XV CURSO AVANZADO DE SARCOMAS GEIS 2023

Diagnóstico en sarcomas. Partes blandas y óseos.

Anatomía Patológica y Diagnóstico Molecular

"Pathology & Molecular diagnosis"

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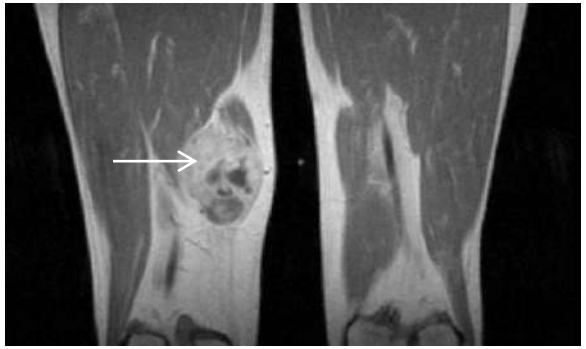
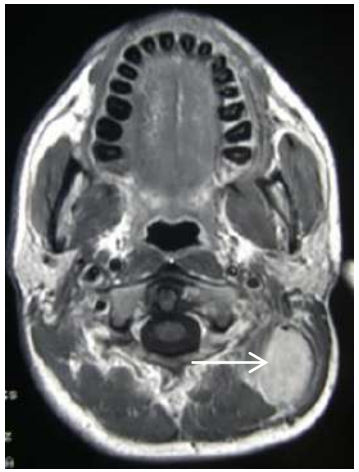
GEIS
GRUPO ESPAÑOL DE INVESTIGACIÓN EN SARCOMAS

Máster en Tumores Musculoesqueléticos

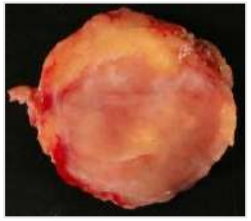
Soft Tissue & Bone Sarcomas: a diagnostic challenge

- Heterogeneous group of rare malignancies (1% of all cancers)
- Any age, any location (somatic, visceral, bone)
- Between 70-100 different histological subtypes / tumor entities
- Morphological overlap
- Limited biopsy material
- Divergent pathological interpretation
- **Both rarity and heterogeneity affect diagnostic accuracy**

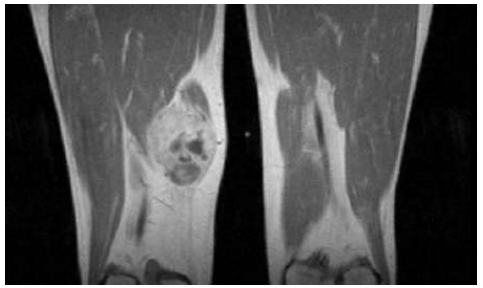




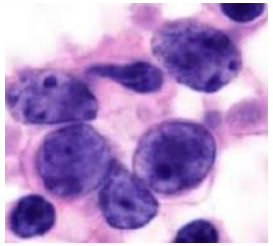

Diagnostic work-up in sarcomas



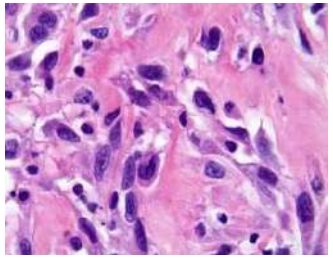

1. Clinical & radiological data



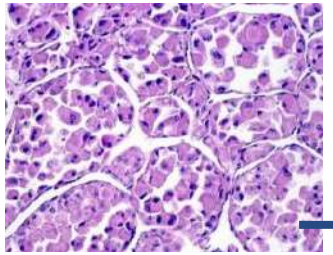

Biopsy (CNB)


FNA

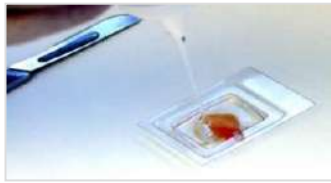

Tru-cut



2. H&E



→ Frozen tissue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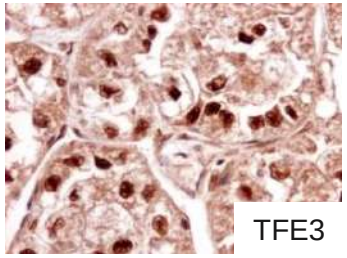
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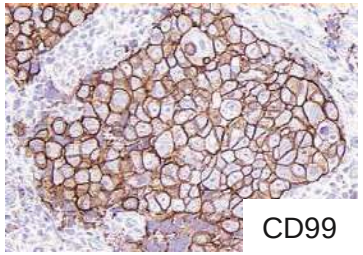
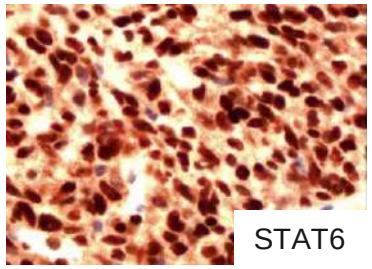

CD99

3.IHC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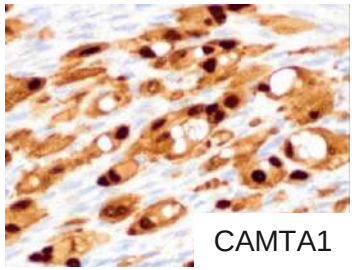
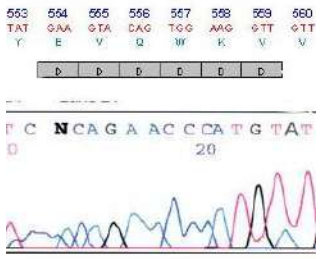
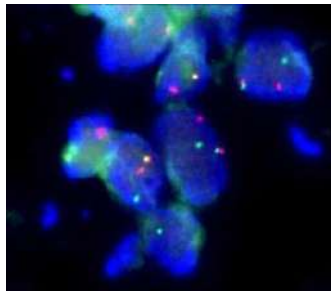

CAMTA1



PCR


FISH

4. Molecular pathology

Diagnosis
Prognosis
Therapy

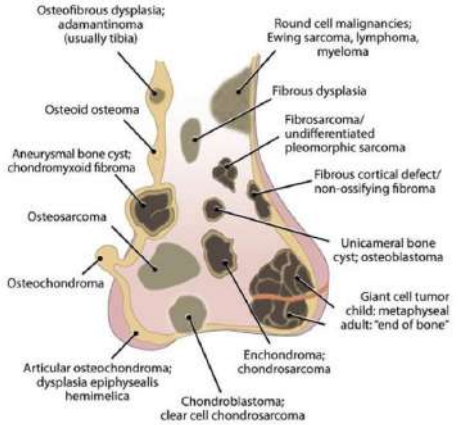
NGS

1. Clinical & radiological data: essential!!

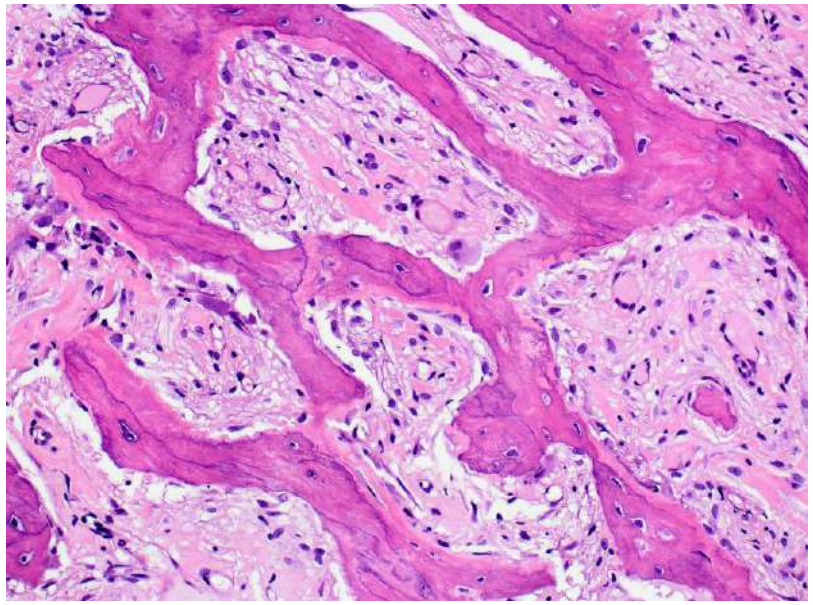
(Mandatory in bone tumours)

- Age, symptoms, site, size, previous history (cancer, RT…)
- Associated syndromes or genetic disorders:

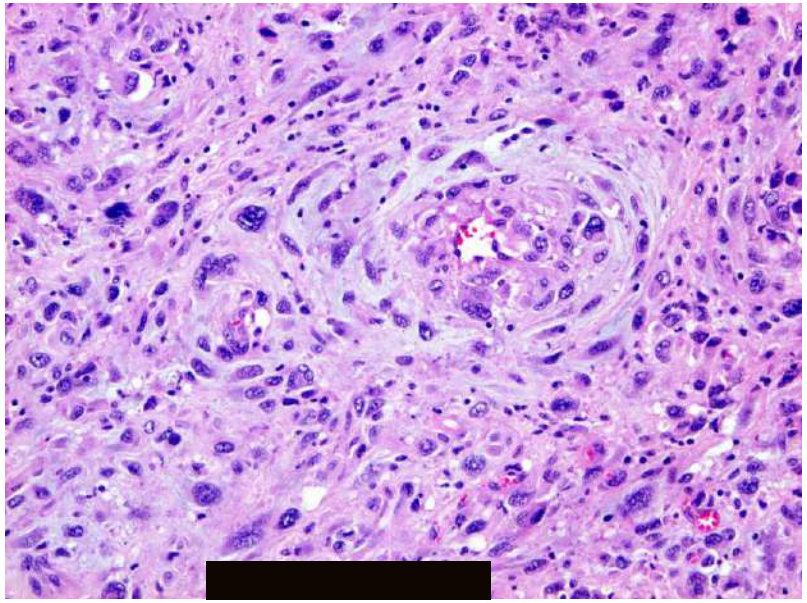
  Li- Fraumeni, Ollier, NF-1, RB1…



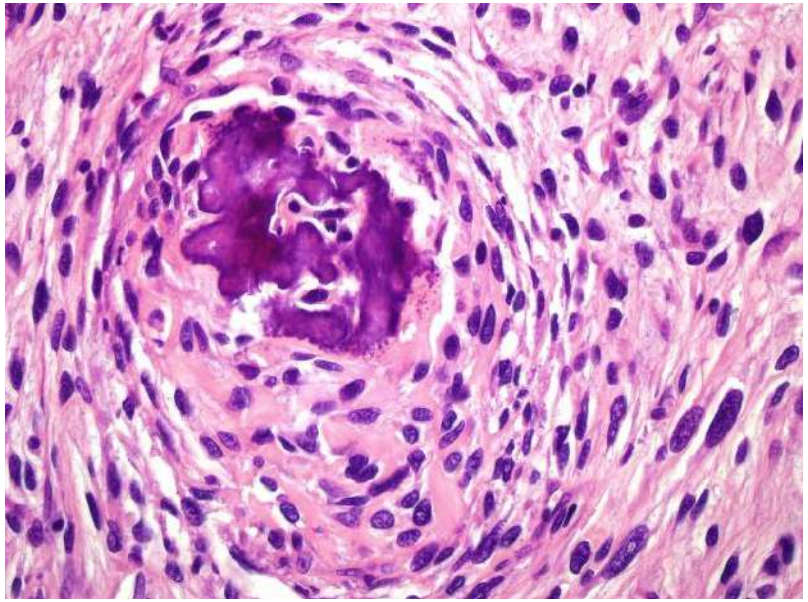
Source: Nielsen GP et al in AFIP atlas. Series 5. Tumors of the Bones and Joints



Osteoblastic tumor (malignant?)



Pleomorphic sarcoma



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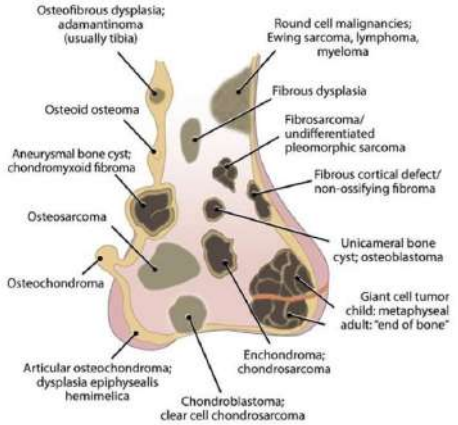

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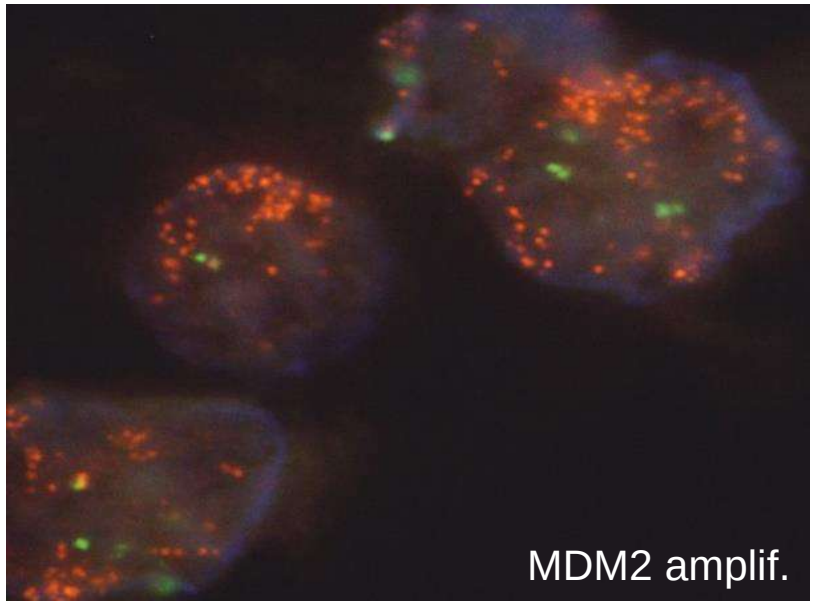

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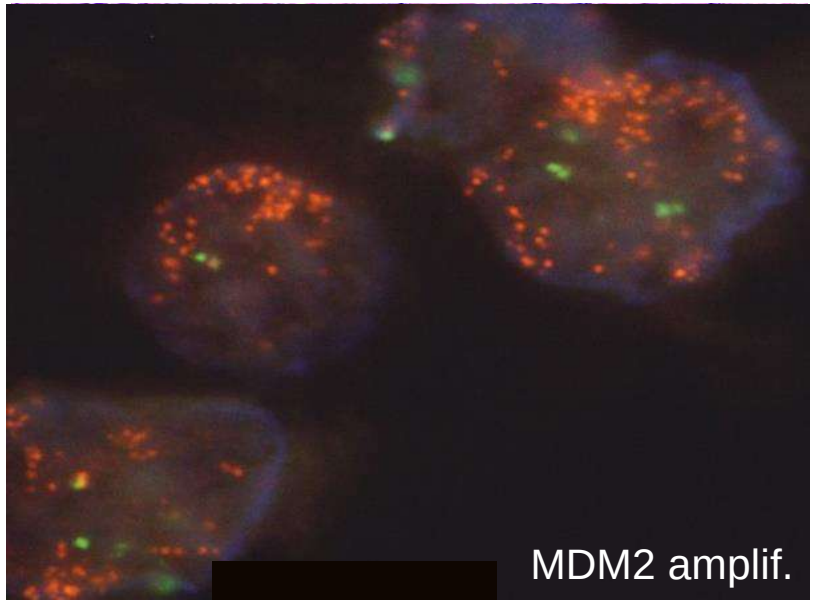

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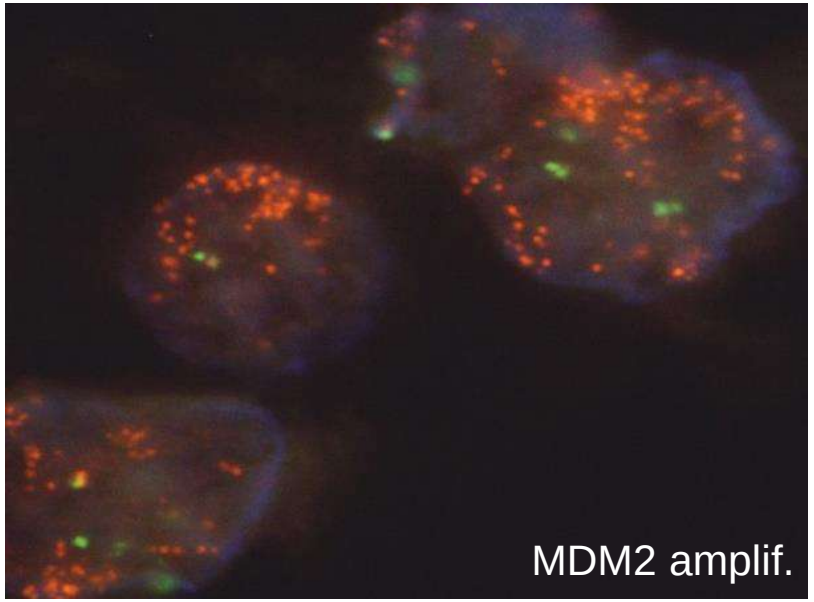

