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Position Paper

Changing concepts in the pathological basis of soft tissue and bone sarcoma treatment

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Abstract

Though soft tissue sarcomas are rare considerable progress has been made in the clinical and biological understanding of these neoplasms. This has led to the launch of a new WHO classification of soft tissue tumours in 2002, which integrate morphological data with tumour specific (cyto-) genetics. Moreover worldwide consensus has grown how to predict clinical behaviour based on a specific grading system and which specific types of tumours seem not to obey these rules. As a consequence entry criteria for multi-institute prospective trials have changed over the last few years. The recent identification of tumour specific drug targets by immunohistochemistry has had impact on specimen requirements and handling as well as laboratory standards. These changes in concepts, classification, and processing of soft tissue sarcomas have had impact on patient selection and treatment and formats of multi-institute trials.

Keywords: Soft tissue tumours; Clinical trials; Pathology; Classification; Genetics

1. Introduction

Soft tissue tumours are relatively rare and account for less than 1% of adult malignancies [1]. Progress in the treatment of these rare tumours has been based on multi-institute prospective phase III trials. This is especially true for treatments concerning a multidisciplinary approach including (experimental) chemotherapy. The soft tissue and bone sarcoma group of the European Organisation for Research and Treatment of Cancer (EORTC) has a long-lasting tradition of successful multi-institute trials, sometimes in collaboration with other disease-oriented groups. These international collaborations necessitate the formulation of standards defining e.g. tumour-types, grading, review processes and immunophenotyping. Since rapid scientific progress is being made in immunophenotyping, the genetics underlying the different disease processes, and drug targeting, a continuous adjustment of criteria used within the field of bone and soft tissue tumour pathology is necessary together with their subsequent communication to the world of clinical oncology. We would like to point out these recent developments and discuss their subsequent consequences for the interpretation of previous trial results and the new ones to come. These developments include (in the order they are discussed below): (a)

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the launching of the new World Health Organisation (WHO) classification of bone and soft tissue tumours [2], (b) the international acceptance of the updated French grading system and the exclusion of its use for certain tumour types, (c) developments in immunophenotyping and (d) genetics of tumours, (e) immuno-identification of drug targets, and finally (f) the redefinition of entry-criteria for clinical trials with (g) their consequences for the future handling of tissue material.

2. The 2002 WHO-classification of soft tissue tumours

The previous World Health Organisation (WHO)-classification of soft tissue tumours dates from 1994 [3]. Since then, considerable new insights have been gained into the molecular pathogenesis and classification of soft tissue tumours. Hence, the new 'blue book' has been expanded and concentrated more on genetic data and prognostic factors [2]. In addition, the epidemiology of the disease, macroscopic and microscopic descriptions are now more understood. A number of chapters have disappeared from the soft tissue issue, since they are/will be, dealt with in other volumes. These include neural tumours, paraganglionic tumours and mesothelial tumours. A large number of vascular, and so-called fibrohistiocytic tumours will be covered in the volume on skin tumours. The following paragraphs highlight the changes in classification in the 2002 WHO-classification per group.

2.1. Adipocytic tumours

Amongst the benign tumours, myolipoma and chondroid lipoma represent two well-defined new entities. The rare fibrolipomatosis of the nerve has been renamed as lipomatosis of the nerve. Angiomyolipoma and myelolipoma have disappeared from the soft tissue fascicle and are discussed in the urogenital and endocrine volumes, respectively. Liposarcomas are the most common soft tissue sarcomas (STS), of which four types are now well characterised:

1. *Atypical lipomatous tumour/well differentiated liposarcoma*. These terms are synonyms, and the latter term is justifiable for lesions that occur in the retroperitoneum/mediastinum. At this specific site, they almost always recur due to surgical irresectability, and hence have a higher morbidity/mortality than extremity lesions. In the 1994 version of the classification, the term 'atypical lipoma' was used for a subcutaneous well-differentiated liposarcoma [3].
2. *Dedifferentiated liposarcoma.* Dedifferentiation occurs in approximately 10% of well differentiated liposarcomas of any subtype, especially in deep-seated tumours, such as those located in the retroperitoneum. Dedifferentiated liposarcoma tends to recur locally in approximately 40% of cases. Originally, dedifferentiation was defined as of high-grade morphology. In the new WHO classification low grade dedifferentiation is also recognised. Overall, approximately 15–20% of cases show distant metastases. Interestingly, dedifferentiated liposarcoma exhibits a less aggressive clinical behaviour that other types of high-grade pleomorphic sarcomas.
3. *Myxoid liposarcoma.* Originally, myxoid liposarcoma was defined separately from round-cell liposarcoma. It became clear that both 'entities' represent the ends of the spectrum of one disease, the round-cell type being the poorly differentiated variant.
4. *Pleomorphic liposarcoma*. Rare combinations of the aforementioned subtypes of liposarcoma are obviously labelled as *mixed-type liposarcomas.*

