Molecular Diagnostics in Sarcoma

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Sarcoma / soft tissue tumors

Benign soft tissue tumors are very common

Sarcomas are rare (~13K per year in the US, 0.8% of cancers; ~5K deaths)

Relatively frequent in pediatric and young adult patients

Morphological and clinical heterogeneity

→ Diagnostically challenging

Applications of molecular studies to sarcoma

1. Diagnose tumors
   Refine current morphologic classification
2. Predict outcome (prognosis)
3. Inform therapeutic decision (predictive markers)

→ Provide biological insights

Molecular genetic categories of sarcoma – two main categories

Simple genome

1. Balanced rearrangements
2. Single gene mutations

= Known oncogenic drivers
= Diagnostic molecular markers

Complex genome

1. Reproducible copy number alterations
2. Apparently random numerical and structural chromosomal alterations

= Unknown oncogenic drivers
= Less/no specific molecular markers

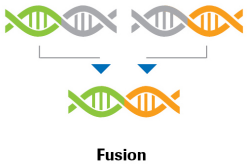
Molecular genetic categories of sarcoma – clinicopathological correlates

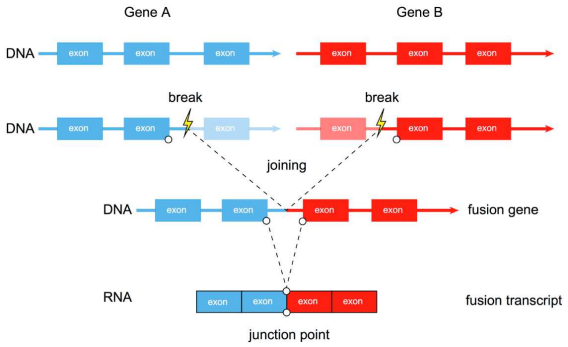
Simple genome	Complex genome
• Monomorphic morphology • Wide range of clinical behavior	• Pleomorphic, high-grade
• Effective targeted therapies against most deregulated kinases • Low mutational rate	• No effective targeted therapies; subsets respond to conventional chemotherapy and radiation • Chromosomal instability /Higher mutational load - Immunotherapy?

Practical molecular classification of sarcoma

- Sarcomas with simple chromosomal rearrangements
- Sarcomas driven by oncogenic SNVs (or small indels)
- Sarcomas with recurrent CNAs
- Sarcomas with complex karyotype

Simple chromosomal rearrangements
in sarcoma





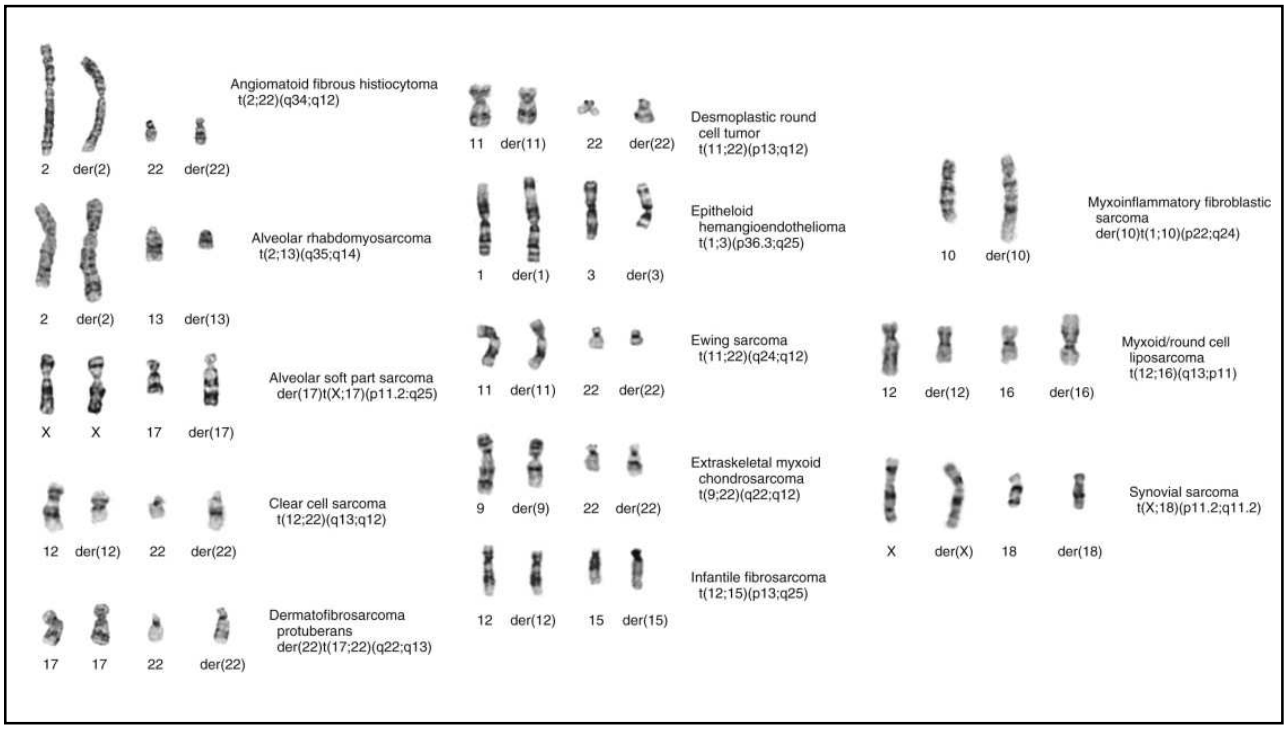
The emerging complexity of gene fusions in cancer. Mertens F, Johansson B, Fioretos T, Mitelman F. 2015. *Nature Reviews Cancer* 15: 371-381

Simple chromosomal rearrangements
in sarcoma

- Strong oncogenic driver mutations
- Initiating / early oncogenic event = present in every tumor cell
- Insights into mechanisms of tumorigenesis

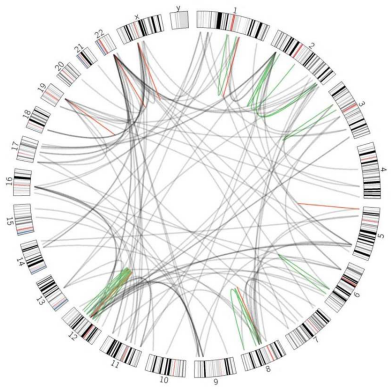
- Highly selective genetic events, restricted to cancer cells
  = ideal **diagnostic** biomarkers

- Some can be inhibited with selective small molecule drugs
  = optimal **targets** for precision therapeutics



SOFT TISSUE TUMOR	TRANSLOCATION	GENE FUSION
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	*PAX3-FOXO1*
	t(1;13)(p36;q14)	*PAX7-FOXO1*
	Other t with 2q35	*PAX3*
Alveolar soft part sarcoma	t(X;17)(p11.2;q25)	*ASPSCR1-TFE3*
Angiofibroma	t(5;8)(p15;q13)	*AHRR-NCOA2*
Angiomatoid fibrous histiocytoma	t(2;22)(q34;q12)	*EWSR1-CREB1*
	t(12;22)(q13;q12)	*EWSR1-ATF1*
	t(12;16)(q13;p11)	*FUS-ATF1*
Clear cell sarcoma	t(12;22)(q13;q12)	*EWSR1-ATF1*
	t(2;22)(q34;q12)	*EWSR1-CREB1*
Dermatofibrosarcoma protuberans/giant cell fibroblastoma	+ring/marker chromosome from t(17;22)(q22;q13)	*COL1A1-PDGFB*
Desmoplastic fibroblastoma	t(2;11)(q31;q12)	Unknown
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	*EWSR1-WT1*
Epithelioid sarcoma-like hemangioendothelioma	t(7;9)(q22;q13)	Unknown
Epithelioid hemangioendothelioma	t(1;3)(p36.3;q25)	*WWTR1–CAMTA1*
	t(X;11)(p11.2;q22.1)	*TFE3-YAP1*
Ewing sarcoma	t(11;22)(q24;q12)	*EWSR1-FLI1*
	t(21;22)(q22;q12)	*EWSR1-ERG*
	Other t with 22q12	*EWSR1* fusion with various ETS partners: *ETV1* (7p22), *FEV* (2q36), *ETV4* (17q21)
	t(16;21)(p11;q22)	*FUS-ERG*
Ewing sarcoma-like	t(20;22)(q13;q12)	*EWSR1-NFATC2*
	t(4;19)(q35;q13) t(10;19)(q26.3;q13)	*CIC-DUX4* *CIC-DUX4*
	inv(X)(p11.4p11.22)	*BCOR-CCNB3*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	*EWSR1-NR4A3*
	t(9;17)(q22;q11)	*TAF15-NR4A3*
	t(9;15)(q22;q21)	*TCF12-NR4A3*
	t(3;9)(q12;q22)	*TFG-NR4A3*
Infantile fibrosarcoma	t(12;15)(p13;q25)	*ETV6-NTRK3*
Inflammatory myofibroblastic tumor	t with 2p23	*ALK* fusion with: *TPM4* (19p13.1), *TPM3* (1q21), *CLTC* (17q23), *RANBP2* (2q13), *ATIC* (2q35), *SEC31A* (4q21), *CARS* (11p15)
Lipoblastoma	t with 8q12	*PLAG1* fusions
Lipoma, ordinary	t with 12q14.3	*HMGA2* fusions
	t with 6p21	*HMGA1 fusions*
Lipoma, chondroid	t(11;16)(q13;p13)	*C11orf95-MKL2*
Low-grade fibromyxoid sarcoma	t(7;16)(q33;p11)	*FUS-CREB3L2*
	t(11;16)(p11;p11)	*FUS-CREB3L1*
Mesenchymal chondrosarcoma	del(8)(q13.3q21.1)	*HEY1-NCOA2*
Myoepithelioma, soft tissue	t(19;22)(q13;q12)	*EWSR1–ZNF444*
	t(1;22)(q23;q12)	*EWSR1–PBX1*
	t(6;22)(p21;q12)	*EWSR1–POU5F1*
	t with 16p11	*FUS*
Myxoid/round cell liposarcoma	t(12;16)(q13;p11)	*FUS-DDIT3*
	t(12;22)(q13;q12)	*EWSR1-DDIT3*
Myxoinflammatory fibroblastic sarcoma/hemosiderotic fibrolipomatous tumor	der(10)t(1;10)(p22;q24)	*TGFBR3–MGEA5*
Nodular fasciitis	t(17;22)(p13;q13.1)	*MYH9-USP6*
Ossifying fibromyxoid tumor	t with 6p21	*PHF1* fusions
Pericytoma	t(7;12)(7p22;q13)	*ACTB-GLI1*
Sclerosing epithelioid fibrosarcoma	t(7;16)(q33;p11.2)	*FUS-CREB3L2*
	t(11;16)(p13;p11.2)	*FUS-CREB3L1*
Solitary fibrous tumor	inv(12)(q13q13)	*NAB2-STAT6*
Spindle cell rhabdomyosarcoma	t with 8q13	*NCOA2* fusion with: *SRF*(6p21),*TEAD1*(11p15)
Synovial sarcoma	t(X;18)(p11.2;q11.2)	*SS18-SSX1*
		SS18-SSX2
		SS18-SSX4
Tenosynovial giant cell tumor	t(1;2)(p13;q37)	*CSF1–COL6A3*
	other t with 1p13	*CSF1*

