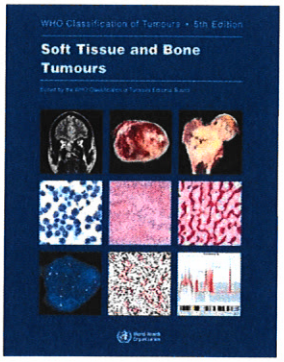
Role of the pathologist in diagnosis, prognostication and therapy of soft tissue and bone tumors

Prof. Dr. David Creytens
Pathologist, Head of Clinics
Professor of Pathology, Soft Tissue and Bone Pathology
Department of Pathology
Ghent University Hospital, Ghent University

Diagnostic challenges in sarcoma pathology

- The **rareness** of sarcoma
- **The significant (continuously growing) number of soft tissue and bone tumor entities** as well as **the dynamically changing nomenclature of soft tissue and bone tumors**
- The **considerable morphological heterogeneity within a soft tissue and bone sarcoma and between different soft tissue and bone sarcomas**; subtle histologic differences can make a big difference in soft tissue and bone tumor diagnostics!
- The **morphologic overlap between benign and malignant soft tissue and bone tumors**; sarcomas can mimick more common benign and malignant non-mesenchymal tumors
- The **trend of limited biopsy material** available for pathologic examination and diagnosis of soft tissue and bone tumors
- **Limitations of diagnosing and grading sarcomas on core needle biopsies**



The 2020 WHO Classification
What's New in Soft Tissue Tumor Pathology?
Michael E. Kallen, MD and Jason L. Hornick, MD, PhD†*

TABLE 1. 2020 WHO Classification: New Soft Tissue Tumor Types

Section/Chapter	Tumor Type
Adipocytic tumors	Atypical spindle cell/pleomorphic lipomatous tumor
	Myxoid pleomorphic liposarcoma
Fibroblastic/myofibroblastic tumors	Angiofibroma of soft tissue
	EWSR1-SMAD3-positive fibroblastic tumor (emerging)
	Superficial CD34-positive fibroblastic tumor
Vascular tumors	EHE with *YAP1-TFE3*
Smooth muscle tumors	Inflammatory leiomyosarcoma*
Tumors of uncertain differentiation	*NTRK*-rearranged spindle cell neoplasm (emerging)
Undifferentiated small round cell sarcomas of bone and soft tissue	*CIC*-rearranged sarcoma
	Sarcomas with *BCOR* genetic alterations
	Round cell sarcomas with *EWSR1*-non-ETS fusions

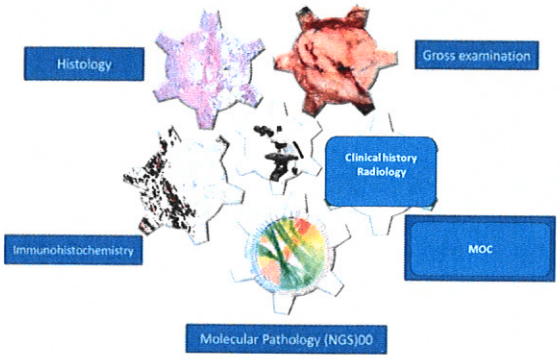
*Formerly included as variant of (conventional) leiomyosarcoma; now separate section.

What should the clinician tell to the pathologist when a sarcoma is considered?

- **Age**
- **Size**
- **Depth** (above/under the fascia)
- **Localisation**
- **Relevant imaging/clinical data**
- **Medical history** (irradiation, previous tumor, recurrence,….)
- If possible deliver fresh tissue

What should the pathologist tell to the clinician when it concerns a sarcoma?

