Molecular characterization of mesenchymal tumors: promises and challenges

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GSF
GETO
- Groupe Sarcome Français -
- Groupe d'Etude des Tumeurs Osseuses -

I, Raul Perret have no conflicts of interest to declare.

Objective of the lecture

To discuss the current value and perspectives of genetics in the diagnosis and management of soft tissue tumors

Take home message

- Molecular biology techniques are valuable <u>tools,</u> in <u>selected cases,</u> but they <u>don't replace our brains</u>
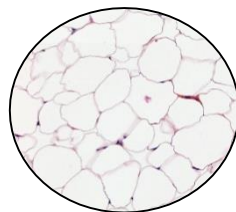
Histopathological features

DIAGNOSIS

Clinical Findings

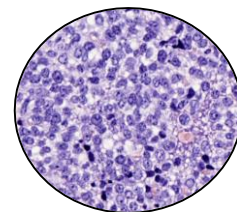**Ancillary techniques**
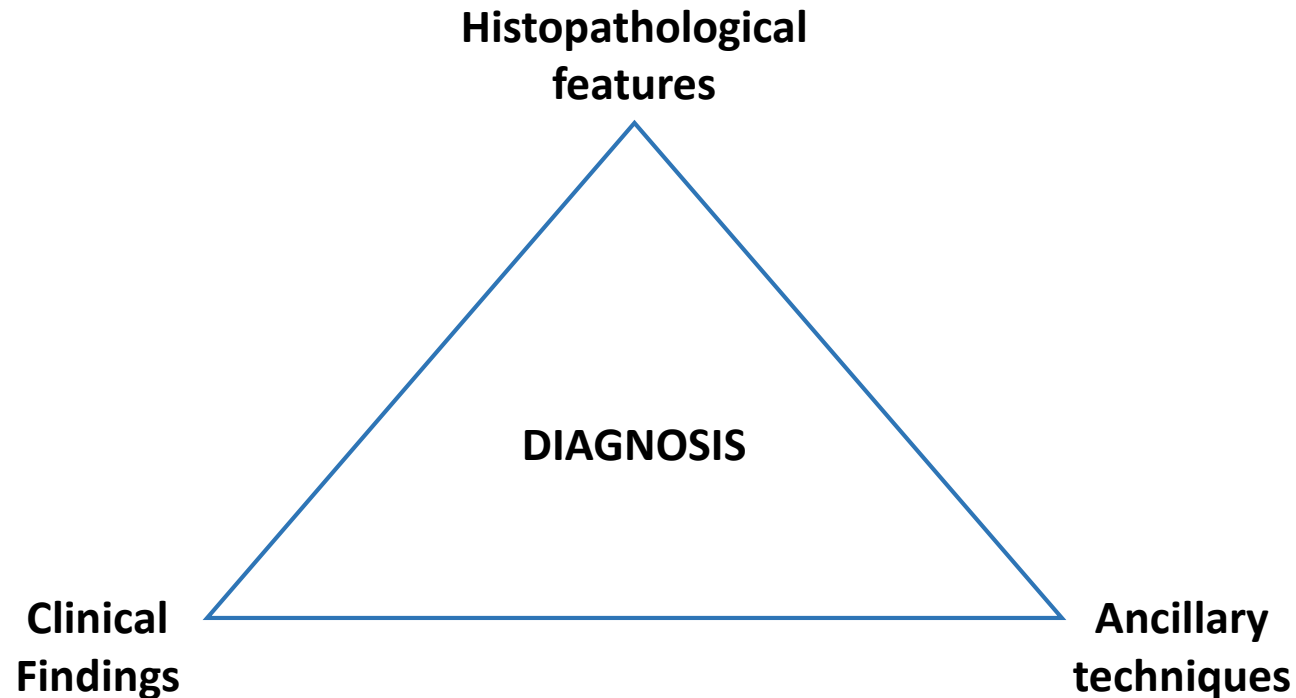
Overview of Soft Tissue Neoplasms

- Sarcomas ≈ 1% adult cancers

- Classification based on histology: **Tumor line of differentiation** *(>100 subtypes)*
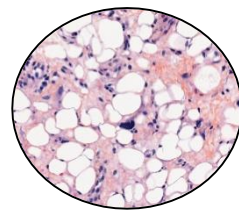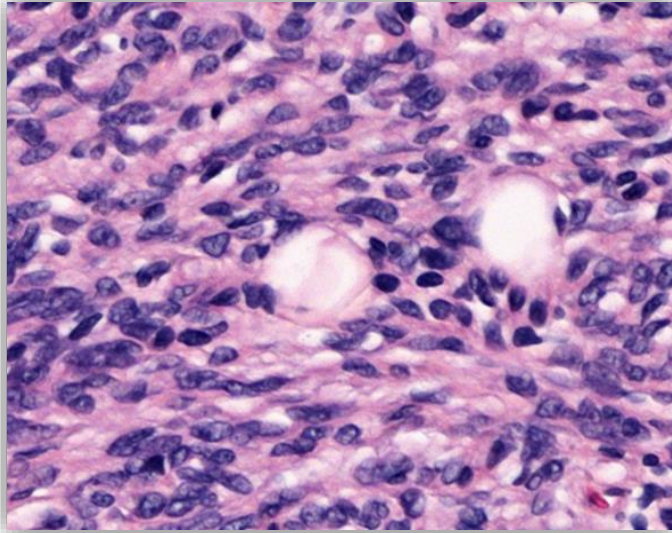

Liposarcoma   Adipose tissue   Ewing Sarcoma

- Classification based on tumour behaviour: **benign, intemediate malignancy, malignant**

- Classification based on genetics: **complex vs simple**

Simple genetics

No/Minimal chromosomal aberrations

Gene translocations   Point mutations
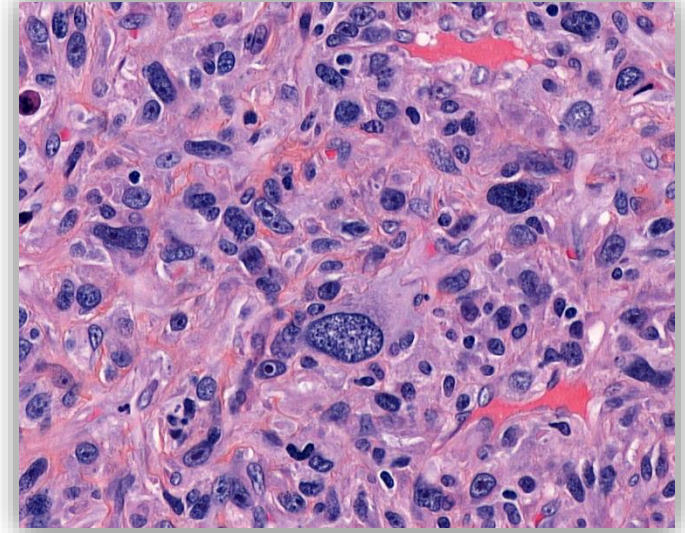

Dermatofibrosarcoma Protuberans
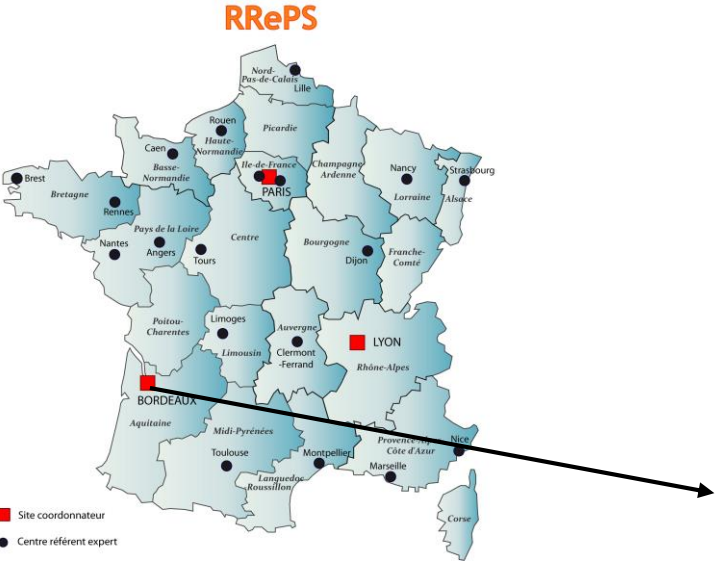
Complex genetics

