Multidisciplinary Treatment of Soft Tissue Sarcomas

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Contents

Preface

The previous volume of this series on soft tissue sarcomas highlighted the importance of the multidisciplinary approach to treatment, the focus of which is continued in the present edition. Proper diagnosis and staging remain the cornerstone of the treatment strategy. Sophisticated histopathology techniques and growing consensus on grading systems have further increased the importance of the histopathologist in providing estimates of the prognosis of the patient as well as providing data for the planning of treatment strategy. The use of cytogenetics is relatively new in this field. This might enable the distinction of subgroups in specific histological tumor types. Furthermore, molecular biological studies not only help to reveal inherited predispositions and details in oncogenesis in tumor development, but they may also provide additional predictive factors for tumor behavior. Further data on treatment strategy will be provided by diagnostic imaging, a field in which the role of magnetic resonance imaging is rapidly developing.

As far as actual treatment is concerned, surgery still provides the major chance for cure. In view of the endeavor to be as sparing as possible, the addition of radiotherapy to surgery is of utmost importance. Usually radiotherapy is given after surgery, but the optimal sequence of the two modalities still needs to be defined. The combined use of surgery with radiotherapy and/or chemotherapy does have an impact on wound healing. In view of the latter development, we thought it might be useful for our readers to add a review on recent data concerning wound healing in these situations.

There appears to be growing consensus about the role of chemotherapy in metastatic disease, where only doxorubicin, ifosfamide, and DTIC are known to be active. Although for certain subsets it may be different, in general single-agent chemotherapy appears to be as effective as standard doses of combination chemotherapy. The lack of efficacy of adjuvant chemotherapy with standard doses of the drugs presently available has been definitively shown.

Just as for radiotherapy, chemotherapy may have an important role in the preoperative treatment of advanced soft tissue sarcomas, where intravenous drug administration appears to be as effective as more com-

plicated intra-arterial administration. Thermochemotherapy, another and still investigational approach to advanced local disease, also yields interesting preliminary results.

Finally, clinical behavior, treatment strategy, and the outcome of treatment in childhood soft tissue sarcomas are quite different from those in adult soft tissue sarcomas. This volume covers all these topics. We would like to thank all the authors for their contributions. Hopefully this book will contribute to a further extension of the multidisciplinary approach to treatment.

J. Verweij, H.M. Pinedo, and H.D. Suit
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List of Abbreviations

ADIC	= Doxorubicin/DTIC
APC	= Adenomatous polyposis coli
CALGB	= Cancer and Acute Leukemia Group B
CDDP	= Cisplatin
CT	= Computer tomography
CTX	= Cyclophosphamide
CYVADIC	= Cyclophosphamide/vincristine/doxorubicin/DTIC
DACT	= Actinomycin D
DFS	= Disease-free survival
DM	= Double minute chromosomes
DSA	= Digital subtraction angiography
DTIC	= Dacarbazine
DX	= Doxorubicin
ECOG	= Eastern Cooperative Oncology Group
EIA	= Etoposide/ifosfamide/doxorubicin
EORTC	= European Organisation on Treatment and Research of Cancer
Epi-DX	= Epidoxorubicin
FASE	= Fast acquisition spin-echo
FHR	= Favorable histological response
FLASH	= Fast low angle shot
FOV	= Field of view
GM-CSF	= Granulocyte-macrophage colony stimulating factor
GOG	= Gynecological Oncology Group
HAP	= Hyperthermic antiblastic perfusion
HSR	= Homogeneously staining region
ICE	= Ifosfamide/carboplátin/etoposide
IFN	= Interferon
IFOS	= Ifosfamide
IHC	= Immunohistochemistry
IRS	= Intergroup Rhabdomyosarcoma Study
IVA	= Ifosfamide/vincristine/actinomycin D
KGMC	= Klinikum Grosshadern Medical Centre

L-PAM	= Melphalan
MAID	= Mesna/doxorubicin/ifosfamide/DTIC
MDR	= Multidrug resistance phenotype
MFH	= Malignant fibrous histiocytoma
MGH	= Massachusetts General Hospital
MMT(s)	= Malignant mesenchymal tumor (study)
MPNST	= Malignant peripheral nerve sheat tumor
MRI	= Magnetic resonance imaging
NF1	= von Recklinghausens neurofibromatosis
NRSTS	= Non-rhabdomyo soft tissue sarcoma
PCNA	= Proliferative cell nuclear antigen
PNET	= Primitive neuroectodermal tumor
RF (energy)	= Radio-frequency
RHT	= Regional hyperthermia
RMS	= Rhabdomyosarcoma
SE (sequence)	= Spin-echo
SIOP	= International Society of Pediatric Oncology
STS	= Soft tissue sarcoma
SWOG	= South-West Oncology Group
TE	= Echo time
TGD	= Tumor growth delay
TNF	= Tumor necrosis factor
TR	= Repetition time
VAC	= Vincristine/actinomycin D/cyclophosphamide
VAI	= Vincristine/actinomycin D/ifosfamide
VCR	= Vincristine
VIE	= Vincristine/ifosfamide/etoposide
WBH	= Whole body hyperthermia

Multidisciplinary Treatment of Soft Tissue Sarcomas

1. Pathology and grading of soft tissue sarcomas

J.M. Coindre

Soft tissue sarcomas (STS) can be defined as malignant tumors of nonepithelial extraskeletal tissue of the body exclusive of the reticuloendothelial system, glia, and supporting tissue of various parenchymal organs. By convention, malignant tumors of the peripheral nervous system are included because they pose similar problems in diagnosis and therapy [1]. In this chapter, we will not discuss bone and visceral sarcomas, for which diagnostic and therapeutic problems are different.

As for surgeons, radiotherapists, and medical oncologists, STS are difficult and rather confusing for the pathologists who have encountered them only rarely. Problems are related to the rarity, ubiquitous location, histologic diversity, and variable outcome of these tumors. They represent less than 1% of all cancers and experience is difficult to obtain in this field. Although most STS arise in the muscles of the extremities, they may occur anywhere in the body, such as in the subcutaneous tissues, the chest wall, the mediastinum, and the retroperitoneum. STS are a heterogeneous group of tumors with a wide range of histologic types and subtypes, and a possible variation in histologic patterns within a given sarcoma phenotype; this explains the frequent problems encountered in diagnosis and classification. STS are locally aggressive tumors, capable of local recurrence and distant metastasis. The ability to metastasize is variable, in part related to the histological type; but for most tumors histological classification is insufficient to predict the advent of metastases and a histological grade must be attributed.

As a member of the multidisciplinary team managing STS patients, the pathologist must accomplish three tasks: 1) establish the diagnosis of sarcoma, 2) classify the tumor, and 3) evaluate the prognosis. Moreover, in some circumstances the pathologist must also appreciate the adequacy of surgical treatment by evaluating resection margins.

Many studies have described the different clinicopathological entities [2–5]; therefore, only the main problems encountered in the fields of diagnosis, classification, and prognosis of STS are dealt with in this paper.

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Diagnosis and classification

To correctly diagnose and classify a sarcoma, the pathologist should insist on two conditions:

- Knowledge of the patient's clinical history. Minimal information includes patient age, tumor site, depth, size and course, and previous medical history.
- Acquisition of a representative sample of the tumor. The optimal method of sampling is an open biopsy, which can be excisional for lesions less than 3 cm in diameter. If the pathologist is very familiar with STS histology, a needle biopsy can be used in certain situations, such as a retroperitoneal mass. However, in these cases there is a chance that the biopsy tissue may not be representative of the entire tumor, and diagnosis and/or classification may not be possible, thus an open biopsy may eventually be necessary. Fine-needle aspiration should not be used for establishing the diagnosis of a soft tissue lesion but can be useful for documenting a recurrence.

The diagnosis of STS can be quite difficult for the pathologist who is not familiar with soft tissue lesions. In a recent study [6] of a large series, the disagreement rate between the referring pathologist's diagnosis and the pathology review panel's diagnosis was 26%. In 10% the case was excluded because it was not a sarcoma, and in the other 16% disagreement concerned the classification of the sarcoma. This level of disagreement emphasizes the need for histologic peer review in all sarcoma studies.

The best way to minimize errors in this vast field is to use an orderly step-by-step approach (called the *skeptical approach*), as proposed by Brooks [5]. A series of crucial questions should be answered by the pathologist: Is the lesion malignant? Is it necessarily a sarcoma? How can the putative sarcoma be classified?

Diagnosis of sarcoma

To make the diagnosis of a sarcoma, the first two questions must primarily be answered. There are numerous benign lesions that can mimick a sarcoma (Table 1). The first group are reactive lesions, such as nodular fasciitis or myositis ossificans, which, because of their rapid growth, worry both the patient and clinician. Histological features, such as cellularity, nuclear atypia, and a high mitotic rate, can suggest malignancy to the pathologist. In fact, extremely rapid growth is more compatible with a reactive lesion than with a sarcoma, and the clinical context should suggest the diagnosis for some of these lesions. Histologically, the most important feature that is apparent at low power is the architectural organization, which has a zonal arrangement and a radial configuration of the vessels. These lesions are absolutely benign and will not recur after proper surgery.

The second group are benign mesenchymal tumors that possess features suggesting malignancy, such as an infiltrative growth pattern, nuclear atypia,

Table 1. Benign lesions that can mimick a sarcoma

Reactive lesions
• Nodular and proliferative fasciitis
• Proliferative myositis
• Myositis ossificans
• Postoperative nodules of the genitourinary tract
• Papillary endothelial hyperplasia
Benign tumors
• Desmoid tumor
• Giant cell tenosynovial tumor, diffuse form
• Atypical schwannoma (ancient and cellular)
• Atypical lipoma
• Atypical fibrous histiocytoma

or cellularity. The pathologist should keep in mind that in the field of soft tissue lesions, the only presumptive sign of malignancy is necrosis; without this, unusual features must be evaluated in context. Moreover, in these atypical benign tumors, the mitotic rate is usually low.

When malignancy is certain, the pathologist may have serious problems differentiating sarcomas from other categories of malignant tumors, such as carcinoma, melanoma, and lymphoma. The pathologist should be careful when there is a previous history of cancer, with certain tumor sites, and with certain histological patterns. At certain sites, a nonmesenchymal malignant tumor is more probable than a sarcoma: cutaneous and mucous areas, lymph node areas (groin, axillary area, retroperitoneum), and around certain organs, such as the kidney, thyroid, breast, and lung. Some histological features are by no means specific of a sarcoma: aspects of spindle, pleomorphic, or round-cell unclassifiable sarcoma, and malignant fibrous histiocytoma, fibrosarcoma, and hemangiopericytoma patterns. These features can be seen in carcinomas and melanomas, and sometimes in lymphomas. Immunohistochemistry is extremely useful in distinguishing nonmesenchymal malignant tumors from sarcomas. The use of a panel of markers, including vimentin, cytokeratin, S100 protein, HMB45, and for certain tumors, common leukocyte antigen, regularly allows a determination of the precise nature of the tumor.

Classification of sarcomas

When the sarcomatous nature of a soft tissue lesion is established, the next question relates to the classification of the tumor. The first universally accepted classification for STS was proposed by the World Health Organization in 1969 [1]. Since this publication, several changes were made, and recently Enzinger and Weiss modified the classification now used [4]. This histogenetic classification is based on proliferating cell type rather than on the type of cell from which the tumor arose. Each tumor is classified according to the appearance of the tissue it has formed.

Table 2. Relative frequency of different types of adult soft tissue sarcomas in two large series

Histological types	Enjoji et al. [7] (n = 664), %	Personal series (n = 711), %
Fibrosarcoma	5.7	4.9
Malignant fibrous histiocytoma	30.8	30.4
Liposarcoma	11.3	13.7
Leiomyosarcoma	10.1	10.4
Rhabdomyosarcoma	6.8	2.4
Synovial sarcoma	5.9	8.4
Malignant schwannoma	5.9	7.2
Angiosarcoma	6.7	3.5
Osteosarcoma	0.5	0.8
Chondrosarcoma	3.8	1.6
Alveolar soft part sarcoma	1.0	0.8
Epithelioid sarcoma	0.8	1.3
Clear cell sarcoma	2.6	0.8
Ewing's sarcoma	0.3	1.3
Malignant mesenchymoma	0.1	0.1
Unclassified	7.6	12.4

This classification is complex, with 15 types and more than 50 subtypes, and is quite confusing for the clinician who is not familiar with it. The relative frequency of the different types of sarcomas has considerably fluctuated in recent decades. Fibrosarcoma was the most frequent sarcoma in older studies, but malignant fibrous histiocytoma (MFH) and liposarcoma are now prominent in adults [7] (Table 2). Even for the pathologist familiar with sarcomas, between 5% and 15% of cases cannot be related to any mesenchymal cell type and therefore remain unclassified.

In fact, STS can be classified into three categories of varying significance:

- Sarcomas whose line of differentiation is clear and for which the proliferating cell line is well identified, such as in liposarcoma, leiomyosarcoma, synovial sarcoma, and rhabdomyosarcoma (Fig. 1). In these cases, the proliferating cell is well defined, with specific morphological, ultrastructural, and sometimes immunohistochemical features, allowing clear identification in the differentiated tumors. About 50% of adult STS fall into this category.
- Sarcomas whose line of differentiation is not clear and for which the proliferating cell line is still debated. This is the case for undifferentiated sarcomas, MFH, and fibrosarcomas, which represent between 40% and 50% of adult STS. MFH and fibrosarcoma are identified mainly on morphological patterns that are not specific and that may be seen in any other sarcomas as a partial component, and even in carcinomas and melanomas (Fig. 2). Dedifferentiated sarcomas are bimorphic neoplasms in which a low-grade, well-differentiated sarcoma is juxtaposed with a high-grade, poorly or undifferentiated sarcomatous component, which is

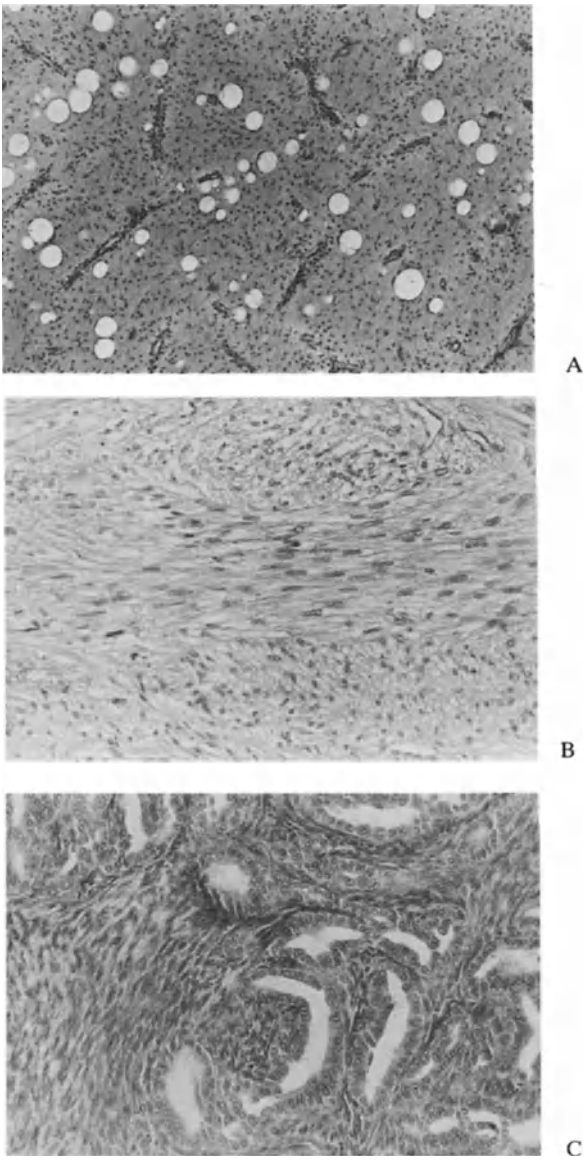


Figure 1. Examples of specific patterns in soft tissue sarcomas. A: Myxoid liposarcoma with a myxoid matrix, a prominent plexiform capillary pattern, and lipoblasts of the 'signet-ring' type. B: Leiomyosarcoma depicting fascicles of cells intersecting each other at right angles. Tumor cells show regular blunt-ended nuclei. C: Synovial sarcoma with distinctive biphasic pattern: Columnar epithelial cells surrounded by fibrosarcoma-like spindle cells.

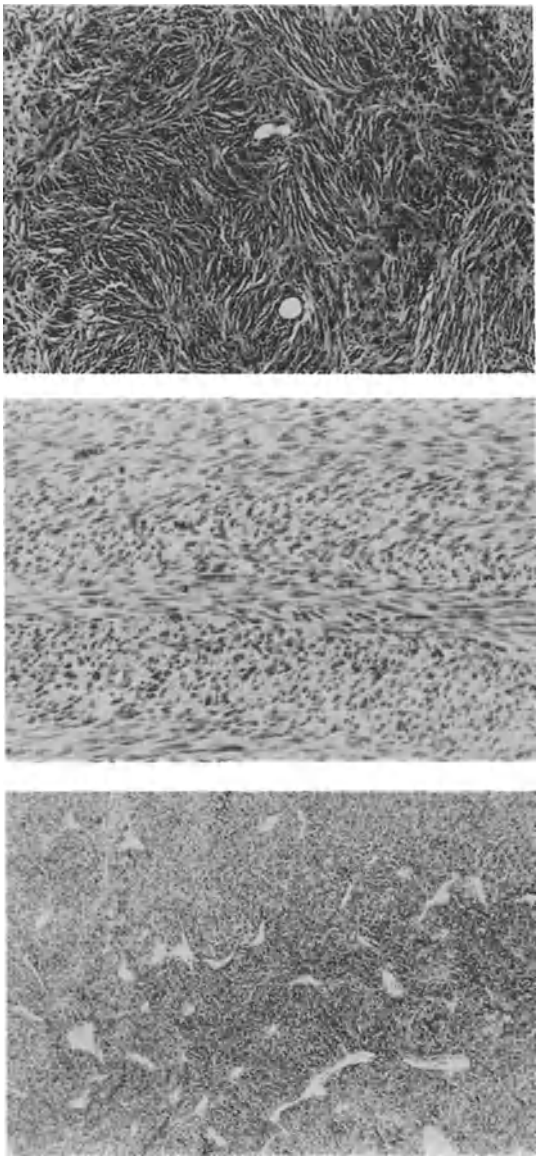


Figure 2. Examples of nonspecific patterns in soft tissue sarcomas. These patterns can be seen in specific sarcoma types, dedifferentiated sarcomas, and nonmesenchymal malignant tumors. A: Storiform pattern from malignant fibrous histiocytoma. B: 'Herringbone' pattern from fibrosarcoma. C: Hemangiopericytoma-like area from monophasic fibrous synovial sarcoma.

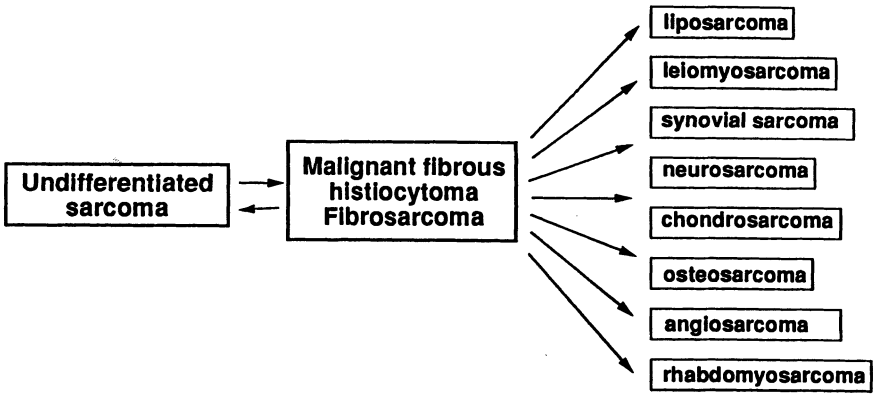


Figure 3. Hypothetical histogenetic scheme of sarcomas.

often of MFH type [8,9]. In MFH, there is no specific ultrastructural or immunohistochemical feature, and different cell types have been found in this tumor, but it most closely resembles primitive fibroblasts [10,11]. Therefore, MFH can be considered both as the terminal transformation of most malignant tumors and as a specific sarcoma type [12]. In the latter case, it can be considered at a crossroads between undifferentiated and differentiated sarcomas [8] (Fig. 3) and is best defined as a pleomorphic spindle cell sarcoma with aggressive clinical behavior.

- Sarcomas of particular types, either of uncertain histogenesis, such as alveolar soft-part sarcoma [13] and epithelioid sarcoma [14,15], or of nonmesenchymal origin, such as clear cell sarcoma (melanoma of soft parts) [16,17], Ewing's sarcoma, and neuroepithelioma, which are related entities (peripheral neuroectodermic tumors or PNET) [18]. These tumors represent about 5% of all adult STS.

Classification of sarcomas is mainly based on the histological aspect after H and E staining. Nevertheless, sarcoma types are variable according to patient age [19] (Tables 3 and 4), tumor location (Table 5), and previous history, such as radiation therapy [20] (Table 6).

Table 3. Sarcoma types in children

Rhabdomyosarcoma	51.4 (%)
Fibrosarcoma	10.8
Synovial sarcoma	5.6
Liposarcoma	4.5
Malignant schwannoma	3.4
Unclassified sarcoma	10.6
Other	13.5

From King et al. [19], with permission.
n = 443.

Table 4. Sarcoma types in the elderly

Malignant fibrous histiocytoma	43.1 (%)
Liposarcoma	13.0
Leiomyosarcoma	12.3
Angiosarcoma	7.1
Malignant schwannoma	4.7
Fibrosarcoma	4.0
Unclassified sarcoma	10.2
Other	5.6

Personal series with 253 patients older than 60.

Table 5. Sarcoma types according to location in adults

Thigh	MFH
	Liposarcoma
Hand and foot	Synovial sarcoma
	MFH
Retroperitoneum	Liposarcoma
	MFH
	Leiomyosarcoma
Skin and subcutaneous	MFH
	Leiomyosarcoma

Table 6. Postradiation soft tissue sarcomas

Malignant fibrous histiocytoma	68 (%)
Osteosarcoma	13
Fibrosarcoma	11
Malignant schwannoma	4
Chondrosarcoma	2
Angiosarcoma	2

From Laskin et al. [20], with permission.
n = 53.

In recent years, much progress has been made in the subtyping of STS by using special techniques, such as immunohistochemistry, electron microscopy, and cytogenetics.

- Immunohistochemistry (IHC) has greatly changed the approach to the classification of STS and is now considered a routine procedure [21,22]. However, for the best results, tissue fixation, procedure, and interpretation should be rigorous. Numerous commercially available markers are useful in the classification of STS (Table 7). IHC is especially useful in confirming a given type suspected on standard histology. For some sarcoma types, IHC is often decisive for diagnosis. This is the case for rhabdomyosarcoma [23], synovial sarcoma [24], clear cell sarcoma [16,17], and epithelioid sarcoma [25] (Fig. 4). For others, it is sometimes useful but less often

Table 7. Immunohistochemistry: Useful markers for sarcoma typing on paraffin sections

Markers	Comments
• Vimentin	Mesenchymal differentiation
• Cytokeratin	Epithelial differentiation (synovial sarcoma, epithelioid sarcoma)
Epithelial membrane antigen	
• Desmin	Muscle differentiation
Muscle actin	Muscle differentiation
Alpha-smooth muscle actin	Smooth muscle differentiation
Myoglobin	Striated muscle differentiation
• S 100 protein	Marker for Schwann cells, chondrocytes, adipocytes, melanocytes.
HMB45	Melanotic differentiation
• Factor VIII R-Ag	
CD 34	Endothelial differentiation
Ulex europaeus	
• Collagen IV	Marker for basement membranes

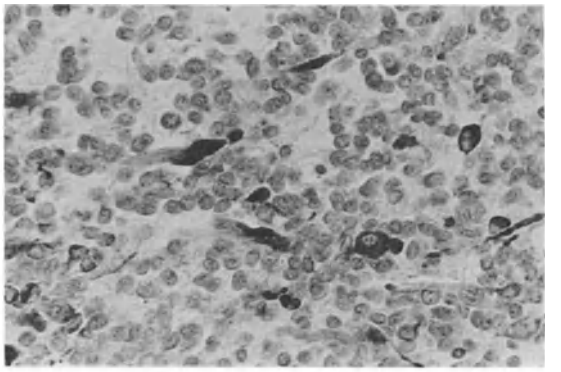
decisive. Such is the case for leiomyosarcoma, malignant schwannoma, angiosarcoma, and chondrosarcoma. For some sarcomas, there are no specific markers; such is the case for MFH, fibrosarcoma, hemangiopericytoma, and alveolar soft part sarcoma.

In fact, IHC is seldom helpful in elucidating the nature of morphologically unclassifiable STS [26]. Moreover, the pathologist must be aware of unexpected positivity related to cross-reactivity or aberrant expression of the antigen. For example, a few positive tumor cells for cytokeratin, desmin, or S100 protein can be seen in MFH. So it is now recommended to use a panel of markers and to interpret the results of IHC on the basis of the histologic aspect.

- In practice, electron microscopy plays a lesser role in the classification of STS, because it is more expensive, time consuming, and occasionally subject to artifact and sampling errors. Moreover, interpretation can be difficult and most pathologists lack expertise in this field. Nevertheless, in the hands of experts this technology is extremely valuable, mainly as a complement for immunohistochemical negative cases [27]. Electron microscopy contributes to the characterization of numerous tumor cells in the field of STS. In fact, the ideal situation would be to use IHC, electron microscopy, and cytogenetics as complementary tools for classification. The role of cytogenetics is described in Chapter 2.

Prognosis of soft tissue sarcomas

Histological typing partly contributes to predicting the clinical course. Some types or subtypes rarely metastasize, such as dermatofibrosarcoma protu-



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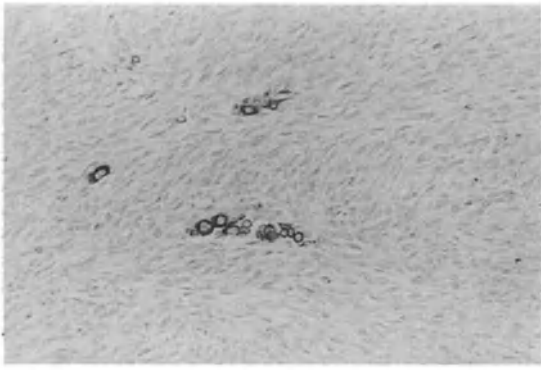

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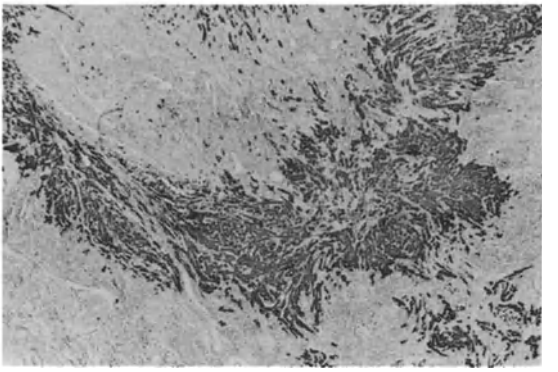

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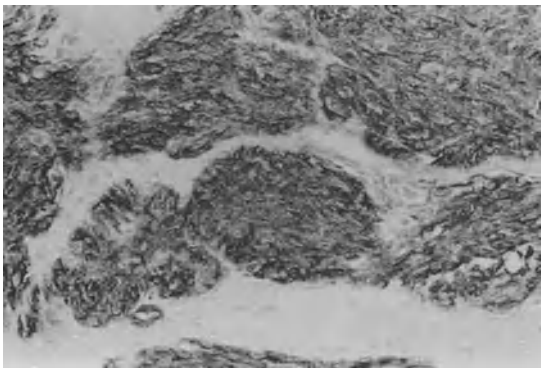

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Figure 4. Soft tissues sarcomas in which immunohistochemistry is often decisive for diagnosis. A: Embryonal rhabdomyosarcoma. A few tumor cells showing positive cytoplasmic reactivity for anti-desmin. B: Monophasic fibrous synovial sarcoma. Cluster of cells staining for cytokeratin. C: Epithelioid sarcoma. Cytokeratin showing positivity in almost all tumor cells. D: Clear-cell sarcoma. Tumor cells demonstrating positive granular cytoplasmic staining with HMB45.

berans, whereas others, such as synovial sarcoma, frequently do. Some types, such as alveolar soft part sarcoma, have a tendency to long-delayed metastases. However, as we have seen, this classification is complex and quite confusing for the clinician. It is subject to periodical variations, and its reproducibility is poor [6,28,29]. Moreover, a percentage of tumors remains unclassified, and for most types the prognostic value of this classification is questionable.

The first coherent and effective system was the clinicopathological classification proposed by Russell et al. in 1977 [30], which separates patients into four stages. This staging system introduced a histological grading applicable to all STS, which is the most important factor. Recently, Henson [31] updated the prognostic value of grading in a very large group of cancers of different types, including 1889 cases of STS. In this category of tumors, grade is strongly correlated with survival, but only 25.2% of cases were graded. This report shows that grading of STS is not taken seriously by some pathologists. There may be legitimate reasons for this attitude: Criteria are arbitrarily selected and may be subjective, conditions of grade attribution are not clearly defined, and the resulting grading is nonscientific and nonreproducible, grading is not applicable to all types of STS, and it depends on tumor sampling.

In contrast to this negative attitude, there is little doubt that most clinicians feel the importance of grade. Grade is today the most important prognostic factor in STS. It is the fastest and most cost-effective method for providing an initial estimate of prognosis and so is the most convenient method providing information allowing patient management.

The qualities required for a good grading system are effectiveness, reproducibility, and usefulness. All the reported grading systems are of

prognostic value, regardless of the way they have been conceived. This fact underlines the strength of grading as a prognostic indicator. Yet to obtain the most effective system, some simple rules should be followed. The first point to be stressed is the method of selecting criteria. These criteria should necessarily include all the prognostic information, so they should be selected after monofactorial and multivariate analysis. Together with Suit et al. [32], we believe that only histological parameters should be retained. By using clinical criteria to assign a grade, the pathologist may be contributing to confusion rather than clarity. To be reproducible, a grading system should be set up with a few simple and well-defined criteria. These criteria should be applicable to all sarcoma types. The method of automatic grade attribution for some types, such as grade 3 for synovial sarcomas, is not supported by the available data. Moreover, it is confusing and should be avoided. To be useful for patient management, the distribution of patients should be well-balanced through the different grades. In fact, a grading system with 90% of patients in one grade is useless for clinician.

Review of the reported grading systems

Since the first report by Russell et al. [30], several grading systems applicable to STS have been reported [33–38]. According to the way criteria have been selected, these systems can be divided into two groups: 1) grading systems for which criteria have been empirically selected [30,33–35] (Table 8). In these systems, mitosis index and tumor cellularity are always retained. In two systems [30,35], a great number of parameters are used. 2) Grading systems for which criteria have been selected after monofactorial and multivariate analysis [36–38] (Table 9). Tumor necrosis is always retained and mitosis index is kept in two of them. Differences between results can be explained by selection of patients and the relatively small number of patients studied.

Trojani et al. [36] tested seven histological parameters among 155 STS: differentiation, cellularity, cellular pleomorphism, the presence of malignant giant cells, necrosis, mitosis count, and presence of vascular emboli. Three

Table 8. Grading systems with "subjectively" selected criteria

	Russell [30]	Hajdu [33]	Markhede [34]	Myhre Jensen [35]
Histological type	+	–	–	+
Mitosis index	+	+	+	+
Necrosis	+	+	–	+
Necrobiosis	–	–	–	+
Cellularity	+	+	+	+
Differentiation	+	+	–	+
Cellular pleomorphism	+	–	+	+
Inflammatory cells	+	–	–	+
Hemorrhage	–	–	–	+

Table 9. Grading systems with "objectively" selected criteria

	Trojani [36]	Costa [37]	Van Unnik [38]
Histological type	–	+	–
Differentiation	+	–	–
Mitosis	+	–	+
Necrosis	+	+	+

criteria were found to be necessary and sufficient in attributing a tumor grade: mitosis count (score 1, 2, or 3), differentiation (score 1, 2, or 3), and necrosis (score 0, 1, or 2). A three-grade system (French Federation of Cancer Centers system) was set up according to the total obtained when summing the scores of the three selected criteria: grade 1 (total: 2 or 3), grade 2 (total: 4 or 5), and grade 3 (total: 6, 7, or 8) (Fig. 5). A multivariate analysis showed that this tumor grade represents the most important prognostic factor and is more important than any clinical factor. The reproducibility of the system was tested by 15 pathologists [39]. The crude proportion in agreement was 75% for tumor grade, 73% for mitosis count, 74% for differentiation, and 81% for tumor necrosis. Agreement for histological type was 61%.

Costa et al. [37] tested six histological parameters among 163 STS: histological type, mitosis, necrosis, pleomorphism, cellularity, and matrix. On the basis of histologic type and certain histologic features, they could identify grade 1 tumors. Among the other tumors, necrosis (absent or minimal versus moderate or massive) was the major criterion for separating grade 2 from grade 3 sarcomas (NCI system) (Fig. 6).

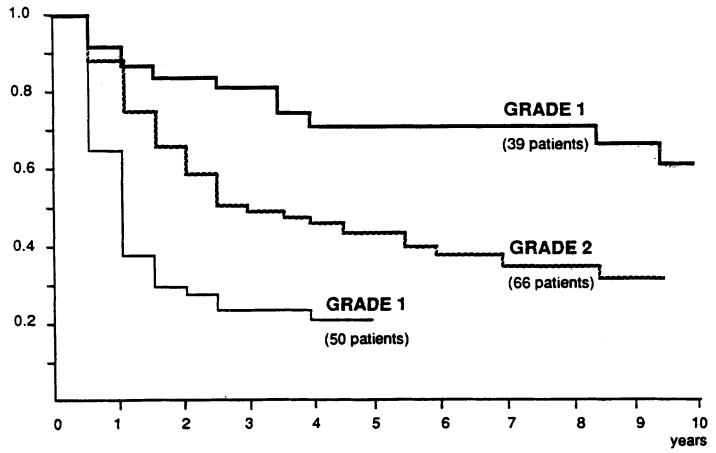

Figure 5. French Federation of Cancer Centers grading system. Survival curves according to tumor grade. From Trojani et al. [36], with permission.

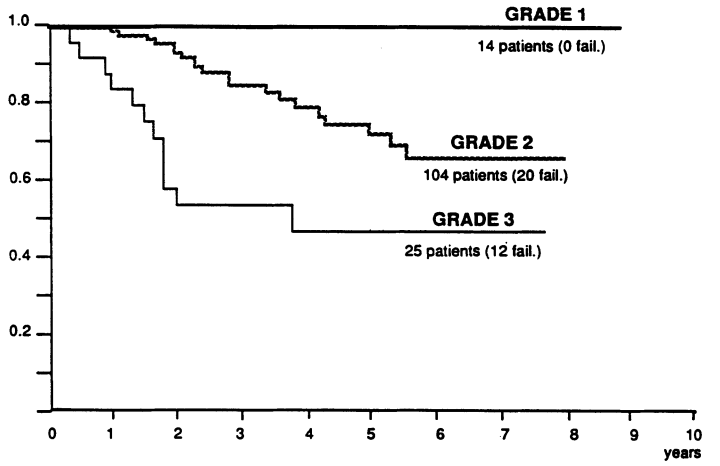

Figure 6. National Cancer Institute grading system. Survival curves according to tumor grade. From Costa et al. [37], with permission.

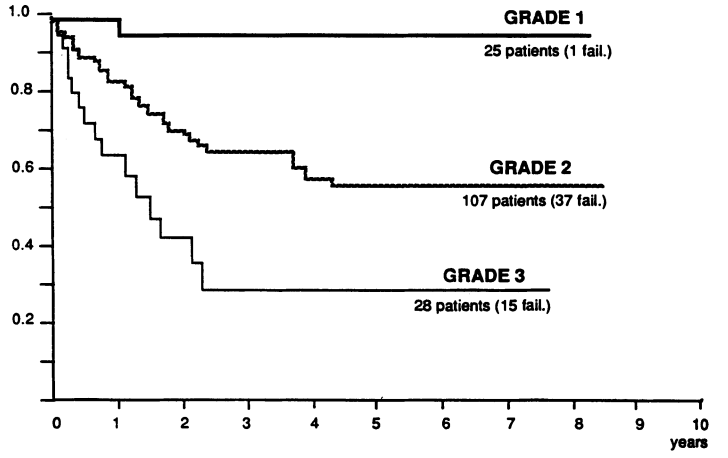

Figure 7. European Organization for Research and Treatment of Cancer grading system. Survival curves according to tumor grade. From Van Unnik et al. [38], with permission.

Van Unnik et al. [38] tested four histological parameters among 169 STS: differentiation, necrosis, myxoid areas, and mitosis count. Mitosis (score 0, 1, or 2) and necrosis (score 0 or 1) were retained after statistical analysis, and a three-grade system (EORTC system) was set up as follows: grade 1 (mitotic score = 0), grade 2 (mitotic score = 1, or mitotic score = 2 and necrosis score = 0), and grade 3 (mitotic score = 2 and necrosis score = 1) (Fig. 7).

Most systems are three grade [30,35–38]. Hajdu [33] reported a two-grade system and Markhede [34] a four-grade system. Usually, three grades are recommended for pathology [40]. Because histological grade is not the only prognostic factor, a two-grade system is not desirable and an intermediate group of malignancy should be kept. A four-grade system usually shows little difference between the two lowermost grades and, moreover, decreases interobserver agreement.

Prognostic value of histological grading in STS

Prognosis of STS is dominated by local recurrence and distant metastasis. Overall survival mainly depends on metastasis, but also on local recurrence, treatment complications, and unrelated causes of death.

Histologic grade particularly indicates the probability of distant metastasis. Some systems have been reported as an indicator of local recurrence [34,41,42], but with the most recent systems, histologic grade is an indicator of metastasis and overall survival. Local recurrence is most often reported to be dependent on surgical margins and/or surgical procedure [34,43–45]. In almost every published multivariate study on metastasis and survival, grade is the most important factor, but it does not summarize all the prognostic information and clinical factors are also to be considered. Clinical factors selected after multivariate analysis are variable from study to study: sex, age, local symptoms, tumor location, size, tumor depth, tumor margins, status of regional nodes, surgical margins, and surgical procedure [34,36,38, 43–49]. For example, tumor size is an independent factor for predicting overall survival in four studies [38,43,44,47] and metastasis in two [44,47]. In a large series [46], factors in addition to grade that exerted a significant influence on metastatic recurrence were local symptoms and tumor size. In this study tumor size was important, specifically for grade 2 and 3 lesions. By combining grade and clinical factors, different systems of prognostic groups have been described [38,43–45]. In a recent study, Ravaud et al. [45] reported a simple and very effective system by combining histologic grade and tumor depth. This system allowed the classification of patients into three prognostic groups: a group with a favorable prognosis, including patients with grade 1 tumor or superficial grade 2 tumor (5-year metastasis-free survival rate = 100%); a group with deep grade 2 tumor or superfical grade 3 tumor (5-year metastasis free survival rate = 48.1%); and a group with the worst prognosis, including deep grade 3 tumor (5-year metastasis-free survival rate = 34.1%).

Contribution of grade to patient management

Since the main value of histological grading is to predict the advent of distant metastasis, its contribution to patient management would be the most efficient use of chemotherapy. Recently, Elias et al. [50] reported the

Table 10. Response to palliative chemotherapy according to grade and mitosis count

	Grade			Mitosis/10 HPF		
	1	2	3	0–10	11–20	>20
N	8	17	80	18	20	23
Complete response (%)	0	6	13	0	0	26
Response rate (%)	13	24	55	11	50	61

From Elias et al. [50], with permission.
n = 105.

results of palliative chemotherapy in 105 patients with advanced sarcoma. The response to chemotherapy was correlated to histologic grade and mitosis rate (Table 10). So in such a situation chemotherapy can be of benefit only in patients with high-grade or high mitotic rate tumors.

Although there is no absolutely convincing evidence for efficacy of adjuvant chemotherapy in STS, some reports have been positive. In one of these [51], including only patients with grade 2 or 3 tumors, the beneficial effect of chemotherapy was evident only in patients with grade 3 tumors and no differences appeared for patients with grade 2 lesions. Since the metastatic potential of grade 1 tumors and superficial grade 2 tumors is almost nonexistent, these patients should not receive adjuvant chemotherapy. Further treatment of this kind is investigational and should only concern patients with high metastatic risk tumors.

In a recent study in a series of 64 patients with a locally advanced STS receiving a neoadjuvant chemotherapy, Cany et al. [52] reported a correlation between tumor grade and clinical response to chemotherapy: Response rate was 50% in grade 3 tumors and 23% in grade 1 or 2 tumors.

Whatever the clinical situation, it seems that the response to chemotherapy is poor with low-grade tumors, and a good reponse can be achieved only with high-grade tumors.

Limitations and pitfalls of grading

Grading does not differentiate benign and malignant lesions, and before grading a soft tissue lesion the pathologist must be sure of its malignancy. In fact, sarcomalike lesions, such as nodular and proliferative fasciitis, display the conventional characteristics of malignancy, and the lesions would be of high grade if grading were used.

Grading is not applicable to all kinds of sarcomas. Grading has been shown to be a predictive factor in STS, but its prognostic value in sarcomas of other primary sites is not proven. For bone sarcomas, prognosis and patient management are mainly related to histological type. Before using

Table 11. Five-year metastasis free rate according to grade in different histological types of adult STS

Histological types	Grade 1	Grade 2	Grade 3
Malignant fibrous histiocytoma	(15)* 100%	(53) 75%	(40) 49%
Liposarcoma	(22) 85%	(24) 64%	(11) 36%
Leiomyosarcoma	(12) 100%	(18) 56%	(13) 40%
Malignant schwannoma	(8) 100%	(16) 60%	(16) 34%
Synovial sarcoma	(0) —	(22) 41%	(17) 0%
Unclassified sarcoma	(0) —	(13) 46%	(41) 18%
Other	(11) 100%	(36) 43%	(33) 39%

* Number of cases.
Personal series.
n = 421.

grading in visceral sarcomas, a study should be done to prove its efficacy. Such a study has been reported in breast sarcomas [53]. In STS, grading is useful in adults. In children, prognosis and treatment are mainly dependent on histological type and staging.

Moreover, grading should be established on untreated primary tumors. In fact, radiotherapy and/or chemotherapy can increase necrosis, decrease mitotic count, and even induce changes in differentiation [54,55], and there is no study reporting the prognostic value of tumor grade on local or distant recurrences.