- **Benign vs malignant**
- **Histologic (sub)type**
- **Grade** if applicable (FNCLCC grading)
- **Dimensions**
- **Resection status, extent and section margins (R0/R1/R2)**
- **Molecular data**



→**correct histopathological diagnosis of the (sub)type sarcoma is crucial for optimal therapy and prognostication**

→**no 'precision medicine' without a correct histopathological diagnosis!!!**

CLASSIFICATION OF SOFT TISSUE TUMORS
according to biological behaviour

- ***BENIGN***
- ***INTERMEDIATE (LOCAL AGGRESSIVE)***
- ***INTERMEDIATE (RARE METASTATIC POTENTIAL)***
- ***MALIGNANT***→SARCOMA

Benign biological behaviour

- **Very low** risk of local recurrence
- **EXTREMELY RARE** metastasis (<<1/50000 of cases), not to predict on the basis of conventional morphology

  e.g. cutaneous benign fibrous histiocytoma

ORIGINAL ARTICLE

Metastasizing "Benign" Cutaneous Fibrous Histiocytoma

A Clinicopathologic Analysis of 16 Cases

Leona A. Doyle, MD and Christopher D.M. Fletcher, MD, FRCPath

Abstract: Cutaneous fibrous histiocytoma (FH) is considered a benign tumor, however, certain types of FH have been shown to have a tendency for local recurrence, and there are rare reported cases of metastasis. In this study, 16 cases of morphologically benign FH with locoregional or distant metastases were identified in consult files. Pathologic features of primary, recurrent, and metastatic tumors, as well as clinical outcome, were evaluated. Nine were male and 7 were female patients; mean age was 42 years (range, 3 to 68 y). Primary tumors arose on the leg in 5 patients, buttock in 1, trunk in 3, shoulder in 3, neck in 2, and

cases of morphologically benign cutaneous FH is an extremely rare but clinically aggressive event. Primary tumors tend to be large and cellular, but aggressive behavior cannot be predicted on morphologic grounds alone, however, early or frequent local recurrence may warrant closer clinical follow-up.

Key Words: soft tissue, tumor, metastasis, fibrous histiocytoma, dermatofibroma, sarcoma

(*Am J Surg Pathol* 2001;25:445–449)

Intermediate (locally aggressive) biological behaviour

- Risk of local destructive recurrence
- No significant potential for metastasis

  e.g. desmoid type fibromatosis

Intermediate (rare metastatic) biological behaviour

- Risk of local destructive recurrence
- Risk of metastasis (<2% of cases), not to predict on the basis of conventional morphology

  e.g. angiomatoid fibrous histiocytoma
  plexiform fibrohistiocytic tumor
  giant cell tumor of bone

Malignant biological behaviour (sarcomas)

- Significant risk of local destructive recurrence
- Significant risk of metastasis
- Grading of sarcomas: FNCLCC of NCI grading systems (see later)
  2-10% grade I
  10-30% grade II
  30-100% grade III

'Diagnostic tools' in sarcoma pathology

- **Conventional morphology** (Hematoxylin&Eosin, 'HE' staining)
- **Immunohistochemistry**
- **Molecular techniques**

Histological grading

- **Must be clearly distinguished from staging and nomograms**
- Histological grade is based on the **histological (intrinsic) qualities of the primary tumor** and is expected to predict tumor aggressiveness
- To obtain the most efficient system, histological parameters should be clearly defined and selected by multivariate analysis so that only the necessary parameters summarising all prognostic histological information are used
- According to the studies followed this approach, the best parameters are *tumor histotype and subtype and/or differentiation*, *tumor necrosis*, *mitotic activity* and (as stated in few) vascular invasion
- The two systems most commonly used are the National Cancer Institute (NCI) grading* and the **French Fédération Nationale des Centre de Lutte contre le Cancer (FNCLCC) grading****

*Costa J, Wesley RA, Glatstein E, et al. Cancer 1984;53:530-41

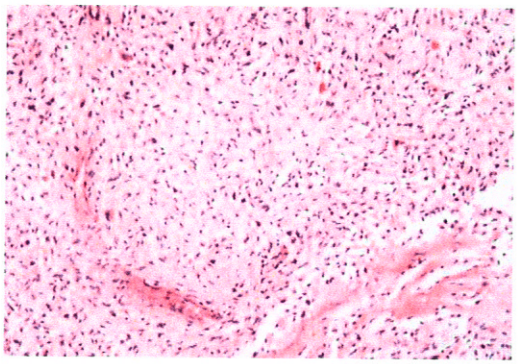
** Trojani M, Contesso G, Coindre JM, et al. Int J Cancer 1984;33:37-42

