Molecular Diagnostics In Mesenchymal Cancers

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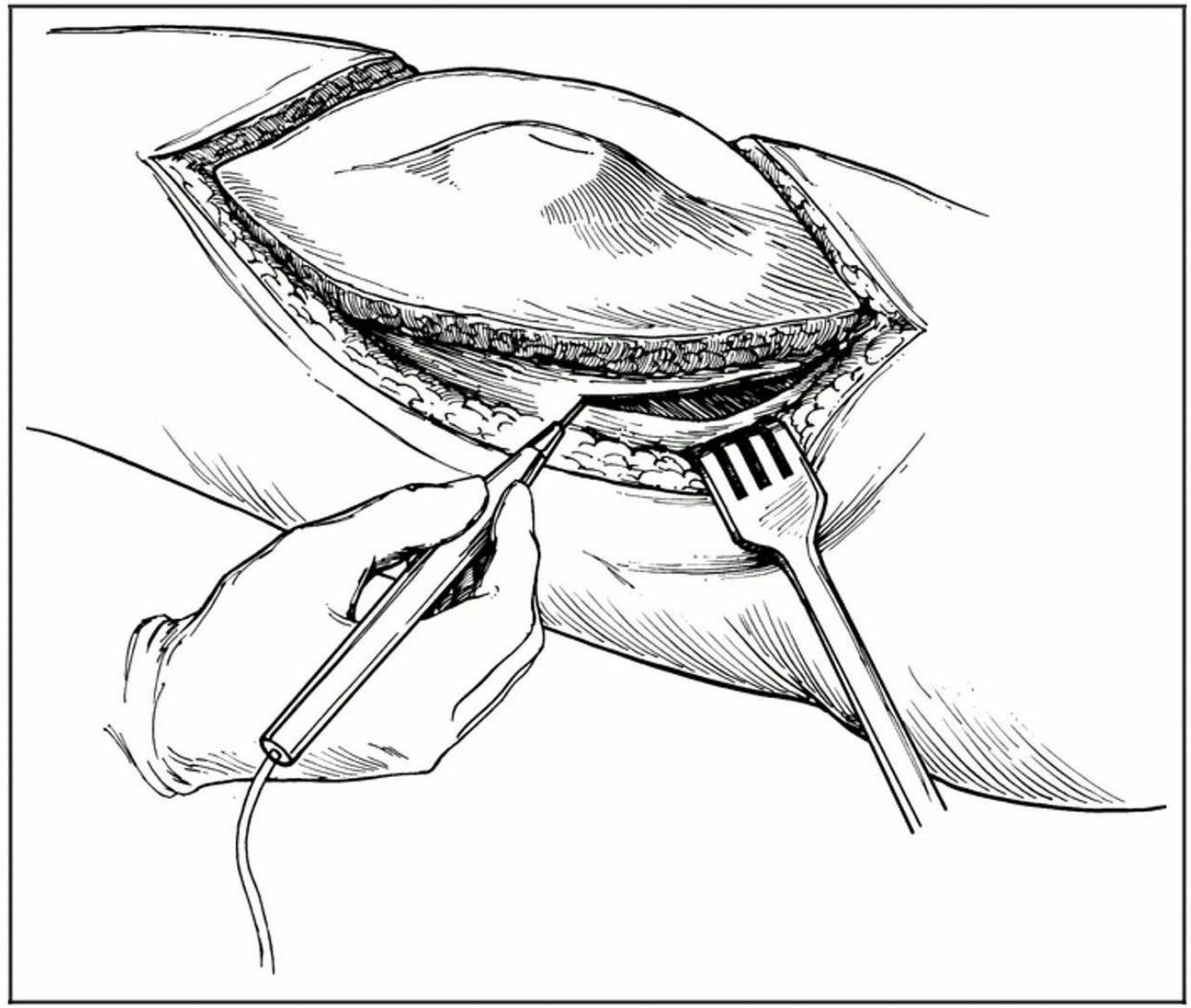
Royal National Orthopaedic Hospital NHS
NHS Trust

Outline

- Mesenchymal tumours– classification.
- Sarcomas and Molecular diagnostic assays (genetic).
- Recent developments in sarcoma diagnosis



Oncological Outcomes of Operative Treatment of Subcutaneous Soft-Tissue Sarcomas of the Extremities*
J Bone Joint Surg Am, 1997 Jun; 79 (6): 888 -97 .

Survival Rates

Figure 6b: Bone sarcoma 5-year rolling 5-year relative survival rates in males and females (UK: 1996-2010)

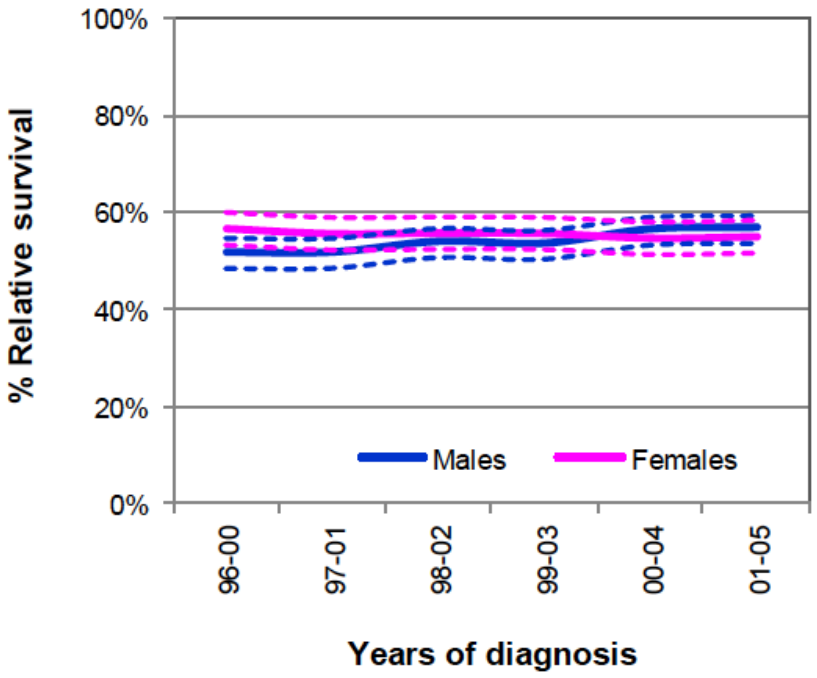
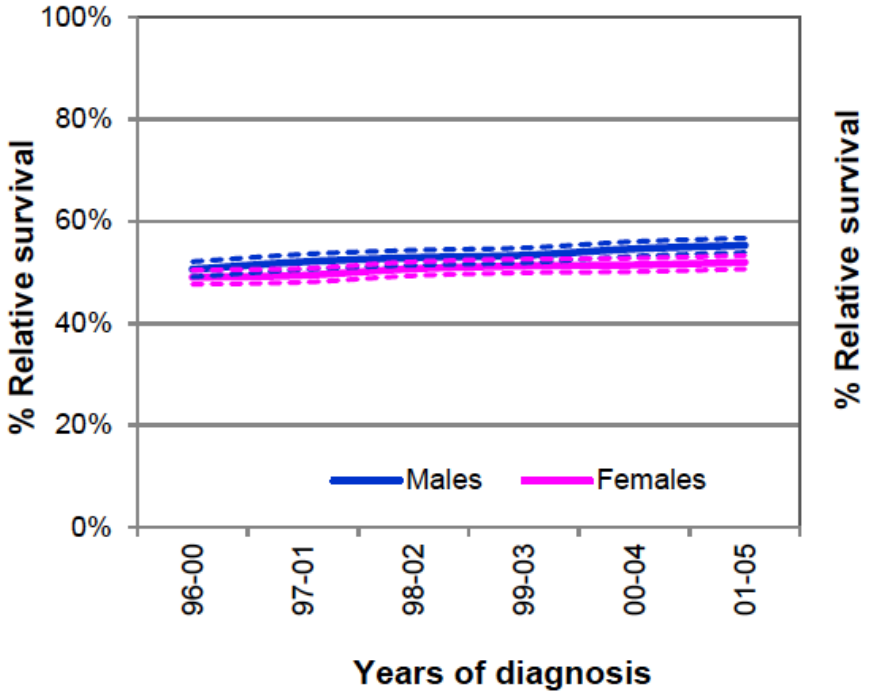
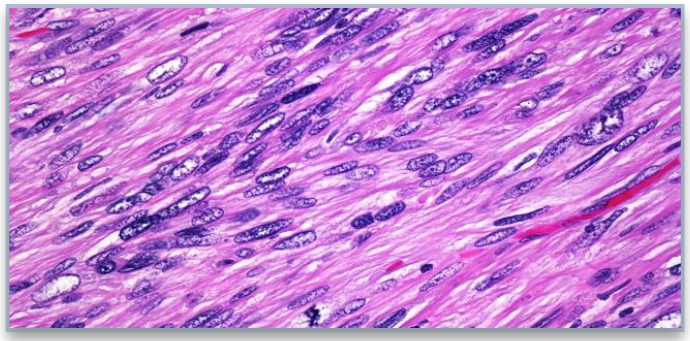