Grading requires representative and well-processed histological material. Grading should be performed on a surgical biopsy. With a needle biopsy, the tissue may not be representative of the entire tumor, and the mitosis count may be underestimated as a result of a sampling error. Moreover, it is not possible to quantify necrosis on a needle biopsy.

Some pathologists believe that grading cannot be applied to the different types of sarcomas because its predictive value differs according to histologic type. This is certainly true for some rare types, such as alveolar soft part sarcoma and clear cell sarcoma, for which grading is useless. However, for most other sarcomas, the advent of metastasis is well correlated with the grade, as observed in 421 adult STS in our center (Table 11).

Tumor grade can be considered as a morphological translation of molecular events that determine tumor aggressiveness; so there is a need to use new technology, such as immunohistochemistry, flow cytometry, and molecular biology, in order to find reliable indicators for tumor growth and chemoresistance. Some reports showed that immunohistochemistry would be a powerful tool in these fields. Tumor proliferative activity can be approached by Ki-67 and/or proliferative cell nuclear antigen (PCNA) immunostaining, which is correlated with the fraction of S-phase cells determined from DNA histograms [56,57] (Fig. 8). In the near future, such a parameter could replace the mitotic count, which constitutes only a small part of the cell

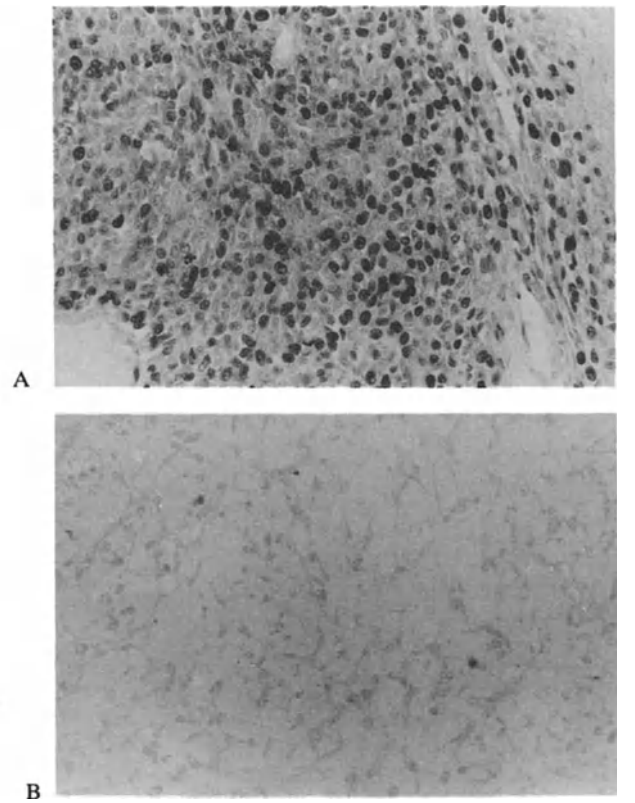

Figure 8. Evaluation of tumor proliferative activity by immunostaining with antiproliferative cell nuclear antigen (PCNA/cyclin monoclonal antibody PC10). A: Ewing's sarcoma with high fraction of S-phase cells determined from DNA histograms. Most of the cells in this field show positive nuclear staining with PC10. B: Extraskeletal myxoid chondrosarcoma with low fraction of S-phase cells determined from DNA histograms. Only two cells in this field show positive nuclear staining with PC10.

proliferation cycle. Altered levels of expression of proto-oncogenes and tumor-suppressor genes can be shown by immunohistochemistry. Recently, Cance et al. [58] reported that sarcomas with a decreased expression of the retinoblastoma (Rb) gene product had a poorer prognosis than tumors with a normal expression. Inactivation of this gene can promote tumor growth, and evaluation of the Rb gene product may be an important prognostic factor in sarcomas. Detection of increased expression of P-glycoprotein associated with multidrug resistance (Fig. 9) could be important for prognostic evaluation and chemoresistance predictability. Chan et al. [59] showed a very strong correlation between immunohistochemical detection of P-glycoprotein and poor prognosis in STS in childhood. However, these results must be confirmed by other studies in larger groups of patients.

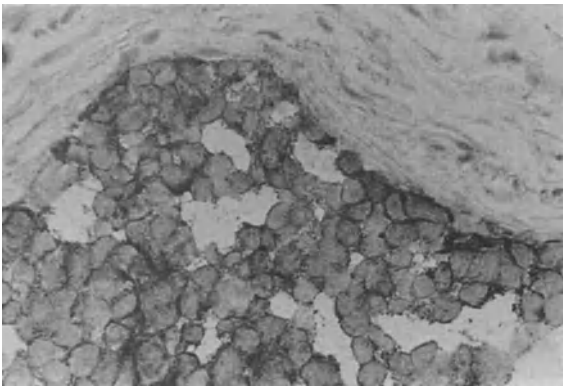

Figure 9. Immunohistochemical detection of P-glycoprotein associated with multidrug resistance. Ewing's sarcoma after chemotherapy. Tumor cells show strong positive membrane staining with monoclonal antibody MDR/JSB-1.

Lastly, several grading systems are now used, and comparison of reported series and trials is not easy. So there is a need for an international consensus with several observers testing different grading systems and histological parameters on a large group of STS. Such a study would achieve a sole international grading system, a kind of working formulation for clinical use [60] in the field of adult STS.

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2. Cytogenetics

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Many soft tissue sarcomas contain characteristic chromosome aberrations that have documented diagnostic relevance [1,2]. These chromosome aberrations most likely reflect loss or altered function of specific genes that contribute to neoplastic transformation. Characterization of genetic aberrations is likely to shed light on the mechanisms of abnormal proliferation in different sarcomas, and this information should enable the development of novel treatment approaches. Because the biologic implications of different sarcoma chromosome aberrations are largely unknown, the present overview will focus on the potential diagnostic relevance of cytogenetic analyses.

Cytogenetic background

Most human cells contain two sex chromosomes along with 22 pairs of autosomes. Until 1970, the different human chromosomes could be distinguished only by gross morphologic features, whereas various staining procedures [3,4] now allow rapid identification of any chromosome based on a characteristic 'banding' pattern. Chromosome banding procedures have enabled the evaluation of consistent cytogenetic aberrations in many neoplastic proliferations. Leukemias and non-Hodgkin's lymphomas were the first neoplasms to be studied systematically using cytogenetic approaches, and it is now clear that many of these hematologic neoplasms contain clonal aberrations of chromosome structure and/or number [5,6]. The hematologic cytogenetic aberrations are of diagnostic and prognostic significance [7–9], and recognition of such aberrations has enabled the cloning and characterization of relevant cancer genes in several leukemias and lymphomas [10,11]. Many investigators have begun to assess benign and malignant soft tissue tumors for the presence of consistent chromosome aberrations, and it has been determined that virtually all soft tissue sarcomas contain consistent chromosome aberrations [1,2]. Certain soft tissue sarcoma aberrations have apparent diagnostic relevance (Table 1) [1,2], and these diagnostic cytogenetic events are discussed herein.

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Table 1. Characteristic cytogenetic aberrations in soft tissue tumors

Histology	Characteristic cytogenetic events	Diagnostic utility?
Peripheral PNET/Ewing's sarcoma	t(11;22)	Yes
Rhabdomyosarcoma—alveolar	t(2;13)	Yes
Rhabdomyosarcoma—Embryonal	+2q, +20	Yes
Synovial sarcoma	t(X;18)	Yes
Malignant fibrous histiocytoma	Complex[a]	?
Malignant nerve sheath tumor	Complex[a]	?
Fibrosarcoma—infantile	+11, +17, +20	Yes
Dermatofibrosarcoma protuberans	Ring chromosomes	?
Leiomyosarcoma	$1p^-$	?
Liposarcoma—myxoid	t(12;16)	Yes
Liposarcoma—pleomorphic	Complex[a]	?
Clear cell sarcoma	t(12; 22)	Yes
Chondrosarcoma—myxoid	t(9;22)	Yes
Mesothelioma	$1p^-$, $3p^-$, −22	Yes

[a] Indicates the consistent finding of extremely complex karyotypes containing multiple numerical and structural chromosome aberrations.

Cytogenetic methods

Classical cytogenetic methods can only be performed during the relatively brief phase of mitosis that is known as metaphase. During metaphase, the chromosomes are extremely contracted, and at this point differential chromosome banding patterns can be induced with several stains [3,4]. Metaphase studies are initiated typically by placing tumor specimens in tissue culture. Because tumor cells can be overgrown in culture by non-neoplastic elements, e.g., fibroblasts, it is important that the cytogeneticist be familiar with characteristic tissue culture morphologies of different neoplastic and non-neoplastic cell types. If tissue cultures are inspected daily, it is possible generally to determine when the tumor cells are growing optimally. At that juncture, the progress of tumor cells through the cell cycle can be blocked using mitotic spindle inhibitors that arrest the cell cycle at metaphase.

Cytogenetic analyses of soft tissue sarcomas require tumor specimens that are fresh, viable, and sterile. Accordingly, it is evident that cytogenetic analyses cannot be carried out on specimens that have been exposed to routine tissue fixatives. It is extremely important that specimens be transported as quickly as possible to the cytogenetics laboratory, because high-grade and/or necrotic specimens often deteriorate rapidly in transit. Careful selection of nonnecrotic tumor specimens is also of paramount importance in the performance of successful cytogenetic analyses. Such analyses can often be carried out on percutaneous fine-needle tumor aspirates [2] if the tumor cells are viable.

Cytogenetic terminology

Cytogenetic aberrations are described using a shorthand system [12] that indicates both the number of copies of each chromosome and the location and mechanism of specific chromosome rearrangements. An example of this shorthand is '46,XX,t(11;22)(q24;q11.2–12),' which describes a female cell with the characteristic chromosome rearrangement of Ewing's sarcomas and peripheral primitive neuroectodermal tumors [12–14]. In the shorthand, '46' denotes the total number of chromosomes in the cell and 'XX' indicates that the cell is from a female ('XY' would indicate a male). The 't' denotes translocation, indicating that exchange of material between two chromosomes is the mechanism of the rearrangement in this case. The first set of parentheses—(11;22)—indicate that chromosomes 11 and 22 are affected by the translocation, whereas the second set (q24;q11.2–12) indicate that the translocation breakpoints were in band 24 on the long arm ('q') of chromosome 11 and in bands 11.2 or 12 on the long arm of chromosome 22. Other common abbreviations include 'p' for the chromosome short arm, 'del' for chromosome deletion, and 'ins' for insertion of material into a chromosome. A comprehensive review of chromosome nomenclature is provided in the International System for Cytogenetic Nomenclature, which was last updated in 1985 [15].

Ewing's sarcoma and primitive neuroectodermal tumor

Ewing's sarcomas and peripheral primitive neuroectodermal tumors (PNET) are both small, round-cell tumors that arise either in soft tissues or bone. Ewing's sarcomas, by definition, are undifferentiated tumors, whereas PNET contain overt evidence of neuroectodermal differentiation. Virtually all Ewing's sarcomas and PNET have a specific chromosome translocation resulting from reciprocal exchange of material between chromosomes 11 and 22 (Fig. 1) [2,12–14]. This translocation is found in tumors of both skeletal and extraskeletal origin. The identical cytogenetic event in Ewing's sarcomas and PNET supports cell biology evidence [14] that these two tumors are closely related entities on a common pathway of differentiation; however, the exact relationship of Ewing's sarcomas and PNET is not clear. Indeed, the normal progenitor cell(s) that give rise to Ewing's sarcomas and PNET have not yet been identified.

Although rare PNET and Ewing's sarcomas lack a classic t(11;22), these cytogenetic variants often contain novel rearrangements of the same region on chromosome 22 that is affected in the typical translocation [12,16]. Of note, t(11;22) has been described in several esthesioneuroblastomas [17], supporting histologic evidence that many esthesioneuroblastomas are actually PNET. Similarly, the recent description of t(11;22) in a soft-tissue small cell osteosarcoma [18] is of interest, because these tumors often resemble

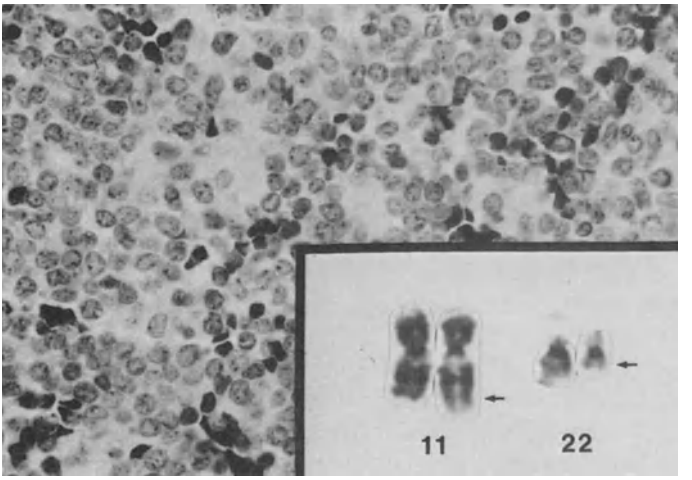


Figure 1. Undifferentiated small round cell neoplasm arising in the pharynx of a 7-month-old boy. Inset: All cells analyzed from this tumor contained a translocation of chromosomes 11 and 22, indicating a diagnosis of Ewing's sarcomas or PNET. Arrows indicate translocation breakpoints on the abnormal chromosomes.

Ewing's sarcomas but are classified as osteosarcomas based on the presence of an osteoid component. The finding of t(11;22) suggests that some small cell osteosarcomas are, if fact, Ewing's sarcomas.

Rhabdomyosarcoma

Most rhabdomyosarcomas (RMS) have either 'embryonal' or 'alveolar' histologies. These two histologies, as described below, appear to result from different genetic aberrations. Embryonal RMS cells resemble the skeletal muscle cells in human embryos, whereas alveolar RMS are generally high-grade tumors composed of undifferentiated small round cells. Alveolar RMS often metastasize widely and can be confused with Ewing's sarcoma, PNET, and even leukemia or lymphoma. Most alveolar RMS contain a specific translocation of chromosomes 2 and 13—t(2;13)(q35–37;q14)—which has not been observed in other varieties of small round-cell tumor [2,19,20]. Embryonal RMS do not have a single diagnostic chromosome translocation, but most cases are characterized by extra copies, or 'trisomies,' of the chromosome 2 long arm and of chromosome 20 [19]. Most embryonal RMS also have loss of genes from the distal end of the chromosome 11 short arm [21,22], which is the same chromosome region that contains the gene(s) for Beckwith-Wiedemann syndrome [23]. Features of the Beckwith-Wiedemann syndrome include predisposition to embryonal rhabdomyosarcoma, Wilms' tumor, and hepatoblastoma. It is interesting that cells in Wilms' tumors and

hepatoblastomas mimic those in embryonic kidney and liver, respectively, and it is possible that the same gene on chromosome 11 contributes to the genesis of each of these 'embryonal' neoplasms [21].

Synovial sarcoma

Synovial sarcomas occur either as monophasic lesions that contain predominantly spindle cells or as biphasic lesions that contain a mixture of spindle cells and epitheloid cells. The monophasic synovial sarcomas, in particular, present diagnostic difficulties; these tumors can be difficult to distinguish from hemangiopericytomas, malignant peripheral nerve sheath tumors, and other spindle-cell sarcomas. Notably, greater than 90% of synovial sarcomas contain a translocation of chromosomes X and 18—t(X;18)(p11.2;q11.2)—

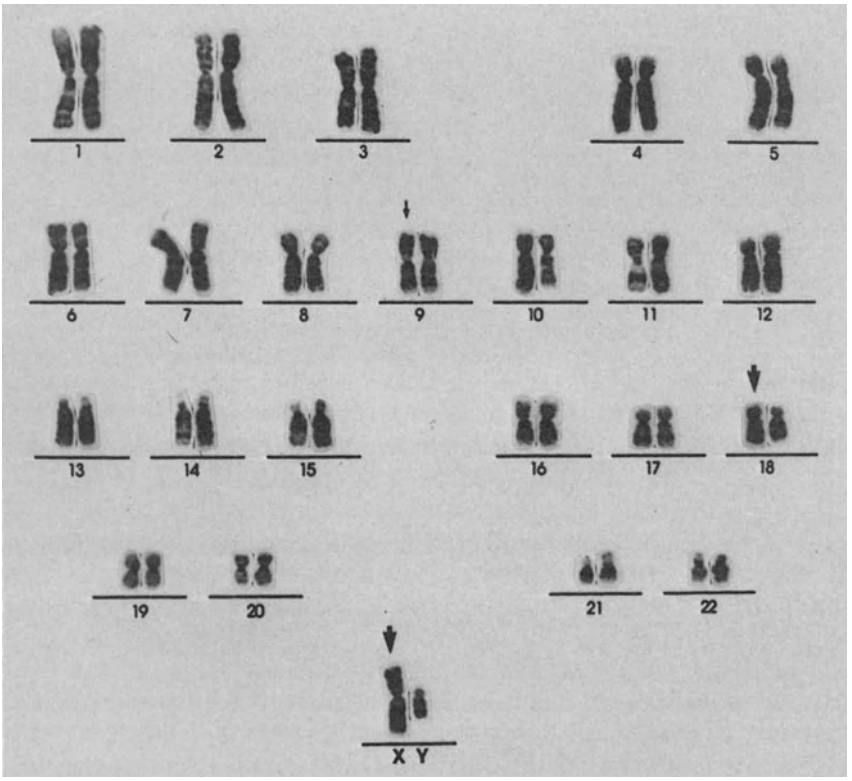


Figure 2. Trypsin-Giemsa banded karyotype of a pyriform mass in an 12-year-old boy. Large arrows indicate translocated copies of chromosomes X and 18, indicating a diagnosis of synovial sarcoma. Small arrow indicates a chromosome 9 with pericentromeric inversion; this inversion is a normal polymorphism that is seen in approximately 1 in 100 normal individuals. Lymphocytes from this boy contained the chromosome 9 inversion but lacked the t(X;18).

(Fig. 2) [1,2,24,25] that has not been described in other varieties of sarcoma. The t(X;18) translocation is characteristic of both monophasic and biphasic synovial sarcomas. Two synovial sarcomas have been described that lacked t(X;18), but one of these had a variant translocation involving the same region on chromosome 18 [26]. The other lacked rearrangement of either chromosome X or 18 [27].

Malignant fibrous histiocytoma

Most malignant fibrous histiocytomas (MFH) are high-grade tumors that contain phenotypically varied cell populations. No diagnostic chromosome aberrations have been identified in high-grade MFH, but preliminary data suggest that MFH with rearrangement of a chromosome 19 short arm have relatively poor prognosis [28,29]. These MFH appear to have an increased frequency of local recurrence and metastatic disease. High-grade MFH have exceedingly complex karyotypes [2,28], which differ from those in certain other soft tissue tumors, including synovial sarcoma, in which the cytogenetic aberrations are relatively simple. Genotypic complexity often results in phenotypic diversity, and the extreme cytogenetic complexity in MFH might explain their histologic pleomorphism and variable immunohistochemical staining patterns [30].

Malignant peripheral nerve sheath tumors

Very few cytogenetic analyses have been reported for malignant peripheral nerve sheath tumors, and no diagnostically useful cytogenetic events have been described in these neoplasms. However, high-grade malignant peripheral nerve sheath tumors often have numerous, complex, cytogenetic aberrations [2,31,32]. Consistent aberrations in malignant peripheral nerve sheath tumors include perturbations of the chromosome 17 short arm that result, in some cases, in loss of the p53 tumor suppressor gene [33,34]. Some malignant peripheral nerve sheath tumors also have aberrations of the recently characterized von Recklinghausen (type 1) neurofibromatosis gene [35], which is located on the long arm of chromosome 17. This finding is of interest because approximately 50% of malignant peripheral nerve sheath tumors occur in individuals affected by von Recklinghausen neurofibromatosis.

Benign schwannomas have noncomplex karyotypes that often demonstrate loss of material from the chromosome 22 long arm [32]. These losses are of interest because benign schwannomas occur with increased frequency in individuals with central (type 2) neurofibromatosis, and the gene for central neurofibromatosis is also located on the chromosome 22 long arm [36]. It is not yet known, however, whether benign schwannomas and central neurofibromatosis result from loss of the same gene.

Fibrosarcoma—Infantile

Although relatively few infantile fibrosarcomas have been karyotyped, these neoplasms appear to be characterized by a consistent pattern of extra chromosomes, including trisomies for chromosomes 11, 17, and 20 [37,38]. It is interesting that these cases lacked structural chromosome rearrangements, because numerical chromosome aberrations, in the absence of structural chromosome perturbations, are virtually always found in benign or low-grade malignant neoplasms. High-grade sarcomas, in contrast, generally contain one or more structural chromosome aberrations [2]. Accordingly, the isolated numerical aberrations in infantile fibrosarcoma are in keeping with the known favorable prognosis of these neoplasms.

Dermatofibrosarcoma protuberans

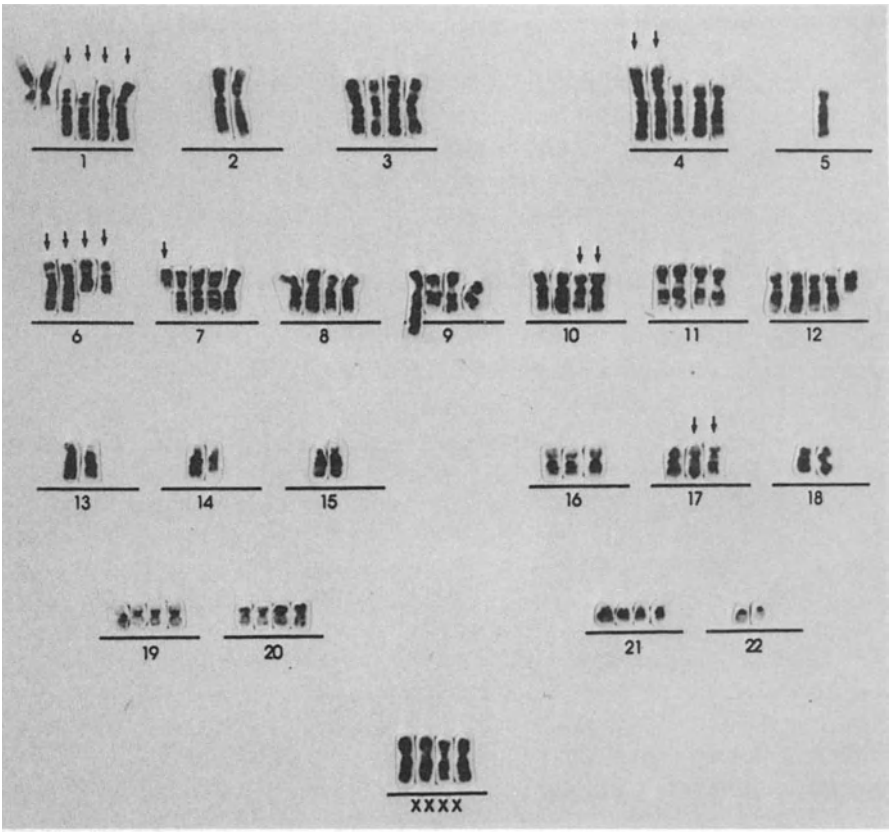
Few of these unusual tumors have been karyotyped, but several examples had quite simple cytogenetic aberrations that included ring chromosomes [39]. 'Ring chromosomes' result when chromosomes lose material at the end of each of their arms; the altered chromosome ends tend to bind to each other, thus creating a circular form. Although ring chromosomes are seen occasionally in many tumor varieties, they appear to occur with particular frequency in dermatofibrosarcoma protuberans and in atypical lipomas [39,40]. The mechanism by which these rings contribute to the neoplastic process remain to be defined.

Leiomyosarcoma

Many leiomyosarcomas, particularly those with high-grade histology, have complex cytogenetic aberrations (Fig. 3) [41,42]. The most consistent aberrations appears to be deletion of the chromosome 1 short arm [41,42]. However, chromosome 1p deletions are found in only 75% of cases, and these deletions are not specific for leiomyosarcoma. It is interesting, nonetheless, that chromosome 1 short arm deletions are also found in extrauterine leiomyomas (JA Fletcher, unpublished data). On the other hand, very few uterine leiomyosarcomas contain a t(12;14), which is found in approximately 20% of uterine leiomyomas [43].

Liposarcoma

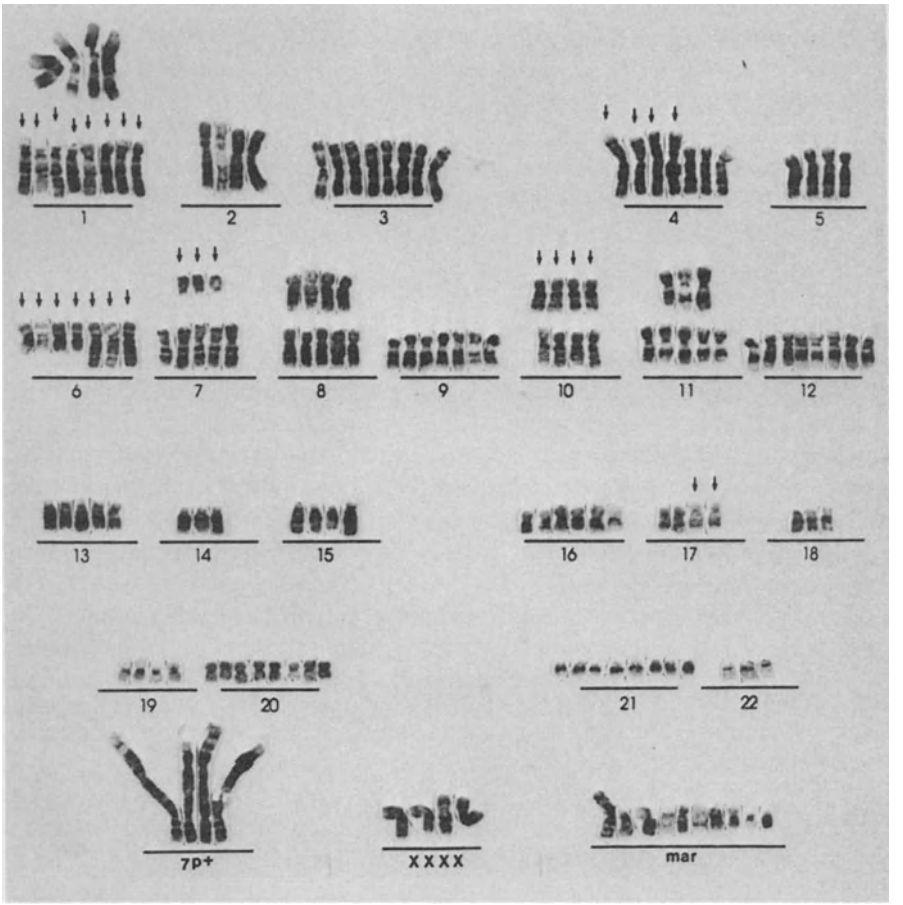
The most striking cytogenetic finding in liposarcomas has been translocation of chromosomes 12 and 16—t(12;16)(q13;p11)—which is found in most myxoid liposarcomas [44,45]. The t(12;16) has not been observed in other



A

Figure 3. A,B: Trypsin-Giemsa banded karyotypes of two cells from a well-differentiated pelvic leiomyosarcoma in a 68-year-old woman. Arrows indicate the numerous clonal chromosome aberrations that were found in all cells from this tumor. Mar is an abbreviation for marker, which indicates a rearranged chromosome of uncertain origin. The metaphase cell in B has more total chromosomes (156 versus 80) than the cell in A. The cell in B also contains several novel aberrations, including the bizarre chromosome 7 rearrangements on the lower left, which were not seen in other cells analyzed from this tumor. Note that the overall cytogenetic complexity is substantially greater than in the synovial sarcoma in Figure 2. (From Fletcher JA, Morton CC, Pavelka K, Lage JM. Cancer Res 50:4092–4097, 1990. Used by permission.)

liposarcoma varieties, including pleomorphic liposarcomas, which contain extremely complex cytogenetic aberrations [2,45]. The t(12;16) has also not been detected in lipomas. However, many lipomas have translocations of chromosome 12 that involve chromosomes other than number 16 [40]. It is unclear whether these lipomas and myxoid liposarcomas arise from perturbation of the same gene on chromosome 12.



B

Clear cell sarcoma

Soft-tissue clear cell sarcoma is an entity that shares many histologic, immunohistochemical, and ultrastructural features with cutaneous malignant melanoma [46]. It is notable that several soft-tissue clear cell sarcomas had a translocation involving chromosomes 12 and 22 that has not been found in melanoma or in other varieties of soft tissue sarcoma [47]. These findings support the classification of clear cell sarcoma as a distinct soft tissue sarcoma entity.

Chondrosarcoma—Myxoid

Extraskeletal myxoid chondrosarcomas are generally low-grade malignant tumors [46]. These tumors can occasionally be difficult to distinguish from other myxoid neoplasms. It is notable, therefore, that a specific translocation involving chromosomes 9 and 22—t(9;22)(q31;q12)—has been reported in several extraskeletal myxoid chrondrosarcomas [48]. The t(9;22) has not been observed in other varieties of myxoid sarcoma.

Mesothelioma

Most malignant mesotheliomas are characterized by loss of genetic material in several chromosomal locations. These genetic deletions typically involve the short arms of chromosomes 1 and 3, and the long arm of chromosome 22 [49,50]. This same pattern of deletions has been noted in approximately 90% of mesotheliomas, irrespective of site (pleural versus peritoneal) or histology (epithelial predominance versus mixed epithelial-sarcomatoid). Few karyotypes have been reported for pure sarcomatoid mesotheliomas, but these neoplasms appear to have more complex cytogenetic aberrations than those with epithelial-type components (JA Fletcher, unpublished data). The invariable presence of deletions suggests that the loss of several genes is important in the genesis of mesotheliomas. In normal mesothelial cells, it is presumed that such genes might serve to constrain proliferation. Detection of clonal deletions can be particularly helpful in pleural or peritoneal fluid specimens, because malignant mesotheliomas are often difficult to diagnose by cytologic methods [52].

Conclusions

Characteristic chromosome aberrations have been identified in benign and malignant mesenchymal tumors, and many of these aberrations have diagnostic relevance. However, with the exception of chromosome 19 aberrations in MFH, the prognostic relevance of specific cytogenetic events has not yet been determined in soft tissue sarcomas. Cytogenetic analyses represent a significant addition to soft tissue sarcoma diagnostic capabilities, but major limitations of the technology include the considerable expertise required for optimal performance of these analyses and the requirement of fresh tumor specimens. Further characterizations of the consistent cytogenetic aberrations in sarcomas might enable the detection of these aberrations in fresh or fixed interphase cells using fluorescent in situ hybridization [53] and other molecular cytogenetic methods. With these refinements, it might be feasible to incorporate genetic detection universally as a routine aspect of soft tissue sarcoma diagnosis.

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3. Molecular biological studies on soft tissue sarcomas

Colin S. Cooper and Jeremy Clark

The new technologies of molecular biology have provided remarkable insights into many aspects of tumor biology. Dramatic improvements have been made in understanding the mechanisms of action of human carcinogens (i.e., virus, radiation, and chemical carcinogens), in elucidating the mechanisms underlying the development of human cancer, in understanding the ways in which chemotherapeutic agents work, and in identifying molecular mechanisms of drug resistances. Although studies on soft tissue sarcoma have in general lagged behind those on the more common malignancies, such as tumors of breast and colon, in the last few years several key advances have been made. First, there have been many reports of cytogenetic abnormalities and of alteration in dominant oncogenes and tumor suppressor genes on soft tissue. Secondly, it is well established that some inherited disorders can predispose to the development of soft tissue sarcomas, and for many of these the genes harboring the inherited mutation have now been cloned and characterized. Thirdly, the discovery of specific chromosomal translocations in some classes of soft tissue tumor and the identification of genes that control differentiation towards striated muscle have provided new markers that can be used in the diagnosis of soft tissue tumors. Finally, studies on tumor ploidy and the expression of genes that control drug resistance have identified a number of important new prognostic indicators. These recent developments are the subject of this review.

Inherited predisposition to sarcoma development

Several types of inherited conditions predispose towards the development of soft tissue sarcoma (Table 1). Most models of tumor development predict that more than one mutation is required to convert a normal cell into a neoplastic clone, and it is generally accepted that in most cases germline abnormalities that predispose towards cancer development may act as one of these mutations, thus reducing the number of genetic 'hits' that need to be acquired by somatic mutation.

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Table 1. Genetic predisposition to sarcoma development

Autosomal dominant disorders	Sarcoma type	Chromosomal location	Gene
Familial retinoblastoma	Many	13q14	RB1
Li–Fraumeni syndrome	Many, particularly rhabdomyosarcoma	17p13	p53
Gardner's syndrome	Fibromatosis	15q21-22	APC
Von Recklinghausens neurofibromatosis	Malignant peripheral nerve sheath tumors	17q11.2	NF1
Beckwith–Wiedemann syndrome	Rhabdomyosarcoma	11p15	Unknown

Sarcomas in retinoblastoma families

Retinoblastoma can present in two forms: a form that is inherited as an autosomal dominant trait in which tumors are often multiple and bilateral, and a form in which tumors are solitary and there is no evidence of inherited transmission. Ten to 20% of patients with the inherited form of retinoblastoma develop a second tumor, mostly osteosarcoma or one of the soft tissue sarcomas, later in life that arise both within and outside the field of therapeutic radiation [1,2]. Based on analysis of the epidemiological characterizations of retinoblastoma, Knudson proposed that development of this tumor required two hits or mutations [3]. He proposed that individuals with the inherited form of the disease carry one hit as a germline mutation and require only one further mutation in a retinal cell for a tumor to develop. By comparison in individuals with the sporadic form of the disease, two hits must occur by somatic mutation in the same retinal cell.

Karyotype analyses of retinoblastomas and of blood from patients with hereditary retinoblastoma has lead to the proposal that the two hits described by Knudson represent deletions or inactivation of both alleles of a gene located at chromosome 13q14 [4–6]. The gene responsible for hereditary retinoblastoma and for predisposition toward the development of sarcomas later in life has now been cloned and is called the *RB1* gene [7,8].

Gardner's syndrome

Inherited predisposition to both superficial and deep fibromatosis has been reported [9]. Families in which deep fibromatoses (also called desmoids) occur as the only manifestation of genetic abnormality are extremely rare. Much more common, however, is the association of desmoids with familial adenomatous polyposis coli (APC) in Gardner's syndrome, a condition that often includes other soft tissue tumors, such as lipomas and leiomyomas, in addition to other neoplastic and non-neoplastic manifestations. It is now generally believed that Gardner's syndrome is a particular expression of

APC rather than a distinct genetic disorder, since both may occur within the same family and both appear to be associated with the same genetic locus on chromosome 5 (5q21-22) [10–15]. The gene located at this locus that is responsible for familial APC has recently been cloned [17–19], and mutations of the APC gene were found in the germline of APC and Gardner's syndrome patients. It has also been reported [18] that individuals with a G→T transition of codon 302 of the APC gene can either have a range of symptoms associated with Gardner's syndrome or have APC with no evidence of extra-codon manifestation. This observation indicates that the clinical abnormalities specifically associated with Gardner's syndrome may be controlled by environmental or genetic factors other than mutations in the APC gene.

Li–Fraumeni syndrome

In 1969 Li and Fraumeni, in a review of medical records of children with rhabdomyosarcoma, identified four families in which there was a higher incidence than expected of soft tissue sarcoma and of breast cancer in female relations [20,21]. Following this initial description it has been established that the spectrum of cancer in this syndrome also includes brain tumors, osteosarcomas, leukemias, and adrenocortical carcinoma. Recently Malkin et al. [22] and Srivastava et al. [23] have detected germline mutations in the p53 suppressor gene (located at chromosome 17p13) in Li-Fraumeni families, suggesting that inherited mutations in this gene are responsible for the development of diverse selection of tumors associated with this syndrome. One of these families examined in these studies is shown in

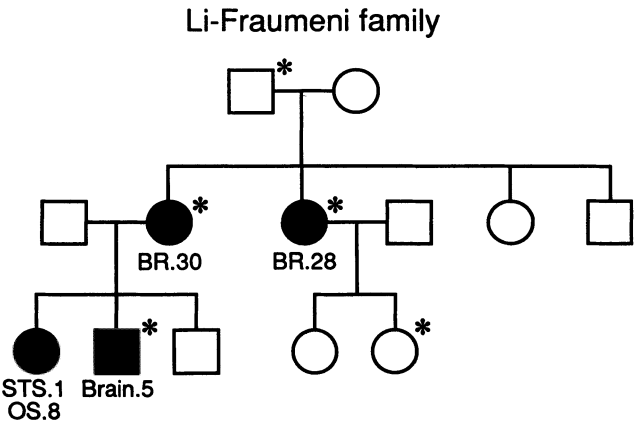

Figure 1. Li–Fraumeni pedigree. Symbols represent females with cancer (●), females without cancer, (○), males with cancer (■), males without cancer (□), and the presence of a constitutional mutation the p53 gene (*). BR = breast; STS = soft tissue sarcoma; OS = osteosarcoma. Ages at which the tumors arose are indicated. Redrawn from Malkin et al. [22].

Figure 1. Direct evidence that inherited mutations in the p53 gene can predispose towards cancer development was provided by studies on transgenic mice harboring a mutated p53 gene [24] and on mice in which the p53 gene was inactivated by gene targeting [25]. In both studies mice with constitutional abnormalities of the p53 gene exhibited a higher incidence of tumors.

von Recklinghausens neurofibromatosis (NF1)

Malignant peripheral nerve sheath tumors (MPNSTs), and to a lesser extent other sarcomas, occur in around 5% of patients with NF1 [26]. The gene predisposing towards NF1 has recently been cloned and characterized [27–29]. Constitutional mutations of the NF1 gene have been detected in NF1 patients, and it would now be interesting to assess the role of mutation of the gene in the development of sporadic MPNST and other sarcomas. Analysis of the NF1 gene sequences has revealed that it has remarkable homology to mammalian GAP, which is responsible for activating the GTPase activity of *ras* proteins.

Beckwith–Wiedemann syndrome

This condition is characterized by an enlarged tongue and abdominal organs, neonatal hypoglycemia, atypical craniofacial features, and certain childhood tumors, including rhabdomyosarcoma. The Beckwith–Wiedemann syndrome is occasionally associated with germline abnormalities of the short arm of chromosome 11, usually involving 11p15, and has recently been mapped by linkage analysis to the same region [30–32]. These observations indicate that a gene located at 11p15 may in its mutated form be responsible for the Beckwith–Wiedemann syndrome and predispose towards the development of rhabdomyosarcoma. Further support for the idea that a gene located at this locus is involved in rhabdomyosarcoma development was provided by the observation that loss of the short arm of chromosome 11 is consistently observed in embryonal rhabdomyosarcoma [33].

The ability to identify predisposing mutations has important implications for the management of soft tissue sarcoma. In particular it should now be possible to screen all potential Li–Fraumeni families for mutations in the p53 gene and to identify carriers who have an exceptionally high risk of developing cancer. Clearly it will be necessary to develop strategies for the counselling, care, and treatment of these individuals. For example, it may be unwise to treat individuals harboring constitutional p53 mutations with high doses of radiation, which may substantially increase the probability of developing a second tumor. In addition, since it is likely that not all Li–Fraumeni families have been identified, and since the frequency at which new germline p53 mutations arise is unknown, it could be argued that all individuals presenting with childhood or adult soft tissue sarcoma should be screened for p53 mutations.

Genes altered during tumor development

It is now generally accepted that the development of tumors involve the accumulation of genetic alterations in two classes of cellular genes, called proto-oncogenes and suppressor genes. Alterations of proto-oncogenes by point mutation, rearrangement, and gene amplification results in their conversion into dominantly acting oncogenes [34]. The products encoded by proto-oncogenes include several classes of protein involved in controlling cell proliferation, such as growth factors (e.g., c-*sis*), growth factor receptors (e.g., c-erbB-1 and c-erbB-2), G-proteins (e.g., the *ras* genes), and nuclear proteins (e.g., *myf* genes), and it is the presence of altered versions of these proteins or their overexpression that contributes to the initiation or maintenance of the malignant phenotype [34]. In the case of tumor suppressor genes (also called recessive oncogenes or antioncogenes), loss or inactivation of both copies of the gene is usually required for tumor development, resulting in removal of the normal gene product. A number of suppressor genes and candidate suppressor genes, including p53, RB1, APC, and NF1, have now been cloned and characterized, and in some cases (e.g., for p53 and RB1) the suppressor function has been formally demonstrated by showing that reintroduction of the suppressor gene into tumor cells results in suppression of the malignant phenotype [35,36].

Oncogenes

DNA transfection into NIH3T3 mouse fibroblasts has been used extensively to screen human tumors for the presence of dominantly acting oncogenes. In this technique tumor DNA is introduced into NIH3T3 cells as a coprecipitate with calcium phosphate, and cultures of cells are monitored for the appearance of foci of morphologically transformed cells that result when the mouse cells stably incorporate an activated oncogene. The majority of the genes detected using this assay are members of the *ras* gene family (H-, K-, and N-*ras*), although non-*ras* genes, such as *met*, *raf*, *trk*, and *ret*, are occasionally observed [34]. Forty-six soft tissue tumors and tumor cell lines, including all major adult tumor classes, have been examined using this assay [37–43]. Activated K-*ras* was detected in a primary embryonal rhabdomyosarcoma [39] and in a leiomyosarcoma [43], while activated N-*ras* was detected in the RD rhabdomyosarcoma cell line and in the HT1080 fibrosarcoma cell line [40]. Activated genes that were unrelated to *ras* were transferred from a liposarcoma and a leiomyosarcoma [41,43]. Although detailed characterization of these genes has not been reported, the gene transferred from a liposarcoma was localized to human chromosome 19 [43].

A different approach has been used to examine a series of childhood rhabdomyosarcomas for the presence of activated *ras* genes [44]. In this study DNA was prepared form formalin-preserved tumors from histopathological archives and the regions of the *ras* genes where activating mutations

usually occur (codons 12, 13, and 61) were amplified using the polymerase chain reaction (PCR). Mutant *ras* genes were then detected by hybridization of amplified DNA to specific oligonucleotide probes. Using this approach, mutated versions of N-*ras* and K-*ras* were detected in 35% (5 of 14) embryonal rhabdomyosarcomas. Taken together these results show that *ras* activation is implicated in the development of at least one class of mesenchymal tumor, but it is a relatively rare event in adult sarcomas.

