdt	HLA-DR
T-ALL	+	+	+ (C)	+	+	+	+	+/–	–/+	+	–/+

SIg = surface immunoglobulin, C = usually cytoplasmic, + = positive, +/– = often positive, –/+ = occasionally positive, – = negative

Table 6.7 Genetic features of lymphoblastic neoplasms

Phenotype	Genetic alteration	Dysregulated gene
Precursor-T	t(1;14)(p32-34;q11)	TAL1
	t(1;7)(p32;q35)	TAL1
	TAL1 deletion	TAL1
	t(7;9)(q34;q32)	TAL2
	t(7;19)(q35;o13)	LYL1
	t(8;14)(q24;q11)	MYC
	t(10;14)(q24;q11)	HOX11
	t(7;10)(q34;q24)	HOX11
	t(11;14)(p15;q11)	RHOM1
	t(11;14)(p13;q11)	RHOM2
	t(7;11)(q34;p13)	RHOM2
-	t(10;11)(p13;q14)	AF10-CALM
	t(1;7)(p34;q34)	LCK
	t(7;9)(q34;p34)	TAN1
	del(9)(p21-22)	MST1/MST2
Precursor-B	t(1;19)(q23;p13)	PBX1-E2A
	21q addition	Unknown

6.8 Lymphoblastic Lymphoma

6.8.1 Pathology

Lymphoblastic lymphomas (LBLs) are part of the spectrum of the precursor blast-cell neoplasms seen in children. These neoplasms may present as disseminated bone marrow and blood disease (acute lymphoblastic leukemia or ALL) or as tissue masses (LBL). Definitions that have been used in the past to help make therapeuticdecisions require <25% bone marrow blasts as well as tissue masses for a process to be identified as a LBL [54, 272, 273, 289, 320]. However, in the current WHO Classification, recognition that this entity represents a spectrum of precursor lymphoid cell disease has led to the diagnostic category of precursor cell lymphoblastic leukemia/lymphoma [3]. Furthermore, many new therapeutic regimes treat LBL similarly to ALL. LBL represents about 30% of lymphomas in children but comprises <10% of NHL seen in adults [338]. As noted above, these immature lymphoid neoplasms frequently have both lymphomatous and leukemic components, making their designation as a lymphoma somewhat arbitrary. Whereas precursor B-cell disease usually presents as ALL, most lymphomatous presentations are of precursor T-cell derivation [3, 289]. Precursor T-cell LBL tends to present as mediastinal or upper torso nodal masses, while precursor B-cell LBL is more

likely to present in skin, soft tissue, bone, tonsil, or as a single peripheral lymph node [12, 48, 212, 337, 378].

Characteristically, LBL may show diffuse or partial effacement of nodal architecture. Particularly in precursor T-cell LBL, the neoplastic cells may infiltrate in an interfollicular zone with sparing of benign, reactive follicles [137, 289, 292]. LBLs are often rapidly dividing neoplasms with a high mitotic rate, and there may be numerous reactive macrophages present, creating a "starry sky" appearance similar to that seen in BL [289]. Cytologically, the neoplastic cells are indistinguishable from the blasts seen in ALL. The cells will have an immature blast-like appearance with fine ("dusty") chromatin and inconspicuous or absent nucleoli. The cytoplasm is scanty and ranges from pale to basophilic in color (see Fig. 6.20) [3, 144]. It should be noted that cytoplasmic vacuoles may occasionally be seen in precursor lymphoblastic lesions and are not specific for BL [289]. Particularly in precursor T-cell disease, the cells may have irregular nuclear contours with multiple infoldings. Precursor B-cell neoplasms tend to have more rounded and smooth nuclear contours. Some "large-cell variants" may be seen with more abundant cytoplasm and more prominent nucleoli (corresponding to the L2 morphologic features described in the French-American-British Classification) [3, 48, 289]. Cytochemical studies may show strong, focal cytoplasmic acid phosphatase reactivity. Terminal deoxynucleotidyl transferase (TdT) will also be positive [3]. Precursor T-LBL may rarely be associated with eosinophilia, myeloid hyperplasia, and increased incidence of a secondary myeloid malignancy (see Fig. 6.21). This particular subtype has been associated with

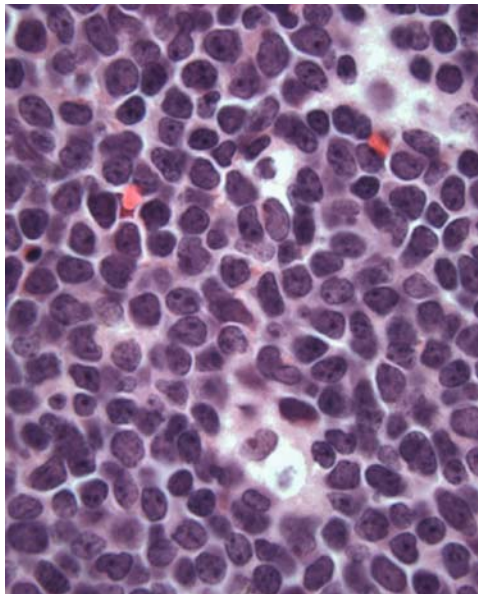


Figure 6.20

Precursor T-lymphoblastic lymphoma. The tumor demonstrates diffuse effacement of lymph node architecture by an infiltrate of blastic cells with minimal cytoplasm, inconspicuous nucleoli, and fine nuclear chromatin. The blasts show variable nuclear contours, and occasional tingible body macrophages are identified (×1000, H&E)

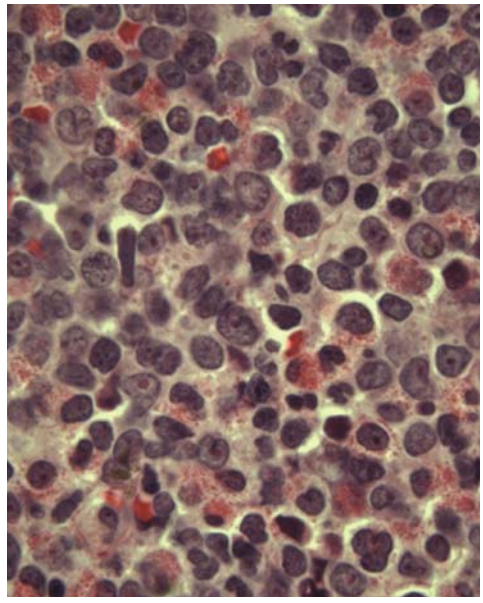


Figure 6.21

Precursor T-lymphoblastic lymphoma with eosinophilia. This represents an unusual variant of lymphoblastic lymphoma associated with a t(8;13)(p11.2;q11–22). Precursor T lymphoblasts are admixed with numerous eosinophils. The patient developed acute myelogenous leukemia 10 months after the diagnosis of T-lymphoblastic lymphoma (×1000, H&E)

specific chromosomal translocations that all involve chromosme 8p11, the most common being the t(8;13) [4, 171].

Precursor T-LBL will typically display a cortical thymocyte immunophenotype, usually reflecting middle to late stages of differentiation (see Table 6.6). Antigenic deletion is common; however, most neoplasms will express some combination of CD1a, CD2, CD5, and CD7 along with coexpression of CD4 and/or CD8. Occasionally, both CD4 and CD8 may be absent. Cell surface CD3 and T-cell receptor antigens are usually not expressed, but CD3 may be seen in the cytoplasm. CD10 is expressed in 15–40% of cases. Occasionally, natural killer (NK) cell antigens, such as CD57 or CD16, may be seen [72, 152, 337, 378]. Precursor B-LBL most often displays an early pre-B or pre-B phenotype (see Table 6.6), with expression of CD19, CD10, and TdT and variable expression of CD20, CD22, and HLA-DR. Usually, cell surface immunoglobulin is absent or weak, but cytoplasmic immunoglobulin may be detected [48, 72, 197, 212, 348, 363]. Precursor B-LBL will often express BCL2, helping to distinguish these cells from BL [364]. TdT will be seen in most cases of precursor B- or T-LBL and may be detected by flow cytometric, immunohistochemical, or cytochemical methods [42, 92, 368]. Demonstration of TdT is an extremely helpful finding in making a diagnosis of LBL [364, 378].

T-LBL and T-ALL are characterized by the many of the same underlying cytogenetic abnormalities, which occur with a frequency of 50–90% [378]. Recombinational events that lead to the diversity in antigen recognition are thought to leave *TCR* genes prone to recombination with oncogenes through chromosomal translocations. Chromosomal translocations are the most frequent cytogenetic abnormalities that occur in T-LBL (Table 6.7). These translocations typically juxtapose promoter and enhancer elements from *TCR* genes next to transcription factor genes such as *HOX11, TAL1,* and *LYL1*, which are normally transcriptionally silent in non-neoplastic T lymphocytes [115, 129, 155, 338]. The most common translocations in T-LBL recapitulate normal T-cell development and involve the *TCRαδ* (14q11), *TCRβ* (7q34), and *TCRγ* (7p15) genes, with the more immature cellular phenotypes favoring the *TCRγ* or *TCRδ* loci. Precursor T-LBLs will display early T-cell receptor gene rearrangements (*TCRδ, TCRγ, TCRα,* and/or *TCRβ*) on molecular studies, although some very immature cells may not demonstrate a rearrangement, and a small percentage have been shown to demonstrate *Ig* gene rearrangements (so-called "lineage infidelity") [295]. While the prognostic significance of these cytogenetic features is unknown in LBL, many of these same cytogenetic findings have not been shown to have prognostic significance in pediatric T-ALL [155].

6.8.2 Biology and Molecular Mechanisms

In contrast to most of the other major subtypes of NHL in which one or a few chromosomal rearrangements alter the function of a single gene locus (e. g., *cMYC* in Burkitt lymphomas and ALK in anaplastic large-cell lymphomas), a number of translocations altering various proto-oncogenes (*TAL1, TAL2, LMO1, LMO2, HOX11, HOX11L2, LYL1, cMYC, NOTCH1, LCK, FGFR1*) occur in T-LBL. A unifying feature of these translocations, with the exception of those involving *FGFR1*, is that they cause deregulated expression of the involved proto-oncogene by placing it near enhancer sequences present in the T-cell antigen receptor (*TCR*) gene loci, as noted above. With the exception of rare chromosomal rearrangements that involve LCK, a cytoplasmic tyrosine kinase involved in interleukin-2 receptor-mediated and TCR-mediated cell signaling in T lymphocytes [52, 148, 331, 332, 381, 385, 405, 415], NOTCH1 (formerly known as TAN1), a transmembrane protein encoded by a member of the *Notch* gene family of membrane-spanning receptor proteins that normally play a role in the control of cell fate decisions during differentiation in a variety of tissues from flies to man [16, 17, 19, 101, 147, 177, 284], and the FGFR1 receptor tyrosine kinase, all of the proteins encoded by genes altered by these rearrangements encode transcriptional regulatory proteins. Further, although NOTCH1 is a membrane-spanning protein, its function ultimately is also as a modulator of gene transcription (vide infra).

The *TAL1, TAL2, MYC,* and *LYL1* genes encode members of the basic helix-loop-helix (bHLH) class of transcription factors and are commonly involved in translocations in the lymphoblastic malignancies [31,

49, 64, 118, 245, 248, 261, 352, 407]. The bHLH domains mediate protein dimerization and sequence-specific binding to DNA of the promoter and enhancer regions of key target genes (most of which have yet to be identified) [68, 82, 183, 208, 277, 278] that normally control cellular differentiation or proliferation. By far the most commonly involved of these loci is *TAL1* (also known as SCL, for stem cell leukemia) [31, 64, 118]. Although few studies have examined lymphomas, involvement of this gene has been reported in up to 30% of T-cell acute lymphoblastic leukemias. In 5% of T-ALL, the *TAL1* locus on chromosome 1 is altered by a t(1;14)(p32-33;q11), whereas the remaining 25% of cases possess rearrangements of the locus that are cytogenetically cryptic and that result in an intragenic deletion of the 5' end of the gene, bringing *TAL1* under the control of the promoter of another chromosome 1 gene called *SIL* that is transcriptionally active[28, 36, 46]. This results in TAL1 ectopic expression in T-lymphoid cells (normal expression of the TAL1 protein is restricted to myeloid and erythroid progenitors, megakaryocytes, mast cells, embryonic brain, and endothelial cells) [31, 135, 170, 182, 211, 257, 307, 387, 388]. Study of *Tal1*-null embryonic stem cells indicates that the gene is essential for the development of all hematopoietic lineages, suggesting a role in very early blood cell development before lineage commitment [103, 105, 131, 252, 298, 299, 324, 326, 357]. The bHLH domain of TAL1 binds with the E-protein transcription factors that include the E2A bHLH transcription factor and related proteins (E12, E47, HEB and E2-2) [123, 261, 310, 378] to form sequence-specific DNA-binding heterocomplexes [164–166]. The exact mechanism underlying TAL1 overexpression in the development of T-cell malignancies is not fully clear, but it is hypothesized that the protein may act as a dominant-negative regulator of transcription, preventing the normal function of the E-proteins, which play key roles in developmental progression [260, 310]. Studies have shown that E-protein inactivation or inhibition can lead to T-cell leukemia/lymphoma development [24, 158], and transgenic mice engineered to express TAL1 develop T-cell malignancies [70, 189, 275]. Interestingly, TAL1-E2A heterodimers can physically interact in cells with the LMO1 and LMO2 proteins as well that are also activated in T-lymphoblastic malignancies by chromosomal translocations [383, 389]. "Double" transgenic mice that express both TAL1 and LMO1 or LMO2 in their T cells develop abnormalities of T-cell differentiation and tumors at a significantly faster rate than mice that express LMO1 or LMO2 only, indicating that TAL1 serves as a synergistic tumor promoter in this system [13, 65, 158, 205].

As noted, two other transcription factor genes altered by translocations found in T-lymphoblastic malignancies encode the cysteine-rich LIM proteins LMO1 and LMO2 (for LIM-only) [23, 311, 313, 409]. LMO1 and LMO2 (formerly known as rhombotin-1 or -2 (RBTN1, RBTN2) and TTG-1 or -2 (for T-cell translocation gene -1 or -2), respectively, each contain an aminoterminal transcriptional activation domain and two tandem LIM domains, the latter of which includes zinc finger-like regions [41, 244, 330, 335]. These LIM domains are structurally related to the DNA-binding zinc finger-like domains of other transcriptional proteins like the GATA factors [271, 279, 287, 291, 336], but there is no evidence that they bind to DNA. In addition, the absence of a homeobox DNA-binding domain (as is found in other LIM family members) suggests that these proteins modulate transcription through protein-protein interactions mediated via their LIM domains. Indeed, LMO1 and LMO2 have the ability to physically associate not only with the TAL1 bHLH protein, but also with the bHLH proteins TAL2 and LYL1 that are activated in rare (2%) cases of T-cell malignancy by chromosome rearrangements [248, 383, 389, 407]. In turn, like TAL1, TAL2 and LYL1 also interact with E2A [253], and all three proteins can bind the same DNA sequences. These observations suggest that the different E2A-TAL1, -TAL2, or -LYL1 heterodimers may functionally inactivate normal E2A activity and/or control the regulation of a common set of as yet unidentified target genes to contribute to T-cell transformation. LMO1 and LMO2 are also involved in this process. For example, LMO2 is found in complexes in T-cell lymphomas that include TAL1, E2A, GATA1, and the ubiquitous nuclear adaptor protein Ldb1/NLI/CLIM2, while LMO1 is known to bind Ldb1 as well [138, 382, 390]. TAL1, TAL2, LYL1, and the LMO proteins likely contribute to T-cell oncogenesis via other mechanisms as well. For example, TAL1, TAL2, and LYL1 can all physically interact

with a novel GTP-binding protein named DRG, which has been shown to stimulate the cotransforming activity of MYC and RAS in an experimental model [230]. Further, LYL1, but not TAL1 or TAL2, interacts with NF-κB1 and reduces its transcriptional activity, perhaps contributing to oncogenic transformation when deregulated by aberrant expression of LYL1 [116]. Interestingly, T-cell malignancies from some patients are known to contain both rearrangements of *LMO2* and *TAL1* gene activation as a result of promoter deletion [389], providing further evidence for the cooperation of these genes in tumor development. Although not as common as *TAL1* alterations, activation of *LMO2* is found in approximately 7% of patients with T-cell lymphoblastic malignancies as a result of the t(11;14)(p13;q11), whereas *LMO1* activation by the t(11;14)(p15;q11) is seen in less than 1% of patients [41, 64, 244, 330]. The oncogenic potential of LMO2 overexpression has been demonstrated recently by the unfortunate development of a monoclonal T-cell lymphoproliferative disorder due to inadvertent insertional mutagenesis of the *LMO2* gene locus following treatment of a patient with X-linked SCID using retrovirally mediated gene therapy [139].

Two additional examples of the group of transcription factors that are altered in T-cell lymphoid malignancies are the *HOX11* and *HOX11L2* homeobox genes, located on chromosomes 10q24 and 5q35, respectively [25, 37, 95, 115, 142, 150, 191, 220, 241]. Homeoproteins have been identified in a wide variety of organisms and appear to play an evolutionarily conserved role as determinants of development of the basic body plan and specific organs, regulating the expression of genes in a spatial, temporal, and tissue-specific fashion. Roughly 50 mammalian homeobox genes are present in a series of four clusters located on different chromosomes [184, 207, 418]. Like other homeoproteins, HOX11 localizes to the cell nucleus, binds to DNA in a sequence-specific manner, and transactivates the expression of specific target genes [87, 375]. *HOX11* is not normally expressed in adult tissues at levels detectable by routine Northern (RNA blot) hybridization analysis, although it can be detected in a variety of tissues (including normal T cells) by more sensitive RT-PCR-based methods [95, 150, 191, 410]. Knockout mice lacking the murine HOX11 homologue, designated Tlx-1 (for T-cell leukemia homeobox 1), are phenotypically normal other than the remarkable exception of being asplenic; thus, *HOX11* appears to be a "master gene" that serves to regulate the normal growth and proliferation of splenic tissue [325]. *HOX11* expression in T-cell lymphoblastic malignancies is found in 5–10% of patients, most often as a result of the t(10;14)(q24;q11) that involves the *TCR α/δ*-chain gene complex at 14q11 but rarely because of the variant translocation t(7;10)(q35;q24) that contains chromosome 7 breakpoints within the *TCR β*-chain gene locus [96, 188]. In both instances, the *HOX11* coding sequence is unaltered, but the gene becomes abundantly overexpressed in T cells. Although the exact role of HOX11 in malignant transformation remains to be elucidated, the high-level expression of the gene in T lymphocytes has been demonstrated to be lymphomagenic in transgenic mice [149]. A mean age of tumor onset of 7.5 months, together with the fact that only about one-third of animals develop malignancy, indicate the necessity for additional genetic changes to cooperate with HOX11 to generate a fully transformed phenotype.

Expression of the *HOX11L2* gene, located at human chromosome band 5q34-q35, is activated in T-cell malignancy by the frequently cryptic t(5;14)(q35;q32), which has been reported in 22% of children and adolescents with T-cell acute lymphocytic leukemia [37], and by the less frequent t(5;14)(q34;q11) that results in recombination of the *HOX11L2* region with the *TCR δ* gene locus [142]. Other studies have reported that *HOX11L2* expression may occur aberrantly in an even higher percentage of T-cell malignancies, suggesting it to be one of the most frequent abnormalities identified in childhood T-cell acute lymphoblastic leukemia to date, and have associated the abnormal expression of the gene with a poor clinical prognosis [25, 115, 241]. Interestingly, HOX11L2 and HOX11 are very similar, and microarray analysis (vide infra) of T-cell malignancies abnormally expressing either gene shows marked similarities in their gene expression profiles; however, while overexpression of HOX11 in T-cell ALL appears to denote a favorable prognosis, HOX11L2 overexpression has been associated with a poor outcome [115].

First described in 1992, the so-called 8p11 myeloproliferative syndrome (EMS)/stem cell leukemia-lymphoma syndrome (SCLL) is a rare chronic myeloproliferative disorder characterized by marked eosinophilia in 90% of patients, generalized lymphadenopathy, and frequent development of T-cell lymphoblastic lymphomas [171, 222, 223]. Of the roughly 30 cases reported to date in the literature, the median age at presentation was 32 years (pediatric cases occur, however, given that the age range is 3–84 years). The appearance of the peripheral blood in EMS/SCLL at presentation resembles chronic myeloid leukemia in some respects, with marked leuckocytosis present in nearly all patients and the major cell types being neutrophils, metamyelocytes, and myelocytes. Most patients rapidly progress to acute myeloid leukemia or, less commonly, B-lineage acute lymphocytic leukemia. The median time to transformation based on available case reports is probably only between 6 and 9 months. In spite of aggressive chemotherapy with either AML or NHL treatment protocols, most patients with EMS/SCLL die from resistant disease or early relapse within 1½ years of their diagnosis, and only aggressive chemotherapy followed by allogeneic transplantation has thus far appeared to offer the hope of cure in a few patients. Intriguingly, lymphoblastic lymphoma, which is uncommonly observed in other myeloproliferative disorders, is seen in more than two-thirds of EMS/SCLL cases, suggesting that the cell targeted for malignant transformation in the disorder is a lymphoid/myeloid bipotential precursor cell. The lymphoma presents at diagnosis in some cases, whereas it appears during the course of the disease in others.

Several chromosomal rearrangements, all involving chromosomal band 8p11, occur in EMS/SCLL cases, with t(8;13)(p11;q12) being the most common. Characterization of the t(8;13) in 1998 showed it to produce a fusion of roughly the N-terminal two-thirds of a novel protein encoded on 13q12 named ZNF198 that contains zinc-finger-like motifs with the entire kinase catalytic domain of the fibroblast growth factor receptor-1 (FGFR1) [297, 323, 360, 408]. Several of the other translocations that occur in EMS/SCLL have also been molecularly cloned [223]; all involve the *FGFR1* gene located at 8p11 and form similar fusions, but with N-terminal partners other than ZNF198. All of the described N-terminal partners of FGFR1 contain putative dimerization domains important for causing the constitutive, unregulated activation of the catalytic function of the kinase, producing an unrelenting growth signal to cells. Such a mechanism of NHL causation is highly reminiscient of the involvement of the ALK receptor tyrosine kinase by its truncation and the generation of various ALK fusions resulting from chromosomal rearrangements in the anaplastic large-cell lymphomas. The positive results recently observed for the ATP-competitive small molecule inhibitor STI-571/imatinib (Gleevec/Glivec, Novartis Pharmaceuticals) for the treatment of chronic myeloid leukemias [88], in which an identical mechanism of oncogenesis is operative (truncation and constitutive activation of the ABL tyrosine kinase due to its fusion with the N-terminus of BCR as a result of the t(9;22) chromosomal rearrangement) holds promise that analogous small molecule inhibitors of the FGFR1 may also be effective for the treatment of EMS/SCLL in the future [63].

The mammalian NOTCH1 protein was initially identified due to its involvement in a t(7;9) chromosomal rearrangement observed in rare cases of human T-cell acute lymphoblastic leukemia/lymphoma [101]. Abnormal, truncated forms of NOTCH1 similar to those encoded by the rearranged *NOTCH1* allele generated by the t(7;9) were shown to uniquely produce T-ALL when expressed in bone marrow progenitor cells in mice [284]. In addition, the expression of activated forms of NOTCH1 in bone marrow progenitor cells can drive thymus-independent T-cell development and is thus sufficient for the induction of the T-cell development program [304]. Subsequent studies revealed NOTCH1 to be normally expressed in T-cell progenitors and essential for the formation, maturation, and function of these cells [99, 315, 316]. Conditional deletion of Notch1 in mice prior to T-cell commitment abolishes T-cell development and causes the appearance of increased number of intrathymic B cells, indicating that the protein serves as a switch for T- versus B-cell lymphoid cell formation [231, 316]. For a number of years, the extent to which NOTCH1 abnormalities contributed to T-ALL was unclear other than in those instances associated with the t(7;9); as described below, recent findings implicate NOTCH1 de-

regulation in the great majority of T-cell acute lymphoblastic leukemia/lymphoma cases [283, 398].

The mammalian NOTCH receptor family, which includes four receptors (NOTCH1-4), are large membrane-spanning proteins that consist (moving from the most N- to C-terminal motifs) of repeated EGF-like motifs, LIN12/NOTCH repeats (LNR), two regions designated the HD-N and HD-C heterodimerization domains (HD), a single transmembrane (TM) segment, a RAM domain, a region of seven reiterated ankyrin repeats, a transcriptional activation domain (TAD), and a PEST protein degradation motif (see Fig. 6.22 for a schematic representation of NOTCH1). Following their translation and during their transit to the cell surface, these receptors are cleaved by a furin-like protease at a site just external to the TM segment, producing the mature heterodimeric NOTCH receptors, which are comprised of noncovalently associated extracellular (N^{EC}) and transmembrane (N^{TM}) subunits associated via their HD-N and HD-C domains. The activation of normal NOTCH signaling depends upon ligand-induced proteolysis of the N^{TM} portion of the receptor; binding of the DSL (Delta, Serrate, and Lag1) NOTCH ligands to the EGF-like region of the receptors leads to cleavage of the N^{TM} at a site internal to the TM segment, releasing the cytoplasmic domain of the N^{TM} that is known as intracellular NOTCH (ICN) and permitting its translocation to the nucleus. Once in the nucleus, ICN associates with a DNA-binding transcription factor that bears homology to transcriptional regulators of the REL family and is known by several names including CBF1, Suppressor of Hairless (Su[H]), Lag-1 (CSL) or RBP-Jkappa. Nuclear ICN binds also to Mastermind-like 1-3, which are large scaffold proteins that recruit coactivators with histone acetyltransferase (HAT) activity, such as p300 and pCAF. The resulting multiprotein complex is responsible for upregulation of the expression of NOTCH target genes. Negative modulation of this NOTCH target gene upregulation occurs in part due to the short half-life of ICN proteins, which are targeted for destruction by F-box proteins of the SEL-10 family through a mechanism that involves the C-terminal PEST sequences common to all four mammalian NOTCH family members.

The protease responsible for ligand-induced NOTCH receptor activation and release of the ICN for translocation to the nucleus consists of a multiprotein complex commonly known as γ-secretase, which contains presenilin-1 or -2, nicastrin, APH-1, and PEN-2 [81, 174]. Interestingly, γ-secretase has generated substantial interest as a target for small-molecule inhibitor development by the pharmaceutical industry due to its ability to catalyze the production of amyloidogenic peptide fragments from β-amyloid precursor protein, a process thought to play an essential role in the genesis of Alzheimer's disease. Unfortunately, the side-effects of γ-secretase inhibitors, including disturbances of lymphocyte development (caused by their inhibition of NOTCH activation) and impaired gut epithelial cell differentiation, have diminished their promise for the long-term chronic therapy required for Alzheimer's dementia [403].

The rare t(7;9) in T-cell acute lymphoblastic leukemia/lymphoma results in the expression of a series of truncated mRNAs that encode N-terminally-truncated, constitutively active NOTCH1 intracellular peptides. Presumbly because the t(7;9)-associated NOTCH1 peptides can access the nucleus independent of the ligand-mediated proteolysis required for normal NOTCH activation that results in ICN formation, γ-secretase inhibitors do not inhibit the growth of t(7;9)-positive T-leukemia/lymphoma cell lines. To better determine the importance of NOTCH1 signaling in T-cell malignancies independent of the t(7;9), Weng and colleagues recently used a functional screen to determine the effects of small-molecule inhibition of γ-secretase, and showed that five t(7;9)-negative T-cell lymphoblastic leukemia/lymphoma cell lines underwent G0/G1 cell cycle arrest in response to inhibitor treatment [398]. Sequence analysis of the *NOTCH1* genes in these cell lines showed frequent mutations, often occurring in cis within single *NOTCH1* alleles, and involving two regions of the locus: (1) those sequences encoding a 50-amino acid segment of the HD-N domain of the N^{EC}, as well as less frequent mutations of the sequences that code for the HD-C of the N^{TM}, and (2) the sequences that encode the C-terminal NOTCH degradation box (PEST) (Fig. 6.21). Mutational analysis of human pediatric as well as adult T-ALL samples showed approximately 55% of cases to

have at least one mutation in the HD or C-terminal PEST portions of NOTCH1, with roughly 20% bearing mutations in both sites. Importantly, NOTCH1 mutations were identified together with all major molecular subtypes of T-acute lymphoblastic lymphoma/leukemia [115], including cases associated with the aberrant expression of *TAL1, HOX11, HOX11L2,* or *LYL1*. The fact that *NOTCH1* mutations occur in T-ALL subtypes corresponding to pro-T (*LYL1*), early cortical (*HOX11*), and late cortical (*TAL1*) stages of normal T-cell development suggests that *NOTCH1* mutations take place in very immature T-lineage cells or even uncommitted pluripotent marrow progenitors and are early events that are permissive and/or cooperative for the acquisition of other genetic aberrations.

The mutations of NOTCH1 found in T-cell acute lymphoblastic leukemia/lymphoma result in increased function, presumably both by enhancing ICN production by destabilizing N^{EC} and N^{TM} association through their respective HD regions and predisposing to N^{TM} proteolytic cleavage and by increasing the stability of the ICN protein due to interference with degradation mediated via the PEST motif. Assays performed with *NOTCH1* cDNAs containing HD and PEST mutations in *cis* confirmed a synergistic ability of the two mutations to activate transcription from a CSL-dependent reporter gene [398]. Although it seems clear that NOTCH1 mutations play a central role in the development of a large percentage of T-cell acute lymphoblastic leukemia/lymphomas, many questions remain as to the exact mechanisms by which this occurs. For example, the critical transcriptional gene targets of NOTCH1 for tumor cell proliferation and survival are still largely unknown. Likewise, it is presently unknown whether NOTCH family members other than NOTCH1 could potentially be mutated, or if other components of the NOTCH signaling pathway might be altered, in some T-cell acute lymphoblastic leukemia/lymphoma cases to contribute to their genesis. Despite these uncertainties, preclinical data [399] support the exciting possibility that γ-secretase inhibitors that were initially developed for the therapy of Alzheimer's disease (for example, see [403]) could be an effective treatment for T-cell neoplasms caused by NOTCH deregulation; clinical trials using these drugs in patients with refractory T-cell acute lymphocytic leukemia/lymphoma are reported to be in the planning stages at the time of the preparation of this text [283].

Cytogenetic and molecular abnormalities in B-LBL are substantially less well characterized than T-LBL. Classical chromosomal translocations that occur in B-precursor ALL, such as hyperdiploidy, t(12;21), t(1;19) and t(9;22), appear to occur less frequently in B-precursor LBL [152, 232]. Reported cytogenetic abnormalities in B-precursor LBL include additional material from the 21q locus [232]. Precursor B-LBLs will usually show monoclonal immunoglobulin gene rearrangements and will lack evidence of somatic hypermutation [161].

The advent of microarray technology has facilitated the identification of new molecular markers in pediatric malignancies and a better understanding of the underlying pathophysiological processes. Array studies have identified underlying genetic pathways that account for the known biological heterogeneity of T-cell hematopoietic disease and uncovered previously unrecognized molecular subtypes. As traditional clinical and cytogenetic features of T-ALL have not been shown to be of prognostic significance, this holds great promise for the identification of poor- and favorable-risk subsets of patients and the design of novel, tailored treatment approaches in the future.

In a recently published microarray study, high levels of oncogene expression were apparent in the majority of T-ALL cases, and frequently occurred even in the absence of activating translocations[115]. Several molecular subtypes of T-ALL were identified, with distinct gene expression profiles that were associated with *HOX11, TAL1,* and *LYL1* activation. These studies also led to the identification of clinical subtypes of T-ALL. For example, the probability of survival at 5 years was 92 ±8% for patients with samples showing aberrant *HOX11* expression, but only 43 ±19% and 33 ±19%, respectively, for the *TAL1*- and *LYL1*- expressing groups [115]. Of further interest, oncogene activation was linked to specific stages of thymocyte development. T-ALL samples characterized by expression of *HOX11* tended to arise from earlier cortical thymocytes, whereas those cases associated with *TAL1* expression arose from more mature, late cortical thymocytes. In examining the genes differentially expressed

in the *HOX11* cluster, most were associated with cell proliferation. Moreover, few antiapoptotic genes were overexpressed in the *HOX11* cluster compared to the *TAL1* and *LYL1* groups, where inferior outcomes were observed. This may explain in part the greater chemosensitivity observed in the *HOX11*-positive group of patients.

Several important clinical applications of such array analyses are likely to emerge. These studies provide an opportunity to better understand the downstream pathways activated by transcription factors in T-cell leukemia/lymphoma, and common pathways may be targets for therapy in the future. Another important clinical extension of this work is the identification of new prognostic markers in T-lymphoblastic disease. As this technology is applied to larger groups of uniformly treated patients within the context of prospective clinical trials, markers that predict response to treatment are anticipated that will help to shape future risk group assignment.

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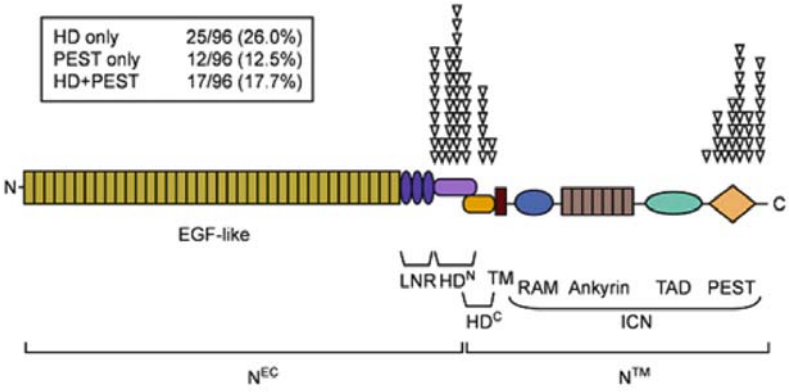

Figure 6.22

Schematic of the NOTCH1 protein and distribution of mutations in human T-cell acute lymphoblastic lymphoma/leukemia. The *upward-pointing arrow* indicates the site at which the full-length NOTCH1 protein is normally cleaved to yield the NOTCH1 extracellular (N^{EC}) and transmembrane (N^{TM}) subunits. The *triangles* indicate the sites at which mutations have been identified in T-cell acute lymphoblastic lymphomas/leukemias; in 96 pediatric T-cell acute lymphoblastic lymphoma/leukemia cases characterized, subsets of patients with mutations only in the HD motifs, solely in the PEST domain, or in both segments of NOTCH1 were identified (note percentages of each subset). EGF-like=EGF-like repeats; LNR=LIN12/NOTCH repeats; HD^N and HD^C=N- and C-terminal heterodimerization domains; TM=transmembrane domain; RAM=RAM domain; ankyrin=ankyrin repeat domain; TAD=transcriptional activation domain; PEST=PEST domain. (Modified and published with permission [398])

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B-Cell Lymphoma/Burkitt Lymphoma

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Contents

7.1 Introduction

B-cell lymphomas constitute an important set of highly curable neoplasms. Although they occur at all ages, their incidence and therefore relative frequency of individual subtypes vary markedly with age and with environmental factors and lifestyle. Approximately half of all non-Hodgkin lymphomas (NHLs) occurring in children and adolescents in North America and Europe are either Burkitt lymphoma (BL) or Burkitt-like lymphomas (BLL). BLL is called atypical Burkitt lymphoma (aBL) if there is a myc-immunoglobulin translocation. Some pathologists, particularly those who follow the German (Kiel) classification, do not recognize BLL, which sits at the histological and biological interface between BL and diffuse large B-cell lymphomas (DLBCL) and classify all such neoplasms as either BL or DLBCL. DLBCL accounts for approximately 10–12% of NHLs. Follicular lymphomas and marginal zone lymphomas of MALT origin have been described in the childhood age group [1, 2], but their rarity has precluded extensive study of either their biology or treatment. BL, originally identified in equatorial Africa, has provided important insights into the epidemiology, pathogenesis, and treatment that have informed and influenced studies of other lymphomas, and even other cancers. It is remarkable that many of the key observations in each of these areas were made in Africa, or on tumor material derived from African patients, although their importance and more general applicability were not always immediately recognized. For this reason, more emphasis than is usually the case will be given in this chapter to the "African lymphoma", as it was first known. Sadly, the majority of children with BL in Africa today receive no or inadequate therapy while approximately 90% of children in affluent nations are cured of their disease.

7.2 Diagnosis and Imaging

B-cell lymphomas are primarily diagnosed on the basis of the histology and immunohistochemistry of tissue obtained at biopsy. Because the common B-cell lymphomas of childhood, particularly BL, can progress extremely rapidly, the presence of symptoms or signs consistent with a B-cell lymphoma should lead to a prompt attempt to establish a tissue diagnosis, either immediately or after a very few weeks, depending upon clinical circumstances, i.e., the likelihood that the signs or symptoms may be caused by a benign condition. Excisional, incisional, or cutting needle biopsy of a mass or lymph node, or aspiration and cytological examination of bone marrow or serous fluid, all provide enough information to make a diagnosis, but consideration should always be given to the provision of unfixed tissue for cytogenetic or molecular studies – either immediately or later, if histology and immunochemistry do not provide enough information, or approved research studies are to be performed. In low socioeconomic circumstances and when the tumor is readily accessible, the diagnosis is often made by fine needle aspiration biopsy. In equatorial Africa, where BL often accounts for half of all childhood cancers and some 80% of all NHLs, and where the diagnosis is often supported by a characteristic clinical syndrome, the accuracy of such an approach is high [3]. With the addition of simple immunophenotyping, e. g., for surface immunoglobulin, to confirm a B-cell immunophenotype, the specificity of the technique would probably be increased further. Cytogenetics (including FISH) or molecular techniques can both refine the diagnosis, but also reveal added heterogeneity, particularly in BLL and DLBCL. BL is relatively homogeneous with respect to both its histology and biological characteristics (including immunophenotype and the presence of a myc-immunoglobulin translocation), but even this disease can be divided into endemic (occurring at relatively high incidence in equatorial Africa and New Guinea and almost always EBV-associated), sporadic (occurring at low incidence outside equatorial Africa), and HIV/AIDS-associated [4].

7.2.1 Burkitt Lymphoma

The term sporadic BL was first applied in the context of histologically defined BL in Europe and the USA, and was expanded to all tumors outside equatorial Africa. Whether this is reasonable is somewhat questionable, since there are no characteristics that are able to provide an absolute distinction between endemic and sporadic tumors, and by some criteria (e. g., EBV as-

sociation) BL in many world regions more closely resembles endemic BL than sporadic. In most developing countries, for example, 50–100% of the tumors contain EBV DNA [5–7], and African-type jaw tumors or other characteristics more often associated with endemic BL have been described in various world regions, including other African regions, Amazonian Brazil, and Turkey [8–13], although these patterns appear to be changing with time. This suggests that both clinical characteristics and EBV association may be environmentally determined, although a role for host factors cannot be excluded. BL in affluent countries is much less frequently associated with EBV (10–20%) [14], and although occasional African-type jaw tumors have been reported from such countries, particularly in the 1960s and 1970s, they are now vanishingly rare. Even HIV-associated BL, the third subcategory defined in the WHO classification, are EBV-associated in only 30–40% of cases [15], although there is limited information in this regard in children [16]. The importance of the environment in pathogenesis is also suggested by differences in the patterns of breakpoint locations on the chromosomes associated with the characteristic myc-immunoglobulin translocations which have been described in tumors from different world regions [6, 17]. These observations suggest that there may be differences in the cell of origin in different world regions. Support for this hypothesis has been recently provided by the observation that there are differences in the pattern of immunoglobulin gene mutations in EBV-positive versus EBV-negative BL [18]. Although all of these subtypes of BL generally carry myc-immunoglobulin translocations, these are characteristic, but not pathognomonic, of BL: such translocations are not confined to histologically defined BL and aBLL, but are also found in a small fraction of DLBCL [19, 20]. Further, occasional, otherwise typical cases (especially in AIDS-associated cases) of BL may lack such a translocation [16]. Thus, a more fundamental definition of BL might be the inappropriate expression of MYC in a cell which phenotypically closely resembles a germinal center cell (whose proliferation utilizes an atypical program which does not involve Myc expression [21]) and which has an appropriate histology.

7.2.2 Diffuse Large B-cell Lymphoma

DLBCL is an even more heterogeneous category – both with respect to molecular markers and, to a degree, immunophenotype. Although a reproducible histological distinction between BL and DLBCL (particularly in the context of agreement among pathologists) is rather poor, there can be no doubt that these two categories represent separate sets of pathological entities, albeit the dividing line between them is imprecise (histologically, immunophenotypically, as well as with respect to molecular abnormalities). In large series, significant differences in median age among children diagnosed as BL vs DLBCL have been described [22], and in spite of the overlap, there are clear differences in the patterns of molecular abnormalities and in gene expression profiles, although once again there is limited information in this regard in childhood DLBCL [20, 23]. In addition to the markedly different frequencies of possession of a myc-immunoglobulin translocation, BL essentially never expresses BCL-2, whereas DLBCL quite frequently expresses this antiapoptotic protein [23]. There may also be differences in the patterns of molecular characteristics in childhood and adult DLBCL. Translocations involving BCL-6 are common in adult DLBCL, but uncommon in children with DLBCL, which suggests that DLBCL in children represents a specific subset of DLBCL [24]. Gene expression profiling of adult tumors has distinguished two or three major categories of DLBCL which relate most closely to normal germinal center cells or activated B cells in terms of their expression patterns. Most childhood DLBCL belong to the germinal center cell subtype [25]. DLBCL in both adults and children, as defined by the WHO classification scheme, also includes clinically and pathologically distinct entities, the two most frequently encountered being primary mediastinal large B-cell lymphoma (PMBL) [26, 27] and T-cell-rich B-cell lymphoma (TCRBL) [28, 29]. PMBL is more common in the adolescent age group and has a somewhat different immunophenotype characterized by the expression of CD45, CD20, and CD79a, with the lack of expression of histocompatability antigens of both class I and II and of surface immunoglobulins [26]. Most characteristic is the diffusely positive CD30 (although less bright than in Hodgkin's disease), and recent gene

expression studies confirm that there is a significant overlap in the molecular profile of PMBL with Hodgkin lymphoma (HL); approximately one-third of the genes expressed in PMBL are also expressed in HL [27]. TCRBL is a rare tumor characterized by the presence of rather few tumor cells in a reactive infiltrate which closely resembles that observed in nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) [28, 29]. Indeed, TCRBL may be preceded by NLPHL, and it is highly probable that at least a fraction of TCRBLs are closely related to NLPHL.

7.2.3 Burkitt-like Lymphoma

BLL is particularly difficult to characterize precisely, since it lies at the interface of BL and DLBCL, and it seems probable that it includes both atypical BL as well as atypical DLBCL – and possibly, a unique entity that deserves a separate diagnosis. BLL may or may not contain myc-immunoglobulin translocations, but the likelihood of their presence is greater in younger patients. This could simply reflect the fact that BL is the predominant tumor of children and adolescents. Some BLLs contain 14;18 translocations, typical of follicular lymphoma (and also found in a fraction of DLBCL of germinal center cell origin), and some may even contain both myc-immunoglobulin and 14;18 translocations, suggesting that one source of histological atypia in BL is its development in a preexisting but often subclinical follicular lymphoma. Transformations from clinically apparent follicular lymphoma (and also marginal zone lymphoma) to BL or to BLL (defined both histologically and cytogenetically) have been described, although exclusively in adults, consistent with the rarity of these more indolent lymphomas in children and, perhaps, their different biological characteristics (e. g., they are more often localized [2]). The possibility that some BLLs develop as the result of the transformation of a subclinical indolent tumor cannot be excluded. In one study, BCL6 was expressed much more frequently in childhood BLL than in BL [30], but BCL2 expression was also frequently observed in BL in this study, while in another study in adults, BCL6 was not observed in BLL [31]. These discrepant results could relate to the age of the patient but may also simply reflect the problem of a reproducible histological diagnosis among different pathologists. In the WHO classification, BLL is defined on the basis of a similarly high proliferative index to BL, i.e., essentially all of the cells (99% with good technique) stain with monoclonal antibodies that bind to the cell Ki-67 surface antigen, such as Ki-67 itself and MIB-1. BLL, then, is a heterogeneous entity that lies between BL, generally characterized by the expression of CD10 and BCL-6 and the possession of a myc-immunoglobulin translocation but no BCL2 or BCL6 translocation, and DLBCL, which quite frequently carries one of the latter translocations but only infrequently a myc-immunoglobulin translocation [32].

7.2.4 HIV-Associated B-cell Lymphomas

All of the mature B-cell lymphomas of children may develop in patients with HIV infection, and the distribution among the three main categories seems to favor BL/BLL over DLBCL, perhaps by a factor of 2 or 3:1 [16]. In contrast, DLBCL is more common in adults (both in HIV-infected and uninfected patients). There is some evidence that the association of these histological entities with specific chromosomal translocations differs in children and adults with HIV infection [16]. Rarely, MALT-associated lymphomas occur in HIV-infected children (they are extremely uncommon in nonimmunosuppressed patients) [16, 33, 34]. Isolated (primary) central nervous system (CNS) lymphoma is more common in HIV-infected patients, but most reports in children are of individual cases. CNS lymphomas in children with AIDS are almost always EBV-associated [35].

While it is apparent that there are considerable difficulties in defining the borderlands of BL, BLL, and DLBCL without recourse to sophisticated studies, this is not necessary for the purposes of treatment. Indeed, the lack of reproducibility provides one reason for using a single approach to all B-cell lymphomas in childhood. This is discussed further below.

7.2.5 Imaging Studies

Imaging studies in children with B-cell lymphoma are performed in order to determine the extent of disease and, in some cases, to identify the cause of emergent or

potentially emergent complications arising from the presence of the tumor. A range of imaging studies will confirm or demonstrate the presence of head and neck, chest, abdominal, or less often, extremity tumors, ultrasound being the most inexpensive and readily available, and CT showing more anatomical detail. In deciding which studies to perform at the time of presentation, there are two primary considerations. The first is to evaluate emergency or potential emergency situations, such as tracheal compression or renal failure, caused by mass effects or the metabolic consequences of the presence of a large tumor burden. The second is to determine the extent of disease and, in conjunction with other criteria, to assign the patient to a particular arm of the treatment protocol being followed. These two considerations may lead to some differences in the set of imaging studies to be carried out in individual patients, although they will often overlap. Imaging studies are also performed during and, if necessary, after treatment, to assess the response to therapy and to detect or to more precisely define progressive disease. Accurate assessment of response requires that the sites of disease at diagnosis are documented. In the majority of cases, the diagnosis will have been established prior to the performance of imaging studies, and decisions regarding imaging studies can thus be based both on the history and physical examination and the most likely sites of involvement. In some patients, the clinical presentation may have already led to particular imaging studies being performed prior to a biopsy, e. g., a chest X-ray and CT scan in a patient presenting with symptoms suggesting a mediastinal mass, or abdominal imaging studies in patients presenting with symptoms referable to the abdomen or with a palpable abdominal mass. The precise imaging studies chosen will also vary in different health care settings. In countries with limited resources, for example, ultrasound may be used more, and CT scans less. In a clinical research setting, a basic set of imaging studies are listed in the protocol document, and every effort is made to study all patients in the same way.

Since B-cell lymphomas frequently involve the abdomen, the single most valuable imaging study is probably a CT scan of the abdomen. This may provide more information about a known abdominal mass, allowing changes that result from therapy to be readily assessed, or it may demonstrate the presence of tumor in the absence of symptoms and/or signs. An abdominal CT scan should normally be performed immediately prior to therapy even if laparotomy has been performed, e. g., in a patient with suspected or proven intussusception, since tumor can, on occasion, recur very rapidly, and a patient believed to have been rendered free of disease at the time of laparotomy a week or two earlier may have developed a new abdominal mass, with implications for optimal therapy. An abdominal CT scan may be supplemented (or sometimes replaced) by ultrasound where this may add value, or where more frequent assessment of a particular mass during therapy is deemed appropriate (repeated ultrasound exams are less expensive and convenient). Renal ultrasound may readily demonstrate renal outflow tract obstruction in patients with a low urine flow and may be used to follow progress.