Numerous chromosomal aberrations

Recurrent   Non-recurrent

Undifferentiated pleomorphic sarcoma

Réseau de Référence en Pathologie des Sarcomes
RRePS

Site coordonnateur
Centre référent expert

Institut Bergonié (Bordeaux, France)
Tertiary care center

Biopathology Department

Available Molecular techniques

Array-Comparative Genomic Hybridization (aCGH)	Fluorescence in-situ Hybridization (FISH)	Massive parallel Sequencing (NGS)	Sanger-Sequencing	RT-PCR
CNVs	MDM2 (Liposarcomas well diff./dediff.) EWSR1 (Ewing sarcoma mainly)	Unclassified or Challenging Tumors	CTNNB1 (desmoid) MYOD1 (spindle cell RMS)	

Molecular Genetics in the pathology department

Aims

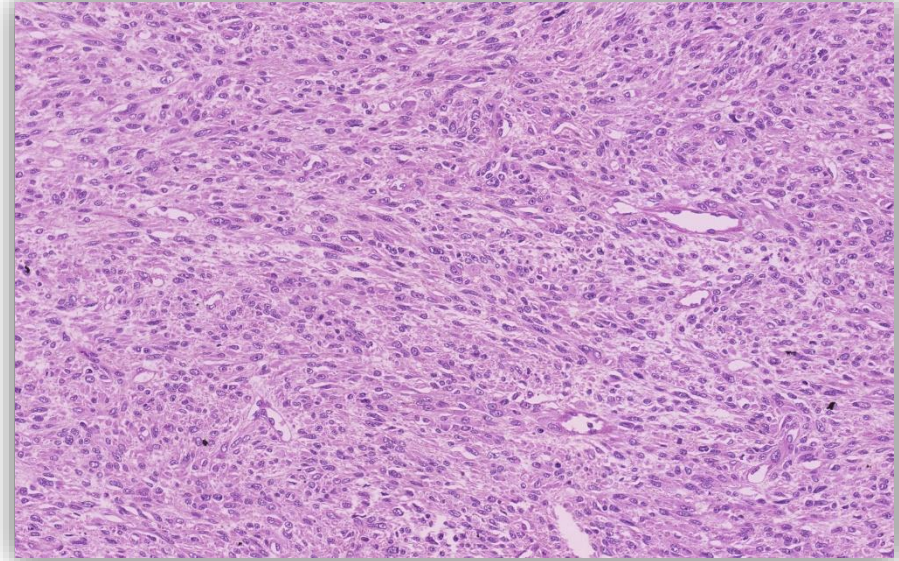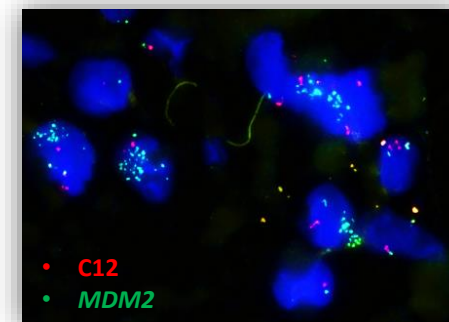| Increase diagnostic accuracy | Identify molecular targets | Predict tumor behavior |

Adipocytic tumors with *MDM2* amplification
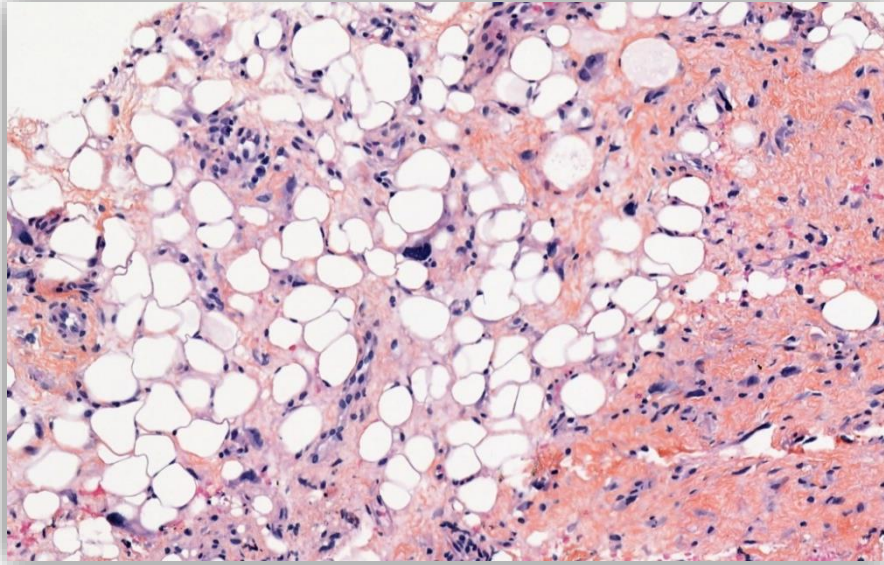
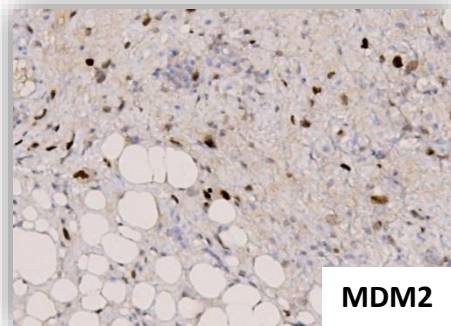**Well differentiated liposarcoma**

Dedifferentiated liposarcoma

Amplification Chr. 12q13-15
(*MDM2, CDK4, HMGA2…*)

MDM2

C12
MDM2

Indications of FISH testing for *MDM2* amplification

- Recurrent adipocytic tumor

- Deep extremity tumors that are >10 cm in patients >50 years

- Adipocytic tumor with equivocal atypia

- Undifferentiated tumors of the retroperitoneum/pelvis/abdomen

- Core needle biopsies of adipocytic tumors*

Clay et al. 2015 PMID: 26146760

*Performed in the French Sarcoma Network

Is the presence of *MDM2* amplification exclusive of well diff./Dediff. Liposarcoma?

- *Intimal sarcoma*
- *Low grade osteosarcoma*
- *Carcinoma*
- *Gyn Tumors*
- *Melanoma*

Forslund A et al. 2008 PMID: 18314481
Michalk M et al. 2016 PMID: 26796597
Howitt BE et al. 2015 PMID: 25231023

