Molecular Pathology of Soft Tissue Sarcomas

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Goals

- Soft tissue sarcomas
  - Background
  - General principles of sarcomagenesis

- Selected examples to illustrate:
  - Molecular pathogenesis
  - Molecular diagnostic techniques
    - Clinical utility
    - Advantages
    - Disadvantages

Soft tissue sarcoma

- Uncommon tumors:
  - ~1% of all cancers (all ages)
  - ~15% of cancers in childhood and adolescence (<20 y/o)
- Lifetime risk:
  - 1 out of 350 people

- Annual incidence, all ages (U.S. SEER data 2005-2009):
  - Soft tissue and bone: 12,000 - 15,000 new cases/year
    - Spindle/pleomorphic sarcoma, NOS 30.2%
    - Liposarcoma 16.5%
    - Leiomyosarcoma 13.7%
    - Fibrosarcoma 6.3%
    - Synovial sarcoma 5%
    - Rhabdomyosarcoma 4.3%
    - Dermatofibrosarcoma protuberans 3.2%
    - Myxosarcoma 0.8%
    - Alveolar soft part sarcoma 0.5%
    - Other 19.5

    - 850-900 new cases/year in children and adolescents (<20 y/o)
      - ~50% are rhabdomyosarcoma

Sarcomahelp.org
SEER.cancer.gov

Cancer “defined”

- Self-sufficiency in growth signals
- Insensitivity to growth-inhibitory signals
- Evasion of programmed cell death
- Limitless replicative potential
- Sustained angiogenesis
- Tissue invasion and metastasis

Hanahan D and Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70

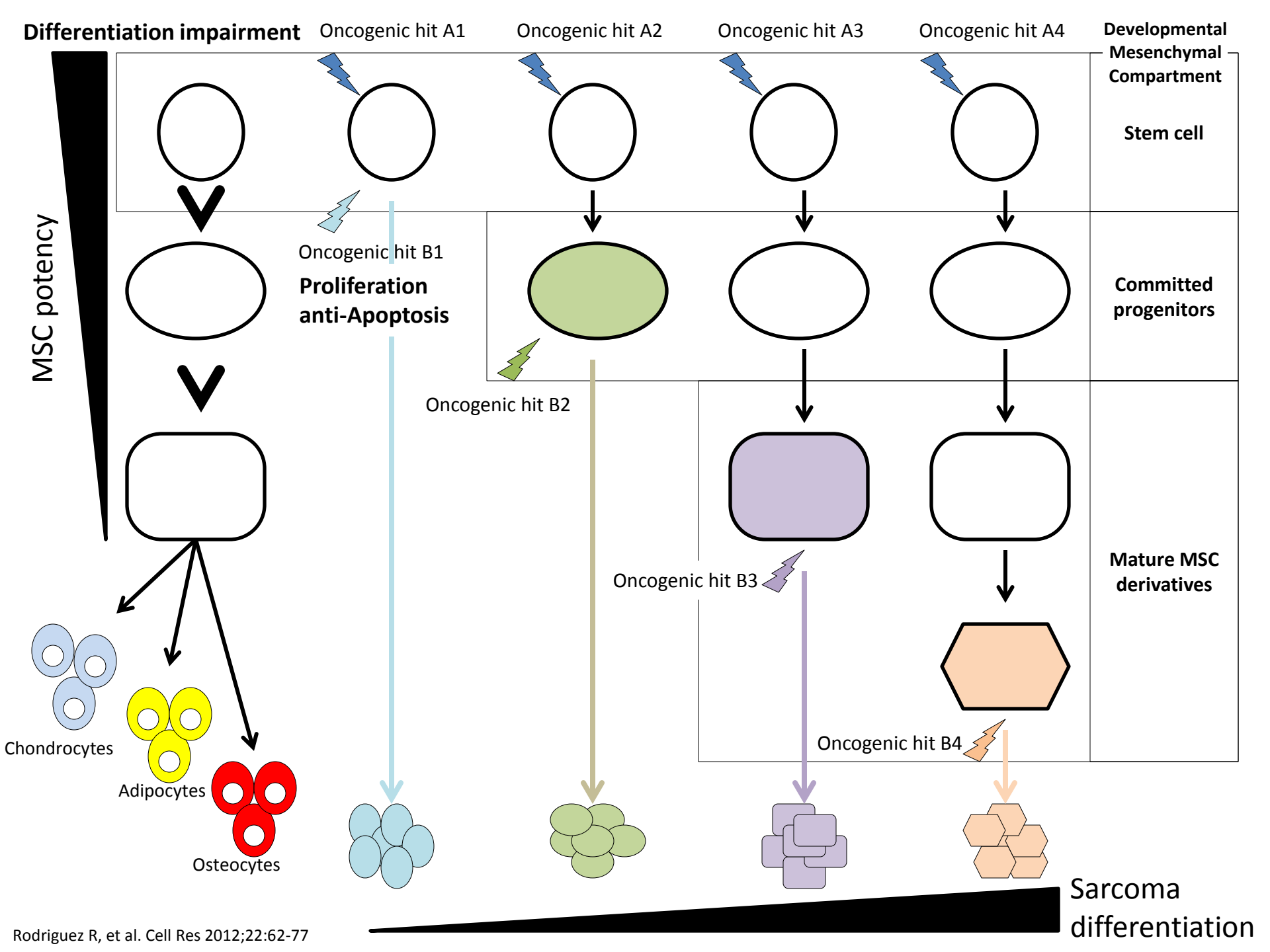
Sarcoma versus carcinoma

- Similar “cancer” criteria for sarcoma…..**but**
- Multistep tumorigenesis seen in epithelial neoplasia (carcinomagenesis) has been more difficult to document
  - Example: translocation associated sarcomas
    - No pre-neoplastic cell identified
    - Translocation may be the only genetic alteration; sufficient for the development of mesenchymal cancer
- Cell type specificity appears to be important in sarcomagenesis
  - Same translocation may be found in different tumors

Mesenchymal stem cells (MSC)

- Mouse MSCs undergo spontaneous tumorigenic transformation after long term ex-vivo culture
  - This does not happen with human MSCs
- Transformation can also be induced by inserting fusion genes, targeted gene mutations, drug or chemical treatment
  - Human MSCs are more reluctant to transform than mouse MSCs
- Specific tumor types appear to depend not only on the pathway targeted, but also the tissue of origin
- Recent evidence suggests a perivascular cell origin for MSCs
  - In long term culture human PVC
    - Display stem cell markers and markers of their tissue of origin
    - Do not display endothelial, hematopoietic or myogenic markers
    - Under proper culture conditions, PVCs can differentiate in to adipocytes, chondrocytes or osteocytes

Crisan M, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;3:301-313
Rodriguez R, et al. Modeling sarcomagenesis using multipotent mesenchymal stem cells. Cell Res 2012;22:62-77



Rodriguez R, et al. Cell Res 2012;22:62-77

Classification of gene mutations

- Driver mutations
  - Directly or indirectly confer a selective growth advantage to the cell in which it occurs

- Passenger mutations
  - No direct or indirect effect on the selective growth advantage of the cell in which it occurs

Vogelstein B, et al. Cancer Genome Landscapes. Science 2013;339:1546-1558

Classification of cancer genes

- Mechanistic classification
  - Tumor suppressor genes
    - Deactivation: deactivating mutation, deletion, or reduced expression
  - Oncogenes
    - Activation: activating mutation, or amplification
  - Caretaker genes
    - More likely to have mutations in tumor suppressor genes or oncogenes
- Functional classification
  - Protein kinases
    - Can function as oncogenes or tumor suppressor genes
  - Transcription factors
    - Can function as oncogenes or tumor suppressor genes
  - DNA maintenance and repair proteins
    - Caretaker genes

Molecular classification of soft tissue sarcomas

1. Variable & complex abnormal karyotype 50%
   - Spindle cell/pleomorphic sarcoma, NOS
   - Leiomyosarcoma
   - Myxofibrosarcoma
   - Pleomorphic liposarcoma
   - Pleomorphic rhabdomyosarcoma
   - Other
2. Reciprocal translocations 15-30%
   - Ewing sarcoma (EWSR1-FLI1)
   - Synovial sarcoma (SS18-SSX)
   - Other
3. Specific mutations
   - Gastrointestinal stromal tumors (KIT & PDGFRA activating mutations)
   - Other
4. Amplifications
   - Well-differentiated liposarcoma (12q13-15 ring chromosome)
   - Other

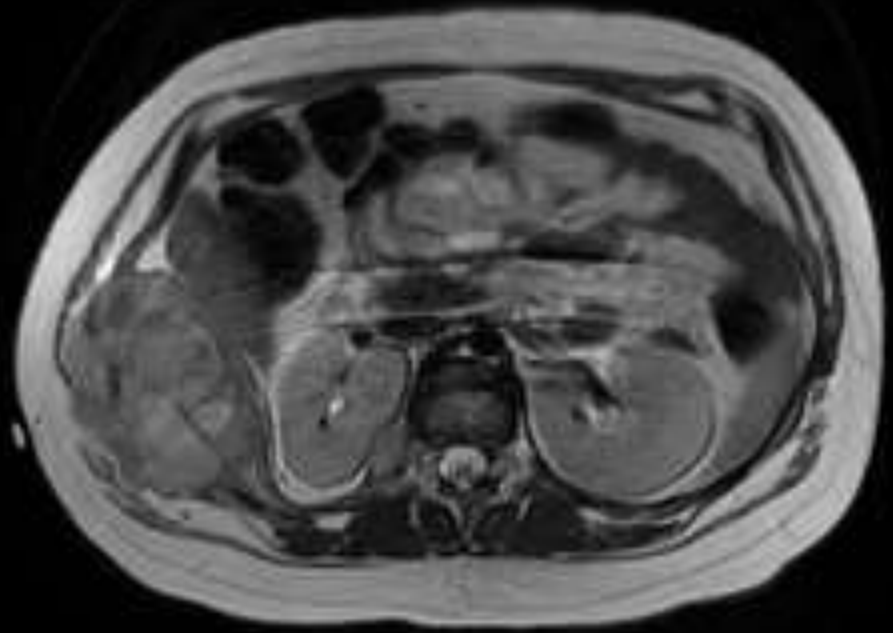
Guillou L, et al. Soft tissue sarcomas with complex genomic profiles. Virch Arch 2010;456:201-217
Fisher C. Soft tissue sarcomas with non-EWS translocations: molecular genetic features and pathologic and clinical correlations. Virch Arch 2010;456:153-166
Jain S, et al. Molecular classification of soft tissue sarcomas and its clinical applications. Int J Clin Exp Pathol 2010;3:416-429

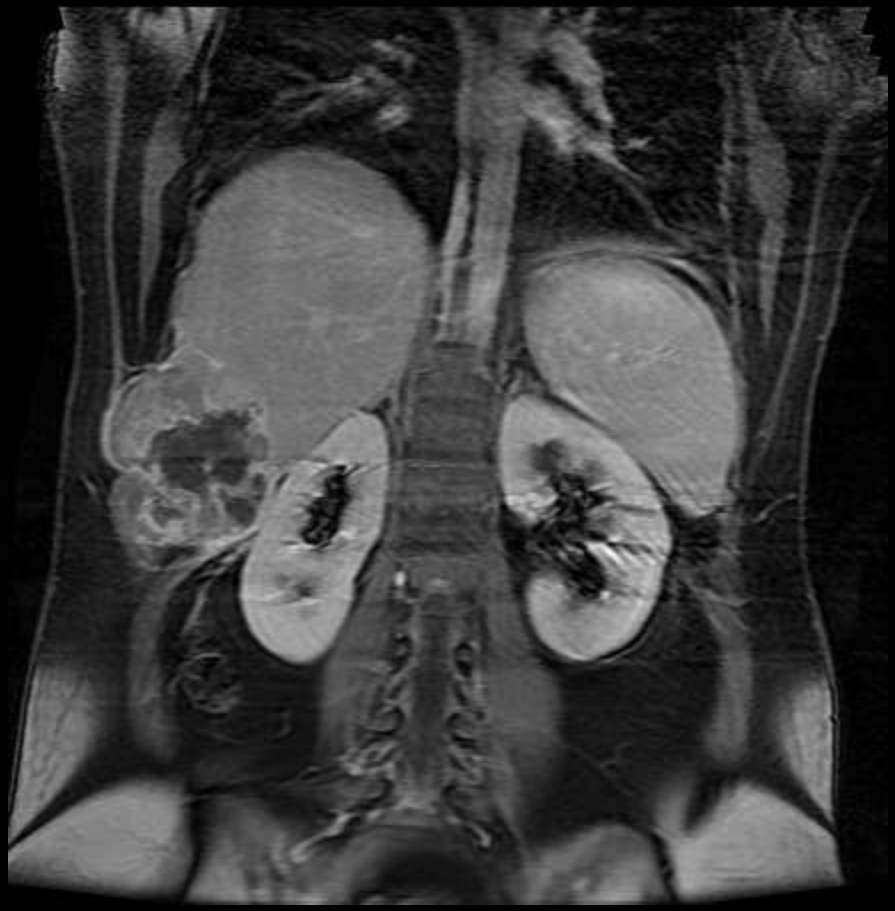
Why test?

- Potential type(s) of information obtained:
  - **Diagnostic**: Aid in rendering a morphologic diagnosis
  - **Prognostic**: Educated guess at a tumor's behavior without the influence of treatment
  - **Predictive**: Response of tumor to therapy

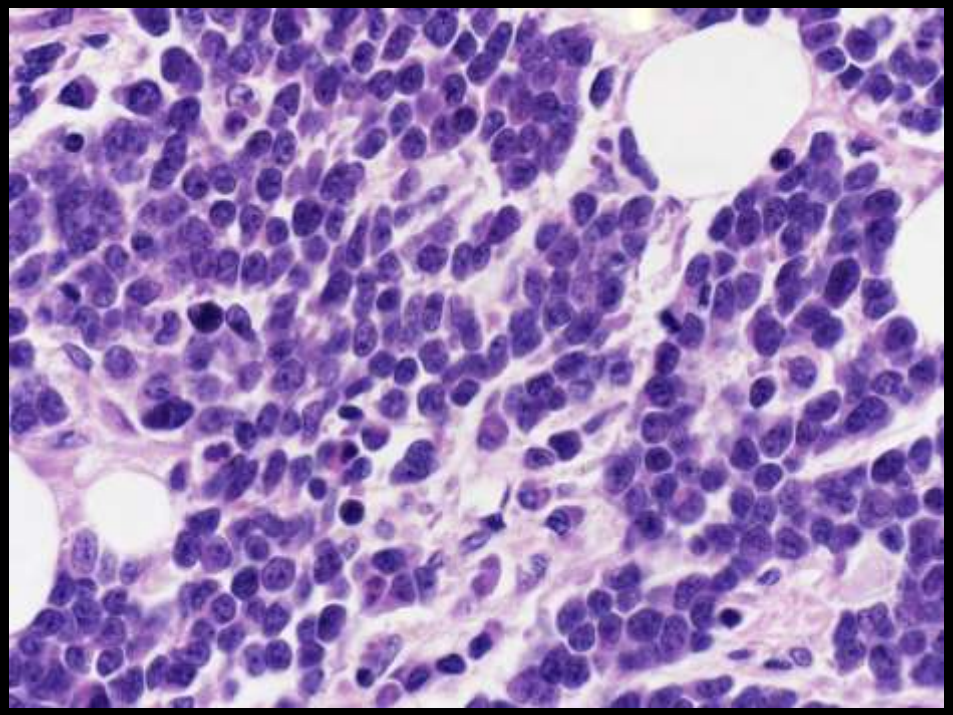
Method	Advantages	Disadvantages
Cytogenetics DNA	Global view Primary and secondary abnormalities identified Does not require knowledge of abnormality or diagnosis May detect abnormalities not seen by FISH or PCR	Requires fresh tissue (dividing cells) Low resolution Cryptic rearrangements Lower sensitivity Slower TAT
FISH DNA or RNA	More targeted view Requires prior knowledge of abnormality or diagnosis Diagnostically specific and sensitive Moderate resolution Moderate analytic sensitivity Multiple tissue types can be used FFPE, frozen, cytology or cultured cells (FISH or iFISH) Can localize abnormality to specific cells Faster TAT	Need for fluorescence microscope Signals fade Does not work on decal tissue
PCR DNA or RNA	High resolution (very targeted view) High sensitivity and specificity, and quantifiable (MRD) Multiplexing possible Can use FFPE, frozen sections, cytology, or fresh Faster TAT	Requires knowledge of abnormality Does not work on decal tissue FFPE may have degraded RNA PCR inhibitors
IHC Protein	Can use FFPE, frozen sections or cytology Morphologic correlation Rapid TAT Relatively inexpensive Mutation specific antibodies available	Interlab variablility
NGS DNA or RNA	High throughput (huge multiplex capability) High resolution (individual nucleotides level)	Cost of equipment (decreasing) Need and cost of bioinformatics

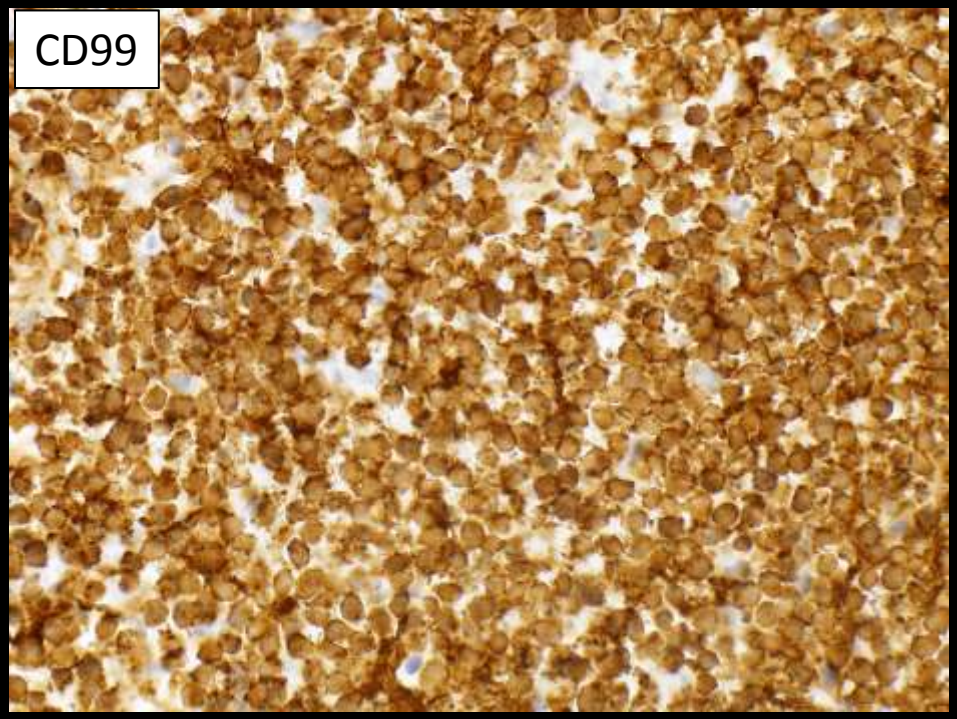
- 17 y/o female, 30 weeks pregnant, presenting with right flank pain.