Perhaps the most exciting results in recent years has been the discovery of specific chromosomal translocations that are unique to particular classes and subclasses of soft tissue sarcoma (reviewed in Chapter 2). For example, a translocation involving chromosomes X and 18 is almost invariably found in synovial sarcoma [45–47]. When specific chromosomal translocations are found in other classes of human neoplasia, they usually correspond at the molecular level to rearrangements involving particular genes. For example, chronic myelogenous leukaemia usually contains translocations between chromosomes 9 and 22 that at the molecular level involves the c-*abl* gene on chromosome 9 and the *bcr* gene on chromosome 22 [48]. By analogy it is presumed that the chromosomal translocations found in soft tissue sarcomas involve alterations of specific genes.

Several studies suggest that gene amplification accompanied by overexpression may be a common mechanism of gene activation in certain types of soft tissue tumors. Double minute chromosomes (DMs) and homogeneously staining regions (HSRs), the cytogenetic hallmarks of gene amplification, are frequently observed in alveolar and embryonal rhabdomyosarcomas as well as in malignant fibrous histiocytomas and liposarcomas [49–53]. Consistent with these reports, the amplification of N-*myc*, c-*myc*, and *gli* protooncogene have been observed in embryonal rhabdomyosarcomas [54–57], and in one study N-*myc* amplification was found in a tumor recurrence but not in the original tumor, suggesting that N-*myc* amplification may be involved in tumor regrowth [54]. Amplification of N-*myc* has also been detected in alveolar rhabdomyosarcoma [58].

In recent studies apparently novel amplified sequences have been isolated from soft tissue tumors. Coccia et al. [59] used an in-gel renaturation technique to identify and isolate sequences that were amplified in an MFH. The isolated sequences did not contain any of 15 known oncogenes, including N-*myc* and c-*myc*, and were amplified in 2 of 8 MFH, 1 of 4 liposarcomas, and 1 of 1 leiomyosarcomas. In one study a high level of amplification of a minisatellite sequence was found in a liposarcoma [60]. This sequence was cloned and mapped to chromosome 7.

Suppressor genes

The observation that soft tissue sarcomas frequently occur in survivors of familial retinoblastomas (see Sarcomas in retinoblastoma families) prompted an assessment of the role of alterations of the RB1 suppressor gene in the

development of sporadic soft tissue sarcomas. Loss of a single copy of chromosome 13 in the general regions of the RB1 gene was observed in 30–40% of sarcomas [61]. More direct evidence for the role of the RB1 gene in sarcoma genes was provided by studies demonstrating abnormalities in expression and homozygous loss (loss of both copies) of the RB1 gene, which in some cases was accompanied by rearrangements within the gene itself of [61–62]. These types of alterations were found in many classes of sarcoma but occurred most commonly in MFH, leiomyosarcomas, and rhabdomyosarcomas.

Recent studies suggest that the p53 gene may also act as a tumor suppressor gene. p53 was originally identified as a protein that was overexpressed in chemically transformed mouse fibroblasts [65] and that bound to the large T antigen of the SV40 DNA virus [66,67]. The p53 gene is located on a region of chromosome 17 that is lost during the development of several types of tumors, including cancer of the lung, breast, and colon. Furthermore the p53 allele that remains in these tumors usually contains point mutations [68,69]. Similar inactivating mutations have recently been observed in the p53 gene in several types of soft tissue sarcoma, particularly in rhabdomyosarcomas and leiomyosarcomas [70,71]. These mutations were usually found in the conserved regions of the p53 gene where mutations are commonly found in other tumor types. However, in contrast to the results obtained for most other tumor types, homozygous deletion of the p53 gene was also frequently observed. Similar gross deletions and rearrangements have also been observed in osteosarcoma and in blast crisis chronic myelogenous leukemia [72,73].

Abnormalities of the p53 gene commonly occur together with alterations of the RB1 gene in soft tissue sarcomas, indicating that coincident loss of two or more suppressor genes may be required for tumor development. This pattern of alteration of p53 RB1 is similar to that achieved following infection by viruses such as SV40 and adenovirus, which can induce soft tissue sarcomas in animal models. For example, in the case of SV40, the large antigen binds both to the P53 and RB1 proteins, while adenovirus encodes the Ela protein, which binds to RB1, and the Elb protein, which binds to p53. It has been suggested that these interactions result in the inactivation of the tumor suppressor function of the RB1 and p53 proteins [74]. Thus the pattern of genetic alteration observed in human soft tissue sarcomas appears to reproduce what occurs at the level of protein–protein interaction during viral infection.

Molecular markers in tumor diagnosis

During the past 15 years there have been major changes in the classification of soft tissue sarcomas and improvements in diagnosis through the use of new immunochemical reagents. For example, the diagnosis of fibrosarcoma,

although originally common, is now restricted to an uncommon tumor type. Furthermore, it has recently been suggested that many tumors diagnosed as MFH may in fact belong to other tumor groups. In addition it is often difficult to distinguish between poorly differentiated rhabdomysarcomas and poorly differentiated examples of other types of childhood tumors. The recent identification of tumor-specific genetic alterations and of genes that are expressed in a lineage-specific fashion offers a completely fresh approach that has the potential to improve still further methods of diagnosis and to improve the consistency of diagnosis between different centers.

The use of myf *gene expression in the diagnosis of rhabdomyosarcoma*

Advances in our understanding of the molecular signals that control the development of striated muscle have yielded several new markers that could potentially be used in the diagnosis of rhabdomyosarcoma. The development of skeletal muscle is a complex process in which multipotent stem cells initially become committed to form mononuclear myoblasts that, in turn, fuse to form myotubes. The myotubes then mature into striated muscle. Transition along this differentiated pathway appears to be determined by a family of transcription factors that are directly involved in controlling the expression of muscle-specific genes. The first genes found to encode muscle determining factors were the mouse MyoD1 gene [75] and the rat myogenin gene [76]. In addition, Braun et al. [77,78] have isolated human genes *myf*3 and *myf*4 [79], the homologues of MyoD1 and myogenin, respectively, and two further genes, *myf*5 and *myf*6. The proteins encoded by these genes are closely related, each containing a highly conserved basic-helix-loop-helix region that is believed to be responsible for the binding of *myf* proteins to enhancer regions in muscle-specific genes. It has also been demonstrated that each member of the *myf* gene family can convert mouse fibroblasts into cells with myogenic characteristics, and in analyses of normal tissue all four genes were expressed exclusively in striated muscle [77–79].

Rhabdomyosarcomas are tumors that show differentiation towards striated muscle. Conventionally, three main histological subtypes are recognized [9]. However diagnosis is often difficult, particularly for poorly differentiated embryonal tumors, which can be difficult to distinguish from other classes of pediatric round cell tumors, such as neuroblastoma, hepatoblastoma, and non-Hodgkins' lymphomas [9]. The final tissue diagnosis frequently requires the use of supplementary techniques, such as electron microscopy and, in particular, the use of immunohistochemical reagents that detect, for example, muscle-associated intermediate filaments, contractile proteins, and myoglobin. The most commonly used antibodies are those directed against desmin, myoglobin, fast myosin, and sarcomeric actin. However their interpretation is sometimes difficult, particularly in poorly differentiated rhabdomyosarcomas, where the expression of muscle-associated proteins is limited

[80–83]. Thus myoglobin, although specific to rhabdomyosarcomas, is only expressed in a modest proportion of tumors (40–60%). Conversely desmin is expressed in most rhabdomyosarcoma but is also expressed in other tumor types. Since rhabdomyosarcomas are thought to be derived from cells that are differentiated towards striated muscle, all rhabdomyosarcomas would be expected to express one or more members of the *myf* gene family. In agreement with this prediction, Cavenee and colleagues found expression of all four *myf* genes in these tumors [84,85]. An independent study by Clark et al. [86] found that *myf*3 and *myf*4 were expressed in a high proportion of tumors but that *myf*5 and *myf*6 were expressed in a lower proportion of tumors (55% and 33%, respectively). The reason for the different results in these two studies is not clear. Both groups failed to observe the expression of *myf* genes in other classes of soft tissue and pediatric tumors, indicating that members of these gene family may provide extremely useful markers in the diagnosis of rhabdomyosarcoma. In these studies the expression of *myf* genes was examined by Northern analysis of tumor RNA. However if this method is to be adopted for routine use in histopathology laboratories, antibodies that recognize the *myf* proteins will be required. Indeed the production of these antibodies should represent a major objective of future studies.

Although these studies have been restricted to the case of *myf* genes in the diagnosis of rhabdomyosarcoma, it is probable that families of tissue-specific transcription factors will also be involved in controlling the differentiation along other soft tissue lineages. The cloning of the genes encoding such factors should in time yield probes that can be used in the diagnosis of other classes of soft tissue tumor.

Tumor-specific chromosomal translocations

As mentioned above, cytogenetic studies have established that several types of soft tissue sarcoma contain specific chromosomal translocations. Moreover, they commonly occur in a high proportion of tumors, thus offering the prospect of use in tumor diagnosis. A specific translocation involving chromosomes 2 and 13, t(2;13)(q37;q14) has been detected in 50% of alveolar rhabdomyosarcomas [87–89]. This translocation has, however, occasionally been observed in embryonal tumors, suggesting that it may not be completely exclusive to the alveolar subclones. A translocation between chromosome X and 18, t(X;18)(p11.2;q11.2) has been found in a high proportion of synovial sarcomas [45–49]. This translocation has been found in both the monophasic and biphasic subtypes, and therefore represents a useful diagnostic marker. The significance of reports of this translocation in a fibrosarcoma [90] and in a malignant fibrous histiocytoma [91] is not clear because using standard histological techniques it is often difficult to distinguish MFH and fibrosarcoma from the menophasic variant of synovial sarcoma.

Cytogenetic analysis of liposarcoma has identified a specific translocation involving chromosomes 12 and 16, t(12;16)(q13-14;p11) [49,92–94]. Since this translocation has only been found in myxoid liposarcomas, it has been proposed that it may have a particular diagnostic use in distinguishing myxoid liposarcomas from other classes of myxoid tumors (e.g., myxoid chondrosarcomas).

Although cytogenetic information is, in some centers, already used as an aid in the diagnosis of soft tissue sarcomas, there are a number of problems associated with the use of this procedure in routine diagnosis. First because of the low mitotic index of many solid tumors, it is often difficult or impossible to obtain a karyotype. Furthermore, since the tumors are grown in culture to help stimulate cell division, it can in some cases take up to 2–3 weeks to complete the cytogenetic analyses; this compares to 3–4 days required to obtain the karyotype for a hematological malignancy. To overcome these problems of reliability and speed of analysis, it will be necessary to identify and clone the genes that are involved in these specific translocations. Once the genes have been sequenced it should be possible to design PCR primers that can be used to specifically amplify and detect the rearranged sequences. This type of analysis is rapid (1–2 days) and also very sensitive, since rearrangements can be detected when tumor cells are mixed with up to a 10^5 excess of normal cells.

Gene loss

It has recently been proposed that loss of the short arm chromosome 11 can be used to distinguish embryonal from alveolar rhabdomyosarcoma. Scrable et al. [33] found that all the embryonal tumors had lost one copy of the short arm of chromosome 11, while all the alveolar tumors retained both copies of this chromosomal region. This region presumably corresponds to the location of a tumor suppressor gene that is lost or inactivated during the development of embryonal tumors, and the results imply that the mechanism of development of embryonal tumors may be distinct from that of alveolar tumors.

Molecular predictors of tumor behavior

An important goal of clinical research is to identify factors that can be used to predict tumor response to treatment and the survival of individual patients. For certain tumor types it has also become apparent that the presence of particular genetic abnormalities correlate with clinical behavior. For example, in cancer of the breast amplification and overexpression of the c-*erb*B-2 gene is correlated with poor survival and shorter times to relapse [95]. In recent studies correlations have also been made between the presence of specific genetic alterations or the expression of particular genes and the behavior of soft tissue sarcomas (Table 2).

Table 2. Predictors of tumor behavior

Tumor type	Marker of poor prognosis	Reference
Malignant fibrous histiocytoma	$19p^+$	52
Malignant fibrous histiocytomas	Absence of ring chromosomes	96
Childhood sarcomas (mostly rhabdomyosarcoma)	P-glycoprotein expression	98
Rhabdomyosarcomas	Diploid DNA content	99
Soft tissue and bone sarcomas	Alterations of the RB1 suppressor gene	97

Cytogenetic abnormalities

A variety of cytogenetic abnormalities have been identified in MFHs. These involve a rearrangement involving 19p13, ring chromosomes, deletions of 11p and 3p, and the presence of the cytogenetic signs of gene amplification (homogenously staining regions and double minute chromosomes). Notably there appears to be a correlation between tumor behavior and 1) the presence of the 19p+ marker chromosome and 2) the presence of ring chromosome [52,96]. Thus metastases and/or local recurrence occurred in 8 of 9 patients with the 19p+ marker but in only 4 of 13 without it. Furthermore, relapse occurred in 2 of 8 patients with ring chromosomes compared to 10 of 14 patients without them.

Alterations of the RB1 suppressor gene

Attempts have been made to correlate the presence of deletions of the RB1 gene with tumor grade. In a combined study of 36 bone and soft tissue sarcomas, Wunder et al. [97] found that the RB1 gene was altered in 10 of 25 high-grade tumors but in only 1 of 11 low-grade tumors. These results indicate that alteration of the RB1 gene may function as a marker of high-grade tumors, and in future studies it may be appropriate to assess RB1 status as an independent prognostic indicator.

Expression of the multidrug resistance gene mdr-*1*

The resistance of malignant cells to chemotherapeutic agents is often associated with the expression of a 170 kDa membrane glycoprotein, called P-glycoprotein, which is encoded by the *mdr*-1 gene. P-glycoprotein is an energy-dependent pump that removes drugs from the cells and that can confer resistance to classes of structurally and functional unrelated drugs. The high levels of P-glycoprotein that can be found in drug-resistant tumor cell lines are frequently accompanied by amplification and overexpression of the *mdr*-1 gene. P-glycoprotein has been assessed as a prognostic marker

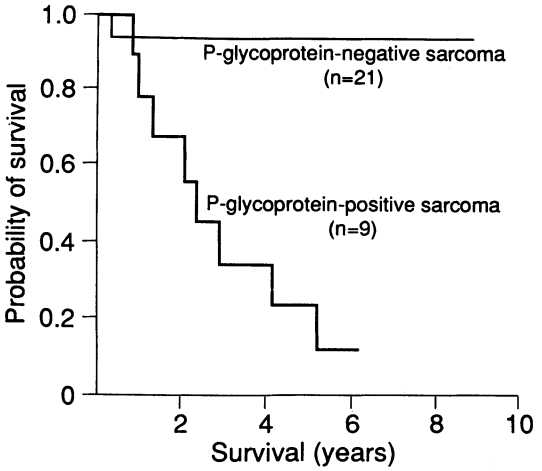

Figure 2. Kaplan–Meier plot of the duration of survival in children with P-glycoprotein-positive and P-glycoprotein-negative soft tissue sarcoma.

in children with soft tissue sarcomas (mostly rhabdomyosarcomas) [98]. Remarkably all nine patients who contained tumors expressing P-glycoprotein relapsed after an initial response, while only 1 of 20 tumors that were consistently P-glycoprotein negative relapsed (Fig. 2). This study indicates that expression of P-glycoprotein is a major factor in determining the long-term response of childhood sarcomas to chemotherapy and suggests that use of chemotherapeutic agents in combination with inhibitors of P-glycoprotein, such as verapamil and cyclosporin, may improve the survival of patients with P-glycoprotein positive tumors.

Tumor DNA content

Shapiro et al. [99] have examined the relationship between tumor cell DNA content (or ploidy) and patient response in a series of unresectable rhabdomyosarcomas. They found that alveolar tumors usually had either a diploid or near-tetraploid DNA content, while embryonal tumors had either a diploid or hyperdiploid DNA content (1.1–1.8 times the DNA content of diploid cells). Notably there was a marked correlation between DNA content and patient survival. Patients bearing tumors with a hyperdiploid DNA content had the longest survival times. Patients with near-tetraploid tumors exhibited intermediate survival, while patients with diploid tumors had the poorest survivals. A similar association between hyperdiploid tumors and a favorable response has also been observed for neuroblastoma and acute lymphoblastic leukemia.

Perspectives

Studies on the transformation of primary mesenchymal cells in culture demonstrated that full transformation requires the cooperation between different classes of activated oncogenes. For example, activated versions of the *myc* and *ras* oncogenes can together result in the transformation of primary embryonic fibroblasts [100]. In contrast, each oncogene on its own is unable to induce transformation. Studies on certain types of human cancer also suggest that stepwise alteration of several different genes is required for malignant conversion. Thus activation of one oncogene (*ras*) and inactivation of three suppressor genes (APC, DCC, and p53) may be required for the development of colon cancer [17,18,68,101]. For most soft tissue sarcomas, only a single consistent genetic alteration has been detected. The identification of other genetic changes and analysis of the ways in which these genes interact are, therefore, important areas for future studies.

A particularly important goal will be the cloning of genes involved in the specific translocation that have been identified through cytogenetic examination of soft tissue tumors. In addition to providing information about the mechanism of sarcoma induction, the cloned genes will yield probes that may be used in the routine detection of this translocation, and hence in tumor diagnosis. Improvements in diagnosis should result from the identification of genes involved in determining the transition along particular soft tissue lineages. This is illustrated above by the use of MyoD1 in the diagnosis of rhabdomyosarcoma, but this general approach may also, in time, be used to improve methods of diagnosis of, for example, liposarcoma and chondrosarcoma, which should express genes determining differentiation towards, respectively, fat and cartilage. Because of the relative rarity of soft tissue sarcoma if treatments of individual sarcoma types are to be improved, it will be necessary to carry out studies on a multicenter basis. As an essential part of these studies there will be a need to develop diagnostic procedures that are easy to carry out and highly reproducible between different centers. Advances in diagnostic procedures through the use of molecular markers may therefore play a key role in improving methods of tumor treatment.

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4. Diagnostic imaging

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During the last decade there has been a significant change in the approach towards the recognition and treatment of soft tissue sarcomas. Pre-operative delineation of soft tissue tumors are available with CT and MRI. Refinements in MRI imaging techniques, the addition of contrast-enhanced imaging [1–4], and MR spectroscopy [5–10] are recent developments that hold promise for the differentiation of soft tissue neoplasms and the monitoring of the response to therapy. Plain film radiography, angiography, scintigraphy, ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS) are the diagnostic tools that may be employed in the evaluation of any tumor. In this chapter we will discuss the present state of the art of diagnostic imaging of soft tissue sarcomas, comparing the possibilities and limitations provided by the different modalities.

Diagnostic imaging approach to soft tissue sarcomas

Assessment of soft tissue sarcomas should be the collaboratative effort of a treatment team in which the radiologist plays an important role [11]. The goal of the radiologic assessment should be

1. To evaluate the aggressiveness and diagnosis of the tumor, which is of importance for the decision on further management
2. To evaluate the internal structure of the tumor (i.e., the presence of tumor necrosis and the location of viable tumor tissue suitable for biopsy)
3. To outline the local extent of the tumor
4. To detect the involvement of adjacent bone
5. To monitor the effect of treatment
6. To detect tumor recurrence
7. To detect metastases

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Imaging techniques

Conventional radiography

Plain film radiography is often used as the first radiologic evaluation of the tumor, but for soft tissue tumors its value is limited, despite recent developments, such as the digital imaging technique with provisions to enhance soft tissue contrast by means of frequency modification [12]. Lipomas, calcifications in a vascular tumor, and seldom, bone erosion caused by a soft tissue tumor may be detected. However, conventional radiographs do provide a convenient baseline for the evaluation of the status of the bone following drug and radiation therapy.

Angiography

Angiography was for a long time indispensible for the assessment of soft tissue sarcomas [13]. However, with the development of CT, and more recently MR imaging, the indications for angiography have diminished considerably. For tumors of vascular origin, such as venous leiomyosarcoma, angiography still is superior to any other imaging modality to define the extent and nature of the lesion. Also, angiography is of value in conjunction with palliative or preoperative measures, such as tumor embolization or local cytostatic infusion [14,15]. The technique does not differ significantly from other diagnostic angiographic examinations. Small catheters (4–5 F) have become increasingly common. Digital subtraction angiography (DSA) enables the use of even smaller catheters and smaller amounts of contrast media, reducing the rate of complication. Although the spatial resolution with this technique is not as good as with film-screen combinations, the tumor blush of vascular tumors is usually better, and the venous phase is also excellent [16].

Ultrasonography

Ultrasonography is of value in guiding fine-needle aspiration biopsy of inpalpable lesions and can be used for routine follow-up in the evaluation of tumor recurrences [17]. Solid and cystic soft tissue masses can be differentiated, but the local behavior of the tumor generally cannot be assessed and there is little information as to the diagnosis [18]. Endoscopic ultrasonography has been reported to be superior to any other imaging modality in the detection, staging, and follow-up of smooth muscle tumors of the bowel, including leiomyosarcoma [19].

Scintigraphy

Presently, scintigraphy is of limited value. Bone scintigraphy, using 99m-technetium, sometimes indicates uptake of the isotope in the primary tumor

[20] and may also aid in the evaluation of possible soft tissue sarcoma invasion of adjacent bone [21]. Scintigraphy using 67-gallium sometimes results in uptake by soft tissue tumors [22]. With modern detection systems, whole body scanning with a dual head technique will be performed in less than 10 minutes. However, additional spot films of specific areas of interest are warranted to provide optimal resolution. The use of specific tumor-seeking agents, such as isotope-labelled monoclonal antibodies, has been reported but does not yet have an established role in the diagnosis of soft tissue sarcomas [23,24]. Recently, Tl-201 single photon emission computed tomography (SPECT) was reported to be superior to CT in the detection of soft tissue tumor recurrence [25], and the use of Tc-99m-labeled methoxy-isobutyl-isonitrile (Cardiolite) has been proposed in the assessment of activity of soft tissue sarcomas [26]. Also, tumor grading of liposarcomas using positron emission tomography (PET) has been suggested in distinguishing between low-grade and high-grade liposarcomas [27].

Computed tomography (CT)

The introduction of CT in the diagnostic imaging arsenal brought a revolution, mainly for three reasons:

1. The much better contrast resolution compared with the previously used methods
2. The ability to provide cross-sectional imaging
3. The possibility for tissue differentiation and characterization, measuring the attenuation values

   To perform an adequate CT examination, the following parameters must be set: scan volume (upper and lower limits), slice thickness and interval, reconstruction algorithm, field of view, and the use of oral and/or intravenous contrast medium. To acquire optimal information, the technique for image evaluation must also be considered.

Scan volume. All modern scanners have equipment for digital radiography (scout view), and such an image must be obtained to define the proximal and distal points of the examination. Enough normal tissue on both sides of the tumor should be included to ensure that the entire tumor is examined. A plastic catheter placed over a scar or a palpable mass will be visible on the digitalized radiograph and may be helpful for localization.

Slice thickness and interval. The choice of slice thickness is mainly determined by the size of the tumor that is examined. However, there should be at least three sections through the lesion in order to minimize the effect of volume averaging. Also, the complexity of the neighboring anatomic structures should be considered. However, the use of thin sections may lead to a large number of CT slices, prolonging the examination and increasing the radiation dose. Then thinner and thicker slices may be combined. As a rule,

the most complex anatomic information will be seen at the belly of an elliptic tumor, and at the end and belly of a teardrop tumor, and in these areas thinner slices should be used. The CT sections should usually be obtained immediately adjacent to each other.

Reconstruction algorithm. Most modern CT equipment provides options for several imaging algorithms. For evaluation of soft tissue tumors, it may be wise to obtain hard film copies, using not only the soft tissue window for the evaluation of tumor contents and their relation to neighboring soft tissues, but also the bone window for the evaluation of the relation between the tumor and neighboring bone.

Field of view. The field of view (FOV) is the diameter of the reconstruction circle used by the CT computer to generate the film image. Reducing the FOV decreases the pixel size, leading to increased spatial resolution. Today, with varying FOVs the pixel size may range between 0.2 and 1 mm. However, even if a small FOV increases the spatial resolution, at the same time it excludes the opposite normal side of the body from comparison, and such comparison may be helpful in detecting subtle soft tissue changes. Also, a small FOV may decrease the contrast resolution.

Oral and intravenous contrast media. Musculoskeletal tumors should be examined both prior to i.v. contrast medium administration and during contrast medium infusion after contrast medium bolus injection. The examination performed before contrast medium administration will reveal, for instance, calcifications in the tumor, and a comparison of the images obtained before and after contrast medium reveals tumor vascularization, as well as the relation between the tumor and neighboring large vessels [28,29]. For example, oral contrast medium outlining the bowel is of importance for the evaluation of pelvic tumors.

Magnetic resonance imaging (MRI)

MRI has proven to be well suited for the examination of soft tissue tumors because of the high contrast resolution, the ease with which imaging in any plane may be performed, and the possibilities (although so far limited) for tissue characterization. However, MRI is a complex imaging modality, requiring careful selection of several technical parameters in order to obtain optimal imaging. Such parameters include pulse sequences, coil selection, slice thickness, scan axis, and field of view. Also, the use of intravenous MR contrast media should be discussed.

Pulse sequences. During the last years, the most widely utilized pulse sequence has been the spin echo. Manipulating with the TR and TE of this sequence, a T1- or T2-weighted image may be obtained. A short TR (less

than 500 msec) and short TE (less than 30 msec) provide a T1-weighted image, while a long TR (more than 1500 msec) and TE (more than 40 msec) sequence is T2 weighted. For most soft tissue tumors, the tumor and muscle often have similar signal intensity in the T1-weighted image, while better tumor-muscle contrast is obtained in the T2-weighted image. In such images, the tumor has a high signal intensity. On the other hand, the distinction between tumor and fat may be poor in a T2-weighted image. Therefore, it has been common to perform both a T1-weighted and a T2-weighted sequence for the evaluation of musculoskeletal tumors.

The possible choices of pulse sequences are theoretically unlimited, and recently several new pulse sequence techniques have been proposed. Using reduced flip angles with gradient echoes allows for image aquisition in much less time than conventional spin-echo sequences, reducing the motion artifacts, which is of interest for increasing patient throughput. It allows for "dynamic imaging" in connection with MR intravenous contrast media enhancement [4]. However, in static imaging a replacement of the T2-weighted SE sequences by flash sequences is not recommended due to the reduction in contrast between the tumor and surrounding tissue. A replacement of the T1-weighted SE sequences by flash sequences seems possible, but does not significantly reduce the examination time [3,4]. Three-dimensional (3-D) spin-echo techniques may be helpful for determining the tumor extent in anatomically complex regions; for example, invasion of the joints. The technique allows for acquisition of volumetric data, which subsequently can be reconstructed into displays of various planes of interest after the examination has been completed. This technique has previously been limited by the relatively long examination time for T2-weighted acquisition.

Fast acquisition spin-echo techniques (FASE) allows for up to 16 times faster acquisition with maintained SE resolution but with increased RF-energy deposition in the tissues [30].

Coil selection. All modern MR units offer not only standard "body coils," but also smaller specialized receiver coils. These specialized coils improve the image quality by 1) increasing the signal strength received from a patient and 2) reducing the volume of irradiated tissue covered by the coil, thus reducing the image noise [31]. Depending on the MR equipment, the design of the coils will vary, but generally, specialized receiver coils designed for different anatomic areas should be used for the imaging of musculoskeletal tumors.

Slice thickness. In most patients a slice thickness varying between 4 mm and 10 mm is appropriate. Using spin-echo imaging, contiguous slices can not be obtained. Theoretically, gaps between the slices may mean that important information could be missed. However, for most MR images available today, the interslice gap is relatively small and should not create a practical problem.

Field of view. The field of view is defined as the diameter of the scan field on which the image matrix is applied. Reducing the field of view increases the spatial resolution, but may also decrease the signal intensity. A reduced field of view is recommended for the distal extremities and anatomic details. A proper balance between the field of view and degradation of the image quality must be determined for each type of equipment.

Tissue characterization. MR provides high soft tissue contrast discrimination. The contrast in the image is mainly determined by T1 and T2 characteristics of the tissue, proton density, and flow. It is also dependent on the pulse sequences used. As different tissues have different T1 and T2 values, the relative contribution of the T1 and T2 to the image contrast may be altered by manipulation of the pulsing sequence. Then, comparing T1- and T2-weighted images may allow tissue characterization.

Concerning the normal bone and soft tissues, muscle and articular cartilage usually have an intermediate signal intensity, both in the T1- and T2-weighted images, except in strongly T2-weighted images, where they become dark. Cortical bones are dark (low signal intensity) in all sequences, and fat is very bright in all sequences.

Most tumors have a prolonged T1 and T2 as compared to muscle and fat. On T1-weighted spin-echo images, tumors usually have the same signal intensity as muscle, but are darker than fat. On the T2-weighted images the tumors become brighter than muscle, and on strongly T2-weighted images tumors usually also become brighter than fat [32].

Much MR equipment today provides possibilities for the calculation of exact T1 and T2 values. However, these values depend both on imaging technique and field strength, and as a whole, the exact T1 and T2 values for tissue characterization have proven to be of little importance, as will be discussed below [33,34].

Intravenous contrast media. The intravenous contrast media commonly used are based on gadolinium (gadolinium-DTPA/Gd-DTPA or gadolinium-DOTA/Gd-DOTA). Gd-DTPA-bismethylamide (Gd-DTPA-BMA) is a new, non-ionic gadolinium compound currently under investigation that offers the potential advantages of similar relaxivity, decreased osmolarity, and lower toxicity than gadolinium-DTPA [2]. MR contrast media are distributed in accordance with traditional intravenous contrast media used in radiology, giving information on vascular permeability and increased blood flow. This gives additional information concerning tumor vascularity, tumor necrosis, and tumor delineation [1]. It allows for differentiation between thrombi and rare vascular sarcomas [35]. With dynamic imaging following intravenous administration of gadolinium-DTPA, the malignant potential of a tumor, the presence of necrosis, and peritumorous edema can be assessed with some overlap [4]. However, the replacement of the conventional T1- and T2-weighted spin-echo sequences with Gd-enhanced sequences, although

possible, will not reduce examination time or increase contrast between the tumor and surrounding tissue [3], and in general contrast enhancement has no firmly established role in the routine MR imaging of soft tissue sarcomas [2]. However, for the evaluation of tumor necrosis as a response to chemotherapy [14] and for the early detection or exclusion of tumor recurrencies [17], its use seems justified.

An interesting observation is the altered biodistribution of gallium-67 found after Gd-enhanced MR-imaging. Reduced liver and bowel activity, and increased renal and skeletal isotope accumulation, have been reported to be attributed to a carrier effect of Gd-DTPA [36].

MR spectroscopy

The metabolic activity within the tumor may be used as a clue to diagnosis, but more importantly, changes in metabolic activity indicate the tumor response to treatment. MR spectroscopy based on ^{31}P has been used by several authors to monitor tumor therapy, as cell death is accompanied by the loss of adenosine triphosphate, an increase in the inorganic phosphate level, and possibly acidification of the cell due to accumulated lactic acid. There are several reports of abnormal spectra, both in animal and human tumors, as well as support of spectral changes following chemotherapy [6–9]. As yet, no characteristic spectra have been found for individual tumor types. Sources of errors relate to regional variabilities of the metabolic parameters with ^{31}P spectroscopy within malignant lesions [7,8]. Also, muscle contamination is a significant problem in the analysis of the spectra, partially overcome by optimal placement of the surface coil guided by the concomitant MR examination. With one-dimensional chemical shift imaging, muscle contamination is further reduced. In the future, three-dimensional chemical shift imaging may be employed, allowing spectra to be obtained from specific voxels of interest [9].

Evaluation of soft tissue tumors

As stated above, the radiologic evaluation of soft tissue tumors should include 1) an assessment of local aggressiveness, 2) a probable diagnosis, 3) the internal structure of the tumor, 4) the local extent of the tumor, 5) possible involvement of adjacent bone, 6) the effect of treatment, 7) possible tumor recurrence, and 8) possible metastases.

Aggressiveness

For the assessment of the aggressiveness of soft tissue tumors, at present all diagnostic imaging modalities are of limited value. Conventional radiographs rarely provide any information. Bone scintigraphy may provide a rough

estimate concerning the aggressiveness, since aggressive lesions may have a considerably increased uptake, while slow-growing lesions have slighter or no uptake at all [20].

A recent report indicates that PET imaging with 18 F2-deoxy-2-fluoroglucose (FDG) can be of value in the grading of liposarcomas [27]. At angiography there is a good correlation between the degree of pathologic vascularization and the differentiation of the tumor, but angiographically avascular masses may be highly malignant [37].

CT and MRI are also of limited value for the evaluation of the aggressiveness of soft tissue tumors. Some authors have reported malignant tumors to be more commonly inhomogeneous, with irregular, poorly defined margins, but there are many exceptions to this general rule [38,39]. Gd-DTPA-enhanced MR imaging using fast low-angle shot (FLASH) sequences have been reported to be useful, giving information about the malignant potential of a tumor, although with certain overlap between benign and malignant tumors [4].

A recent study combining magnetic resonance imaging with magnetic resonance spectroscopy indicates that tumor grading may be possible in the future with improvements in spectroscopic technique [9], but as a whole, presently there are no methods that significantly contribute to the evaluation of aggressiveness in established clinical practice. Therefore, with the exception of lipoma (see below), all deep soft tissue tumors should be approached as if they were aggressive [40].

Diagnosis

In establishing the diagnosis of a soft tissue tumor, the aggressiveness of the tumor, its location, the possible multiplicity of the tumor, and specific radiologic patterns of the tumor matrix must be determined. To this several important clinical data should be added: the age of the patient, the history, findings at clinical examination, laboratory data, etc. To obtain this information, different combinations of the diagnostic imaging modalities have been used.

Plain film examinations generally yield little diagnostic information. However, the low density of a lipoma may be recognized [41] (Fig. 1), and calcified phlebolites may be seen in a tumor of vascular origin. Ultrasound may be used for differentiation between solid and cystic lesions, but the greatest value of ultrasound is to serve as a guide for the biopsy of soft tissue tumors (Fig. 2) [17,42].

Scintigraphy is an excellent tool for the evaluation of the multiplicity of a lesion. Otherwise, the information gained from scintigraphy is nonspecific for the diagnosis [43]. The diagnostic capability of angiography is limited. Vascular tumors, such as angiolipoma and cavernous venous hemangioma, can be diagnosed at angiographic examination, but the overlap between different tumor types is considerable.

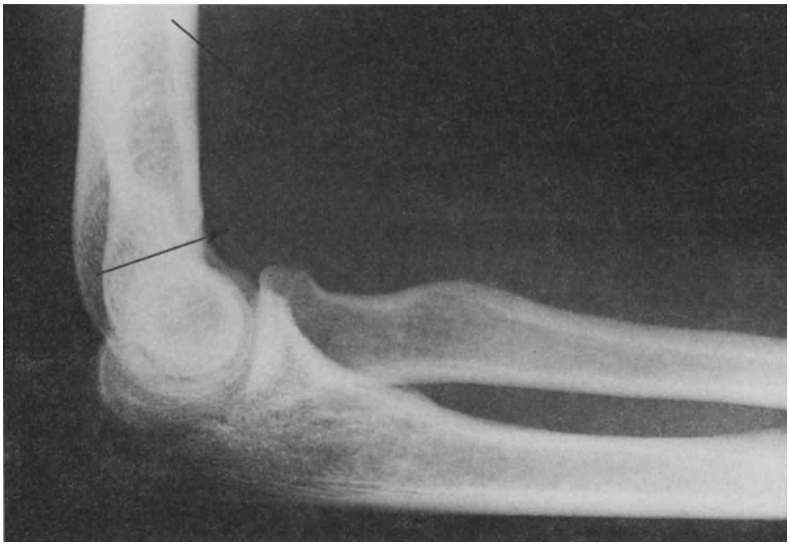

Figure 1. Plain film examination of the elbow, lateral view. Relative radiolucency is seen in the soft tissues anterior to the elbow (arrows), representing an area of less attenuation of the x-rays in the fatty tissues of a lipoma.

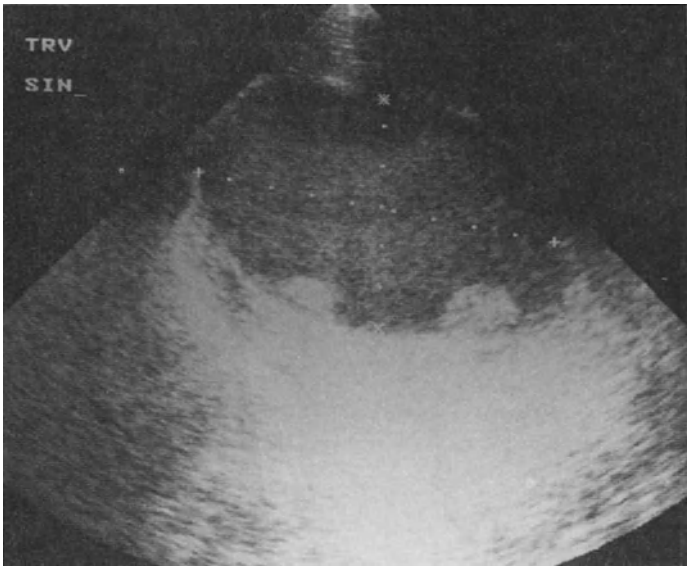

Figure 2. Ultrasound as a guide for biopsy: Malignant fibrous histiocytoma. At ultrasound examination, tumor nodules are seen adjacent to the capsule. The central area of homogenous echogenicity represents a large hematoma. If biopsy is performed, it should be directed towards the tumor nodules in the periphery.

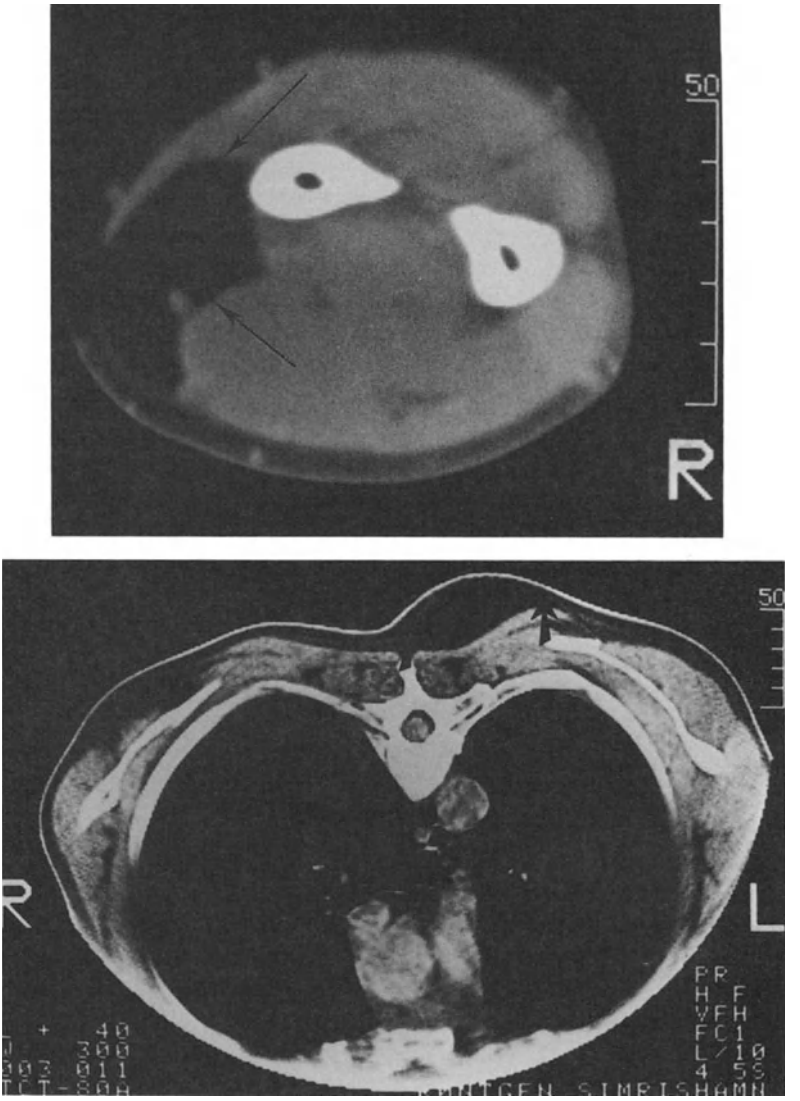

Figure 3. CT pattern; soft tissue lipoma. A: Subfascial lipoma, right forearm. The lesion is distinctly outlined by the adjacent flexor and extensor muscles of the forearm. It is transversed by a vessel but is otherwise of homogenous low density (arrows). B: Subcutaneous lipoma dorsally in the left chest wall. In this superficial location lipomas are less clearly seen, being isodense with the surrounding fat. A thin capsule surrounds the lipoma (arrows).

Occasionally, CT may reveal clues that lead to a tissue diagnosis. This especially concerns vascular tumors and lipomas (Fig. 3). If a tumor is homogenous, with a density varying between −60 and 100 Hounsfield units, the diagnosis of a lipoma is certain [44]. Otherwise, the density of soft tissue

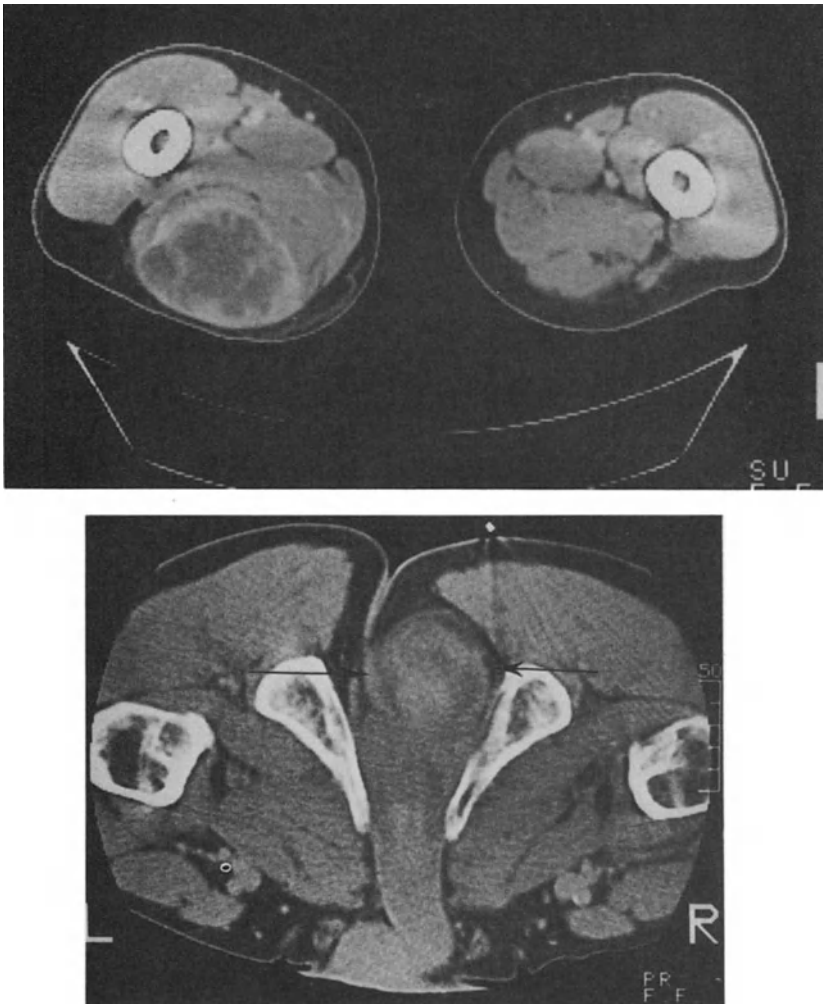

Figure 4. Two different types of soft tissue tumors with similar pattern at CT. A: Malignant fibrous histiocytoma in the biceps femoris muscle of the thigh. Note the irregular enhancement after contrast administration, suggestive of central necroses. B: Perineal angioleiomyoma — a benign smooth muscle tumor (arrows). The internal structure is similar to that of the lesion in A. In both cases the irregular contrast enhancement reflects the uneven vascular supply to the tumor.

tumors is not useful for distinguishing benign from malignant lesions. However, malignant soft tissue tumors tend to be more inhomogeneous, involve multiple muscles, have irregular borders, and blurr the adjacent fat, while benign lesions tend to be homogeneous, involve only one muscle, and do not blurr adjacent fat [28]. But in the individual case, CT will not be reliable for distinguishing malignant from benign lesions (Fig. 4). CT is,

however, helpful in guiding biopsy or aspiration of bone or soft tissue tumors.

