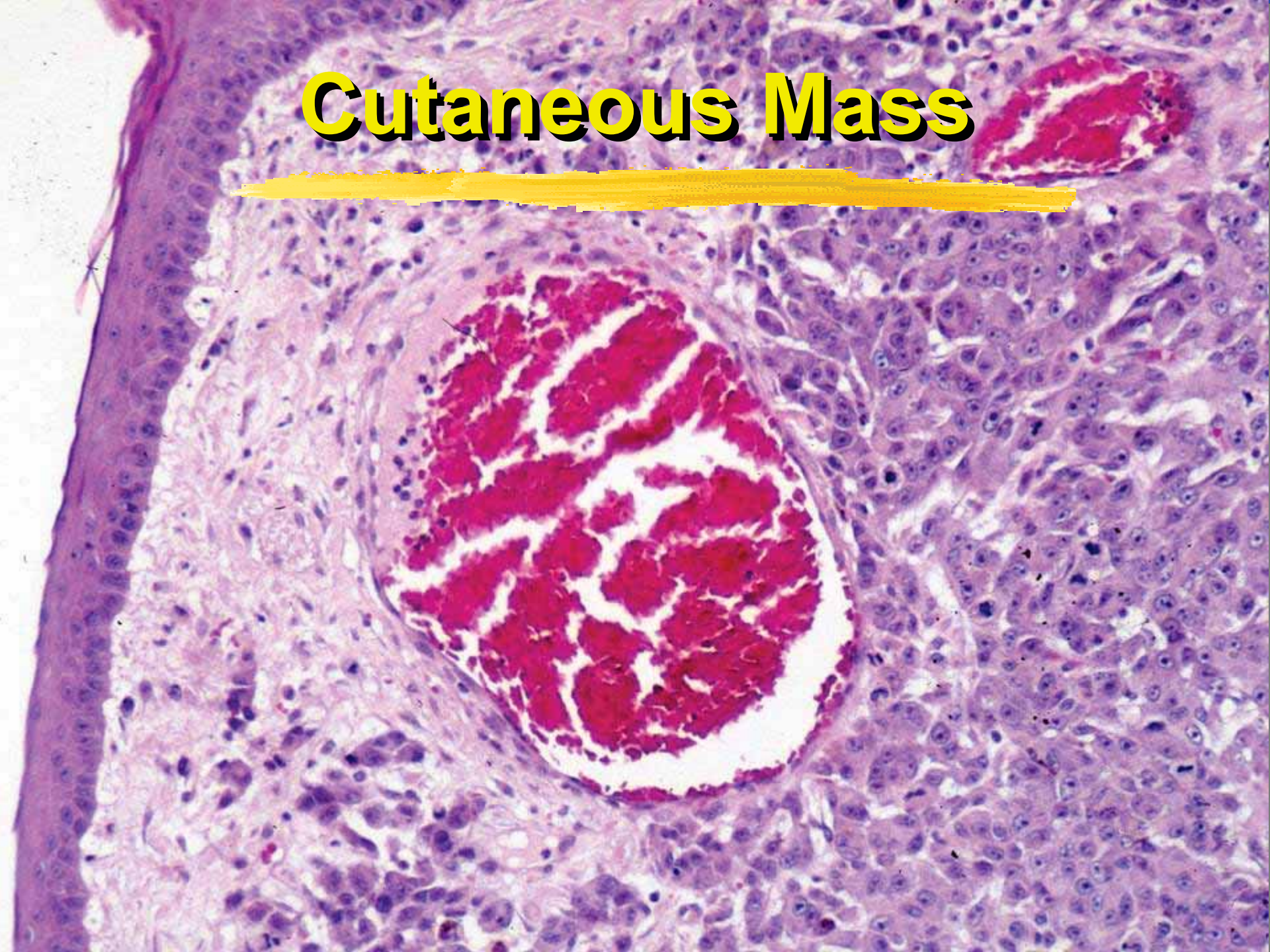
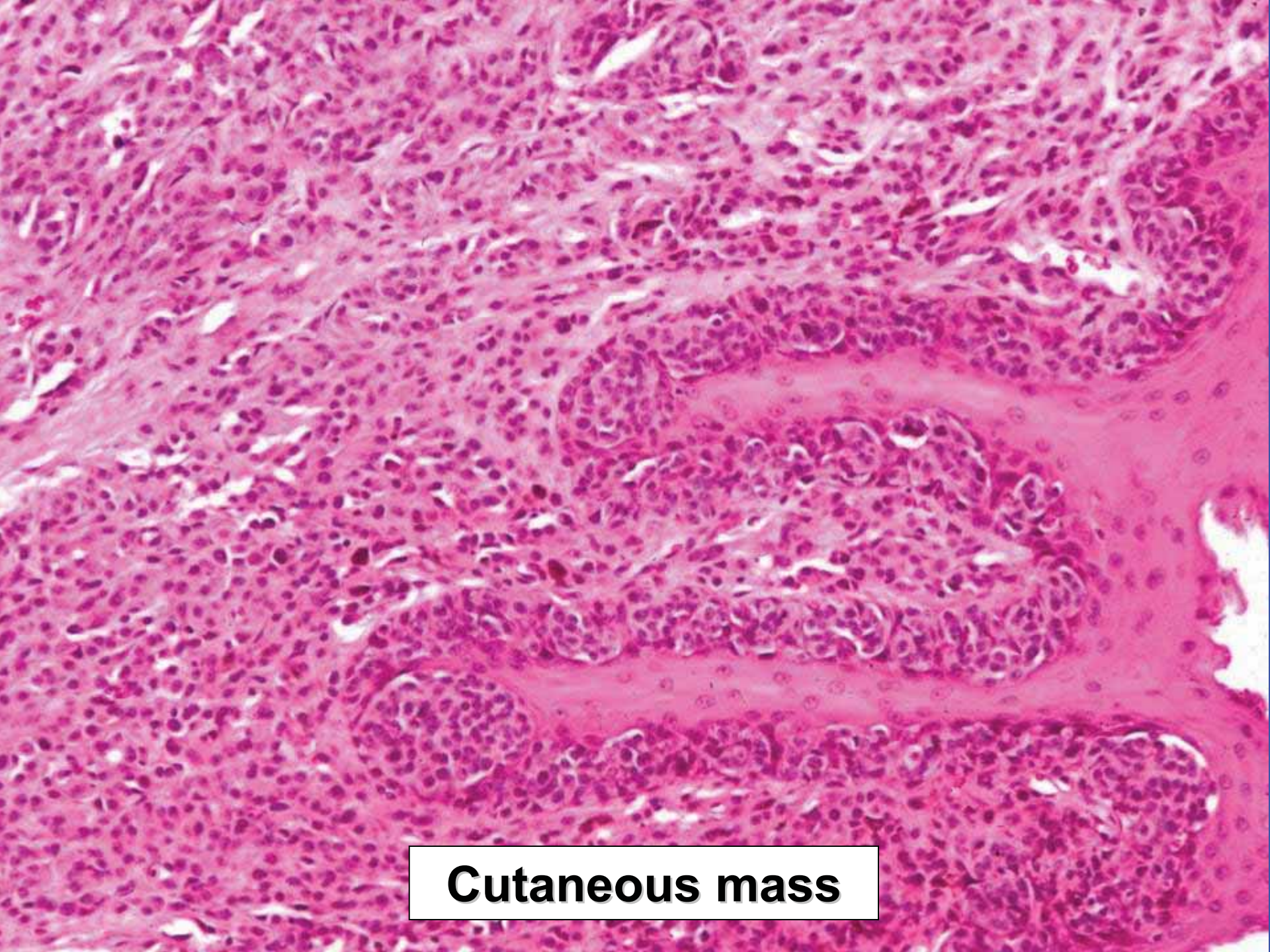
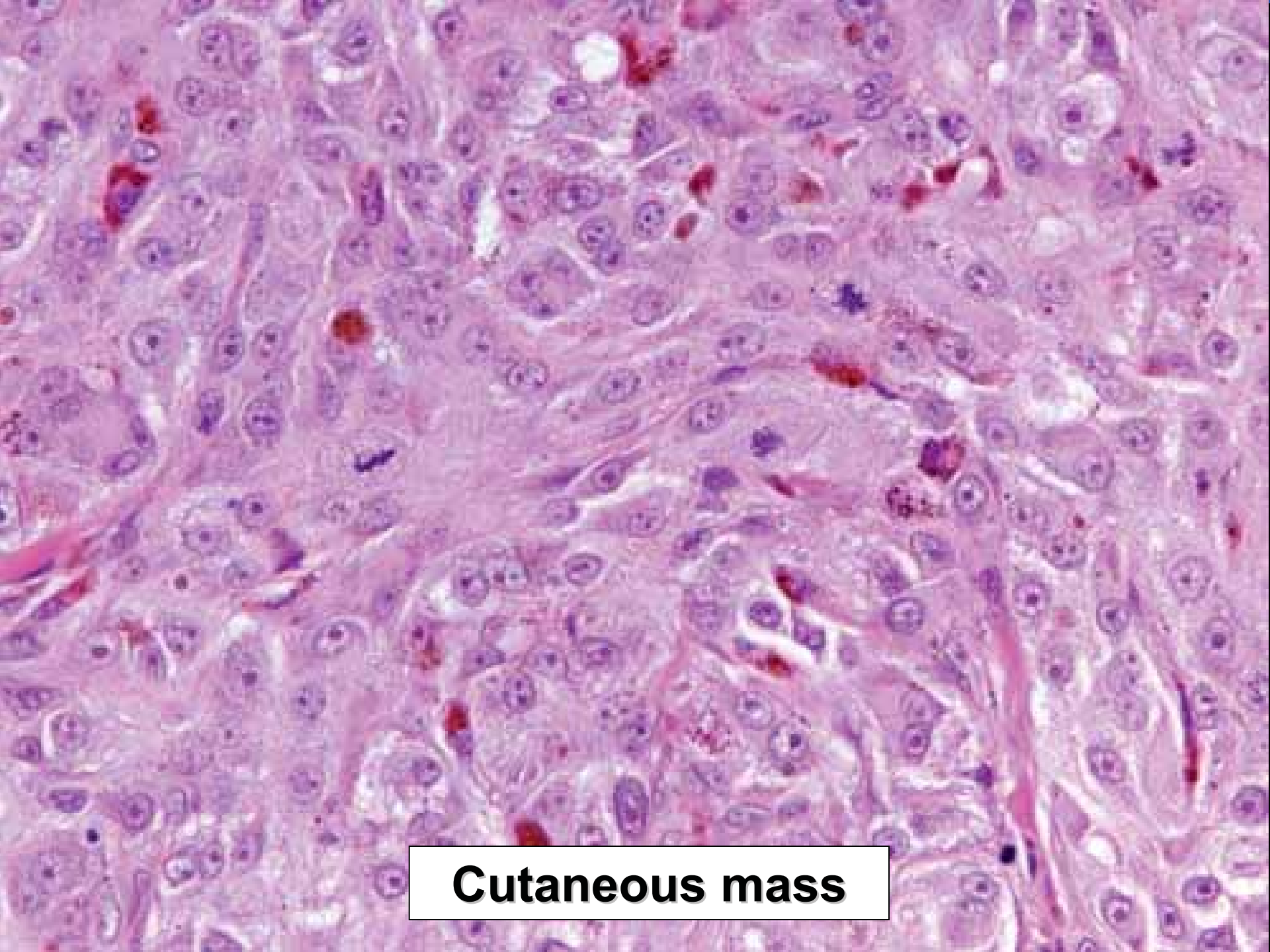
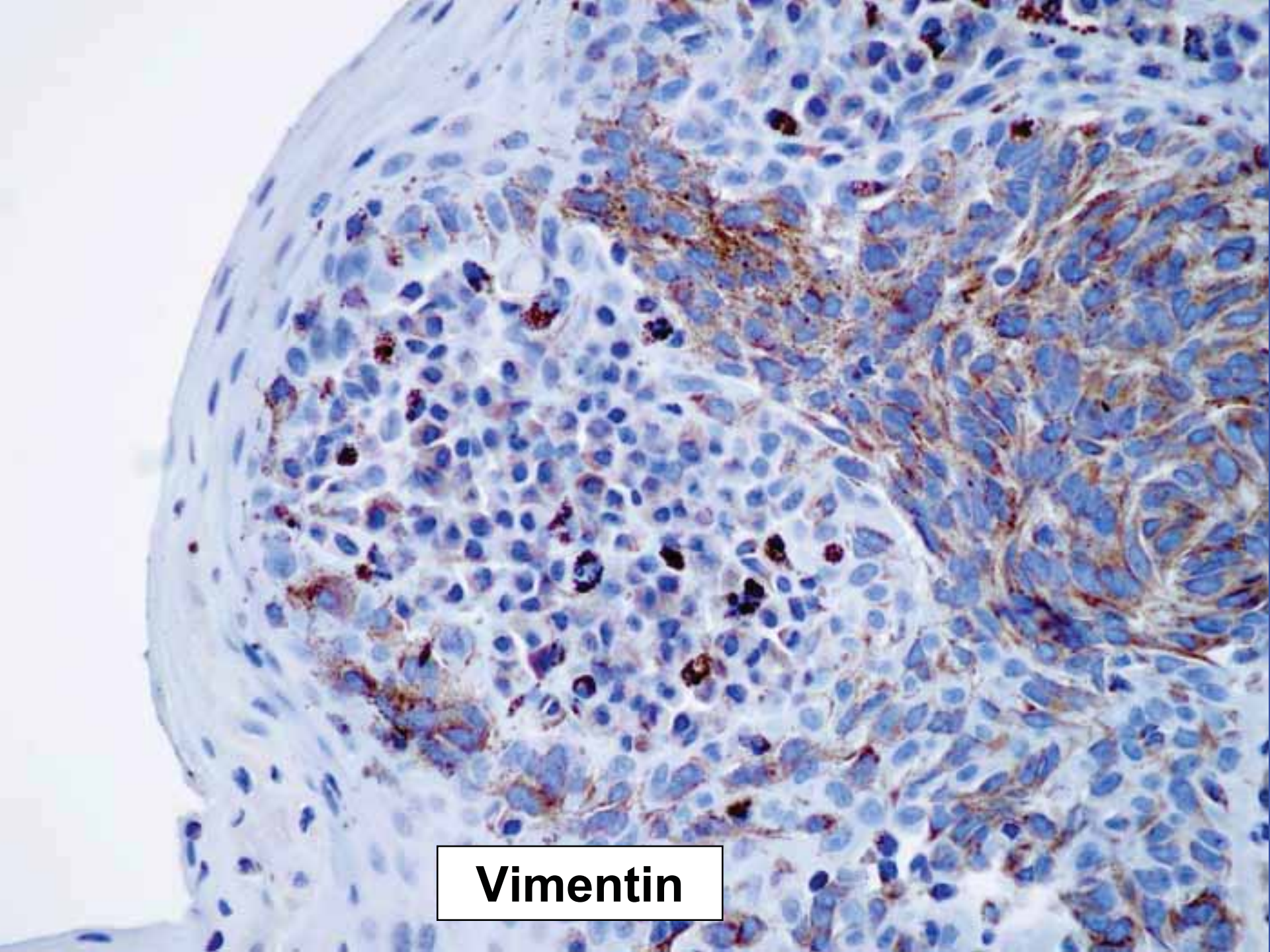
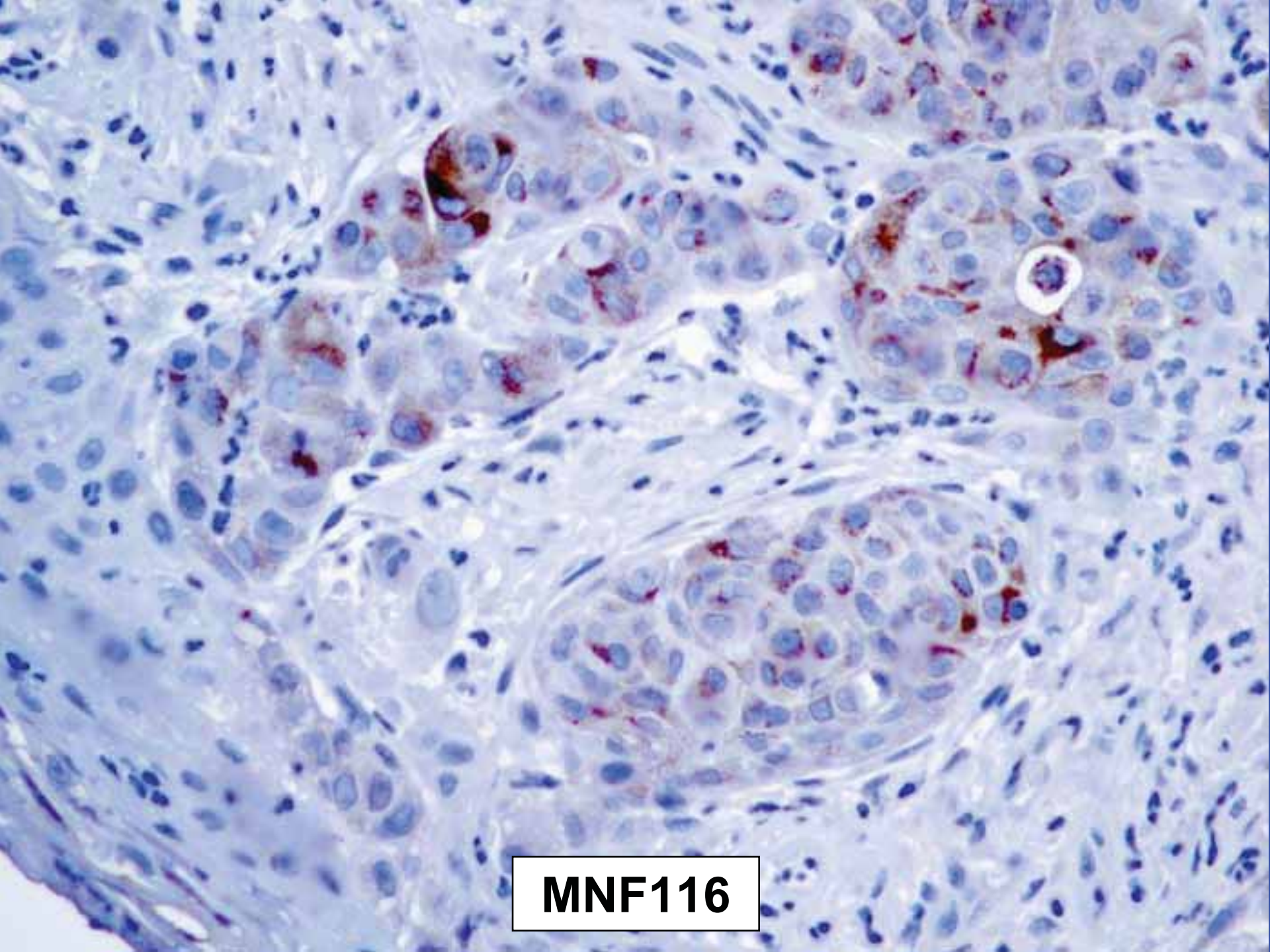
Cutaneous Mass