Figure 6a: Soft tissue sarcoma (excluding skin) 5-year rolling 5-year relative survival rates in males and females (UK: 1996-2010)



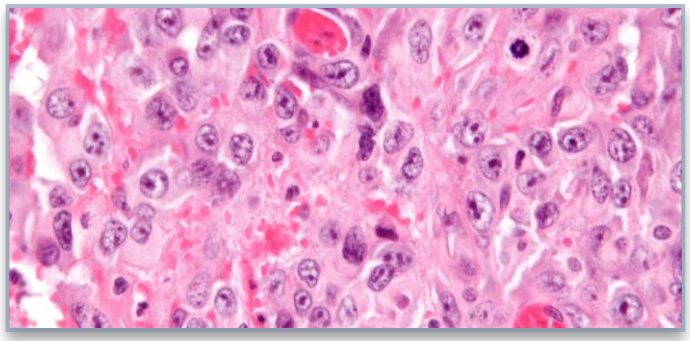
NCIN national cancer intelligence network
Using information to improve quality & choice

Classification of mesenchymal tumours



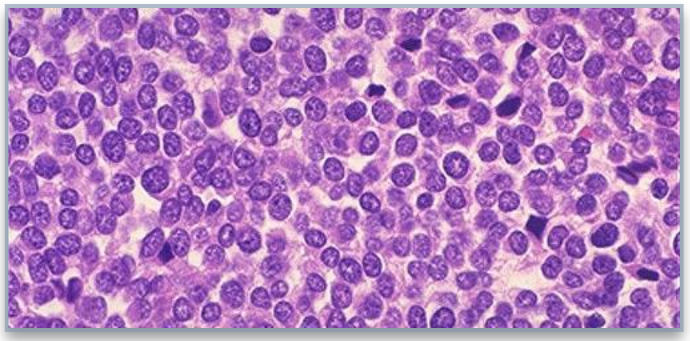
SPINDLE CELL

- Leiomyosarcoma
- Spindle cell rhabdomyosarcoma
- Fibrosarcoma
- Spindle cell sarcoma NOS



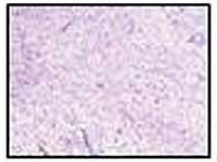
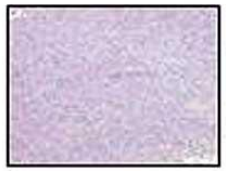
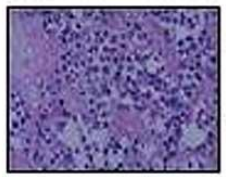
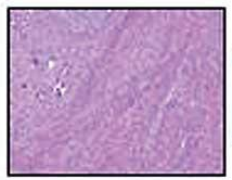
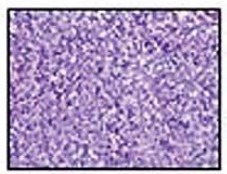
EPITHELIOID CELL

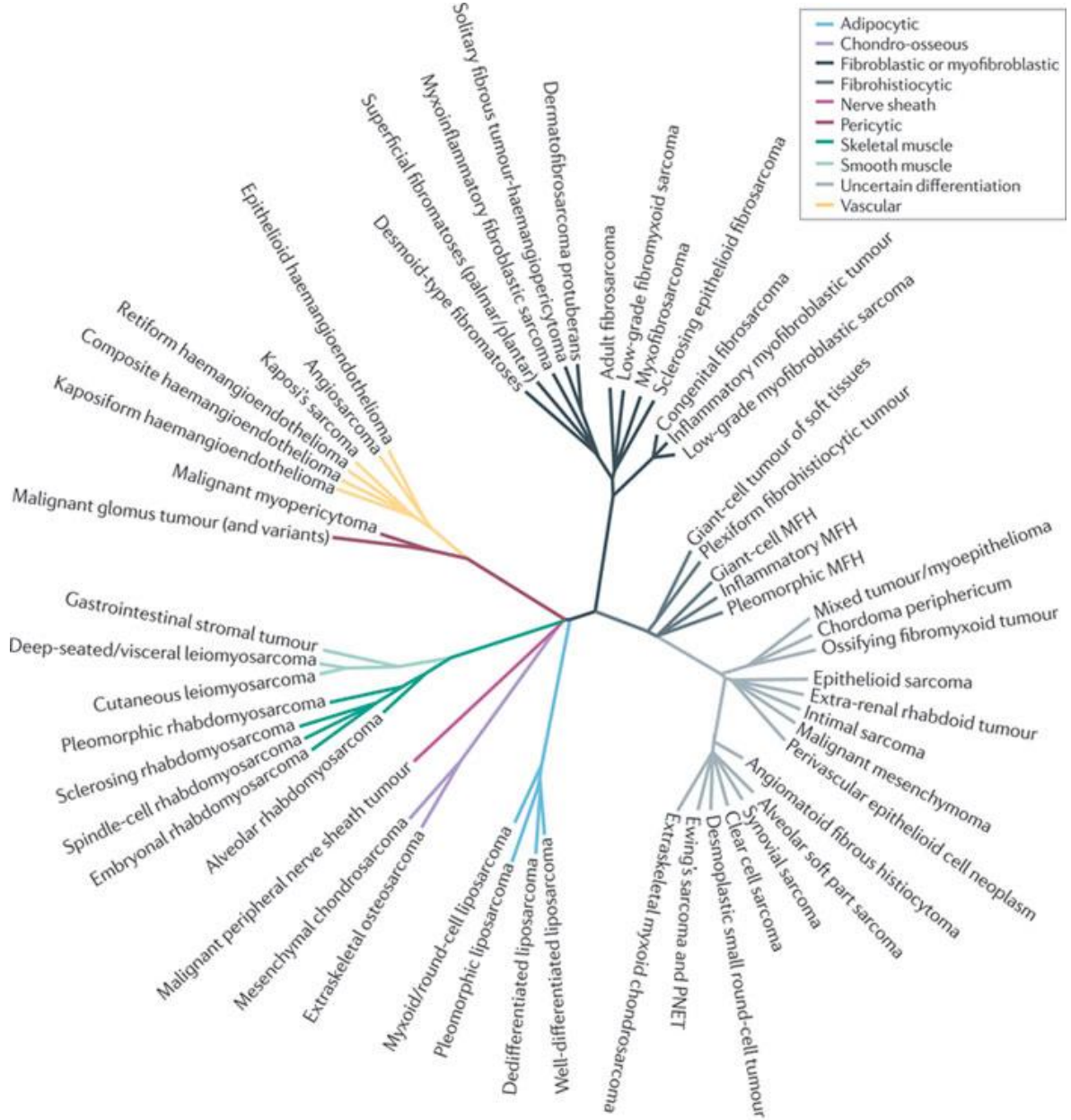
- Epithelioid sarcoma
- Epithelioid MPNST
- Epithelioid angiosarcoma
- Malignant rhabdoid tumour
- Metastatic Melanoma
- Metastatic carcinoma