At present, MRI is incapable of reliably distinguishing between most soft tissue tumors and allowing a specific diagnosis in soft tissue sarcomas. Also, with unenhanced imaging the signal intensities of viable tumor, tumor necrosis, edema, hemorrhage, and necrosis overlap [14,45]. MR imaging may, however, aid in the representative sampling of tumors following surgery by directing the pathologist to representative areas of the tumor [46].

The ability to obtain images in multiple planes often clarify anatomic relationships and facilitate anatomic localization of tumors. This is sometimes helpful in the characterization of lesions that occurs in specific anatomic locations or in relation to certain anatomic structures, such as tendons, for example, in synovial sarcoma [47].

Most musculoskeletal tumors have a similar appearance on MR images: low signal intensity (dark, about the same as muscle) on T1-weighted spin-echo images and high signal intensity (white) on T2-weighted images (Fig. 5). Exceptions to this pattern exist, and such exceptions may be a clue to diagnosis. Tumors with high signal intensity both on T1- and T2-weighted images may contain fat, hemorrhage, or slowly flowing blood. Conversely, lesions with low signal intensity on the T2-weighted images are probably acellular and contain mostly collagen, are calcified, or have a rapid blood flow. MRI has a low capability of detecting calcifications [48]. Although the T1 and T2 values of the tumor tissue may be calculated in each patient, there is growing experience that such values are of little or no value for suggesting a specific histopathologic diagnosis [33,34]. Occasionally, however, substances with special paramagnetic properties, such as melanin in clear cell sarcoma, will allow for tissue characterization on the basis of their T1- and T2-relaxation values [47].

At combined MR imaging and spectroscopy of bone and soft tissue tumors, no characteristic spectra have been found for the individual tumor types [9].

Internal structure

The presence of necrosis as demonstrated on contrast-enhanced CT examinations of untreated patients has been found to be an unfavorable prognostic sign in soft tissue sarcomas [29] (Fig. 6). Inversely, the most favorable prognostic indicator in patients with musculoskeletal sarcomas treated with intra-arterial chemotherapy is the development of necrosis. With the unenhanced spin-echo technique MR imaging cannot be used to predict the percentage of tumor necrosis [14], whereas contrast-enhanced MRI will be able to differentiate between tumor, necrosis, and peritumoral edema [1,4]. Preliminary data also suggest that MR spectroscopy in combination with MR imaging may be helpful in the evaluation of tumor necrosis as a response to

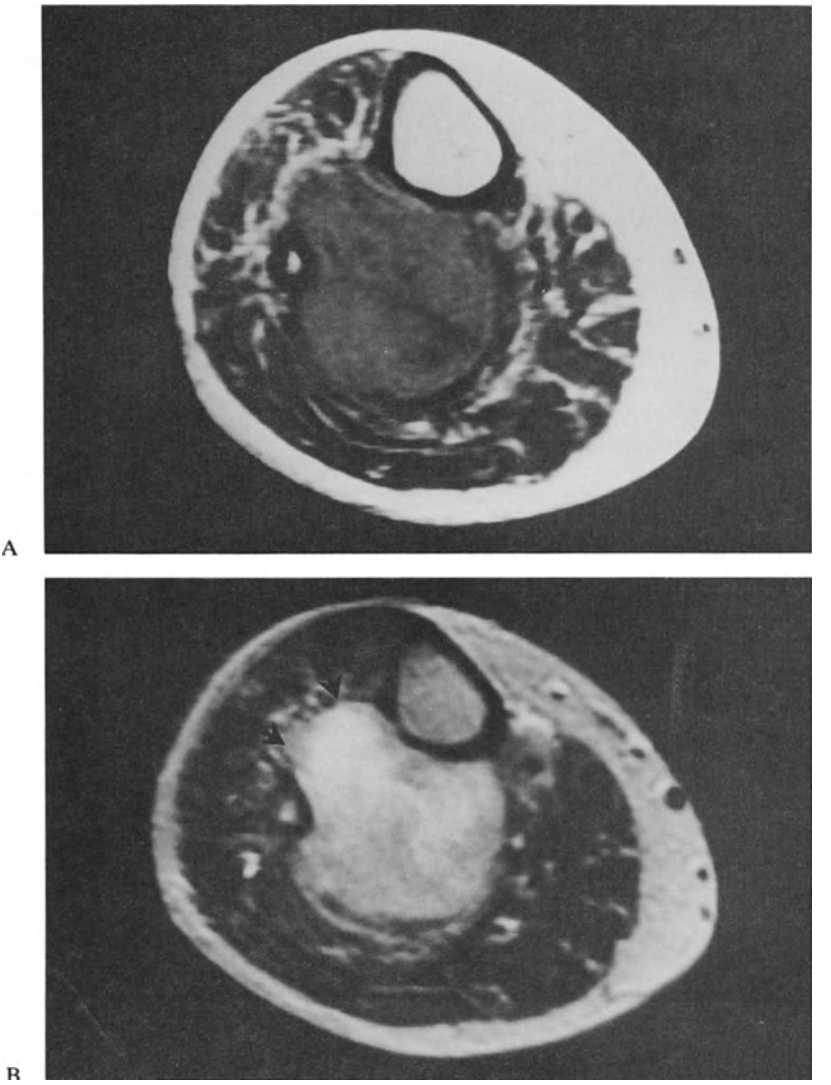

Figure 5. MRI of a malignant fibrous histiocytoma deep in the leg. A T1-weighted spin-echo sequence. The whole tumor has a low signal intensity. B T2-weighted spin-echo sequence. In this sequence the tumor has a high signal intensity, providing excellent contrast with the bone and surrounding muscles. The aggressive nature of the lesion can be inferred from the marked displacement of the interosseous membrane (arrows).

therapy in soft tissue sarcomas [8]. Ultrasonography and CT is helpful in directing preoperative fine-needle aspiration biopsy towards viable areas of the tumor [17]. MRI correlation may be helpful in the postoperative sampling of representative tumor areas in large tumor specimen [46].

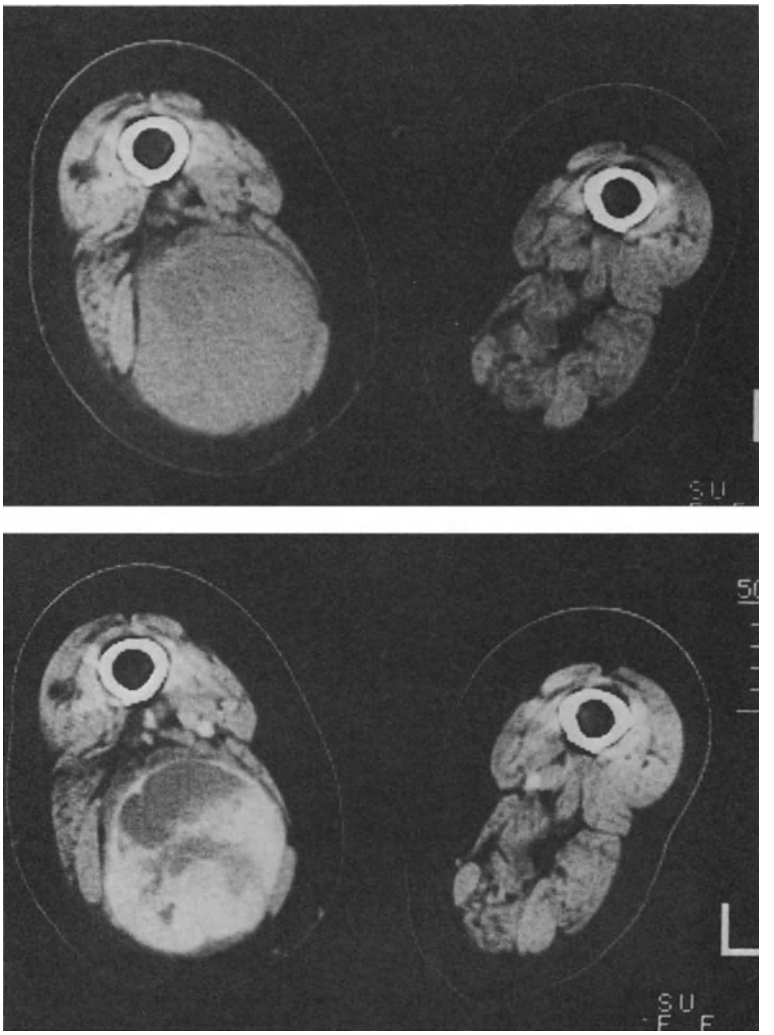

Figure 6. CT pattern; tumor necrosis. A: Nonenhanced CT-scan. A homogenous mass is present in the right semimembranosus muscle. B: After i.v. contrast administration, nonenhancing areas are clearly outlined, which on subsequent biopsy were found to represent necroses.

Local extent of the tumor

There is a strong positive correlation between tumor size and subsequent development of metastases in soft tissue sarcoma (Fig. 7), and with MR imaging or CT the tumor volume can easily be assessed. Evaluation of the local extent of the tumor is also of utmost importance for the preoperative planning. For this planning, as well as for purposes of surgical staging, the

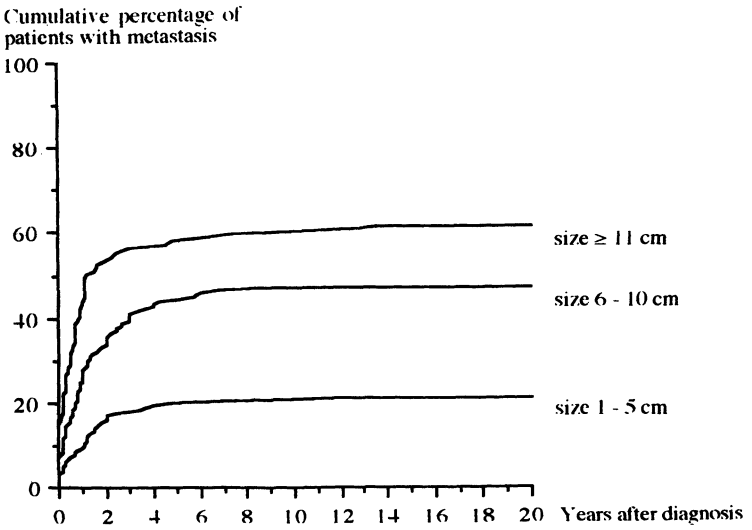

Figure 7. Cumulative percentage of patients with metastases in 477 patients with soft tissue sarcoma. (From Rydholm A, Gustafson P, Lund Orthopedic Sarcoma Group, Sweden. Reprinted with permission.)

extremities are considered to be divided into various anatomic compartments [49].

A compartment is an anatomic structure surrounded by natural barriers for tumor growth. Such barriers are the cortical bone and the periosteum, the epiphyseal plate, and the articular as well as the epiphyseal cartilage. In soft tissue, the muscle fascia is a barrier towards tumor growth. Therefore, each bone is considered a compartment, as are the major muscle groups. Also, an isolated muscle may be considered a compartment. Certain regions of the body have no natural barriers to tumor growth and are consequently classified as primary extracompartmental anatomic sites.

An important goal with the evaluation of the local extent is to identify the compartment of origin, to depict the extent of a tumor within the compartment, and to note if the lesion has escaped this compartment. Also, it is important to evaluate the relation between the tumor and neighboring large vessels and nerves, as well as bone.

CT is a good method for identifying the compartment of origin and the possible extension outside this compartment [50,51]. However, some tumors may be nearly isodense with the muscle. Artifacts, peritumoral edema, and inflammatory reaction around the tumor may blurr adjacent soft tissue planes, making tumor delineation difficult or impossible [28,52] (Fig. 8a). Even if the intravenous contrast medium may improve the tumor visibility, CT is inferior to MR.

MRI is an excellent method for the examination of soft tissue tumors

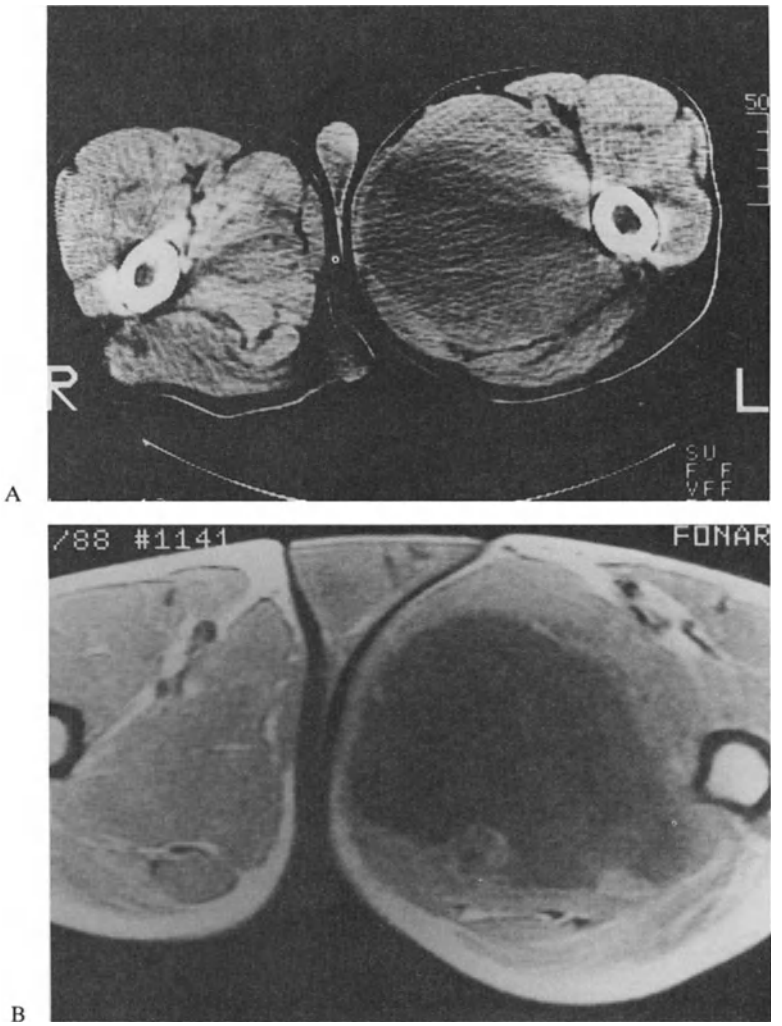

Figure 8. Internal structure and local extent using CT and MRI: malignant fibrous histiocytoma, left adductor magnus muscle in axial view. A: CT examination through the vertex of the lesion without contrast enhancement. A rather poorly defined soft tissue mass is seen in the compartment of the adductor muscles. Streak artifacts are caused by the dense cortical bone. B: T1-weighted spin-echo sequence, at the same level. A tumor capsule with irregular excrescenses is seen peripheral to a homogenous mass representing a hematoma. The outline of the internal structure of the mass allows for an optimal choice of the biopsy site.

because of its high soft tissue contrast resolution and facilities for imaging in multiple planes. There is no doubt that MRI today is far superior to any other method in defining the extent of soft tissue sarcoma (Fig. 8b) [32,48,53–56]. However, in certain cases it may be impossible to differentiate exactly between peritumoral edema, inflammatory reaction, and tumor using

SE sequences. For better differentiation, the use of intravenous gadolinium-DTPA has been tried (Fig. 9). With traditional SE sequences, the use of gadolinium-DTPA seems only to reveal the vascularization of the tumor and of the peritumoral zone, giving no additional information on the differentiation between these structures [4,34]. However, dynamic studies using FLASH-gradient-echo sequences may aid in the differentiation between tumor, edema, and normal tissue, as the edema has a slower gradual increase in signal intensity than the tumor [4].

Evaluation of the involvement of the great vessels and nerves is crucial, as this may determine if a limb-salvage procedure is possible or not. At CT, involvement of the neurovascular bundle is detected in most cases. For such evaluation, intravenous contrast medium should be administered, preferably as a bolus. This is especially important when small vessels are imaged [16].

MRI is superior to other imaging modalities, because the contrast between the tumor and the vessels is generally high [32,34,53]. Also, the possibility of volume imaging and subsequent reconstruction in planes of interest may add information concerning the relationship between the tumor and the neurovascular bundle.

Involvement of adjacent bone

Scintigraphy is rarely useful for the evaluation of bone involvement, but sometimes there is an uptake of the radionuclide, both in the bone and the soft tissue tumor. Increased bone uptake, or direct continuity between tumor uptake and the bone, is considered positive for bone involvement, whereas normal uptake between the tumor and the bone is negative [21]. The value of CT is limited when the tumor is situated very near the bone, but not invading it. Here beam-hardening artifacts can interfere with the determination, as can the false 'widening' of bone on soft tissue window settings.

MRI may be an accurate method for delineation of the tumor–bone relationship in some cases [48]. If there is a sheet of normal tissue between the tumor and the bone, the bone is obviously not involved, and there is cortical bone invasion when there is a tumor signal seen within the normal cortex that normally has no signal. When none of these conditions exist and the tumor is situated adjacent to the bone, MRI is not accurate in determination of tumor invasion [57] (Fig. 10).

Treatment response

A combination of MR imaging and MR spectroscopy holds promise for predicting the sensitivity to hyperthermia treatment and radiotherapy, and for monitoring tumor response. However, the utility of this method in a clinical setting awaits the results of study of larger patient groups and advances in spectroscopic techniques [9,14]. As stated above, gadolinium-

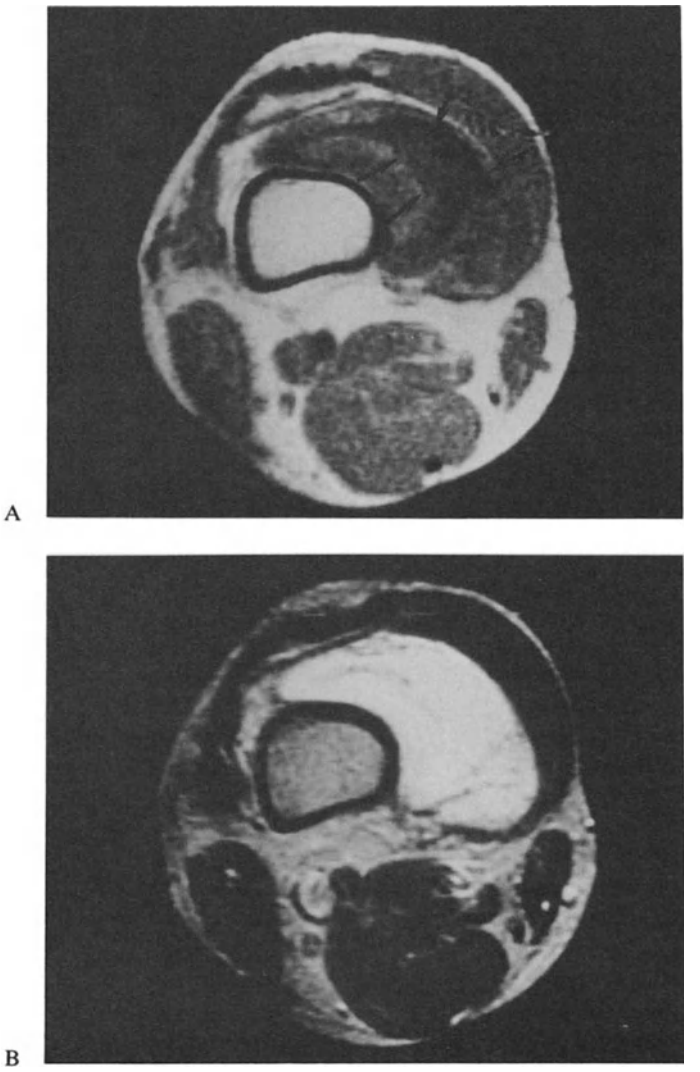

Figure 9

enhanced MR imaging is of value monitoring the tumor response, in the form of necrosis, to intra-arterial cytostatic infusion.

Tumor recurrence

Soft tissue sarcomas are known to have a high tendency for local recurrence, depending on the tumor type, size, grade, and location. The rate of local recurrence reported is as high as 90% after simple excision and 30% after

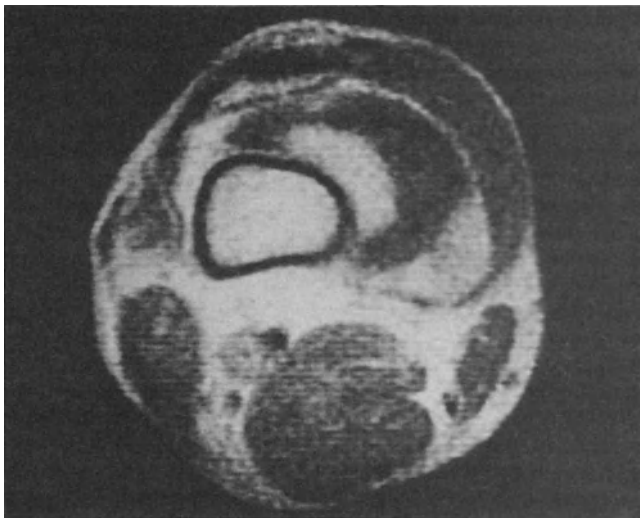

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Figure 9. Contrast medium enhancement at MRI: Extramuscular liposarcoma, right knee in axial view. A: T1-weighted spin-echo sequence. Note the central low signal area representing tumor necrosis (arrows). B: T2-weighted spin-echo sequence. The entire tumor has a high signal intensity, obscuring the central necrosis. C: T1-weighted Gd-enhanced spin-echo sequence. There is contrast enhancement of the vascularized portions of the tumor, providing better delineation of the tumor necrosis than in A and B, and better delineation of the tumor periphery than in A.

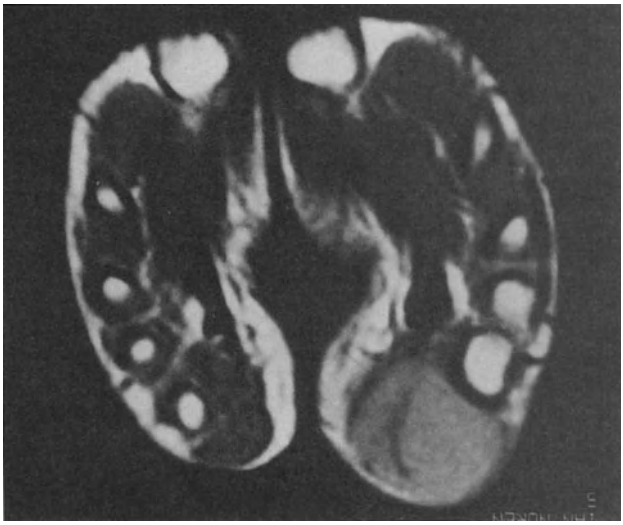

Figure 10. Alveolar rhabdomyosarcoma of the right hypothenar region. T1-weighted spin-echo sequence. A mass is evident adjacent to the cortex of the fifth metacarpal bone. However, on subsequent histopathological examination normal tissue was found interposed between the tumor and bone at all levels.

wide resection [2]. Clinical detection of these recurrent tumors is difficult, especially when the tumor recurs in the scar or deep tissue. MR is superior to CT to detect tumor recurrences because of the high contrast resolution [58]. MRI and sonography are approximately equally useful in the detection of local recurrence of soft tissue sarcoma, usually presenting as a nodular high signal intensity lesion on T2-weighted spin-echo sequences and a discrete hypoechoic nodule at ultrasonography. Sonography can be used for guiding needle biopsies at the examination, but may be more difficult to interpret than MR during the early postoperative period [17].

Tumor metastases

Pulmonary metastases are the most common ante-mortem recognized manifestations of generalized disease in soft tissue sarcoma. Plain films of the chest and/or CT are the modalities of choice to demonstrate these lesions. Rarely, skeletal metastases are recognized on ante-mortem studies, whereas they are more frequently noted post mortem. This may be due to a low sensitivity of plain film examinations and of radionuclide studies in the detection of these lesions compared to MR imaging [59].

Conclusions

The development of new imaging modalities has meant a dramatic improvement in the radiologic evaluation of musculoskeletal tumors, and among those, also soft tissue sarcomas. During the last few years, several controlled studies have been published on the accuracy of the different imaging modalities, and there is a consensus that MRI is superior to all in the evaluation of soft tissue tumors. At present, a specific diagnosis usually cannot be made be means of diagnostic, non-interventional imaging of soft tissue sarcomas. In the near future, refinements in the techniques of dynamic contrast-enhanced magnetic resonance imaging and magnetic resonance spectroscopy can be anticipated to enable in vivo tissue differentiation and monitoring of therapy response in these tumors [3,4,7–9,34,53–56].

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5. Surgical wound healing

Dempsey S. Springfield

Surgeons usually take wound healing for granted. We assume that after the tumor has been removed and the wound closed the patient will heal the wound within a week or two, and that the tissues will be restored with only a scar and the absence of resected tissues, indicating that an operation has been done. For the most part, wound healing can be taken for granted, and it is unusual for a patient to have difficulty healing a clean, elective operative wound unless there is another condition compromising wound healing. There are a number of known conditions that compromise the ability of a patient to heal a wound, including: excessive trauma to the tissues during surgery, inadequate vascularization of tissues due to pre-existing vascular disease or poor flap design, a postoperative wound infection, extreme nutritional deficit, and most importantly for the surgical oncologist, prior irradiation or concomitant chemotherapy.

Wound healing has been studied for as long as there have been surgeons, and our understanding of how wounds heal is reasonably complete [1–3]. The sequence of events that occur as a wound heals is known. The cell types necessary for wound healing are known, their actions are understood, and the effect of their absence on wound healing have been defined. In addition, the materials that the cells must produce for all the events of wound healing to occur have been analyzed. The one major aspect of wound healing that needs to be more thoroughly explored is how the different cell types and individual cells are controlled so that they arrive at the wound at the proper time, produce just the right amount of material or do whatever job is required, and then disappear. Recent research has revealed that growth factors are important in this regulation process, and it is likely that as we understand the actions of the numerous growth factors we will gain insights into how the actions of the individual cells are orchestrated.

The effects of irradiation and chemotherapy on healing wounds has become of increasing interest to the surgical oncologist. This is due to the fact that during the past two decades, the use of radiation and chemotherapy in conjunction with surgery to treat malignant tumors has greatly increased. As surgical resections were combined with irradiation and chemotherapy, especially when these adjunctive agents were used in the preoperative period,

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wound healing problems increased, and it became clear that surgical wounds made in patients who received irradiation or chemotherapy needed special attention if they were to heal without complications. These wound-healing problems have lead to renewed interest in wound-healing biology and, more specifically, in how irradiation and chemotherapeutic agents alter wound healing. This chapter will discuss the healing of normal tissues and how irradiation and chemotherapy affect the normal healing process.

Normal wound healing

Complete healing of a clean surgical wound takes at least 2 years. During this time the wound goes through the process of joining the tissues that were separated during the operation, building strong bonds between the once separated tissues, and then remodeling these bonds to produce a mature scar. A wound immediately after it has been made has no strength against separation, but at the end of healing process, some 2 years later, it will have its maximal strength, although maximum strength is only 80% normal (Fig. 1). The strength of the wound (measured by stressing it to failure) remains at this level indefinitely, never reaching the strength of nonwounded tissues.

It is convenient to think of the healing process as taking place in three stages. These three stages are not separated by definite events, but overlap in time and activity as one stage merges into the next. Dividing the healing process into three stages makes it easier to understand what happens as a wound heals. The three stages are referred to as 1) inflammatory, 2) proliferation, and 3) remodeling. Each of these stages and all parts of each

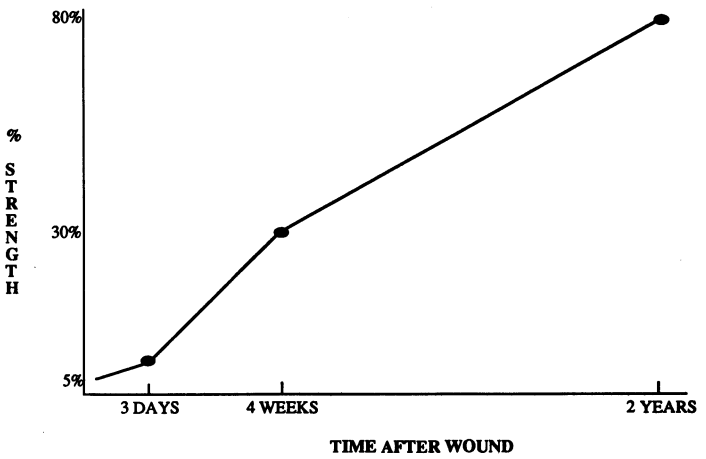

Figure 1. The wound only gains a minimal amount of its strength early, but this is sufficient to hold the tissues together. It increases in strength quickly after 3 weeks but takes 2 years to gain its maximal strength. The maximal strength is only 80% of the normal strength of skin.

stage must be completed if the wound is to heal properly, and anything that interferes with even the smallest step in the healing process will result in abnormal wound healing, usually manifested clinically as a delay in the rate at which the wound achieves its strength. When the delay is minimal and the wound is not stressed, no clinically observed wound-healing problems occur; however, when the delay is significant or when the wound is stressed by a large seroma or excessive activity by the patient, the wound will dehisce or the resultant reduction in the wound's bacteriostatic quality will lead to an infection. Either of these two latter problems results in a significant postoperative complication for the patient, and the wound-healing morbidity makes management of the patient more difficult. More importantly, a postoperative complication often interferes with subsequent therapy and reduces the chance for cure.

Inflammatory stage

The inflammatory stage of wound healing begins as soon as the wound is closed and continues for approximately 4 days. During these 4 days the wound is infiltrated with platelets and acute inflammatory cells, which are critical for the initiation of wound healing. The initiating event in the inflammatory stage is the exposure of normal pre-existing collagen bundles to blood products that were released into the wound by bleeding capillaries. This interaction between collagen bundles and free blood products initiates the coagulation cascade and specifically stimulates the release of thromboplastin, which leads to clotting of the blood.

This clot has a high concentration of platelets, which are critical to the next phase of wound healing; anything that significantly reduces the number of platelets or that interferes with platelet function will lead to abnormal wound healing and increased wound-healing complications. The platelets provide platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β), two of the important regulating materials necessary for completing the early phase of wound healing.

Once the clot has formed and platelets have released their growth factors, vasodilatation of the local vascular bed begins and the number of monocytes, polymorphonucleocytes (PMNs), and lymphocytes in the wound increases dramatically. The PMNs and lymphocytes do not seem to have a direct role in wound healing, but they reduce the number of bacteria present and, presumably, the incidence of wound infections, obviously an extremely important function. The monocytes, on the other hand, have a direct role in wound healing and are critical to the wound-healing process. Without the monocytes, the wound would not gain the strength necessary to be clinically healed. After the monocytes infiltrate the wound, they become macrophages and ingest the local debris, while at the same time releasing growth factors that stimulate mitogenesis and angiogenesis, as well as stimulating local fibroblasts. The fibroblasts are stimulated to increase their production of

collagen, the material necessary for the wound to regain its final strength. It seems that monocytes are responsible for the control of collagen production, and they control collagen production through their regulatory control of fibroblastic activity. In the inflammatory stage of wound healing, only minimal amounts of collagen are produced, and during the first few days the wound is held together by a fibrin clot. The tensile strength is less than 5% of normal during the first few days.

Proliferative stage

On days 2 or 3 after wounding, the proliferative stage begins even before the inflammatory stage ends. This stage lasts for at least 3 weeks, and during this time the wound gains considerable strength. We usually remove sutures during the middle of this stage, and it is towards the end of this stage that the patient considers the wound healed. The major event of this stage is the accelerated production of collagen and the early maturation of that collagen from collagen fibrils with limited crosslinks to fibers with many crosslinks. Crosslinking of collagen fibrils requires the formation of aldehydes from the E-amino group of hydroxylysines and lysines on the polypeptide chains. Although the increased amount of collagen is necessary for the wound to strengthen, it is the crosslinks that provide the principal strength characteristic of the collagen. It is the increased number of crosslinks that occur with maturation that account for the majority of the increase in wound strength with maturing of the wound. Collagen production can be considered the end product of all events associated with wound healing, as it is the final and most important material needed for wound healing. The production of collagen must be continuous because even as the wound heals the normal, constant breakdown of collagen also continues.

Early in the proliferative stage, angiogenesis, initiated by the monocytes during the inflammatory stage, is further stimulated by both the local conditions (local tissue hypoxia) and the continued presence of monocytes. As the new vessels grow into the hypoxic healing wound, the oxygen level increases and the stimulus for additional new vessels is decreased. Fibroblasts continue to be stimulated to produce collagen, and their production of collagen increases with maturation of the wound. In addition, during this phase of the proliferative stage myofibroblasts appear. It is not clear what causes the myofibroblasts to appear, but they are stimulated by mechanical stress to produce contractile proteins containing actin and myosin. They will produce increasing amounts of their contractile protein as the wound is subjected to stretch. The contractile proteins bring the wound together and reduce the distance that needs to be filled by the newly formed collagen.

Collagen production peaks on or about day 7 after wound closure, but continues at this rate for 2–4 weeks. During this time the wound's strength increases dramatically. While the total amount of collagen increases some (more collagen is produced than destroyed), the increase in total collagen is

insufficient to explain the increase in the strength of the wound. As previously mentioned, most of the increase in strength observed after day 7 is due to the increased number of crosslinks within the collagen. The increased crosslinking is a function of collagen maturation, and only when the collagen is maximally crosslinked does it have its maximal strength. The tensile strength of the wound is only about 30% of normal by the fourth week, indicating that crosslinking is far from completed.

Remodeling stage

The remodeling stage begins around the third week and continues for at least 2 years. During this stage the wound gradually regains most but not all of the strength of normal tissues. Although the gain in strength is, for the most part, due to further maturation of collagen with additional crosslink formation, continued collagen production by fibroblasts is required because collagen breakdown continues. As the collagen matures and the crosslinks increase, the tensile strength of the wound increases from approximately 30% to 80% of normal skin, but the wound never returns to the strength of normal skin.

Effect of irradiation on wound healing

Total dose, rate of administration, and timing of irradiation are all important variables determining the effect of irradiation on the wound-healing process. Irradiation given in therapeutic doses administered after the wound is 3 or 4 weeks old must have an effect on the remodeling stage of wound healing, but its effect is not observed clinically and, in general, the effects of irradiation administered after the wound has completed the proliferative stage are not clinically significant.

On the other hand, irradiation administered in the more immediate postoperative period will slow the healing process and can result in clinical wound-healing problems. In the rat, 1800 roentgens given immediately after wound production decreased the strength of the wound and delayed fibroblasts proliferation, but when given 24 hours after the production of the wound it did not seem to alter the wound-healing process [4]. Others agree and from their work have suggested that in experimental animals the first 24–48 hours after wound production are the most critical hours with respect to successful wound healing. The clinical observation is that wound-healing problems in patients who receive their irradiation in the immediate postoperative period, usually administered as interstitial irradiation (brachytherapy), have more complications than those who receive their postoperative irradiation later. In a series of patients reviewed by Arbert, there was only a 3% incidence of wound-healing complications in patients whose irradiation was given with an external beam after the wound had completed the proliferative stage of

healing, compared to an incidence of 22% in patients who received their postoperative irradiation within the first week with interstitial implants [5]. It seems safe to assume that at 5–7 days after wound production, irradiation can be administered without significantly altering the clinically observed healing process.

Irradiation administered later than a few days after wound production must have an effect on the fibroblasts and their collagen production, but this effect has not been observed clinically. When there is a delay in wound healing due to a postoperative infection or dehiscence, the effect of irradiation will be to prevent further healing, but despite this, the author has seen postoperative irradiation given to patients with an open wound without complications, but a skin graft was eventually required. As long as the wounds were kept dry and clean, no adverse effects have been noted, but the wounds did not heal by secondary intention during or after the irradiation, as one would expect if the wounds had not been irradiated. These unhealed wounds were managed by applying a split-thickness skin graft after completing the total dose of postoperative irradiation.

Preoperative irradiation has an effect on wound healing, except when it is administered in very low does. The effects are observed whether the irradiation is given as a single dose or in multiple doses. In experimental animals, a single dose of 300 rads given in the preoperative period did not alter wound healing, but doses over 1000 rads given as long as 3 weeks before wound production slowed wound healing measurably [4]. This is what we would expect based on our clinical observations. Numerous authors have reported significant wound-healing problems in patients who have received preoperative irradiation, and our own experience agrees with this [6,7].

The two common clinical situations in which preoperative irradiation has been given are 1) when patient has received irradiation 6 months or more prior to the operation and the operative incision is to be made through the previously irradiated tissues and 2) when the patient has received planned preoperative irradiation during the month preceding when the tissues are to be operated on. There have been few experimental studies in animals examining the effect of irradiation given months to years before wound production. Our clinical experience is that wound-healing complications are more common in patients who have received their preoperative irradiation within a few weeks to months prior to surgery, compared to patients whose irradiation was more than 6 months prior to surgery.

The available research and limited clinical observations suggest that irradiation given within a few weeks before wound production and that given immediately after wound production have similar effects on wound healing [4,5,8,9]. The studies that have been done indicate that irradiation given from up to 12 weeks before wound production until immediately after wound production results in a decreased rate of wound healing, as measured by the rate at which the wound regains its tensile strength. The

most pronounced effect is observed when the irradiation was given either immediately before wound production or immediately afterwards [4,9–11]. These wounds eventually regained the same tensile strength as the control wounds, but the irradiated wounds were significantly weaker for the first 3 weeks, after which the tensile strength becomes equal. These studies did not demonstrate histologic changes explaining the reduced rate of wound healing, and it is assumed that the effect of irradiation is on cellular function rather than cellular morphology. The ability of the cells to proliferate may also be diminished, adding to the delay in wound healing.

We do not know exactly how the recent preoperative or immediate postoperative irradiation damages the ability of the wound to heal, but it seems to be a direct effect of irradiation on the fibroblasts [12,13]. Irradiated fibroblasts do not function properly. They do not produce sufficient collagen, or the collagen produced does not mature quickly enough to keep up with the demands of the healing wound. This results in a wound with reduced tensile strength at any given time. The collagen produced by the irradiated fibroblasts probably is eventually normal, as the wounds do regain normal wound strength, but it takes twice as long for an irradiated wound to regain its strength compared to a non-irradiated wound. The effects of irradiation on fibroblasts seems to be permanent.

A wound produced in tissue irradiated months to years previously also has an altered wound-healing capacity. The irradiated tissues are usually abnormal in appearance and to palpation. The skin and subcutaneous tissues are atrophic, and the tissues are less pliable than normal. This tissue will have a higher incidence of wound-healing complications than normal tissues. The most common explanation offered for the changes in the appearance and the difficulty that this irradiated tissue has in healing is the lack of adequate blood flow, with resultant tissue hypoxia [14]. Tissue hypoxia has been suggested as the cause of reduced healing because of the observed vascular changes seen in irradiated tissue. The capillaries are often occluded and vessel walls are thickened.

Recently other factors have been suggested, and it is now not clear what role the observed vascular changes play [15]. Tissue oxygen tension in irradiated tissues in a resting state seems to be normal and the tissue is not hypoxic, as had been predicted, but it is not known whether the irradiated tissue can respond normally to the increased oxygen demands after the production of a wound. Relative tissue hypoxia may be present in the irradiated tissues during healing, although this has not been shown to be the case. Thus currently the importance of the observed vascular changes on wound healing are not known.

What has been demonstrated is that the fibroblasts that are injured by irradiation do not recover all of their abilities, even years after being irradiated, and it is suspected that the fibroblasts are permanently altered and that the alterations are transmitted to the daughter fibroblasts [15]. This suggests that the effect of irradiation is on the fibroblast's DNA and is a permanent

alteration. The permanently damaged fibroblasts are more likely to be the cause of both the altered tissue characteristics and the delayed wound-healing ability of the previously irradiated wound [15,16]. Fibroblasts harvested from irradiated tissue of humans who had received therapeutic irradiation from 18 to 2 years previously had slower growth rates in vitro, longer doubling times, and reduced plating ability [14]. Even after numerous passages in tissue culture, the fibroblasts demonstrated these same defects. Fibroblasts harvested from the same patient but outside the irradiated field behaved normally. The cause of this reduction in fibroblast function is not known. It may be the result of damage to the fibroblast's ability to multiply, damage to the ability of the fibroblasts to produce collagen, or a reduction in the fibroblast's response to the stimulatory effects of local growth factors. Any of these mechanisms would result in the observed wound-healing delays. Before a complete understanding is available, more research must be done.

Effects of systemic chemotherapy on wound healing

It is increasingly common for patients who have a musculoskeletal tumor to have chemotherapy before surgery. The most important thing for the surgeon to know is the drugs used, when the last dose of chemotherapy was administered, how the patient has responded to prior doses of the same drug, and the patient's white cell count, and more specifically, the absolute number of polymorphonucleocytes. The most common side effect of chemotherapy is neutropenia, and a surgical procedure performed just before the patient's white cell count reaches its lowest number is dangerous. Usually this occurs between 10 and 14 days after the administration of the drugs, and so it is usually safe to do an operation 2 weeks after the administration of chemotherapeutic agents. It has been our experience, although not prospectively studied, that patients with a neutrophil count of 500 or greater have sufficient polymorphonuclear cells to have a normal postoperative course, heal surgical wounds without difficulty, and do not have an increased risk of infection.

