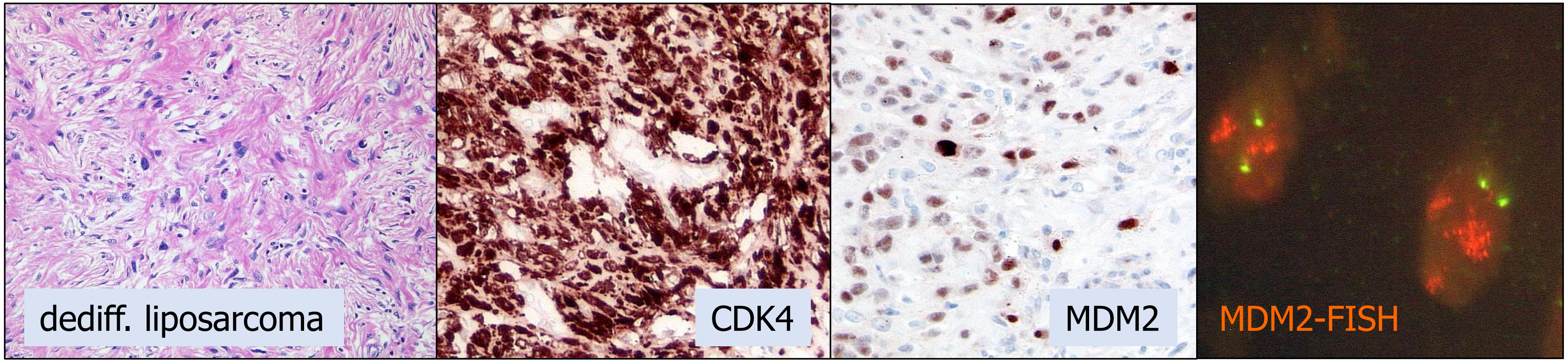
medizinische fakultät
Westfälische Wilhelms-Universität Münster

Medizinische Universität Graz



Understanding the biology and genetics in Sarcomas/GIST

Metastases in cancer (in Sarcomas/GIST) and why/how/where they arise

The role of pathologists in Sarcomas/GIST

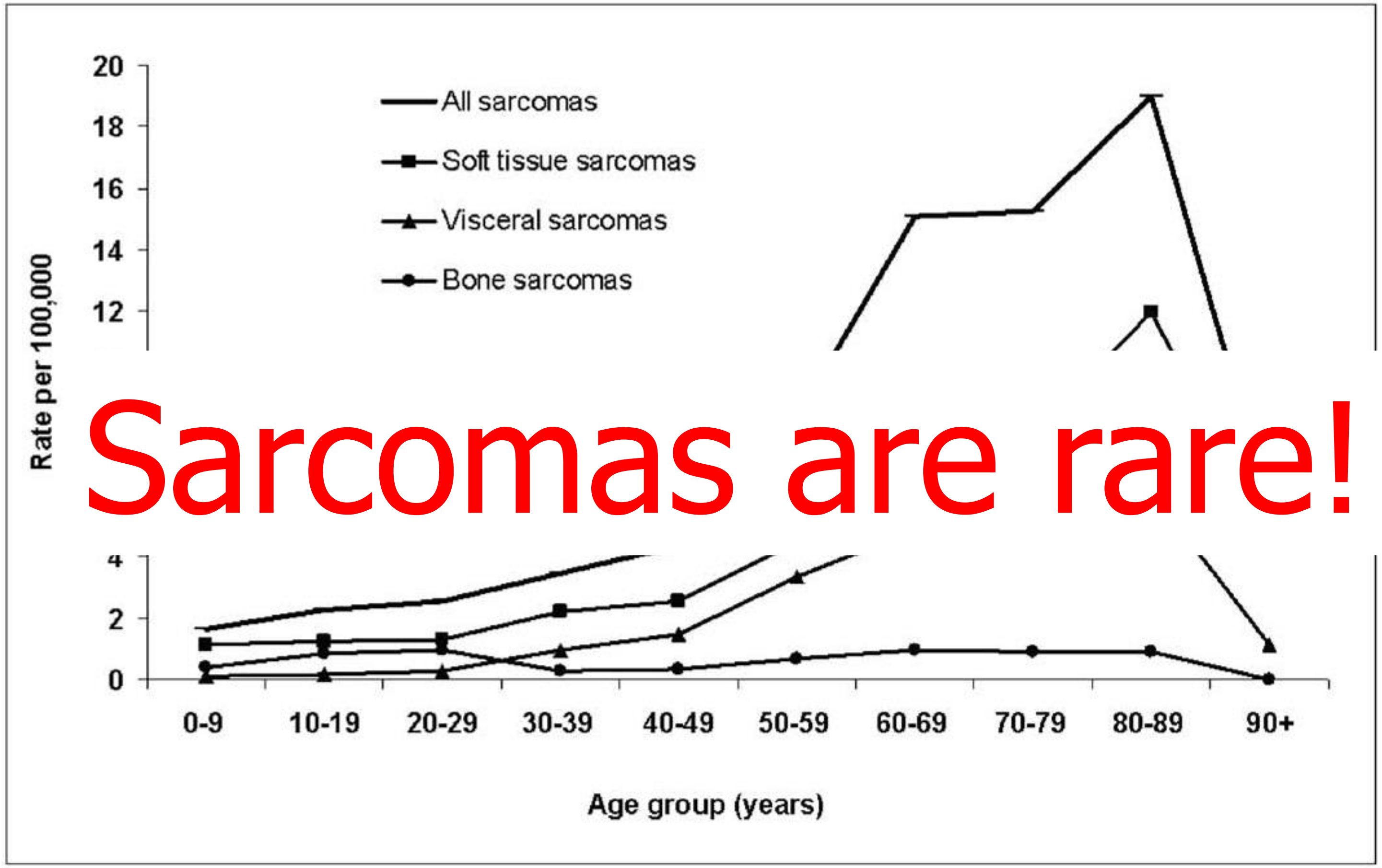
Eva Wardelmann and Bernadette Liegl-Atzwanger

Contents of today:

- Epidemiology and Incidence (France and Germany)
- WHO classification 2013
- Molecular aberrations and biological effects
- Translocations and RNAsequencing
- Mutations and DNAsequencing
- Gene amplifications
- Role of other mechanisms (stemness, TME, metastasis)
- Future issues

Epidemiology of Sarcomas

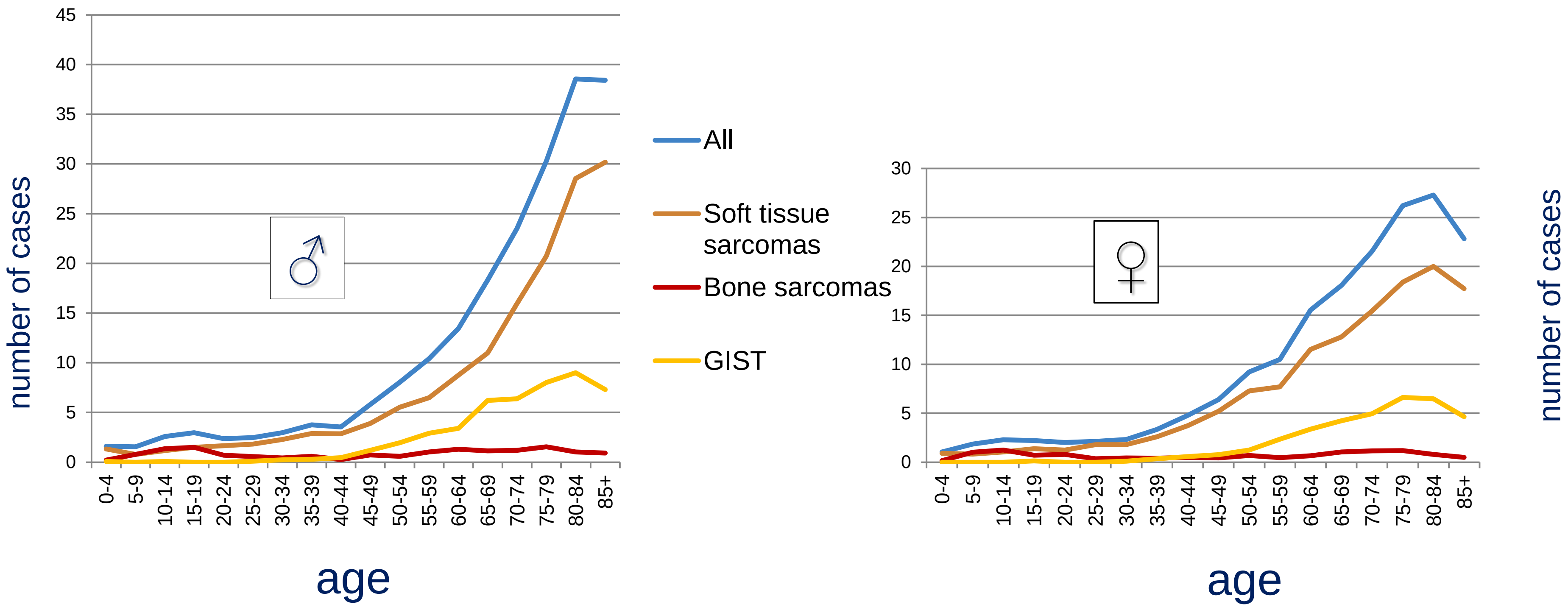
(Rhône-Alpes 2005-2007; 6.000.000 inhabitants)



Ducimetière et al., PLoS ONE 6(8); 2011

Incidence of Sarcomas/year: ~5/100.000
~ 4000 new sarcomas in Germany/year

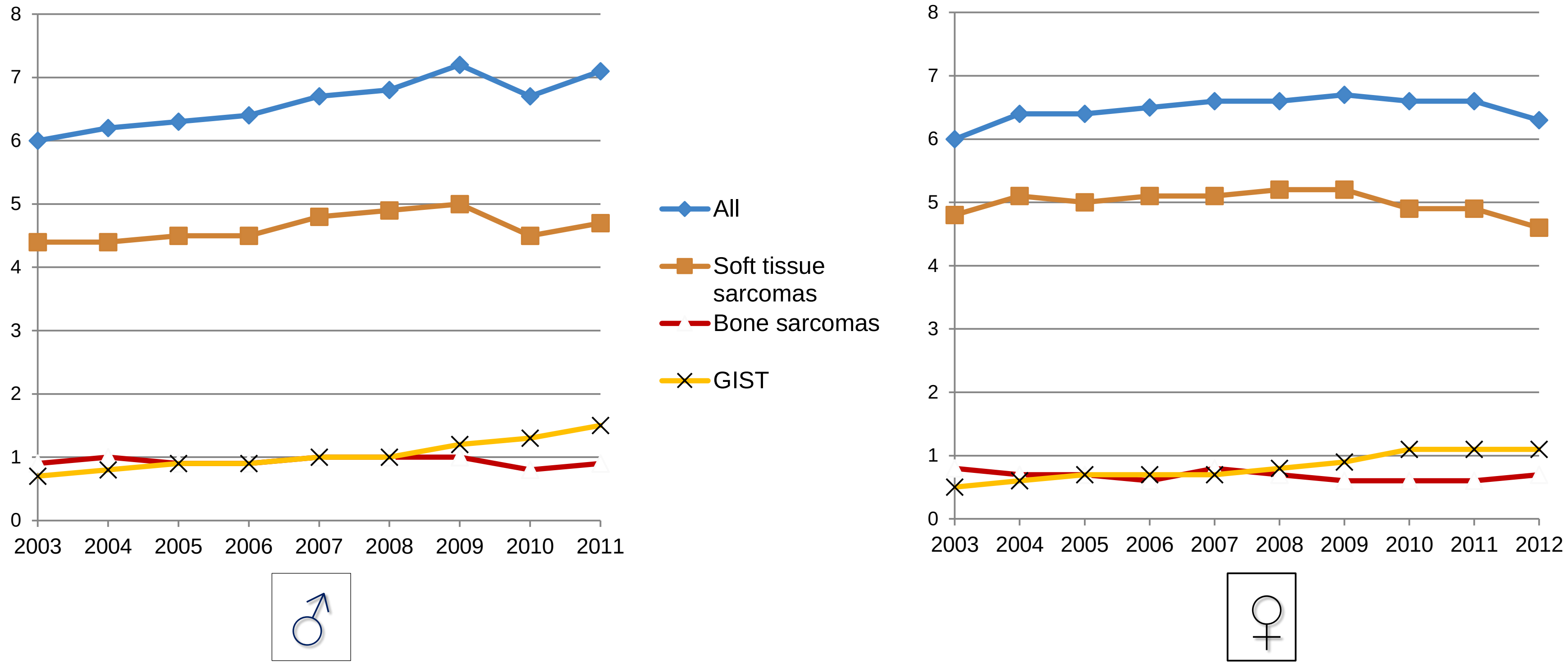
Incidence of sarcomas in Germany 2013



Incidence of sarcomas/year: ~7,4♂/100.000 and 6,6♀/100.000 inhabitants

Ressing et al. 2016

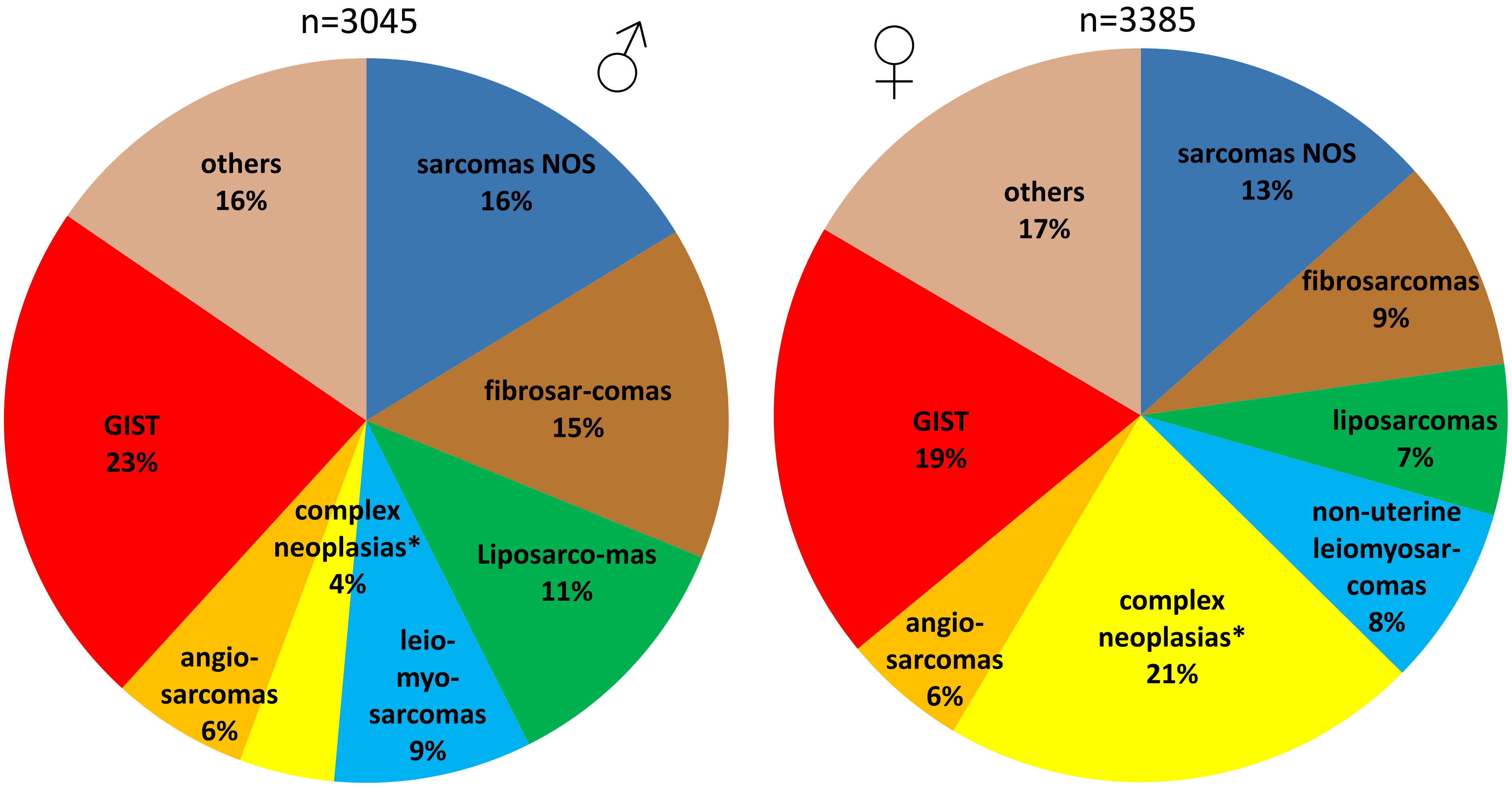
Incidence changes in sarcomas between 2003 and 2011



