Molecular Pathology of Sarcomas: Diagnostic and Therapeutic Utility

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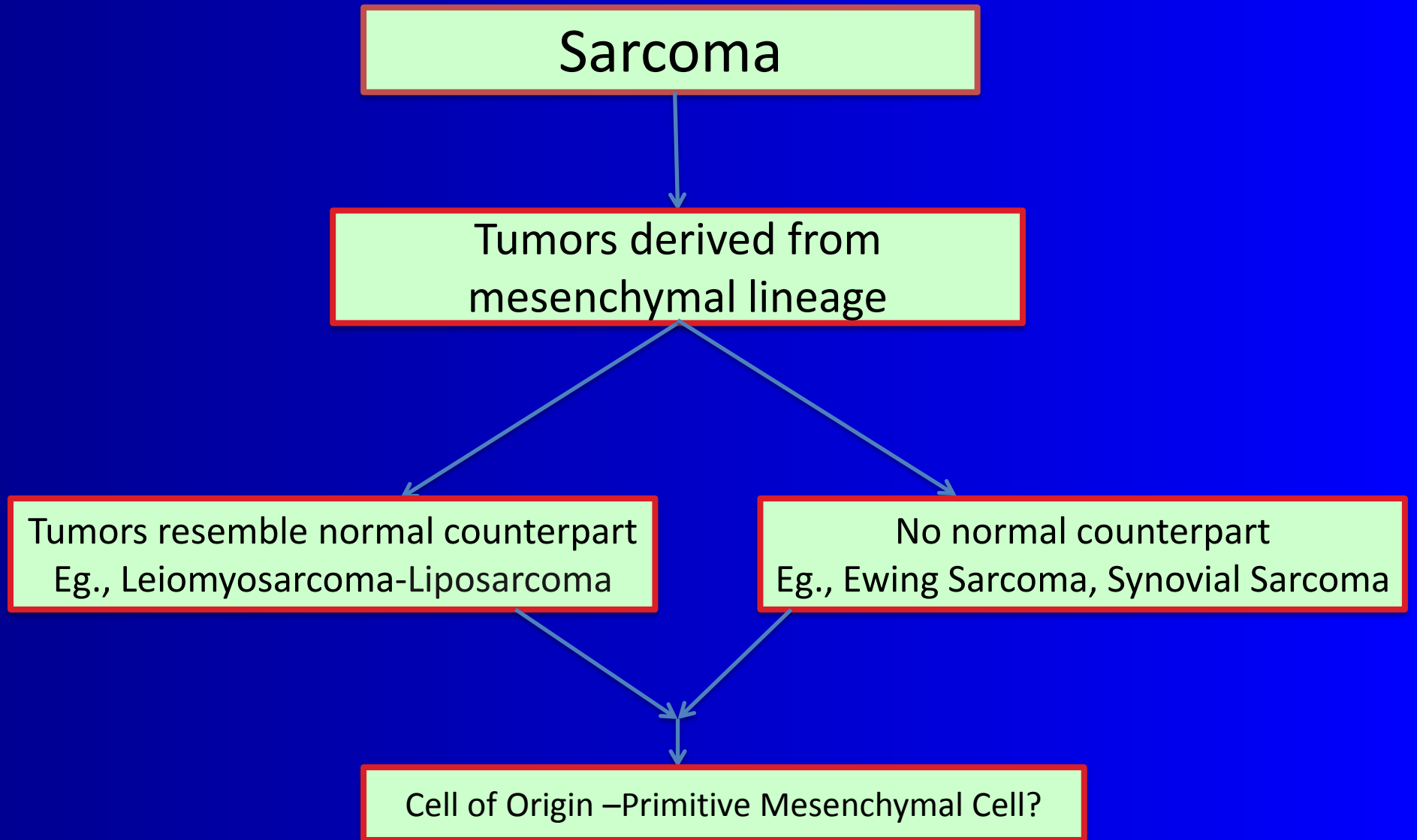
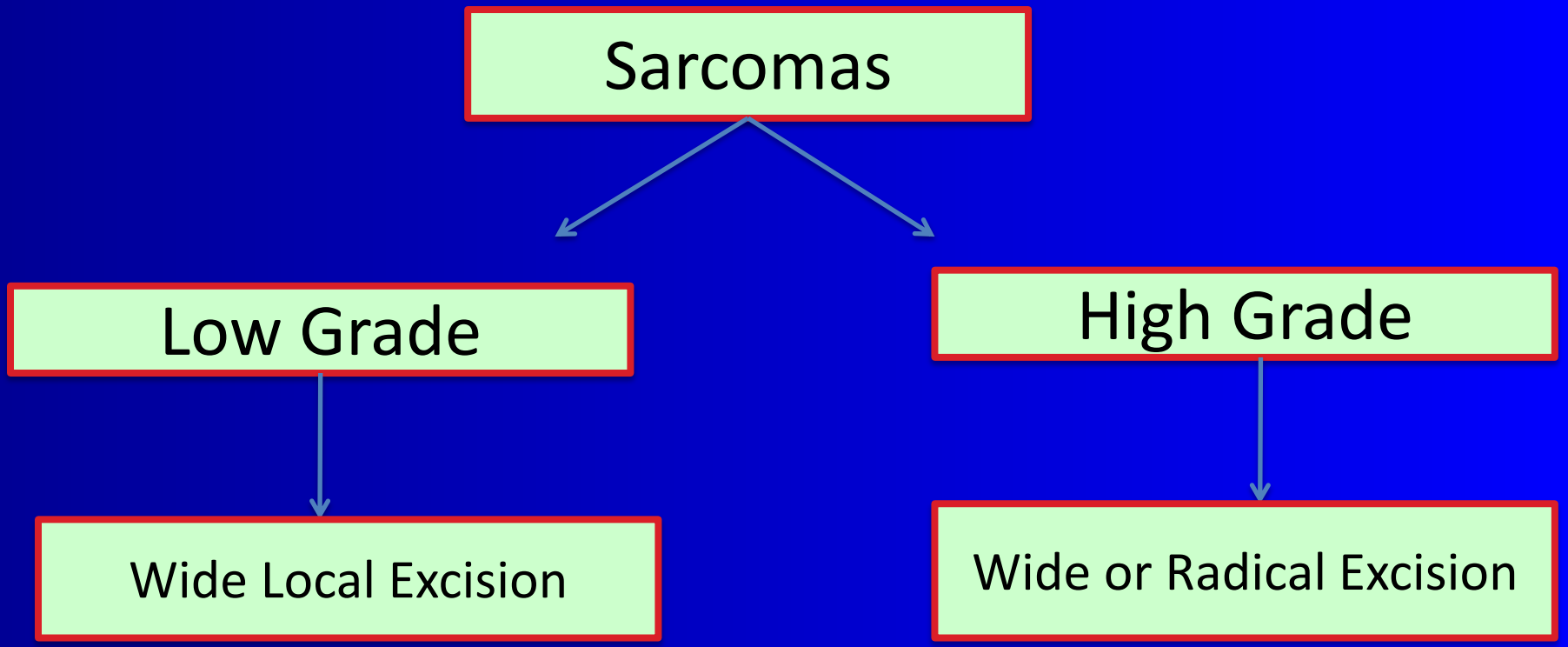
Role of a Pathologist

- Custodians and Curators of tumor diagnostic specimens
- Appreciation of Pathogenesis
- Determination of sub types
- Appropriate Molecular Testing for Diagnosis, Prognosis and Therapeutics
- Critical Role in Sarcoma clinical team to streamline therapeutic strategies

Conventional Pathology-Sarcomas



Sarcomas-Clinical Management



Behavior: Indolent to highly invasive to metastatic

Prognosis: Age, tumor size, grade, depth, histological subtypes

Chemotherapy

Sensitive

Gastrointestinal Stromal Tumor (GIST)
Ewing Sarcoma
Rhabdomyosarcoma
Synovial Sarcoma
Myxoid Liposarcoma
Malignant Fibrous Histiocytoma

Intermediate

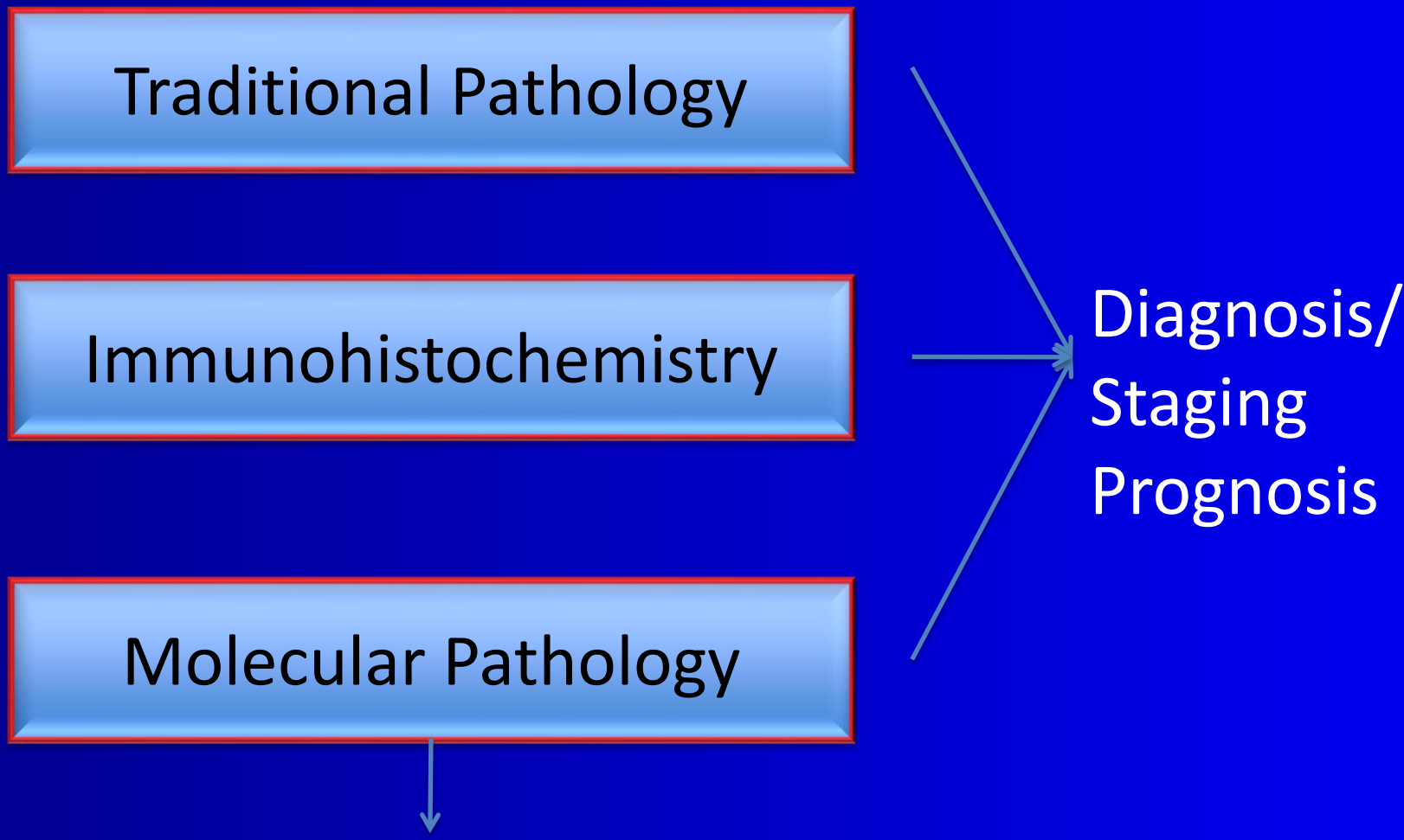
Liposarcoma(other types)
Myxofibrosarcoma
MPNST
Leiomyosarcoma

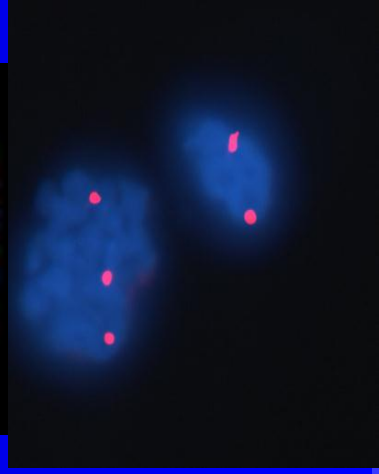
Resistant

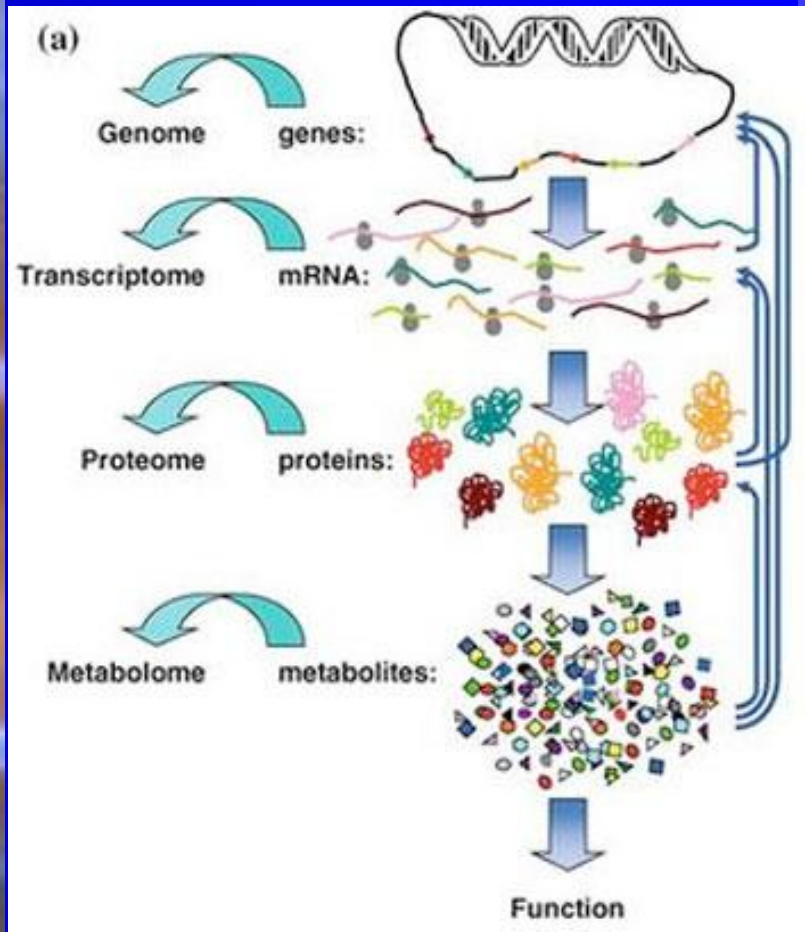
Chondrosarcoma
Clear Cell Sarcoma
Epithelioid Sarcoma
Rhabdoid Tumor
Alveolar Soft Part Sarcoma

Histology driven Chemotherapy- Important for targeted and non-targeted therapy

Diagnosis of Sarcomas






(a)
Genome
genes:
Transcriptome
mRNA:
Proteome
proteins:
Metabolome
metabolites:
Function

Molecular Pathology of Sarcomas

Specific Translocations or gene amplification

Defined by Oncogenic gene mutations

Complex karyotypes/genomic rearrangements

Diverse tumors in each class

Molecular Pathology-Sarcomas

Translocation associated Sarcomas and relatively simple Karyotypes

Sarcomas with complex karyotypes and no specific translocations

Ewing Sarcoma/PNET
Desmoplastic Small round cell tumor
Alveolar Rhabdomyosarcoma
Myxoid Liposarcoma
Extraskeletal myxoid Chondrosarcoma
Clear cell sarcoma (soft tissue)
Angiomatoid fibrous histiocytoma
Congenital/infantile Fibrosarcoma
Low grade fibromyxoid sarcoma
Inflammatory myofibroblastic tumor
Alveolar Soft Part Sarcoma
Synovial Sarcoma
Epithelioid Hemangioendothelioma

Epithelioid Sarcoma
Leiomyosarcoma
Myxofibrosarcoma
Adult fibrosarcoma
Liposarcomas other than myxoid liposarcoma
Embryonal and pleomorphic rhabdomyosarcoma
Osteosarcoma
Angiosarcoma
Undifferentiated Pleomorphic sarcoma

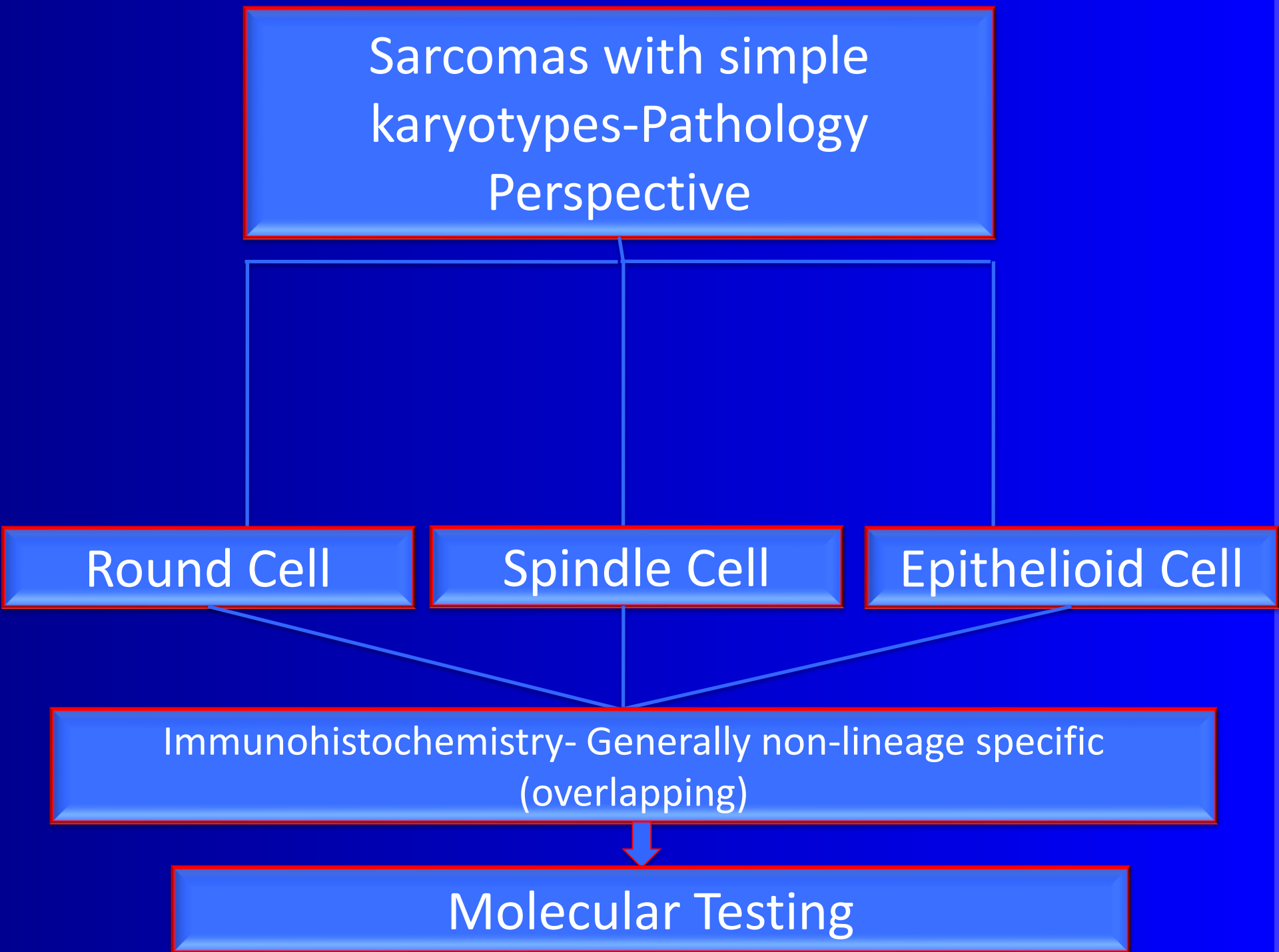
Mutation Driven- Gastrointestinal Stromal Tumor

Amplification associated- Liposarcoma-Angiosarcoma

Molecular Pathology-Sarcomas

- Ewing Sarcoma/PNET- First discovered translocation associated sarcoma t(11;22)(q14;q22) (EWS-FLI1) New Eng J Med, 309; 496-498, 1983

Sarcoma Type	Translocation	Fusion gene	Year
Ewing Sarcoma	t(21;22)	EWSR1-ERG	1993
Clear Cell Sarcoma Myxoid/round cell Lipoarcoma	t(12;22) t(12;16)	EWSR1-ATF1 FUS-DDIT3	1993 1993
ARMS DSRCT	t(2;13)&(1;13) t(11;22)	PAX3&PAX7-FOXO1A EWS-WT1	1994
Extraskeletal Myxoid Chondrosarcoma	t(9;22) t(9;17)	EWSR1-NR4A3 EWSR1-TAF2N	1995 1999
Synovial Sarcoma	t(X;11)	SYT-SSX1 and SSX2	1995
DFSP	t(17;22)	COL1A1-PDGFB	1997
Congenital fibrosarcoma	t(12;15)	ETV6-NTRK3	1998
IMT	t(2p23)	Alk Fusions (many partners)	2000
Alveolar Soft Part Sarcoma	t(X;17)	ASPL-TFE3	2001
Low Grade Fibromyxoid sarcoma	t(7;16)	FUS-ATF1	2003
Angiomatoid Fibrous Histiocytoma	t(12;16) t(12;22)	FUS-ATF1 EWSR1-ATF1&CREB1	2000 2007


Sarcomas with simple karyotypes-Pathology Perspective
Round Cell
Spindle Cell
Epithelioid Cell
Immunohistochemistry- Generally non-lineage specific (overlapping)
Molecular Testing