A chest CT scan is indicated in children with DLBCL, but a plain chest X-ray will suffice to exclude or identify significant disease in the chest in a patient with BL. A chest CT scan is also important to determine the presence of incipient or overt tracheal or superior vena cava compression or lung/pericardial invasion in a patient with a mediastinal mass. Information of this kind may be used to determine the risk of heavy sedation or anaesthesia, which may be required for additional procedures (see Chapter 9). In some cases, echocardiography may be indicated, e. g., to measure cardiac function in the presence of a pericardial effusion.

The presence of head and neck disease (suggested by symptoms or signs) may determine the need for head and neck imaging, which is not normally performed in all patients. MRI is generally the most useful imaging procedure in this circumstance, since it is also the best modality to examine the nervous system, orbit, and soft tissues. A baseline MRI of the brain in the absence of clinical indications or a research study is generally not useful.

In addition to obtaining more information about known sites of disease, or examining sites with a high likelihood of involvement, whole-body screening may be considered in order to detect sites of disease that would otherwise remain unrecognized, but which may influence the treatment to be used. The value of whole-body MRI using fast spin-echo (FSE) short inversion

time inversion recovery (STIR) sequences has recently been reported [36]. This may be a more sensitive screening test than conventional imaging in the identification of all sites of disease, including bone and bone marrow involvement at presentation. Since bone marrow disease may at times be patchy, this approach could help to refine the detection of bone marrow involvement, although large clinical trials would be required to evaluate the impact of this on treatment results.

A second approach to whole-body screening is the use of nuclear medicine scanning, which has the additional advantage that it also provides some functional information. Most aggressive B-cell lymphomas avidly take up gallium-67, part of which is transported intracellularly via transferrin receptors, which are present at high levels on the surface of rapidly proliferating cells. Some investigators find whole body ^{67}Ga scintigraphy of value, both in identifying otherwise unrecognized sites of disease, and also in patient follow-up: residual masses detected by CT scan are unlikely to consist of viable tumor in the absence of ^{67}Ga uptake [37]. Gallium-67 is also taken up by bony sites of disease, although it is probably a less sensitive test in this regard than technetium-99m bone scanning, even in BL and BLL, and for technical reasons, it is usually necessary to choose between a gallium scan and ^{99m}Tc methylene diphosphonate bone scan. The value of ^{67}Ga scintigraphy at any site is increased by the use of single photon emission tomography (SPECT) and may be further enhanced by the use of combined (hybrid) SPECT/CT [38]. ^{18}Fluorodeoxyglucose (FDP) positron emission tomography (PET) is probably more sensitive than ^{67}Ga SPECT, but there is limited comparative information on the use of these various imaging techniques in B-cell lymphomas in children [39]. PET is becoming particularly popular in the evaluation of the viability of a residual tumor mass after therapy and in this regard is probably superior to ^{67}Ga, although it is presently less widely available [40, 41]. The value of other imaging techniques, e. g., the use of radiolabelled monoclonal antibodies such as rituximab, directed against CD20, for the detection either of disease sites at presentation or of persistent or recurrent disease are still in an exploratory phase [42].

While increasingly sophisticated imaging techniques are able to detect small amounts of disease at presentation and residual disease after therapy with a reasonably high degree of certainty, such tests may have relatively little impact on EFS rates; surgical resection of small volume disease, serum LDH, and the presence of CNS disease and/or bone marrow disease, coupled to the detection of extensive thoracic or abdominal disease (which is likely to be confirmed by physical examination as well as relatively simple imaging studies) are the most important parameters for risk assignment. Similarly, most patients do not relapse, while the early detection of recurrent disease, which in any event has a poor prognosis, is of unproven value. Thus, interval follow-up imaging studies are also likely to have little impact on the outcome. The detection of a slow response to therapy early enough to be able to switch patients to a higher risk arm (if available) could, however, improve the outcome for the small fraction of patients in the lower risk categories destined to do poorly.

7.3 Clinical Features

B-cell NHLs in children are predominantly extranodal tumors. Since an absolute distinction among the B-cell lymphomas in children and adolescents cannot be made on the basis of sites of disease alone, the clinical features are dealt with here together, mention being made when particular sites are more or less frequently observed in the various entities included under this heading. The most common presentation is with intra-abdominal disease; peripheral lymph node involvement is relatively uncommon, although more likely to be present in DLBCL than in BL. In equatorial Africa, however, as high a fraction as 60% of children with BL (maximal incidence is in 3–4 year olds, with a gradual reduction in the likelihood with age) will have a characteristic jaw tumor at presentation with loosening or even premature eruption of teeth, particularly the molar teeth around which (and within) the tumor readily grows. An overt mass is generally present, and there is frequently involvement of multiple jaw segments. There may be associated orbital involvement, or the latter may occur as an apparently separate lesion, sometimes causing compression of the cranial nerves that pass into the orbit (III, IV, VI). Ophthalmoplegia

may be present even in the absence of an overt orbital or intracranial (e. g., parasellar) mass. Equatorial African patients also have a remarkably high frequency (as high as 15%) of presentation with paraplegia caused by an extradural mass. In the USA and Europe, such a presentation is exceedingly uncommon. Extradural masses may cause paraplegia either by cord compression alone or by occlusion of the spinal arteries or both. Infarction of the cord leads to permanent paraplegia, but many patients can recover partially or fully if the cord compression is treated early enough.

An uncommon but well-known presentation of patients with limited BL is intussusception of the small bowel, which is best diagnosed by ultrasound examination [43]. Emergency laparotomy with intestinal resection is normally indicated. Abdominal masses may also present with local symptoms, or compression of adjacent structures, including the biliary tract, ureters, gastrointestinal tract (although relatively rarely giving rise to intestinal obstruction, perforation, or gastrointestinal bleeding), inferior vena cava, or sacroiliac plexus. Ascites is not infrequently observed in BL (paracentesis will reveal tumor cells). Occasionally, presentation with appendicitis may occur, due to involvement of the appendix, although more often there is a right iliac fossa mass – the terminal ileum and ascending colon are particularly frequent sites of intra-abdominal disease [44, 45]. Regional nodes in the mesentary are frequently involved, although many enlarged nodes removed at the time of surgery may be free of tumor even at a microscopic level. Ovarian involvement is common in girls.

Involvement of the chest is much less common in BL and BLL, and mediastinal involvement, although described, is sufficiently rare that the diagnosis should be seriously questioned [46]. In PMBL, a mediastinal mass is the presenting feature but a high fraction of patients with this disease have one or more additional extranodal sites of disease, the most frequently involved sites being the lungs, kidneys, liver, adrenals, ovaries, pancreas, and bone. All of the B-cell lymphomas may present with involvement of the pleura and consequent serous effusion, but lung parenchymal involvement is uncommon except in PMBL. Involvement of the heart muscle is exceptionally rare but has been described [47]; pericardial involvement is also uncommon, but cardiac involvement may be more common in immunocompetent patients [48], as are other unusual sites of involvement.

Outside equatorial Africa, head and neck involvement may include pharyngeal tumor, occasionally presenting as unilateral hypertrophy of a tonsil, nasal sinuses, lymph nodes, salivary glands, and thyroid. Jaw tumors in sporadic BL, which differ clinically from those observed in Africa since they seem to have no predilection for the developing molar teeth, are frequently associated with generalized bone marrow involvement (rarely the case in endemic BL) and often mulitple sites of bony disease [49]. Parasellar or other extradural intracranial or paraspinal masses are occasionally seen, and any of the cranial nerves may be involved, even in the absence of overt masses in the head and neck. A characteristic finding is numbness of the chin, presumably due to involvement of the mental nerve. Generalized lymphadenopathy is uncommon in BL, but more common in DLBCL and intermediate in BLL. It may occasionally be associated with extensive bone marrow involvement and hepatosplenomegaly in what most pediatric oncologists refer to as acute B-cell leukemia. While bone marrow involvement has implications for stage and requires intensive treatment, the patient does not have a different disease and ideally should not be given a different name. If the need is felt to refer separately to these patients, then a better term might be Burkitt leukemia. Bone marrow and CNS involvement are very rare in children with DLBCL.

Other sites occasionally involved at the time of presentation include the meninges (manifested as cerebrospinal fluid pleocytosis), testis, breast, skin, uterus, gall bladder, bone, and brain, although intracerebral disease is particularly uncommon in the absence of HIV infection or chronic refractory involvement of the craniospinal meninges. Primary presentation with CNS disease has been described in occasional case reports [50, 51]. Breast involvement is most frequently observed in pubertal, pregnant, or lactating women [52–54].

7.4 Staging

Staging classifications provide a means of describing succinctly the extent of disease, and of estimating prognosis. In the B-cell lymphomas, the primary presentation, unlike for example the majority of epithelial neoplasms, is not confined to a particular organ, nor does it have (with the exception of HL) a characteristic pattern of invasion of adjacent structures. Thus, staging systems in lymphoid neoplasms reflect the tumor volume more than the degree of spread from the primary site. Indeed, B-cell lymphomas frequently involve multiple organs and, given the historic pattern of recurrence outside the radiation field prior to the use of systemic chemotherapy, are assumed to be widespread from the outset. This is consistent with the distribution of lymphoid tissue throughout the body and the potential of lymphoid cells to survive in a broad range of organs and tissues. Except in patients with a single site of disease, defining the "primary" tumor location may be very difficult. The most important subdivision of tumor burden is "limited" versus "extensive". Various devices have been used to further subdivide these categories and to define them somewhat more precisely. When the disease is limited, the number of sites can be assessed, and staging systems subdivide patients with single or multiple sites into stage I and stage II, respectively. However, patients occasionally present with a single, very large, extra-abdominal, extrathoracic mass and a correspondingly worse prognosis (depending upon the treatment given) than most patients in stage I or II. Such patients require more intensive therapy. Patients with completely resected intra-abdominal BL, which is nearly always of small volume, have long been known to have an excellent prognosis, probably better than most stage I patients, and require the least intensive therapy, although in the most commonly used staging system (see below), they are categorized as stage II.

When the disease is obviously extensive, the dividing line has generally been the presence of bone marrow involvement which generally signifies the most widespread category of disease, although different degrees of bone marrow invasion may signify large differences in the tumor burden. CNS involvement has also long been considered to indicate a particularly poor prognosis, perhaps initially stemming from the lack of effective CNS therapy, but in the USA at least, also because it is most often associated with extensive disease elsewhere, particularly in the bone marrow. Thus, CNS involvement results in assignment of the patient to the highest stage and risk group.

Staging systems for lymphoma have been influenced by HL. Since this disease has a rather predictable pattern of spread and is also sensitive to radiation, the first successful treatment approach was radiotherapy – hence the primary importance of *location* of disease sites in the evolution of staging systems for HL. HL predominantly involves lymph nodes, and spread to extranodal tissues has an impact on the prognosis. NHLs in children and young adults are primarily extranodal, and thus the underlying premises on which HL staging (Ann Arbor) is based do not apply. In HL, for example, disease both above and below the diaphragm signifies widespread disease. Although this parameter was incorporated into several staging systems for childhood NHL, its significance in NHL is questionable, particularly since in the most commonly used staging system (see below), all primary intrathoracic and extensive intra-abdominal tumors are assigned to stage III anyway, and only resected intra-abdominal tumors are generally considered not extensive.

A number of staging systems have been developed specifically for NHL in children, either for BL in Africa or for childhood NHL in general. These are quite similar, the African classification differing particularly with respect to limited disease, which in Africa is usually facial (jaw or orbital tumor). In the staging system for BL (Table 7.1), therefore, limited disease was divided into a single facial tumor versus multiple facial tumors [55, 56]. Stage III, in the systems used in the 1960s and 1970s, included patients with abdominal BL, paraspinal or bone tumor (outside the face) and stage IV, patients with CNS or bone marrow involvement. Extradural tumor causing paraplegia was sufficiently commonly encountered in Uganda to warrant mention in this system, and patients presenting with extradural tumor were assigned to stage III because although they frequently had a single small tumor mass, their prognosis, which would otherwise have been excellent, was marred by their high risk of developing CNS relapse (cranial nerve palsies or CSF pleocytosis).

It was subsequently shown that therapy of CNS relapse could be effective, and a revised scheme was developed for BL, based on a survival analysis in which CNS disease was assigned to one of two stages, according to the extent of disease elsewhere [57]. It was subsequently shown that patients with completely resected intra-abdominal tumor, even if relatively extensive prior to resection (e. g., bilateral ovarian disease, which was usually quite easily resected), had an excellent prognosis and, accordingly, such patients were assigned a separate stage (AR). This system is still used, in slightly modified form, in some centers in equatorial Africa.

Subsequently, staging systems similar to the earlier systems used for BL in Africa were developed in the USA for all childhood NHL, the best known being those of Wollner [58] and St Jude (Table 7.2) [59]. Murphy, who described the latter staging system which continues to be used and has become almost universally accepted, pointed out a number of its shortcomings, one being that it encompasses all pediatric NHL although the pattern of organ and tissue involvement differs in each lymphoma subtype. Stage III includes patients with primary intrathoracic (most often associated with lymphoblastic lymphoma, less often PBML) or extensive intra-abdominal tumor (most often a B-cell lymphoma), disease on both sides of the diaphragm (based on the Ann Arbor system for HL), as well as all patients with primary extradural tumor, as in the earlier African system, although this presentation is remarkably rare outside equatorial Africa. The word "extensive" is not defined and may well have had a different connotation in the pre-CT and nuclear medicine era. In the case of intra-abdominal disease, it presumably refers to patients who would not be included in stage II by virtue of resection of a primary gastrointestinal tumor. Particularly problematic – and, again, pointed out by Murphy – is the arbitrary determination that patients with NHL cannot have more than 25% bone marrow involvement (if so, the diagnosis of "leukemia" would be merited). This creates an artificial subdivision between lymphoblastic lymphoma and acute lymphoblastic leukemia, and also led most pediatric oncologists to refer to BL (or BLL) with bone marrow involvement exceeding 25% as acute B-cell leukemia – a term which is not included in the WHO classification scheme – since such patients are not included in the staging system. As they do, in fact, have B-cell lymphoma, according to the WHO classification system, it may be appropriate to allow all patients with bone marrow disease to be classified as stage IV. The German Berlin-Frankfurt-Munster group (BFM) now includes multifocal bone involvement as a criterion for inclusion in stage IV. Such patients frequently also have disseminated bone marrow disease and presumably always have at least patchy marrow involvement [60]. The BFM group has also shown that in patients

Table 7.1 Clinical staging of Burkitt's Lymphoma used initially by Ziegler in Uganda [56]

I	Single facial tumor
II	Two or more separate facial tumor masses
III	Intrathoracic, intra-abdominal, paraspinal, or osseous tumor (excluding facial bones)
IV	CNS (malignant cells in the CSF) or BM involvement

Table 7.2 The St Jude staging system for childhood non-Hodgkin lymphoma

I	A single tumor (extranodal) or single anatomic area (nodal), excluding mediastinum or abdomen
II	A single tumor (extranodal) with regional node involvement On same side of diaphragm: a) Two or more nodal areas b) Two single (extranodal) tumors with or without regional node involvement A primary gastrointestinal tract tumor (usually ileocecal) with or without associated mesenteric node involvement, grossly completely resected
III	On both sides of the diaphragm: a) Two single tumors (extranodal) b) Two or more nodal areas All primary intrathoracic tumors (mediastinal, pleural, thymic) All extensive primary intra-abdominal disease; unresectable All primary paraspinal or epidural tumors regardless of other sites
IV	Any of the above with initial CNS or bone marrow involvement (<25%)

Table 7.3 Staging studies for B-cell lymphomas

Physical examination[a]	
Complete blood count[a]	
Laboratory tests	Serum electrolytes
	Liver and renal serum tests
	Serum LDH
	Serum uric acid
Imaging studies	Chest X-ray[a]
	Chest CT scan (if chest X-ray film abnormal or suspiciously abnormal)
	Thoracic ultrasound (e. g., for following thoracic tumor)[b]
	Abdominal ultrasound examination (include liver/spleen, kidneys, abdomen, pelvis)[b,d]
	Abdominal CT scan[c]
	Gallium^{67} scan[b]
	MRI of head and neck region/brain[b]
	Whole body MRI
	FDP-glucose PET
Bone marrow examination[a]	
CSF examination (cytology)[a]	

Useful or sometimes indicated investigations

- Serum lactate (especially in the presence of a large tumor)
- Bone scan (for more precise documentation of bony lesions)
- Endoscopy (e. g. for GI bleeding)

CSF=cerebrospinal fluid; LDH=lactate dehydrogenase

[a] Obligatory

[b] Often performed in affluent countries

[c] Routinely performed by most institutions in affluent countries

[d] May be the better study to perform in children with little intra-abdominal fat and can be useful in differentiating between bowel and tumor

classified as stage III, serum LDH is a valuable parameter for defining patients with a markedly different prognosis: the poor prognosis of stage III patients with a serum LDH above 500 IU/L was much improved in successive studies when their treatment was intensified, primarily through a 10-fold increase in the dose of methotrexate (see below) [60]. More recently, an additional LDH parameter (above or below 1000UI/L) has been used by the BFM group to further subdivide risk groups (see below). One could argue that the word "extensive" has been replaced by an objective, if surrogate marker of tumor burden.

7.4.1 Staging Procedures

The primary purpose of staging studies today is to determine how intensive treatment should be. Accurate classification of the clinical characteristics, particularly those potentially able to influence the outcome is, of course, critical to clinical research in order to assess the comparability of different series with respect to disease extent and hence effectiveness of therapy. A complete history and physical examination constitutes the first element of any staging workup. This may reveal potential sites of disease and thus influence the set of investigations that are performed, and information from the examination may be used in determining the disease stage (which is not dependent upon the method used to determine disease sites and tumor size). A minimum set of studies would include complete blood count, renal and liver function tests, bone marrow, and CSF examination. Imaging studies will normally include a CT scan of the abdomen and a chest X-ray, with a chest CT scan being performed only if indicated. If CT scanning is not possible (e. g., in Africa), ultrasound examination may be substituted, but where there is an obvious, palpable, intra-abdominal tumor, relatively little additional information pertinent to staging is actually obtained by either a CT or ultrasound examination. Ultrasonography or CT scanning may reveal the presence of outflow tract obstruction. Imaging of the head and neck region is normally undertaken only if indicated by symptoms or signs suggesting the presence of disease in this region. Whole-body imaging, e. g., by MRI or nuclear medicine scanning, either ^{67}Ga scintigraphy or ^{18}fluorodeoxyglucose (FDG) PET may be used to screen for unrecognized sites of disease.

All abnormal staging studies are normally repeated at suitable intervals (usually, for the first time, after a single cycle of therapy) to assess the response to treatment. A summary of studies to be considered for assigning stage is provided in Table 7.3. Only a few studies are designated as obligatory, and it should be born in mind that stage may be differently assigned according to which studies are performed (e. g., obligatory studies only vs abdominal CT scan vs a whole-body screen). In most affluent countries, multiple studies, including radiology, ultrasound, and nuclear medical scans are performed. Serum LDH may be required for risk assignment but is, technically, not a part of staging.

7.5 Treatment

The foundations of the modern treatment of BL, and therefore of B-cell lymphomas in children, were largely derived from the early studies of pioneer therapists in equatorial Africa, aided by colleagues from the USA and Europe. Since radiation therapy was not available and patients were rarely amenable to complete surgical resection, there was a strong incentive to examine the value of chemotherapy. Although early studies were not conducted with the rigor of modern clinical trials, the dramatic response to chemotherapy was immediately apparent, since essentially all patients treated prior to the chemotherapy era died (rare exceptions included spontaneous remissions). In Uganda, where Burkitt himself worked, as well as in Kenya and Nigeria, the remarkable observation was made in the 1960s that a fraction of BL can be cured with a single drug administered once or a very few times [61–63]. Investigators in Africa strongly believed that an antitumor immune response was a critical element in patients who were cured and studiously avoided lengthy treatment durations (i.e., more than one or two doses of drug) which they feared would have deleterious effects on the outcome as a result of immunosuppression. Although this was based on rather limited information and, in retrospect, was probably incorrect, prolonged therapy (beyond a few months) has not been shown to be advantageous and is, therefore, undesirable. The optimal therapy duration may depend upon the extent of disease and is likely to vary from one patient to another, but in the absence of a means to individualize therapy, decisions can only be made for defined patient groups. Patients with no measurable tumor burden after surgery are routinely treated with 2 or 3 cycles of simple therapy (usually a combination of 4 or 5 drugs), while those with more extensive disease receive more intensive therapy (higher drug doses and additional drugs) for several months. Although the purpose of risk adaptation of therapy is to minimize toxicity to patients, it is clear that many patients receive more therapy than they need in the absence of better predictors of treatment outcome. This is unavoidable if high survival rates are to be achieved. Fortunately, with good supportive care, the toxicity of even the most intensive regimens is manageable – the percentage of toxic deaths is very small (and decreasing) – and long-term effects also appear to be acceptably low, although further follow-up is needed.

7.5.1 Early Studies in Africa

In Burkitt's series of 90 patients with jaw tumors treated at Mulago Hospital, Uganda [61], patients received either one or two doses of cyclophosphamide (CTX 30–40 mg per kg i.v., or the same dose orally over 3–4 days), methotrexate (MTX 1 mg per kg daily for 4–5 days), or vincristine (VCR 0.07–0.15 mg per kg i.v.). Seventy-four patients (82%) had complete (CR) or partial responses (PR). Those with small tumors were much more likely (10 of 10) than those with large tumors (10 of 40) to achieve a CR, suggesting a relationship between response and tumor size, which was subsequently confirmed [55–60, 64, 65]. Burkitt also noted that recurrent disease did not occur after 11 months of remission; patients in remission after this time could, for all practical purposes, be considered cured. This has also been amply confirmed in BL at all ages and in all countries; rare late relapsing patients, particularly adults, could be variants of DLBCL. Longer follow-up in Uganda indicated that approximately 20% of patients could be expected to achieve long duration remissions after one or two doses of CTX, VCR, or MTX [55, 64]. Burkitt also observed a high frequency – approximately 50% – of CNS relapse in patients who had previously achieved CR.

Ngu at the University Colleage Hospital in Ibadan, Nigeria [62], and Clifford and colleagues at the Kenyatta Hospital in Nairobi [63, 64] also reported dramatic responses and long-term survivors after treatment with similar doses of CTX or, in Clifford's hands, melphalan, sometimes with orthomelphalan in addition. Ngu attracted attention to the fact that serum uric acid levels were often raised in patients with extensive tumors, and sometimes became even more elevated following therapy. He described a patient who died from what would now be called acute tumor lysis (serum uric acid on the day of death was 54 mg per 100 ml) and reported that this and other complications, such as perforation of the bowel and, in one unusual case, of the arch of the aorta, may ensue from rapid dissolution of the tumor following therapy – probably a consequence of the very high growth fraction of tumor cells.

In the late 1960s and early 1970s, a collaboration between the Uganda Cancer Institute of Makerere University in Kampala and the National Cancer Institute (NCI) of the USA led to several other important observations [66, 67].

7.5.2 Non-Cross-Resistant Drugs and Combination Therapy

In the first clinical trial conducted at the Lymphoma Treatment Center (LTC) of the Uganda Cancer Institute, patients who were in CR 2 weeks after a single dose of CTX at 40 mg/kg were randomized to either no more therapy or five more doses of CTX. Intrathecal (i.t.) therapy was given only to patients with malignant cells in the CSF, either at presentation or relapse. Stage III patients who received a single dose of CTX (11) tended to develop relapse in the same site as the original tumor – all except one within 2 to 8 weeks of randomization. However, such patients responded to further CTX, seven of seven achieving a second CR, indicating that a single dose of CTX represented inadequate therapy for the majority of stage III patients. Patients who received multiple doses of CTX relapsed at approximately the same frequency, but only after several doses of CTX and usually while still receiving the drug [68]. Ten of these CTX-resistant patients were treated with a combination of VCR and MTX followed by Ara-C, a regimen known as BIKE regimen, and nine achieved a CR, eight remaining free of disease for 30–102 weeks (i.e., most were probably cured). These data indicated that CTX and the drugs included in the BIKE regimen are not cross-resistant. The next logical step was to explore the treatment of BL from the outset with multiple drugs.

In a second clinical trial conducted at the LTC, patients with stage III or IV disease were randomized at the time of presentation to receive either six doses of cyclophosphamide (40 mg/kg every 2–3 weeks) or two cycles of TRIKE, in which patients received CTX followed by VCR and MTX, followed by Ara-C, each of the three components being spaced at approximately 2-week intervals for a total of two cycles [68, 69]. Although the overall relapse rate between the two arms of the study were similar, patients receiving CTX alone invariably recurred at the same location (with or without CNS disease), as in the previous study, while those receiving TRIKE regimen most often recurred with isolated CNS relapse (ten of 16 relapses). Such patients usually achieved CR after IT therapy with continuation of systemic TRIKE. These data suggested that TRIKE was an effective regimen for controlling systemic disease and potentially superior to CTX. However, since numbers were small and patients who relapsed on CTX alone or those with isolated CNS recurrence could be salvaged by intrathecal therapy [68–70], there was initially a trend rather than a statistically significant advantage to TRIKE in terms of overall survival. Subsequent analysis with additional follow-up suggested that TRIKE was, in fact, superior to CTX alone [66].

In the mid-1970s, it remained unclear whether sequential drug combinations were superior to CTX alone. To examine this question, a randomized study was initiated in which two cycles of a simultaneous drug combination, COM (a combination of CTX, VCR, and MTX repeated after 2 weeks), were studied in comparison to CTX alone (40 mg/kg repeated after 2 weeks). Since an effective means of preventing CNS disease had still not been identified, no intrathecal therapy was given unless CNS disease was present (at presentation or relapse). However, patients who achieved remission were randomized to receive no further therapy or craniospinal irradiation, 20–24 Gy, in 30 fractions over 2 weeks, to explore its efficacy in the prevention of CNS disease. In the event, craniospi-

Table 7.4 Treatment outcome in 185 previously untreated patients managed at the Lymphoma Treatment Center in the late 1960s and early 1970s

Treatment	Number of patients	Limited disease (stages I, II)	Extensive disease (stages III, IV)	Long-term survival	Follow-up
All patients treated initially with CTX alone	163	44 (27%)	119 (73%)	80 (51%)	5–73 months[a]
Toxic death	15 (9%)	0	15	0	
Death, cause unknown	10 (6%)	2	8	0	
PR	17 (10%)[b]	4	13	0	
CR	121 (74%)	38 (86%)	83 (70 %)	80 (66%)	
Outcome in CR patients					
No relapse (1–7 doses)	42 (26%)	22	20	42	12–71 months
Relapse - more CTX[c]	20 (12%)	7	13	20 (12%)	10–72 months[d]
Systemic	*13*	*5*	*8*	*13*	
CNS and systemic	*1*	*0*	*1*	*1*	
CNS only	*6*	*2*	*4*	*6*	
Relapse - BIKE	47 (29%)	5	42	17 (36%)	20–73 months
Systemic	*22*	*3*	*19*	*7*	
CNS and systemic	*7*	*1*	*6*	*1*	
CNS only	*18*	*1*	*17*	*9*	
Relapse - other treatment	2	0	2	1	5 months
Relapse - death/no treatment	10 (8%)	4	6	0	
Patients treated initially with TRIKE[e]	22	0	22	16 (72%)	7–37 months[f]
Not evaluable					
Died prior to therapy	7				
Lost to follow-up	4				
All patients	185	44	141	96 (51%)	5–73 months

a Only four patients were followed for less than a year such that additional relapses are unlikely
b Four patients had CR after MTX/VCR, but all subsequently died
c These patients are "selected" by the fact that they responded to CTX alone (e. g., they had received a single dose, or relapsed late or in a sanctuary site). The data should not be interpreted as indicating that treatment of relapse with CTX is highly effective, but rather that 20 out of 121 patients who achieved complete response relapsed, and achieved prolonged remission after one or more relapses (two patients had two relapses, one had three)
d Follow-up is measured from the date of the last relapse. In all except one patient, it was >12 months
e 20 patients achieved CR, and 16 patients relapsed, 10 with isolated CNS disease
f Two patients were followed for less than a year (7 and 10 months) from the last relapse
Staging used here was that in use at the Uganda Cancer Institute, see Table 7.1

nal irradiation failed to prevent CNS recurrence [71], which led to an observation similar to that made with TRIKE – the proportion of patients who relapsed in each arm was similar over the follow-up period, but seven of the eight recurrences among patients treated with CTX alone involved both systemic and CNS disease, while eight of the ten recurrences in the COM arm were confined to the CNS [72].

7.5.3 Therapy Directed Towards CNS Disease

Various regimens of intrathecal chemotherapy given only in the first cycle had not been shown to be able to prevent CNS relapse [70], yet a review in 1972 of all patients at the LTC who had had CNS disease, either at presentation or at relapse, revealed that 23 of 42 (55%) were long-term survivors [73]. Although most patients had limited disease in addition to CNS involvement (e. g., a jaw tumor or isolated CNS relapse), this finding contrasted dramatically with the perspective from the USA at that time, where CNS disease was believed to be an obstacle to cure [74]. Most patients with CNS disease in the USA, however, tended to have extensive disease elsewhere. Ugandan patients with simultaneous CNS and systemic relapse also had a worse outcome [66], suggesting that the poor prognosis of patients with CNS disease in the USA was largely due to the associated extensive systemic disease [75]. This conclusion is supported by the fact that more effective systemic therapy subsequently led to greatly improved event-free survival (EFS) in patients with CNS disease in both the USA and Europe. The inability to prevent CNS disease in Uganda seemed at odds with the results of treatment of overt CNS disease, but the likely explanation for the apparent discrepancy is that patients with overt CNS disease generally received multiple cycles of CNS-directed therapy, suggesting that the failure of prophylactic intrathecal therapy was probably due to the fact that it was only given in the first treatment cycle. It was also observed that patients who had previously received intrathecal MTX and subsequently relapsed in the CSF had a high probability of achieving a complete response to intrathecal Ara-C, suggesting non-cross-resistance with intrathecal MTX and arguing for the inclusion of both drugs in treatment and prophylactic regimens (which subsequently became standard practice).

Approximately two decades of treating BL in Africa indicated that 40–50% of all patients with BL and over 80% of patients presenting with a single extra-abdominal site of disease achieved long-term survival with rather simple therapy, even without the benefit of CNS prophylaxis [66, 67]. More than one-third of survivors had been treated with CTX alone, and the remainder had received additional drugs, including MTX, VCR, and Ara-C, either as part of primary therapy or for treatment of relapse. Some of this information is summarized in Table 7.4, which is based on my recent review of data from the Uganda series. No further progress could be made in Uganda because of political strife, and although variants of COM have been used widely in sub-Saharan Africa since that time, its efficacy when combined with an effective CNS prophylactic regimen has not been documented in Africa because of inadequate follow-up.

7.5.4 Early Studies in the USA

The Ugandan data, although based on small numbers of patients, strongly supported the use of drug combinations from the outset. The COM regimen was, therefore, adopted at the NCI, USA, in an attempt to improve upon earlier results obtained in the treatment of BL at the NCI with CTX alone [76], which had resulted in some long-term survivors. The NCI studies were open to both children and adults, the oldest patient being 38 years. A slightly different COM regimen to that used in Uganda was developed, in which three cycles of therapy were given. This included intrathecal methotrexate in all therapy cycles. After 15 patients had been treated, high doses of prednisone were added, and a double dose of CTX was substituted for COM in the third course. Patients with intra-abdominal disease, even if completely resected, received total abdominal radiation to a total dose of 2100 rads over 18 elapsed days. Overall survival was similar to that observed in Africa [77], but now that combination regimens were used from the outset, relapse was rarely associated with long-term survival, although transient responses to treatment were observed. Cure of relapse, however, was documented in a small number of patients treated with autologous bone marrow infusion after very high-dose CTX, BCNU, Ara-C, and thio-

guanine (BACT) – the first demonstration of the potential value of such an approach [78]. This led some oncologists, particularly in the context of adult patients, to use high-dose therapy and stem cell rescue (HD-SCR) in patients who achieved a CR after treatment with low intensity regimens. Whether or not this approach would have significantly improved survival rates remains unknown, since the results of HD-SCR are generally reported in terms of the selected subset of patients who actually received HD-SCR [79]. Even these selected results, however, appear to be inferior to those obtained with the most effective high-intensity treatment protocols used in recent years.

7.5.5 Comparison with Leukemia Treatment

In the 1970s, the Children's Cancer Study Group (CCSG) undertook a study which proved to be particularly influential in determining the direction of treatment of non-Hodgkin lymphomas in children [80, 81]. A modified COMP regimen (four drugs) was compared to another regimen developed at Sloan Kettering Memorial Cancer Center, LSA_2L_2 (10 drugs), from a regimen used for the treatment of acute lymphoblastic leukemia that had been reported to give excellent results (for the era) in all non-Hodgkin lymphomas. All patients continued therapy for a total of 18 months, and in both study arms radiation was also given to sites of bulk disease in all patients (both long duration therapy and radiation are no longer recommended). The main question of this study was whether histology (patients were divided into lymphoblastic lymphoma vs non-lymphoblastic lymphoma) was an important determinant of the outcome for each of the regimens used. In early stage disease, this was not the case (84% EFS, at 5 years), but in patients with advanced disease, there was a clear advantage to the LSA_2L_2 regimen in lymphoblastic lymphoma, and a similarly clear advantage to patients with other types of non-Hodgkin lymphoma with the COMP regimen [81]. When the latter results were further broken down, the advantage occurred particularly in patients with undifferentiated lymphoma (Burkitt's and non-Burkitt's), i.e., essentially what would now be diagnosed as BL or BLL. Disseminated undifferentiated lymphoma patients had a 50% survival rate with COMP at 5 years, and 29% when treated with LSA_2L_2. No advantage of either regimen was observed in large-cell lymphoma, although there was a trend towards the better survival of patients treated with COMP (52% vs 43% at 5 years) [81]. In retrospect, this result must be interpreted in the knowledge that both patients with anaplastic large-cell lymphoma and DLBCL were included in this group. In Germany, the Berlin-Frankfurt-Münster (BFM) group obtained similarly poor results in patients with extensive B-cell lymphoma with a treatment protocol designed for acute lymphoblastic leukemia [82].

Other treatment protocols conducted in the era of the late 1970s to early 1980s gave similar results [82–87]. While excellent results were obtained in patients with limited disease, and even extensive disease, patients with bone marrow and CNS involvement continued to have poor treatment outcomes. Because of the large differences among patients according to disease extent, comparison of these series should be made with care, and ideally by stage rather than by comparison of reported overall EFS or survival rates. Even subsets of patients with stage III disease, which encompasses a broad range of tumor burdens, may have had different outcomes in different protocols. Results reported in the late 1980s to the turn of the millennium, however, demonstrated clear increments in survival in both the USA and Europe [60, 86–96]. The elements of these latest treatment protocols, which continue to be refined, are discussed below.

7.5.6 Critical Components of Modern Treatment Protocols

7.5.6.1 Basic Drugs

There can be no doubt from the early literature that an effective backbone of therapy for B-cell lymphomas in children (developed largely for historical reasons, but certainly including the most active drugs among those studied in the pioneering days of the treatment of BL in Africa) includes CTX, MTX, and VCR. Used in combination at the dose ranges originally tested in Africa, and in concert with CNS prophylaxis, these drugs alone are capable of curing a significant fraction of children with BL and probably BLL and DLBCL as well. Results in stage I and II patients overall are likely

to be above 80% EFS and in patients with small volume, completely resected disease, probably over 90%, although patients with stage III and particularly stage IV disease (and patients with bone marrow involvement greater than 25%) have a poor prognosis in the absence of additional therapy elements. This means that if the best results are to be obtained, few patients can be treated with the least intensive regimens.

7.5.6.2 High-Dose MTX

High-dose MTX was reported in 1976 by Djerassi and Kim as able to induce durable remission in children with recurrent lymphoma [97]. It was subsequently incorporated into almost all regimens used for the treatment of advanced childhood BL. Earlier protocols in which high-dose MTX doses of up to 3 g/m^2 were used in addition to the standard drugs still achieved relatively poor results in patients with stage IV disease (bone marrow and/or CNS involvement), and subsequent protocols employed doses of between 5 and 8 g/m^2 with considerable improvement in the outcome in these patients. These protocols, however, also incorporated additional drugs, such that the importance of the higher doses of MTX, at least in patients with the highest tumor burdens, was difficult to discern. Some light is shed on this issue by the results of two successive BFM studies. In the first, stage III patients were treated with 0.5 g MTX and had a relatively poor prognosis: 43% EFS at 6 years. In a subsequent study, the dose of MTX was elevated to 5 g/m^2 (some minor additional treatment changes were made), and the EFS rate at 6 years improved to 81% [60]. In a subsequent randomized study, the BFM group examined the importance of the duration of the 5 g/m^2 MTX infusion (24 h vs 4 h). A worse outcome was observed for high-risk patients who received 4 h infusions, although low-risk patients had an excellent outcome when treated with both lower doses (1 g/m^2) and a shorter infusion period (4 h) of MTX [98]. While the association of serum MTX levels with outcome has not been rigorously explored, a survey of the results of various protocols suggests that the best results are achieved in high-risk patients when MTX doses of at least 5 g/m^2 are given. The optimal duration may depend on the total dose given and the timing of the initiation of leucovorin rescue, and both optimal dose and infusion duration may also be a function of the overall protocol design. The Society Français d'Oncology Pediatrique (SFOP), for example, achieves excellent results with an infusion duration of 4 h in the highest risk group (C), but the total dose is 8 g/m^2 [95], and there are many other differences between the SFOP and BFM protocols.

High doses of MTX may not be required for patients with limited disease treated according to BFM regimens, but even patients with completely resected disease receive ifosfamide, Ara-C, and etoposide (ETOP) in addition to the basic drugs in BFM95, while in the SFOP and NCI protocols, this same patient subset receives only basic drugs, but MTX is given in high doses (3 and 6.7 g/m^2, respectively). In the Pediatric Oncology Group (POG) and the later CCSG, MTX was not administered to patients with limited disease, and although results were very good, EFS was approximately 10% less with the POG protocols, while the CCG "Orange" protocol included additional drugs [60, 95, 98–101]. Excellent results, however, were achieved with standard COMP therapy in patients with limited stage disease in CCG501 [100]. Since all of these regimens are well tolerated, over-treatment of some patients may be a small price to pay to ensure close to 100% EFS in this patient group.

7.5.6.3 The Role of Additional Drugs

Anthracyclines were not available at the time of the first studies in Africa and were not included in the earlier chemotherapy protocols. There are few if any data regarding the efficacy of single-agent anthracyclines, but doxorubicin (DOX) was incorporated into BL regimens once it became available. Although only a single randomized study with the objective of examining the role of anthracycline (daunorubicin) has been performed, with no benefit being shown [102], it is of interest that the reported results for COMP as used by CCSG in patients with limited non-lymphoblastic lymphoma (overall survival 91% and 98% in two successive protocols) are comparable to, perhaps better than, those achieved by POG (87% at 4 years), in which an anthracycline was used in place of MTX. In addition, the HiC-COM protocol reported by Schwenn et al. did not include anthracycline and gave similar re-

sults to protocols of the same era that included DOX, even in patients with extensive disease [89]. In view of the late cardiac toxicity encountered with anthracyclines, even at relatively low cumulative doses [103], and the added acute toxicity [102], the use of DOX in modern combination drug regimens in children with B-cell lymphomas, particularly those with early stage disease, may be worthy of further exploration.

Similarly, corticosteroids were not used in African patients, and although they are included in most combination regimens, there is no clear evidence of their value. In fact, one of the successful high intensity protocols does not include a corticosteroid [92, 93]. Although the toxicity of corticosteroids is not high, they may add to the likelihood of the development of opportunistic infections during chemotherapy and are associated with late effects.

The use of effective drugs in addition to CTX, VCR, and MTX (±DOX and corticosteroid), however, does appear to be important, at least in patients with extensive disease. Studies in France and the NCI demonstrated responses to IFOS with or without ETOP and high-dose Ara-C in patients with recurrent disease after regimens that included CTX, VCR, MTX, and DOX [104–106]. While it is difficult to assess the value of any one of these drugs in particular combinations, it is likely that the achievement of high EFS rates in patients with extensive disease requires the use of regimens that contain sufficiently high-dose MTX, high-dose Ara-C (both of which address both systemic and CNS disease), and one or two more effective drugs in addition to CTX and VCR, all at adequate dose levels and dose densities. A randomized study conducted by the POG, albeit in an era of less intensive chemotherapy, in which combined high-dose MTX and high-dose Ara-C were superior to CTX and high-dose MTX, VCR, and prednisone, supports this conclusion [107].

7.5.6.4 Dose Density

In Uganda as well as in the USA, relapse was occasionally observed prior to commencement of the next cycle of therapy [68, 92], and pediatric protocols have emphasized the importance of commencing each therapy cycle at the earliest possible time – usually as soon as the neutrophil count has recovered to 500 or 1000 per mm^3. In a tumor in which essentially all cells are cycling, this may be important to success, whether or not such success can be attributed to dose intensity [108].

7.5.6.5 Risk Adaptation

Patients are normally divided into two to four risk groups for the purposes of treatment. Staging, although still included in the definition of such groups, has a diminishing role in treatment assignment. This is because each stage is heterogenous, and the divisions between them appear to be less than optimal parameters for the definition of treatment subgroups. While molecular genetic factors pertaining to either the host or the tumor may also be important, those examined to date appear to exert a sufficiently small effect and to complicate the logistics of measurement enough that they have not been introduced into the risk group classifications. Patients with the most limited extent of disease (completely resected abdominal disease or stage I) enjoy an excellent prognosis approaching 100%, and here the concern is not to over-treat patients to the point of risking excessive and potentially fatal toxicity or serious late effects. Patients with completely resected disease, particularly those with stage I or small volume disease in the abdomen (e. g., presenting as intussusception) (classified as stage II in St Jude) enjoy the best prognosis, and in both the SFOP and BFM risk classifications (Tables 7.5 and 7.6) are separated off as the lowest risk group. Unresected stage I and II patients are assigned to R2 in BFM and group B in SFOP. BFM risk group R2 also includes patients with stage III disease and serum LDH less than 500 IU/L, while R3 includes patients with stage III or IV disease (as well as patients with >25% bone marrow involvement) and serum LDH ≥500 and <1000 IU/L. The highest risk groups are defined slightly differently by BFM and SFOP. The BFM R4 category includes patients with stage III or IV disease, or >25% involvement of the bone marrow, and serum LDH >1000 IU/L. SFOP group C is for patients with CNS involvement or >70% blast cells in the bone marrow (in LMB89; in LMB96, a protocol currently being conducted in France, the UK, and the USA, only 25% of blasts is required for assignment to group C). SFOP group B includes all pa-

Table 7.5 Definitions of therapy groups in French-American-British Protocol LMB96 for B-cell lymphomas presently ongoing in France, UK, and USA[a]

Therapy group	
Group A	Complete surgical resection stage I or abdominal stage II
Group B	All patients not eligible for group A or C
Group C	Any CNS[b] involvement and/or bone marrow involvement ≥ 25% blasts[c]

a See [157]
b CNS involvement is defined as any blasts in CSF; cranial nerve palsies not explained by extracranial tumor; clinical spinal cord compression; isolated intracerebral mass; parameningeal extension, cranial and/or spinal
c Bone marrow involvement of 70% is required for assignment to group C in LMB89

Table 7.6 Definitions of therapy arms for B-cell lymphoma in BFM protocol NHL-BFM 95[a]

Burkitt and large B-cell lymphoma	
Risk group 1	Stage I and II, completely resected
Risk group 2	Stage I and II, not completely resected Stage III and LDH < 500 U/L
Risk group 3	Stage III and LDH 500-999 U/L Stage IV or B-cell leukemia and LDH <1000 U/L and CNS -ve
Risk group 4	Stage III and IVor B-cell leukemia and LDH ≥1000 U/L and/or CNS +ve

[a] See [98]

Table 7.7 Definitions of therapy groups in NCI protocol 89-C-41 for B-cell lymphomas[a]

Therapy group	
Low-risk patients	Stage I or II and LDH <150% of normal
High-risk patients	All other patients

[a] See [92]

Table 7.8 Outline of therapy in protocol FAB LMB 96[a]

Therapy group	
Group A	Two cycles of COPAD
Group B	COP COPADM 1 COPADM 2 COPADM 2 (reduced cyclophosphamide) CYM 1 and 2 COPADM 3
Group C[b]	COP COPADM 1 COPADM 2 CYVE 1 and 2 Mini-CYVE 1 and 2 CYVE and intrathecal therapy and high-dose methotrexate Mini-CYVE and intrathecal therapy and high-dose methotrexate Maintenance 1 Maintenance 2–4 COPADM 3

Intrathecal therapy is administered in all cycles except COPAD, CYVE, mini-CYVE, and maintenance courses 2–4, except where indicated.
C=cyclophosphamide; O=vincristine; P=prednis(ol)one; AD=doxorubicin; M=methotrexate with leucovorin rescue; CY=cytarabine (Ara-C); VE=etoposide
[a] See [157]
[b] Patients with CNS disease receive additional high-dose methotrexate after CYVE 1 or mini-CYVE 1 and additional intrathecal drugs at the beginning of CYVE or mini-CYVE

tients not included in groups A or C. In the NCI protocol, patients with limited stage disease (a single mass or completely resected intra-abdominal disease and LDH not above 150% of normal) were defined as low risk and all others as high risk (Table 7.7).

Clearly, in BFM and particularly SFOP studies, classical staging does not provide sufficient information for treatment triage. Indeed, in SFOP protocols, staging is not needed to decide treatment as long as there is information on the bone marrow and CNS status (and whether or not all disease was resected in patients with limited disease). In BFM, apart from the limited

Table 7.9 Outline of therapy in protocol BFM-NHL 95[a]

Burkitt and large B-cell lymphomas	
Risk group 1	Two cycles: A-B
Risk group 2	Prephase followed by 4 cycles: A-B-A-B
Risk group 3	Prephase followed by 5 cycles: AA-BB-CC-AA-BB
Risk group 4	Prephase followed by 6 cycles: AA-BB-CC-AA-BB-CC

A cycles consist of dexamethasone, vincristine, ifosfamide, Ara-C, VP16, intermediate dose methotrexate (1g/m^2), and intrathecal methotrexate, Ara-C and prednisolone.
B cycles consist of dexamethasone, vincristine, cyclophosphamide, doxorubicin, intermediate dose methotrexate, and intrathecal methotrexate, Ara-C and prednisolone.
In AA and BB cycles, high-dose methotrexate (5gm/M^2) is given instead of intermediate dose methotrexate, and doses of intrathecal therapy are modified.
CC cycles consist of dexamethasone, vindesine, high-dose Ara-C, VP-16, and intrathecal methotrexate, Ara-C, and prednisolone.
[a] See [98]. In this study, patients were randomized to receive methotrexate over 24 h or over 4 h in cycles A, B, AA, and BB

stage group and the presence of CNS disease, the major determinant of risk group is LDH. Risk assignment might well be feasibly based almost entirely on serum LDH since as long as adequate CNS therapy – whether prophylactic or therapeutic – is given, LDH may be all that is necessary to assign the remaining patients to appropriate treatment arms, although occasional patients with extensive disease and low or normal LDH are seen. Whether such patients would have a good prognosis with less intensive therapy is unknown. Conversely, not all patients with an elevated serum LDH require more intensive therapy: 43% of patients with LDH ≥500 IU/L in BFM studies, for example, achieved long-term survival with an intermediate rather than high dose of MTX. Whether such patients could be identified via gene expression profiling of their lymphoma cells or through pharmacokinetic or pharmacogenomic studies is a topic for research (see below). Conversely, it may be possible to identify, by similar means, the smaller fraction of patients with limited stage disease destined to do poorly when treated with the least intensive therapy. Whether this is a worthwhile endeavor is open to debate.