Incidence of sarcomas has not increased between 2003 and 2012 – with the exception of GIST

Ressing et al. 2016

Frequency of different sarcoma subtypes



*complex neoplasias: ESS, MMMT, SySa, carcinosarcomas, adenosarcomas

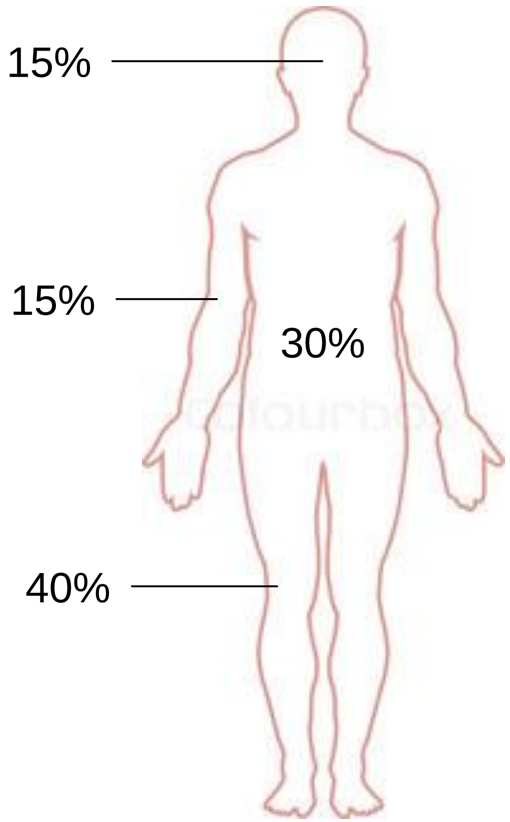
Krebsregister Rheinland-Pfalz

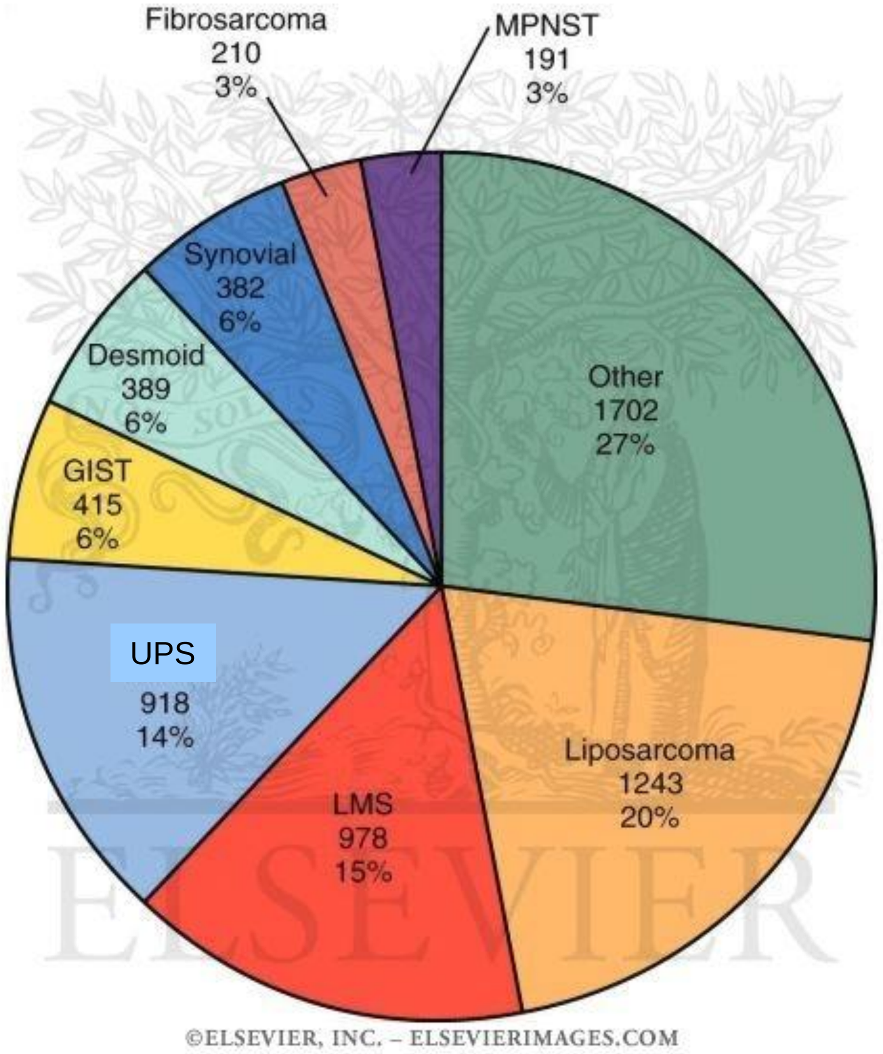
Incidence of sarcomas and histological subtypes in Germany in 2012

Ressing M.[1], Wardelmann E.[2], Blettner M. [3], Emrich K.[1], Hohenberger P. [4], Jakob J.[4], Kasper B.[5], Zeissig S.R.[1]

Poster at the DKK 2016
Data from 10 of 11 Cancer registries, 69,8 Mio. inhabitants

Distribution and frequency of different sarcoma subtypes



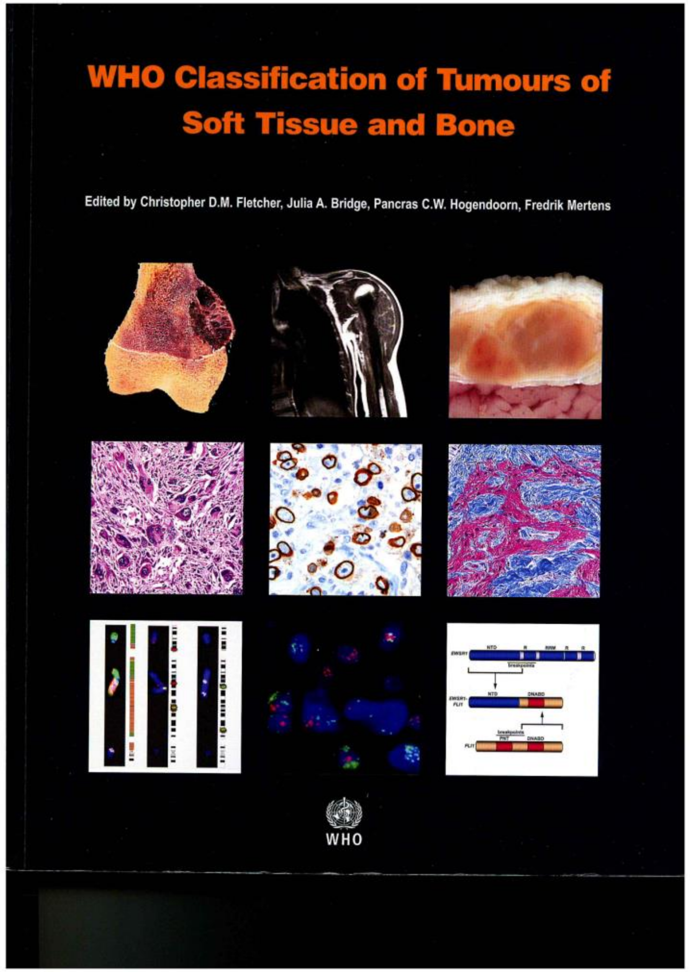


Problems with the classification of soft tissue sarcomas

- rare cancer (compared to other malignancies such as carcinomas, lymphomas, leucemia…)
- > 60 different subtypes
- not always clearly defined diagnostic criteria
- partly overlapping definitions/morphology
- low experience of the single pathologist due to the low frequency
- not enough sarcoma centers with STS expertise in all relevant medical subspecialities

The efficacy of treatment in sarcomas depends on the correct histopathologic diagnosis!

WHO classification of sarcomas 2013



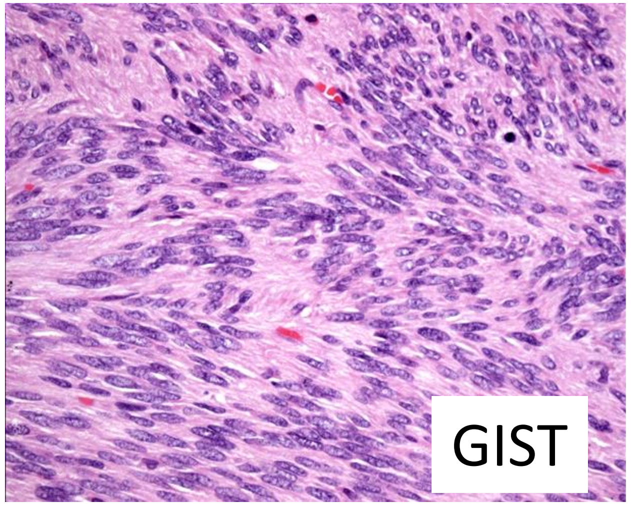
WHO classification of tumours of soft tissue[a,b]

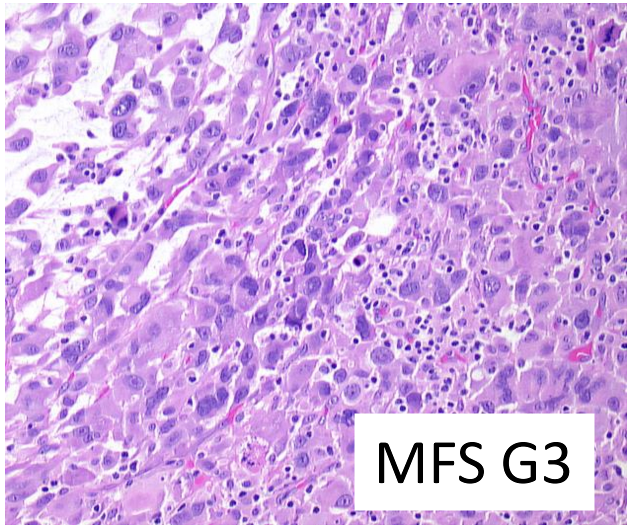
ADIPOCYTIC TUMOURS	
Benign	
Lipoma	8850/0
Lipomatosis	8850/0
Lipomatosis of nerve	8850/0
Lipoblastoma/lipoblastomatosis	8881/0
Angiolipoma	8861/0
Myolipoma	8890/0
Chondroid lipoma	8862/0
Extra-renal angiomyolipoma	8860/0
Extra-adrenal myelolipoma	8870/0
Spindle cell/pleomorphic lipoma	8857/0
Hibernoma	8880/0
Intermediate (locally aggressive)	
Atypical lipomatous tumour/	8850/1
well differentiated liposarcoma	8850/3
Malignant	
Dedifferentiated liposarcoma	8858/3
Myxoid liposarcoma	8852/3
Pleomorphic liposarcoma	8854/3
Liposarcoma, not otherwise specified	8850/3
FIBROBLASTIC / MYOFIBROBLASTIC TUMOURS	
Benign	
Nodular fasciitis	8828/0*
Proliferative fasciitis	8828/0*
Proliferative myositis	8828/0*
Myositis ossificans	
Fibro-osseous pseudotumour of digits	
Ischaemic fasciitis	
Elastofibroma	8820/0
Fibrous hamartoma of infancy	
Fibromatosis colli	
Juvenile hyaline fibromatosis	
Inclusion body fibromatosis	
Fibroma of tendon sheath	8813/0
Desmoplastic fibroblastoma	8810/0
Mammary-type myofibroblastoma	8825/0
Calcifying aponeurotic fibroma	8816/0*
Angiomyofibroblastoma	8826/0
Cellular angiofibroma	9160/0
Nuchal-type fibroma	8810/0
Gardner fibroma	8810/0
Calcifying fibrous tumour	8817/0*
Intermediate (locally aggressive)	
Palmar/plantar fibromatosis	8813/1*
Desmoid-type fibromatosis	8821/1
Lipofibromatosis	8851/1*
Giant cell fibroblastoma	8834/1
Intermediate (rarely metastasizing)	
Dermatofibrosarcoma protuberans	8832/1*
Fibrosarcomatous dermatofibrosarcoma protuberans	8832/3*
Pigmented dermatofibrosarcoma protuberans	8833/1*
Solitary fibrous tumour	8815/1*
Solitary fibrous tumour, malignant	8815/3
Inflammatory myofibroblastic tumour	8825/1
Low-grade myofibroblastic sarcoma	8825/3*
Myxoinflammatory fibroblastic sarcoma/ Atypical myxoinflammatory fibroblastic tumour	8811/1*
Infantile fibrosarcoma	8814/3
Malignant	
Adult fibrosarcoma	8810/3
Myxofibrosarcoma	8811/3
Low-grade fibromyxoid sarcoma	8840/3*
Sclerosing epithelioid fibrosarcoma	8840/3*
SO-CALLED FIBROHISTIOCYTIC TUMOURS	
Benign	
Tenosynovial giant cell tumour	
localized type	9252/0
diffuse type	9252/1*
malignant	9252/3
Deep benign fibrous histiocytoma	8831/0
Intermediate (rarely metastasizing)	
Plexiform fibrohistiocytic tumour	8835/1
Giant cell tumour of soft tissues	9251/1
SMOOTH MUSCLE TUMOURS	
Benign	
Deep leiomyoma	8890/0
Malignant	
Leiomyosarcoma (excluding skin)	8890/3
PERICYTIC (PERIVASCULAR) TUMOURS	
Glomus tumour (and variants)	8711/0
Glomangiomatosis	8711/1*
Malignant glomus tumour	8711/3
Myopericytoma	8824/0
Myofibroma	8824/0
Myofibromatosis	8824/1
Angioleiomyoma	8894/0
SKELETAL MUSCLE TUMOURS	
Benign	
Rhabdomyoma	8900/0
Adult type	8904/0
Fetal type	8903/0
Genital type	8905/0
Malignant	
Embryonal rhabdomyosarcoma (including botryoid, anaplastic)	8910/3
Alveolar rhabdomyosarcoma (including solid, anaplastic)	8920/3
Pleomorphic rhabdomyosarcoma	8901/3
Spindle cell/sclerosing rhabdomyosarcoma	8912/3

- adipocytic
- fibroblastic/myofibroblastic
- fibrohistiocytic
- smooth muscle
- pericytic
- skeletal muscle
- vascular
- chondroosseous
- uncertain differentiation
- nerve sheath
- undifferentiated/unclassified
- GIST

Role of sarcoma morphology in respect to their genotype

- no/low nuclear pleomorphism-> specific genomic aberrations
- strong nuclear pleomorphism-> complex genotype



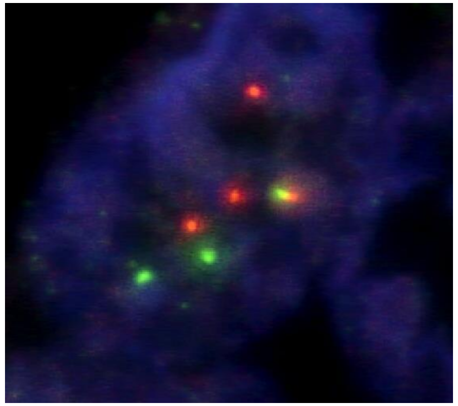


Molecular analysis as a key for diagnosis and therapy

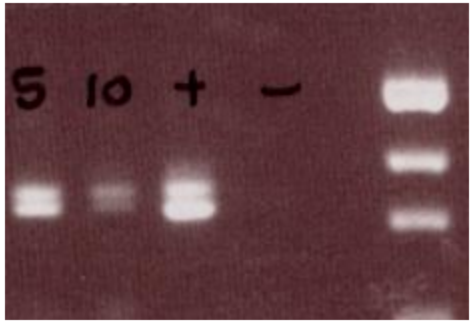
- specific reciprocal translocations
- specific somatic mutations
- more or less specific amplifications
- unspecific complex karyotypes

