Through the Microscope: Adventures in IHC Land

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The H&E is home

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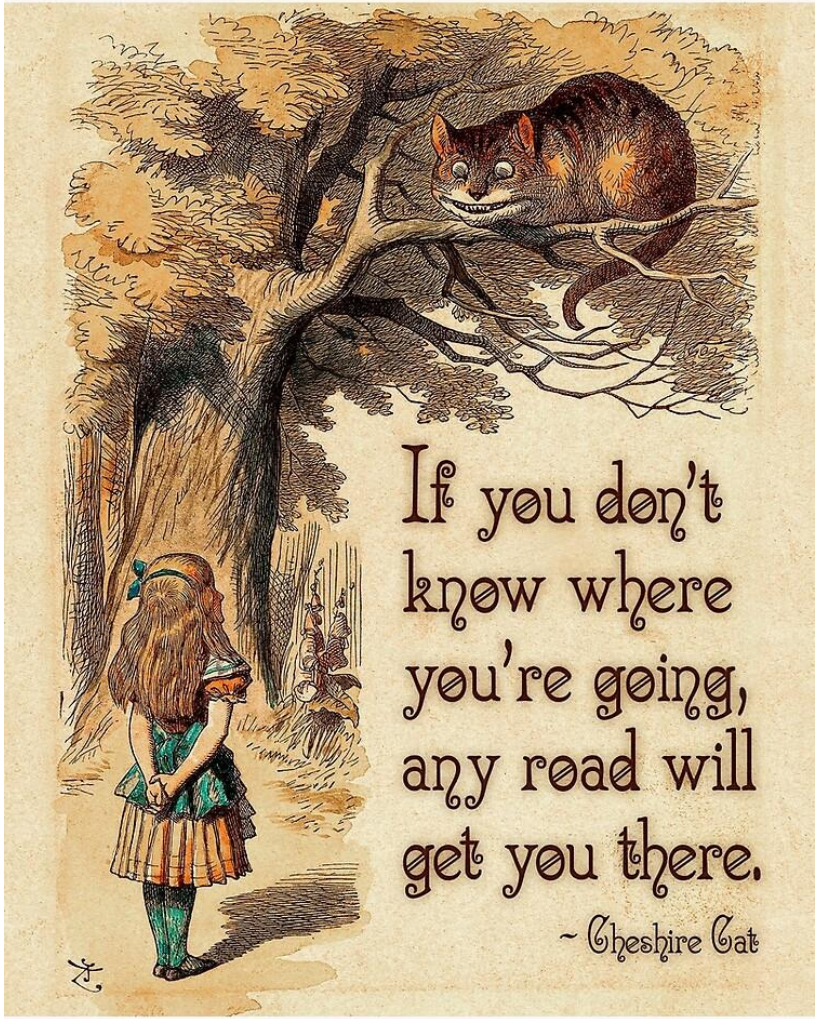
IHCs selected depend on the differential diagnosis generated from the H&E examination and knowledge of clinical & radiologic features



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Sometimes the immunostains don’t perform as expected (sometimes without us realizing it)

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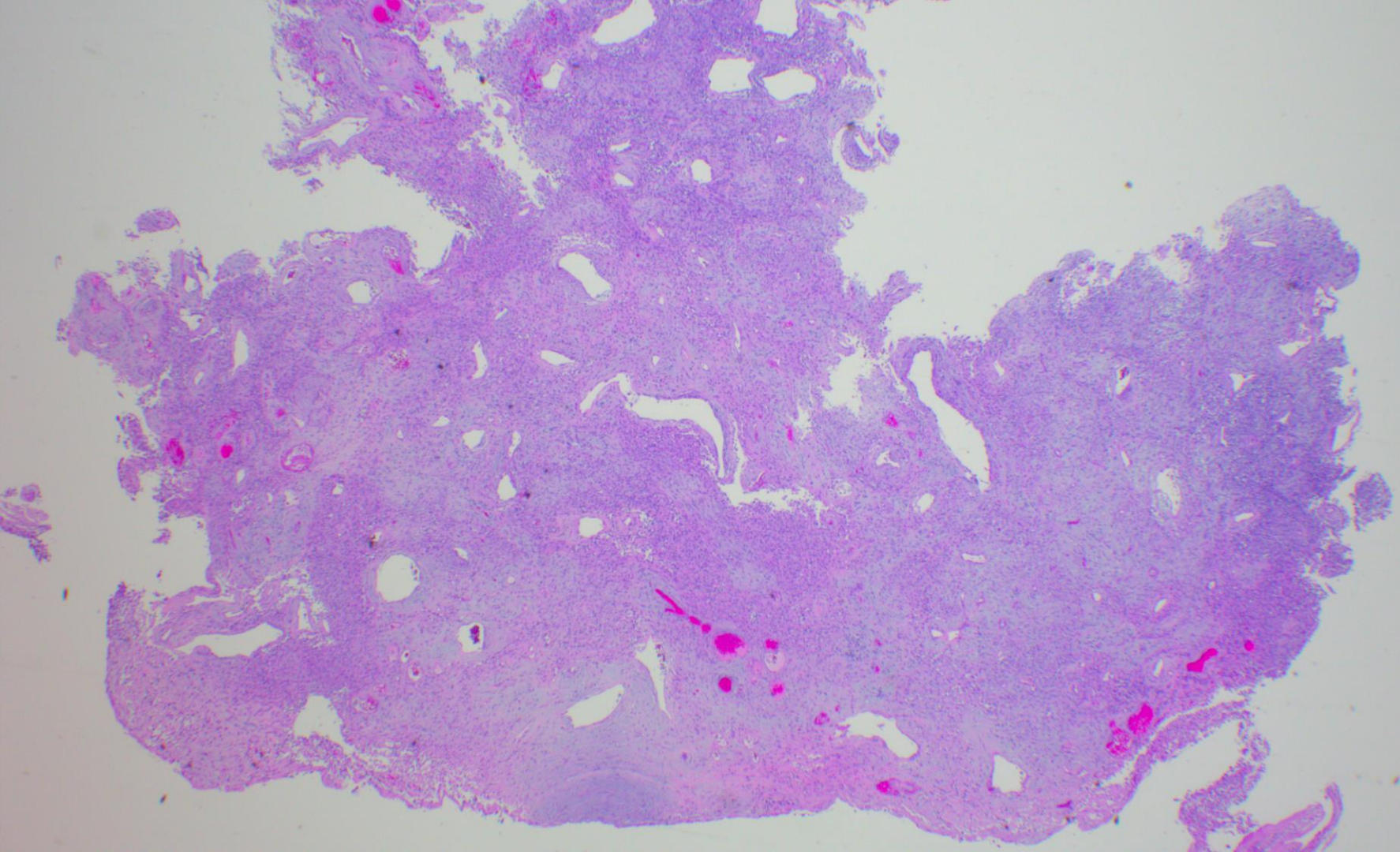
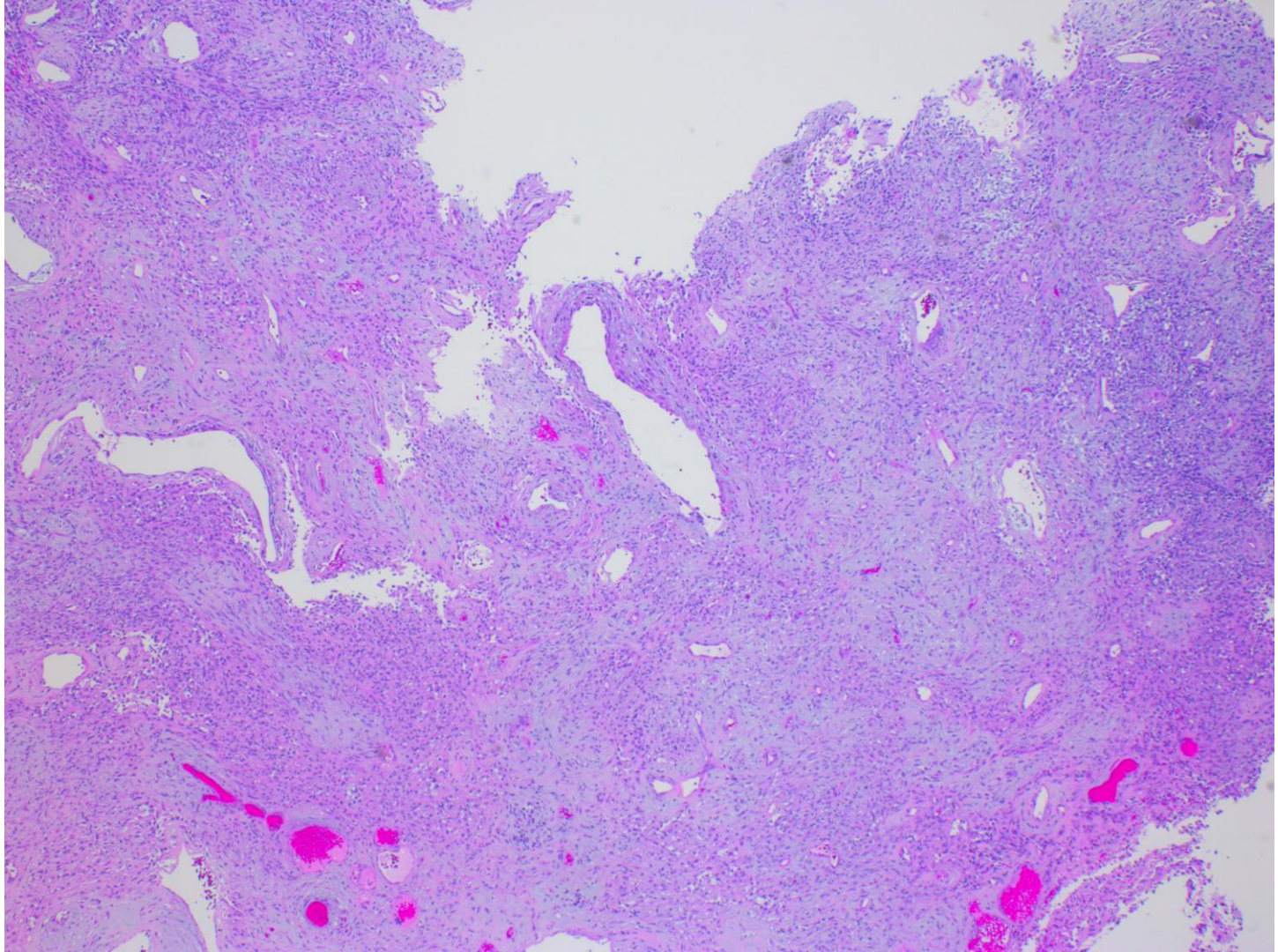
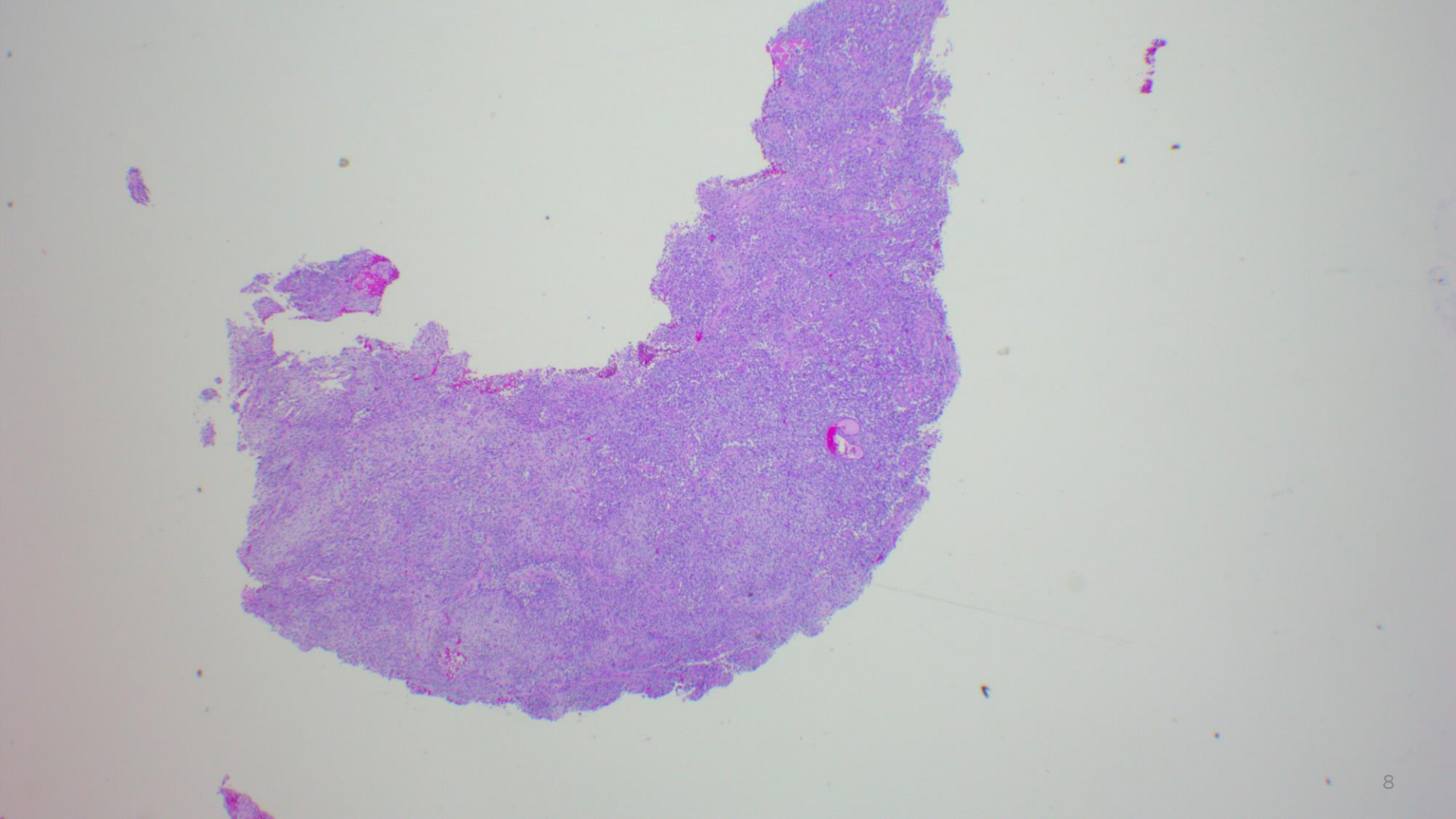
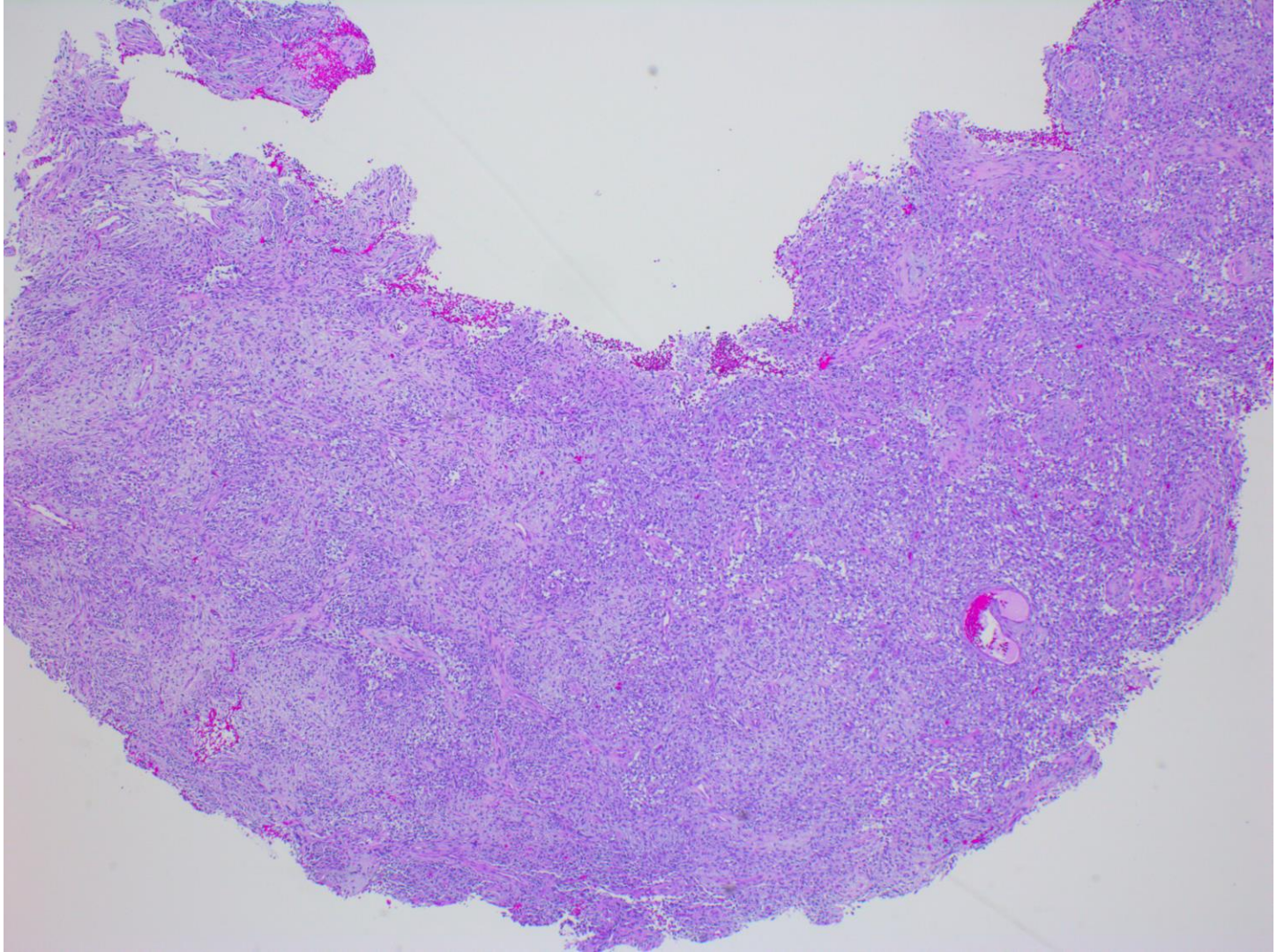
Case 1

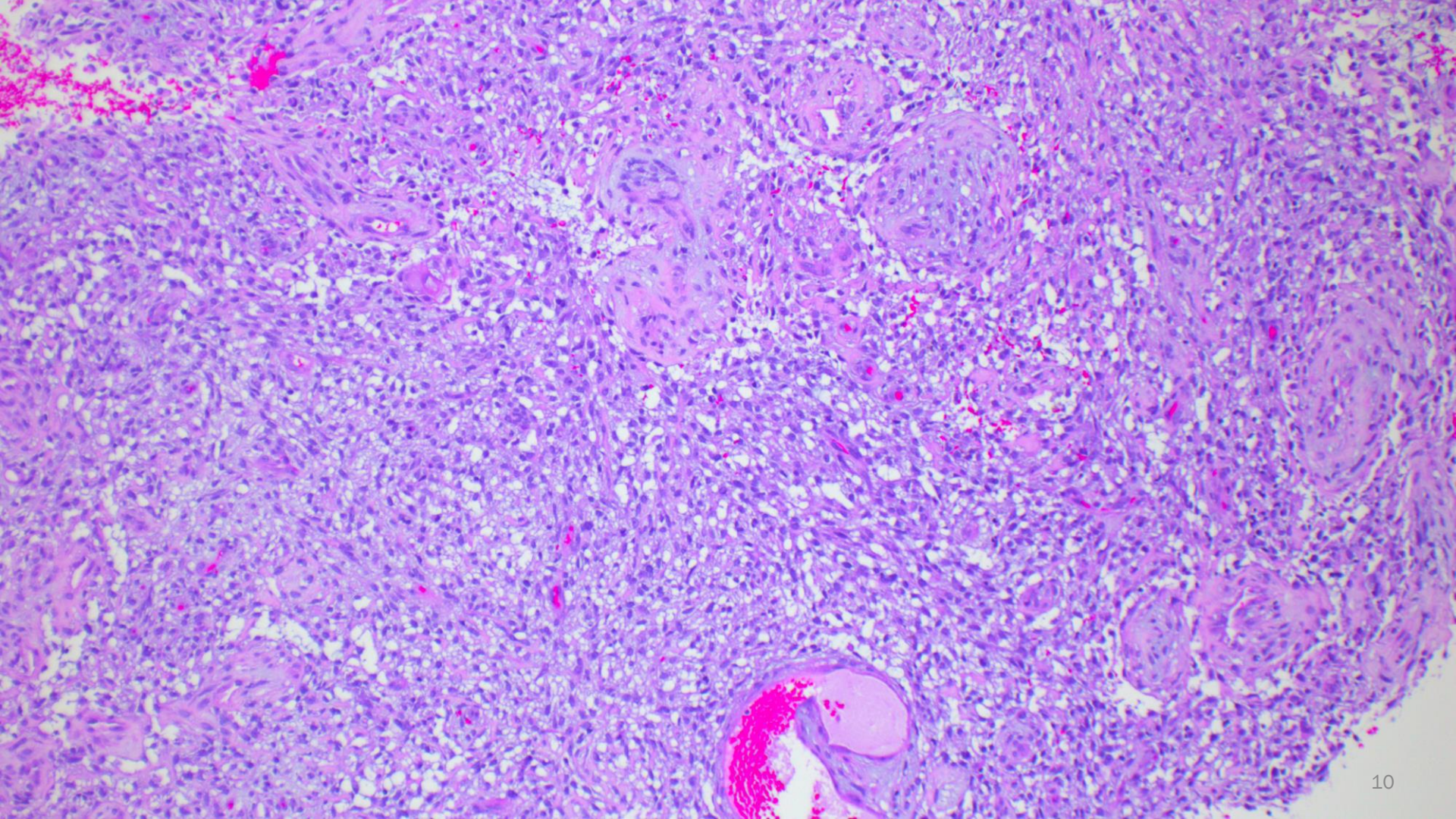
- 35 yo underwent colposcopic examination
- Cervical mass
- Biopsy was performed

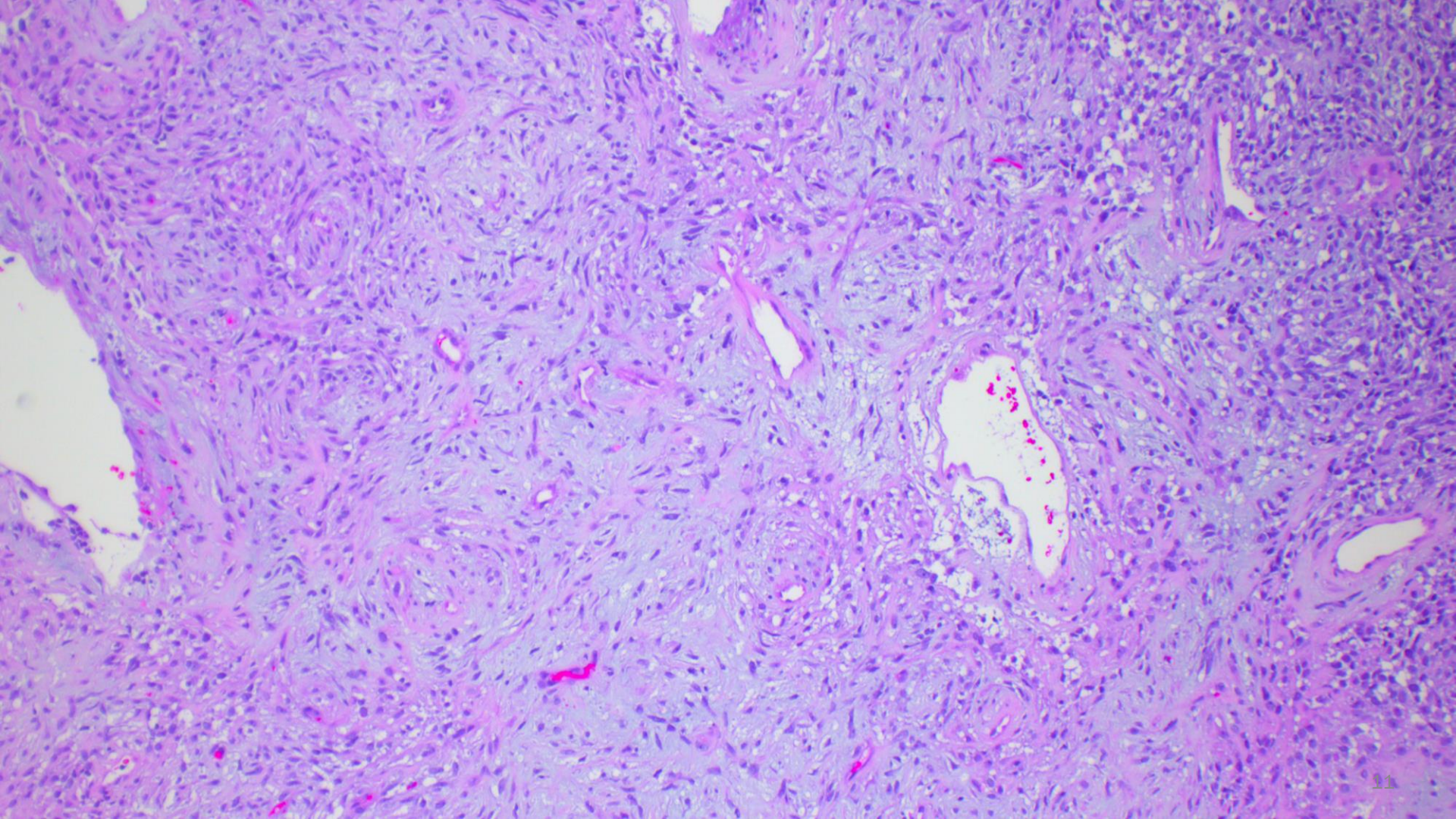


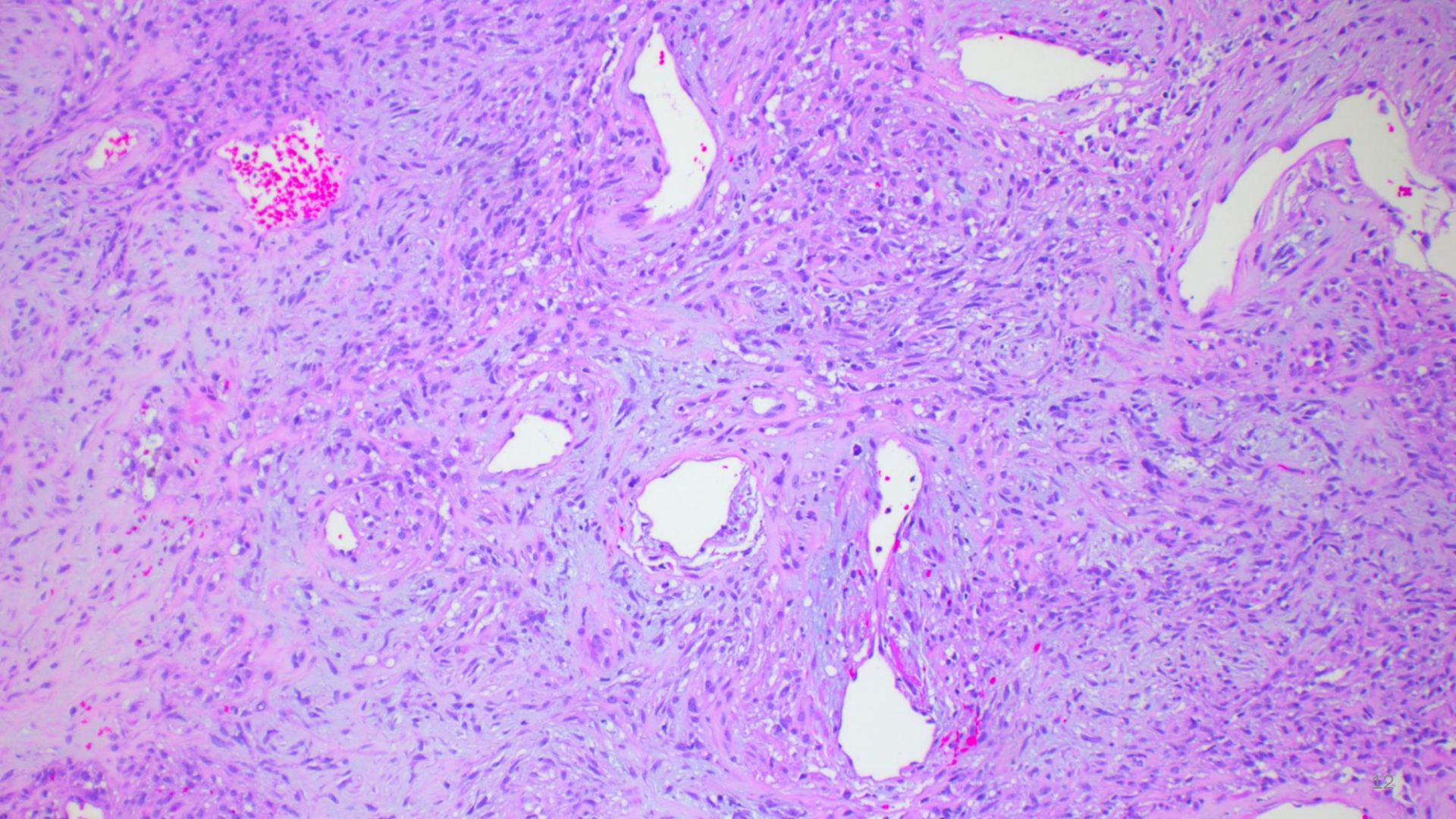






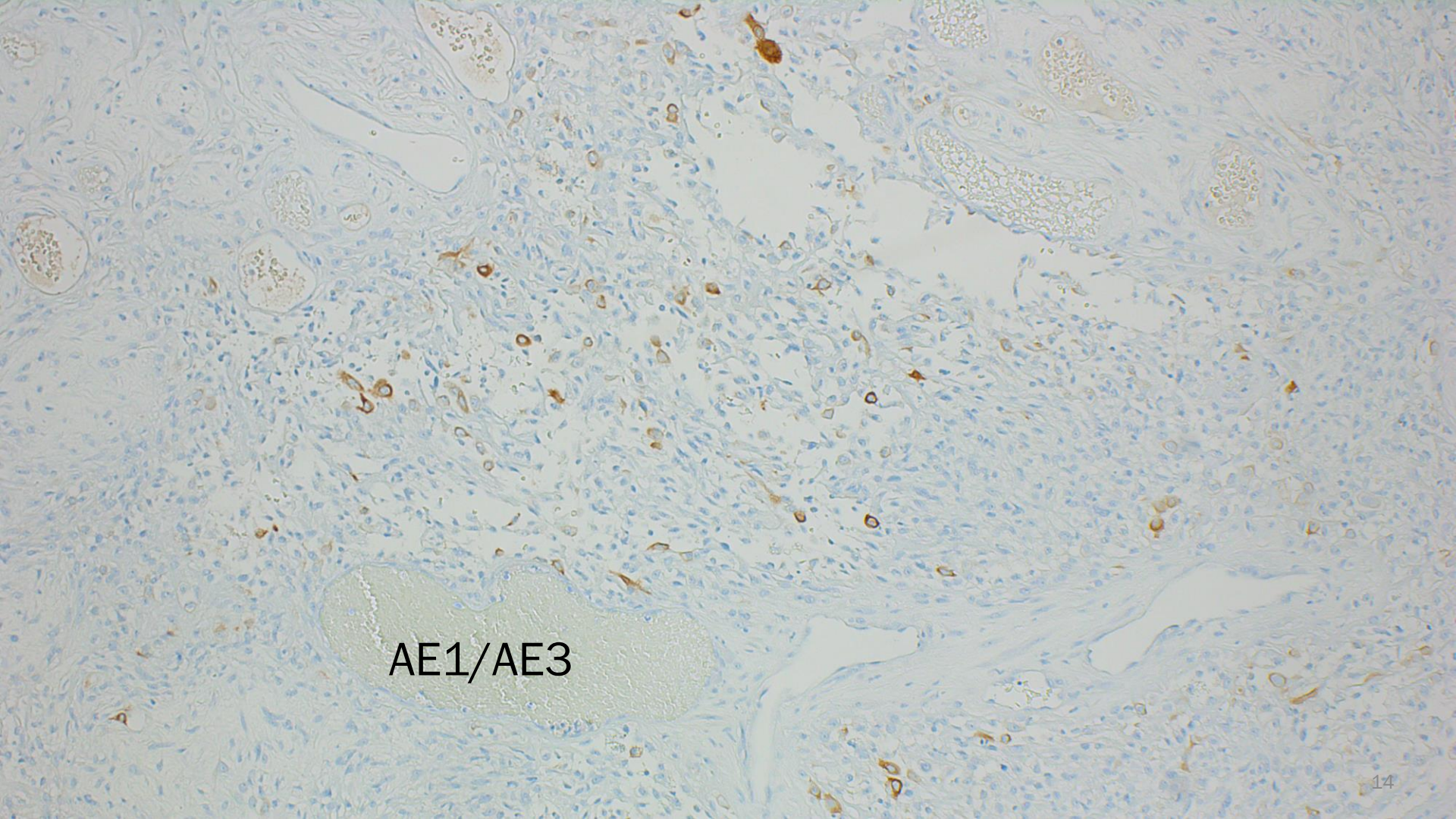






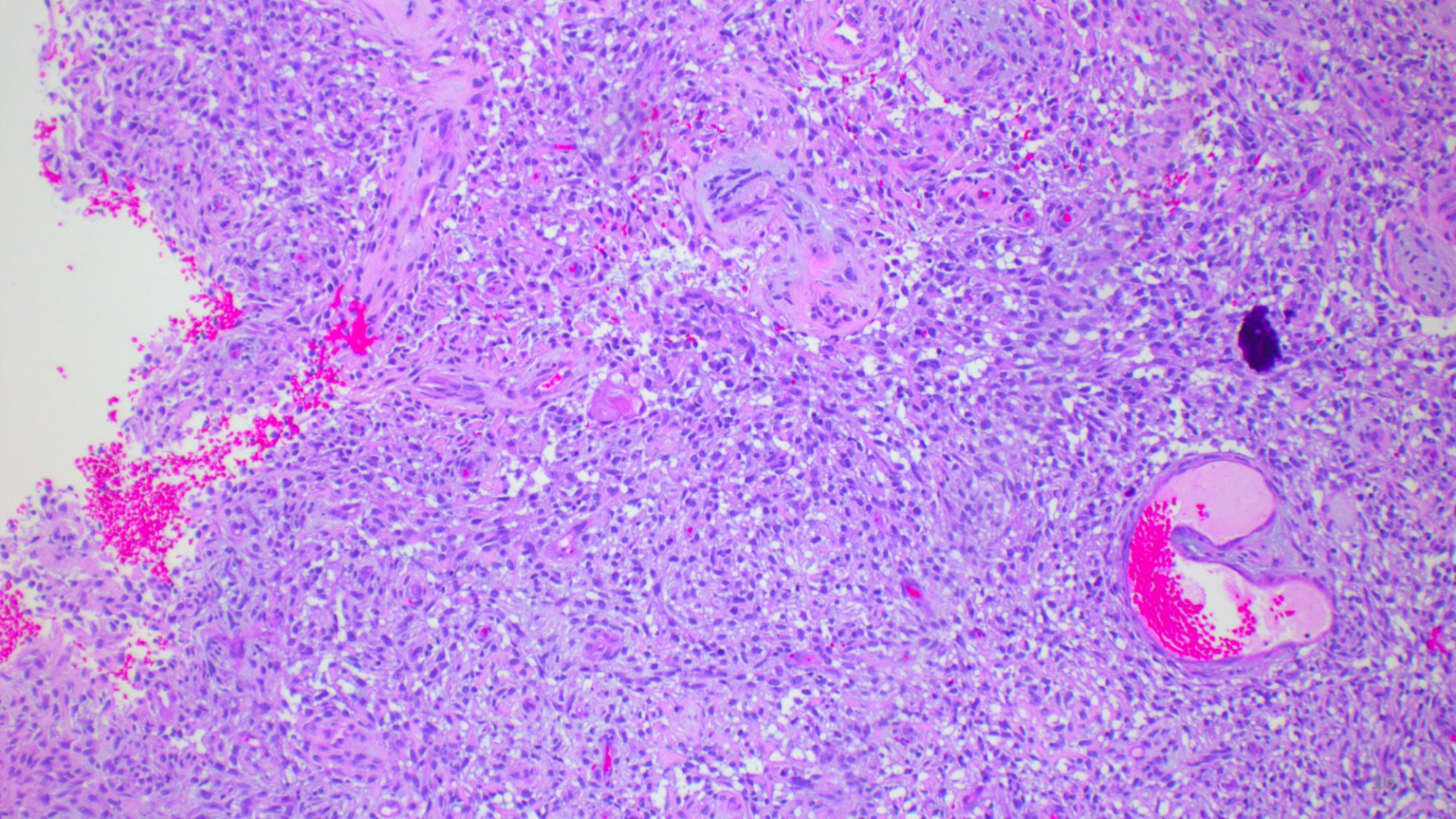
Differential Diagnosis

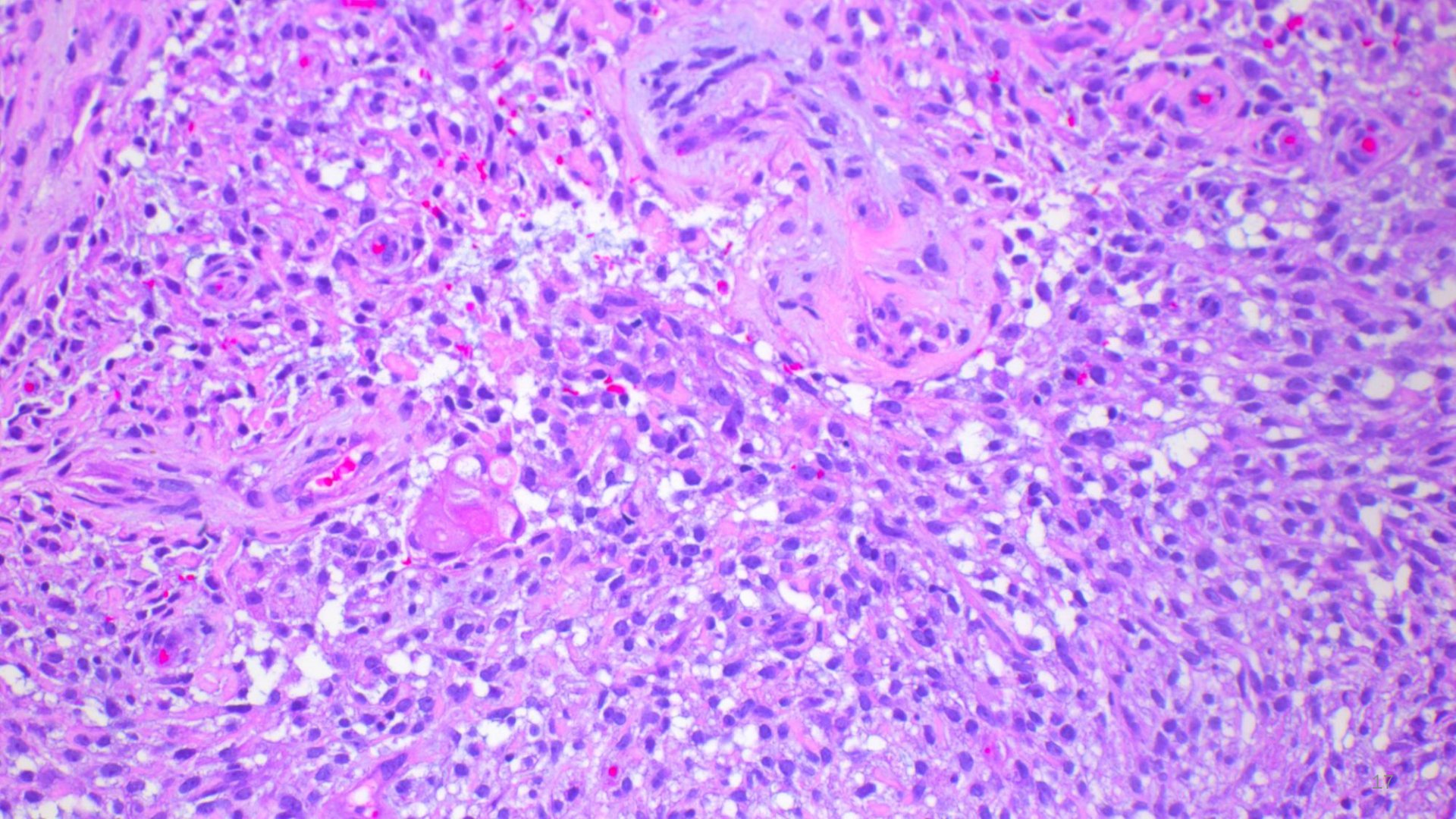
- Carcinoma
- MMMT (Carcinosarcoma)
- Melanoma
- Sarcoma

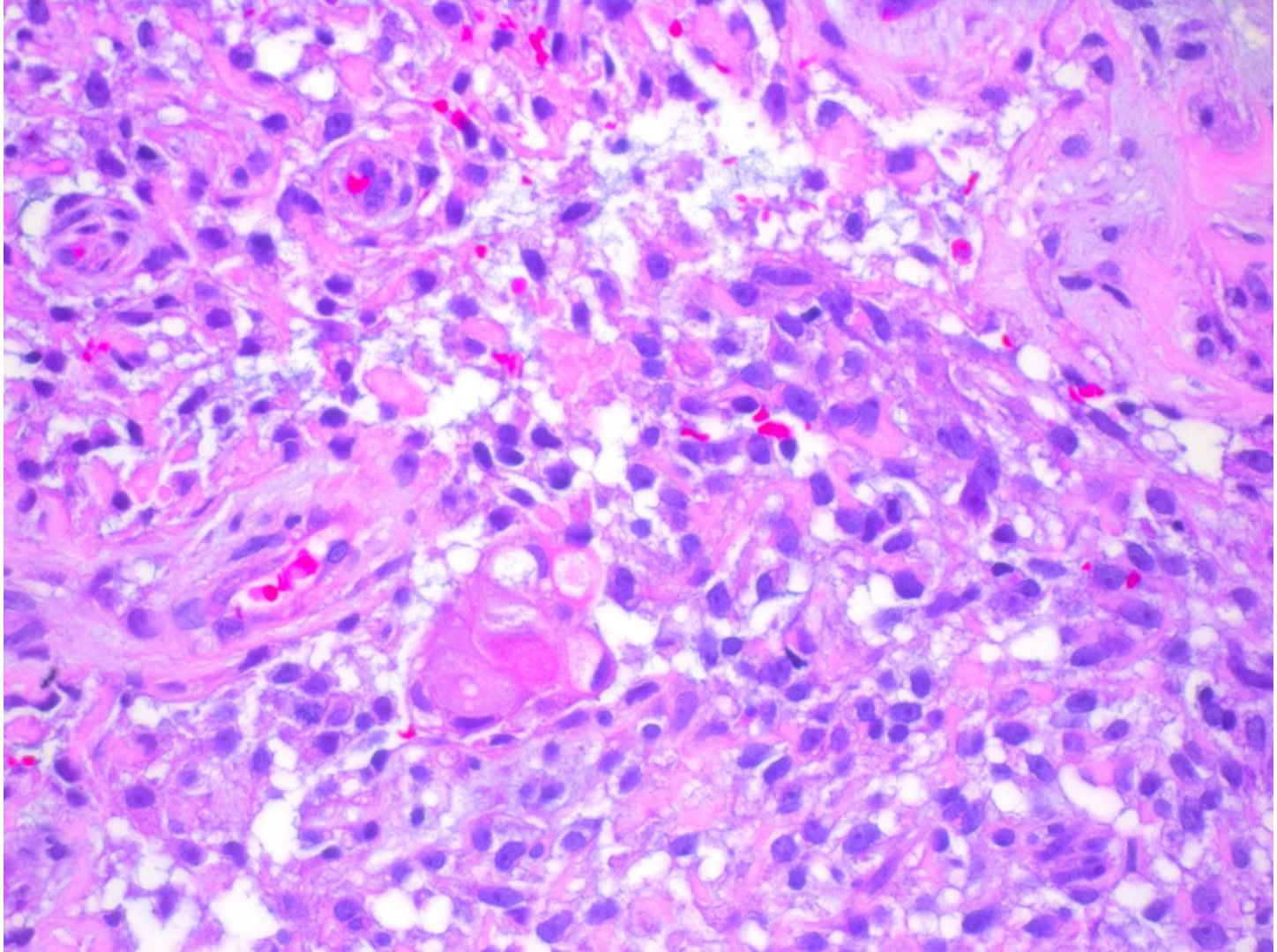

AE1/AE3

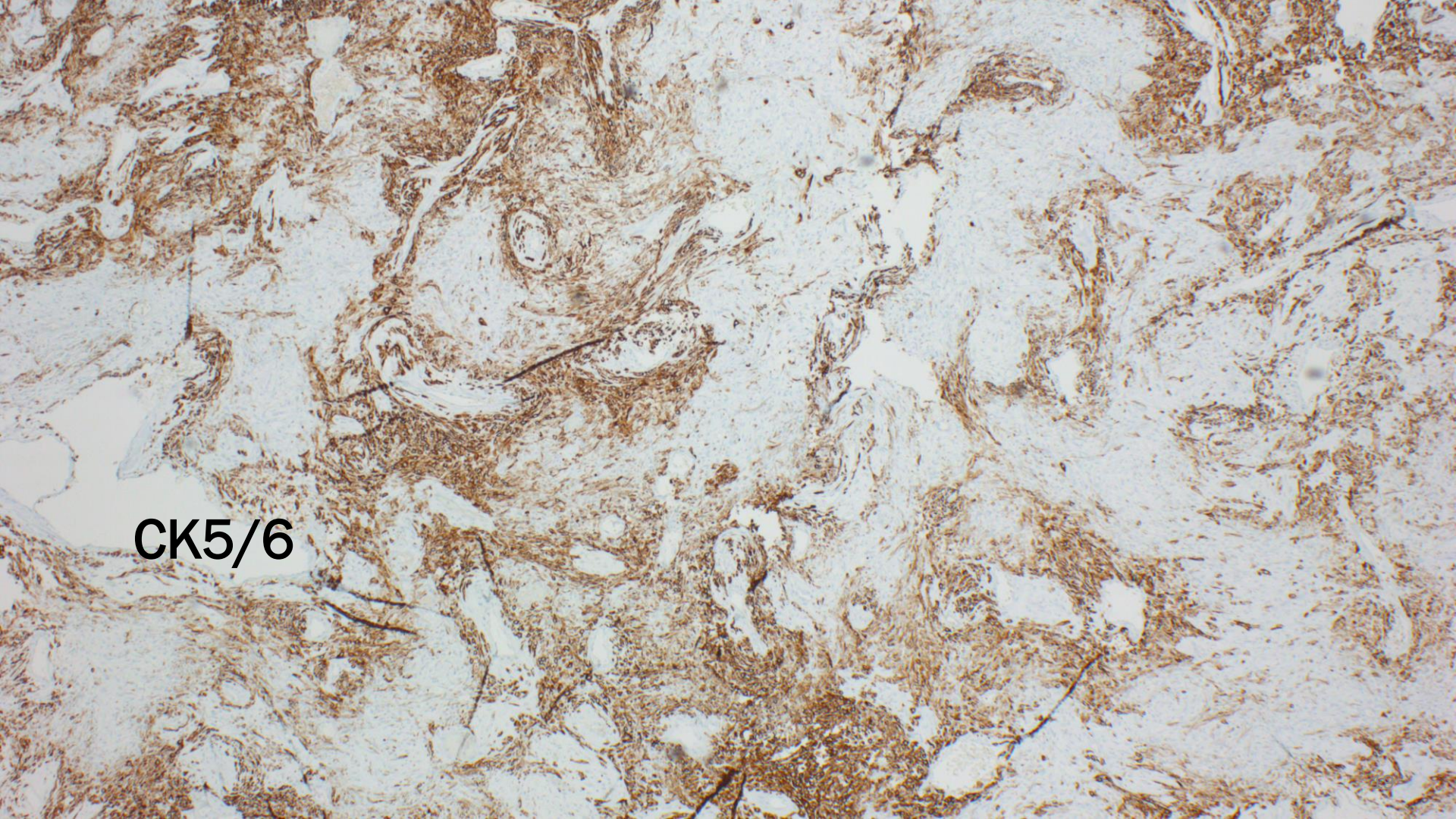
Differential Diagnosis

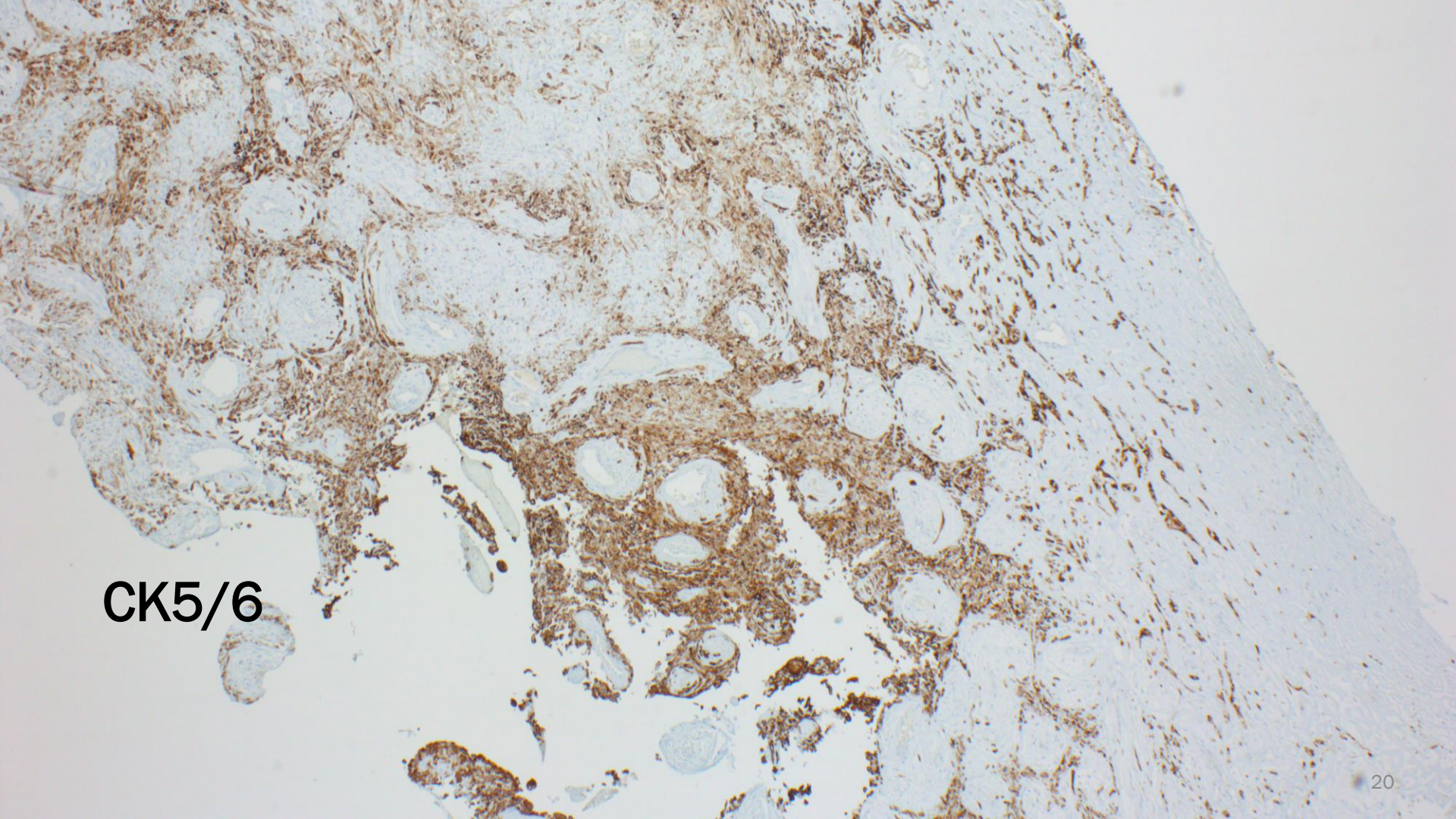
- ≠ Carcinoma
- ≠ MMMT (Carcinosarcoma)
- ≠ Melanoma (S100 -)
- Sarcoma