The effects of chemotherapeutic agents on wound healing have been studied extensively in experimental animals, and it has been assumed that the findings in these animals (usually mice or rats) is applicable to humans. Although the effect of drugs is somewhat different, the effect of chemotherapeutic agents on wound healing, as is the case with irradiation, is related to the type of drug administered, the timing of drug administration in relation to wound production, and the dose of drug administered. In general, the higher the dose and the closer it is given to wounding (especially in the immediate postoperative period), the greater the effect. Again, as is the case with irradiation, the effect of chemotherapy is to reduce the rate at which the wound regains its strength as compared to wounds in subjects not

receiving chemotherapy. In contrast to the effects of irradiation, the effects of chemotherapeutic agents on wound healing are transient and thus a few weeks after the administration of chemotherapy wounds heal normally [17].

At one time it was thought that the effect of chemotherapy on wound healing was due to the associated weight loss (reflecting a negative nitrogen balance) and anemia. Recent experimental studies have revealed that weight loss is not associated with reduced wound healing unless the animal has lost more than 15% of its body weight [18]. This amount of body weight loss is unusual in patients receiving chemotherapy, and it is unlikely that the clinically observed wound healing delays are related to the patient's weight loss. In experimental studies of chemotherapy's effect on wound healing, the animal's weight loss has been kept below this figure and, despite keeping the animal's body weight up, chemotherapy does adversely effect wound healing; therefore, weight loss is not sufficient to explain the effects of chemotherapy on wound healing. Anemia has also been shown not to adversely affect wound healing [18]. Thus, neither weight loss nor anemia is an explanation of the effects of chemotherapy on wound healing.

The mechanism of action of the chemotherapeutic agents has not been fully explored. It is suspected that the drugs interfere with cell division and cellular functions through their actions on the reparative cell's DNA. The eventual results of these effects seems to be reduced production of collagen, but whether this is due to a reduction in the number of monocytes, a reduced number of fibroblasts, a reduction in the function of monocytes or fibroblasts, or some combination of these effects is not known.

In their review of the effects of chemotherapeutic agents on wound healing published in 1981, Shamberger and associates suggested that although the effects of chemotherapeutic agents on monocytes had not been demonstrated, suppression of monocytes was a potential site of action of chemotherapeutic agents, particularly corticosteroids [19]. They also state that the reduced production of collagen, demonstrated in animals receiving Adriamycin or corticosteroids, was the primary defect measured and the cause of the reduced wound healing. Because collagen production is one of the last steps in wound healing, any of a number of prior steps could be the specifically affected process producing altered collagen production. Thus, as with irradiation, the exact mechanism by which collagen production is altered is not known.

Devereux and associates studied wound healing in rats receiving Adriamycin and found a reduction in wound tensile strength when the Adriamycin was given on the day of wounding [20,21]. Their study revealed that the histologic appearances of the wounds in rats who had received Adriamycin were identical to those of rats who had not received Adriamycin, but the amount of collagen was less. They found that the reduction in total collagen was due to a reduction in collagen production, not an increase in collagen

Table 1. Effects of chemotherapeutic agents on wound healing

	Adverse effect on wound healing	
Chemotherapeutic agent	Yes	No
BCNU	X	
5-fluorouracil		X
6-mercaptopurine		X
Actinomycin-D	X	
Adriamycin	X	
Azathioprine		X
Bleomycin	X	
Cisplatin	X	
Corticosteroids	X	
Cyclophosphamide	>150 mg/kg	<100 mg/kg
Methotrexate	X (leucovorin reverses)	
Mitomycin-C		X
Nitrogen mustard	X	
Thio-tepa		X
Vincristine		X

The effect is greatest when the drug is given just after wounding, and, in most cases, if the wound is allowed to heal for 7–10 days the drug will have no clinical effect. Adriamycin is unique because when it is given to a patient who has had irradiation, the tissue previously irradiated will become inflamed. This effect has been referred to as a recall effect.

degradation. Reduced production has been suggested by others as well [22,23]. In all likelihood the reduction in collagen production is a final common pathway of the effect of chemotherapeutic agents on wound healing strength, although each agent's specific effect may be different.

Table 1 summarizes the effects of different chemotherapeutic agents on wound healing. Further research is needed to better understand how these agents affect wound healing, but currently we assume that their action is through alteration of cellular DNA function to produce reduced collagen production.

Adriamycin has been studied the most, and its effect is probably the most clinically significant. Adriamycin will reduce wound healing strength if given within the week before or the week after producing the wound [18,19]. A surgical procedure should not be done within this 2-week period, but before or after this time frame wounds will heal normally. Adriamycin probably directly interferes with fibroblasts function, as it has an additive effect to the adverse effects of irradiation. In addition, Adriamycin has a recall effect on irradiated tissues. It is not known why or how this works, but the clinical observation is that a patient who has received radiation and is given Adri-

amycin may develop an inflamed and even blistering reaction on the skin of the previously irradiated treatment ports. This observation suggest that the irradiated tissues are more sensitive to Adriamycin than non-irradiated tissues [22,24]. Patients receiving irradiation and Adriamycin at the same time will also have increased sensitivity in the tissues irradiated, but this phenomenon is not observed when the irradiation is administered more than 1 week after the Adriamycin has been given. As has been stated previously in this chapter, it is thought that the irradiation produces permanent alterations in the fibroblast's DNA, and therefore the Adriamycin must potentiate the effect of these alterations. With the exception of Adriamycin, other chemotherapeutic agents do not exhibit this recall phenomenon, so their specific mechanism of action is probably not the same as that of Adriamycin.

All of the chemotherapeutic agents probably have a dose-dependent effect, with a critical dose with respect to wound healing. The dose of cyclophosphamide at which adverse effects are observed has been found [18,19]. Cyclophosphamide causes abnormal crosslinking in DNA, and when given in sufficient doses will reduce fibroblast proliferation. Probably by reducing the number of fibroblasts in the healing wound, cyclophosphamide slows the wound-healing process. When it is given in doses equal to or below 100 mg/kg no adverse effect is observed, but at higher doses given during the first week after wound production, it will reduce the rate at which the wound regains its strength.

Methotrexate slows wound healing slightly, and when the tensile strength of a wound in an animal that received methotrexate at the time of wound production is tested within the first week after wound production, there is a reduction, but the effects of the methotrexate is transient and when tested 3 weeks later the strength of the wound is normal [18,19]. The effects of methotrexate on wound healing are not usually a clinically significant factor.

Corticosteroids have a clinically significant effect on wound healing [18]. If given early (within the first 3 days after wound production), they will reduce the strength of the wound, but if given more than 11 days after wound production, they increase wound strength. The dose of corticosteroids is also critical, and in the rat doses of less than 5 mg/kg/day of cortisone acetate will produced no adverse effect but higher doses will [18,19]. As is the case with other chemotherapeutic agents, the reduced wound strength associated with corticosteroids is due to reduced collagen production by the healing tissues.

Nitrogen mustard, actinomycin-D, bleomycin, BCNU [1,3-bis (2 chloroethyl) 1 nitrosourea], and cisplatin have adverse effects on wound healing. The details of their effect have yet to be evaluated and little is known about their actions as related to wound healing. Thio-TEPA (triethylenethiophosphoramide), 5-FU (5-fluorouracil), azathioprine, 6-MP (6-mercaptopurine), vincristine, and mitomycin C have no known adverse effects on wound healing.

Clinical observations

Irradiation and wound healing

There are numerous clinical reports of the effects of preoperative irradiation on wound healing [5,7,25–28]. All report that there is an increase in wound-healing problems, but there are no controlled studies and it is difficult to know how many are due to the irradiation and how many are due to the magnitude of the surgery. We have reviewed a consecutive series of 202 of our own patients treated with preoperative irradiation between 1971 and 1989 [6]. These patients received a preoperative mean dose of 47.5 Gy, and 71% received a postoperative boost of around 15 Gy. The routine was for the patient to receive external beam megavoltage irradiation as a single daily treatment of either 1.8 or 2.0 Gy over a 5-week period. The patient was then given a 2- to 3-week break in treatment before having a surgical resection. Those patients who received a postoperative boost began the therapy between 2 and 4 weeks after surgery and completed it in 10–15 days. Thirty-six patients had their postoperative treatment administered with implant brachytherapy. Seventy-four (37%) patients had some type of wound-healing morbidity. One patient died in the immediate postoperative period due to a wound infection. Thirty-three (16%) required surgical

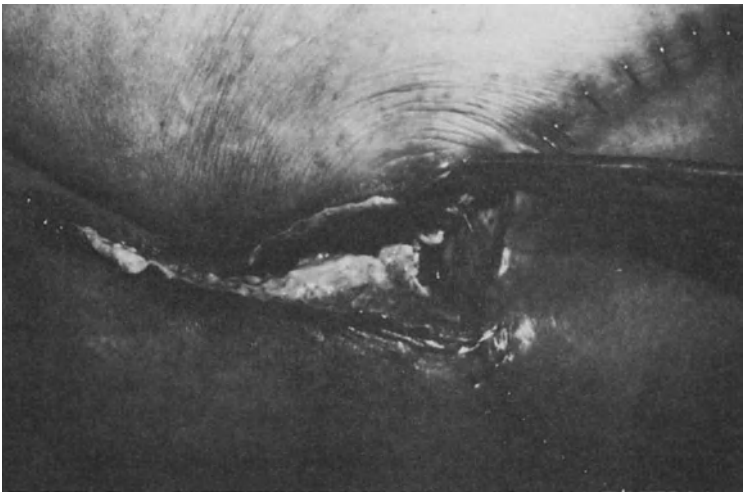

Figure 2. A patient who had preoperative irradiation (50 Gy) and developed a major postoperative wound-healing problem. As usual, the wound appeared to hold well for the first week but then opened and began to drain. A wound hematoma and the lack of well-vascularized muscle deep to the subcutaneous tissue is associated with an increased incidence of major wound-healing complications. This patient required a surgical debridement and a free muscle flap before the wound was successfully closed.

treatment of their wound complication, including 6 amputations, 19 reconstructions, and 8 debridements with secondary closures (Fig. 2). The other 40 patients were treated without surgery, usually with observation alone (Fig. 3). Three patient-related parameters were found to be associated with an increased risk of a wound-healing complication: 1) location of the tumor, with tumors in the lower extremity having a higher incidence of complications; 2) age, with patients over 60 years of age having a higher incidence of wound-healing morbidity than younger patients; and 3) pathologic grade of tumor, with grade III (highest grade) having the highest incidence of wound healing morbidity. Two treatment-related variables were found to be associated with increased wound healing morbidity: 1) greater than 100 ml of blood lost with the surgical resection and 2) twice-a-day preoperative irradiation. The larger tumors had a tendency to be associated with increased wound-healing morbidity, as did postoperative brachytherapy, but neither of these variables reached statistical significance.

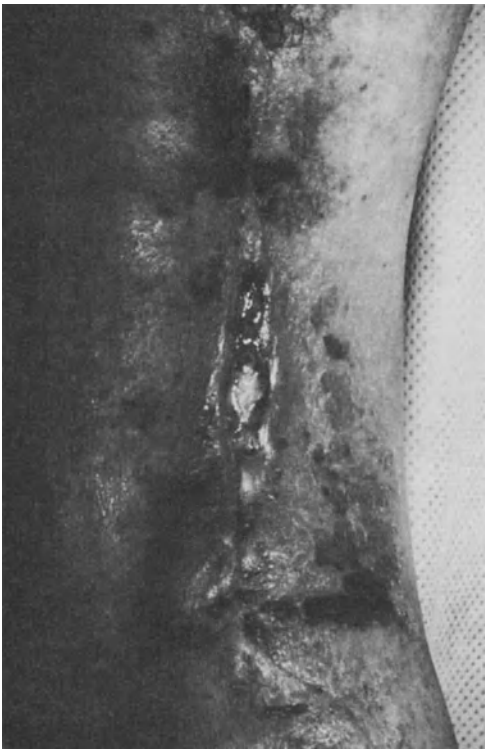

Figure 3. A patient who had received preoperative irradiation (50 Gy) and who had a minor wound complication 4 weeks postoperative. No surgical debridement was done and the wound healed within 2 months. This type of wound complication is the result of skin edge necrosis in a wound with good deep tissues and without a residual deep hematoma.

During the 18 years of the study many aspects of the treatment protocol changed, and the radiation oncologist and surgeons gained experience with patients receiving preoperative irradiation and the special problems of their surgical wounds. Despite these changes and a perception that the incidence of wound-healing morbidity decreased over the 18 years, the incidence of complications was unchanged from 1971 to 1989. It is surmised that the lack of evidence of our perceived improved healing rate is a reflection of an increase in the use of the combination of irradiation and surgery in the management of soft tissue sarcomas. In the 1970s patients selected for the combination of preoperative irradiation and surgery tended to have small- to moderate-sized soft tissue sarcomas in a favorable anatomic location, while those with very large tumors or those whose tumors were in difficult anatomic locations were treated with amputation. Through the years with our success in controlling these soft tissue tumors, and at the same time, in saving functional extremities, we have broadened the criteria, so that now it is the very rare patient who is felt not to be a candidate for the combined treatment of irradiation and limb-salvage surgery. Currently, we are attempting to combine preoperative irradiation and limb-sparing surgery for patients who we would not have even attempted 18 years ago. Thus we have kept the incidence of wound-healing problems about the same while increasing the difficulty of the cases.

We believe the relative low incidence of wound-healing complication is due to better planned preoperative irradiation, technical aspects of the surgical procedure, and the postoperative care. The irradiation is planned so that a minimum of normal uninvolved tissue is irradiated, as wide a strip of skin and subcutaneous tissue as possible are spared from irradiation, and only one treatment a day is given. Occasionally for large tumors in areas with a particularly high risk of wound complication (buttocks and groin), only 20 Gy is given preoperatively and the majority of the dose is given after the surgical resection. At the time of surgery the irradiated tissues are handled carefully and skin flaps are avoided. Whenever possible the fascia and underlying muscle are left attached to the skin and subcutaneous tissue as they are reflected from the tumor. After the tumor and a small cuff of surrounding tissue has been removed (increasingly less and less surrounding tissue is being removed with the irradiated tumors), all potential 'dead' space is filled. When local tissue is not available to fill this 'dead' space, local muscle pedicle flaps or even free muscle pedicle flaps are used to provide well-vascularized tissue to fill the defect. Split-thickness skin grafts can be used when necessary, but should be placed on viable muscle (Fig. 4). All the wounds are drained and the drains are left in place until there is minimal drainage (<10 ml/24 hrs). Patients with lower extremity tumors are not allowed out of bed for at least 7 days and often not until 10 days after surgery. Rehabilitative activities are begun slowly after 2 weeks. We recommend slow mobilization of the patient to allow the wound additional time to start its healing. We believe this protects the wound from dehiscence and

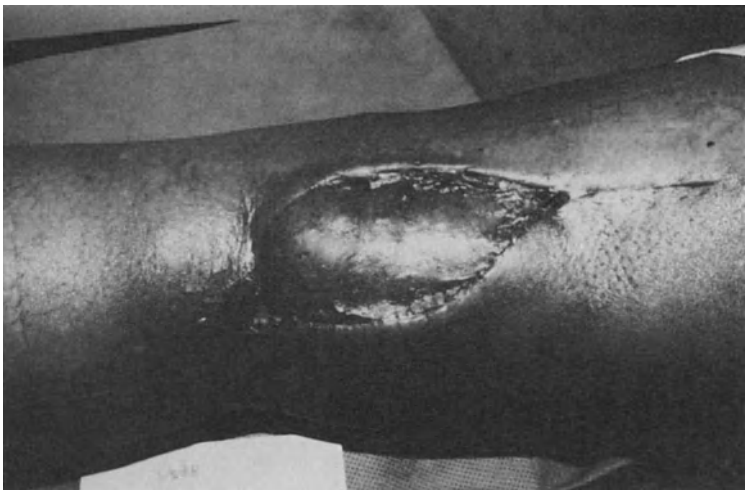

Figure 4. A patient whose resection (after preoperative irradiation) required the removal of so much skin that a primary skin closure could not be done. The medial gastrocnemius muscle was transferred on its own vascular pedicle to fill the void left after the resection, and this muscle was then skin grafted. Split skin grafts heal well, and this type of wound management is superior, with the hope that the large 'dead' space will fill in on its own.

reduces the risk of seroma formation. We suspect that it is the wound seroma that increases the incidence of a late (10–30 days postoperative) wound breakdown and subsequent wound infection. We have used ultrasound and CT scans in the early postoperative period as a means of screening for seroma but have not routinely done so.

The sutures are left in for at least 1 month from the date of surgery. If the patient develops a wound complication within the first 2 weeks after surgery, it is better to return the patient to the operating room early to correct the problem, which usually requires bringing in a vascularized muscle, than to wait, hoping the wound will heal on its own. The sutures are left in for at least 1 month from the date of surgery. Finally, if the patient develops a late (after 30 days postoperative) minor wound-healing problem, observation is the treatment of choice, as surgical debridement usually results in a greater loss of tissue. These wounds will heal, although it usually takes 4–6 weeks (Fig. 5).

Summary

It is clear that the alterations in wound healing caused by irradiation and chemotherapy are due to reduced rates of collagen production, probably from adverse effects of irradiation and chemotherapy on fibroblasts. The

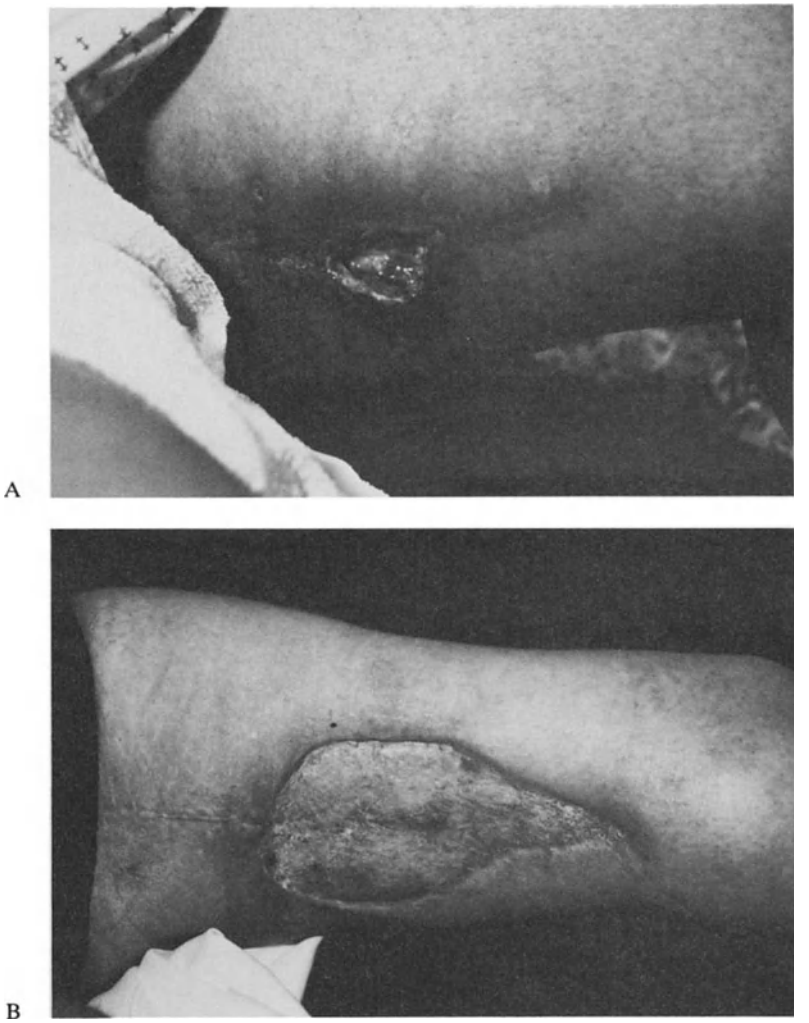

Figure 5. A: This patient had received 60 Gy of preoperative and postoperative irradiation and had a healed wound. One year after her treatment she presented with a stitch abscess, and the stitch was removed and a minor debridement was done. This wound enlarged and required multiple debridements over 3 months before the tissues were able to heal. B: This is the size of the wound before it was healthy enough to heal a split-thickness skin graft. The irradiated tissues are often able to function normally unless stressed, and surgical debridement is often enough trauma to lead to further necrosis and wound breakdown. Irradiated tissues should be handled carefully.

effect is to slow the process of wound healing so much that there is significantly more time for a complication to occur. If no complication occurs in the first 3–4 weeks after wound production, healing seems to proceed reasonably normally. The specific mechanisms by which irradiation and

chemotherapy cause their damage is not known and additional research is needed.

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6. Preoperative radiation therapy for patients with sarcoma of the soft tissues

Herman Suit and Ira Spiro

Successful treatment of patients with soft tissue sarcoma by surgery alone requires the removal of large volumes of grossly normal tissue in order that the subclinical extensions of tumor beyond the evident lesion be included in the surgical specimen. Although radical surgery achieves high local control rates on well-selected patients, there is, for many patients, a high cost in terms of loss of function and cosmesis. To reduce the morbidity of radical surgery, radiation is now often combined with comparatively conservative surgery so that less functionally intact tissue need be removed. The underlying concept is that less than radical dose levels (50–60 Gy over 5–6 weeks) inactivate the sarcoma cells that have infiltrated into the tissues adjacent to the clinically detected tumor [1,2]. The consequence is that the patient is spared the sequelae of either radical surgery or ultra-high-dose radiation therapy and has a probability of local control at least as good as that using radical surgery alone.

This approach has been widely utilized and has, indeed, yielded local control rates that are fully comparable to those obtained by radical surgery alone [1–13]. Most importantly, these local control rates are realized with greatly improved functional results. For example, amputation as the initial treatment of patients with soft tissue sarcoma of the extremities has now become quite uncommon. Barr et al. [14] have reported that at the Royal Marsden Hospital the amputation rate was only 2.4% (six patients) among a total of 253 patients treated for primary soft tissue sarcoma of the extremities. Karakousis et al. [4] performed amputation in only 4% of 85 patients with sarcomas of an extremity in the period 1977–1983; in the prior decade, 40% of similar patients had an amputation.

In considering the combination of radiation and surgery, the optimal sequence of the two modalities needs to be defined. The principal advantage of administering radiation before rather than after surgery is that the volume of tissues irradiated is smaller [1,2]. This is due to the fact that the plan for radiation therapy of the unoperated patient is designed to irradiate only those tissues involved by the grossly evident tumor and those tissues suspected of involvement on a subclinical basis. In comparison, the plan for postoperative radiation therapy must include not only tissues suspected of

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involvement by tumor, but also all tissues manipulated during the surgical procedure.

The consequent decreased irradiation of grossly normal tissues in the preoperatively treated patient reduces the frequency and severity of late radiation injury. Despite the advantages for lesser late tissue damage (tissue atrophy, necrosis, pathological fracture, induced neoplasm, etc.), the preoperatively irradiated patient does experience some increase in the frequency of wound-healing problems. There is a good basis for expecting that surgical and radiation therapy techniques will evolve such that the wound morbidities will rapidly decrease and closely approach those for postoperative radiation therapy.

Planning of the surgery and radiation in this combined modality strategy requires a detailed assessment of the optimal volume for both the surgical resection and the radiation treatment. Clearly, the target volume is exactly the same for both surgery and radiation therapy. This patient study is directed to the question of the extent to which the surgical margin can be reduced in the patient who receives radiation prior to surgery. Preoperative radiation may offer the advantage of a lesser treatment volume by both radiation and surgery.

Radiation treatment volumes

Data from the Ontario Cancer Institute demonstrate that the field sizes employed in radiation treatment for soft tissue sarcomas are smaller for radiation given preoperatively rather than postoperatively [15]. In their study 26 patients were treated by preoperative radiation. Following the surgical resection, the same patients were planned for postoperative radiation therapy as though they were previously unirradiated. The field sizes were 241 cm^2 and 391 cm^2 for the preoperative and the postoperative treatments, respectively ($p < 0.001$). This is a large effect and means that the late postradiation changes will be less frequent and severe following radiation given before rather than after surgery. There are no comparable data regarding the volumes of the resected specimen in patients who have had the surgery before or following irradiation.

Local control and surgical margins

Sadoski et al. have analyzed the margin status of the resected specimen and local control results in a consecutive series of 132 patients with soft tissue sarcoma of an extremity. These patients were treated at the Massachusetts General Hospital from 1974 and 1988 by preoperative radiation therapy and relatively conservative resection [16]. In their series, the resections removed all visible tumor. The radiation treatment was generally 50 Gy, given at

1.8–2.0 Gy/fraction and 5 fractions/week, followed by surgical resection ≈3 weeks later. In 100 patients this treatment was supplemented by a 'boost' dose of ≈15 Gy given by brachytherapy, intraoperative electron beam radiation therapy techniques, or more frequently, by postoperative small-field external beam radiation therapy. The reasons for not giving the 'boost' dose to 32 patients were usually those of poor general condition of the patient, concern regarding the status of the surgical wound, the patient's wish for no further therapy, etc. The aim for the dose to be ≈65 Gy was based on the assumption that the cells of sarcomas were at least as resistant as those of carcinomas of the breast. For the latter tumors, dose levels have traditionally been approximately 65 Gy for surgery and radiation as the treatment of lesions that are smaller than the usual soft tissue sarcoma.

In fact, the size distribution for sarcomas differs drastically from that for carcinomas of the breast. For example, in the Sadoski series, 83% of the sarcomas were greater than 5 cm in maximum dimension. For carcinoma of the breast, only 33% of 2648 lesions seen at the Gustav Roussay Institute were more than 5 cm in diameter [17]. This preponderance of the large tumors in the sarcoma group inclined us to plan for dose levels at least as high as is commonly utilized in patients with carcinoma of the breast.

For the analysis of the MGH material, a negative margin was defined as no recognizable tumor cells at the inked margins. The margins were scored as negative (104 specimens) or positive (28 specimens). The negative margins were classed as 1) cells 1 mm or closer to the inked margin, 2) cells greater than 1 mm from the margin, and 3) no tumor cells detected in the specimen.

Results of their study are presented in Table 1. The actuarial local control result at 5 years was 93% for the entire group of 132 patients. For the patients with negative margins, local control was achieved in 97%; the result was 81% for those whose specimen had positive margins ($p = 0.01$). Among the 104 patients whose specimen had negative margins, the local control rates were not dependent upon the extent of the negative margin, i.e., 94% and 97% for specimens with cells at ≤1 mm and those with cells at >1 mm from the inked margin. This finding indicates that there is an advantage to

Table 1. Local control and margin status in 132 patients treated by preoperative radiation for extremity STS

Margin	Local control
Positive (28)[a]	82%
Negative (104)	97% ($p = 0.01$)
Margin well defined	
≤1 mm (46)	94%
>1 mm (36)	97%
No tumor in specimen (22)	100%

[a] N = number of patients.

the patient in obtaining a negative margin, even though that margin is remarkably thin.

The relationship between radiation dose and the local control probability of soft tissue sarcomas in patients treated by radiation and surgery is not well defined. In the Sadowski series, the observed local control result was 32 of 32 for patients who received only the 50 Gy and no 'boost' dose; these were 30 with negative margins and three with positive margins. Although there may be a temptation to interpret this finding as indicating that dose levels above 50 Gy contribute little to local control rates, there are the following facts: 1) 19% of the patients who had specimen with positive margins failed locally, and 2) only 3 of the 27 patients with positive margins did not receive a boost dose. Hence, there is an obvious need for further investigation of the role of dose, at least among the patients with positive margins.

Local control results of $\geqslant$90% have been obtained by other centers that have utilized doses of $\approx$50 Gy. For example, Barkley et al. [7] reported that the local control rate was 91% among 110 patients treated with 50 Gy [$\approx$2 Gy/fraction] preoperatively for locally advanced soft tissue sarcoma at the MD Anderson Cancer Center. Brant et al. from the University of Florida [9] used $\approx$50 Gy given at 1.2–1.25 Gy/fraction on a bid dose schedule preoperatively and also obtained a local control rate of 90% in 48 patients. With reference to the status of the surgical margins, their local control results were 100% (15 of 15) for negative margins, 92% (22 of 24) for marginal resections, and 6 of 9 for positive margins. Atkinson et al. reported in 1963 from Sydney [3] that local control was achieved in 14 of 15 patients treated with 45 Gy/4–5 weeks followed by 'block resection.'

Lower doses (30–40 Gy) were employed preoperatively (with no postoperative 'boost') and with less good results by McNeer et al., who obtained local control in only 22 of 34 patients [18]. Mansson et al. [4] observed local control in 21 of 23 patients treated preoperatively; the results were 4 of 5 [80%] for nonradical resection and 17 of 18 [94%] following radical resection. Abbatucci et al. of the Centre Baclesse [6] gave 6.5 Gy $\times$ 2 preoperatively, and then postoperatively the dose was carried to a total of $\approx$60 Gy. They reported local control in 98% of 54 patients with negative margins, but in only 56% of 23 patients with positive margins and in 75% of 12 patients with undefined margins.

A positive margin is also an important parameter for local control among patients treated postoperatively. Reference is made here to two reports from centers using doses of $\approx$50 Gy. For instance, Bell et al. [8] described the experience at the Ontario Cancer Institute with 100 patients treated with $\approx$50 Gy postoperatively for locally advanced soft tissue sarcoma. Local control was 93% (48 of 52) when the margins were negative but only 50% (24 of 48) for positive margins; in their analysis, a positive margin meant that the tumor was visualized during the surgical procedure, was 'shelled out,' or the margins were positive pathologically. Thus, the overall local

failure rate was 28 of 100 or 28%. Pao and Pilepich described the results from the University of Minnesota [10]: Local control was obtained in 34 of 41 patients (83%) who had a grossly complete resection. The results were 9 of 10 for negative margins but 25 of 31 for positive margins (80%).

The efficacy of radical surgery alone is dependent upon securing negative margins. In the recent analysis of the series at Gothenburg, Berlin et al. [19] reported that local failures were observed in 17 of 26 (65%) marginal resections/amputations as compared with only 11 of 111 (10%) radical or wide resections/amputations. For additional data on this point see reports by Simon and Enneking [20], Abbas et al. [22], and Rydholm and Rööser [21].

The size of the sarcoma was not a recognizable determinant of tumor control probability in the MGH series treated by radiation preoperatively. Amongst patients with negative margins, local control rates were 100%, 93%, 100%, 100%, 86%, and 100% for sarcomas measured to be $\leq$25 mm, 26–50 mm, 51–100 mm, 101–150 mm, 151–200 mm, and >200 mm, respectively. The observed excellent local control rates in patients with the large lesions, i.e., >10 cm (maximum diameter), is an especially attractive feature of treatment with radiation administered before surgery.

Local control rates were similar for primary and recurrent sarcomas, when examined in patients with a comparable margin status, i.e., 81% and 80% for the positive margin tumors. The results in the negative margin tumors were 97% and 91% for the primary and recurrent sarcomas, respectively.

Treatment categories included treatment by 1) radiation plus surgery (106 patients), 2) local excision followed by radiation and then re-excision of the tumor bed (19 patients), and 3) radiation followed amputation by (7 patients). The local control outcomes in patients with negative margins were 97%, 100%, and 100% for the three groups, respectively.

Anatomic site may be a factor in positive margin patients. In the MGH series, results appeared to be less satisfactory for patients with sarcomas of the upper extremity, namely, local control was realized in only 4 of 8 patients. This contrasts with a 94% local control rate among 20 patients with sarcomas of the lower extremity who had positive margins. Because of the small numbers involved, this is clearly not a significant finding.

Conclusions

In patients irradiated preoperatively, the data presented here indicate that there is a reasonable basis for planning the surgical margin to be close, but negative, i.e., intact cells not actually at the inked margin. This would result in a smaller surgical specimen, with the consequences of easier closure without tension, less tissue void to fill with grafts and/or flaps, and higher rates of uncomplicated wound healing. This, combined with irradiation of

smaller volumes of uninvolved tissue, should increase the quality of long-term functional and cosmetic results.

We do understand that the actual experience of preoperative radiation therapy to date is with surgical procedures that achieved negative margins over most of the specimen. Those that had positive margins had the positive margin limited to a relatively small portion of the surface of the specimen. Were the surgery planned to achieve a negative margin, but quite close over much of the lesion surface, there would be some increment in the risk of local failure. That appears to us to constitute a very small risk. Because of the potential clinical gain from the use of the smaller resectional volume, testing of such an approach appears highly attractive.

An important question in such a study is the appropriate radiation dose in patients whose surgical specimens have positive margins. Further, an additional appropriate subject for future study is the importance of the extent of necrosis seen in the operative specimen for local control probability. The very low local failure rate means that a definitive assessment of this parameter will take time and a substantial number of patients.

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7. Changing concepts in the systemic treatment of locally advanced or metastatic soft tissue sarcomas

J. Verweij and H.M. Pinedo

For many years chemotherapy has been applied for the treatment of metastatic soft tissue sarcomas. Because of the rather disappointing success rate and due to the lack of active drugs, in recent years there have been some changes in the use of chemotherapy for metastatic disease. In addition, systemic chemotherapy is now being studied as a neoadjuvant prior to surgery.

Is single-agent chemotherapy equal to combination chemotherapy?

In previous volumes of this book we have indicated that there are only three cytotoxic drugs with more than minor activity in soft tissue sarcomas. These drugs are doxorubicin (DX) [1–3] and ifosfamide (IFOS) [4–7], both with a response rate of 20–25% in nonpretreated patients, and DTIC [8,9], yielding a response rate of 17% in pretreated patients but not tested as single agent up front. As different studies on these drugs performed by different groups all produced comparable results, the data on the activity of these drugs can be considered as very solid. All studies performed on other drugs have shown no, or hardly any, activity [10], with the exception of epidoxorubicin (Epi-DX) [11], which at equivalent doses of 75 mg/m^2 every 3 weeks was found to be somewhat, though not significantly, less active than DX (18% versus 25%), while the toxicity of Epi-DX (especially the myelotoxicity) was significantly less. Equitoxic doses of the two drugs have not yet been compared. In order to try to improve response rates, many studies on combination chemotherapy have been performed. However, these studies may have been performed in patients with a better performance score in view of the anticipated higher toxicity of combination chemotherapy. As performance score is the most important prognostic factor of response [2,12–15], it becomes evident that only randomized studies will prove whether combination chemotherapy is more effective than single-agent treatment (Table 1). The Eastern Cooperative Oncology Group (ECOG) performed a randomized study comparing DX 70 mg/m^2 every 3 weeks with DX 50 mg/m^2 plus cyclophosphamide (CTX, or Cytoxan) 750 mg/m^2 plus vincristine (VCR)

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Table 1. Single agent versus combination chemotherapy

Group	Regimen	No. of pts.	Response rate (%)	Ref.
ECOG	DX	66	27	16
	vs.			
	DX/CTX/VCR	70	19	
	vs.			
	CTX/VCR/DAC	64	11	
ECOG	DX (q 3 wks)	112	18	3
	vs.			
	DX (weekly)	109	16	
	vs.			
	DX/DTIC	110	30	
EORTC	DX	244	24	22
	vs.			
	DX/IFOS	233	27	
	vs.			
	CTX/VCR/DX/DTIC	135	27	

1.4 mg/m^2, all by i.v. bolus on day 1 every 3 weeks, and with CTX 750 mg/m^2 plus VCR 1.4 mg/m^2 plus actinomycin-D (DACT, or dactinomycin), 0.4 mg/m^2 by i.v. bolus on day 1 every 3 weeks [16]. In a total of 200 patients, the response rates were 27%, 19%, and 11%, respectively. The difference between the single-agent regimen and the doxorubicin-free regimen was significant ($p = 0.003$) in terms or response, but not in terms of survival. The results obtained with the latter regimen suggest that CTX, VCR, and DACT are not effective drugs in this disease. These observations were confirmed for CTX in an EORTC study [6] and were also supported by data from the Gynecological Oncology Group (GOG) [17] and the South-West Oncology Group (SWOG) [18]. The latter study also confirmed that DACT is inactive.

In another more recent ECOG study, 331 evaluable patients were randomized to receive DX 70 mg/m^2 i.v. bolus on day 1 every 3 weeks; DX 20 mg/m^2 i.v. bolus on days 1–3 and 15 mg/m^2 i.v. bolus on day 8 and weekly thereafter; or DX 60 mg/m^2 on day 1 plus DTIC 250 mg/m^2/day on days 1–5, repeated every 3 weeks [3]. This trial thus questioned the role of the DX dose and the value of adding DTIC. The two DX regimens resulted in comparable response rates (18% and 16%, respectively), while the addition of DTIC significantly ($p < 0.02$) increased the response rate to 30%, mainly by increasing the number of partial remissions. However, toxicity was also more pronounced, while the higher response rate of the combination failed to affect the overall survival.

Long before it became clear that CTX and VCR do not have substantial activity in soft tissue sarcomas, these drugs had been incorporated into the earlier mentioned ADIC regimen, resulting in a regimen called CYVADIC.

Together with the ADIC regimen, the CYVADIC regimen is the most extensively studied regimen in metastatic soft tissue sarcomas. The original CYVADIC regimen consisted of CTX 500 mg/m^2 i.v. on day 1, VCR 1.5 mg/m^2 i.v. on days 1 and 5, DX 50 mg/m^2 i.v. on day 1, and DTIC 250 mg/m^2 i.v. on days 1–5 [19]. The side effects from this regimen are manageable, but include nausea/vomiting, alopecia, leucocytopenia, and thrombocytopenia. In addition, as with ADIC, CYVADIC had the disadvantage of necessitating hospitalization. Therefore, a shorter schedule of CYVADIC has been studied, resulting in toxicity and activity comparable to those of the original regimen [20], although proof from a randomized trial is not available. After confirming the activity of DX plus IFOS [21], in a very large phase II study [15] the EORTC has performed a randomized study comparing DX with DX/IFOS and with a short schedule of CYVADIC. In the phase II study on DX/IFOS, 51 of 175 (35%) evaluable patients responded [15]. Toxicity consisted mainly of nausea/vomiting, alopecia, and myelosuppresion, and appeared similar to that of CYVADIC.

In the phase III study patients were randomized to receive DX 75 mg/m^2 i.v. bolus on day 1 or DX 50 mg/m^2 i.v. bolus on day 1 plus IFOS 5 g/m^2 as a 24-hour i.v. infusion on day 1, or CTX 500 mg/m^2 i.v. bolus on day 1 plus VCR 1.5 mg/m^2 i.v. bolus on day 1, plus DX 50 mg/m^2 i.v. bolus on day 1 plus a DTIC 750 mg/m^2 i.v. short-term infusion on day 1. Cycles were to be repeated every 3 weeks. A total of 749 patients were entered. This study is now approaching its final evaluation, and preliminary analyses have already been reported [22]. Response rates in this interim report were similar for the three arms: DX 24%, DX/IFOS 27%, and CYVADIC 27%. Also, there was no significant difference in overall survival or disease-free survival. This study also confirmed the importance of a good performance score to achieve a response, regardless of the treatment given.

Although subset analyses should be performed before any final conclusion can be drawn, the results of these studies appear to add to those of the ECOG studies [3,16]. Overall, none of these studies show improved survival rates for patients receiving combination chemotherapy, while 2 of the 3 even failed to indicate improved response rates after combination chemotherapy. Thus for regimens including 'Standard' doses of drugs, single-agent doxorubicin appears to be equivalent to combination chemotherapy and is less toxic.

Is more better?

The results of the first ECOG study [16] and the EORTC study [22] also suggest that reduction of the dose of DX from 70–75 mg/m^2 to 50 mg/m^2 every 3 weeks in order to add one or more myelotoxic drugs will result in reduced activity. This adds to the early data of O'Bryan et al. [23] obtained in 98 soft tissue sarcoma patients indicating that there is a dose-response

Table 2. Intended doxorubicin dose intensity

Regimen	Protocol DX dose	DX dose intensity ($mg/m^2/wk$)	No. of pts.	Response rate (%)	Ref.
DX	$75\,mg/m^2/9\,wks$	25	41	37	23
DX	$60\,mg/m^2/3\,wks$	20	10	20	
DX	$45\,mg/m^2/3\,wks$	15	28	18	
CYVADIC	$50\,mg/m^2/4\,wks$	12,5	84	38	13
CYV alt ADIC	$50\,mg/m^2/8\,wks$	6,25	78	14	

relationship for DX in soft tissue sarcomas (Table 2). In that early study, in which treatment was given every 3 weeks, doses of $60\,mg/m^2$ or more produced higher response rates than doses of $50\,mg/m^2$ or less. Furthermore, the EORTC previously performed a randomized study comparing CYVADIC with a schedule alternating VCR/CTX and ADIC in similar doses as used with CYVADIC, at 4-week intervals [13]. With CYVADIC 17% complete remissions (CR) and 21% partial remissions (PR) were achieved in 84 patients, while in the cycling arm a significantly lower response rate of 5% CR and 9% PR was achieved in 78 patients ($p = 0.001$), indicating that DX should be administered every 3 or 4 weeks, instead of every 8 weeks.

Although not necessarily an aim of the study, all these studies addressed the concept of DX dose intensity. Thus the observation that combination chemotherapy appears not to improve response rates in comparison to single-agent DX may be related to the reduction of the dose of doxorubicin in combination chemotherapy regimens, through which activity is lost. However, myelotoxicity previously precluded the use of higher doses of DX in combination with other cytotoxic drugs (Table 3). The recent introduction of hemopoietic growth factors made it possible to study high-dose DX combinations. Subsequent to its randomized phase III study, the EORTC has performed a Phase II study with DX $75\,mg/m^2$ i.v. bolus day 1 plus ifosfamide $5\,g/m^2$ as a 24-hour i.v. infusion on day 1 and GM-CSF $250\,\mu g/m^2$/day s.c. days 3–17, with cycles to be repeated every 3 weeks. A total of 111 patients were entered. The first 52 patients received GM-CSF $250\,\mu g/m^2$ as a single daily s.c. injection, and the next 59 patients had their daily GM-CSF divided into 12-hourly injections [25] to see whether the dosing schedule of

Table 3. Doxorubicin dose in combination with fixed ifosfamide relatative to myelotoxicity and infection

Group	DX dose (mg/m^2)	No pts.	Leucopenia	Infection (%)	Ref.
RMH	60	27	45% grade 4	55	21
Indiana	60	42	Median nadir 1.3	43	24
EORTC	50	400	34% grade 4	6	15,22

GM-CSF had any influence on the reduction of myelotoxicity. The latter was not the case. The aimed dose intensity of chemotherapy was achieved in the majority of patients for up to seven courses. The preliminary overall response rate was 45%. Obviously these data need to be confirmed in a randomized study, which is at present being performed by the group.