Simple chromosomal rearrangements in sarcoma



2015:

All cancers: 11,124 unique fusions

Sarcoma: 142 unique fusions, **78 recurrent**

Gene fusions in soft tissue tumors: Recurrent and overlapping pathogenetic themes. Mertens F, Antonescu CR, Mitelman F. 2016. *Genes Chromosome Cancer* 55:291-310

Simple chromosomal rearrangements in sarcoma

158 recurrent gene fusions in soft tissue tumors

ACTB-GLI1	pericytoma
AHRR-NCOA2	Angiofibroma of soft tissue
ALDH2-HMGA2	mesenchymal tumor
ASPSCR1-TFE3	alveolar soft part sarcoma
ATIC-ALK	inflammatory myofibroblastic tumour
BCOR-CCNB3	Ewing-like sarcomas
BCOR-MAML3	Small Blue Round Cell Sarcomas
BRD8-PHF1	endometrial stromal sarcoma(ESS)
C11orf95-RELA	ependymoma, sarcoma, neoplasm, osteosarcoma
CARS-ALK	inflammatory myofibroblastic tumour
CEBPZ-FUS	liposarcoma
CIC-DUX4	sarcoma, trisomy 8
CIC-LEUTX	Angiosarcomas
CLTC-ALK	inflammatory myofibroblastic tumour
COG5-HMGA2	uterine leiomyoma
COL1A1-PDGFB	dermatofibrosarcoma
COL1A2-PLAG1	lipoblastoma
COL6A3-CSF1	localized giant cell tumor of tendon sheath
CREB3L2-FUS	fibrosarcoma
CREBBP-BCORL1	Ossifying fibromyxoid tumor (OFMT)
CSF1-S100A10	three tenosynovial giant cell tumors (TSGCT)
DCTN1-ALK	inflammatory myofibroblastic tumour
DDIT3-FUS	liposarcoma
DVL2-TFE3	Perivascular epithelioid cell neoplasm (PEComa)
EBF1-LOC204010	lipoma
EML4-NTRK3	fibrosarcoma, nephroma, congenital mesoblastic nephroma
EPC1-PHC2	hemangioendothelioma
EPC1-PHF1	endometrial stromal sarcoma
ETV6-NTRK3	congenital fibrosarcoma
EWS-FLI1	Ewing's sarcoma family of tumors (ESFT)
EWSR1-ATF1	clear cell sarcoma
EWSR1-CHN1	sarcoma of cartilage derivation
EWSR1-CREB1	clear cell sarcoma,Angiomatoid fibrous histiocytoma
EWSR1-CREB3L1	sclerosing epithelioid fibrosarcoma
EWSR1-CREB3L3	sclerosing epithelioid fibrosarcoma
EWSR1-CREM	mesenchymal tumor, Angiomatoid fibrous histiocytoma
EWSR1-DDIT3	liposarcoma
EWSR1-DUX4	sarcomas
EWSR1-ERG	ewing sarcoma
EWSR1-ETV1	ewing sarcoma
EWSR1-ETV4	ewing sarcoma
EWSR1-FEV	ewing sarcoma
EWSR1-FLI1	biphenotypic sarcoma
EWSR1-NFATC2	ewing sarcoma
EWSR1-NR4A3	chondrosarcoma
EWSR1-OSCP1	extraskeletal myxoid chondrosarcoma
EWSR1-PBX1	myoepithelioma
EWSR1-POU5F1	sarcoma, myoepithelioma
EWSR1-SMARCA5	ewing sarcoma
EWSR1-WT1	leiomyosarcoma
FIP1L1-PDGFRB	eosinophilia, reticulolymphosarcoma, cerebellar syndrome
FN1-ACVR2A	chondrosarcoma, osteochondromatoses (osteochondromatosis)
FN1-ALK	endometrial stromal sarcoma
FN1-EGF	neoplasm
FN1-FGF1	phosphaturic mesenchymal tumor
FN1-FGFR1	phosphaturic mesenchymal tumor
FOXO1-FGFR1	alveolar rhabdomyosarcoma
FOXO1-PAX3	rhabdomyosarcoma
FOXO1-PAX7	alveolar rhabdomyosarcoma
FUS-ATF1	angiomatoid fibrous histiocytoma
FUS-CEBPZ	malignantliposarcoma
FUS-CREB3L1	fibrosarcoma
FUS-CREB3L2	fibrosarcoma
FUS-CREB3L2	low grade fibromyxoid sarcoma
FUS-CREB3L2	hyalinizing spindle cell tumor
FUS-DDIT3	liposarcoma
FUS-ERG	ewing sarcoma
FUS-FEV	ewing sarcoma
FUS-KLF17	Soft tissue neoplasm
GREB1-NCOA2	sarcoma, neoplasm
HEY1-NCOA2	chondrosarcoma
HMGA2-ACKR3	FML(lipoma)
HMGA2-ALDH2	mesenchymal tumor
HMGA2-C9orf92	lipomas
HMGA2-CCNB1IP1	leiomyoma
HMGA2-COX6C	leiomyoma
HMGA2-DYRK2	FML(lipoma)
HMGA2-DYRK2	FML(lipoma)
HMGA2-EBF1	lipoma
HMGA2-EFCAB6	lipoma
HMGA2-FHIT	angiomyxoma
HMGA2-LHFPL6	lipoma, pulmonary chondroid hamartomas
HMGA2-LPP	lipoma
HMGA2-NFIB	lipoma
HMGA2-PLPP3	lipomas
HMGA2-RAD51B	leiomyoma
HMGA2-SETBP1	neoplasm, FML(lipoma)
IGFBP5-ALK	leiomyosarcoma, tumors, leiomyoma, smooth muscle tumor
IGH-FOXP1	reticulolymphosarcoma, lymphoma
IRF2BP2-CDX1	chondrosarcoma
JAZF1-PHF1	endometrial stromal sarcoma
JAZF1-SUZ12	endometrial stromal sarcoma
KCNMB4-CCND3	osteosarcoma
KDM2A-WWTR1	Ossifying fibromyxoid tumor (OFMT)
KIF13B-NRG1	epithelioid sarcoma
KIRREL1-PRKCA	fibrous histiocytomas (FH)
KMT2B-GPS2	undifferentiated spindle cell sarcoma (UDS)
LAMTOR1-PRKCD	aneurysmal benign fibrous histiocytoma
LRP1-SNRNP25	osteosarcoma
MBTD1-EZHIP	endometrial stromal sarcoma
MEAF6-PHF1	endometrial stromal sarcoma, OFMT
MED12-PRDM10	epithelioid sarcoma
MYH9-USP6	nodular fasciitis (NF)
NAB2-STAT6	solitary fibrous tumour
NOTCH2-MIR143	myopericytoma, hemangiopericytoma, GT, myofibroma
NUMA1-SFMBT1	aneurysmal benign fibrous histiocytoma
NUP160-SLC43A3	angiosarcoma
NUTM2A-CIC	neoplasm, HMR(Ewing-like sarcomas)
NUTM2B-YWHAE	endometrial stromal sarcoma(ESS), neoplasm
OSCP1-EWSR1	extraskeletal myxoid chondrosarcoma
PAX3-FOXO1	rhabdomyosarcoma
PAX3-MAML3	alveolar rhabdomyosarcoma, tumor
PAX3-NCOA1	rhabdomyosarcoma
PAX3-NCOA2	rhabdomyosarcoma
PAX7-FOXO1	rhabdomyosarcoma
PDGFB-COL1A1	dermatofibrosarcoma
PLAG1-HAS2	FML(lipoma), lipoblastomatoses(lipoblastoma)
PLAG1-HAS2	FML(lipoma), lipoblastomatoses(lipoblastoma)
PLAG1-RAD51B	lipoblastoma
PPFIBP1-ALK	inflammatory myofibroblastic tumour
PTBP1-MAML2	hemangioendothelioma
RANBP2-ALK	inflammatory myofibroblastic tumour
RBBP1-ALK	epithelioid sarcoma
SEC31A-ALK	inflammatory myofibroblastic tumour
SERPINE1-FOSB	penile mesenchymal tumor
SET32-NF1	pediatric spindle cell tumor
SFPQ-TFE3	perivascular epithelioid cell tumour
SS18L1-SSX1	synovial sarcoma
SS18L1-SSX2	synovial sarcoma
SS18L1-SSX4	synovial sarcoma
SS18-SSX1	epithelioid sarcoma
SS18-SSX2	synovial sarcoma
SS18-SSX4	synovial sarcoma
TAF15-CHN1	extraskeletal myxoid chondrosarcomas
TAF15-NR4A3	chondrosarcoma
TAF15-OSCP1	extraskeletal myxoid chondrosarcoma
TCF12-CHN	myxoid chondrosarcoma
TCF12-NR4A3	chondrosarcoma
TCF12-OSCP1	extraskeletal myxoid chondrosarcoma
TFG-MET	infantile spindle cell sarcoma
TFG-NR4A3	chondrosarcoma
TFG-ROS1	inflammatory myofibroblastic tumor(IMT), anaplastic lymphoma
TFG-TEC	myxoid chondrosarcomas
THBS1-ALK	myxoid leiomyosarcoma, neoplasm
TIMP3-ALK	myxoid leiomyosarcoma, neoplasm
TMED6-COG8	sarcoma
TPM3-ALK	inflammatory myofibroblastic tumour
TPM4-ALK	inflammatory myofibroblastic tumour
TPR-MET	osteosarcoma
TRPS1-PLAG1	myxoid leiomyosarcoma
USP15-CNTN1	spindle cell sarcoma
WWTR1-CAMTA1	vascular neoplasm
WWTR1-FOSB	penis neoplasm, cerebellar syndrome
YAP1-TFE3	Ewing sarcomas
YWHAE-NUTM2A	endometrial stromal sarcoma
YWHAE-NUTM2B	endometrial stromal sarcoma
ZC3H7A-BCOR	Ewing sarcoma
ZC3H7B-BCOR	endometrial stromal sarcoma
ZFP36-FOSB	vascular neoplasm, cerebellar syndrome

ChimerDB 4.0

Sarcomas with simple chromosomal rearrangements

- Chimeric transcription factors → transcriptional deregulation
  - *EWSR1-FLI1* in Ewing sarcoma
- Aberrant kinase signaling
  - *ETV6-NTRK3* in Infantile fibrosarcoma
- Mutational epigenetic deregulation
  - *SS18-SSXn* in Synovial sarcoma