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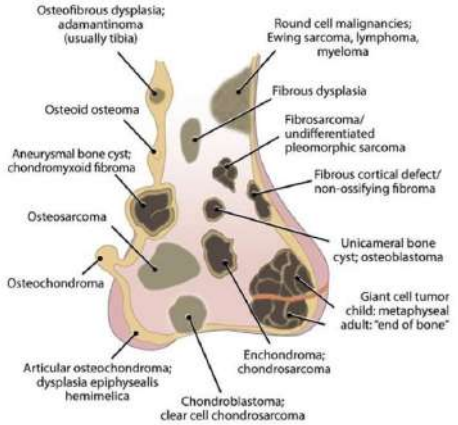

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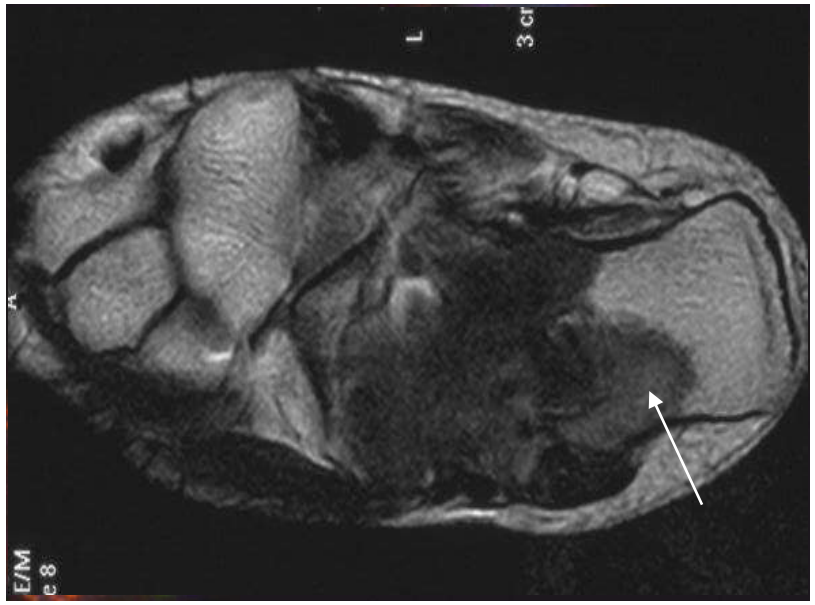
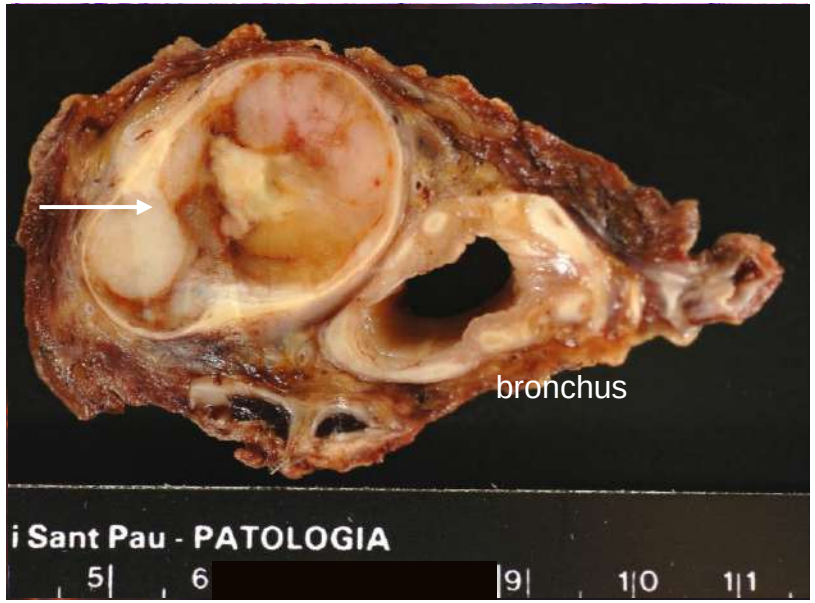


Low-grade intramedullary OS (astragalus)

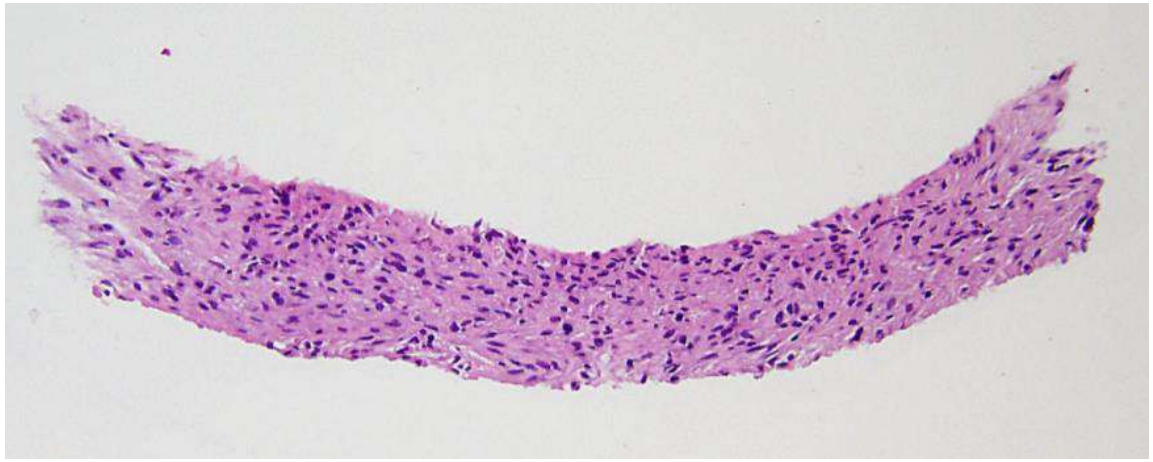


Intimal sarcoma (pulmonary artery)

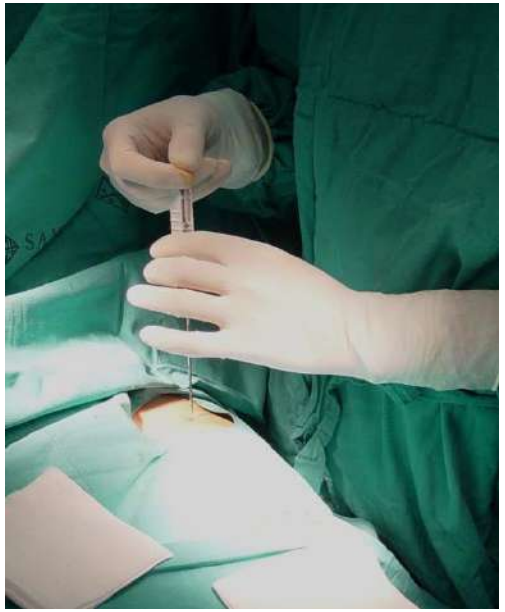
Dediff liposarcoma (retroperitoneum)

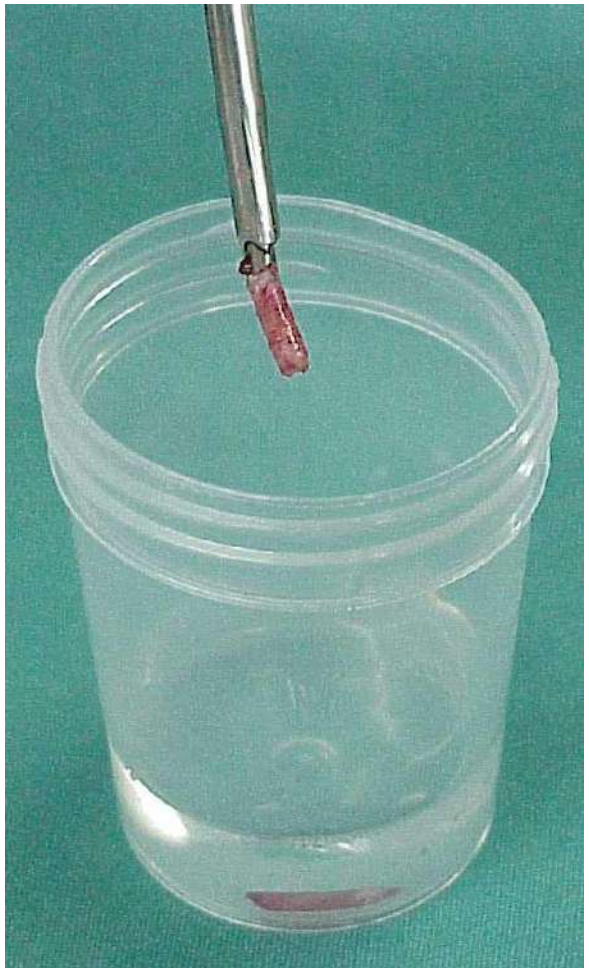
2. Conventional histology (H&E): role of core needle biopsy

- Is the lesion mesenchymal?
- If mesenchymal…is it benign or malignant (sarcoma)?
- What type of sarcoma? (histotype & grading_when possible)



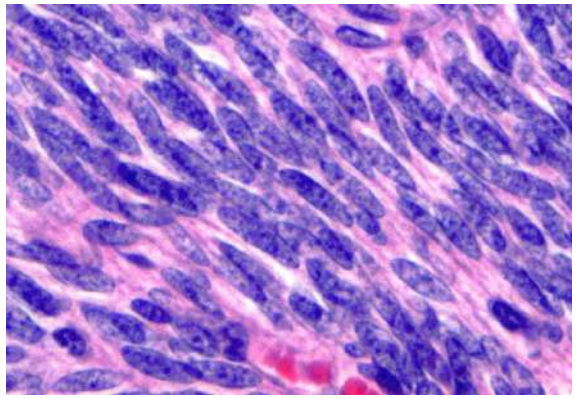
Limited biopsy material



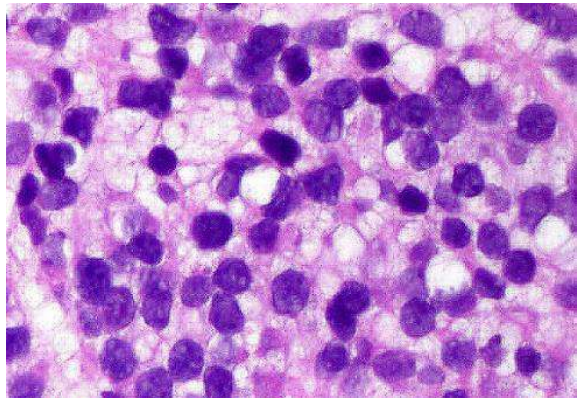


Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. https://doi.org/10.106/j.annonc.2021.07.006

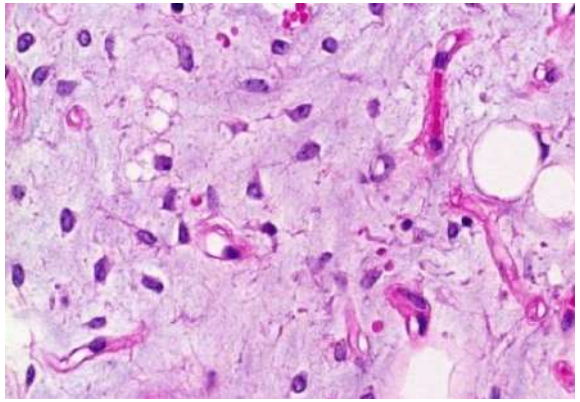
2. Conventional histology (H&E):
cellular morphology, matrix & others



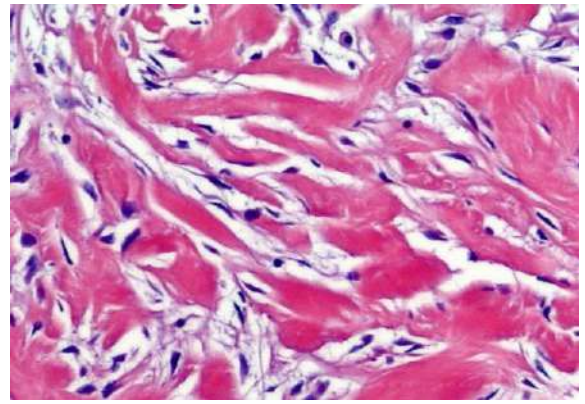
Spindle cell



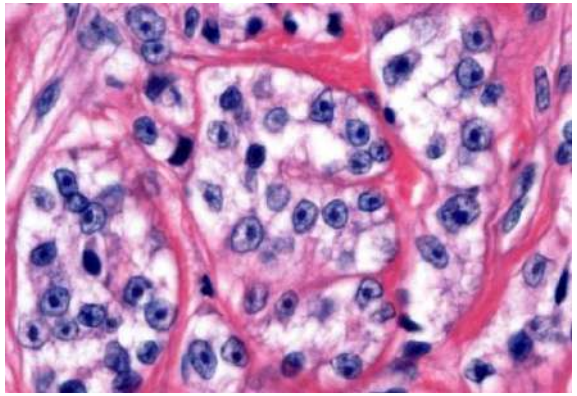
Round cell



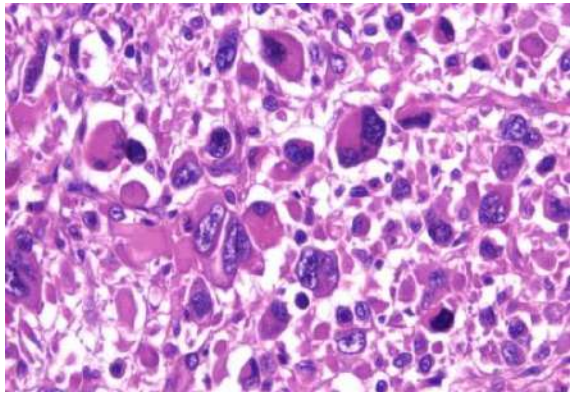
Myxoid



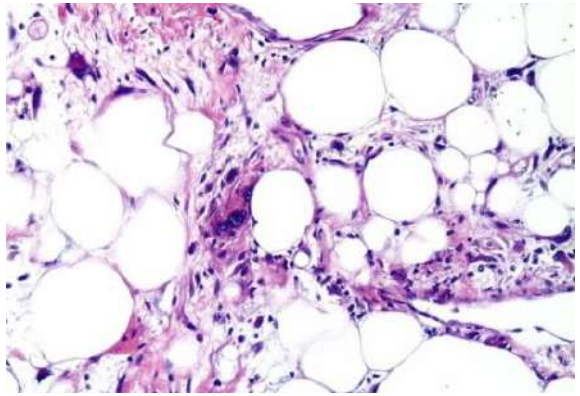
Collagenous



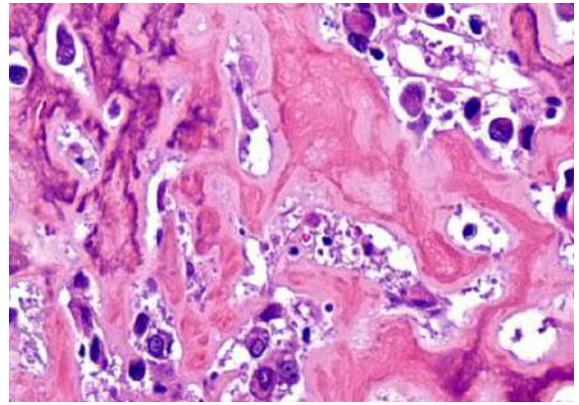
Epithelioid



Pleomorphic

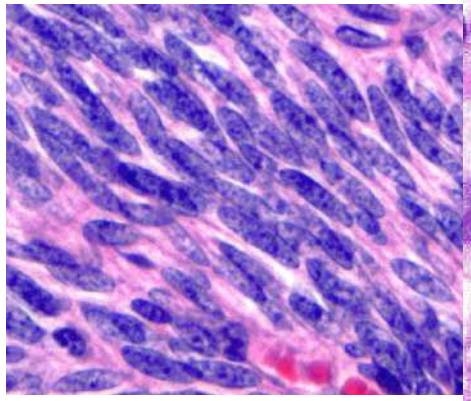


Lipomatous

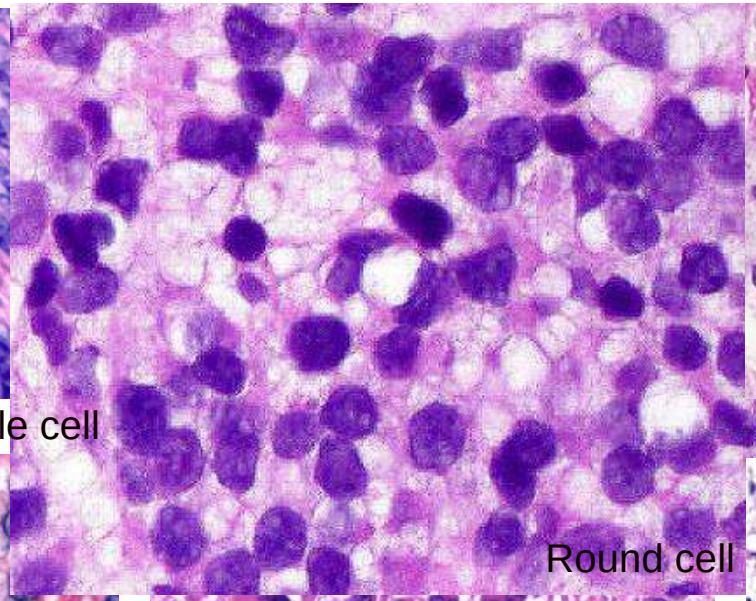


Osteoid

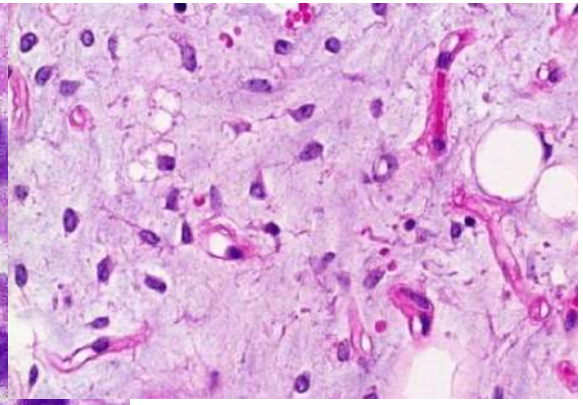
2. Conventional histology (H&E):
cellular morphology, matrix & others



