European Reference Network
for rare or low prevalence complex diseases
Network Paediatric Cancer (ERN PaedCan)

January 19th 2022

Jimena de Pedro & Daniel Orbach

LEARNING FROM MISDIAGNOSIS

Moderation:
Roelof van Ewijk

Co-funded by the European Union's Health Programme

COI declaration

- Jimena: Nothing to declare
- Dr. Orbach:
  - Consultant pour Bayer (2018-, *larotrectinib*) and Roche
  - IDMC member for a Lilly product
  - Various: Consultant for Novartis Pharma France, Eusapharm

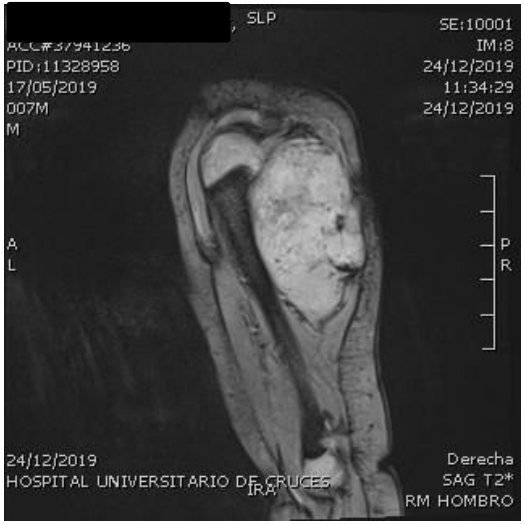
CLINICAL CASE

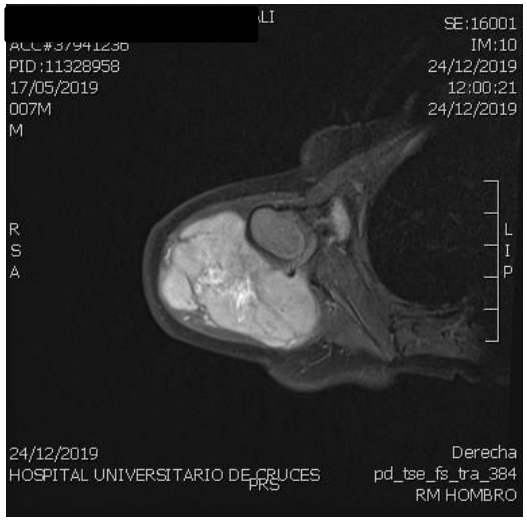
- 7 Month-old healthy male
- Lump in the right shoulder noticed the same day
- No relevant past medical history
- Physical exam:

  Shoulder asymmetry, palpable hard mass (6x9cm) in right deltoid region.

CLINICAL CASE

MRI





Heterogeneous solid mass, developed in the deltoid muscle. Approximately 55x53x30mm. Axillary adenopathy of 6mm in diameter with the same intensity as the mass.

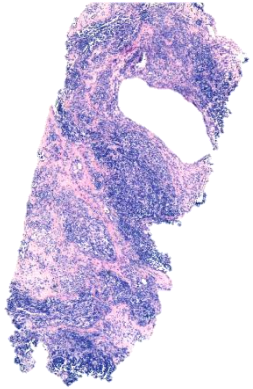
Which tumor would you suspect?

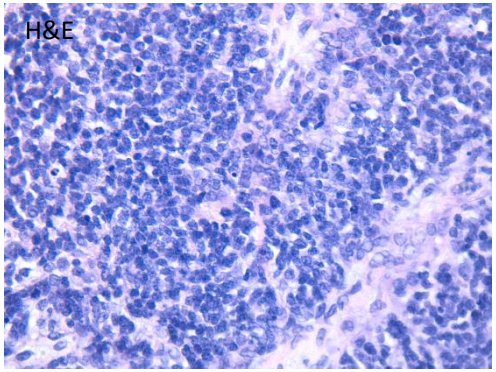
1. Rhabdomyosarcoma
2. Synovial Sarcoma
3. Rhabdoid Tumor
4. Ewing Sarcoma
5. Infantile Fibrosarcoma

Histology

Morphology:

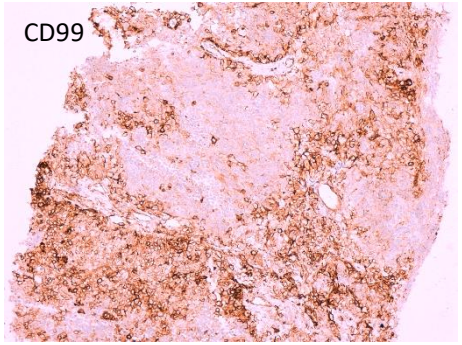
Skeletal muscle and connective tissue infilitrated by a densely immature cellular tumor.