In order to reduce the number of variables, this study randomizes treatment with 'standard' doses of DX plus IFOS with the high-dose regimen. In view of the preliminary results of their study comparing single-agent DX with combination chemotherapy [22], the EORTC initiated a dose-intensity study with single-agent anthracyclines. As indicated, the optimal dose of single agent DX without growth factor support is 70–75 mg/m^2 every 3 weeks [23], while equivalent doses of epidoxorubicin are slightly less active. On the other hand, we now know that higher doses of epidoxorubicin can be applied [26,27]. A French study testing single-agent epidoxorubicin at doses ranging from 100 to 130 mg/m^2 showed the anticipated increase in myelotoxicity but found these doses to be manageable [26].

Amazing results were reported from Belgrade [27]. The investigators used the very high dose of epirubicin of 60 mg/m^2/day, days 1–3, combined with cisplatin, which is hardly active in this disease, at a dose of 30 mg/m^2/day, days 1–4. This schedule was repeated every 4 weeks. In 35 patients the response rate was claimed to be as high as 57%, including 20% CRs. Although myelotoxicity did apparently not impress the investigators, 63% of patients had grade 4 leucocytopenia, with neutropenic fevers in 51%, while 37% of patients had (uncomplicated) grade 4 thrombocytopenia. The authors suggest that epidoxorubicin should be tested in soft tissue sarcomas at a single-agent dose of at least 150 mg/m^2 per cycle.

The latter two studies, in fact, apart from addressing the question of the relevance of epidoxorubicin dose intensity, also include the question of the relevance of dose scheduling. In view of these and previous studies, the EORTC initiated a phase II study randomizing DX 75 mg/m^2 i.v. bolus day 1 versus Epi-DX 160 mg/m^2 i.v. bolus day 1 versus Epi-DX 60 mg/m^2 i.v. bolus day 1–3, with cycles to be repeated every 3 weeks. However, after a total of 30 patients had been entered in this study, the latter two schedules were changed into 150 mg/m^2 i.v. bolus day 1 and 50 mg/m^2/day i.v. bolus 1–3 because of unacceptable toxicity observed at the higher doses. The results of this study will indicate whether these high doses of epirubicin are manageable without growth factor support and, moreover, whether activity is better than the activity of optimal doses of doxorubicin.

A slightly different approach to the concept that more might be better is to look at treatment intensification by combining three active drugs (Table 4). At the Dana Farber Cancer Institute, Antman et al. performed three interesting studies related to this topic. In a phase I study of a continuous infusion of mesna, DX, IFOS, and DTIC (MAID), they observed a 44% response rate [28]. Subsequently a phase II dose was selected to produce a WBC nadir of 0.5–1.5 $\times$ 10^9/l. The phase II study was performed by using

Table 4. Anthracycline/ifosfamide/DTIC combination chemotherapy

Regimen	No pts.	Response rate (%)	Grade 4 leucopenia (%)	Toxic deaths (%)	Ref.
MESNA/DX/IFOS/DTIC	108	47	59	1	29
MESNA/DX/IFOS/DTIC	160	31	73	4	30
MESNA/EPI-DX/ISOF/DTIC	40	49	>85	4	31

DX 20 mg/m^2/day, IFOS 2.5 g/m^2/day, and DTIC 300 mg/m^2/day, all given as a 72-hour continuous infusion. Mesna was given at a dose of 2.5 g/m^2/day, as a continuous infusion on days 1–4. Cycles were repeated every 3 weeks. The high response rate from the phase I study was confirmed [29]. With 11 CRs and 38 PRs, the overall response rate was 47%. Toxicity was substantial: 59% of 471 evaluable courses were coincided by grade 4 leucocytopenia, with 23% neutropenic fever, including 3% proven sepsis; 21% of all cycles resulted in grade 3–4 thrombocytopenia. Nonhematologic toxicity consisted predominantly of anorexia and vomiting, and infrequent but severe mucositis, hematuria, renal failure, and CNS toxicity. There was one toxic death due to sepsis. Though toxic, this treatment appeared feasible with careful monitoring.

Subsequently, to test the additive value of ifosfamide, an intergroup study of CALGB and SWOG was initiated, randomizing DX plus DTIC versus the above-discussed regimen of DX + DTIC + IFOS. This study included 338 patients, 327 of whom were evaluable: 167 treated without ifosfamide and 160 treated with the regimen including ifosfamide [30]. The percentages of grade 4 toxicities were 22% and 73%, respectively, and the toxic death rates were 0.6% and 4%. The response rates were 19% and 31%, respectively, with only 2% and 1% CRs. Despite the significant increase in toxicity with the addition of ifosfamide, the related response rate was disappointing, and there was no survival benefit. A comparable regimen was designed by the Milan group [31]. They currently study an outpatient regimen combining epidoxorubicin 90 mg/m^2 i.v. bolus day 1, ifosfamide 2.5 g/m^2/day, days 1–3 as a 2-hour infusion and dacarbazine 300 mg/m^2/day, days 1–3 as a 30-minute infusion, with cycles to be repeated every 3 weeks. In a preliminary analysis in a highly selected group of 45 patients with either locally advanced disease or a few pulmonary metastases, but amenable to surgery, they report a 49% response rate. However, these results do not compare favorably with those obtained by the EORTC with high-dose DX + ifosfamide + GM-CSF in a much less selected group of patients [25]. Moreover the >85% incidence of grade 4 neutropenia is worrisome, as is the fact that 2 of 45 patients (4%) had a toxic death. A preliminary conclusion from all these studies may be that more intensive chemotherapy indeed yields more res-

ponses in metastatic disease. However, this is achieved at the cost of increased and, in some studies unacceptable, toxicity, while in the only randomized study at present [30] there is no survival benefit. More randomized studies will have to be performed and more subset analyses have to be reported before more definitive conclusions can be drawn.

Drug testing

Something that becomes quite evident from the data above is that there is certainly a need for new and hopefully more active drugs. As indicated, there are only three drugs with established activity: DX [1–3], IFOS [4–7], and DTIC [8,9]. In previous volumes we extensively reviewed the phase II studies performed with other drugs, which all were found to lack activity in soft tissue sarcomas. There has been some discussion as to whether new drugs should be tested up front or as a second-line treatment. DX was established as an active drug in an era when there were no other active drugs, and thus the drug could easily be tested up front [1,2]. In the past the EORTC performed a limited study with DX as a second-line treatment after failure with either IFOS or cyclophosphamide. In these pretreated patients the response rate was 17% [32]. Also IFOS did show some activity in second-line treatment [6], while the two Phase II studies on DTIC were both performed in pretreated patients [8,9]. These studies all indicate that a truly active drug in soft tissue sarcomas should be able to prove its activity in phase II studies in pretreated patients, although in this design one should take possible cross-resistance into account. We will also have to realize that CR patients may potentially be cured [33]. As we know that CRs may be achieved by existing chemotherapy, it becomes difficult to justify new drug testing up front. On the other hand, the present achievements of chemotherapy in metastatic disease, in general, are far from optimal. Therefore it may be concluded that new drug testing should preferentially be performed in second-line chemotherapy and that only drugs yielding a response rate of at least 25% in this setting should be considered for further studies.

Neoadjuvant (induction) chemotherapy

In view of the high response rates to preoperative chemotherapy, even in tumors that can be considered as non-chemotherapy-responsive when metastasized, recent studies in soft tissue sarcomas addressed the same topic. Intra-arterial chemotherapy is discussed elsewhere in this book, but there is quite convincing evidence that with the drugs that can be used, at present intravenous administration can be as effective and less complicated [34]. A study performed at the Institute Gustave Roussy reported a 38% response rate with different chemotherapy schedules in 34 patients; 24 of

these patients underwent surgery after 2–7 cycles of chemotherapy, which in 12 proved to be radical [35]. Patients who could not undergo surgery were irradiated. While 2-year survival was only 18% in non-CR patients, it was 80% in patients in CR after chemotherapy plus surgery plus radiotherapy. This may suggest a benefit of preoperative systemic chemotherapy. In the study from Boston [29], 22 patients with inoperable primaries but without distant metastases were included. In these patients the response rate was 64%. This suggests that primary soft tissue sarcomas may be more chemotherapy responsive than metastatic disease. Apart from the fact that we should take this into account when evaluating study results on chemotherapy in general, it indicates that neoadjuvant systemic chemotherapy should be further explored. This can only be done properly in a prospective randomized design. At present only one such study is ongoing, being performed by the EORTC and randomizing preoperative DX/IFOS for three cycles with no preoperative chemotherapy in patients with resectable tumors with poor prognostic factors.

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8. Adjuvant chemotherapy of soft tissue sarcomas

Wilson C. Mertens and Vivien H.C. Bramwell

Adequate surgical resection, with or without radiotherapy, remains the primary potentially curative treatment of soft tissue sarcomas, as most patients present without clinically evident metastases. Unfortunately, metastases subsequently occur in up to half of these cases [1–4], despite adequate local control, and it is only with the elimination of hematogenously borne micrometastases that further improvements in survival will occur.

The modest response rates achieved with single-agent or combination chemotherapy for advanced or metastatic soft tissue sarcomas have been disappointing, and this therapy is not administered with curative intent. As a result, interest has developed in the use of chemotherapy as an adjunct to local treatments (adjuvant chemotherapy), with the underlying rationale that because tumor burden is low at the time of initiation of adjuvant therapy a higher cure rate could be achieved [5]. In addition, chemotherapy may be better tolerated when the tumor burden is low. The primary indicator of effectiveness in advanced disease, tumor response rate, is not applicable in the adjuvant setting as the primary tumor has already been removed. The important endpoints of adjuvant therapy, relapse-free survival (RFS) and overall survival (OS), require prolonged follow-up, as the therapy must be judged by its ability to reduce the number of tumor recurrences and deaths over time. Comparison with a randomly assigned control group is essential to adequately document treatment effects in these patients, as the survival of patients with soft tissue sarcoma appears to be improving, irrespective of the administration of chemotherapy [1,2,4,6], thus making comparison with historical controls inappropriate.

Successful adjuvant chemotherapy strategies have been defined for a number of human cancers, including osteosarcoma, Ewing's sarcoma, and rhabdomyosarcoma. However, the role of adjuvant chemotherapy in adult soft tissue sarcoma remains controversial. Few published clinical trials in soft tissue sarcoma have accrued more than 100 patients. Random allocation does not assure that imbalances, resulting from the complex interactions of variables, such as the numerous pathologic subtypes, histologic grades, primary tumor locations, and different local control procedures, will be avoided. Indeed, the probability that several prognostic factors will be

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evenly distributed between two groups of patients in trials with small sample sizes is low. Imbalances between known prognostic factors may not be entirely compensated for by prerandomization stratification or postrandomization adjustments.

Since the last volume in this series on soft tissue sarcomas was published, no new randomized studies of adjuvant chemotherapy have been reported, although some trials have been updated. This chapter reviews randomized clinical trials of adjuvant chemotherapy that include a concurrent control group.

Randomized trials of adjuvant chemotherapy

Doxorubicin versus control

Table 1 summarizes the results of five trials, each of which examines the outcome of a group receiving adjuvant doxorubicin in comparison to a randomized control group treated by surgery with or without radiotherapy.

Boston/ECOG

The results of two randomized studies, one conducted at the Dana Farber Cancer Institute (DFCI) and the Massachusetts General Hospital (MGH), and the other conducted by the Eastern Cooperative Oncology Group (ECOG study EST2377), have been reported jointly [7]. Both studies accrued patients with stage IIB to IVA (AJC) tumors stratified for stage, surgical margins, and primary size. Both conservative surgery with radiotherapy or radical surgery were permitted, and patients with locally recurrent disease were eligible. Patients treated at DFCI were randomized to five cycles of doxorubicin, 90 mg/m^2 every 3 weeks, versus no adjuvant treatment postsurgery. Those patients treated at MGH received two cycles of doxorubicin 90 mg/m^2 preoperatively and three cycles postoperatively. Patients treated on the ECOG study received doxorubicin 70 mg/m^2 for seven cycles, with all cycles given after surgery. No significant differences were found with respect to local control, metastasis-free survival, relapse-free survival (RFS), and overall survival (OS). Subgroup analysis did not demonstrate a benefit from doxorubicin therapy for either extremity or for nonextremity sarcomas.

Intergroup

The Intergroup Sarcoma Study Group (ISSG) randomized patients to doxorubicin 35 mg/m^2 daily for 2 days, repeated every 3 weeks for a total of six cycles, or to no further treatment. Ninety-two patients were randomized, with 86 patients available for analysis. With a median follow-up of 44 months [8], no differences in disease-free or overall survival were seen. No

Table 1. Adjuvant chemotherapy: Randomized studies of doxorubicin versus control

Center	Treatment	No. evaluable patients	Median FU months	LR[d]	Mets.[e]	RFS (%)[e]		OS (%)[e]	
Boston/ECOG[b] [7] (1978–1985)	DX[a]	37	49 (16–80)	3	NS[e]	74	p = 0.11	68	p = 0.23
	Control	38		4	NS	62		68	
Intergroup[b] (USA) [8,9] (1983–1987)	DX	32	20 (1–39)	NS	NS	67	n.s.	82	n.s.
	Control	32		NS	NS	67		89	
Scandinavia[b] [10] (1981–1986) radical resection	DX	77	40	6	NS	62	n.s.	75	n.s.
	Control	77		6	NS	56		70	
Scandinavia[b] [10] marginal resection plus RT	DX	16	40	2	NS	62	n.s.	69	n.s.
	Control	11		1	NS	64		73	
UCLA[c] [6] (1981–1984)	DX	56	28	2[e]	NS	58	n.s.	85	n.s.
	Control	63		7[e]	NS	54		80	
Bologna[c] [11–13] (1981–1986)	DX	33	68 (38–96)	4[f]	7	67	p < 0.05	84	p < 0.05
	Control	44		7[f]	22	47		64	

[a] DX = doxorubicin.
[b] Sarcomas, all sites.
[c] Extremity sarcomas only.
[d] FU = follow-up; LR = local recurrence; Mets. = metastases; n.s. = not significant; NS = not stated; OS = overall survival; RFS = relapse-free survival; RT = radiotherapy.
[e] LR alone. Six patients had simultaneous LR plus mets.
[f] From earlier report with median FU ~40 months.

benefit from doxorubicin therapy was seen for nonextremity sarcomas, but in the preliminary report it was suggested that in patients with extremity tumors there was a trend toward improved relapse-free survival (RFS) for the doxorubicin-treated group ($p = 0.06$), but OS was not significantly different [9].

Scandinavian sarcoma group

This study is the largest evaluating adjuvant doxorubicin. From 1981 to 1986, 240 patients with high-grade (Broder grade III or IV) sarcomas were randomized to receive doxorubicin 60 mg/m^2 monthly for nine courses or no systemic treatment. Sixty-nine patients (29%) were not evaluable, with the most common reasons for exclusion being ineligible histologies (22 patients) or marginal excisions not followed by radiotherapy (25 patients). It should be noted that exclusion of the latter patients, who might have a higher rate of local recurrence and possibly distant metastasis, might be expected to improve the results for the study as a whole. This high rate of ineligibility is similar to that reported for the EORTC trial (see below). Thirty-four patients who underwent marginal resection received radiotherapy postoperatively before being randomized and were analyzed separately. Chemotherapy was commenced within 6 weeks after surgery, except for patients receiving postoperative radiotherapy, who were commenced on therapy within 10 weeks of surgery. No patients were lost to follow-up, and the median duration of follow-up was 40 months. No significant differences between the treatment and the control groups were seen with respect to local recurrence, RFS, or OS, either for the 181 evaluable or the 240 total randomized patients [12]. Amputation was performed in 26 of 155 (17%) patients with extremity sarcomas. Radical surgery was preferred where feasible, and radiotherapy was only given to patients undergoing regional excision (27 patients, 17%). The local recurrence rates were low for radically excised patients (8% in both doxorubicin and control groups) but slightly higher in patients treated by marginal excision and radiotherapy (14% in the doxorubicin group and 10% in the control group $p = 0.72$) [10].

UCLA

One hundred and nineteen patients with grade III extremity soft tissue sarcoma received preoperative intra-arterial doxorubicin (20–30 mg/24 hours for 3 days), followed by external beam radiation therapy to the primary tumor, with surgical excision of the tumor 1 week after the completion of radiation. Patients were then randomized within 6 weeks of surgery to doxorubicin 45 mg/m^2 for 2 days each month for 5 courses or no further therapy. With a median follow-up of 28 months, there were no significant differences in terms of RFS or OS. A slightly lower local recurrence rate in those patients who received adjuvant systemic doxorubicin did not reach

statistical significance. The infusion of doxorubicin intra-arterially to all atients may have obscured any differences between the treatment groups [6].

Bologna

This study, first reported in 1986 [11] and recently updated [12,13], evaluated 77 patients with high-grade extremity sarcomas. These patients had undergone 1 of 3 local control procedures:

1. Amputation (28 patients): Patients were randomized after ablative surgery to doxorubicin 25 mg/m^2/day for 3 days every 3 weeks for a total of six cycles, commencing 3 days postoperatively, or no further treatment.
2. Conservative surgery (29 patients): Patients underwent biopsy, followed by radiotherapy (4500 cGy over 3 weeks) with doxorubicin 75 mg/m^2 repeated once. Conservative surgery was performed and patients were then randomized to doxorubicin for four cycles (300 mg/m^2) for a total doxorubicin dose of 450 mg/m^2 or to no further treatment.
3. Re-excision (20 patients): If persistent tumor was suspected in patients who had been treated at another hospital within the previous 3 months, a further excision of the tumor bed was performed and then these individuals were randomized as in 1. Patients received postoperative external beam radiation therapy (4500 cGy) if residual tumor was demonstrated histologically.

The earlier results revealed that RFS was significantly better for the doxorubicin-treated group (79.1% versus 54.3%; $p < 0.005$), with a 28-month median follow-up and an accrual of 59 patients. Updated figures for 77 patients with a median follow-up of 68 months revealed persistent differences of RFS ($p < 0.05$) and OS ($p < 0.05$), favoring the doxorubicin-treated group. Interestingly, in this most recent report these differences were only apparent in the groups that underwent amputation and scar re-excision, with no differences demonstrable between the doxorubicin and control groups for patients treated by conservative surgery, who also had a poorer outcome. The conservative surgery group had a high incidence of local recurrence, but the report did not elaborate further. This study attempted to utilize a pair-matched stratification for age, site, size of tumor, and stage. The technique of randomization resulted in a large imbalance in patient numbers between the doxorubicin (33 patients) and control (44 patients) groups, and has been criticized [14]. In addition, the control group included an excess number of pelvis/thigh tumors (57% versus 37.5%), which are generally larger and have a poorer prognosis than distal or upper extremity tumors. Despite similar durations of follow-up, the RFS for control patients in this study is worse (47%) than that for comparable control groups of other studies shown in Table 1. Conversely the RFS (68%) for the treatment group is similar to that achieved by chemotherapy and control groups of other studies.

Combination chemotherapy versus control

Table 2 summarizes the chemotherapy regimens, and Table 3 summarizes the results of randomized studies evaluating adjuvant combination chemotherapy.

Table 2. Adjuvant multiagent chemotherapy regimens

1. M.D. Anderson
   Vincristine 2 mg i.v. every week for 9 weeks, day 1 of regimen only
   Doxorubicin 60 mg/m^2 i.v. day 2 (max. 420 mg/m^2)
   Cyclophosphamide 200 mg/m^2 orally days 3–5
   Dactinomycin 0.3 mg/m^2 i.v. days 1–5 (max. 0.5 mg/m^2) after maximum doxorubicin delivered
   Courses repeated every 28 days until dactinomycin replaces doxorubicin, and cycles occur every 8 weeks; chemotherapy delivered for 2 years
2. Mayo Clinic
   Vincristine 1.2 mg/m^2 i.v. days 1 and 5
   Cyclophosphamide 250 mg/m^2 i.v. days 1, 3, and 5
   Dactinomycin 0.325 mg/m^2 i.v. days 1–5
   Alternating every 6 weeks with
   Vincristine 1.2 mg/m^2 i.v. days 1 and 5
   Doxorubicin 50 mg/m^2 i.v. day 3
   Dacarbazine 250 mg/m^2 i.v. 1–5
   Total of 8 cycles of chemotherapy given
3. NCI
   High-dose regimen
      Doxorubicin 50 mg/m^2 day 1, escalated each cycle as tolerated by 10 mg/m^2 to a maximum of 70 mg/m^2 (max. cumulative dose of 500–550 mg/m^2)
      Cyclophosphamide 500 mg/m^2 i.v. day 1, repeated every 28 days, escalated by 100 mg/m^2 per course to a maximum of 700 mg/m^2
      At maximum dose of doxorubicin, change to 6 courses of
         Methotrexate 50 mg/kg i.v. over 6 hours day 1, repeated every 28 days, escalated each course as tolerated by 50 mg/kg to a maximum of 250 mg/kg
      Leucovorin 15 mg/m^2 2 hours after completion of methotrexate and every 6 hours × 8
   Low-dose regimen
      5 cycles of doxorubicin and cyclophosphamide as above; no methotrexate
4. EORTC
   Cyclophosphamide 500 mg/m^2 i.v. day 1
   Vincristine 1.4 mg/m^2 i.v. day 1
   Doxorubicin 50 mg/m^2 i.v. day 1
   Dacarbazine 400 mg/m^2 i.v. days 1–3
   Repeated every 28 days for 8 cycles
5. Fondation Bergonie
   Cyclophosphamide 500 mg/m^2 i.v. day 3
   Vincristine 1.5 mg/m^2 (max. 2 mg/dose) day 1
   Doxorubicin 50 mg/m^2 day 2
   Dacarbazine 400 mg/m^2 days 1–3
   Repeated every 21 days for 9 cycles

Table 3. Adjuvant chemotherapy: Randomized studies of combination chemotherapy versus control

Center[b]	Treatment	Site	No. evaluable patients	Median FU months	LR[e]	Mets.[e]	RFS (%)[e]	OS (%)[e]
M.D. Anderson [16] (1973–1976)	Chemotherapy	Limb	20	>120	2	9	54	65
	Control		23		8	11	35	36
							p = 0.04	p = 0.25
Mayo Clinic [17] (1975–1981)	Chemotherapy	Limb	26	64	17	NS[e]	NS[e]	90
		Abdomen[c]	4					
	Control	Limb	26					
		Abdomen	5			NS	NS	77
								p = 0.55
NC [18–22] (1977–1981)	Chemotherapy	Limb	39	85	1	NS	75	82
	Control		28		4	NS	54	60
							p = 0.04	p = 0.12
	Chemotherapy	Head[d], neck, trunk	17	35	NS	NS	77	68
	Control		14		NS	NS	49	58
							p = 0.08	p = 0.38
EORTC [23,24] (1977–1988)	Chemotherapy	All	150	72	24	45	67	67
	Control		179		55	64	52	59
							p = 0.01	p = 0.53
Fondation Bergonie [25,26] (1980–1988)	Chemotherapy	All	31	52.4 (14–120)	1	5	NS	87
	Control		28		5	16	NS	53
								p = 0.002

[a]

[b] Mayo Clinic, 25% low grade; NCI, no low grade; EORTC, 16% low grade; and Fondation Bergonie, no low grade.

[c] Includes retroperitoneal.

[d] Excludes retroperitoneal.

[e] LR = local recurrence; Mets. = metastases; NS = not stated; OS = overall survival; RFS = relapse-free survival; FU = follow-up.

M.D. Anderson

Between 1973 and 1976, a randomized clinical trial of combination chemotherapy versus observation was performed for patients with soft tissue sarcomas. The chemotherapy consisted of doxorubicin 60 mg/m^2, vincristine, and cyclophosphamide administered for 7 cycles. Doxorubicin was then replaced by dactinomycin, for a total of 18 months of treatment. Patients with Stage IIB and IIIB (AJC) lesions were entered, and patients were stratified for histology but not for grade or size of tumor. All patients underwent local resection and radiotherapy. When results were reviewed at 18 months median follow-up, unacceptable toxicity and a poor RFS for the chemotherapy group (76% versus 83% for controls) led to early termination of the study [19]. An imbalance between treatment and control arms, particularly for tumor grade, might have been responsible for the initial poor results in the chemotherapy group. In view of results obtained by the National Cancer Institute Surgery Branch in extremity soft tissue sarcoma (see below), this trial was re-analyzed after 10 years of follow-up. The 20 patients with extremity lesion sarcoma treated with chemotherapy had significantly better RFS ($p = 0.04$) compared with the 23 patients who received no further therapy. This could almost entirely be accounted for by a difference in the rate of local recurrence (see Table 3), whereas the incidence of metastasis and OS were not significantly different [16].

Mayo Clinic

Sixty-one patients, stratified according to the nature of disease excised (primary tumor or locally recurrent disease), site of origin (somatic or visceral), and histologic grade (Broder's I & II or III & IV) were randomized to therapy with vincristine, cyclophosphamide, and dactinomycin, alternating with vincristine, doxorubicin (50 mg/m^2), and dacarbazine. Chemotherapy was administered every 6 weeks for a total of 8 courses. Six control and seven doxorubicin-treated patients were also given methanol extraction residue (MER) of BCG intracutaneously in five dorsal sites on day 1 of each of the first 8 postoperative follow-up cycles (or an equivalent time in the control group). Local excision or amputation was permitted, but adjuvant radiation therapy was not offered. Patients relapsing after therapy were offered surgical resection of metastases and local recurrence. There was no statistically significant survival benefit for chemotherapy. Adjuvant chemotherapy was associated with a reduced requirement for salvage surgical excision of pulmonary metastases (30% of chemotherapy-treated patients versus 55% of the untreated group) and metastases at other sites. However, the rate of salvage surgery for local recurrences was not reduced by adjuvant chemotherapy. It is likely that the high local recurrence rate (28%) reflects the omission of adjuvant radiotherapy after local excision [17].

National Cancer Institute

Patients with stage IIA-IIIB (AJC) extremity soft tissue sarcoma underwent either amputation or limb-sparing resection of tumor followed by radiotherapy. Patients were then randomized to receive an aggressive chemotherapy regimen of doxorubicin and cyclophosphamide, with the institution of high-dose methotrexate and leucovorin rescue after a maximum cumulative dose of doxorubicin (500–550 mg/m^2) was administered. Patients randomized to chemotherapy who required radiation therapy received their first chemotherapy course 3 days prior to the initiation of radiation, with the second course administered during radiation therapy. Other patients received their chemotherapy within 3–4 weeks following surgery. At a median follow-up of 4.5 years [18,19], improvements in RFS and OS were reported in patients who received chemotherapy ($p = 0.008$ and 0.01, respectively; one-sided p values used). Patients who received adjuvant chemotherapy had a decreased incidence of local recurrence that was also statistically significant (one-sided $p = 0.01$).

This study was updated recently with a median follow-up of 7.1 years [20,21]. Five-year RFS remained better for patients who received chemotherapy (two-sided $p = 0.04$). The difference in OS was no longer statistically significant, although a trend toward improvement was seen in the chemotherapy arm (82% versus 60%, two-sided $p = 0.124$). Local recurrence rates continue to be favorably influenced by chemotherapy (two-sided $p < 0.05$). Criticisms of this study include its relatively small size, as well as an unusual randomization procedure, leading to an imbalance in the total numbers of patients entered into each treatment arm. In addition, a higher proportion of chemotherapy-treated patients had distal extremity lesions (41% compared with 25% for the no-treatment arm).

A similar trial examining soft tissue sarcomas of head and neck, breast, and trunk was reported in 1985 [22]. Thirty-one patients were entered into this study after complete resection of gross tumor and postoperative radiotherapy. The chemotherapy regimen was similar to that used for patients with extremity sarcoma. Six patients also received immunotherapy with *Corynebacterium parvum*, and analysis of the data with and without these subjects did not influence the results. A trend toward improved 3-year actuarial RFS in the chemotherapy arm was seen (77% versus 49% in the control arm; $p = 0.075$), but the 3-year OS was not different (68% for chemotherapy arm compared with 58% of controls; $p = 0.38$). In a further study, 37 patients with resectable retroperitoneal sarcomas were randomized to receive the same adjuvant chemotherapy, or no further treatment, after definitive surgical resection followed by adjuvant radiation therapy, and there was no difference in RFS ($p = 0.54$) [19].

A later study randomized 88 patients to the same chemotherapy regimen or a lower dose regimen that eliminated methotrexate, and administered

only 5 courses of doxorubicin and cyclophosphamide to a maximum cumulative dose of doxorubicin of 350 mg/m^2. RFS and OS were similar between the two arms, but the statistical power to detect an important difference was limited [21].

European Organization for Research and Treatment of Cancer (EORTC)

With 468 patients accrued between 1977 and 1988, this is the largest trial of adjuvant chemotherapy in soft tissue sarcoma [23,24]. Patients were randomized to either CYVADIC or no chemotherapy. Patients were eligible if a new primary or locally recurrent tumor had undergone complete microscopic removal, but external beam radiotherapy (5000 cGy in 4 weeks or biologic equivalent) was administered if the surgical margin was <1 cm. Chemotherapy had to commence within 13 weeks of initial surgery. Thirty percent of patients randomized in this study were subsequently found to be ineligible, most commonly because of inappropriate radiotherapy. With a median follow-up of 6 years, although RFS was significantly better in the chemotherapy arm (67% CYVADIC versus 52% control, $p = 0.01$), this was entirely accounted for by a reduction in the rate of local recurrence in the chemotherapy arm (16% versus 31%, $p = 0.001$), and differences in OS were not significant (67% versus 59%, $p = 0.53$). The reduced local recurrence rate was confined to head, neck, and trunk tumors, whereas the local recurrence rate in extremity sarcomas was not influenced by chemotherapy. Although this study has been criticized because of the high rate of ineligibility and poor compliance with chemotherapy (50% of patients completed the full 8 courses; 12% did not receive any chemotherapy), when the results were reanalyzed 1) including ineligible patients and 2) including only those patients who completed a full course of treatment, the main conclusions of the study were not altered (V. Bramwell, personal communication).

Fondation Bergonie, France

Between 1980 and 1988, 65 patients were randomized to chemotherapy with CYVADIC or no further therapy [25,26]. Stratification according to known prognostic variables was not performed, and most patients underwent conservative surgery and radiotherapy. The chemotherapy regimen and drug doses were similar to those used in the EORTC study, but chemotherapy was commenced no later than 30 days after surgery, and most patients received their first course of chemotherapy during the first 3 days of therapy with radiation (which commenced on the 14th postoperative day). Chemotherapy was administered in a planned 21-day cycle in the Fondation trial, compared to a less intensive 28-day cycle for the EORTC trial. However, the dose intensity received has not been compared between the two studies. With a median follow-up of 4.4 years, statistically significant differences

were found in terms of metastasis-free survival (84% versus 43%, p = 0.0003) and OS (87% versus 54%, p = 0.002), both favoring the chemotherapy arm. Although there was a delay in the development of local recurrences in the chemotherapy arm, and the actual number of local recurrences was lower (3% in the chemotherapy arm versus 18% for controls), this difference was not significant (p = 0.17), perhaps because of the overall small size of the study.

The most important defects of this study are its relatively small size as well as an imbalance in histologic subtypes between the two treatment arms. In addition, a higher proportion of patients on the chemotherapy arm had extremity primaries, which usually have a better prognosis. The most recent report [28] restricts OS to disease-related deaths, leaving unaccounted for four deaths initially reported. As in the Bologna trial, the OS of the control group appears to be lower than that found in other trials.

Pooled results of randomized trials

Two recent reports, one published in a symposium proceedings [8] and the other in abstract form [27], describe the pooled results of the three randomized adjuvant studies—Boston, ECOG, and intergroup—all of which randomized patients to adjuvant doxorubicin or no further treatment. Approximately 168 patients out of a total of 185 were felt to be fully eligible. Considering RFS and OS, no statistically significant difference could be detected between the doxorubicin and control groups for all entered patients, nor for the extremity or nonextremity subgroups. No information was given on local control. Although combined results may provide greater statistical power to address clinical questions, this analysis is weakened by inconsistent reporting of numbers of eligible patients and their stages, patient deaths, and the inherent problems of combining protocols employing different drug doses and schedules, and possibly differing study populations [28].

Meta-analysis of outcome in 13 randomized trials of adjuvant chemotherapy versus control in soft tissue sarcoma has been published in abstract form. This analysis includes a study of uterine sarcomas [29] not reviewed in this chapter and considers the three sites (extremity; head, neck, and trunk; and retroperitoneal) identified in the National Cancer Institute publications as separate trials. These trials include 1400 patients. The authors conclude that overall survival is increased by 9% (odds ratio 0.71, p = 0.01). Both distant (odds ratio 0.62, p = 0.001) and local recurrence (odds ratio 0.42, p = 0.0003) are reduced. However, trials vary as to the types of outcome data reported, and the number of trials evaluated for outcome in terms of survival, distant failure, and local failure were 10, 8, and 6, respectively. This analysis included trials evaluating single-agent doxorubicin and combination chemotherapy, with the trend favoring combination chemotherapy

[30]. Firm recommendations cannot be made until further information is available in a full publication, in order to critically evaluate the methods used to analyze the data. Even then, this will not provide guidance as to the most appropriate drug combination. Further individual trials of specific promising Doxorubicin combinations versus control are still required to clarify this issue.

Treatment-associated toxicity

Cytotoxic chemotherapy as used in the trials described above can cause a number of side effects, including alopecia, nausea and vomiting, myelosuppression, and the possibility of granulocytopenic-associated sepsis, and mucositis, as well as less frequent toxicities, such as hemorrhagic cystitis and pulmonary abnormalities. However, in some studies the frequency of doxorubicin-associated cardiomyopathy has caused concern as this is difficult to treat, often irreversible, and frequently lethal. The incidences of clinical doxorubicin-associated congestive heart failure were 7%, 10%, and 4% in the Intergroup [8], Boston [8], and Scandinavian studies [10], respectively. No information is available for the ECOG or UCLA studies, and clinical congestive heart failure did not occur in the Bologna study [13].

Investigators from the NCI Surgery Branch reported a high incidence of doxorubicin-associated cardiomyopathy in patients treated on their first study [31]. Of 101 prospectively assessed patients, 14 developed symptomatic congestive heart failure and 52% of radionuclide angiograms in asymptomatic patients were abnormal. The same investigators went on to assess prospectively 118 patients with soft tissue sarcoma treated on various NCI protocols [32]. Sixty-two patients received high-dose doxorubicin ($50\,mg/m^2$/month, increased by $10\,mg/m^2$ to a maximum of $70\,mg/m^2$) with cyclophosphamide and high-dose methotrexate, to a planned cumulative doxorubicin dose of $530\,mg/m^2$. Fifty-six patients received low-dose doxorubicin ($70\,mg/m^2$/month) with cyclophosphamide, reaching a planned cumulative doxorubicin dose of $350\,mg/m^2$. As doses were adjusted for myelosuppression, the actual cumulative doses for doxorubicin for high-and low-dose groups were $452 \pm 156\,mg/m^2$ and $319 \pm 77\,mg/m^2$, respectively. Symptomatic congestive heart failure was not seen in this study, in contrast to the first series, but the average left ventricular ejection fraction declined in both groups; changes from normal to abnormal occurred in a higher proportion (22 versus 11) of patients in the high-dose doxorubicin group, although the differences were not statistically significant.

Comment

The only studies, reported in full papers, that have shown significant differences in RFS and OS (Bologna and NCI) have been criticized on statistical

grounds. The contrasting results from Fondation Bergonie and the EORTC trials are intriguing. In both studies CYVADIC was the adjuvant chemotherapy regimen used, although delivery appears to have been more intensive in the Fondation trial. The shorter time to initiation of chemotherapy after surgery in the Fondation trial may have enhanced the effect of chemotherapy. On the other hand, the small size of the Fondation study may have resulted in imbalances in prognostic factors between the treatment arms. This makes comparison of these studies difficult, and definitive publications of these trials are awaited with interest.

Reductions in local recurrence rates have been noted in several studies, particularly those using combination chemotherapy, although in most cases this has not been accompanied by significant reductions in metastases or improvements in overall survival. The only study of combination chemotherapy that does not show this effect (Mayo Clinic) differed from the others in omitting local radiotherapy, leading to a high overall rate of local recurrence, and based on current information it is likely that the chemotherapy administered was inadequate. This effect is less clearly seen for adjuvant single-agent doxorubicin. Although the UCLA and Bologna studies show some decrease in rates of local recurrence following chemotherapy, no such effect is evident in the Boston/ECOG and Scandinavian studies, and insufficient information is available for the Intergroup trial. The variable patterns of improvement in local control also complicate interpretation. The EORTC study found reduced local recurrence rates in head, neck, and trunk sarcomas. In contrast, a significant reduction in the local recurrence rates in patients with extremity sarcoma was noted in the study from the National Cancer Institute. A reduction in local recurrence rates in extremity sarcoma was reported for the Fondation Bergonie trial, although these data must be interpreted cautiously in view of its small size and the possible imbalance in prognostic factors between treatment arms.

In considering the use of adjuvant chemotherapy outside a clinical trial setting, the modest clinical gain in local control and the equivocal results in terms of survival must be counterbalanced by the often substantial toxicity of these regimens.

Although adjuvant chemotherapy is often given to young patients with high-grade extremity sarcomas, the data to support this type of treatment are scanty, and the largest study of adjuvant chemotherapy (EORTC) supports its use in an entirely different group of patients. Even so, such treatment may only reduce the unpleasant consequences of local recurrence in difficult sites in the head, neck, and trunk, without influencing survival.

Future prospects for research in adjuvant chemotherapy

Intensive clinical research efforts have produced conflicting results, and it is difficult to make firm recommendations on the use of adjuvant chemotherapy outside the setting of well-constructed research protocols. However,

the introduction of new agents into combination chemotherapy and tantalizing, if inconclusive, results from published studies provide the stimulus for further research in this area. Investigators at the NCI Surgery Branch are currently randomizing patients with extremity soft tissue sarcoma who have received surgery and adjuvant chemotherapy with cyclophosphamide (700 mg/m^2) and doxorubicin (70 mg/m^2) monthly for five courses to postoperative radiotherapy or no further treatment. The radiotherapy in the treatment arm will begin within 2–3 days of the commencement of chemotherapy. The aims of this study are to evaluate the combined toxicities of chemotherapy and radiation, and to document any differences in the rates of local control. For patients who have tumors arising in the head and neck regions, breast, or trunk, all patients will be treated by surgery and radiation therapy, and then randomized to a combination of cyclophosphamide and doxorubicin, or to no chemotherapy.

Neoadjuvant chemotherapy (chemotherapy delivered before primary or local therapy) has some advantages over postoperative treatment, as the presence of evaluable tumor allows the determination of chemotherapy sensitivity clinically as well as pathologically, and valuable prognostic information may be obtained. The primary tumor may also be downstaged, and some lesions that were technically unresectable may be more amenable to surgical intervention. In addition, drug delivery to the primary tumor may be improved, as surgery and radiation therapy have not yet been delivered and the tumor's vascular supply remains undisturbed. The EORTC is evaluating neoadjuvant chemotherapy in a randomized trial of patients with soft tissue sarcomas of the extremities, head, neck, and trunk. Only poor-risk patients are eligible, based on tumor size (>8 cm) or intermediate/high histological grade (mitotic count $\geq$3/10 high power fields). Three courses of chemotherapy with doxorubicin, ifosfamide, and mesna are administered before definitive surgery and radiotherapy. Chemotherapy must commence within 6 weeks of biopsy or an attempt at definitive surgery. Patients in the control group proceed to immediate surgery and postoperative radiotherapy.

An Intergroup trial, in which doxorubicin, dacarbazine, ifosfamide, and mesna administered after definitive surgery was compared to no treatment, has been described in detail in the previous volume. Unfortunately, the study has closed as a result of poor accrual.

Recently there has been renewed interest in immunotherapy with the development of biological response modifiers with noval and interesting modes of action. In general these agents have proved disappointing in soft tissue sarcoma [33]. Muramyl tripeptide phosphatidylethanolamine (MTP-PE), a synthetic analogue of the bacterial cell wall component, muramyl dipeptide, has been developed as a liposomal preparation with a prolonged half-life [34]. Both the parent agent, as well as liposome-encapsulated MTP-PE, increase the cytotoxic activity of monocytes against tumor cell lines from both normal subjects and cancer patients [35]. Studies have shown that treatment with muramyl dipeptide or MTP-PE can eradicate spontaneous

pulmonary metastases in a murine melanoma model [34], and liposome-encapsulated MTP-PE improved survival in a murine fibrosarcoma model [36]. A randomized trial of adjuvant liposome-encapsulated MTP-PE compared with empty liposomes administered after amputation to dogs suffering from spontaneous osteosarcoma resulted in an improved median survival for the MTP-PE treated group; four dogs in the treatment group were free of disease at 1 year, whereas none of the control animals survived [37]. ECOG is proposing a randomized phase III trial of MTP-PE liposomes in patients with grade IIB-IVA (AJC) soft tissue sarcoma. MTP-PE will be administered weekly for 6 months as a 1-hour intravenous infusion. The ability of MTP-PE to activate pulmonary macrophages capable of destroying tumor, and the particular propensity of patients with high-grade soft tissue sarcomas to develop pulmonary metastases, is the rationale underlying this protocol. It is unconventional to mount a randomized adjuvant trial, in the absence of Phase II data in advanced disease, but Phase I studies have been completed [38,39], and its theoretical benefit in the control of micrometastastic disease may justify this unusual proposal. In addition, the EORTC is undertaking a Phase II study of this agent in metastatic soft tissue sarcoma. The ECOG study would be the first large, randomized adjuvant trial of a biologic response modifier in this disease, and its results may shed further light on the biology and immunotherapy of pulmonary micrometastases.

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9. Preoperative chemotherapy for soft tissue sarcomas of the extremities: The experience at the University of California, Los Angeles

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The evolution of current treatment

Surgery

The traditional approach to extremity sarcomas was surgical excision. This was associated with a local failure rate of approximately 95% [1], as tumors were simply 'shelled out' of their pseudocapsule. More radical muscle group and compartment excisions were then performed that reduced the local failure to approximately 40% [2]. Even with amputation, local failure was approximately 35%. Without effective additional therapy to control microscopic residual disease, radical surgery alone resulted in both poor functional results and high recurrence rates.

Chemotherapy for metastatic disease

The present data on the activity of cytotoxic drugs in metastatic disease are reviewed in Chapter 7 [see also refs. 3–14]. Doxorubicin, ifosfamide, and DTIC appear to be the best dugs available for this disease.

Preoperative chemotherapy

The use of preoperative or neoadjuvant therapy has many practical and theoretic advantages. Chemotherapy or radiotherapy is delivered to a virgin tumor bed with an undisturbed blood supply and excellent oxygenation, both of which are essential for drug delivery and radiation effectiveness. The main cause of death in patients with soft tissue sarcomas continues to be distant metastatic disease, particularly in patients with high-grade or extremity lesions [3]. Administration of systemic preoperative chemotherapy allows early eradication of micrometastases.