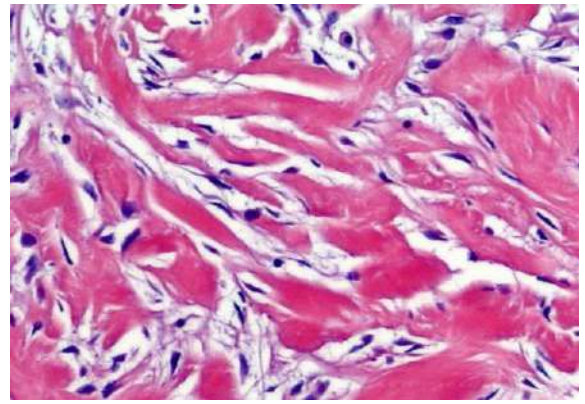
Spindle cell



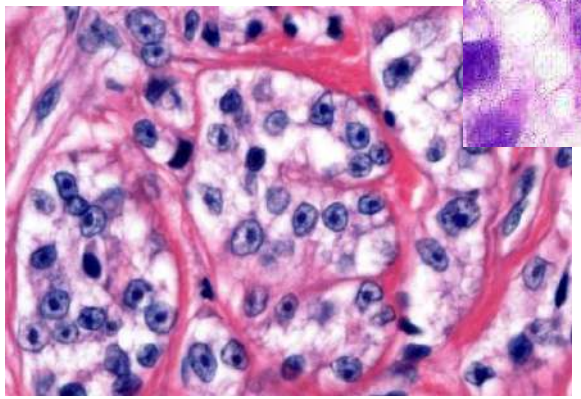
Round cell



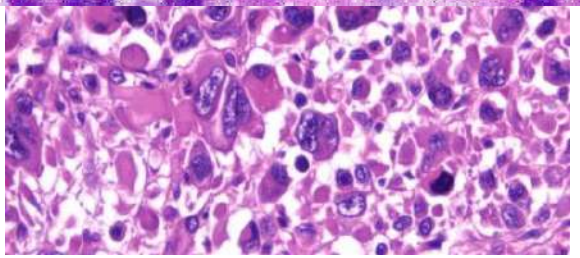
Myxoid



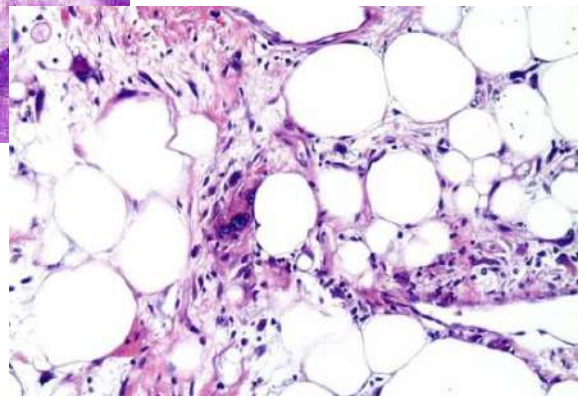
Collagenous



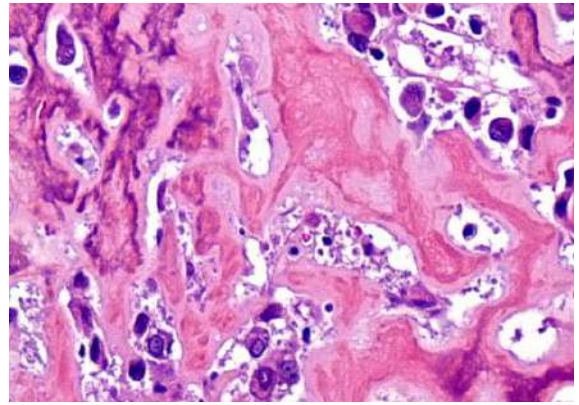
Epithelioid



Pleomorphic



Lipomatous

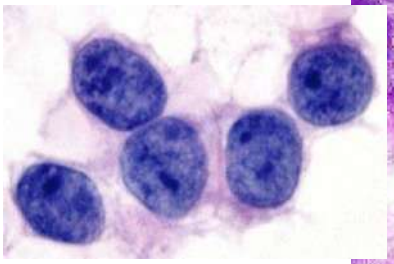


Osteoid

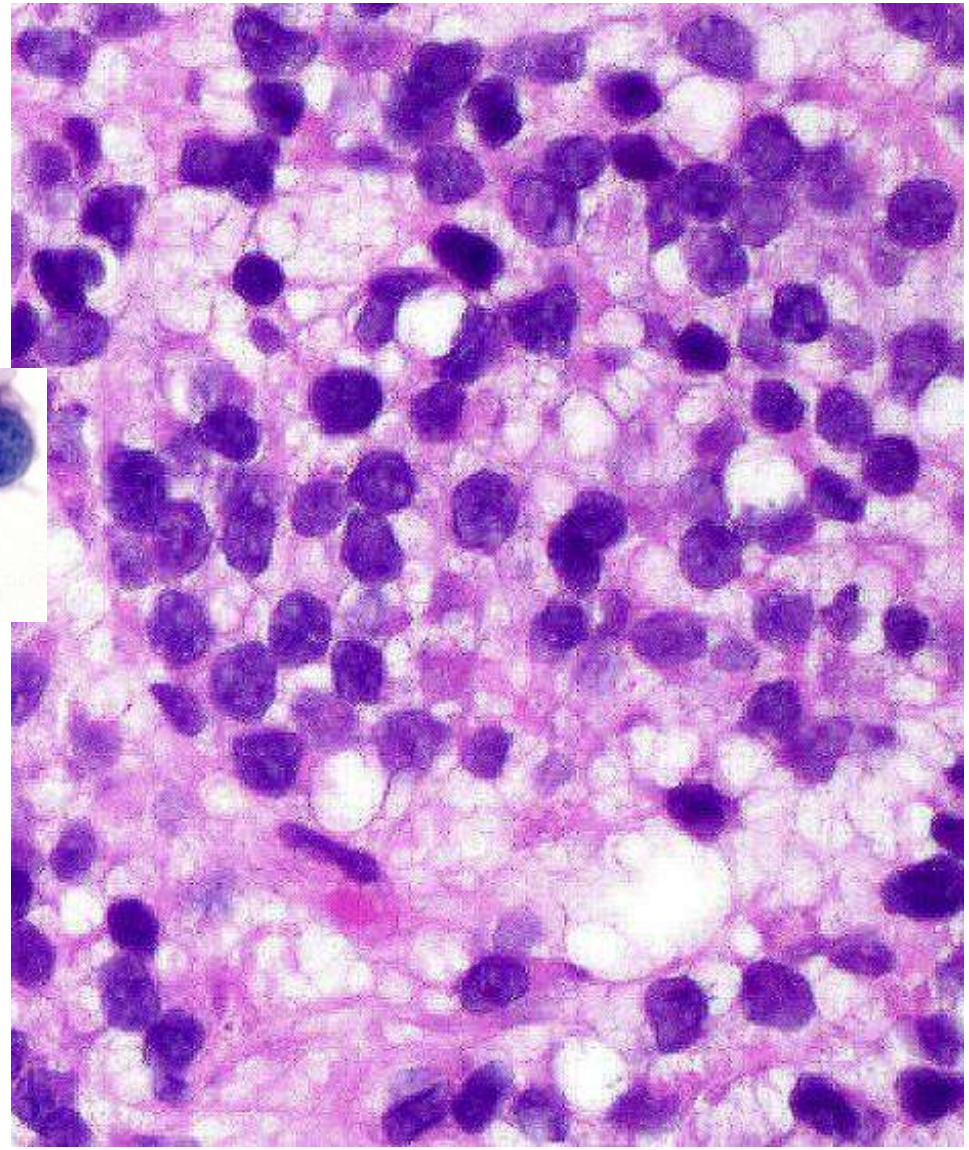
2. Conventional histology (H&E):

Small round cell tumors

- Ewing sarcoma
- Undiff small round cell sarcomas (CIC, BCOR…)
- Alveolar rhabdomyosarcoma
- High-grade myxoid liposarcoma
- Poorly differentiated synovial sarcoma
- Myoepithelial tumors
- Mesenchymal chondrosarcoma
- Desmoplastic small round cell tumor (DSRCT)
- Differential diagnosis: lymphoma, neuroblastoma, small cell carcinoma (adults!)…



- Mainly in children & adolescents
- High-grade by definition
- Often “translocated-sarcomas”



DSRCT

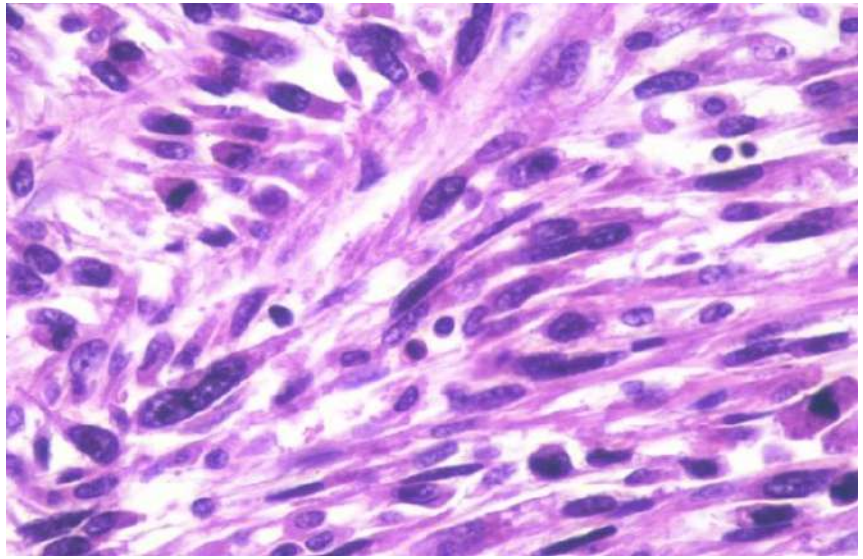
2. Conventional histology (H&E): histological grade

- Tumor aggressiveness
- Most important independent prognostic factor; guide for treatment
- Staging system: (TNM) + **G 1-3**
- French grading system_FNCLCC
- Three histological parameters assessed:

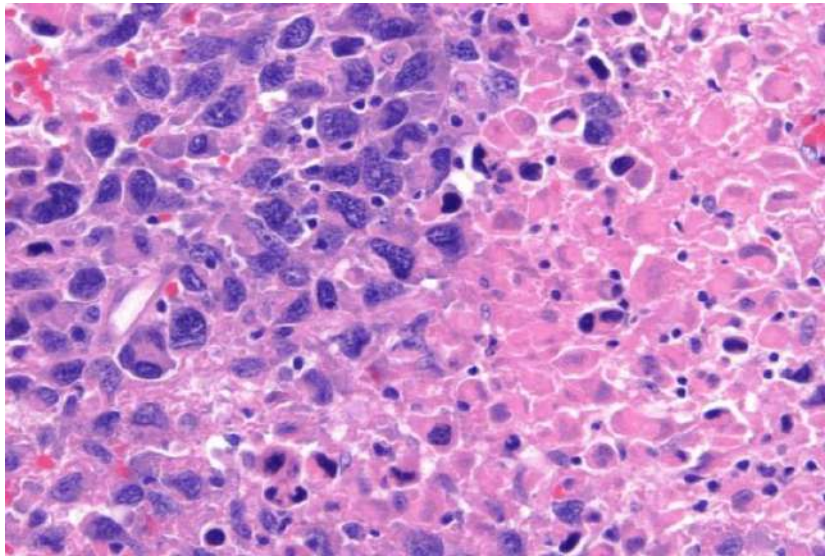
    1. Tumor differentiation

    2. Number of mitoses per 10 high-power fields

    3. Percentage of tumor necrosis

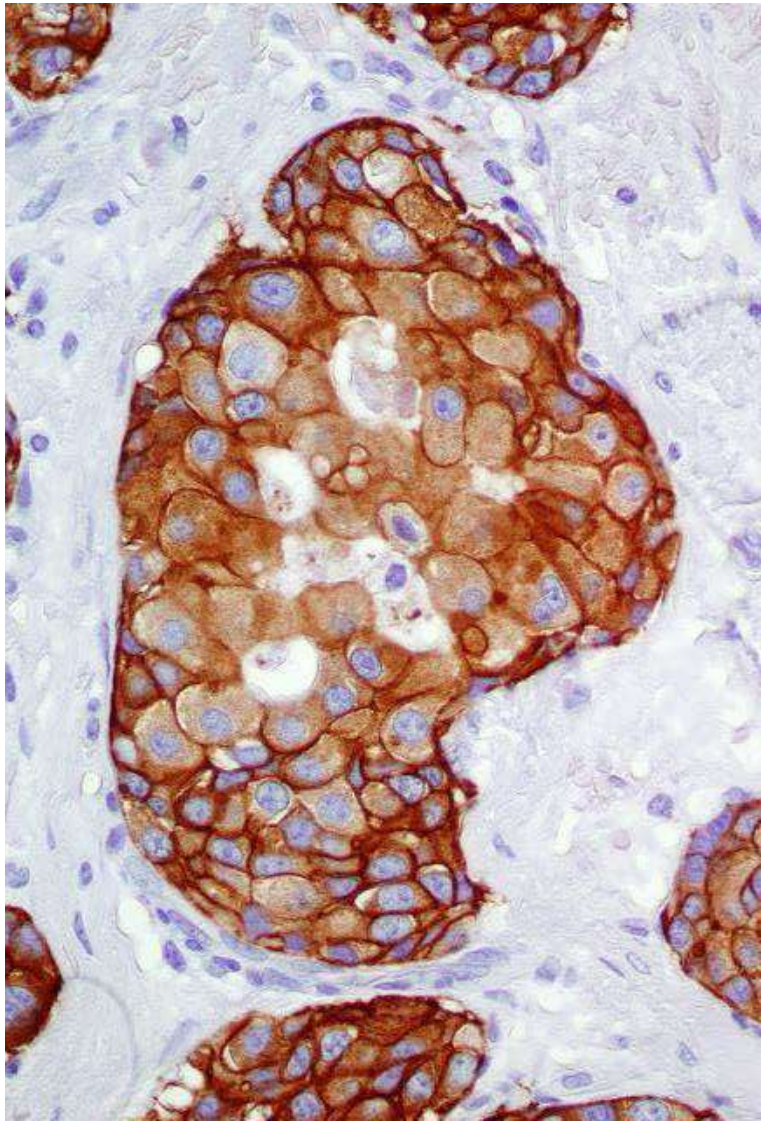


Hypercellularity, atypia, few mitoses - G1



Severe atypia, extensive necrosis - G3

3. Integrate an IHC panel: role of immunohistochemistry



a) D.D. between **mesenchymal / non-mesenchymal** tumors (carcinoma, melanoma, lymphoma)

b) Standard method for establishing **differentiation (lineage):** myofibroblastic, vascular, neural, smooth muscle, skeletal muscle…

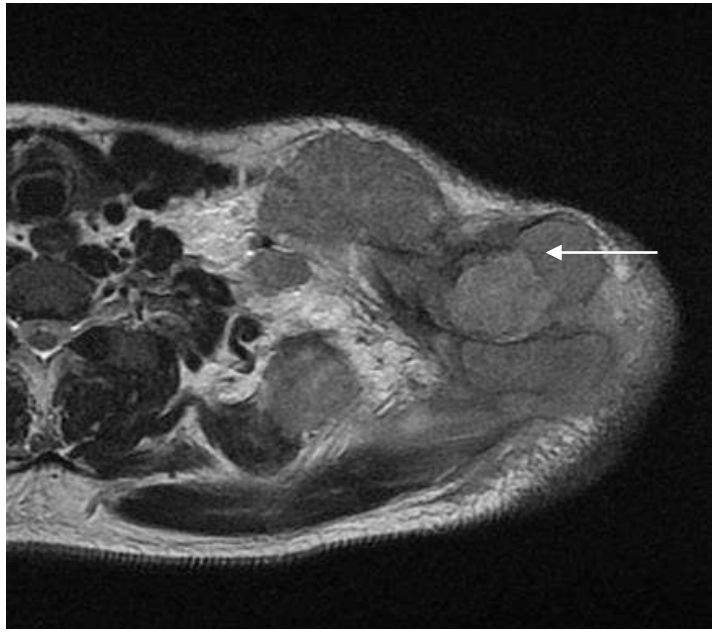
c) Facilitates D.D. and classification of a spindle and/or round cell neoplasm