IHQ:

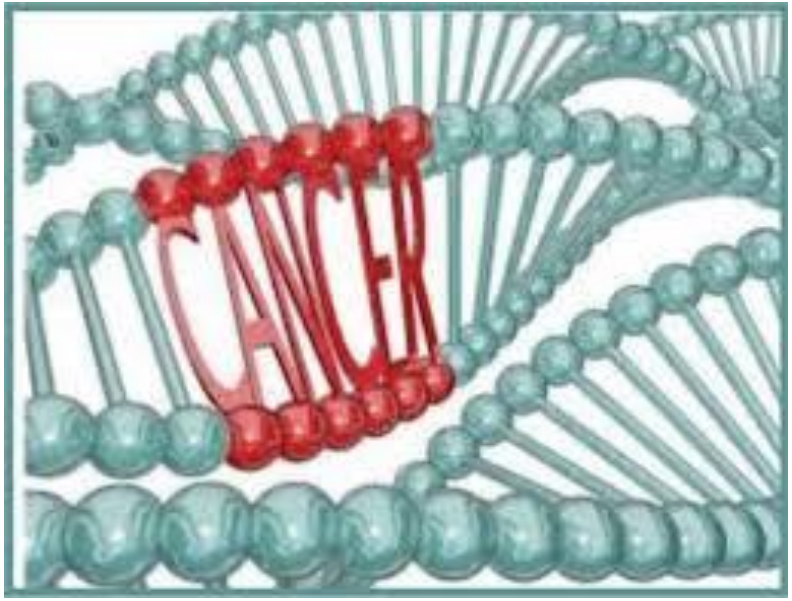
- Positive: CD99 diffuse, TLE-1 y Enolase weak
- Negative: AE1/AE3, desmina, MYoD1, miogenina WT-1, S-100, CD3 y CD20



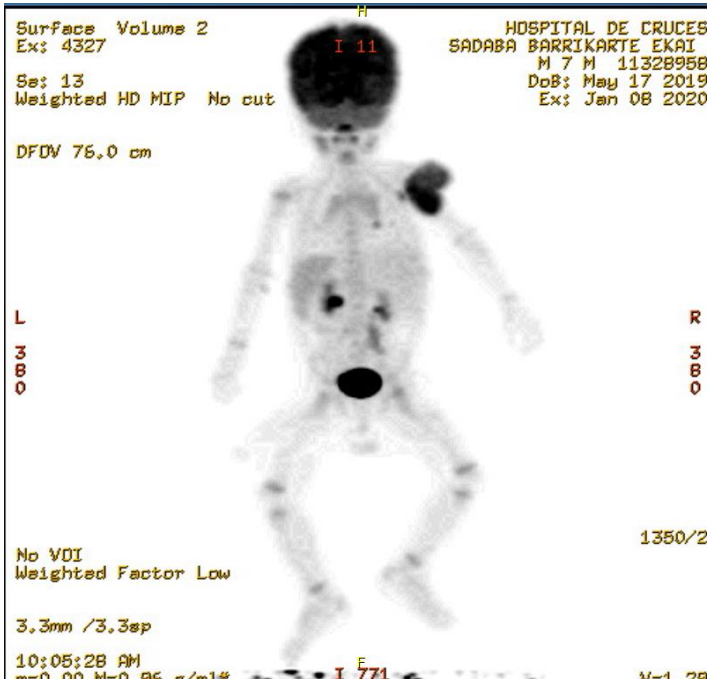
FISH: Positive for *EWSR1* (22q12.2) rearrangement (Zyto Light SPEC EWSR1 Dual color break apart)

EWING SARCOMA

Histology

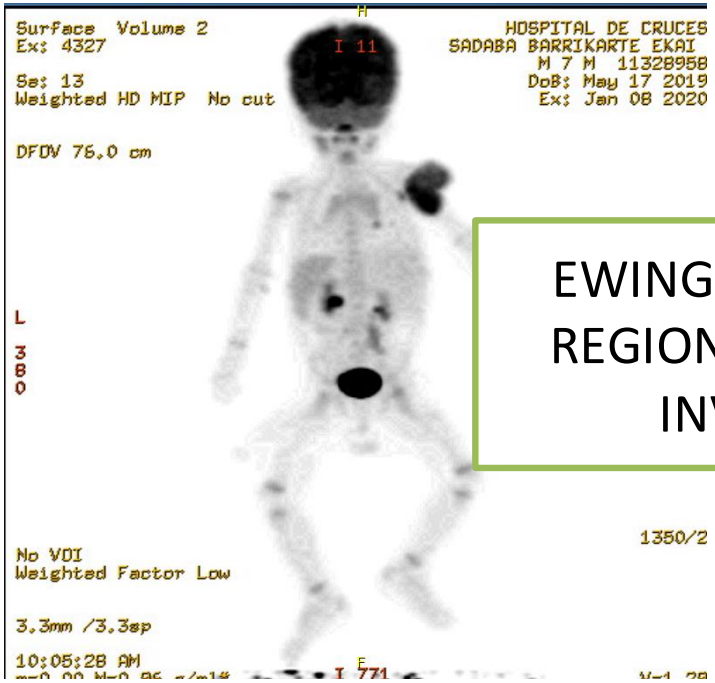


STAGING



Elevated metabolic activity in the right deltoid mass and ipsilateral axilar lymphadenopathy. Possible uptake in right hilium.