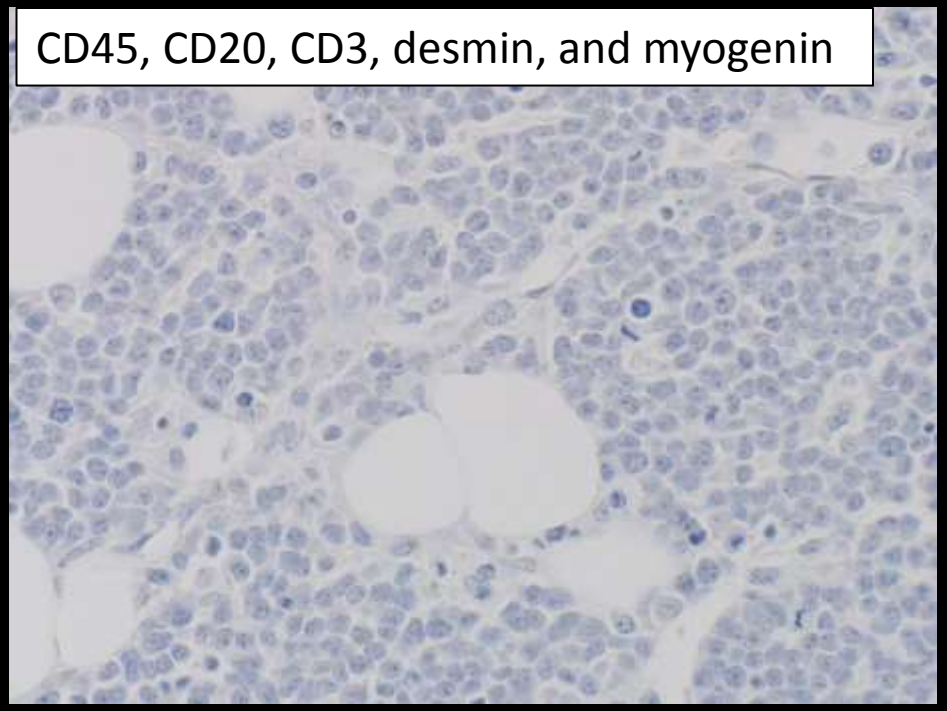


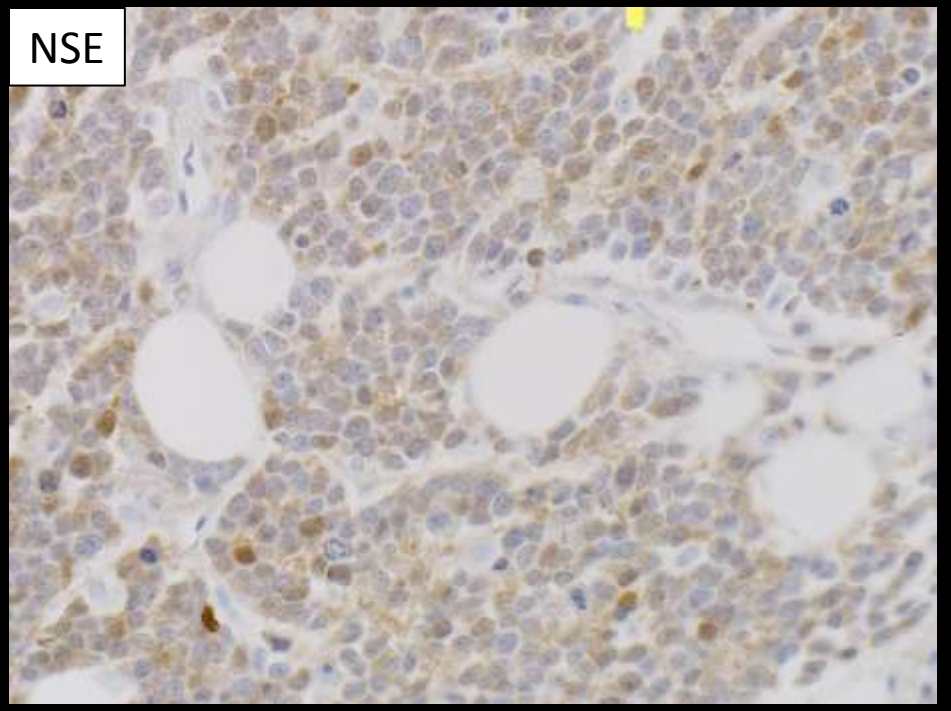


MRI: Large 10.5 x 8.7 x 6.5 cm lobulated extraperitoneal mass in the posterolateral mid and upper right abdomen, showing heterogeneous enhancement and multiple enhancing septations surrounding nodular areas with variable degrees of enhancement. It displaces the adjacent liver medially and the outer layer of adjacent thoracic/abdominal wall musculature laterally. It surrounds portions of the lower right ribs, and appears to be arising from the lower aspect of the intercostal muscles, suggesting a sarcoma.

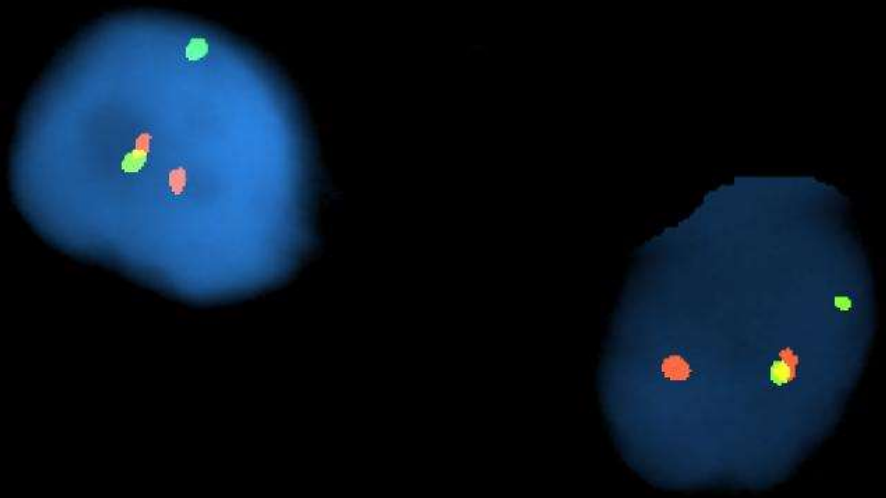



CD99


CD45, CD20, CD3, desmin, and myogenin


NSE

EWSR1 (22q12) Break Apart Probe



Positive for EWSR1 gene rearrangement

Image from Dr. Mark Micale, William Beaumont Hospital, Royal Oak, MI

Diagnosis:

- Ewing sarcoma/Primitive Neuroectodermal Tumor (ES/PNET)
  - Lung and bone metastases present
- Delivered healthy 33-week-old baby
- Treated

ES/PNET

- **Definition:**
  - Malignant small round blue cell sarcoma with variable neuroectodermal differentiation as assessed by light microscopy, immunohistochemistry, and EM
- **Recurrent translocations:**
  - EWSR1 22q12 fusions with a variety of fusion partners (promiscuous)
    - EWSR1-FLI1 t(11;22)(q24q12) 85%
    - EWSR1-ERG t(21;22)(q21;q12) 10%
    - Other

Reciprocal translocations in sarcomas

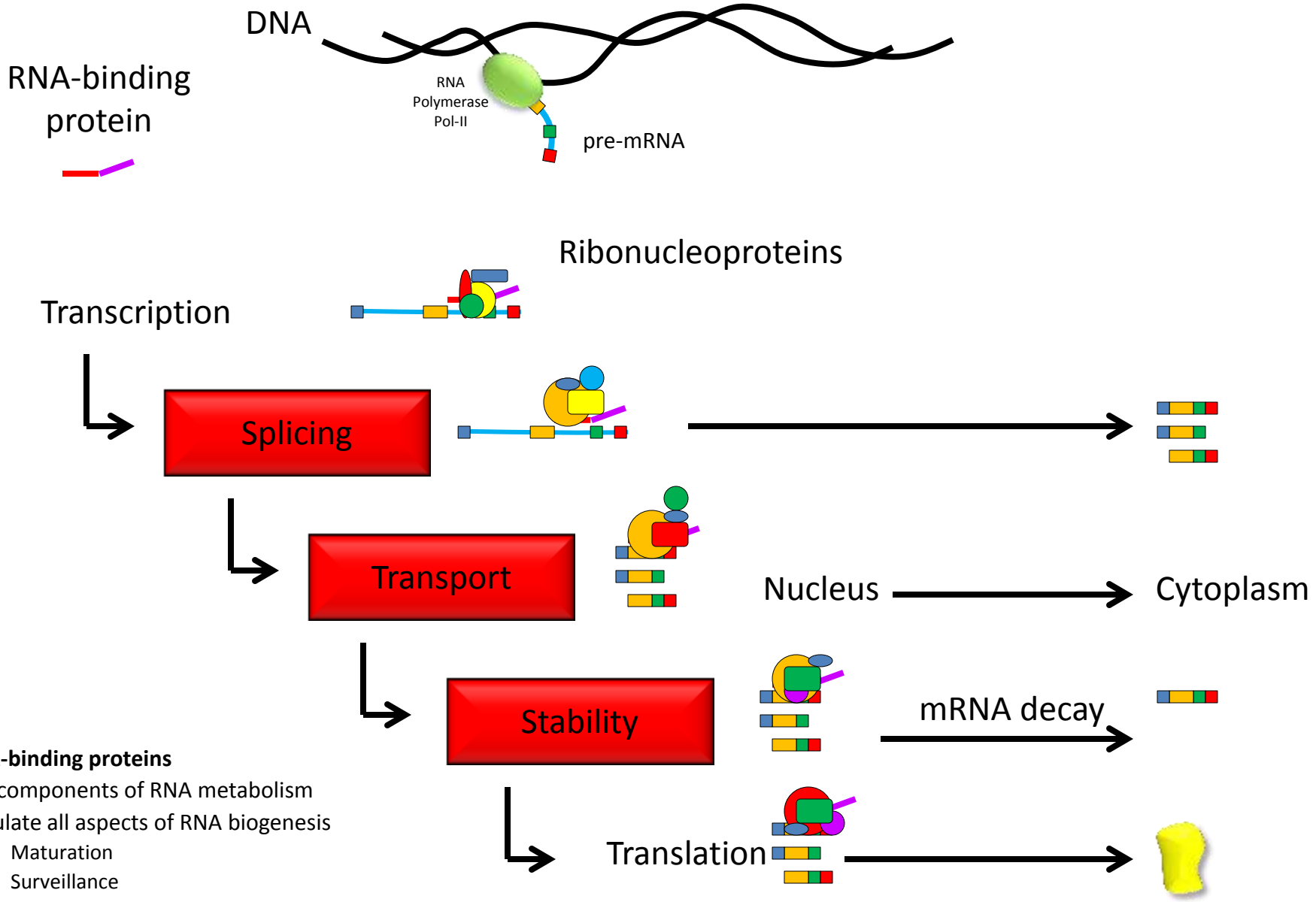
- RNA-binding protein genes
  - FUS, EWSR1, TAF15 (FET family of genes)
- Receptor tyrosine kinase (RTK) genes
  - ALK
- Growth factor genes
  - PDGFB
- Other
  - SS18 (formerly SYT)

General principles of translocations

- **Translocations occur within introns (DNA)**
  - Difficult to detect due to variability of break points (PCR)
- **RNA splicing joins chimeric mRNA exons**
  - Easier to detect with rtPCR (cDNA) due to more consistent splicing of exons
- **Frequency of specific translocations appears to be a random event related to gene size, average intron length, length of longest intron**
  - Exons comprise only 1-2% of human genome
  - Recombinogenetic DNA sequence elements are not more frequent in frequently translocated genes
- **Additional factors:**
  - Accessibility of DNA for rearrangement (open/actively transcribed > close/silent)
  - Proximity in nucleus (nuclear domains)

Ewing sarcoma breakpoint region 1 (EWSR1)

- RNA-binding protein
  - FET (previously TET) family of RNA-binding proteins FUS(TLS), EWSR1 (EWS), and TAF15(TAFII68)
    - N-term activation domain (SYGQQS hexanucleotide repeat domain)
      - serine, tyrosine, glycine, glutamine, glutamine, serine
    - RNA-binding domain (RRM: RNA recognition motif)
    - RG-rich regions (arginine-glycine-glycine)
    - Cys_2-Cys_2 Zinc finger nucleic acid binding site
- Locates predominantly in nucleus; shuttles to cytoplasm
  - Nucleolus and coiled body
- Functions
  - RNA transcription
  - mRNA processing (splicing)
  - DNA repair



RNA-binding proteins

Key components of RNA metabolism

Regulate all aspects of RNA biogenesis

- Maturation
- Surveillance
- Nucleocytoplasmic transport
- Translation
- Degradation

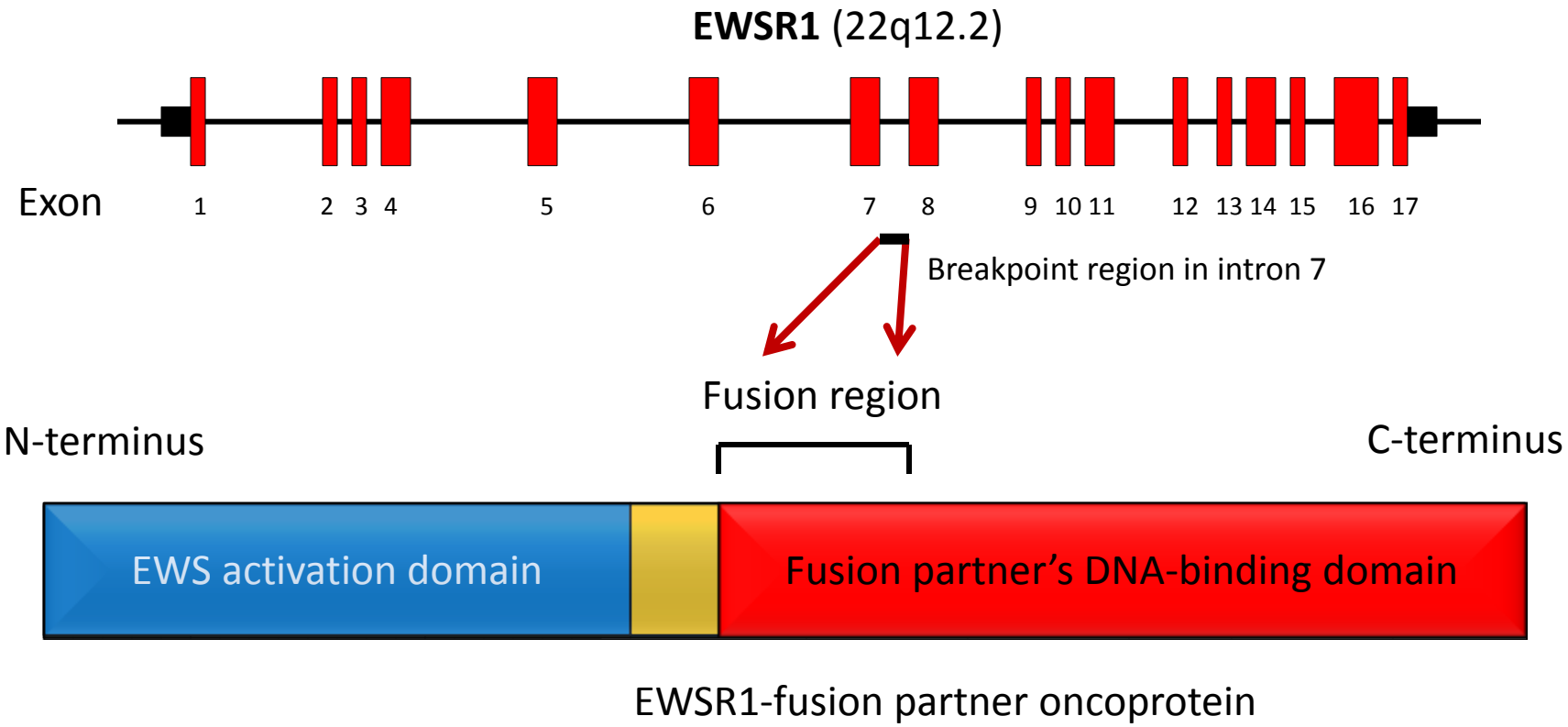
ES/PNET

- Native FLI1 transactivating domain is tightly regulated and lineage restricted
- Native EWSR1 transactivating domain is strongly and broadly activated

- EWSR1-FLI1 unrestricted high level expression of the fusion gene product
  - Structurally heterogeneous
    - Up to 18 possible in-frame chimeric transcripts
    - Two main types (85-90%)
      - Type 1: EWS exon 7 fused to FLI1 exon 6
      - Type 2: EWS exon 7 fused to FLI1 exon 5
      - Fusions always contain intact EWS exons 1-7 (TAD) and intact FLI1 exon 9 DNA-binding domain
      - Type 1 fusions initially reported to show somewhat better survival than other types

- Other genetic alterations involving cell progression and apoptosis are commonly present (25%) and define a subset of more aggressive chemoresistant tumors
  - p16/p14ARF (CDKN2A gene)
  - p53 (TP53 gene)

Cytogenetic and molecular genetic pathology of soft tissue tumors. In Enzinger & Weiss's Soft Tissue Tumors. 5th Ed. Mosby Elsevier 2008; pages 73-102



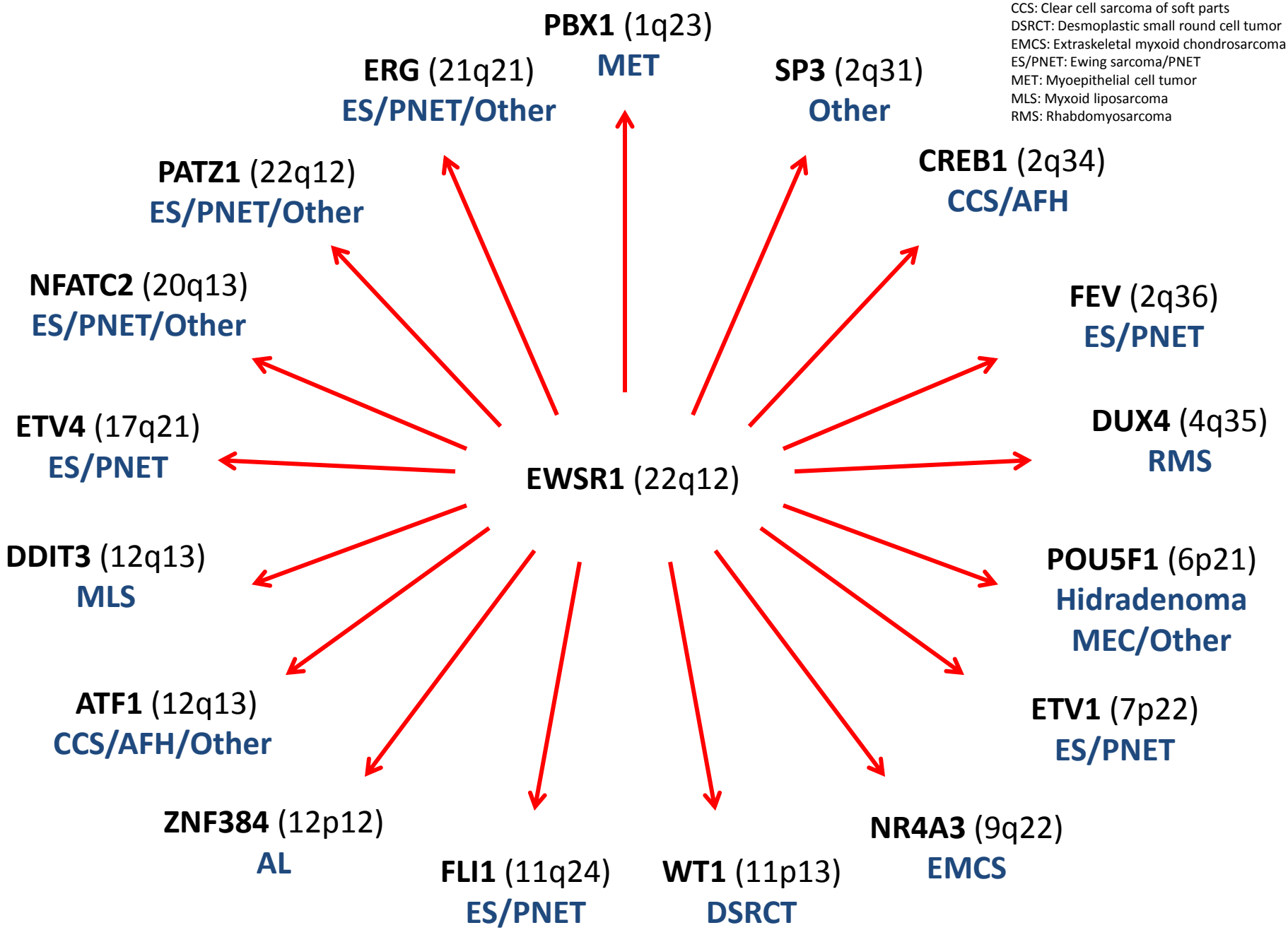
R7BD: RPB7-binding domain
DHR: Degenerate hexanucleotide repeat domain (serine-tyrosine-glycine-glutamine-rich (SYGQQS)
ZFM1: IQ binding domain (binds calmodulin)
RGG1, 2 and 3: Arginine/Glycine/Glycine rich areas
RRM: RNA recognition motif
ZF: Zinc finger domain
NLS: Nuclear localization signal