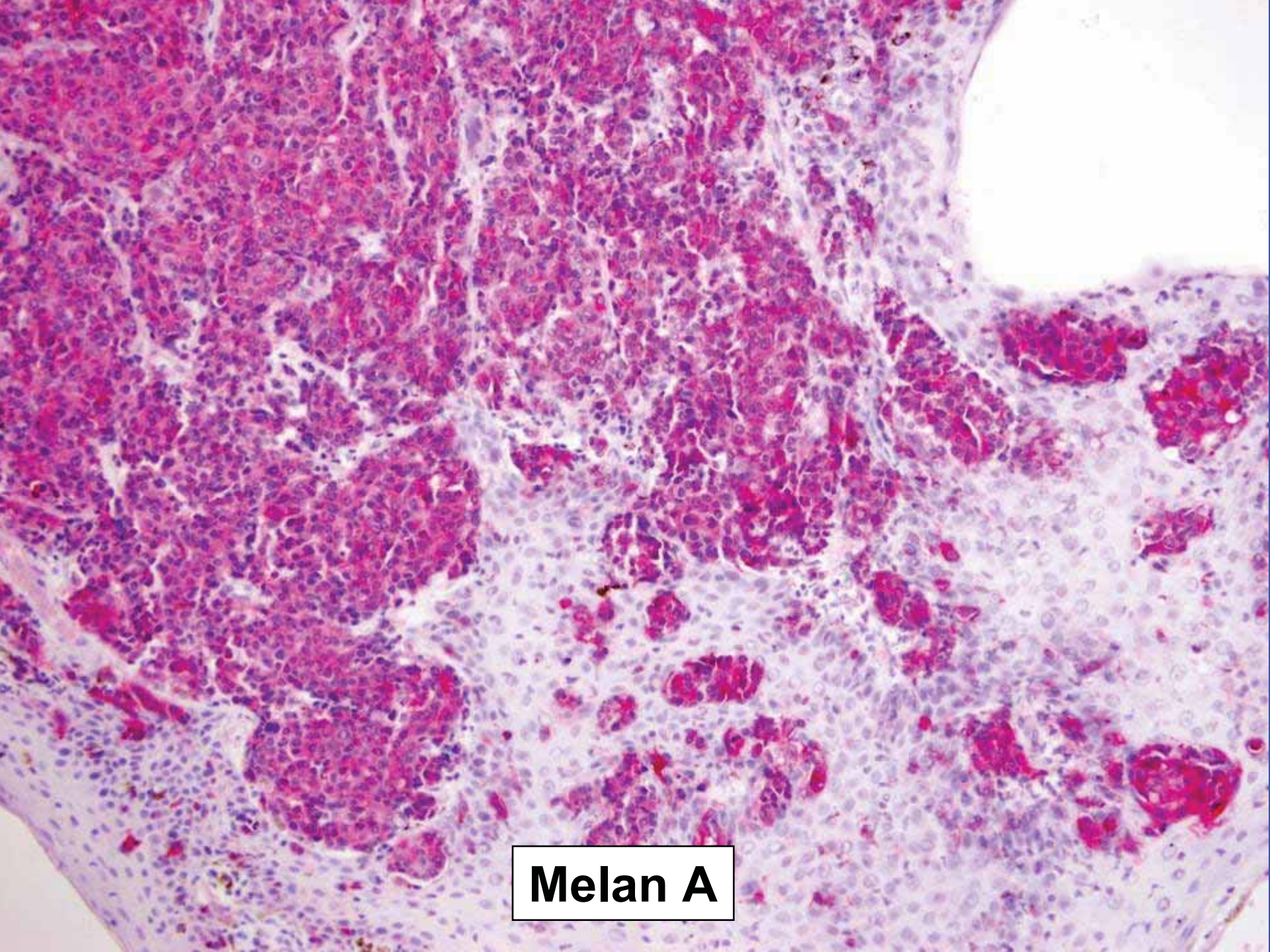


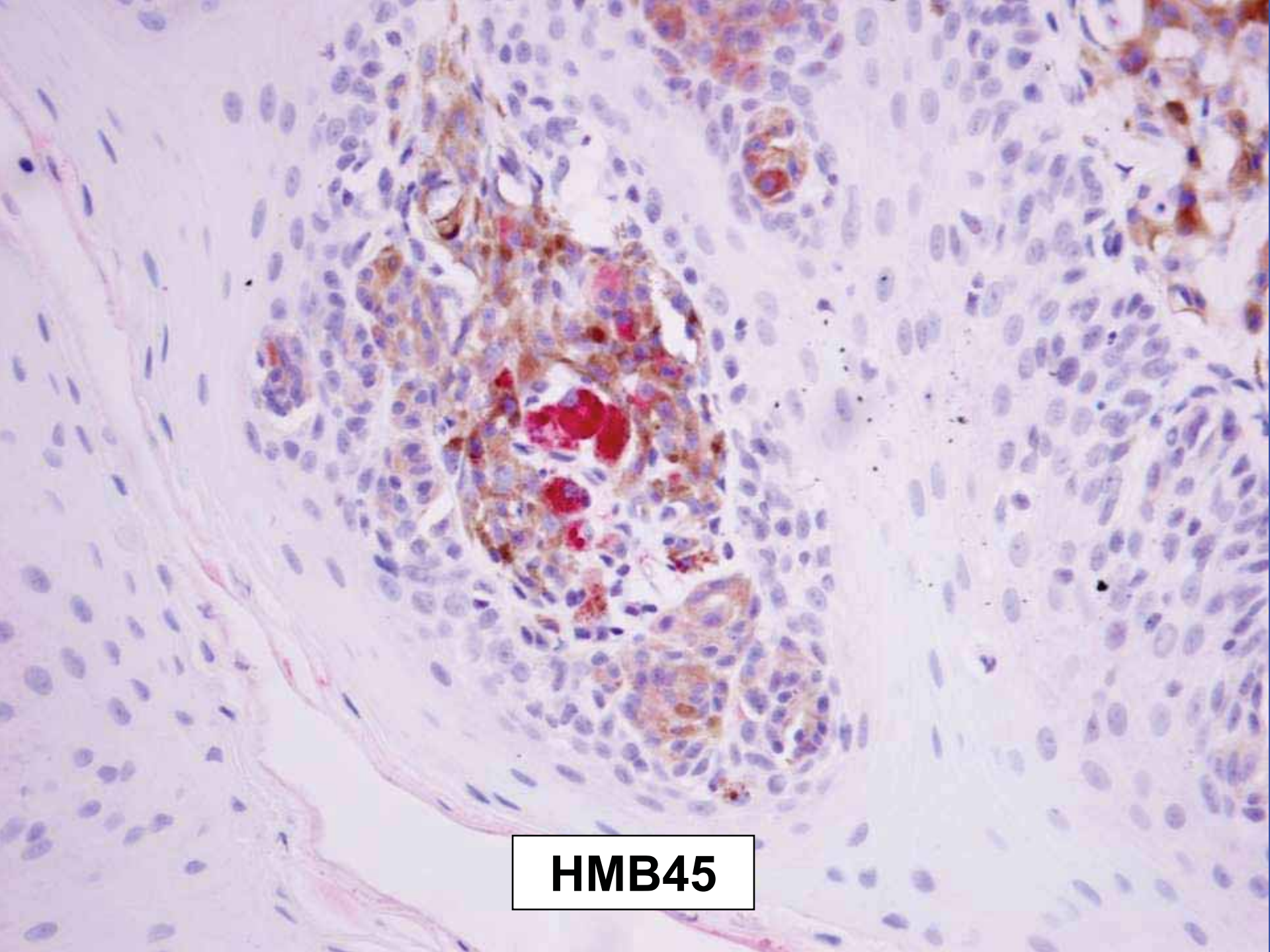

Cutaneous mass

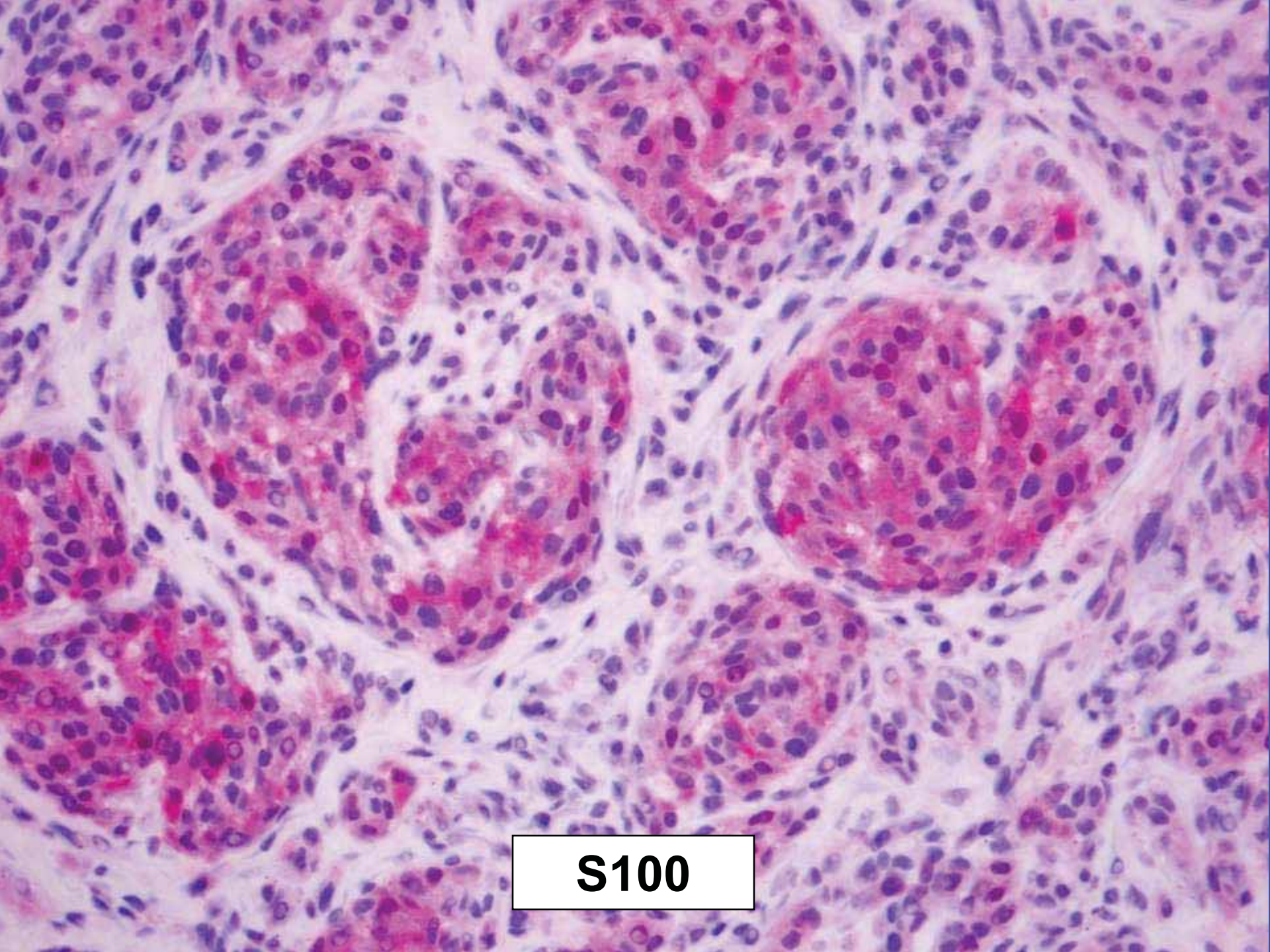

Cutaneous mass

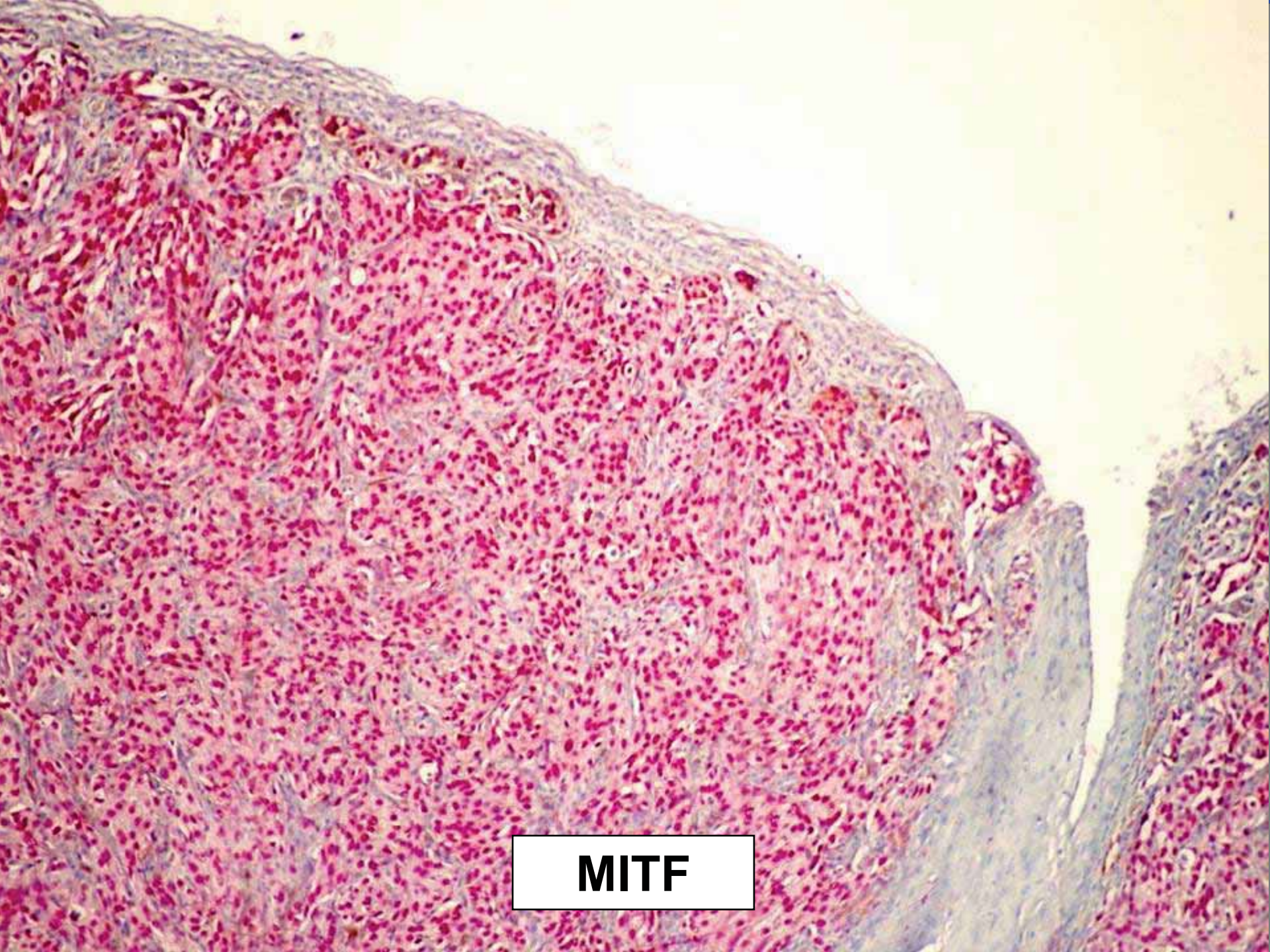

Vimentin

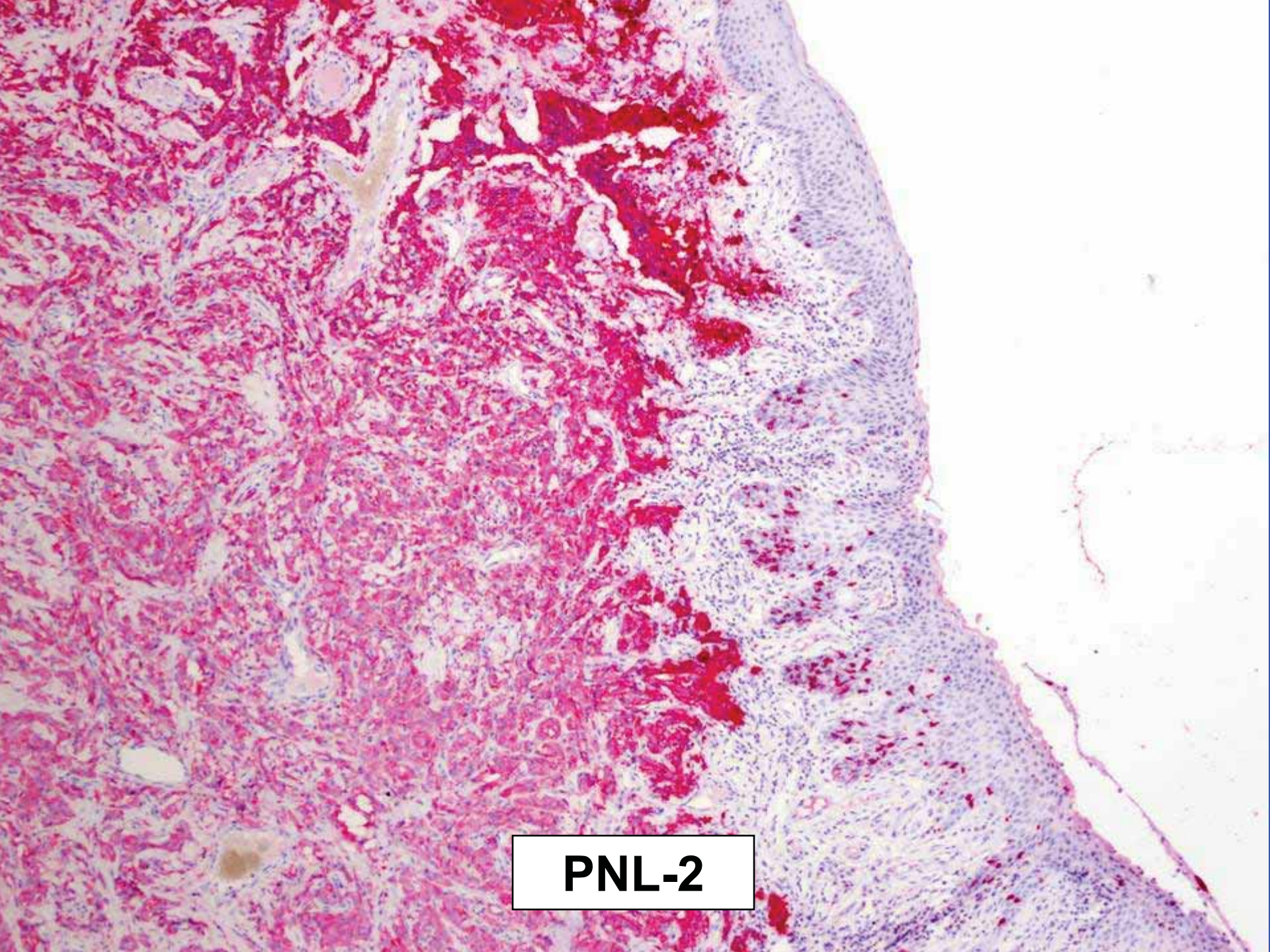

MNF116

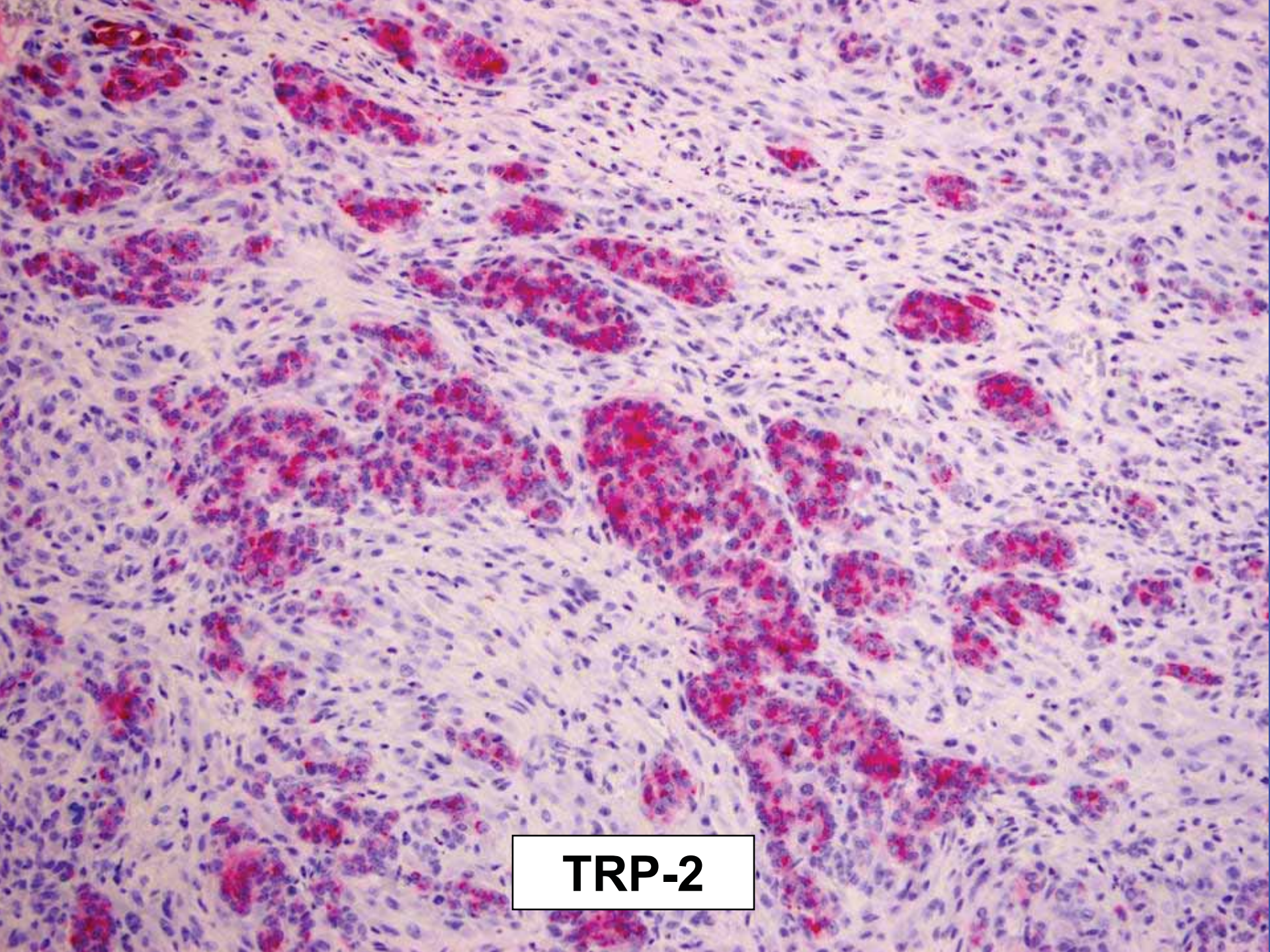

Melan A


HMB45


S100

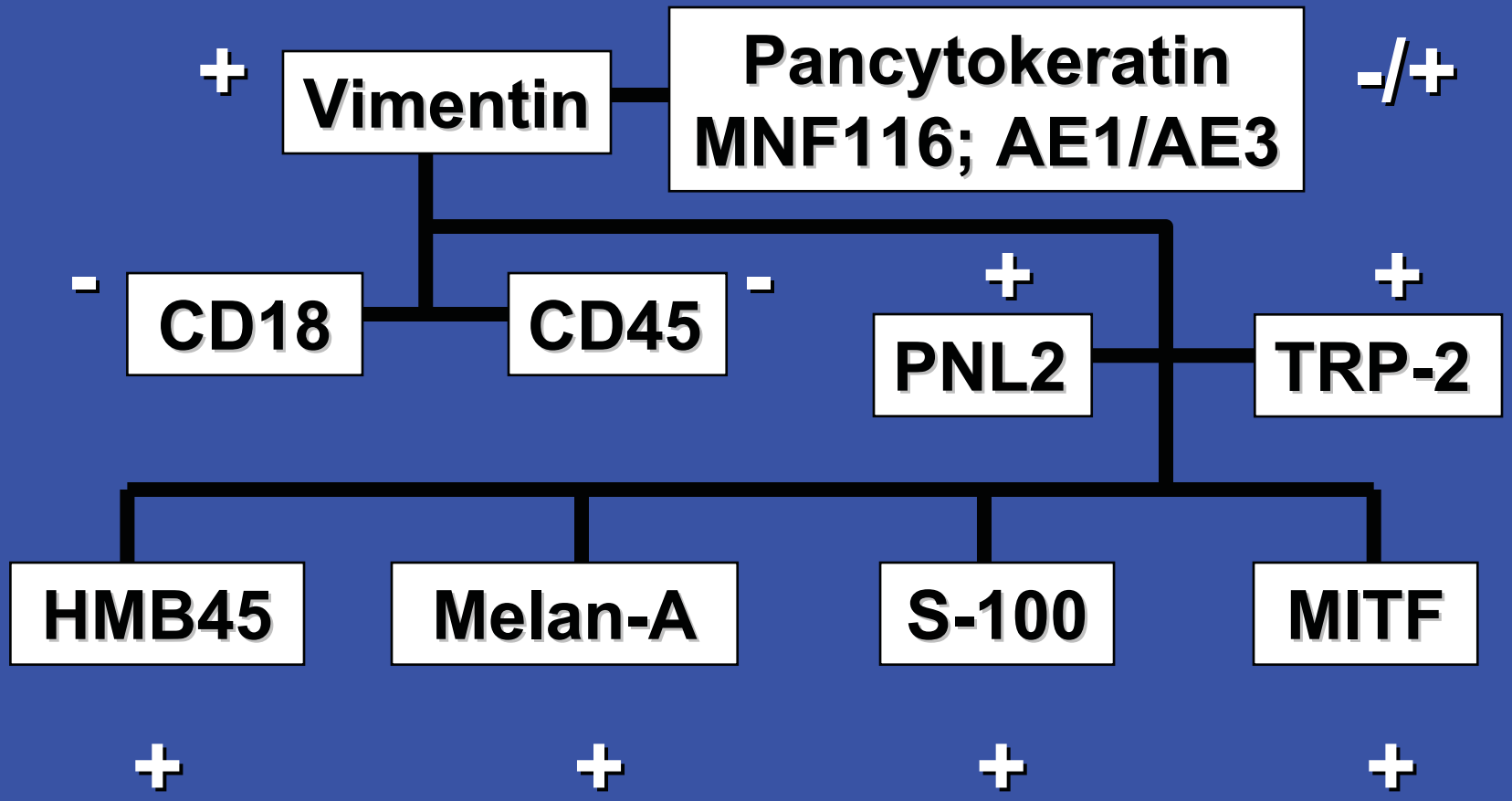

MITF


PNL-2


TRP-2


What's your
Diagnosis

Cutaneous and Oral Melanomas

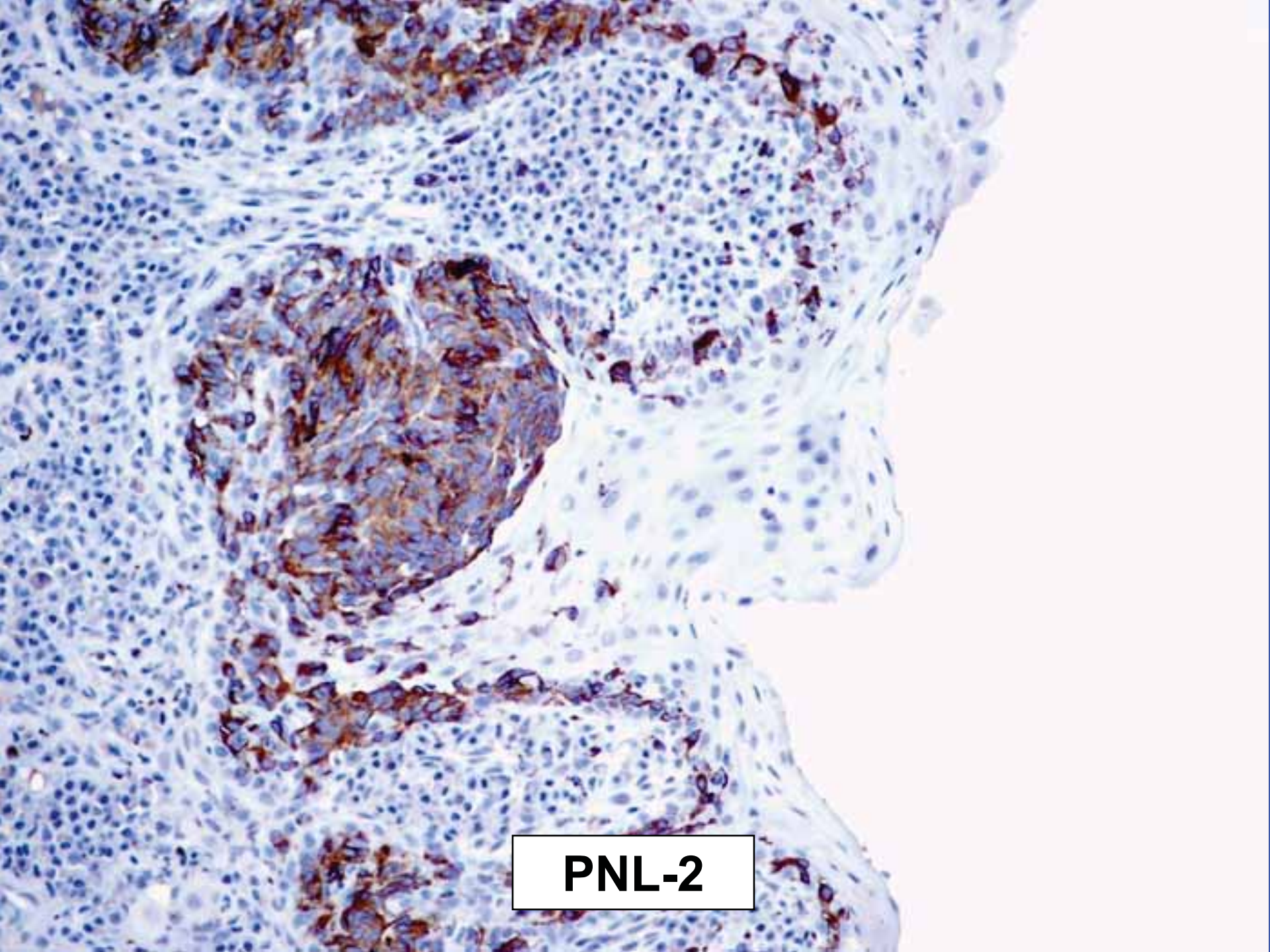


Cutaneous and Oral Melanomas

- Melan A - product of the MART-1 gene (Melanoma antigen recognized by autologous cytotoxic T-lymphocytes)
  - melanocytes, sex cord stroma, adrenal cortex
  - sensitive (85%) and specific marker for melanomas
  - sensitivity lowest in spindle cell amelanotic melanomas
- HMB45 - Melanosomal glycoprotein gp-100
  - melanocytes, other neural crest cells
  - very specific, but less sensitive (70%) than melan A
  - sensitivity is very low in amelanotic melanomas
- S-100 - Staining is nuclear and cytoplasmic:
  - glial, ependymal cells, Schwann cells, melanocytes, dendritic cells, sweat glands of the skin, myoepithelial cells, chondrocytes, adrenal, thyroid, neuroendocrine
  - sensitive for poorly differentiated melanomas
  - approximately 90% of amelanotic melanomas

Cutaneous and Oral Melanomas

- MITF - Microphthalmia associated transcription factor
  - melanocytes
  - nuclear expression
  - nearly 100% sensitivity
  - preliminary studies detected it in soft tissue sarcomas
- TRP-2 - Melanosomal glycoprotein gp-100
  - melanocytes, other neural crest cells
  - very specific, but less sensitive (70%) than melan A
  - sensitivity is very low in amelanotic melanomas
- PNL-2 - Staining is nuclear and cytoplasmic:
  - detects normal and neoplastic melanocytes
  - sensitivity superior to Melan-A and MITF, similar to S-100


PNL-2

Study Design

- 50 canine oral amelanotic melanomas
  - location within the oral cavity or on the lip
  - evidence of epitheliotropism or at least junctional activity
  - minimal to no melanin pigment
  - cellular morphology suggestive of melanocytic origin (polygonal to spindle shaped cells).
- 10 well-differentiated, well pigmented canine oral melanomas (positive controls)
- 10 cases of well-differentiated, subcutaneous soft tissue spindle cell sarcomas (negative controls)
- IHC for Melan-A, HMB-45, MITF, S-100 , PNL-2, tyrosinase, tryptophane hydroxylase, tyrosine hydroxylase, and TRP-1 and TRP-2

Preliminary Data

- Melan A, HMB45, PNL-2, TRP-2 are highly specific for canine melanomas
- S-100, MITF, PNL-2, TRP-2 and Melan A have high sensitivity for canine melanomas
- S-100 and MITF are less specific
- Cocktail of Melan A, PNL-2 and TRP-2 will detect approximately 95% of oral amelanotic melanomas
- Use alkaline phosphatase with red chromogen for nuclear markers and pigmented tumors
- Bleach after the immunostaining
- Strongest expression in cells with junctional activity
- Always sample overlying epithelium (non-ulcerated tumor portion), more differentiated cells

Canine Melanocytic Neoplasia

- Haired skin: Most commonly benign
- Oral, subungual: Most commonly malignant
- Mechanisms underlying these differing clinical outcomes are not understood

Oncogene-induced Senescence Accounts for the Biological Behavior of Benign Canine Melanocytic Neoplasia

- Some benign tumors rarely ever undergo malignant transformation - mechanism?
- For some tumors it is difficult to distinguish benign from malignant lesions - markers?
- Mechanisms that limit growth may be of future therapeutic utility

Normal Cells

- Replicative senescence
- Senescence: irreversible exit from the cell cycle with preservation of metabolic function
  - Shortening of telomeres with each cell division
  - Critical length – triggers cell cycle arrest
  - Can be prevented by over-expression of telomerase (stops telomere shortening)

Cancer cells: limits on proliferation?

