Molecular biology of sarcomas

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What molecular abnormalities are encountered in sarcomas?

There are two types of sarcomas with regards to the type of molecular alterations: sarcomas with relatively simple, usually specific lesions and those with complex unspecific lesions. A molecular classification for sarcomas can therefore be proposed (Table 1).

Recurrent chromosomal translocations: about 10-15% of all sarcomas bear a recurrent chromosomal translocation (Table 2). The most frequent sarcomas with such an abnormality are dermatofibrosarcoma protuberans (DFSP), myxoid liposarcoma, Ewing's sarcoma, and synovial sarcoma.

Each of these translocations generates a fusion gene believed to be directly related to the pathogenesis of the sarcoma in which it is expressed[1,2] (Fig. 1). Most of these gene fusions encode aberrant chimeric transcription factors by combining a strong promoter (e.g. *EWSR1),* which confers the expression level, and a DNA-binding domain (e.g. *FLI1, ERG, ATF1, WT1)*, which confers the target specificity of the transcriptional activation. A second mechanism describes results in an autocrine growth factor, as seen in DFSP and giant cell fibroblastoma with *COL1A-PDGFB* gene fusion. This fusion puts platelet-derived growth factor receptor beta *(PDGFRB)* under the control of the *COL1A1* promoter, thus removing all elements repressing *PDGFRB* transcription. A third mechanism involves the fusion of the catalytic domain of a tyrosine kinase receptor with an expressed protein, resulting in a ligand-independent activated chimeric tyrosine kinase, as seen in congenital fibrosarcoma due to *ETV6-NTRK* fusion.

Specific activating or inactivating mutations: about 20% of sarcomas show a specific oncogenic mutation, which is the central event in gastrointestinal stromal tumors (GIST) and in malignant rhabdoid tumors.

Mutation of either *KIT* or *PDGFRA* leads to a constitutive activation KIT or PDGFRA tyrosine kinase receptors, with subsequent activation of signal transduction cascades regulating proliferation, apoptosis, and differentiation[2]. The most frequent sites of mutation are exon 11 of *KIT* (juxtamembrane domain), exon 18 of *PDGFRA* (activation loop) and exon 9 of *KIT* extracellular

Table 1. *Molecular classification of sarcomas*

– Sarcomas with a recurrent translocation
– Sarcomas with a specific activating mutation
– Sarcomas with a specific inactivating mutation
– Sarcomas with a simple genomic profile with a 12q14-15 amplicon
– Sarcomas with a complex genomic profile

Table 2. *Recurrent chromosomal translocations associated with soft tissue sarcomas*

Sarcoma type	Translocation	Genes involved	Prevalence
Ewing's sarcoma/ primitive neuroectodermal tumor	t(11;22) (q24;q12)	EWSR1-FLI1	85-95%
	t(21;22) (q22;q12)	EWSR1-ERG	5-10%
	t(7;22) (p22;q12)	EWSR1-ETV1	rare (< 1%)
	t(17;22) (q12;q12)	EWSR1-ETV4 (E1AF)	rare
	t(2;22) (q33;q12)	EWSR1-FEV	rare
	t(1;22) (p36;q12)	EWSR1-ZSG	rare
	t(16;21) (p11;q22)	FUS-ERG	rare
Synovial sarcoma	t(X;18) (p11;q11)	SS18(SYT)-SSX1	65%
		SS18(SYT)-SXX2	35%
		SS18(SYT)-SSX4	rare
Myxoid liposarcoma	t(12;16) (q13;p11)	TLS(FUS)-DDIT3(CHOP)	95%
	t(12;22) (q13;q12)	EWSR1-DDIT3(CHOP)	rare
Alveolar rhabdomyosarcoma	t(2;13) (q35;q14)	PAX3-FOXO1A(FHKR)	60-80%
	t(1;13) (p36;q14)	PAX7-FOXO1A(FHKR)	10-20%
	t(X;2) (q13;q35)	PAX3-AFX	rare
	t(2;2) (q35;p23)	PAX3;NCOA1	rare
Clear cell sarcoma	t(12;22) (q13;q12)	ATF1-EWSR1	> 90%
	t(2;22) (q32;q12)	EWSR1-CREB1	rare
Extraskeletal myxoid	t(9;22) (q22;q12)	EWSR1-TEC(NR4A3/CHN/TEC)	75%
chondrosarcoma	t(9 ;17) (q22;q11)	TAF2N(RBP56)-TEC/CHN	25%
	t(9 ;15) (q22;q21)	TCF12-TEC(CHN)	rare
Desmoplastic small round cell tumor	t(11;22) (p13;q12)	WT1-EWSR1	> 90%
Low-grade fibromyxoid	t(7;16) (q32-34;p11)	TLS(FUS)-CREB3L2	90%
sarcoma	t(11;16) (p11;p11)	TLS(FUS)-CREB3L1	10%
Dermatofibrosarcoma	t(17 ;22) (q22;q13)	COL1A1-PDGFB	> 90%
protuberans/giant cell fibroblastoma	ring 17q, ring 22q, der(22)	COL1A1-PDGFB	75%
Alveolar soft-part sarcoma	t(X ;17) (p11.2;q25)	ASPL-TFE3	> 90%
Infantile fibrosarcoma (cell. mesoblastic nephroma)	t(12 ;15) (p13;q25)	ETV6(TEL)-NTRK3(TRKC)	80-90%
Inflammatory myofibroblastic	t(2;19) (p23;p13.1)	TPM4-ALK	
tumor	t(1;2) (q22-23;p23)	TPM3-ALK	
	t(2;17) (p23;q23)	CLTC-ALK	
	t(2;11) (p23;p15.5)	CARS-ALK	
	t(2;2) (p23;q13)	RANBP2-ALK	
	other 2p23 rearrangements	ALK-other partners	
Angiomatoid fibrous histiocytoma	t(2 ;22)(q34 ;q12)	EWSR1-CREB1	90%
	t(12 ;22)(q13 ;q12)	EWSR1-ATF1	10%

