

# GIST 201: Understanding Your Pathology Report with *KIT* / *PDGFRA* Genotyping



**GSI Patient Summit – Saturday 14 September 2013**

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**Section of Soft Tissue/Sarcoma Pathology**

**Faculty, Sarcoma Research Center**

# ***GIST Pathology: Lecture Overview***

- 1. What happens to my tumor in pathology?**
- 2. What information is in my pathology report?**
- 3. Why is this information there?**
- 4. What is the evidence that the information is useful?**
- 5. What is mutational testing?**

*What happens to my tumor in  
pathology?*

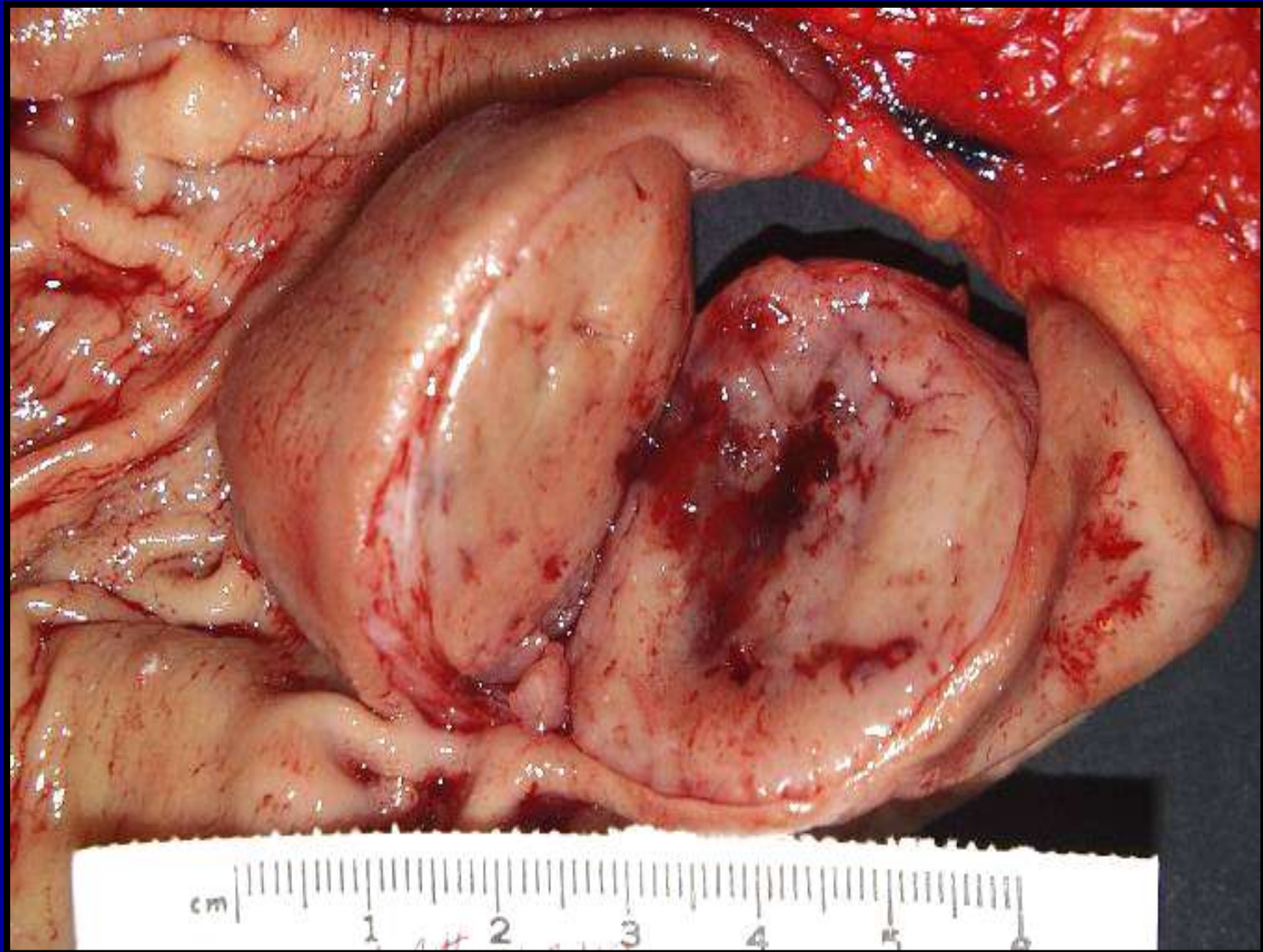


**Tumor sample is received from the OR and logged into computer.**

**Tumor is examined by a pathologist.**



# *GIST - Gross Appearance*



Courtesy of Brian Rubin, Cleveland Clinic



STUDENT NUMBERS

1. J. Adams	11. S. Johnson
2. B. Baker	12. T. King
3. C. Carter	13. U. Lee
4. D. Davis	14. V. Miller
5. E. Evans	15. W. Nelson
6. F. Fisher	16. X. Ortiz
7. G. Garcia	17. Y. Patel
8. H. Harris	18. Z. Quinn
9. I. Ingram	19. A. Reed
10. K. Kelly	20. B. Ross







**Tumor is sampled and placed in plastic cassettes for further processing.**

**Tumor is also given to cytogenetics, tumor bank, molecular diagnosis and electron microscopy when appropriate.**





**The tissue blocks are fixed in formalin and then loaded on a tissue processor overnight.**



Tissue processing is done overnight and utilizes graded treatments of formalin, ethanol, xylene and paraffin.





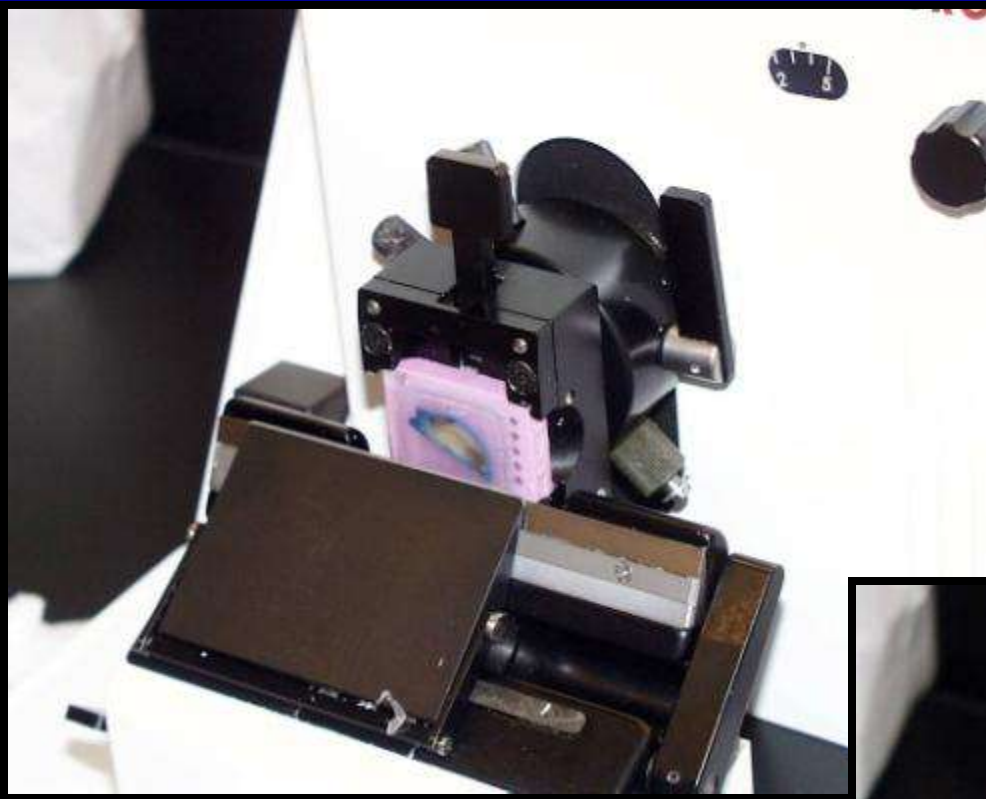
**Blocks are retrieved from the tissue processor.**





**The tissue fragments are embedded in a paraffin mold and cooled – the result being a tissue block.**





**The paraffin-embedded blocks are loaded and cut using a microtome.**



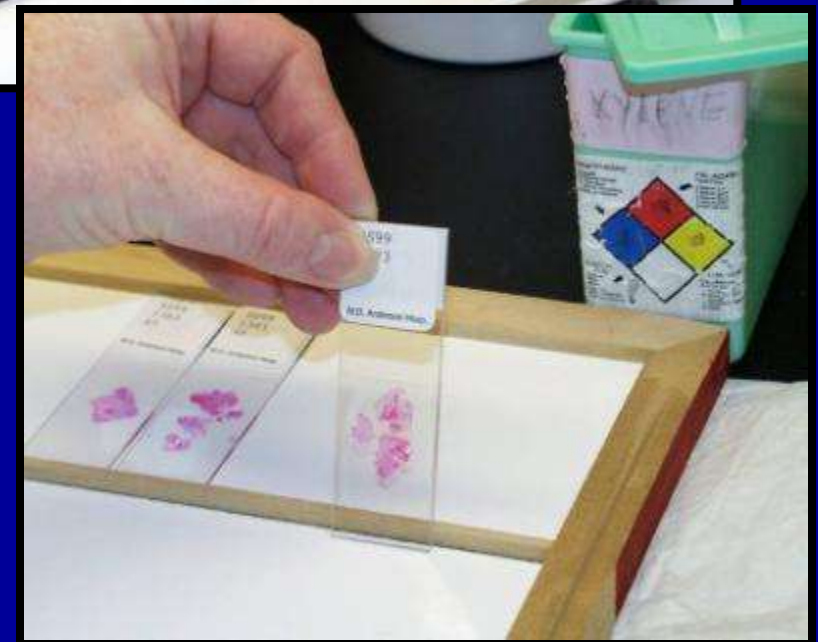


**Tissue paraffin ribbons are placed in a warm waterbath and the picked up on glass slides.**



The unstained slides can be used for H&E, special stains, immuno-histochemistry, molecular studies, etc.





**Most slides are H&E (hemotoxin & eosin) stained, given coverslips, organized and delivered to the proper pathologist.**





**Additional unstained slides  
can be cut at a later time.**





**After final diagnosis, both slides and the paraffin blocks from which they are cut are cataloged and stored for future use.**



***What information is in my  
pathology report?***



cap

## Protocol for the Examination of Specimens From Patients With Gastrointestinal Stromal Tumor (GIST)

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Based on AJCC/UICC TNM, 7<sup>th</sup> edition  
Protocol web posting date: June 2012

### Procedures

- Biopsy
- Resection

### Authors

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For the Members of the Cancer Committee, College of American Pathologists

\* Denotes primary author. † Denotes senior author. All other contributing authors are listed alphabetically.

## Surgical Pathology Cancer Case Summary

Protocol web posting date: June 2012

### GASTROINTESTINAL STROMAL TUMOR (GIST): Resection

Select a single response unless otherwise indicated.

#### Procedure

Excisional biopsy

Resection

Specify type (eg, partial gastrectomy): \_\_\_\_\_

Metastasectomy

Other (specify): \_\_\_\_\_

Not specified

#### Tumor Site

Specify (if known): \_\_\_\_\_

Not specified

#### Tumor Size

Greatest dimension: \_\_\_ cm

+ Additional dimensions: \_\_\_ x \_\_\_ cm

Cannot be determined (see "Comment")

#### Tumor Focality

Unifocal

Multifocal

Specify number of tumors: \_\_\_\_\_

Specify size of tumors: \_\_\_\_\_

#### GIST Subtype

Spindle cell

Epithelioid

Mixed

Other (specify): \_\_\_\_\_

**Mitotic Rate**

Specify: \_\_\_ /50 HPF

*Note: The required total count of mitoses is per 5 mm<sup>2</sup> on the glass slide section. With the use of older model microscopes, 50 HPF is equivalent to 5 mm<sup>2</sup>. Most modern microscopes with wider 40X lenses/fields require only 20 HPF to embrace 5 mm<sup>2</sup>. If necessary please measure field of view to accurately determine actual number of fields required to be counted on individual microscopes to count 5 mm<sup>2</sup>.*

**+ Necrosis**

+ \_\_\_ Not identified

+ \_\_\_ Present

+ Extent: \_\_\_%

+ \_\_\_ Cannot be determined

**Histologic Grade (Note B)**

- GX: Grade cannot be assessed
- G1: Low grade; mitotic rate  $\leq 5/50$  HPF
- G2: High grade; mitotic rate  $> 5/50$  HPF

**Risk Assessment (Note C)**

- None
- Very low risk
- Low risk
- Intermediate risk
- High risk
- Overtly malignant/metastatic
- Cannot be determined

**Margins**

- Cannot be assessed
- Negative for GIST  
Distance of tumor from closest margin: \_\_\_ mm or \_\_\_ cm
- Margin(s) positive for GIST  
Specify margin(s): \_\_\_\_\_

**Pathologic Staging (pTNM) (Note G)**

TNM Descriptors (required only if applicable) (select all that apply)

- m (multiple)
- r (recurrent)
- y (posttreatment)

**Primary Tumor (pT)**

- pTX: Primary tumor cannot be assessed
- pT0: No evidence for primary tumor
- pT1: Tumor 2 cm or less
- pT2: Tumor more than 2 cm but not more than 5 cm
- pT3: Tumor more than 5 cm but not more than 10 cm
- pT4: Tumor more than 10 cm in greatest dimension

**Regional Lymph Nodes (pN) (Note D)**

- Not applicable
- pN0: No regional lymph node metastasis
- pN1: Regional lymph node metastasis

**Distant Metastasis (pM) (Note D)**

- Not applicable
- pM1: Distant metastasis  
+ Specify site(s), if known: \_\_\_\_\_

**+ Additional Pathologic Findings**

+ Specify: \_\_\_\_\_

**Ancillary Studies (select all that apply) (Note E)**

Immunohistochemical Studies

- KIT (CD117)
  - Positive
  - Negative
- Others (specify): \_\_\_\_\_
- Not performed

Molecular Genetic Studies (eg, KIT or PDGFRA mutational analysis)

- Submitted for analysis; results pending
- Performed, see separate report: \_\_\_\_\_
- Performed
  - Specify method(s) and results: \_\_\_\_\_
- Not performed

**Preresection Treatment (select all that apply)**

- No therapy
- Previous biopsy or surgery
  - Specify: \_\_\_\_\_
- Systemic therapy performed
  - Specify type: \_\_\_\_\_
- Therapy performed, type not specified
- Unknown

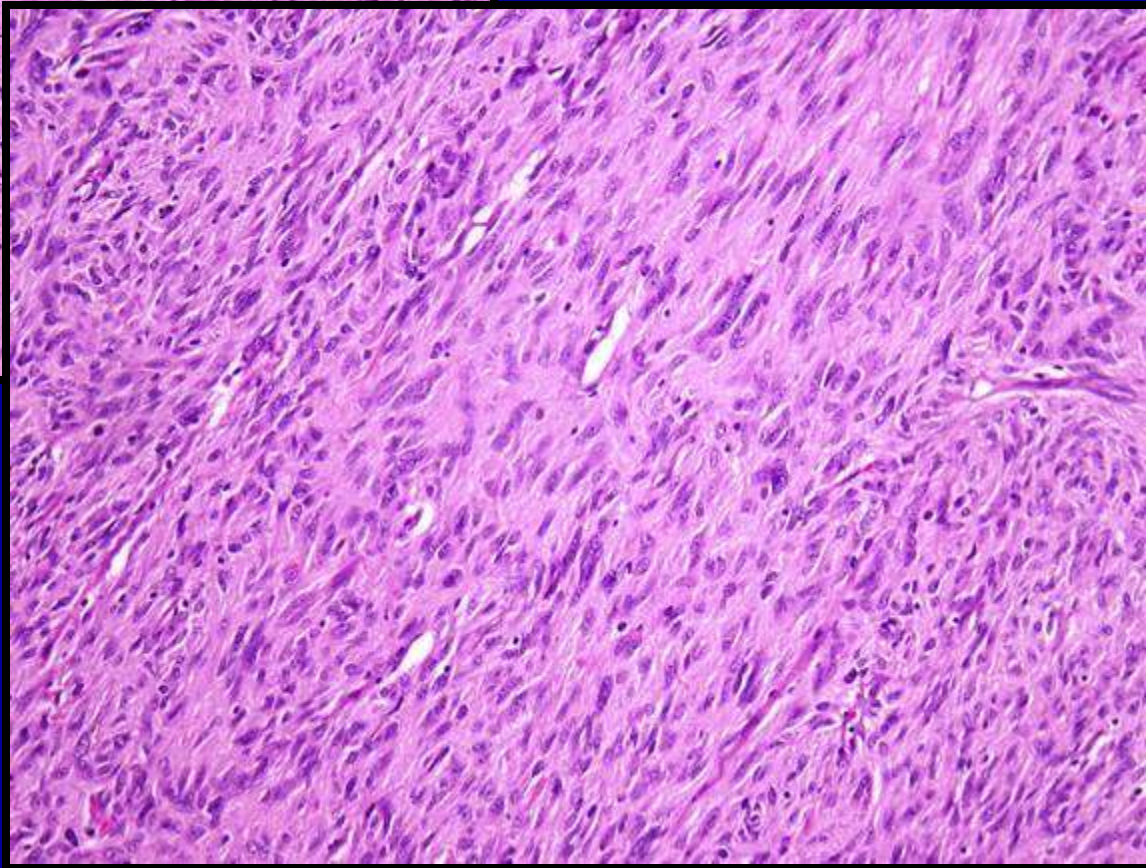
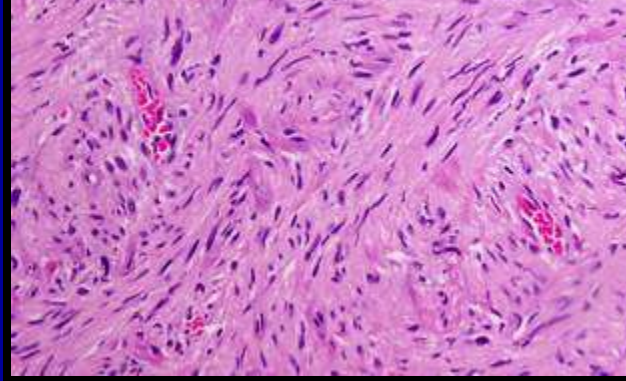
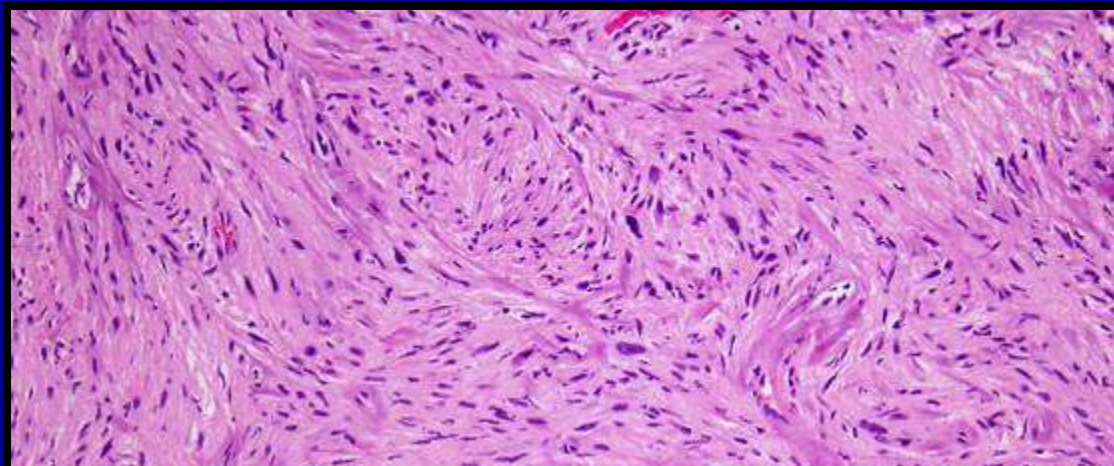
**+ Treatment Effect (Note F)**

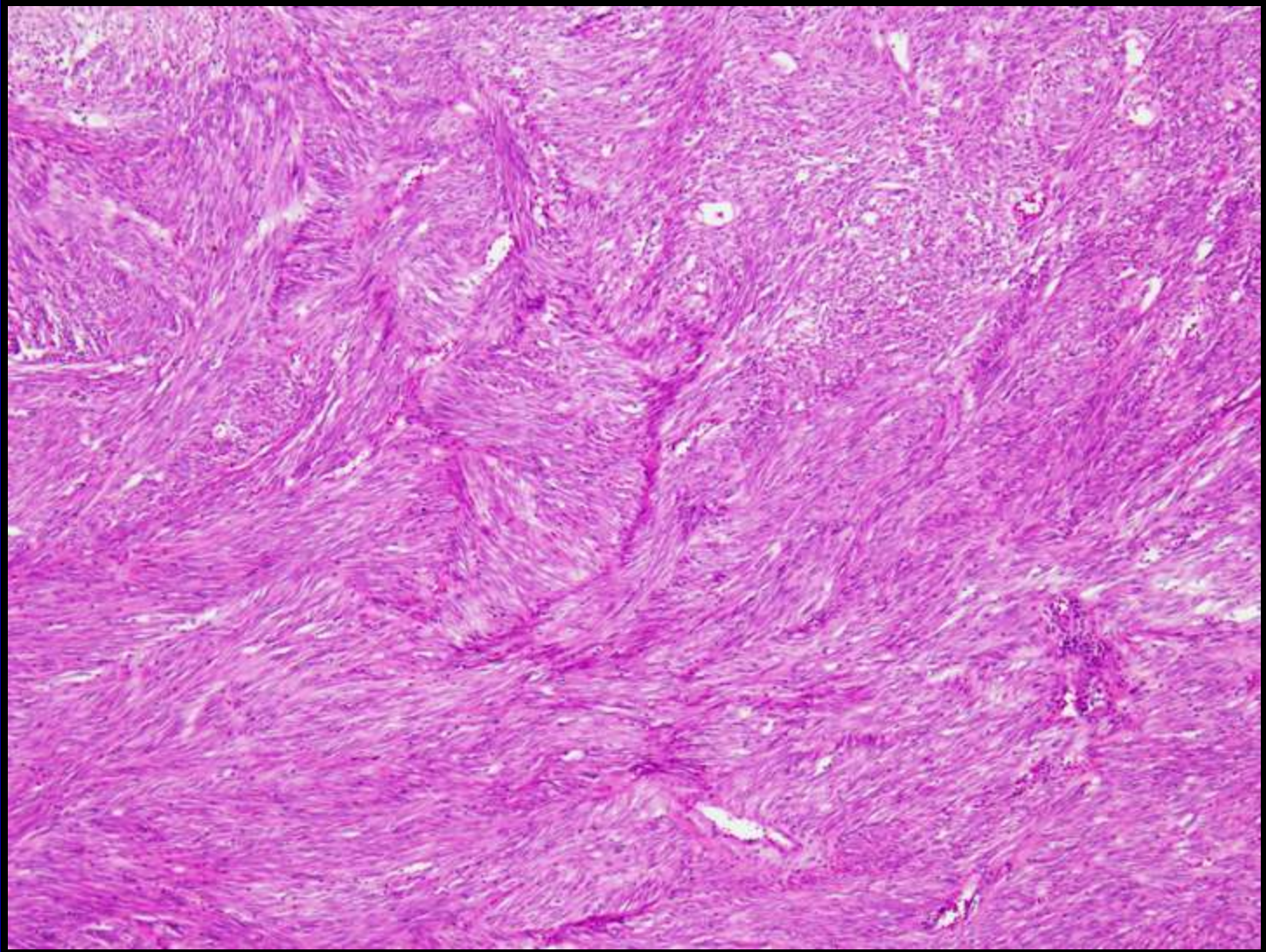
+ Specify percentage of viable tumor: \_\_\_%