Table 7.10 Outline of therapy in NCI protocol 89-C-41 for B-cell lymphomas[a]

Therapy group	
Low-risk patients	Three A cycles
High-risk patients	Four cycles: A-B-A-B

A cycles consist of cyclophosphamide, doxorubicin, vincristine, and high-dose methotrexate, B cycles of high-dose ara-C, ifosfamide, and etoposide.
[a] See [92, 93]

An outline of the therapy arms for SFOP, BFM, and NCI risk groups is shown in Tables 7.8, 7.9, and 7.10, respectively.

7.5.6.6 Prevention of CNS Disease

Intrathecal therapy is a critical component of treatment and is generally given to patients in all risk groups, even though the likelihood of relapse in patients with small volume, completely resected intra-abdominal disease is small. From the early studies in Africa, it seems reasonable to conclude that intrathecal therapy should be given over several cycles, and also that both intrathecal MTX and intrathecal Ara-C should be included. The administration of these drugs is separated by 12 h in BFM protocols and by days in other protocols. Some also give a corticosteroid intrathecally with MTX or Ara-C to reduce the risk of arachnoiditis. The total number of injections varies from one protocol to another, and also according to risk group. In essentially all protocols, high-dose MTX and usually high-dose Ara-C is included for the highest risk patients. These elements provide additional protection against CNS disease as well as providing a systemic effect. CNS radiation is no longer given to prevent CNS disease (see below).

7.5.6.7 Treatment of DLBCL with Different Regimens

Although the treatment protocols discussed above are equally applicable and, apparently, equally successful in all of the three main categories of B-cell lymphoma in childhood and adolescents, some investigators have pursued a "histology-based" approach to therapy, using different regimens for BL/BLL and DLBCL. In particular, POG used separate protocols for BL/BLL and large-cell lymphoma (including both anaplastic large-cell lymphoma and DLBCL) for many years. In a recently reported study, 180 eligible patients with advanced (stage III and IV) large-cell lymphoma treated with the APO regimen (DOX, VCR, predinisone, 6-MP, and MTX) were randomized to receive or not to receive intermediate-dose MTX and high-dose Ara-C during mainentance therapy [109]. In this study, the total planned therapy duration was 12 months. The 4-year EFS was 67% for all patients (which included anaplastic large-cell lymphoma), with no significant difference being observed between the two arms, which was similar to data published earlier by the same group, in which APO was compared to ACOP+ (CTX, DOX, VCR, 6-MP, and prednisone). The latter study suggested that APO is somewhat superior to ACOP+ [110]. There was also no significant difference in outcome in patients with anaplastic large-cell lymphoma vs DLBCL. These results for DLBCL (70% EFS at 4 years with the intermediate-dose MTX and high-dose Ara-C vs 64% for standard APO) appear to compare unfavorably to other recently reported results, e. g., those reported by the SFOP and BFM groups [95, 98]; in both studies, patients with DLBCL had a similar outcome to patients with BL. Furthermore, in these SFOP and BFM studies, patients with stage IV disease had comparable survival rates (possibly better in SFOP) to the overall result of APO. Thus, the APO data at best do not make a strong argument for a histologically based approach to the treatment of B-cell lymphomas in children and adolescents. Given the problems of diagnostic reproducibility among pathologists and the long duration of the APO protocol, it would seem preferable at the present time to use identical protocols for BL/BLL and DLBCL, although as more data accrue for subgroups of the latter (e. g., PMBL and TCRBCL), this approach may need to be re-examined [111]: a recent report from BFM, for example, suggested that PMBL may have a somewhat worse prognosis than other types of DLBCL (70% at 5 years) [26], although this included patients treated with several protocols. There is presently insufficient information on TCRBCL to draw any meaningful conclusions.

7.5.6.8 Role of Surgery

The value of complete or almost complete surgical resection, particularly of intra-abdominal lymphoma, has been debated since the pioneering studies in Africa. In some patients, notably those who present with small volume intestinal disease (frequently in the terminal ileum), complete resection is readily accomplished or even inevitable in order to provide emergency care. In others, complete resection of large volume disease may be possible, e. g., patients with ovarian disease (and no other sites of disease) or with a single large-bowel mass that is not adherent to adjacent structures, particularly the retroperitoneum. These situations are probably not comparable, although with modern intensive therapy, the outcome may not be greatly different. Patients with completely resected, small volume disease have long been known to have an excellent prognosis with relatively unintensive short duration chemotherapy alone [57, 87, 112], and there is general agreement that such patients require the least intensive chemotherapy. However, it is also possible that resection of a large mass could allow less intensive chemotherapy to be used without jeopardizing the outcome. Evidence that this may be so was provided by a retrospective study conducted in Africa many years ago, in which patients in whom at least 90% of the tumor was resected (the clearest situation being with ovarian disease) enjoyed an excellent prognosis even with the simple therapies being used in Africa at the time [57]. In a multivariate analysis performed by the CCSG in 84 patients with abdominal lymphoma, only the extent of disease, but not complete surgical resection, was a significant risk factor [113]. In BFM studies 81, 83, and 86, in which the outcome in relationship to the extent of surgery was examined in 177 patients, EFS was 95%, 69%, 62%, and 67% for patients with complete resection, subtotal resection (36), partial re-

section (21), or biopsy only. In this study, patients in stage III/IV disease in whom complete resection was achieved had a better outcome than those in whom complete resection was not performed (EFS 92% vs 63%), but patients with stage II disease had a similar excellent outcome (97–100%) whether or not complete resection was performed. Nearly all patients in whom complete resection was performed had a localized bowel mass (40 of 43). These data suggest that tumor burden at the start of chemotherapy is the best determinant of outcome. Thus, in some circumstances (e. g., in Africa) in which only less intensive therapy can be given, such that patients with a large tumor mass have a rather poor prognosis, there may be some value to complete resection, particularly of large tumor masses, when this is readily accomplished. However, it is generally true that those patients with resectable tumors have a lower tumor burden. Since there are rather few patients in whom large tumors were resected, there are insufficient data to be sure whether their outcome with minimal chemotherapy would be the same as that of patients in whom a small tumor is removed. As such patients have a good prognosis with more intensive chemotherapy but are also at greater risk for postsurgical complications, attempts at surgical removal of large abdominal masses is not recommended in situations where the required intensity of chemotherapy can be administered [113–115].

7.5.6.9 Role of Radiation

Today, radiation has a limited roll in the treatment of B-cell lymphomas of childhood. Since radiotherapy was not available in equatorial Africa at the time of the early chemotherapy studies, radiation was not initially included in the therapy of BL in Africa. Upon the establishment of a radiotherapy unit in Nairobi, a small series of previously untreated patients were given primary radiation therapy. Local control was very poor, and although hyperfractionation improved the response [116], radiation was not incorporated into the standard treatment approaches because of the poor results of standard dose fractionation, the inconvenience of hyperfractionation, and the poor long-term outcome [117]. In the USA, radiation was traditionally used in the treatment of lymphomas as one of the few available approaches prior to the introduction of chemotherapy. Since it did enjoy limited success in some situations and rather significant success in HL, it continued to be used by some investigators even though, after the introduction of chemotherapy, its role was not formally evaluated for many years. Glatstein et al. reported their results in 41 children with NHL treated with radiation alone at Stanford University between 1961 and 1971 in which high-dose (minimum 3500 rads) radiation was delivered to the areas of tumor involvement in patients with stage I, II, or III disease (Ann Arbor) [118]. Although it is difficult to accurately reassign the cases diagnosed according to the Rappaport classification system to the WHO classification, this study, which is comparable to other published studies, is of interest in that it demonstrates that radiation alone is a poor treatment modality compared with chemotherapy, although some patients, particularly with limited stage disease, may do well. Among stage I patients, only 55% were alive and free of disease at 2 years, and among stage II patients, 38%. There were only four stage III patients, and one remained free of disease at 2 years. No stage IV patient survived. Failures were due to regrowth of tumor outside the radiation field, particularly in the bone marrow, although only four patients among the 20 with disease not involving the mediastinum developed subsequent bone marrow recurrence. The question remained whether local radiation would complement chemotherapy.

Murphy and Hustu reported 90% disease-free survival in children with stage I and II NHLs (using the St Jude staging system) treated with CTX, VCR, and prednisone and involved field irradiation, followed by oral maintenance therapy (6MP and MTX). Patients with stage III and IV disease received similar chemotherapy but were randomized to receive involved field radiation. No benefit of radiation was observed in this group, and the investigators suggested that the role of involved field radiation in conjunction with combination chemotherapy should be reexamined, particularly in view of the long-term toxicity of radiation therapy. A number of investigators removed radiation from standard therapy (although it was not infrequently used for patients with residual masses), without any obvious deleterious effect, while the POG performed a randomized study to examine the use of local radia-

tion in patients with limited disease [119]. All 129 patients received chemotherapy with CTX, VCR, prednisone, and DOX followed by maintenance therapy with 6MP and MP for 24 weeks, and were randomized to receive or not to receive 27 Gy to the involved field. There was no demonstrable benefit of radiation therapy – the disease-free survival at 4 years was 87% in both groups – although the immediate toxicity was increased in those who received radiation.

Following the example of acute lymphoblastic leukemia, cranial irradiation was initially used for CNS prophylaxis in children with NHL, including B-cell lymphomas, in a number of institutions and cooperative groups. Only one small randomized study has been reported. This was conducted in African patients with BL. Ugandan patients with no previous CNS disease and who achieved complete remission with chemotherapy were sent to Nairobi for craniospinal irradiation. Equal numbers of patients relapsed in the CNS in each arm, in some cases even before or immediately after completion of the radiation, suggesting no efficacy of radiation delivered in this way [71]. In the USA and Europe, the use of neuraxis radiation for the prevention of CNS disease continued to be variable. Nonrandomized studies from Memorial Sloan Kettering Hospital and CCSG have not provided evidence of an advantage of cranial radiation over intrathecal therapy (with or without high-dose MTX and/or high-dose Ara-C) as CNS prophylaxis [120, 121], and the weight of evidence, coupled to the long-term toxicities of cranial radiation and the introduction of high-dose methotrexate and/or Ara-C (with its added potential neurotoxicity), plus the excellent results obtained in patients who do not receive neuraxis radiation have led to the elimination of CNS irradiation as an approach to the prevention of CNS disease. The good prognosis enjoyed by patients with CNS involvement at presentation not treated with radiation [60, 98] has also led to the elimination of radiation for the treatment of overt CNS disease by the major cooperative groups.

7.5.6.10 Tumor Lysis Syndrome

Uricosemia (and the associated risk of potentially fatal consequences during initial therapy) was first noted in Nigerian patients with extensive BL [62]. In the 1970s, sudden death from hyperkalemia immediately after the start of therapy in BL was reported in several patients [122], and hyperphosphatemia and hypocalcemia were also observed to occur in the first 24–48 h of treatment in patients with extensive disease [123]. A number of descriptions of the acute tumor lysis syndrome followed [124–126]. Particularly frequent in tumors with high proliferative fractions and in patients with extensive disease, this syndrome is more likely to occur in the presence of renal insufficiency prior to therapy that is usually a consequence of uric acid nephropathy, not infrequently exacerbated by renal outflow tract obstruction from tumor or, occasionally, involvement of the kidney with lymphoma. After therapy is initiated, hyperphosphatemia may ensue with resultant hypocalcemia (occasionally leading to cardiac arrythmias) and precipitation of amorphous phosphates and sometimes calcium in the renal tubules (rarely, extrarenal soft-tissue calcification can occur). This results in tubular obstruction and azotemia. Hyperhydration and the administration of allopurinol during the early part of therapy effectively reduce the risk of clinical tumor lysis, but inhibition of xanthine oxidase allows xanthine and hypoxanthine to accumulate which, rarely, may contribute to intrarenal tubular obstruction [127]. The rapid uricolytic effect of urate oxidase or its recombinant form, rasburicase, administered prior to therapy and/or up to a week after therapy has commenced in high-risk patients (i.e., patients with BL and advanced stage/high serum LDH), more effectively reduces the risk of tumor lysis syndrome [128, 129] and has several additional advantages over allopurinol. It reduces the uric acid level to normal within hours, thus allowing for more rapid recovery of renal function, and obviates the need for alkalinization, thus facilitating phosphorus excretion and diminishing the risk of hypercalcemia. Alkalinization, even when allopurinol is used to prevent renal failure from tumor lysis, should be stopped at the start of therapy because it may increase the risk of precipitation of amorphous phosphates and, through the induction of alkalosis, further increase the risk of arrythmias. Since urate oxidase acts by converting uric acid to allantoin, its use in preference to allopurinol also avoids the increased excretion of xanthine and hypoxanthine which occur with allopuriol. Although there is a small risk of anaphylaxis

with urate oxidase, this is decreased even further with the use of a recombinant form of the enzyme, rasburicase [128]. Interestingly, the use of a "prephase" of low-dose chemotherapy in the German and French protocols, putatively to reduce the risk of tumor lysis syndrome, may or may not make a difference to its frequency, but certainly does not eliminate it, and patients with extensive marrow involvement remain at high risk [128, 129]. Controlled studies of the value of the prephase have not been performed.

7.6 Prognostic Factors

The most important prognostic factor for B-cell lymphomas – as for almost all cancers – is, of course, the treatment used. As improvements in the results of therapy are achieved, formerly powerful prognostic factors (e. g., involvement of bone marrow and/or CNS) become less significant and may eventually lose all significance. Such factors have, however, undergone a transition from being a prognostic factor to a risk factor, i.e., they are used in determining the optimal therapy. Thus, the diminishing ability to demonstrate prognostic factors can be seen as a sign of success. The BFM studies, in particular, have now accrued sufficient numbers of patients to examine prognostic factors in more detail and potentially to identify factors which have a smaller influence on prognosis, although, in drawing conclusions, the possible influence of treatment changes and modifications in the risk group assignment of particular subgroups over this period should be borne in mind. Some of these factors may be taken into consideration by mathematical modeling techniques.

7.6.1 Tumor Burden and Stage

The second most important prognostic factor is the tumor burden which can be measured in various ways, the most widely used being stage and serum LDH. Patients with extensive bone marrow involvement (greater than 25%) have high tumor burdens, and approximately half of these patients will relapse unless treated with more intensive protocols than those in use in the early 1980s. In three sequential studies of the BFM group, for example, EFS for B-cell leukemias improved from 40% to 50% in ALL-BFM-81 and 83% to 78% in ALL-BFM-86 [130]. The ALL-BFM-86 result has not been improved upon with more recent protocols, including the latest, BFM95, (77% EFS) suggesting that in this group of patients, the addition of CC cycles, which include drugs not used in other cycles (high-dose Ara-C, vindesine and ETOP), has not been beneficial [60, 95, 98]. However, these drugs do appear to be of value in other risk groups. Patients in BFM-R3 of BFM-90, who were divided into R3 and R4 in BFM95 on the basis of serum LDH ≤1000 IU/L, did not do as well as patients in R3 and R4 combined in BFM95 (treated with 24-h high-dose MTX; there was a randomization to 4 h vs 24 h). This appears to be due to the fact that all R3 and R4 patients (i.e., all patients with stage III and IV disease and serum LDH ≥500 IU/L) received at least one CC cycle in BFM95, whereas CC was included only in R3 patients with an incomplete response after two therapy courses) in BFM-90 [60]. Since there was no improvement in the outcome of patients with extensive bone marrow disease, this result must be primarily due to the improved survival of patients in stage III with LDH ≥500 IU/L.

Patients with serum LDH >1000 IU/L have, in the past, had a particularly poor prognosis [87], which was the reason for creating a new risk group, R4 (patients with advanced (stages III and IV) B-cell lymphoma/leukemia and a serum LDH ≥1000 IU/L with or without CNS disease), in the most recent BFM study, BFM95 [98]. Serum LDH has long been recognized as a risk factor that correlates with tumor burden [65, 87, 131], and the BFM results confirm its importance as both a prognostic factor and a valuable parameter for risk categorization. LDH-A expression is influenced by MYC [132], which may account for the occasional extremely high levels in relapse patients, and possibly for the occasional patients in whom it is not elevated in spite of advanced disease; this could relate to molecular abnormalities in the MYC protein, although this has not been examined. Thus, although LDH correlates well with tumor burden, other factors, such as the level of expression of MYC, may be important, too. Whether this is relevant to the treatment outcome is unknown.

Other serum markers that probably reflect tumor burden, at least to a considerable degree, include soluble IL-2 receptors [133, 134] and, in African EBV-pos-

itive BL, antibodies against the early antigens of EBV. Early antigens are expressed when the virus enters the lytic cycle and were long ago shown to be a powerful prognostic indicator, probably because they also correlate with tumor burden [65]. Recent preliminary data suggest that antibodies against a key protein involved in the switch to the lytic cycle (Zta) may also be of prognostic significance [135]. However, since the number of EBV genome copies per tumor cell varies from one tumor to another, and also antibody responses are not solely dependent upon the antigen load, this, like all other serum markers, cannot be considered a precise measure of the tumor burden. Serum EBV DNA is also likely to reflect the number of genome copies of EBV per tumor cell as well as the total tumor burden. Although shown to be a useful marker in some other EBV-associated diseases, including HL, this has yet to be studied systematically in EBV-positive BL [136]. In BFM studies, no significant difference in outcome according to the presence or absence of EBV DNA in tumor cells was observed in 222 patients with BL, although only 26 were EBV-positive [14].

CNS disease continues to be a prognostic factor, e. g., in group C patients treated with the LMB89 protocol of the SFOP, but EFS at 5 years in patients with CNS disease was 80% compared to 90% in patients without CNS disease [95]. In BFM95, 40 CNS patients had an EFS of 69% at 3 years.

7.6.2 Rapidity of Response

Since treatment is the most important prognostic factor, it might be expected that an in vivo test of treatment responsiveness early in therapy might predict the ultimate outcome, as is the case in acute lymphoblastic leukemia, for example. In B-cell lymphomas, the rapidity of response is also likely to be an important prognostic [60,114,130]. The slowest responders are, of course, patients who never achieve CR, whose outcome is almost universally poor. However, it is possible that at least some of these patients may achieve long-term survival if treated from the outset (switching after 2 or 3 therapy courses is probably too late [60]) with more intensive therapy or additional drugs. This is supported by the SFOP experience, in which patients assigned to group B were switched to group C in the event of nonresponse to the low-dose prephase (CTX, VCR, and prednisone). Nonresponders had a very poor outcome in the previous treatment protocol, LMB84 (22%), but the outcome was much better (72%) in LMB89 in which nonresponders were switched to group C therapy [95].

7.6.3 Age and Sex

Ascertaining the impact of age and sex on the prognosis not only requires careful analysis, in part because the peak age is different in different lymphoma subtypes, but may also differ according to the treatment given. In a recent analysis of over 2000 patients treated with BFM protocols between 1986 and 2002, the median age at diagnosis for patients with BL (including those categorized as having leukemia) was 8.4 years, for DLBCL (subcategorized as centroblastic type) 11.4 years, and for PMBL patients, 13.2 years [22]. Male DLBCL patients had a significantly superior EFS, while adolescent girls with DLBCL had a worse outcome than younger girls. In the SFOP series of B-cell lymphomas, age over 15 years in patients in risk group B was also a significant risk factor [95]. In NCI studies, which included adult patients up to the age of 60 years, and in an Italian study, no prognostic impact of age could be discerned [92, 93].

7.6.4 Histology

In the BFM, SFOP, and NCI studies of patients with B-cell lymphomas, the histological diagnosis was not a significant prognostic factor. The BFM group, however, subdivides DLBLC into several histological groups. In an analysis of 1004 B-cell lymphomas treated since 1986 with three protocols, which included 173 DLBCL of centroblastic type and 40 PMBL, boys with centroblastic lymphoma had a significantly better prognosis than all of the other patients [22]. Since this diagnosis was not used in studies outside BFM, and a diagnosis of BLL was not made in BFM studies, this finding is not easy to translate to other contexts. In an earlier analysis, patients with PMBL had an EFS of only 70% at 5 years, and patients with a serum LDH ≥500 IU/L had a particularly poor prognosis (EFS 40% at 5 years) [26]. A smaller number of patients with PMBL (20)

treated by the CCSG with four different protocols had an EFS of 75% at 5 years – significantly better than that of all large-cell lymphoma patients (including anaplastic large-cell lymphoma) whose EFS was 50% at 5 years [137]. It is difficult to draw firm conclusions from these studies, given the rather small numbers of PBML cases and pooling of all large-cell cases, but taken together, these data suggest that there may be differences in the therapeutic response of PBML compared with other large B-cell lymphomas, and that more research will be required to define the optimal therapy.

7.6.5 Molecular Characteristics

In recent years, considerable attention has been paid to the potential importance of molecular markers to prognosis. In the B-cell lymphomas of childhood, several potential cytogenetic or molecular markers related to the myc-immunoglobulin translocations, e. g., the chromosomal breakpoint locations, may be worthy of exploration. Similarly, the prognostic impact of the presence or absence of EBV has been explored to a very limited extent [14].

Molecular profiling may be a more convenient way to measure the biological differences that relate to the gross molecular lesions, or to the precise cell or origin, but although information of this type exists for B-cell lymphomas in adults, such information is lacking in children. Evidence that single nucleotide polymorphisms may be of importance to the prognosis has been reported for tumor necrosis factor, lymphotoxin alpha, and glutathione-S-transferase [138–140].

7.7 Outcome

The outcome of treatment in children and adolescents with B-cell lymphoma when treated with risk-adapted therapy is excellent. Similar EFS rates of approximately 90% have been obtained by several groups in the last 10 years or so, with even stage IV and leukemic patients having having EFS rates in excess of 70–80% [92, 93, 95, 96, 96A] (Table 7.11). In the case of BMF and SFOP protocols, several thousand children and adolescents have now been treated, and these results would appear to be solid. These excellent results come at the expense of sig-

Table 7.11 Results of various protocols in the treatment of B-cell lymphomas

	No patients	EFS at 3 years or more
LMB 89:[a]		
Stage I and II patients	122	96±4%
Stage III patients	280	93±3%
Stage IV	97	95±4%
Leukemic patients	67	79±8%
BFM 95:[b]		
Stage I	53	98±2%
Stage II	119	98±2%
Stage III	221	87±2%
Stage IV	33	81±7%
Leukemic patients	79	77±8%
POG protocols:		
Stage I and II[c]	>100	88%
Stage III[d]	64	79±6%
Stage IV[e]	59	79±9%
Leukemic patients[e]	74	65±8%
NCI 89-C-41:		
Low-risk patients[f]	18	100%
High-risk patients[g]	66	85%

a See [95]
b See [98]
c See [101]
d Results are those of the modified Total Therapy B protocol for small noncleavced cell lymphoma (BL and BLL) : see [107]
e Results are those of the modified Total Therapy B protocol for small noncleaved cell lymphoma (BL and BLL) and B-cell acute lymphoblastic leukemia: see [165]
f See [92]
g See [93]

nificant toxicity, particularly in terms of fever and neutropenia, and it is still too early to evaluate the late toxic cost. However, early results in one of the more intensive protocols suggest that these will be less than in earlier patients, almost certainly due to the elimination of radiation, but perhaps also to the use of multiple agents with broadening of the spectrum of toxicity rather than deepening, and the short duration of therapy which imposes limits on the cumulative exposure [103].

7.8 Management of Relapse

The highly successful results of the treatment of children and adults with B-cell lymphomas has led to a considerable reduction in the frequency of relapse and to a poor outcome of the treatment of relapse in patients initially managed with appropriately intensive therapy. The shrinkage of large masses by chemotherapy frequently gives rise to residual masses which contain no viable tumor; in the BFM90 study, 46 of 167 (28%) patients in risk group R2 had residual masses, as did 73 of 175 (44%) patients in R3. Progression occurred in 2 and 13 of these patients, respectively, demonstrating that the risk of progression was clearly related to the extent of tumor at diagnosis and LDH level, but also, that the majority of these patients must have had non-viable tumor, but also, that the majority of these patients must have had non-viable tumor. It is important that the high possibility of nonviable tumor residuum is recognized in order not to treat patients unnecessarily. Imaging studies including MRI, ^{67}Ga scintigraphy, and PET scanning may be valuable in this regard, and biopsy may sometimes be indicated. However, the BFM experience with "second look" laparotomy does not support the routine use of this procedure to determine whether there is persistent viable tumor. In a high proportion of patients, no viable tumor was found in the residual masses, and in those in whom viable tumor was histologically identified, the outcome was generally very poor, and there was no apparent advantage of early detection (vs waiting until there is clear progression). In general, the best policy seems to be to avoid relapse to the extent possible by ensuring that patients are assigned to the appropriate risk group. Identifying those who may fail in spite of this may not currently be possible except in the case of nonresponders to the first cycle of therapy. Such patients can be reassigned to a more intensive therapy arm very early in treatment (a potential value of a prephase). The value of autologous bone marrow transplantation in this circumstance has not been proven. In fact, some evidence exists, albeit in adult patients, that ABMT in place of intensive chemotherapy may lead to a worse outcome [141].

Regimens designed for patients with progressive disease [104–106] have, for the most part, been incorporated into primary regimens in the hope of preventing relapse. This hope has been substantially realized. Regimens containing platins have not been used widely in NHL. One exception is the DECAL regimen of the CCSG, which includes dexamethasone, ETOP, cisplatin, high-dose Ara-C, and L-asparaginase [142]. This regime produced a 23% EFS at 5 years for 68 patients with NHL in first or subsequent relapse. Eight of the 16 patients alive and disease-free at 3 years had lymphoblastic lymphoma, two had small noncleaved cell lymphoma and five, large-cell lymphoma. Most, if not all, of these patients were presumably treated on less intensive therapies than, for example, recent BFM or SFOP protocols, so the value of this approach in more intensively treated patients remains unknown. Of interest is the lack of a demonstrable benefit to patients who underwent bone marrow transplantation after achieving remission with DECAL rather than receiving the planned four cycles of maintenance therapy, consisting of ifosfamide/mesna and ETOP alternating with DECAL. DECAL was incorporated into the „Orange" protocol of the CCG, in which the reported EFS in high risk patients was 61% at 5 years.

Relapse after the most intensive treatment protocols is generally associated with a poor outcome. Recently, the Austrian Cooperative Study Group reported that eight of nine patients with B-cell lymphoma/leukemia who failed their initial therapy, four of whom underwent stem cell transplantation, died of their disease [143]. There is no accepted treatment approach for such patients. Intensive therapy including drugs not previously administered, such as a platinum compound, should be considered as an initial salvage regimen. Although there is no evidence that stem cell transplantation is useful in patients who respond, al-

logeneic transplantation (including haplotype matches), which has been successfully used, should be considered [147], even in patients who have failed autologous marrow transplantation [145]. In this setting non-myeloablative regimens have been used [146]), although only occasional patients are likely to be salvaged [148]. Whether transplantation is more effective than continued chemotherapy in patients who respond to salvage regimens is unknown, but the possibility of a graft-versus-tumor effect at least provides a rationale for the use of allogeneic transplantation.

7.9 Follicle Center Cell and Marginal Zone Lymphomas

Most NHLs in children are aggressive malignancies, but an increasing number of reports include pediatric patients with indolent B-cell lymphomas [1]. It is not possible to estimate the incidence of these lymphomas because a central database does not exist for these rare pediatric lymphomas. In some series of pediatric NHL, follicle center lymphomas comprise approximately 2% of all cases. The majority of reported cases of follicle center cell lymphomas in children involve the peripheral nodes, especially in the head and neck area, and tend to be early stage without the typical BCL-2 overexpression that characterizes adult cases [166]. Interestingly, there are several small series of testicular follicle cell lymphomas that have been reported in children [2, 167]. The overall survival for all sites of follicle center cell lymphoma in children exceeds 90%. Therapy has ranged from excisional biopsy alone to radiation therapy with or without chemotherapy. The chemotherapy regimens have varied but often include CHOP [166]. There have been no controlled clinical trials and only anecdotal information about the use of anti-B-cell monoclonal antibodies such as Rituximab. Surgery alone seems to be a reasonable option for stage 1 nodal or testicular disease [168]. Given the unknown natural history of follicle cell lymphomas in children, conservative management seems prudent.

Nodal and extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT) are very rare in children and are sometimes reported in association with HIV and autoimmune disorders [169–171]. In children, nodal marginal zone lymphoma (NMZL) is more common than extranodal disease. The histopathology of NMZL in children frequently shows disruption of the residual lymphoid follicles resembling progressive transformation of germinal centers. NMZL often involves lymph nodes in the head and neck region and an excellent prognosis. Extranodal sites of disease include the ocular adnexal structures and the salivary glands [170]. Local treatment with surgery with or without local irradiation is recommended, but there are too few reported cases to give definitive treatment guidelines.

7.10 Future Prospects

New techniques for studying gene expression profiles and genetic polymorphisms holds promise for an improved diagnosis, risk assessment, and development of novel targeted therapies [25, 138-140, 149]. A more precise definition of risk assessment would probably reduce over-treatment of patients and identify upfront the rather small number of patients who do poorly. Such patients could be reassigned from the outset to a higher risk category, or to an experimental arm if already in the highest risk category. There is little doubt that these techniques will become automated and streamlined with time; meanwhile, it will be important to determine whether molecular analysis either of the lymphoma cells or of normal tissues (e. g., for pharmacogenomic studies or the identification of polymorphisms relevant to pharmacokinetics or pharmacodynamics) will, in fact, permit more precise risk assignment.

Both improved technology and more detailed understanding of the molecular pathogenesis raise the potential for developing targeted approaches to therapy, particularly perhaps in BL where molecular abnormalities are more predictable, and at least outside the affluent countries, EBV (particularly the invariably expressed gene, EBNA1) may represent an appropriate target. Proof-of-principle studies have already demonstrated the potential efficacy of targeting either the myc-immunoglobulin translocation [150, 151] or EBV [152, 153]. However, once again, it will be many years before these approaches become realistic. The introduction of targeted nanoparticle systems for in vivo

imaging, molecular characterization, and targeted therapy, now in the early stages of development [154, 156], could move this field forward rapidly since nanoparticles appear able to deliver agents designed to inhibit specific genes (e.g., via RNAi), to replace defective genes, or even to permit targeting of predrugs that require enzymatic activation, to tumor cells with much greater efficiency than viral vectors. The latter are also more difficult to construct.

One form of targeting which is presently available is the use of monoclonal antibodies directed towards surface proteins, such as CD20 and CD22. At present, anti-CD20 antibodies, particularly Rituximab, have been primarily used in lymphoproliferative diseases arising after organ or bone marrow transplantation [157, 158] (see Chapter 11). Their use in other B-cell lymphomas in children and young adults, whether at presentation or relapse, is largely anecdotal [159, 160], although essentially all B-cell lymphomas express both CD20 and CD22 [161], such that there is a potential for benefit. Some in vitro studies provide grounds for optimism [162]. The therapeutic value of the addition of anti-B cell monoclonal antibodies to intensive chemotherapy or to allogeneic transplantation in very high-risk groups or patients who relapse remains unknown. Similarly, whether anti-B-cell monoclonal antibodies would allow some reduction in the intensity of therapy for selected patient groups can only be determined by randomized clinical trials. Such trials are currently in development by the Children's Oncology Group but must be tempered by the unknown added risk of serious infection in heavily treated patients that may arise from prolonged B-cell immunosuppression caused by Rituximab [163].

For the immediate future, it is likely that risk-adaptation schemes in the context of a specific set of therapeutic regimens will continue to be refined, perhaps by the inclusion of information gained from the gene expression profile or the presence of specific polymorphisms (although this will not occur for some years). It should also be recognized that while intensive, expensive, but highly effective combination chemotherapy is widely used in affluent countries, such protocols may not be feasible in low-income and many middle-income countries. In such countries, a high fraction of patients with curable B-cell lymphomas do not receive appropriate therapy, and many may die without any therapy at all. This is particularly true in high-incidence regions in Africa where many crucial early observations were made and where resources are particularly limited. Thus, in addition to developing increasingly high technology approaches to diagnosis and treatment, consideration should also be given to the other end of the spectrum-improving existing facilities for therapy in developing countries in order to bring feasible and reasonably effective therapy to as many patients as possible. This is the approach most likely to improve global survival rates.

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Anaplastic Large-Cell Lymphoma

Alfred Reiter

Contents

8.1 Introduction

Anaplastic large-cell lymphoma (ALCL) accounts for approximately 10–15 % of non-Hodgkin lymphoma (NHL) of childhood and adolescents (Burkhardt et al. 2005). ALCL was first described by Stein et al. in 1985 (Stein et al. 1985). This new entity was characterized by large anaplastic cells with strong reactivity to the monoclonal antibody, Ki-1 (later assigned CD30), originally raised against the Reed-Sternberg cell line L428 (Schwab et al. 1982), and by the presence of activation markers such as interleukin-2 (Il-2) receptor and HLA-DR antigens (Stein et al. 1985). The expression of the CD30 antigen is not restricted to ALCL. This antigen can also be detected on Reed-Sternberg cells of Hodgkin lymphoma, and various large-cell non-Hodgkin lymphomas such as peripheral T-cell lymphoma (Stein et al. 1985). A major step in the recognition of ALCL as a distinct category of NHL was the detection of the nonrandom chromosomal translocation t(2;5)(p23;q35) in the malignant cells of the majority of cases (Fischer et al. 1988). This translocation causes the nucleophosmin (NPM) gene located at 5q35 to fuse with a gene at 2p23 encoding the receptor tyrosine kinase anaplastic lymphoma kinase ALK (Morris et al. 1994). The production of monoclonal antibodies directed against fixative-resistant epitopes of ALK allowing its detection by immunohistochemistry has significantly improved the strength of the diagnosis of ALCL (Pulford et al. 1997).

Prior to its recognition, ALCL was misdiagnosed as a number of other diseases. In childhood, ALCL was especially confused with malignant histiocytosis, regressing atypical histiocytosis, Hodgkin lymphoma or even reactive lymphadenopathy (Stein et al. 1985; Kadin et al. 1986; Agnarsson and Kadin 1988; Bucsky et

Table 8.1 Subtypes of anaplastic large-cell lymphoma according to the WHO classfication and their distribution in childhood and adolescence

Reference	Brugieres et al. 1998	Seidemann et al. 2001	Williams et al. 2002
No. of patients	82	89	72
Histological subtype		not specified	not specified
• Common type	65%		
• Lymphohistiocytic variant	17%		
• Small-cell variant	7%		
• Common+small-cell	8%		
• Giant-cell variant	1%		
• Mixed	1%		
Immunophenotype			
• T-cell	87%	53%	51%
• Null	13%	41%	36%
• B-cell	0%	6%	8%
• CD30	100%	100%	100%
• EMA	100%	na	na
ALK-1 positive	93%	81%[a]	na
ALK-1 negative	7%	19%[a]	

na, not available
[a]of 43 patients evaluated for ALK-1

al. 1989; Delsol et al. 1988; Greer et al. 1991). The pathology, genetics, and molecular pathogenesis of ALCL are discussed in more detail in Chapter 6. Because of the wide morphologic spectrum of the tumor cell population and the admixture of reactive cells, several subdivisions of ALCL, based on cytomorphological criteria, have been described (Table 8.1). However, the significance of such subclassification remains uncertain. The multiple histologic appearances of ALCL has sometimes led to misdiagnosis.

For example, the lymphohistiocytic variant is characterized by a large number of reactive histiocytes which may mask the anaplastic tumor cell population leading to adiagnosis of reactive histiocytic diseases or malignant histiocytosis (Pileri et al. 1990). In the past, the small-cell variant, characterized by a predominant population of small to medium-sized neoplastic cells with irregular nuclei, was often misdiagnosed as peripheral T-cell lymphoma (Kinney et al. 1993; Benharroch et al. 1998). In rare cases, the small-cell variant transforms during the course of the disease into the common type and vice versa (Reiter et al. 1994; Hodges et al. 1999). The sarcomatoid form of ALCL, characterized by CD30+ large, bizarre, and often spindle-shaped neoplastic cells, can mimic soft-tissue tumors, especially of the malignant fibrous histiocyte type (Chan et al. 1990). Other rare subforms of ALCL are characterized by an abundant admixture of eosinophils or neutrophils (McCluggage et al. 1998). Such cases may easily be mistaken as Hodgkin lymphoma, true histiocytic malignancies, or even an acute inflammatory process. This is especially valid for the neutrophil-rich subform because it may mimic an acute inflammation due to abscess formation in both the skin and bone (Mann et al. 1995; Simonart et al. 1999; Mira et al. 2003).

As discussed in Chapter 10 it is important to distinguish the primary cutaneous ALCL from systemic forms of ALCL. Primary cutaneous ALCL is rare in childhood, and these patients seem to have a favorable prognosis (Tomaszewski et al. 1999; Vermeer et al. 2001). Some of them may not even need therapy at all (Bekkenk et al. 2000; personal unpublished observations). Another rare group of patients are children in

which ALCL evolves from pre-existent lymphomatoid papulosis. Anecdotal observations suggest that these patients may survive long-term with a waxing and waning course without chemotherapy even if nodal involvement concomitantly exists (Reiter et al. 1994). Several studies suggest that lymphomatoid papulosis and ALCL may originate from the same clone (Aoki et al. 2001). The relationship between cutaneous ALCL and lymphomatoid papulosis is discussed in more detail in the Chapter 10.

The clonal nature of ALCL was confirmed in the late 1980s by the detection of the nonrandom chromosomal translocation t(2;5)(p23;q35) in the malignant cells (Fischer et al. 1988; Rimokh et al. 1989; Kaneko et al. 1989; Le Beau et al. 1989; Mason et al. 1990). A milestone in the understanding of the biological nature of ALCL was the demonstration by Morris et al. in 1994 that the 2;5 translocation causes the NPM gene located at 5q35 to fuse with a gene at 2p23 encoding the receptor tyrosine kinase anaplastic lymphoma kinase ALK (Morris et al. 1994). Transfection of murine hematopoetic cells with the NPM-ALK fusion gene induces transplantable lymphoid tumors (Kuefer et al. 1997). The implications of this genetic aberration in the pathogenesis of ALCL are discussed in more detail in Chapter 6 and two recent reviews (Pulford et al. 2004a,b). In the majority of cases, ALCL occurs as a de novo malignant lymphoma. In rare occasions, however, ALCL appears as a secondary lymphoma which evolves from a low-grade or high-grade malignant T-cell lymphoma, Hodgkin's lymphoma or lymphomatoid papulosis (Kadin et al. 1986; Reiter et al. 1994; Kaudewitz et al. 1989; Davis et al. 1992; Nijsten et al. 2004). The pathological and molecular features of ALCL were extensively discussed in recent reviews (Stein et al. 2000; Falini 2001).

8.2 Diagnosis and Imaging

The establishment of the diagnosis ALCL is still based on histopathology and immunophenotyping (see Chapter 6). However, a large enough biopsy sample should be obtained for complete characterization of the lymphoma tissue including chromosomal analysis, FISH, and molecular genetic studies. The above studies can be of particular value in the differentiation of ALCL from reactive or other benign lymphoproliferative disorders as well as from malignancies of nonlymphoid origin. Major difficulties may arise in the separation of ALCL from Hodgkin lymphoma of the nodular sclerosis type, soft-tissue sarcomas, primary bone tumors, lymphomatoid papulosis, and even reactive lymphoproliferative processes. In particular, the distinction between virus-associated or other hemophagocytic syndromes (Janka 2005) and ALCL or peripheral T-cell lymphomas accompanied by marked hemophagocytosis in the bone marrow may also be difficult (Falini et al. 1990). Also, in the rare case of exclusively skin lesions, the distinction between primary cutaneous ALCL and lymphomatoid papulosis may be difficult (Van Neer et al. 2001; Willemze et al. 2005). Although basic immunohistochemistry studies are possible on conventional paraffin sections using paraffin-resistant antibodies against CD30 (Schwarting et al. 1989), anti-Alk-1 (Pulford et al. 1997), EMA, CD 15, T- and B-lineage specific antigens as well as NK-cell antigens, the preservation of snap-frozen tumor tissue for immunophenotyping is advantageous.

Imaging procedures are similar for all children with NHL. In children suffering from ALCL, however, special attention should be directed to the examination of soft tissue and skin, due to their relatively frequent involvement as discussed below. Chest X-ray is routinely performed and is sufficient to detect a mediastinal mass and/or pleural effusions. A CT scan of the neck, thorax, abdomen, and pelvis should be routinely obtained for staging. Skeletal scintigraphy is sensitive for detecting the involvement of bone. Areas of increased activity in the skeletal scintigraphy should be further examined by X-ray and MRI. Skeletal scintigraphy is recommended for patients with symptoms of bone involvement such as pain and swelling. Missing asymptomatic bone lesions is not crucial since bone involvement is not used for stratification of therapy due to the lack of prognostic impact in large series (Reiter et al. 1994; Brugieres et al. 1998; Seidemann et al. 2001; Williams et al. 2002).

A brain MRI or CT scan is indicated if a patient has signs or symptoms suggestive of CNS involvement, but this is very rare in ALCL. Gallium-67 scintigraphy has been used by some centers for staging but is being re-

placed by PET scans (Parker 1989) in children with lymphoma.

Positron emission tomography (PET) with fluorine-18 fluorodeoxyglucose (FDG) has become a widely used diagnostic tool for staging and restaging of malignant lymphomas (Reske and Kotzerke 2001; Kostakoglu and Goldsmith 2000). Increased FDG uptake in lymphoma is based on elevated glycolysis and a longer residence time of FDG in malignant cells compared with most normal tissues (Som et al. 1980). In contrast to the extensive data on the role of FDG-PET in the management of lymphomas in adults, there are only a few studies evaluating the possible role of FDG-PET in childhood lymphoma. All reports so far have published data on retrospective studies comparing results of FDG-PET with conventional imaging methods such as MRI and/or CT (Montravers et al. 2002; Depas et al. 2005). In a retrospective single-center study, Herman et al. compared FDG-PET with CT findings at initial staging (Hermann et al. 2005). Some 662 regions were evaluated in 25 consecutive pediatric patients (17 Hodgkin`s lymphoma and 7 NHL). Concordant findings were found in 92% of the 662 analyzed regions (78% concordant negative, 14% concordant positive). In 7% of the analyzed regions, discordant findings were found, 4% were classified as PET+/CT−, while 3% were classified as PET-/CT+. Discordant findings were more frequent in extranodal regions compared with nodal regions. Staging based on PET findings resulted in a change of staging based on CT findings in 6 of the 25 patients. However, whether staging according to PET is more appropriate than staging based upon conventional imaging alone for the guidance of therapeutic modalities remains unclear. Discordant findings between FDG-PET and CT may be due to different limitations of FDG-PET and CT. Increased uptake of FDG is not restricted to malignant cells but can be observed in other nonmalignant tissues with high level but physiological glucose metabolism, including the thymus gland (Brink et al. 2001) and brown fat (Yeung et al. 2003). The limitations of both methods may be reduced by dual-modality PET/CT fusion images (Lardinois et al. 2003; Yeung et al. 2005). However, even with concordant findings in PET and conventional imaging, differentiation between reactive hyperplasia (e. g., due to inflammation) of lymph nodes and nodal tumor spread is not always possible (Hermann et al. 2005). A main limitation of most studies comparing findings of FDG-PET with findings of conventional imaging methods is the lack of histological validation of lesions classified as tumor, especially in those judged discordant in PET and conventional imaging (Buchmann et al. 2001; Hermann et al. 2005). Thus, prospective studies are required not only to evaluate and verify the diagnostic role of FDG-PET in staging but also to assess whether different findings of FDG-PET compared with conventional imaging translate to modifications of therapy and whether in turn this results in improved outcome and/or reduction of the treatment burden for the patients.

8.3 Clinical Features

The clinical presentation of children and adolescents with ALCL is characterized by several distinctive features. Extranodal involvement including skin, bone, soft tissue, and the presence of B-symptoms are more frequent in ALCL patients than children and adolescents with other NHL subtypes (Fig. 8.1) (Kadin et al. 1986; Reiter et al. 1994; Brugieres et al. 1998; Seidemann et al. 2001; Sandlund et al. 1994 a,b). There are also some other unique features in the clinical history of ALCL patients. Patients may have an indolent phase consisting of lymphadenopathy or a longer illness characterized by fever and, most intriguing, a waxing and waning course of lymphadenopathy before a diagnosis is made. (Reiter et al. 1994; Greer et al. 1991). The waxing and waning of disease may be mediated by cytokines such as transforming growth factor beta. (Newcom et al. 1988; Kadin et al. 2001).

