

Molecular Pathology of STT.

P.Pauwels, UGent.

GO HOME!!!!!!

Metaphase Analysis

- Genome wide screen to detect
 - aneuploidy
 - structural abnormalities
 - clonal evolution

What Is FISH?



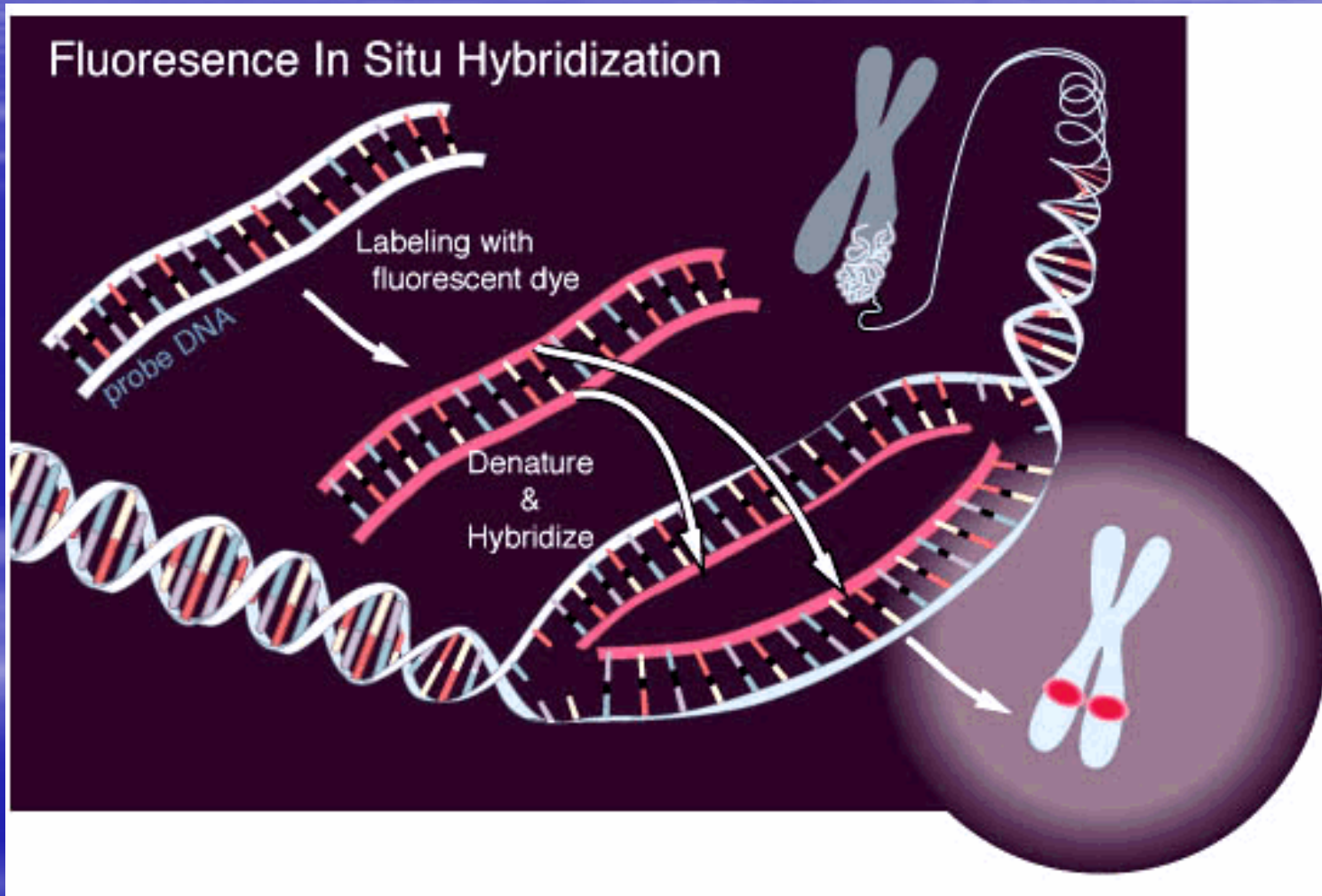
FISH: A definition

“ ... is a technique that enables the morphological demonstration of specific DNA or RNA sequences in individual cells in tissue sections, single cells or chromosome preparations.”

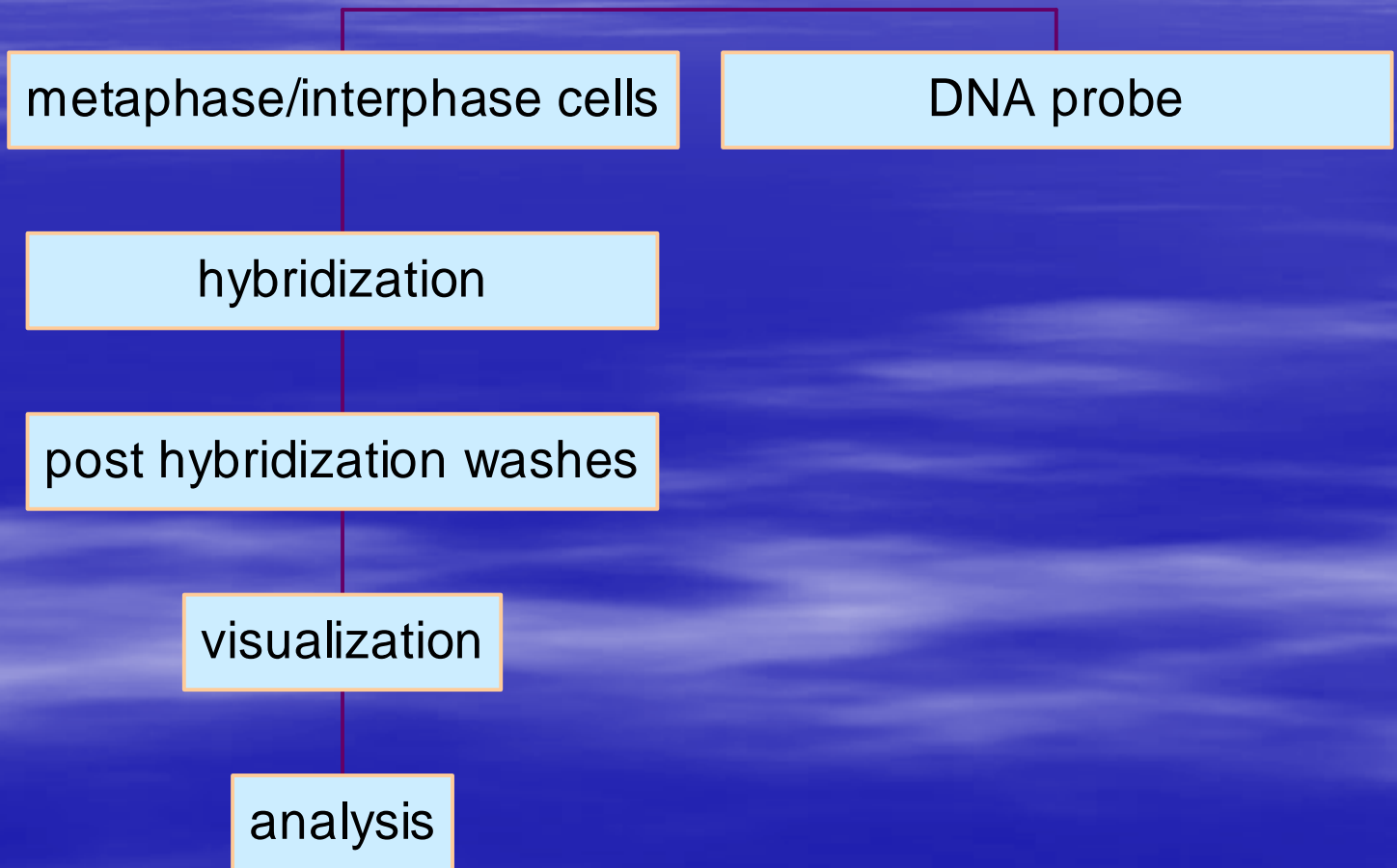
Heinz Hofler

from “Principles of in situ Hybridization” (1990)