EML4-ALK Fusions in various tumor subtypes
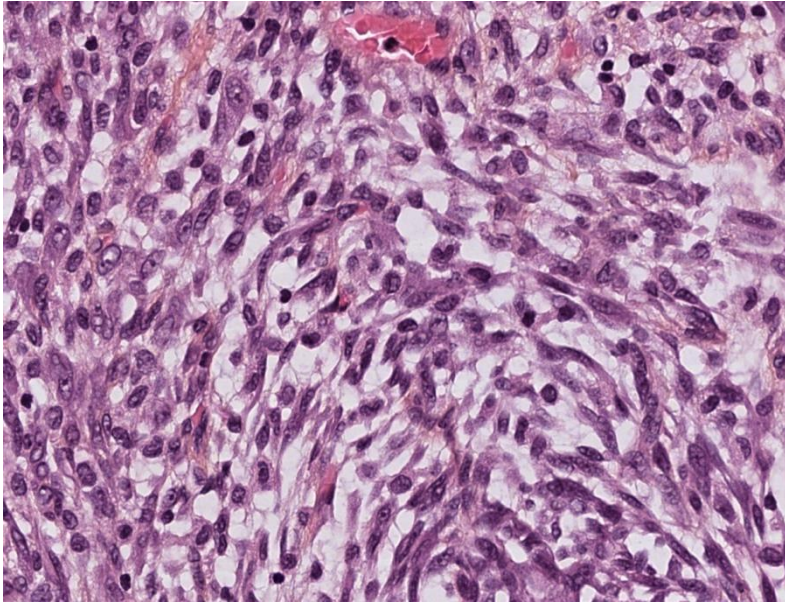
Inflammatory Myofibroblastic Tumor
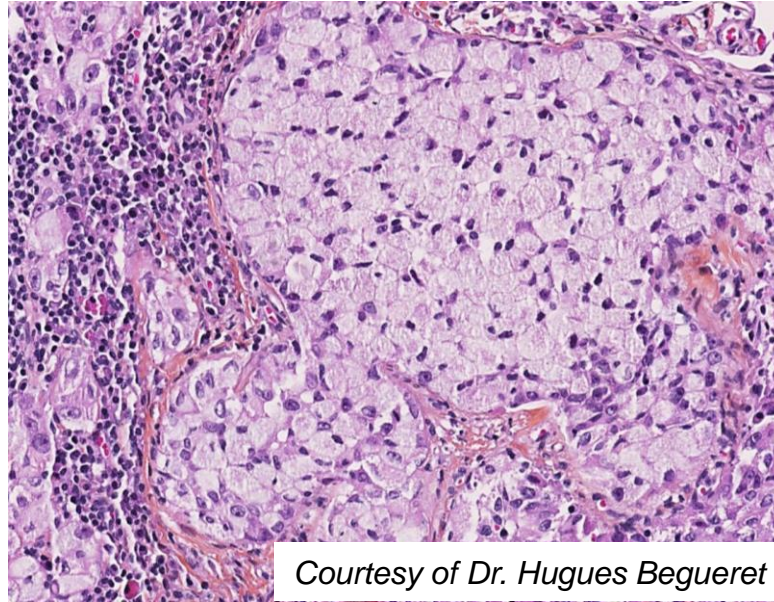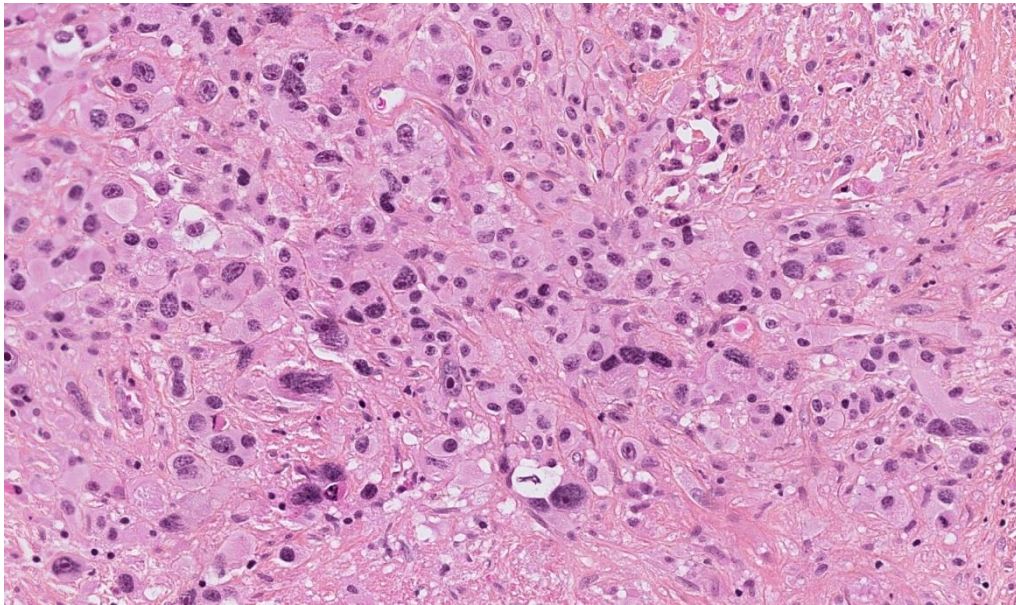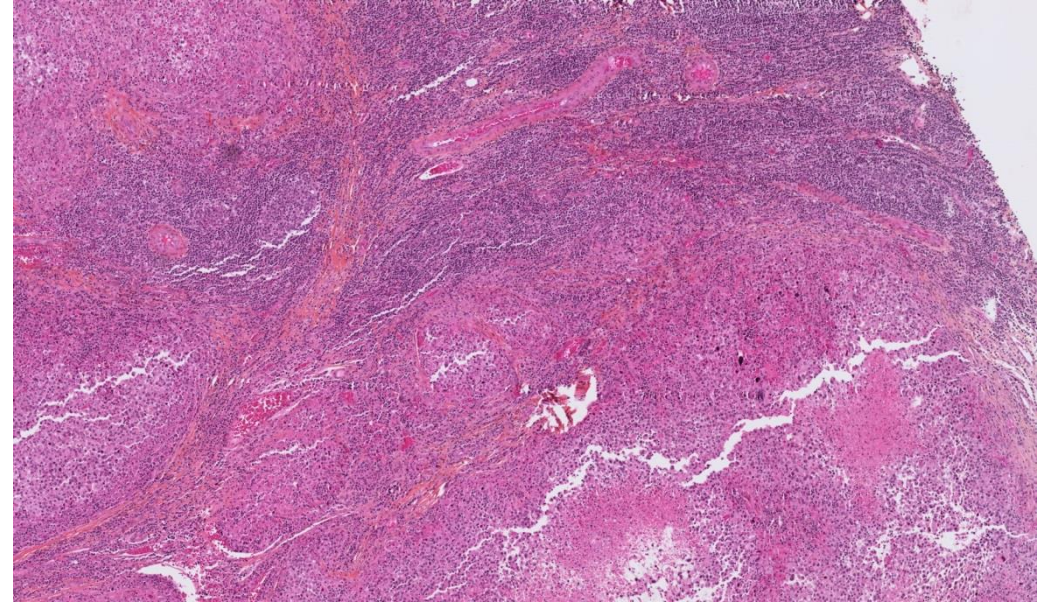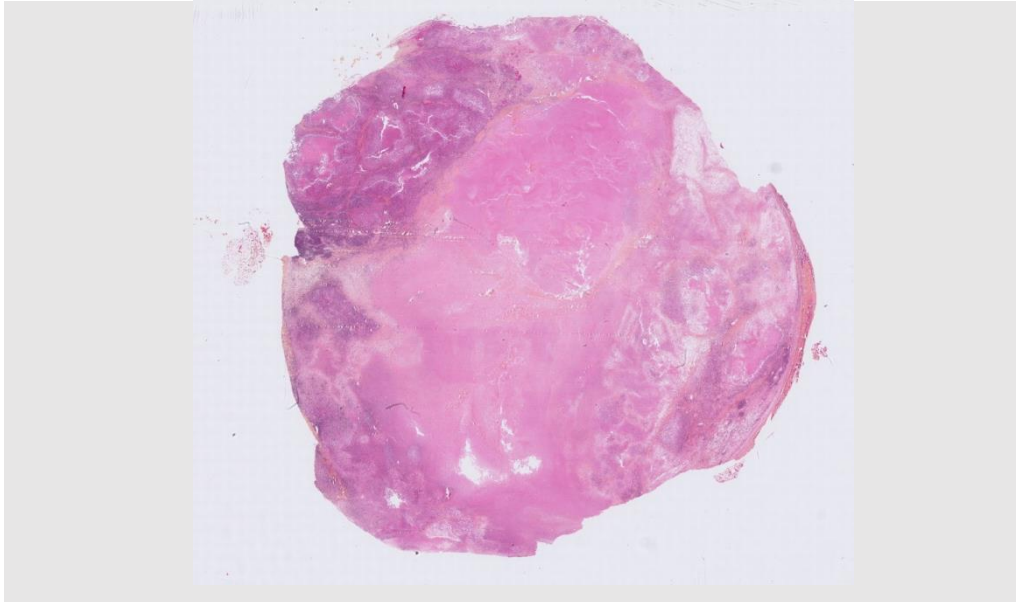
Courtesy of Dr. Hugues Begueret