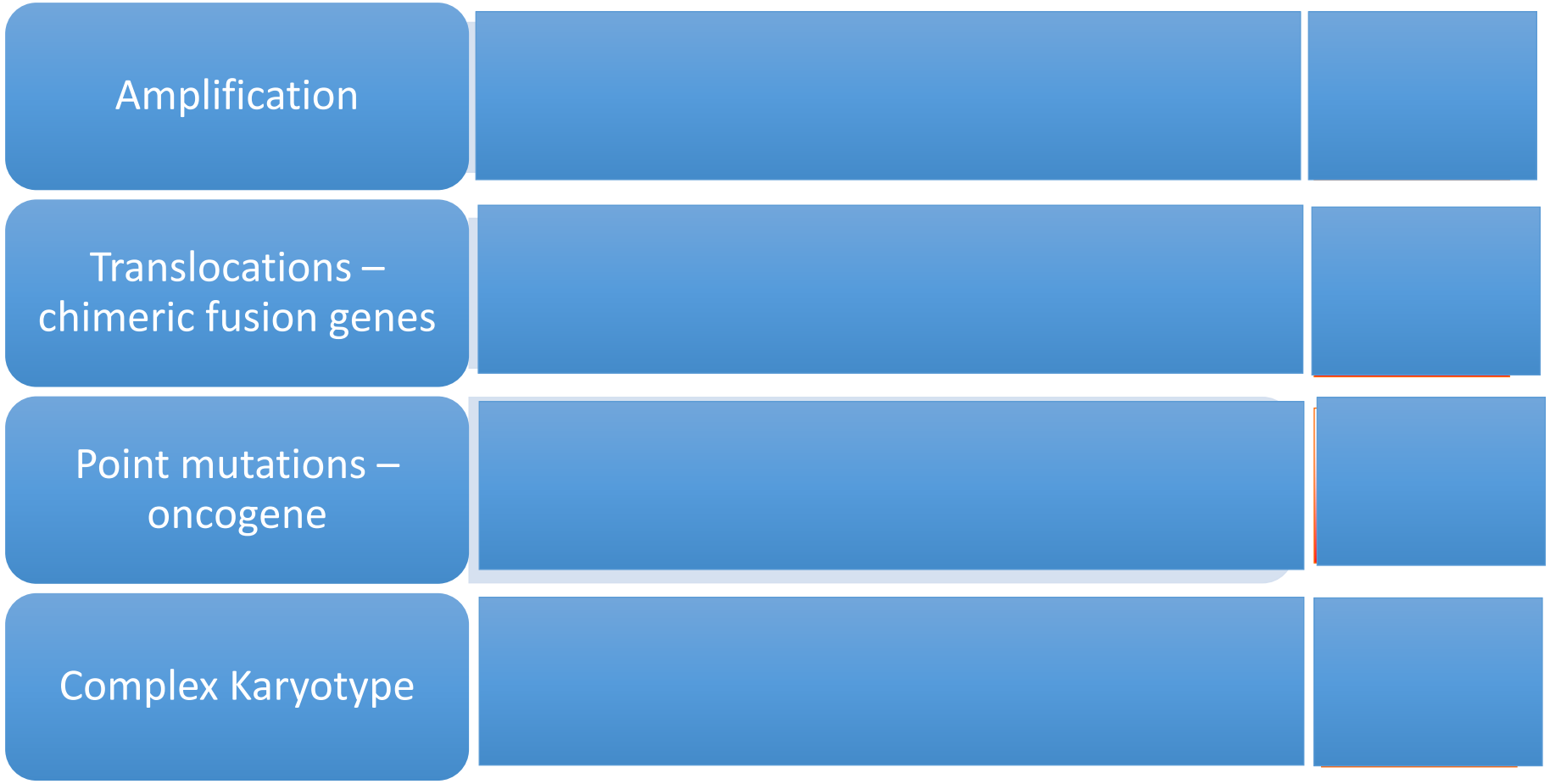
ROUND CELL

- Ewing Sarcoma
- Desmoplastic round cell tumour
- Alveolar rhabdomyosarcoma
- Neuroblastoma
- Lymphoma
- Organ specific – Wilm's tumour, hepatoblastoma, pleuropulmonary blastoma

DIFFERENTATION	Subtypes	Chromosomal traslocations	Fusion trascripts
 ADIPOCYTIC TUMORS	*Lipoblastoma*:	t(7;8)(q31;q13); t(8;8)(q24;q13)	PLAG1-COL1A2;PLAG1-HAS2
	Myxoid liposarcoma	t(12;16)(q13;p11); t(12;22)(q13;q12)	CHOP-TLS; CHOP-EWS
 FRIBLOBLASTIC/ MYOFIBROBL.TUMORS	*Inflammatory myofibroblastic tumor*	t(1;2)(q25;p23); t(2;19)(p23;q13); t(2;17)(p23;q23)	TPM3-ALK; ALK-TPM4; ALK-CLTC
	Infantile fibrosarcoma	t(12;15)(p13;q25)	ETV6-NTRK3
	Dermatofibrosarcoma protuberans/ Giant cell fibroblastoma	t(17;22)(q22;q13)	COL1A1-PDGFB
 SKELETAL MUSCLE TUMORS	*Alveolar rhabdomyosarcoma*	t(2;13)(q35;q14); t(1;13)(p36;q14)	PAX3-FKHR; PAX7-FKHR
 TUMORS OF UNCERTAIN DIFFERENTIATION	*Angiomatoid fibrous histiocytoma*	t(12;22) (q13;q12); t(12;16) (q13;p11)	
	Synovial sarcoma	t(X;18)(p11.2;q11.2)	SYT-SSX1/2/4
	Alveolar soft part sarcoma	t(X;17)(p11;q25)	TFE3/ASPL
	Clear cell sarcoma	t(12;22)(q13;q12)	EWS-ATF1
	Extraskeletal myxoid chrondrosarcoma	t(9;22)(q22;q12); t(9;15)(q22;q21)	EWS-TEC; CHN-TFC12
	Desmoplastic small round cell tumor	t(11;22)(p13;q12)	EWS-WT1
 EWING SARCOMA		t(11;22)(q24;q12);t(21;22)(q22;q12); t(17;22)(q12;q12); t(7;22)(p22;q12);	FLI1-EWS; ERG-EWS E1AF-EWS; ETV1-EWS


Adipocytic
Chondro-osseous
Fibroblastic or myofibroblastic
Fibrohistiocytic
Nerve sheath
Pericytic
Skeletal muscle
Smooth muscle
Uncertain differentiation
Vascular
Solitary fibrous tumour-haemangiopericytoma
Myxoinflammatory fibroblastic sarcoma
Superficial fibromatoses (palmar/plantar)
Desmoid-type fibromatoses
Dermatofibrosarcoma protuberans
Adult fibrosarcoma
Low-grade fibromyxoid sarcoma
Myxofibrosarcoma
Sclerosing epithelioid fibrosarcoma
Congenital fibrosarcoma
Inflammatory myofibroblastic tumour
Low-grade myofibroblastic sarcoma
Giant-cell tumour of soft tissues
Plexiform fibrohistiocytic tumour
Giant-cell MFH
Inflammatory MFH
Pleomorphic MFH
Mixed tumour/myoepithelioma
Chordoma periphericum
Ossifying fibromyxoid tumour
Epithelioid sarcoma
Extra-renal rhabdoid tumour
Intimal sarcoma
Malignant mesenchymoma
Perivascular epithelioid cell neoplasm
Angiomatoid fibrous histiocytoma
Alveolar soft part sarcoma
Synovial sarcoma
Clear cell sarcoma
Desmoplastic small round-cell tumour
Ewing's sarcoma and PNET
Extraskeletal myxoid chondrosarcoma
Well-differentiated liposarcoma
Dedifferentiated liposarcoma
Pleomorphic liposarcoma
Myxoid/round-cell liposarcoma
Extraskeletal osteosarcoma
Mesenchymal chondrosarcoma
Malignant peripheral nerve sheath tumour
Alveolar rhabdomyosarcoma
Embryonal rhabdomyosarcoma
Spindle-cell rhabdomyosarcoma
Sclerosing rhabdomyosarcoma
Pleomorphic rhabdomyosarcoma
Cutaneous leiomyosarcoma
Deep-seated/visceral leiomyosarcoma
Gastrointestinal stromal tumour
Malignant glomus tumour (and variants)
Malignant myopericytoma
Kaposiform haemangioendothelioma
Composite haemangioendothelioma
Retiform haemangioendothelioma
Kaposi's sarcoma
Angiosarcoma
Epithelioid haemangioendothelioma