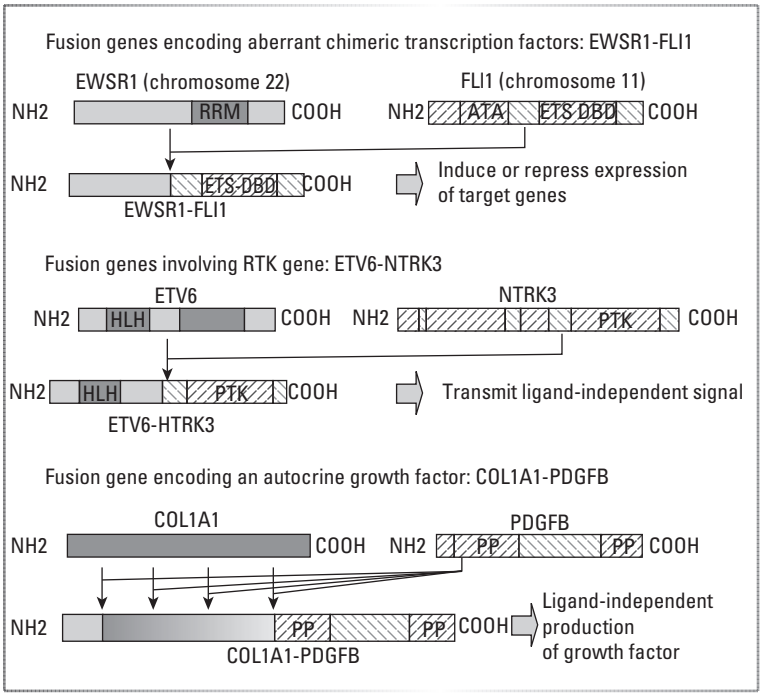

Figure 1. *Categories of reciprocal chromosomal translocations encountered in sarcomas. RRM: RNA recognition motif; ATA: amino-terminal transactivation domain; ETS-DBD: Ets DNA-binding domain; HLH: helix-loop-helix domain; PTK: protein tyrosine kinase domain; PP: propeptide.*

domain) (Fig. 2). However, about 10% of GIST show no detectable mutation in either *KIT* or *PDGFRA*. A *BRAF* mutation has been reported in a small subset of these "wild-type" GIST.

Malignant rhabdoid tumors are very rare sarcomas characterized by a specific biallelic inactivation of *SMARCB1* also known as *hSNF5* or *INI1*.

Simple genomic profile with 12q14-15 amplicon: atypical lipomatous tumors/well-differentiated liposarcomas (ALT/WDLPS) and dedifferentiated liposarcomas (DDLPS) as well as intimal sarcomas are characterized by this particular amplicon involving *MDM2* and often (90%) *CDK4*[3]. The DDLPS is characterized by additional amplicons involving genes whose overexpression may explain dedifferentiation and aggressiveness. These tumors represent about 10-15% of all sarcomas.

Complex genomic profile with frequent loss of *RB1* and mutations of *P53*[4]: about 50-60% of sarcomas show such a complex profile, characterized by several gene losses and gains. These tumors are mainly represented by leiomyosarcomas, myxofibrosarcomas, and poorly differentiated sarcomas (or so-called malignant fibrous histiocytomas) (Table 3).

What is the specificity of these molecular abnormalities?

Apart from a few exceptions, recurrent chromosomal translocations are specific of a tumor type[5].

Although a few papers have questioned the specificity of t(X;18) *(SS18-SSX)* in synovial sarcomas, it has been demonstrated that this translocation is specific and sensitive for the diagnosis of synovial sarcoma. These papers described the reverse transcription-polymerase chain reaction (RT-PCR) detection of

