KNIGHT DIAGNOSTIC LABORATORIES

Pioneering Personalized Diagnostics

Diagnosing and Predicting the Behavior of GI Stromal Tumors

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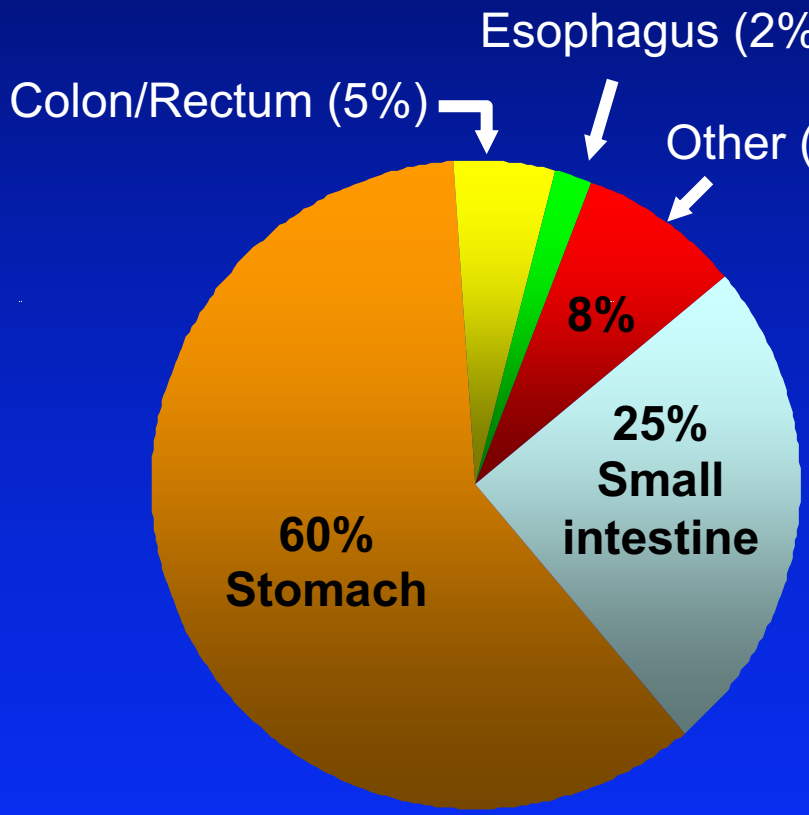
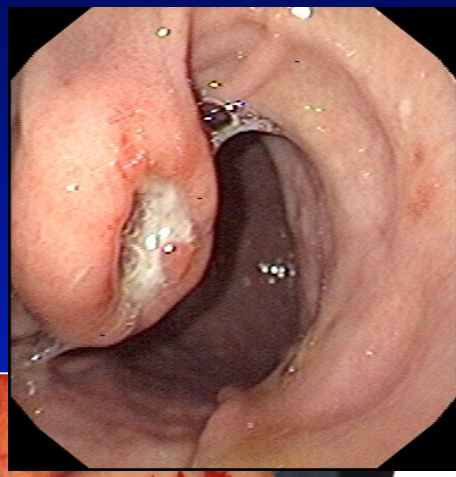
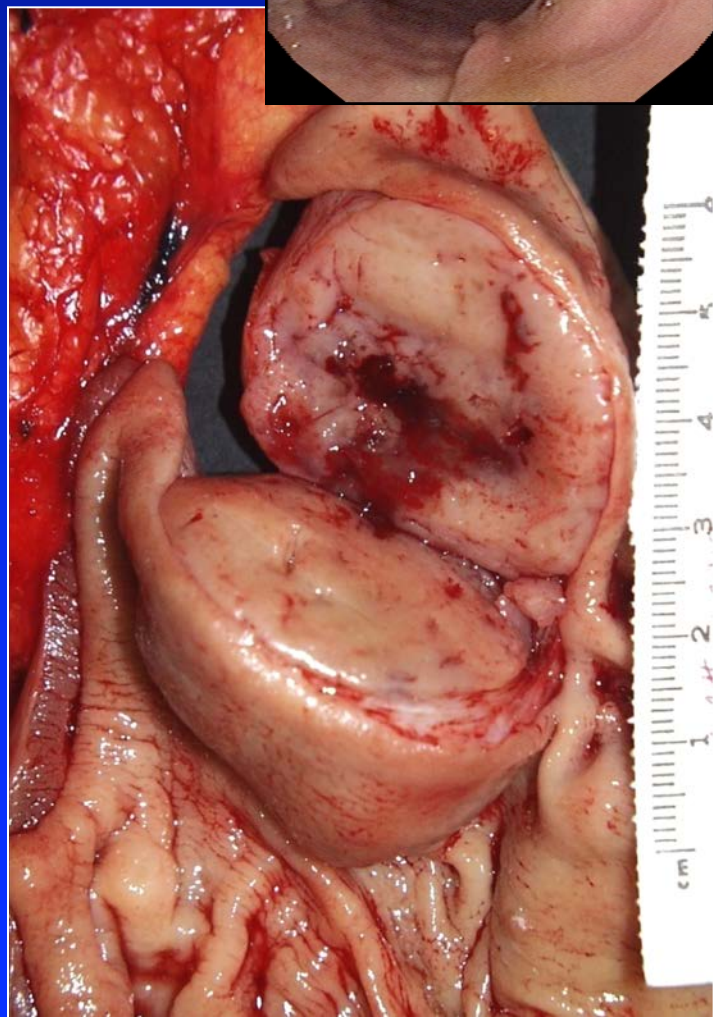
Director, Knight Diagnostic Laboratories

KNIGHT CANCER INSTITUTE
Oregon Health & Science University

GI Stromal Tumor



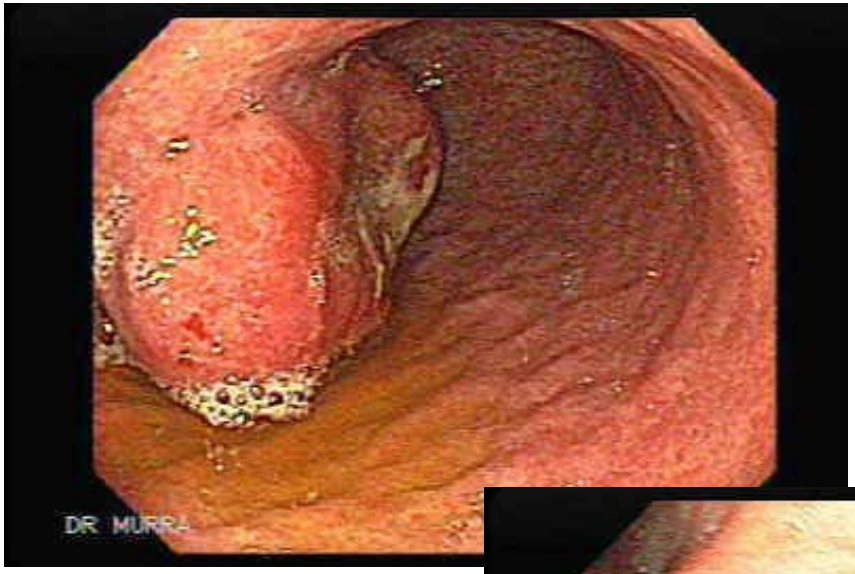


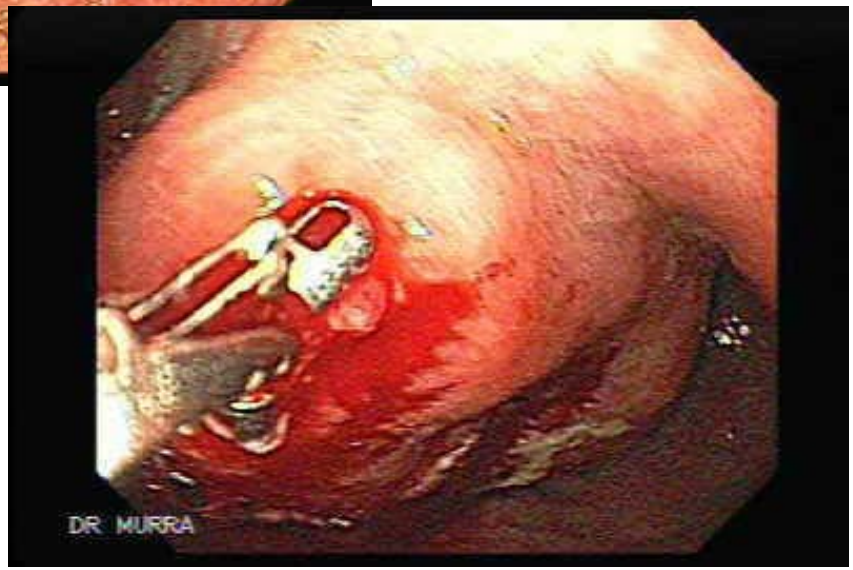


- Comprise only 0.2% of all GI tumors, but 80% of GI sarcomas
- 5000 – 6000 new cases per year in the U.S.