2.2. Fibroblastic/myofibroblastic tumours

Keloid as an entity has disappeared from the scene, as it is no longer regarded as a true neoplasm, and a large number of new benign entities were included in the classification: Ischaemic fasciitis, desmoplastic fibroblastoma, mammary-type fibroblastoma, angiomyofibroblastoma, cellular angiofibroma, Gardner fibroma, inclusion body fibromatosis, calcifying fibrous tumour, giant cell angiofibroma and lipofibromatosis. An important conceptual change is represented by the inclusion of 'haemangiopericytoma' into the chapter on solitary fibrous tumours, since the border between those lesions became increasingly blurred. The formerly labelled 'myxoid variant of malignant fibrous histiocytoma' (MFH) has now been definitively allocated to the fibroblastic category and has been renamed as myxofibrosarcoma. Low-grade fibromyxoid sarcoma, (acral) myxoinflammatory fibroblastic sarcoma, sclerosing epithelioid fibrosarcoma, and low-grade myofibroblastic sarcoma represent new malignant entities.

2.3. So-called fibrohistiocytic tumours

Tenosynovial giant cell tumours were formerly covered in a separate chapter on synovial tumours [3]. Since they have descriptively more in common with 'fibrohistiocytic tumours, these lesions are now allocated to this chapter. The concept of 'MFH' has been challenged and is still debated. Pleomorphic MFH is now synonymous with high-grade undifferentiated sarcoma. In addition, the morphological features of giant cell MFH and inflammatory MFH are shared by a variety of other tumour types. This will have major impact on the classification of pleomorphic sarcomas and the comparison of trial results.

2.4. Smooth muscle tumours

Smooth muscle tumours occurring in the skin, genital system and gastro-intestinal (GI)-tract, – most of the

latter 'smooth muscle tumours' representing CD117-positive GI stromal tumours –, are covered in the respective volumes. It is of note that soft tissue leiomyosarcoma is more frequent than its benign counterpart and that smooth muscle tumours occurring in immunocompromised patients are often Epstein-Barr virus-related.

2.5. Pericytic/perivascular tumours

Only glomus tumours and myopericytoma are retained in this category. The latter forms a morphological continuum with myofibroma, angioleiomyoma and so-called infantile haemangiopericytoma.

2.6. Skeletal muscle tumours

Three malignant types are included: embryonal (encompassing the spindle cell, botryoid and anaplastic subtypes), alveolar and pleomorphic rhabdomyosarcoma.

2.7. Vascular tumours

Since 1994, various new entities have been characterised, particularly in the intermediate malignancy category, including the kaposiform, retiform and composite types of haemangioendothelioma. Epithelioid haemangioendothelioma is the only haemangioendothelioma classified as malignant, due to its considerable metastatic rate. Endovascular papillary angioendothelioma has been renamed as papillary intralymphatic angioendothelioma.

2.8. Chondro-osseous tumours

Only soft tissue chondroma and extra-skeletal osteosarcoma are retained in this group. Myositis ossificans and fibro-osseous pseudotumour are now regarded as variants of nodular fasciitis (see group III) and fibroplasia ossificans progressiva seems to be a non-neoplastic lesion. Since extra-skeletal myxoid chondrosarcoma does not show convincing cartilaginous differentiation, this entity is now placed in the 'tumours of uncertain differentiation' category. Well-differentiated and mesenchymal chondrosarcoma, although occurring in the soft tissues as well, are discussed in the bone tumour section [2].

2.9. Tumours of uncertain differentiation

This category contains tumours without a clear line of differentiation or without a normal cellular counterpart. Obviously, several new entities have been described since 1994, including pleomorphic hyalinising angiectatic tumour (PHAT), mixed tumour/myoepithelioma, and neoplasms with perivascular epithelioid cell differentiation (PEComas). Clear cell sarcoma was originally included in the peripheral nerve tumour section, but the line of differentiation is still unknown. Angiomatoid fibrous histiocytoma, formerly present in the fibrohistiocytic tumour category, is now also allocated to this section. Since we now know more about divergent differentiation in various sarcomas, the category of malignant mesenchymoma is gradually leaving the stage, while intimal sarcoma is introduced as a new entity. Finally, Ewing's sarcoma (ES)/peripheral neuroectodermal tumour (PNET), including its soft tissue variant, is discussed in the bone tumour section.

3. Grading

STS are aggressive tumours which metastasise in a large percentage of cases. Tumour size, location, depth and histological type are all prognostic factors in terms of metastatic risk and overall survival. Grading systems based on histological parameters were introduced to provide more accurate information on the degree of malignancy of tumours. Nonetheless, they are of poor predictive value regarding local recurrence, which is mainly correlated with suboptimal surgical procedures.

