PATHOLOGY OF GISTs

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GISTs recent but long history

- Defined as the most frequent mesenchymal tumor of the gastrointestinal tract (GIT)
- They have a long history in a short time
- **1962** first tumor described– Stout AP: "Bizarre smooth muscle tumor of the stomach" (Cancer)
- **1983** firstly named GIST – Mazur MT & Clark HB: "Gastric stromal tumors: reappraisal of histogenesis" (Am. J. Surg. Pathol.)

GISTS history

- 1988 First IHC – Miettinen M.: "Gastrointestinal stromal tumors. An immunohistochemical study of cellular differentiation" (Am. J. Clin. Pathol.)
- 1994 WHO's GIST - Weiss SW, Sobin LH: "World Health Organization Classification of Soft Tissue tumors" (Springer Verlag, Berlin)
- 1996 – Mentztel T, Katenkamp D.: "Gastrointestinal stromal tumor with skenoid fibers and bidirectional immunohistochemical differentiation" (Histopathology)
- 1996 – Suster S, Fletcher CDM: "Gastrointestinal stromal tumors with prominent signet ring cell features" (Mod. Pathol)
- 1998 clearing histogenesis – Kindblom LG et al.: "Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal" (Am. J. Pathol.)
- 1999 – Sircar K, Huizinga JD et al.: "Interstitial cells of Cajal as precursors of gastrointestinal stromal tumors" (Am. J. Surg. Pathol.)

GISTs history

- 1999 identificarea GANT ca subtipuri de GIST la MET – Damiani S., Pasquinelli G.: "GANT-like gastrointestinal pacemaker cell tumors with oncocytic features" (Virchows Arch.)
- 2000 CD34 identification in GIST – Robinson TL, Huizinga JD et al.: "GIST may originate from a subset of CD34-positive interstitial cells of Cajal" (Am. J. Pathol.)

GIST history

- 2001– Chambonniere ML et al.: "Expression of *microtubule associated protein tau* by gastrointestinal stromal tumors" (Hum. Pathol.)
- 2001 identification of nestin in Cajal cell and GIST – Tsujimura et al., Am. J. Pathol.
- 2002 identification of c-kit gene mutations in GIST – Heinrich MC et al.: "Biology and genetic aspects of gastrointestinal stromal tumors: kit activation and cytogenetic alterations" (Hum. Pathol.)

The Cajal cells dilemma

- Present debate regarding interstitial Cajal cells (ICC) and Cajal-like cells (ICLC) – morphology, immunophenotype, location and functions;
- Incertitude surrounding the origin of GIST cells → express both CD34 and c-kit → possible origin: ICC, ICLC, fibroblasts, myofibroblasts or even stem cells or telocytes

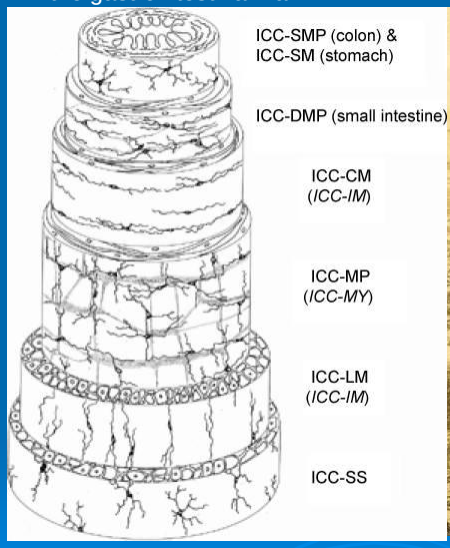
GIST' Histogenesis: ICC / ICLC

- Initial gastrointestinal location, extended to many others
- Gut pacemaker cells
- Form intramural networks
- Develop from intrinsic gut mesenchyme? Or from bone marrow CD 34 positive stem cells ?
- IHC: CD34, CD117, Nestin, Tau, S-100 protein, Leu 7, DOG1,PDGFRA, PGP9.5.

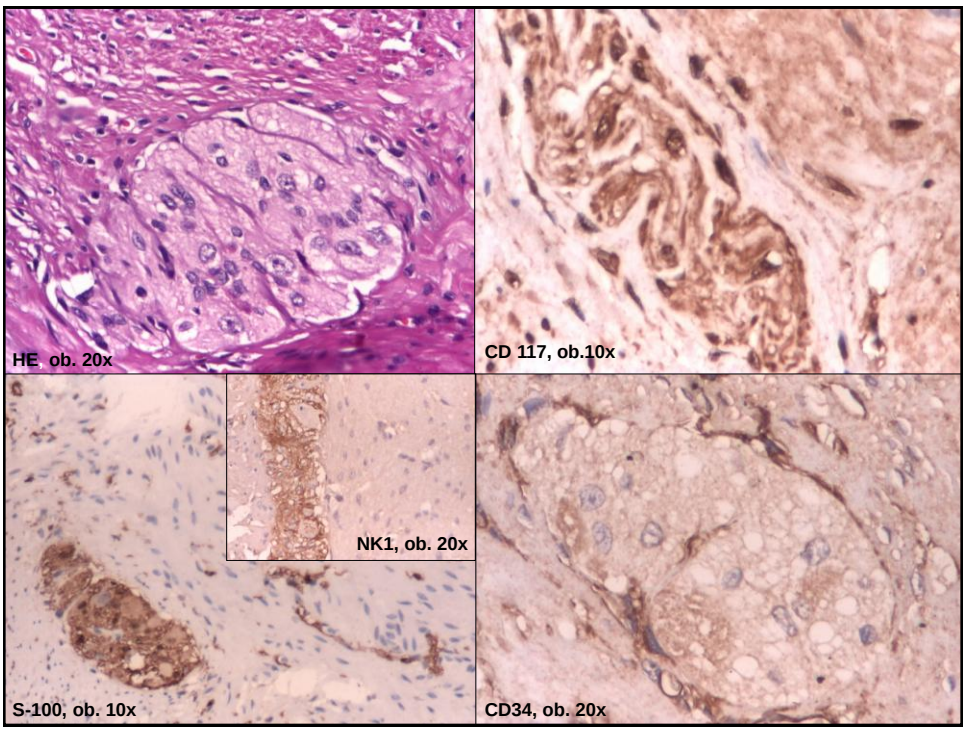
ICC location

- ICC described initially by Cajal as interstitial neurons in the gut and thereafter identified all over the gastrointestinal tract
- The ICC network is distributed within the submucosal, intramuscular and intermuscular (myenteric) layer and deep muscular plexus
- Only ICC or also ICLC? (Pieri et al 2008).

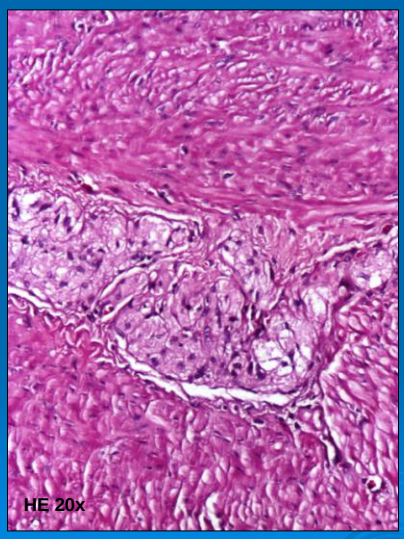
Types of ICC with different locations in the gastrointestinal wall

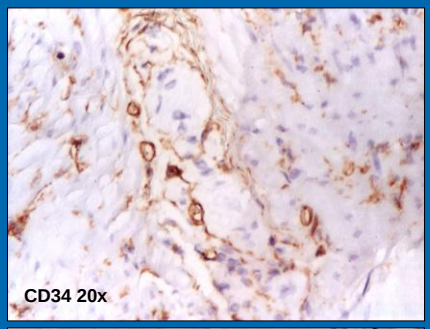


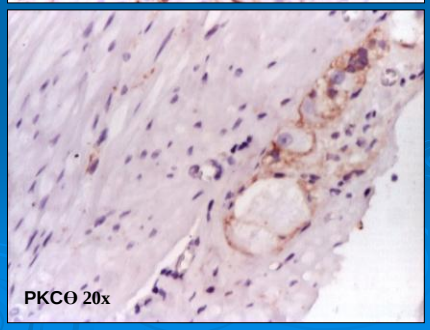
Komuro T, J Physiol 576.3 (2006) pp. 653-8