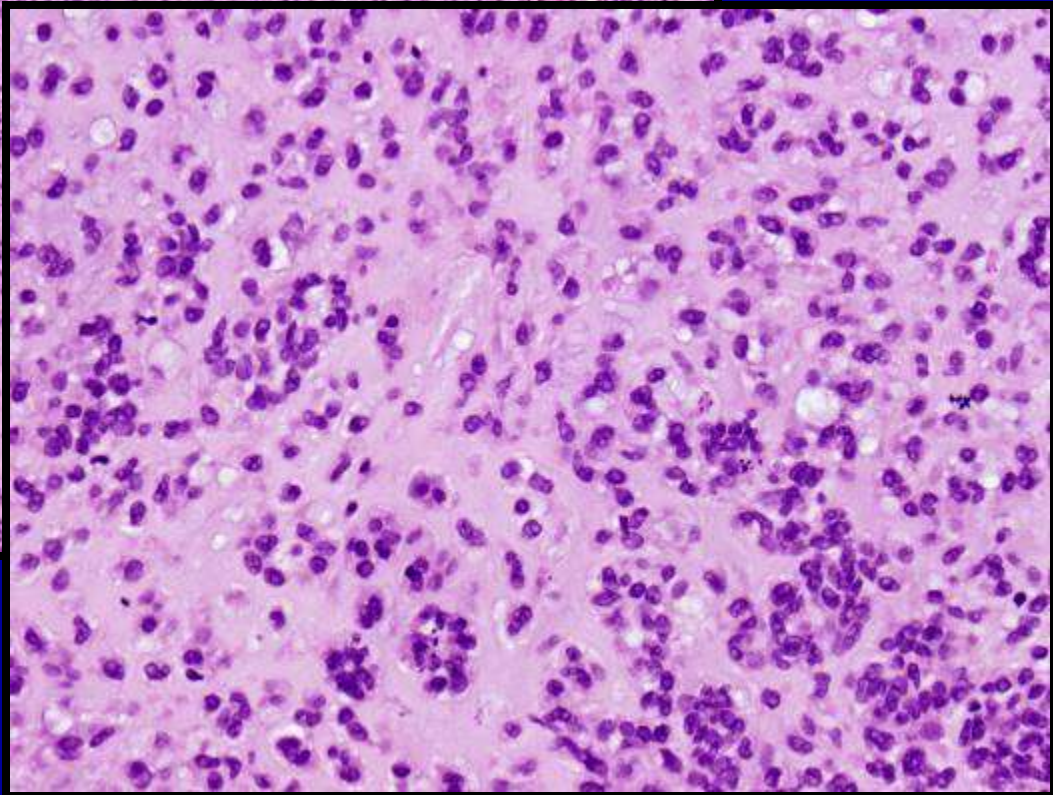
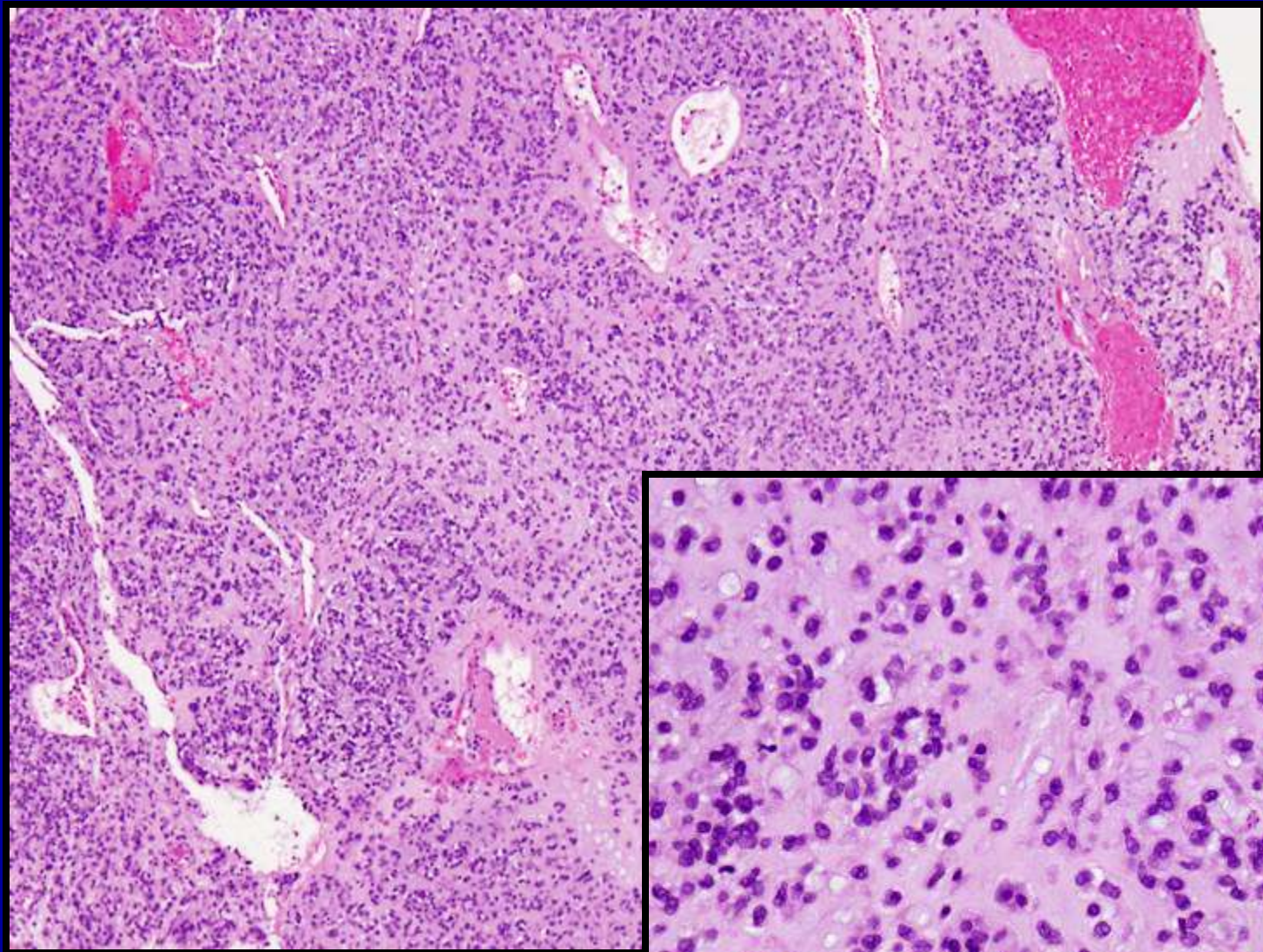
**+ Comment(s)**

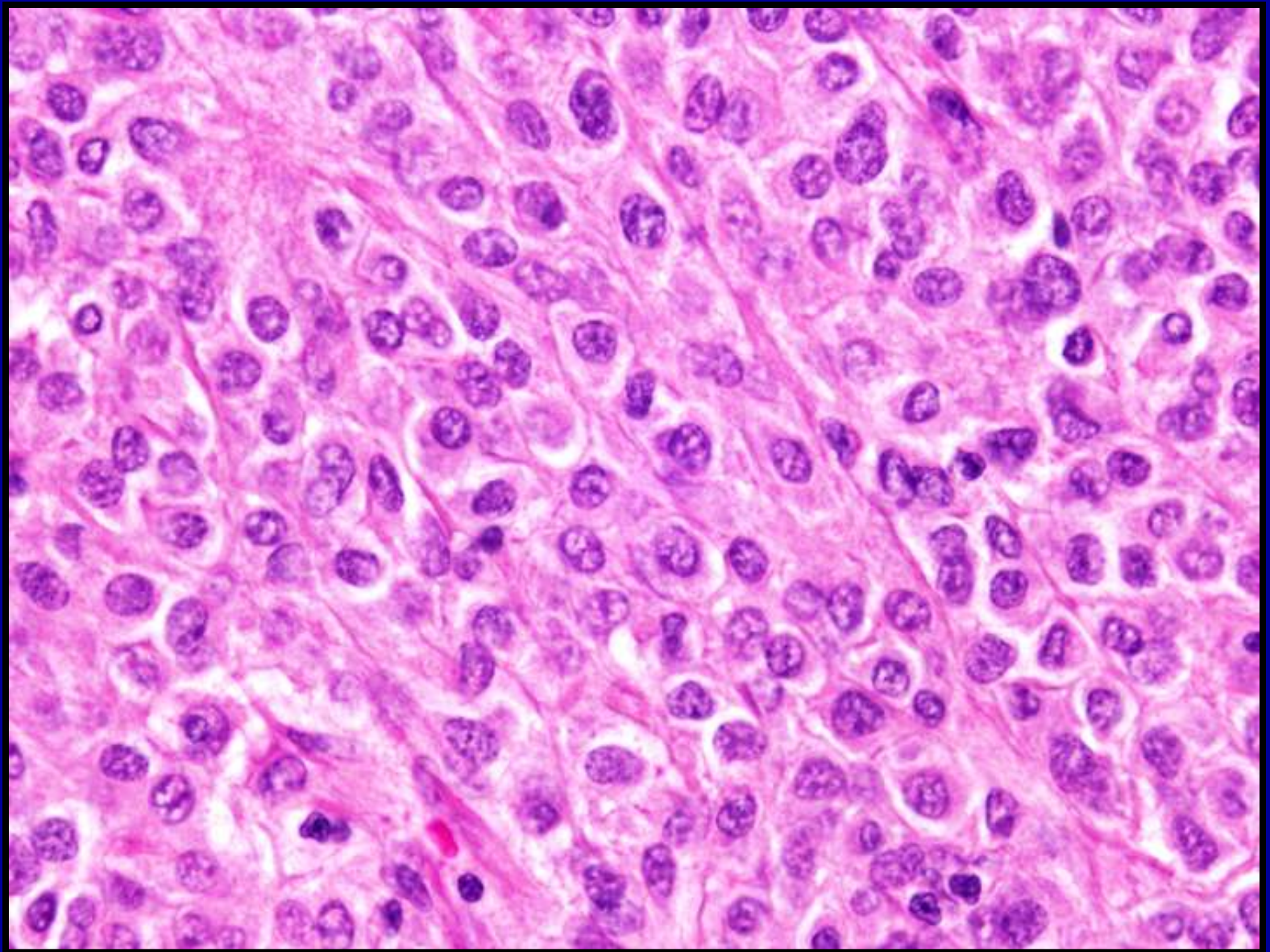


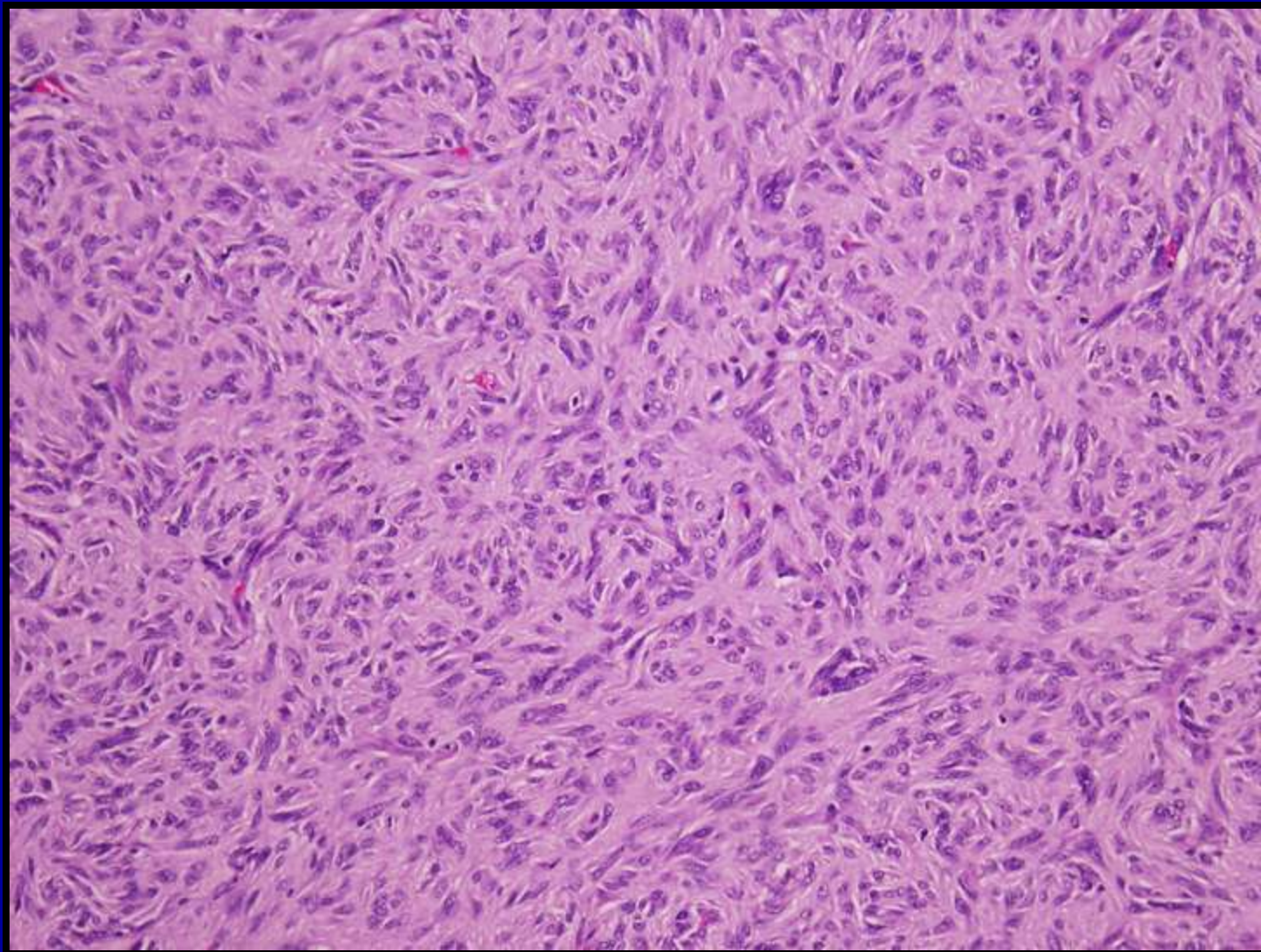
# *The many faces of GIST*

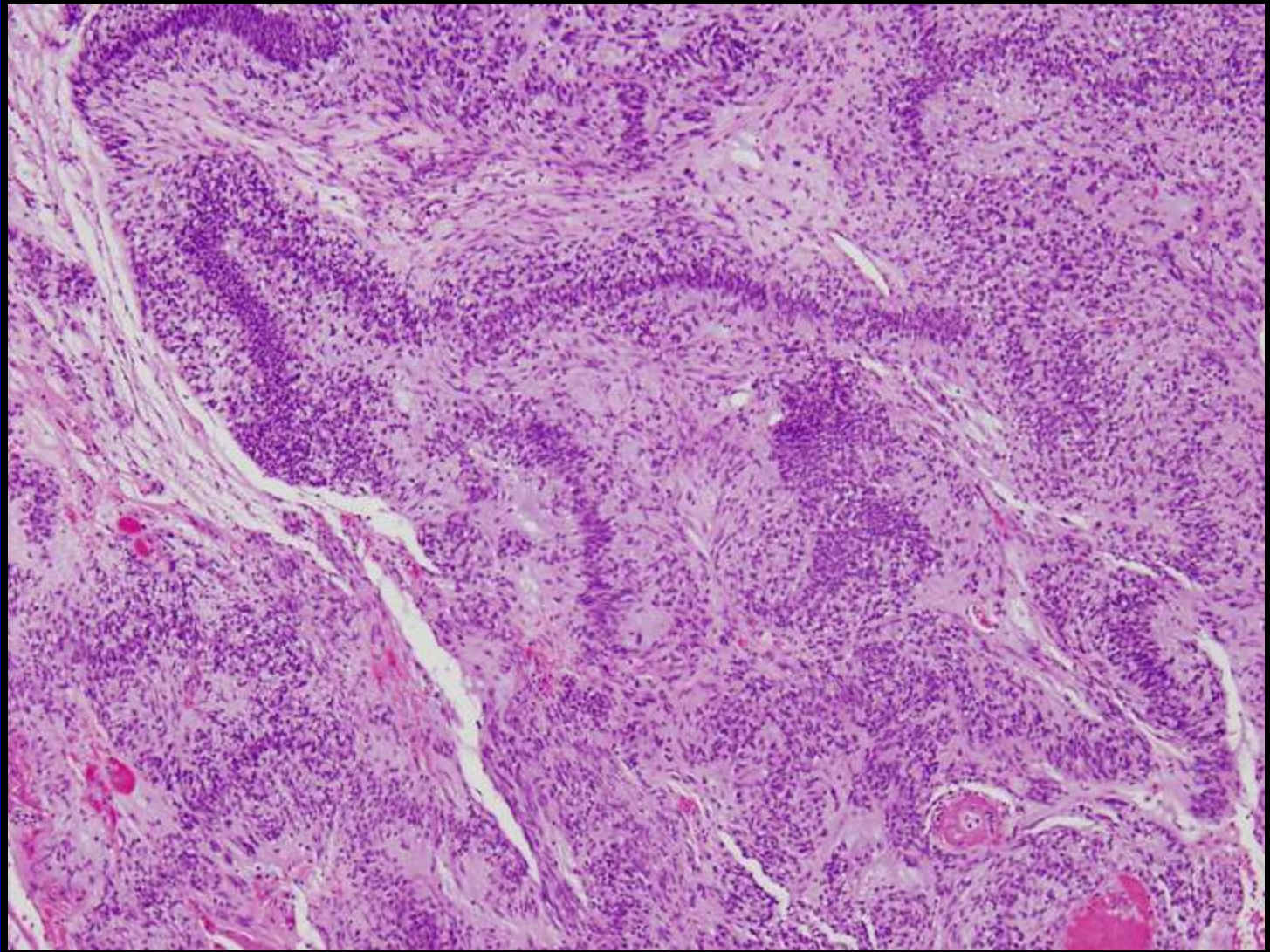


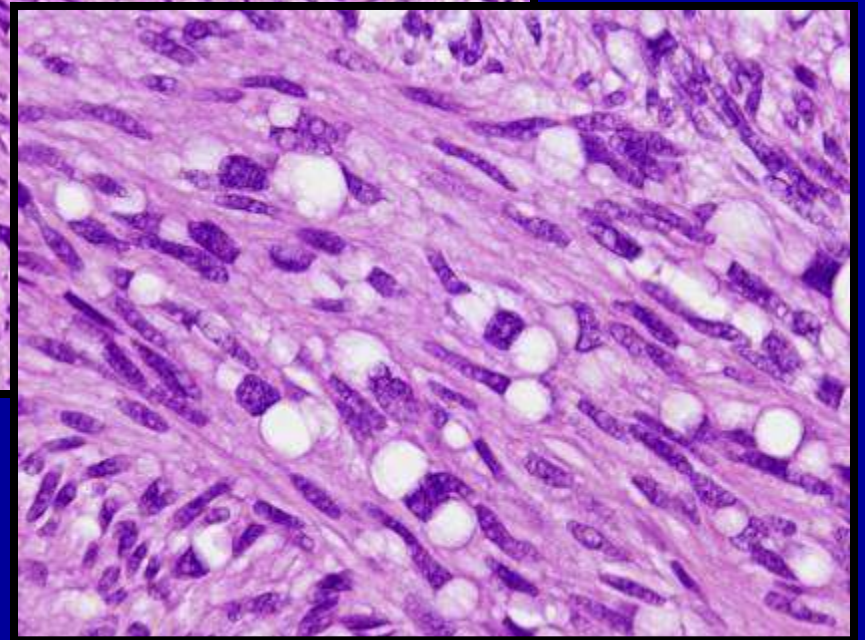
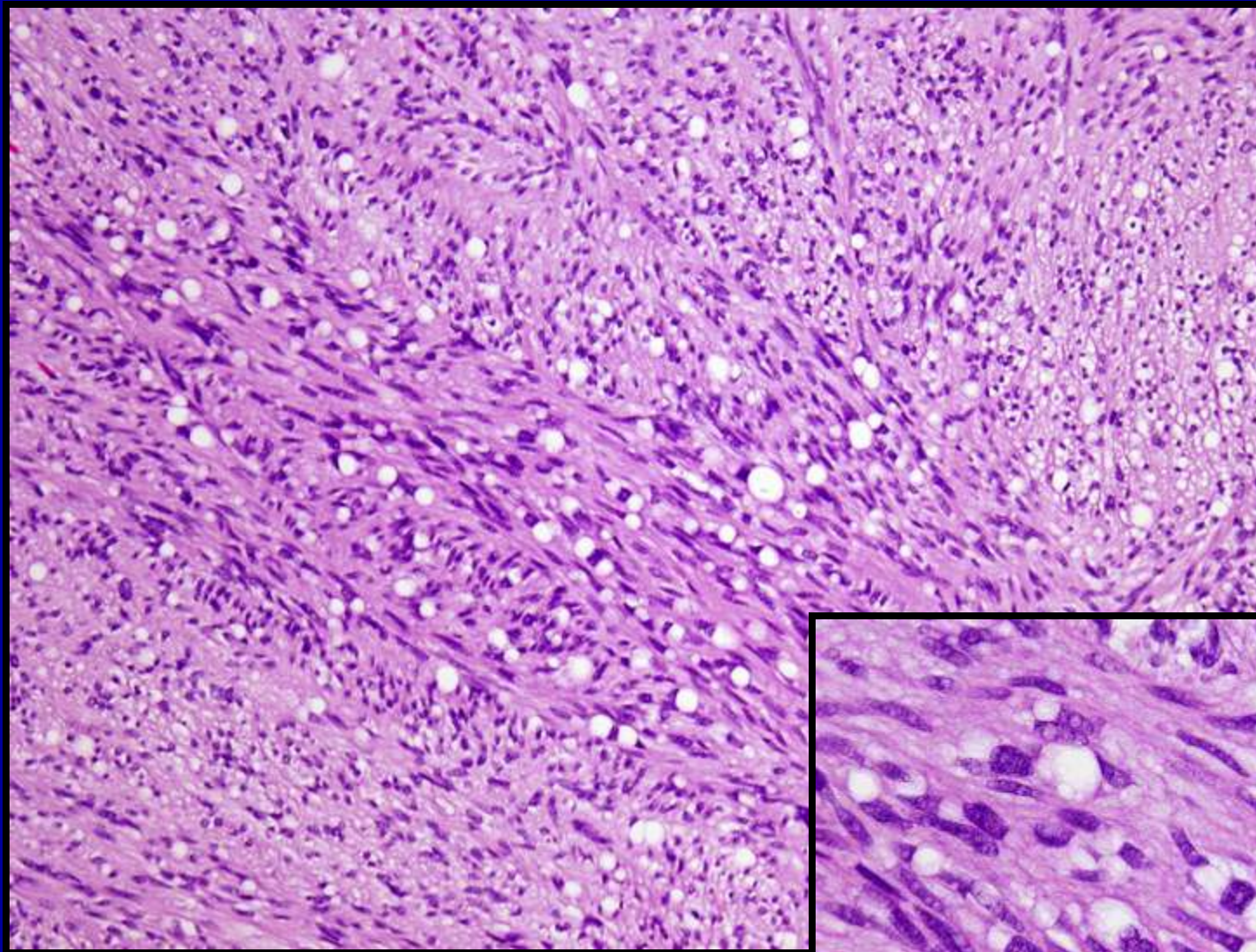