Many grading systems were developed in the past in order to increase the discrimination between low-grade tumours (with a good prognosis) and high-grade tumours (with a poor prognosis). Two systems are mentioned in the Third Edition of the WHO classification of soft tissue tumours [2], and are currently used: the National Cancer Institute (NCI), and the French Federation of Cancer Centres Sarcoma Group (FNCLCC) systems. Both are three-tiered systems. The FNCLCC system was chosen for use in European Organisation for Research and Treatment of Cancer (EORTC) trials and advocated in the 2002 version of the WHO classification of international standards. It offers slightly better discrimination between low- and high-grade sarcomas, the intermediate group being smaller. Additionally, it seems to be more easily reproducible between pathologists, as it is based on three histological parameters, two of which are measurable (amount of necrosis and mitotic rate), while the last one, differentiation, is more subjectively assessed. These parameters were selected after multivariate analysis performed in a large series of patients. The FNCLCC system was created in 1984 [4], then updated in 1997 [5].

Performing accurate grading requires awareness of some limitations. Only untreated primary STS may be graded, thus excluding recurrences. In addition, tumours which have been previously treated by radiotherapy or chemotherapy are excluded. These treatments can alter parameters like the extent of necrosis or mitotic count. Visceral sarcomas must also be

excluded. Gastro-intestinal stromal tumours (GISTs) can be separated into subgroups based on other prognostic factors, mainly location, size and mitotic activity. The FNCLCC grading system has not yet been tested in other visceral sarcomas. In a number of entities, grading is no longer recommended according to the new WHO classification: these include angiosarcoma, extraskeletal myxoid chondrosarcoma, alveolar soft part sarcoma, clear cell sarcoma and epithelioid sarcoma).

Grading should be performed on representative material. One sample should be taken for every centimetre in the largest diameter of the tumour. Needless to say that in heterogeneous tumours there can be differences in grade within one tumour. Therefore, biopsies are recommended from those parts on magnetic resonance imaging (MRI) that argue most for a high-grade compartment. The histological diagnosis of sarcoma must be accurate. Grading is not a substitute for diagnosis: it cannot distinguish between benign lesions and malignant tumours.

Performing grading on needle-core biopsies is controversial. The representativity of small samples can be a problem, even when sufficient material is provided for a diagnosis. High-grade tumours can often be accurately recognised and graded, while the pathologist is more likely to miss one or more components in a seemingly low-grade tumour.

Grading provides solid information on the risk of metastasis and overall survival rate. Nevertheless, when using this information for therapeutic management, clinicians must be aware of its limits. Patient selection for adjuvant therapies is based not only on grade, but also on histological type or subtype, and on staging [2], which includes various criteria such as size, depth, regional lymph node involvement and distant metastatic spread.

Grade is the most important prognostic factor for sarcomas when they are studied as a whole. However, further analyses have shown that the prediction was more accurate for STS which showed greater histological variation from tumour to tumour, e.g. high-grade pleomorphic sarcomas (such as the in the past the popular diagnosis of MFHs) or leiomyosarcomas [6]. In some tumour groups, histological diagnosis is more informative than grade in terms of prognosis, e.g. various subtypes of liposarcomas. Another study showed that grade has no prognostic value in malignant peripheral nerve sheath tumours, or rhabdomyosarcomas [7]. Paediatric tumours are better prognosticated by other parameters, such as age or resectability, which emphasises that the FNCLCC grading system must be restricted to adult STS. Lastly, cytogenetics and molecular biology techniques are likely to provide new factors for predicting the clinical course and therapeutic response of STS.

4. Immunophenotyping

Immunohistochemical characterisation plays a key role in the diagnostic work-up of STS. The determination of the line of differentiation is not only crucial in order to ensure proper classification, but also to provide prognostic and/or predictive information. A detailed description of the immunophenotype of soft tissue tumours is beyond the aims of this manuscript [8]. We will therefore focus upon those differentiation markers showing major clinical relevance. Importantly, as the vast majority of immunoreagents are very sensitive but not very specific, immunohistochemical characterisation should always be performed using a panel of markers (Tables 1–3). Secondly, in order to avoid diagnostic pitfalls, immunostains should be evaluated strictly in the context of the morphology of the tissue used.

4.1. Myogenic differentiation markers

Demonstration of myogenic differentiation is important not only in order to differentiate between rhabdomyosarcoma (RMS) and non-RMS paediatric soft tissue tumours, but also to recognise within the undifferentiated (ex-MFH) pleomorphic sarcoma category both pleomorphic leiomyosarcoma (LMS) and RMS, which represent prognostically unfavourable subtypes [9].