Sarcomas with simple chromosomal rearrangements

Diagnostic considerations

Disease-defining: fusion gene = tumor type (*SS18-SSX1* = SS, *NAB2-STAT6* = SFT)

Sarcomas with simple chromosomal rearrangements

Diagnostic considerations

Disease-defining: fusion gene = tumor type (*SS18-SSX1* = SS, *NAB2-STAT6* = SFT)

Genetic heterogeneity (one tumor type, multiple gene fusions)

EWSR1-DDIT3 / *FUS-DDIT3* in MLPS; *PAX3-FOXO1* / *PAX7-FOXO1* in aRMS; *TPM3-ALK* / *TFG-ROS1* in IMT)

Genetic pleiotropy (one gene fusion, multiple tumors)

EWSR1-CREB1 in angiomatoid fibrous histiocytoma, clear cell sarcoma, mesothelioma, (primary pulm m sarc)

Sarcomas with simple chromosomal rearrangements

Diagnostic considerations

→ identification of a fusion gene in a soft tissue tumor often has diagnostic value

Molecular characterization of genomic rearrangements

Low-throughput

~~Karyotype~~

FISH

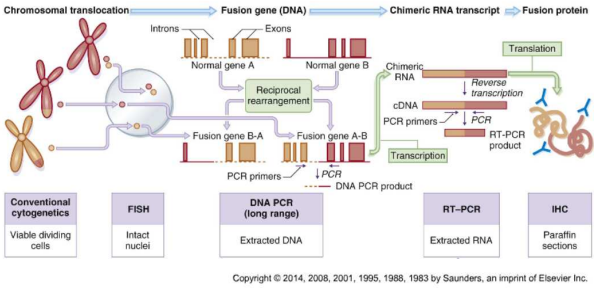
~~RT-PCR~~

(IHC surrogates)

High-throughput (multiplex)

Sequencing-based (DNA/RNA)

Hybridization-based



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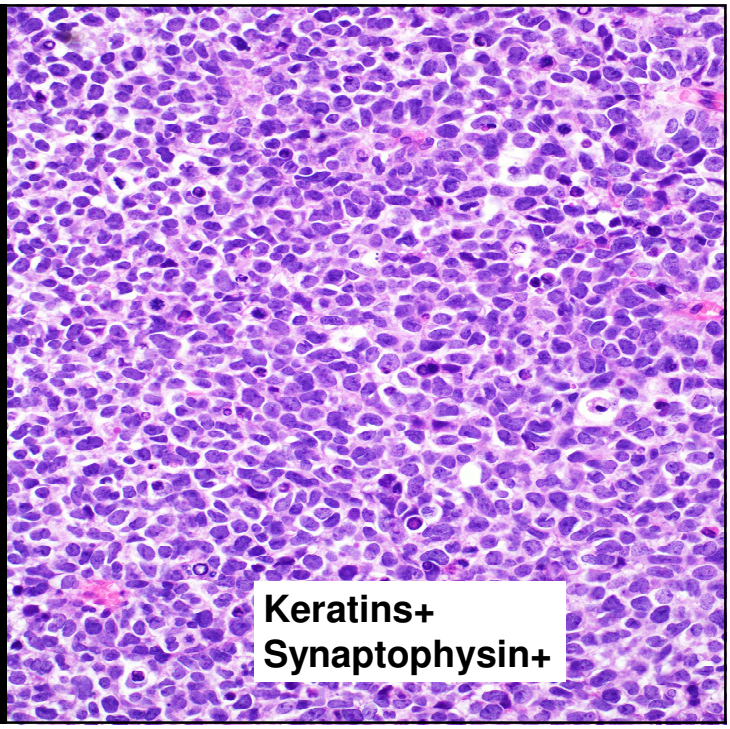
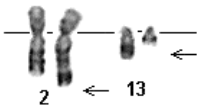
36 y/o M of Kenyan origin
Presents with brisk epistaxis

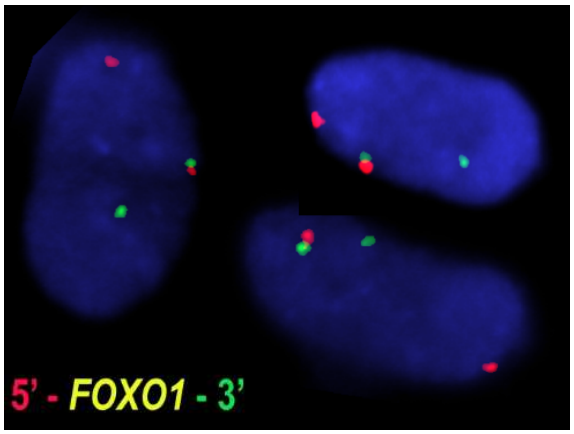
Mass on nasal endoscopy



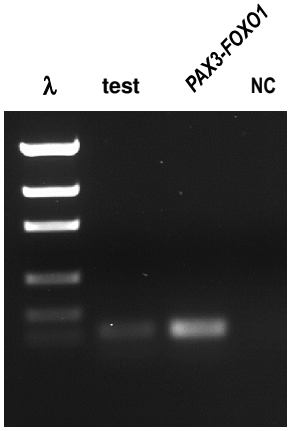
Karyotype = FISH = RT-PCR



46,XY,t(2;13)(q35;q14)[15]



.nuc ish(FOXO1x2)(3'FOXO1sep 5'FOXO1x1)[26/50]



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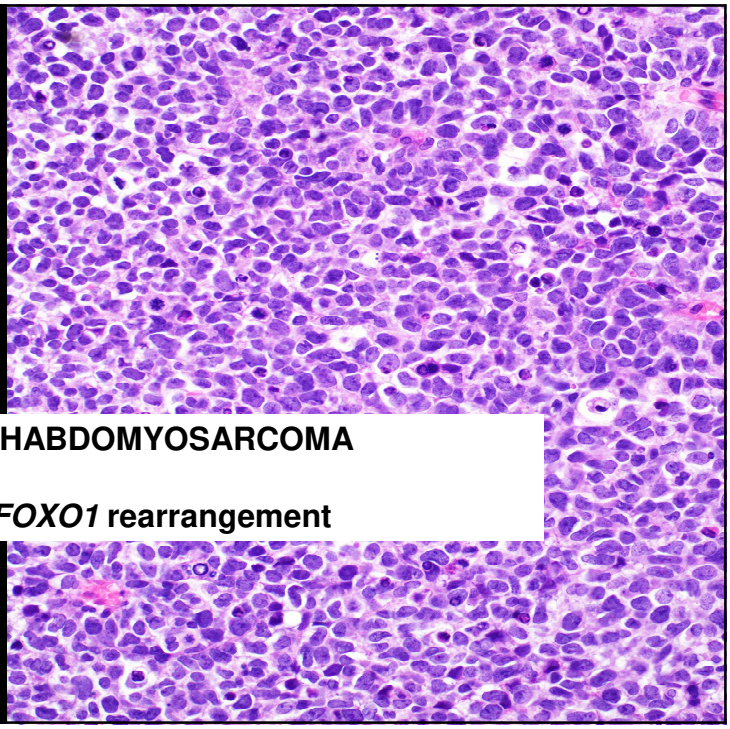
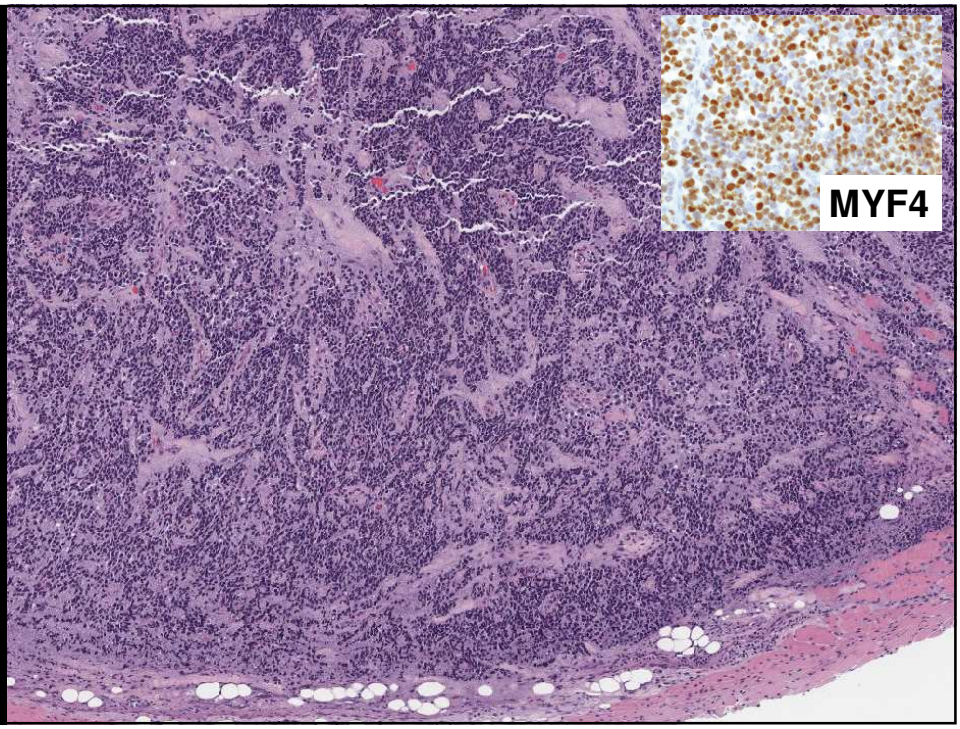