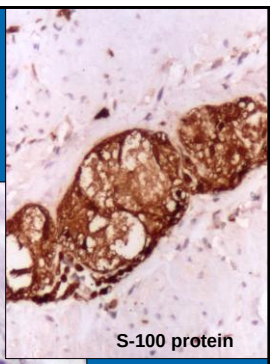
Small intestine

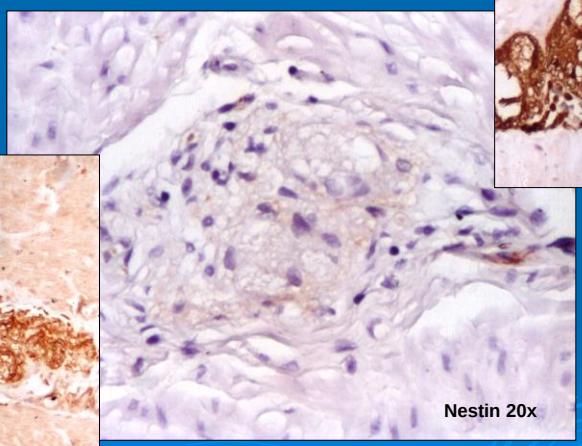

HE 20x

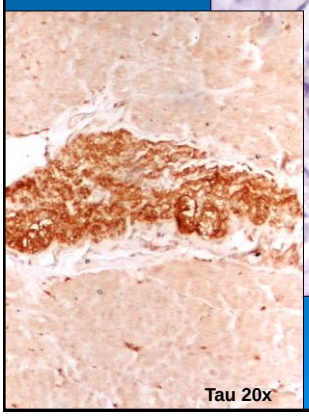

CD34 20x


PKCΘ 20x

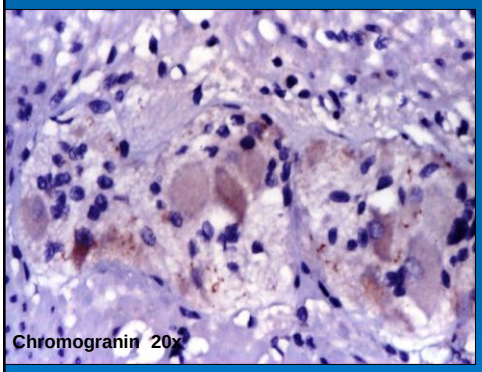
Small intestine

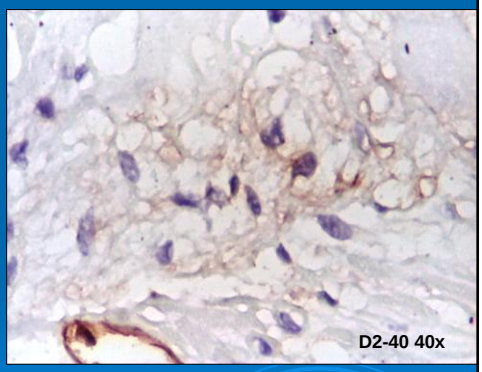

S-100 protein


Nestin 20x

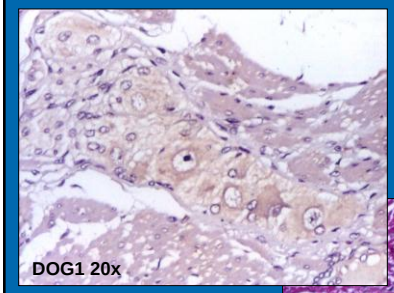

Tau 20x

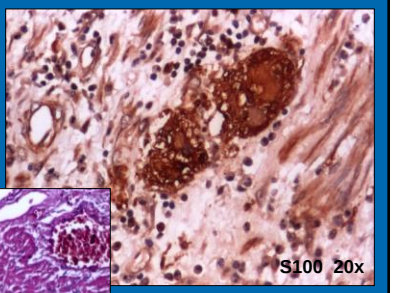
Small intestine

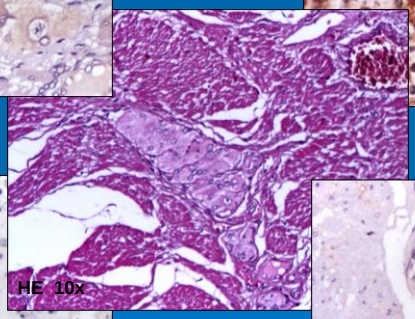


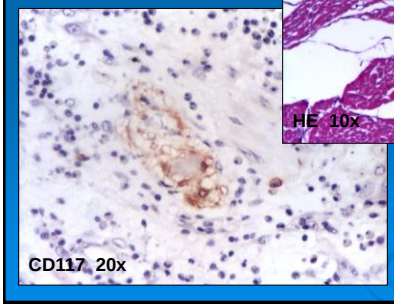


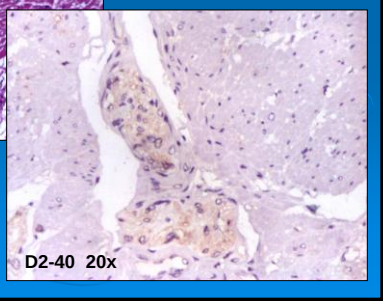
Stomach











GIST

- The most frequent mesenchymal tumour of the gastrointestinal tract
- Thought to arise from the interstitial cells of Cajal (ICC) and, more recently, from the interstitial Cajal-like cells (ICLC)
- IHC: CD117, CD 34,PDGFRA, DOG1, PKCϴ, D2-40, Nestin, Tau

Histogenetic considerations

- GISTs do not originate exclusively from ICC, since these tumors can occur in sites where ICC do not exist (omentum, peritoneum, retroperitoneum).
- ICLC and other candidates, including a multipotent progenitor, can also constitute the origin of these tumors.
- CD34 positivity, considered an expression of immaturity for ICC, has not the same significance in GISTs: >70% are CD34+, but only 25-30% have a biologically malignant behavior.

Clinical features

- most of the patients are elderly persons of both genders,
- sometimes GISTs can arise in children, having particular features and evolution.
- these tumors can be single or multiple sporadic, the last type being sometimes misinterpreted as recurrent or metastatic disease.

Location

- They may arise throughout the gut, but the commonest sites are the stomach (60-70%), the small bowel (20-30%), the colorectum (5%), the esophagus (up to 5%).
- GISTs developed in the retroperitoneum, the omentum and the mesentery are considered extraGISTs.

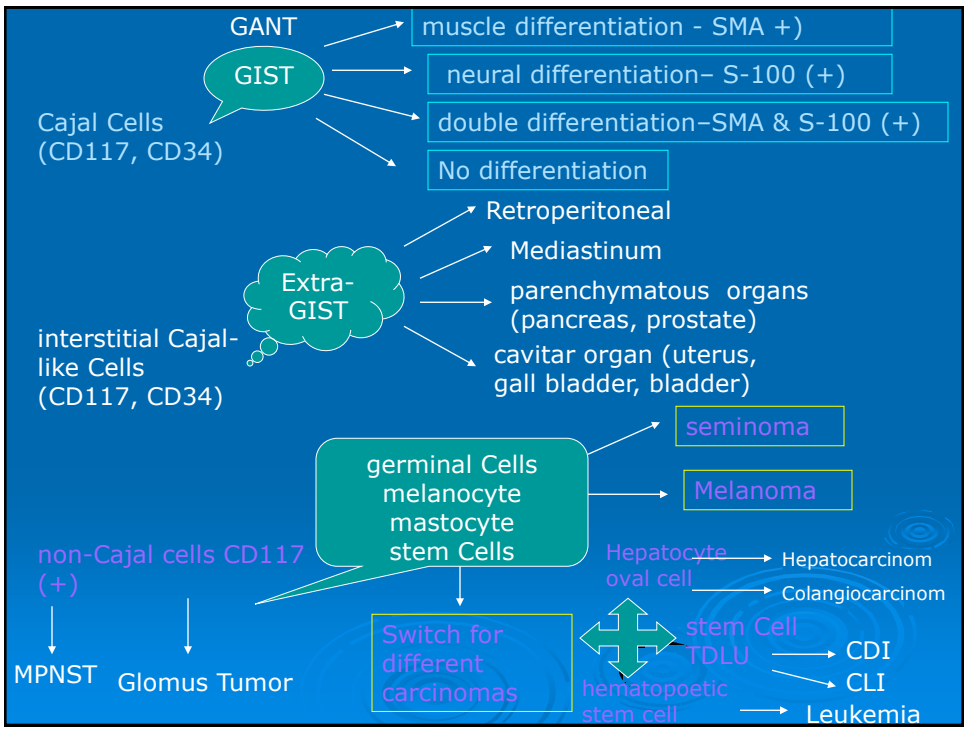
Grossly

- usually polypoid masses situated in the external coat of the bowel, with or without infiltration and ulceration of the mucosa.
- tumor size is variable, from several millimetres to 10-15 cm.
- well delimitated, but not encapsulated, with a white-greyish parenchyma, sometimes with necroses and hemorrhages. The superjacent mucosa can be intact or, in ~30% of cases, ulcerated.