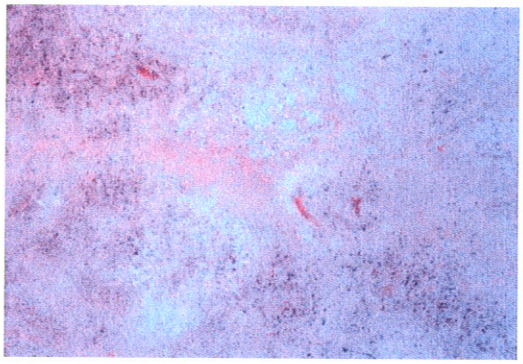
Table 1 Definition of parameters in the French grading system

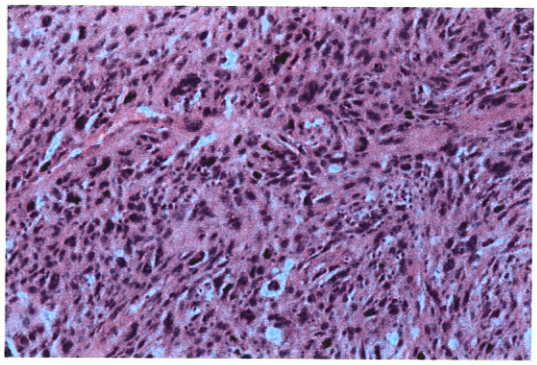
Definition of parameters
Tumour differentiation (see Table 2)
Score 1: Sarcomas closely resembling normal adult mesenchymal tissue and potentially difficult to distinguish from the counterpart benign tumour (e.g. well-differentiated liposarcoma, well-differentiated leiomyosarcoma). Score 2: Sarcomas for which histological typing is certain (e.g., myxoid liposarcoma, myxofibrosarcoma). Score 3: Embryonal and undifferentiated sarcomas, synovial sarcomas, sarcomas of doubtful type.
Mitotic count established on 10 HPFs[1]
Score 1: 0–9 mitoses per 10 HPF. Score 2: 10–19 mitoses per 10 HPF. Score 3: More than 19 mitoses per 10 HPF.
Tumour necrosis
Score 0: No necrosis. Score 1: Less than 50% of tumour necrosis. Score 2: 50% or more than 50% of tumour necrosis.
Histological grade
Grade 1: Total score 2, 3. Grade 2: Total score 4, 5. Grade 3: Total score 6, 7, 8.

[1] Modified from Trojani *et al.*[4]
A HPF measures 0.1734 mm²
HPF: high-power field

Neuville A, Chibon F, Coindre JM. Pathology 2014;46(2):113-120







Grading boring but rewarding: value of grading

- **Histological grade is the most important prognostic factor for adult soft tissue sarcoma**
- As the best predictor of metastasis development and tumor mortality, histological grade is **a key parameter of the currently used TNM clinicopathological staging system**
- Since grading permits prediction of metastasis, and because the response rate to chemotherapy is better in patients with high grade sarcoma, **the grading system can be used to select patients for adjuvant chemotherapy**

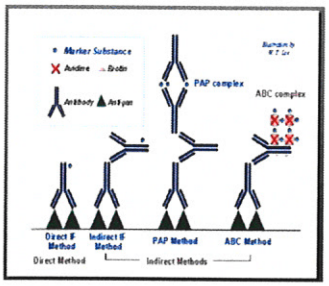
Limitations of histological grading systems

- Histologic (sub)typing is a prerequisite to grading. **Grading does not replace histotyping**
- **Never grade a treated tumor**
- **Moderate reproducibility**
- **Not every tumor is graded according to FNCLCC!**
- Grading is less informative than histological type in dedifferentiated and round cell liposarcomas, alveolar soft part sarcoma, clear cell sarcoma, epithelioid sarcoma, low-grade fibromyxoid sarcoma and primitive neuroectodermal tumor (PNET)
- **The current universal use of core needle biopsies**: the smaller the biopsy, the more problematic the (sub)typing and grading can be ('sample error', problem of heterogeneity in soft tissue tumors and sarcomas!!)
- **Grading should not be used on tumors of 'intermediate malignancy'** such as atypical fibroxanthoma and dermatofibrosarcoma protuberans
- Less informative for intermediate (grade 2) sarcoma (which represents about 40% of cases!)

