The British Association of Gynaecological Pathologists

Lecturer – Dr Ayoma Attygalle

Consultant Gynaecological Pathologist at the Royal Marsden Hospital for 16 years and Lead Gynaecological Pathologist for 13 years.



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Beyond Morphology - An immunohistochemical and molecular approach to gynaecological malignancies

Case based lecture: Immunohistochemical approach to ovarian epithelial malignancies

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London

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The British Association of Gynaecological Pathologists

Speaker disclosures

- ❑ I have no financial conflicts of interest to disclose

WHO list of ovarian epithelial malignancies

- High grade serous carcinoma
- Low grade serous carcinoma
- Carcinosarcoma
- Mucinous carcinoma
- Endometrioid carcinoma
- Clear cell carcinoma
- Malignant Brenner tumour
- Mesonephric-like adenocarcinoma
- Undifferentiated/de-differentiated carcinoma
- Mixed carcinoma

Value of IHC in diagnosis of ovarian carcinomas

‘Morphology remains the mainstay in diagnosis’ WHO 2020

Immunohistochemistry plays a supportive but at times a crucial role in aiding diagnosis.

- To confirm a diagnosis already made/suspected on morphology
- To help distinguish between two possible diagnoses
- Morphology is non-specific

To maximise value of immunohistochemistry

- Recognise it is an adjunct to diagnosis – not a stand alone diagnostic modality
- Appropriate sampling and morphological evaluation is crucial
- Use morphology to decide on a focused immunopanel

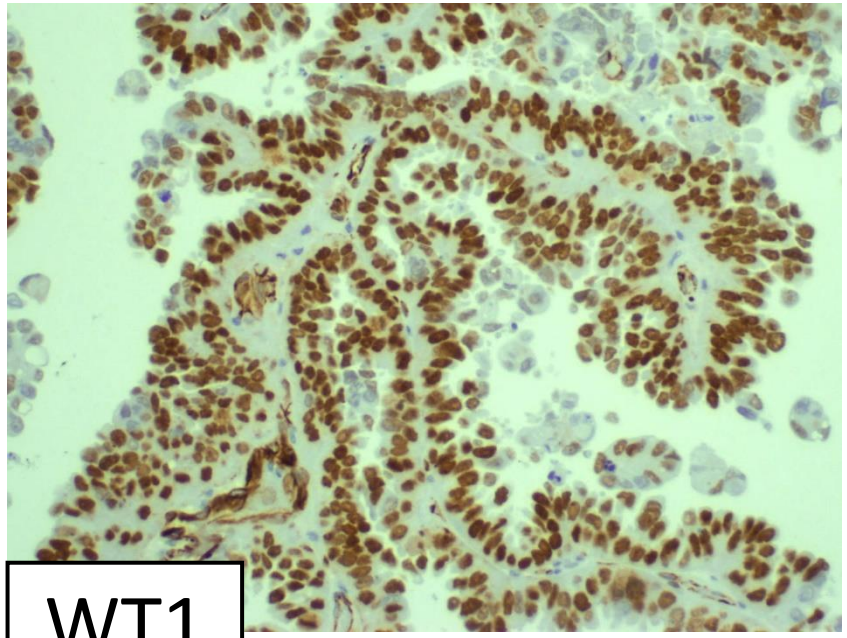
A few basic principles

- Use of panels in diagnosis– don’t rely on one marker only, but some markers are better discriminators in certain settings

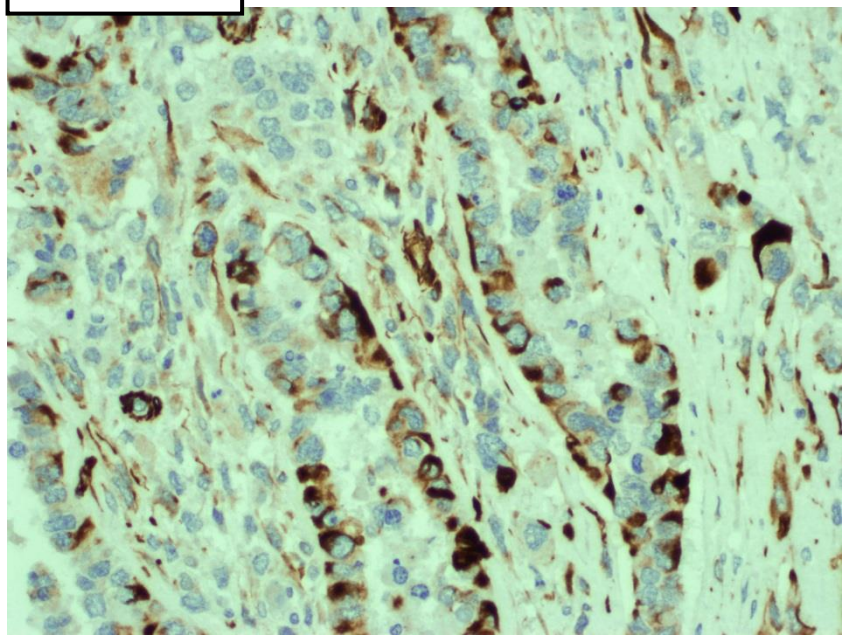
  -NO antibody is total specific

A few basic principles

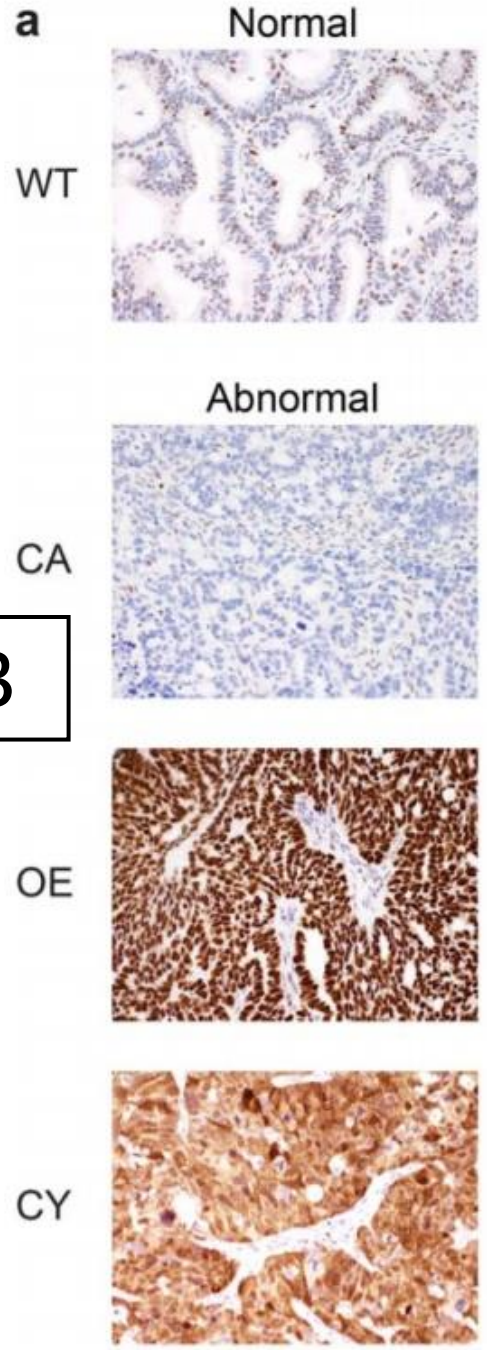
- Use of panels in diagnosis– don’t rely on one marker only
- Be aware of the pattern of specific staining – membrane, cytoplasmic or nuclear or combinations and what they may mean.



WT1



p53



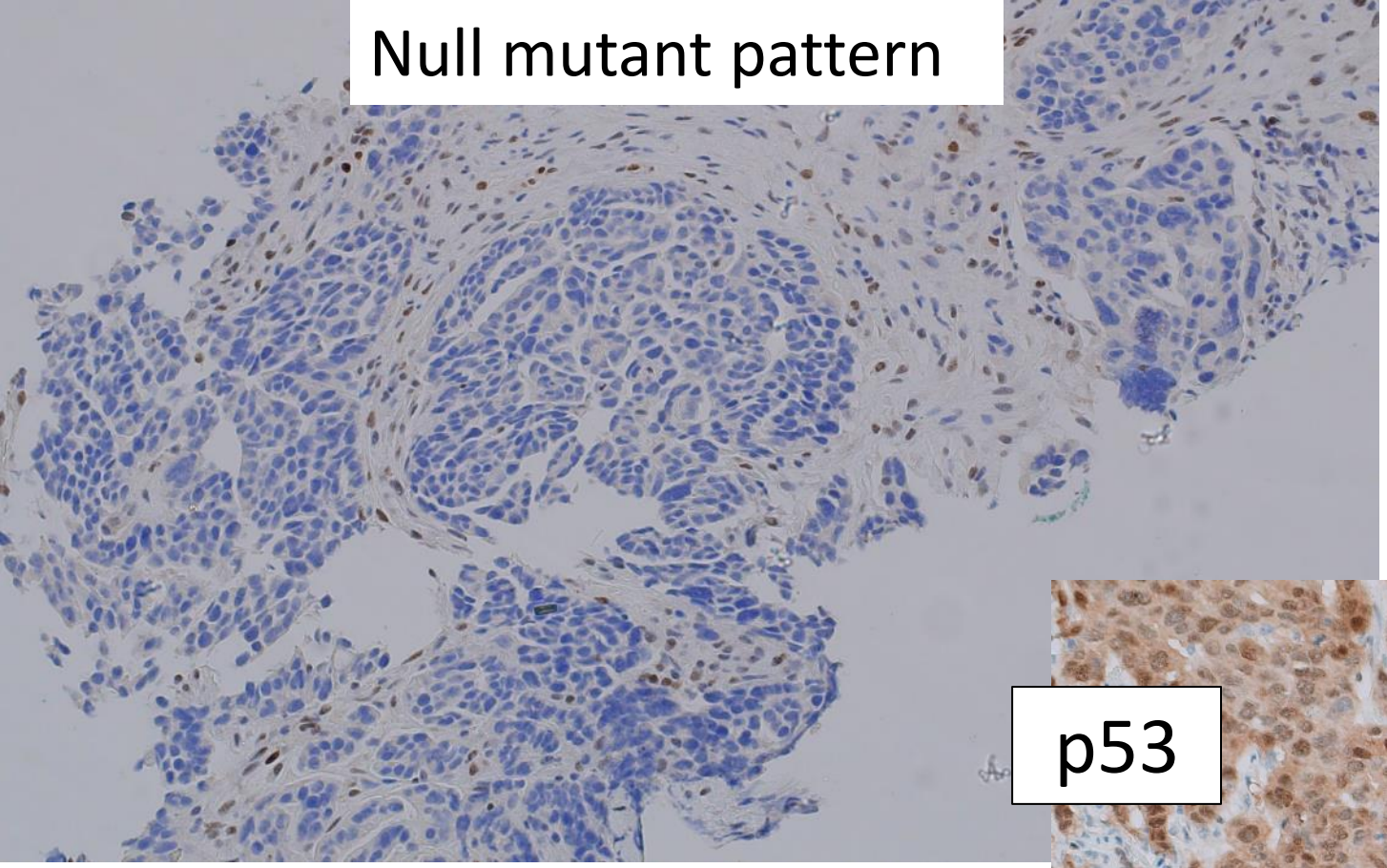
Normal, but also seen in up to 4% high grade serous carcinomas harbouring TP53 loss of function mutation

Complete absence/null

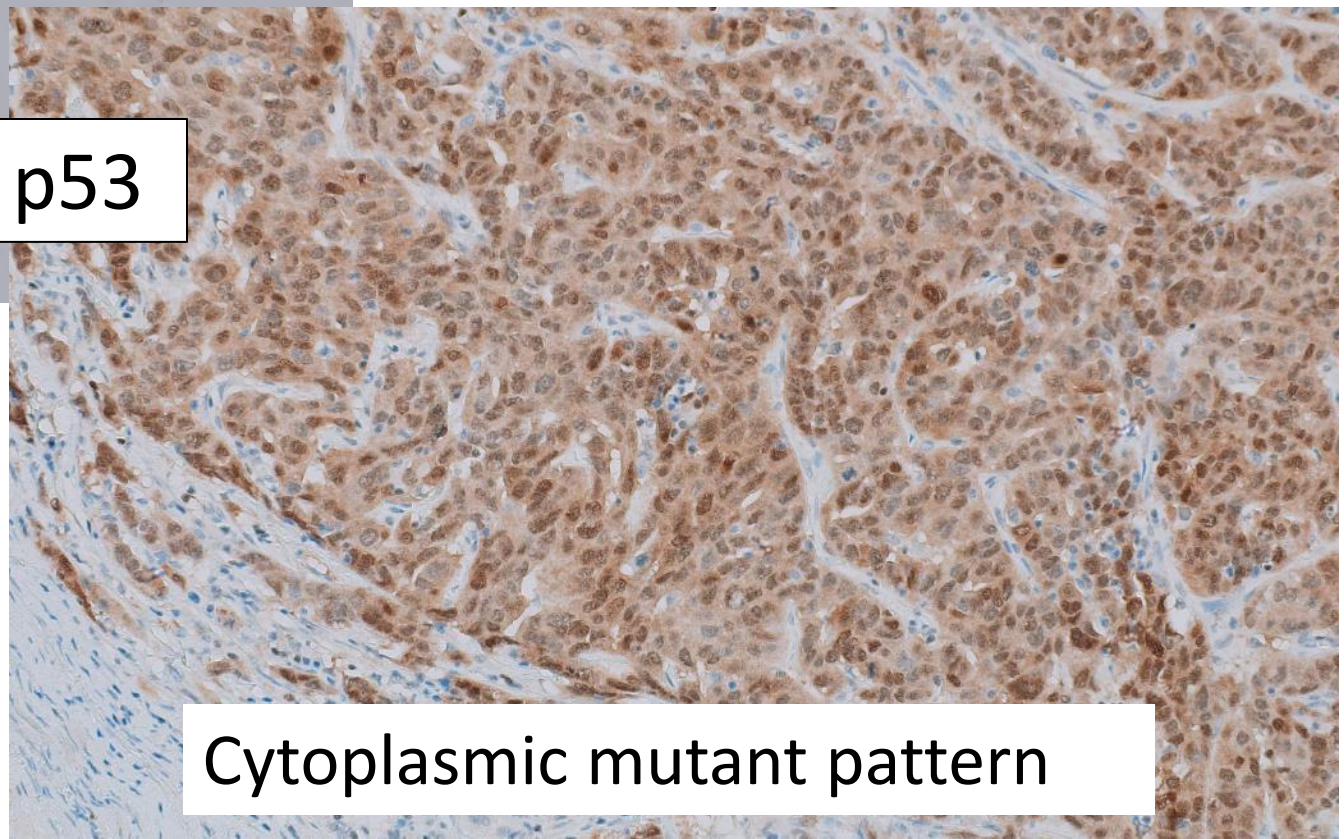
Over-expressed

Cytoplasmic with no nuclear over-expression

Kobel et al 2016



p53



A few basic principles

- Use of panels in diagnosis– don’t rely on one marker only
- Be aware of the pattern of specific staining – membrane, cytoplasmic or nuclear or combinations and what they may mean.
- Knowledge about the antibody (e.g. binding epitope, monoclonal vs polyclonal and specific clone) used in your laboratory

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Original Article

Calibration and Optimization of p53, WT1, and Napsin A Immunohistochemistry Ancillary Tests for Histotyping of Ovarian Carcinoma: Canadian Immunohistochemistry Quality Control (CIQC) Experience

Sandra Lee, M.D., Anna M. Piskorz, Ph.D., Cécile Le Page, Ph.D., Anne-Marie Mes Masson, Ph.D., Diane Provencher, M.D., David Huntsman, M.D., Wenqian Chen, M.D., Ph.D., Paul E. Swanson, M.D., C. Blake Gilks, M.D., and Martin Köbel, M.D.

WT1

- 9/1187 (0.76%) errors, all due to false negative results
- All errors occurred in labs using 6Fh2 clone (error rate 2.4)
- No errors using WT-49 clone

Napsin A

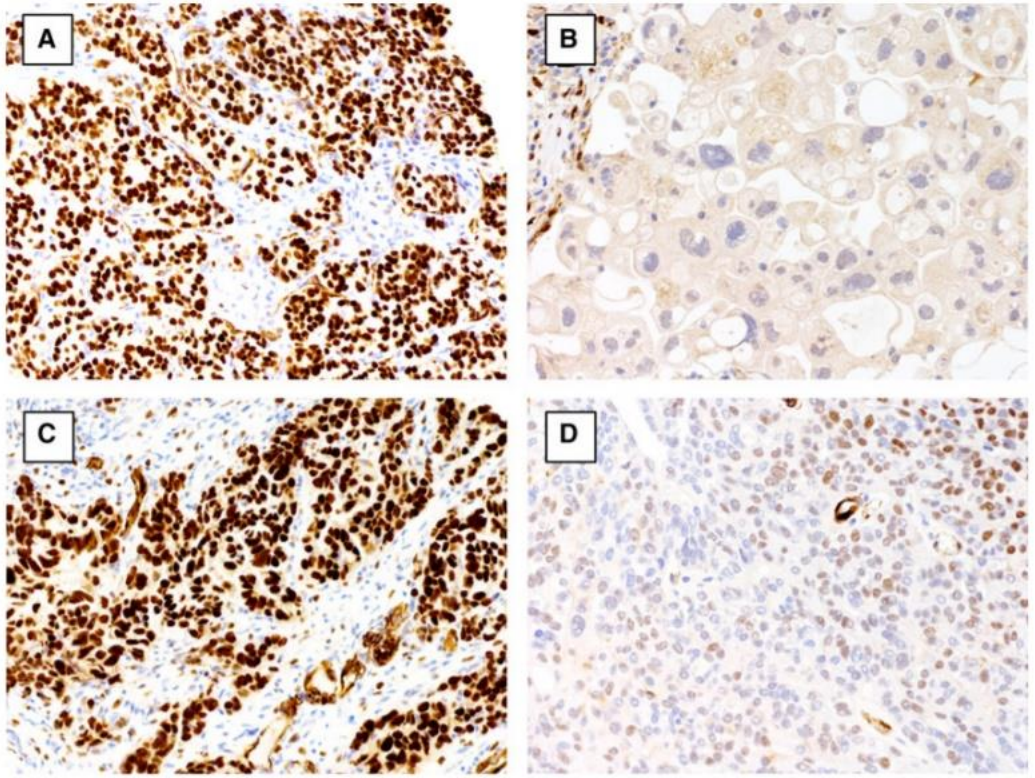
- 40/1067 (4%) errors, 16 false positives (FP) and 24 false negatives (FN)
- Error rate higher for polyclonal compared to monoclonal ab (3 vs 1.1)
- Polyclonal ab caused FP in 12 cases (endometrioid and mucinous carcinoma) and FN in 6 cases of clear cell Ca

Lee et al 2015

A few basic principles

- Use of panels in diagnosis– don’t rely on one marker only
- Be aware of the pattern of specific staining – membrane, cytoplasmic or nuclear or combinations and what they may mean.
- Knowledge about the antibody (monoclonal vs polyclonal and specific clone) used in your laboratory
- Be aware of the significance of positive staining and possible effect that chemotherapy may have on expression of markers

Comparison of WT1 expression in tubo-ovarian high grade serous carcinoma, before and after neoadjuvant chemotherapy



Pre-chemotherapy

Post-chemotherapy

Casey et al *Histopathology 2017*

WT1 expression in high grade serous carcinoma – ?tubo-ovarian vs uterine serous Ca

- Tubo-ovarian high grade serous Ca - typically strong and diffuse positive
- Uterine serous Ca (USC) – typically negative
- However occasionally USC may show diffuse positivity
- Rarely tubo-ovarian high grade serous WT1 negative

WT1 expression in high grade serous carcinoma – ?tubo-ovarian vs uterine serous Ca

- Tubo-ovarian high grade serous Ca - typically strong and diffuse positive
- Uterine serous Ca (USC) – typically negative
- However occasionally USC may show diffuse positivity
- Rarely tubo-ovarian high grade serous WT1 negative

WT1 useful but be aware of occasional/rare cases that may not stain as expected!

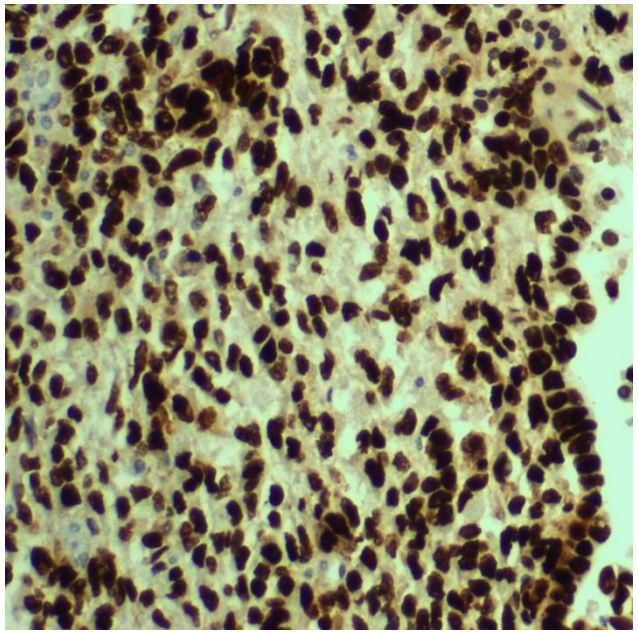
A few basic principles

- Use of panels in diagnosis– don't rely on one marker only
- Be aware of the pattern of specific staining – membrane, cytoplasmic or nuclear or combinations
- Be aware of the possible effect that chemotherapy may have on expression of markers
- Be aware of the significance of positive staining and possible effect that chemotherapy may have on expression of markers
- Use of appropriate controls and titrate according to need and knowledge about the antibody (monoclonal vs polyclonal and specific clone) used.

Titrate according to need

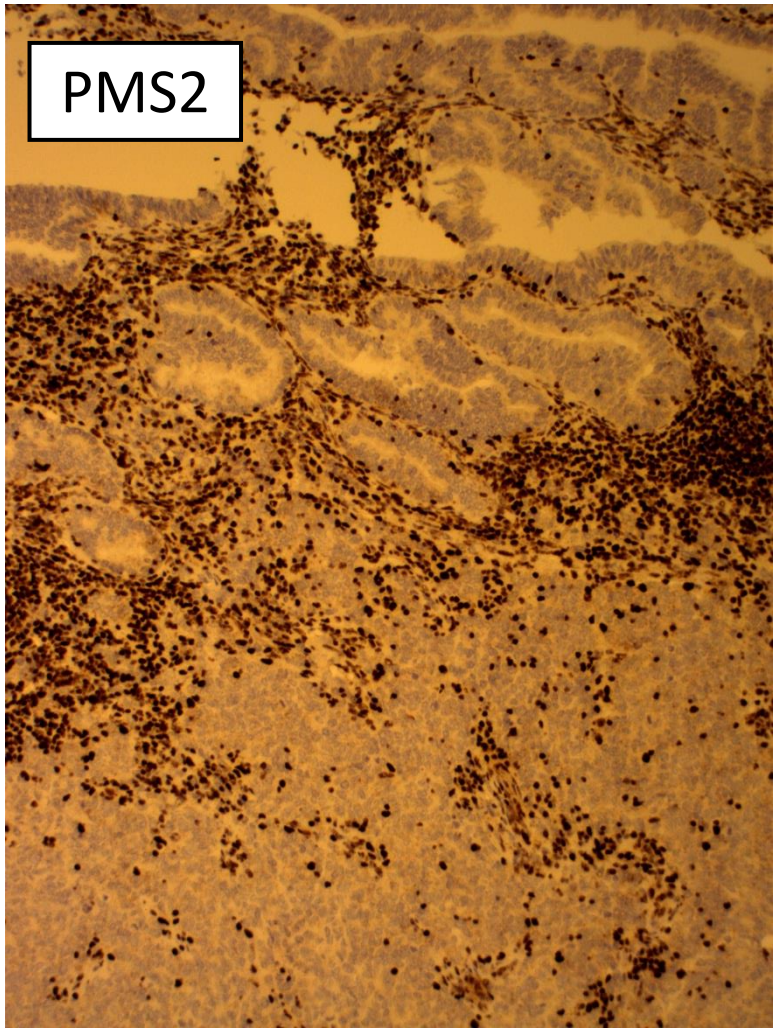
p53

- Titration towards stronger staining preferred for better interpretation at lower cut off and also to discriminate between high wild-type and over expressed mutant phenotype at upper cut off.
- Stronger staining moves the cut off definition away from % positive cells to pattern of staining – virtually all tumour cells strongly positive in over-expressed mutant phenotype

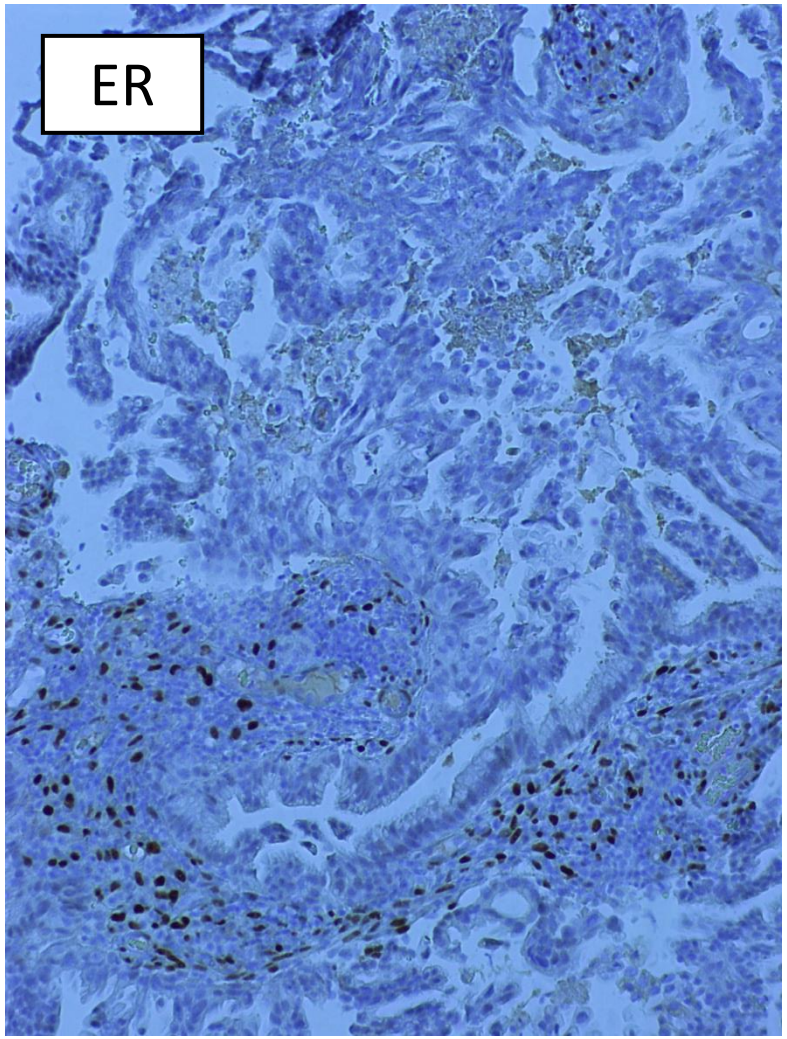


A few basic principles

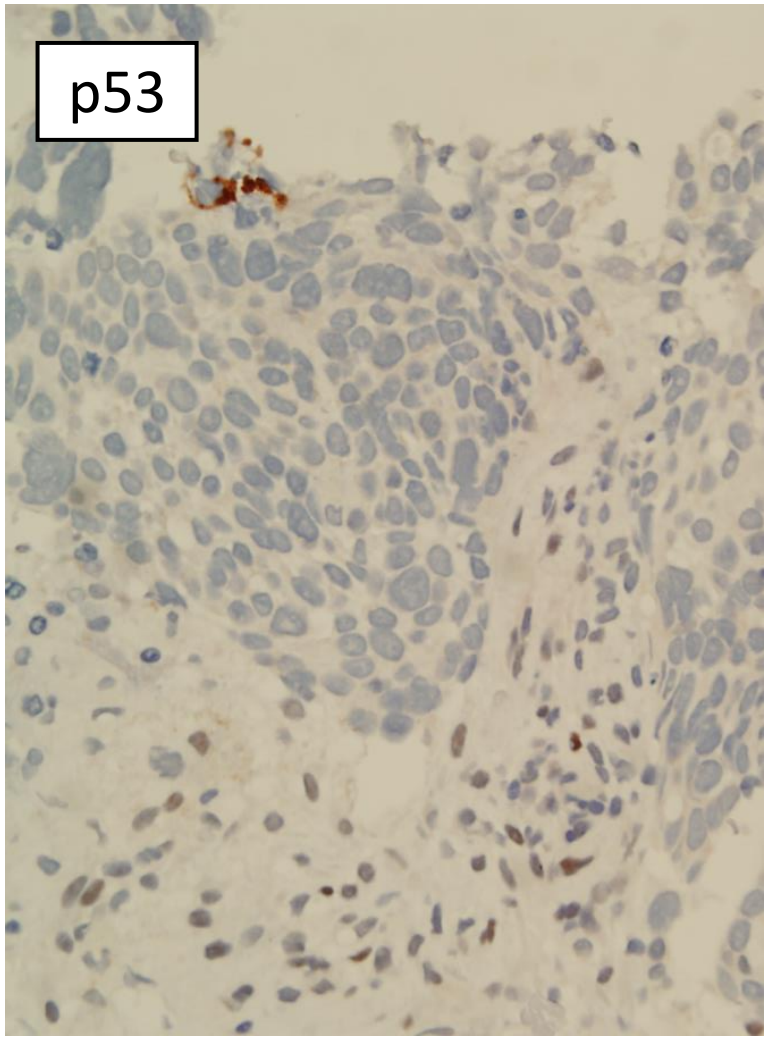
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- Use of appropriate controls, titrate according to need
- Check control – ideal if internal control present.