Macroscopy - GIST

Special types of GISTs

- **Familial GIST**
- It is rare and characteristically associated with germline dominant mutation of the KIT or PDGFRA gene with high penetrance .
- In this context,GIST is usually multifocal and variably associated with hyperplasia of the interstitial cells of Cajal and abnormalities of mast cells such as urticaria pigmentosa or systemic mast cell disease, together with pigmentary abnormalities,including perioral, neck, axillary and perineal freckling and pigmentation at the gastro-esophageal junction.
- **Syndromic GISTs**
- Association of GIST in the context of neurofibromatosis type I **(NF-1**), the commonest cancer-predisposition syndrome, which carries up to 25% risk for developing GIST. Multifocal.
- **Pediatric GISTs:**
- GISTs arising in children, adolescents, or young adults have a much lower frequency of mutations in KIT or PDGFRA (less than 10% of cases). In the classic pediatric setting, GIST shows evidence of KIT or PDGFRA mutations in only 11% cases; 5% occur in exon 11 of KIT, 9% in exon 9 and 3% in PDGFRA.



Histopathology

- Compact tumor proliferation with a delicate fibrovascular stroma +/- lax areas.
- cell type: spindle-cell, epithelioid or mixed.
- The cell type also has a prognostic significance, the epithelioid and mixed cell type GISTs being associated with a poorer prognostic.

Risk stratification according to the number of mitoses, localization and tumor size

Risk stratification for aggressive behavior in GISTs
Fletcher et al., Hum Pathol 2002

	Max size	Mitotic Index
Very low risk	<2 cm	<5/50 HPF
Low risk	2 to 5 cm	<5/50 HPF
Intermediate risk	<5 cm	6-10/50 HPF
	>5 to 10 cm	<5/50 HPF
High risk	>5 cm	>5/50 HPF
	>10 cm	Any
	Any	>10/50 HPF

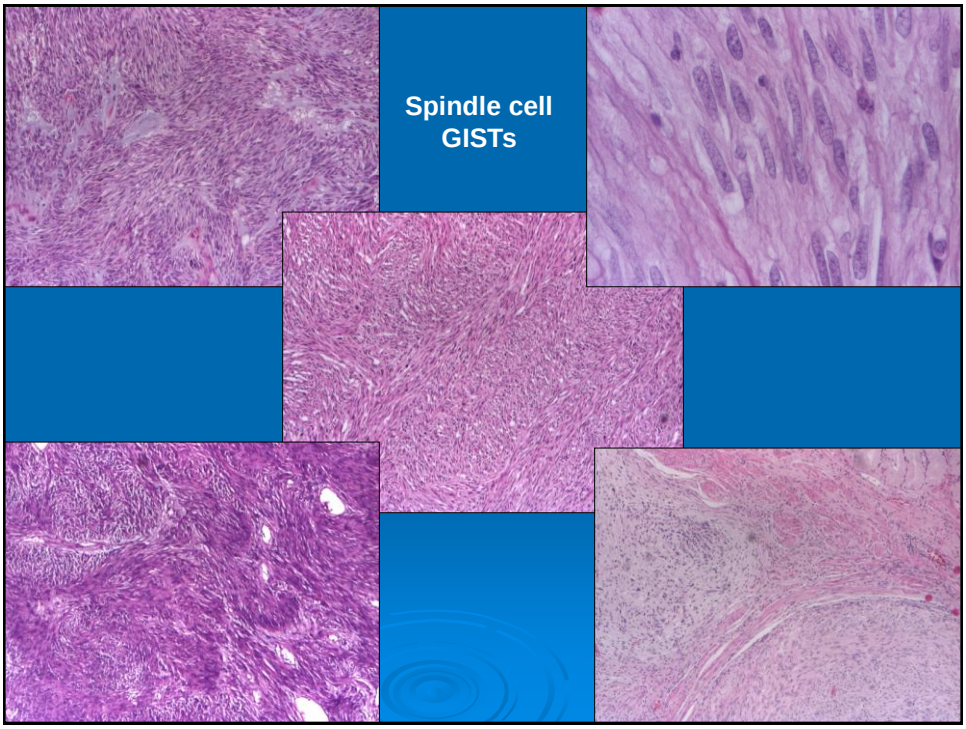
Risk stratification (Demitri 2007)

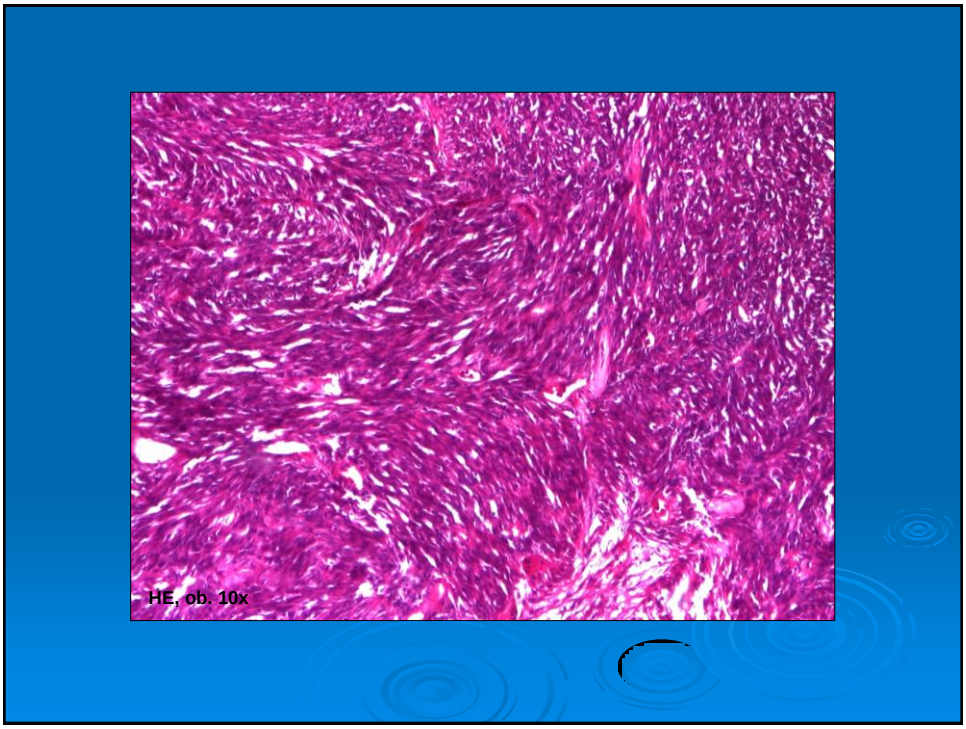
Table 1 Risk Stratification of Primary GIST by Mitotic Index, Size, and Site*

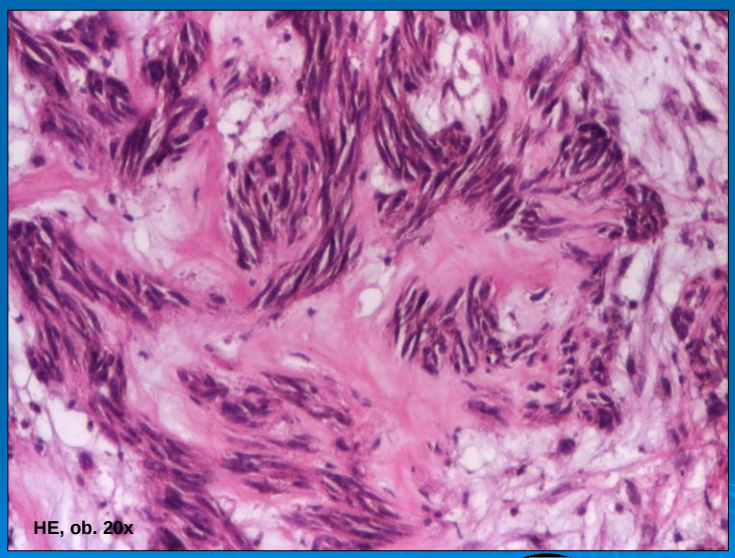
		Risk for Progressive Disease* (%)			
Mitotic Index	Size	Gastric	Duodenum	Jejunum/Ileum	Rectum
≤ 5 per 50 hpf	≤ 2 cm	None (0%)	None (0%)	None (0%)	None (0%)
≤ 5 per 50 hpf	> 2 ≤ 5 cm	Very low (1.9%)	Low (4.3%)	Low (8.3%)	Low (8.5%)
≤ 5 per 50 hpf	> 5 ≤ 10 cm	Low (3.6%)	Moderate (24%)	(Insuff data)	(Insuff data)
> 5 per 50 hpf	> 10 cm	Moderate (10%)	High (52%)	High (34%)	High (57%)
> 5 per 50 hpf	≤ 2 cm	None†	High†	(Insuff data)	High (54%)
> 5 per 50 hpf	> 2 ≤ 5 cm	Moderate (16%)	High (73%)	High (50%)	High (52%)