Immunohistochemistry (IHC)

- IHC plays a **central role in the diagnosis** of soft tissue and bone tumors
- 'traditional' and widely available IHC are used to identify specific proteins in tumor cells indicating **a specific line of differentiation**, e.g. desmin and MyoD1 expression in tumors with skeletal muscle differentiation
- However, new molecular techniques enabled detection of **novel highly specific markers for identifying several specific types of soft tissue and bone tumors**, e.g. MUC 4 for low grade fibromyxoid sarcoma or STAT6 for solitary fibrous tumor
- Other IHC stains are used to **screen for treatment targets**, e.g. PD-L1 and panTRK
- The availability of a broad spectrum of IHC stains, the careful interpretation of these stains and the **awareness of overlapping IHC in different soft tissue and bone tumors** is essential in soft tissue and bone tumor diagnostics!

Immunohistochemistry



"soft tissue tumor practical triage panel"

- **CD34**
- **Desmin**
- **SMA**
- **S100**
- **EMA**
- **CKBs**

→differential diagnosis of fibroblastic, myoid, nerve sheath, vascular and perineurial cell tumors, synovial and epithelioid sarcoma

→exclude 'non sarcomas' with a sarcomatoid morphology

Other useful markers (1)

- **CD117(C-KIT)** and **DOG1** (gastrointestinal stromal tumors) (abdominal tumors)
- **MDM2, CDK4** (well-differentiated and dedifferentiated liposarcoma) (abdominal and retroperitoneal tumors)
- **Caldesmon** (leiomyosarcoma)
- **Myogenin (MYF4)** (rhabdomyosarcoma)
- **Microphalmia Transcription Factor (MITF)** (desmoplastic and spindle cell melanoma)
- **SOX10** (melanoma)
- **ERG** (angiosarcoma)
- **Beta-catenin** (desmoid fibromatosis)
- **HHV-8** (Kaposi sarcoma)
- **SATB2** (osteosarcoma)

Other useful markers (2)

- **cMYC** (radiation (angio)sarcoma)
- **MUC4** (low-grade fibromyxoid sarcoma)
- **TLE1** (synovial sarcoma)
- **NKX2.2** (Ewing sarcoma)
- **STAT6** (solitary fibrous tumor)
- **H3.3G34W** (giant cell tumor of bone)
- **H3K27me3** (sporadic and radiation induced malignant peripheral nerve sheath tumor)
- **BCOR** (BCOR Ewing-like sarcoma)
- **DUX4** (DUX4 Ewing-like sarcoma)
- **Brachyury** (chordoma)
- **CAMTA1** (epithelioid hemangioendothelioma)

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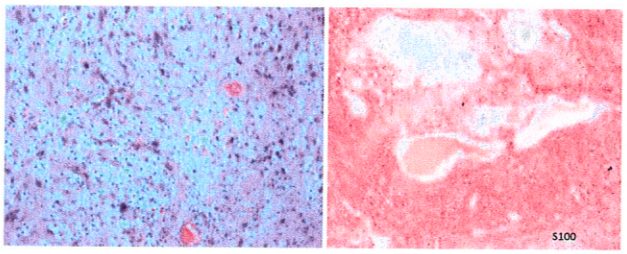
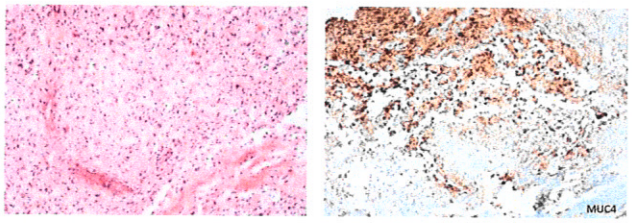
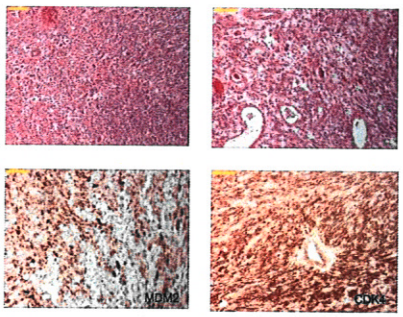