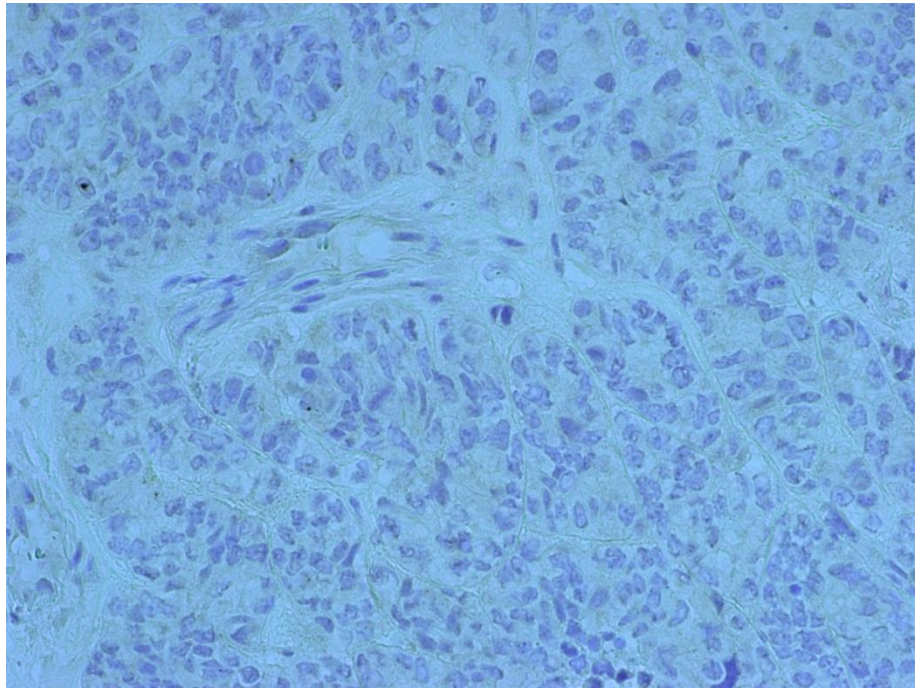
Loss of expression/absent

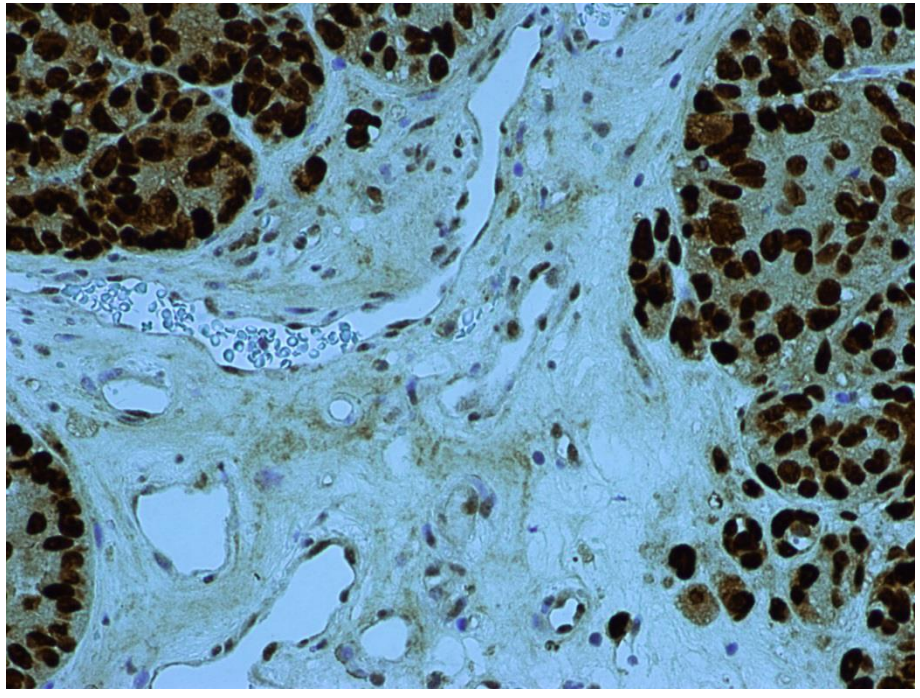


ER negative

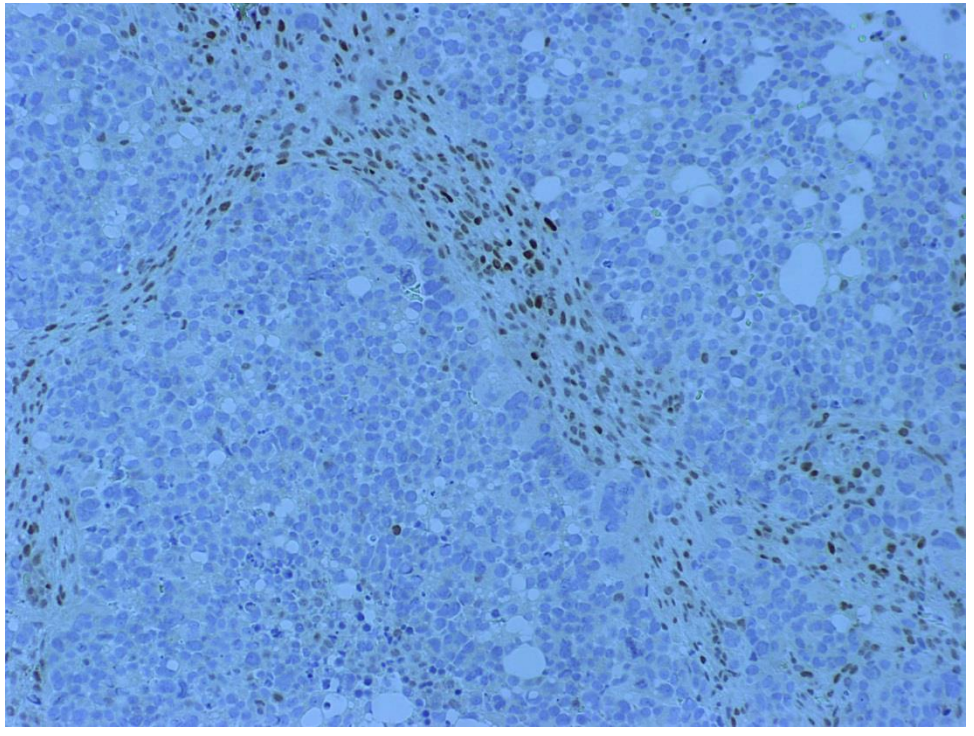


null phenotype



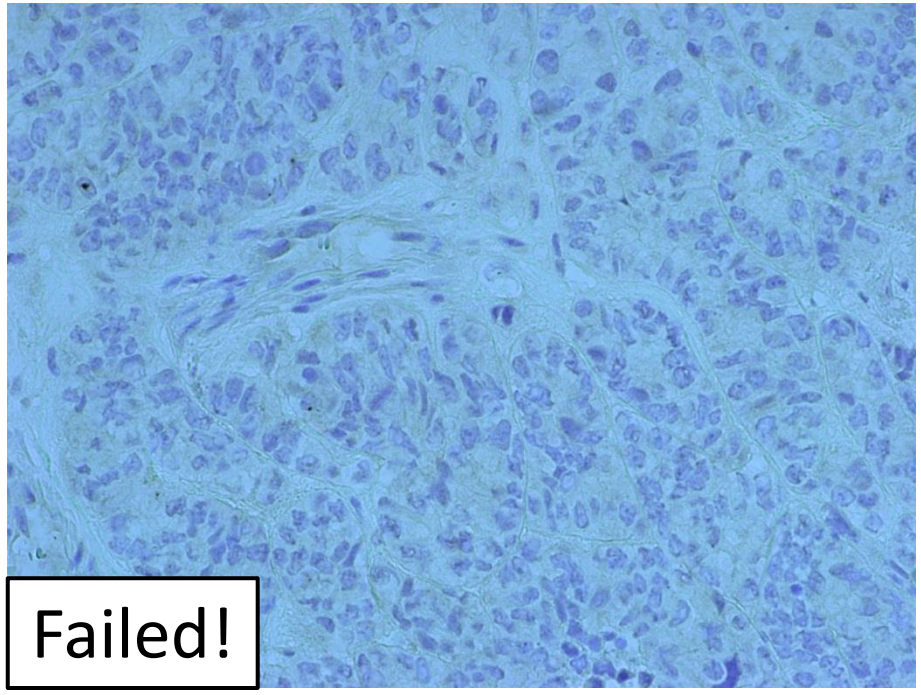


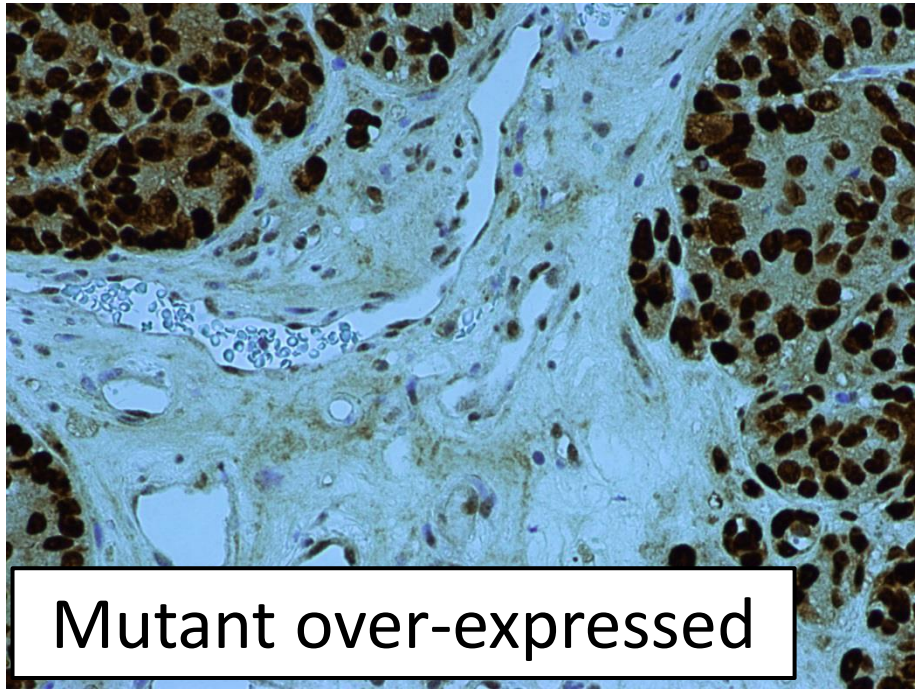
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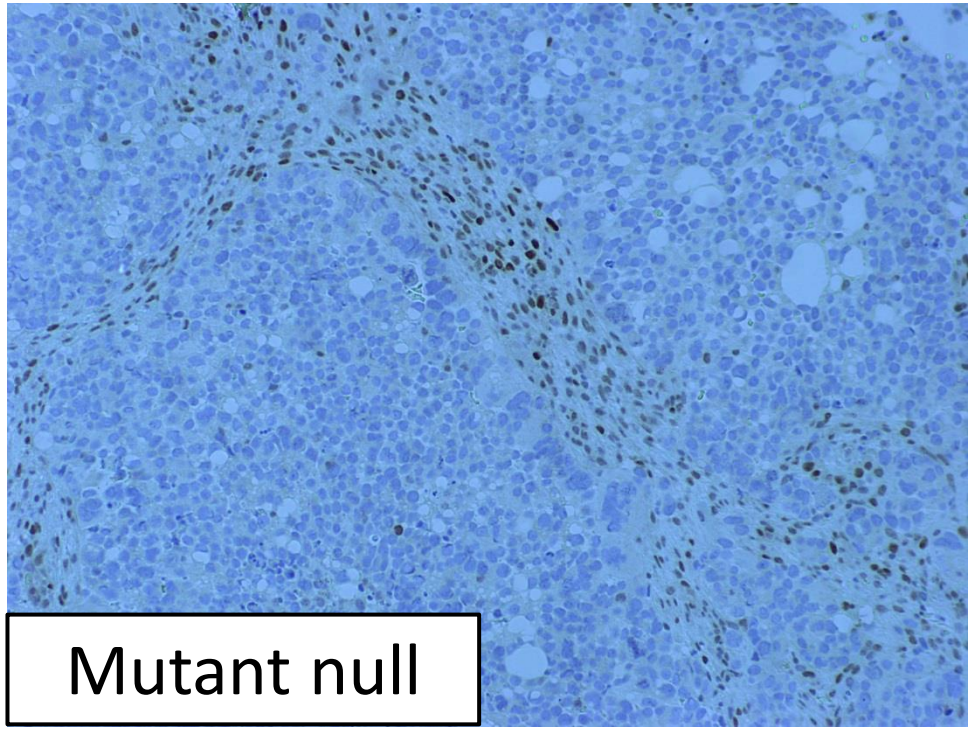


p53

Same case







A few basic principles



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- Be aware of the possible effect that chemotherapy may have on expression of markers
- Be aware of the significance of positive staining and possible effect that chemotherapy may have on expression of markers
- Use of appropriate controls
- Check control – ideal if internal control present.
- Continuous loading IHC platforms, controls should ideally be used with every slide, if no internal control.

Be aware of situations when IHC may **not** help

E.g.

- Primary ovarian mucinous ca vs metastatic pancreatic/biliary adenocarcinoma
- No reliable marker (DPC4 not routinely available – is deleted in proportion of pancreatic ca and therefore if negative would help to diagnose pancreatic origin)
- If Ca125 positive – more likely to be metastatic

Confirming diagnosis….

- Beware that IHC may confirm misdiagnosis!
- Good clinical/radiologic correlation and morphological evaluation

IHC panel

Non-mucinous tumours

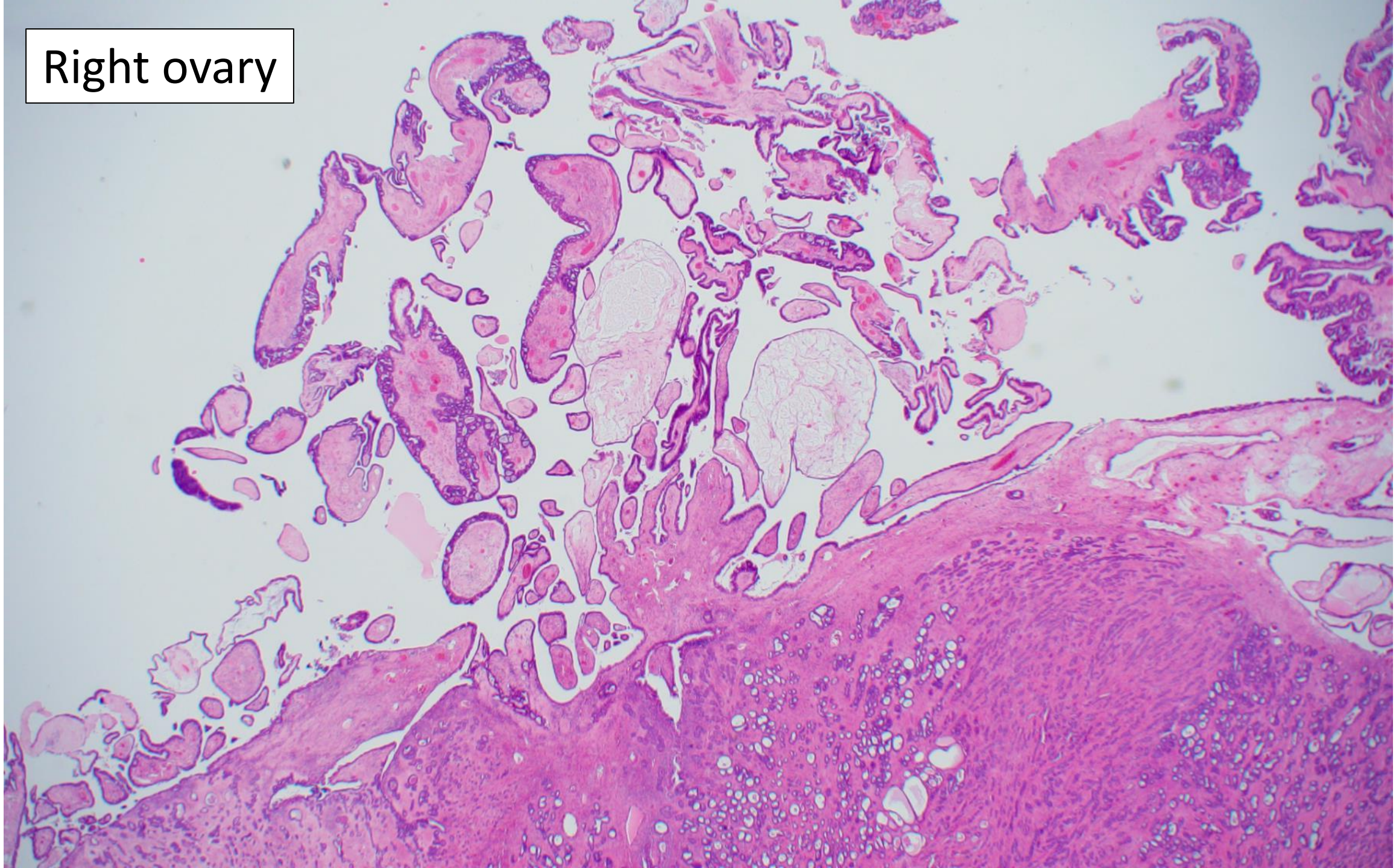
- Basic panel: PAX8, p53, p16, ER, PgR, WT1, Ki 67
- Additional stains: Ca125, Ber EP4, calretinin
- Napsin A, AMACR (if clear cell carcinoma suspected on morphology) Endometrioid with secretory changes may be Napsin A positive
- TTF1, GATA3, CD10 (if mesonephric-like adenocarcinoma suspected)
- If endometrioid/clear cell carcinoma: MMR IHC
- If carcinosarcoma: desmin, myogenin – to exclude heterologous rhabdomyosarcomatous elements
- E-caderin, BRG1, INI1: undifferentiated /de-differentiated Ca

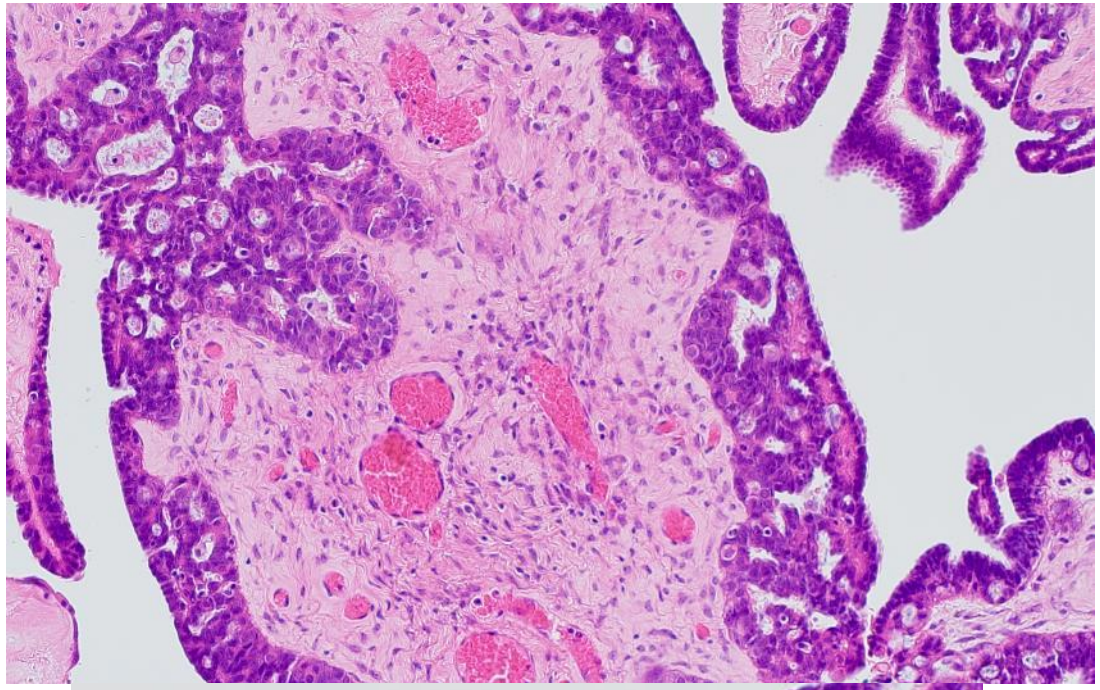
Be aware of how to report specific staining

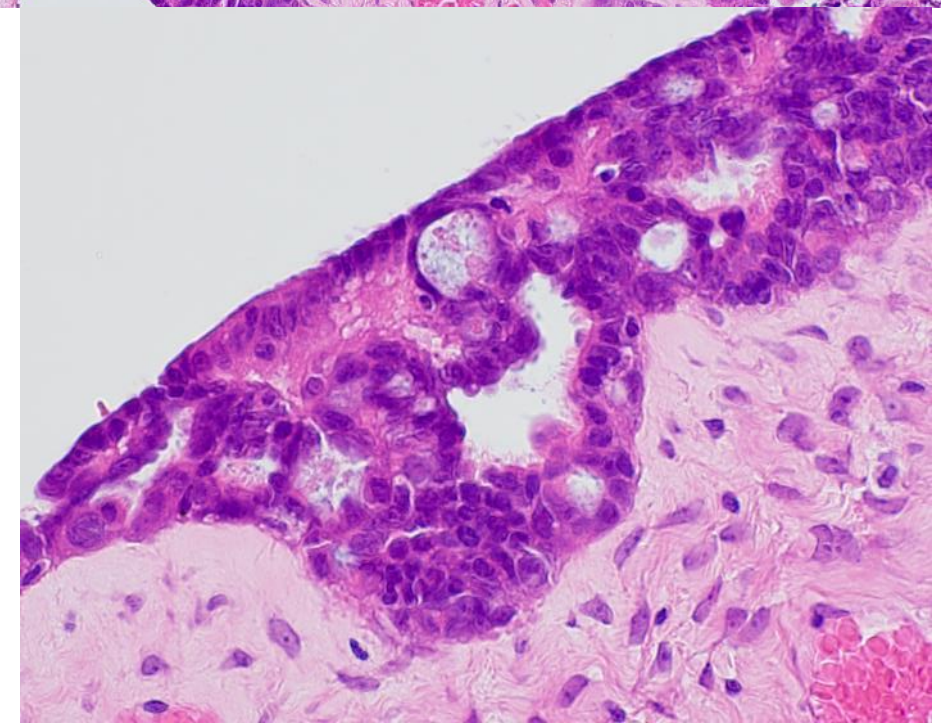
- When do we say + and – (e.g. WT1, ER)
- p53 wild or mutant
- p16 mosaic, null, diffuse/block
- Mismatch repair proteins – intact/present vs absent

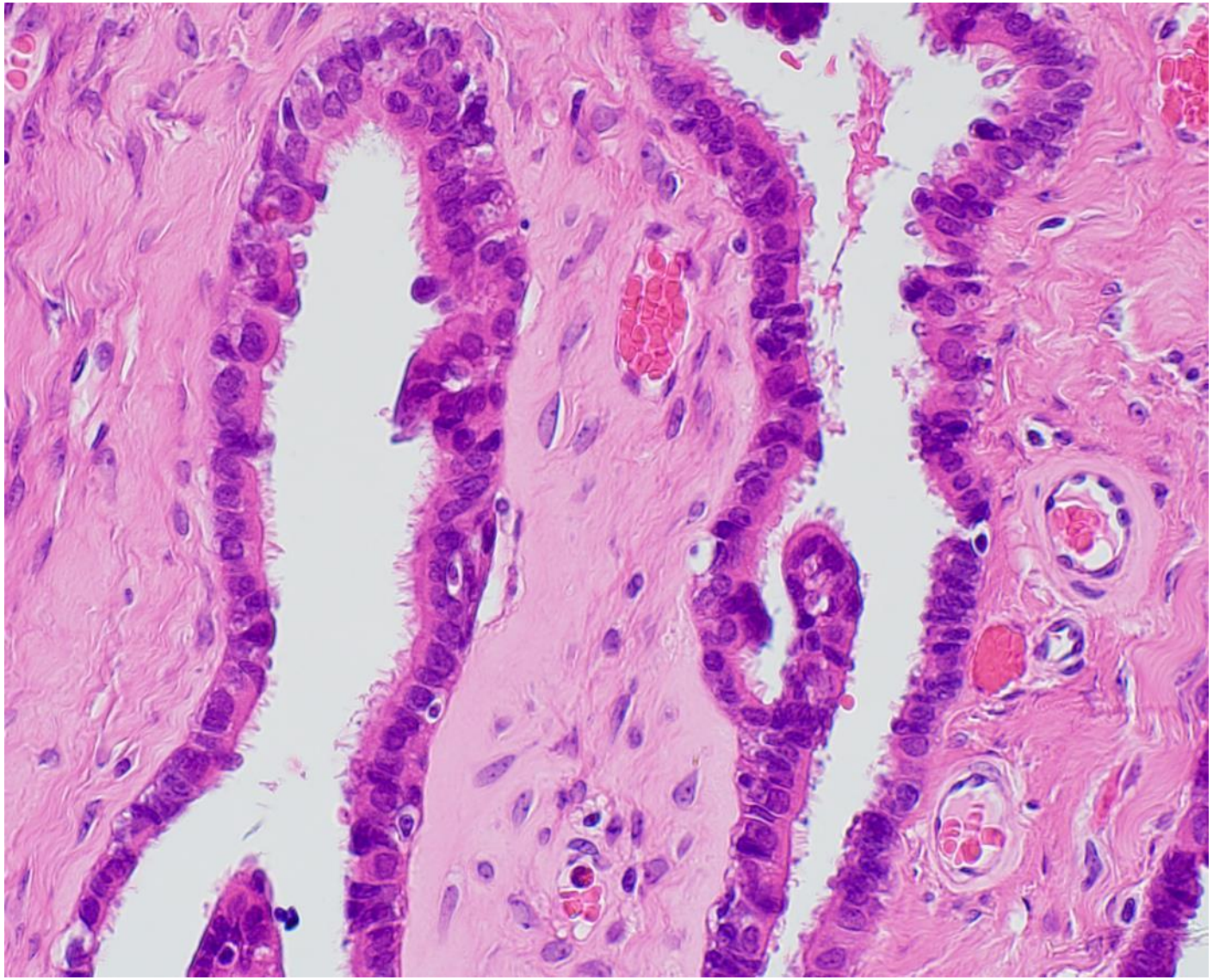
Case 1

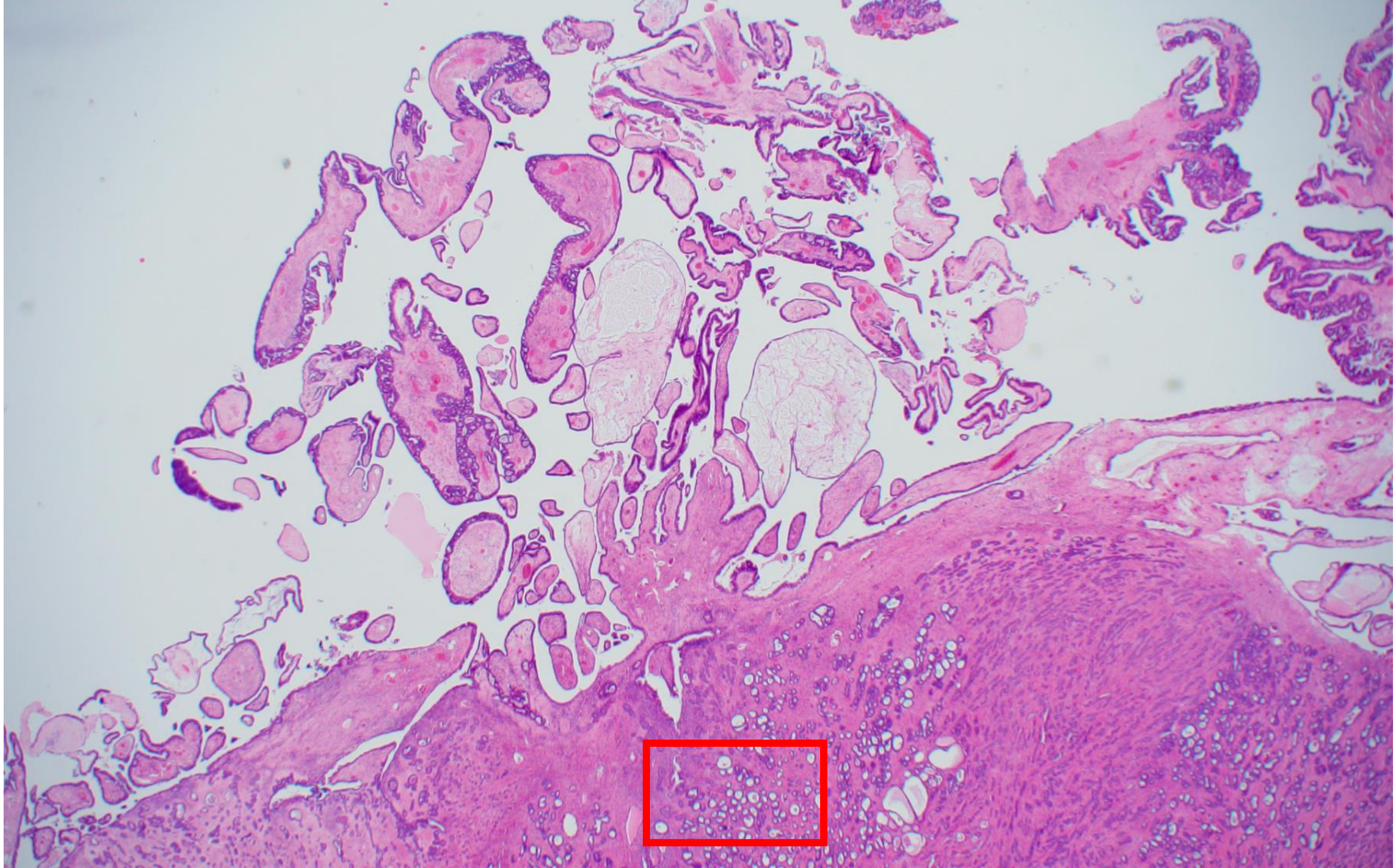
- **71-year old female**
- **Dec 2020:** Probably ovarian cancer with para-aortic lymphadenopathy and peritoneal metastasis. Started Carboplatin & Paclitaxel
- **01.03.2021:** Interval debulking surgery to 5mm residual diease