Methods in molecular pathology

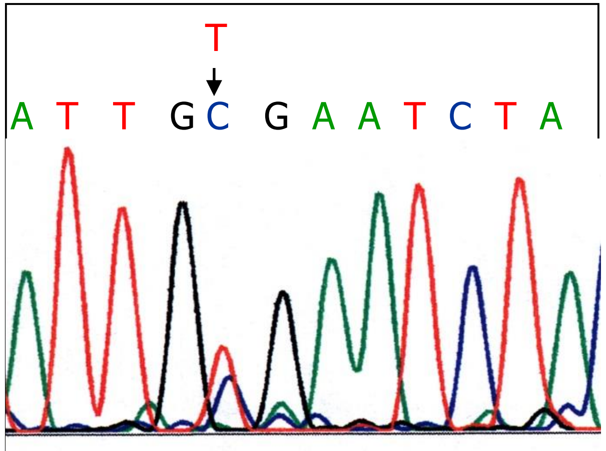
Multicolor FISH analysis to detect translocations and gene amplifications



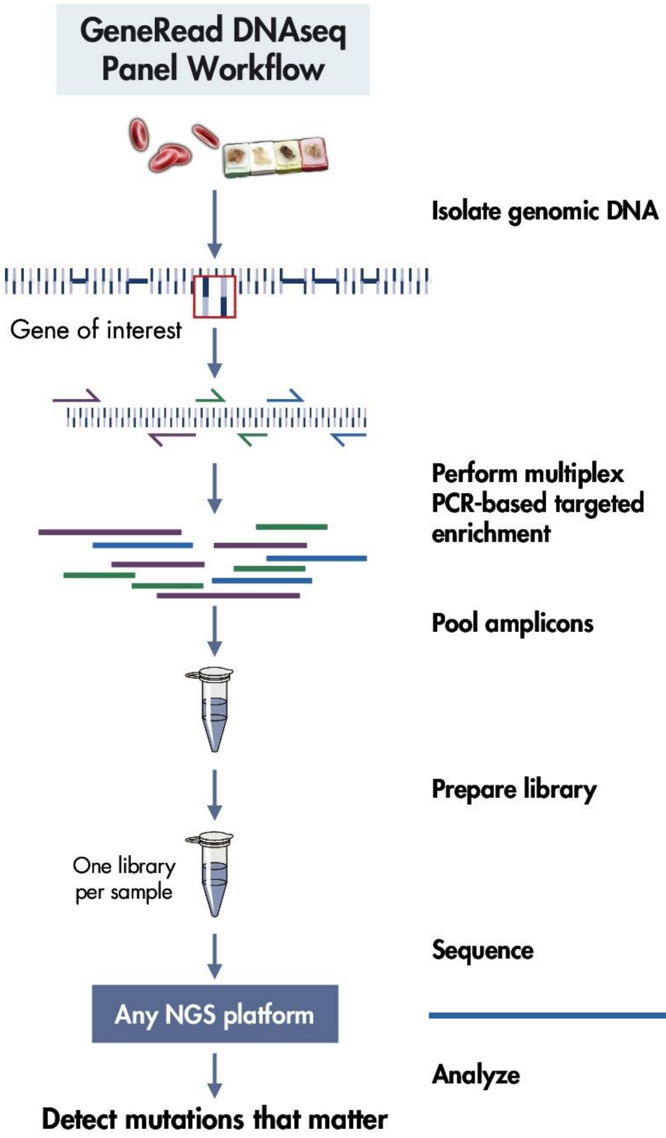
rt-PCR to detect fusion transcripts

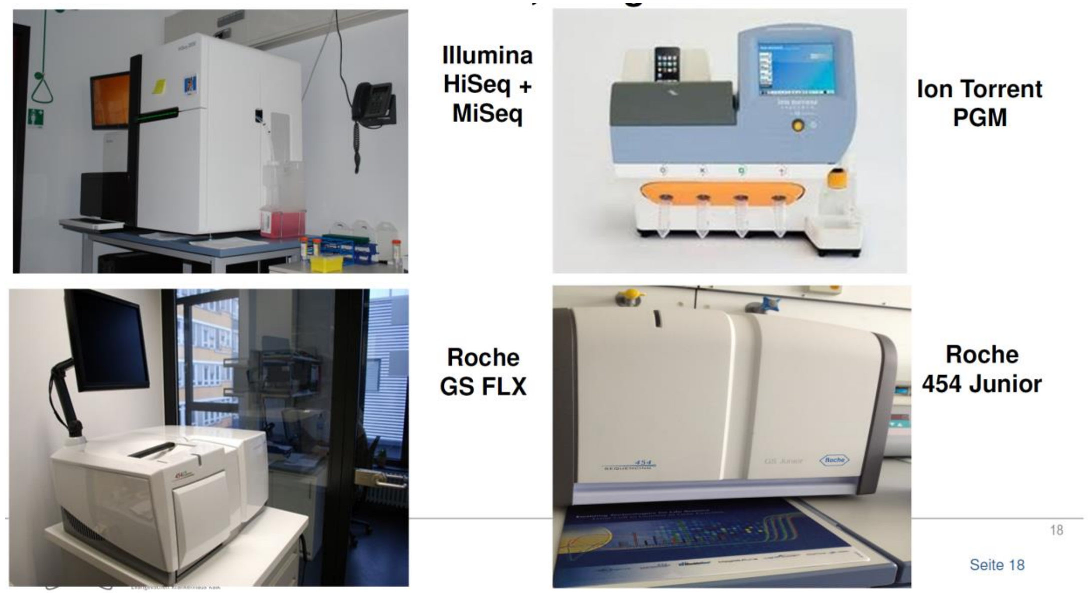


DNA sequencing of single gene regions to detect specific mutations



Next Generation Sequencing - Workflow



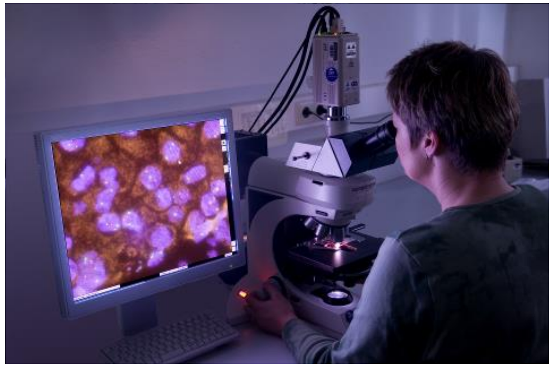


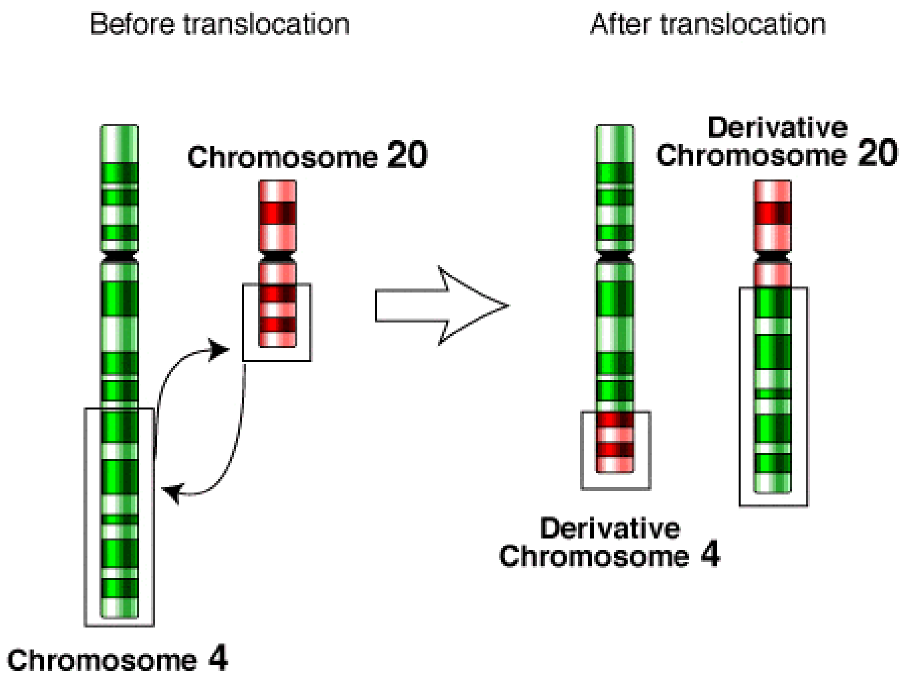
MiSeq - Illumina

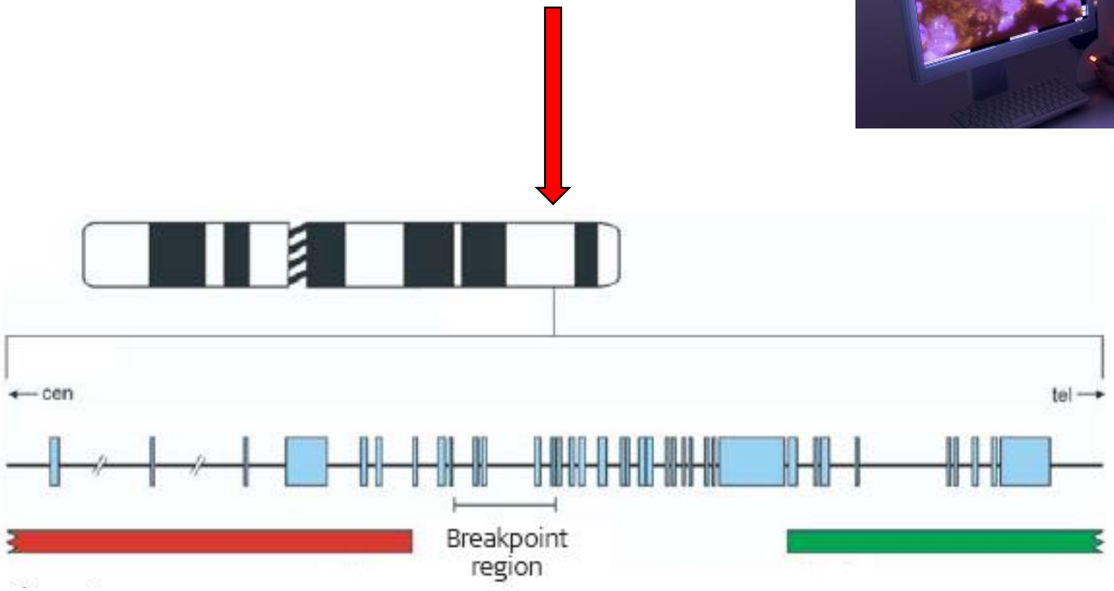
Molecular analysis as a key for diagnosis and therapy

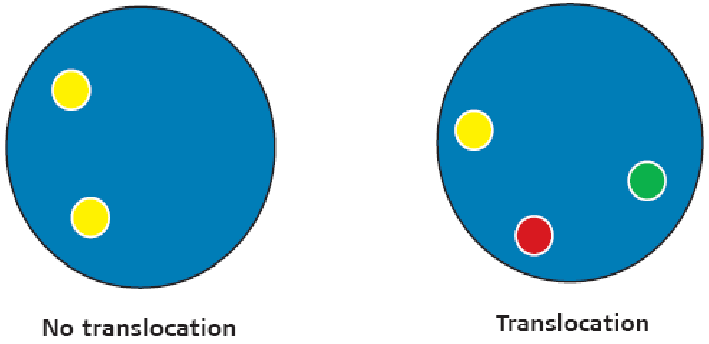
- specific reciprocal translocations
- specific somatic mutations
- more or less specific amplifications
- unspecific complex karyotypes

What is a translocation and how to proof it?





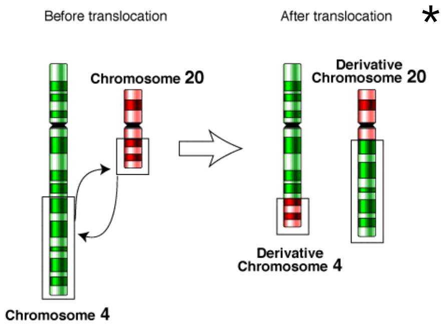




Translocations may pinpoint potential targets in sarcomas

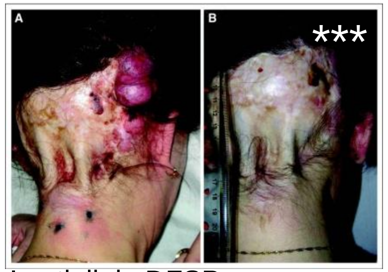
Chromosomal translocation

chimeric fusion gene

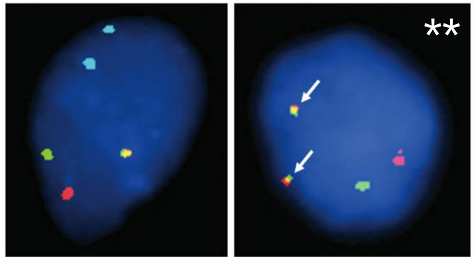


Chimeric transcription factor:
Ewing sarcoma
Synovial sarcoma
Alveolar rhabdomyosarcoma
Myxoid liposarcoma
Clear cell sarcoma
Solitary fibrous tumor
….

Chimeric autocrine growth factor:
Dermatofibrosarcoma protuberans
Giant cell tumor of tendon sheath/
Pigmented villonodular synovitis



Imatinib in DFSP

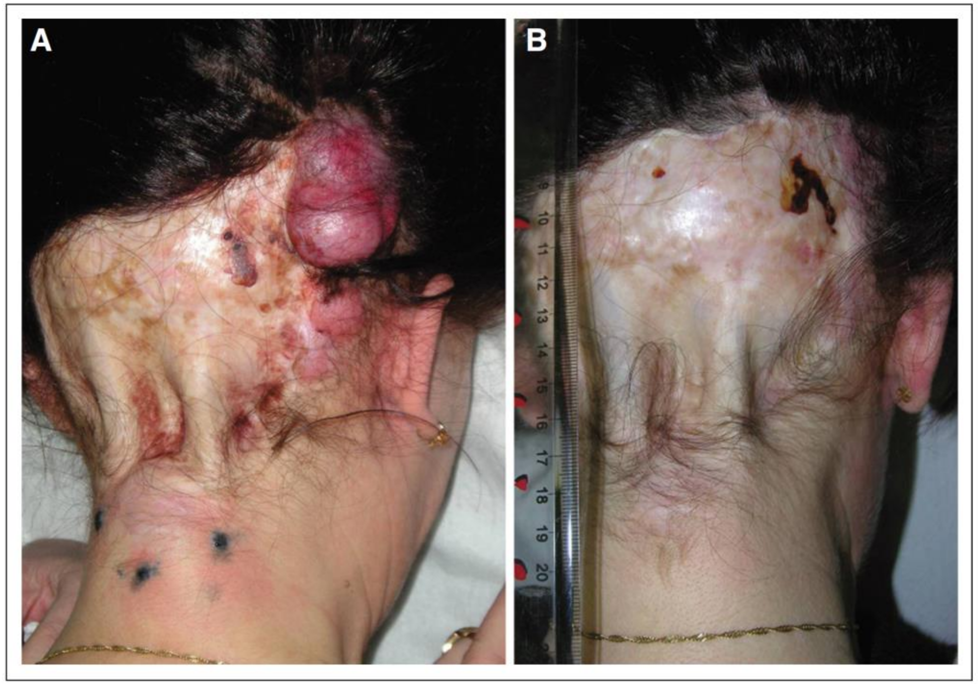


CSF1-COL6A3 in PVNS

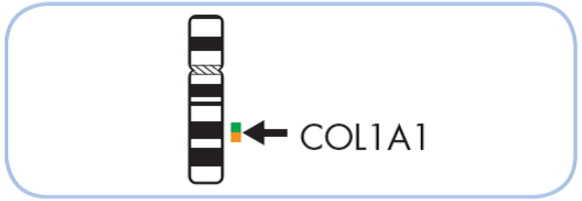
Chimeric tyrosine kinase:
Inflammatory myofibroblastic tumor
Infantil fibrosarcoma

*wikipedia
** Nishio J Oncol Lett 2013
***Rutkowski P et al., JCO 2010

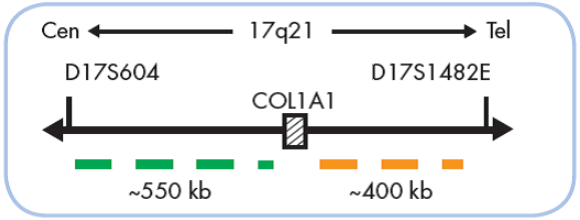
Dermatofibrosarcoma protuberans treated with Imatinib (target PDGFB-COL1A1)



Rutkowski et al. JCO 2010

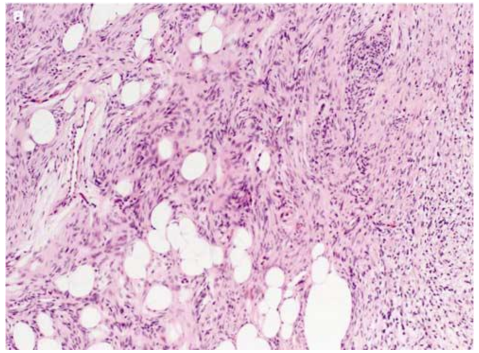


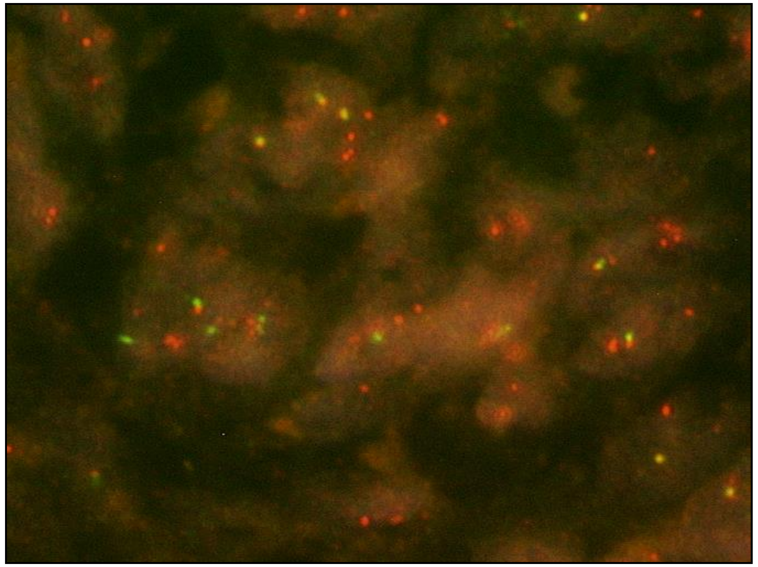
Ideograms of chromosome 17 indicating the hybridization locations.