Translocation associated sarcomas

Tumor Type	Translocation	Fusion gene(s)
Alveolar Soft Part Sarcoma	der(17)t(x;17)	ASPL-TFE3
Alveolar Rhabdomyosarcoma	t(2;13) &t(1;13)	PAX3-FOXO1 &PAX7-FOXO1
Angiomatoid Fibrous Histiocytoma	t(2;22) , t(12;22) &t(12;16)	EWSR1-CREB1, EWSR1-ATF1 &FUS-ATF1
Clear Cell Sarcoma	t(12;22) &t(2;22)	EWSR1-ATF1 &EWSR1-CREB1
Congenital infantile fibrosarcoma	t(12;15)	ETV6-NTRK3
Dermatofibrosarcoma Protuberans	t(17;22)	COL1A1-PDGFB
Desmoplastic small round cell tumor	t(11;22)	EWSR1-WT1
Endometrial Stromal sarcoma	t(7;17), t(6;7) &t(6;10)	JAZF1-SUZ12, JAZF1-PHF1 &EPC1-PHF!
Ewing Sarcoma/PNET	t(11;22), t(21;22), t(7;22), t(2;22), inv(22), t(6;21)	EWSR1-FLi1, EWSR1-ERG, EWSR1-ETV1. EWSR1-E1AF. EWSR1-FEV. EWSR1-ZSG &FUS-ERG
Extraskeletal myxoid chondrosarcoma	t(9;22), t(9;17), t(9;15)	EWSR1-NR4A3, RBP56-NR4A3, TCF12-NR4A3, TFG-NR4A3
Inflammatory myofibroblastic tumor	t(3;9), t(1;2), t(2;19), t(2;17), t(2;2), t(2;17)	TPM3-ALK, TPM4-ALK, CLTC-ALK,RANBP2-ALK
Low grade Fibromyxoid sarcoma	t(7;16) &t(11;16)	FUS-CREB3L2 &FUS-CREB3L1
Myxoid-Round cell Liposarcoma	t(12;16) &t(12;22)	FUS-DDIT3 &EWS-DDIT3
Epithelioid Hemangioendothelioma	t(1;3)(p36;q25)	WWTR1-CAMTA1
Synovial Sarcoma	t(X;18) &t(X;20)	SS18-SSX1, SS18-SSX2, SS18-SSX4 &SS18L1-SSX1

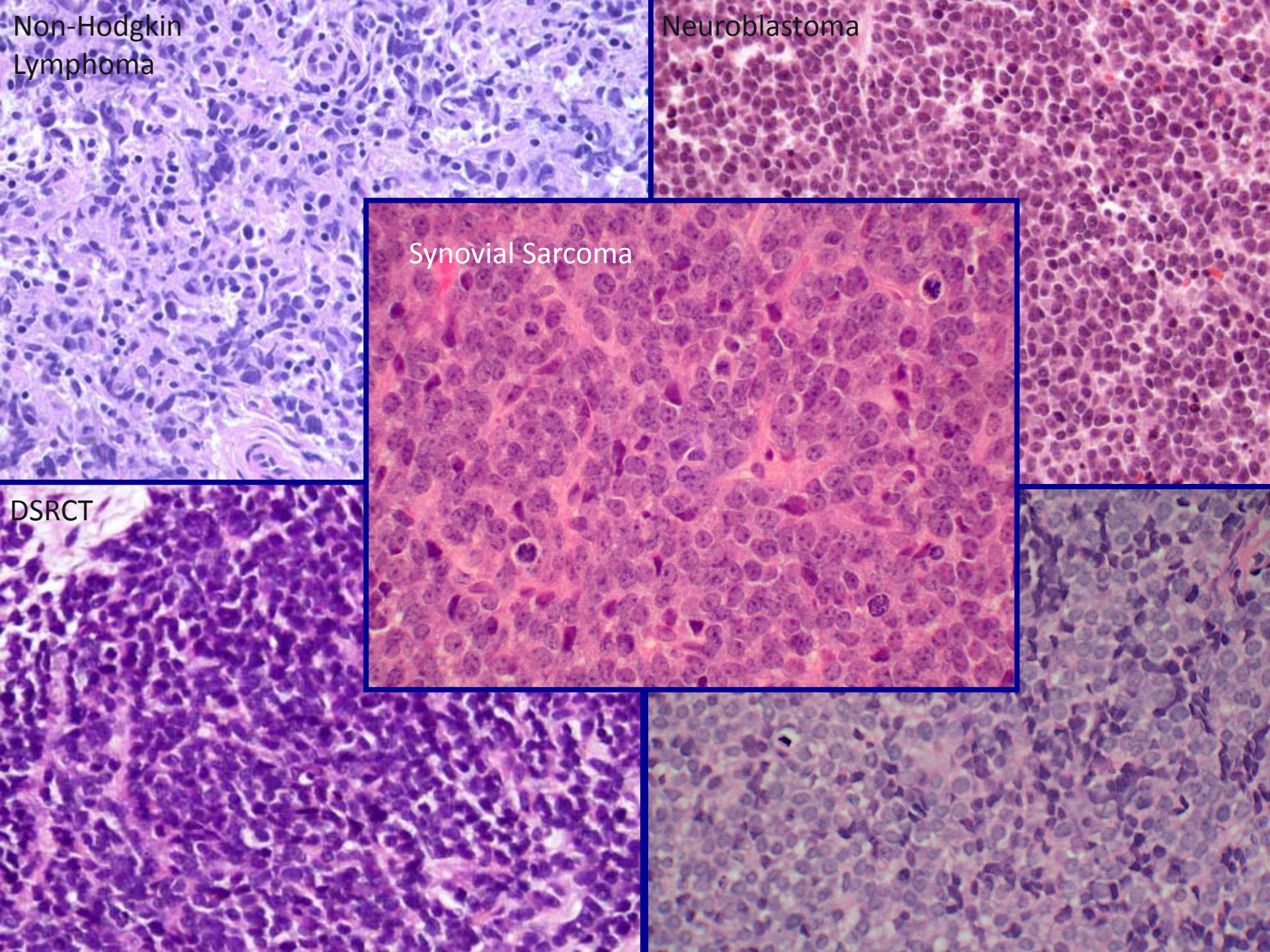
Molecular Testing in Sarcomas- Caveats

Infidelity in Chromosomal Translocations

Translocation	Gene Fusion	Tumor Type	Site/Organs
t(12;15)	ETV6-NTRK3	Infantile Fibrosarcoma Mesoblastic Nephroma Secretory carcinoma breast Salivary gland carcinoma-mammary analog AML	Soft Tissue Kidney Breast Salivary Gland Blood &BM
t(X;17)	ASPL-TFE3	Alveolar Soft Part Sarcoma Renal Cell Carcinoma Subset of PECOMAs	Soft Tissue Kidney Soft tissue
t(1:2)	TPM3-ALK	Inflammatory myofibroblastic tumor (IMT) Anaplastic Large Cell Lymphoma (ALCL)	Soft tissue, abdomen Skin, Lymph nodes
t(12;22) t(2;22)	EWSR1-ATF1 EWSR1-CREB1	Angiomatoid Fibrous Histiocytoma &Clear Cell Sarcoma	Soft Tissue

EWSR1 is a sort after Gene for Sarcoma Translocations

Tumor Type	Translocation	Fusion genes
Ewing Sarcoma	t(11;22)(q24;q12) t(21;22)(q22;q12) t(7;22)(q22;q12) t(17;22)(q12;q12) t(2;22)(q31;q12) t(2;22)(q33;q12) t(1;22)(p36.1;q12) t(20;22)(q13;q12) (inv)22	EWSR1-FLI-1 EWSR1-ERG EWSR1-ETV1 EWSR1-E1AF EWSR1-SP3 EWSR1-FEV1 EWSR1-ZNF278 EWSR1-NFATC2 EWSR1-ZSG
Angiomatoid Fibrous Histiocytoma	t(12;22)(q13;q12)	EWSR1-ATF1
Clear cell Sarcoma	t(12;22)(q13;q12)	EWSR1-ATF1
Desmoplastic Small Round Cell Tumor	t(11;22)(p13;q12)	EWSR1-WT1
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	EWSR1-NR4A3
Myxoid/round cell liposarcoma	t(12;22)(q13;q12)	EWSR1-DDIT3
Myoepithelioma	t(19;22)(q13;q12) t(1;22)(q23;q12) t(6;22)(p21;q12)	EWSR1-ZNF44 EWSR1-PBX1 EWSR1-POU5F1

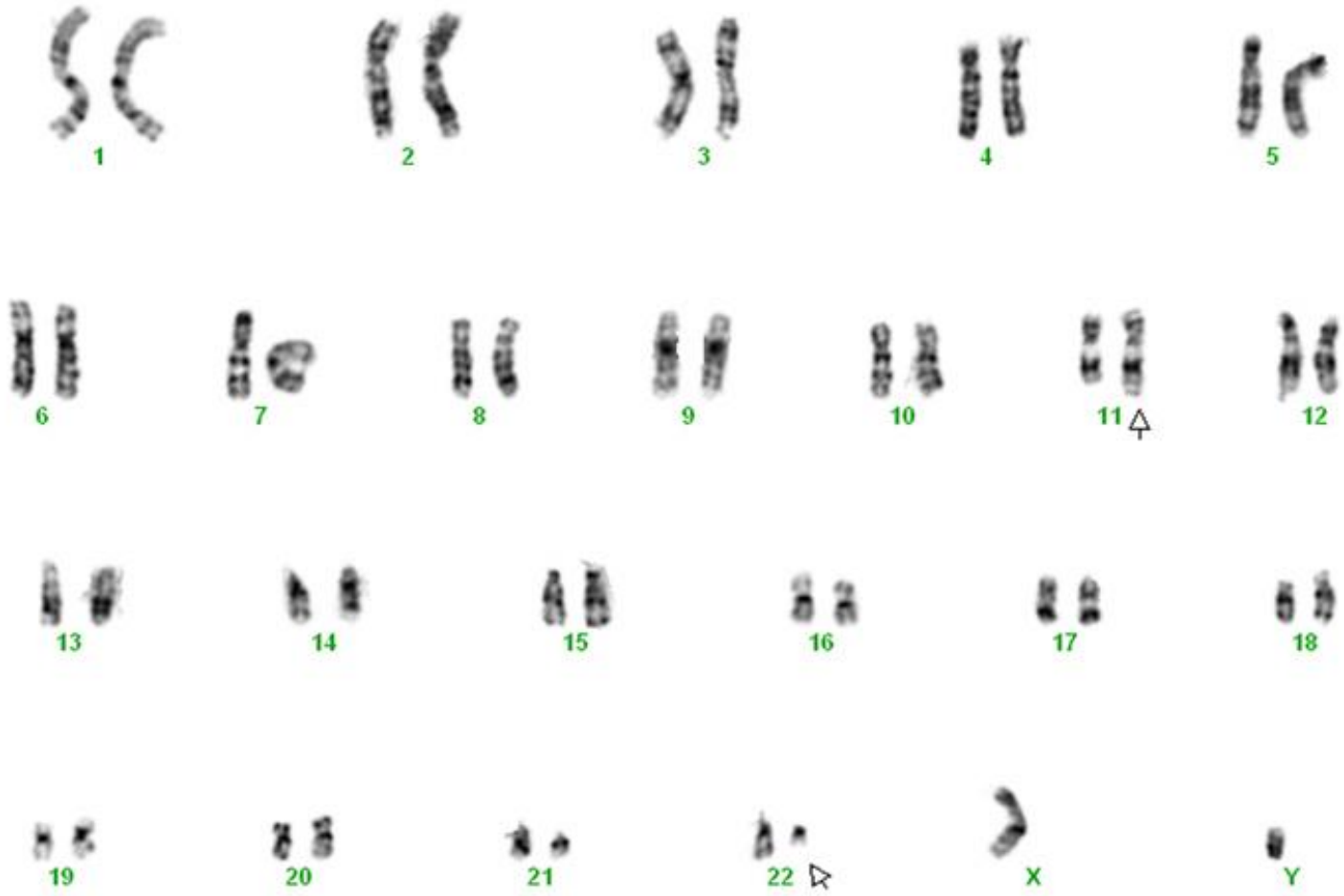

Non-Hodgkin Lymphoma
Neuroblastoma
Synovial Sarcoma
DSRCT


CD99
Sensitive but not a specific Marker

Immunohistchemical Profile of Round Cell Tumors

Tumor	CD99	Fli1	LCA	B	T	TdT	CK	Chr	S-100	Des	Myogenin	WT1
Ewing/ PNET	+	+	-	-	-	-	+/-	-	+/-	-	-	-
LBL/ALL	+	+	+/-	+/-	+/-	+	+/-	-	-	-	-	+/-
NHL-other	+/-	+	+	+	+	-	-	-	-	-	-	-
Mesen.Chondrosarcoma	+	-	-	-	-	-	-	-	+	+/-	+/-	-
Small Cell OS	+/-	-	-	-	-	-	+/-	-	+/-	+/-	-	-
Rhabdomyosarcoma	+/-	-	-	-	-	-	-	-	-	+	+	+/- cyto)
DSCRT	+	-	-	-	-	-	+	-	+/-	+	-	+
Neuroblastoma	-	-	-	-	-	-	+/-	+	+/-	-	-	-

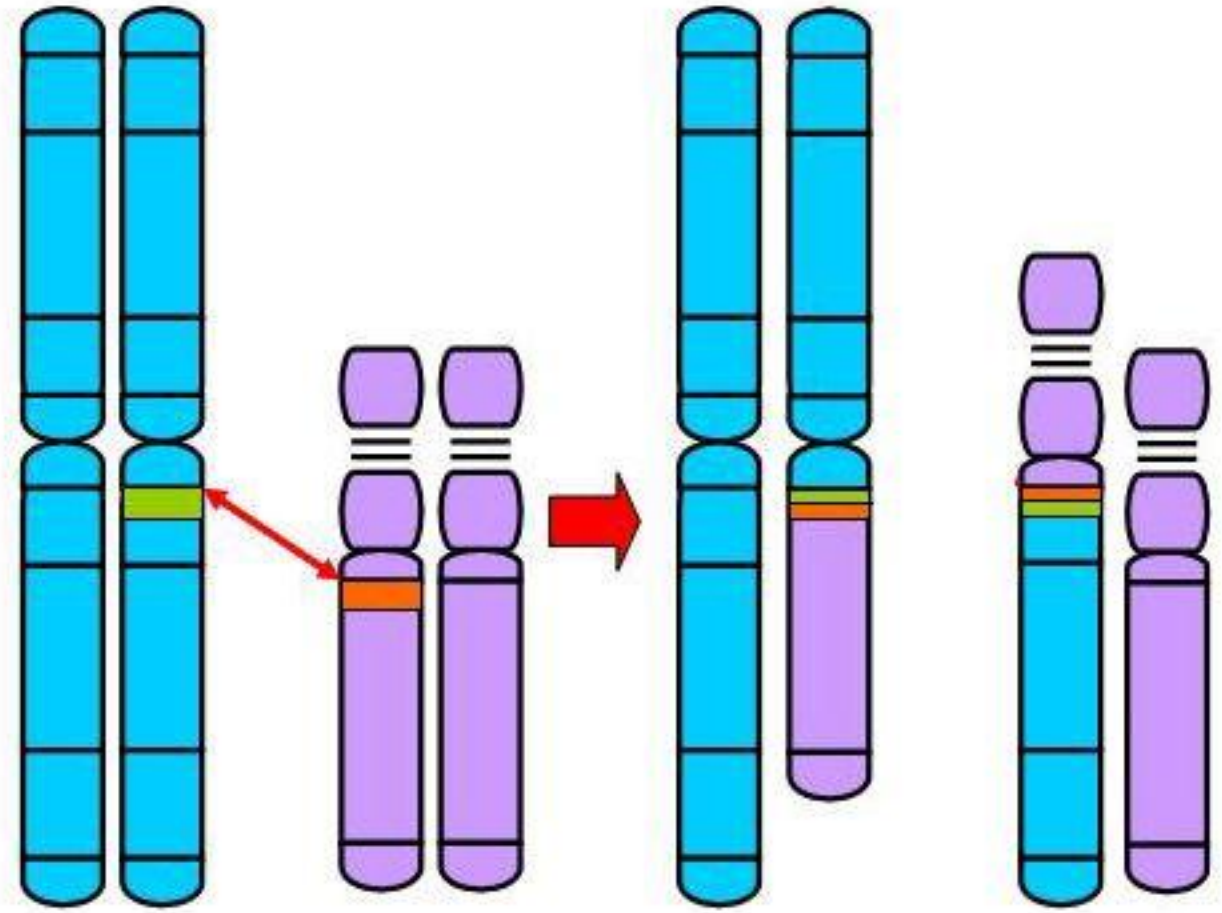
Ewing Sarcoma



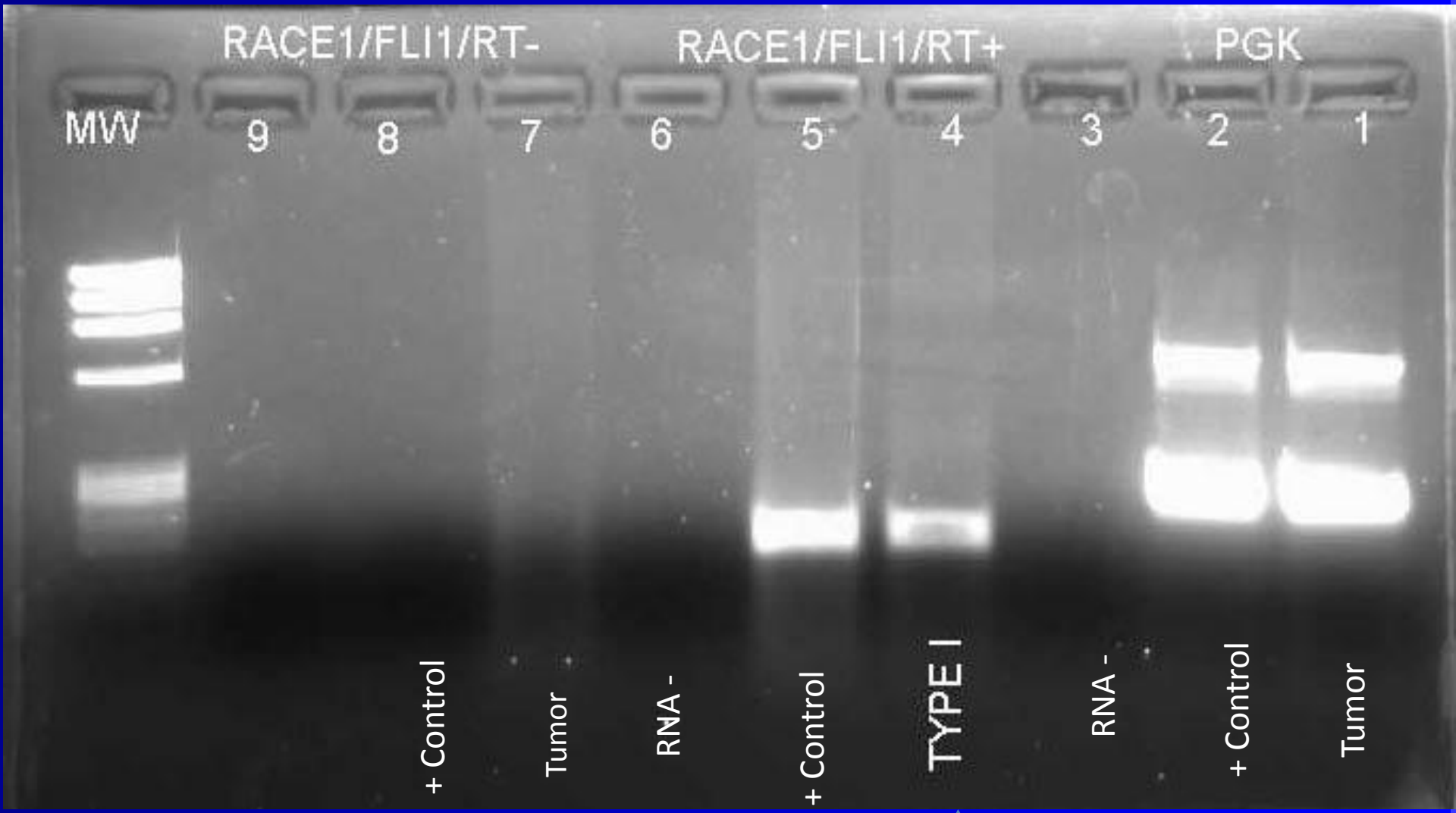
46,XY,t(11;22)(q24;q12)

t(11;22)(q24;q12)