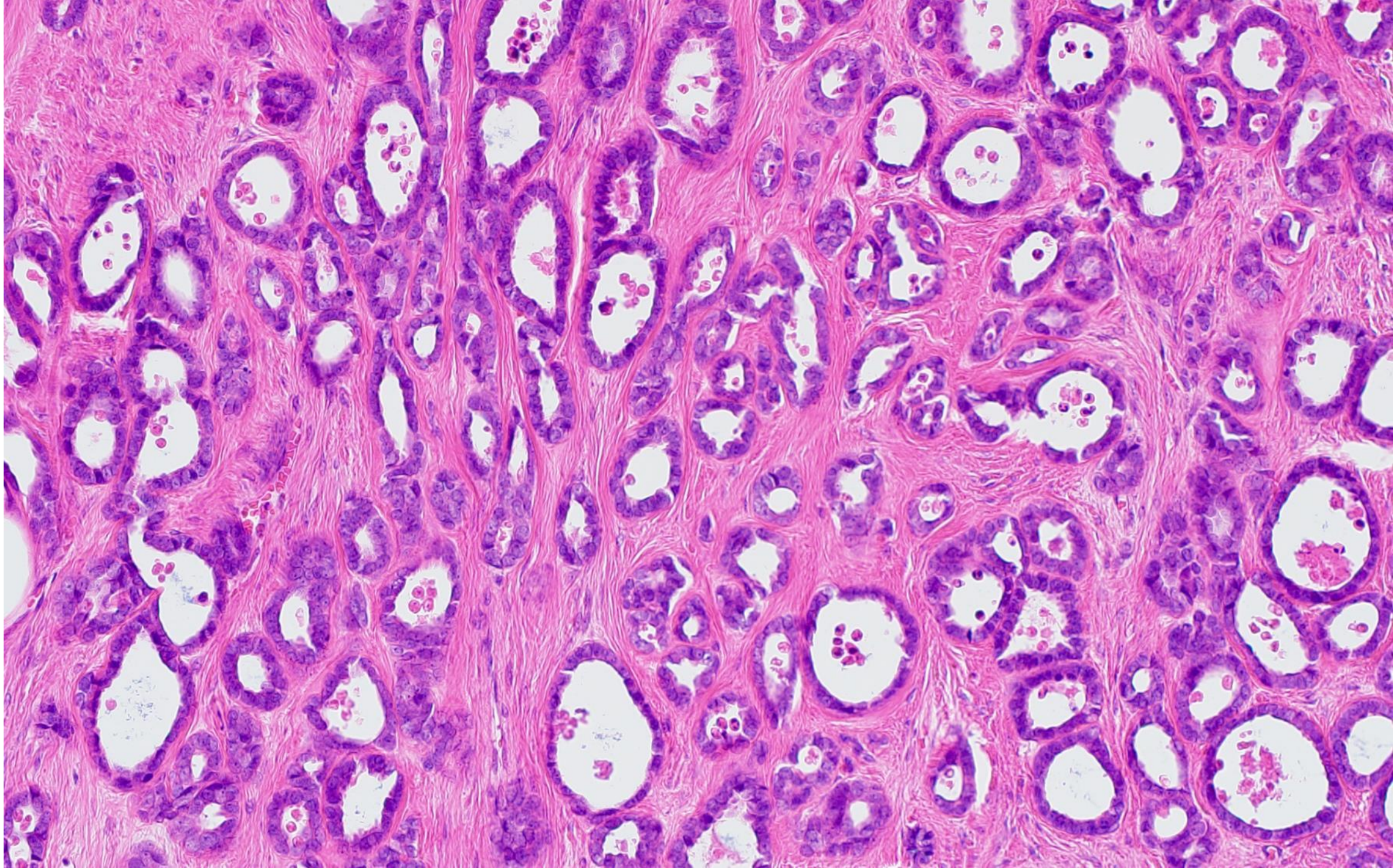

Right ovary

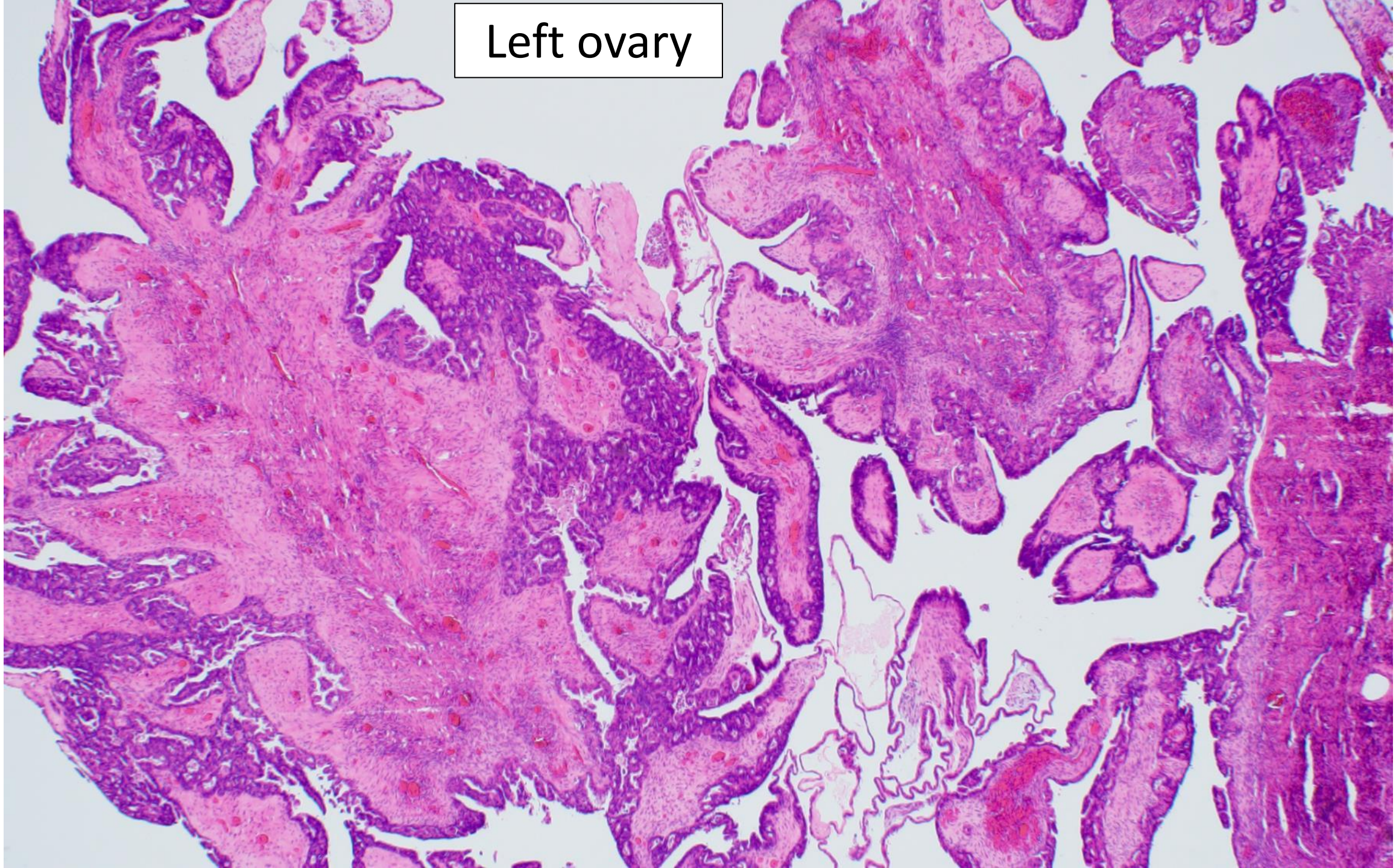


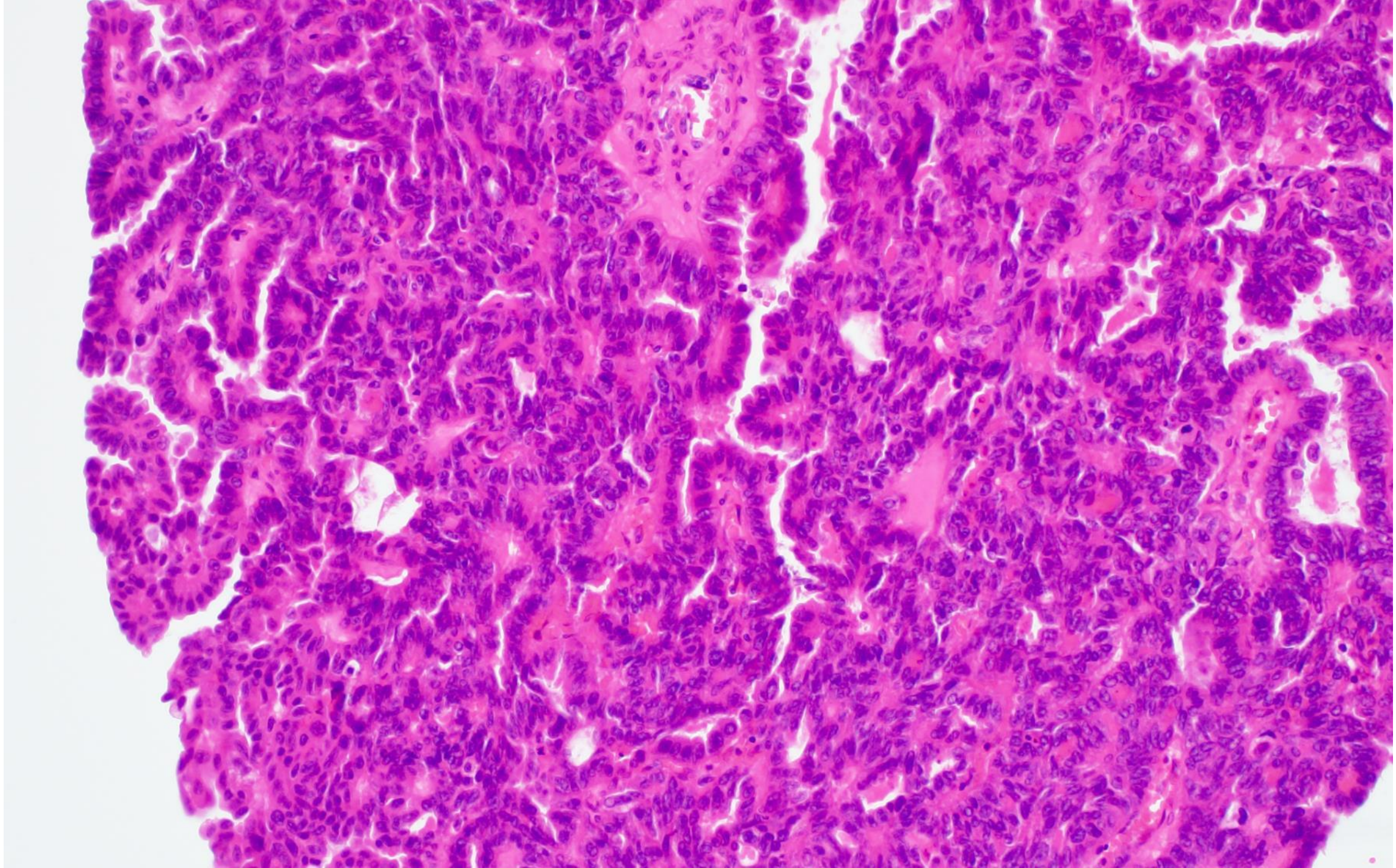


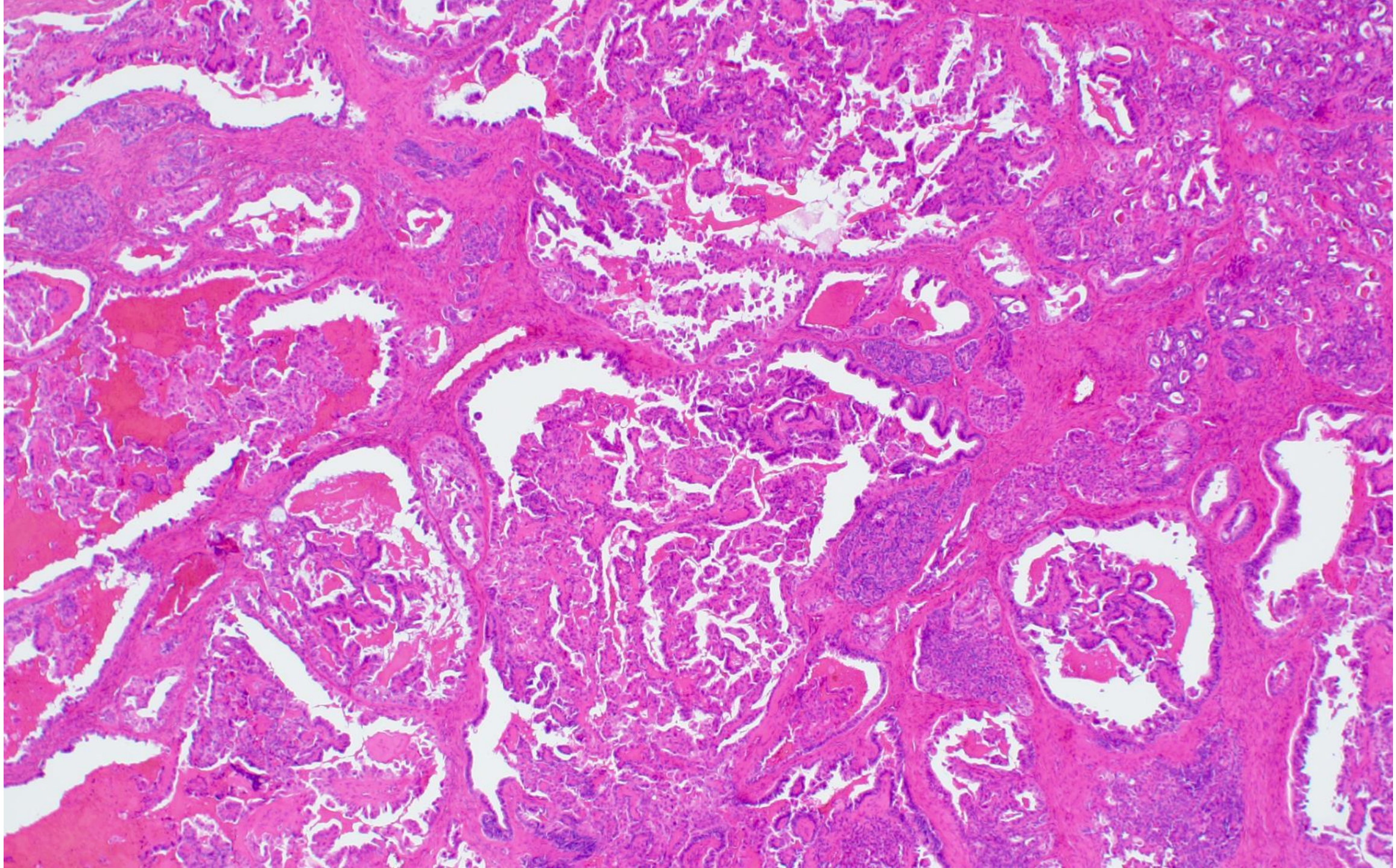


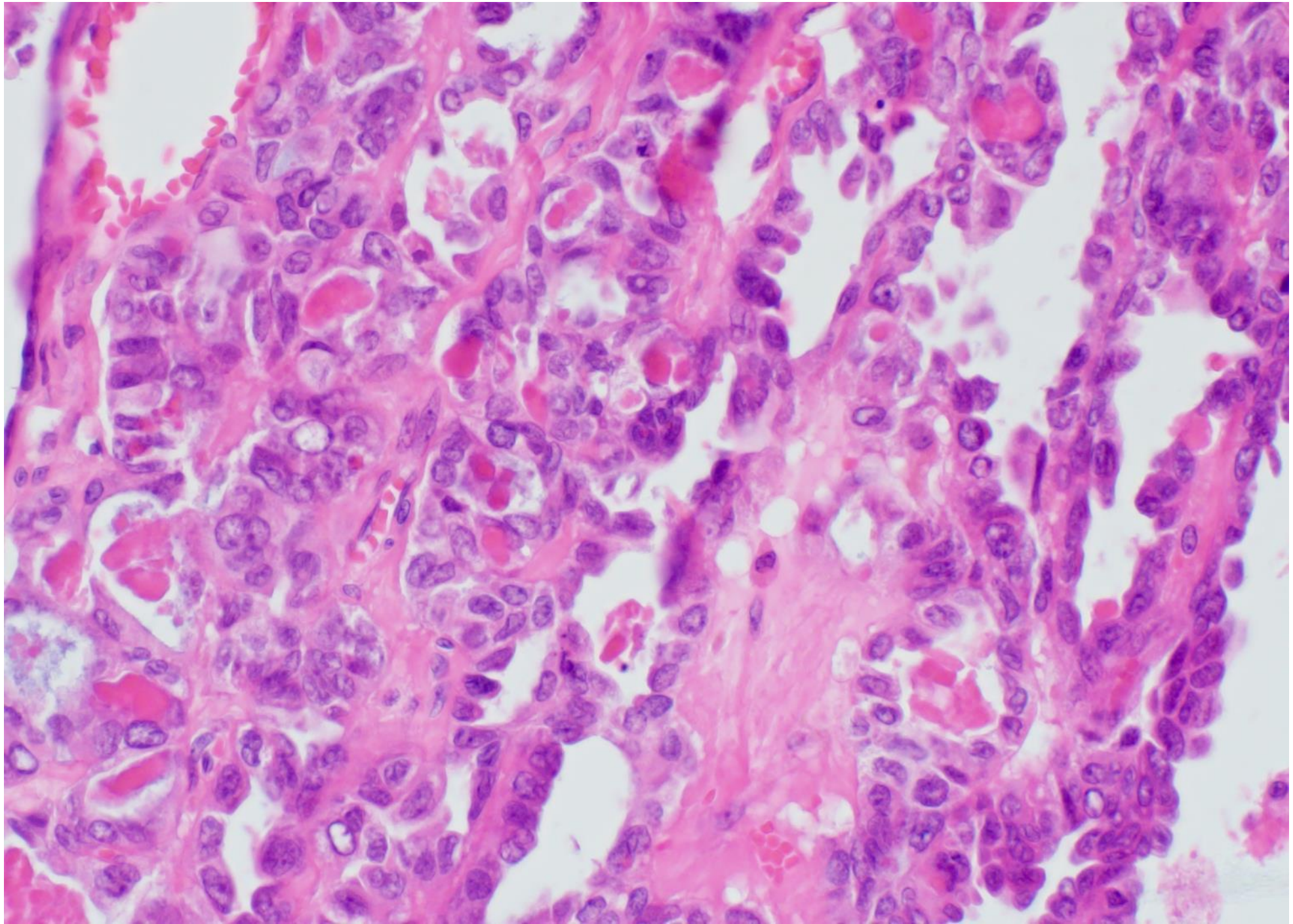


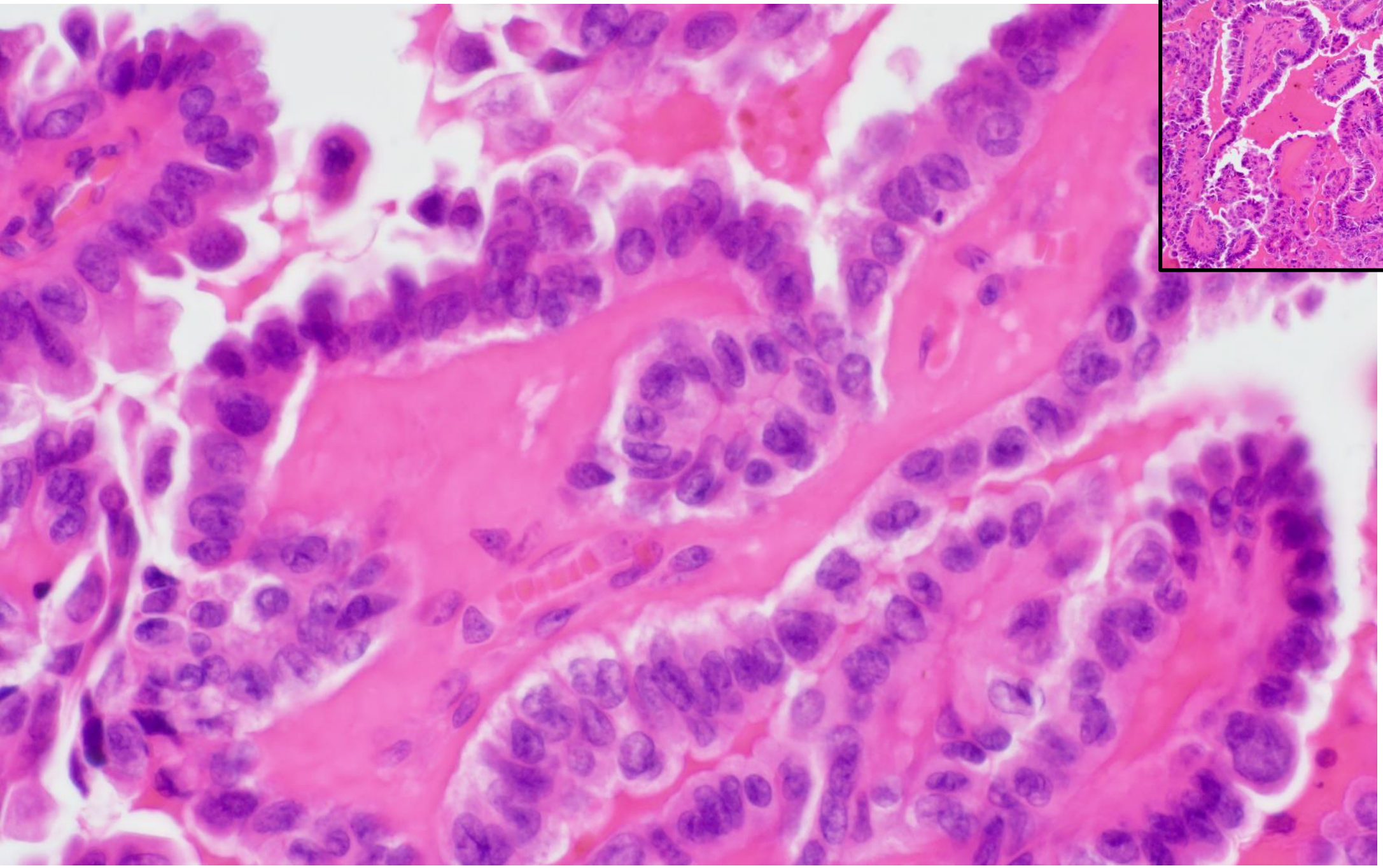


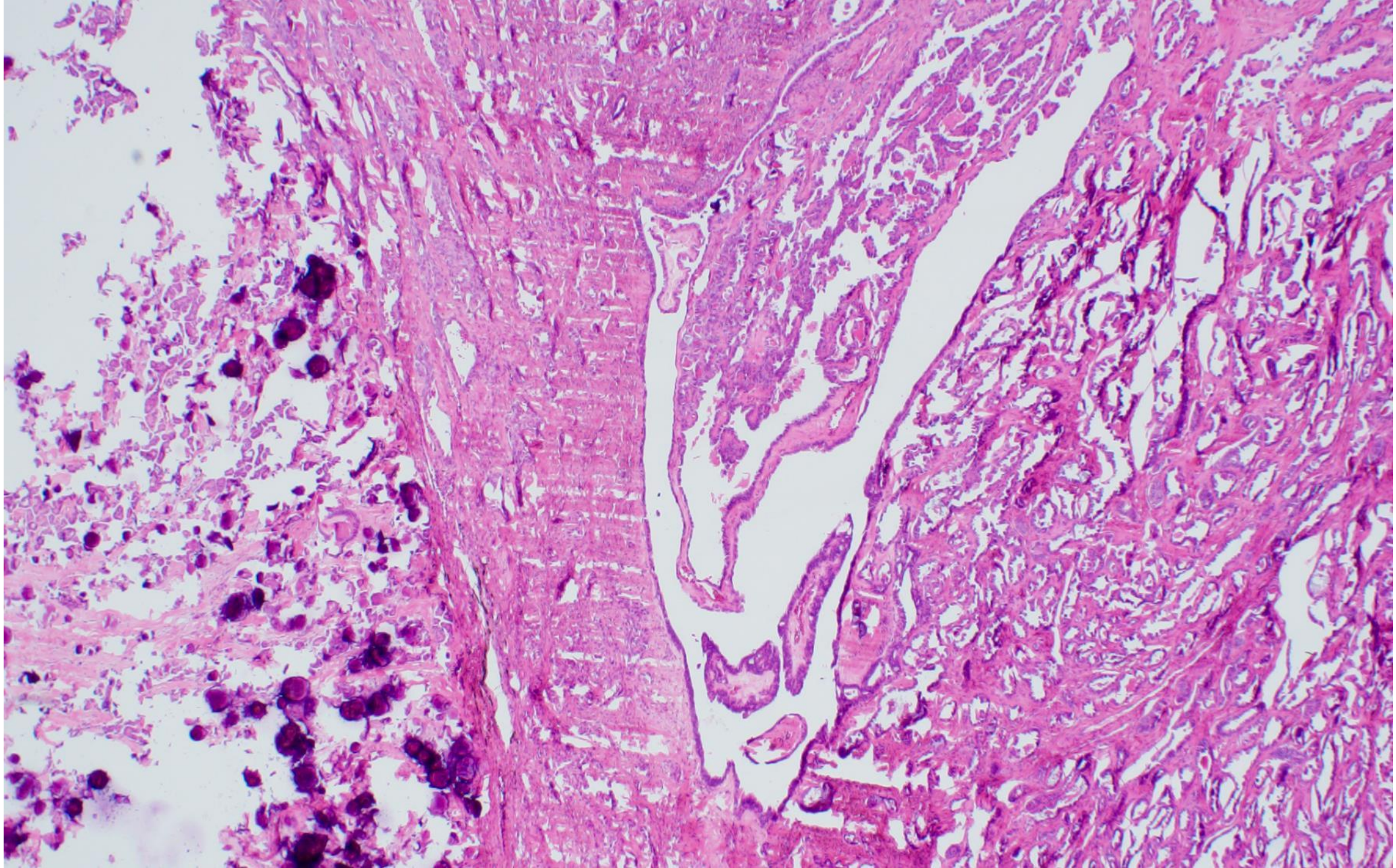

Left ovary

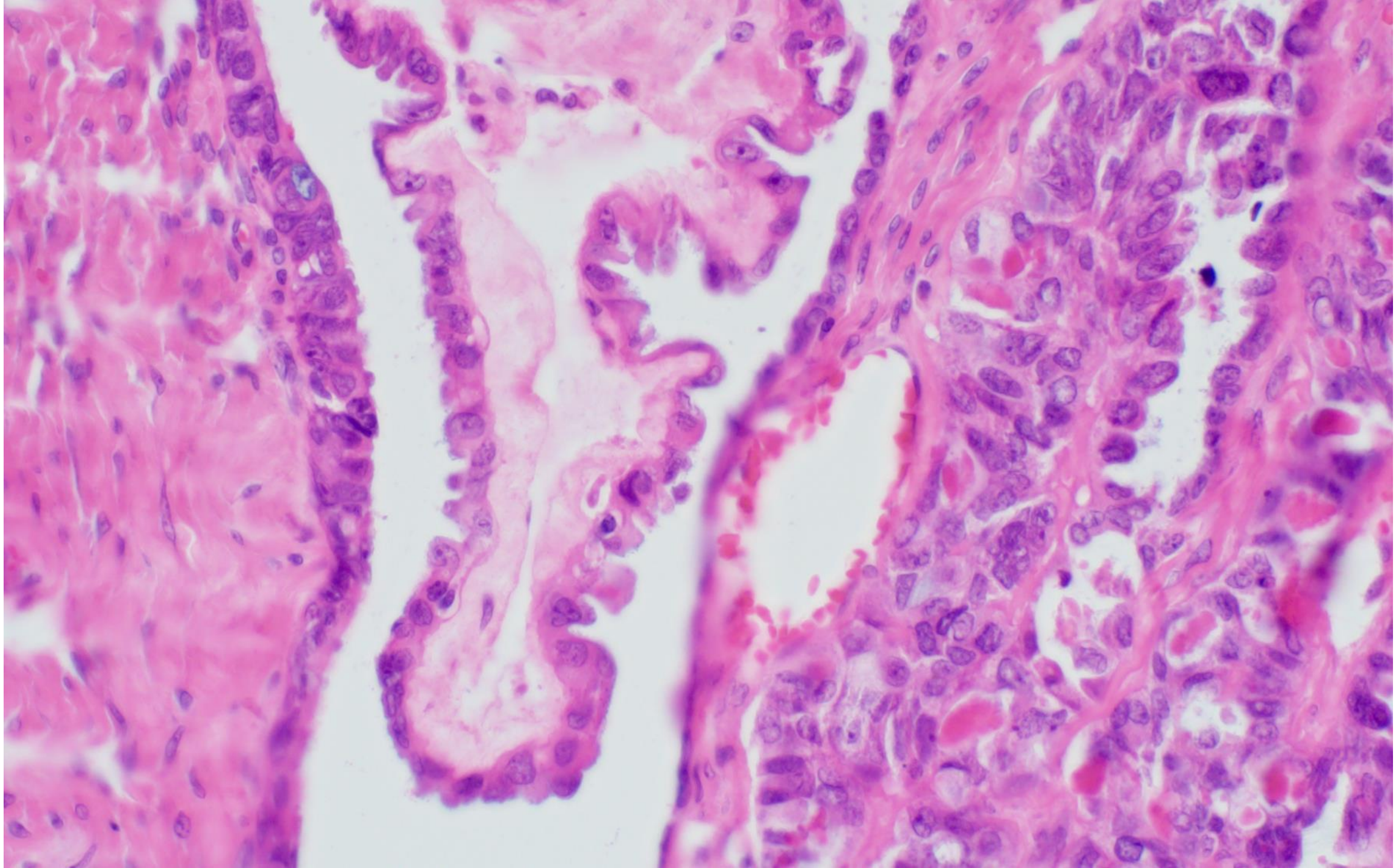