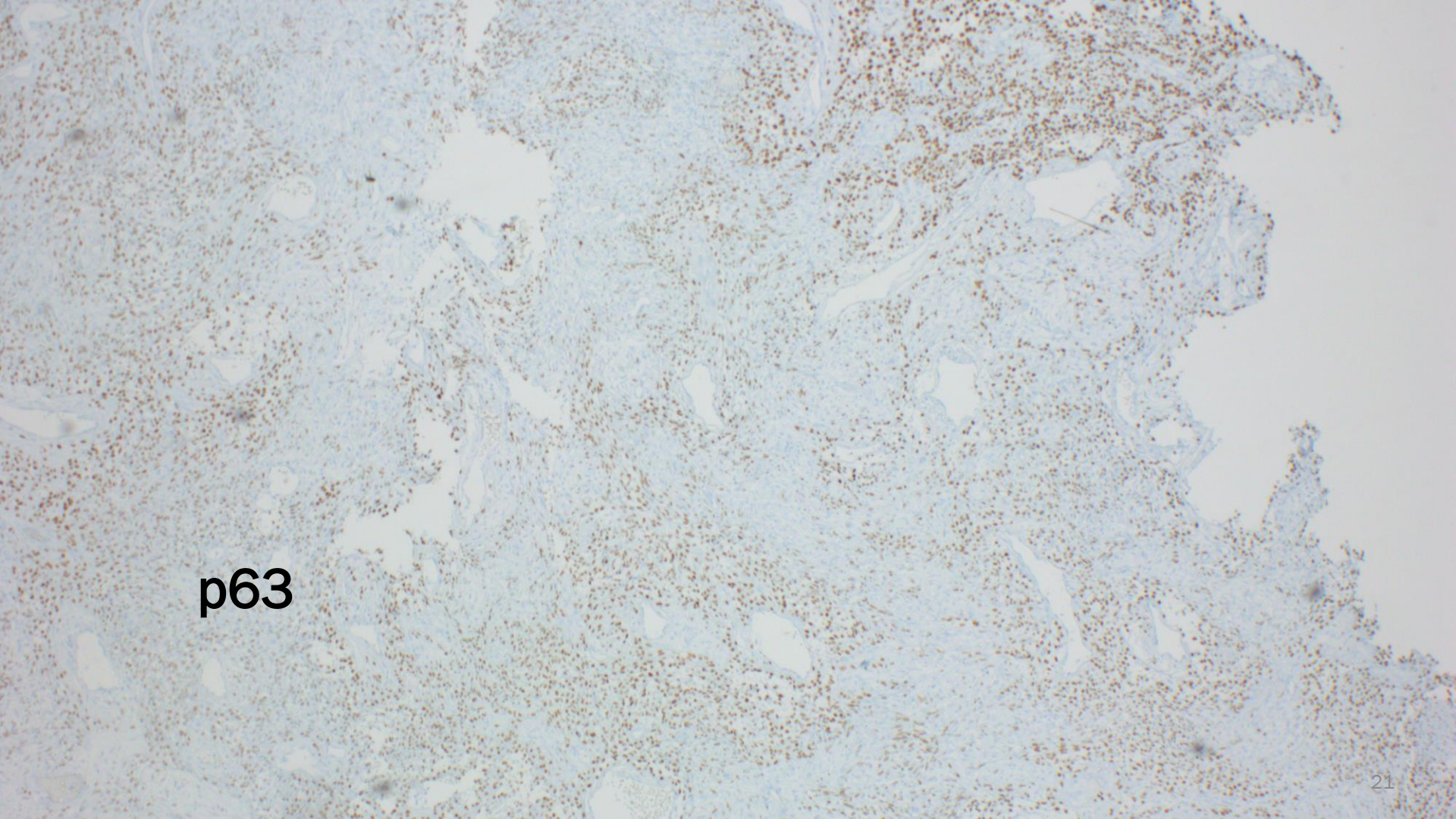







CK5/6


CK5/6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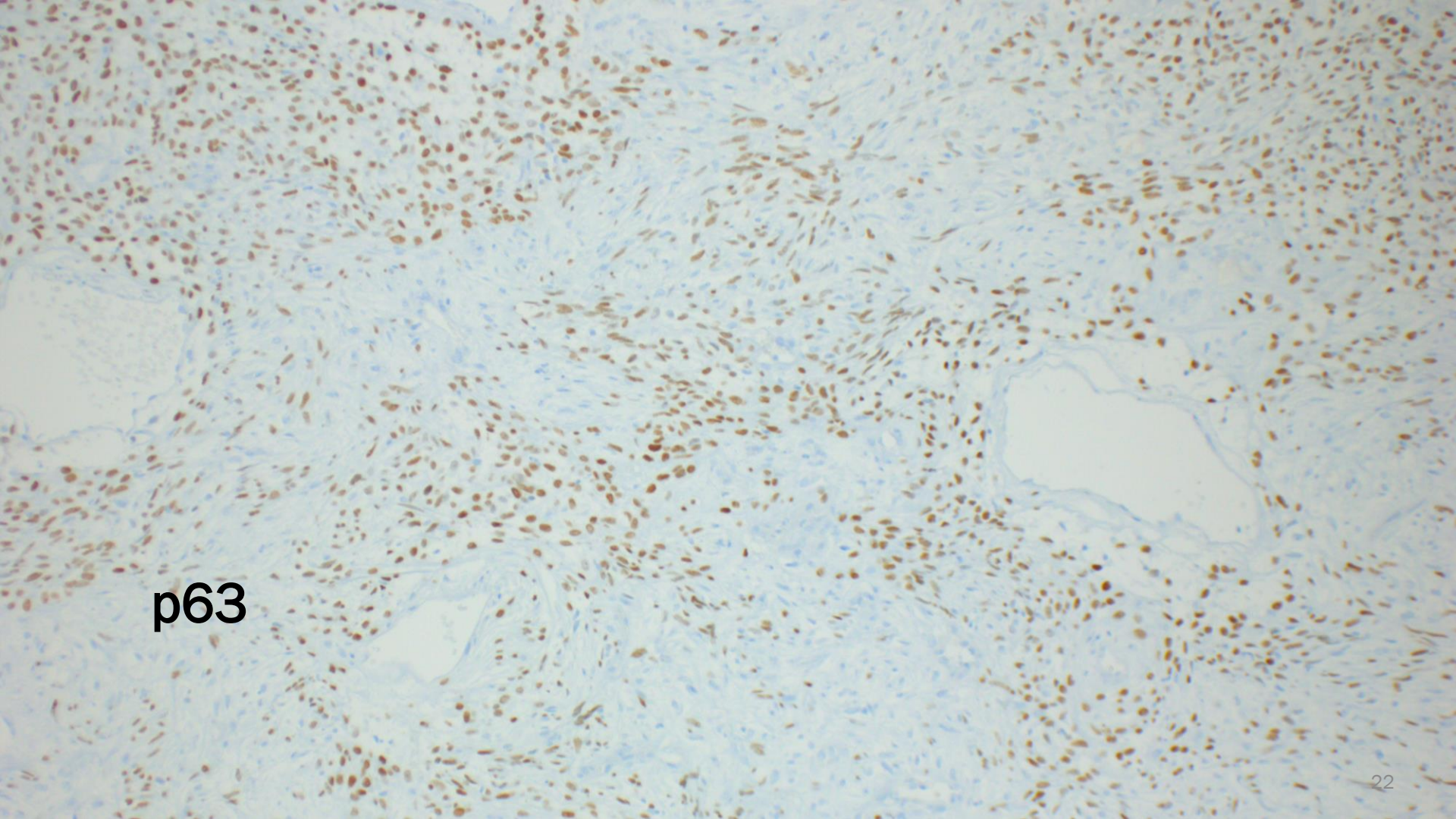
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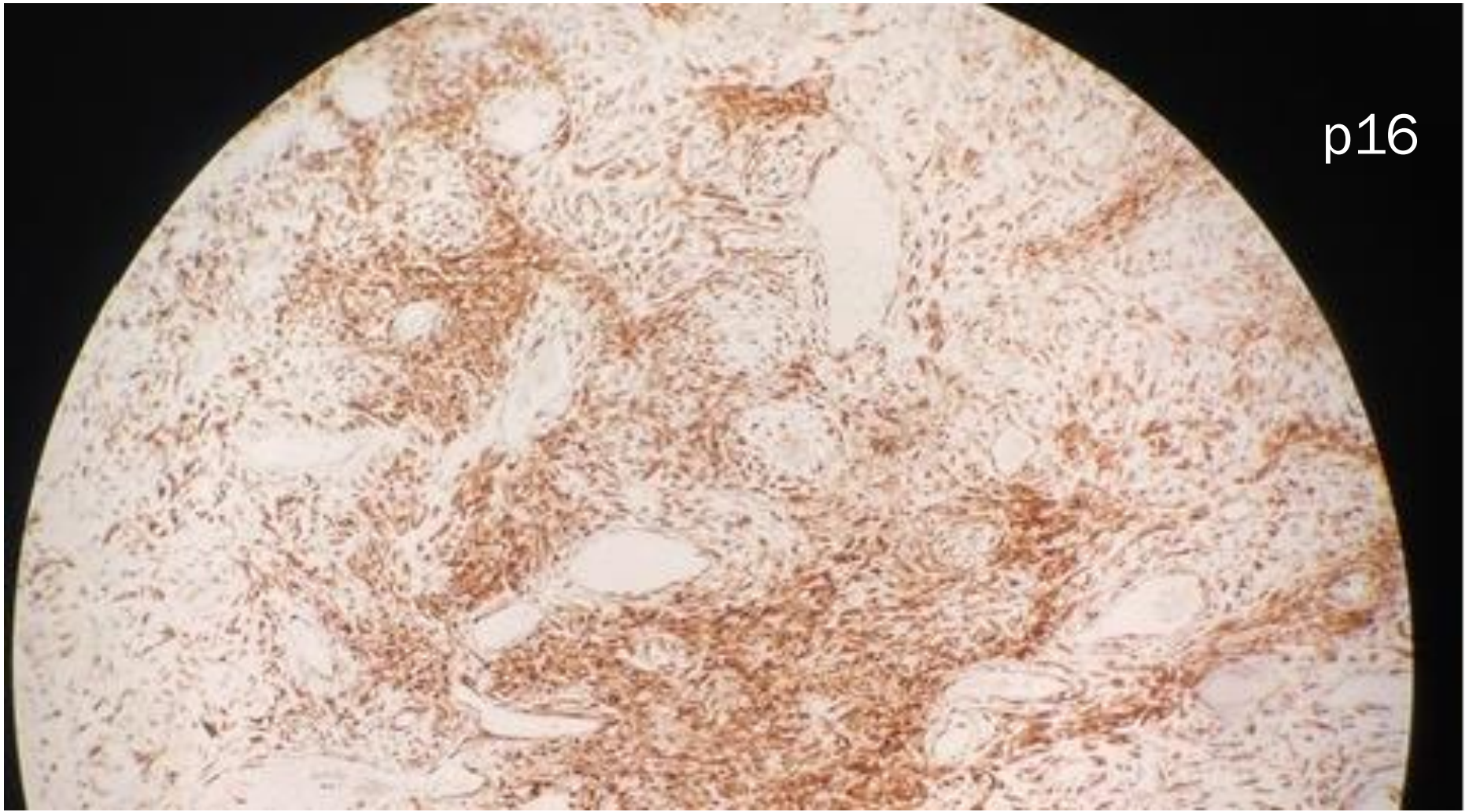
p16

Sarcomatoid Squamous Cell Carcinoma

Cervical Sarcomatoid (Spindle Cell) Carcinoma

- Squamous cell carcinoma constitutes approximately 90% of cervical cancers
- Sarcomatoid subtype is rare
- Mean age 40s
- HPV 16, 33
- Vimentin, SMA staining reported

Cervical Sarcomatoid (Spindle Cell) Carcinoma

- Aggressive, short disease free intervals
- Patients who remained disease free had stage I or II disease and received radiation therapy

AE1/AE3, aka “pankeratin”, does not highlight all carcinomas

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Keratins

- Main intracytoplasmic intermediate filament proteins of epithelial cells
- 54 functional human keratins
- ~ Half (26) are restricted to the hair follicle
- Important for mechanical stability and integrity of epithelial cells
- Divided based on molecular weight and pH

Keratins

- Moll catalog number 1 (high molecular weight) to 19 (low molecular weight)
- Type I – acidic (CK9-CK10, CK12-20, 23-24, 25-28, 31-40)

  Type II – neutral to basic (CK1- 8, 71-86)
- Keratin polymers contain equimolar amounts of types I and II keratins

Keratins

- Expression depends on cell type and differentiation
- Stratified squamous epithelium: 1 - 6, 9 – 17
- Simple epithelium: 7 - 8, 18 – 20
  - Most abundant in malignancies: 8, 18, 19

- Keratins are also found in some mesenchymal cells
  - Myometrial smooth muscle
  - Endothelial cells

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Keratin antibody clones

Affinities for individual keratins may vary for each clone

AE1/AE3: 1, 2, 3, 4, 5, 6, 7, 8 (type II) (AE3)

10, 14, 15, 16, 19 (type I) (AE1)

- 17 missing (squamous), 18 missing (adenocarcinoma)
- May be negative in Hepatocellular Carcinoma (30% +), Adrenal Cortical Carcinoma (10% +), Renal Cell Carcinoma (65% +)

Keratin antibody clones

Affinities for individual keratins may vary for each clone

OSCAR: 7, 8, 18, 19

MNF116: 5, 6, 8, 17, 19

CAM5.2: 8 (7, +/-18)

Hepatocellular carcinoma (90% +)

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Cocktails of antibodies providing a broader spectrum of keratin coverage, individual keratins, or other epithelial markers may reveal the true nature of the tumor

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Markers for epithelial screening

Solutions:

- Keratin cocktail: AE1/AE3, CK8/18 (higher affinity 8, +18; adenocarcinoma), MNF116 (17 squamous carcinoma)
- Other keratins: CK5/6, HMW keratin (34βE12), CK8/18
- Epithelial markers: p63, p40
- EMA
- Ep-CAM: Ber-EP4, MOC-31

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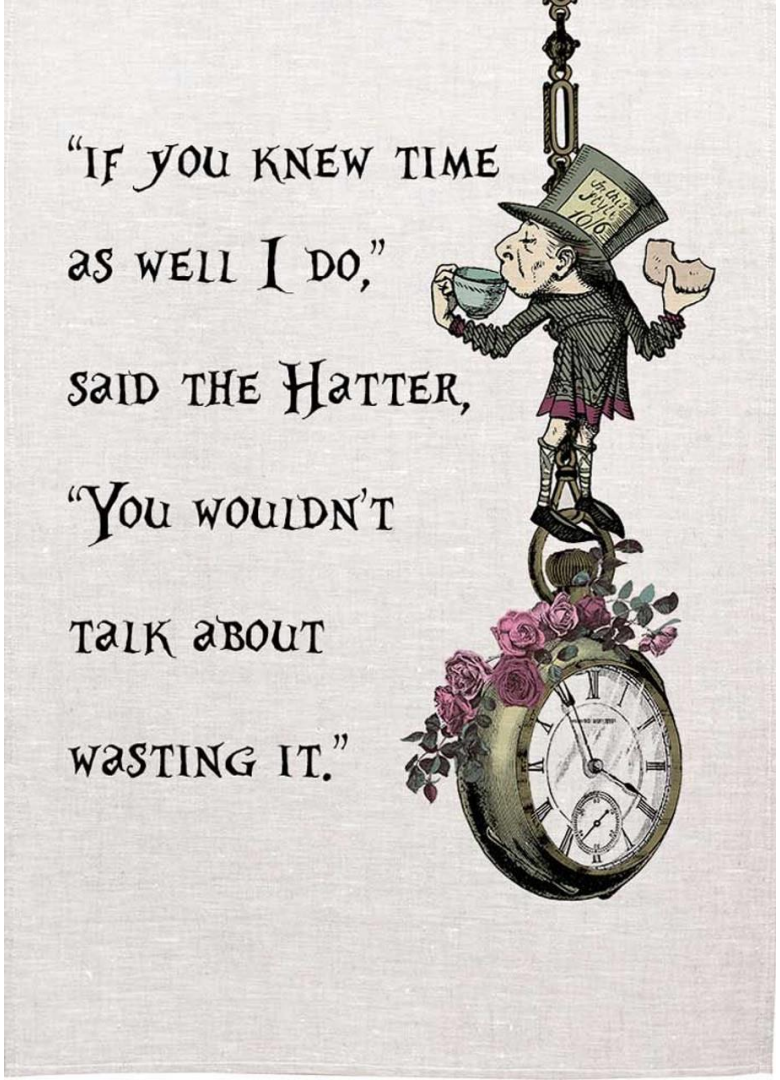
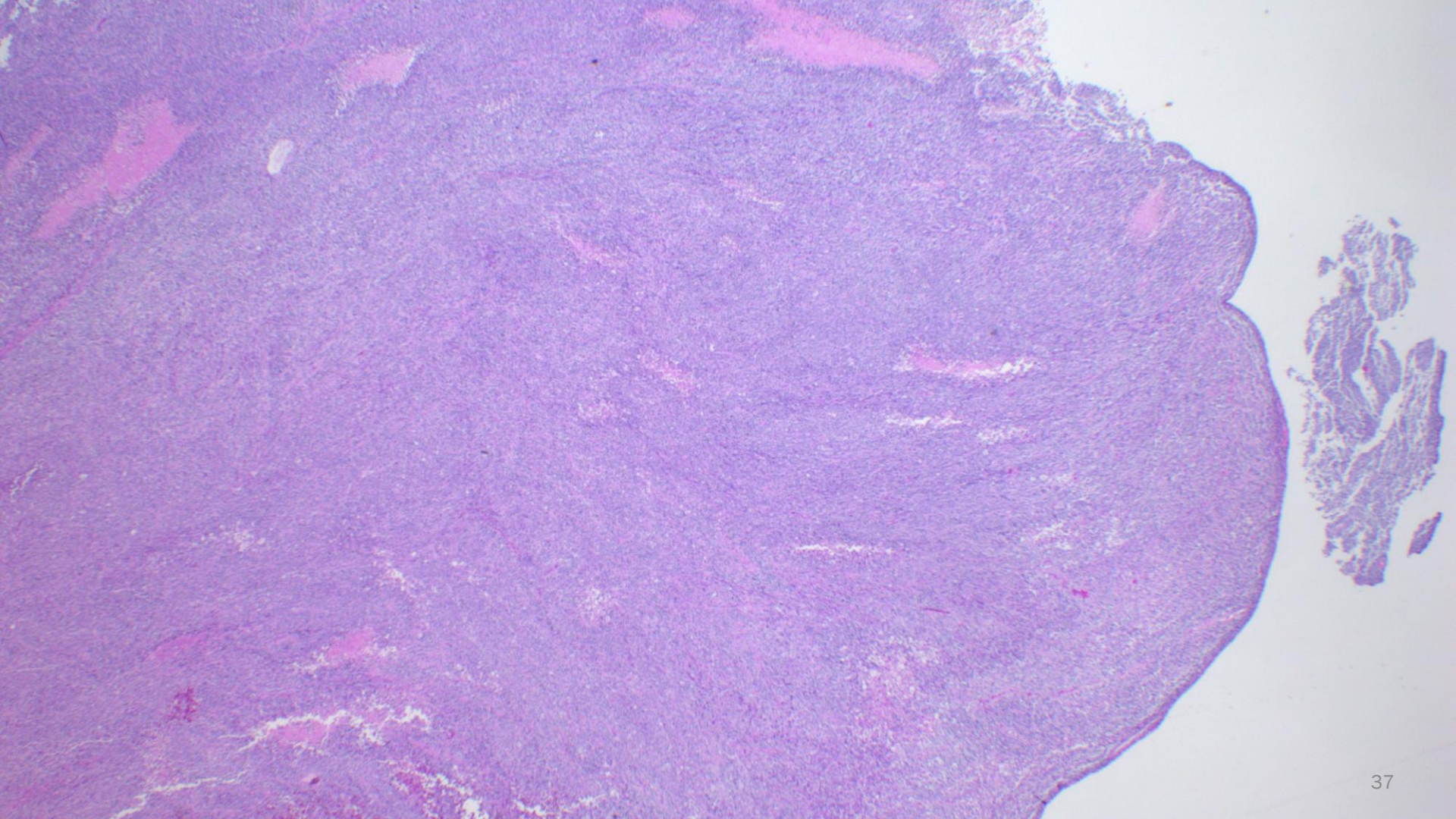
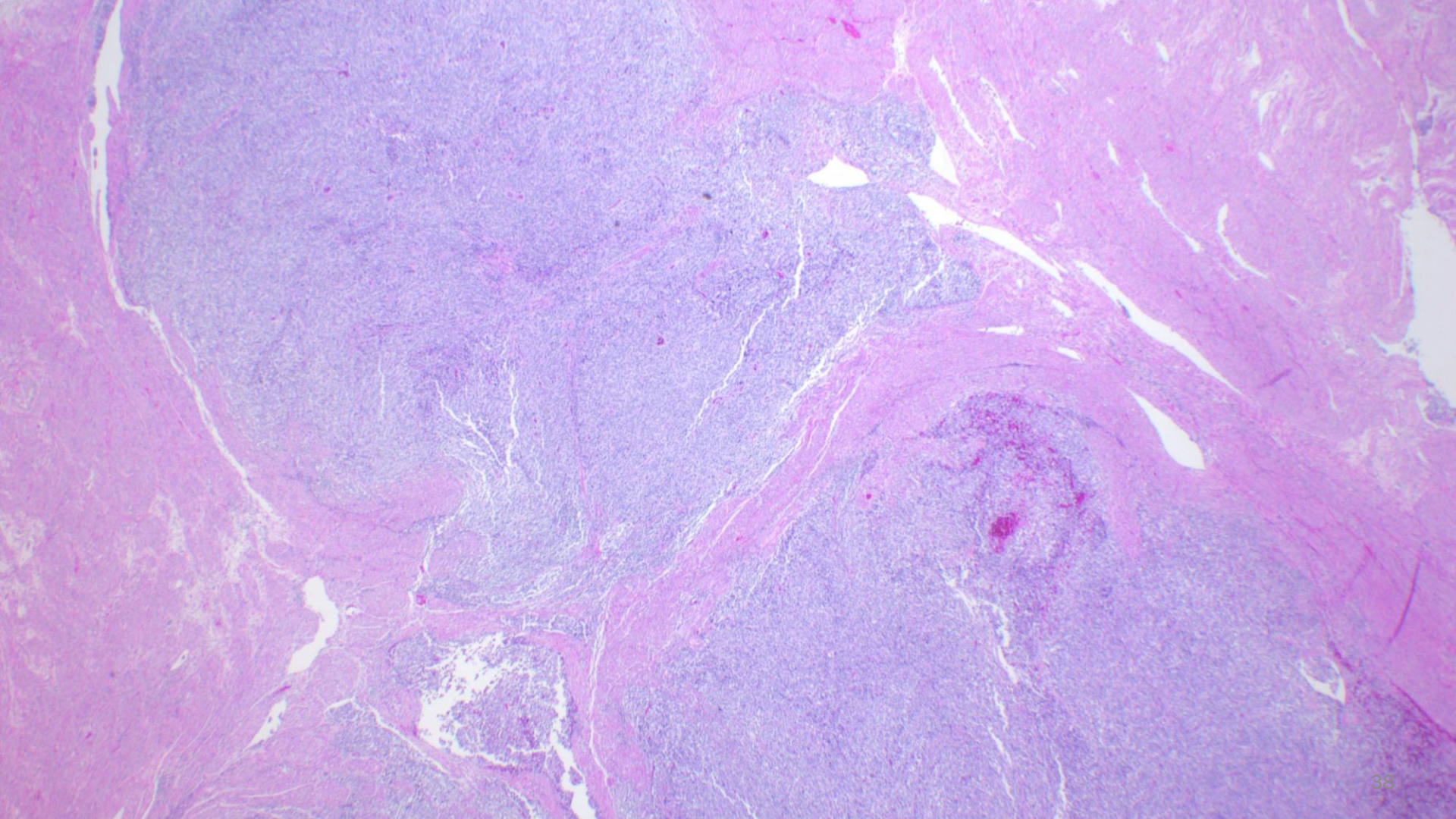
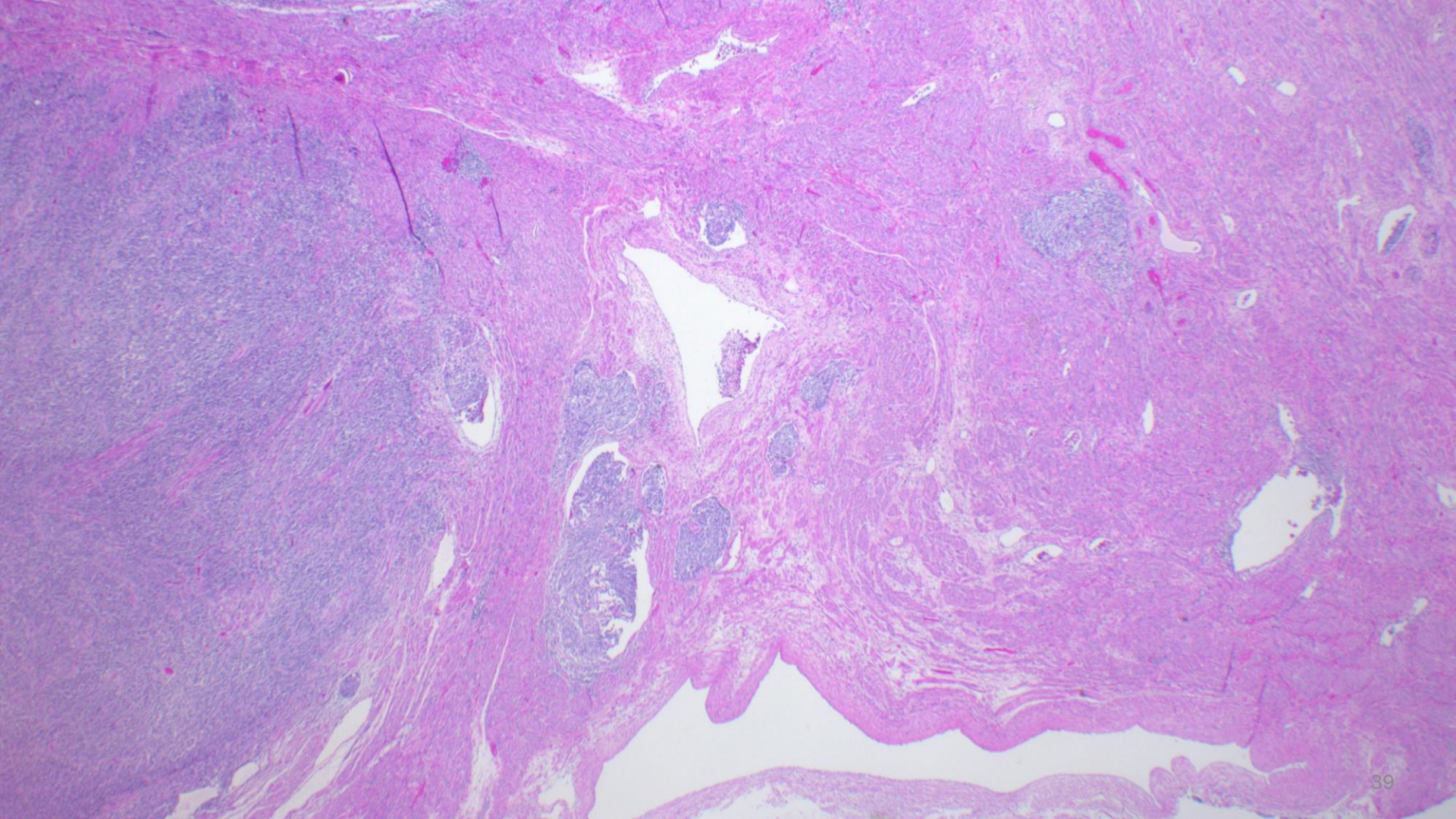

"IF YOU KNEW TIME
AS WELL I DO,"
SAID THE HATTER,
"YOU WOULDN'T
TALK ABOUT
WASTING IT."

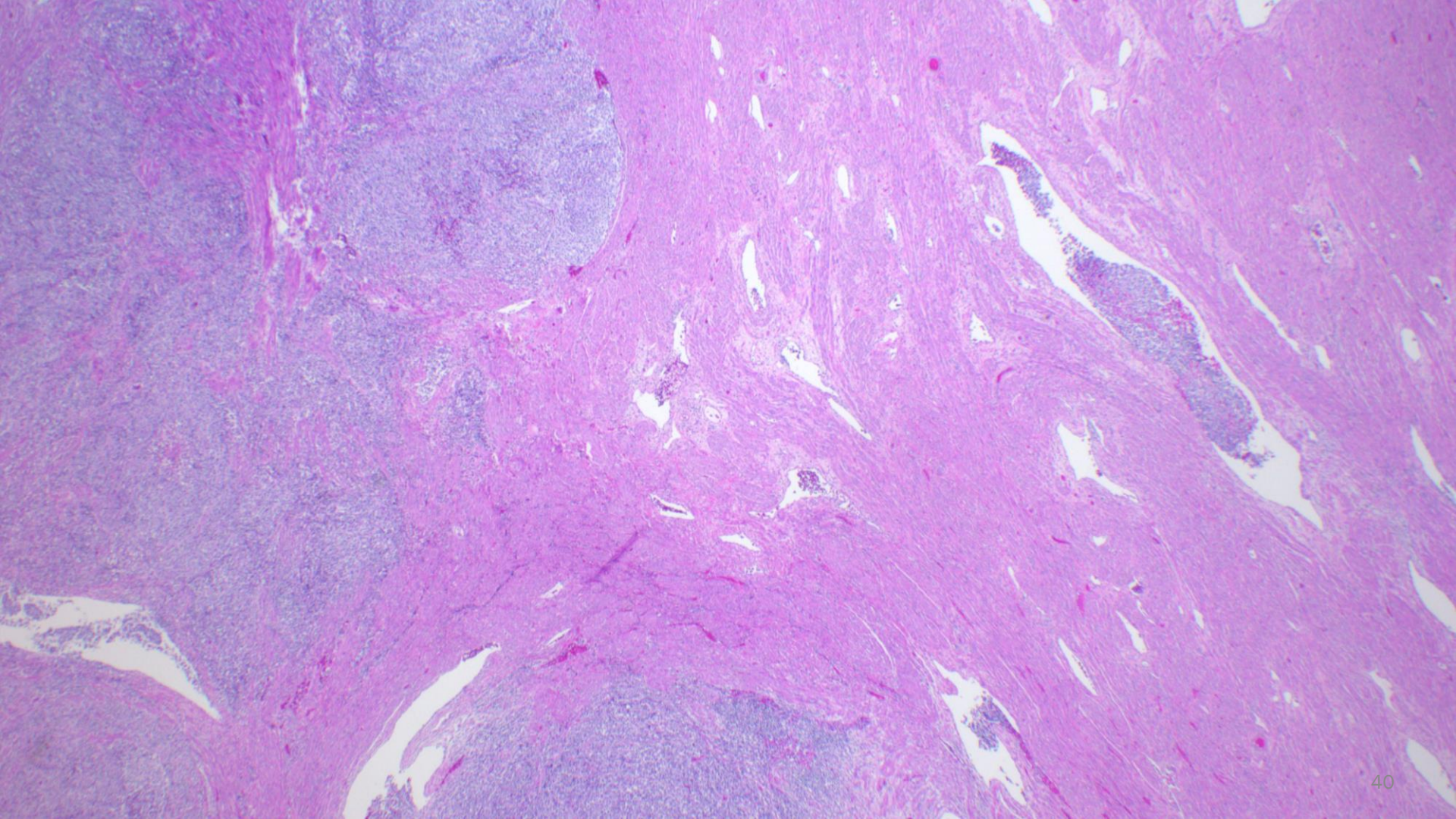
Case 2

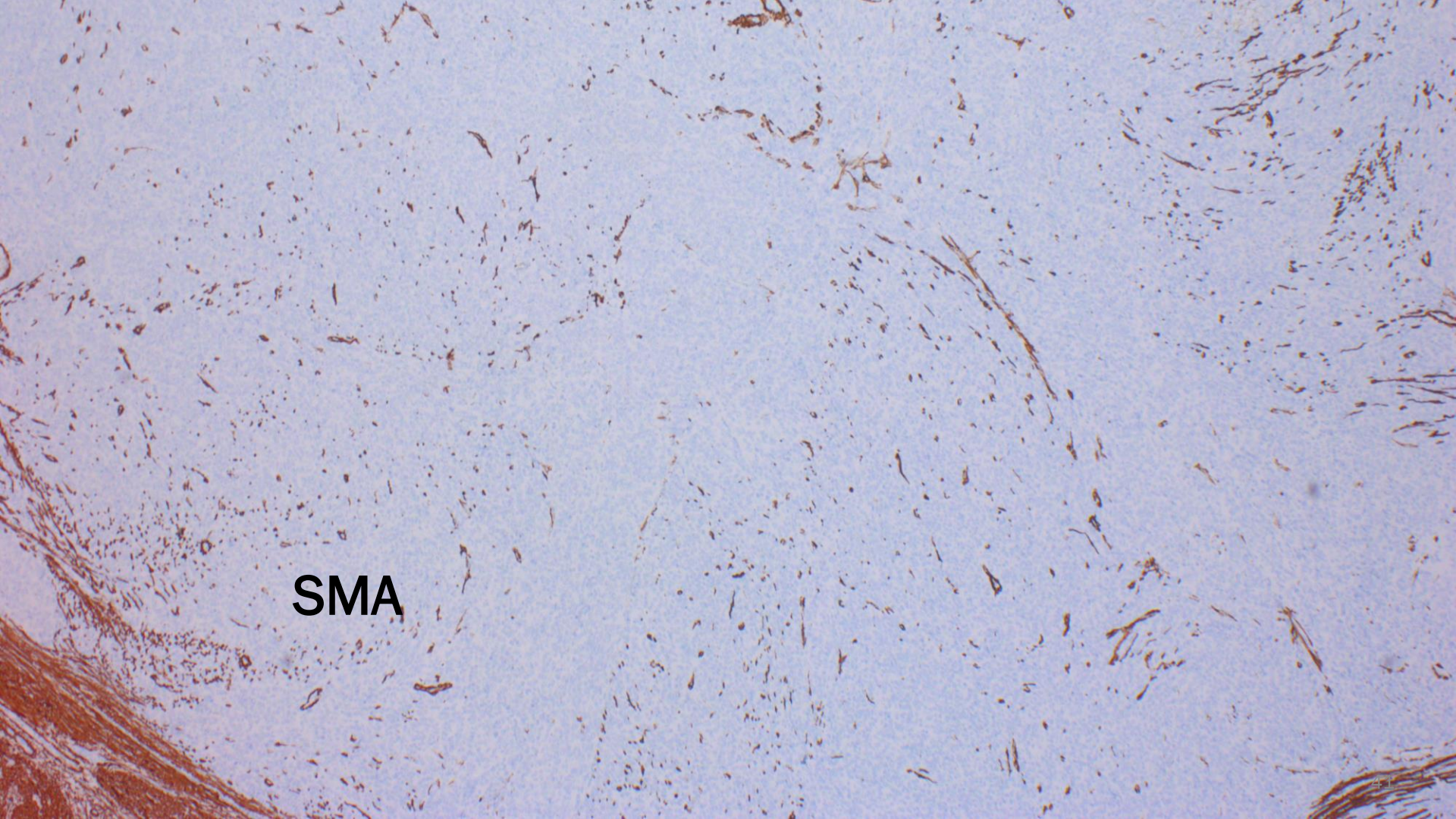
- 71 yo with a malignant endometrial neoplasm
- Endometrial Stromal Sarcoma, High Grade is favored
- Endometrial Stromal Tumor FISH is Negative
  - JAZF1, PHF1, YWHAE

