Diagnostic Immunohistochemistry in Gynecologic Pathology

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IHC in Gynecologic Pathology

- Vulva
  - Squamous dysplasia
  - Paget's disease
- Vagina
  - Squamous dysplasia
  - Metastatic carcinomas
- Cervix
  - Squamous dysplasia
  - Glandular lesions

IHC in Gynecologic Pathology

- Uterine corpus-epithelial
  - Tumor types
- Uterine corpus-stromal
  - Endometrial stromal
  - Smooth muscle
- Tube
  - STIC
- Ovary

IHC in Gynecologic Pathology

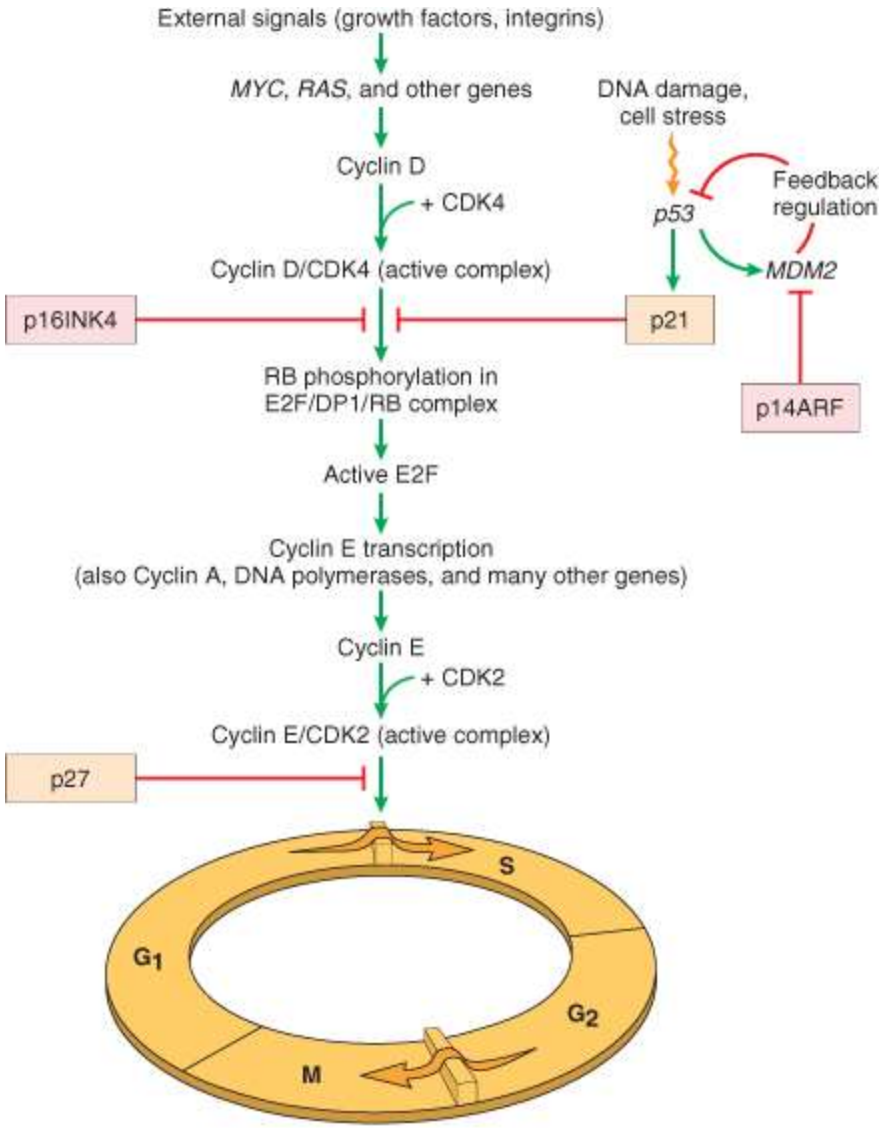
- Ovary
  - Epithelial
  - Stromal
  - Germ cell
  - Metastases
- Peritoneum
  - Epithelial versus mesothelial
  - Mullerian versus other sites

HPV related squamous dysplasia

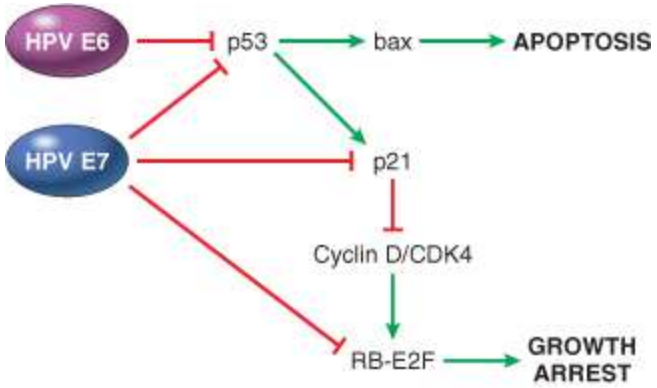
- Cervix, vagina, vulva, perineum, anal canal and peri-anal skin
- Stains (for establishing high grade dysplasia)
  - P16
  - Ki-67
- CIN1 versus normal
  - Stains cannot reliably distinguish between CIN1 and normal/reactive changes

HPV related squamous dysplasia

- CIN2, CIN3, VAIN2, VAIN3, VIN2, VIN3, AIS
  - Diffuse strong p16 reactivity
  - Increased Ki-67 labeling index
- What to do when p16 and Ki-67 are discordant
  - If Ki-67 labeling index is not increased, p16 is less reliable
    - P16 could be occasionally strong in metaplastic epithelium
  - If p16 is negative, diagnose high grade dysplasia only when
    - Ki-67 staining is in "atypical" epithelium and not in inflammatory cells
    - Ki-67 staining seen beyond the basal/para-basal layer



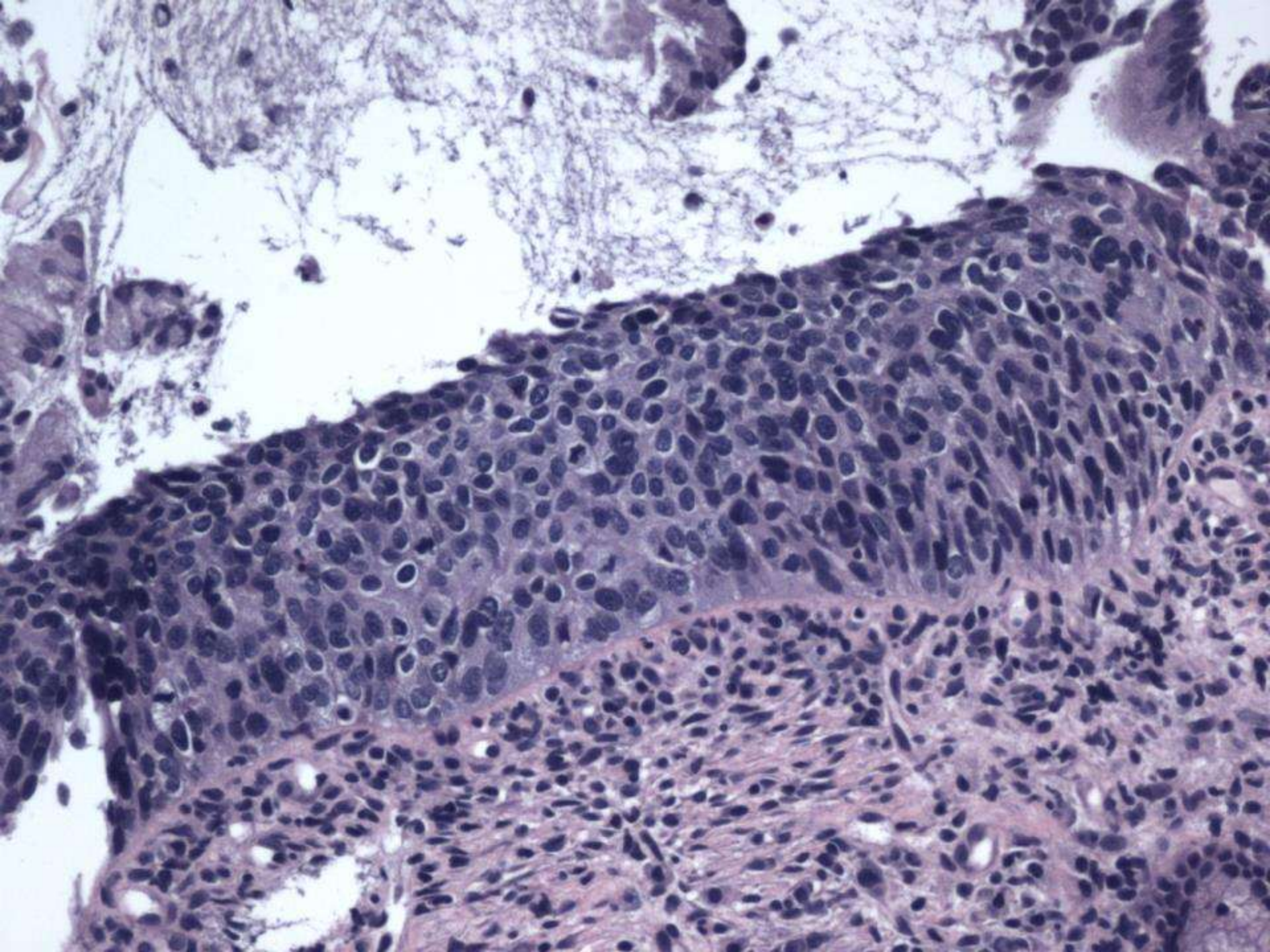
Role of p16 in cell cycle

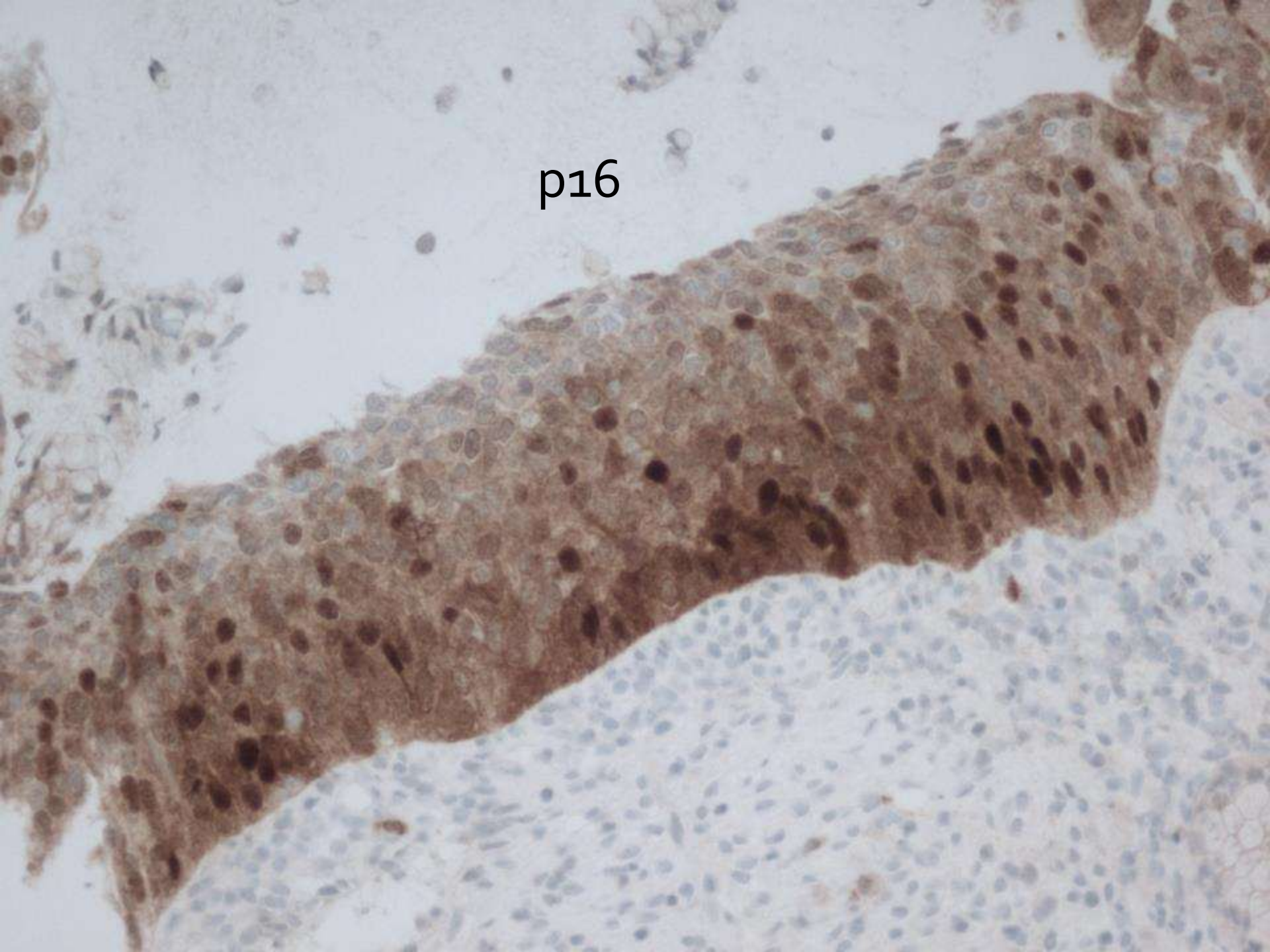


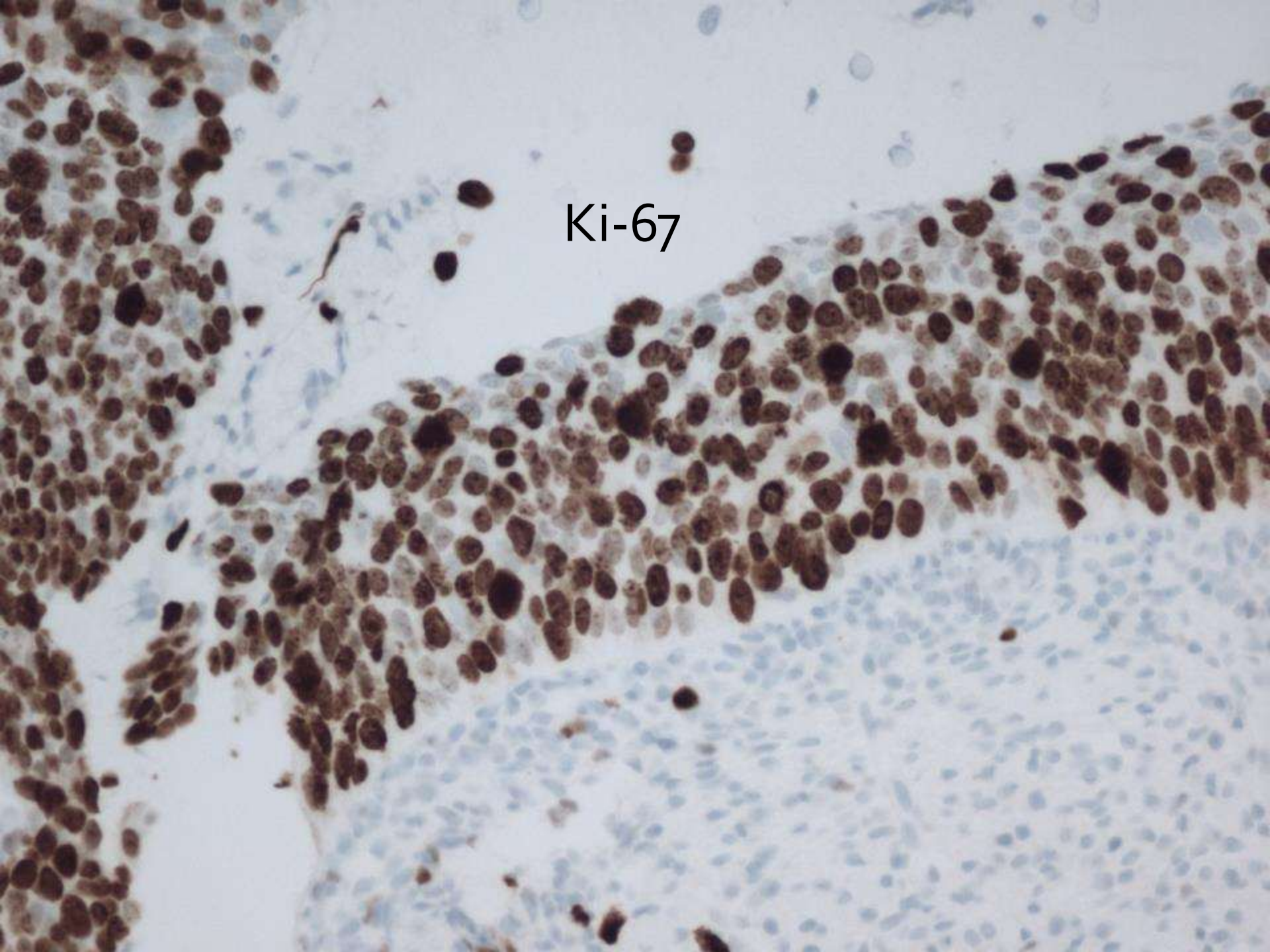
Effect of HPV on Cell Cycle

Increased expression of p16 in cervical carcinoma is possibly a compensatory response to cell disturbances brought on by the viral oncogenes

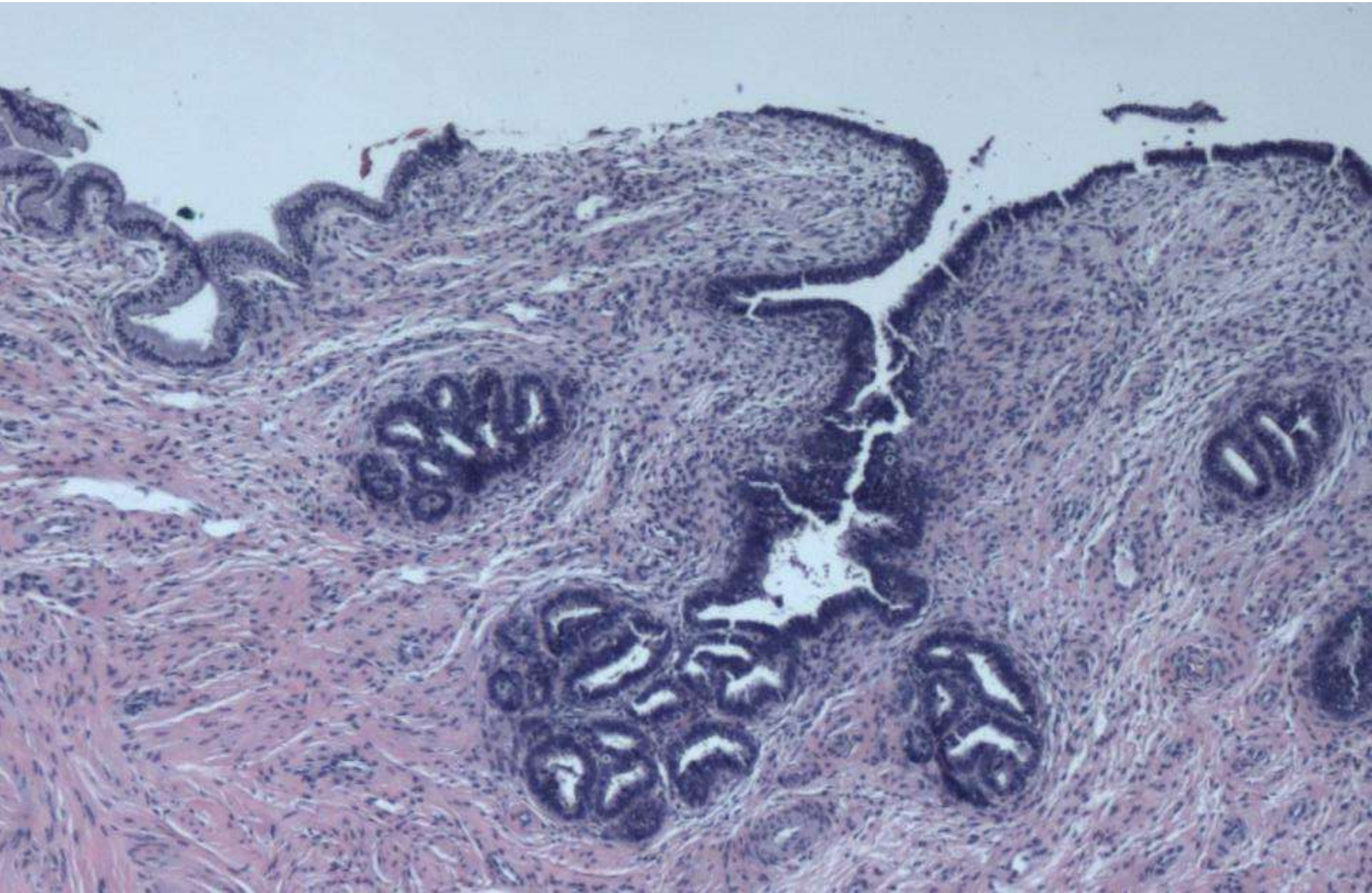
From: Robbins and Cotran Pathologic Basis of Disease. 7th Edition. 2005