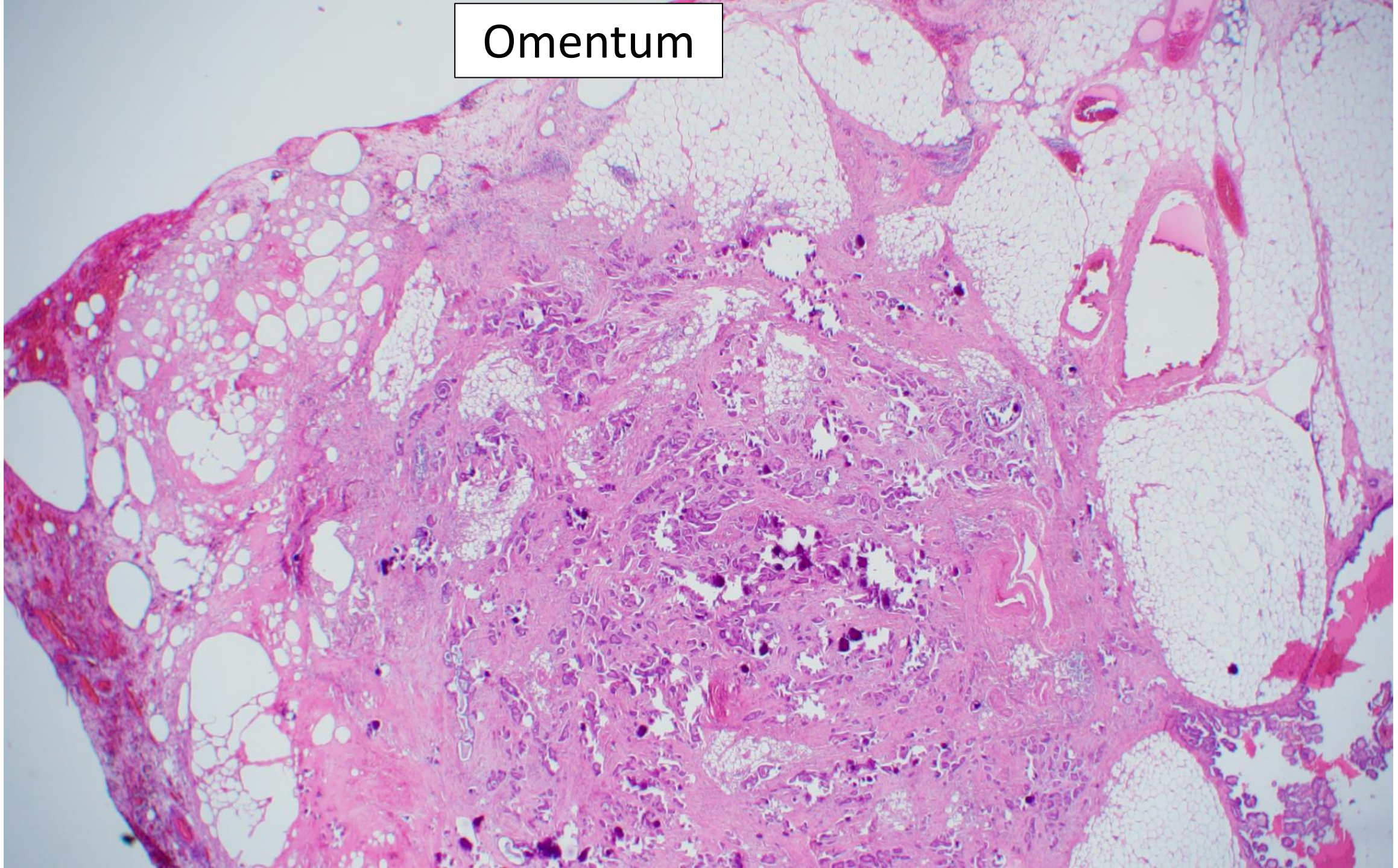


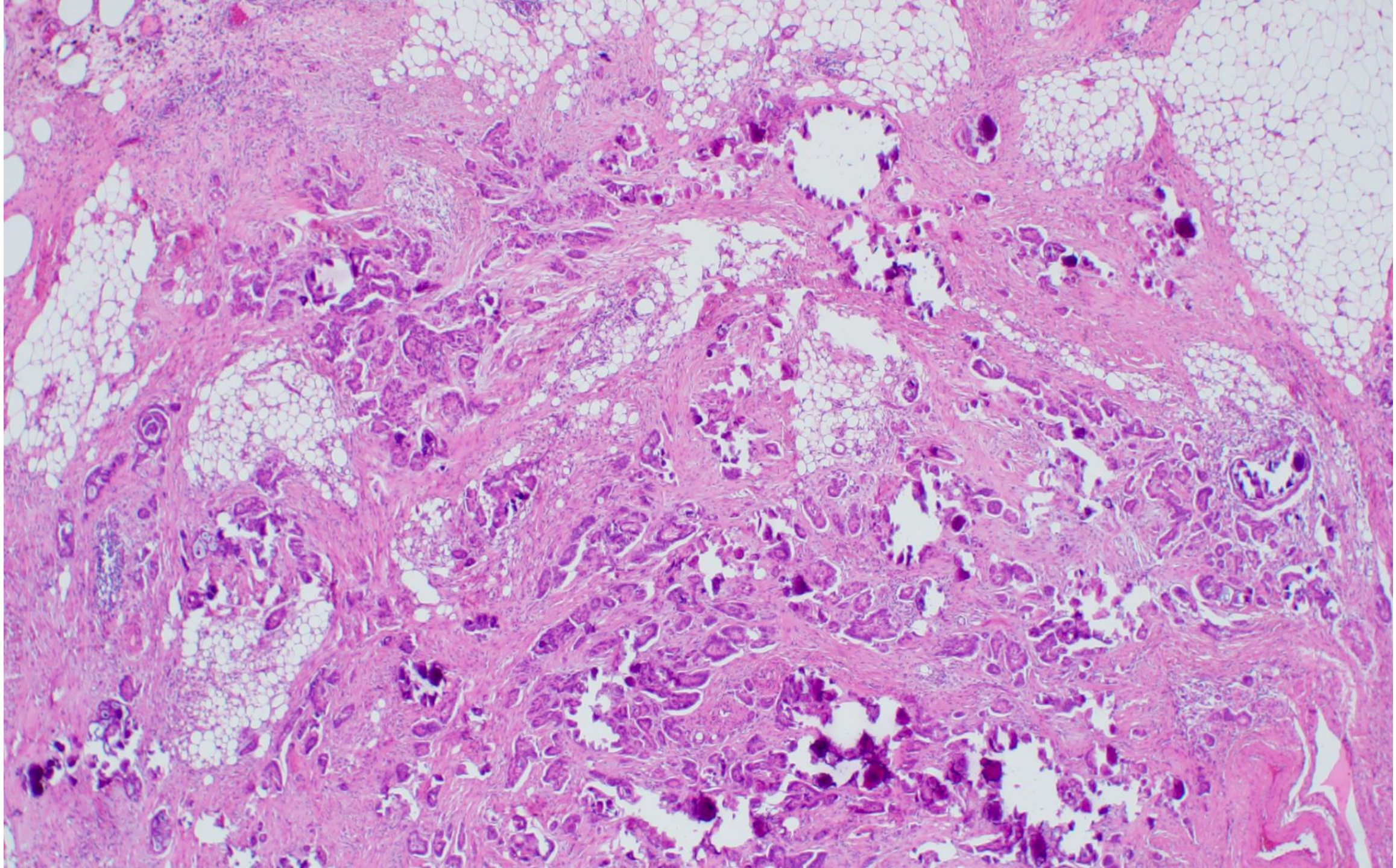


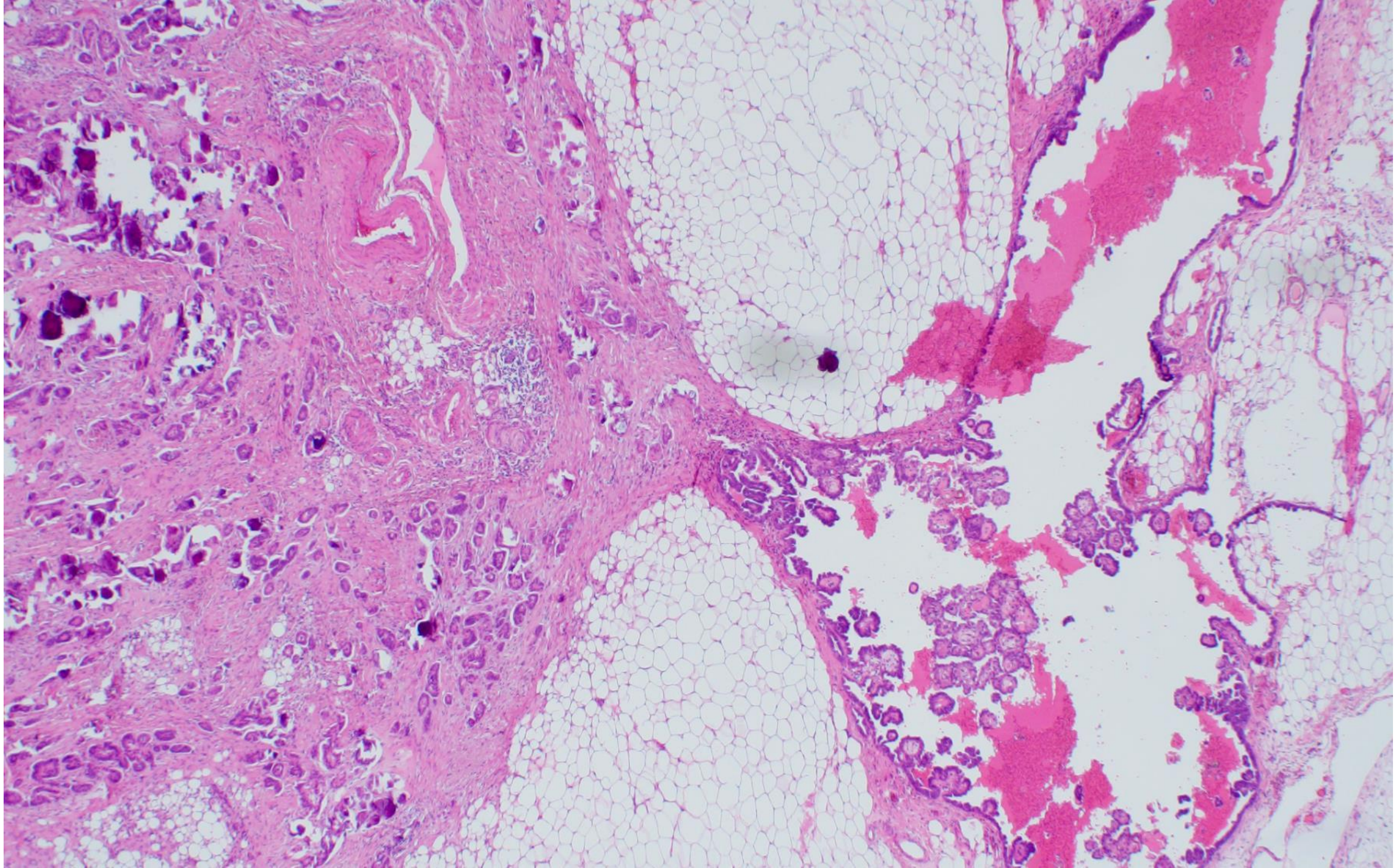


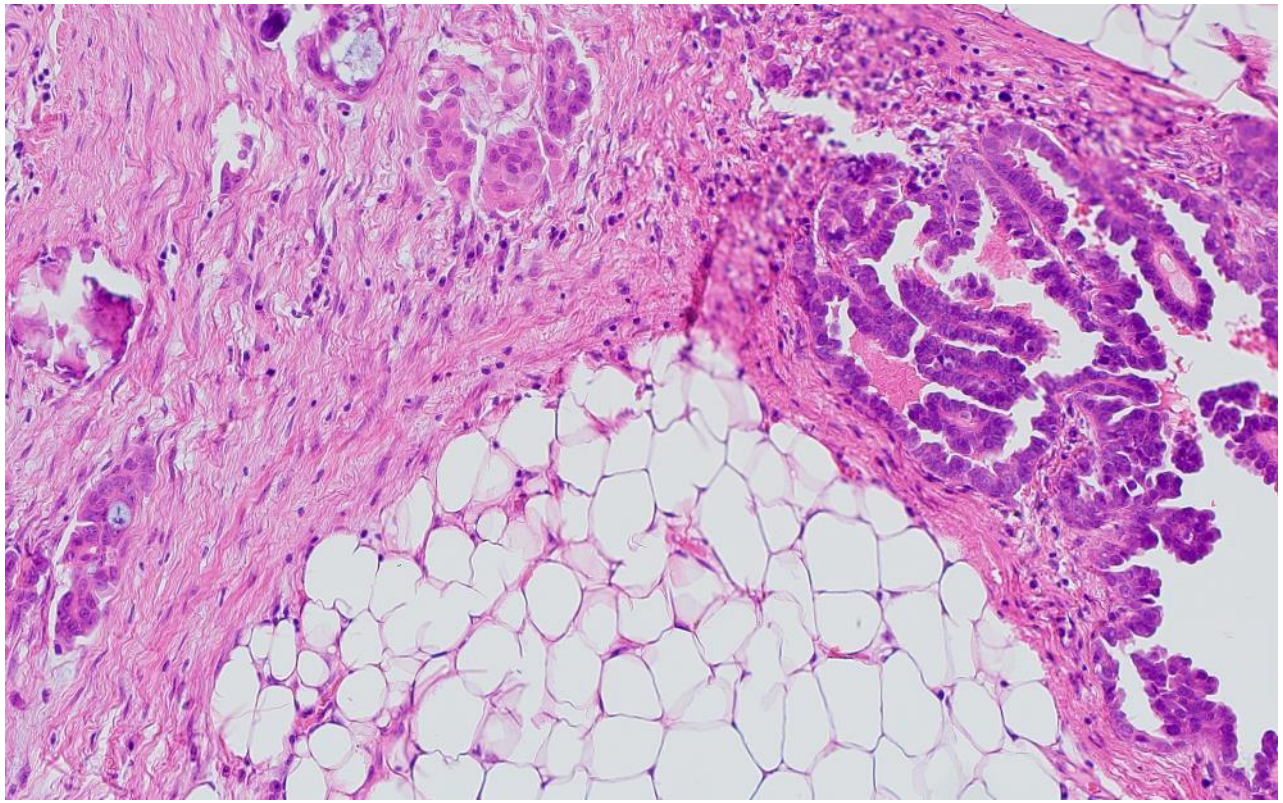


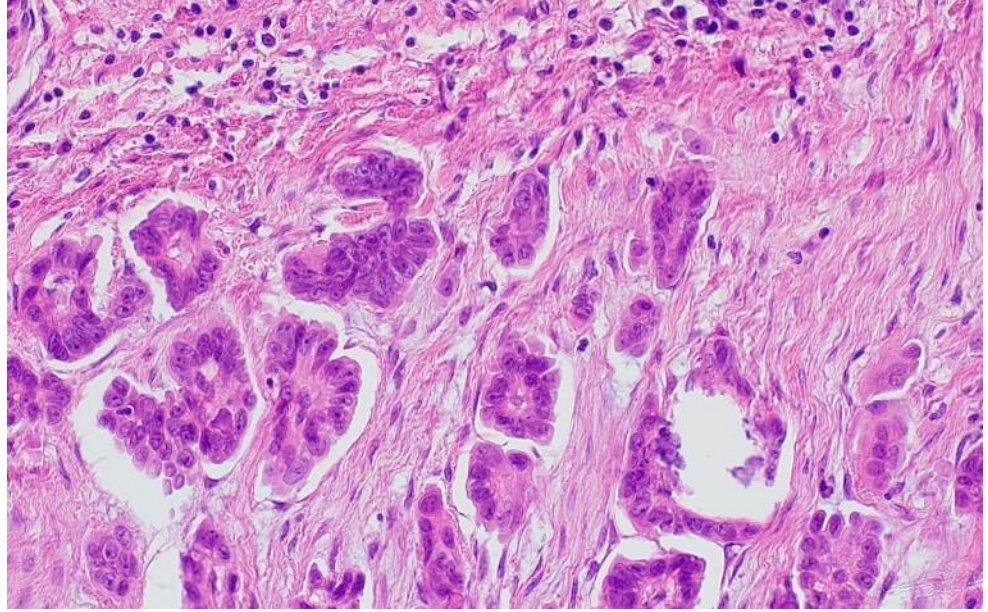


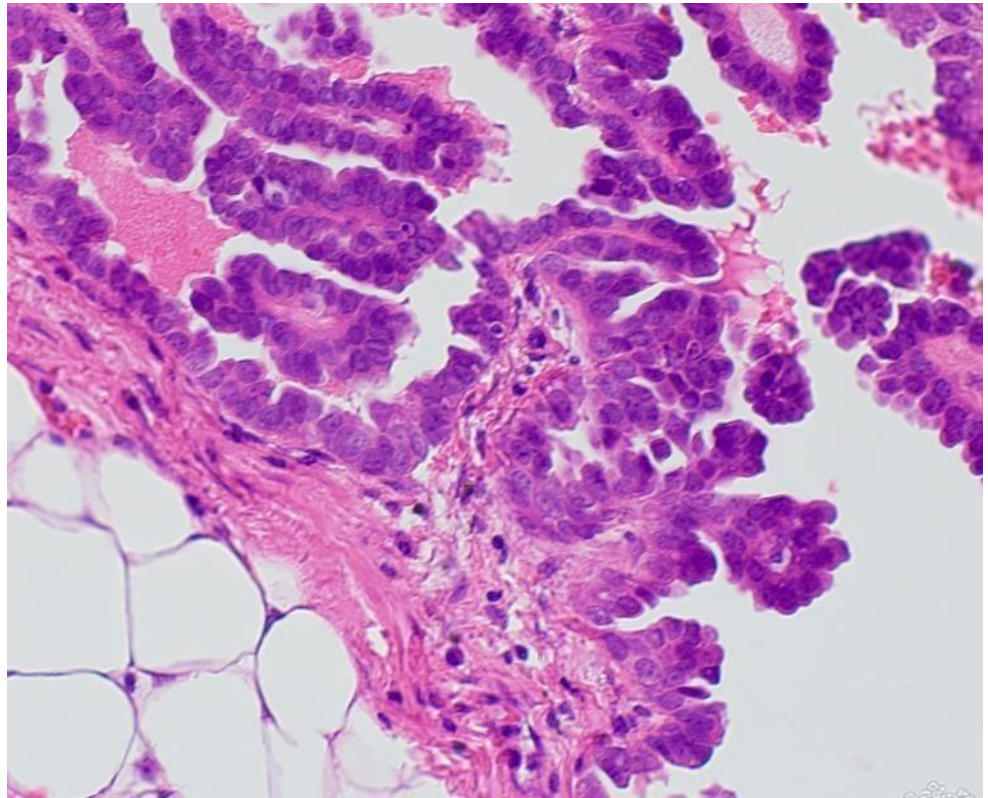

Omentum











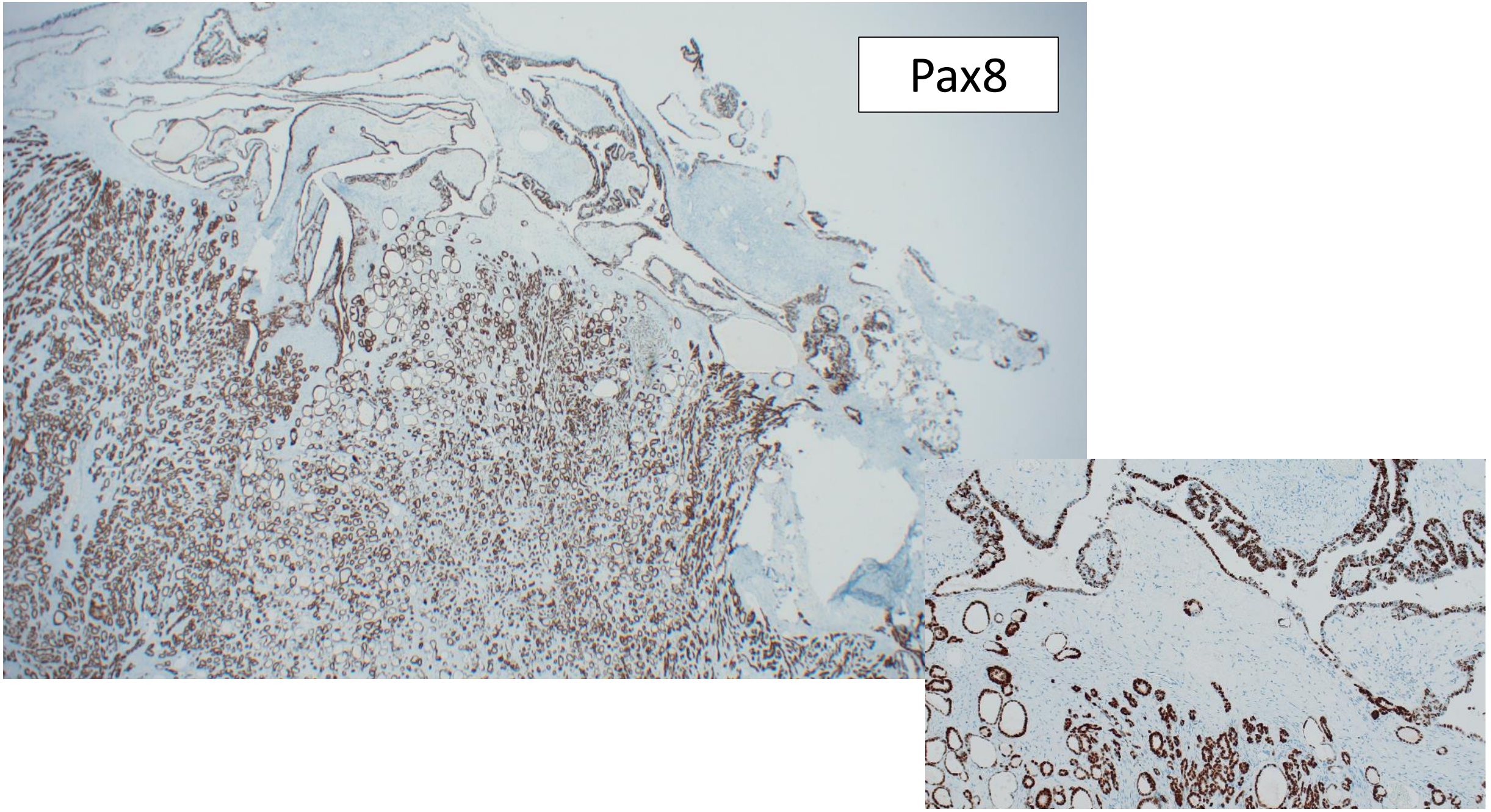
Poll question 1

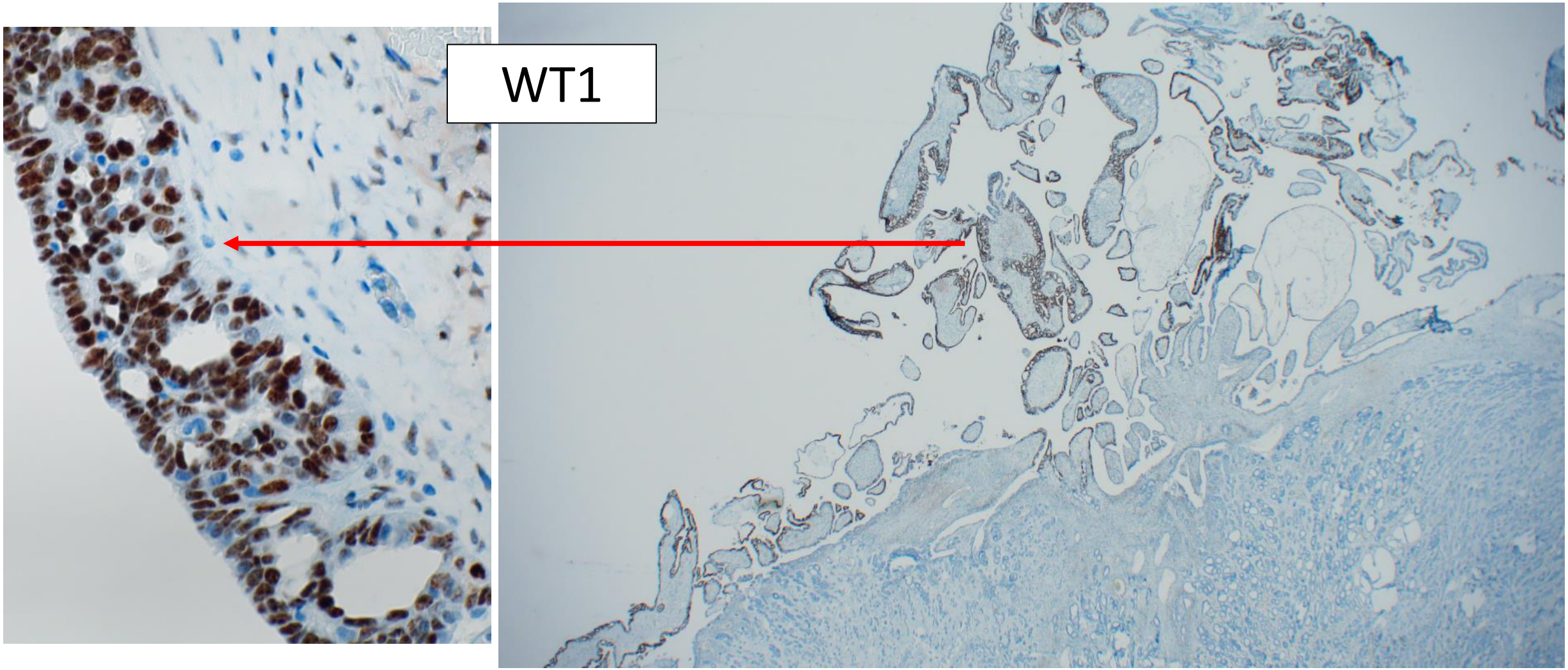
Diagnosis?