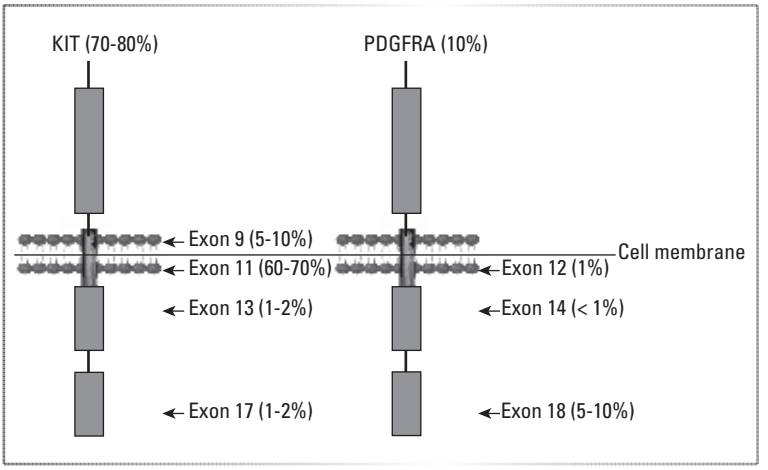

Figure 2. *Mutations of* KIT *and* PDGFRA *in gastrointestinal tumors leading to a ligand-independent activation of a receptor tyrosine kinase protein.* KIT *contains 21 exons but mutations cluster within four exons: exon 11 encoding the intracellular juxtamembrane domain, exon 9 encoding the extracellular transmembrane domain, exon 13 encoding the first portion of the split kinase domain, and exon 17 encoding the kinase activation loop.* PDGFRA *is a member of the same family of receptor tyrosine kinases as* KIT *with mutations in 3 exons: exon 18 (kinase activation loop), 12 (juxtamembrane region) and 14 (split kinase domain). Ten to 20% of cases show no mutation of* KIT *or* PDGFRA. *PDGFRA: platelet-derived growth factor receptor alpha.*

fusion transcripts in other tumors without being validated by other techniques or reproduced by others, and most likely correspond to PCR contamination.

A few fusion transcripts are common to different entities: *ETV6-NTRK3* is present in congenital fibrosarcoma/mesoblastic nephroma, but also in secretary breast carcinoma and acute myelogenous leukemia; *ASLP-TFE3* is present in alveolar soft-part sarcoma and in some renal cell carcinomas, arising preferentially in children and young adults; *TPM3-ALK* is present in both inflammatory myofibroblastic tumor and anaplastic lymphoma; both *EWSR1-ATF1* and *EWSR1-CREB1* are present in clear cell sarcoma and in angiomatoid fibrous histiocytomas. These data can be explained either by the occurrence of a second, unknown, tumor-specific molecular event or, more likely, by a divergent differentiation program of distinct precursor cells.

Specific activating or inactivating mutations: although *KIT* mutations are known in mast cell disease, seminoma, acute myeloid leukemia, and sinonasal natural killer/T-cell lymphoma, the type of mutation encountered

Table 3. *Sarcoma histotypes with a complex genomic profile*

– Leiomyosarcoma
– Myxofibrosarcoma
– Pleomorphic liposarcoma
– Pleomorphic rhabdomyosarcoma
– Osteosarcoma
– Malignant peripheral nerve sheath sarcoma
– Angiosarcoma
– Poorly differentiated sarcoma (so-called malignant fibrous histiocytoma)

is clearly different from those found in GIST. The mutations demonstrated in GIST are nearly disease-specific, but with two important exceptions: mutations of exons 11, 13, and 17 of *KIT* have been reported in acral and mucosal melanomas, as well as in melanomas arising on skin with chronic sun damage, and mutations of exons 12 and 18 of *PDGFRA* have been documented in inflammatory fibroid polyps.

Biallelic inactivation of the *SMARCB1/INI1* tumor-suppressor gene has been identified as the specific event of malignant rhabdoid tumors, whatever their location, but it has also been reported in some cases of proximal-type epithelioid sarcomas, suggesting a link between both tumor types.

Simple genomic profile with 12q14-15 amplicon is present in ALT/WDLPS, DDLPS, intimal sarcoma, and in parosteal osteosarcoma. Although the presence of an *MDM2* amplification detected by fluorescence in situ hybridization (FISH) is highly suggestive of one of these tumors, it is not absolutely specific, since such amplification has been reported in other malignant tumors including sarcomas such as rhabdomyosarcoma. In fact, the whole genomic profile as shown by array-comparative genomic hybridization (array-CGH) is much more specific and should be used in difficult cases[3].

Complex genomic profile has no specificity for a tumor type.

What has molecular biology contributed to the classification of sarcomas?

Some molecular abnormalities have been shown to be highly specific diagnostic markers, and their prevalence in most sarcomas is such that they come to define these entities[6]. This is particularly true for most sarcomas with recurrent translocations or with a simple genomic profile with 12q14-15 amplicon.

Molecular pathology has helped to gather previously distinct histological entities such as Ewing's sarcoma and neuroepithelioma, myxoid and round cell liposarcoma, infantile fibrosarcoma and cellular mesoblastic nephroma, and inflammatory malignant fibrous histiocytoma and dedifferentiated liposarcoma.

Molecular pathology has helped to differentiate previously linked tumors due to histological overlap, such as poorly differentiated embryonal rhabdomyosarcoma and solid alveolar rhabdomyosarcoma, "myxoid" sclerosing WDLPS and myxoid liposarcoma, Ewing's sarcoma and olfactory neuroblastoma, skeletal and soft tissue myxoid chondrosarcoma.

Molecular pathology has helped to extend entities to unusual forms in terms of patient age, tumor site, histological aspect, and immunohistochemical profile, particularly for Ewing's sarcoma, synovial sarcoma, and dedifferentiated liposarcoma. For example, thanks to the demonstration of *MDM2* amplification, we now know that most so-called malignant fibrous histiocytomas or poorly differentiated sarcomas, adult type rhabdomyosarcomas and malignant mesenchymomas developed in the internal trunk and particularly in the retroperitoneal space correspond to dedifferentiated liposarcomas.

What techniques can be used to demonstrate these molecular abnormalities?