SPEC COL1A1 Probe map (not to scale).

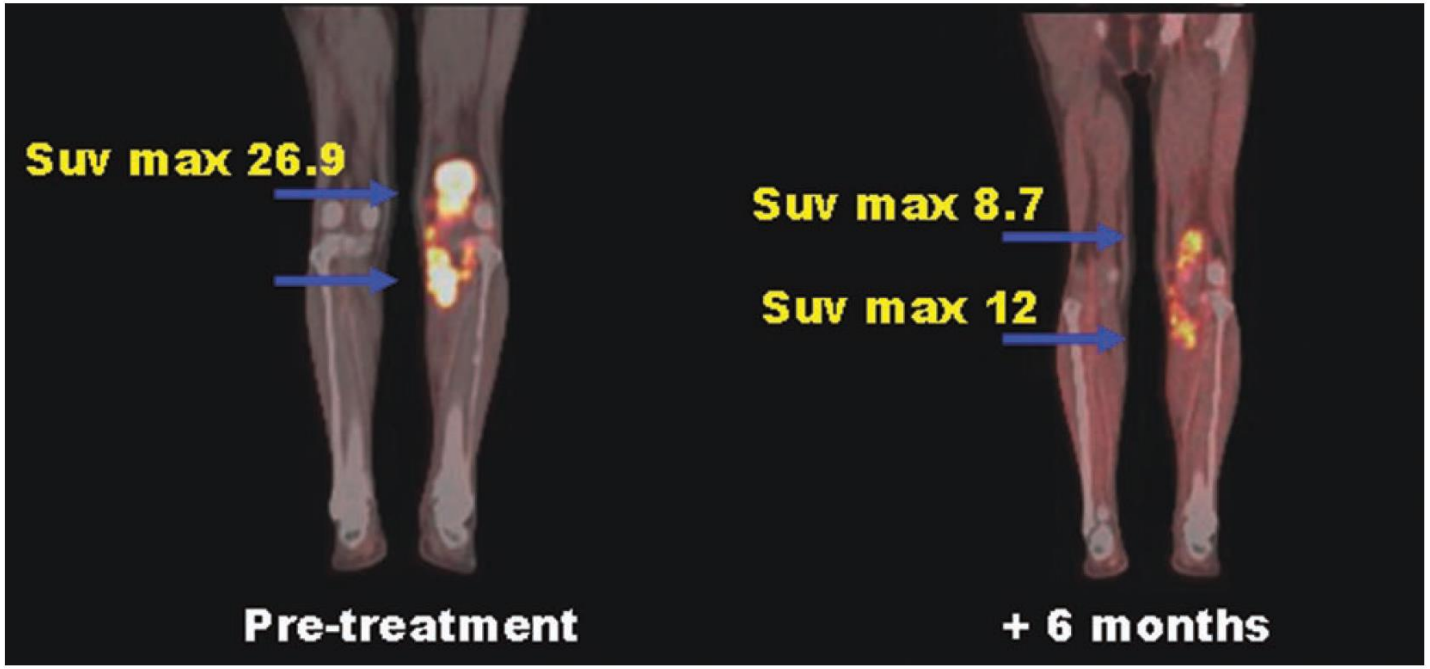
Abbott et al. Mod Pathol 2011





Provided by HU Schildhaus

Response of pigmented villonodular synovialitis to imatinib (target CSF1-COL6A3)



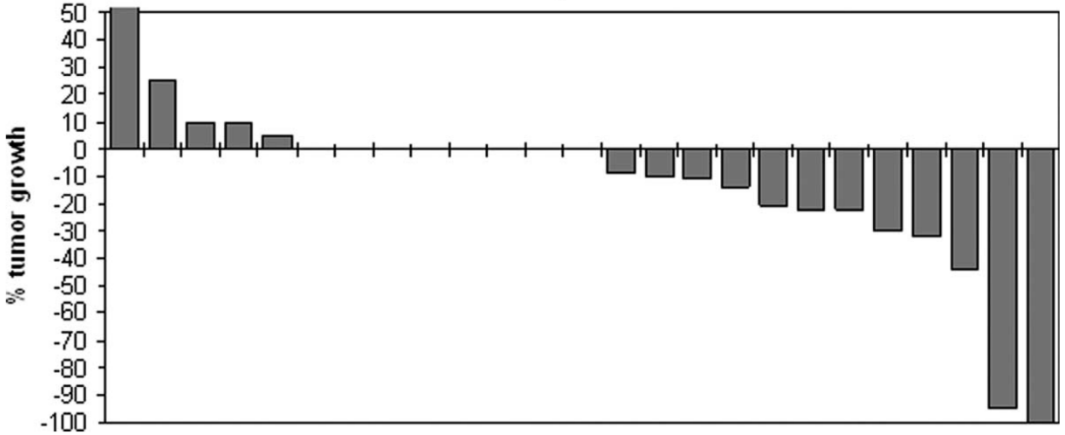


Figure 1. The best tumor shrinkage is illustrated according to Response Evaluation Criteria in Solid Tumors (RECIST).

Parameter	No. of Patients (%)
RECIST best response	
CR	1 (3.7)
PR	4 (14.8)
SD	20 (74.1)
PD	2 (7.4)
NE	2 (—)
Symptomatic response	
Assessable	22
Response	16 (72.7)
Median IM treatment duration [95% CI], mo	8 [4-12]
Median PFS, mo	20.9

Tumor entity	Genomic aberration	Fusion gene, mutated gene
Alveolar rhabdomyosarcoma (ARMS)	t(2;13)(q35;q14) t(1;13)(p36;q14) t(2;2)(p23;q36) t(X;2)(q13;q36)	PAX3-FOXO1A PAX7-FOXO1A PAX3-NCOA1 PAX3-FOXO4
Alveolar soft part sarcoma (ASPS)	t(X;17)(p11;q25)	ASPSCR1-TFE3
Angiomatoid fibrous histiocytoma (AFH)	t(12;16)(q13;p11) t(2;22)(q33;q12) t(12;22)(q13;q12)	TLS-ATF1 EWS1R-CREB1 EWS1R-ATF 1
Angiosarcoma (ASA)	missense mutation amplification	KDR, FLT4 c-MYC
Clear cell sarcoma (CCS)	t(12;22)(q13;q12) t(2;22)(q33;q12)	EWS1R-ATF1 EWS1R-CREB1
Congenital fibrosarcoma (CGFS)	t(12;15)(p13;q25)	ETV6-NTRK3
Dermatofibrosarcoma protuberans (DFSP)	t(17;22)(q22;q13) der(22)t(17;22) ring chromosome	COL1A1-PDGFB
Desmoplastic small round cell tumor (DSRCT)	t(11;22)(p13;q12)	EWS1R-WT1
Endometrial stromal sarcoma (ESS)	t(7;17)(p15;q21) t(10;17)(q22;p13)	JAZF1-JJAZ1 YWHAE-FAM22A/B
Epithelioid hemangioendothelioma (EHE)	t(1;3(p36.3;q25) t(X;11)	WWTR1-CAMTA1 YAP1-TFE3
Epithelioid sarcoma (ES)	intragenic deletions	SMARCB1/INI1
Ewing sarcoma (ES)	t(11;22)(q24;q12) t(21;22)(q22;q12) t(7;22)(p22;q12) t(17;22)(q12;q12) t(2;22)(q33;q12) t(16;21)(p11;q22	EWS1R-FLI1 EWS1R-ERG EWS1R-ETV1 EWS1R-E1AF EWS1R-FEV FUS-ERG
Extrarenal rhabdoid tumor (ERT)	Homozygous inactivation by deletion	hSNF/INI1/SMARCB1/BAF47 or SMARCA4 (BRG1) loss

Extraskelettal myxoid chondrosarcoma (EMCS)	t(9;22)(q22;q12) t(9;17)(q22;q11) t(3;9)(q12;q22) t(9;17)(q22;q11)	EWS1R-CHN TAF2N-CHN TFG-NR4A3 TCF12-NR4A3
Fibromatosis (desmoid type)	CTNNB1 mutations, APC mutations	missense mutations
Gastrointestinal stromal tumor (GIST)	mutations	KIT, PDGFRA, SDH, NF1, BRAF or other genes
Inflammatory myofibroblastic tumor (IMFT)	t(2p23)	div. ALK fusion partners
Low grade fibromyxoid sarcoma (LGFS) **Sclerosing epithelioid fibrosarcoma (SEF)**	t(7;16)(q33-34;p11) t(11;16)(p11;p11)	FUS-CREB3L2 FUS-CREB3L1 EWS1R-CREB3L1
Myxoinflammatory fibroblastic sarcoma (MIFS)	t(1;10)(p22;q24) Ring chromosome	deregulation of FGF8+NPM3 amplification of VGLL3
Solitary fibrous tumor/hemangiopericytoma (SFT)	der(12)(q13-15)	NAB2-STAT6
Synovial sarcoma (SS)	t(X;18)(p11;q11) t(X;18)(p11;q11) t(X;18)(p11;q11) t(X;20)(p11;q13)	SS18-SSX1 SS18-SSX2 SS18-SSX4 SS181-SSX1
Tenosynovial giant cell tumor (TGCT)	t(1;2)(p13;q37)	CSF-COL6A3
Well differentiated liposarcoma (WDLS)/atypical lipomatous tumor (ALT)	Ring chromosome/giant marker	amplification of MDM2, CDK4, HMGA2, GLI-SAS

Wardelmann and Hartmann in:
I. Schrijver and G. Netto, Genomic applications in Clinical and Anatomical Pathology

The list of translocations is growing every day…

EWSR1 and FUS are often involved in STS translocations

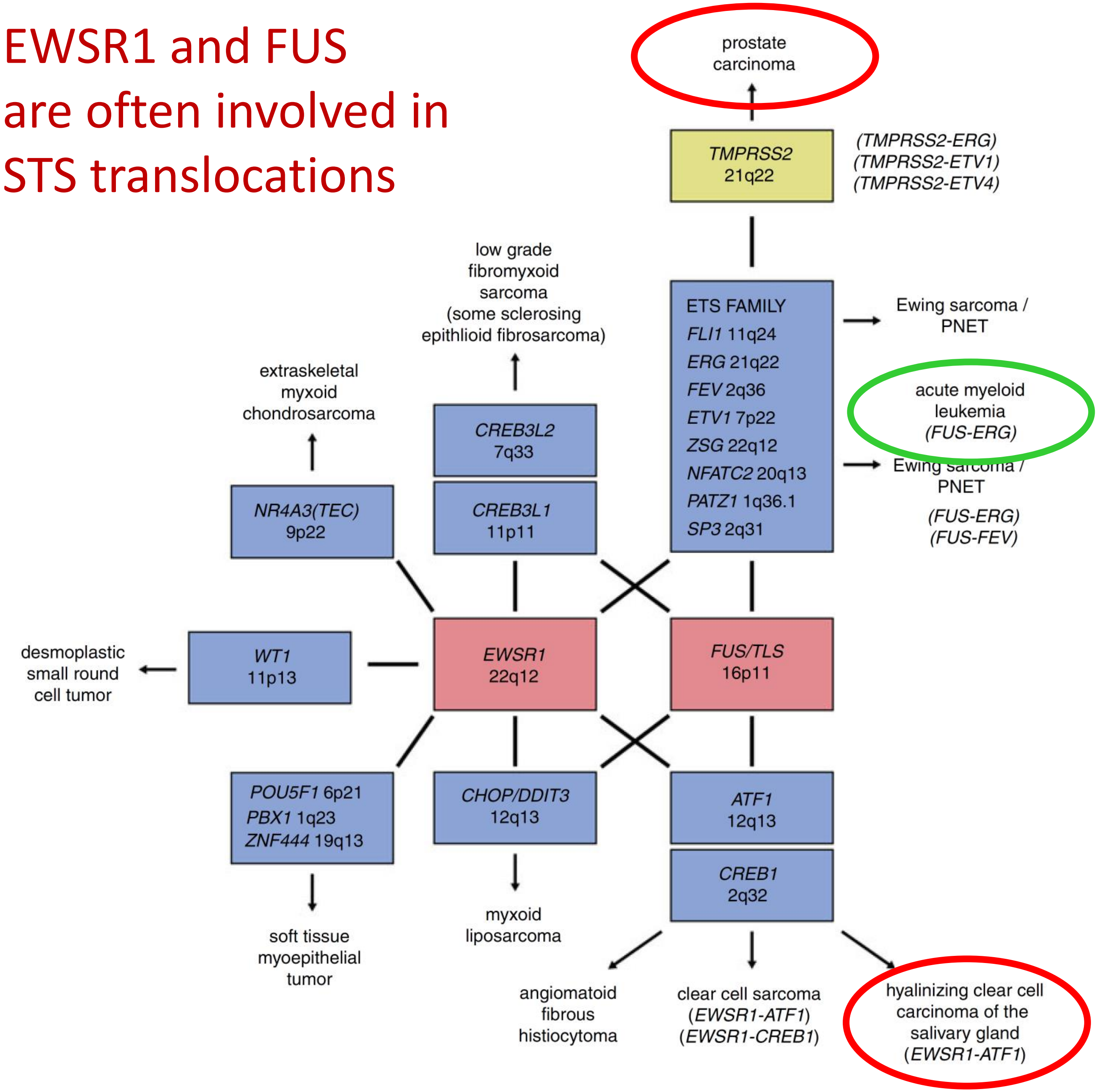
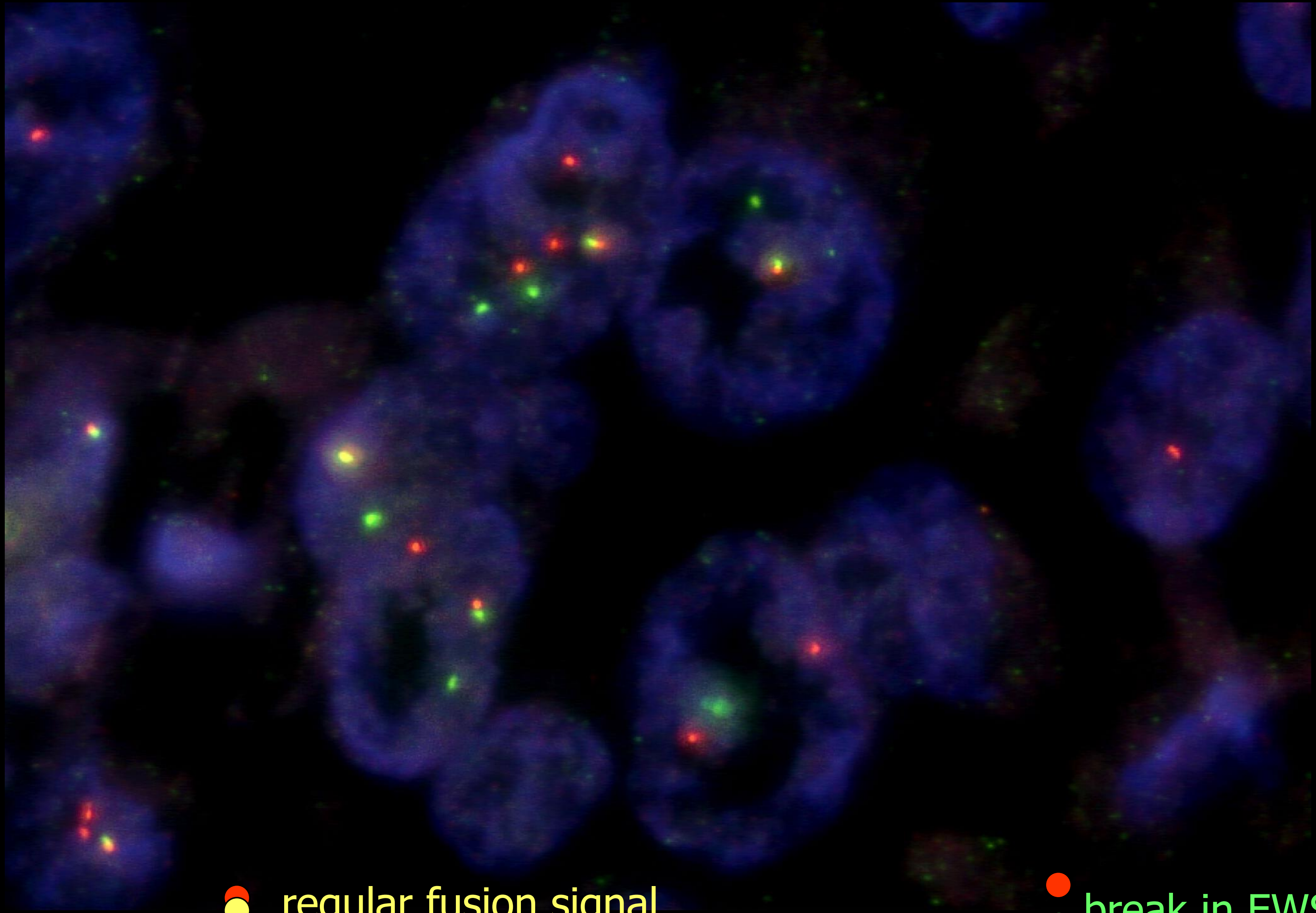