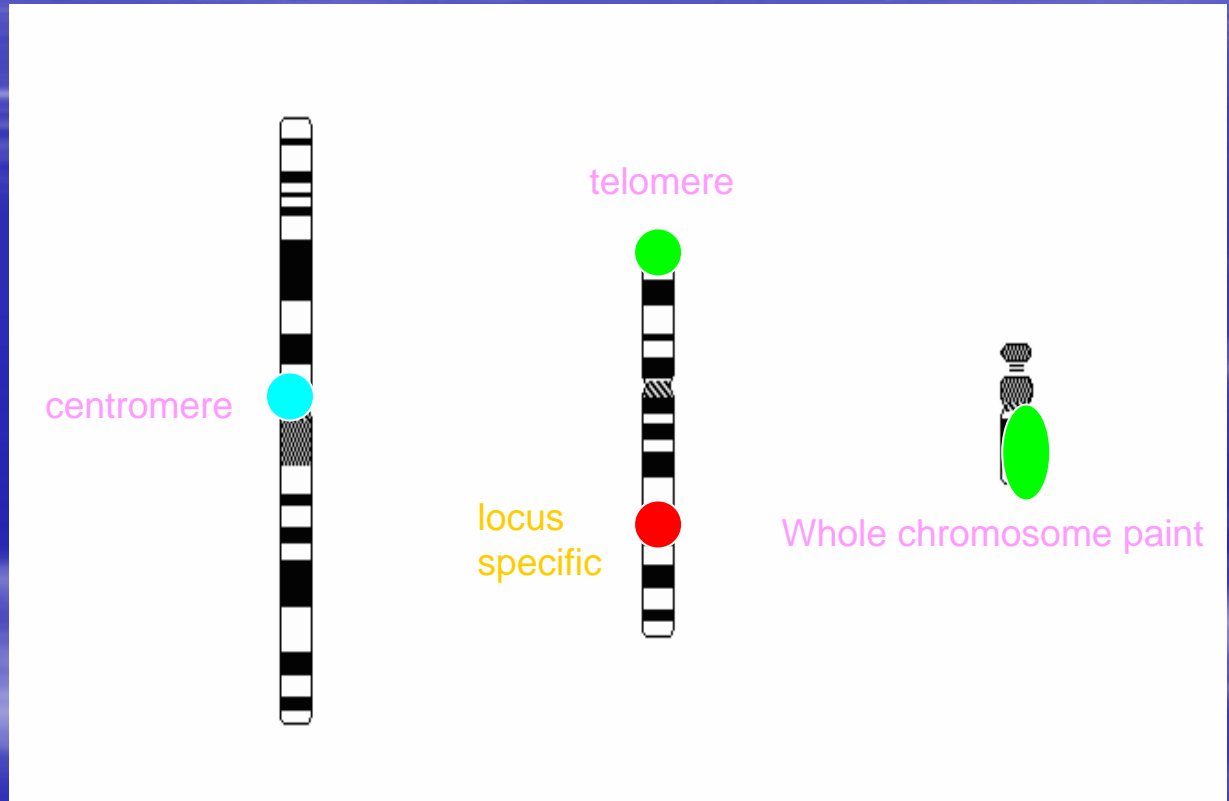
FISH Methodology



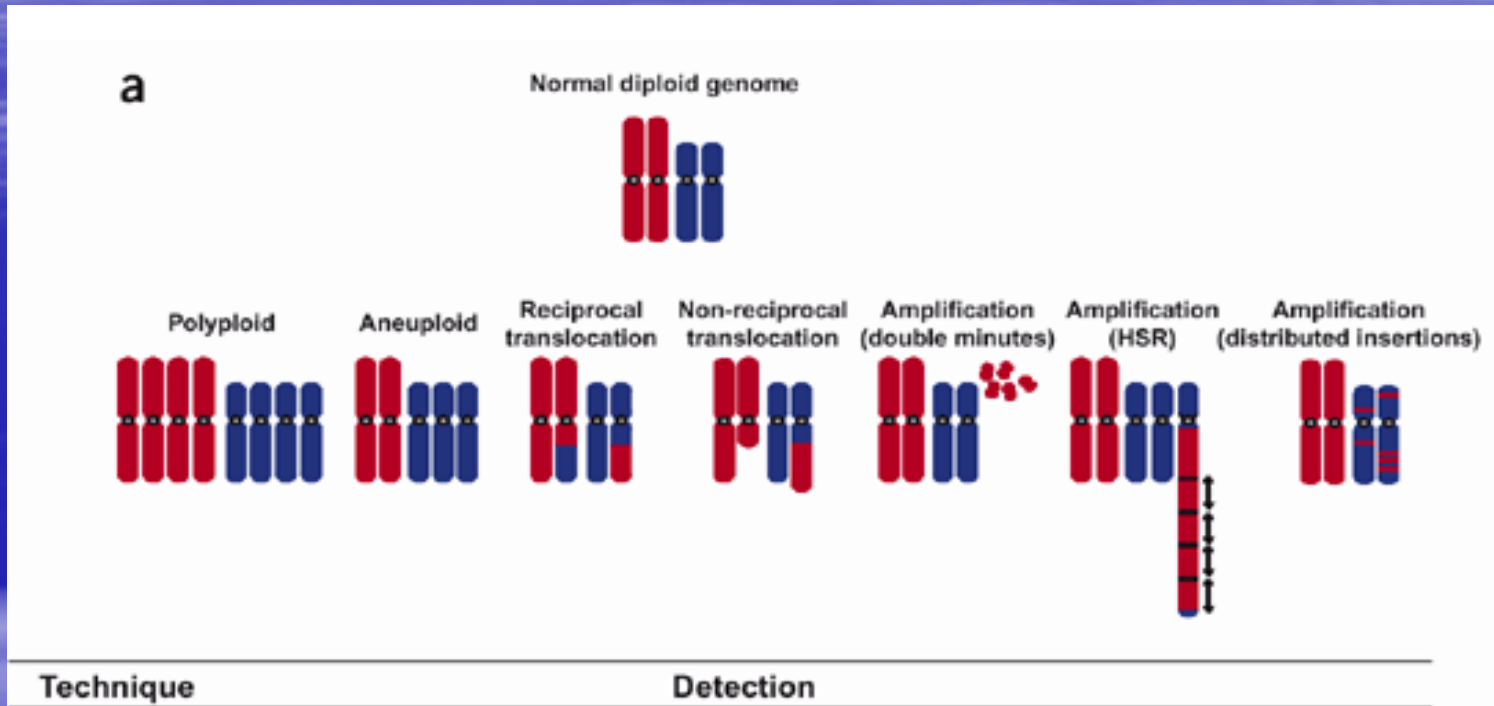
FISH



FISH Probes



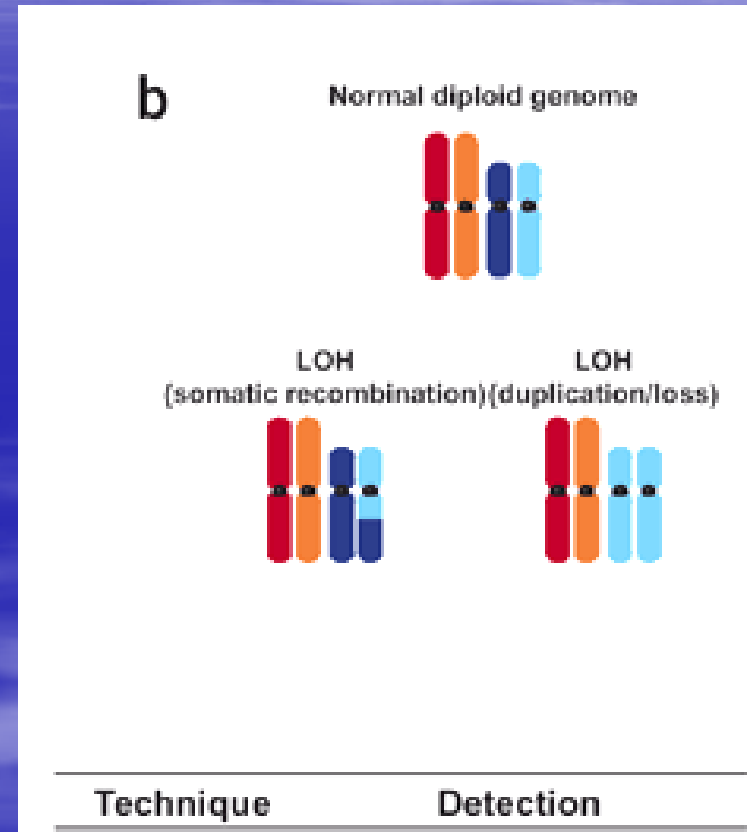
Types of Chromosome Abnormalities



FISH + + + + + + +

- Adapted from Albertson et al 2003 Nature Genetics 34:369-376

Types of Chromosome Abnormalities



FISH

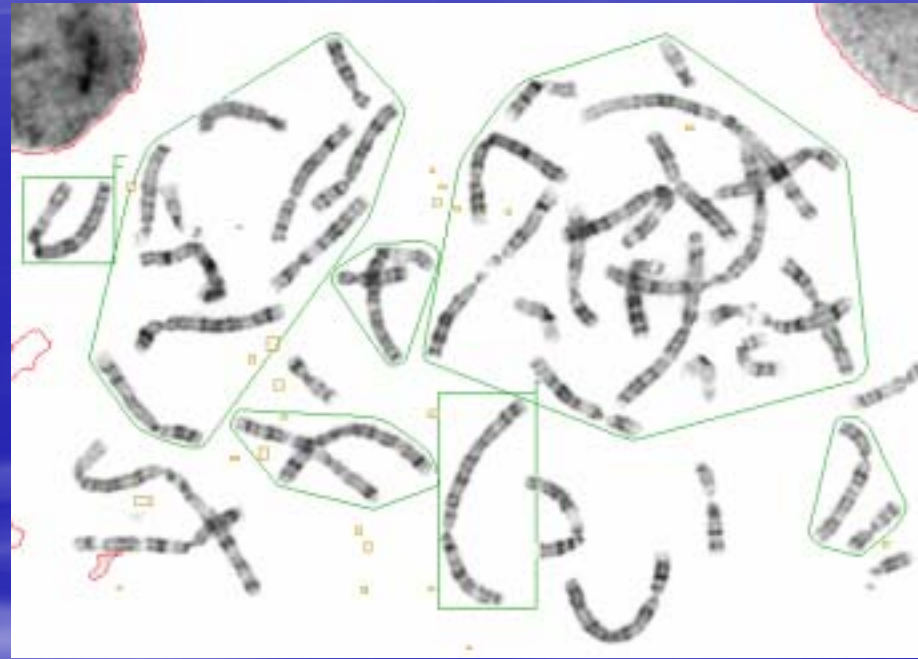
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Metaphase examples:



Bone marrow



Blood

HER-2/*neu* Amplification and Overexpression in Breast Cancer

- Amplification and overexpression: key to
 - prognosis
 - predictive response
 - therapy type and response

HER2-neu Oncogene Expression

- HER2-neu (human epidermal growth factor receptor 2)
 - expression levels altered by gain in copy number or mutations that result in increased levels of expression
 - overexpression associated with
 - poor prognosis
 - predicted therapy response

Laboratory evaluation of HER-2/neu status:

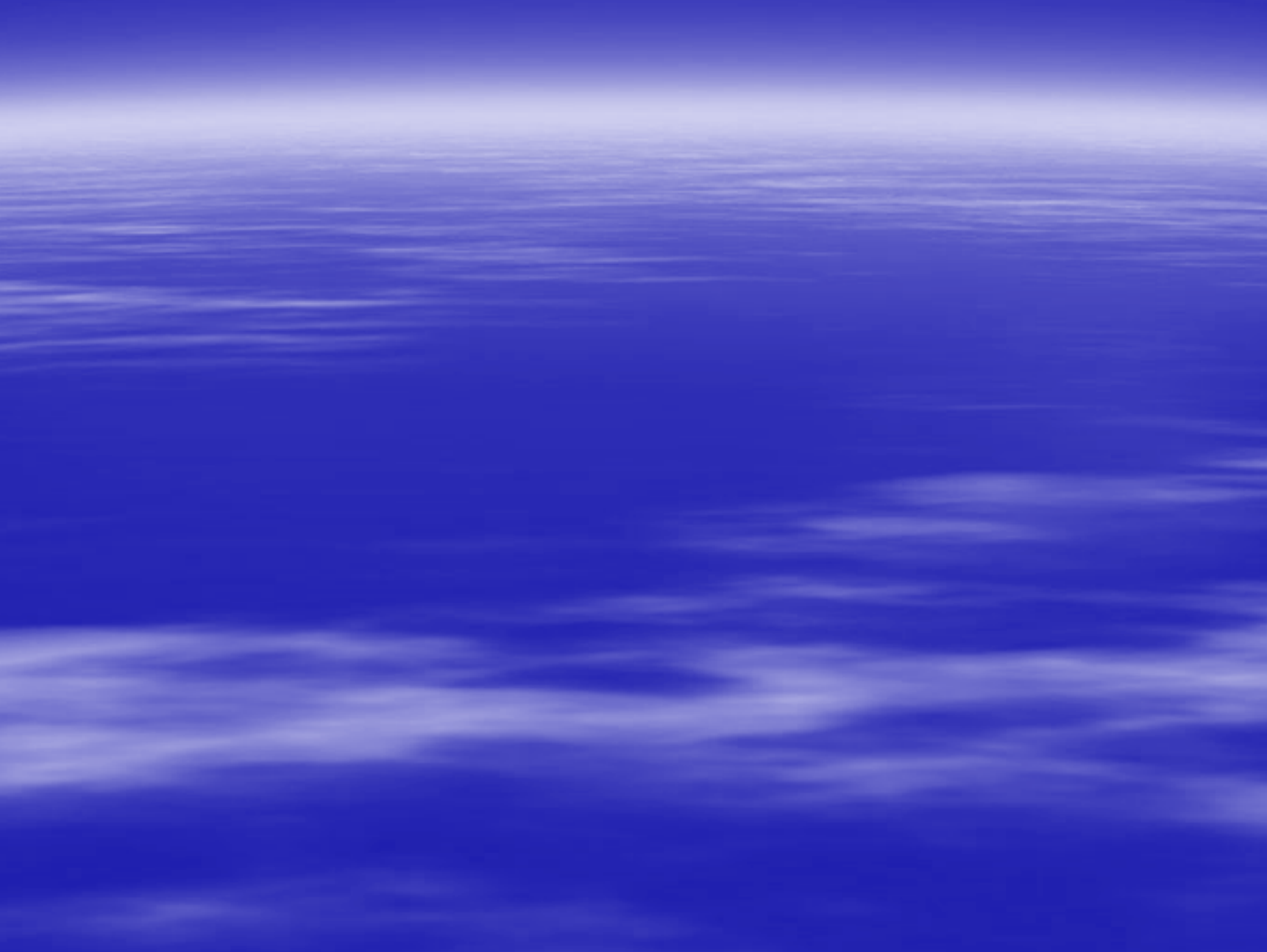
- Protein overexpression
 - Immunohistochemistry (IHC)
 - ELISA (serum)
- Gene amplification
 - FISH, PCR
- mRNA levels
 - RT-PCR

IHC: HER-2/*neu* oncoprotein detection

- Monoclonal antibodies: CB11, Tab 250
 - 77-91% concordance with FISH
 - equivocal IHC result: weak to moderate intensity partial cell membrane staining around a minority of tumour cells
 - retest with FISH

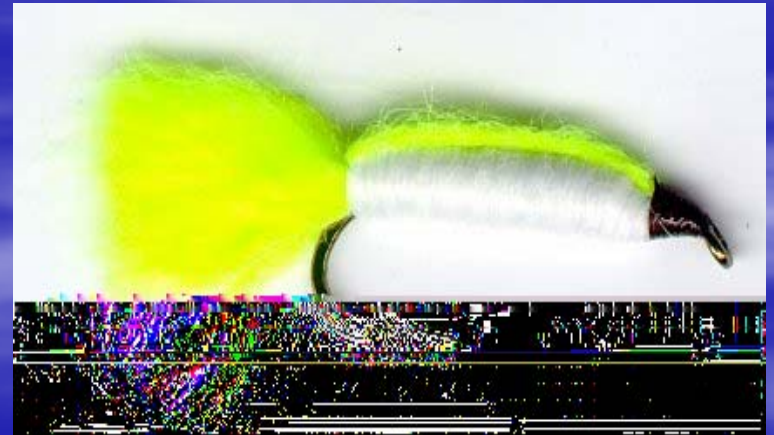
FISH analysis for HER2-neu gene amplification:

- 4-6 μm paraffin-embedded, formalin-fixed tissue sections
 - preselected area of hybridization on stained slide
- FISH using the Vysis PathVysion HER-2 Probe set with pretreatments