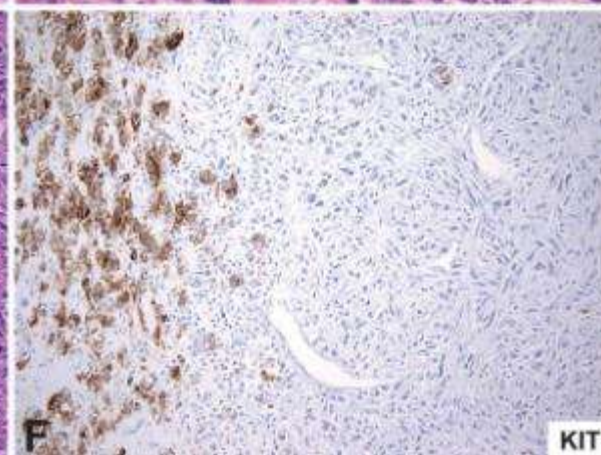
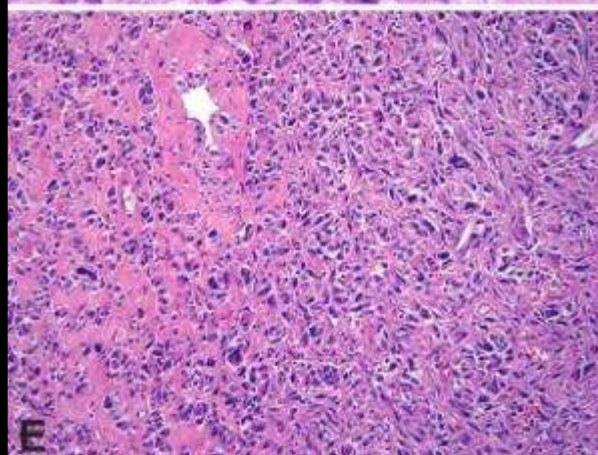
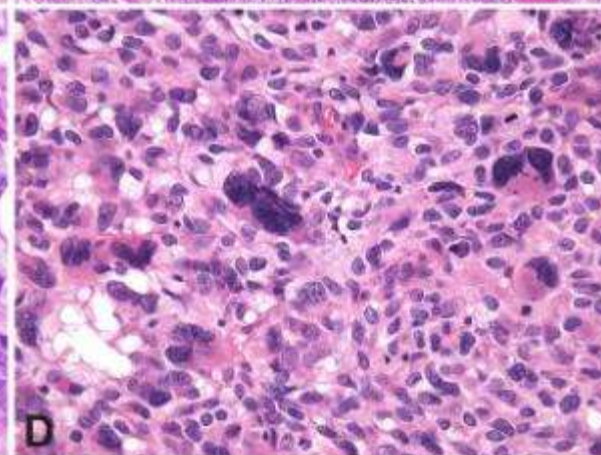
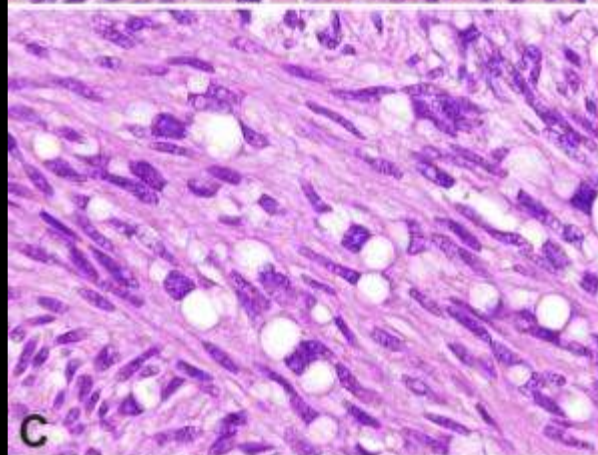
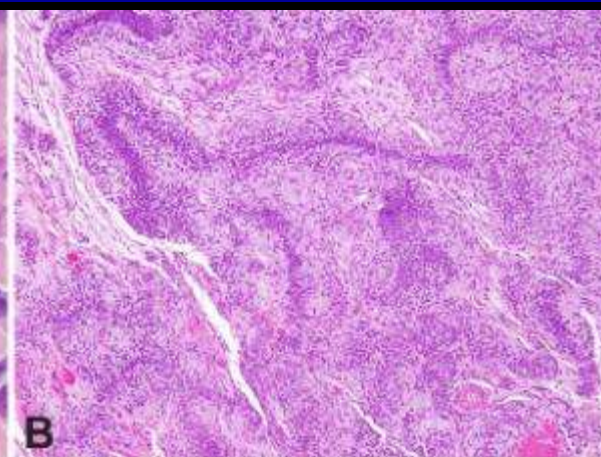
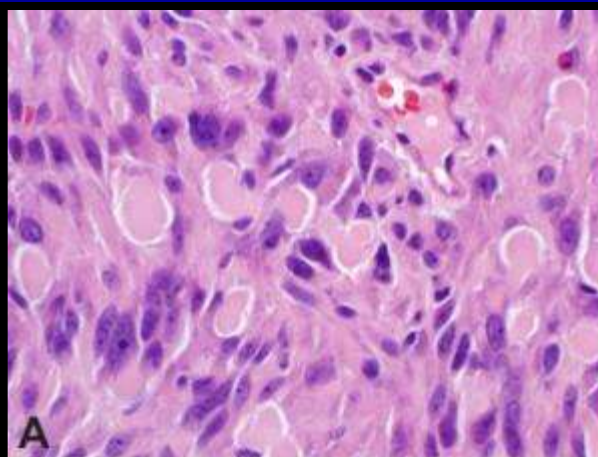


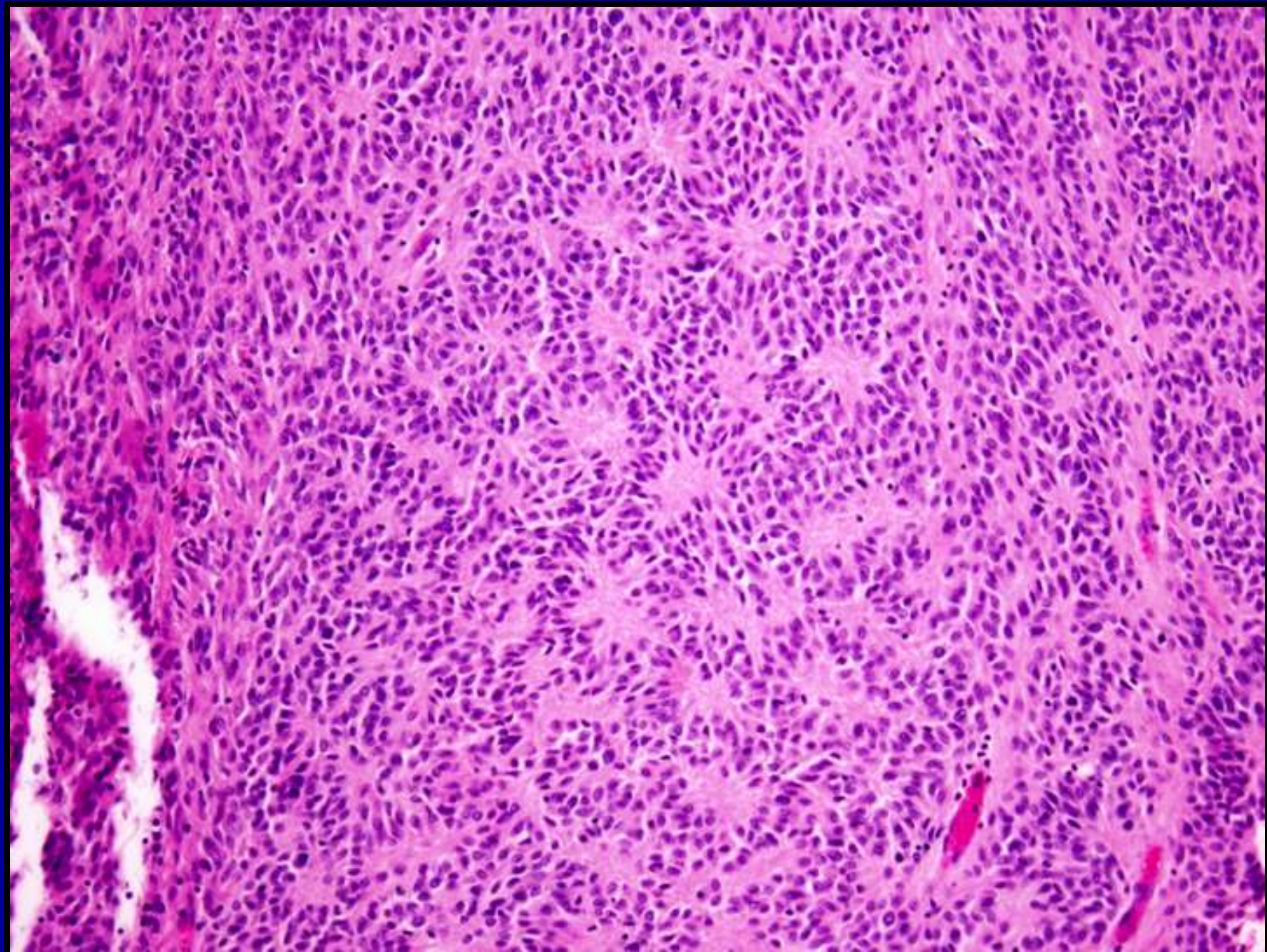













# Immunohistochemical Profile of GIST

H&E	CD117 (KIT)	CD34	Smooth muscle actin	S100 protein	Desmin	Pan-keratin
	95%	70%	30%	5%	2%	<1%
	+	+	+	+	+	+

**KIT (CD117) +ve (95%)**

**CD34 +ve (70%)**

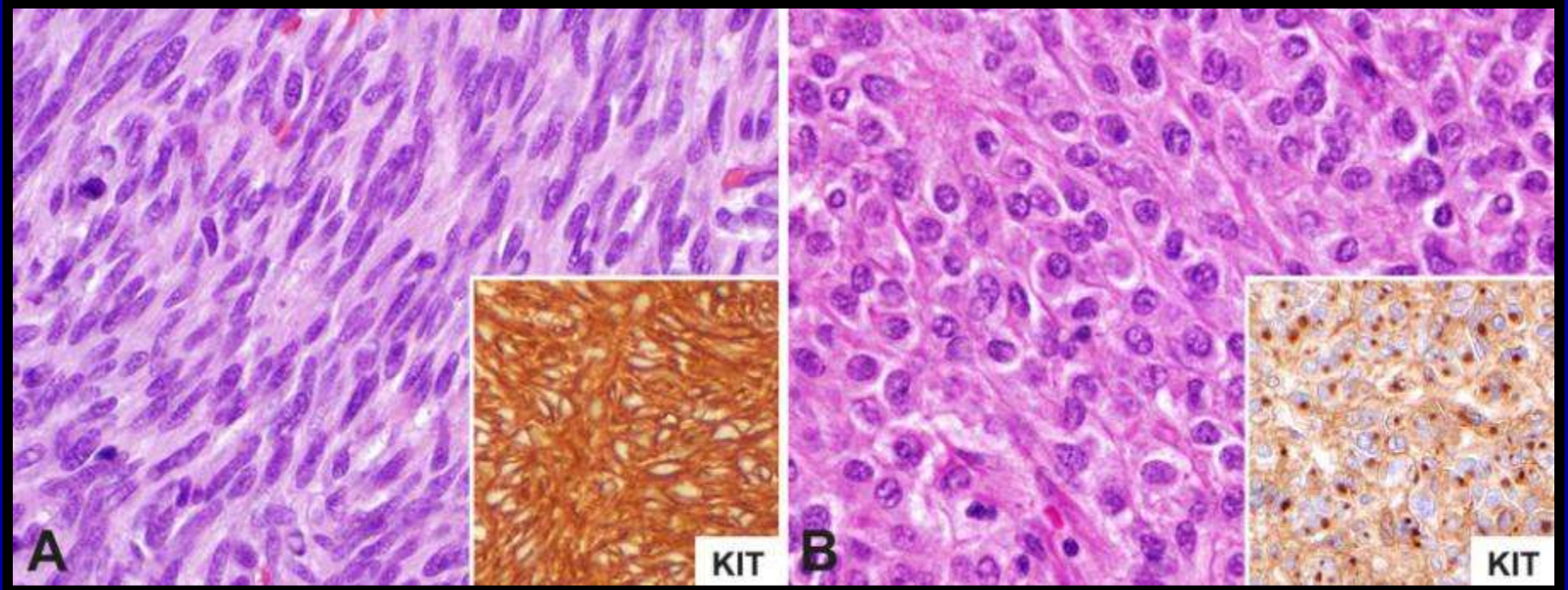
**SMA +ve (30-40%)**

**Desmin -ve**

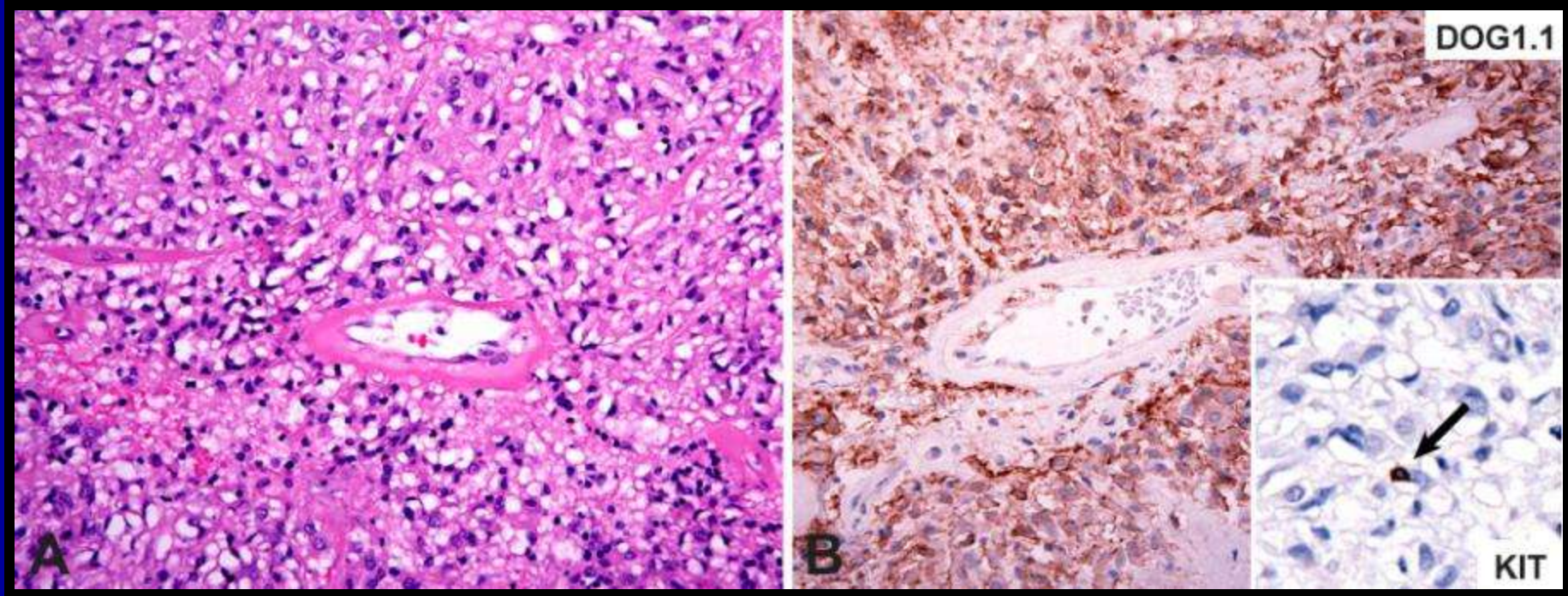
**S-100 protein -ve**

**Keratin -ve**

# *KIT immunoreactivity in GIST*



# *KIT-negative GIST*



# ***Risk assessment in GIST***

# *GIST – Prognostic Factors*

Size

Mitotic Rate

Anatomic Location

Pleomorphism

Cellularity

Necrosis

**Mucosal Invasion**

Proliferation Markers (Ki-67, Mib-1, PCNA, etc)

DNA Flow Cytometry

Image Analysis

Nuclear Organizer Regions

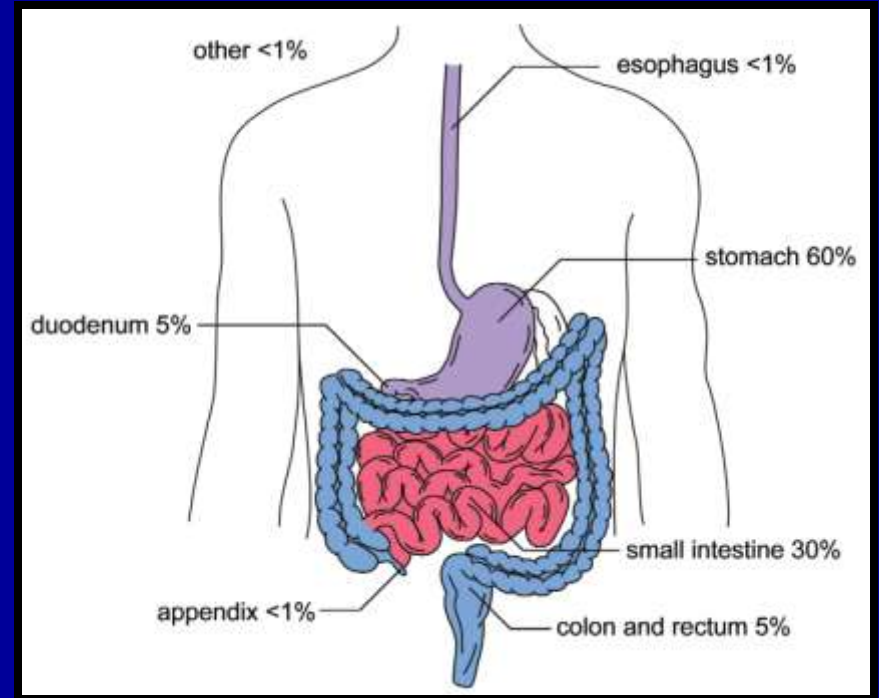
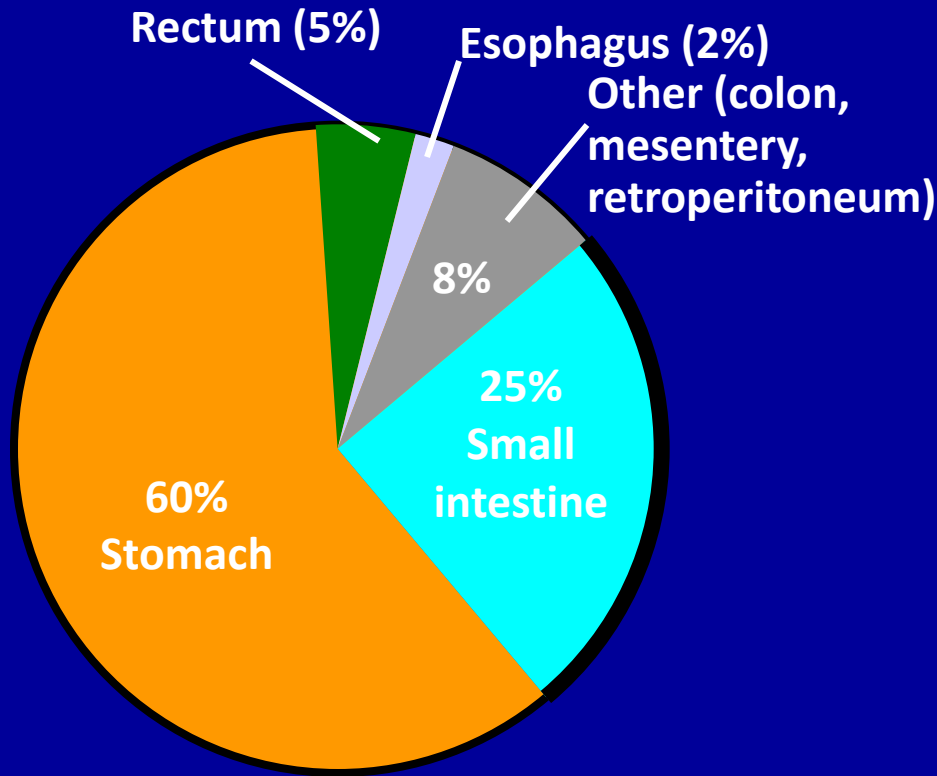
**Problem** – Small GISTs without mitoses  
can metastasize!