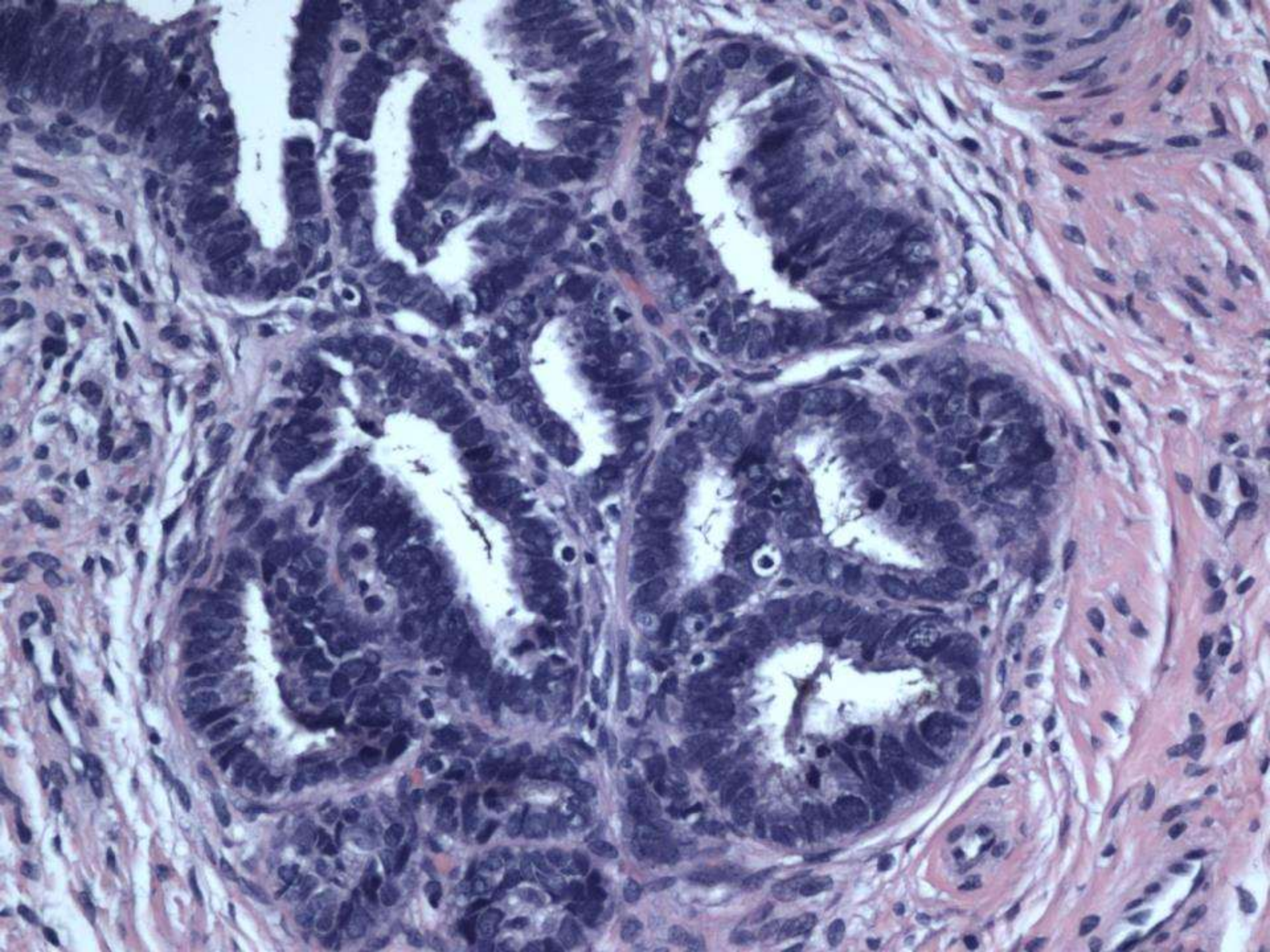


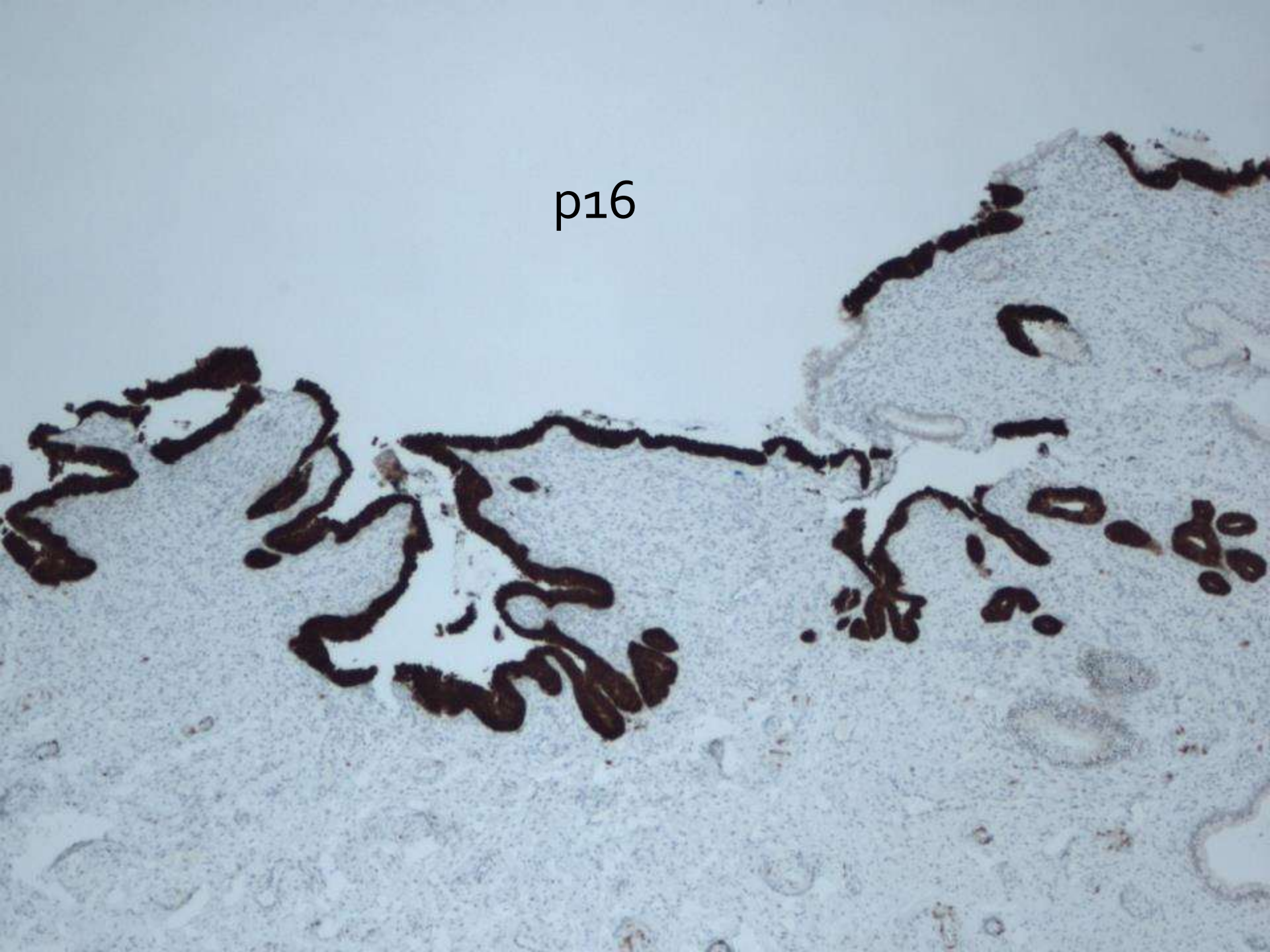

p16

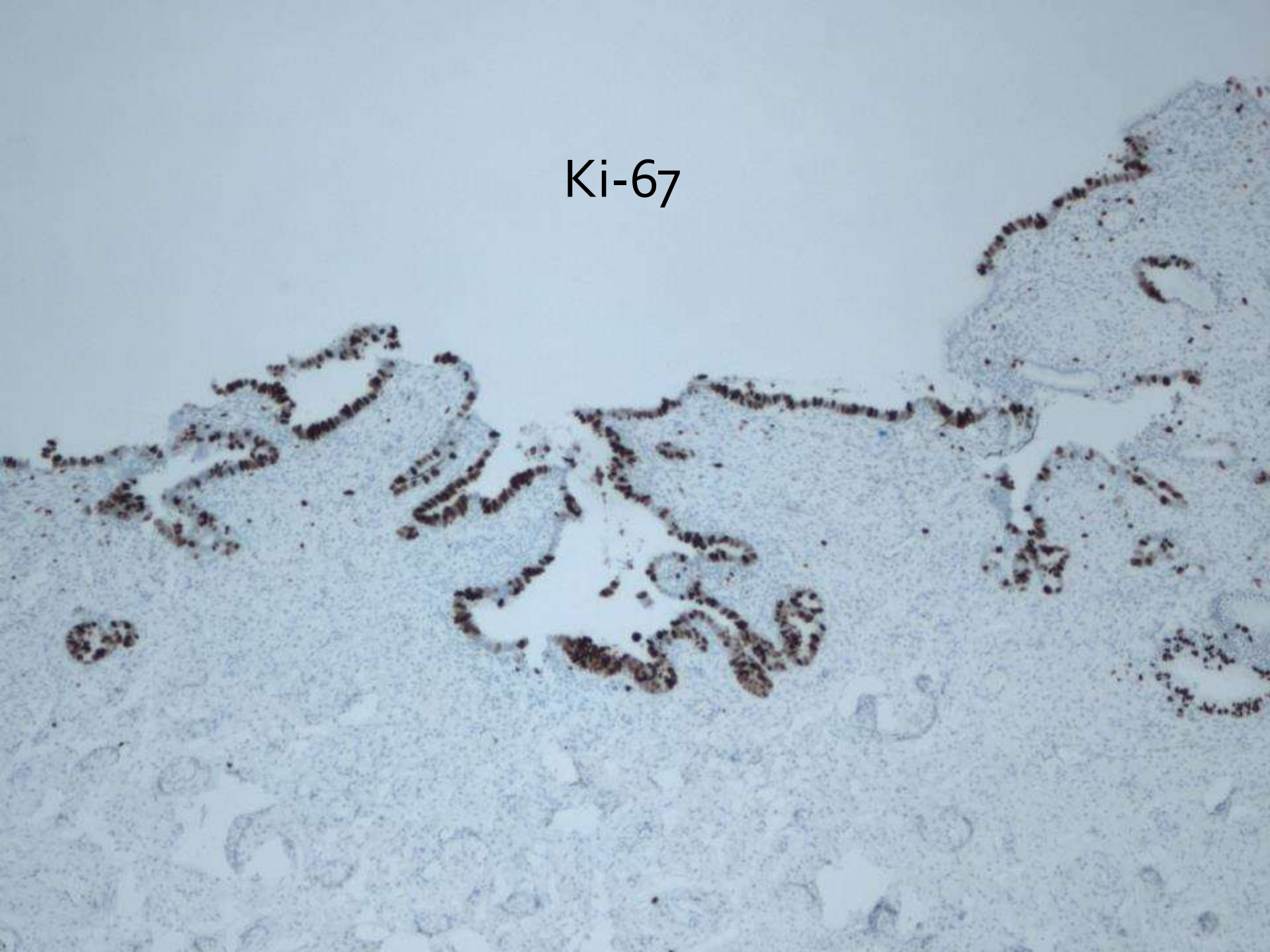

Ki-67

Endocervical adenocarcinoma in-situ



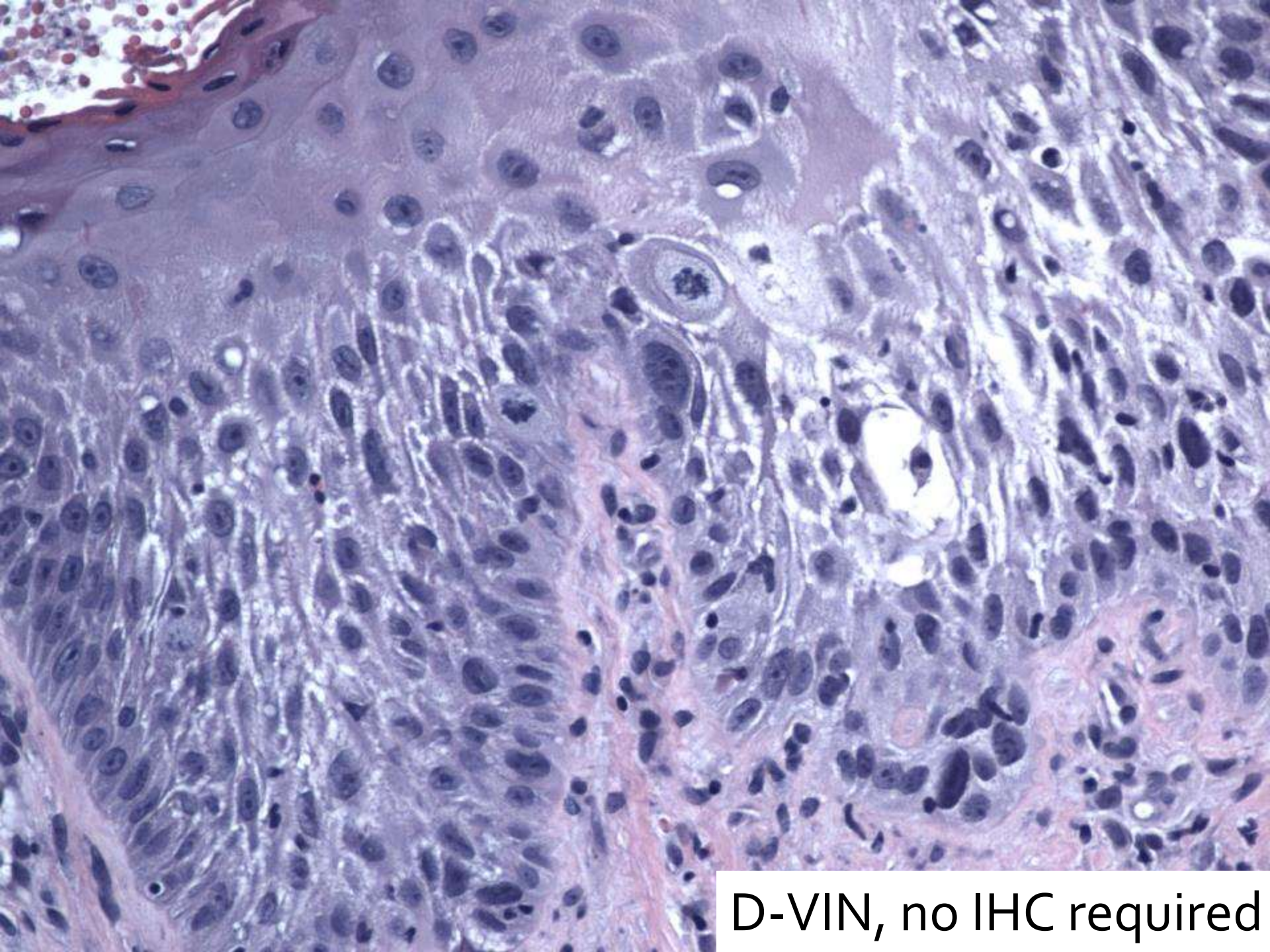


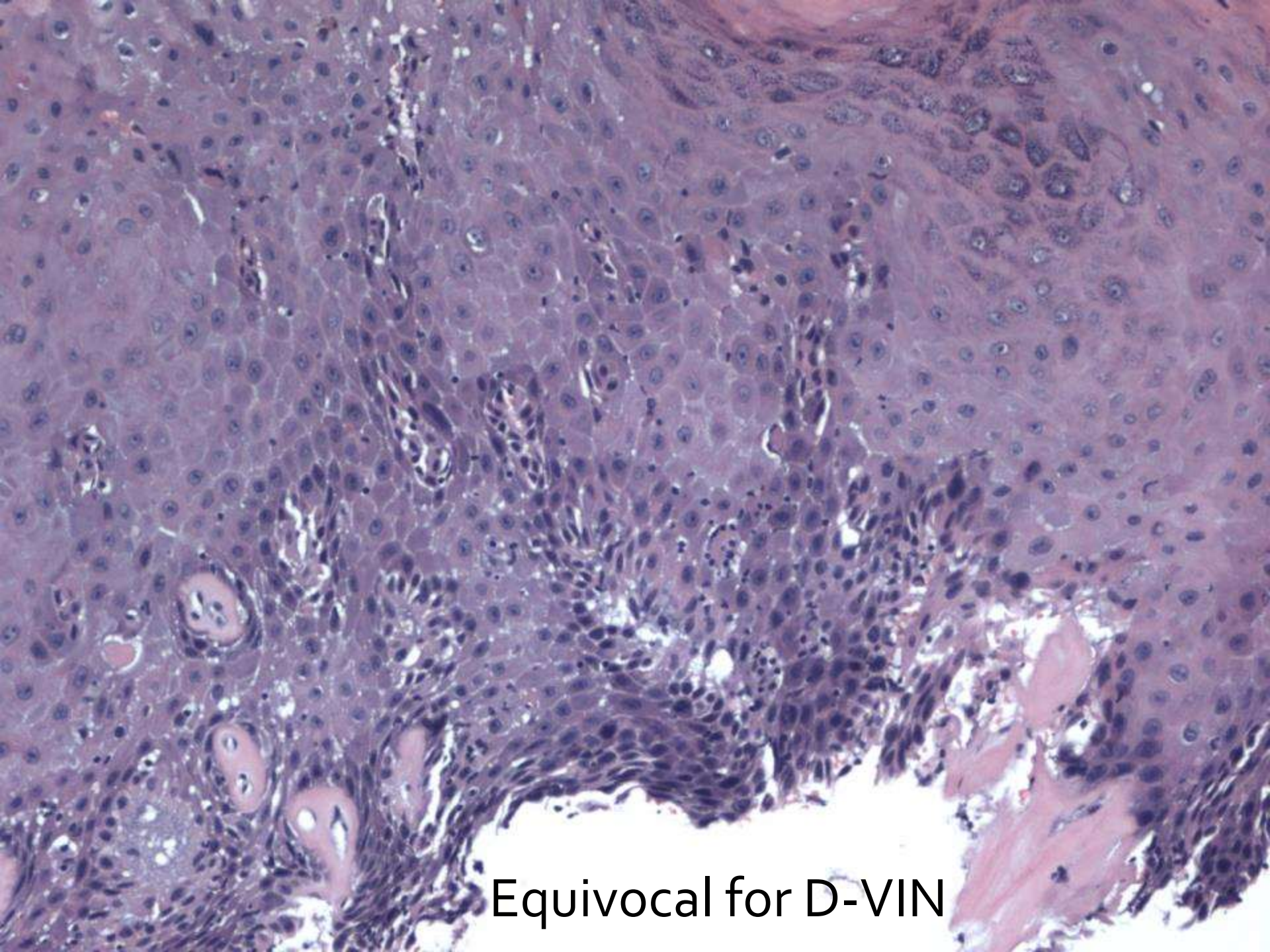

p16

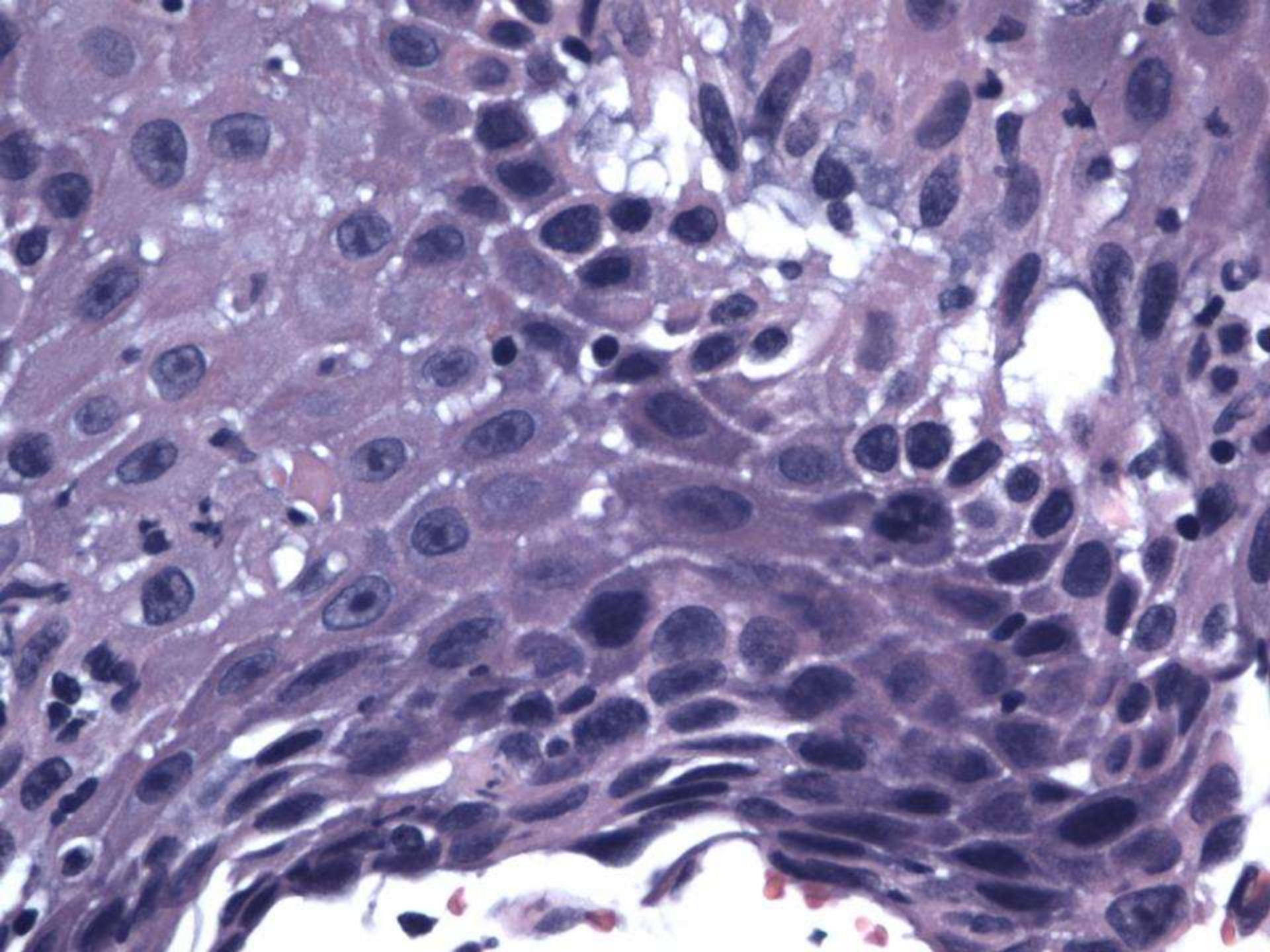

Ki-67

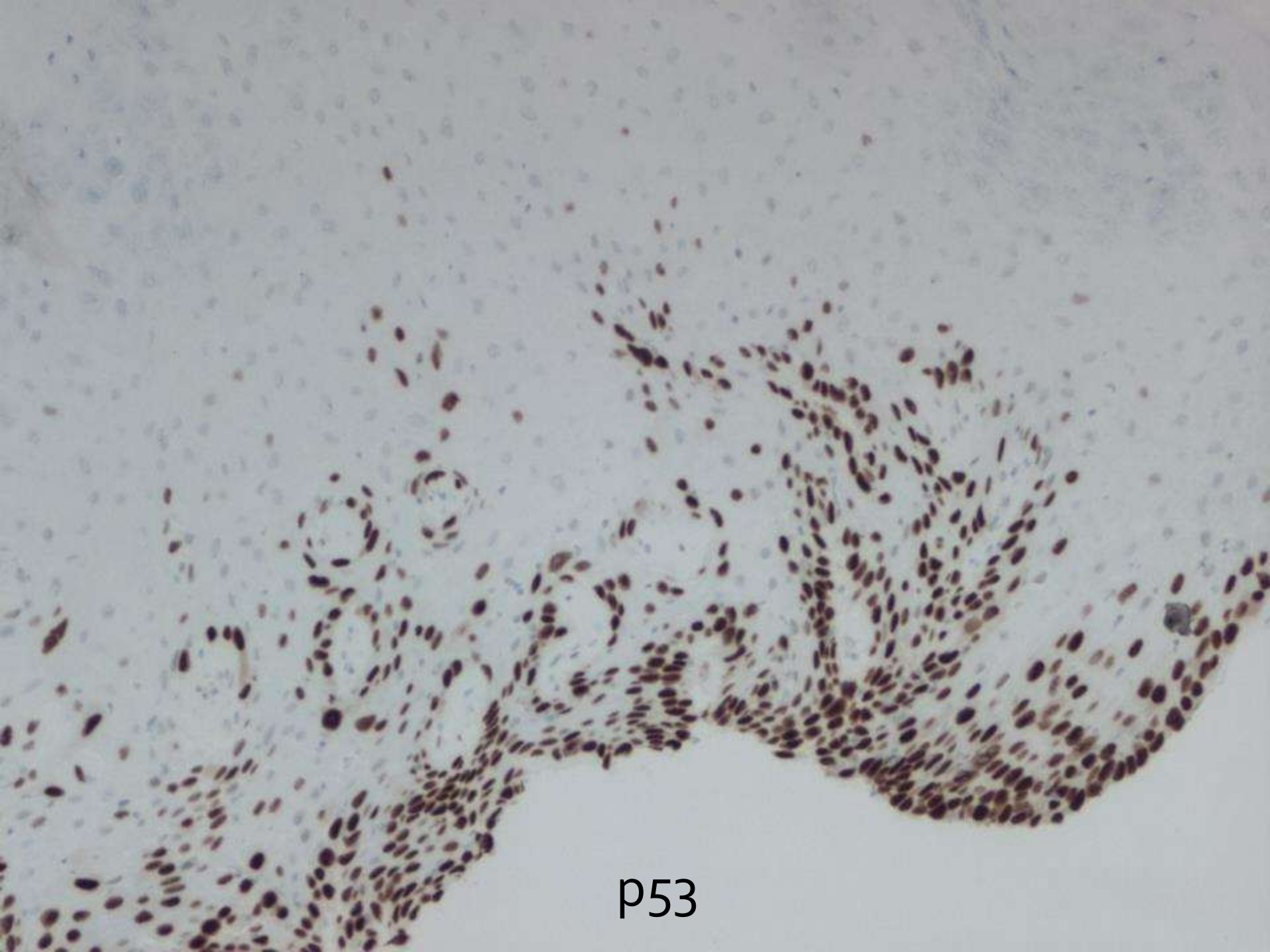
Non-HPV Dysplasia

- Differentiated type of vulvar intraepithelial neoplasia (d-VIN)
  - Not graded as all are considered pre-invasive/high grade
  - P16 negative or scattered reactivity
  - Ki-67 increased in basal/parabasal region
  - P53 strong reactivity in basal/parabasal region
    - Negative or wild-type reactivity for p53 does not rule out the diagnosis of d-VIN


D-VIN, no IHC required


Equivocal for D-VIN




p53

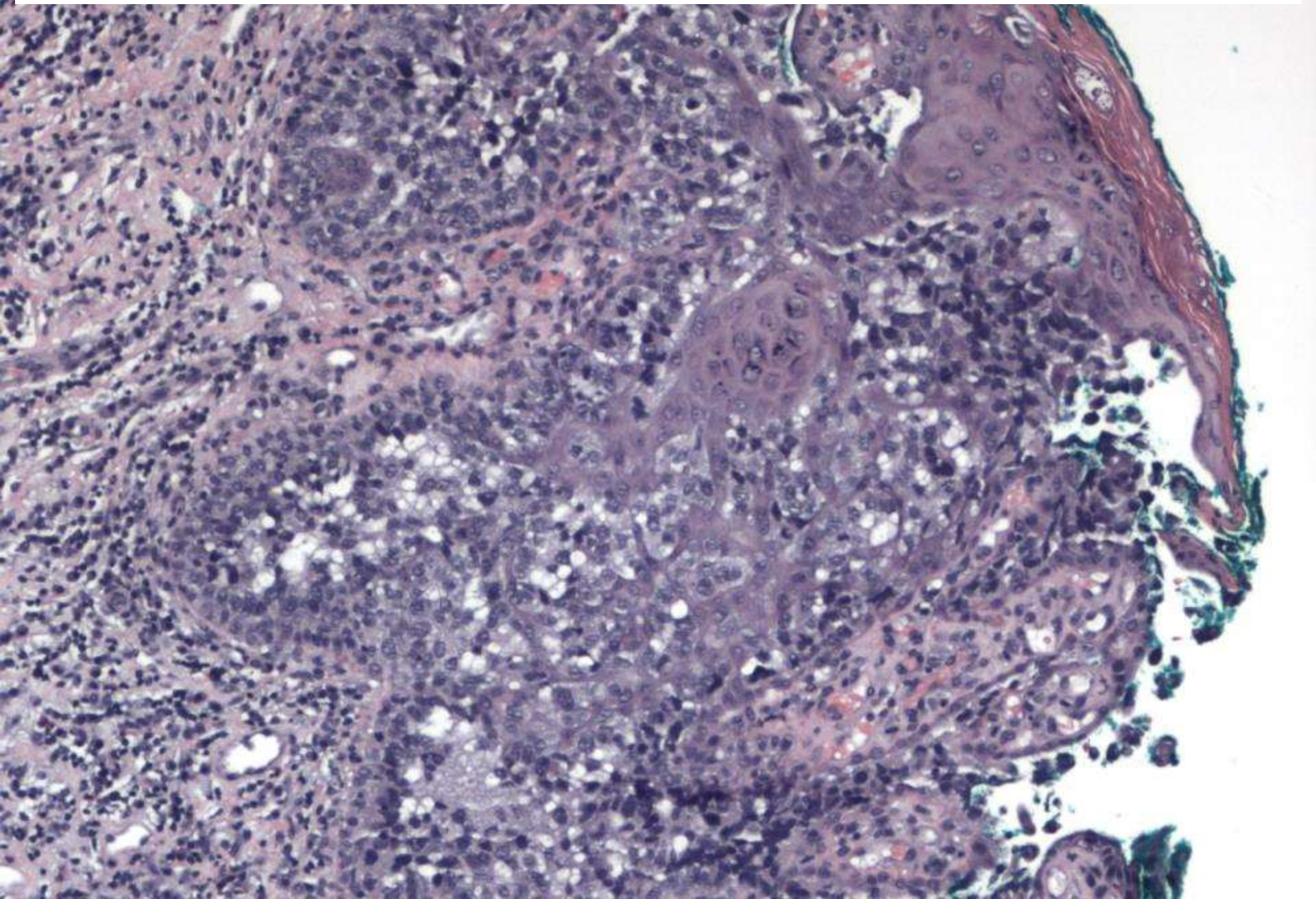
Vulva-Paget's Disease

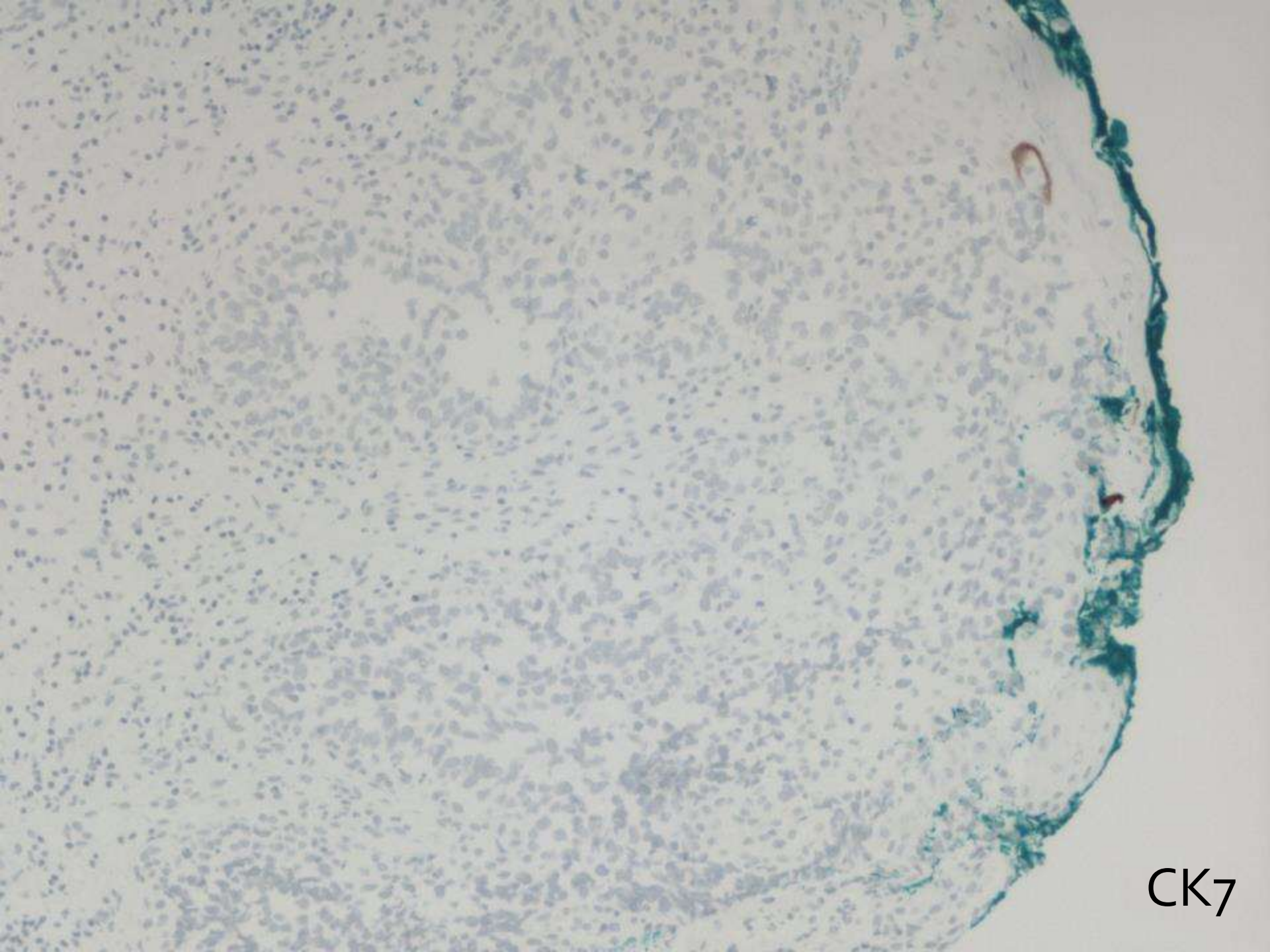
- Primary vulvar
  - Stains like mammary Paget's disease
- Secondary
  - Uncommon
  - Gastrointestinal (GI) or urinary tract source
  - Use IHC for lower GI and urothelial differentiation

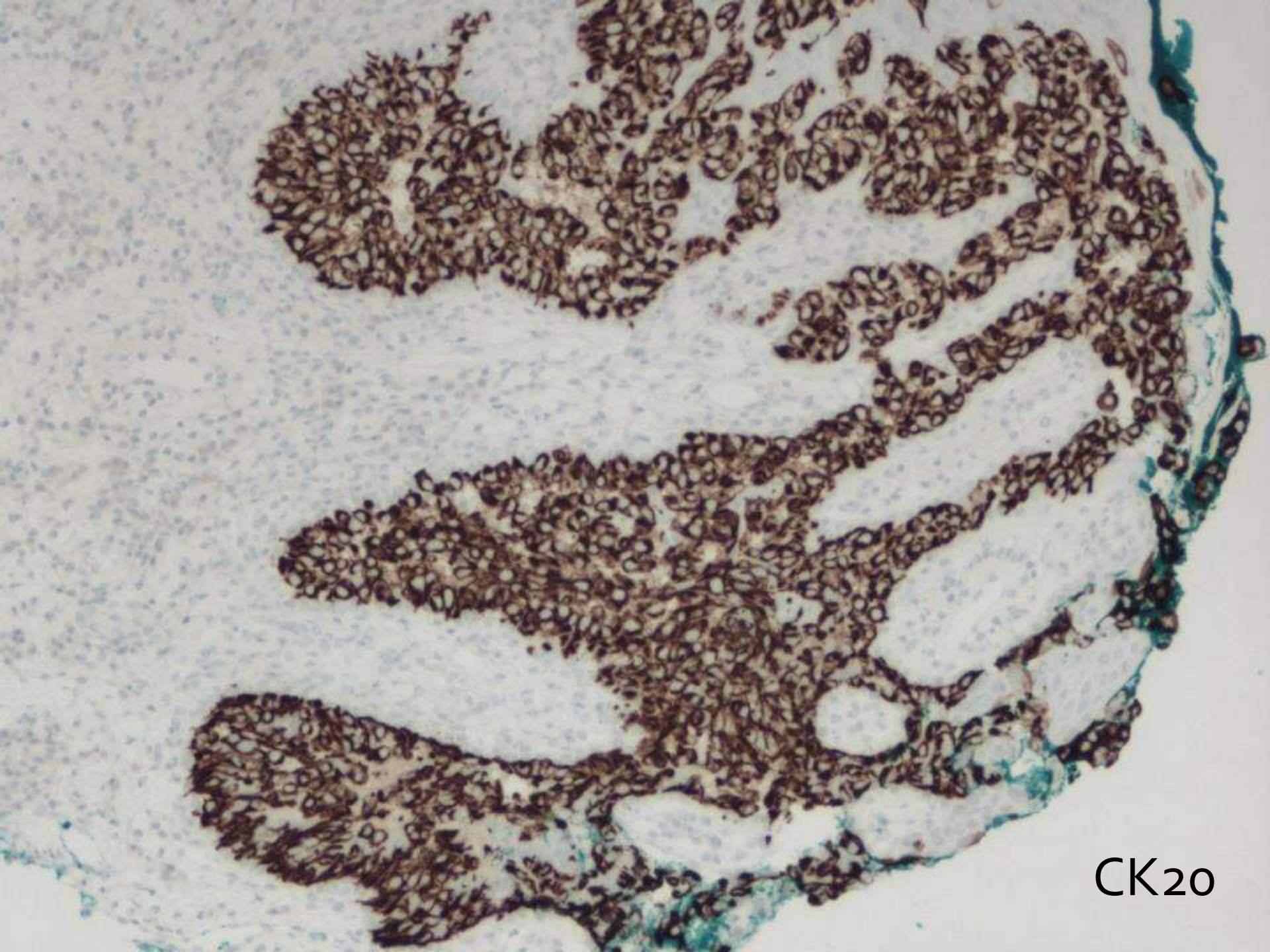
Vulva-Paget's Disease

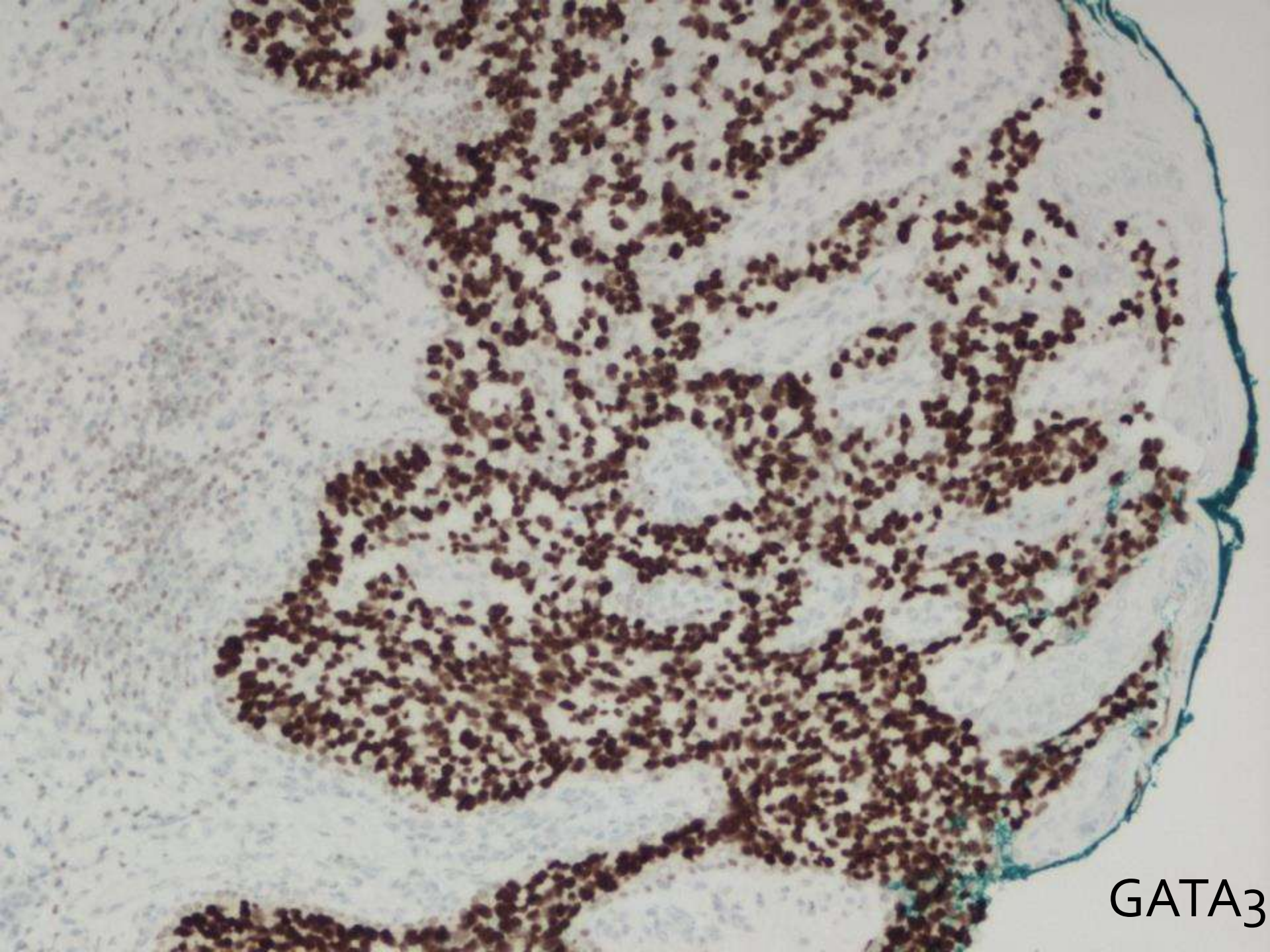
- Expected staining of primary vulvar Paget
  - Mammary markers positive
    - CK7, ER, GCDFP-15, mammaglobin, GATA3, HER2
  - Also positive for CEA
  - Negative for squamous markers (CK5, p63) and melanocytic markers (S100, HMB45, Melan A)
- Secondary Paget
  - Think about it if mammary markers are negative and positivity for CK20 plus history!

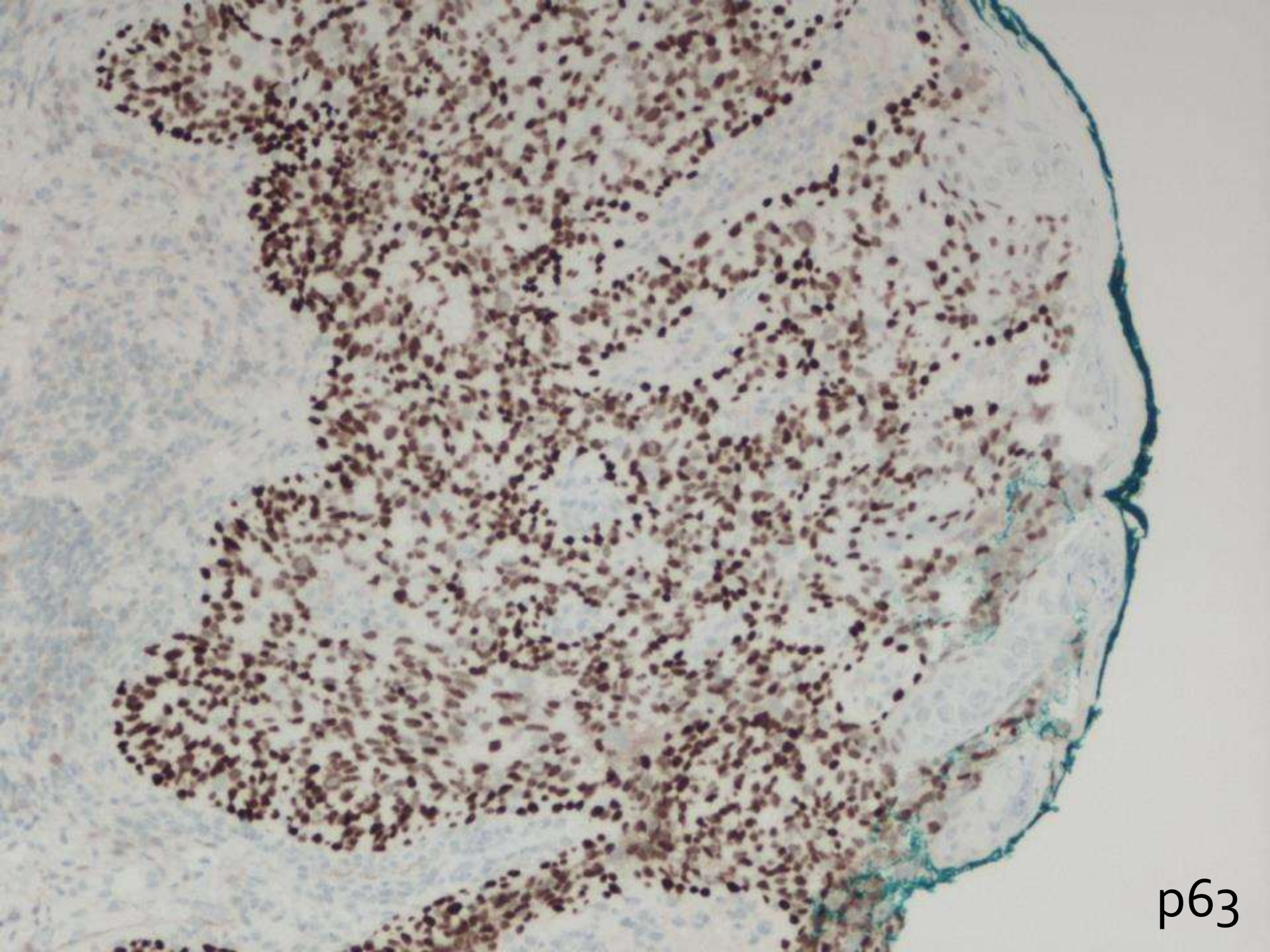
60 female with pelvic mass, vulvar bx performed. Prior hx of possible microscopic invasion of high grade urothelial ca in bladder bx 9 years ago; no residual invasion in cystectomy




CK7


CK20


GATA3


p63

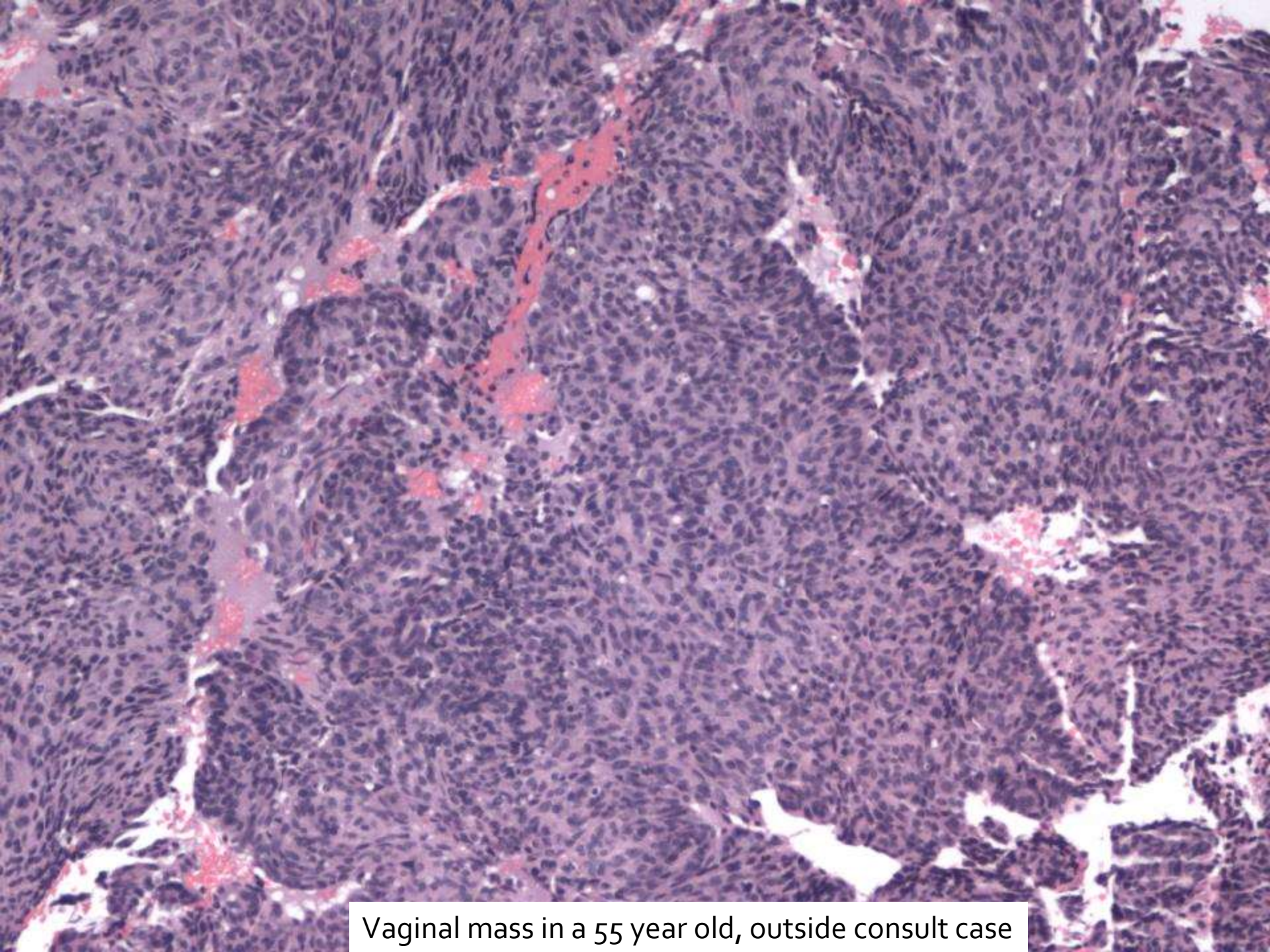
Vagina-Metastatic Tumors

- Vagina is a common site of metastasis
- Common secondary tumors
  - Endometrial
  - Tubal/ovarian
  - Breast
  - Colon
  - Local: Cervix, vulvar, urinary bladder

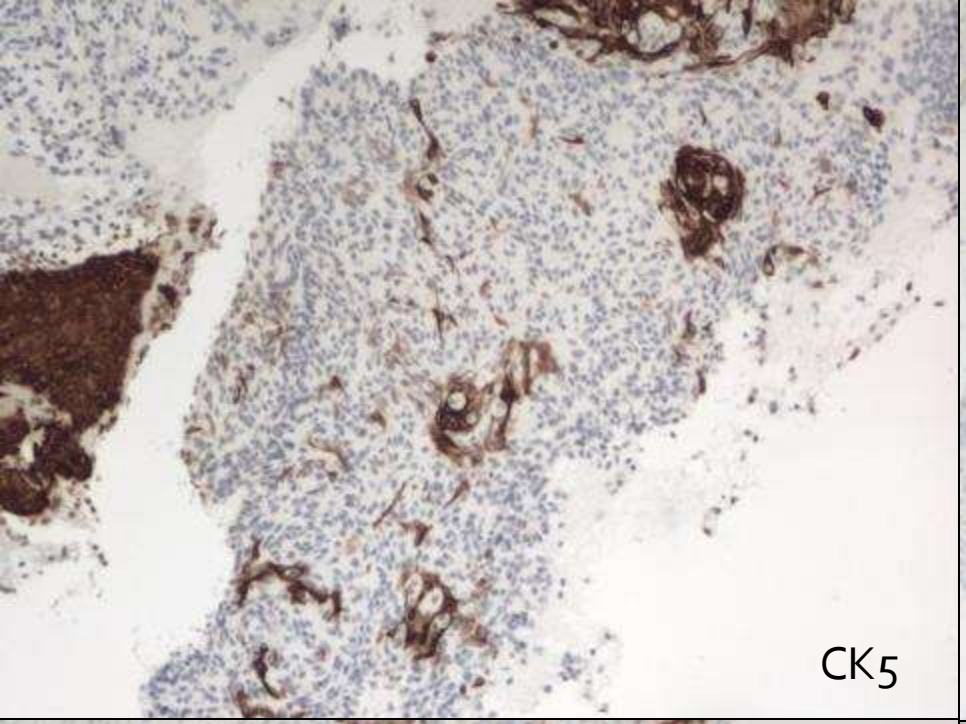
Vaginal Tumors-IHC Panel

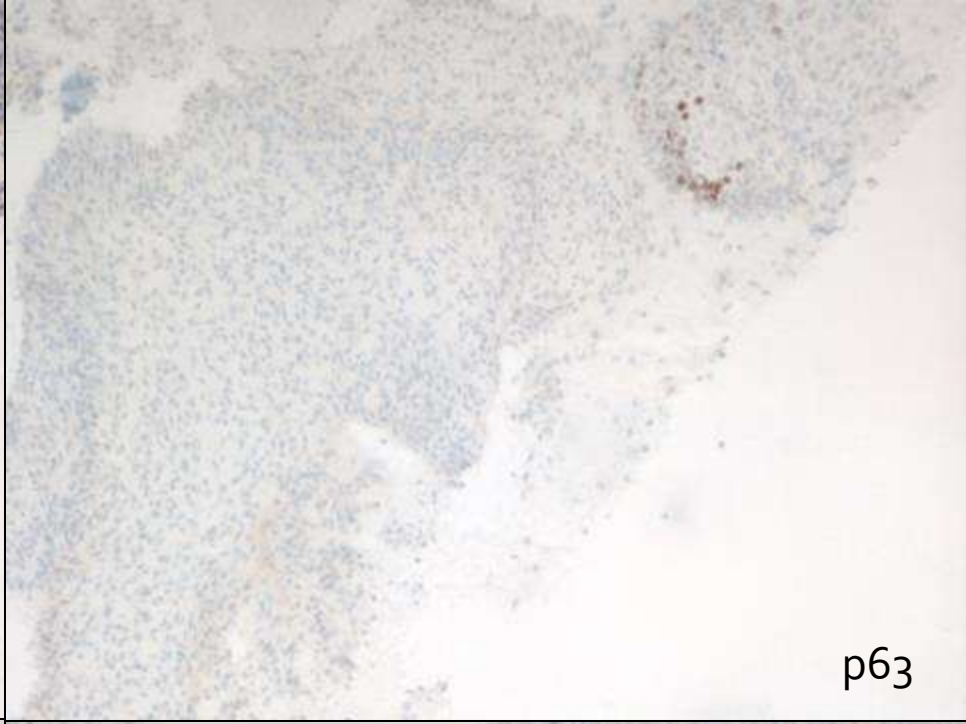
Antibodies	Gyn-adeno	Gyn-squamous	Urothelial	Breast	Colon
CK5	-/patchy+	+	+	- (unless TN)	-
P63	-	+	+	-	-
CK7	+	+/patchy+	+	+	-
CK20	-	-	+/-	-	+
PAX8	+	-	-	-	-
ER	+/-	-	-	+	-
Vimentin	+ (endomet)	-	-	-	-
SATB2/CDX2	-	-	-	-	+
UPII/UPIII	-	-	+	-	-
P16	-/+	+ (DS)	-/+	-/+	-/+
GATA3	-	-/weak+	+ (DS)	+ (DS)	-

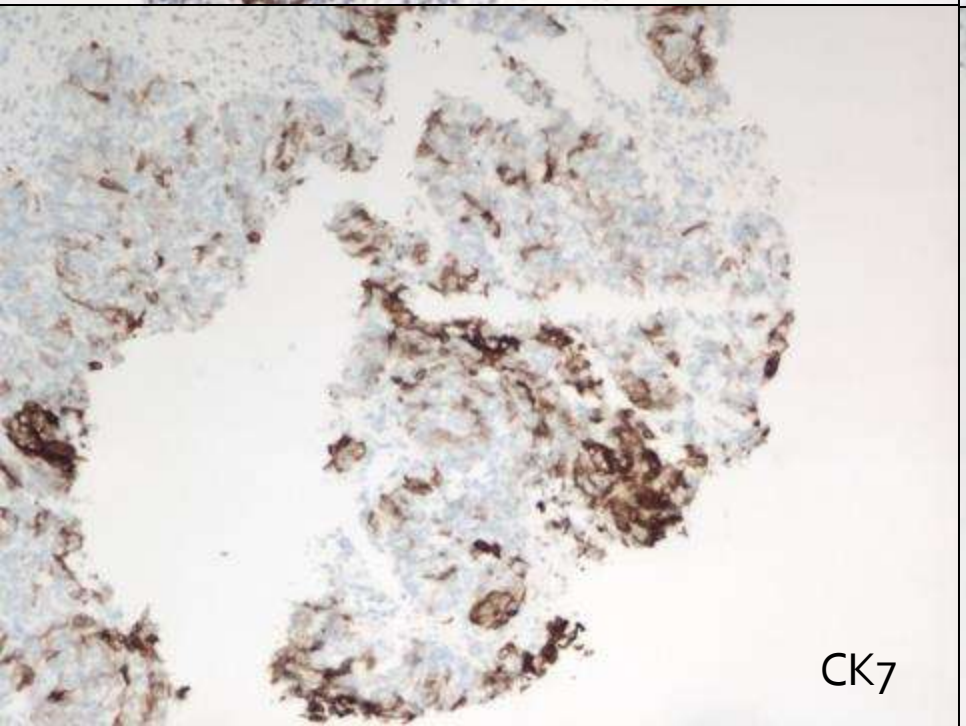
DS: diffuse strong; endomet: endometrioid type; TN: triple negative

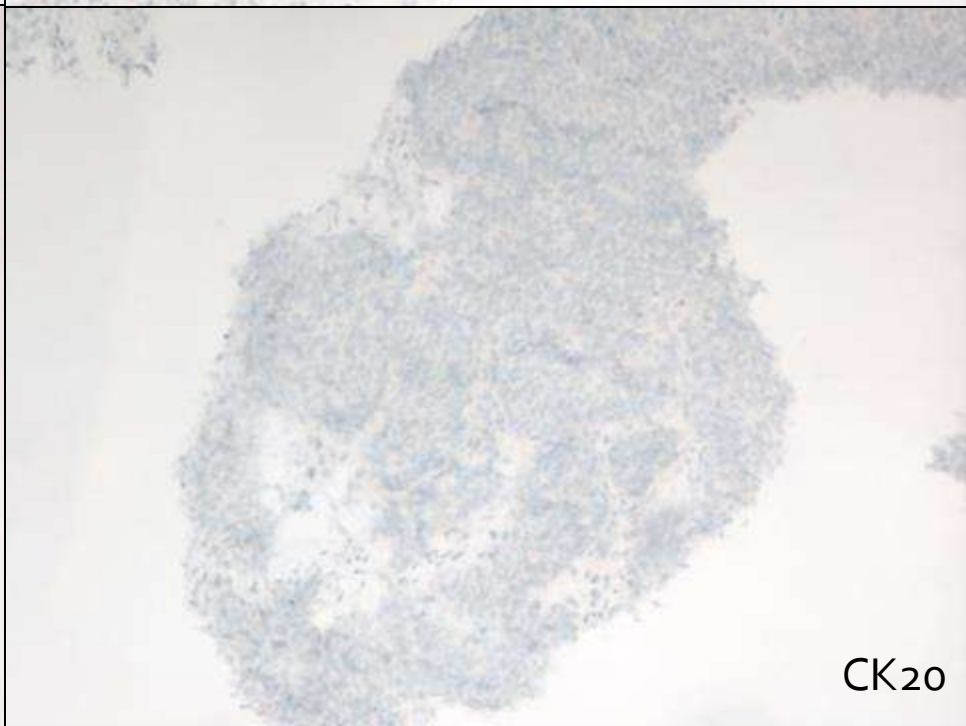


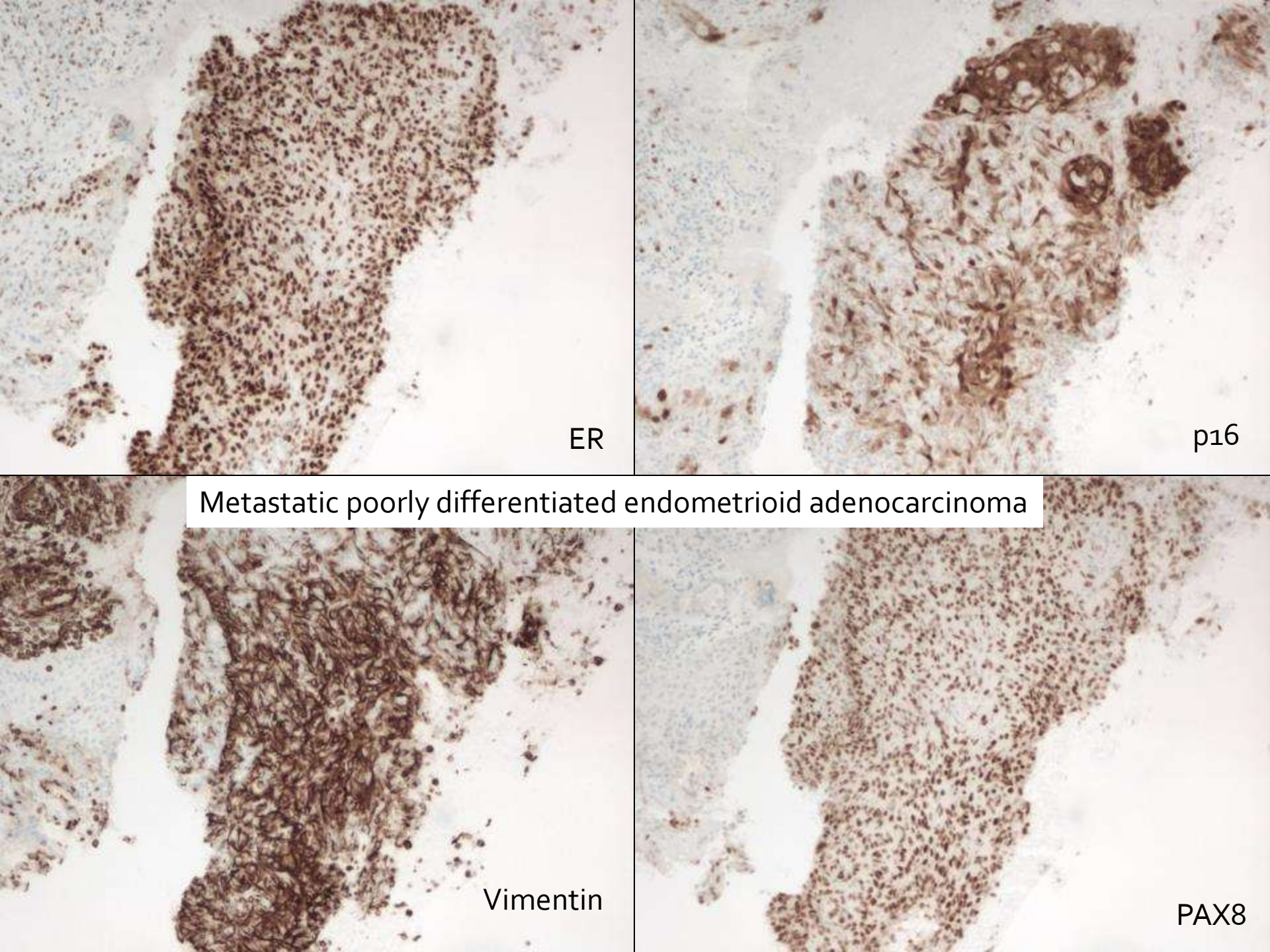
Vaginal mass in a 55 year old, outside consult case