Smooth muscle markers are basically represented by smooth muscle actin, desmin and h-caldesmon. Smooth muscle actin immunopositivity is observed in most LMS. Desmin and h-caldesmon immunoreactivity is observed in approximately 70% of cases [10]. Focal smooth muscle immunoreactivity can be seen in a

Table 1
Immunohistochemical panel for spindle cell sarcomas

Tumour type	Cytokeratin (%)	EMA (%)	Desmin (%)	SMA (%)	h-Caldesmon (%)	S-100 (%)
LMS	5	5	70	90	70	–
SS	80	>90	–	–	–	30
MPNST	–	5	–[a]	–	–	50

LMS, leiomyosarcoma; SS, synovial sarcoma; MPNST, malignant peripheral nerve sheath tumour; SMA, smooth muscle actin; EMA, epithelial membrane antigen.

[a] Positive in MPNST with heterologous rhabdomyosarcomatous differentiation.

Table 2
Immunohistochemical panel for round-cell sarcomas

Tumour	CD99 (%)	Myogenin (%)	Desmin (%)	Keratins (%)	EMA (%)	SYN (%)	FLI-1 (%)
PNET/ES	95	–	–	20	5	20	>90
ARMS	10	>90	>90	Rare	–	–	–
DSRCT	10	–	80	90	90	5	–
PDSS	90	–	–	50	90	–	–
MCHS	80	–	–	–	–	–	–

PNET/ES, peripheral neuroectodermal tumour/Ewing's sarcoma; ARMS, alveolar rhabdomyosarcoma; DSRCT, desmoplastic small round-cell tumour; PDSS, poorly differentiated synovial sarcoma; MCHS, mesenchymal chondrosarcoma.

Table 3
Immunohistochemical markers for epithelioid sarcomas

Tumour	Cytokeratin (%)	EMA (%)	CD34 (%)	CD31 (%)	S-100 (%)	FLI-1 (%)
ES	>90	>90	50	rare	–	–
EAS	50	30	80	80	–	100
EMPNST	20	–	–	–	100	–

ES, epithelioid sarcoma; EAS, epithelioid angiosarcoma; EMPNST, epithelioid malignant peripheral nerve sheath tumour.

variety of soft tissue tumours, as well as in non-sarcomatous lesions (e.g. sarcomatoid "spindle cell" carcinoma) and therefore should not interpreted "per se" as unequivocal evidence of smooth muscle differentiation.

Desmin and muscle-specific actin also represent very sensitive markers of striated muscle differentiation staining up to 90% of RMS of all subtypes (embryonal, alveolar and pleomorphic). However, all these markers are overshadowed by myogenin, a nuclear transcription factor involved in striated muscle differentiation, which specifically identifies striated muscle differentiation in all RMS subtypes [11]. Importantly, myogenin is more abundantly expressed in alveolar RMS than in embryonal and pleomorphic subtypes.

4.2. Neural markers

S-100 immunopositivity, despite a total lack of specificity still represents the most sensitive marker of neural differentiation. Approximately 50% of malignant peripheral nerve sheath tumours (MPNST) exhibit S-100 positivity, which is usually limited to less than 30% of neoplastic cells. Epithelioid MPNST represents an important exception, as most neoplastic cells will express this marker. It has to be remembered that approximately 30% of monophasic synovial sarcoma (SS) may also exhibit S-100 immunopositivity, making differential diagnosis with MPNST somewhat challenging [12]. Glial fibrillary acidic protein (GFAP) is expressed in up to 30% of MPNSTs. Neuron-specific enolase (NSE) and PGP 9.5 are too non-specific to play useful roles.

4.3. Epithelial differentiation markers

SS, as well as any soft tissue neoplasm featuring epithelioid morphology, is characterised by variable expression of cytokeratin and epithelial membrane antigen (EMA). Recognition of SS among spindle cell sarcomas is extremely important, as this neoplasm exhibits significant chemo-sensitivity. Cytokeratin expression is observed in up to 80% of classic SS and in approximately half of the poorly differentiated ones [12–14]. Cytokeratin is expressed in virtually all epithelioid sarcomas [12,14], and is also observed in 50% of epithelioid angiosarcoma (EAS) [15]. EMA also stains most epithelioid sarcomas, and 90% of SS (including poorly differentiated ones); it therefore represents the most sensitive marker of epithelial differentiation in this context.

4.4. Endothelial differentiation markers

Demonstration of endothelial differentiation appears crucial when dealing with poorly differentiated vascular neoplasms, in particular epithelioid angiosarcoma, an AS variant than can mimic a carcinoma. Classic markers are represented by CD34, CD31 and Factor VIII-RA. CD34 is very sensitive, but is also expressed in approximately 50% of epithelioid sarcomas [16], in addition to a endless list of spindle cell neoplasms including dermatofibrosarcoma protuberans and solitary fibrous tumour. CD31 is far more specific and CD31 immunopositivity can be detected within intratumoral histiocytes. FVIII-RA is also very specific, but tends to be less sensitive. A promising marker is represented by Fli-1, a nuclear transcription factor involved in endothelial differentiation, which appears to stain both normal and neoplastic endothelium [17,18]. Fli-1 immunoreactivity is also observed in ES/PNET as a result of *FLI-1* gene rearrangements [19]. An important diagnostic adjunct is represented by nuclear detection of human herpes virus (HHV-8) in Kaposi's sarcoma [20].