Fluorescence *in situ* hybridization (FISH) detects a specific DNA target in the nuclei of interphase cells and can be used to demonstrate a gene rearrangement such as a translocation, an amplification, or a deletion[7]. Nowadays, it is the most frequently used technique for showing translocation and *MDM2* amplification. Commercially available

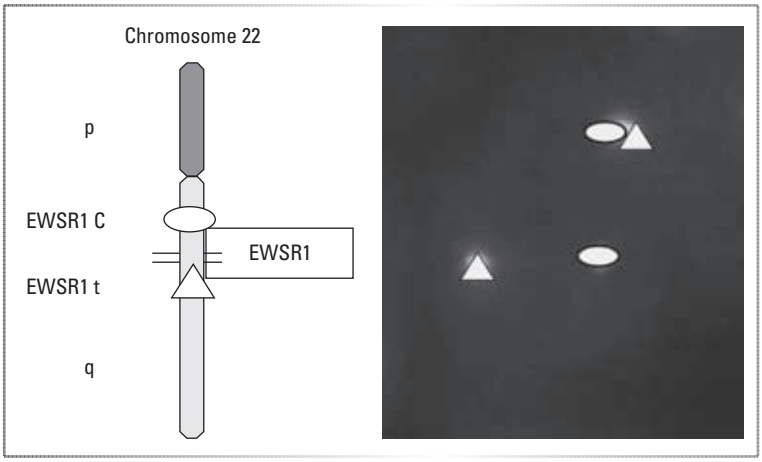

Figure 3. *Break-apart probes are the most used for detecting reciprocal translocations in sarcomas. Example of* EWSR1 *break-apart probes which can be used to detect a* EWSR1 *rearrangement in Ewing's sarcomas, desmoplastic round cell tumors, clear cell sarcomas, angiomatoid fibrous histiocytomas, and some myxoid extraskeletal chondrosarcomas. Two probes flanking* EWSR1 *gene are used, one centromeric (red, here ellipse) and one telomeric (green, here triangle). Gene rearrangement is shown by a splitting of one pair of green (triangle)-red (ellipse) signals.*

break-apart probes (Fig. 3) are regularly used for demonstrating rearrangement of *EWSR1, SS18 (SYT), DDIT3 (CHOP), FOXO1A (FKHR), TLS (FUS), ETV6,* and *ALK* (Table 4). Some probes can be used for several tumor types, such as *EWSR1* probes for Ewing's sarcoma, clear cell sarcoma, desmoplastic small round cell tumor and some myxoid/round cell liposarcoma, extraskeletal myxoid chondrosarcoma, and angiomatoid fibrous histiocytoma, and *TLS (FUS)* for low-grade fibromyxoid sarcoma and most myxoid/round cell liposarcoma. For DFSP, homemade fusion probes (*COL1A* and *PDGFB*) and break-apart probes for *PDGFB* are used. Commercially available probes for *MDM2* associated with probes for the centromeric region of chromosome 12 can be used to show 12q14-15 amplicon.

The FISH technique has several advantages: it can be used on fresh or fixed tissue, it can be performed fast (overnight procedure) and can be used on core needle biopsies or on touch preparations with a few cells. The

Table 4. *Commercially available probes useful for fluorescence in situ hybridization in sarcomas*

Probes	Sarcoma type
EWSR1	Ewing sarcoma Desmoplastic round cell tumor Clear cell sarcoma Extraskeletal myxoid chondrosarcoma Myxoid/round cell liposarcoma (< 5%) Angiomatoid fibrous histiocytoma
SS18 (SYT)	Synovial sarcoma
FOXO1A	(FKHR) Alveolar rhabdomyosarcoma
DDIT3 (CHOP)	Myxoid/round cell liposarcoma
TLS (FUS)	Low-grade fibromyxoid sarcoma Myxoid/round cell liposarcoma
ETV6	Infantile fibrosarcoma Cellular mesoblastic nephroma
ALK	Inflammatory myofibroblastic tumor
MDM2	Atypical lipomatous tumor/well-differentiated liposarcoma Dedifferentiated liposarcoma Intimal sarcoma

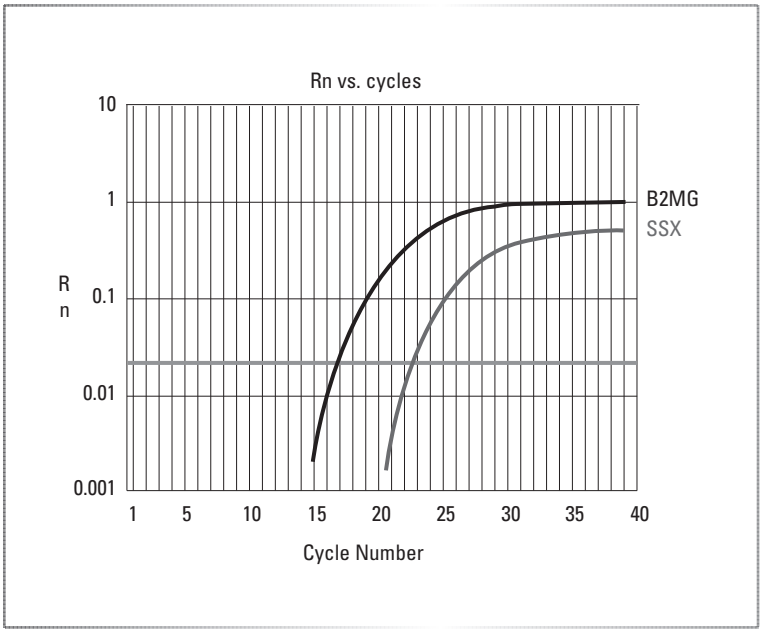

Figure 4. *Quantitative RT-PCR technique is well-adapted to the daily practice because it is an automated real-time analysis performed in a single tube, which decreases the risk of contamination. Moreover, there is no gel and it is highly sensitive and specific. Graphs show fluorescence emission data during each PCR cycle. The fluorescence shown is directly related to the quantity of PCR product. B2MG: graph for amplification of beta2-microglobulin which is used as an internal control gene; SSX: graph for SS18-SSX transcript. RT-PCR: reverse transcription-polymerase chain reaction.*

main limitation is unsuccessful hybridization or detection in 10-20% on fixed tissues.