CK5


p63


CK7


CK20

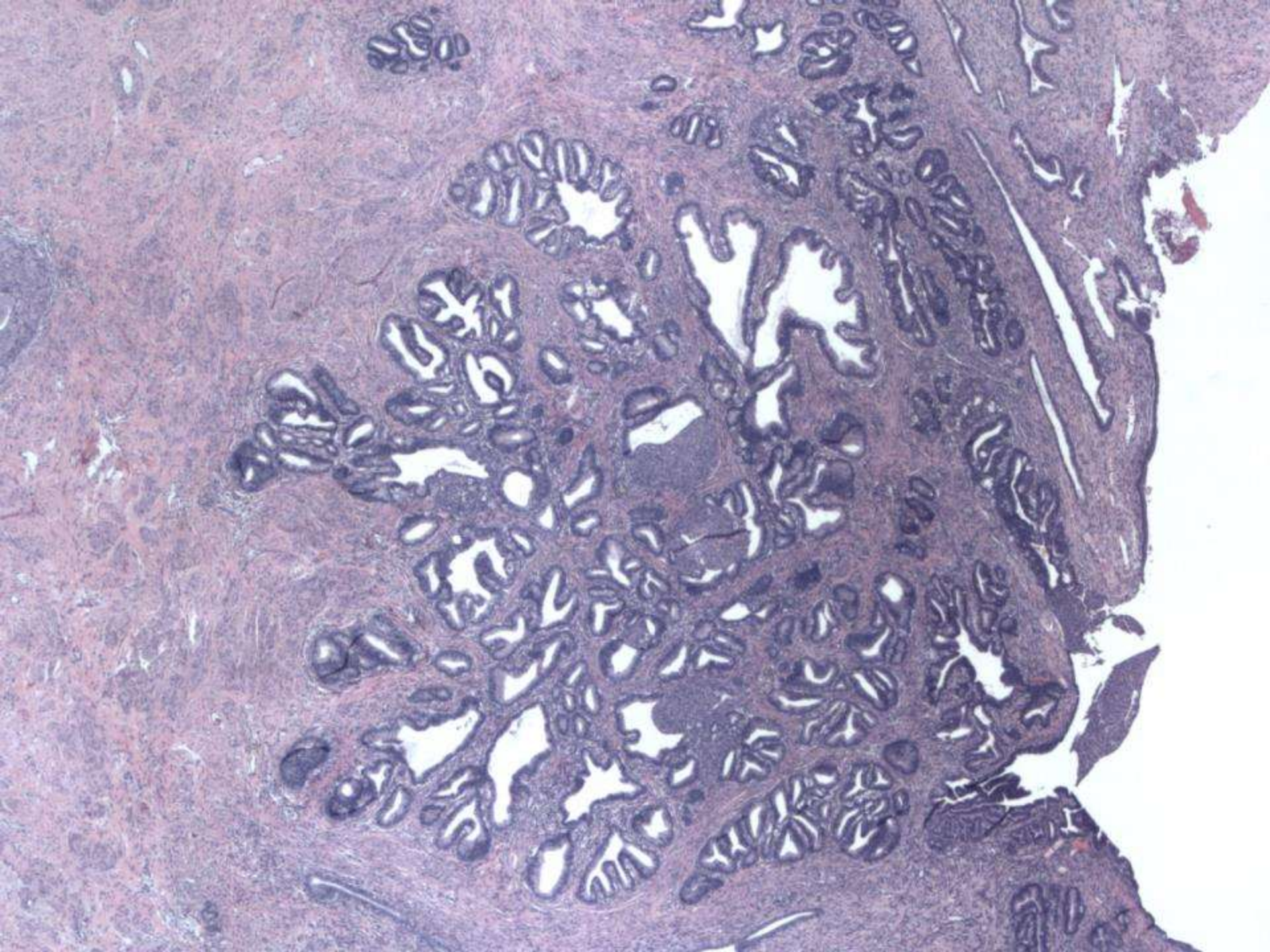

ER
p16
Metastatic poorly differentiated endometrioid adenocarcinoma
Vimentin
PAX8

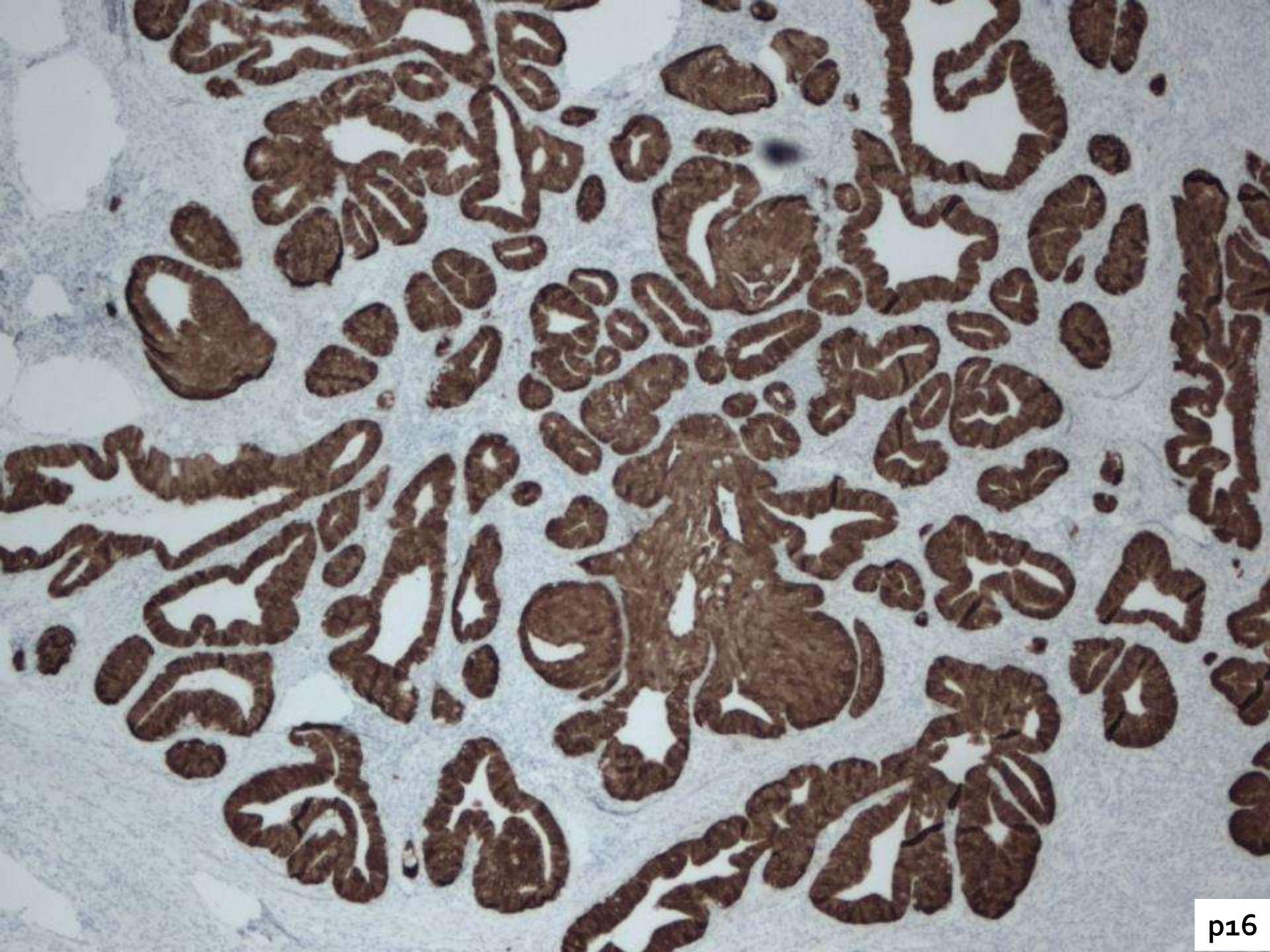
Cervical Adenocarcinoma

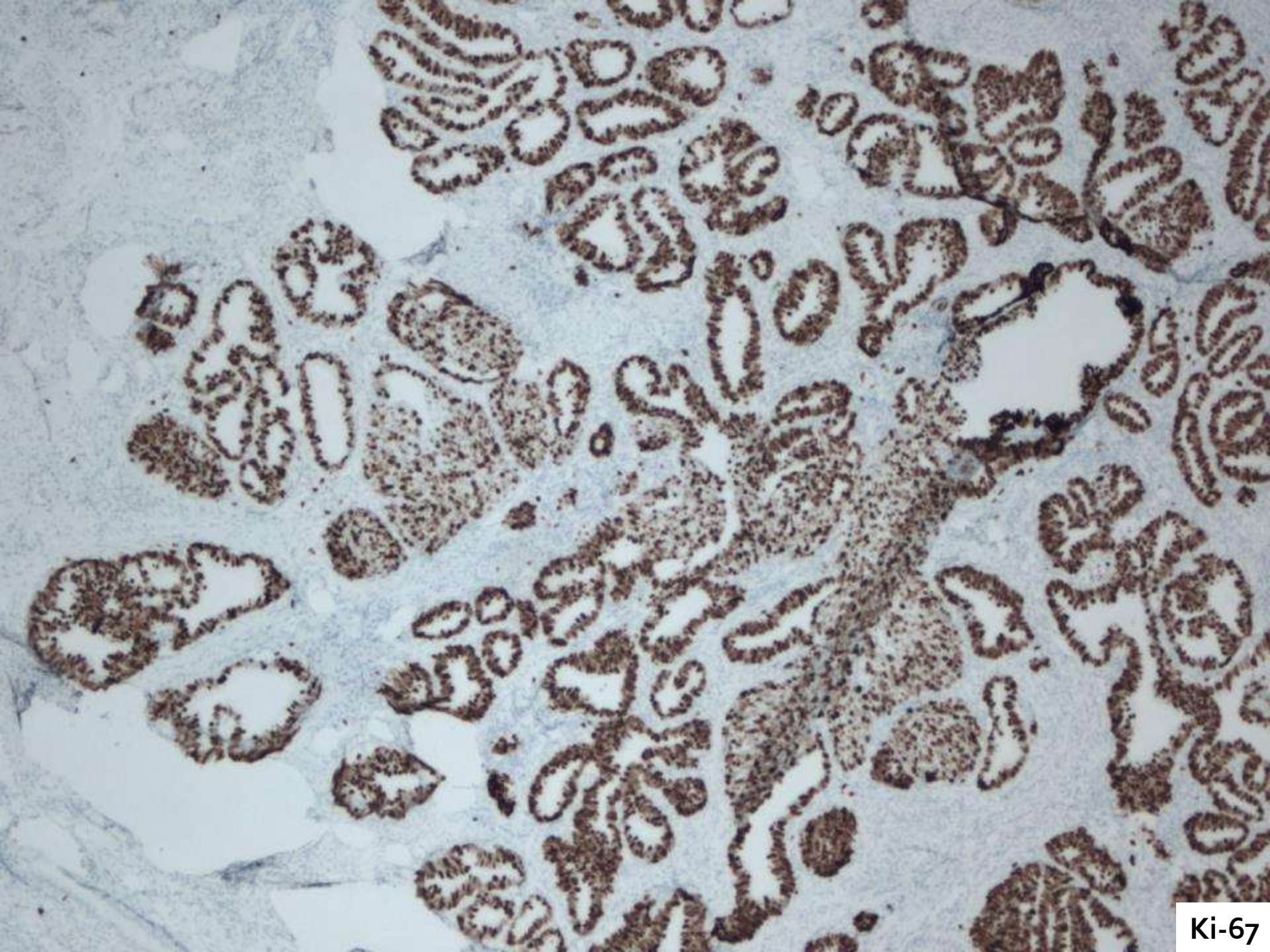
- Subtle and can be missed in ECC and even in LEEP specimens
  - AIS (in-situ lesions) can sometimes be difficult to distinguish from invasive adenocarcinomas but both have similar IHC profile
  - Even small volume invasive disease can result in metastasis
  - Most adenocarcinomas are HPV16/18 related

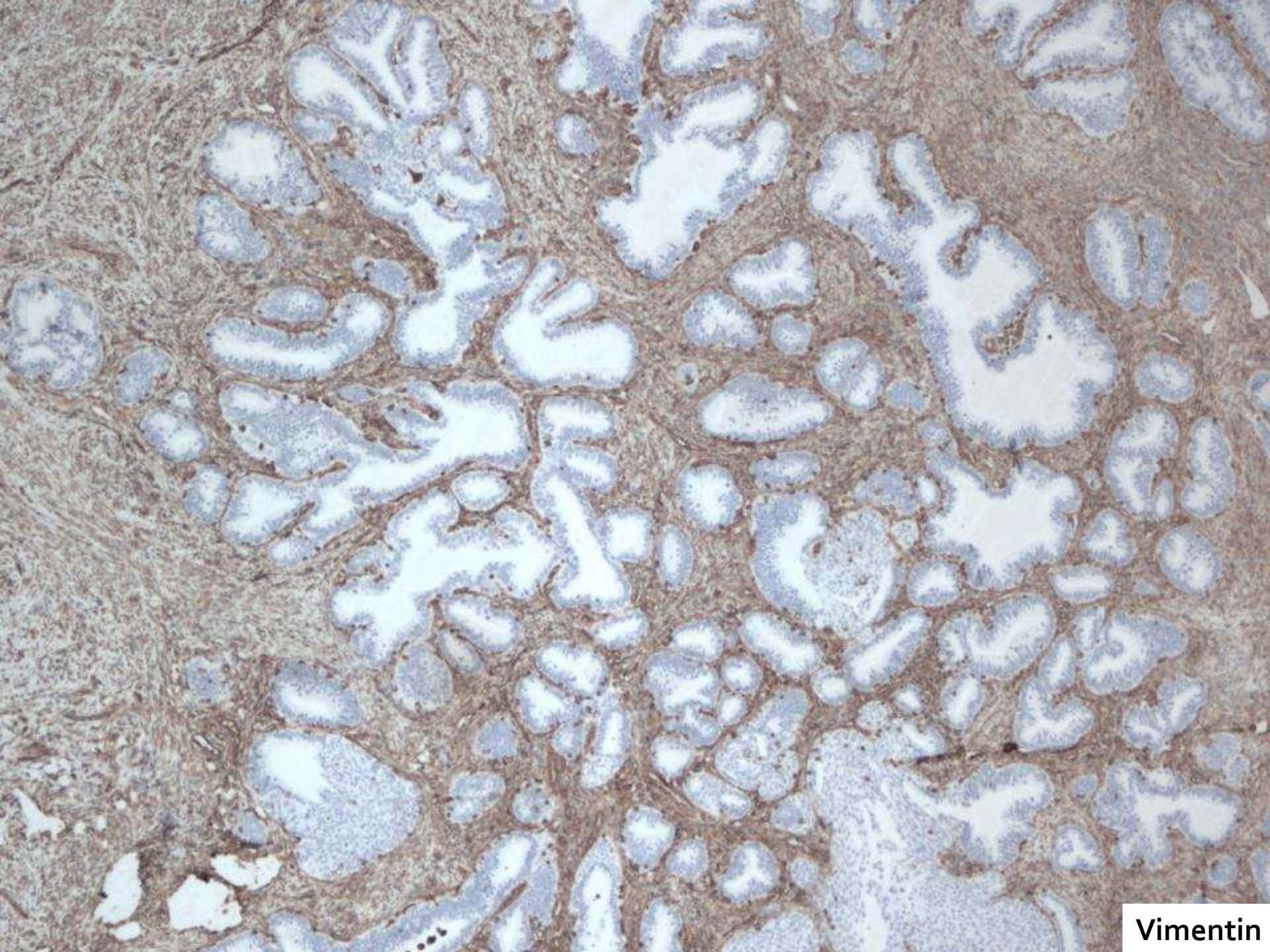
Cervical Adenocarcinoma

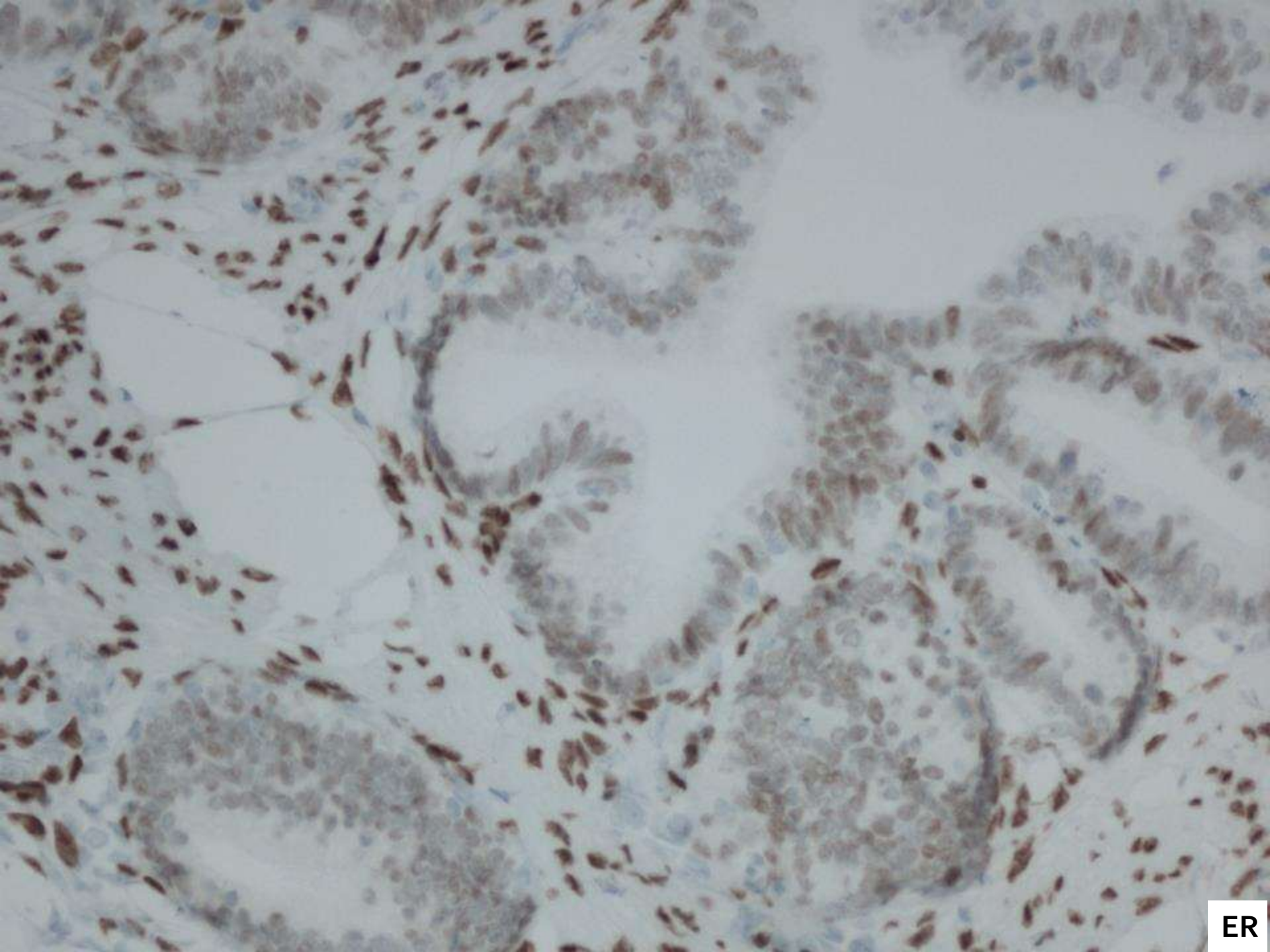
Antibodies	Cervical adenocarcinoma	Endometrial Endometrioid
PAX8	+ (~60-70% cases)	+ (~90% cases)
Vimentin	-	+
ER	-/weak	+
CEA(m)	+ (~40-50% cases)	-/+ (squamous areas)
P16	+ (diffuse strong)	+ (but not 100% cells)
Ki-67	Very high (~90% index)	Variable
MMR proteins	Preserved	Lost in few cases
HPV in-situ hybridization	+	-

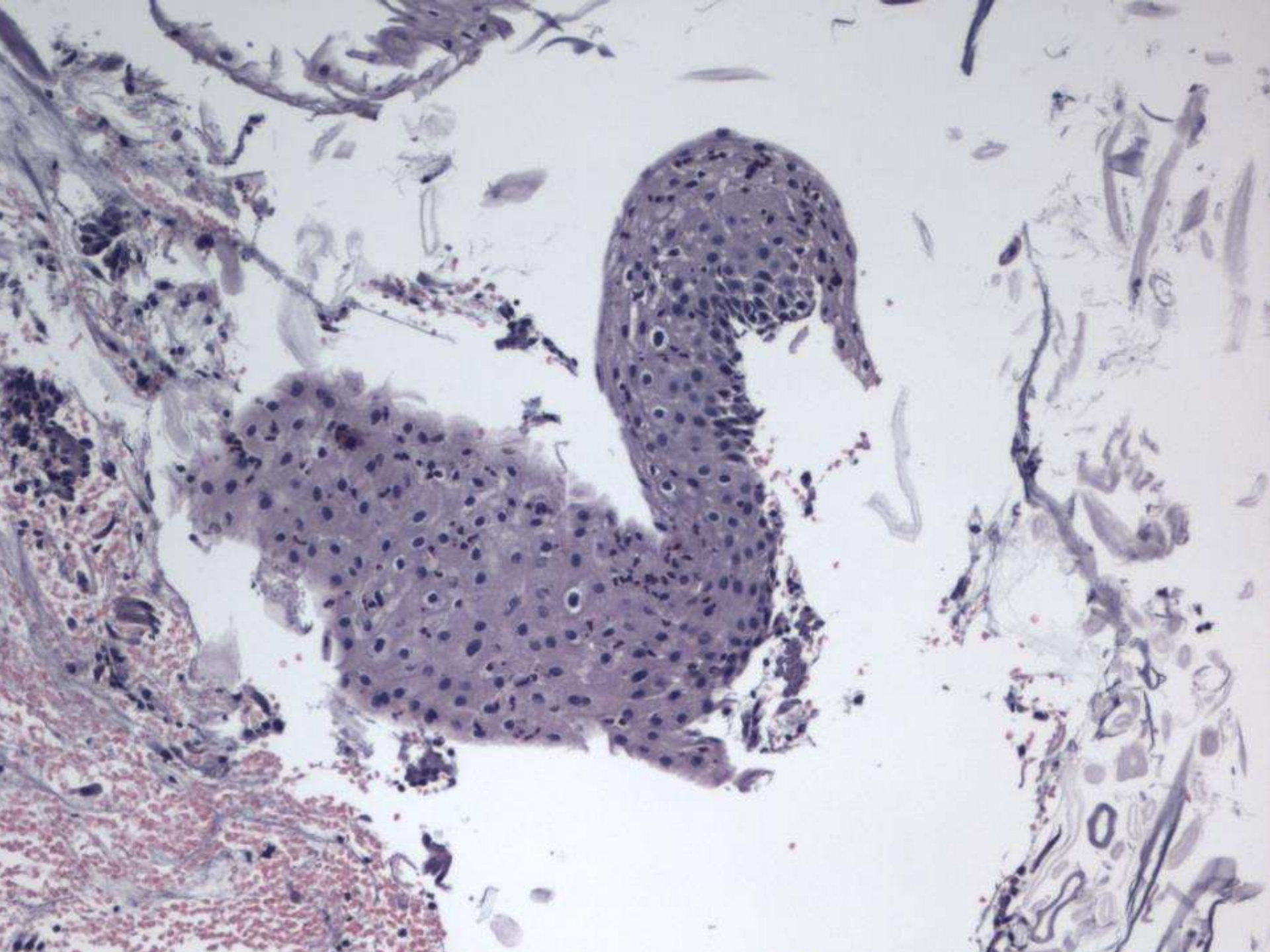





Ki-67


Vimentin


ER

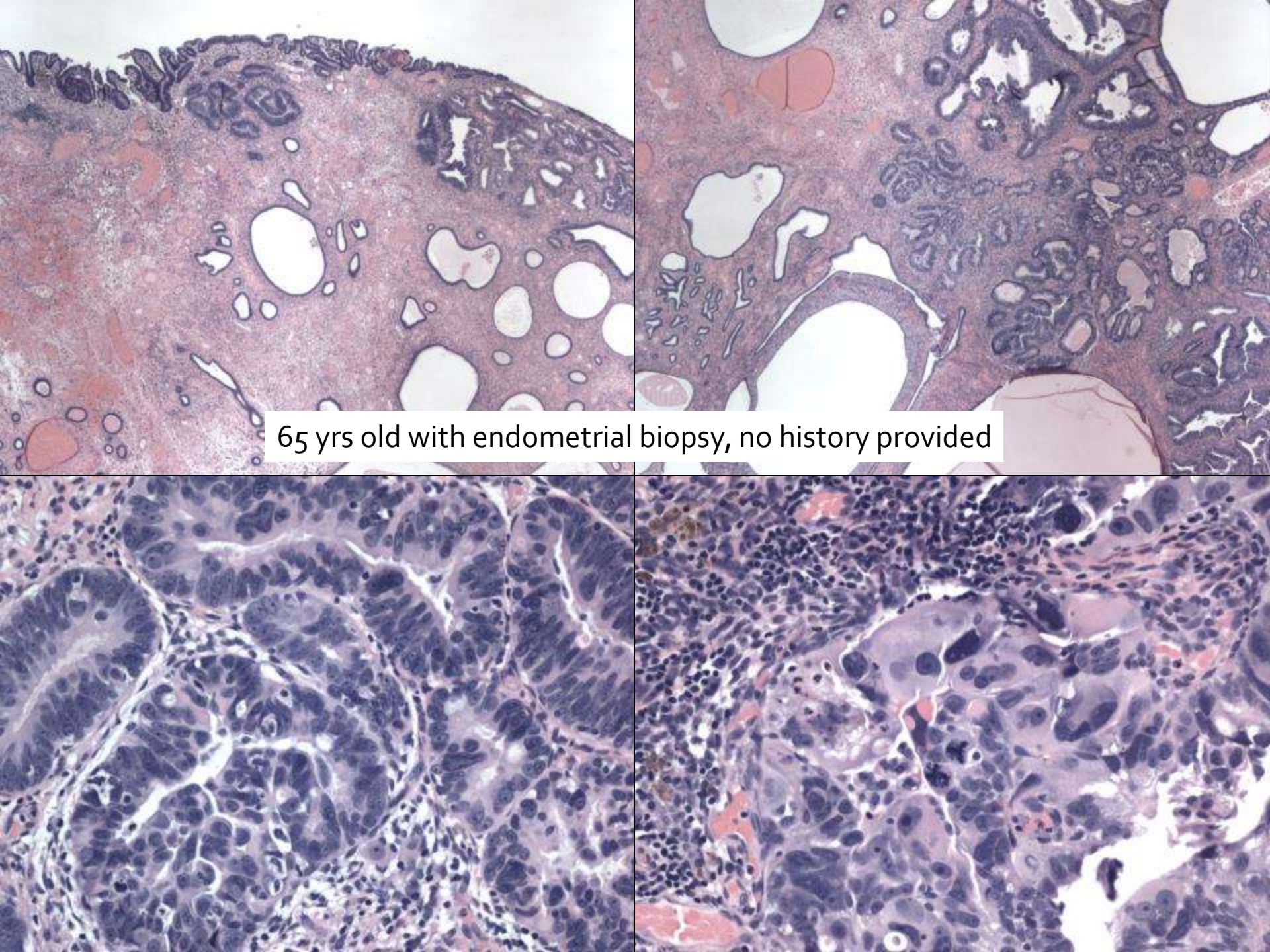


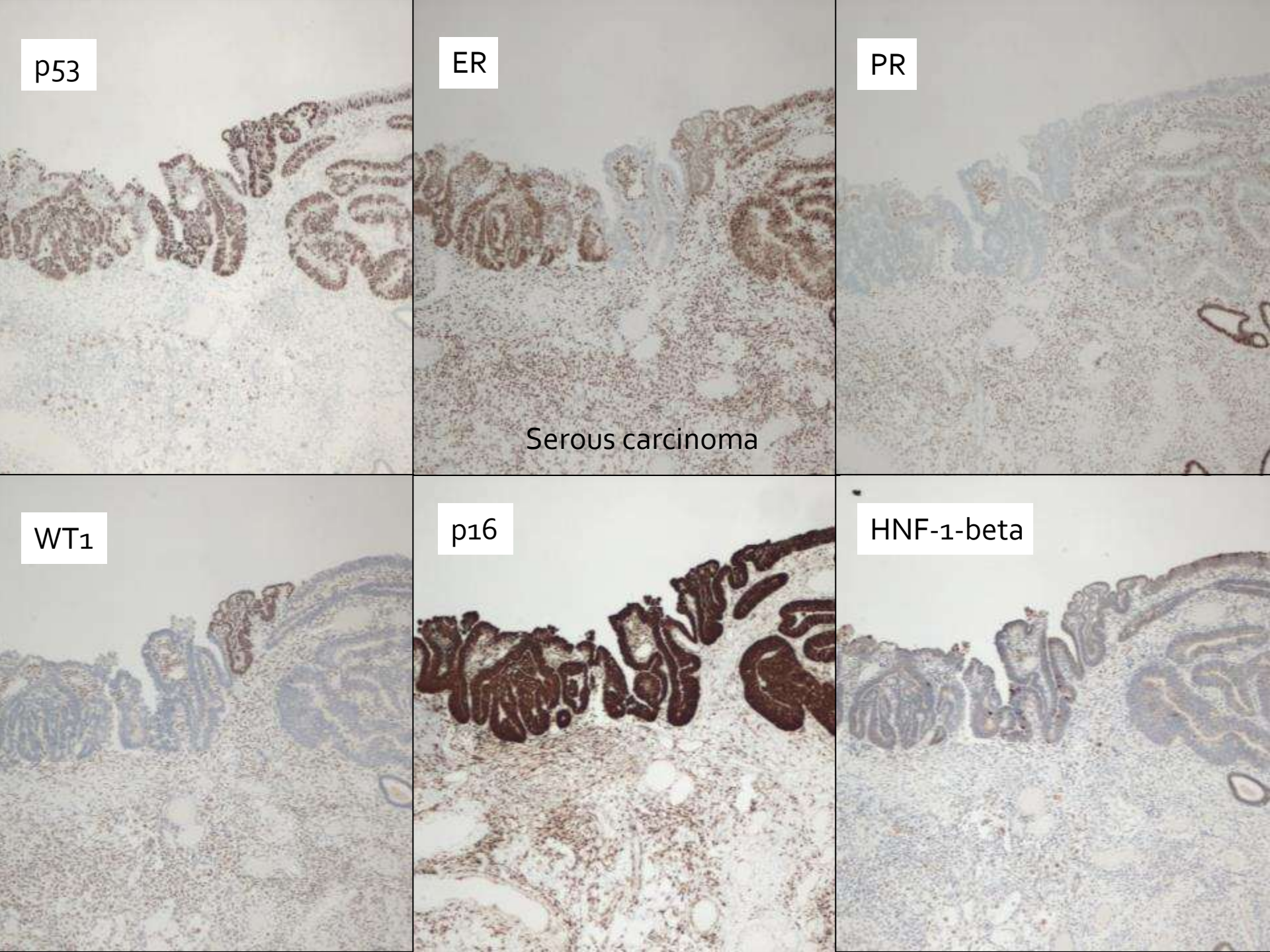
Uterine Corpus-Tumor Types

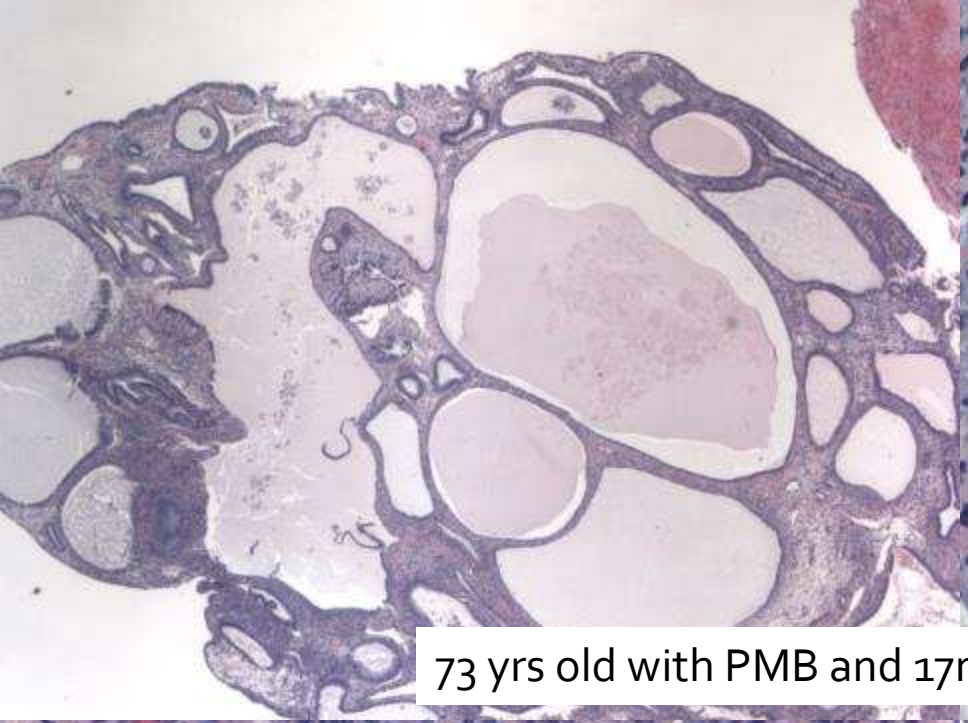
- Traditional classification
  - Endometrioid (with mucinous and squamous diff)
  - Clear cell
  - Serous
  - Carcinosarcoma (MMMT)
  - Undifferentiated carcinoma

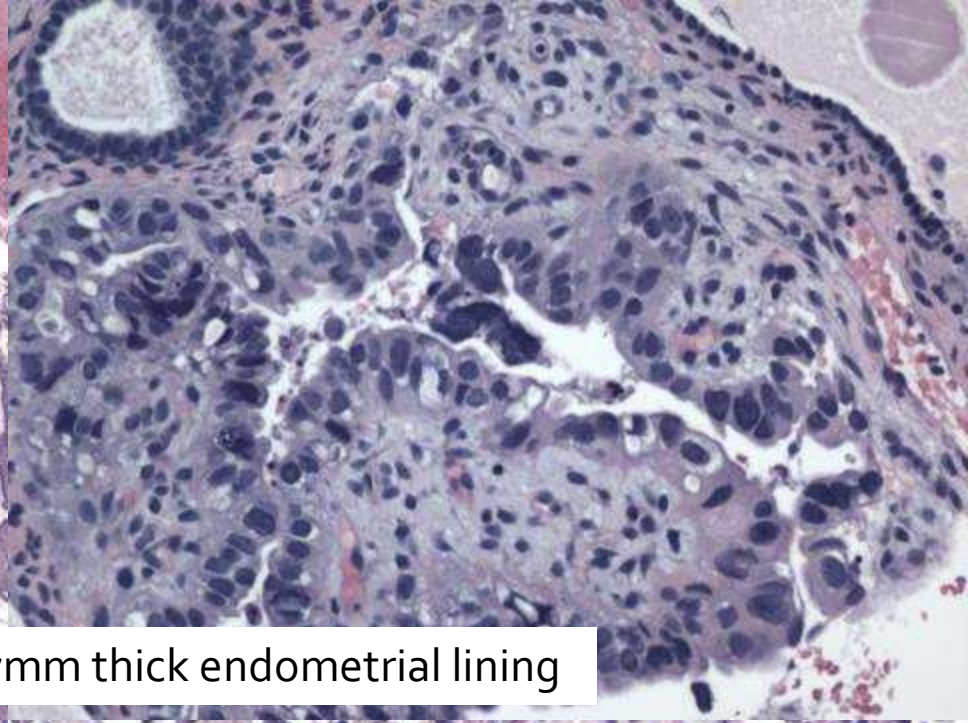
Uterine Corpus-Tumor Types

Antibodies	Endometrioid	Serous	Clear Cell
P53	Wild type	+	Wild type/+
P16	Patchy +	+ (diffuse strong)	+ (diffuse strong)/-
HNF-1 beta	-/+ (weak-mod)	-	+ (strong)
Napsin A	-	-	+
ER	+	Patchy +	-
PR	+	-	-
Vimentin	+	Patchy +/-	Patchy +/-
PTEN	Loss	+	+/-
MMR proteins	Loss in some cases	Preserved	Loss in some cases
WT1	-	-/+ (in ~1/3rd cases)	-
ARID1A (BAF250)	Loss in some	Preserved	Loss
Beta-catenin	Nuclear in 50%	No nuclear staining	Not well studied