Table 8.2 compares the clinical features of ALCL patients from five large multicenter studies. The age at diagnosis ranges from 0.8 to 18 years with a median of about 10 years. There is an increased male to female ratio with 56 % to 70% of patients being boys. Approximately half of the patients had B-symptoms. The majority of patients have advanced stage disease at the time of diagnosis. Lymphadenopathy was present in 88–97% of patients. Peripheral nodes were most frequently involved, followed by retroperitoneal nodes and a mediastinal mass (Reiter et al. 1994; Brugieres et

al. 1998; Seidemann et al. 2001; Williams et al. 2002; Mori et al. 2003). The pattern of spread of nodal disease in ALCL patients can be contiguous as well as noncontiguous. Extranodal involvement is present in about 60% of patients. Soft tissue and skin were the most frequent sites of extranodal disease, followed by bone and lung. Lung disease may appear as either a nodular or infiltrative pattern and is associated with a malignant effusion in 14–26% of patients. Rarely, focal lesions of the pancreas, kidney, liver, and the intestinal tract are observed. Soft-tissue involvement may occur as multiple tumors in the subcutaneous tissue and muscles, or as a single larger tumor resembling a soft-tissue sarcoma. Bone manifestations vary from small osteolytic lesions to large tumors simulating bone tumors. In our own series, the disease was confined to the bone in 5% of patients. Skin lesions can be single or multiple cutaneous/subcutaneous nodules, large ulcerated lesions (see Fig. 10.8), or multiple or disseminated papulomatous lesions of red-yellow color (Fig. 8.2). As previously mentioned, primary cutaneous ALCL is rare in children. Compared to patients with Burkitt lymphoma or lymphoblastic lymphoma, children with ALCL have less frequent CNS and bone marrow involvement at diagnosis (Fig. 8.1). However, the diagnosis of bone marrow involvement depends on the procedure applied. Bone marrow biopsy is more sensitive than a bone marrow aspirate for detecting

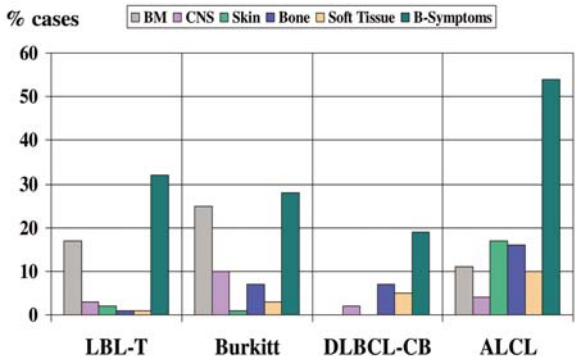

Figure 8.1

Organ involvement and presence of B-symptoms in children with anaplastic large-cell lymphoma (ALCL) compared to other histologic subtypes of NHL of childhood and adolescence. BM, bone marrow; CNS, central nervous system; LBL-T, lymphoblastic lymphoma, T-cell type; DLBCL-CB, diffuse large-cell lymphoma, centroblastic subtype. Source: BFM study group registry, *n*=1602 consecutive patients

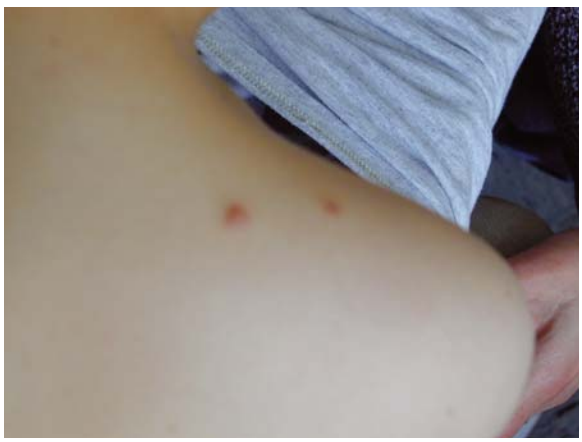

Figure 8.2

Papulomatous skin lesions of ALCL

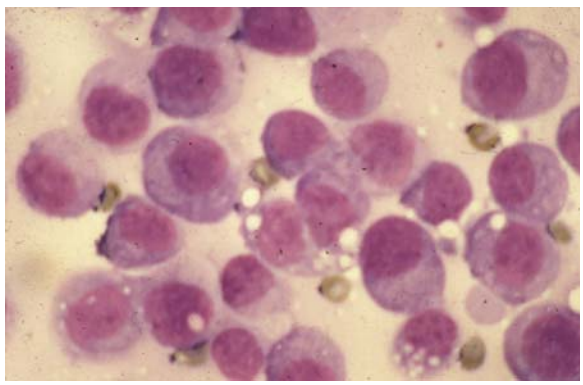

Figure 8.3

Cytospin preparation of cerbrospinal fluid of a patient with ALCL and mengingeal disease. The histology of skin lesions revealed a lymphohistiocytic subtype of ALCL. In the cerebrospinal fluid, the lymphoma cells resembled the small-cell variant of ALCL characterized by rather monomorphic smaller cells with deep basophilic cytoplasm and only discrete irregularity of nuclei. The cells were carrying the chromosomal translocation t(2;5)(p23;q35) as detected by two-color fluorescence in situ hybridization

Table 8.2 Clinical characteristics of ALCL patients in multicenter studies

Reference	Reiter et al. 1994	Brugieres et al. 1998	Seidemann et al. 2001	Williams et al. 2002	Mori et al. 2003
No. of patients	62	82	89	72	31
Boys	66%	56%	70%	57%	61%
Female	34%	44%	30%	43%	39%
Age (years) median	9.7	10	10.5	11.8	11
range	0.8–17.6	1.4–17	0.8–17.3	1.1–16.4	2–18
Stage, St. Jude					
I	6	I+II 28%	9%	6	10%
II	26		22%	15	6%
III	56	III+IV 72%	62%	73	74%
IV	11[a]		7%	6	10%
Stage, Ann Arbor			na	na	na
I	13	I+II 30%			
II	29				
III	21	III+IV 70%			
IV	37				
B-symptoms	42%	68%	52%	50%	na
Splenomegaly	21	21%	11	22%	23%
Hepatomegaly	27	17%	25	15%	26%
Tumor sites:[b]					
lymph nodes	90	94%	90	88%	97%
mediastinal mass	29	39%	31	40%	52%
skin	14	33%	18	25%	na
bone	21	12%	16	12%	19%
lung	6	13%	15	12%	na
soft tissue	21	12%	15%	8%	na
effusions	14%	7%[d]	15%	19%[d]	26%
bone marrow	0%[c]	16%[e]	6%	1%[c]	10%
CNS	1%		1%	4%	0%
LDH	na	≥800 U/L 18%	≥500 U/l 9%	na	na

na, not available; [a]mulitfocal bone counted as stage IV; [b]multiple sites may be affected in one patient; [c]based on bone marrow aspirations; [d]data only for pleural effusions; [e]based on bone marrow biobsy

ALCL (Reiter et al. 1994; Seidemann et al. 2001; Williams et al. 2002; Brugieres et al. 1998). Applying RT-PCR technology to detect ALCL-specific fusion gene products (Downing et al. 1995) increases the proportion (up to 50%) of patients showing ALCL cells either in the BM or peripheral blood (Rosolen et al. 2004). Not infrequently, marked hemophagocytosis by macrophages can be observed in the BM of ALCL patients.

CNS disease may occur as meningeal involvement, diagnosed by the presence of ALCL cells in the CSF (Fig. 8.3) and/or as an intracerebral mass. In rare cases, ALCL may present as isolated meningeal disease (Reiter et al. 1994). Testicular involvement is rare in ALCL. Among more than 270 ALCL patients enrolled in the BFM studies since 1990, there were only two boys with initial involvement of the testis. In rare cases, the neu-

trophil-rich ALCL may mimic inflammatory processes (abscess) in bone, skin, and soft tissue (Tamiolakis et al. 2004; Mann et al. 1995; Simonart et al. 1999; Mira et al. 2003).

Some of the distinctive clinical features of ALCL may be due to cytokine production by the tumor cells. ALCL cells have been shown to produce Il-6, which may be responsible for fever, bone lesions, and thrombocytosis, Il-9, Il-4, interferon-gamma, as well as GM-CSF and G-CSF, which may induce leukocytosis (Agematsu et al. 1991; Merz et al. 1991a,b; Nishihira et al. 1992). In NHL-BFM 90, 12% of the ALCL patients had a leukocyte count of more than 20,000/μL at the time of diagnosis. Thrombocytosis of more than 500,000/μL was noticed in 21% of the patients, while thrombocytopenia of less than 100,000/μL was seen in 6% of patients, although none of them had detectable bone marrow invasion. Despite the presence of rather large tumors, fewer patients with ALCL than those with Burkitt or lymphoblastic lymphoma have significantly increased serum lactate dehydrogenase (LDH) concentrations. This is in accord with a lower risk for acute tumor lysis syndrome after the start of chemotherapy in these patients.

In contrast to patients with advanced stage Burkitt lymphoma or lymphoblastic lymphoma, patients with ALCL rarely present with life-threatening complications at diagnosis. Exceptions are patients with large mediastinal masses who are prone to respiratory distress and superior vena cava syndrome due to compression of mediastinal structures. In rare cases, disseminated intravascular coagulation of an as yet unknown pathogenesis has been observed (Arber et al. 1991).

8.4 Staging

The St. Jude staging system is widely used for staging NHL in childhood (Murphy 1980) (Chapter 9). This staging system is based on the clinicoanatomical pattern and the more frequent involvement of bone marrow and CNS in lymphoblastic lymphoma and Burkitt lymphoma and has proved useful for the stratification of therapy intensity for the treatment of these entities (see Chapters 7 and 9). Due to the more unusual distribution pattern (e. g., extranodal disease) in ALCL, the validity of the St. Jude staging system for the stratification of treatment in this context is less well defined. For example, the staging of patients with multifocal bone lesions without bone marrow involvement, or with multiple skin or soft-tissue lesions lacking BM or/and CNS involvement, is difficult because these sites have no special consideration in the St. Jude staging classification. Staging according to the modified Ann Arbor classification for Hodgkin lymphoma (Lister et al. 1989) assigns patients with involvement of extralymphatic organs such as skin, bone, liver, and soft tissue to stage IV even if they have no BM or/and CNS disease. Thus, the proportion of patients assigned to stage IV differs remarkably between the St. Jude staging system and the Ann Arbor staging classification (Table 8.2). In addition, the Ann Arbor classification is not totally satisfactory because the frequent bone involvement in ALCL patients has no prognostic impact with current treatment protocols (Reiter et al. 1994; Seidemann et al. 2001; Brugieres et al. 1998; Williams et al. 2002) while visceral involvement confers an adverse prognosis (Brugieres et al. 1998; Le Deley et al. 1999). In addition to the above imaging procedures described in section 8.2, a complete blood count, cerebrospinal fluid CSF analysis, and BM aspiration from at least two sites are mandatory.

The diagnosis of initial CNS disease in ALCL patients is confirmed by the presence of morphologically identifiable ALCL cells in the CSF on cytospin preparations, especially if they demonstrate Alk-1 staining, and/or the presence of intracerebral infiltrates on cranial CT or MRI. In difficult cases, a stereotactic guided needle biopsy may be considered. In cases where only single suspicious cells are observed on CSF cytospin preparations, immunocytochemistry staining with Alk-1 antibody or/and FISH analysis using 2p23-specific probes may be useful for clarification.

8.5 Treatment and Outcome

The approach to treatment of children and adolescents with ALCL since the late 1980s has been quite variable. Some groups treated these children with acute lymphoblastic leukemia protocols, whereas other investi-

Table 8.3 Treatment results in multicenter therapeutic studies for childhood anaplastic large-cell lymphoma

Protocol	Stratification of treatment	No. courses/ duration	No. of patients	Outcome[a]	Remission failure	Toxic death	Relapses	Time diagnosis to failure (months)	Ref
HM89/91 multicenter	No	10 courses 8/7 months	82	pEFS 3y 66%	4	0	21	10 (7–49)	Brugieres et al. 1998
NHL-BFM 90 multicenter	Stage I-,II-r I-,II-nr, III IV	3 courses/2 mo 6 courses/5 mo 6 courses/5 mo	89	pEFS 76% at 5y	2	0	20	6 (5–65)	Seidemann et al. 2001
UKCCSG-NHL 9001/9002/9003 multicenter	Stage I Stage II, III, no-CNS-IV	8 courses/nd 5 courses/nd	72	pEFS 59% at 5y	11[b]	3	13	5 (3–14)	Williams et al. 2002
	IV-CNS-pos	9 courses/nd							
APO-POG multicenter	No	17 courses 12 months	86	pEFS 72% at 5 y	a	a	a	a	Laver et al. 2005[a]
LSA_2-L_2-mod multicenter	No	24 months	34	pEFS 65%	3	1	8	26 (2–40)	Rosolen et al. 2005

Abbreviations: nd, no data; CPM, Cyclophosphamid; Ifo, Ifosfamid; Dox, Doxorubicin; Epipod, Epipodophyllotoxin; pEFS, probability of event-free survival; CRT, cranial radiotherapy; [a]data not specified for ALCL patients; [b]plus to toxic death before determination of response

gators used short, pulse B-cell lymphoma-type chemotherapy (Vecchi et al. 1993; Reiter et al. 1994; Massimino et al. 1995). Local radiotherapy 30–35 Gy was applied to bulky disease in some patients.

Table 8.3 summarizes the results of treatment in children and adolescents with ALCL from several multicenter studies. The overall results were comparable with very different treatment strategies. With a modified LSA_2-L_2 protocol, a probability of event-free survival of 65% was achieved in a multicenter trial in Italy (Rosolen et al. 2005). In the study NHL-BFM 90 of the German-Austrian-Swiss BFM group, patients with ALCL were treated according to a regimen derived from the treatment program for B-cell lymphoma. The treatment consisted of 5-day courses of combination chemotherapy which were given with short intervals of approximately 3 weeks. The Kaplan-Meier estimate for 9-year event-free survival in 89 patients enrolled into Study NHL-BFM 90 was 76% (95% CI 67–85%) (Seidemann et al. 2001). The pattern of failure was characterized by early occurrence (18 of 20 relapses occurred within 15 months after completing therapy). A similar observation was made in the UKCCSG study where ALCL patients were treated according to the French B-cell lymphoma protocols (Williams et al. 2002). These protocols are described in Chapter 7. The EFS was 66% in the French studies HM89/91 using modified B-cell lymphoma protocols. Relapses occurred at a mean of 10 months from diagnosis (Brugieres et al. 1998) in HM89/91. An EFS of 72% at 5 years for 86 ALCL patients was achieved in a POG study with the APO regimen which features anthracyclines without alkylating agents or epipodophylotoxins (Laver et al. 2005).

Table 8.4 Number of drugs and cumulative doses in multicenter therapeutic studies for anaplastic large-cell lymphoma of childhood

Protocol	Branch	Pred (g/m^2)	Dexa (g/m^2)	CP (g/m^2)	Ifo (g/m^2)	VCR (mg/m^2)	Doxo (mg/m^2)	HD-MTX (g/m^2)	MTX Infusion time	ARA-C (g/m^2)	Epipo (mg/m^2)	Other	Reference
HM91	No	4.8	0	10.3	0	13	360	3 ×6	3h	0	1,200	VBL 48 mg/m^2 Bleo 160 mg/m^2	Brugieres et al. 1998
NHL-BFM 90	Stage I-,II-r	–	0.19	1.4	8	–	50	0.5 ×3	24h	0.15 ×8	400	–	Seide-mann et al. 2001
	I-,II-nr, III	–	0.34	3.4	12	–	150	0.5 ×6	24h	0.15 ×12	600	–	
	IV	–	0.34	3.4	12	9	150	5 ×6	24h	0.15 ×12	1300	–	
UKCCSG NHL 9001/9002/9003	Stage I	1	0	0	0	3	100	1 ×4	3h	0.2 ×10	400	TG 0.75 g/m^2	Williamse et al. 2002
	Stage II, III, IV	1.32	0	5.8	0	6	180	3 ×5	3h	0.1 ×10	0	–	
	CNS-disease	1.32	0	7.3	0	8	240	8 ×3	3h	4 ×8 0.1 ×10	2800	–	
APO-POG	No	10.72		0	0	27	300	0.06 ×11	nd	0	0	6-MP 18 g/m^2	Laver et al. 2005
LSA_2-L_2-mod		-	1.23	7.5	-	50	120	2 ×2	2 h	0.15 × 15 0.3 ×21	9450	L-Asparagina-se 10,000 IU/m^2 ×12 6-TG [a], 6-Mp[a]	Rosolen et al. 2005

CP, cyclophosphamide, Ifo, ifosfamide, VCR, vincristine, MTX, methotrexate, Doxo, doxorubicin, ARA-C, cytarabine, Epipo, teniposide or etoposide; VBL, vinblastin; Bleo, bleomycin;TG, thioguanin; 6_MP, 6-mercaptopurin; r, resected; nr, not resected

[a]Thioguanine during consolidation, 6-mercaptopurine during maintenance

Table 8.5 CNS prophylaxis in multicenter therapeutic studies for anaplastic large-cell lymphoma of childhood

Study/Protocol	HD-MTX (g/m^2)	i.th. therapy	CRT	Dexamethasone	Patients	CNS-pos	Relapses	CNS Relapse alone	CNS Relapse combined	Reference
HM89/91	3 ×6 3h i.v. infusion	No	No	No	82	0	21	0	0	Brugieres et al. 1998
NHL-BFM 90	0.5 ×6 stage IV 5 ×6 24h i.v. infusion	Triple ×6 Stage IV Half dose triple ×12	No	Yes	89	1[a]	20	1[d]	0	Seidemann et al. 2001
UKCCSG-NHL 9001/9002/9003	Stage I: 1 ×4	For head and neck only	No	No	72					Williams et al. 2002
	Stage II-IV 3 ×5 3h i.v. infusion	MTX/HC ×10	No			3[c]	13[b]	nd	nd	
APO-POG	Randomized +/- 1 g/m^2 24h i.v. infusion	MTX ×6	No	No	86	d	d	d	d	Laver et al. 2005[d]
LSA_2-L_2-mod AIEOP LNH92	2g/m^2 ×2, 2h i.v. infusion	Triple ×11 stage I-II Triple×18 stage III-IV	No	Yes	34	0	8	nd	nd	Rosolen et al. 2005

Abbreviations: nd, no data

[a]patient received fractionated intraventricular chemotherapy during courses no. 2–6 and cranial radiaotherapy 24 Gy

[b]not specified for initial stage and CNS status of patients

[c]CNS-pos patients receive MTX 8 g/m^2 over 3h ×5, Ara-C 3 g/m^2 × 4, and triple i.th ×3, one of them received CRT 24 Gy

[d]data not specified for ALCL patients

8.5.1 Stratification of Treatment Intensity

In the BFM and UKCCSG studies, the treatment intensity was stratified for presumed risk features, while in the other studies referenced in Table 8.3, all patients received the same treatment intensity. In the study NHL-BFM 90, therapy intensity was stratified according to stage (Seidemann et al. 2001). Patients with stage I and II disease and complete tumor resection received three courses of therapy while all others received six courses. The therapy courses for patients with stage I, II, and III disease contained an intermediate dose 0.5 g/m² of MTX, while high-dose MTX 5 g/m² was given to patients with stage IV disease and/or multifocal bone involvement. Furthermore, the latter received a third therapy element composed of high-dose cytarabine, etoposide, dexamethasone, and i.t. therapy. All therapy was delivered within 2 (stage I, and II-resected) to 5 months. In the UKCCSG study, treatment intensity was also stratified for stage (Williams et al. 2002). Patients with stage I received a less intensive although longer treatment sparing alkylating agents, while patients with stages II-IV/non-CNS disease were treated with a shorter but more intensive regimen including anthracyclines. The few patients with initial CNS disease received a higher dose of MTX and additionally HD-Ara-C and etoposide.

Steroids, vincristine, doxorubicin, and MTX were common components in all regimens (Table 8.4). However, the individual doses, the number of doses, and the cumulative doses of these drugs differed considerably. In the BFM regimen as well as in the modified LSA_2-L_2 of the Italian group, dexamethasone was used instead of prednisone. Doxorubicin was given up to a total cumulative dose of 300 mg/m² and 360 mg/m² in the APO regimen and the French HM 91 regimen, respectively, whereas doxorubicin was given at a much lower cumulative dose in the UKCCSG, the BFM protocol, and the modified LSA_2-L_2 of the AIEOP. Low-dose MTX was applied in the APO regimen, whereas medium- and high-dose MTX was given in the other studies. Except for the APO regimen, cyclophosphamide was part of the treatment in all studies, with a wide range of the cumulative doses. Other drugs such as cytarabine, epipodophyllotoxins, 6-mercaptopurine, thioguanine, and bleomycin were included with varying individual and cumulative doses in some, but not all regimens. Vinblastine was only given in the French HM 89/91 study.

These regimens also differed considerably in the duration of treatment (Table. 8.3). The BFM regimen was delivered within 2 (stage I+II with resected tumors) to 5 months. Total therapy duration was similarly short in the UKCCSG study. In the modified LSA_2-$L2_2$ protocol of the Italian group, therapy was given over 24 months, while in the HM 89/91 regimen and the APO regimen treatment duration was 8 and 12 months, respectively. The majority of tumor failures occurred within the first year from diagnosis. Late recurrences, some more than 5 years after diagnosis, were observed in all studies regardless of the duration of therapy (Table. 8.3). Due to the heterogeneity of these regimens with respect to the use of individual drugs and drug dosages and the different schedules of administration, only limited conclusions can be drawn as to key components of the protocols. From the POG study (5-drug APO regimen), one might conclude that doxorubicin, vincristine, and steroids are key drugs (Laver et al. 2005). Alkylating agents, high-dose MTX, and etoposide were the main components of most regimens, but these agents were absent in the APO regimen at the expense of rather high cumulative doses of doxorubicin, vincristine, and prednisone (Laver et al. 2005). An interesting observation was the efficacy of vinblastine in the management of recurrent ALCL (Brugieres et al. 2000). The role of vinblastine in front-line therapy is currently under investigation in a large European intergroup randomized trial and North Amercian COG study.

8.5.2 CNS Prophylaxis and CNS Therapy

The CNS is rarely involved in ALCL patients at the time of diagnosis (Table 8.2) or at relapse (Table 8.5). No CNS relapses were observed among 170 CNS-negative patients in the French HM and German-Austrian-Swiss BFM studies in which patients did not receive prophylactic cranial radiotherapy (Brugieres et al. 1998; Seidemann et al. 2001) (Table 8.5). Thus, reliable evidence exists that cranial radiotherapy is not needed for the prevention of CNS relapses in ALCL patients without overt CNS disease at presentation. In the HM 89/91 studies, patients received MTX 3 g/m² as i.v. in-

Table 8.6 Local therapy modalities and site of failure in multicenter therapeutic studies for anaplastic large-cell lymphoma of childhood

Protocol	Local Radiotherapy	No. of patients	Remission failure	Relapses	Restricted to initial sites	New sites invoved	Local involved	BM involved	CNS involved	Ref
HM-89/91	no	82	4	21	3	18	nd	nd	0	Brugieres et al. 1998
NHL-BFM 90	no	89	2	20	11	9	15	1	1	Seidemann et al. 2001
UKCCSG-NHL 9001/9002/9003	no	72	11	13	4	9	nd	nd	nd	Williams et al. 2002
APO-POG	Restricted to patients with viable residual tumor after induction	86	nd	nd	nd	nd	nd	nd	nd	Laver et al. 2005
LSA_2-L_2-mod	Restricted to patients with residual tumor ≥ 5cm after consolidation	34	3	8	nd	nd	nd	nd	nd	Rosolen et al. 2005

fusion over 3 h but no intrathecal (IT) therapy, while in the BFM regimen, CNS-directed therapy consisted of IT MTX alone or triple IT therapy in combination with intermediate dose 0.5 g/m² MTX for stage I-III patients or high-dose MTX 5g/m² for stage IV patients. In addition, the BFM patients received dexamethasone as the steroid form. Thus, from the French HM 89/91 studies it can be concluded that even IT therapy may be omitted provided systemic MTX is administered in a schedule which results in cytotoxic MTX levels in the CSF. Whether IT chemotherapy can safely be replaced by systemic MTX 3 g/m² as i.v. infusion over 3 h is currently being tested in a randomized fashion in a European intergroup trial.

Due to the small numbers of patients with overt CNS disease at diagnosis, no conclusions can currently be drawn as to the optimal treatment in this circumstance (Seidemann et al. 2001).

8.5.3 Role of Local Therapy Modalities and Pattern of Therapy Failure

Complete resection of localized disease may be advantageous. In NHL-BFM 90, all nine patients with completely resected localized tumor survived event-free after having received only three 5-day courses of chemotherapy. In patients with large tumors or widespread disease, surgical debulking is of no apparent value. The role of radiotherapy is difficult to determine from the available published information from multicenter trials. Local radiotherapy was only included in the modified LSA_2-L_2 protocol of the Italian group and the APO protocol of the POG for patients with residual tumor after consolidation or induction therapy, respectively (Table 8.6). Nevertheless, some observations are possible from an analysis of the pattern of tumor failure observed in these studies (Table 8.6). In the HM 89/91, BFM, and UKCCSG studies in which local radiotherapy was not part of the protocol, only one-third of the relapses observed were restricted to initial sites, while in two-thirds of patients, the relapses

Abbreviations: nd, no data

[a] in one patient new manifestations appeared after one course of therapy whereas the initial tumors disappeared

[b] one patient with initial meningeal disease suffered from CNS relapse

were in new sites of disease (Reiter et al. 1994). In POG studies using CHOP chemotherapy with or without involved field radiation (RT) for early stage NHL, there was no survival advantage with the addition of RT for any histologic subtype of NHL including ALCL (Link, personal communication).

The patient with ALCL who does not achieve a complete clinical or radiologic response to chemotherapy poses a diagnostic and therapeutic dilemma in some instances. There are limited data to assess the role of second-look surgery in these situations. In the French study HM 89/91, eight patients underwent second-look surgery for suspected residual tumor after the third course of therapy, and seven had completely necrotic tumor (Brugieres et al. 1998). All seven patients with necrotic tumor remained long-term disease-free survivors. Similar findings were noted in a previous BFM series (Reiter et al. 1994). In NHL-BFM 95, of 85 ALCL patients 17 were reported to have residual tumor after the 5th course of therapy. Only one of them underwent second-look surgery, which revealed a complete necrotic mass. In 16 of these patients, no surgery or biopsy was undertaken. Twelve of them remained free of disease, while two suffered from local relapse and two other patients suffered from CNS relapse (unpublished data). A special challenge with respect to incomplete tumor resolution during chemotherapy arises in patients with mediastinal tumors. In study NHL-BFM 90, 14 of 28 patients with a mediastinal mass at diagnosis had a residual mediastinal mass at the end of the treatment. Three of these patients suffered local progression, ten remained free of progression, and one patient was lost to follow-up (Seidemann et al. 2001).

Theses observations suggest that in the majority of cases persistent tumor by imaging during and after chemotherapy do not confer an increased risk for subsequent local progression of the disease, provided there has been a good response (>PR) to chemotherapy. Imaging methods such as PET may contribute to the detection of persistent active tumor during and after chemotherapy in order to allow for early adjustment of treatment modalities before clinical progression of the disease takes place. In addition, monitoring of residual clonal lymphoma cells in the PB and/or BM using molecular methods may help to identify those patients at risk for subsequent tumor progression.

8.6 Prognostic Factors

In patients suffering from ALCL, a number of variables have been reported to be associated with the risk of treatment failure, but the prognostic impact of such features depends on the treatment applied. Table 8.7 summarizes the results of the analysis of the prognostic impact of clinical and biological features in three larger multicenter studies of childhood and adolescent ALCL. In none of these series did the age and gender of the patients or the stage of disease have a significant impact on the outcome in multivariate analysis. In the BFM study NHL-BFM 90, there was no statistically significant difference in outcome among patients with St. Jude stage II, III, and IV (Fig. 8.4). The French HM 89/91 studies might be the most informative because all patients received the same treatment. In these studies only patients with a mediastinal mass, any visceral involvement, or a LDH > 800 U/L had a significantly higher risk for failure in a multivariate analysis (Brugieres et al. 1998). In study NHL-BFM 90, only the presence of B-symptoms were adverse prognostic factors, while in the UKCCSG study, no single parameter was associated with an increased risk for failure (Seidemann et al. 2001; Williams et al. 2002). In a multivariate analysis of the pooled data of 235 patients from all three studies, the presence of a mediastinal mass, visceral involvement of the lung, focal lesions of liver or spleen, and/or enlargement of liver and/or spleen >5 cm below the costal margin, and skin involvement were significantly associated with an increased risk for failure (Le Deley et al. 1999). Whether BM involvement and/or CNS disease confers a worse prognosis cannot yet be determined due to the low numbers of patients with these features in reported studies.

A T-cell versus null-cell phenotype of ALCL and ALK-1 reactivity had no prognostic impact in the HM and BFM studies. However, most of the pediatric cases of ALCL are T-cell and ALK-1 positive. In the HM 89/91 study, the lymphohistiocytic variant was associated with an increased risk for failure in the univariate but not in the multivariate analysis (Seidemann et al. 2001; Brugieres et al. 1998)

The prognostic significance of elevated serum levels of LDH is not consistent between studies. (Brugieres et al. 1998; Seidemann et al. 2001). Serum levels of soluble

Table 8.7 Adverse prognostic impact of clinical and biological features in childhood anaplastic large-cell lymphoma in three multicenter studies

Reference	Brugieres et al. 1998	Seidemann et al. 2001	Williams et al. 2002
No. of patients	82	89	72
Boys	–	–	–
Girls	–	–	–
Age ≥ 10 years	–	nd	–
Stage, St. Jude		–	–
I + II			
III +IV	u 0.006/m 0.5		
B-symptoms	–	u 0.04/m 0.05	–
Splenomegaly	u 0.02/m0.5	u 0.04/m >0.05	–
Hepatomegaly	u 0.005/m0.5	–	–
Tumor sites			
• lymph nodes	–	–	
• mediastinal mass	u 0.0002/m 0.01	–	u 0.05/m 0.1
• skin	u 0.01/m 0.16	–	–
• bone	–	–	–
• lung	u 0.00008/m 0.09	u 0.03/m >0.05	–
• soft tissue		–	–
• effusions		–	–
• bone marrow		–	–
• CNS		–	–
LDH	≥800 U/L	–	nd
	u 0.009/m 0.05		
Immunophenotype	–	–	nd
T			
null			
Alk-1 pos	–	–	nd
Alk-1 neg		–	

Abbreviations: u, univariate analysis; m, multivariate analysis; -, p >0.05; nd, not done

IL-2 receptor have been found to correlate with stage and prognosis in NHL (Pui et al. 1987) and may also be of prognostic value in ALCL (Janik et al. 2004). An even more specific parameter of the total tumor burden may be the serum concentration of the CD30 antigen which is released from ALCL cells (Younes et al. 1997; Nadali et al. 1995; Zinzani et al. 1998). However, data on the prognostic value of these parameters are available only from adult ALCL patients. Anti-ALK antibodies were identified in varying titers in the plasma of patients with ALK-1-positive ALCL, implying an immune response to the fusion protein. The prognostic significance of this antibody remains to be determined (Pulford et al. 2000). The prognostic value of molecular monitoring of minimal residual disease in peripheral blood or bone marrow is currently under investigation.

8.7 Management of Relapse

A distinct feature of ALCL is that the majority of patients who relapse respond well to salvage therapy. However, the course after relapse seems to be variable. Some patients have a rapidly fatal course after relapse, whereas other patients achieve long-term second remissions after treatment with conventional chemotherapy alone. Most intriguing, even for patients who relapse following ABMT, are reports of long-lasting remissions with vinblastine monotherapy. Spontaneous regression and an indolent long-term waxing and waning course without salvage therapy have also been observed in select patients, especially those with a prior history of lymphomatoid papulosis.

There are only a few reports detailing treatment results after relapse following front-line therapy of ALCL. Chakravarti et al. reported in 1990 the long-term survival of two children with CD30-positive ALCL who had suffered a first or second relapse by use of autologous bone marrow transplantation (Chakravarti et al. 1990). Of the 20 patients who relapsed during study NHL-BFM 90, nine patients remained in long-lasting second remission after rescue chemotherapy alone (one patient) or after autologous (six patients) or allogeneic (two patients) hematopoetic blood stem cell transplantation (HSCT) using a TBI-based conditioning regimen (Seidemann et al. 2001). Most of these relapsed patients achieved a second remission with a combination chemotherapy consisting of high-dose Ara-C, etoposide, vindesine, dexamethasone, and i.t. therapy followed by the same therapy courses as given during front-line treatment but intensified with high-dose MTX (Attarbaschi et al. 2005; Williams et al. 2002).

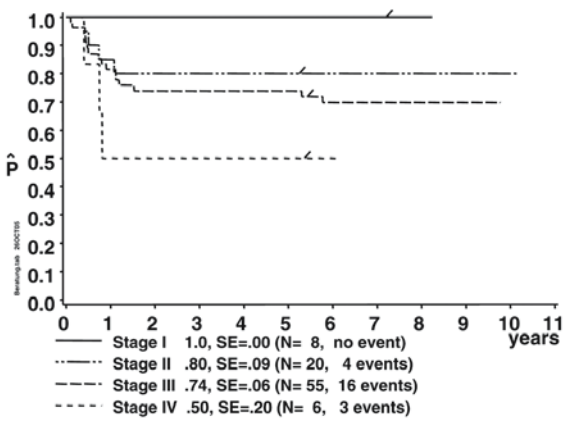

Figure 8.4

Kaplan-Meier estimate of event-free survival at 5 years according to St. Jude stages of ALCL patients treated in study NHL-BFM 90.The p log.rank > 0.1 in all comparisons except stage I vs stage IV ($p = 0.027$). SE, standard error.

Brugieres et al. reported the largest series to date of 41 children with recurrent ALCL in three consecutive front-line studies (Brugieres et al. 2000). In the first 20 patients, salvage chemotherapy was based upon 3-week courses of CCNU, vinblastine, and bleomycin delivered every 6 weeks for 12 months (Table 8.8). In the following 21 patients, Ara-C was given as a substitute for bleomycin (course VCA, Table 8.8). Of the total group of 41 patients, 36 patients achieved second CR. Of these, 21 continued with chemotherapy alone, and 11 of them were in second CR at the time of the report. Ten patients had a second relapse. Of the 15 CR patients who underwent autologous BMT or HSCT, nine patients remained in second CR at the time of the report, while five suffered from a second relapse, and one died of toxicity. In terms of the probability of disease-free survival, there was no statistically significant difference between patients receiving chemotherapy alone or intensification with ABMT. However, the role of ABMT in the treatment of children with ALCL relapse cannot be discerned unequivocally from this study. Examining the prognostic parameters, apart from the time of the occurrence of relapse from first diagnosis of ALCL (patients suffering from relapse within 12 months from first diagnosis of ALCL did worse), the intensity of the front-line protocol had a significant impact on the outcome after relapse. The probability of disease-free survival of patients who relapsed after the less intensive cyclophosphamide/vincristine/prednisone/doxorubicine(COPAD) regimen was 58% and was 30% ($p = 0.02$) for those patients who were initially treated according to the more intensive HM89/91 regimens. Patients who did not undergo ABMT in second CR were mostly those treated with COPAD or the HM 89 protocol as first-line treatment whereas almost all the patients who relapsed after the

more intensive HM91 regimen underwent ABMT in second CR. Therefore, it cannot be excluded that the latter group would have had a worse outcome with chemotherapy alone.

An intriguing observation was made in the report of Brugieres et al. (Brugieres et al. 2000). Twelve patients were treated with weekly vinblastine 6 mg/m^2, 6–18 months for a first (four patients), second (five patients), or a subsequent (three patients) relapse including six relapses occurring after ABMT. Ten of 11 evaluable patients achieved a complete remission. Five of eight responders who received only weekly vinblastine remained in remission for more than 15 months after stopping the drug.

Thus, the management of children who fail first-line treatment for ALCL remains a challenge. Due to the rarity of the disease, international cooperation is required to evaluate different strategies in a prospective trial. The probability of disease-free survival after a first relapse seems to depend on the time to reccurrrence and the intensity of the front-line treatment. Early occurrence and high intensity of the front-line therapy seem to confer a higher risk for failure of salvage therapy. Of note, even after second or even third relapse, patients appear to survive for prolonged periods, which is quite unusual in other subtypes of childhood NHL.

Table 8.8 Relapse protocols CVBA and CVA of the French Society of Pediatric Oncology (Brugieres et al. 1998)

CBVA[a]			
CCNU	100 mg/m^2	p.o.	D1
Vinblastine	6 mg/m^2	i.v.	D1, 8, 15, 22
Bleomycin	20 mg/^{2}	i.v.	D1, 8, 15, 22
Ara-C (replaces bleomycin when a total dase of 200 mg/m^2 of bleomycin is reached	100 mg/m^2	i.v.	D1–5
CVA[a]			
CCNU	100 mg/m^2	p.o.	D1
Vinblastine	6 mg/m^2	i.v.	D1, 8, 15, 22
Ara-C	100 mg/m^2	i.v.	D1–5

[a] Courses every 6 weeks for 12 months
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8.8 Future Prospects

According to published data, ALCL remains the subgroup of childhood NHL with a rather high proportion of patients in whom first-line therapy fails to induce durable remission. Roughly two-thirds of patients can be cured by remarkably different treatment protocols, suggesting that the optimal chemotherapy regimen remains to be defined. Due to the rarity of the disease, randomized trials aimed at optimizing treatment should be carried out in an international collaboration in order to accrue the required number of patients in a reasonable time period. Current randomized trials are testing the impact of vinblastine added to a proven regimen of front-line therapy. However, considering the large heterogeneity of ALCL in terms of histology, genetics, clinical manifestations, and response to therapy, one could hypothesize that there are multiple subsets of patients with differing tumor cell biology and prognoses. Therefore, a new look at prognostic parameters in large prospective trials including clinical features, histological variants, cytogenetics, gene expression profiles, anti-ALK antibodies, molecular monitoring of minimal residual disease, and cytokine production profiles is necessary. Prospective evaluation of the sensitivity and specificity of PET technology for staging patients and assessing response to therapy is also needed.

New therapy options are becoming available. 13-cis-retinoic acid has induced remission in patients with relapse of ALCL (Chou et al. 1996). Since the CD30 antigen is absent in most normal tissues (Stein et al. 1985; Durkop et al. 2000), this antigen is an ideal tumor-specific target for immunotherapy. Different anti-CD30 immunotoxins were shown to exert antitumor activity in ALCL xenograft models in mice (Pasqualucci et al. 1995; Terenzi et al. 1996; Barth et al. 2000). In phase I clinical studies, anti-CD30 immunotoxins demonstrated activity in patients with chemotherapy-refractory CD30+ Hodgkin and non-Hodg-

kin lymphoma in adults (Schnell et al. 2002, 2003). Another alternative approach may be the coupling of cytostatic drugs to anti-CD30 moAbs which exert potent and selective antitumor activity in vitro and in an ALCL xenograft model (Francisco et al. 2003). Bi-specific antibodies against the CD30 antigen and lineage-specific antigens of immune effector cells, such as CD16 on NK cells or CD64 (high-affinity FcγRI receptor on neutrophils and macrophages) may be capable of targeting immune effector cells to CD30+ lymphoma cells (Renner et al. 2001; Borchmann et al. 2002). In addition, binding of anti-CD30 moAbs to the antigen may also induce direct effects on cell growth and survival of CD30+ lymphoma cells. Potent antitumor effects were observed in ALCL xenograft models using naked anti-CD30 moAbs derived from mice (Tian et al. 1995; Pfeifer et al. 1999) and with the mouse-human chimeric anti-CD30 moAb SGN-30 (Wahl et al. 2002). With the latter, antitumor activity was also observed in a clinical phase I study (Bartlett et al. 2002). CD30 is a 120-kd transmembrane glycoprotein belonging to the TNF receptor superfamily (Durkop et al. 1992; Falini et al. 1995), and the corresponding ligand has been cloned and found to be a member of the cytokine family, with homology to tumor necrosis factor (Smith et al. 1993). The ligand which is expressed on activated T cells has been shown to induce apoptotic cell death in ALCL cell lines upon binding but enhanced the proliferation of Hodgkin lymphoma-derived cell lines (Mir et al. 2000). The same authors could show similar in vitro effects with anti-CD30 antibodies. Whether binding of anti-CD30 antibodies to the CD30 receptor induces apoptosis of ALCL cells or only growth inhibition by inducing cell cycle arrest is not yet completely clarified (Levi et al. 2001; Hubinger et al. 2001, 2004). While the exact mechanism of the inhibitory effect of the ligation of the CD30 receptor on ALCL cells remains to be clarified, an important finding was that NPM-ALK fusion protein binds to the intracellular domain of the CD30 receptor of ALCL cells carrying the chromosomal translocation t(2;5) (Hubinger et al. 1999). Although the same investigators could not find any evidence for activation of the tyrosine kinase NPM-ALK upon stimulation of CD30, a CD30-mediated inactivation of NPM-ALK on CD30 stimulation cannot be ruled out.

The ALK fusion protein constitutively activates the ALK kinase and plays a key role in lymphomagenesis (Kuefer et al. 1997; Pulford et al. 2004b). Tyrosine kinase inhibitors such as imatinib have been found to have a great therapeutic potential in other malignancies in which oncogenic tyrosine kinases have a pathogenic function. (O'Dwyer and Druker 2000). Another ATP-competitive small molecule UCN-01 which inhibits numerous tyrosine kinases as a single agent has induced long-lasting stable disease in a patient with multiple relapses of ALCL including relapse after autologus BMT (Sausville et al. 2001). Currently, more ALK-selective inhibitors are under development (Cheng et al. 2004). However, by analogy to the observation of the use of imatinib in the treatment of CML (Druker 2002), continuous treatment with any of the ATP-competitive inhibitors for ALK will likely select for resistant ALK kinase mutant proteins. Therefore, combinations of small compounds will probably be more promising. Another strategy aims at increased degradation of ALK-tyrosine kinases. Herbimycin A was found to induce caspase-dependent apoptosis in ALCL cell lines (Ergin et al. 2001; Turturro et al. 2002). Herbimycin acts as an antagonist of the heat shock protein Hsp 90 which in turn interacts with the kinase domain of tyrosine kinases, preventing them from being degraded by proteasomes (Neckers et al. 1999). Ribozyme-mediated cleavage of NPM-ALK fusion transcripts has so far failed to suppress NPM-ALK levels sufficiently to affect cell growth significantly, presumably due the relatively long half-life of the NPM-ALK fusion protein of approximately 48 h (Hubinger et al. 2003). Similar observations were made by Ritter et al. using chemically synthesized small interfering RNAs for the downregulation of the NPM-ALK fusion mRNA in SR786 ALCL cells. Although endogenous NPM-ALK mRNA expression was decreased by 50–60%, a single transfection of the cells did not affect the amount of NPM-ALK fusion protein. Only after repeated transfections over 8 days was a significant reduction of the amount of NPM-ALK protein seen, but without induction of apoptosis (Ritter et al. 2003). Thus, the long half-life of the NPM-ALK fusion protein, together with high steady-state levels of the fusion protein expressed in ALCL cells, are factors that will hamper therapy approaches aimed at reduction of the chimeric protein.

ALK expression in normal cells is restricted mainly to rare cells in the brain. The restriction of ALK in an immunoprivileged site raises the possibility that ALK proteins of ALK-positive tumors could act as nonself antigen and could evoke a tumor-specific immune response in the patient. The detection of circulating anti-ALK IgG antibodies in patients with ALK-positive ALCL supports this possibility (Pulford et al. 2000, 2002). The preliminary evidence suggesting a more favorable prognosis of patients with high levels of circulating autologous anti-ALK antibodies lends promise for a future role of engineered anti-ALK antibodies in the treatment of ALK-positive ALCL patients (Pulford et al. 2000). Recently, Ait-Tahar et al. have confirmed the presence of a T-cell response in the blood of patients with ALK-positive ALCL (Ait-Tahar et al. 2005), and Passoni et al. have identified the ability of ALK peptide sequences to stimulate a MHC-restricted cytotoxic T-cell response in normal individuals (Passoni et al. 2002). Due to its restriction to the tumor and its causative pathogenic role in ALK-positive ALCLs, ALK-fusion proteins would be an ideal target for immunotherapy strategies. Thus, these provocative findings of preliminary studies of ALK-specific T- and B-cell responses in patients and normal individuals may open new horizons of highly specific immunotherapy strategies including vaccine trials, antibody therapy, and cellular-based therapy strategies in the autologous and allogeneic setting.

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Precursor B and Precursor T-Cell Lymphoblastic Lymphoma

J.T. Sandlund

Contents

9.1 Introduction

The malignant lymphomas are the third most common malignancy in childhood (Young et al. 1986; Bleyer 1990; Parker et al. 1996; Percy et al. 1999). Among children less than 15 years of age, there is a slight predominance of non-Hodgkin lymphoma over Hodgkin lymphoma (Percy et al. 1999). The most commonly encountered histologic subtypes of non-Hodgkin lymphoma in children according to the WHO classification include Burkitt lymphoma, diffuse large B-cell lymphoma, anaplastic large-cell lymphoma, and precursor B and precursor T lymphoblastic lymphoma (Murphy et al. 1989; Jaffe et al. 2001). Among these, the lymphoblastic lymphomas account for approximately 30% of childhood NHL (Murphy et al. 1989). The clinical features and advances in the diagnosis and treatment of lymphoblastic lymphoma will be reviewed in this chapter.

9.2 Diagnosis and Imaging

9.2.1 Diagnosis

The differential diagnosis of lymphoblastic lymphoma includes both malignant and benign conditions. In a child who presents with enlarged cervical lymph nodes and a normal complete blood count and chest X-ray (i.e., no mediastinal mass), it is reasonable to treat for a presumed bacterial adenitis with a 10- to 14-day course of antibiotics. Younger children (<4 years of age) and those individuals with a sore throat or upper respiratory symptoms and cervical adenopathy are the least likely to have a malignancy (Slap et al. 1984). Serologic and skin testing may be helpful in diagnosing

other infectious conditions including Epstein-Barr virus, histoplasmosis, toxplasmosis, brucellosis, and tuberculosis. The presence of enlarged supraclavicular nodes should increase the suspicion of a malignant process.

The diagnosis of lymphoblastic lymphoma is most readily made by a biopsy of the involved tissue. Enough tissue should be obtained for a comprehensive pathologic evaluation, including standard immunohistochemical stains of histologic slides, flow cytometric determination of immunophenotype, cytogenetic analysis, and molecular pathologic screening (see Chapter 6). These studies will help differentiate lymphoblastic lymphoma from other types of lymphoma and from small, round, blue cell tumors including neuroblastoma, rhabdomyosarcoma, and the Ewing family of tumors.

In some clinical settings, it is not possible to safely perform an open biopsy. For example, children with precursor T-cell lymphoblastic lymphoma often present with airway compression secondary to a large anterior mediastinal mass, putting them at very high risk during general anesthesia. Symptoms of orthopnea or a CT-calculated tracheal cross-sectional area less than 50% for age have been identified as significant risk factors for anesthetic complications (Shamberger et al. 1995). In this case, the diagnosis may be established by a parasternal fine needle aspirate or fine needle core biopsy with local anesthesia; an anesthesiologist should be in attendance, even if only light or conscious sedation is considered (Garrett et al. 2002). In situations of extreme respiratory compromise, it may be prudent to treat with steroids for 24 to 48 hours before considering any diagnostic procedure. However, there is a small risk of losing the diagnosis with pre-biopsy use of steroids (Loeffler et al. 1986). Many patients with lymphoblastic lymphoma involving the mediastinum also have a large pleural effusion. The cytological examination of pleural fluid obtained by a thoracentesis establishes the diagnosis in most cases. This is in contrast to Hodgkin lymphoma and diffuse large B-cell lymphoma in which it is unusual to make the diagnosis by examination of the pleural fluid. The examination of a bone marrow aspirate or biopsy may also yield a diagnosis and thus preclude the need for a more invasive procedure. However, in a patient with a normal complete blood count and differential, there is only a small likelihood of making a definitive diagnosis by examining the bone marrow.

The World Health Organization (WHO) Classification of Tumors of Haematopoietic and Lymphoid Tissues specifies criteria for the diagnosis of lymphoblastic lymphoma (Jaffe et al. 2001). This group of malignancies is divided into two broad groups on the basis of their immunophenotype: precursor B-cell neoplasms (including both precursor B lymphoblastic leukemia and lymphoma) and precursor T-cell neoplasms (including both precursor T lymphoblastic leukemia and lymphoma). The clinical distinction between acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma is determined on the basis of the degree of bone marrow involvement: those with >25% replacement by lymphoblasts are designated as ALL, whereas those with a lesser degree of replacement are considered to have advanced stage lymphoblastic lymphoma with marrow involvement (Murphy 1980).

The histopathology, immunophenotype, cytogenetics, and molecular pathogenesis of the lymphoblastic lymphomas are discussed in detail in Chapter 6. The lymphoblasts in ALL and lymphoblastic lymphoma have overlapping morphologic, immunophenotypic, and cytogenetic features, prompting many to speculate that the distinction between the two is largely arbitrary (Head and Behm 1995). Attempts to distinguish T-cell lymphoblastic lymphoma from T-ALL on the basis of immunophenotype have for the most part been unsuccessful. However, with respect to normal intrathymic maturation stage, some studies have reported a higher rate of early stage thymocyte differentiation among T-ALL cases as compared to T-lymphoblastic lymphoma, where an intermediate or late stage of differentiation predominates (Roper et al. 1983; Mori et al. 1988; Shikano et al. 1994). It has also been suggested that T lymphoblastic lymphomas are characterized by a more frequent expression of T-cell receptor αβ than γδ, compared with T-ALL (Gouttefangeas et al. 1990). Current molecular studies (e. g., DNA microarrays) are aimed at better defining the relationship between lymphoblastic lymphoma and ALL.