STAGING

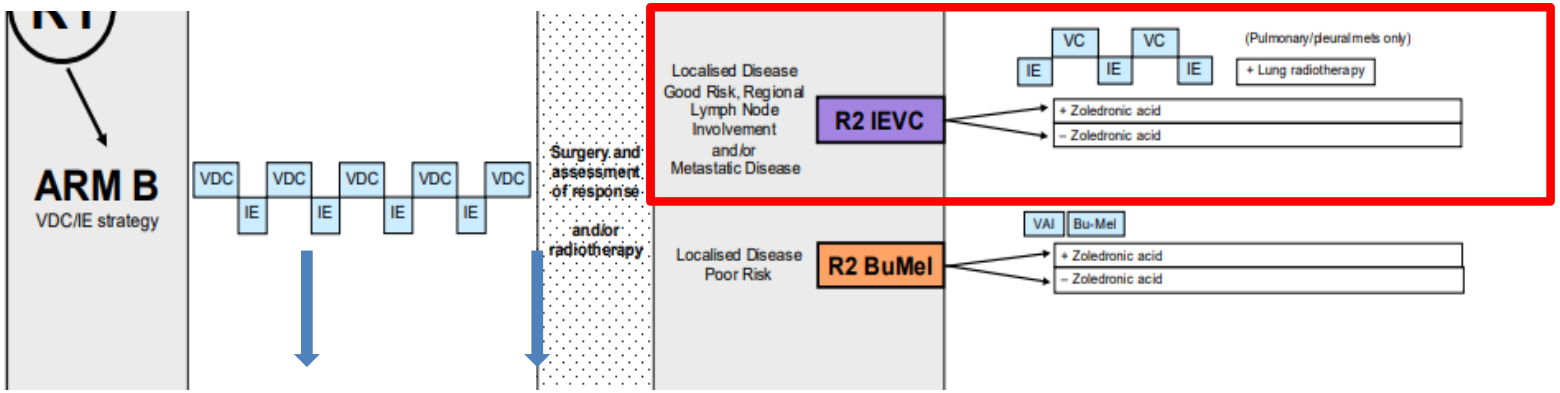


EWING SARCOMA WITH REGIONAL LYMPH NODE INVOLVEMENT

Elevated metabolic activity in the right deltoid mass and ipsilateral axilar lymphadenopathy. Possible uptake in right hilium.

TREATMENT

EUROEWING PROTOCOL 2012 (ARM B)

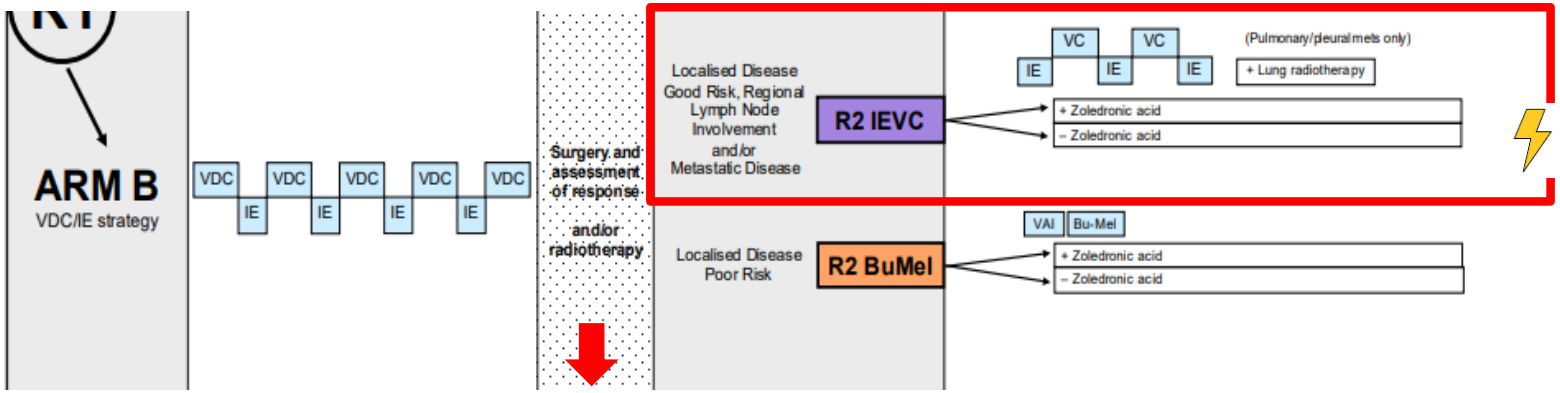


Radiological Assesment:
Partial Response. (Reduction of more than 50% of the tumor volume)

Radiological Assesment:
Complete Metabolic response
Partial Morphological response (stability)

TREATMENT

EUROEWING PROTOCOL 2012 (ARM B)



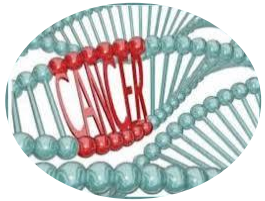
+ RT : 45 Gy + 54 Gy boost

(tumor bed and axillar adenopathy)

SURGERY: Wide exeresis of the middle and posterior portions of the deltoid muscle. Axillar adenopathy not resected

>Histology: Necrosis of the **75% of the tumor specimen**.

MEDIAL BORDER WAS AFFECTED.



NGS PANEL

CODE	DIAGNOSIS	TYPE OF ALTERATION	GENE	PROTEIN	NUCLEOTIDE	%	LECTURES
SARCOPED25	Rhabdoid Tumor?	Mutations	*SMARCB1*	p.Arg158Ter	c.472C>T	42%	1440x
			SMARCB1	p.Arg40Ter	c.118C>T	42%	1068x
							Oncomomine (Thermofisher)

EWSR1 rearrangements not found

Biallelic SMARCB1 nonsense mutations
Loss of function of SMARCB1 in the tumor

Does this finding changes our original diagnosis ?