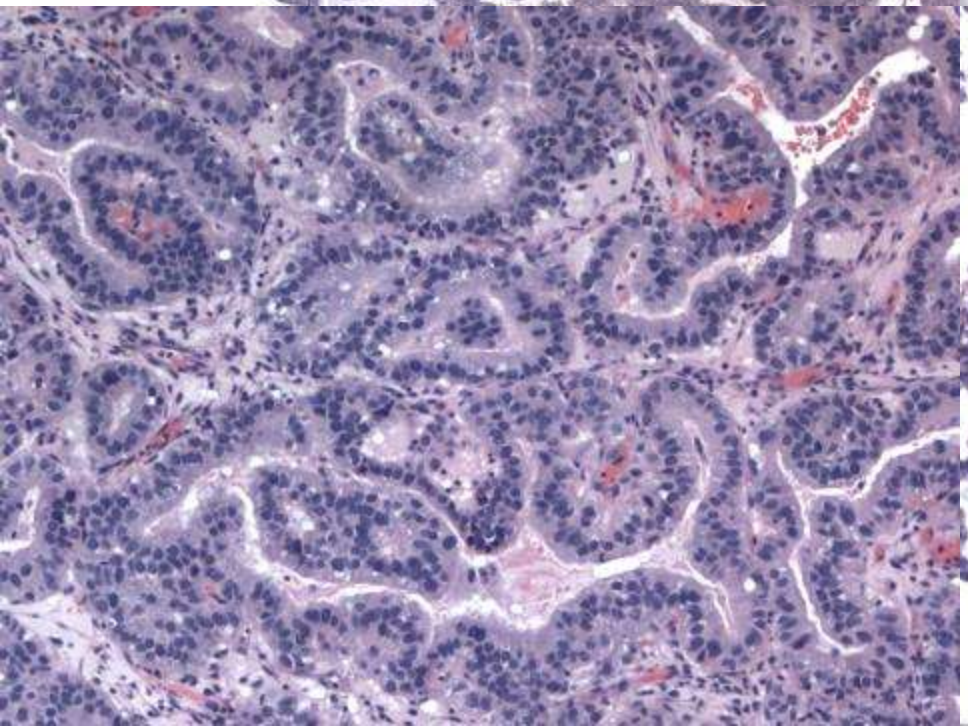

65 yrs old with endometrial biopsy, no history provided

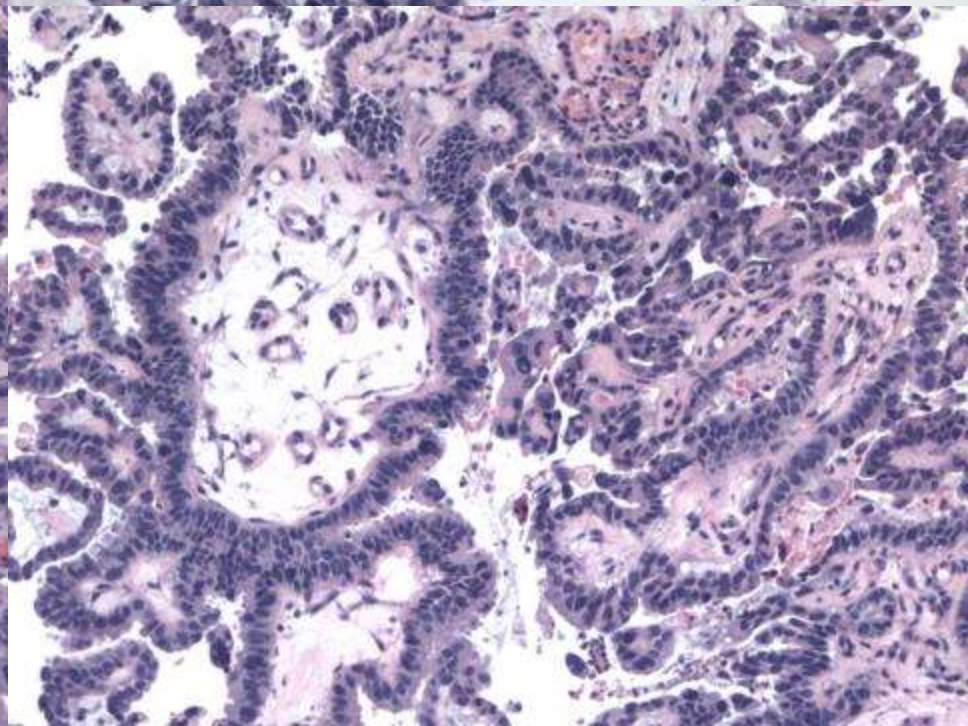

p53
ER
PR
Serous carcinoma
WT1
p16
HNF-1-beta

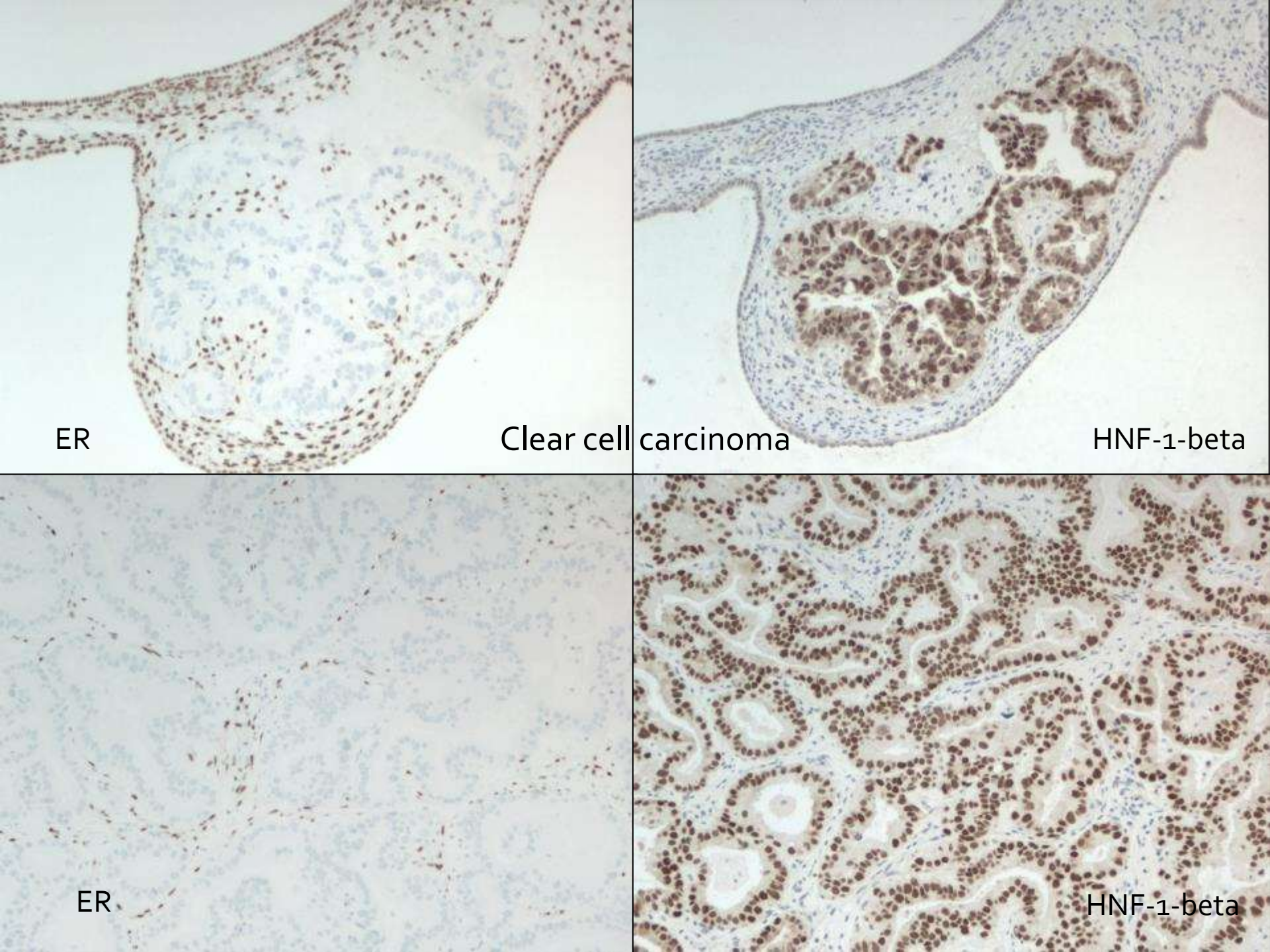




73 yrs old with PMB and 17mm thick endometrial lining

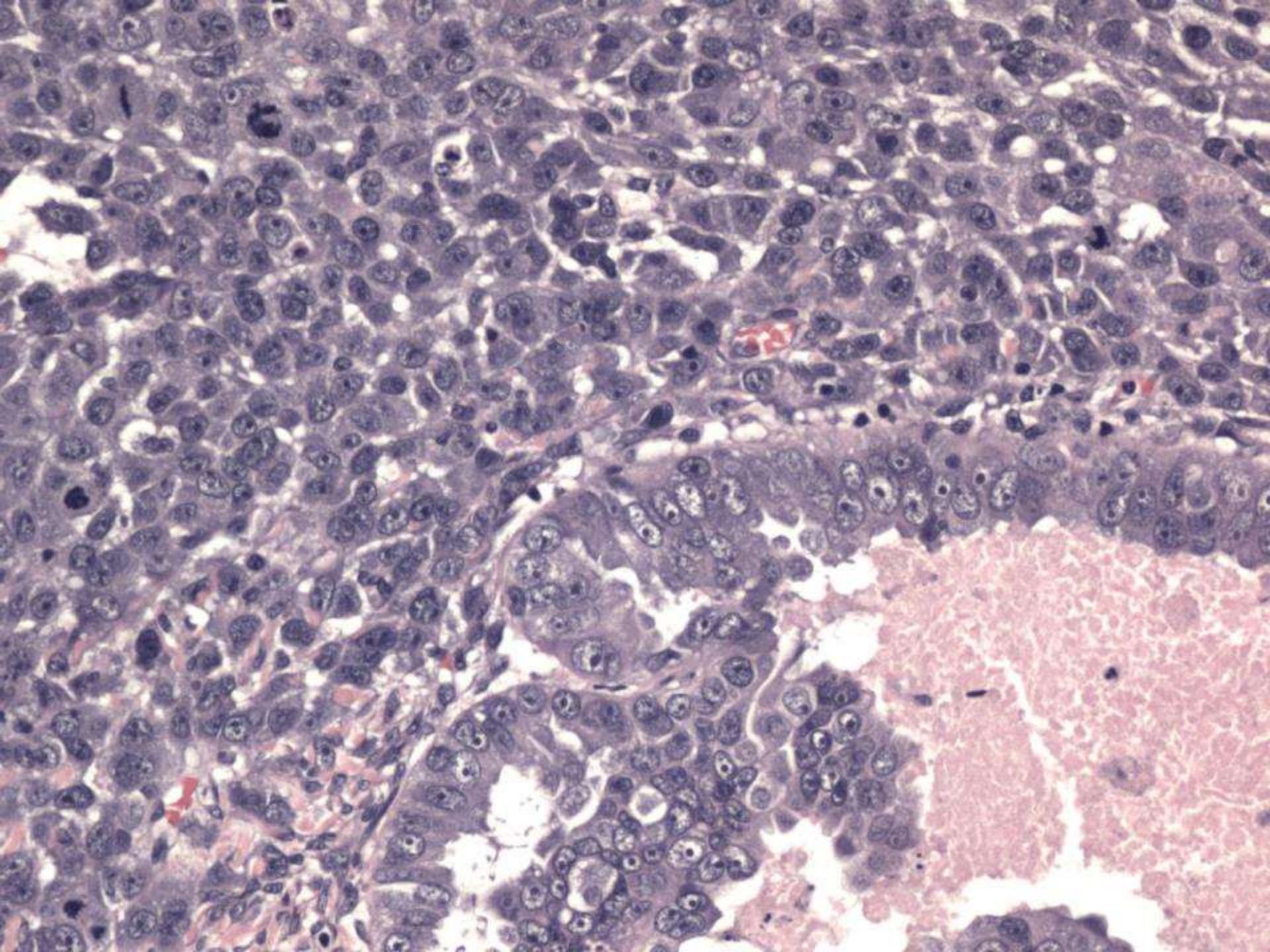


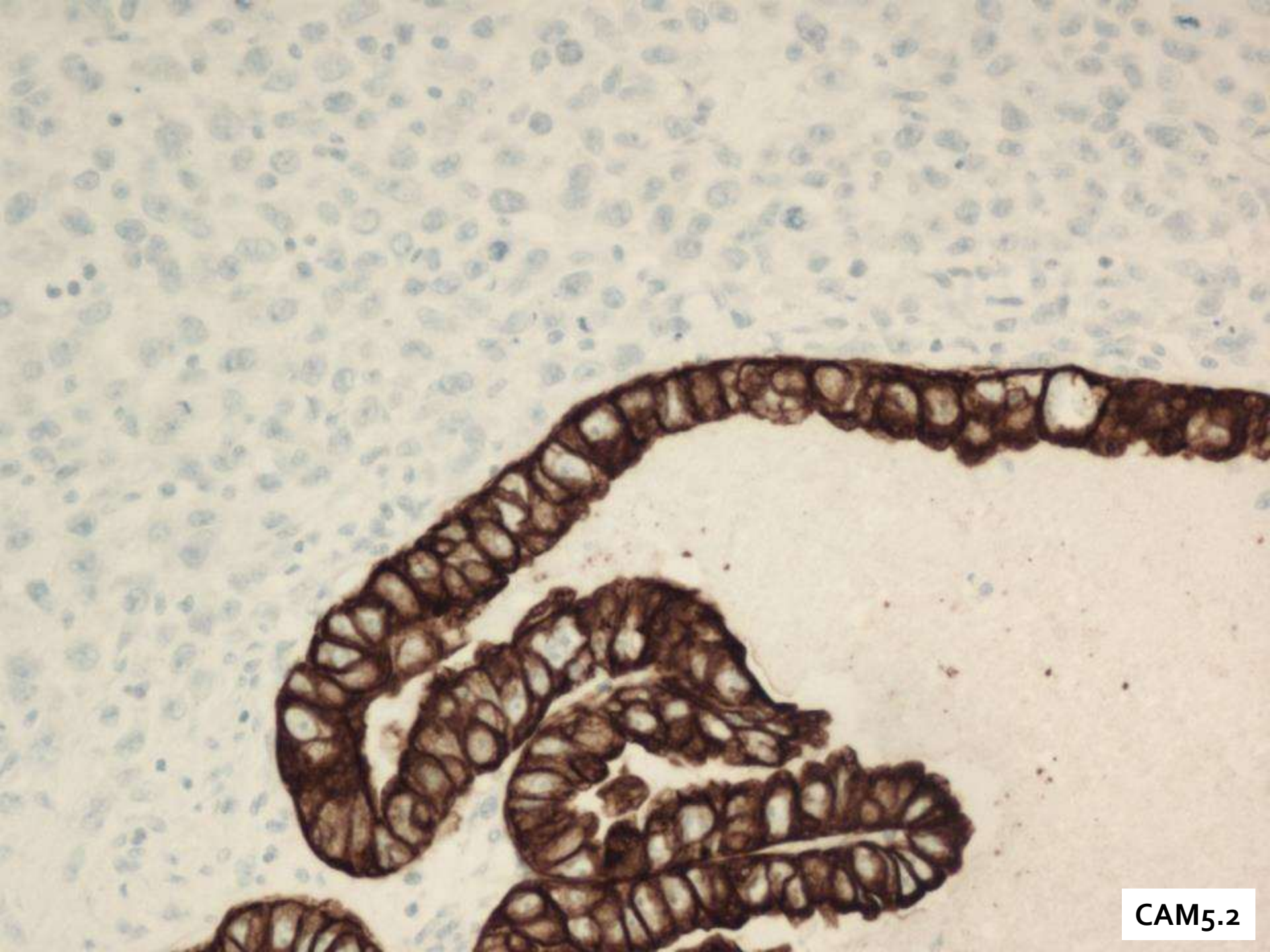


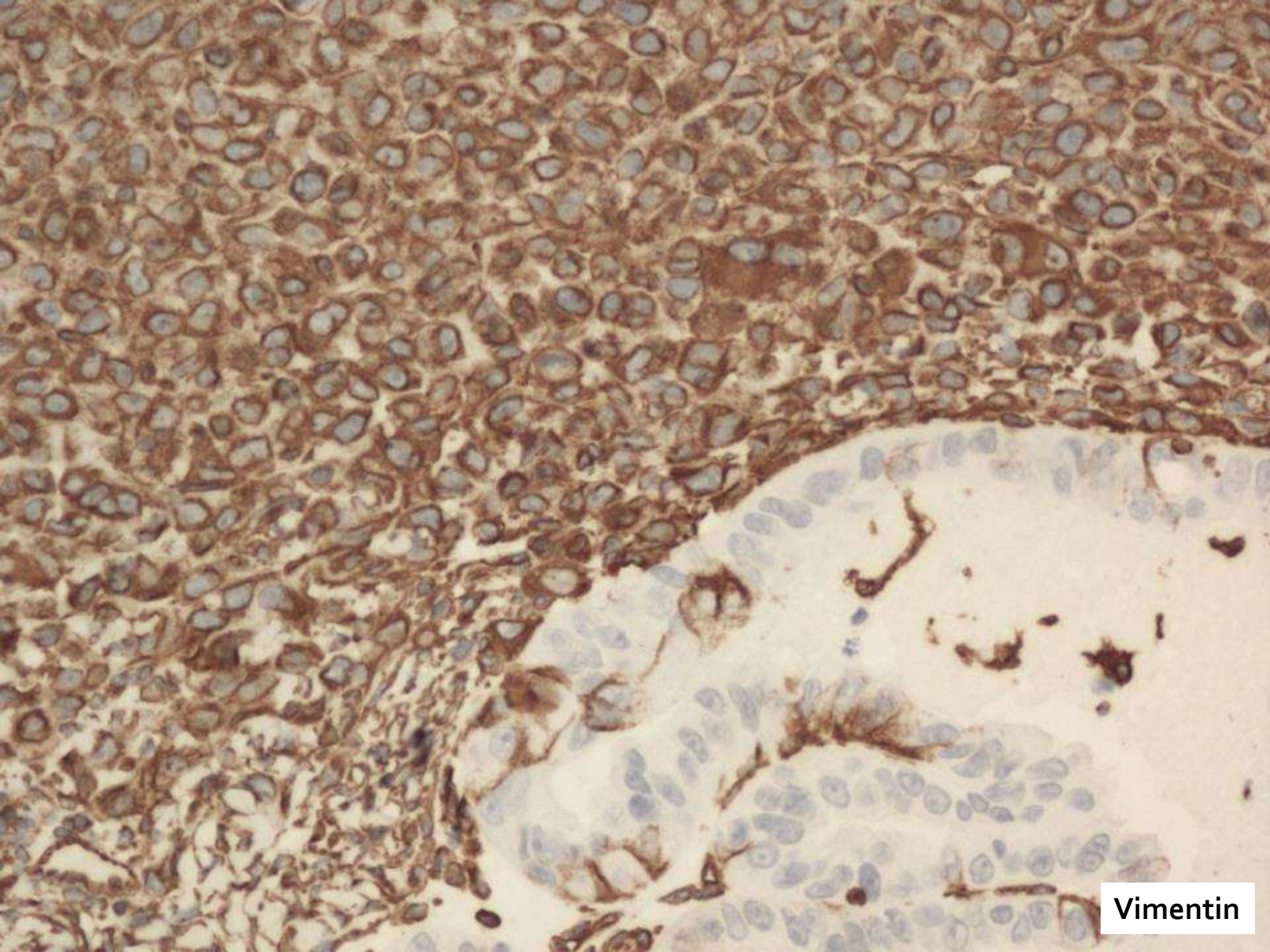

ER
Clear cell carcinoma
HNF-1-beta
ER
HNF-1-beta

Uterine Corpus-Tumor Types

- Carcinosarcoma
  - Often the carcinoma component is a high grade serous carcinoma and will stain like other serous cancers
  - Homologous type of sarcomatous component generally does not stain for anything apart from vimentin
  - Often AE1/AE3, CAM5.2 and Vimentin are used to identify/confirm the biphasic architecture

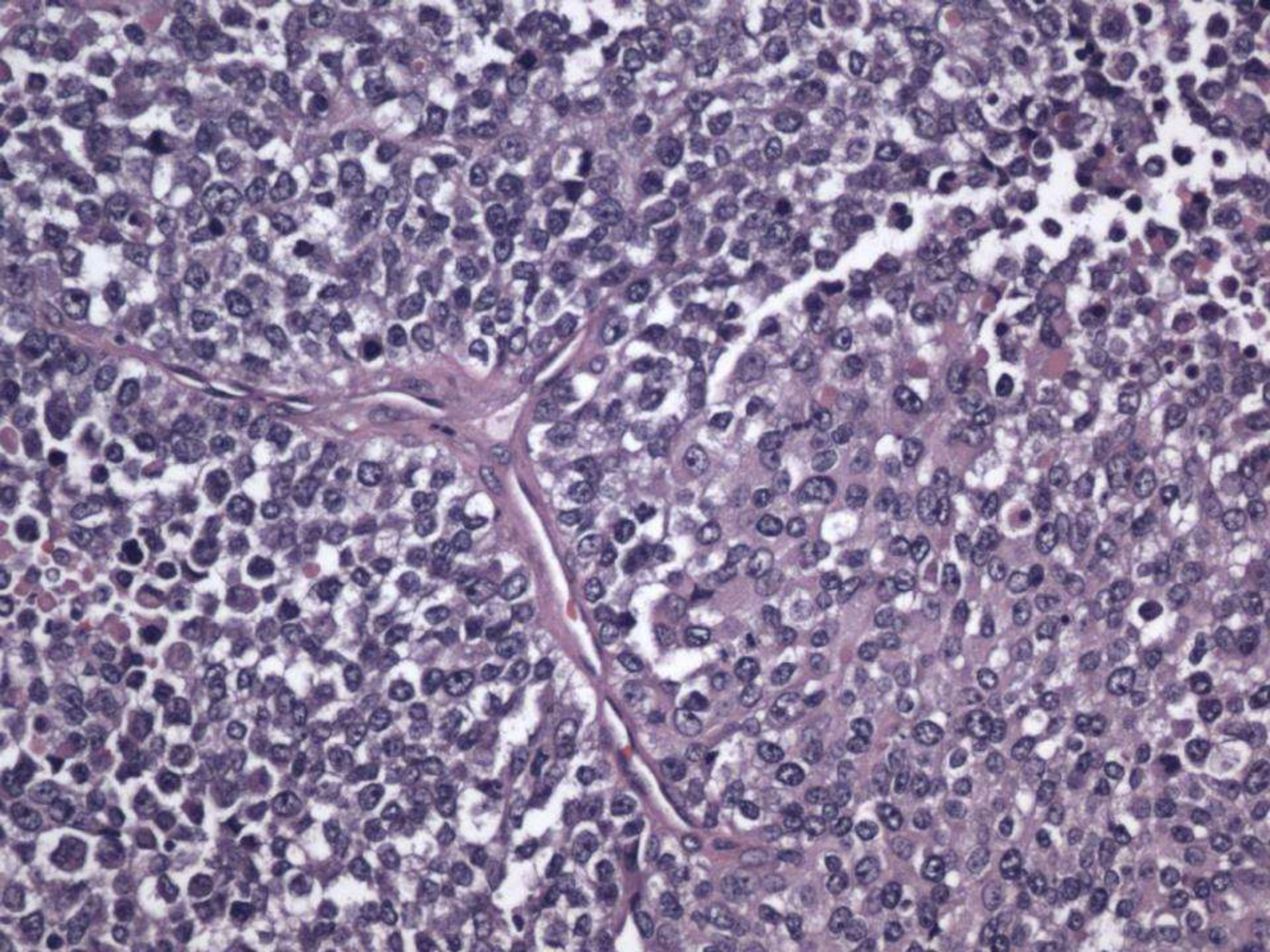


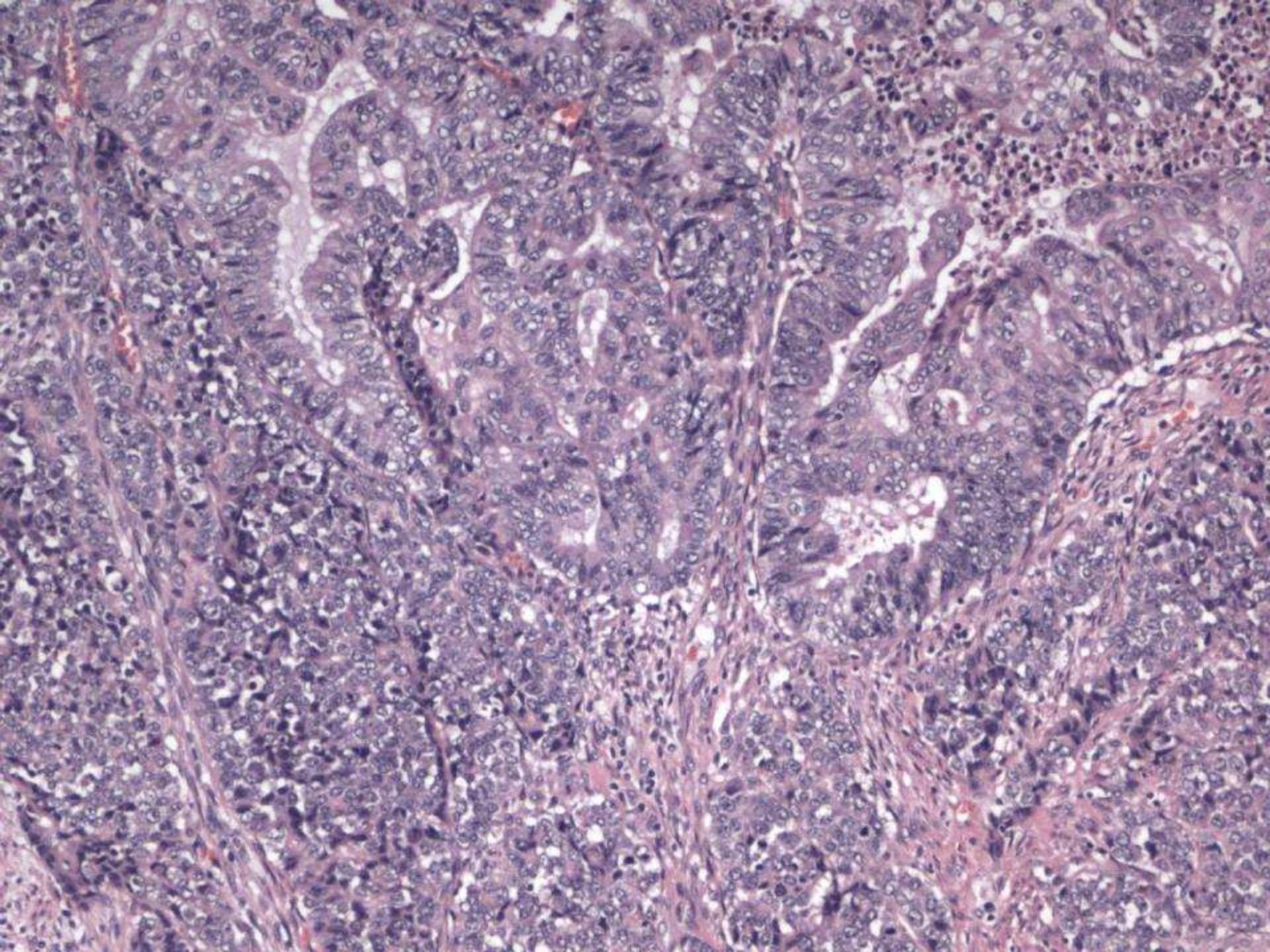

CAM5.2

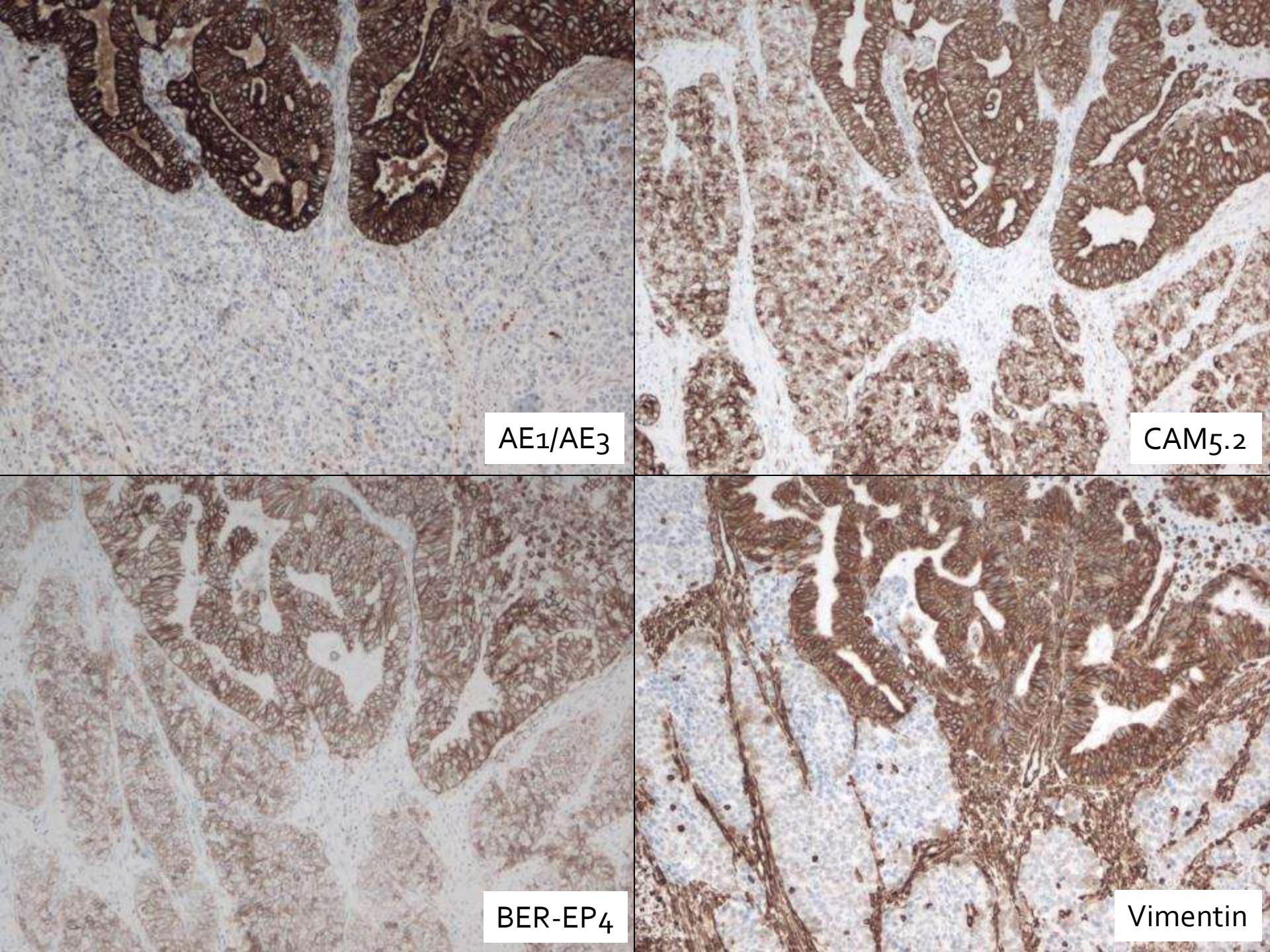

Vimentin

Uterine Corpus-Tumor Types

- Undifferentiated carcinoma
  - Can be present by itself or in combination with well-differentiated endometrioid adenocarcinoma
  - The undiff component is patchy positive with pan-cytokeratin markers (more with CAM5.2 and less with AE1/AE3)
  - Often reactive for EMA
  - Diagnosis confirmed by lack of biphasic morphology, undifferentiated medium sized cells and patchy keratin reactivity






AE1/AE3
CAM5.2
BER-EP4
Vimentin

Endometrial Cancer-Molecular Classification

- The Cancer Genome Atlas (TCGA)
  - Whole genome sequencing, exome sequencing, microsatellite instability (MSI), and copy number analysis
  - Classified 232 endometrioid and serous cancers into 4 groups
    - POLE ultramutated
    - MSI hypermutated
    - Copy number high
    - Copy number low

Endometrial Cancer-Molecular Classification

- POLE Ultramutated
  - POLE encodes the major catalytic and proofreading subunits of the polymerase epsilon DNA polymerase enzyme complex
    - Responsible for leading strand DNA replication
  - Ultramutated cases were characterized by POLE exonuclease domain mutations (EDM)
    - In TCGA, these mutations were detected by whole genome and exome sequencing
    - Can also be detected by Sanger sequencing or PCR
    - No IHC surrogate at present

Endometrial Cancer-Molecular Classification

TCGA category	Molecular changes	Morphology	Outcome
POLE ultramutated	Very high mutation rate,* all with POLE mutation, MSS	Endometrioid (high grade) or ambiguous	Favorable despite aggressive clinical features
MSI hypermutated	High mutation rate, low copy number alteration, MMR-D	Endometrioid	Intermediate
Copy number high	Low mutation rate but p53 mutation, extensive copy number alteration	Serous and some endometrioid (high grade) carcinomas	Worst outcome
Copy number low	Low mutation rate, low copy number alteration, MSS	Endometrioid (low to intermediate grade)	Favorable (? less favorable than POLE mutated tumors)

*Mutation in PTEN (94%), FBXW7 (82%), ARID1A (76%), PIK3CA (71%), TP53 (35%), and MSS (65%)
MSS: microsatellite stable; MMR-D: mismatch repair protein deficient.

Strategy for Endometrial Carcinoma

- Mismatch repair protein testing by IHC for MSH6 and PMS2
  - MMR deficient (MMR-D) if lost
- *POLE* exonuclease domain mutations by PCR
  - *POLE*-EDM
- IHC for p53
  - Wild type (p53wt)
  - No expression or diffuse strong expression (p53abn)

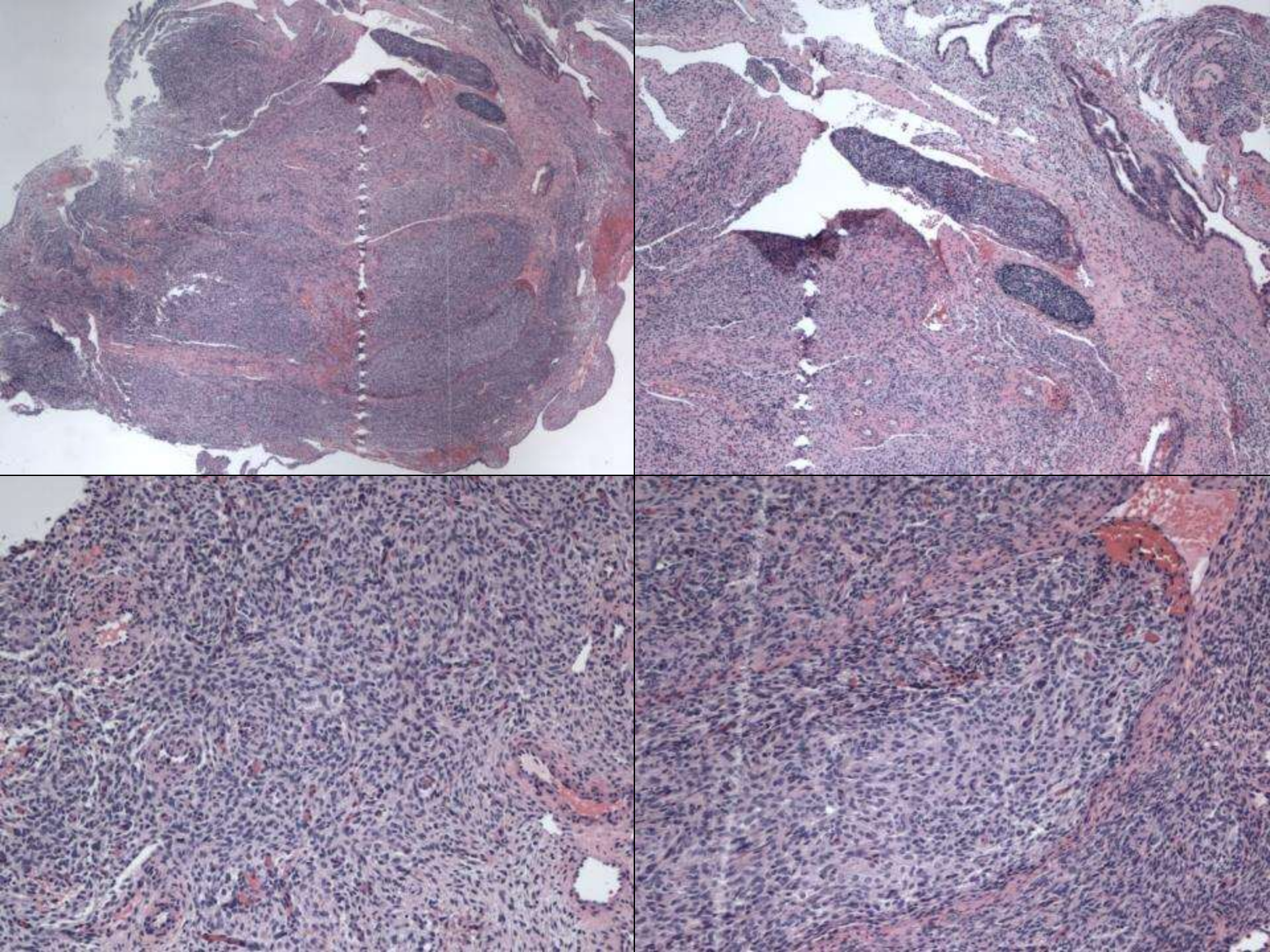
Talhouk A and McAlpine JN. Gynecologic Oncology Research and Practice. 2016;3:14.