Reverse transcription-polymerase chain reaction (RT-PCR) is the standard method for detecting specific fusion transcripts and should be used first when a translocation is suspected. It is more suitable with RNA extracted from frozen tissue, but can also be used with RNA extracted from paraffin-embedded tissue, provided amplified fragments of less than 150 bp are used. It is highly recommended to use quantitative or real-time RT-PCR (Fig. 4), which is more adapted to daily diagnosis than conventional RT-PCR. This technique is highly specific, but has several limitations when used in poorly experienced laboratories; variable success rate of RNA extraction from paraffin-embedded material, possible difficulty of primer design, and high risk of PCR contamination[5]. However, when used in strictly controlled conditions, it is a specific and sensitive technique with about 90% of interpretable results on formalin paraffin-embedded tissues.

Sequencing with or without prescreening by denaturing high performance liquid chromatography (dHPLC) of PCR-amplified exons from DNA extracted from frozen or paraffin-embedded tissue is used for the detection of mutations of *KIT* and *PDGFRA* in GIST.

Immunohistochemistry can be used for detecting fusion gene proteins in sarcomas with a translocation when one portion of a given protein is overexpressed. In practice, it is used with success in alveolar soft-part sarcoma with TFE3 antibody (Fig. 5) and in inflammatory myofibroblastic tumor with ALK antibody. It is also a good technique for detecting inactivating mutations of the *SMARCB1/INI1* gene in rhabdoid tumors (Fig. 6). On the other hand, antibodies for carboxy-terminal WT1 in desmoplastic round cell tumor and FLI1 in Ewing's sarcoma are not very useful given their poor specificity. Immunohistochemistry is also used for detecting overexpression of MDM2 and CDK4 in sarcomas with a 12q14-15 amplicon. However, specificity and sensitivity

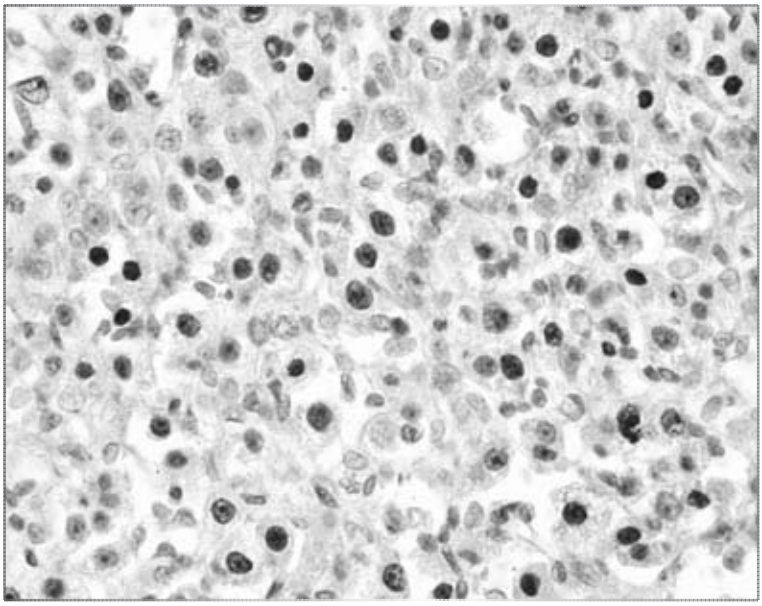

Figure 5.
Immunohistochemical analysis can be used for detecting TFE3 protein in cell nuclei in soft part alveolar sarcoma in which a t(X;17)(p11;q25) involving TFE3 and ASPL genes leads to a TFE3 overexpression.

are between 80 and 90% and this technique is less efficient than FISH.

Quantitative PCR can be used for detecting *MDM2* and *CDK4* amplification from frozen as well as from paraffin-embedded tissues, but is less sensitive than FISH.

Array-CGH can be considered as the best technique for demonstrating simple genomic profile with 12q14-15 amplicon when FISH is unsuccessful (Fig. 7). Array-CGH should be performed from frozen material, but it can be used from paraffin-embedded tissues in some cases.

Which material can be used to detect molecular abnormalities in sarcomas?

Frozen tissue is the best for demonstrating molecular abnormalities and it is strongly recommended to freeze a piece of tumor when the diagnosis of sarcoma is clinically suspected[8,9] (Table 5). This recommendation underlines the importance of an organized network of tumor banks at the national level. However, frozen tissue is not always available, particularly in consultative second opinion practice, which is important in the field of soft tissue tumors.