1. YES
2. NO
3. I DON'T KNOW

Which tumour would you suspect now?

1. Rhabdomyosarcoma
2. Synovial Sarcoma
3. Rhabdoid Tumor
4. Ewing Sarcoma
5. Infantile Fibrosarcoma

NGS PANEL

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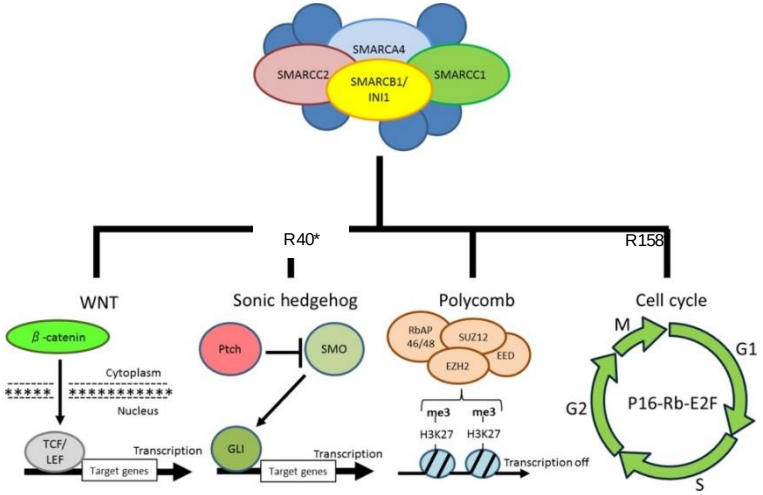
Oncomomine (Thermofisher)

SMARCB1 (22q11): Tumor suppressor gene



SMARCB1-deficient Tumors

Kohashi and Oda, 2017

Table 1. *SMARCB1*-Deficient Tumors of Childhood.

Tumor	Age range	Site	SMARCB1 staining pattern	*SMARCB1* genetic alteration
Malignant rhabdoid tumor	Usually <3 years	Kidney, soft tissue, and viscera	Complete loss	Biallelic deletions/mutations Up to 20% with germline deletion or mutation[15]
Atypical teratoid rhabdoid tumor	Usually <3 years	Brain and spinal cord	Complete loss (98%) Loss of BRG1 (2%)	Biallelic deletions/mutations Up to 35% with germline deletion or mutation[15]
Cribriform neuroepithelial tumor	<11 years	Brain (periventricular regions and medulla)	Complete loss	Partial exon duplications, deletions, and point mutations
Renal medullary carcinoma	Mainly adolescents and young adults	Kidney	Complete loss	LOH or hemizygous deletions, possible epigenetic silencing of remaining allele[16]
Epithelioid sarcoma	Mainly adolescents and young adults	Skin and subcutaneous tissue, mainly extremities (classic form) Deep soft tissue, mainly perineum and proximal limb girdles (proximal form)	Complete loss in 90% of cases	Homozygous deletions in most[17]
Epithelioid malignant peripheral nerve sheath tumor	Adults, may be seen in young children	Most in dermis and subcutaneous tissue, and some in deep soft tissue	Complete loss in 25% to 66% of tumors	Unknown, possibly due to loss of 22q11.3 material encoding *SMARCB1* locus
Schwannomas in familial schwannomatosis	Any	Peripheral nerves and spinal nerve roots	Mosaic pattern of retention and loss	Germline nontruncating splice-site mutations and missense mutations in exon 1 with the deletion of second allele[18]
Chordoma	Any	Skull base, spine; cervical and spheno-occipital origin common in children	Complete loss in a subset of poorly differentiated tumors	Deletions more common than point mutations
Myoepithelial carcinoma	Any	Soft tissues and viscera	Complete loss in 40% of pediatric tumors	Homozygous deletions[19]
Sinonasal carcinoma	Adults, rare cases in adolescents	Sinonasal region	Complete loss in a subset of tumors	Homozygous and heterozygous deletions[20]
Synovial sarcoma	Any, most common in adoescents and young adults	Mainly in deep soft tissues of extremities, reported in wide variety of other locations	Reduced expression in 88% to 94% of tumors	Epigenetic phenomenon resulting from displacement of *SMARCB1* by the SSX-SS18 fusion protein

LOH: loss of heterozygosity.

Pawel, 2017

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Histology

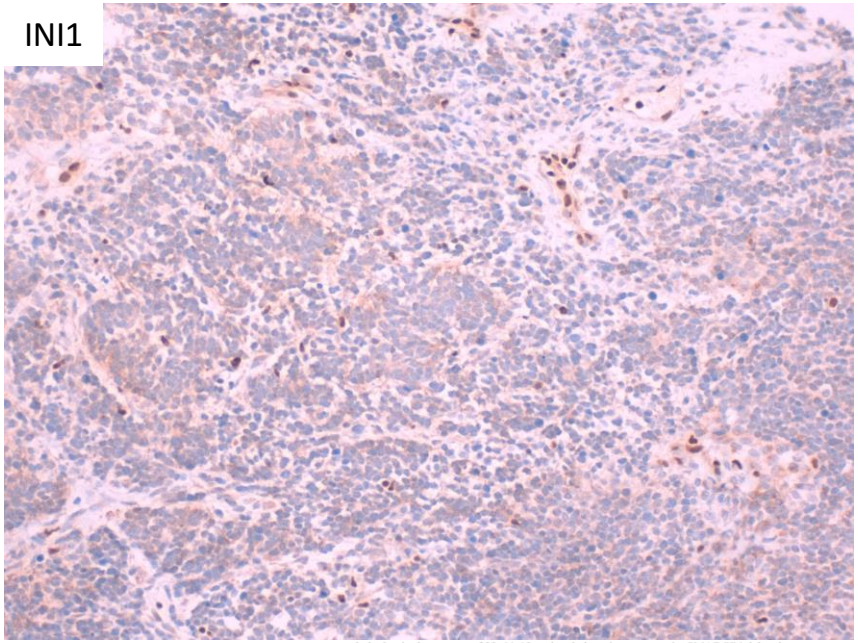
Morfology:

Skeletal muscle and connective tissue infilitrated by a densely immature cellular tumor.

IHQ:

- Positive: CD99, TLE-1 y Enolasa debil
- Negative: SMARCB1/ INI-1 (loss of nuclear expression)

FISH: Negative for *EWSR1* (22q12.2) rearrangement



conclude that in the context of 22q11-12 regional alterations present in *SMARCB1*-deleted tumors, simultaneous *EWSR1* involvement may be misinterpreted as equivalent to *EWSR1* rearrangement. A detailed clinicopathologic correlation and supplementing the *EWSR1* FISH assay with complementary methodology is mandatory for correct diagnosis.

Rhabdoid Tumor

Histology

Morfology:

Skeletal muscle and connective tissue infilitrated by a densely imı cellular tumor.

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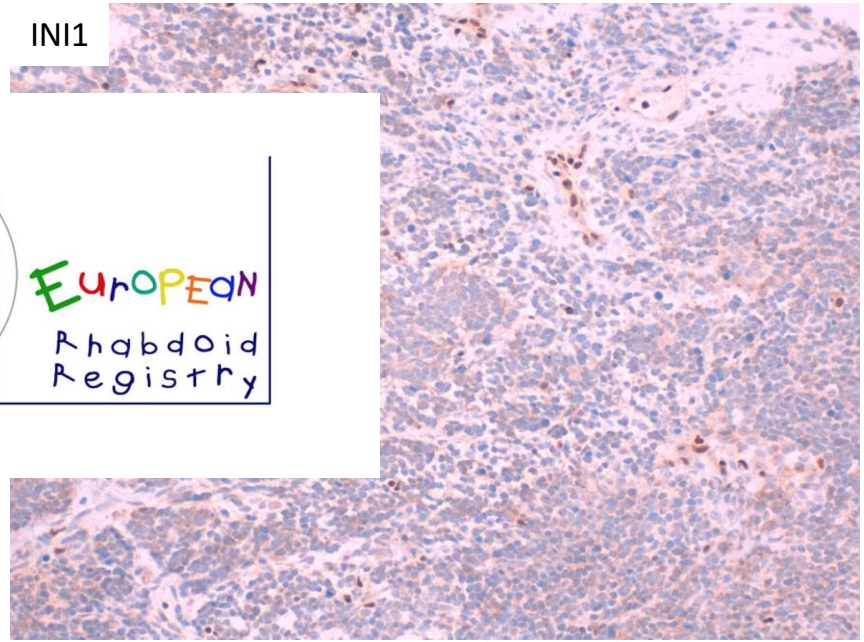

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Rhabdoid Tumor

Malignant Rhabdoid Tumors

- Rare, highly agressive embryonal neoplasm
- Most common in infancy and childhood
- Dismal prognosis (5 year OS 20-30%)
- Some MRT histologically indistinguishable from Ewing Sarcoma (SMARCB1 stain is critical for diagnosis)
- Rhabdoid Tumor predisposition syndrome

New diagnosis?

Inform the parents

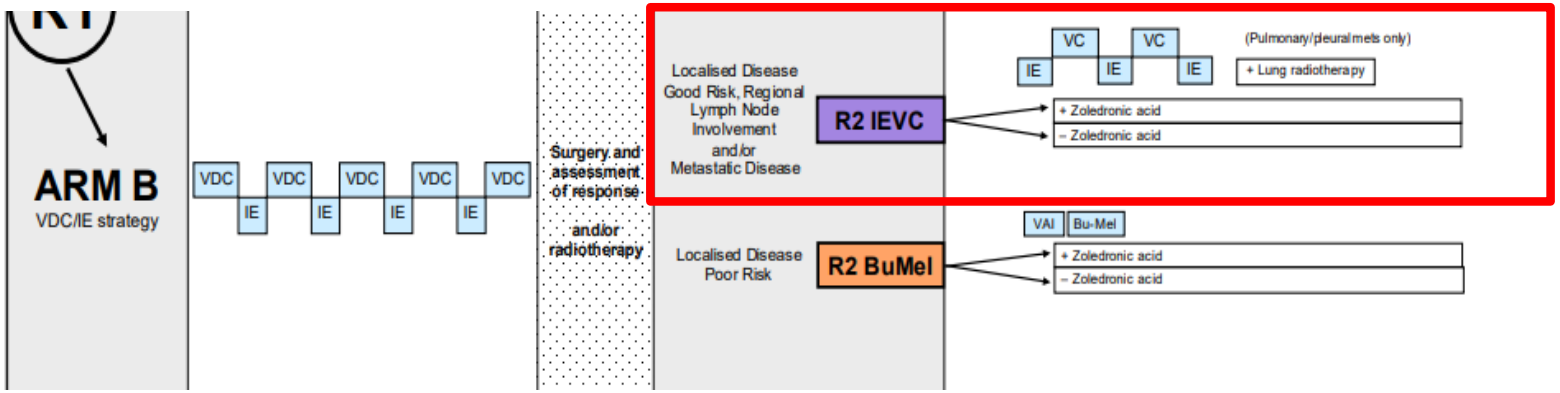
Finishing Chemotherapy….