4.5. Other useful markers

CD99 represents a powerful diagnostic marker in the differential diagnosis of small round-cell sarcomas. In fact, the vast majority of ES/PNET exhibit strong membrane CD99 immunopositivity [21]. However, it has to be underlined that among potential mimics of ES/PNET, CD99 is also expressed in 90% of SS (including the poorly differentiated round-cell variant), in 40% of Merkel cell carcinomas [22], in most examples of lymphoblastic lymphomas and in mesenchymal chondrosarcomas [23].

C-kit (CD117) has recently become one of the most clinically relevant phenotypic markers. In fact, its expression in GIST permits the accurate recognition of this once orphan tumour, as well as the proper selection of patients for treatment with tyrosine kinase inhibitors [24,25]. It should be underlined that widespread and unnecessary application of heat-induced antigen retrieval in this context has led to indiscriminate reports of c-kit immunopositivity in a variety of mesenchymal neoplasms [26] and possibly irrational application of the targeted-therapy. In fact, it has to be underlined that c-kit expression "per se" does not predict sensitivity to Imatinib mesylate (STI-571, Glivec).

HMB-45 not only plays an important role in the differential diagnosis of malignant melanoma, but has also proved extremely helpful in recognising those entities belonging to the recently coined family of PEComas, which include angiomyolipoma, lymphangioleiomyomatosis and other rarer entities [27].

In addition to myogenin and Fli-1, other nuclear transcription factors appear to represent extremely promising phenotypic markers. WT-1 has proved useful in the recognition of desmoplastic small round-cell tumour [28], an extremely aggressive neoplasm characterised by polyphenotypic expression of neural, epithelial and myogenic markers [29]. Even more recently, it has been shown that TFE3 is expressed in the neoplastic cell population of alveolar soft part sarcomas [30]. It has to be stressed that, with the exception (until now) of myogenin none of these markers appears to be entirely specific. However, when evaluated together with the morphology of the tissue, they not only significantly increase the diagnostic accuracy, but also predict the presence of the underlying genetic aberration. The expression of proto-oncogenes and tumour suppressor genes products has also proved useful in the differential diagnosis of STS. It has been recently shown that both well-differentiated liposarcoma and dedifferentiated liposarcoma are characterised by amplification and overexpression of mdm2, cdk4 and HMGIC. mdm2 overexpression has been recently proposed as a useful diagnostic tool in distinguishing between dedifferentiated liposarcoma and other retroperitoneal high-grade sarcomas [31,32]. p53 overexpression may also play a role in the differential diagnosis of atypical fibroxanthoma (AFX), which includes malignant melanoma, spindle cell carcinoma and leiomyosarcoma. p53 expression in AFX is due to ultra violet (UV)-induced *TP53* mutations and is almost always found [33].

4.5.1. Obsolete Markers

In parallel with the conceptual evolution of the classification of soft tissue tumours, several immunohistochemical markers have lost most of their utility. This has proved true for "fibrohistiocytic" markers such as lisozyme and alpha-1-antichimotrypsin, but also for vimentin, whose diagnostic application is now minimal. Several differentiation markers may still be valid, but have been practically replaced by reagents showing better reproducibility. The replacement of MyoD1 by myogenin represents an illuminating example. In general, it has to be underlined that tumour immunophenotyping represents a dynamic process. Pathologists need to be aware of the consolidated advances in the field in order to ensure diagnostic accuracy. Participation in external quality control programmes is also strongly advised, as technical as well as clinical validation is mandatory, particularly in the context of clinical trials.

5. Genetics

Although traditional morphological and immunohistochemical assessment remains the foundation of clinical decision-making, adjunctive data from genetic studies can improve the precision of diagnosis and accuracy of subtyping in clinically important soft tissue tumour areas. Increasingly, genetic data are useful for predicting behaviour and response to therapy, and in some areas, such as paediatric small round-cell tumours, are likely to be mandatory [34]. Cytogenetic and molecular genetic aberrations have been described in many benign and malignant soft tissue and bone tumours. These include chromosomal deletions, duplications and rearrangements, gene amplification by rearrangement or mutation, and oncogene activation and suppression. Examples of the latter include the *TP53* gene located at 17p13, *MDM2* gene at 12q, *RB1* gene at 13q14, *WT1* gene at 11p13, and the *NF1* gene at 17q11.2. Mutations in the *TP53* gene are common abnormalities in human cancers, and loss of the short arm of chromosome 17, point mutation of *TP53*, and homozygous loss of both alleles have been reported in STS, including rhabdomyosarcoma, leiomyosarcoma and undifferentiated pleomorphic sarcoma (formerly MFH). Inheritable mutations of germ-line *TP53* (in Li–Fraumeni syndrome) or *RB1* (hereditary retinoblastoma) are found in familial sarcomas. Of particular importance, a number of sarcomas have consistent specific translocations,