Formalin fixed paraffin-embedded tissue is always available. Although most of the useful molecular abnormalities can be demonstrated on this kind of material, it has limitations due to fragmentation of nucleic acids. Therefore, it is necessary to select PCR primers that produce PCR products of less than 150-200 bp. Among formalin-derived fixatives, buffered formalin is the best, provided the duration of fixation is less than 24 to 36 hours. Addition of acids such as picric acid or acetic acid should be avoided because of their harmful effect on the quality of nucleic acids. New fixatives based on dehydration without formalin give very good results in terms of quality of nucleic acid and could represent a good alternative to frozen tissue, at least for diagnostic purposes.

Isolated cells obtained by fine needle aspiration or touch preparation from fresh or

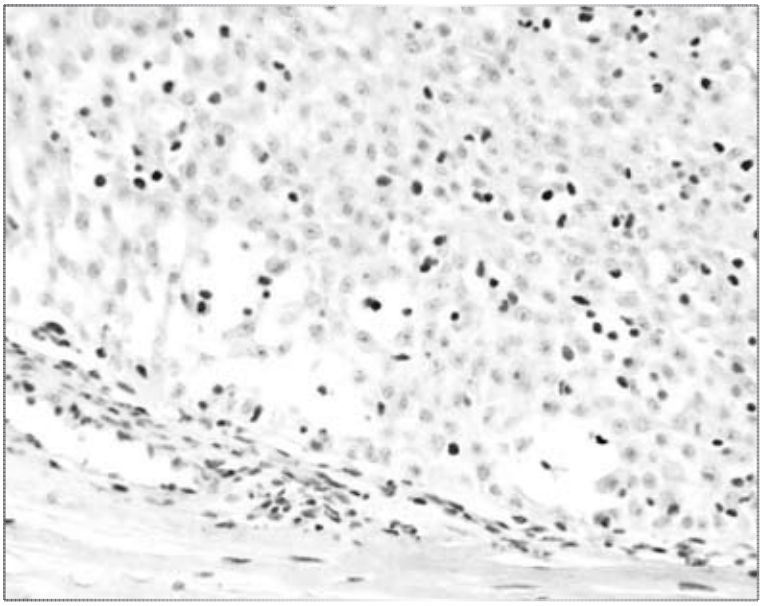

Figure 6.
Immunohistochemistry is also a very good technique for demonstrating SMARCB1/INI1 *inactivation in malignant rhabdoid tumor, which is characterized by a biallelic inactivation of this gene. There is a loss of nuclei staining in tumor cells, whereas this staining is still present in reactive and normal cells.*

frozen tumor represent very good material for molecular analysis.

What is the reproducibility of molecular techniques in sarcomas?

Only a few data are available in the literature, but a pilot study performed within the Conticanet European Network of Excellence gives an insight into inter-laboratory, inter-technique, and inter-material variability.

Inter-laboratory reproducibility: reproducibility for detecting fusion transcripts by real-time RT-PCR was 89% among three laboratories, with discordance only for myxoid liposarcomas. Reproducibility for detecting *KIT* and *PDGFRA* mutations in GIST was also evaluated among three laboratories with a concordance of 96%. However, these laboratories have good experience in molecular pathology of sarcomas with a large throughput.

Another study conducted with six laboratories by the French Sarcoma group showed a good reproducibility for detecting fusion transcripts by RT-PCR in synovial sarcoma, but a discordance of 16.5% for detecting mutations in GIST.

Inter-technique reproducibility: FISH and RT-PCR have been compared for detecting translocations. Concordance between both techniques is excellent, with the possibility of negative RT-PCR and positive FISH when the primer design does not cover all the fusion gene possibilities. When using both techniques on paraffin-embedded tissue, RT-PCR usually gives more interpretable results than FISH. However, FISH is more appropriate for DFSP, myxoid/round cell liposarcoma and Ewing's sarcoma, at least on paraffin-embedded tissue.

Inter-material reproducibility: comparison of frozen tissue and paraffin-embedded tissue has been performed for the detection of fusion transcripts and mutations of *KIT* and *PDGFRA*. Concordance between both materials is excellent, but with lesser efficiency for paraffin-embedded tissue owing to more non-interpretable cases than for

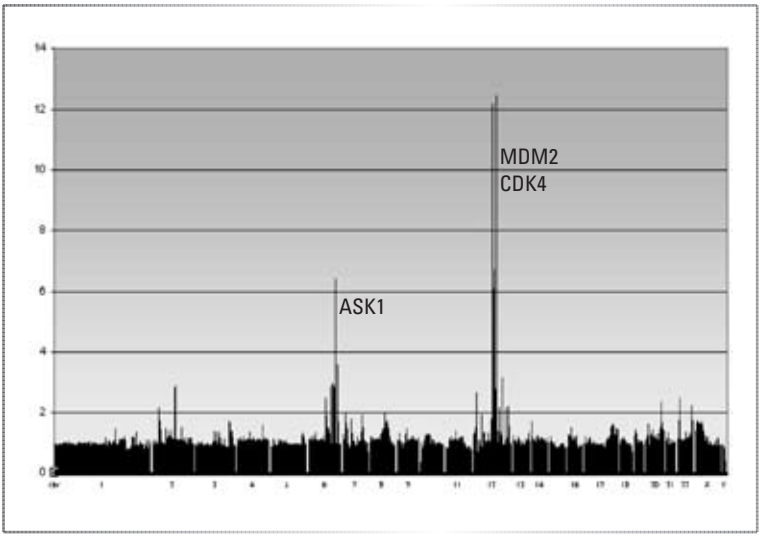

Figure 7. *Array-comparative genomic hybridization is the best technique for demonstrating the typical genomic profile of dedifferentiated liposarcoma which is characterized by a 12q14-15 amplicon involving* MDM2 *and* CDK4 *genes associated with other amplicons such as in 6q23 involving* ASK1 *gene.*

frozen tissue, particularly for Ewing's sarcoma and GIST.