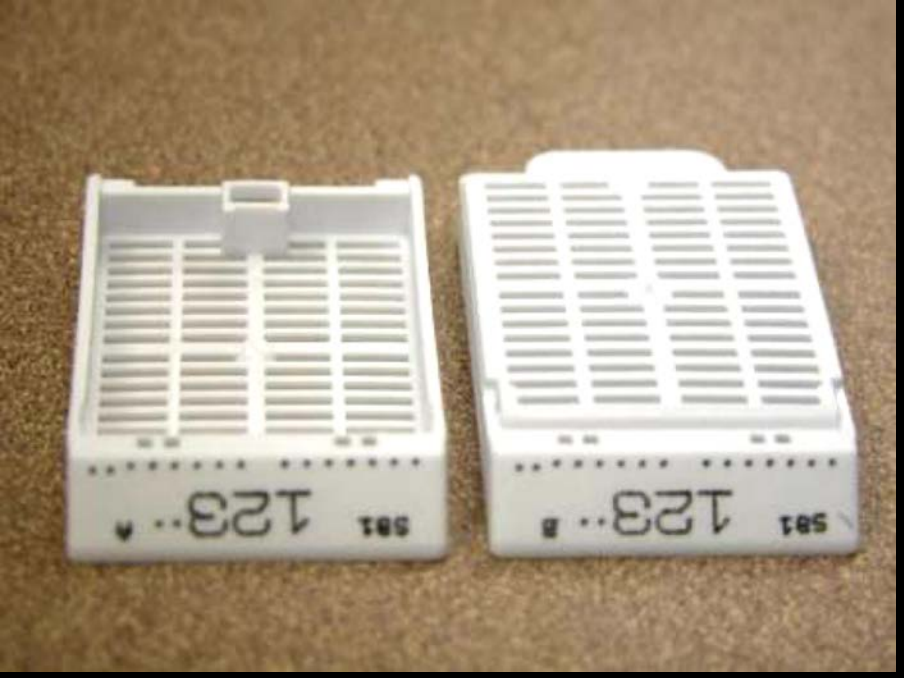
Tumor Biopsy

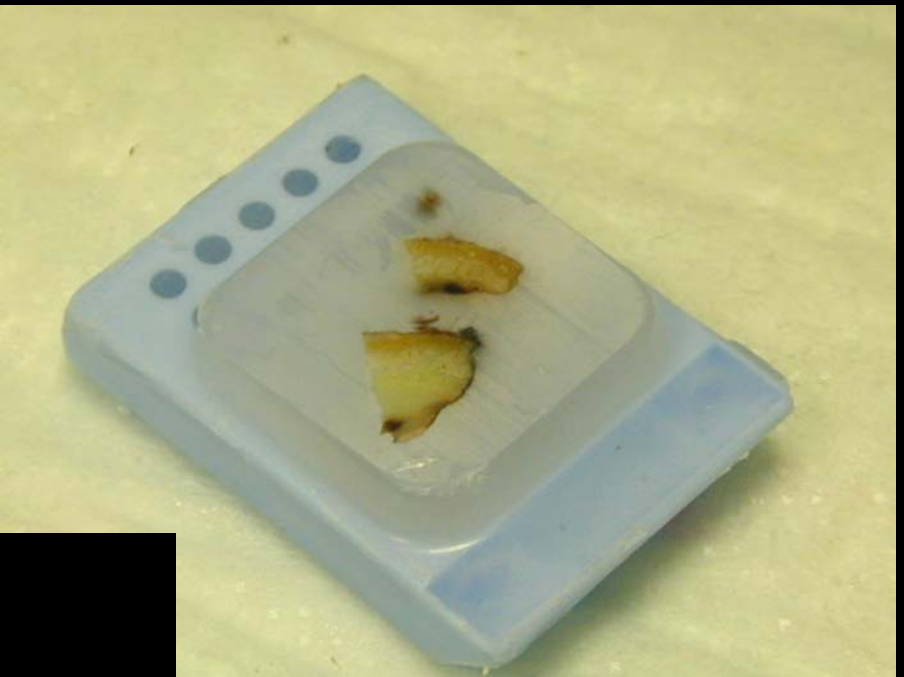
Endoscopic Biopsy





Specimen sent to Pathology lab in formalin

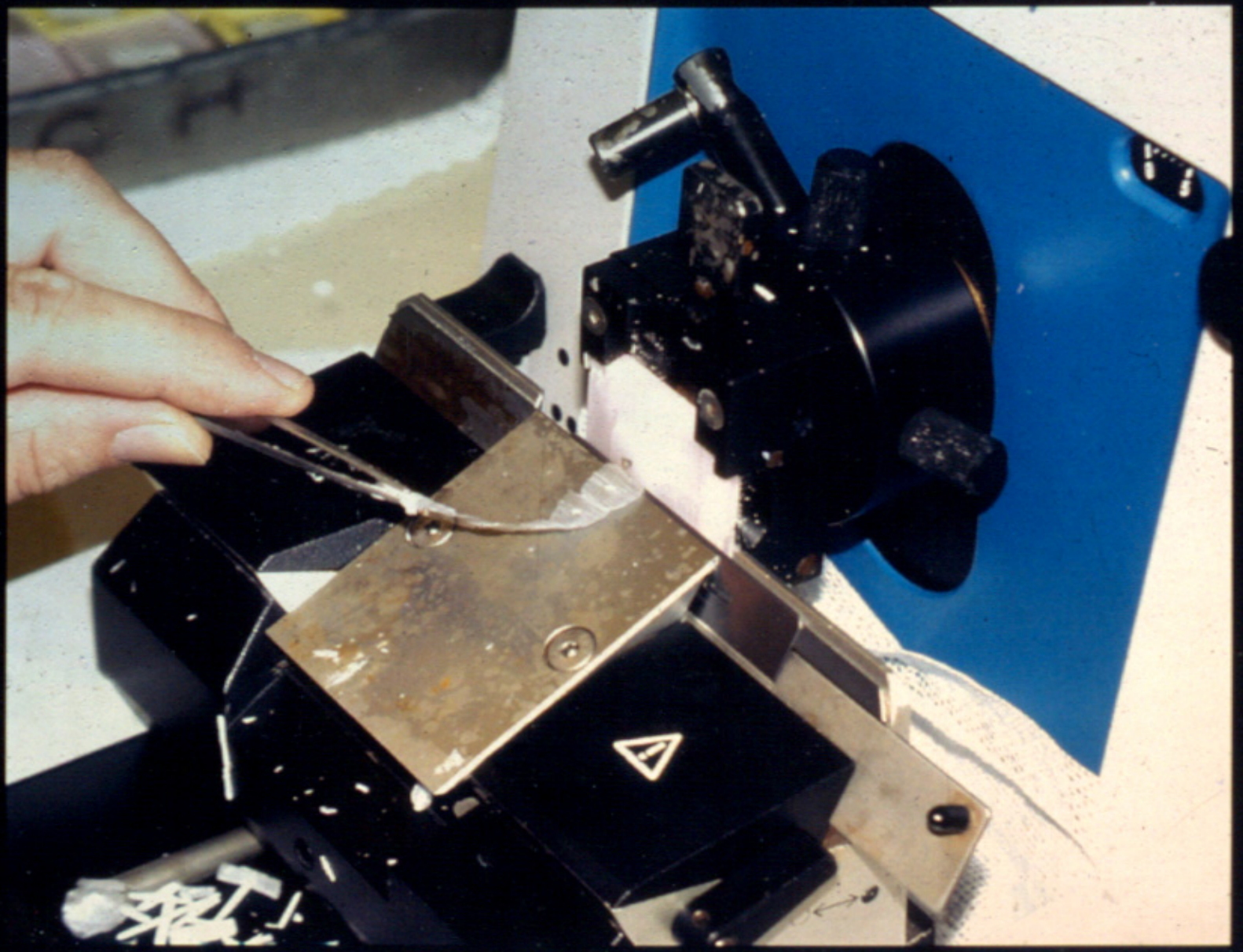




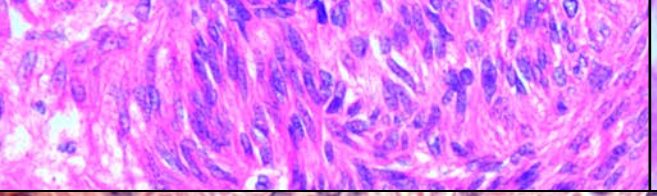
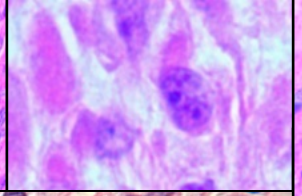
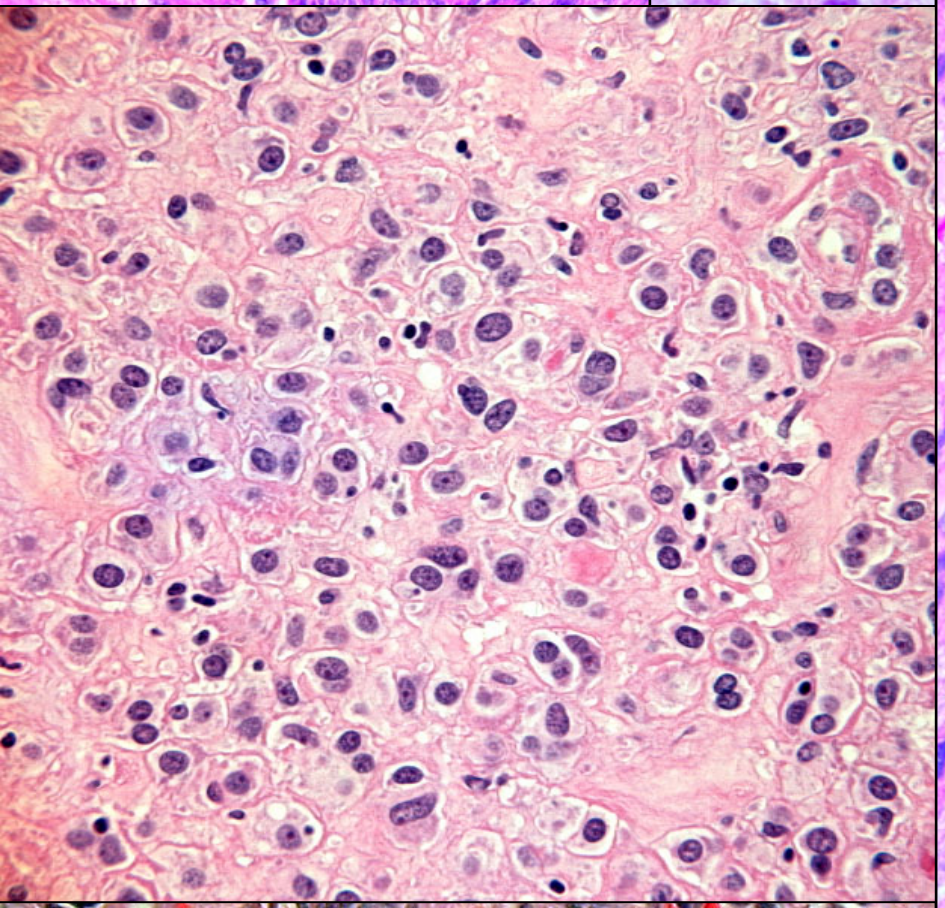
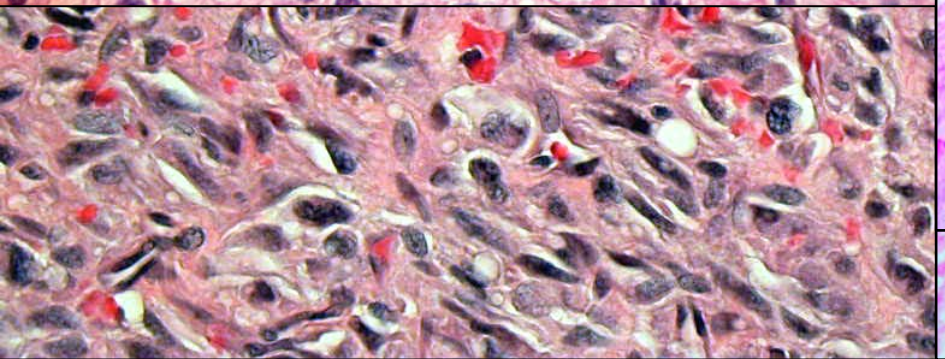
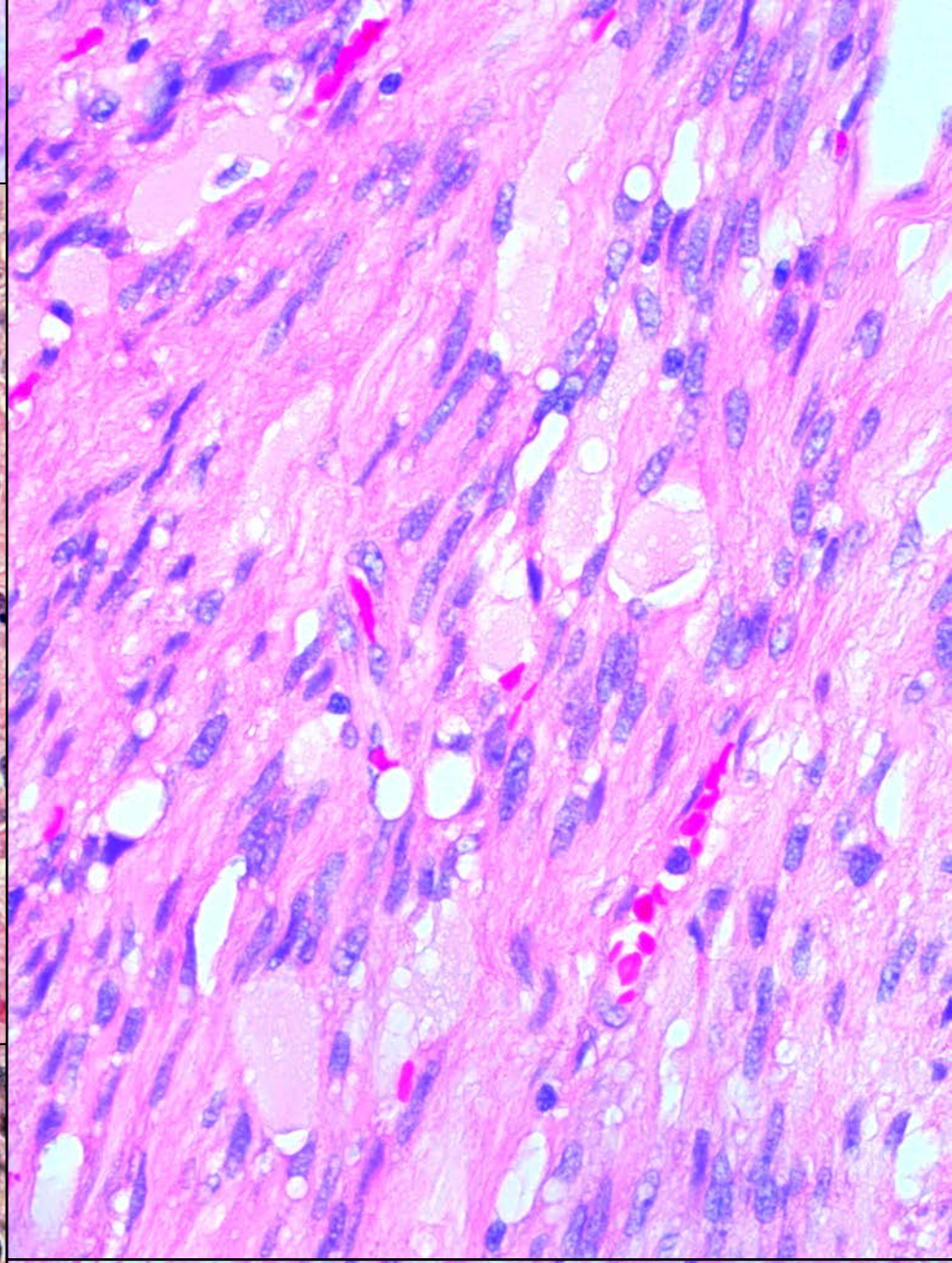
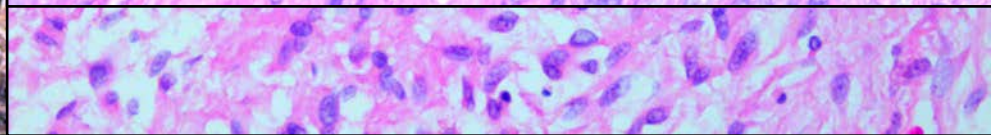
Standard (Rotary) Microtome