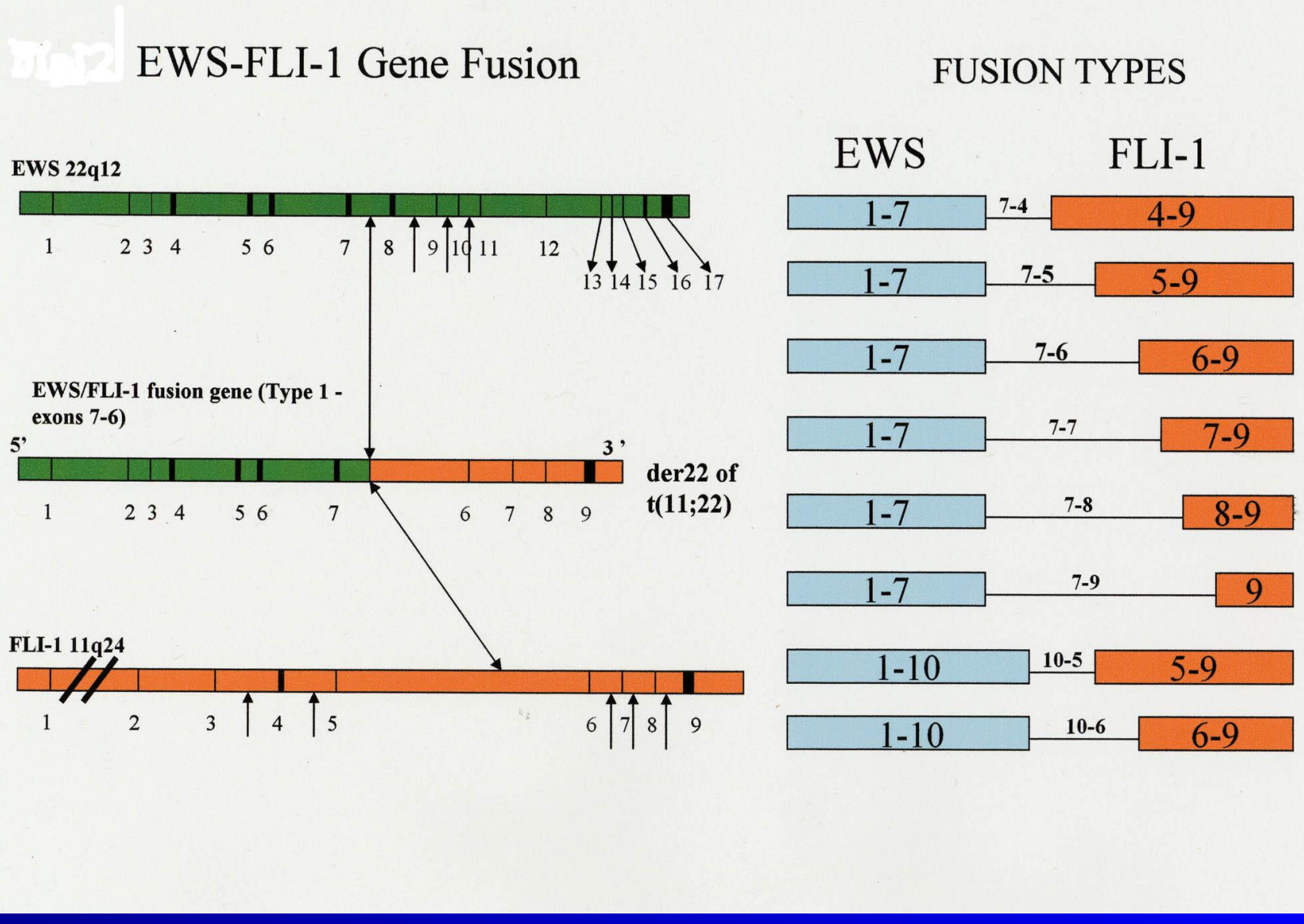
Chromosomal Translocation



EWING SARCOMA-RT-PCR

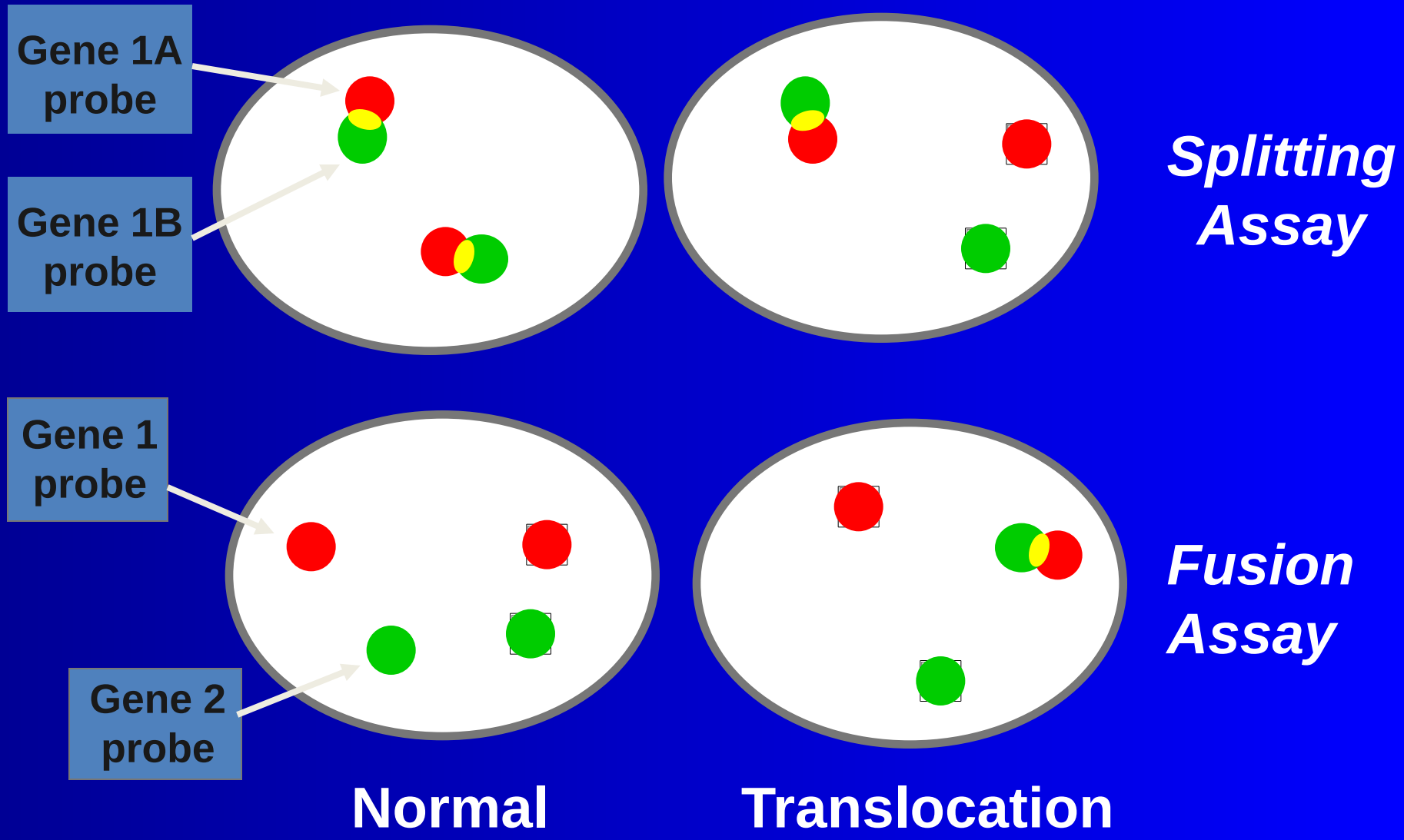


Ewing Sarcoma with Type I fusion

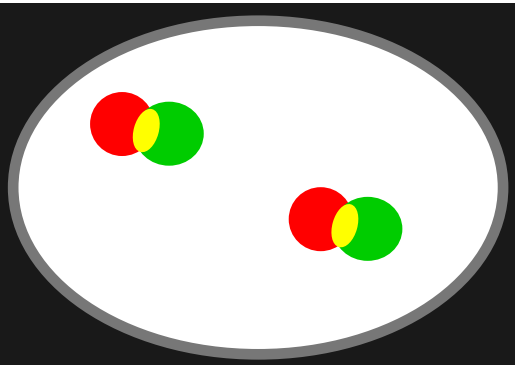


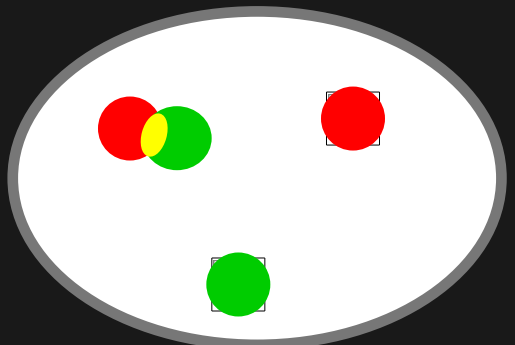
Variant Fusions: EWSR1-ERG, EWSR1-ETV1, EWSR1-E1AF, EWSR1-FEV, EWSR1-ZSG &FUS-ERG

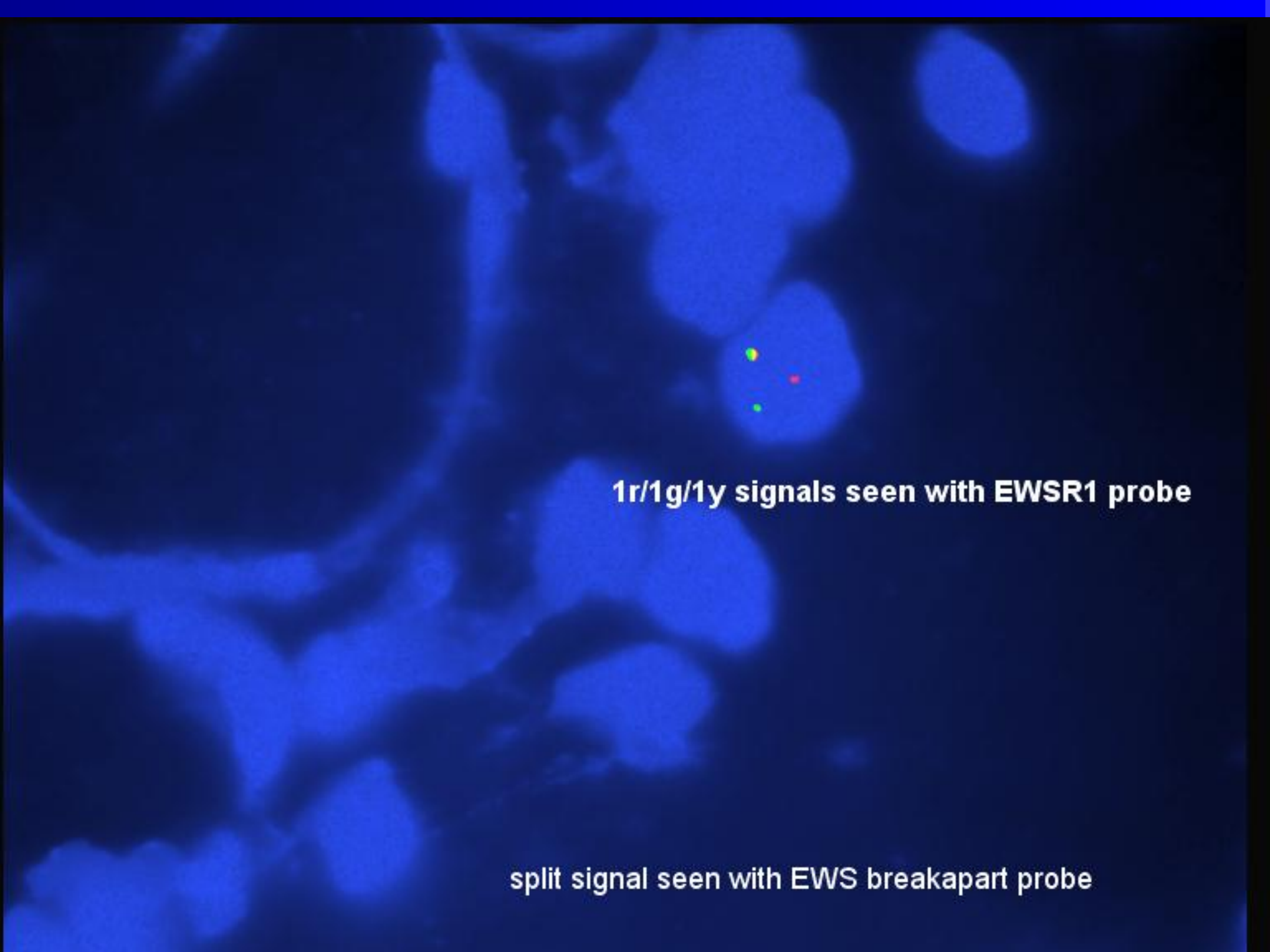
Fluorescence *in situ* hybridization detection of chromosomal translocations



Ewing Sarcoma diagnosis by Fluorescent in-situ Hybridization





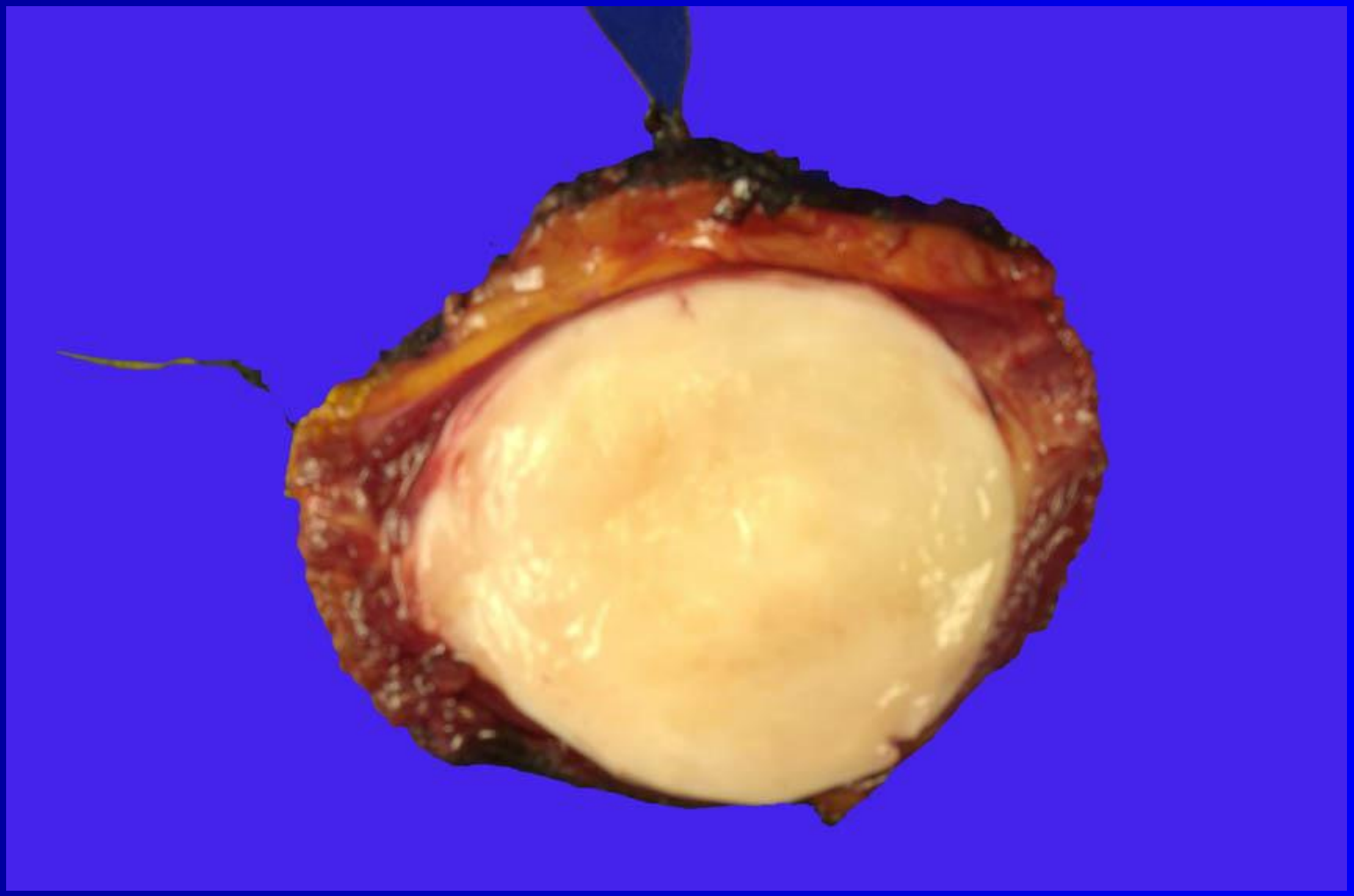

1r/1g/1y signals seen with EWSR1 probe
split signal seen with EWS breakapart probe

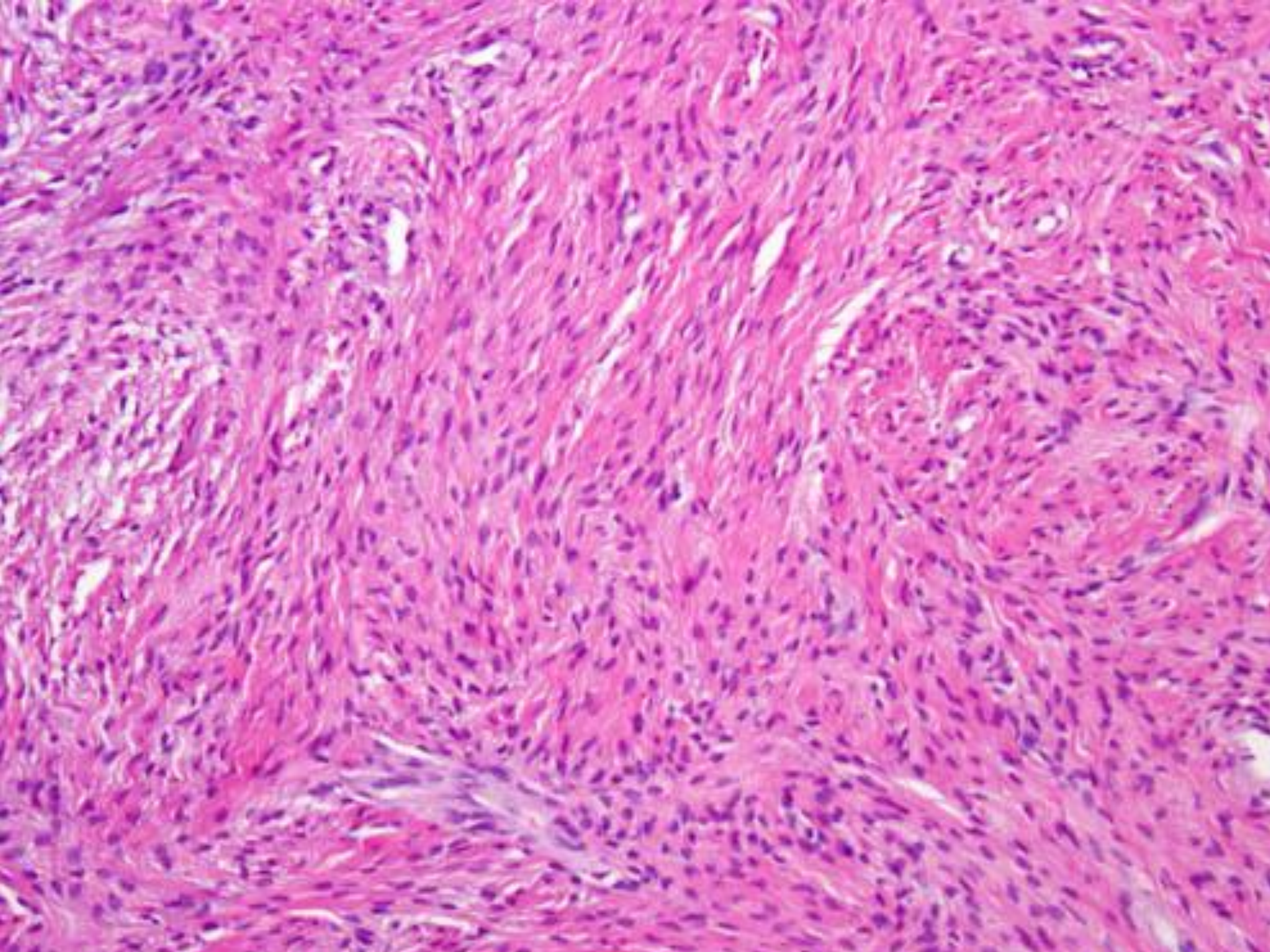
Molecular Testing for Sarcomas-*What can the Pathologist provide?*

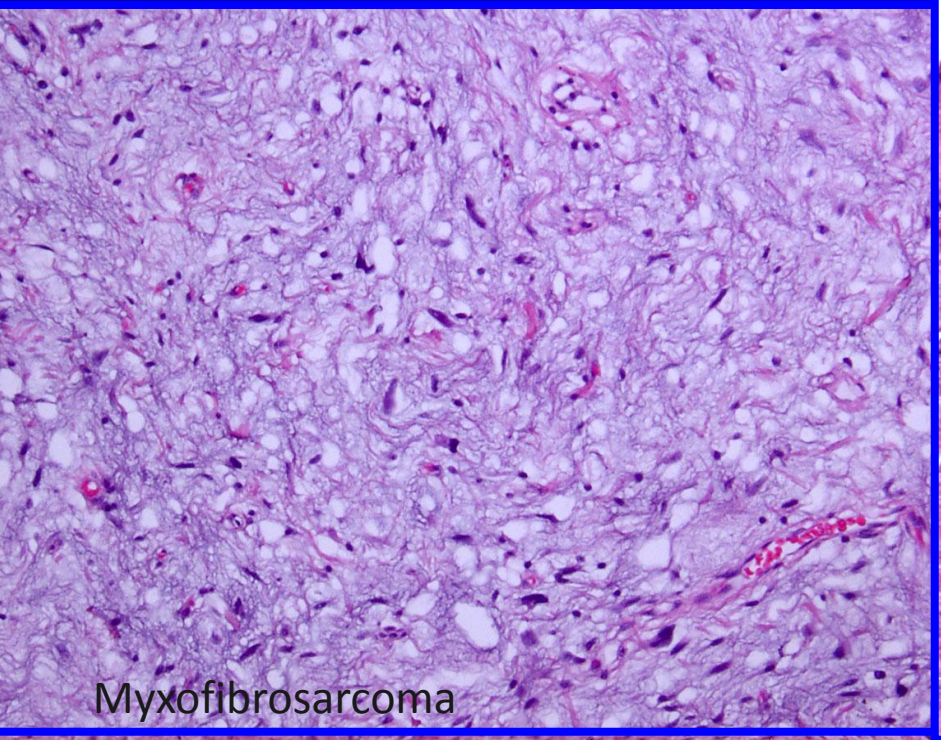
- Diagnosis
- Round Cell Neoplasms: Ewing vs DSRCT vs Small Cell OS vs Poorly Differentiated Synovial Sarcoma vs. Alveolar Rhabdomyosarcoma vs. Neuroblastoma
- Spindle cell neoplasms- Confirm Synovial Sarcoma