There is no prognostic significance associated with the specific chromosomal abnormalities in lympho-

Table 9.1 Stages of Non-Hodgkin Lymphoma (based on the classification proposed by Murphy 1980)

Stage I
A single tumor (extranodal) or involvement of a single anatomical area (nodal), with the exclusion of the mediastinum and abdomen
Stage II
A single tumor (extranodal) with regional node involvement
Two or more nodal areas on the same side of the diaphragm
Two single (extranodal) tumors, with or without regional node involvement on the same side of the diaphragm
A primary gastrointestinal tract tumor (usually in the ileocecal area), with or without involvement of associated mesenteric nodes, that is completely resectable
Stage III
Two single tumors (extranodal) on opposite sides of the diaphragm
Two or more nodal areas above and below the diaphragm
Any primary intrathoracic tumor (mediastinal, pleural, or thymic)
Extensive primary intra-abdominal disease
Any paraspinal or epidural tumor, whether or not other sites are involved
Stage IV
Any of the above findings with initial involvement of the central nevous system, bone marrow, or both

blastic lymphoma that were enumerated in Chapter 6. This may in part be due to the relatively small number of cases of lymphoblastic lymphoma for which the results of cytogenetic studies have been reported. Microarray analyses for gene expression profiling of lymphoblastic lymphoma cases may identify subgroups at increased risk for treatment failure,as has been reported for T-ALL (Ferrando et al. 2002, 2004; Yeoh et al. 2002; Ferrando and Look 2003).

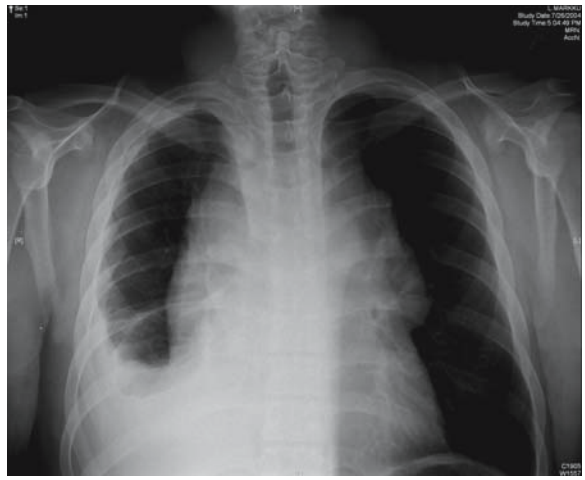

Figure 9.1

Chest radiograph showing large anterior mediastinal mass and pleural effusion in a child with a precursor T-cell lymphoblastic lymphoma

9.2.2 Imaging/Staging

There are a number of imaging studies which should be included in the workup of a child with lymphoblastic lymphoma. A screening chest X-ray should be performed on every child who is being evaluated for a possible malignant lymphoma. This is critical, especially before any anesthesia is given, because the presence of a mediastinal mass may result in airway compromise at the time of anesthesia/sedation. If a mediastinal mass is appreciated on chest X-ray, a CT scan of the neck and chest with intravenous contrast should be obtained before any invasive procedure is performed. Once the diagnosis has been confirmed, a CT scan of the abdomen and pelvis should be performed. Nuclear imaging studies (e. g., gallium and bone scans) are sometimes included in the diagnostic imaging workup of these patients. Bone scans may be useful in localizing bone pain associated with pathologic fractures or lytic lesions from lymphoblastic infiltration. Gallium and more recently PET scans have been used to both stage patients and assess the response to chemotherapy (Kostakoglu et al. 2002). However, there is a paucity of published data about the sensitivity and specificity of gallium seen and PET

scans in lymphoblastic lymphoma as well as data correlating response by PET or gallium and overall outcome. Brain imaging is not routinely indicated in the workup of these children unless there are cranial nerve palsies. Even in the setting of a cranial nerve palsy, an MRI with gadolinium may not show evidence of lymphomatous infiltration. An examination of a cytocentrifuged specimen of CSF is still the best diagnostic test to confirm CNS involvement by lymphoblastic lymphoma. Examination of the bone marrow obtained from a posterior iliac crest aspirate and biopsy should include morphology, flow cytometry for immunophenotyping, cytogenetics, and molecular screening. Upon completion of an expedited workup, a disease stage is assigned, usually according to the St Jude system described by Murphy (Murphy 1980) (see Table 9.1). Less than 20% of children with lymphoblastic lymphoma have stage 1 or 2 disease, and these children are likely to have a precursor B-cell phenotype; the majority of patients with stage 3 and 4 disease have a precursor T-cell phenotype.

9.3 Clinical Features

Children with precursor T-cell lymphoblastic lymphoma typically present with a mediastinal mass and/or bulky cervical/supraclavicular adenopathy with a normal complete blood count. By definition, they have less than 25% blasts in the bone marrow. In contrast, children with precursor B-cell lymphoblastic lymphoma often present with more limited disease involving skin, soft tissue, bone, tonsil, or a single peripheral lymph node site (Lin et al. 2000).

The presenting signs and symptoms of patients with lymphoblastic lymphoma are a manifestation of the primary sites of involvement. A mediastinal mass, which is typically in the anterior compartment, may be associated with a wide spectrum of respiratory symptoms, ranging from no symptoms at all or minor cough, to severe respiratory distress with arrest in some cases (Fig. 9.1). In some cases, associated malignant pleural effusions may worsen the respiratory compromise. Involvement of the mediastinum may result in impaired venous return and associated swelling of the neck and supraclavicular region, referred to as "superior vena cava (SVC) syndrome" (Ingram et al. 1990) – this condition may predispose to thrombus formation. These lymphomas may also invade the pericardium, resulting in a malignant pericardial effusion and possible tamponade (Magrath 2002).

Children may also present with painless swelling of lymph nodes in the head and neck region, axilla, or inguinal area. In more disseminated cases, hepatosplenomegaly may be detected on physical examination. Renal involvement is frequently detected on abdominal CT scans but is not usually associated with impaired kidney function. CNS involvement may be associated with cranial nerve palsies, symptoms of increased intracranial pressure including headache, nausea/vomiting, and papilledema with vision changes, or no symptoms as all (Sandlund et al. 2000). Because the bone marrow is infiltrated with less than 25% blasts, it is unusual for patients to manifest anemia, thrombocytopenia, neutropenia or have blasts in the peripheral blood (Sandlund et al. 1994). Bone involvement may cause pain or a limp. Skin involvement is uncommon, but as previously mentioned, it is often associated with precursor B disease and affects the scalp more often than other areas of the body (Link et al. 1983; Meyers and Hakami 1984). Overt testicular disease is rare at presentation but was a common site of relapse prior to the era of effective systemic therapy.

9.4 Treatment

9.4.1 Initial Management

The initial management of a patient with lymphoblastic lymphoma, especially in advanced stage disease, should address potential oncologic emergencies such as respiratory compromise from a mediastinal mass or metabolic derangements. Careful attention should be given to the initial laboratory results including a complete blood count and serum chemistry panel including electrolytes, blood urea nitrogen (BUN), creatinine, calcium, phosphorus, lactic dehydrogenase, liver function tests, and uric acid. Children with bulky adenopathy, especially in the mediastinum, may present with hyperuricemia, hyperphosphatemia, and associated renal dysfunction. These metabolic abnormalities

may develop or be exacerbated by the administration of chemotherapy due to massive lysis of tumor cells and subsequent release of purines, potassium, and phosphorous into the bloodstream. This results in deposition of uric acid, xanthines, and phosphates in the renal tubules, which further compromises renal function. This condition is referred to as "tumor lysis syndrome" (Magrath 2002). Historically, the approach in such settings is to administer vigorous intravenous hydration (D5 ½ NaCl with sodium bicarbonate at ~2–3 liters/m^2/day with no added potassium) and allopurinol (xanthine oxidase inhibitor) to block further production of uric acid. The introduction of new uricolytic agents (e. g., Uricozyme, Rasburicase) has significantly improved our ability to manage hyperuricemia and thus prevent or minimize tumor lysis syndrome (Pui et al. 1997, 2001). These agents cause a precipitous drop in uric acid by converting it to allantoin. This is important because it both limits the metabolic abnormalities and facilitates delivery of chemotherapy. Uricozyme has been used for decades in Europe, but is associated with significant allergic reactions in some patients. More recently, a recombinant form (Rasburicase) which is less immunogenic has become commercially available in the USA (Pui et al. 2001).

9.4.2 Treatment Overview

One of the major breakthroughs in the treatment of children with non-Hodgkin lymphoma was the upfront use of ALL-type chemotherapy by investigators at St. Jude Children's Research Hospital (Pinkel et al. 1975). Prior to this strategy, children with NHL were often treated with radiation therapy, single-agent chemotherapy, and surgical excision of localized disease (Lemerle et al. 1975). There was a subset of patients who had a rapid response to radiation therapy but within several months developed "leukemia". Many of these patients probably had lymphoblastic lymphoma involving the mediastinum and certainly had systemic disease with occult marrow involvement at diagnosis. The realization that these patients all required systemic therapy set the stage for curative therapy of NHL in children, especially for those with lymphoblastic histology. The early leukemia regimens utilized by St. Jude investigators included vincristine, prednisone, and antimetabolites (mercaptopurine and methotrexate) and were referred to as "Total Therapy» protocols (Pinkel et al. 1975). They included involved field radiation therapy and some form of preventive CNS therapy. CNS preventive therapy was included because the patterns of failure for some children with NHL were similar to those observed in ALL and included the CNS (leptomeningeal relapse). These initial protocols were only curative in a small percentage of patients with NHL. Children with mediastinal disease continued to have a poor prognosis. During this time period, chemotherapy protocols that included alkylating agents were showing promising results for the treatment of Burkitt lymphoma (Ziegler 1977). The Childrens Cancer Study Group initiated a randomized clinical trial to determine whether a lymphoma or leukemia regimen was most effective for children with all histologies of NHL. They selected COMP as the lymphoma regimen and LSA_2L_2 as the leukemia-type therapy (Wollner et al. 1979; Anderson et al. 1983, 1993). The LSA_2L_2 regimen is a multiagent regimen that was originally developed by Wollner and colleagues at Memorial Sloan Kettering for the treatment of ALL, whereas the COMP regimen is alkylator-based (i.e., cyclophosphamide). The results of this clinical trial led to a number of very important observations (Anderson et al. 1993). For example, amongst patients with Murphy stage 3 or 4 disease, there was a significant outcome relationship between treatment arm and histology. The LSA_2L_2 regimen was superior to COMP for lymphoblastic lymphoma (5-year EFS 64% vs 35%), whereas the COMP regimen was superior to LSA_2L_2 for Burkitt lymphoma (5-year EFS 50% vs 29%). For patients with Murphy stage 1 and 2 disease, there was an excellent outcome for all histologic subtypes regardless of treatment arm (5-year EFS 84%). The results of this study established an important foundation for subsequent NHL trials in children.

9.4.2.1 Limited Stage Disease

The excellent results achieved for patients with early stage non-Hodgkin lymphoma, and the goal to minimize both the acute and late effects of therapy, prompted several pediatric oncology cooperative groups to

conduct trials to determine whether some components of therapy could be omitted without compromising the excellent treatment outcome. Several other cooperative groups were achieving excellent results using ALL protocols for patients with early stage lymphoblastic lymphoma (Neth et al. 2000). Because of the rarity of early stage lymphoblastic lymphoma, most of the published outcome data combines the precursor B-cell and precursor T-cell cases together. The Pediatric Oncology Group performed two sequential clinical trials that addressed the role of involved field radiation and maintenance therapy, respectively (Link et al. 1990, 1997). In the first study, patients were randomized to receive chemotherapy alone [three courses of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)] followed by a 24-week maintenance phase of 6-mercaptopurine and low-dose methotrexate or the same chemotherapy with involved field radiation. This study demonstrated that there was no advantage to including involved field radiation. In the second study, patients were randomized to receive the same 9 weeks of CHOP chemotherapy with or without the 24-week maintenance phase (Link et al. 1997). This study demonstrated that the maintenance phase could be safely eliminated for those with Burkitt or diffuse large-cell lymphoma. However, there was a benefit of maintenance for patients with lymphoblastic lymphoma. Even with maintenance therapy, the event-free survival was only 50%, but the overall survival was closer to 90% because most patients were successfully salvaged with multiagent ALL chemotherapy. The results of the French and BFM cooperative group regimens using a more aggressive approach (LMT81) were associated with event-free survivals over 90% (Patte et al. 1992). Patients with B-precursor lymphoblastic lymphoma on NHL-BFM 86 and 90 were treated with either an intensive "ALL-type" or shorter "Burkitt lymphoma-type" regimen. The ALL-type approach, similar to that used in T-cell lymphoblastic lymphoma (BFM 90), was superior to the short-pulse B-NHL strategy (Neth et al. 2000). Several years ago the Children's Oncology Group (COG) adopted the BFM approach for patients with limited stage lymphoblastic lymphoma .

9.4.2.2 Advanced Stage Disease

The majority of successful regimens designed for the treatment of advanced stage lymphoblastic lymphoma are derived from regimens designed to treat ALL (see Tables 9.2 and 9.3). They are multiagent regimens comprising induction, consolidation, and maintenance phases delivered over 18–30 months. Although involved field irradiation of the primary tumor site was included to treat residual mediastinal masses in earlier trials, it is generally not recommended in contemporary treatment protocols (see Table 9.3).

The multiagent nature of most of the successful current regimens makes it difficult to identify which agents or blocks of therapy are most important in the study design. One of the early St. Jude studies demonstrated the efficacy of the addition of cytarabine and teniposide to an otherwise standard antimetabolite-based ALL treatment regimen (Dahl et al. 1985). Epipodophyllotoxins were subsequently used in several other protocols (Amylon et al. 1999; Abromowitch 2000) but have been omitted from recent studies because of the risk of secondary ALL. There has been continued controversy over the role of high-dose methotrexate in the management of advanced stage lymphoblastic lymphoma. The LMT81 regimen of the French cooperative group (SFOP) achieved excellent results by adding multiple courses of high-dose methotrexate (3 g/m^2/course) into an LSA_2L_2 backbone (Patte et al. 1992). Moreover, the BFM 90 regimen featured four courses of high-dose methotrexate (5 g/m^2/dose) as a consolidation phase and has resulted in one of the best outcomes to date (Reiter et al. 2000). Interestingly, studies from childhood ALL protocols have reported that higher doses of methotrexate are required to achieve maximal concentrations of intracellular polyglutamates in T-cell lymphoblasts (Synold et al. 1994). The POG 9404 regimen for T-cell leukemia and lymphoma addressed the role of high-dose methotrexate (Asselin et al. 2001) in a backbone of intensive doxorubicin and weekly L-asparaginase. The study was terminated early because of an inferior outcome for children with T-ALL in the arm without high-dose methotrexate. The study was not powered to examine the impact of high dose methotrexate in advanced stage precursor T-cell lymphoblastic lymphoma. A recently completed COG study (A5971) also examined

Table 9.2 Treatment outcome for advanced-stage lymphoblastic non-Hodgkin lymphoma

Protocol	Stage	No. of patients	Event-free survival rate	Reference
BFM 90	III IV	82 19	5 yr EFS = 90% ± 3% 5 yr EFS = 95% ± 5%	Reiter et al. 2000
POG8704: no extra Asp vs extra Asp	III/IV III/IV	83 84	4 yr CCR = 64% (SE, 6%) 4 yr CCR = 78% (SE, 5%)	Amylon et al. 1999
SFOP LMT81	III IV/ALL	33 43	57 mo EFS = 79% (SE, 4%) 57 mo EFS = 72% (SE, 4%)	Patte et al. 1992
CCG: LSA_2L_2 (modified) vs ADCOMP	I–IV I–IV	243 138	5 yr EFS = 74% 5 yr EFS = 64%	Tubergen et al. 1995
LSA_2L_2 (modified) CCG-551	III/IV	124	5 yr EFS = 64%	Anderson et al. 1983, 1993
X-H SJCRH	III/IV	23	4 yr DFS = 73%	Dahl et al. 1985
APO (Dana Farber)	III/IV	21	3 yr DFS = 58% ± 23%	Weinstein et al. 1983
A-COP + (POG)	III	33	3 yr DFS = 54% ± 9%	Hvizdala et al. 1988

the role of high-dose methotrexate in a BFM backbone. The results of that study are pending. L-asparaginase is also thought to be an important component of effective T-lymphoblastic lymphoma therapy. It has been featured to some degree in most published regimens for lymphoblastic lymphoma (Amylon et al. 1999; Goldberg et al. 2003). The Pediatric Oncology Group (POG) performed a randomized trial to address whether the use of weekly L-asparaginase after induction was beneficial. This study demonstrated a survival advantage in the arm that featured additional L-asparaginase (Amylon et al. 1999). The anthracylines represent another class of drugs that is commonly used and thought to be important in precursor T-cell ALL and lymphoblastic regimens (Goldberg et al. 2003). Re-induction (delayed intensification) phases may also contribute to a superior outcome – this strategy is featured in the BFM90 regimen (Reiter et al. 2000). The recently completed COG A5971 study featured a delayed intensification phase and randomized patients to an initial intensification block of cyclophosphamide and anthracycline.

9.4.2.3 Central Nervous System Prophylaxis and Treatment

CNS prophylaxis is an integral component of all lymphoblastic lymphoma protocols (see Table 9.3) (Weinstein et al. 1983; Dahl et al. 1985; Hvizdala et al. 1988; Anderson et al. 1993; Tubergen et al. 1995; Amylon et al. 1999; Reiter et al. 2000). Intrathecal chemotherapy alone is adequate for children with early stage disease . Most protocols for advanced stage lymphoblastic lymphoma have safely omitted cranial irradiation and rely only on IT chemotherapy for CNS prophylaxis. IT methotrexate is either given alone or combined with cytarabine, hydrocortisone, or both. The use of systemic high-dose methotrexate also contributes to the prevention of CNS relapse (Asselin et al. 2001). Recent protocols that included cranial RT as a component of CNS prophylaxis used doses between 1200 and 1800 cGy (Asselin et al. 2001; Reiter et al. 2000). BFM 90 incorporated 1200 cGy cranial RT in all patients for prophylaxis (Reiter et al. 2000). In a subsequent trial by the BFM, prophylactic cranial RT was eliminated

Table 9.3 Components of lymphoblastic lymphoma protocols

Protocol	Agents	Dur	CNS		Re-ind	HD-MTX	Anthracycline	L-Asp	VP-16	CTX	RT	Reference
			IT	CRRT								
BFM 90	Pred, VCR, Dauno, L-Asp, CTX, Ara-C, 6MP,MTX, Dex, Adria, 6-TG	2 yrs	IT MTX	Proph: 12 Gy CRRT CNS+: age 1-2 yrs: 18Gy CRRT age >3 yrs: 24 Gy CRRT	Yes	5 g/m^2 × 4	Adria: 120mg/m^2 Dauno: 120 mg/m^2	Ind: 10,000 IU/m^2 ×8 Re-Ind: 10,000 IU/m^2 ×4	0	3 g/m^2	Yes, if residual tumor in testes	Reiter et al. 2000
POG 8704	VCR, DOX,CTX, Pred L-Asp, VM26, Ara-C, 6-MP, ± HD L-Asp	2 yrs	IT MHA	CNS +: CRSPRT	0	0	Adria: 390mg/m^2	Ind: 10,000 IU/m^2 × 3 Consol: ± 25,000 IU/m^2 × 20	VM-26 7200 mg/m^2	6100 mg/m^2	0	Amylon et al. 1999
LMT 81	MTX, dauno, CTX, VCR, Pred, Asp, 6-TG, Ara-C, BCNU, HU, CCNU	2 yrs	MTX/ HC	Proph: 0 CNS +: 24 Gy CRRT	0	3 g/m^2 × 10	Dauno Ind.: 120mg/m^2 Maint.: 315–360mg/m^2 Total: 415–480mg/m^2	6000 IU/m^2 × 12	0	5.4 - 6.0 g/ m^2	IFRT for residual tumor in testes	Patte et al. 1992
LSA$_2$L$_2$	CTX, VCR,MTX, Pred, Dauno, Ara-C, 6TG, L-Asp, carmustine, HU, BCNU	18 mo	IT MTX	Proph: 0 CNS+: CR RT 24 Gy	0	0	Dauno: Ind: 120mg/m^2 Maint: 45mg/m^2/ cycle #2 ×?	6000 IU/m^2 ×14	0	Ind: 1.2g/m^2 Maint: 600mg/m^2/cycle #1 ×?	>3 cm 20–30 Gy	Anderson et al. 1993
AD COMP	CTX, VCR, Dauno, L-Asp, MTX, Pred	18 mo	IT Ara-C IT MTX	Proph: 0 CNS +: CRSPRT 24 Gy Cr. 12 Gy Sp.	0	0 (300 mg/m^2 × 10)	Dauno 360mg/m^2	6000 IU/m^2 × 9	0	11.2g/m^2	>3 cm 10–30 Gy	Tubergen et al. 1995

Protocol	Agents	Dur	CNS		Re-ind	HD-MTX	Anthracycline	L-Asp	VP-16	CTX	RT	Reference
			IT	CRRT								
SJCRH 10H	Pred, VCR, Asp, VM26, Ara-C, MTX, 6MP	2 ½ yrs	IT MTX	Proph: 24Gy CRRT	0	0	0	10,000 IU/m^2 ×8	Vm-26 4620 mg/m^2	0	0	Dahl et al. 1985
APO	VCR, Adria, Pred,6- MP, L-Asp, MTX	2 yrs	IT MTX	Proph: 24 Gy CRRT	0	0	450mg/m^2	28,000 IU/m^2 if >6 yrs; 56,000 IU/m^2 if <6 yrs, ×5	0	0	0 (except for ind. failure)	Weinstein et al. 1983
ACOP+	Adria, VCR, Pred, CTX, MTX	2 yrs	IT MTX, HC	Proph: 24 Gy CRRT	0	0	Adria: Ind: 60mg/ m^2 ×1 Maint: 60mg/ m^2/ Cycle ×?	0	0	Ind: 1.2 g/m^2 Maint: 1.2g/m^2/ cycle ×?	Ind. failure >2 cm	Hvizdala et al. 1988
POG 9404	VCR, DOX, MTX, Pred, 6-MP, Asp		IT Ara-C MTX	Proph: 1800 Gy	0	5 g/m^2 ×4	Dox 360mg/ m^2	25,000 IU/ m^2 ×20	0	0	Testicular disease	Asselin 2001
CCG 5941	VCR, dauno,CTX, VP-16, Ara-C, Asp, Dox, 6TG, MTX	12 mo	IT MTX Ara-C	CNS +: CRSPRT 1800/600Gy	0	1 gr/ m^2 ×7	Daun/Dox 240/m^2	PEG-Asp 2000 IU/m^2 ×1 1750 IU/ m^2×1 1750 IU/m^2 ×6	2800 mg/ m^2	8.4 g/m^2	0	Abromowitch 2000

without compromising the excellent treatment outcome (Pusill-Wachtsmuth et al. 2003).

The recommended treatment for overt CNS disease at diagnosis usually includes a combination of intrathecal chemotherapy (e. g., methotrexate alone or combined with hydrocortisone and cytarabine) and cranial or craniospinal RT (see Table 9.3).

9.5 Prognostic Factors

In a single institution review of non-Hodgkin lymphoma, stage, serum lactate dehydrogenase, and era of therapy were all shown to have prognostic significance (Murphy et al. 1989). It has been a challenge to identify reproducible prognostic factors for the lymphoblastic lymphoma subtype of NHL. This is in part a reflection of the relatively small number of failures that occur with modern therapy. In this regard, no prognostic factors were identified in a comprehensive analysis of potential prognostic factors (e. g., gender, age, stage of disease, serum LDH, immunophenotype, day 33 complete tumor response, and residual tumor at the end of induction) associated with the very successful BFM 90 regimen (Reiter et al. 2000).

Other potential prognostic factors, including early response and biologic markers, are discussed in the last section.

9.6 Outcome

Earlier treatment regimens, such as the LSA_2L_2 protocol, resulted in a 5-year event-free survival of approximately 60% (Mora et al. 2003). Various strategies to improve upon this result have included intensification of therapy, dose escalation of certain agents (e. g., high-dose methotrexate; Patte et al. 1992; Reiter et al. 2000), additional courses of active agents (e. g., L-asparaginase; Amylon et al. 1999), incorporation of new agents (e. g., epidpodophyllotoxins; Dahl et al. 1985), and incorporation of re-induction or delayed intensification phases. These approaches have contributed to the 80–90% event-free survival at 5 years that has been achieved with current regimens. The outcomes achieved over time are summarized in Table 9.2.

9.7 Management of Relapse

The prognosis for children with recurrent or refractory precursor T-cell lymphoblastic lymphoma is thought to be quite poor, particularly after receiving modern aggressive therapy. Salvage generally consists of two phases: re-induction therapy followed by an intensification phase featuring hematopoietic stem cell support (Sandlund et al. 2002a). Initially, it is critical to establish chemosensitivity (preferably a second complete response) with multiagent/single-agent chemotherapy. This may be achieved using combinations of high-dose therapy which were not part of the initial treatment plan (e. g., high-dose cytarabine, etoposide, high-dose methotrexate, etc.) or some other known active salvage regimen such as ICE (ifosfamide, carboplatin, etoposide; Kung et al. 1999) or DECAL (Kobrinsky et al. 2001). Another alternative is the compound 506U (Ara-G) which has been shown to have activity as a single agent in relapsed T-cell lymphoblastic disease (Berg et al. 2005; Kurtzberg et al. 2005). In those patients who achieve a second complete response, an intensification phase with hematopoietic stem cell support should be considered. There are relatively few published data on the role of hematopoietic stem cell transplantation in children with recurrent or refractory lymphoblastic lymphoma, particularly with respect to autologous versus allogeneic approaches. The risk of relapse is high after autologous stem cell transplants for T-cell ALL, and therefore most pediatric oncologists recommend an allogeneic hematopoietic stem cell transplant if a suitable donor is identified. Since the overall survival is greater than 90% for children with early stage lymphoblastic lymphoma, there are few published data on the outcome after relapse. Children whose original therapy included CHOP followed by methotrexate and 6-mercaptopurine maintenance did well with salvage that included either ALL regimens or allogeneic stem cell transplantation (Link et al. 1997).

9.8 Future Prospects

Through systematic refinement in protocol-directed therapy, there have been significant improvements in the treatment outcome for children with lymphoblas-

tic lymphoma; however, approximately 10–20% of patients with advanced stage precursor T-cell lymphoblastic will have recurrent or refractory disease. Additionally, the intensification of therapy that has improved cure rates may also place patients at increased risk for late sequelae of therapy, such as cardiotoxicity, infertility, and second malignancies. Therefore, safer and more effective treatment strategies are needed.

A clearer identification of high-risk and low-risk patients at the time of diagnosis would enable investigators to further refine risk-adapted therapy. More intensive (including hematopoietic stem cell transplants) or novel approaches could be implemented to target those at very high risk of failure, whereas those at a much lower risk of failure could have therapy tailored to reduce the risk of treatment-related complications. A more accurate determination of risk for treatment failure may be accomplished in a number of ways. Multiple studies of children with ALL have clearly demonstrated that poor early response to therapy is the strongest independent predictor of treatment outcome (Gaynon et al. 1990, 1997; Reiter et al. 1994; Gajjar et al. 1995; Steinherz et al. 1996; Kidd et al. 1997; Schultz et al. 1997; Coustan-Smith et al. 1998, 2002a,b, 2004; Sandlund et al. 2002b). Early response in the therapy of ALL has historically been determined by morphologic examination of blood and bone marrow, and more recently by MRD (minimal residual disease) flow cytometric and PCR methodologies (Coustan-Smith et al. 1998, 2002a,b, 2004). Campana et al. have used flow cytometry to demonstrate that comparable levels of MRD are detected in both the peripheral blood and bone marrow at the time of diagnosis in T-ALL patients, indicating that peripheral blood can be used to accurately follow MRD in T-ALL patients (Coustan-Smith et al. 2002a,b). Historically, the evaluation of an early response in patients with lymphoblastic lymphoma has proven problematic. Routine diagnostic imaging studies are not reliable – biopsies of residual mediastinal masses at the end of induction often reveal only necrosis and no evidence of tumor. MRD technology may provide helpful insights in determining an early response in these patients. In this regard, a recent COG study of children with newly diagnosed lymphoblastic lymphoma (A5971) performed by Campana et al. demonstrated that equivalent levels of MRD were detected in the peripheral blood and bone marrow of all patients (Campana et al. 2005), and most importantly, MRD was detected in the setting of bone marrows that were morphologically normal. Future studies are planned to examine the prognostic significance of these findings.

It may also be possible to identify high-risk and low-risk patients on the basis of molecular markers. There has been a paucity of biologic studies on tissue from newly diagnosed lymphoblastic lymphoma patients, in large part because of the limited tissue available to study. Gene array profiling studies may permit the identification of high-risk patients, as has been accomplished in adults with diffuse large B-cell lymphomas (Alizadeh et al. 2000; Rosenwald et al. 2002) and T-cell ALL (Ferrando et al. 2002, 2004; Ferrando and Look 2003).

Novel immunotherapeutic approaches are also being considered for patients with T-cell lymphoblastic lymphoma. Examples include Campath-1H, a humanized monoclonal antibody which targets CD52, that has been shown to have activity in T-cell lymphomas, (Pawson et al. 1997) and ONTAK, an interleukin-2 receptor-targeted diphtheria toxin, that has been shown to have activity in peripheral T-cell lymphomas (LeMaistre et al. 1998; Olsen et al. 2001; Talpur et al. 2002).

There is clearly a need for additional biologic studies of fresh tumor obtained from patients with newly diagnosed lymphoblastic lymphoma. Much of what we assume to be true for T-cell lymphoblastic lymphoma comes from studies of leukemia cells from patients with T-cell ALL. A clearer understanding of the pathogenesis of lymphoblastic lymphoma may lead to therapeutic strategies which directly target the molecular lesion.

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Cutaneous T-Cell Lymphomas and Rare T-Cell Non-Hodgkin Lymphomas

M.E. Kadin

Contents

10.1 Introduction

Cutaneous lymphomas are rare in children and adolescents (Fink-Puches et al. 2004). Skin involvement in children with non-Hodgkin lymphoma may represent primary cutaneous lymphoma or a secondary manifestation of widespread, systemic disease. In adults, about 80% of skin lymphomas are cutaneous T-cell lymphomas (CTCL) (Willemze et al. 2005). CTCL are a heterogeneous group of diseases most commonly represented by mycosis fungoides, and CD30-positive lymphoproliferative disorders (lymphomatoid papulosis and anaplastic large-cell lymphoma). Mycosis fungoides, albeit rare in childhood, is the most common form of cutaneous lymphoma in children and adolescents (Fink-Puches et al. 2004). T-cell lymphoblastic lymphoma may sometimes present with cutaneous lesions, but in most cases there is associated widespread nodal and bone marrow disease (Murphy and Fairclough 1989). Primary cutaneous B-cell lymphomas are also rare in childhood except for the occasional case of precursor B-cell lymphoblastic lymphoma presenting with localized skin disease (Lin et al. 2002; Kahwash and Qualman 2002). In adults, the most common subtypes of primary cutaneous B-cell lymphoma include follicular centre cell, marginal zone, and diffuse large B-cell lymphoma (Hoefnagel et al. 2005). The diagnosis and treatment of localized lymphoblastic lymphoma is discussed in Chapter 9. This chapter will focus on the cutaneous T-cell lymphomas that have been reported in childhood. Most of the literature in this area includes small patient series or isolated case reports

10.2 Mycosis Fungoides and Sezary Syndrome

Mycosis fungoides (MF) is a chronic cutaneous lymphoma originating from usually epidermotropic T cells with highly convoluted (cerebriform) nuclear morphology (Nickoloff 1988; Smoller et al. 2003). MF is the most common type of cutaneous T-cell lymphoma (CTCL) and accounts for approximately 50% of all cutaneous lymphomas (Willemze et al. 1997, 2005). It is mostly a disease of older patients with approximately 75% of patients diagnosed after the age of 50 years. Onset of MF in childhood and adolescence is rare. The tumor cells in MF originate from mature CD4+ (helper-inducer) T lymphocytes expressing cutaneous lymphocyte antigen (CLA) in >95% of cases. In less than 5% of cases, particularly in children, MF originates from CD8+ cytotoxic/suppressor T cells (Agnarsson et al. 1988; Berti et al. 1999). Sezary syndrome is a leukemic variant of CTCL in which there is a triad of erythroderma, lymphadenopathy, and cerebriform cells in the blood (Wieselthier et al. 1990; Vonderheid et al. 2002). The following discussion will emphasize the unique features of juvenile onset MF.

10.2.1 Clinical Features

MF usually affects adults greater than 50 years of age but can occur in children and adolescents (Groves et al. 2000). Nineteen of 34 patients in one pediatric series were diagnosed at a median age of 13 years (range 6–16 years), and 15 patients were diagnosed after age 16 years (median age, 23 years) (Wain et al. 2003). There is a male predominance of 1.6–2.0:1in both pediatric and adult series. The clinical course is usually indolent with initial lesions often resembling eczema. The differential diagnosis of MF includes parapsoriasis (question of a preneoplastic condition), chronic dermatitis of various causes, eczema, psoriasis, hypersensitivity/drug reactions, systemic lupus erythematosis, atopic dermatitis, contact dermatitis, and actinic reticuloid. Most of these conditions can be excluded by history and histologic examination supplemented by judicious use of immunohistochemistry and clonal analysis. Some conditions, e. g., actinic reticuloid, are conditions of advanced age and easily excluded in childhood cases of cutaneous lymphoid infiltrates (Toonstra et al. 1989).

MF may present over long periods (average of 2–10 years) with waxing and waning cutaneous eruptions. A definitive diagnosis of MF was made a median of 5 years after the onset of symptoms in the pediatric series described above. The 5-year and 10-year survival

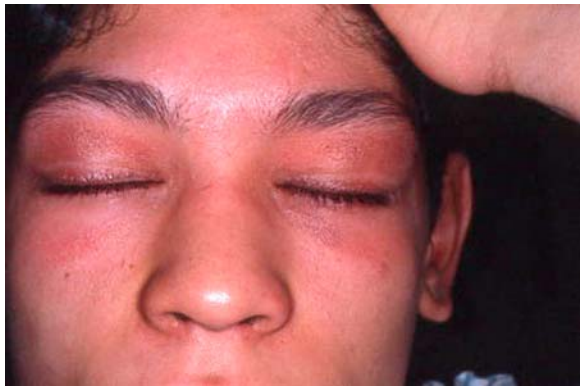

Figure 10.1

Clinical appearance of cutaneous T-cell lymphomas (CTCL). Periorbital patch lesions of mycosis fungoides (MF) in 12-year-old boy

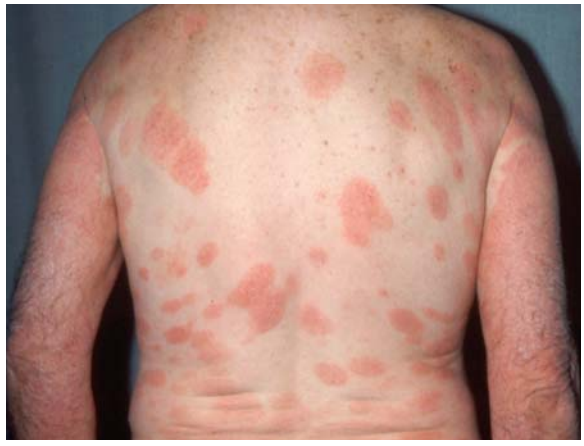

Figure 10.2

Plaque lesions of MF

in the pediatric series was 95% and 93%, respectively (Wain et al. 2003). Hypopigmented and poikilodermatous lesions are overrepresented in juvenile onset MF (Whittam et al. 2000; Wain et al. 2003). Initial skin lesions of MF have a predilection for sun-protected areas such as the buttocks and breasts. Skin lesions usually begin as erythematous scaly patches (Fig. 10.1) and become progressively thicker plaques (Fig. 10.2). Eventually, tumors may form.

10.2.2 Pathology

MF typically has a lichenoid appearance with a variably thick band of atypical lymphocytes at the interface of the epidermis and upper papillary dermis (Fig. 10.3). The distinction of MF from benign chronic dermatitis or other inflammatory dermatoses may be difficult at times. Diagnostic features of MF include lining up of atypical lymphocytes in the basal layer of the epidermis, clear spaces or haloes around the atypical lymphocytes, and collections of three or more atypical lymphocytes within the epidermis, refered to as Pautrier microabscesses (Fig. 10.4) (Smoller et al. 2003). The most reliable feature for making the diagnosis of MF is the detection of complex convoluted nuclei which resemble the complex gyri of the brain – hence the term cerebriform nuclei.

The epidermis often shows hyperplasia or acanthosis, and ulceration may be present in advanced disease. Follicular tropic MF is due to infiltration of hair follicles by neoplastic lymphocytes, often with mucinous change (follicular mucinosis) best visualized with the aid of the Alcian blue stain (Flaig et al. 2001). Inflammatory cells are generally not prominent in uncomplicated MF and when present in abundance should lead to a pause in making the diagnosis. Papillary dermal fibrosis is a noteworthy feature, but it is not specific. Spongiosis or papillary dermal edema is more often associated with benign inflammatory disease but does not exclude a diagnosis of MF.

Sezary syndrome may present particular difficulties in the histologic diagnosis because the lymphocytic infiltrate is often not epidermotropic and there is often edema and inflammatory cells (Sentis et al. 1986; Wieselthier et al. 1990; Trotter et al. 1997; Vonderheid et al. 2002). The detection of cerebriform cells in the skin and/or blood and clonal analysis supplemented with flow cytometry and immunohistochemistry will usually be sufficient to establish the correct diagnosis (Fig. 10.5).

In advanced MF, particularly the tumor stage, there can be a large-cell transformation (Diamandidou et al. 1998). This is defined as large cells comprising 20% or more of the neoplastic cells or clusters of large cells. The large cells have large nuclei and prominent nucleoli and can resemble immunoblasts. Nuclear convolu-

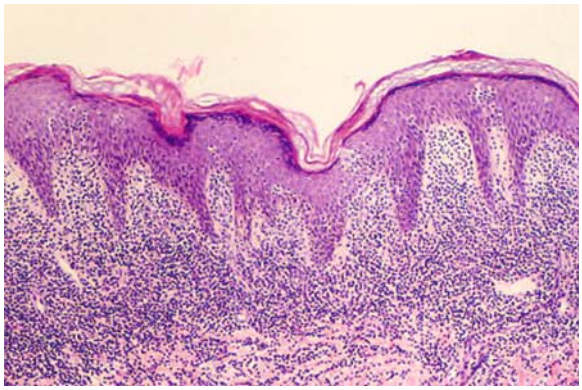

Figure 10.3
Morphologic appearance of cutaneous T-cell lymphomas. Band-like lymphocytic infiltrate at dermal-epidermal junction in MF

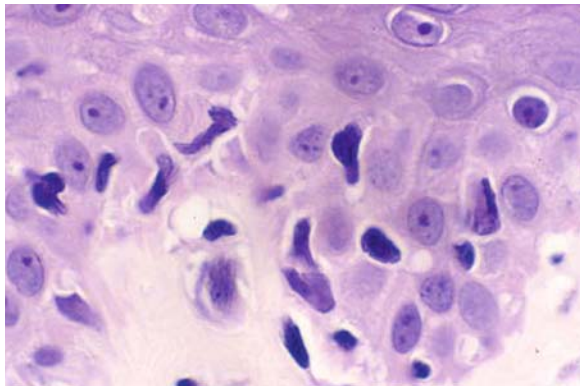

Figure 10.4
Cerebriform lymphocytes with halos in basal layer of epidermis in MF

tions may be lost but can be seen in some residual smaller neoplastic cells. Mitoses, which are infrequent in chronic MF, are usually markedly increased in large-cell transformation. Large-cell transformation is often accompanied by a change in immunophenotype with loss of common T-cell antigens and increased expression of CD25, CD30, CD71 (transferrin receptors), and cytotoxic proteins, e. g., TIA-1 (Vermeer et al. 1999).

Lymphadenopathy is common in advanced disease. Lymph nodes may be infiltrated with neoplastic lymphocytes or may show only dermatopathic lymphadenopathy in which there is an expansion of the interfollicular T-zones with numerous melanin-laden macrophages, Langerhans cells, and interdigitating histiocytes, and variable numbers of atypical lymphocytes. Palpable lymphadenopathy is associated with an unfavorable prognosis regardless of whether the histology reveals only dermatopathic changes or clear evidence of lymphoma. The distinction between dermatopathic lymphadenopathy and lymphoma may be difficult by only histologic examination (Scheffer et al. 1986). This may be due to the fact that histologically negative lymph nodes may contain clonal T cells identical to those in the skin (Weiss et al. 1986; Assaf 2005). Detection of T-cell clonality in lymph nodes is associated with a shorter survival. The systemic nature of the disease is reflected by evidence that there is commonly a gross disturbance in the peripheral T-cell repertoire in patients with MF, manifested by an imbalance of the normal T-cell subset distribution (Yawalkar N et al; Kupper and Fuhlbrigge 2004).

10.2.3 Immunophenotype

Neoplastic T lymphocytes usually show focal paranuclear/Golgi region staining for tartrate-sensitive acid phosphatase and alpha-naphthyl butyrate esterase. Moreover, a coarse granular staining for periodic acid Schiff diastase-sensitive material is seen in blood smears of Sezary cells. This is in contrast to the diffuse, finely granular staining of PAS-positive material in neutrophils and monocytes. These staining patterns may be helpful in differentiating neoplastic T lymphocytes (Sezary cells) from monocytes which are of similar size and also have convoluted nuclei. The diagnosis of MF may be facilitated by the use of immunohistochemistry, but there are pitfalls. There may be deletion of certain T-cell antigens, such as CD2, CD3, CD5, and/or CD7. Among these, CD7 is most often absent in early disease. However, this finding is not specific and may occur in some benign dermatoses. Activation antigen CD25 (IL-2R) is often expressed on neoplastic lymphocytes in early disease and CD30 in advanced disease. Cytotoxic molecules TIA-1, granzyme-B, and perforin are expressed in increasing amounts as the disease progresses (Vermeer et al. 1999).

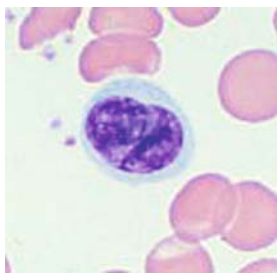

Figure 10.5
Sezary cell with nuclear convolutions in peripheral blood

While in most MF cases the neoplastic cells are derived from CD4+ cells, in about 5% the neoplastic cells express a CD8+ phenotype. These CD8+ cases may display unusual clinical features such as hypopigmentation or nodular ulcerative lesions and be associated with juvenile onset MF. In one large pediatric series, the atypical lymphocytes were CD8-positive in 38% of patients (Wain et al. 2003). Mucous membranes may be involved, and a poor response to therapy with short survival has been noted (Agnarsson et al. 1988).

10.2.4 Genetic Features

Clonal rearrangements of T-cell antigen receptor genes can be detected in 75–85% of MF cases using Southern blot, reverse transcriptase polymerase chain amplification (RT-PCR), or microcapillary techniques. There is evidence that early MF is oligoclonal and that with time a dominant T-cell clone emerges. The same clone may be detected in the skin and peripheral blood, and even in lymph nodes which are histologically nondiagnostic. There is some indication that detection of a clone in the blood may be associated with a worse prognosis. Detection of a T-cell clone is of the utmost importance in making the distinction of malignant lymphoma, e. g., Sezary syndrome, from benign erythroderma.

Several common cytogenetic abnormalities have been discovered in MF and Sezary syndrome (Thangavelu et al. 1997; Mao et al. 2002, 2003a,b). Notable losses at specific regions of chromosomes 10q and 13q in Sezary syndrome have been detected by comparative genomic hybridization (Karenko et al. 1999). Chromosomal loss of tumor suppressor gene PTEN at 10q24 and deletions and/or hypermethylation of tumor suppressor genes p15 and p16 are commonly found in MF. Isochromosome 17p is associated with the loss of one allele of the tumor suppressor gene p53, and often with mutation of the other allele. Inactivation of p53 is associated with tumor progression. The most characteristic finding of MF is amplification of the JunB gene, a transcription factor, detected by genomic hybridization (Mao et al. 2003a,b). Mutations of the Fas gene have been detected in a minority of MF cases, and may provide an explanation for resistance to apoptosis of slowly dividing neoplastic T lymphocytes in some MF cases (Dereure et al. 2002).

10.2.5 Prognosis

The prognosis of CTCL is based on the extent of disease at presentation (stage) as defined by the TNM classification (Table 10.1) (Sausville et al. 1988). Some studies suggest that patients can be divided into three prognostic groups: Stages IA, IB, and IIA, with excellent 5-year and 10-year survival; stages IIB and III with intermediate survival; and stages IVA and IVB with poor survival. The prognosis for juvenile-onset MF is similar to that of adult-onset disease. The majority of pediatric patients have stage IA and IB disease, and most series report no disease progression beyond stage IB. Fortunately, Sezary syndrome is rare in children, if it occurs at all.

A follicular tropic form of MF leads to alopecia which can be very disturbing, particularly in children (Flaig et al. 2001; van Doorn et al. 2001). The loss of hair and breakdown of skin function as a result of neoplastic infiltration of the epidermis and hair appendages increases the susceptibility to infection. MF patients are often thermodynamically very labile and suffer from cold intolerance. Pruritis is another common and disturbing symptom which may be attributable to cytokines.

Table 10.1 Stages of cutaneous T-cell lymphoma according to TNM definitions

Primary tumor (T)		
	T1	Eczematous patches, papules, or limited plaques covering less than 10% of skin surface
	T2	Erythematous patches, papules, or limited plaques covering ≥ 10% of skin surface
	T3	Tumors, one or more
	T4	Generalized erythroderma
Nodal involvement (N)	N0	No clinically abnormal peripheral lymph nodes; pathology negative for CTCL
	N1	Clinically abnormal peripheral lymph nodes; pathology negative for CTCL
	N2	No clinically abnormal peripheral lymph nodes; pathology positive for CTCL
	N3	Clinically abnormal peripheral lymph nodes; pathology positive for CTCL
Distant metastasis (M)	M0	No involvement of visceral organs
	M1	Visceral involvement (must have confirmation of pathology; organ involved should be specified
Stage I	1A	T1, N0, M0
	1B	T2, N0, M0
Stage II	IIA	T1 or 2, N1, M0
	IIB	T3, N0 or N1, M0
Stage III		T4, N0 or N1, M0
Stage IV	IVA	T1–T4, N2 or N3, M0
	IVB	T1–T4, N0–N3, M1

Table 10.2 Clinical and pathological features of CD30+ cutaneous lymphoproliferative disorders

Disorder	Clinical	Pathology	Treatment	Prognosis	Comment
Lymphomatoid papulosis	Continuous or recurrent spontaneously regressing papules and nodules, <2cm	Perivascular (early) to wedge-shaped (late) cutaneous lymphoid infiltrates. Inflammatory cells surrounding large atypical lymphoid cells (immunoblasts) sometimes resembling Reed-Sternberg cells	Observation; PUVA or weekly low-dose methotrexate (median dose 20 mg)	Excellent	Associated with development of systemic lymphoma (mycosis fungoides, Hodgkin's disease, or anaplastic large-cell lymphoma) in 15–20% of patients
Primary cutaneous anaplastic large-cell lymphoma	One to several nodules/tumors greater than 2 cm in diameter; partial regression is possible	Sheets of large CD30+ pleomorphic to anaplastic cells in dermis generally extending into subcutis	Surgical removal or irradiation for focal disease; methotrexate or multidrug chemotherapy for multifocal disease	Very good	Tumor cells lack anaplastic lymphoma kinase (ALK) in most but not all cases. Distinction from ALK+ systemic ALCL with secondary skin lesions is important
Borderline lesions	Nodular lesions difficult to distinguish between LyP and ALCL by clinical appearance	Sheets of large atypical cells usually confined to dermis	Excision or local irradiation for lesions that do not regress	May be associated with extracutaneous spread in 25%	Dutch cutaneous lymphoma group defines borderline lesions as (1) LyP lesions with histology of ALCL or (2) ALCL clinical lesions with histology of LyP

10.2.6 Therapy

In patients with focal patch stage disease, topical steroids or retinoids, e. g., bexarotene gel, may be useful (Duvic and Cather 2000). For more extensive skin-restricted disease, PUVA photochemotherapy, topical application of nitrogen mustard or chlormustine (BCNU), and total skin electron beam are effective (Kaye et al. 1989; Jones et al. 2002; Whittaker et al. 2003). Most pediatric patients can be sucessfully treated with topical steroids and emolients or psoralen and ultraviolet A irradiation (PUVA) or local radiotherapy. Biological reagents, e. g., interferon alpha and IL-2 diptheria toxin (DAB_{389}IL-2) directed against the IL-2 receptor, are also used for the treatment of MF (Olsen et al. 1995). In patients with dermatopathic lymph nodes and detection of clonal T-cell populations, addition of alpha-interferon or retinoids should be consid-

ered (Assaf et al. 2005). In adults, multiagent chemotherapy may be necessary for the treatment of multiple tumors, lymph nodal and/or visceral disease (Kim et al. 2003; Dummer et al. 2003). Unfortunately, no increase in survival has been shown from the use of multiagent chemotherapy for MF. Extracorporeal photophoresis is particularly useful for the treatment of patients with circulating lymphoma cells, including Sezary syndrome (Edelson et al. 1987; Russell-Jones 2000). Anti-CD52 (CAMPATH-1H) monoclonal antibody therapy has been useful for some patients with advanced MF (Lundin et al. 2003).