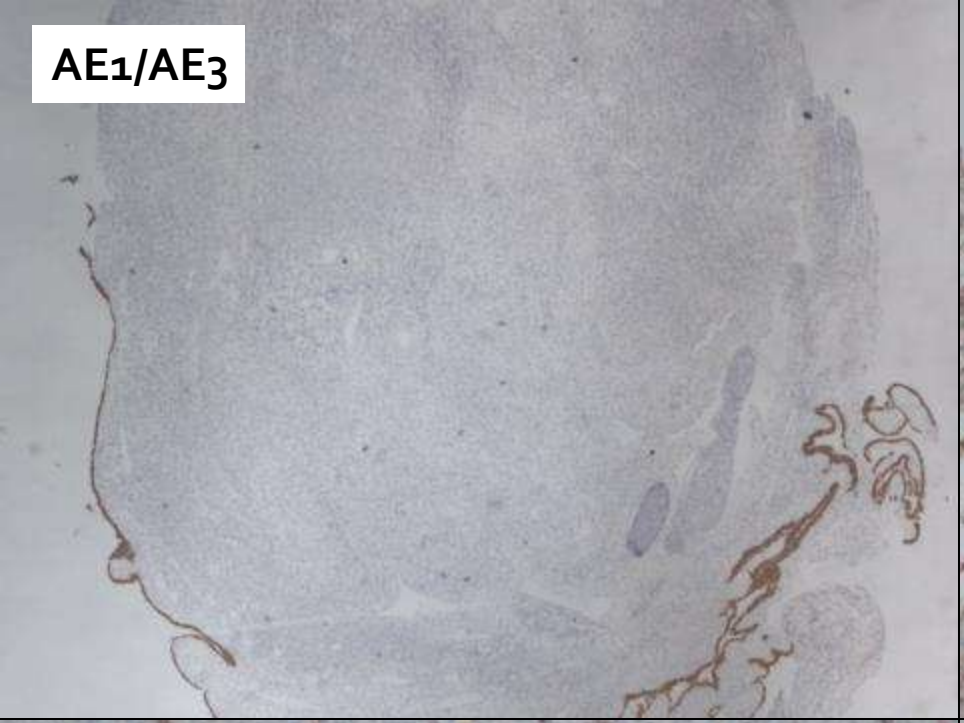
Uterine Mesenchymal Tumors

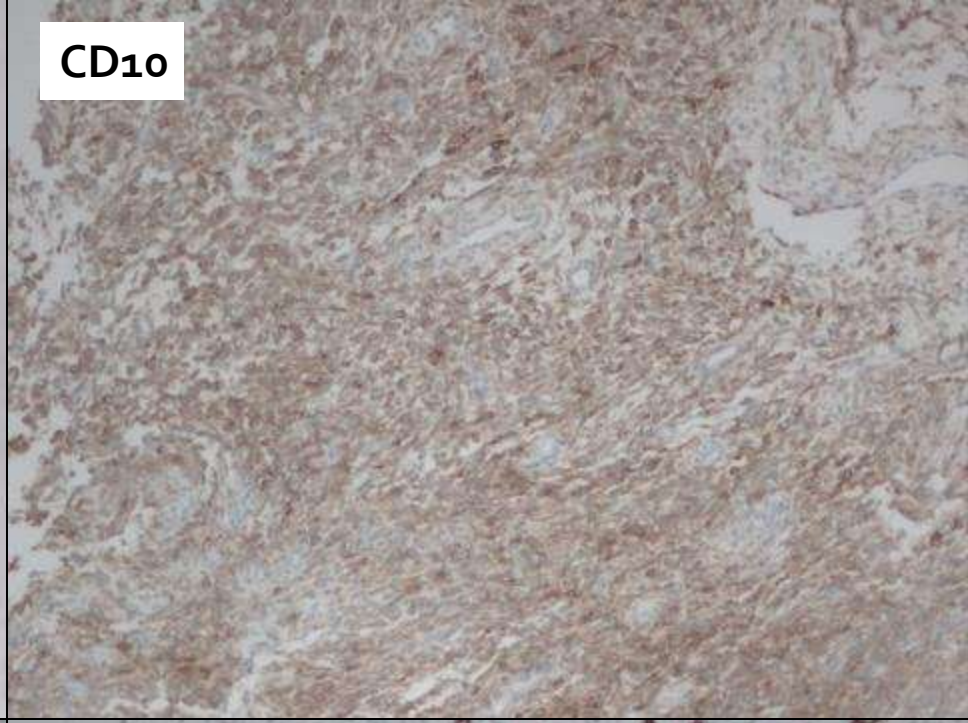
- Smooth muscle tumors
  - Leiomyoma
  - Atypical leiomyoma
  - STUMP
  - Leiomyosarcoma
- Endometrial stromal tumors
  - Nodule
  - Stromal sarcoma
    - Predominantly low grade ESS
    - Rare high grade ESS

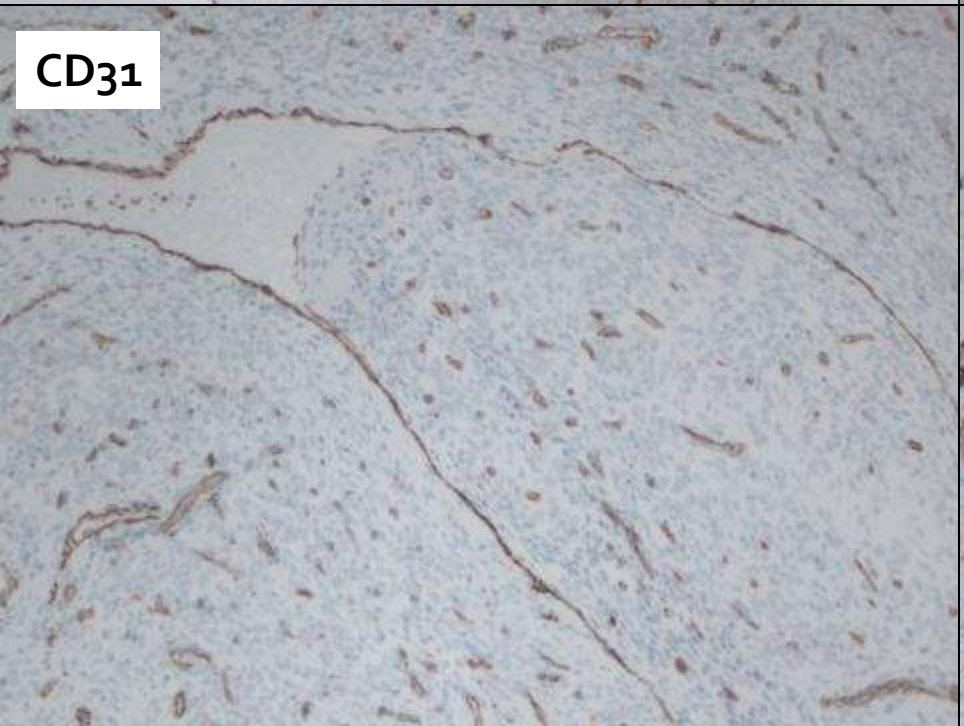
Uterine Mesenchymal Tumors

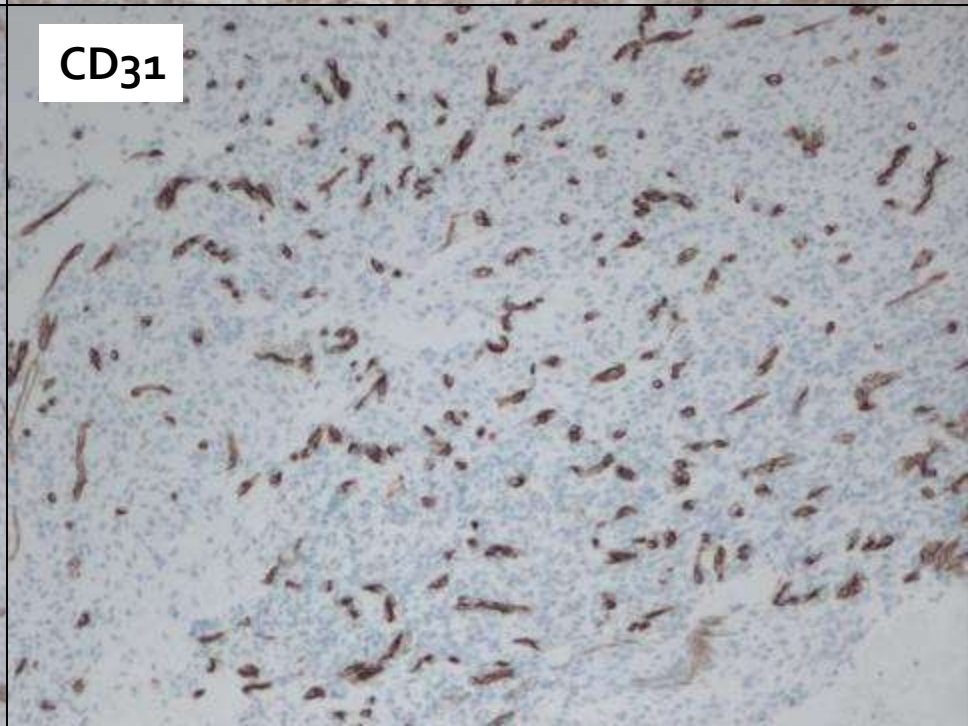
- Smooth muscle markers
  - Smooth muscle actin
  - Common muscle actin
  - Desmin
  - Caldesmon
  - WT1
- Stromal markers
  - CD10
  - WT1
  - Interferon induced transmembrane protein 1(IFITM-1)




AE1/AE3

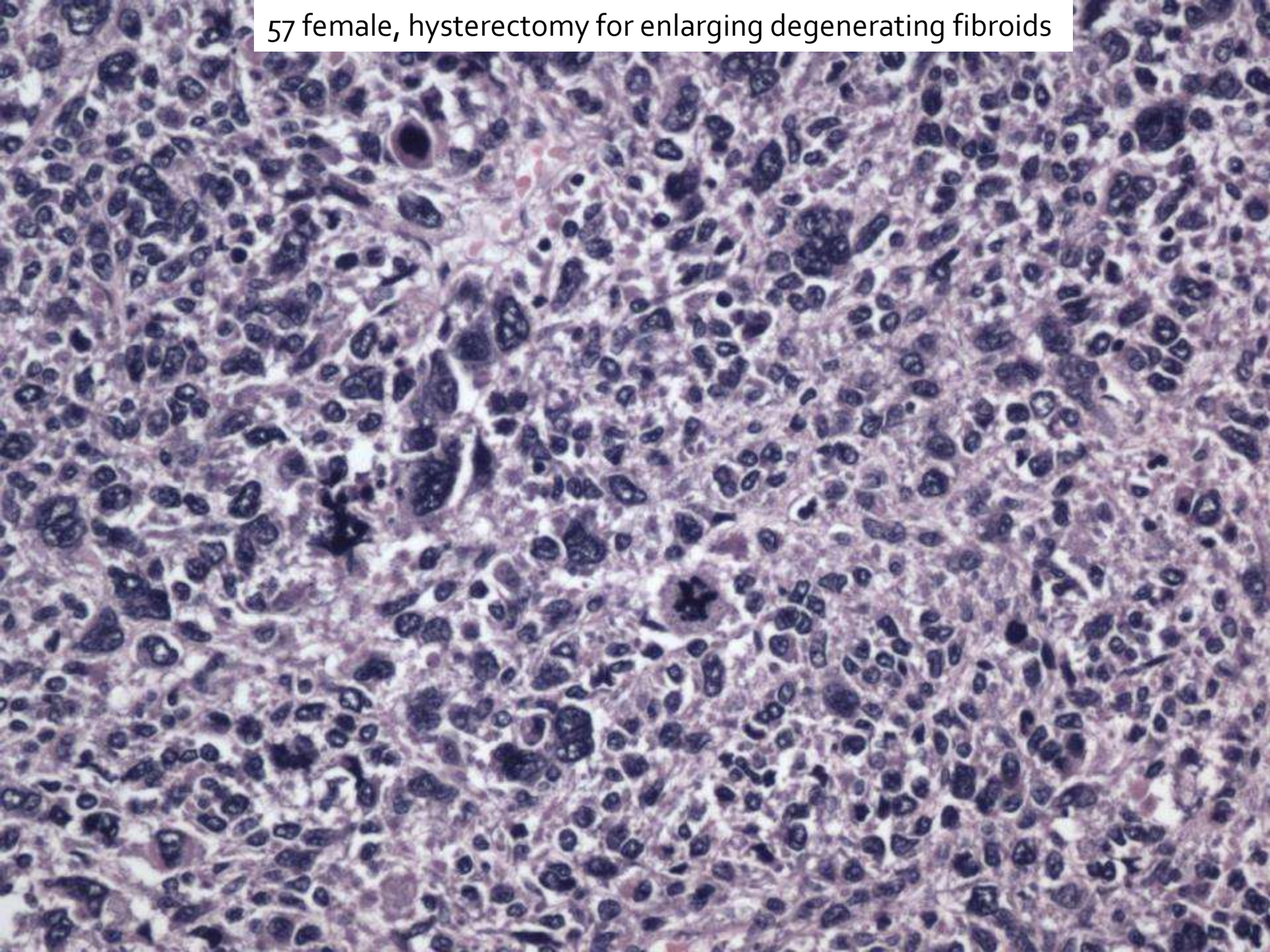

CD10

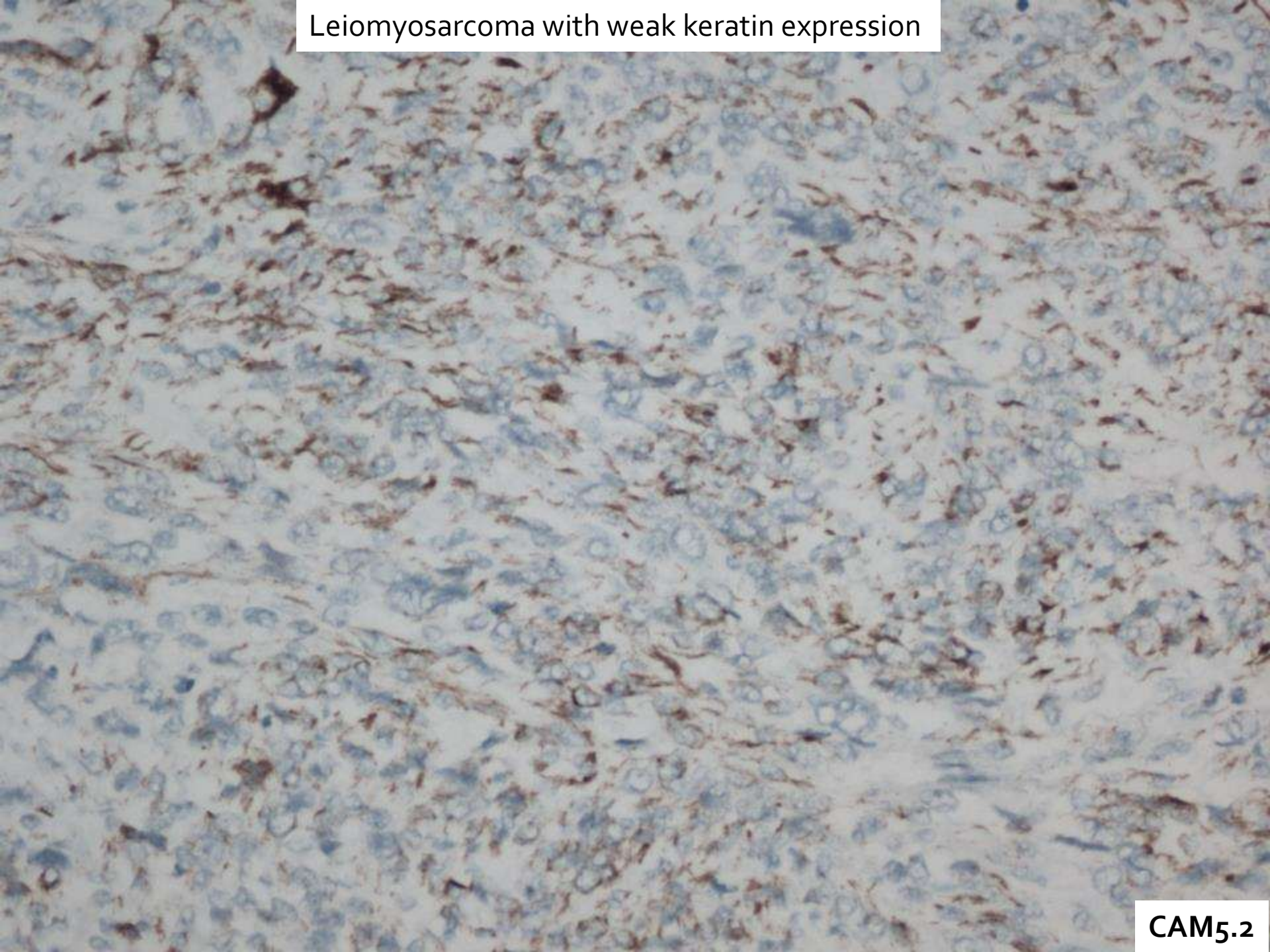

CD31


CD31

Uterine Mesenchymal Tumors-IHC pitfalls

- Leiomyosarcomas can mark for pan-cytokeratin stains in a patchy fashion
- Actin and desmin are less specific than caldesmon
- Myxoid and epithelioid leiomyosarcomas may show unusual immunoreactive patterns
- CD10 can be positive in smooth muscle tumors
- Endometrial stromal sarcomas with smooth muscle differentiation can show reactivity for smooth muscle markers
  - Mixed tumors when at least 30% of each component
  - Otherwise tumors with ESS morphology and staining for smooth muscle markers have clinical outcome similar to ESS


57 female, hysterectomy for enlarging degenerating fibroids

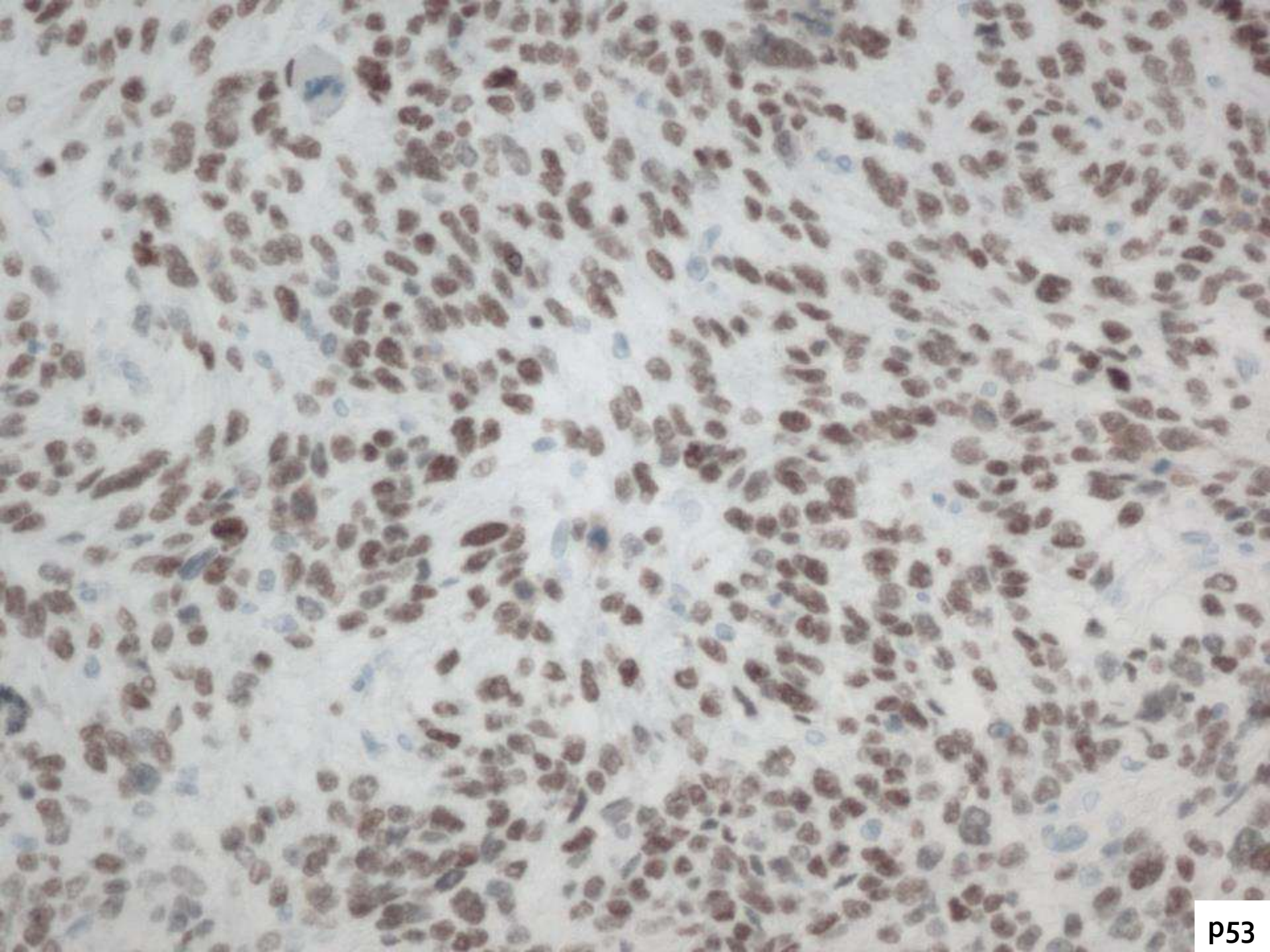

Leiomyosarcoma with weak keratin expression
CAM5.2

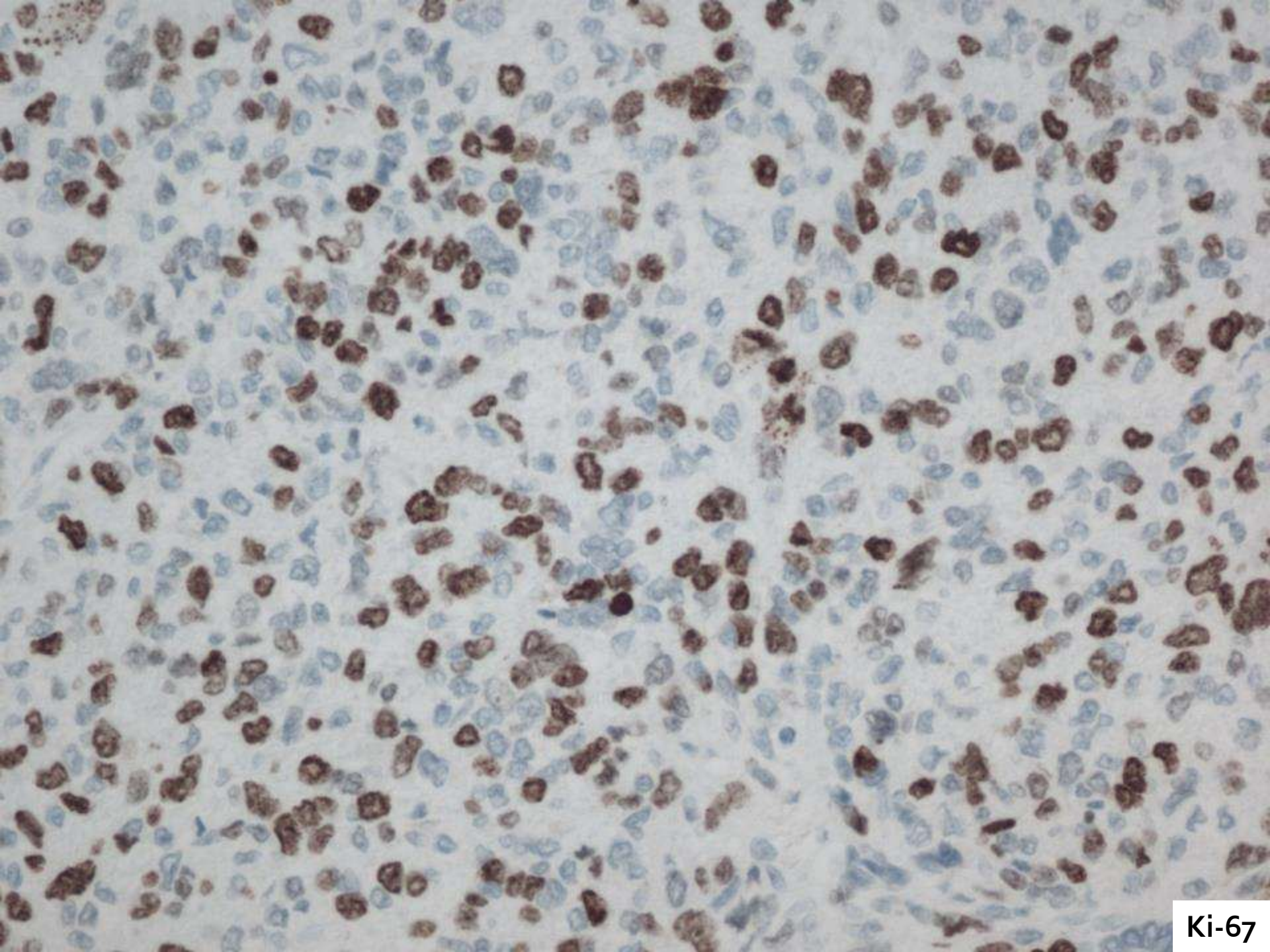
STUMP versus Leiomyosarcoma

- Morphologic criteria
  - Atypia, Necrosis, mitotic activity
- IHC can be complementary but should not be used alone
  - P16
    - Not very reliable as most STUMP and atypical leiomyoma cases show diffuse expression
  - P53
    - Diffuse strong nuclear reactivity favors leiomyosarcoma
  - Ki-67
    - Can be helpful when there is marked atypia but mitoses are either absent or difficult to score
  - Fascin
    - Most leiomyosarcoma and 50% of STUMP are positive, but expressed only rarely in leiomyomas
  - ATRX* and DAXX**
    - Loss of expression associated with poor prognosis in smooth muscle tumors

*ATRX: Alpha-thalassemia/mental retardation syndrome X-linked

**DAXX: death-domain-associated protein

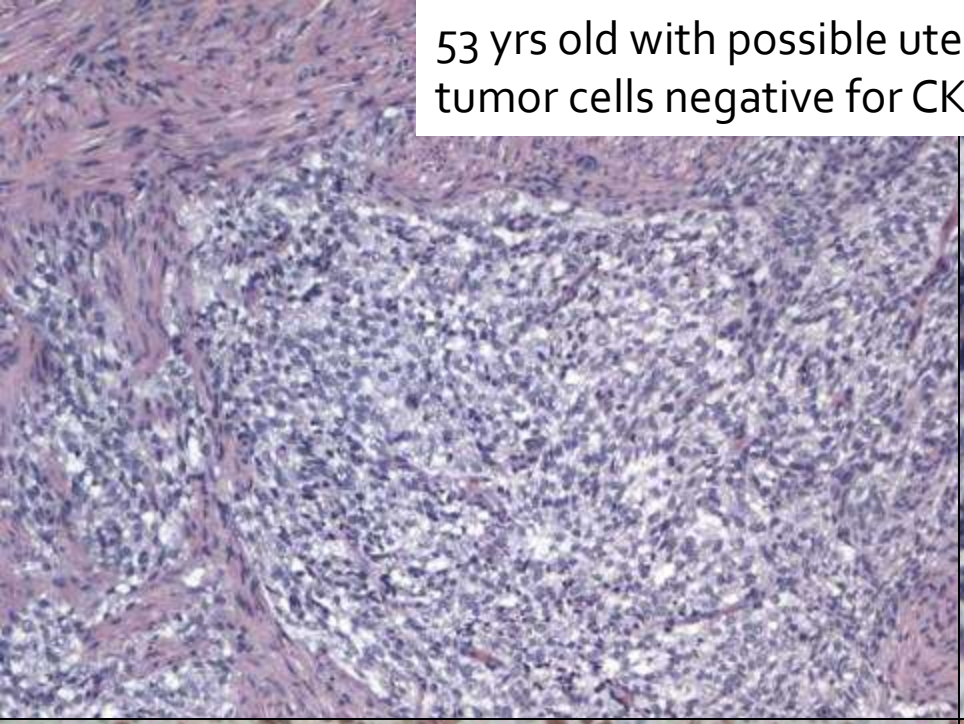

p53

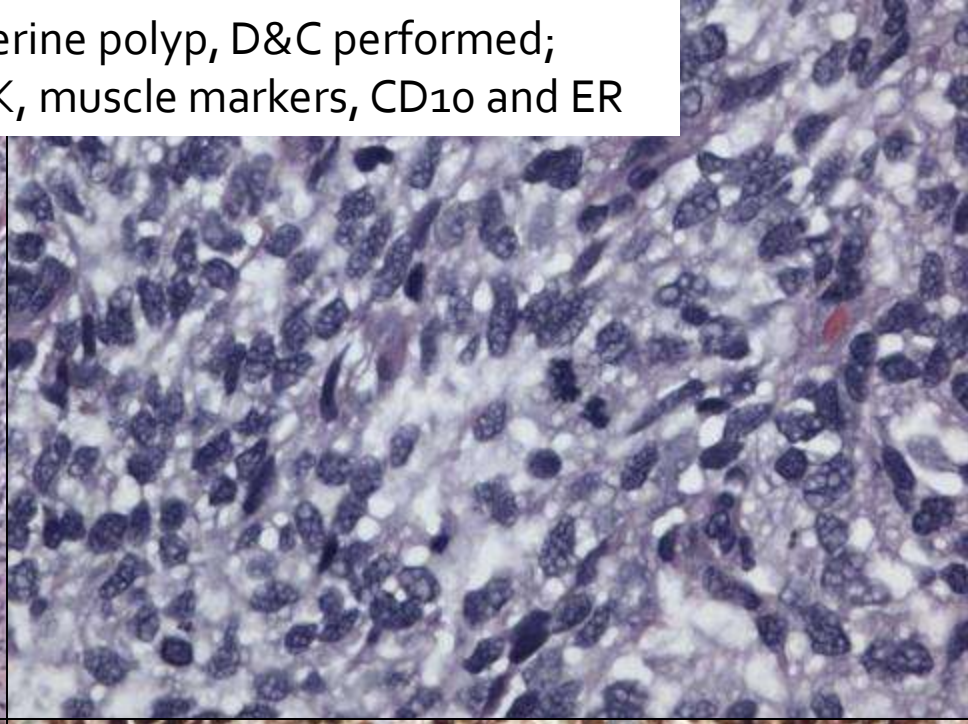

Ki-67

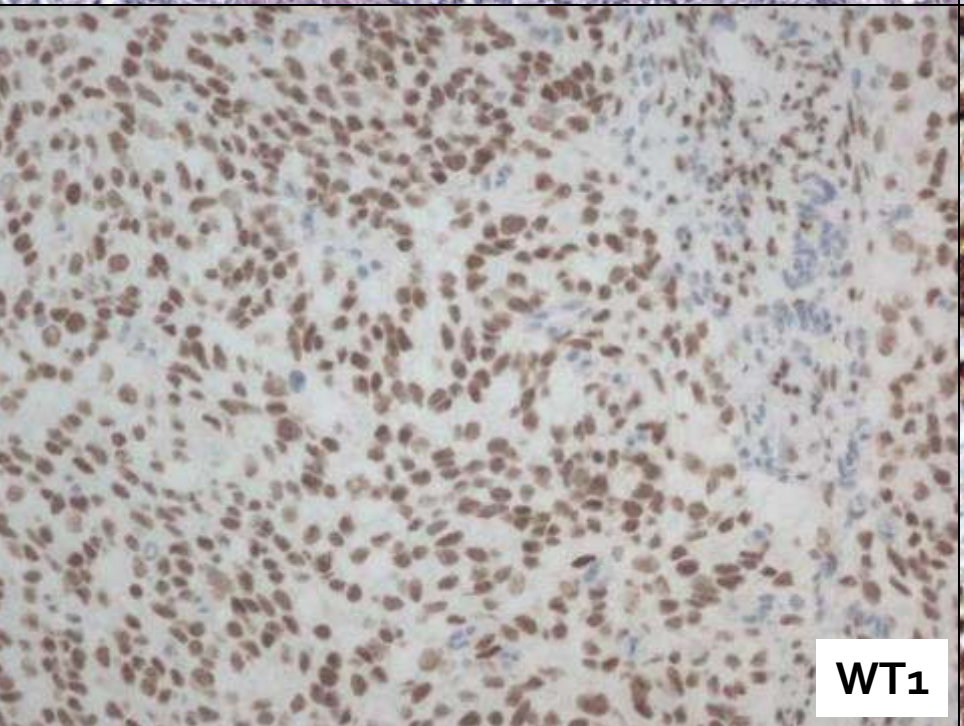
High grade ESS

- Very rare
- May still have some degree of stromal differentiation
  - Patchy CD10 and hormone receptors
  - Often cyclin D1 positive
    - Correlates with YWHAE-NUTM2A/B fusion [t(10;17)(q22;p13)]

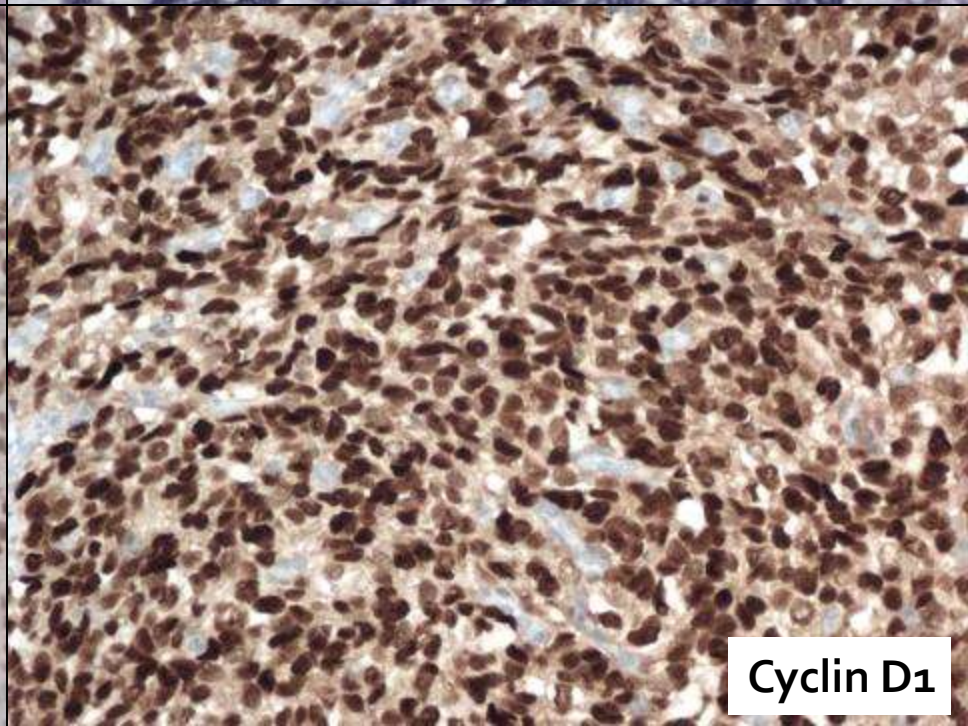
53 yrs old with possible uterine polyp, D&C performed; tumor cells negative for CK, muscle markers, CD10 and ER







WT1



Cyclin D1

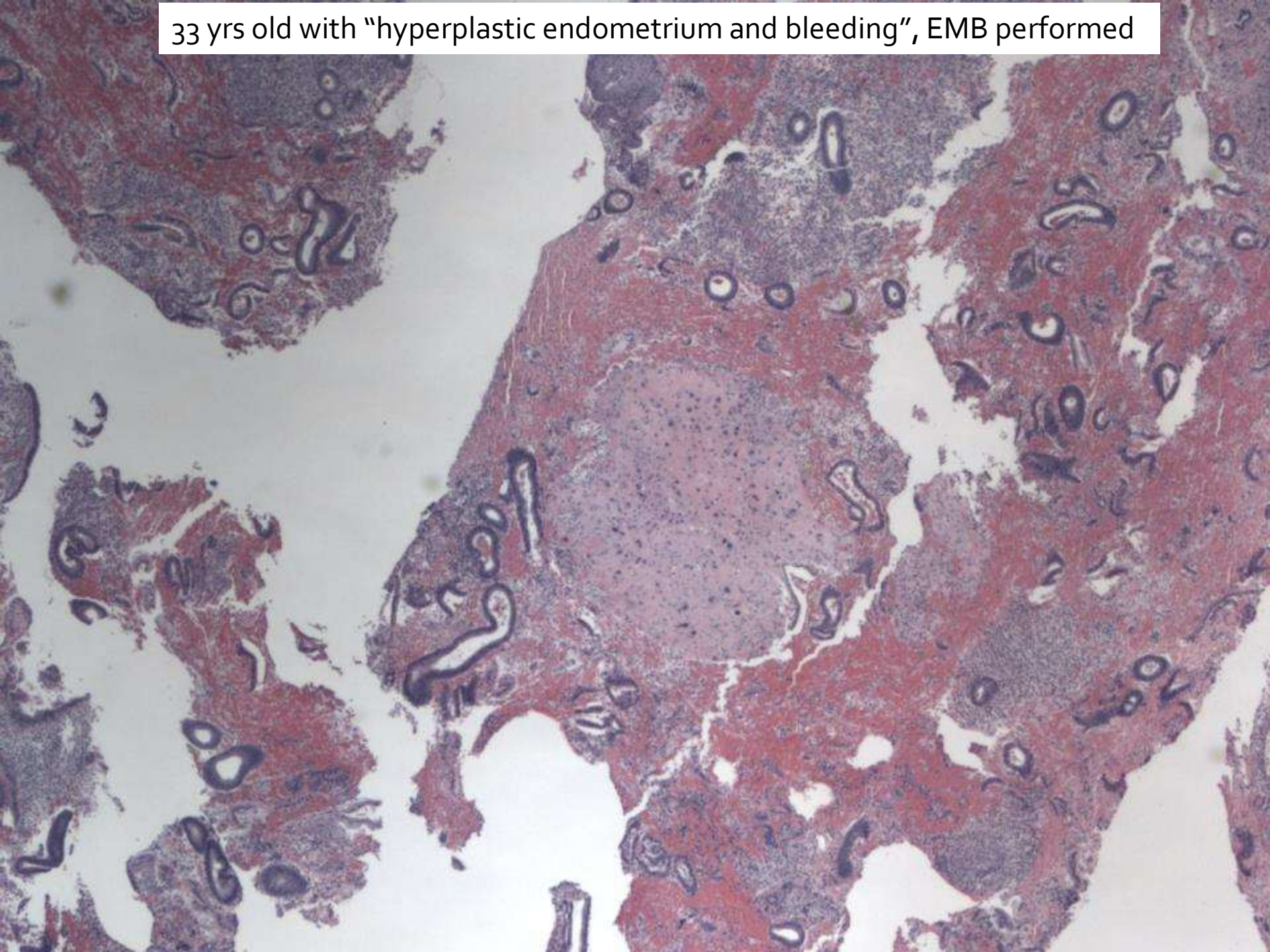
Perivascular Epithleioid Cell Tumor (PEComas)

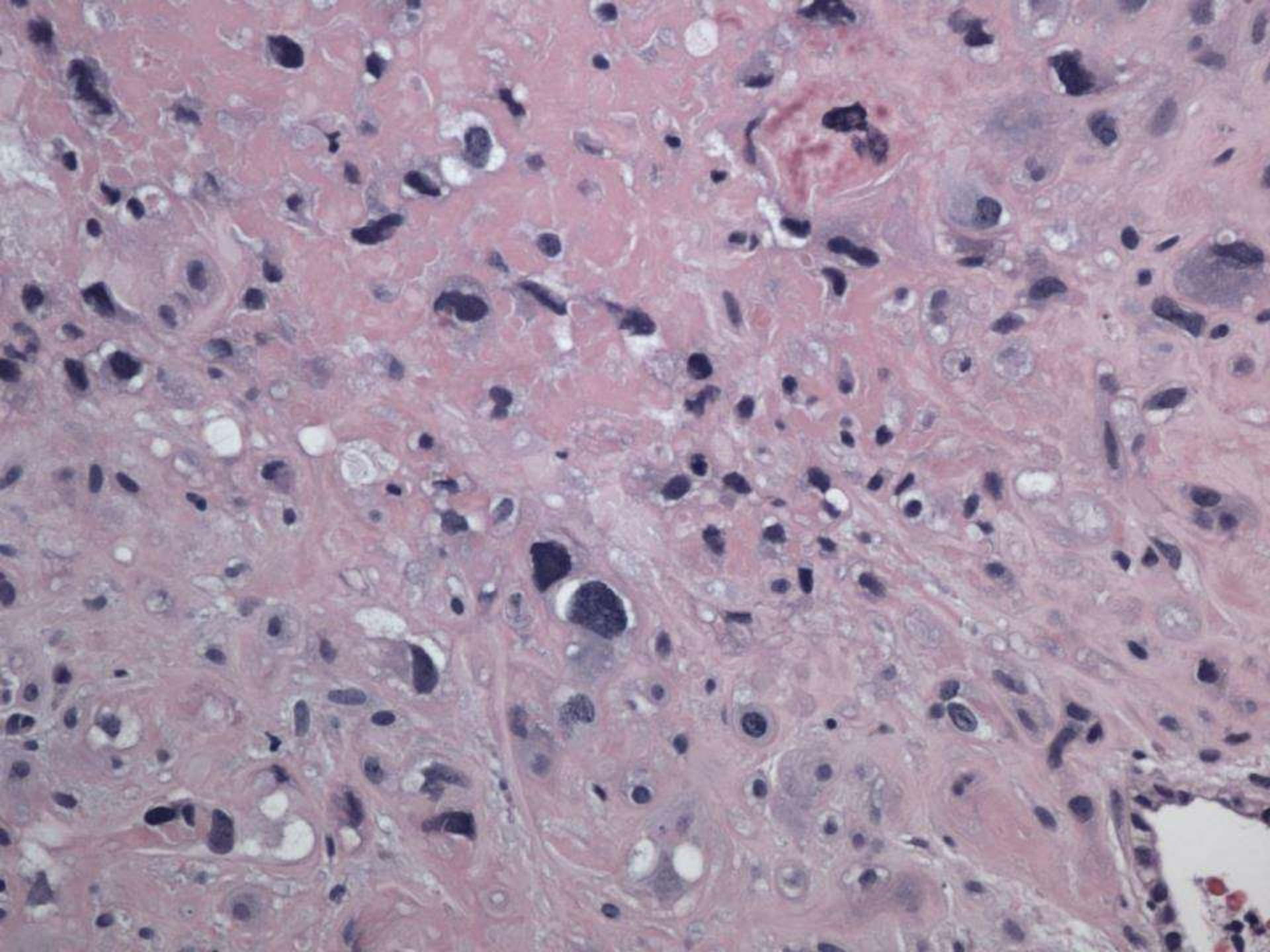
- Rare to present as primary in the uterus
- Spindle and epithelioid cells with clear and granular cytoplasm
  - Morphologic overlap with smooth muscle and endometrial stromal tumors
- Positive for SMA, desmin, HMB45, and Melan A but often negative (or weak pos) for S100
  - Typical smooth muscle tumors and endometrial stromal tumors are negative for melanocytic markers