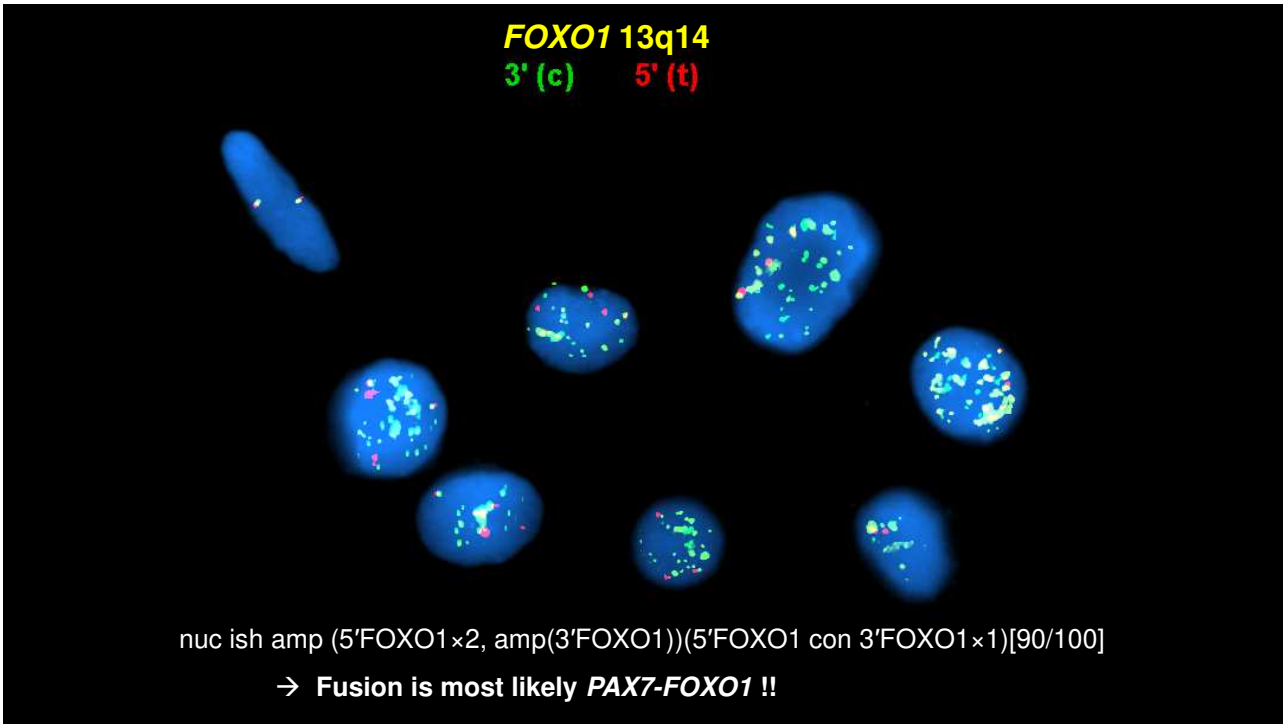
ALVEOLAR RHABDOMYOSARCOMA

with *PAX3-FOXO1* rearrangement

Female 17

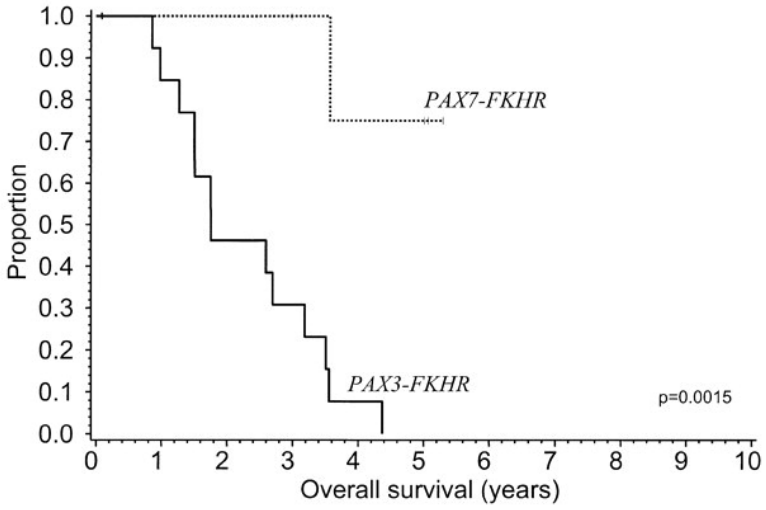
Cervical lymph node biopsy





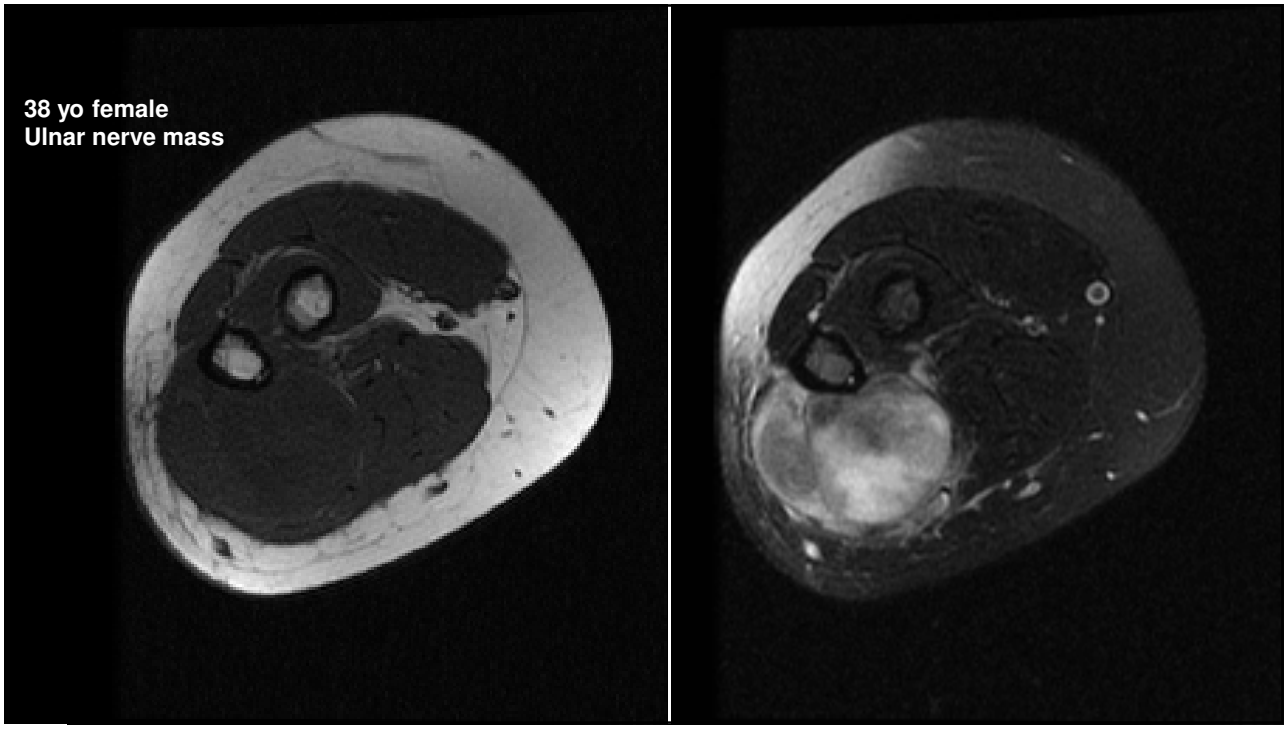
nuc ish amp (5′FOXO1×2, amp(3′FOXO1))(5′FOXO1 con 3′FOXO1×1)[90/100]

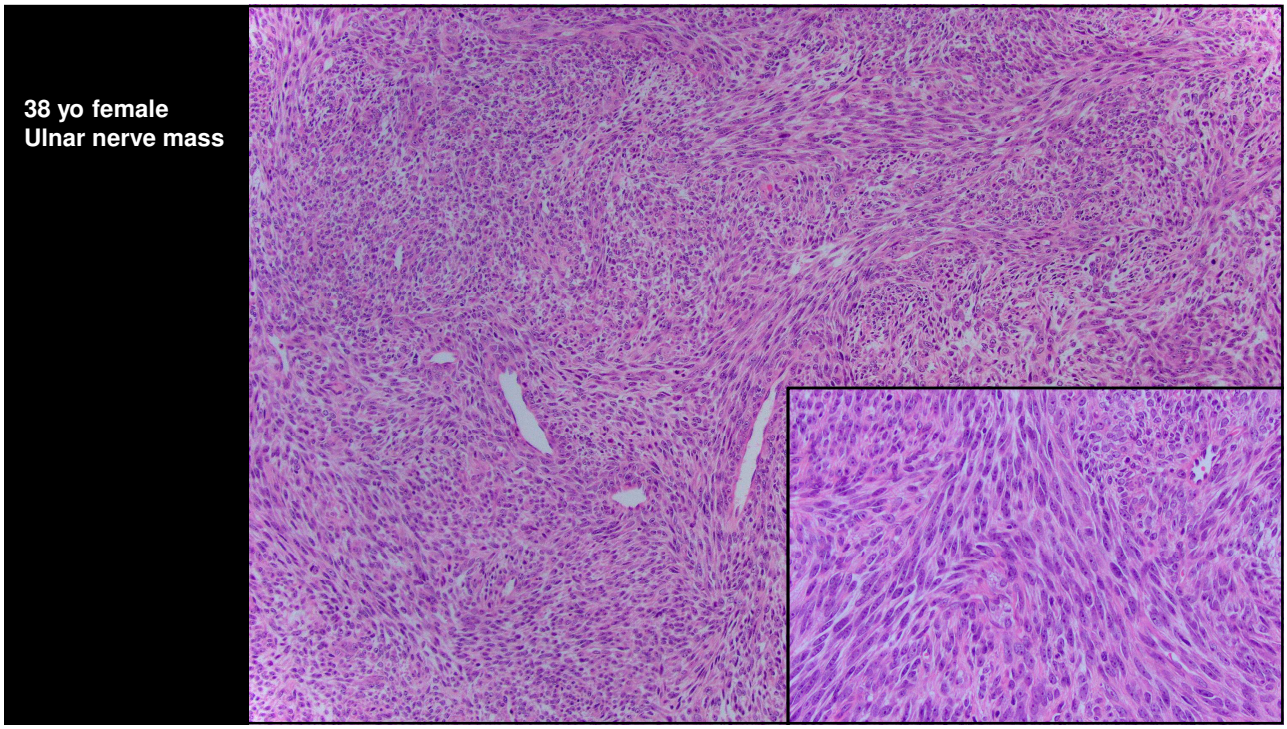
→ **Fusion is most likely *PAX7-FOXO1* !!**