Atlascytogeneticsoncology
Kovar H. Sarcoma 2011

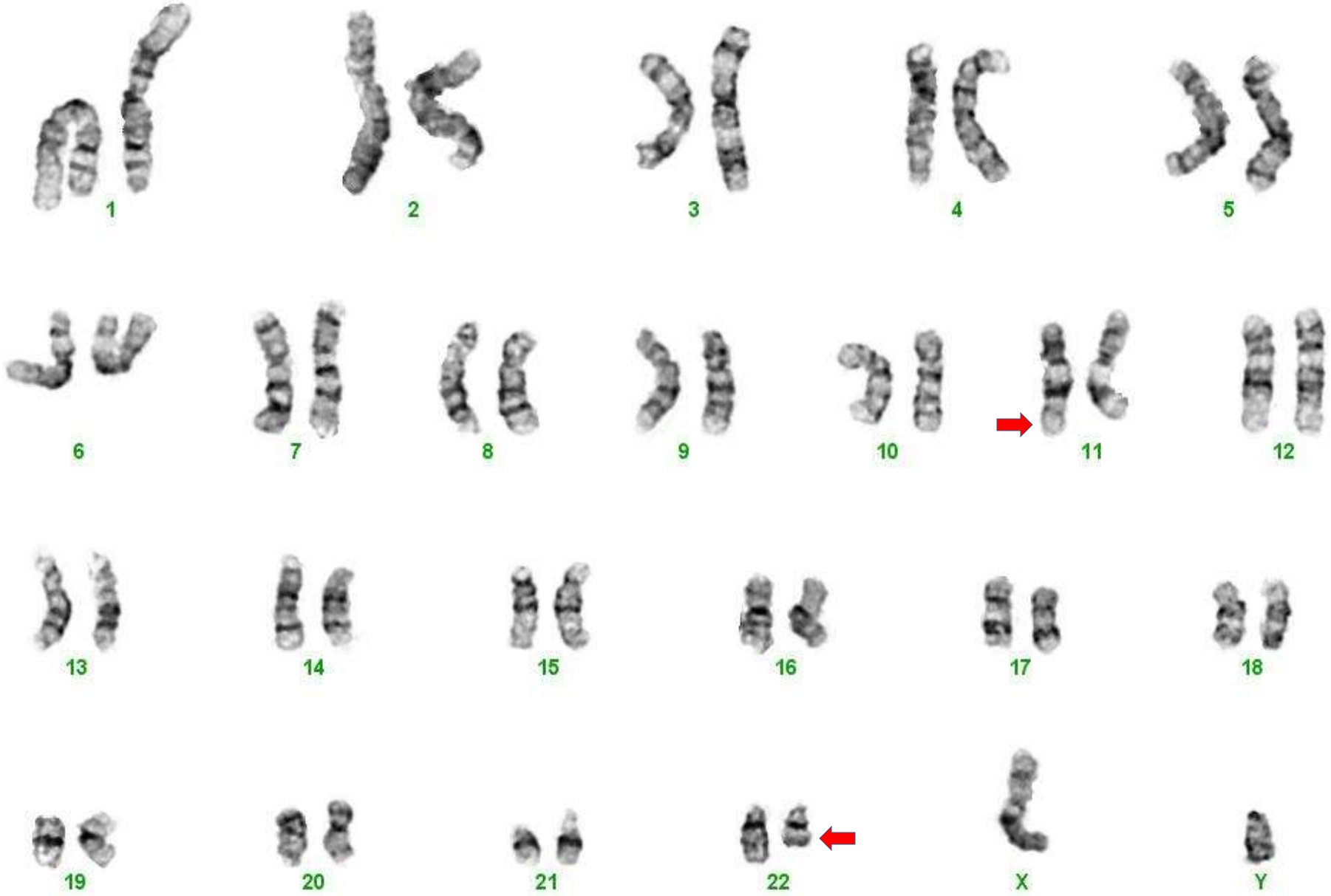
ES/PNET translocations

- EWSR1-FLI1 t(11;22)(q24q12) 85-90%
  - EWSR1 exons 7 to 11 fused to FLI1 exons 3 to 8
  - Usually EWSR1 exons 7 or 8 fused to FLI1 exons 5 or 6
    - Type 1: EWSR1 exon 7 to FLI1 exon 6 (51%)
    - Type 2: EWSR1 exon 7 to FLI1 exon 5 (27%)
- EWSR1-ERG t(21;22)(q21;q12) 10%
- EWSR1-ETV1 t(7;22)(p22;q12)
- EWSR1-ETV4 t(17;22)(q21;q12)
- EWSR1-FEV t(2;22)(q36;q12)
- EWSR1-NFATC2 t(20;22)(q13;q12)
- EWSR1-PATZ1 inv(22)(q12q12)

- FUS-ERG t(16;21)(p11;q22) <1%
- FUS-FEV t(2;16)(q35;p21)

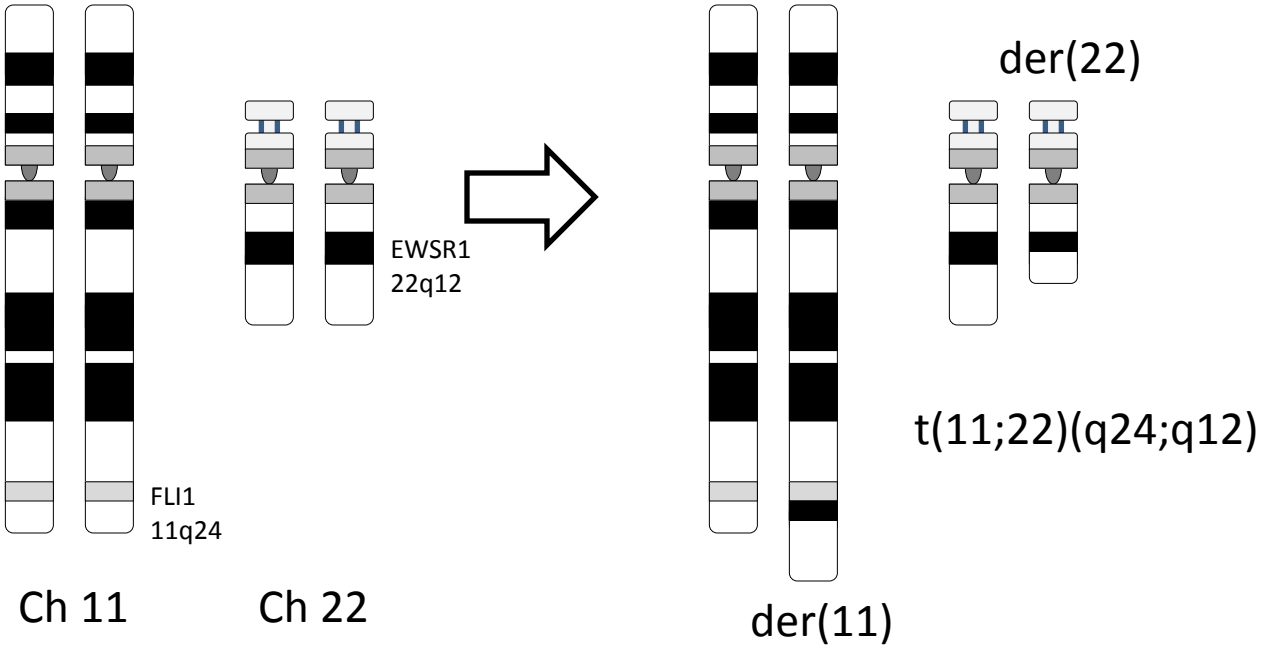


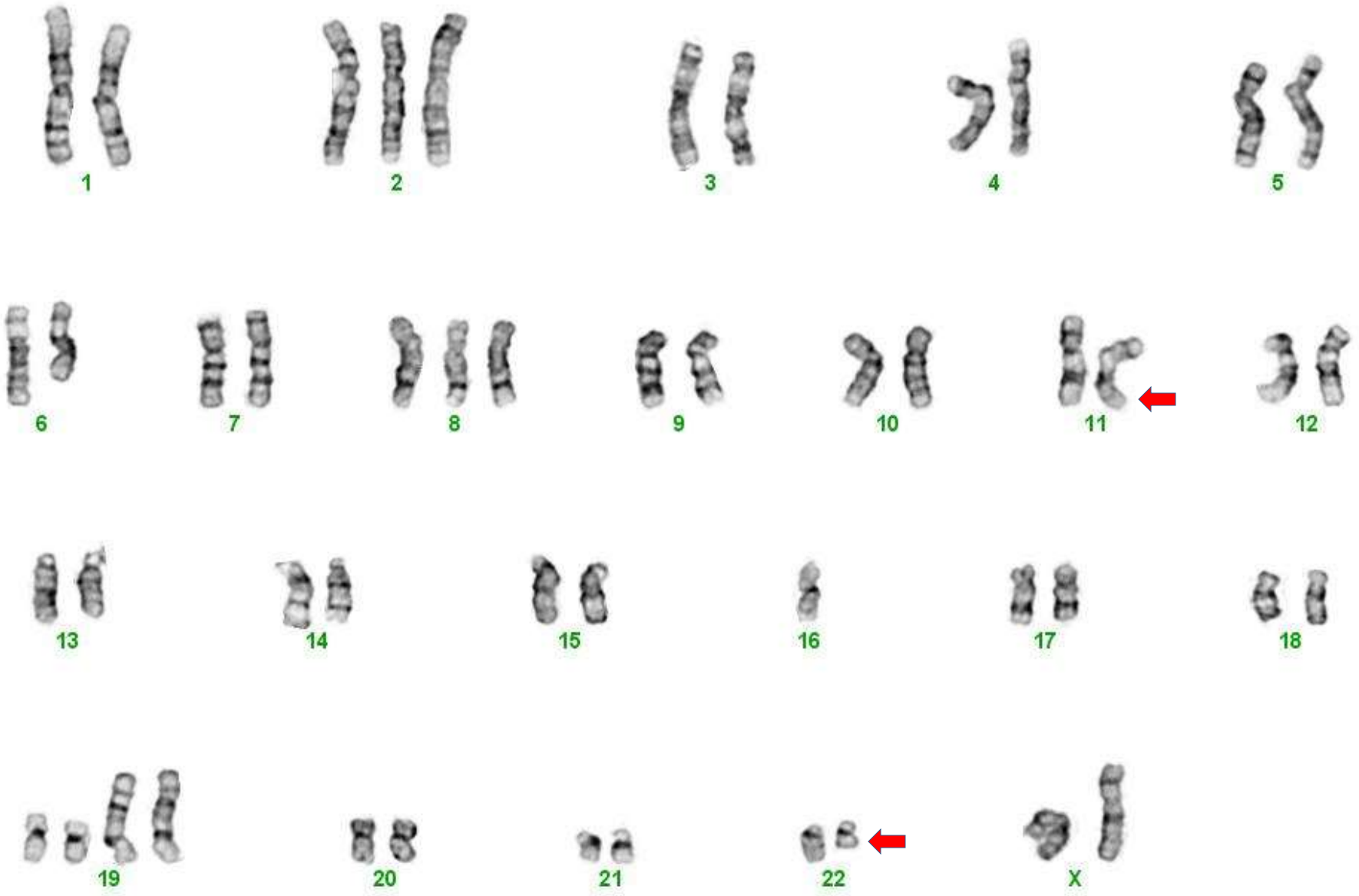
Huret JL, et al. EWSR1, partners, and tumours. 2010



46,XY,t(11;22)(q24;q12)

Image from Dr. Mark Micale, William Beaumont Hospital, Royal Oak, MI


FLI1
11q24
EWSR1
22q12
der(22)
t(11;22)(q24;q12)
Ch 11
Ch 22
der(11)

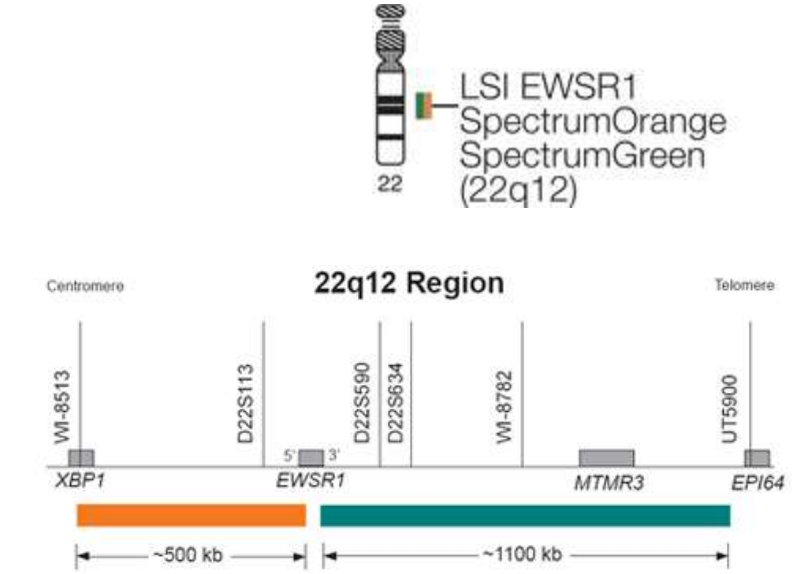


50,XX,t(2;13)(p13;q12)del(6)(q23),+8,t(11;22)(q24;q12),-16,der(19)t(1;19)x2,+r

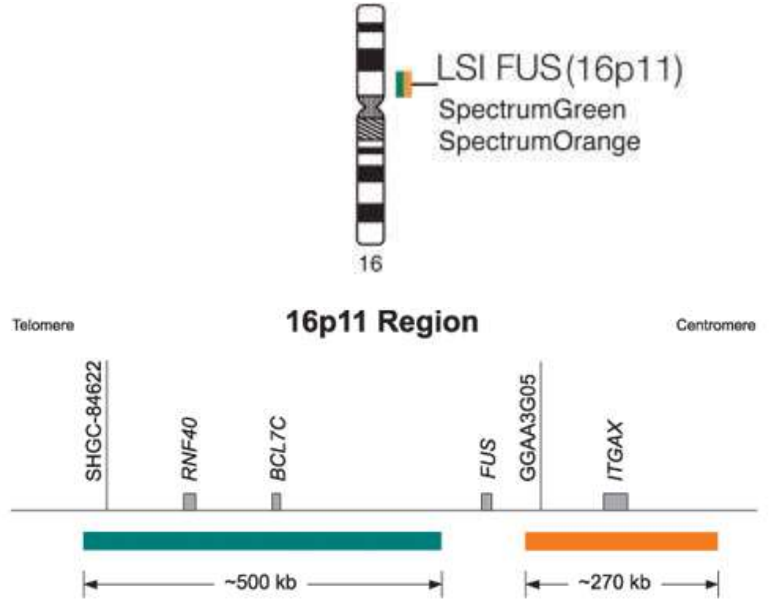
A

Image from Dr. Mark Micale, William Beaumont Hospital, Royal Oak, MI

FISH dual color break apart probes for EWSR1 & FUS

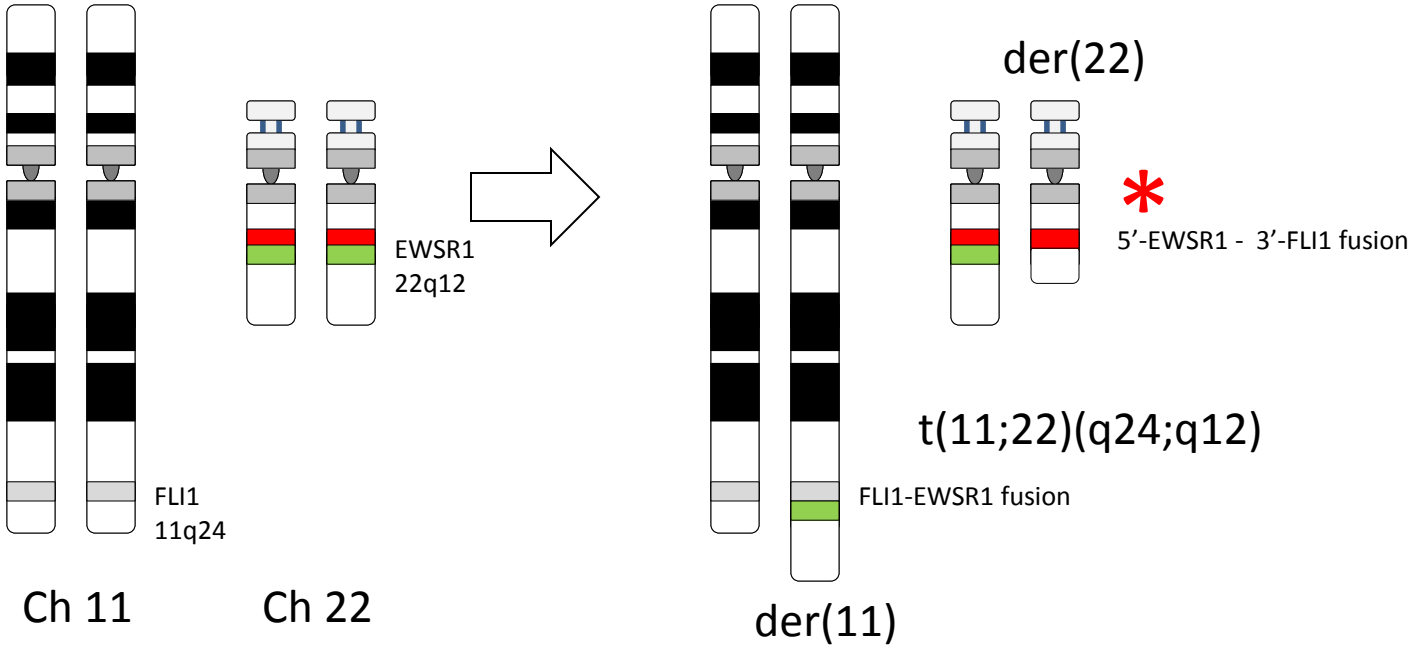


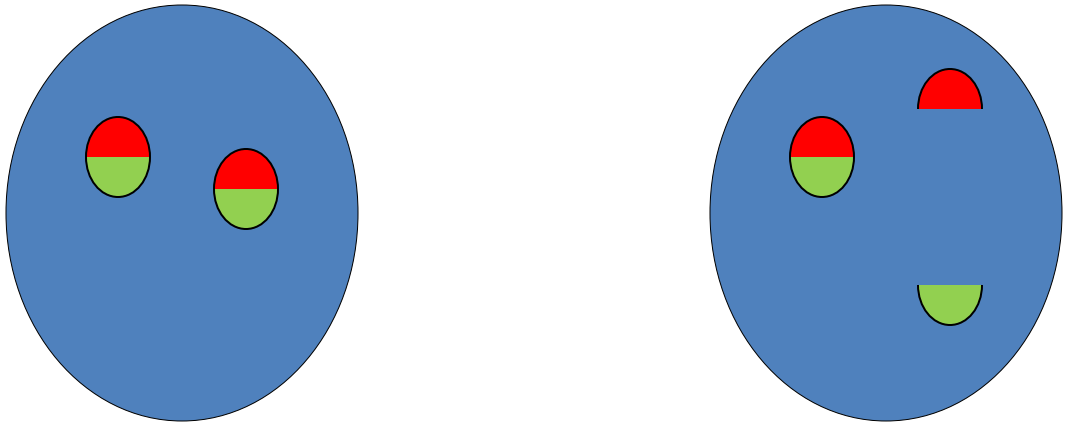
LSI EWSR1 Dual Color, Break Apart Rearrangement Probe