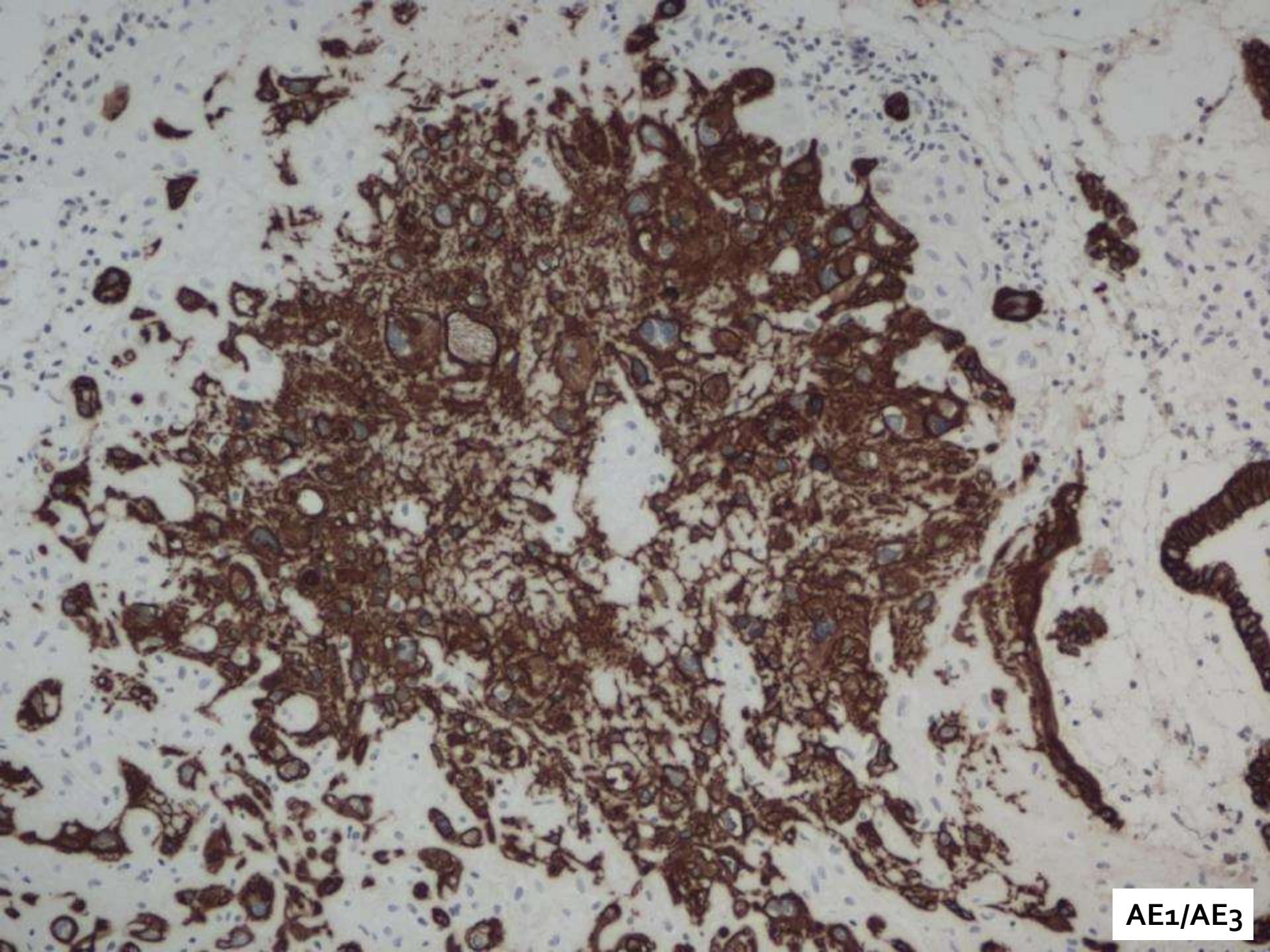
Trophoblastic Markers

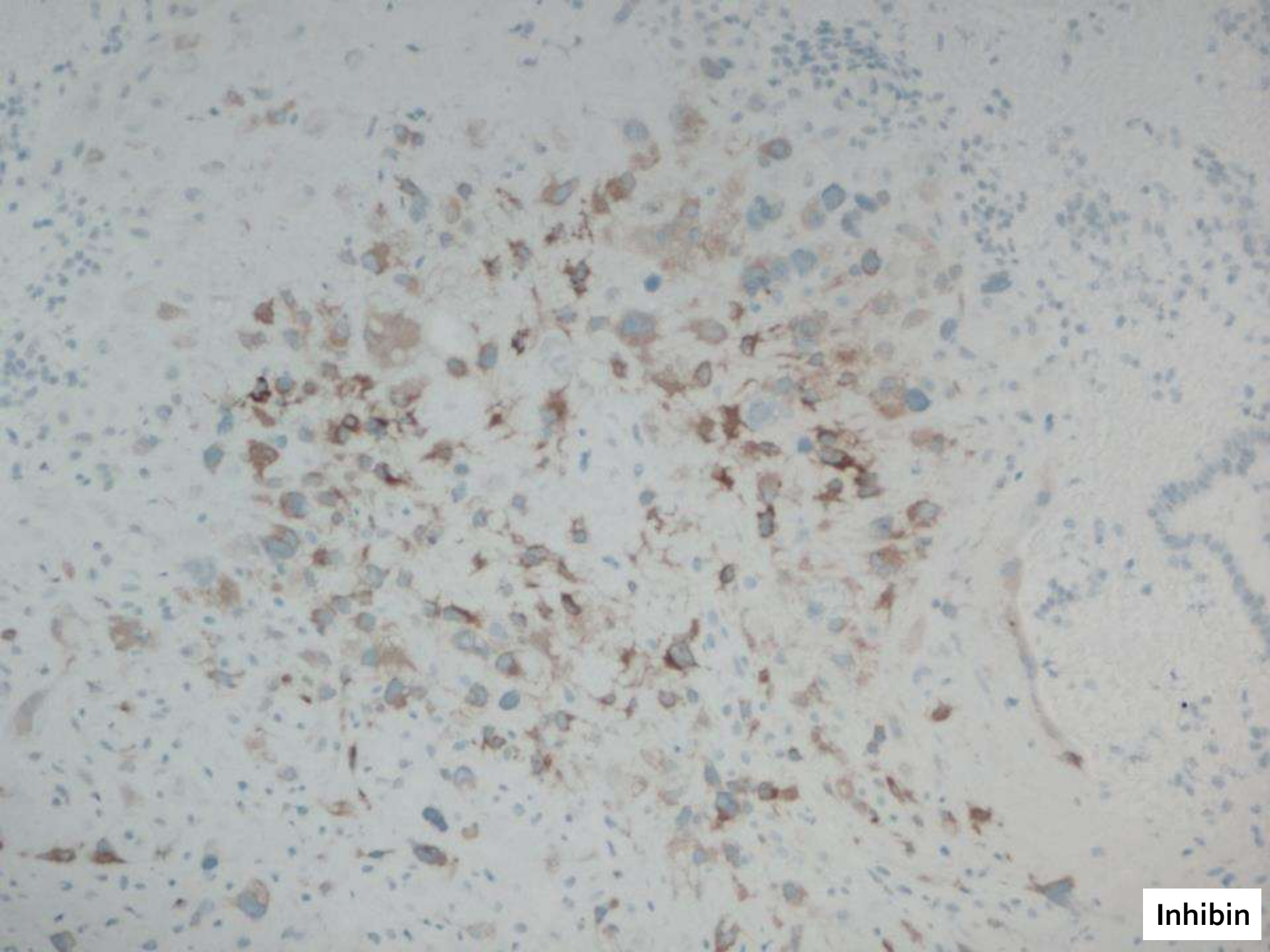
- Keratins: (AE1/AE3), CK18
- General marker: Inhibin
- Cytotrophoblast markers: p63 and β-catenin
- Implantation site trophoblast: hPL, hCG, HLA-G, CD146 (Mel-CAM)
- Syncytiotrophoblast markers: hCG, hPL, PLAP

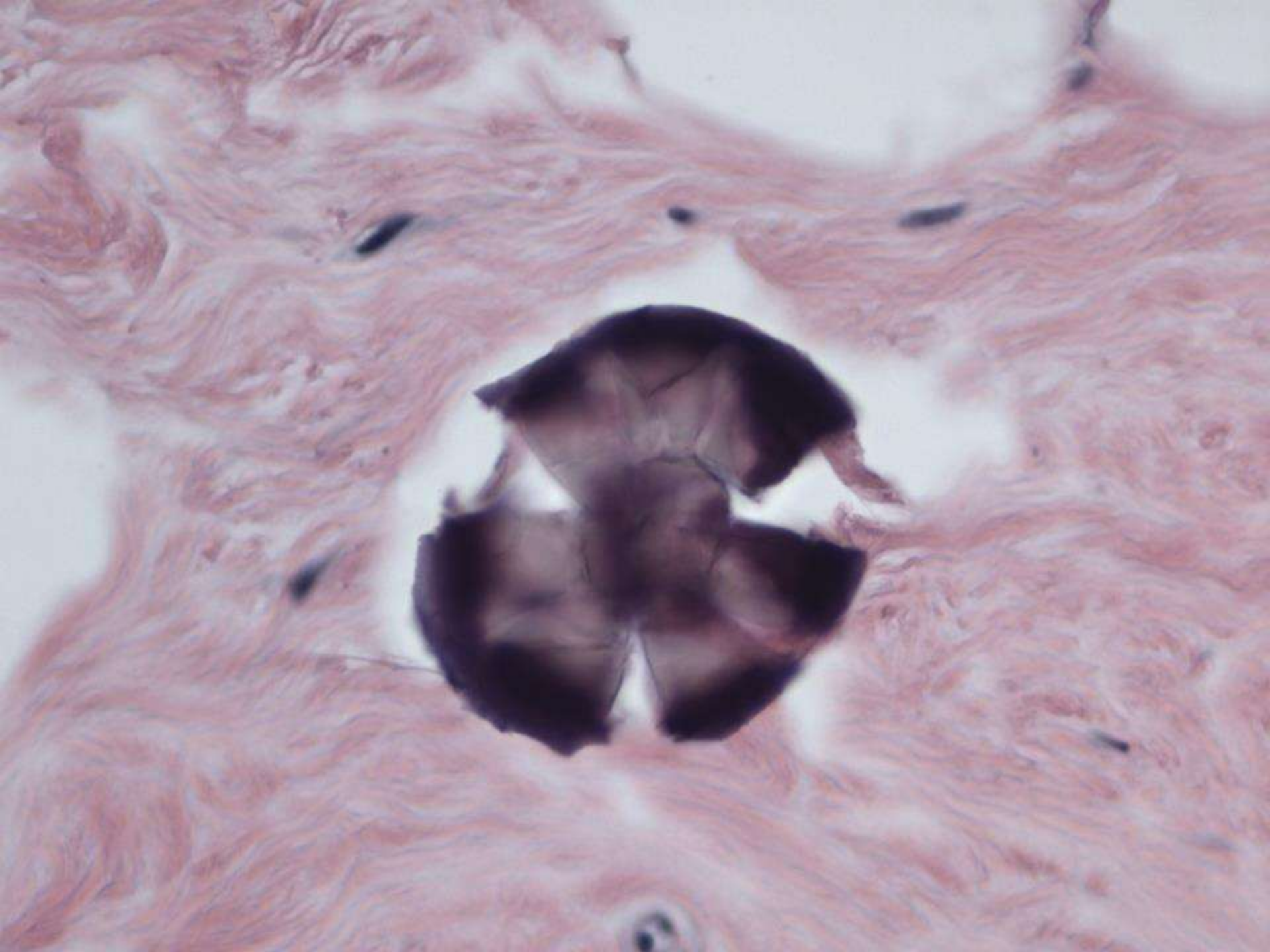
33 yrs old with “hyperplastic endometrium and bleeding”, EMB performed





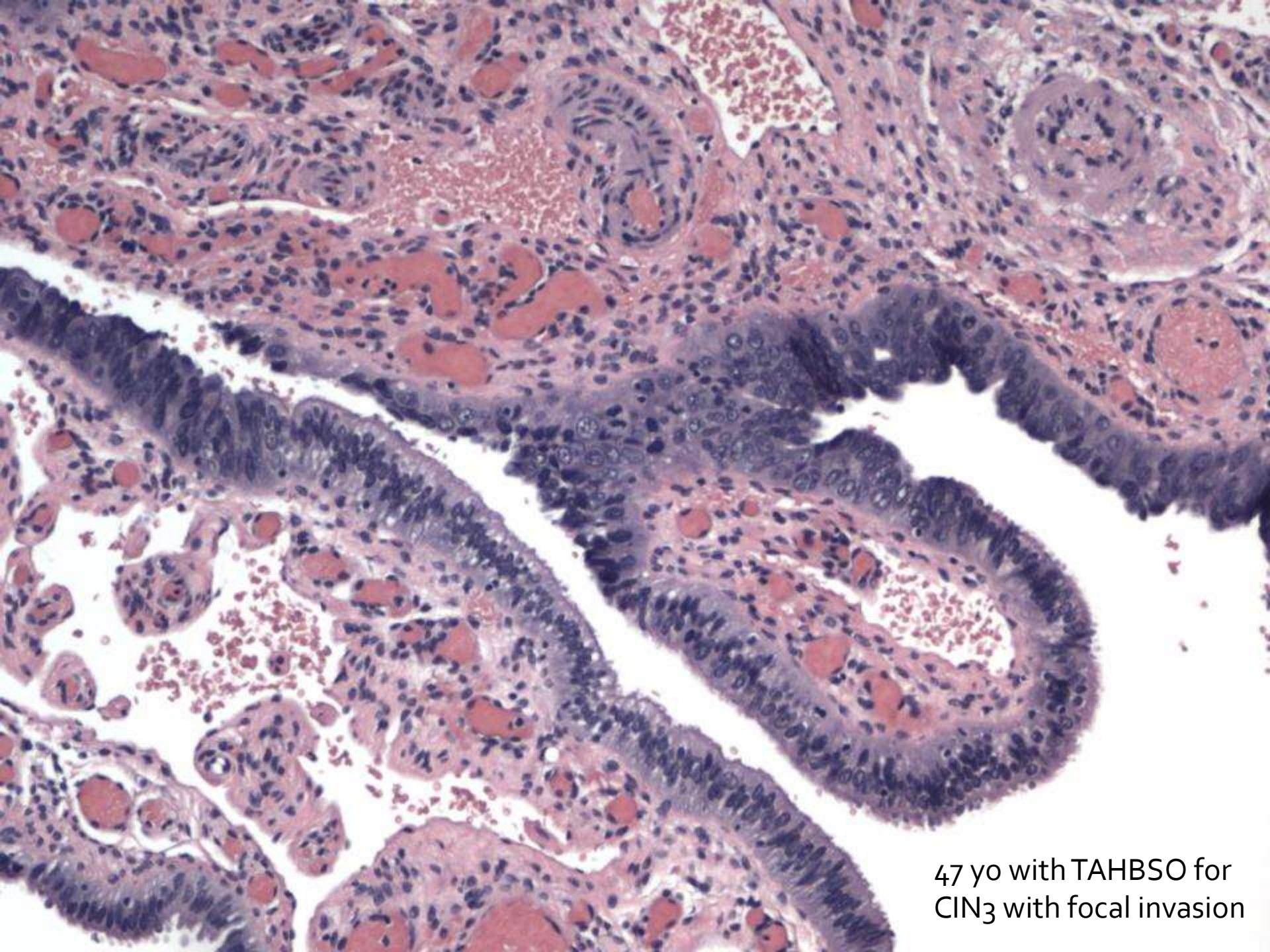

AE1/AE3


Inhibin

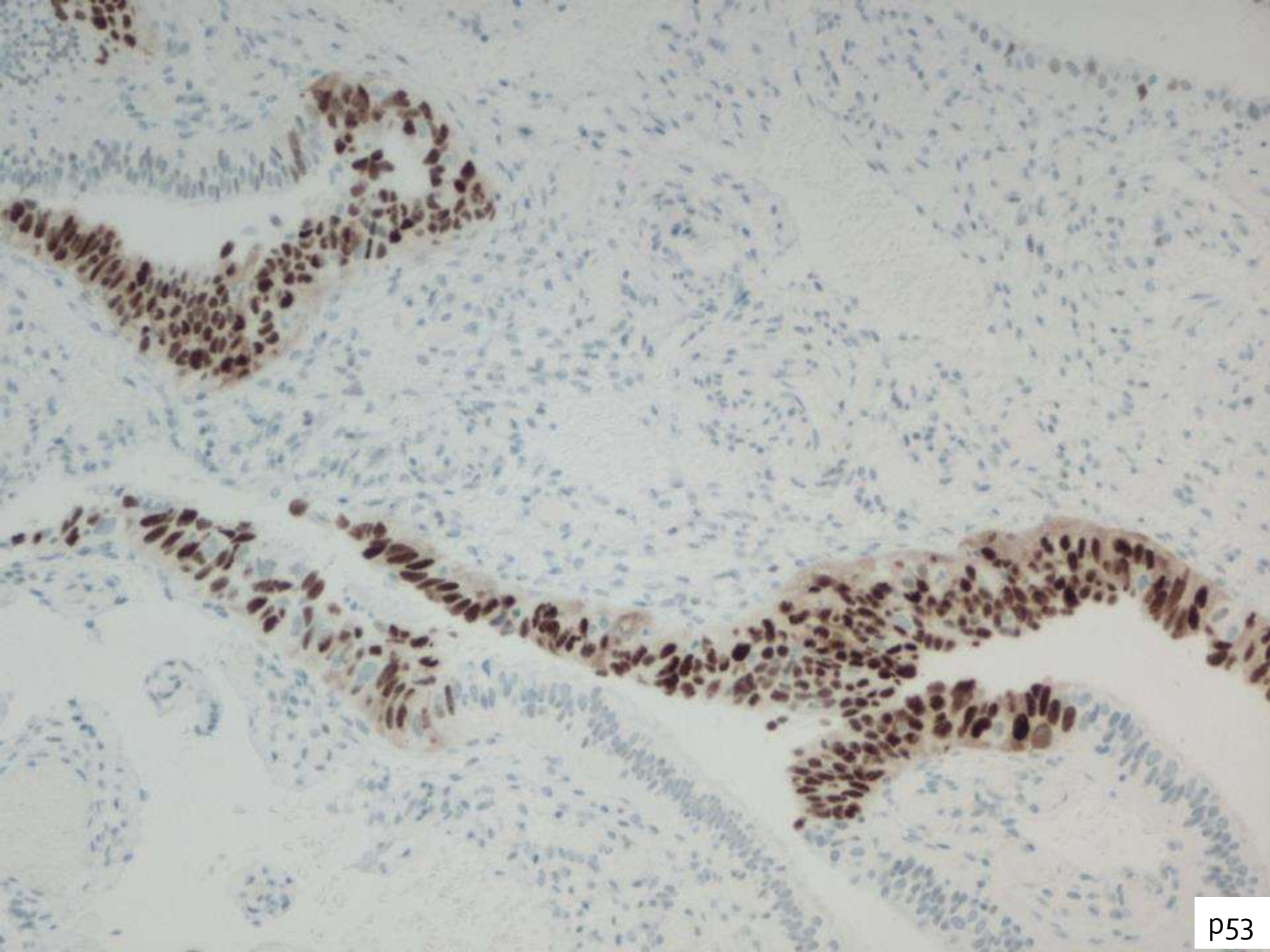


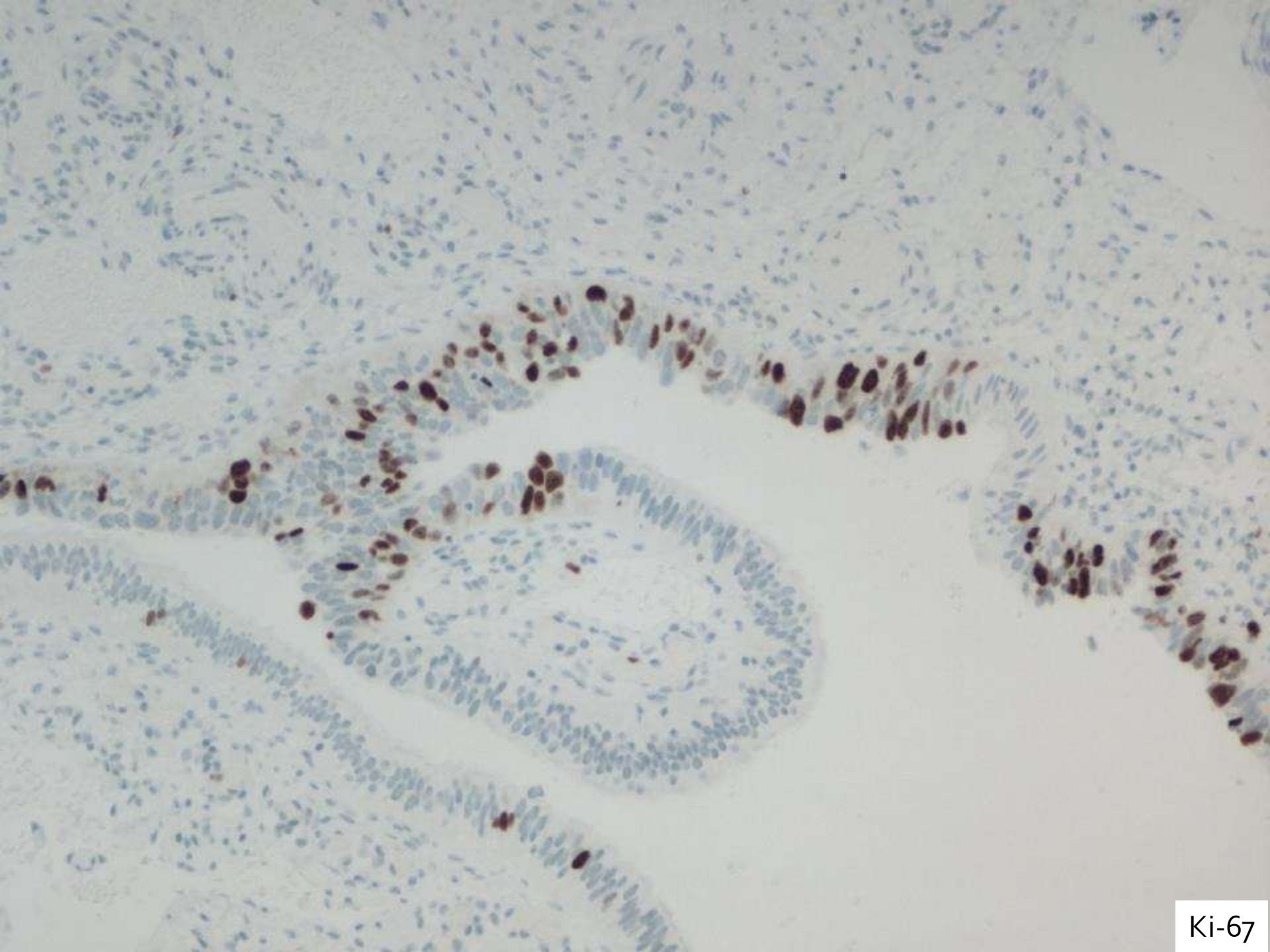
Fallopian Tube

- Serous tubal intraepithelial carcinoma
  - Incidental
  - BRCA carrier
- Now routine to submit distal 2 cm portion of fallopian tube in every case
  - Fimbriated end is longitudinally sectioned to maximize surface area for examination



47 yo with TAHBSO for CIN3 with focal invasion


p53


Ki-67

Ovarian tumors

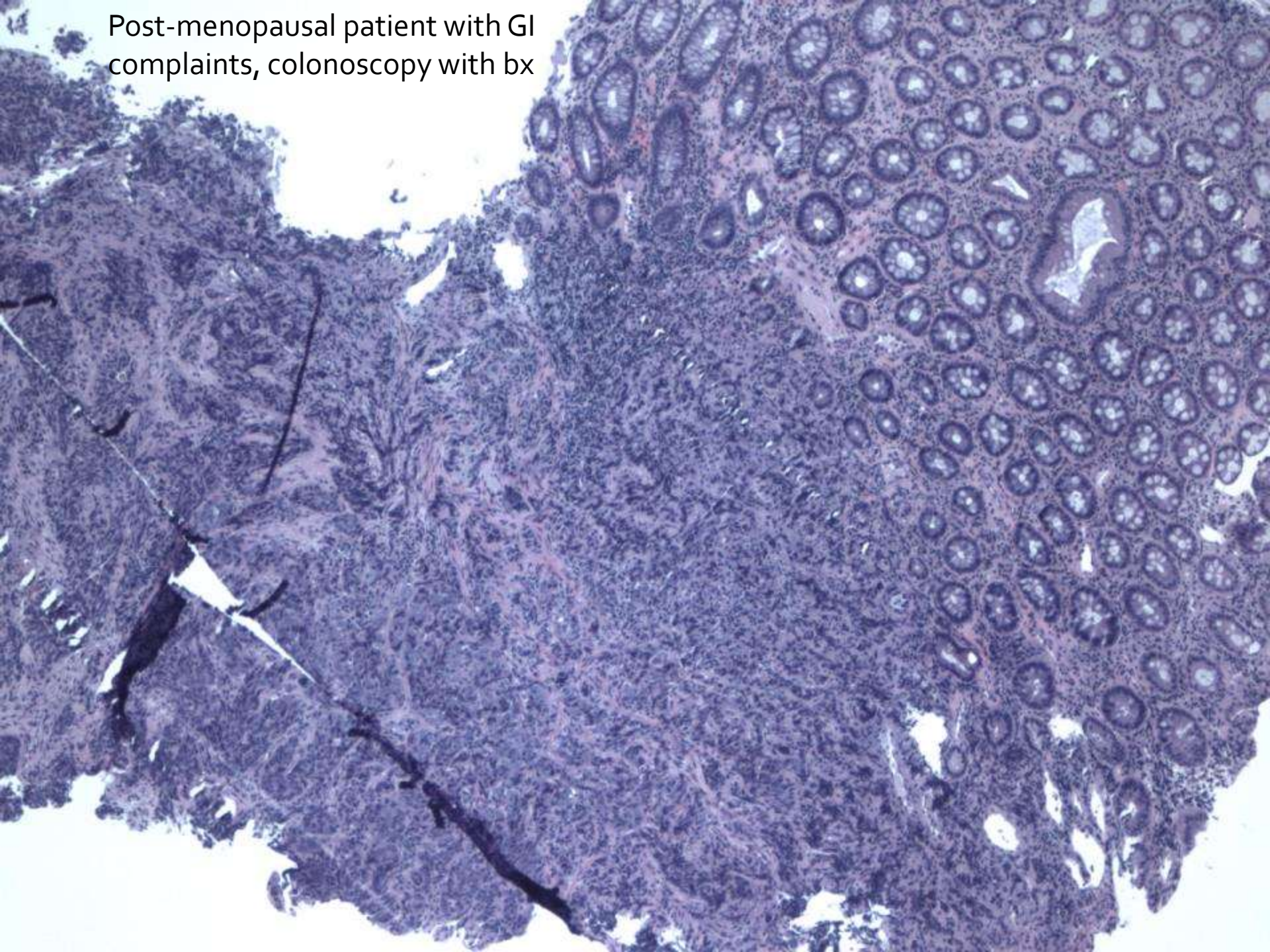
- Epithelial
  - Serous, clear cell, endometrioid, mucinous
- Stromal
  - Fibrothecoma, AGCT, Sertoli-Leydig cell
- Germ cell
  - Dysgerminoma, Yolk sac, Embryonal, Teratoma
- Others

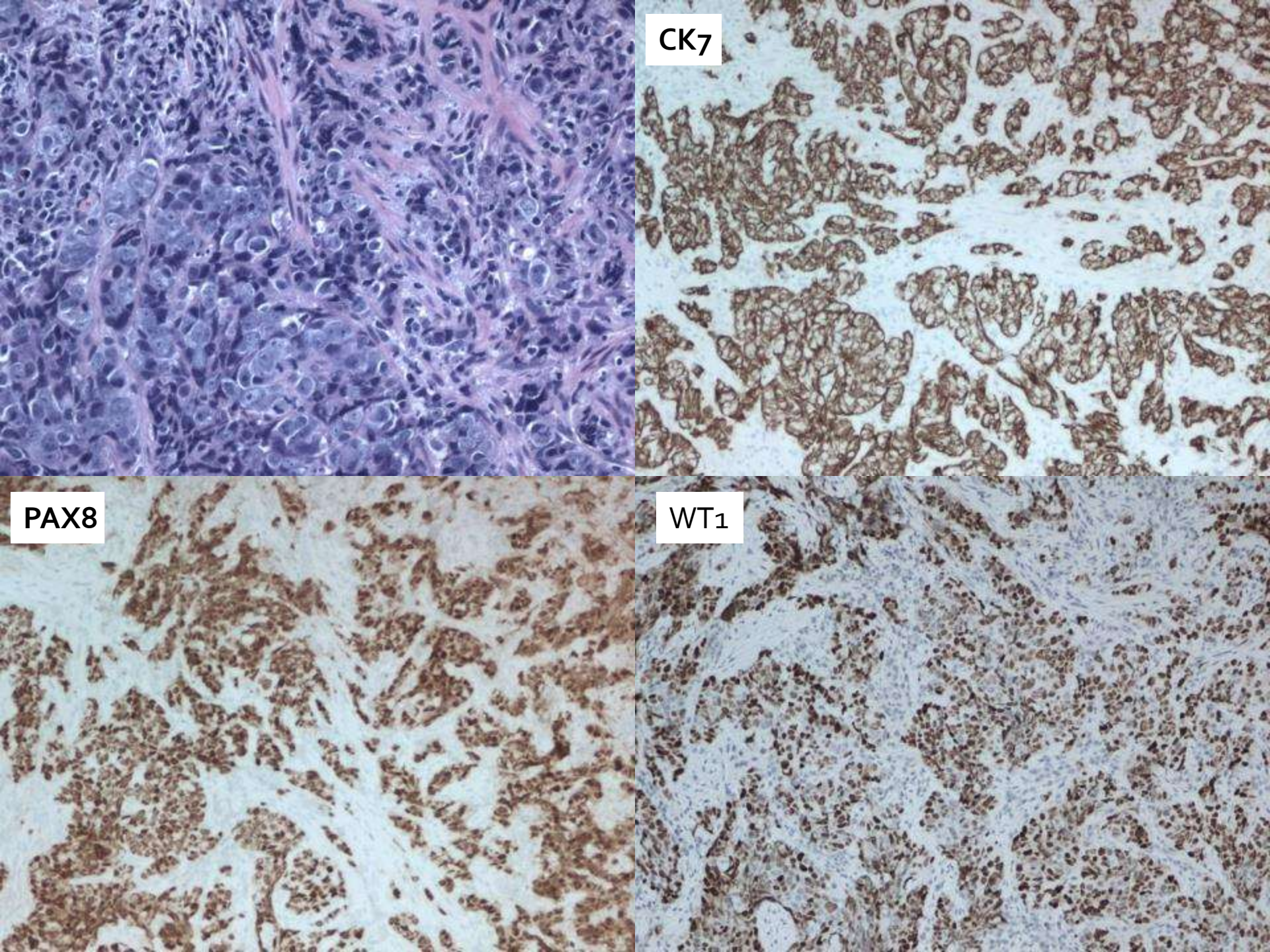
Ovarian Epithelial Tumors

Antibodies	Endometrioid	Serous-high grade	Clear Cell
P53	Wild type	+	Wild type/+
P16	Patchy +	+ (diffuse strong)	+ (diffuse strong)/-
HNF-1 beta	-/+ (weak-mod)	-	+ (strong)
Napsin A	-	-	+
ER	+	Patchy +	-
PR	+	-	-
Vimentin	+	Patchy +/-	Patchy +/-
WT1	- / scattered	+ (diffuse strong)	- / scattered
PAX8	+	+	+
BER-EP4 / MOC31	+	+	+

Low grade serous carcinomas and serous borderline tumors are positive for WT1, but show "wild type" reactivity for p53. They are often strong positive for ER and PR.

Post-menopausal patient with GI complaints, colonoscopy with bx




CK7
PAX8
WT1

Ovarian Mucinous Tumors

- Favor primary
  - Unilateral
  - Large (often >10 cm)
  - Presence of endometriosis
  - Sero-mucinous type epithelial lining
  - Negative appendix
  - IHC profile

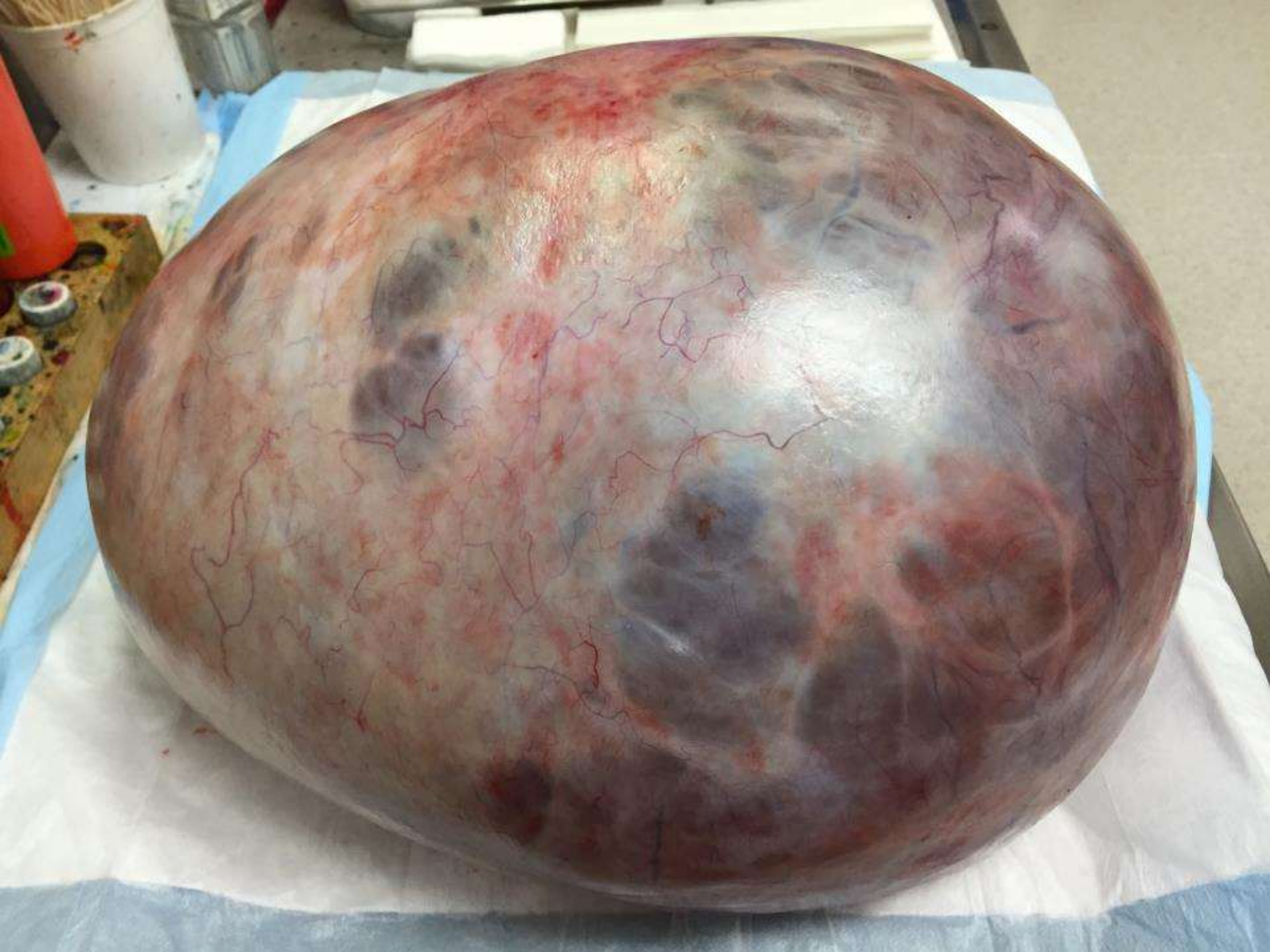
Ovarian Mucinous Tumors

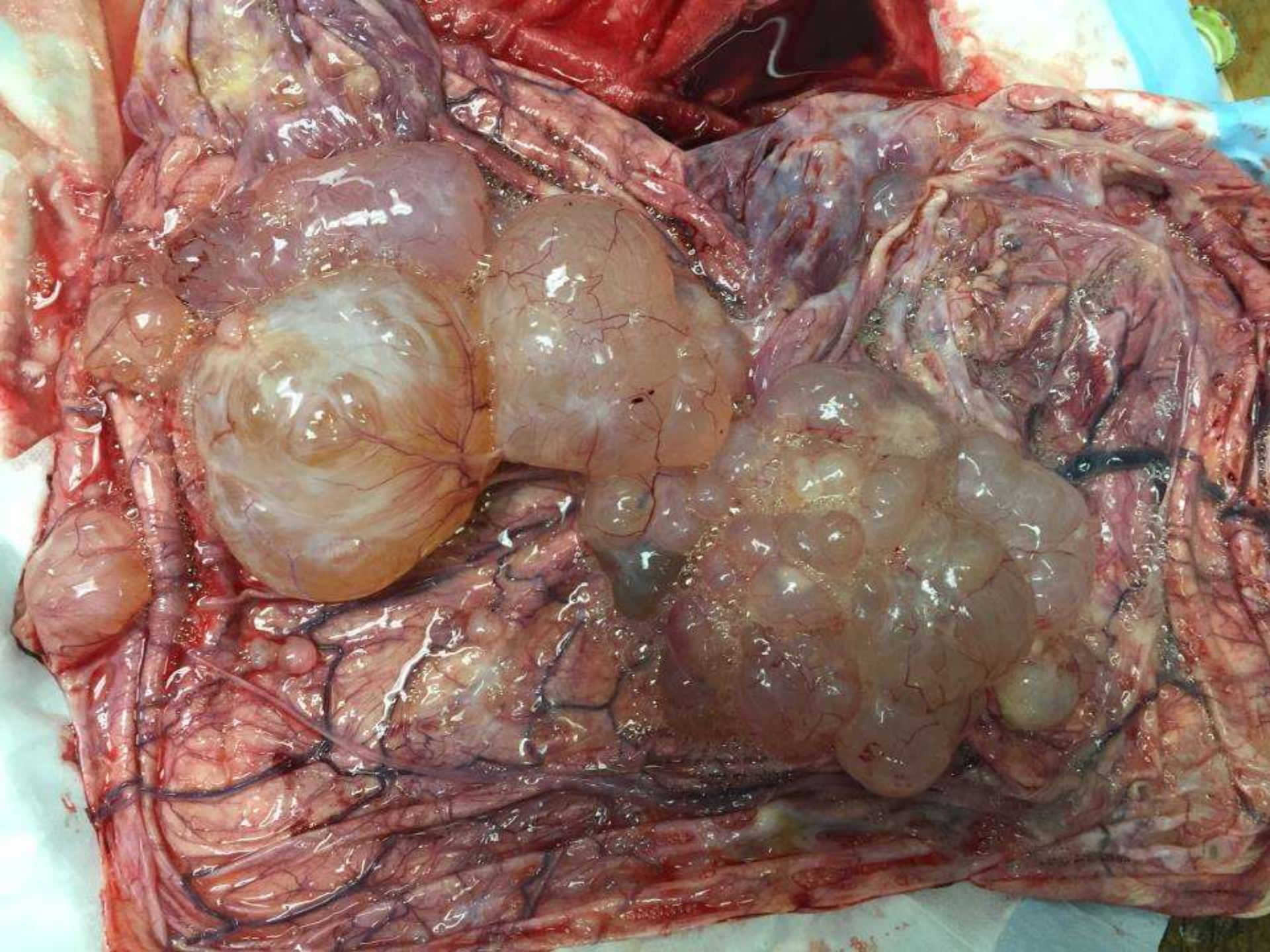
- Favor secondary
  - Bilateral
  - Size less than 10 cm
  - Multi-nodular growth
  - GI-type epithelium
  - Appendix with mucinous lesion
  - IHC profile

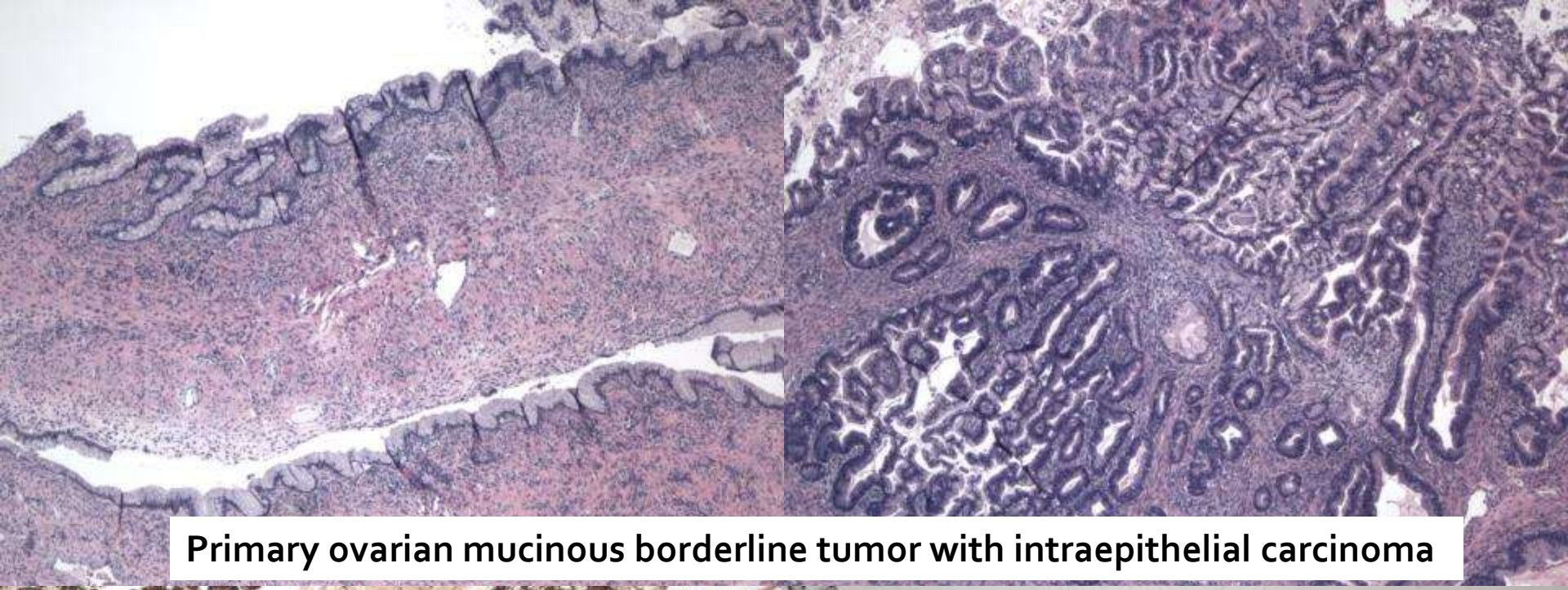
Ovarian Mucinous Tumors-IHC

Antibody	Primary Ovarian	Colo-rectal	Appendix	Small bowel	Upper GI	Pancreas/ biliary	Cervix
CK7	+	-	- / +	- /+	+	+	+
CK20	- /+	+	+	+	- / +	- / +	-
ER	+ (25%)	-	-	-	-	-	- /+ (w)
PAX8	+ (40%)	-	-	-	-	-	+ (70%)
SMAD4	+	+ / -	+	+	+	- (50%)	+
P16	Not DS	Not DS	Not DS	Not DS	Not DS	Not DS	+ (DS)
CDX2	- / +	+	+	+	- / +	- / +	- /+
SATB2	-	+	+	+ /-	-	-	-