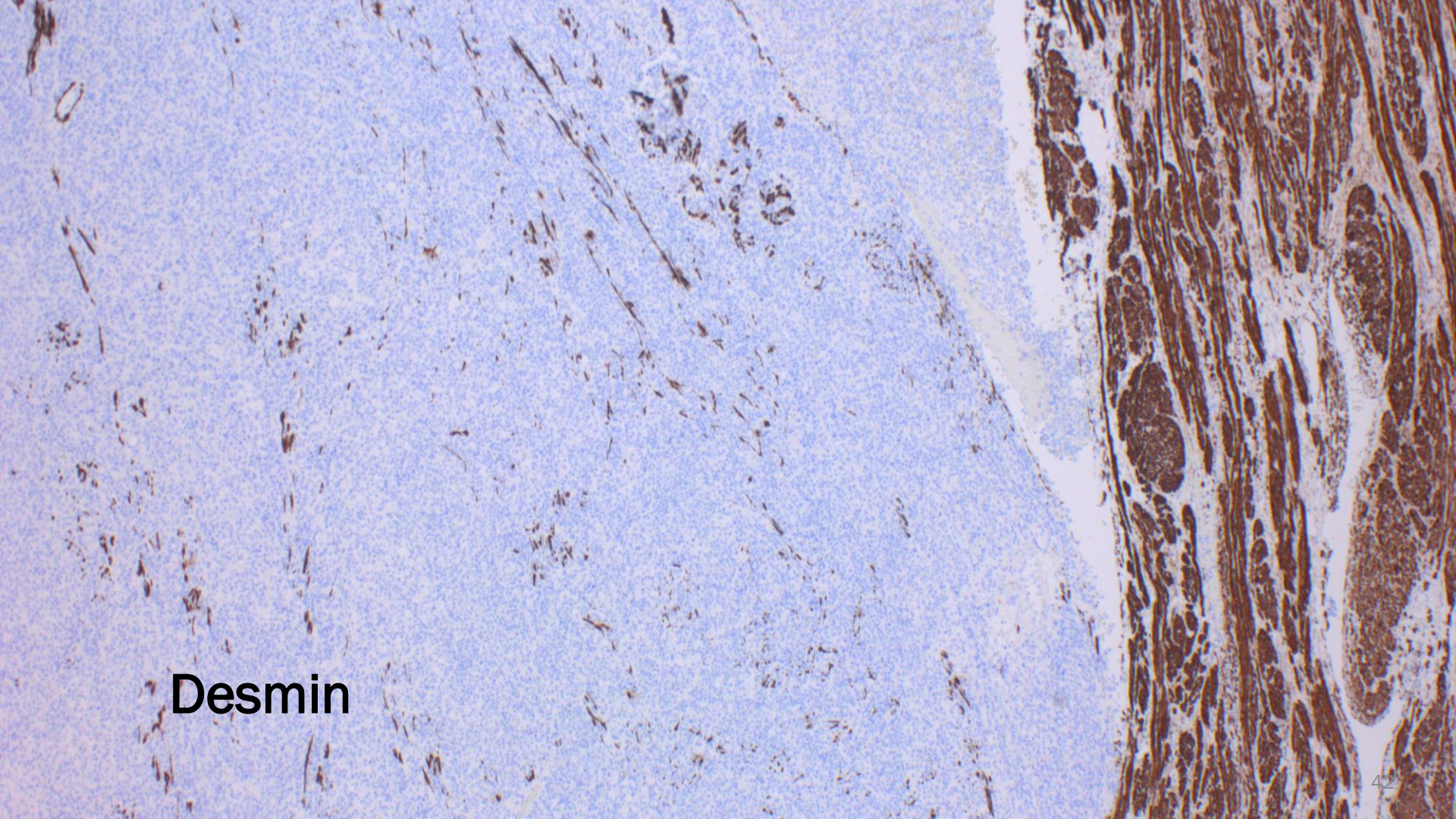









SMA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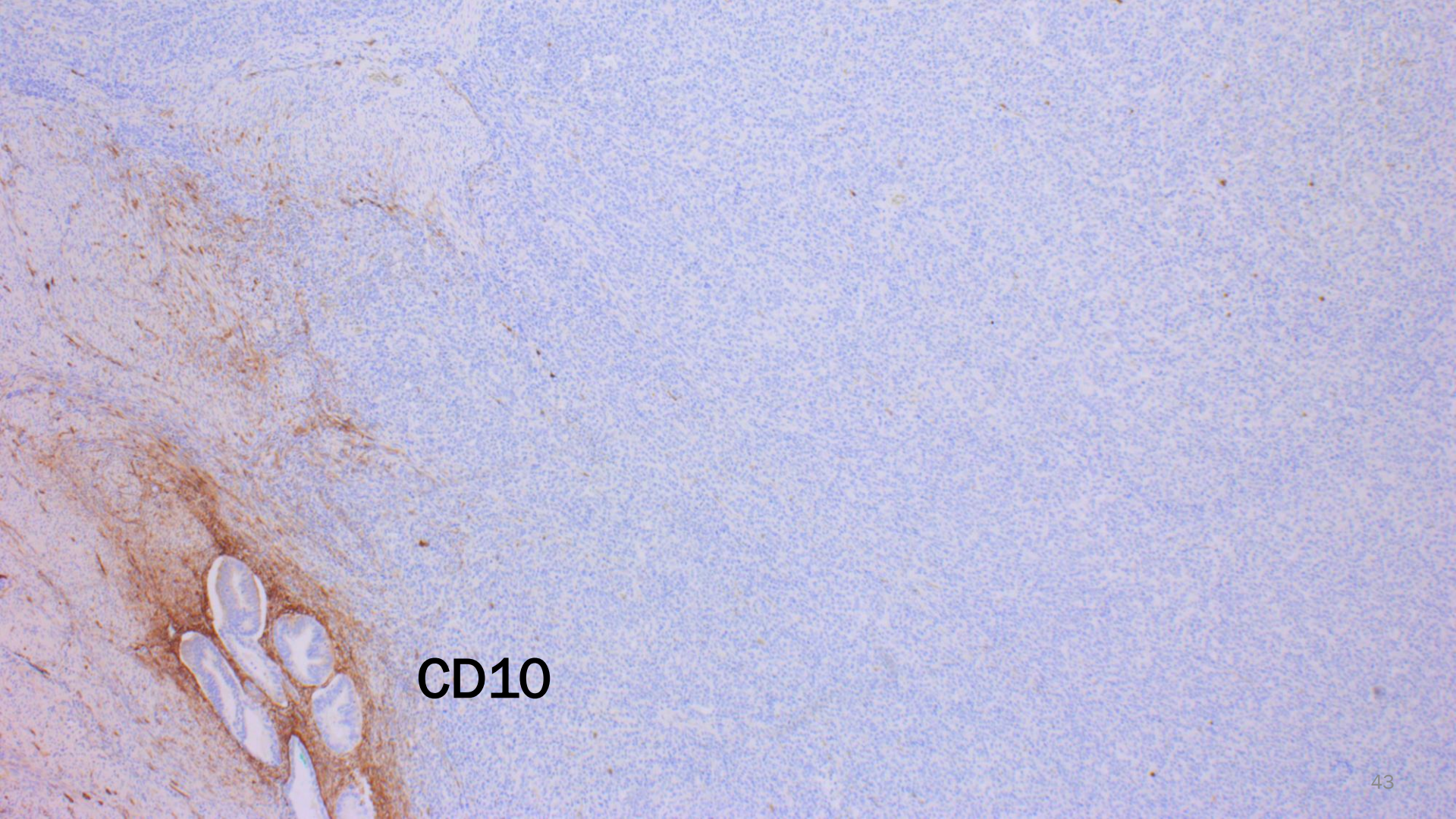
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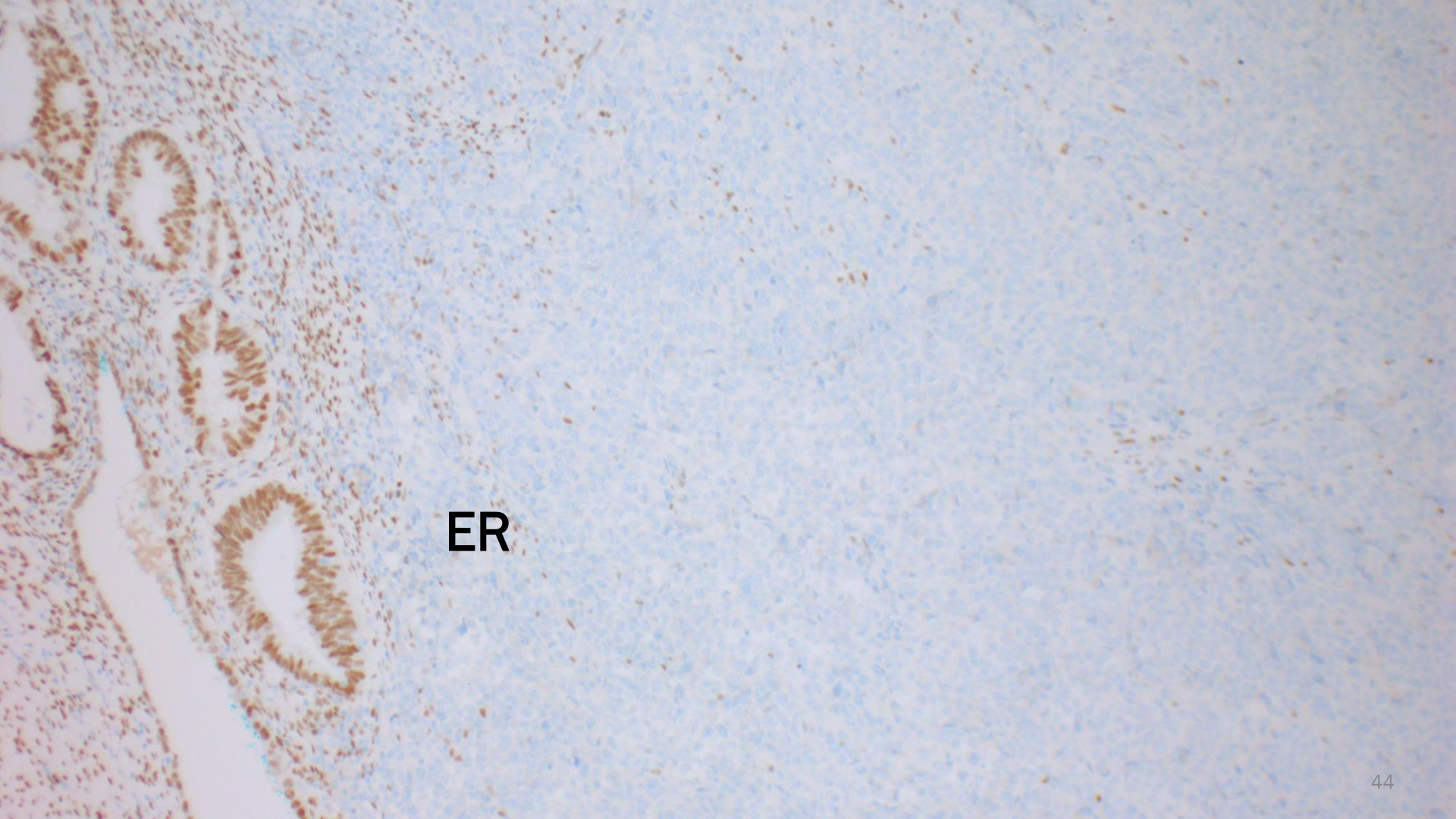
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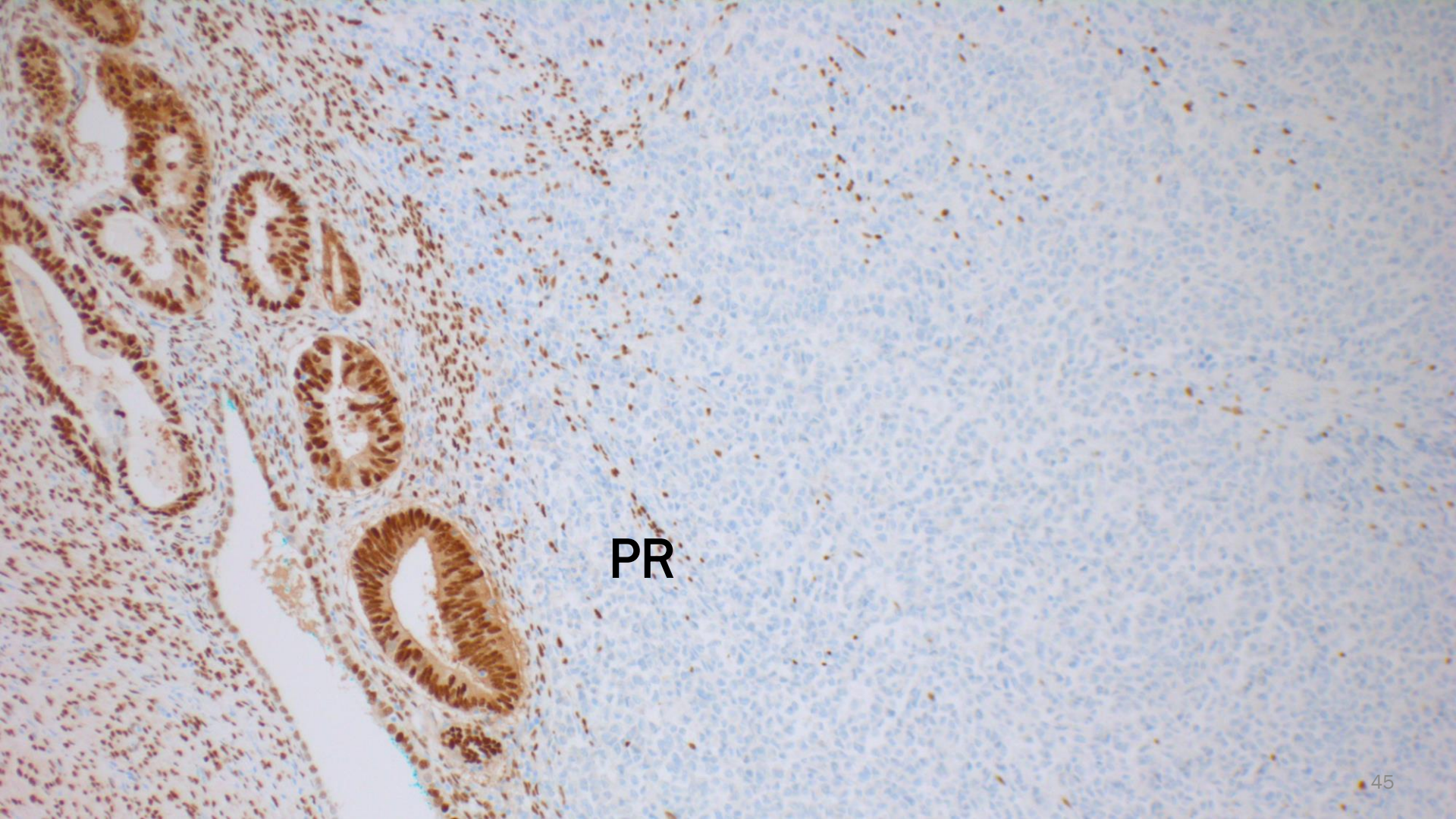
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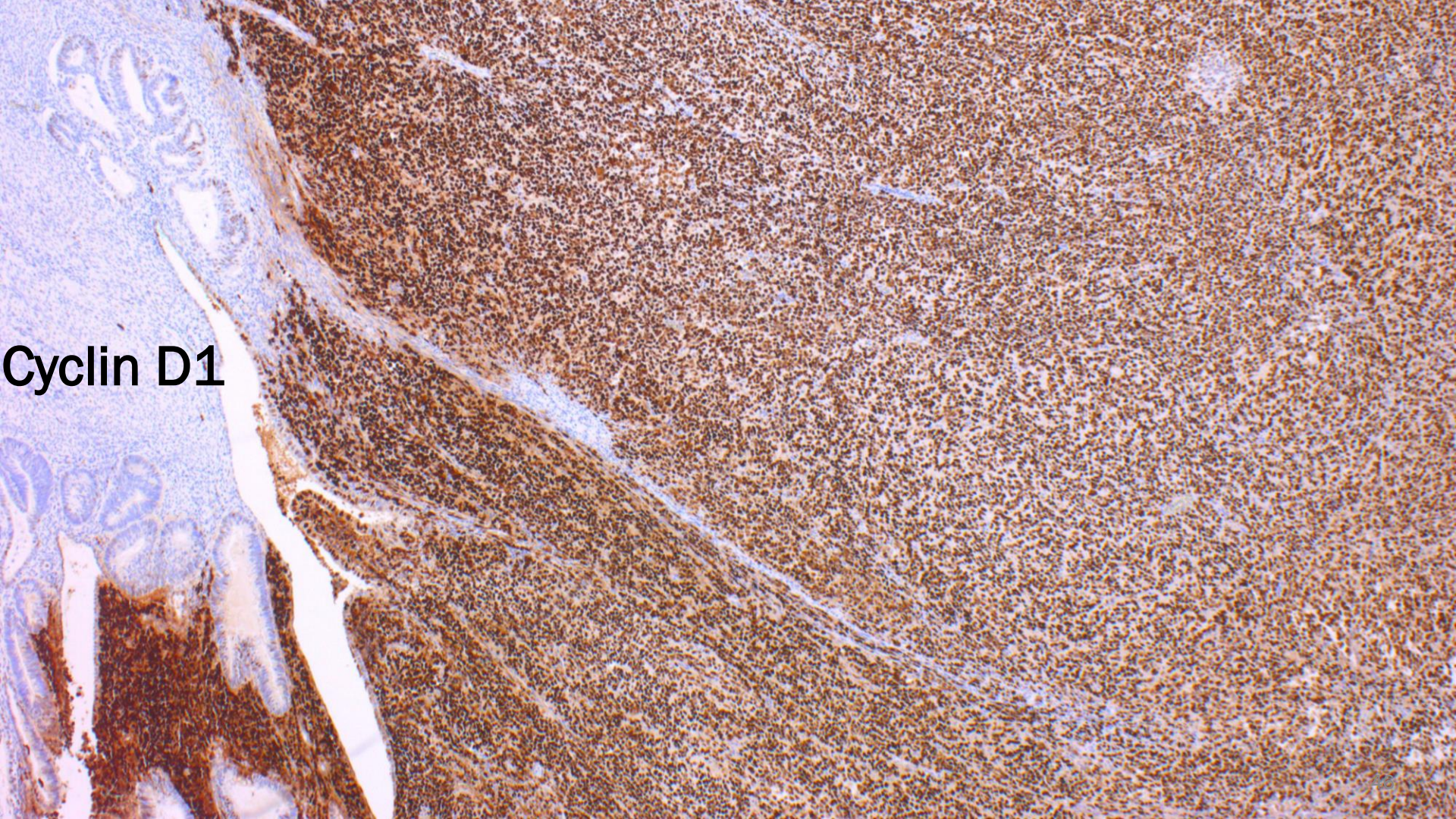
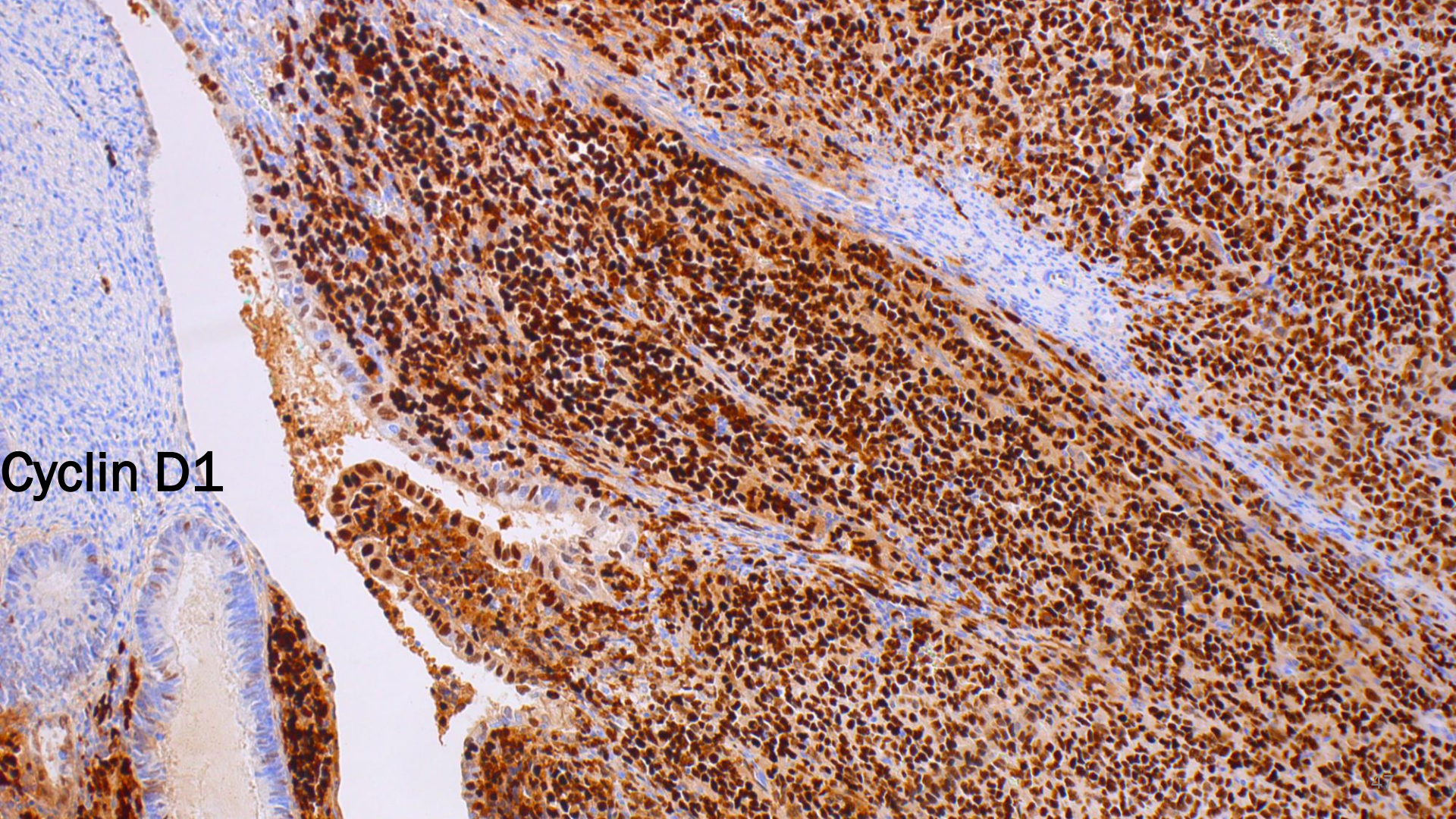
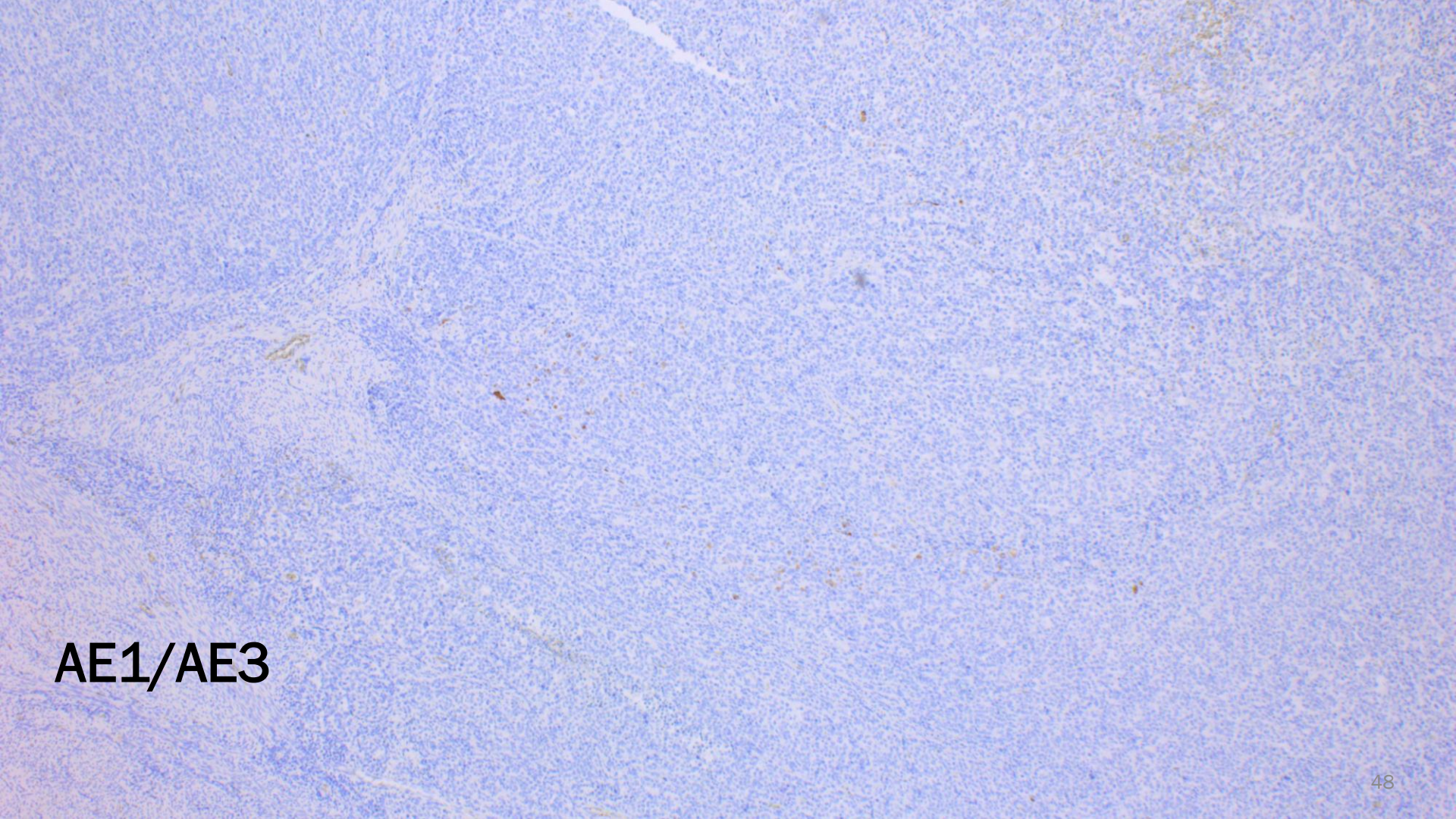
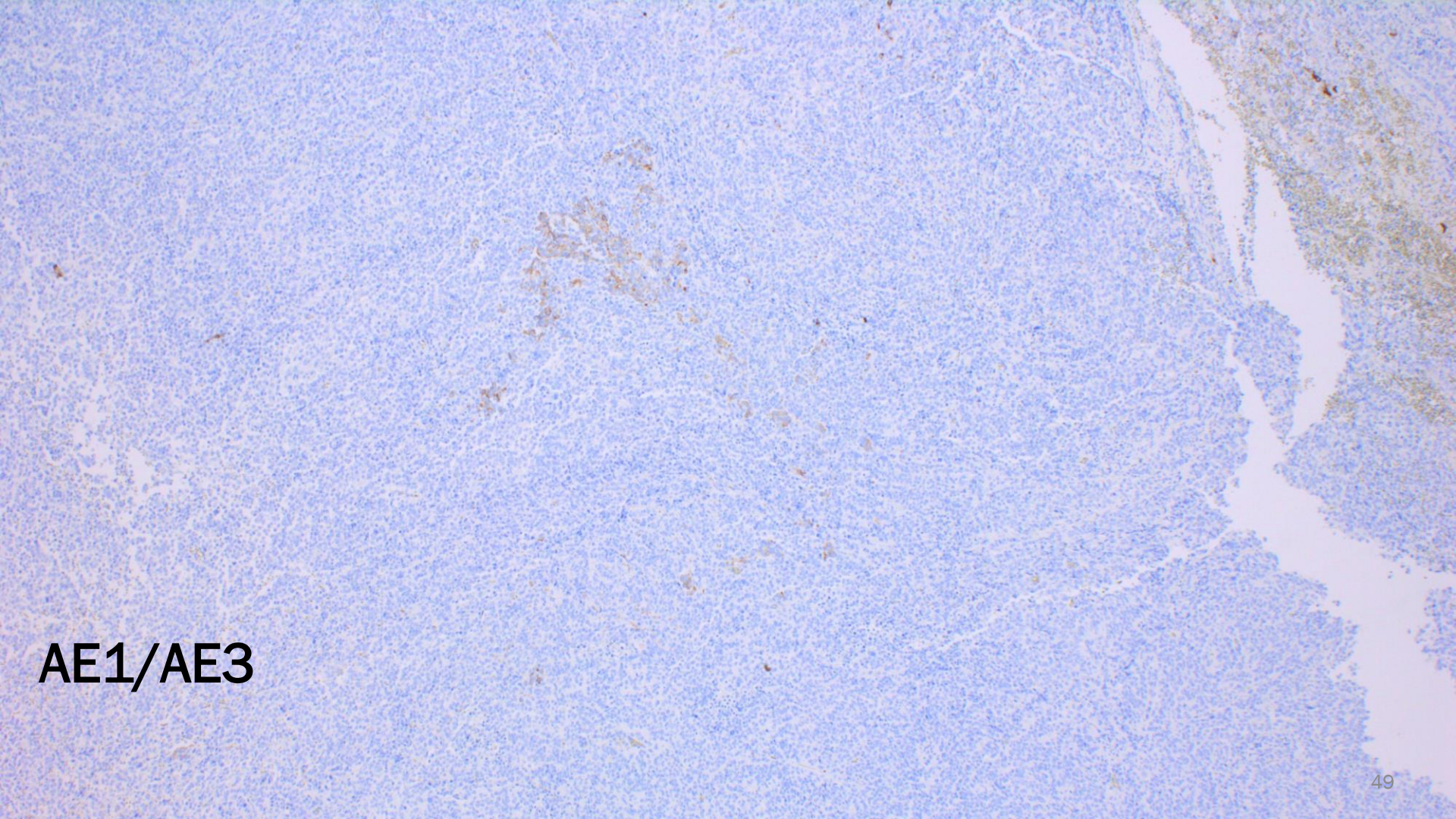
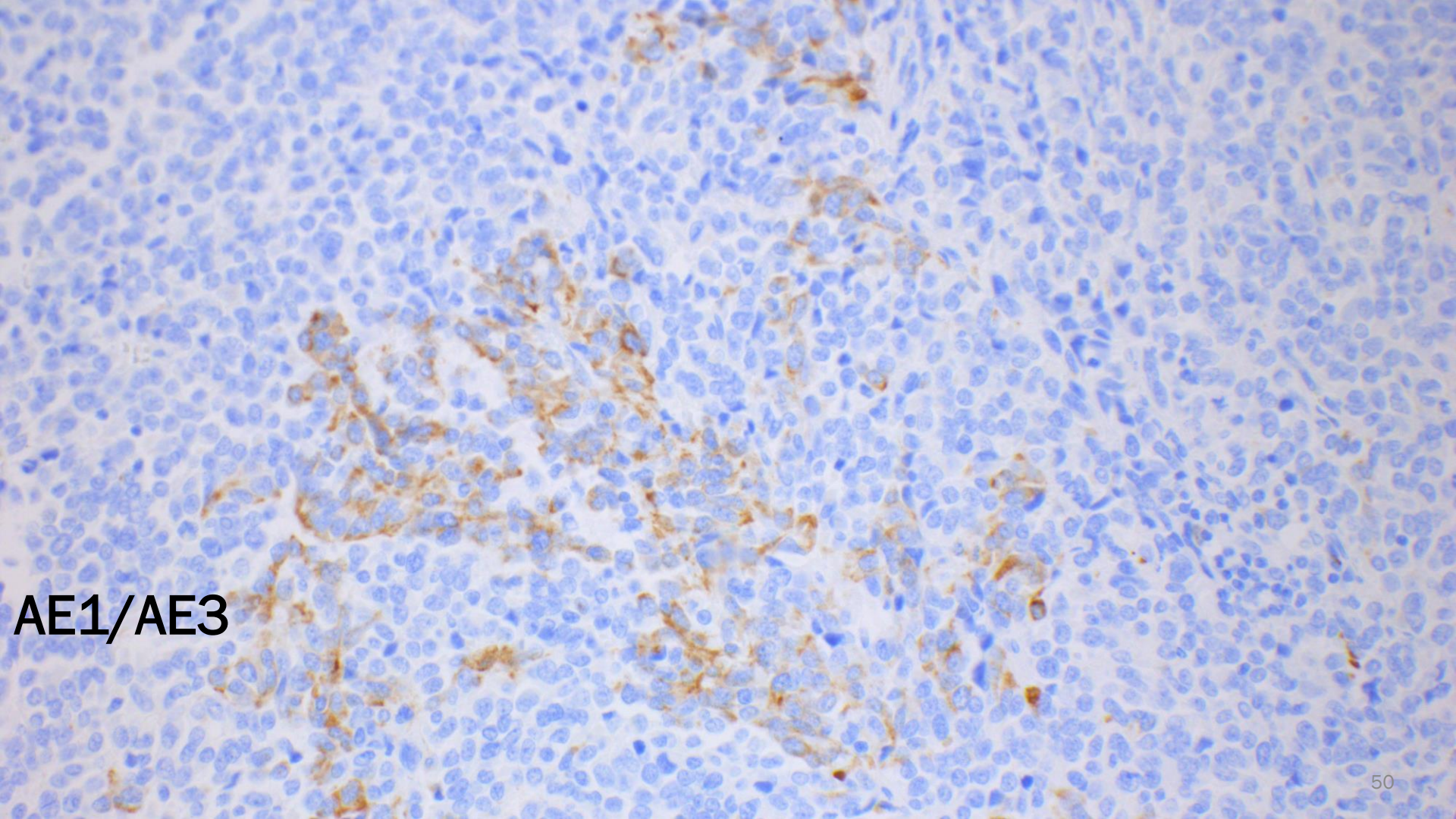
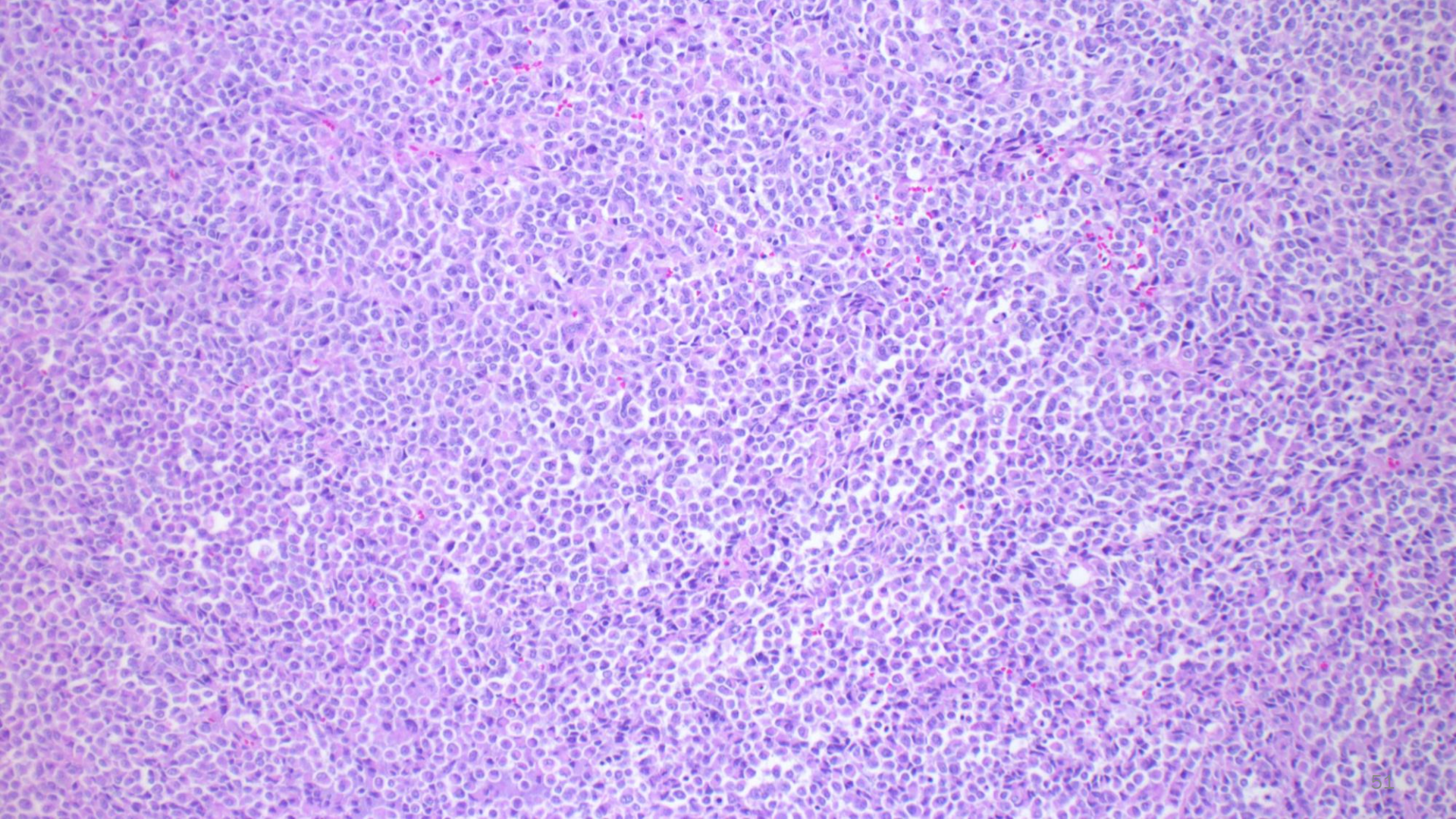