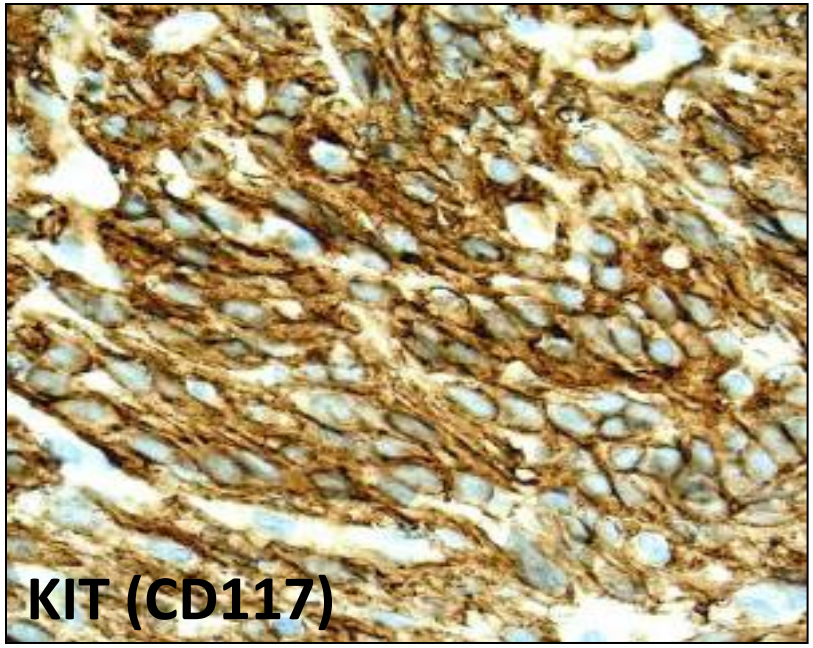
For sectioning paraffin-embedded tissues

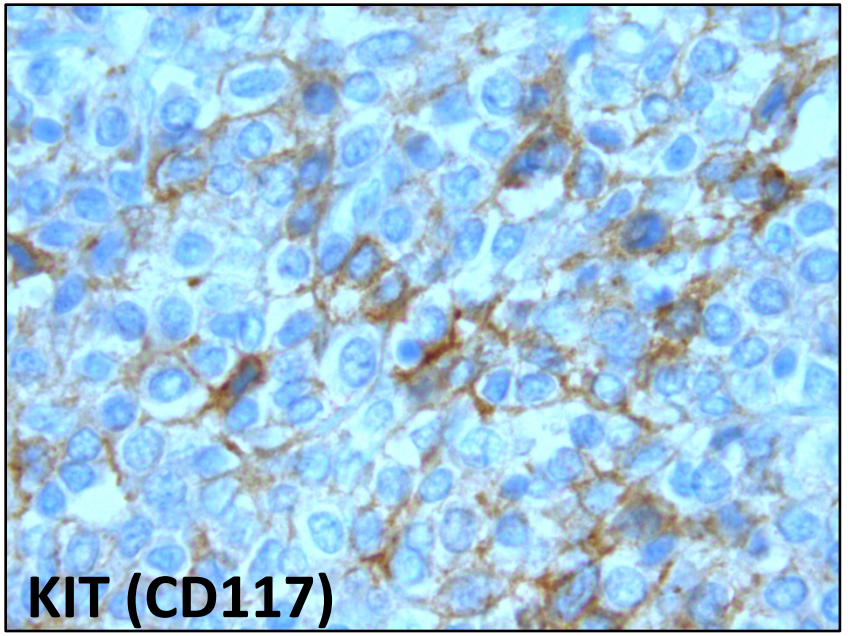
Sectioning a paraffin-embedded biopsy

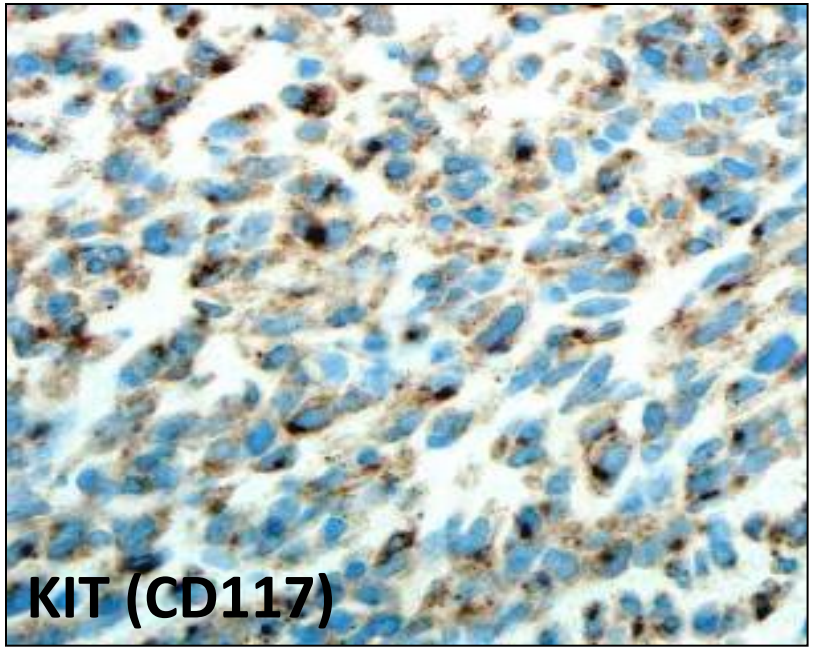


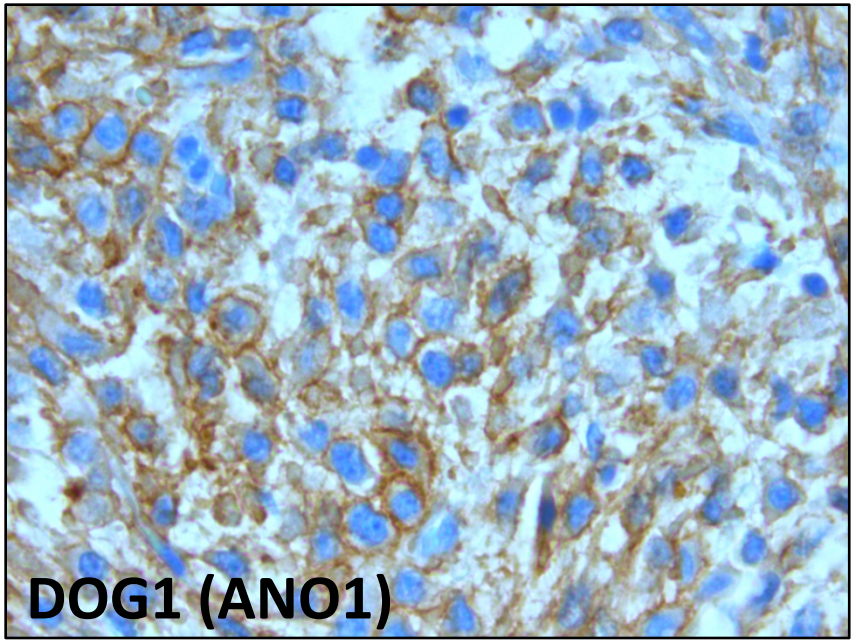
Ribbon of biopsy sections floated on waterbath



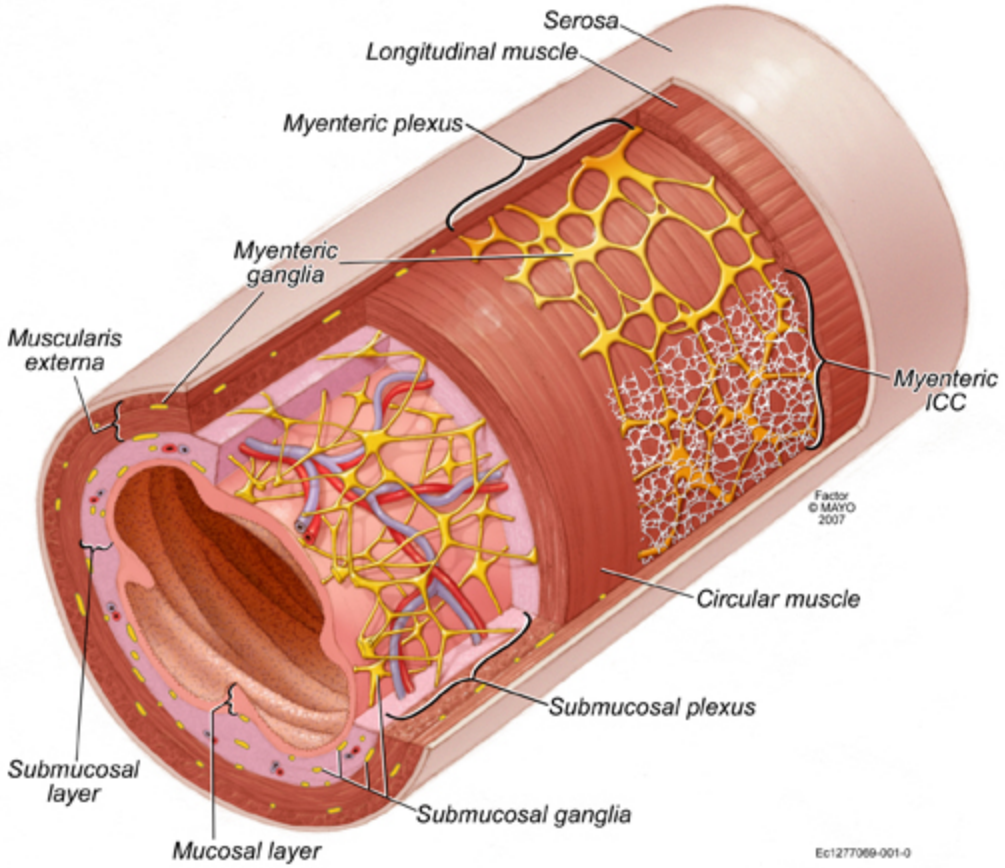


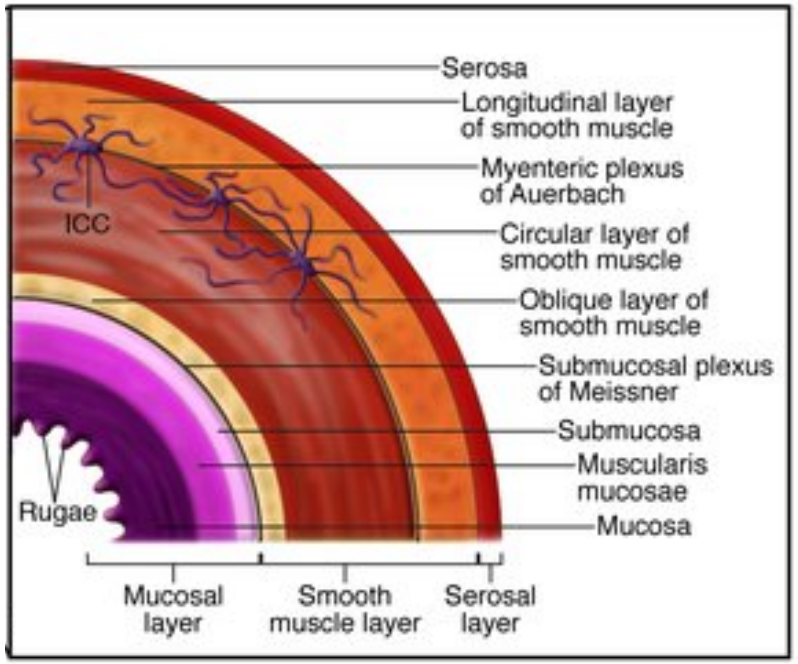




Novelli M. et al. Histopathology 2010; 57(2):259-270.