Cell morphology

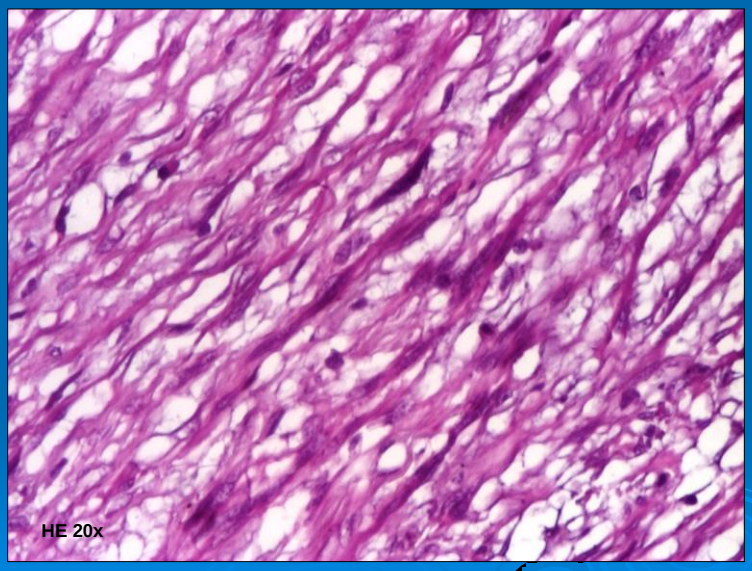
- Spindle cells in GISTs: heterogeneous
  - very large, with two or more polar branching processes,
  - flat with radial thin processes and variably stained cytoplasm.
  - the nuclei are either rod-like with rounded extremities, or fusiform.
  - Nuclear chromatin can be granular, fine or coarse, with inconspicuous nucleoli.
  - characteristic paranuclear cytoplasmic vacuolae.
  - Mitoses are usually typical and scarce.
  - Rarely, metaplastic chondroid or distrophic calcifications can occur.