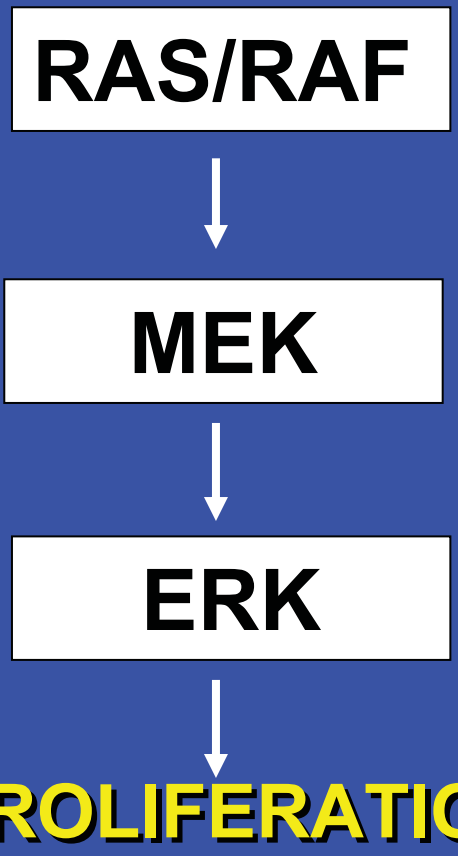
- Ischemia/hypoxia - necrosis
- DNA damage – cell cycle arrest (p53)– apoptosis
- Replicative senescence – (telomerase)
- Premature senescence:
  - Not dependent on replication number
  - Telomere-independent (telomerase does not prevent)
  - Specifically induced by certain oncogenes

Overexpression of an Oncogene: RAS Proliferation Signal

BRAF, HRAS mutations:
Constitutive activation

Intact tumor suppressor pathway: p16INK4A

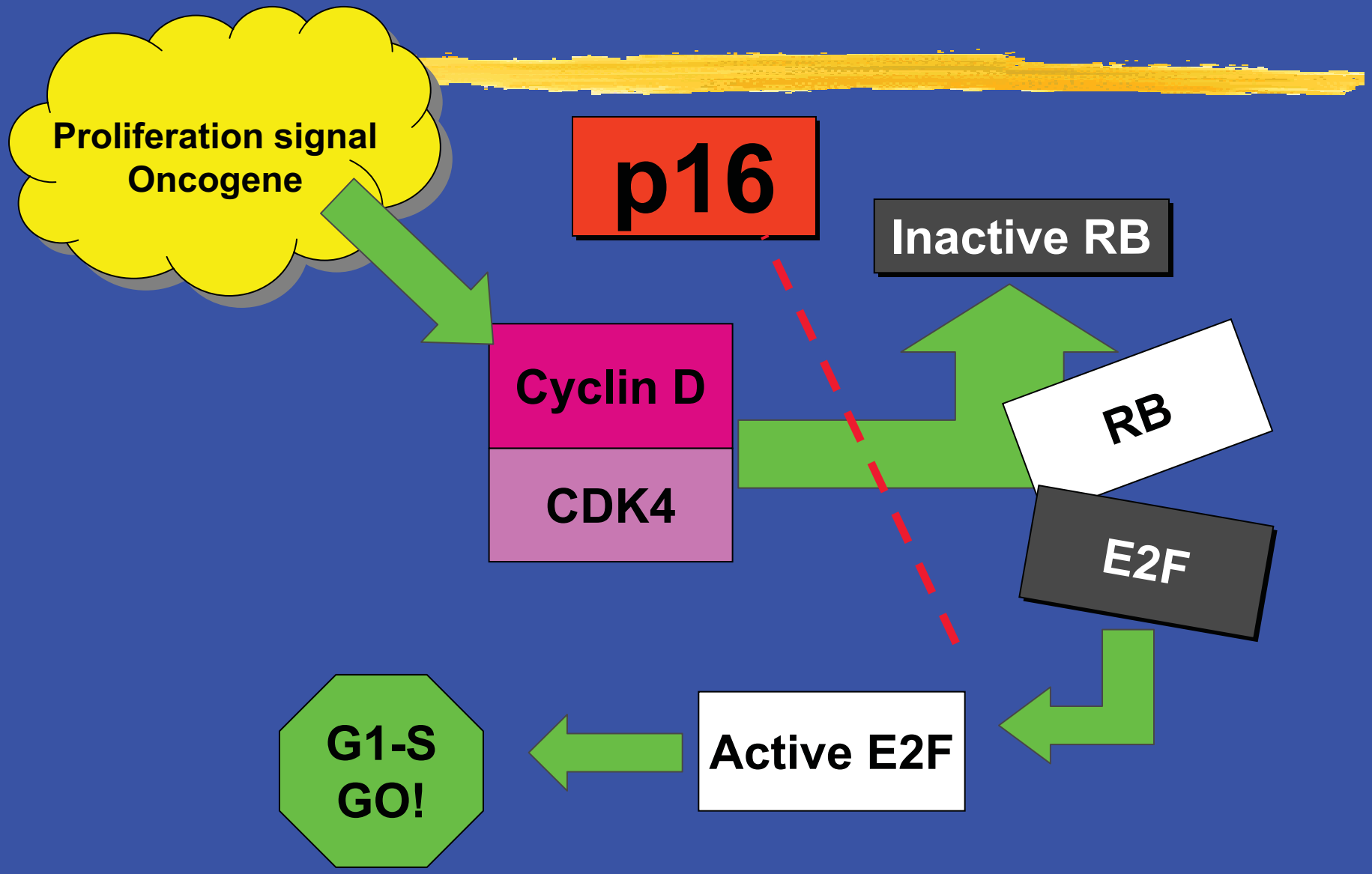
- Key role in regulation of the cell cycle
- Increased expression in NORMAL senescent cells
- Increased expression in some benign tumors
- Expression induced by overexpression of certain oncogenes



MAP kinase pathway

***BUT… benign melanomas eventually STOP proliferating!!!

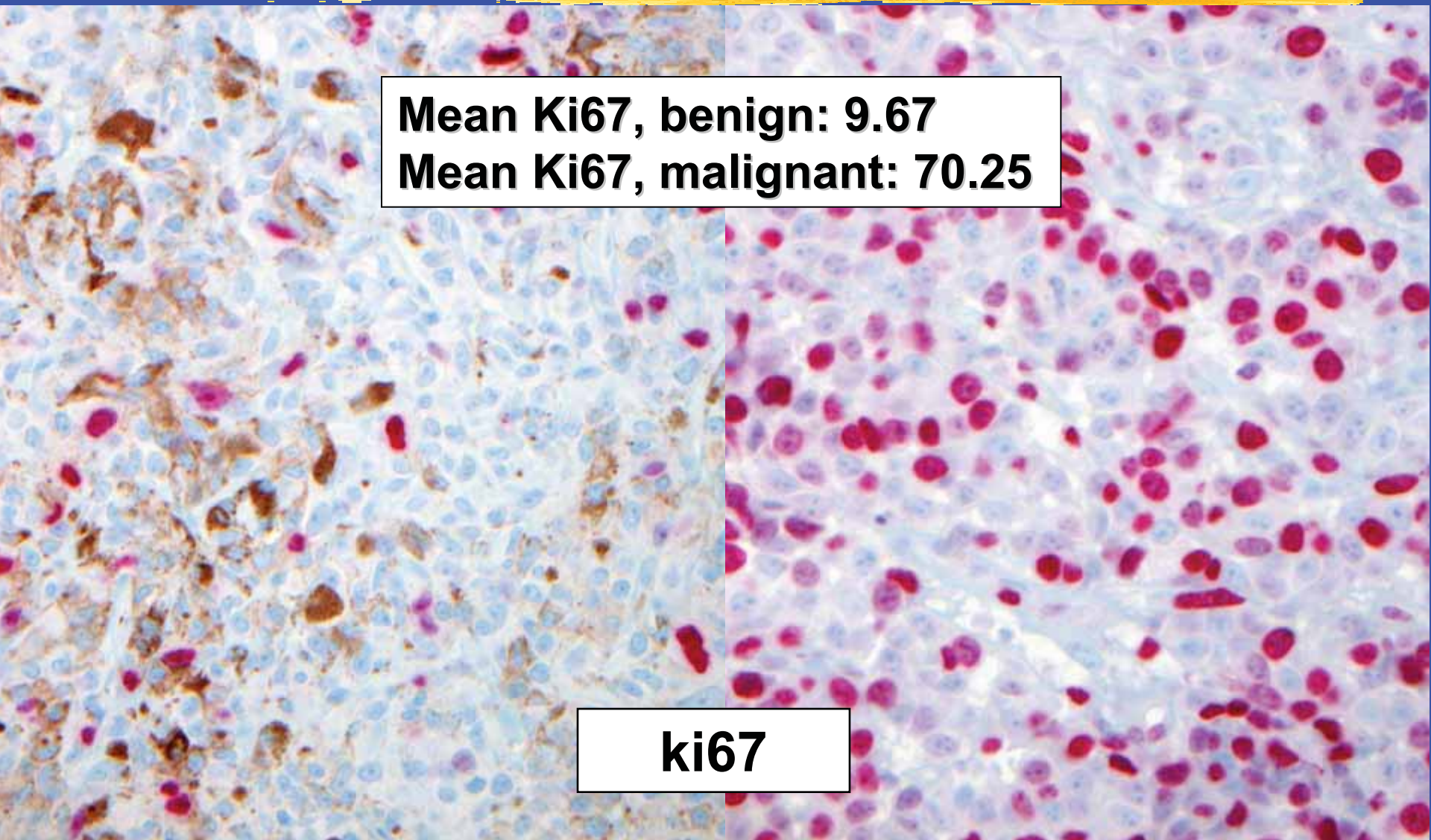
Control of the Cell Cycle at G1-S



Established Markers of Oncogene-Induced Senescence

1. Activation of an oncogene
   - *N-Ras* mutations
   - *B-Raf* mutations
   - H-Ras mutations/copy number increase
   - ERK phosphorylation
2. Total or near-total loss of proliferation
3. Intact tumor suppressor pathway
   - p16
   - p53
4. Senescence-associated β-galactosidase
   - Need frozen sections

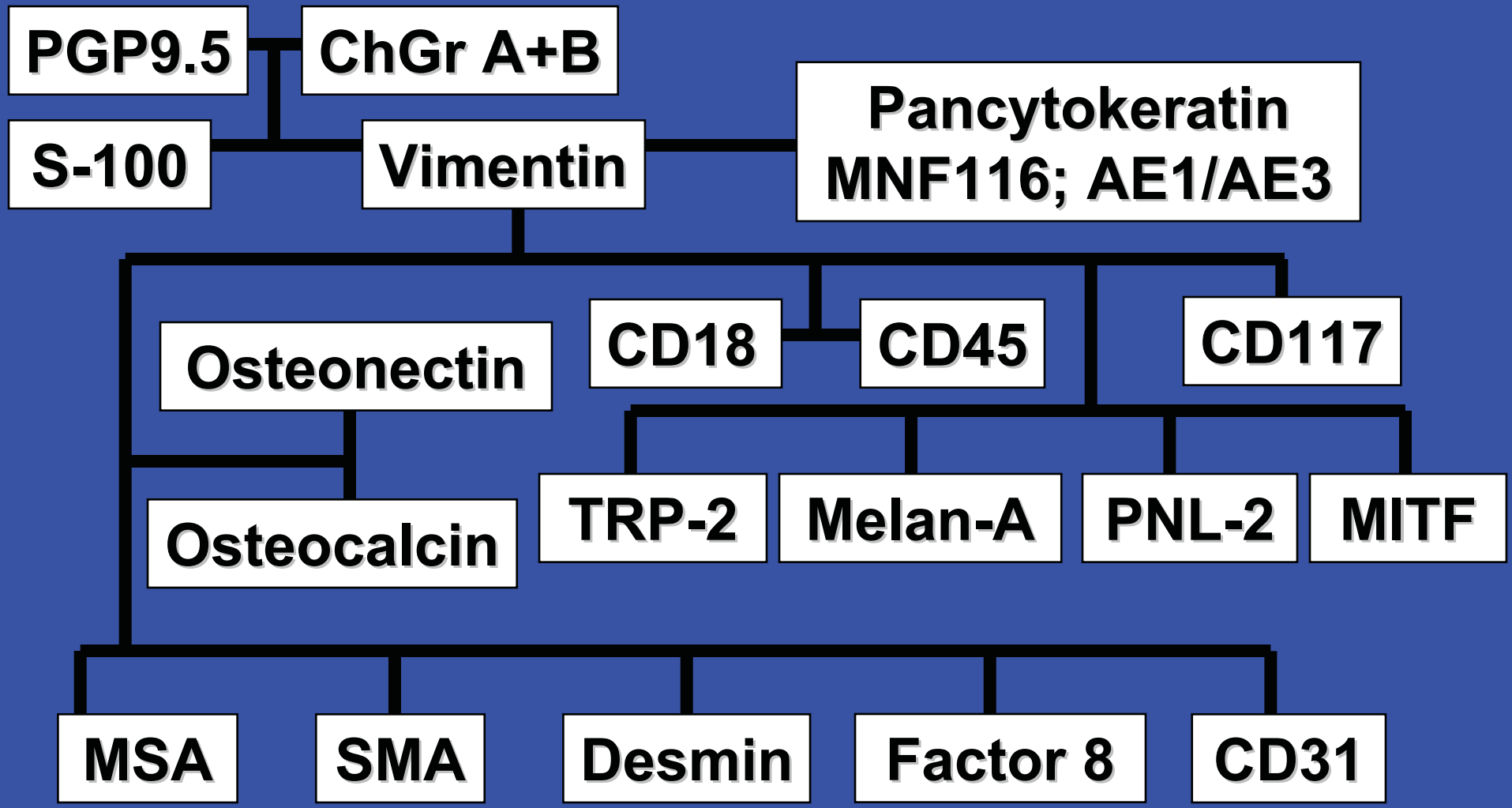
Benign Melanocytic Lesions Have Lower Proliferation Markers than Malignant Lesions



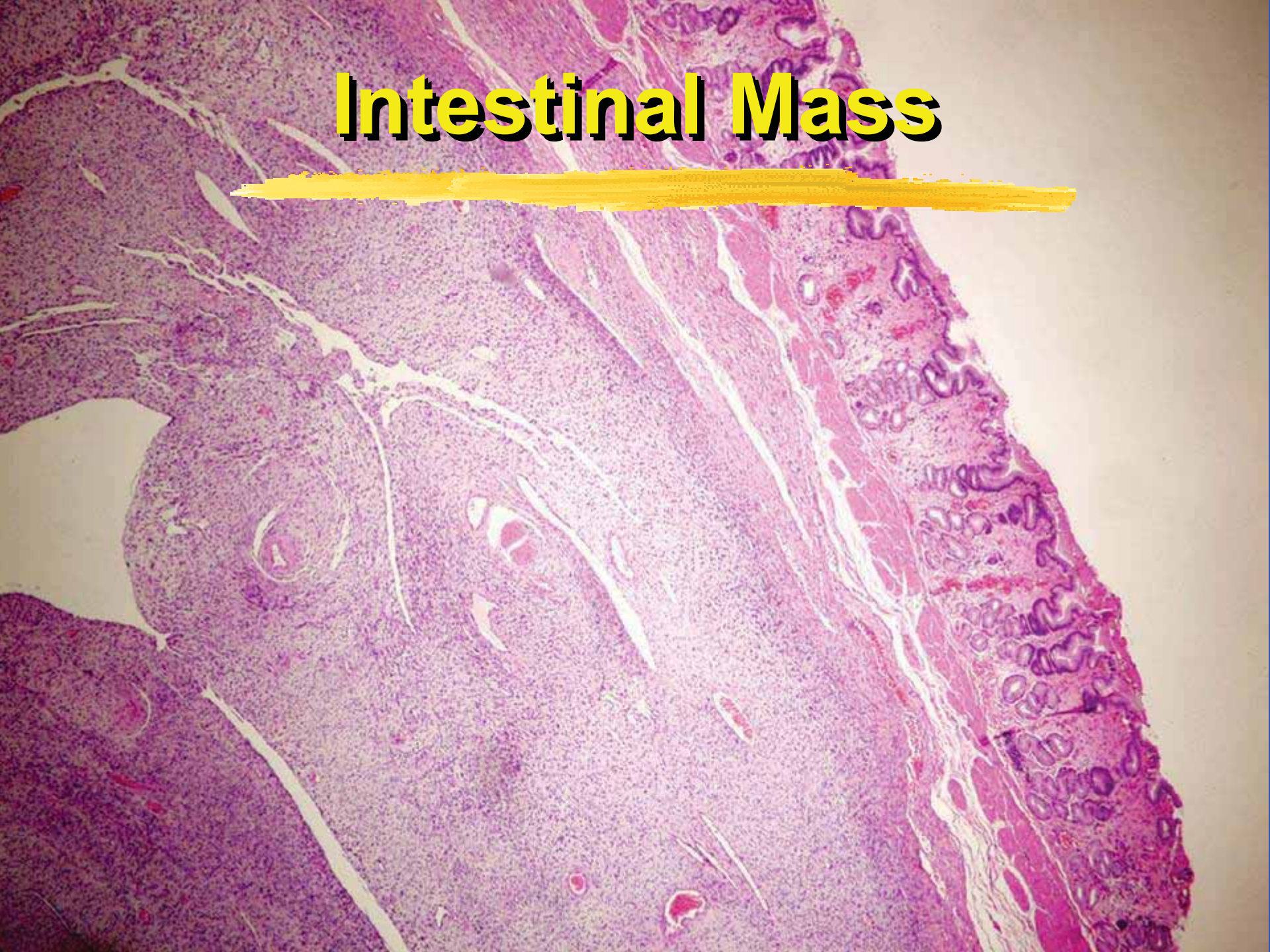
Soft Tissue Sarcomas

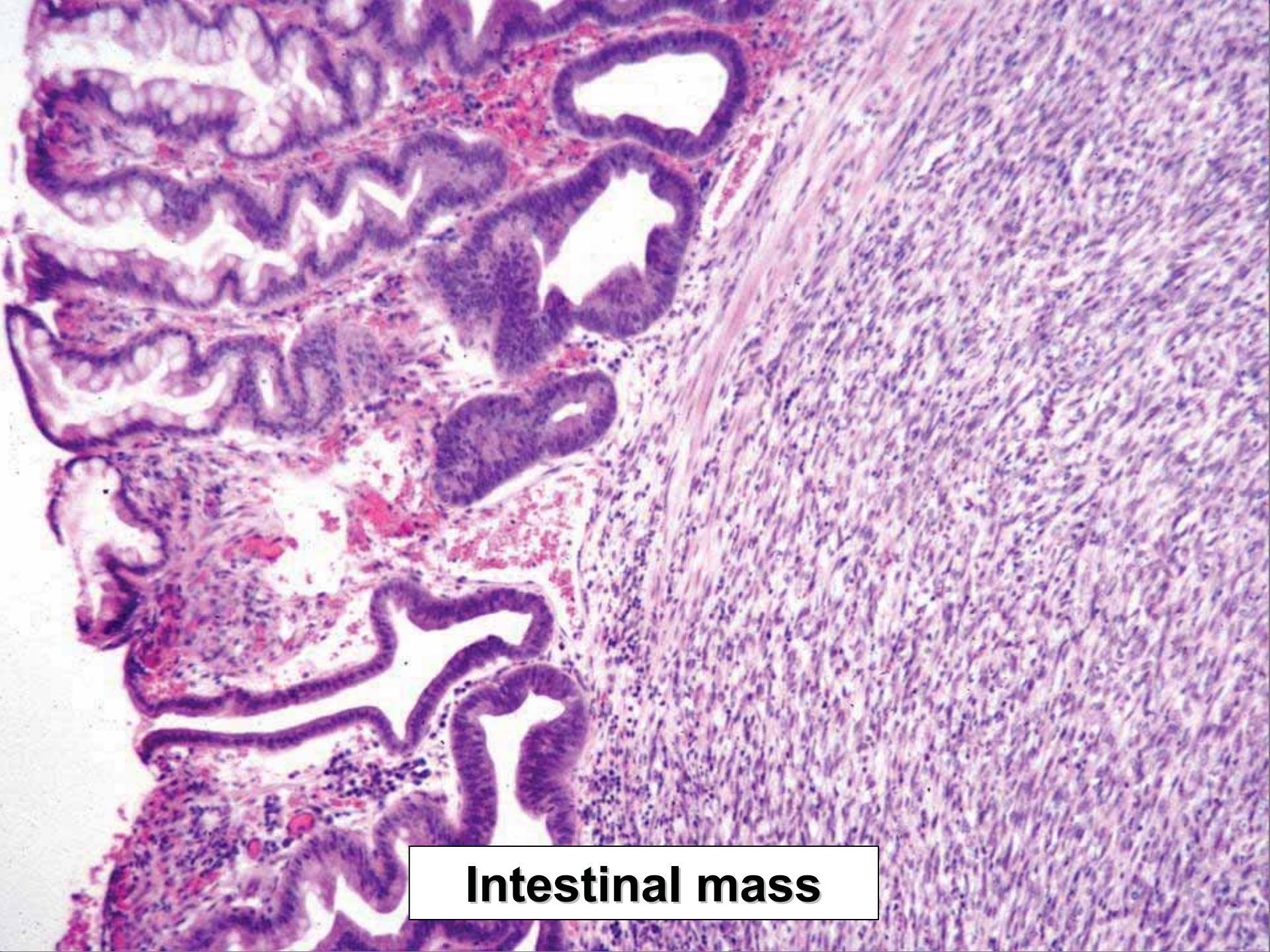
- Vimentin +
- Fibrosarcomas: SMA +/-, Desmin -
- Rhabdomyosarcoma: Desmin +, SMA -, MSA + myoglobin +
- Leiomyosarcoma: Desmin +/-, SMA +, MSA +
- PNST: S-100 +, PGP 9.5 +/-
- Liposarcoma: S-100 +
- Hemangiopericytoma: Desmin +/-, MSA +/- , S-100 -, Factor 8 -
- MFH: Desmin +/-, MSA +/-
  - Differentiate from histiocytic sarcomas: CD18, CD45

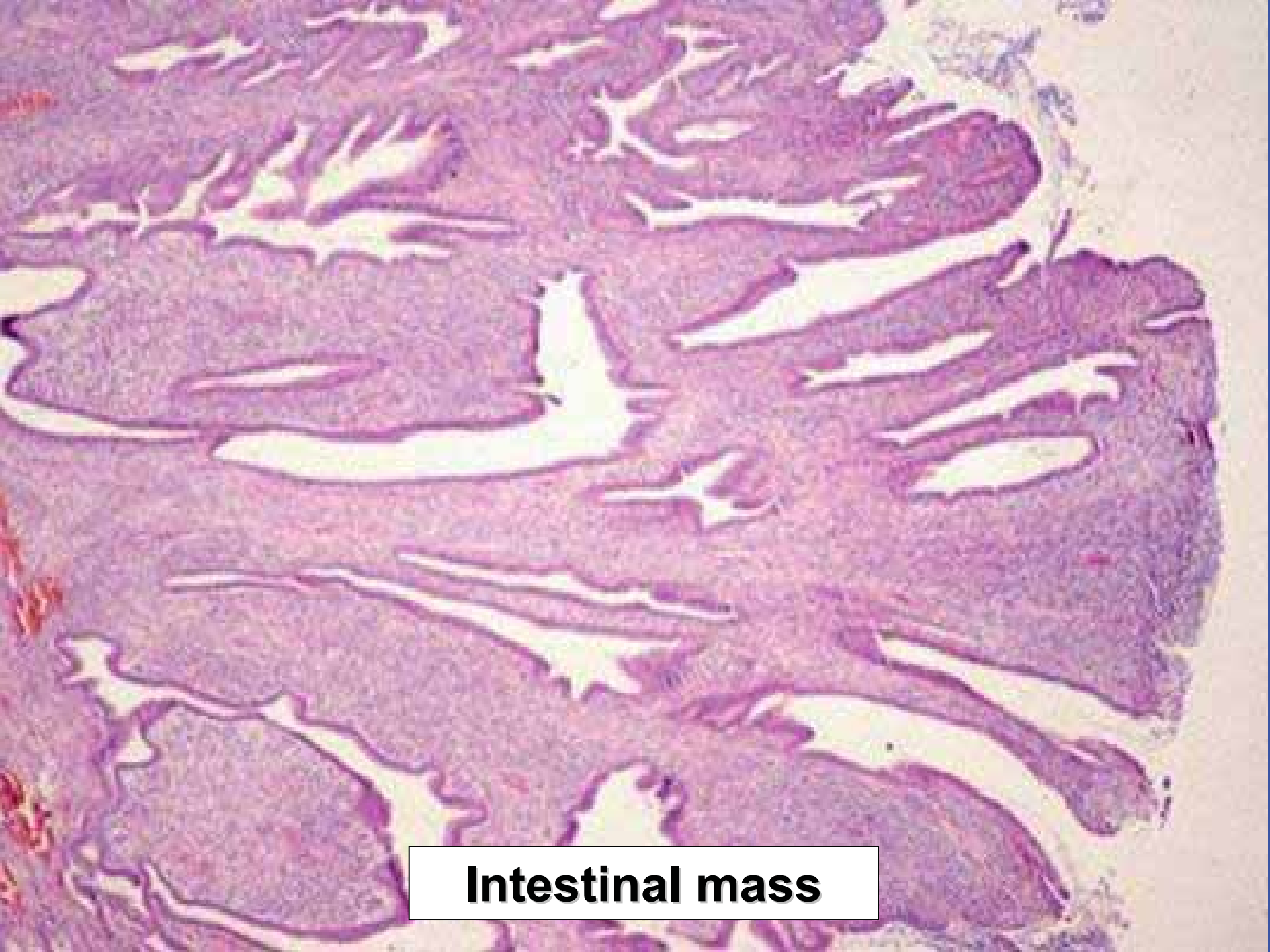
Non-angimatous, Non-lymphomatous Gastrointestinal Sarcomas