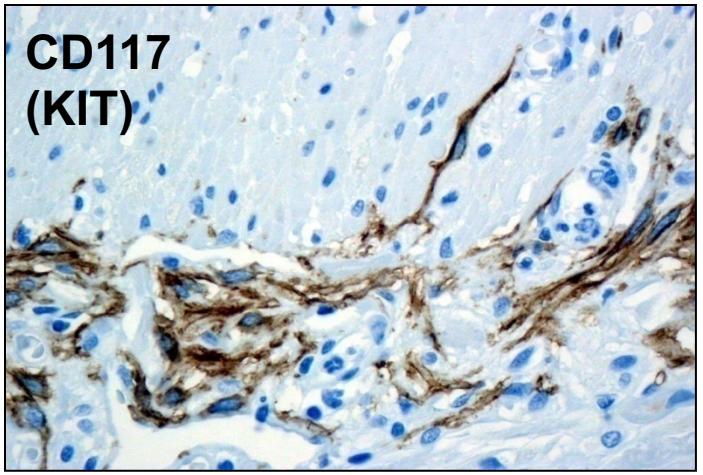
Interstitial Cells of Cajal





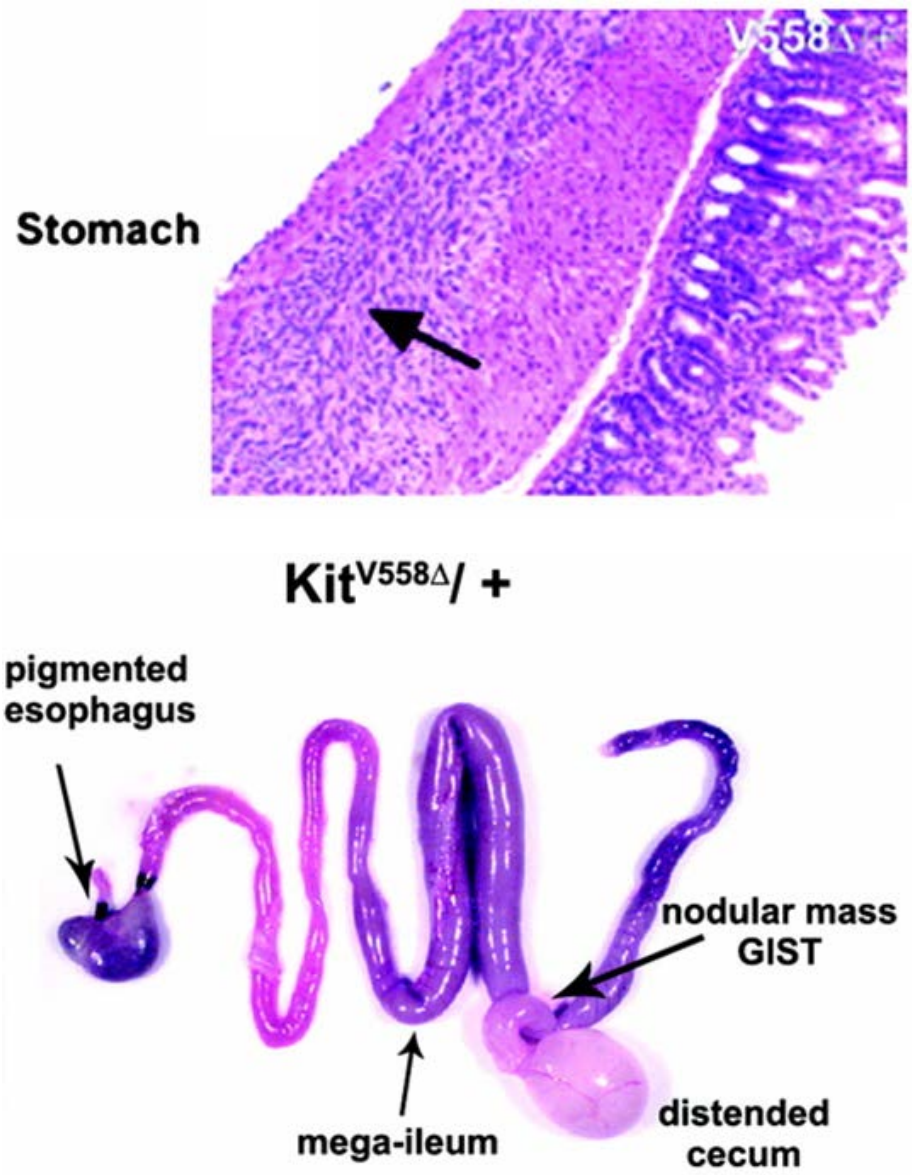
Like GISTs, ICC cells:

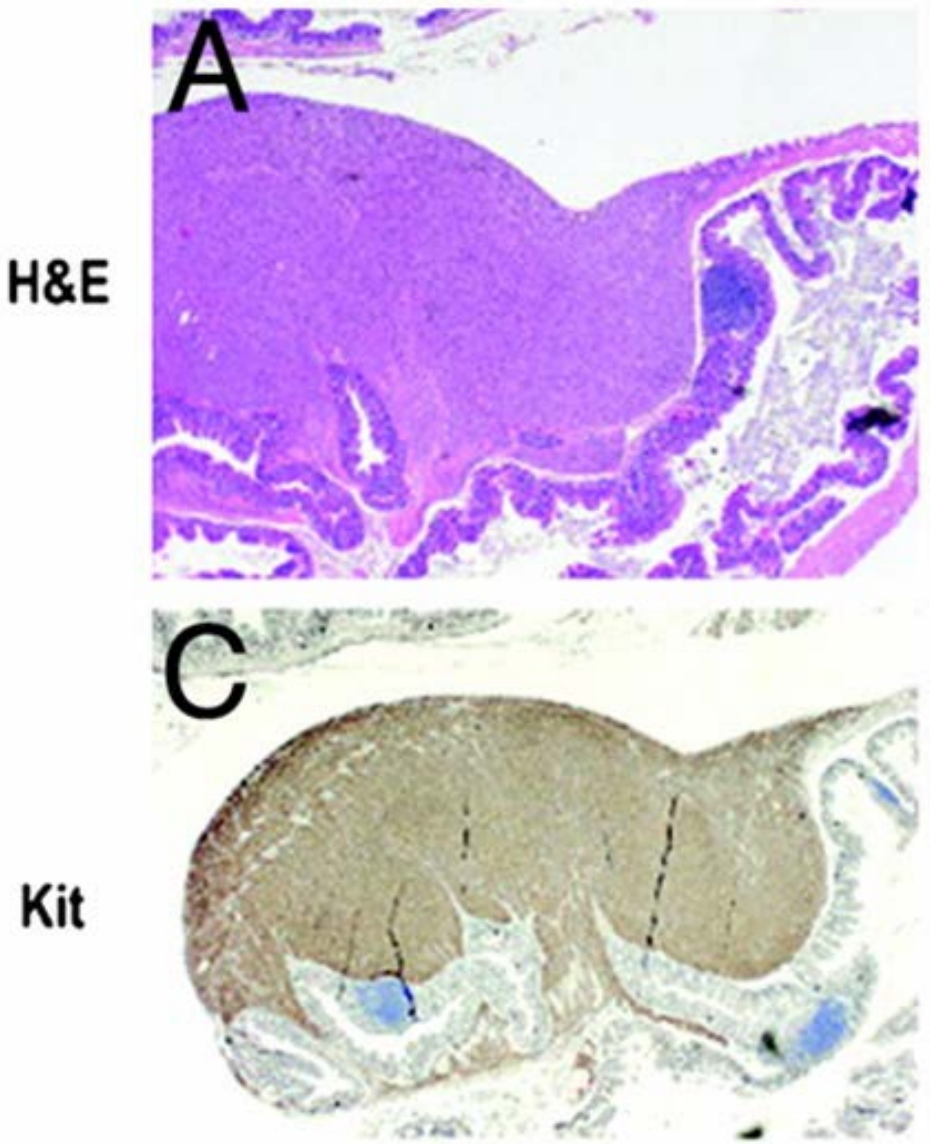
- express KIT
- express DOG1
- express ETV1



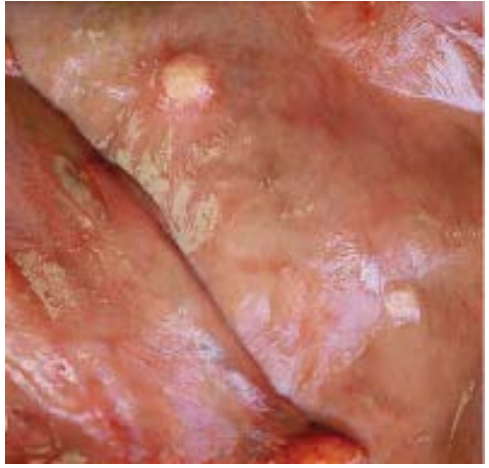
Linden DR, Farrugia G. Autonomic Control of Gastrointestinal Function. In: Low PA, Benarroch EE, eds. Clinical Autonomic Disorders. 3rd ed. Baltimore, MD: ©Lippincott Williams & Wilkins; 2008:88-105

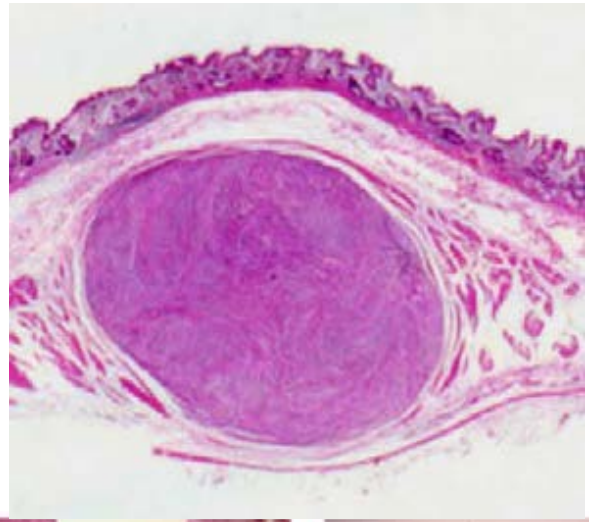
Mouse Model Of GIST

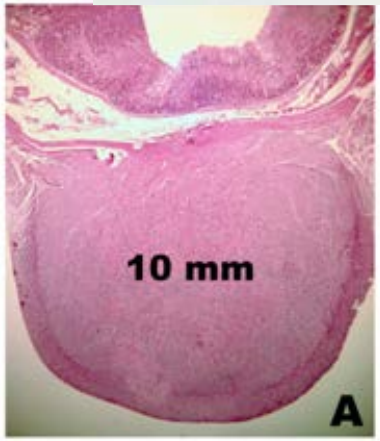


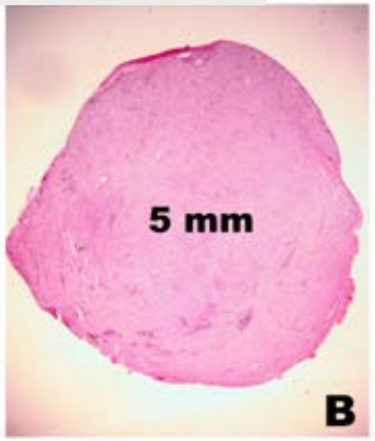


Sommer et al., PNAS *100*: 6706-11, 2003









Micro-GISTs

- GISTs < 1 cm are common in the general population
  - 10-30% in the stomach
  - <0.2% in the colon & appendix
- Compared with larger GISTs, micro-GISTs have:
  - Similar *KIT* mutations
  - Lower mitotic index
  - Benign morphology