Spindle cell
GISTs

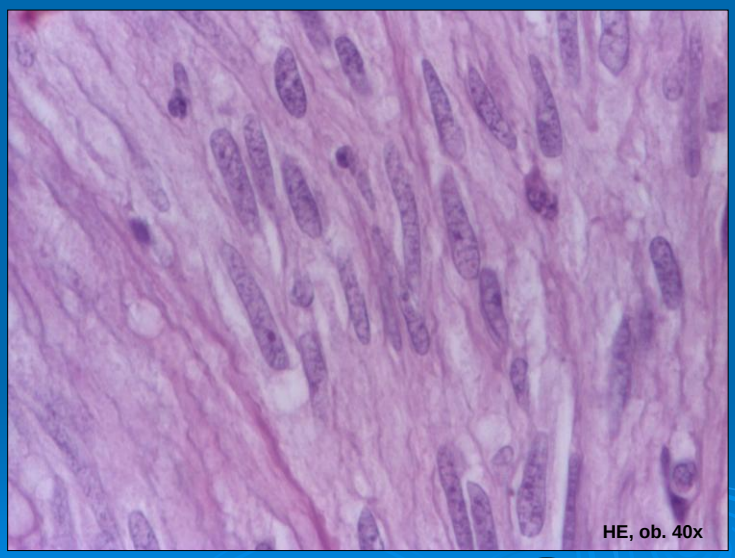

HE, ob. 10x



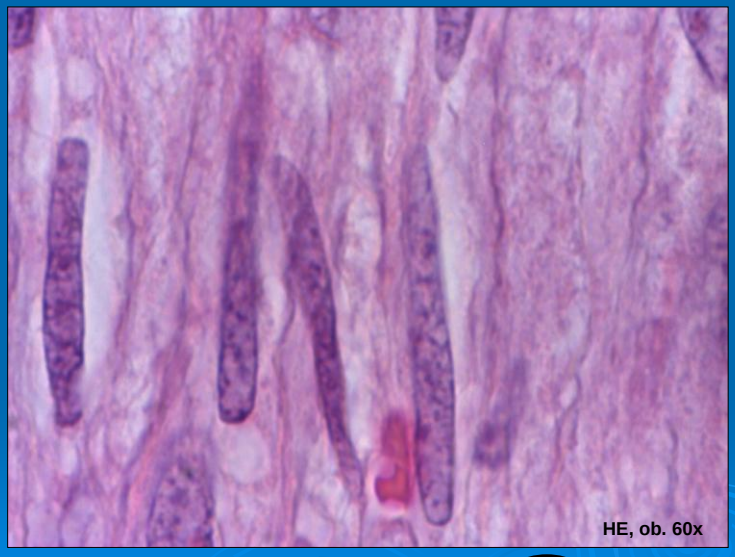
HE, ob. 20x



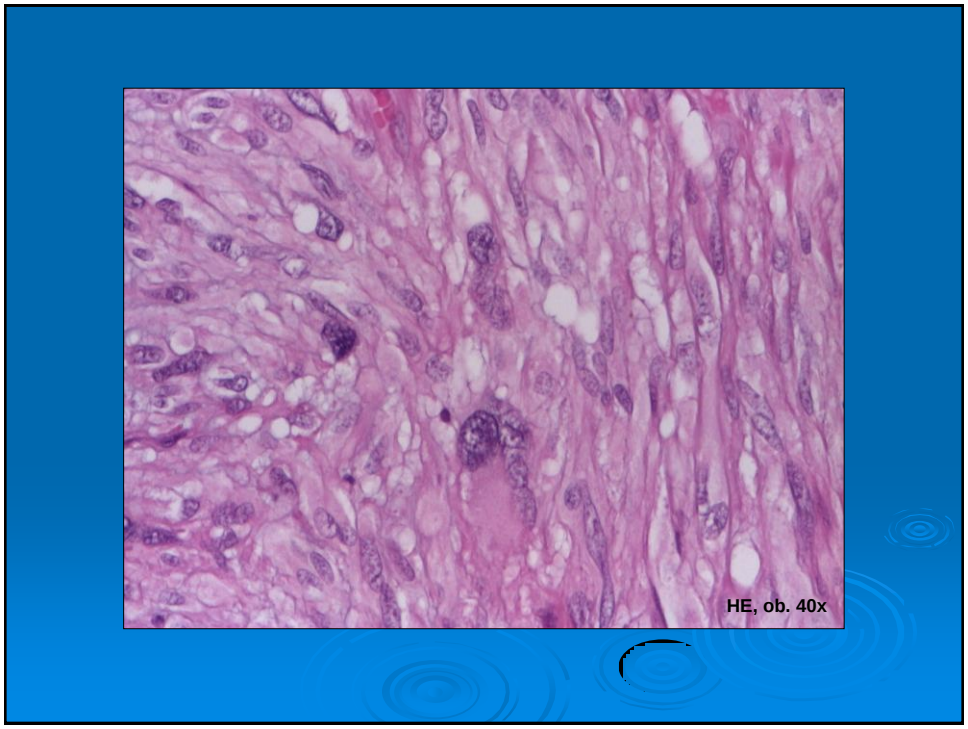
HE 20x

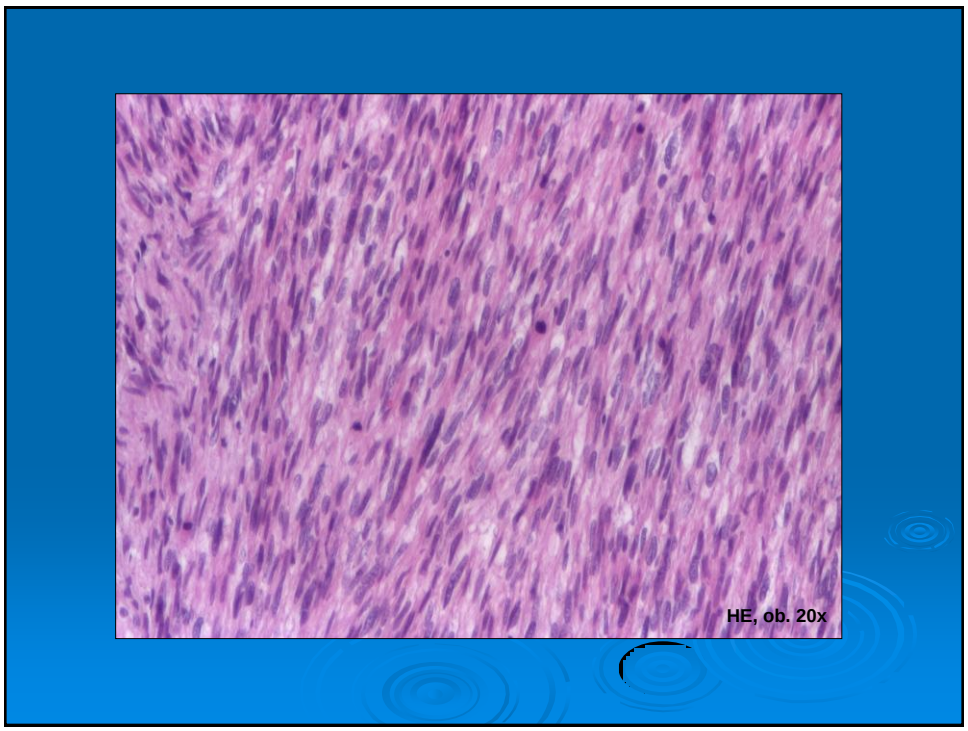


HE, ob. 40x



HE, ob. 60x

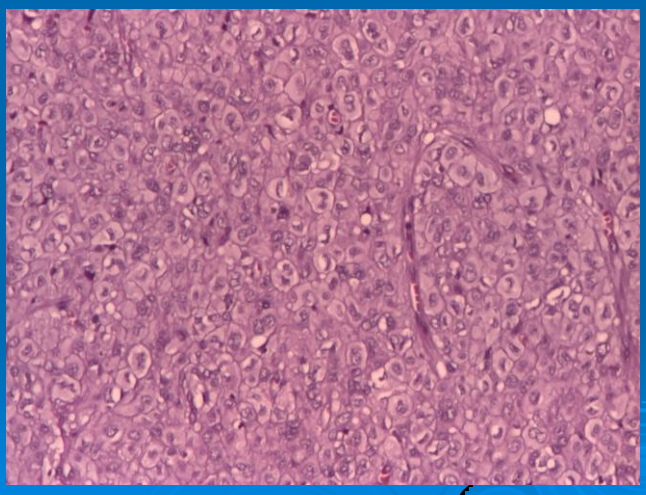

HE, ob. 40x


HE, ob. 20x

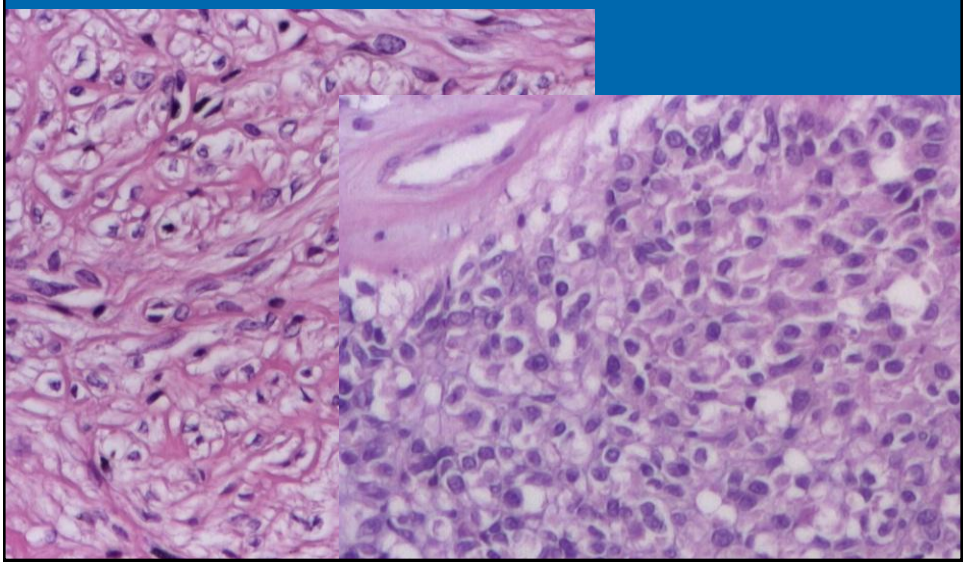
Cell morphology

- Epithelioid type (5-10% of cases)
- proliferate as islets or sheaths of large rounded cells with pale eosinophilic cytoplasm, sometimes foamy or plasmacytoid,
- rounded vesiculous nuclei and visible nucleoli.
- the tumor stroma can be finely fibrovascular or more abundant, myxoid or edematous.
- A significant number of mitoses can be considered as prognostic factor.

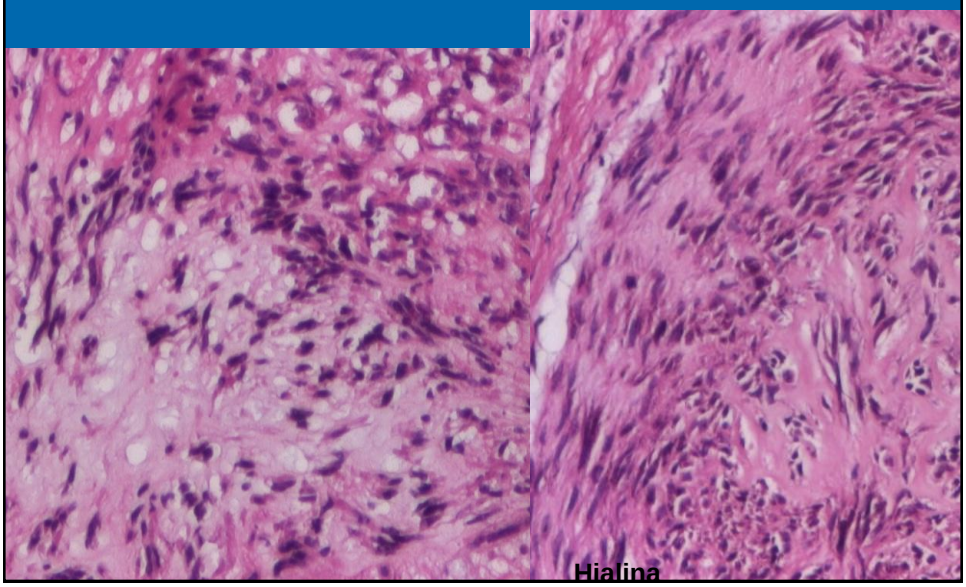
Epithelioid type GIST



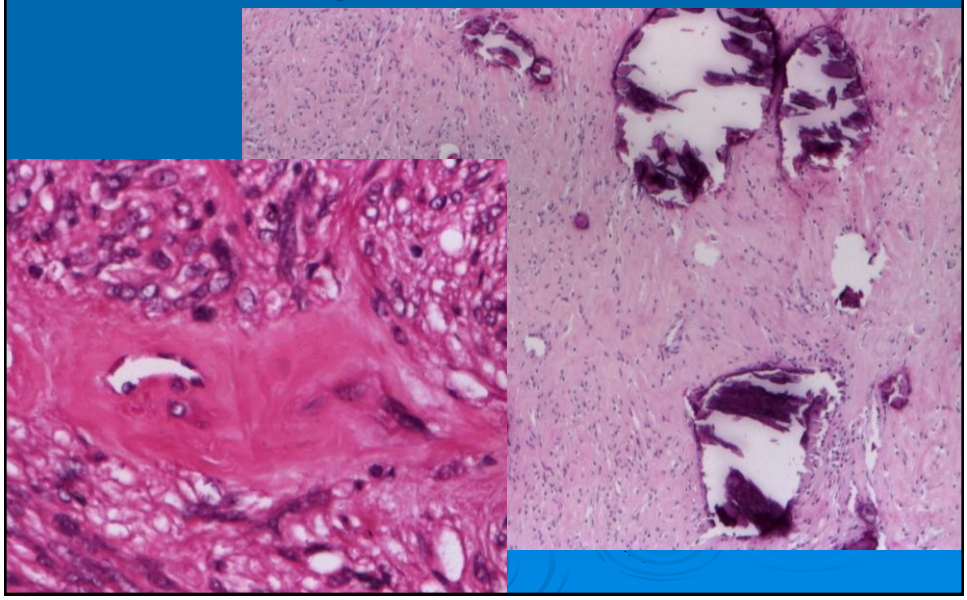
Epithelioid pattern, clear cells plasmocytoid pattern