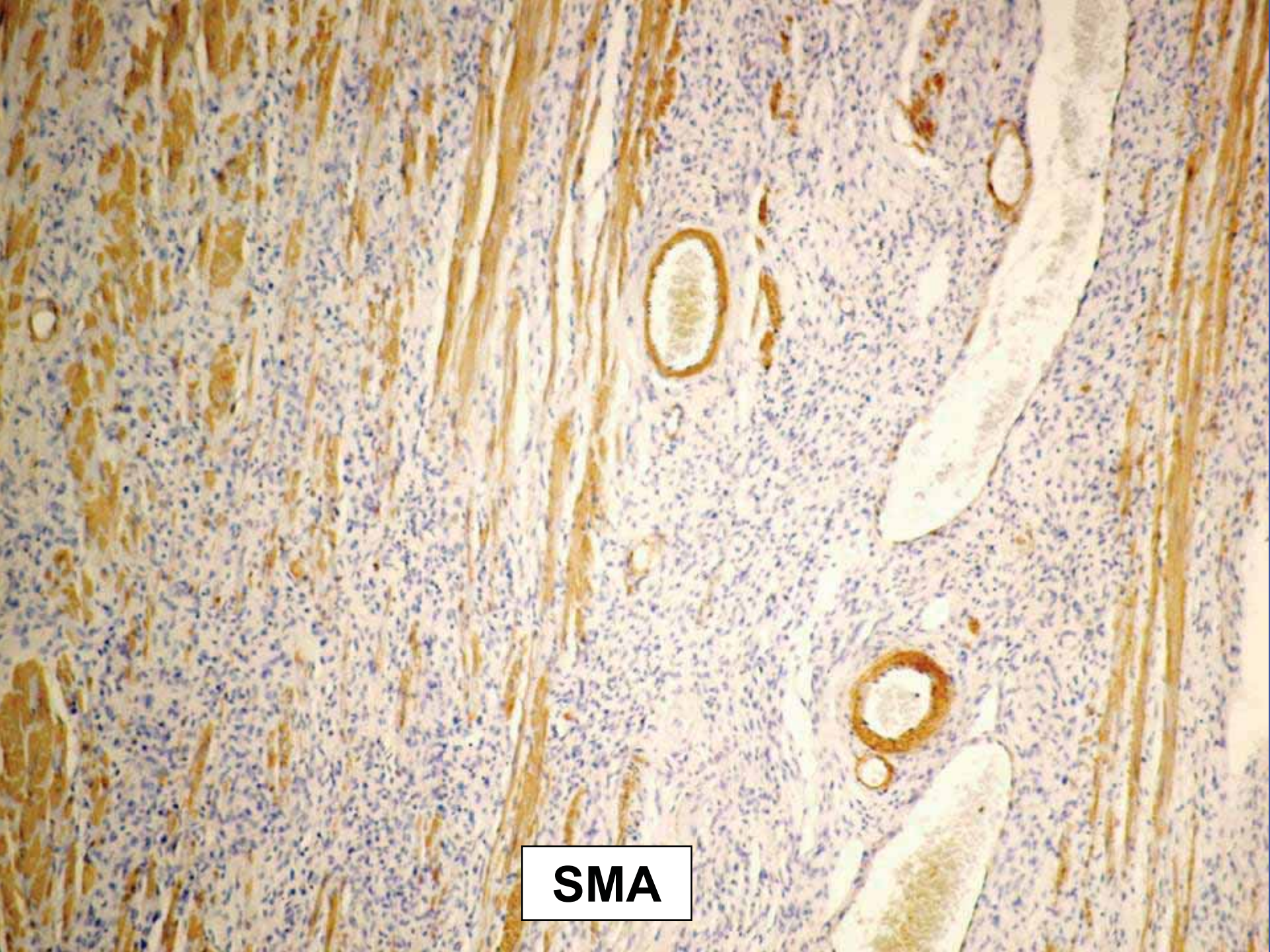


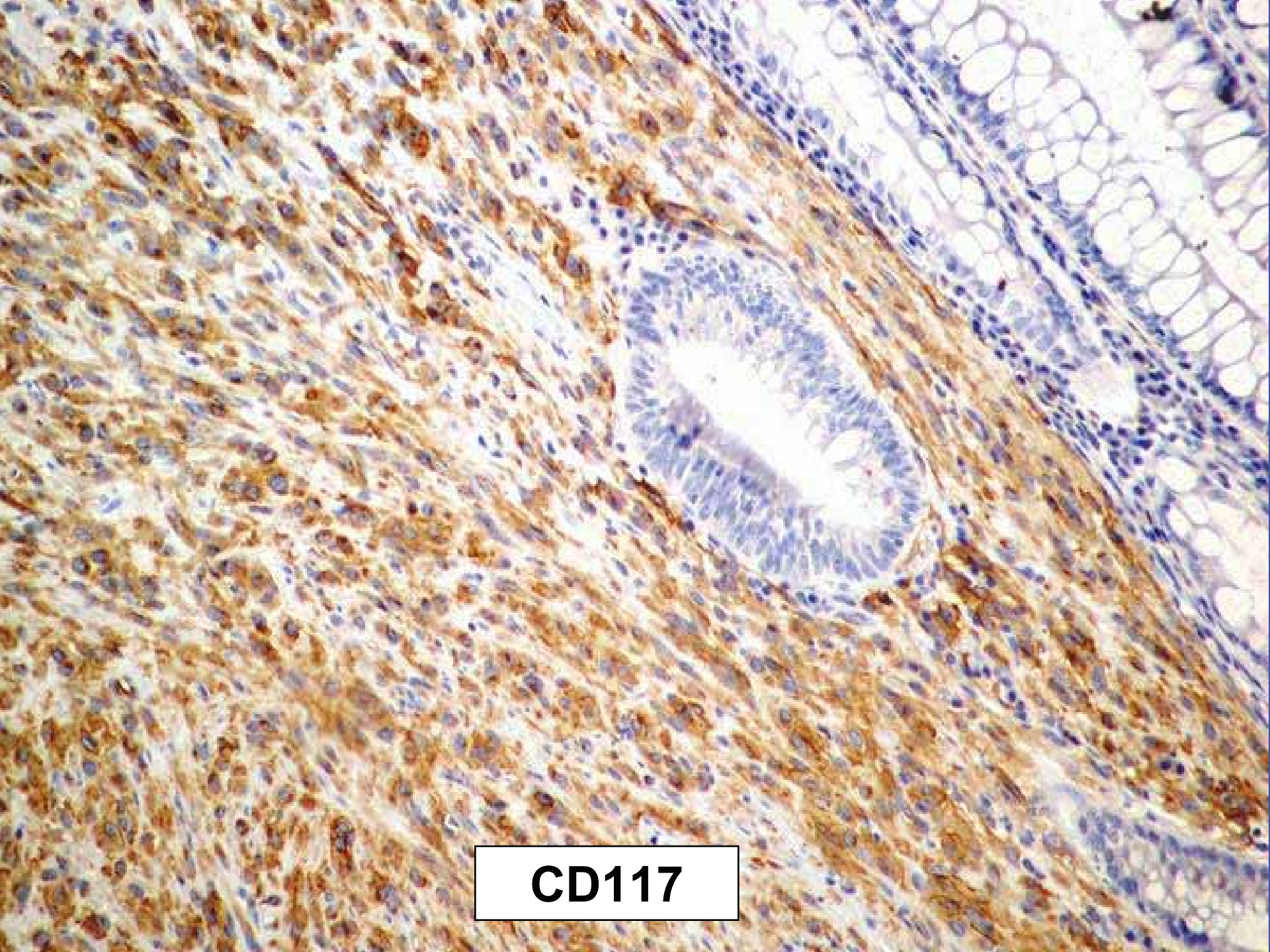
Intestinal Mass

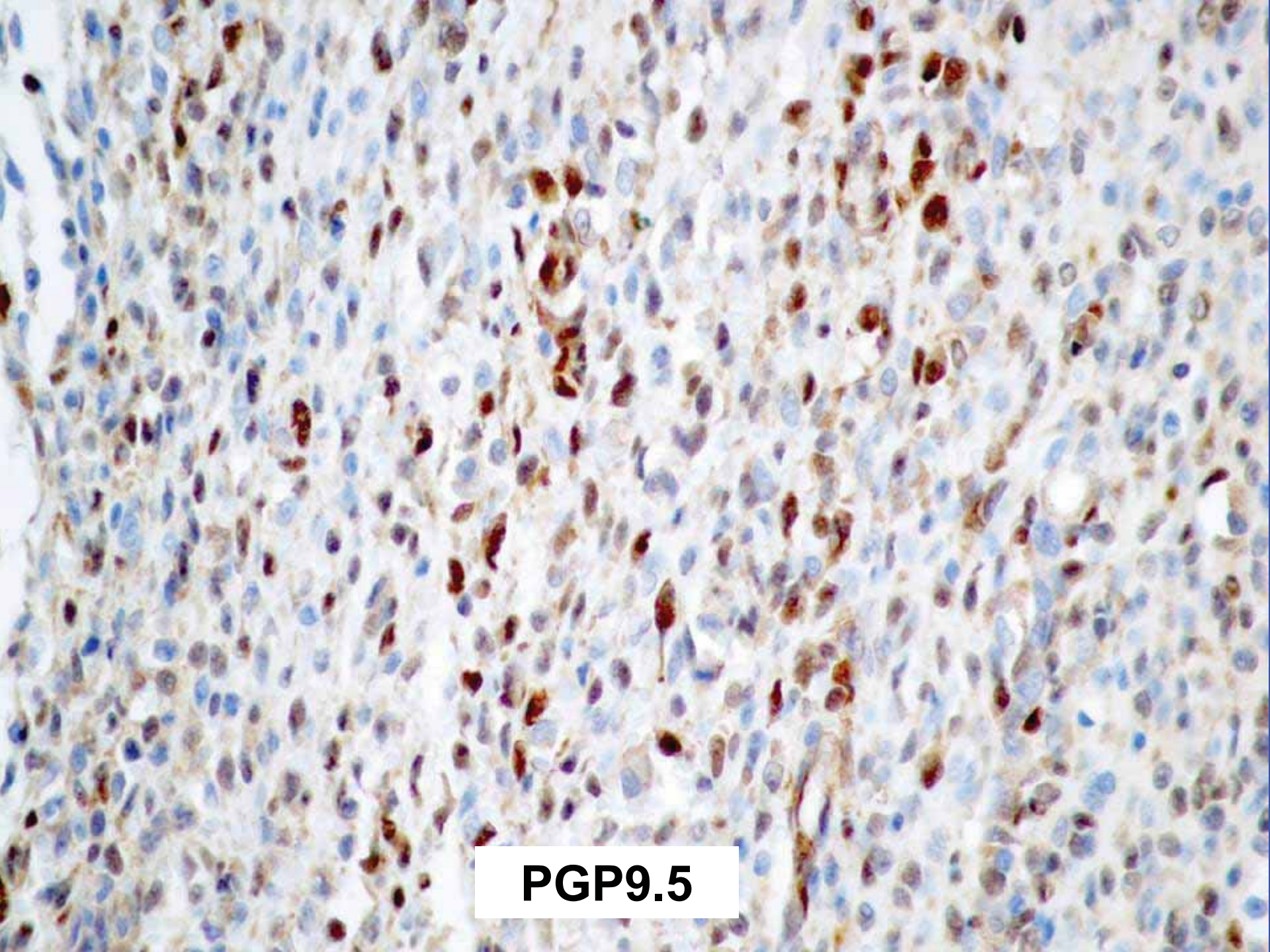



Intestinal mass


Intestinal mass

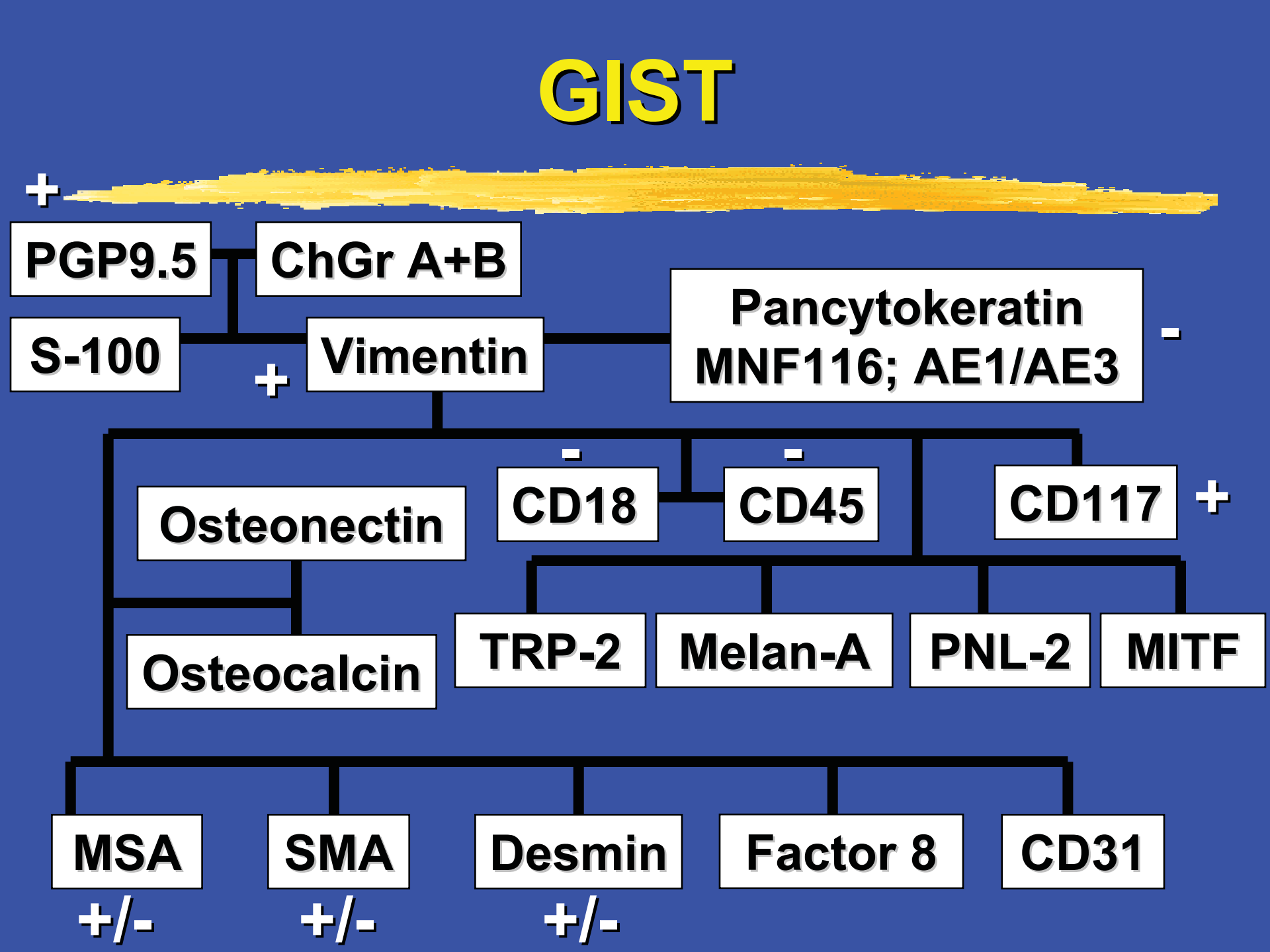

SMA


CD117


PGP9.5

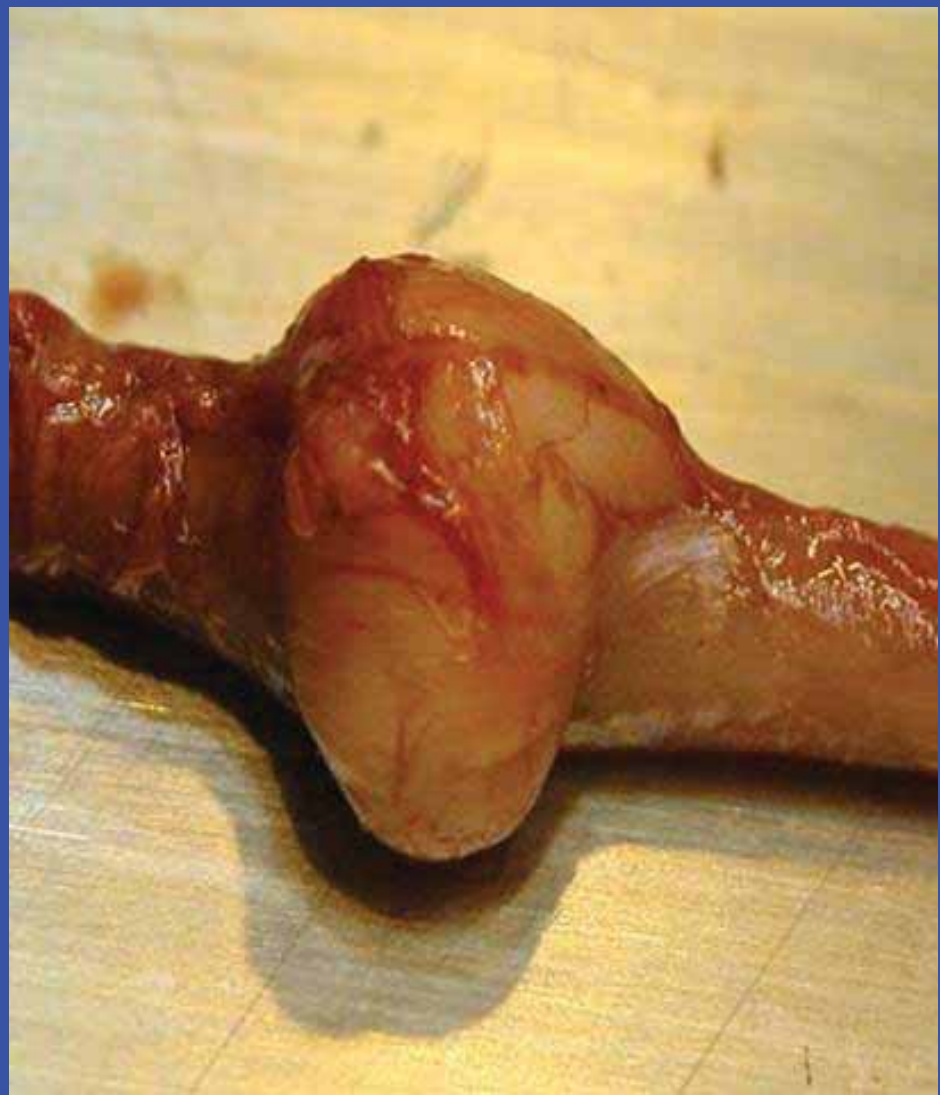
What's your Diagnosis

GIST


+
PGP9.5
ChGr A+B
S-100
Vimentin
+
Pancytokeratin
MNF116; AE1/AE3
-
Osteonectin
CD18
-
CD45
-
CD117
+
Osteocalcin
TRP-2
Melan-A
PNL-2
MITF
MSA
+/-
SMA
+/-
Desmin
+/-
Factor 8
CD31

Non-angimatous, Non-lymphomatous Gastrointestinal Sarcomas

- Included:
  - Fibrosarcoma
  - Leiomyosarcoma
  - Neurofibrosarcoma
  - Spindle cell sarcoma
- Originate from the wall of the gastrointestinal tract
- Mesenchymal/spindle cells
- Smooth Muscle Origin
  - Most common in dogs



What are GIST?

- Distinct tumor entity
  - Histologic characteristics
  - Express tyrosine kinase KIT (CD117)
- Interstitial Cells of Cajal
  - Pacemaker for autonomic movement
- Prognosis
  - Invasive
  - Metastasis

Study Population

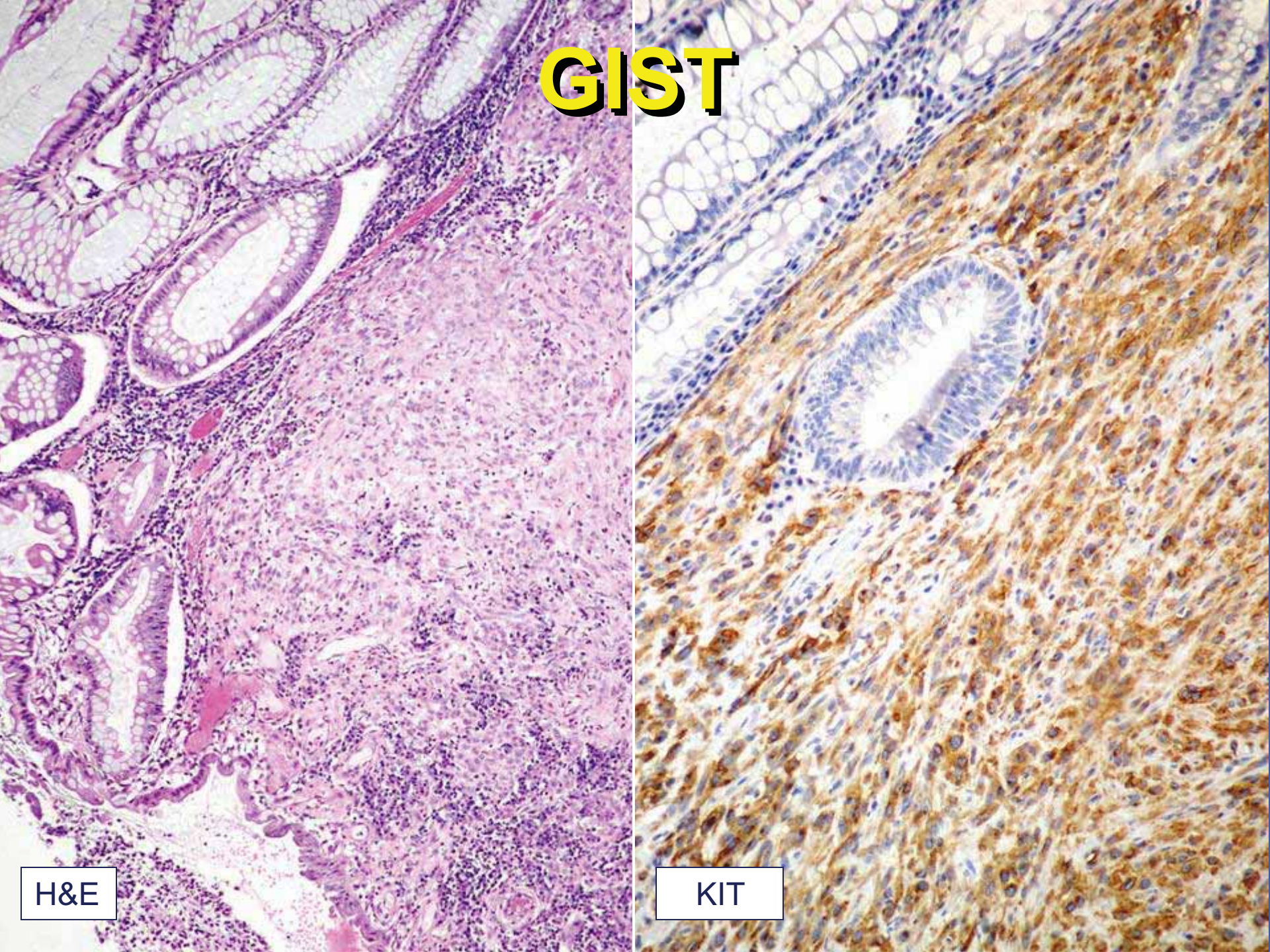
- 41 non-angiomatous, non-lymphomatous gastrointestinal mesenchymal sarcomas
- Leiomyosarcoma, fibrosarcoma, anaplastic sarcoma, spindle cell sarcoma
- Leiomyomas and other benign tumors excluded from study population

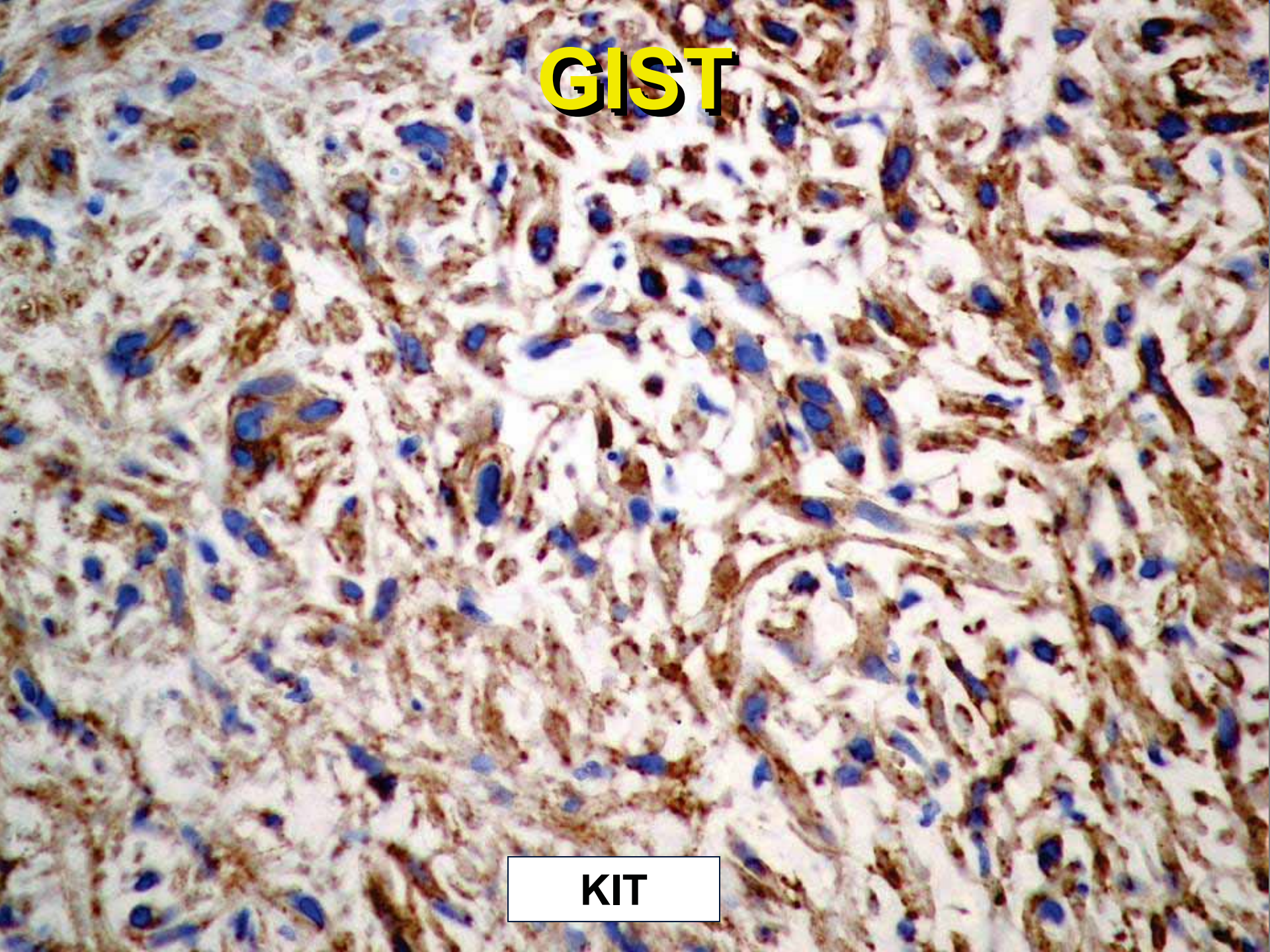
Study Design

- Histochemical Staining
  - Cellular pattern
  - Cell morphology
  - Evidence of vascular/lymphatic invasion
  - Necrosis
- Immunohistochemistry
- DNA isolation/amplification
  - Polymerase Chain Reaction
  - Sequencing and sequence analysis