S100 expression in ancient schwannoma



MUC4 expression in low-grade fibromyxoid sarcoma



MDM2 and CDK4 expression in dedifferentiated liposarcoma

Molecular profiling of sarcomas

- For **diagnosis and classification**
- For **prognosis**
- For **treatment**

Molecular (diagnostic/prognostic)classification of sarcomas

- Sarcomas can be classified in **4 large 'genetic' groups**

1/sarcomas with specific translocation

(e.g. Ewing sarcoma, synovial sarcoma; myxoid liposarcoma; clear cell sarcoma; dermatofibrosarcoma protuberans;..)

2/sarcomas with specific mutations

(e.g. gastrointestinal stroma cell tumors-GISTs; desmoid fibromatosis; spindle cell rhabdomyosarcoma; myxoma; fibrous dysplasia; giant cell tumor of bone;..)

3/sarcomas with amplifications/deletions

(e.g. well-differentiated liposarcoma; dedifferentiated liposarcoma; atypical spindle cell/pleomorphic lipomatous tumor;..)

4/sarcomas with complex genomic profile (>50% of all soft tissue sarcomas!)

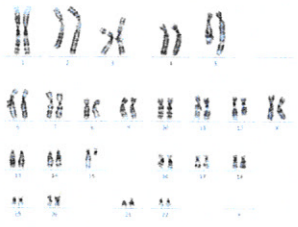
(e.g. leiomyosarcoma; myxofibrosarcoma; pleomorphic rhabdomysarcoma; pleomorphic liposarcoma;..)

Tools available for molecular profiling of sarcomas in clinical diagnostics

- **Conventional cytogenetic karyotyping**
- Fluorescence in situ hybridization (**FISH**) and reverse transcription polymerase chain reaction **(RT)-PCR**
- Array comparative genomic hybridization (**aCGH**)
- Next generation sequencing (**NGS**)

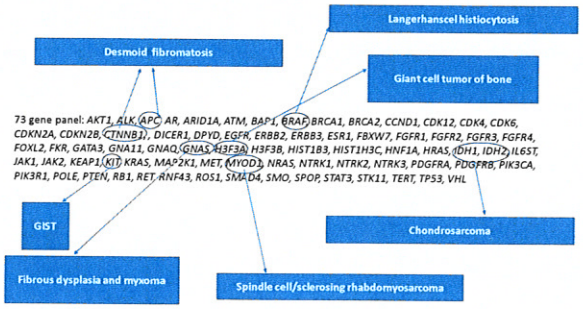
Conventional cytogenetic karyotyping

- A useful screening tool **to detect gross chromosomal alterations**
- However, it has limitations such as the **need for fresh tissue** which is not always available
- In addition, it is **time consuming** and **requires expertise** in primary cell culture
- It can also be difficult to detect small genetic alterations using this method



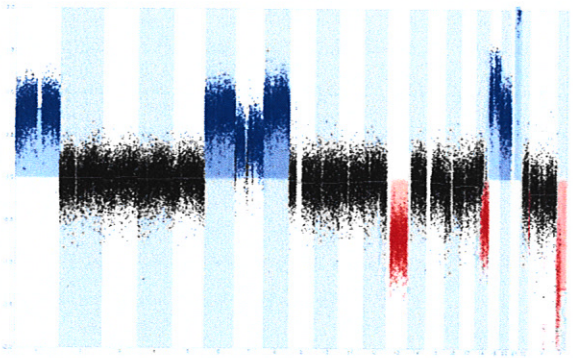
DNA Next Generation Sequencing

- The advent of clinical Next Generation Sequencing (NGS) allows for broad characterization of the genome
- **Analysis** can be performed on the entire genome (all DNA), complete exome (genes expressed in the genome), or **targeted genes and regions**
- The latter is most widely used in clinical testing (commercial NGS gene oncopanels), but **many such panels are not optimized for sarcomas** (cannot be used to detect deletions/amplifications and fusion transcripts produced by gene fusion!)