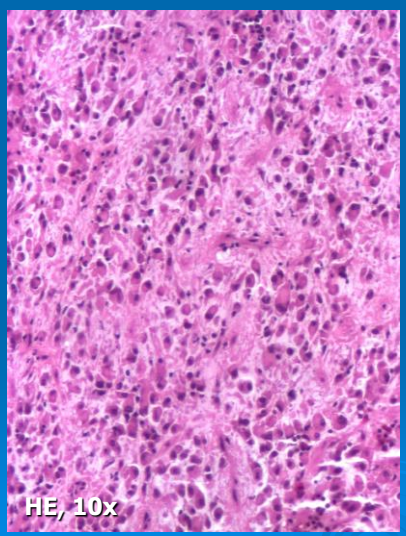
Stroma hyalinization

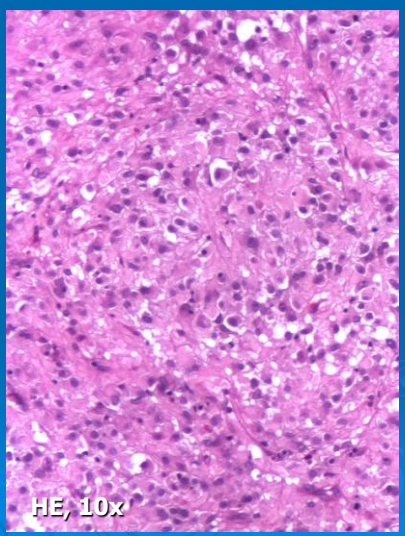


Distrophic calcifications

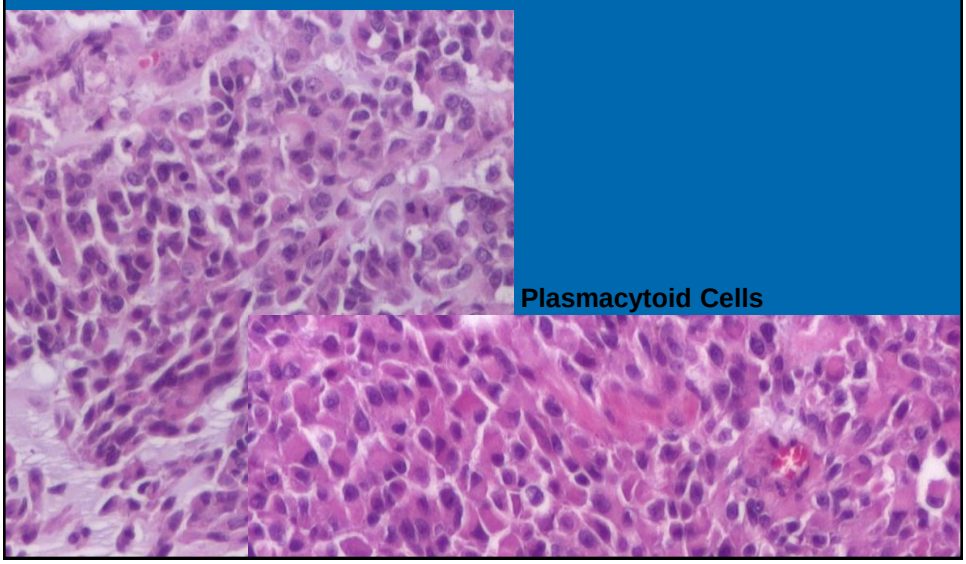


Morfologic features: plasmacytoid cells; paranuclear vacuolization

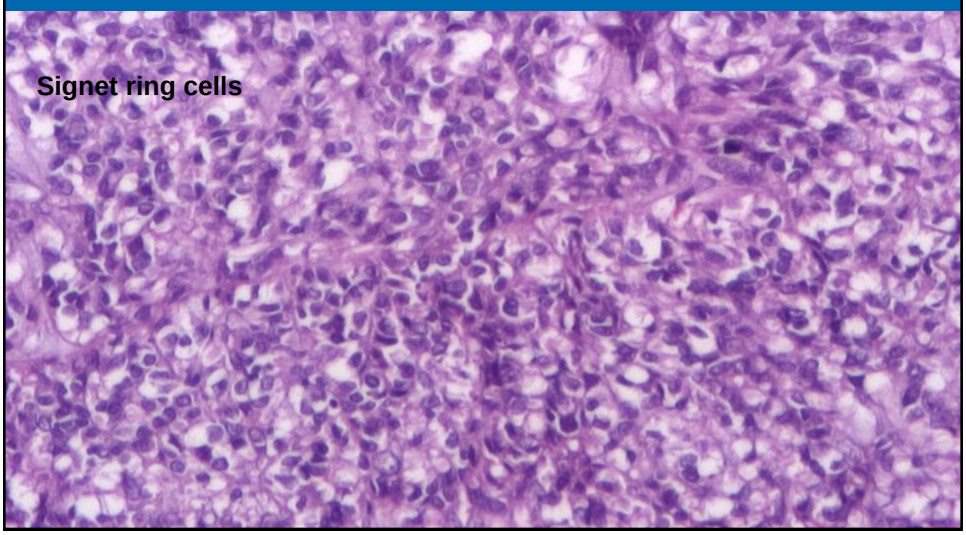




HP aspects suggesting malignity



HP aspects suggesting malignity



Immunohistochemical profile

- CD34 – the first marker used
- CD117 – c-kit gene protein
- PDGFRA - platelet derived growth factor receptor A
- DOG1 - discovered on GIST
- D2-40 / podoplanin
- Nestin, Caveolin 1, Tau, PKC teta

C-kit protein (CD117)

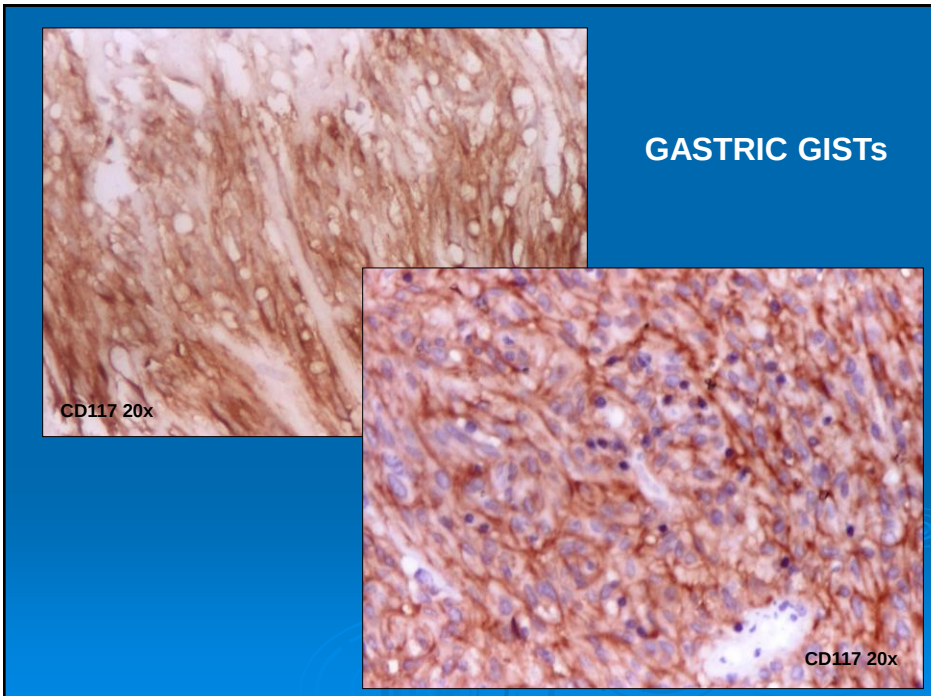
- - it is a 145-kD glycosylated transmembranary receptor with tyrosine kinase activity

structure :

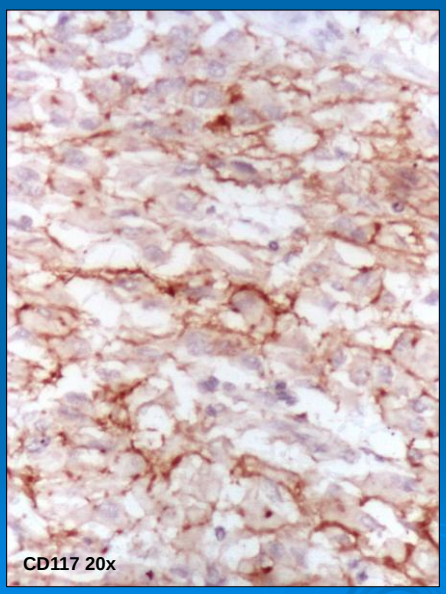
- * an extracellular domain (consists of 5 Ig-like domains)
- * a transmembrane region (TM)
- * a juxtamembrane (JM) domain
- * an intracytoplasmic tyrosine kinase domain
- - its specific ligand is the stem cells growth factor (SCF)

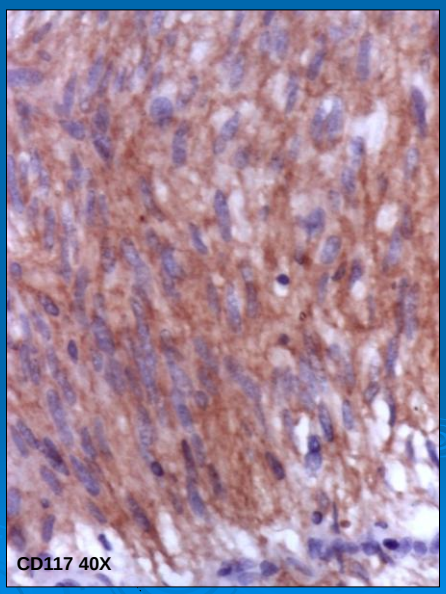
Heterogeneous c-kit staining patterns

- CD117 is expressed in >95% of GISTs
- IHC patterns in tumor cells:
  - Membrane
  - Cytoplasm: Homogenous
    Coarse granular
    Fine granular
    Paranuclear dot
- CD117 staining patterns can be associated with certain c-kit gene mutations (homozygous or heterozygous) involved in the response to imatinib (*Tabone-Eglinger S et al. Clin Cancer Res, 2008, 14(8):2285-2294*)