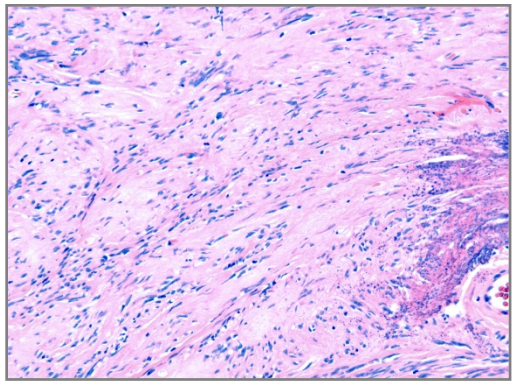
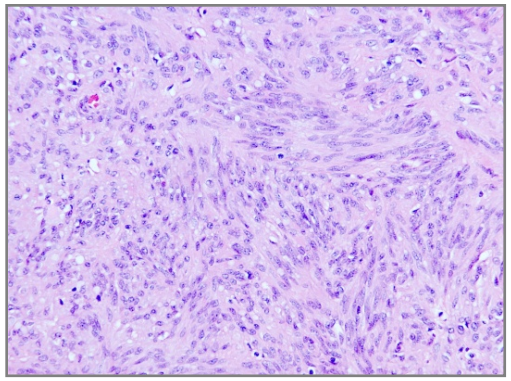
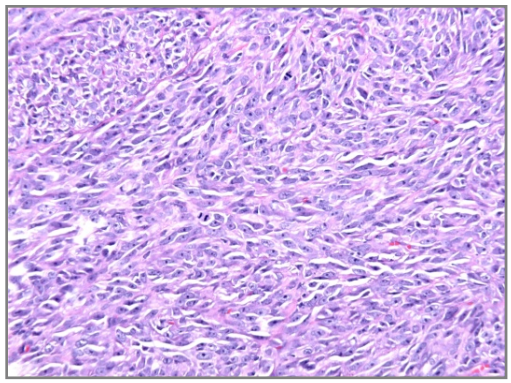
Corless et al. Am J Pathol, 2002

Rossi et al. 34:1480-1491, 2010

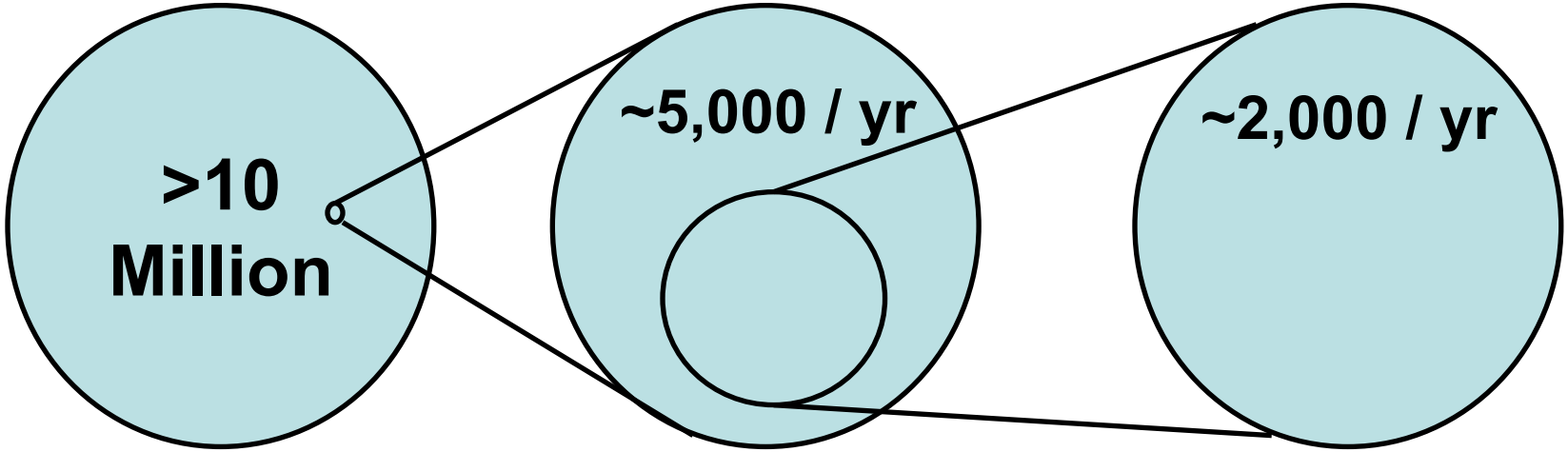
Agaimy et al. Am J Surg Pathol 31:113-130, 2007

Agaimy et al. Am J Surg Pathol 32:867-873, 2008

GIST Progression

ICC Cells → Micro-GIST → Low risk GIST → Malignant GIST



KIT or *PDGFRA* mutation

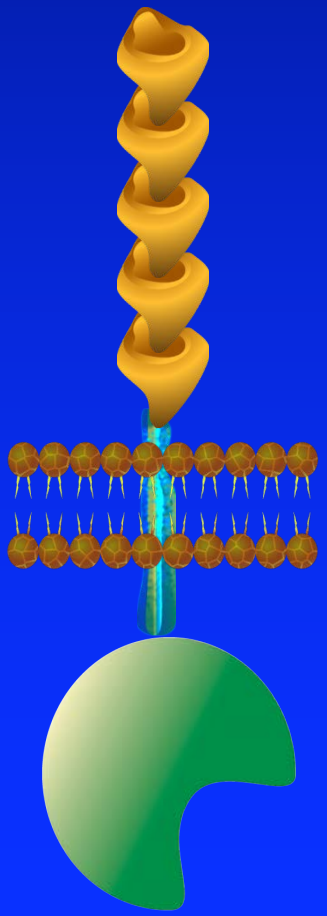
Loss of 14q, 22q
MAX

Loss of 1p
CDKN2A, RB1, TP53

KIT and *PDGFRA* Mutations in GIST

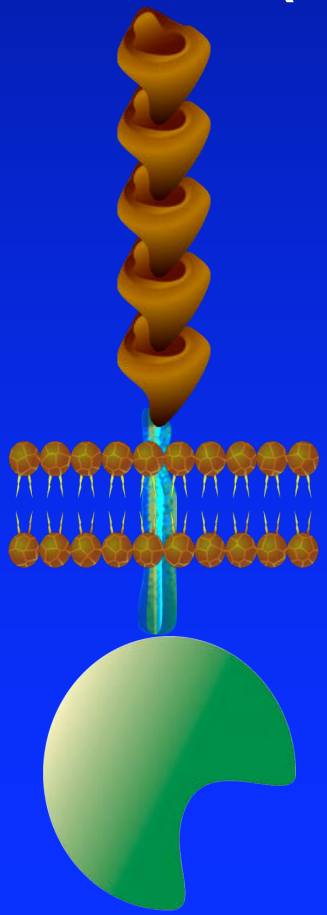
'Wild-type' tumors: 15%

KIT (75%)



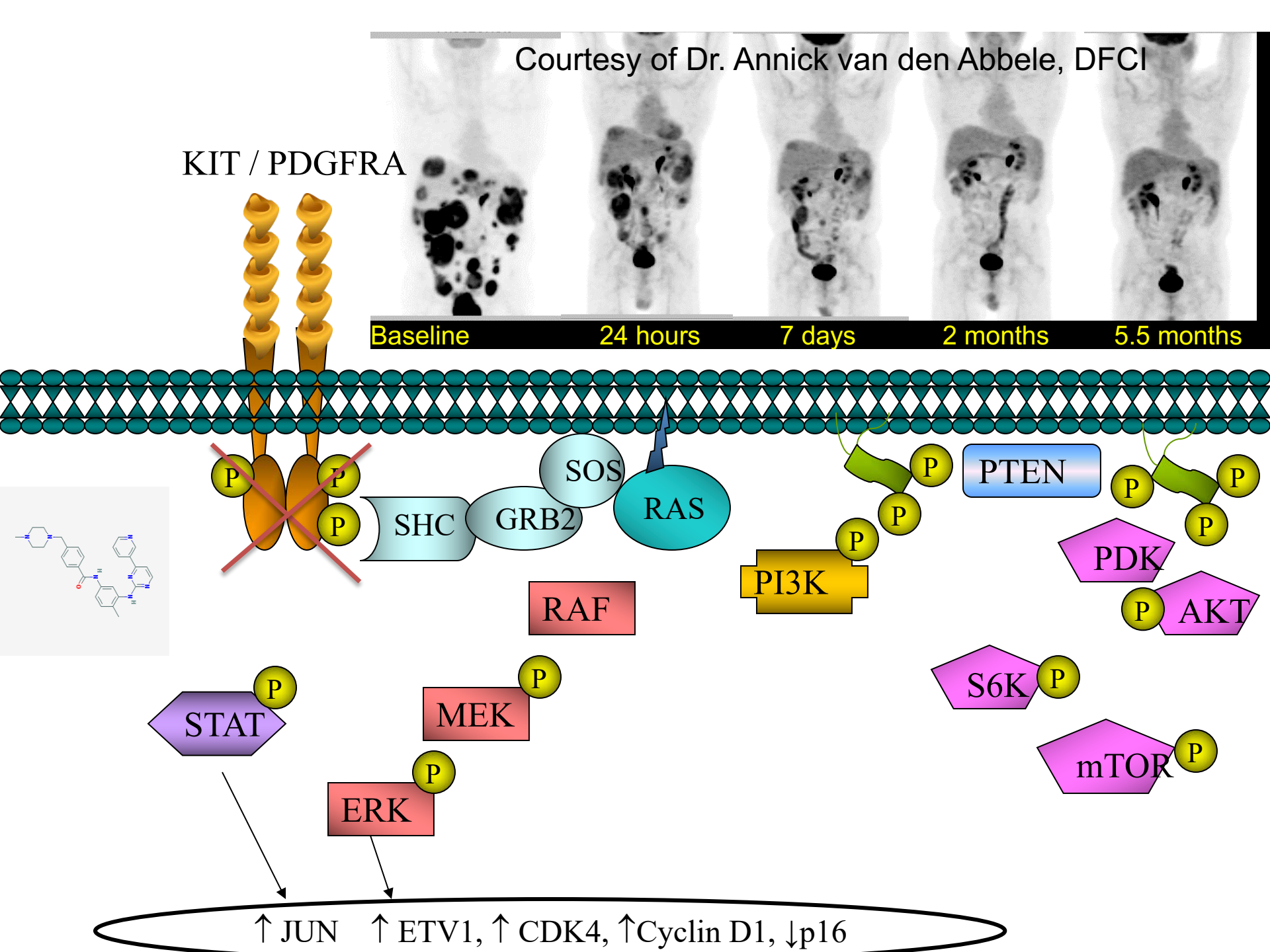
- Exon 9 (8%)
- Exon 11 (65%)
- Exon 13 (1%)
- Exon 17 (1%)

PDGFRA (10%)