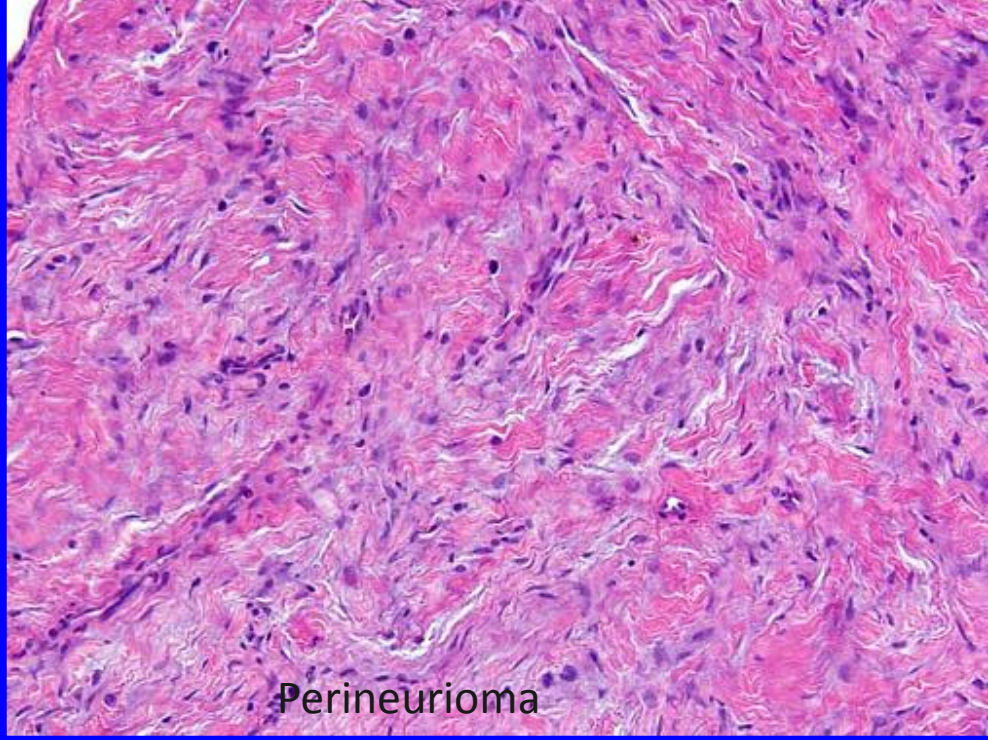
- Benign or Malignant:
- Low Grade Fibromyxoid Sarcoma vs Perineurioma vs low grade myxofibrosarcoma vs. fibromatosis

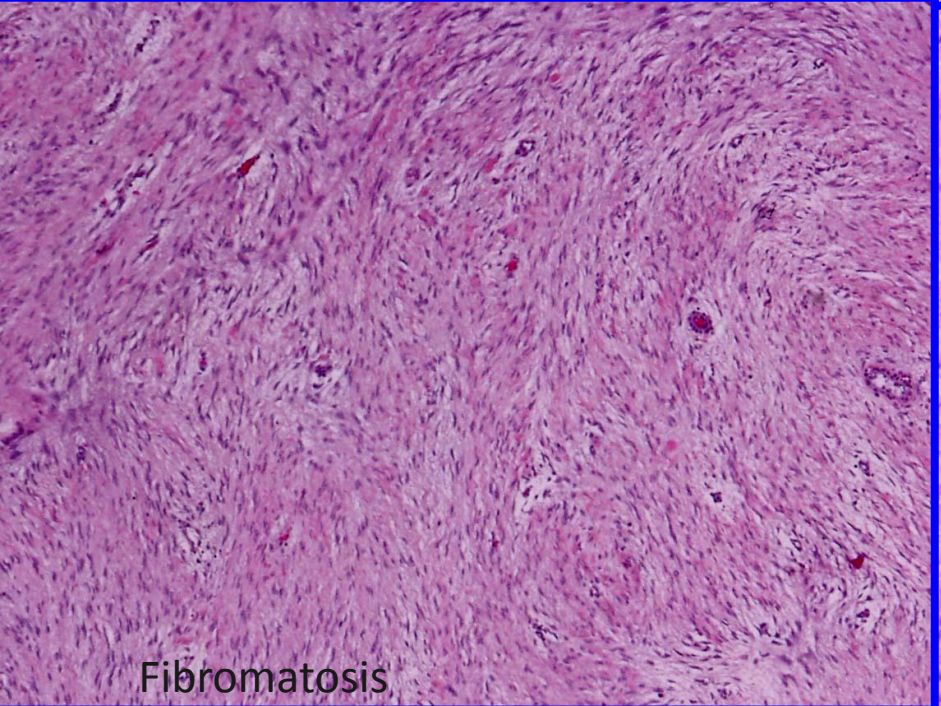
25 year old female with a soft tissue mass of left leg

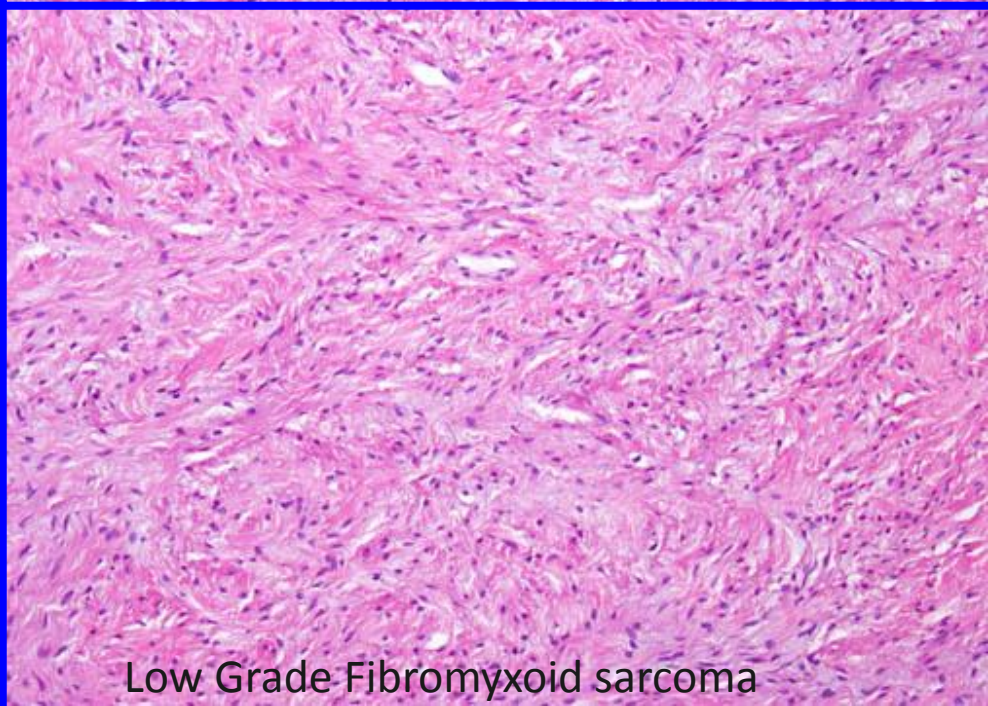





Myxofibrosarcoma


Perineurioma

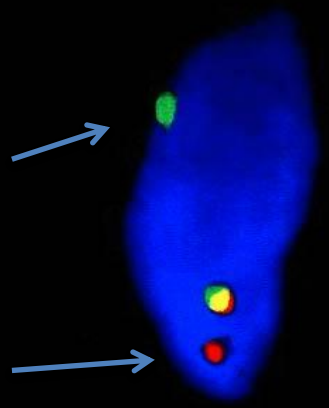

Fibromatosis


Low Grade Fibromyxoid sarcoma

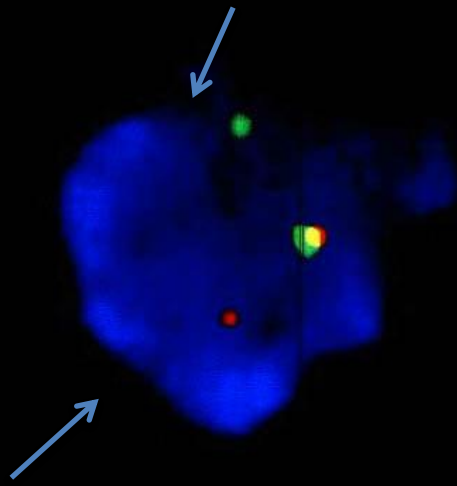
Low Grade Fibromyxoid Sarcoma

Translocation associated sarcoma-t(7;16)(q33;p11)- FUS-CREB3L2

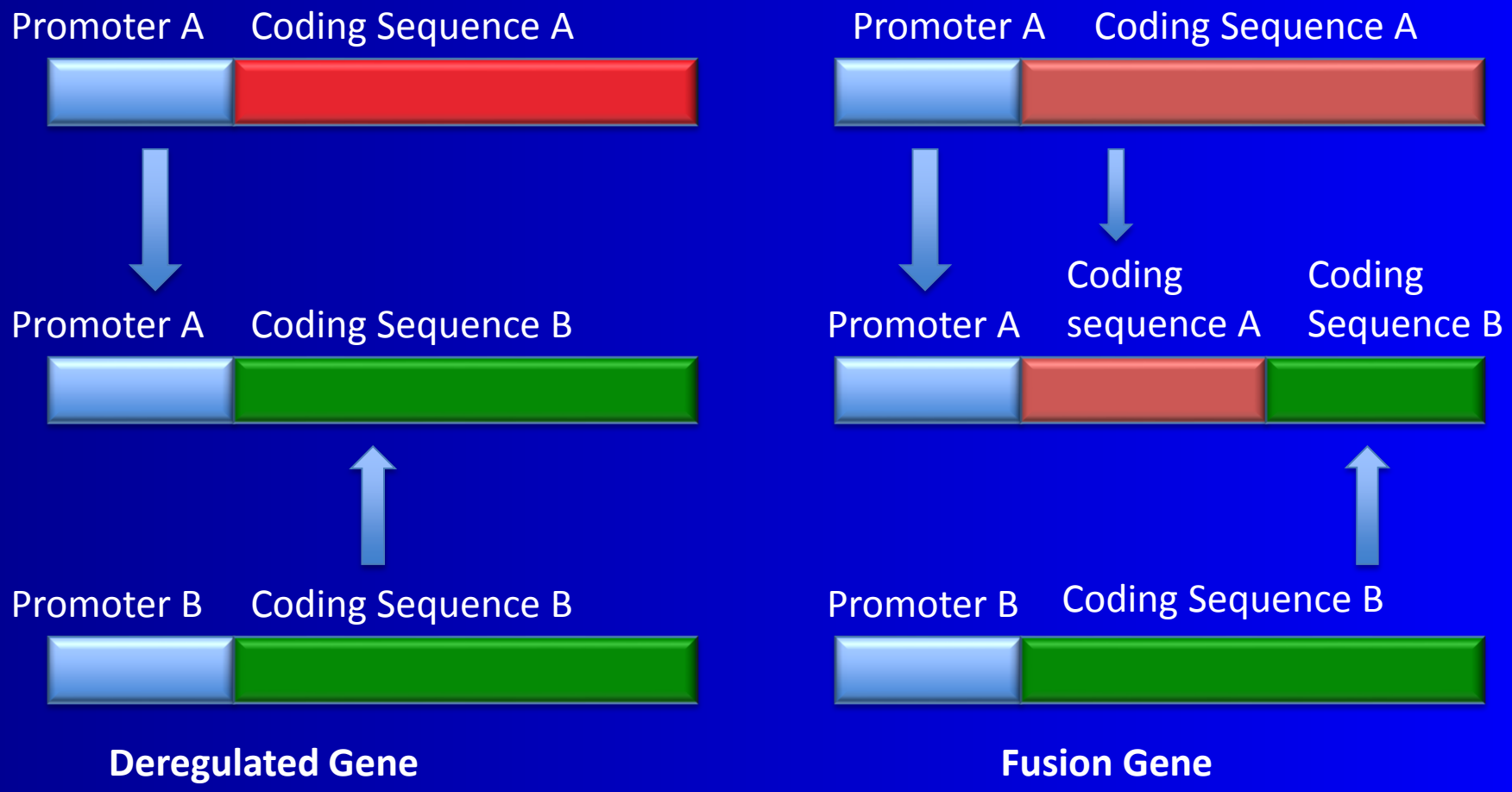
FUS break apart Probe with Split Signal



FUS break apart Probe with Split Signal



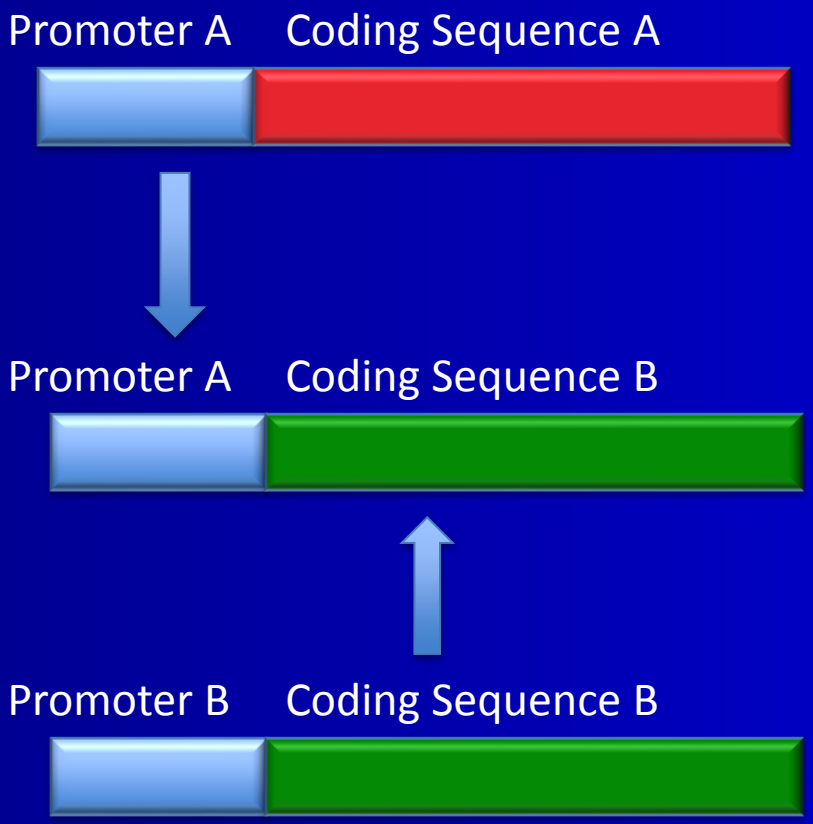
Outcome of Chromosomal rearrangements



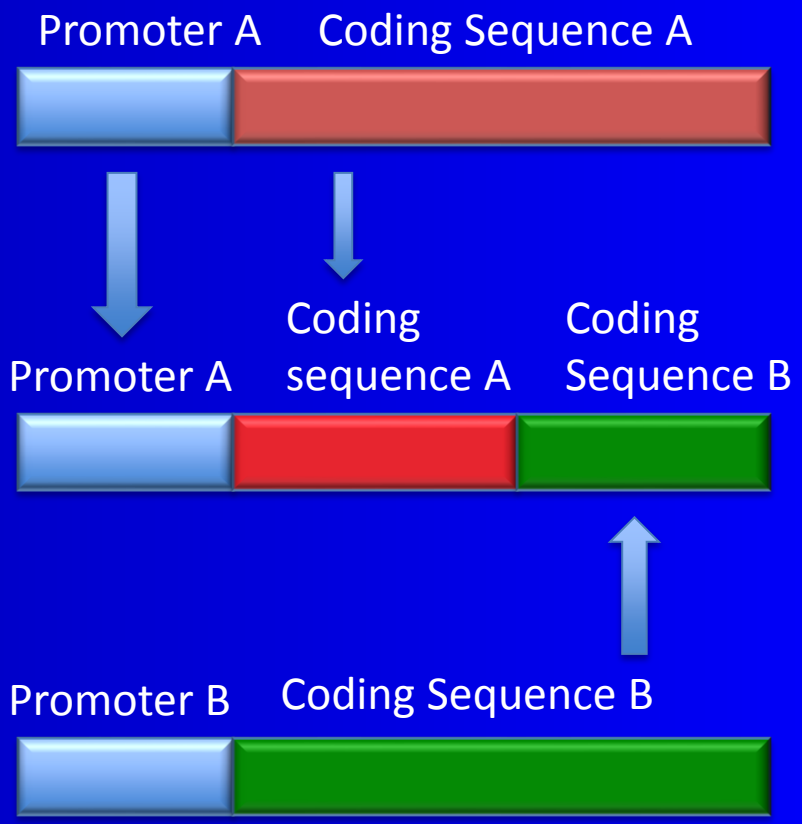
Adapted from Mertens et al., Sem in Oncol 36,(4):2009.

Can Fusion genes be Targeted?

Deregulated Gene ✔



Fusion Gene ✖



Targeted Therapy –Translocation-associated sarcomas

Tumor Type

- Dermatofibrosarcoma Protuberans (DFSP)
- Inflammatory Myofibroblastic Tumor (IMT)
- Diffuse type Tenosynovial Giant Cell Tumor (PVNS)

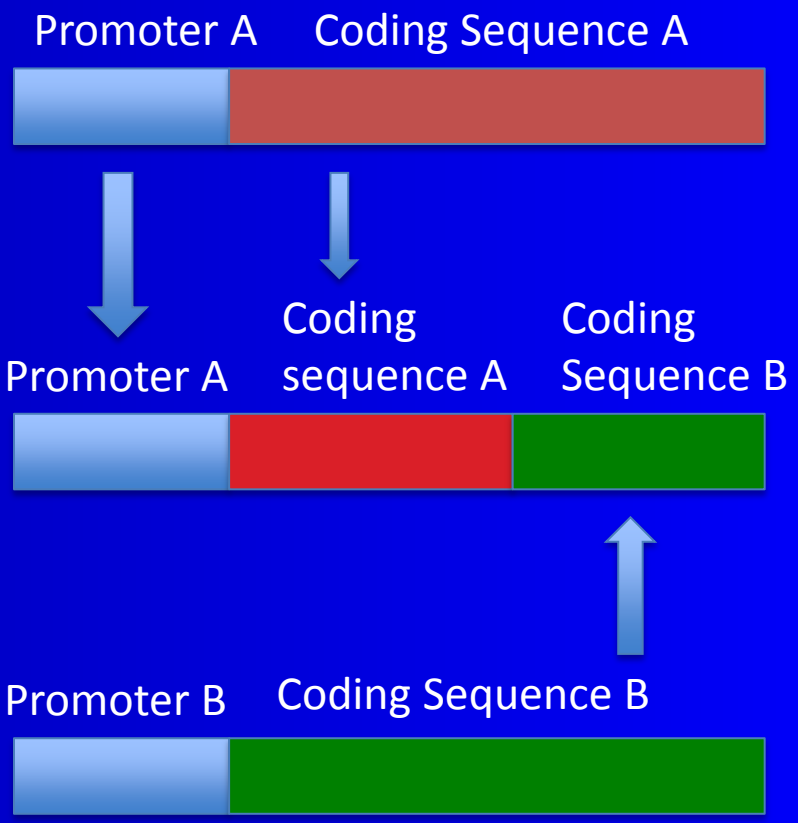
Fusion gene- Target

- *COL1A1-PDGFB* – Imatinib
- *Multiple Partners-ALK-* Crizotinib
- *COL6A3-CSF1-* Imatinib

Targeted Therapy –Translocation associated sarcoma

Elusive

Fusion Gene



Few down stream Targets

Alveolar Rhabdomyosarcoma- *PAX3-FOXO1A*

Ewing Sarcoma EWSR1 and FUS-

IGF1R

Alveolar Soft Part Sarcoma- *ASPS-TFE3*

Clear Cell Sarcoma- *EWSR1-ATF1 or CREB1*

MET

Synovial Sarcoma –*SS18-SSX1 or SSX2*

FGFR - PDGFRA

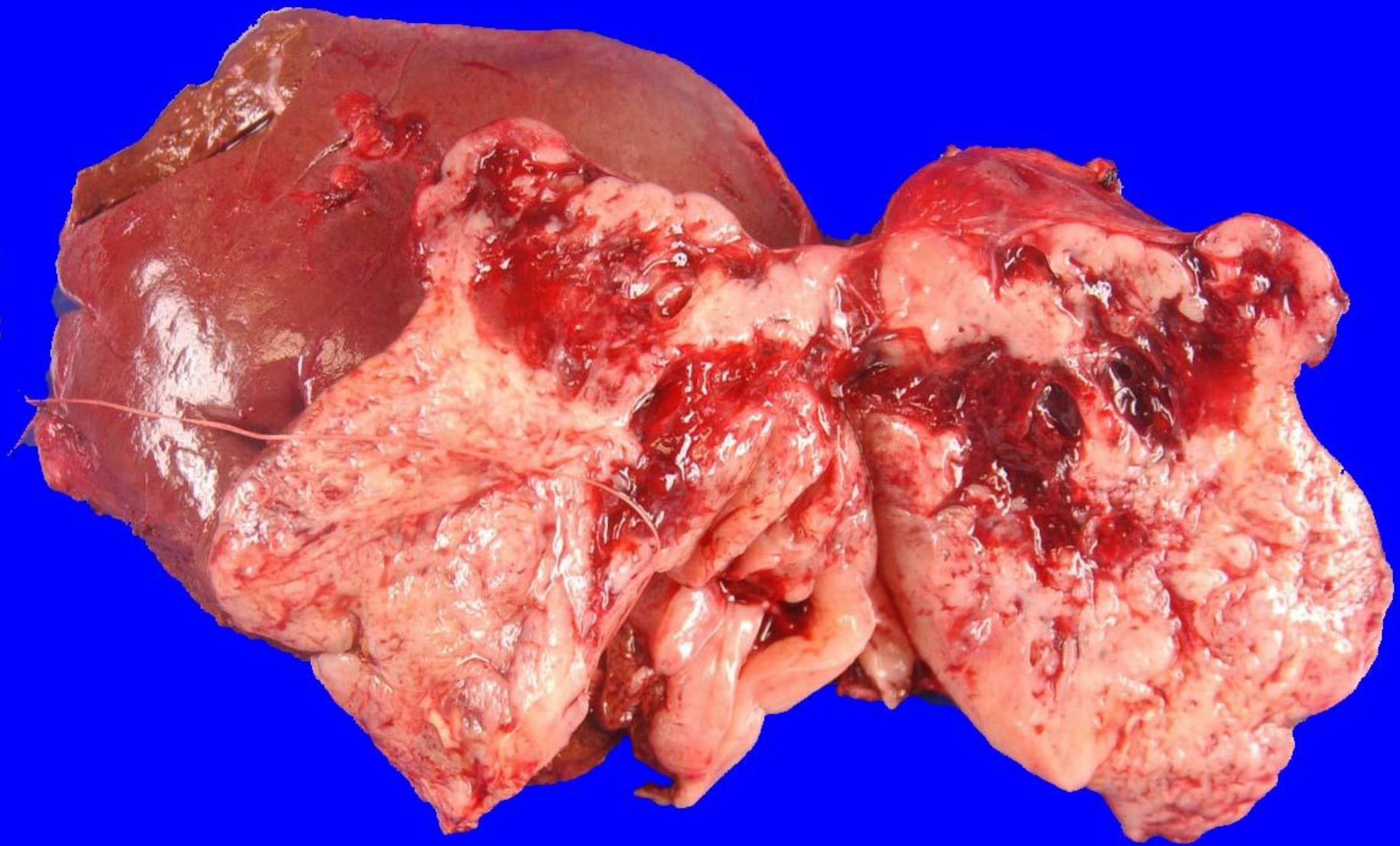
Molecular Sarcomas with Driver Mutations