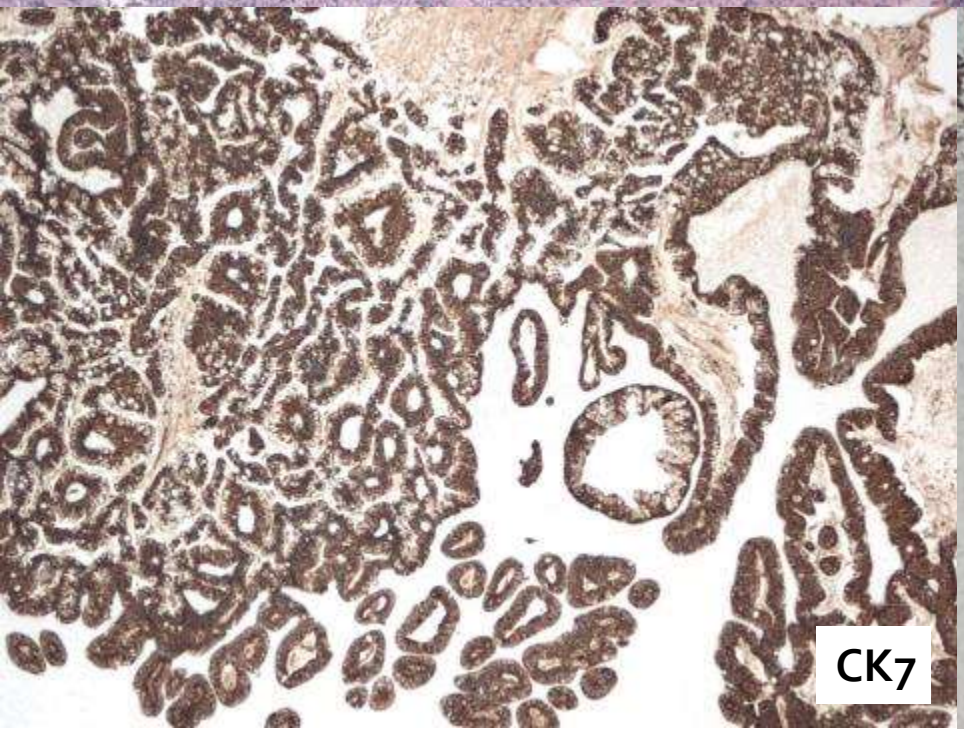
DS: diffuse strong; w: weak

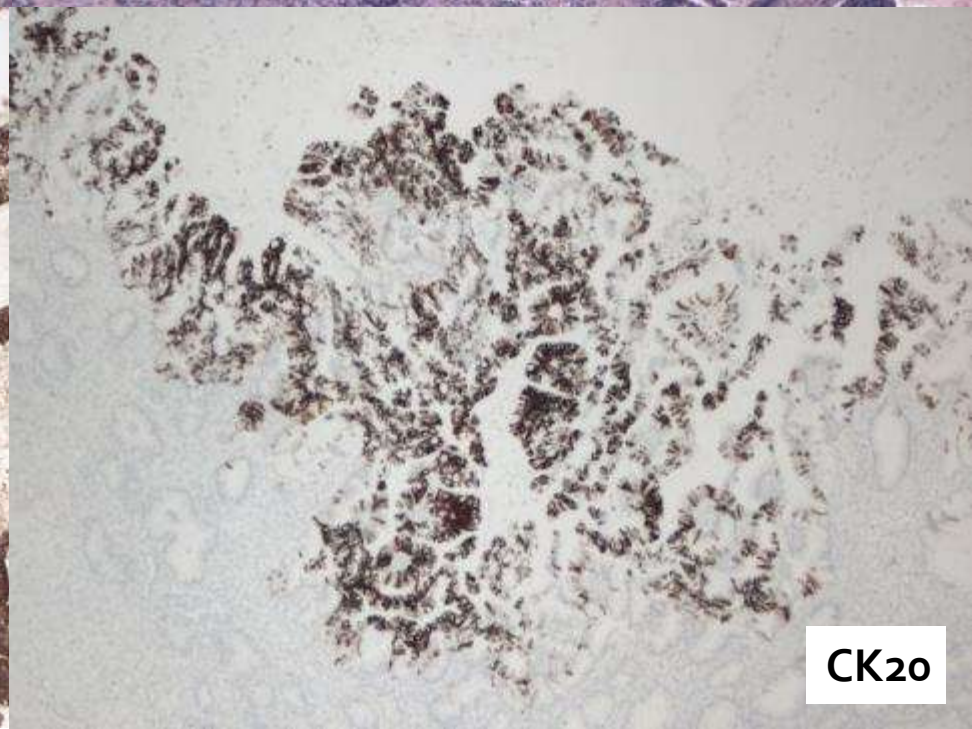




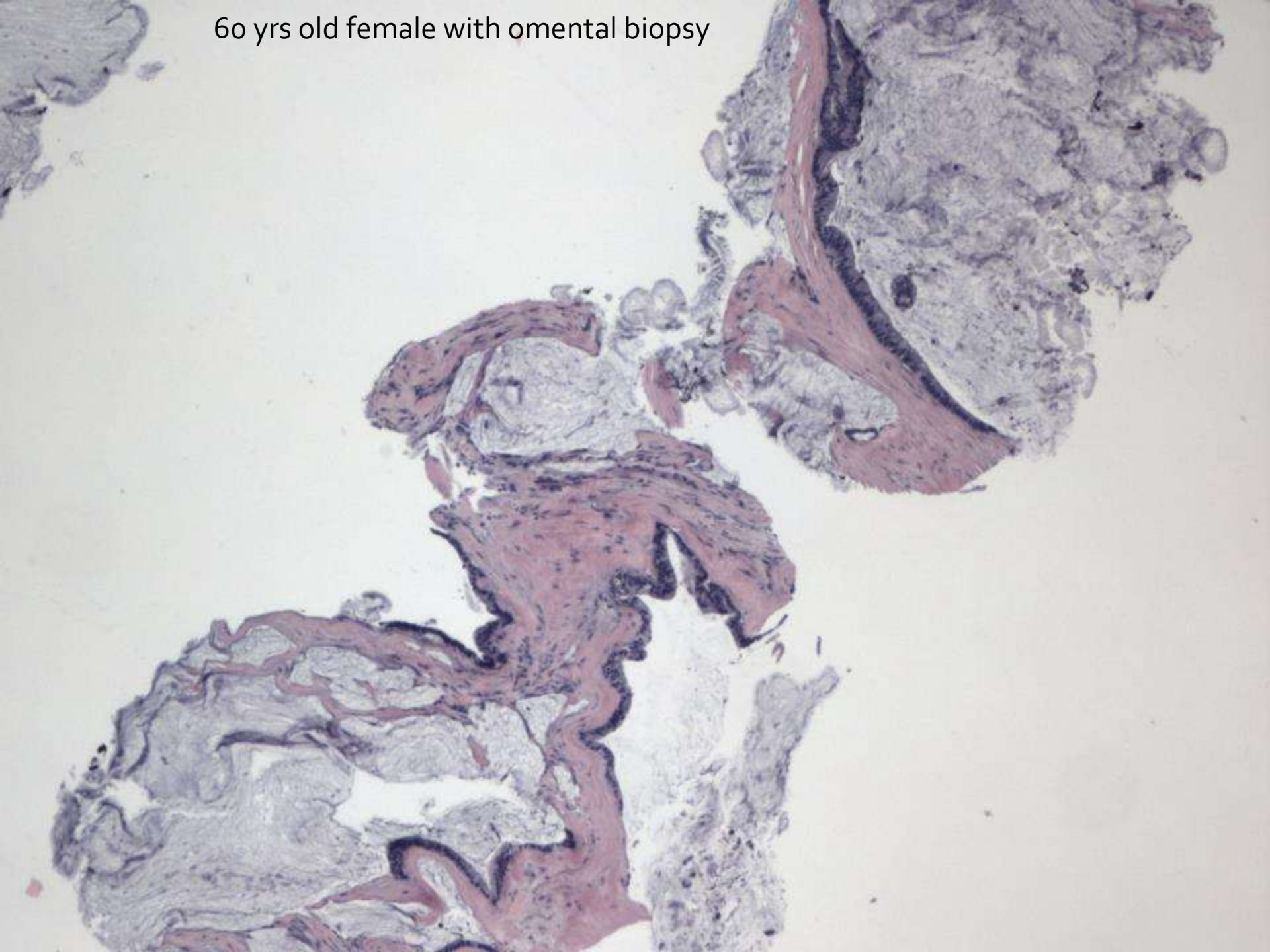


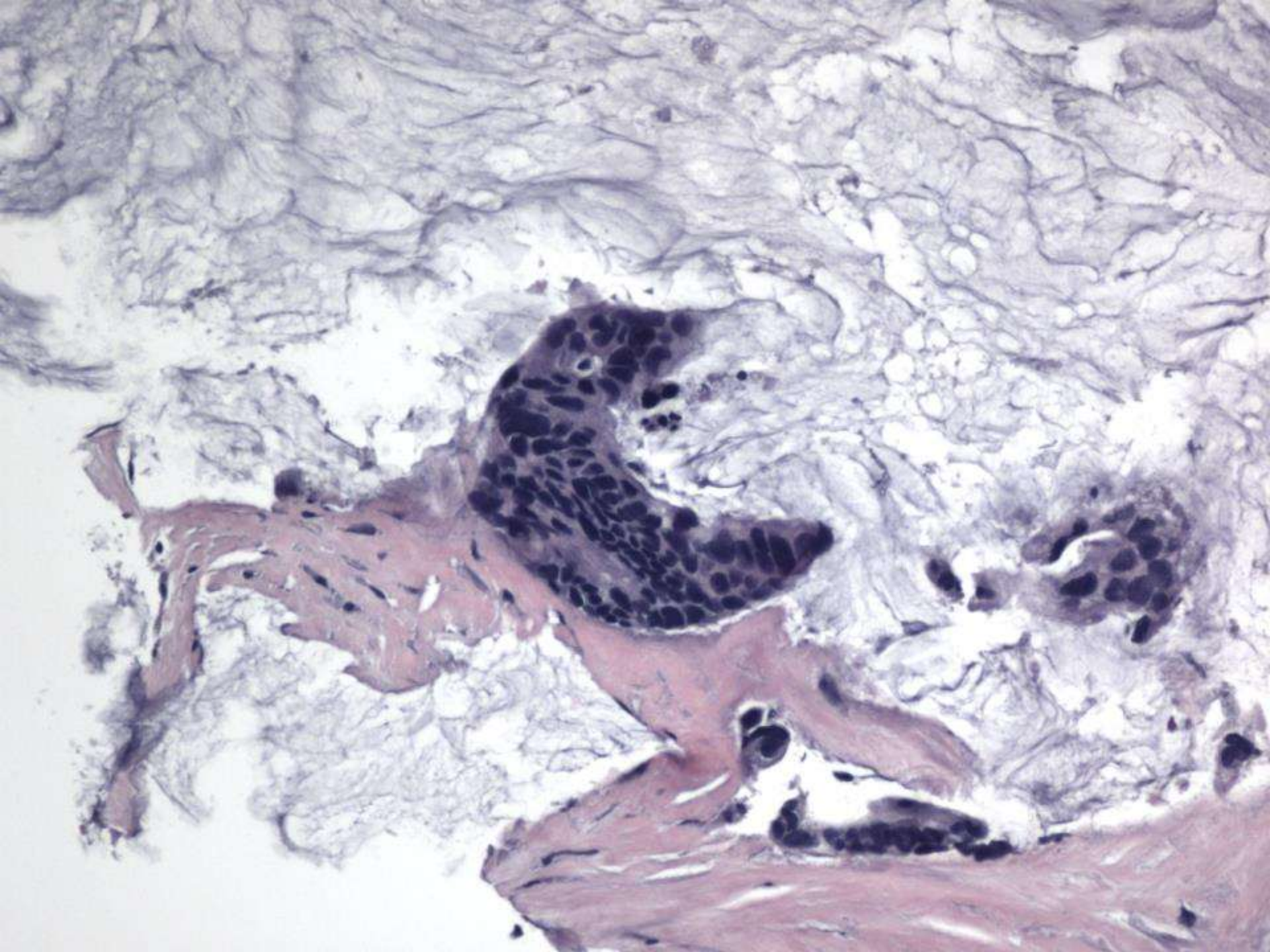
Primary ovarian mucinous borderline tumor with intraepithelial carcinoma

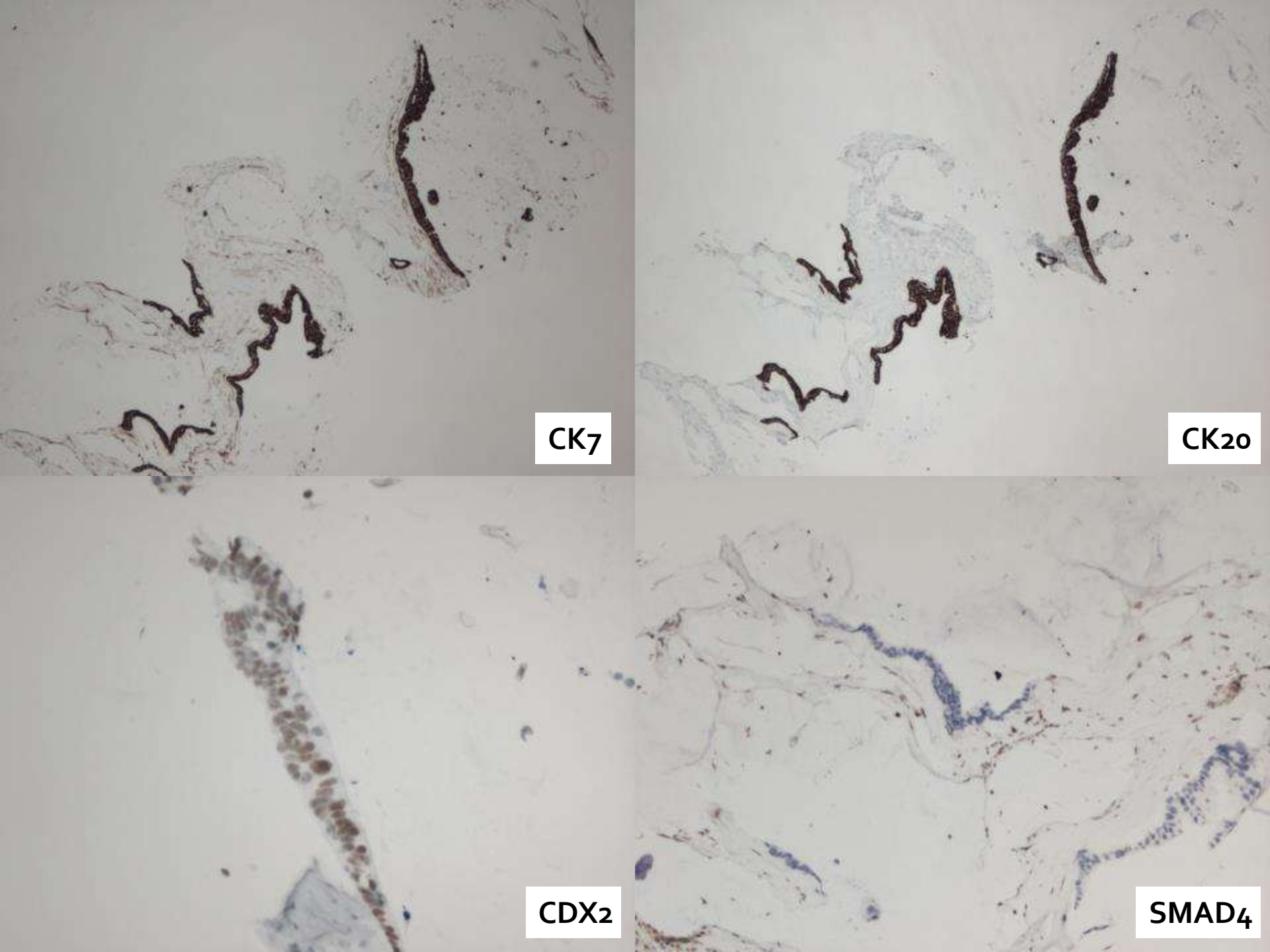




60 yrs old female with omental biopsy






CK7
CK20
CDX2
SMAD4

Immunohistochemistry

POSITIVE STAINS

- CK7
- CK20
- CDX2 (moderate)
- P16 (patchy mod to strong)
- HNF-1 beta (weak)

NEGATIVE STAINS

- PAX8
- ER
- PR
- GATA3
- TTF-1
- SMAD4 (aka DPC4)

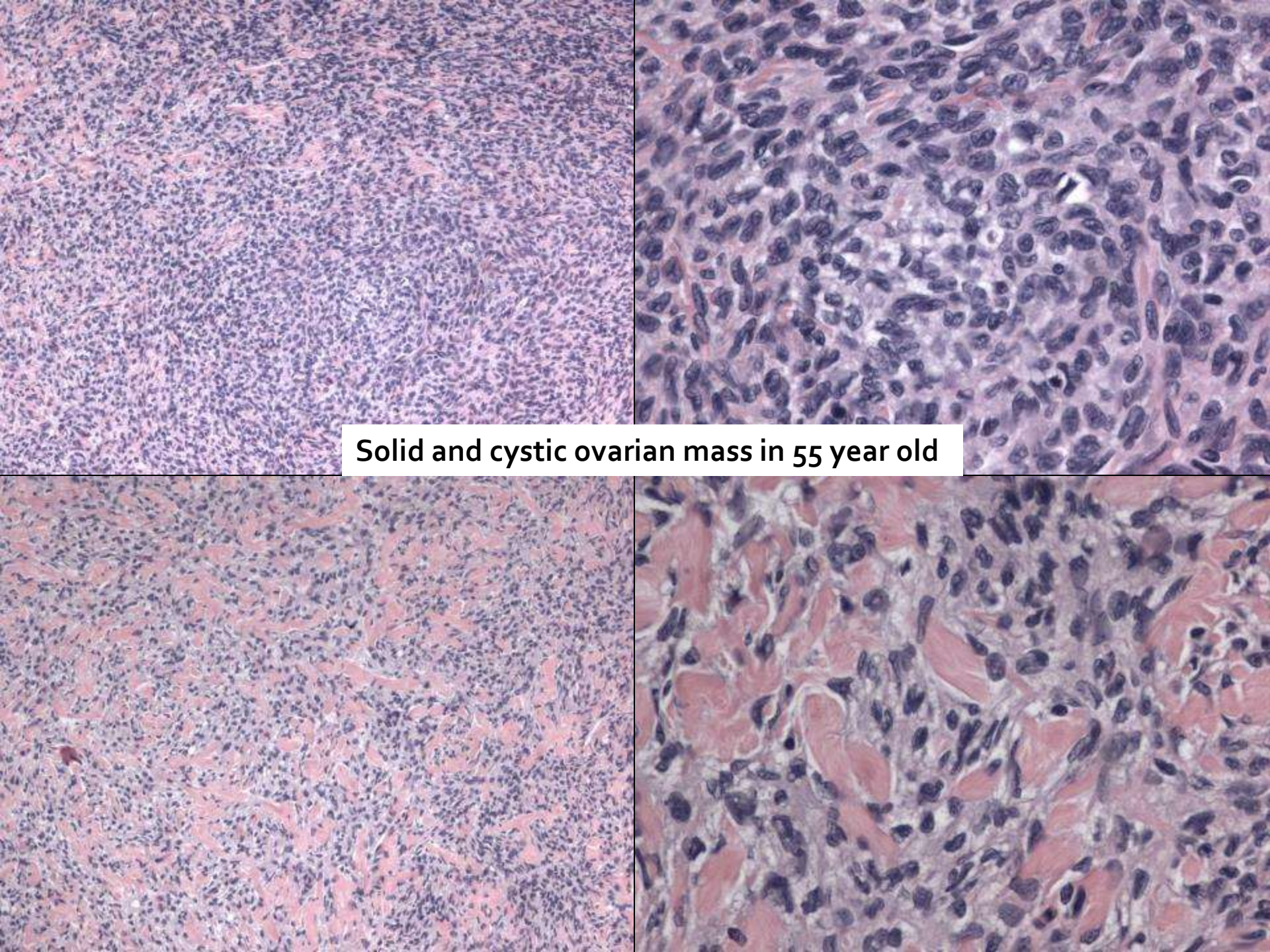
Diagnosis

<u>Mucinous adenocarcinoma</u>

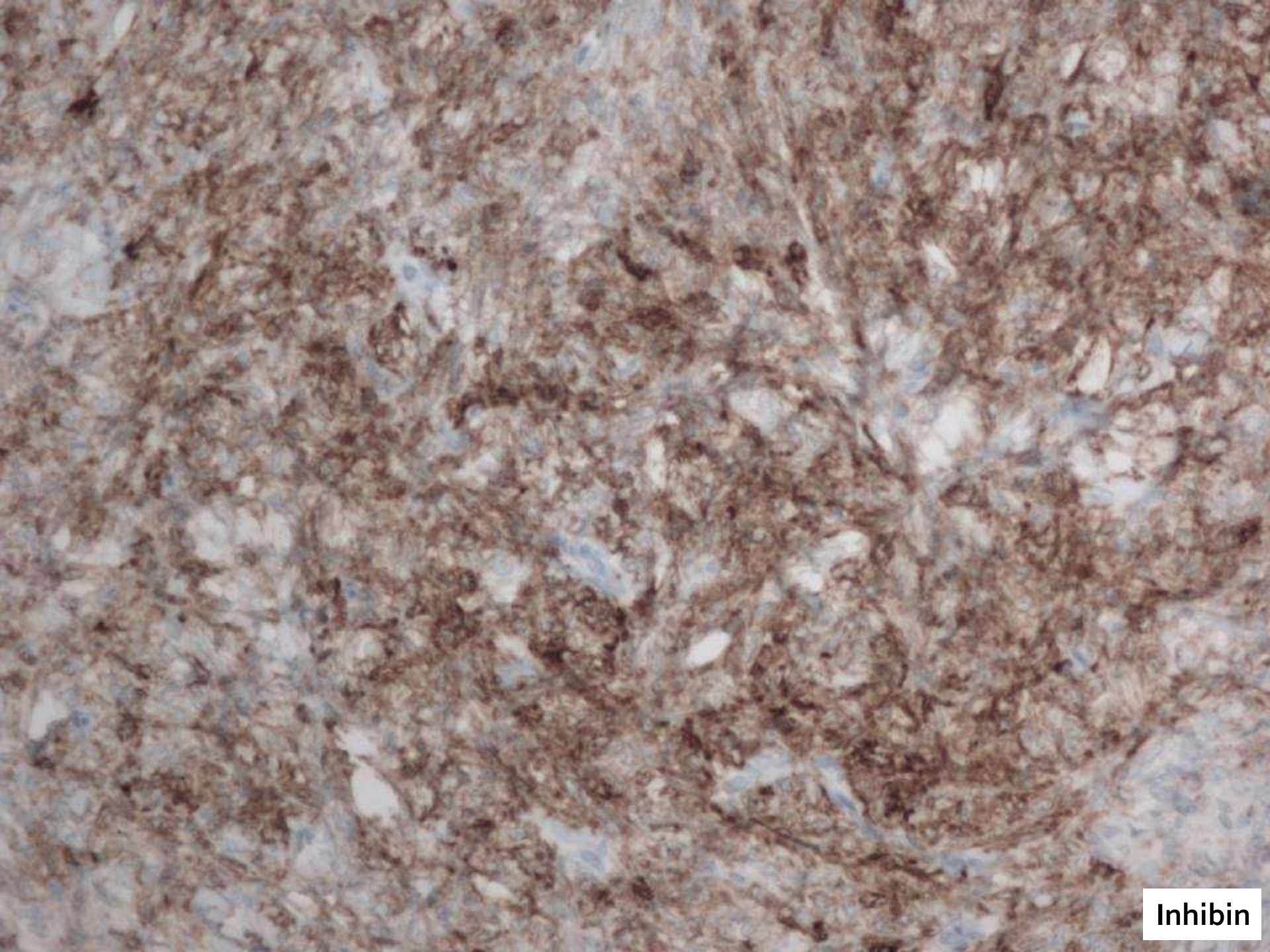
Most likely sites: Pancreatic-biliary tract
Less likely sites: Upper GI, appendix
Other less likely sites: Mullerian, lower GI

Ovarian Stromal Tumors

- Ovarian stromal markers
  - Inhibin, CD99, Calretinin, WT1, Melan A (steroid cell tumors often positive)
  - FOXL2
    - Positive in most granulosa cell tumors
    - Positive in ~50% of Sertoli-Leydig cell tumors
- Stromal tumors are almost always negative for EMA
- Can use reticulin to distinguish AGCT from fibrothecoma
  - AGCT: Reticulin around clusters of cells
  - Fibrothecoma: Reticulin is around individual cell



Solid and cystic ovarian mass in 55 year old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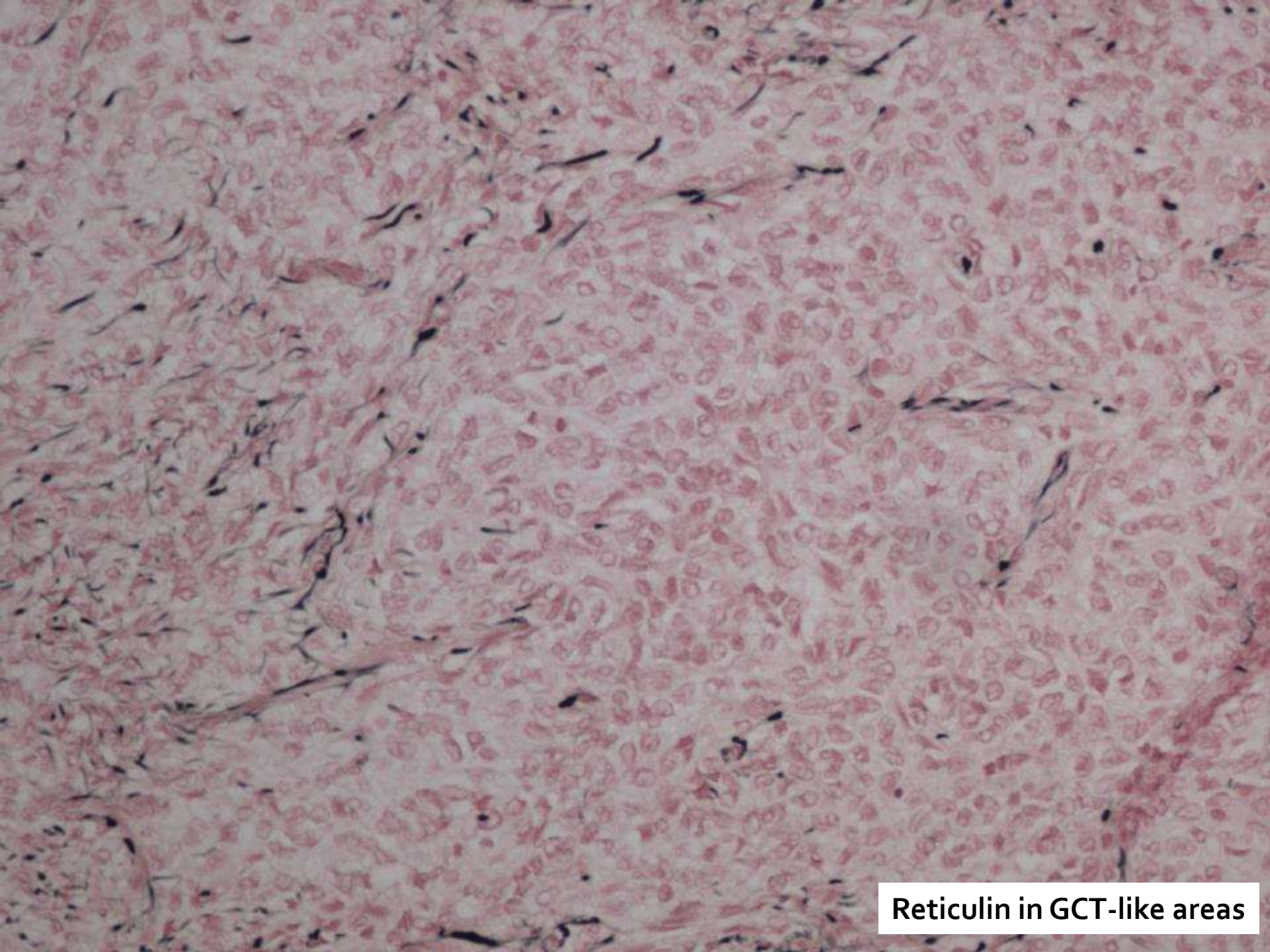
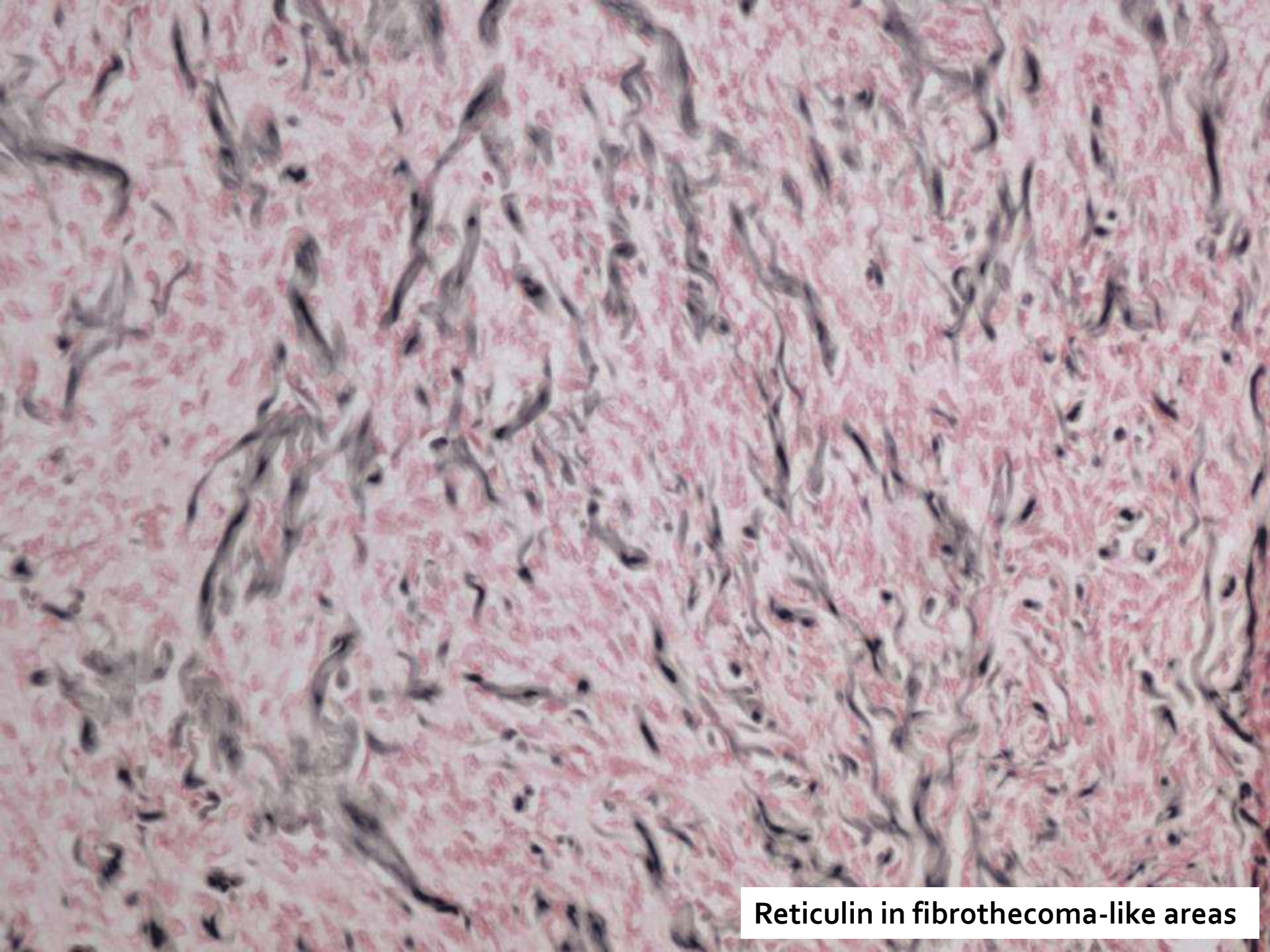
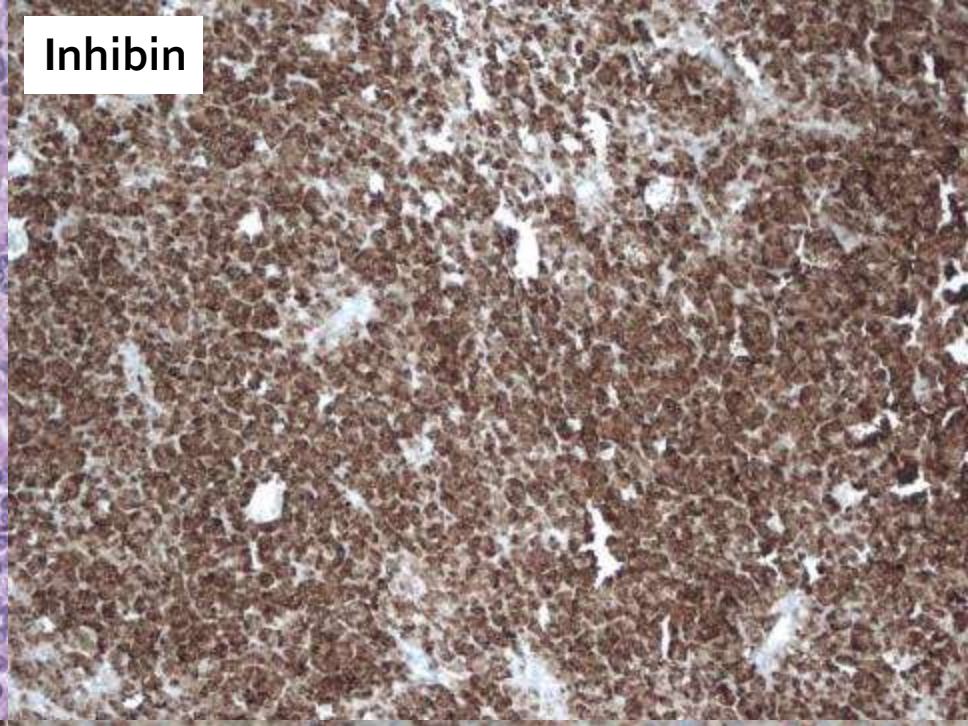

Reticulin in GCT-like areas


Reticulin in fibrothecoma-like areas


Steroid cell tumor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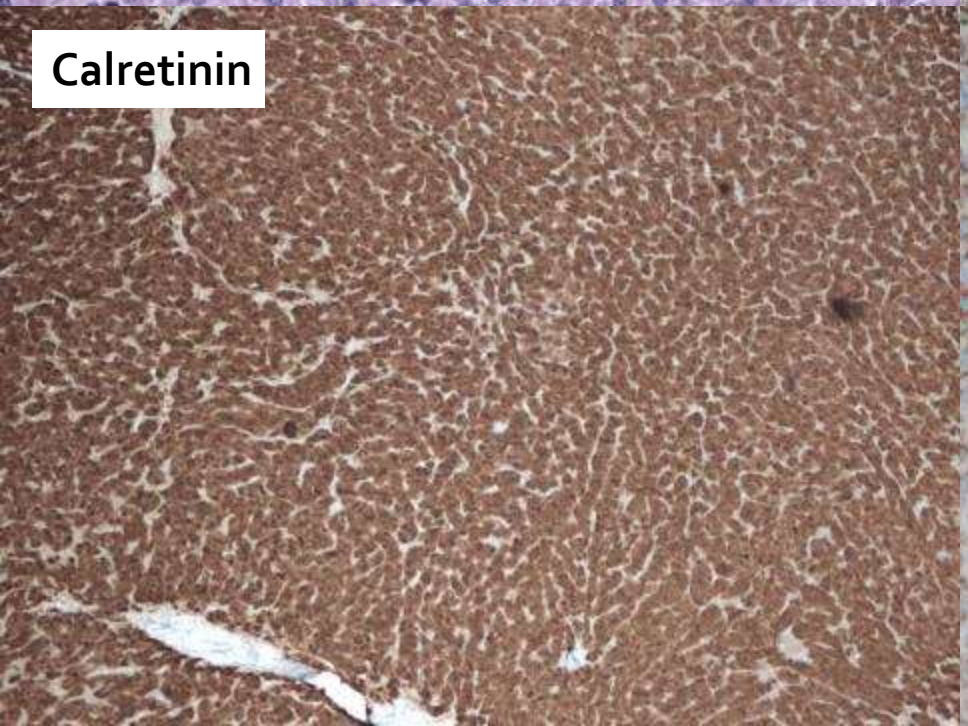
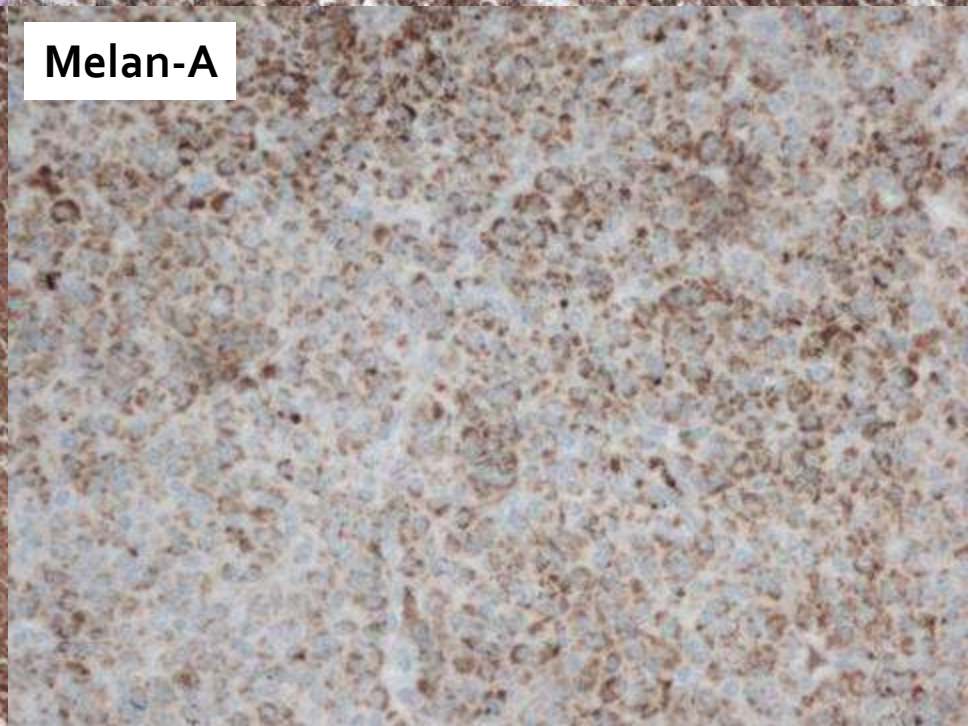

Calretinin


Melan-A

Ovarian Stromal Tumors

- Ovarian endometrioid stromal sarcoma
  - Resembles ESS of the endometrium
  - Stains like ESS of the endometrium
    - CD10+, ER/PR+, negative for smooth muscle markers and inhibin (or patchy expression)
  - Association with endometriosis (< 50% cases)
  - Often uterus previously removed for "benign" reasons

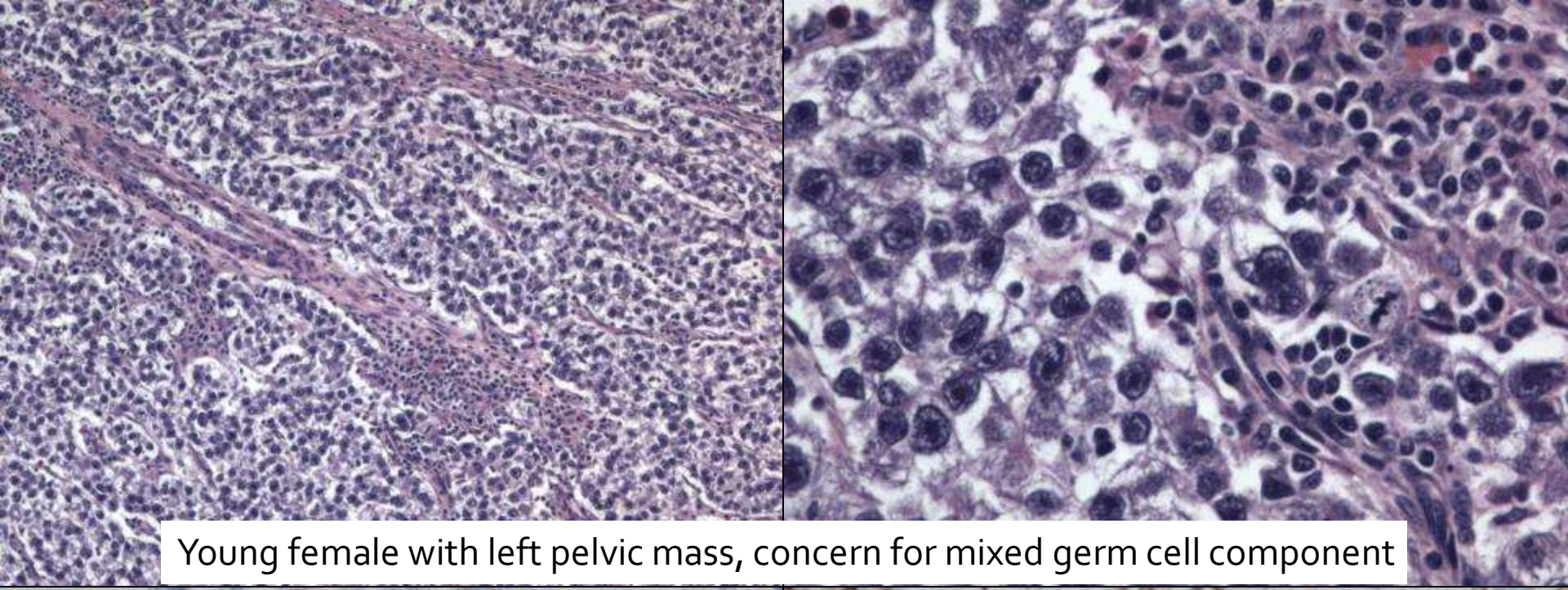
Ovarian Germ Cell Tumors

- Age often less than 20
  - Only exception is mature cystic teratoma which can be seen in much older patients
- Immature teratoma is easy to diagnose as many different tissue components can be identified
  - If only immature component is present, then neural/neuro-epithelial markers (S100, GFAP) can be used for diagnosis