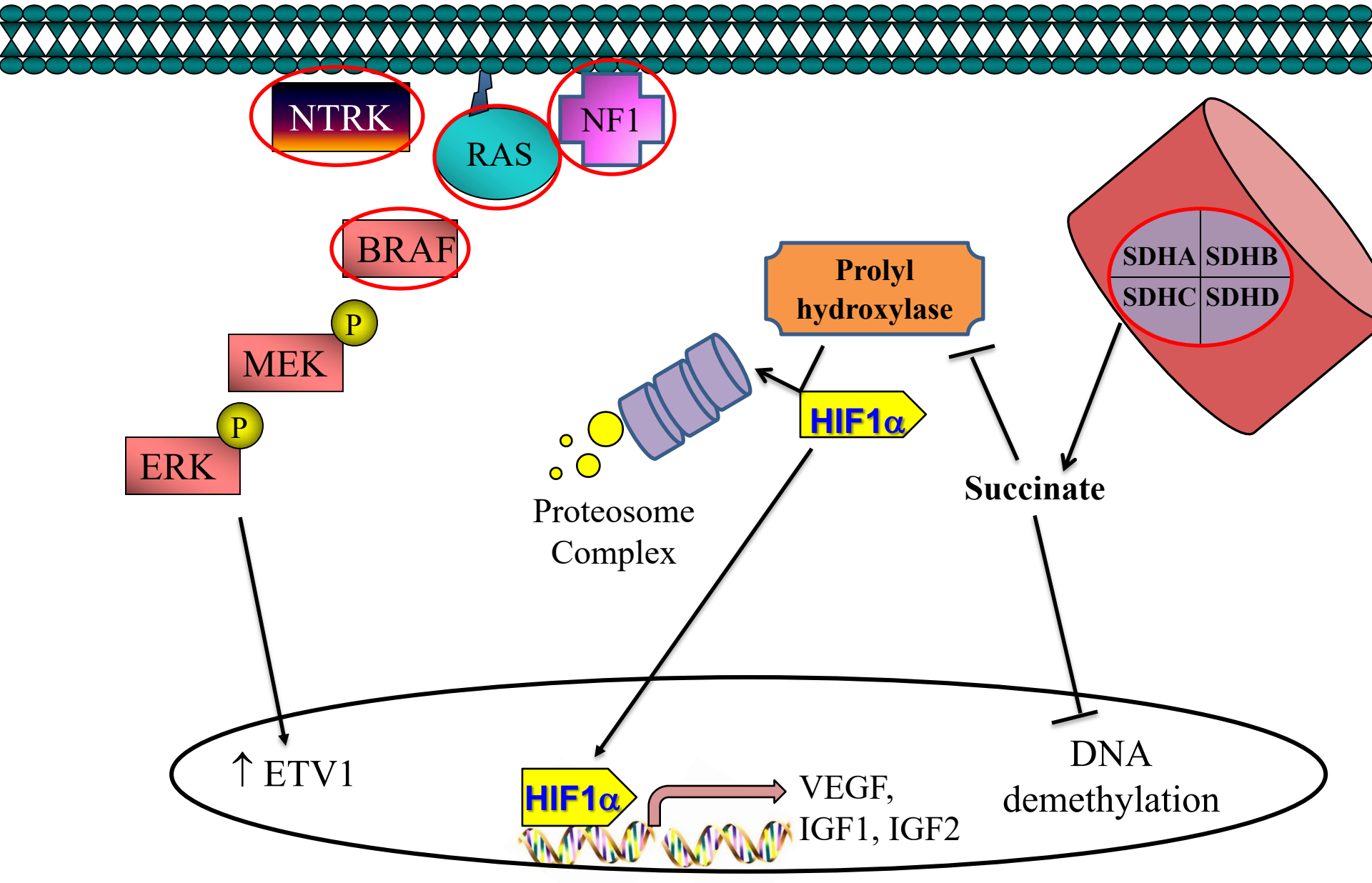


- Exon 12 (2%)
- Exon 14 (rare)
- Exon 18 (8%)

(Includes D842V)

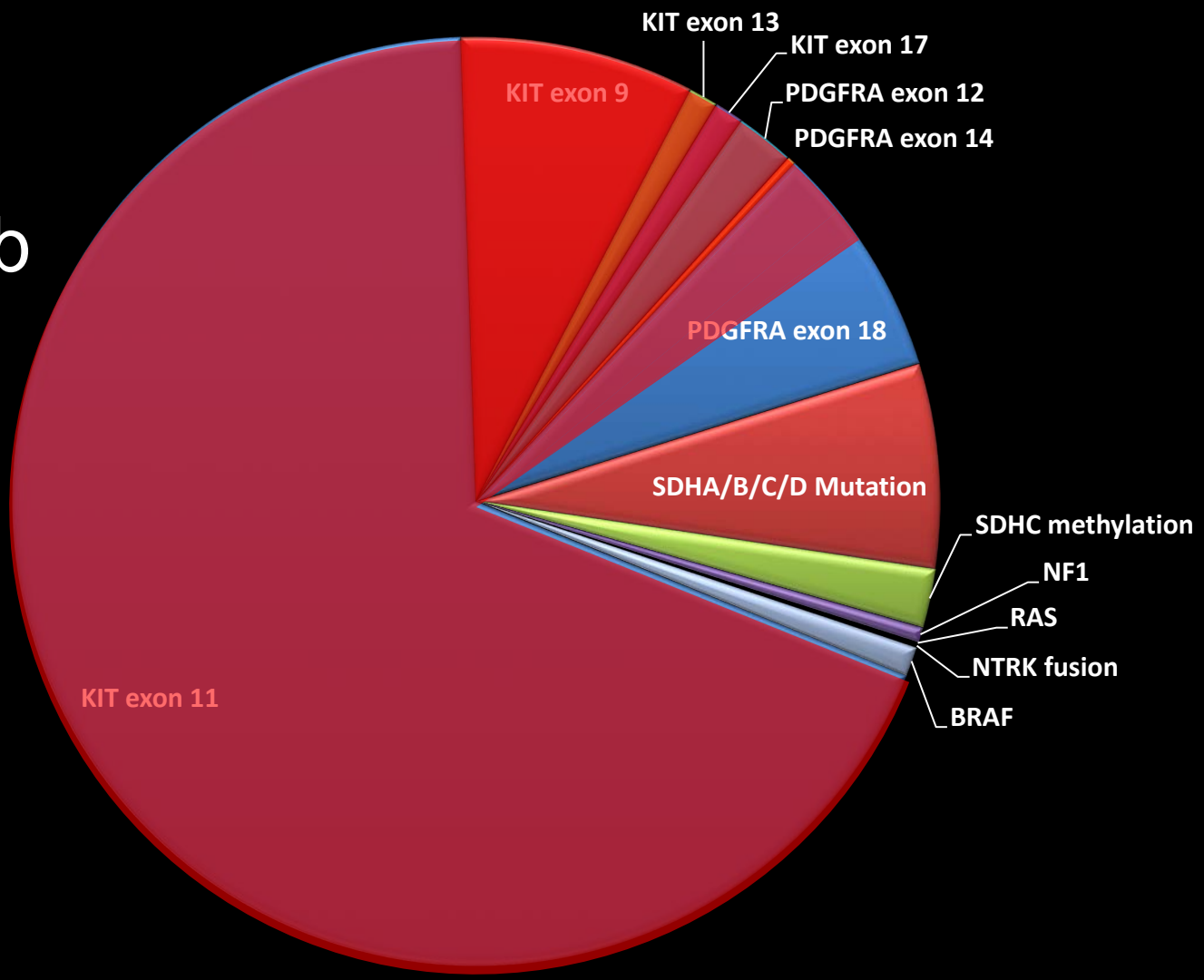

Courtesy of Dr. Annick van den Abbele, DFCI
Baseline
24 hours
7 days
2 months
5.5 months
KIT / PDGFRA
P
SHC
GRB2
SOS
RAS
PTEN
PI3K
PDK
AKT
RAF
MEK
ERK
STAT
S6K
mTOR
↑ JUN ↑ ETV1, ↑ CDK4, ↑Cyclin D1, ↓p16

Genetic Alterations in Wild-type GISTs



Molecular Subtypes of GIST

Imatinib



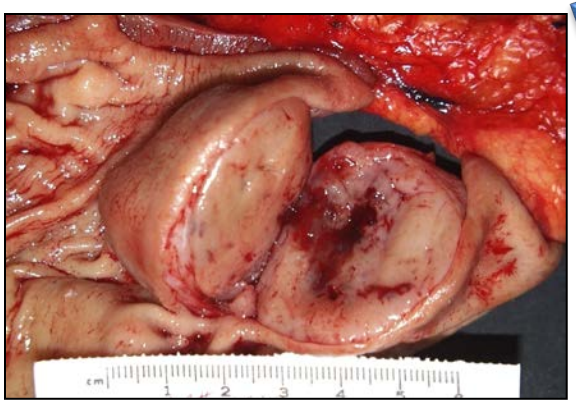
GIST Stage And Risk At Presentation Prospective Population-Based Study

- During a 2 year period, 115 GISTs were diagnosed in the Rhone Alps region of France.
- Among these:
  - 88% had not spread
    - 36.5% were low or very low risk
    - 35.6% were intermediate risk
    - 27.7% were high risk
  - 12% were metastatic

Cassier et al. British Journal of Cancer, 2010; 103:165–170

GIST Management

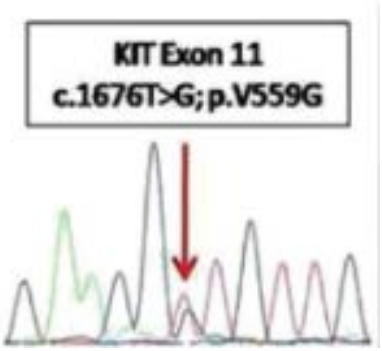
Morphology
CD117+
DOG1+



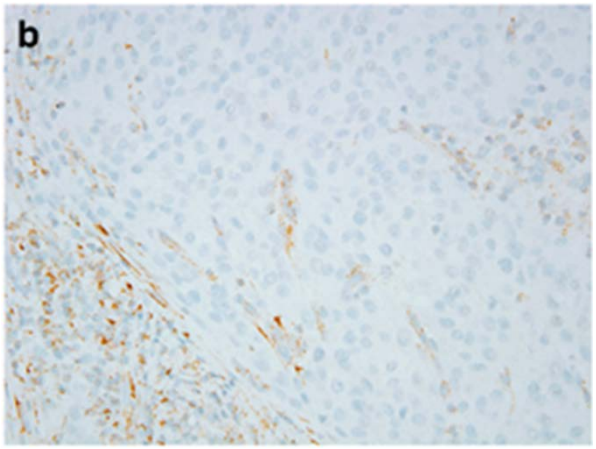
Low risk of recurrence

High risk of recurrence or metastatic
Treat with imatinib?