Cyclin D1

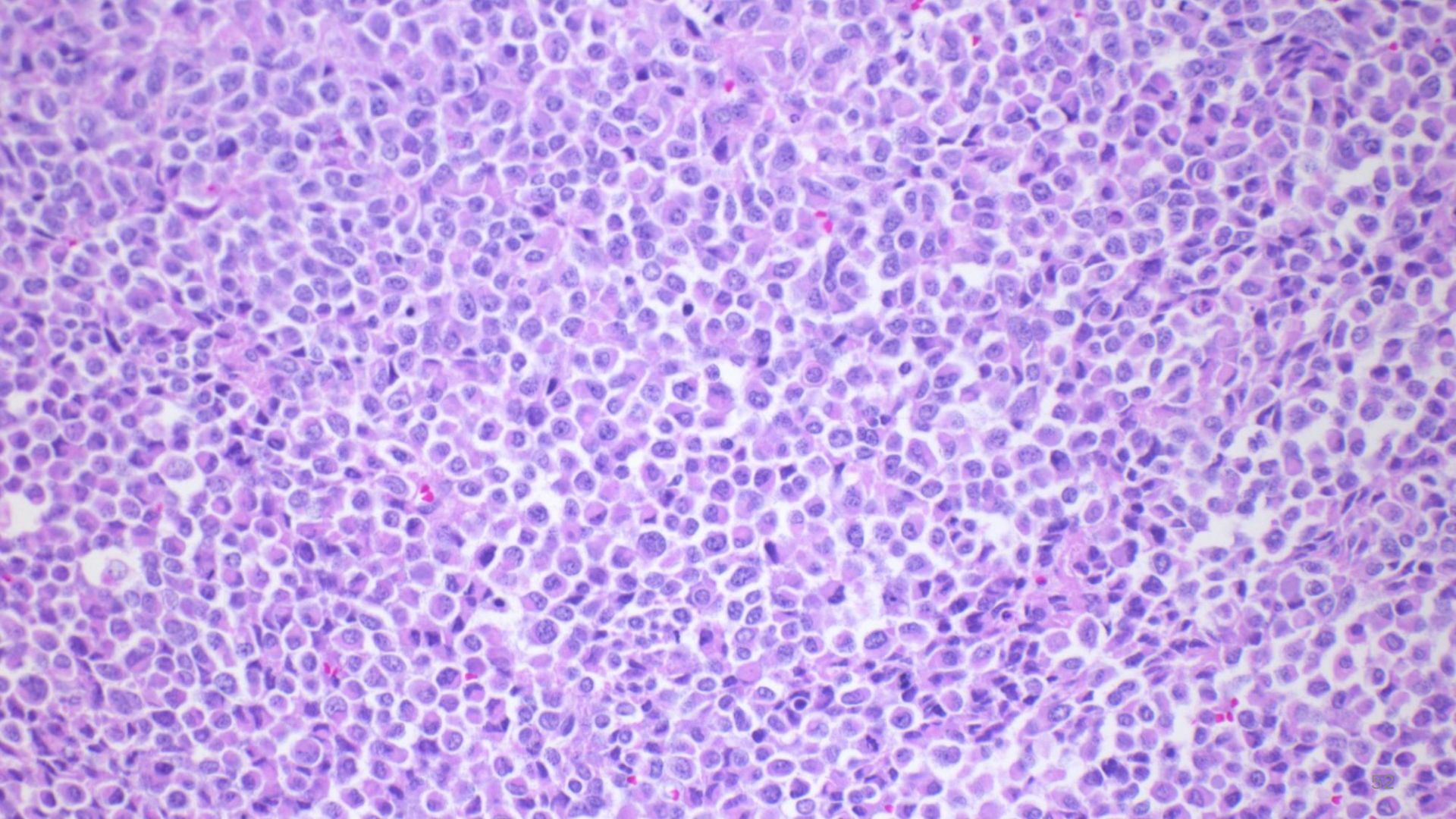

Cyclin D1

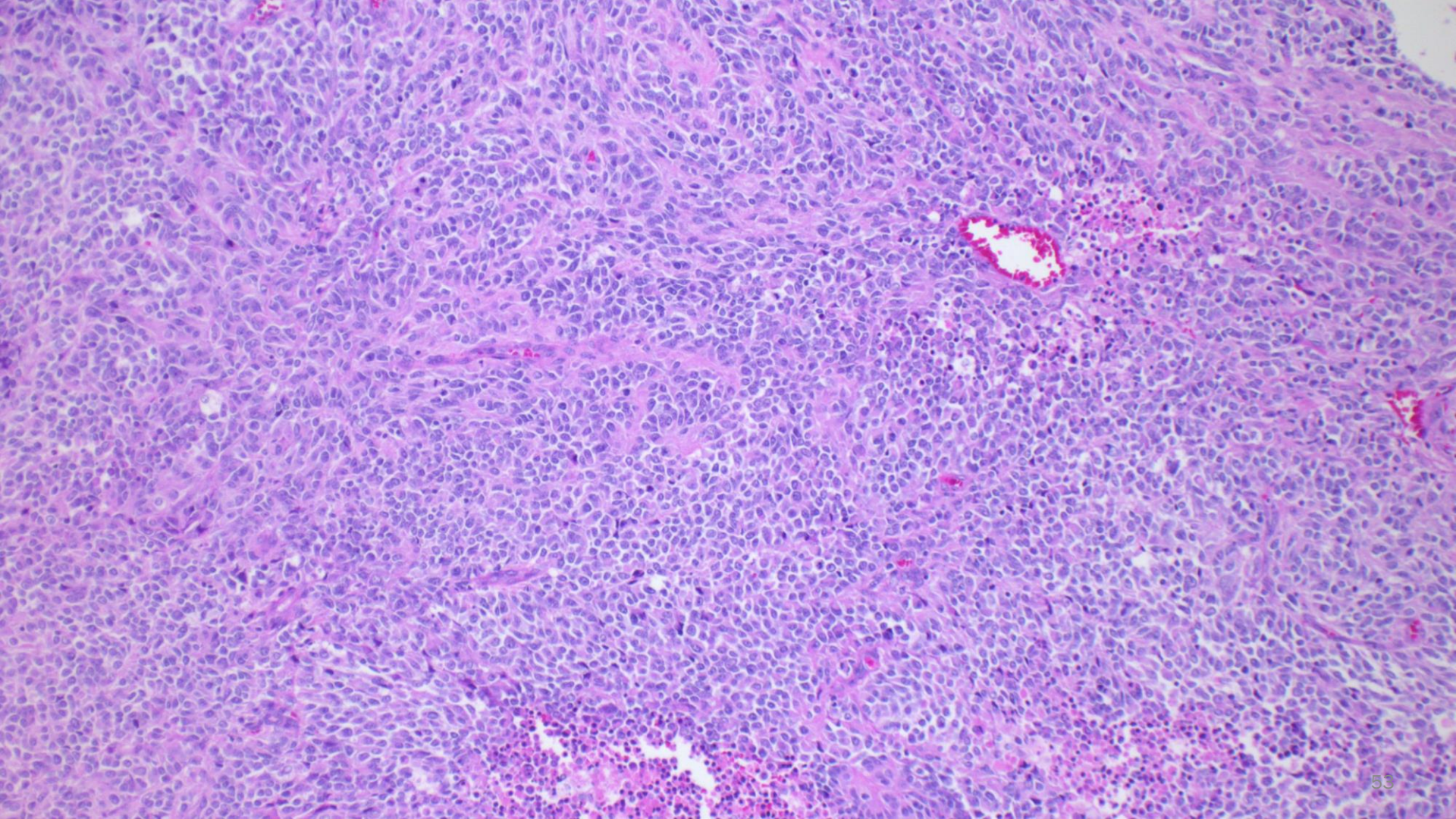

AE1/AE3

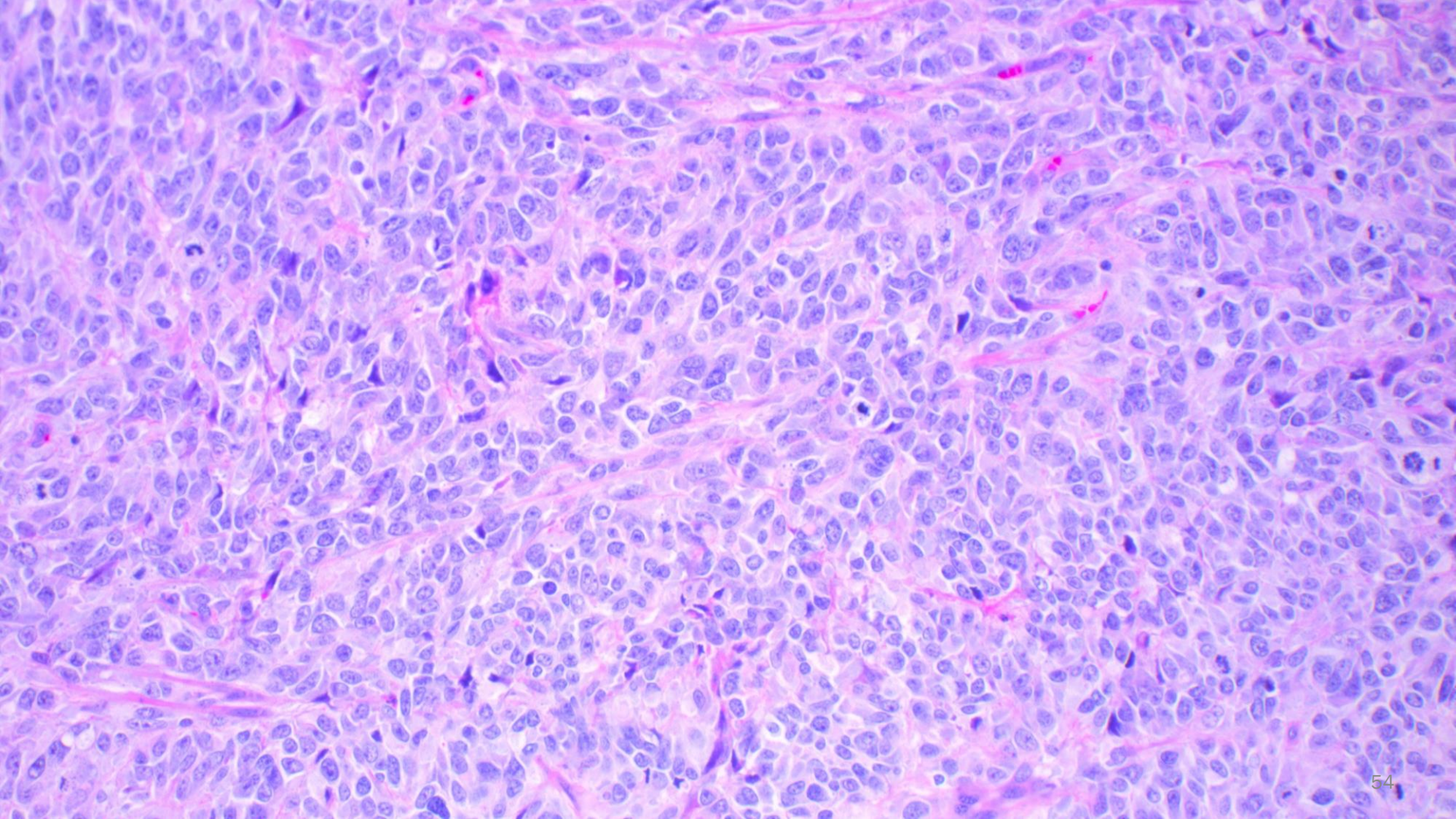

AE1/AE3

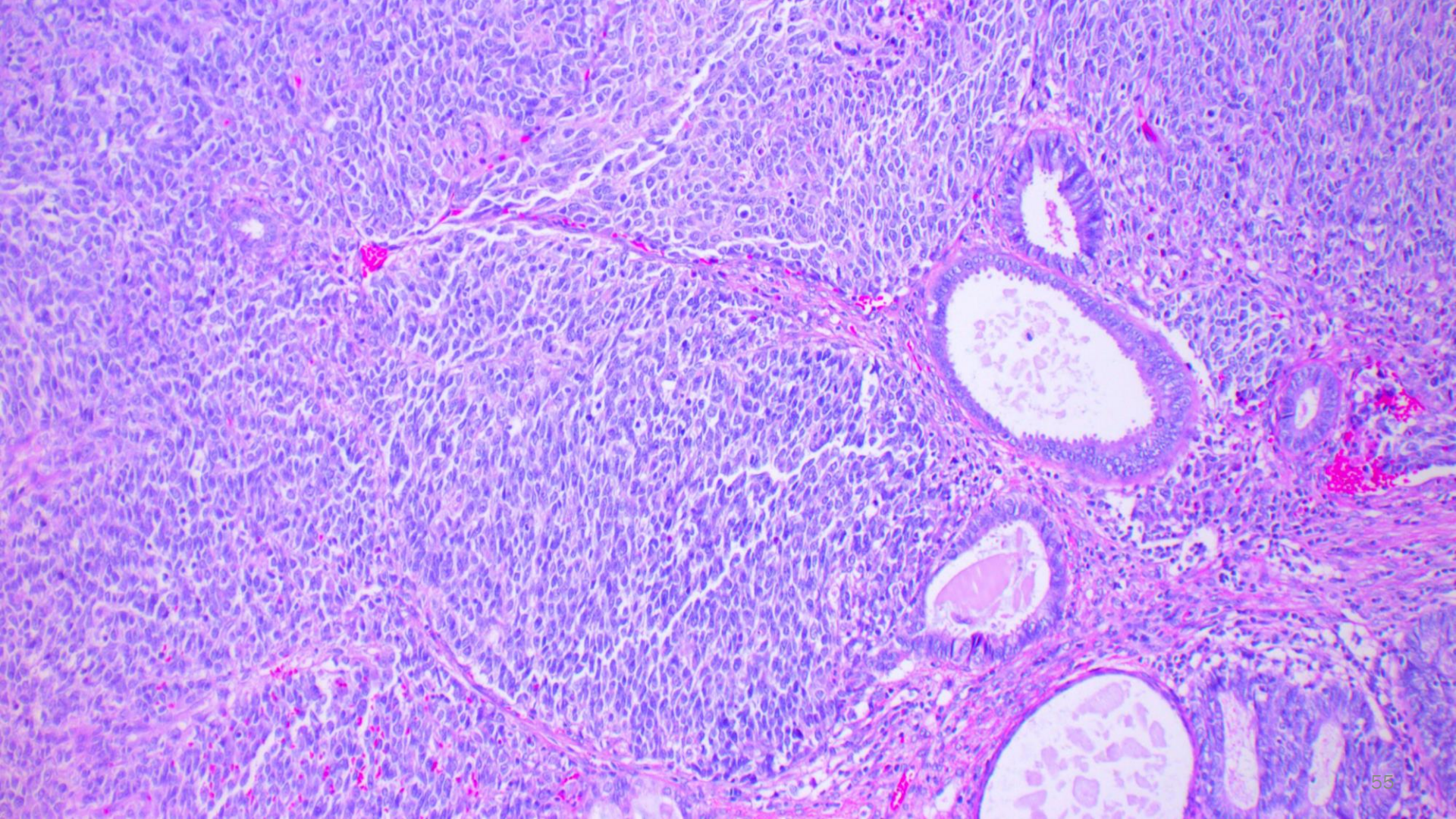

AE1/AE3

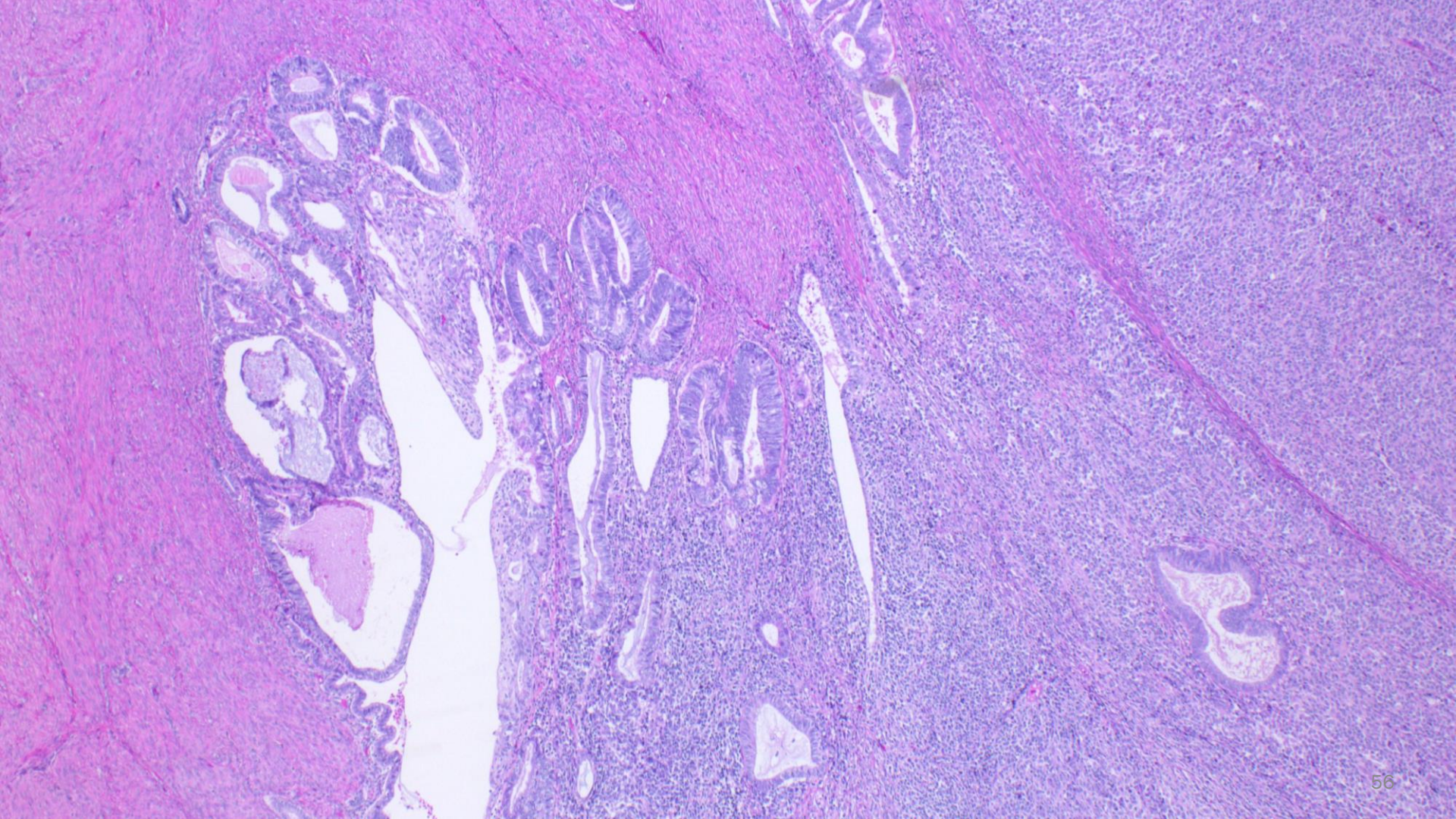


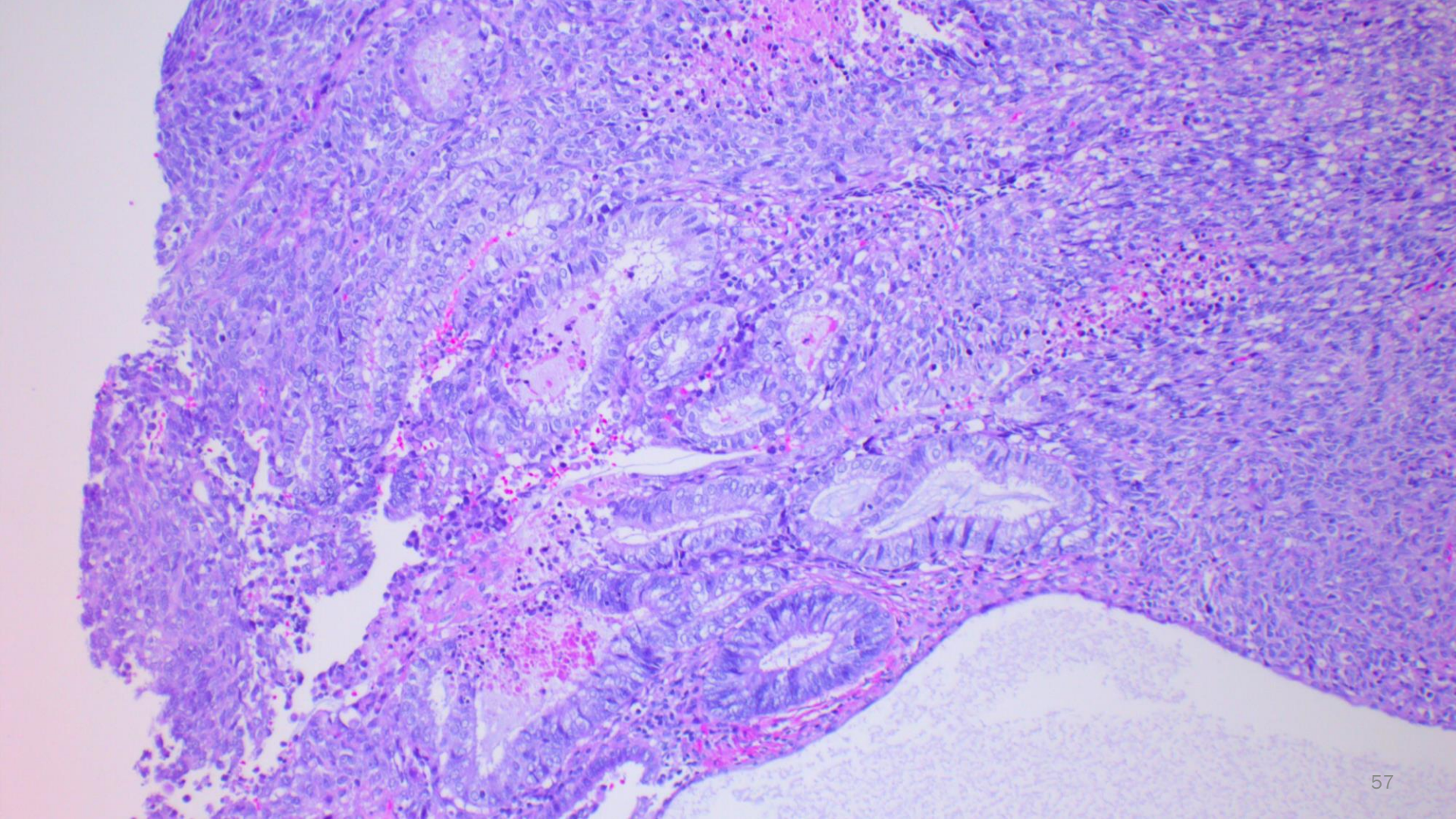


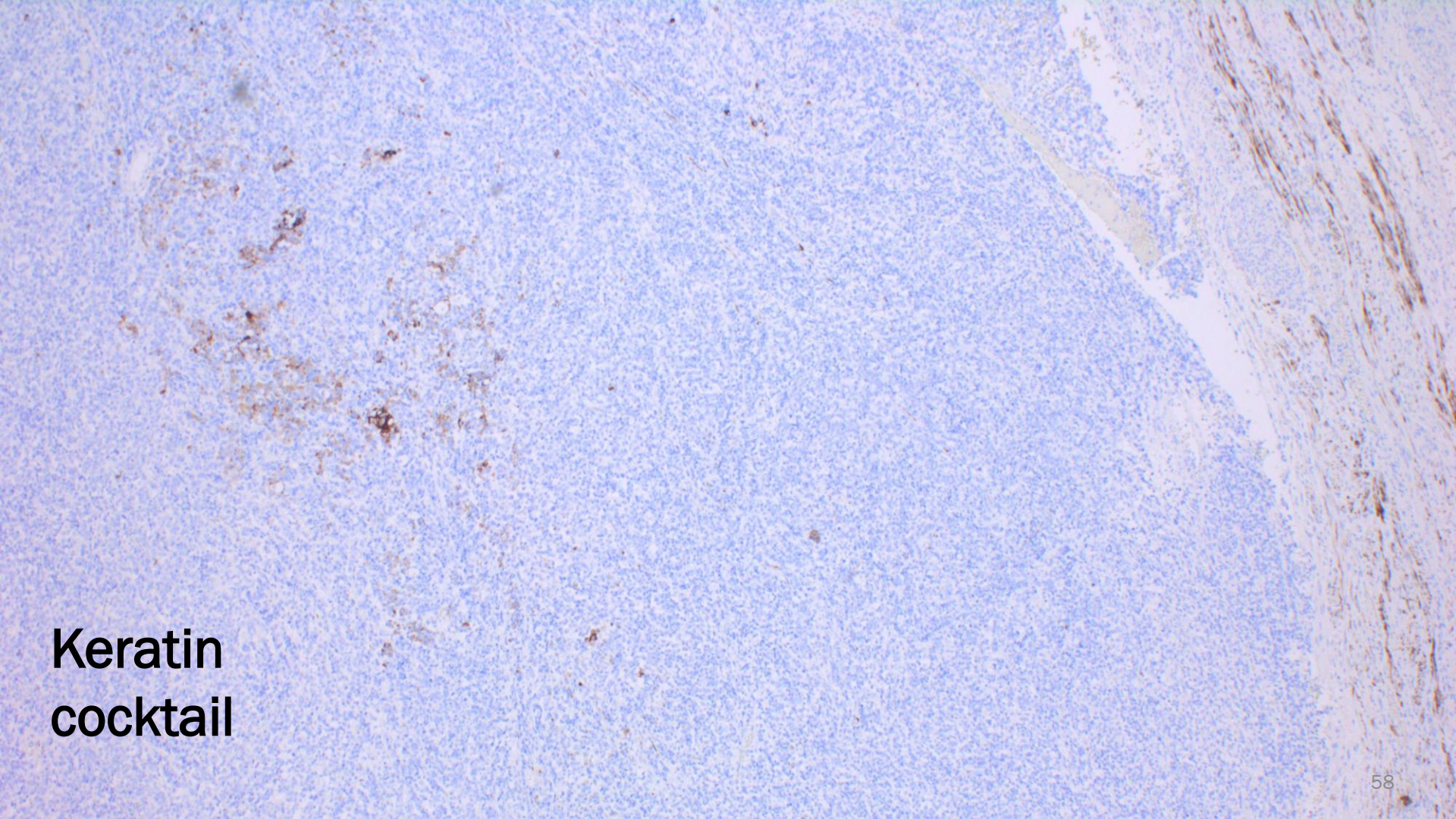


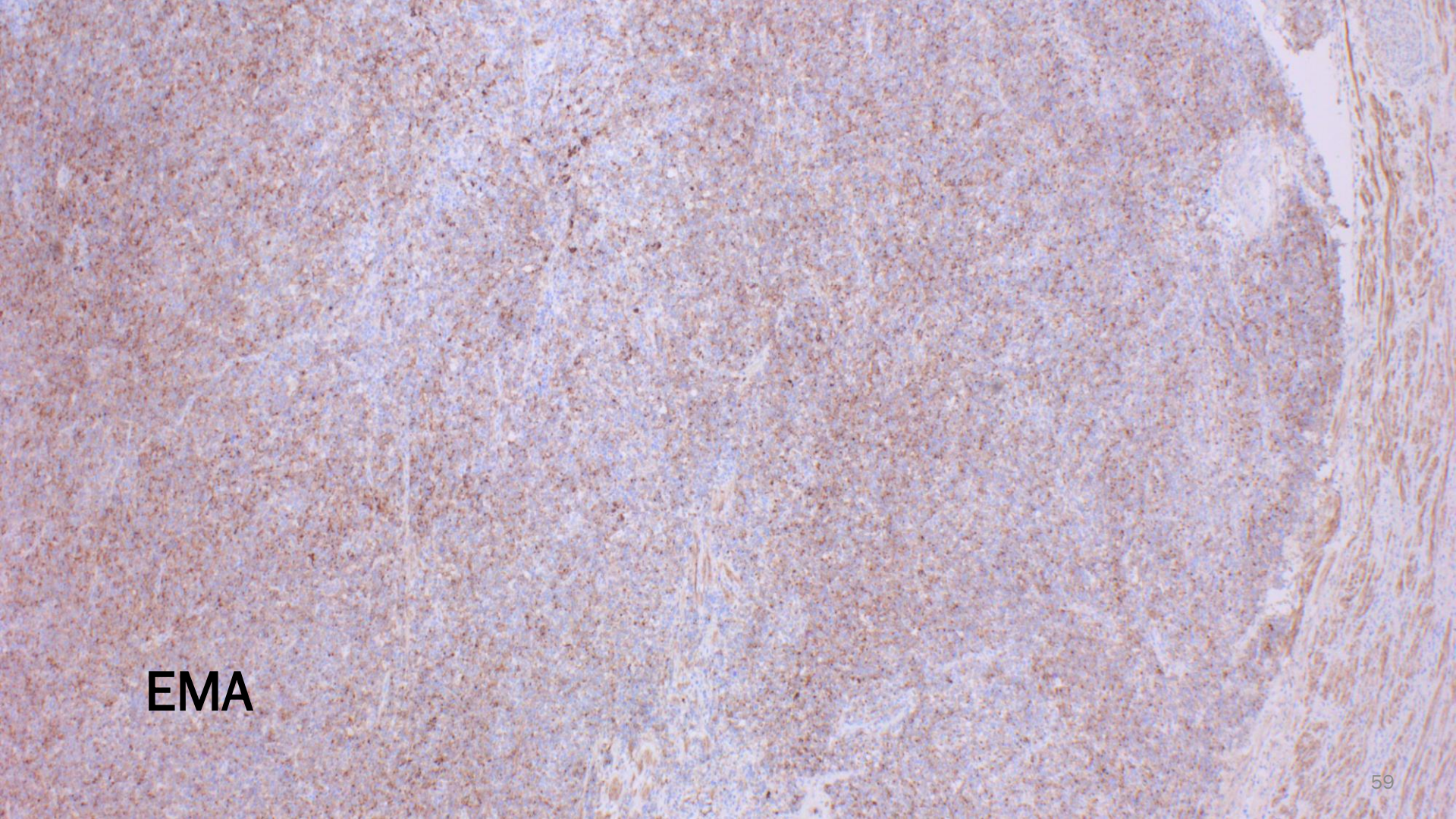









Keratin
cocktail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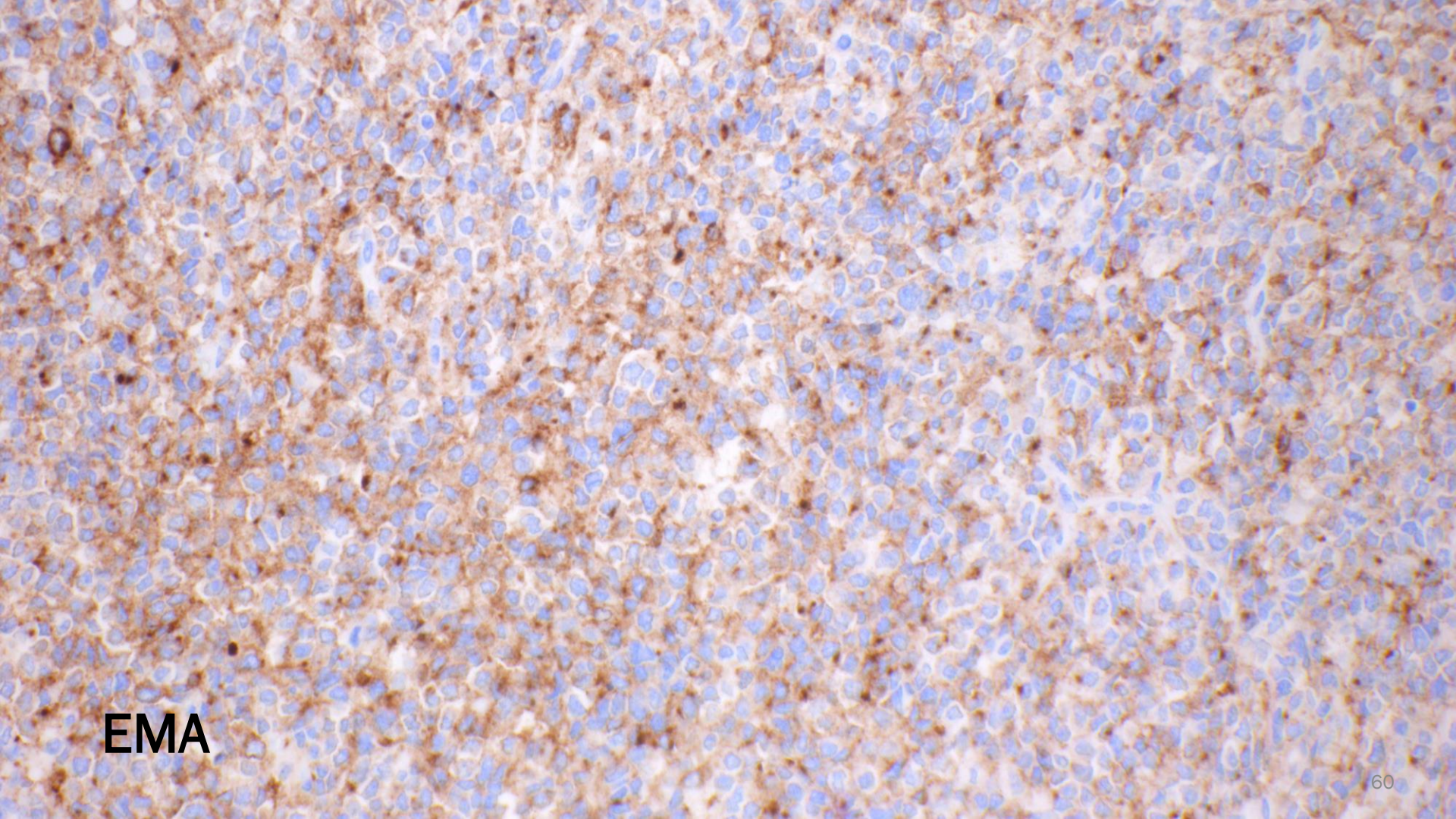
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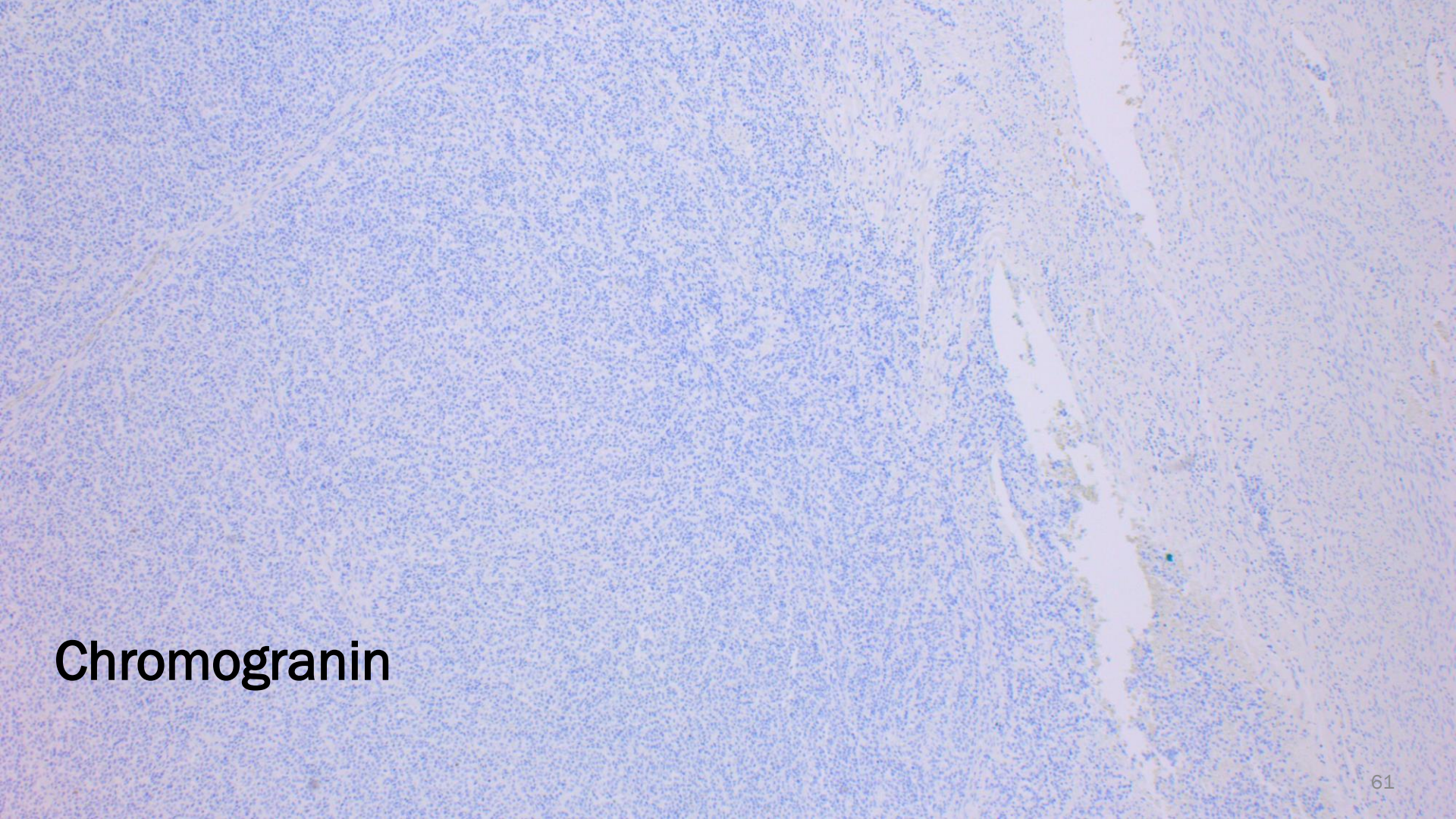
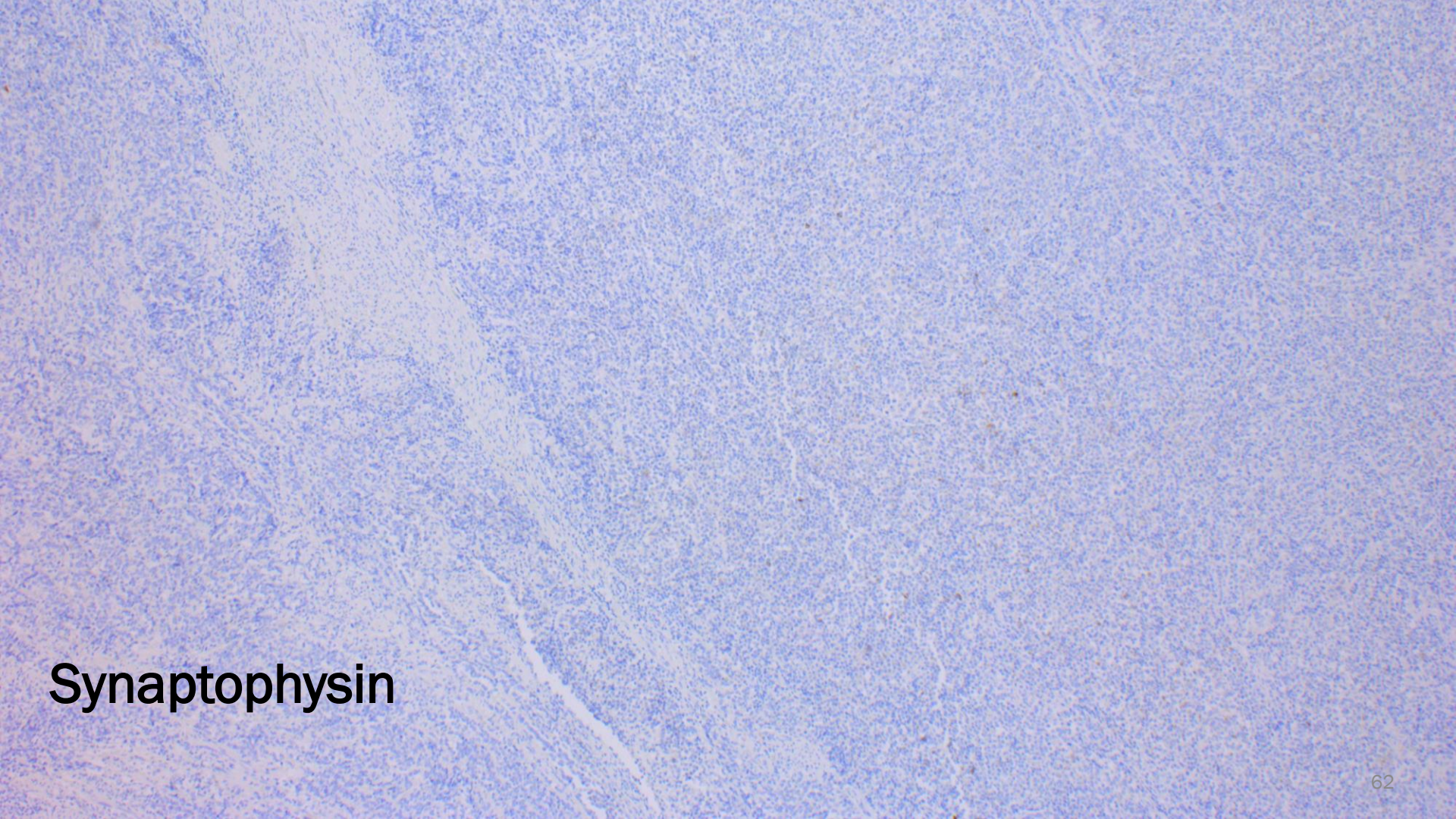
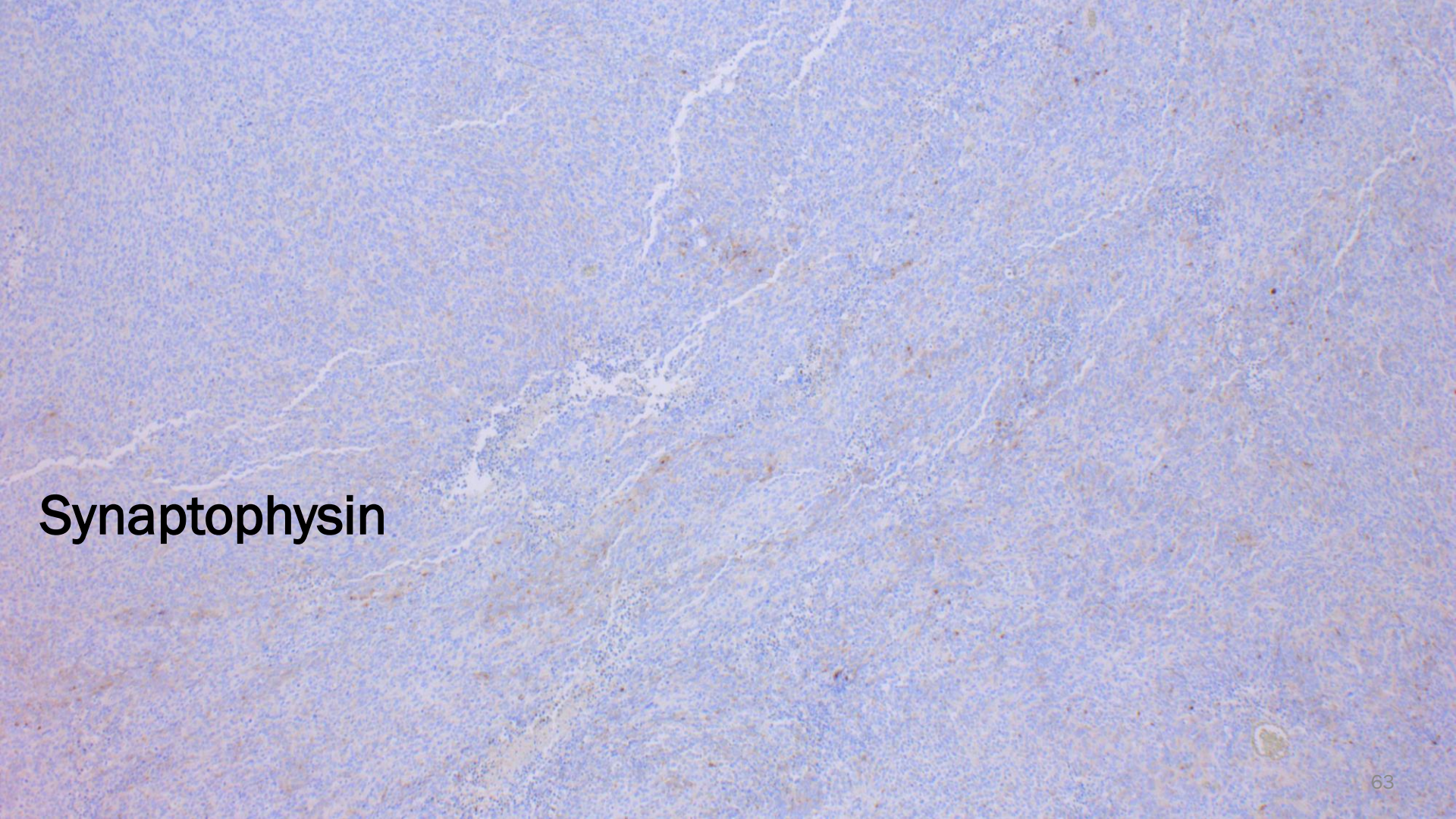
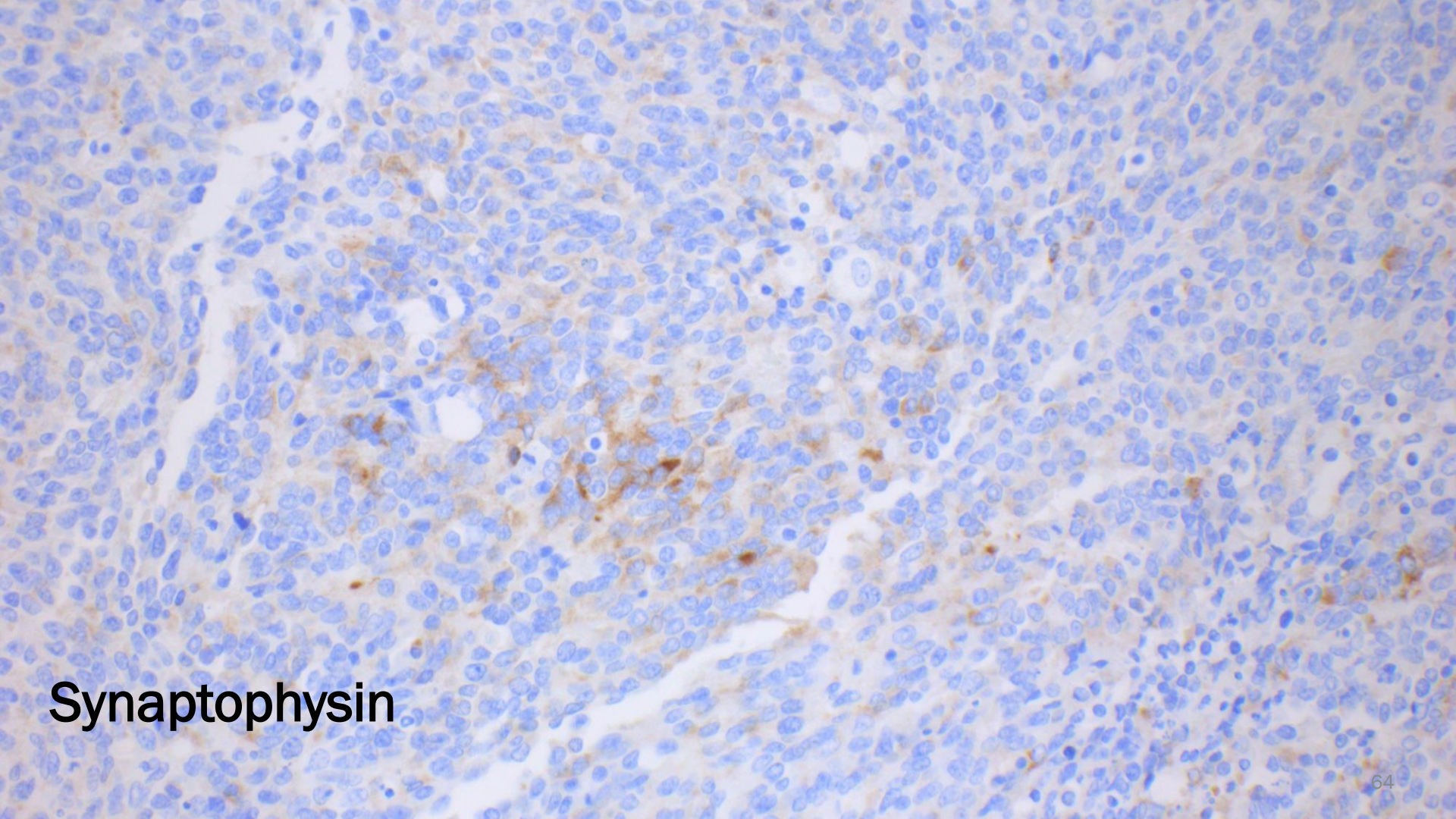


Chromogranin

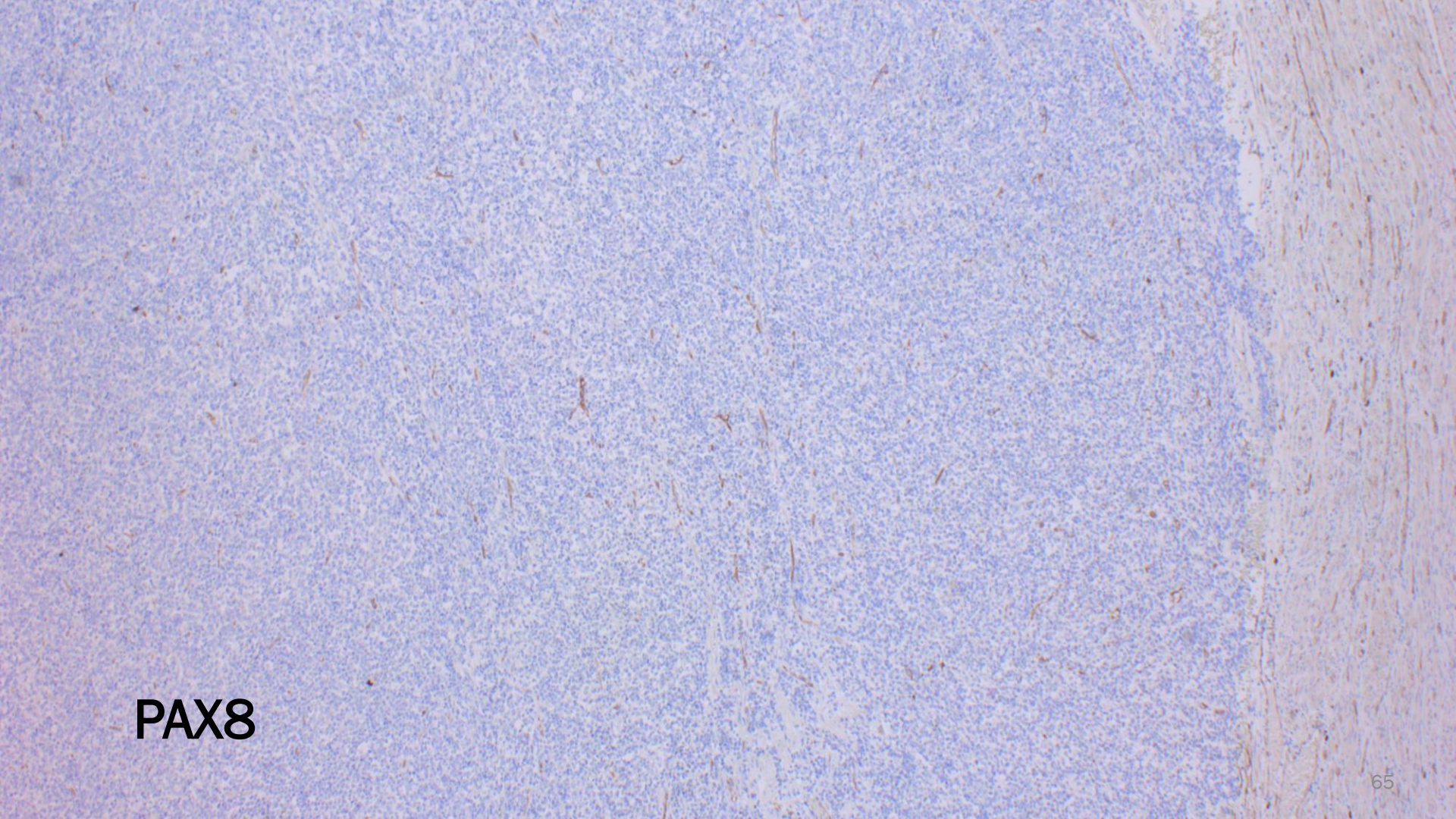


Synaptophysin


Synaptophysin



Synaptophysin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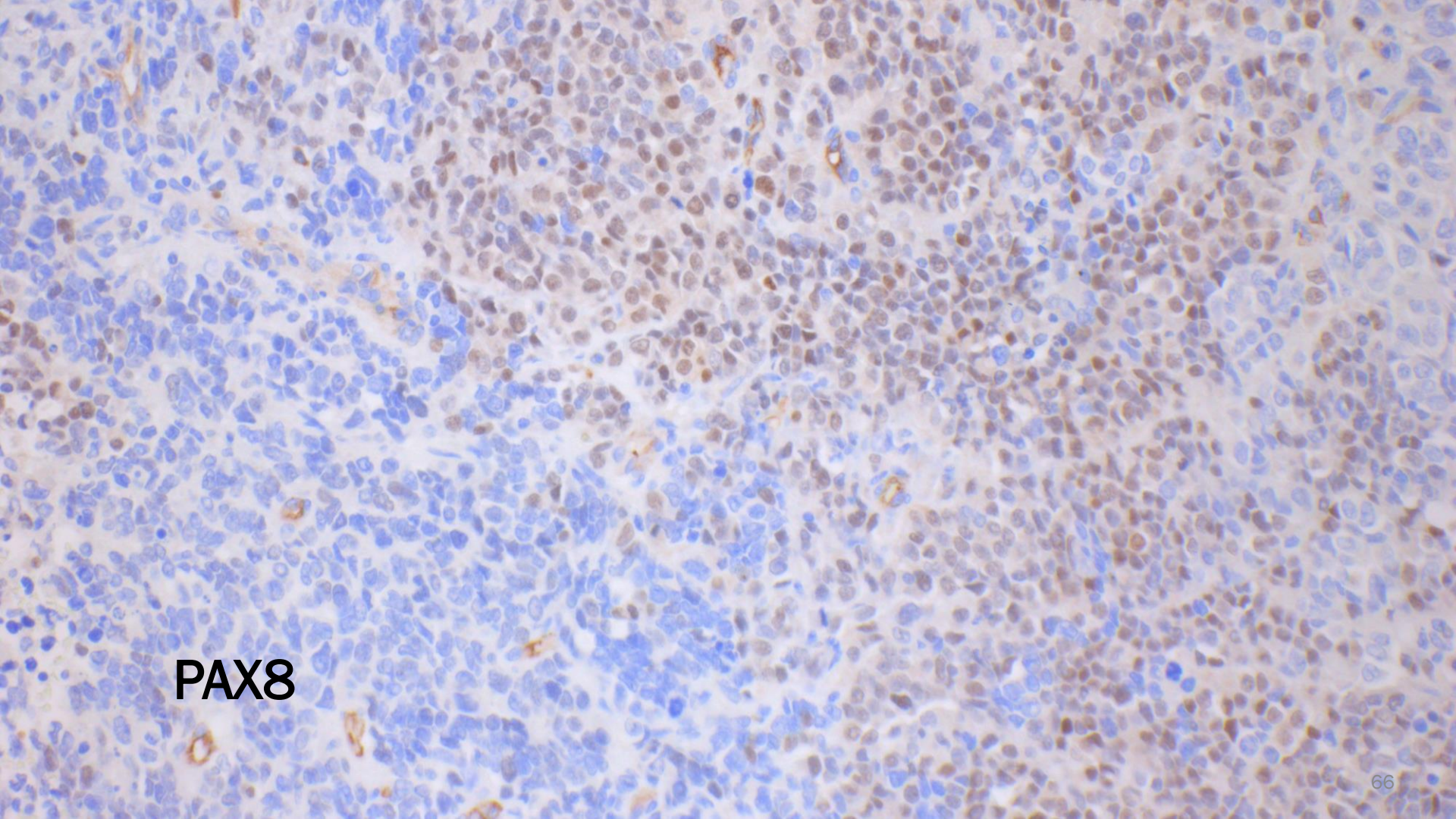
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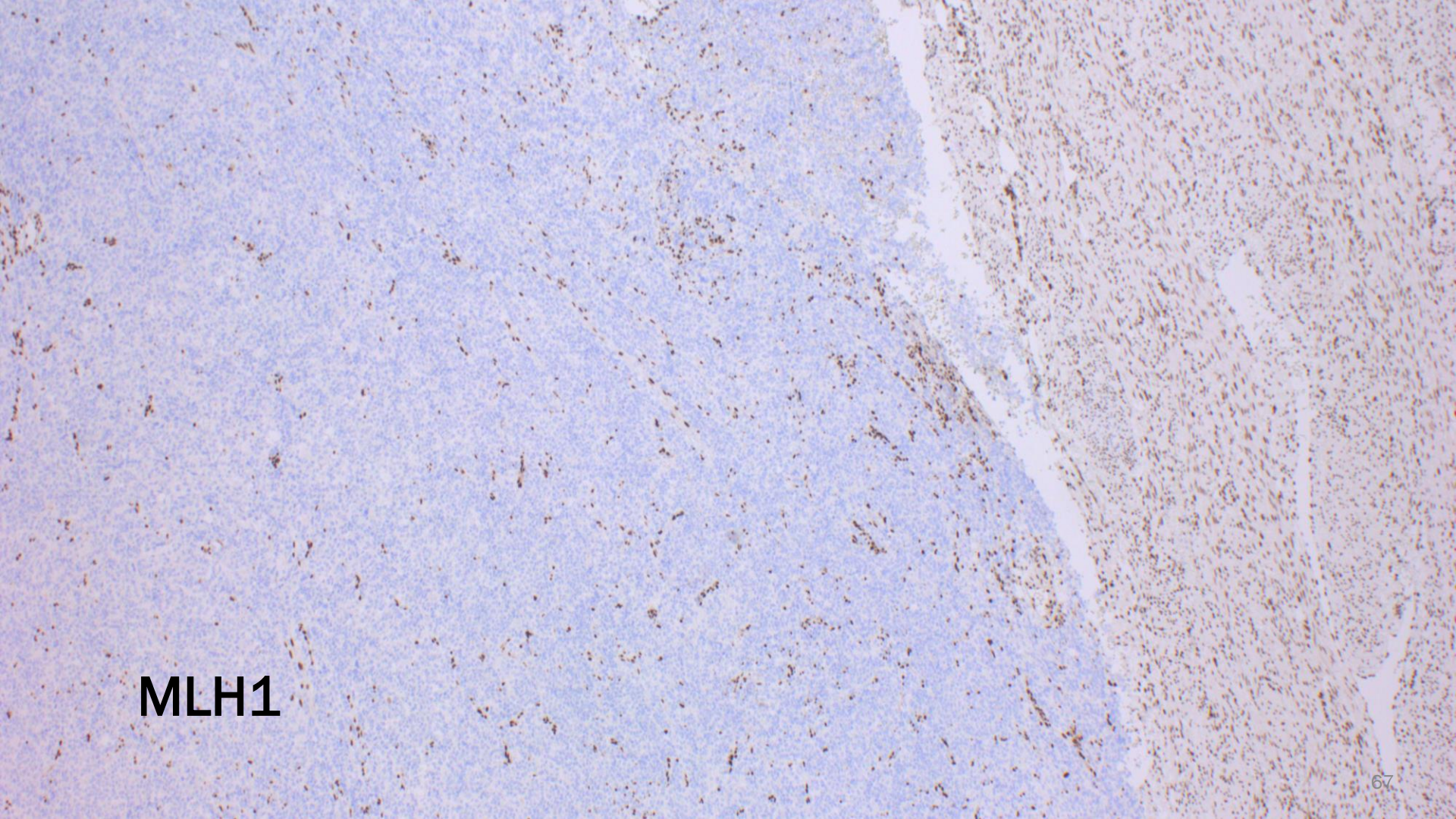
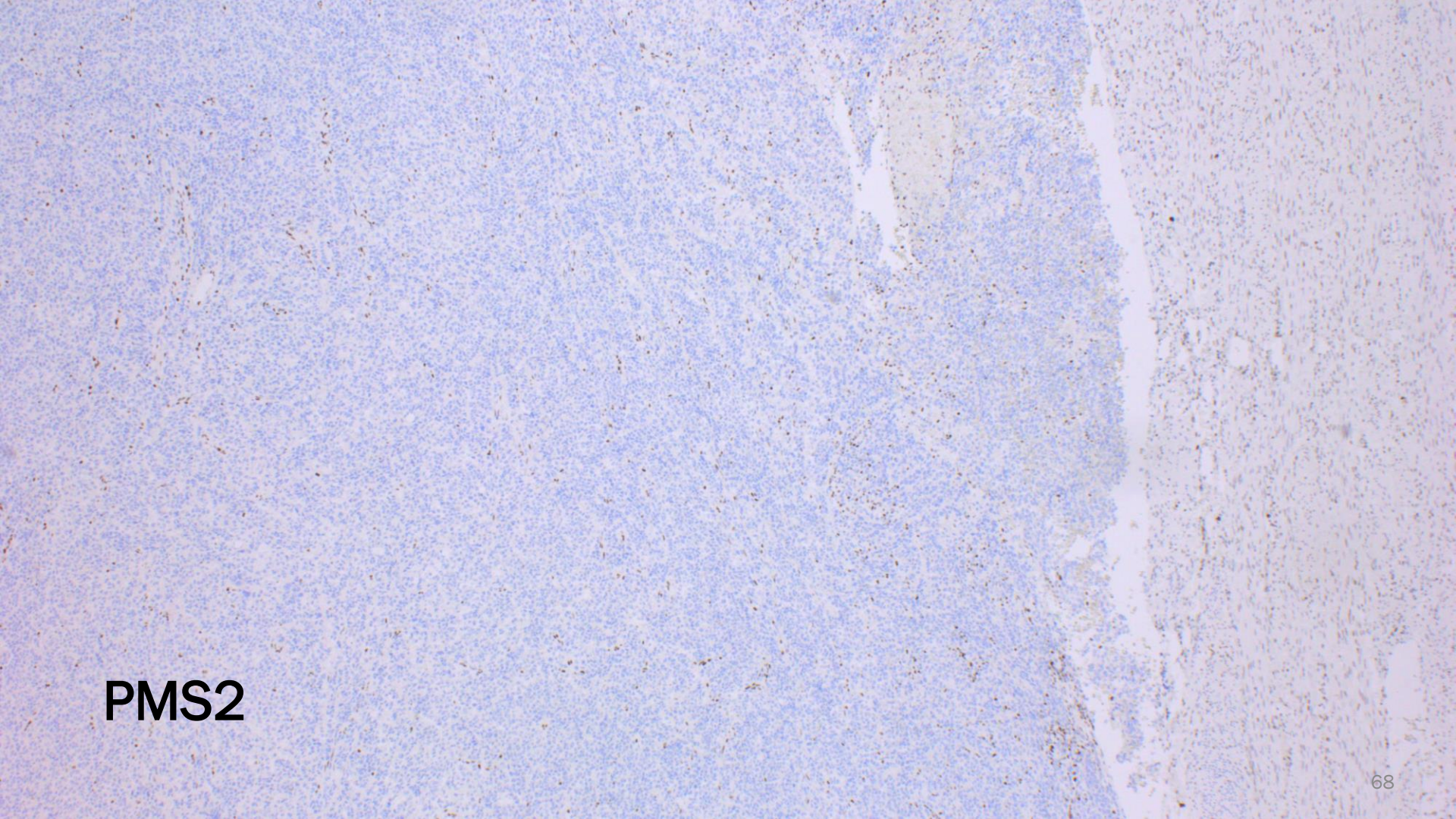
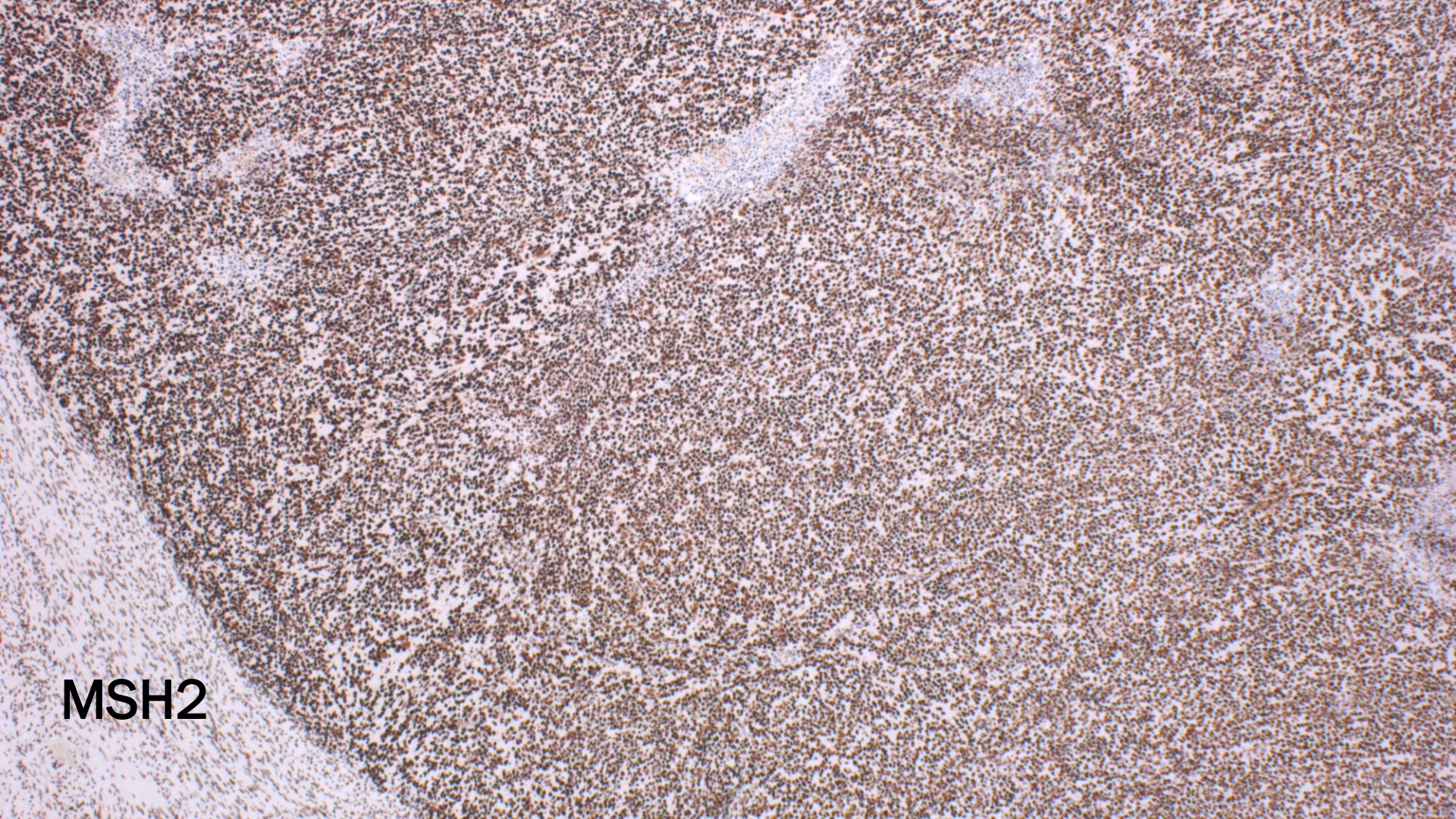

MLH1


PMS2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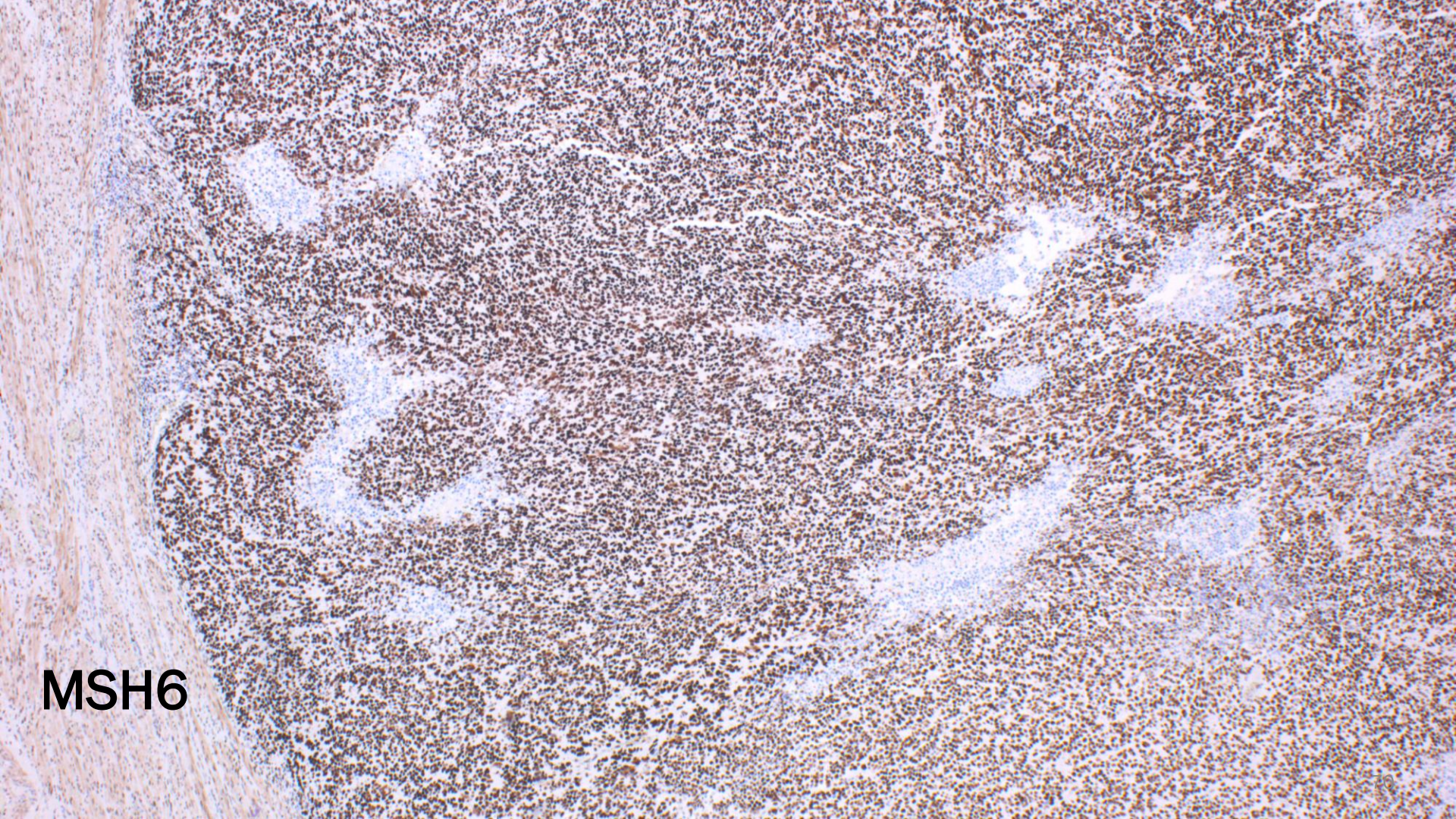
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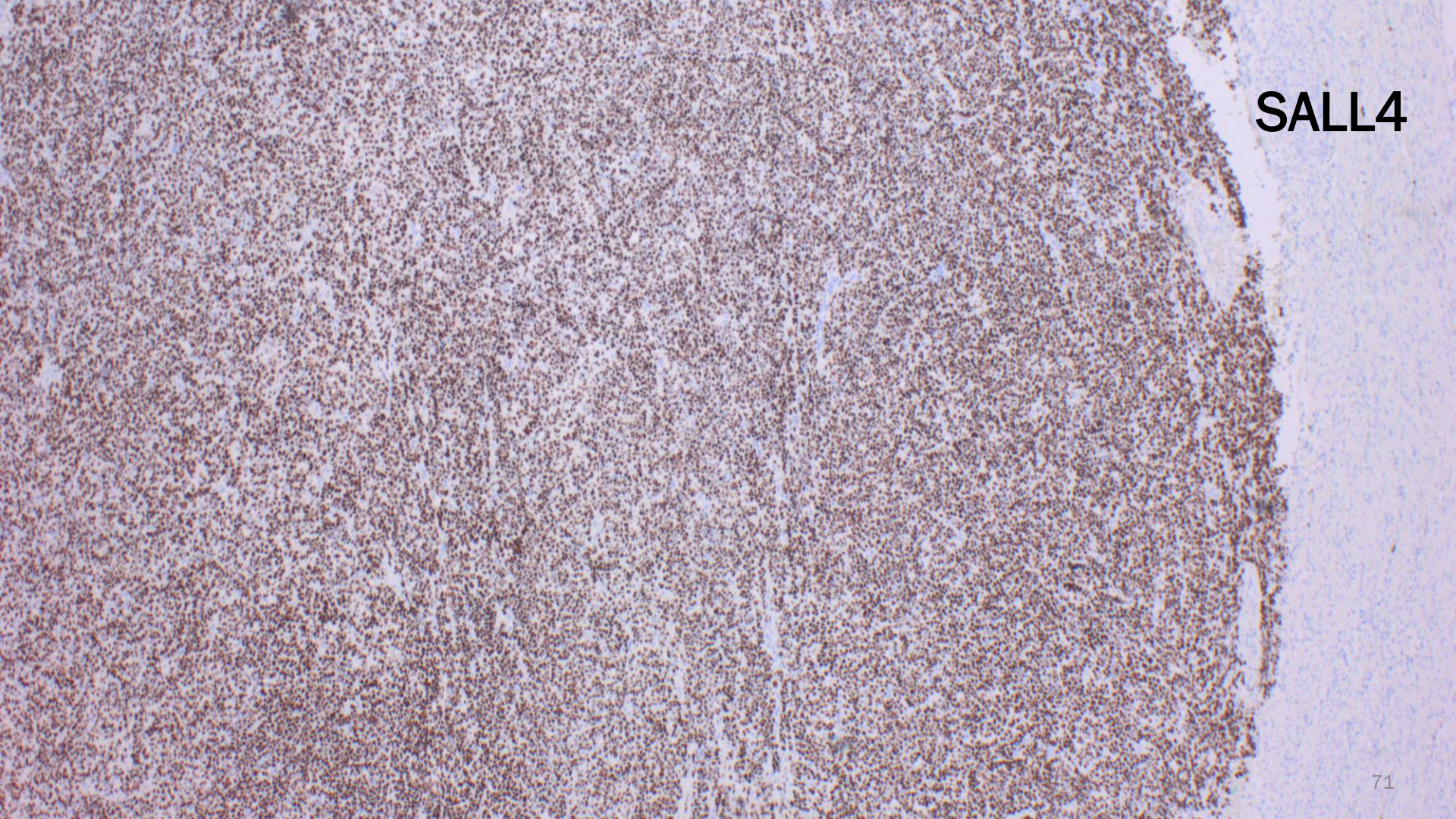
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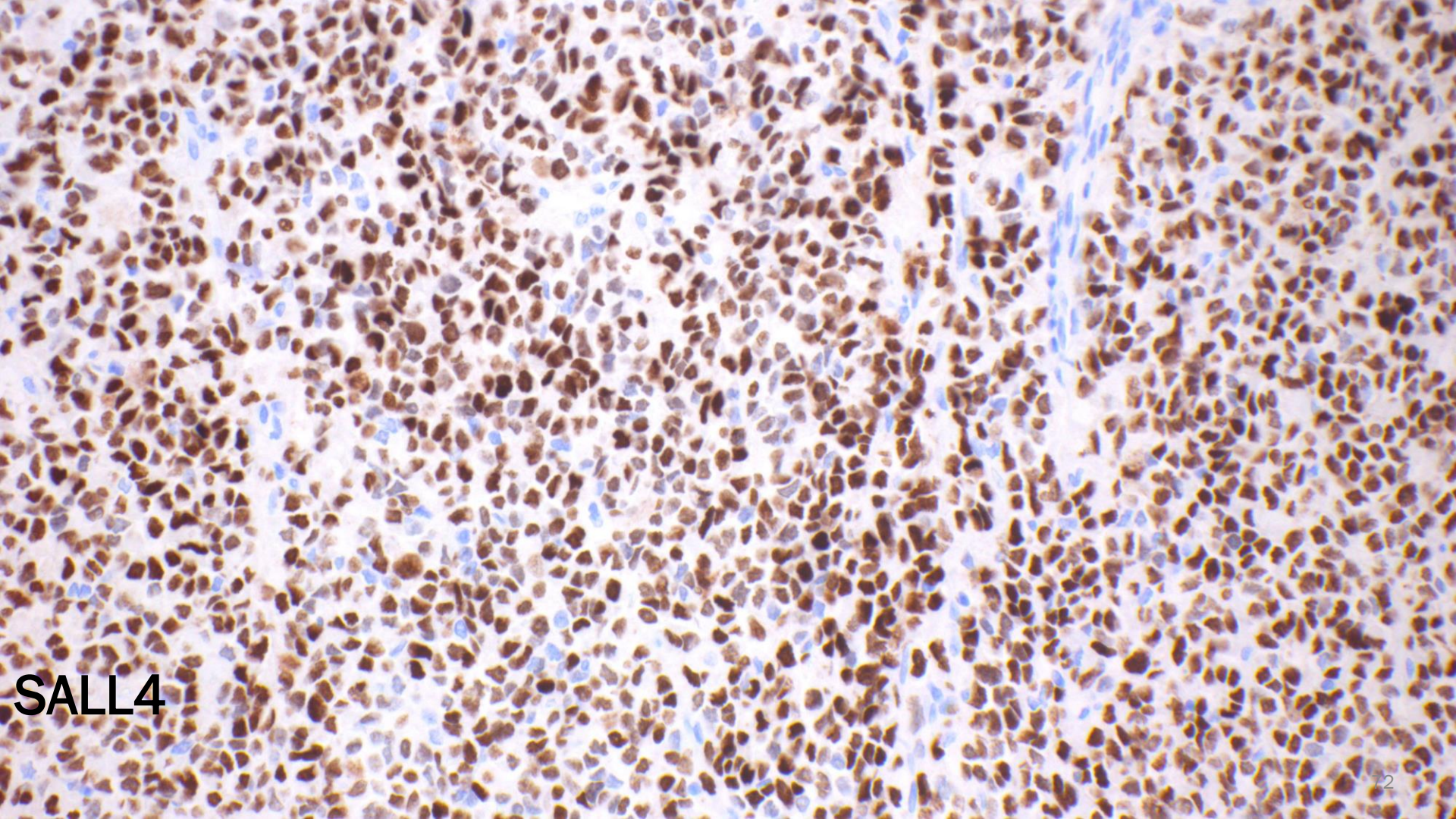
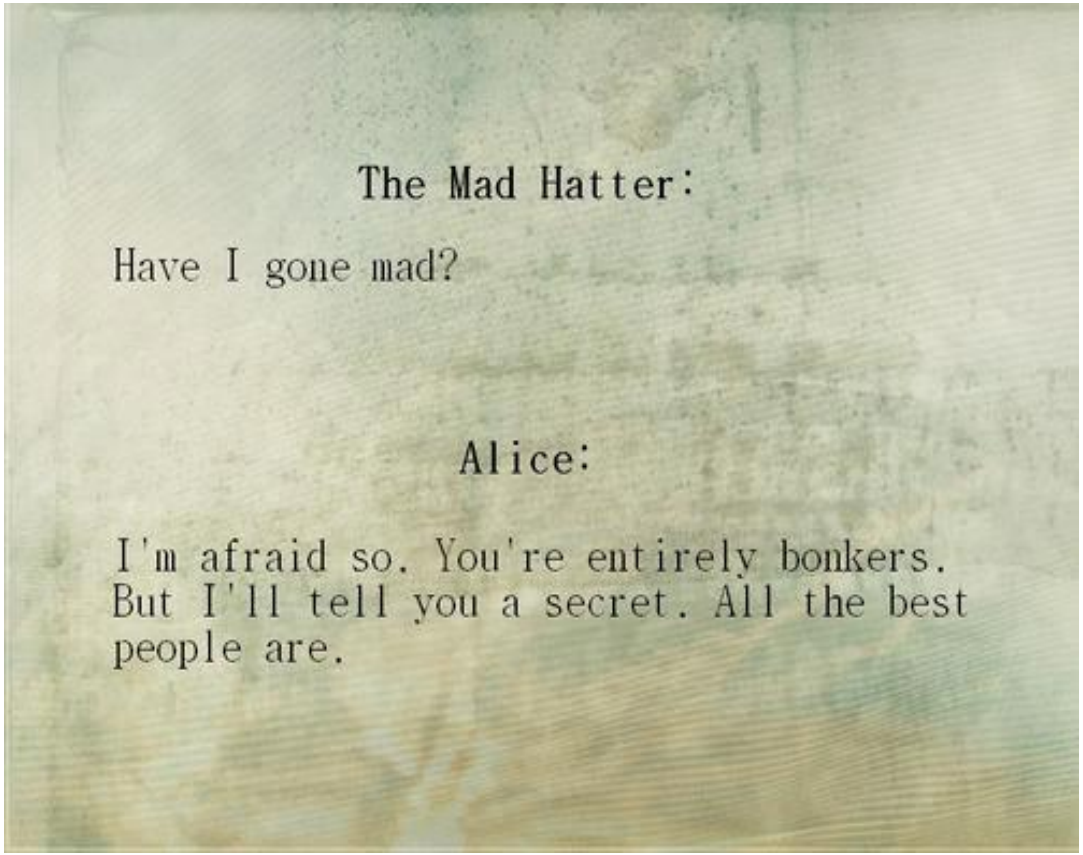

SALL4

Undifferentiated Carcinoma

SALL4 in Carcinoma?



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Undifferentiated Carcinoma of the Endometrium

Basel Altrabulsi, MD, Anais Malpica, MD,† Michael T. Deavers, MD,† Diane C. Bodurka, MD,‡ Russell Broaddus, MD, PhD,† and Elvio G. Silva, MD†*

Abstract: Undifferentiated carcinoma arising in the endometrium is considered a rare neoplasm with only a few studies published thus far. This limited number of studies is most likely a reflection of the underrecognition of this tumor because of a lack of diagnostic criteria to separate it from endometrial endometrioid adenocarcinoma, FIGO grade 3. In this study, we present the clinicopathologic features of 16 cases of endometrial undifferentiated carcinoma. In addition, we review the clinicopathologic features of 33 cases of endometrial endometrioid adenocarcinoma, FIGO grade 3, and compare them with the undifferentiated cases. The age of the 16 patients with undifferentiated carcinoma of the endometrium ranged from 40 and 69 years (mean, 59 years). Stage was known in 13 patients. Six (46%) patients presented with early stage disease (4 stage I and 2 stage II). Seven (54%) patients presented with advanced stage disease (2 stage III and 5 stage IV). Staging information was not available for 3 patients. Undifferentiated carcinoma was characterized by a proliferation of medium-sized, monotonous, epithelial cells growing in solid sheets with no specific pattern. Glands were not identified. Keratin immunostaining was focally positive in 11 of 12 cases, and EMA was focally positive in all 12 cases. The age of the 33 patients with endometrial endometrioid carcinoma, FIGO grade 3, ranged from 40 to 90 years (mean, 68 years). Twenty-three (70%) patients presented with early stage disease (21 stage I and 2 stage II), and 10 (30%) patients presented with advanced stage disease (8 stage III and 2 stage IV). Focal glandular differentiation was seen in all cases. The solid component was different from the one seen in the undifferentiated carcinomas because well demarcated trabeculae, cords, or groups of cells were identified in all cases. The tumor cells in the solid areas resembled the cells in the glandular component of the tumor. Immunoperoxidase studies for keratin and EMA were positive in 23 of 23 cases. Twelve of the 16 (75%) patients with undifferentiated carcinoma died of disease; 10 (62.5%) of them within 5 years after diagnosis. In contrast, 13 of 33 (39.4%) patients with endometrial endometrioid carcinoma, FIGO grade 3, died of disease. Twelve (36.4%) died within 5 years after diagnosis. In summary, undifferentiated carcinoma of the endometrium appears to be more aggressive than endometrial endometrioid adenocarcinoma, FIGO grade 3. Its proper recognition is important for prognosis and potentially for therapy.

Key Words: endometrium, undifferentiated carcinoma, endometrioid carcinoma, endometrial carcinoma, carcinoma

(*Am J Surg Pathol* 2005;29:1316–1321)

Undifferentiated carcinoma of the endometrium is a poorly recognized neoplasm because of the fact that the histologic features that separate this neoplasm from high-grade endometrioid adenocarcinoma have not been clearly defined. The World Health Organization defines undifferentiated carcinoma of the endometrium as a "malignant tumor of epithelial structure that is too poorly differentiated to be placed in any other category of carcinomas."[16] However, this neoplasm has also been included together with grade 3 endometrioid carcinoma since 1971 when the general assembly of FIGO defined grade 3 adenocarcinoma as a neoplasm "predominately solid or entirely undifferentiated carcinoma."[5] Subsequent revisions defined grade 3 carcinoma as a neoplasm with more than a 50% nonsquamous or nonmorular solid growth pattern, which implies that an entirely solid carcinoma is a grade 3 endometrioid adenocarcinoma.[4] This overlaps with the definition of undifferentiated carcinoma. In addition, it is not known whether it is clinically significant to separate grade 3 endometrioid endometrial adenocarcinoma from undifferentiated carcinoma. For several years, we have observed in our clinical practice that undifferentiated carcinomas of the endometrium are extremely aggressive neoplasms. We have also noted that the available literature contains significant discrepancies reporting the survival of women with grade 3 endometrial adenocarcinomas. Some studies reported a 5-year survival of 40%, whereas other studies found the 5-year survival to be 70%.[10,14,19] We think that this discrepancy is most probably the result of inclusion of grade 3 endometrioid adenocarcinoma together with undifferentiated carcinoma in some studies.