1. Low grade serous carcinoma
2. Mixed low grade serous carcinoma and endometrioid carcinoma
3. Mixed low grade serous carcinoma and another type of carcinoma

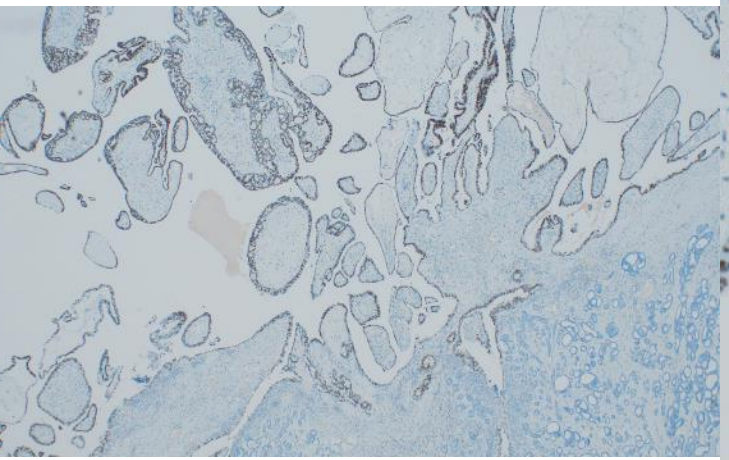
Case 1

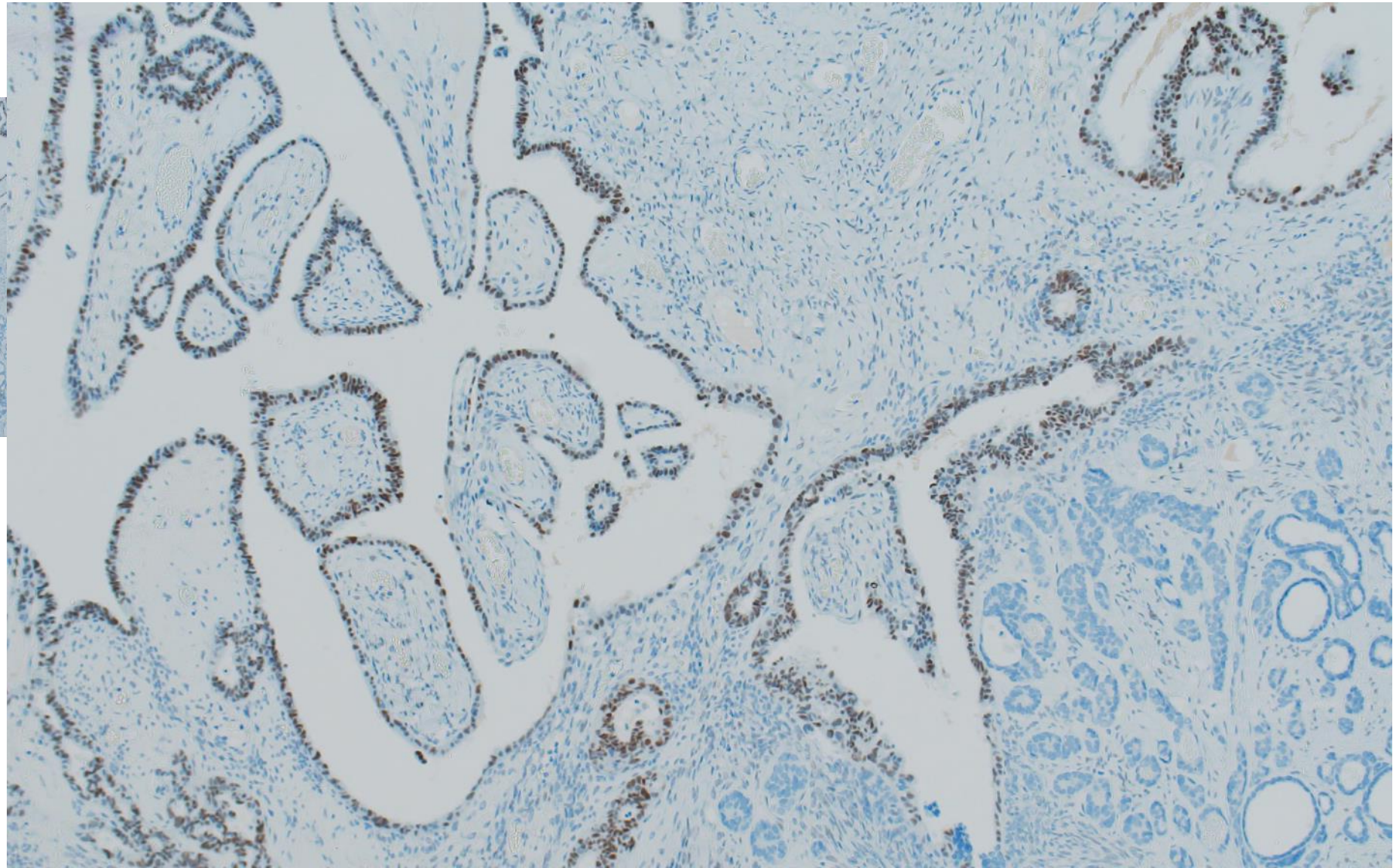
- **71-year old female**
- **Dec 2020:** Probably ovarian cancer with para-aortic lymphadenopathy and peritoneal metastasis. Started Carboplatin & Paclitaxel
- **01.03.2021:** Interval debulking surgery to 5mm residual disease. Pathology: predominantly low grade serous carcinoma with foci of endometrioid adenocarcinoma all consistent with ovarian origin. (local pathologist)
- **25.05.2021:** C6 Carboplatin + Paclitaxel + Bevacizumab --> maintenance Bevacizumab
- **15.07.2021:** CT TAP: progressive disease (platinum-resistant)
- **23.07.2021**: Ca125=40
- **Aug 2021:** Referred to RMH for the consideration of clinical trials