# *NIH Consensus Risk Assessment*

	<b>Size</b>	<b>Mitotic Count</b>
<b>Very Low Risk</b>	< 2 cm	< 5/50 HPF
<b>Low Risk</b>	2-5 cm	< 5/50 HPF
<b>Intermediate Risk</b>	< 5 cm	6-10/50 HPF
	5-10 cm	< 5/50 HPF
<b>High Risk</b>	> 5 cm	> 5/50 HPF
	> 10 cm	Any Mitotic Rate
	<b>Any Size</b>	<b>&gt; 10/50 HPF</b>



# GIST: Sites of Involvement

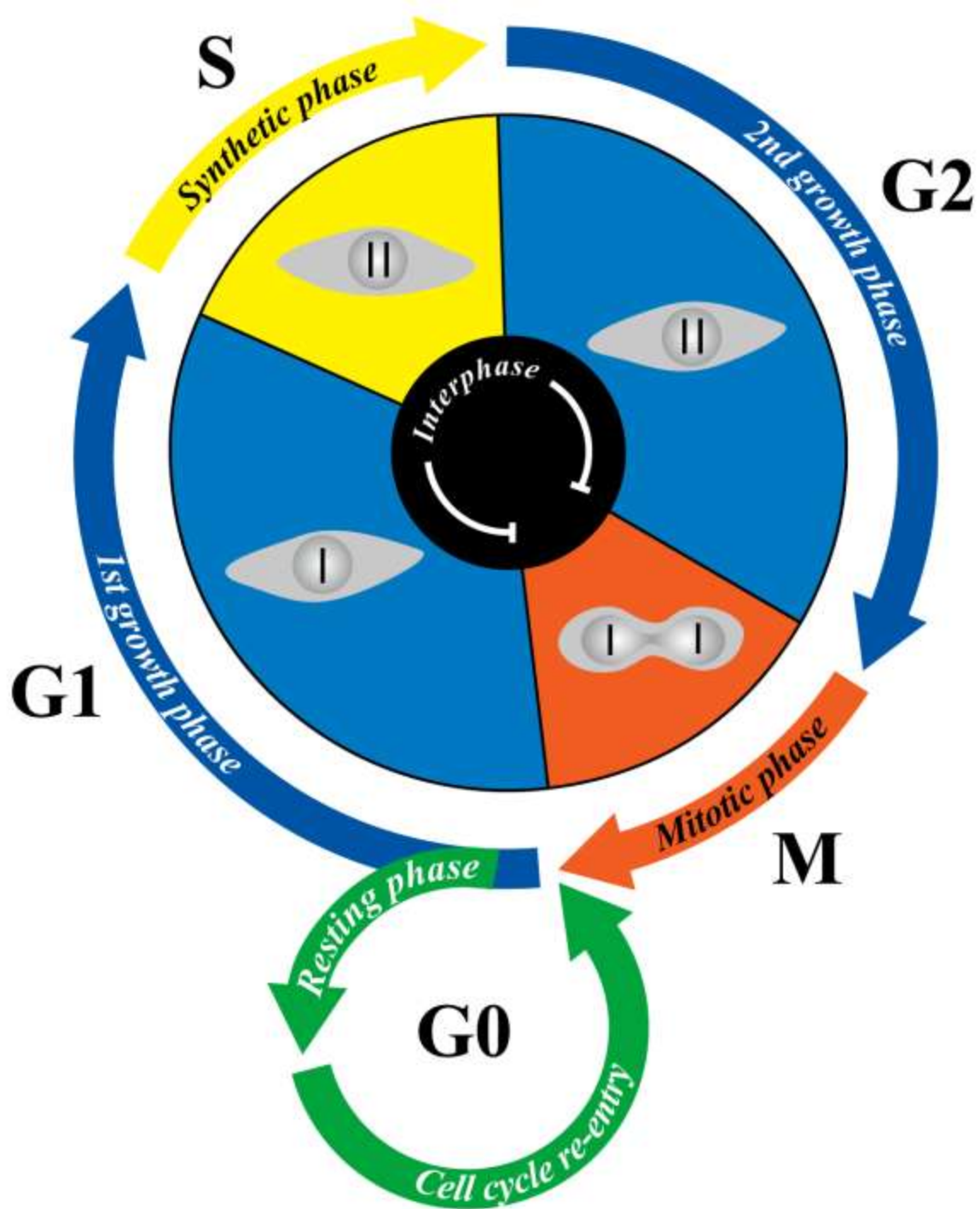


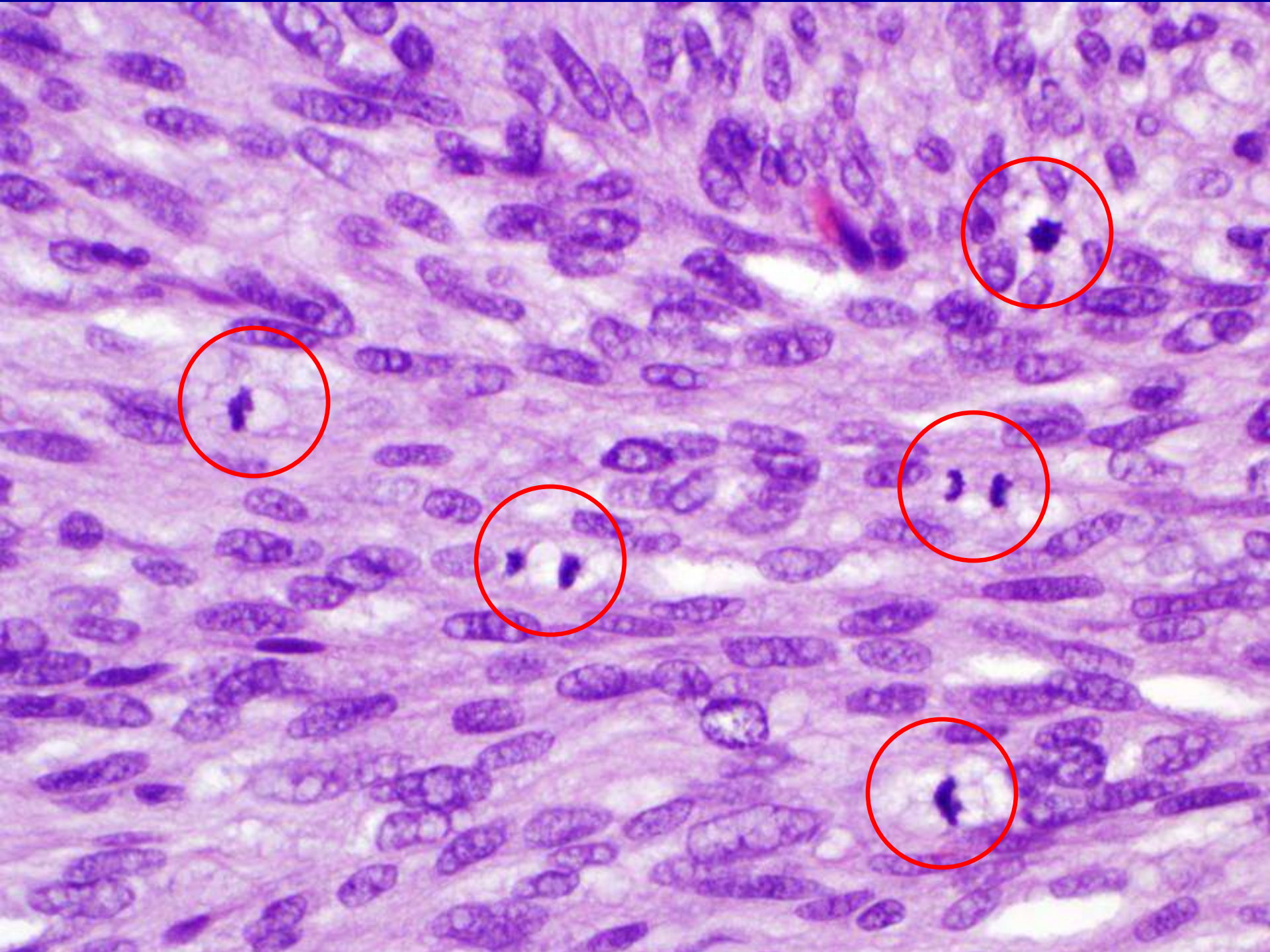
**Omentum, mesentery, pelvis and retroperitoneum = EGIST (<1%)**

# 2007/2010 NCCN GIST Risk Assessment Guidelines\*\*\*

Tumor	Parameters	Risk of	Progressive	Disease# (%)	
	Size	Gastric	Duodenum	Jejunum/Ileum	Rectum
Mitotic	≤ 2 cm	None (0%)	None (0%)	None (0%)	None (0%)
Index	> 2 ≤ 5 cm	Very low (1.9%)	Low (8.3%)	Low (4.3%)	Low (8.5%)
≤ 5 per 50 hpf	> 5 ≤ 10 cm	Low (3.6%)	(Insuff. data)	Moderate (24%)	(Insuff. data)
	> 10 cm	Moderate (10%)	High (34%)	High (52%)	High (57%)
Mitotic	≤ 2 cm	None*	(Insuff. data)	High*	High (54%)
Index	> 2 ≤ 5 cm	Moderate (16%)	High (50%)	High (73%)	High (52%)
> 5 per 50 hpf	> 5 ≤ 10 cm	High (55%)	(Insuff. data)	High (85%)	(Insuff. data)
	> 10 cm	High (86%)	High (86%)	High (90%)	High (71%)

\*\*\* Modified from Miettinen & Lasota, *Semin Diagn Pathol*, 2006 by Dr. Chris Corless, OHSU  
 Data based on long-term follow-up of 1055 gastric, 629 small intestinal, 144 duodenal and 111 rectal GIST





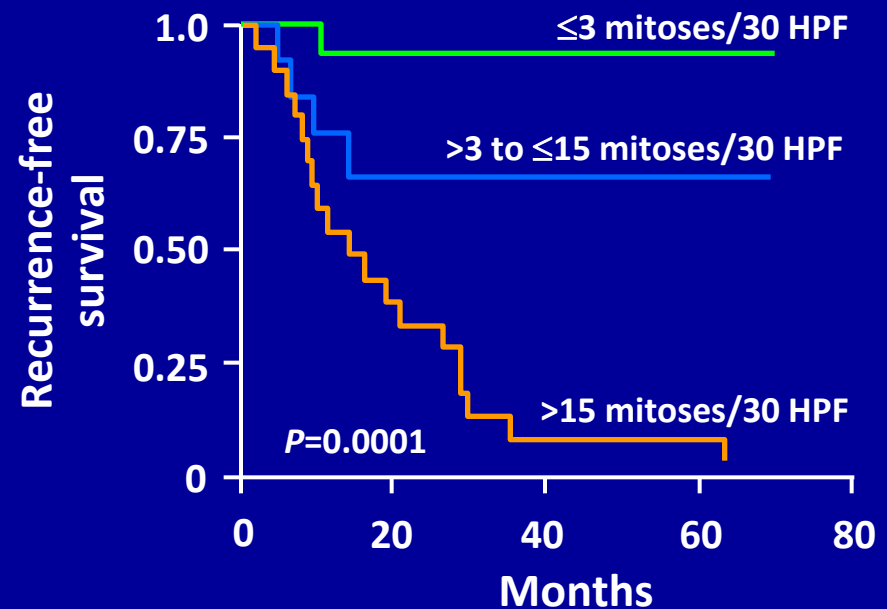
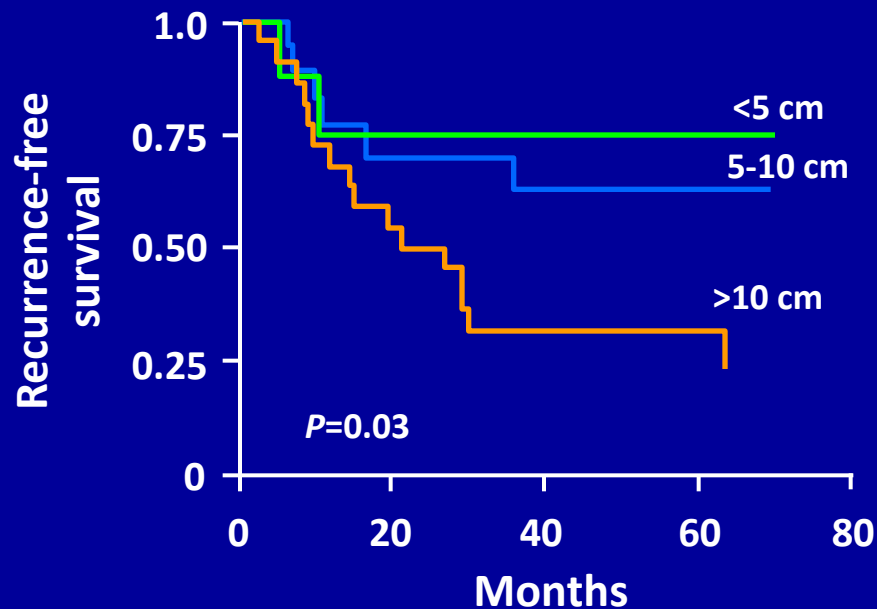
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\*\*\* Modified from Miettinen & Lasota, *Semin Diagn Pathol*, 2006 by Dr. Chris Corless, OHSU  
 Data based on long-term follow-up of 1055 gastric, 629 small intestinal, 144 duodenal and 111 rectal GIST

# GIST - Recurrence-Free Survival Following Surgical Treatment of Primary GIST

- Recurrence-free survival is predicted by tumor size and mitotic index



# FNCLCC Grading

- All three numbers are summated to determine degree of differentiation

Grade 1 : 2-3

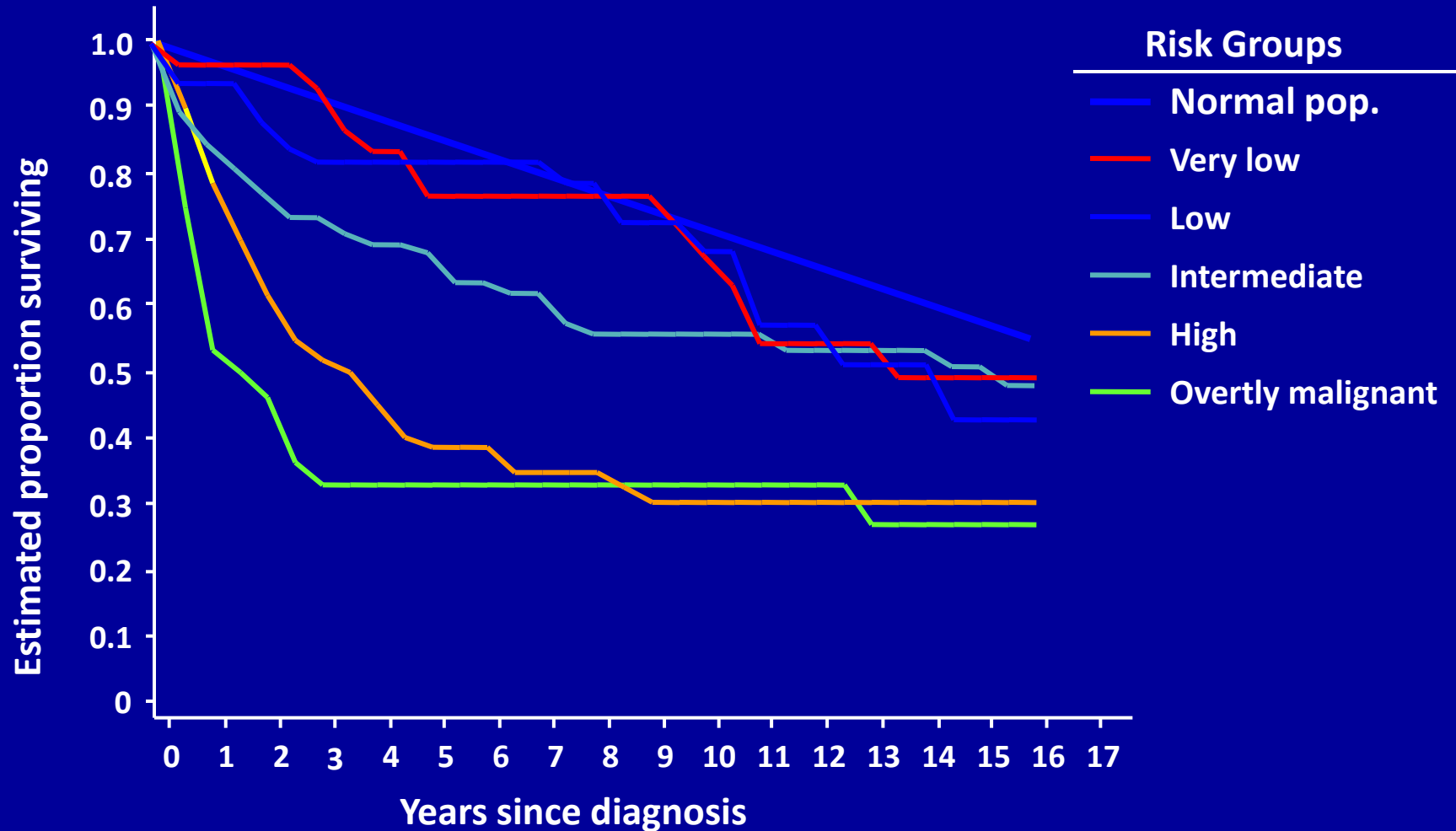
Grade 2 : 4-5

Grade 3 : 6-8

- Proven to correlated well with survival

- Mitotic Count. In the most mitotically active area, ten successive high-power fields (at 400x magnification=0.1734 mm<sup>2</sup>) using a 40x objective.
  - 1 0-9 mitoses per 10 HPFs
  - 2 10-19 mitoses per 10 HPFs
  - 3 >20 mitoses per 10 HPFs
- Tumor necrosis. Evaluated on gross examination and validated with histological sections
  - 0 No tumor necrosis
  - 1 <50% tumor necrosis
  - 2 >50% tumor necrosis
- Degree of Differentiation. 1-3