Table 2 Histologic promiscuity of chromosome translocations

Translocation	Tumor type
ETV6-NTRK3/t(12;15)	Infantile fibrosarcoma Acute myeloid leukemia Secretory breast carcinoma
ALK gene fusions	Inflammatory myofibroblastic tumor Anaplastic large cell lymphoma
FUS-ERG/t(16;21)	Ewing's sarcoma Acute myeloid leukemia
ASPL-TFE3/t(X;17)	Alveolar soft part sarcoma Subset of pediatric renal carcinomas
EWS-ATF1/t(12;22) and EWS-CREB1/t(2 : 22)	Clear cell sarcoma Angiomatoid fibrous histiocytoma

- most translocations are not specific for a given tumor entity
- translocations exist in different cellular lineages and lead to all kinds of cancer

History: Fluorescence in-situ hybridisation (FISH)



regular fusion signal

break- apart probe chromosome 22 EWSR1

break in EWSR1

Clear cell sarcoma

RNA sequencing…

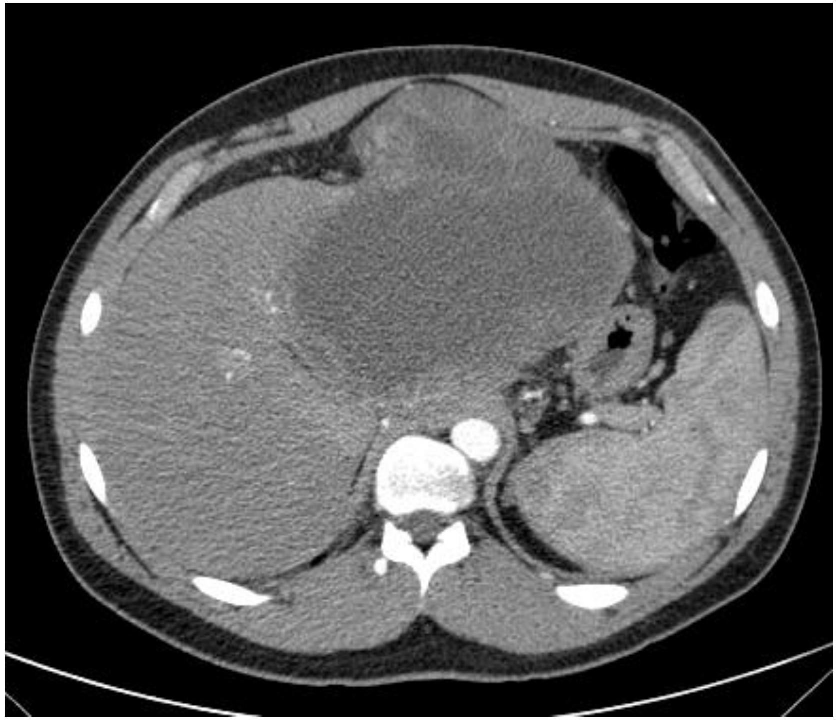
- is helpful to detect translocations (especially in sarcomas)
- allows synchronous analysis of multiple potential translocation partners
- is possible in FFPE material
- depends on RNA quality in FFPE material
- is more expensive and time consuming than FISH analysis

10 patient cases:

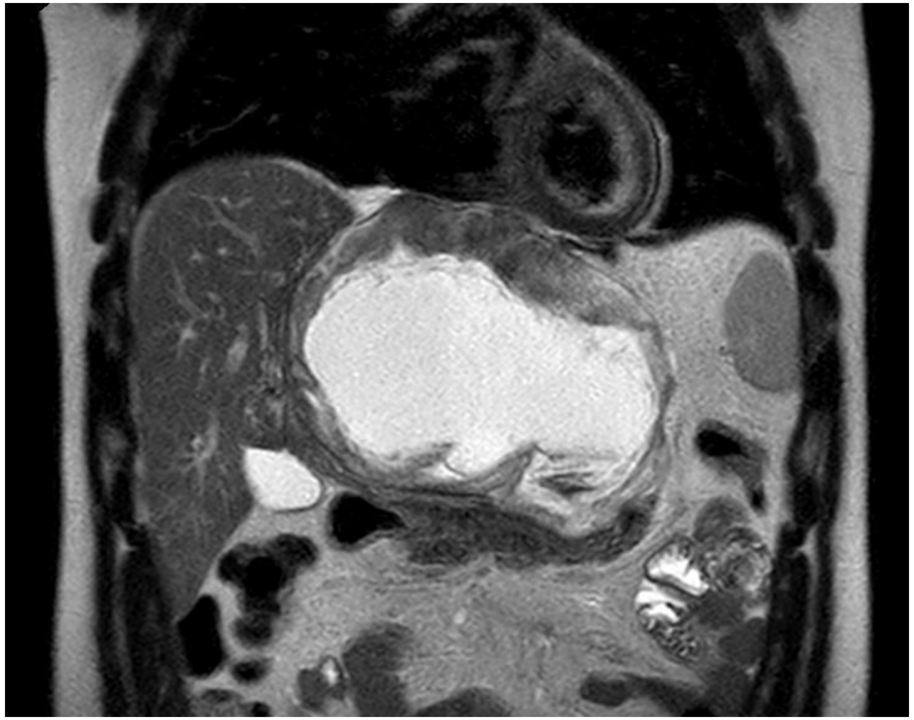
Case #:	Remarks/patient history:	Fusion transcripts (RNA-seq):
Patient 1 H/17/12546	Tumor location: left liver lobe First diagnosis: Solitary fibrous tumor (NAB2-STAT6 not evaluated)	**FUS-CREB3L2** (ex6/5)
Patient 2 H/15/18095	Tumor location: gastric wall IHC: no lineage-specific differentiation	**PTCH1-GLI2** (ex1/8)
Patient 3 H/17/6711	Tumor location: colon transversum descriptive diagnosis; no signs for malignancy	**RAF1-TRIM24** (ex7/10)
Patient 4 H/17/16	Tumor location: right knee Diagnosis 2013: myxoid liposarcoma (FUS FISH negative) Diagnosis 2016: synovial sarcoma (SS18 FISH positive, but RT-PCR negative)?	**SS18-SSX2** (ex3/6); **KDM6A-GRIA3** (ex2/8); **MAP2K4-MAP2K4P1** (ex7/2)
Patient 5 H/17/525	-/-	**ANXA7-CAMK2G** (ex5/ex4)
Patient 6 H/16/24338	First diagnosis: Solitary fibrous tumor (NAB2-STAT6 not evaluated)	**FGFR1-TACC1** (ex17/7); **ARHGAP26-CASC16** (ex11/3)
Patient 7 H/16/18837	Metastatic sarcoma, unknown primary	**TPR-NTRK1** (ex21/10); **TFG-GPR128** (ex3/2)
Patient 8 H/15/12110		**TFG-GPR128** (ex3/2) **COL1A1-USP6** (ex1/1)
Patient 9 H/17/15305	-/-	**MAP2K4-MAP2K4P1** (ex7/2); **RPSAP52-HMGA2** (ex1/2)
Patient 10 H/15/20277	Tumor: brain metastasis; klein-rund-blauzelliger Tumor FISH: SS18, EWSR1, FUS negative; CIC 25/100 pos; RT-PCR: BCOR-CCNB3 negative	**MAP2K4-MAP2K4P1** (ex4/2); **RPSAP52-HMGA2** (ex1/2)

Case report demonstrating the relevance of a specific translocation detection to establish the diagnosis

21-years old patient with 15 cm large tumor in the left liver lobe



CT

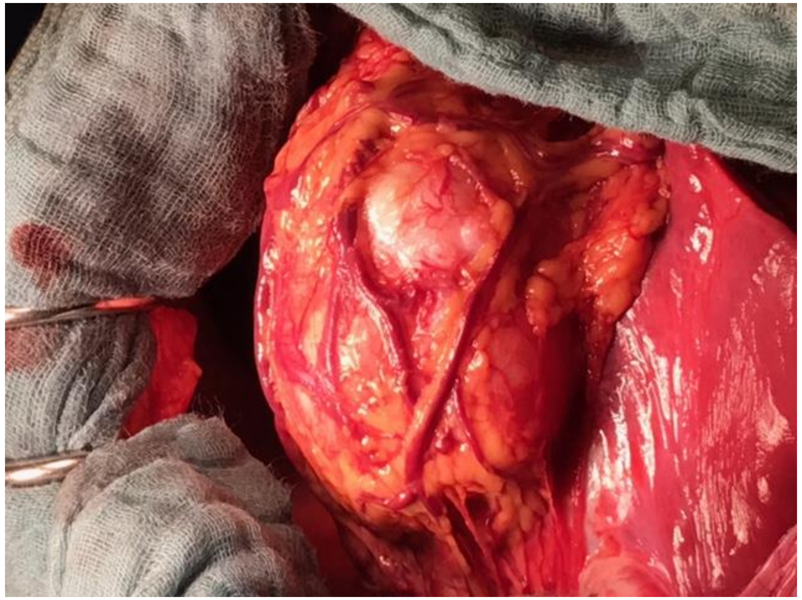


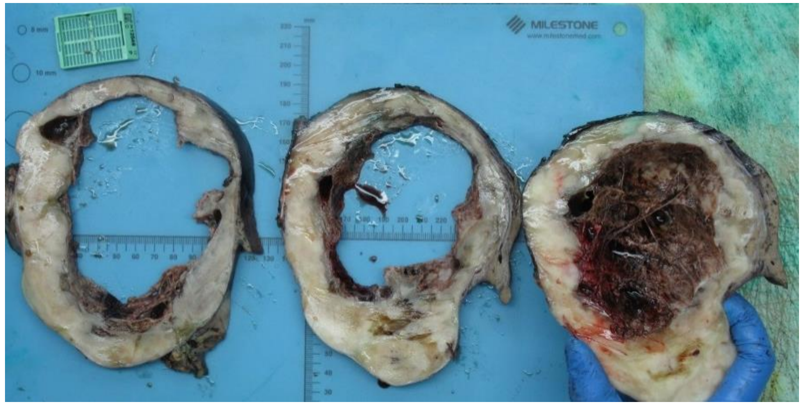
MRI

Courtesy PD Dr. C. Schülke, Radiologische Klinik (Direktor Prof. W. Heindel)

Intraoperative aspect H12546/17

Courtesy Prof. D. Palmes, Chirurgische Klinik
UK Münster (Direktor: Prof. N. Senninger)



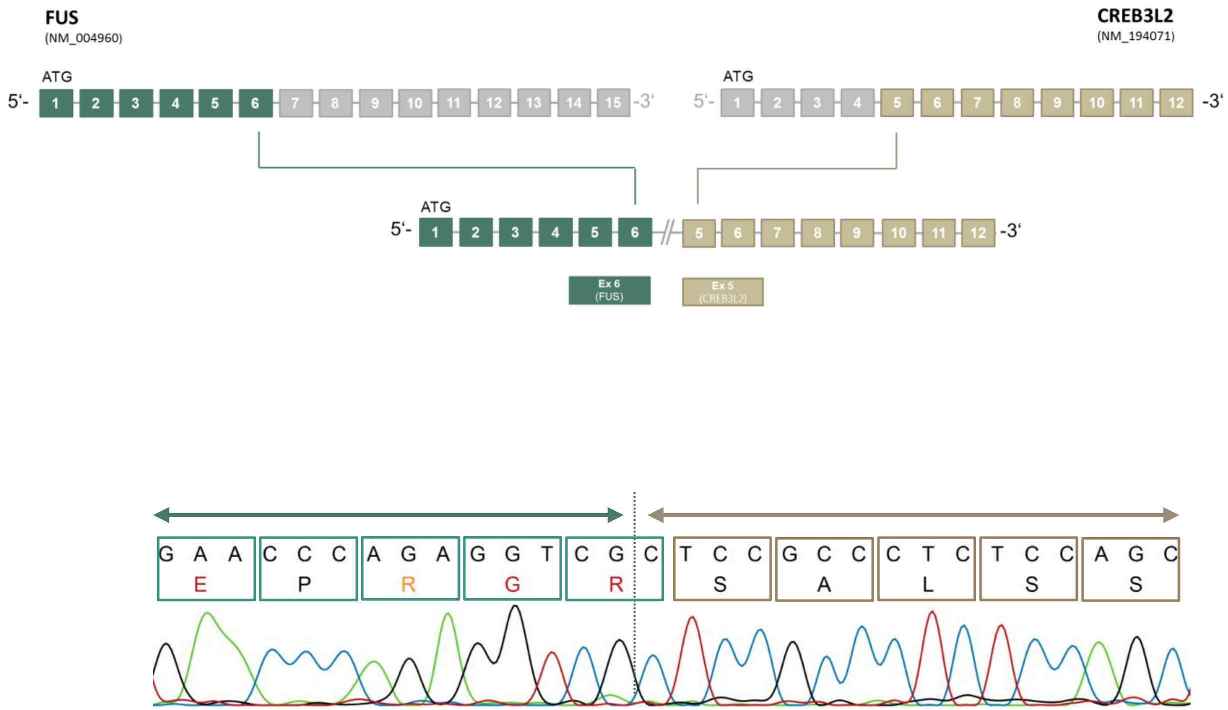


Macroscopy after fixation

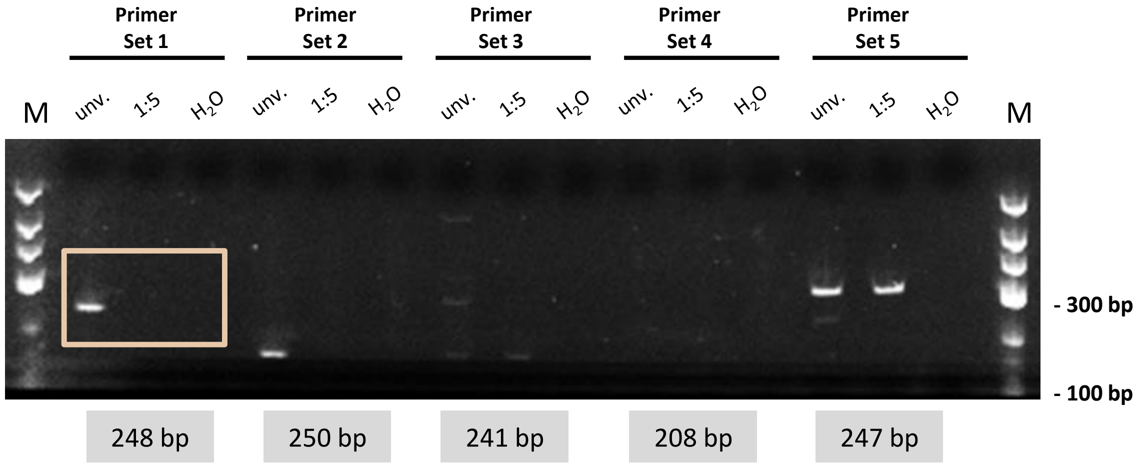
Patient 12546/17
FUS-CREB3L2

FUS-CREB3L2

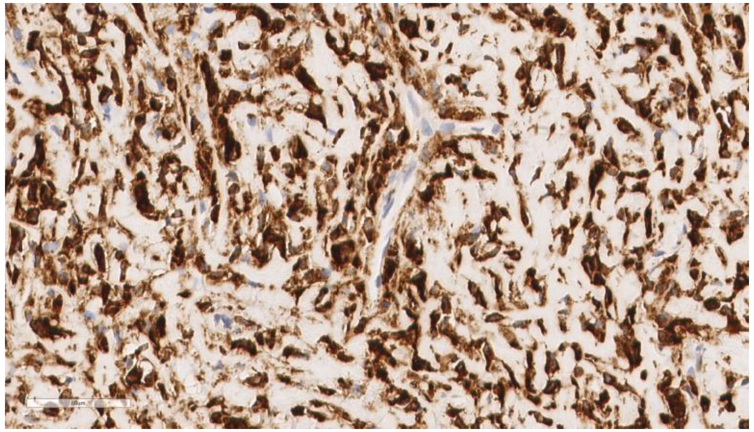
t(16;7) (p11.2;7q33)