Array comparative genomic hybridization (aCGH)

- aCGH is cytogenetic technique to **analyze copy number variations (CNVs) in the cancer genome at high resolution**, it is possible to map the changes directly onto the genomic sequence
- This method can identify recurrent chromosomal changes such as microdeletions and duplications in neoplastic lesions
- **An important limitation of aCGH is the inability to detect alterations that do not result in copy number changes**, such as balanced translocations common in many sarcoma types



(RT)-PCR and FISH

- They are **commonly employed to detect specific genetic alterations including gene rearrangements, fusions, deletions and amplifications**
- They are relatively widely available and can be done **on formalin-fixed paraffin-embedded (FFPE) tissue**
- However, these tests **cannot be used to screen for unknown genetic abnormalities** and they are limited by the available probe and primer sets which are targeted to known events
- **You must know precisely what you are looking for to order the proper test** and some rare fusion variants (different genes involved in fusions in the same neoplasm such as *EWSR1-FLI1* and *EWSR1-ERG* in Ewing sarcoma) may not be detected or even have test available for them

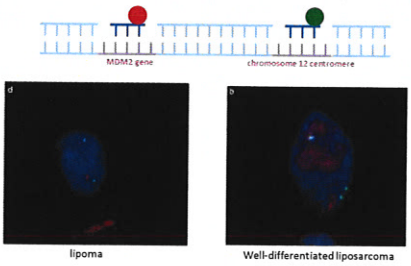
"basic" ("clinical") molecular soft tissue FISH platform

- *FKHR gene rearrangement*
  (alveolar rhabdomyosarcoma)
- *cMYC gene amplification*
  (radiation angiosarcoma)
- *EWSR1 gene rearrangement*
  (Ewing sarcoma, clear cell sarcoma, myoepithelioma/myoepithelial carcinoma of soft tissue, extraskeletal myxoid chondrosarcoma, desmoplastic round cell tumor,.....)
- *FUS gene rearrangement*
  (low-grade fibromyxoid sarcoma, myxoid/round cell liposarcoma)
- *DDIT3 (CHOP) gene rearrangement*
  (myxoid/round cell liposarcoma)

"basic" ("clinical") molecular soft tissue FISH platform

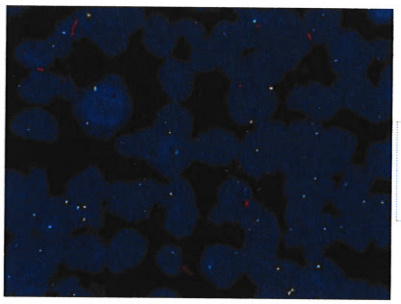
- *SYT gene rearrangement*
  (synovial sarcoma)
- *MDM2/CDK4 gene amplification*
  (well and dedifferentiated liposarcoma, some osteosarcoma subtypes)
- *ALK gene rearrangement*
  (inflammatory myofibroblastic tumor, epithelioid fibrous histiocytoma)
- *COL1A1-PDGFRB fusion*
  (dermatofibrosarcoma protuberans)
- *RB1 deletion*
  (spindle/pleomorphic lipoma, 'atypical spindle cell/pleomorphic lipomatous tumor', mammary type myofibroblastoma, cellular angiofibroma)

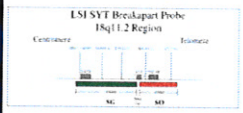
MDM2 in situ hybridization (fluorescent: FISH)



lipoma

Well-differentiated liposarcoma





SYT gene rearrangement in synovial sarcoma

Limitations of FISH and role of RNA sequencing testing for gene fusion testing in sarcoma diagnostics

- Many newly described tumor entities, 5th edition WHO

(BCOR 'Ewing-like' sarcoma, CIC 'Ewing-like' sarcoma, 'NTRK spindle cell neoplasms', NCOA2 rearrangements in 'angiofibroma of soft tissue', TFCP2 rearrangements in spindle cell rhabdomyosarcoma, FOSB rearrangement in pseudomyogenic hemangioendothelioma, PAX3 rearrangement in biphasic sinonasal sarcoma,...)