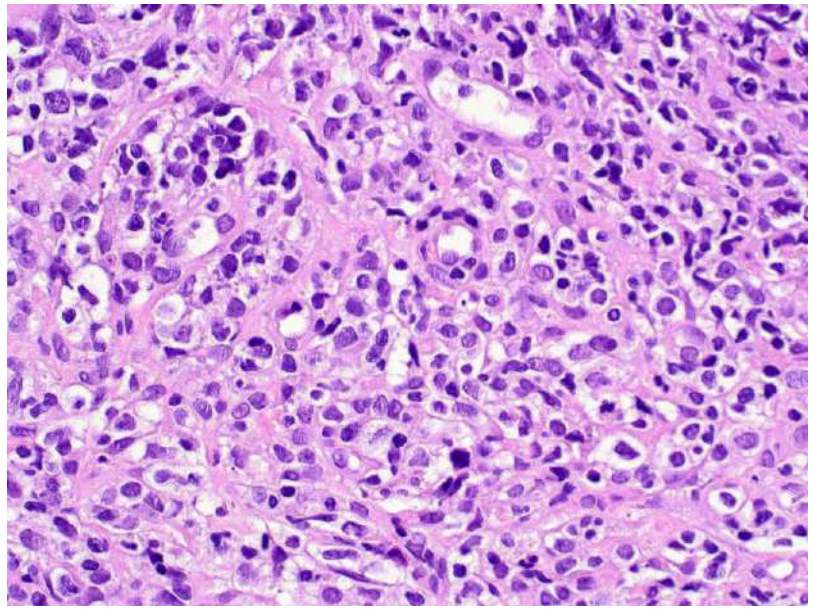
d) Correlation with **molecular genetic** alterations (gene fusions, amplifications, deletions, mutations…)

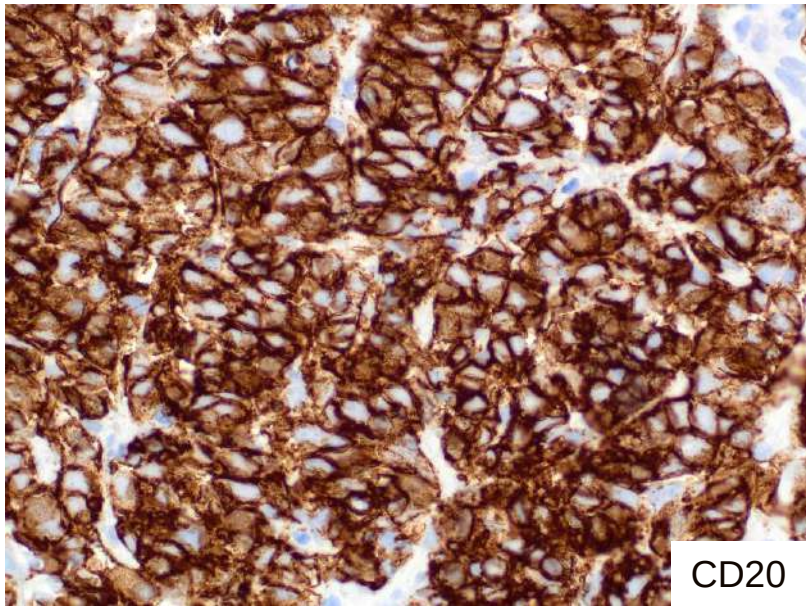
Differential diagnosis between mesenchymal / non-mesenchymal tumors

Clinical history

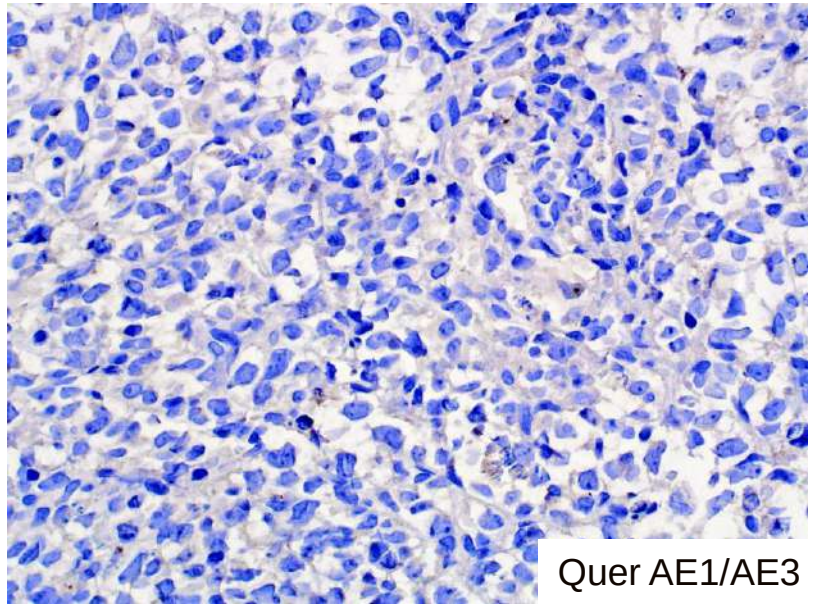
Male, 39 y-o
Left scapular & soft tissue mass
CNB: small round cell tumor
IHC: CD20+
Dx: Diffuse large B-cell lymphoma



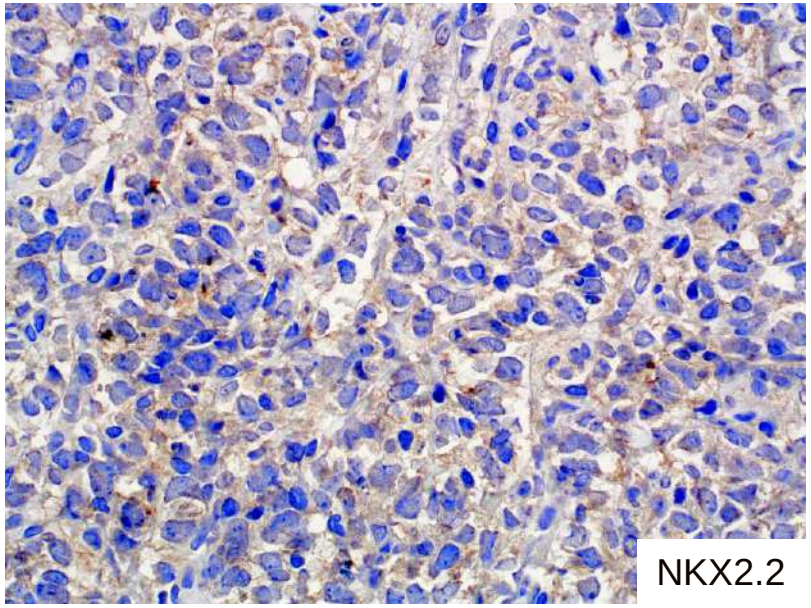




CD20



Quer AE1/AE3



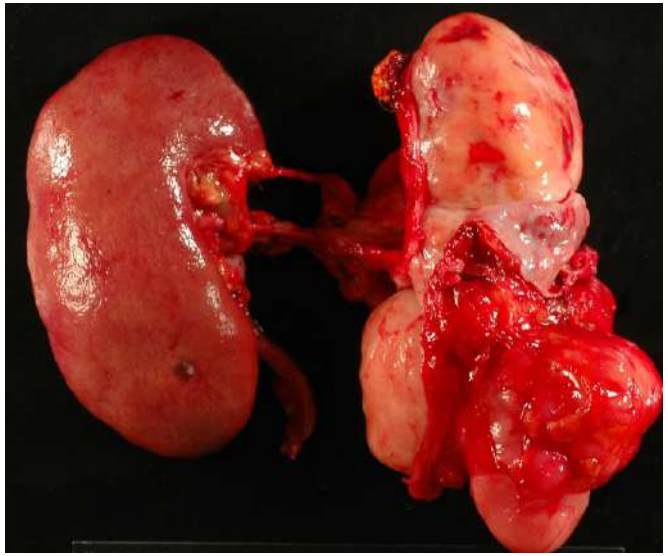
NKX2.2

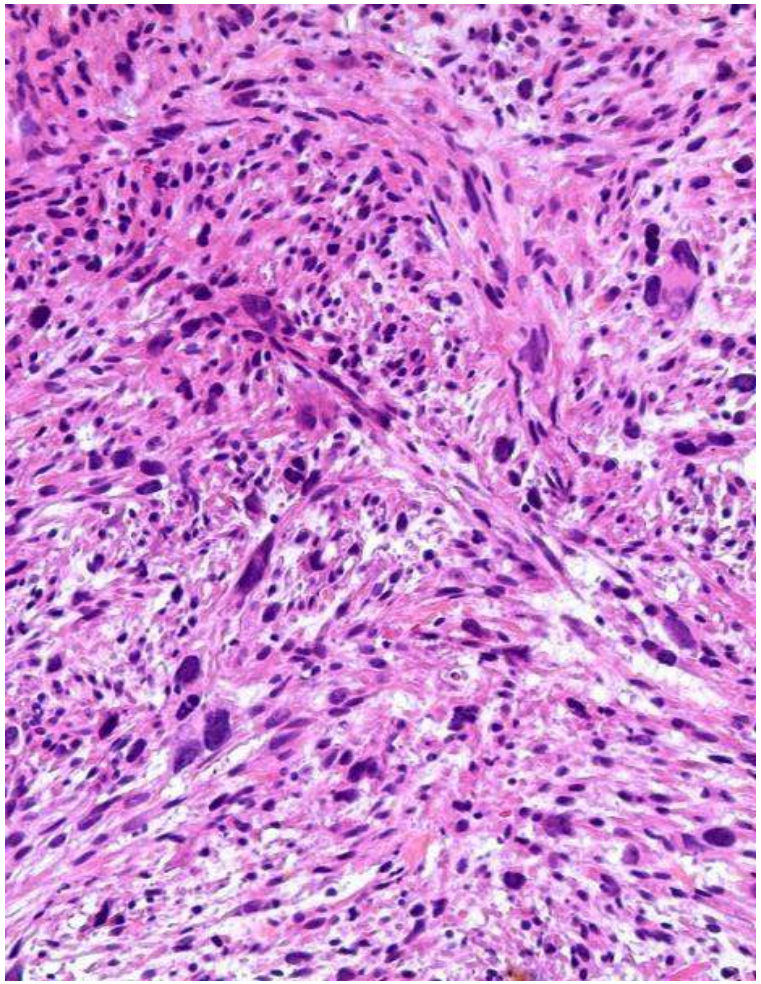
Establish differentiation (lineage)

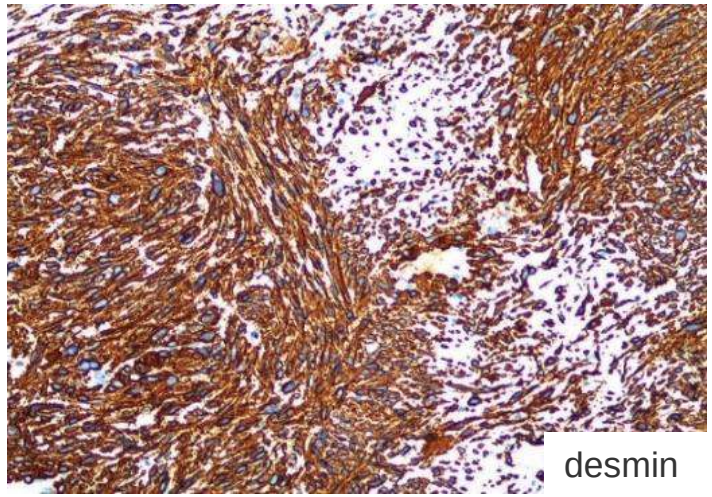
Clinical history

Male, 73 y-o

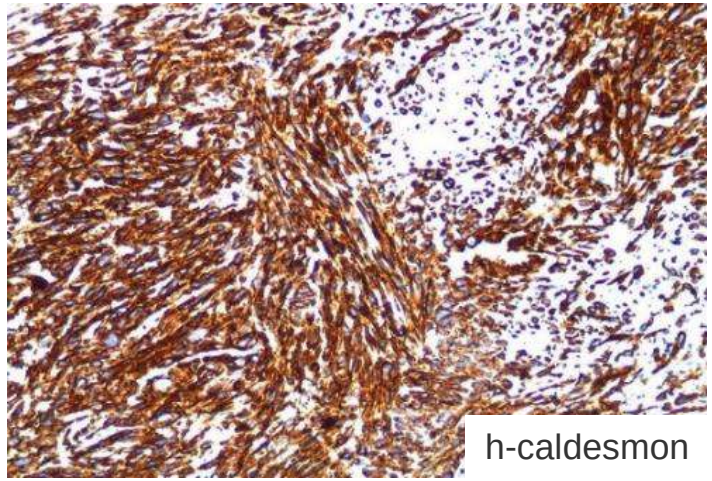
Retroperitoneal mass. Dediff liposarcoma??







desmin



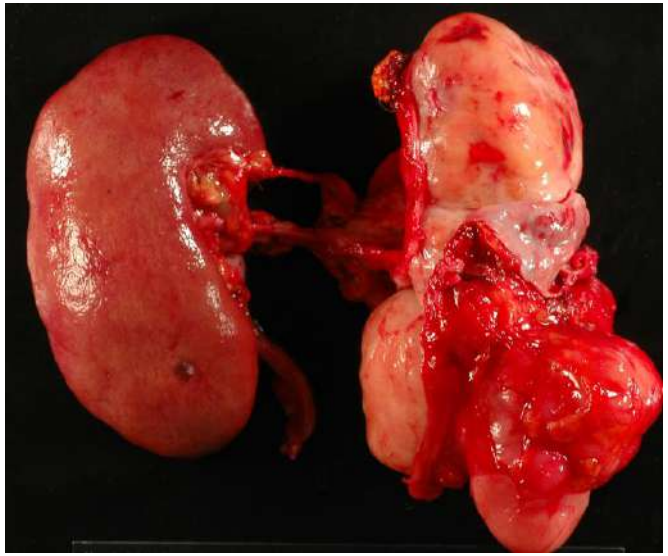
h-caldesmon

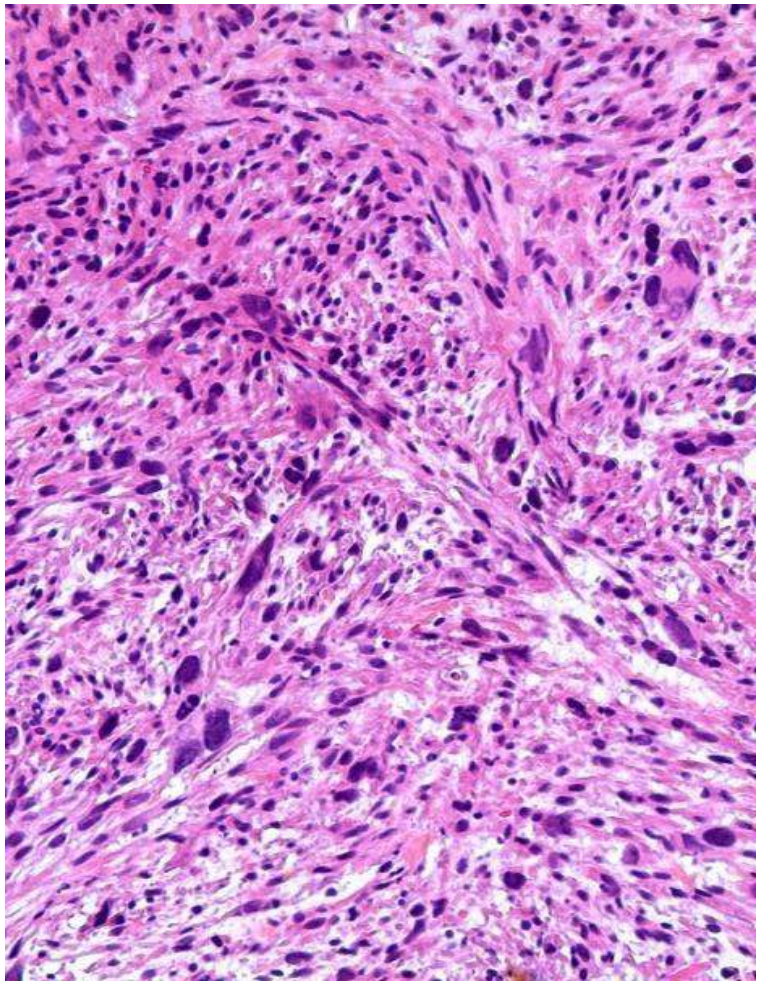
Establish differentiation (lineage)

Clinical history

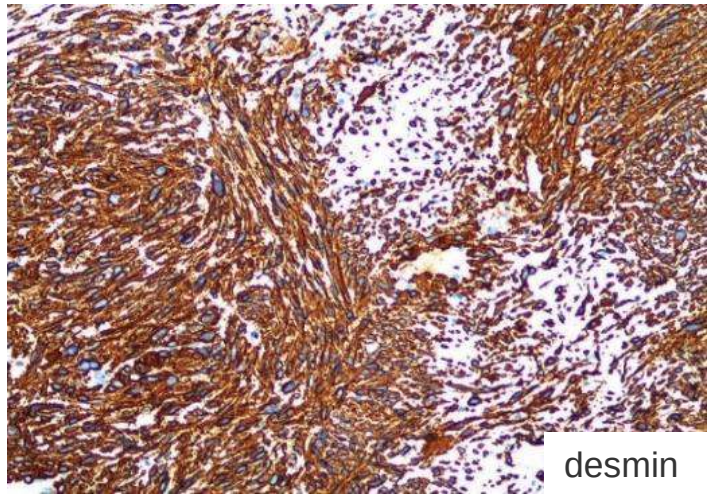
Male, 73 y-o

Retroperitoneal mass. Dediff liposarcoma??