In-frame fusion



Diagnosis: sclerosing epitheloid fibrosarcoma of the liver; IHC: MUC-4 positive



Courtesy Ruth Berthold

Molecular analysis as a key for diagnosis and therapy

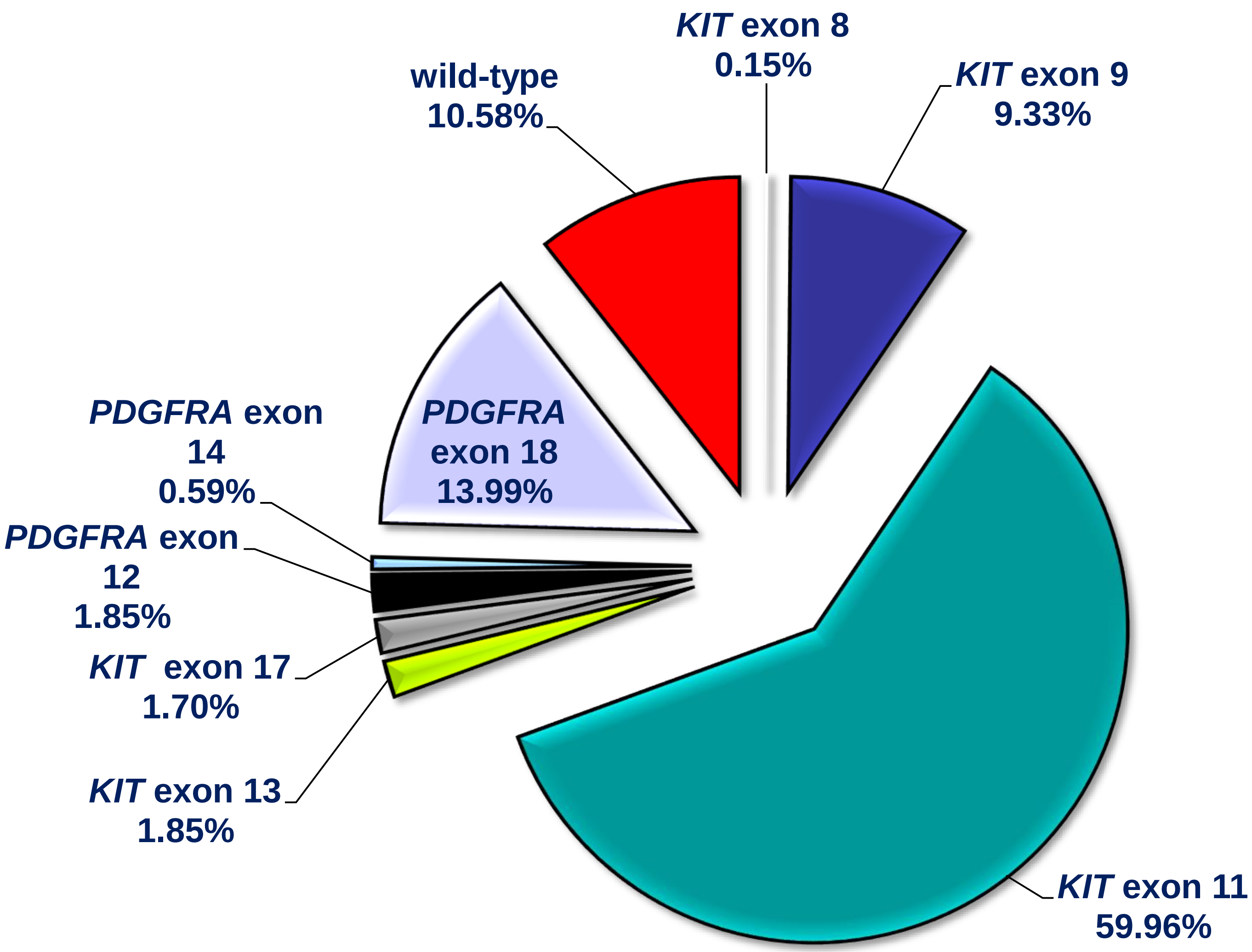
- specific reciprocal translocations
- specific somatic mutations
- more or less specific amplifications
- unspecific complex karyotypes

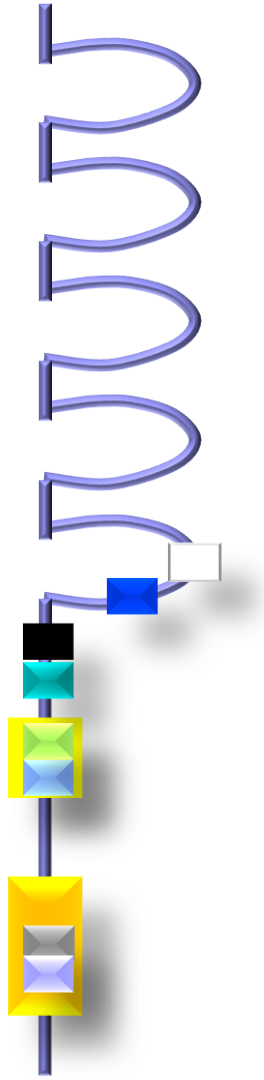
Sarcomas with specific somatic mutations

- Gastrointestinal stromal tumors (GIST) (KIT/PDGFRA)
- Fibrous dysplasia and myxomas (GNAS1)
- Rhabdoid tumors (hSNF5/INI1)

> - used to be determined by Sanger-Sequencing
> - More sensitive technology is Next Generation Sequencing (NGS)

KIT and *PDGFRA* mutations occur in 85% to 90% of all GIST (n=1351)





Frequency of different molecular subtypes in GIST

The image part with relationship ID rId2 was not found in the file.

Schaefer IM et al. Adv Anat Pathol 2017

Molecular classification of GIST

+ paraganglioma

+ paraganglioma
+ Pulmonary chondroma

mod. acc. to Boikos and Stratakis, Endocrine 2014

Genomic progression in GIST

MAX – Myc associated factor X
DMD – gene encoding dystrophin
Losses of p16, p53 and Rb1

Schaefer IM et al. Adv Anat Pathol 2017

GIST-relevant genes

KIT exons 8, 9, 11, 13, 17
PDGFRA exons 12, 14, 18
SDHA-D (genetic and epigenetic alterations)
KRAS, BRAF
NF1
MAX (Myc associated factor X)
DMD (gene encoding dystrophin)

> other genes will pop up due to NGS

Next Generation Sequencing in GIST

COMPARISON OF TECHNOLOGIES

Next-Generation sequencing

VS.

SANGER sequencing

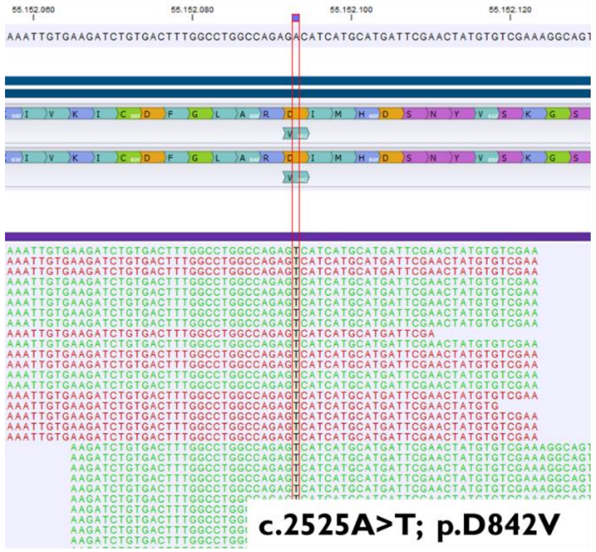
>detection limit (+/- 15-20%)

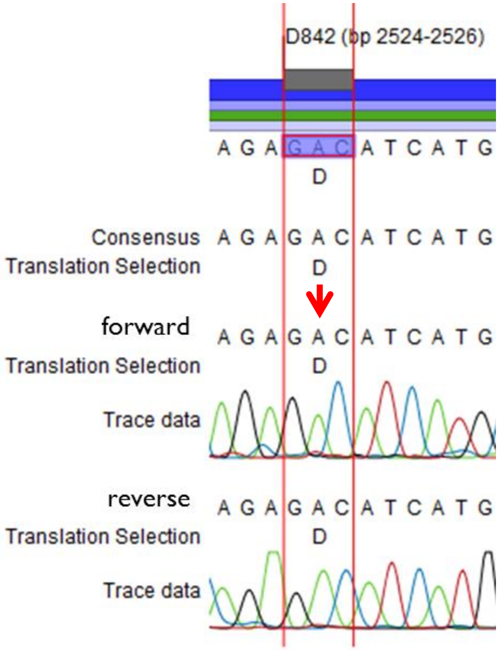
PDGFRA
Exon 18

RESULT: p.D842V

Chromosome	Region	Reference	Allele	Zygosity
4	55152093	A	T	Heterozygous

Count	Coverage	Frequency	COSMIC	dbSNP
72	629	11,45	736	121908585





PDGFRA
Exon 18

RESULT: WILDTYPE

Next Generation Sequencing in MYXOMA

COMPARISON OF TECHNOLOGIES

Next-Generation sequencing

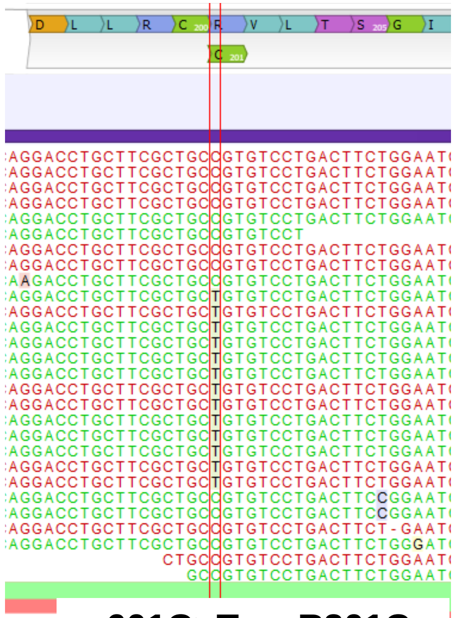
VS.

SANGER sequencing

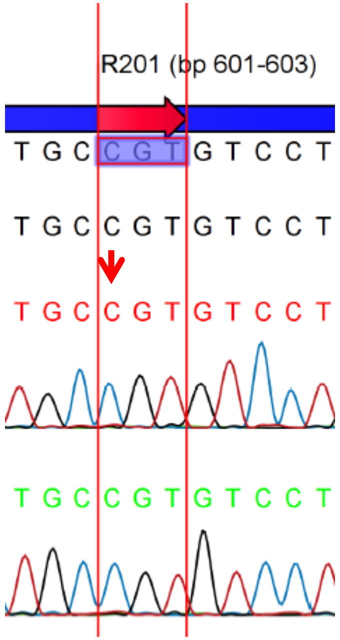
GNAS1
Exon 8

RESULT: p.R201C

Count	Coverage	Frequency
14	223	6,28



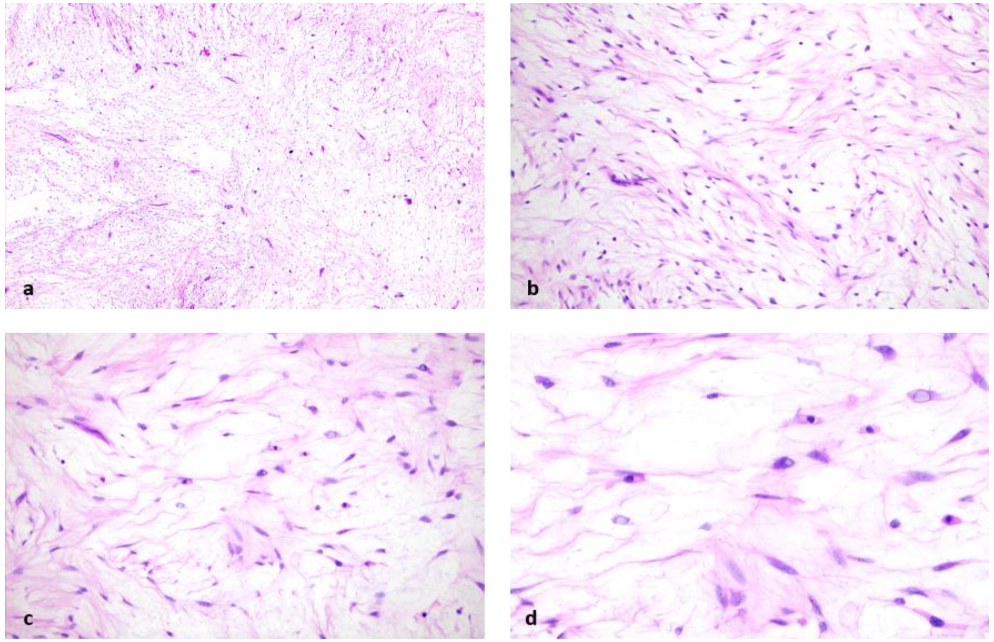
c. 601C>T; p.R201C

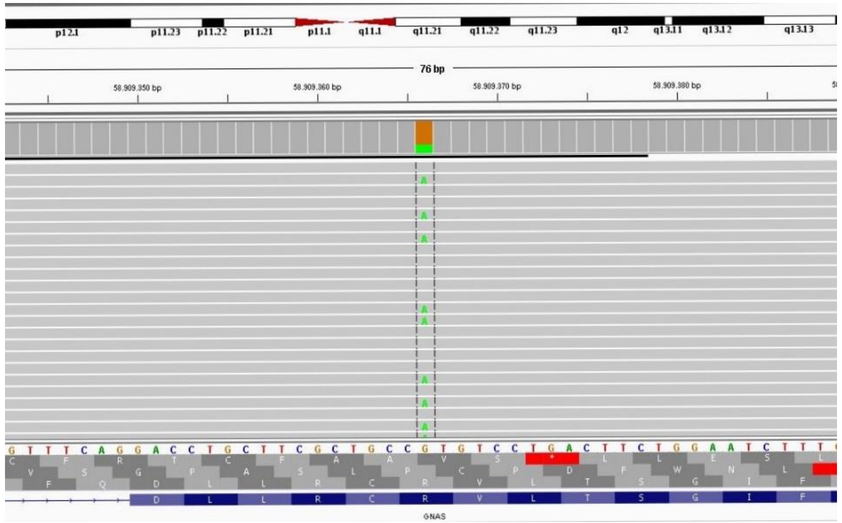


GNAS1
Exon 8

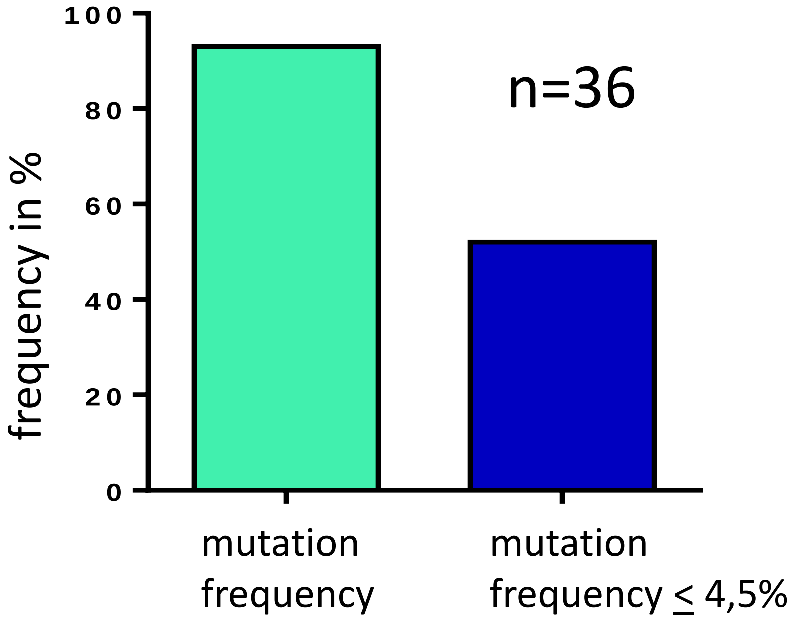
RESULT: WILDTYPE

Myxoma





GNAS1 mutation in exon 8 (p.R201C)