In this study, we present the clinicopathologic features of 16 cases of undifferentiated carcinomas of the endometrium. We also present the clinicopathologic features of 33 cases of endometrial endometrioid adenocarcinoma, FIGO grade 3, for comparison. So far, this is the first published study in which this comparison has been made, thus reflecting the importance

Undifferentiated Carcinoma

- High grade carcinoma with no morphologic evidence of differentiation
- ~ 2% of endometrial cancers
- Median age 55 years
- 40 – 60% present with extrauterine disease

Undifferentiated Carcinoma

Monomorphic

- Discohesive, generally uniform, medium cells arranged in sheets
- Frequent mitoses, geographic necrosis
- Rhabdoid morphology, myxoid background, tumor infiltrating lymphocytes
- DDx: Sarcoma (e.g. HG ESS), Lymphoma, Neuroendocrine Carcinoma

Undifferentiated Carcinoma

Monomorphic

- In many cases (~40%) admixed with a differentiated component: hyperplasia, endometrioid adenocarcinoma

Undifferentiated Carcinoma

Molecular

- MSI High
- TP53 mutation
- POLE mutation
- PI3K mutation
- SWI/SNF mutation (loss of SMARCA4/BRG1; SMARCB1/INI1; ARID1A, ARID1B)

Undifferentiated Carcinoma

Immunohistochemistry

- Diffuse, strong keratin staining is not present
- E-cadherin often lost
- EMA, CK8/18 more often positive (scattered)
- PAX8, ER, PR are usually negative (more frequently present in p53 mutated tumors)
- Chromogranin, synaptophysin should be negative or only focal (<10%)

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Undifferentiated Carcinoma

Immunohistochemistry

- MMR loss (usually MLH1, PMS2) 50 – 75%
  - Rare in sarcomas (~1%)
- SALL4 (36%; ? More frequent)
- Vimentin

SALL4

- Regulator of embryonic stem cell pleuripotency
- Marker of Germ Cell Tumors (Dysgerminoma, Yolk Sac Tumor, Embryonal Carcinoma, Choriocarcinoma)
- Rhabdoid Tumors, Wilms Tumor
- Serous Carcinoma (~30%)

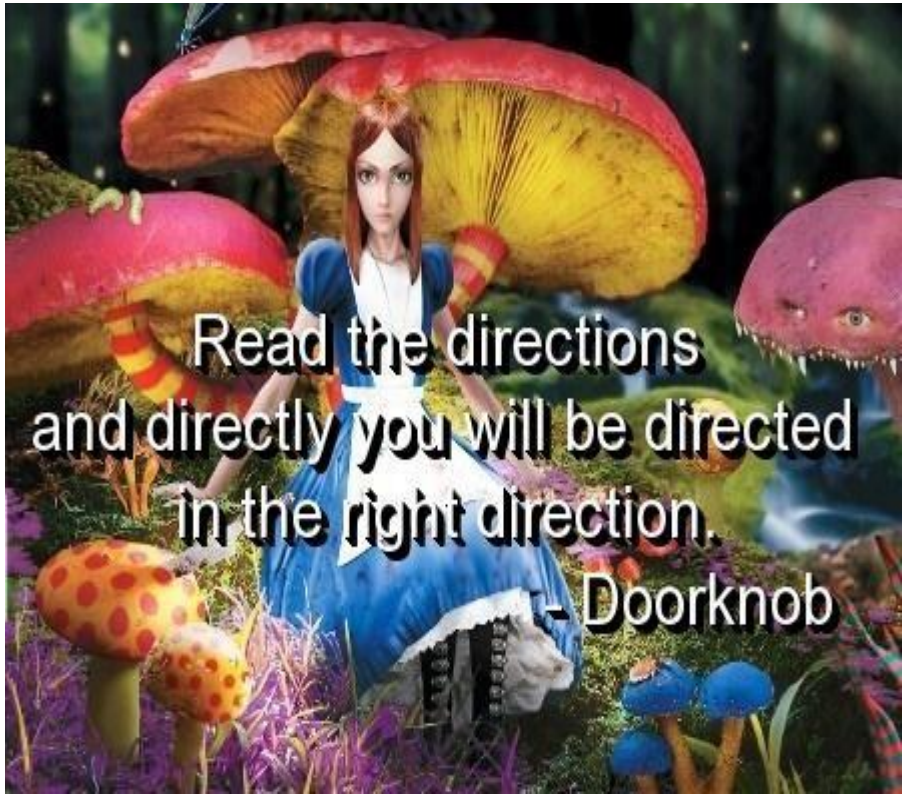
SALL4

- Marker of stem cell-like dedifferentiation in non germ cell tumors
  - Undifferentiated Carcinoma

Cyclin D1

- Regulator of cell cycle progression
- Dysregulation (e.g. mutation) leads to increased proliferation, tumorigenesis
- Overexpressed in up to 50% of Breast Cancer
- Overexpressed in 36% of Grade 3 Endometrioid Adenocarcinomas
- Mantle Cell Lymphoma

Be aware of the spectrum of staining possibilities, especially if the results don't fit DDx



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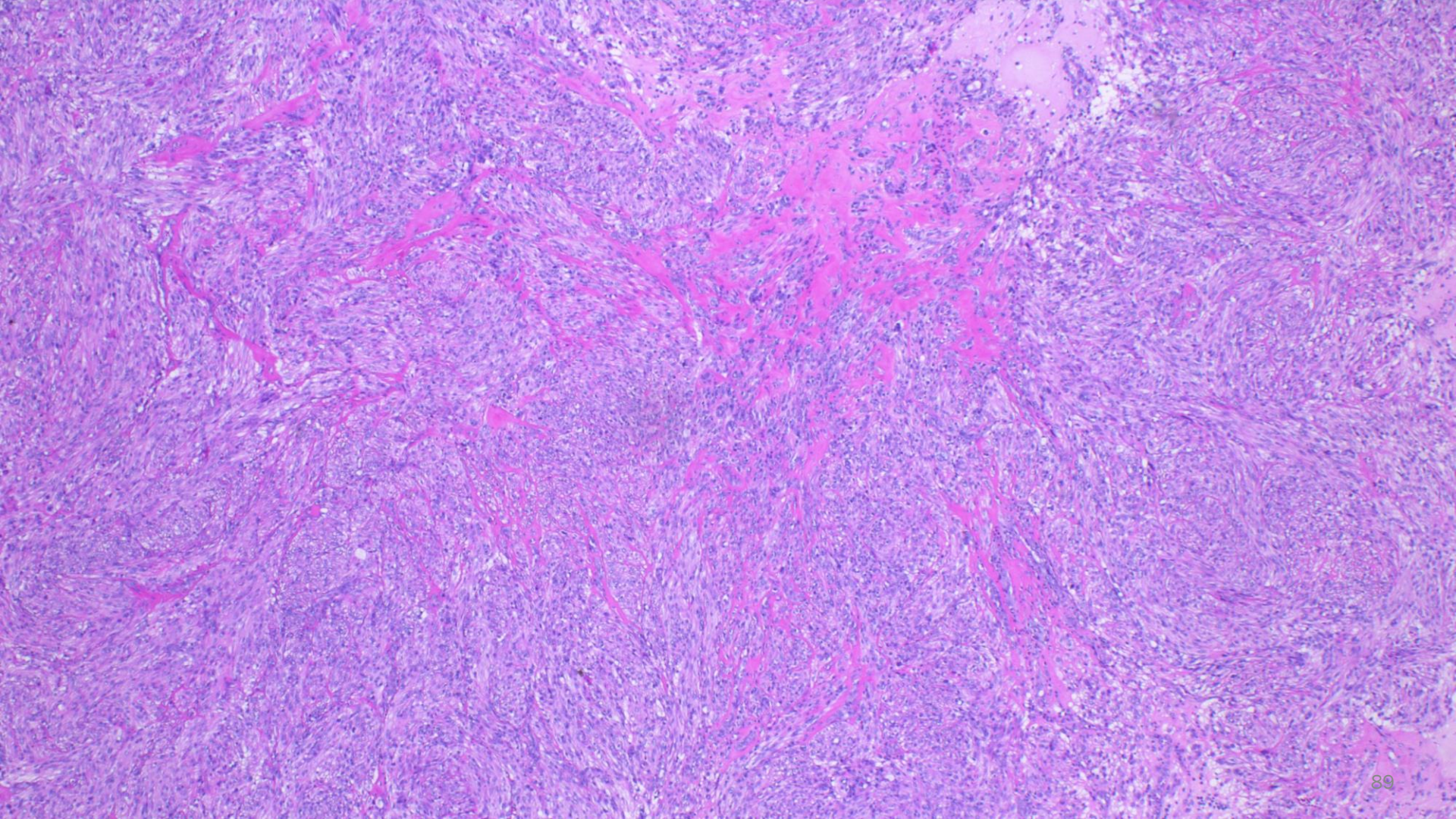
Case 3

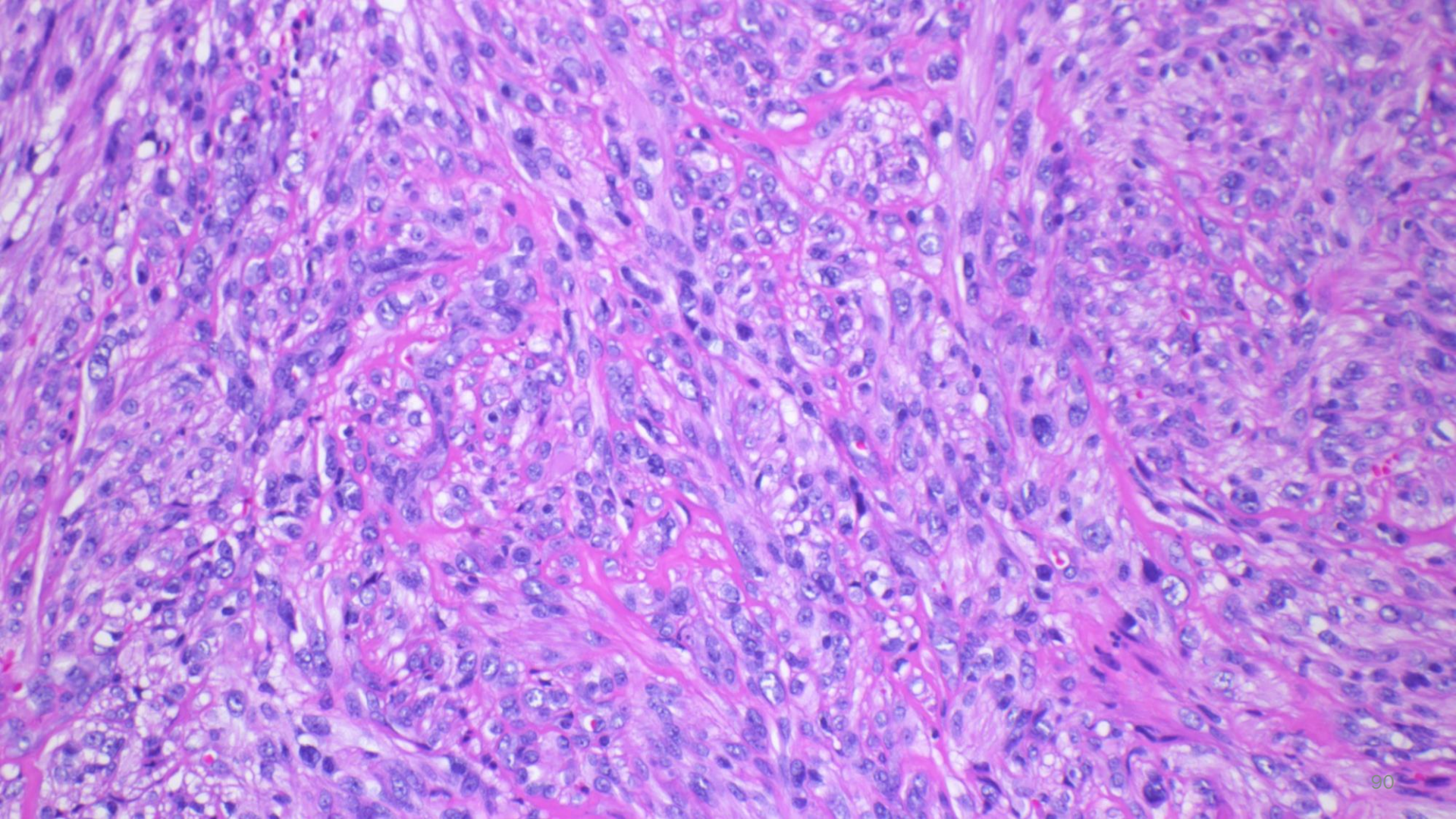
- 48 y.o. with pelvic mass, fibroid uterus, ventral hernia
- Fibroids removed in 2011
- Hysterectomy
  - Op Note: Masses arising from fundus and nodules in the cul-de-sac and omentum
  - Case sent for consultation with resultant diagnosis of Leiomyosarcoma

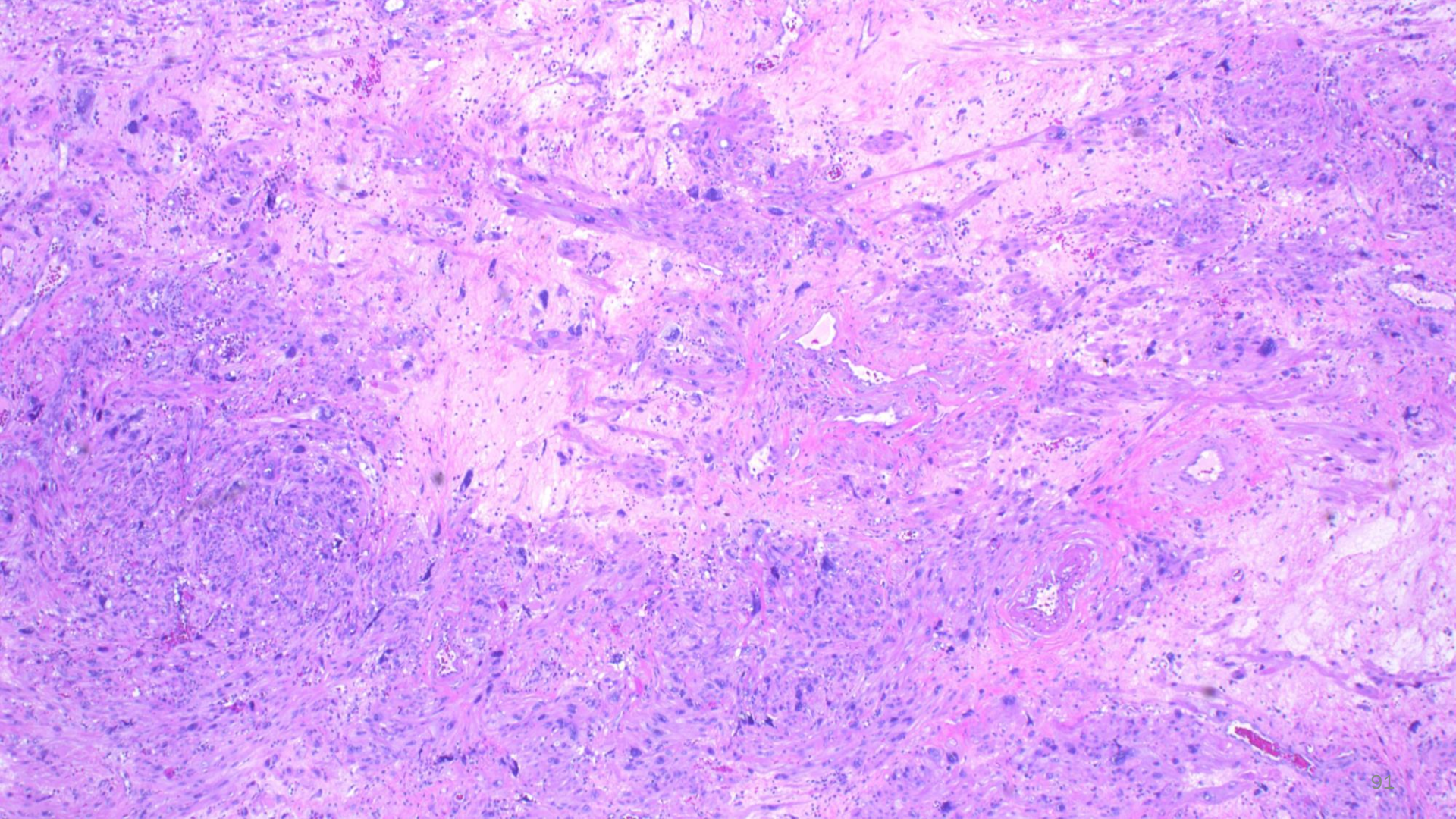
- Consultation: “However, pathologic classification of the primary “uterine mass” is challenging as the tumor fragments were received separate and/or detached from the main hysterectomy specimen. It is therefore difficult to determine with certainty if gynecologic or soft tissue tumor criteria should be used for classification. If the tumor is truly arising in the myometrium, then stricter gyn criteria should be used – in which case the neoplasm would lack the tumor cell necrosis or mitotic activity of greater than 10 mitotic figures per 10 hpf to reach a diagnosis of LMS. However, smooth muscle tumors of deep soft tissue would be classified as LMS based on the atypical cytologic features alone.”

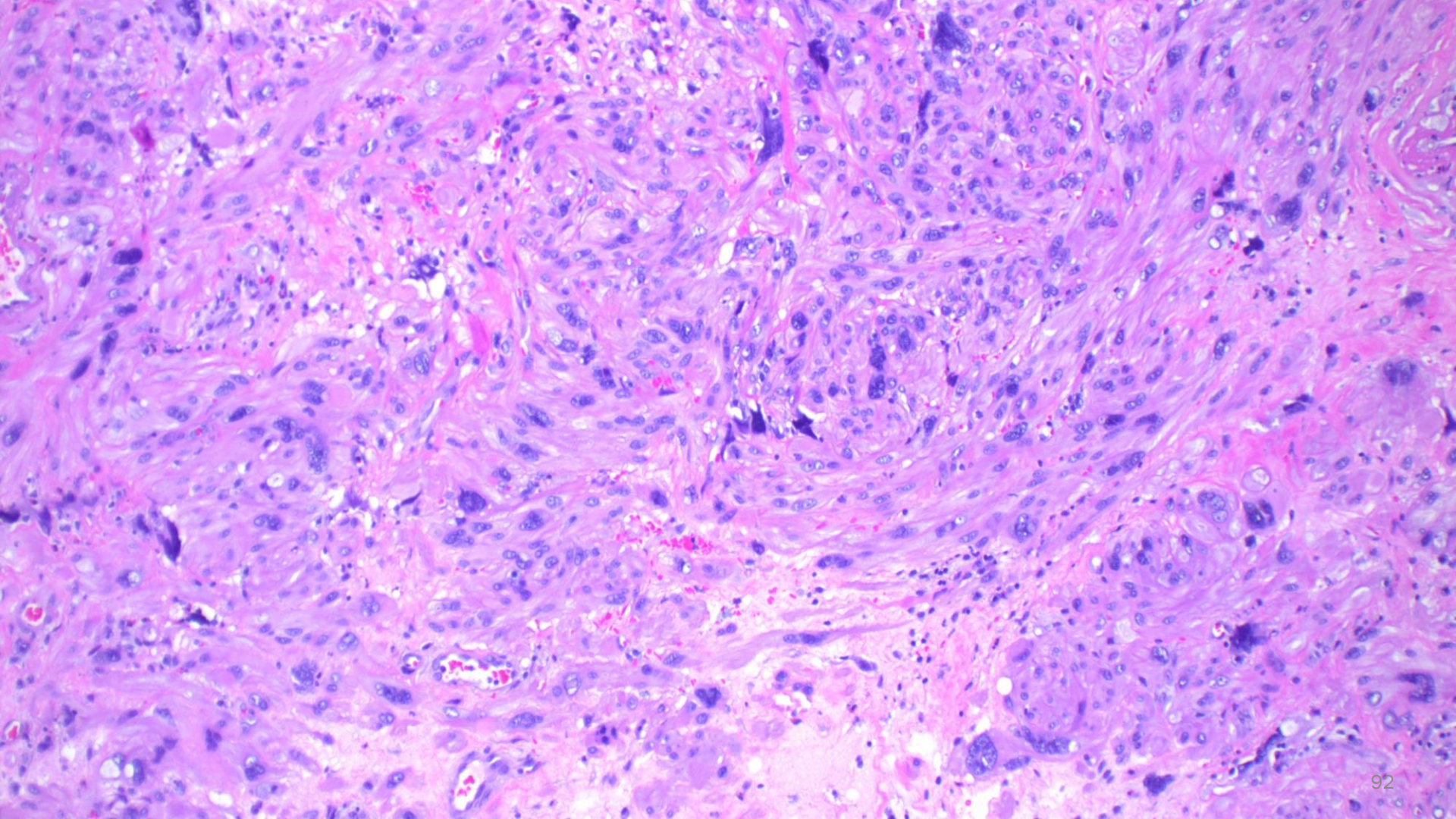
Leiomyosarcoma Diagnosis

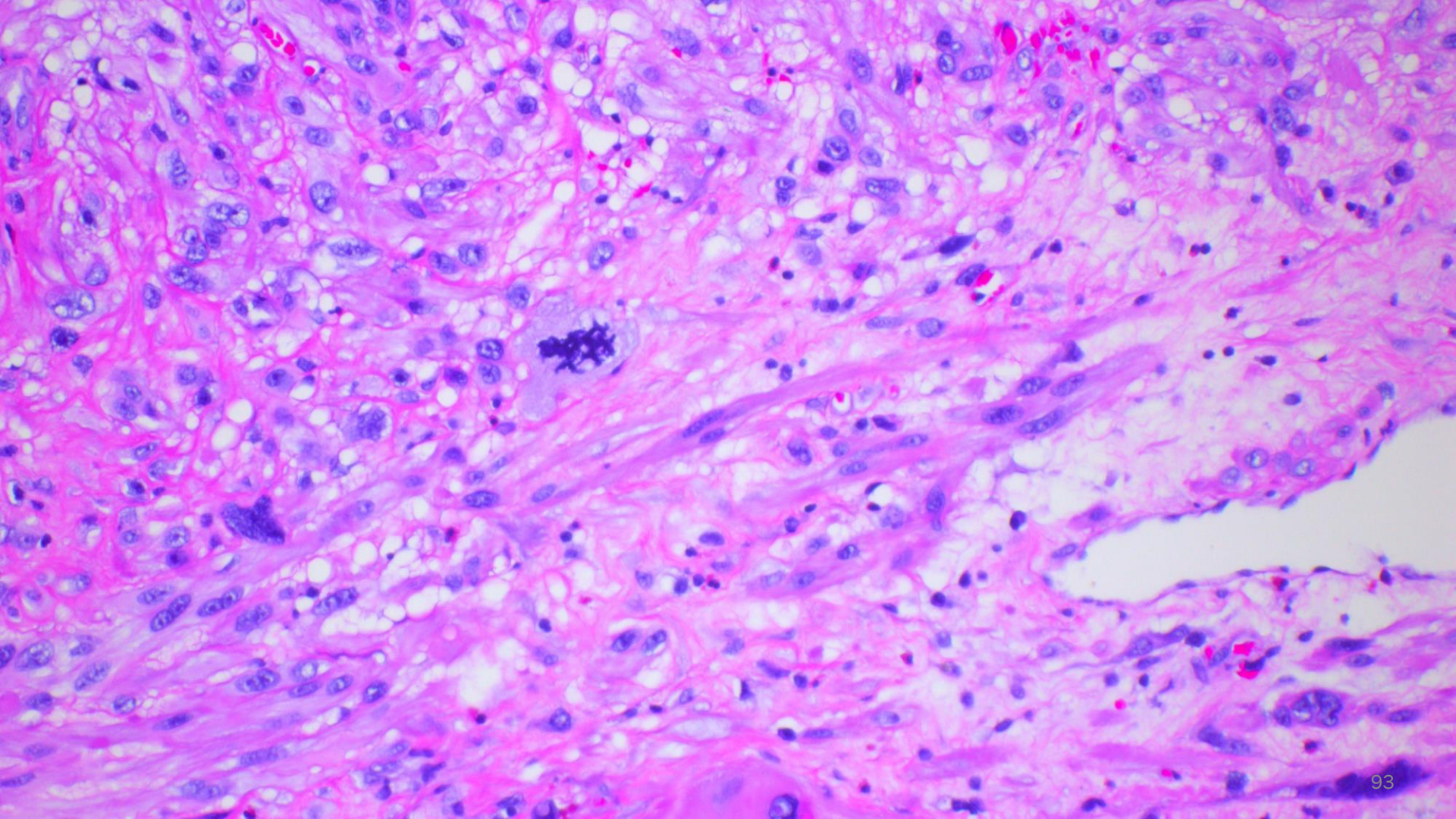
- Soft tissue: in general, smooth muscle tumors with nuclear atypia and any mitotic activity
- Gynecologic: SW Bell, RL Kempson, MR Hendrickson. Problematic Uterine Smooth Muscle Neoplasms: A Clinicopathologic Study of 213 Cases. AJSP 18(6): 535-558, 1994.
  - Diffuse significant atypia, ≥ 10 mf/10 hpf, coagulative tumor cell necrosis

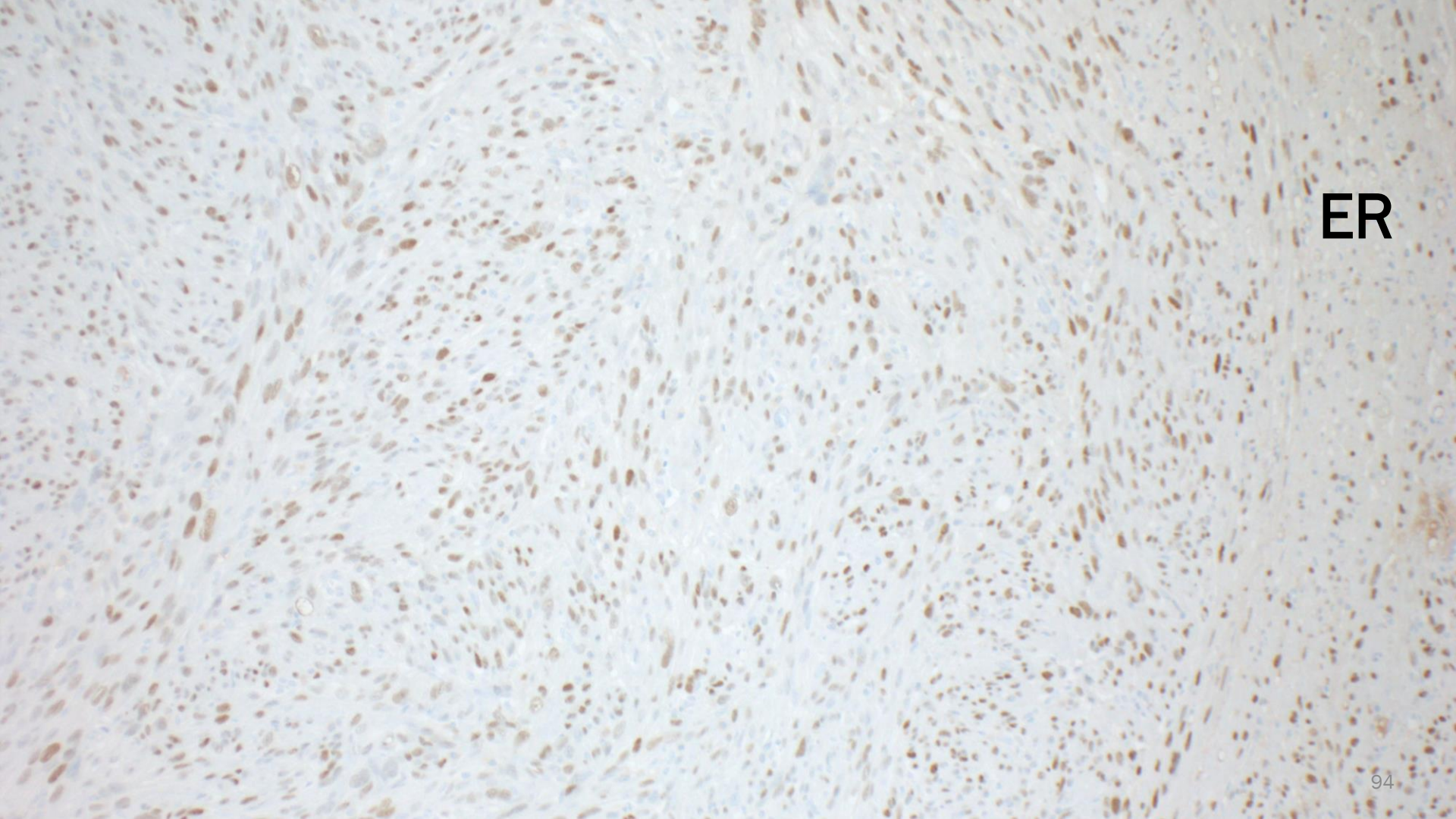








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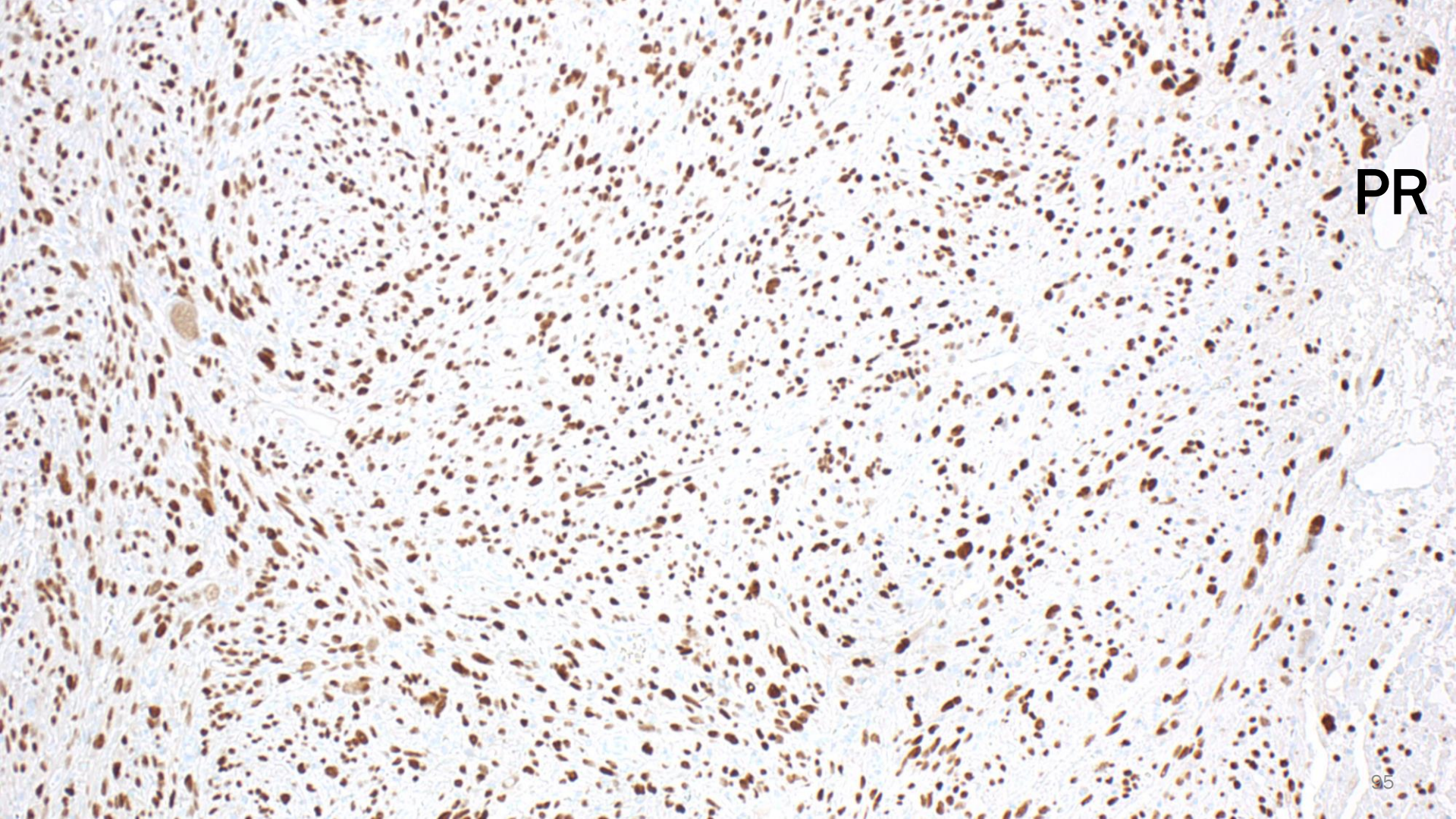
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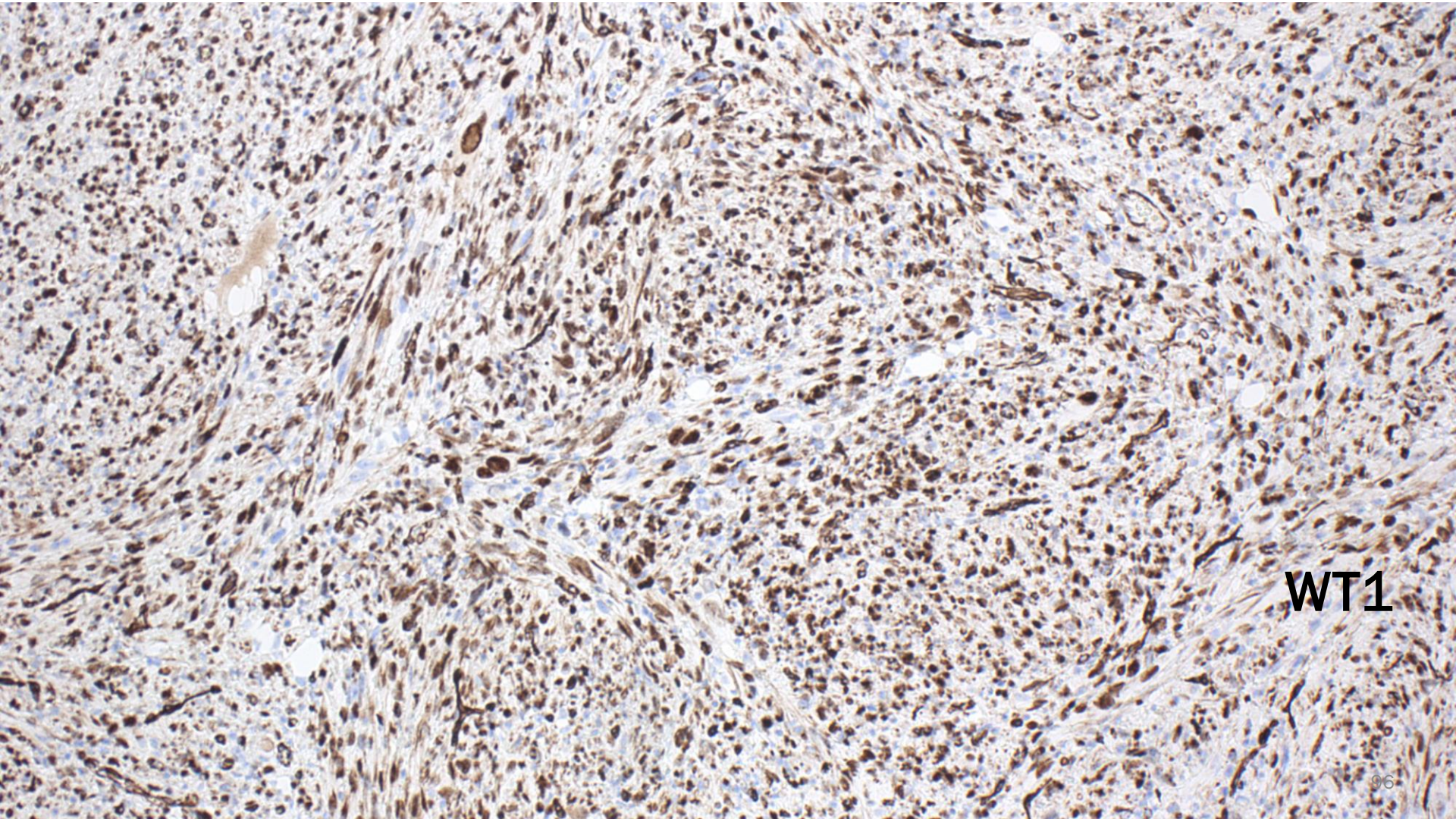
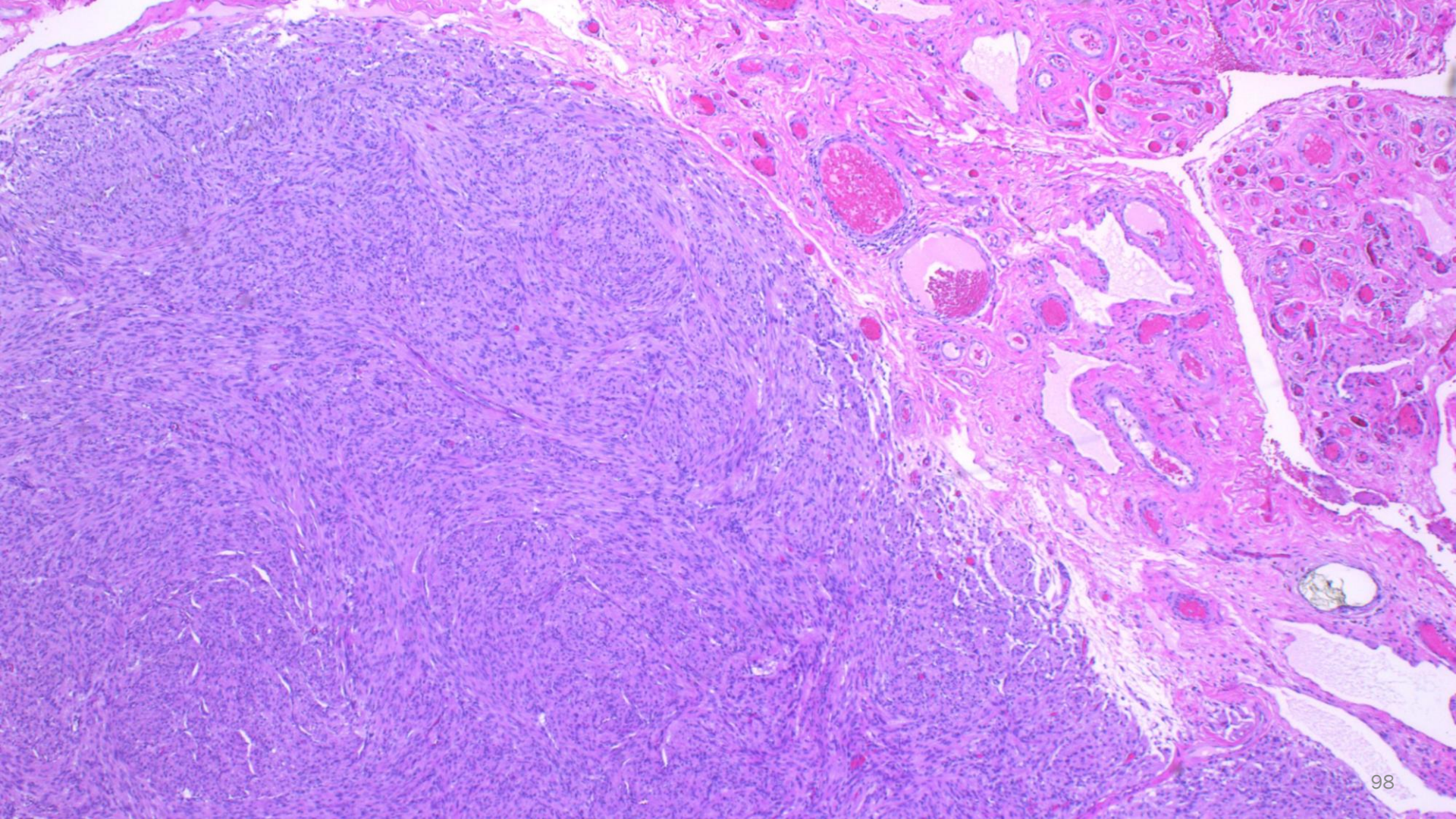
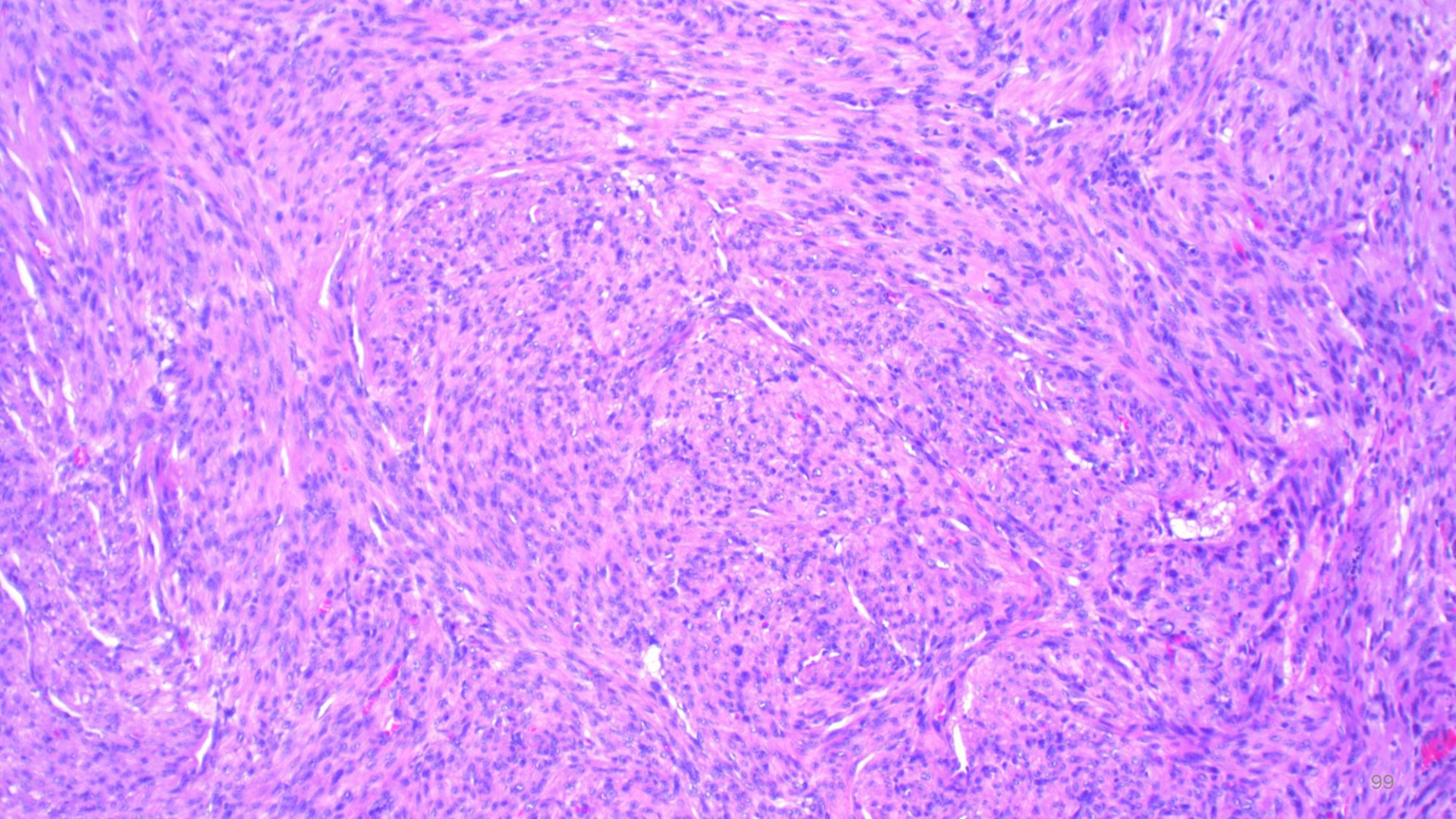

WT1

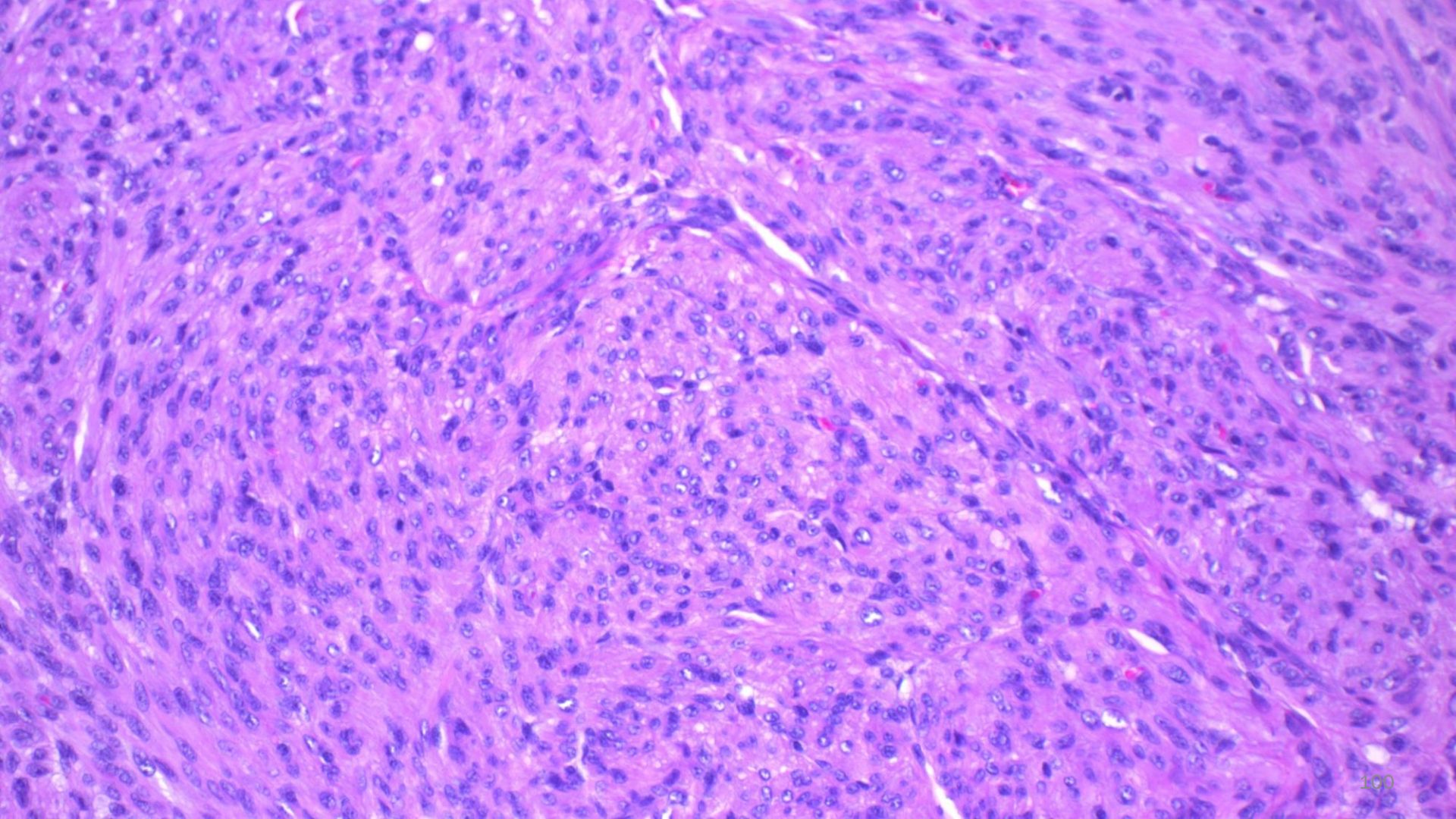
Case 3

- Uterine mass:
  - Significant atypia
  - 4 mitoses/10 hpf
  - No coagulative tumor cell necrosis