Preoperative therapy also offers the possibility of salvaging some patients with bulky tumors that are initially too large for limb-sparing surgery. If effective, there may be a reduction in tumor size, which also results in better definition of the interface between the tumor and normal tissue. This facilitates

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wide local excision with a margin of normal tissue. In some cases effective chemotherapy may not result in dramatic reduction in the size of the tumor, despite inducing extensive tumor necrosis.

The administration of chemotherapy preoperatively allows one to establish the in-vivo sensitivity of the primary tumor to the chemotherapeutic agents used. This provides valuable prognostic information and allows for the identification of high-risk groups that would benefit from additional chemotherapy [11,15,16]. Postoperative adjuvant chemotherapy is then offered to those patients with tumors that responded preoperatively, sparing nonresponsive patients the morbidity of continuing on a chemotherapeutic agent to which their tumor does not responded.

UCLA's multidisciplinary preoperative therapy

At the UCLA Medical Center advances in the treatment of soft tissue sarcomas of the extremities have come from the development and implementation of sequential protocols utilizing multimodality therapy (Table 1). Between 1974 and 1991, 407 patients with soft tissue sarcomas of the extremities were treated at UCLA and were evaluable. This group consisted of 226 males and 181 females. The mean age was 47.45 years, the median 46 years, with an age range of 4–90 years. Proximal lesions (78%) were more common than distal lesions, and lesions of the lower limb (76%) were more common than lesions of the upper limb. The majority of tumors (61%) occurred in the lower limb, proximal to the knee joint. The most common histologies encountered were liposarcoma (27%), malignant fibrous histiocytoma (22%), synovial sarcoma (16%), fibrosarcoma (6%), neurofibrosarcoma (5%), undifferentiated sarcoma (5%), and leiomyosarcoma (4%).

Tumors were predominantly grade 3 (73%) and grade 2 (25%). At the time of diagnosis 80% of patients were stage 1, 17% were stage 2 (locally recurrent), and 3% were stage 3 (metastatic). Patients were treated according to the limb-salvage protocol in use at the time of their diagnosis. Thirteen patients had treatment that deviated from one of our protocols.

Our first pilot study, in 1973, involved ten patients and demonstrated the

Table 1. UCLA Medical Center's preoperative protocols

Group	Years	Radiation dose	Chemotherapy
A	1974–1980	3500 cGy	Doxorubicin i.a.
B	1980–1984	1750 cGy	Doxorubicin. i.a.
C	1984–1988	2800 cGy	Doxorubicin i.a. vs. i.v.
D	1988–1990	2800 cGy	Doxorubicin, cisplatin
E	1990	2800 cGy	Doxorubicin, cisplatin, ifosfamide

feasibility of continuous intra-arterial infusion of doxorubicin prior to radical local surgery [4]. A percutaneous intra-arterial catheter was placed by the Seldinger technique into the major artery supplying the tumor. Doxorubicin was then infused continuously at a rate of 30 mg/day for 3 consecutive days. Pathological examination of the resected specimens showed approximately 50% necrosis. This study demonstrated several important technical points: Heparin precipitates doxorubicin, the catheter tip must be placed in a high-flow vessel to avoid marked skin erythema and muscle necrosis, and injection of fluorescein through the arterial line outlined its distribution of flow when a Woods lamp was used.

Recognizing that doxorubicin was an effective radiosensitizer, it was felt that adding radiation immediately after the infusion of doxorubicin would enhance the cytotoxic effect. The next ten patients received a total dose of 3500 cGy of external beam radiotherapy, in ten fractions, immediately following the intra-arterial (i.a.) infusion of doxorubicin [12,13]. These patients underwent en bloc resection of the tumor approximately 1 week later. Tumor necrosis was increased to 85% with this treatment. Based on our experiences with these early pilot studies, the first protocols evolved.

In 1974, a prospective trial of neoadjuvant therapy began (Table 2) [12,13]. Seventy-five patients were treated with i.a. doxorubicin followed by radiation therapy of 350 cGy/day for 10 days to the entire area of the tumor. Sixty-four patients (85%) had grade 3 lesions and 11 (15%) had grade 2 lesions. Three patients (4%) required amputation and the remaining 72 (96%) underwent a limb-salvage procedure. After a mean follow-up of 80.67 months, 8 patients (11%) developed local recurrences, 30 (40%) developed metastases, and 34 (45%) died. There were 44 complications of therapy in 28 patients (37%), including 7 bony fractures. This high complication rate was felt to be primarily due to the radiation dose.

In 1981 a second prospective trial was developed that involved giving the identical regimen of i.a. doxorubicin, but the subsequent dose of radiation was decreased to 1750 cGy [20,21]. One hundred thirty-five patients were treated on this protocol. Ninety-four patients (70%) had grade 3 tumors, 39 (29%) had grade 2 tumors, and 2 (1%) had grade 1 tumors. Four patients (3%) required amputation and the remaining 131 patients (97%) were

Table 2. Results of the multimodality preoperative protocols used at the UCLA Medical Center

Group	N	Complications	Local recurrence	Metastasis	Mortality
A	75	28 (37%)	8 (11%)	30 (40%)	34 (45%)
B	135	31 (23%)	19 (14%)	46 (34%)	43 (32%)
C i.a.	49	11 (22%)	4 (8%)	15 (31%)	15 (31%)
C i.v.	60	13 (22%)	6 (10%)	12 (20%)	10 (17%)
D	52	15 (29%)	5 (10%)	19 (36%)	11 (21%)
E	23	7 (30%)	1 (4%)	5 (21%)	2 (8%)

treated by limb salvage. After a mean follow-up of 60.83 months, 19 patients (14%) developed local recurrences, 46 (34%) developed metastases, and 43 (32%) died. The number of complications decreased, with 36 complications occurring in 31 patients (23%), one requiring amputation. The number of fractures was reduced by more than half, with only three occurring. However, associated with this diminished dose of radiation and reduced complication rate was an increase in the number of local recurrences. The original reviews of these protocols found that this difference in local recurrence rates between the two groups was significant, but with longer follow-up this difference is no longer statistically significant.

In an attempt to improve the local control rate, while still avoiding the serious complications seen with 3500 cGy, the dose of radiation was increased to 2800 cGy, and this has proved satisfactory. Excellent local control rates were obtained with this protocol, but then questions arose regarding the systemic antitumor benefit derived from intra-arterial therapy, as a significant amount of doxorubicin can bind on the first pass through the tumor bed.

In 1984 a protocol was developed to compare the results of i.a. versus systemic i.v. infusion of doxorubicin in the preoperative period. Patients were randomized to receive a 72-hour continuous infusion of doxorubicin via either the i.a. or i.v. route, followed by radiation therapy to a total dose of 2800 cGy. Limb-sparing surgery was then performed. Forty-nine patients received i.a. doxorubicin and 60 received i.v. doxorubicin. Only one patient required amputation and this was in the i.a. group. Complications resulting from therapy occurred in 11 (22%) of the i.a. group and 13 (22%) of the i.v. group. The distribution of grade and stage of disease was similar between the two groups. Pathological examination of the resected specimens revealed mean necroses of 38% and 26%, respectively, for i.a. and i.v. therapy. Mean follow-ups were 36.76 and 42.35 months, respectively, for the i.a. and i.v. groups. Local control was similar between the two groups, local recurrence occurred in four (8%) of the i.a. group and in six (10%) of the i.v. group; however, i.a. doxorubicin did not control distant micrometastases as effectively as i.v. administration. Metastases occurred in 15 (30%) of the i.a. group and 12 (20%) of the i.v. group. There were 15 (31%) deaths in the i.a. group and 10 (17%) in the i.v. group. For these reasons, as well as the increased cost and risks associated with i.a. administration, we stopped administering doxorubicin via the intra-arterial route and now give all our chemotherapy intravenously.

In 1988 a second chemotherapeutic agent, cisplatin, was added to our neoadjuvant regimen in an effort to increase tumor response. Fifty-two patients received i.v. doxorubicin and cisplatin (120 mg/m^2), followed by 2800 cGy of external beam irradiation. Thirty-nine tumors (75%) were grade 3, 12 (23%) were grade 2, and 1 (2%) was grade 1. Thirty-eight patients (73%) were stage 1, 10 (19%) were stage 2, and 4 (8%) were stage 3. Complications of therapy were not significantly increased and occurred in 15 patients (29%). Resected specimens showed an average necrosis of 57%.

After a mean follow-up of 26.5 months, local recurrence occurred in five patients (10%), metastases occurred in 19 patients (36%), and 11 patients (21%) died. The addition of cisplatin did increase the amount of tumor necrosis but did not significantly alter survival.

Ifosfamide had been reported to have considerable activity in soft tissue sarcomas, and in 1990 ifosfamide (14 g/m^2) was added as a third agent in our preoperative protocol. Patients are now given doxorubicin, cisplatin, and ifosfamide intravenously, followed by 2800 cGy of radiation. At the time of this review 23 patients had been treated with this protocol. Twenty-one tumors (91%) were grade 3 and two (9%) were grade 2. Fifteen patients (65%) were stage 1, six (26%) were stage 2, and two (9%) were stage 1. The mean tumor necrosis was 46%. Posttherapy complications occurred in seven patients (30%), most of these being local wound problems. After an average of 5 months follow-up, only one local recurrence (4%) had occurred, five patients (22%) developed metastasis, and two (9%) died. Too few patients have been treated on this protocol to make any definitive conclusions.

Conclusions

In the treatment of soft tissue sarcomas of the extremities, surgery alone, even radical surgery, is unable to adequately control local or distant disease. Systemic chemotherapeutic agents reduce or eliminate both local and systemic tumor cell metastasis, while radiation can effectively reduce the local tumor cell burden.

Multimodality preoperative therapy has been effective in allowing limb-salvage surgery in 95% of patients treated at UCLA. Overall the local failure rate in our patients was 11%, while metastatic disease developed in 32%. Our overall 1-year Kaplan–Meier survivorship is 91%, 5-year survivorship is 68%, and 10-year survivorship is 65%.

We found the intravenous administration of doxorubicin to be as effective at controlling local disease as intra-arterial administration, while at the same time providing a marginally significant ($p = 0.075$) improvement in survival. As well, intravenous administration is both safer and less expensive.

Using log rank statistic analysis, there was not a statistically significant difference between our treatment groups with regard to the incidence of local recurrence, metastasis, or survival.

Our multimodality preoperative therapy protocols have been very effective at controlling local disease, but one third of patients have gone on to develop metastastic disease. Future neoadjuvant therapy protocols must be linked to effective adjuvant therapy in an effort to reduce or eliminate metastastic disease. As new multiagent neoadjuvant and adjuvant chemotherapy evolves, we should continue to see an improvement in our treatment of soft tissue sarcomas.

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10. Hyperthermia and thermochemotherapy

Rolf D. Issels

The combination of anticancer drugs with hyperthermia was only recently introduced as an experimental clinical strategy. This approach within the field of medical oncology is based on the convincing evidence that hyperthermia enhances the therapeutic effect of several chemotherapeutic agents, even in drug-resistant cells. Such a combined modality is of particular interest as a means of overcoming the limited therapeutic efficacy of conventional anticancer agents in the case of large, resistant human tumors.

High-grade soft tissue sarcomas, like many other solid tumors, present a dual management problem: local control and prevention of distant metastases. In the last decade the current standard of either limb-sparing surgery plus radiation therapy or radical resection (often amputation) has largely improved local control rates for extremity lesions. However, the anatomic location and invasiveness of sarcomas (e.g., retroperitoneal) often prevents resection with adequate margins, and the toxicity of radiotherapy limits the use of potentially therapeutic doses. In addition, patients with large, high-grade sarcoma or recurrent local disease are at high risk for developing metastases. Despite multimodality management for this disease, currently 40–60% of patients die within 5 years. In cases of complete resection, adjuvant chemotherapy, theoretically intended to eradicate occult micrometastatic disease already present at diagnosis, has not been shown to benefit patients in terms of overall survival. The rationale for thermochemotherapy in sarcomas rests on the assumption that heat exposure (40–44°C) will increase tumor-cell kill by direct thermal cytotoxicity and/or thermal chemosensitization within different areas of the same tumor, depending on blood perfusion. The result should be an improvement in the functional and cosmetic results at comparable or higher local control rates than achieved by standard treatment procedures, as well as an improvement in the overall survival.

As many readers may not be familiar with the current status of hyperthermia in the context of chemotherapy, a brief overview related to the special topic of this chapter will be given. Based upon clinical results of chemotherapy alone in soft tissue sarcoma, the most active single agents will be defined, and preclinical data on their potential for thermochemotherapy, including several other drugs, will be summarized. Second, different heating techniques

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used for the application of thermochemotherapy in sarcomas, with an overview on the clinical data of several institutions, will be discussed including our own results.

Preclinical aspects—a search for the most appropriate drug schedule

Hyperthermia (temperature range 40–45°C) shows supra-additive interactions with selected chemotherapeutic drugs or causes toxicity of compounds that are nontoxic at 37°C (thermosensitizer). Excellent reviews upon the interactions of hyperthermia with a wide range of drugs (e.g., doxorubicin, bleomycin, cisplatin, nitrosureas, cyclophosphamide) in models employing cells in tissue culture and animal tumors have been presented by Hahn [1], Marmor [2], Engelhardt [3], and Dahl [4], and the phenomenon of thermal chemosensitization dependent upon timing or sequencing is well documented. Maximal effects occurred when the chemotherapeutic agents were scheduled simultaneously with hyperthermia either in vitro (endpoint: clonogenic cell survival) or in vivo (endpoint: tumor growth delay, TGD), respectively. In tissue-culture studies, hyperthermia has also been demonstrated to enhance drug cytotoxicity in cell lines made resistant to individual drugs, including Adriamycin [5,6], cisplatin [7], and mitomycin C [8]. In regard to the chemosensitivity of soft tissue sarcomas, doxorubicin (Adriamycin) is clinically the most important drug, with an average response rate of 25% [9], but with wide variations in different studies due to a suggested dose-effect relationship (10; Chapter 8). Unfortunately the duration of doxorubicin therapy is limited by cumulative cardiotoxicity.

In vitro studies with CHO cells have shown thermal enhancement of doxorubicin only for temperatures above 42.0–43°C and heat exposure durations shorter than 30 minutes [11]. In rat cells, at 41°C synergism with doxorubicin was present for exposure durations shorter than 1 hour, but at 42°C synergism persisted even for 2-hour heat treatments [12]. More generally, such differences might reflect the heterogeneity in the intrinsic thermochemosensitivity of different cell lines. In vitro thermochemosensitivity screening of human tumors has been reported more recently by Calabro and colleagues [13]. Hyperthermia alone at 42.5°C, but not at 40.5°C, decreased the in vitro growth of 83% (15 of 18) of all tumors, including the six biopsy specimens obtained from human sarcomas at a maximum of heat exposure (120 minutes). Doxorubicin plus heat was synergistic in only 15% of heat-drug combinations that were randomly distributed among the tumors; i.e., no specific pattern was evident for either temperature level or exposure time. Development of heat-doxorubicin antagonism occurred with longer hyperthermic exposure. In contrast, Akiyoshi et al. reported a much higher rate (31%) of synergistic interaction in biopsies of 13 human tumors treated with 42°C hyperthermia plus doxorubicin at three different doses [14]. Although the data cannot be extrapolated to clinical practice without con-

sidering the possible influence of the in vivo effects of hyperthermia, in vitro thermo-chemosensitivity screening of human tumors may assist the selection of appropriate chemotherapeutic agents, including doxorubicin, prior to the clinical application of heat combined-treatment regimens.

Similar to doxorubicin, clinical studies using ifosfamide during the last decade have shown this to be a promising agent in soft tissue sarcoma [15,16] (see Chapter 8). A study by the EORTC combined ifosfamide ($5\,g/m^2$) and doxorubicin ($50\,mg/m^2$) as first-line treatment in 175 cases of advanced soft tissue sarcoma and showed a response rate of 35% [17]. The fact that the addition of ifosfamide to the lower dose of doxorubicin resulted in a response rate that was similar to that produced by the most effective dose of doxorubicin ($75\,mg/m^2$) suggests that ifosfamide is active in this disease.

The ability of hyperthermia to increase the sensitivity of human-derived tumor xenografts in nude mice to ifosfamide has been extensively investigated by Wiedemann et al. [18]. Both ifosfamide or cyclophosphamide alone caused only a transient growth delay of the xenografted tumors, comparable with the effect of hyperthermia (43°C, 1 hour), while the combination of the same dose of the chemotherapeutic agents with heat resulted in complete remissions ($p = 0.001$), respectively. Moreover, the authors could demonstrate that tumor oxygenation was improved during hyperthermia, rather than decreased, most likely due to an increase of tumor blood flow. An extension of this study using human sarcoma xenografts has been recently reported by the same authors [19]. The results for ifosfamide at different doses showed a strong synergistic effect with hyperthermia at clinical relevant temperatures (40–43°C) using tumor-free survival of treated mice as the endpoint for their analysis. These in vitro data confirm previous results on the enhancement of ifosfamide toxicity at mild (41°C) temperature elevations [20].

The only other actual agent for the treatment of soft tissue sarcoma, either as single agent or in combination with chemotherapy, is dacarbazine (DTIC) [21–25]. Thermal enhancement of the cytotoxic effects of DTIC [26], and epirubicin [27] has been reported, whereas no synergistic effect could be observed in this disease for the inactive drugs actinomycin D [28] and methotrexate [29]. Of the other inactive chemotherapeutic agents, several drugs should be also mentioned because of their potential in thermochemotherapy trials.

For trials with regional hyperthermic perfusion, melphalan, actinomycin D, cisplatin, and DTIC have been used in most of the studies. At temperature of 41°C, melphalan toxicity is greatly enhanced, most likely due to increased drug uptake, as shown in vitro [30]. When the increase of antitumoral effects was compared with the increase of normal tissue side effects (e.g., bone marrow) in mice treated with melphalan systemically at 41°C, a therapeutic gain could be observed [31]. In recent work with melphalan-sensitive and -resistant human rhabdomyosarcoma xenografts in athymic mice, thermal enhancement of melphalan-induced tumor growth delay was

observed to be 1.5 and 1.7, respectively, at 42°C for 70 minutes [32]. Hyperthermia has been shown to increase the cellular uptake of cisplatin [33], the amount of DNA crosslinking [34], and the cytotoxic actions of cisplatin [35,36]. More recent results on pharmacokinetics and toxicity also provide a rationale for hyperthermic enhancement of cisplatin effects in vivo [37].

Although the drug etoposide (VP-16, epipodophyllotoxin) had been previously shown to have only minor activity against sarcomas, its combination with alkylating agents can result in synergistic effects [38]. For etoposide combined with heat exposure, a positive effect has been observed in vitro [39], and a recent report showed the potential of etoposide to increase the effectiveness of cisplatin under heat conditions in a preclinical in vivo study [40].

In conclusion, based upon results obtained during the last decade with combination chemotherapy in soft tissue sarcoma of adults, the most active agents are doxorubicin, ifosfamide, and dacarbacine (DTIC). There is substantial evidence from preclinical data that the antitumoral cytotoxicity of each of these chemotherapeutic agents can be enhanced by combining them with appropriate heat exposure to cells or the tumor tissue. In addition, several other drugs with only minor activity in soft tissue sarcoma show a strong potential for the specific strategy of combined thermochemotherapy.

Clinical applications

Systemic thermochemotherapy

As most cancers refractory to convential therapy are systemic diseases, the proposal that whole-body hyperthermia (WBH) in combination with systemic chemotherapy be used to treat metastatic disease is an inherently attractive approach. Several different physical techniques have been utilized for induction of WBH. One technique involved the placement of an arteriovenous shunt and heating of the shunted blood through a countercurrent heat exchanger [41]. The most common techniques induce WBH by transfer of heat through the skin surface [42]. The temperature distributions that develop during WBH are presumably more uniform than those that can be achieved with loco-regional heating, although they are limited to a maximum of 41.8–42°C. A recent detailed study on the temperature distributions in dogs undergoing WBH procedures has verified this general conclusion [43]. Importantly, and depending upon the method used, the several monitored internal temperatures (e.g., tumor, nasal, esophagal, bladder, rectal) can be less than the maximum by one or more degrees Centigrade. The measured intratumoral temperatures were found to be statistically lower ($p = 0.0028$) and more variable than rectal temperature during the plateau phase of WBH [44]. From clinical experience, systems for WBH have warm-up times (time to reach the body target temperature of 41.8°C) of about 1–2 hours,

and this period may likely induce the phenomenon of thermotolerance in tumors, as has been shown experimentally [45]. The physiologic and metabolic stresses of WBH have been described in detail [46], and recent improvement in anesthetic management have resulted in less severe direct complications of this therapy [47].

Rather limited information is currently available on the antitumor effects of WBH combined with chemotherapy. This is largely because phase I studies comprise the majority of the studies published [48]. Only limited information has been provided on the effects of systemic thermochemotherapy for sarcomas. An objective response rate of 36% in 11 patients with soft tissue sarcomas has been observed when doxorubicin (45 mg/m^2) was administered at the beginning of WBH (41.8–43.0°C for 2 hours) followed by cyclophosphamide (1000 mg/m^2) after 6 hours and courses repeated 4-weekly [49]. Besides the two nonresponding patients with mesothelioma, of note is that 3 of 3 patients (1 CR, 2 PR) with liposarcoma and 1 of 2 patients with leiomyosarcoma (1 CR) showed objective responses. However, the observed response rate for these patients who had not received prior chemotherapy is similar to what might be expected from chemotherapy alone. The authors also reported that the mean leukocyte nadir of patients receiving systemic thermochemotherapy was not significantly different from patients treated subsequently with chemotherapy alone. This is in contrast to what has been reported by Engelhardt et al. [50], who observed a significant increase ($p = 0.044$) of bone marrow toxicity with the addition of WBH to systemic chemotherapy (doxorubicin, cyclophosphamide, and vincristine) in a small randomized study on small cell lung cancer.

At the University of Texas, Bull and colleagues [51] have treated 17 patients with metastatic soft tissue sarcomas and osteosarcomas who had previously failed surgery, radiotherapy, and prior chemotherapy regimens. WBH (41.8–42.0°C for 2 hours) was given for a median of three treatments at 6- to 8-week intervals plus BCNU chemotherapy. A tumor response was seen in four patients (1 CR, 3 PR), but the duration of the response was only 4 months.

More recently the potential positive interaction between interferon (IFNαLy) and hyperthermia was clinically tested by Robins et al. at the Wisconsin Clinical Cancer Center [52]. The results of this Phase I trial using WBH (40.5°C for 75 minutes) combined with escalated doses of IFN (maximum 10×10^6 units/m^2) in advanced cancer patients, including five sarcomas, showed no statistically significant difference in toxicity or biological response modulation (e.g., natural killer cell cytotoxicity) between IFN alone or combined modality therapy. Two patients refractory to conventional treatments showed an objective response (2 PR), and three other patients (including two leiomyosarcomas) showed longlasting stable disease. Prior preclinical data demonstrating the thermal enhancement of the antiproliferative effects of IFN [53], together with the clinical safety reported in this Phase I study, encourage further investigations.

The first experience with the combination of WBH and chemotherapy used for refractory cases of malignant tumors in 17 children (including five children with sarcomas) has been reported by Willnow et al. [54]. WBH (41.8–42.0°C for 2–3 hours) was induced by extracorporal blood warming. Vincristine (1.5 mg/m^2) and actinomycin D (0.4 mg/m^2) were given during WBH and cyclophosphamide (600–800 mg/m^2) was applied 1 hour before starting the WBH procedure. Objective regression of metastatic disease was found in 6 of 12 evaluable cases (1 CR, 5 PR). The observed systemic toxicity was severe in two children in whom the body temperature exceeded 42°C (42.3–42.7°C). One of the two children died within 48 hours after the WBH procedure (irreversible cardiac arrest). The authors also noted acute cardiotoxicity in the first two children receiving doxorubicin (20 mg/m^2) plus WBH.

Clinical evaluations of WBH using an extracorporal circuit combined with chemotherapy (cisplatin, doxorubicin, mitomycin C) for advanced miscellaneous cancers in Japan, in a multi-institutional trial recruiting 168 patients was reported by Maeta et al. [55]. Of these patients, 88% had received prior chemotherapy and/or radiation therapy. The overall response rate was 29.5% (39 of 132 evaluable patients), which is most favorable in the subgroup of 14 patients with soft tissue sarcomas. Seven of these patients showed objective regression of their advanced disease. Performance status was the most critical prognostic factor for either response or the occurrence of complications, respectively. Due to the high incidence of severe complications (19.6%) with treatment-related death in 24 patients, the authors stated: 'We should reconsider the bases for selection of patients for treatment with total body hyperthermia and we should pay greater attention to the possible development of fatal complications'.

In reviewing these WBH trials it is difficult to draw any firm conclusions due to the variation in tumor grade, histology, drug(s) administered, drug dose and intensity, heating techniques, and patient history. More carefully designed Phase I/II studies are warranted in order to give an indication of the value of systemic thermochemotherapy.

Isolated hyperthermic antiblastic perfusion

The administration of cytotoxic drugs by hyperthermic perfusion is currently used predominantly for the treatment of malignant melanoma but also for soft tissue sarcoma of the limbs. Treatment techniques for hyperthermic antiblastic limb perfusion, its specific rationale, clinical trials at several institutions and their results for different stages of melanoma, as well as the complications and side effects of the procedure have been recently reviewed [56]. The same rationale has guided the extension of this management to the treatment of soft tissue sarcomas. The concept and technique of regional chemotherapy using intra-arterial infusion of a cytotoxic drug—as first proposed in 1950 by Klopp [57]—was introduced into clinical use for the

first time in 1957 by Creech and colleagues [58]. Stehlin et al. [59] proposed the association of hyperthermia with the concept of regional perfusion chemotherapy, stressing the importance of a synergistic effect of both hyperthermia and chemotherapy. The technique of isolated hyperthermic limb perfusion using an extracorporal circuit—as already described for WBH—allows high doses of a selected drug to be brought to a precise region. At the same time no systemic toxicity (e.g., bone marrow) should occur because blood escape via collateral circulation towards the general circulation is prevented by placing a tourniquet above the arterio-venous access.

There are optimistic early reports of perfusion of limbs with L-PAM (L-phenylalanin mustard = melphalan) and actinomycin D for soft tissue sarcomas. A total of 37 patients (9 lipo-, 7 synovial-, 6 rhabdomyo-, and 4 fibro-sarcomas, and 11 other histologic subtypes) with soft tissue sarcomas of the extremities were entered in a multidisciplinary approach utilizing hyperthermic perfusion and external irradiation combined with radical local excision [60]. Improvement of local tumor control, permitting limb-sparing procedures, has been claimed, but no data on objective regressions at the time of local treatment have been presented. The overall local control rate was 80% and the amputation rate was 10%. As a reference treatment for comparison of these results, the authors discussed their previous results [59] as historical controls in which the perfusion technique was not altered to include heated blood. Lethi et al. [61] reported on 64 patients with soft tissue sarcomas of the extremities using regional hyperthermic perfusion (40.0°C for 1 hour) with L-PAM and actinomycin D, either before (37 patients) or after (27 patients) surgical resection of the tumor. Postoperatively, about half of the patients received (50 Gy) external beam radiation. For all patients the local recurrence rate at 2 years was 3.4% and at 5 years was 11.1%. The overall 5-year survival rate was 67%. Unfortunately, no data on objective tumor response were presented for patients who received hyperthermic antiblastic perfusion before definitive surgery. The group at the University of Groningen [62] treated 14 patients (8 primary and 6 recurrent high-grade soft tissue sarcomas) with isolated hyperthermic (39–40°C for 1 hour) or normothermic (two cases) perfusion (L-PAM + actinomycin D) followed by wide excision. None of the patients received systemic adjuvant chemotherapy or external-beam radiotherapy. During the follow-up (median, 13 years) five patients developed distant metastases and one other patient showed recurrent local disease after 48 months. The actuarial 5- and 10-year survivals were 69%.

In a small series [63] of 15 patients with limb sarcomas treated by hyperthermic perfusion (39–40°C for 1 hour) with L-PAM + actinomycin D, local recurrence was seen in 4 of 8 patients with recurrent disease at presentation, but in none of 6 patients with primary sarcomas after 30 months of follow-up. In an attempt to search for agents other than L-PAM or actinomycin D that can be safely utilized, Fletcher and colleagues [64] used cisplatin for

hyperthermic limb perfusion in soft tissue sarcoma and melanoma of the extremities. Of 35 patients with primary soft tissue sarcoma, 15 had high-grade tumors and 20 tumors were greater than 5 cm in diameter. The applied doses of cisplatin ranged from 0.75 to 2 mg/kg during perfusion at 40°C for 1 hour. Among the 29 patients who underwent hyperthermic perfusion before definitive resection was performed (approximately 1 month later), only 17 patients were evaluable for local response due to the lack of measurable disease in the other patients. An objective response has been observed in eight patients (3 PR and 5 MR), where a minimal response (MR) was defined as less than 50% reduction in the product of the dimensions of the measured lesion. At last follow-up (mean 16 months), none of the 35 sarcoma patients had loco-regional recurrence but five patients had developed distant metastases.

A detailed description of the clinical results at the Regina Elena Cancer Institute using isolated hyperthermic limb perfusion for extremity sarcomas has been previously reported [65]. In a pilot study recruiting 22 patients, hyperthermic perfusion without chemotherapy but at a minimum temperature of 42°C for at least 2–4 hours was performed with satisfactory tumor regressions (11 of 22 patients) but unacceptable mortality (four patients with postoperative toxic deaths). In consecutive studies, L-PAM (0.8 mg/kg) and actinomycin D (0.015 mg/kg) or cisplatin (2.5–5 mg/kg) were employed as antineoplastic drugs, combined with a less aggressive regimen of hyperthermic perfusion (41–41.5°C for 2 hours). In 68 patients treated, two toxic deaths occurred (2.9%) and two other patients underwent amputations due to severe late complications. After cisplatin doses of 3.2 mg/kg during hyperthermic perfusion, the patients showed no significant local or systemic toxicity, while high-dose cisplatin (5.0 mg/kg) was associated with severe complications in two patients.

In a first series of 30 patients, following hyperthermic antiblastic perfusion (HAP) definitive surgery was performed with an amputation rate of 42%. As the local tumor response to HAP and the duration of loco-regional control were unsatisfactory, the protocol was modified to include continous intraarterial (i.a.) doxorubicin infusion (10 mg/24 hr for 10 days), generally initiated within 10 days after HAP followed by delayed surgery (at the tenth week) after three cycles. Alternatively, patients received only 45–60 Gy of external-beam radiation within 4 weeks after HAP administration prior to surgery. According to the most recent retrospective analysis on 70 evaluable patients presented by Di Filippo et al. [66], the radiotherapy-including protocol resulted in an amputation rate of zero and locoregional control of 94%, which is slightly better than the HAP + i.a. doxorubicin regimen, but the overall 5-year survival of patients is comparable (70% versus 73%, respectively). From these results it also became clear that HAP alone is insufficient for local control and for the improvement of long-term survival for stage III–IVA recurrent soft tissue sarcomas.

In general, because there was no control group treated with normothermic

antiblastic perfusion in either of the reported studies, we do not know how much the hyperthermic enhancement of drug effects or direct thermal cytotoxicity of hyperthermia has contributed to the overall therapeutic effect of the combined modalities. The role of hyperthermia in perfusion therapy for soft tissue sarcomas needs to be more clearly defined in controlled trials.

Regional hyperthermia (RHT) combined with systemic chemotherapy

With the exception of arterio-venous perfusion, clinical hyperthermia is usually achieved by exposing malignant tissues to high-intensity electromagnetic or ultrasonic fields [67]. Using electromagnetic (EM) energy for noninvasive heating, the field sources are external to the patient and the heating modality and techniques are dictated by the characteristics of the clinical problem (e.g., size, depth, anatomical location). A coherent array of radiating sources can be used for EM energy deposition in deep-seated tissues, and there are several reasons for using the phased-array approach for deep heating. The energy sources can be arranged so that the electric field is along the body axis, and therefore predominantly tangential to the subcutaneous fat/muscle interface, avoiding the problem of excessive fat heating. It also allows a limited amount of energy spent on focusing or steering within the exposed tissue region. This focusing of energy can be accomplished by manipulating the relative phase and amplitude of the external sources.

The array device most widely used is the annular phased-array system (BSD Corp., Salt Lake City, Utah), and its technical design and clinical potential have been well documented [68,69]. The body region to be treated, such as the pelvis, is surrounded by an annular configuration (60 cm diameter) of the radiating antennae, which are coupled to the patient contour with a water bolus. The frequency used for phased-array devices suitable for heating tissue deep in the pelvis is in the range of 70–100 MHz. Smaller array designs for limb heating (30 cm diameter) or for the treatment of children (40 cm diameter) have been developed recently and are also capable of being operated in a phase- and amplitude-controlled mode.

Accurate assessment of the treatment-induced temperature distributions in tumor and surrounding tissues has been essential for the evaluation of RHT treatments. At present, only invasive techniques are possible. These are routinely performed in the clinic by percutaneous or intra-operative insertion of one or more Teflon® thermometry catheters across the diameter of the tumor within the field of the planned RHT. Insertion of thermistor probes into the lumen of the catheters immediately before RHT and the use of a semiautomatic thermal mapping system [70] allow measurements of temperatures mapped along the axis of each thermometry catheter at fixed intervals (0.5 or 1 cm), repeated every 5 minutes. These allow temperature-time profiles to be obtained for a larger number of spatial points for each probe insertion.

Regional hyperthermia (RHT) combined with radiation, using the BSD system for deep-seated tumors, has been extensively studied at several institutions in Phase I trials [71]. Because of the potential to target heat delivery to deep-seated tumors, including the adjacent surrounding tissue (tumor bed) while simultaneously applying conventional doses of systemic combination chemotherapy to patients, the Klinikum Großhadern Medical Center (KGMC) started the first pilot study in 1986 employing the BSD system for RHT with systemic chemotherapy [72].

Twelve patients with advanced deep-seated tumors, including seven retroperitoneal soft tissue sarcomas, were entered who had shown progressive disease under conventional treatment protocols. Patients with soft tissue sarcomas received 4-weekly cycles of ifosfamide (1500 mg/m^2, days 1–5) plus etoposide (100 mg/m^2, days 1, 3, and 5), combined with RHT given only on days 1 and 5. In 68% of the RHT treatments for pelvic tumors, the intratumoral temperature exceeded 42.5°C, with a minimum temperature of at least 40°C. Subacute toxicity and the complication rate were acceptable, although pain within the field of the applicator (44% of RHT treatments) was a limiting factor to achieve the prescribed 1-hour treatment duration. Of note, myelosuppression was not increased by the addition of RHT.

From these encouraging results in regard to feasibility and toxicity, the RHT-86 protocol has been activated at the KGMC as a Phase II study combining ifosfamide plus etoposide with RHT in patient with locally advanced sarcomas. An interim report on 40 patients (27 pretreated with multi-agent chemotherapy, 10 with surgery and/or radiation), including 25 soft tissue sarcomas, detailed the information on histology, tumor grade, size and localization of the treated tumors, and patient history [73]. In 38 assessable patients, the overall objective response rate was 37% (6 CR, 4 PR, and 4 pathohistologic responses, i.e., >50% necrosis found at the time of subsequent complete surgical resection). More importantly, since the local tumor response related to the combined treatment was defined as the endpoint for this study, comparison of the time-averaged temperature parameters (T_{20}, T_{50}, and T_{90}) of objective responders (14 patients) with patients showing local progressive disease under thermochemotherapy (13 patients) showed a significant difference ($p < 0.01$). These data suggest the activity of the combined regimen in pretreated patients with advanced sarcomas and that clinical outcome was dependent upon the intratumoral temperatures achieved during RHT.

A more recent updated report on the Phase II study [74] confirmed the previous results on this combined treatment modality for 65 patients (mean observation time for all patients, 10.1 months). The results of the tumor response (overall response, 34%) for these patients, including 43 soft tissue sarcomas, are summarized in Table 1. Sixty-two percent (40 patients) of these patients had received ifosfamide-containing drug regimens before entering the RHT study, 26% (17 patients) were pretreated by surgery and/or radiation, and 12% (8 patients) were treated primarily. A total of

Table 1. Tumor response

Histology	N	No. treatments	NED	PR	NC	PD	n.e.	Alive
Soft tissue sarcoma	43	268	11	2	15	14	1	21/43
Leiomyosarcoma	11	63	1 pCR/1 FHR	1	3	4	1	5/11
Liposarcoma	9	56	2 pCR/2 FHR	1	2	2		5/9
Rhabdomyosarcoma	8	66	2 pCR/2 FHR		3	1		6/8
Malignant fibrous histiocytoma	5	32	1 FHR		3	1		2/5
Others	10	51			4	6		3/10
Ewing's sarcoma	12	82	3 pCR		7	2		6/12
Chondrosarcoma	7	54	1 pCR/1 FHR	2		1	2	3/7
Osteosarcoma	3	22	1 FHR			1	1	1/3
Total	65	426	$17^{\text{pCR } 9}_{\text{FHR } 8}$	4	22	18	4	31/65

NED = no evidence of disease; CR = complete response; PR = partial response; FHR = favorable histological response; p = pathohistological; n.e. = not evaluable.

426 RHT treatments (mean 6.6. RHTs/patient) were applied predominantly for pelvic tumors (82%). The duration of response was satisfactory; i.e., following CR the patients showed a disease-free survival of 15.6 months at the cutoff date for the analysis. Of the patients with PR and FHR, three died from metastatic and/or local disease after 4, 17, and 39 months, and one patient died from other disease (AML) after 27 months. The other eight patients remained stable for a mean of 14.1 months.

Again, the time-averaged temperature achieved in 20%, 50%, and 90% of all measured tumor sites taken from the RHT treatments differed significantly between responders and nonresponders ($p < 0.01$). The correlation between the tumor response with the averaged temperatures in a given fraction of measured tumor points ($T_{\%}$ values) is shown in Figure 1. By taking into account tumor temperature (T_{20}) and temperature difference or gradient as measured within the tumor ($T_{50}-T_{90}$) of each individual patient of both groups, this retrospective analysis allowed the correct classification of responders and nonresponders in most cases. An interesting observation from the analysis of these temperature data is that the descriptors that were most predictive of treatment outcome are not in a temperature range that is known to be particularly cytotoxic (i.e., $T_{20} = 41.5°C$, $T_{50} = 40.9°C$, and $T_{90} = 40.0$) but well in the range of thermal chemosensitization.

The data are in accordance with the model relating the efficacy of RHT treatments to spatial characteristics (T_{50}-, T_{90}-analysis) of the temperature distribution developed at Duke University by Oleson and collegues [75]. In a series of 45 patients with soft tissue sarcomas using preoperative hyperthermia combined with conventional radiation therapy, the percentage of necrosis (>80%) of the sarcoma upon extensive histopathologic examination of the resected specimen was considered to be a successful treatment outcome. The authors demonstrated in a previous report [76] that two RHT treatments

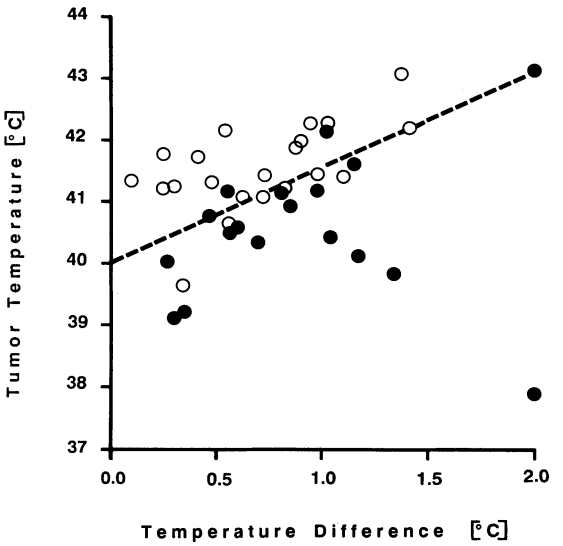

Figure 1. Plot of time-averaged tumor temperature (T_{20}) versus temperature difference ($T_{50} - T_{90}$) calculated for 20%, 50%, and 90% of all measured tumor points within the tumor of each individual patient (n = 39). ○, responders (n = 21); ●, nonresponders (n = 18); . . . . , arbitrarily chosen to separate responders (above) and nonresponders (below).

per week (arm B) were superior to one RHT treatment (arm A) with regard to the degree of histopathological changes, and that the spatial temperature distribution (T_{50}-, T_{90}-analysis) was related to this biological endpoint. More recently, the same group showed that a descriptor of both the frequency distribution of intratumoral temperatures (such as $T_{\%}$) and the mean averaged treatment time (cumulative minutes $T_{\%} >$ °C) was the strongest predictor of the histopathologic outcome [77].

At the KGMC, a pilot study (RHT-91) was started in November 1990 for high-risk soft tissue sarcoma patients using preoperative systemic EIA-chemotherapy combined with RHT. The design of the study is shown in Figure 2. By definition, tumor size >8 cm and/or extracompartmental tumor location (group A) or recurrent disease at presentation (group B) were considered as high-risk factors, in addition to tumor grade (II or III). For EIA systemic chemotherapy, patients receive etoposide (125 mg/m^2 × 3 × 3, days 1 and 4), ifosfamide (1.25 g/m^2, days 1–4), Adriamycin (50 mg/m^2, day 1), and mesna (250 mg/m^2, days 1–4) with RHT only given on days 1 and 4 in repeated EIA/RHT cycles every 3 weeks. Evidence that support the use of systemic, preoperative induction chemotherapy alone to render soft tissue sarcomas resectable or to improve local control has been presented previously [78,79]. Our first experience with the RHT-91 study [80] on 22 evaluable patients (20 soft tissue sarcomas and 3 chondrosarcomas) show an objective clinical response of 27% (6 PR) before surgery, and in another

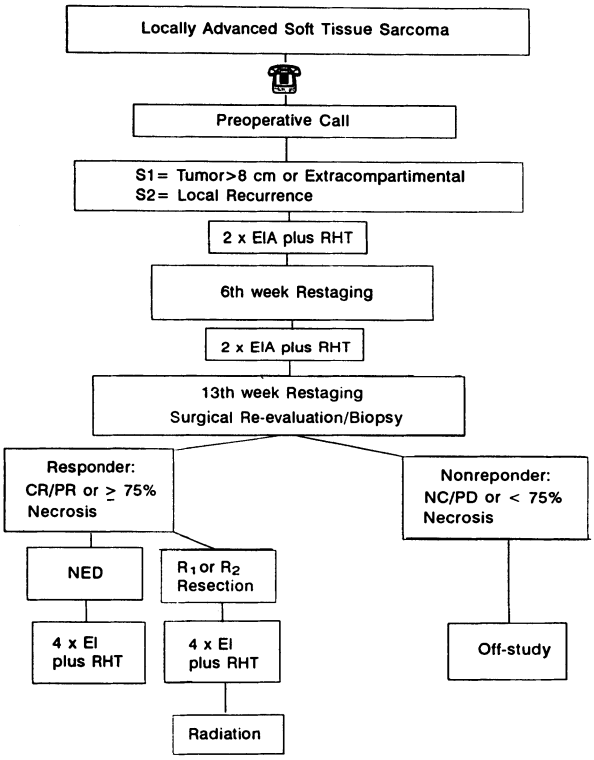

Figure 2. RHT-91 Study.

four patients (4 FHR) a pathologic response to preoperative chemotherapy (>50% necrosis). Because the goals of this study are the improvement of local control and long-term survival, follow-up will be essential for further evaluation.