- NGS allows the detection of a *GNAS1* mutation in 93% of cases (frequency in the literature is 30-60%)
- NGS facilitates differential diagnosis of myxoma and myxofibrosarcoma

Courtesy Finnja Zurek-Leffers

Molecular analysis as a key for diagnosis and therapy

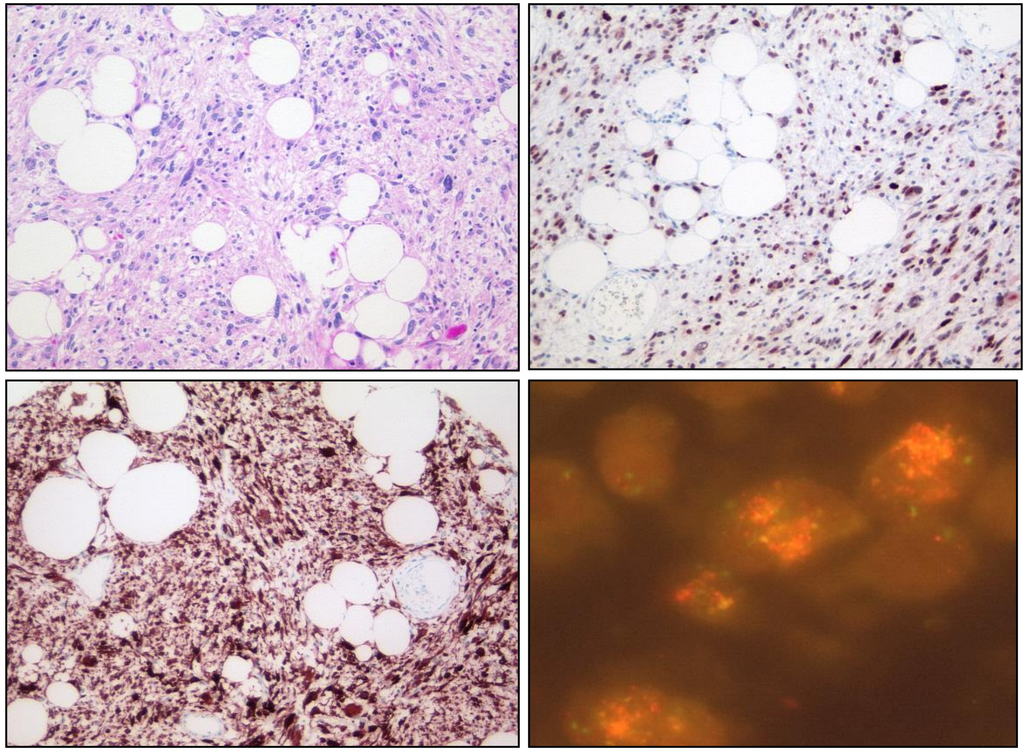
- specific reciprocal translocations
- specific somatic mutations
- more or less specific amplifications
- unspecific complex karyotypes

Sarcomas with more or less specific amplifications

- dedifferentiated/well differentiated liposarcomas/atypical lipomatous tumors (MDM2/CDK4)

H&E

MDM2



CDK4

MDM2-FISH

Molecular analysis as a key for diagnosis and therapy

- specific reciprocal translocations
- specific somatic mutations
- more or less specific amplifications
- unspecific complex karyotypes

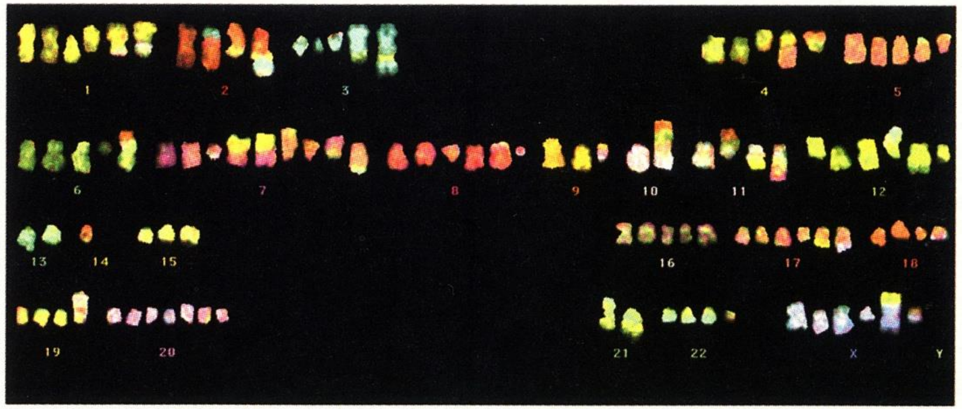
Sarcomas with unspecific complex karyotypes

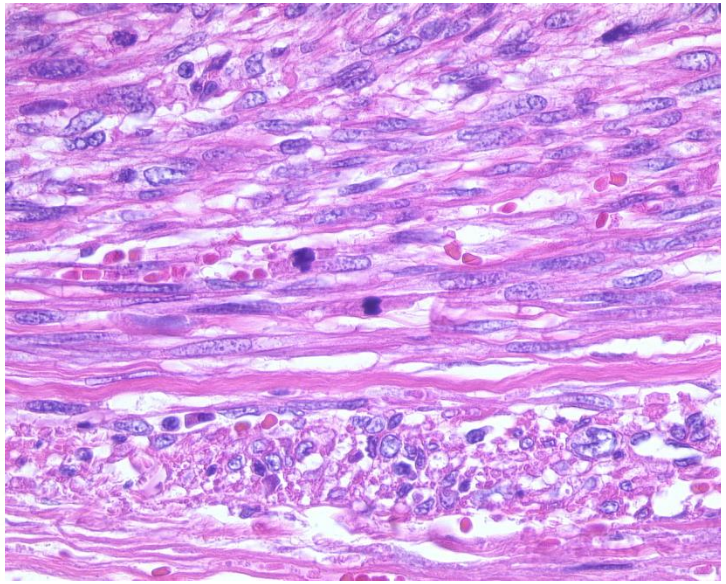
- undifferentiated pleomorphic high-grade sarcomas (UPS; formerly MFH)
- myxofibrosarcomas
- leiomyosarcomas

– complex structural and numeric chromosomal aberrations

– Cellular metabolism not predictable

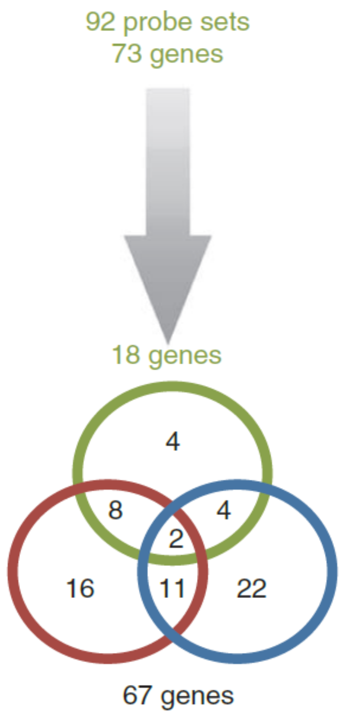
– mostly grade 3 sarcomas



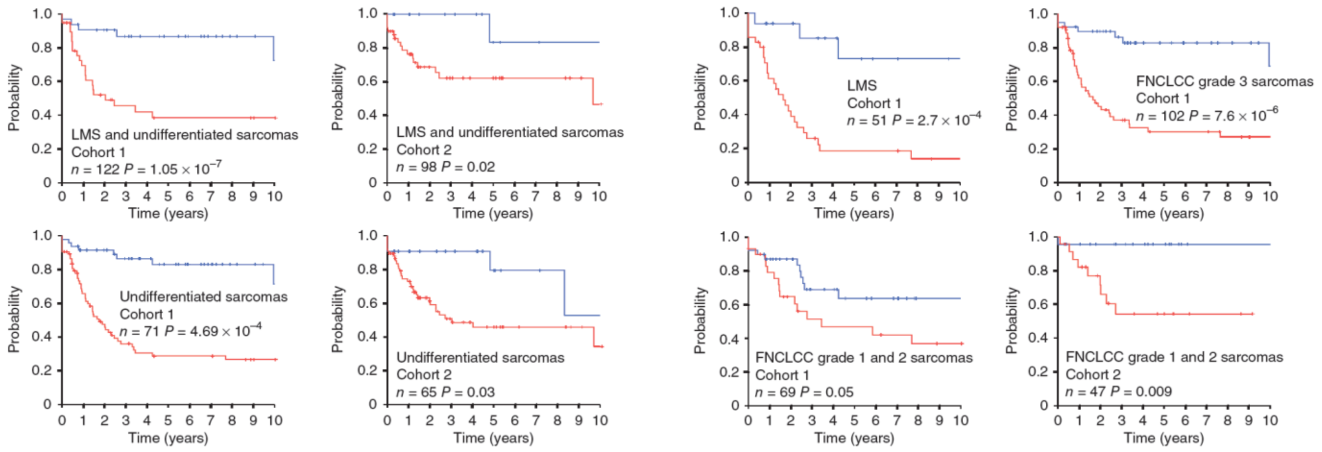


leiomyosarcoma

Metastatic signatures in sarcomas



Chibon et al. Nature Med 2010



CINSARC = complexity index in sarcomas

Table 2. Prognostic Value of CINSARC Signature, Genomic Index, and *CDCA2* and *KIF14* Expression Evaluated Against the FNCLCC Grading System

	Univariate Analyses			Multivariate Analyses		
Variable	HR	95% CI	*P*	HR	95% CI	*P*
CINSARC	**6.2**	**1.88 to 20.71**	**6.3×10^{-4}**	**6.58**	**1.57 to 61.01**	**.007**
FNCLCC	3.4	1.36 to 8.9	5.6×10^{-3}	2.19	0.72 to 8.70	.17
Genomic Index	**21.34**	**2.08 to 20.68**	**2.8×10^{-4}**	**4.20**	**1.49 to 14.26**	**.006**
FNCLCC	**3.4**	**1.36 to 8.9**	**5.6×10^{-3}**	**3.38**	**1.12 to 13.41**	**.03**
CDCA2	**7.6**	**2.63 to 22**	**1.1×10^{-5}**	**4.83**	**1.49 to 20.37**	**.007**
FNCLCC	3.4	1.36 to 8.9	5.6×10^{-3}	1.66	0.51 to 7.03	.42
KIF14	**8.04**	**2.77 to 23.37**	**5.9×10^{-6}**	**6.58**	**1.92 to 34.27**	**.0016**
FNCLCC	3.4	1.36 to 8.9	5.6×10^{-3}	1.98	0.63 to 7.97	.25

NOTE. Analyses used Cox regression model. Significant results are indicated in bold.

Abbreviations: *CDCA2*, cell division cycle A2; CINSARC, Complexity Index in Sarcoma; FNCLCC, Fédération Nationale des Centres de Lutte Contre le Cancer; HR, hazard ratio; *KIF14*, kinesin family member 14.

Lagarde et al. JCO 2013

What is the role of stem cell characteristics for sarcoma biology (for local aggressive-ness and metastatic potential)?