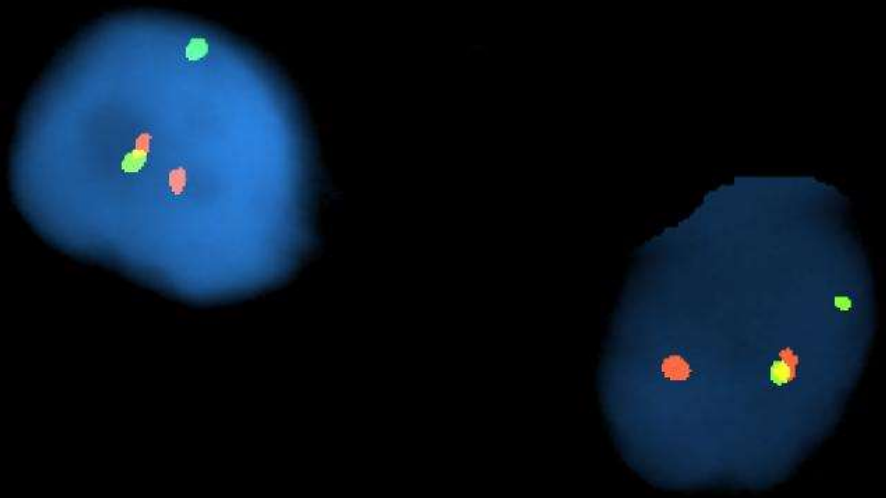
LSI FUS Dual Color, Break Apart Rearrangement Probe

FISH dual color break-apart probe for EWSR1 rearrangement (in this case with FLI1)



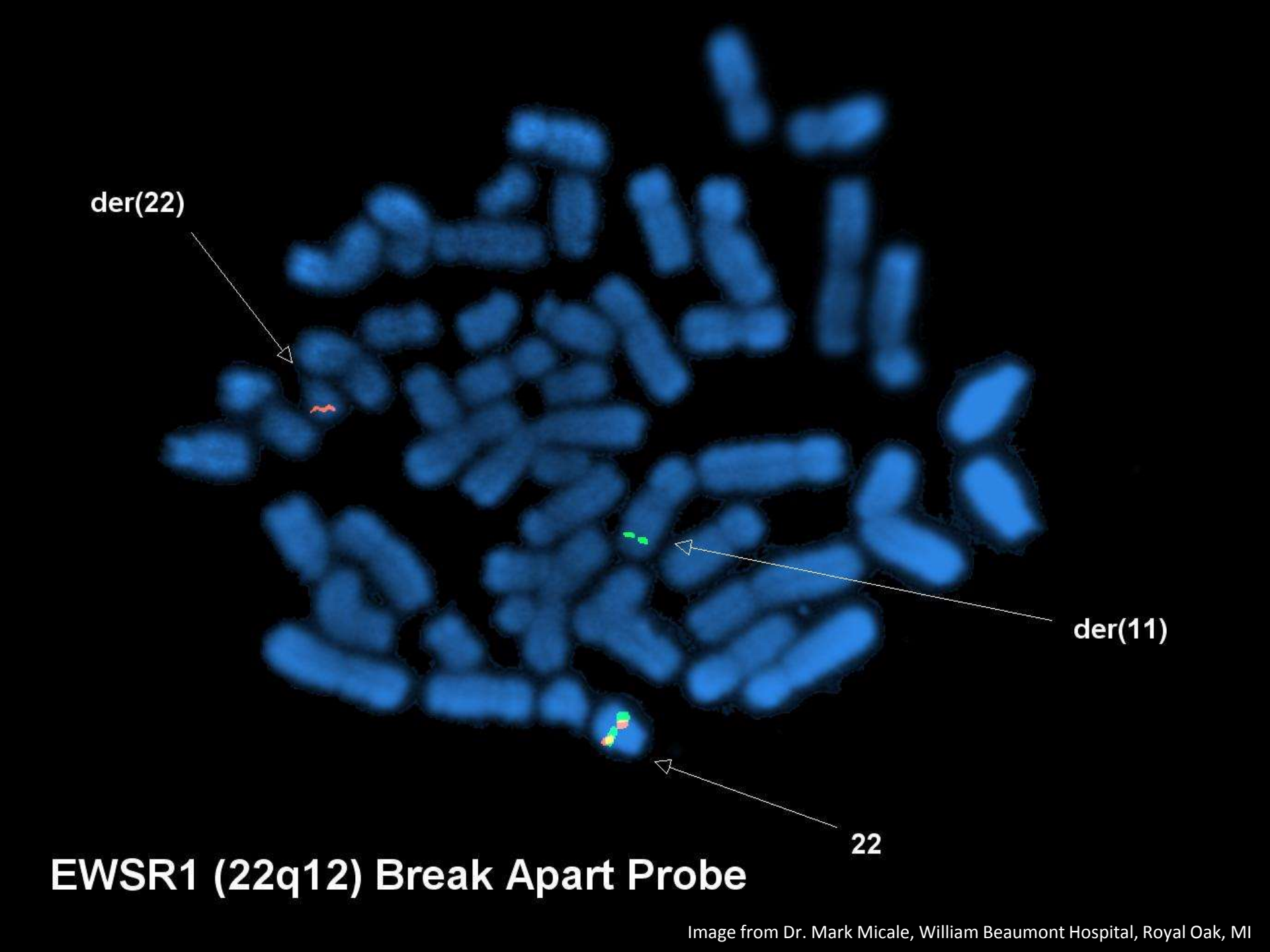


EWSR1 (22q12) Break Apart Probe



Positive for EWSR1 gene rearrangement

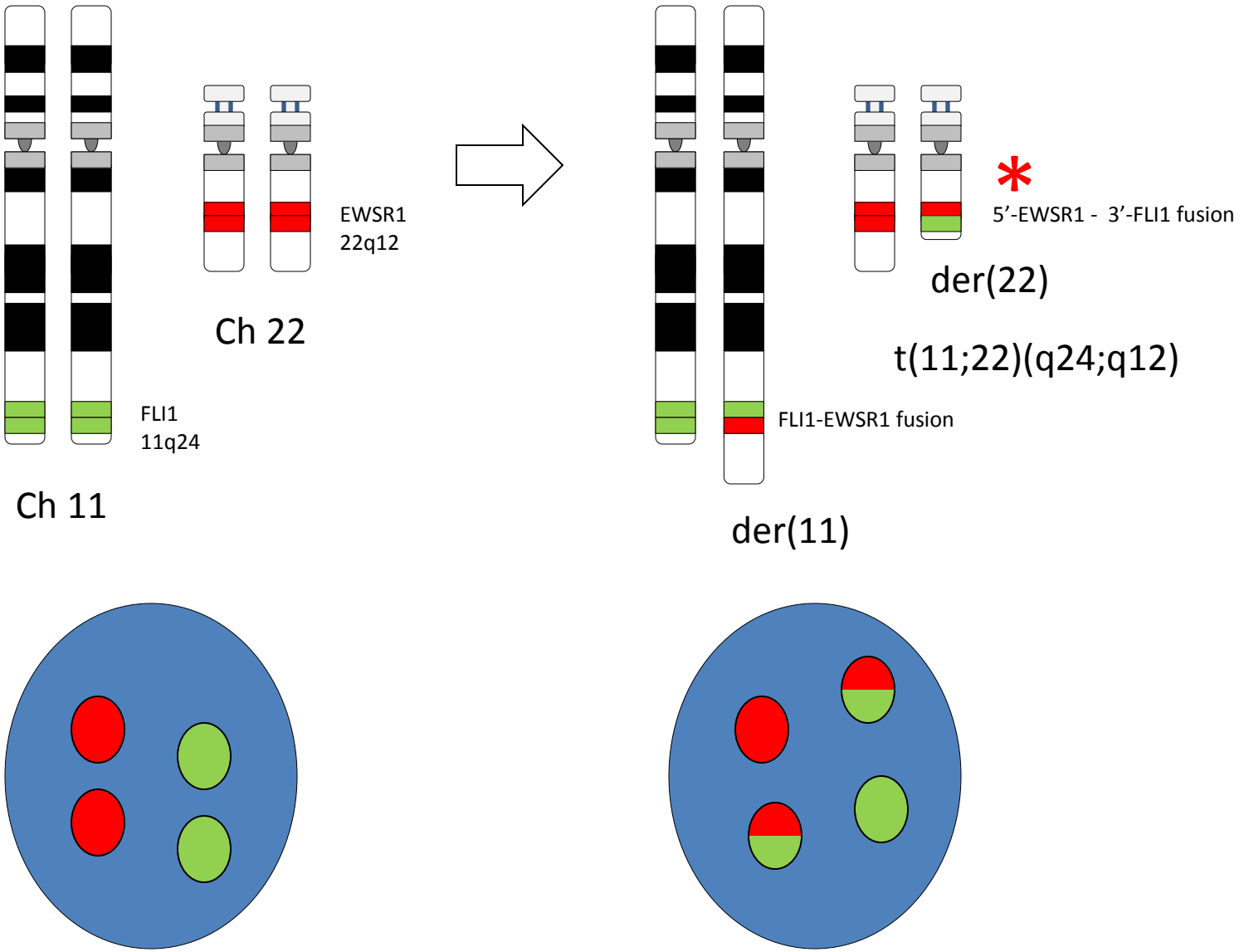
Image from Dr. Mark Micale, William Beaumont Hospital, Royal Oak, MI



EWSR1 (22q12) Break Apart Probe

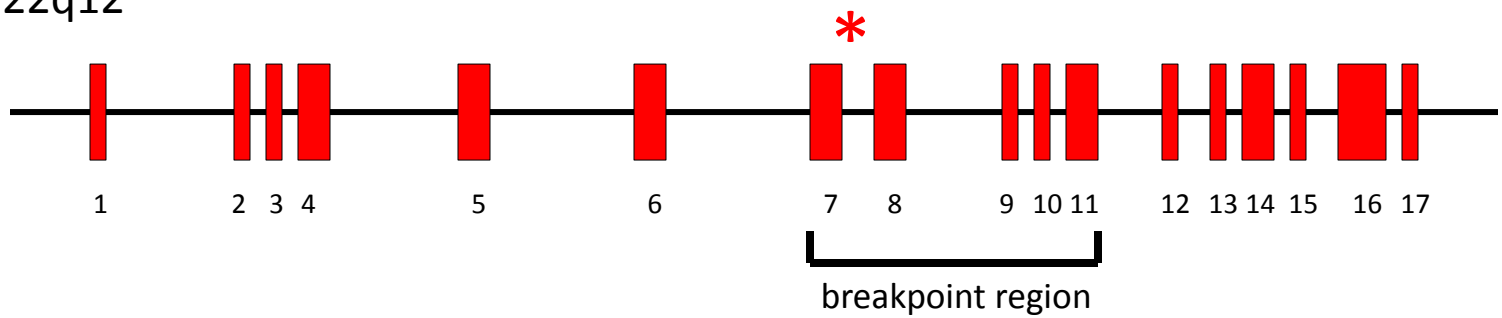
Image from Dr. Mark Micale, William Beaumont Hospital, Royal Oak, MI

FISH dual-color dual-fusion probe for EWSR1 rearrangement (in this case with FLI1)

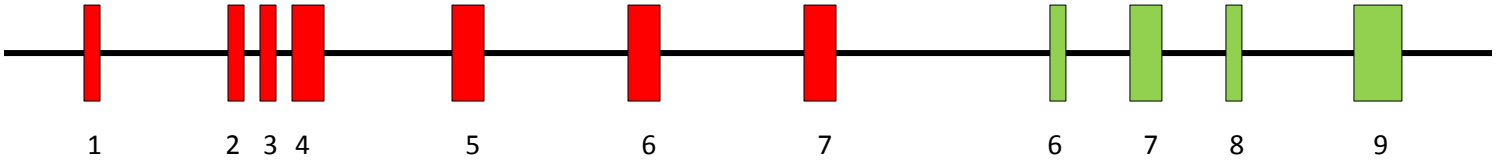


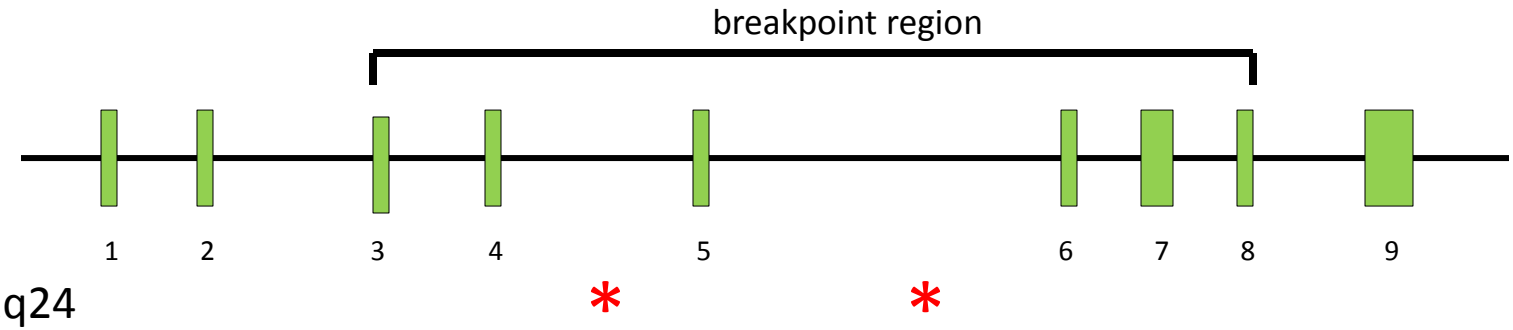
rtPCR for EWSR1-FLI1 fusions

EWSR1 22q12



EWSR1-FLI1 t(11;22)(q24;q22)

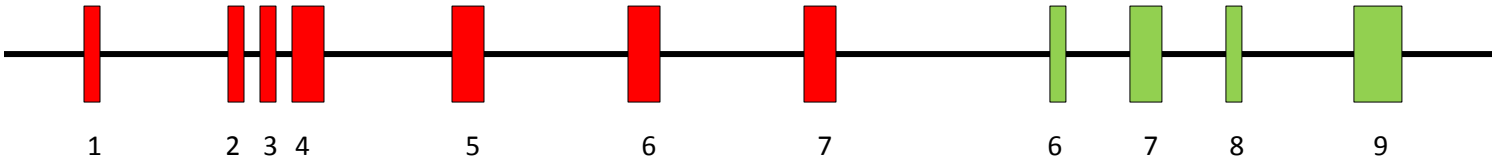


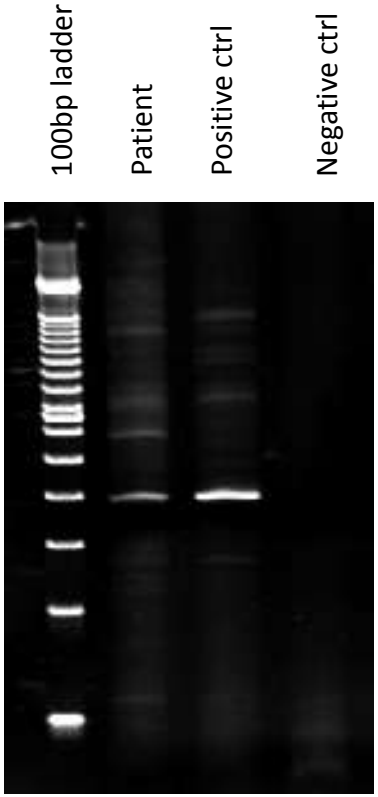


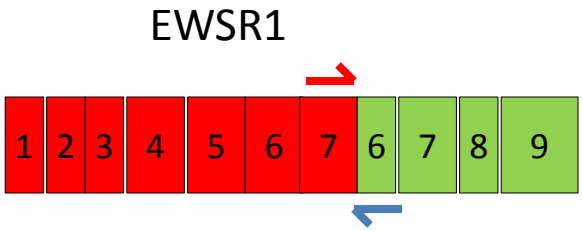
FLI1 11q24

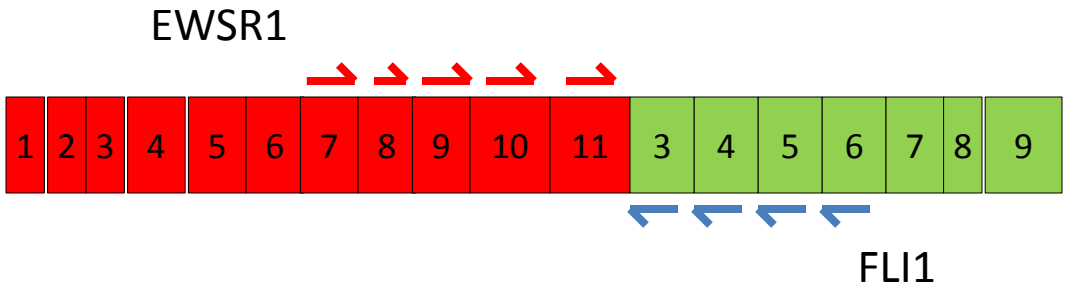
rtPCR for EWSR1-FLI1 fusions

EWSR1-FLI1 t(11;22)(q24;q22)









Ewing sarcoma/PNET

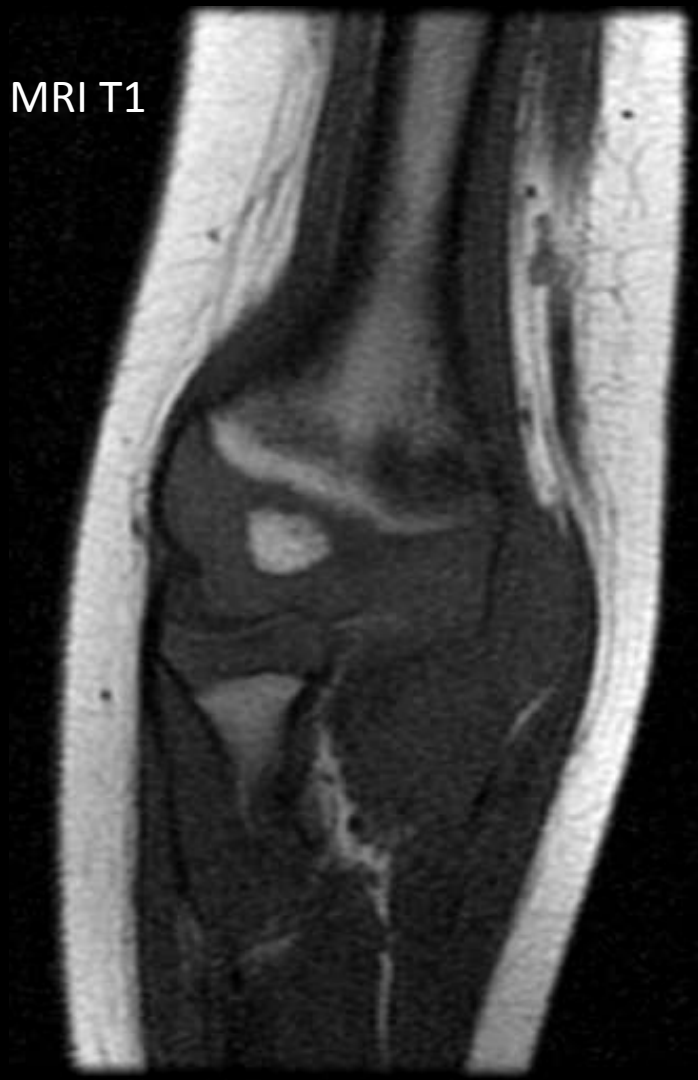
- **Utility of molecular diagnostics:**
  - Diagnosis
  - Minimal residual disease detection (MRD)