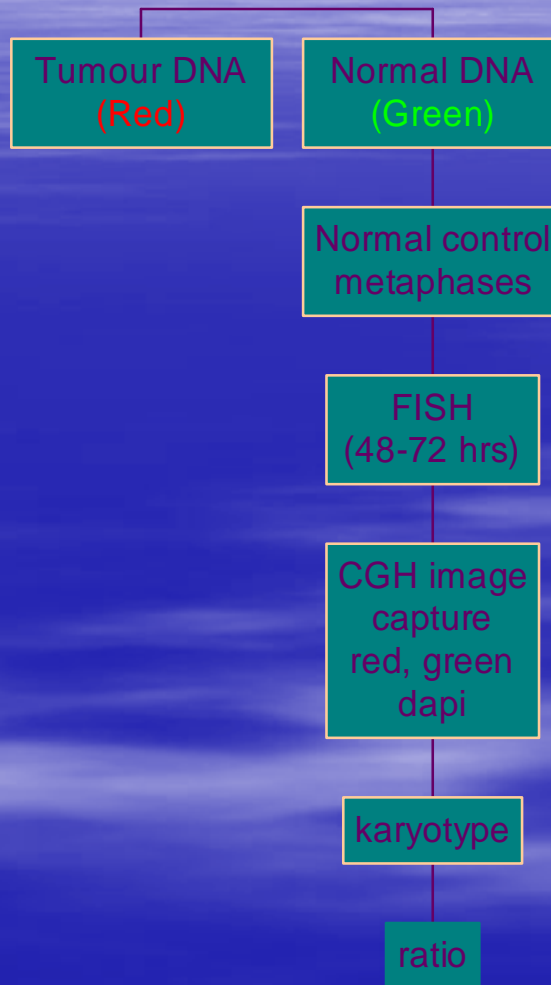


CGH probes

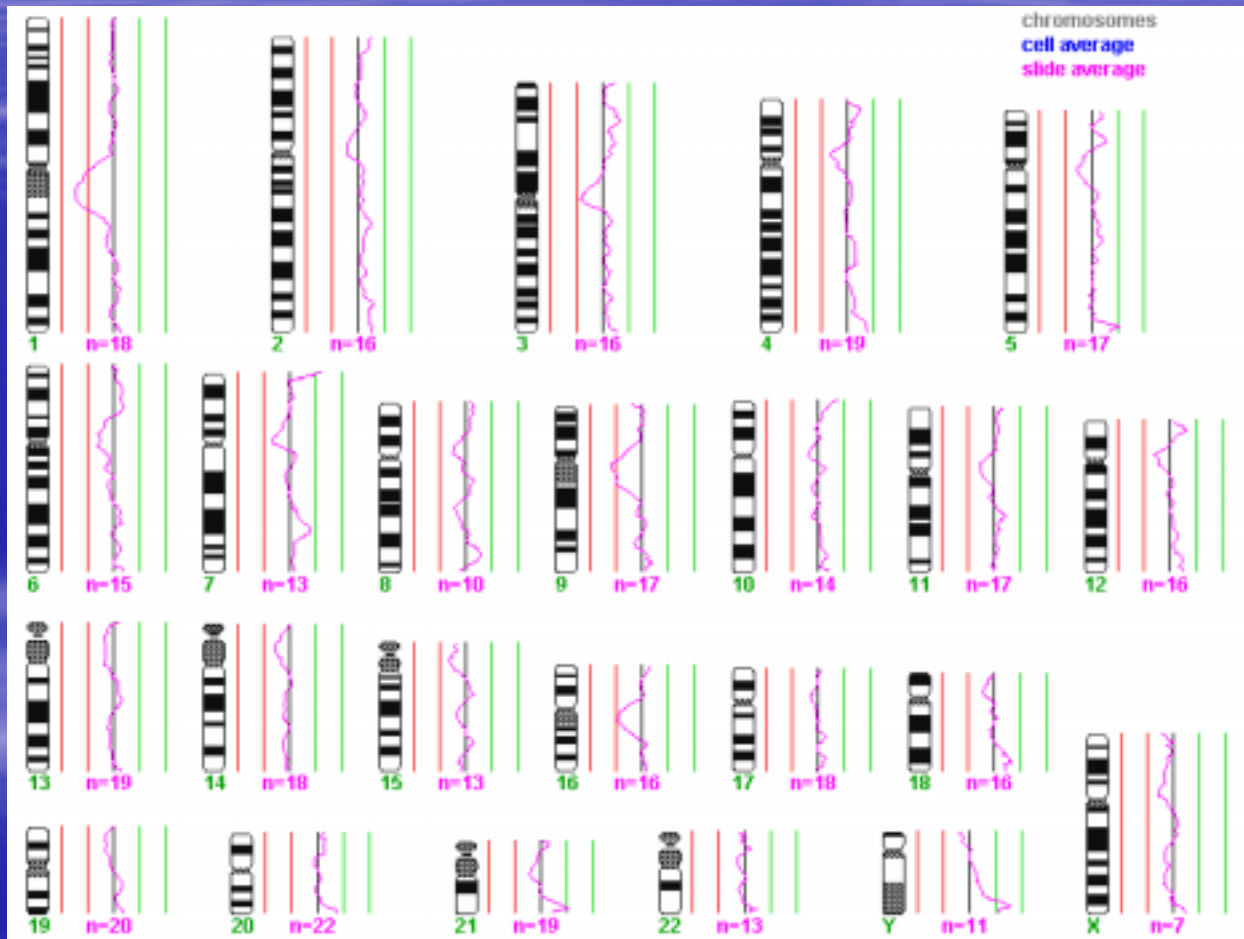
- test/patient DNA
- control/normal DNA
- single hook streamers lure from The English Fly Fishing Shop <http://freespace.virgin.net/fly.fishing/lures.html>



CGH methodology

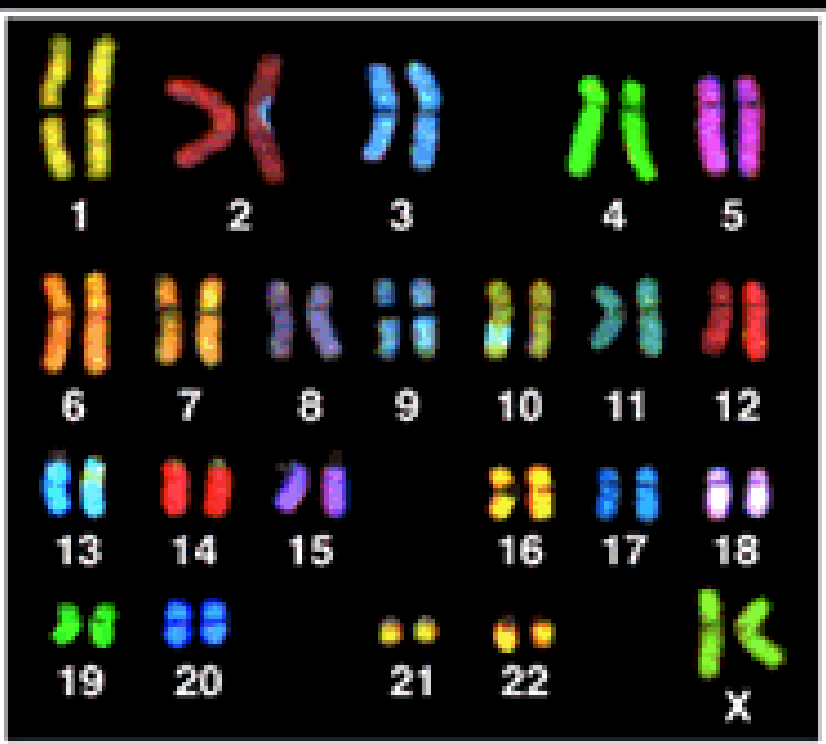
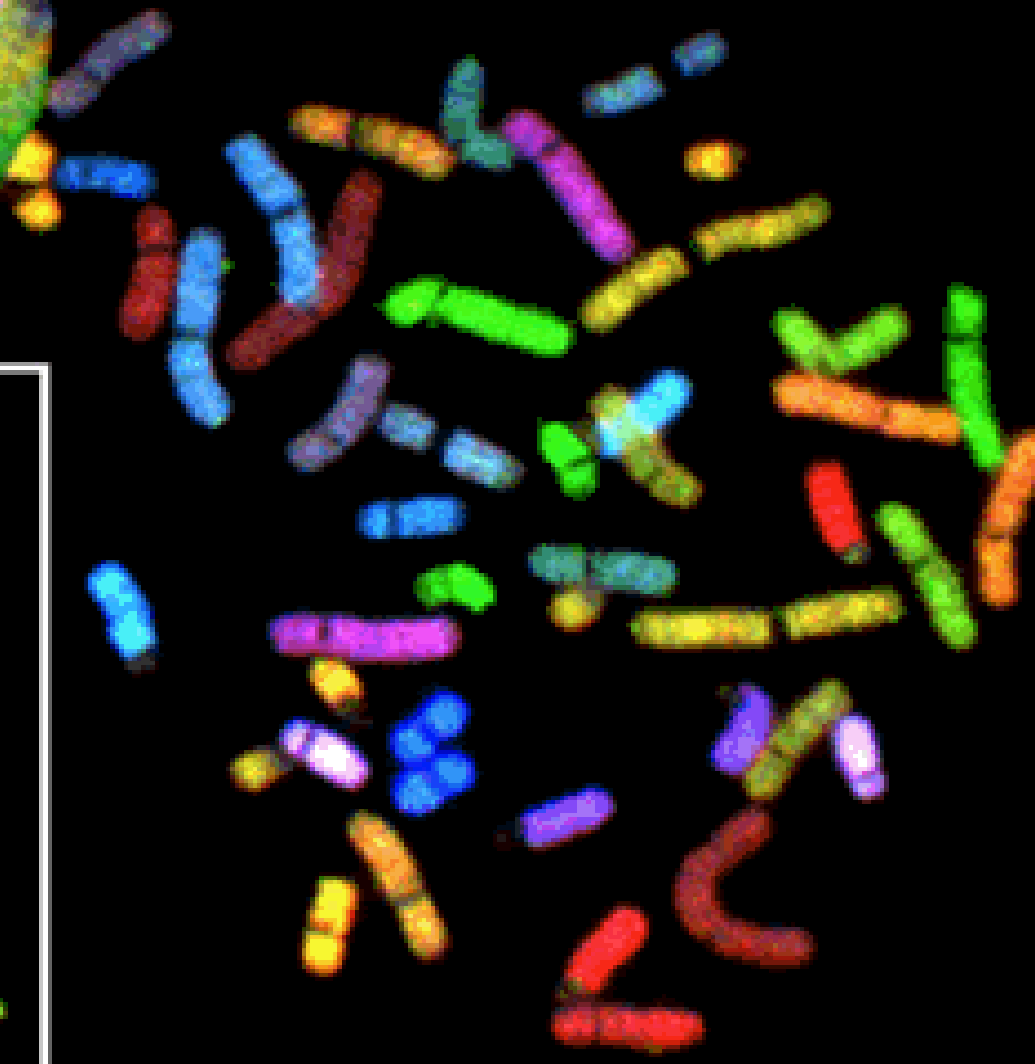
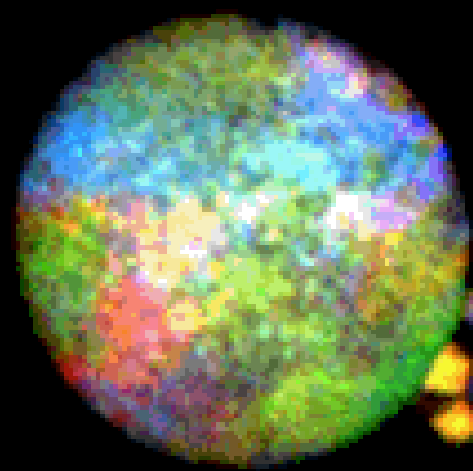


CGH Ratio Interpretation



CGH: Limit Of Resolution

- 3-10 Mbp chromosome imbalance
- abnormality present in ~50% cells
- no identification of balanced chromosome rearrangements



Methods for Identifying Chromosome Aberrations

- Detected using cytogenetic and molecular methods
 - ✓ chromosome banding
 - ✓ molecular cytogenetics
 - Loss of heterozygosity
 - restriction landmark genome scanning
 - representational difference analysis

Genetic Changes in Tumours

- Gene dosage effect
 - gene amplification
 - gene deletions

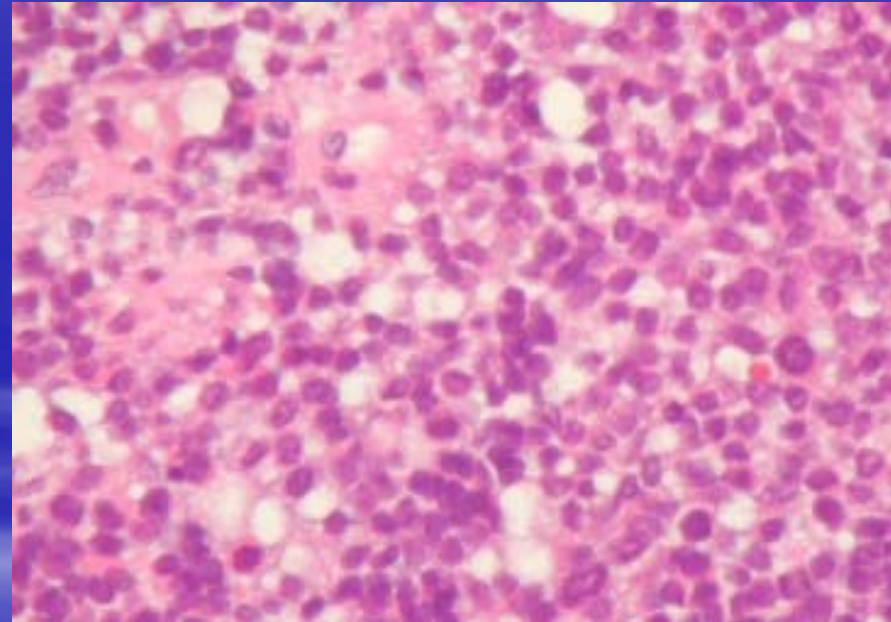
Ewing Sarcoma (EWS)

- Represents a family of tumors including
 - Ewing sarcoma of bone
 - extraosseous Ewing sarcoma and
 - peripheral neuroectodermal tumor (PNET) of bone or soft tissue
- 2nd most common bone tumor in children

Pathology

EWS

- One of many 'small round blue cell' tumors seen in pediatrics
- Thought to be of neural origin, derived from post-ganglionic parasympathetic primordial cells
 - tumor cells synthesize acetylcholine transferase

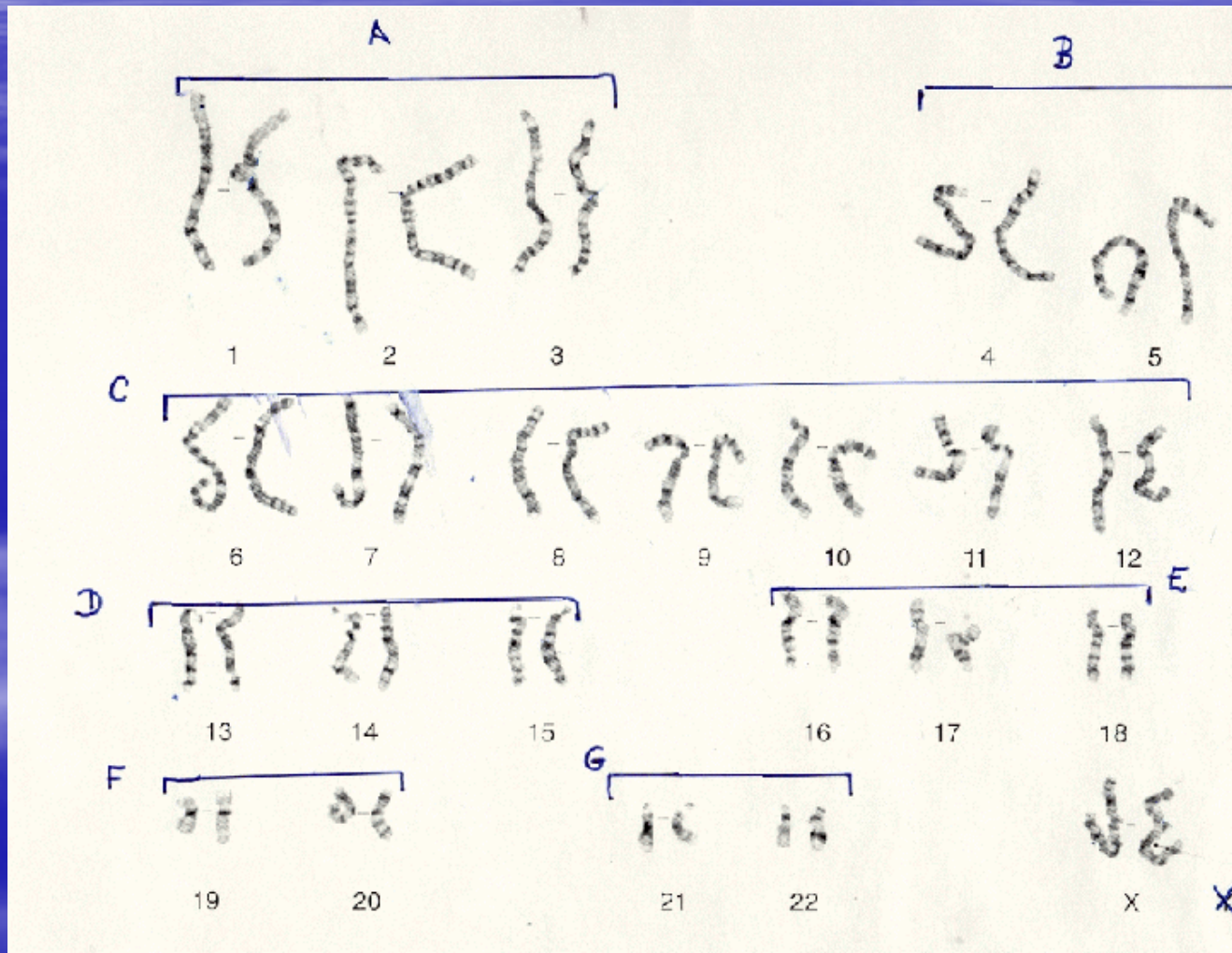


Small, Round, Blue Cell Tumor

Differential Diagnosis

- Lymphoma/Leukemia
 - Rhabdomyosarcoma
 - Metastatic Carcinoma
 - Neuroblastoma
 - **PNET/Ewing Sarcoma**
 - Small Cell Osteosarcoma
-
- Ewing
 - Tumor without differentiation
 - PNET
 - Tumor *with* neural differentiation