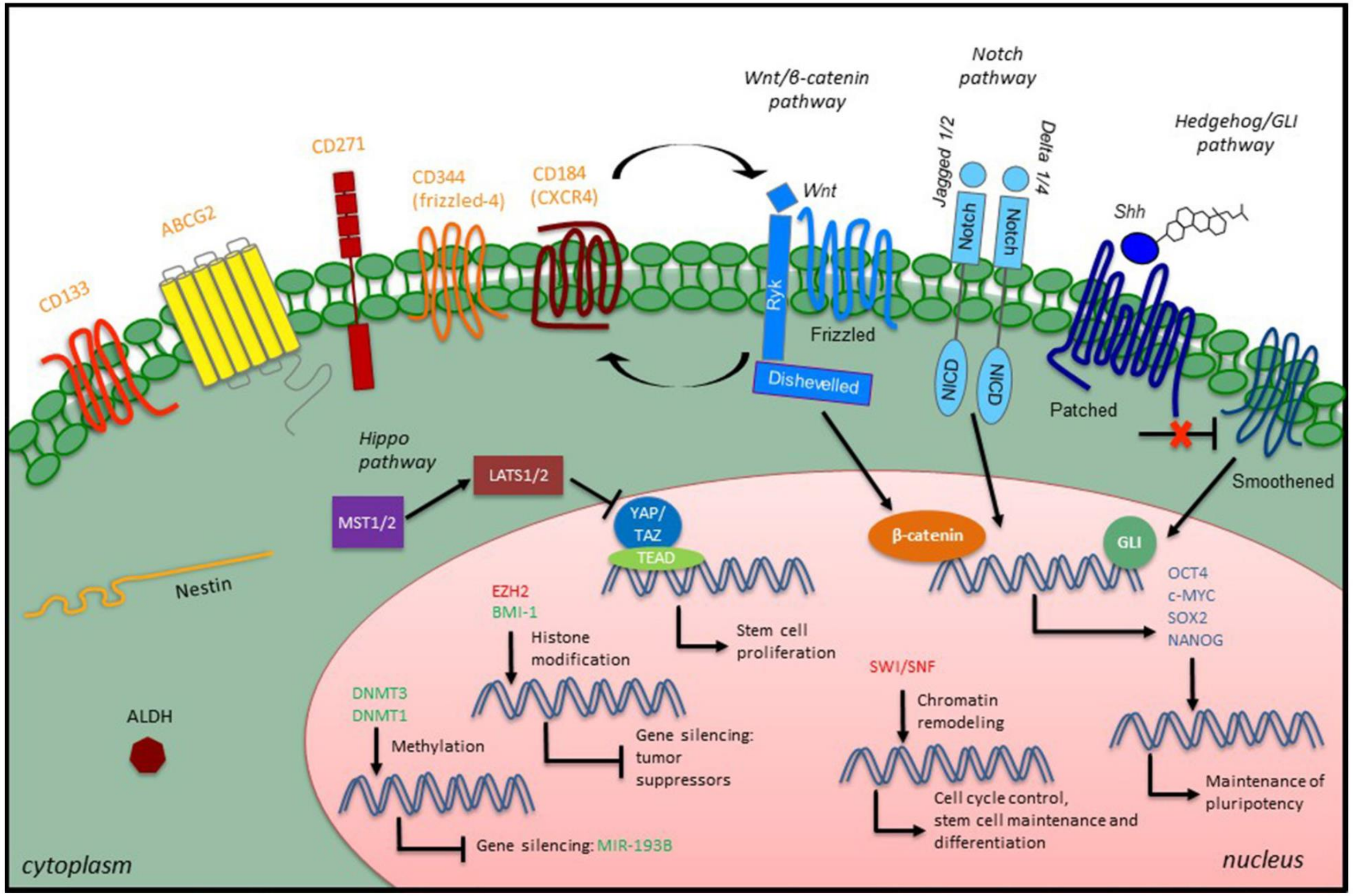


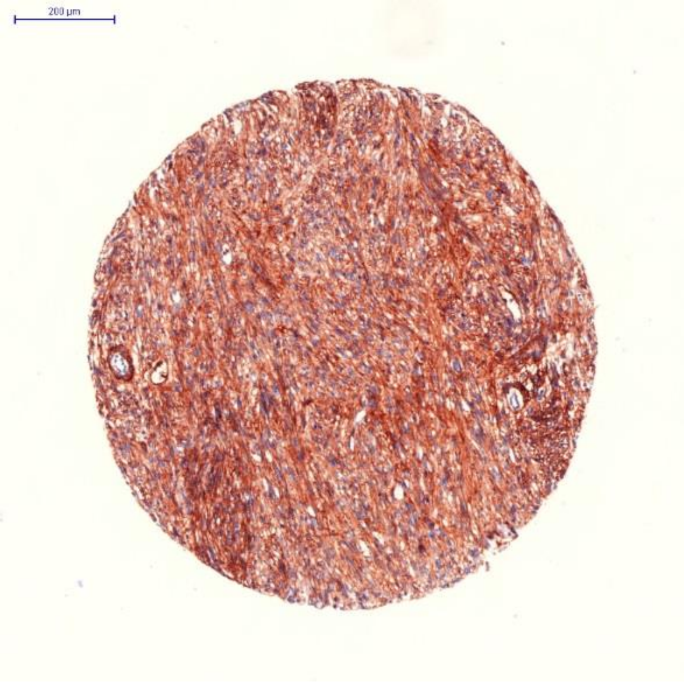
FIGURE 1 | Overview of CSC characteristics and signaling in STS. STS CSCs express specific stem cell surface markers (orange), which have been used as CSC identifiers, along with some intracellular markers such as the intermediate filament Nestin or the enzyme ALDH. Developmental signaling pathways play a role in the CSC phenotype by promoting the expression of embryonic transcription factors (blue). Epigenetic modulators (green, confirmed modulators; red, putative modulators) also participate in the CSC phenotype through different mechanisms: maintenance of existing methylation patterns or *de novo* methylations at CpG islands, histone modification and chromatin remodeling.

Soft Tissue Sarcoma Cancer Stem Cells: An Overview

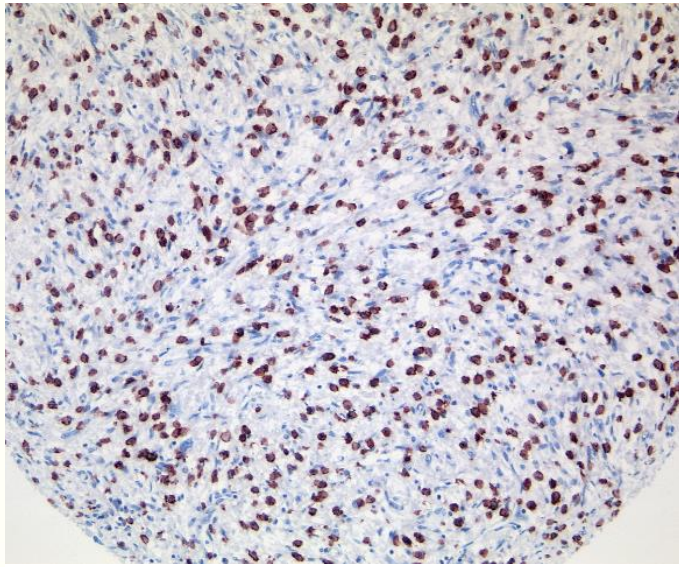
Katia C. Genadry[1†], Silvia Pietrobono[2†], Rossella Rota[2‡] and Corinne M. Linardic[1,3*‡]

REVIEW
published: 26 October 2018
doi: 10.3389/fonc.2018.00475

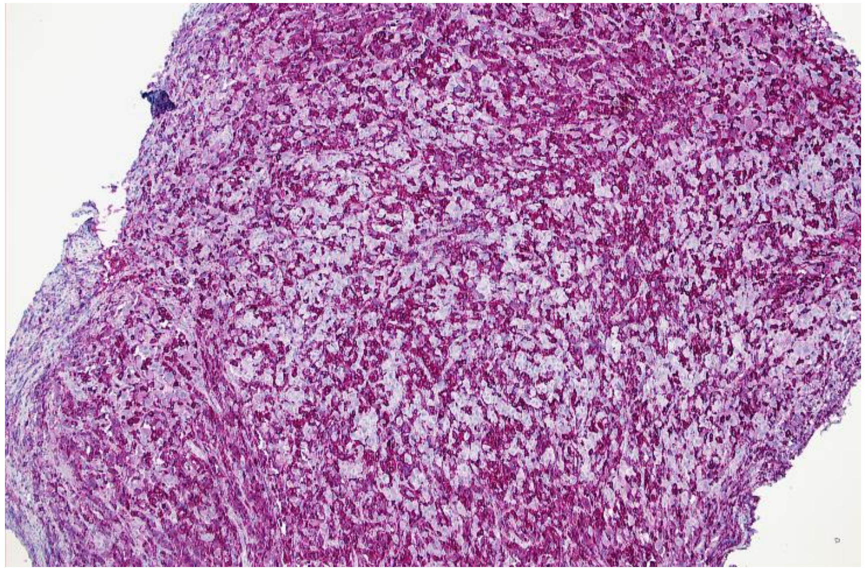
What is the role of tumor microenvironment in sarcomas?



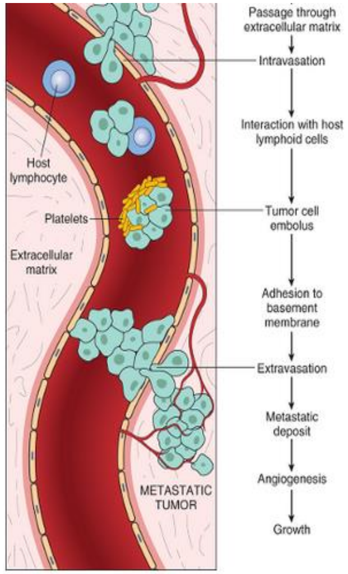
collagen type IV (DDLS)



CD117+ mast cells (DDLS)



CD163+ macrophages (UPS)



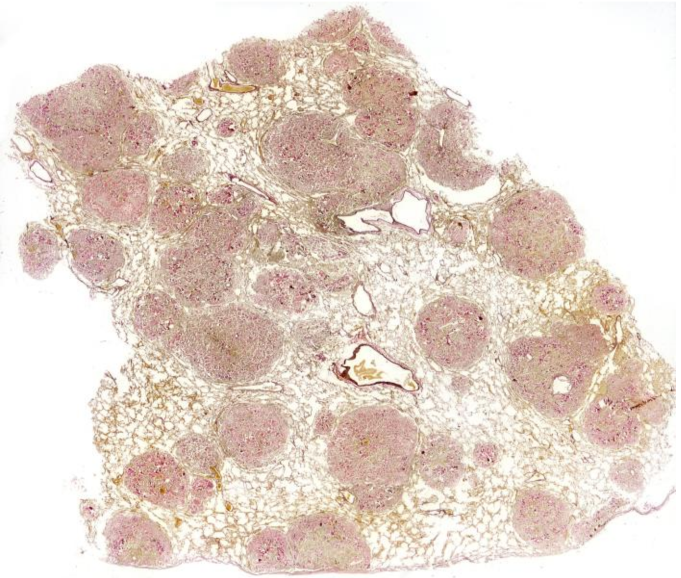
Robbins Pathologic Basis of Disease, 8/E

Metastatic cascade

1. migration
2. vessel invasion
3. adhesion
4. growth and angiogenesis

Potential targets

Src
MMPs
HIF1α
PLOD2
gp78
Chemokine receptors
CD44
Notch
SK3
....



Lung metastases in osteosarcoma

Some open questions:

- Why do some sarcoma subtypes metastasize preferentially to lymphogenic and not hematogenic?
- What is the role of the patient‘s immune system to prevent metastasis?
- Are circulating tumor cells/is circulating tumor DNA a proof of metastatic disease?

NATURE REVIEWS | CLINICAL ONCOLOGY

Using biology to guide the treatment of sarcomas and aggressive connective-tissue tumours

Armelle Dufresne[1], Mehdi Brahmi[1], Marie Karanian[2] and Jean-Yves Blay[1]*

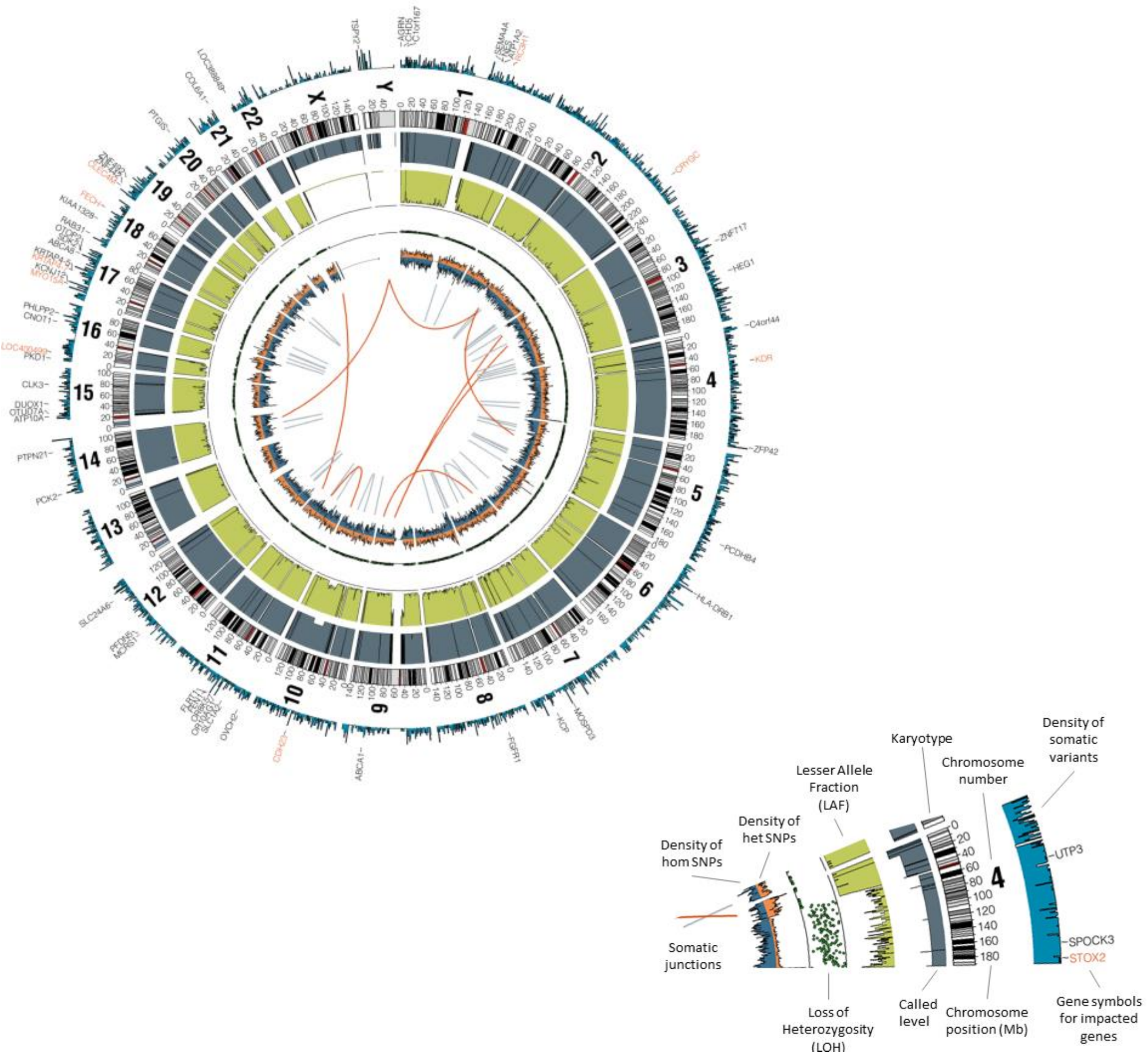
Nat Rev Clin Oncol. 2018 Jul;15(7):443-458. doi: 10.1038/s41571-018-0012-4

Table 2 | **Targetable biological alterations in sarcomas**

Biological dysregulation	Actionable target	Histotype	Agent	ORR	Study design	PFS (months)	Refs
Protein kinase receptor activation							
KIT and/or *PDGFR* activating mutation	KIT and/or PDGFR	GIST	Imatinib	51%	Phase III	20.4	13
			Sunitinib	33%	Phase III	6.0 versus 1.4	23
			Regorafenib	4.5%	Phase III	4.8 versus 0.9	24
BRAF activating mutation	BRAF	Langerhans cell histiocytosis	Vemurafenib	43%	Basket study	NR	34
Translocation involving a growth factor or kinase							
COL1A1–PDGFB fusion transcript	PDGFR	DFSP	Imatinib	46–70%	Phase II	20.4	41
COL6A3–CSF1 fusion transcript	CSF1R	PVNS	Imatinib	19%	Retrospective	21.0	44
ALK fusion transcript (various partners)	ALK and/or ROS1	IMT	Crizotinib	1/1	Case report	NR	49
ETV6–NTRK fusion transcript	NTRK	Infantile fibrosarcoma and others	Entrectinib	100%	Phase I	NR	152
Translocation involving transcription factors							
EWRS1 fusion transcript (various partners)	IGF1R	Ewing sarcoma	R1507 (IGF1R inhibitor)	10%	Phase II	7.3	61
ASPL–TFE3 fusion transcript	VEGFR	ASPS	Pazopanib	27%	Retrospective	13.6	67
			Cediranib	35%	Phase II	NR	69
Inactivation of tumour suppressor gene							
TSC1 and/or *TSC2* inactivating mutation	mTOR	PEComas	mTOR inhibitor	5/5	Case report	NR	85,86
PTEN expression loss	mTOR	Leiomyosarcoma	mTOR inhibitor	5%	Phase II	<2.0	94
Gene amplification							
MDM2 amplification	MDM2	Liposarcoma	MG7112	5%	Phase II	NR	101
Epigenetic alterations							
SMARCB1 loss	EZH2	Epithelioid sarcoma	Tazemetostat	19%	Phase II	NR	113
SSX–SS18 fusion transcript	EZH2	Synovial sarcoma	Tazemetostat	0/33	Phase II	NR	114
H3F3	RANKL	GCTB	Denosumab	70%	Phase II	NR	119
Protein overexpression							
Unknown	PDGFR and/or ligands	Sarcoma	Olaratumab	18.2%	Randomized phase II	6.6 versus 4.1 (OS 26.5 versus 14.7)	124
Unknown	VEGFRs and/or ligands	Sarcoma (except liposarcoma)	Pazopanib	6%	Phase III	4.6 versus 1.6	126
Hormone receptors	Hormone receptors	ESS	Aromatase inhibitors	46%	Retrospective	36.0	134
Hormone receptors	Hormone receptors	Uterine leiomyosarcoma	Aromatase inhibitors	0	Phase II	3.0	135

ASPS, alveolar soft part sarcoma; DFSP, dermatofibrosarcoma protuberans; ESS, endometrial stromal sarcoma; EZH2, enhancer of zeste homologue 2; GCTB, giant cell tumour of bone; GIST, gastrointestinal stromal tumour; IMT, inflammatory myofibroblastic tumour; MDM2, E3 ubiquitin-protein ligase MDM2; NR, not reported; ORR, overall response rate; OS, overall survival; PEComas, perivascular epithelioid cell neoplasms; PFS, progression-free survival; PVNS, pigmented villonodular synovitis; RANKL, receptor activator of nuclear factor-κB ligand; VEGFR, VEGF receptor.

Future Issues in Sarcoma and GIST



- to introduce novel diagnostic tools
- to facilitate centralized and standardized pathology
- to identify novel targets
- to develop new drugs
- to test new combinations
- to perform innovative statistics
- to modify regulations