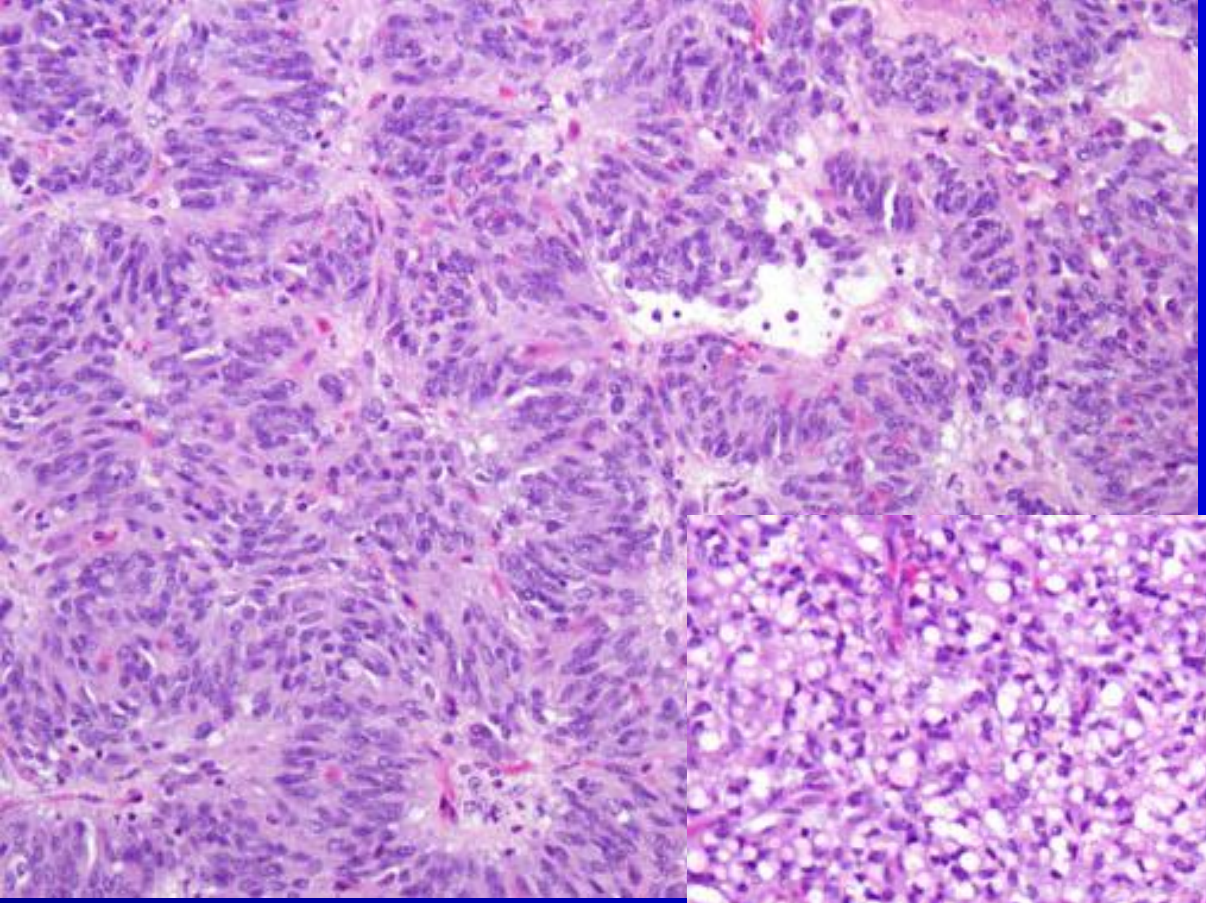

TOROS
RAMÓN Y CAJAL

Gastrointestinal Stromal Tumor (GIST)

- The most common sarcoma of GI tract
- Most Common Location: Stomach, Small Bowel
- Also abdomen, mesentery and extra-GI GIST
- Annual Incidence-4000-5000 cases in United States
- Cell of Origin- Interstitial Cells of Cajal ("GI pacemaker cells")
- Mutations in C-kit gene, less commonly PDGFR-Alpha and rare BRAF mutation (7% of GIST pts)

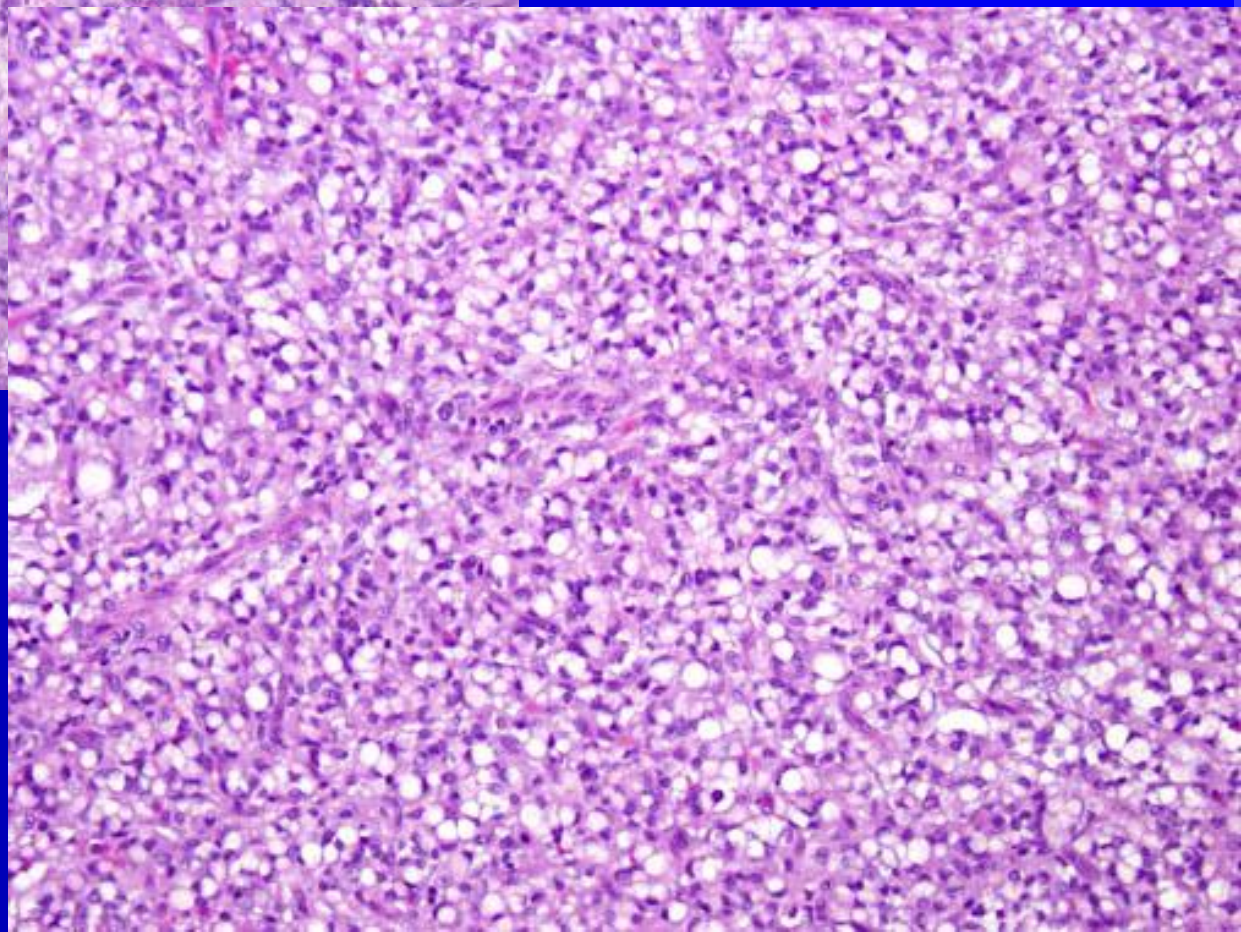
Leading model for kinase-targeted therapy

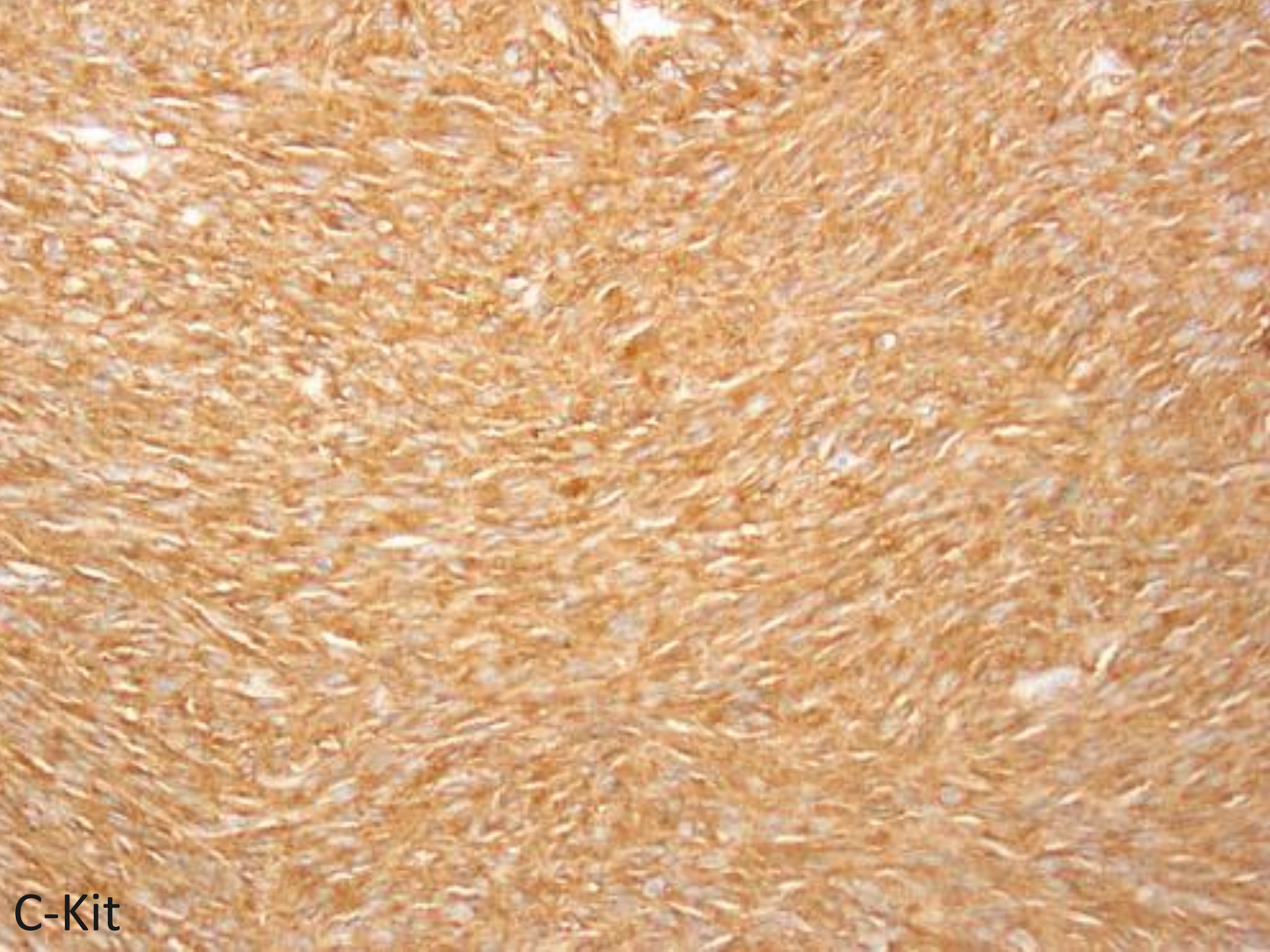




GIST-Spindle Cell Type

GIST-Epithelioid Type



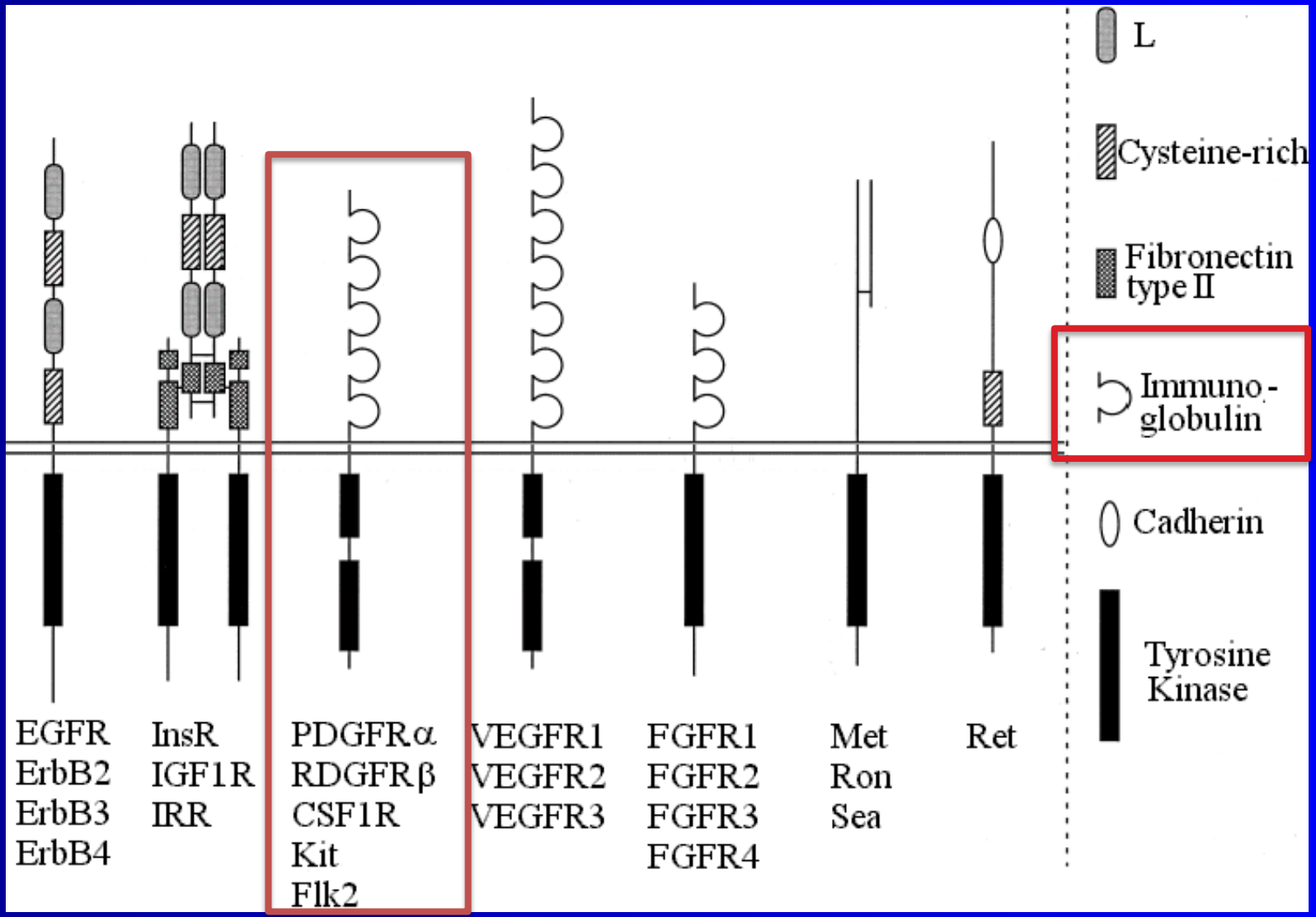

C-Kit

C-kit/CD-117

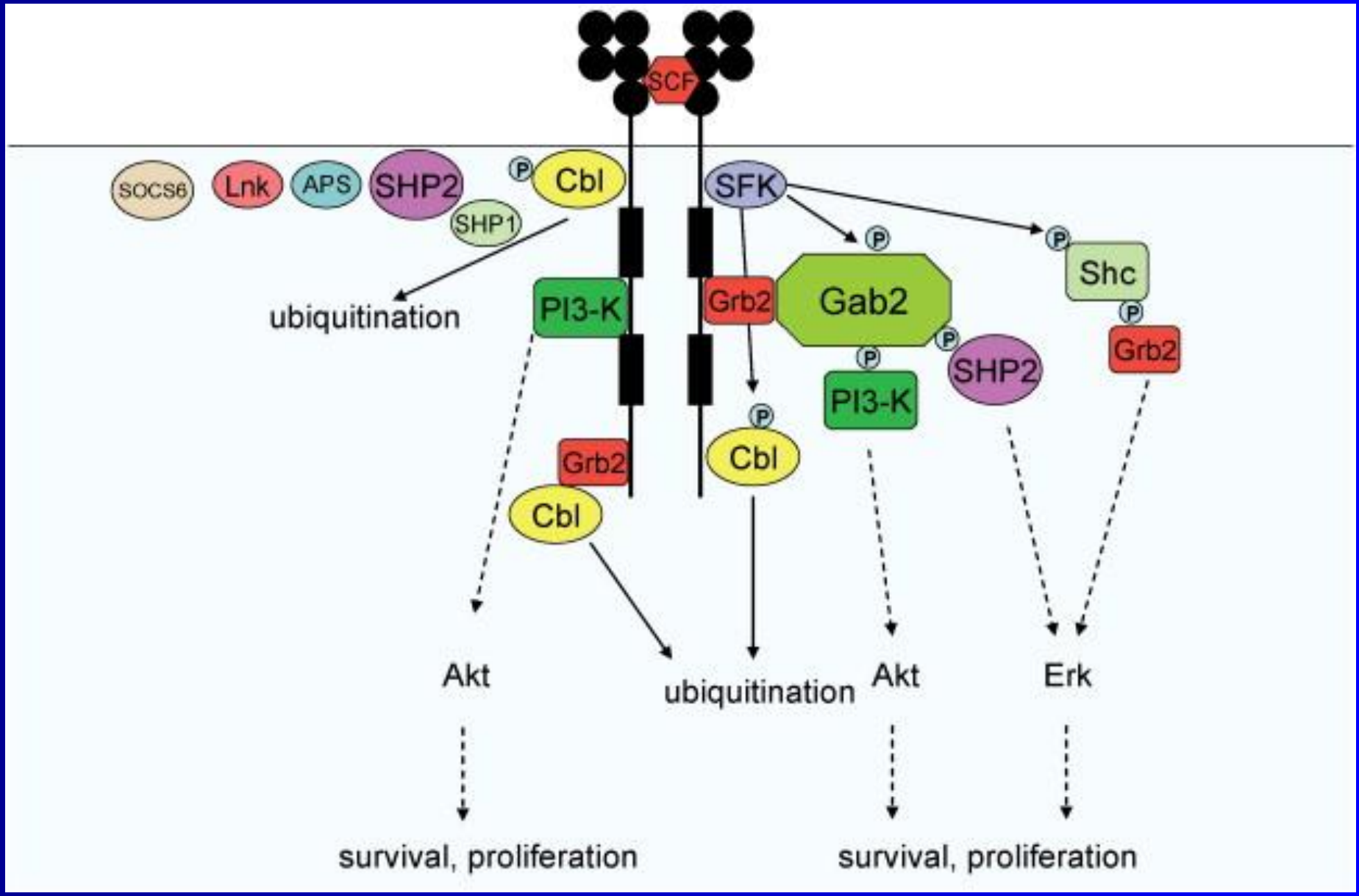
- CD-117/C-Kit is a class III receptor tyrosine kinase
- Location: chromosome 4q12-13 close to PDGFRA and FLK1 receptor tyrosine kinases
- Role: normal development and function of ICC, hematopoiesis, gametogenesis and melanogenesis

- Activating kit mutations : GIST, seminomas, AML, melanomas, mastocytosis and some thymomas

Membrane Receptor Kinases

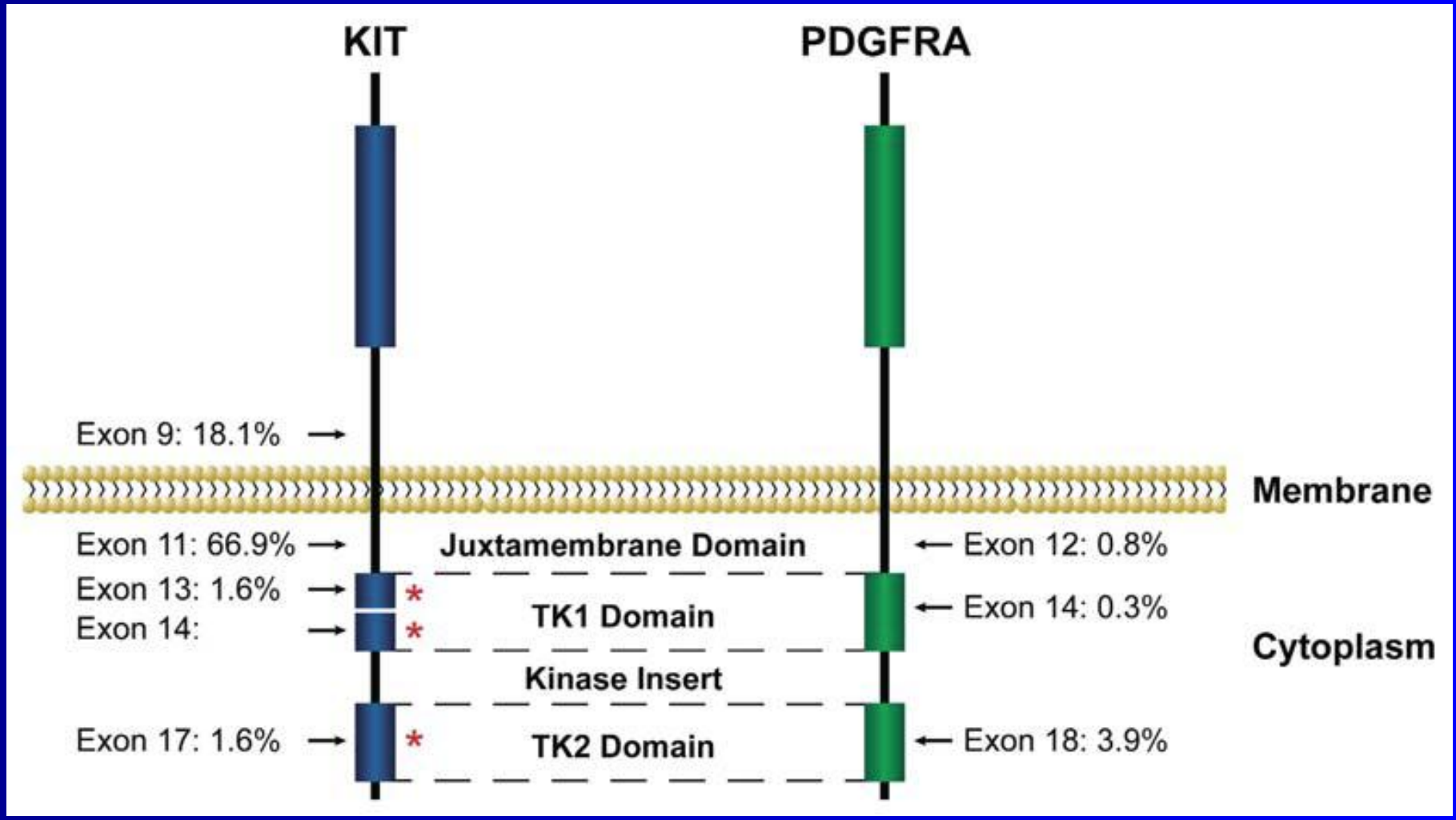


C-Kit Signaling Pathway



Cell Signal. 2009 Dec;21(12):1717-26. Epub 2009 Jun 18.