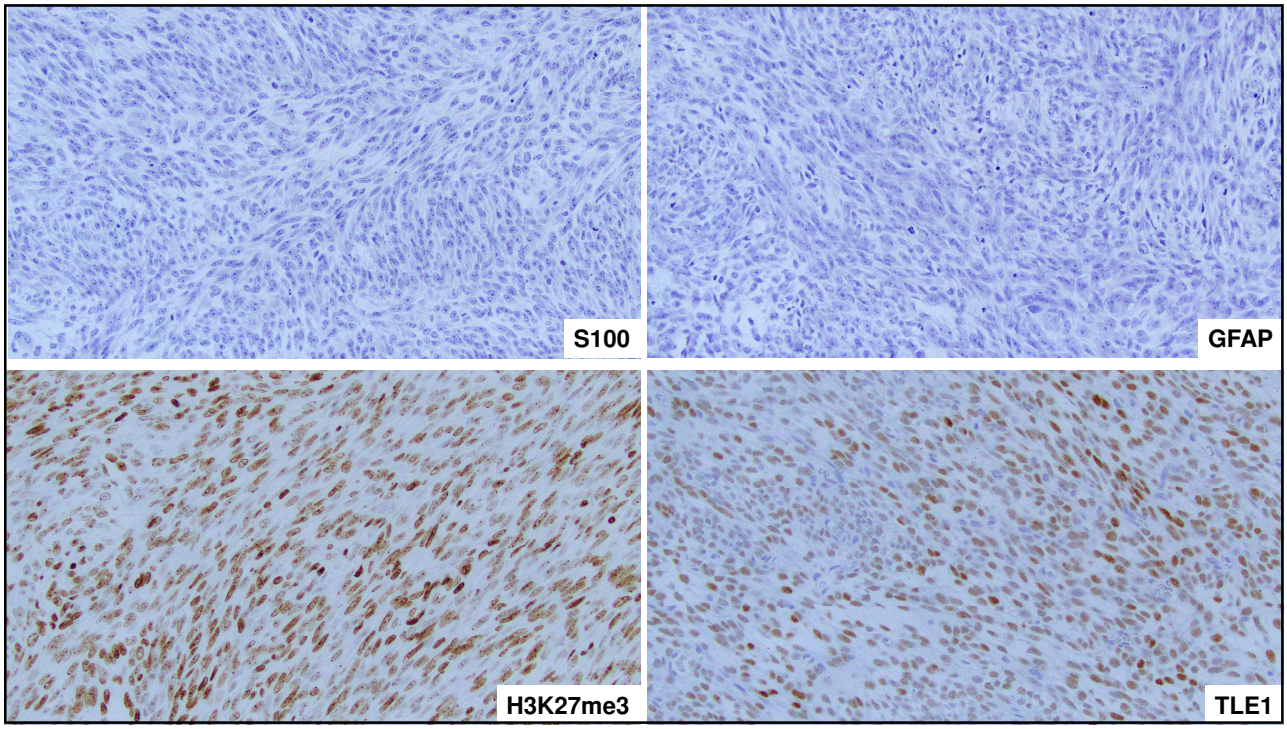


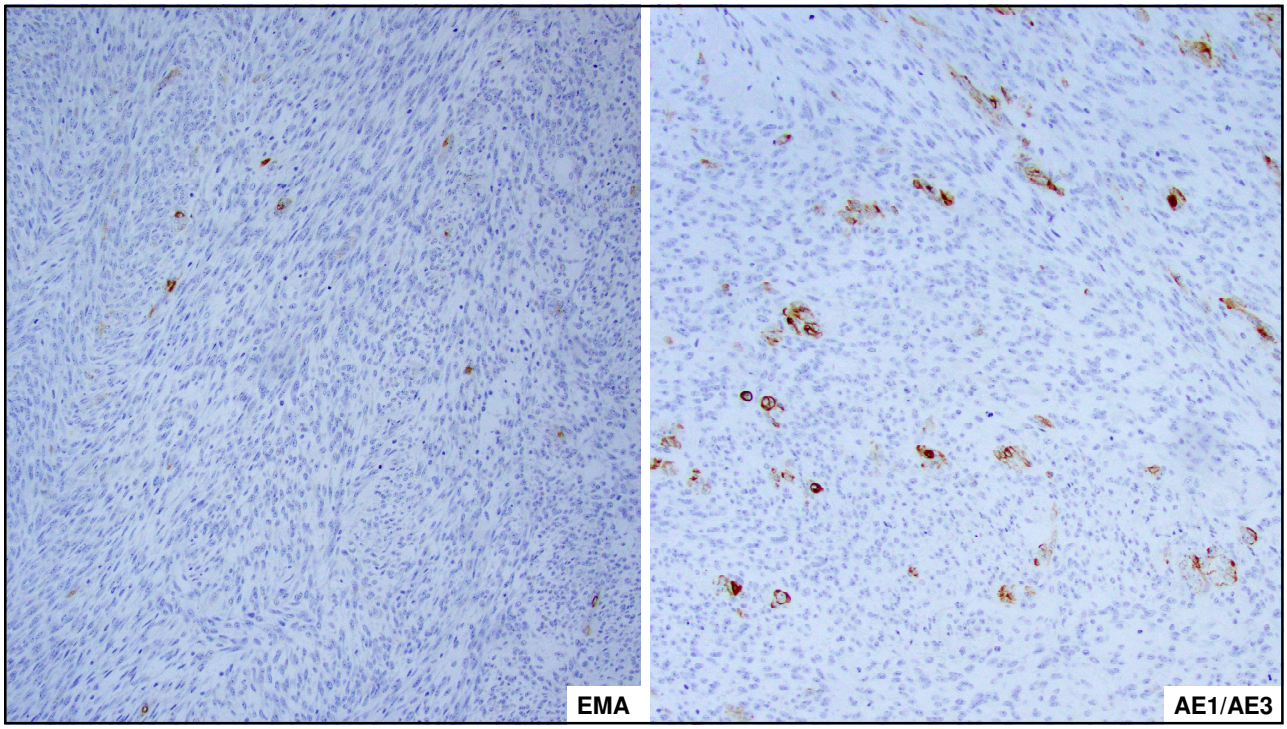
→ In ARMS, specific fusion provides prognostic information

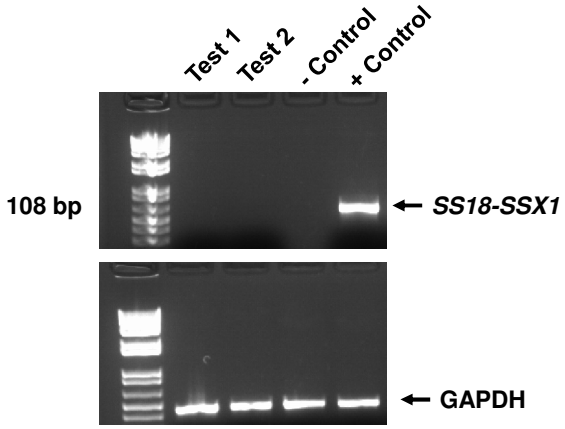
Poul H.B. Sorensen et al. DOI: 10.1200/JCO.2002.03.137
Journal of Clinical Oncology 20, no. 11 (June 2002) 2672-2679.

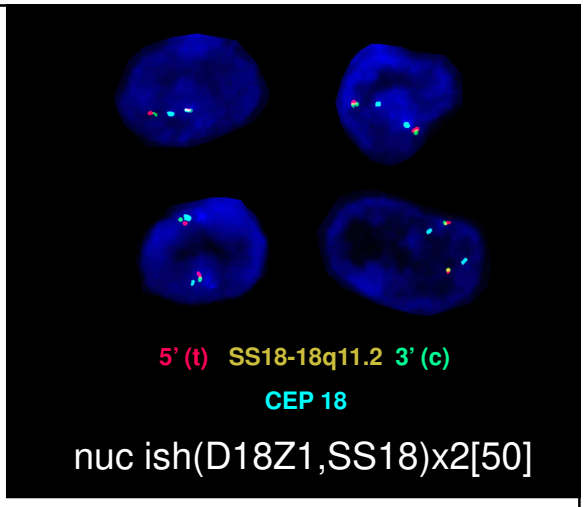



38 yo female
Ulnar nerve mass


S100
GFAP
H3K27me3
TLE1







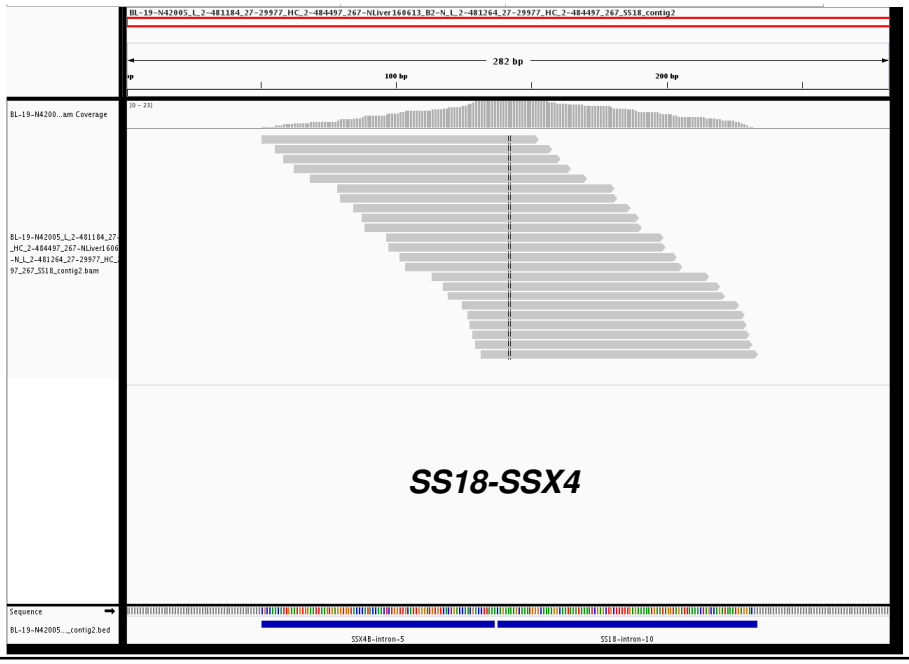
SS18-SSX1 **and** ***SS18-SSX2*** **transcripts were not identified**

No evidence of ***SS18*** **rearrangement by FISH**

HIGH-GRADE SPINDLE CELL SARCOMA **MOST CONSISTENT WITH**

MALIGNANT PERIPHERAL NERVE SHEATH TUMOR

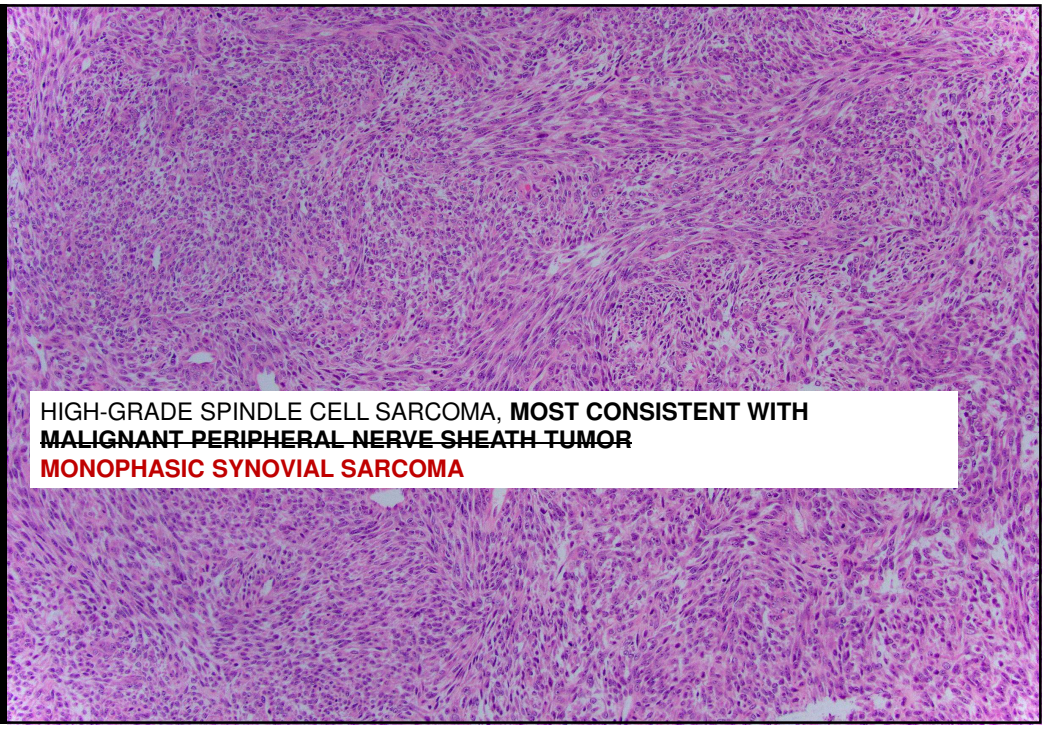
Cancer gene panel (Oncopanel)



38 yo female
Ulnar nerve mass

EMA+
AE1/AE3 focal
H3K27+

S100 -
SOX10 -



HIGH-GRADE SPINDLE CELL SARCOMA, **MOST CONSISTENT WITH**
~~**MALIGNANT PERIPHERAL NERVE SHEATH TUMOR**~~
MONOPHASIC SYNOVIAL SARCOMA

Molecular characterization of genomic rearrangements - how and when to test?