No standard Treatment

Treatment based on Sarcoma-like protocols

TREATMENT

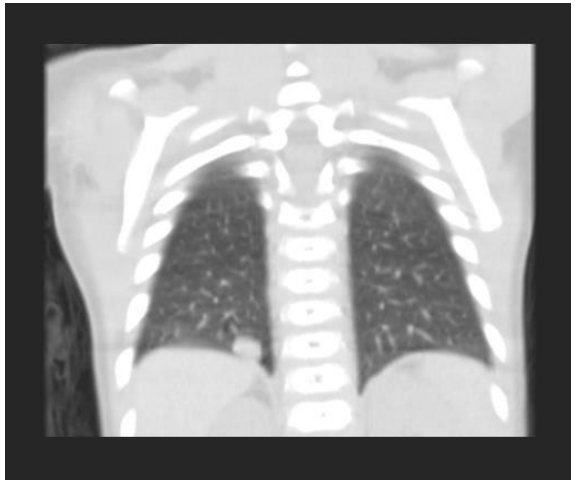
EUROEWING PROTOCOL 2012 (ARM B)

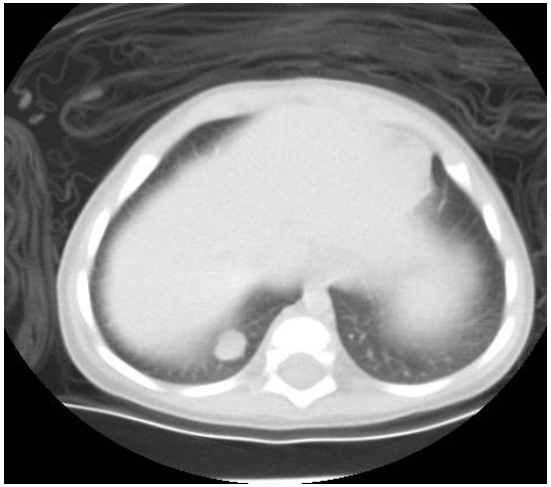


END OF TREATMENT

Radiological assessment (6 weeks after EOT)

EARLY RELAPSE





Single Pulmonar Nodule in inferior Right lobe

PET-CT

Treatment Options

- Chemotherapy 
- Radiotherapy 

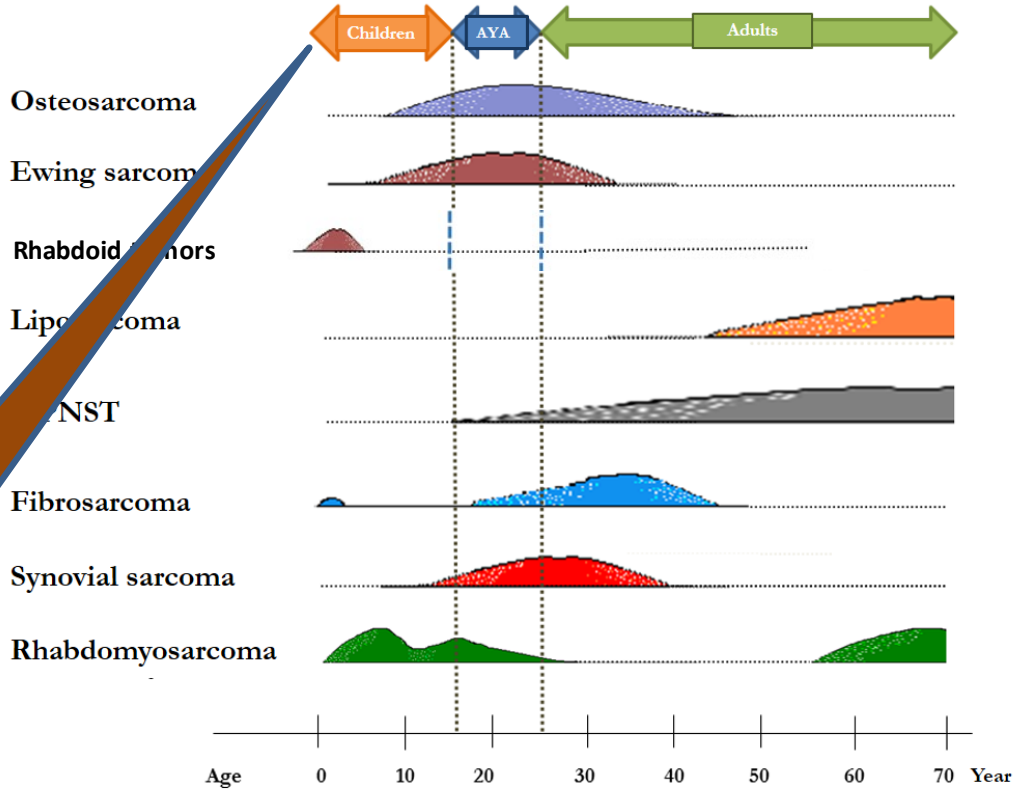
  Whole Lung RT: 15 Gy + Boost 54 Gy tumor bed
- New Drugs 