Molecular classification of mesenchymal tumours



Gene Amplification – diagnostic tests

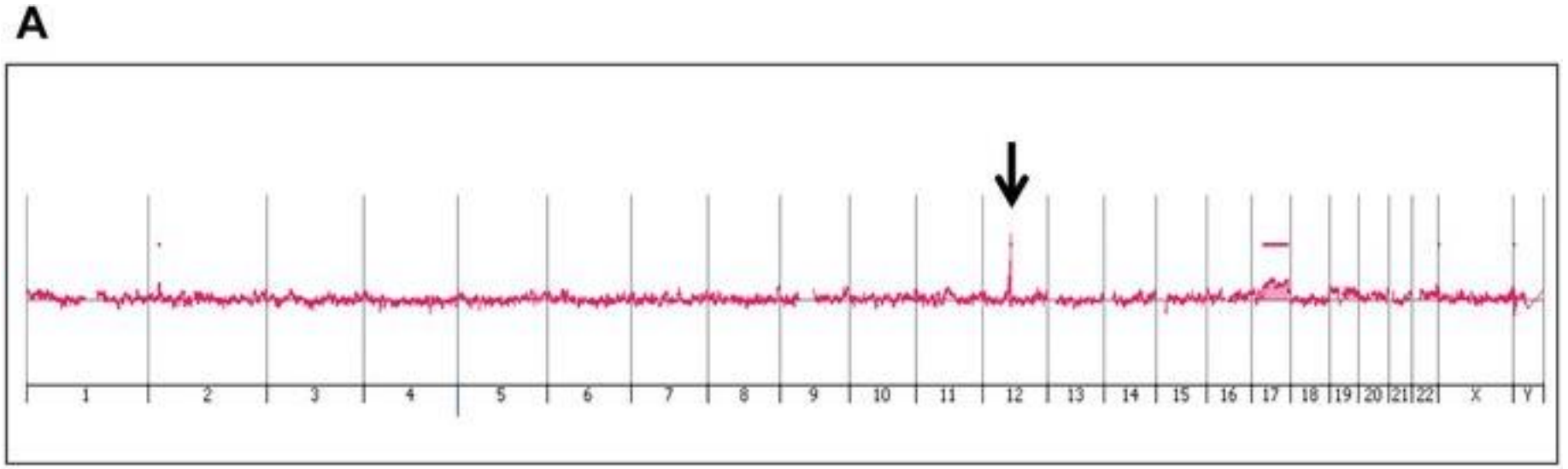
MDM2 amplification

- Atypical lipomatous tumour/ dedifferentiated liposarcoma
- Low grade osteosarcoma / parosteal osteosarcoma