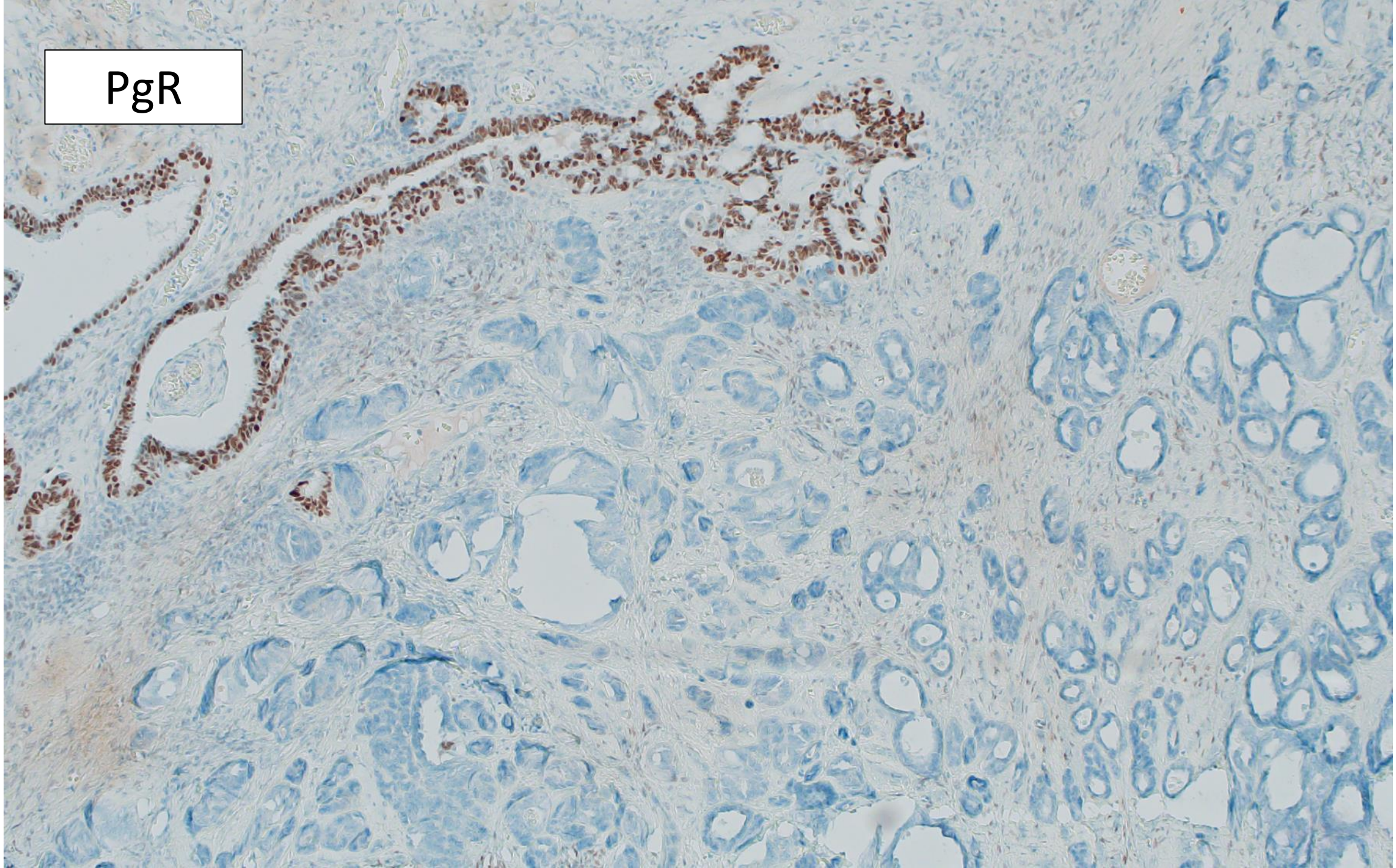

Pax8

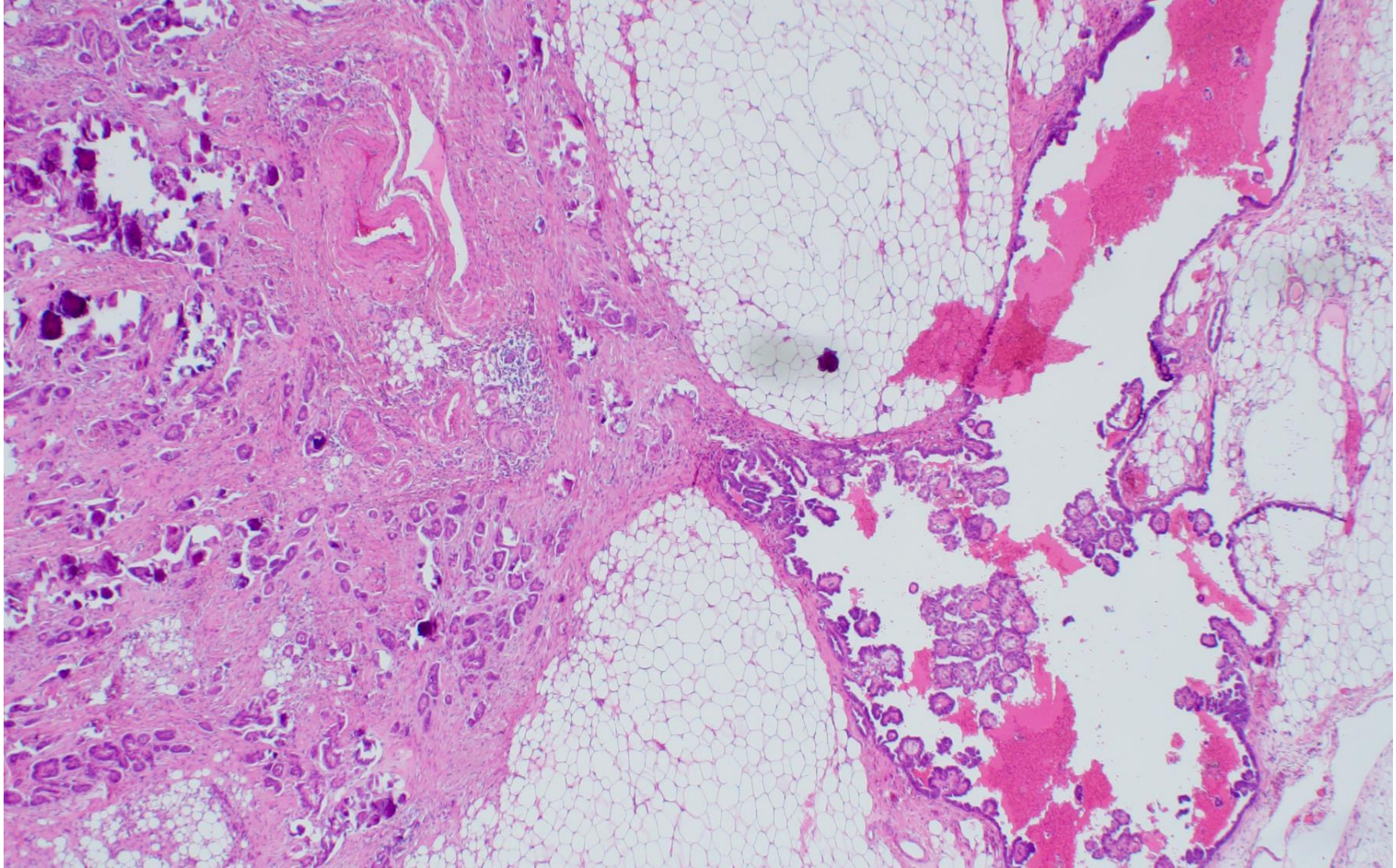

WT1

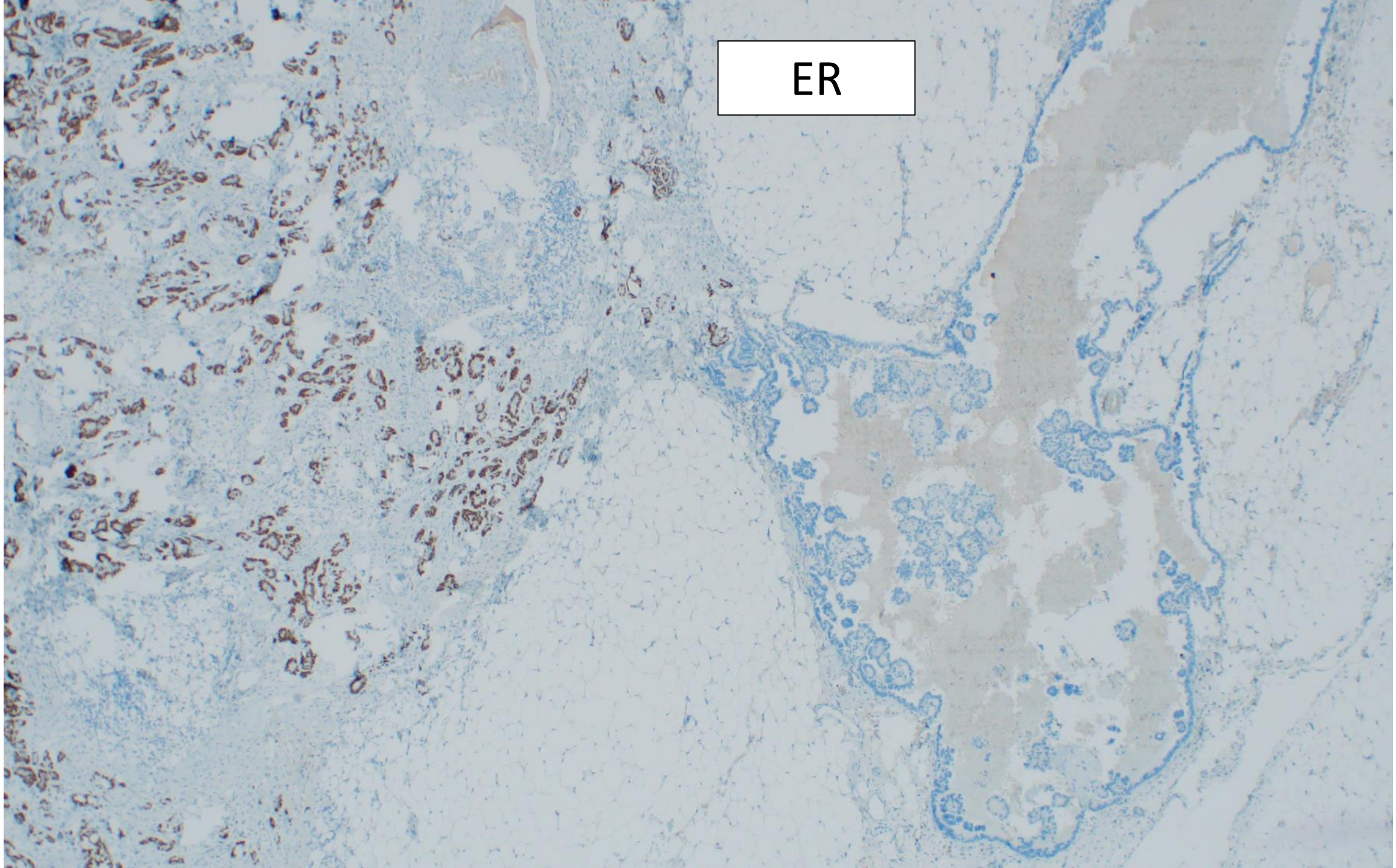
ER

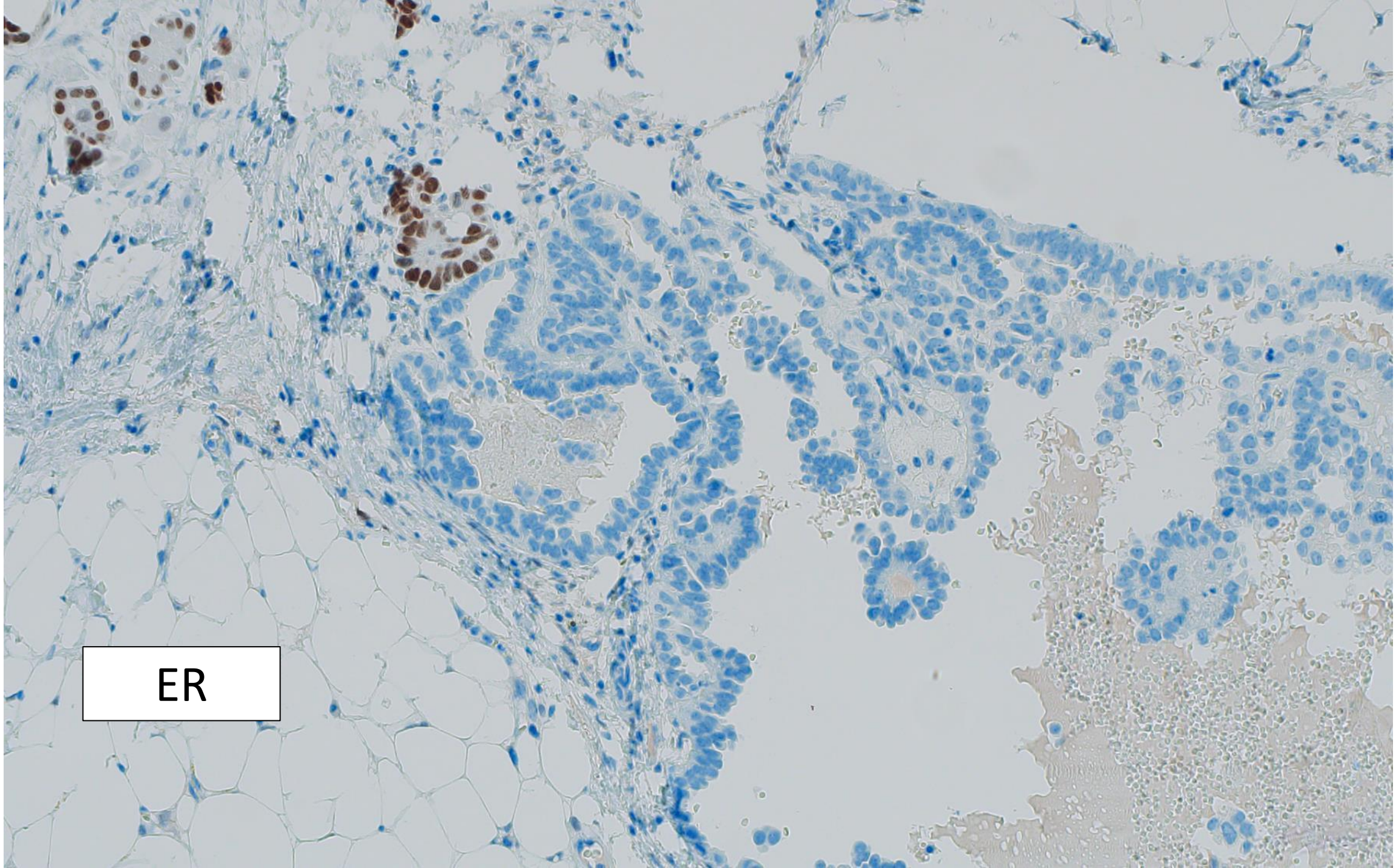


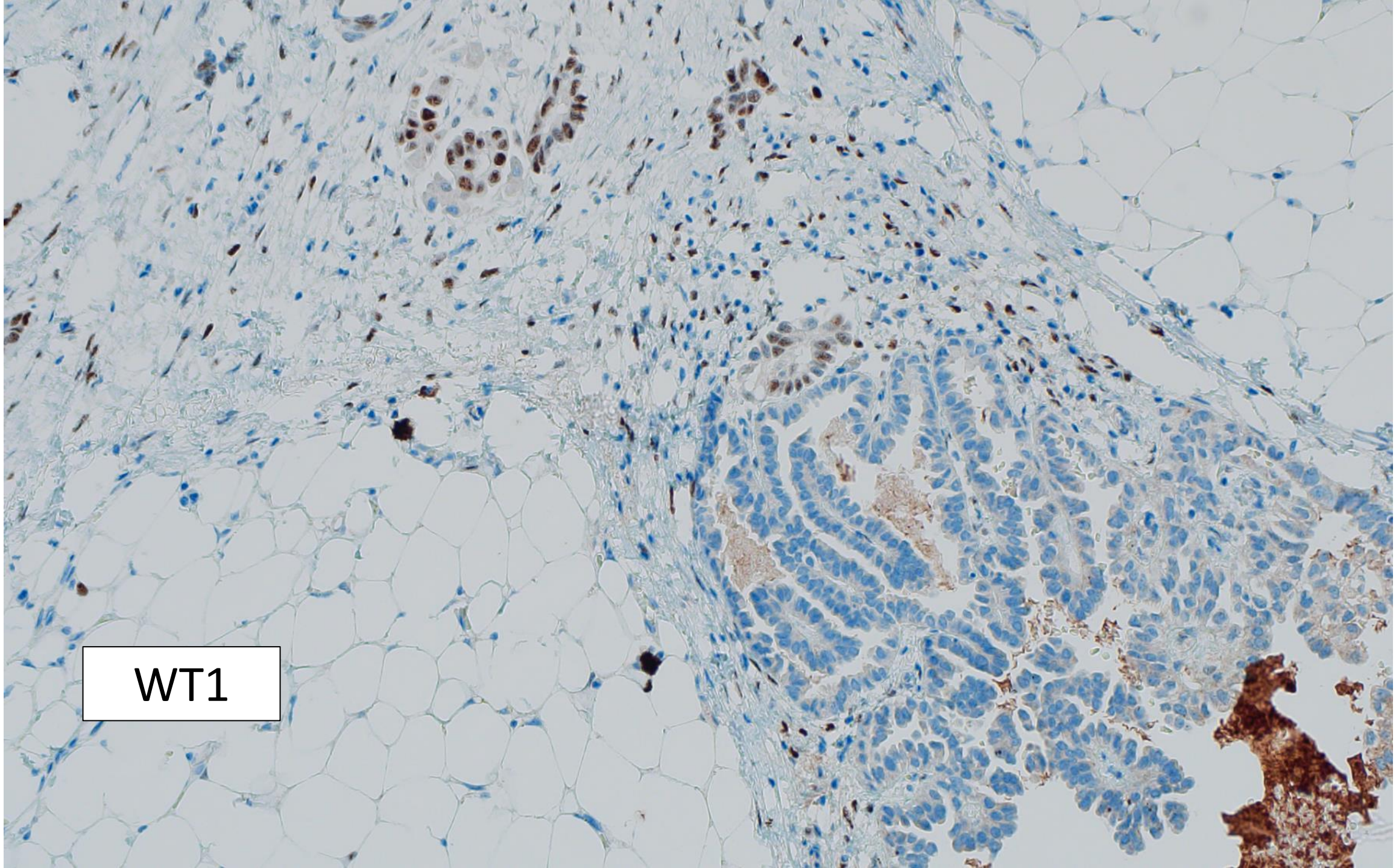


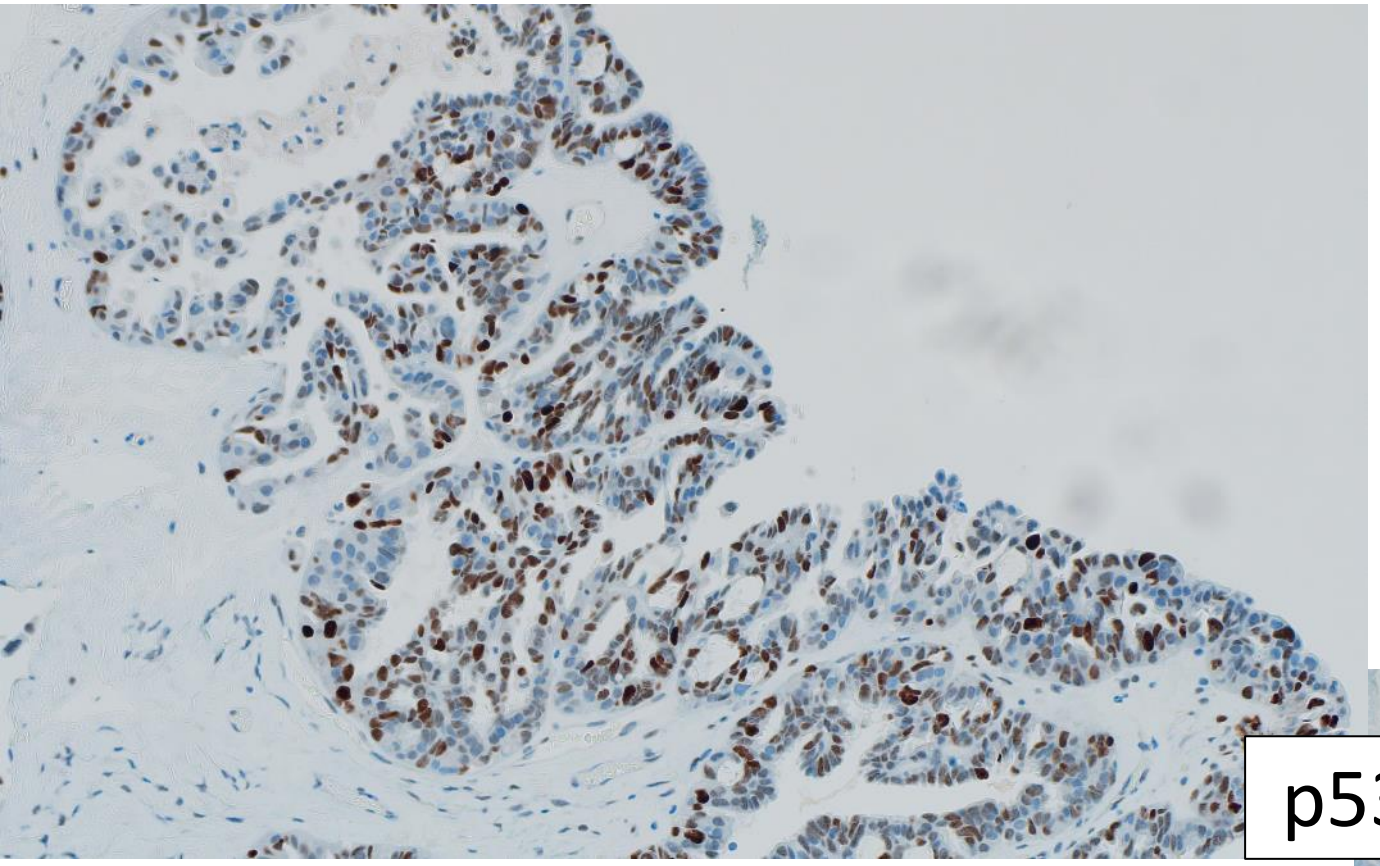

PgR



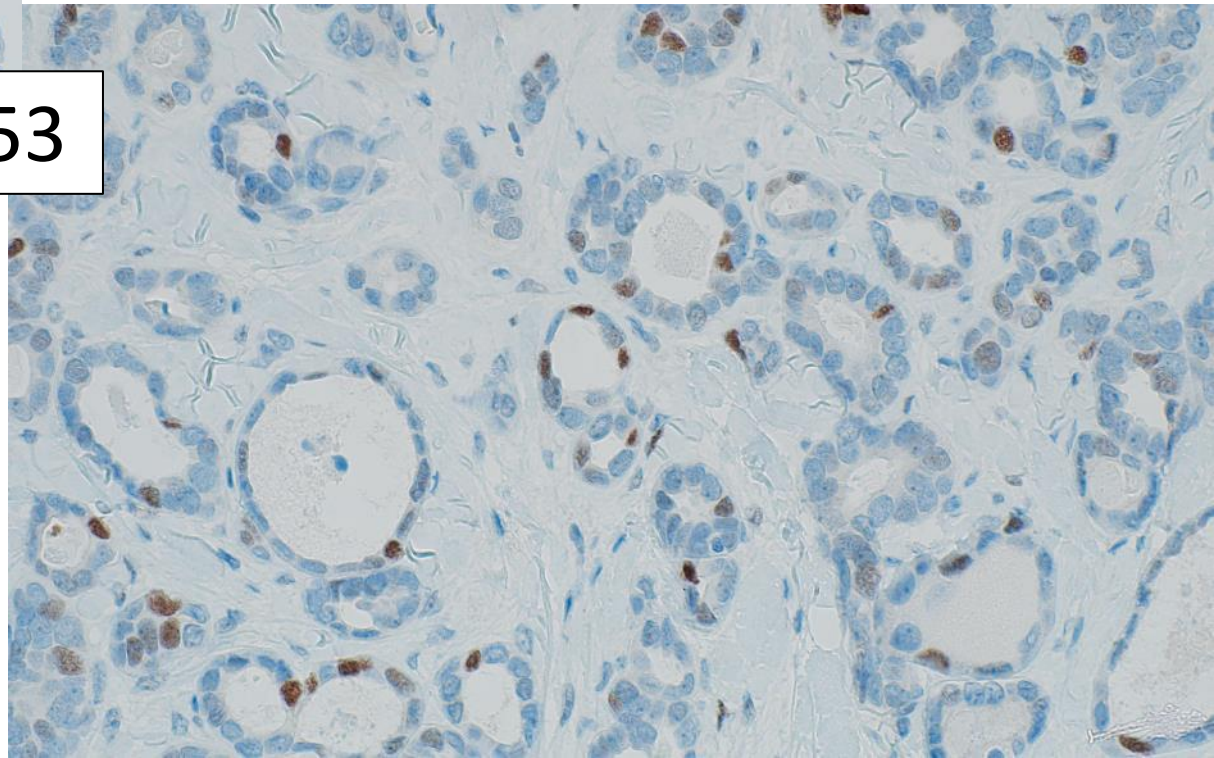

ER

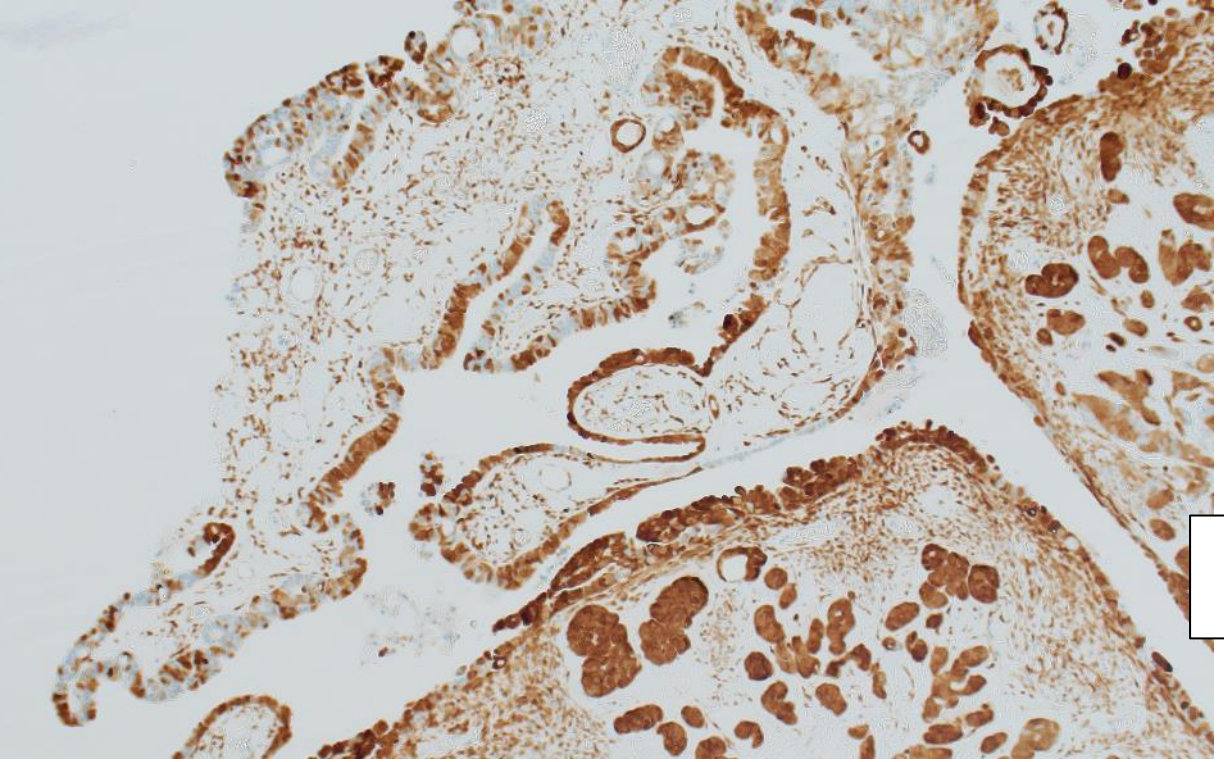

ER


WT1

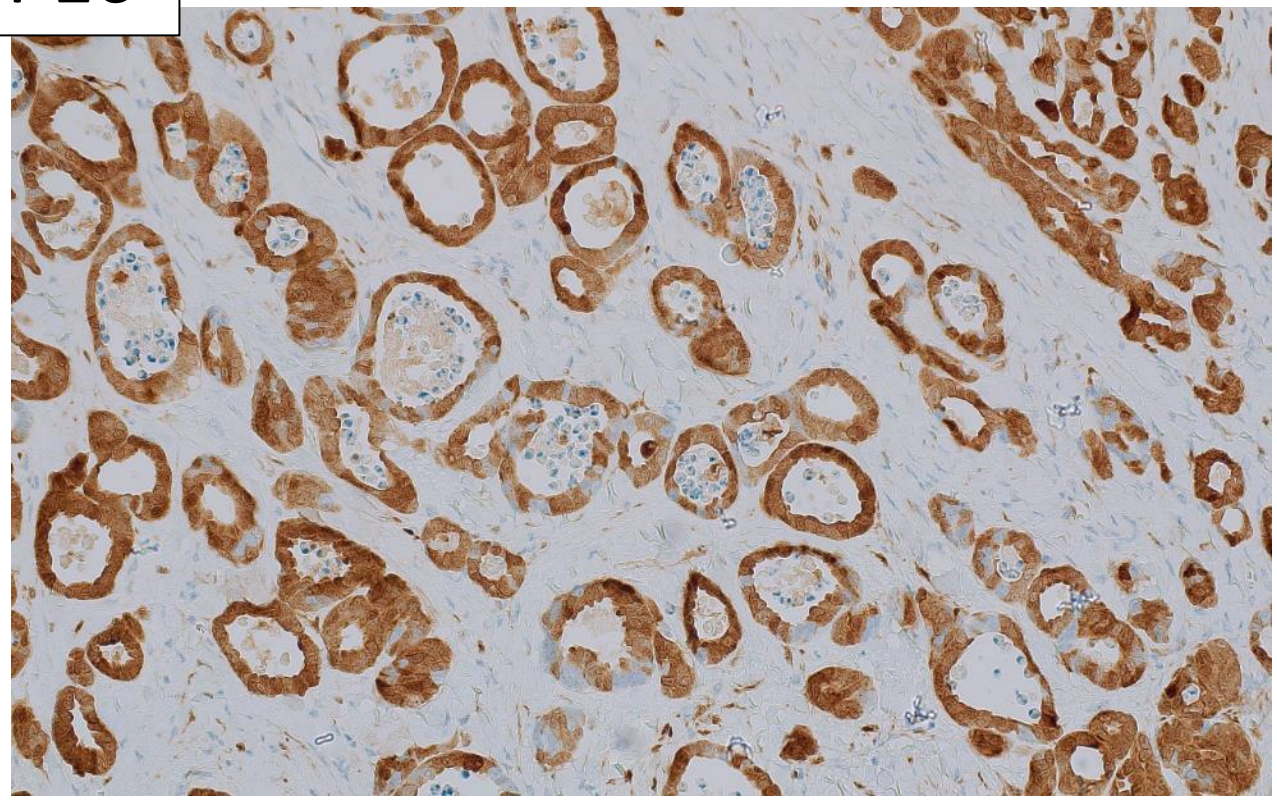


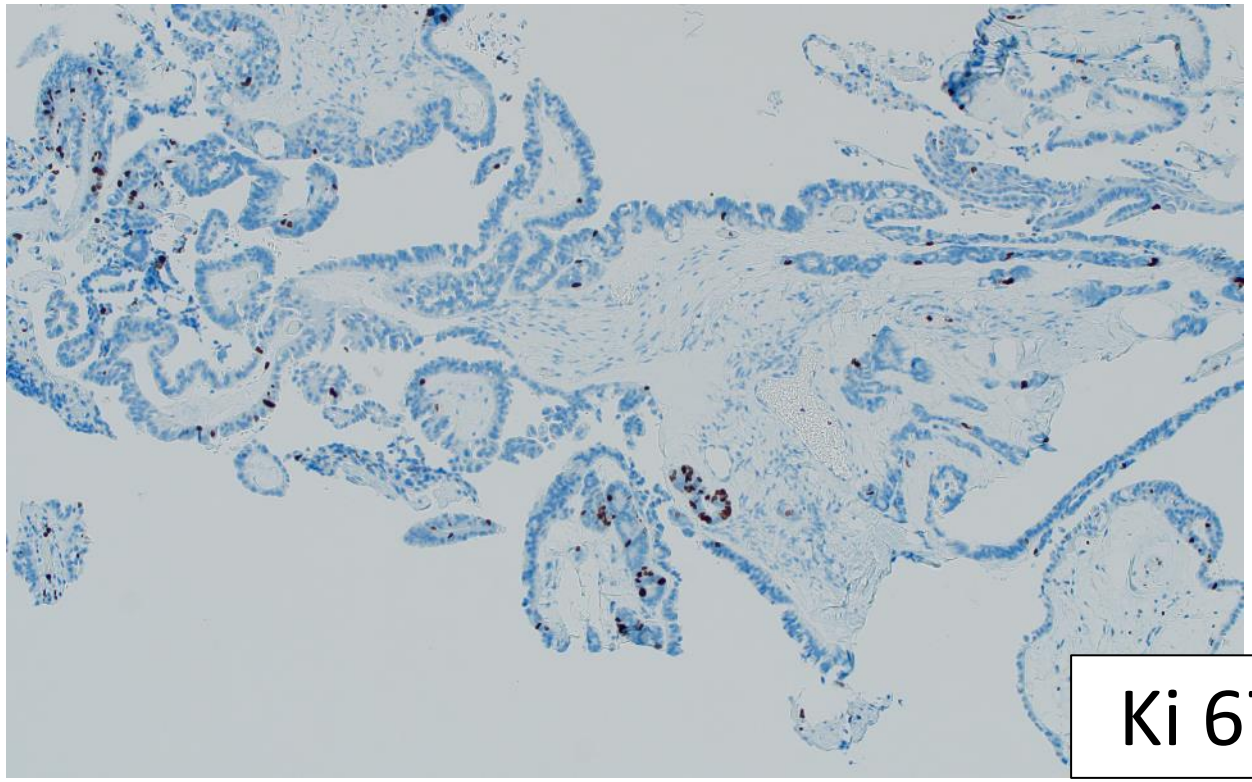
p53



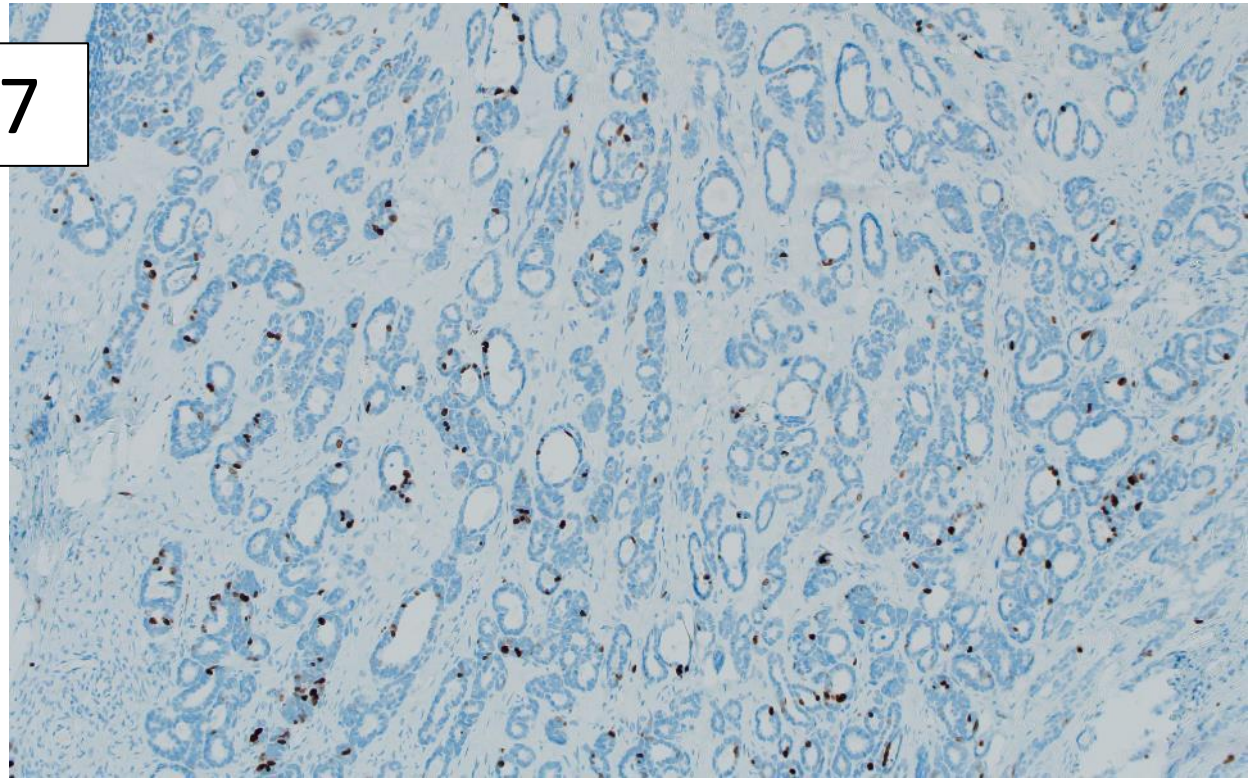


P16





Ki 67



Poll question 2

Diagnosis?

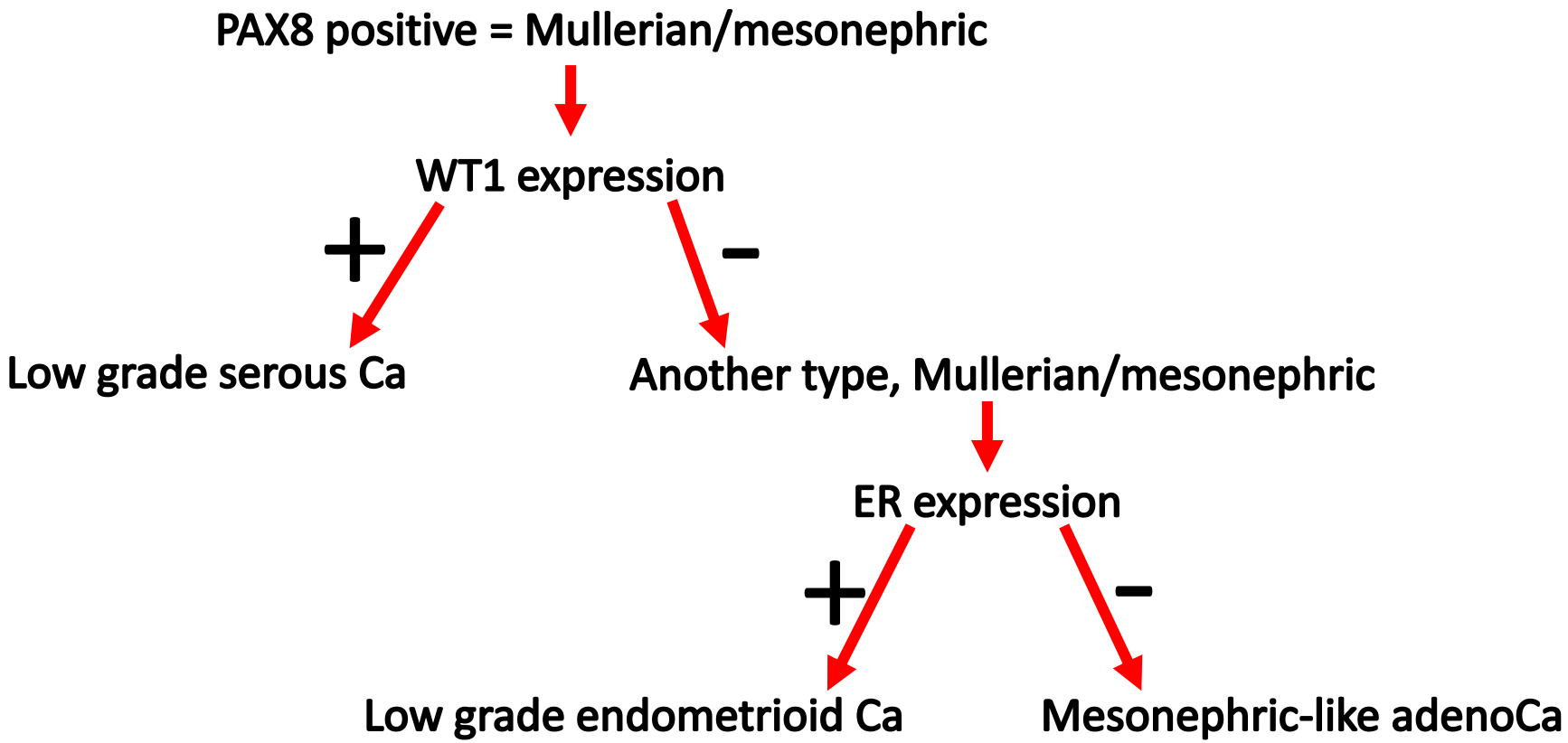
1. Low grade serous carcinoma
2. Mixed low grade serous carcinoma and endometrioid carcinoma
3. Mixed low grade serous carcinoma and another type of carcinoma

Diagnosis?

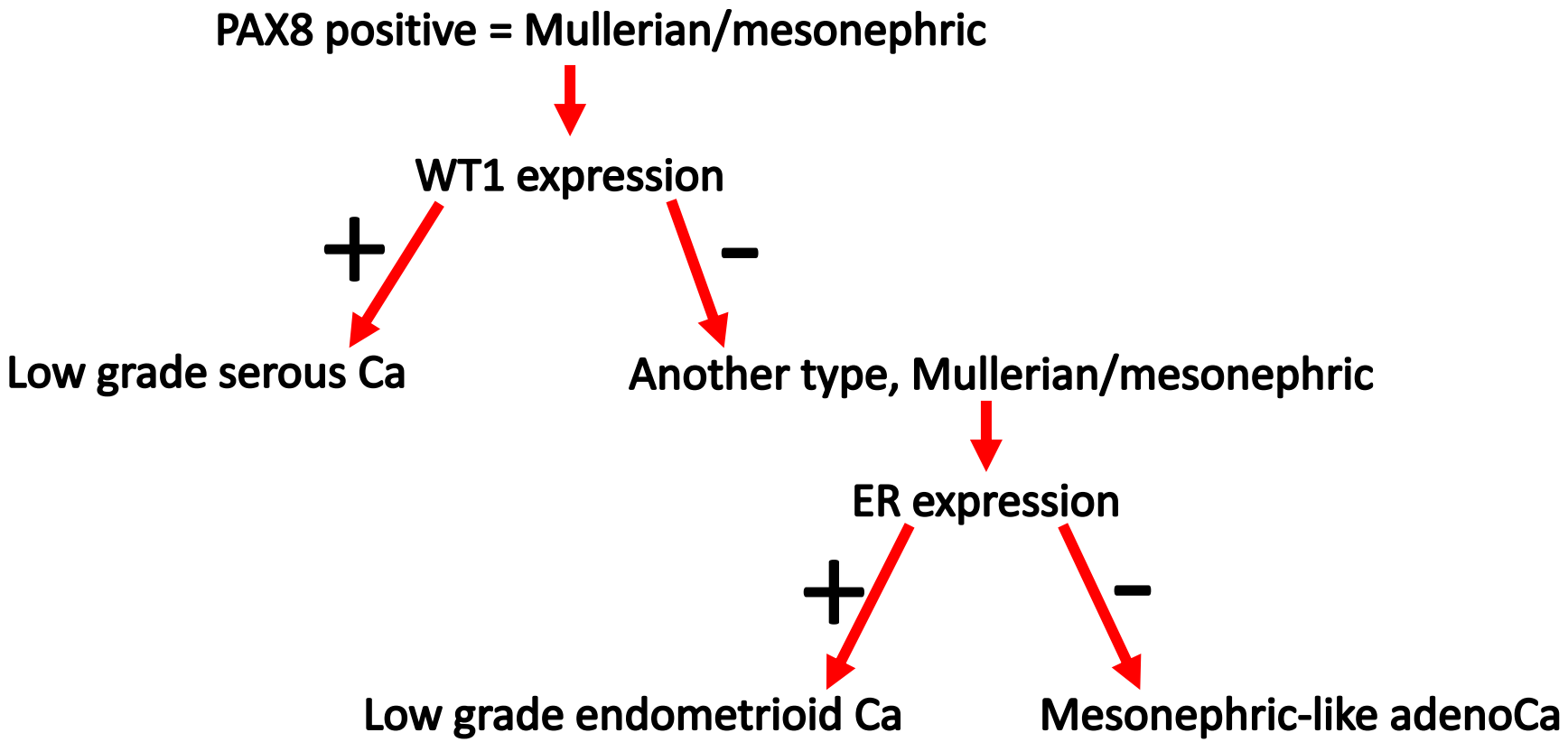
1. Low grade serous carcinoma
2. Mixed low grade serous carcinoma and endometrioid carcinoma
3. Mixed low grade serous carcinoma and another type of carcinoma

Those of you who voted (3) please use chat function to provide your answer for what you think ‘another type’ is.

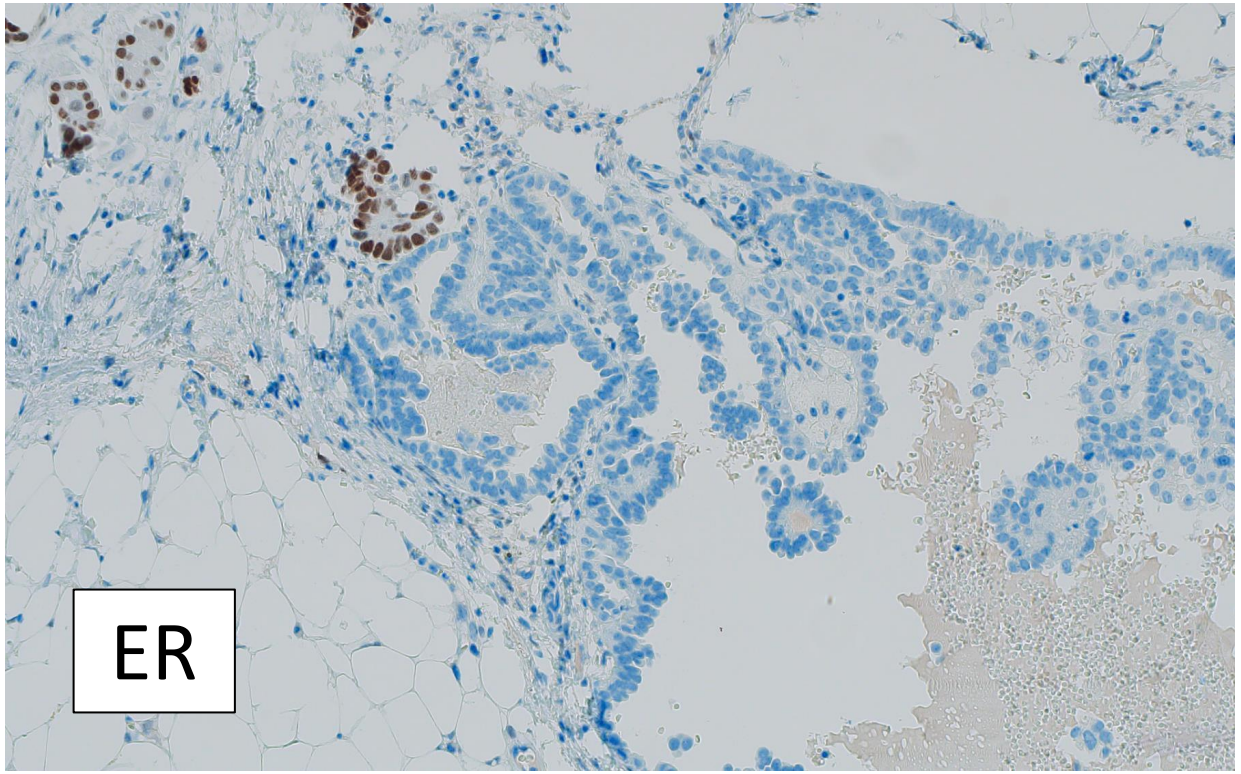
Immunohistochemical clues (always correlate with morphology)

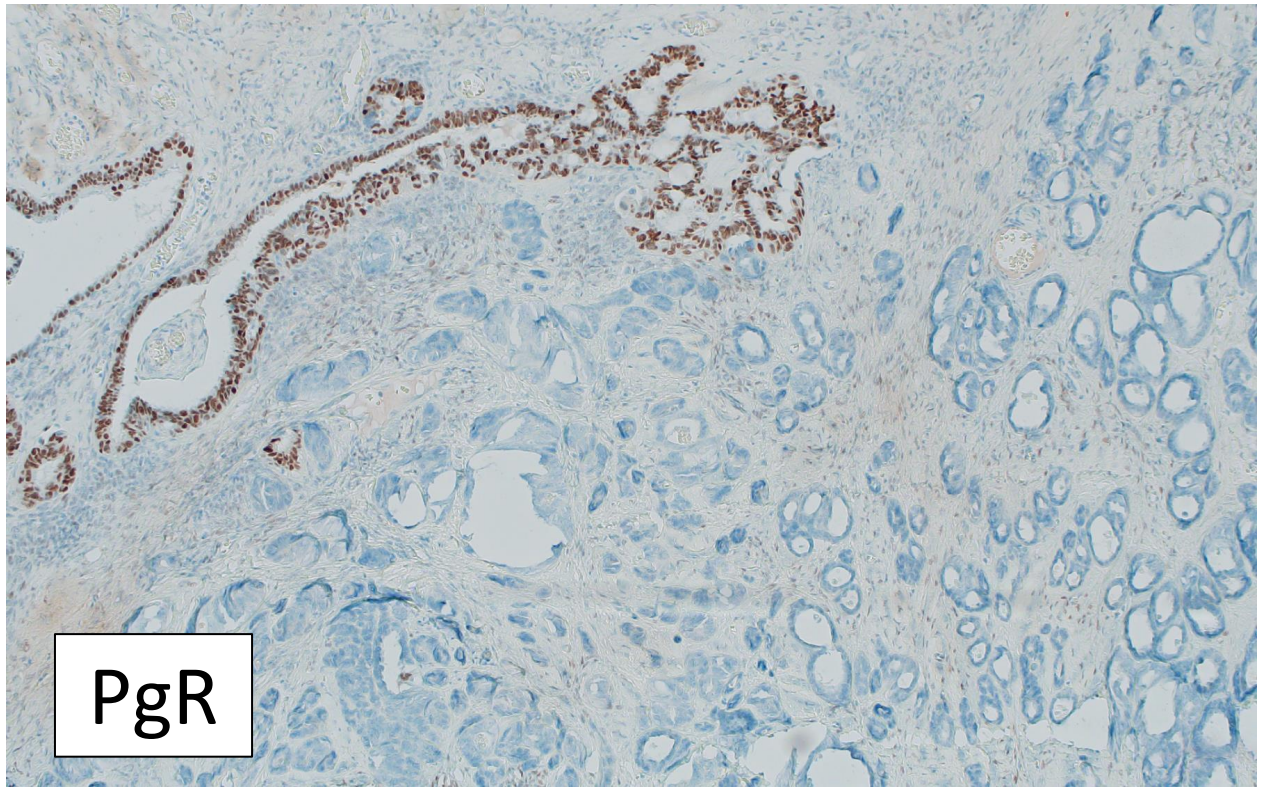


Immunohistochemical clues (always correlate with morphology)



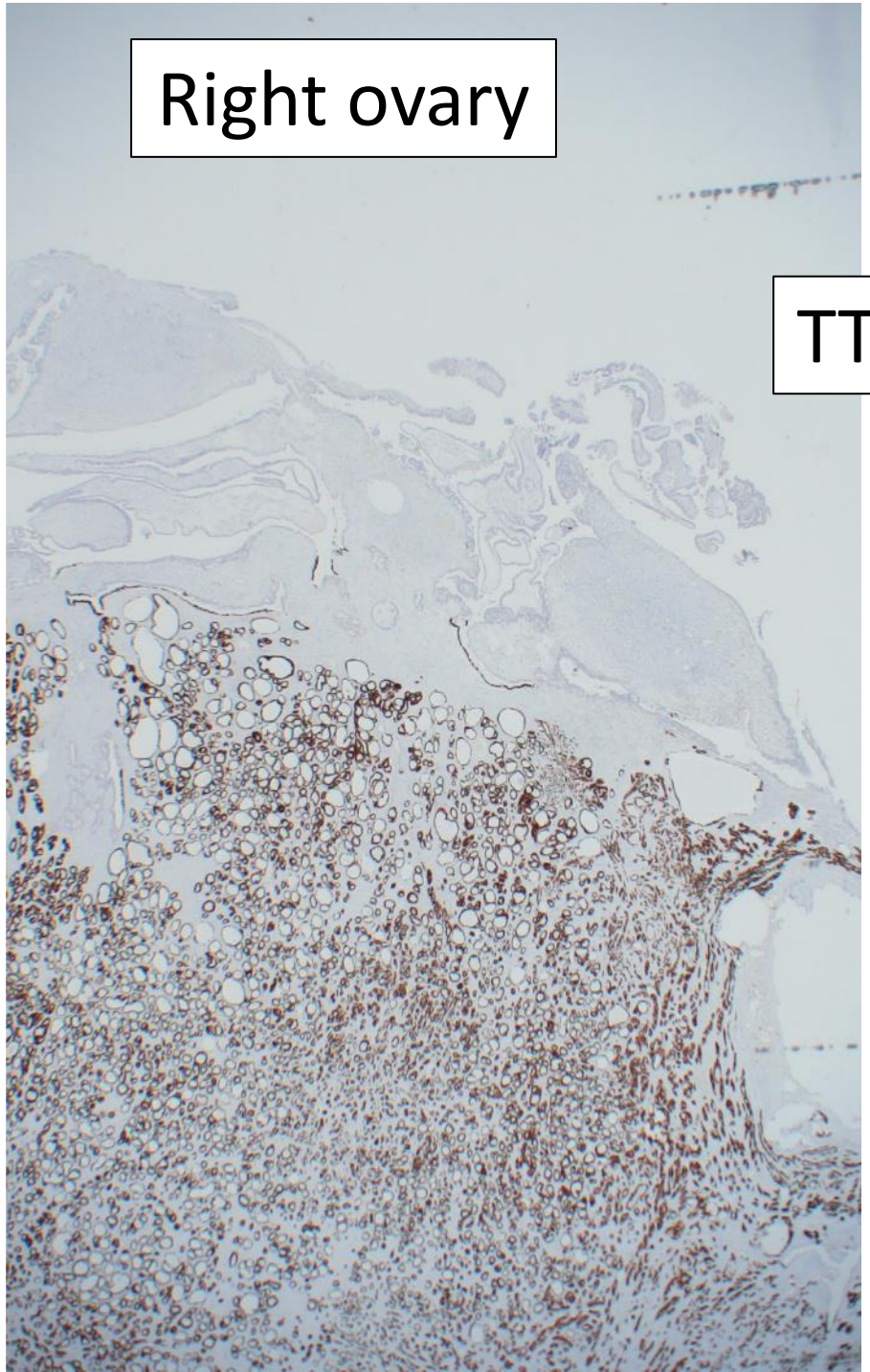
PAX8+, ER neg : clear cell carcinoma – morphology does not fit!
high grade serous carcinoma (typically p53 mutant pattern,WT1 and ER often positive, but occasionally negative), but morphology does not fit!!