Small intestine GISTs

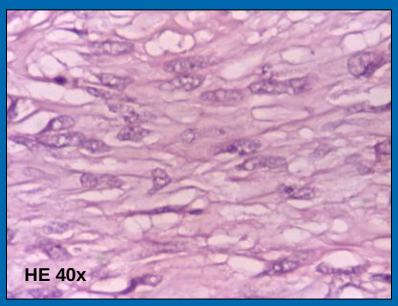


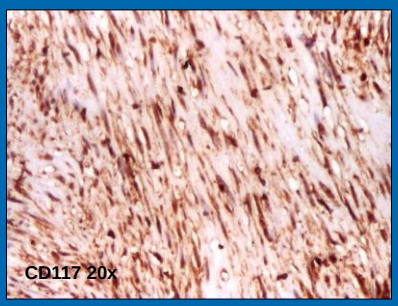


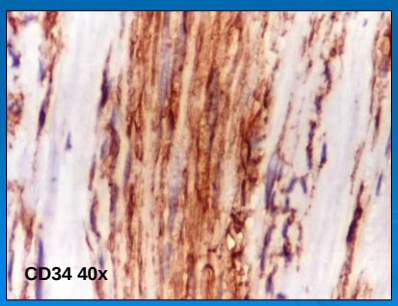
Heterogeneous GISTs immunoprofile

- Classically: CD34 and CD117 positive
- Variations: CD34+ CD117-
  CD34- CD117+
  CD34- CD117-
- Other biomarkers: S-100 protein, SMA, DOG1, PDGFRA, Nestin, Tau, PGP 9.5, D2-40, Caveolin 1
- Some of these IHC markers also have prognostic value

Gastric GIST


HE 40x


CD117 20x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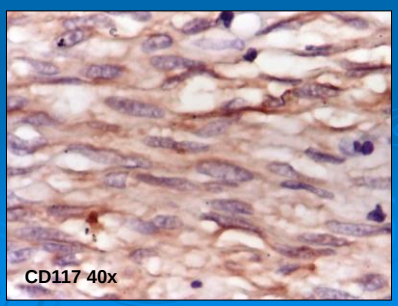
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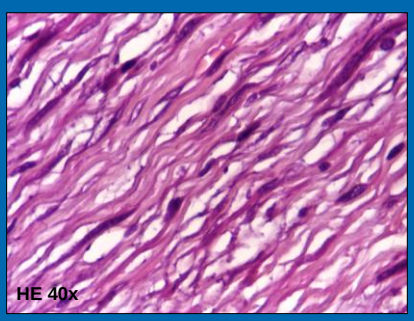
Gastric GIST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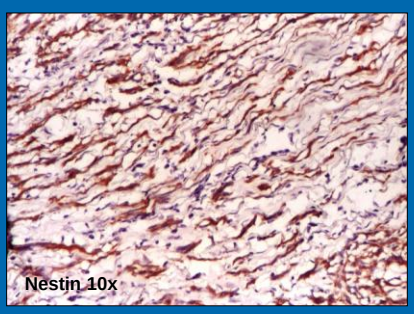
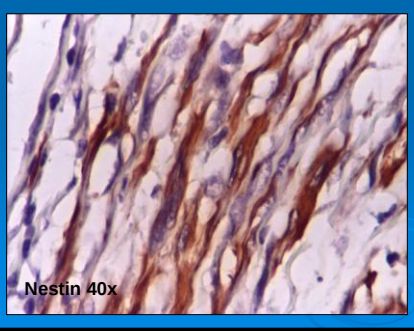

Nestin 10x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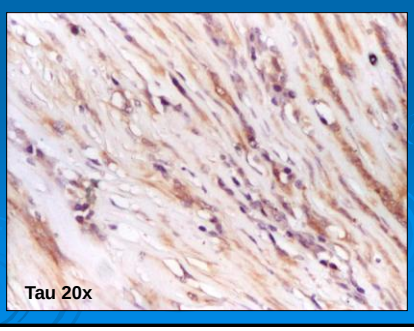
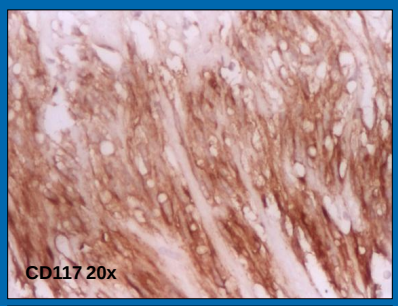
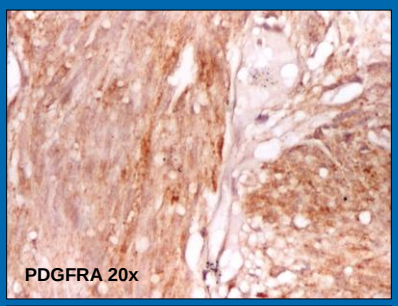
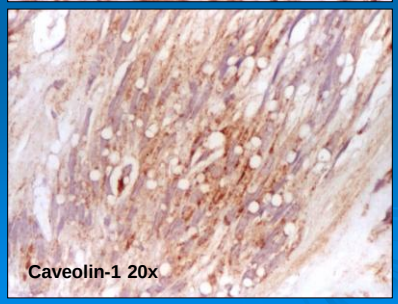
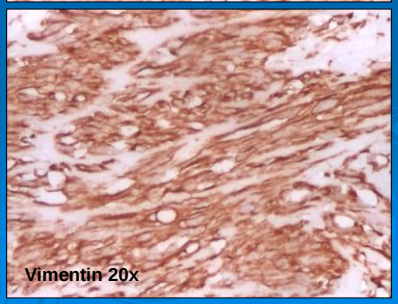