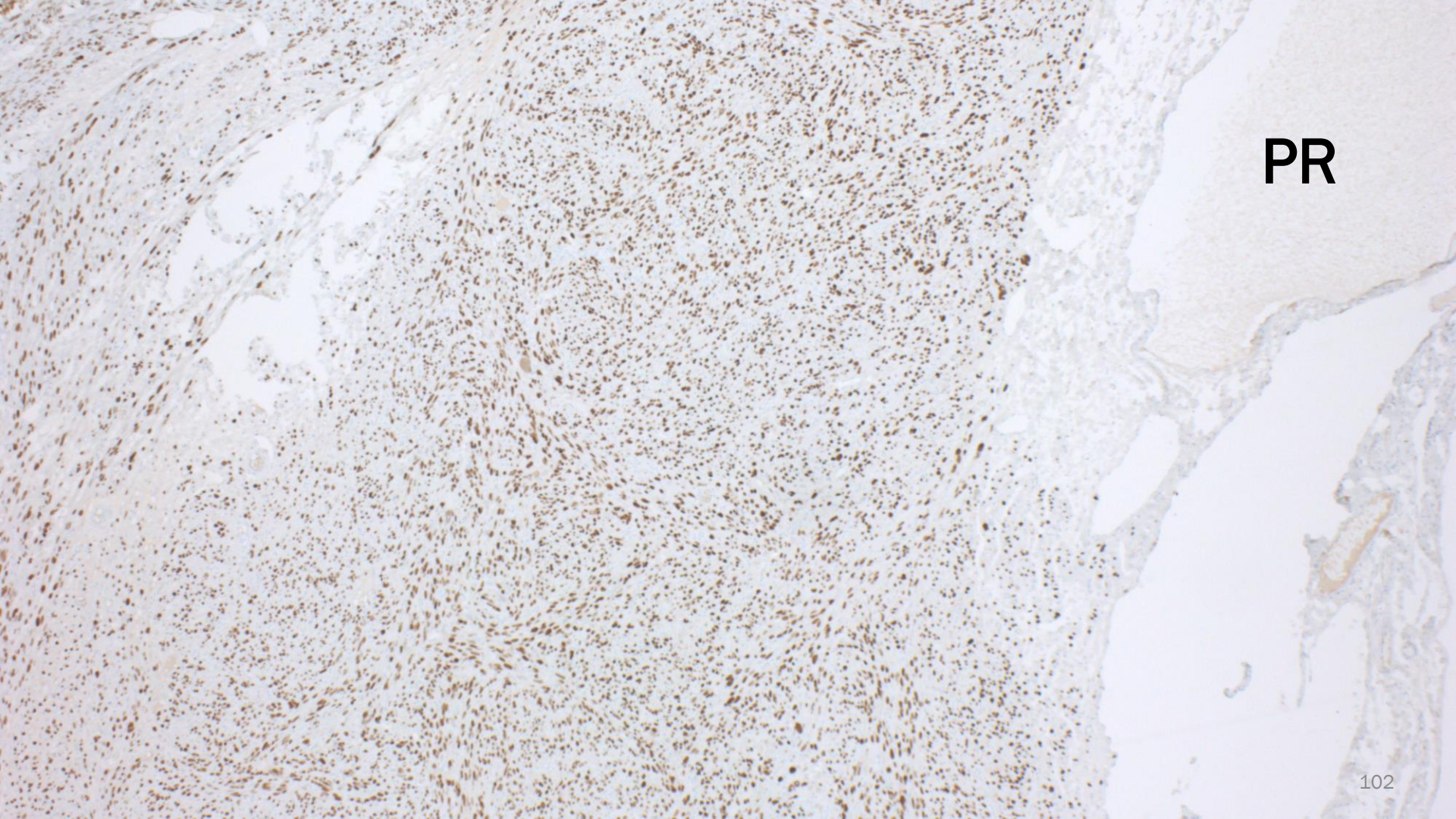
  - Atypical leiomyoma

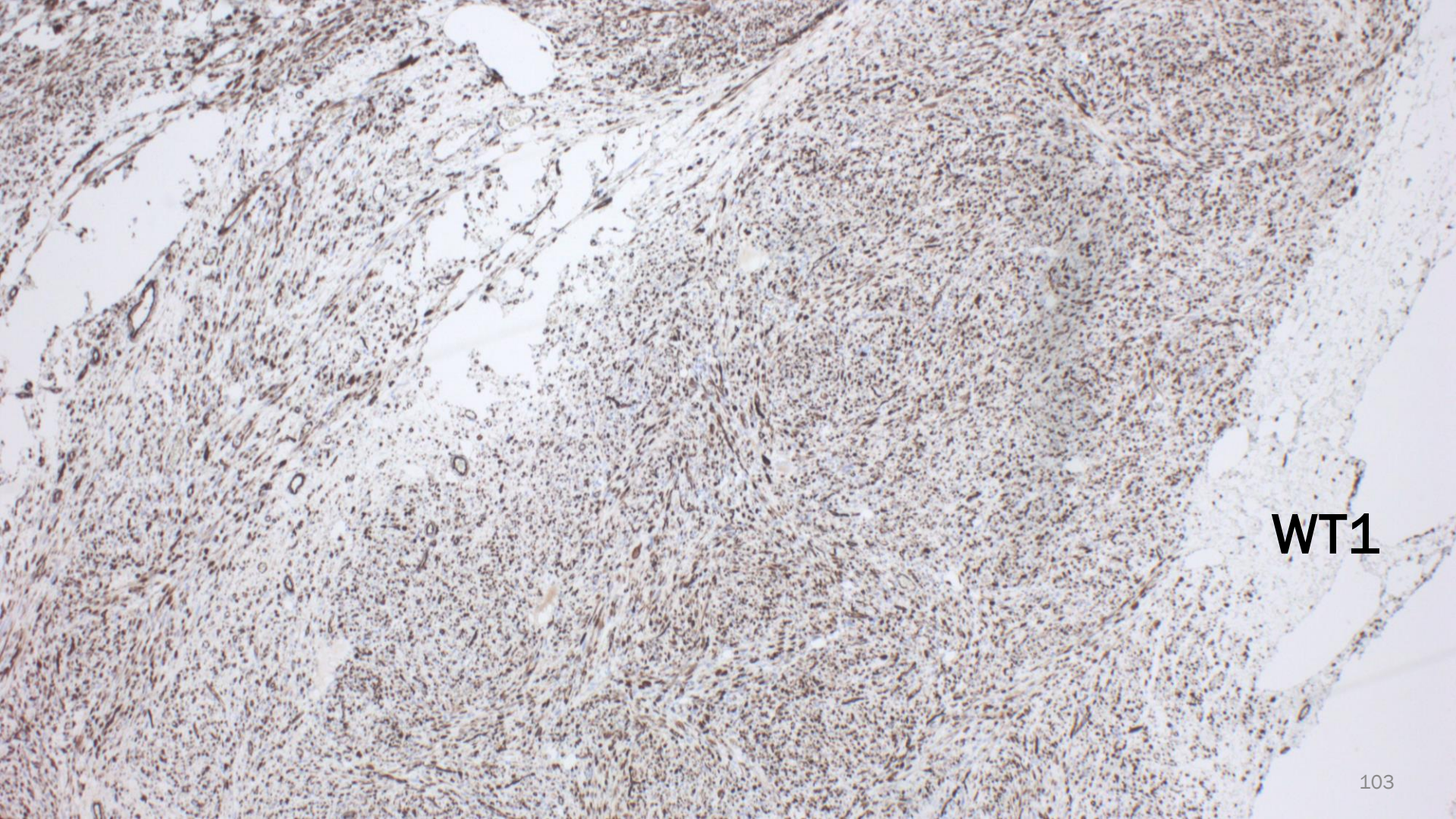


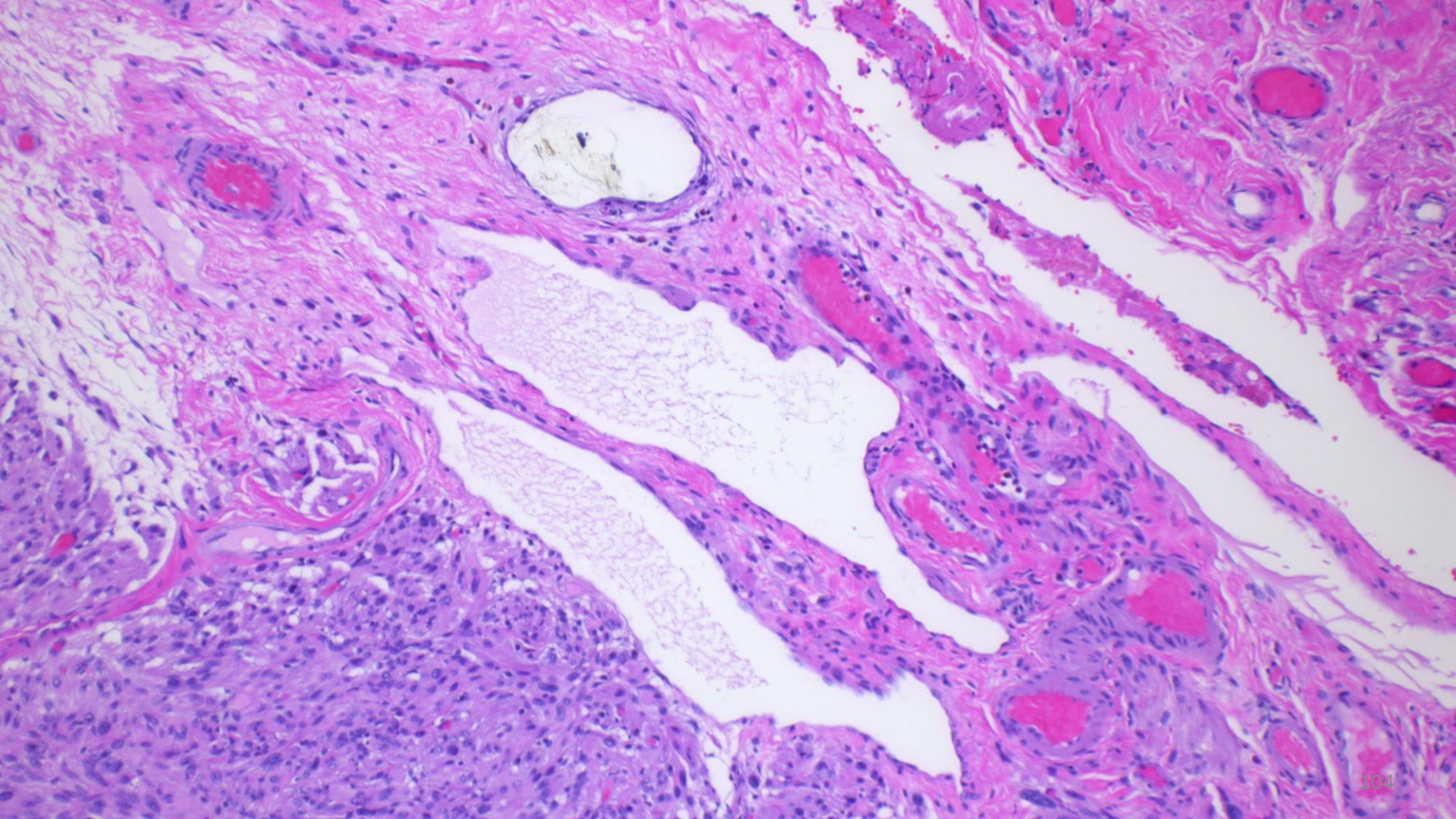


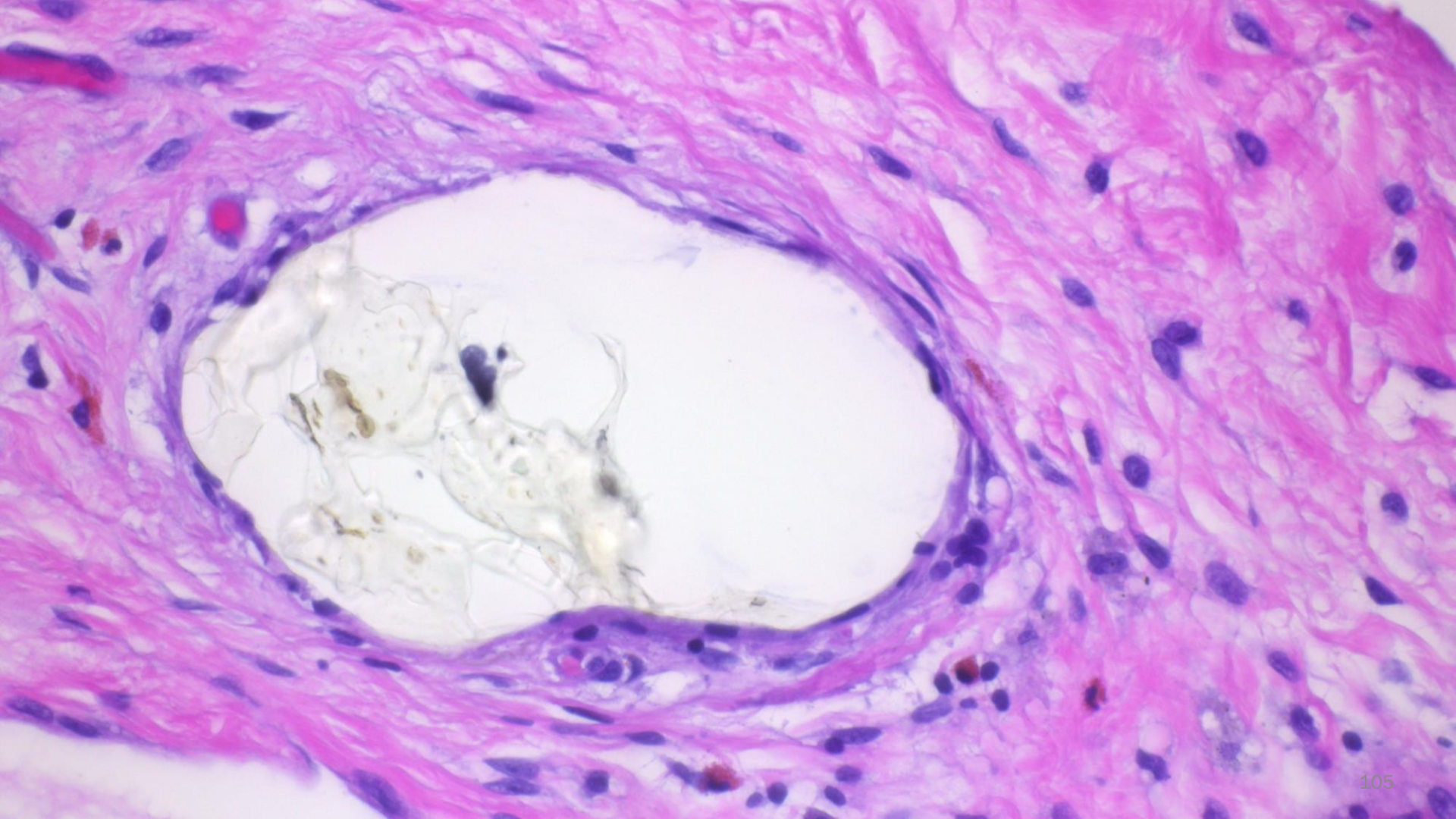


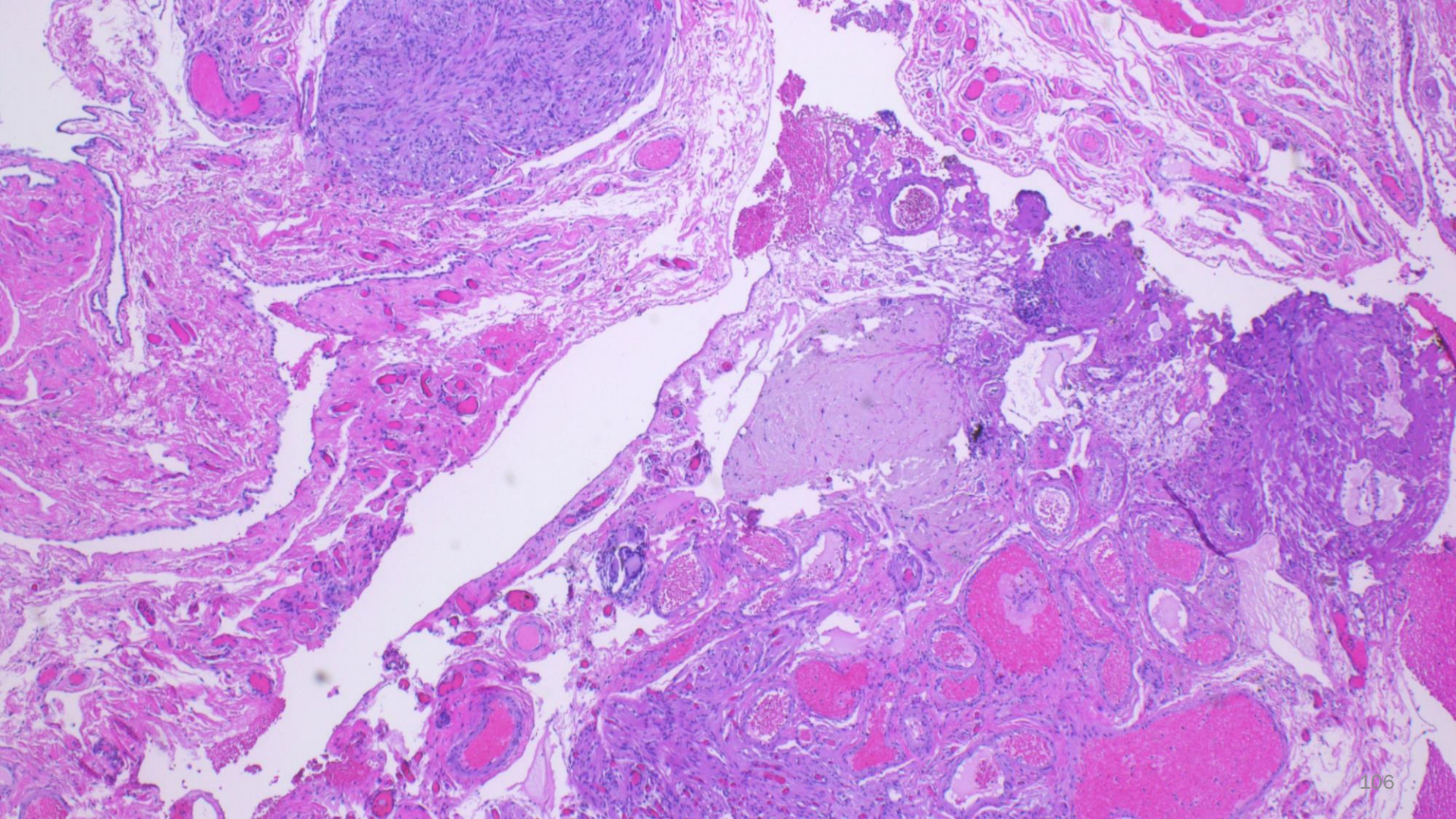

ER

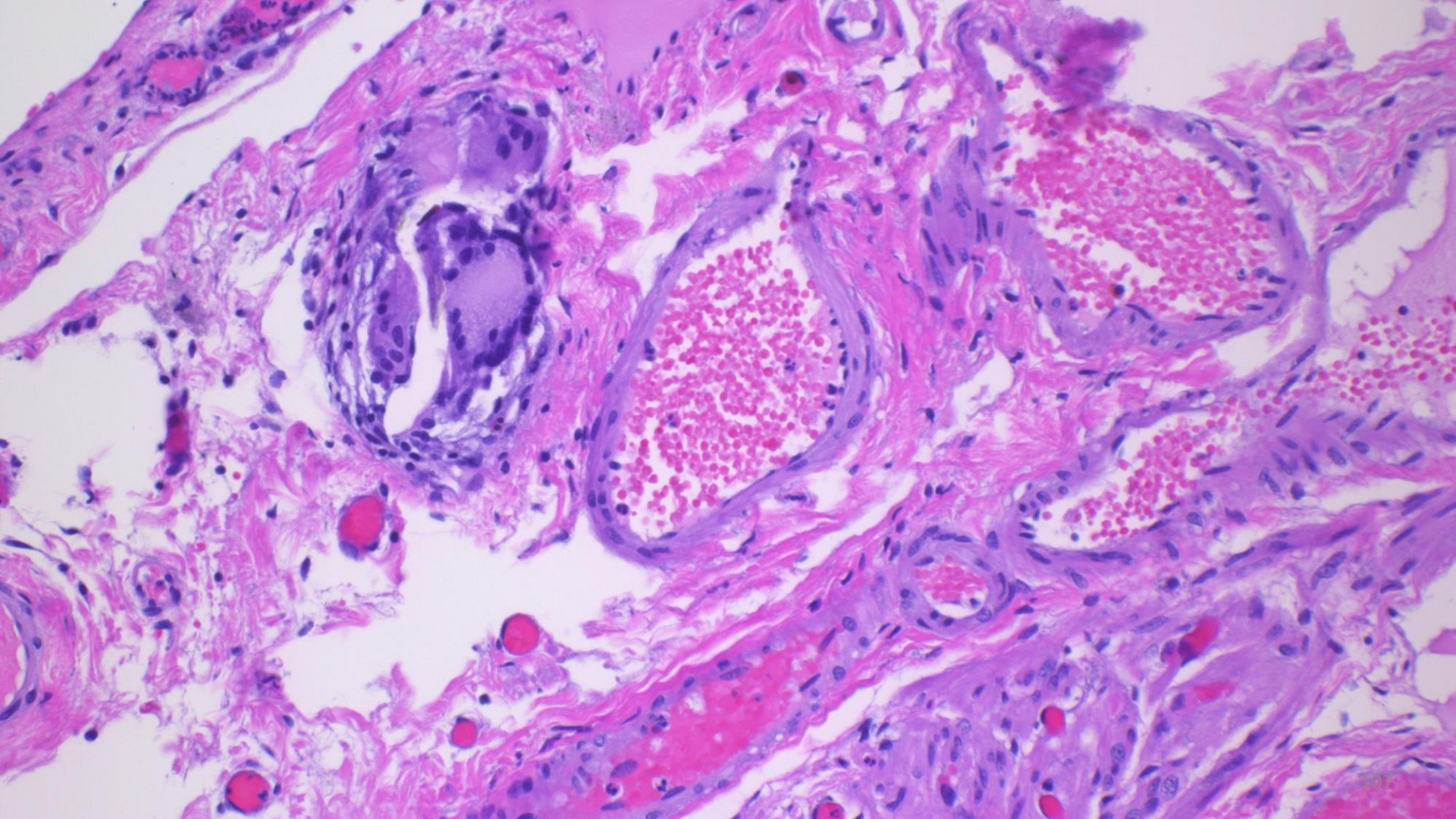

PR


WT1









Case 3

- Morcellation, power (1993) or open:
  - Dissemination of sarcoma
  - Dissemination of benign tissue
  - 2014 FDA issued a warning against the use of laparoscopic power morcellators in the majority of women undergoing myomectomy or hysterectomy for treatment of fibroids.
  - 2020 FDA: Power morcellation only be performed with a tissue containment system in selected patients

Case 3

STUMP

Observation, hormone therapy

Gyn vs Soft Tissue Smooth Muscle Tumors

Immunohistochemistry

- Kelley TW, Borden EC, Goldblum JR. Estrogen and progesterone receptor expression in uterine and extrauterine leiomyosarcomas: an immunohistochemical study. Appl Immunohistochem Mol Morphol 2004;12:338-41.
- Deavers M, Silva E, Euscher E, Liu J, Broaddus R, Malpica A. WT-1 expression may differentiate mullerian from non-mullerian smooth muscle tumors. Mod Pathol 2006;19:176A.
- Patil DT, Laskin WB, Fetsch JF, Miettinen M. Inguinal smooth muscle tumors in women – a dichotomous group consisting of Mullerian-type leiomyomas and soft tissue leiomyosarcomas: an analysis of 55 cases. Am J Surg Pathol 2011;35:315–24.

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Gyn vs Soft Tissue Smooth Muscle Tumors

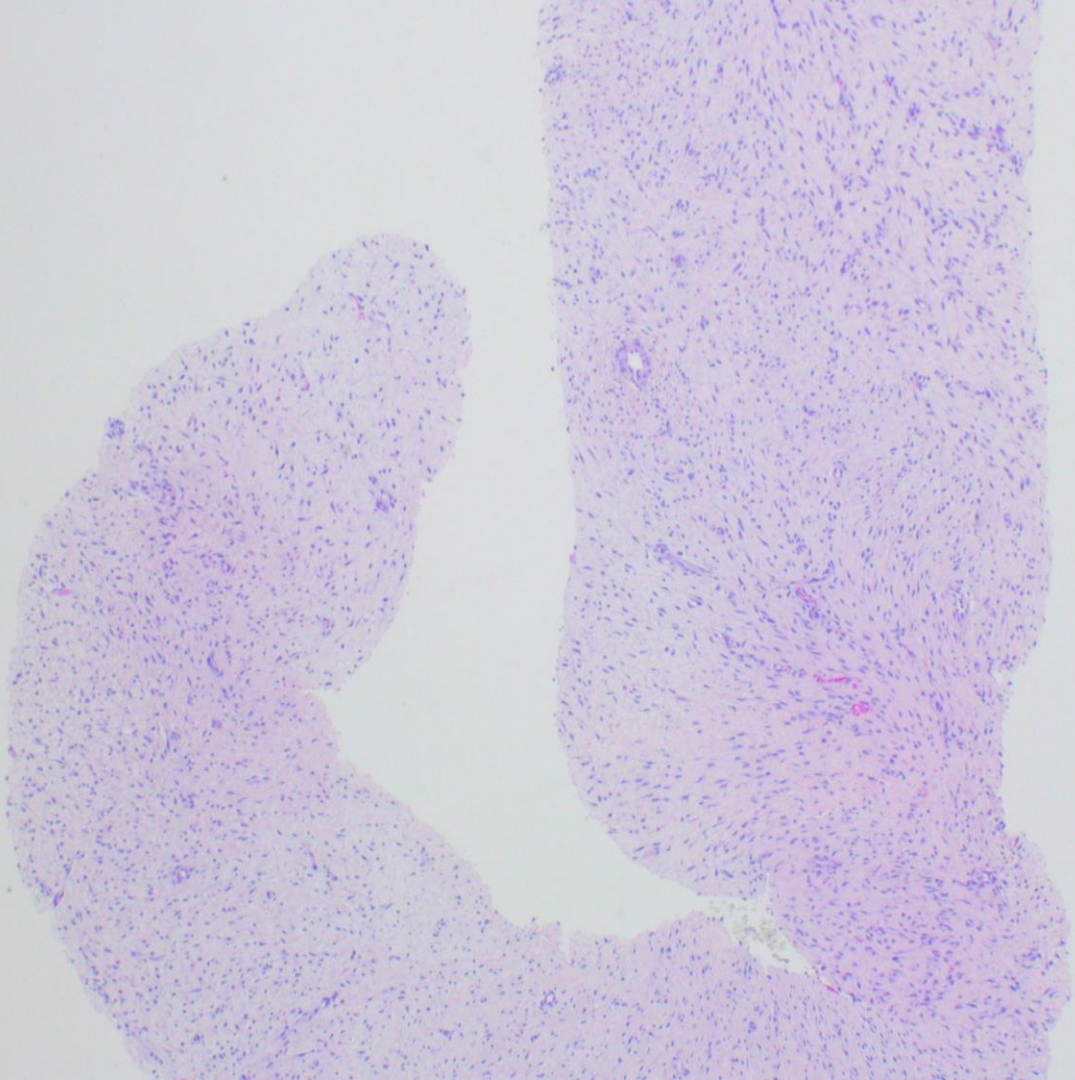
ER, PR, WT1 Immunohistochemistry in SMTs

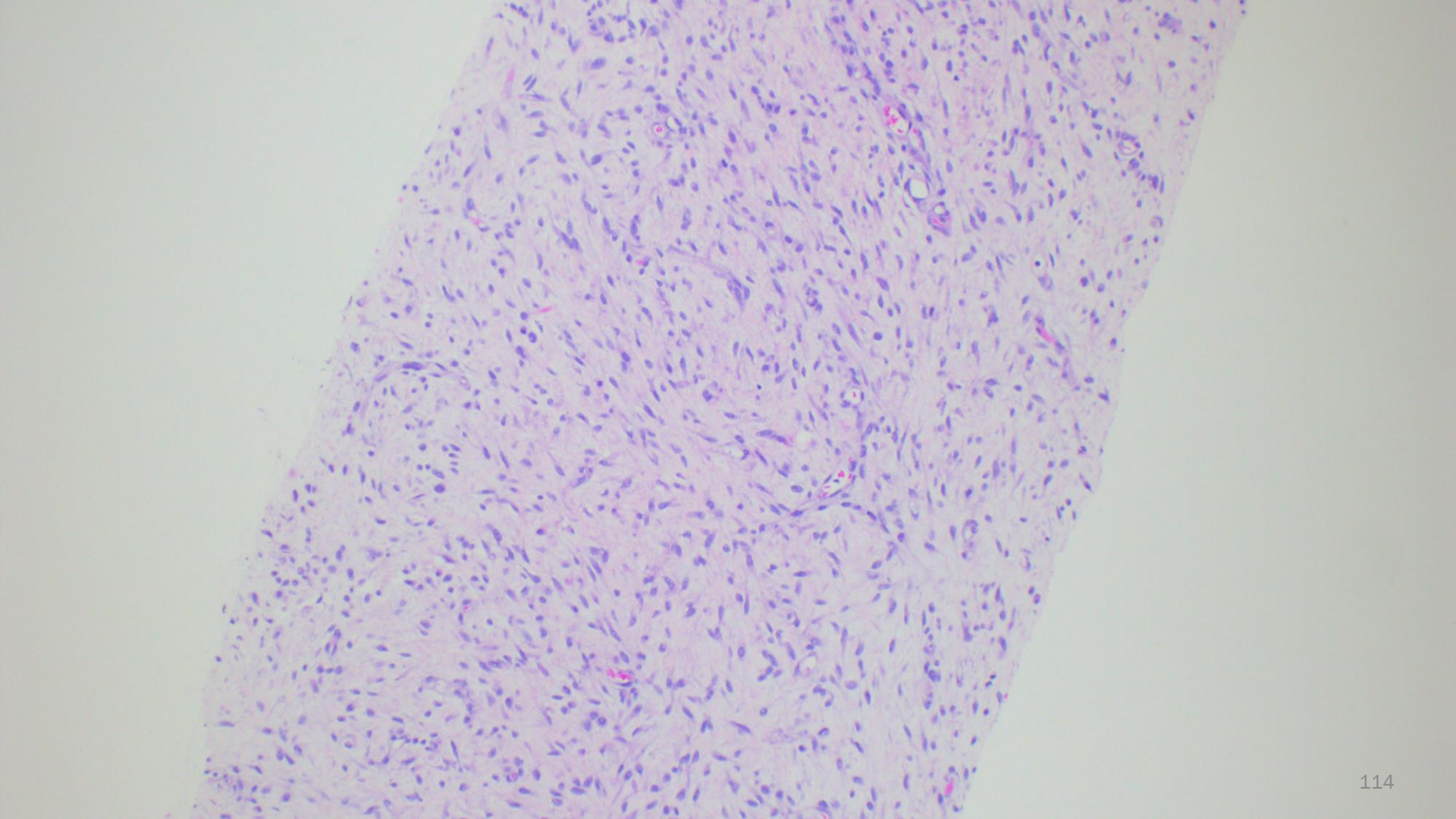
- Majority of gynecologic LM and STUMP express ER, PR, WT1 (83 – 93%)
- Majority of soft tissue LM are negative for ER, PR, WT1 (exceptional cases – ER and PR + in thigh LM of a woman)
- Most gynecologic LMS express ER, PR, WT1 (66 – 80%)
- Most soft tissue LMS are negative for WT1, and lack diffuse ER, PR (focal [1+] ER and PR can be seen in up to a third of cases – women and some men; rare cases with more diffuse expression – retroperitoneal, lower extremity LMS in women)

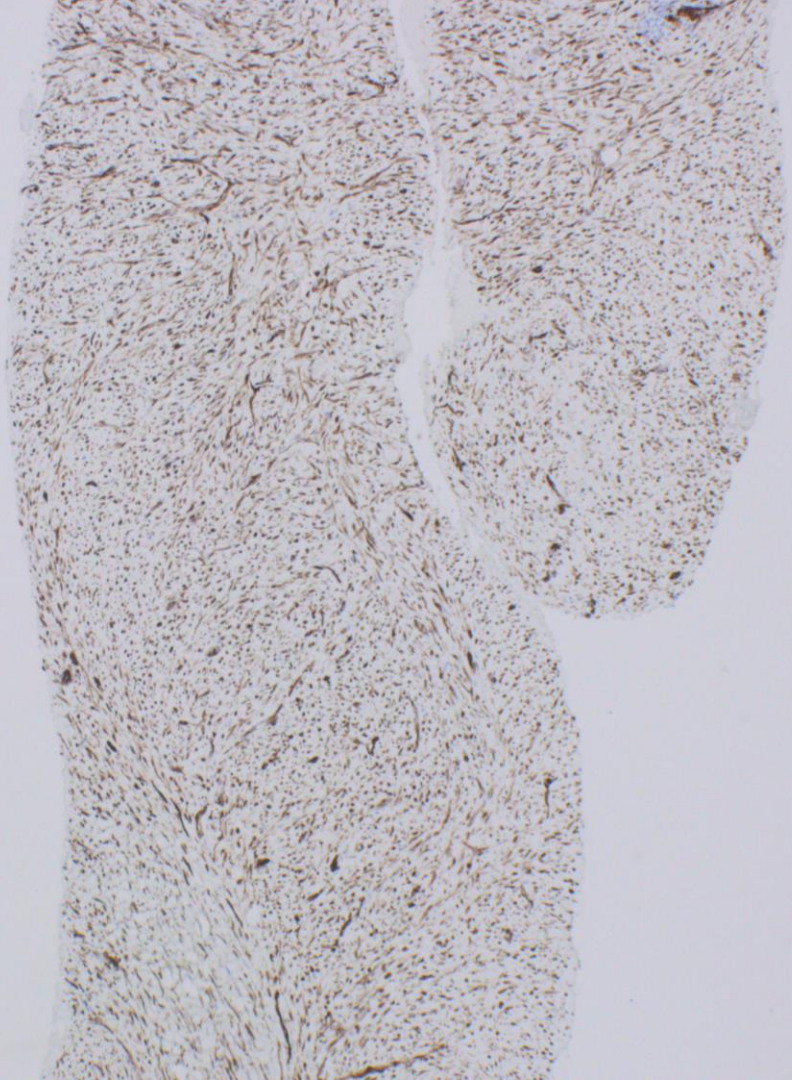
Lagniappe

WT1 in Liposarcoma

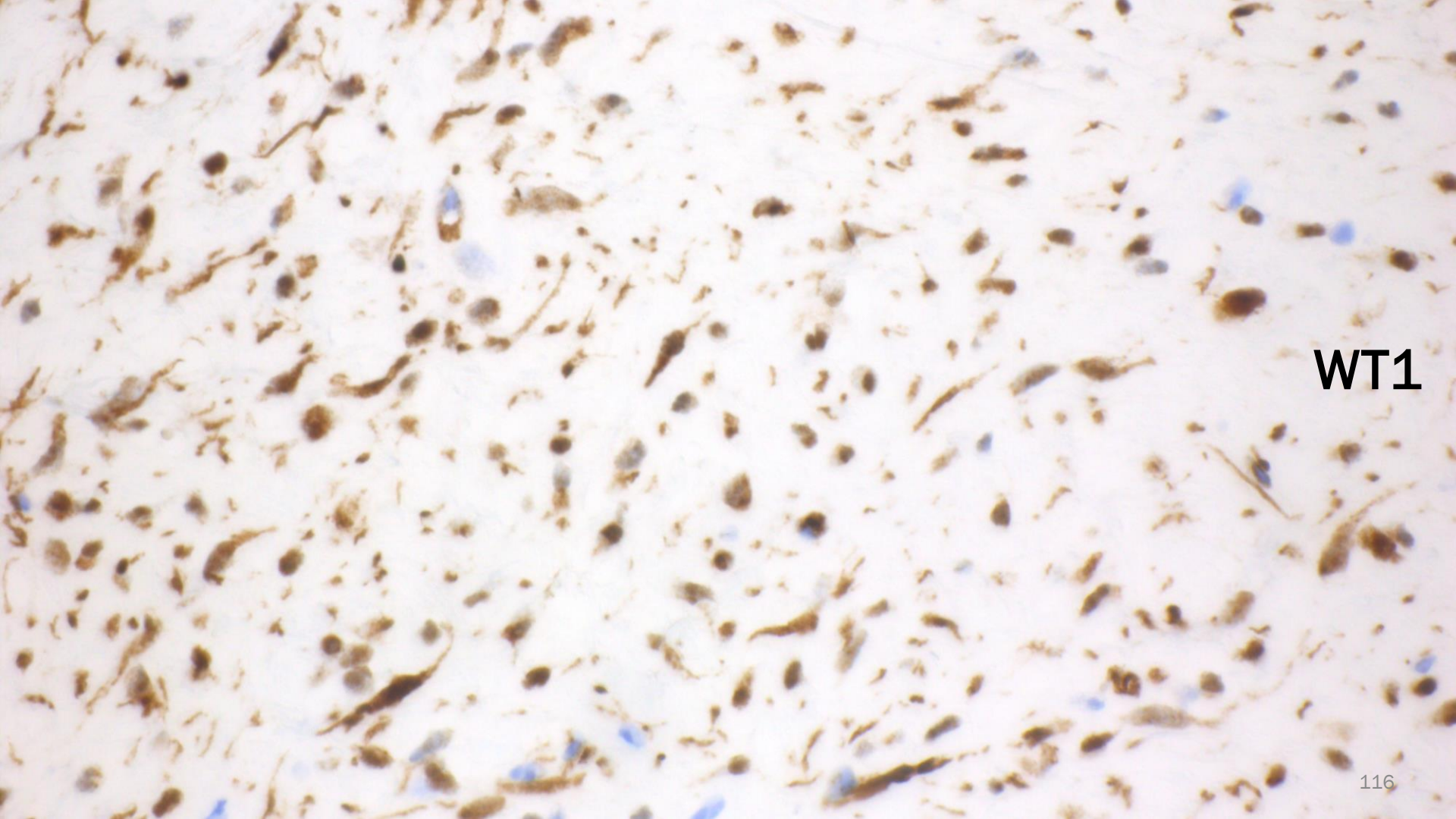
- Desmin can be expressed in fibrous and cellular areas of Well Differentiated LS, and in Dedifferentiated LS (40%)
- WT1 may be positive





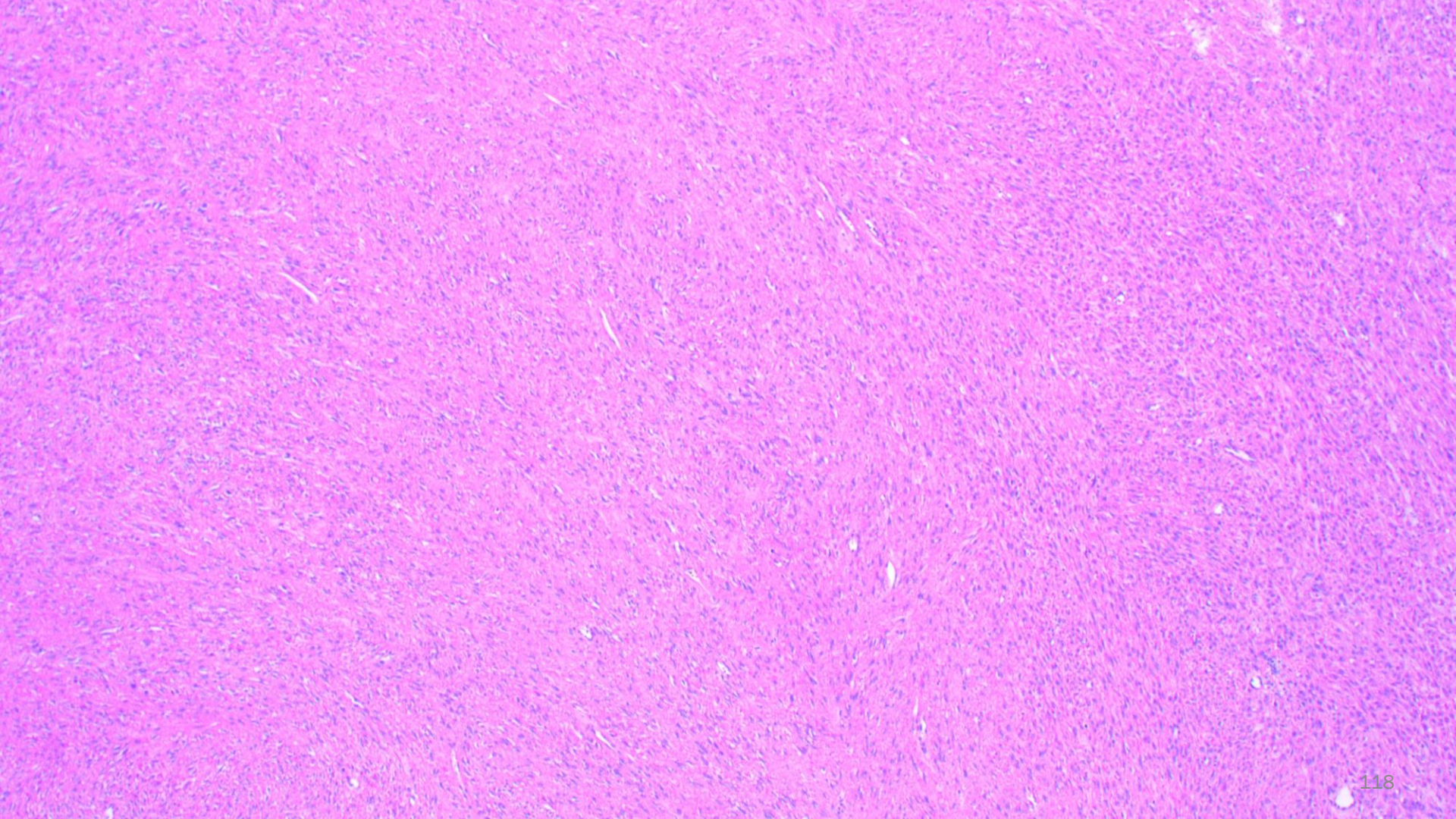


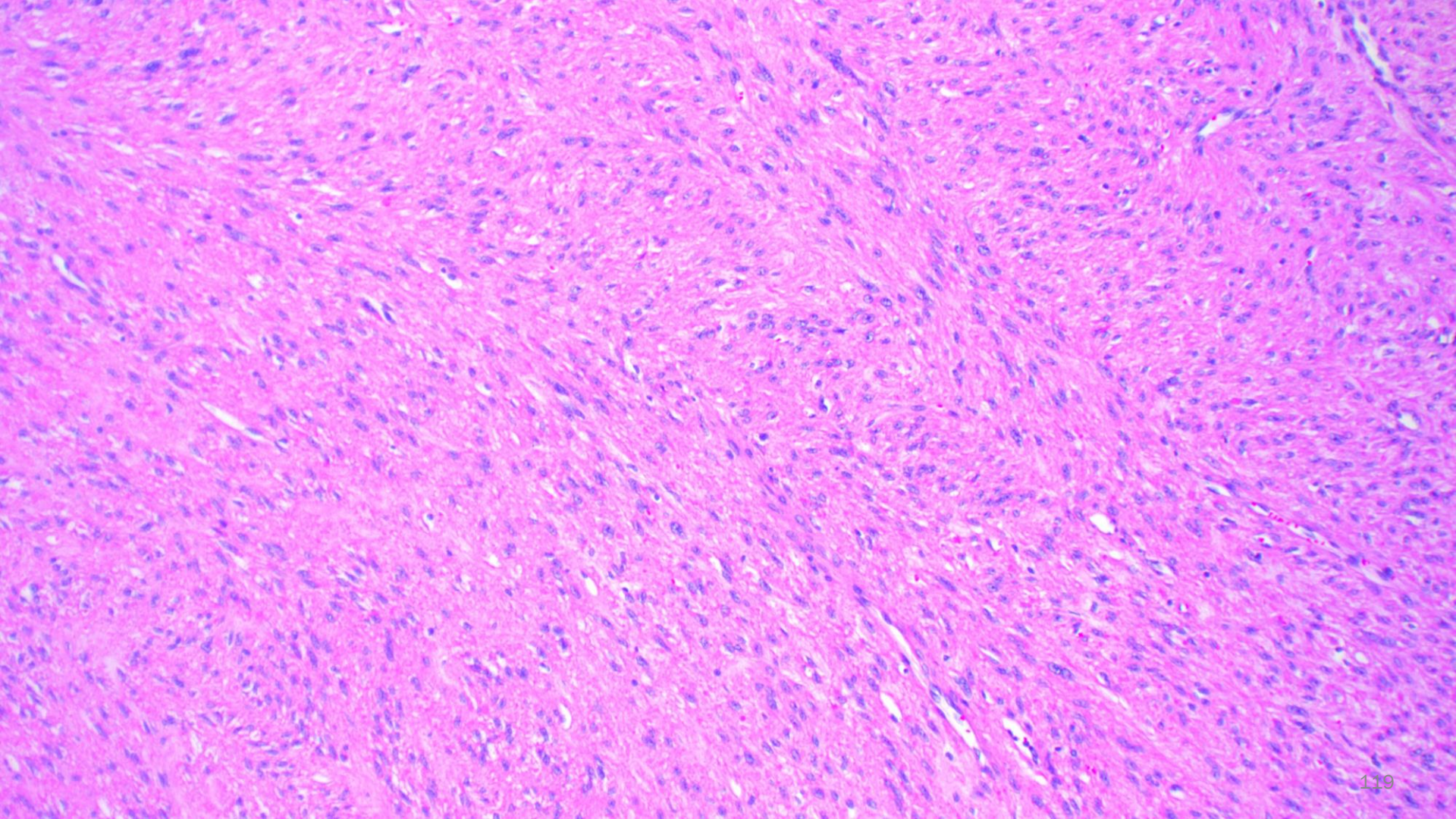
WT1

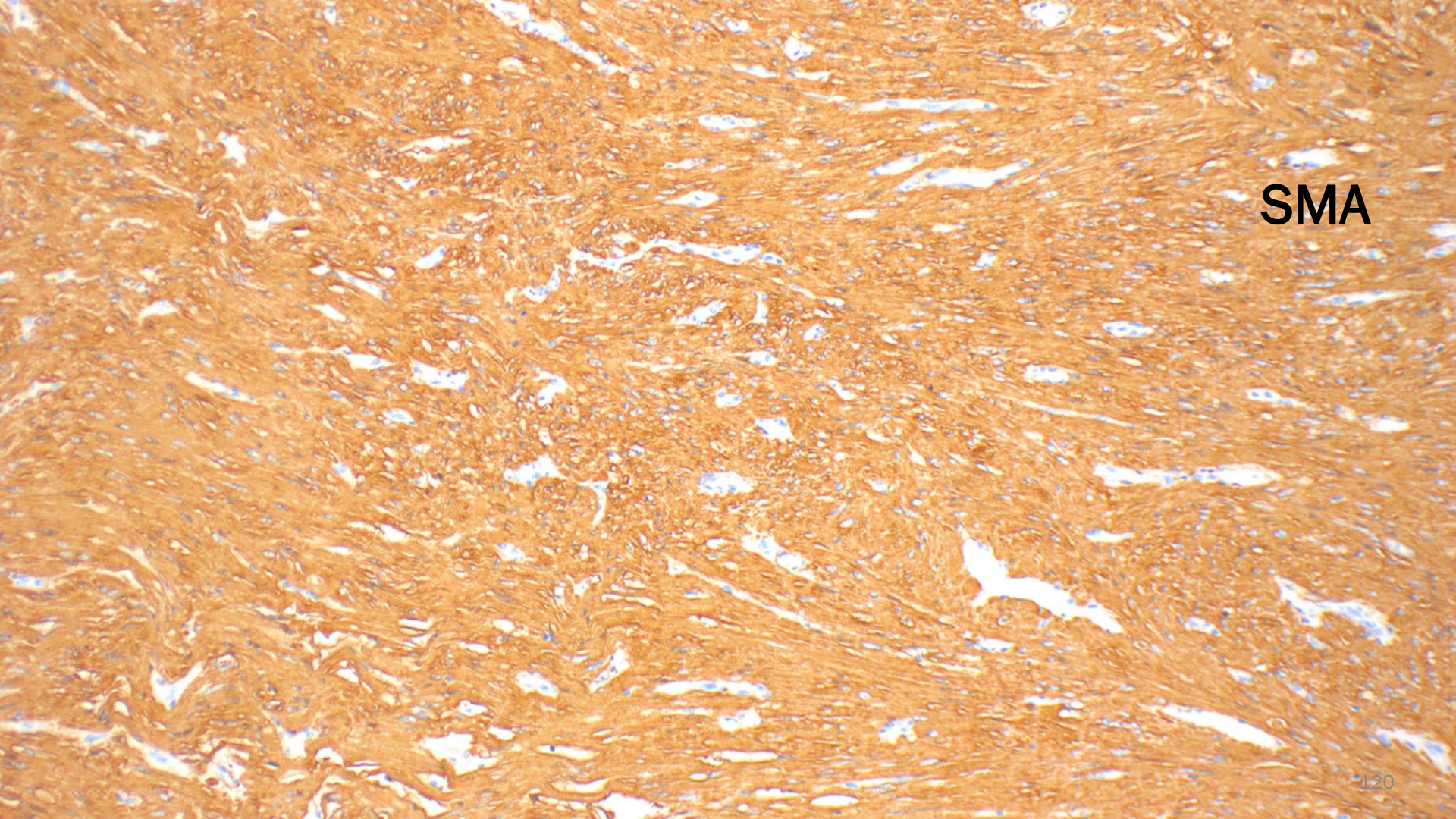

WT1

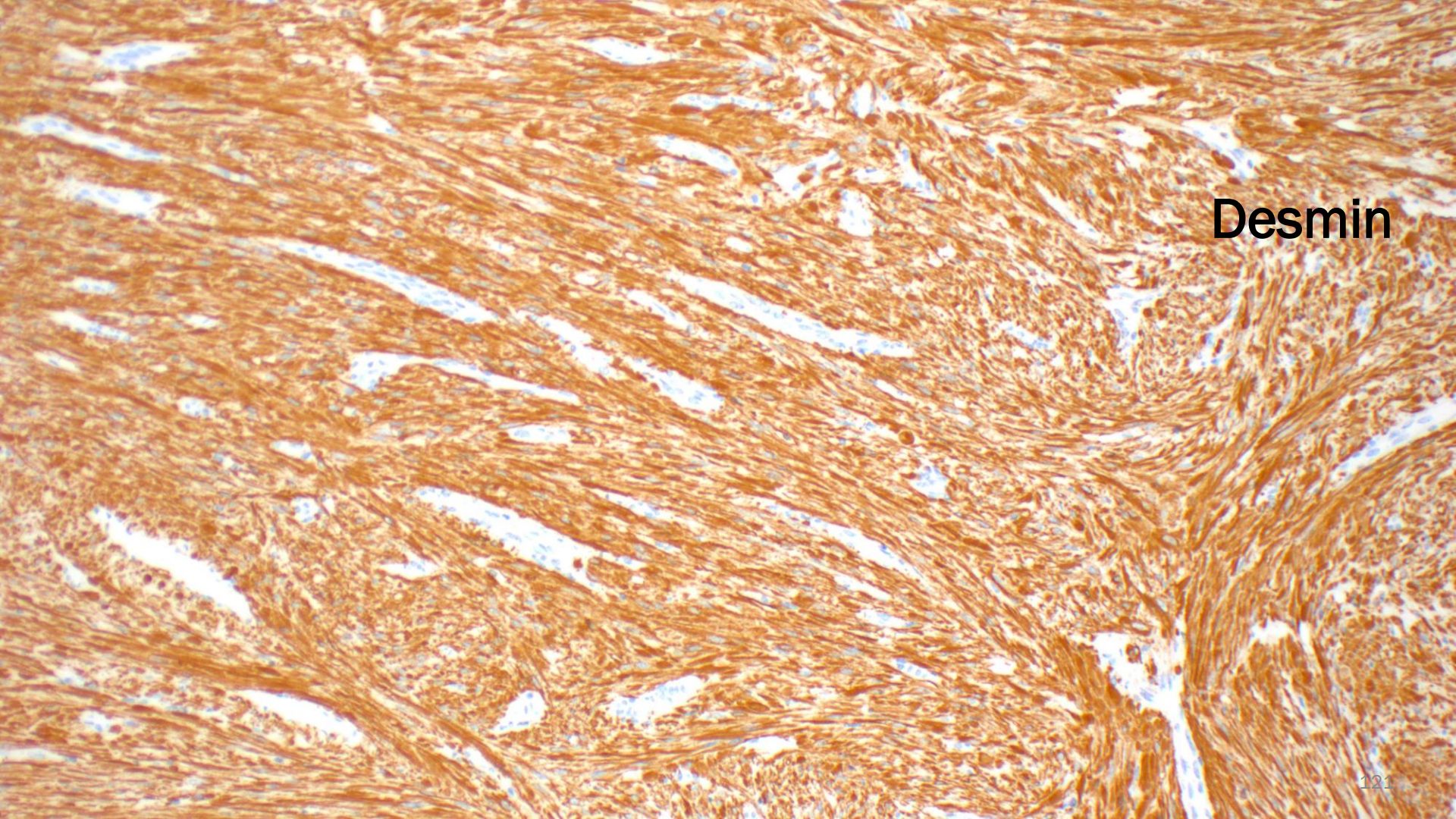
Case 4

- 37 y.o. with a history of esophageal GIST
- Prelabial and paraurethral masses
- Excised






SMA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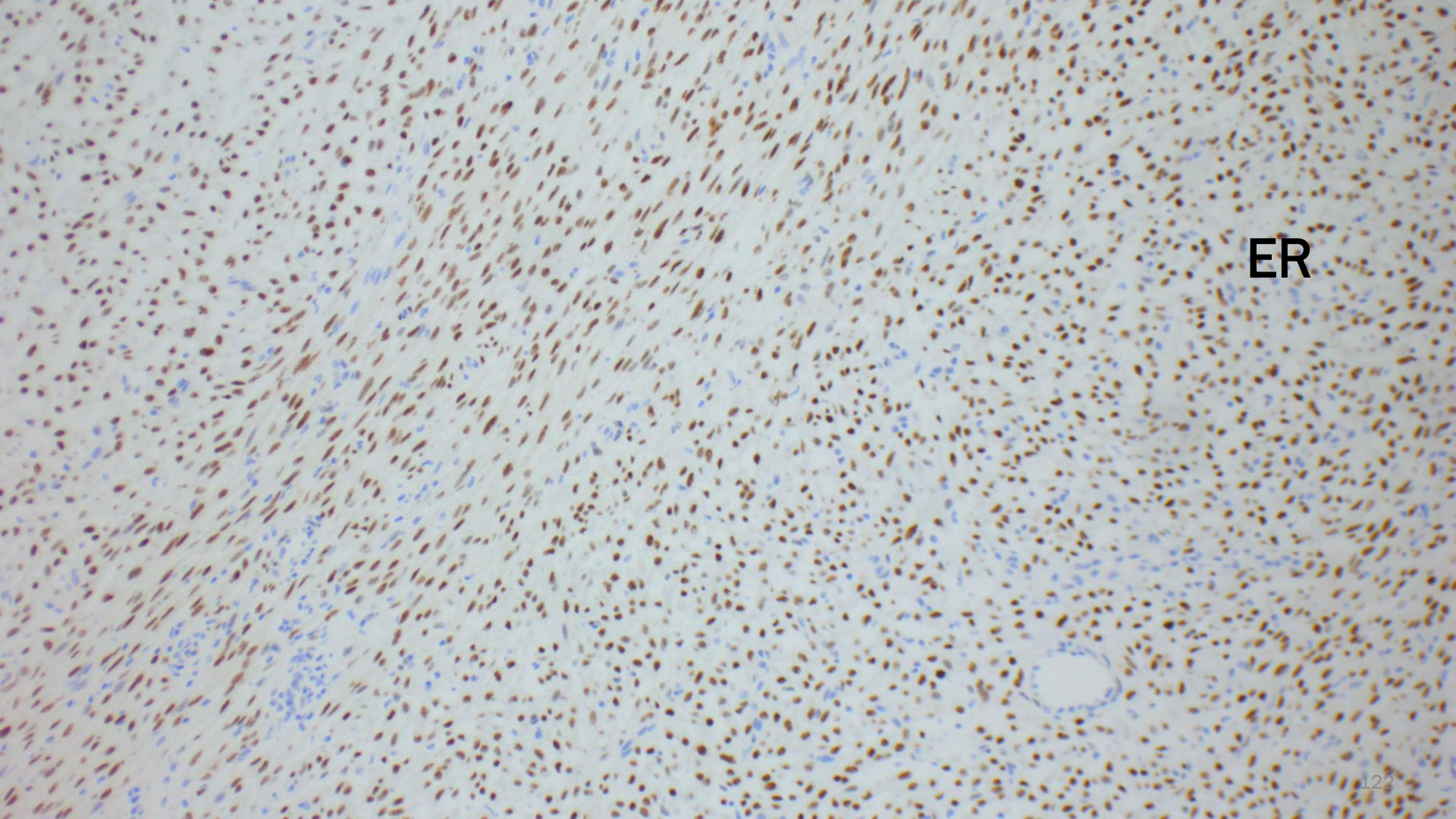
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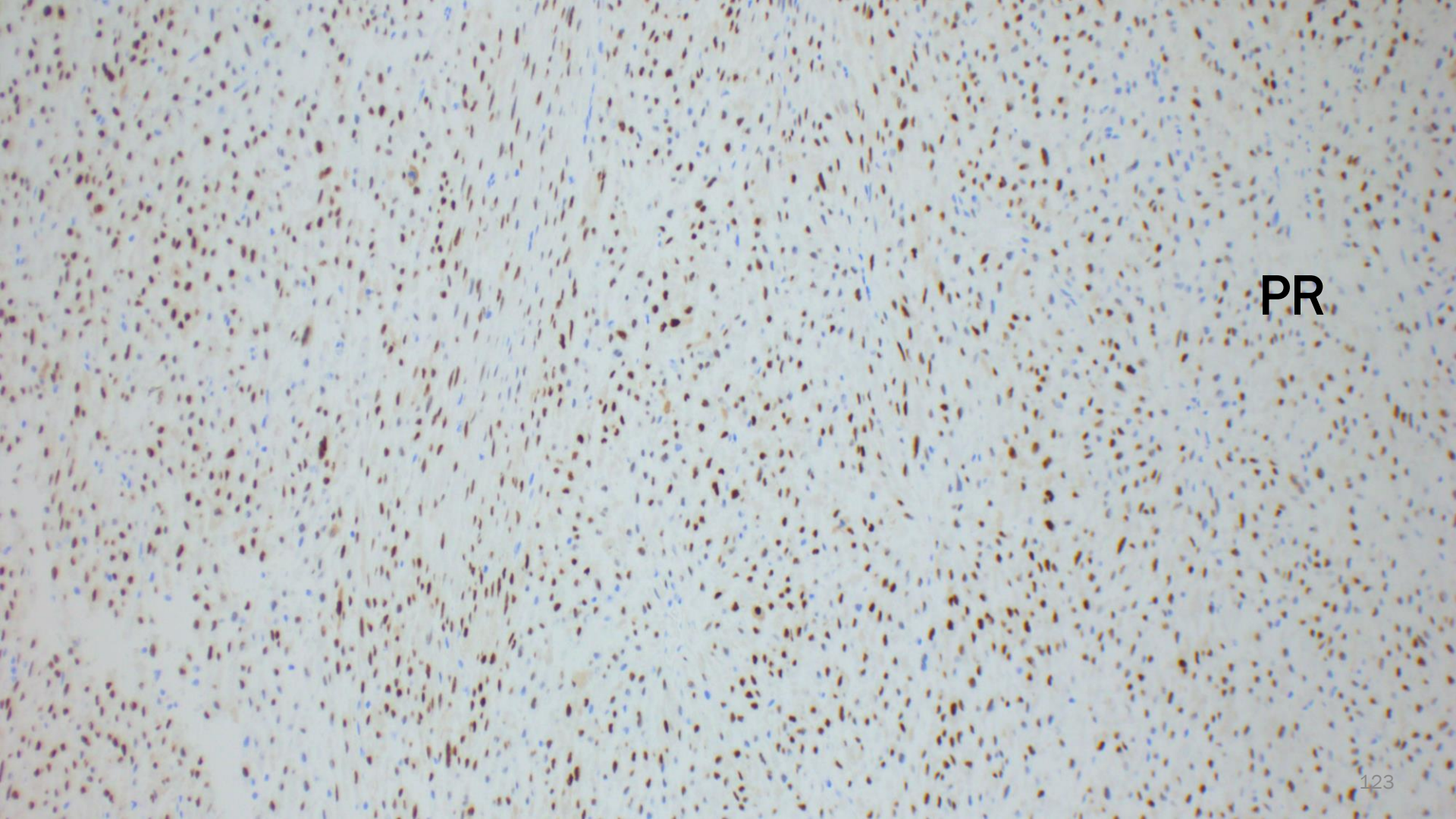
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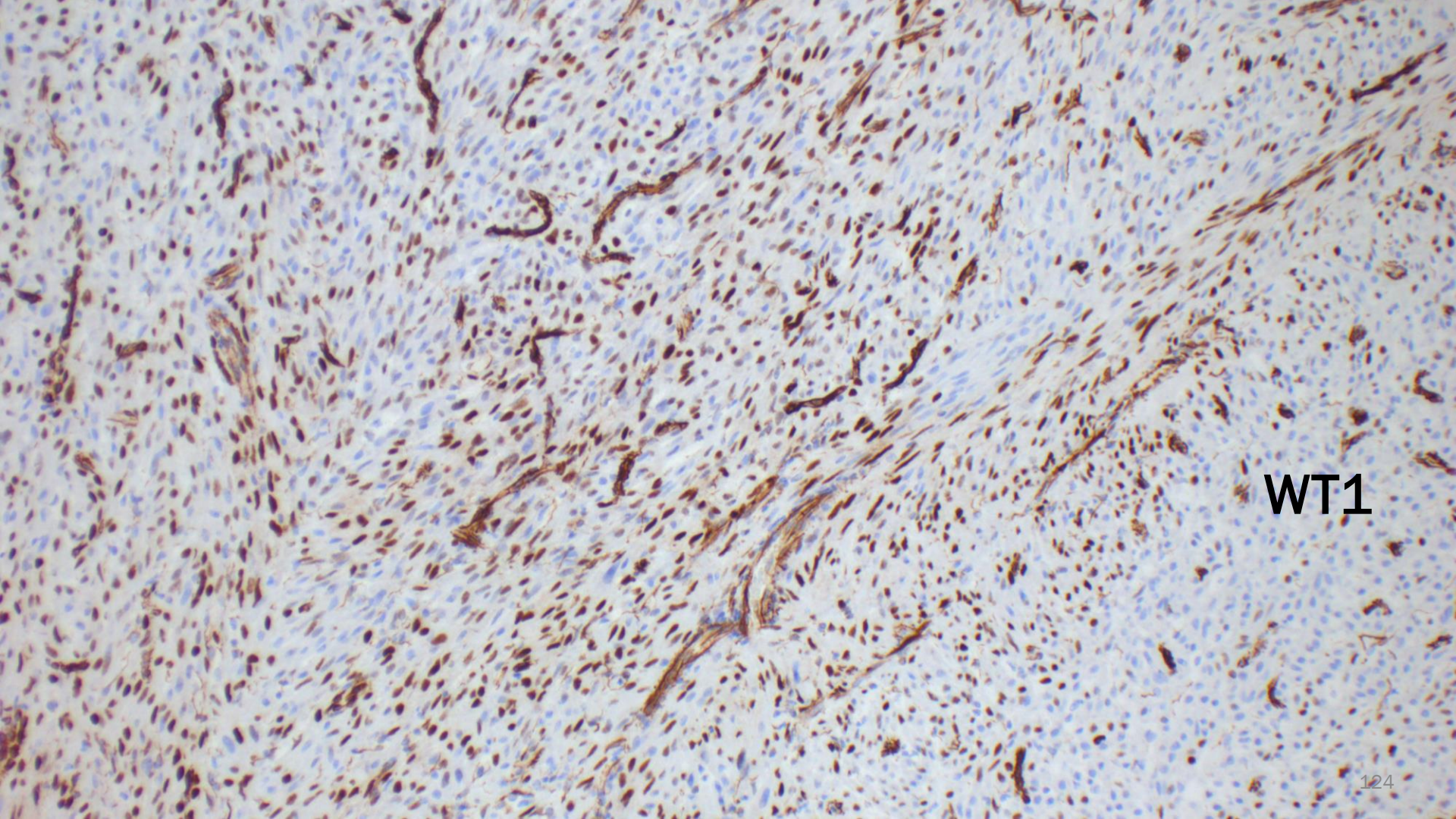
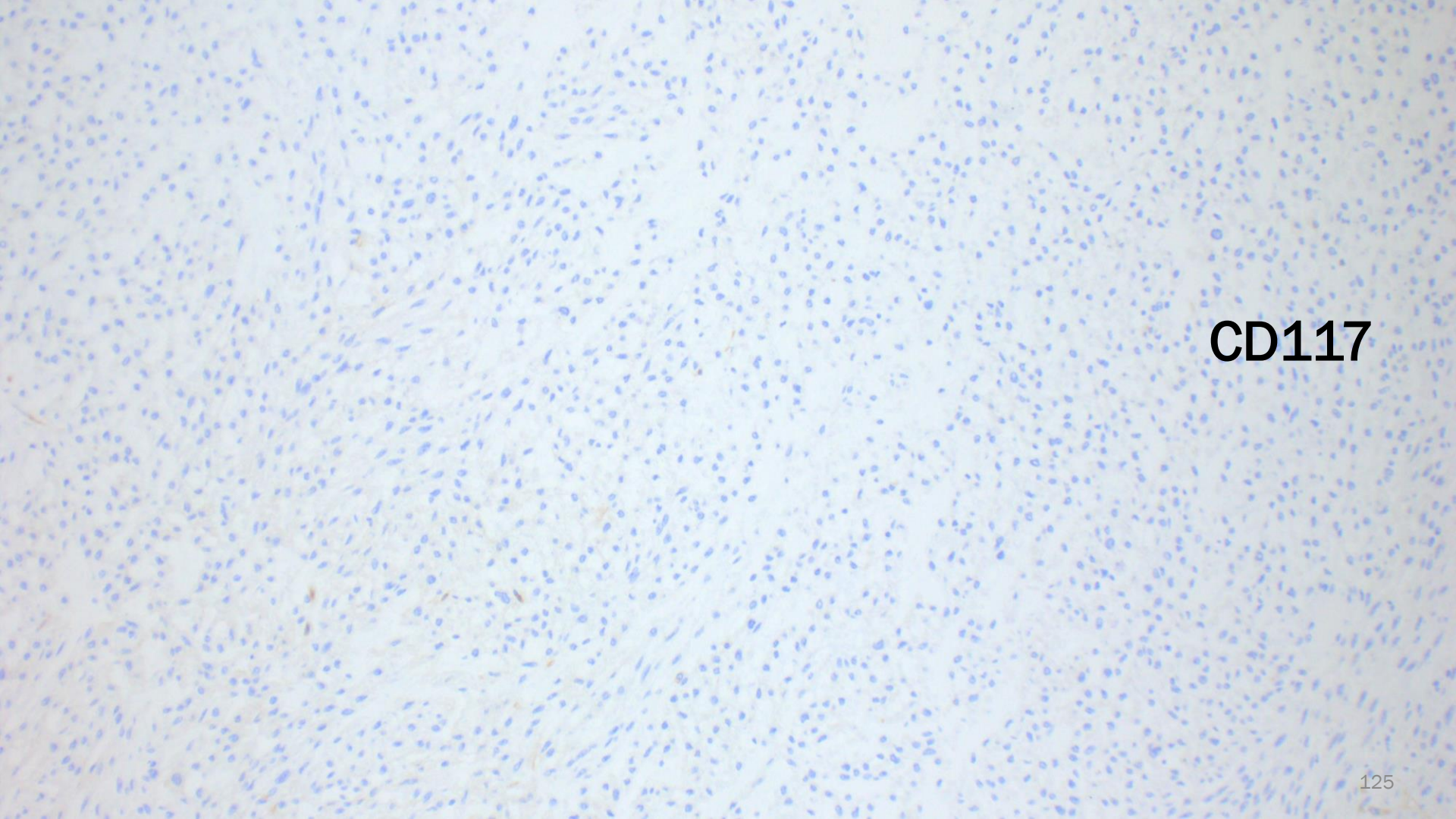
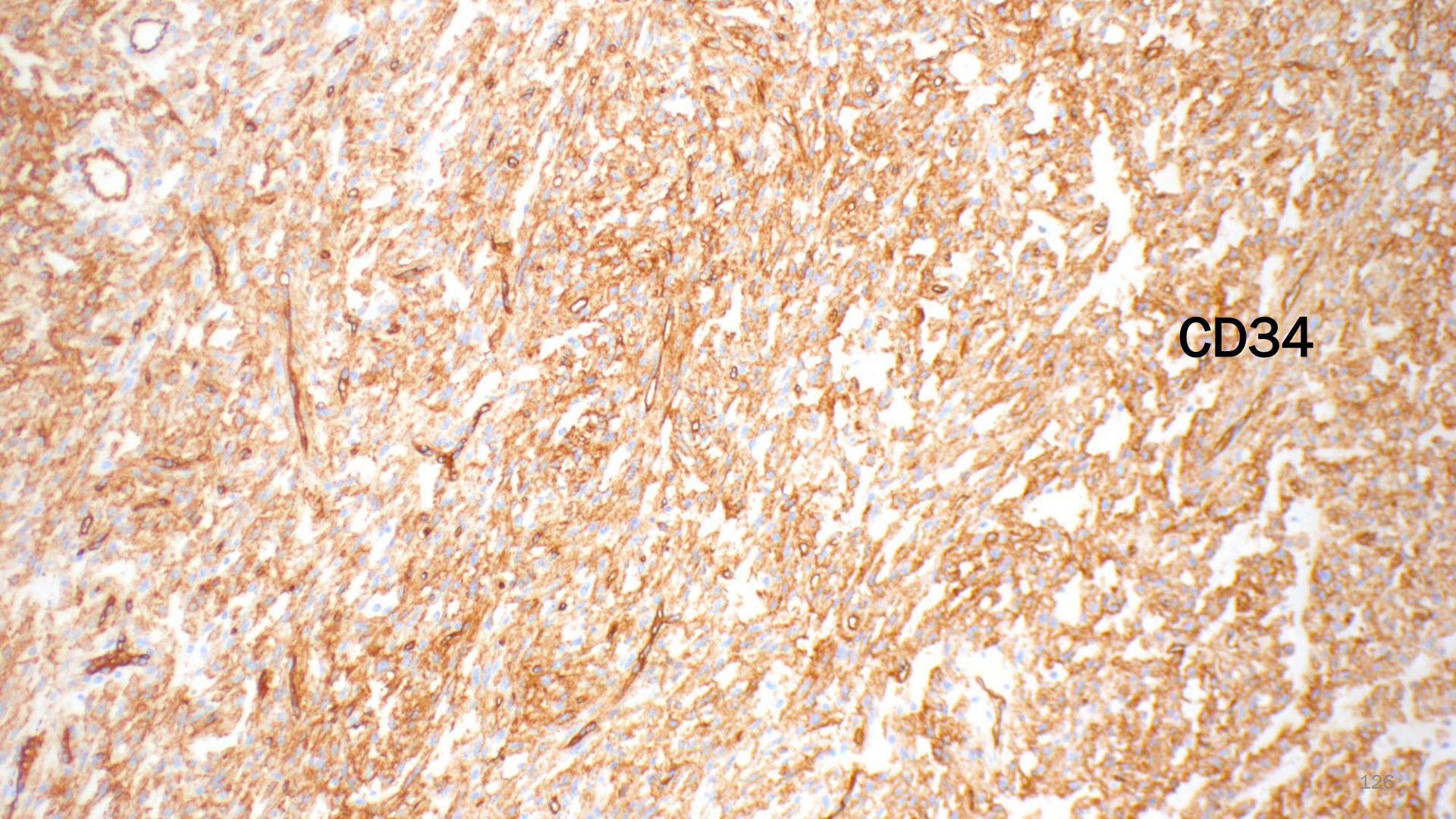