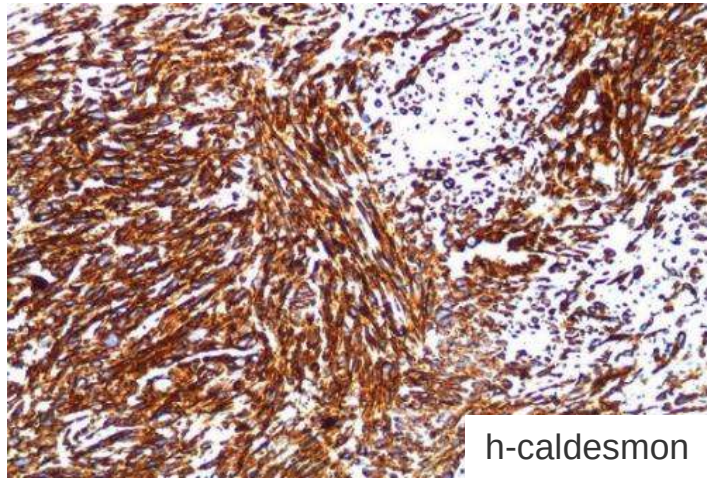




Dx: Leiomyosarcoma



desmin



h-caldesmon

DD between spindle cell neoplasms

	CD34	CK	ERG	S100	DESM	Other markers
Angiosarcoma	+	+/-	++	-	-	CD31
Spindle cell rhabdomyosarc	-	-	-	-	+ diffuse	Myogenin+ve , MyoD1+ve
Leiomyosarcoma	-	+/-	-	-	+	Caldesmon, actin HHF35, calponin
Synovial sarcoma	-	+ (7/19)	-	+/-	-	**SS18**, EMA, CD99, CK7
GIST	+ve 50 %	-	-	+/-	-	CD117, DOG-1, Caldesmon, **SDHB** (loss)
IMT	-	+/-	-	-	+/-	ALK (50%) Calponin
MPNST	+	+/-	-	+ (focal)	+ Triton	**H3K27m3 loss;** CD56, GFAP, SOX10 25-50%
Solitary fibrous tumor	++	-	-	-/+	-	**STAT6**, BCL2, CD99

Use of IHC markers as surrogates for molecular genetic testing

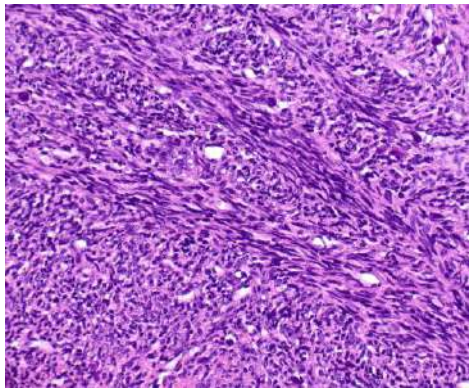
ORIGINAL ARTICLE

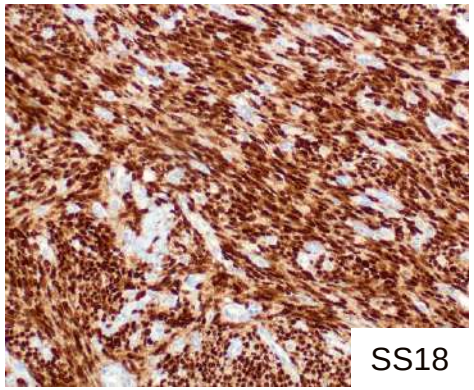
A Novel SS18-SSX Fusion-specific Antibody for the Diagnosis of Synovial Sarcoma

Esther Baranov, MD, Matthew J. McBride, PhD,† Andrew M. Bellizzi, MD,‡ Azra H. Ligon, PhD,* Christopher D.M. Fletcher, MD, FRCPath,* Cigall Kadoch, PhD,† and Jason L. Hornick, MD, PhD**

Clinical history

Male, 6 y-o. Left popliteal mass





SS18

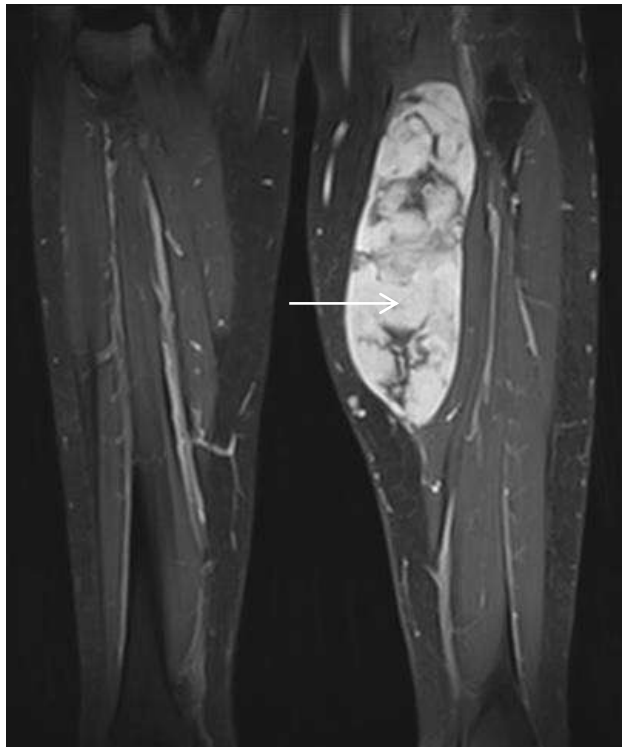
Modern Pathology
https://doi.org/10.1038/s41379-021-00782-1

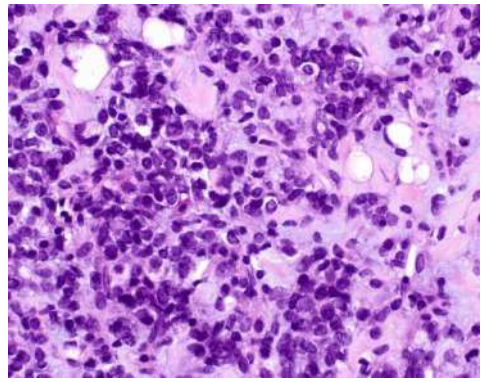
ARTICLE

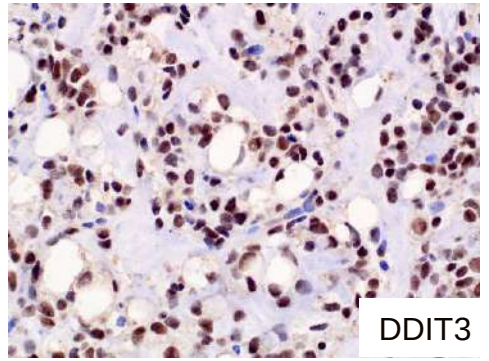
Nuclear expression of DDIT3 distinguishes high-grade myxoid liposarcoma from other round cell sarcomas

Clinical history

Female, 46 y-o. Right thigh (soft tissue) tumor

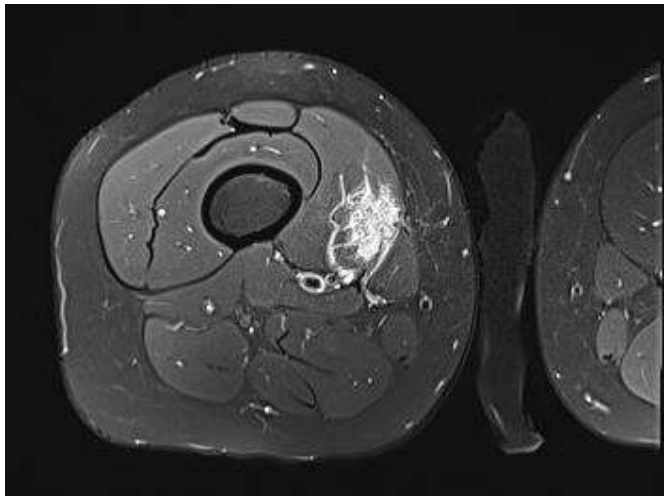






DDIT3

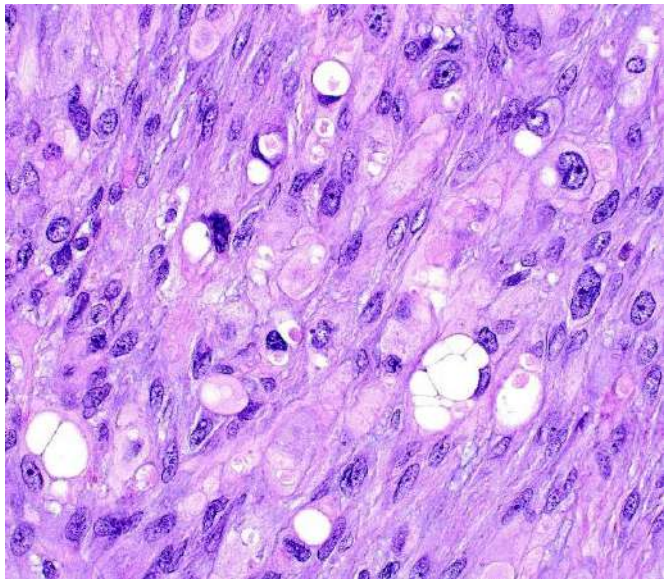
Use of IHC markers as surrogates for molecular genetic testing



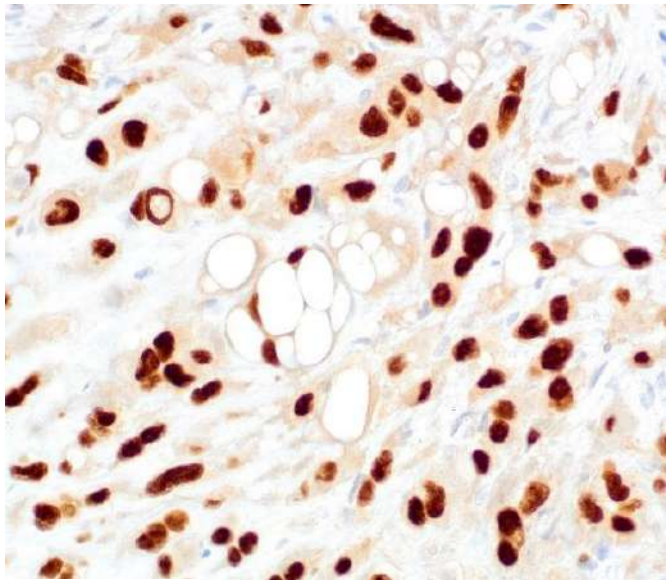
Clinical history

Female, 38 y-o

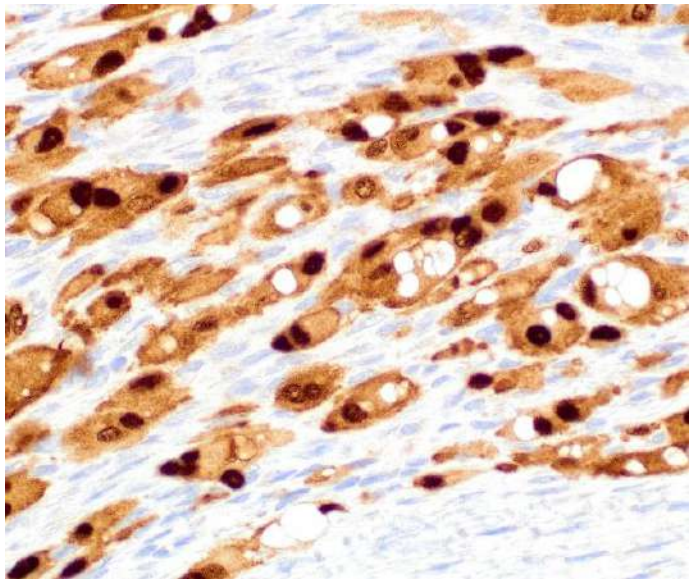
Right thigh tumor



Epithelioid neoplasm:
AdenoK? Liposarcoma? Vascular ?

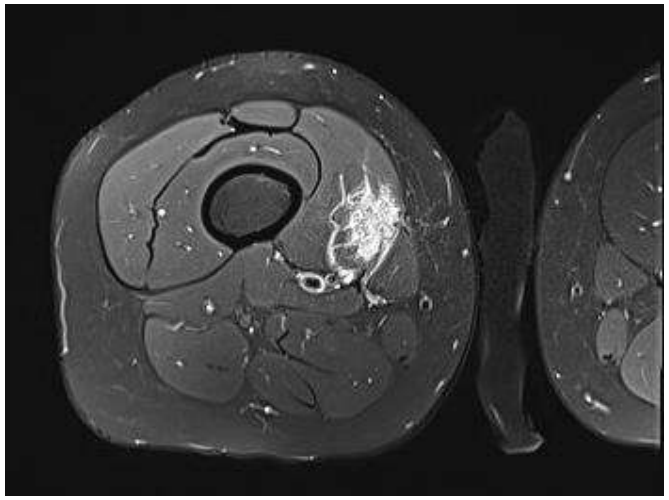


ERG+ve: Indicates vascular tumor
HE? EHE? Epithelioid AS?



CAMTA1+ve → EHE

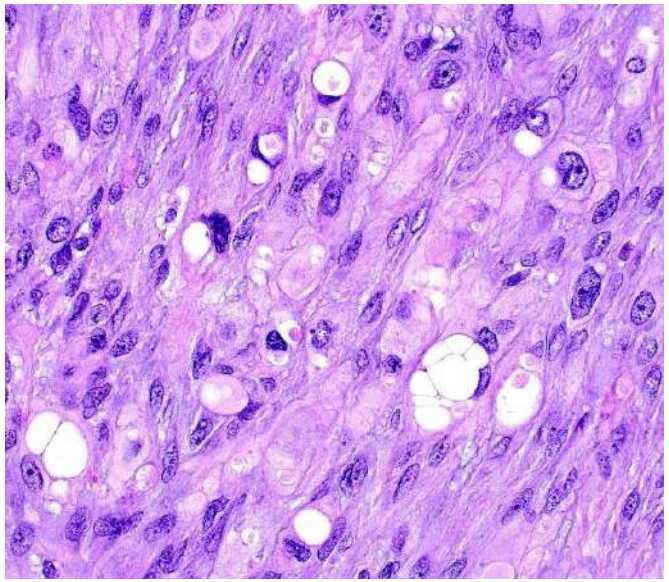
Use of IHC markers as surrogates for molecular genetic testing



Clinical history
Female, 38 y-o
Right thigh tumor

Epithelioid hemangioendothelioma

[*WWTR1::CAMTA1 gene fusion]*



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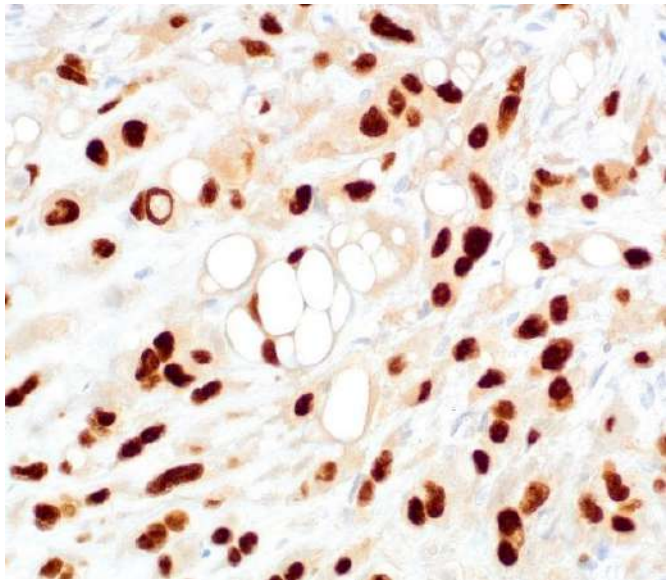
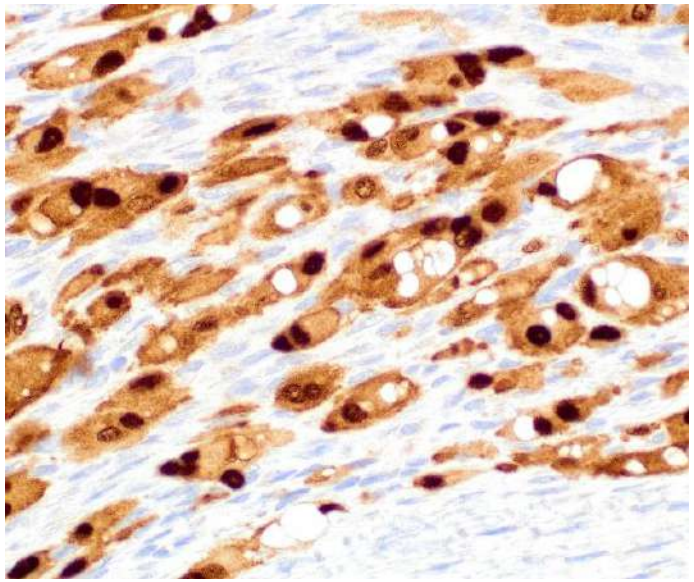


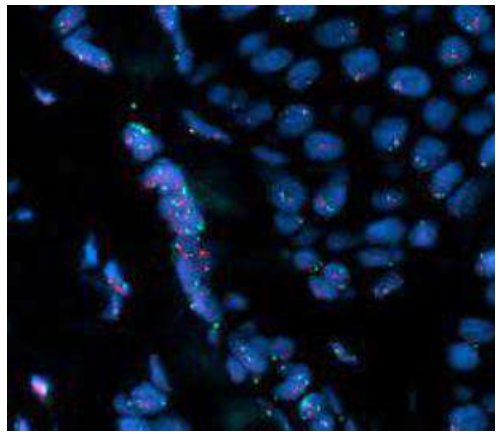
ERG+ve: Indicates vascular tumor
HE? EHE? Epithelioid AS?