MYC amplification

- Radiation induced angiosarcoma

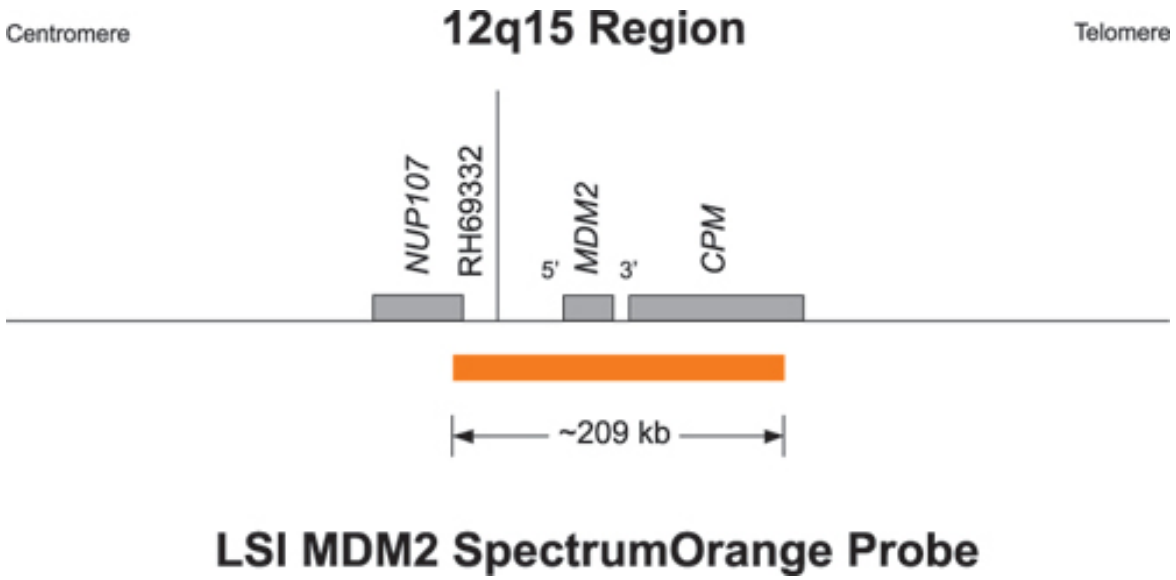
Copy number profile



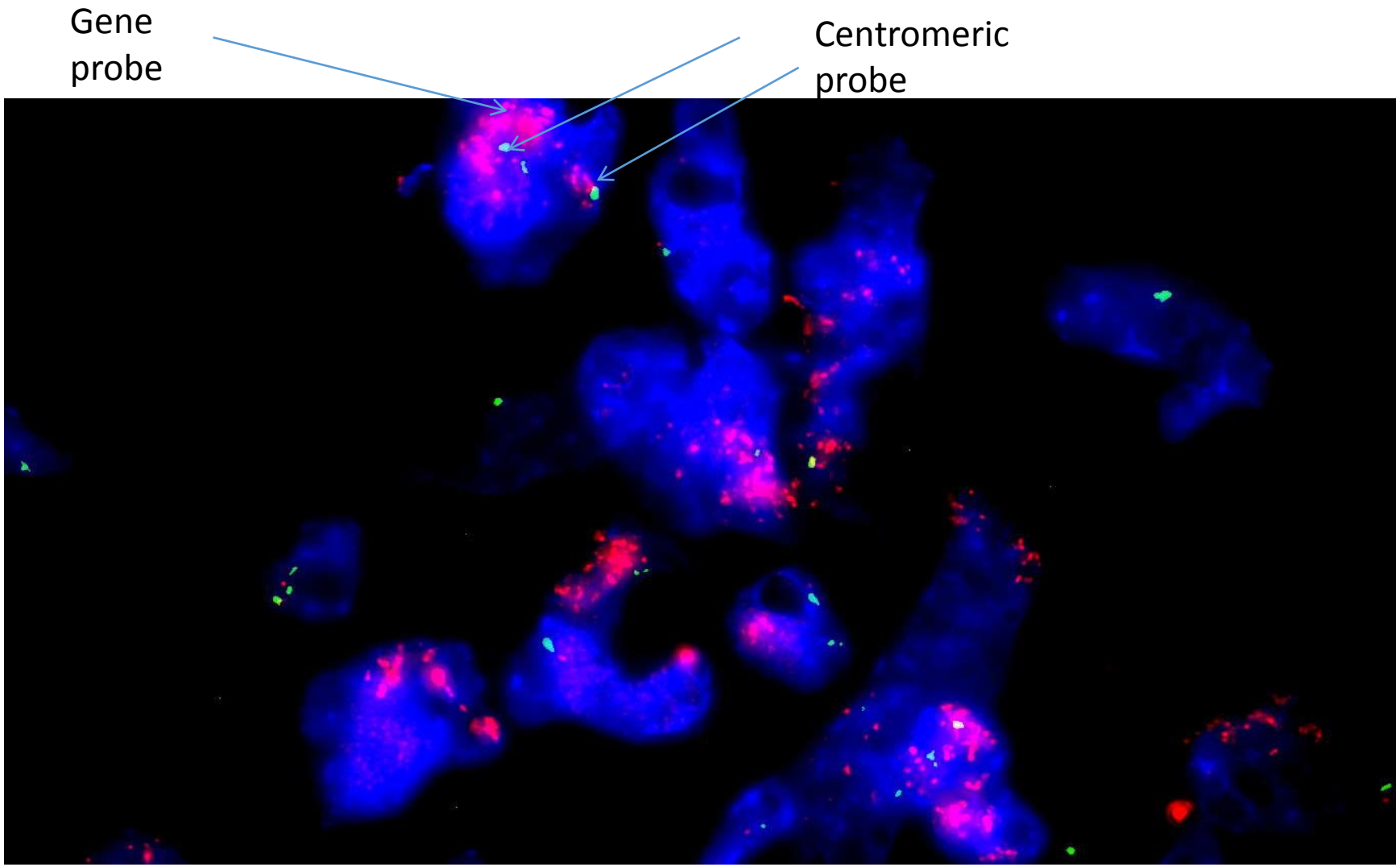
Chr12q – MDM2 locus

FISH
(Design for amplification)

- Assay design –
- centromeric probe (enumerate the chromosomes)
- gene probe (enumerate the gene copy number)



MDM2 amplification – Well differentiated liposarcoma

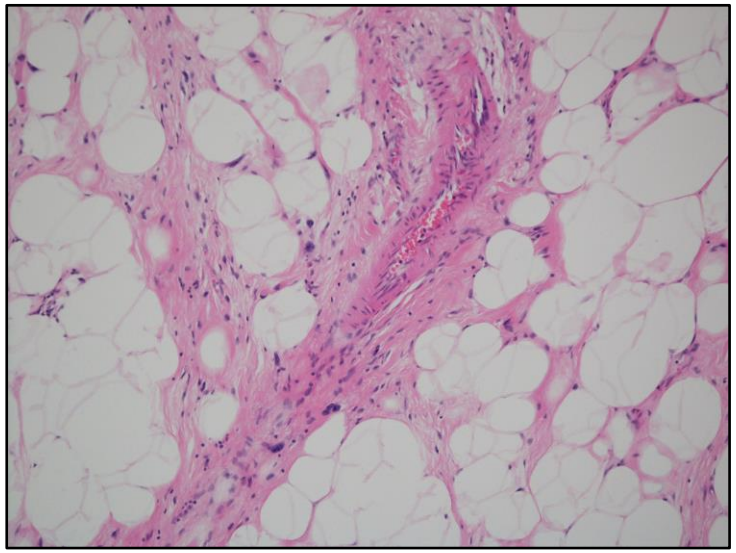


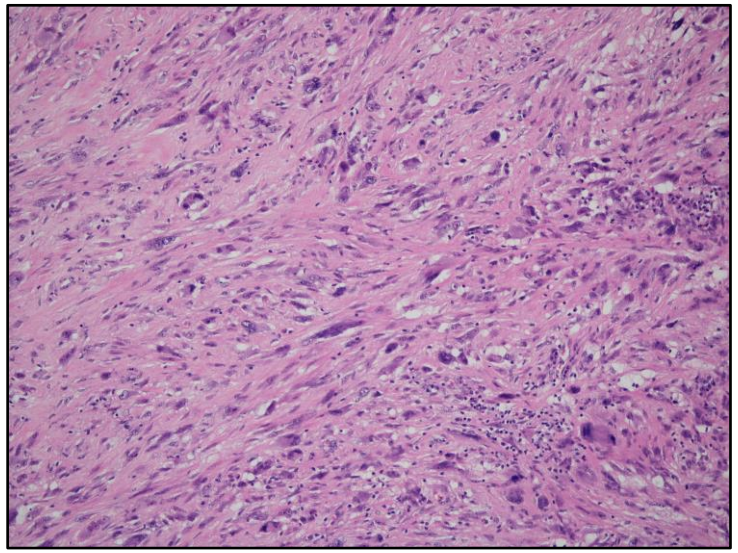
- Addition of centromeric probe for the chromosome of interest– enables one to count number of chromosomes present.
- Diploid,
- aneuploid,
- low level copy gain,
- high level amplification

Atypical lipomatous tumour
Well differentiated Liposarcoma
Dedifferentiated liposarcoma

Well-differentiated –
slow growing, does not metastasize, multiply recurrent, no response to chemotherapy

Dedifferentiated–
aggressive, can metastasize, limited and transient benefit to chemotherapy, median survival about 12 months





MDM2/CDK4 amplification – medical treatment options

- RG7112
- Oral – inhibitor of *MDM2*
- Good response to well – dedifferentiated liposarocma

- PD0332991
- SMI – Acts against *CDK4/6*
- Good progression free survival at 12 weeks.