Chromosome groups: A - G



Chromosome Abnormalities - Aneuploidy:

- $N = 23$ chromosomes
 - Euploidy:
 - Diploid ($2n$)
 - Triploid ($3n$)
 - Tetraploid ($4n$)
- Aneuploidy
 - A chromosome number that's not an exact multiple of n (23 chromosomes)
 - Due to meiotic non-dysjunction
 - E.g. Trisomy (T13, 18, 21); Monosomy (Turner syndrome)

Chromosomal Abnormalities: Structural Rearrangements

- Due to chromosome breakage and reunion in an abnormal way
- Either:
 - **Balanced** (*usually* normal phenotype)
 - Inversion, Reciprocal translocation, Robertsonian translocation
 - **Unbalanced** (abnormal phenotype)
 - Deletion, Duplication, Ring, Isochromosome

Balanced Structural Rearrangements

Inversion:

- ***Inversion:***
 - Chromosome undergoes 2 breaks
 - Segment between the breaks is inverted
- 2 types:
 - Paracentric
 - 2 breaks on one side of centromere; arm ratio unchanged
 - unbalanced offspring (recombinants) usually not viable (acentric or dicentric)
 - Pericentric
 - one break in each arm; often arm ratio changed
 - recombinants have duplications and deficiencies of ch segments
 - risk of carrier having viable recombinant: 5-10%
- 46, XY, inv(3) (q21q26)

Balanced Structural Rearrangements - Translocations

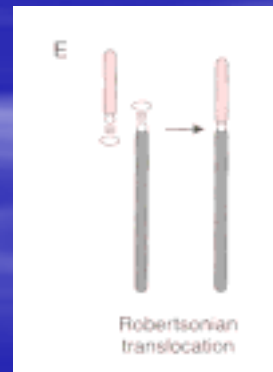
■ ***Reciprocal translocation:***

- Breakage of non-homologous chromosomes
- Reciprocal exchange of broken-off segments
- 46, XX t (3;11) (q13;p14)



■ ***Robertsonian translocation:***

- 2 acrocentrics fuse near centromere
- Loss of short arms
- Still balanced because multiple copies of rRNA genes
- 45, XX der (14;21) (q10;q10)



Unbalanced Structural Rearrangements - Deletion & Duplication:

- ***Deletion:***

- Loss of a chromosome segment (5p-)
- Terminal or interstitial
- Must be at least 5 Mb to be seen cytogenetically
- 46, XX del (5p) (p15)

- ***Duplication***

- 46, XY dup (1) (q22q25)

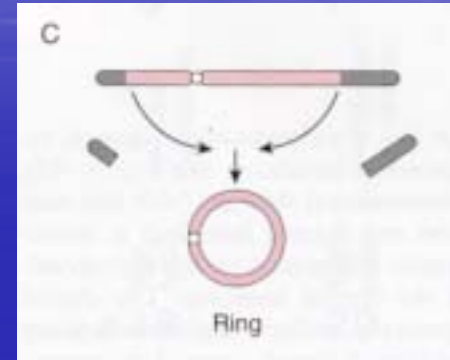
Unbalanced Rearrangements - Deletions



Unbalanced Structural Rearrangements: Ring and Isochromosome

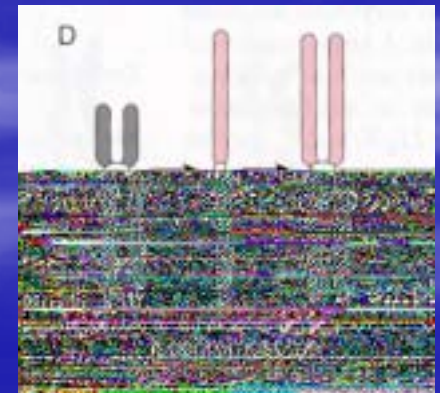
- ***Ring chromosome:***

- Chromosome undergoes 2 breaks
- Broken ends reunite in a ring
- 46, XX, r (7) (p22q36)



- ***Isochromosome:***

- One arm missing; other arm duplicated
- Breakpoints are assigned to the centromere
- 45, X, i(X) (q10) = isochromosome for Xq



GIST : the never ending story.

P.Pauwels

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Operation: Partial Gastrectomy, Pancreatectomy, and Splenectomy

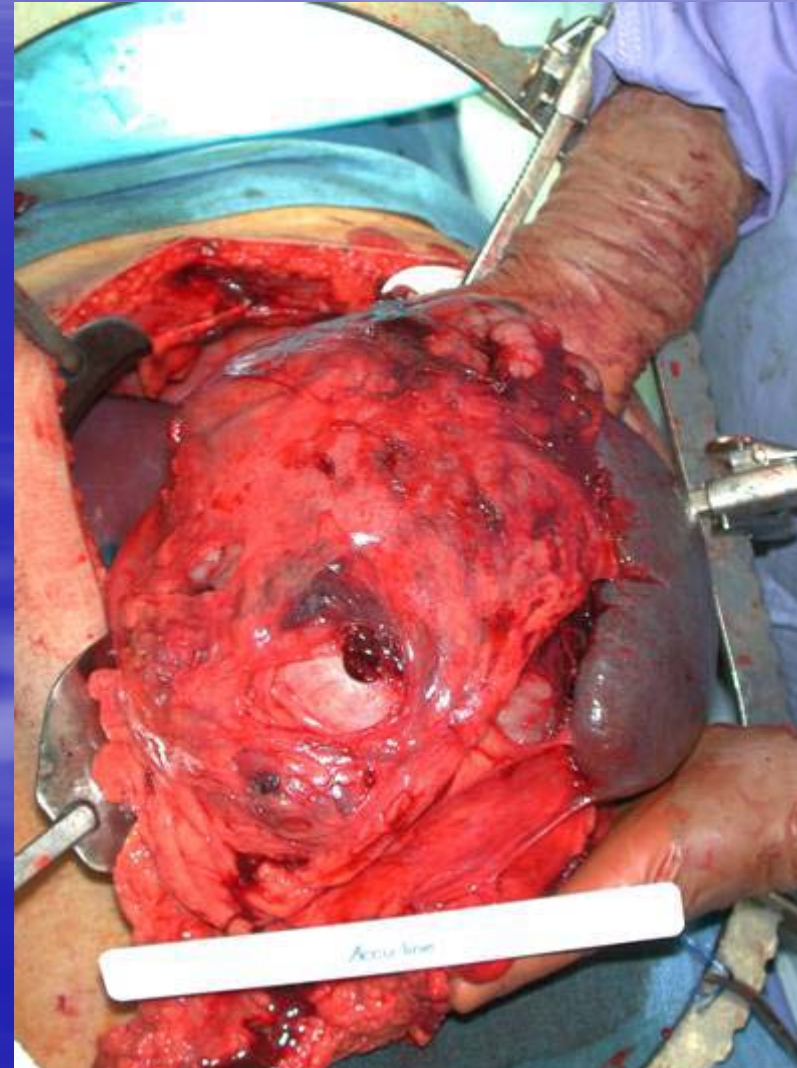


GIST : the never ending story.

P.Pauwels



Operation: Partial Gastrectomy, Pancreatectomy, and Splenectomy



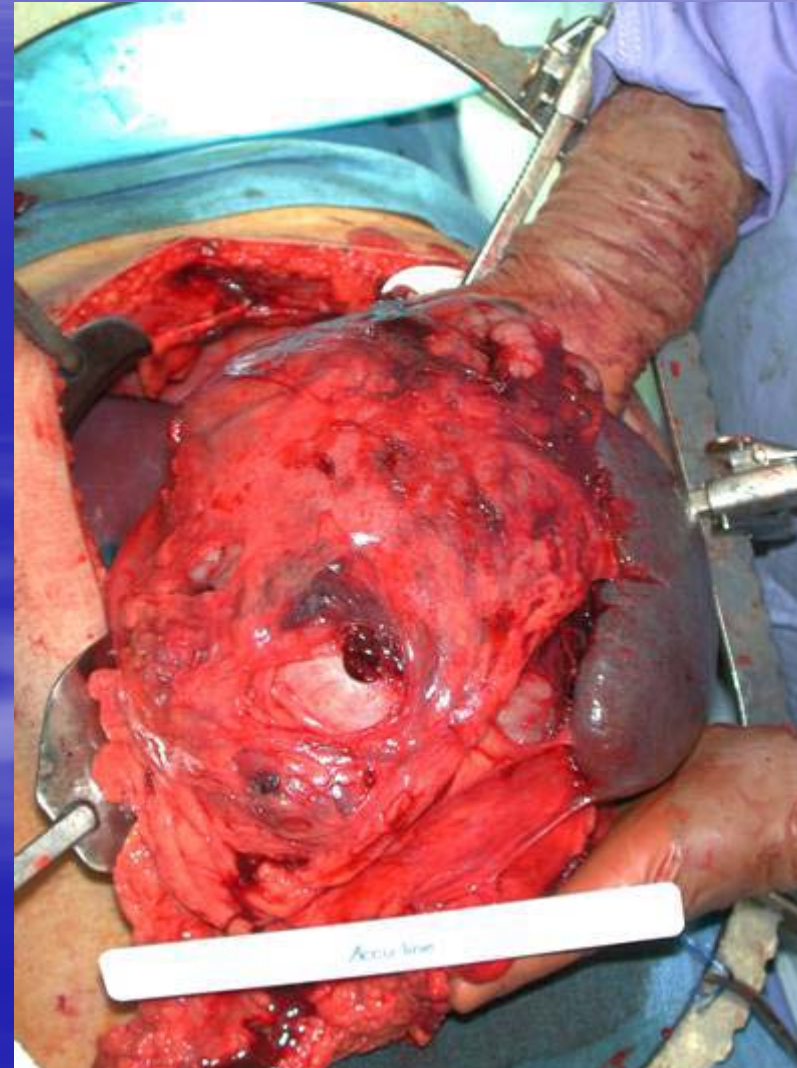
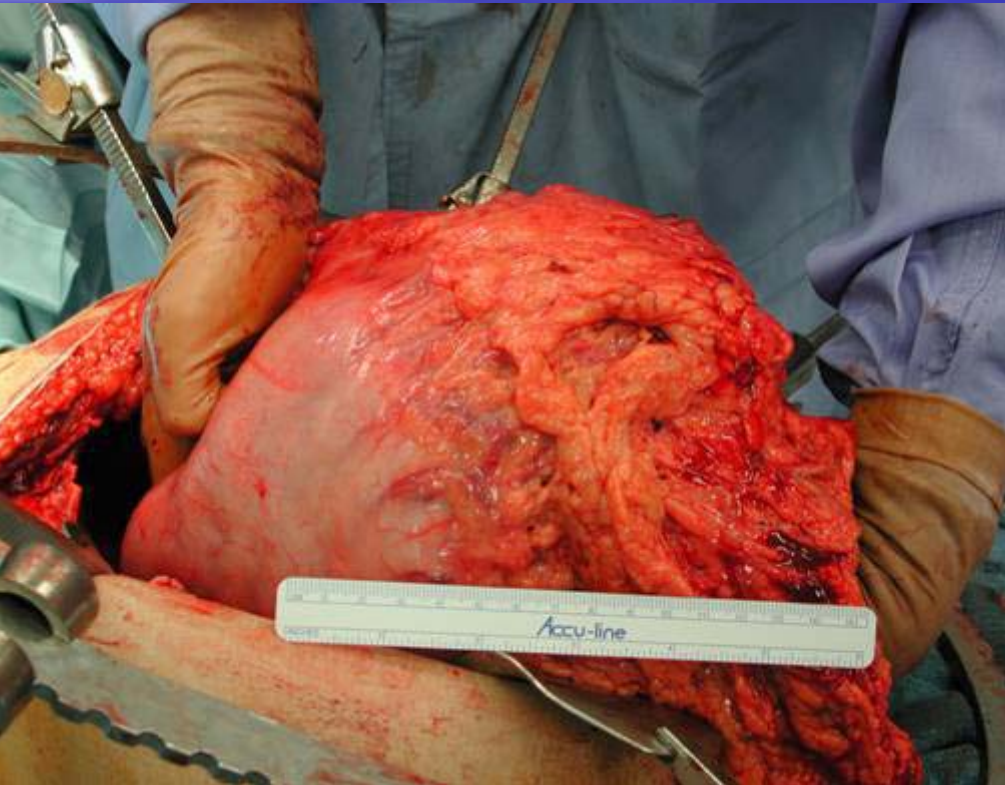
- Originally classified as other tumours—leiomyoma, leiomyoblastoma, or leiomyosarcoma—because of their histological appearance
- Advances in modern molecular biology and immunohistochemistry have allowed GISTs to be distinguished reliably from these other histopathological subtypes of GI tumours

GIST : the never ending story.