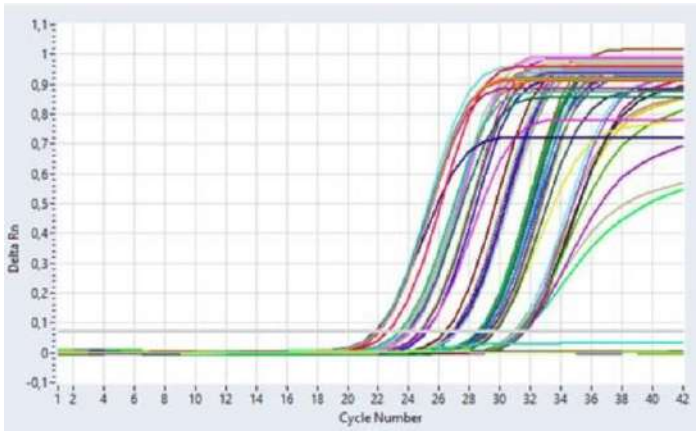


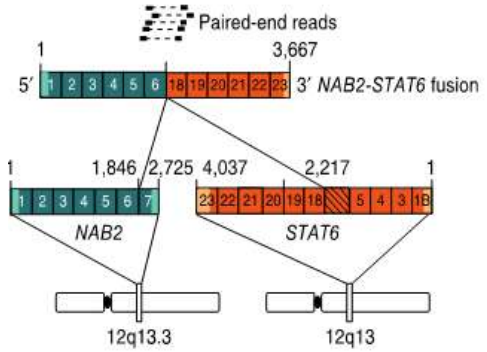
CAMTA1+ve → EHE

4. Integrate genetics_ when necessary. Molecular pathology

- **FISH** (Fluorescent in situ hybridization)
- **RT-PCR** (reverse transcription polymerase chain reaction)
- **NGS** (“next generation sequencing”)







Use of molecular techniques in sarcoma diagnosis (clinical practice)

- Differential diagnosis between lipomatous tumors (FISH)
- Confirmation of the alveolar subtype of rhabdomyosarcoma (FISH)
- Diagnostic confirmation in complex cases (FISH, PCR, NGS)
- Confirmation of a sarcoma with unusual histological and/or immunohistochemical features (FISH, NGS)
- Differential diagnosis between undifferentiated round cell sarcomas (Ewing's sarcoma, non-STD EWSR1 sarcomas, CIC, BCOR sarcomas) vs DSRCT vs poorly differentiated SS vs rhabdomyosarcoma… with important prognostic and therapeutic implications (FISH, **NGS**)
- **Prediction of treatment response** (FISH, PCR, NGS)
- **Selection of targeted therapies** (i.e. NTRK-rearranged neoplasms) (FISH, **NGS**)…

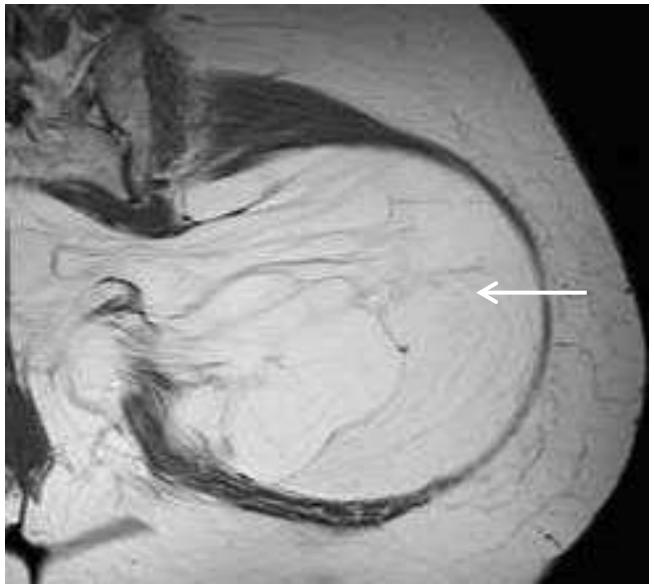
Use of MDM2 amplification by FISH in lipomatous tumors

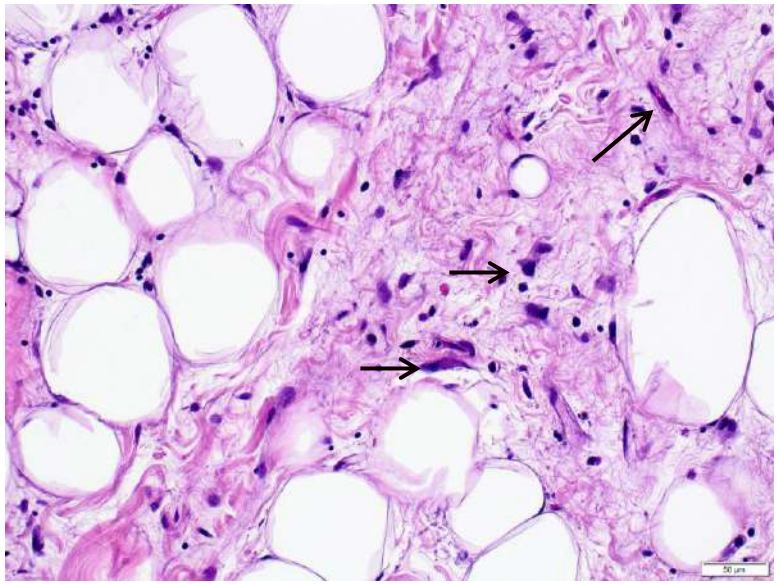
- Deep tumors >10 cm in patients >50 years
- Recurrent tumors
- Equivocal cytological atypia
- Location in retroperitoneum, pelvis, abdomen
- Any spindle cell sarcoma in the retroperitoneum (rule out DDLPS)

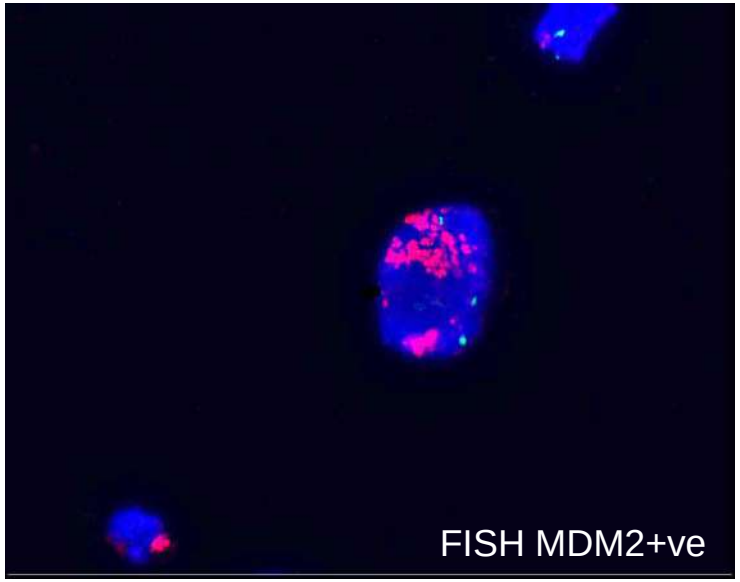
Clay MR et al. Am J Surg Pathol 2015

Clinical history

Female, 66 y-o. Buttock. Imaging: Im Lipoma vs ALT





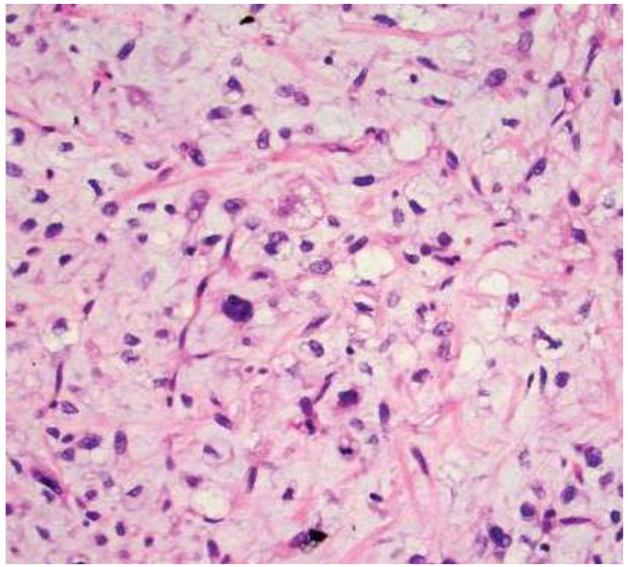


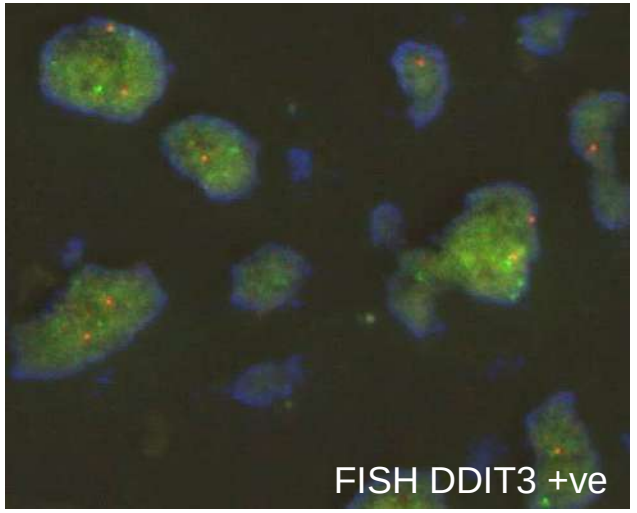
Confirmation of a sarcoma in an unusual clinical and morphological context (FISH)

Clinical history

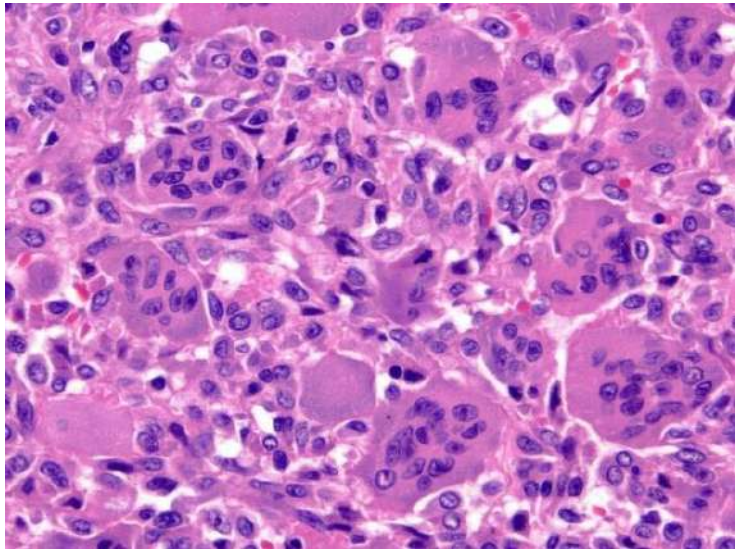
Male, 63 y-o. Anterior mediastinum mass

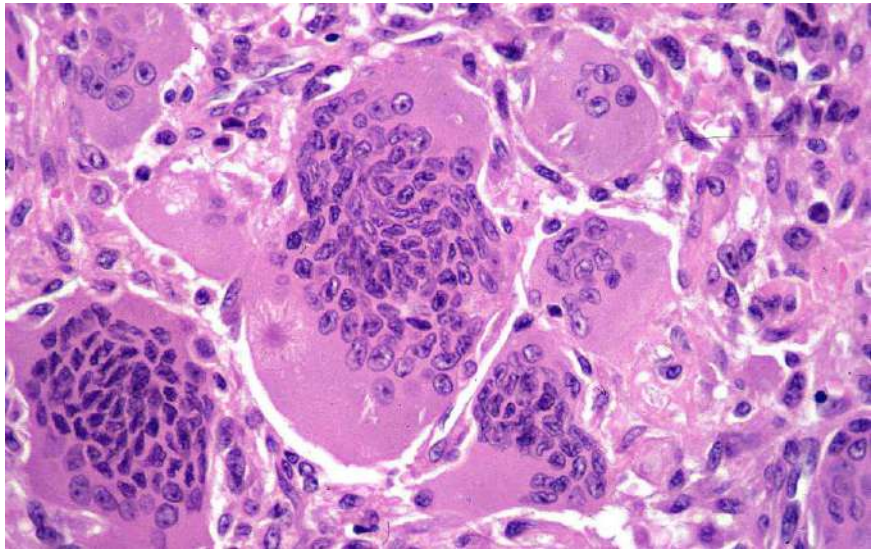
- Histology of myxoid liposarcoma with pleomorphism. DD: myxoid pleomorphic liposarcoma (frequent in the mediastinum!) vs myxofibrosarcoma
- FISH: DDIT3+ve
- In this case: unusual morphology and location.





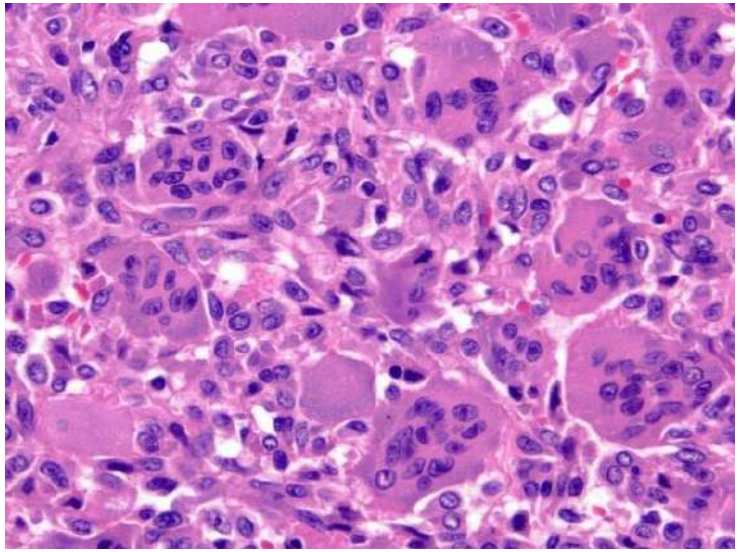
Differential diagnosis between giant cell-rich tumors (GCTB, chondroblastoma) (PCR)

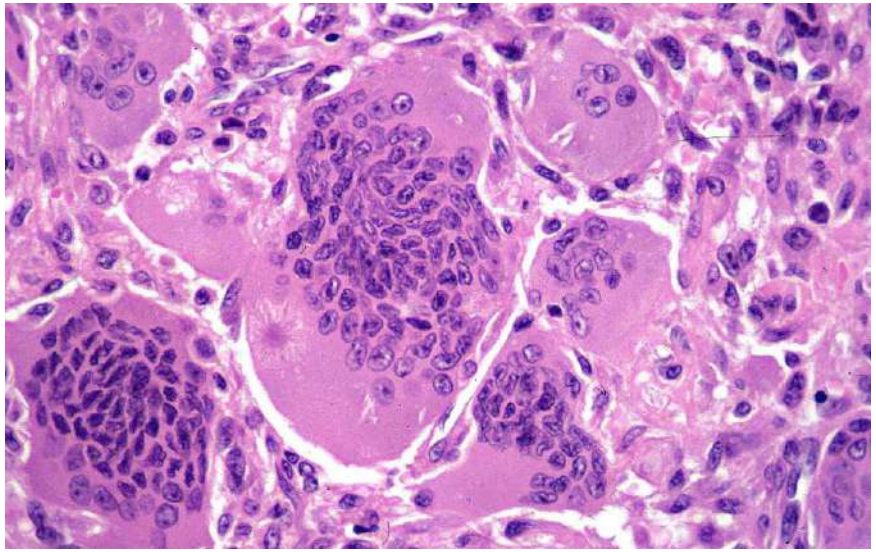




H3F3A p.G34W gene mutation

Differential diagnosis between giant cell-rich tumors (GCTB, chondroblastoma) (PCR)





H3F3A p.G34W gene mutation

H3F3A (Histone 3.3) G34W Immunohistochemistry

A Reliable Marker Defining Benign and Malignant Giant Cell Tumor of Bone

Fernanda Amary, MD, PhD,† Fitim Berisha, MSc,* Hongtao Ye, PhD,* Manu Gupta, PhD,† Alice Gutteridge, MSc,† Daniel Baumhoer, MD, PhD,‡ Rebecca Gibbons, BSc,* Roberto Tirabosco, MD,* Paul O'Donnell, MD,* and Adrienne M. Flanagan, MD, PhD*†*