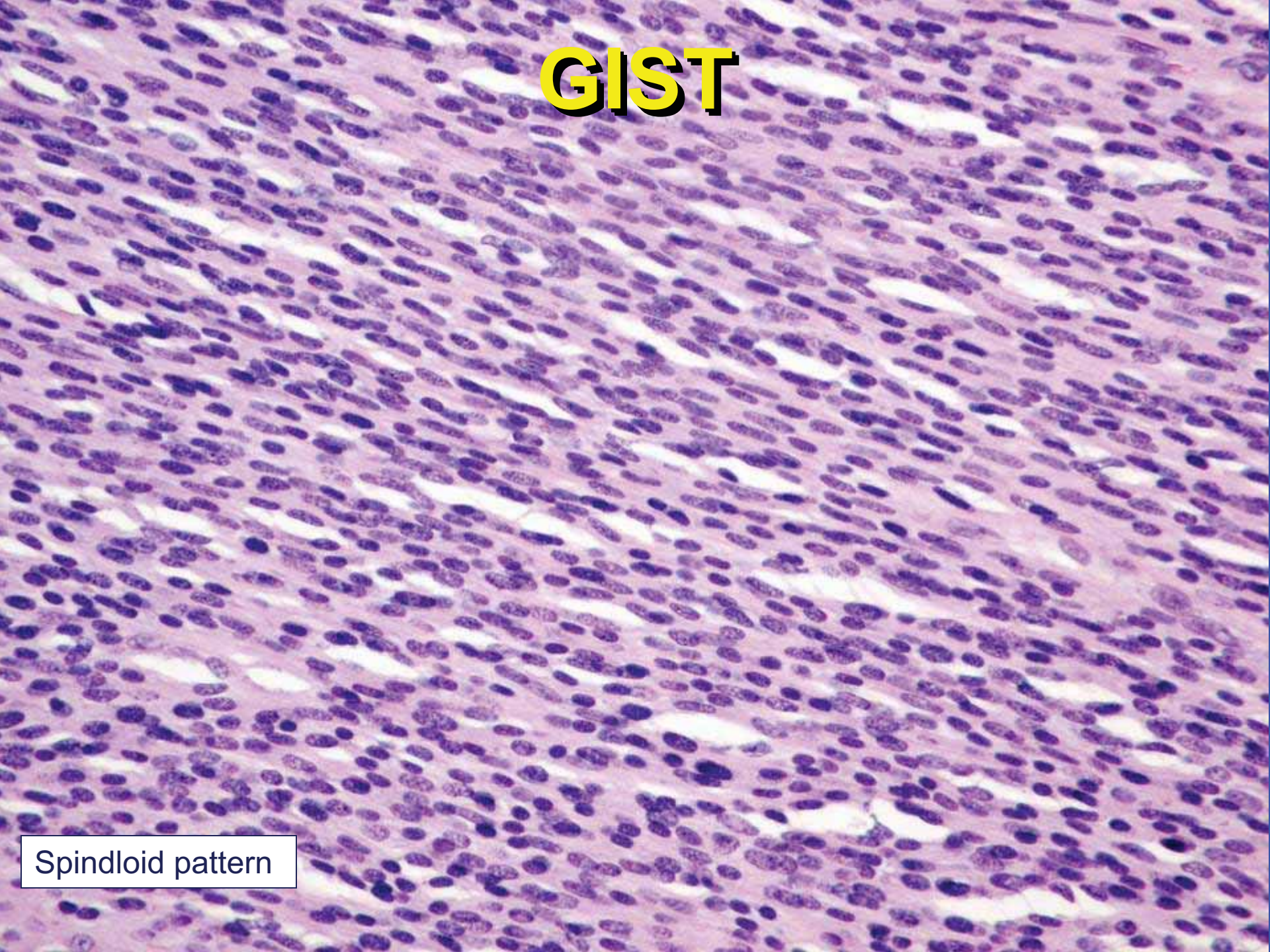
Immunohistochemistry

- KIT
- Vimentin
- Desmin
- Smooth Muscle Actin (SMA)
- S-100
- Pgp 9.5
- Other stains used: Factor 8 related antigen, pancytokeratin, CD18


GIST
H&E
KIT

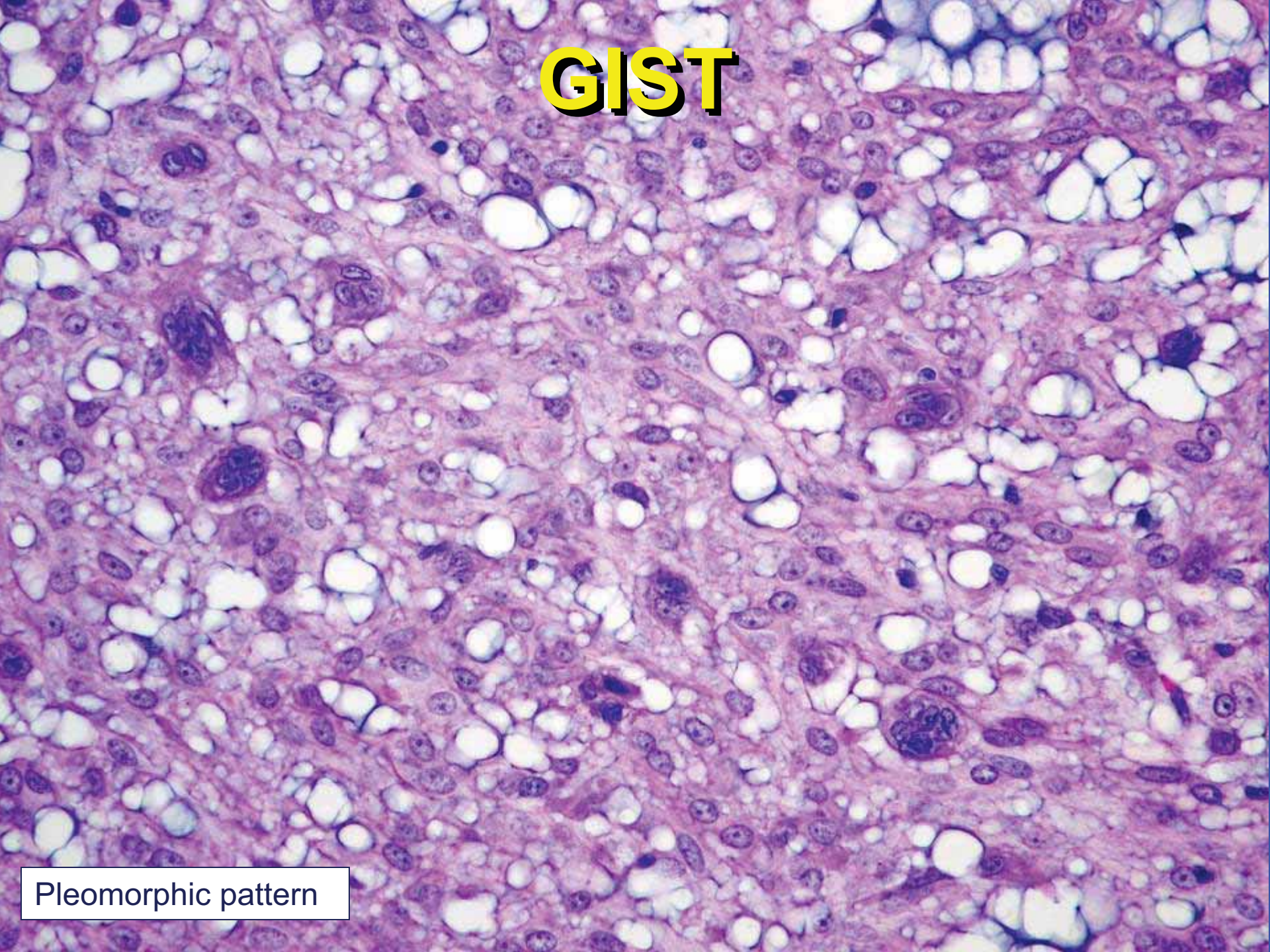

GIST
KIT

GIST



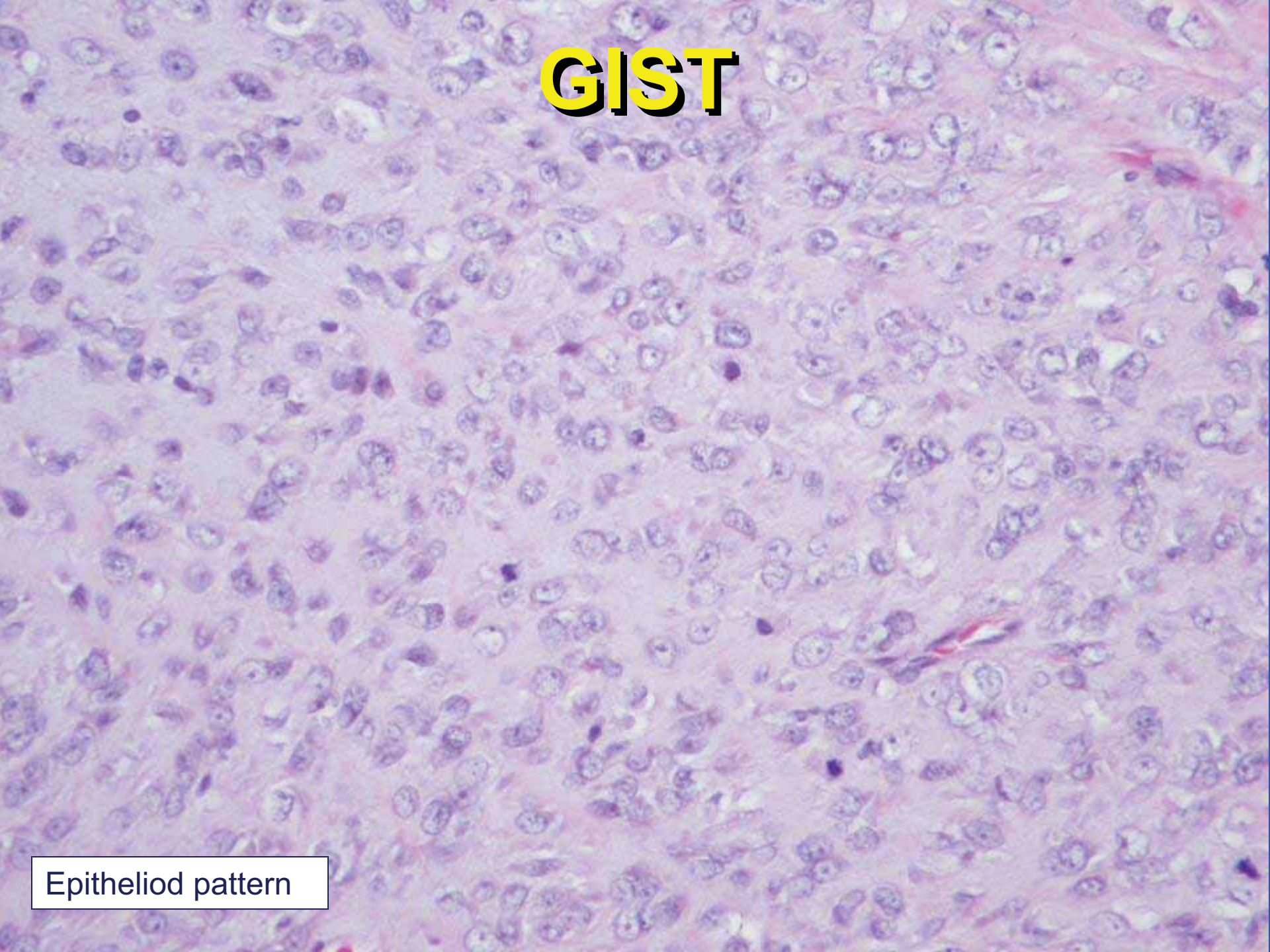
Spindloid pattern

GIST

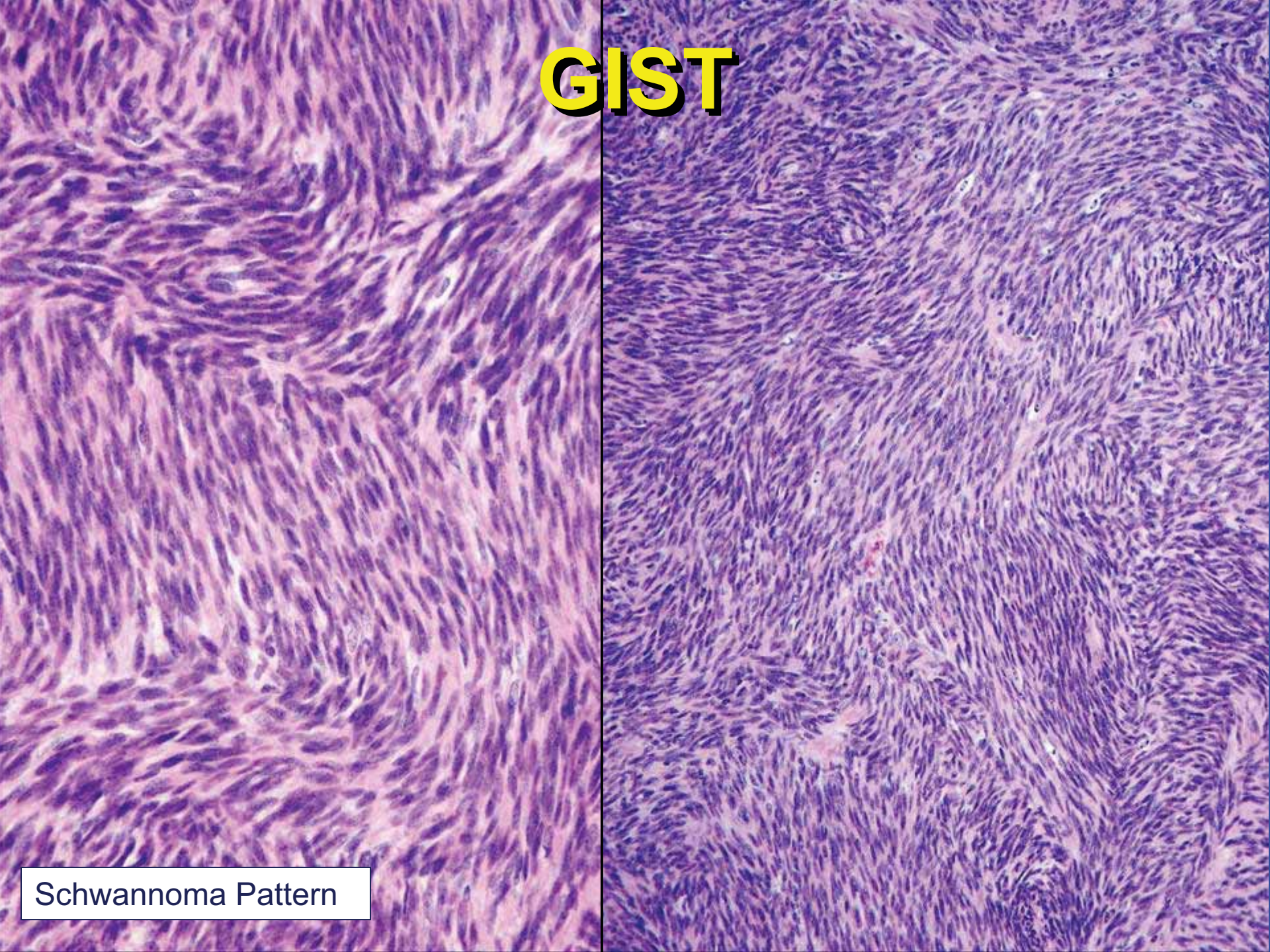


Pleomorphic pattern

GIST



Epitheliod pattern

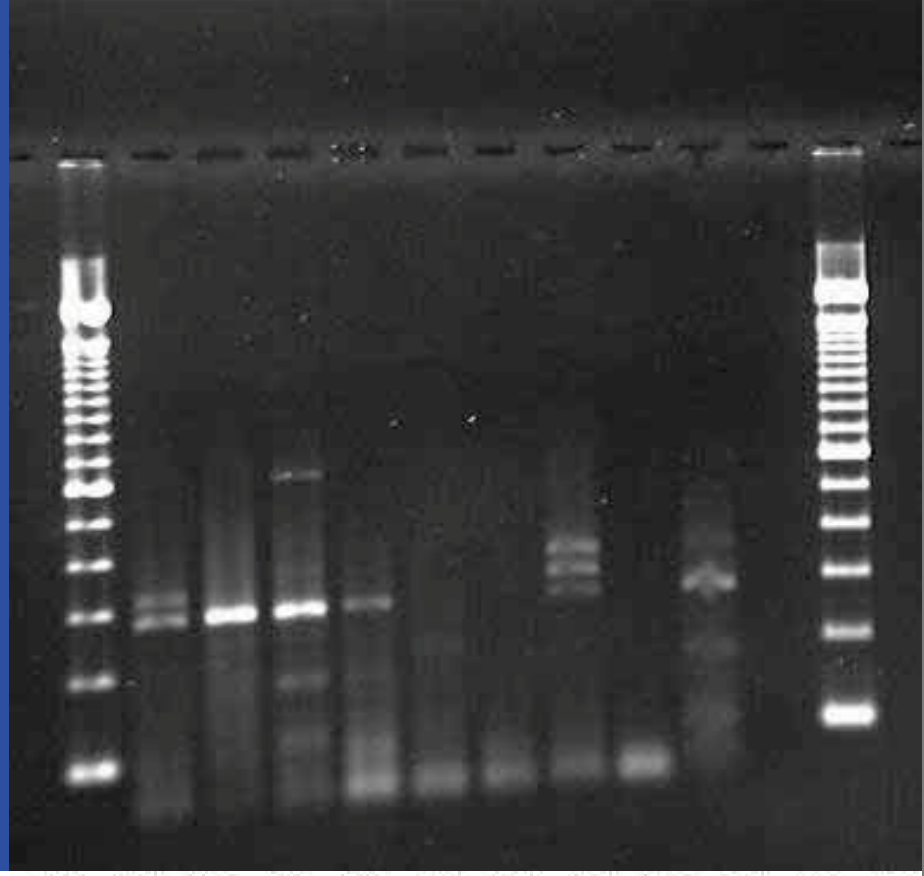

GIST
Schwannoma Pattern

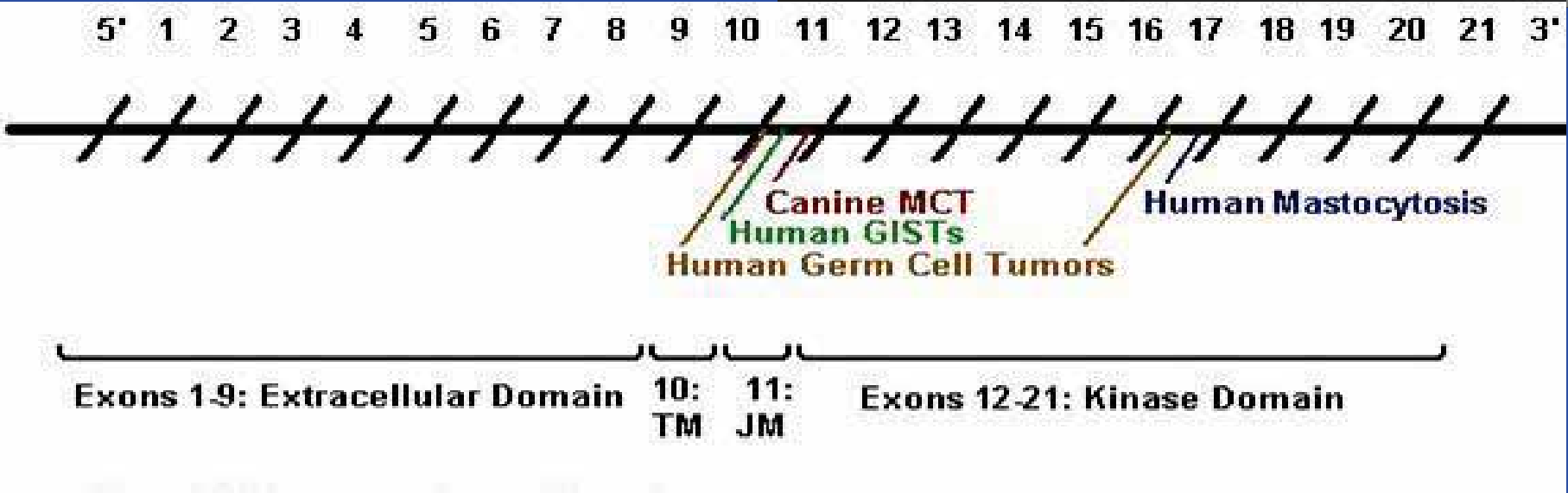
Results

- 18 (44%) KIT positive
  - 72% female, 28% male
  - 4-15 years old, mean age ~11 years
  - Small intestine (67%)
  - 5/18 metastasis to the liver, lymph node or omentum
- Positive immunoreactivity for:
  - Vimentin: (100%)
  - S-100: 14 (78%)
  - Smooth Muscle Actin: 6 (33%)
- Categories
  - Neurogenic, Myogenic, Bi-directional

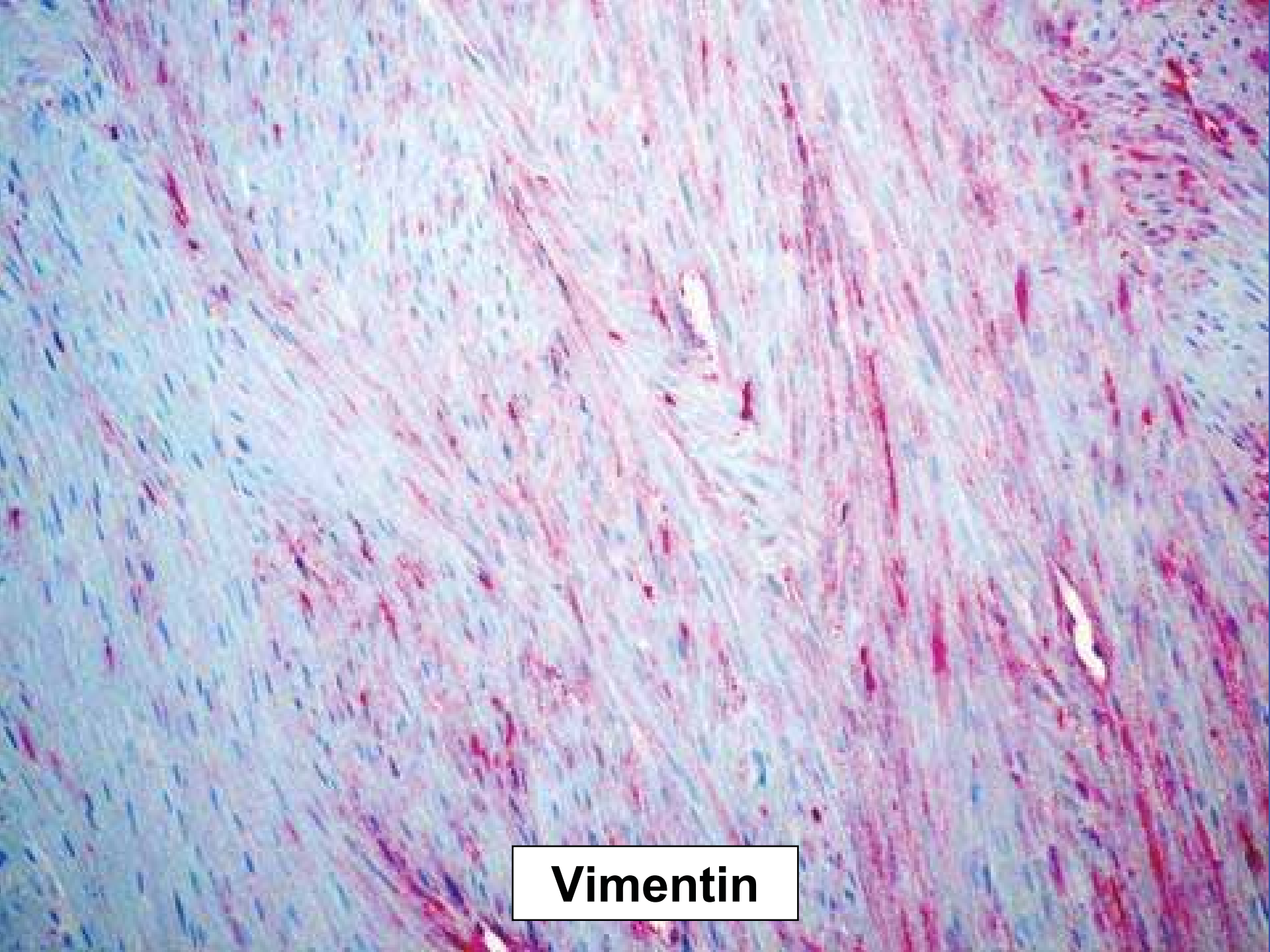
c-*kit* Mutations

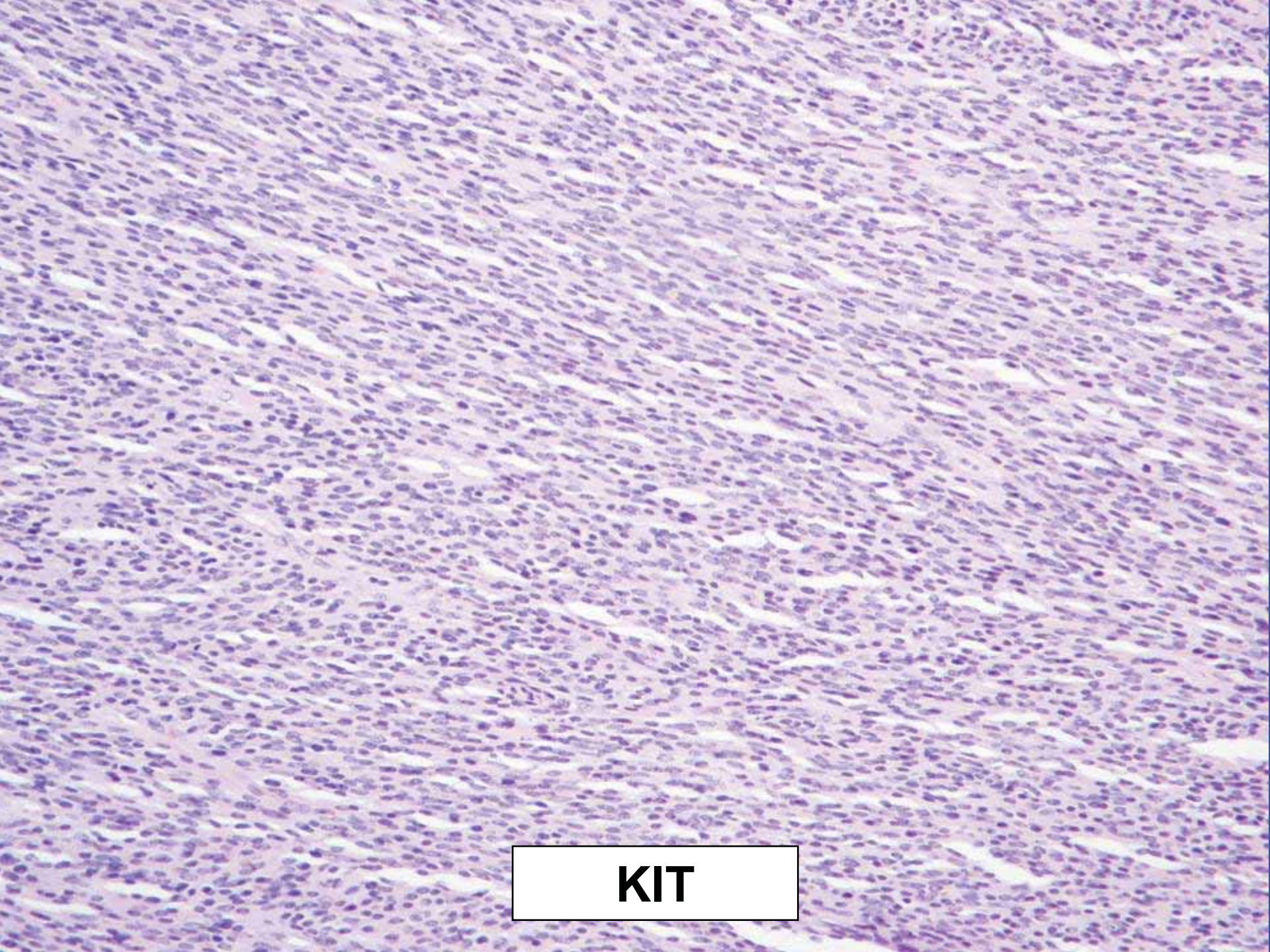
- 16 of these cases yielded amplification products
- 6 cases showed activating mutations in exon 11 of JM