Kit and PDGFRA mutations in GIST



(adapted from Heinrich et al ASCO, 2003)

Diagnosis of GIST

- Strong C-kit immunoreactivity
- Activating mutations exons 9 and 11 - 80%
- Rare mutations – exons 13 and 17
- PDGFRA (exons 12,14,18)- 10-15%
- No mutation ~ 10%
- C-Kit IHC negative GIST (4%)- usually PDGFRA mut

- GIST with no known mutations
- Pediatric GIST – multifocal gastric- indolent
- GIST associated with Neurofibromatosis -1

Types of Kit Mutations

- Common Site is 5' end of exon 11-"hot-spot"
- Point Mutations
- In-frame deletions
- Deletions → Do worse
- Substitutions
- Less common site-3'end of exon 11-
  Internal tandem duplications (ITD) → Indolent
- Exon 9- at EC domain-insertion of two AA,
  AY502-3- small bowel → More Aggressive

PDGFRA mutations

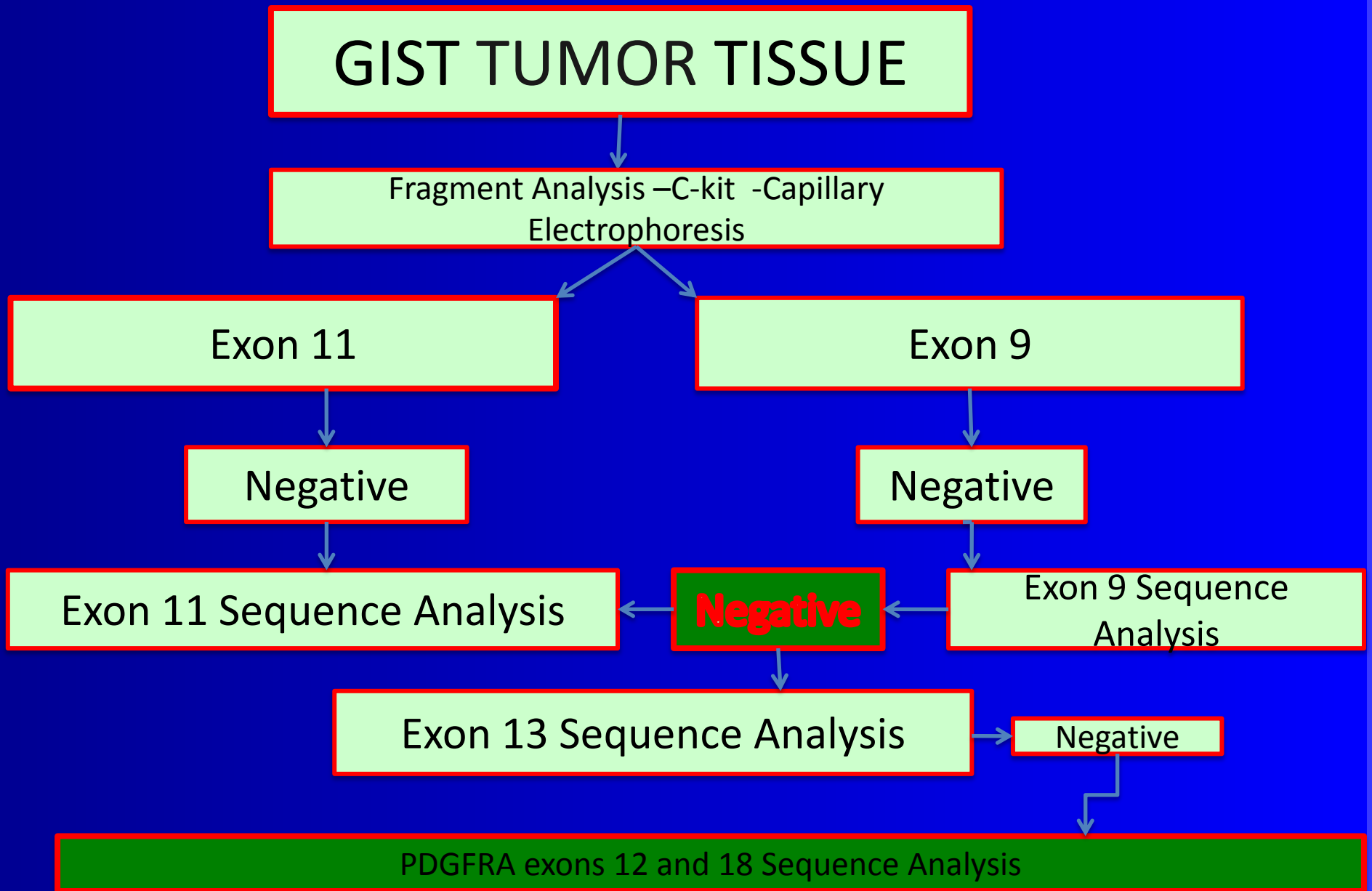
- One-third of GISTS lacking in Kit mutations
- Exons 12, 14 or 18
- Gastric Location, epithelioid morphology
- Variable IHC expression for C-kit
- Indolent behavior

- Hot spot- second kinase domain (exon 18- D842V) → Insensitive to Imatinib therapy

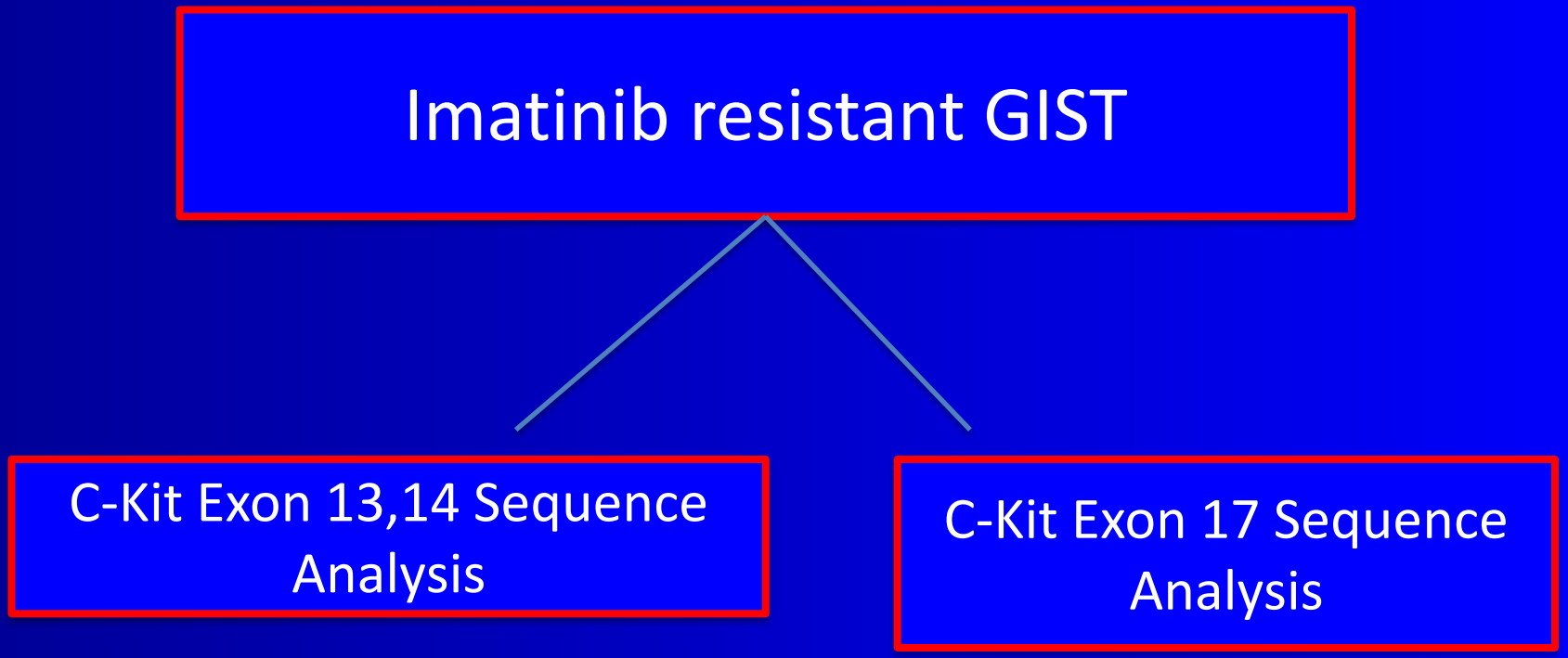
GIST-Indications for mutation Testing

- NCCN Task Force Report -2010
- High-Risk GISTs –(>5 cm and >5 mitoses/50 high power fields)
- Metastatic GISTs
- GISTs which are negative for C-kit by immunohistochemistry
- GISTs with epithelioid morphology
- Small bowel GISTs
- GISTs resistant to imatinib

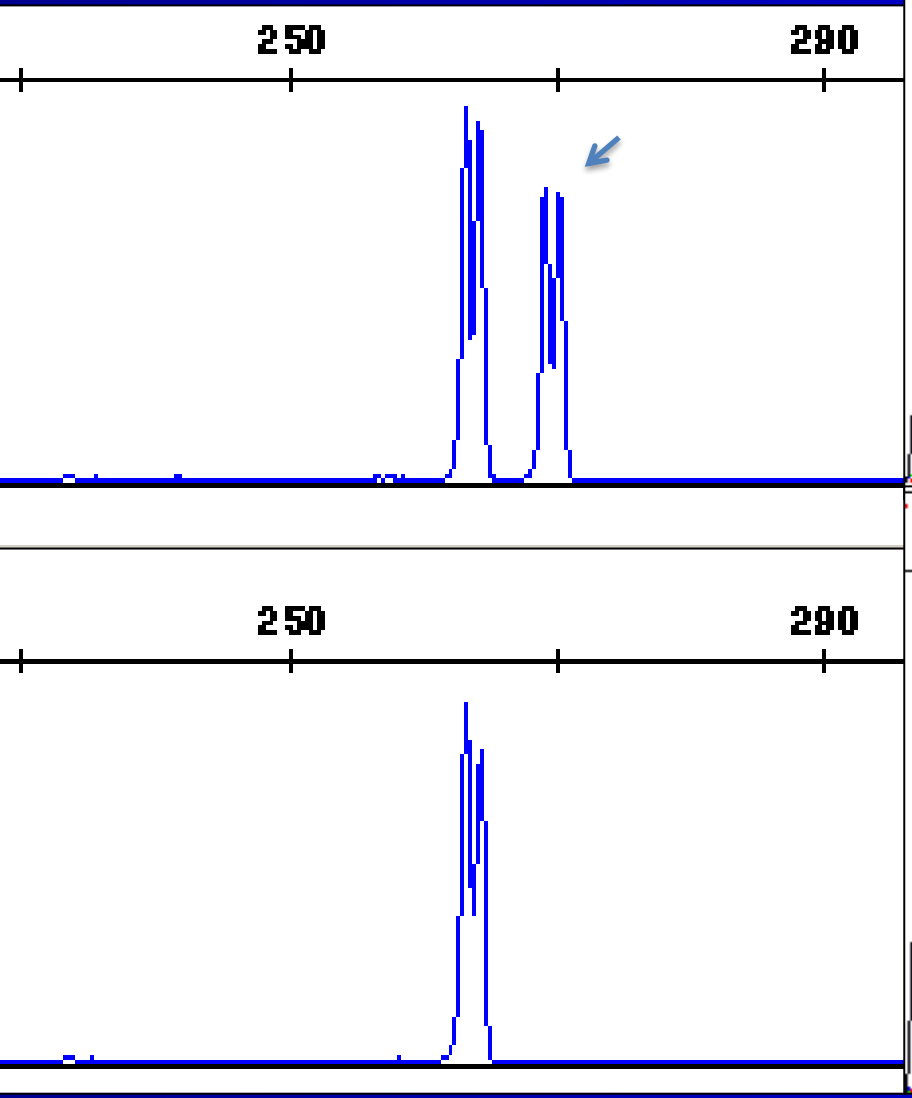
Testing Algorithm for C-kit and PDGFRA



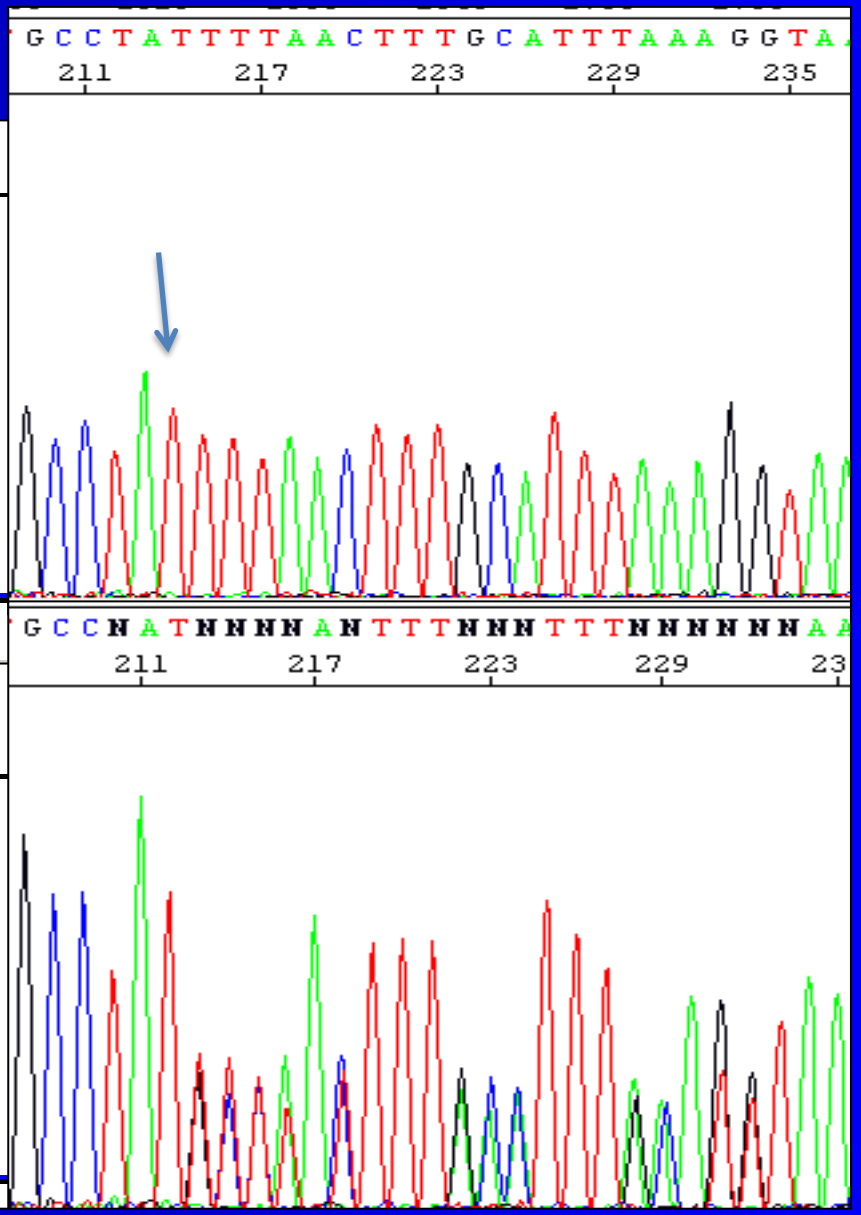
Molecular Testing for Second Site Mutation



Exon 9 Fragment Analysis

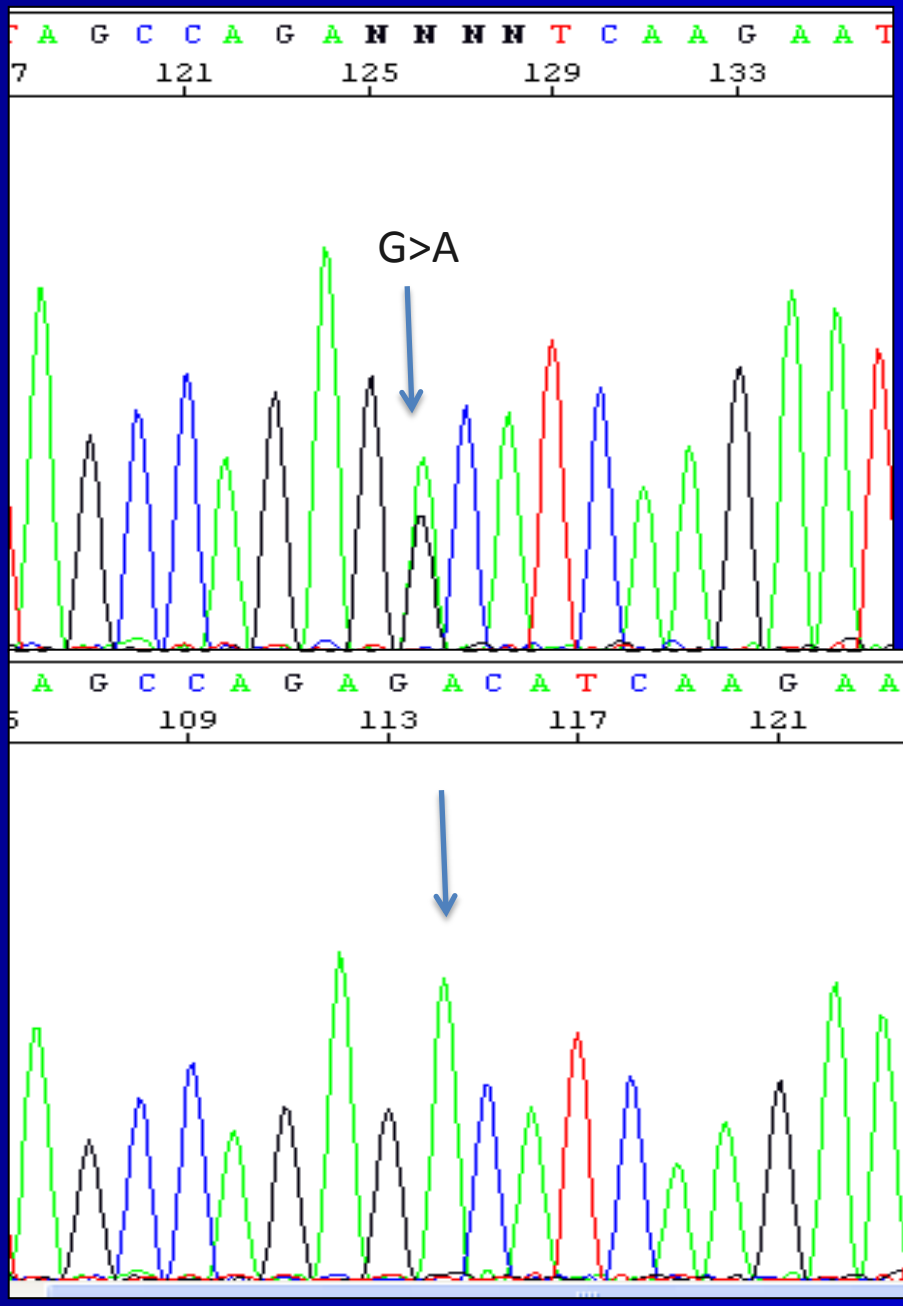


Exon 9 Sequencing



c.1509ins6, (GCCTAT)