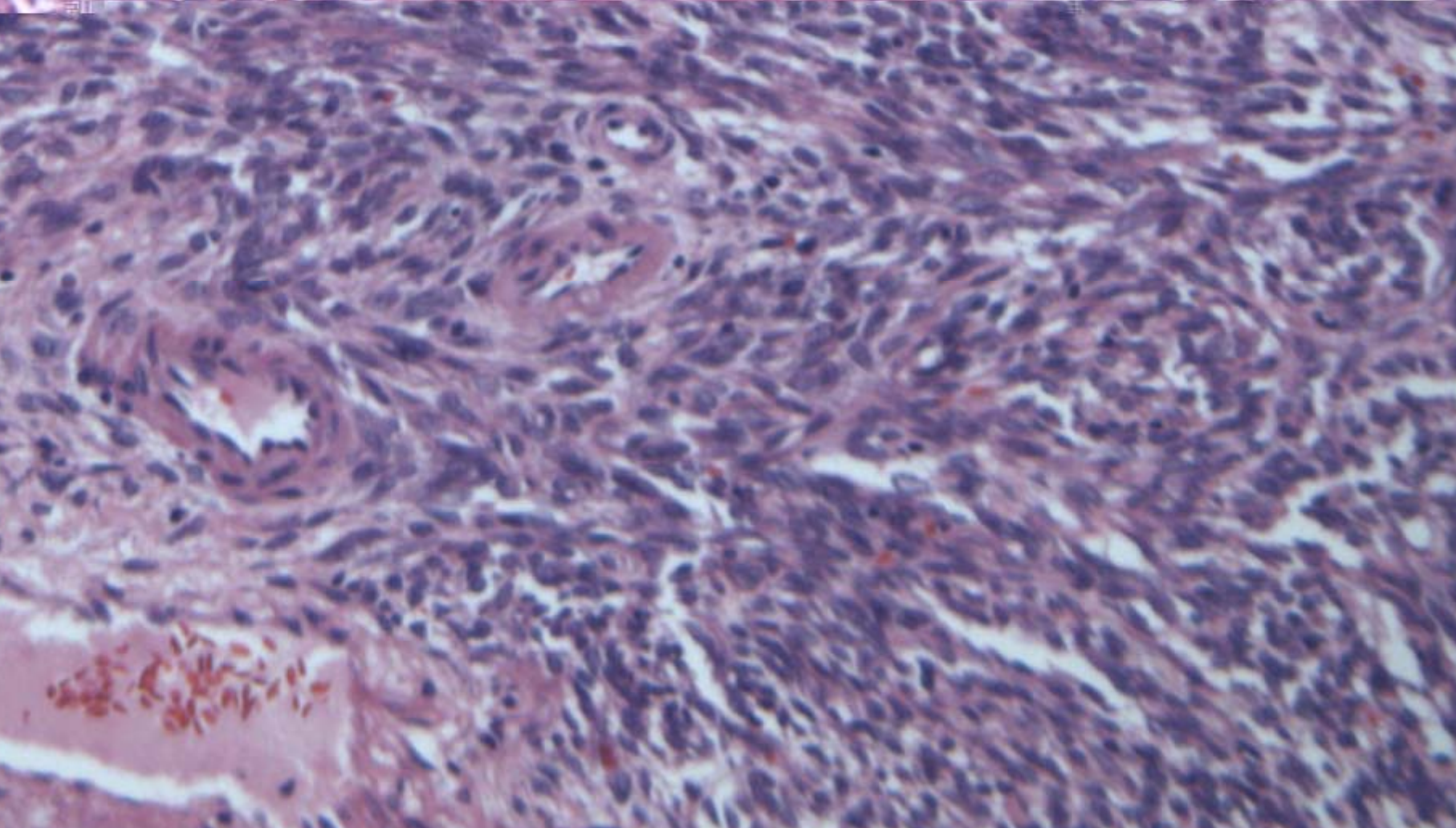
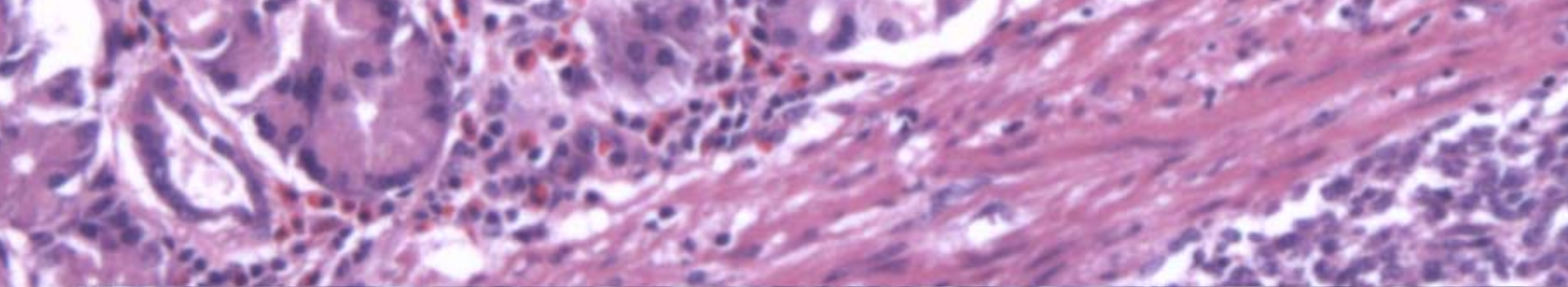
P.Pauwels