- Newly described tumor entities with *EWSR1* rearrangements: importance of information about the *EWSR1* fusion partner!

(EWSR1-SMAD3 positive fibroblastic tumor, 'Ewing sarcoma-like' round cell sarcomas with EWSR1-NFATc2/PATZ1 fusions,...)

- Known tumor entities where it becomes more and more prognostically and therapeutically important to know the entire gene fusion product

(e.g. PAX3-FOXO1 vs PAX7-FOXO1 in alveolar rhabdomyosarcoma)

- To further characterize 'undifferentiated' spindle and round cell sarcomas, research setting

TABLE 4. 2020 WHO Classification: Recently Identified Genetic Alterations in Soft Tissue Tumors

Section	Tumor Type	New Genetic Alterations
Fibroblastic/myofibroblastic tumors	Fibrous hamartoma of infancy	*EGFR* mutations
	Calcifying fibrous tumor	*FN1-EGF*
	Lipofibromatosis	*FN1-EGF*; other receptor tyrosine kinase or EGFR ligand fusions
	Dermatofibrosarcoma protuberans	*COL6A3-PDGFD, EMILIN3-PDGFD*
	SFT	*NAB2-STAT6*
	Myxoinflammatory fibroblastic sarcoma	*BRAF* fusions
	Infantile fibrosarcoma	*EML4-NTRK3, NTRK1, NTRK2, BRAF,* and *MET* fusions
Pericytic/perivascular tumors	Glomus tumor	*MIR143-NOTCH1/2/3*
	Myopericytoma/myofibroma	*PDGFRB* mutations, *SRF-RELA* (cellular myofibroma)
Vascular tumors	Epithelioid hemangioma	*FOS* and *FOSB* fusions
	Pseudomyogenic hemangioendothelioma	*SERPINE1-FOSB, ACTB-FOSB*
Skeletal muscle tumors	Spindle cell/sclerosing rhabdomyosarcoma	*MYOD1* mutations (adolescent/adults)
		SRF-NCOA2, TEAD1-NCOA2, VGLL2-NCOA2, VGLL2-CITED2 (congenital/infantile)
		EWSR1-TFCP2, FUS-TFCP2 (intraosseous, spindle cell and epithelioid)
Peripheral nerve sheath tumors	Epithelioid schwannoma	*SMARCB1* mutations
	Granular cell tumor	*ATP6AP1* or *ATP6AP2* mutations
	Benign triton tumor (neuromuscular choristoma)	*CTNNB1* mutations
	MPNST	*SUZ12* or *EED* mutations (loss of H3K27me3)
Tumors of uncertain differentiation	Phosphaturic mesenchymal tumor	*FN1-FGFR1, FN1-FGF1* (rare)

RNA NGS sequencing

NGS-based sequencing can also be applied to RNA and is commonly referred to as **RNA sequencing (RNA seq).**

-the entire transcriptome (all RNA expression) can be sequenced and correlated to provide gene expression data

-RNA seq can also been used **to detect point mutations** (but this is not nearly as widely used as direct DNA sequencing)

-Most importantly for sarcoma, RNA seq can **detect fusion transcripts** produced by gene fusion

→**TruSight RNA Pan-Cancer**: 1385 cancer genes, research setting

→**Archer fusion plex platform**: clinical practice

Conclusion

- Important role of the pathologist in diagnosis, prognostication and therapy of soft tissue and bone tumors
- Soft tissue and bone tumor diagnostics=challenge for the pathologist, importance of centralisation of this pathology
- Special techniques (immunohistochemistry and molecular pathology) are essential for (sub)typing of sarcomas
- Value and limitations of grading in sarcomas
- Role of molecular profiling in sarcomas