Lung adenocarcinoma
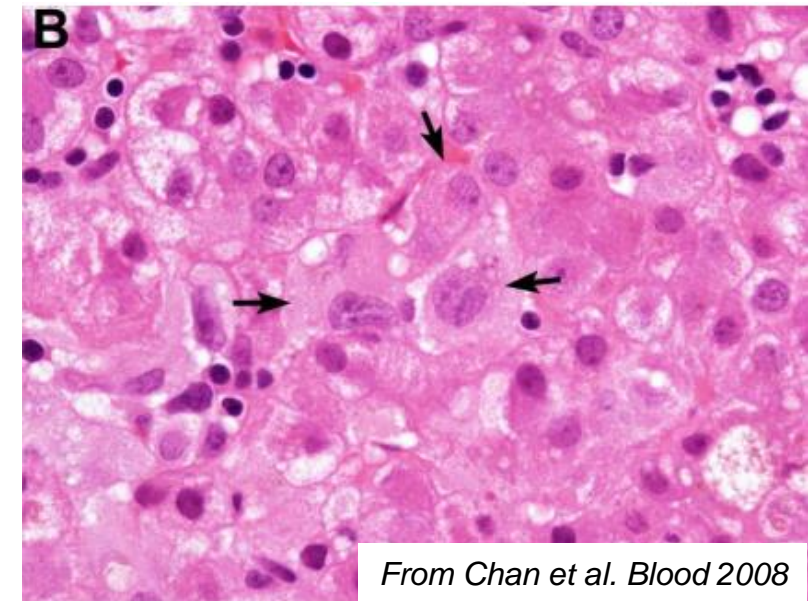
From Chan et al. Blood 2008

Non-Langerhans cell histiocytosis

A molecular alteration does not certify a diagnosis, CPC is mandatory

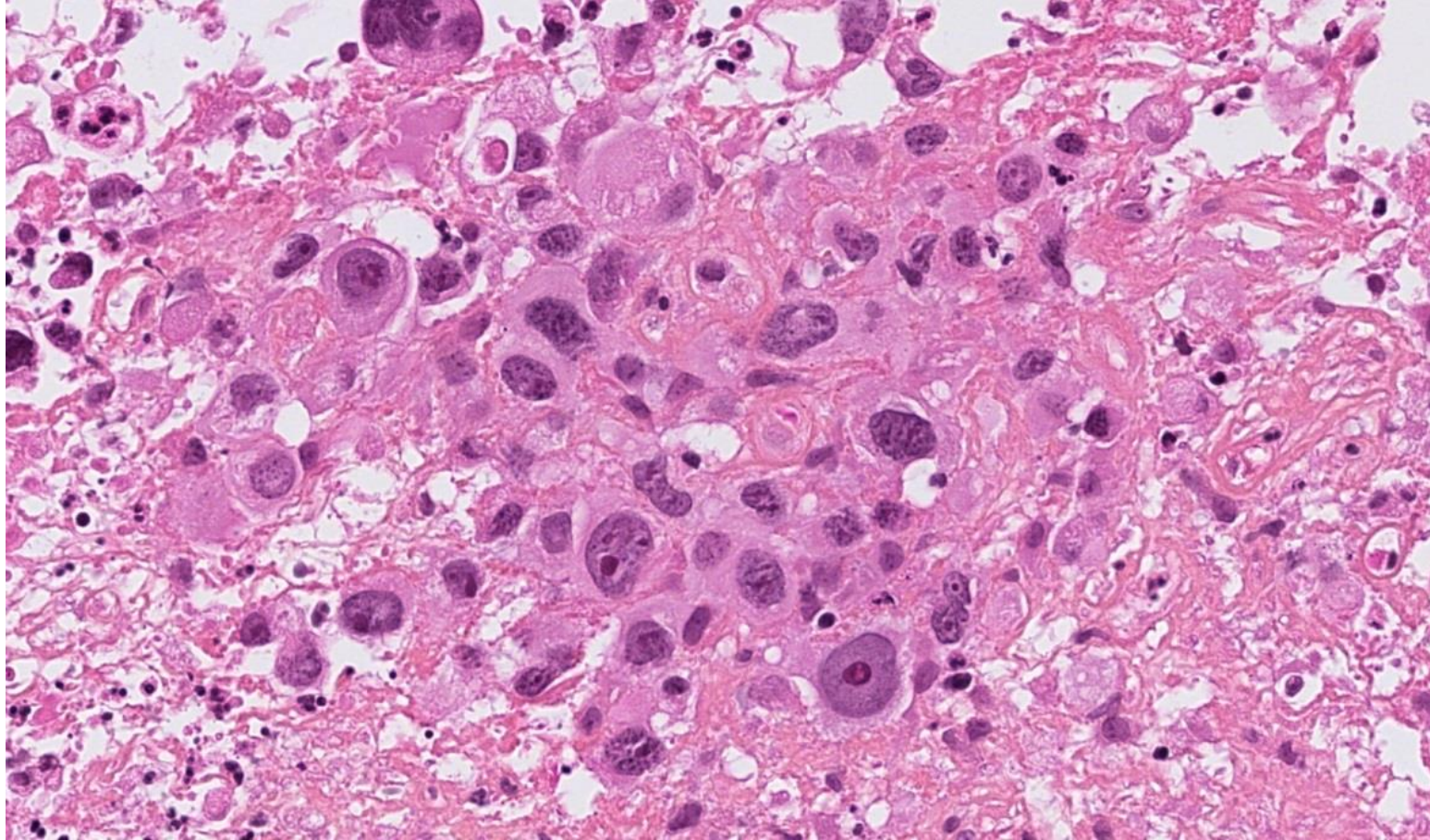*Adult male, axillary adenopathy. Metastasis? Lymphoma?*