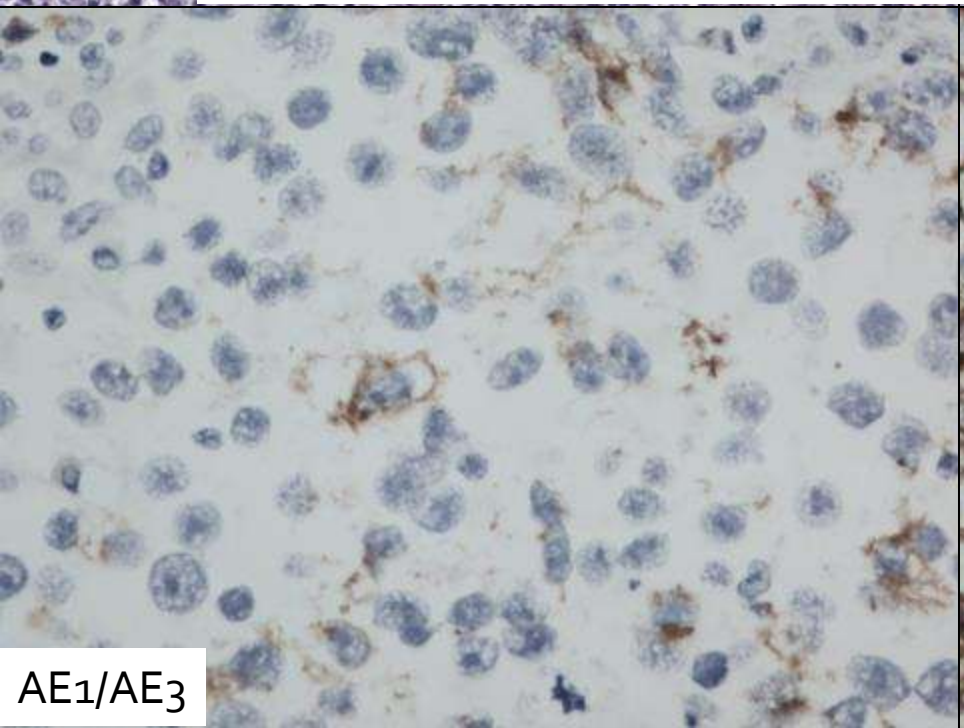
Ovarian Germ Cell Tumors-IHC

Antibody	Dysgerminoma	Embryonal CA	Yolk Sac Tumor
SALL4	+	+	+
OCT3/4	+	+	-
AE1/AE3	-	+	-/+
CAM5.2	+/-	+	+/-
CK7	-	-	-
CD30	-	+	-
AFP	-	-	+
SOX2	-	+	-
SOX17	+	-	Variable

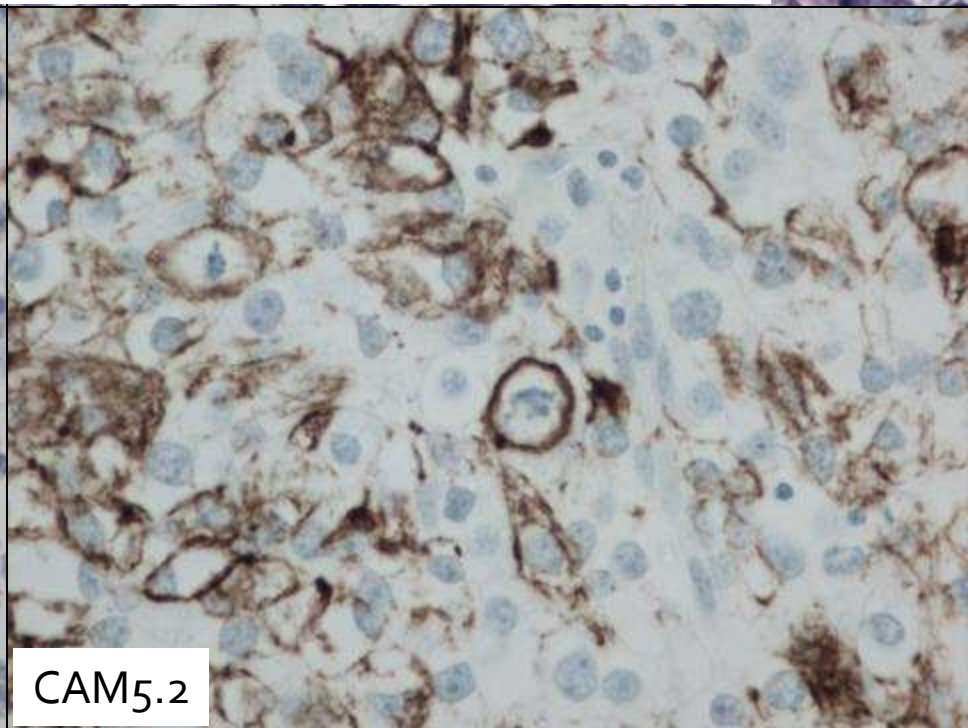
Ovarian choriocarcinoma is rare but positive for HCG, EMA, CAM5.2, variable for SALL4 but negative for OCT3/4, SOX2, and SOX17



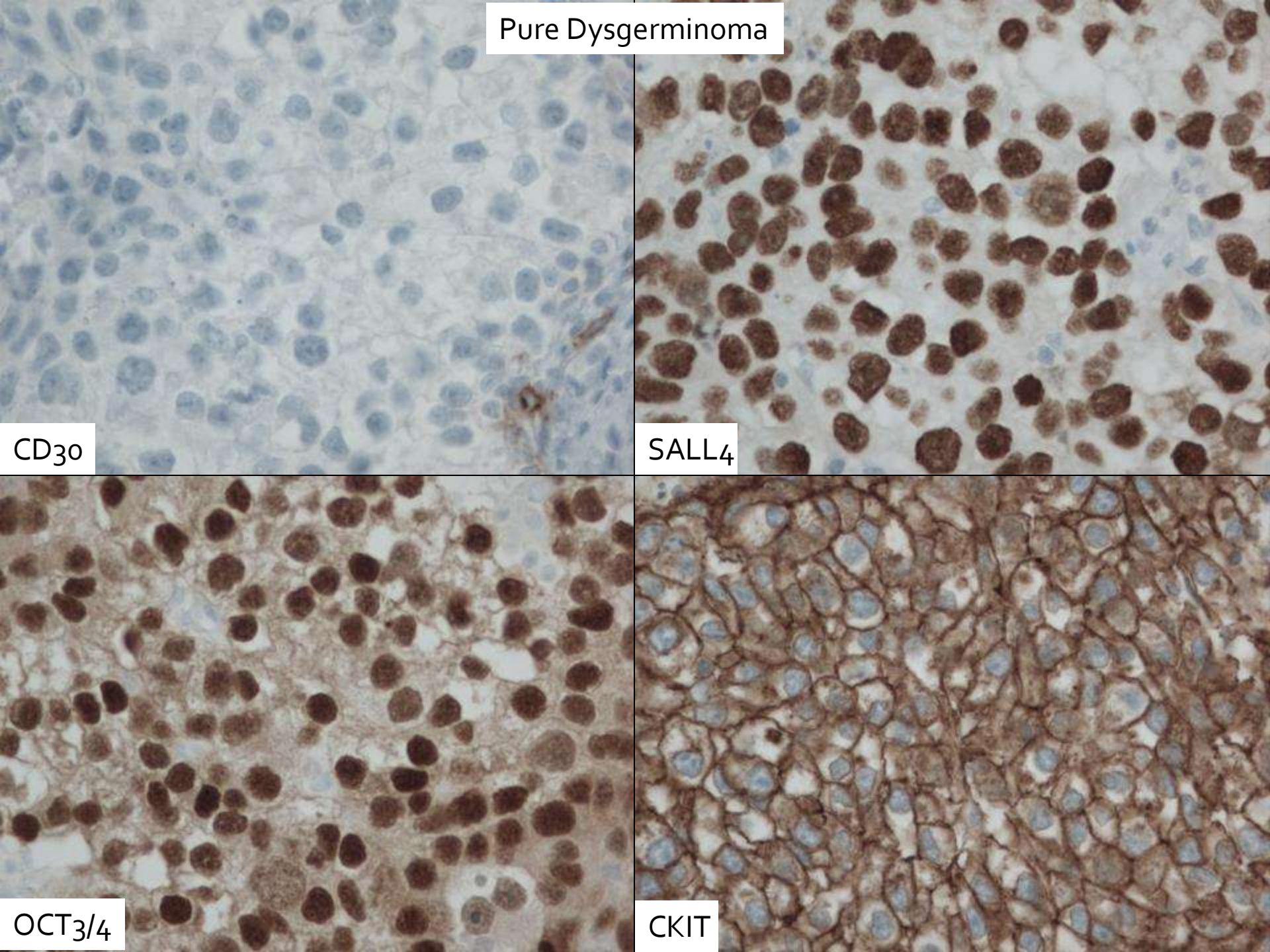
Young female with left pelvic mass, concern for mixed germ cell component

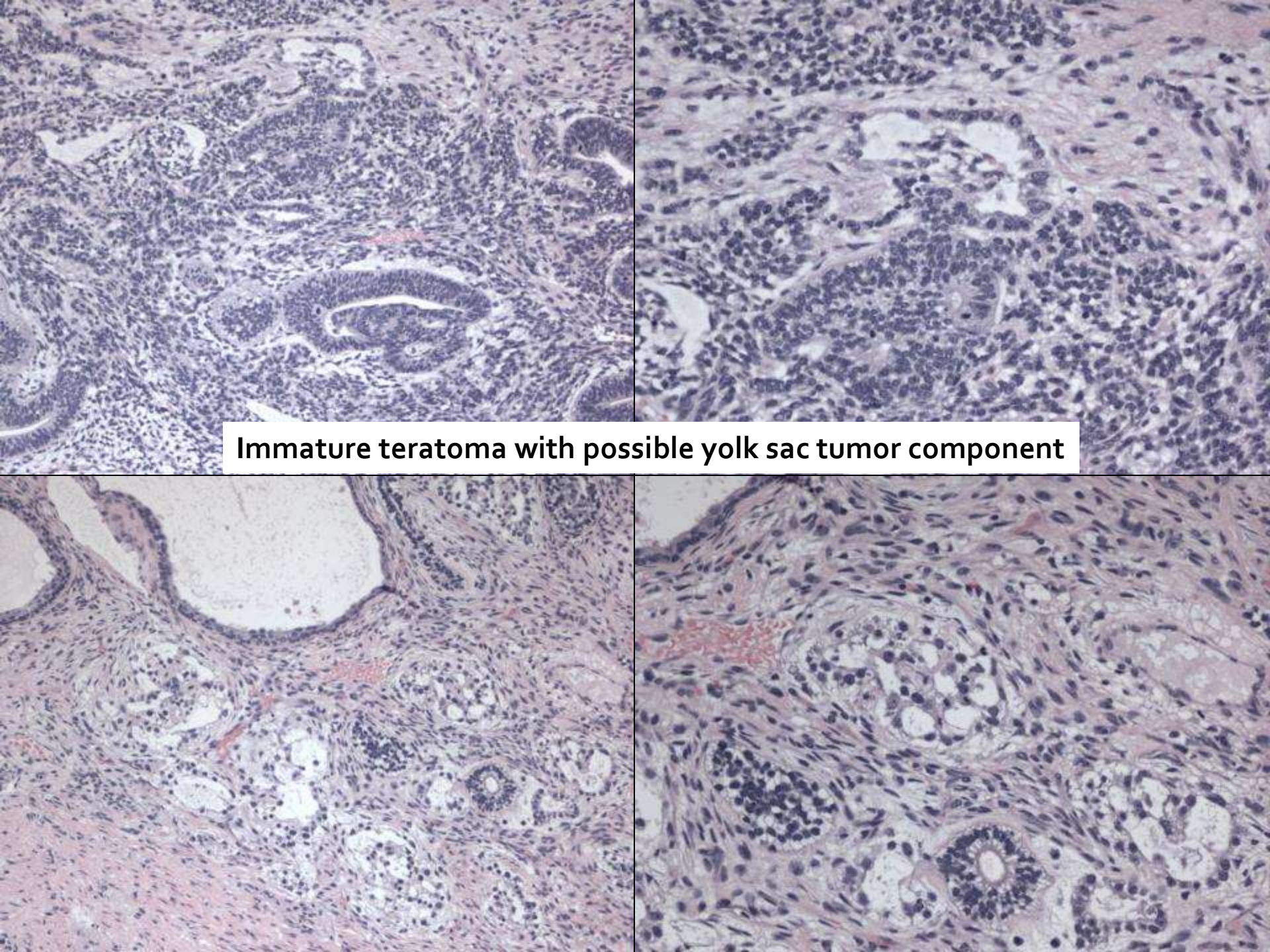


AE1/AE3

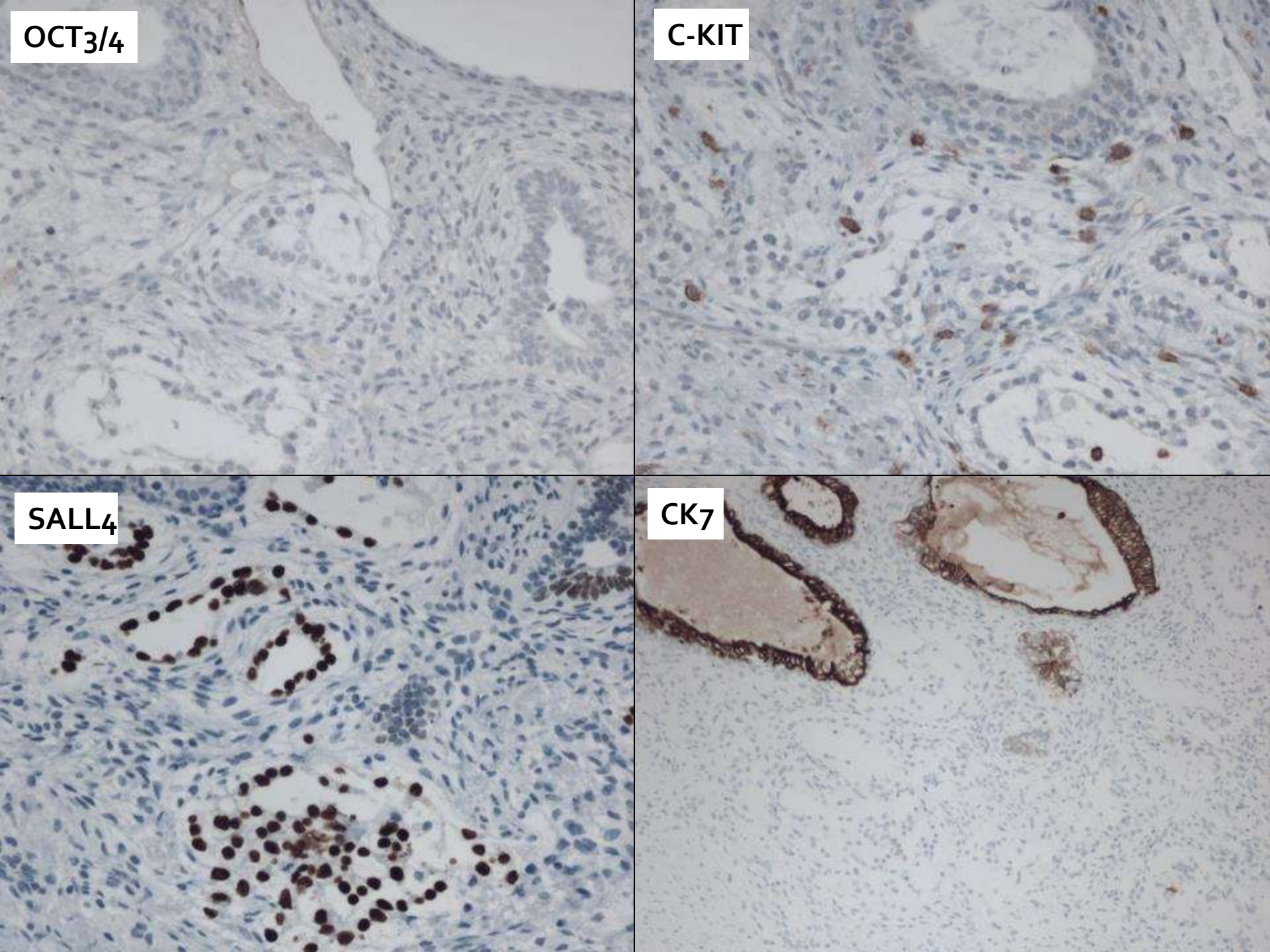


CAM5.2


Pure Dysgerminoma
CD30
SALL4
OCT3/4
CKIT

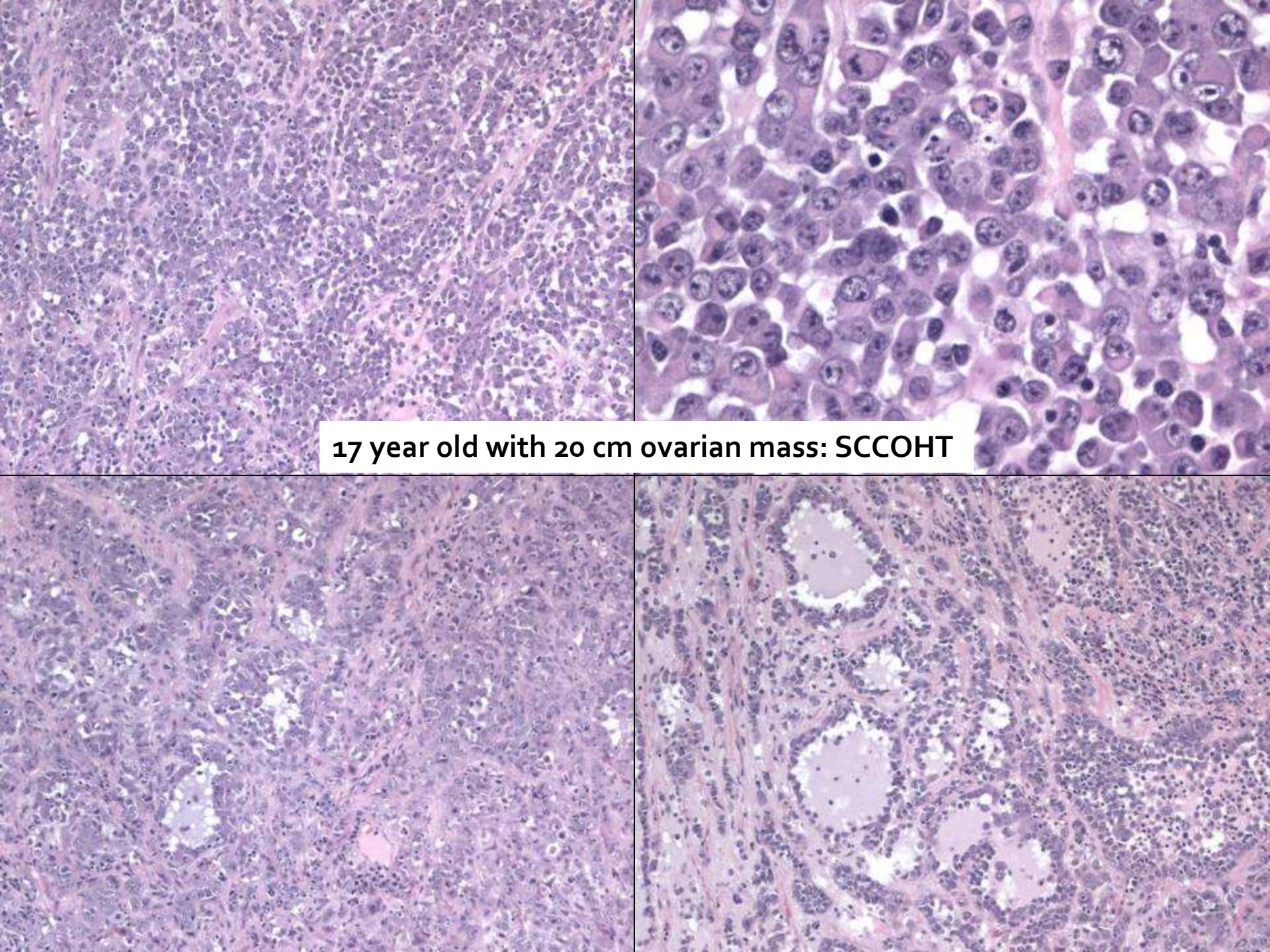


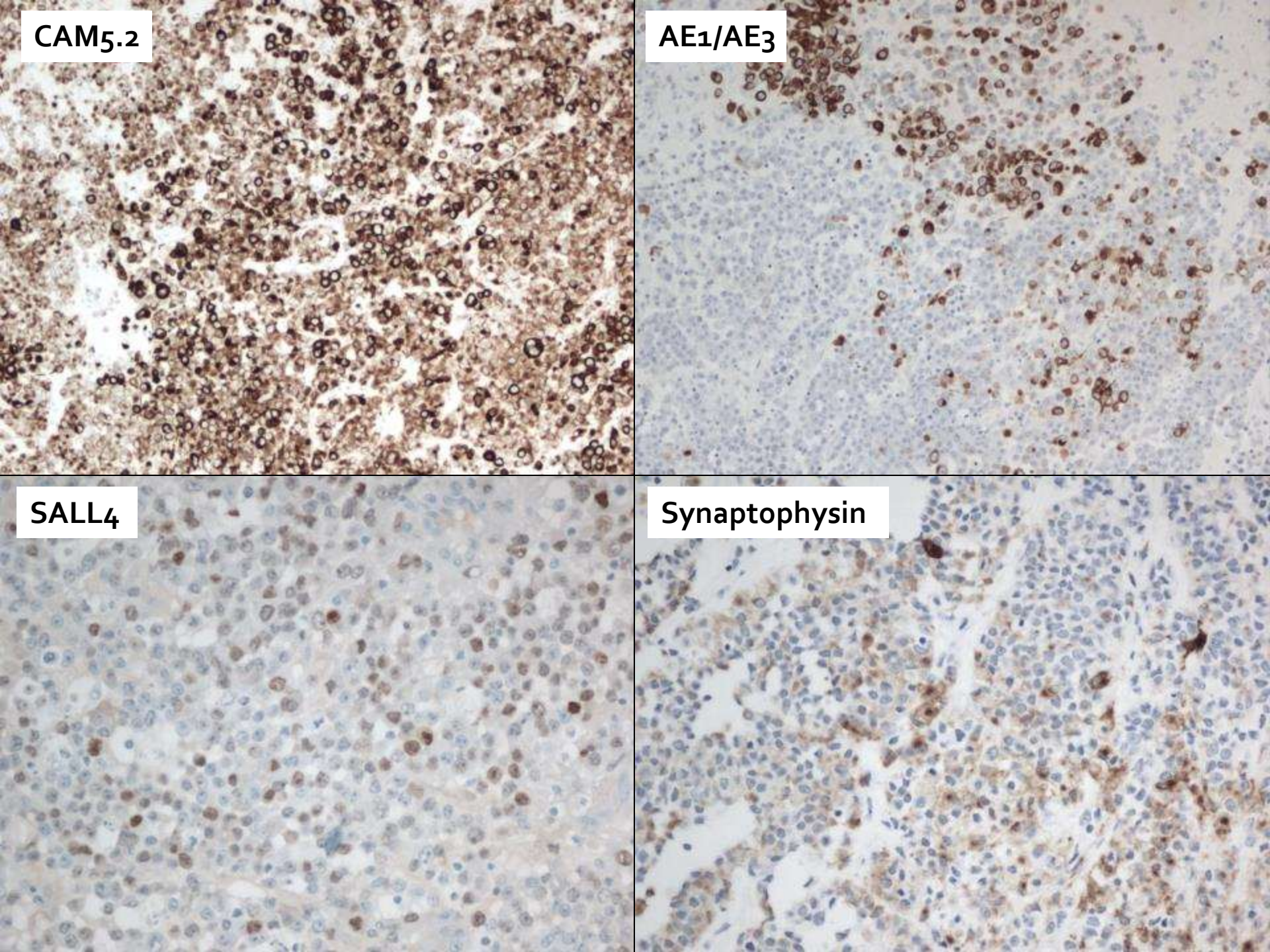
Immature teratoma with possible yolk sac tumor component

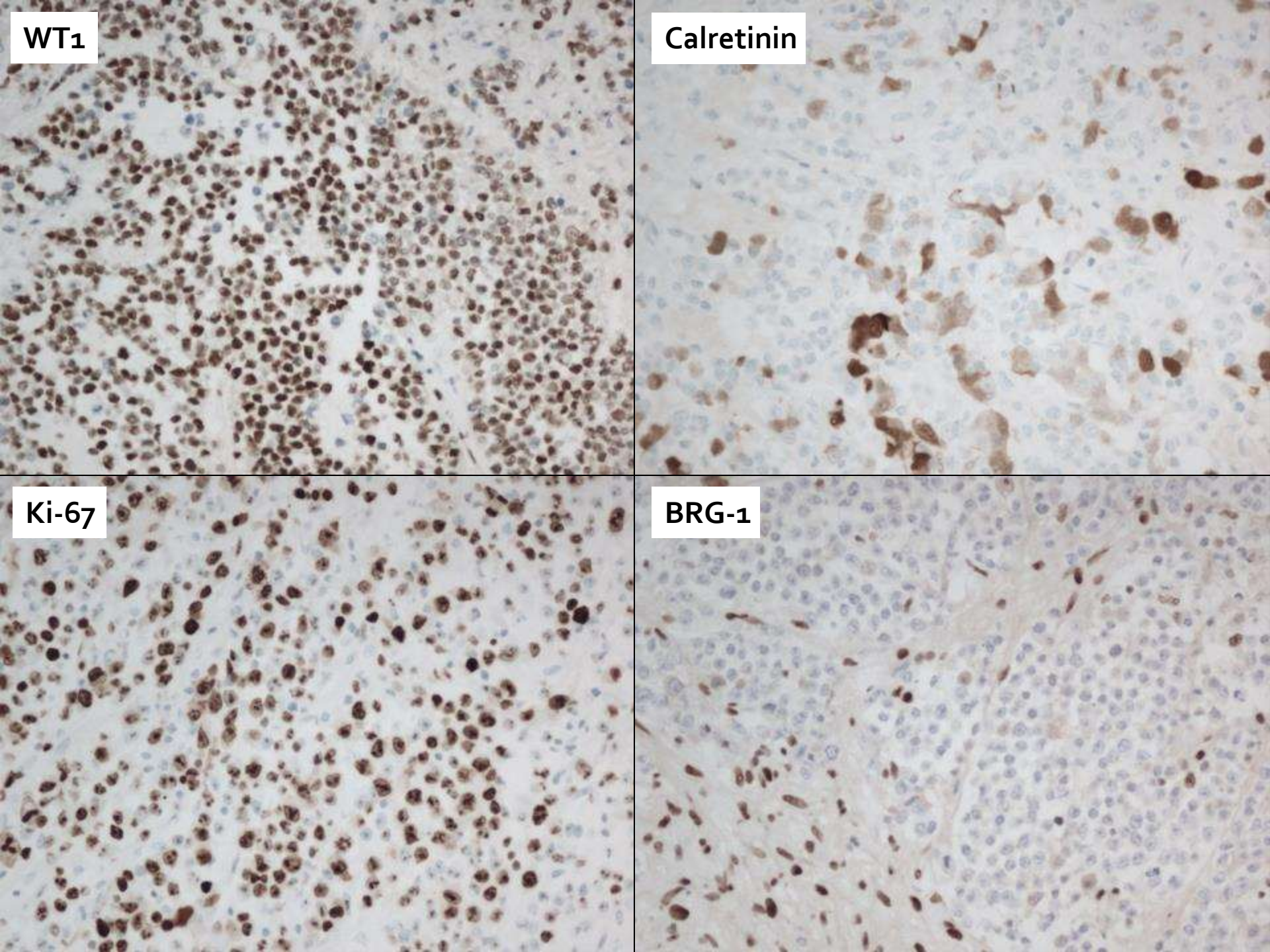

OCT3/4
C-KIT
SALL4
CK7

Ovarian Tumors-Cell of origin debatable

- Small cell carcinoma of hypercalcemic type
  - Not always small (large cell variant)
  - Hypercalcemia in 60-70% cases only
  - Young patients
  - Cystic "follicle-like" areas, solid areas, "rhabdoid" morphology
    - Difficult to classify into defined groups on H&E exam
  - WT1+, Calretinin+, EMA+, CD10+, AE1/AE3+, p53+
  - Scattered reactivity for neuroendocrine markers but often negative for chromogranin
  - Loss of BRG-1 (encoded by *SMARCA4* gene)


17 year old with 20 cm ovarian mass: SCCOHT

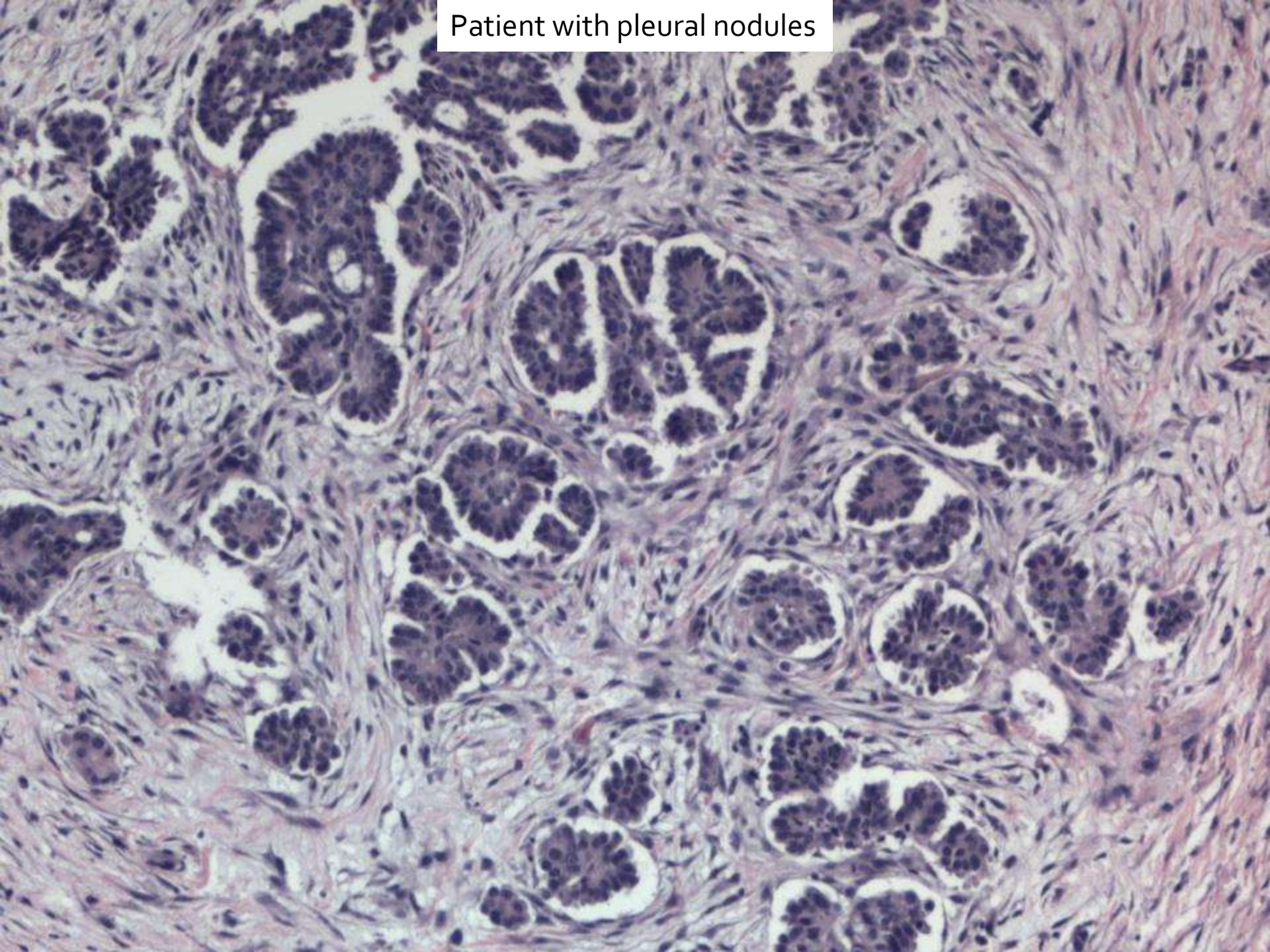

CAM5.2
AE1/AE3
SALL4
Synaptophysin

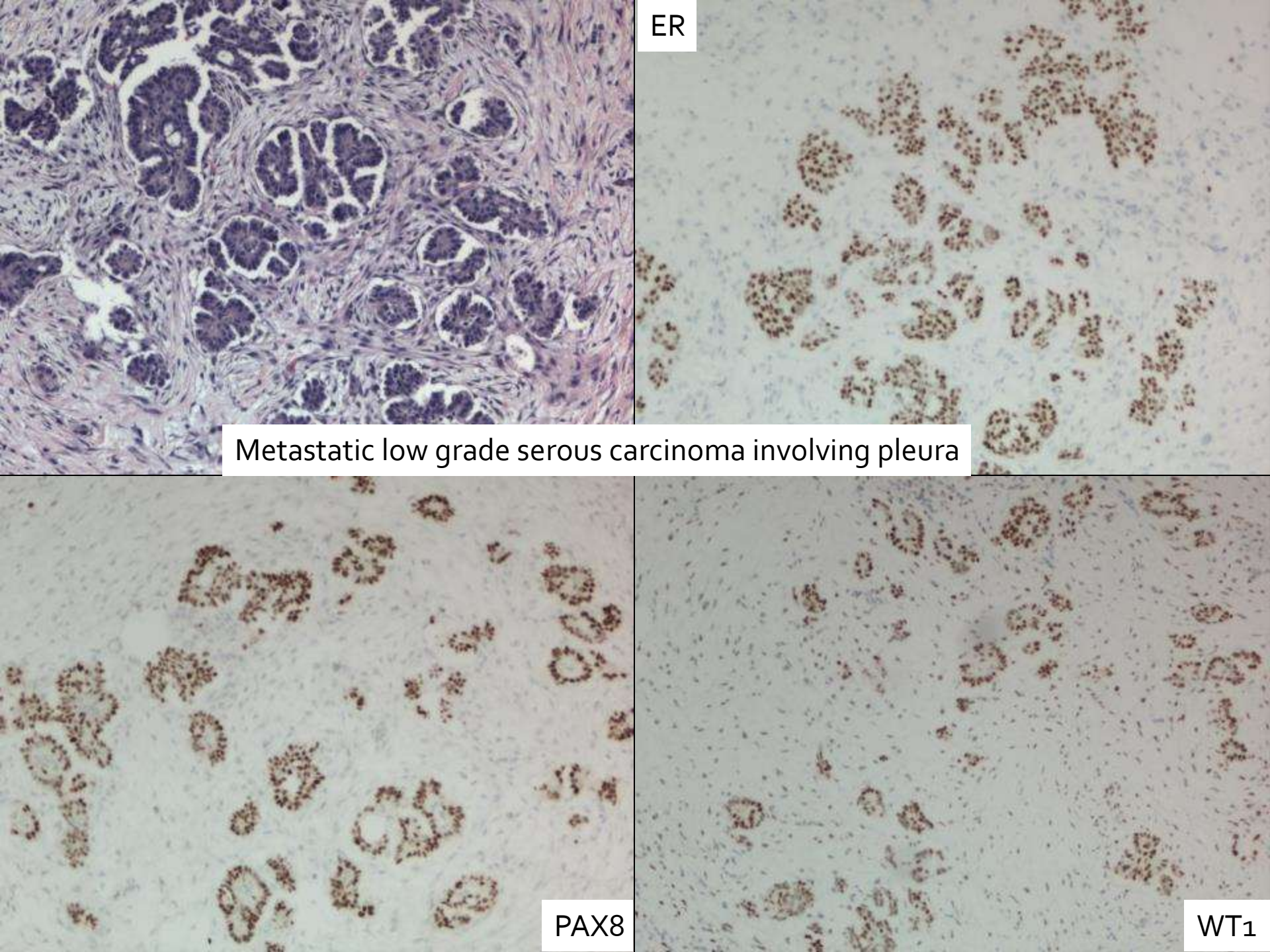

WT1
Calretinin
Ki-67
BRG-1

Mesothelium v/s Mullerian v/s Others

Antibody	Mesothelial	Mullerian	Other
PAX8	mostly neg	+	neg (unless renal or thyroid)
WT1	+	neg or + (serous)	neg
Calretinin	+	neg or patchy	neg
BER-EP4	neg	+	+
MOC31	neg	+	+
ER	mostly neg	+	neg (unless breast)
Bg8	neg	+	+
BAP1	neg in ~50% of mesothelioma	+	+
GATA3	Weak positive	Variable	neg (unless bladder or breast)
TTF1	Neg	Neg	Neg (unless lung)
CDX2	Neg	Neg	Neg (unless GI)

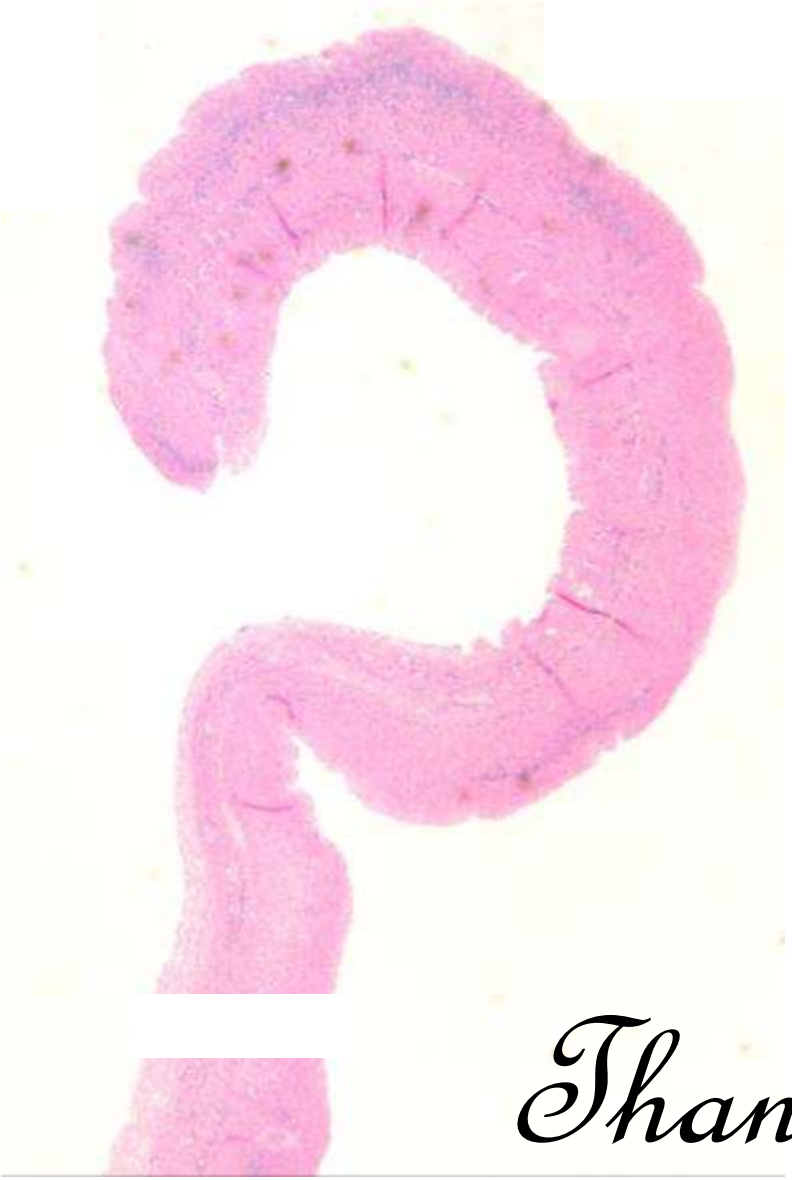
Patient with pleural nodules




ER
Metastatic low grade serous carcinoma involving pleura
PAX8
WT1

Summary

- Gynecologic pathology is vast and quite variable
  - Application of IHC stains require knowledge of different entities
  - Good to have a differential diagnosis before requesting IHC
    - "Antibody bashing" is not helpful
- Research an unusual reactivity or show / ask someone with gyn path interest



Thank you