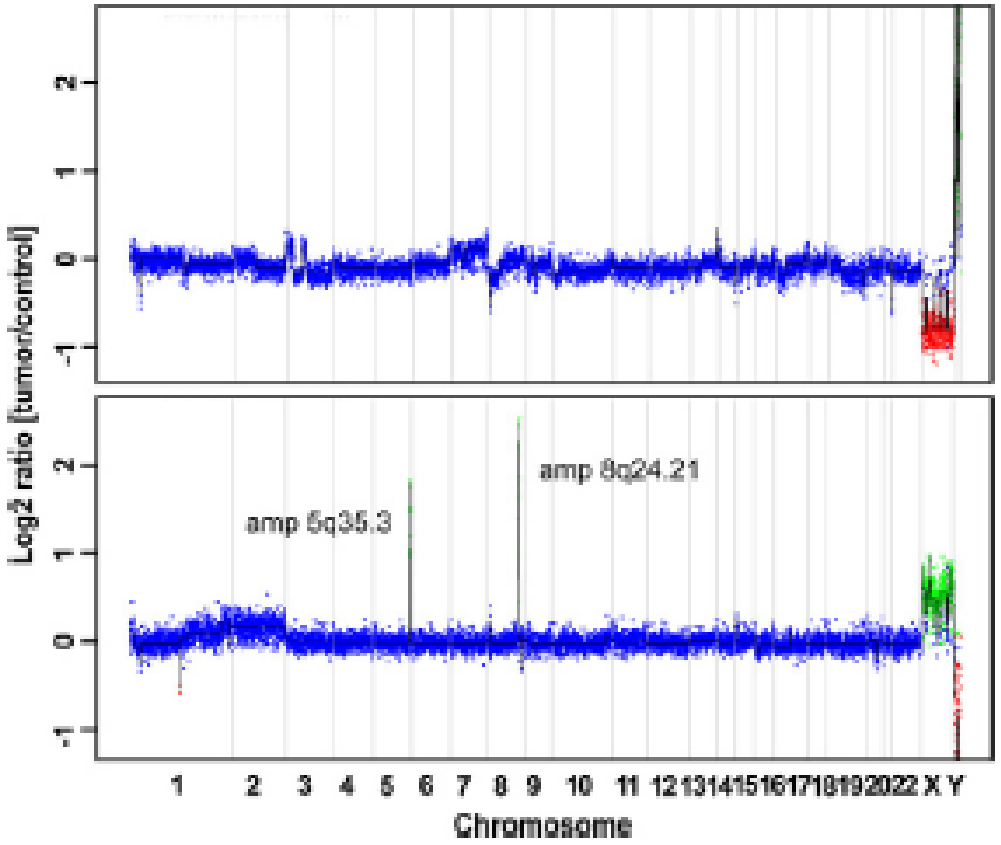
MYC – radiation induced angiosarcoma

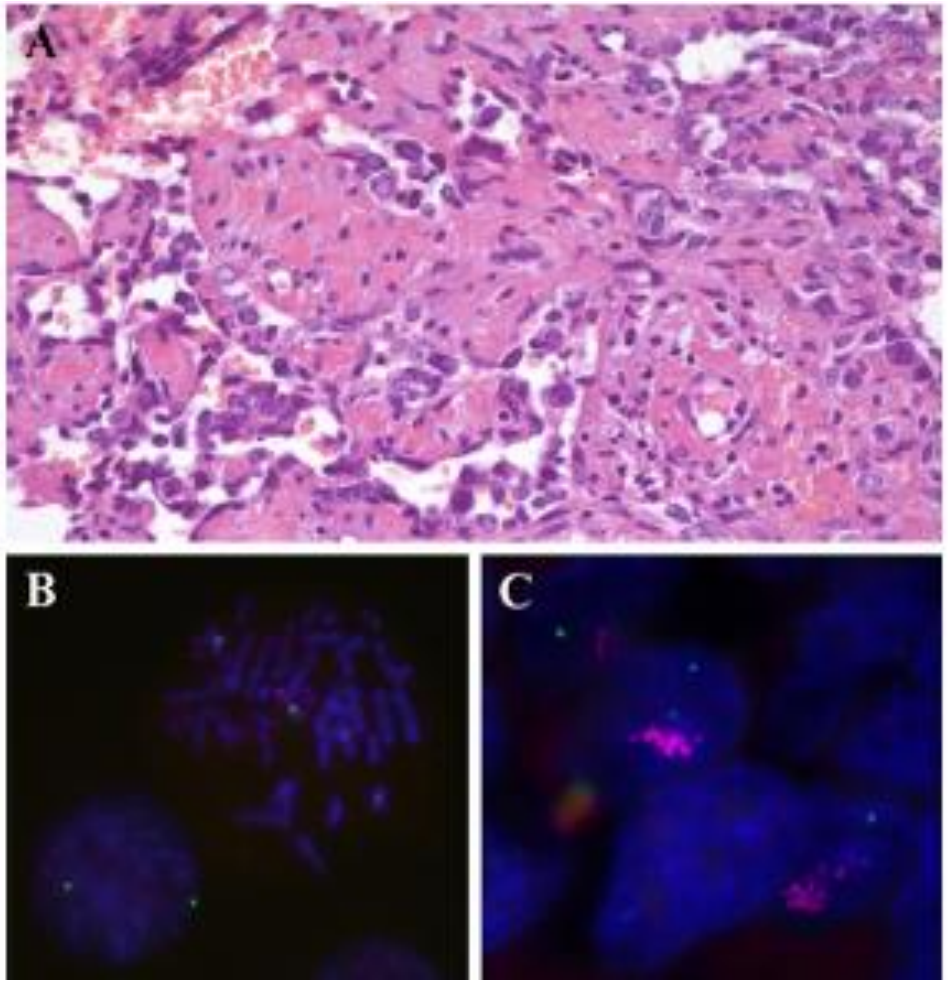
The American Journal of Pathology, Vol. 176, No. 1, January 2010

DOI: 10.2353/ajpath.2010.090637

Short Communication

MYC High Level Gene Amplification Is a Distinctive Feature of Angiosarcomas after Irradiation or Chronic Lymphedema


Log2 ratio [tumor/control]
amp 5q35.3
amp 8q24.21
Chromosome


A
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Cancer Medicine

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ORIGINAL RESEARCH

Fibroblastic growth factor receptor 1 amplification in osteosarcoma is associated with poor response to neo-adjuvant chemotherapy

M. Fernanda Amary[1,2], Hongtao Ye[1], Fitim Berisha[1], Bhavisha Khatri[1], Georgina Forbes[1], Katie Lehovsky[1], Anna M. Frezza[2,3], Sam Behjati[4], Patrick Tarpey[4], Nischalan Pillay[1,2], Peter J. Campbell[4], Roberto Tirabosco[1], Nadège Presneau[2], Sandra J. Strauss[2,3] & Adrienne M. Flanagan[1,2]

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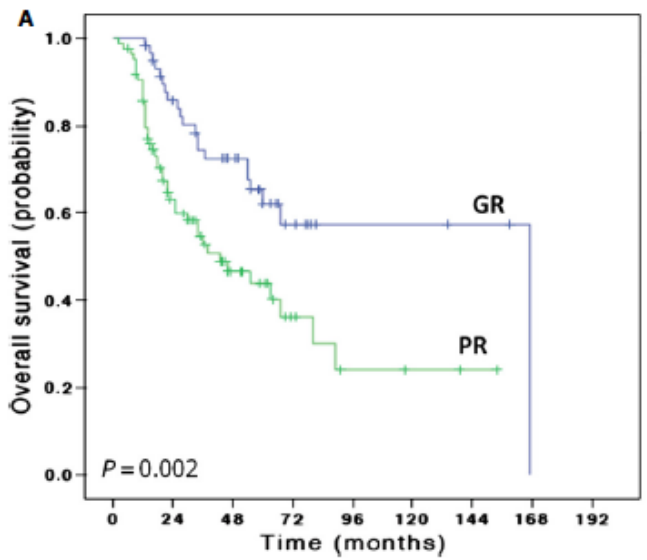
[2]UCL Cancer Institute, Huntley Street, London, WC1E 6BT, U.K.
[3]University College London Hospitals Foundation Trust, London Sarcoma Service, 250 Euston Road, London, NW1 2PG, U.K.
[4]Cancer Genome Project, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SA, U.K.



Molecular classification of mesenchymal neoplasms

Amplification	
Translocations – chimeric fusion genes	• Ewing's sarcoma – EWSR1-FLI1 • Synovial Sarcoma – SYT-SSX1
	• Undifferentiated sarcoma • Pleomorphic liposarcoma

Fusion gene detection – diagnostic assays

- RT-PCR
- RNA-seq
- FISH
- Immunohistochemistry – e.g. *NAB2—STAT6* fusion in Solitary Fibrous Tumour.