WT1


CD117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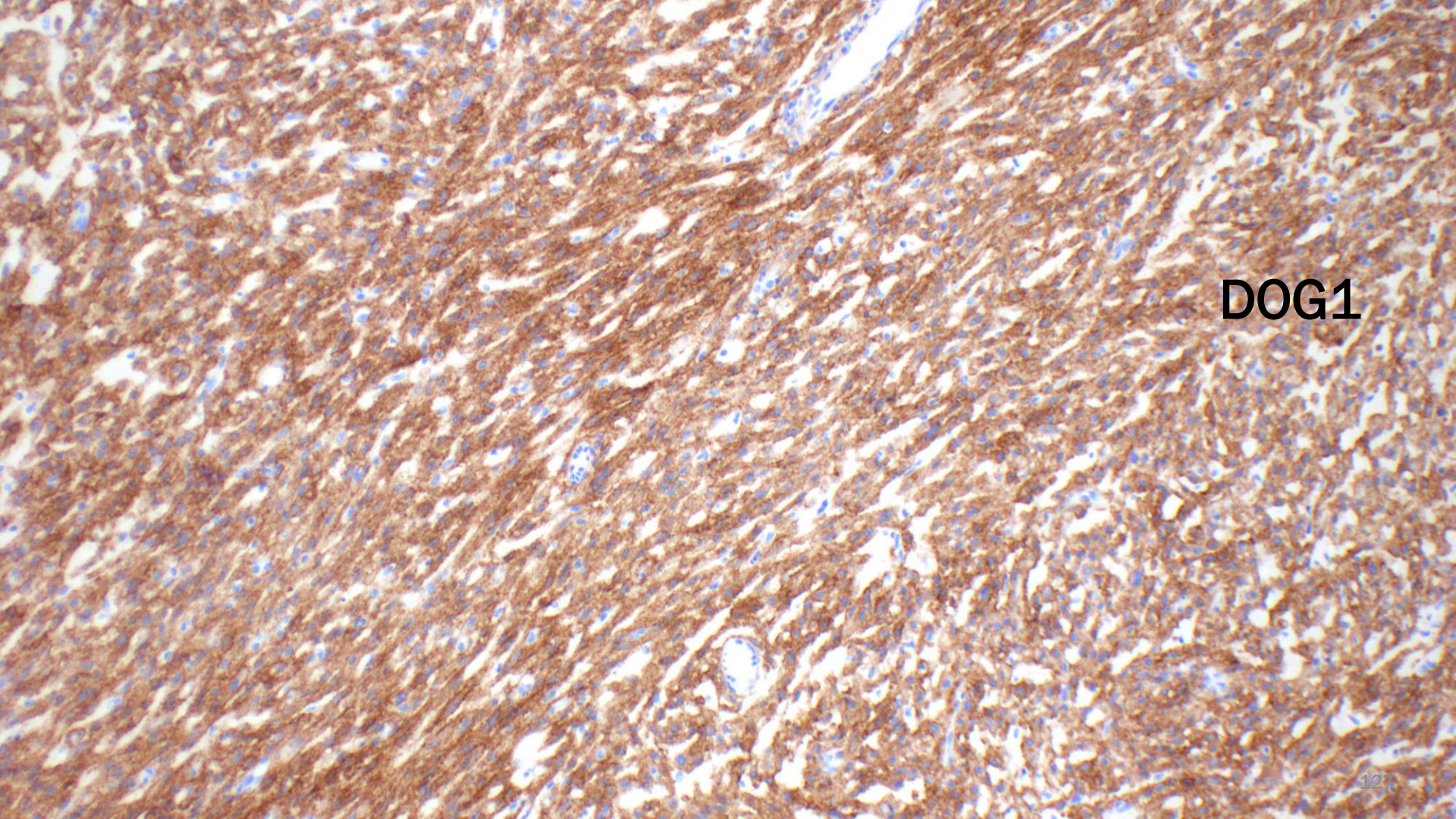

DOG1

Case 4

- ? Metastatic GIST
- ? Leiomyoma

Case 4

DOG1 in gynecologic smooth muscle tumors

- Miettinen M, Wang Z-F, Lasota J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases. Am J Surg Pathol 2009;33:1401-1408.
- Sah S, McCluggage WG. DOG1 immunoreactivity in uterine leiomyosarcomas. J Clin Pathol 2013;66:40-43.

Case 4

DOG1 in gynecologic smooth muscle tumors

- Uterine type leiomyomas (abdomen and retroperitoneum): 12%
- Disseminated peritoneal leiomyomatosis: 24%
- GI leiomyomas: 0%
- Uterine leiomyosarcoma: 27%
- Soft tissue leiomyosarcoma: 0%

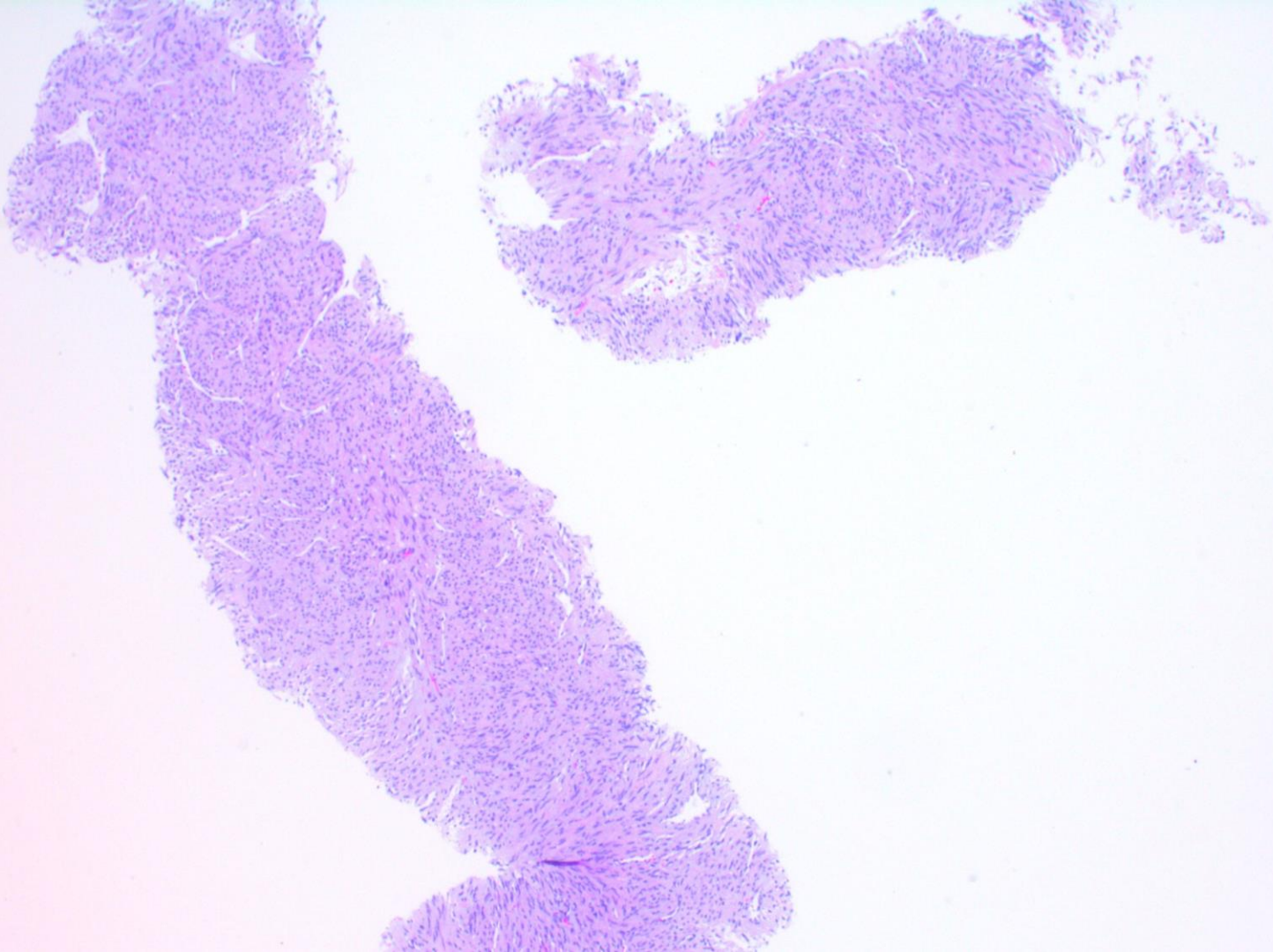
Case 4

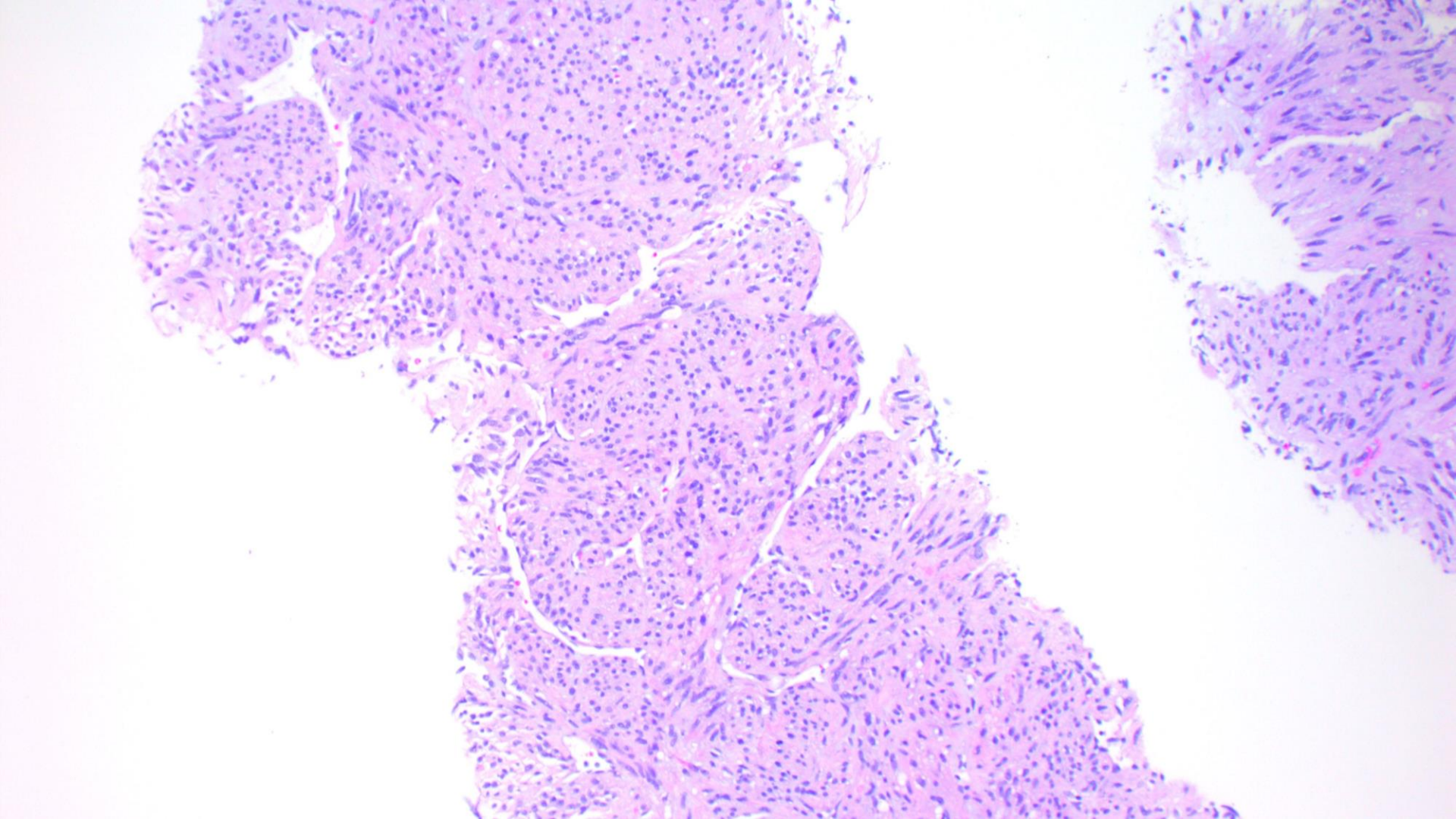
- Leiomyoma
  - Not metastatic GIST

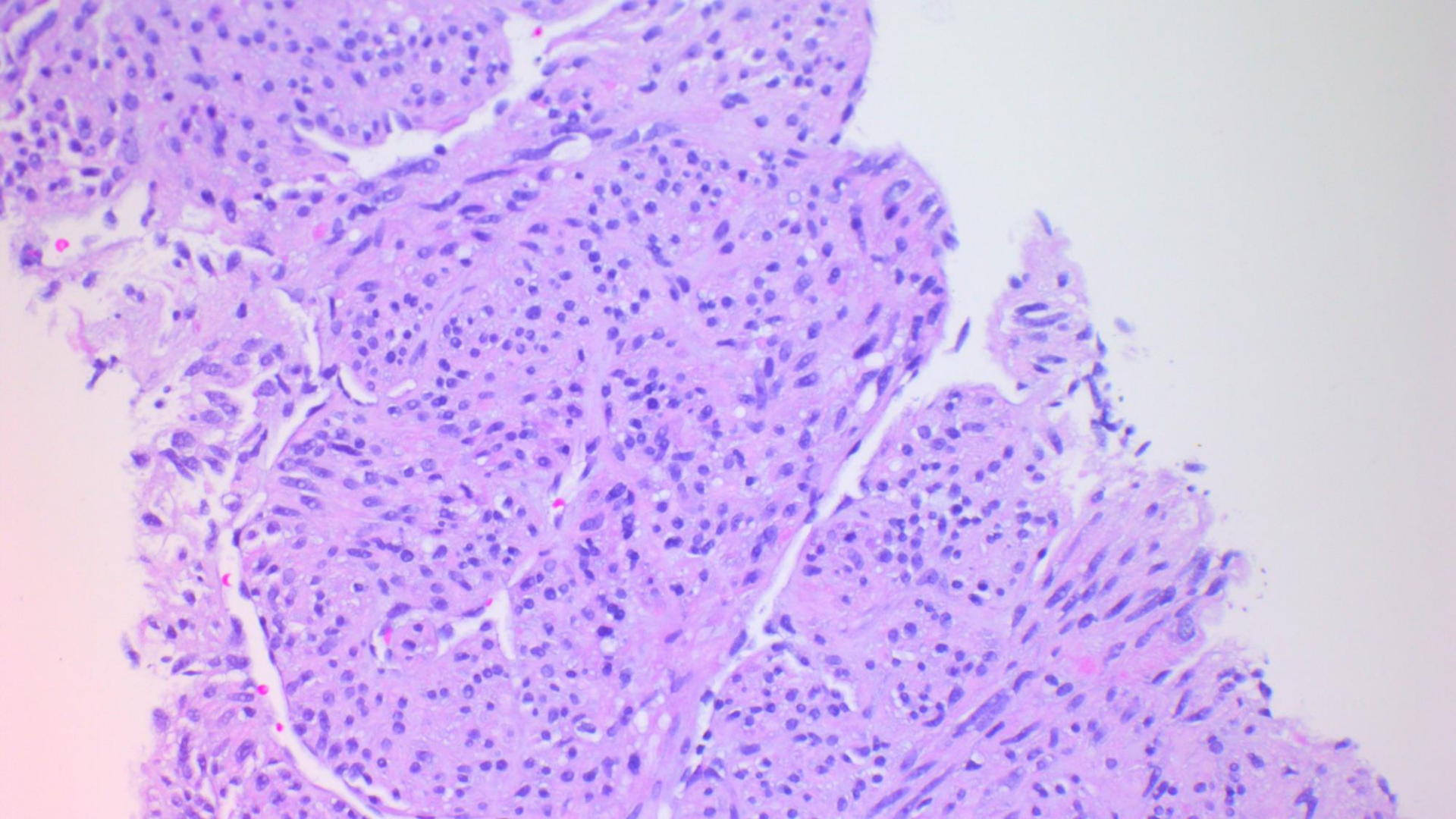

Alice:
"HOW LONG IS FOREVER?"
White Rabbit:
"SOMETIMES, JUST ONE SECOND."

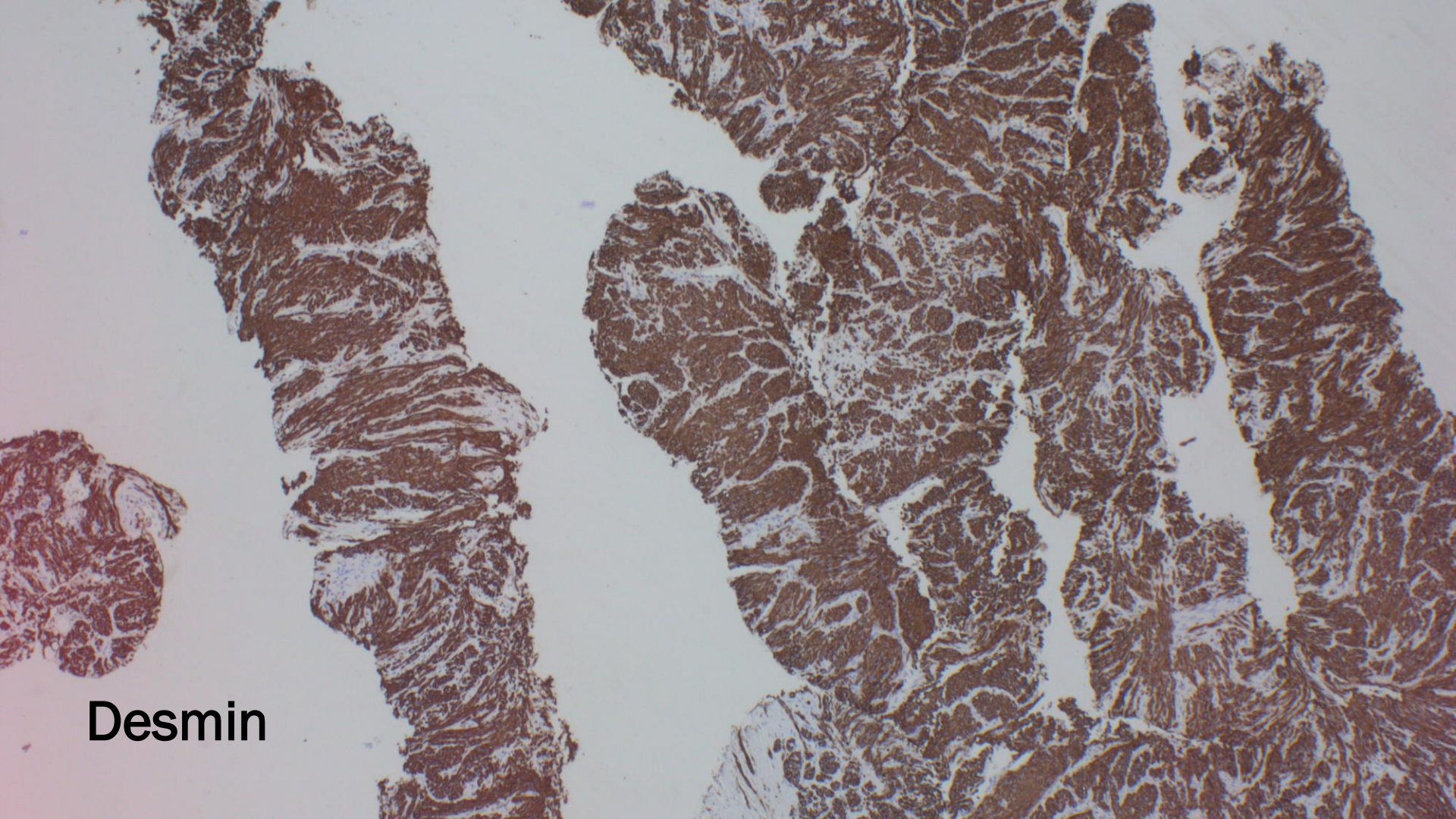
Case 5

- 75 y.o. with pelvic mass likely of uterine origin, left inguinal lesion (? left adnexa protruding into inguinal canal vs separate mass), and right iliac crest and left mid iliac bone lesions (myeloma vs metastases)
- The inguinal canal lesion was biopsied





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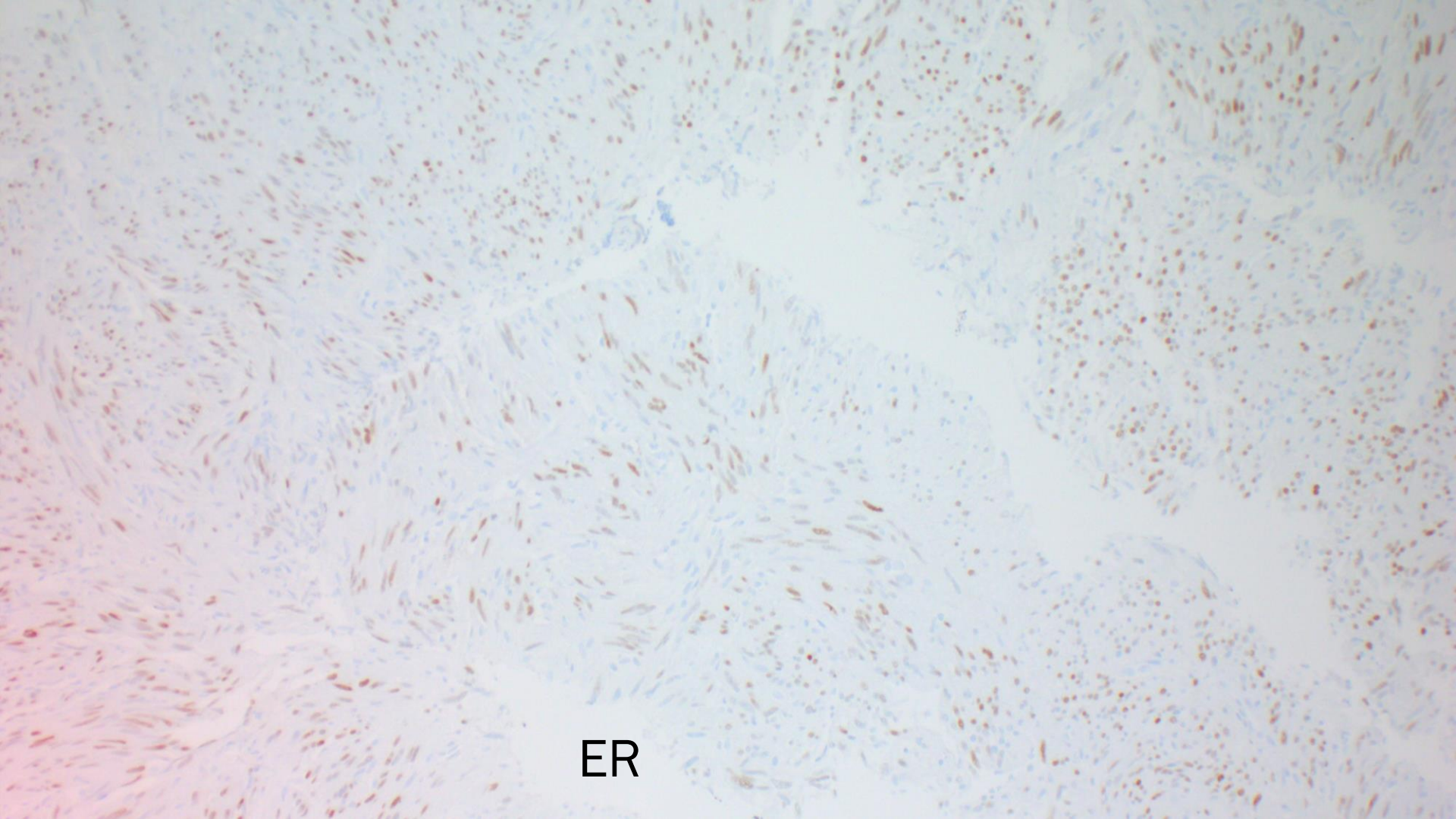
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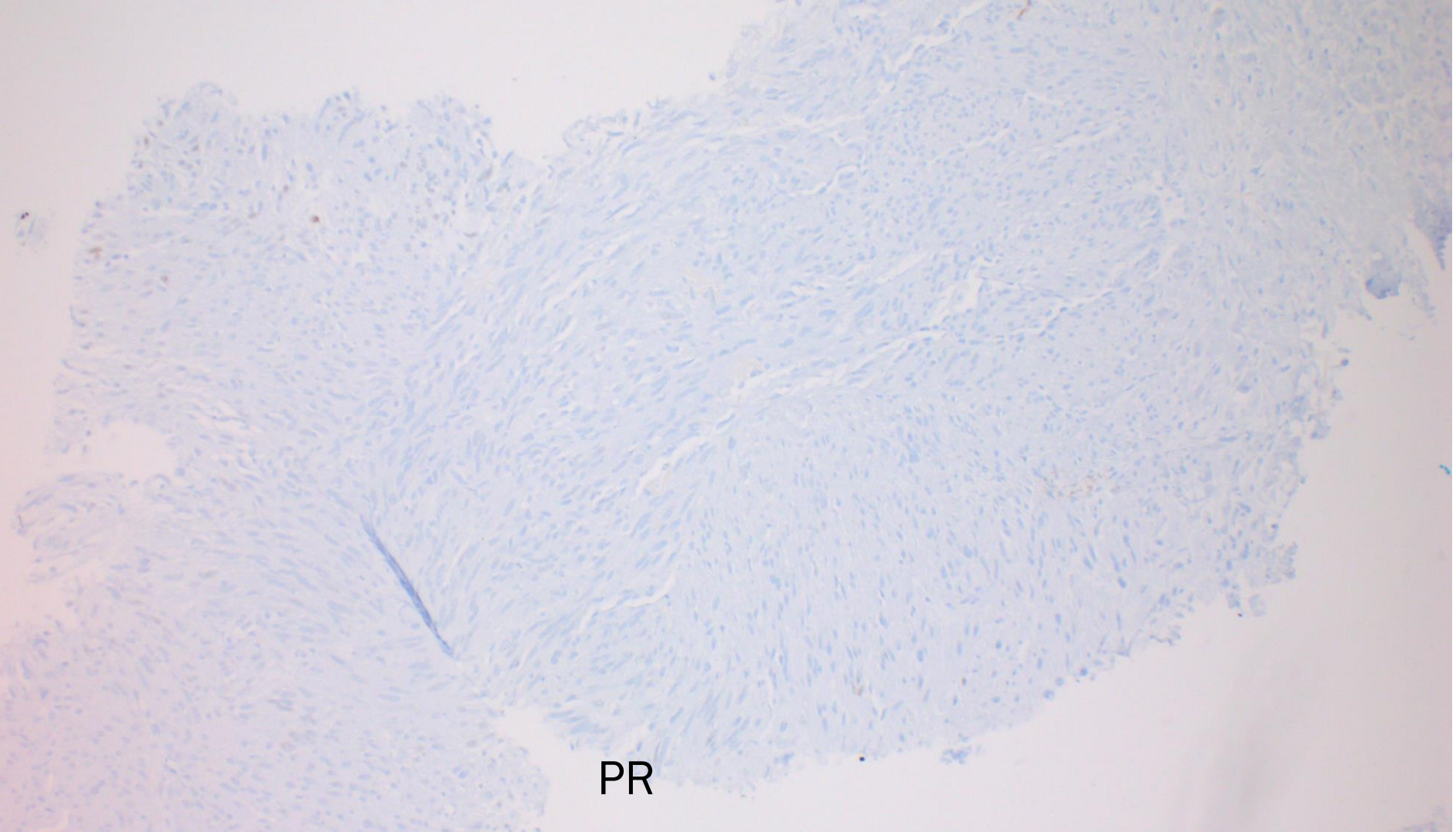
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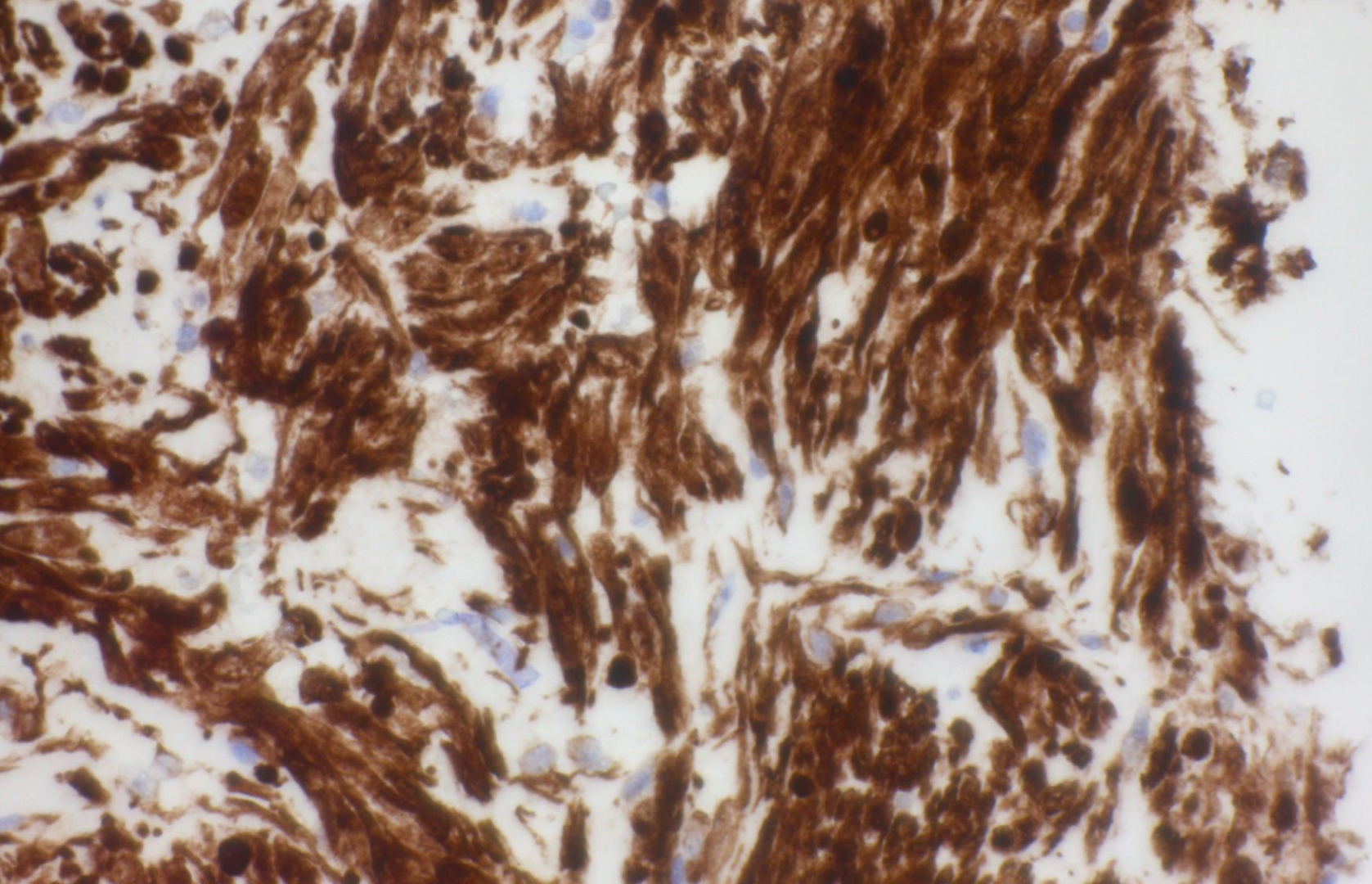
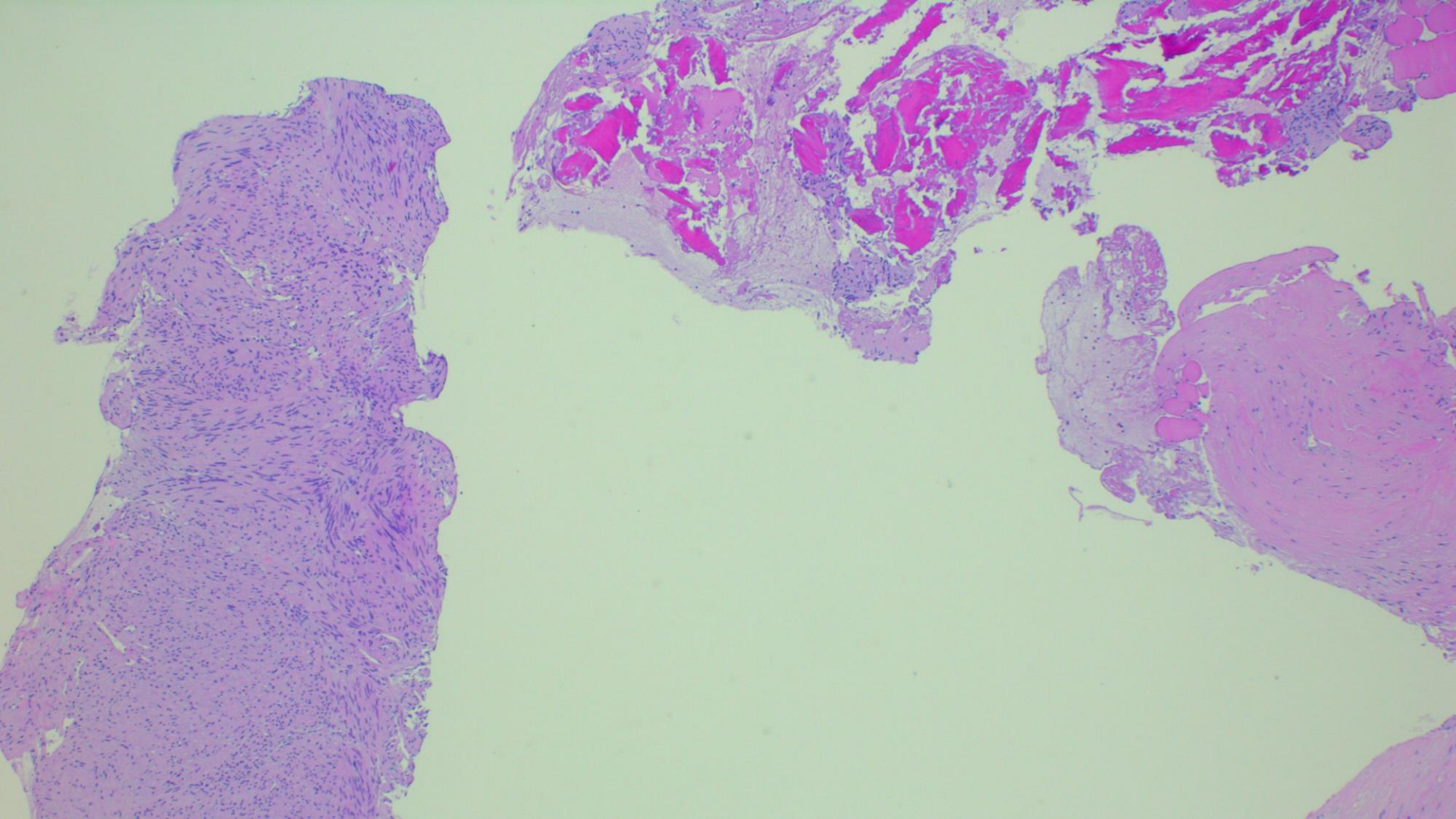


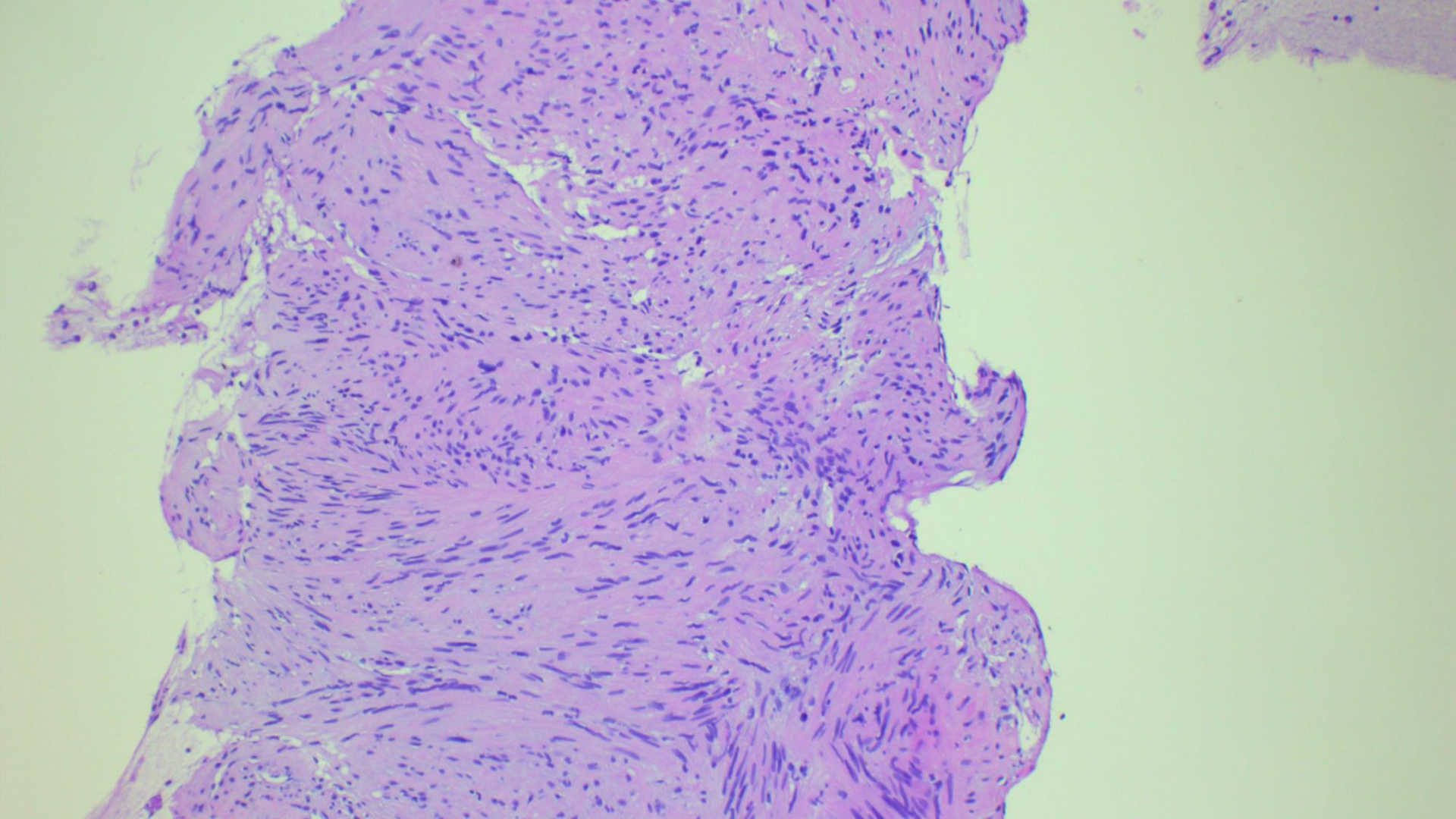
WT1

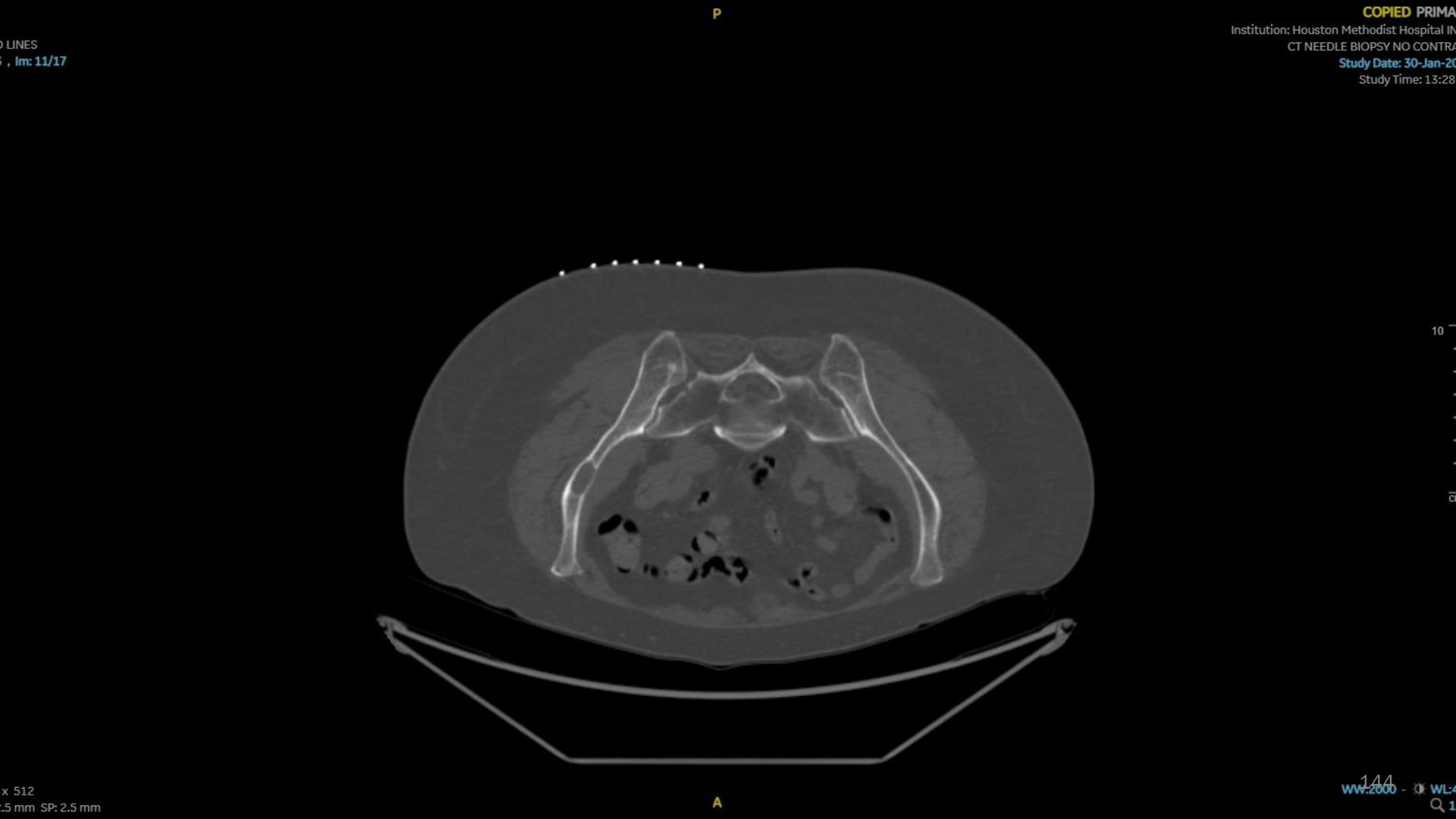
Case 5

- Smooth muscle neoplasm with no malignant features
- Favor gyn-type leiomyoma

- The left iliac bone lesion was biopsied






P
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Institution: Houston Methodist Hospital IN
CT NEEDLE BIOPSY NO CONTRA
Study Date: 30-Jan-20
Study Time: 13:28
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144
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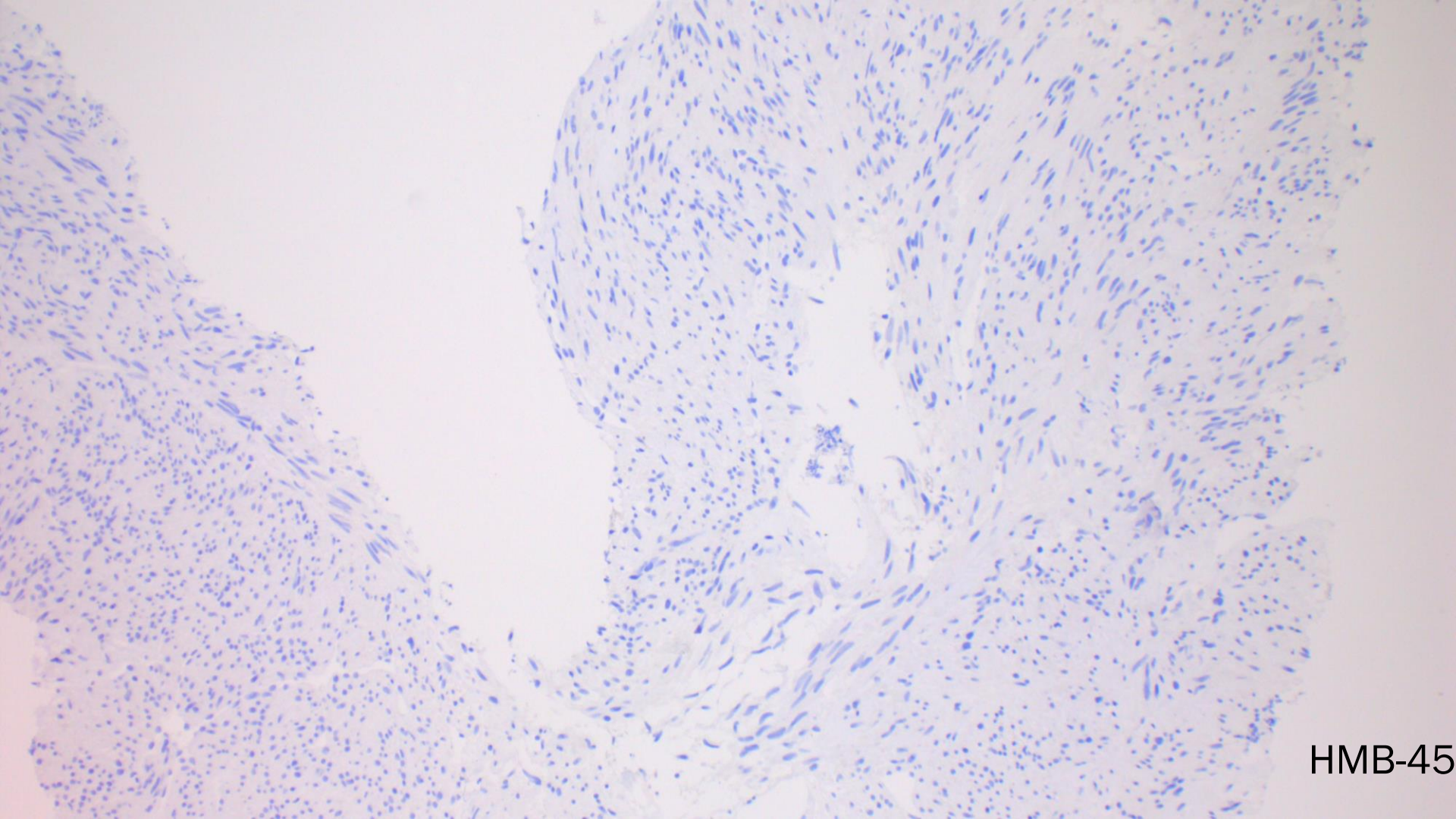
Who Are You?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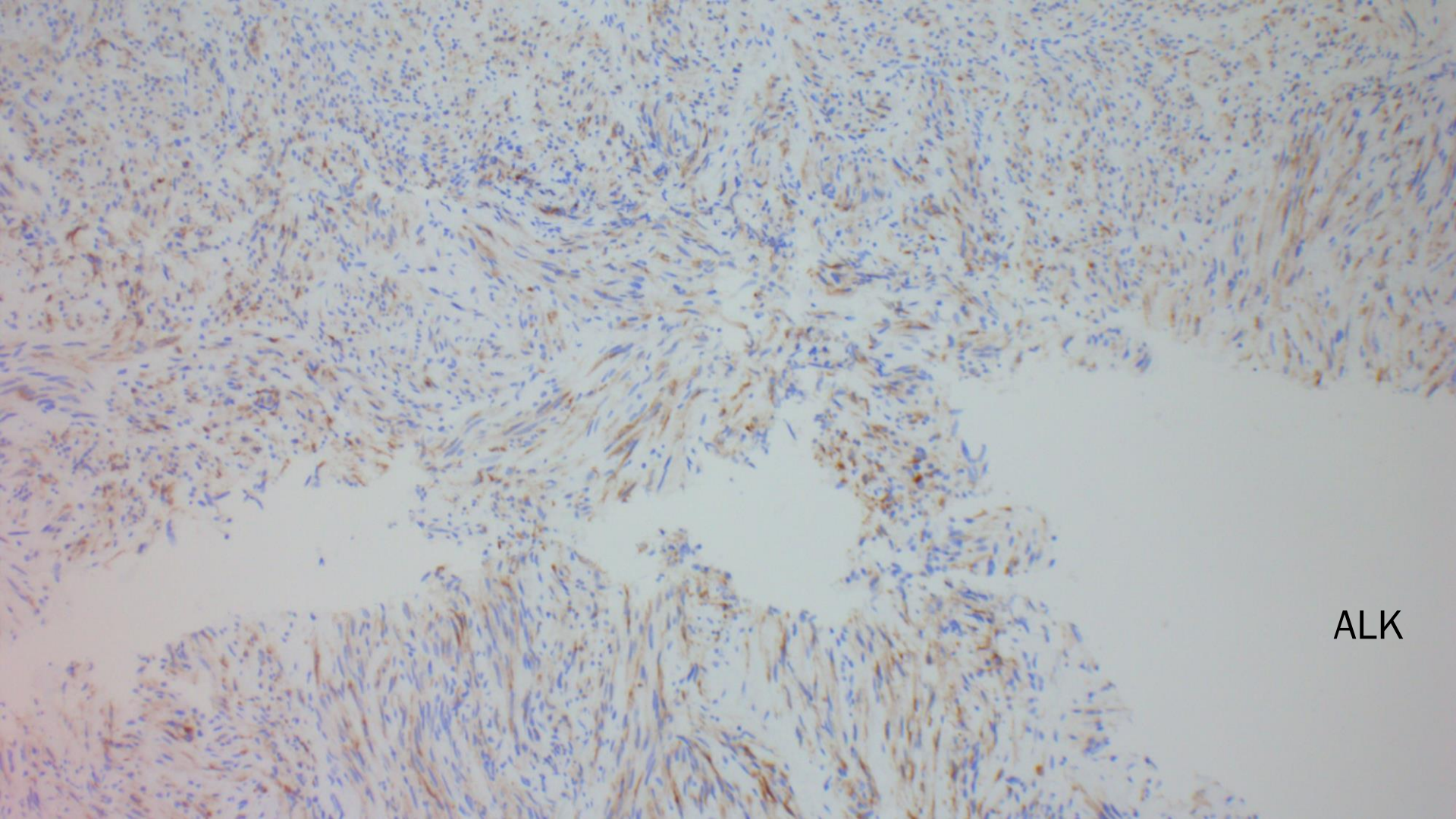