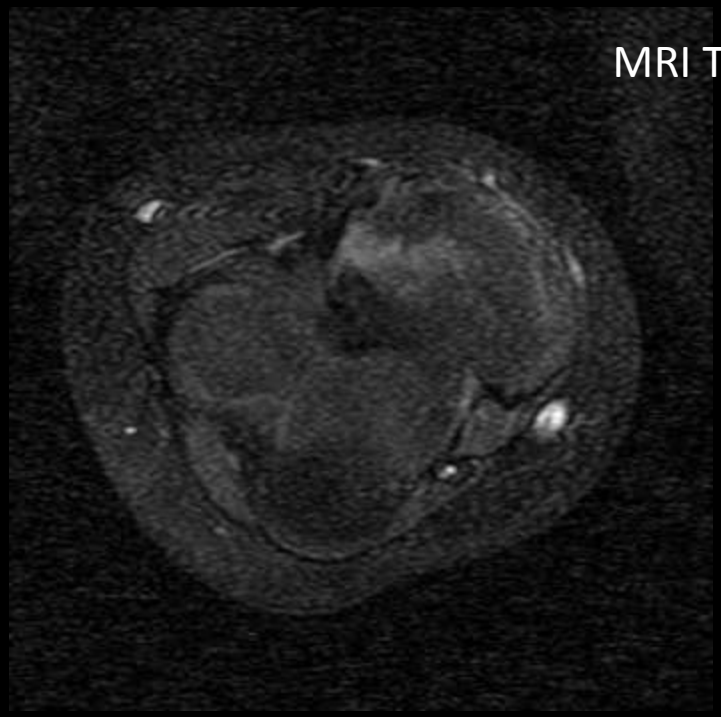
- **Differential diagnosis:**
  - Rhabdomyosarcoma
  - Poorly differentiated synovial sarcoma t(X;18)(p11.2;q11.2) SS18-SSX
  - Lymphoblastic lymphoma
  - Neuroblastoma
  - Mesenchymal chondrosarcoma t(8;8)(q21;q13)/del(8)(q21-q13)) HEY1-NCOA2
  - Small cell osteosarcoma

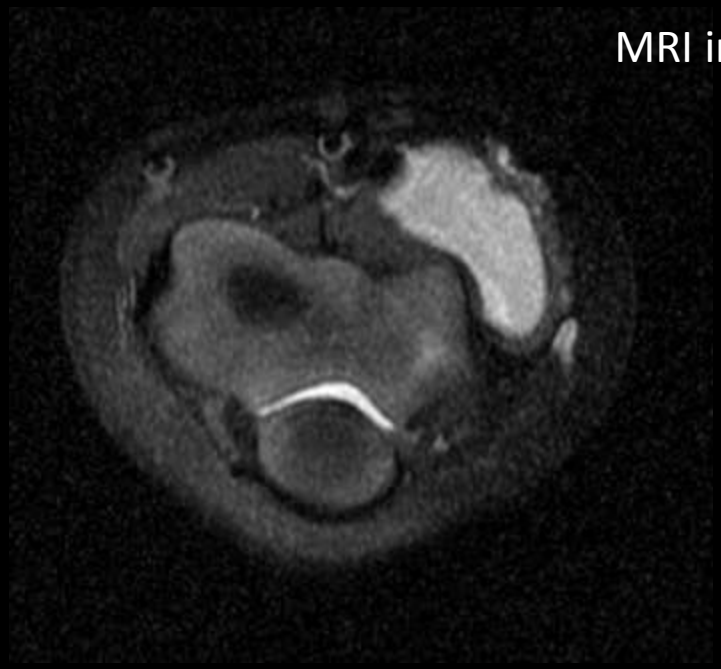
Non-ES/PNET sarcomas with EWSR1 rearrangements

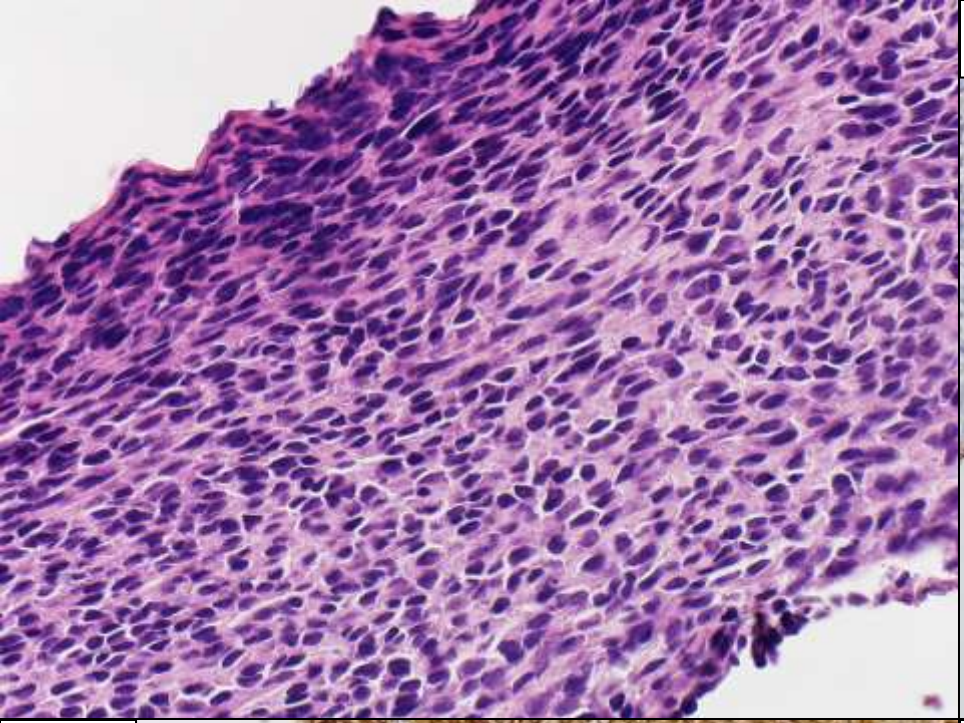
- Desmoplastic small round cell tumor (DSRCT)
  - t(11;22)(p13;q12) EWSR1-WT1
- Myxoid liposarcoma
  - t(12;16)(q13;p11) FUS-DDIT3
  - t(12;22)(q13;q12) EWSR1-DDIT3
- Extraskeletal myxoid chondrosarcoma
  - t(9;22)(q22;q12) EWSR1-NR4A3
  - t(9;17)(q22;q11) TAF15-NR4A3
- Clear cell sarcoma
  - t(12;22) (q13;q12) EWSR1-ATF1
  - t(2;22)(q33;q12) EWSR1-CREB1
- Angiomatoid fibrous histiocytoma
  - t(2;22)(q34;q12) EWSR1-CREB1
  - t(12;22)(q13;q12) EWSR1-ATF1
  - t(12;16)(q13;p11) FUS-ATF1

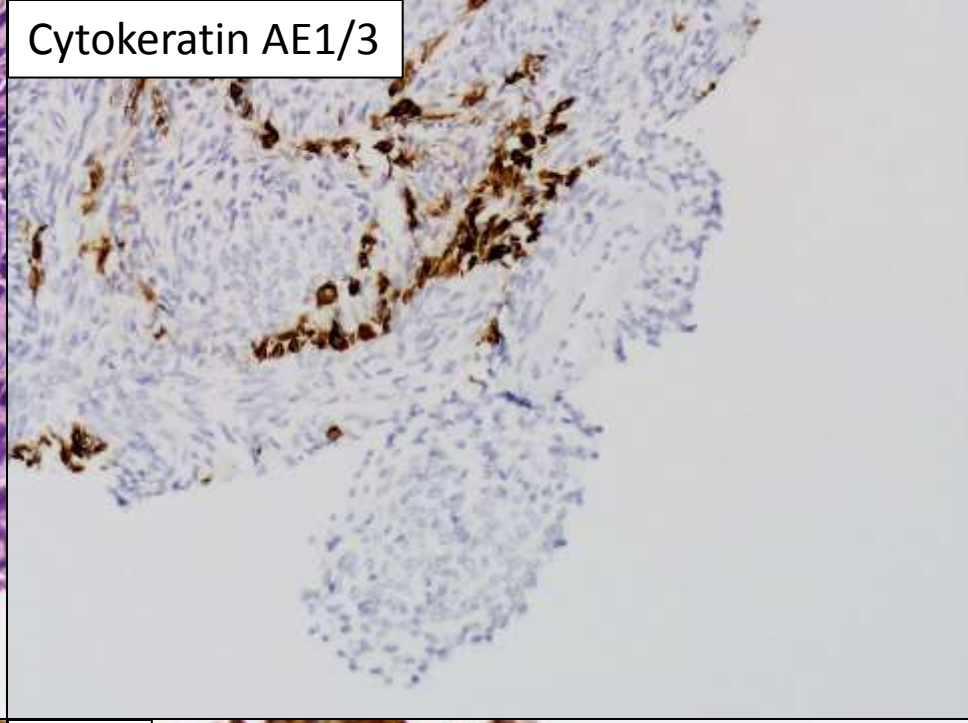
2 y/o male with soft tissue mass in proximal right forearm. He underwent biopsy, followed by neoadjuvent chemotherapy and radical resection.