Kit mutation exon 17- Resistance Mutation

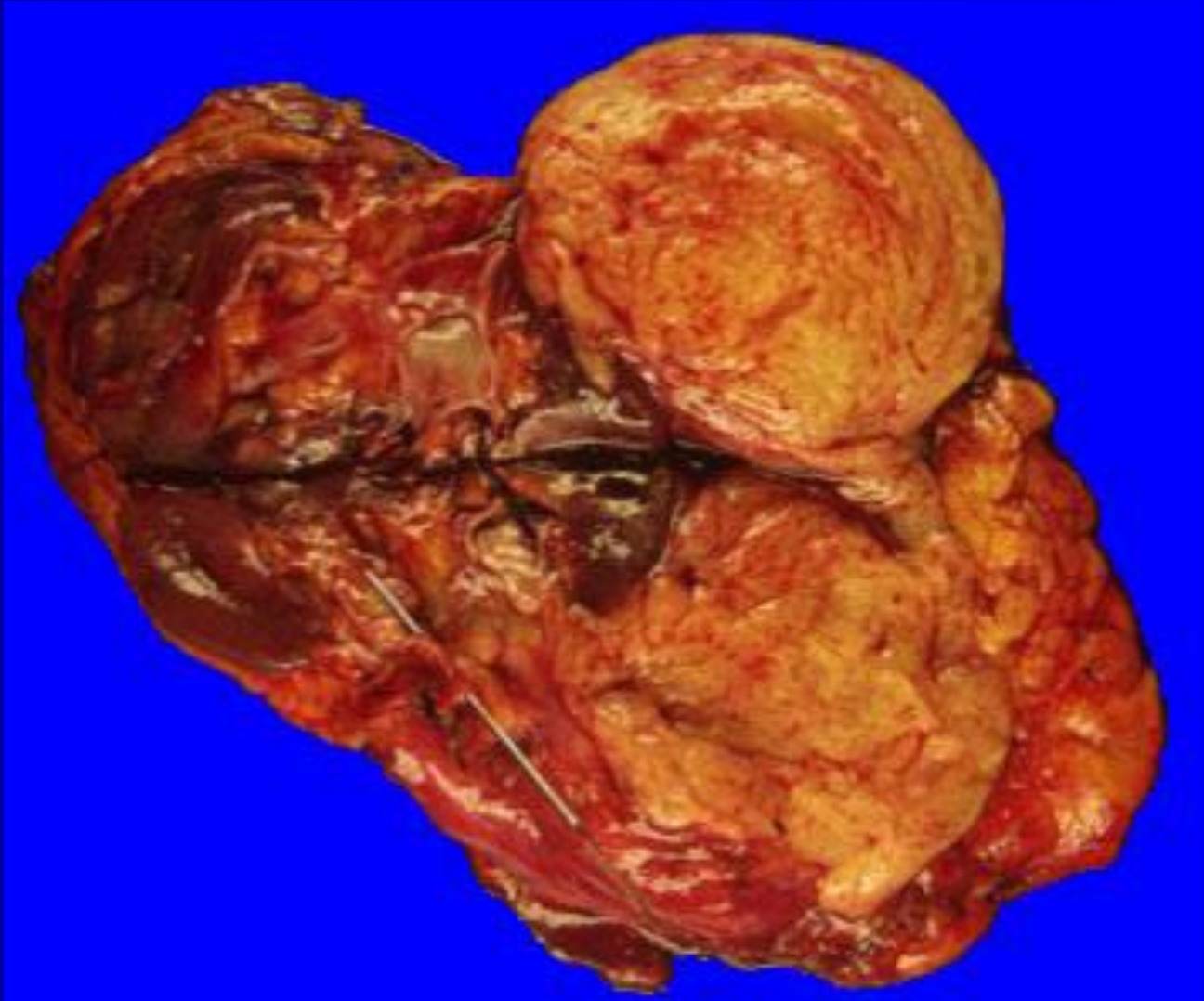


GIST- Targeted Therapy

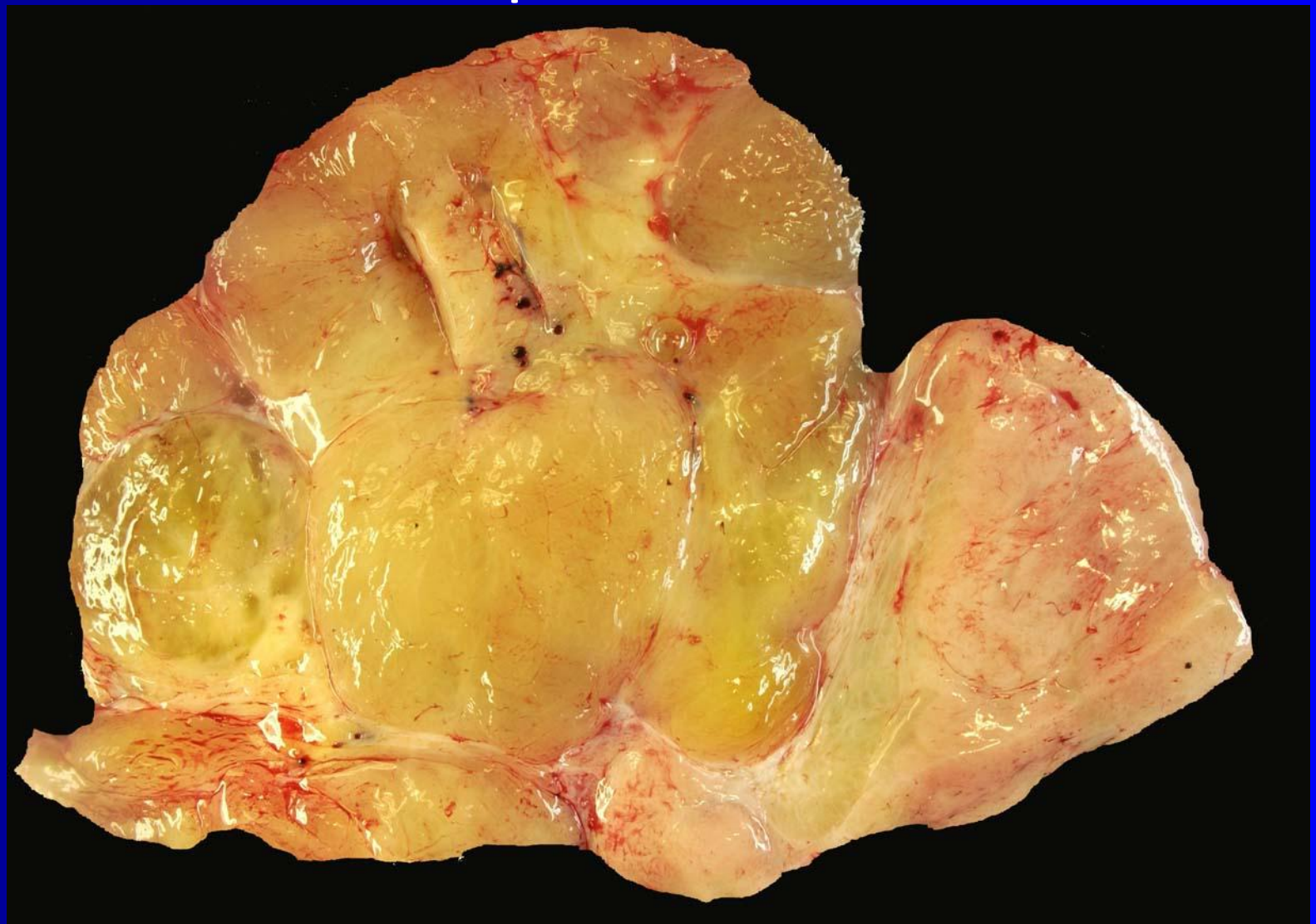
- Imatinib mesylate (STI571, Gleevec) is a selective tyrosine kinase inhibitor
- Targets Kit and PDGFRA
- Partial response or stable disease in 80% of patients with metastatic GIST
- Pathological response is necrosis and fibrosis and is heterogeneous
- Half of the patients will develop drug resistance
- Usually due to second site kit mutation
- Half of the resistant cases, no secondary mutations are identified

Sarcomas with Specific Amplifications

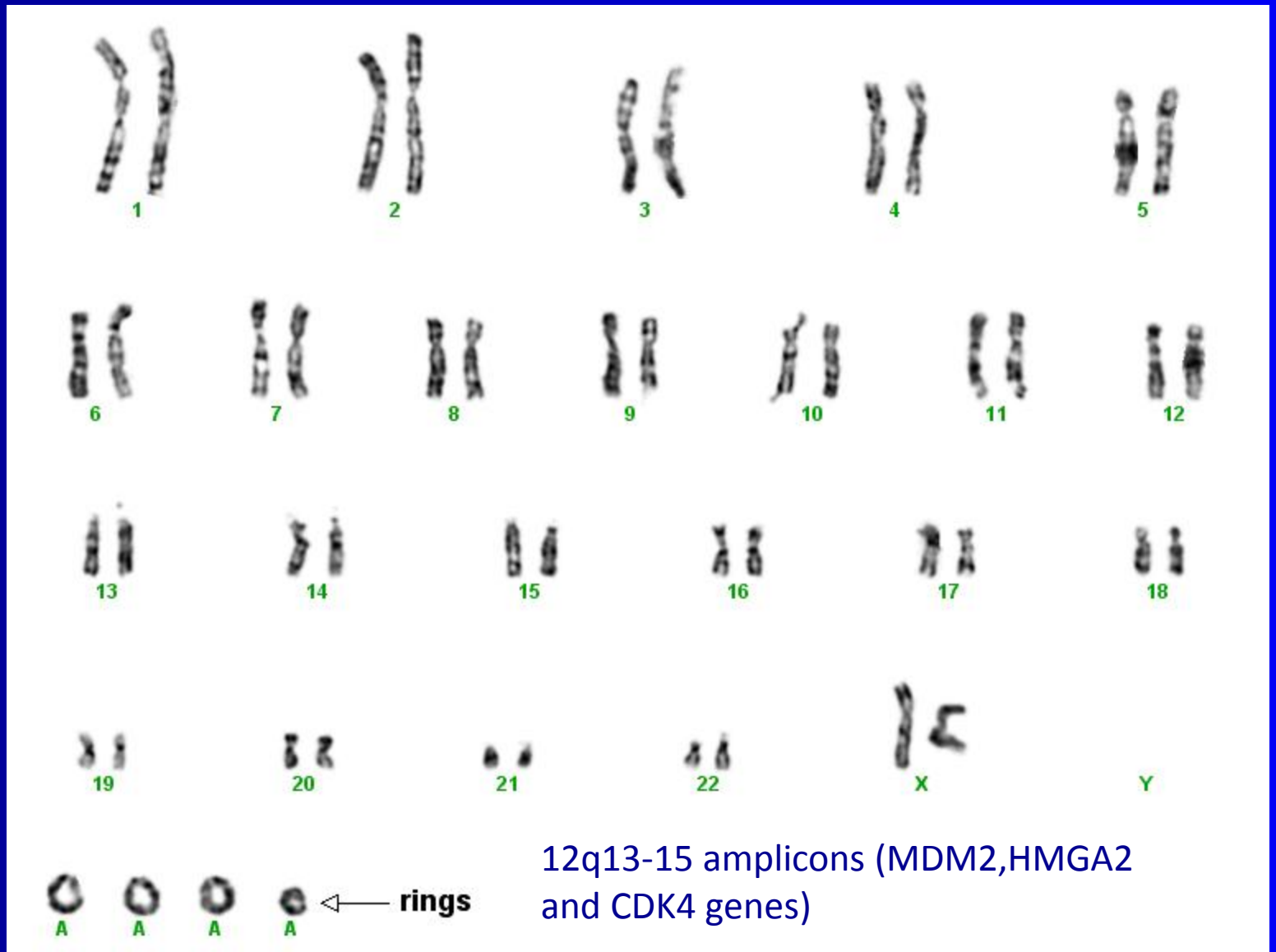
Retroperitoneal Liposarcoma



Sarcomas with Specific Amplifications- Well-Differentiated and Dedifferentiated Liposarcoma

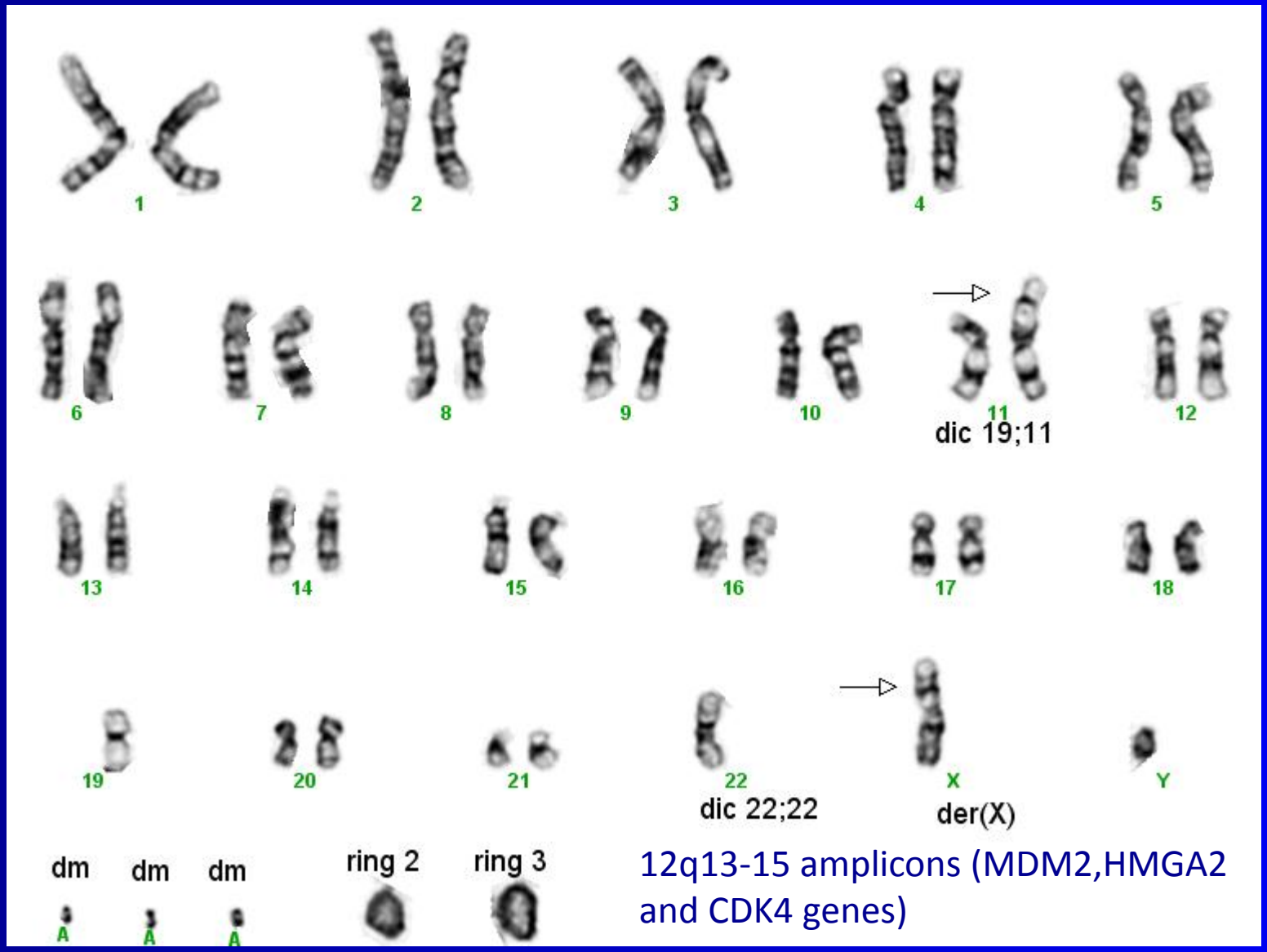


Retroperitoneal Liposarcoma-WDLS

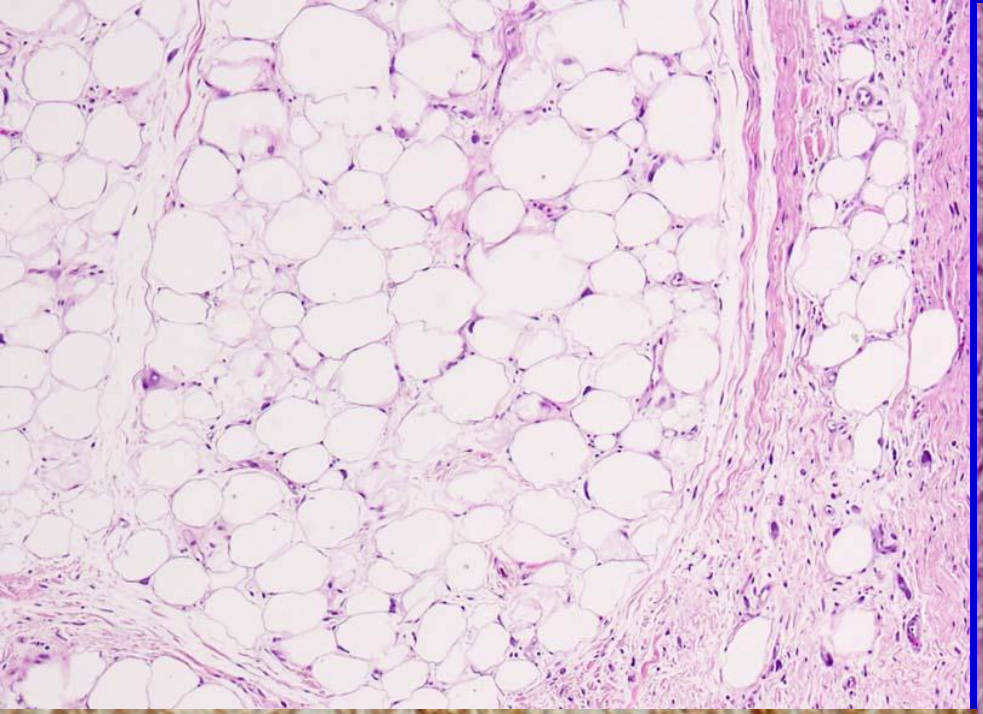


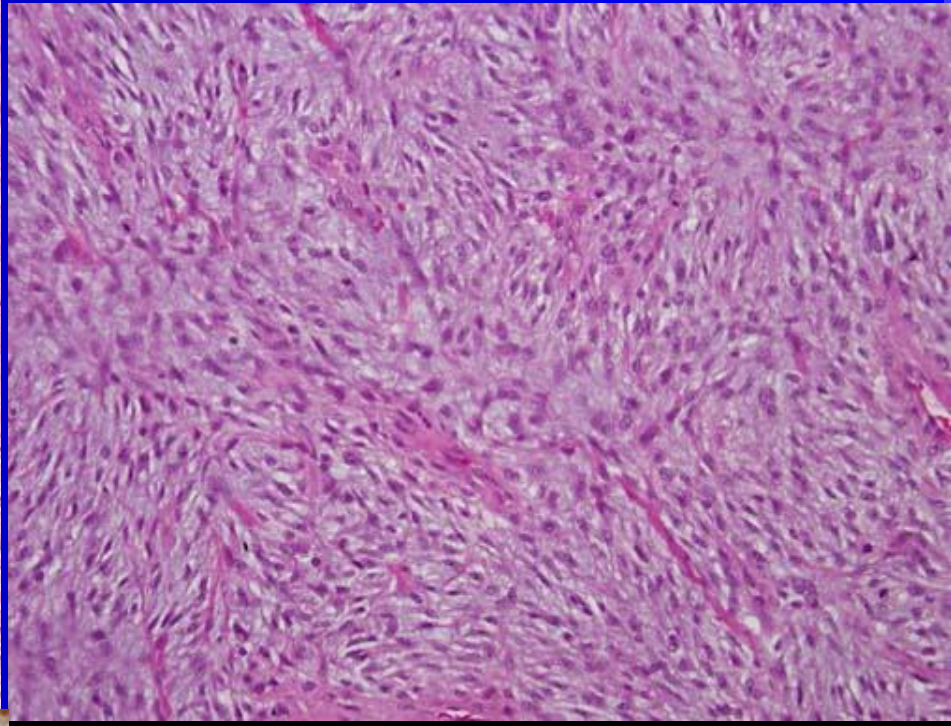
12q13-15 amplicons (MDM2,HMGA2 and CDK4 genes)

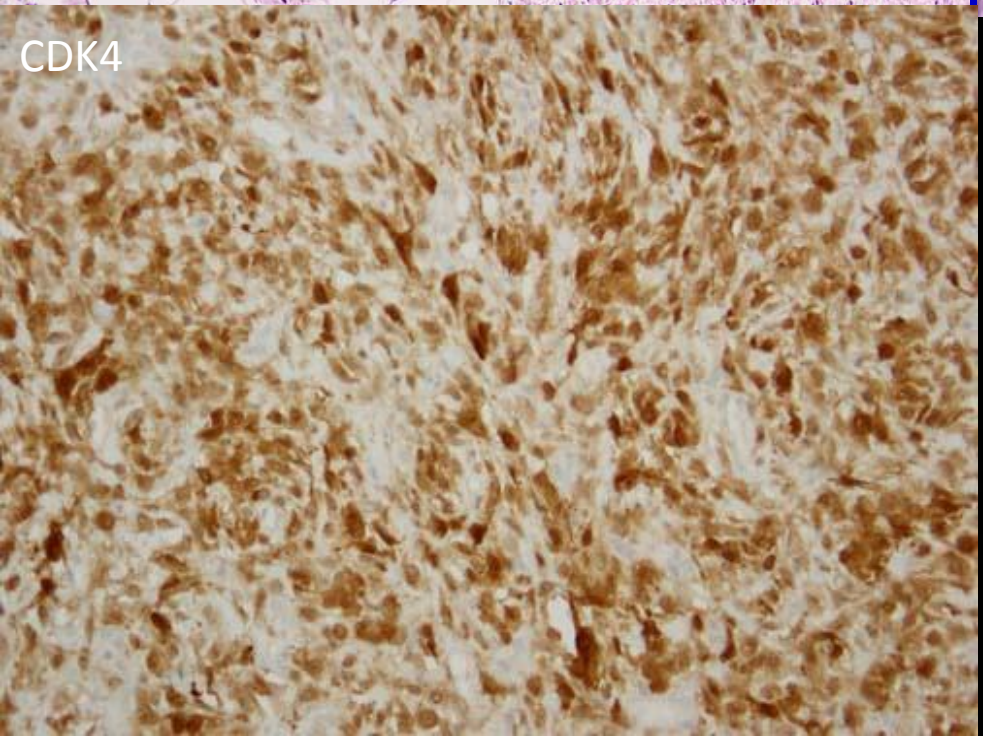
Retroperitoneal liposarcoma -Dedifferentiated