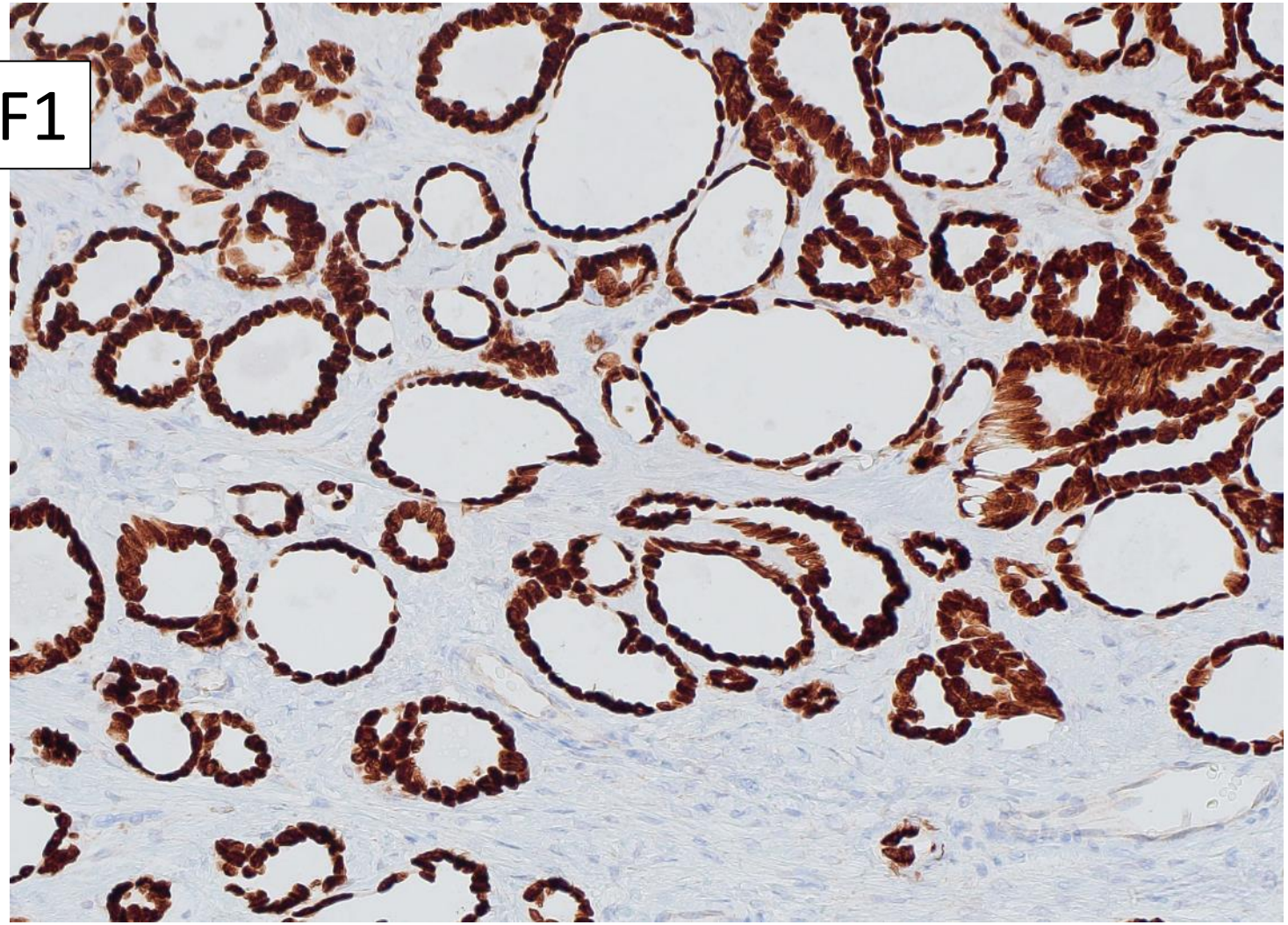


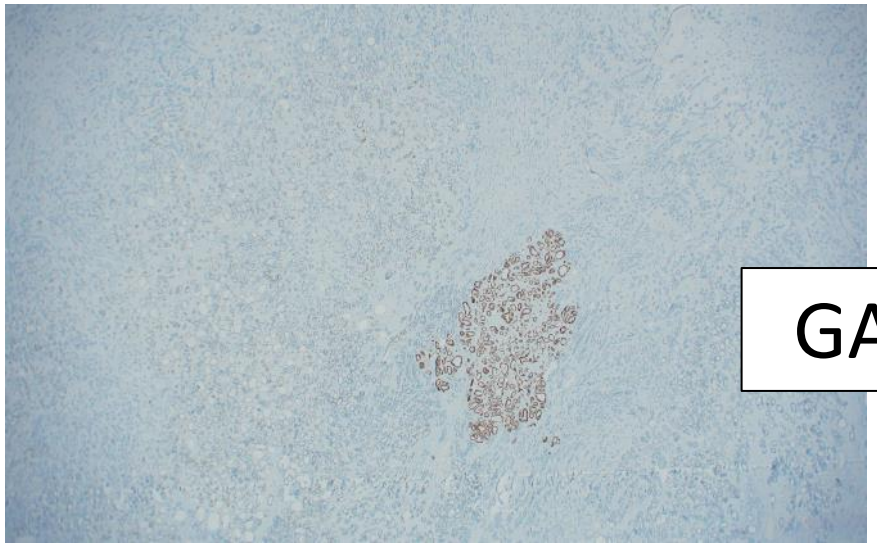
Most important clues: morphology and **flat negative ER and PgR**