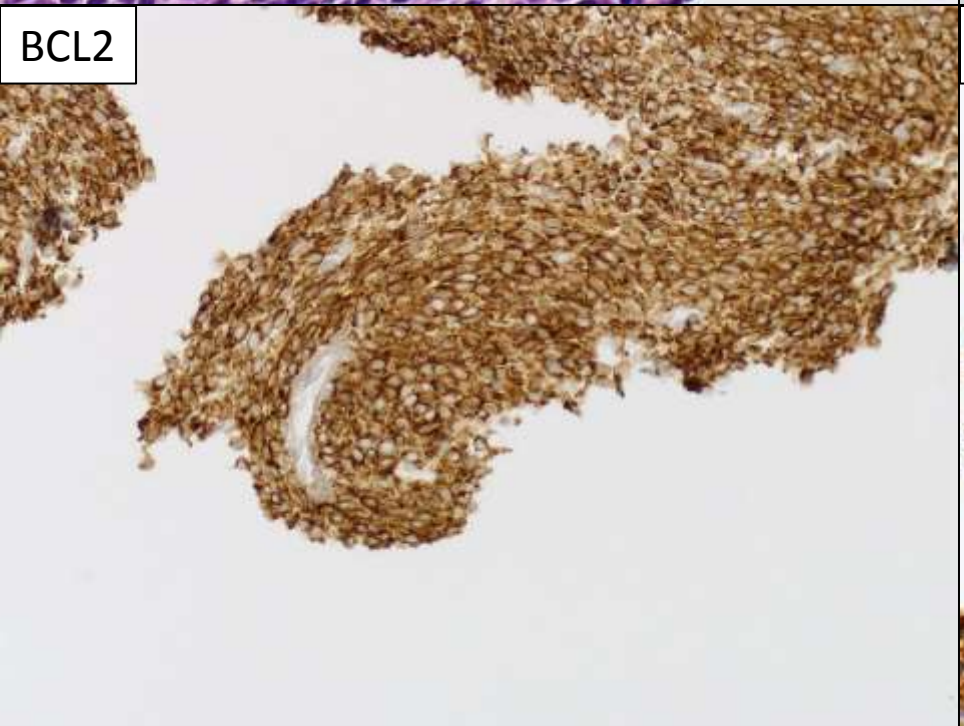


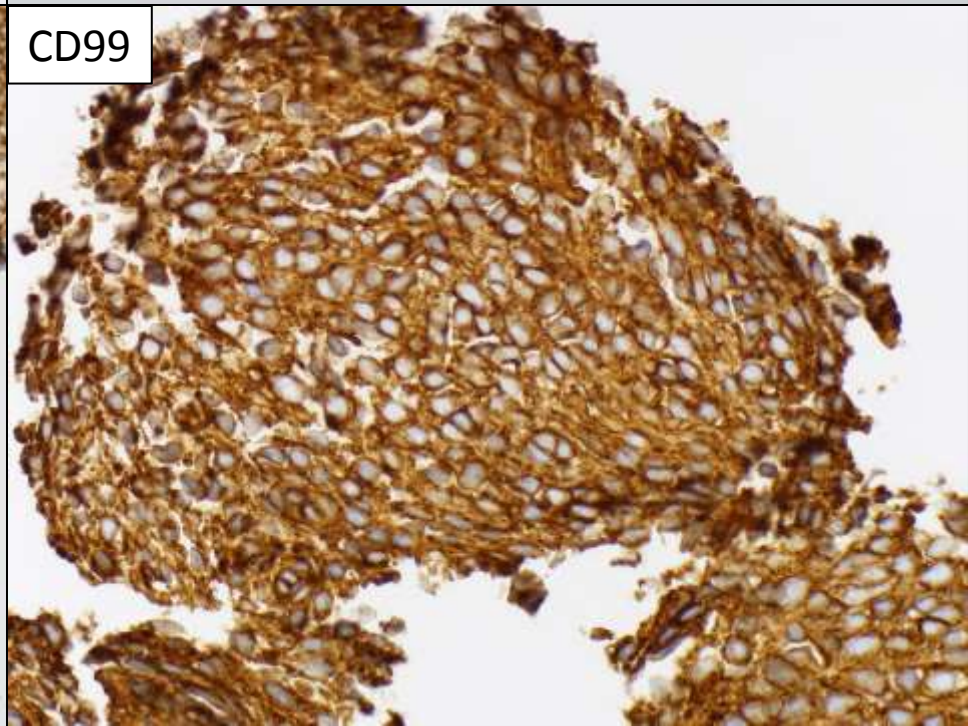




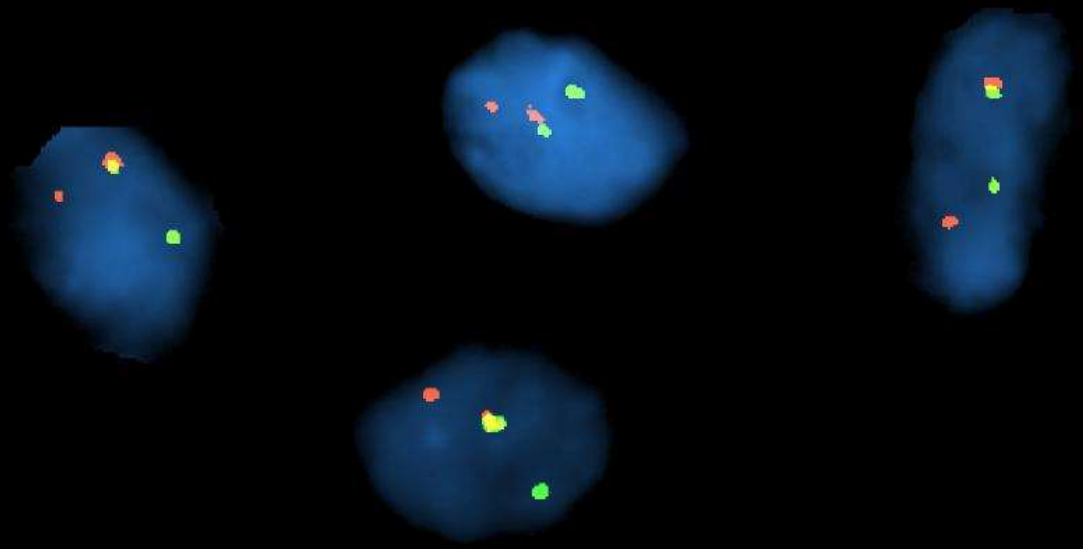



Cytokeratin AE1/3


BCL2

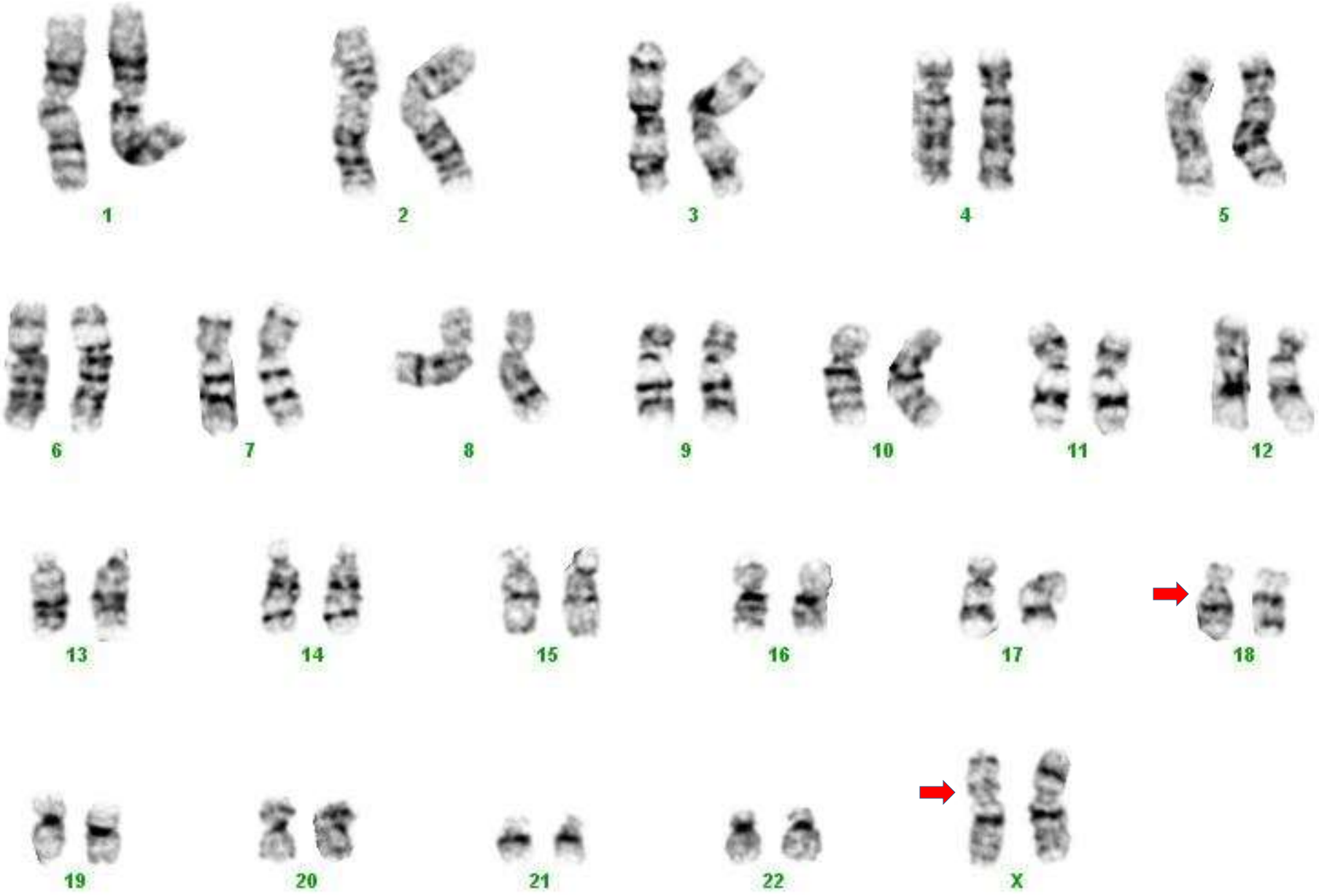

CD99

SYT (18q11.2) Break Apart Probe



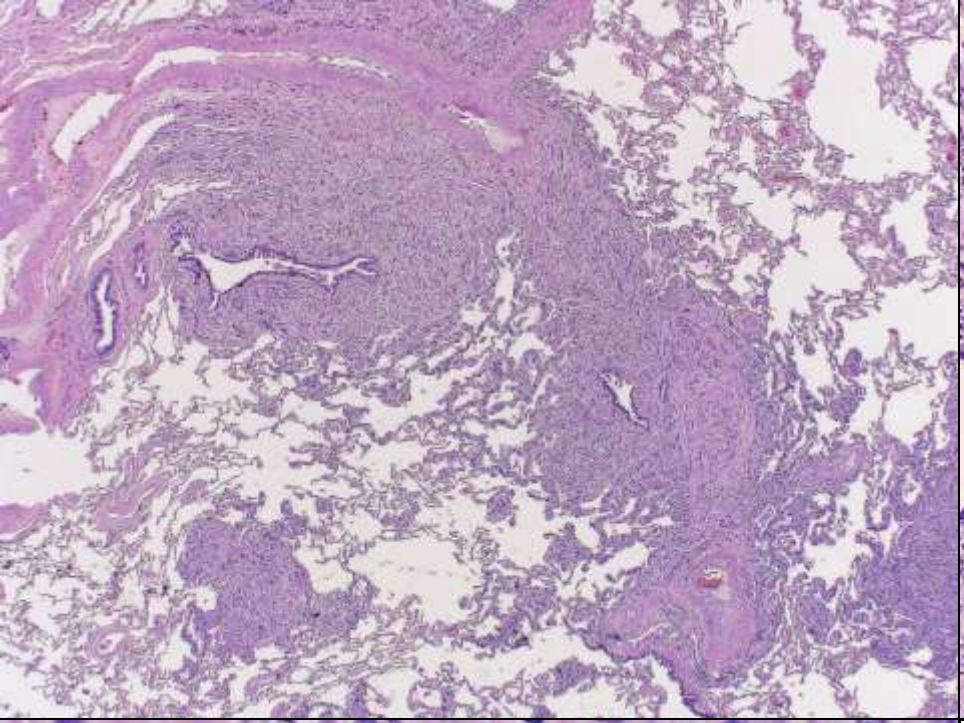
Positive for SYT gene rearrangement

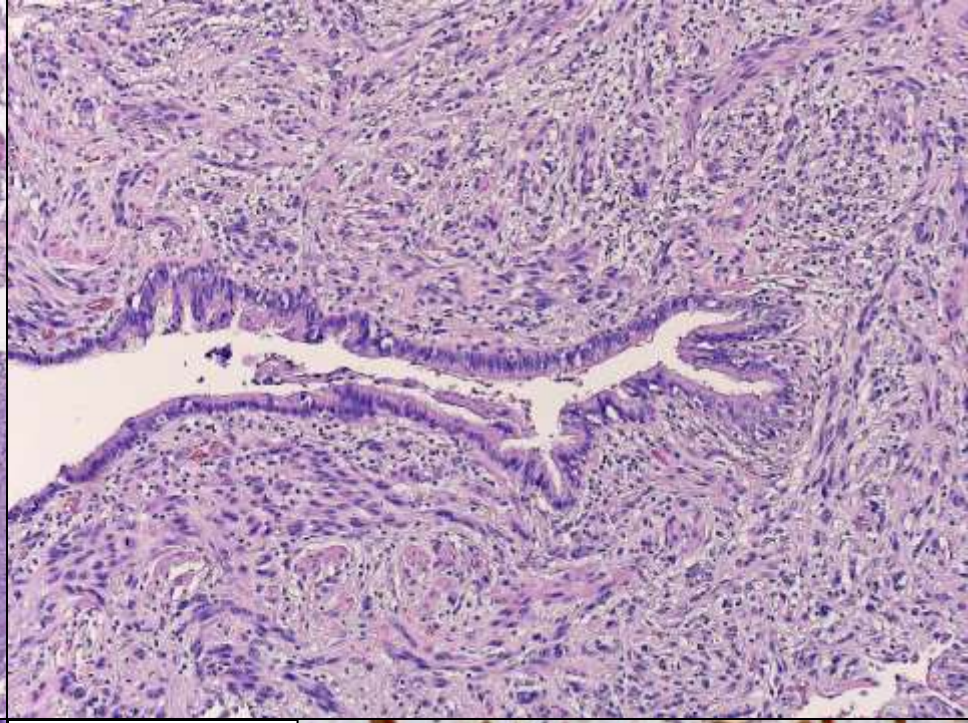
Image from Dr. Mark Micale, William Beaumont Hospital, Royal Oak, MI

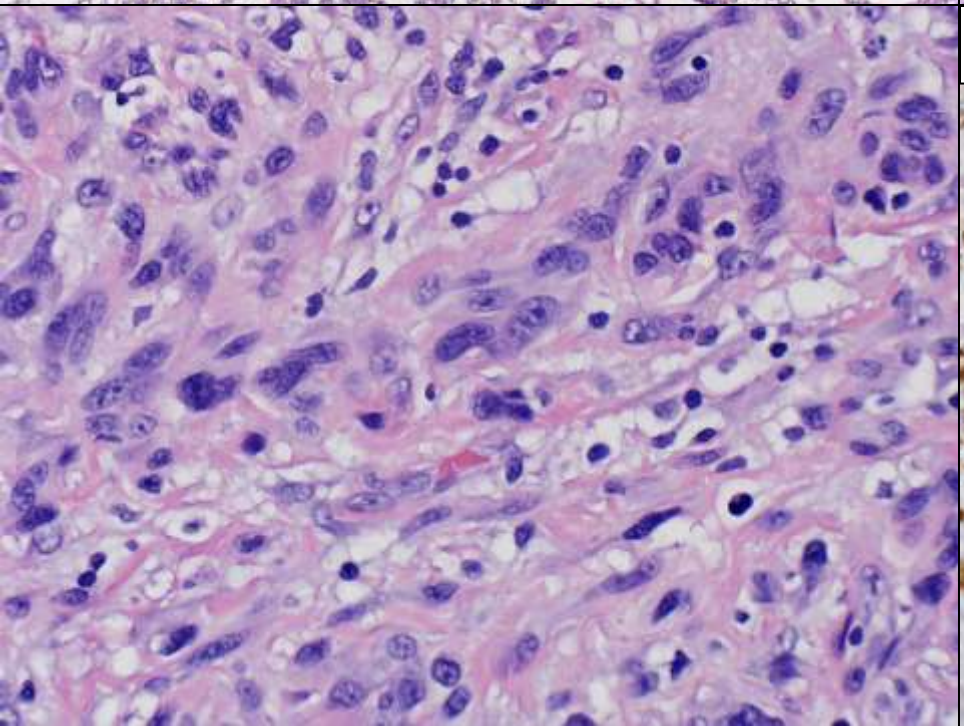


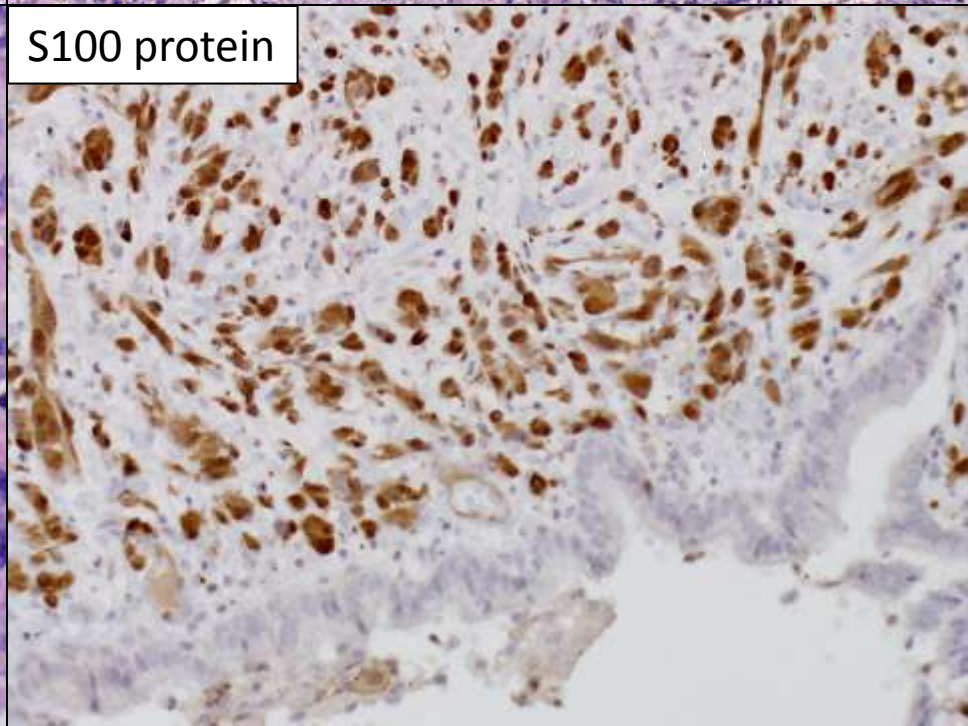
46,X,t(X;18)(p11.2;q11.2)

Image from Dr. Mark Micale, William Beaumont Hospital, Royal Oak, MI








S100 protein

Diagnosis:

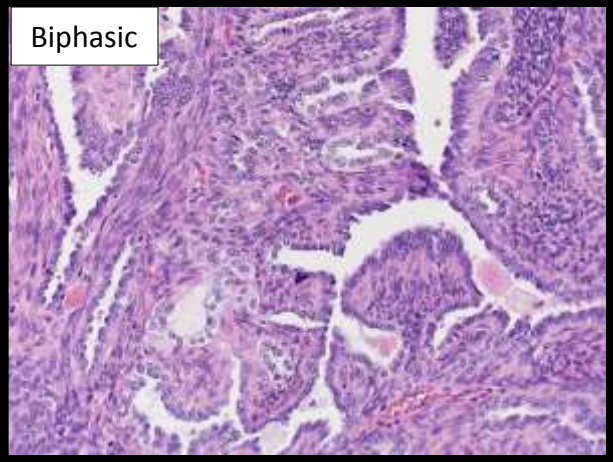
- Malignant spindle cell neoplasm, favor metastatic melanoma

- FISH which had been previously ordered, returns positive for SS18 rearrangement, consistent with synovial sarcoma

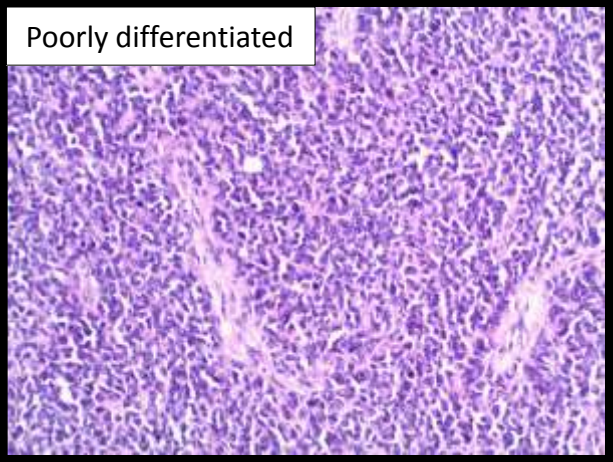
Synovial sarcoma (SS)

- **Definition:**
  - Sarcoma with variable epithelial differentiation. May arise at any site, and despite its name does not arise from or differentiate towards synovium.
- **Recurrent translocations:**
  - t(X;18)(p11.2;q11.2) SS18-SSX
    - SSX1 & SSX2 (synovial sarcoma X breakpoint 1 & 2)
      - Only 13 amino acids apart
    - SS18 (synovial sarcoma translocation, chromosome 18)
      - SS18-SSX1 biphasic 35-50%
      - SS18-SSX2 biphasic <10% (reported more favorable prognosis)
      - SSX4 to SS18 rare
  - Differ in that neither SSX or SYT have DNA-binding domains; appear to be transcriptional regulators working through protein-protein interactions (favored to be housekeeping genes, but exact function is unknown)

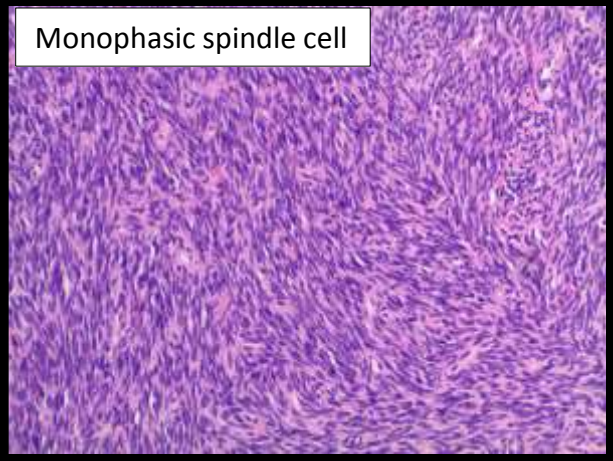
Biphasic



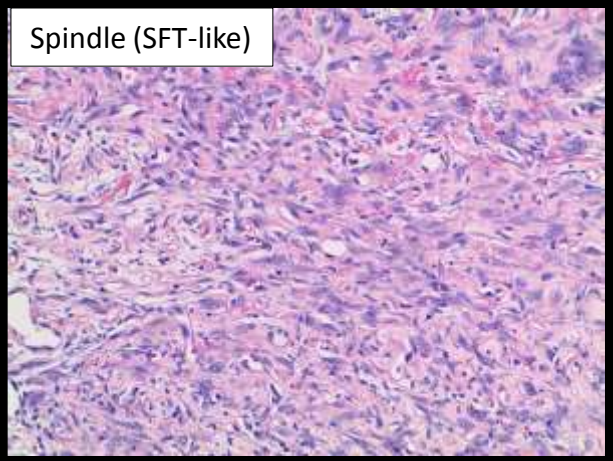
Poorly differentiated



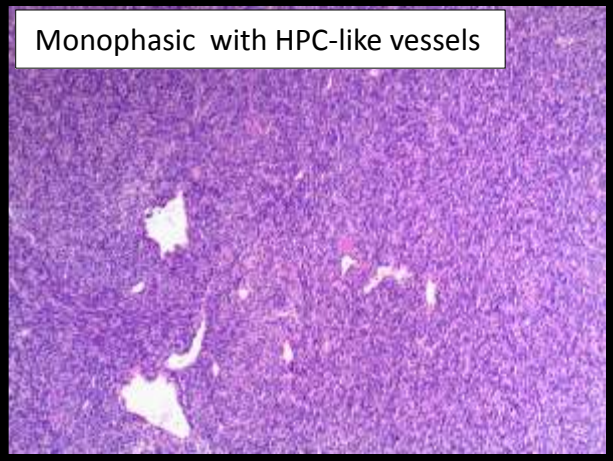
Monophasic spindle cell



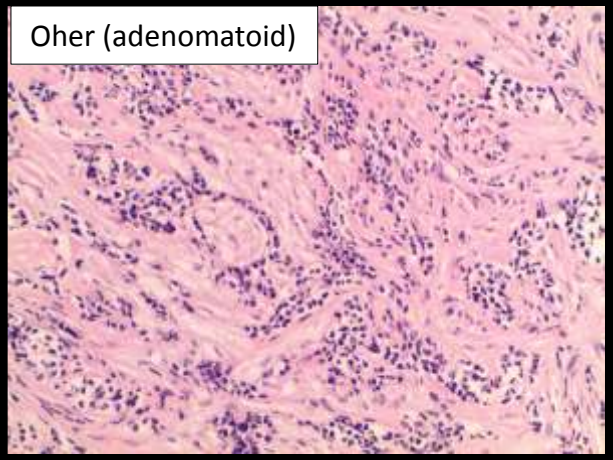
Spindle (SFT-like)



Monophasic with HPC-like vessels



Oher (adenomatoid)



Synovial sarcoma (SS)

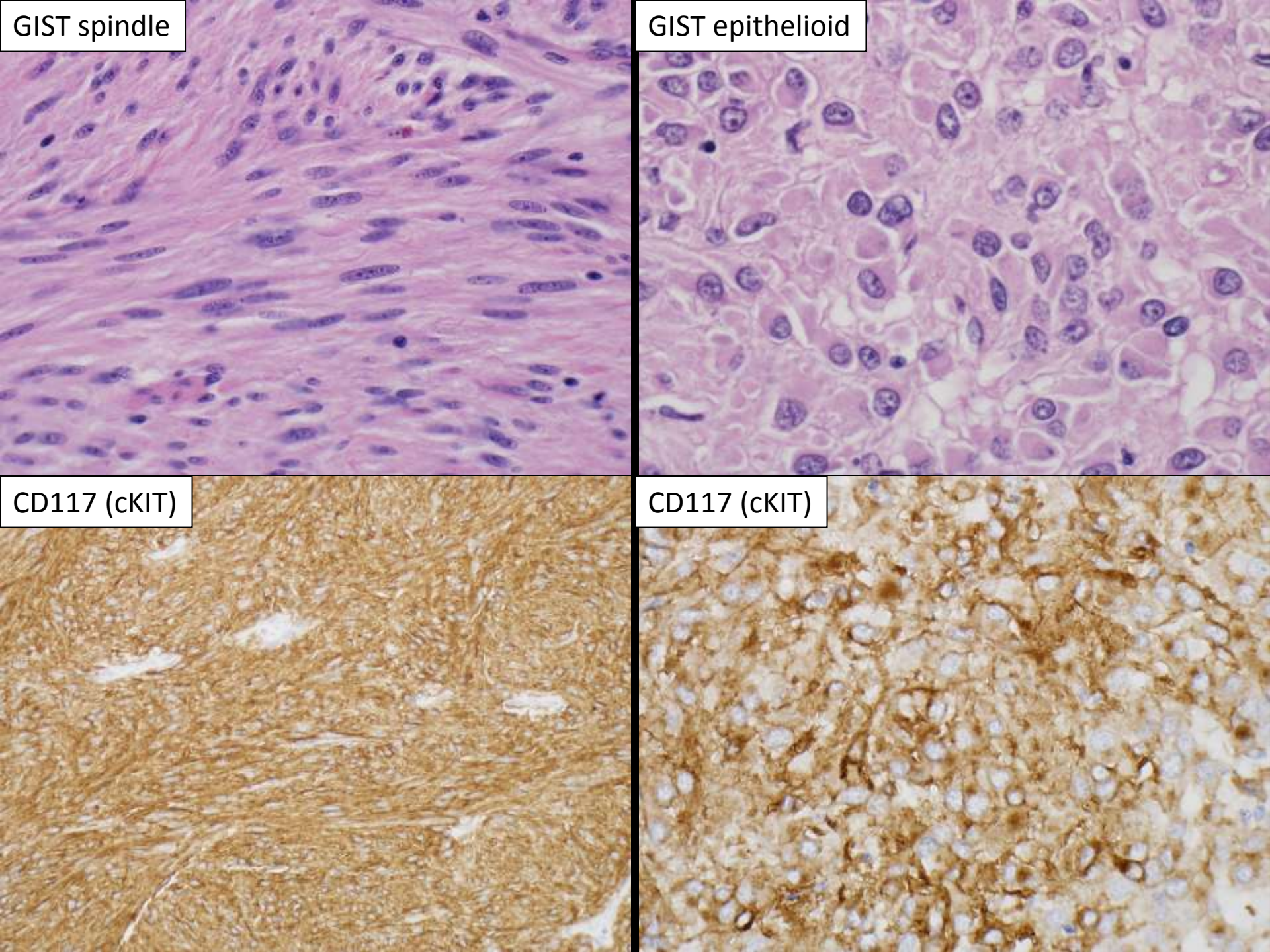
- **Utility of molecular diagnostics:**
  - Diagnosis

- **Differential diagnosis:**
  - Poorly differentiated (small round blue cell tumor)
    - Ewing sarcoma/Primitive neuroectodermal tumor
    - Rhabdomyosarcoma
    - Neuroblastoma
    - Cellular solitary fibrous tumor (hemangiopericytoma)
    - Mesenchymal chondrosarcoma
    - Lymphoblastic lymphoma
  - Biphasic
    - Carcinosarcoma
    - MPNST with glandular differentiation
  - Monophasic fibrous
    - Fibrosarcoma
    - Leiomyosarcoma
    - Cellular solitary fibrous tumor (hemangiopericytoma)
    - Malignant peripheral nerve sheath tumor (MPNST)
    - Spindle cell carcinoma
    - Malignant peripheral nerve sheath tumor
  - Monophasic epithelial
    - Carcinoma

Extragastrointestinal stromal tumor (EGIST)

- **Description:**
  - Spindle cell proliferation arising from gut wall, with phenotypic features of interstitial cell of Cajal. Spectrum of biologic behavior from benign to malignant. They are the most common mesenchymal tumor of the gastrointestinal tract. A small number of GISTs arise from mesentery or retroperitoneum and show no connection with the gut (EGISTs).
- **Recurrent mutations:**
  - Activating mutations of tyrosine kinase
    - KIT 70-80%
    - PDGFRA 5-8%

    Mutually exclusive
  - Other 12-15%
    - SDH
    - BRAF
    - HRAS
    - NRAS
- KIT is expressed in >95% of GISTs, including those without KIT or PDGFRA mutations.