SOX10

MITF
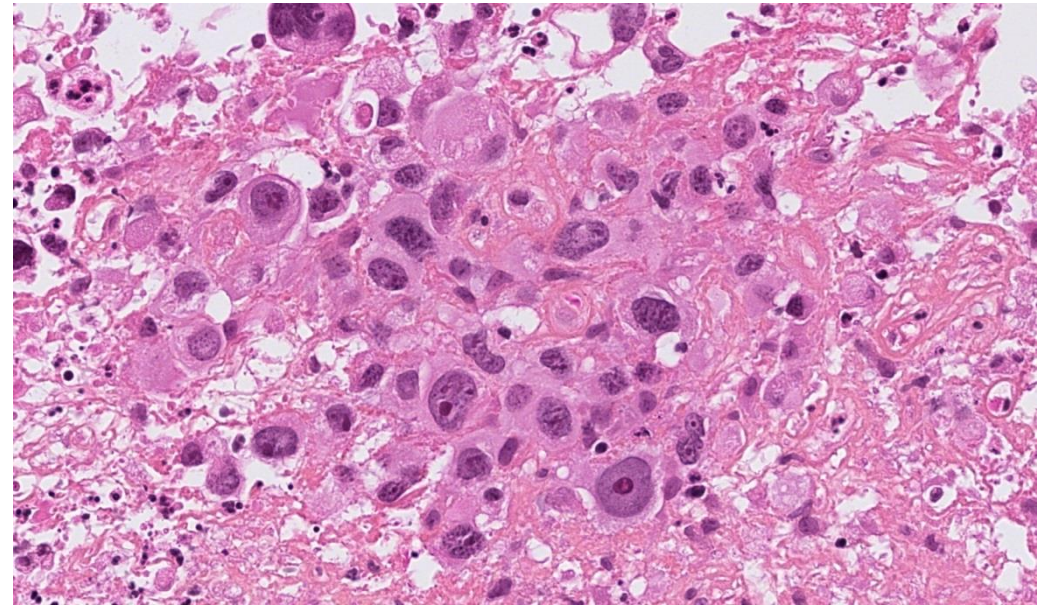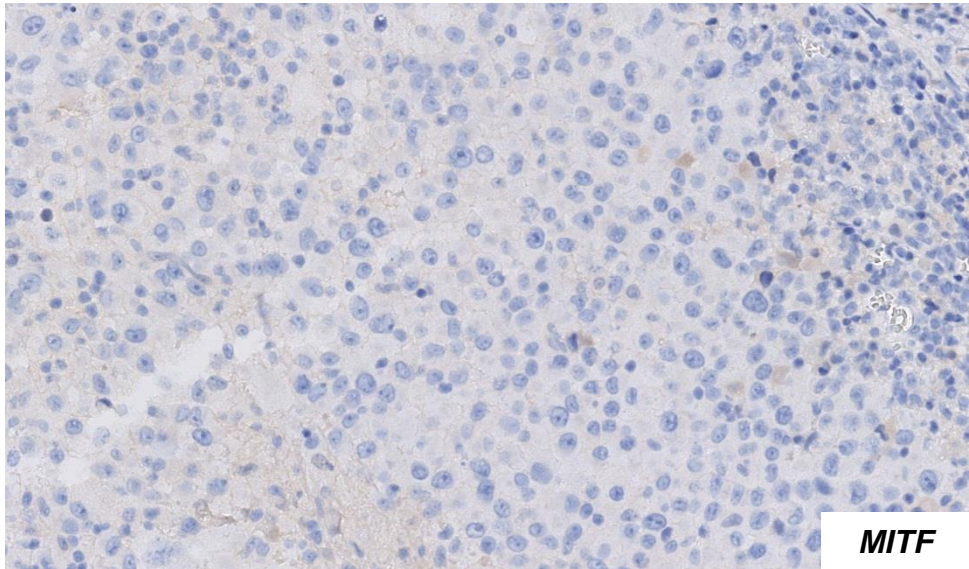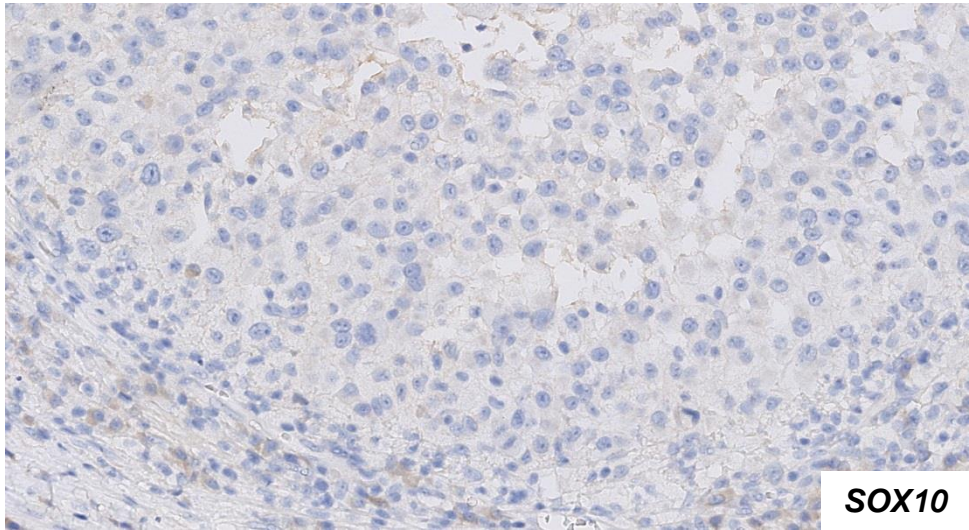
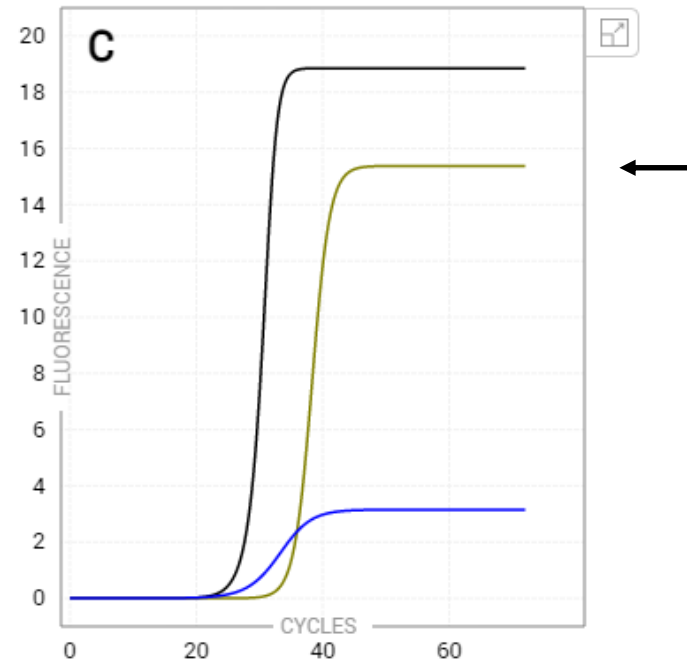| Other negative markers		
S100	Myogenin	CD68
HMB45	MDM2	P40
MelanA	CD45	OCT4
CD34	CD20	MPO
ERG	CD3	Pan-keratin
Desmin	CD30	CK7
H-Caldesmon	CD5	CK20
ALK	CDX2	CD138
TTF1	PAX8	CD21
INSM1	Chromogranin	Synaptophisin
MUC4		

Ki67 75%	INI1 and BRG1 conserved expression

Next Step?

Molecular screening for *BRAF/NRAS* mutations

TARGET		CQ	ΔCQ
● BRAF Total	⊕	30.35	-
● NRAS Q61R	⊕	36.79	7.61
● NRAS Total	⊕	29.18	-