~~Karyotype / RT-PCR~~ : fading

FISH: Clinically useful. May miss complex rearrangements. Sampling issues

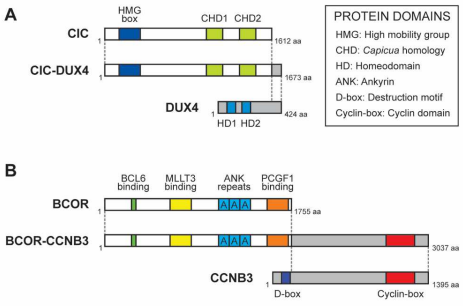
NGS (DNA/RNA)? 3 groups:

- Unclassified high-grade sarcomas

  (dedifferentiated t-associated sarcomas)
- Undifferentiated small round cell sarcomas
- Unclassified distinctive spindle or epithelioid cell neoplasms ("molecularly defined entities")

"Molecularly-defined" soft tissue tumors

- *CIC*-rearranged sarcomas
- Sarcomas with *BCOR* alterations
- Sarcomas with *EWSR1-NFATC2* fusions
- Sarcomas with *EWSR1-PATZ1* fusions
- Sarcomas with *FUS-NFATC2* fusions
- Sarcomas with *EWSR1-POU5F1* fusions

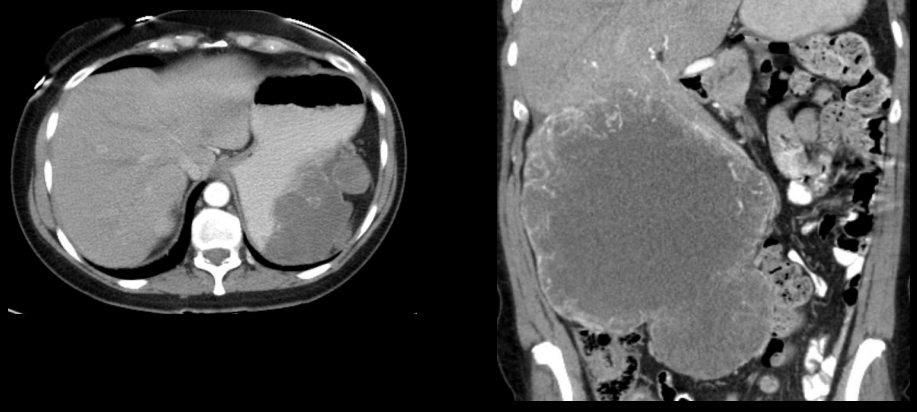


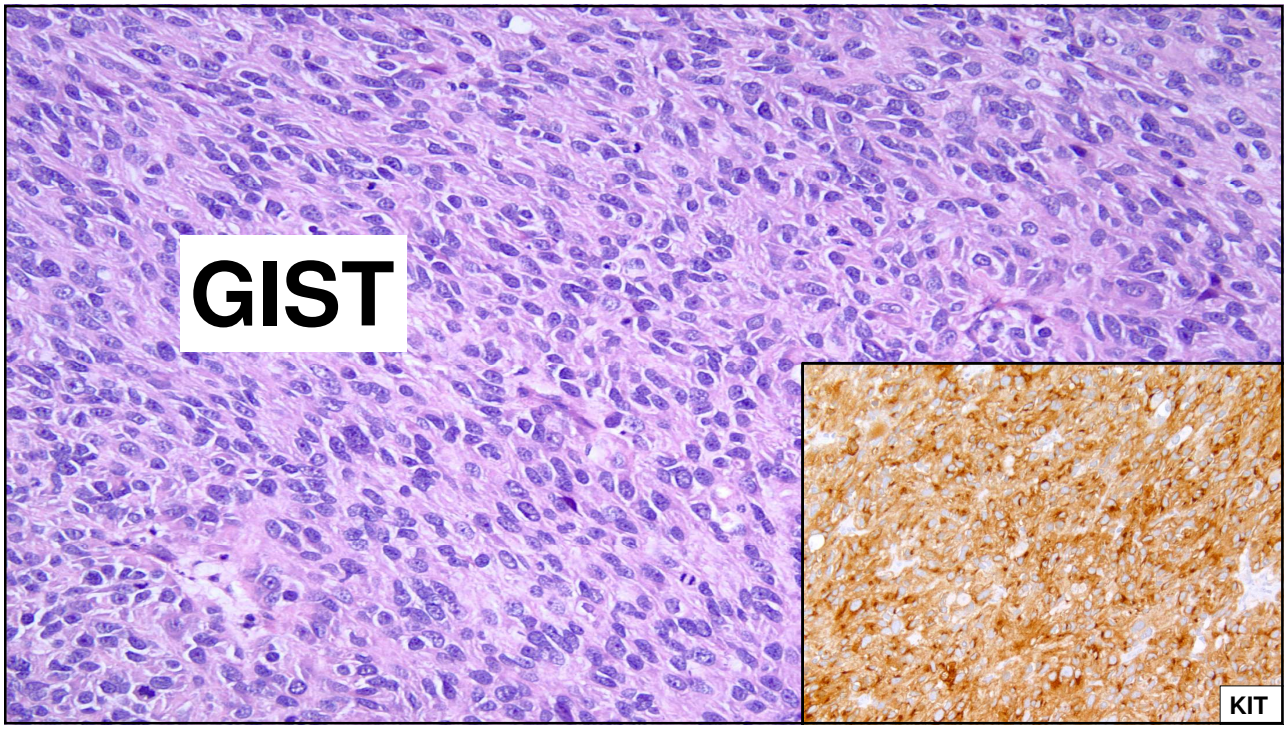
- *NTRK*-rearranged spindle cell neoplasms
- *PDRM10*-rearranged soft tissue tumor (superficial CD34-positive fibroblastic tumor)
- *EWSR1-SMAD3* positive fibroblastic tumor

Practical molecular classification of sarcoma

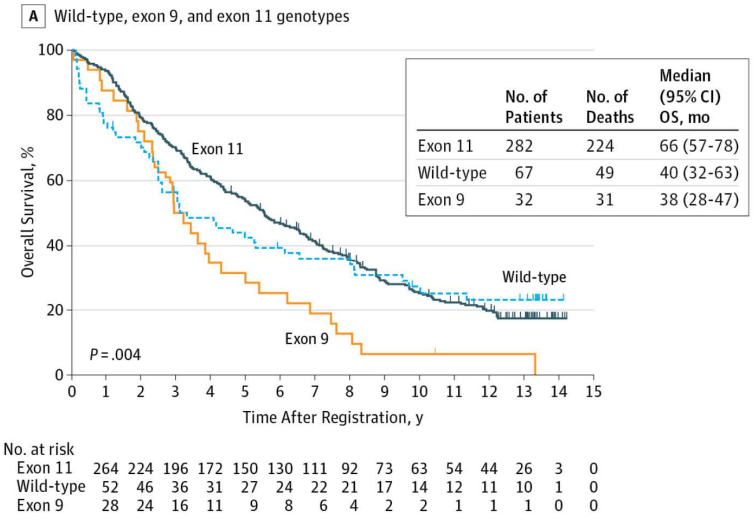
- Sarcomas with simple chromosomal rearrangements
- Sarcomas driven by oncogenic SNVs (or small indels)
- Sarcomas with recurrent CNAs
- Sarcomas with complex karyotype

54 yo woman
Perigastric pain, abdominal discomfort





GIST response to TKIs depends on KIT/PDGFRA mutation

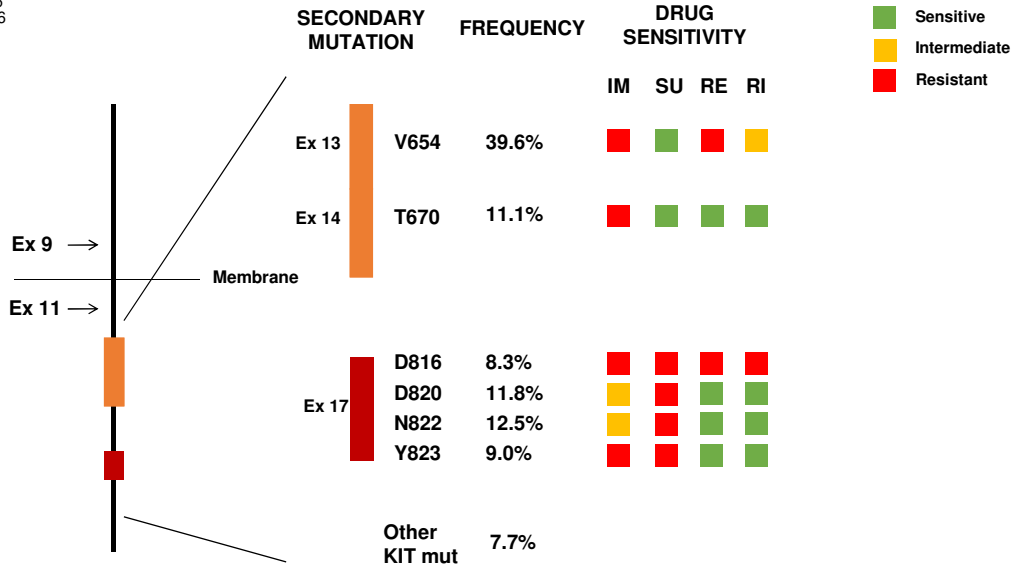


	No. of Patients	No. of Deaths	Median (95% CI) OS, mo
Exon 11	282	224	66 (57-78)
Wild-type	67	49	40 (32-63)
Exon 9	32	31	38 (28-47)