Conclusions

The clinical application of hyperthermia combined with chemotherapy has a strong, well-documented biological rationale. Clinical results using 'thermochemotherapy' in far-advanced, either locally (surgery, radiation) or systemically (chemotherapy) pretreated patients with soft tissue sarcomas clearly indicate therapeutic activity of the combined modality in several of the studies referenced. Currently continuous multipoint thermometry is available, which indicates that it is not justified to speak about the tumor temperature, but rather a distribution of temperatures, which is heterogeneous not only within the tumor tissue but also in treatment time. The time/temperature descriptors of hyperthermia treatments showing a strong cor-

relation to treatment outcome (e.g., histopathological, clinical response) can now be tested prospectively along with other prognostic important factors in patients on the risk for local or metastatic disease.

The use of WBH combined with systemic chemotherapy has to be further tested in patients with advanced disease (Phase I/II trials). However, in the future WBH might play its most significant role in an adjuvant or even preoperative (neoadjuvant) setting, in order to enhance the cytotoxicity of the earliest possible chemotherapy treatment against occult systemic micrometastasis. For the more loco-regional applications of hyperthermia combined with chemotherapy (either using isolated limb-perfusing or noninvasive regional heating), controlled prospective trials (Phase II/III) for high-risk groups of patients with soft tissue sarcomas are warranted. The potential for isolated limb perfusion might be the use of chemotherapeutic agents (e.g., cisplatin) at a high dose but an acceptable temperature (e.g., 41°C). The histopathological outcome of resected sarcomas after thermochemotherapy could be an important guideline for further systemic treatment.

As the RHT approach allows full-dose systemic standard chemotherapy combined with tumor temperature elevation, this strategy may have an important role in the preoperative treatment of soft tissue sarcomas. Prospective trials are warranted.

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11. Soft tissue sarcomas in children

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Investigators from Europe and the United States meet periodically to define prognostically significant classifications for the soft tissue sarcomas (STS) of children. International teams of pathologists have developed classification systems based on classic histologic features, including that of the International Society of Pediatric Oncology (SIOP). Accurate classification facilitates both the diagnosis and assessment of the probability of metastasis [1–5].

This chapter focuses on recent reports from the literature regarding rhabdomyosarcoma (RMS), the most common STS in persons under age 21, and nonrhabdomyosarcoma soft tissue sarcomas (NRSTS). Ongoing clinical trials, late effects of treatment, molecular biologic tests, and syndromes associated with childhood STS are discussed.

Rhabdomyosarcoma

Histologic and biologic studies

The conventional histologic classification for childhood RMS comprises embryonal (60% of cases), alveolar (20%), pleomorphic (<5%), and undifferentiated tumors (15%). Several biologic features of tumor cells were recently shown to improve the classification of RMS and to correlate with outcome. For example, Shapiro and colleagues [6] studied the DNA content (ploidy) of RMS cells in relation to histology and response to therapy. About one third of their patients had diploid tumor stem lines, regardless of histologic subtype. Hyperdiploid tumors (1.10–1.80 times the DNA content of normal diploid cells) are usually embryonal, whereas near-tetraploidy (1.80–2.60 times normal DNA content) is strongly associated with alveolar histology. In patients with unresectable metastatic tumors, hyperdiploidy conferred the best prognosis and diploidy the worst. Tumor cell ploidy was the best predictor of treatment outcome for patients with alveolar or embryonal RMS. Patients with unresectable diploid RMS are thought to have an unacceptably high risk of treatment failure.

A specific chromosomal abnormality, t(2;13)(q35;q14), is common in

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childhood alveolar RMS [7]. Patients with this translocation often present with widespread metastatic disease. Roberts et al. [8] studied the *GLI* gene, originally identified as amplified in cells from a human malignant sarcoma. The *GLI* gene sequences that might be amplified appear restricted to tumors with primitive histologic features, perhaps reflecting overproduction of a gene product able to influence gene expression during early mesenchymal cell development. Other investigators have indicated that nearly half of all embryonal RMS contain mutations in the N-*myc* [9] and N-*ras* or K-*ras* genes [10], suggesting that *ras* gene mutation is implicated in the development of epithelial and hematologic neoplasms. There is no statistically significant correlation between N-*myc* amplification and age, site, gender, stage, or survival of individuals with alveolar RMS [9].

Rhabdomyosarcoma and skeletal muscle have been shown to express the muscle regulatory gene MyoD1 [11,12]. The MyoD1 protein has been shown to be restricted to normal tissues of fetal skeletal origin and to RMS in surgical samples and laboratory cell lines. Its expression indicates commitment to myogenic differentiation and may be diagnostic of RMS and helpful in the classification of pediatric STS in general.

Reuland et al. evaluated the Fab fragment of a radiolabeled monoclonal antimyosin antibody [13]. Tracer uptake was observed in tumor sites in 7 of 13 RMS patients. Those with complete responses who had weak residual positive uptake in the primary tumor site were considered to be false-positive. Diagnosis of antimyosin-positive tumors with the aid of the immunoscan was more predictive of tumor control than were clinical findings in combination with ultrasound and computed tomography or histologic diagnosis by tumor biopsy.

Parham et al. [14], for the Intergroup Rhabdomyosarcoma Study (IRS), evaluated various immunohistochemical stains against 109 pediatric solid tumors, primarily RMS. Immunostaining was performed using monoclonal antibodies to desmin, neurofilaments, vimentin, cytokeratin, and leukocyte common antigen. Other studies included stains with anti-muscle-specific actin and polyclonal antibodies to desmin, creatinine kinase M subunit, myoglobin, and neuron-specific enolase. It was concluded that the combined use of anti-desmin and anti-muscle-specific actin enhanced the diagnosis of childhood sarcomas, especially when used with other techniques, such as electron microscopy.

The incidence of various unclassified soft tissue sarcomas decreased in the Kiel Pediatric Tumor Registry from 1982 to 1989, mainly because of the availability of antibodies to different types of intermediate filaments, muscle-specific actin, myoglobin, and the markers for neuron-specific enolase and protein S-100 [15]. Despite these achievements, not all cases could be diagnosed with certainty.

Chan and colleagues [16] demonstrated that immunohistochemical detection of P-glycoprotein at diagnosis correlates positively with a poor outcome for patients with RMS and undifferentiated sarcomas. Although most patients

responded to chemotherapy, early development of tumor recurrence was associated with P-glycoprotein positivity at diagnosis. Tumors that were P-glycoprotein negative at diagnosis were often positive for the stain at relapse. Other studies have failed to demonstrate a definitive association among P-glycoprotein positivity at diagnosis, response to therapy, and outcome [17]. Additional studies using tumor material from the IRS Committee and from St. Jude Children's Research Hospital are in progress.

Recent clinical trials

Head and neck RMS. The group from the University of Milan [18] reported on primary intracranial RMS involving two patients with lesions arising in the posterior fossa and frontotemporal region, respectively. Both underwent surgical removal of a mass, had an early recurrence of tumor, and died shortly thereafter.

Gasparini and colleagues [19] from Milan reported on the questionable role of central nervous system (CNS) radioprophylaxis in the therapeutic management of children with meningeal extension of childhood RMS. Among 15 consecutive patients with nonorbital RMS with meningeal spread, combination chemotherapy followed by radiation therapy (60 Gy) to the primary tumor plus intrathecal methotrexate yielded responses in 13, 4 of whom developed non-CNS relapse. The 3-year progression-free survival was 59%. These results led the investigators to question the need for whole-brain irradiation for these patients and to suggest that children with RMS with meningeal extension who receive combination chemotherapy plus wide local field irradiation have progression-free survival similar to that of patients who receive whole-brain irradiation.

The third IRS study (IRS III) evaluated the efficacy of reducing the volume of irradiation for children with cranial parameningeal RMS [20]. In patients with base of skull erosion or cranial nerve palsy (but no intracranial soft tissue extension), the use of wide local volume irradiation resulted in a 3-year survival of 82%. IRS II, using the full cranial volume for similiar cases, had resulted in a 3-year survival of 68%. Thus, decreasing the volume of irradiation in patients without an intracranial extension of tumor did not jeopardize local tumor control or survival.

Representatives of SIOP reported a 4-year event-free survival of 62% for 34 patients with nonmetastatic head and neck RMS or other sarcomas treated with a primary ifosfamide/vincristine/dactinomycin (IVA) regimen [21]. Eleven of 34 patients failed with local recurrences, nine of whom were salvaged with local irradiation and additional chemotherapy. Three 'salvaged' patients later developed distant metastases, and two of these patients died. These investigators emphasized the occurrence of local failure and the apparent lack of compromise in ultimate survival rates while avoiding the late effects of irradiation in two thirds of cases.

An update of orbital RMS treated without enucleation with combination

chemotherapy and radiotherapy described cataract development 1–4 years after completion of radiation therapy in most patients [22]. These children generally do not tolerate contact lenses well and are best served by primary intraocular lens implantation if they cannot wear lenses.

Five of 126 patients with RMS of the head and neck at the Institut Gustave Roussy had primary disease located in the larynx [23]. These patients were between $5\frac{1}{2}$ and $13\frac{1}{2}$ years of age and presented with dysphonia and/or dyspnea. Treatment included chemotherapy and radiation therapy (45 Gy over 5 weeks). All five remained disease-free, although several developed significant sequelae. None required laryngectomy.

The results of the treatment of head and neck sarcoma at the Children's Hospital in Philadelphia indicated that among 60 children aged 3 months to 18 years treated between 1970 and 1987, the mortality was 50% for those treated 1970–1979, which with improved management decreased to 23% for those treated from 1980 to 1987 [24].

Wide-field CNS radiation therapy with repeated lumbar intrathecal medications was used to treat patients with parameningeal RMS on the IRS protocols from 1977 to 1987 [25]. Five patients developed ascending transverse myelitis 5–9 months after the completion of radiation therapy. Limiting the administration of intrathecal methotrexate-hydrocortisone-ara-C therapy to four doses avoided further complications.

In general, it can be commented that head and neck RMS sites, which account for about one third of the primary sites of this tumor, have been treated more successfully in recent years in comparison to the past. Enucleation is no longer required for orbital RMS, which rarely metastasizes. Small irradiation therapy portals are now possible because of more accurate delineation of tumor by computerized tomography and magnetic resonance imaging.

Genitourinary RMS. The paratesticular region continues to be one of the more prognostically favorable sites for RMS [26,27]. Hamilton et al. [28] described 15 patients treated at the Royal Marsden from 1974 to 1986. Most presented with stage I disease; 6 of 10 remained continuously disease-free. The authors commented that efficacious chemotherapy has diminished the roles of surgery and radiotherapy after radical excision for stage I disease. The authors caution that aggressive multimodality approaches are relevant for metastatic disease.

The Memorial Sloan-Kettering Cancer Center reported on 28 patients with paratesticular RMS. All received chemotherapy, and 20 received radiation therapy; 16 have survived more than 5 years without evidence of disease [29]. Multivariate analysis showed that unresectability was the most important predictor of mortality.

The IRS I and II reported sequelae of therapy for paratesticular RMS in 86 children and adolescents [30]. Bowel obstruction was noted in some patients, loss of normal ejaculatory function in eight, development of

hydrocele in five, and lymphedema of the leg in five; four additional patients had chronic diarrhea, two had urethral strictures and urethritis, and four had bone or soft-tissue hypoplasia in the field of radiation therapy. One third of those receiving cyclophosphamide developed hemorrhagic cystitis.

The group from Dana-Farber Cancer Center indicates a high survival rate for patients with paratesticular RMS but warned that chemotherapy and radiation therapy are not substitutes for radical surgical procedures in children with bladder or prostatic RMS [31]. Reports from the IRS [32,33] indicate that 33 of 154 patients with primary bladder RMS admitted from 1972 to 1976 underwent total excision by partial cystectomy and also received multiagent chemotherapy; 18 of the 33 patients classified with groups II–IV disease received radiation therapy. Twenty-five of 26 survivors of partial cystectomy had a functional bladder 3 or more years after diagnosis. The estimated 3-year survival after partial cystectomy was 79%.

Raney et al. [34] evaluated primary chemotherapy with and without radiation therapy and/or surgery for children with localized genitourinary tract tumor and compared the results of IRS I and II. Chemotherapy comprised of vincristine/dactinomycin/cyclophosphamide (VAC). The survival of patients treated with primary chemotherapy was 70%, compared to the 78% survival of patients who received primary surgical treatment in IRS I. However, 3-year disease-free survival was inferior in IRS II, and treatment with primary chemotherapy followed by radiation therapy and/or surgery failed to improve the bladder salvage rates for patients on IRS II.

At Memorial Sloan-Kettering, 25 patients with bladder and bladder prostate RMS were treated from 1970 to 1985 [35]. Complete surgical resection was performed by a total cystectomy in 14 patients and a partial cystectomy in two. All patients received chemotherapy in dosages defined by the T2 and T6 protocols. There were 11 disease-free survivors, most of whom had been followed for more than 6 years.

Hays et al. [36] evaluated the improved survival and bladder preservation among patients with bladder/prostate primary tumors in IRS III. Patients were treated with pulse VAC and radiation (2500 or 4500 cGy). Preliminary 3-year disease-free survival was 90% compared to 70% for similar patients in IRS II.

The IRS committee reported normal bladder function in 38 of 52 patients for whom information was available 5–15 years after chemoradiation; nine patients were incontinent and five had urinary frequency. Of a total of 109 patients, 29% had hematuria. Almost all patients retained adequate renal function [37].

The many reports on the results of treatment of genito-urinary RMS detail the progress that has been made in treatment of these patients. Paratesticular RMS rarely requires a retroperitoneal lymph node dissection, which may be associated with significant late morbidity. RMS of the dome of the bladder is generally amenable to surgery, without sacrifice of the bladder and its attendant morbidity. In spite of bladder salvage of increas-

ing numbers of children with bladder-prostate RMS, morbidity remains significant.

Truncal and extremity RMS. Review of perineal RMS treated on IRS I or II indicated that most of the 36 cases had an alveolar histology [38]. Six of 35 patients had metastatic disease at diagnosis. Twenty-eight of 36 patients achieved a complete response, with a 3-year disease-free survival of 42%, compared to 52% for all of the patients on the combined IRS I and II trials.

Hudson et al. [39] reported on 11 patients with perineal-perianal RMS, most of whom had an alveolar histology and metastatic disease. Six are long-term survivors. The Memorial Sloan-Kettering group evaluated 35 patients with extremity RMS treated from 1970 to 1985 [40,41]. Alveolar and embryonal histologies were equally distributed. Patients were treated with similar chemotherapy protocols. Variables evaluated included invasiveness, size of primary tumor, anatomic location, regional lymph node involvement, distant metastases at diagnosis, completeness of surgical resection, amputation, and alveolar histology. All variables but anatomic locations and amputation were significant. By multivariate analysis, local tumor invasiveness was the most significant predictor of fatal outcome.

RMS of the trunk remains particularly troublesome in relationship to the treatment that is required and the results obtained. Tolerance of the bowel to irradiation significantly affects delivery of the radiation dosages required to cure retroperitoneal tumors. Many of these lesions have alveolar histology, which, irrespective of the stage of the primary tumor and age of the patient, is associated with a poor prognosis. The alveolar histologic pattern is more often associated with extremity lesions, with early metastases to lymph nodes and other sites.

General studies

Outcomes for infants and older children treated at the Institut Gustave Roussy from 1955 to 1984 were reviewed and compared [42]. No significant differences in male/female ratio, primary sites, or clinical stage were noted. However, there was a higher frequency of alveolar and poorly differentiated histology in infants. The overall 5-year survival was similar for infants and older children, regardless of histology.

The Italian Cooperative Studies of RMS indicated the prognostic significance of histology related to overall outcome, irrespective of the clinical grouping [43]. The most favorable outcome was associated with embryonal histology. Heyn et al. [44] evaluated for the IRS the effects of intensive chemotherapy on children with localized alveolar RMS. Patients given more intensive chemotherapy with pulse VAC in IRS II had a longer disease-free survival (69% versus 43% at 3 years) than individuals with the same diagnosis treated on IRS I, but this was of marginal significance, as was the overall

survival difference (77% versus 57% at 3 years). There was only limited statistical evidence that more intensive therapy improved the prognosis for children with localized alveolar RMS of the extremity.

In a SIOP protocol involving 63 patients with alveolar histology and varying primary sites, local recurrence was observed in 21 patients and distant metastases (including local simultaneous relapse) in three at a median of 15 months after achieving remission [45]. Lymph node involvement and metastatic disease at diagnosis were unfavorable prognostic factors.

A review of 103 patients treated for RMS at Memorial Sloan-Kettering from 1970 to 1990 indicated that embryonal RMS metastasized almost exclusively to the lung. No patients with embryonal RMS developed marrow disease as the sole site of metastasis. Alveolar RMS frequently metastasized to the bone and marrow [46].

A report from the Japanese Pediatric Registry [47] indicated that clinical stage was significantly correlated with overall survival, and that age, sex, histology, and primary site had no independent prognostic significance for tumor-free survival. Patients who had undergone total excision of their tumors had a better outcome than those who had not. Survival rates improved with the introduction of more effective chemotherapy.

Etcubanas et al. [48] reviewed the incidence of RMS presenting as a disseminated malignancy from an unknown site. The authors concluded that the approach to such pediatric patients should be directed toward the histopathologic diagnosis rather than toward establishing the primary site. Results of these studies were updated by Kuttesch et al. [49], who extended the evaluation to patients with other types of malignant neoplasms, including small round-cell tumors. They suggested that certain biologic subtypes of solid tumors may disseminate in the absence of a recognizable primary site and that chemotherapy should be tumor specific rather than site specific.

A St. Jude Children's Research Hospital study indicated the value of magnetic resonance imaging (MRI) in detecting residual disease after initial chemotherapy and/or radiation therapy for RMS and other soft tissue sarcomas [50].

Results of specific chemotherapy trials

Pinkerton et al. [51] treated 43 patients with malignant soft tissue sarcomas, categorized as clinical group II–IV, with six courses of VAC. Thirty-six patients received high-dose mephalan with autologous bone marrow rescue. Twenty-six patients ultimately received irradiation. The VAC chemotherapy, administered rapidly on an outpatient basis, was well tolerated. It was hypothesized that a conservative approach to radiation and surgery would minimize late sequelae.

The IRS IV evaluated ifosfamide/doxorubicin and IVA [52]. In the study, which was designed to test the toxicity and response rates of patients with

clinical group III RMS, 47% of the patients obtained a complete response and 35% a partial response. Common toxicities included neutropenia, mucositis, seizures, peripheral neuropathy, and nephrotoxicity.

An additional pilot study by the IRS [53] evaluated ifosfamide/doxorubicin followed by VAC and hyperfractionated irradiation (110 cGy twice daily to a total dosage of 5940 cGy). The overall response rate to the drug pair was 75%. Toxicity, which consisted primarily of neutropenia, was deemed acceptable.

Kung et al. [54] followed up their earlier study of ifosfamide/etoposide using escalating dosages of carboplatin [55]. Dose-limiting toxicity was severe myelosuppression, hematuria, and bacterial infections. There were no toxic deaths. The earlier evaluation indicated a greater response rate with the ICE therapy than with ifosfamide/etoposide alone.

Schmidt et al. [56] reported on the treatment of mesenchymal tumors that failed to respond to treatment with IVA in the malignant mesenchymal tumor study (MMTs) 84. Twenty-eight of 216 patients who failed to respond to IVA received cisplatin/doxorubicin. Five of these 28 children achieved complete responses, five attained a partial response, and in three patients tumor size increased. Only 2 of 28 patients remained in complete remission after chemotherapy alone.

Weiner et al. [57] evaluated responses in 257 group III patients enrolled in IRS III, excluding patients with certain pelvic sites. There were 109 second operations performed in 21 patients with a clinical complete response, 66 with a clinical partial response, and 52% of those with no clinical responses to chemotherapy prior to radiation therapy. Of those patients with a partial or no response based on clinical criteria, 74% were recategorized as pathologic complete responders. This recategorization increased the survival percentages and established a more valid response status.

Koscielniak et al. [58,59] reported on the treatment of 344 previously untreated patients with soft tissue sarcomas who were enrolled in the German soft tissue sarcoma study CWS81. Most had RMS. Chemotherapy for Stages I–III comprised vincristine/dactinomycin/cyclophosphamide/doxorubicin. Patients who failed to respond and who had metastatic disease received ifosfamide instead of cyclophosphamide. There was no difference in outcome between patients with Stage I and II disease (88% disease-free survival in both groups); however, the disease-free survival for Stage III was 54% and for Stage IV it was 11%. The most important prognostic factors were tumor size and degree of tumor regression after primary chemotherapy. Lack of tumor control was the main cause of failure. Patients with primary unresectable tumors who achieved complete remission after 7–9 weeks of chemotherapy had a disease control rate similar to those having total resection.

Carli et al. [60] reported the experience of the German and Italian Cooperative Studies for childhood RMS, which used various dosages of ifosfamide/dactinomycin/doxorubicin/vincristine. Preliminary data indicated a trend toward an improved response rate, with regimens including higher

doses of ifosfamide (10 g/m^2 versus 6 g/m^2) for patients with clinical group III RMS.

Jaffe et al. [61] questioned whether there was a safe 'therapeutic window' during which chemotherapy could be delivered before radiotherapy or surgery for RMS. Chemotherapy consisted of VAC ± doxorubicin, given with the intent of reducing tumor burden, rendering inoperable tumors operable, delaying radiation therapy as long as possible, and permitting an increase in linear growth. Forty-five of 52 patients received chemotherapy before radiation and/or surgery; 93% responded. Primary chemotherapy converted five inoperable tumors to an operable status. Overall survival after salvage therapy was 80% and disease-free survival was 66%, indicating that it is safe to withhold radiation therapy and/or surgery up to 6 months in patients who respond to primary chemotherapy.

Diez and Mur [62] treated 20 patients with IVA. Eight of 15 patients with measurable disease achieved complete or partial responses. Suarez et al. [63] reported for SIOP the results of renal function studies of 74 children with soft tissue sarcomas enrolled in MMTS 84. Total cumulative dosages of ifosfamide were 36 g/m^2 or 60 g/m^2, given with vincristine and dactinomycin. Renal function was measured by plasma and urinary electrolytes, glucose, protein, amino acids, urinary pH, urinary osmolarity, creatinine clearance, phosphate tubular reabsorption, β_2-microglobulinuria, and lysozymuria. Four of the 74 patients developed Fanconi renal syndrome, and five had elevated levels of β_2-microglobulin or low phosphate reabsorption. Severe toxicity was correlated with the higher cumulative dosages of ifosfamide, younger age, and vesicoprostatic primary tumor.

The combination of vincristine, actinomycin-D, and cyclophosphamide remains the standard to which other chemotherapy regimens are compared. Doxorubicin has been used with VAC for many years, with suggestions in improvement in results obtained by several groups of investigators. Delivery of vincristine for more than 6 consecutive weeks, as advocated by the IRS, has been associated with significant neurotoxicity. Ifosfamide, the latest alkylating agent to be used in the combination chemotherapy schemes for RMS, has been associated with significant renal impairement, and thus its role in prolonged therapy should be questioned. Whether ifosfamide is better than cyclophosphamide can be determined only after the results of comparative combination chemotherapy studies are obtained.

Other studies

Crist et al. [64] reviewed the survival of patients entered onto IRS I and II between 1972 and 1984. The estimated survival at 5 years from initiation of treatment was 56% in IRS I and 62% in IRS II, with the largest survival increment noted in patients in clinical group III. The degree of initial resection was the most important prognostic factor in both studies. Survival rates decreased as clinical grouping ascended from I to IV. Histology was

the only patient characteristic consistently related to survival within the clinical groups. Patients with alveolar tumors had the poorest survival, and those with botryoid/embryonal lesions had the best.

Preliminary results of IRS III indicate a 73% estimated survival for the 835 enrolled patients, which is superior to the survival of both IRS I (60%) and IRS II (67%) [65]. Similar improvements were noted for continuous response duration, particularly in patients with group III disease.

The SIOP RMS-84 study reported on 286 patients treated with IVA, which was followed by cisplatin/doxorubicin in the absence of a complete response [66]. All patients with incompletely excised tumor received radiation therapy. The overall 4-year survival for patients with nonmetastatic disease was 69%, compared to 52% in a previous study. Toxicity accounted for fewer than 2% of the deaths, and only one patient has developed a second malignant neoplasm. The authors believe that the percentage of late effects will be lower, compared to other studies, because only 25% of the patients were irradiated.

Ongoing studies

The IRS IV protocol defines pretreatment staging classification, using the TNM (tumor/regional node/metastasis) system for the first time (HM Maurer, personal communication). Pretreatment tumor size is determined by external

Table 1. IRS IV tumor/regional node/metastasis (TNM) pretreatment staging classification

Stage	Sites	Tumor	Size	Node	Metatases
I	Orbit Head and neck (excluding parameningeal RMS) Genitourinary (nonbladder, nonprostate)	T_1 or T_2	a or b	N_0 or N_1 or N_x	M_0
II	Bladder/prostate Extremity Cranial (parameningeal RMS) Other (includes trunk, retroperitoneum, etc.)	T_1 or T_2	a	N_0 or N_x	M_0
III	Bladder/prostate	T_1 or T_2	a	N_1	M_0
	Extremity		b	N_0 or N_1 or N_x	M_0
	Cranial (parameningeal) Other (includes trunk, retroperitoneum, etc.)				
IV	All	T_1 or T_2	a or b	N_0 or N_1	M_1

T_1 = tumor confined to site of origin; T_2 = tumor extension/fixation to surrounding tissue; a = <5 cm in diameter in size; b = >5 cm in diameter in size; N_0 = no regional nodal involvement; N_1 = nodes clinically involved; N_x = nodal status not known or site not clinically evaluable; M_0 = no distant metastasis; M_1 = metastasis present.

measurement, computed tomography, or MRI, depending on location. Metastatic sites are identified by imaging rather than by histologic confirmation, except for bone marrow evaluation. Stages I–IV are defined by various sites with assigned T, N, and M categories (Table 1). The chemotherapy regimen is determined by stage, whereas radiation therapy is determined by group (i.e., extent of postoperative residual tumor). Groups II and IV receive conventionally fractionated radiation therapy, and group III cases are randomized to conventional versus hyperfractionated radiation therapy. The use of VAC, vincristine/dactinomycin/ifosfamide (VAI), and vincristine/ifosfamide/etoposide (VIE) to treat patients with stage I–III disease is being compared. Ifosfamide and etoposide followed by VAC is being tested in stage IV patients. Exclusions are as noted in Table 2.

The German Pediatric Cooperative Oncology Group CWS-91 protocol divides patients into three risk groups (J Treuner, personal communication). Group A includes all but paramenigeal and extremity Stage I tumors. Group B includes stage I parameningeal and extremity tumors, all stage II tumors, and stage III orbital and nonbladder, nonprostate genitourinary tumors. Group C includes all stage III tumors except those above, with stratification for low-risk categories depending on response to chemotherapy at week 13 or 14, and pretreatment tumor and nodal status. The treatment for groups A and B comprises vincristine/dactinomycin/cyclophosphamide/doxorubicin. Etoposide/vincristine/dactinomycin/ifosfamide/doxorubicin therapy is used in high-risk (group C) patients. Radiation therapy consists of 32 Gy for group B patients and 48 Gy for group C.

The SIOP protocol for malignant mesenchymal tumors, MMT-89, utilizes

Table 2. Stage- site related treatment by the IRS IV protocol

Stage	Chemotherapy	Stage	I	II	III
1[a]	VAC vs. VAI vs. VIE	1	No RT	ALL	All[b]
2	VAC vs. VAI vs. VIE	2	No RT	↓	↓
3	VAC vs. VAI vs. VIE	3	RT - - - - -	↓	↓
4	VM vs. IE, followed by VAC			Conv.	Conv. Hyperfx-RT
		4- Conventional RT			
		IRS-II Local relapse rate:			GroupII—10% Group III—16% Group IV—21%

[a] Excludes Group I paratesticular and orbit/eyelid patients who will receive VA and Group II orbit/eyelid patients who will receive VA + conventional RT.

[b] Vulvar and vaginal tumors receive only conventional RT.

From HM Maurer, personal communication.

Conv. = conventional; Hyperfx = RT = radiotherapy.

vincristine and dactinomycin to treat group A (completely resected localized tumor); VAI to treat group B, which includes favorable sites (vaginal, paratesticular, bladder, prostate) and nonparameningeal head and neck tumors; and IVA to treat group C, such as extremity and other sites (D Sommerlet, personal communication). For parameningeal tumors, IVA is given.

Radiation therapy studies

In 1984, the Memorial Sloan-Kettering Institute began a trial alternating chemotherapy and radiation therapy [67]. Hyperfractionated irradiation was given to compress the time to deliver radiotherapy interposed with chemotherapy (vincristine, dactinomycin, cyclophosphamide, doxorubicin, bleomycin, and methotrexate). Radiation therapy to the primary site was delivered in 150 cGy fractions twice daily to a total dose of 5400 cGy in two courses of 3000 cGy and 2400 cGy. All patients received a full dose of radiation therapy without unplanned interruptions due to toxicity. Of 25 patients, 88% had a complete response. Local failure occurred in only one patient, indicating a lack of compromise in local control or survival rate.

The same authors evaluated the radiocurability of microscopic RMS in relation to total dose [68]. Twelve of 15 patients who received less than 4000 cGy and 13 of 17 who received 4000 cGy or more achieved relapse-free survival, suggesting that doses below 4000 cGy, in conjunction with multiagent chemotherapy, may be sufficient for local control of microscopic disease.

The Italian Cooperative Rhabdomyosarcoma 88 protocol treated patients with RMS or NRSTS with intensive chemotherapy and hyperfractionated radiation therapy to a total dose of 40 Gy (patients with microscopic disease) or 54.4 Gy (patients with macroscopic disease) [69]. Outcome comparisons to the previous Rhabdomyosarcoma 79 protocol, which used standard radiation therapy schedules, were not possible. Better tolerance for the completion of the recommended radiation dose without unplanned interruptions was observed.

Fontanesi and associates [70] recently reported their experience with conventional radiation therapy (40 Gy) for microscopic disease and hyperfractionated radiation therapy (60 Gy) for measurable residual RMS. Control was maintained in 13 or 14 patients with microscopic disease who received conventionally fractionated irradiation and in 12 of 14 with hyperfractionated external beam irradiation. No differences in skin, mucosal, or gastrointestinal toxicities were seen for either type of irradiation.

Late effects

Pediatric, surgical, and radiation oncologists have become more aware of the late effects of therapy in long-term survivors of RMS [71]. Late effects of radiation and chemotherapy are related to the anatomic sites and doses of

radiation therapy, as well as to the chemotherapeutic agent(s) used. The most serious late effect is the occurrence of secondary neoplasms [71–74]. Reports from the IRS I and II indicate that 17 malignant neoplasms occurred in more than 1000 children [74]. The most common second malignant neoplasms are bone tumors (most at the site of primary irradiation) and acute myeloblastic leukemia. Most of the second malignant neoplasms occurred in the first 5 years after chemotherapy.

Nonrhabdomyosarcoma soft tissue sarcomas (NRSTS)

Maurer and associates [75], reporting for the Pediatric Oncology Group, discussed 83 eligible patients admitted to a protocol designed to study the efficacy of the adjuvant chemotherapy after primary tumor resection of NRSTS. Chemotherapy consisted of alternating cycles of vincristine/doxorubicin/cyclophosphamide with VAC for 1 year. Adjuvant chemotherapy was randomized for patients in clinical Groups I and II. IRS clinical Group I patients received no postoperative radiation therapy, whereas Group II patients received postoperative radiation therapy, and Group III patients with operable tumors received chemotherapy and preoperative radiation prior to surgery. Fifty-three patients refused randomization and chose chemotherapy (19 patients) or observation only (34 patients). Disease-free survival was 74% in the group treated with chemotherapy and 76% in those observed. Overall survival was 95% and 97%, respectively. Outcome did not differ considerably between randomized and nonrandomized patients. Of significance, however, patients with grade III tumors fared worse than did those with tumors graded I–II. Disease-free survival was 61% for patients with grade III and 89% for patients with grade I–II lesions. It was concluded that patients with completely or grossly resected grade I and II NRSTS do not benefit from adjuvant chemotherapy, that patients with completely resected grade I NRSTS do not need postoperative radiation therapy, that the various histologies can be classified by tumor grade into two prognostic groups (grades I and II versus grade III), and that chemotherapy trials should be developed for all patients with grade III and Group III inoperable and metastatic tumors.

Rao et al. [76] reviewed the management and prognosis of head and neck RMS and other sarcomas in 50 patients with nonparameningeal tumors. Disease-free survival was achieved by 28 patients. The authors indicated that for patients not undergoing complete resection the addition of radiation and chemotherapy may result in improved survival. Rao and associates [77] also indicated that surgery alone or with supplemental radiation therapy is effective in local control for the majority of patients with NRSTS of the extremities. The patients were categorized by the American Joint Committee on Cancer Staging System. Those with larger lesions (>5 cm, T_2) with histologic grade III were at greatest risk of failure. The authors concluded

that patients with grade III T_2 lesions may benefit from the addition of chemotherapy.

Few studies of large numbers of pediatric patients with NRSTS have been reported. The studies of Maurer [75] and Rao et al. [77] are to be complimented in that these studies have indicated which patients may benefit from additional treatment following surgery. Details of treatment results for these less common soft tissue sarcomas are necessary to define additional prognostic variables for them.

Fontanesi et al. [78] evaluated the impact of surgery, postoperative chemotherapy, and radiation in 112 consecutive patients with NRSTS. They concluded that the prognosis of patients with unresectable tumors remained unsatisfactory because of the difficulty in securing local control.

A review of 190 neoplasms in 103 children and adolescents diagnosed between birth and 12 months of age at the St. Paul-Ramsey Medical Center found these tumors comprised approximately 20% of the center's entire pediatric soft tissue series [79]. More than 75% of the cases were pathologically benign. Congenital infantile fibrosarcoma was considered a borderline tumor; none of 13 such cases in this series behaved like malignant lesions.

Young et al. [80] at the National Cancer Institute discussed the treatment of sarcomas of the chest wall with intensified combination modality therapy. Most of the 31 patients had peripheral neuroepithelioma. Other tumors included Ewing's sarcoma, RMS, and undifferentiated sarcomas. Treatment consisted of vincristine/cyclophosphamide/doxorubicin ± dactinomycin and imidazole carboxamide; high-dose conventionally fractionated radiation therapy was given to treat the primary tumor and nonpulmonary metastases. Patients who achieved a complete response received radiation therapy or intensive cycles of chemotherapy followed by autologous bone marrow transplantation. Twenty-five patients had a complete response. There were five local/regional recurrences, three failures in the radiation field, and two failures in the regional pleura. No treatment-related deaths occurred. One patient developed acute leukemia 42 months after therapy had begun.

Synovial sarcoma was reported in 49 patients registered with the Kiel Pediatric Tumor Registry [81]. Thirty-five patients had lower extremity lesions, most of which were classified as biphasic. The 7-year survival was 63%. Histologic subtype did not correlate with prognosis.

Paulus et al. [82] reported on 19 patients with primary intracranial sarcomas. The most common was malignant fibrous histiocytoma (six patients), followed by leiomyosarcoma, RMS, angiosarcoma, and single cases of six other types of sarcoma. Results indicated that intracranial and extracranial sarcomas can be classified similarly.

Malignant peritoneal mesothelioma of childhood is rare and thus is most often described in single case reports [83]. The biologic behavior of these tumors may be that of low-grade peritoneal serous neoplasms or of the well-differentiated papillary mesothelioma that occurs in adult women.

Rhabdoid tumors represented 26 malignant soft-tissue tumors in patients

entered onto IRS I–III [84]. Eleven were less than 1 year of age at diagnosis. The tumors affected predominantly the proximal extremities, trunk, and retroperitoneum-pelvis-abdomen. Nineteen of the 26 patients died within 82 months (median, 6 months); five patients survived 2–13 years. Despite various single agents given to six patients with extracranial malignant rhabdoid tumors at St. Jude Children's Research Hospital, no patient achieved a complete response [85]. However, 3 of 5 patients who received ifosfamide alone or in combination with etoposide and carboplatin had a partial response. Ifosfamide-containing regimens to treat patients with this inherently chemoresistant tumor merit further evaluation.

A total of 223 patients with undifferentiated soft tissue sarcomas were entered onto IRS I, II, and III before 1990 [86]. Their survival was 65% at 2 years and 58% at 3 years, compared to 74% and 67%, respectively, for patients with RMS. Tumor size was prognostically significant.

Of 21 cases of malignant fibrous histiocytoma (MFH) of bone (six patients) or soft tissue (15 patients) diagnosed at St. Jude Children's Research Hospital, 17 were primary tumors and four were second malignancies [87]. Fifteen patients had localized disease, and six had locally extensive or pulmonary metastatic disease. Most of the lesions were located on extremities. Eight patients with postsurgical residual disease died without achieving remission, despite multiagent chemotherapy and/or radiotherapy. The role of adjuvant chemotherapy in completely resected soft tissue MFH remains unclear because all 13 patients who underwent complete wide resection survived disease-free.

Dermatofibrosarcoma protuberans is an extremely uncommon tumor in infants and children. Patients presenting at St. Thomas Hospital in London ranged in age from 14 months to 12 years [88]. None of the patients who had undergone complete resection had evidence of recurrence.

Cases of an intra-abdominal desmoplastic small round-cell tumor with a predilection for adolescent males and abdominal sites were recently described [89–91]. Focal rhabdoid features and intense desmoplasia are often noted, as is expression of Leu-7, keratin, neuron-specific enolase, vimentin, and desmin. This tumor, which has mesenchymal and epithelial markers, has demonstrated a highly aggressive behavior despite chemotherapy and radiotherapy.

New chemotherapeutic agents

Ifosfamide, as isomer of the alkylating agent cyclophosphamide, is among the most promising agents evaluated during the past 10 years for activity against RMS and other soft tissue sarcomas. In early studies in Europe, ifosfamide, given in combination with vincristine and dactinomycin, showed considerable activity against RMS. Single-agent studies performed in the United States in the mid-1980s confirmed the drug's activity against a broad

spectrum of pediatric tumors, including RMS and other sarcomas [92,93]. Ifosfamide is now part of the primary treatment protocols for Ewing's sarcoma, osteosarcoma, and RMS.

However, European studies also documented nephrotoxicity associated with the agent's attendant enzymuria, proteinuria, glucosuria, and rickets [64,94,95]. Encephalopathy, because of its self-limiting nature, is no longer considered a significant problem. The nephrotoxic effects of ifosfamide have been associated with high total dosages, and there is evidence that the nephrotoxic features are more frequent in younger patients and those with renal dysfunction [63,94]. The MMT 84 [63] reported a significant increase in nephrotoxicity in patients receiving 10 courses of ifosfamide with other agents, as compared to six courses with other agents. Because some nephrotoxic features do not become evident until 6 or more months after completion of chemotherapy, it has been recommended that the total dosage of ifosfamide given to all patients be limited and that the treatment period be carefully monitored to prevent additional nephrotoxic changes.

Among the promising agents for which clinical trials have been, or will soon be, initiated are the topoisomerase I inhibitor topotecan, a water-soluble camptothecin derivative, and the diarylsulfonylurea sulofenur (PJ Houghton, personal communication). Both agents have shown considerable activity against RMS in xenograft models [96,97] and in other sarcomas and colon carcinomas. The precise role of these agents in the treatment of RMS and other pediatric sarcomas remains to be defined. Taxol is also being investigated in early clinical trials.

The combination of ifosfamide and etoposide, a topoisomerase II inhibitor, has been popular in recent years because of its ability to produce a response in most childhood tumors [54,98]. The addition of carboplatin to ifosfamide and etoposide in the ICE protocols [99] has resulted in significant myelosuppression; responses have been seen in individuals previously unresponsive to either ifosfamide or etoposide.

The expanded use of human tumor xenografts has, as noted, facilitated the identification of drugs active against RMS and other sarcomas [97]. Evaluation of investigational agents in a phase II setting prior to delivery of standard chemotherapeutic agents has yielded valuable information regarding the responsiveness of various childhood sarcomas to these new agents [100].

Li–Fraumeni and other syndromes associated with sarcomas

Epidemiologists and molecular biologists have cooperated in recent years to call attention to various conditions linked to cancer. The association of RMS and neurofibromatosis type I has been recognized for a number of years. Prostatic RMS has been described in a 7-month-old boy whose mother and maternal grandmother both had previously developed peripheral nerve sheath malignancy [101].

The familial syndrome of breast cancer, sarcomas, and other neoplasms has intrigued investigators for over two decades. In 1969, Li and Fraumeni described four families in which there appeared to be a dominantly inherited cancer propensity [102,103]. Since the original description of the syndrome, the associated neoplasms have been expanded to include brain tumors, osteosarcoma, adrenocortical carcinoma, and leukemia. Other tumors may also be associated with this syndrome, including lung, prostatic, pancreatic, colorectal, and stomach carcinomas, as well as melanoma and lymphomas. More recently the syndrome has been linked to the p53 tumor suppressor gene, which is located on the short arm of human chromosome 17 and is involved in the control of cellular proliferation. Germline mutations of the p53 gene have been identified in families with the Li–Fraumeni syndrome, which involves young patients who have at least several first-degree relatives who were diagnosed with cancer before the age of 45 [104–106]. It has been suggested that more than 90% of the p53 carriers would develop cancer by age 70. Recent information also suggests that survivors of primary cancers associated with p53 gene abnormalities may be at greater risk of developing multiple or second malignancies [107].

The 'at-risk' screening of individuals for p53 gene abnormalities and the accumulation of relevant data have not been easy. The syndrome is not yet clearly defined, and the mortality associated with p53 abnormalities is high. As genetic studies become more readily available to families with various hereditary/familial syndromes, counseling will become essential in an attempt to reconcile family history with the realities of cancer susceptibility and occurrence.

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