10.3 CD30+ Cutaneous T-Cell Lymphomas Including Lymphomatoid Papulosis

CD30+ cutaneous lymphomas comprise a spectrum of papules, nodules, and tumors comprised of activated T cells expressing the lymphocyte activation antigen CD30 (Willemze and Beljaards 1993). The spectrum includes clinically benign lymphomatoid papulosis (LyP), malignant anaplastic large-cell lymphoma (ALCL), and intermediate or borderline lesions (Table 10.2). There is a tendency to spontaneous regression which is consistent in LyP and variable in ALCL (Paulli et al. 1995). At the time of diagnosis, there is no evidence of MF or extracutaneous disease.

10.4 Lymphomatoid Papulosis

10.4.1 Clinical Features

Lymphomatoid papulosis (LyP) is a recurrent or continuous papulonodular eruption characterized by spontaneous regression of skin lesions. LyP presents as multiple cutaneous papules and nodules up to 2 cm in diameter (Fig. 10.6). Generally, the papules are only a few millimeters in diameter and develop a central area of necrosis preceding spontaneous regression. However, necrosis is not evident in all cases. The lesions appear erythematous and may be surrounded by a paler halo area. Lesions vary in duration but generally last 4–6 weeks and are often succeeded by an area of hypo- or hyperpigmentation. Lesions occur in all areas including the face, digits, trunk, and genitals. Involvement of mucous membranes does not occur. Recurrent lesions can appear in the same or different sites. There may be a tendency for clustering of lesions (Liu et al. 2003).

LyP occurs with similar frequency in males and females, with some series showing a male prevalence and others a female prevalence. All races and ages are affected. The peak age incidence is the 5th decade. Children as young as 1 year of age can be affected (Nijsten et al. 2004).

About 20% of LyP patients will develop lymphoma at some time during their life (Bekkenk et al. 2000; Wang et al. 1992). In about 90% of these patients, the lymphoma follows the earliest manifestations of LyP. In 10%, the lymphoma occurs nearly simultaneously or precedes all visual manifestations of LyP. The most common lymphomas associated with LyP are MF, ALCL, and Hodgkin lymphoma. Less often, pleomorphic T-cell lymphomas, nodular B-cell lymphomas, or other non-Hodgkin lymphomas have been associated with LyP. There is no known association with leukemias. One study revealed a significant increase in non-lymphoid solid tumors in LyP patients (Wang et al. 1999). A major concern is whether children who have more years at risk will eventually develop the highest frequency of lymphomas as suggested by one study (Cabanillas et al. 1995).

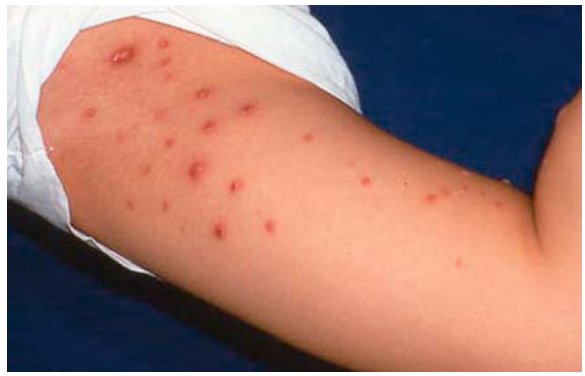

Figure 10.6

Clinical appearance of lymphomatoid papulosis (LyP). Multiple ulceronecrotic lesions on arm of 10-year-old girl

10.4.2 Pathology

Early lesions have a perivascular distribution of lymphocytes. Mature lesions have a wedge-shaped configuration (Fig. 10.7). An intravascular accumulation of neutrophils is a constant and therefore essential diagnostic feature. The epidermis often shows an accumulation of neutrophils with central necrosis. The lymphocytes vary in morphology, with some resembling immunoblasts with amphophilic to basophilic cytoplasm with prominent nucleoli to smaller atypical lymphocytes with convoluted, sometimes cerebriform nuclei. Mitoses are frequent, and apoptotic bodies are usually present although variable in number. Admixed with the atypical lymphocytes are smaller, normal-appearing lymphocytes and variable numbers of neutrophils and eosinophils. Histiocytes may be present but rarely form anything resembling a granuloma. Plasma cells are usually not conspicuous.

There are three major histologic types of LyP (El Shabrawi-Caelen et al. 2004). Type A resembles Hodgkin's disease based on plentiful inflammatory cells and the presence of Reed-Sternberg-like cells. Type B resembles MF because of the predominance of intermediate-sized cells with convoluted nuclei resembling Sezary cells. Type C resembles ALCL because of the predominance of large immunoblasts with relatively few inflammatory cells.

LyP can closely resemble pityriasis lichenoides et varioliformis acuta (PLEVA) both clinically and histologically. PLEVA is more frequent in children. It is especially important to distinguish LyP from PLEVA since PLEVA has no known association with lymphomas. Atypical lymphocytes are seen less often and neutrophils and eosinophils are less frequent in PLEVA. PLEVA more frequently shows individual keratinocyte degenerative changes or necrosis. Extravasation of erythrocytes is more common in PLEVA. Immunohistochemically, CD30+ cells are infrequent or absent in PLEVA. CD8+ lymphocytes predominate in PLEVA, whereas CD4+ lymphocytes are prevalent in LyP (Varga et al. 1990).

Nodular scabies can be mistaken clinically for LyP. There is more often an intratriginous distribution of scabetic lesions. Scabies contains an inflammatory infiltrate, and there may be immunoblasts present. Some immunoblasts stain for CD30. B cells are more frequent in scabies than in LyP. The most distinctive finding is the scabetic mite which may be visible by microscopy. LyP type B can be difficult to distinguish from MF. In LyP type B, there is epidermotropism of cerebriform lymphocytes similar to MF. CD30+ intermediate-sized cells are more common in LyP type B. Unlike MF, LyP type B lesions are papules which regress spontaneously. LyP type C resembles ALCL. The principal differences are the size of the lesion, which is less than 2 cm in LyP, and the more rapid regression. Histologically, LyP type C remains confined to the dermis with sparing of the subcutaneous fatty tissue, which is infiltrated by atypical cells in ALCL.

Since LyP can occur in patients with Hodgkin's disease, skin lesions of LyP type A may be difficult to distinguish from secondary skin lesions of systemic Hodgkin's disease. Clinically, secondary skin lesions of Hodgkin's disease tend to occur proximal to involved regional lymph nodes, by retrograde spread through the lymphatics. They are usually a late manifestation of disease. In LyP, the Reed-Sternberg-like cells are positive for leukocyte common antigen (CD45) and negative for CD15 (Leu-M1), whereas Reed-Sternberg cells in classical Hodgkin's disease are CD45– and CD15+ in more than 70% of cases.

LyP can resemble skin lesions of the small-cell variant of systemic anaplastic large-cell lymphoma which frequently occurs in children and adolescents. In the

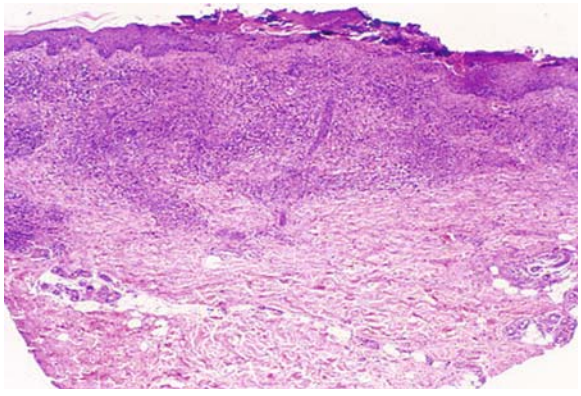

Figure 10.7

Histopathology of LyP. Wedge-shaped infiltrate in advanced lesion

small-cell variant of systemic ALCL, CD30+ cells are often confined to or concentrated around blood vessels, similar to LyP. Tumor cells in the small-cell variant are nearly always positive for ALK protein, which is invariably negative in LyP.

10.4.3 Immunophenotype

The atypical cells in LyP have the phenotype of activated helper T cells expressing CD30, CD25, CD71, and HLA-DR antigens (Kadin et al. 1985). The atypical cells also express CD45 and are negative for CD15. About 80% of cases reveal co-expression of T-cell antigens, although one or more common T-cell antigens are often deleted. Most reports indicate that the atypical cells are derived largely from CD4+ lymphocytes, whereas one recent report describes a higher frequency of cells expressing CD8 alone or together with CD4 (double-positive cells) and some cases with an natural killer cell phenotype (CD56+ and/or CD57+) (El Shabrawi-Caelen et al. 2004). About one-half of cases reveal cytotoxic proteins TIA-1, granzyme B or perforin in the atypical cells (Kummer et al. 1997).

10.4.4 Genetic Features

Clonal rearrangements of TCR genes have been detected in about 60% of LyP lesions analyzed (Weiss et al. 1986). Several studies have shown a clonal relationship between the regressing skin lesions of LyP and MF or ALCL of the same patient (Kaudewitz et al. 1989; Chott et al. 1996). One study of polymorphic microsatellite DNA revealed genomic instability in the LyP clone (Ruebben et al. 2004). In Hodgkin's disease of the rare T-cell variant, a clonal relationship between Reed-Sternberg cells and LyP cells has been shown (Davis et al. 1992). An identical IgH chain gene rearrangement has been detected by single-cell analysis of CD30+ cells in LyP and CD30+ Reed-Sternberg cells in a patient with B-cell type Hodgkin's disease (Gellrich et al. 2004). In this latter study, single-cell analysis of LyP uncomplicated by lymphoma indicated that the clonal T cells correspond to small CD30+ cells and the CD30+ cells are commonly polyclonal. This is in contrast to an earlier study of CD30+ cells which found them to be clonal T cells. Further studies are needed to clarify the histogenesis of the CD30+ cells and smaller T cells in LyP.

Only few karyotypic studies have been done in LyP. Direct chromosome analyses have revealed both structural and numerical abnormalities, in support of the premalignant nature of LyP (Peters et al. 1995). Additional chromosome abnormalities occur in the progression of LyP to malignant lymphoma. In a karyotypic study of LyP from a child, only diploid cells without structural abnormalities were found. In aggregate, the few genetic studies done to date suggest the possibility that LyP may begin as a benign hyperplastic lymphoid hyperplasia with an accumulation of genetic abnormalities during progression to lymphoma.

10.4.5 Therapy

Low-dose methotrexate, median dose 20 mg weekly, is the single most effective treatment for LyP in adults (Vonderheid et al. 1996). Other effective therapies include PUVA and oral bexarotene. Topical steroid therapy appears to have little benefit in LyP. Local irradiation has little rationale since the LyP lesions are likely to appear elsewhere. Little is known about appropriate therapy for LyP in children. Expectant observation is recommended in most cases. Occasional reports claim some beneficial effect of tetracycline or erythromycin, but there is little supporting evidence. We obtained a durable 14-year complete remission in a 10-year-old native American Indian girl who was treated for 9 weeks with 20mg/m^2 of oral methotrexate because of resistance to prednisone and persistance of numerous LyP lesions on the face and extremities as well as a large disfiguring tumor nodule on the nose. This case raises the possibility of using methotrexate for treatment in selected aggressive cases of LyP alone or together with cutaneous ALCL.

There is no convincing evidence that treatment of LyP to remission will prevent the occurence of secondary lymphomas, although this seems likely in those cases where a clonally related ALCL follows childhood LyP. The prevention of MF and Hodgkin's disease in patients with LyP is less likely. One study demonstrated occult T cells bearing the LyP unique clonal TCR signature in the bone marrow of two LyP patients who had been staged previously for B-cell lymphomas

(Gniadecki et al. 2003). It is of particular interest that LyP may reoccur in patients successfully treated for Hodgkin's disease. These results suggest the possibility that an occult precursor/progenitor stem cell is of extracutaneous origin and may help to explain the frequent recurrence of LyP in most patients.

10.5 Primary Cutaneous Anaplastic Large-cell Lymphoma

10.5.1 Clinical Features

Primary cutaneous ALCL originates in the skin as one or more tumor nodules typically greater than 2 cm in diameter (Bekkenk et al. 2000). In some instances, the tumors derive from the coalescence of smaller nodules. Ulceration is common. The lesions can occur anywhere on the body, including the face, extremities, hands, and feet. The initial presentation can be of a single tumor or multiple tumors in the same region as a cluster or less often in separate sites (Fig. 10.8). After the initial presentation, tumors may appear at the same or distant sites. The tumors more often affect adults but also occur in children, usually 10 years or older. They may be associated with papules of LyP. ALCL usually does not regress or shows only partial regression. However, the tumors may regress after weeks or months, justifying a period of observation in some cases. It is important to exclude extracutaneous disease since systemic ALCL with nodal and visceral involvement can be histologically indistinguishable from primary cutaneous ALCL.

10.5.2 Pathology

Tumors are comprised of sheets of large anaplastic cells with large nuclei and prominent nucleoli (Fig. 10.9). The nuclei are oval to round with some irregularities or indentations. The indentations give rise to the appearance of half-moon cells in profile or doughnut shapes en face (Agnarsson and Kadin 1988). Multinucleated cells are usually present in a minority. They may be distributed in a horseshoe or wreathlike arrangement. The cytoplasm is abundant and pale eosinophilic to basophilic in H&E-stained sections. In some cases, the tumor cells resemble histiocytes or Langerhans cells. These cytologic appearances led early observers to coin the term of "regressing atypical histicytosis" for some primary cutaneous ALCL. In Giemsa stains of imprints, the cytoplasm often appears deep blue with multiple vacuoules, and a diagnosis of ALCL can be made on needle aspirate cytology, especially when combined with immunostaining for CD30.

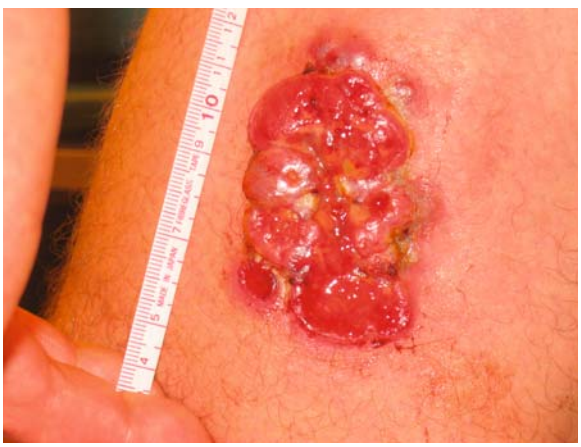

Figure 10.8

Clinical appearance of anaplastic large-cell lymphomas (ALCL). Multilobular ALCL on chest wall

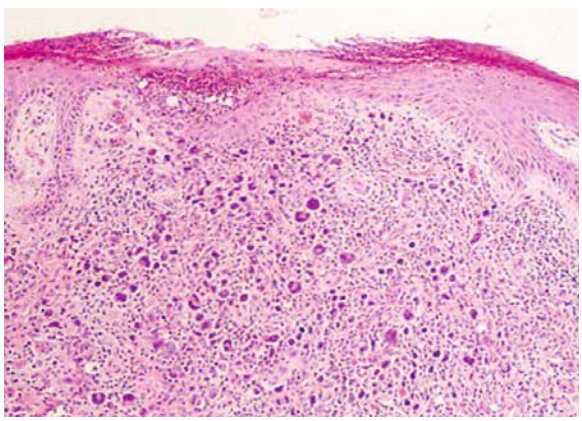

Figure 10.9

Histology of ALCL. Tumor cells with abundant cytoplasm and complex irregular nuclei including ring or doughnut forms; note admixed neutrophils

In many cases, the tumor cells near the surface or epidermis appear smaller and show nuclear convolutions resembling tumor cells of MF. Hence, the distinction between primary ALCL and tumor stage MF can be difficult and may be necessarily made on clinical correlations, i.e., in the absence of patches and plaques, the diagnosis of ALCL is more likely the correct one. In children, primary cutaneous ALCL is more likely than initial presentation of MF in the tumor stage.

Tumor cells usually appear in sheets with smaller lymphocytes concentrated at the periphery or depth of the lesion. Inflammatory cells, particularly neutrophils and/or eosinophils, can be numerous, and in some instances the tumor cells are so infrequent as to make the diagnosis of lymphoma difficult, and raising the differential diagnosis of infectious etiology. Such cases have been refered to as neutrophil-rich ALCL or pyogenic ALCL (Burg et al. 2003). In a few cases, elevated levels of interleukin-8 were found in the serum and in one case in a tumor cell culture supernatant, suggesting that this cytokine was responsible for the recruitment of neutrophils to the tumor. Serum levels of IL-8 decreased following treatment. Immunohistochemical staining for CD30 antigen is helpful in recognizing the tumor cells in neutrophil- or eosinophil-rich cases. The epidermis may be ulcerated and/or it can show extensive hyperplasia (pseudoepitheliomatous hyperplasia) mimicking squamous carcinoma.

10.5.3 Differential Diagnosis

The most important differential diagnosis is systemic ALCL, which has a significantly worse prognosis (Beljaards et al. 1989; de Bruin et al. 1993). The distinction can be made clinically in most instances. However, about 20–30% of systemic ALCL present with secondary skin lesions. These secondary skin lesions generally lack the cells near the surface of the lesion with convoluted nuclei resembling MF cells. The presence of ALK protein by immunohistochemistry or t(2;5) by FISH or karyotyping favors systemic ALCL (De Couteau et al. 1996).

Malignant melanoma is another important differential diagnosis. Melanoma cells may not contain pigment at the light microscopy level. Melanoma cells can stain positive for CD30 antigen. Melanomas have an intraepidermal component which is usually absent in ALCL. Staining for T-cell antigens will help to distinguish ALCL from malignant melanoma.

10.5.4 Immunophenotype

The most consistent finding is the expression of CD30 antigen, a member of the tumor necrosis family receptor proteins. Cross-linking of CD30 can induce apoptosis or cell-cycle arrest in some cell types, which may contribute to the regression of skin lesions (Mori et al. 1999; Willers et al. 2003). The tumor cells also express one or more T-cell antigens in most cases, most often CD4. However, CD3 and/or TCR beta antigens are often deleted as is Zap-70, indicating that T-cell receptor signaling is dysfunctional, which may play a role in the pathogenesis of ALCL (Bonzheim et al. 2004). Importantly, most primary cutaneous ALCL are negative for the ALK tyrosine kinase associated with systemic ALCL. However, a small minority of primary cutaneous ALCL may express ALK, so the distinction between primary cutaneous and systemic ALCL cannot rely entirely on the absence or presence of ALK protein.

10.5.5 Genetic Features

Nearly all primary cutaneous ALCL have clonal rearrangement of the TCR antigen receptor gene. This includes cases with a immunohistochemical null cell phenotype. Rare cases of primary cutaneous CD30+ B-cell ALCL have been observed. The translocation t(2;5)(p23;q35) associated with genesis of the chimeric oncoprotein NPM-ALK has only rarely been reported in primary cutaneous ALCL. No recurrent chromosomal abnormalities have been reported.

10.5.6 Therapy

Most primary cutaneous ALCL can be removed surgically or treated by local irradiation. However, recurrences are not uncommon. Because of the spontaneous regression of some cases and the excellent prognosis with 5-year survival >90%, a period of expectant observation of 1–3 months appears justified in selected cases (Bekkenk et al. 2000).

10.6 Subcutaneous Panniculitis-like T-Cell Lymphoma

Subcutaneous Panniculitis-like T-cell lymphoma (SPTCL) is a rare lymphoma derived from cytotoxic T cells and affecting primarily the subcutaneous tissue with extension to the dermis and epidermis in some cases (Gonzalez et al. 1991; Burg et al. 1991). Clinically, there are distinctive ulcerating lesions which may appear as erythematous to violaceous nodules, plaques, or both.

10.6.1 Clinical Features

SPTCL usually presents as plaques on the lower extremities. SPTCL commonly affects middle-aged patients, but children can be involved. The male to female ratio is equal. Advanced lesions ulcerate, revealing underlying yellow fatty tissue (Fig. 10.10). The clinical course is variable, and some cases originally diagnosed as benign panniculitis can progress to obvious malignant lymphoma (Marzano et al. 2000). Other cases can remain clinically indolent, and spontaneous remissions do occur. There is some evidence that the tumor cells can produce interferon-gamma, which could explain some of the clinical symptoms (Burg et al. 1991). It is often associated with a hemophagocytic syndrome and is characterized by fevers, lung infiltrates, hepatosplenomegaly, coagulation abnormalities, and pancytopenia.

10.6.2 Pathology

The histopathology is distinctive, with rimming of individual fat cells by atypical malignant lymphocytes (Fig. 10.11) (Gonzalez et al. 1991). The lymphocytes are medium sized with hyperchromatic nuclei and usually small nucleoli. Cytoplasm is sparse. Mitoses are seen, and frequent apoptotic bodies are noted. Areas of fat necrosis are common (Weenig et al. 2001). In some cases, particularly those of gamma-delta origin, there is an extension of the atypical lymphocytic infiltrate into the dermis and occasionally the epidermis. Pautrier microabscesses and other features of MF are lacking. In most cases, the dermis and epidermis remain uninvolved by the lymphocytic infiltrate.

10.6.3 Immunophenotype

The tumor cells are derived from CD8+ cells which contain cytotoxic proteins TIA-1, granzyme B and perforin. They may be derived from either alpha-beta T cells or gamma-delta T cells (Salhany et al. 1998). As described above, dermal and epidermal changes are largely restricted to gamma-delta cases. Epstein-Barr virus is not detected.

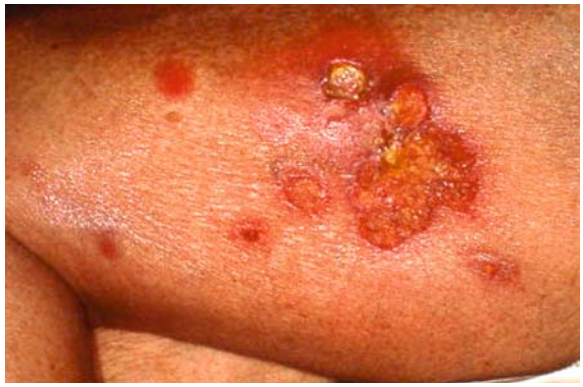

Figure 10.10
Clinical appearance of subcutaneous panniculitis-like T-cell lymphoma. Ulcerating lesions revealing subcutaneous fatty tissue

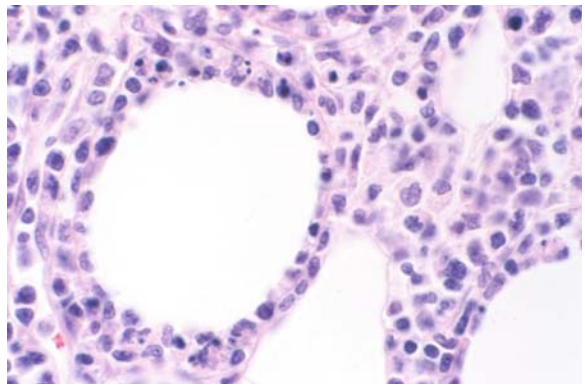

Figure 10.11
Histology of subcutaneous panniculitis-like T-cell lymphoma. Note apoptotic bodies of dying cells and rimming of fat cells by atypical lymphocytes

10.6.4 Genetic Features

Clonal rearrangements of TCR genes are detected (Hoque et al. 2003). No specific cytogenetic abnormalities have been found in limited studies.

10.6.5 Therapy

The clincial course is quite variable, ranging from indolent disease to a rapidly fatal hemophagocytic syndrome. Most treatment regimens involve multiagent chemotherapy, which is usually successful in achieving short-term responses. However, the median survival is typically less than two years. Treatment is also complicated by the breakdown of affected tissues with resulting infections (Santucci et al 2003).

10.7 Hepatosplenic T-Cell Lymphoma

Hepatosplenic T-cell lymphoma(HSTCL) is an uncommon T-cell lymphoma that presents in young males. Patients often have B-symptoms and hepatosplenomegaly with minimal lymphadenopathy (Kadin et al. 1981; Gaulard et al. 1986; Cooke et al. 1996). Tumor cells appear in a distinctive sinusal/sinusoidal pattern.

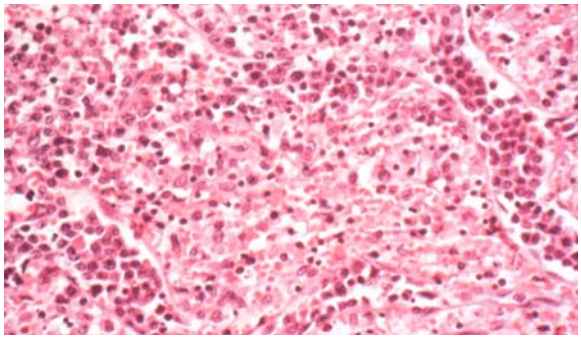

Figure 10.12

Histology of hepatosplenic T-cell lymphoma in spleen. Note sinusoidal infiltrate of neoplastic lymphocytes. Red pulp shows neoplastic infiltrate and extramedullary erythopoiesis in patient with anemia, a frequent complication of hepatosplenic T-cell lymphoma

10.7.1 Clinical Features

Interestingly, HSTCL is most common in males (median age, 35 years) but has been reported in children. In addition to systemic symptoms, patients often have pancytopenia and peripheral blood lymphocytosis. Lymphadenopathy is usually not found. Patients may develop cachexia and jaundice due to liver failure. The clinical course is aggressive with a median survival of less than 2 years (Belhadj et al. 2003).

10.7.2 Pathology

Tumor cells are found in the sinuses of the spleen, liver, and when affected, the sinuses of lymph nodes and bone marrow (Fig. 10.12) (Kadin et al. 1981; Farcet et al. 1990). Tumor cells are medium sized lymphocytes without prominent nucleoli and moderate cytoplasm. Erythrophagocytosis by tumor cells or reactive histiocytes is frequent and a hemophagocytic syndrome can occur (Kadin et al. 1981; Cooke et al. 1996). Mitoses are not frequent. The spleen may show evidence of extramedullary hematopoiesis and portal tracts in the liver are spared. Bone marrow hematopoiesis appears normal and tumor cell infiltration of sinuses may be obscure and best seen in immunohistochemical stains.

10.7.3 Immunophenotype

Tumor cells are T cells which are often negative for both CD4 and CD8. CD56 is typically positive. Cytotoxic marker protein TIA-1 is usually positive, but stains for granzyme B and perforin are usually negative, indicating that the cells are not biologically active. Most cases were found to be derived from gamma-delta T cells, but recent reports disclose similar cases of alpha-beta origin (Gaulard et al. 1990; Macon et al. 2001).

10.7.4 Genetic Features

Two distinctive cytogenetic markers associated with HSTCL have been described, trisomy 8 and isochromosome 7q (Kadin et al. 1981; Wang et al. 1995; Macon et al. 2001).

10.7.5 Therapy

The clinical course of HSTCL is usually very aggressive despite the use of multiagent chemotherapy. Rare long-term survivals have been observed following allogeneic bone marrow transplantation (Domm et al. 2005).

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Lymphoproliferative Disorders Related to Immunodeficiencies

T.G. Gross • B. Shiramizu

Contents

11.1 Introduction

The concept of tumor was first put forward by Thomas in 1959 [1]. The hypothesis was that the immune system is an active process controlling the emergence of malignant clones from somatic cells during the lifetime of a normal, immune-competent individual. The increased incidence of cancer observed in patients with immune deficiencies compared to the general population strongly supports this hypothesis [2, 3]. The cause of such immune deficiencies may be a genetically inherited defect, secondary to human immunodeficiency type 1 (HIV-1) infection, or iatrogenic following solid organ transplantation (SOT) or hematopoietic blood or marrow transplantation (BMT).

Retrospective surveys of patients with primary and secondary immune deficiencies have revealed an increased risk for specific cancer types. Lymphoproliferative disorders are much more common than observed in immune-competent children and represent the majority of cancers in immunocompromised children. De novo, reactivated, or chronic infections play a pivotal role in promoting the development of lymphoproliferative disease observed in children with immunodeficiency. Patients with primary (genetically determined) or secondary (acquired) immune deficiencies, which primarily affect T-cell function, are at the highest risk of developing lymphomas, often associated with Epstein-Barr virus (EBV). Primarily due to the growing number of transplants, it is estimated that over 150 cases of EBV-associated lymphoproliferative disease (EBV-LPD) are diagnosed in children in the USA each year [4]. This compares to the approximately 750 cases of childhood non-Hodgkin lymphoma (NHL) diagnosed per year in the USA [5], including 300 cases of Burkitt lymphoma, 200 cases of lympho-

blastic lymphoma, 100 cases of anaplastic large cell lymphoma, 100 cases of diffuse large B-cell lymphoma, and 50 unspecified cases of NHL [6].

In addition to lymphomas, patients with secondary immune deficiencies are at increased risk for carcinomas that are linked to infection with viruses such as human herpes virus-8 (HHV-8) and human papilloma virus (HPV). Additionally, not all lymphomas are associated with infectious agents, such as EBV. Therefore, the defect in immune surveillance may be in identification and/or elimination of cells with abnormalities of proliferation, function, and/or apoptosis; and more than just the inability to properly control infections.

In general, children who are immunodeficient and develop a malignancy, including lymphoma, have a worse prognosis compared to other individuals with histologically similar malignancies. For children who develop a localized NHL, the outcome can be quite favorable after surgery with or without radiation therapy. However, many lymphomas in this population are disseminated and require systemic cytotoxic therapy. These patients usually tolerate cytotoxic therapy poorly, with increased morbidity and mortality secondary to infectious complications and end organ toxicities. The overall goal of therapy is to enhance immunity, i.e., reduction of immunosuppression in transplant recipients, effective antiviral therapy for HIV-1-infected patients or immune replacement with hematopoietic stem cell transplantation in patients with a primary immunodeficiency. If these results can be achieved, then there is a better chance of achieving remission and decreasing the risk of recurrence. In this chapter, we will focus on advances made in our understanding of the etiology, pathogenesis, and the treatment of lymphomas in this unique population of patients that are being increasingly cared for by pediatric oncologists.

11.2 Epstein-Barr Virus

Epstein-Barr virus (EBV) is clearly associated with much of the lymphoproliferative disease observed in immunodeficient children. EBV is one of eight known human herpes viruses and is subgrouped into the gamma herpes virus subfamily. Like all herpes viruses, EBV is able to persist in the host for life, but in the vast majority of healthy carriers, the virus causes no disease. As will be discussed later in this section, disruption of the fine balance between the host immune system and the virus may lead to the development of EBV-related disease. The only natural host for EBV is man. EBV infects B lymphocytes and squamous epithelium of the oral and nasopharnyx. EBV initially infects B cells in lymphoid tissue of Waldeyer's ring [7, 8]. These B cells may remain latently infected. Latently infected B cells may disseminate throughout the body as resting memory B cells in secondary lymphatic organs, i.e., lymph nodes, spleen, and bone marrow [9], and become the reservoir for EBV infection [10]. The number of latently infected B cells is approximately 10^{-5} to 10^{-6} of all B cells, and this number remains stable for most of the life of the individual [10, 11]. If viral reactivation and replication occur, then cell lysis and death with shedding of the virus into the saliva occurs. The outcome of salivary EBV is either horizontal transmission to another host or infection of the oronasal epithelium resulting in virus replication, which then can infect other B cells.

Latent infection is characterized by the expression of nine virally encoded proteins: EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, leader protein (LP), latent membrane protein (LMP)-1, LMP-2A, and LMP-2B [12]. EBV-encoded RNAs (EBERs), EBER-1 and EBER-2, are seen in all latently infected cells, but they do not code for any proteins, and their function has not been determined. Expression of EBV genes varies among the spectrum of EBV-associated diseases and often differs from in vitro immortalized lymphoblastoid B-ell lines (LCL) or normal human resting B cells infected by EBV [13, 14]. Briefly, EBV-positive Burkitt lymphoma cells commonly express only EBNA-1, EBER-1, and EBER-2, which defines type I latency. Type I latency is also observed in a portion of EBV-positive gastric carcinoma. Type II latency, as defined by EBNA-1, LMP-1, LMP-2, EBER-1, and EBER-2 expression and is found in EBV-positive nasopharyngeal carcinoma, T-cell NHL, and the Reed-Sternberg cells of some patients with Hodgkin lymphoma. The EBV-LPD-infected cells observed in immunodeficient patients resemble in vitro immortalized LCL and generally express all nine EBV-related latent proteins (type III latency). It has been shown that peripheral resting

memory, post-germinal center, CD23-positive B cells are the source of EBV latency in seropositive healthy individuals. These EBV-infected resting memory B cells express only LMP-2, EBER1, and EBER2 (type IV latency) [15]. Again, it is important to keep in mind that viral replication always results in the lysis and death of the host cell, so when discussing therapies for EBV-associated B cell proliferation, inhibition of viral replication would appear to be counterproductive.

Understanding the immune response to EBV infection is essential in understanding the pathogenesis of EBV-related diseases. EBV is a very potent immune stimulus. The immune system controls lymphoproliferation in the normal host and maintains a host/virus symbiosis. Figure 11.1 illustrates the delicate balance between the host T-cell immune response and control of B-cell proliferation of latently infected B cells. In a healthy individual, while only 10^{-5} to 10^{-6} B cells are latently infected with EBV; approximately 1–5% of all circulating $CD8^+$ T cells are capable of reacting against EBV [11, 16] (Fig. 11.2). Initially, there is a B-cell proliferation producing both EBV-specific and non-specific antibodies. The number of these virus-containing B cells rises during the acute phase of the infection but never exceeds 0.03–0.1% of the circulating mononuclear cells [17].

Natural killer (NK) cells and $CD4^+$ T cells have been shown to play some role in controlling EBV infections, but it is the CD8+ memory cytotoxic T cells (EBV-CTL) that are the primary defense against B cell proliferation [18–22]. Initially, cytotoxic T cells are polyclonal and are neither EBV-specific nor HLA-restricted [19]. They resemble activated killer cells induced in vitro by exposure of T cells to nonspecific mitogens or IL-2 [19]. These $CD8^+$ lymphocytes account for the majority of the cells during the classic lymphocytosis characteristic of infectious mononucleosis and are the large, pleomorphic, atypical lymphocytes or "Downey cells" commonly observed in the peripheral blood [18, 23]. The quantity of these $CD8^+$ T cells in the peripheral blood correlate better with symptoms seen in infectious mononucleosis (IM) than do the number of EBV-infected B cells or viral load [24]. These $CD8^+$ lymphocytes disappear during convalescence [19], to be replaced by EBV-specific CTL, which maintain a latent viral infection and control EBV-driven B-cell proliferation [19–22]. This symbiosis of EBV and the infected host is maintained by interactions between viral gene expression in latently infected B cells and host EBV-CTL surveillance. A deficient CTL response, either quantitative or qualitative, results in an EBV-driven B-cell proliferative process. The lack of an appropriate CTL response can also result in an aggressive, predominantly T cell and histiocytic reaction that is not EBV-specific. This reaction is characterized by extensive infiltration of lymphoid and parenchymal organs with hemophagocytosis and tissue destruction which can be rapidly progressive and usually fatal [25, 26].

Although it is the cellular response that is critical for controlling EBV infections, neutralizing antibodies produced following primary infection may play a role in thwarting the spread to additional B cells. The humoral response to EBV is well characterized [27–30]. Viral capsid antigen (VCA) antibodies are the earliest to appear, beginning with IgM and followed by IgG. IgM antibodies usually arise during the incubation period, peak with symptoms, and then decline rapidly. IgG anti-VCA antibodies reach a peak 2–3 weeks after IgM and persist for life. The majority of patients also have a transient response to the EBV early antigen (EA), peaking usually within a month of infection. Antibodies to EBNA may appear several weeks after the onset of the illness in some patients, but in general take several months to appear, with titers rising slowly over 1–2 years and persisting for life. The majority of normal individuals will have detectable IgG to EBNA by 6 months following EBV infection, although it may take years to develop detectable anti-EBNA titers. In young children, the anti-VCA and anti-EA responses may be much less intense, and anti-EBNA may take much longer to appear [31].

The diagnosis of EBV infection is not trivial, especially in the immunodeficient patient. The gold standard has been serology consistent with a primary infection. The monospot test detects heterophil antibodies, and hence this test is not specific for EBV infection [32]. Serology for EBV infection has limited value in many immune compromised patients, as they may not have the ability to respond normally or may have detectable anti-EBV antibodies, acquired passively from intravenous gammaglobulin or other blood

products. The detection of EBV DNA in the peripheral blood or serum by polymerase chain reaction (PCR) provides good evidence that the patient has been infected by EBV. Increased levels of EBV DNA detected by PCR have been associated with EBV-associated diseases. However, interpretation of quantitative EBV DNA PCR or viral load results can be problematic, since immunocompromised patients tend to have higher amounts of EBV DNA than normal healthy individuals with latent EBV infections, and the results are not standardized in different laboratories. Therefore, the best method to diagnosis EBV as an etiologic agent is tissue biopsy using in situ hybridization.

11.3 Post-transplant Lymphoproliferative Disease

The risks of developing cancer after receiving a transplant is increased about 5–10-fold over the general population. The cumulative risk of cancer in solid organ transplant (SOT) recipients rises to more than 50% at 20 years after transplant [33]. Cancer is now one of the most frequent causes of death in this population [34, 35]. Compared to the general population, the risk of developing cancer post-transplant is much higher for children than adults. Excluding non-melanoma skin cancer, transplant recipients less than 25 years of age carry as high as a 50-fold risk of cancer compared to about 4-fold for recipients >25 years of age [36]. The cumulative incidence of a new malignancy following blood or marrow transplant (BMT) is about 5–12% [37, 38]. Children, especially ones <10 years of age, have >50-fold increased risk over the general pediatric population [37]. The cumulative incidence of cancer following transplantation increases with age [33, 39], making children receiving transplants at very high risk of developing a malignancy throughout their lifetime. Post-transplant malignancies do not mirror the types of cancer seen in the non-transplant pediatric population but are quite similar to what is observed in other immunodeficient populations. In addition to the defect in immunosurveillance, this population may be at increased risk for cancer from exposure to potential carcinogenic agents, such as alkylating agents and/or total body irradiation used in BMT, or chronic use of antimetabolite agents for immunosuppression, e. g., azathioprine, methotrexate, or mycophenolate mophetil.

Post-transplant lymphoproliferative disease (PTLD) represents a spectrum of clinically and morphologically heterogeneous lymphoproliferations and is the most common post-transplant malignancy seen in children. Almost all PTLD following BMT is associat-

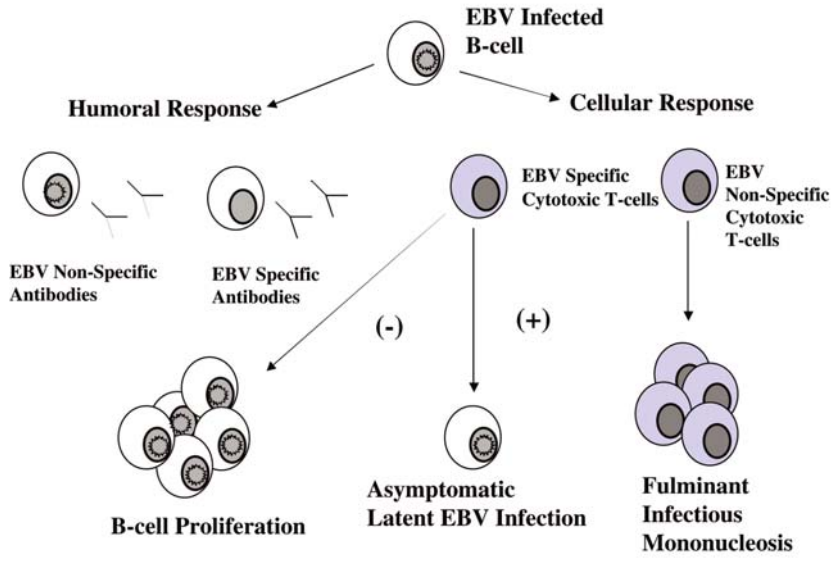

Figure 11.1
Immune response to EBV infection (From [232])

ed with EBV [26, 40]. Less than 20–30% of PTLD following SOT in children is not EBV-positive[40]. EBV lymphoproliferative disease post-transplant may manifest as isolated hepatitis, lymphoid interstitial pneumonitis, meningo-encephalitis, or an infectious mononucleosis-like syndrome. Frequently, the definition of PTLD is limited to lymphomatous lesions (localized or diffuse) that are often extranodal (frequently in the transplanted organ). Though less common, PTLD may present as a rapidly progressive, disseminated disease with multiorgan system failure, which almost always results in death despite therapy [26, 41–43].

In 2001, the World Health Organization published the most recent classification of lymphomas and leukemias, which included recommendations for the classification of PTLD from a 1997 Society for Hematopathology workshop [40]. PTLD is divided into three subtypes: early lesions, polymorphous and monomorphic PTLD. Presence of infiltrating T cells, disruption of nodal architecture, and necrosis are the major features in distinguishing polymorphic PTLD from early lesions. However, depending upon the status of the immune system, the number of T cells and the amount of necrosis in the lesions will vary in the polymorphous subtype. Histologies observed in the monomorphic subtype are similar to de novo NHL, with diffuse large B-cell lymphoma being the most common histology and, less frequently, Burkitt or Burkitt-like lymphoma [40]. This is in contrast to what is observed in NHL in children outside the transplant setting, where Burkitt or lymphoblastic histologies predominate [6]. A rare monomorphous B-cell subtype of PTLD is multiple myeloma or plasmacytoma [40]. In PTLD, as opposed to de novo NHL, there appears to be no difference in the histologies observed between pediatric and adult cases of PTLD. PTLD may be polyclonal, oligoclonal, or monoclonal, and both the polymorphous and monomorphous histologies may be present in the same patient or even within the same lesion of PTLD [44]. Thus, the histology of a single biopsied site may not be representative of the entire disease process.

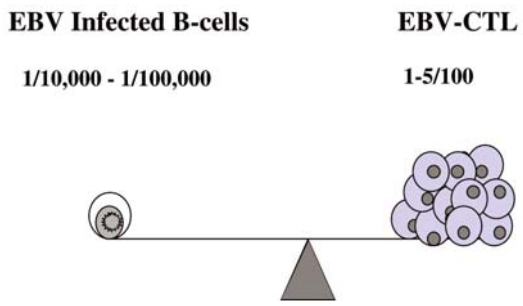

Figure 11.2

Balance of immune response to EBV infection (From [232])

The immunophenotype of the B cells in PTLD may be quite variable. Typically, CD10 and *bcl-6* expression are absent with MUM-1 positivity in the majority of cases. This suggests that most PTLD is derived from post-germinal center (GC) B cells [45]. Analyses of somatic hypermutation in immunoglobulin genes confirm that most monomorphous PTLD (>90%) have been through the germinal center [45]. Because of the propensity for PTLD to exhibit plasmacytoid differentiation, CD20 expression can be extremely variable compared to lymphomas in the general population. In some instances, CD20 expression is absent, whereas other B-cell antigens such as CD79a and/or CD22 may be present. Approximately two-thirds of PTLD cases have diffuse expression of CD20, but the remaining cases express only focal or undetectable CD20 expression [46].

In addition, not all cases of PTLD are of B lineage. Non-B-cell histologies are rare following BMT. T-cell PTLD tends to occur late, often more than 10 years after transplantation [47]. Of interest is that approximately 25% of T-cell PTLD are EBV-positive [48]. PTLD with Hodgkin lymphoma histology also tends to occur late, i.e., more than four years post-transplant, but is generally EBV-positive [49]. Some have questioned whether post-transplant Hodgkin lymphoma may be a variant of polymorphic B-cell PTLD [50, 51]. The reason for the long latency in non-B-cell PTLD is unknown. B-cell PTLD can also occur late after transplant, i.e., more than one year, but it tends to be EBV-negative [52, 53].

The diagnosis of EBV-associated PTLD requires a tissue biopsy plus in situ hybridization with probes for EBV sequences (EBERS). Reliance upon immunohistochemical staining for the latent membrane protein 1 (LMP-1) is not sufficient, as LMP-1 is positive in only 75% of EBERS-positive cases [54]. Caution must be

taken in making a diagnosis of PTLD, as there are typically increased background numbers of EBV-positive cells in lymphoid tissue in the post-transplant period. For example, in post-transplant patients, there are typically one to five EBER-positive cells per high powered field (hpf), compared to the nontransplant patient where zero to one EBER-positive cell/hpf is usually observed [55]. Perhaps the greatest difficulty in making the diagnosis of PTLD occurs when there is no mass lesion, but disseminated, infiltrative disease in various tissues and organs. The diagnosis can sometimes be suspected upon examination of the peripheral blood, bone marrow, cerebrospinal fluid (CSF), or other body fluids for the presence of plasmacytoid cells or large B cells. The disseminated type of PTLD may first be discovered at autopsy. An autopsy study of pediatric PTLD demonstrated that PTLD can be widely disseminated at the time of death with the antemortem cause of death thought to be severe graft vs host disease (GVHD) and/or infection [37, 42, 43].

There is no unifying cytogenetic abnormality in PTLD. Published small series or case reports have reported chromosomal abnormalities including t(14; 18), t(13; 14), t(8; 14), and t(3; 22), trisomy 3 and 12, amplifications of MYCN and other complex abnormalities [56, 57]. Comparative genomic hybridization analyses have demonstrated about half of the cases of PTLD to have amplifications and deletions, with gains in chromosomes 3q and 8q being most frequent, followed by losses in 17p, 1p, and 4q [58]. Clonality as defined by immunoglobulin gene rearrangements occurs with a greater frequency in the monomorphous subtype of PTLD compared to the polymorphous subtype. Mutations in known tumor suppressor genes and oncogenes such as *p53, c-myc,* and *ras* genes have been identified in rare cases of PTLD [59, 60]. The most common mutations involve *bcl-6*, which is consistent with the data indicating that most PTLD is derived from post-germinal center B cells. Mutations in *bcl-6* have been reported in 40% of cases overall and up to 90% of monomorphous PTLD [45, 59]. The role of *bcl-6* in the pathogenesis or outcome of PTLD is not clear. One study demonstrated that *bcl-6* mutations predicted poor survival [61], but this was not confirmed in another analysis (personal communication, T. Greiner, University of Nebraska Medical Center).