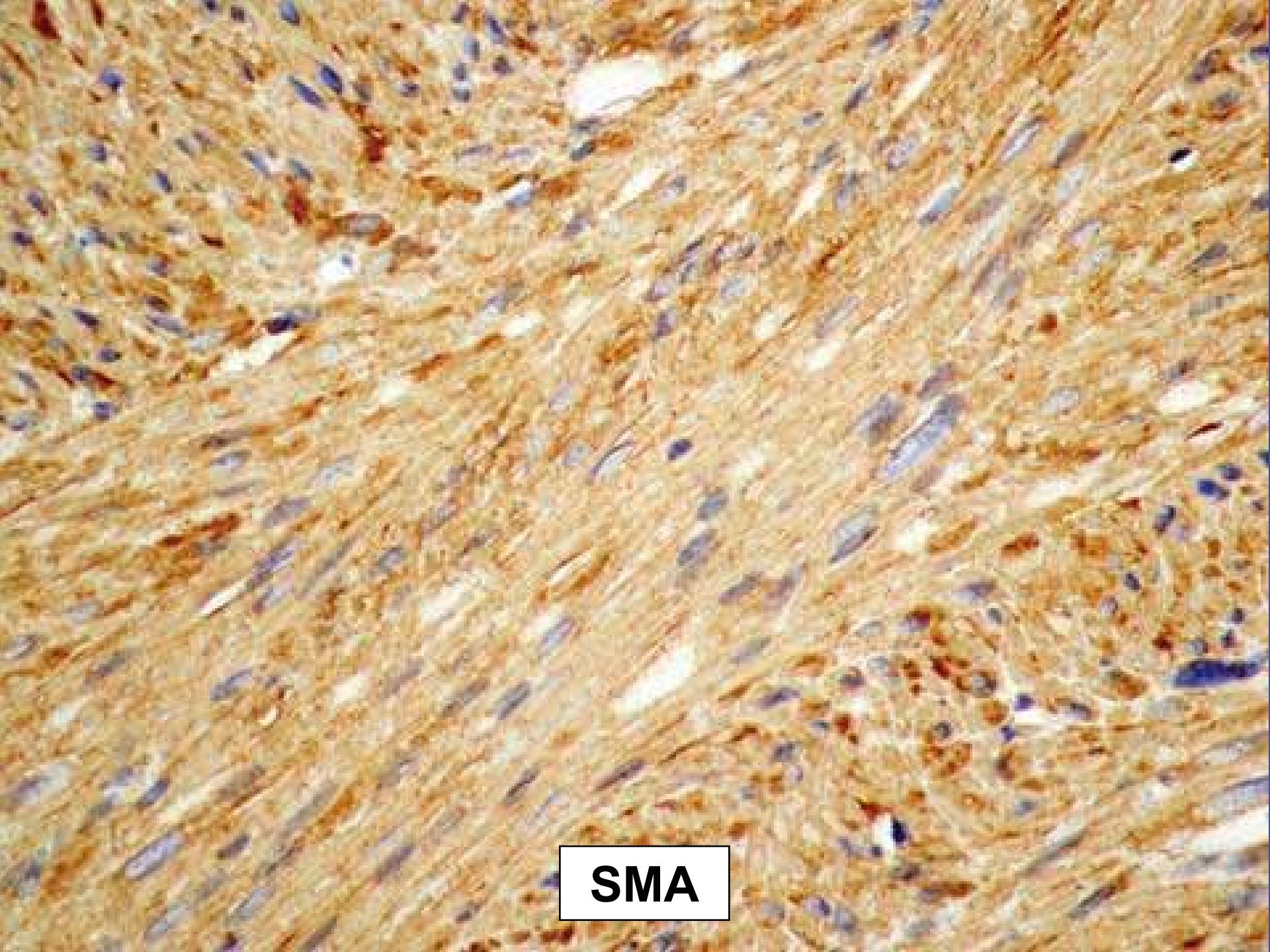


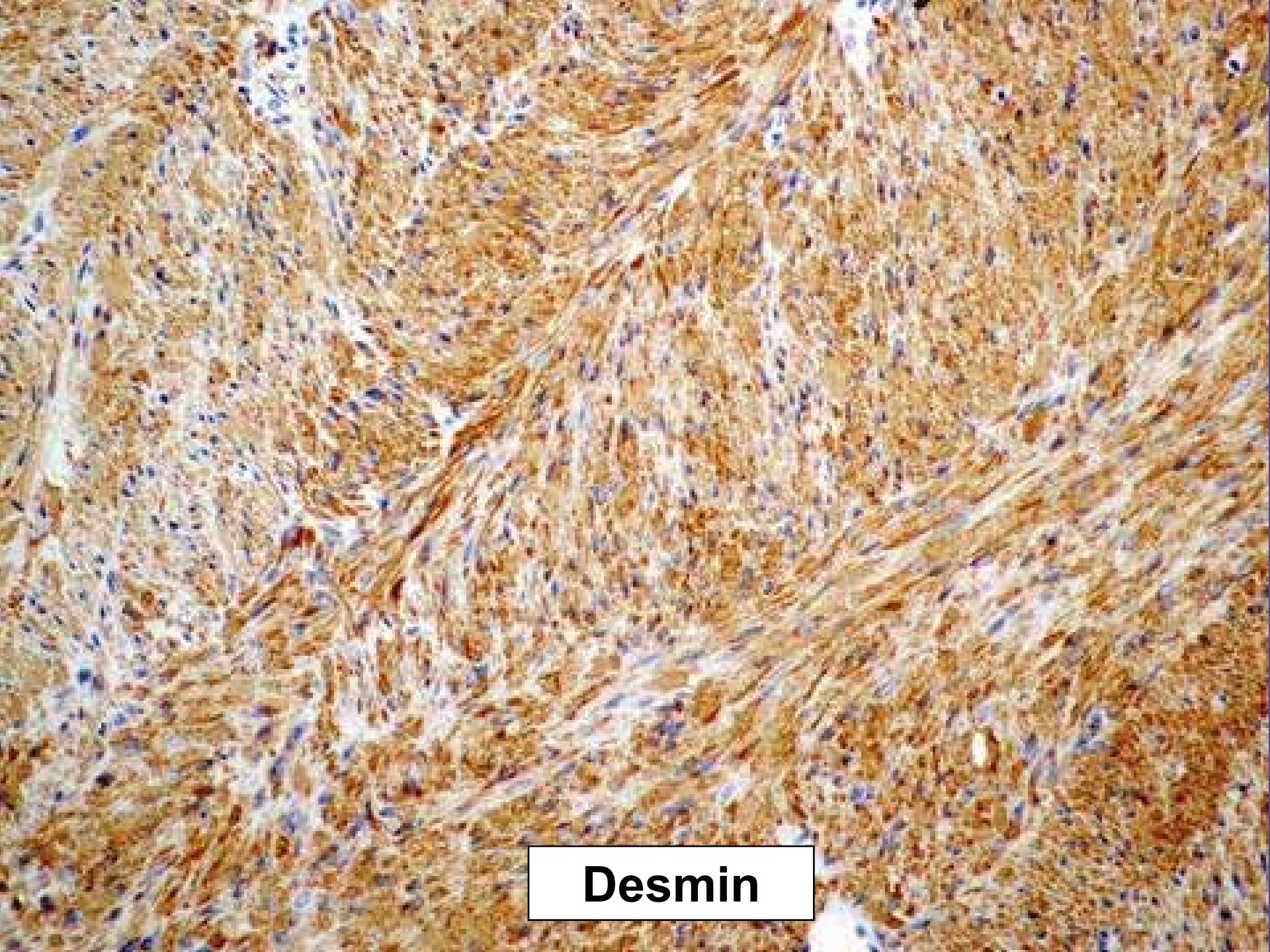


Intestinal Mass

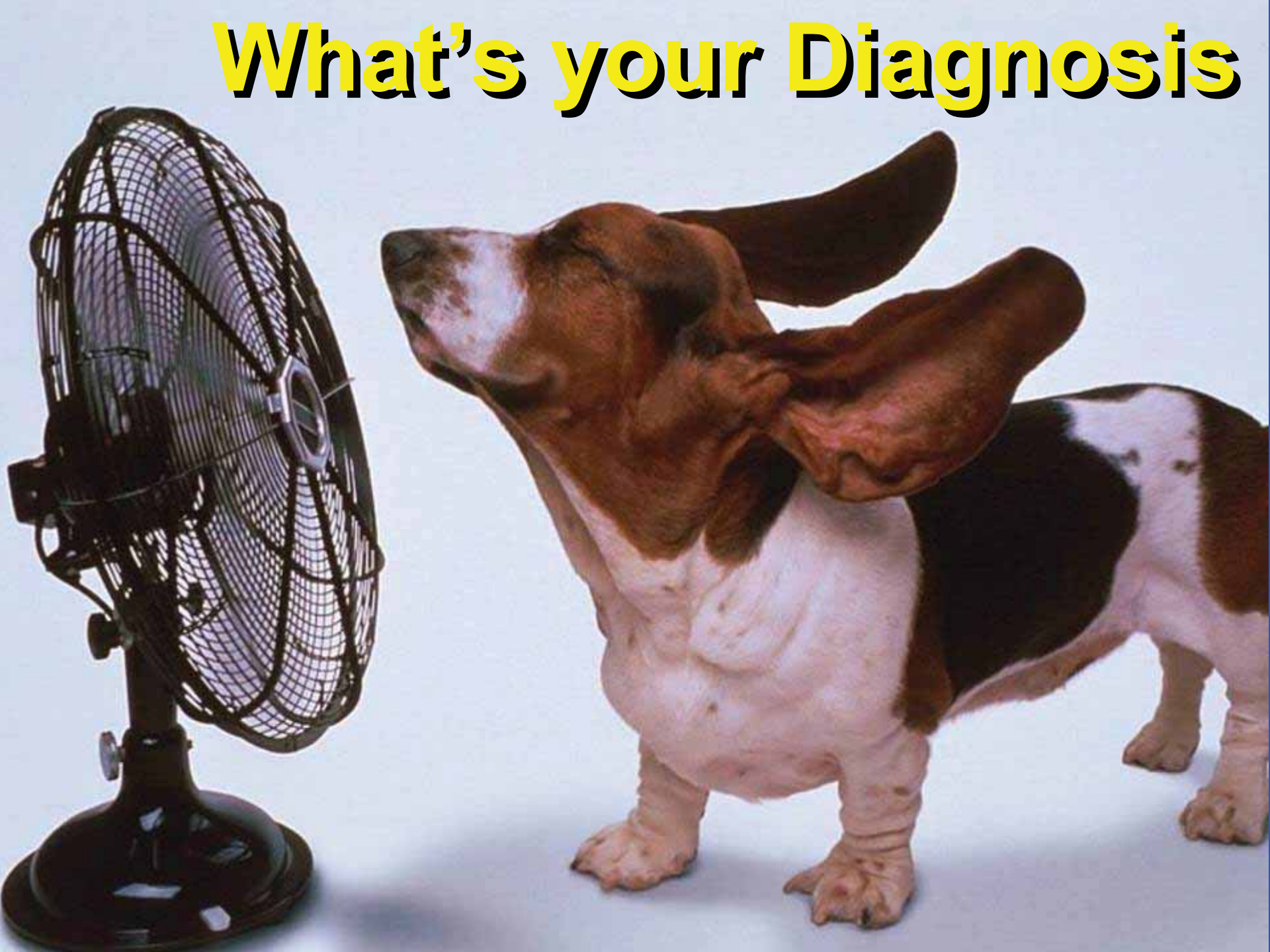

Vimentin


KIT

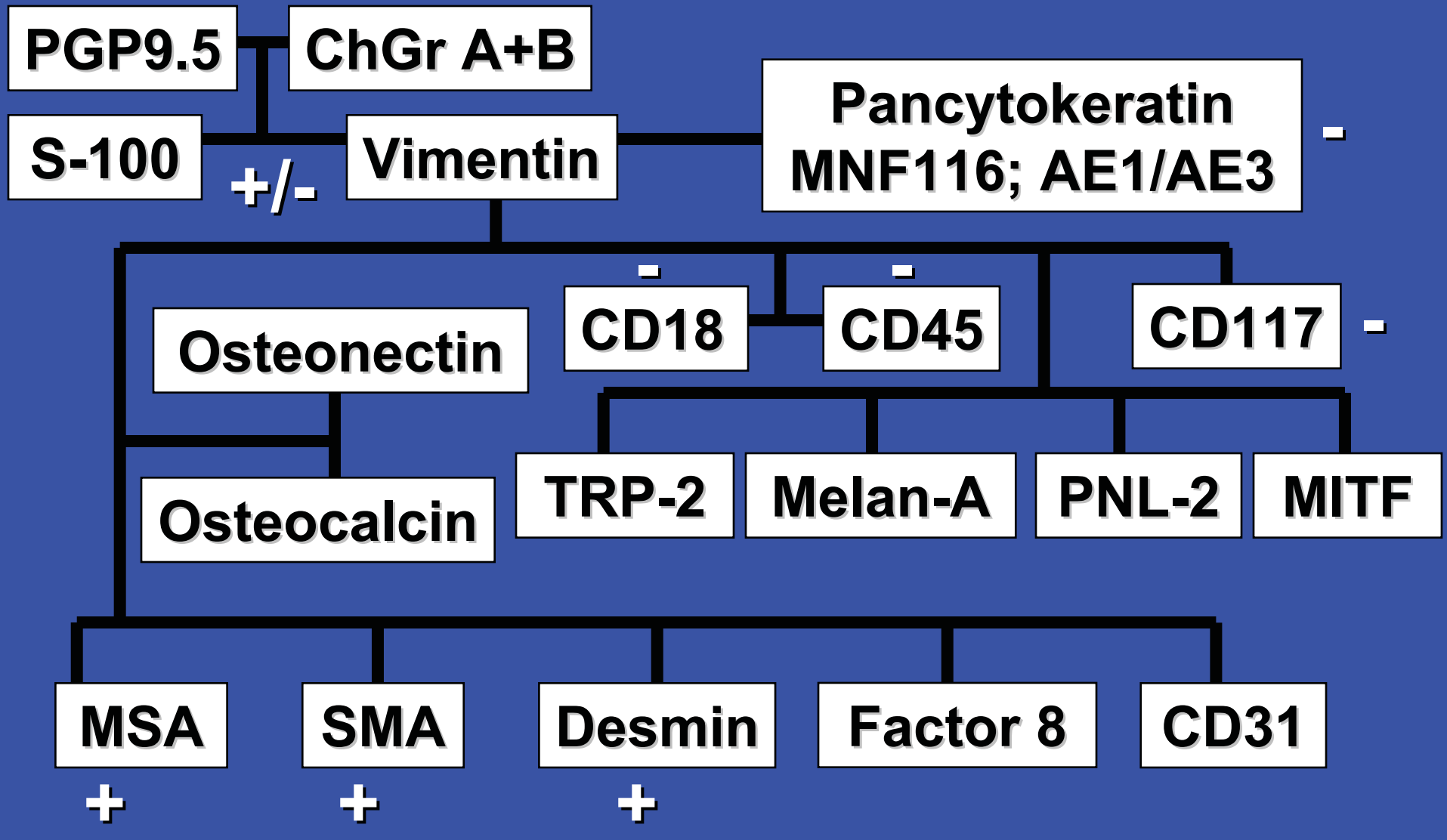

SMA


Desmin

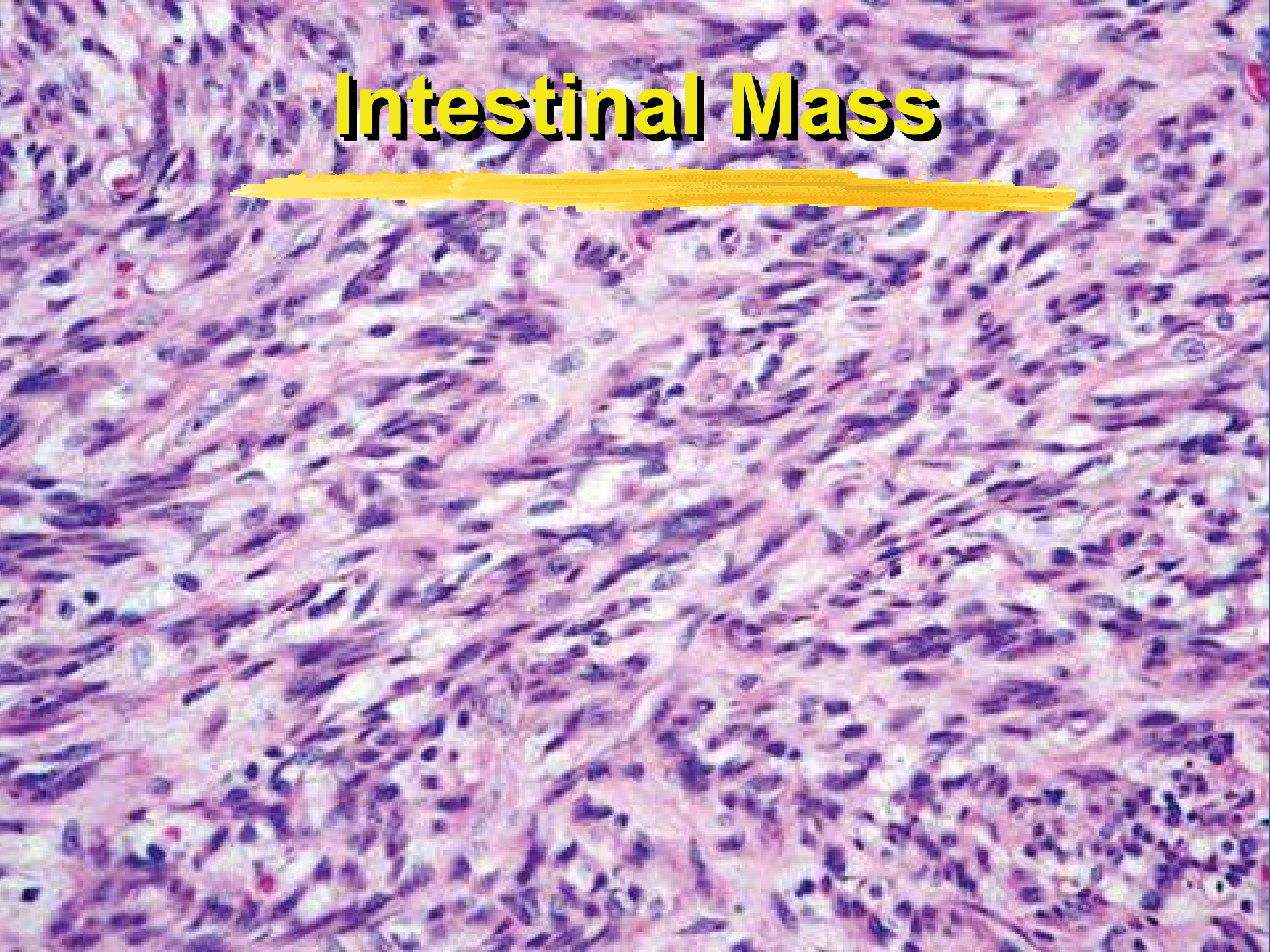
What's your Diagnosis

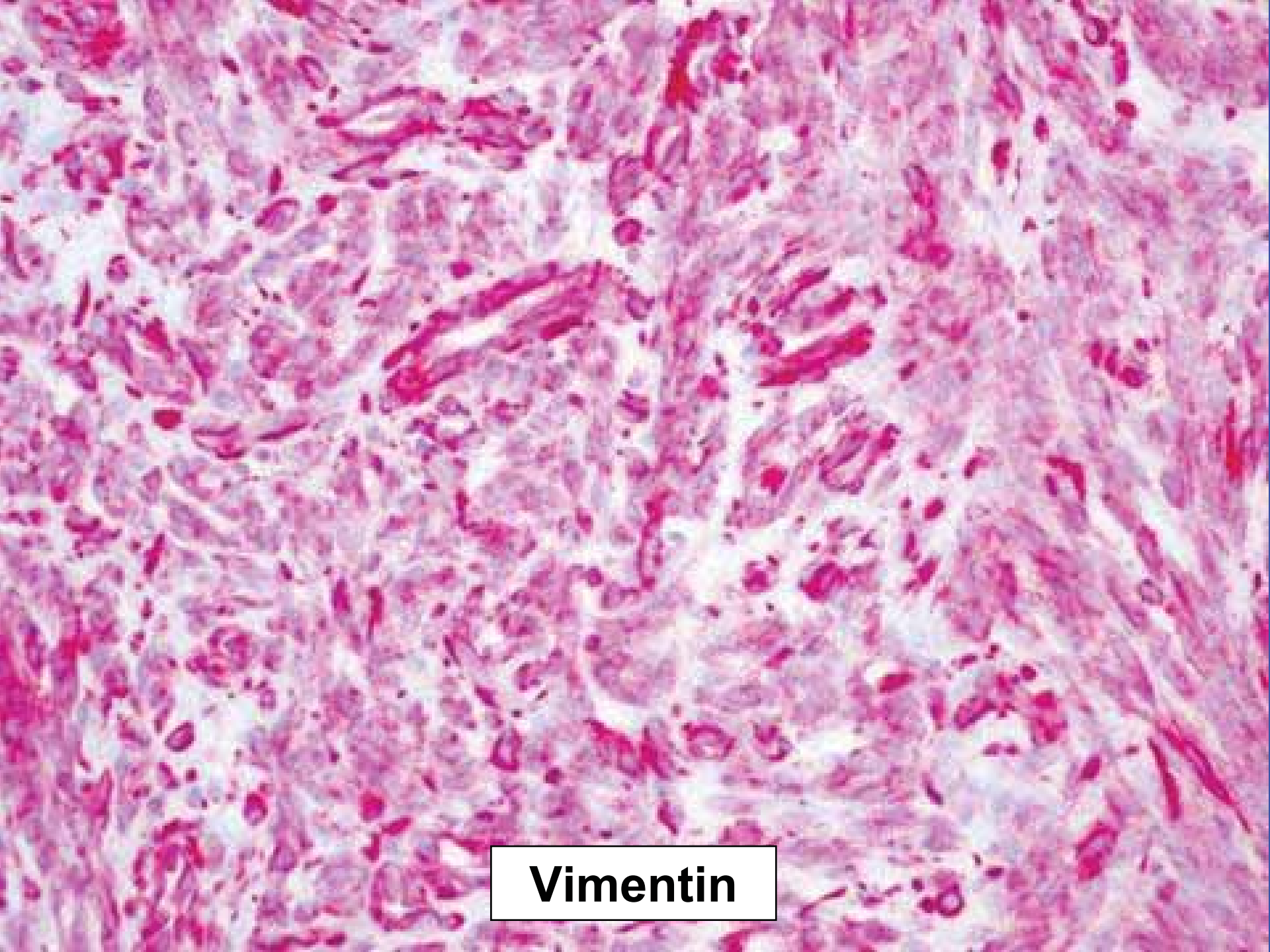


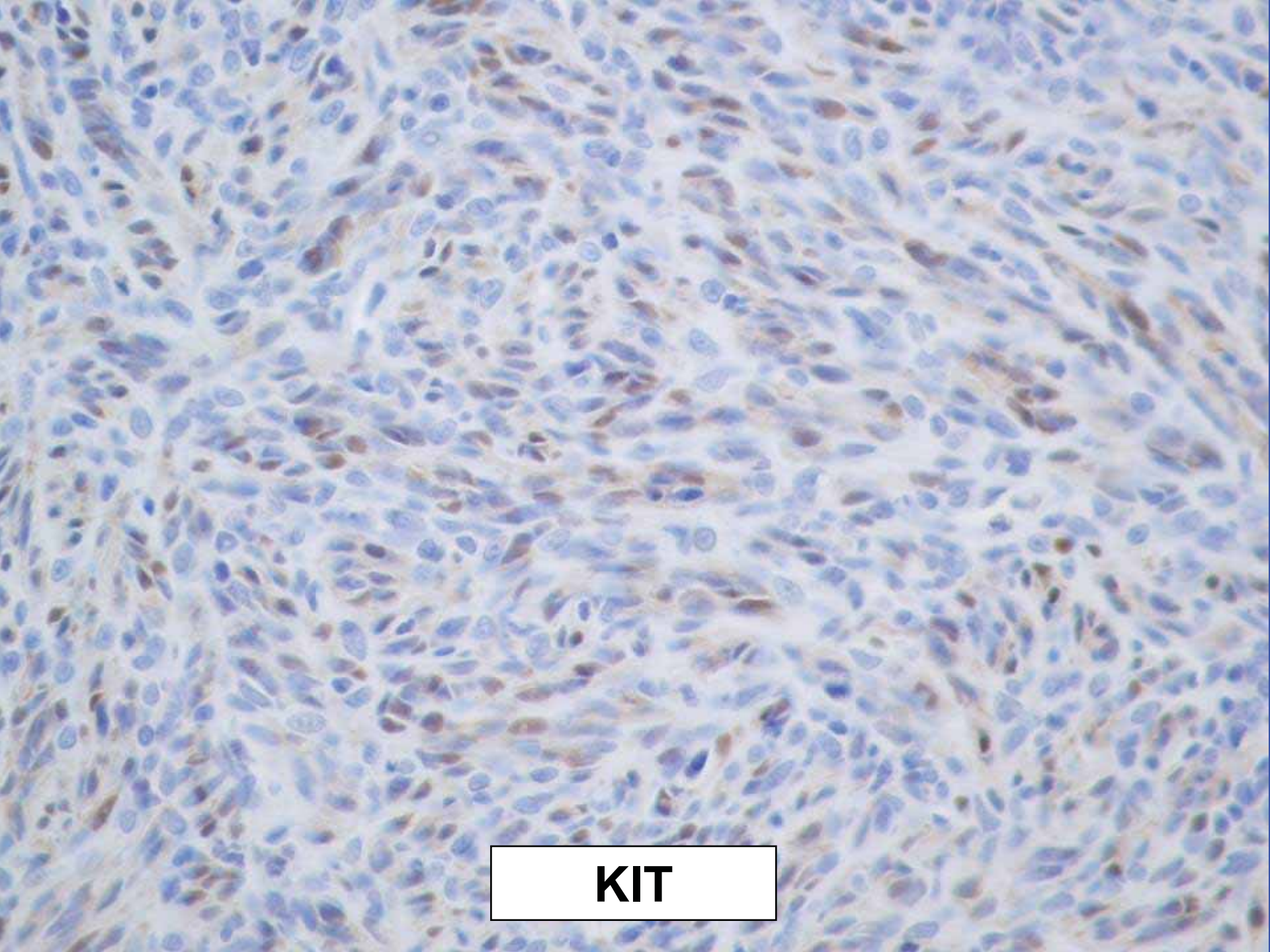
Well Differentiated Leiomyosarcoma

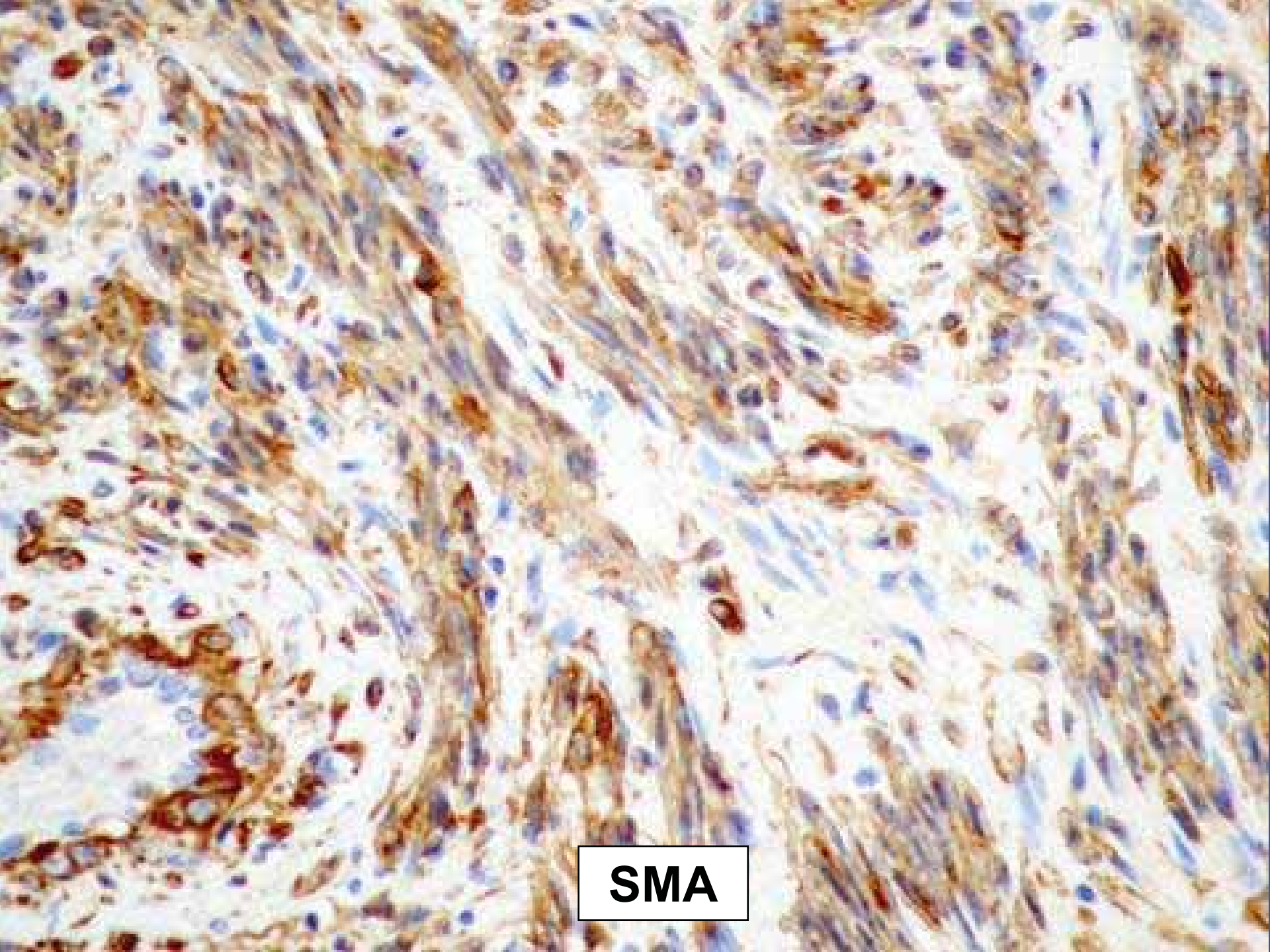


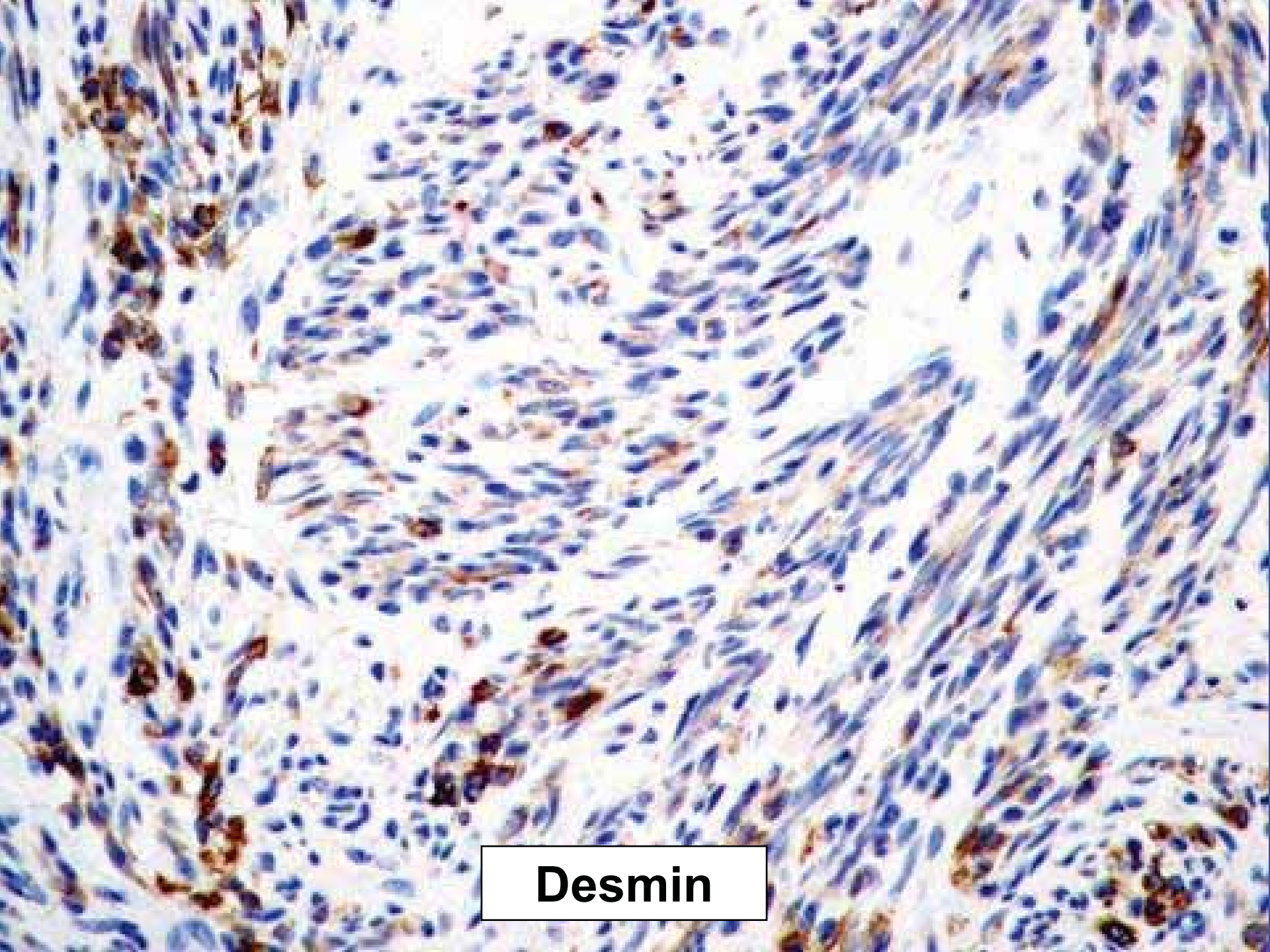
Intestinal Mass




Vimentin

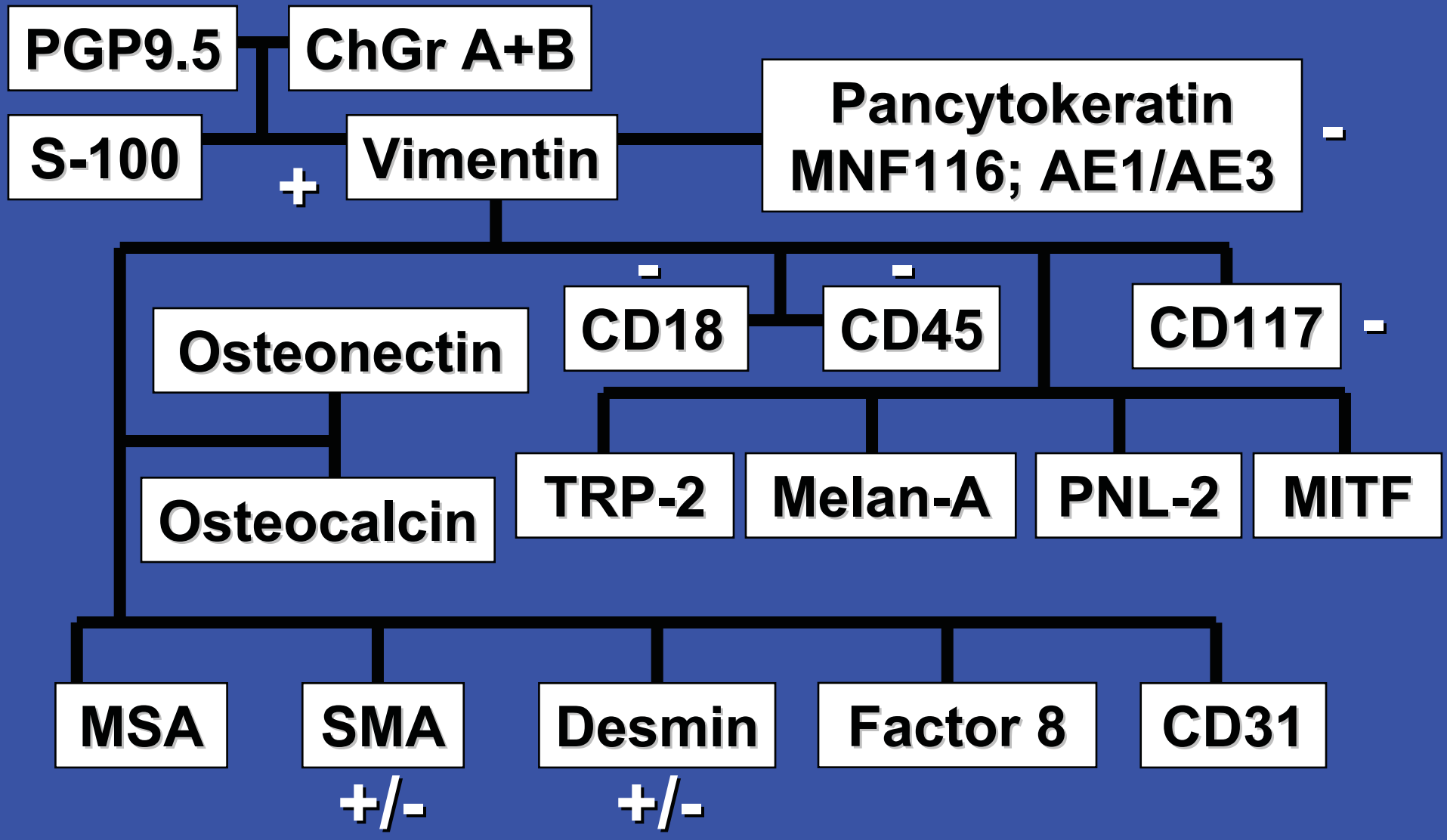

KIT


SMA


Desmin


What's your Diagnosis