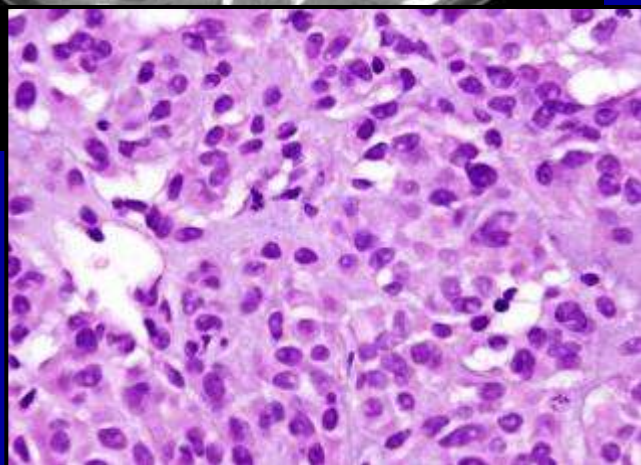
# GIST - Overall Survival by Risk Group



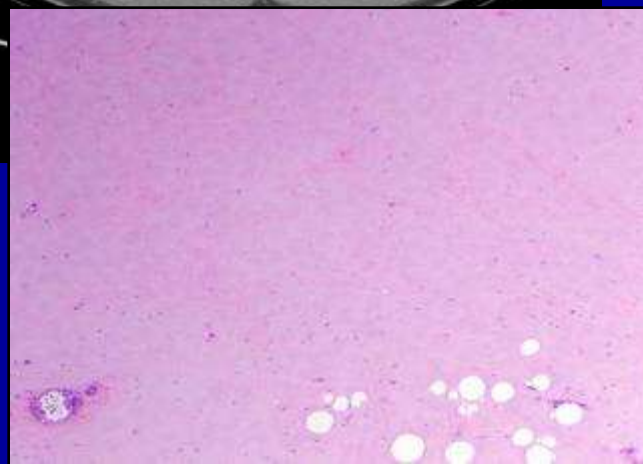


***Treatment can cause big changes.***

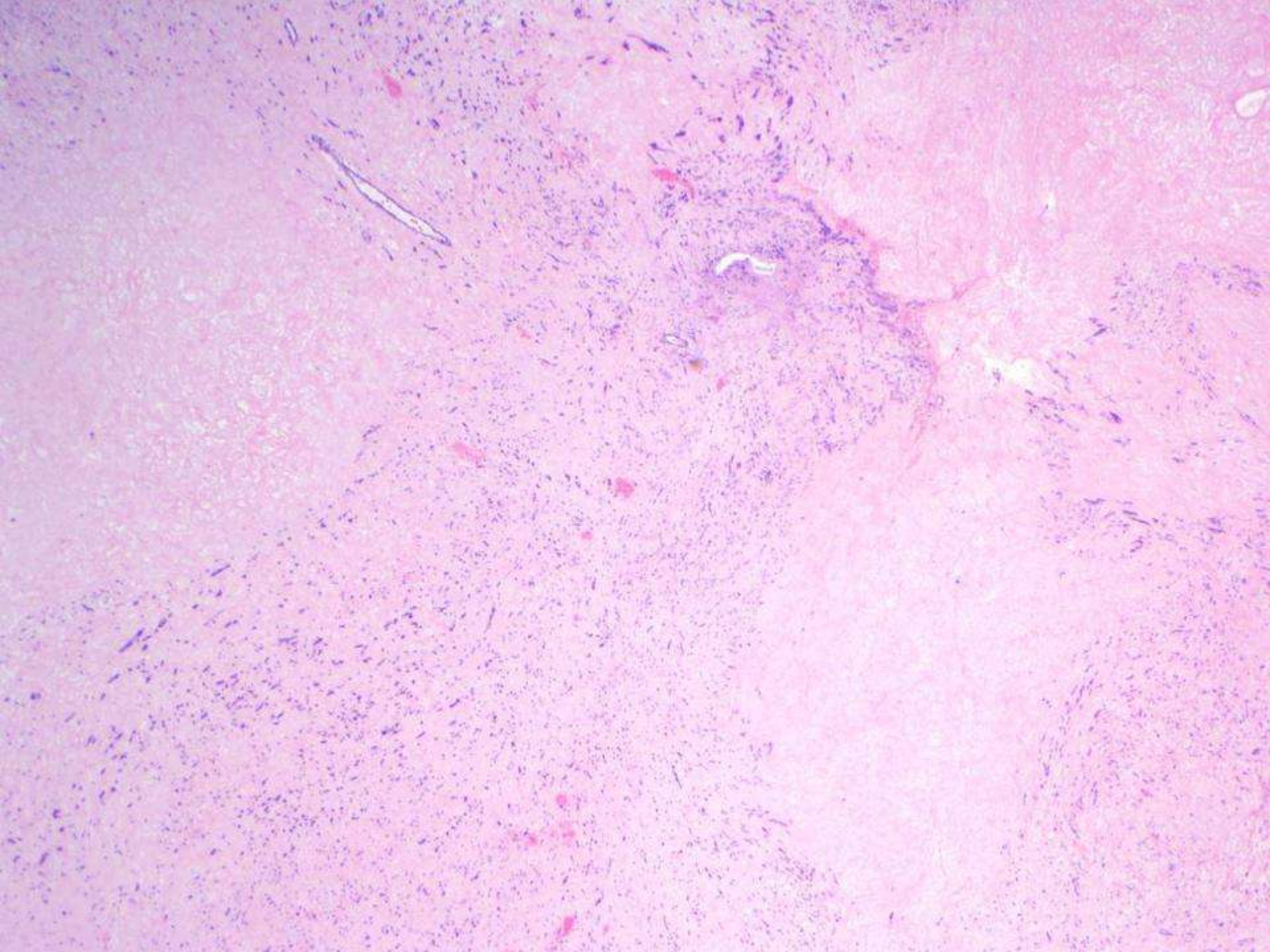
# *Treatment effect*



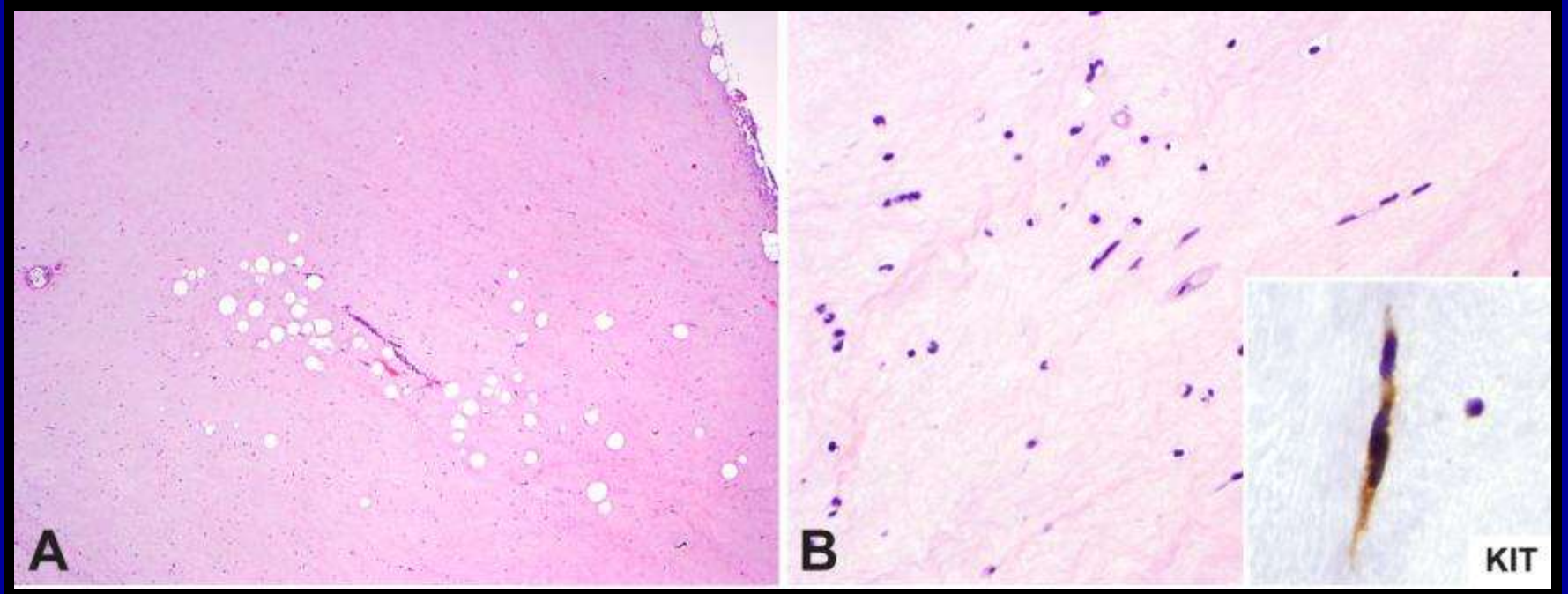
**Pre-Imatinib**



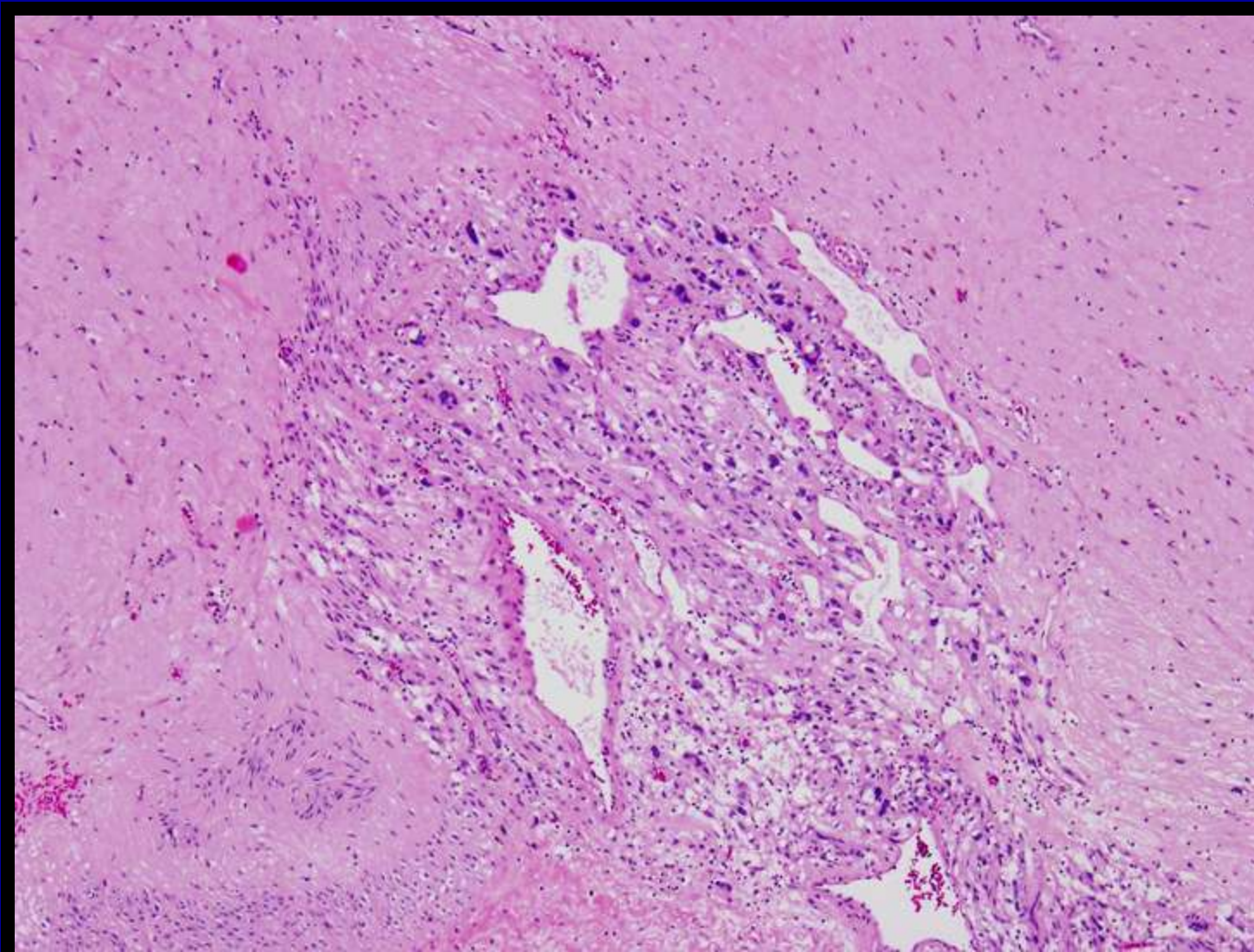
**Post-Imatinib (8 weeks therapy)**

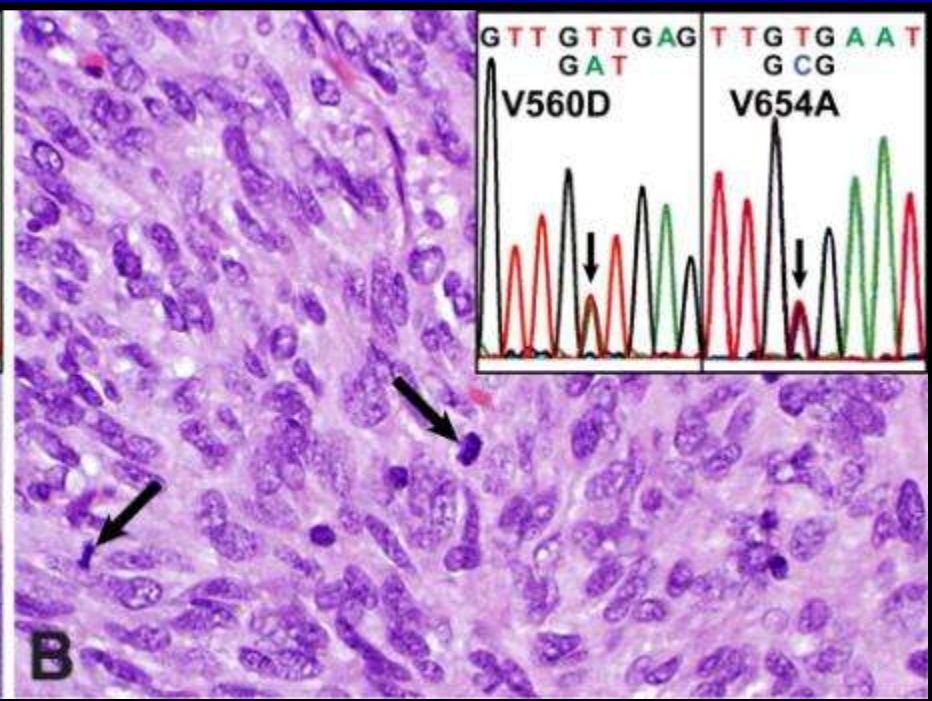
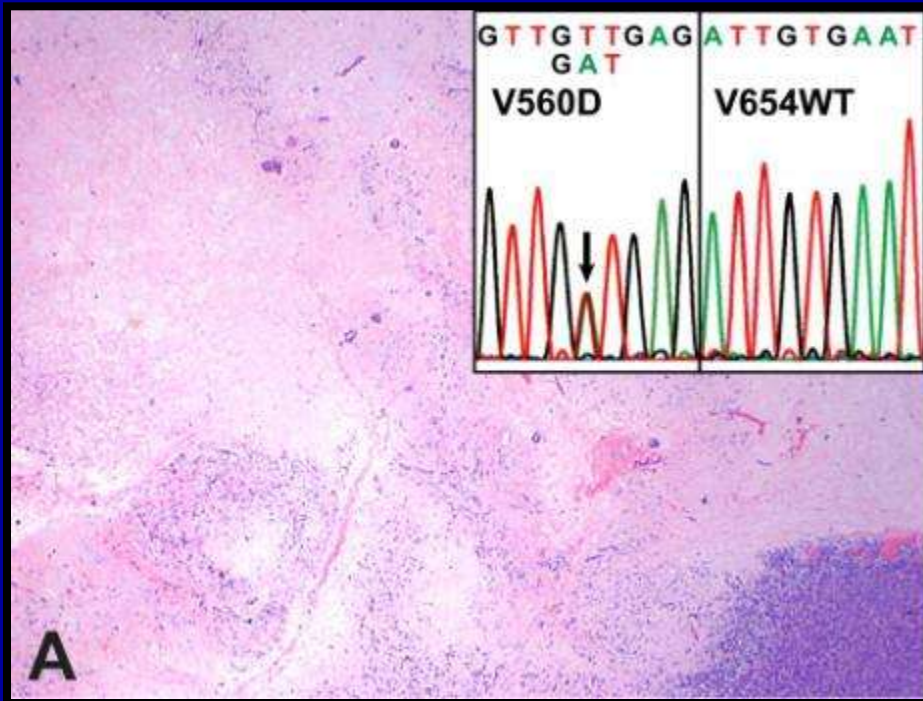


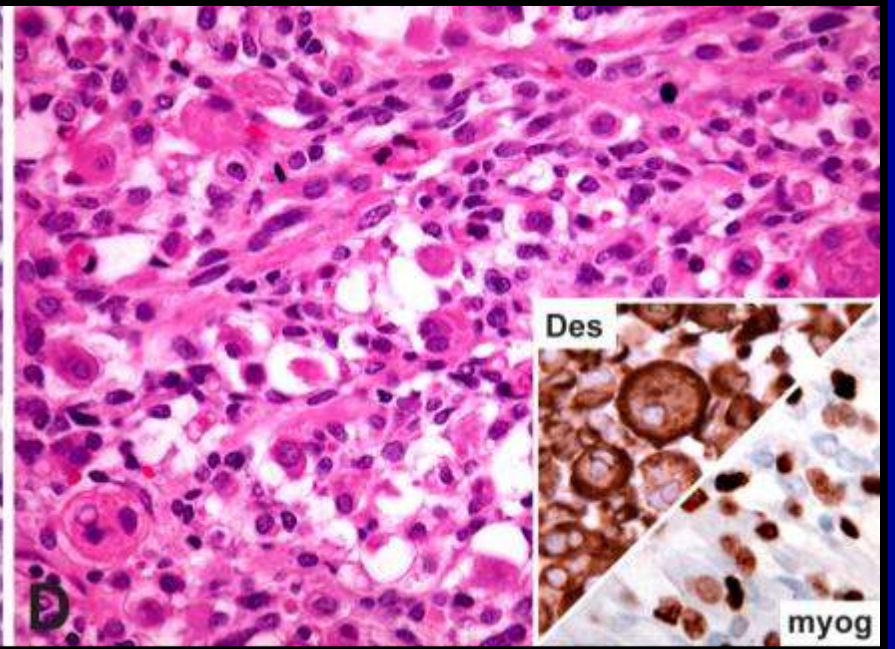
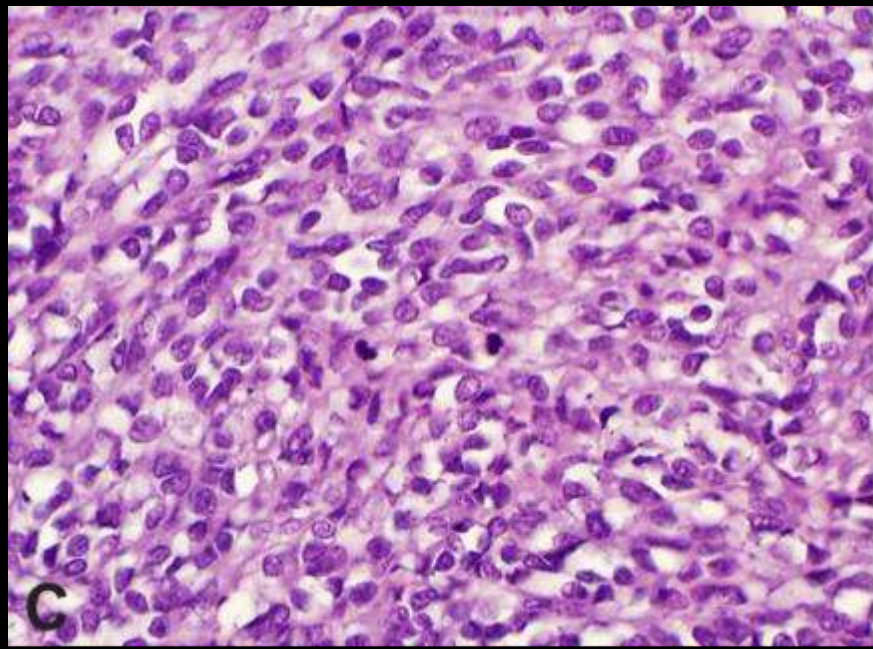
# *Long term Imatinib Tx*



# *Long term Imatinib Tx*



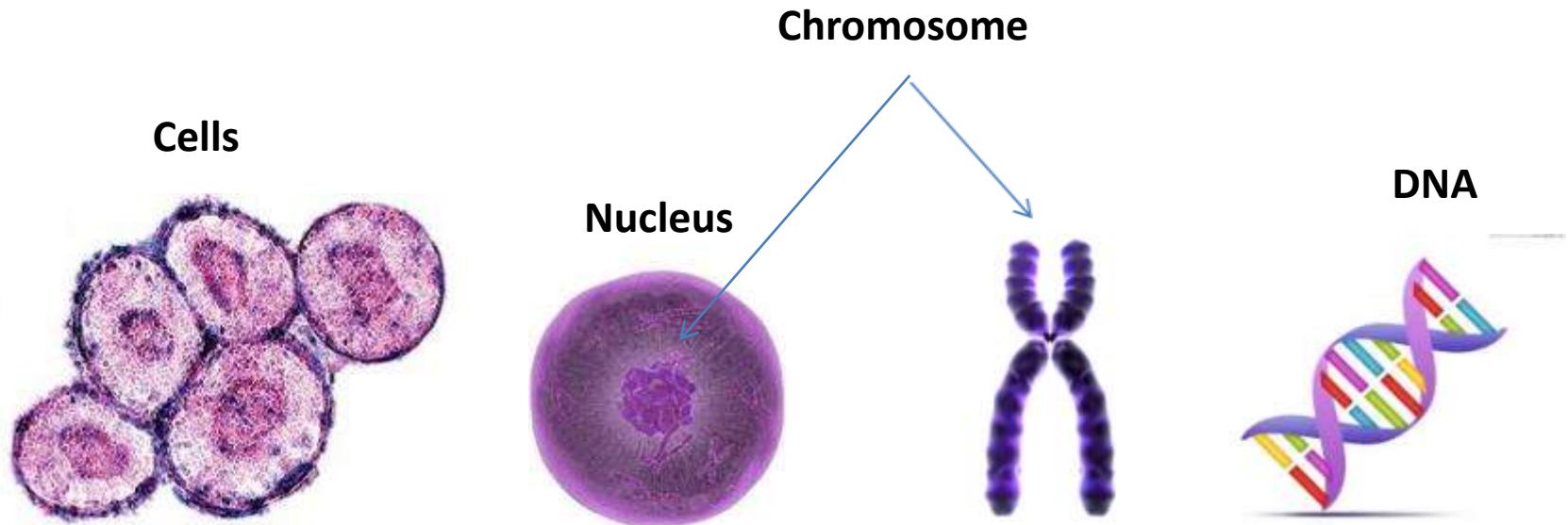




*What do we mean by Genotyping  
or Mutational Testing?*

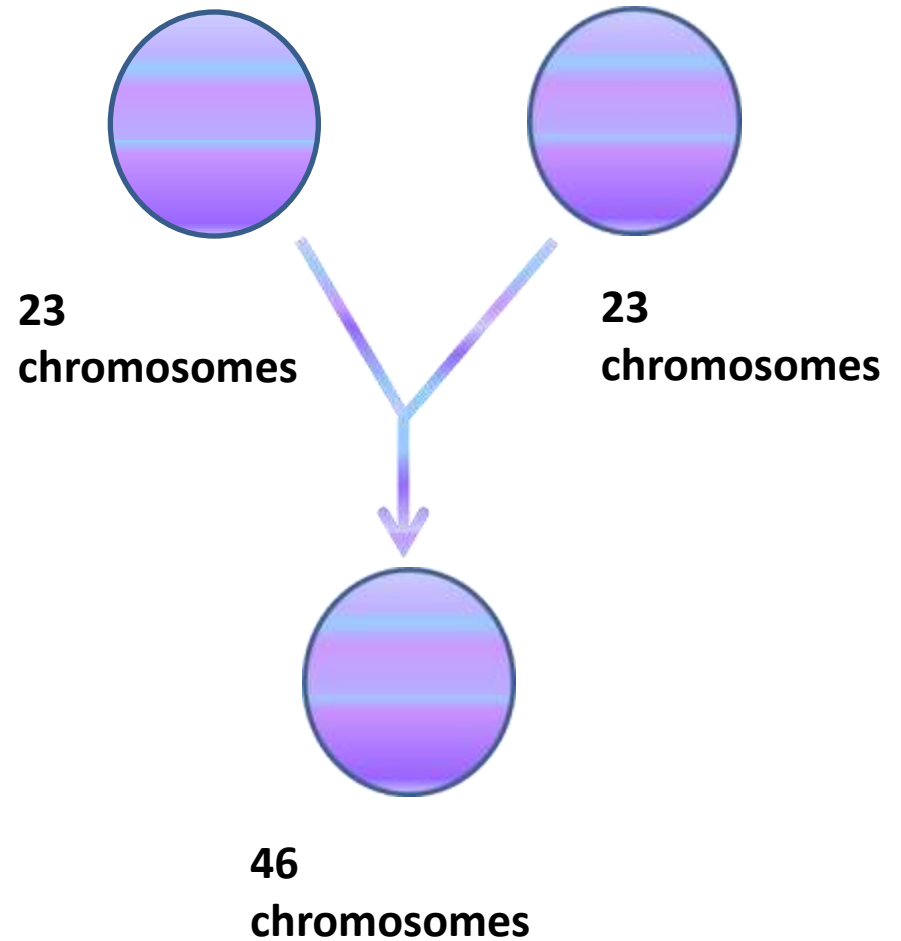
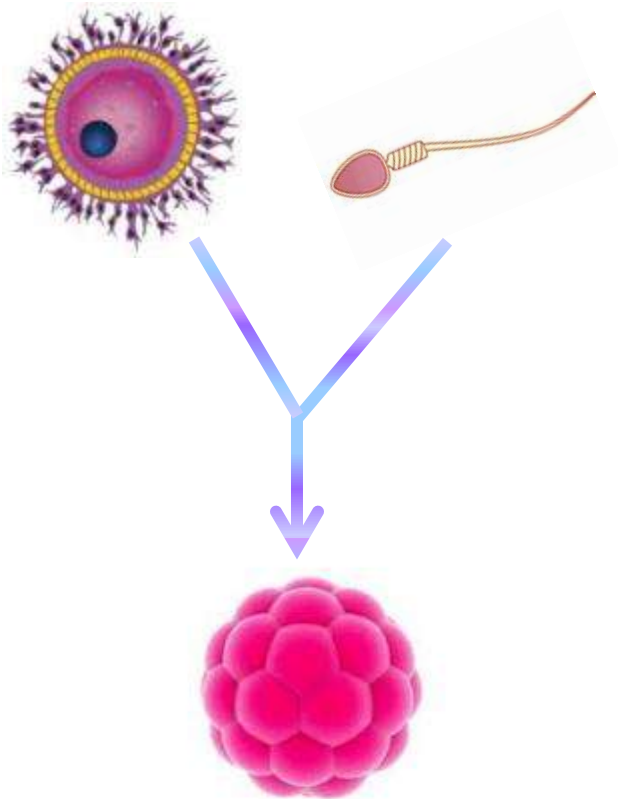


# What Are Genes?



- Tissues: specialized structures made up of cells
- Cells: building units, made up of cytoplasm and nucleus
- Nucleus: instructions/blueprint for cells
- Genes: carry the hereditary characteristic of cells
- Chromosome: made up of DNA and other proteins
- DNA: molecule encodes genetic data

# Human Genetics

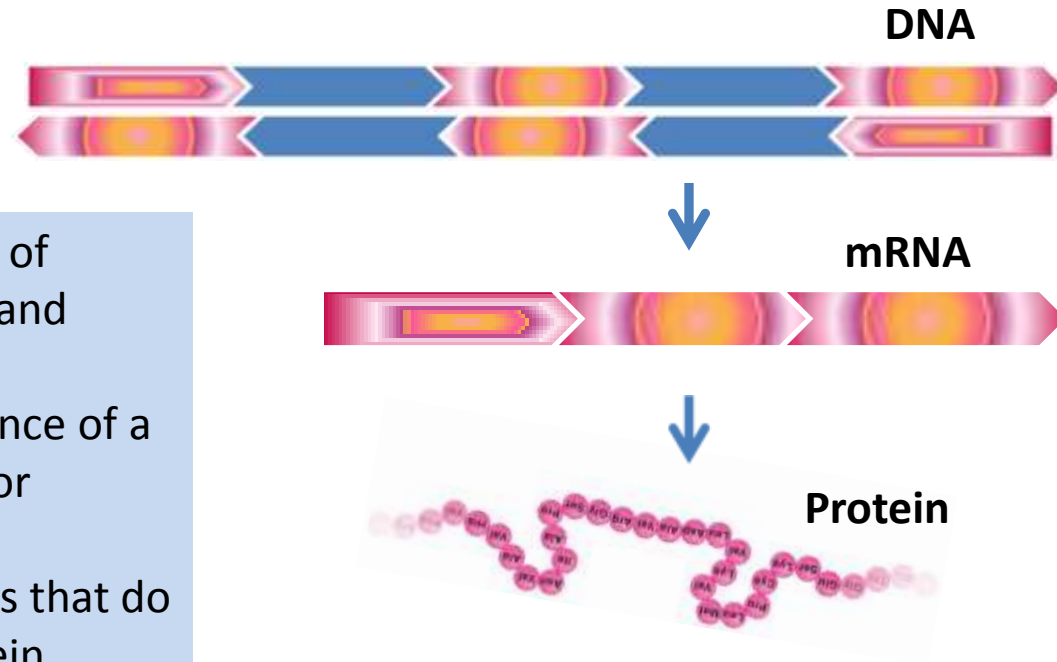


# Human Genetics





# Chromosome Structure And Expression



**Genes:** are made up of sequences of exons and introns

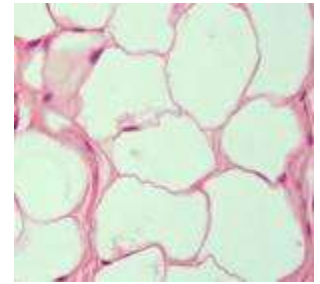
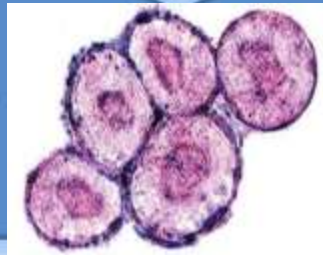
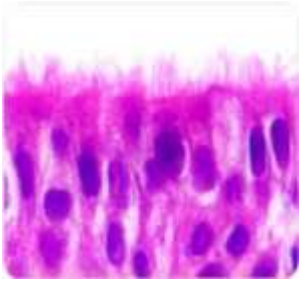
**Introns:** DNA sequence of a gene that encodes for protein