- Tumor size
- Tumor location
- Mitoses per 5 mm^2

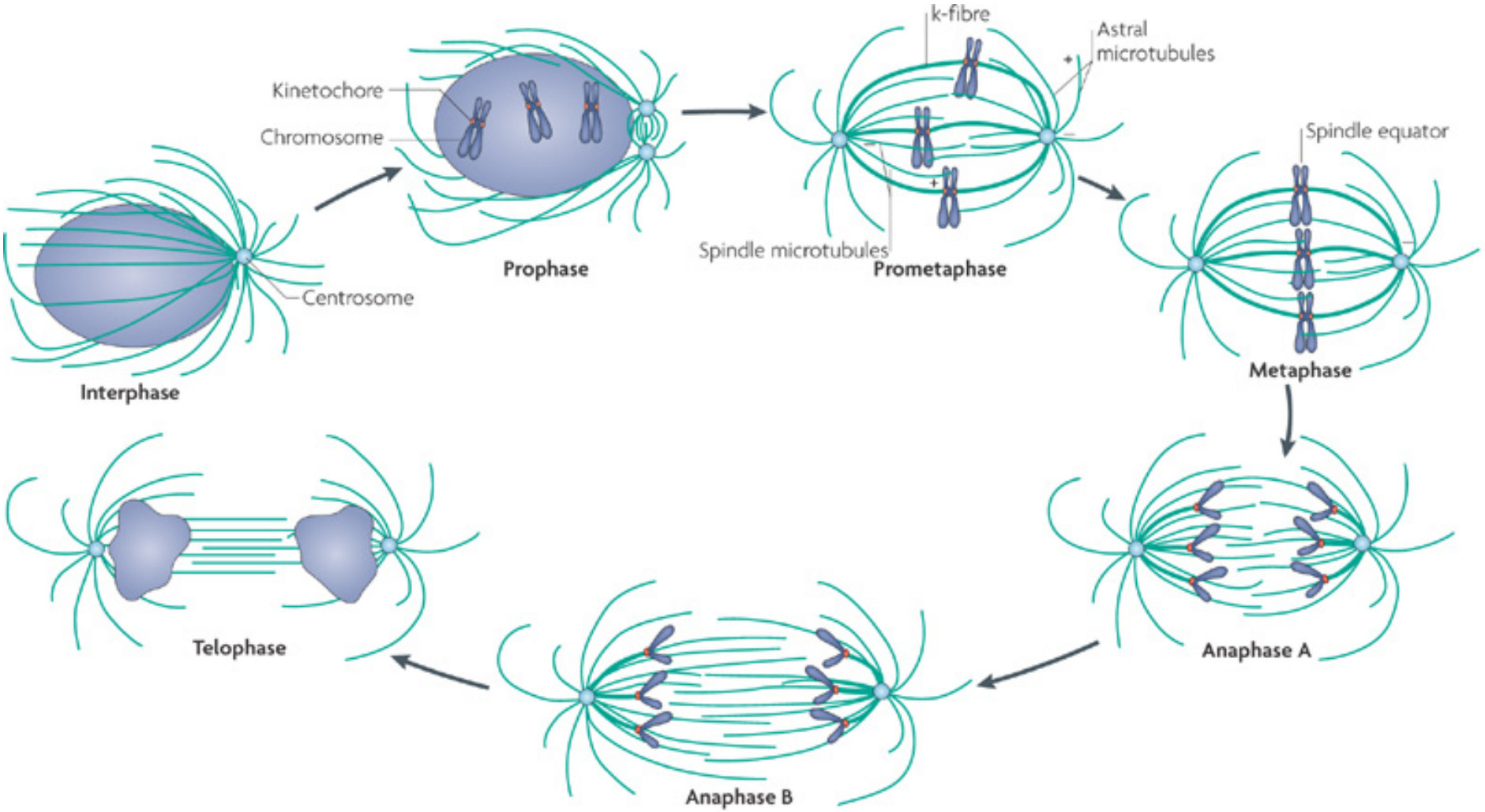


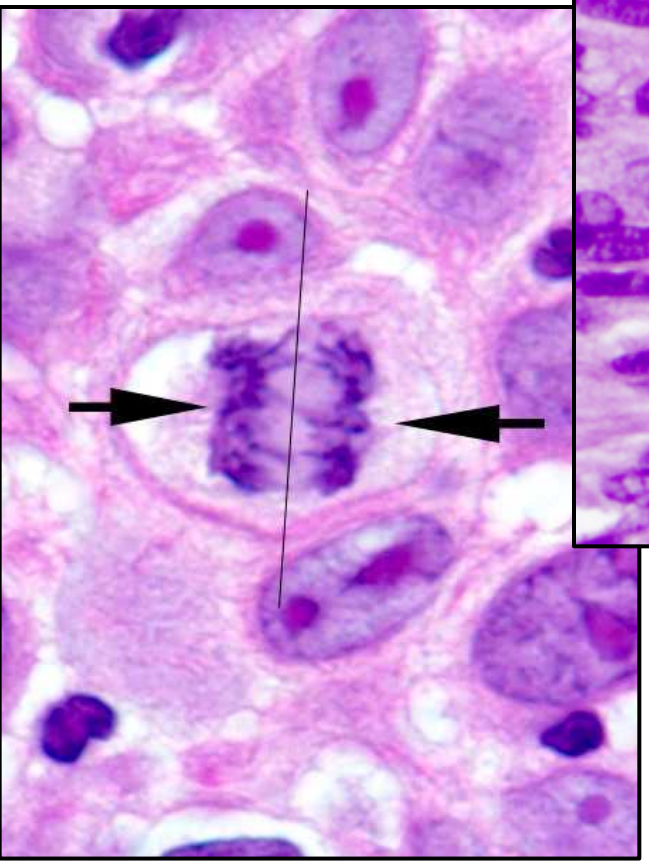
SDHB Stain

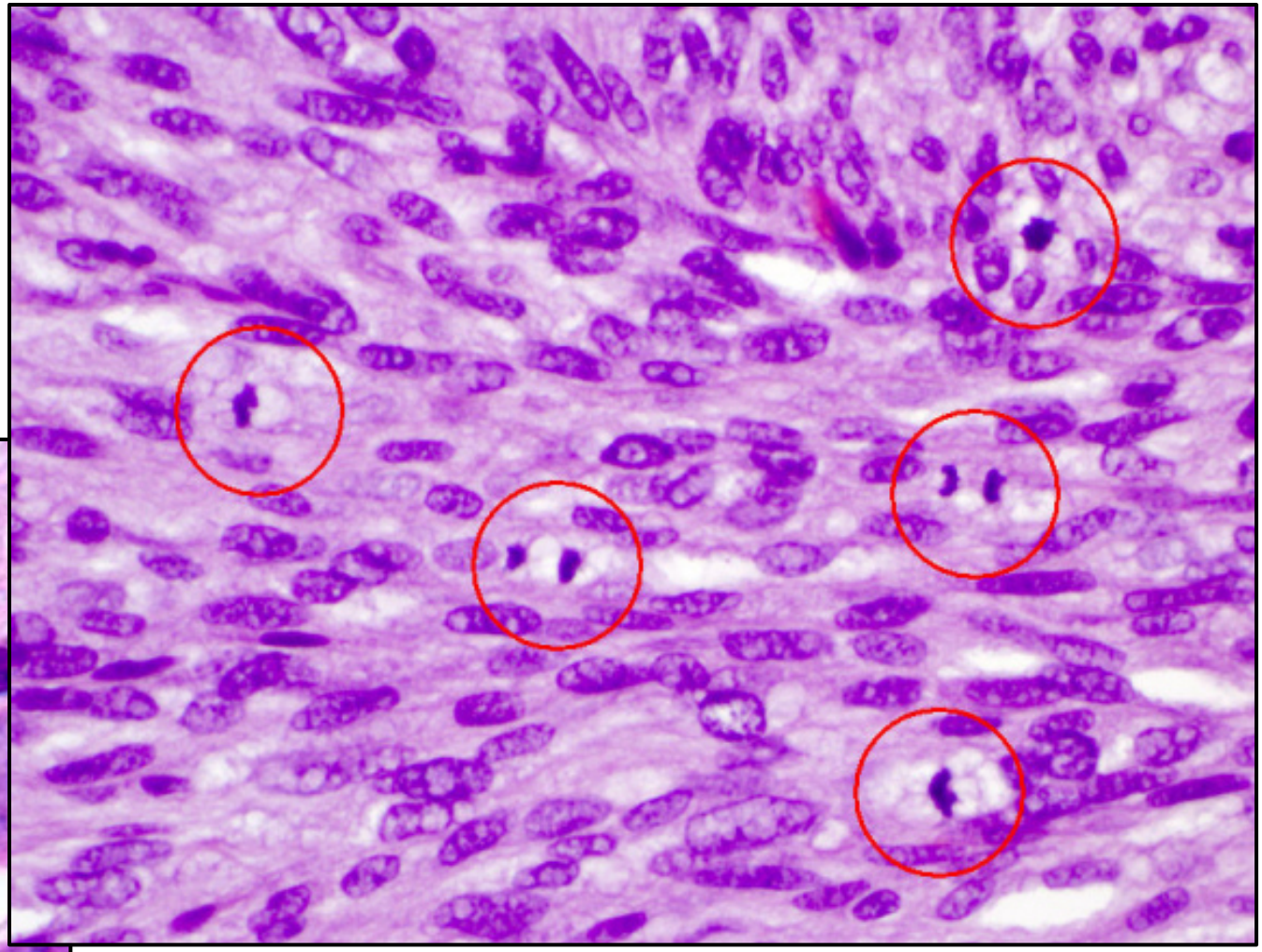


KIT exon 11 mutation → 400 mg imatinib
KIT exon 9 mutation → 800 mg imatinib
PDGFRA D842V → no imatinib (clinical trial)

Imatinib –> No


Kinetochore
Chromosome
Centrosome
Interphase
Prophase
k-fibre
Astral microtubules
Spindle microtubules
Prometaphase
Spindle equator
Metaphase
Anaphase A
Anaphase B
Telophase





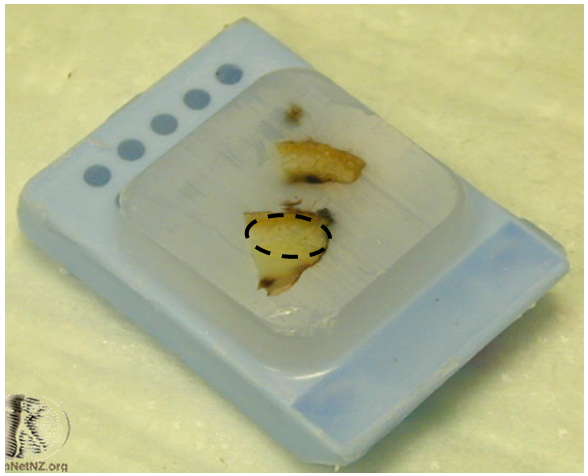
Primary GIST – Risk of Recurrence

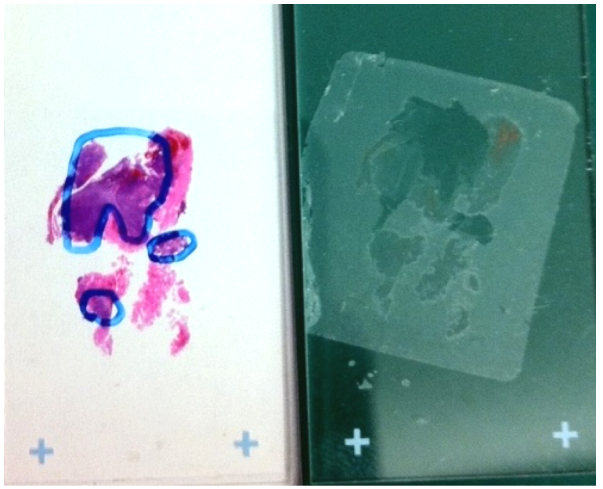
	Size	Gastric (n=1055)	Jejunum/Ileum (n=629)	Duodenum (n=144)	Rectum (n=111)
Mitotic Index ≤5 per 5 mm^2	≤ 2 cm	0%	0%	0%	0%
	> 2 ≤ 5 cm	1.9%	4.3%	8.3%	8.5%
	> 5 ≤ 10 cm	3.6%	24%	Insuff. data	Insuff. data
	> 10 cm	10%	52%	34%	57%
Mitotic Index >5 per 5 mm^2	≤ 2 cm	(None)	(High)	Insuff. data	54%
	> 2 ≤ 5 cm	16%	73%	50%	52%
	> 5 ≤ 10 cm	55%	85%	Insuff. data	Insuff. data
	> 10 cm	86%	90%	86%	71%

Miettinen & Lasota, Semin Diagn Pathol, 23(2):70-83, 2006

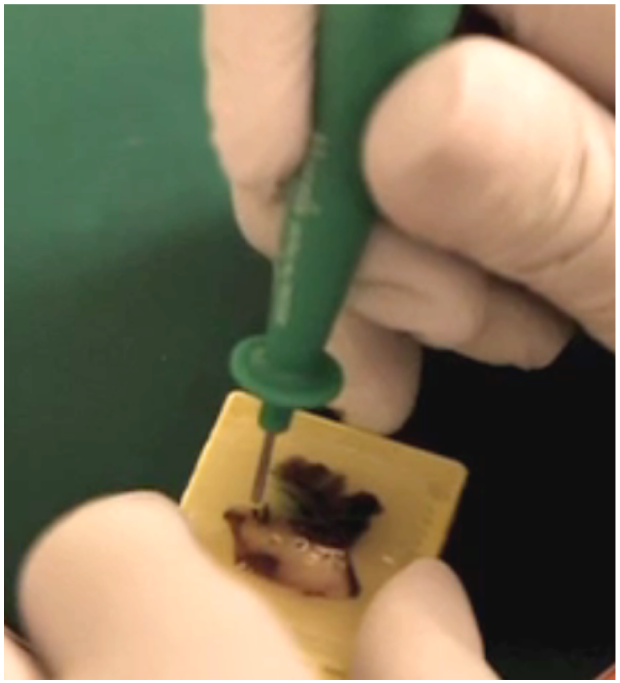
Selecting Tumor-Rich Material for DNA Extraction