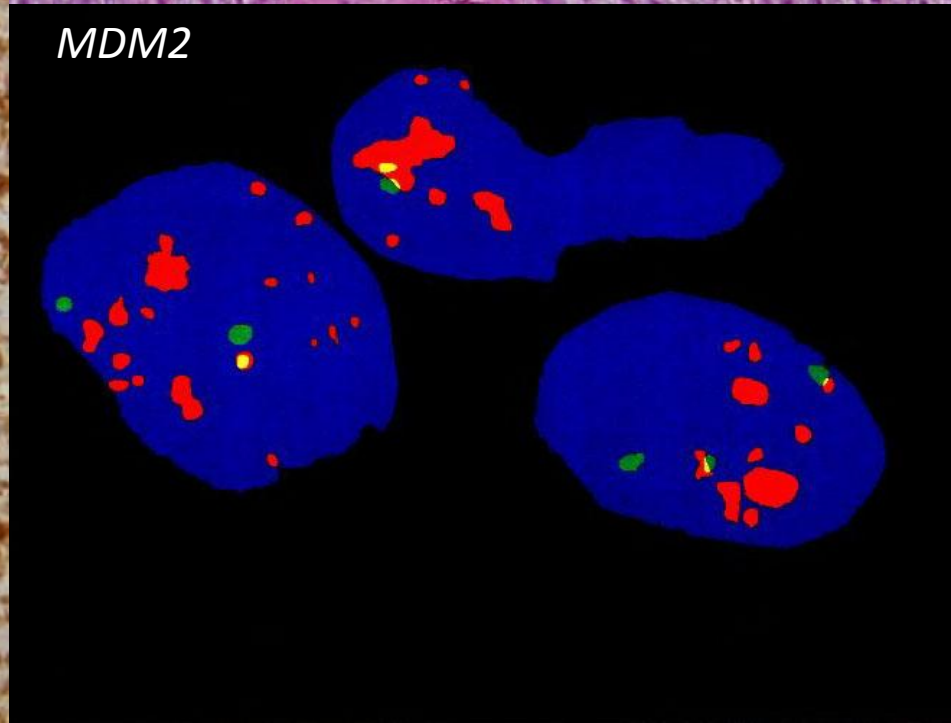


12q13-15 amplicons (MDM2,HMGA2 and CDK4 genes)

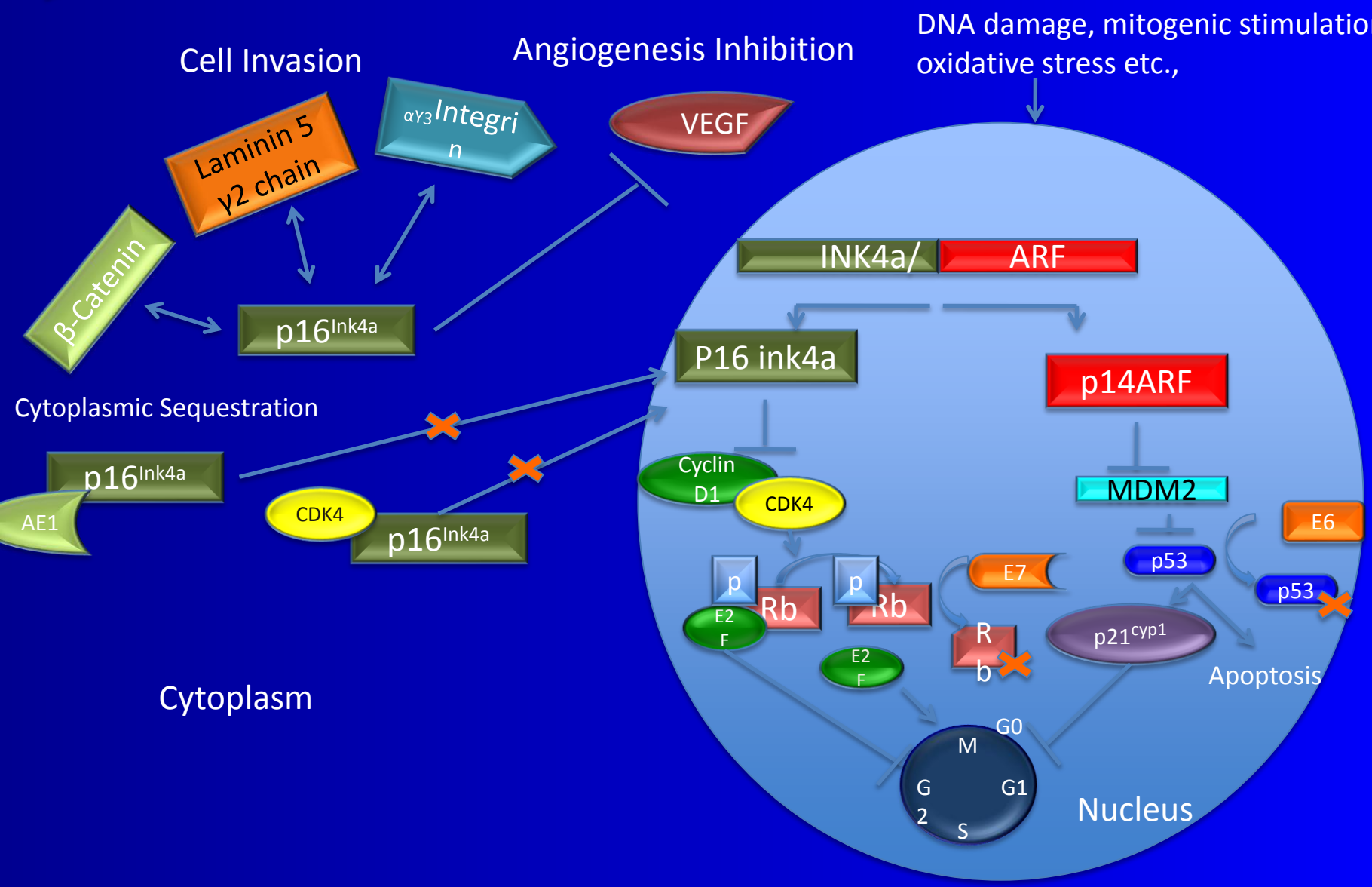





CDK4


MDM2

Retroperitoneal Liposarcoma



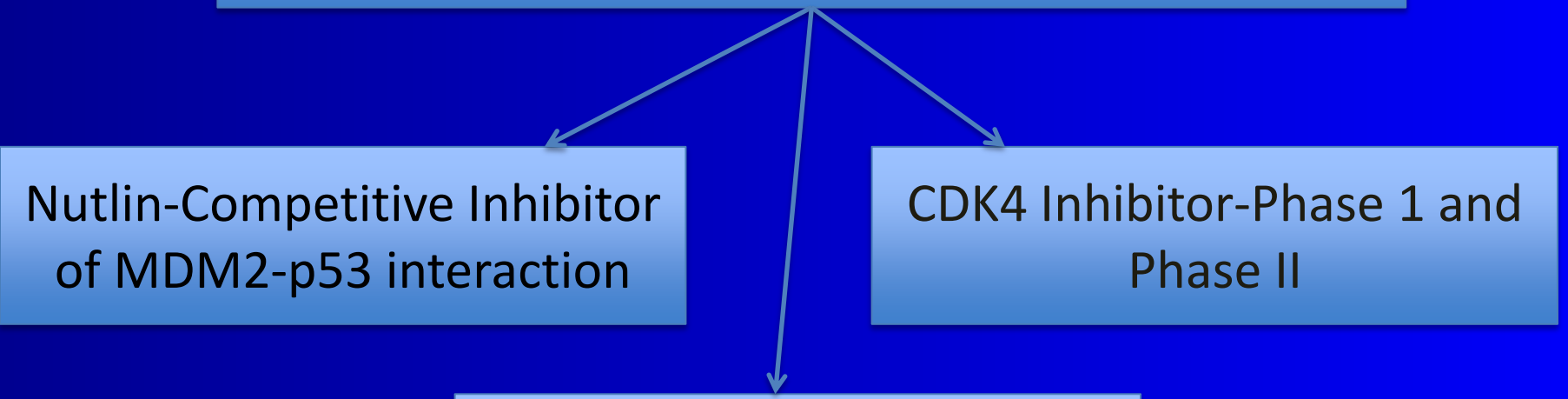
Retroperitoneal Liposarcoma

Outcome depends on completeness of surgical resection and histology

Poor outcomes in patients with rapidly growing or incompletely resected tumors

Poor response to Radiation and chemotherapy

Clinical Trials based on genomic targets



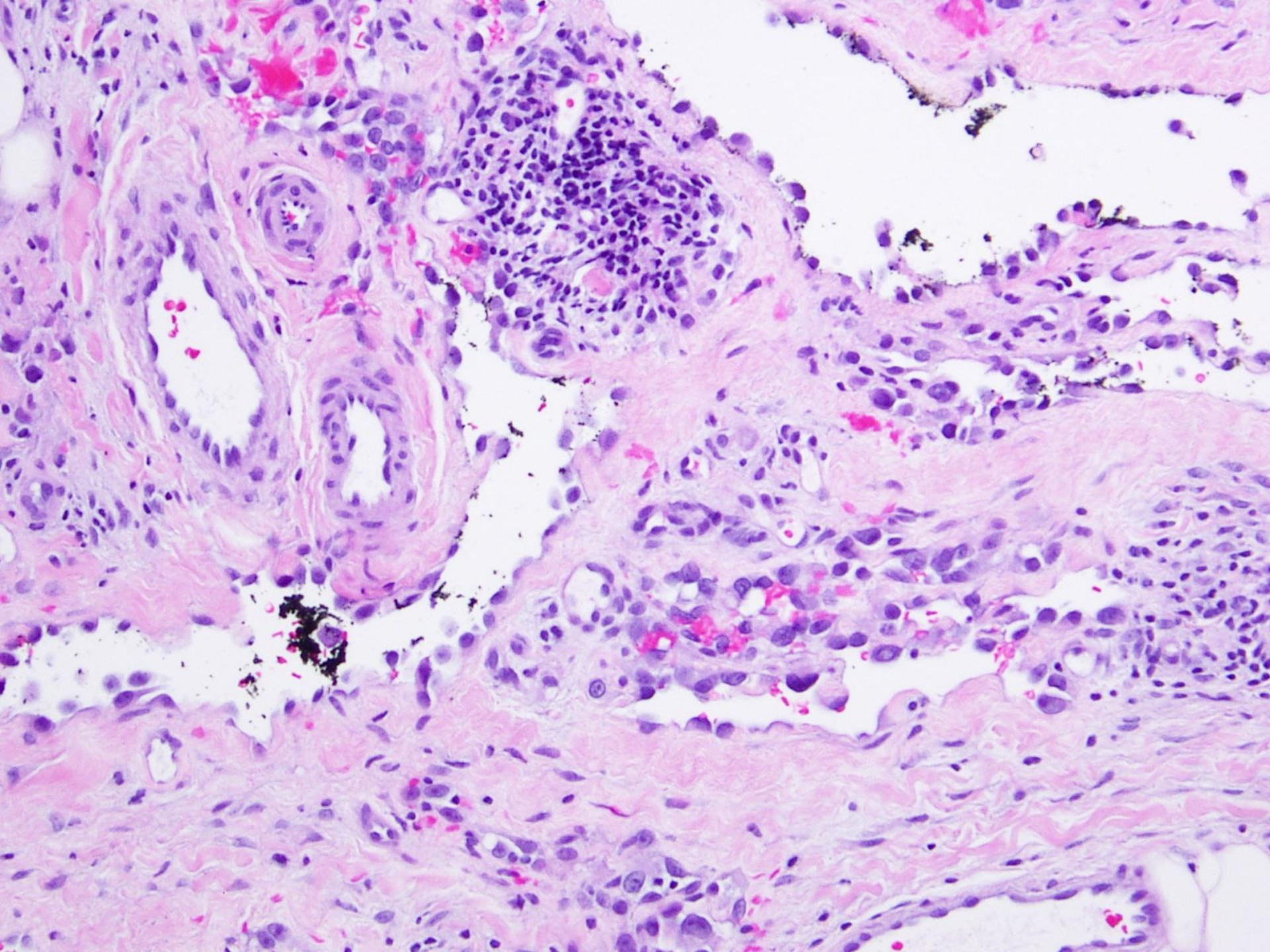
Subtype Specific genomic Alterations

Tumor Type	Gene Mutation
• Pleomoprhic Liposarcoma	• TP53 (17%) and NF1 (8%)
• Myxofibrosarcoma • Myxoid /round cell Liposarcoma	• NF1 (10.5%) • PIK3CA (18%)

Barretina J et al., Sub-type specific genomic alterations define new targets for soft-tissue sarcoma therapy. Nature Genetics 2010; 42(8): 715-721.

Amplification Associated Sarcomas

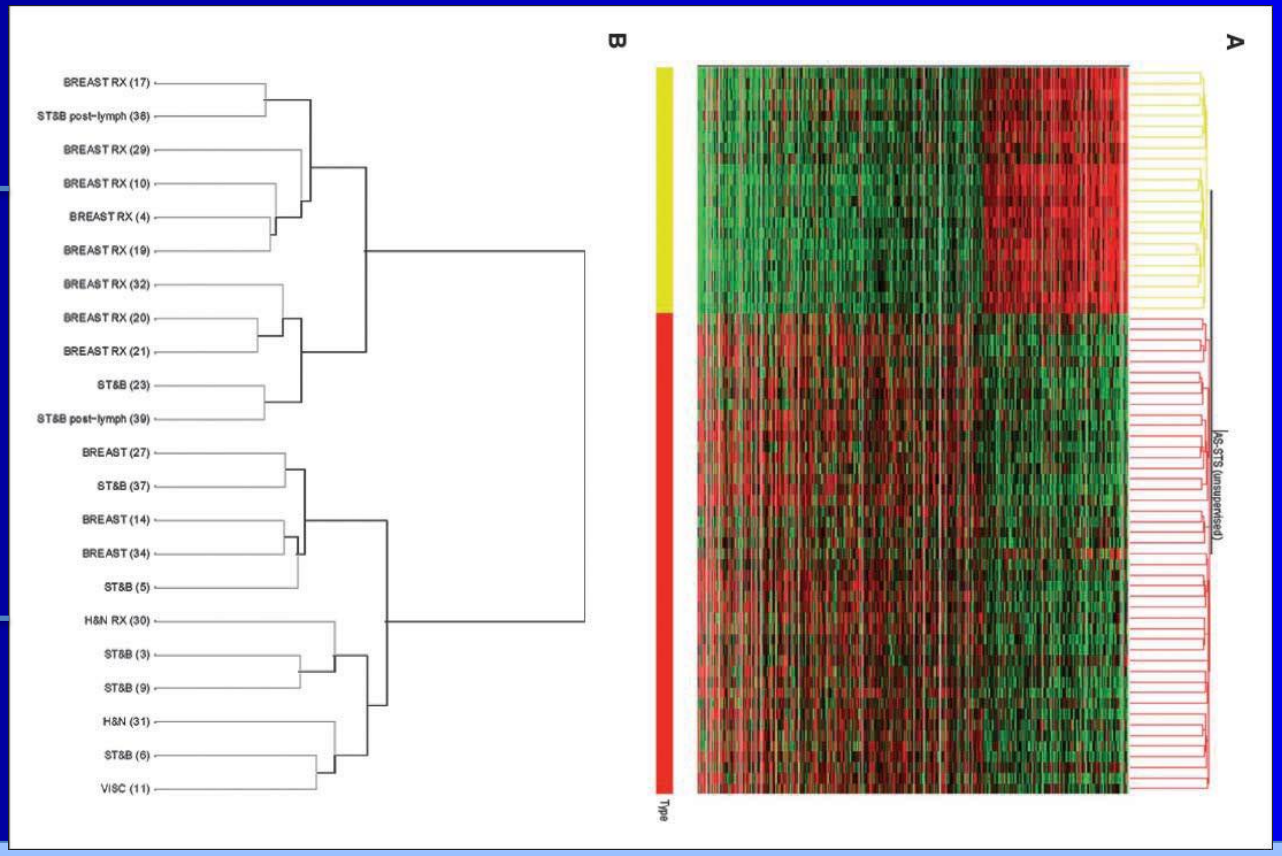
- Angiosarcoma
- Biologically heterogeneous-Anatomical Site based
- Multifocal, local recurrence and early hematogeneous spread
- 30%- 5 year survival
- 40% of radiation induced sarcomas are angiosarcoma developing after RT therapy for breast cancer



KDR Activating Mutations in Angiosarcomas are sensitive to specific kinase inhibitors. Antonescu et al., Cancer Res 2009;69:7175-7179

Secondary

Primary



AS

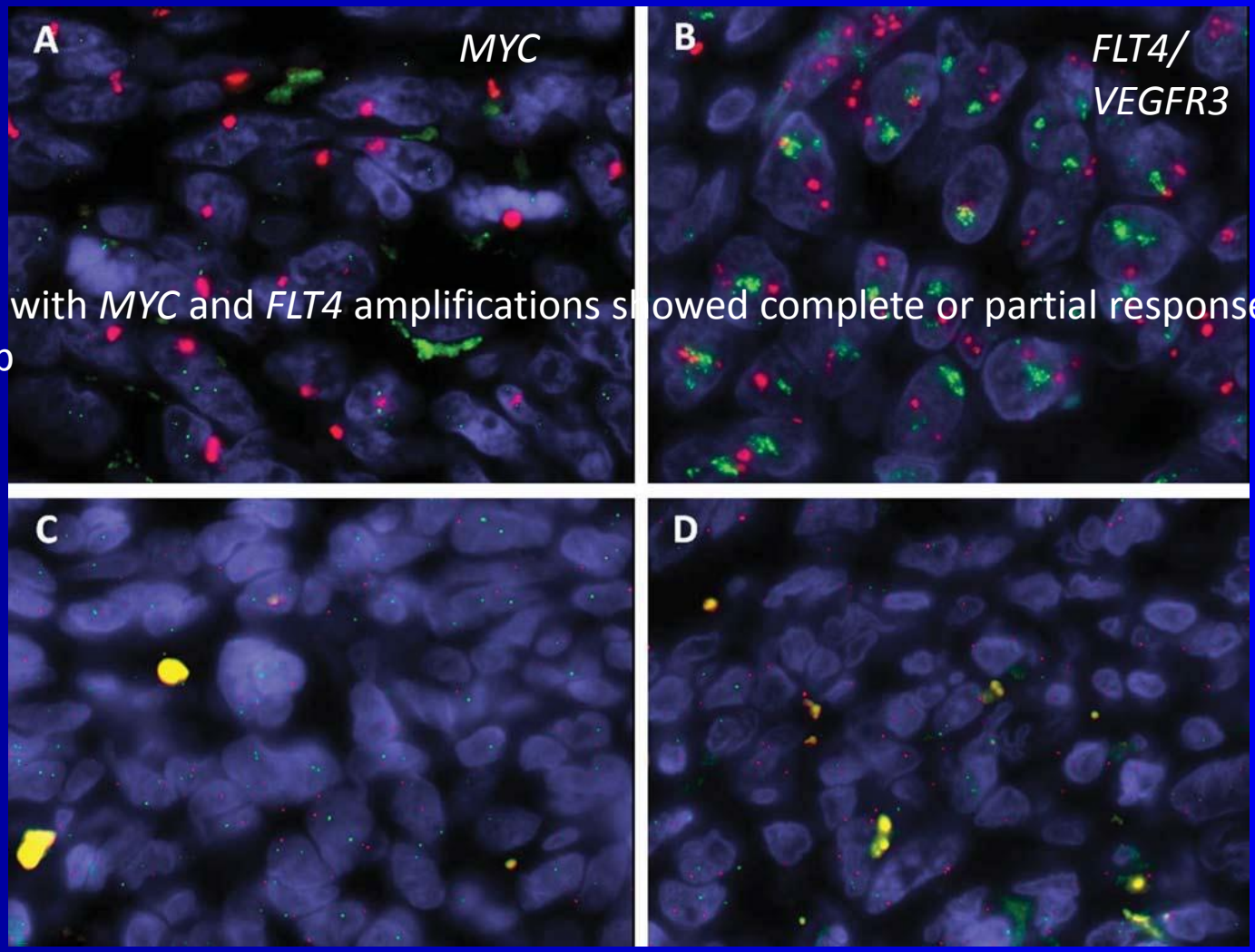
others

Up-regulation of vascular specific receptor tyrosine *kinases (TIE1, KDR /VEGFR2, SNRK, TEK, FLT1 /VEGFR1 in AS- in breast/chest wall AS both primary and secondary*

10% showed *KDR* mutations – sensitive to Sorafinib and Sunitinib

Consistent MYC and FLT4 gene amplifications in Radiation induced angiosarcoma but not in other radiation associated atypical vascular lesions.Guo et al., Genes, Chromosomes, Cancer 50:25-33,2010

Radiation associated Angiosarcoma



Patients with *MYC* and *FLT4* amplifications showed complete or partial response to Sorafinib

Primary Angiosarcoma

RT-induced Sarcoma- not AS

Targeted therapy driven molecular testing

- Drug and Mutation Test
- Imatinib Mesylate-kit/PDGFRA
- Imatinib mesylate- COL1A-PDGFB - t(17;22)
- Imatinib mesylate-CSF1-COL6A3 fusion
- PIK3CA inhibitors –PIK3CA mutation
- Crizotinib- Alk translocation
- Sorafinib-VEGFR2/KDR mutations
- Ridaforolimus- mTOR inhibitor-TSC1 and 2 loss and increased level of p70S6K

- Tumor Type
- Gastrointestinal stromal Tumor (GIST)
- Dermatofibrosarcoma Protuberans (DFSP)
- Giant cell tumor of tendon sheath
- Myxoid/round cell liposarcoma
- Inflammatory myofibroblastic tumor
- Angiosarcoma
- PEComa and related tumors

Molecular Pathology of Sarcomas: Diagnostic and Therapeutic Utility

Is Molecular Testing useful?

Pediatric Round Cell and Spindle Cell Tumors: Diagnostic Utility

Benign vs Malignant Tumors

Targeted Therapy: GIST, DFSP, Angiosarcoma

Clinical Trials

A meaningful outcome of these trials require accurate histopathological and appropriate molecular studies of these tumors by an experienced pathologist

Adapted from Nature Reviews


Thank You