**Exons:** parts of genes that do not encode for protein

Gene expression:

1. Transcription: formation of mRNA
2. Translation: formation of protein

# Cellular Differentiation



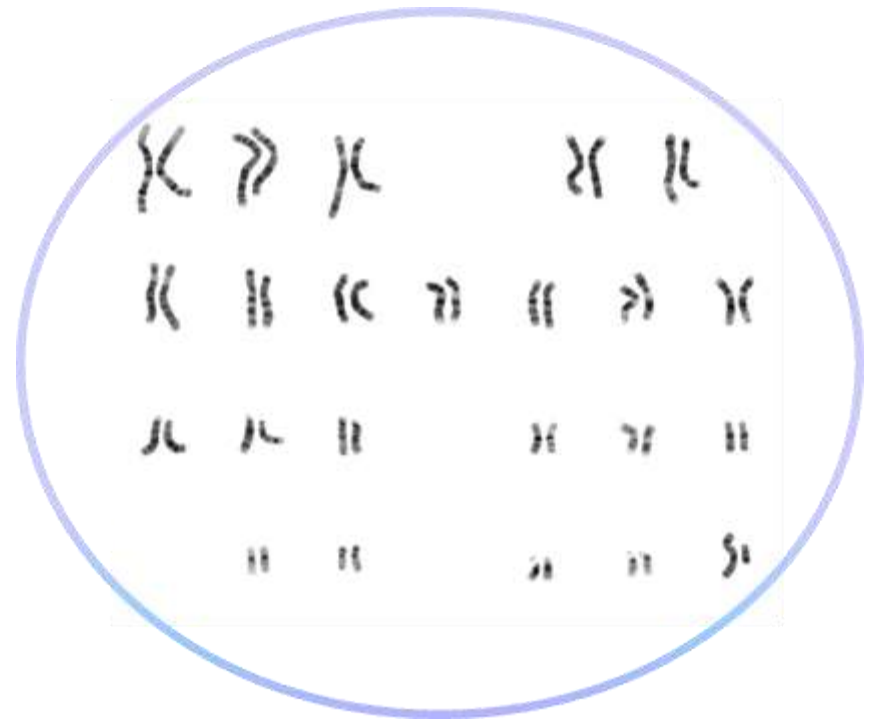
# Same Genes Different Cells



- Somatic cells share similar genetic composition
- Some genes are expressed, others are not
- Gene expression determines shape and function of cells

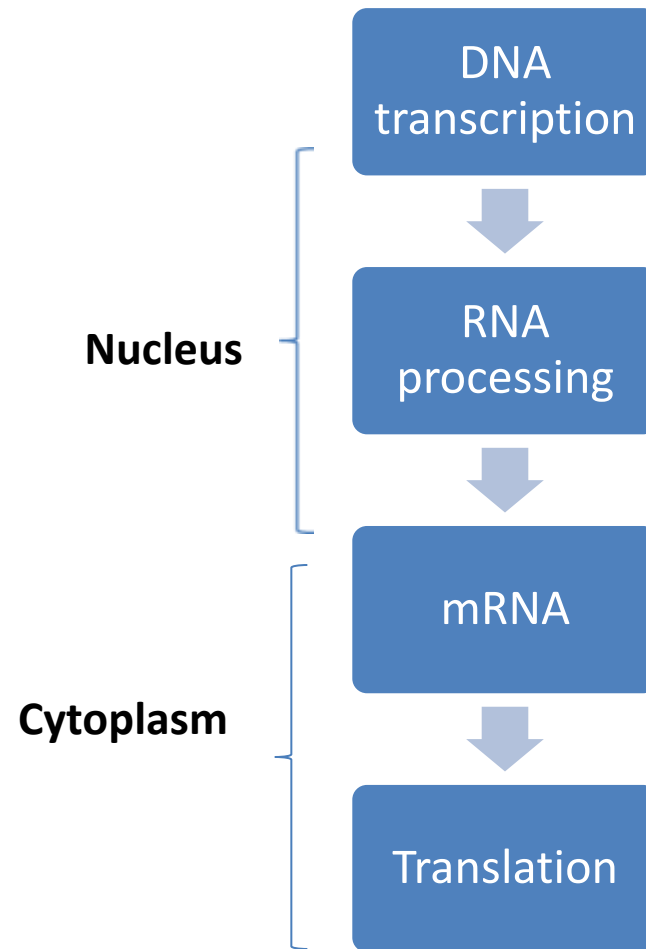
# Human Genome Project

- All DNA sequence in the 23 pairs of chromosomes
- <2 % of genome encode for proteins
- >98% of genome do not encode for proteins



# Regulation Of Gene Expression

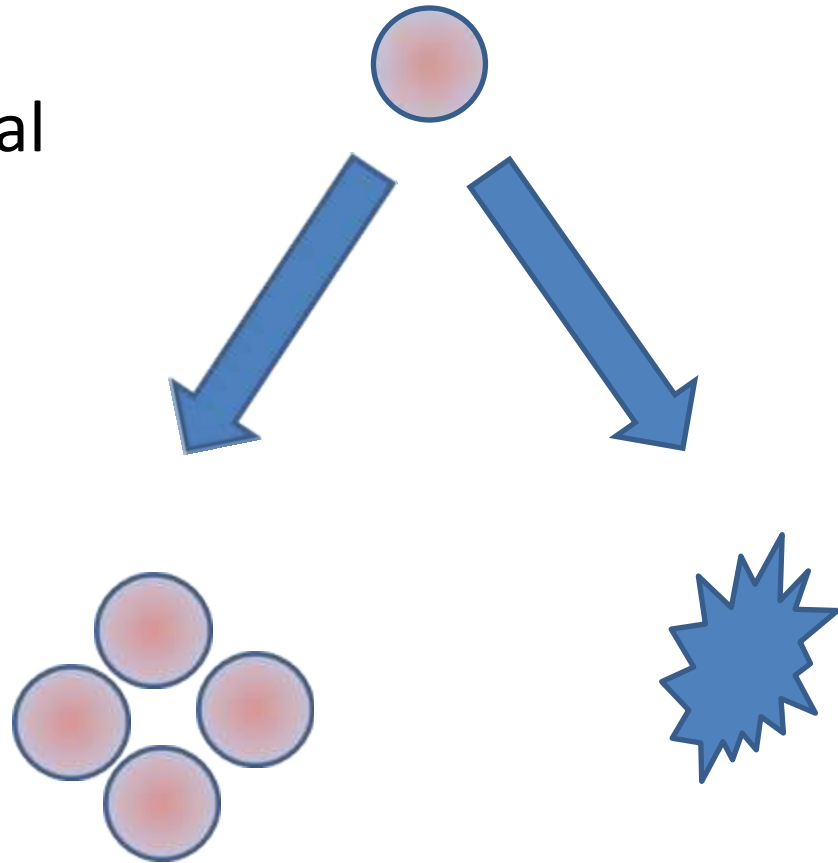
- Level of regulation:
  - Transcription of genes
  - Post transcription of genes
  - Translation of mRNA
  - Protein degradation





# Regulation Of Proliferation

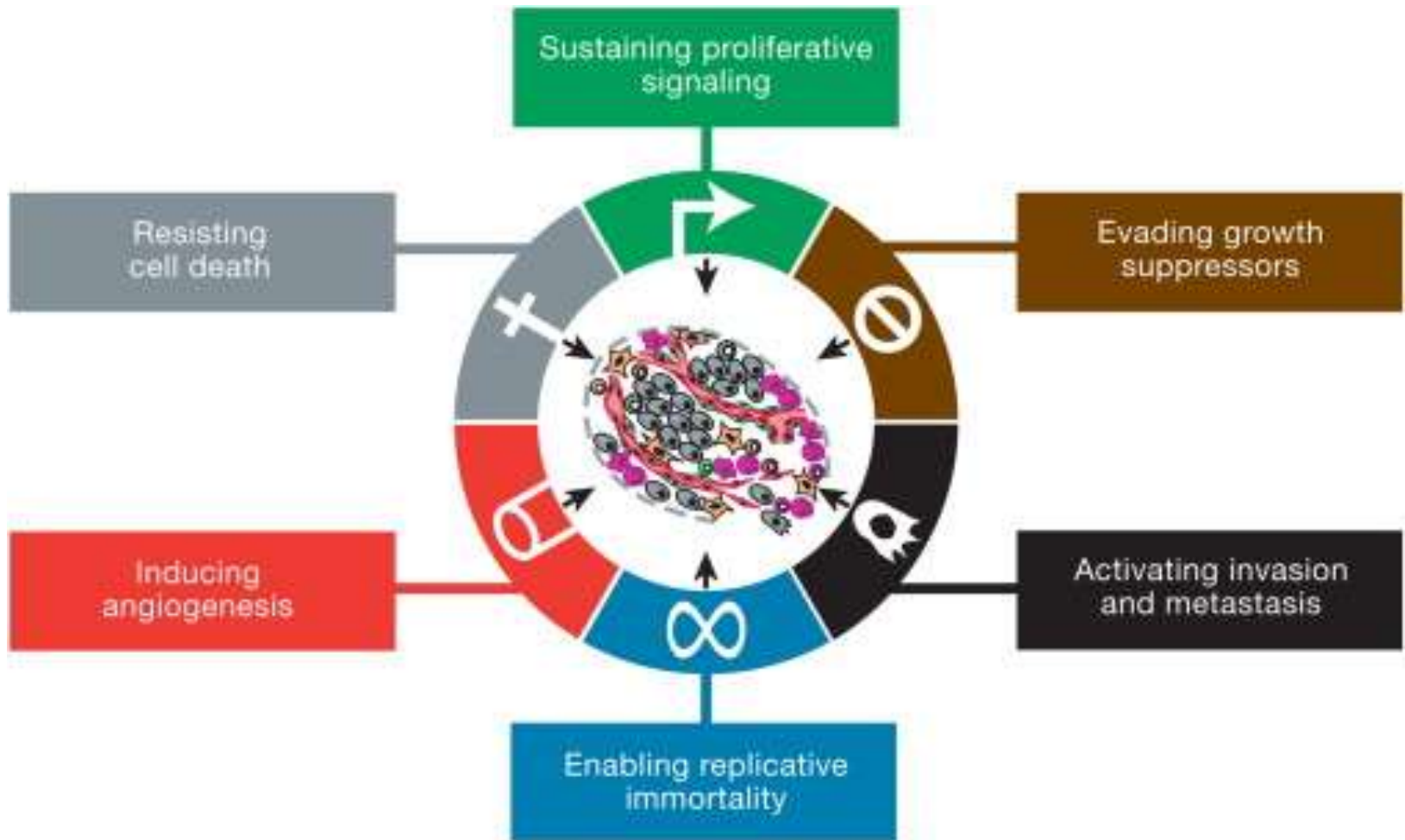
- Cell proliferation restricted under normal circumstances. Cells enter **cell cycle** to proliferate.
- **Apoptosis** to remove excess and damaged cells.
- Genes regulate cell cycle and apoptosis.



# Mutation

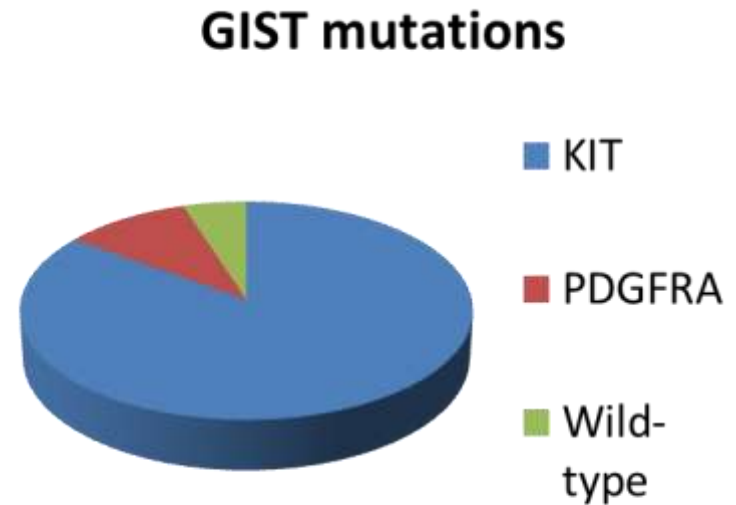
- Mutation: change in genome structure of a cell that may or may not alter its phenotypic properties.
- Consequences:
  - None
  - Loss of function
  - Gain of function
- Causes:
  - Radiation
  - Chemicals
  - Viruses
  - Genetic aberrations





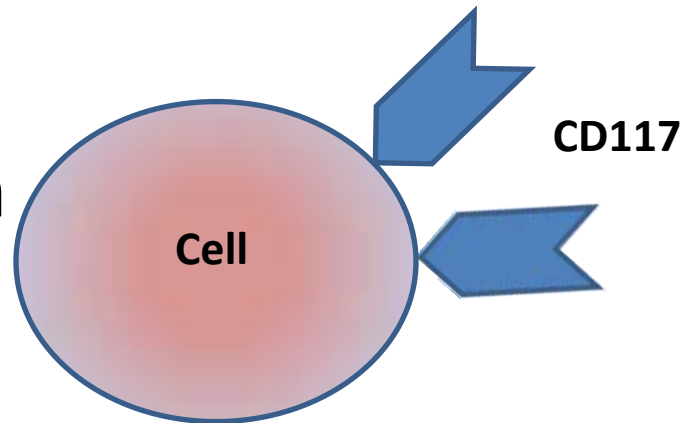
# Genotyping In GIST

- Genotyping: studying genetic constitution by determining differences in the genetic make-up of an individual and comparing it to a reference sequence.
- Genotyping work up for GIST cases:
  - *KIT* (muts in 80-85%)
  - *PDGFRA* (muts in 10-15%)
  - Wild type



# ***KIT* Gene Mutation In GIST**

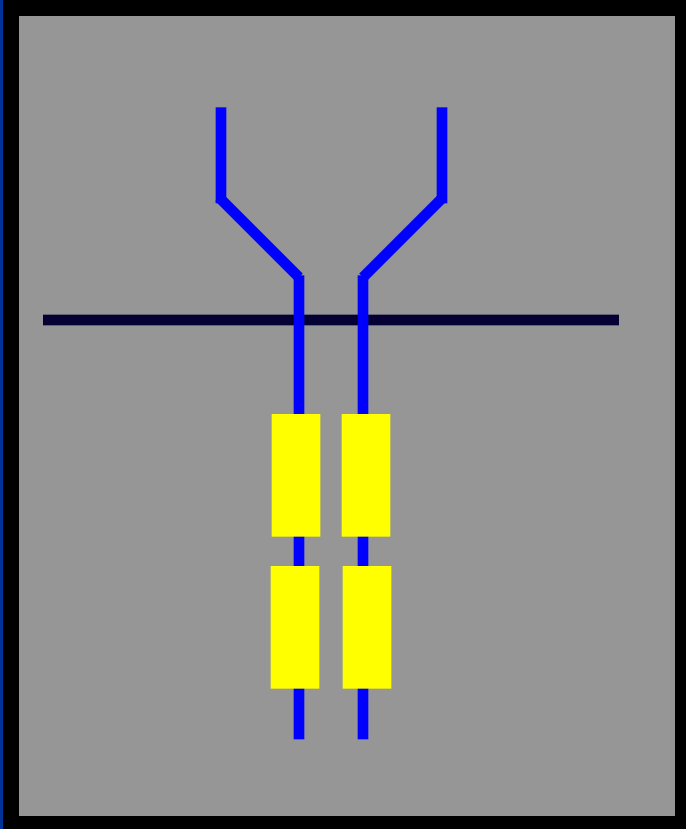
- KIT (also known as CD117) is a cell surface protein.
- It plays a role in cell survival, proliferation and differentiation.
- It is found to be mutant in 80-85% of GIST tumors.
- Mutation leads to gain of function.



# PDGRA Mutation In GIST

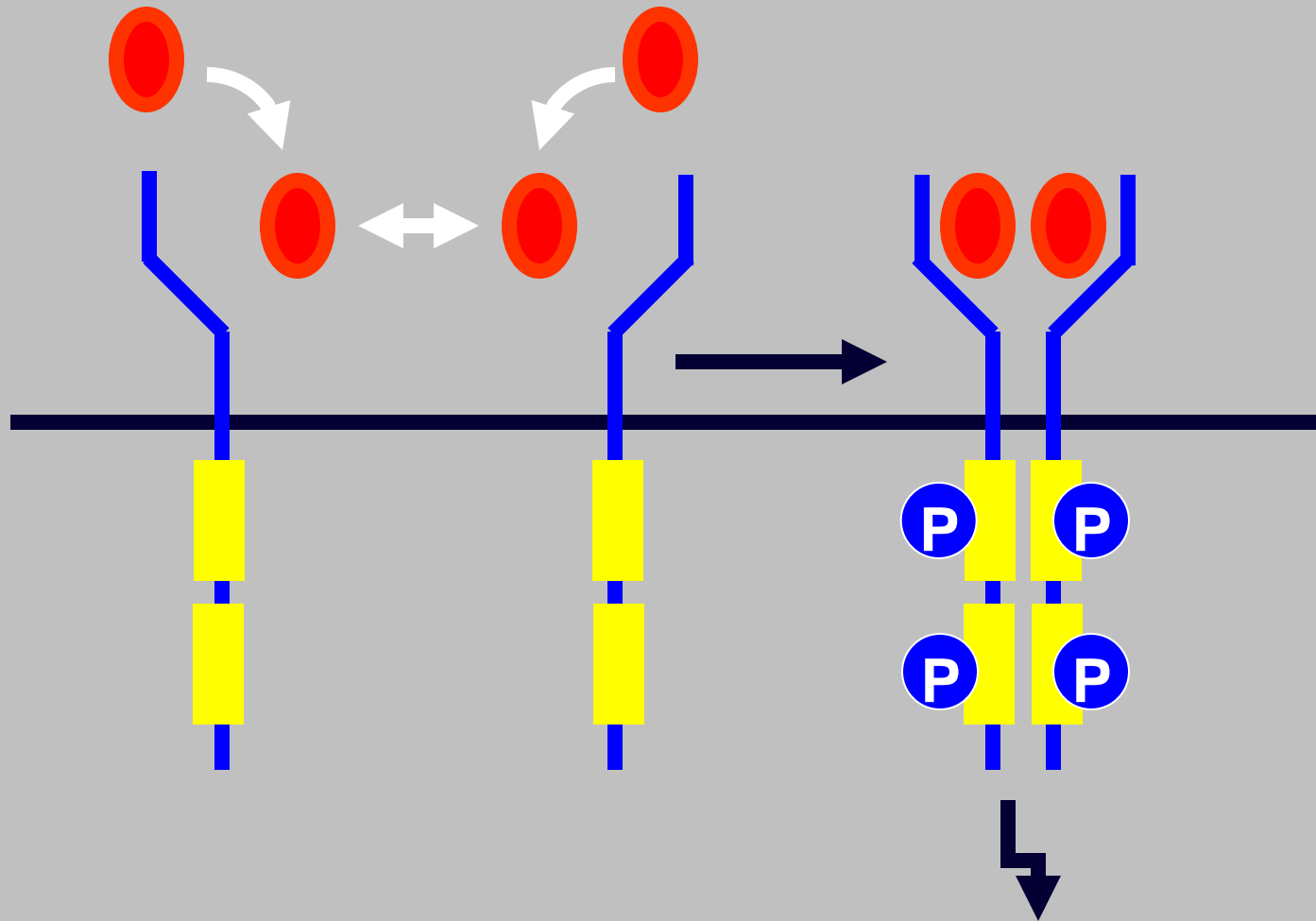
- Platelet derived growth factor receptor that binds GFRA and promote proliferation of blood vessel cells and other mesenchymal cells.
- The gene is mutated in 10-15% of GIST tumors.

# What is KIT?



- Type III receptor tyrosine kinase
- Chromosome 4q
- Proliferation & maintenance
  - germ cells
  - hematopoietic (mast) cells
  - melanocytes
  - interstitial cells of Cajal.