Previous investigations of EBV substrains have been based on the hypothesis that a "super tumorigenetic" EBV strain exists. The 30-base pair deletions in the LMP-1 oncogene have been demonstrated to be present in about 30% of PTLD cases, but this finding was not predictive of outcome [62, 63]. Similar nonpredictive findings have been shown with EBNA-1 substrain analysis. Thus, it is most likely that the distribution of substrains of EBV in PTLD is reflective of the geographic and ethnic distribution of EBV and does not play a role in tumorigenesis [60, 63].

Microarray analyses have been successful in subdividing diffuse large B-cell lymphoma (DLBCL) in adults in the nontransplant population and are of prognostic value [64, 65]. Preliminary results of microarray analyses in PTLD have demonstrated that the expression profiles of polymorphous PTLD cluster with cases of infectious mononucleosis. Infectious mononucleosis and polymorphic PTLD lesions express numerous T cell and macrophage-associated genes, whereas monomorphous PTLD expresses more abnormalities in cell cycle regulatory genes. However, when T cell and macrophage genes are excluded, PTLD cases segregate into two large clusters. One cluster is composed entirely of monomorphous PTLD (about 50% of all monomorphic lesions), and the second cluster contains the cases of infectious mononucleosis, all polymorphous PTLD cases, and the remaining monomorphous PTLD cases [66]. The importance of these findings remains to be determined.

Since risk factors, therapy, and outcomes differ between PTLD observed following BMT and SOT, each will be discussed separately. As previously mentioned, reduction of B-cell proliferation and/or enhancement of T-cell immunity are the goals of therapy. The outcome is highly dependent on the stage of the disease. Local disease that is readily controlled by surgery and/or radiation has an excellent outcome, but in nonlocalized disease requiring chemotherapy, the outcome has been poor, primarily due to increased infectious and treatment-related mortality. Historical data from the University of Minnesota demonstrate only 25% one-year survival for patients with PTLD following BMT [37]. Data from the Israel Penn Transplant Tumor Registry (IPITTR) demonstrate greater than 50% mortality for patients with PTLD following SOT [33].

11.3.1 PTLD Following Blood or Marrow Transplantation

The incidence of PTLD post-BMT has been reported to be low, between 1.0% and 1.6% [37, 43, 67–69]. Any factor(s) that either stimulates B-cell proliferation and/or decreases or delays T-cell immunity will increase the risk of PTLD. For allogeneic BMT recipients, the risk of PTLD has consistently been found to be strongly associated with HLA disparity, T-cell depletion of the stem cell graft, and the use of antithymocyte globulin (ATG) [37, 43, 67–69]. Data from the University of Minnesota that included 43 cases of PTLD found that patients were diagnosed at a median of 0.3 years post-stem cell transplant (SCT) (range 0.1–7.3 years) and that the incidence plateaued at 5 years post-BMT [37]. All but one case were diagnosed in patients receiving allogeneic BMTs. Only 9 of the 43 patients in this series survived, and in 15 patients, the diagnosis of PTLD was made postmortem. Median survival was 36 days, and the one-year survival rate was 25%.

Methods of T-cell depletion (TCD) that selectively remove T cells, e. g., sheep red blood cell (SRBC) rosetting and anti-T-cell monoclonal antibodies, confer a higher risk of PTLD than methods that "pan-lymphocyte" deplete the stem cell graft, e. g., CAMPATH-1 monoclonal antibodies or elutriation [68]. Since pan-lymphocyte depletion methods decrease the number of EBV-infected B cells as well as T cells, this may delay B-cell proliferation until EBV-CTL function recovers. The use of CD34-positive selection has become a popular method for TCD of allogeneic peripheral blood stem cell (PBSC). Early results suggest that the incidence of PTLD is similar to other pan-lymphocyte depletion methods. PTLD has been recently reported following the use of highly immunosuppressive therapy with CD34-selected autologous peripheral blood stem cell rescue to treat autoimmune disease or malignancy [70, 71]. One study suggested that the incidence of PTLD may be higher in recipients of non-myeloablative preparative regimens, again presumably due to the increased immune suppressive therapy [72].

It is important to note that the diagnosis of PTLD following BMT is not always obvious, demonstrated by the fact about a third of cases from the Minnesota experience were diagnosed on postmortem examination [37]. PTLD in the BMT setting frequently presents with a disseminated or fulminant form of the disease that lacks a well-defined tumor mass or adenopathy. For this reason, methods of reliably identifying patients who are at high risk before they develop disease, such as semiquantitative viral load determination utilizing PCR methods on peripheral blood to detect EBV infection/reactivation, are frequently utilized. There are many reports that correlate increased viral load with the development of PTLD [73–75]. However, there are no blinded, prospective studies to determine the predictive value of quantitative PCR for the development of PTLD. One must be cautious in interpreting EBV viral load results since there is great variability between different methods and/or laboratories, necessitating the use of one laboratory to serially monitor a particular patient [76]. High viral loads in a symptomatic patient, i.e., fever, adenopathy, appear to predict the development of PTLD. In an asymptomatic patient, however, a single elevated EBV level appears to correlate poorly with the development of PTLD. On the other hand, in recipients of T-cell-depleted grafts, high viral loads are predictive of PTLD, even in an asymptomatic patient [75]. Persistently positive or an increasing EBV load may be helpful, but unfortunately, some patients developed PTLD without any detectable EBV in the peripheral blood, or developed PTLD so rapidly that weekly monitoring failed to identify patients early.

Preemptive therapeutic approaches, such as the anti-CD20 monoclonal antibody rituximab, have been utilized by some investigators as a means to reduce B cell proliferation until EBV-CTL activity recovers in patients who have evidence of persistently increased EBV viral loads [77]. For some patients, a simple reduction in immunosuppressive therapy, if possible, may result in improvement of EBV viral load levels, although this has rarely been successful in treating established PTLD after BMT [43]. Currently, there are no standardized treatment approaches for BMT recipients who develop PTLD and frequently a combination of therapies have been utilized. Surgical resection and/or radiotherapy can be effective for localized PTLD, but this presentation of PTLD is uncommon following BMT. Success has been reported in a small number of patients treated with interferon-α [43]. Cy-

totoxic chemotherapy has been utilized in SOT patients with PTLD, but its usefulness after BMT has been limited due to concerns regarding toxicity when administered early after BMT [78]. Several reports demonstrate that patients treated with rituximab can achieve a complete remission (CR) [77, 79, 80]. The infusion of donor leukocytes (DLI) has been demonstrated to be successful in the treatment of PTLD post-BMT [74, 81]. However, severe graft-vs-host disease (GVHD) has occurred, and DLI is not always successful at controlling PTLD [73]. To circumvent the GVHD problem, investigators have inserted a suicide gene, i.e., herpes thymidine kinase, into donor lymphocytes or produced ex vivo EBV-specific CTL [74, 82]. These strategies have proven very successful at preventing and treating PTLD, but these approaches are not feasible for most centers, due to the cost and regulatory oversight. In summary, many centers today perform regular screening for EBV by real-time PCR of the blood and administer rituximab pre-emptively for predetermined levels based on the assay used. If PTLD develops, rituximab is administered again to control B-cell proliferation until T-cell immunity can be restored with time or infusion of DLI.

11.3.2 PTLD Following Solid Organ Transplantation

The primary risk factor for PTLD after SOT appears to be EBV seronegativity at the time of transplant [83]. Therefore, younger age recipients have the highest risk of PTLD [84, 85]. The type of organ transplanted has also been identified as a risk factor with an incidence of 1–5% in low-risk patients, e. g., renal, heart, and liver, to 10–30% in the high-risk patients, e. g., lung, small bowel, and multiple organ grafts. It is difficult to assess the risk of specific immunosuppressive agents, since it is usual for multiple agents to be used simultaneously. In general, it appears that the risk increases with the intensity and duration of immunosuppression [33]. Anti-T-cell-specific antibodies appear to confer the highest risk of PTLD [86]. Calcineurin inhibitors, cyclosporine, and FK506 (tacrolimus) are less risky with regard to increasing the risk of PTLD, and there is some evidence that FK506 (tacrolimus) predisposes to a higher risk than cyclosporine [84, 85, 87]. Recent studies, however, have shown that if serum levels are monitored closely, there is no increased risk when using FK506 as compared to cyclosporine [88]. It appears that the risk of PTLD is not increased with the use of mycophenolate mofetil (MMF) or the mTOR inhibitors such as sirolimus [89, 90].

Prognostic factors are difficult to ascertain since most studies report small numbers of patients with various therapies. Some studies suggested that monomorphic subtypes of PTLD had a worse prognosis than polymorphic subtypes, but this has not been found in other series [52, 53, 91, 92]. Clonality has not consistently been found to be a prognostic factor [52, 92]. It appears the combination of monomorphic histology, clonal immunoglobulin gene rearrangement, and/or abnormal karyotype may predict a poor prognosis. The stage of the disease is an important prognostic factor. Classical NHL staging systems, however, have had limited application in PTLD due to the high predilection for extranodal involvement [52, 93]. Most studies indicate that multiple (>2) sites of disease are associated with a poorer prognosis, and CNS involvement portends a dismal outcome [52, 93–95]. PTLD associated with primary EBV infection appears to have a favorable prognosis. Finally, PTLD diagnosed more than 1 year post-transplant with non-B-cell phenotype and/or EBV-negative has a very poor prognosis [52]. The exception to this rule is HD, which is often associated with EBV and responds well to standard HD therapy [49].

Prophylaxis and preemptive strategies to reduce the incidence of PTLD in SOT are controversial. Since antivirals, i.e., acyclovir or ganciclovir, do not suppress EBV-driven B-cell proliferation, their role in PTLD has been questioned [96]. Theoretically, antivirals may play a role in reducing the incidence of PTLD by reducing the number of infected B cells in high-risk patients, i.e., EBV+ donor into an EBV- recipient [97]. There have been numerous trials using antiviral therapy either as a prophylaxis or preemptive therapy for CMV (reviewed in [98]). In randomized trials using antivirals to prevent CMV disease, it has been difficult to demonstrate a decreased incidence of PTLD.

Attempts to prevent PTLD after SOT by acting preemptively at the time levels of EBV DNA increase in the peripheral blood have provided inconsistent re-

sults [88, 97, 99]. The most common intervention has been to reduce immunosuppression with or without antiviral therapy. Some investigators have used rituximab preemptively when an increased EBV DNA viral load is noted [99, 100]. However, there does not appear to be a good correlation between the reduction of the EBV viral load in the peripheral blood by rituximab and the clinical response of PTLD [11]. Another possible surrogate for preemptive therapy involves following the numbers of EBV-CTLs in the peripheral blood after SOT. It has been shown that a low number of EBV-CTLs may be a better predictor of PTLD than EBV viral load [101, 102].

The treatment of PTLD after SOT presents several therapeutic challenges. These patients are very susceptible to regimen-related toxicity, i.e., infections and end organ toxicity, as well as being at risk of rejecting the transplanted allograft. The ideal therapy for PTLD would reduce B-cell proliferation, prevent/treat allograft rejection, minimize inhibition of the immune response required to control EBV driven B-cell proliferation, and result in minimal host or organ toxicity.

The approach most widely used as the initial therapy is reduction or withdrawal of immunosuppression [103]. The response to reduction/withdrawal of immunosuppression varies widely from 20% to 86%. This range of response is attributable to the differences in practice of reduction/withdrawal of immunosuppression and the heterogeneous nature of PTLD, with localized or polymorphic disease more likely to respond to this approach [91, 96]. In one study of pediatric PTLD, 58% of patients responded to a reduction of immunosuppression, with 16/17 patients with polymorphic histology responding, but only 5/17 patients with monomorphic histology having a response [91]. Even for patients who have a disease that is responsive to reduction of immunosuppression, there is an increased risk of organ rejection. This is most dramatically seen in cardiac transplant recipients where complete cardiovascular collapse and death have been reported in more than 20% of patients with PTLD [104].

Local control with surgery and/or radiotherapy is very effective in curing localized disease, but only a small percentage of patients have early stage PTLD [95]. If the disease is not amenable to local control and/or reduction/withdrawal of immunosuppression has failed, either due to progressive disease and/or development of allograft rejection, the outcome is poor. The outcome for such patients with refractory PTLD is again variable, with disease-free survival reported to be between 0 and 70% [52, 53, 91, 93, 105–109].

Strategies to enhance EBV T-cell immune responses have been utilized. Interferon-α was reported to be efficacious in treating some cases of PTLD [109–111]. Partial responses were observed in about 80% of patients. However, only half of the patients survived more than 6 months, with many failures due to infection and rejection, as well as recurrent disease. Adoptive T-cell therapy is an attractive approach, but it is complex in an organ transplant recipient. First, cadaveric organs are typically utilized; therefore, donor leukocytes are often not available. Second, in contrast to BMT, PTLD following SOT is usually of recipient origin [112], making the immunologic recognition, specificity, and efficacy of donor leukocytes uncertain. Third, the use of closely matched relative's leukocytes runs the risk of both rejection and GVHD [113]. HLA-typed EBV-specific T-cell lines have been established from healthy volunteers and banked for use in treating PTLD patients with some success [114]. Though ex vivo generation of EBV-specific CTL is possible and several groups are investigating this approach, a major obstacle to this approach, as opposed to BMT, has been the limited survival of the adoptively transferred EBV-CTLs [114, 115].

The initial studies employing anti-B-cell monoclonal antibodies (anti-CD21 and anti-CD24) for the treatment of PTLD in SOT recipients suggested a high response rate, but long-term follow-up demonstrated only a 50% disease-free survival [106]. Recent studies have utilized the anti-CD20 humanized monoclonal antibody known as rituximab. There are numerous anecdotal case reports and small series demonstrating the efficacy of rituximab in the treatment of PTLD. One single center experience showed an improved outcome of PTLD compared to historical controls in the "rituximab era" [100]. Though rituximab is very effective at depleting B cells, it is not specific for PTLD and also depletes normal B cells. A clinical trial that combined rituximab with chemotherapy to treat lymphoma in patients with acquired immunodeficiency

syndrome (AIDS) demonstrated an increased infection risk and worse outcome in patients receiving rituximab [116]. Another study of rituximab demonstrated progression of PTLD despite reduction or elimination of the detectable EBV viral load [11]. The largest published experience using rituximab demonstrated a response rate of 65%, with an 18% relapse rate, 4% death rate due to allograft rejection, and 12% infectious deaths [79]. The best strategy for the use of rituximab in PTLD remains unknown. It is often used in conjunction with the reduction of immunosuppression, making it difficult to assess the effect of rituximab vs reduction of immunosuppression. One study using rituximab in patients with monomorphic PTLD who failed to obtain a reduction of immunosuppression demonstrated a response rate of only 46%, with only a 32% complete response rate [117]. Other antibodies, such as anti-IL-6, show some promise in the treatment of PTLD [118].

Using chemotherapy to treat patients with PTLD who fail a reduction of immunosuppression is attractive because of the potent cytotoxicity against aberrant lymphoproliferation and the concurrent immunosuppressive effect which is usually sufficient to prevent or treat allograft rejection. However, treatment-related morbidity/mortality due to end organ toxicity and infections can be problematic. The utility of chemotherapy in treating PTLD is difficult to determine because the published reports contain small, single-center retrospective experiences and lack large, prospective trials using uniform treatment. Results from published studies demonstrate that standard chemotherapy regimens for treating NHL, e. g., CHOP, ProMACE-CytaBOM, ESHAP, etc., result in complete remissions as high as 60% for patients with PTLD, with low rates of relapse and graft rejection, i.e., <5%. However, the 2-year survival remains less than 50%, with therapy-related mortality (TRM), i.e., death due to causes other than PTLD while on chemotherapy, ranging from 25% to 70% [47, 52, 53, 91, 105, 108, 109, 119]. The largest experience of chemotherapy in PTLD patients comes from the Israel Penn International Transplant Tumor Registry. A total of 193 patients with PTLD received chemotherapy, and one-third of the patients were reported to be alive at 3 years. Failures included 37% deaths from progressive PTLD and about one-third succumbing to toxicity [119]. In general, children with PTLD appear to tolerate standard NHL chemotherapy regimens better than adults.

The largest pediatric series using standard dose chemotherapy to treat PTLD demonstrated 9/10 achieving CR, and 7/10 being disease-free and alive at a median follow-up of 19 months [91]. Another recent study treated 36 children with PTLD that failed a reduction of immunosuppression with a low-dose cyclophosphamide and prednisone regimen [93]. With this regimen, the 2-year overall survival, relapse-free survival, and failure-free (without PTLD and with functioning original allograft) survival was 73%, 69%, and 67%, respectively. However, the relapse rate observed (19%) was higher than that observed with standard NHL chemotherapy regimens, suggesting a subset of patients may require more intensive therapy. Additionally, the outcome after relapse was very poor, with only 1/5 patients salvaged with chemotherapy and irradiation. Four patients who presented with disseminated, rapidly progressing disease and multiorgan failure or fulminant PTLD (F-PTLD) did very poorly, with only 1/4 achieving any response (PR), and all died of progressive PTLD. Intensification of chemotherapy has resulted in better control of F-PTLD, but all patients died of organ toxicity or infection [108]. Successful treatment of children with F-PTLD has been achieved with the addition of rituximab to the low-dose chemotherapy regimen [120]. Rituximab was added to this chemotherapy backbone in attempts to increase efficacy without added toxicity and is currently under investigation in the Children's Oncology Group (COG).

11.4 HIV-Related Malignancies

The morbidity and mortality associated with the human immunodeficiency virus type 1 (HIV) continues to increase globally with similar dire consequences seen in North America. By the end of 2003, an estimated 40 million people were living with HIV or AIDS, including 1,000,000 in North America, with 5 million new cases of HIV infection in 2003, of which 800,000 were children [121]. By the end of 2002, some 9,300 children younger than 13 years were diagnosed

with AIDS in the USA [122]. One of the impacts of the HIV/AIDS epidemic is that the risk for malignancy is increased in individuals infected with the virus [123, 124]. For many pediatric and adolescent AIDS cases with hemophilia, malignancy was the first indication of HIV infection [125]. A number of studies demonstrated that children infected with HIV are at increased risk for developing cancer. In a survey of Children's Cancer Group institutions, an estimated 100-fold increased risk of non-Hodgkin lymphoma (NHL) or Kaposi's sarcoma (KS), as AIDS-defining malignancies, was seen in HIV-infected children [126]. In a separate study from a US surveillance cohort of 4,954 children with AIDS, 124 children (2.5%) were identified as having a malignancy with an estimated relative risk (RR) of 651 for children who were 2 or more years beyond their AIDS diagnosis [124].

As patients live longer with HIV infection or are diagnosed with AIDS, an increase in malignancies has been observed [127]. The epidemiology of HIV-related malignancies (HRM), which includes Kaposi's sarcoma (KS) and HIV-associated non-Hodgkin lymphoma (H-NHL), has changed as antiretroviral therapies improved [128–130]. While there has been a decrease in the incidence of HRM with the introduction of highly active antiretroviral therapy (HAART) in resource-rich countries, the incidence of some specific types of cancers is still relatively higher than in non-HIV-infected individuals, both in adults and in children [128, 129]. The risk of lymphoma in an HIV-infected individual is 100 times that of an uninfected person [131]. Lymphomas associated with HIV are usually aggressive, with the majority occurring in extralymphatic sites [132]. Although, the use of HAART is correlated with a significant decline in KS, the decrease in H-NHL cases appears to be less dramatic [127]. The increased risk for KS and H-NHL are particularly noted in adults, but children with AIDS are at risk as well [124, 126, 133, 134]. In contrast to adults, there is also an apparent increased risk for leiomyosarcoma seen in HIV-infected children [125, 126, 133–135].

Specific risk factors associated with the development of malignancies in HIV-infected children were studied in a large multicenter cohort study and identified a high viral burden with EBV as being associated with HRM, although the effect was modified by CD4 cell count [134]. In contrast, route of HIV infection, demographic characteristics, and zidovudine use were not associated with the development of malignancy. However, specific pathogenic mechanisms of pediatric HRM remain unclear in the setting of immune dysfunction associated with HIV infection [134]. The immunocompromised state induced by HIV leads to a lack of surveillance of proliferative cells and/or incomplete control of other viral infections. One of the consequences of this process results in virus-induced or virus-associated cancers as is seen with EBV (B-cell lymphoma, nasopharyngeal carcinoma, leiomyosarcoma); human herpes virus type 8/Kaposi's sarcoma herpes virus (HHV8/KSHV) (Kaposi's sarcoma, body cavity-based lymphoma); human papilloma virus, human papilloma virus (HPV) (cervical and anal neoplasm, skin cancer); or even HIV itself (large-cell NHL, Burkitt NHL) [136–144]. The malignant pathways involving viral agents in HIV infection are likely multifactorial, ranging from cellular transformation, dysregulation of the immune system, to chromosomal abnormalities. Thus in HRM, the pathological heterogeneity is reflected in the different histologies observed amongst the various types of malignancies.

NHL of B-cell origin is a recognized AIDS-associated malignancy, but other lymphoproliferative diseases, including HD, are increasingly being reported [124, 133–135, 145–148]. H-NHL can be broadly grouped into three subcategories: systemic (nodal and extranodal), primary central nervous system (PCNSL), and body cavity-based lymphoma, or also referred to as primary effusion lymphoma (PEL) (Table 11.1). Approximately 80% of all H-NHL are considered to be systemic, defined as not being limited to the CNS (PCNSL) or other anatomic regions (PEL) [149]. In children, within the first 2 years after AIDS diagnosis, the incidence of H-NHL was 510 per 100,000 person-years, with the median time for developing H-NHL after AIDS diagnosis being 14 months [124]. The relative risk (RR) for developing H-NHL, with the most common type being Burkitt lymphoma, was 651. In contrast, the risk for PCNSL (91 per 100,000 person-years) was particularly high with a RR of 7,143 [124]. PEL, a unique lymphomatous effusion associated with HHV8/KSHV, is primarily observed in adults infected with HIV and rarely in children [150, 151]. HD also

appears to be increased in HIV-infected individuals, including children [124, 146, 147, 152]. The explanation for an increase in HD in HIV-infected children is still unknown; however, EBV has been speculated to play a role [147, 153]. The introduction of HAART has lowered the incidence of systemic lymphomas; however, the decrease has not been as successful as noted with the incidence of KS. Globally, H-NHL remains one of the most common HRM with a high morbidity and mortality rate [123, 154–157]. Similarly, in children, H-NHL is also one of the most common HRM with over 80% in one study being H-NHL [133]. Others report similar findings with H-NHL, either systemic or PCNSL being predominantly represented [124, 134, 135, 145, 146, 158]. HAART has decreased the incidence of NHL in HIV-positive individuals, similar to the effect seen in KS, particularly for PCNSL cases [154, 155, 159].

The pathology of the majority of childhood H-NHL is consistent with a B-cell origin [160]. The pathological spectrum seen in childhood H-NHL ranges from mucosa-associated lymphoid tissue (MALT), small non-cleaved cell lymphoma (Burkitt lymphoma) to diffuse large-cell lymphoma (DLCL) [145, 147, 152, 161, 162]. In limited molecular studies, abnormalities in known specific B-cell-associated neoplastic genes have been found in pediatric cases: *c-myc* oncogene mutations, immunoglobulin gene mutations, as well as the presence of EBV in tumor tissue [134, 147, 163]. The existence of infectious agents distinct from HIV that promote the development of NHL in HIV-infected individuals is plausible. The primary pathogenic agents other than HIV that have been studied in H-NHL are EBV and HHV8/KSHV. These pathogenic agents probably act in concert during the differentiation and transformation processes, leading to the various histological subtypes of H-NHL. The histological characterization of H-NHL can provide some clues as to the pathways involved in development [129, 143]. Naïve B cells evolve into memory B cells and plasma cells that produce immunoglobulin. Upon entering the germinal center (GC), these naïve B cells become proliferating centroblasts and progress towards maturation into nonproliferating centrocytes. At the same time, this process is associated with somatic hypermutation of the immunoglobulin variable genes and BCL6 mutations. The immunoglobulin heavy and light chains accumulate point mutations as rearranged genes. At various stages during differentiation, B cells undergo transformation, resulting in various types of NHL. Markers such as BCL6, multiple myeloma oncogene (MUM1), or syndecan-1 are used to identify histological subtypes. H-NHL Burkitt's lymphoma (BL) and diffuse large-cell lymphoma (DLCL) express BCL6+ but are MUM1- and syndecan-1- as GC centroblasts and early centrocytes. In contrast, as post-GCB cells, primary effusion lymphoma (PEL) express MUM1+ and syndecan-1+, but not BCL6-. Both EBV and KSHV/HHV8 interact with the immunological picture along the way, and ultimately, the presence of either or both of the viruses is characteristic of the histological subtypes. Thirty percent of systemic H-NHL cases are positive for EBV. In contrast, PEL is characterized by the presence of both viruses, 100% are positive for HHV8/KSHV and approximately 70% are also positive for EBV. While there are some similarities between EBV and HHV8/KSHV, there are significant differences between the two infectious agents that may account for the different disease patterns associated with each. Factors such as EBV and *c-myc* oncogene rearrangement are known entities and are well established as being closely associated with H-NHL, particularly with primary CNS lymphoma [123, 148, 149, 153, 164–166]. While EBV is detected in the majority of primary CNS H-NHL, only 50–60% of large-cell H-NHL have detectable EBV and frequently express EBV latency type III antigens, i.e., EBNA-1,2,3 and LMP-1 and LMP-2 [167–170]. In contrast, the association of EBV with Burkitt lymphoma (BL) is slightly less (30%), and typically only EBNA-1 (latency type I) is expressed [60, 169–172]. The majority of people worldwide have been exposed to EBV with B lymphocytes acting as the reservoir for latent infection. Immunosuppression as a result of HIV infection promotes the development of EBV-infected and immortalized B-cell clones. A second hit resulting in genetic changes can lead to an EBV-containing monoclonal proliferation. Because the EBV genome can only be detected in 60% of large-cell H-NHL, other factors are implicated in the etiology, including impairment of T-cell immunosurveillance and polyclonal B-cell expansion which may result in gene rearrangements.

Table 11.1 Features of HIV-associated lymphoma [233]

H-NHL[a]	Cells	Clonality	Characteristics	Co-infection	Location
Systemic	B cells	Monoclonal & polyclonal	Lack genetic mutations associated with most malignancies (*p53*, *Ras*, *RB1*); Burkitt's and diffuse large-cell lymphoma: express BCL6, and *c-myc* translocations	Not typically associated with oncoviruses	80% Extranodal involvement
PCNSL	B cells	Mostly monoclonal	Large cell lymphomas lacking *c-myc* translocations; low CD4 cell counts	Most EBV-associated	CNS
PEL	B cells	Monoclonal & polyclonal	Lack B-cell antigens (CD19 and/or CD20) or *c-myc* translocations; CD45, MUM1, CD38, & Syndecan-1	HHV8 100% EBV 70%	Body cavities (pleural, peritoneal, pericardial)

[a]H-NHL = HIV-associated non-Hodgkin lymphoma; Systemic=H-NHL not localized to PCNSL or body cavity (PEL); PCNSL= primary central nervous system lymphoma; PEL = primary effusion lymphoma

Gene rearrangements involving oncogenes and immunoglobulin genes have been extensively studied amongst H-NHL. While the exact role of *c-myc* is still unclear, rearrangements of the oncogene are clearly documented in H-NHL [166, 169, 170, 173–176]. Studies of the molecular genetics of H-NHL provided some insight into the pathogenesis of the malignancies. In part, our understanding of the molecular events leading to H-NHL was strengthened by analyses of the immunoglobulin region, *c-myc, p53, bcl-6* regions [173, 177–180]. Marked B-cell proliferation in the setting of HIV-associated immunosuppression may be an important factor in the pathogenesis of H-NHL [181–185]. The proliferation may lead to patterns of translocation of part of the *c-myc* gene on chromosome 8 and the immunoglobulin heavy chain gene on chromosome 14 with defective recombination during isotype class switching of the constant region with preferential V_H immunoglobulin usage [175, 182, 186–190]. The vast majority of studies demonstrate evidence for monoclonality based on immunoglobulin rearrangements or by other means such as EBV or light-chain analysis, but there are unique cases in which monoclonality cannot be established [147, 157, 163, 191, 192]. While these so-called "polyclonal" H-NHL cases appear to be unique, more studies are necessary to confirm the significance of the H-NHL subtype [147, 148, 163].

Even though HD is rare, the incidence appears to be slightly higher than in HIV-seronegative children. Lymphocyte depletion and mixed cellularity histologies are the most common in adults, and there is some information to support the same histology subtypes in children [193, 194].

The clinical presentation of H-NHL in children can vary depending on the age, CD4 cell count, and sites of involvement [152]. Symptoms range from vague complaints (fatigue, loss of appetite) to those related to specific organ involvement (hepatosplenomegaly, pleural effusion). HIV-infected children diagnosed with HD also present similarly to non-HIV-infected children with HD. Prior to instituting treatment, careful and complete staging of H-NHL is necessary to assess tumor burden and organ involvement. Depending on the suspected sites, the staging studies can include: site-specific radiographic imaging (CT, MRI, gallium scan, bone scan, plain X-ray), bone marrow examination, and lumbar puncture. Therapeutic options for pediatric H-NHL have primarily been based on the adult experience [127, 195, 196]. Like adult HRM, cancer-directed treatment is recommended for children, who have a reasonable chance of survival with concurrent HAART [133, 158, 197]. Optimal treatment strategies and recommendations continue to evolve for childhood HRM as the treatment for HIV improves, allowing for better tolerability of chemotherapy regi-

mens. HIV-infected children with H-NHL have been treated with combination chemotherapy consisting of different regimens including cyclophosphamide, methotrexate, cisplatin, vinblastine, vincristine, prednisone, mercaptopurine, and intrathecal chemotherapy [133, 148, 198]. Although the prognosis is not as good as seen in pediatric non-HIV-NHL, improvements are being seen with the 5-year event-free survival approaching 50% [133]. The experience in HD for HIV-infected children is similarly limited with respect to chemotherapy strategies. From the successful treatment regimens used for HD in immunocompetent adult patients, the same drug combinations and doses have been tried in HIV-infected cases with less success [199, 200]. Even less experience has been reported in childhood HIV-associated HD, although the disease, too, is responsive to therapy utilizing treatment for localized disease and for systemic disease, thus advocating the need for prompt diagnosis and intervention [133, 152, 158].

11.5 Lymphoproliferative Disease in Primary Immunodeficiencies

Due to advances in the prevention and treatment of opportunistic infections, patients with primary immunodeficiencies now enjoy longer lives than ever before. However, neoplastic disorders, particularly lymphoproliferative complications, remain the second most common cause of premature mortality (still preceded by infections) [201]. The incidence of tumors in certain immunodeficiency diseases such as Wiskott-Aldrich syndrome (WAS), ataxia telangiectasia (AT), and common variable immunodeficiency (CVID) is estimated to be between 15% and 25%, and this risk increases with age [202, 203]. NHL observed in primary immunodeficiency have the following characteristics: (1) male predominance, even in autosomal recessive disorders, (2) young median age at diagnosis, and (3) high frequency of extranodal presentation, particularly in the gastrointestinal tract and CNS. Though EBV has been identified as a common co-factor predominantly in the B-cell phenotypes, the presence of EBV DNA and latent viral proteins have been found in cases of Hodgkin disease (HD), as well as T-cell NHL. However, not all NHL, including B-cell NHL are EBV-associated. Even in X-linked lymphoproliferative disease (XLP) where affected boys have a very aberrant immune response to EBV infection, many of the NHL have been found to be EBV-negative [204, 205].

Hodgkin disease (HD) accounts for up to 10% of tumors arising in patients with immunodeficiencies and occurs at an early median age [206]. A case control study in the late 1980s comparing the immunodeficiency HD cases with other pediatric HD cases from the Children's Cancer Group (CCG) demonstrated that immunodeficient patients with HD presented earlier in life (mean 7.8 years vs 11.5 years), were significantly less likely to achieve remission, and if remission was achieved, the 5-year probability of survival was inferior for immunodeficient patients (53% vs 86%). There is some speculation about whether the majority of HD diagnosed in this population is truly HD. Histologies other than nodular sclerosing, i.e., mixed cellularity, lymphocyte-predominate and lymphocyte-depleted are more common in immunodeficient patients [206]. HD in immunodeficient patients is often associated with EBV and may reflect a spectrum of B-cell lymphoproliferation with a mixture of cells that reflect an abnormal or inadequate immune response [50].

Historically, treatment with conventional doses of chemotherapy and radiation has met with limited success in primary immunodeficient patients [207]. This is primarily due to opportunistic infections and an increase in treatment-related mortality due to end organ dysfunction. In the current era, improved antiviral and antifungal therapies have allowed many patients with immunodeficiencies who have lymphoma to be treated more aggressively [162]. However, even for patients who achieve complete remission, relapses are common. It is unclear if relapse is always truly a recurrence of the same clonal process or a "new" disease, as "recurrences" with different clonal origin compared to the original lymphoma have been documented [208]. Since the primary risk factor for cancer is the underlying immunodeficiency, immune system replacement with allogeneic hematopoietic stem cell transplantation is ideal, if a suitable donor is available.

The increased understanding of specific defects has added to our understanding of the pathogenesis of

lymphoma in primary immunodeficiencies. A brief discussion of the current understanding of lymphogenesis in specific immunodeficiencies is provided.

Severe combined immunodeficiency (SCID) is comprised of a number of genetically distinct disorders with severe impairment of both cellular and humoral immune responses. These disorders typically result in death during infancy from opportunistic infections [209]. NHL is almost exclusively the cancer observed in patients with SCID. Generally, only SCID patients with the ability to make B cells are felt to be at risk for developing NHL. Examples of such conditions include: (1) XSCID in which loss of function through mutations in the X-linked common gamma-chain gene of multiple interleukin receptors blocks T-cell development, but B-cell numbers are generally plentiful, (2) purine nucleoside phosphorylase (PNP) deficiency, where T-cell expansion and function are impaired by the accumulation of toxic intracellular metabolites, with lesser effects on B cells, and (3) Omenn's syndrome, where DNA repair is defective due to mutations in RAG1 genes predominantly [210] or ARTEMIS mutation [211]. In contrast, the majority of adenosine deaminase (ADA)-deficient SCID patients will not have B cells, and NHL occurs less frequently. B-cell NHL without evidence of EBV infection has been observed, suggesting the defect predisposing to lymphoma may be a generalized defect in the ability to control B-cell proliferation [212].

Wiskott-Aldrich syndrome (WAS) is an X-linked disorder of broad range and variable immunodeficiency and characterized by microthrombocytopenia, resulting from mutations in the Wiskott-Aldrich syndrome protein (WASP) gene [213]. The WASP gene encodes a large intracellular protein with several functional domains involved with cytoskeletal integrity and signal transduction. Several molecules reported to be associated with WASP are involved in the normal progression through the cell cycle. WASP is expressed in cells of hematopoietic origin and in the thymus. Experimental evidence suggests that WASP-negative B cells are relatively resistant to apoptosis. If patients live long enough, all will develop lymphoma. Though rare, there are reports of EBV-negative B-cell lymphomas in WAS, and these appear to be more frequently seen in adult males with clinically milder forms of WAS or what has been referred to in the past as X-linked thrombocytopenia (XLT).

Males affected with X-linked lymphoproliferative disease (XLP) were originally recognized for fatal complications of EBV infection and a high risk of lymphoma [204]. The defective gene has been identified as SH2D1A or SAP (SLAM Associated Protein) [214]. SAP is a small adaptor protein of only a SH2 binding domain and short C- and N- terminus. Though the function of SAP is still being delineated, it has been shown to bind to at least four regulatory molecules known to alter T and NK cell functions by both activation and suppression, and it is thought to be involved in T-B cell interactions through cytokine regulation [215]. Clinical features of XLP include an excessive immune reaction to EBV associated with hemophagocytosis and liver failure that is clinically indistinguishable from other forms of hemophagocytic syndromes and has been called fulminant infectious mononucleosis (FIM) or EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH) [25]. Both FIM and EBV-HLH can be seen in patients without XLP. XLP patients may also present with lymphoproliferative disease, hypogammaglobulinemia, or hematologic cytopenias [204, 205]. Patients who develop FIM/EBV-HLH may initially present with the usual signs and symptoms of IM, but these symptoms are often more severe. The course and progression of the disease are variable, ranging from presentation in multiorgan failure developing over hours, to persistent or recurring symptoms of IM for months. An atypical lymphocytosis is usually present at early stages of the disease, but patients subsequently develop severe, persistent pancytopenia, hepatic dysfunction resulting in fulminant hepatitis, meningoencephalitis, and varying degrees of myocarditis [26, 204]. The development of hepatic dysfunction, often with coagulation abnormalities secondary to liver failure or disseminated intravascular coagulation, and pancytopenia are ominous signs, as are other signs and symptoms of hemophagocytic syndromes, such as hypofibrinogenemia and elevated triglycerides [25, 204].

As discussed previously, EBV is a potent stimulus to the immune system, resulting in a massive EBV-specific and nonspecific response of both the humoral and cellular immune systems. Here the EBV-nonspecific response, primarily the cellular immune response, is

uncontrolled and is characterized by extensive infiltration of parenchymal organs by lymphoid cells, primarily $CD8^+$ cells in varying degrees of transformation and histiocytes with surprisingly few B cells. If this aggressive immune reaction goes unabated, ultimately all organs and even vessels will sustain extensive damage. This reaction culminates in phagocytosis, tissue destruction, and cellular depletion with death usually following shortly thereafter due to multisystem organ failure. Once this develops, therapy is difficult and usually unsuccessful, with a median survival time of approximately 4 weeks [205]. Antiviral drugs, immunoglobulins, IL-2, IFNα, IFNγ, plasmapheresis, corticosteroids, and most cytotoxic drugs have been ineffective [204]. The most consistent success in treatment is the early use of etoposide and immunosuppression with corticosteroids and cyclosporin or tacrolimus (FK506) [216]. Therapy may be required for 6–12 months. These patients are profoundly immunocompromised from their disease and the therapy. Therefore, a successful outcome is dependent on control of FIM/EBV-HLH symptoms while preventing and treating life-threatening infectious complications. Even with remission of symptoms, recurrences are common and tend to be more difficult to control, resulting in an extremely poor prognosis. When control of symptoms can be achieved, allogeneic BMT is recommended and is the felt to be the only curative therapy [217, 218].

A lymphoproliferative disorder (LPD) develops in approximately one-fourth of XLP patients [204, 205]. Most lymphomas are of B-cell phenotype, but approximately 10% of LPD are of a non-B-cell phenotype. Non-B-cell lymphoproliferative diseases include Hodgkin disease, T-cell NHL, lymphomatoid granulomatosis, or angiocentric immunoproliferative lesions [25, 204]. As in other immunodeficiencies, the role of EBV in the LPD is unclear, since about half of the boys with LPD have had no evidence of prior EBV infection, and EBV is detectable in only 25% of tumor specimens [205]. Again as in other immunodeficiency, allogeneic hematopoietic stem cell transplant should be considered if remission can be achieved.

Chediak-Higashi syndrome (CHS) is an autosomal recessive disorder characterized by recurrent bacterial infections, oculocutaneous albinism, abnormal platelets, varied neurologic dysfunction, and a 90% probability prior to the age 20 of developing a lethal hemophagocytic complication associated with EBV infection (referred to as the accelerated phase, again clinically indistinguishable from other forms of hemophagocytic syndromes and should be treated similarly) [219]. CHS is caused by mutations in the LYST gene (lysosomal trafficking regulator), and giant lysosomes are the characteristic findings in leukocytes on blood smear. Since lysosomes are the key storage compartments for cytolytic proteins including perforin and granzyme B, the cytotoxic effector function of NK and T cells is typically impaired in CHS, which is felt to be the reason for recurrent infections and inability to respond appropriately to EBV infection [220]. A transport defect inhibiting peptide loading and antigen presentation by HLA class II molecules on EBV-transformed B lymphocytes from CHS patients has also been proposed as a mechanism contributing to the pathogenesis of EBV-LPD in CHS patients.

X-linked hyper IgM syndrome (XHIM, X-linked CD40 ligand deficiency) results in a failure of immunoglobulin switching by B cells which requires signaling through CD40 and in decreased development and maintenance of type 1 cell-mediated responses (including NK cell function) due to impaired responsiveness of CD40-expressing, monocyte-derived, antigen-presenting cells (APCs) [221]. Patients with XHIM appear to have an increased risk of lymphomas, but particularly HD associated with EBV infection. Presumably, depressed cell-mediated function required for control of EBV is the mechanism. Patients with XHIM are also at increased risk for biliary carcinomas, as there is a high rate of sclerosing cholangitis in patients with a history of chronic cryptosporidiosis [222].

Autoimmune lymphoproliferative syndrome (ALPS) represents a constellation of genetic apoptosis defects associated with mutations in FAS, Fas ligand and caspase 8 genes [223]. Most of the cases described have heterozygous, dominant negative mutations involving FAS. Characteristic clinical features present in early childhood or even at birth. These include chronic multifocal lymphadenopathy, splenomegaly, autoimmune hemolytic anemia (and often other immune cytopenias), with increased proportions of circulating senescent T cells (CD3+, CD4-, CD8-), so-called dou-

Table 11.2 Comparison of lymphoproliferative disease between types of immunocompromised children [233]

	Histology	EBV-associated	Treatments	Outcome
Primary immunodeficiencies	• B cell (>80%) • T cell • HD* • MALToma	Majority, but depends on specific diagnosis	• Chemotherapy • ?Anti-B-cell antibody • allogeneic BMT	• Increased toxicity to chemotherapy • Relapses common
HIV-related	• B cell (90%) (including PCNSL, PEL) • HD*	~50% (100% in PCNSL)	• Chemotherapy + HAART	• ~50% DFS
Post-transplant	• B cell • T cell • HD*	>90% (BMT) ~70% (SOT)	• Reduced immunosuppression • Anti-B-cell antibody • Chemotherapy • DLI (BMT)	• 60–80% DFS

*HD – Hodgkin disease (nodular sclerosing subtype rare), PCNSL – primary central nervous system lymphoma, PEL – pleural effusion lymphoma, BMT – blood or marrow transplant, SOT – solid organ transplant, HAART – highly active retroviral therapy, DLI- donor lymphocyte infusion, DFS – disease free survival

ble-negative T cells. The majority of patients experience symptomatic improvement with steroid therapy, and generally, the autoimmune complications lessen in severity with age. However, the estimated risk of lymphoma in such patients is around 30%, and some patients have developed more than one lymphoid tumor over time. Patients who have the most severe forms of ALPS should be considered for correction with allogeneic hematopoietic stem cell transplantation [224]. Recently, the use of Rituximab and Fansidar [225], agents which induce apoptosis in the senescent lymphocytes bypassing the FAS/FAS ligand signal, have been shown to reduce lymphadenopathy and autoimmune symptoms in patients with ALPS. Whether such strategies will ultimately reduce the risk of lymphomas remains to be determined.

Ataxia telangiectasia (AT) is an autosomal disorder with cancer predisposition that has variable and profound immunologic and other systemic manifestations, principally cerebellar degeneration [226]. For some time it has been recognized that AT cells fail to activate cell-cycle checkpoints normally after exposure to γ-irradiation or radiomimetic agents. The mutant gene in AT (ATM) is a member of the phosphatidyl inositol kinase (PIK) family of molecules involved in signal transduction, and has also been implicated in meiotic recombination [227]. ATM appears to act as a sensor of double-stranded DNA breakage, e. g., in response to oxidative stress, activating numerous damage repair pathways including cell-cycle checkpoint control, *p53* activation, and DNA repair. Mutations in ATM lead to accelerated telomere loss and premature aging [228]. In the context of normal lymphopoiesis, ATM is clearly involved in the control of productive gene rearrangements of the B and T cell immune receptor molecules, since AT lymphocytes demonstrate a 25-fold increase in nonrandom rearrangements of immunoglobulin and TCR genes compared to lymphocytes from normal individuals [229]. Thymic output in AT is greatly reduced, and the subsequently restricted T-cell repertoire results in oligoclonal postthymic expansion [230]. Some of the nonrandom rearrangements involve translocation of Ig chains with *c-myc* reflecting, in magnified proportion, commonly seen cytogenetic rearrangements in lymphomagenesis. Though lymphoid tumors (both lymphomas and leukemias) predominate, patients with AT also experience high rates of epithelial cancers involving the skin, gastrointestinal tract, genitourinary tract, and CNS. Multiple tumors can be present simultaneously or develop sequentially. Early reports discussed a concordance of histologies in tumors affecting AT siblings from the same family – an intriguing but still mysterious observation [231]. The extent of response of tu-

mors in AT patients to conventional chemotherapy remains controversial; however, the frequent development of chronic lung disease in AT and inability to maintain chemotherapy intensity may contribute to poorer outcomes.

11.6 Summary

Increasing numbers of pediatric oncologists are now being faced with the difficult task of caring for immunodeficient children with malignancies, with lymphoproliferative disease/lymphoma predominating. Treating these patients presents numerous challenges. Regardless of the etiology of the immune defect, immunodeficient patients with cancer fare worse than the general population, even those with histologically similar malignancies. General principles for the treatment for these patients are summarized in Table 11.2. With advances in supportive care, particularly the prevention and treatment of opportunistic infections, patients with lymphoma associated with immunodeficiencies now enjoy longer lives than ever before, but morbidity and mortality remain problematic. Despite the therapeutic challenges, these patients provide a remarkable opportunity to study and explore how the immune system functions to prevent cancer. Though many malignancies observed in immunocompromised patients are associated with infectious agents, a substantial number are not. Therefore, anticancer immune surveillance must be more than just an inability to control infections. Immune surveillance must also protect against the development of neoplasia by the identification and/or elimination of cells with abnormalities in proliferation, function, and/or apoptosis.

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Late Effects Following Lymphoma Treatment

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Contents

12. 1 Introduction

Multimodality therapy for childhood lymphomas has resulted in markedly improved survival over the past several decades. For the period 1985–1994, the five-year survival rate for childhood cancer reported by the National Cancer Institutes Surveillance and End Results (SEER) section was 91% for Hodgkin lymphoma (HL) and 72% for non-Hodgkin lymphoma (NHL) (Ries et al. 1999). The multimodal therapy, including the multiple chemotherapeutic agents responsible for this improved survival, has been shown to result in adverse long-term health-related outcomes. These outcomes may manifest months to years after the completion of treatment and are commonly referred to as "late effects". Late effects include second malignant neoplasms, organ dysfunction, and adverse psychosocial sequelae. Treatment-related late effects can be due to surgery, radiation therapy, chemotherapy, or their combination. For radiotherapy, total dose and fraction size, organ or tissue volume, and machine energy are the most critical parameters. For chemotherapy, the important factors are agent type, single and cumulative dose, and schedule. Host factors such as genetic predisposition, inherent tissue sensitivities, capacity for normal tissue repair, premorbid organ function, and health behaviors may modify treatment-related toxicities, but have not been well studied.

Considerable data regarding the late effects of therapy have been published by the Childhood Cancer Survivor Study (CCSS) and will be reported in this review. The CCSS, a cohort study of 14,054 individuals, was established to address specific hypotheses related to long-term health-related outcomes in childhood cancer survivors (Robison et al. 2002). The cohort was constructed from rosters of all children treated at each

of the 26 participating institutions in the USA and Canada. Inclusion criteria included: (1) diagnosis of one of the following forms of childhood cancer before 21 years of age: leukemia, HL, NHL, neuroblastoma, soft-tissue sarcoma, bone cancer, malignant central nervous system (CNS) tumor, or kidney tumor; (2) initial treatment at one of the collaborating institutions between January 1, 1970, and December 31, 1986; and (3) survival for at least 5 years following diagnosis. Participants completed several comprehensive self-report questionnaires concerning medical and psychosocial outcomes, and detailed treatment data were collected on all participants from the treating institutions.