Quality assurance programs should be organized for evaluating laboratories involved in molecular analyses of sarcomas and particularly of GIST. Although FISH can be performed by surgical pathologists, some techniques such as RT-PCR, quantitative PCR, sequencing, and array-CGH should be performed by molecular pathologists.

What is the impact of molecular analysis on the final diagnosis?

Molecular analysis can be very useful for distinguishing benign and malignant tumors: the two main applications are deep lipoma with histological changes versus ALT/WDLPS (lipoma-like subtype) by using FISH for detecting *MDM2* amplification, and low-grade fibromyxoid sarcoma versus perineurioma or other benign proliferations by using RT-PCR for detecting *FUS-CREB2L3* or FISH for detecting *FUS* rearrangement. It can also be useful for the diagnosis of myxoid liposarcoma versus lipoblastoma in young patients and for differentiating infantile fibrosarcoma versus myofibromatosis or cellular fibromatosis in children on core needle biopsy.

Molecular diagnosis is an ancillary method for the routine work-up of pediatric round cell tumors, particularly for identifying Ewing's sarcoma, desmoplastic small round cell tumor, alveolar rhabdomyosarcoma, and poorly differentiated synovial sarcoma. It is particularly useful for the diagnosis of Ewing's sarcoma given the non-specificity of CD99 immunoreactivity.

Molecular testing is highly recommended to validate the histological diagnosis when dealing with unusual clinical presentations such as synovial sarcoma occurring in the

Table 5. *Indications for freezing sarcomas*

– All soft tissue tumors suspected of malignancy should be frozen and stored in a tumor bank.
– Tumor banking is recommended for the following soft-tissue tumors: • Deep tumors of any size • Subcutaneous tumors larger than 5 cm • Tumors in patients younger than 30 years of age • All other tumors suspected of being malignant

lung or Ewing's sarcoma developed in an older patient, or unexpected histological or immunohistochemical findings.

Both FISH and array-CGH may sometimes be necessary to diagnose a dedifferentiated liposarcoma when the well-differentiated liposarcomatous component is missing.

Detection of *KIT* and *PDGFRA* mutations is rarely needed for the diagnosis of GIST, but may be useful for the few CKIT immunonegative cases.

In a pilot study conducted by the Conticanet European Network of Excellence, a systematic molecular analysis for any suspicion of a sarcoma with a specific genetic lesion showed that molecular analysis was helpful, i.e. confirmation of a probable diagnosis, in 4% of GIST and 32% of sarcomas with a suspicion of translocation. It was also useful for suspected ALT/WDLPS and DDLPS, whereas it was necessary, i.e. confirmation of a possible diagnosis, in 1% of GIST, 8% of sarcomas with a suspicion of translocation, and in 3% of suspected ALT/WDLPS and DDLPS.

What is the prognostic impact of molecular analyses in sarcomas?

The molecular heterogeneity of fusion transcripts is considered to have a prognostic implication in some sarcomas. The presence of *PAX7-FOXO1A* is associated with a better prognosis in alveolar rhabdomyosarcoma as compared to the more common *PAX3-FOXO1A* fusion type. A less-clear impact was noted for *SS18-SSX1* and *SS18-SSX2* in synovial sarcomas and for type 1 *EWSR1-FLI1* versus other fusion transcripts in Ewing's sarcoma with conflicting results. The detection of fusion transcripts in bone marrow is of poor prognosis in Ewing's sarcoma. In other tumor types, such as myxoid liposarcoma and clear cell sarcoma, the fusion transcript variant has no prognostic value[5].

In GIST, the type of mutation seems to have a prognostic value: *KIT* exon 11 deletions involving codons 557 to 558 and homozygous *KIT* exon 11 mutations were reported to be associated with a poor prognosis, whereas the presence of *PDGFRA* mutations in exon 18 has been associated with a low potential for metastasis.

What is the impact of molecular analysis on the therapeutic decision?

Thanks to its diagnostic impact, molecular testing is decisive when the treatment is histotype-specific, particularly in childhood sarcomas such as Ewing's sarcoma, alveolar rhabdomyosarcoma, desmoplastic round cell sarcoma, and infantile fibrosarcoma. Some adult sarcoma histotypes may also show specific sensitivity to different cytotoxic agents such as trabectedin in myxoid/round cell liposarcoma. Identification of dedifferentiated liposarcoma among poorly differentiated adult sarcomas is important because of its low metastatic risk and therefore the uselessness of adjuvant chemotherapy in this setting.

The mutational status of GIST is a key factor for the therapeutic response to imatinib mesylate and other tyrosine kinase inhibitors[10]. Patients with *KIT* exon 11 mutations have significantly better response to imatinib than those with *KIT* exon 9 mutations. In the latter, a double dose of imatinib is often necessary to achieve a good response. Patients with *PDGFRA* D842V mutation are primary resistant to imatinib. Secondary resistance to imatinib may occur, particularly in patients with *KIT* exon 11 mutations. In most cases, this is related to secondary mutations. Therefore, the use of other tyrosine kinase inhibitors with different efficiency according to the mutational status is important.

Imatinib is also efficient in DFSP with a proved translocation best demonstrated by FISH.