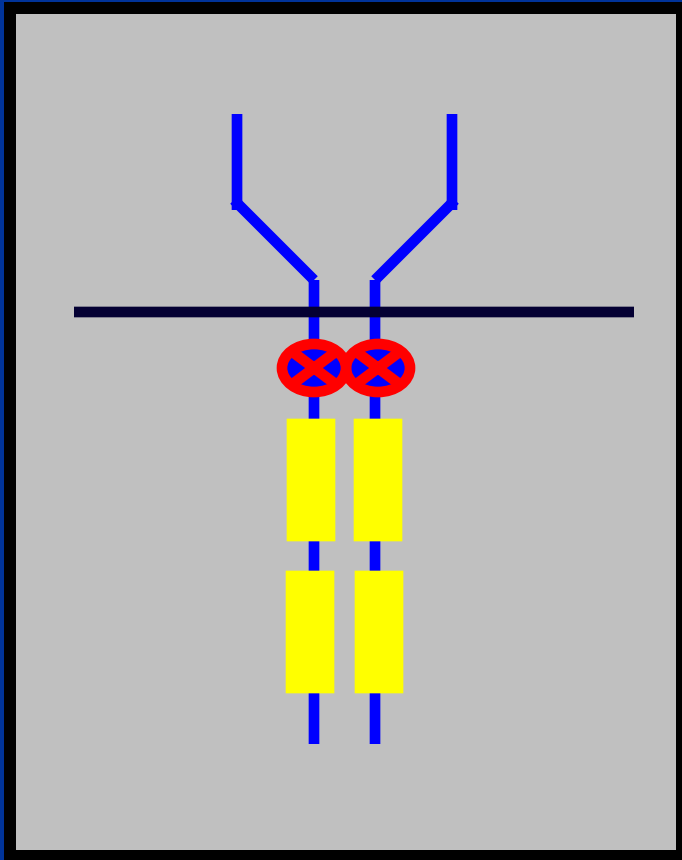
# Normal KIT Function



Ligand Dependent Activation



# GISTs Possess Ligand Independent Activating Mutations in *KIT* Exon 11



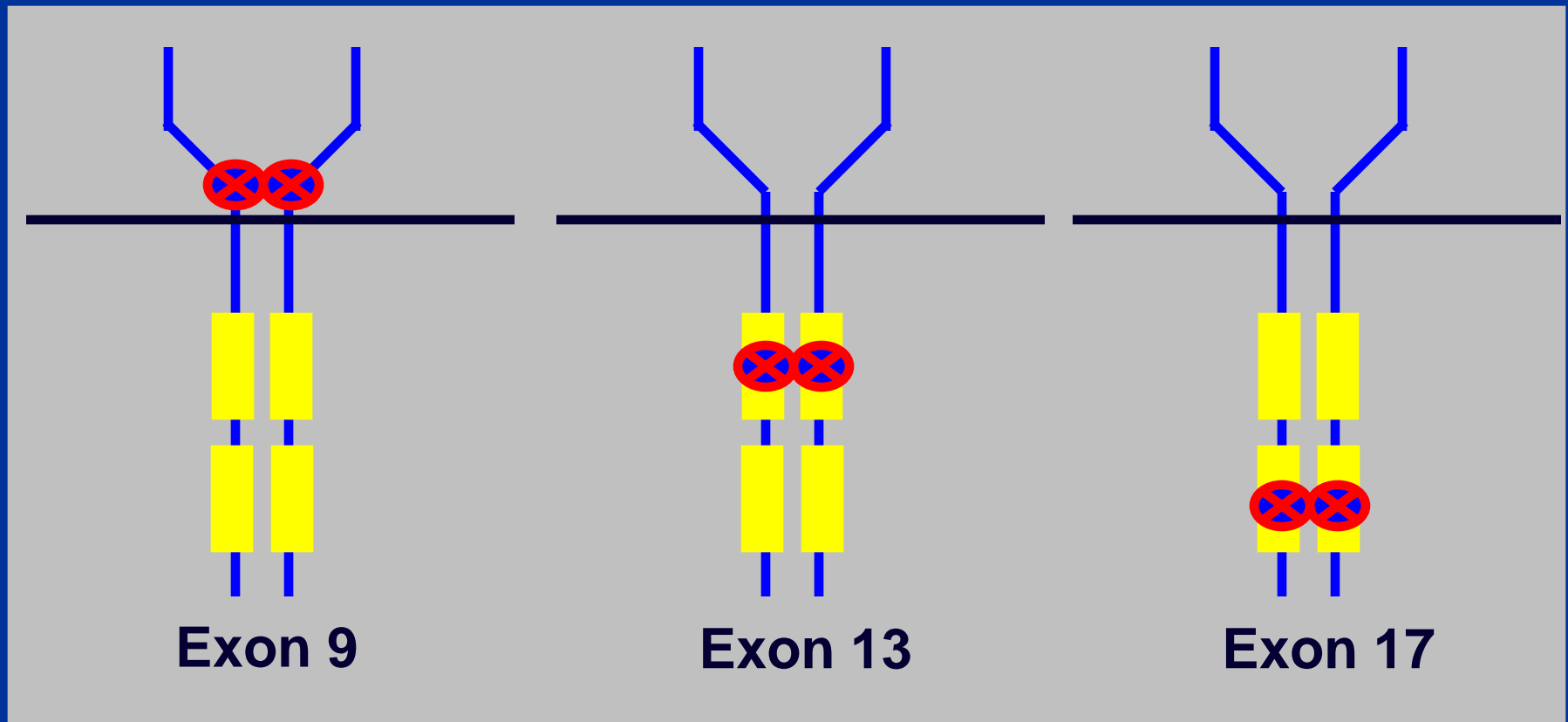
All mutations -whether involving base pair substitutions, deletions or duplications - preserve the open reading frame.

# ***KIT* mutations are activating and oncogenic!**

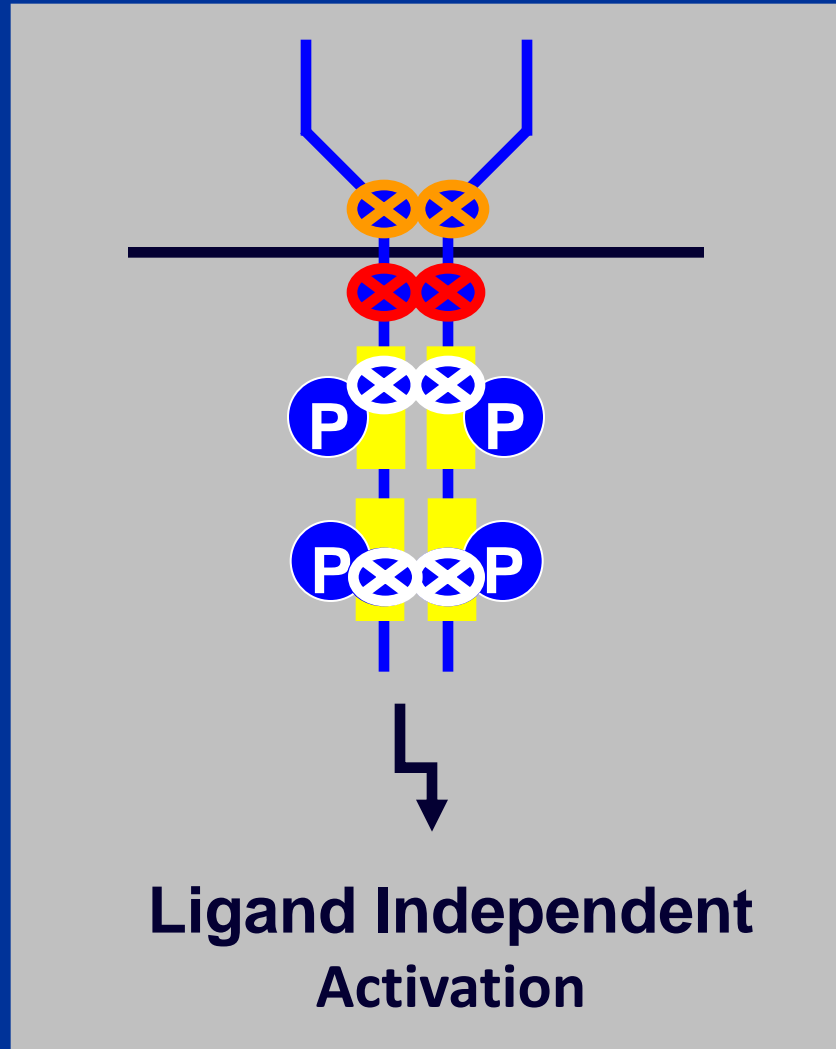
Constructs harboring *KIT* mutants are constitutively phosphorylated and kinase is constitutively activated in Ba/F3 cells.

Ba/F3 cells harboring *KIT* mutant constructs grow autonomously in culture (normally growth factor dependent) and cause tumors in nude mice.

# A minority of GISTs possess mutations in *KIT* exons 9, 13, & 17



# Activating *KIT* Mutations in GISTs



# Imatinib Mesylate



Formula:  $C_{30}H_{35}N_7SO_4$

MW: 589.7

- Rational drug design
  - 2-phenylamino pyrimidine
  - Based on structure of ATP binding site
  - Highly water soluble
  - Oral bioavailability

Inhibitor of selective tyrosine kinases

bcr-abl

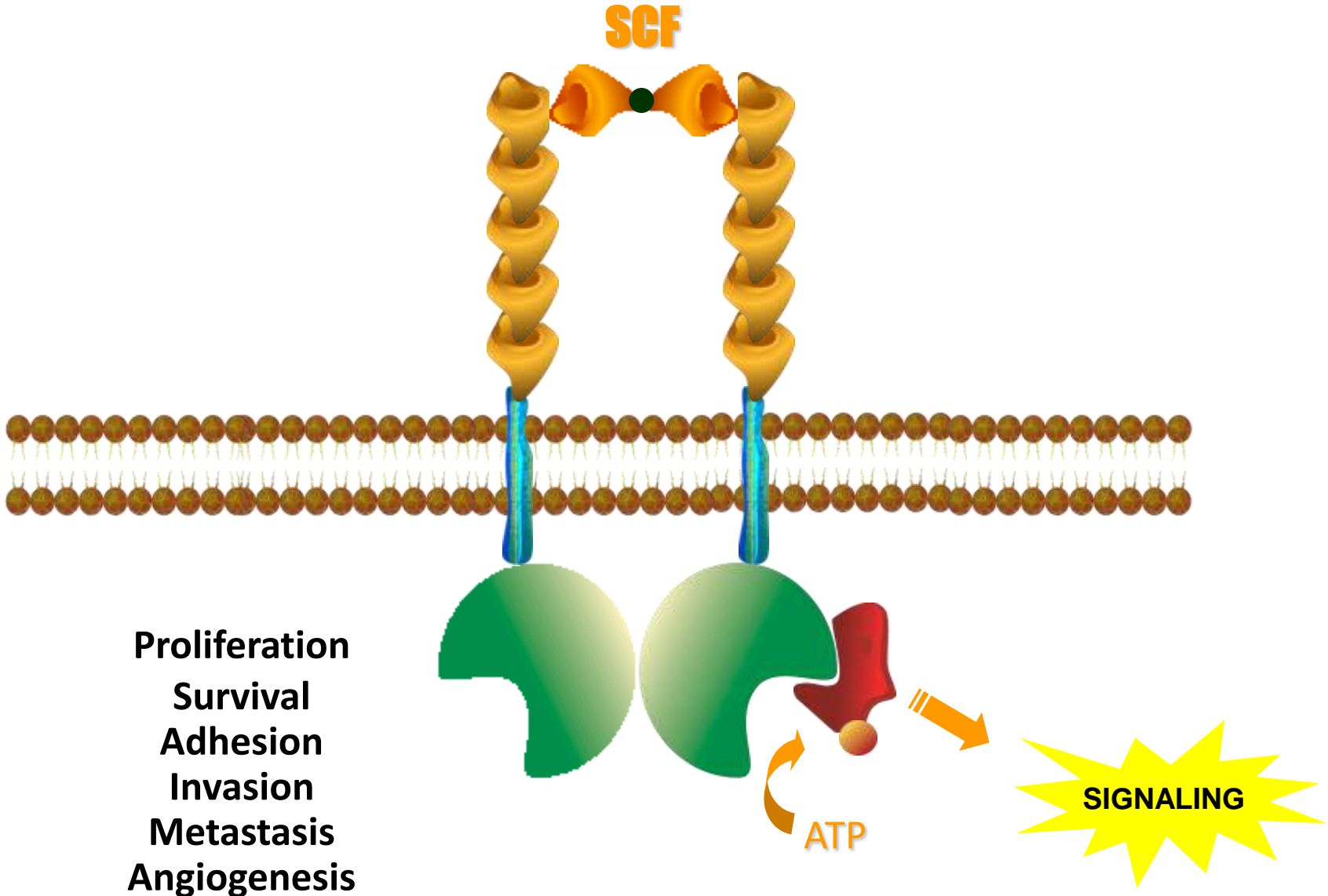
PDGF-R

c-kit

} Potent ( $IC_{50} \approx 0.1 \mu M$ )

# KIT Signaling – WT Cells

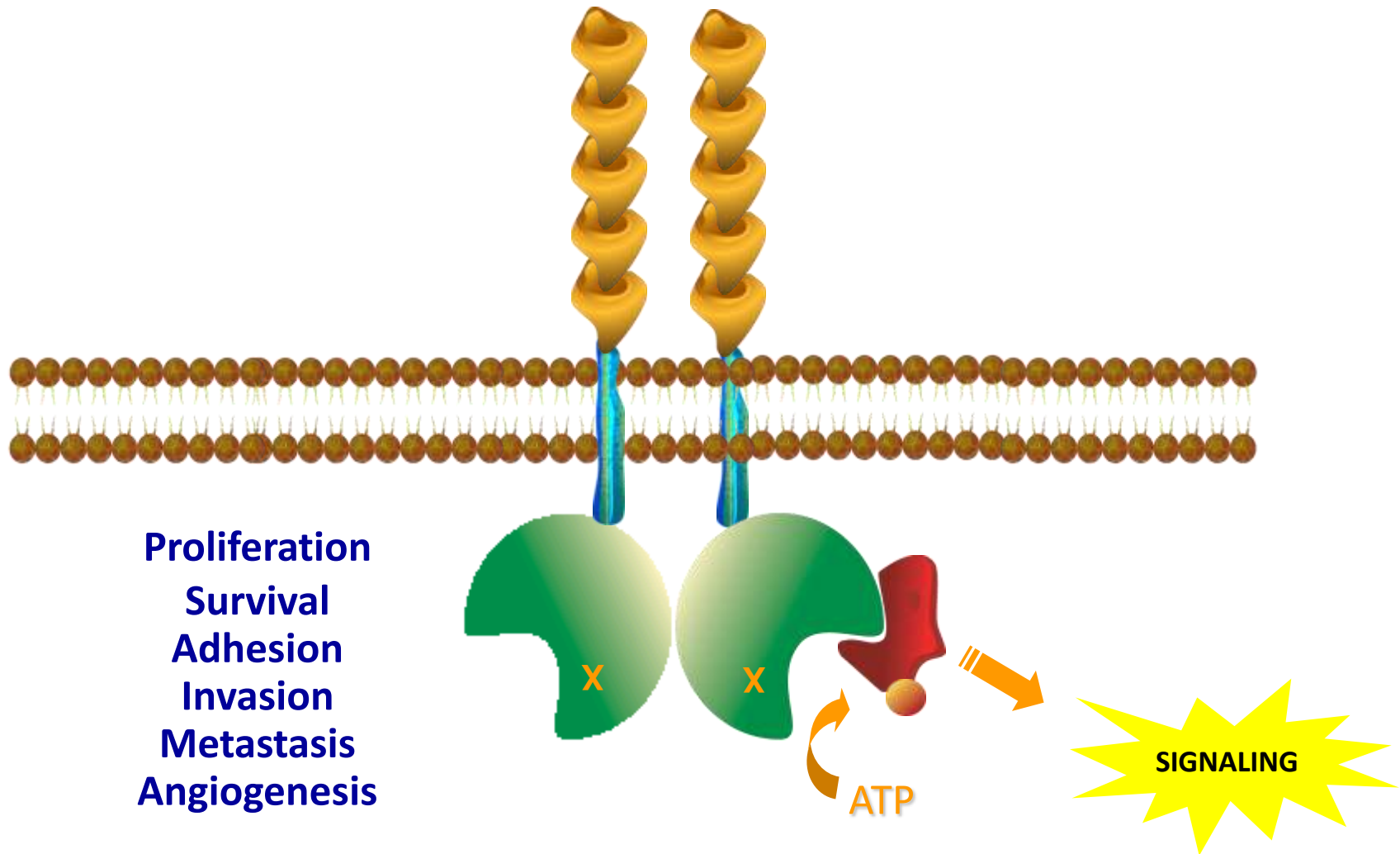
SCF



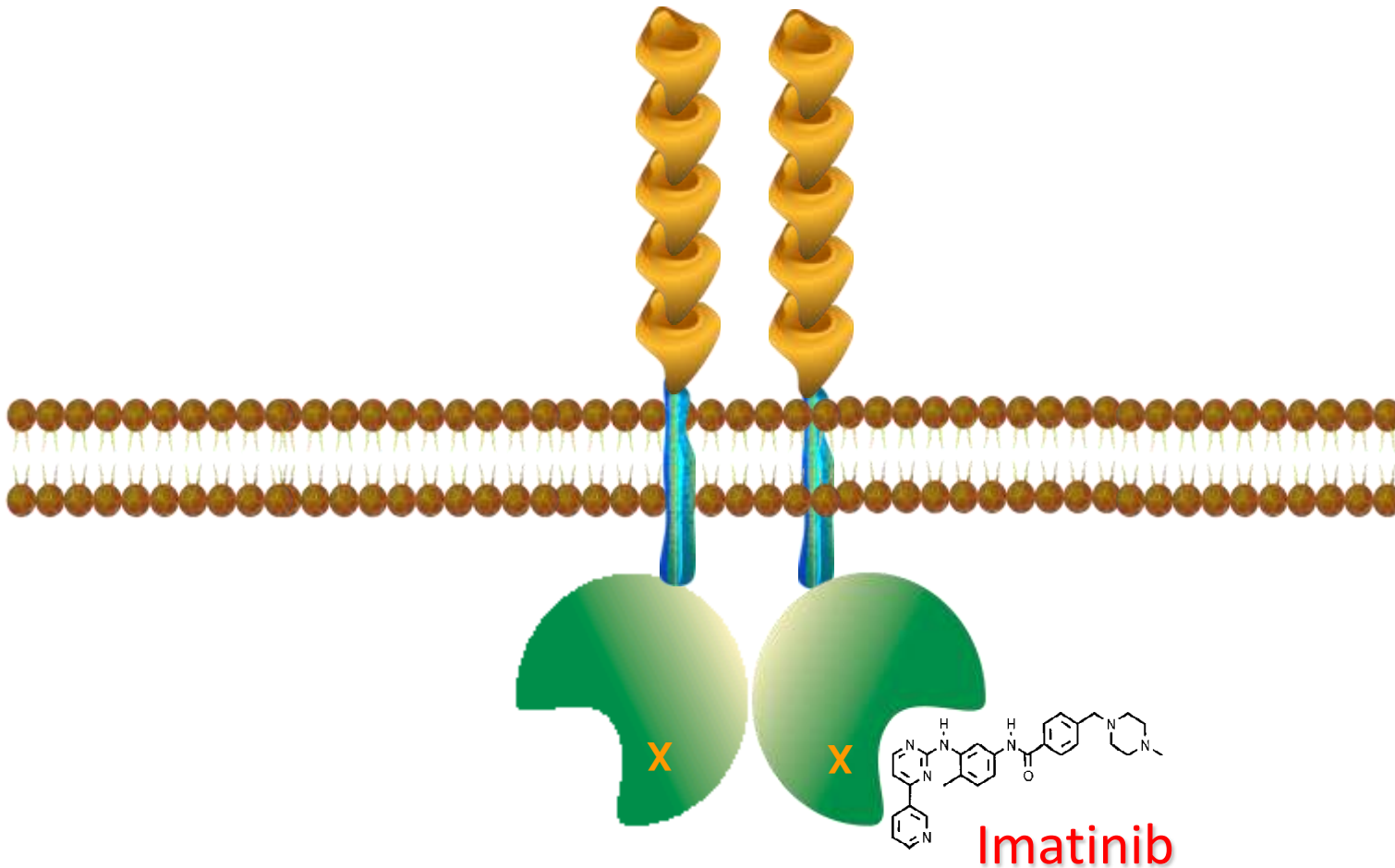
Proliferation  
Survival  
Adhesion  
Invasion  
Metastasis  
Angiogenesis

# Oncogenic KIT Signaling

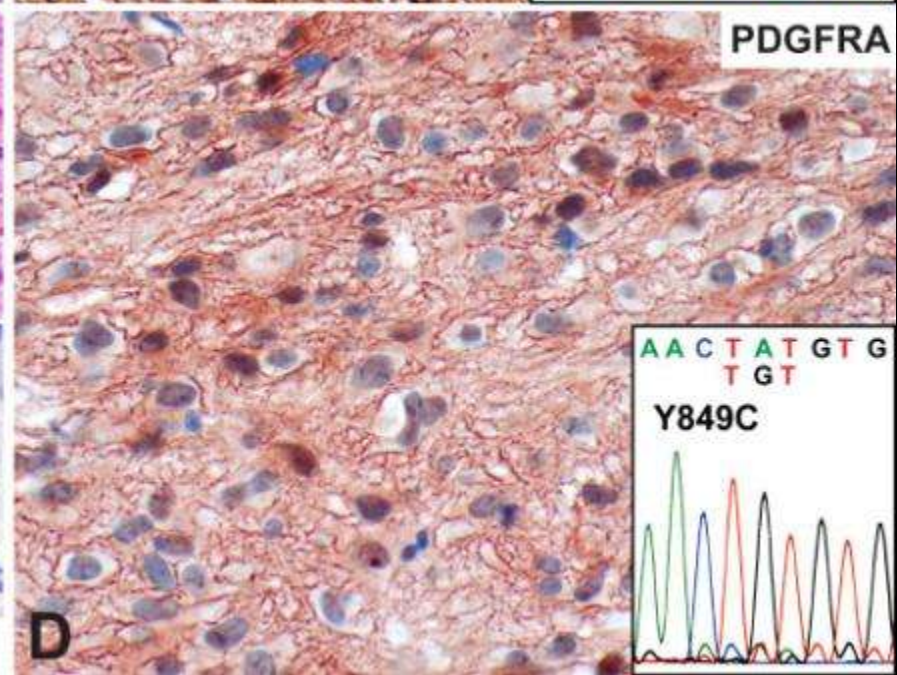
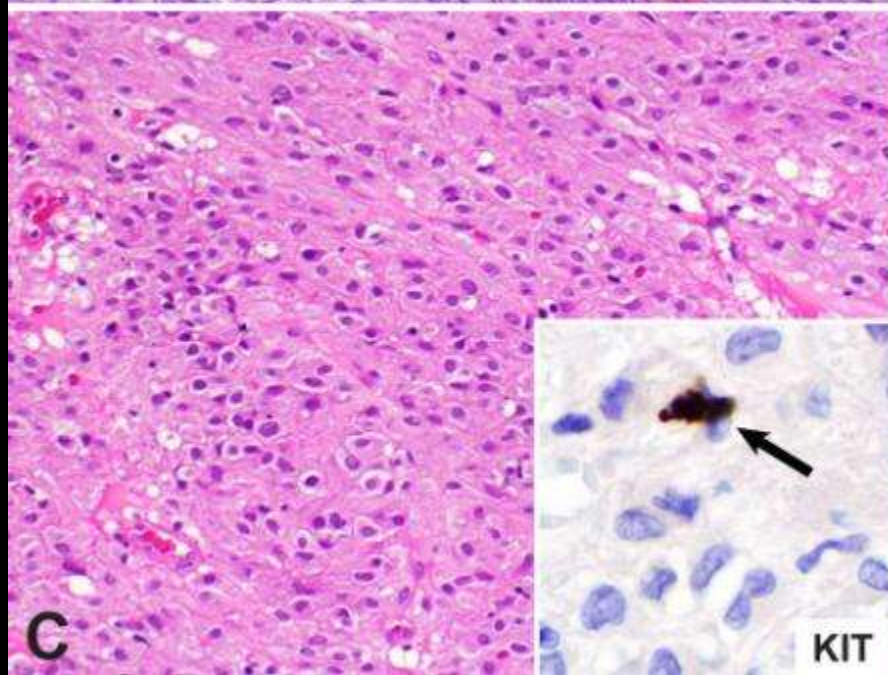
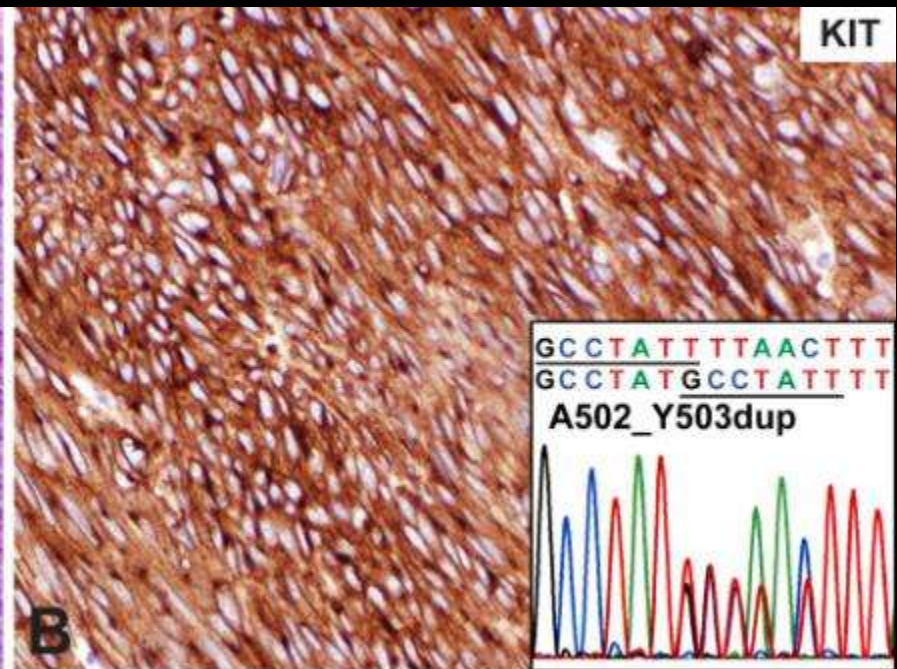
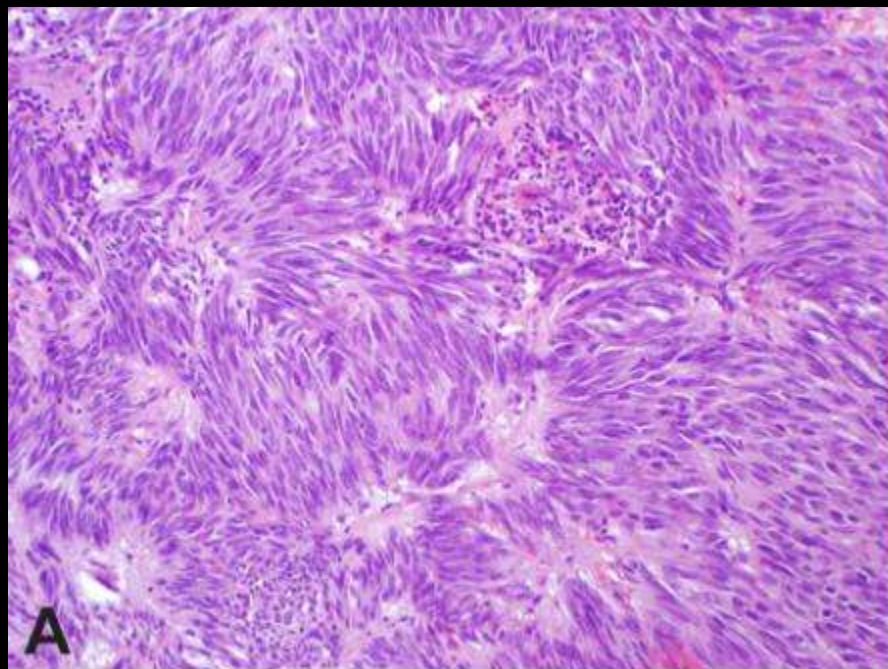
## GI Stromal Tumors