Poorly Differentiated Leiomyosarcoma



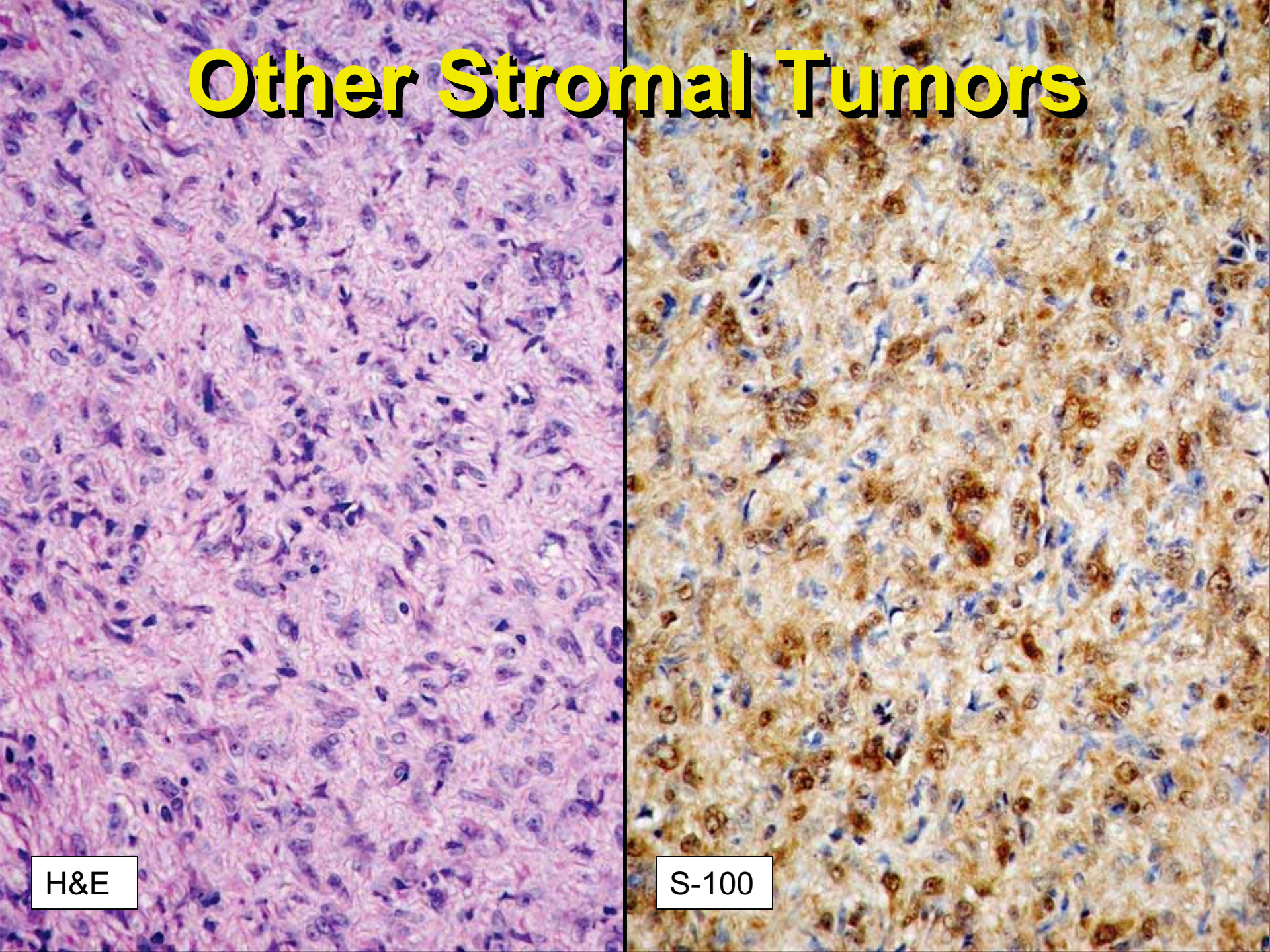
Results

- 12 (29%) Smooth Muscle Origin
  - SMA positive, KIT negative
  - 50% females, 50% males
  - 1-14 years old, mean ~7.5
  - Small intestine (92%)
  - 2/12 Metastasis (omentum)
- Positive immunoreactivity for:
  - Vimentin
    - 4 negative to minimal staining: well differentiated leiomyosarcoma
  - Desmin
  - S-100

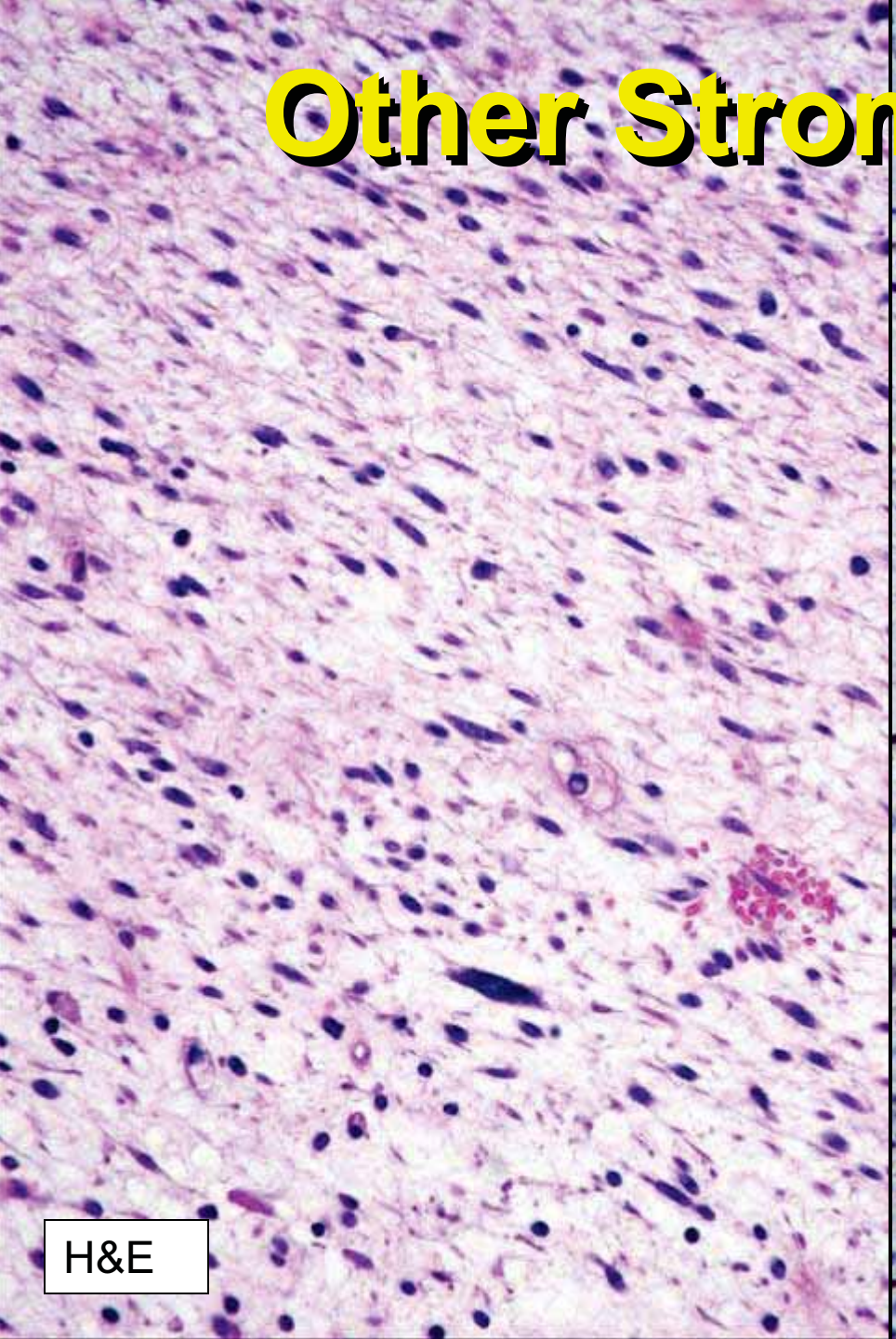
Results

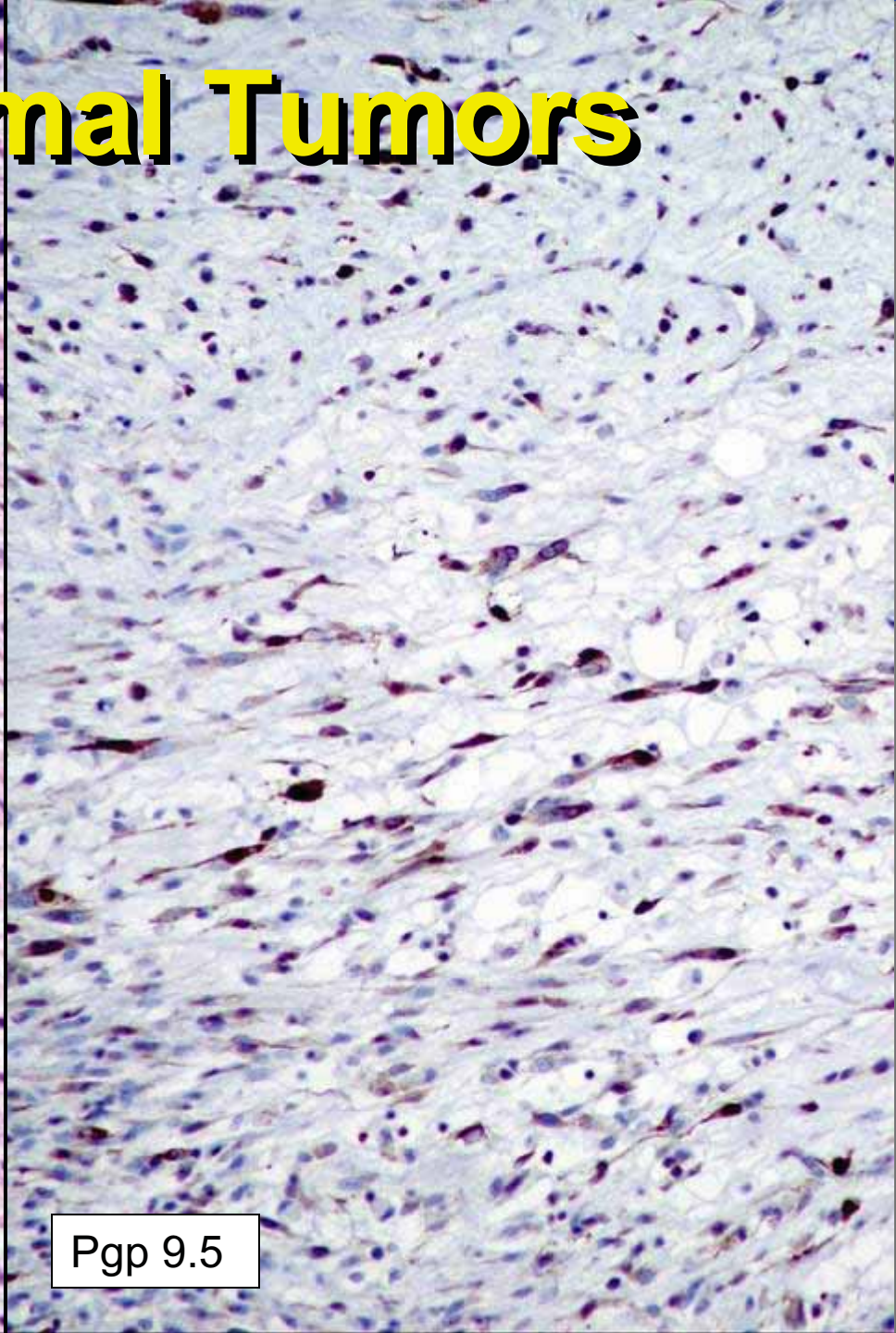
- 11 (24%) Non-GIST/non-smooth muscle origin
  - Vimentin: 100%
  - Desmin
  - S100
  - Pgp 9.5