Table 4
Chromosomal translocations in malignant soft tissue tumours

Tumour type	Translocations	Involved genes
Synovial Sarcoma	t(X;18)(p11.2;q11.2)	*SSX1*or *SSX2*,*SYT*
MRC liposarcoma	t(12;16)(q13;p11)	*CHOP*,*TLS*
	t(12;22)(q13;q11–q12)	*CHOP*,*EWS*
Ewing's sarcoma/PNET	t(11:22)(q24;q12)	*FLI1*,*EWS*
	t(21:22)(q22;q12)	*ERG*,*EWS*
	t(7;22)(p22;q12)	*ETV1*,*EWS*
	t(2;22)(q33;q12)	*FEV*,*EWS*
	t(17;22)(q12;q12)	*E1AF*,*EWS*
Desmoplastic SRCT	t(11;22)(p13;q12)	*WT1*,*EWS*
Alveolar rhabdomyosarcoma	t(2:13)(q35;q14)	*PAX3*,*FKHR*
	t(1;13)(p36;q14)	*PAX7*,*FKHR*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q21–31;q12.2)	*CHN*,*EWS*
	t(9;17)(q22:q11)	*CHN*,*RBP56*
Clear cell sarcoma	t(12;22)(q13;q12)	*ATF1*,*EWS*
Alveolar soft part sarcoma	t(X;17) (p11;q25)	*TFE3*,*ASPL*
Dermatofibrosarcoma/GCF	t(17;22)(q22;q13)	*COL1A*,*PDGFB1*
Infantile fibrosarcoma	t(12;15)(p13;q25)	*ETV6*,*NTRK3*
Low grade fibromyxoid sarcoma	t(7;16)(q34;p11)	*FUS*,*BBF2H7*

MRC, myxoid/round-cell; PNET, peripheral primitive neuroectodermal tumour; SRCT, small round-cell tumour; GCF, giant cell fibroblastoma.

which result in new fusion genes (Table 4). The specific functions of these genes are largely unknown, but their tumour-specific RNA transcripts generally encode proteins which represent transcription factors (derived from one partner gene) or nucleic acid binding domains (from the other); the latter include the *EWS* gene, which is involved in several different translocations. Most translocations appear to act through the production of abnormal proteins with altered control of cell proliferation. Identifying the rearrangement or the gene products enables a precise diagnosis. Techniques for the detection of these and other clinically relevant abnormalities such as *MYCN* gene amplification in neuroblastoma include fluorescent in situ hybridisaiton (FISH), polymerase chain reaction (PCR), reverse transcriptase (RT)-PCR, mutation screening and gene sequencing, and usually require fresh or frozen tissue, but are increasingly applicable to formalin-fixed, paraffin-embedded material. A molecular diagnostic service for sarcomas should be available in all specialist centres. Genetic findings can confirm relationships between morphological subtypes, improve diagnostic accuracy and prediction of behaviour in specific sarcomas beyond the general features of size, depth and grade, and lead to improved therapy. Thus, in fatty tumours, the morphological classification has been validated. Abnormalities in the 12q13–15 region (which includes the *MDM2* and *CDK4* genes) and giant marker and ring chromosomes are found in both atypical lipomatous tumours and dedifferentiated liposarcomas, and identical translocations are seen in myxoid and round-cell liposarcoma (Table 4). However, pleomorphic liposarcomas are a distinct subgroup having complex rearrangements with numerous extra chromosomes, as in many pleomorphic unclassified sarcomas. Similarly, the genetic identities of dermatofibrosarcoma protuberans and giant cell fibroblastoma, and of low-grade fibromyxoid sarcoma with hyalinising spindle cell tumour, have been shown, again confirming morphological suppositions. Extra-skeletal myxoid chondrosarcoma, which is probably not a cartilaginous tumour, has translocations, which are not found in skeletal myxoid chondrosarcoma, a separate entity. On the other hand, Ewing's sarcoma/PNET has the same genetic abnormalities, irrespective of the site of origin. No specific genetic features relating to diagnosis or prognosis have been identified in pleomorphic sarcomas. In diagnosis, the finding of a specific translocation is especially useful for distinguishing small round-cell tumours, including PNET, poorly differentiated SS, and desmoplastic small round-cell tumours. Additionally, in some tumours with variable translocations, the gene involved in the rearrangement (e.g. whether *PAX3* or *PAX7* in alveolar rhabdomyosarcoma, or *SSX1* or *SSX2* in SS) relates to prognosis and potential response to chemotherapy, independent of tumour site, stage and size. In ES/PNET with t(11:22)(q24;q12), the *EWS-FLI1* rearrangements show great diversity; the so-called type I gene fusion, in which *EWS* exon 7 is fused to *FLI1* exon 6, is reportedly associated with an improved prognosis compared with other fusion types. Mutational analysis is proving particularly relevant in the clinicopathological assessment of GIST. In most GISTs, there are activating mutations in the *KIT* gene, located on chromosome 4q11–21, which encodes a type-III receptor tyrosine kinase protein (CD117), a diagnostically useful immunohistochemical marker. In GISTs, the *KIT* gene mutations are mostly in the juxtamembrane domain at exon 11, with a smaller number at exons 9 and 13. Familial and multiple GISTs are associated with a germline mutation at exon 11. It appears that the type of