Tau 20x

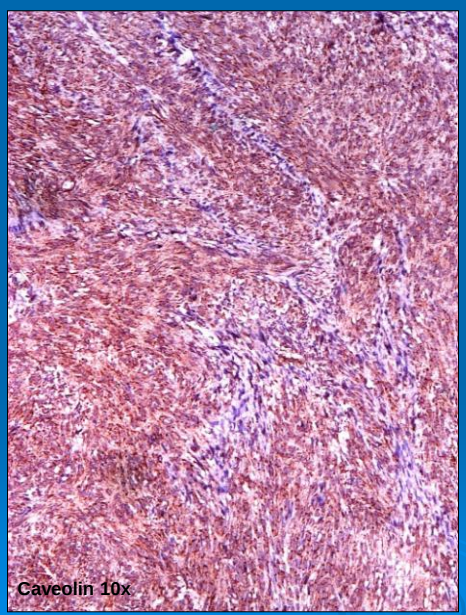
Gastric GIST

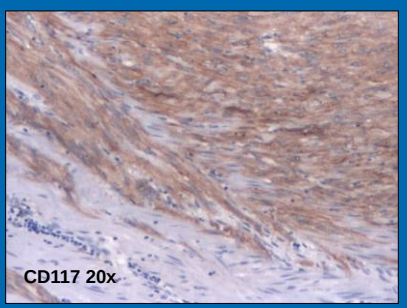

CD117 20x

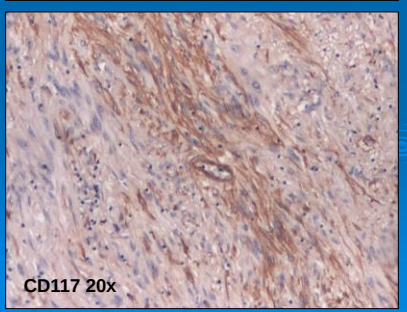

PDGFRA 20x


Caveolin-1 20x

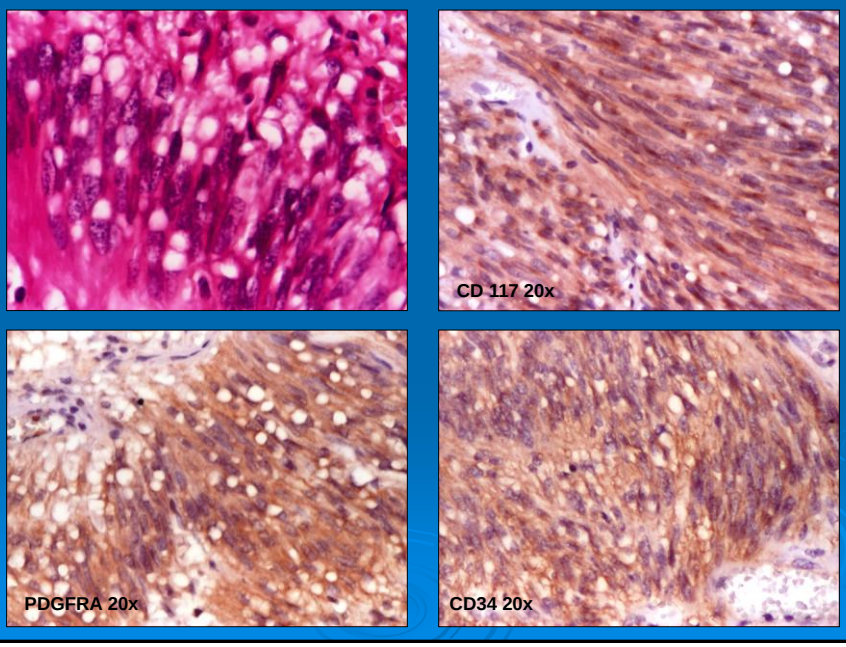

Vimentin 20x


Caveolin 10x

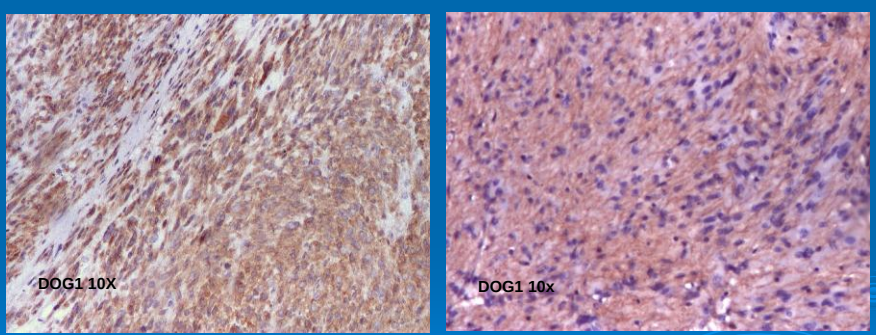

CD117 20x


CD117 20x

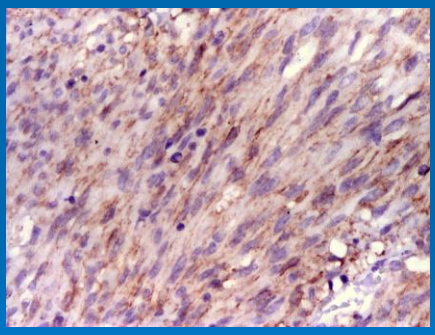
Intestinal GIST

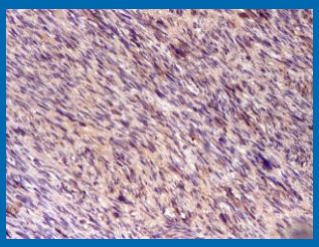


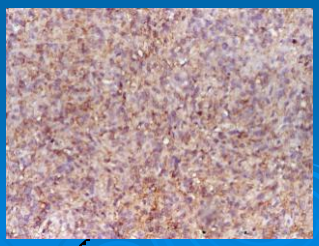
New markers



D2-40



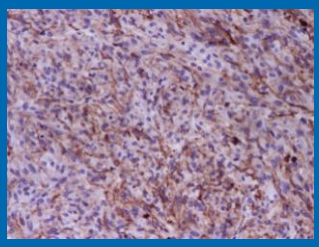




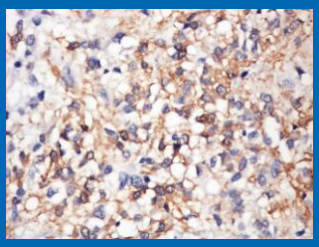
Markers of special differentiation

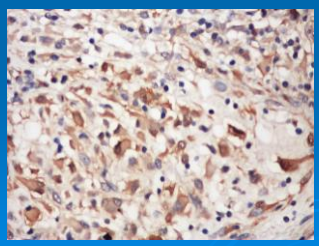
- - smooth muscle actin (SMA), mostly in the spindle cell type, with a cytoplasmic distribution;
- - S100 protein (S100), cytoplasmic, associated with a neuroid pattern;
- - Desmin, rarely, with a zonal distribution;

Special differentiation

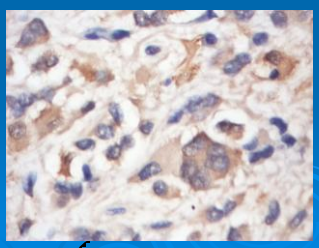


SMA
10x/ 20x





S-100
10x/20x



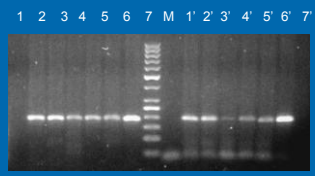
Molecular features- ckit mutations

- occur on exon **11** which codifies a juxtamembranary domain with regulatory function; it is the most frequently encountered, in >70% of GISTs.
- Other mutations, in decreasing order of frequency, are located on exons 9, 13 and 17.
- Recent studies: GISTs with mutations on exon 11 are the most sensitive to the specific treatment with Imatinib, compared with mutations on exons 9 or 13. Also, deletions compared with point mutations in exon 11 have been found to be a significant unfavorable factor in patients with gastric GISTs.

PDGFRA mutations

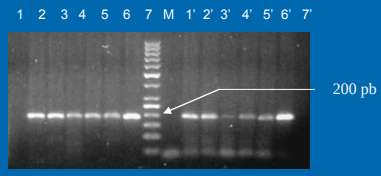
- located on exon **18** (over 80%), exon 12 (much less frequent - 6%) or exon 14 (the rarest - 1%). PDGFRA mutations on exon 18 characterize the category of GISTs with primary resistance to Imatinib, while mutations on exon 14 are usually associated with a less favorable prognosis. Exon 12 mutations are the most sensitive to specific treatment

PCR pentru c-Kit Exoni 9 si 11



Verificare electroforetica pe gel de agaroza 2% a reactiilor PCR pentru gena C-KIT-exonii 9 si 11
Probe (1-7=exon 9 si 1'-7'=exon 11):
1 – ADS (control negativ)
2- 123750
3 – 126904
4 – 128048
5 – 130530
6 – 174048
7 – proba de control pozitiv 174934-2
M – 50bp DNA Ladder (Fermentas)

PCR pentru c-Kit Exoni 9 si 11



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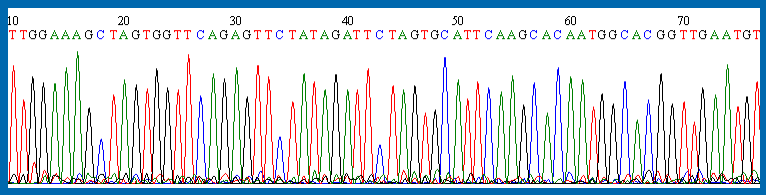
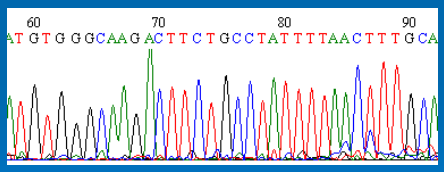
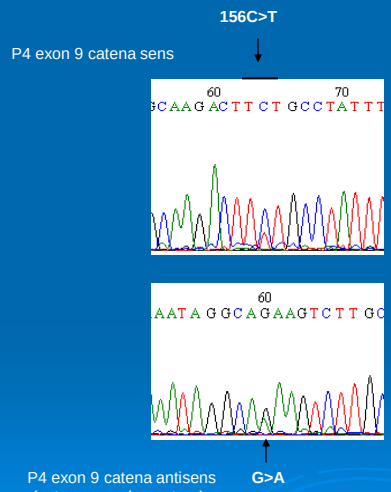
Secventiere gena *C-KIT*, exonul 9





Secventiere (bidirectionala) cu kitul *BigDye Terminator v3.1 Cycle Sequencing Kit* si echipamentul Analyzer 310 (Applied Biosystems)

Analiza secventiere bidirectionala



Mutatie punctiforma (substitutie-tranzitie) in exonul 9 al genei C-KIT(pozitia nucleotidica 156 a exonului 9,) notata astfel: **c.1502C>T,** care induce la nivelul proteinei substitutia aminoacidului serina din pozitia 501 (codonul 501) cu fenilalanina **S501F**
(c=coding sequence, C=citozina, T=timina)

Codonul 501 TCT (subliniat in imagine) este inlocuit prin mutatie cu **codonul TTT** (in ADN);
conform codului genetic ARN:
codonul UCU (codifica S/Ser = Serina) este inlocuit (substituit) prin mutatie cu codonul UUU (codifica F/Phe = Fenilalanina)

New approach in GISTs

- SDHA immunohistochemistry and loss-of-function mutations analysis ; mainly in ckit and PDGFRA wild-type
- SDHB – immunohistochemistry
- CD133 – IHC
- Natural killer isoform p30 -as prognostic factor.

Victor Babes Institute GIST team

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- Cristina iosif
- Florina Vasilescu
- Dana Terzea
- Florin Andrei
- Georgeta Cardos
- Alina Grigore