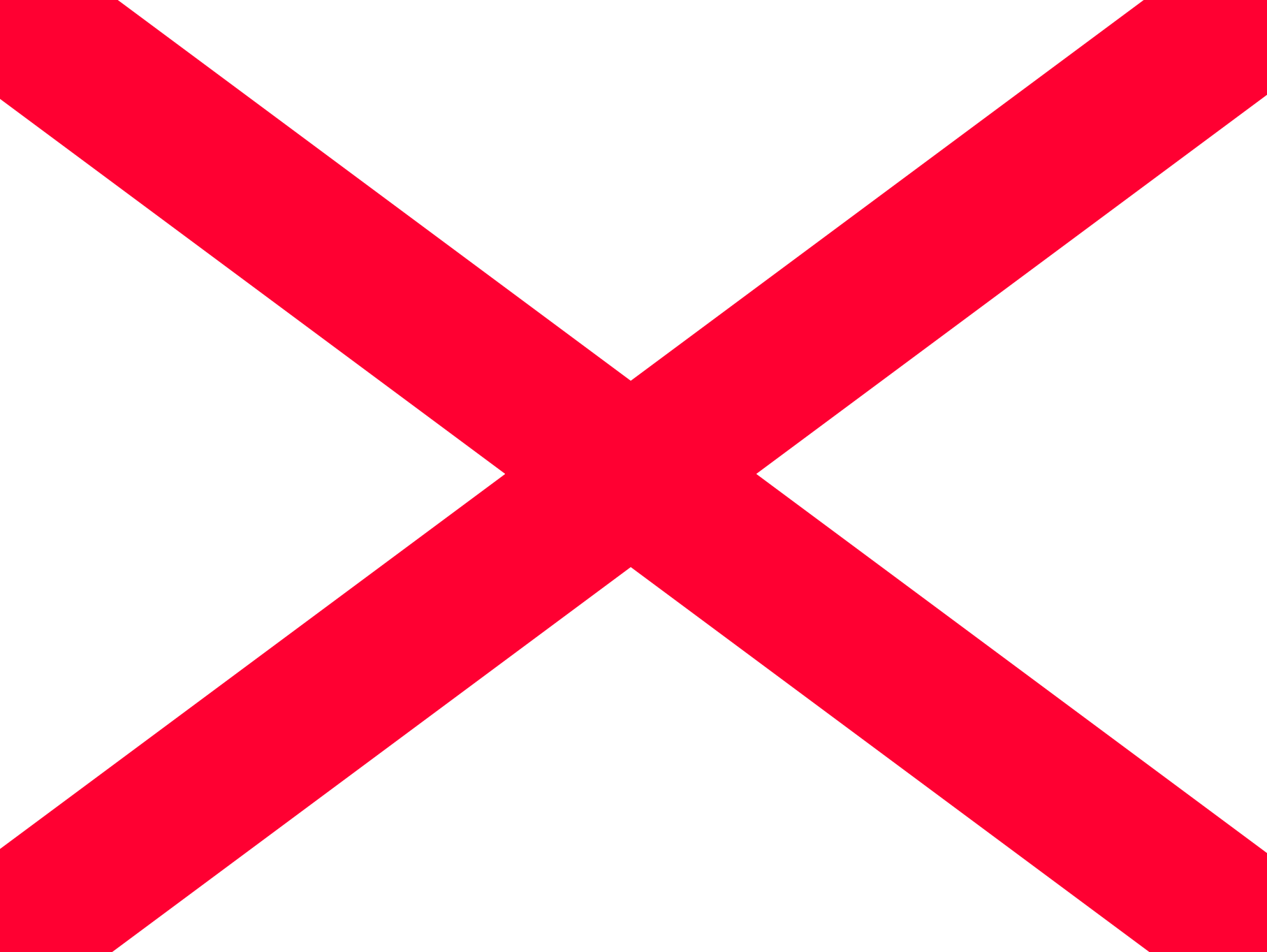


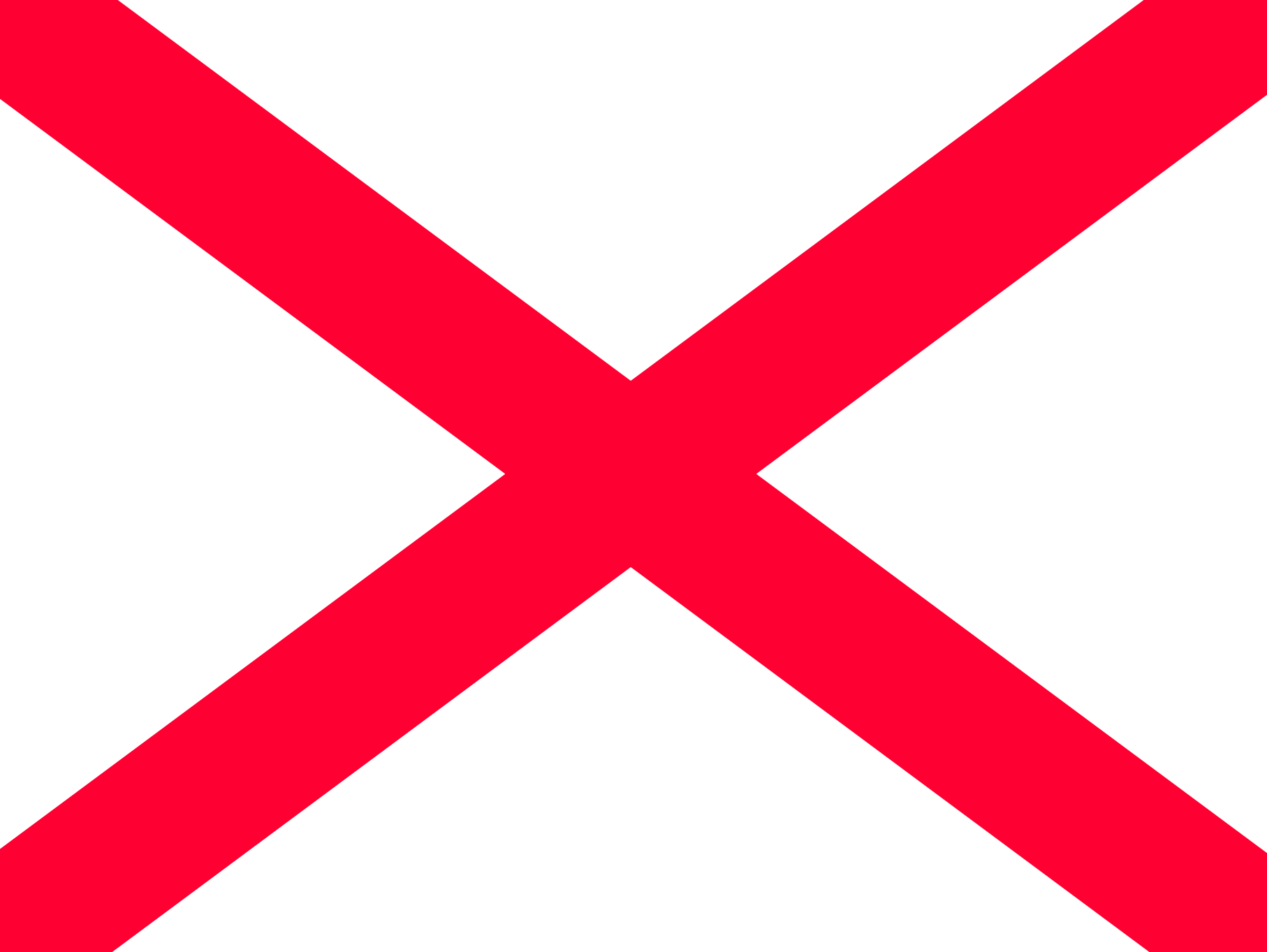
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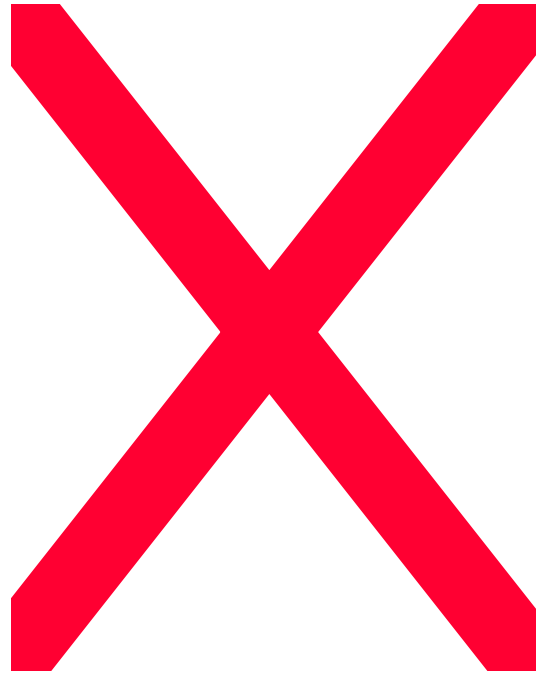
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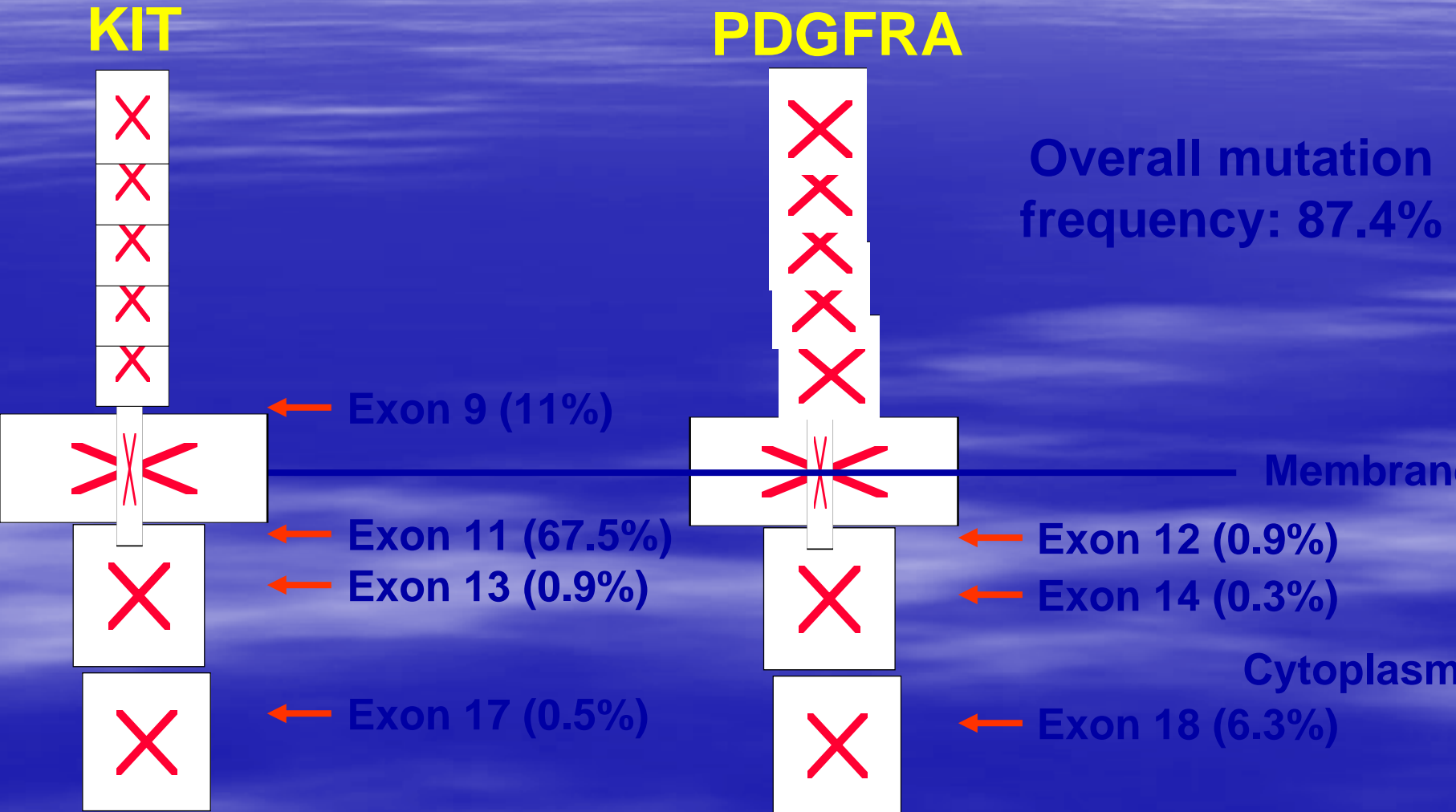