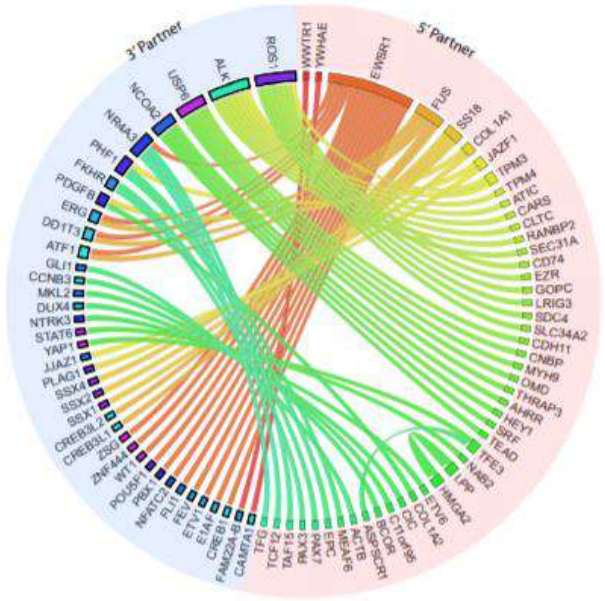
NGS and Sarcomas

Assay Targets

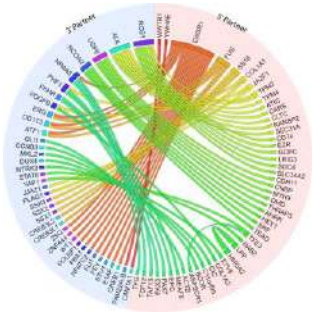
Includes the following genes and their fusion partners:

ALK	FUS	NTRK3	TCF12
CAMTA1	GLI1	PDGFB	TFE3
CCNB3	HMGA2	PLAG1	TFG
CIC	JAZF1	ROS1	USP6
EPC1	MEAF6	SS18	YWHAE
EWSR1	MKL2	STAT6	
FOXO1	NCOA2	TAF15	

Sarcoma Fusion Map



Archer Sarcoma Fusion Plex® v1: 26 target genes



Archer Sarcoma Fusion Plex Expanded® v2: 63 target genes [RNA-based platform]

ALK	BCOR	BRAF	CAMTA1	CCNB3	CIC	CSF1	CTNNB1
EGFR	EPC1	ERG	ESR1	ETV1	ETV4	ETV5	ETV6
EWSR1	FGFR1	FGFR2	FGFR3	FOS	FOSB	FOXO1	FUS
GLI1	HMGA2	JAZF1	MBTD1	MDM2	MEAF6	MET	MGEA5
MKL2	MYOD1	NCOA1	NCOA2	NCOA3	NR4A3	NTRK1	NTRK2
NTRK3	NUTM1	PAX3	PDGFB	PDGFRA	PHF1	PLAG1	PRKCA
PRKCB	PRKCD	RAF1	RET	ROS1	SS18	STAT6	TAF15
TCF12	TFE3	TFG	USP6	VGLL2	YAP1	YWHAE	

Impact of NGS in sarcomas

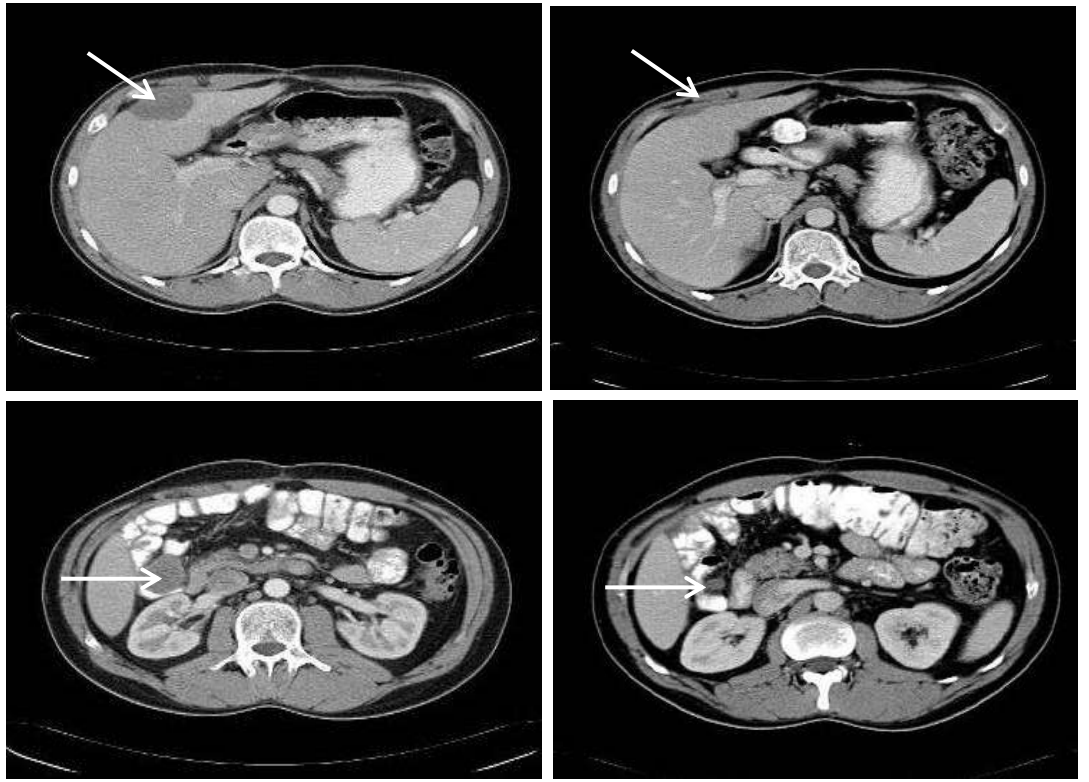
- Diagnostic accuracy (“precision medicine”)
  - accurate diagnosis provides appropriate clinical decision making
- Identification of “new morphologic entities”
  - i.e. novel gene fusions in round cell sarcomas (+ others)
- Prediction of tumor behaviour and treatment response (prognosis)
  - i.e. KIT exon 9 mutant GIST: dose optimization
- Identification of new potential targets (“basket trials”)
  - i.e. NTRK / ALK-rearranged neoplasms (+ others)

Histotyping and (molecular) targeted therapy

Diagnostic accuracy

- GIST: Imatinib/Sunitinib/Regorafenib
- DFSP: Imatinib
- GCTTS: anti-CFS1 (Pexidartinib, Vimseltinib)
- IMT: Crizotinib, Entrectinib
- WD/DDLPS: MDM2 inhibitors (Miladotaman)
- Angiosarcoma: Sirolimus, Everolimus
- Malignant PEComa: mTOR inhibitors (Sirolimus, Everolimus)
- SFT: Sunitinib
- ASPS: Sunitinib, Pazopanib, Degorafenib
- Myxoid liposarcoma: Trabectedin/Eribulin
- GCT/ABC: Denosumab
- NTRK-rearranged neoplasms: NTRK inhibitors (Larotrectinib, Entrectinib)

Multifocal Recurrent IMT Treated with ALK-iInhibitor Crizotinib

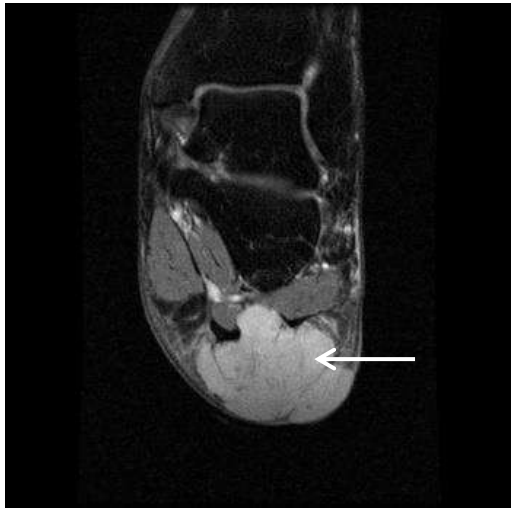


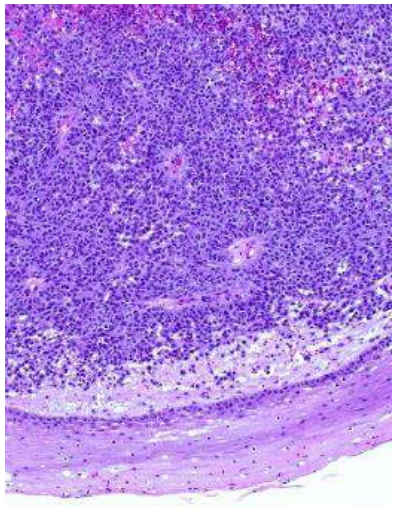
N Engl J Med 2010;363:1727-33

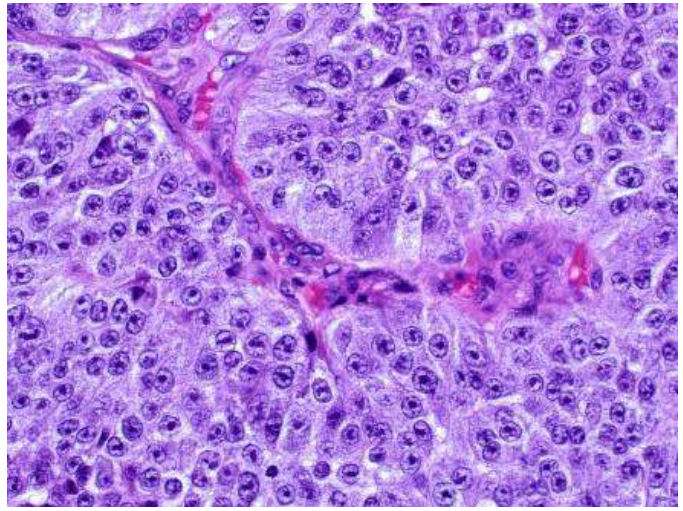
Diagnostic accuracy in complex cases

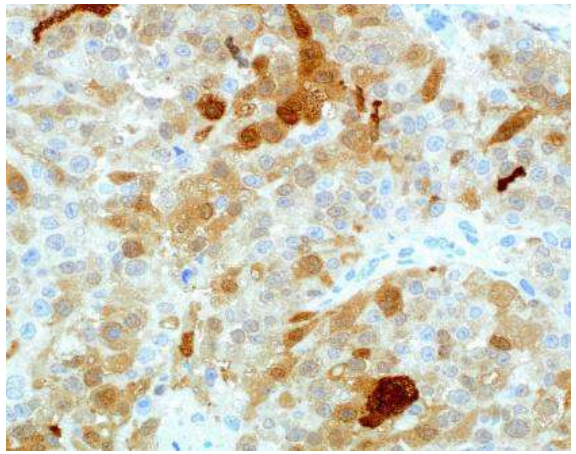
Clinical history

Female, 67 y-o. Plantar soft tissue tumor (5 cm) with multiple bone and lung metastases at initial diagnosis

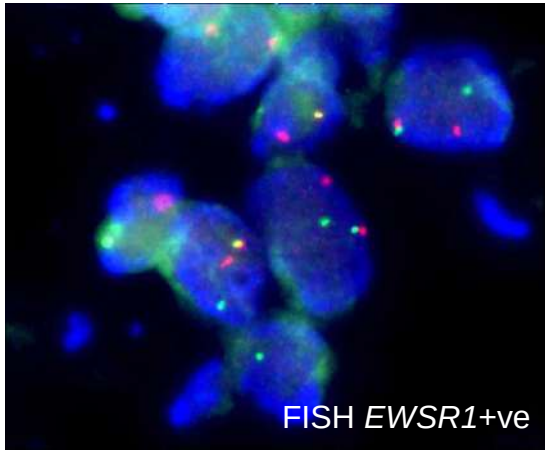








S100+ve
CK +/-
Melan A_ve
HMB45_ve
INI1 retained
Desmin_ve
Myogenin_ve
ERG_ve



Courtesy: M. Ferré. Molecular Lab H. Sant Pau

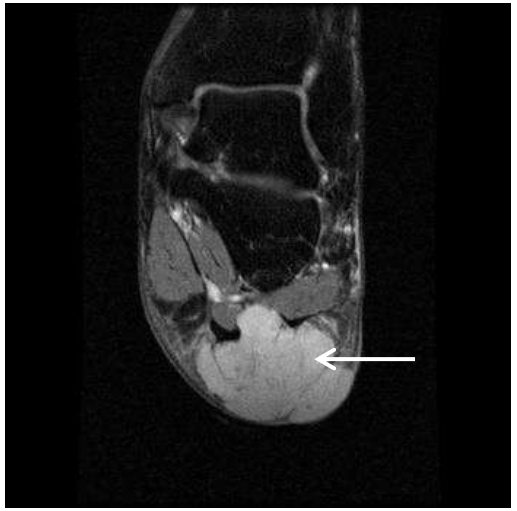
- **Clear cell sarcoma** vs MM

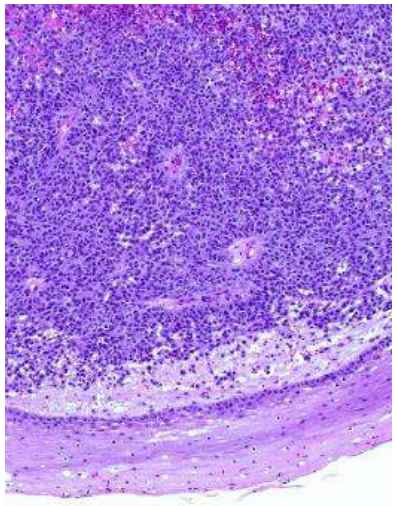
- Myoepithelial ca?
- Extraskeletal myxoid chondrosarc?

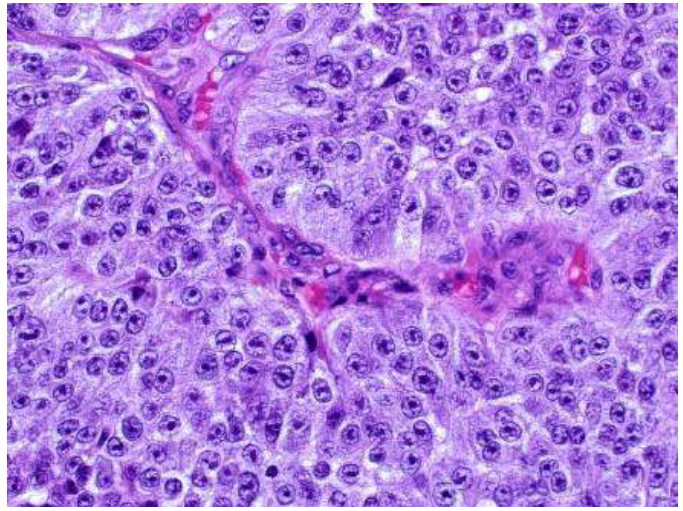
Diagnostic accuracy in complex cases

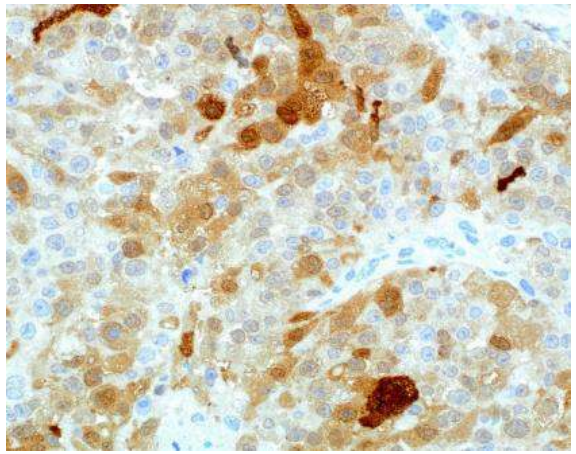
Clinical history

Female, 67 y-o. Plantar soft tissue tumor (5 cm) with multiple bone and lung metastases at initial diagnosis

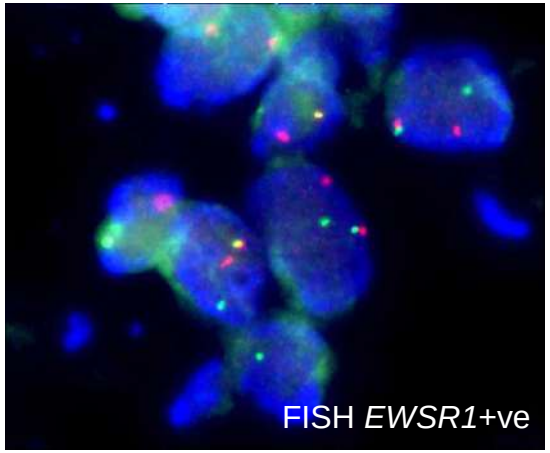








S100+ve
CK +/-
Melan A_ve
HMB45_ve
INI1 retained
Desmin_ve
Myogenin_ve
ERG_ve



Courtesy: M. Ferré. Molecular Lab H. Sant Pau

- **Clear cell sarcoma** vs MM

- Myoepithelial ca?
- Extraskeletal myxoid chondrosarc?