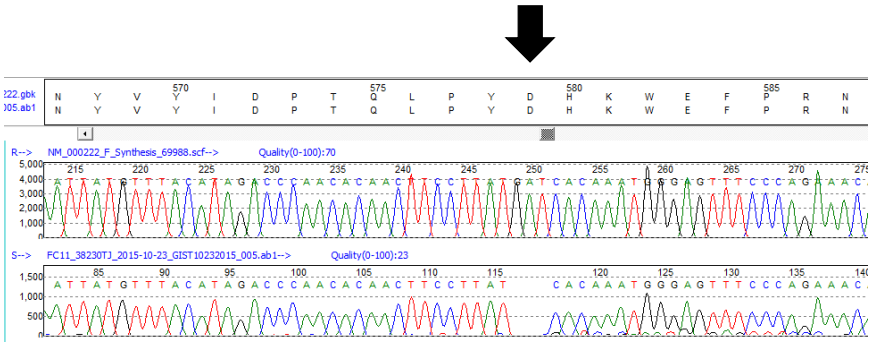
From: **Correlation of Long-term Results of Imatinib in Advanced Gastrointestinal Stromal Tumors With Next-Generation Sequencing ResultsAnalysis of Phase 3 SWOG Intergroup Trial S0033**
JAMA Oncol. Published online February 09, 2017. doi:10.1001/jamaoncol.2016.6728

Progression of KIT-mutant GIST under TKI treatment

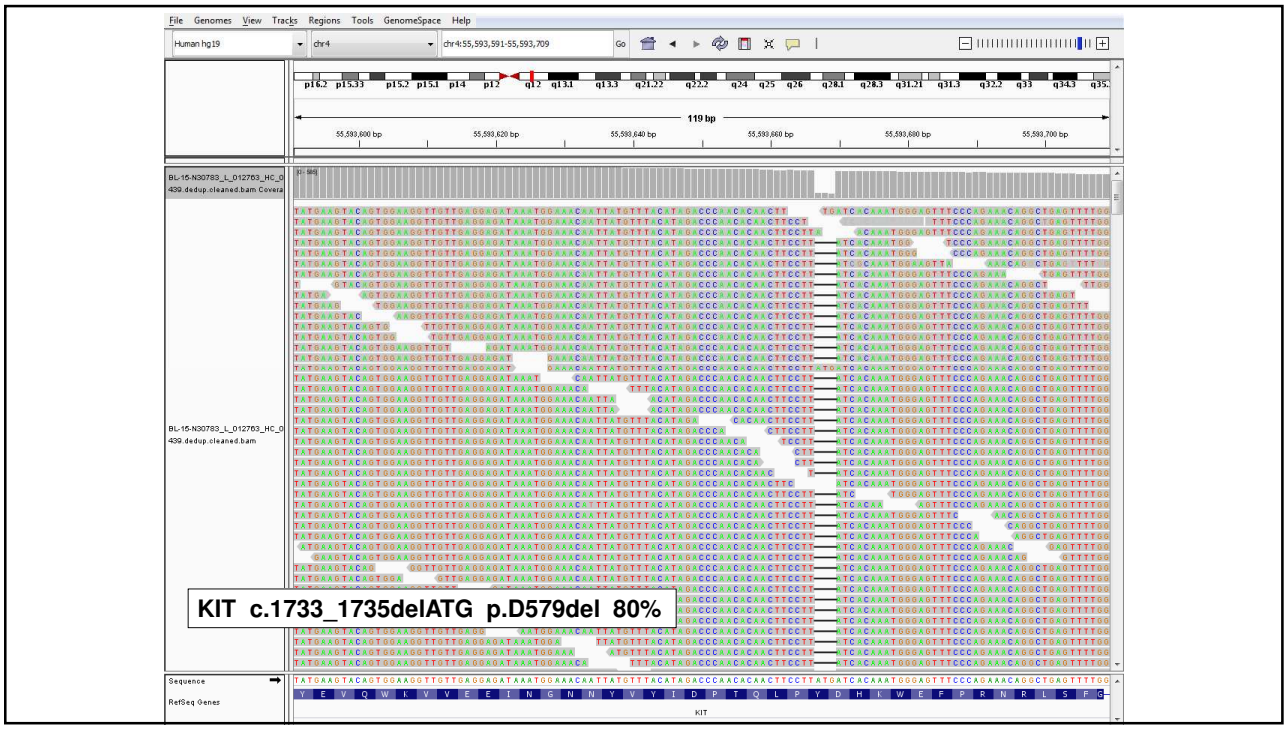
Debiec-Rychter M, 2005
Antonescu CR, 2005
Wardelmann E, 2006
Desai J, 2008
Heinrich MC, 2008
Liegl B, 2008



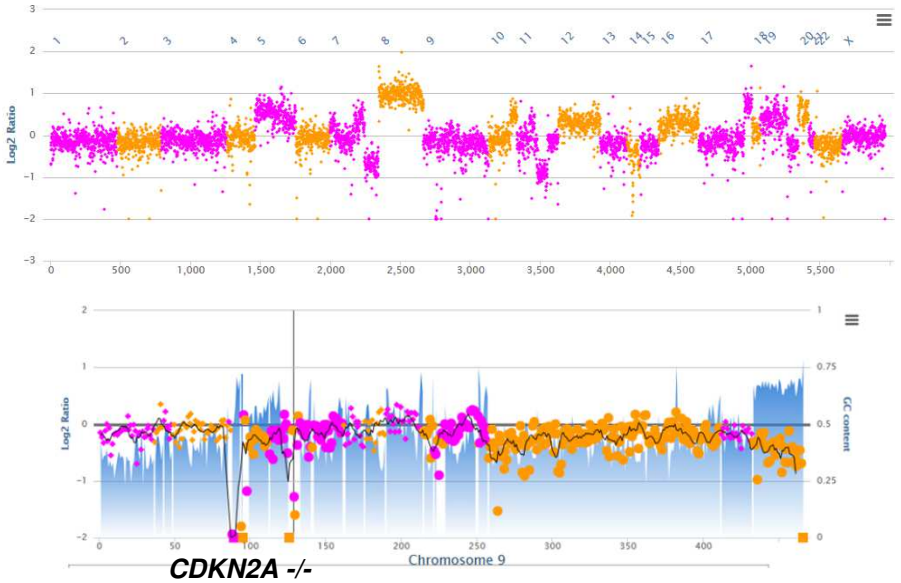
Modified from Heinrich *et al.* JCO 2006, JCO 2008



KIT p.D579del
(exon 11 activating mutation)



Cancer gene panel (Oncopanel) – CNA plot



CDKN2A -/-

Sarcomas driven by oncogenic SNVs (or small indels)

- Transcriptional deregulation
  - *MYOD1* in spindle cell rhabdomyosarcoma
- Epigenetic deregulation (oncometabolites)
  - *IDH1/IDH2* in Enchondroma / chondrosarcoma
- Aberrant kinase signaling
  - *KDR* in angiosarcoma, *BRAF* in LCH

Practical molecular classification of sarcoma

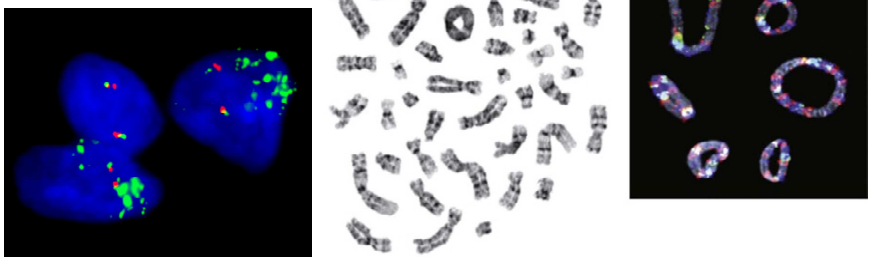
- Sarcomas with simple chromosomal rearrangements
- Sarcomas driven by oncogenic SNVs (or small indels)
- Sarcomas with recurrent CNAs
- Sarcomas with complex karyotype

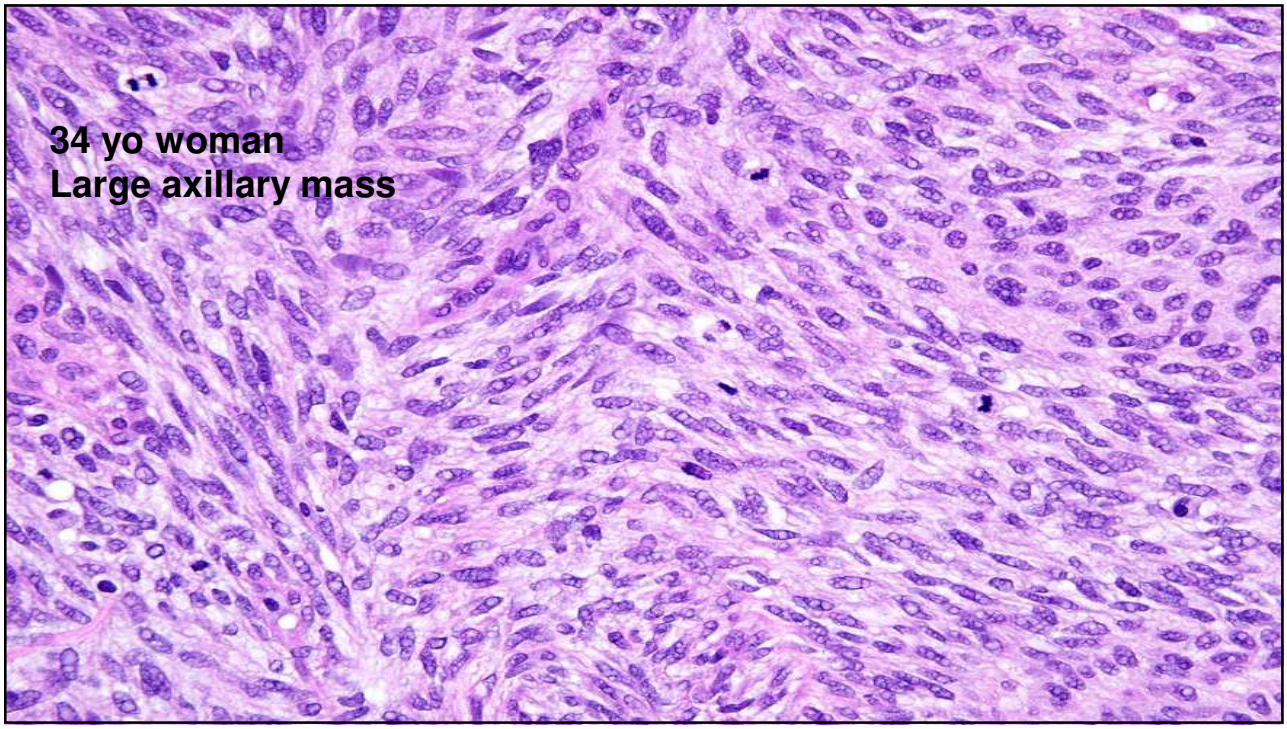
Molecular diagnostics of sarcomas with recurrent CNAs