Break-apart FISH

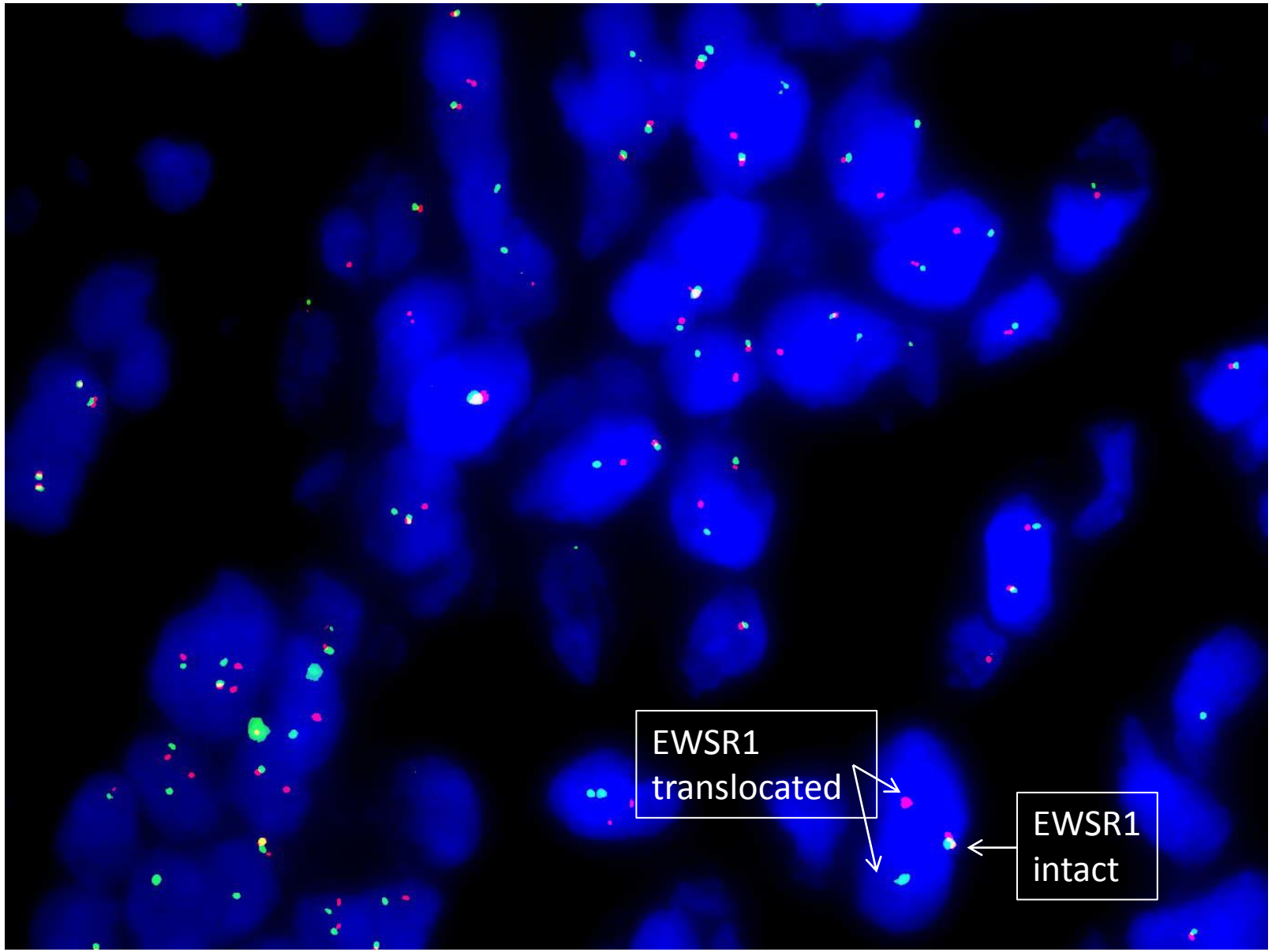
- Fusion detection: break-apart probe corresponding to the most commonly rearranged partner.

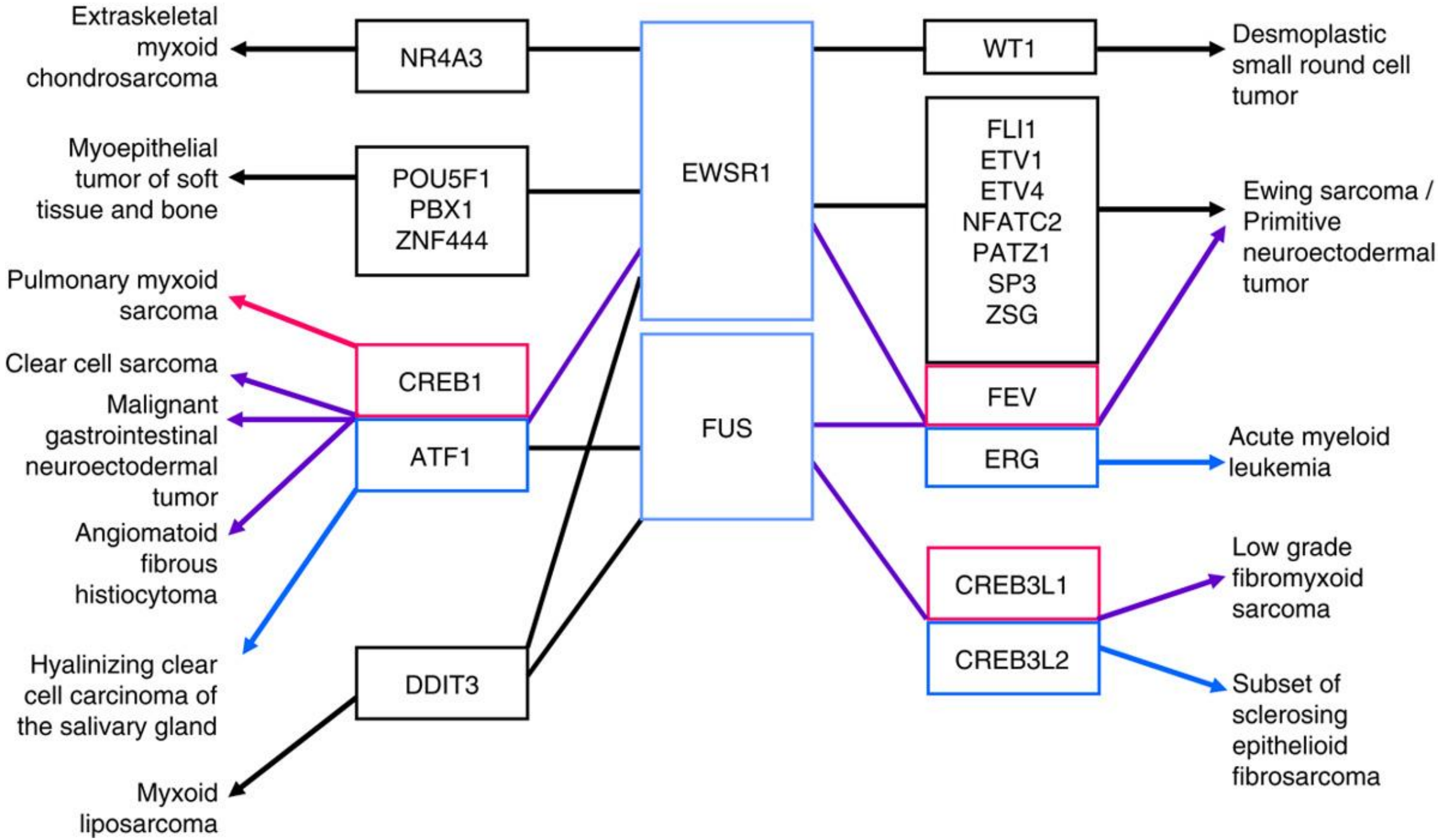
- E.g. FISH for Ewing sarcoma or myxoid liposarcoma:

- EWSR1 or FUS

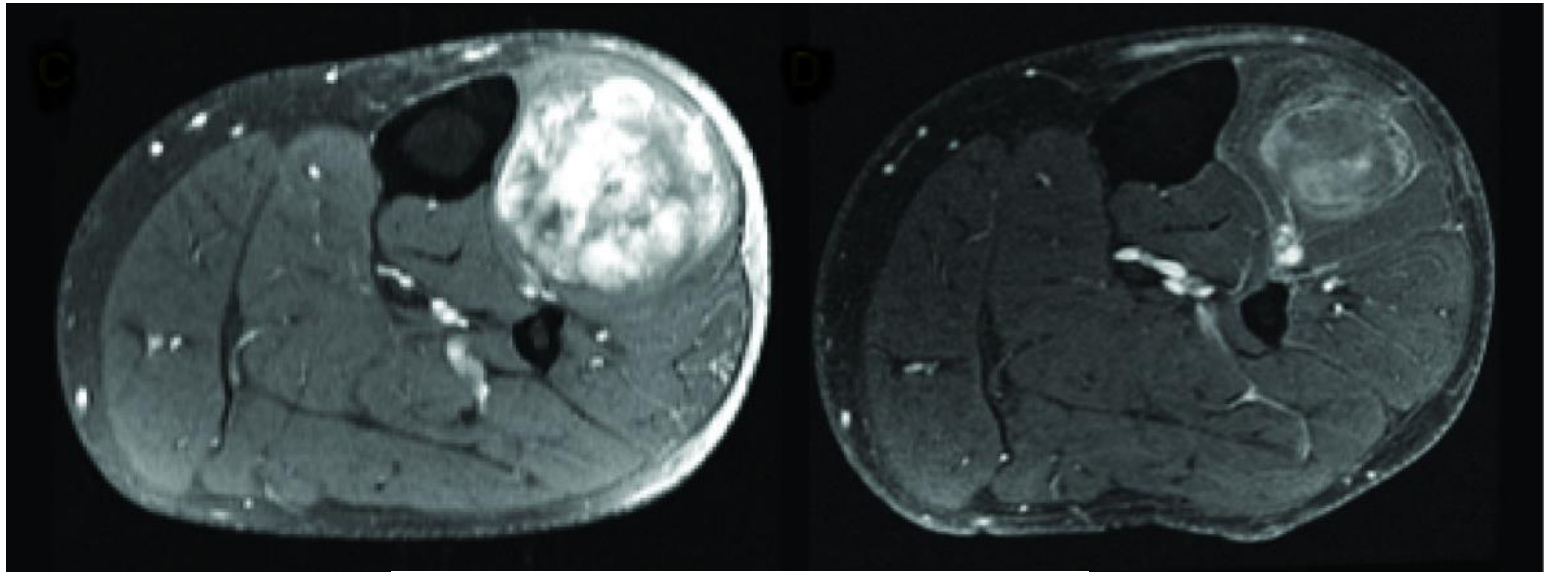
EWSR1 BREAK APART DESIGN – DUAL COLOUR

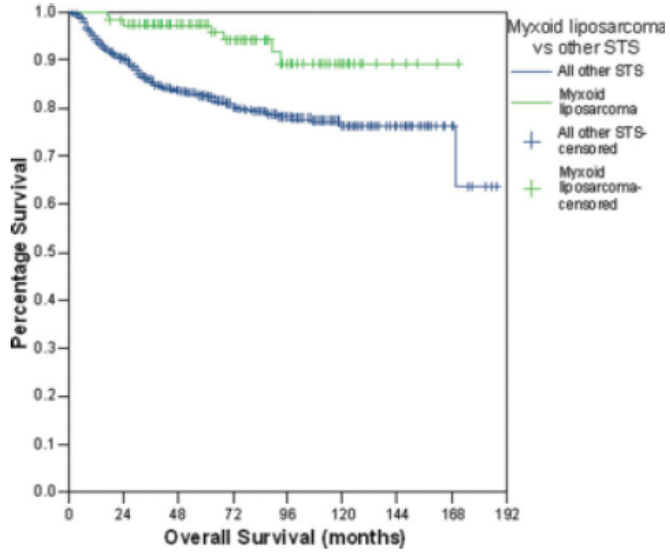
LSI EWSR1 Dual Color, Break Apart Rearrangement Probe


EWSR1
translocated
EWSR1
intact

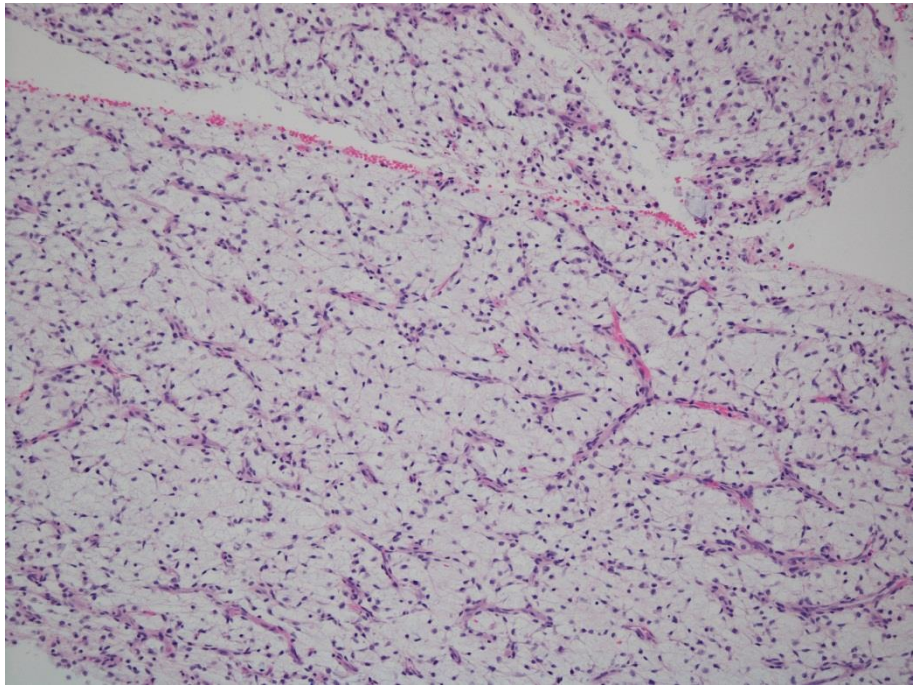

Extraskeletal myxoid chondrosarcoma
NR4A3
Myoepithelial tumor of soft tissue and bone
POU5F1
PBX1
ZNF444
Pulmonary myxoid sarcoma
Clear cell sarcoma
Malignant gastrointestinal neuroectodermal tumor
Angiomatoid fibrous histiocytoma
CREB1
ATF1
Hyalinizing clear cell carcinoma of the salivary gland
Myxoid liposarcoma
DDIT3
EWSR1
FUS
WT1
FLI1
ETV1
ETV4
NFATC2
PATZ1
SP3
ZSG
FEV
ERG
CREB3L1
CREB3L2
Desmoplastic small round cell tumor
Ewing sarcoma / Primitive neuroectodermal tumor
Acute myeloid leukemia
Low grade fibromyxoid sarcoma
Subset of sclerosing epithelioid fibrosarcoma

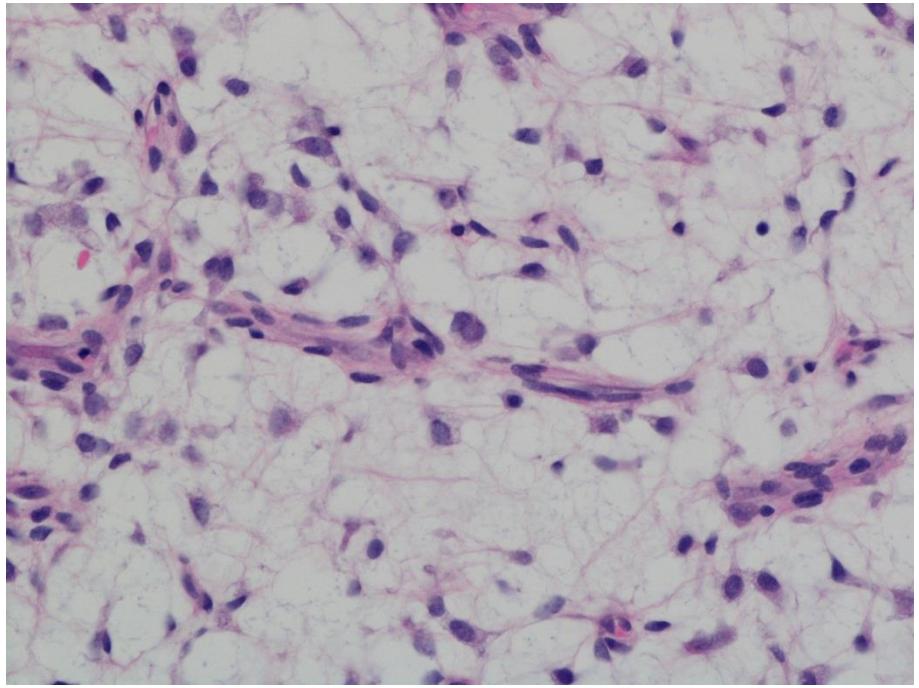
Myxoid liposarcoma





Myxoid/round cell liposarcoma





FISH – some cons

- Single gene assay
- Microscopic Interpretation can be complex
- Low throughput – one rearrangement at a time e.g. NSCLC (need to test for ALK, ROS1 and RET rearrangements.)

PLEXIFORM NEUROFIBROMA