As therapy for HL and NHL often utilize similar classes of chemotherapy agents and both may utilize radiotherapy, we discuss potential late effects for both lymphomas combined, by organ system. Where there are significant differences for late effects by type of lymphoma, this has been noted.

12.2 Second Malignant Neoplasms

Several large studies have examined the incidence and spectrum of second malignant neoplasms (SMNs) in childhood lymphoma survivors, particularly for HL (Bhatia et al. 2003; Inskip 2001; Metayer et al. 2000; Neglia et al. 2001; Swerdlow et al. 2000; Wolden et al. 1998). In an analysis of SMN in the CCSS, which excluded patients with retinoblastoma, the SIR for SMN in the cohort was 6.4, with a 20-year incidence of 3.2% and an absolute excess risk of 1.88 malignancies per 1000 years of patient follow-up. Risk of SMN was elevated for all primary childhood cancer diagnoses, with the lowest SIR reported for NHL (3.2) and the highest for HL (9.7). In multivariate analyses, adjusted for radiation exposures, SMNs were independently associated with a childhood cancer diagnosis of HL and exposure to alkylating agents, among other variables (Neglia et al. 2001). In large cohorts of HL survivors followed over time, the risk of leukemia appears to plateau at 10–15 years posttherapy, while the risk of second solid malignancies, sarcoma, melanoma, lung, thyroid and gastrointestinal, and particularly breast cancer rises with ongoing follow-up, with a lifetime risk still unknown (Bhatia et al. 2003; Neglia et al. 2001; Wolden et al. 1998). Although data exist that suggest an increased risk in female survivors, even accounting for breast cancer, results are not consistent. While the gender effect is not consistent among studies, diagnosis at younger age and therapy for relapsed disease are consistently associated with increased risk (Acharya et al. 2003; Bhatia et al. 2003; Green et al. 2000; Metayer et al. 2000; Neglia et al. 2001; Swerdlow et al. 2000; van Leeuwen et al. 2000b; Wolden et al. 1998).

Patients who have undergone autologous stem cell transplantation for lymphoma have a risk of developing second malignancies. Information regarding risk factors predisposing to secondary carcinogenesis is largely derived from studies of cohorts including adolescent and adult patients. Metayer and colleagues conducted a case-control study of 56 patients with secondary MDS/AML and 168 matched controls within a cohort of 2,739 patients receiving autologous transplants for HL or NHL. In multivariate analyses, risks of MDS/AML significantly increased with the intensity of pretransplantation chemotherapy and with mechlorethamine or chlorambucil, compared with cyclophosphamide-based therapy. The use of TBI at doses of 12 Gy or less did not appear to increase the leukemia risk, but TBI doses of 13.2 Gy or more increased the risk significantly. There is a suggestion that peripheral blood stem cells may increase the risk of MDS/AML compared with bone marrow grafts (Metayer et al. 2003). In a series of 493 patients treated for NHL at The University of Texas M.D. Anderson Cancer Center, 22 patients developed MDS or AML. Multiple logistic regression analyses showed that TBI was independently associated with an increased risk of developing MDS/AML, and patients receiving TBI in combination with cyclophosphamide and etoposide were more likely to develop MDS/AML than patients who received TBI with cyclophosphamide or thiotepa (Hosing et al. 2002). In a series from the City of Hope National Medical Center, among 612 patients treated for lymphoma, 22 developed MDS or acute leukemia, with an estimated cumulative incidence of 8.6%±2.1% at 6 years. Multivariate analyses revealed stem cell priming with etoposide and pretransplant radiotherapy to be significant risk factors (Krishnan et al. 2000).

Data related to 467 French patients treated with autologous transplantation for HL were matched with 1,179 conventionally treated patients listed in international databases. There were 18 secondary cancers, leading to a 5-year cumulative incidence of 8.9%. Risk factors for second cancer were age 40 years or older, the use of peripheral blood as a source of stem cells, and treatment for relapsed disease. Solid tumors were more frequent in patients treated with transplantation, although the incidence of MDS and AML was similar in the two groups (Andre et al. 1998).

12.3 Neurocognitive

Survivors of NHL may be at risk for neurocognitive late effects if they have received CNS-directed therapies, such as cranial radiation or intrathecal chemotherapy. Due in part to the heterogeneity of type of treatment for NHL, this has not been well studied among lymphoma survivors. When neurocognitive toxicity has been addressed in reports of clinical trials for NHL, the occurrence is relatively uncommon (Cairo et al. 2002; Sposto et al. 2001). This contrasts with acute lymphoblastic leukemia (ALL), where neurocognitive late effects have been well studied. However, as similar therapies are utilized, specifically intravenous or intrathecal methotrexate and cranial radiotherapy, we will briefly review the toxicities, with much of the data from ALL survivors.

Toxicities include educational deficits in both global IQ and in attention and nonverbal cognitive skills (Hill et al. 1997; Meadows et al. 1981; Waber et al. 1995; Williams et al. 1991), related to cranial radiotherapy or intrathecal chemotherapy, and leukencephalopathy related to intravenous and intrathecal methotrexate (Bleyer 1981; Bleyer et al. 1990; Iuvone et al. 2002). However, the impact on lymphoma survivors does not appear as significant as in ALL survivors, as is demonstrated by data from the Childhood Cancer Survivor Study. Mitby and colleagues demonstrated a small increased risk for the need for special education for survivors receiving intrathecal chemotherapy with (OR, 2.6; 95%CI, 2.30–2.95) or without (OR, 1.3; 95%CI, 1.09–1.78) cranial radiotherapy. However, NHL survivors were not at increased risk of requiring special education services, whereas survivors of ALL did exhibit an increased risk. Thus, some caution should be taken in extrapolating adverse long-term outcomes from the ALL survivor group. Despite the fact that NHL survivors did not have an increased need for special education, compared with siblings, they did have an increased risk of not completing high school (OR = 1.8; 95%CI 1.15–2.78). Interestingly, survivors of HL in this study who received no specific CNS-directed therapy also required more special education services than their siblings (OR = 4.4; 95%CI 2.64–7.24) (Mitby et al. 2003).

12.4 Cardiovascular

Lymphoma survivors exposed to doxorubicin are at risk for long-term cardiac toxicity. The risks to the heart are related to individual and cumulative anthracycline dose, the total and fractional irradiation dose, the volume delivered to different depths and specific areas of the heart, age at exposure, latency period, and gender, as discussed below.

The effects of thoracic radiotherapy are difficult to separate from those of anthracyclines, since most children now treated for lymphoma are exposed to anthracyclines whether or not they also receive thoracic radiotherapy. The pathogenesis of injury differs, however, with radiation primarily affecting the fine vasculature of the heart, and anthracyclines directly damaging myocytes (Fajardo et al. 1976). Late effects of radiation to the heart include: delayed pericarditis; pancarditis, which includes pericardial and myocardial fibrosis, with or without endocardial fibroelastosis; myopathy; coronary artery disease (CAD); functional valve injury and conduction defects (Adams et al. 2003; Hancock et al. 1993; King et al. 1996). A recent series from the Harvard Joint Center for Radiation Therapy reported on a cohort of 48 survivors of pediatric and young adult HD who received a median dose of mediastinal radiotherapy of 40.0 Gy (27–51.7 Gy), with only four having also received anthracycline therapy. In 43 for whom echocardiography results were available, all had significantly decreased mean left ventricular mass, end-diastolic dimension and end-diastolic wall thickness, with 17 also exhibiting abnormal end-systolic

wall stress. In addition, 20 had a significant valve defect. Autonomic dysfunction was evident in 50% of the patients, and peak oxygen uptake during exercise was decreased in 30% (Adams et al. 2004). However, with current techniques and reduced doses of radiotherapy, these effects are unlikely following treatment for childhood cancer. In a study of 635 patients treated for childhood HL, 12 patients died of cardiac disease, including seven deaths from acute myocardial infarction, but these deaths occurred in patients treated during childhood with 42–45 Gy. In the same population of survivors, the actuarial risk of pericarditis requiring pericardiectomy was 4% at 17 years (occurring only in children treated with higher radiation doses). Among children treated with 15–26 Gy, none developed radiation-associated cardiac problems (Hancock et al. 1993).

Increased risk of doxorubicin-related cardiomyopathy is well documented among survivors of HL and NHL, as well as other forms of childhood cancer such as ALL and sarcoma where anthracyclines are utilized. Anthracycline-related cardiomyopathy is associated with female sex, cumulative doses greater than 200–300 mg/m^2, younger age at time of exposure, and increased time from exposure (Kremer and Caron 2004; Kremer et al. 2001; Lipshultz et al. 2000; Nysom et al. 1998b; Sorensen et al. 2003). Cardiac abnormalities related to doxorubicin exposure may not manifest for many years after exposure. The Institut Gustave Roussy recently evaluated cardiac abnormalities in 229 15+-year survivors of childhood cancer treated with doxorubicin at a mean dose of 344 mg/m^2 (range 40–600 mg/m^2) between 1968 and 1982. Significant cardiac disease with a fractional shortening <25%, ejection fraction <50%, end-systolic wall stress >100 g cm^{-2}, or clinical heart failure was seen in 89 patients (39%) of whom 65 were asymptomatic. (Pein et al. 2004) There was no threshold for the anthracycline dose in this study, although risk increased with increasing dose. Neither age at time of treatment nor gender influenced the risk. Radiotherapy exposure increased risk 4-fold (95%CI 1.0–17.5), as did longer time since treatment, even after 15 years (Pein et al. 2004). Kremer and colleagues recently systematically reviewed the literature on subclinical cardiotoxicity following treatment with anthracyclines for childhood cancer. A clear dose-response was noted with ranges of subclinical cardiotoxicity of 15.5–27.8% and abnormal afterload of 19–52%, for those who received doses in excess of 300 mg/m^2. In those who were treated with cumulative doses below this level, the reported range for abnormal left ventricular function was 0–15.2% (Kremer and Caron 2004).

These data support the need for clinical protocols that evaluate lower doses of anthracyclines and employ other methods of cardioproctection when reduction in dose is not possible. In addition, long-term cardiac surveillance for survivors who have been exposed to anthracyclines is clearly indicated. For women, this is of additional importance during pregnancy, when the cardiac volume increases dramatically in the last trimester and during labor, and therefore, cardiac workload increases dramatically. Overt congestive heart failure has been reported, although due to small case series, the magnitude of the risk remains undetermined (Hinkle et al. 2004a; Pan and Moore 2002).

Other strategies to decrease the risk of cardiotoxicity from anthracyclines have including altering the route of administration. To date, albeit with relatively short follow-ups, there does not appear to be a benefit compared to what was expected from consecutive divided daily doses or continuous infusion, as compared with bolus administration of a single dose (Ewer et al. 1998; Gupta et al. 2003; Levitt et al. 2004; Lipshultz et al. 2002).

Similarly, the use of dexrazoxane (DZR) has been evaluated as a potential cardioprotectant agent. Despite early adult and pediatric data that this agent decreased acute toxicity (Anderson 2005; Lipshultz 1996; Schiavetti et al. 1997; Swain et al. 1997; Venturini et al. 1996; Wexler et al. 1996), the long-tem benefit is unclear. While a deleterious antitumor effect of dexrazoxane has not yet been reported in pediatric trials, it has been reported in one adult trial (Swain and Vici 2004), resulting in more restricted recommendations for its use in adult trials (Schuchter et al. 2002). In addition, it may contribute to increased acute hematologic and infectious disease toxicity and increased risk of second cancers (Schwartz et al. 2003). Given the latency time to development of severe cardiac abnormalities following doxorubicin exposure, it will be many years before it is known whether dexrazoxane is

successful in reducing long-term cardiotoxicity in pediatric patients.

Another approach in the amelioration of doxorubicin-induced left ventricular (LV) dysfunction has been the use of the angiotensin-converting enzyme inhibitor enalapril. However, the results are still too preliminary to make any conclusions regarding long-term benefit. While early improvement in LV function and structure have been reported, longer-term maintenance of this effect has not been consistent across studies (Lipshultz et al. 2002; Silber et al. 2004).

Rhythm disturbances are also reported following doxorubicin exposure. Schwartz and colleagues studied electrocardiograms in 52 long-term survivors of childhood cancer who had been treated with anthracyclines. Prolongation of QTc of >0.43 were noted in six of 22 patients who had received cumulative anthracycline doses of >300 mg/m^2, compared with 0 of 15 patients who had received lower anthracycline doses. Thoracic radiotherapy increased the risk in both groups, although the higher anthracycline dose group still demonstrated a greater frequency of prolongation of QTc. Exercise further prolonged the QTc in 6 of 10 patients evaluated (Schwartz et al. 1993).

12.5 Pulmonary

Pulmonary fibrotic disease is seen as a late complication following radiation therapy. In the modern management of pediatric lymphoma, radiotherapy is often given in combination with chemotherapy, including agents with independent pulmonary toxicity, such as bleomycin, utilized for the treatment of HL. Acute pneumonitis, manifested by fever, congestion, cough, and dyspnea, can follow radiotherapy alone at doses of >40 Gy to focal lung volumes, or after lower doses when combined with anthracyclines (Mah et al. 1987). Although it is uncommon with contemporary therapy, asymptomatic reduction in lung function, manifested by a reduction in diffusion capacity or restrictive or obstructive patterns on formal testing, may occur.

Bleomycin-associated pulmonary fibrosis with permanent restrictive disease is dose-dependent, usually occurring at doses >200–400 U/m^2, higher than those currently used in pediatric HL (Bossi et al. 1997; Fryer et al. 1990; Kreisman and Wolkove 1992). Mefferd and colleagues evaluated lung function in 20 pediatric HL patients treated with MOPP/AVBD and 15–25 Gy mantle radiation and found 55% to have abnormal diffusion capacity (Mefferd et al. 1989). Marina and colleagues evaluated serial pulmonary function in children treated with COP/ABVD and mantle radiotherapy and found 65–73% to have only mildly decreased or normal diffusion capacity (Marina et al. 1995). Similar abnormalities of pulmonary function were reported by Nysom and colleagues in survivors of childhood ALL, HL, and NHL (Nysom et al. 1998a,c). Since symptomatic pulmonary disease was rare in all of these studies, it is difficult to predict what will be the impact of mild abnormalities of pulmonary function as the survivors age. A prospective study of pulmonary function from Stanford University of 145 teenagers and adults with HL treated from 1980 to 1990 evaluated patients prior to treatment, early after treatment (<15 months), and more than 36 months after therapy (Horning et al. 1994). Some 32% of patients treated with mediastinal radiotherapy alone (median dose 44 Gy), 37% treated with mediastinal radiotherapy (median dose 44 Gy) and bleomycin (median dose 112 U), and 19% treated with bleomycin (median dose 120 U) without mediastinal radiotherapy had a reduction in forced vital capacity (FVC) below 80%; only 7% of the entire study population had a reduction in diffusion capacity below 70%. Mediastinal radiotherapy was the only therapeutic factor that increased the risk. No patients had significant disease to warrant hospitalization (Horning et al. 1994).

Despite these data, it is difficult to estimate the prevalence of pulmonary dysfunction in childhood lymphoma survivors. Clinical evaluations coupled with functional and quality of life assessments are needed in very long-term survivors in whom treatment for cancer is well known. An analysis of self-reported pulmonary complications of 12,390 survivors of common childhood malignancies (including HL and NHL) has been reported by the CCSS. Compared with a sibling control group, survivors were more likely to have lung fibrosis, recurrent pneumonia, chronic cough, pleurisy, use of supplemental oxygen therapy, abnormal chest wall, exercise-induced shortness of breath, and bronchitis with relative risks ranging from

1.2 to 13.0, highest for lung fibrosis and lowest for bronchitis. The 25-year cumulative incidence of lung fibrosis was 5% for those who received chest radiotherapy and <1% for those who received pulmonary toxic chemotherapy. Treatment-related risk factors included chest radiation for lung fibrosis, supplemental oxygen therapy, recurrent pneumonia, exercise-induced shortness of breath, and chronic cough. Cyclophosphamide was associated with exercise-induced shortness of breath, supplemental oxygen therapy, chronic cough, bronchitis, and recurrent pneumonia; bleomycin with need for supplemental oxygen therapy, bronchitis, and chronic cough; and doxorubicin with an increased risk of emphysema, supplemental oxygen therapy, chronic cough, and shortness of breath. One survivor treated for NHL had undergone a lung transplant, and three HL survivors developed adenocarcinoma of the lung as a second malignancy. It is expected that second cancers and other clinical manifestations of treatment affecting the lungs will continue to increase as the survivors age (Mertens et al. 2002).

12.6 Bone and Body Composition

Survivors of pediatric lymphoma may be at an elevated risk for decreased bone mineral density. Risk factors include increased age at time of exposure, estrogen deficiency, female gender, corticosteroid use and type, growth hormone deficiency, and cranial radiation. Prevalence, chronicity, and severity are not consistent across studies, and therefore, the risk remains poorly defined (Aisenberg et al. 1998; Kim et al. 2004; Nysom et al. 2001; Ratcliffe et al. 1992; van Leeuwen et al. 2000a; Vassilopoulou-Sellin et al. 1999). An increased incidence of fractures and osteonecrosis may also be present in these patients, but as was true for osteopenia and osteoporosis, this has not been systematically studied in large groups of HL survivors (Enrici et al. 1998; Hancock et al. 1978; Hanif et al. 1993; Mould and Adam 1983; Prosnitz et al. 1981, 1982; Thornton et al. 1997; Tombolini et al. 1992). Bisphosphonates, calcium supplements, and hormone replacement therapy, used in the general population with reduced bone mineral density, are potential interventions (Greenspan et al. 2000; Kim et al. 2004; Sherman 2001). Further research into the type and frequency of screening, the population at highest risk, and interventions are clearly indicated for survivors of HL and NHL, where attempts have been made over the past decade to decrease doses of corticosteroids (HL & NHL), alkylating agents (HL & NHL), methotrexate (NHL), and cranial radiotherapy (NHL). For lymphoma survivors who have undergone HSCT, there is a lack of consensus regarding the risk and incidence of decreased bone mineral and the recommendations for intervention (Ebeling et al. 1999; Gandhi et al. 2003; Nysom et al. 2000; Schimmer et al. 2001).

12.7 Endocrine

12.7.1 Thyroid Gland

Thyroid dysfunction, manifested most often by primary hypothyroidism but also hyperthyroidism, goiter, or nodules, is a common delayed effect of radiation therapy fields that include the thyroid gland, mantle, and neck, such as HL. In children treated with radiation therapy, most who develop hypothyroidism do so within the first 2–5 years following treatment, but new cases can occur later. Thyroid dysfunction varies with the dose of radiation, the length of follow-up, and the biochemical criteria utilized to make the diagnosis (Gleeson et al. 2002). For example, criteria for the diagnosis of hypothyroidism, the most frequently reported abnormality, may include either elevated thyroid-stimulating hormone (TSH), depressed thyroxine (T4), or both (Hancock et al. 1995; Oberfield et al. 1997; Shalet 1996; Sklar 1999).

The incidence of hypothyroidism has decreased with the lower cumulative doses of radiotherapy employed in the newer protocols. In a study of 1677 children and adults with HL who were treated with radiation therapy between 1961 and 1989, the actuarial risk at 26 years for overt or subclinical hypothyroidism was 47%, with a peak incidence at 2–3 years after treatment (Hancock et al. 1991). In a study of HL patients treated between 1962 and 1979, hypothyroidism occurred in four of 24 patients who received mantle doses of <26 Gy, but in 74 of 95 patients who received >26 Gy. The peak incidence occurred at 3–5 years after

treatment with a median of 4.6 years (Constine et al. 1984). A cohort of childhood HL survivors treated between 1970 and 1986 were evaluated for thyroid disease by use of a self-report questionnaire in the CCSS (Sklar et al. 2000). Among 1791 survivors, 34% reported that they had been diagnosed with at least one thyroid abnormality. For hypothyroidism, there was a clear dose response with a 20-year risk of 20% for those who had received less than 35 Gy, 30% following 35–44.9 Gy and 50% following >45 Gy to the thyroid gland. Compared to the general age-matched population, the relative risk for hypothyroidism was 17.1, for hyperthyroidism 8.0, and for thyroid nodules 27.0. The time elapsed since the diagnosis was a risk factor for both hypo- and hyperthyroidism, where the risk increased in the first 3–5 years after the diagnosis. For nodules, the risk increased 10 and more years after the diagnosis. Women were at increased risk for hypothyroidism and thyroid nodules (Sklar et al. 2000).

12.8 Reproductive Endocrine

12.8.1 Male Gonadal Function

Spermatogenesis is highly sensitive to cyclophosphamide and other alkylating agents, such as procarbazine and nitrogen mustard, drugs commonly used to treat HL (Ben Arush et al. 2000; Gerres et al. 1998; Hill et al. 1995; Kulkarni et al. 1997). There is a dose-response effect for oligospermia and azoospermia, so that infertility is a common complication of therapy with COPP, MOPP, and other combinations. Only four cycles of COPP can lead to germ cell aplasia in the majority of boys treated with this combination for HL (Hobbie et al. 2005). In a report by Bokemeyer and colleagues, long-term gonadal toxicity was compared between groups of survivors of HL and NHL. Both groups had received comparable median cumulative doses of cyclophosphamide, but only the patients with HL had received procarbazine. The incidence of gonadal toxicity among the HL survivors was more than 3-fold greater than in the NHL survivors. In fact, the only men in the NHL group who had elevation of FSH had received far higher doses of cyclophosphamide than the mean (Bokemeyer et al. 1994). Endocrine function is not affected by the doses that destroy germ cells, and boys can progress through puberty and have normal level of testosterone in spite of having received very high doses of alkylating agents (Chapman et al. 1979; Cicognani et al. 2003; Qureshi et al. 1972).

Prepubertal boys are not spared, although boys who are older at the time of treatment may be more sensitive to germ cell ablation (Dhabhar et al. 1993). Reduction in total doses of alkylating agent therapy in multiagent protocols may be expected to result in less male infertility (Hill et al. 1995; Kulkarni et al. 1997; Relander et al. 2000; Schellong et al. 1999). Review of the available studies suggests that males who receive less than 4 g/m^2 of cyclophosphamide, without testicular or cranial radiation or any other alkylating agent, are likely to retain their fertility. Conversely, cumulative doses above 9 g/m^2 confer a greater risk of infertility.

The degree and permanency of radiotherapy-induced damage to the male reproductive system are dependent upon dose, field, and schedule. The germinal epithelium is damaged by much lower doses (<1 Gy) of radiotherapy than are Leydig cells (20–30 Gy) (Thomson et al. 2002). Doses less than 30 Gy are unlikely to affect testosterone production, so boys treated with these doses usually progress through puberty normally. Although temporary oligospermia can occur after these very low radiation doses, permanent azoospermia results from doses of >3–4 Gy. The potential for a return of spermatogenesis in the intermediate dose range of 1–3 Gy is variable (Ash 1980; Lushbaugh and Casarett 1976). Scatter from abdominal radiation with doses >20 Gy for HL can cause transient elevation in follicle-stimulating hormone (FSH) and oligospermia, but no effect was seen with lower doses (Kinsella et al. 1982).

12.8.2 Female Gonadal Function

Unlike the situation in males, hormonal function and potential for fertility are synchronous in females. Prepubertal females possess their lifetime supply of oocytes with no new oogonia formed after birth. Risk of menstrual irregularity, ovarian failure, and infertility increase with age at treatment (Bath et al. 2002; Hill et al. 1995; Mayer et al. 1999; Thomson et al. 2002). Therefore, amenorrhea and premature ovarian failure

occur more commonly in adult women treated with cyclophosphamide and other alkylating agents than in adolescents, with prepubertal females tolerating cumulative doses as high as 25 g/m^2 (Damewood and Grochow 1986; Kreuser et al. 1992). However, several large studies of survivors treated through the 1980s have shown elevated relative risks for infertility and premature ovarian failure in female survivors of lymphoma (Byrne et al. 1987; Chiarelli et al. 1999). In a study of childhood cancer survivors treated between 1945 and 1975, the relative fertility of married survivors of childhood HL and NHL was 0.77 (95%CI 0.64–0.92) and 0.81 (95%CI 0.56–1.16), respectively, compared with sibling controls. Among female survivors of adolescent HL, the relative risk for premature ovarian failure between 21 and 30 years was 3.35 and between 31 and 40 years, 1.27, compared with sibling controls. Relative risk between the ages of 21 and 30 rose to 9.6 for those treated with radiotherapy below the diaphragm and alkylating agents (Byrne 1999). In another study of 719 survivors treated between 1964 and 1988, of whom 29% were lymphoma survivors, overall there was a 15.5% failure to conceive. Increasing doses of abdominopelvic radiotherapy and increasing doses of alkylating agents resulted in an increase in premature ovarian failure and a fertility deficit in the entire cohort (Chiarelli et al. 1999).

Risk-adapted protocols for HL with avoidance of long-term toxicities as a primary study goal have been developed in the last decade. Substitution of cyclophosphamide for mechlorethamine appears to have significantly reduced the risk of ovarian dysfunction, which is then further lessened by a reduction in total dose of both agents (Hudson 2002; Linch et al. 2000; Schwartz 2003). Investigators are now collecting data on premature menopause in the group of women treated more recently in order to determine the full impact of these modifications in therapy.

12.8.3 Reproduction

With more childhood cancer survivors retaining their fertility, pregnancy outcome data are now becoming available. The CCSS cohort reported 1082 pregnancies among HL survivors and 300 among NHL survivors. The relative risks for live births, compared with female siblings, were 0.79 (95%CI 0.66–0.95) and 0.80 (95%CI 0.59–1.1) between HL and NHL survivors, respectively. The risk for medical abortion was increased among HL survivors, compared with siblings (RR = 1.46; 95%CI 1.12–1.91). The risk for stillbirths or miscarriage was not significantly elevated for either group. Chemotherapy did not increase the risk of an adverse outcome (Green et al. 2002). In the same cohort, Green and colleagues also evaluated the pregnancy outcomes of partners of male survivors in this cohort. Compared with partners of male siblings, there was an increased risk for stillbirths among partners of male NHL survivors (RR = 4.19; 95%CI 1.13–15.5). There was no difference in live births, still births, or medical abortions among the partners of the male lymphoma survivors and partners of male siblings (Green et al. 2003).

Progress in reproductive endocrinology has resulted in the availability of several options for preserving or permitting fertility in patients about to receive potentially toxic chemotherapy or radiotherapy (Bath et al. 2002; Thomson et al. 2002). For males, cryopreservation of spermatozoa before treatment is an effective method to circumvent the sterilizing effect of therapy. Although pretreatment semen quality in patients with cancer may be less than that noted in healthy donors, the decline in semen quality and the effect of cryodamage to spermatozoa from patients with cancer are similar to those of normal donors (Agarwal 2000; Hallak et al. 1998; Khalifa et al. 1992; Muller et al. 2000). For those unable to bank sperm, newer technologies such as testis sperm extraction may be an option, as demonstrated for male survivors of germ cell tumors who had postchemotherapy nonobstructive azoospermia (Damani et al. 2002). Further micromanipulative technologic advances such as intracytoplasmic sperm injection and similar techniques may be able to render sperm extracted surgically, or even poor-quality cryopreserved spermatozoa from cancer patients, capable of successful fertilization (Damani et al. 2002; Pfeifer and Coutifaris 1999).

In pre- and postpubertal females, cryopreservation of ovarian cortical tissue or enzymatically extracted follicles and in vitro maturation of prenatal follicles are of potential clinical use. To date, most of this technology has been performed in laboratory animals (Bahadur and Steele 1996; Donnez et al. 2000; Newton 1998).

Another option available to the postpubertal female is the stimulation of ovaries with exogenous gonadotropins and retrieval of mature oocytes for cryopreservation. However, only a few oocytes can be harvested after stimulation of the ovaries (Donnez et al. 2000). In vitro fertilization and subsequent embryo cryopreservation have also been successful. Ethical issues regarding risk and benefits, assent and consent, and disposition of gametes are yet to be worked out (Dudzinski 2004). Furthermore, all these approaches harbor the risk that malignant cells will be present in the specimen and reintroduced in the patient at a later date. Those with hematologic or gonadal tumors would be at greatest risk for this eventuality (Donnez et al. 2000; Newton 1998).

With increased use of assisted fertility techniques in survivors of childhood cancer, the risk of congenital anomalies will need to be followed closely, due to reports of increased anomalies in offspring born by in vitro fertilization or intracytoplasmic sperm injection (Bonduelle et al. 2002; Ericson and Kallen 2001; Hansen et al. 2002; Serafini 2001; Simpson and Lamb 2001).

12.9 Dental

In a study of the effects of chemotherapy on oral and dental structures and craniofacial growth in 30 survivors of childhood lymphoma, eruption status, root malformations, premature apexification, agenesis, crown anomalies, soft-tissue abnormalities, gingival and periodontal status, enamel defects and discolorations, and craniofacial growth status of the subjects were documented and compared with findings in 20 healthy children. Statistically significant differences between the study and control groups were found for plaque index, enamel hypoplasia, discolorations, and agenesis (Alpaslan et al. 1999). In another case control study of 36 long-term survivors of NHL treated with multiagent chemotherapy and 36 controls, the survivors had significantly higher plaque index, more enamel discolorations, and root malformations (Oguz et al. 2004).

Salivary gland irradiation incidental to treatment HL causes a qualitative and quantitative change in salivary flow, which can be reversible after doses of <40 Gy, but may be irreversible after higher doses, depending on whether sensitizing chemotherapy, such as doxorubicin, is also administered. Dental caries are the most serious consequence. The use of topical fluoride can dramatically reduce the frequency of caries, and saliva substitutes and sialagogues can ameliorate sequelae, such as xerostomia (Makkonen and Nordman 1987; Maxymiw and Wood 1989).

These findings give further impetus to the need for routine dental and dental hygiene evaluations for survivors of childhood lymphoma. However, despite known risk factors, dental practices among survivors are less than recommended for the general population (Yeazel et al. 2004).

12.10 Spleen

Splenectomy increases the risk of life-threatening invasive bacterial infection (American Academy of Pediatrics 2003). Since it is no longer standard practice to perform a staging laparotomy for pediatric HL, the previously described long-term complications, both related to surgery and altered immune function, should no longer be an issue for most survivors of childhood HL (Jockovich et al. 1994; Kaiser 1981). However, children may be rendered asplenic by radiation therapy to the spleen in doses of >40 Gy (Coleman et al. 1982; Weiner et al. 1995). Low-dose involved field radiation (21 Gy) given together with multiagent chemotherapy does not appear to adversely affect splenic function (Weiner et al. 1995).

For patients with surgical or functional asplenia, prophylactic antibiotics, generally penicillin, are recommended to be taken daily for life. These recommendations are based on general pediatric practice, but the benefit of prophylactic antibiotics in asplenic cancer survivors has not been studied. Older survivors often report discontinuing antibiotics and should therefore be instructed to take penicillin at the onset of febrile illness. Medical care should be sought promptly for fevers $\geq$38.5°C. Patients should receive antibiotic prophylaxis for dental work and should be immunized against meningococcus, hemophilus influenzae B, and

Streptococcus pneumoniae (American Academy of Pediatrics 2003).

12.11 Psychosocial

Survivors of pediatric lymphoma are at risk for adverse psychologic outcomes. Among 4914 survivors of leukemia and lymphoma in the Childhood Cancer Survivor Study, 5.4% reported symptoms of depression, with equal reporting across diagnostic groups. In addition, 15% of the 1843 HL survivors and 11.4% of the 902 NHL survivors reported somatic distress. Reports of symptoms of depression or somatic distress were higher among the lymphoma survivors than a cohort of siblings. For survivors of HL, female gender, lower household income, less than a high school education, and lack of current employment increased the risk for depression and somatic distress symptoms, with older age also increasing the risk for somatic distress. Among the NHL survivors, lower educational attainment and lack of current employment increased the risk of both depression and somatic distress, with lower household income also increasing the risk for somatic distress (Zebrack et al. 2002). Results from the Italian Multicentric Study on Long-Term Survivors of Childhood Cancer support the finding of psychological distress. In this cohort, survivors of leukemia and lymphoma reported that feelings of anxiety, depression, panic attacks, and fear of recurrence were increased, with differences more marked when compared with friends or other relatives of the case compared to siblings (Cuttini et al. 2003).

These data underscore the need to address both physical and psychosocial factors that may impact the health status and quality of life in long-term survivors of lymphoma.

12.12 Mortality

Patients who have initially been cured of HL are at increased risk of early mortality from disease, treatment, and external causes. In a series from St. Jude Children's Research Hospital, Hudson and colleagues studied late events in 387 pediatric HL patients treated from 1968 to 1990 treated on four consecutive trials, with a median follow-up of 15.1 years (Hudson et al. 1998). Cumulative incidence of 25-year cause-specific mortality was 9.8±1.6% for Hodgkin disease, 8.1%±2.6% for second cancers, 4.0%±1.8% for cardiac disease, 3.9%±1.5% for infection, and 2.1%±0.8% for accidents. Standardized mortality ratios showed excess mortality from cardiac disease (SMR = 22, 95%CI 8–48) and infection (SMR=18, 95%CI 7–38) (Hudson et al. 1998). In a recent analysis of 1261 patients treated for HL in the Netherlands between 1965 and 1987, which included 329 patients under the age of 21 years, with a median follow-up of 17.8 years, the relative risk of death from all causes other than HL was 6.8 times that of the general population. Of note, in patients treated under the age of 21 years, the relative risk of death from solid tumors was 14.8 and from cardiovascular disease, 13.6 (Aleman et al. 2003). In the Childhood Cancer Survivor Study, there was an elevated risk of death for initial 5-year survivors of HL and NHL from second malignancies (HL: SMR = 24.0, 95%CI 19.2–29.7; NHL: SMR = 15.6, 95%CI 9.6–23.7), cardiac disease (HL: SMR = 12.0, 95%CI 6.5–22.4; NHL: SMR = 6.5, 95%CI 2.3–14.0), and pulmonary disease (HL: SMR = 13.8, 95%CI 9.3–19.4; NHL: SMR = 14.7, 95%CI 6.1–35.4) (Mertens et al. 2001). In a similar study from the Nordic countries using registry data, HL patients experienced the highest proportion of deaths (19.4%) and were noted to have excess mortality from second malignancy, pulmonary disease, and disease of the heart and circulatory system. However, this study also demonstrated the importance of changes in therapy over time to decrease the risk of late effects. In the HL patients, comparing patients diagnosed from 1960 to 1979 with those diagnosed from 1980 to 1989, the overall death hazard ratio was decreased (0.34, 95%CI 0.23–0.52) (Moller et al. 2001). This is related to a decrease in deaths from the primary cancer without an associated increase in mortality from second cancers or treatment-related toxicities. The former reflects improvements in therapeutic efficacy, and the latter reflects changes in therapy made as a consequence of the study of the causes of late effects.

12.13 Monitoring for Late Effects

The American Society of Pediatric Hematology/Oncology, the International Society of Pediatric Oncology, and the American Academy of Pediatrics support the need for long-term monitoring of adverse long-term sequelae for all childhood cancer survivors. This is especially important for survivors of pediatric lymphoma. Tables 12.1 and 12.2, adapted from the Children's Oncology Group (COG) Long-term Follow-up Guidelines (Landier et al. 2004), summarize the common late effects seen in lymphoma survivors and general guidelines for monitoring studies. Survivors should seek care from professionals with expertise in the recognition and management of late effects (Arcecci 1996; Harvey et al. 1999; Masera et al. 1997; Meadows et al. 1998; Sanders et al. 1997). To support this, more information on the late effects of childhood cancer is being published in both oncology and general pediatric, nursing, and family practice journals (Eshelman et al. 2004; Friedman and Meadows 2002; Oeffinger 2003; Oeffinger and Hudson 2004). The Children's Oncology Group and others have published guidelines for survivors and health care providers. The COG Long-term Follow-up Guidelines are accompanied by detailed health links about exposures, risks for late effects, and monitoring, and all are available on a public website (Landier et al. 2004). In the United Kingdom, both the SIGN guidelines and the UKCCSG "After Cure Booklet" are available on a public website. The "After Cure Booklet" provides patient information including health education and information leaflets on a range of late sequelae (Blacklay et al. 1998).

It is well acknowledged that survivors need to be educated about their diagnosis, treatments received, need for monitoring, and recommended disease prevention practices (Blacklay et al. 1998; Hudson et al. 1999, 2004; Landier et al. 2004; Oeffinger and Hudson 2004). Health promoting behaviors should be stressed for survivors of childhood lymphoma, and patient information has been shown to enhance attitude to follow-up and influence adverse life style behaviors (Eiser et al. 2000; Hudson et al. 1999, 2004).

12.14 Research Challenges for Lymphoma Survivors

Although outcomes research in pediatric oncology has been carried on for over 25 years, there continue to be considerable challenges. Many late effects have long and variable latency periods, and lifetime risks remain undetermined. Changes in therapeutic approaches, designed in part to decrease late effects, combined with improved survivorship mandate the need for ongoing studies that focus on health-related outcomes of newer treatment approaches that are not limited to a simple analysis of cure. Monitoring guidelines currently in place are based, in part, on long-term data from survivors treated with therapies that are no longer being used (Landier et al. 2004). Perhaps the greatest challenge is that in order to clearly quantify the magnitude of risk, studies of late effects require ascertainment of affected and unaffected survivors. Incomplete follow-up can result in a reduction in study power and selection bias. Patients who participate in long-term follow-up may differ in systematic and important ways from those who do not participate.

Risk factor analyses require a quantitative rather than qualitative approach in documenting and reporting late effects. However, despite efforts to the contrary, to date we do not yet have a satisfactory and uniformly accepted manner of quantifying late effects, as is evidenced by the efforts of the Radiation Therapy Oncology Group (RTOG) Late Effects Working Group (Rubin et al. 1995). As childhood cancer survivors enter adulthood and are followed by a combination of pediatric, radiation oncology, and primary care specialists, such scoring systems must be usable by a broad variety of health care professionals.

Outcomes-based research may be difficult to carry out in an acute care clinic model, where acute clinical needs take precedence. It is challenging to spend the time required to enroll patients on research protocols, conduct unbiased research, and balance the needs of the individual patient with those of the scientific community. In addition, funding boundaries for clinical and research goals may become blurred, and in an era of emphasis on cost containment, these must be delineated as clearly as possible. If research is to lead to changes in the standard of care for patients, it is im-

Table 12.1 General guidelines for radiation late effects: assessment and management

System	Potential effects	Monitoring guidelines
Central nervous system	Cognitive dysfunction	**History (annually):** Assessment of educational and/or vocational progress Cognitive, motor or sensory deficits, seizures, and other neurologic symptoms. Neurocognitive testing baseline, then periodically as clinically indicated for patients with evidence of impaired educational or vocational progress **Physical examination (annually):** Neurologic exam
	Leukencephalopathy	CT or MRI brain plus MR angiography
Dental	Salivary gland dysfunction	Regular dental care including fluoride applications. Use of sialogues
Cardiac	Cardiomyopathy Pericarditis Coronary artery disease Valvular disease	**History (annually):** SOB, DOE, orthopnea, chest pain, palpitations. If under 25 years: abdominal symptoms (nausea, vomiting). **Physical exam (annually):** Cardiac exam **Diagnostic studies:** EKG for evaluation of QTc interval at entry into long-term follow-up and then as clinically indicated dependent on dose and volume of RT and anthracyclines **Additional considerations:** Cardiology consultation in patients with subclinical abnormalities on screening evaluations, left ventricular dysfunction, dysrhythmia, or prolonged QTc interval. Additional cardiology evaluation in patients who received $\geq$ 300 mg/m^2 or < 300 mg/m^2 plus chest radiation or TBI who are pregnant or planning pregnancy to include an EKG and echocardiogram before and periodically during pregnancy (especially during 3rd trimester) and monitoring during labor and delivery due to risk of cardiac failure.
Pulmonary	Pulmonary fibrosis	**History (annually):** Cough, SOB, DOE, wheezing **Physical exam (annually):** Pulmonary exam **Diagnostic studies:** Pulmonary function tests (PFTs) (including DLCO and spirometry) and CXR baseline at entry into long-term follow-up and prior to general anesthesia. Repeat as clinically indicated in patients with abnormal or progressive pulmonary dysfunction
Thyroid	Overt or compensated hypothyroidism Thyroid nodules or cancer Hyperthyroidism	**Physical examination (annually):** Thyroid palpation **Laboratory tests (annually):** Free T4; TSH

System	Potential effects	Monitoring guidelines
Gonadal (female)	Delayed/arrested puberty Early menopause Ovarian failure	**History (annually):** Pubertal (onset, tempo), menstrual, pregnancy; sexual function (vaginal dryness, libido), medication use impacting sexual function. **Physical exam (annually):** Height, weight, Tanner stage **Laboratory (baseline):** FSH, LH, estradiol at 13 years and as clinically indicated in patients with: – Delayed puberty, irregular menses or primary or secondary amenorrhea – Clinical signs and symptoms of estrogen deficiency
Gonadal (male)	Germ cell failure Infertility/azoospermia Leydig cell dysfunction Hypogonadism Delayed/arrested puberty	**History (annually):** Pubertal (onset, tempo), sexual function (erections, nocturnal emissions, libido), medication use impacting sexual function **Physical exam (annually):** Height, weight, Tanner stage, testicular volume by Prader orchiometry **Laboratory (baseline):** FSH, LH, testosterone at age 14 and as clinically indicated in patients with: – Delayed puberty – Clinical signs and symptoms of testosterone deficiency Semen analysis
Bone	**Osteopenia** Bone mineral density $\geq$ 1 and < 2.5 SD below mean **Osteoporosis** Bone mineral density $\geq$ 2.5 SD below mean **Osteonecrosis** (avascular necrosis – AVN)	Bone density evaluation (DEXA or quantitative CT) baseline at entry into long-term follow-up and then as clinically indicated **History (annually):** Joint pain, swelling, immobility, limited range of motion **Physical exam (annually):** Musculoskeletal exam **Imaging studies:** MRI as clinically indicated by signs or symptoms suggestive of AVN
Second malignancies	Sarcomas CNS tumors Breast cancer Melanoma Non-melanoma skin cancer Thyroid cancer Other solid tumors	Complete physical examinations annually Attention paid to organs in radiation field Diagnostic imaging as clinically indicated Mammography to screen for female breast cancer

portant to assess what types of evaluation will provide the most valuable information for the greatest number of patients in the most cost-effective fashion. Survivorship research carried out within a long-term follow-up clinic can be either institution-initiated, cooperative group-sponsored, or population-based. Maintaining a current database will facilitate data collection analysis. In addition, having a means for tracking survivors is vital for collecting long-term data on individuals whose care has been transferred to a community-based setting. Approaches for integrating research activities into institutional models of survivor care have been described (Hinkle et al. 2004b; Hudson et al. 2004).

Primary reliance on patients can be an effective approach to obtaining data, for both retrospective and prospective analyses, as is evidenced by the CCSS (Robison et al. 2002). Independent studies of the efficiency, reliability, validity, and utility of self-reported me-

Table 12.2 General guidelines for chemotherapy late effects: assessment and management

System	Agents	Potential effects	Monitoring guidelines
Dental	Chemotherapy	Root, enamel and gingival changes	Dental exam and cleaning every six months Regular dental care including fluoride applications. Baseline panorex prior to dental procedures to evaluate root development.
Cardiac	Anthracyclines	Cardiomyopathy Arrhythmias	**History (annually):** SOB, DOE, orthopnea, chest pain, palpitations. If under 25 years: abdominal symptoms (nausea, vomiting). **Physical exam (annually):** Cardiac exam Diagnostic studies: EKG for evaluation of QTc interval at entry into long-term follow-up and then as clinically indicated **Additional considerations:** Cardiology consultation in patients with subclinical abnormalities on screening evaluations, left ventricular dysfunction, dysrhythmia, or prolonged QTc interval. Additional cardiology evaluation in patients who received $\geq$ 300 mg/m^2 or < 300 mg/m^2 plus chest radiation or TBI who are pregnant or planning pregnancy to include an EKG and echocardiogram before and periodically during pregnancy (especially during 3rd trimester) and monitoring during labor and delivery due to risk of cardiac failure.
Pulmonary	Bleomycin	Restrictive lung disease	**History (annually):** Cough, SOB, DOE, wheezing **Physical exam (annually):** Pulmonary exam **Diagnostic studies:** PFTs (including DLCO and spirometry) and CXR baseline at entry into long-term follow-up and prior to general anesthesia. Repeat as clinically indicated in patients with abnormal or progressive pulmonary dysfunction
Gonadal (female)	Alkylating agents	Delayed/arrested puberty Early menopause Ovarian failure	**History (annually):** Pubertal (onset, tempo), menstrual, pregnancy; sexual function (vaginal dryness, libido), medication use impacting sexual function. **Physical exam (annually):** Height, weight, Tanner stage **Laboratory (baseline):** FSH, LH, estradiol at 13 years *and* as clinically indicated in patients with: – Delayed puberty, irregular menses or primary or secondary amenorrhea – Clinical signs and symptoms of estrogen deficiency

System	Agents	Potential effects	Monitoring guidelines
Gonadal (male)	Alkylating agents	Germ cell failure Infertility/ azoospermia Leydig cell dysfunction Hypogonadism Delayed/ arrested puberty	**History (annually):** Pubertal (onset, tempo), sexual function (erections, nocturnal emissions, libido), medication use impacting sexual function **Physical exam (annually):** Height, weight, Tanner stage, testicular volume by Prader orchiometry. **Laboratory (baseline):** FSH, LH, testosterone at age 14 *and* as clinically indicated in patients with: – Delayed puberty – Clinical signs and symptoms of testosterone deficiency Semen analysis
Bone	Corticosteroids+/– methotrexate Alkylating agents (due to hypogonadism)	Osteopenia Bone mineral density $\geq$ 1 and < 2.5 SD below mean	Bone density evaluation (DEXA or quantitative CT) baseline at entry into long-term follow-up and then as clinically indicated
	Corticosteroids	Osteoporosis Bone mineral density $\geq$ 2.5 SD below mean Osteonecrosis (avascular necrosis – AVN)	**History (annually):** – Joint pain, swelling, immobility, limited range of motion – Physical exam (annually): – Musculoskeletal exam **Imaging studies:** – MRI as clinically indicated by signs or symptoms suggestive of AVN
Second malignancies	Alkylating agents: Topoisomerase II inhibitors	Leukemia	**History (annually for 10 years):** Fatigue, bleeding, easy bruising **Physical exam (annually for 10 years):** Dermatologic exam (pallor, petechiae, purpura) **Laboratory (annually for 10 years):** CBC/differential
	Cyclophosphamide	Transitional bladder carcinoma	**History (annually):** Hematuria, frequency, urgency **Laboratory (annually):** Urinalysis

dical and psychologic complications will be necessary in the assessment of this approach for long-term follow-up.

12.15 Future Directions

Although associations between certain therapeutic exposures and adverse physiologic outcomes of childhood lymphoma are well known, the mechanisms underlying many of these outcomes remain largely unknown and are ripe for study. In spite of having received similar treatment for specific forms of lymphoma, there is considerable variability in the proportion of survivors that develop treatment-related toxicities. Are these merely stochastic or chance events, or do host-related factors, such inherited differences in drug metabolism and activation, radiation sensitivity, and DNA repair have an impact on the risk of long-term toxicity? While morbidity and premature mortality

have been examined with respect to treatment exposures, their relation to other factors such as environmental exposures that are etiologically related to the major problems in an aging population have not been explored. These too require creative research efforts. New patterns of late morbidity and mortality may emerge as survivors continue to age, and it is only through continued study that such patterns will be identified, and interventions for treatment and prevention designed.

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