NRAS Q61R is present in 0.77% of AACR GENIE cases, with cutaneous melanoma, melanoma, thyroid gland papillary carcinoma, poorly differentiated thyroid gland carcinoma, and colon adenocarcinoma having the greatest prevalence[4].
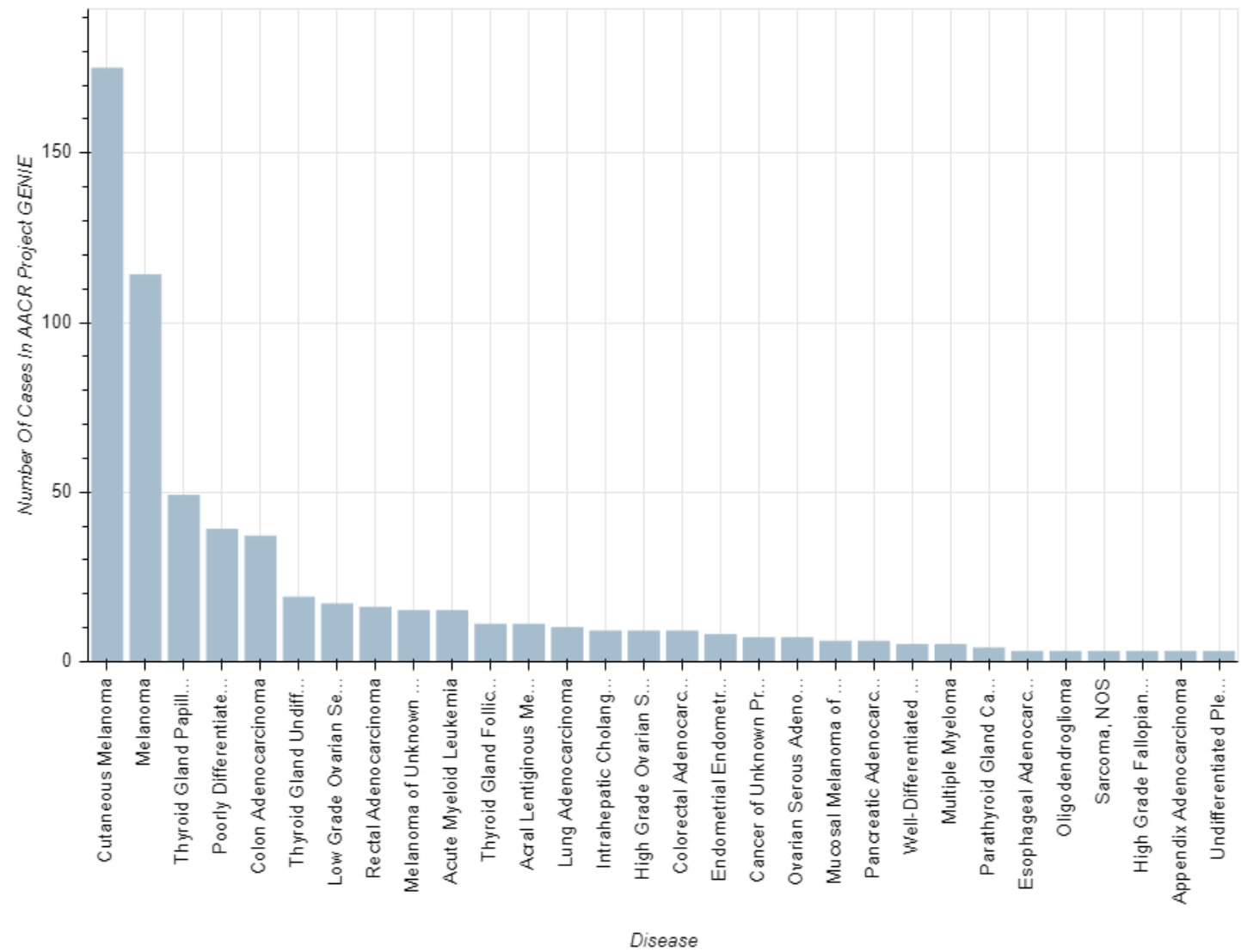
Diagnosis: undifferentiated malignant epithelioid tumor, favor undifferentiated melanoma (probable metastatic location)

Clues to the diagnosis of dedifferentiated and undifferentiated melanoma :

- Presence of minimal differentiated clone in dedifferentiated melanoma

- Earlier history of melanoma

- Undifferentiated histology that does not fit any defined entity

- Locations at sites that are unusual for undifferentiated/unclassified pleomorphic sarcoma (axilla, inguinal, neck, digestive system, etc.)

- Unusual multifocal disease typical of melanoma spread

- Detection of a melanoma-compatible gene mutation

- Absence of another genuine primary (eg, anaplastic carcinoma) in other organs.
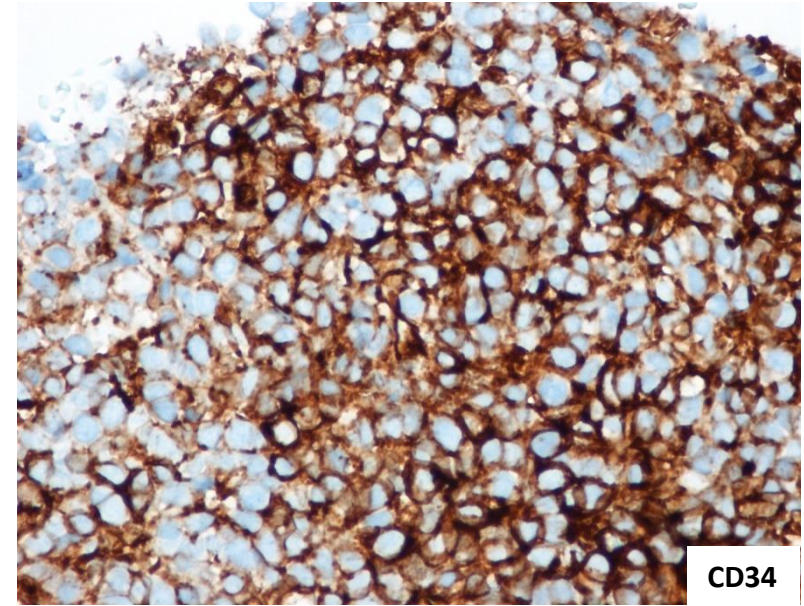
Adult male, popliteal fossa