mutation might determine response to therapy with imatinib mesylate, a selective inhibitor of ABL and KIT tyrosine kinases. Receptors for the platelet-derived growth factor receptor (PDGFR) belong to the same subfamily, suggesting a potential role for the same drug in the therapy of dermatofibrosarcoma protuberans and related fibrosarcomas, in which the PDGFR gene is rearranged.

Gene expression profiling is a recently introduced technique for the simultaneous examination of thousands of genes in cDNA microarrays containing hundreds of sarcomas. Some tumour types – SS, GISTs, neural tumours, and a subset of leiomyosarcomas, show distinct gene expression patterns. Other tumours, such as pleomorphic sarcoma and liposarcoma, share molecular profiles. Marked expression of known genes, such as *KIT* in GISTs, occurs within gene sets that distinguish the different sarcomas. However, many uncharacterised genes also contribute to the distinction between tumour types. Analysis of the huge amount of data derived from these studies might reveal further useful markers for diagnosis, specific prognostic factors and identify possible targets for molecular therapy.

6. Immuno-identification of drug targets

Several primary genetic events in tumours, such as translocations or mutations subsequently lead to expression of downstream drug targets such as C-kit, epidermal growth factor receptor (EGFR)-1, or Her-2/Neu. Given the vast amount of new and unexpected genes expressed, which are nowadays identified as a result of cDNA expression microarrays, this list is most likely to rapidly expand. The expression of these molecules can be monitored either at the RNA level or, more simply, and may be more effectively, at the protein expression level by immunohistochemistry. However, immunohistochemistry has its limits with regards to specificity, both as a result of primary antibody cross-reactivity, as well as the detection techniques used such as antigen retrieval. Currently, clinical trials are being performed based on selective interactions with these molecules. Despite the clinical success of targeting C-kit in GISTs, caution should be used in response to over-enthusiastic reactions based upon presumed expression of C-kit in miscellaneous tumours. Although there are several reports of C-kit expression in an array of tumours, this is largely based on immunohistochemical techniques using antigen retrieval, and may include false-positive results. In a trial comparing GIST and other sarcomas, no benefit with regard to the survival rates was observed for non-GIST patients after the administration of imatinib mesylate [35]. In addition, in osteosarcomas, for example despite original reports of HER2 overexpression [36–38] [39], more and more critical papers have now appeared [40–42] challenging the original observations. However, Phase II trials have tested the efficacy of trastuzumab (Herceptin) in patients with relapsed or refractory osteosarcoma [37,43]. Obviously standardisation in technology is necessary to avoid the overoptimistic interpretation of preliminary results. It means pathogenesis must not only be careful in diagnosing tumours, but also in identifying potentially useful treatment options. EGFR-1 has been found to be expressed in SS using micro-array technology [44–46]. This led to the rapid design of a trial targeting EGFR-1 using the drug gefitinib (ZD1839, Iressa). It remains to be proven whether this approach is effective since preliminary data show, at least at the immunohistochemical level, that the expression of EGFR-1 is restricted to subsets of tumour cells, thereby leading to the question of how the drug is to target the other cells?

7. Entry criteria

The major efforts of the EORTC Soft Tissue and Bone Sarcoma Group (STBSG) have been within the field of medical oncology (phase I, II and III trials), with less emphasis on locoregional treatments. The STBSG has always considered pathological review to be necessary to ensure quality control. At present, only patients with histologically-proven sarcomas are eligible for trials, i.e. cytology alone is not sufficient. This may seem overly rigorous to cytopathologists with experience in this area, but histology is necessary for reliable subclassification and grading and thus correct stratification with regard to prognostic factors. In the future, other diagnostic modalities may be accepted, e.g. the demonstration of specific translations or gene fusion products.