  Tazemetostat: 520 mg/m^2/dose

Expert Discussion

- Dr Daniel Orbach

Age distribution of main sarcomas according to age



Newborn /infant :

- Rhabdoid tumor
- Infantile fibrosarcoma
- Rhabdomyosarcoma
- Undifferentiated sarcoma

Biology to confirm/help pathologic diagnosis

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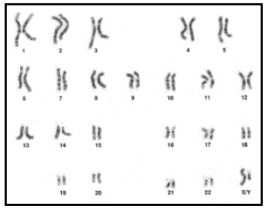
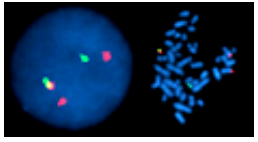
Banding

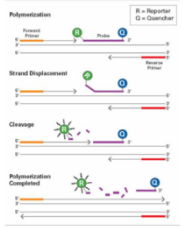


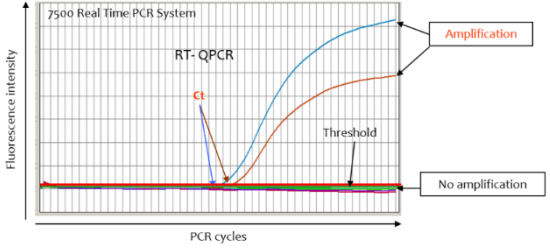
FISH



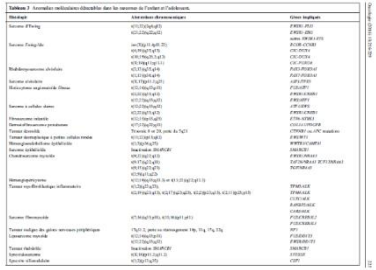
FISH technics

- Taqman TR QPCR:
  Fluorescent probes & Multiplex approach
  High sensibility : semi quantitative PCR
  Avoiding contaminations



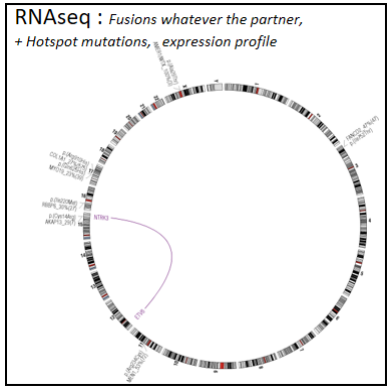


Multiplex RT-PCR



List of sarcomas with molecular abnormalities (2018)