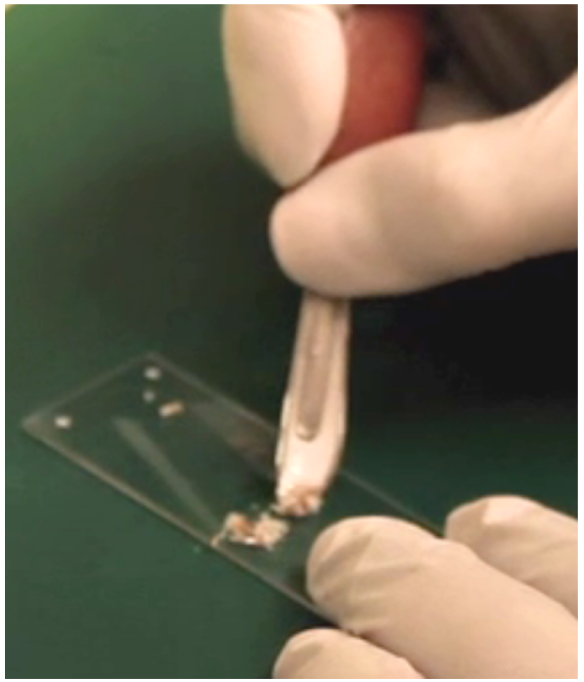
Coring a block



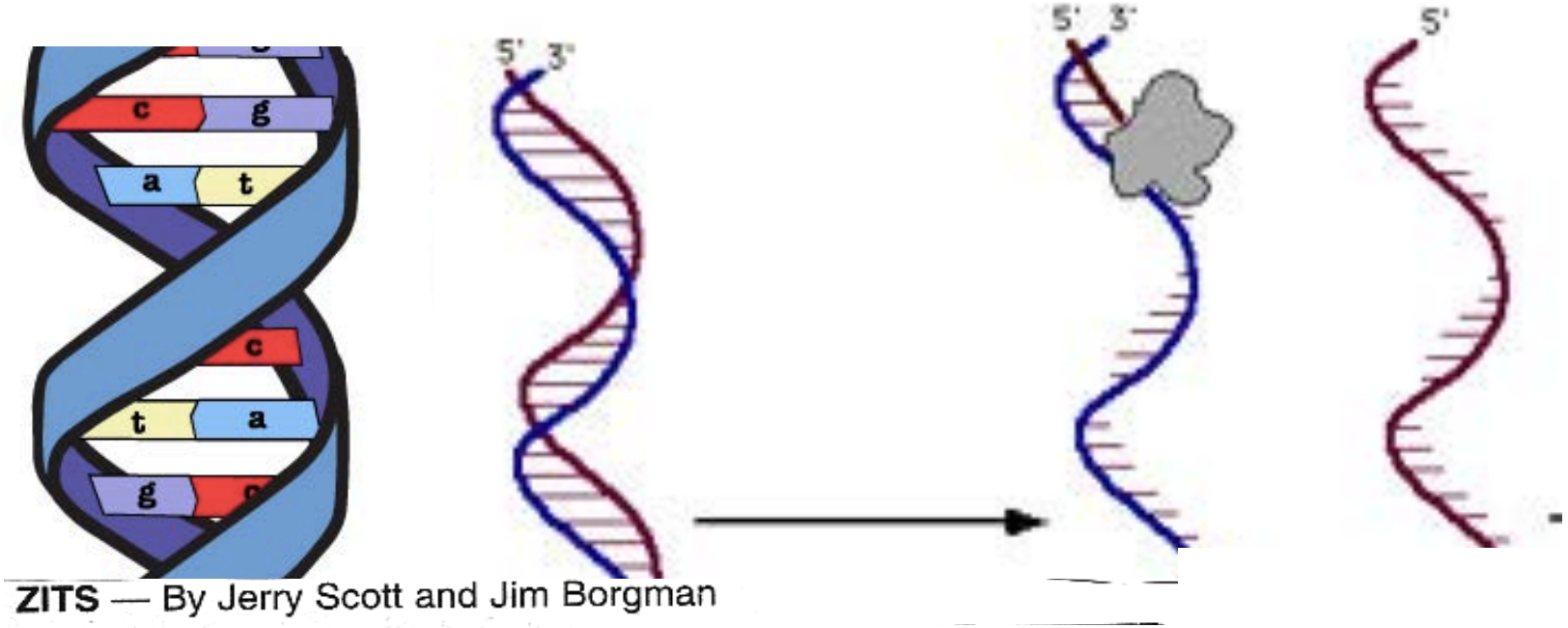


Scraping slides





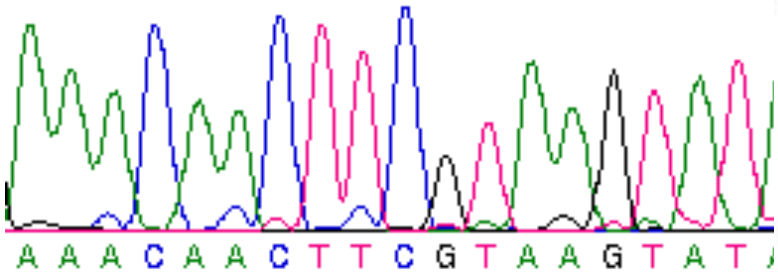
Looking for Mutations: DNA Sequencing



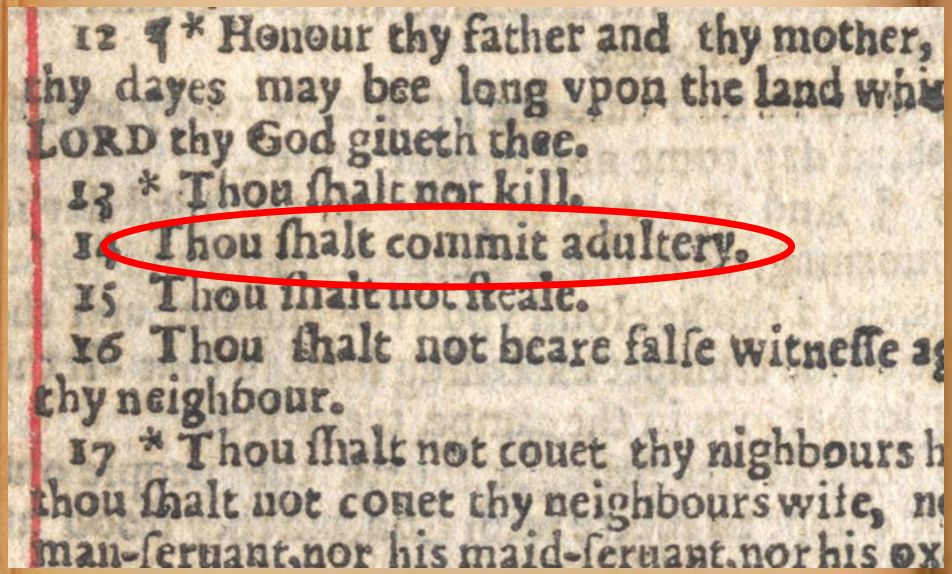
ZITS — By Jerry Scott and Jim Borgman

Next-Generation DNA Sequencing

- Massively parallel sequencing (many sequencing reactions performed simultaneously)



Bible Replication Errors


12 ¶ * Honour thy father and thy mother,
thy dayes may bee long vpon the land whi
LORD thy God giueth thee.
13 * Thou ſhalt not kill.
14 Thou ſhalt commit adultery.
15 Thou ſhalt not ſteale.
16 Thou ſhalt not beare falſe witneſſe a
thy neighbour.
17 * Thou ſhalt not couet thy nighbours h
thou ſhalt not couet thy neighbours wife, n
man-ſeruant,nor his maid-ſeruant,nor his ox

1632 Edition
The 'Wicked Bible'

GIST Genome Errors

KIT Gene Mutation

[Pro Tyr Val His Lys]

CCT TAT GTT CAC AAA

↓

CCT TAT --- CAC AAA

[Pro Tyr --- His Lys]

Activated KIT leads to development of GI stromal tumors

Bible Replication Errors

1795 Edition *Mark 7:27*

'Let the children first be filled'

'Let the children first be killed'

GIST Genome Errors

KIT Gene Mutation

[Ala Thr Val Lys Ser]

GCT ACA GTT AAA TCT

GCT ACA GAG AAA TCT

[Ala Thr Asp Lys Ser]

Summary

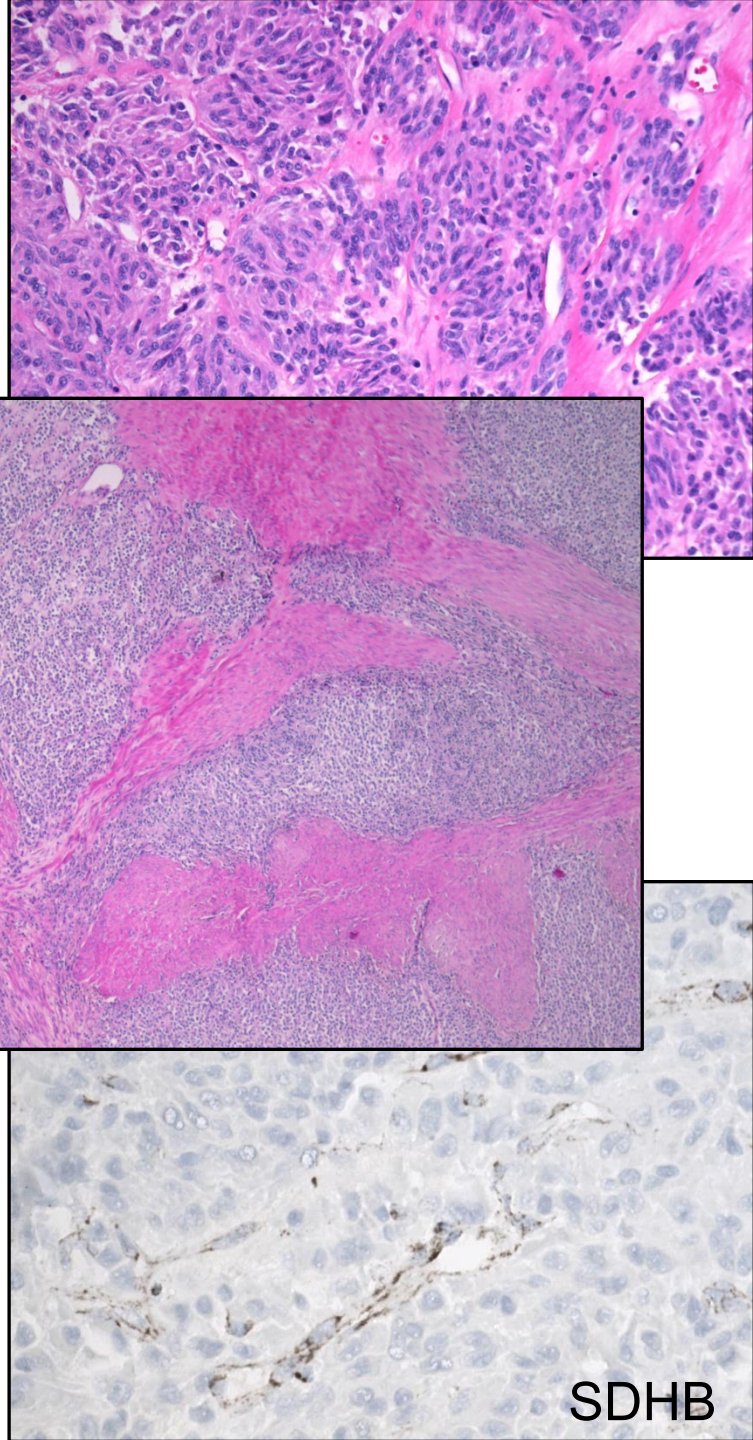
- GISTs are a family of tumors arising from mutations in a number of different genes
- Most GISTs probably arise from ‘micro-GISTs’ through acquisition of mutations or other genetic alterations beyond KIT/PDGFRA/SDH
- Next-gen sequencing is helpful in molecularly subtyping GISTs
- Mitotic index, tumor size and tumor location are the 3 most important factors in determining the likelihood of disease recurrence

Predictive Value of Kinase Genotype In Metastatic GIST Patients On Imatinib

- Exon 11-mutant tumors:
  - Better progression-free and overall survival compared to exon 9 and WT tumors
  - 400 mg is adequate dose
- Exon 9-mutant tumors:
  - Improved progression-free survival when treated with 800 mg imatinib
- PDGFRA D842V-mutant tumors:
  - Resistant

SDH-Deficient GISTs

- Deficient in an enzyme called succinate dehydrogenase
- Nearly always gastric origin
- Multi-nodular growth pattern
- Low mitotic rate, but high rate of recurrence and metastasis
- Poor response to imatinib



Molecular Classification of GISTs

Genetic type	Relative Frequency	Anatomic Distribution	Germline Examples
KIT Mutation	**75%**		
Exon 8	Rare	Small bowel	1 Kindred
Exon 9 (insertion 502-503AY)	8%	Small bowel, colon	None
Exon 11 (deletions, single nucleotide substitutions, insertions)	65%	All sites	Several kindreds
Exon 13 (K642E)	1%	All sites	3 Kindreds
Exon 17 (D820Y, N822K, Y823D)	1%	All sites	Several kindreds
PDGFRA Mutation	**10%**		
Exon 12 (deletions, single nucleotide substitutions, insertions)	1%	All sites	2 Kindreds
Exon 14 (N659K)	Rare	Stomach	None
Exon 18 D842V	6%	Stomach, mesentery, omentum	None
Exon 18 (deletions)	2%	All sites	1 Kindred

SDH-Deficient GIST

- Most are due to mutations in *SDHA, SDHB, SDHC* or *SDHD*
  - At least half of these mutations are germline
  - Propensity to develop:
    - Paraganglioma and GIST (Carney-Stratakis syndrome)
    - Pancreatic neuroendocrine tumor
    - Renal cell carcinoma, Pituitary adenoma
  - Penetrance varies, even among family members