Other Stromal Tumors



Other Stromal Tumors

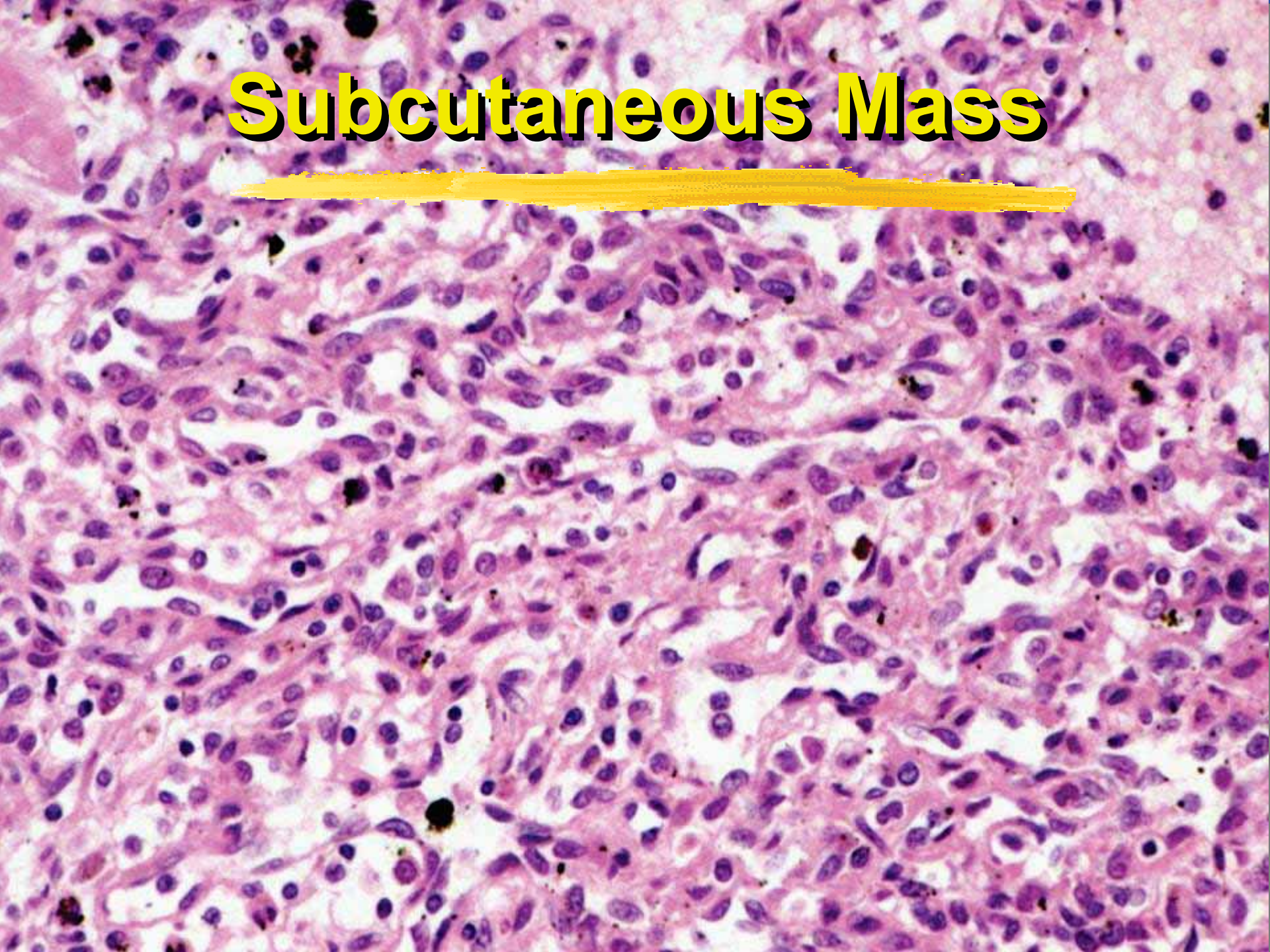


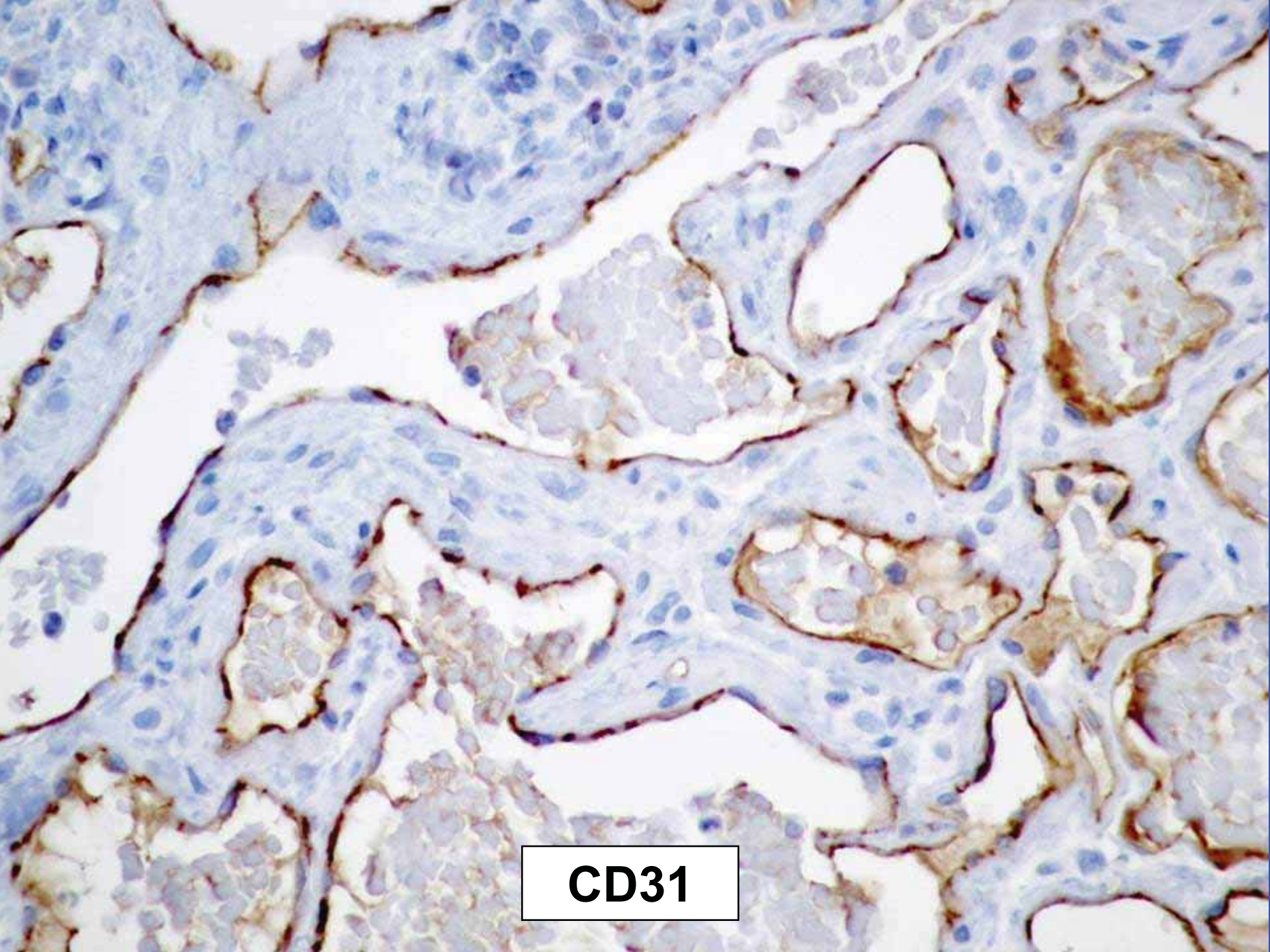


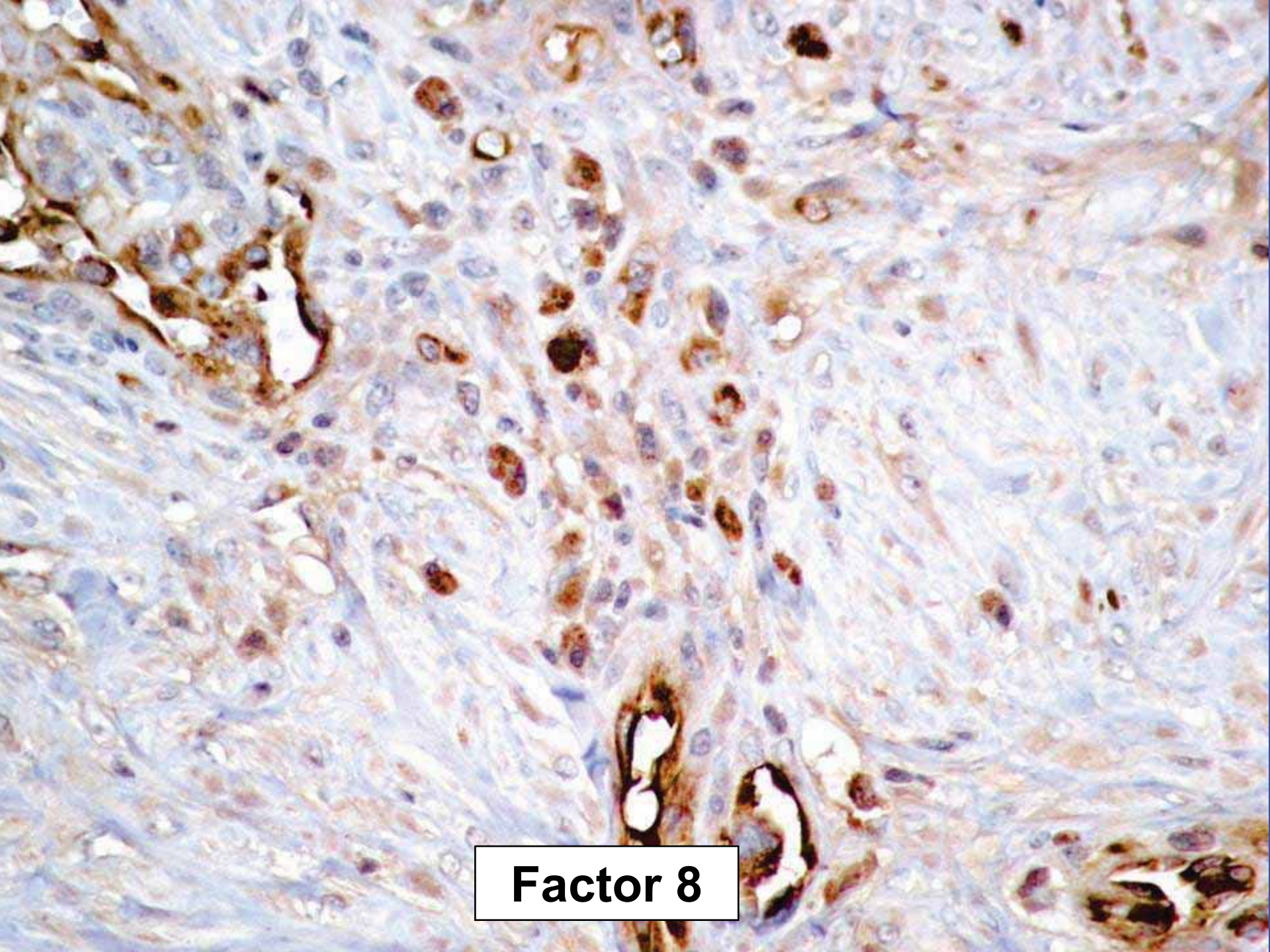
Non-angimatous, Non-lymphomatous Gastrointestinal Sarcomas

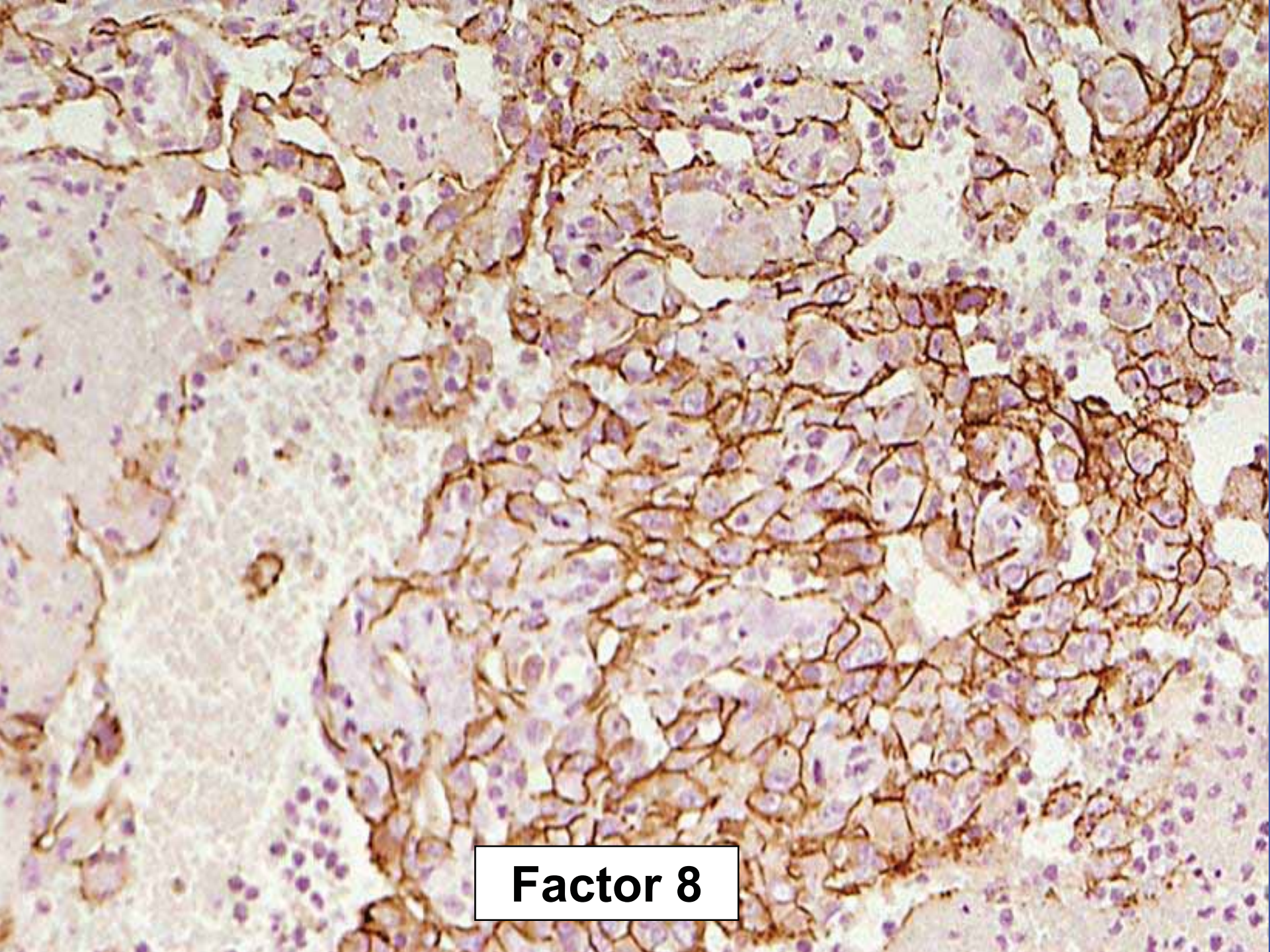
- Immunohistochemistry essential for diagnosis of non-lymphoid intestinal mesenchymal tumors
- Gastrointestinal stromal tumors and leiomyosarcomas have similar incidence
- Gastrointestinal stromal tumors tend to be more aggressive and have an increased potential for metastasis compared to leiomyosarcomas
- The expression of KIT and the presence of these mutations in *c-kit* implicate KIT in the pathogenesis of these tumors
- Indirect proportional staining for vimentin ans smooth muscle action reflects differention of leiomyosarcomas

Subcutaneous Mass



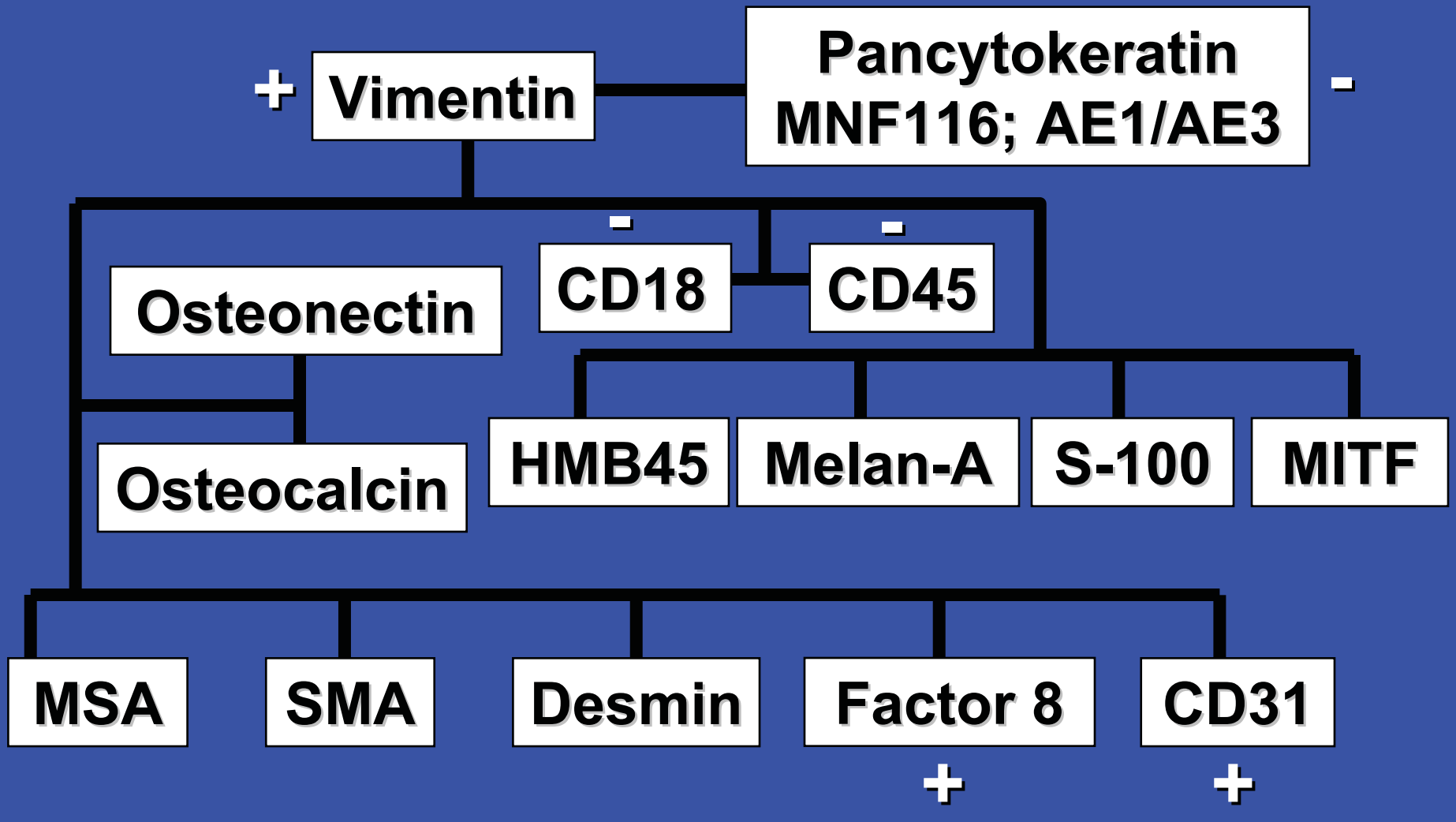

CD31


Factor 8


Factor 8

What's your Diagnosis

Hemangiosarcoma



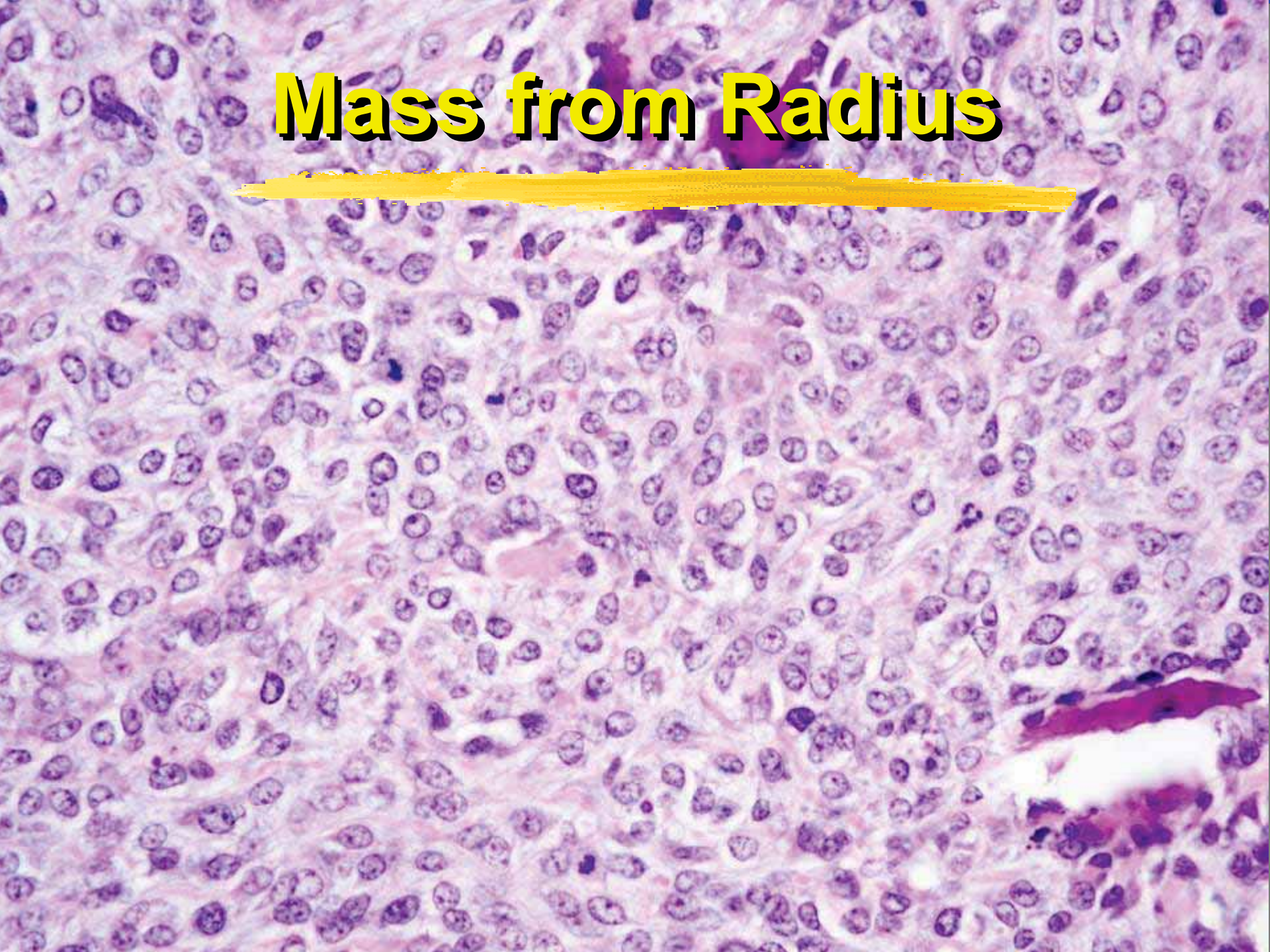
Hemangiosarcoma

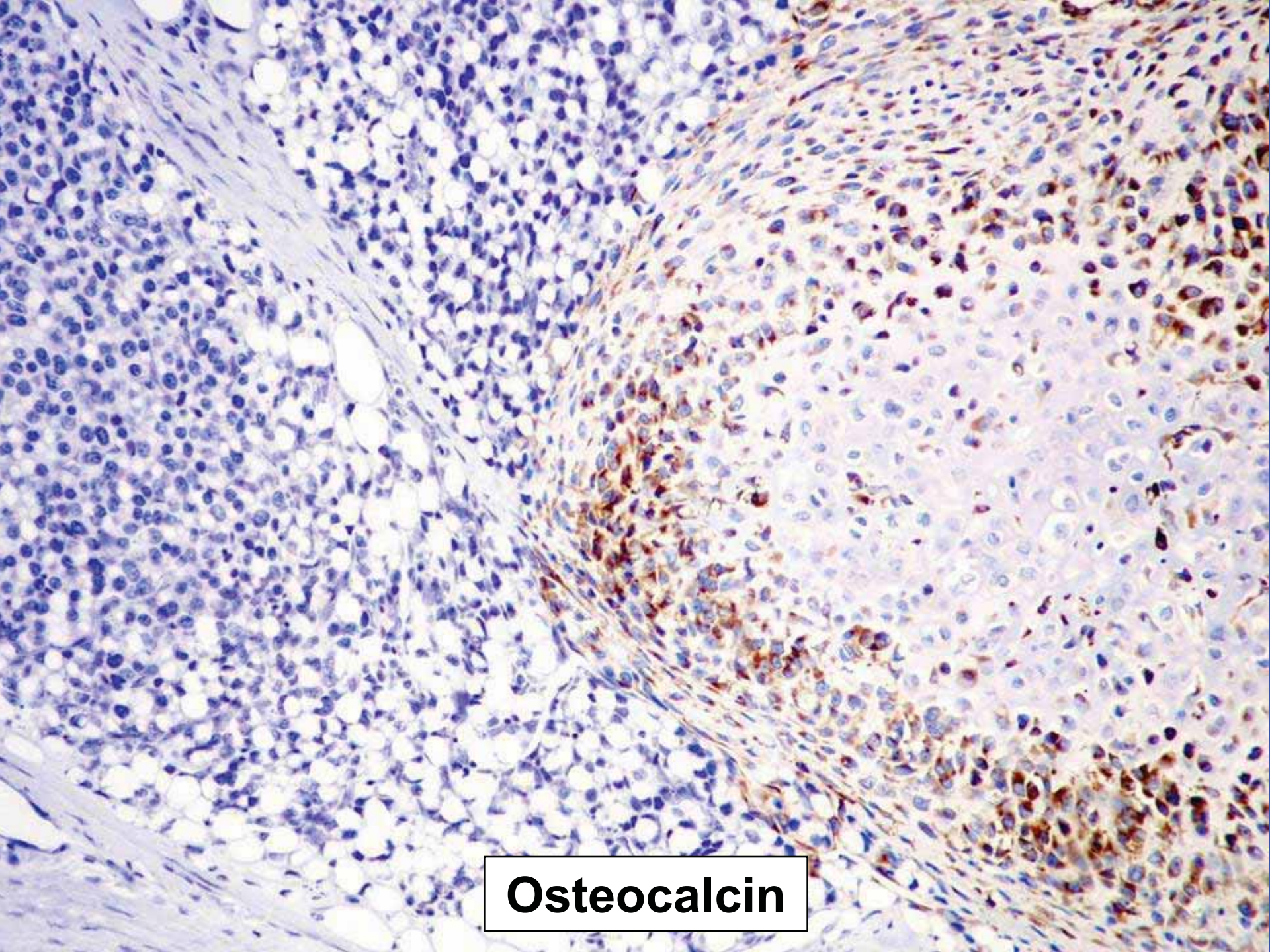
- Vimentin, CD31, factor 8
- Factor 8-related antigen and CD 31 (PECAM-1)
  - endothelial cells, megakaryocytes, platelets (CD31)
    - Factor 8: most vascular tumors
    - difficult to interpret with abundant blood
    - CD31: easier to interpret than Factor 8
    - more fixation sensitive

Other Vascular Markers

- LYVE-1
  - Receptor for extracellular matrix glycosaminoglycan hyaluronan (HA), homologue of CD44 in vessels
  - Specific for lymphatic endothelium (highly controversial: demonstrated in canine lymph nodes, but problems in inflammation and cancer)
- CD 34 (Hematopoietic progenitor cell antigen)
  - Stem cells of the hemopoietic system, endothelium
  - Not a diagnostic marker for vascular tumors
- Endoglin (CD105)
  - Receptor for TGF-β1 and TGF- β3
  - Almost no expression in normal blood vessels
  - High expression in proliferating vessels
- *Ulex Europaeus I* agglutinin (lectin), Thrombomodulin
  - Questionable use for vascular tumors

Mass from Radius




Osteocalcin