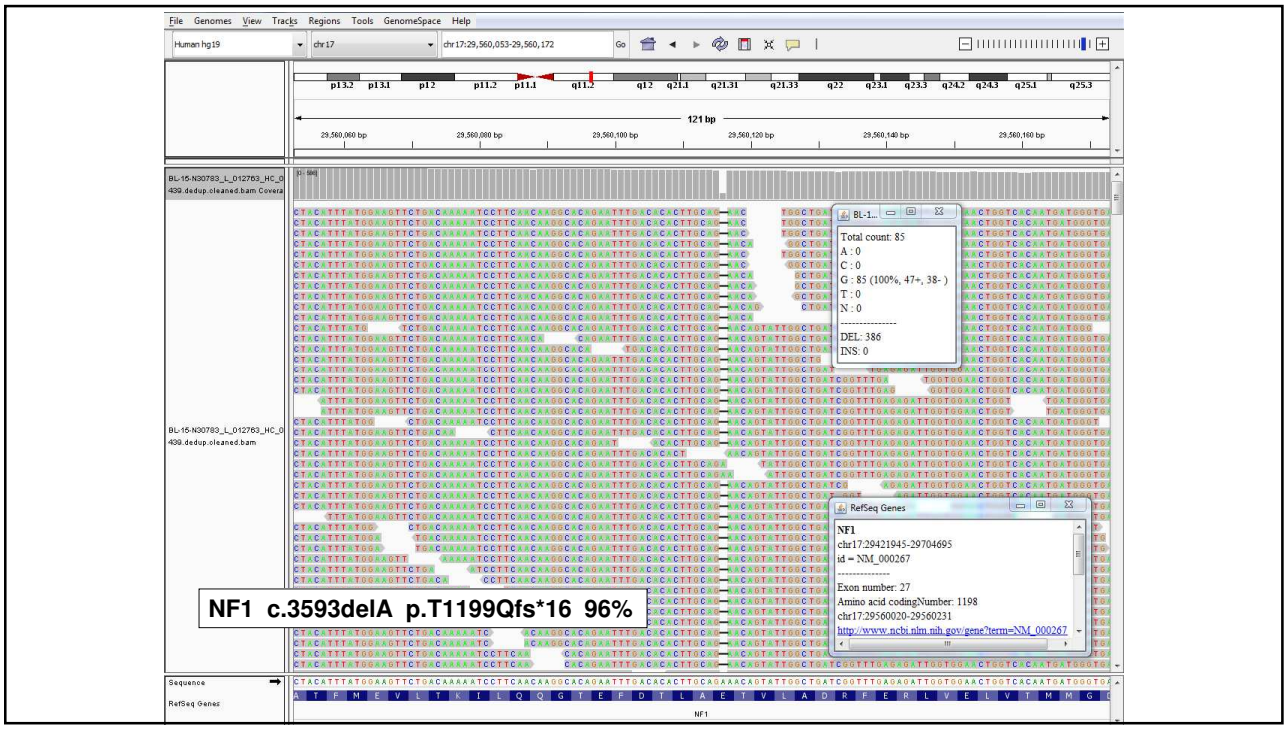
Well-differentiated/dedifferentiated liposarcoma

High level amplification of 20-25 chromosomal regions

12q12-q15 including *MDM2* + *CDK4*







34 yo woman
Large axillary mass

Clinical diagnosis of neurofibromatosis type I

→ Malignant peripheral nerve sheath tumor

Molecular diagnostics of sarcomas with recurrent CNAs

Diagnostic confirmation:

Dedifferentiated liposarcoma (vs myxofibrosarcoma)

MPNST (vs synovial sarc vs melanoma)

SMARCA4-deficient sarcoma

Practical molecular classification of sarcoma

- Sarcomas with simple chromosomal rearrangements
- Sarcomas driven by oncogenic SNVs (or small indels)
- Sarcomas with recurrent CNAs
- Sarcomas with complex karyotype

Sarcomas with recurrent CNAs or complex genome

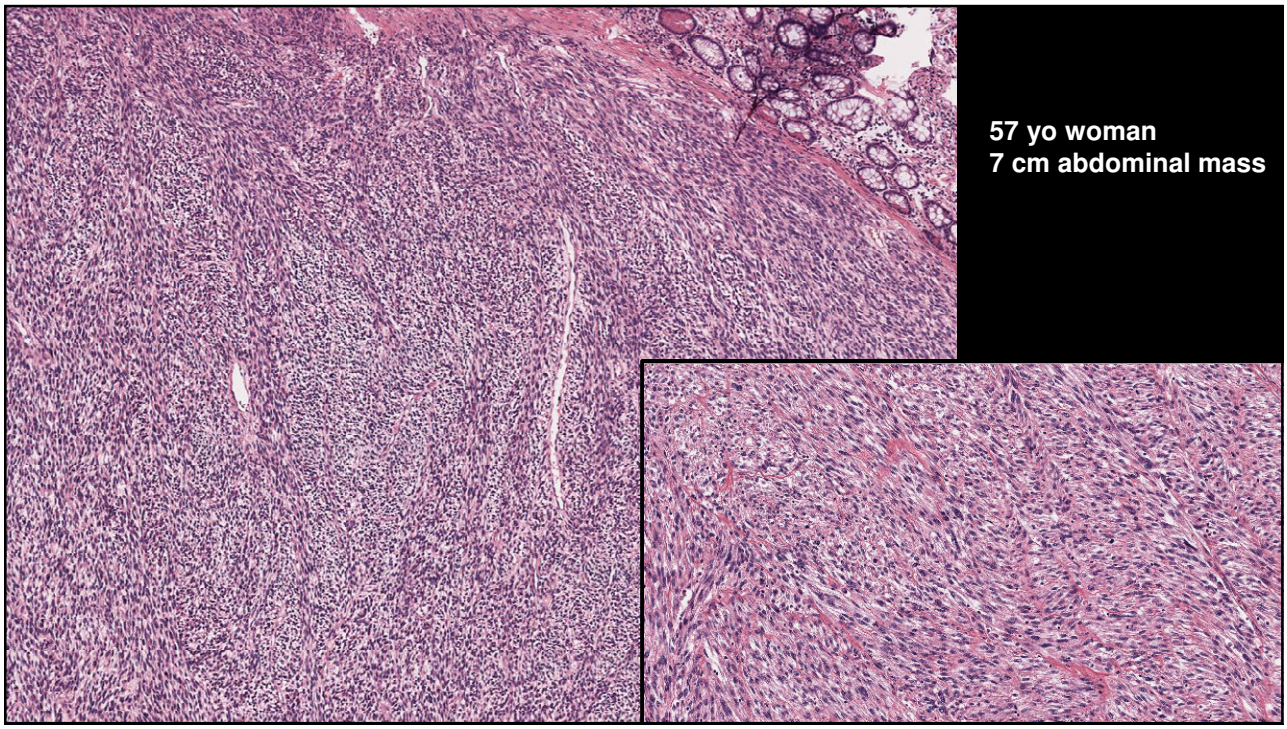
Chromosomal instability (apparently random events):

- Oncogene amplification
- Loss of tumor suppressor genes

Chromothrypsis, chromoplexy, break-fusion-bridge
→ subclonal heterogeneity

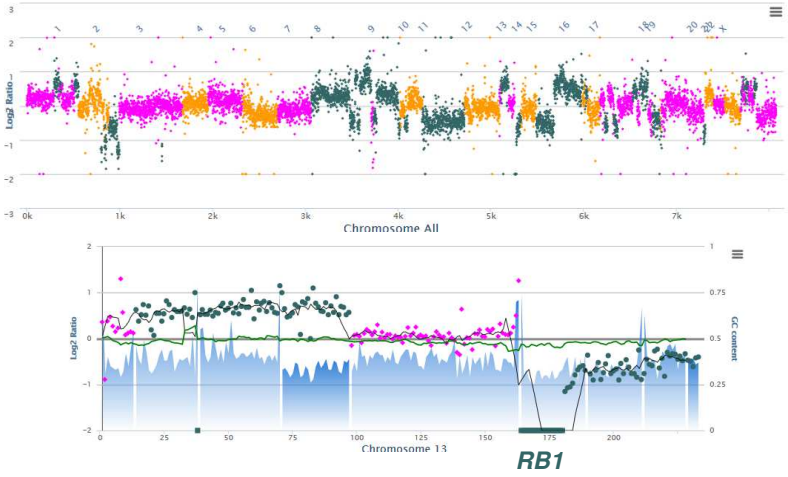
Sarcomas with complex genome

Leiomyosarcoma
Myxofibrosarcoma
Osteosarcoma
Chondrosarcoma
Undifferentiated pleomorphic sarcoma



Cancer gene panel (Oncopanel)

5	IGV	17	7572987	TP53	c.1121delG	p.G374Vfs*48	2	90%	251	Frameshift
6	IGV	17	7578436	TP53	c.494A>C	p.Q165P		44%	357	Missense
5	IGV	X	76939472	ATRX	c.1274_1275delAA	p.K425Rfs*8	2	30%	243	Frameshift



RB1

Genomic hits in leiomyosarcoma

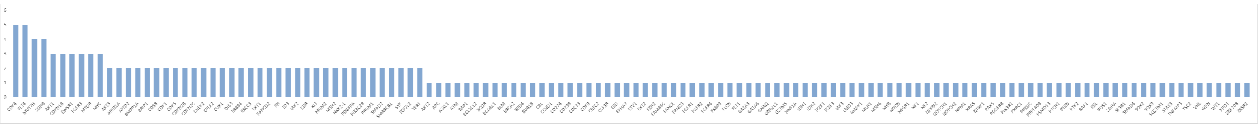
RB1 biallelic inactivation in ~95% (!)

TP53 biallelic inactivation in 92%

ATRX mutations in ~25%

Chudasama P et al, Nat Commun 2018 (WES 49 LMS)

CNAs detected in 192 sarcomas by a 300+ gene NGS panel: long tail



Molecular diagnostics of sarcomas with complex kayotype

Limited diagnostic value

Identification of mutational signatures (UV exposure)

Identification of targetable mutational processes (HRD, MMR)

Germline analysis (familial / hereditary component)

Molecular diagnostics in sarcoma

- Sarcomas with simple chromosomal rearrangements

  URCS - **FISH** / **multiplex NGS** ✓✓✓

- Sarcomas driven by oncogenic SNVs (or small indels)

  GIST, desmoid, LCH - Targeted / exome NGS

- Sarcomas with recurrent CNAs

  DDLPS, NF1-associated MPNST - diagnostic confirmation

- Sarcomas with complex karyotype

  HRD, MMR / UV signatures

IHC surrogates

Molecular Diagnostics in Sarcoma

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