RNAseq : *Fusions whatever the partner, + Hotspot mutations, expression profile*



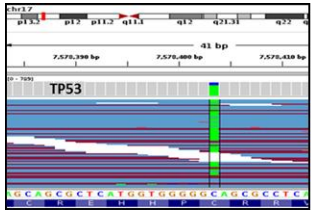
RNA sequencing

➢ For well known fusion transcripts

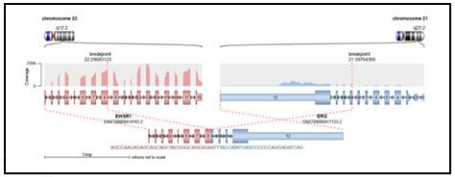
… and unknown ones

Co-funded by the European Union's Health Programme

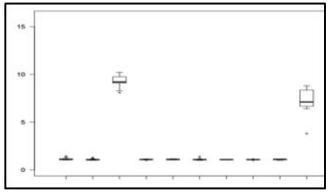
RNAseq and pediatric tumors



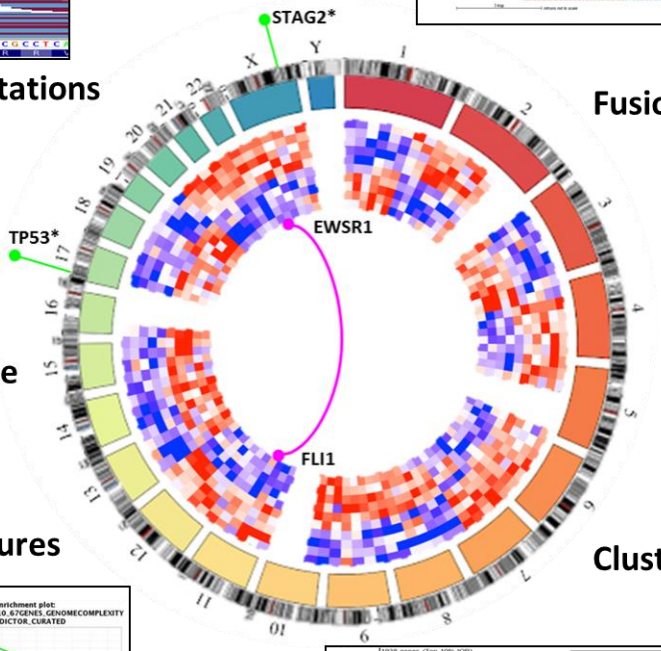
Mutations

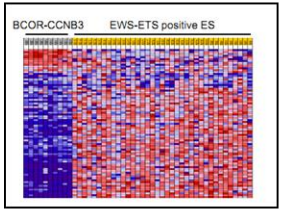


Fusion

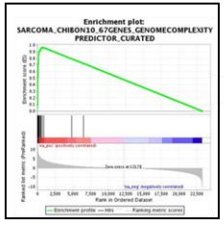


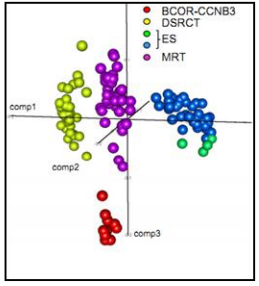
Expression différentielle





Signatures





Clustering

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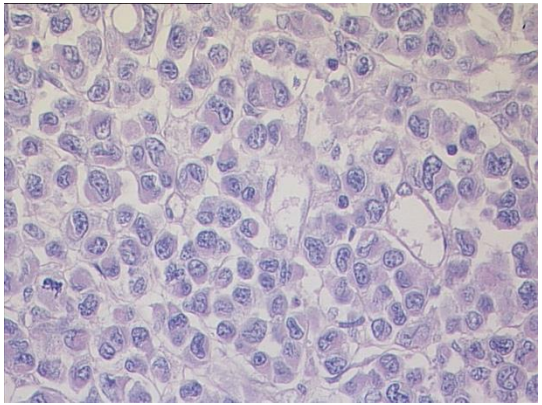
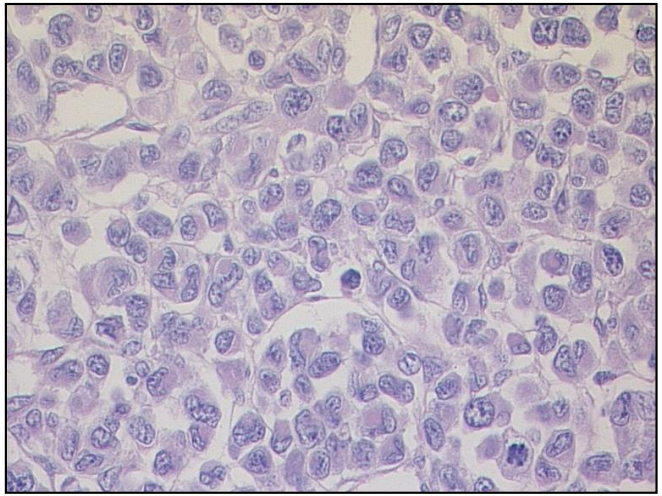
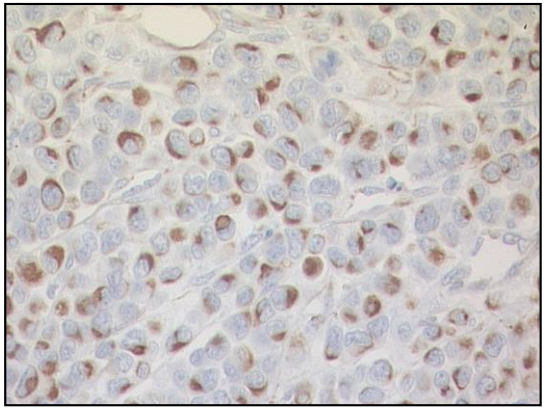
Rhabdoid tumor histology



HES





Vimentine

- Large round cells
- Eosinophil cytoplasm
- Excentric nucleus with huge nucleoles
- Intra-cytoplasmic inclusions

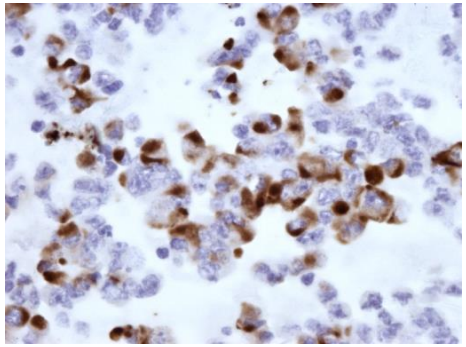
institutCurie

P Fréneaux

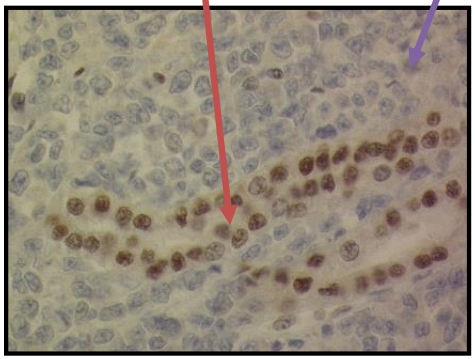
Rhabdoid tumor: immuno-histologic pattern

- IHC : Vimentine +
  Keratine/EMA +
  CD99+ (non specific)
  Desmine -
  INI 1 -

« dot » staining



AE1/AE3



Anti-INI1 Ab

➢ Importance of systematic INI 1 staining in all infants' sarcomas

Take Home Messages

- Reconsider the diagnosis in case of doubts…. (Infant, nodal involvement, soft tissue mass…)
- Systematic INI 1 staining in all infants sarcomas
- EWSR1 rearrangment is not necessarily a Ewing sarcoma
- Integrate molecular characterization in the diagnostic workup
- Patient not mistreated

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Thank you for your attention