For practical purposes, all subtypes of STS have previously been lumped together for inclusion in clinical trials. This rather crude approach has been dictated by the rarity of these tumours, but with accumulating experience, it has become clear that treatment must be adapted according to the histological subtypes. Thus, malignant mesotheliomas are now generally excluded from STS trials because they respond to other chemotherapeutic agents (cisplatin and gemcitabine)[47]. The same applies to the juvenile types of rhabdomyosarcomas (embryonal and alveolar), neuroblastomas, and the soft tissue variants of bone sarcomas. Malignant mixed mesodermal tumours and carcinosarcomas are also not included; most investigators now consider them to be variants of carcinomas [48]. The recent re-definition of GISTs by their CD117 (c-kit) positivity also illustrates this evolution, within both pathobiological and clinical research fields: these tumours were previously classified as leiomyosarcomas (a caveat in the interpretation of the results of trials before 1999). However, it was noted that intra-abdominal leiomyosarcomas never responded to

treatment. The 'designer drug' imatinib mesylate, a tyrosine inhibitor directed against KIT (CD117 epitope), proved to be surprisingly effective in the treatment of GISTs, thus necessitating the separation of these tumours from the other STS. In the future, further subtypes will be singled out for specific treatments, and as mentioned above, the choice of therapy may come to depend on the demonstration by various methods of specific therapeutic targets.

The situation is slightly different with regard to bone sarcomas. Chondrosarcomas are rare and have (so far) proven to be chemoresistant; However, a trial for this tumour is under development. Chemotherapy is of proven efficacy in osteosarcoma and ES/PNET. For these two tumour types, histological confirmation is mandatory; moreover, the whole primary tumour should be available for review of the treatment response after adjuvant chemotherapy, requiring a section of the whole tumour through its largest dimension. Molecular biology has-so far-not proven to be particularly useful in the diagnosis of osteosarcomas, which are anyway usually readily recognisable in their high-grade versions (low-grade osteosarcomas do not require chemotherapy). However, within the group of 'small blue round-cell tumours (SBRCT)' several variants and relatives of ES/PNET have been identified, and it will become a requirement for future trials that fresh/frozen material is available for molecular genetics. Actually, this situation may soon become pertinent for all sarcomas as well as other tumour types, e.g. lymphomas and it should therefore become a routine procedure in all pathological departments to ensure the availability of frozen tissue samples from all tumours.

8. Importance of tissue blocks, frozen material and tissue arrays for trial management

As mentioned above, the STBSG considers pathological review of paramount importance for quality control. Depending on the trial in question, this review process deals not only with the tumour subclassification and grading, but also with the identification of molecular targets for therapy. With the development of new target-specific drugs, the latter will become increasingly important in the future. To date, conventional immunohistochemistry is still considered crucial in this process. Therefore, the availability of adequately fixed [49], representative paraffin-embedded tissue is of utmost importance. The standardised performance of immunohistochemical procedures in the reference centres can avoid variations in the reproducibility of staining. Paraffin-embedded material as well as fresh-frozen tissue of STS will be collected at primary diagnosis and follow-up after therapy, tumour progression or metastasis. The tissue will be stored in a tissue bank with the possibility to link tumour-related pathological data to the clinical databases of multi-centre EORTC-trials. This powerful tool will provide more insight in to the biology of these rare neoplasms and detect new therapeutic approaches. Tissue bank material provides the basis for a variety of innovative technologies. With the use of Tissue Microarray (TMA) technology [50], large numbers of tumours can be screened for many known or new molecular markers which might have an impact on future trial planning or stratification. TMA analysis requires only small amounts of paraffin-embedded tissue, leaving enough material for other research or diagnostic needs (e.g. translational research projects). However, it should be kept in mind that there may be a need for additional tissue for validation studies of the TMA technology, especially in STS with known heterogeneity. Additionally, the TMA technique might not be applicable for complex phenotypes and this may require standard full-section analysis. TMA technology detects DNA, RNA or protein targets in paraffin-embedded tissues. However, the quality of FISH experiments is more uniform and reliable after cold ethanol fixation of fresh-frozen tumour samples preserving high molecular weight DNA/RNA [50]. Another way to counteract the limitations of RNA-detection due to fixation-/tissue processing-effects might be the construction of micro-arrays using fresh-frozen tissue [51]. The increasing application of cDNA micro-array techniques poses an additional need for fresh-frozen tissue samples. This technique increases molecular understanding of tomour pathogenesis and identifies genes that may serve as markers for a new molecular classification of STS. Gene expression profiling might be helpful in pointing out target tumour subgroups for different therapeutical approaches and identify other entities that can be lumped together – as in previous work – because they have no discriminating characteristics [52]. In addition, it may create new ways of monitoring treatment efficacy and resistance mechanisms, as well as detecting new targets for drugs. The combined application of cDNA and tissue micro-arrays provides the possibility to validate gene expression profiles at the protein level which should impact on the design of new, more individualised trial protocols. The centralised collection of as many tissue samples as possible, especially of rare STS entities, will facilitate the validation of individualised or target-specific therapeutic approaches through the application of these different diagnostic technologies to a relevant number of cases. Therefore, the collection of fresh-frozen tissue should become routine for every STS (diagnostic biopsies as well as resection specimens) across all centres.

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