MRI STIR TSE
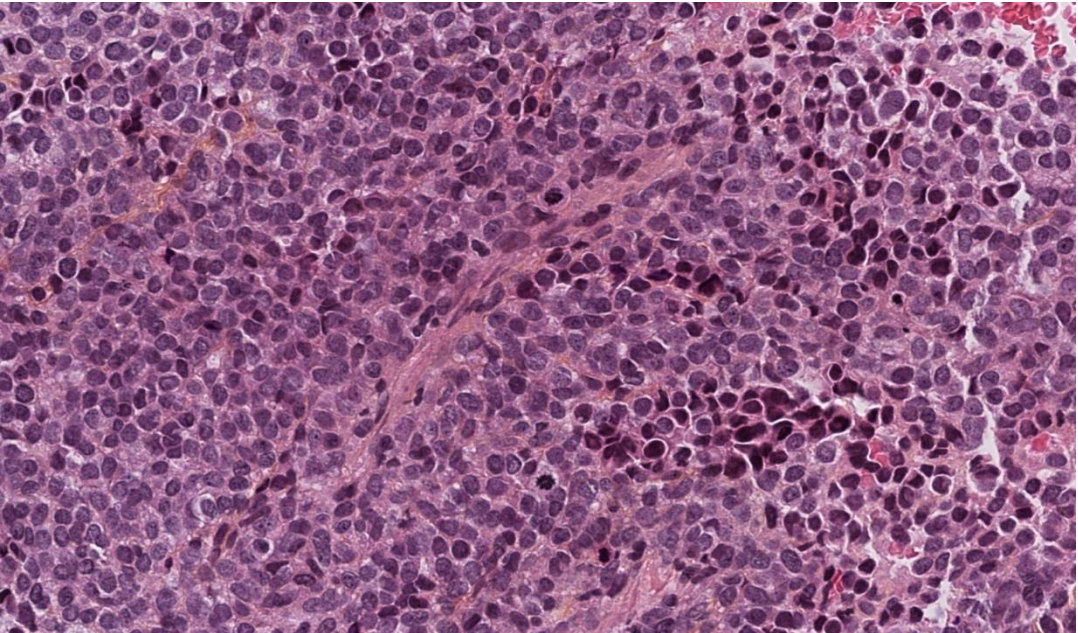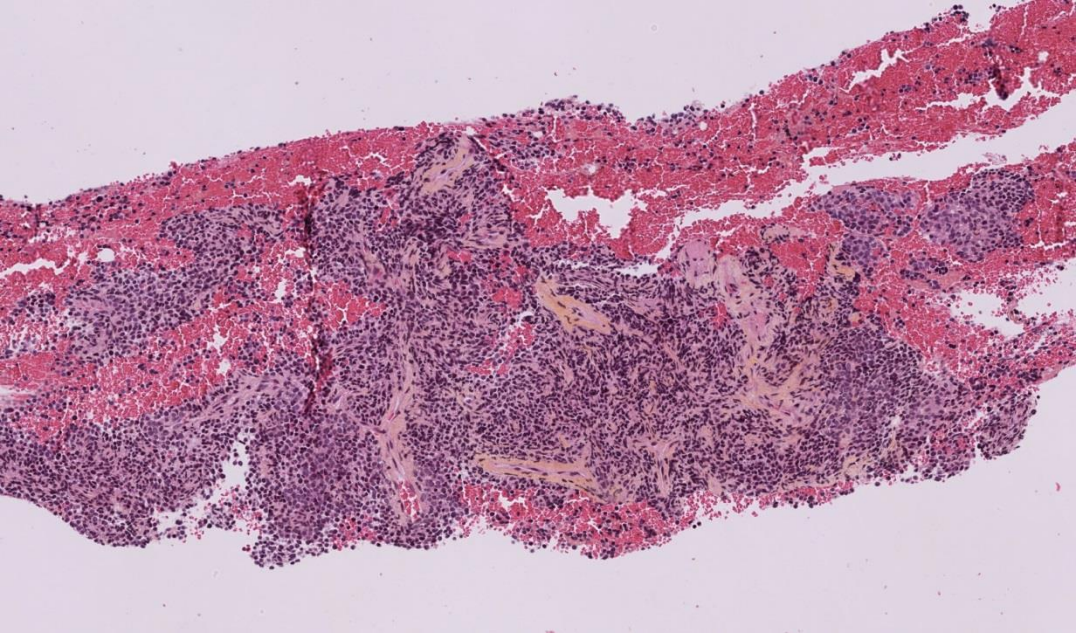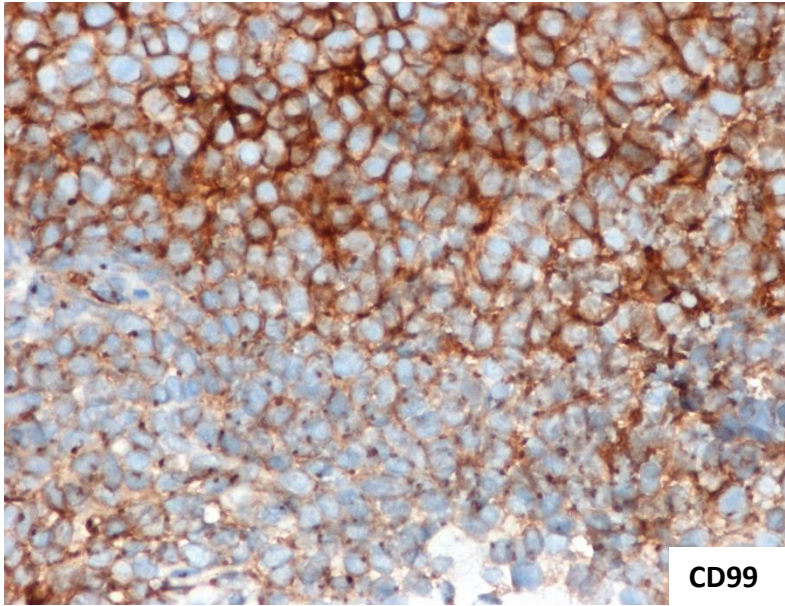
CD99
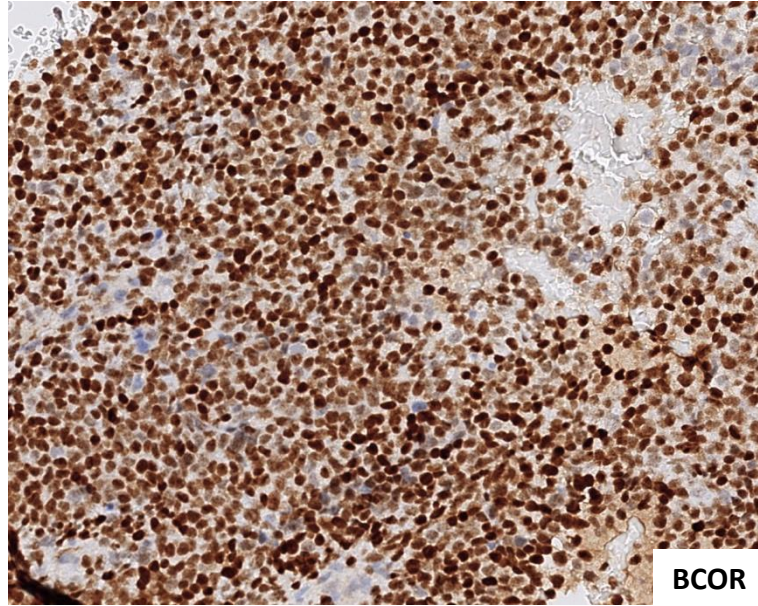
BCOR

CD34

Other markers		
S100 -	Desmin -	CD3 -
Pan-keratin +/-	WT1 -	CD20 -
EMA -	ETV4 -	CD79A -
ERG -	NKX2-2 -	MPO -
NUT -	SATB2 -	Synaptophysin -
SMA -	SS18-SSX -	Chromogranin -
INI1 and BRG1 conserved expression		

10 -14 days later...

Targeted RNA-Sequencing results

NAB2-STAT6 fusion

STAT6

Diagnosis: Solitary fibrous tumor, high-risk based on Demicco et al. (PMID: 28731041)

Molecular genetics can be very helpful for characterizing diagnostically challenging tumors

Other potentially useful applications of molecular profiling

- Prediction of tumor aggressiveness :

Validated prediction of clinical outcome in sarcomas and multiple types of cancer on the basis of a gene expression signature related to genome complexity.

Chibon F[1], Lagarde P, Salas S, Pérot G, Brouste V, Tirode F, Lucchesi C, de Reynies A, Kauffmann A, Bui B, Terrier P, Bonvalot S, Le Cesne A, Vince-Ranchère D, Blay JY, Collin F, Guillou L, Leroux A, Coindre JM, Aurias A.

- Sarcoma classification based on RNA or DNA-methylome tumor profiling:

Transcriptomic definition of molecular subgroups of small round cell sarcomas

Sarah Watson [1] [2], Virginie Perrin [1] [2], Delphine Guillemot [3], Stephanie Reynaud [3], Jean-Michel Coindre [4] [5], Marie Karanian [6], Jean-Marc Guinebretière [7], Paul Freneaux [8], François Le Loarer [4] [5], Megane Bouvet [3], Louise Galmiche-Rolland [9] [10], Frédérique Larousserie [11], Elisabeth Longchampt [12], Dominique Ranchere-Vince [6], Gaelle Pierron [3], Olivier Delattre [1] [2] [3] [13], Franck Tirode [1] [2] [14]

Sarcoma classification by DNA methylation profiling

Christian Koelsche [#] [1] [2] [3], Daniel Schrimpf [#] [1] [2], Damian Stichel [#] [2], Martin Sill [#] [4] [5], Felix Sahm [1] [2], David E Reuss [1] [2], Mirjam Blattner [4] [6], Barbara Worst [4] [6] [7], Christoph E Heilig [8], Katja Beck [8] [9], Peter Horak [8], Simon Kreutzfeldt [8], Elke Paff [4] [6] [7], Sebastian Stark [4] [6] [7], Pascal Johann [4] [6] [7], Florian Selt [4] [7] [10], Jonas Ecker [4] [7] [10], Dominik Sturm [4] [6] [7], Kristian W Pajtler [4] [5] [7], Annekathrin Reinhardt [1] [2], Annika K Wefers [1] [2], Philipp Sievers [1] [2], Azadeh Ebrahimi [2], Abigail Suwala [1] [2], Francisco Fernández-Klett [1] [2], Belén Casalini [2],

CINSARC

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- Cancer grading system based on a gene expression signature related to genome complexity