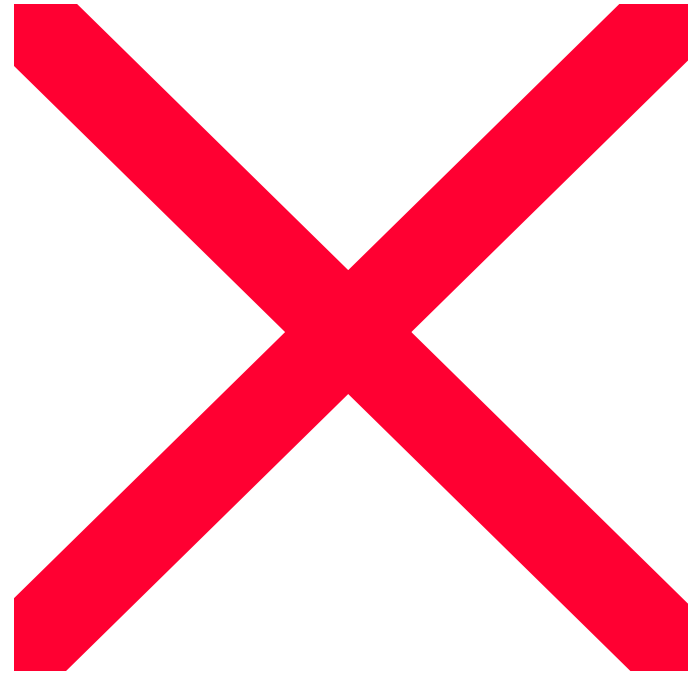




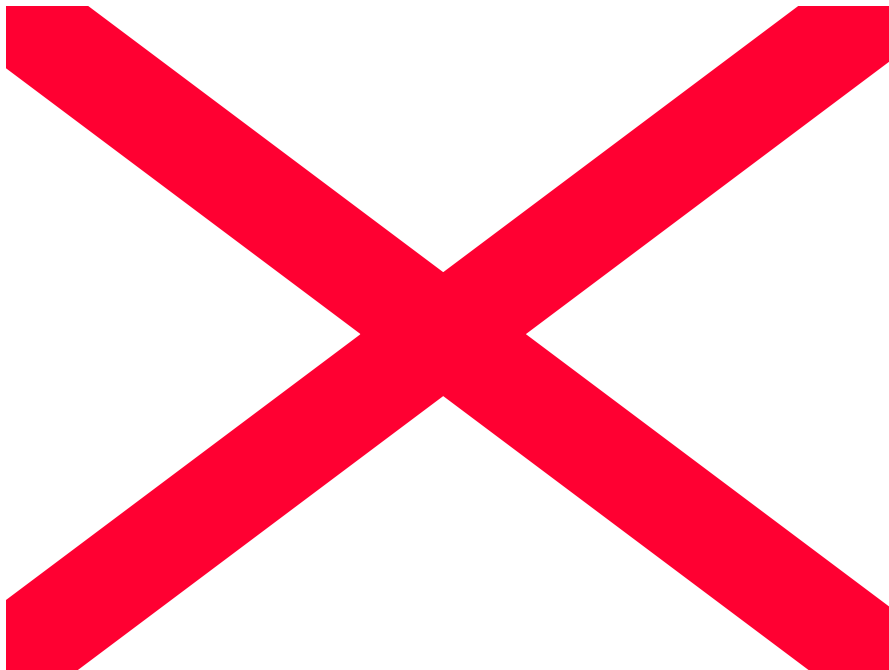


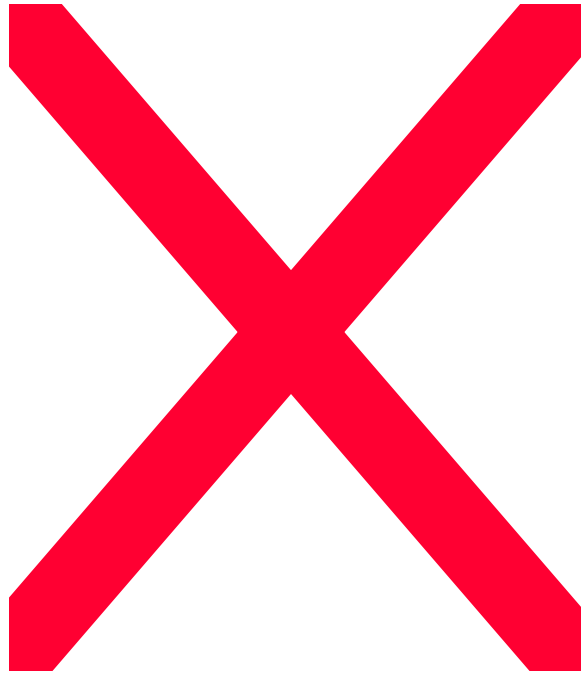
KIT and PDGFRA Mutations in GIST

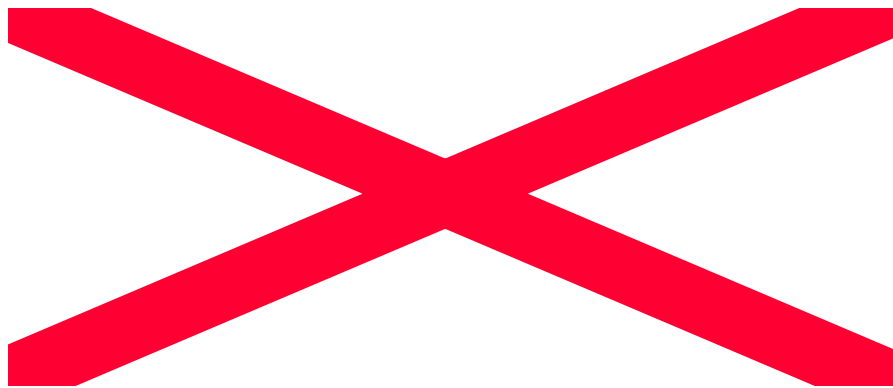


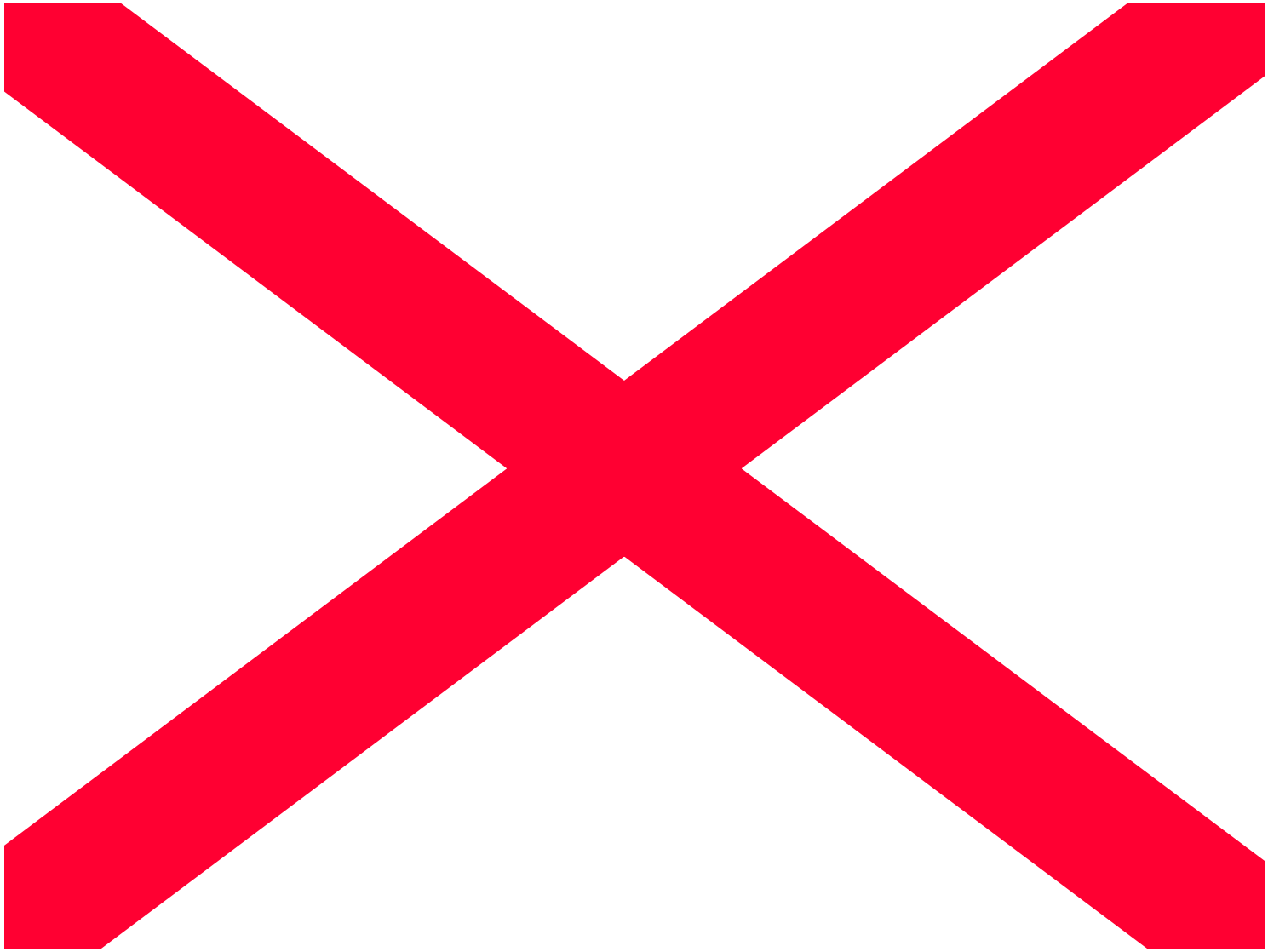




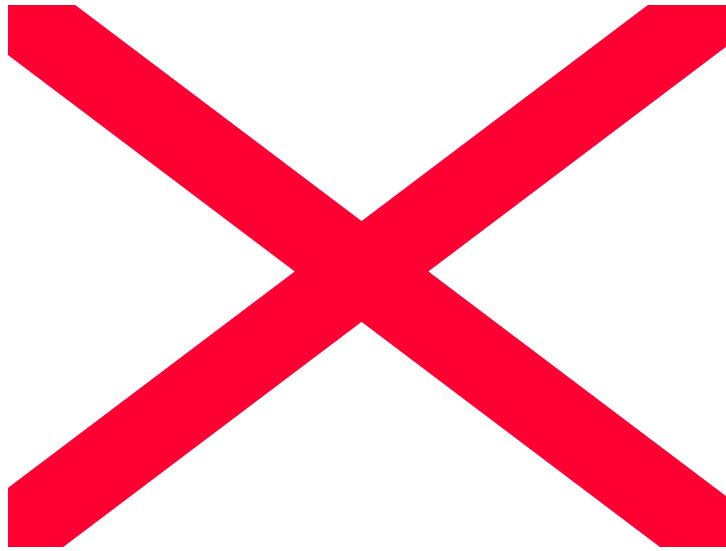








Histopathology- a great source of DNA



Detection of Translocations in Sarcomas

- Cytogenetics
- Southern blotting
- FISH
- IHC (over-expression, expression of specific domains only)
- RT-PCR (RNA to cDNA)

CYTOGENETICS

Specific Translocations

- Ewing's Sarcoma/PNET
FLI1)
(EWS-ER)
ETV1) $t(11;22)(q 24, q 12)$ (EWS-
 $t(21;22)(q22, q12)$
 $t(7;22)(p22;q12)$ (EWS-
- Desmoplastic Small Cell Tumor $t(12;22)(q13;q12)$ (EWS ATF1)
aka (EWS-WT1)
- Extraskeletal myxoid chondrosarcoma $t(9;22)(q22;12)$ (EWS-TEC)
aka (EWS-CHN)
- Myxoid Liposarcoma $t(12:16)(q13;p11)$ (FUS-CHOP)
 $t(12:22:20)$ (EWS-CHOP)
- Synovial Sarcoma $t(X:18)(p11.2;q11.2)$ (SYT-SSX1)
and (SYT-SSX2)
- Alveolar rhabdomyosarcoma $t(2:13)(q35;q14)$ (PAX3-FKHR)

Histologic Diagnoses

Sarcomas of Fibrous Tissue

- Malignant Fibrous Histiocytoma (MFH)
- Low-grade Fibromyxoid Sarcoma (Fibrosarcoma)
- Dermatofibrosarcoma Protruberans
- Desmoid Fibromatosis
- Nodular Fasciitis

Sarcomas of Peripheral Nervous Tissue

- Malignant Peripheral Nerve Sheath Tumor
- AKA
 - Malignant Schwannoma
 - Neurofibrosarcoma
 - Neurogenic Sarcoma

Sarcomas of Smooth Muscle

- Leiomyosarcoma
 - GI
 - GU
 - Skin
 - Vessel
 - Other

Sarcomas of Adipose Tissue

- Liposarcoma
 - Atypical Lipomatous Tumor
 - Myxoid Liposarcoma
 - Cellular Myxoid Liposarcoma
 - Dedifferentiated Liposarcoma
 - Pleomorphic Liposarcoma

Sarcomas of Blood and Lymph Vessels

- Angiosarcoma
 - Hemangiosarcoma
 - Lymphangiosarcoma
- Epithelioid Hemangioendothelioma
- Hemangiopericytoma
- Kaposi's Sarcoma

Sarcomas of Skeletal Muscle

- Embryonal Rhabdomyosarcoma
- Alveolar Rhabdomyosarcoma
- (Pleomorphic Rhabdomyosarcoma)

Sarcomas of Unknown Tissue

- Synovial Sarcoma
 - Monophasic
 - Biphasic
- Alveolar Soft Part Sarcoma
- Epithelioid Sarcoma
- Unclassified Sarcoma

Extraskelletal Sarcomas of Bone

- Extraskelletal Osteosarcoma
- Extraskelletal Chondrosarcoma
- Extraskelletal Ewing's Sarcoma (PNET)

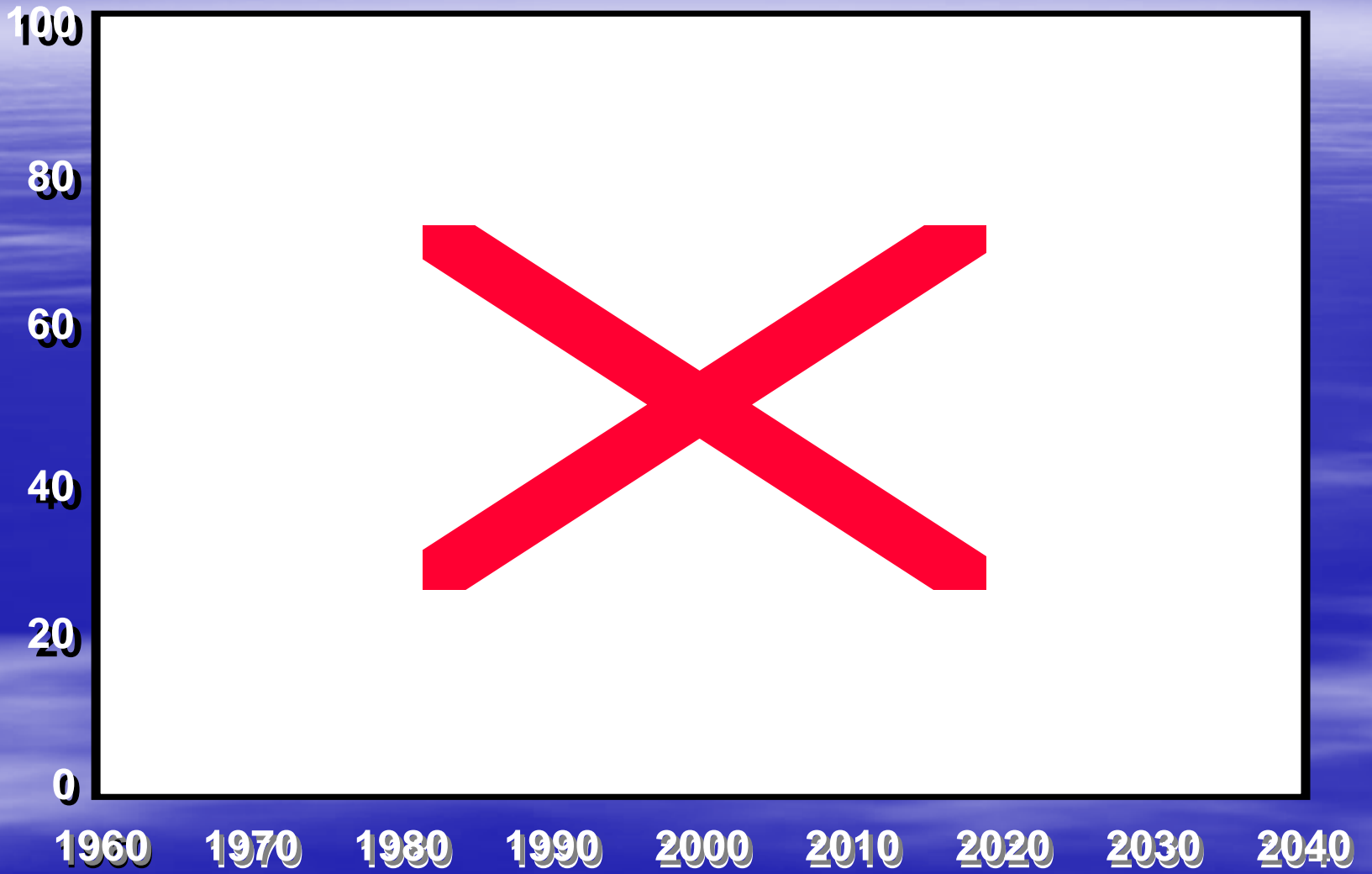
Soft-tissue Tumors of Melanocytic Tissue

- Melanoma of Soft Parts
 - AKA - Clear Cell Sarcoma

ACS ESTIMATES - 2001

CANCER INCIDENCE

	<u>NEW CASES</u>	<u>DEATHS</u>
ALL SITES	1,268,000	553,400
BONE & JOINTS	2,900	1,400
SOFT TISSUES	8,700	4,400
PROSTATE	198,100	31,500
BREAST	193,700	40,600
LUNG	169,500	157,400
COLO-RECTAL	135,400	50,400



Myxoid Liposarcomas

- Defined by a specific translocations

t (12:16) (q13;p11) (FUS-CHOP)

t (12:22:20) (EWS-CHOP)

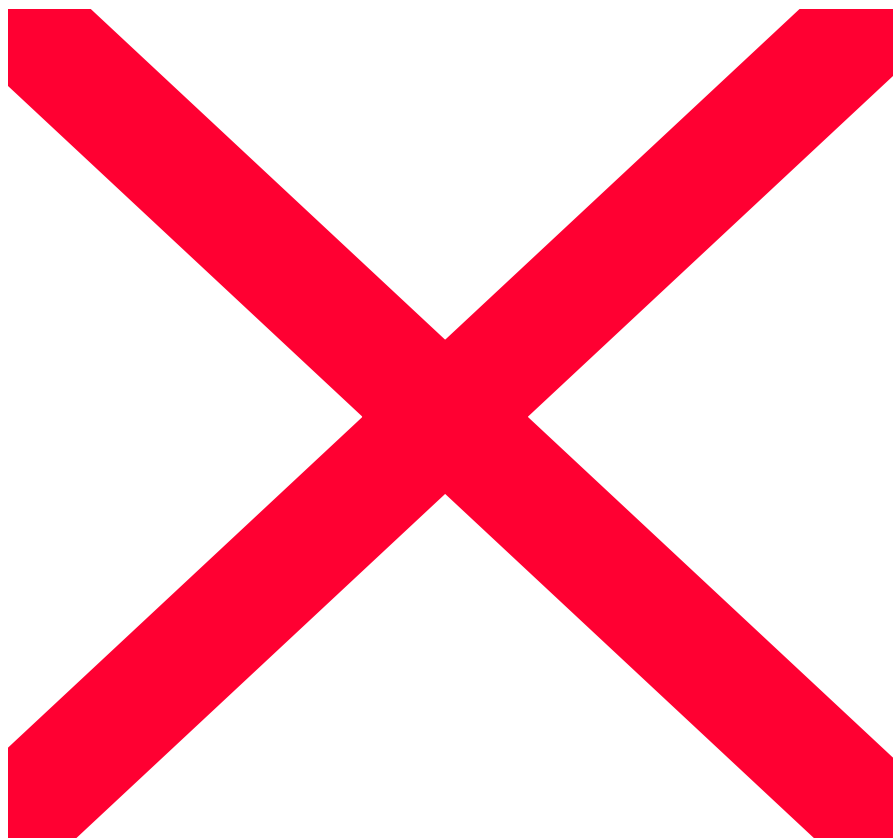
- Metastasize to Fat

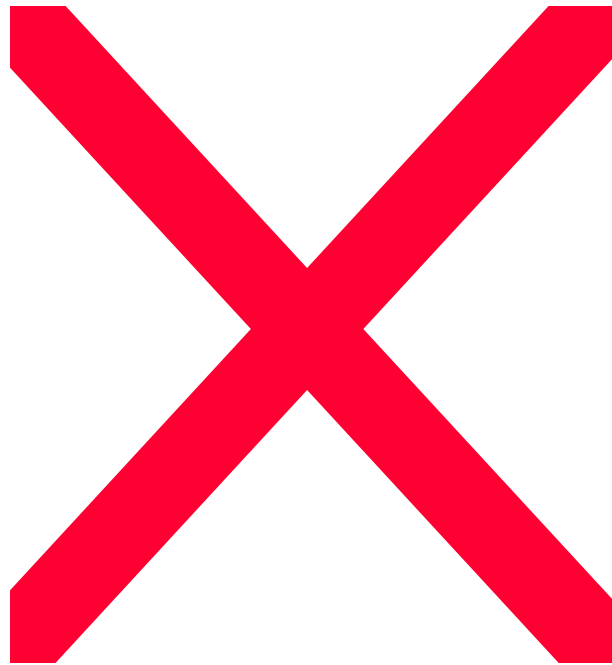
- Pelvis, retroperitoneum, mediastinum

- Sensitive to standard chemotherapy

- Differentiation induced by PPAR-gamma or retinoid-X agonists

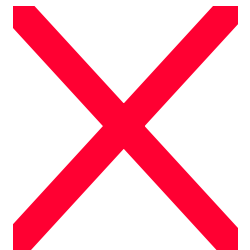
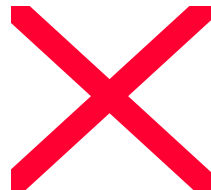
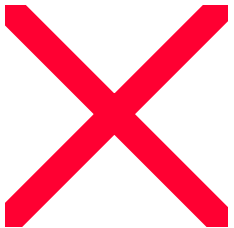
- Translocation a target





Nibs & Mabs

- Imatinib
- Sunitinib
- Sorafenib
- Erlotinib
- Bevacizumab
- Cetuximab
- Trastuzumab