GIST spindle
GIST epithelioid
CD117 (cKIT)
CD117 (cKIT)

kit oncogene KIT (*AKA* CD117)

- Member of type III receptor tyrosine kinase (RTK) family
  - Other members: PDGFRA, CSF1R, and FLT3
- Critical to normal development & function of interstitial cells of Cajal
- Also involved in hematopoiesis, gametogenesis, and melanogenesis.
- Activating KIT mutations identified in multiple tumors: seminomas, mast cell tumors, acute myelogenous leukemia, and melanoma.

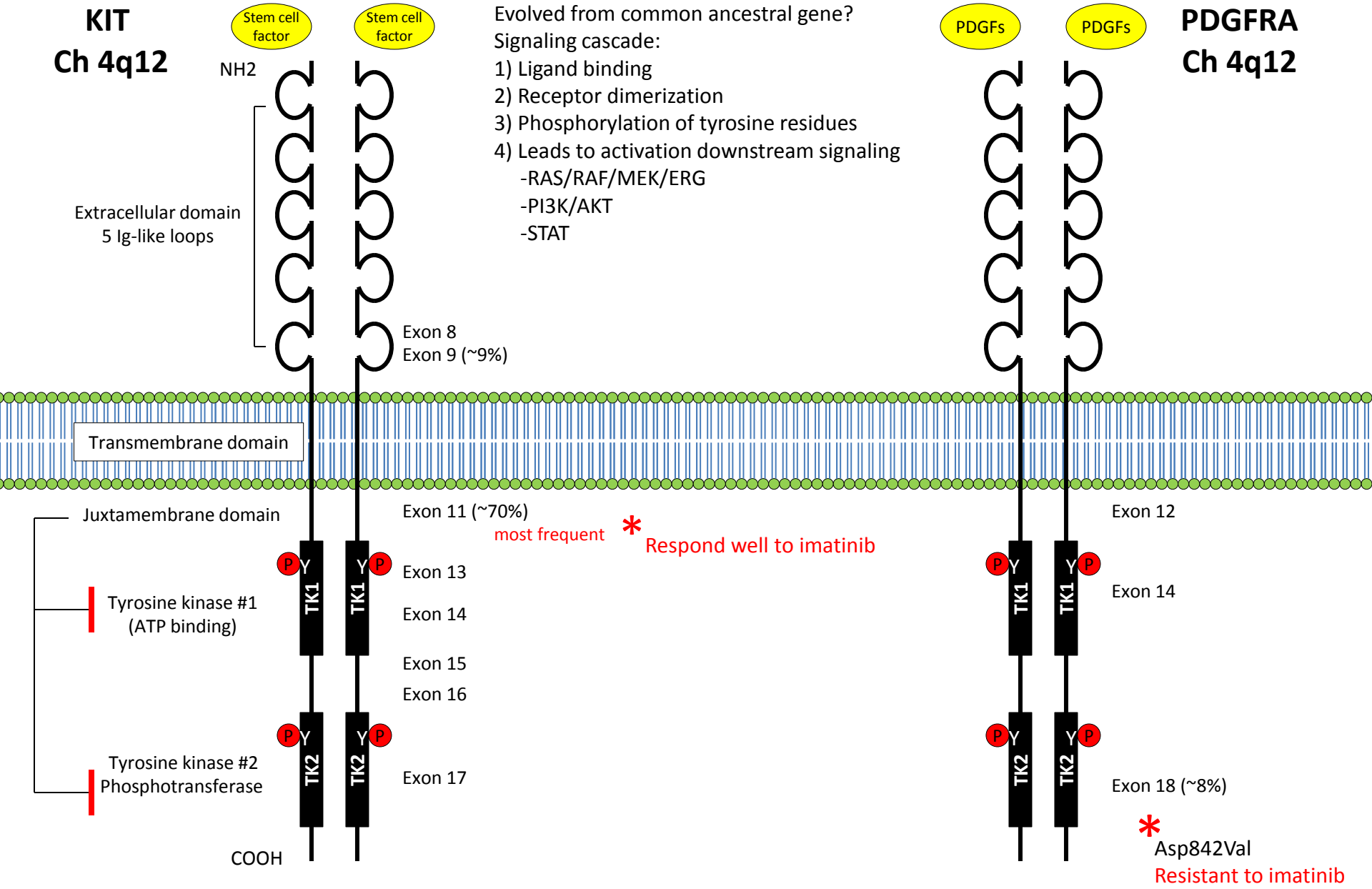
Platelet derived growth factor-alpha (PDGFRA)

- RTK closely related to KIT (homologue)
- PDGFRA mutated GISTs
  - Tend to arise in stomach, have epithelioid morphology, variable to absent KIT IHC staining, and more indolent behavior

Gastrointestinal stromal tumor (GIST)

- KIT and PDGFRA mutated GISTs
  - Sporadic = Majority (solitary tumors)
  - Familial = Rare (often multiple tumors and background ICCH)

- Wild-type GISTs (no KIT or PDGFRA mutations identified)
  - 10% of adult GISTs and 85% of pediatric GISTs
  - Most are associated with hereditary syndromes
    - Neurofibromatosis type 1 (NF1)
    - Carney triad (CT)
    - Carney-Stratakis syndrome (CSS)
    - Hereditary paraganglioma/pheochromocytoma syndrome (HPGL/PCC)
  - BRAFV600E in 13% of cases
  - HRAS
  - NRAS



Modified from Lasota J & Miettinen M. Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal tumors. Histopathology 2008;53:245-266

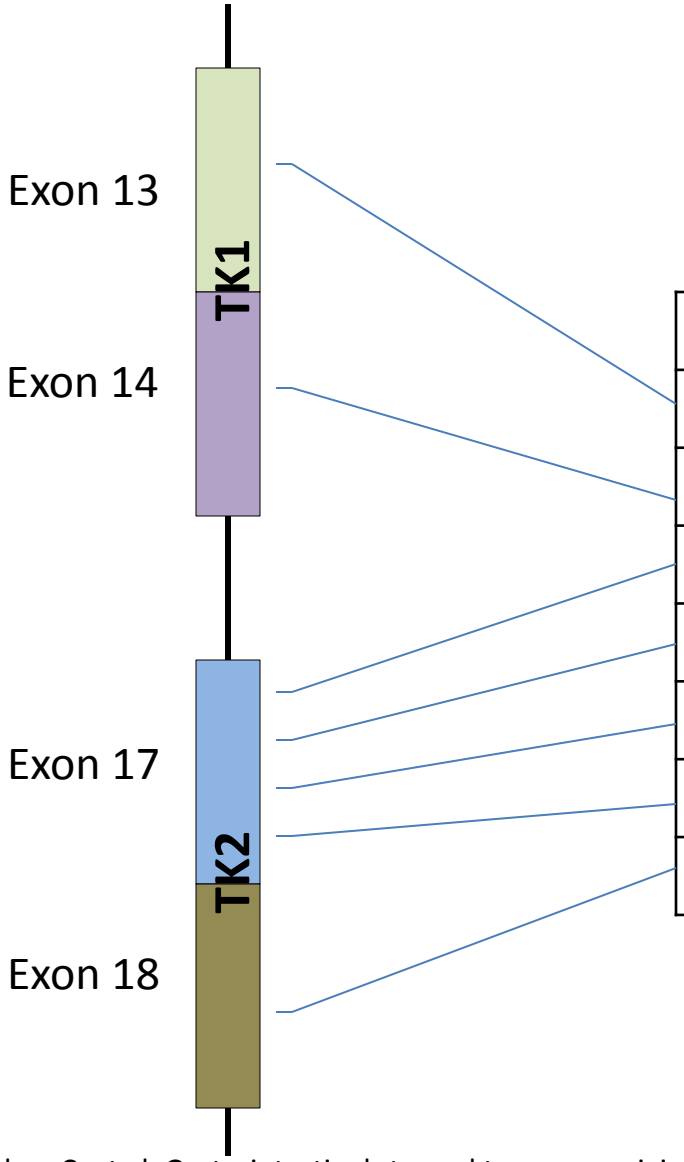
Gene mutation	Frequency	Site	Familial
KIT	75-80%		
Exon 8	Rare	Small bowel	One kindred
Exon 9 insertion AY502-503	10%	Small bowel & colon	None
Exon 11 (del, sub,ins)	67%	All sites	Several kindreds
Exon 13 K642E	1%	All sites	Two kindreds
Exon 17 D820Y, N822K and Y823D	1%	All sites	Five kindreds
PDGFRA	5-8%		
Exon 12 (such as V561D)	1%	All sites	Two kindreds
Exon 14 N659K	<1%	Stomach	None
Exon 18 D842V	5%	Stomach, mesentery & omentum	None
Exon 18 (ex: del IMHD p.842-846 IMHD)			
KIT & PDGFR wild-type	12-15%		
BRAF V600E	~7-15%		
SDHA, SDHB, SDHC, SDHC	~2%	Stomach & small bowel	Carney-Stratakis
HRAS or NRAS	<1%		
Sporadic pediatric	~1%	Stomach	Not heritable
Carney triad with GISTs	~1%	Stomach	Not heritable
NF1 with GISTs	Rare	Small bowel	Numerous

Corless C, et al. Gastrointestinal stromal tumours: origin and molecular oncology. Nature Reviews Cancer 2011;11:865-878

imatinib mesylate for GIST

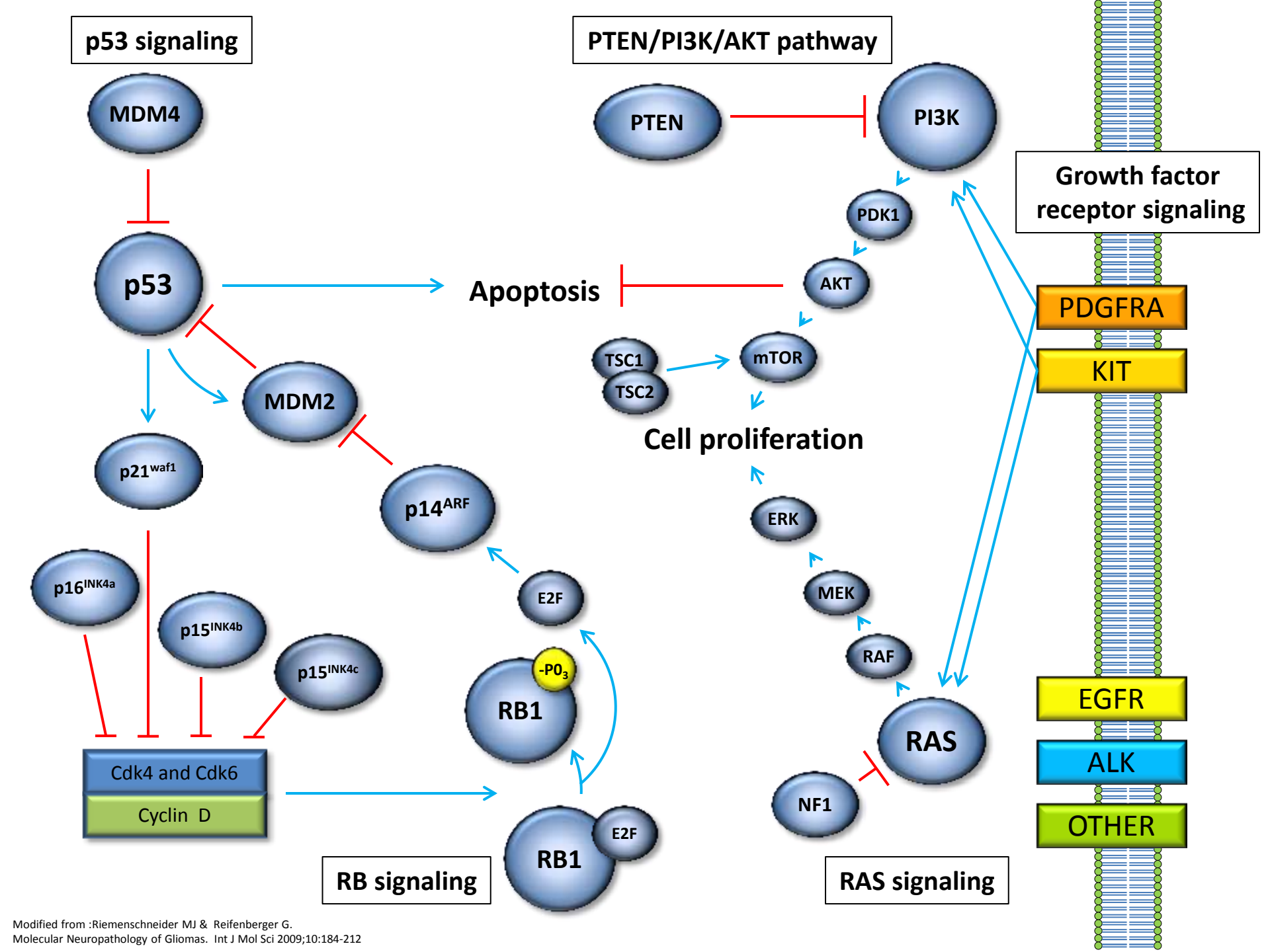
- Imatinib mesylate is a selective tyrosine kinase inhibitor (TKI) whose targets included KIT, PDGFRA, PDGFRB and ABL
  - Partial or stable response in 80% of patients
  - Complete lasting responses are rare
    - ~ 50% of initial responders develop drug resistance
      - Most commonly a second KIT mutation in the kinase domain
      - Disrupts imatinib binding by stabilizing the receptor in a constitutively active form

Secondary KIT mutations



Mutations	imatinib	sunitinib	sorafenib	nilotinib
V654A	Resistant	Sensitive	Sensitive	Intermediate
T670I	Resistant	Sensitive	Sensitive	Resistant
D816A/G/H/V	Resistant	Resistant	Resistant	Resistant
D820A/E/G/Y	Resistant	Resistant	Sensitive	Unknown
N822H/K	Intermediate	Resistant	Sensitive	Resistant
Y823D	Resistant	Resistant	Unknown	Unknown
A829P	Intermediate	Resistant	Unknown	Unknown

Corless C, et al. Gastrointestinal stromal tumours: origin and molecular oncology. Nature Reviews Cancer 2011;11:865-878



Modified from :Riemenschneider MJ & Reifenberger G.
Molecular Neuropathology of Gliomas. Int J Mol Sci 2009;10:184-212

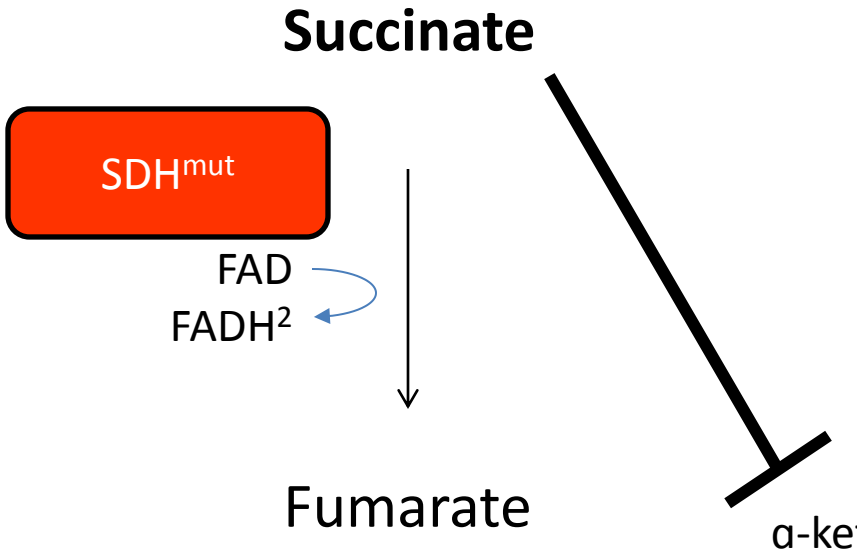
SDH in GIST

- Multi-subunit complex of nuclear encoded genes
- Component of mitochondrial Krebs cycle and ETS
- Inactivating mutations affect global epigenomic methylation pattern
  - Failure in DNA methylation maintenance (hypermethylation)
  - Succinate accumulation inhibits TET2 dioxygenase which converts 5-MC to 5-hMC (initial step in demethylation)
  - Similar to IDH1/2 in gliomas
  - Can use IHC to detect SDH-null GISTs (SDHA & SDHB) and decrease in 5-hMC
- SDH mutant GISTs have more stable genomes
  - Do not have the numerous chromosomal gains and losses (CNVs) seen in KIT and PDGFR GISTs

Janeway KA, et al. Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. Proc Natl Acad Sci USA 2011;108:314-318
Killian JK, et al. Succinate Dehydrogenase Mutation Underlies Global Epigenomic Divergence in Gastrointestinal Stromal tumors. Canc Disc 2013; June Epub

Krebs cycle and electron transport chain (ETS)

INACTIVATING/LOSS OF FUNCTION MUTATIONS



α-ketoglutarate dependent dioxygenases

TET2 5-methylcytosine dioxygenase:
- ↓ 5-hydroxymethylcytosine
- → Glioma CIMP (hypermethylation)

SDHA 5p15
SDHB 1p36.1-p35
SDHC 1q23.3
SDHD 11q23

Malzkorn B, et al. Unraveling the Glioma Epigenome – From Molecular Mechanisms to Novel Biomarkers and Therapeutic Targets. Brain Pathology 2011;21:619-632