Table 6. *Clinical impact of molecular testing in sarcomas*

Molecular type	Histotype	Diagnostic impact	Prognostic impact	Therapeutic impact
Recurrent translocation	Ewing's sarcoma	+++	?	+
	Synovial sarcoma	++	?	–
	Alveolar rhabdomyosarcoma	+++	++	+
	Myxoid liposarcoma	+	–	±
	Dermatofibrosarcoma protuberans	+	–	+
	Low-grade fibromyxoid sarcoma	+++	–	+
	Desmoplastic round cell tumor	++	–	+
	Clear cell sarcoma	+	–	±
	Infantile fibrosarcoma	+++	–	+
	Alveolar soft-part sarcoma	+	–	–
	Inflammatory myofibroblastic tumor	+	–	–
	Angiomatoid fibrous histiocytoma	++	–	
Activating mutations	Gastrointestinal stromal tumor	+	+	++
Inactivating mutations	Rhabdoid tumor	+++	–	–
Simple genomic profile	ALT/WDLPS	++	–	+
with 12q14-15 amplicon	Dedifferentiated liposarcomas	++	–	+
	Intimal sarcoma	++	–	–
Complex genomic profile (see table 2)		–	–	–

ALT/WDLPS: atypical lipomatous tumors/well-differentiated liposarcomas.

There is no doubt that, in the near future, treatment of sarcomas will be molecular-targeted, with an increasing role played by molecular pathology[11]. In DDLPS and other sarcomas with a simple genomic profile with the 12q14-15 amplicon, it will be possible to use anti-MDM2 agents and drugs targeting the JNKinase pathway. In translocation-related sarcomas, specific gene fusions could be used as therapeutic targets. Furthermore, special drugs could be used to target proteins in activated signaling pathways, such as the PI3K/AKT/mTOR pathway, which plays a key role in cancer and particularly in sarcomas. Analysis of the polymorphisms of DNA repair genes will also be important, given their increasingly understood role in drug response.

What are the indications for molecular analyses in sarcomas?

Since detection of specific translocations, activating or inactivating mutations and amplifications can be used reliably as histotype-specific, prognostic or predictive markers (Table 6), an increasing number of

surgical pathologists and clinicians are now relying on molecular validation and this sort of validation will certainly be required in the near future for any sarcoma with a suspicion of specific molecular abnormality. This strategy definitively classifies tumors belonging to the group of sarcomas with a specific genetic abnormality. However, drawbacks such as high cost, low turnaround time, and lack of quality assurance have limited the expansion of genetic analyses on a large scale. Nevertheless, given the value of genetic analyses for deciding treatment and the relatively low cost of these techniques as compared to the cost of treatment, it is highly recommended to use them whenever possible. A national network of laboratories specialized in molecular pathology of sarcomas would improve the turnaround time and help in the organization of quality assurance programs.

Most sarcomas with a suspicion of specific translocation should be tested molecularly, except perhaps for the obvious biphasic synovial sarcomas, myxoid/round cell liposarcomas, and DFSP. A specific translocation should be suspected when faced with a sarcoma exclusively composed of monotonous round or spindle cells, with no cellular pleomorphism. These tumors most often arise in young patients, but may be seen in older individuals.

Mutations of *KIT* and *PDGFRA* should be detected in any GIST, at least in GIST of intermediate and high-risk malignancies.

MDM2 amplification is required for the diagnosis of DDLPS without any ALT/WDLPS component and for the diagnosis of ALT/WDLPS when dealing with an unusual case. However, with the new therapeutic possibilities for these tumors, genetic characterization will probably be required.

References

1. Riggi N, Cironi L, Suvà ML, Stamencovic I. Sarcomas: genetics, signalling, and cellular origins Part 1: the fellowship of TET. J Pathol. 2007;213:4-20.
2. Suvà ML, Cironi L, Riggi N, Stamencovic I. Sarcomas: genetics, signalling, and cellular orignins. Part 2: TET-independent fusion proteins and receptor tyrosine kinase mutations. J Pathol. 2007;213:117-30.
3. Coindre JM, Pédeutour F, Aurias A. Well-differentiated and dedifferentiated liposarcomas. Virchows Arch. 2009 [Epub ahead of print].
4. Guillou L, Aurias. Soft tissue sarcomas with complex genomic profiles. Virchows Arch. 2009 [Epub ahead of print].
5. Antonescu CR. The role of genetic testing in soft tissue sarcoma. Histopathology. 2006;48:13-21.
6. Fletcher CDM, Unni KK, Mertens F. Tumours of soft tissue and bone. Pathology and genetics. World Health Organization. IARC Press: Lyon 2002.
7. Tanas MR, Goldblum JR. Fluorescence in situ hybridization in the diagnosis of soft tissue neoplasm: a review. Adv Anat Pathol. 2009;16:383-91.
8. Gulley ML, Kaiser-Rogers KA. A rational approach to genetic testing for sarcomas. Diagn Mol Pathol. 2009;18:1-10.
9. Osuna D, de Alava E. Molecular pathology of sarcomas. Rev Recent Clin Trials. 2009;4:12-26.
10. Rubin BP. Gastrointestinal stromal tumours: an update. Histopathology. 2006;48:83-96.
11. Toguchida J, Nakayama T. Molecular genetics of sarcomas: applications to diagnoses and therapy. Cancer Sci. 2009;100:1573-80.