ALK

Case 5

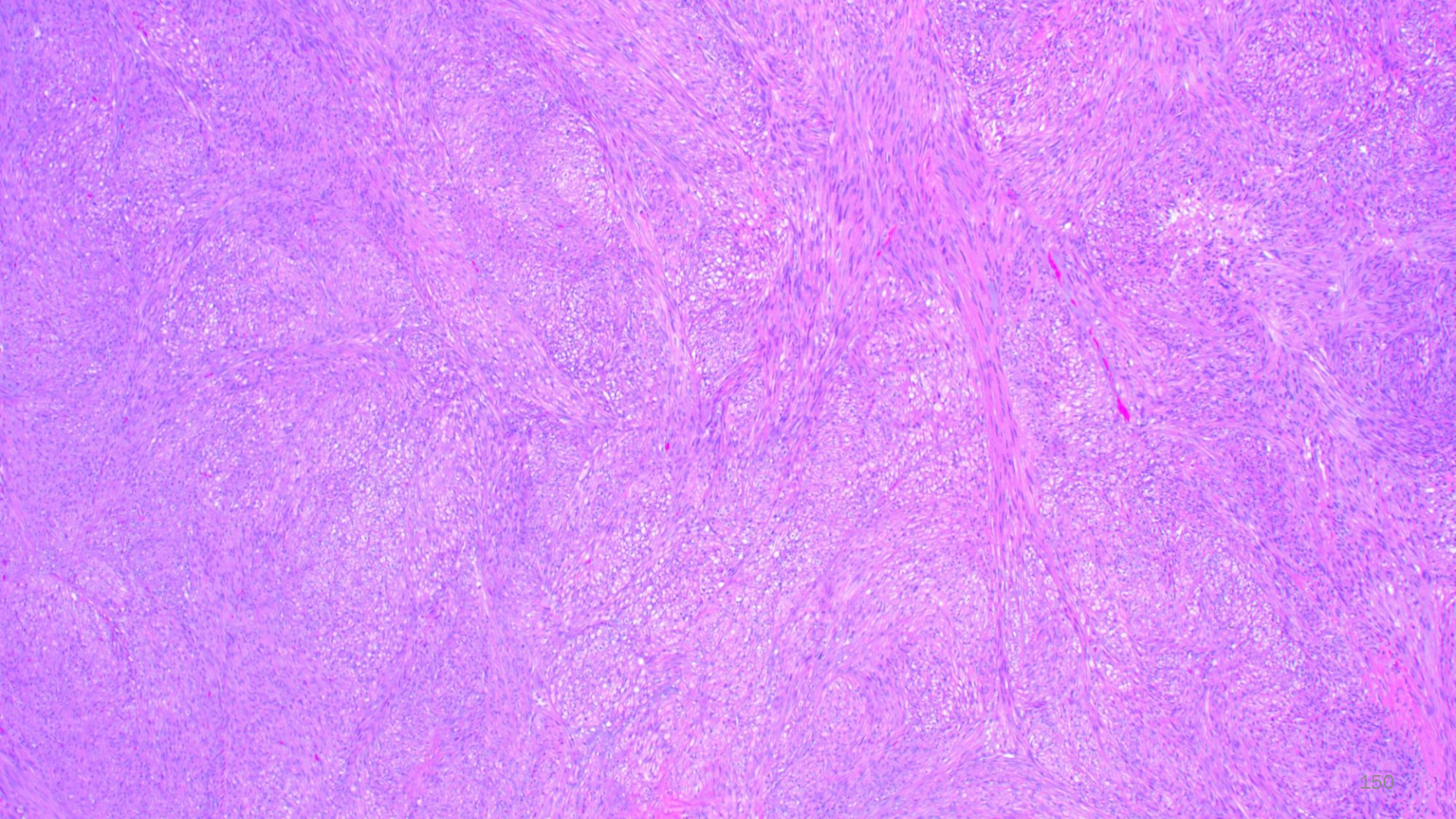
- ALK FISH Positive

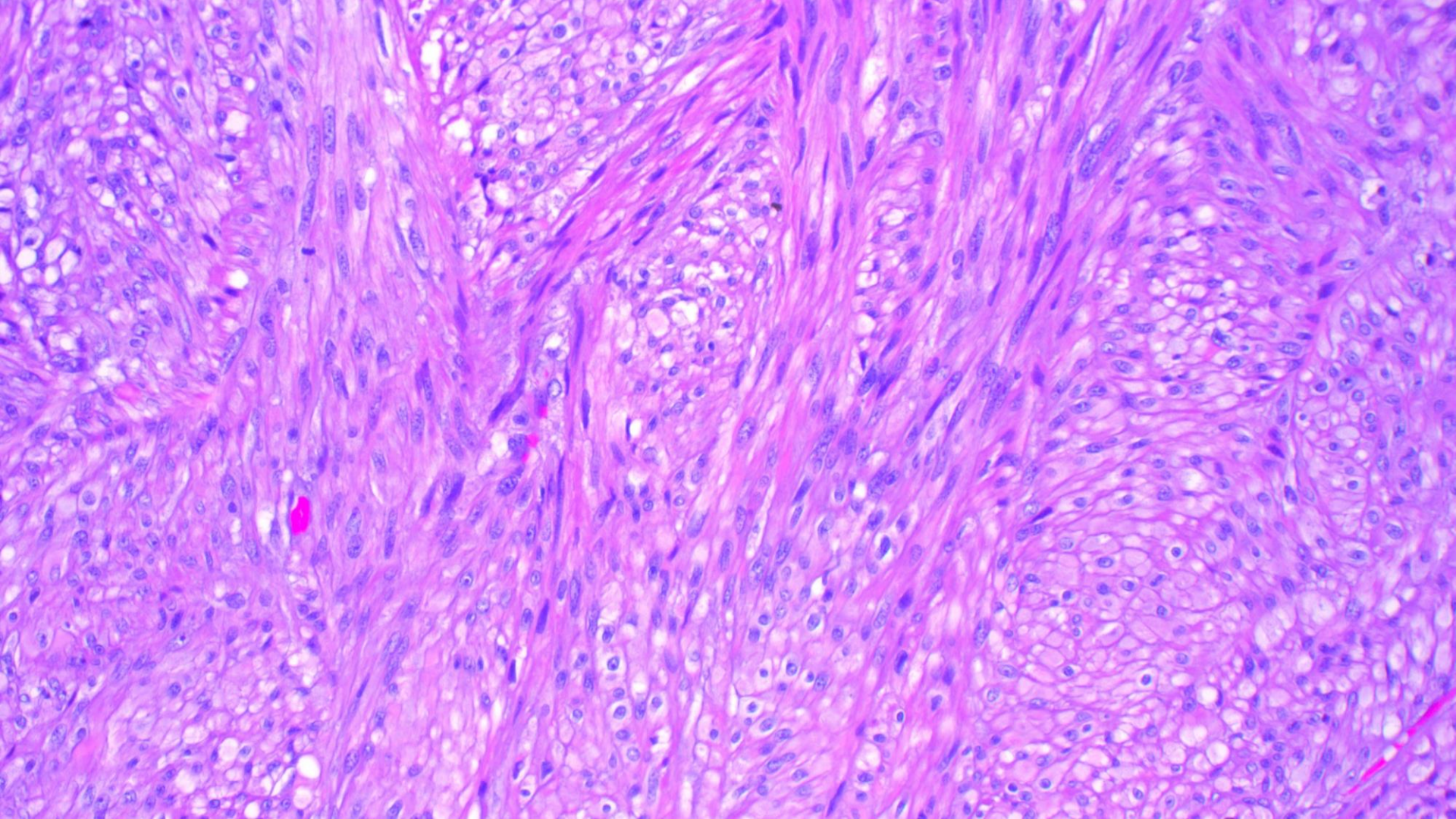
- Inflammatory Myofibroblastic Tumor

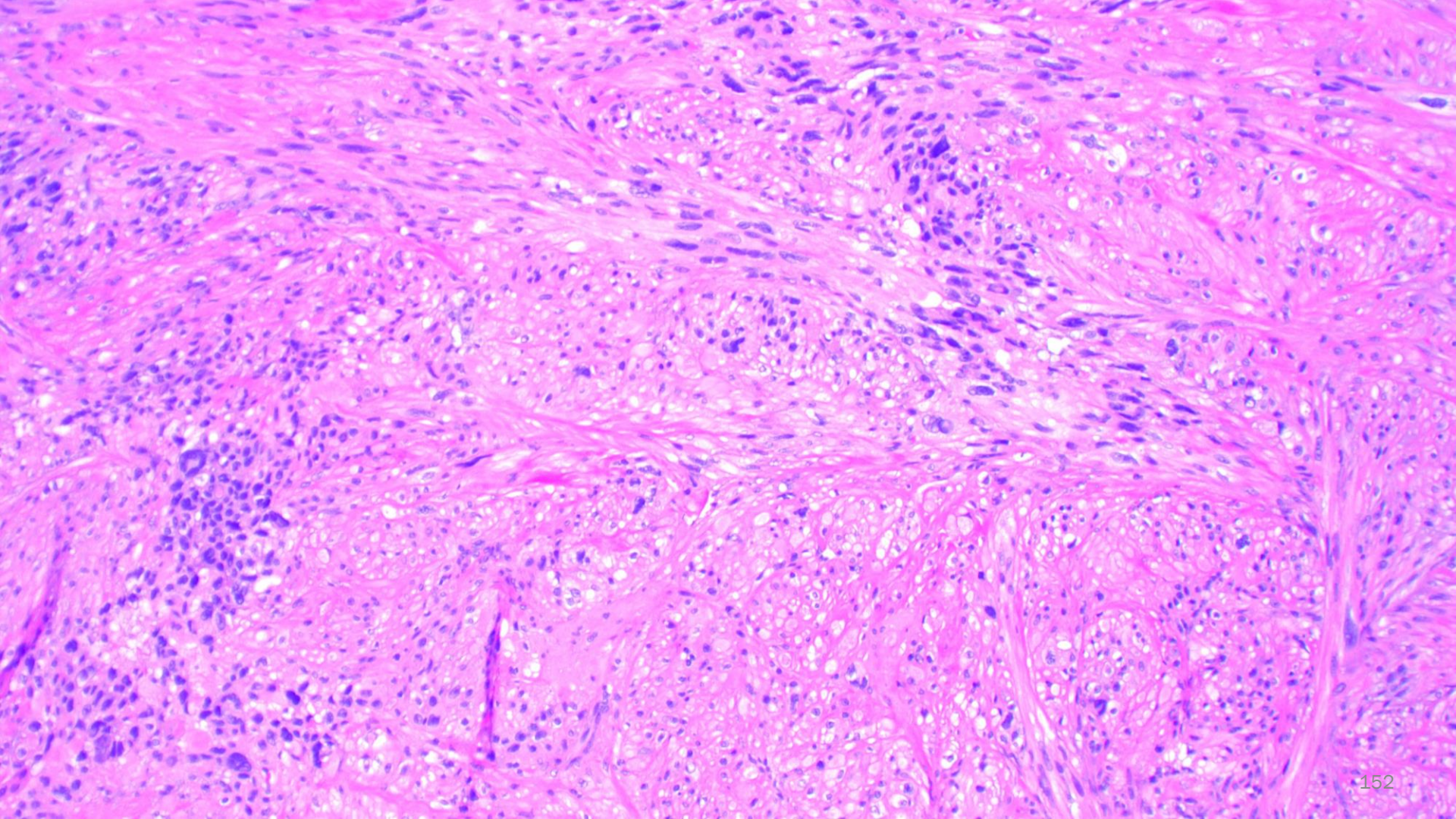
Case 5

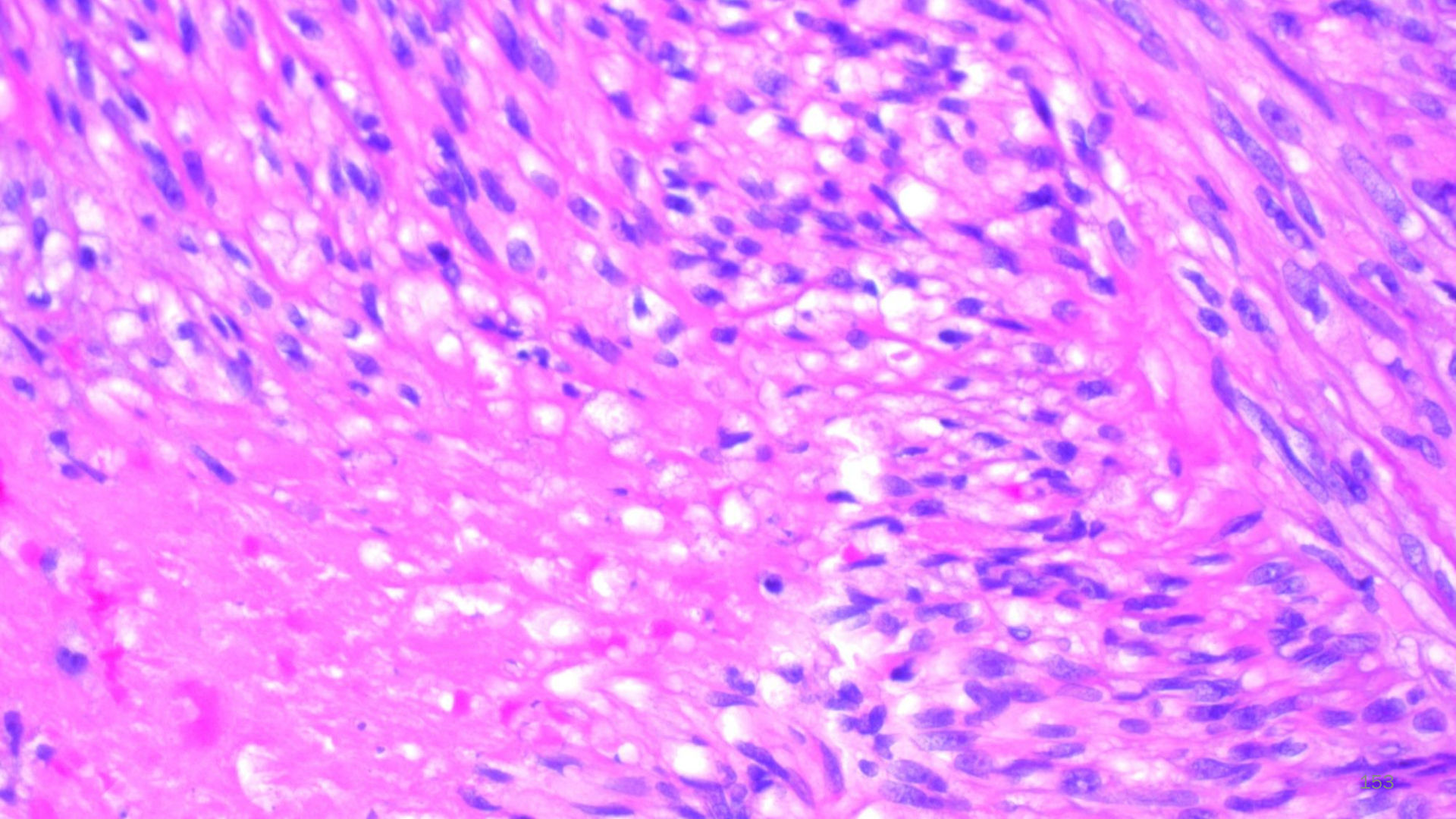
- Hysterectomy performed

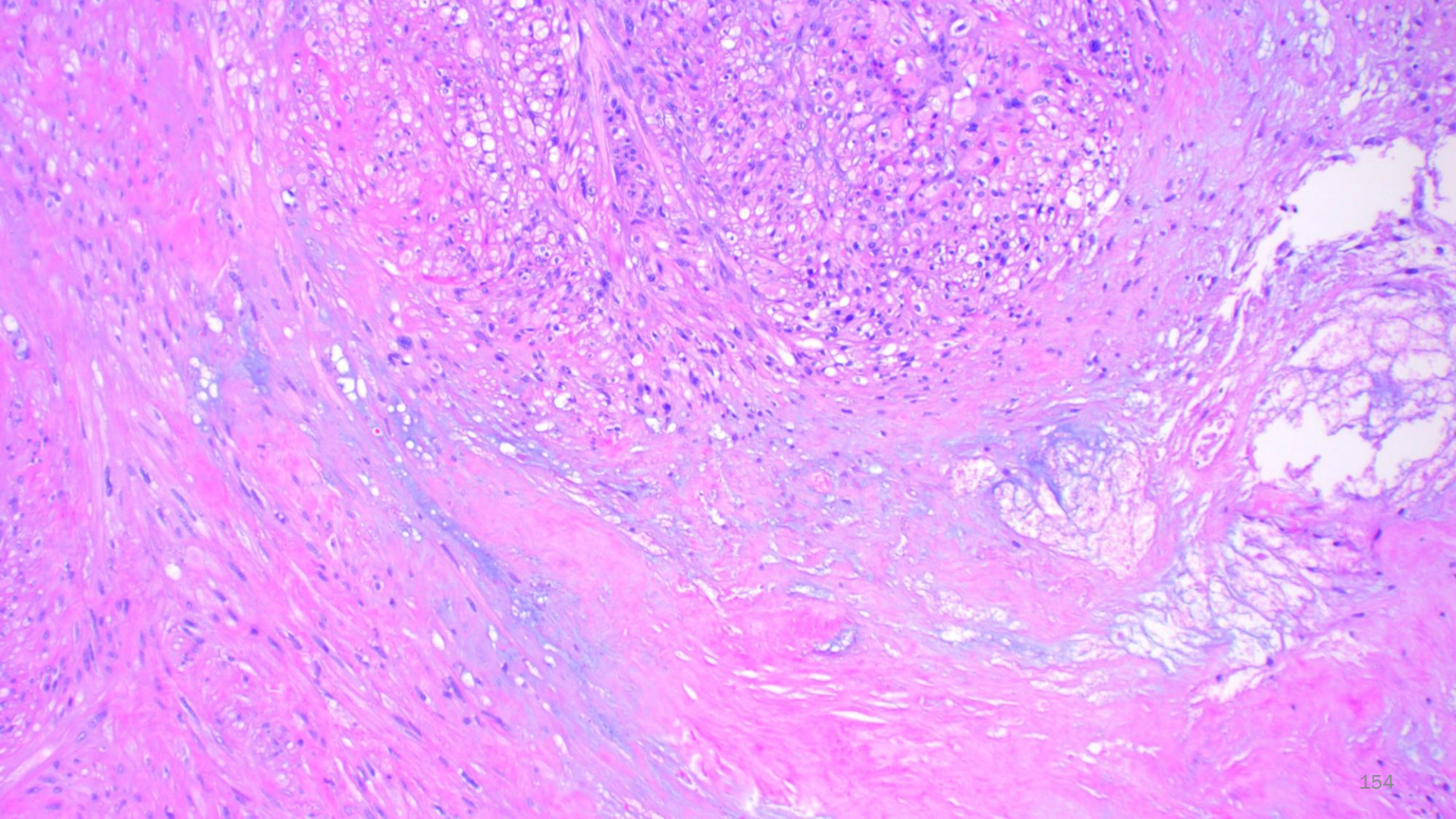
- Fusion Panel: TNS1::ALK fusion, predicted to be activating











Uterine Inflammatory Myofibroblastic Tumor

- Pickett JL, Chou A, Andrici JA, et.al. Inflammatory myofibroblastic tumors of the female genital tract are under-recognized: a low threshold for ALK immunohistochemistry is required
  - 0.3% previously diagnosed leiomyomas → IMTs
  - 2.3% previously diagnosed LMS → IMTs
- Tumors with ALK activation may be sensitive to Alk inhibitors

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Uterine Inflammatory Myofibroblastic Tumors

Immunohistochemistry

- SMA: 89 – 100%
- Desmin: 67 – 100%
- ER: 25 – 67%
- PR: 75 – 100%
- CD10: 33 – 100%
- ALK: 87.5 – 100%

Uterine Inflammatory Myofibroblastic Tumor

- Hypocellular, fascicular, hyalinized patterns
- Variable degree of myxoid change, often with a loose nodular fasciitis-like appearance
- Variable degree of lymphoplasmacytic inflammation

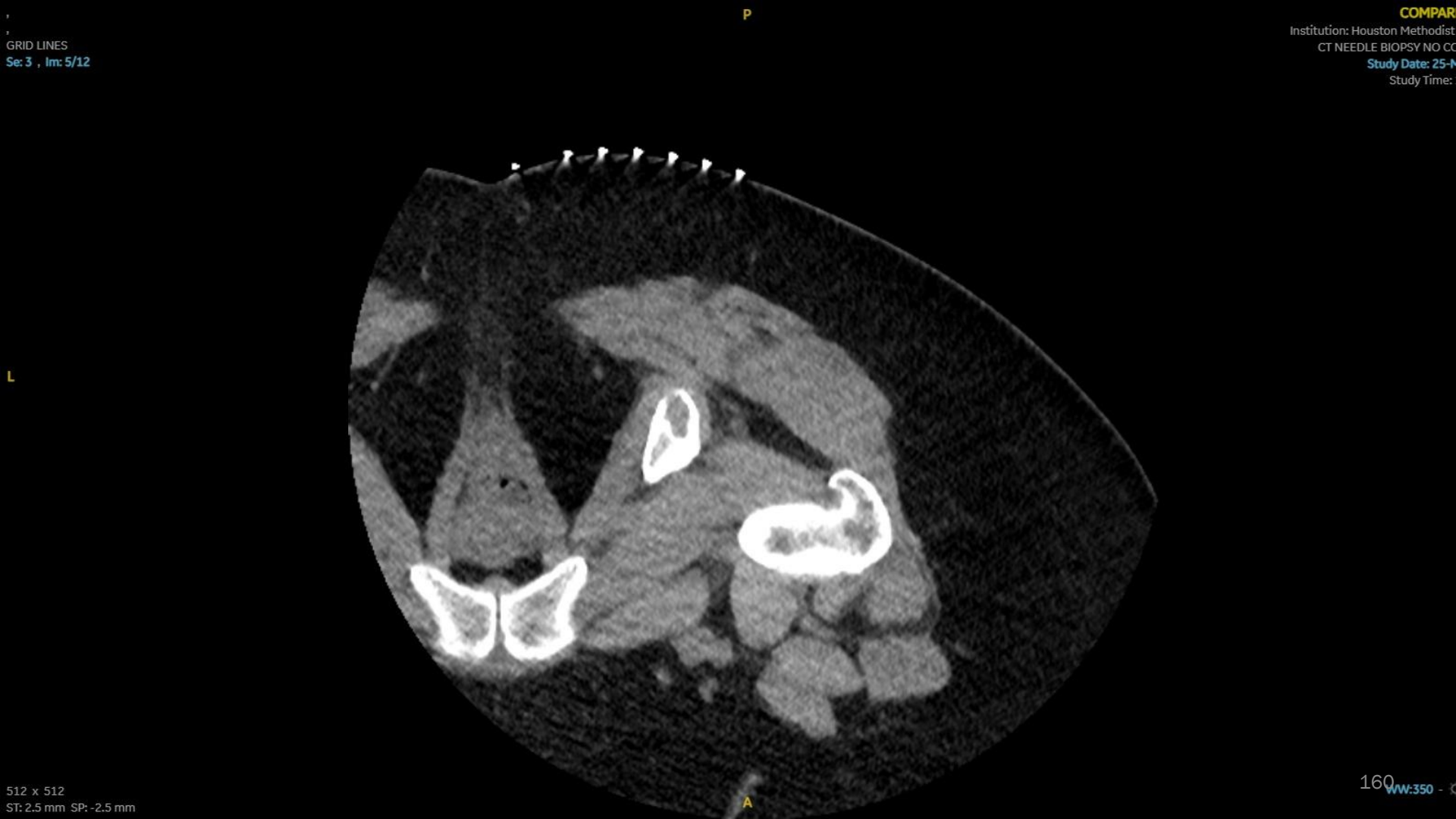
Uterine Inflammatory Myofibroblastic Tumor

Features associated with aggressive behavior

- Age > 45 years
- Size ≥ 5 cm
- ≥ 4 mitoses / 10 hpf
- Infiltrative borders

Lagniappe

- 32 y.o. with SLE on prednisone, tacrolimus, and mycophenolate
- Leiomyoma, lung 2022
- 6.6 cm central liver mass, 1.6 cm right ischial bone lesion with cortical breakthrough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GRID LINES
Se: 3 , Im: 5/12
P
COMPAR
Institution: Houston Methodist
CT NEEDLE BIOPSY NO CO
Study Date: 25-M
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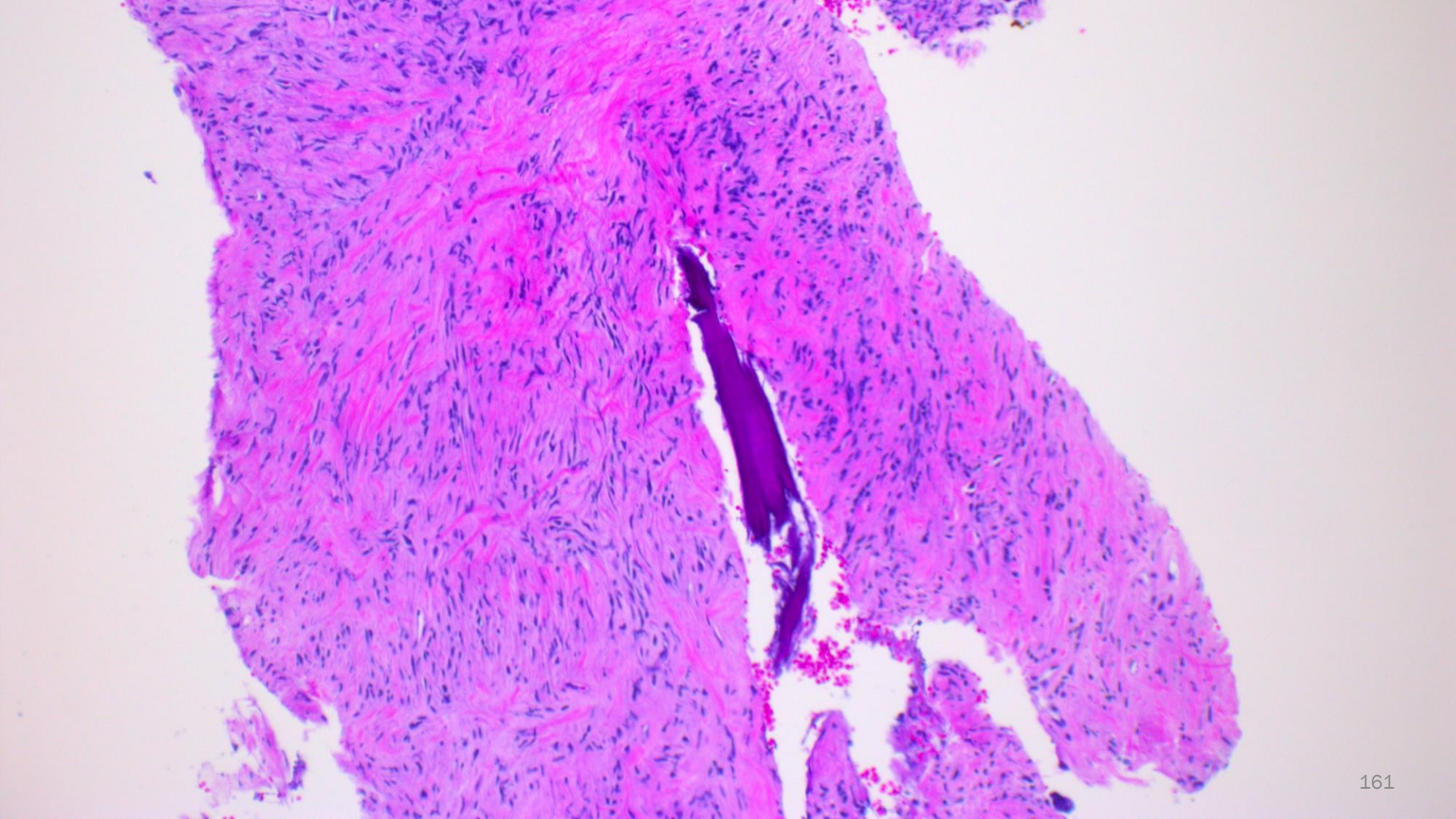
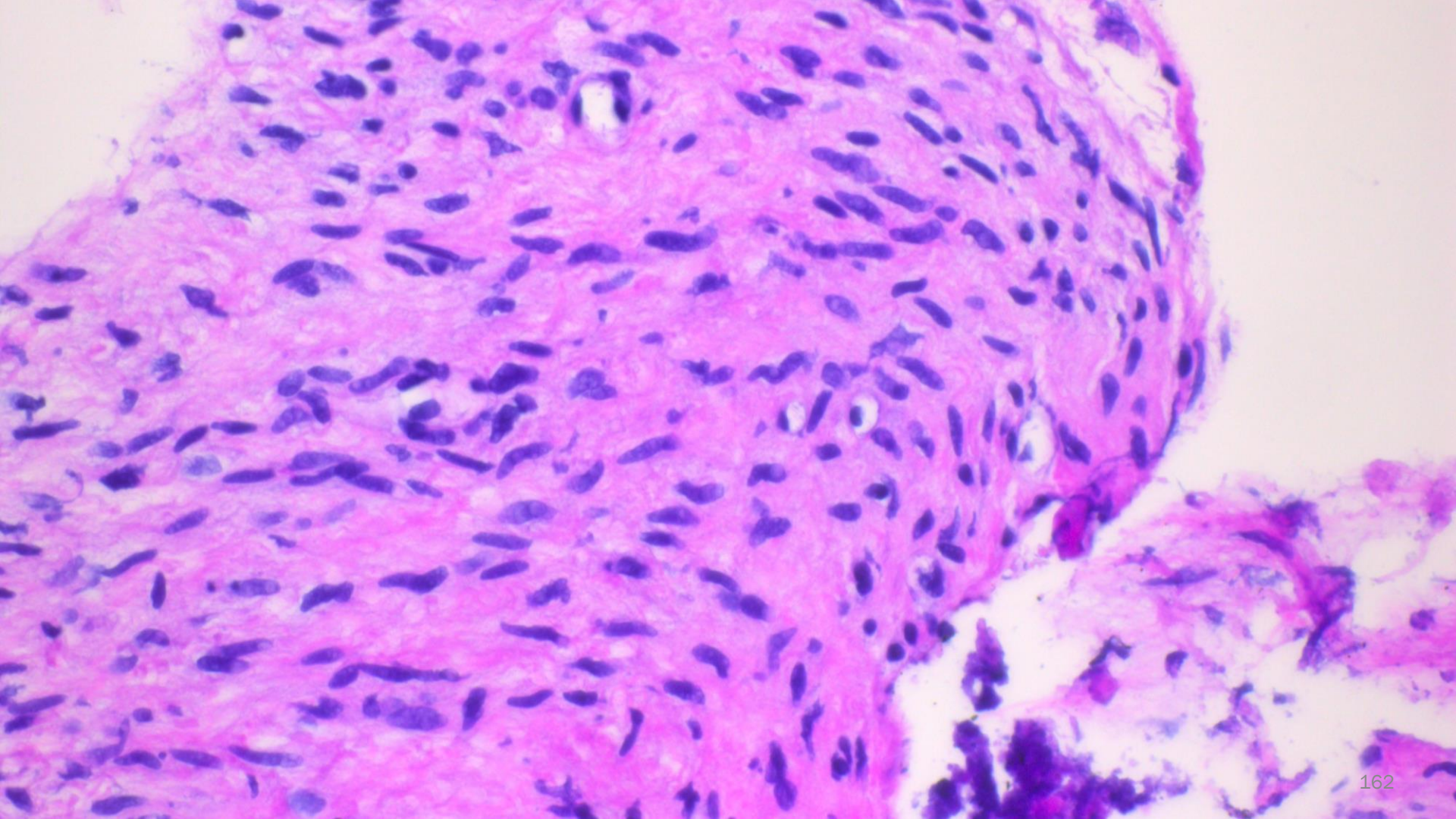
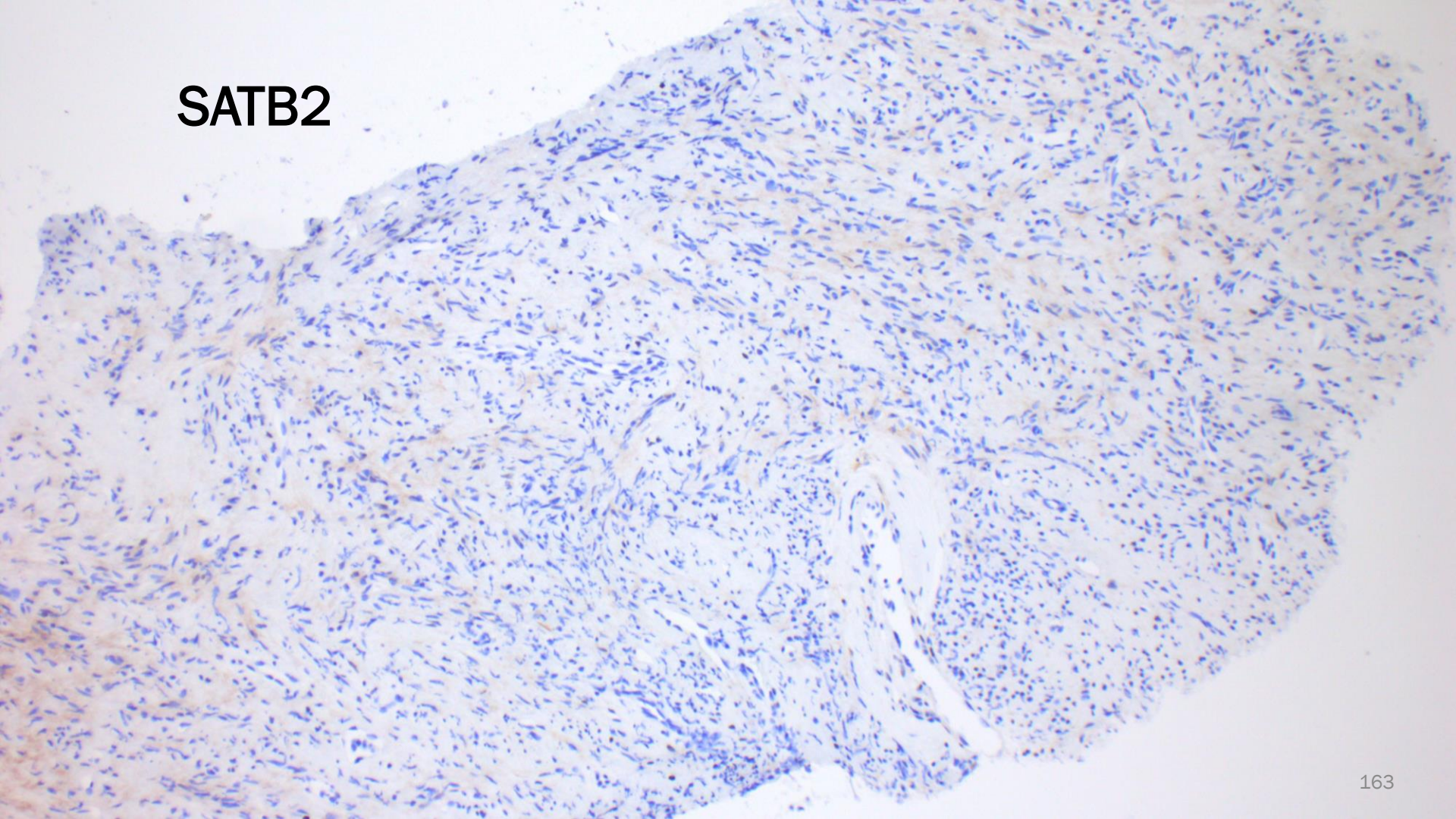
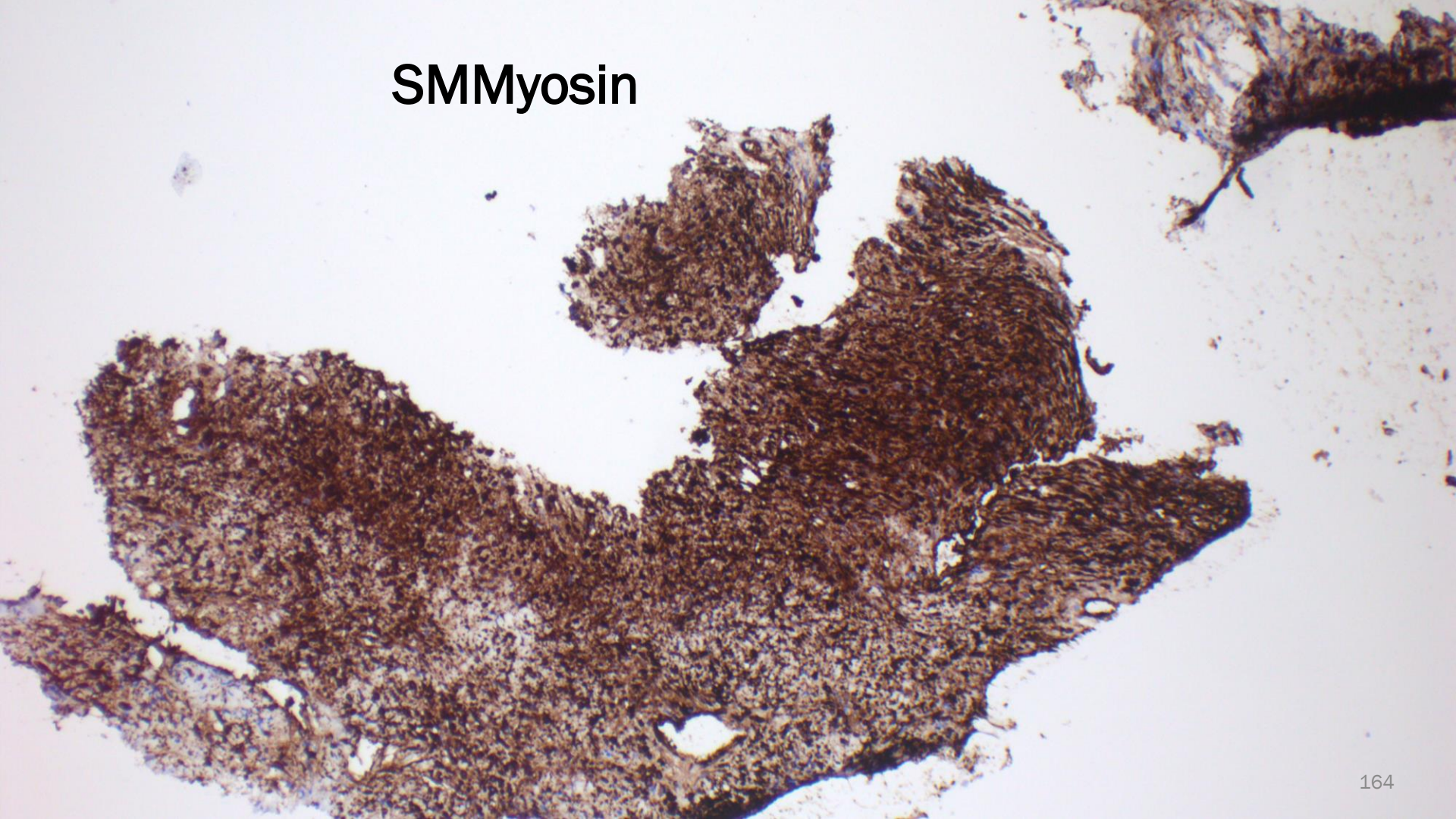
512 x 512
ST: 2.5 mm SP: -2.5 mm
A
160
WW:350

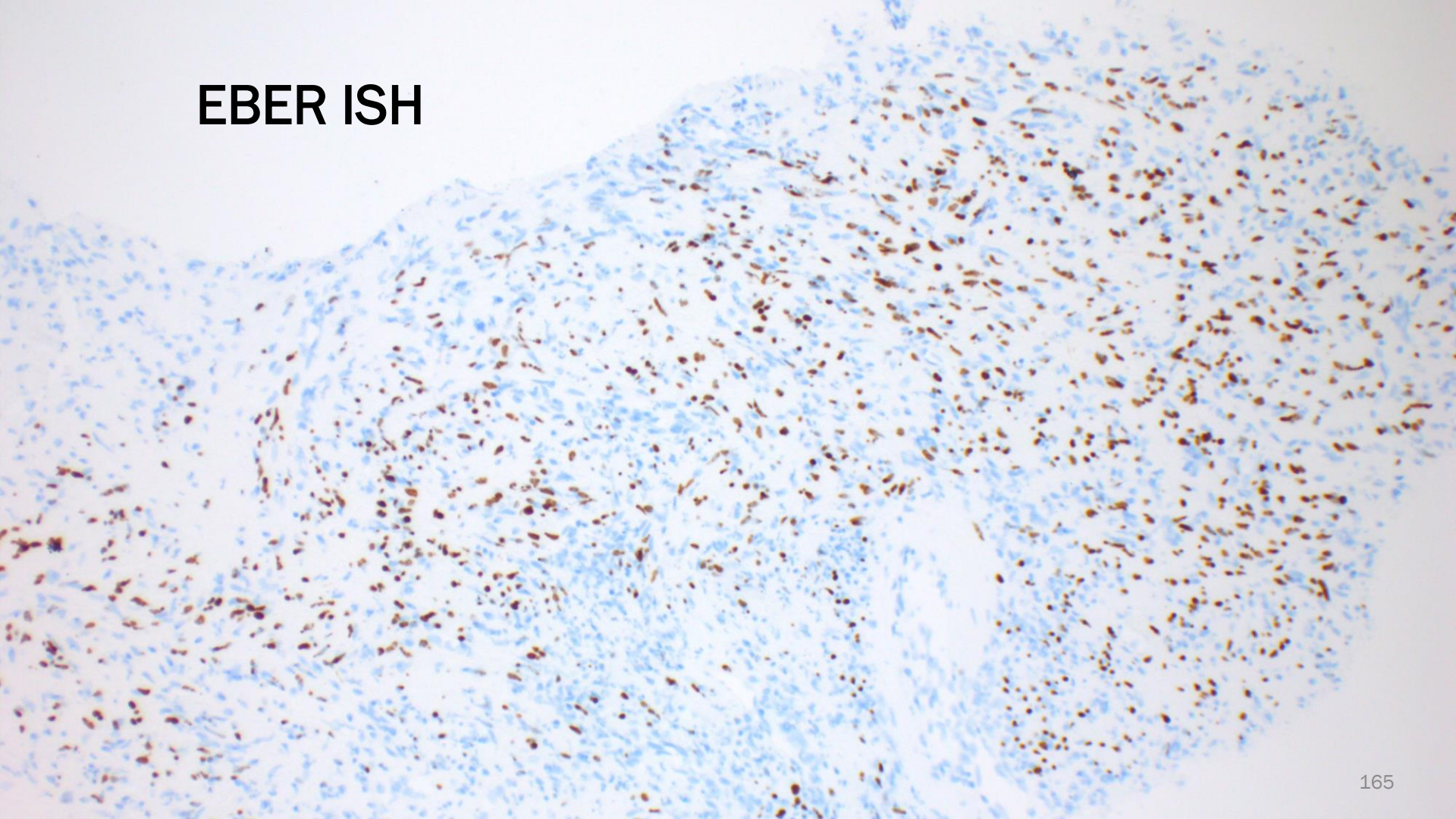





SATB2


SMMyosin

EBER ISH



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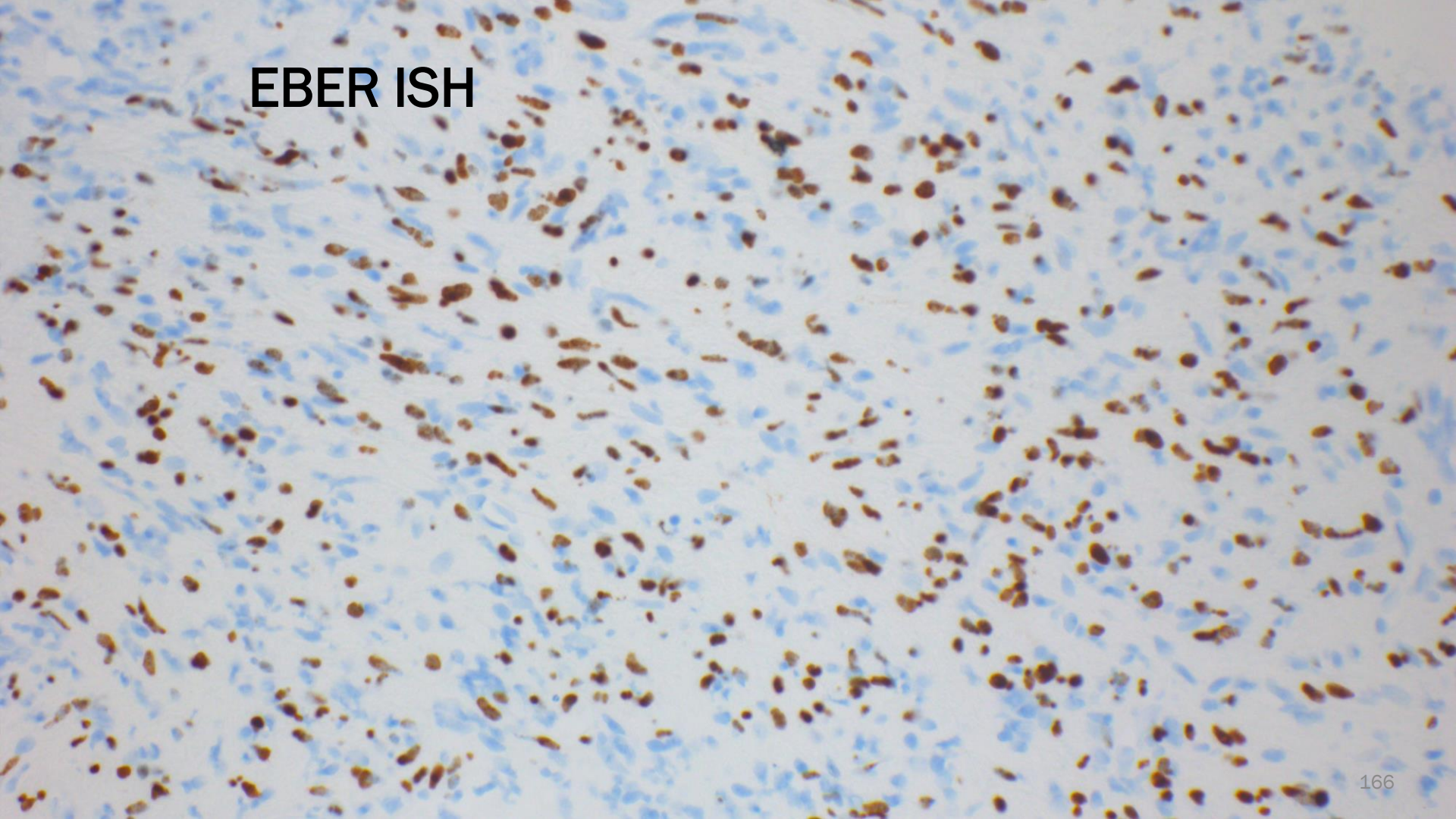


Lagniappe

- EBV associated smooth muscle tumor

Adventures in IHC Land

Stay out of the rabbit hole

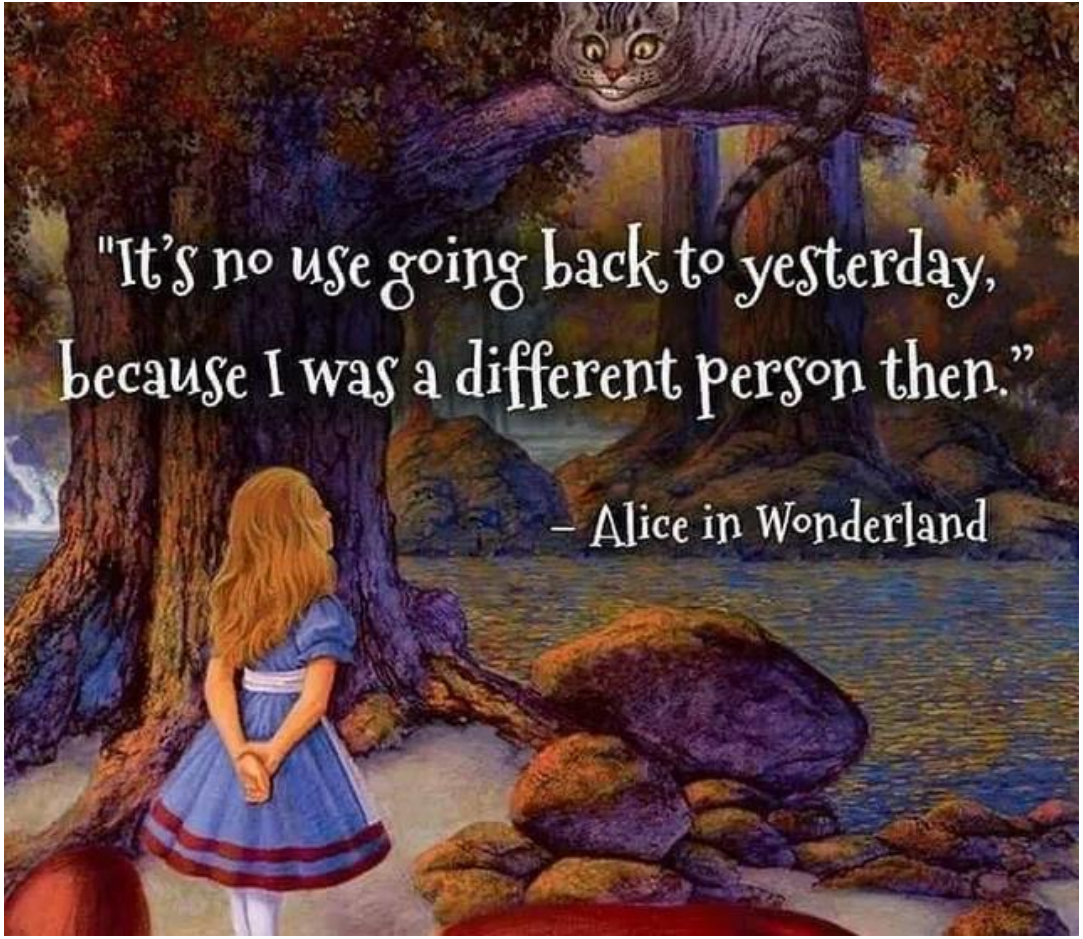
- “Pankeratin” (e.g. AE1/AE3) may not highlight all carcinomas. Consider cocktails that have a broader spectrum, other keratins (CK5/6, CK8/18), other epithelial markers (EMA, p63)
- Undifferentiated carcinoma of the endometrium morphologically can mimic other tumors and may not stain diffusely for keratin. Consider EMA, MMR, SALL4

Adventures in IHC Land

Stay out of the rabbit hole

- Gynecologic smooth muscle tumors often express ER, PR, and WT1 (nuclear), while soft tissue SMTs do not
- DOG1 can be positive in gynecologic smooth muscle tumors
- Inflammatory Myofibroblastic Tumors can mimic gynecologic smooth muscle tumors, but treatment may be different. Have a low threshold for considering ALK IHC

THANK YOU!



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