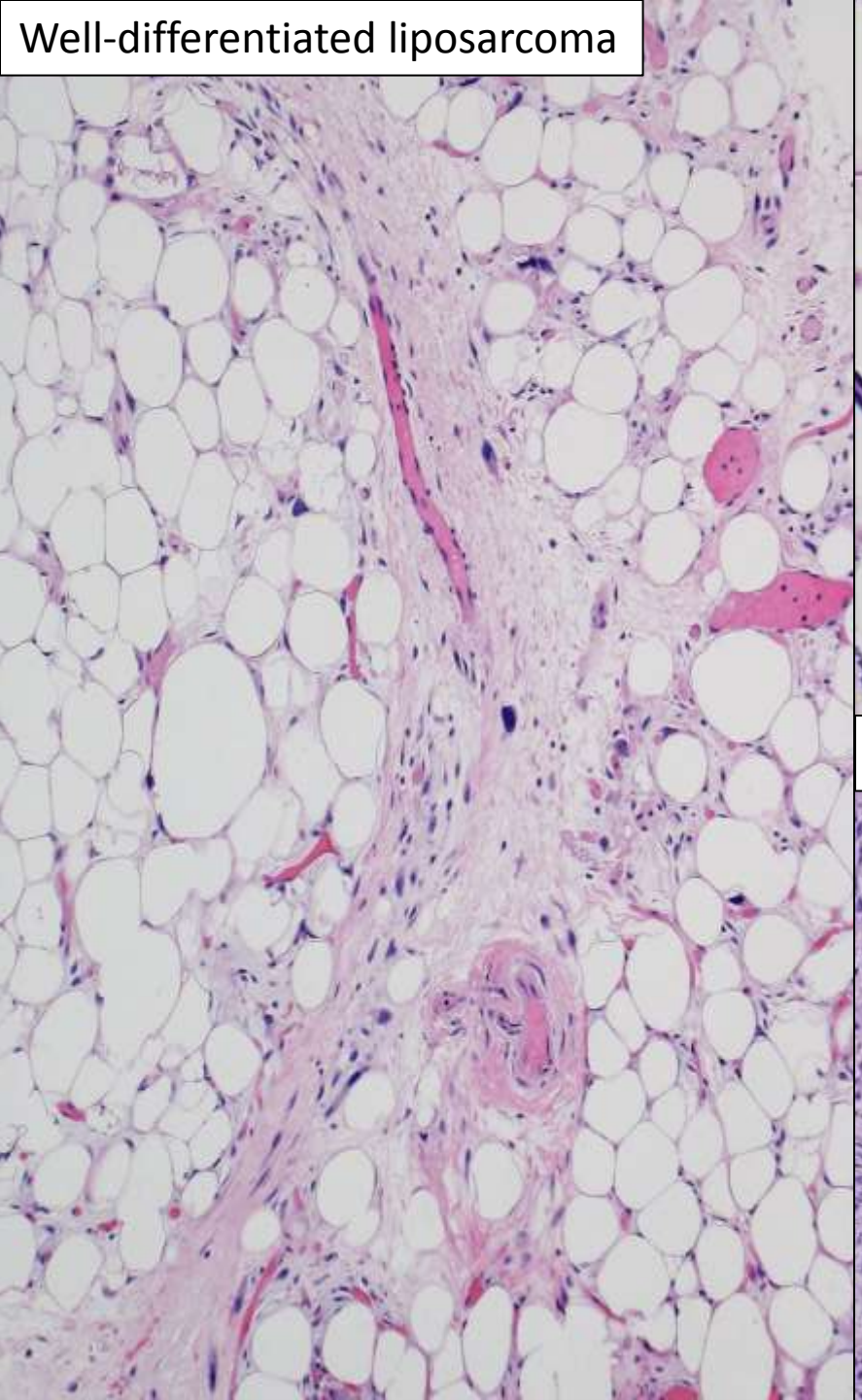
Gastrointestinal stromal tumor (EGIST)

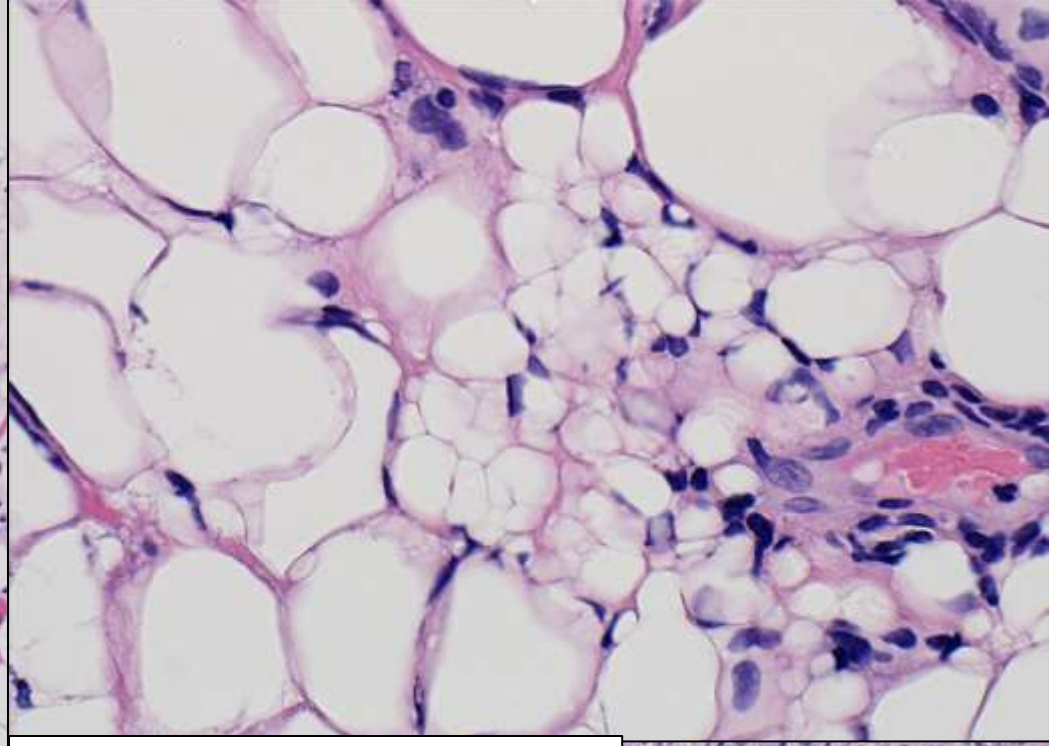
- **Utility of molecular diagnostics:**
  - Diagnosis
  - Prognostic (specific mutations)
  - Predictive (targeted therapy available)

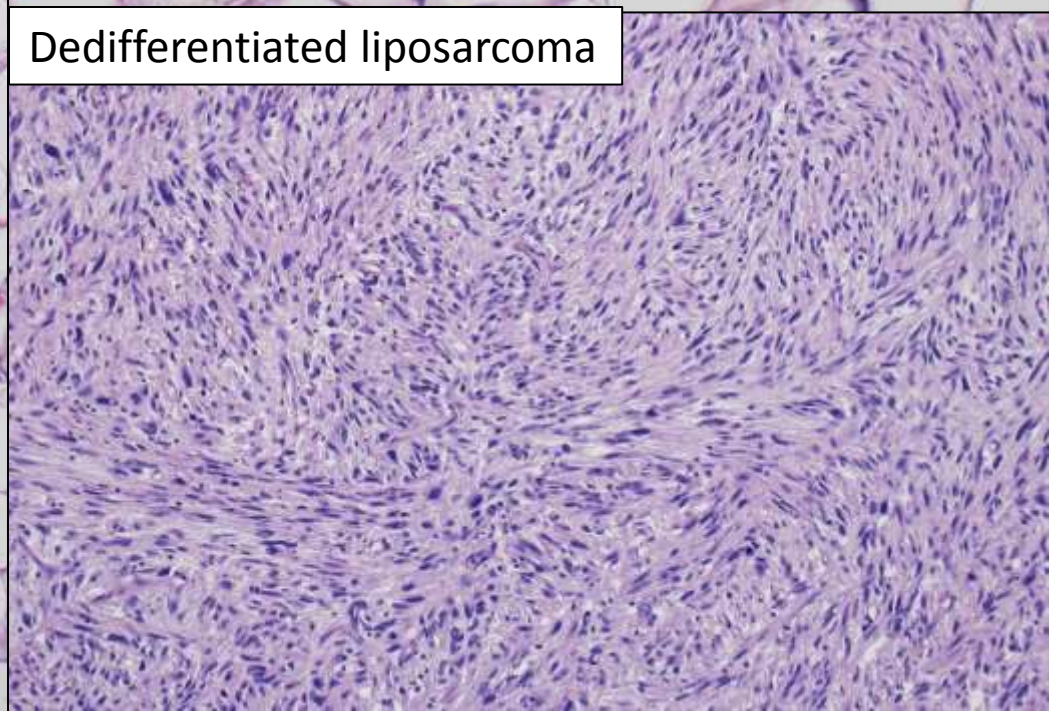
- **Differential diagnosis:**
  - Spindle cell and epithelioid neoplasms, including:
    - Schwannoma
    - Leiomyoma
    - Leiomyosarcoma

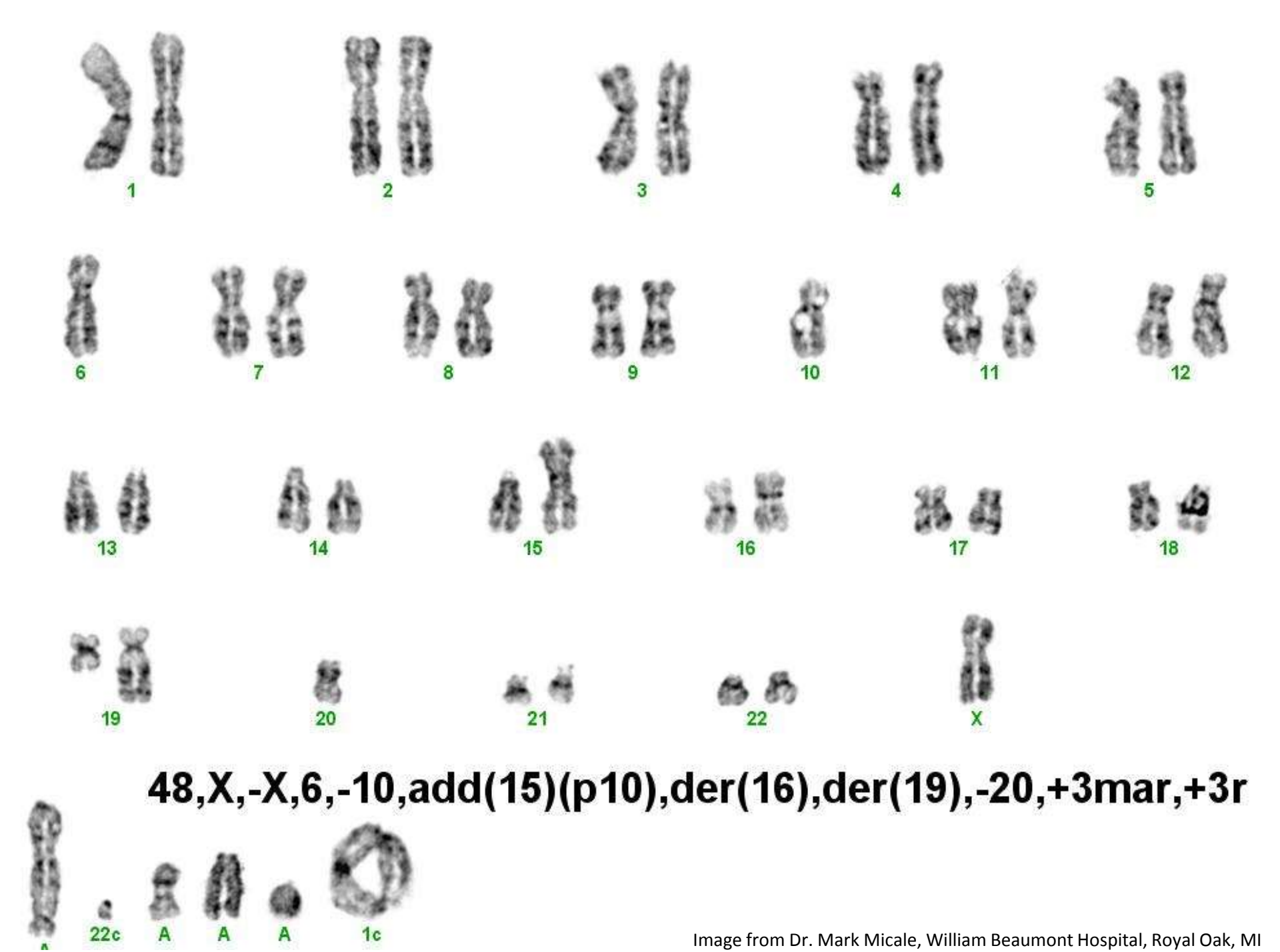
Well-differentiated and de-differentiated liposarcoma (WDLPS & DDLPS)

- **Definition:**
  - Locally aggressive sarcoma showing variably-developed adipocytic differentiation, variation in cell size, nuclear atypia, and variable numbers of lipoblasts. Morphology categorized into adipocytic, sclerosing, spindle cell and inflammatory subtypes.
  - May progress to de-differentiated liposarcoma (low & high grade)
    - Retroperitoneal tumors >20%
    - Limbs <2%
- **Recurrent genetic abnormality:**
  - Amplification of 12q13-15 within ring and/or supernumerary chromosomes
    - Potential oncogenes
      - MDM2, CDK4, HMGA2, TSPAN31, OS1, OS9, CHOP and GLI1
      - Most evidence for MDM2, CDK4, HMGA2, TSPAN31
    - MDM2 most consistently amplified (99%)


Well-differentiated liposarcoma

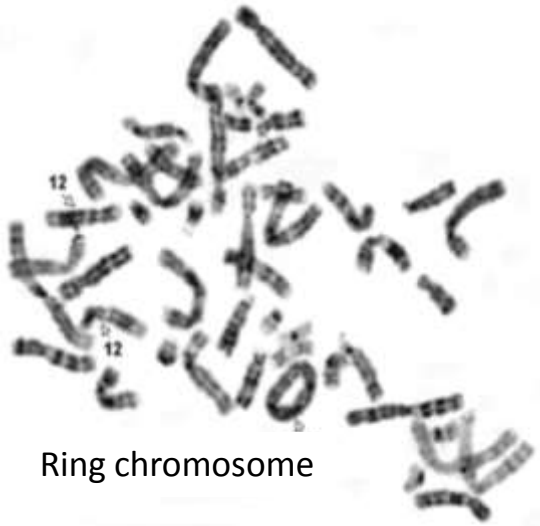



Dedifferentiated liposarcoma



48,X,-X,6,-10,add(15)(p10),der(16),der(19),-20,+3mar,+3r

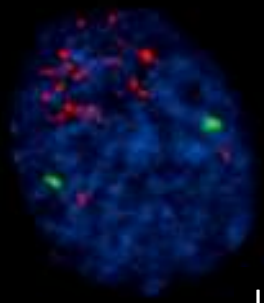
Image from Dr. Mark Micale, William Beaumont Hospital, Royal Oak, MI



Ring chromosome

Giant marker/supernumerary chromosome

metaphase FISH
MDM2 SO
CDK4 SG

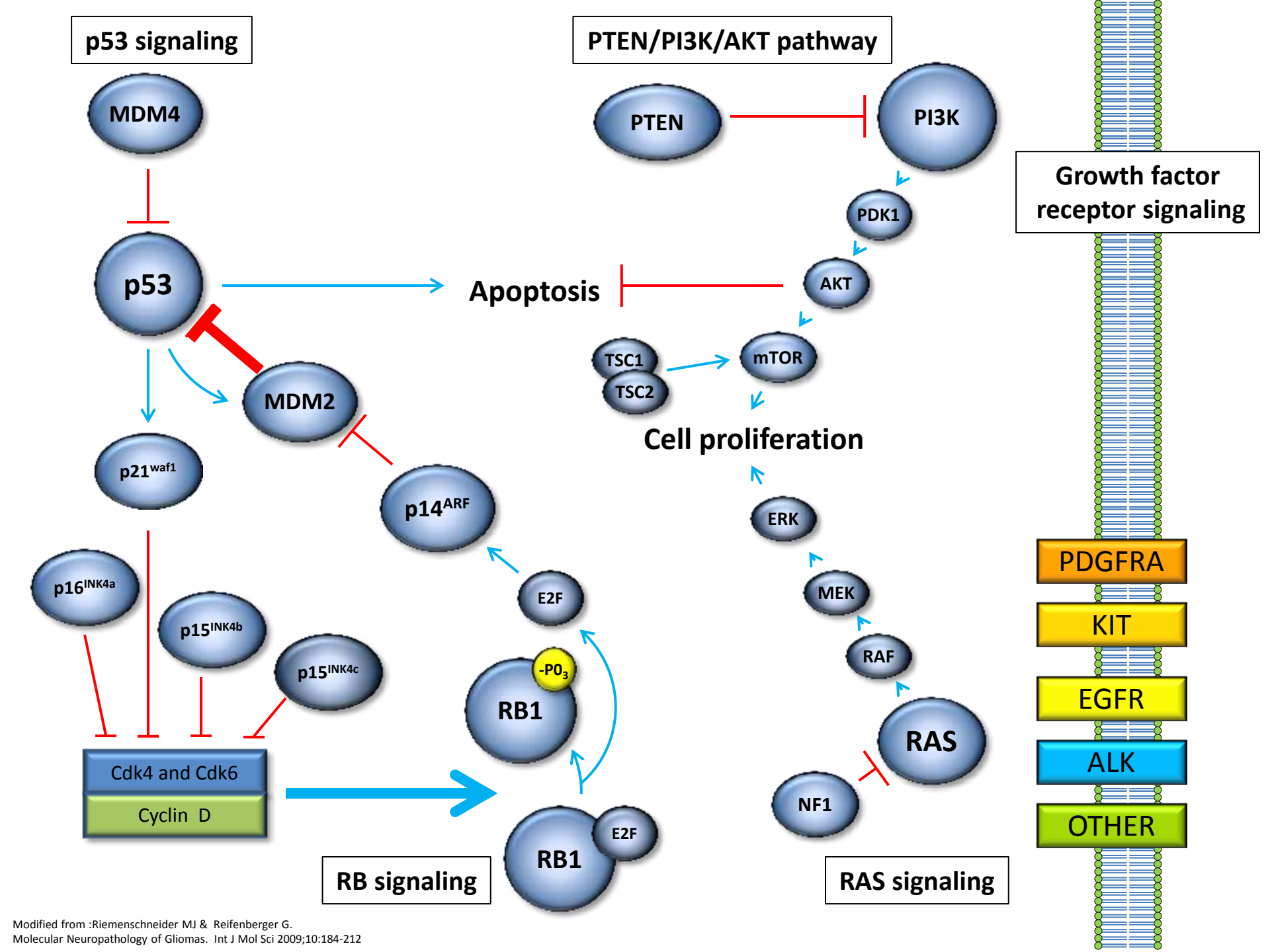


Interphase FISH
MDM2 SO
CEP SG

Atlasgeneticsoncology.org
Zhang H, et al. Am J Surg Path 2010;34:1304-1311
Bridge J, et al. Molecular Diagnostics of Soft Tissue Tumors. 2011;135:588-601

MDM2 oncogene, E3 ubiquitin protein ligase

- Ch 12q14.3-q15
- Negative regulator of p53
  - Negative feedback loop:
    - Elevated p53 levels activate MDM2 transcription
    - MDM2
      - Binds to transcriptional activating domain of p53, blocking p53 dependent transcription
      - Acts as a ubiquitin ligase, targeting p53 for degradation



Modified from :Riemenschneider MJ & Reifenberger G.
Molecular Neuropathology of Gliomas. Int J Mol Sci 2009;10:184-212

Well-differentiated and de-differentiated liposarcoma (WDLPS & DDLPS)

- **Utility of molecular diagnostics:**
  - Diagnosis

- **Differential diagnosis:**
  - Lipoma
  - Pleomorphic sarcoma infiltrating adipose tissue
  - Normal adipose tissue
  - Fat necrosis
  - Atrophy of fat
  - Localized massive edema

Summary

- Molecular diagnostics can provide diagnostic, prognostic and predictive types of information in the clinical work-up of soft tissue sarcomas.
- Each method has its own strengths and weaknesses.
- Appropriate triage of tissue while the specimen is fresh allows for potential access to the full array of diagnostic testing.

Summary

- The field of molecular diagnostics is rapidly evolving, both in the area of new methodologies and discovery of recurrent genetic abnormalities in cancer.
- Molecular testing will increasingly become standard of care with further development and availability of targeted therapies.
- Immunohistochemical methods to detect protein products of chimeric gene fusions or mutated genes can provide a cost effective way to screen for well-characterized genetic alterations in the setting of cancer.

Suggestions

- Submit tissue for cytogenetic analysis. You can always cancel the testing, and incur only the charge for setting up the culture.
- Make air dried cytology preparations of tumor samples prepared at the time of frozen section and/or the grossing of fresh tissue. They are easier and faster to process, avoid the problem of nuclear truncation inherent to FISH performed on paraffin block tissue, and provide excellent quality DNA and/or RNA for molecular testing modalities.
- In the setting of partially bony or calcified tissue, consider submitting non-decalcified tissue for processing into FFPE to preserve DNA and RNA integrity

Thank you.