- Stratififaction of tumors in two groups: low risk and high risk of metastasis

CINSARC

Le Guellec et al. 2018 PMID: 29860427
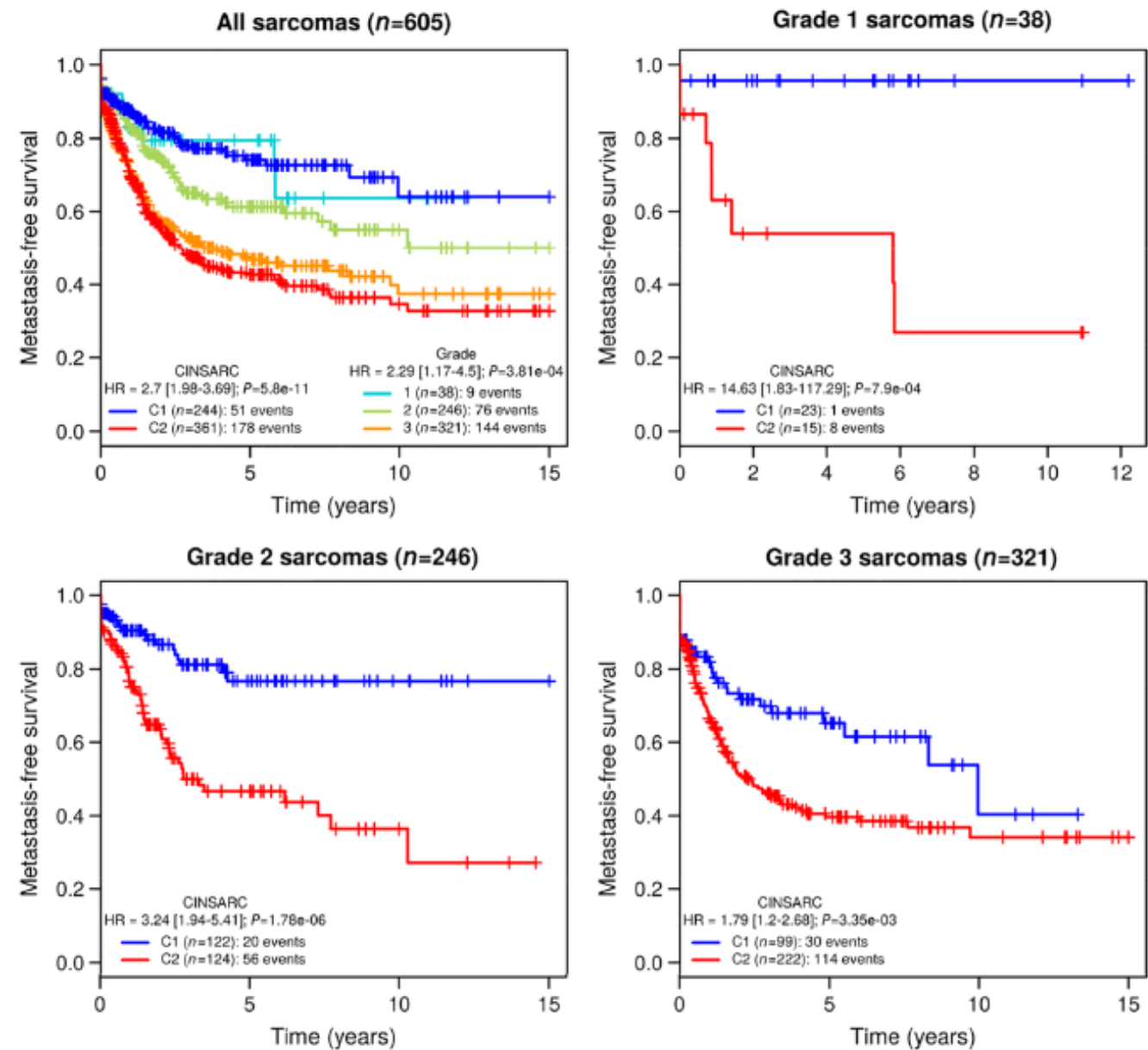
CINSARC

Advantages

- Probably gives additional prognostic information

- Reproducibility

- Dichotomic

- Can be performed in very small samples and after neo-adjuvant treatment

Disadvantages

- Expensive

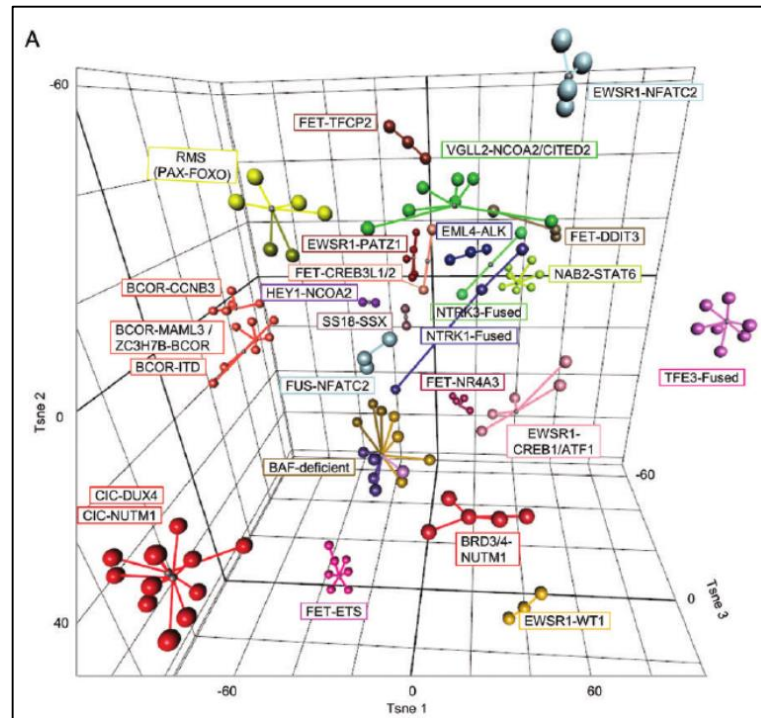- Not a perfect system

- Not useful for every sarcoma subtype

Utility in clinical practice?   Don't know yet…

Sarcoma classification based on on RNA or DNA-methylome tumor profiling

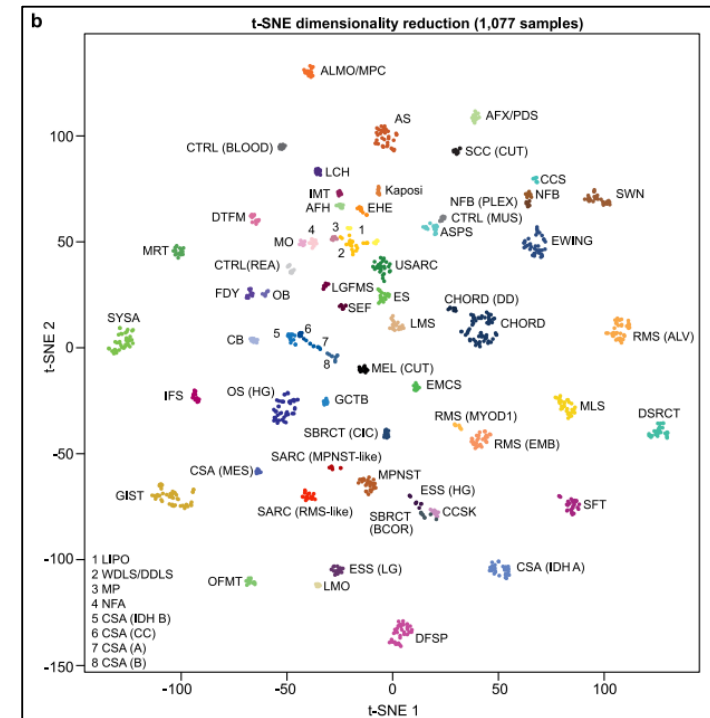## Transcriptomic definition of molecular subgroups of small round cell sarcomas

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Useful techniques to interpreted with caution (CPC)

Conclusion

- Molecular techniques are valuable <u>tools</u> that can provide additional diagnostic, prognostic and therapeutic data

- <u>Multidisciplinary expertise</u> is needed for its correct implementation

- Molecular genetics <u>don't replace</u> the basic diagnostic process of disease

La cité du vin – Bordeaux, France

Le pont de Pierre – Bordeaux, France

Thank you

Gracias

Merci

Glaciar Perito Moreno – Santa Cruz, Argentina

Saltos del Moconá – Misiones, Argentina