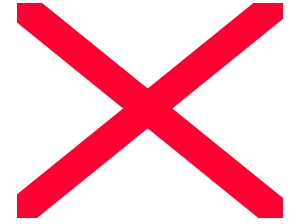


Drug-types and targets

- Mab's: monoclonal antibodies
- Nib's: (tyrosine) kinase inhibitors

Targets

- C-kit: imatinib
- EGF receptor pathways
EGFR1 & EGFR2=HER2/neu
- Angiogenesis: VEGF



Sunitinib (SU11248, Sutent)

FDA Januari 2006: Geregistreerd
voor GIST & niercelcarcinoom

EMEA: recent goedgekeurd

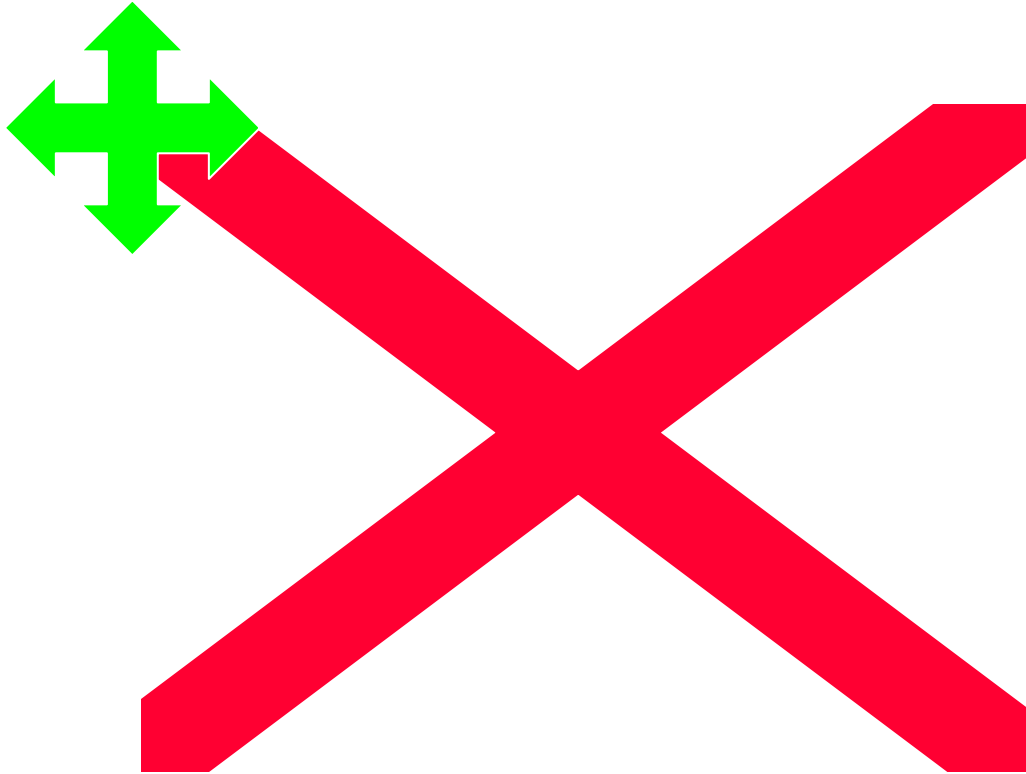
Imatinib en GIST: Problemen

- bloeding
- oedeem
- comedicaatie! (CYP 3A4, 2D6)
- grote OK's

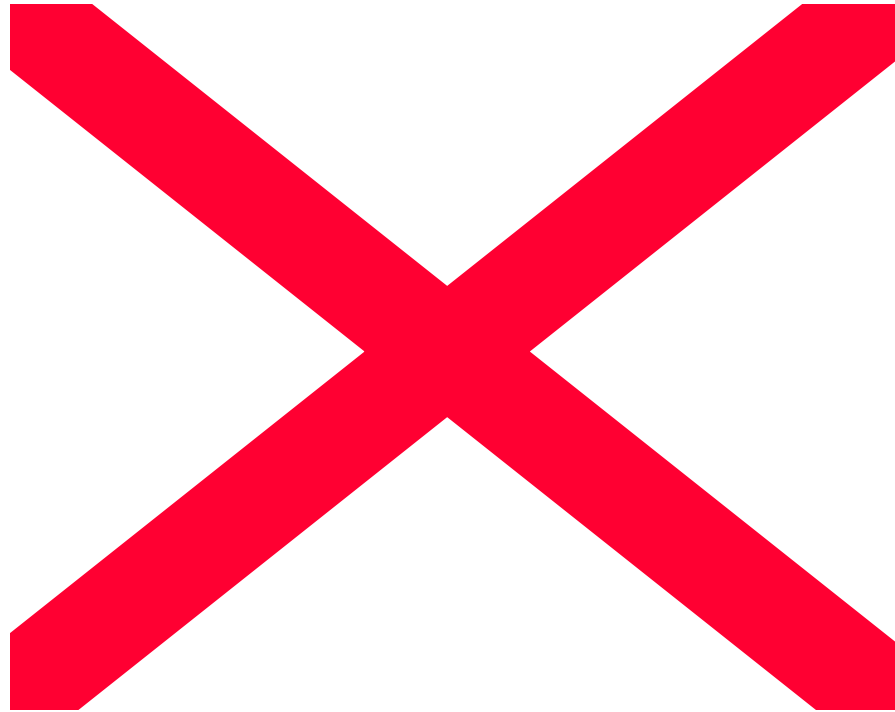
→ behandeling in centrum!

Probleem:

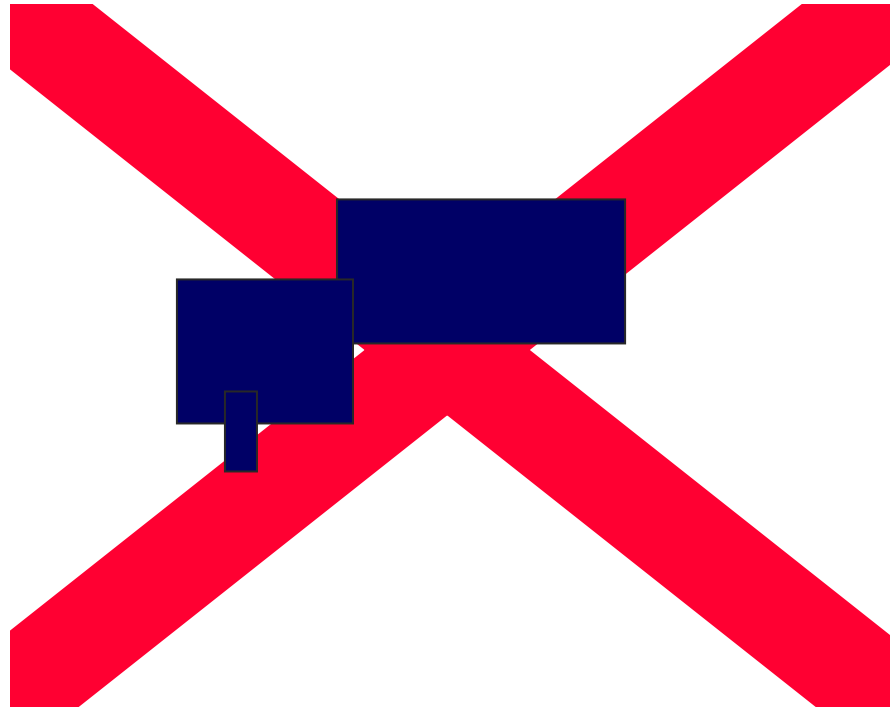
→ resistentie tegen imatinib



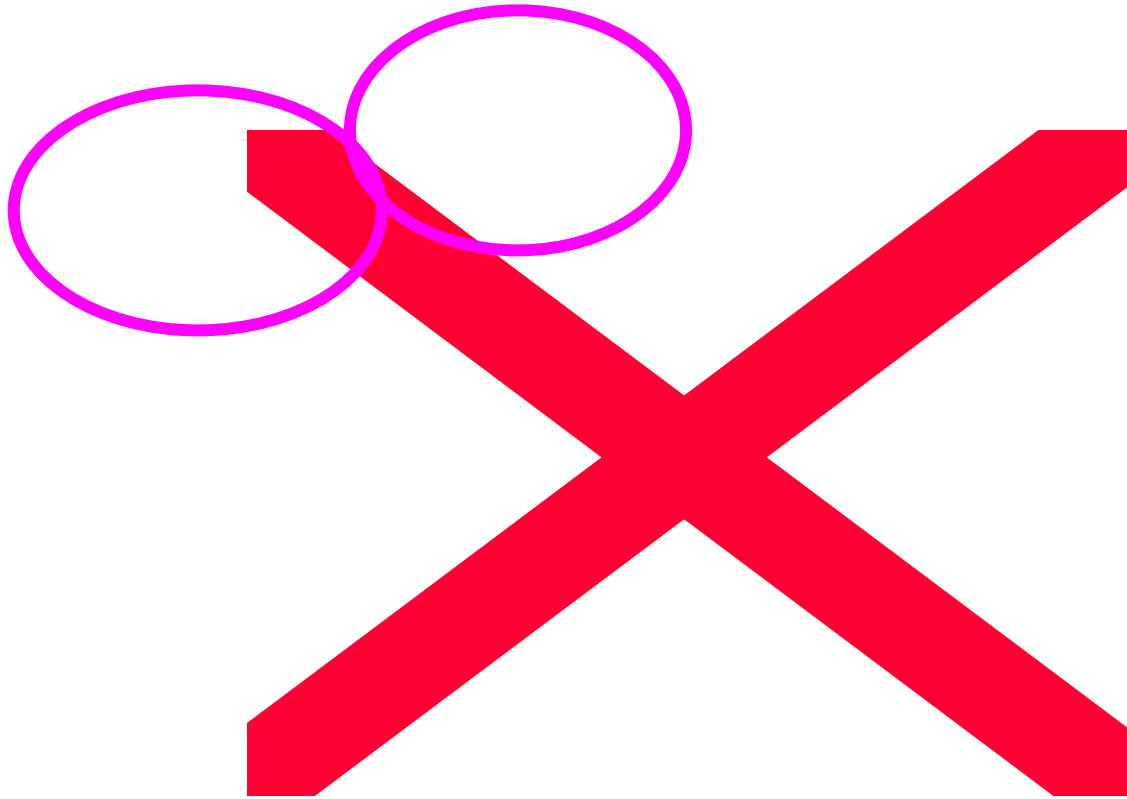
FDG-PET bij GIST met sunitinib als patienten resistent tegen imatinib



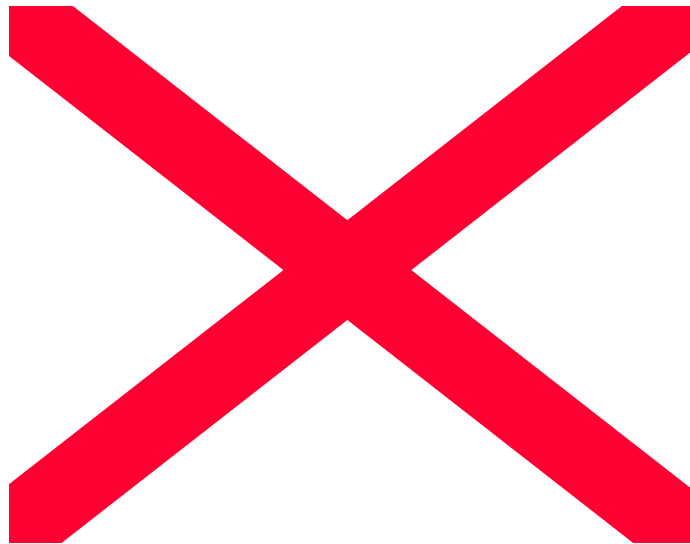
Remming VEGF signaal



Epitheliale receptor (HER) familie



Aangrijpingspunt erlotinib en cetuximab



Reasons for failure

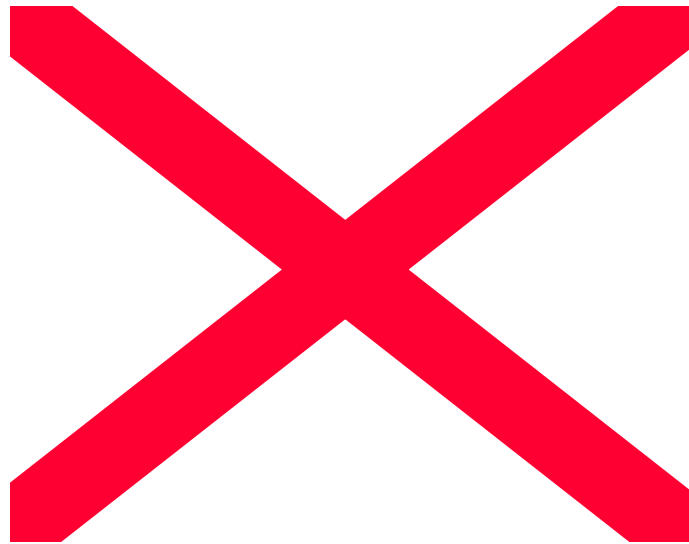
heterogeneity of tumors

- subpopulations
- complex tumors

connections with other pathways

- downstream effectors for each pathway

Budgettaire bijwerkingen



Budgettaire bijwerkingen



You'll never believe it, but....

Nature Medicine 2007

US \$ 100.000,- voor 1 jaar Avastin bij NSCLC

Winst: 2 maanden toename mediane overleving

16% toename van verkoop oncolytica in 2005

US \$ 1.100 M verkoop van Avastin in 2005

US \$ 69 M in stocks in 2005 uitgekeerd aan

Arthur Levinson, Chairman Genentech

Effects of Chromosome Abnormalities

- Net gain or loss of chromosome material
 - whole chromosome or segmental imbalances
- Position effect
 - relocation of sequences
 - gene disruption/disregulation