NGS: gene fusion *EWSR1::CREM* → **Clear cell sarcoma of soft tissue**

STT other than Ewing carrying EWS translocation

Histotype	Cytogenetic alterations	Molecular alterations
Angiomatoid fibrous histiocytoma	t(12;22)(q13;q12)	*EWSR1-ATF1*
	t(2;22)(q33;q12)	*EWSR1-CREB1*
Clear cell sarcoma	t(12;22)(q13;q12)	*EWSR1-ATF1*
	t(2;22)(q33;q12)	*EWSR1-CREB1*
Low-grade fibromyxoid sarcoma/sclerosing epithelioid fibrosarcoma	t(11;22)(p11;q12)	*EWSR1-CREB3L1*
Angiosarcoma	t(12;22)(q13;q12)	*EWSR1-ATF1*
Hemangioma of bone	t(18;22)(q23;q12)	*EWSR1-NFATC1*
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	*EWSR1-WT1*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	*EWSR1-NR4A3*
Myoepithelial tumor of soft tissue	t(6;22)(p21;q12)	*EWSR1-POU5F1*
	t(19;22)(q13;q12)	*EWSR1-ZNF444*
	t(1;22)(q23;q12)	*EWSR1-PBX1*
	t(9;22)(q33.2;q12)	*EWSR1–PBX3*
	t(1;22)(p34.1;q12)	*EWSR1-KLF17*
Myxoid liposarcoma	t(12;22)(q13;q12)	*EWSR1-DDIT3*

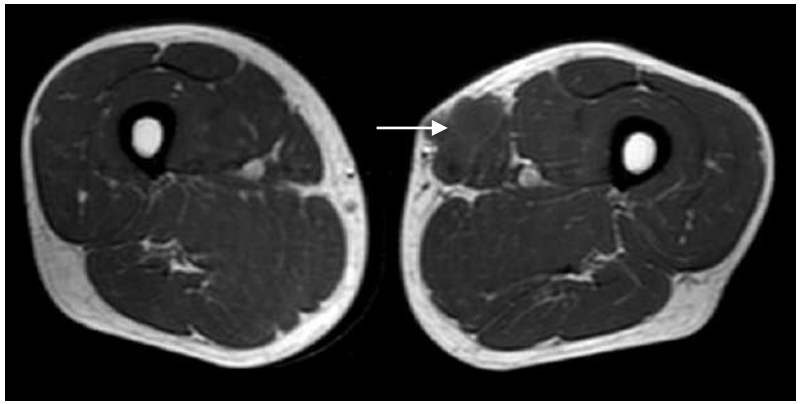
Sbaraglia M et al. Virchows Archiv 2019

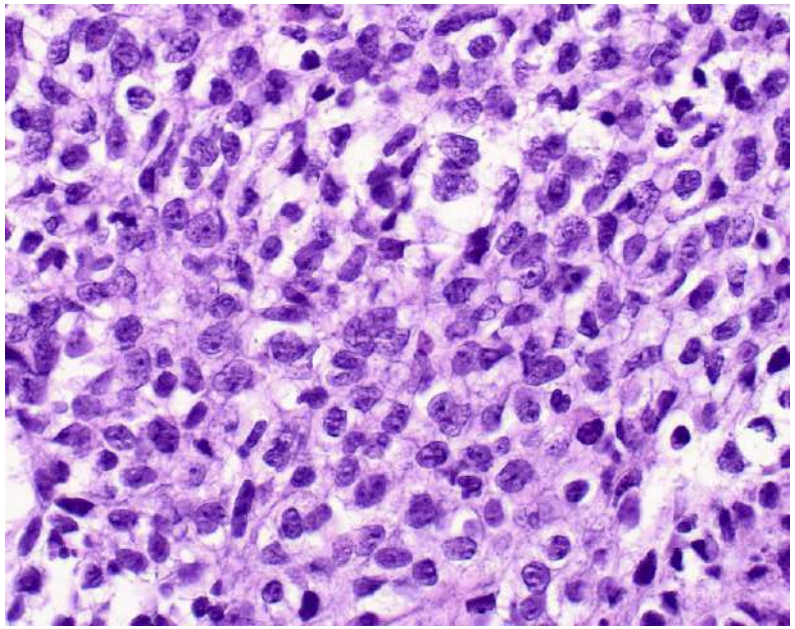
Identification of new tumor entities

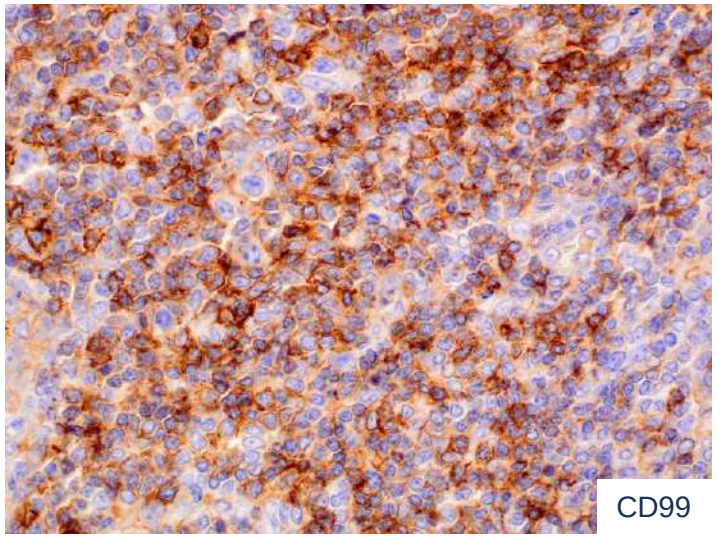
+ prognosis

Clinical history

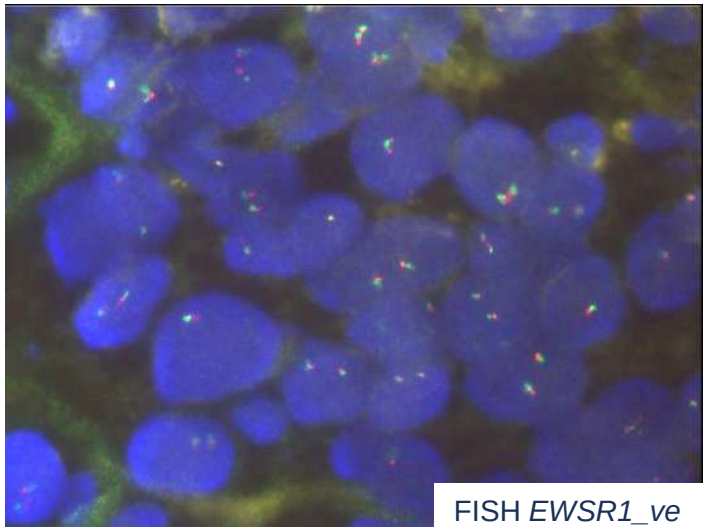
Male, 31 y-o .Subcutaneous mass (left thigh)







CD99



FISH *EWSR1_ve*

NGS (Archer Sarcoma Fusion Plex® expanded v2)

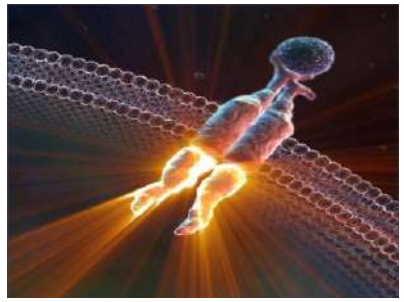
CIC::DUX4 gene fusion

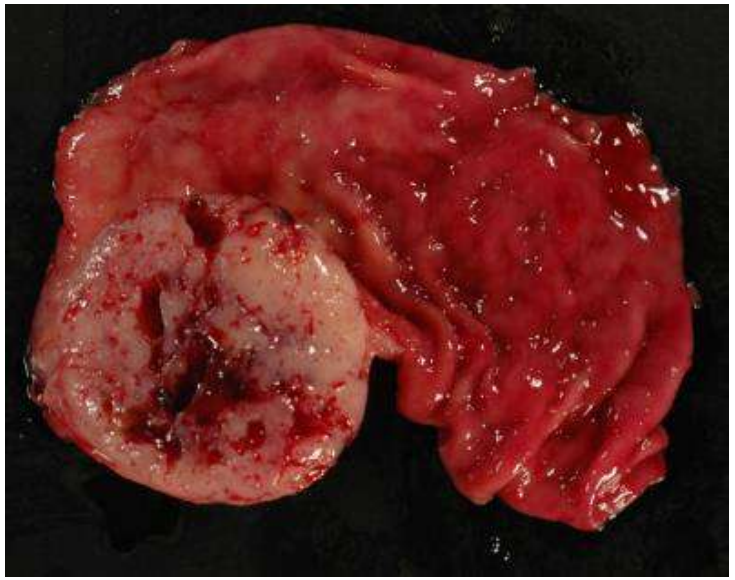
Courtesy: Ll Catasús, A González, M Ferré. Mol Lab H. Sant Pau

Prediction of treatment response

Clinical uses of GIST genotyping:

- To **confirm the diagnosis** (in a CD117 and/ or DOG-1 immuno -ve suspected GIST)
- Critical to making a clinical decision in several settings: predictive value for sensitivity to molecular-targeted therapy (**response to treatment**):
  - KIT exon 11 mutations Imatinib-responsive in 90%
  - PDGFRA exon 18 mutation D842V resistant to Imatinib
  - NF-1 related GIST insensitive to Imatinib
  - KIT exon 9 mutant tumor: dose optimization
- **Prognostic** value

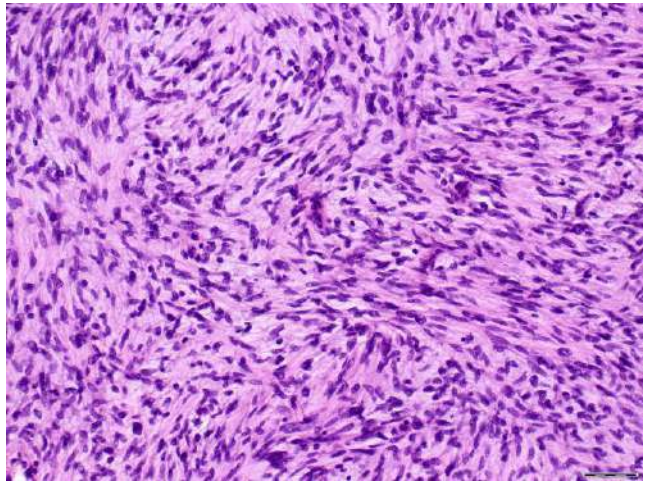




Identification of new potential targets

ALK-rearranged mesenchymal neoplasms

Clinical history
Male 35 y-o.
Localized thoracic wall soft tissue mass: “MPNST”
Excision
Progression (distant M1)



NGS (Archer Sarcoma Fusion Plex® expanded v2:

PLEKHH2:ALK gene fusion

Courtesy: Ll Catasús, A González. Mol Lab H. Sant Pau

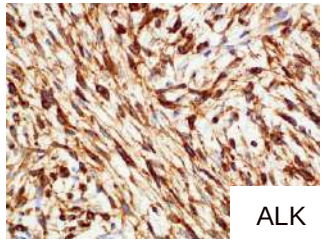
PLEKHH2-ALK: A Novel In-frame Fusion With Durable Response to Alectinib: Utilizing RNA Sequencing in Search for Hidden Gene Fusions Susceptible to Targeted Therapy

Nagasaka M et al. Clin Lung Cancer 2021

ALK-rearranged Mesenchymal Neoplasms: A Report of 9 cases Further Expanding the Clinicopathologic Spectrum of Emerging Kinase Fusion Positive Group of Tumors

Dermawan J et al. Genes Chromosome Cancer 2023

ALK IHC +ve
ALK FISH +ve



ALK

Limitations of NGS

- Still most sarcomas (~60%), particularly those in adults, lack a specific molecular signature
- New fusion genes are identified every day; many are meaningless, with no clinical benefit or therapeutic implications
- Genetic overlap!
- Availability → only few centers; complex method

Limitations of NGS: Genetic overlap

- Same genetic alterations in unrelated entities and tumors of different lineages (sarcomas, low malignant potential STT, carcinomas…)
- Impact on prognosis and treatment. **Evaluate in the morphological context !!**

- ***EML4::ALK* fusions in:**
  - IMT
  - Lung **adenocarcinoma**
  - Non-Langerhans cell histiocytosis
- ***EWS::ATF1* / t(12;22) and *EWS::CREB1* / t(2;22) in:**
  - CCS and AFH
  - CCS “GI” type
  - Primary pulmonary myxoid sarcoma
  - Hyalinizing clear cell **salivary gland carcinoma**
  - Myoepithelial tumors of soft tissue
  - Rare angiosarcomas, mesotheliomas...

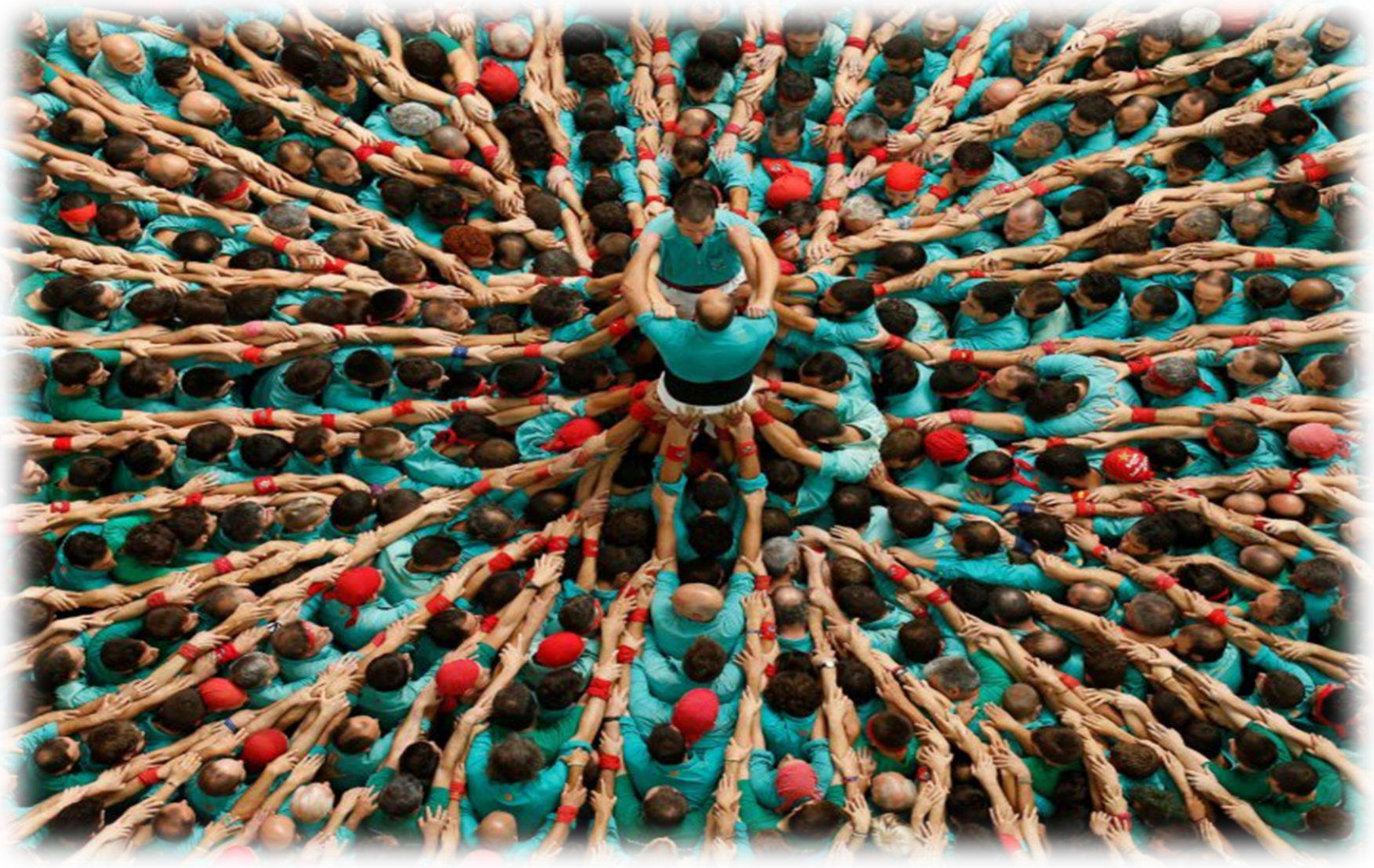
- ***ETV6::NTRK3* / t(12;15) in:**
  - Infantile fibrosarcoma
  - Secretory breast **carcinoma**
  - Post RT thyroid carcinoma
  - Cellular mesoblastic nephroma
  - Rare cases of IMT, **glioma**...
- ***ASPL::TFE3* / t(x;17) in:**
  - ASPS
  - Subset of pediatric renal **carcinomas**

Remarks: the benefits of a correct classification

- **Accurate Dx: essential for clinical decision making**
- Histotype (diagnosis) and grading → predicts behaviour and outcome (prognosis)
- Conventional morphology remains as a powerful tool
- Integration with immunohistochemistry (diagnostic standard)
- Molecular pathology increasingly helpful:
  - *Diagnosis (not always required)*
  - *Progress in understanding pathogenesis of STT, new entities*
  - *Prediction of treatment response*
  - *Target identification*
- Molecular tumor board

Thank you for your attention

sbaguer@santpau.cat



Castellers. Human towers of Catalonia.