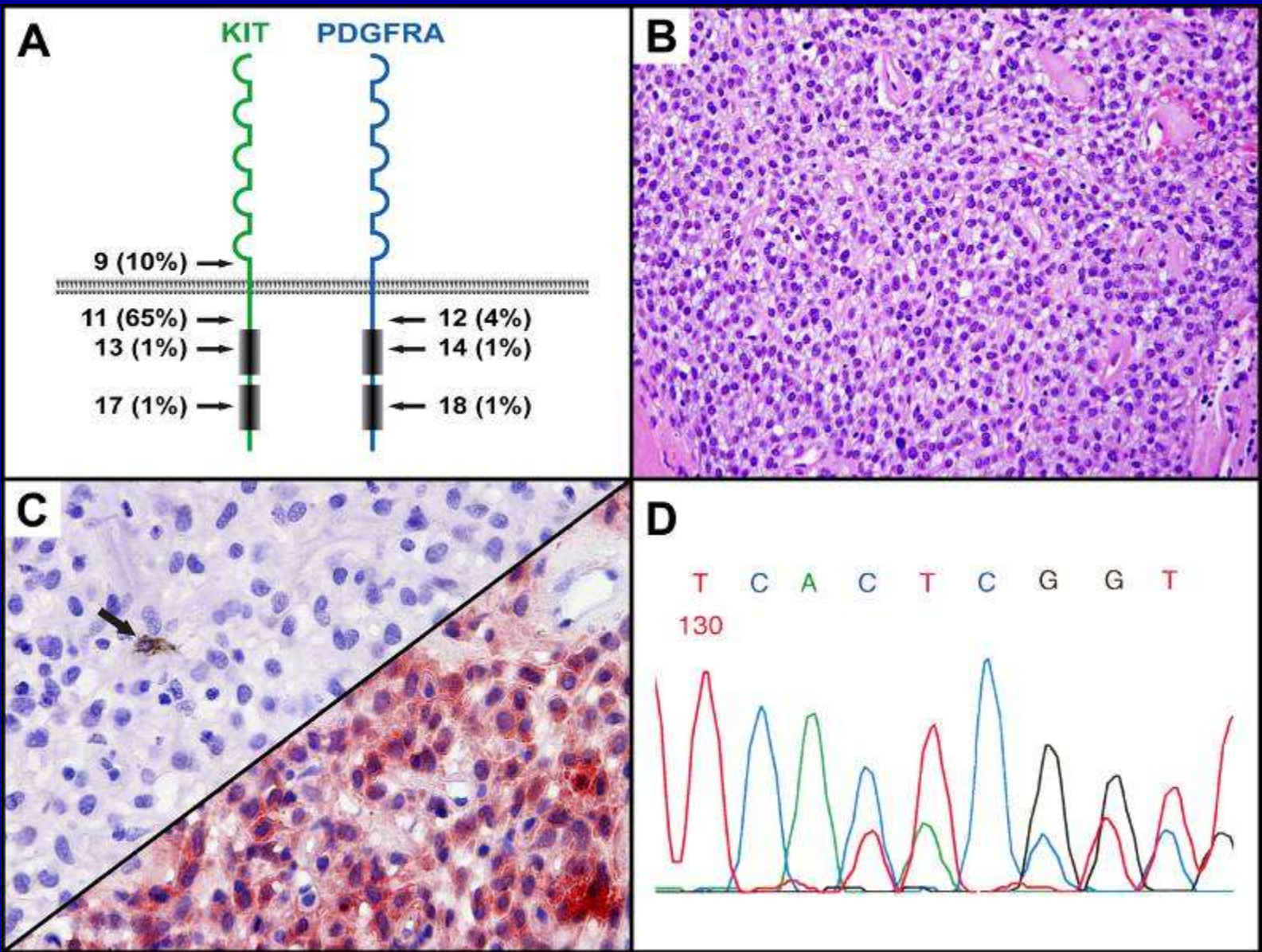


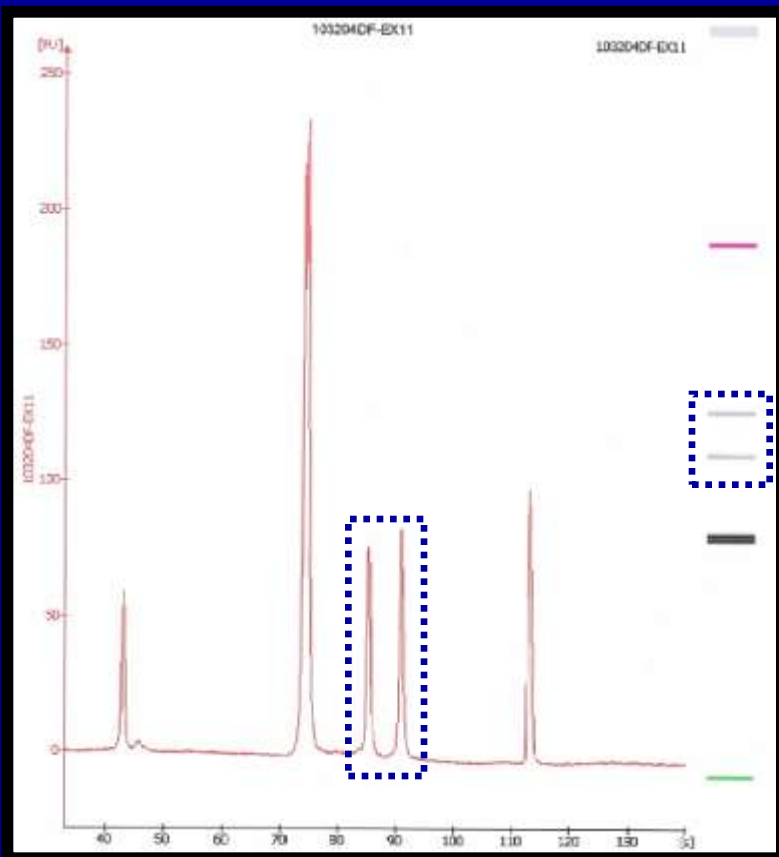
# Imatinib Inhibits KIT Signaling





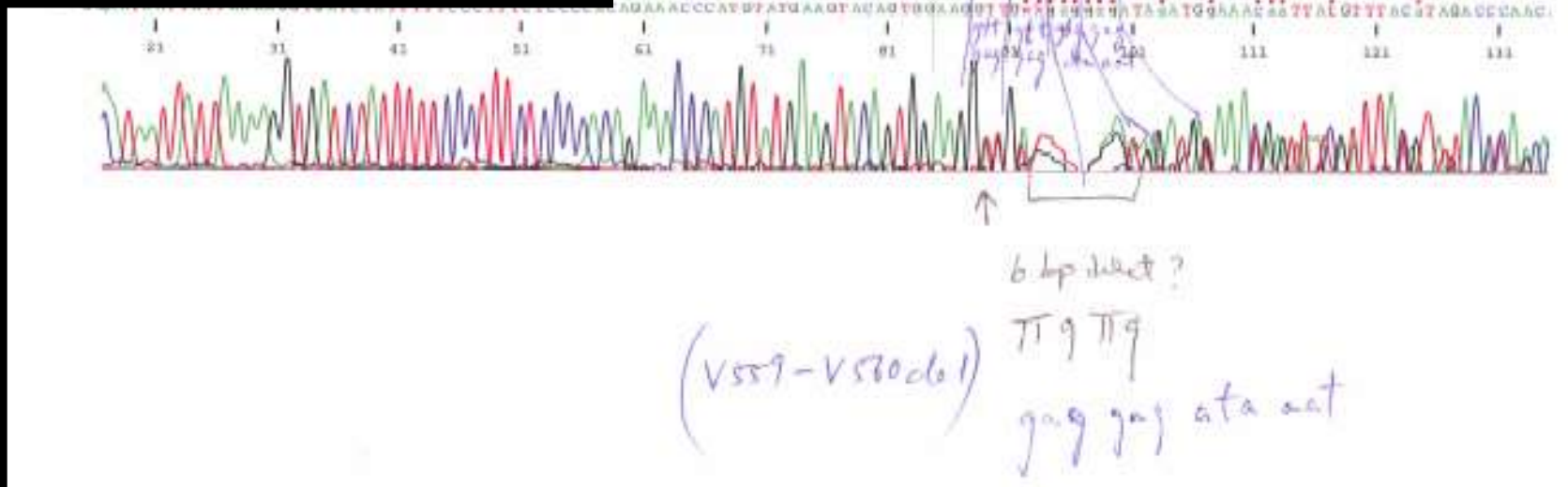






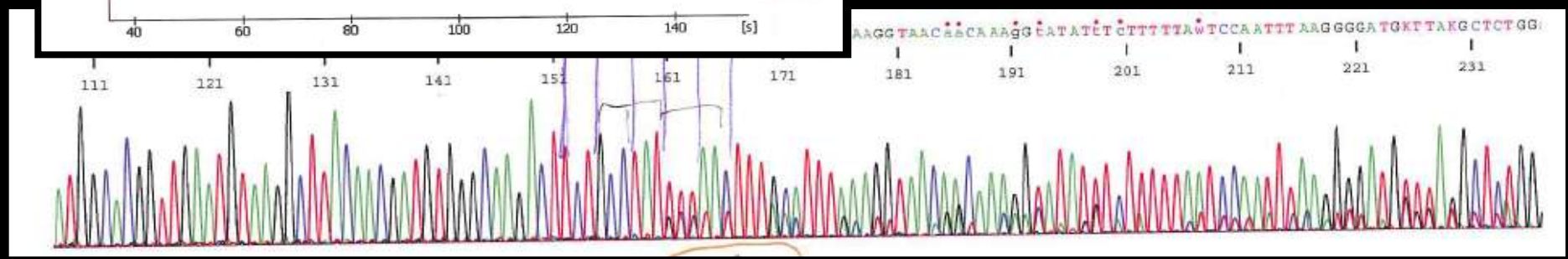
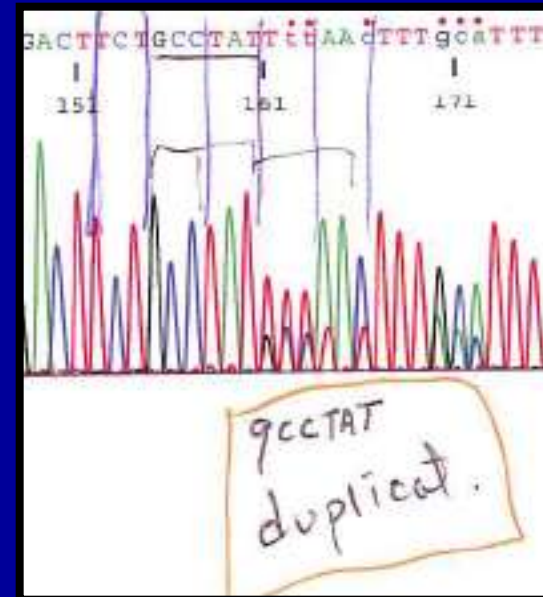
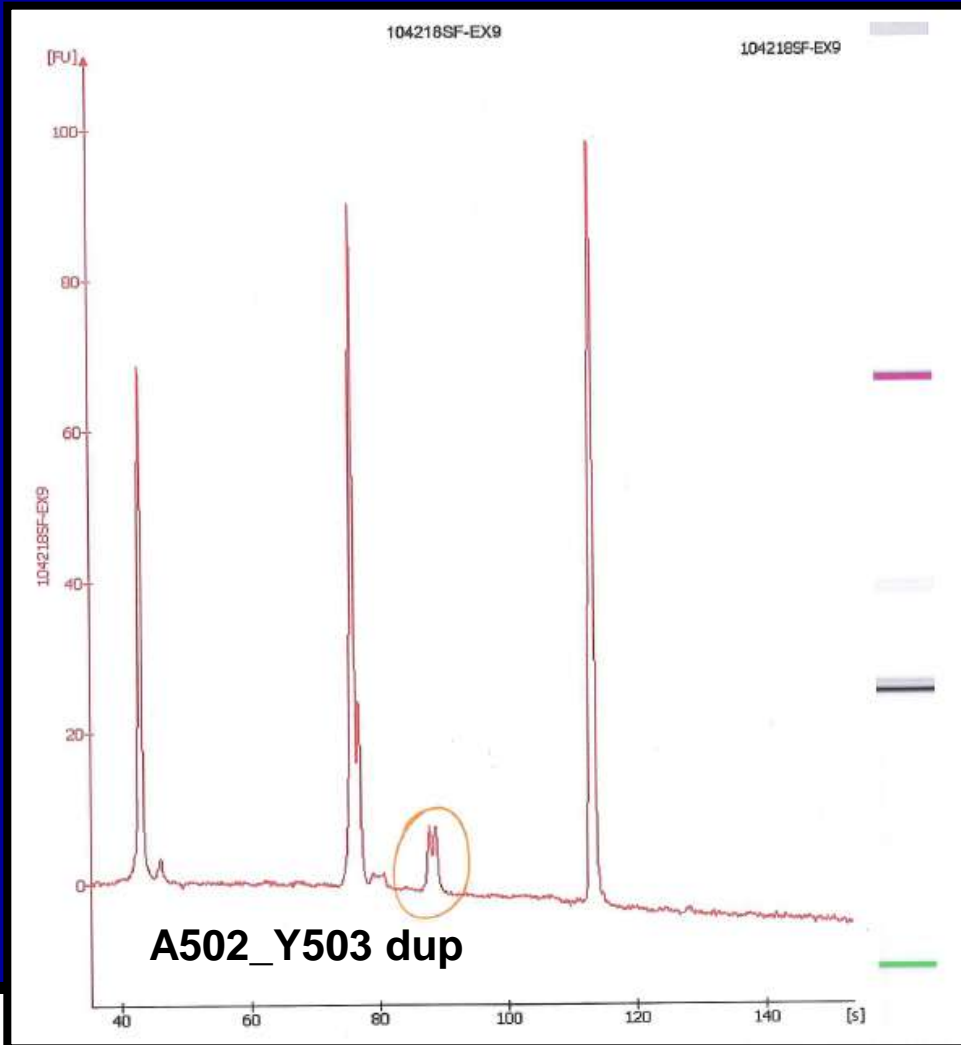
# Exon 11

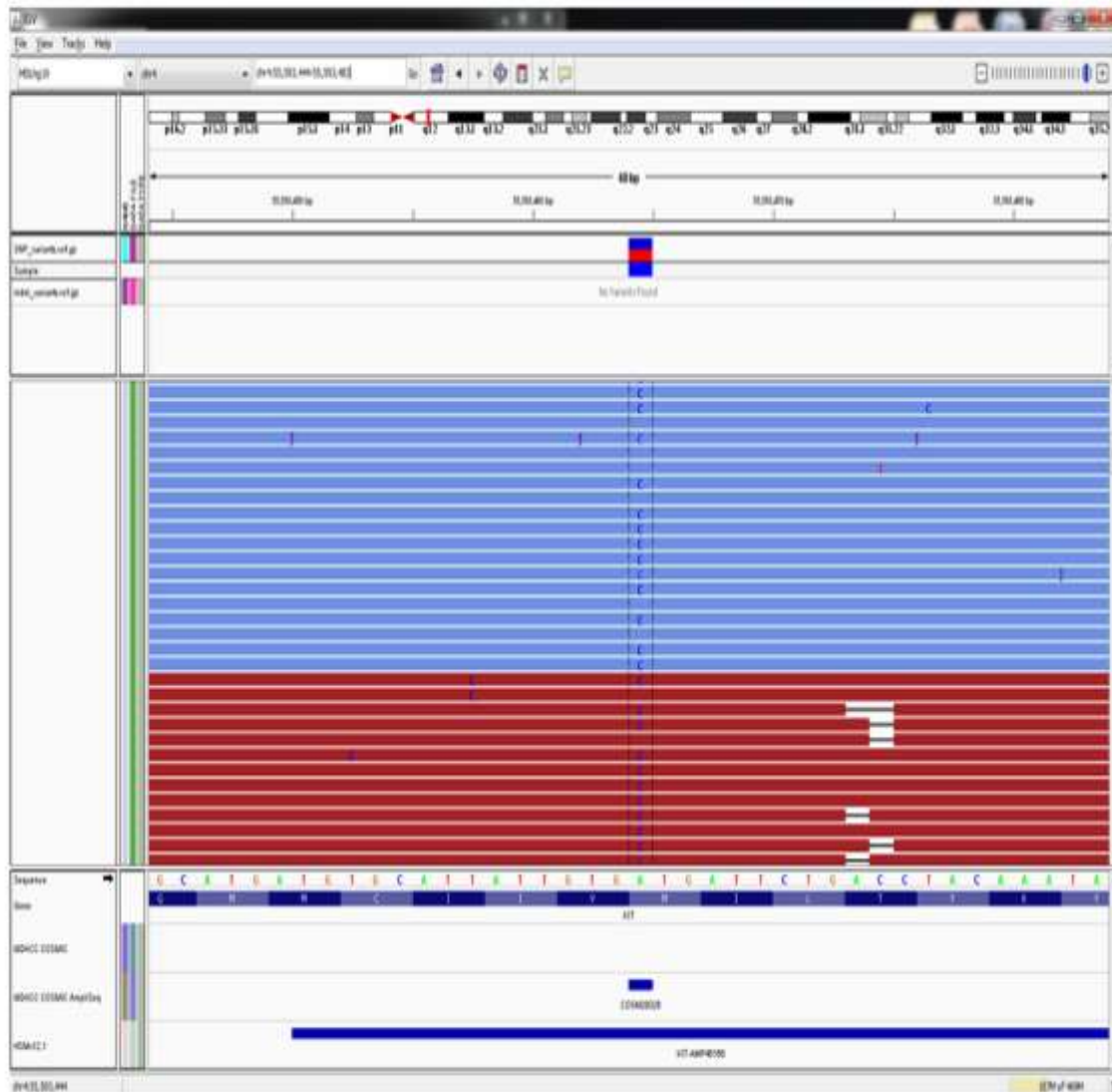
## V559\_V560del



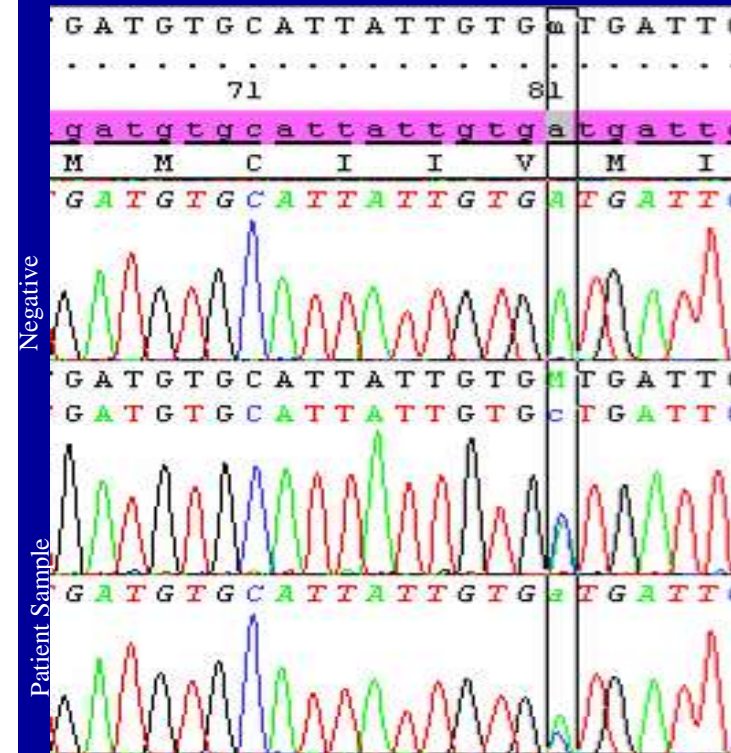
# Exon 9

## A502\_Y503dup





Detection of SNV in KIT Exon 10, currently not covered by Sanger



Confirmation by Sanger  
ATG→CTG, M541L  
KIT EXON 10

Chromosome	Position	Gene Symb	Ploidy	Ref	Variant	VarFreq	Coverage	RefCov	VarCov
chr4	5559346 4	KIT	Het	A	C	63.42	1077	389	683

75% Tumor

# *Thank You*

- **Brian Rubin, Cleveland Clinic**
- **Jason Hornick, Brigham & Women's Hospital/Harvard**
- **Michael Heinrich & Chris Corless, University of Oregon**
- **Jon Trent, University of Miami**
- **Ghadah Al-Saanna, Sarcoma Path Visiting Faculty**
- **Many colleagues at UTMDACC**