Right ovary

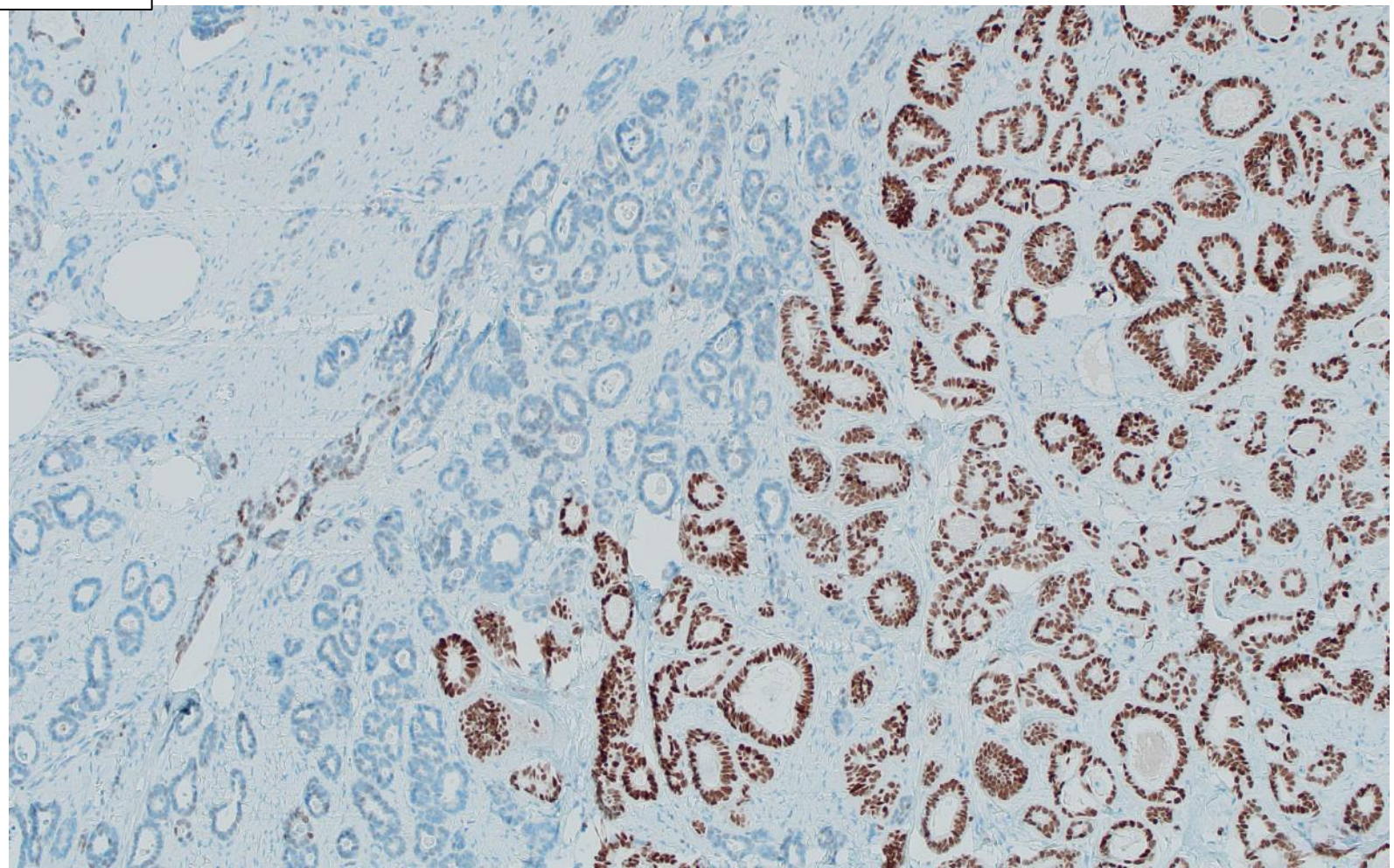


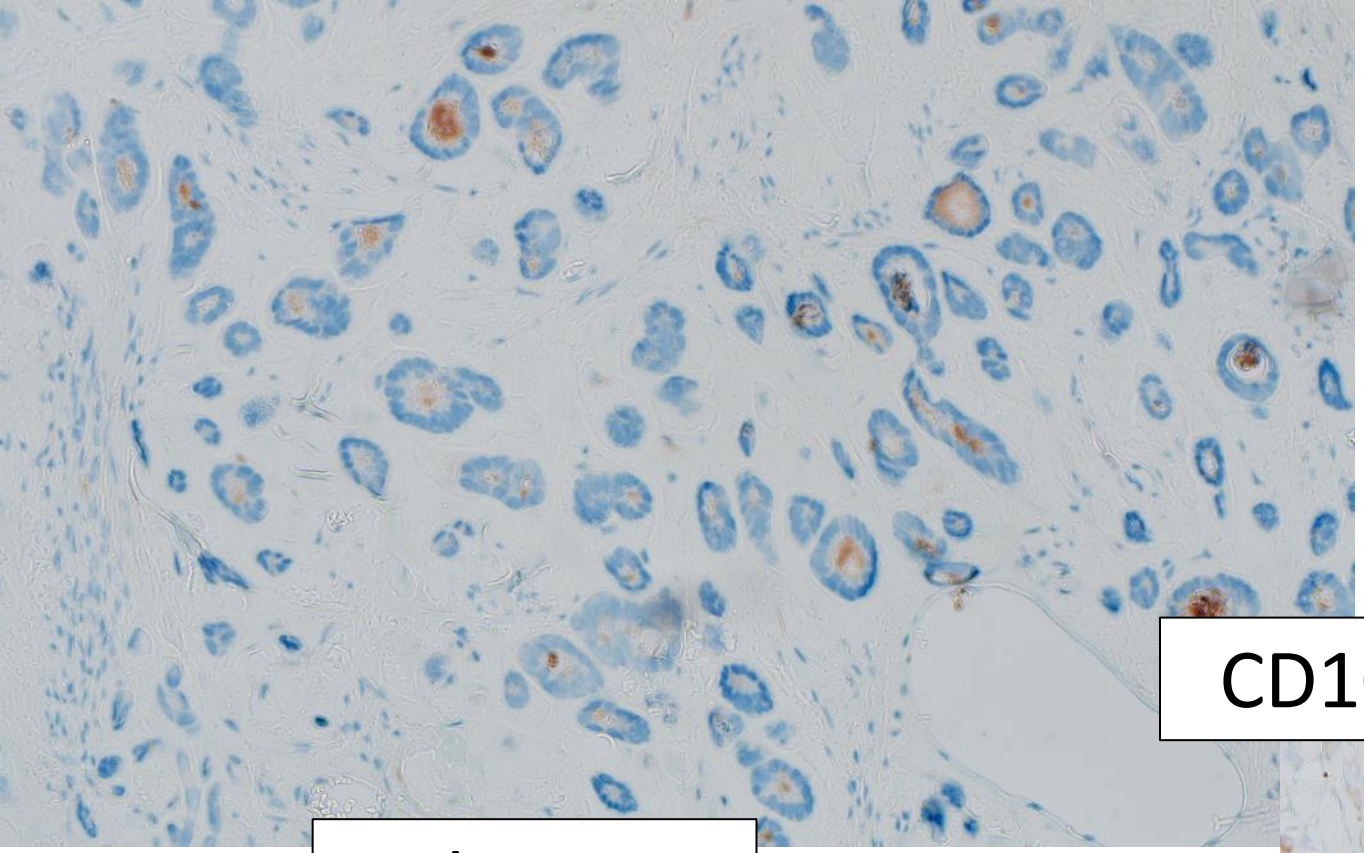
TTF1





GATA3

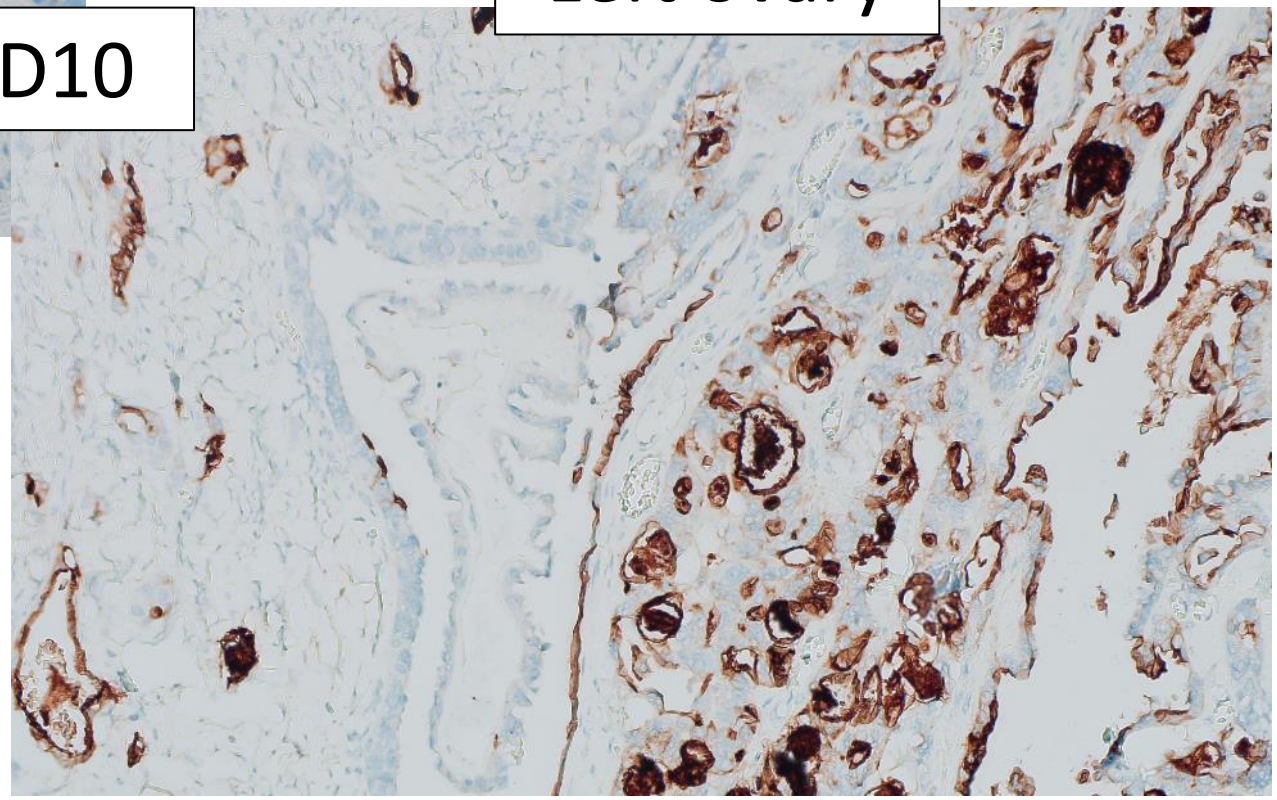




Right ovary

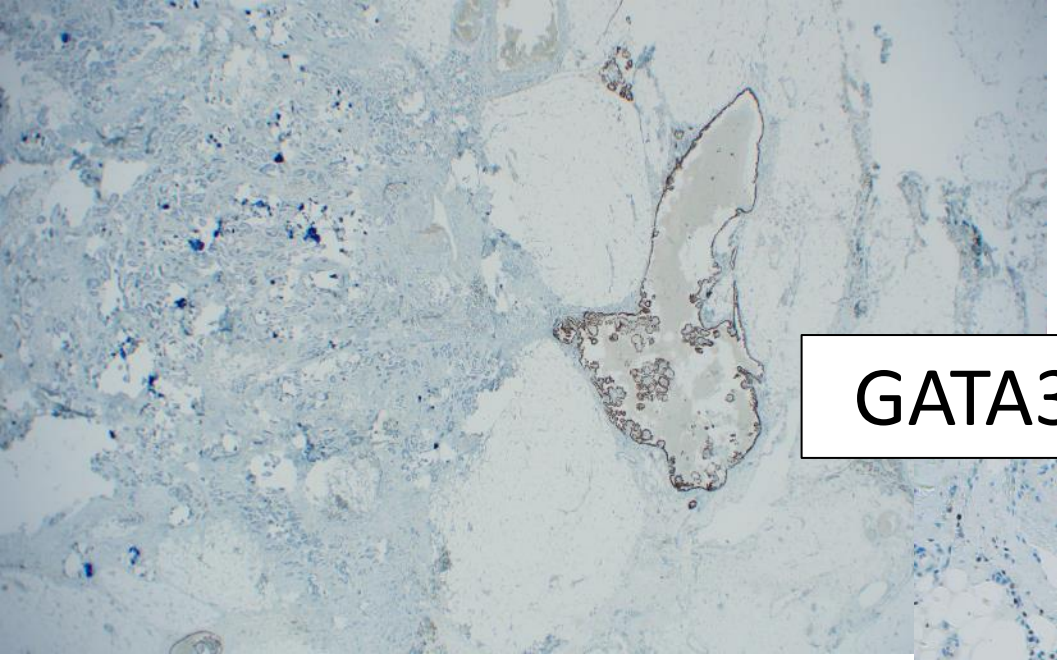
CD10

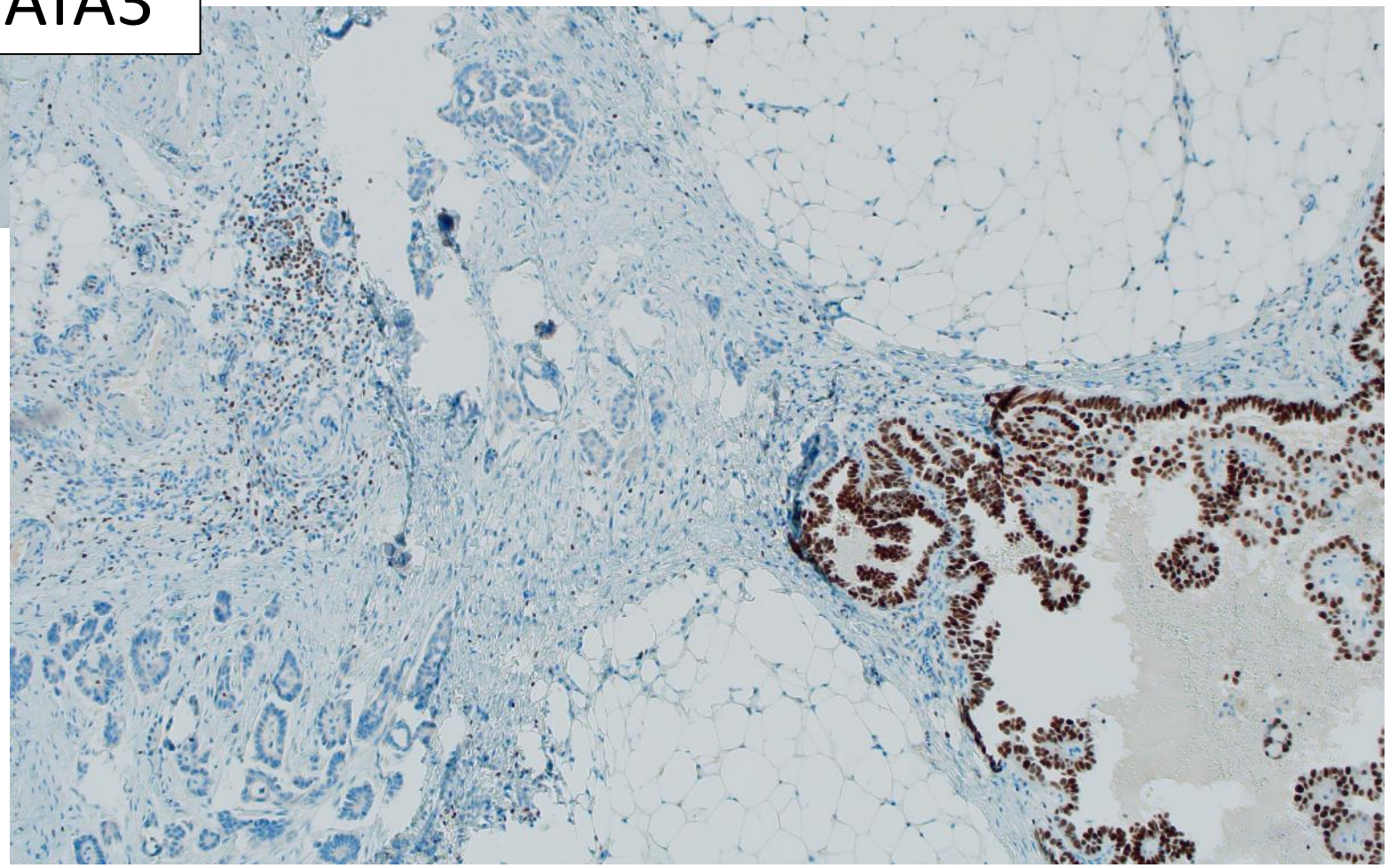
Left ovary

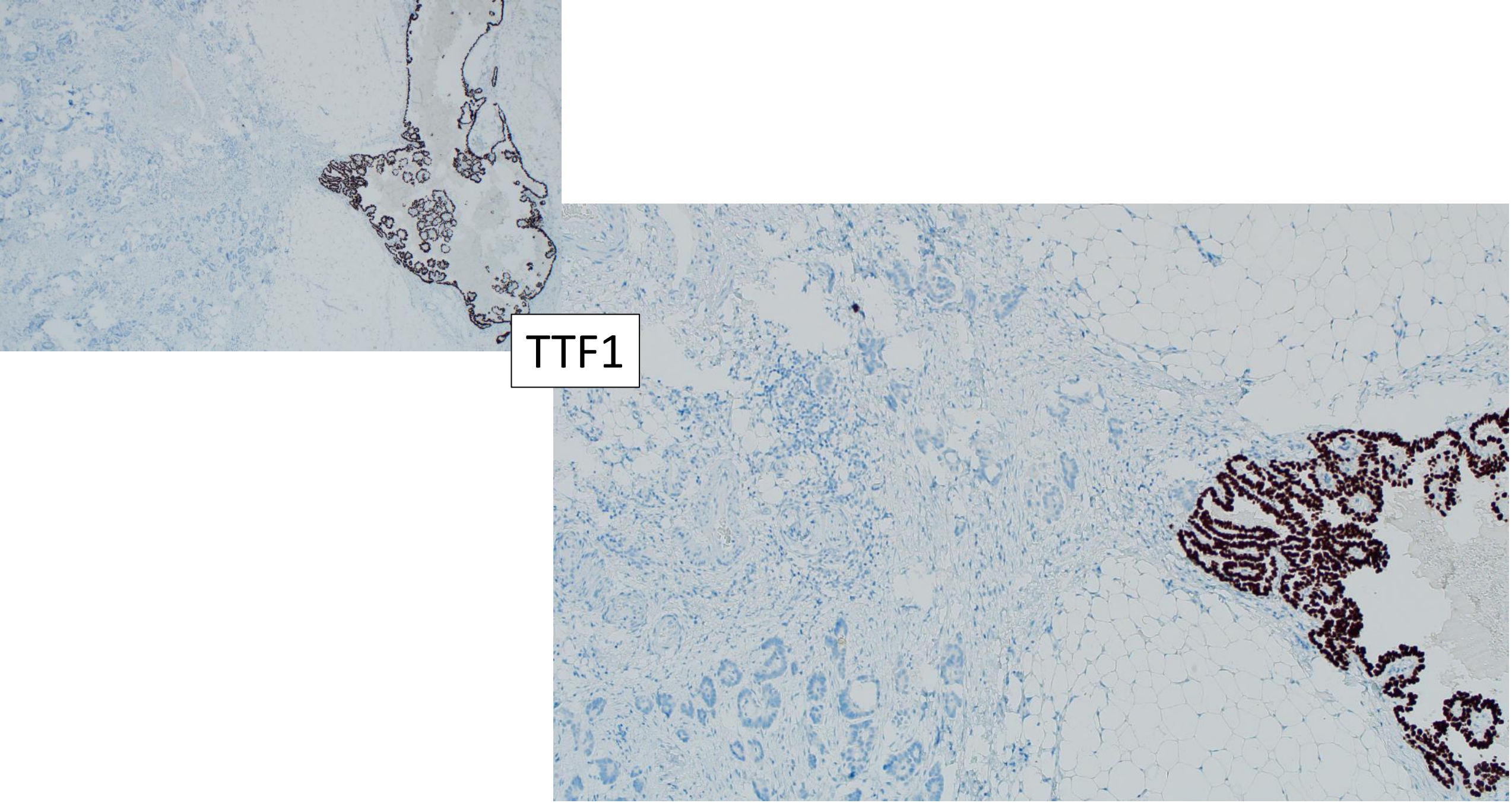


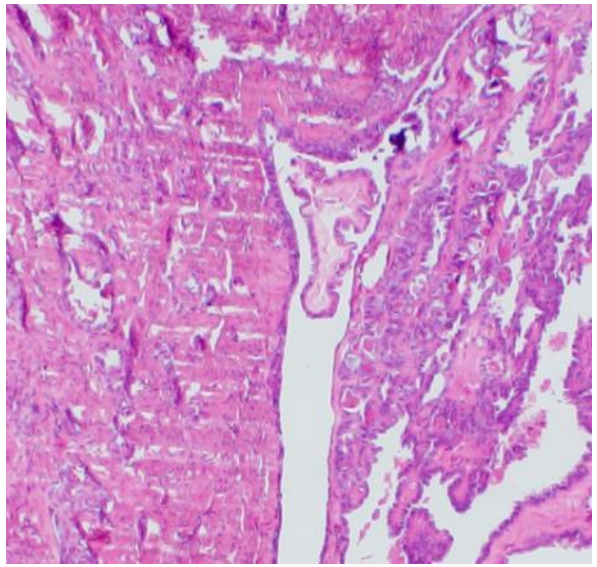
Omentum

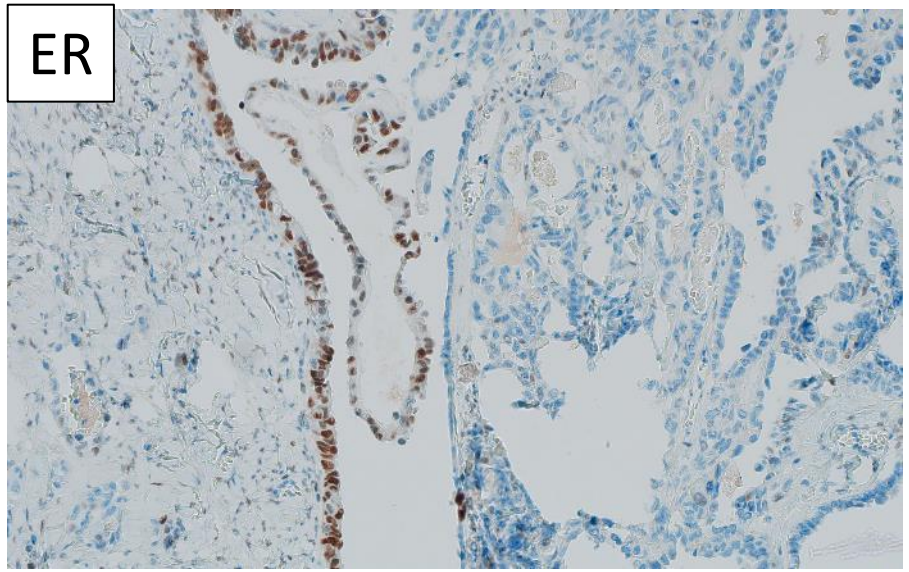
GATA3

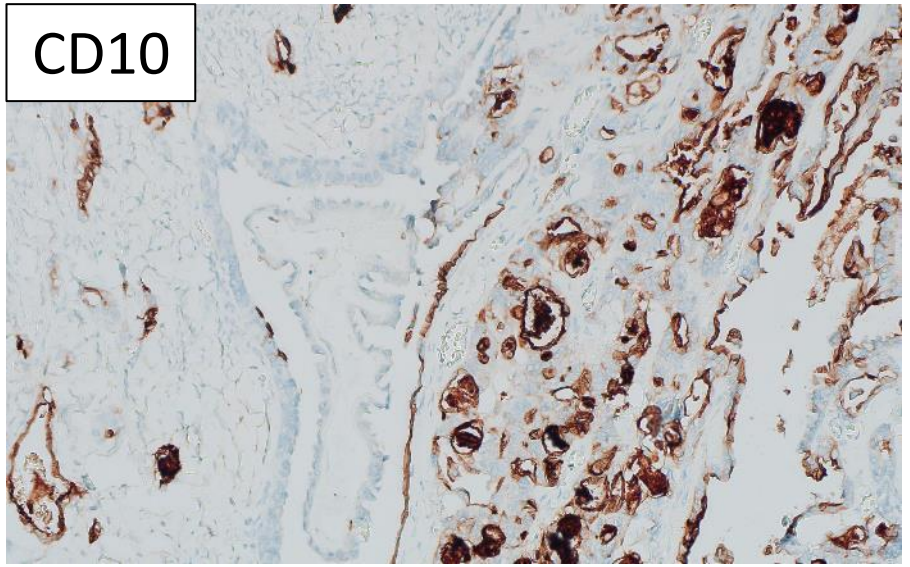


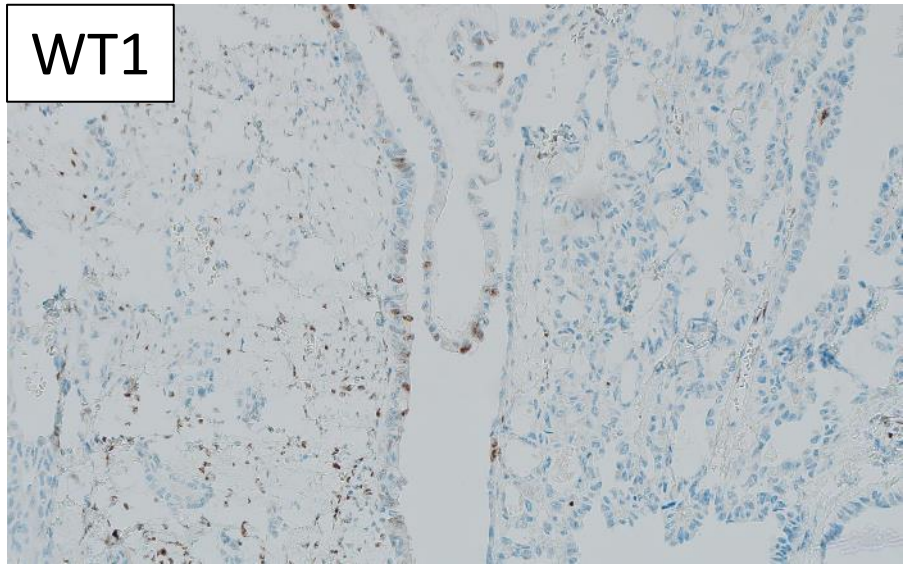


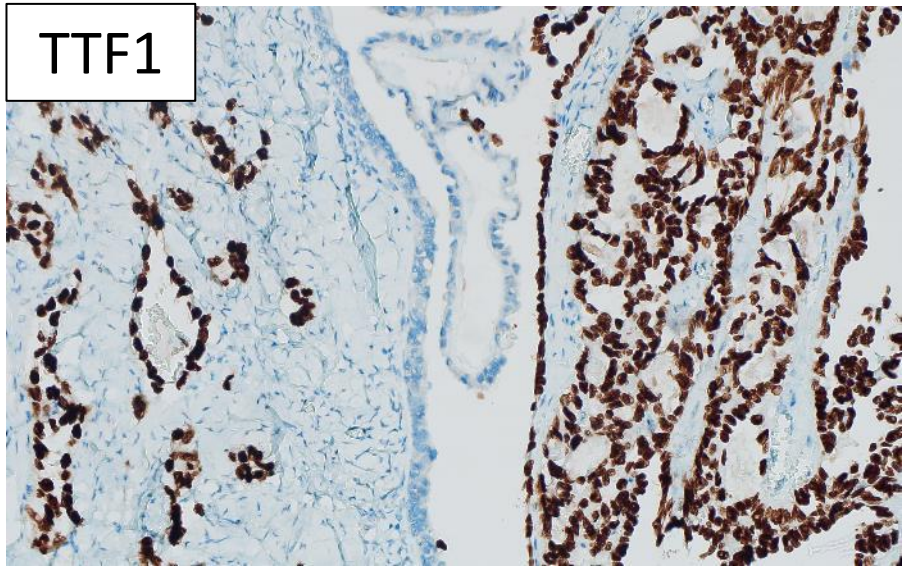

TTF1











Left ovary

WHO criteria: mesonephric-like adenocarcinoma

Essential: histological features prototypical for mesonephric carcinoma

Desirable: positivity for GATA3 or TTF1; negativity for ER and PgR

WHO criteria: HPV-independent adenocarcinoma mesonephric-type, of uterine cervix

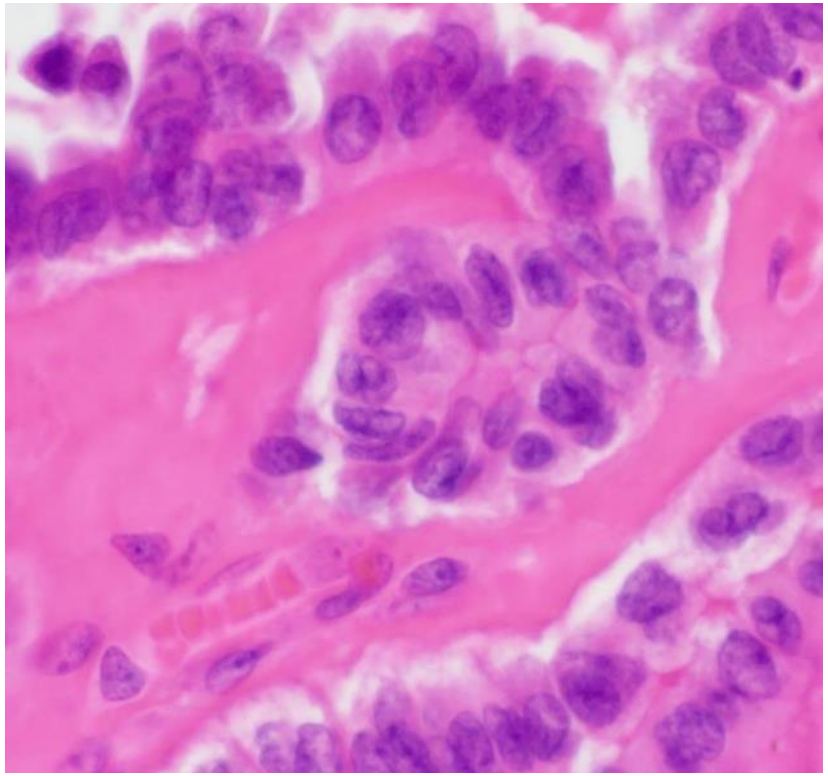
Essential: an admixture of architectural patterns, optically clear nuclei with grooves; p16 not diffuse

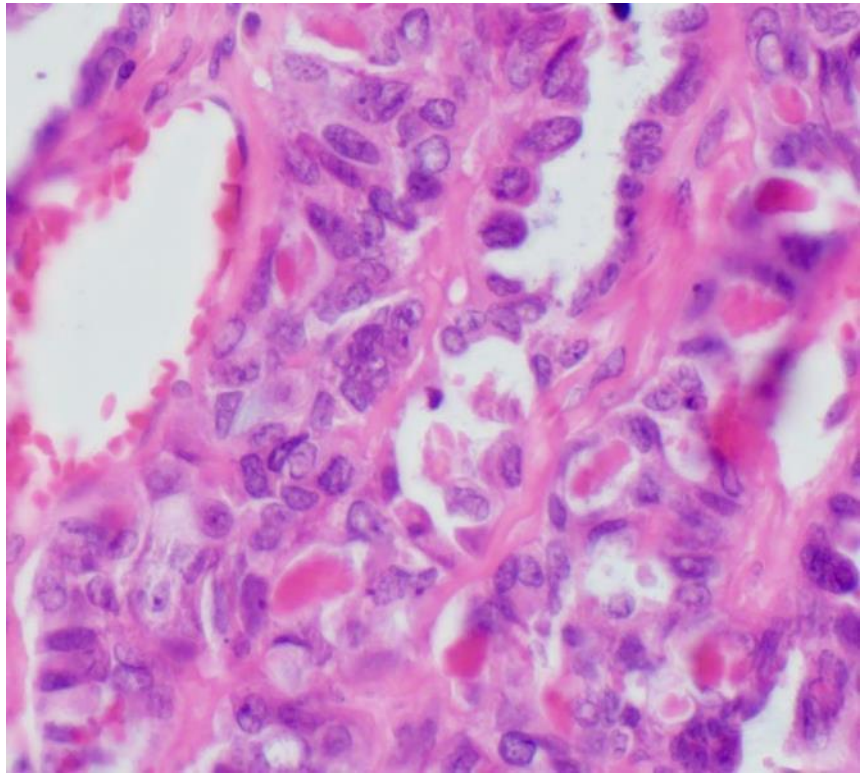
Patterns:

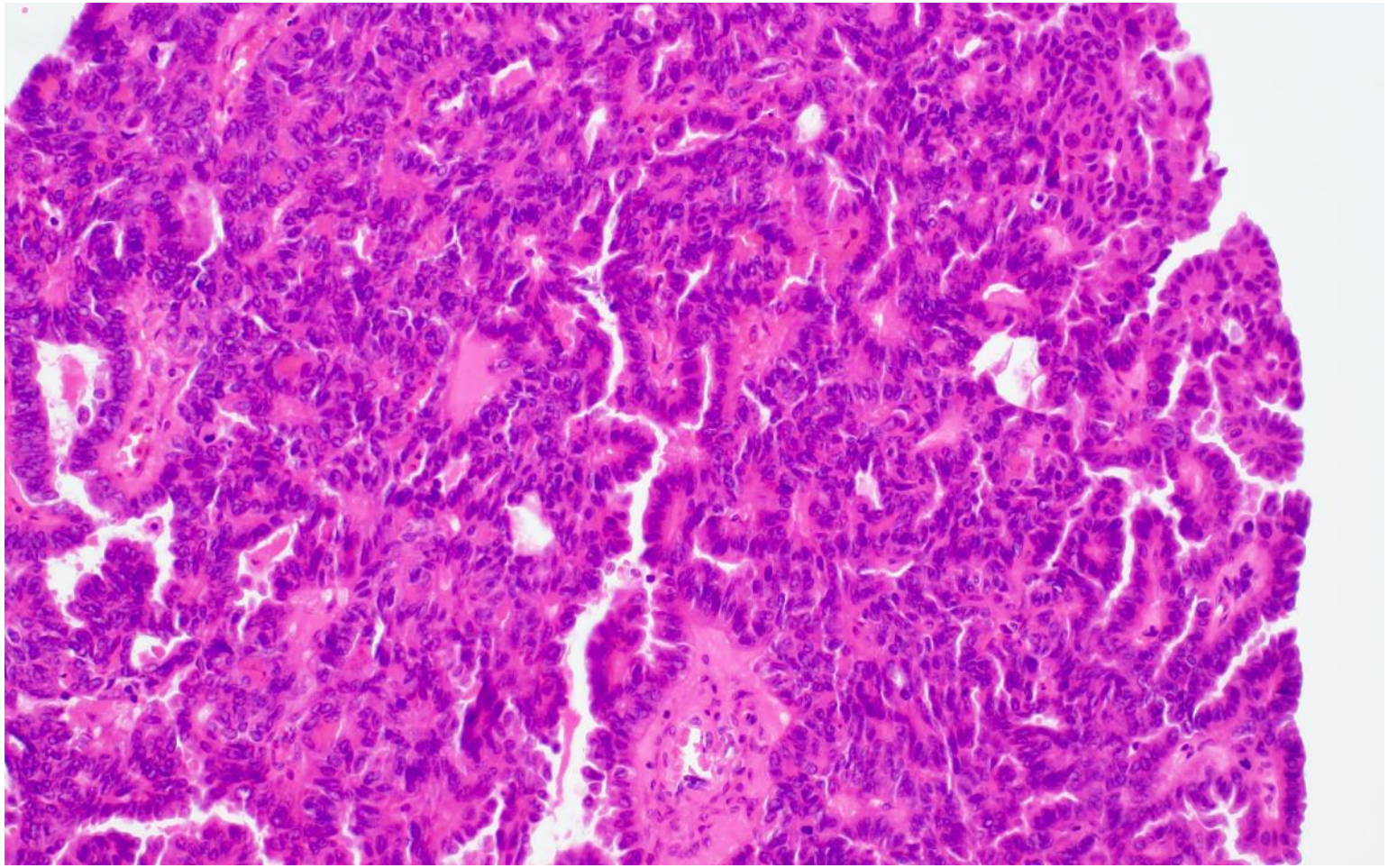
- back to back tubules filled with dense eosinophilic material
- pseudoendometrioid pattern –angulated glands and columnar cells
- papillary, retiform, sex cord-like, hobnail, glomeruloid, sieve-like, spindled and solid

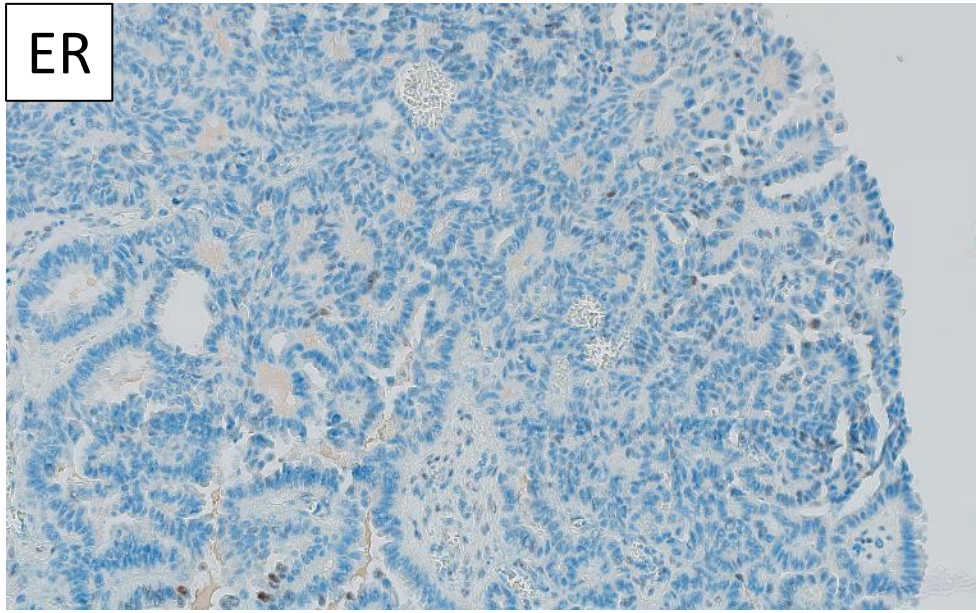
WHO criteria: mesonephric-like carcinoma

Essential: histological features prototypical for mesonephric carcinoma








ER

Case 1
Diagnosis

Mixed

Low grade serous carcinoma

and

Mesonephric-like adenocarcinoma

Hypotheses regarding pathogenesis of Mesonephric-like adenocarcinoma in ovary

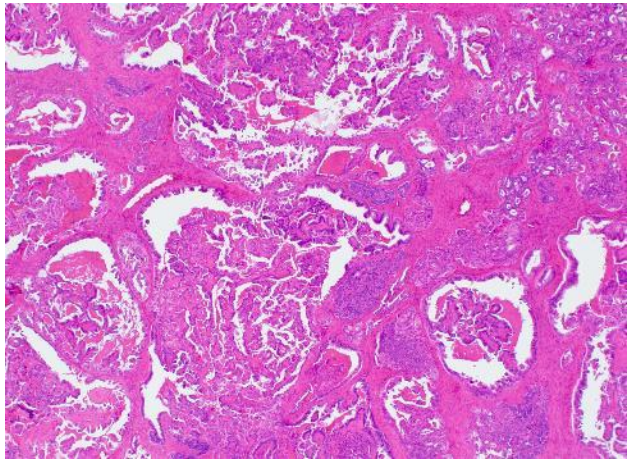
1. Arise from Mullerian carcinomas showing secondary mesonephric transdifferentiation (hence mesonephric-like rather than mesonephric)

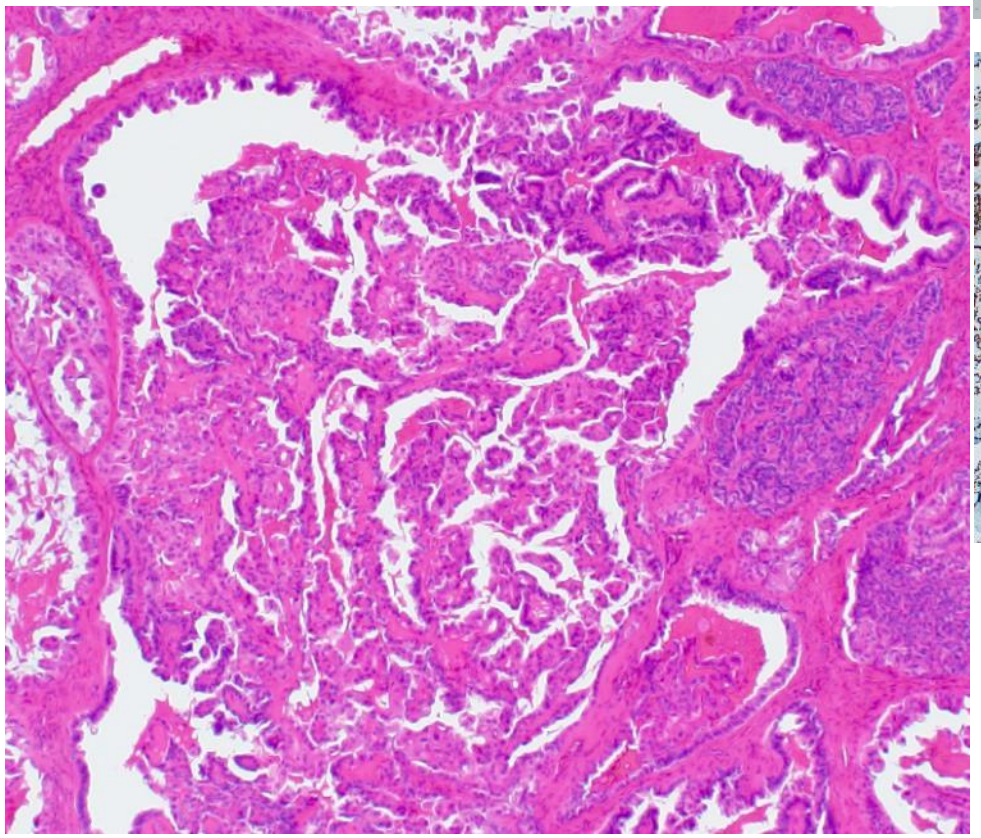
- may explain the association of mesonephric-like carcinomas with low grade serous carcinoma/borderline serous tumours

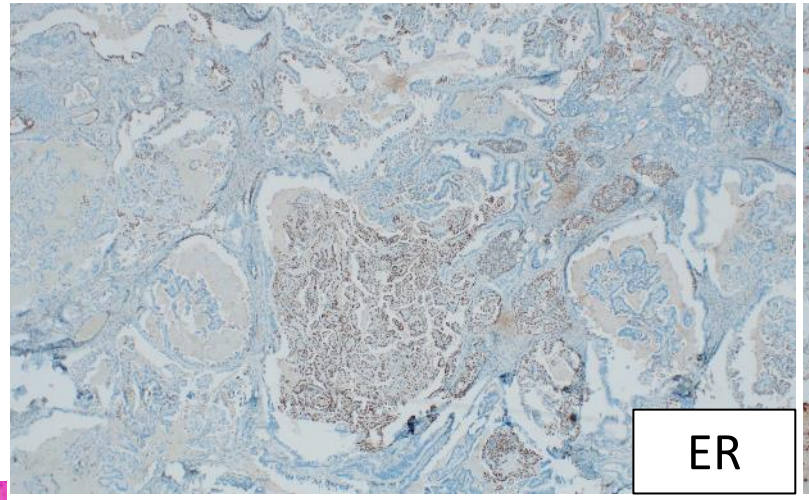
- both components harbour shared *KRAS/NRAS* mutations *(McCluggage et al 2020, Chapel et al 2018)*

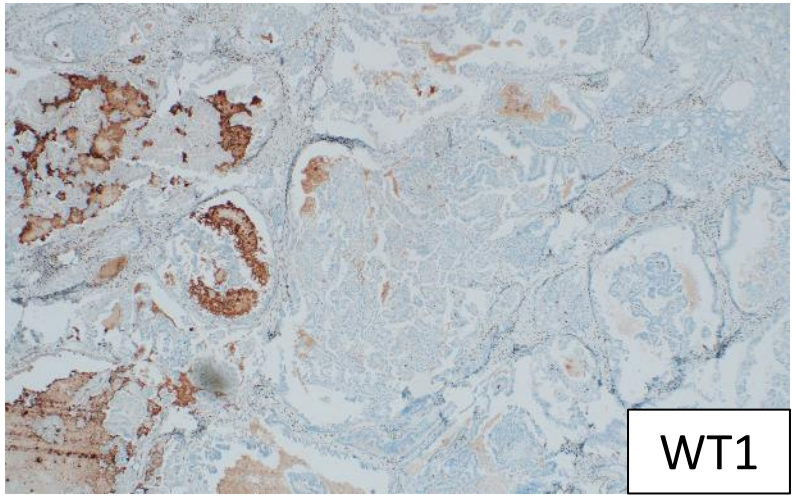
- arise in background of endometriosis (endometriotic cyst) +/- seromucinous cystadenomas, endometrioid borderline tumour. (*McCluggage et al 2020)*

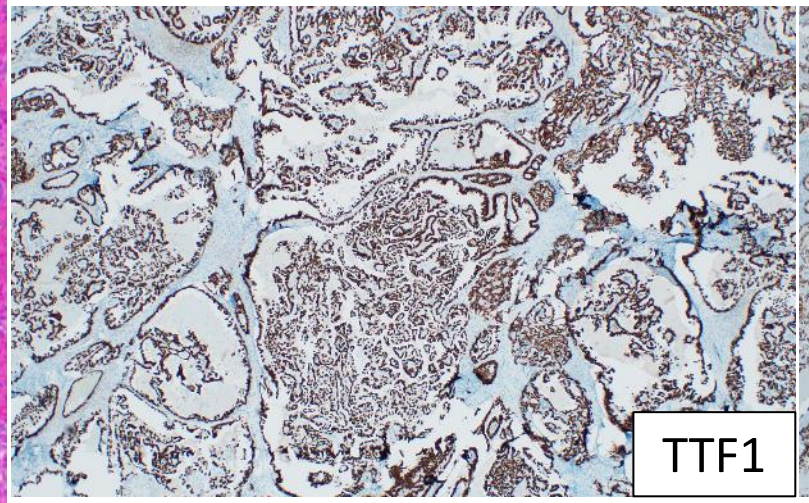
2. Some are thought to arise from mesonephric remnants in para-ovarian area

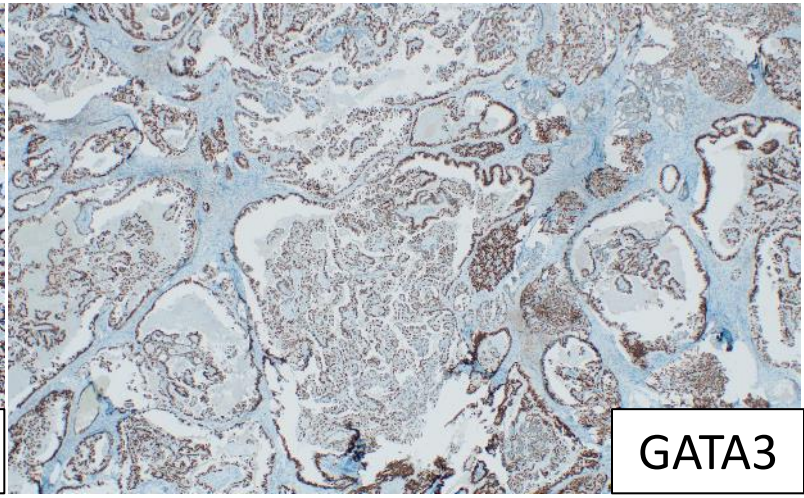








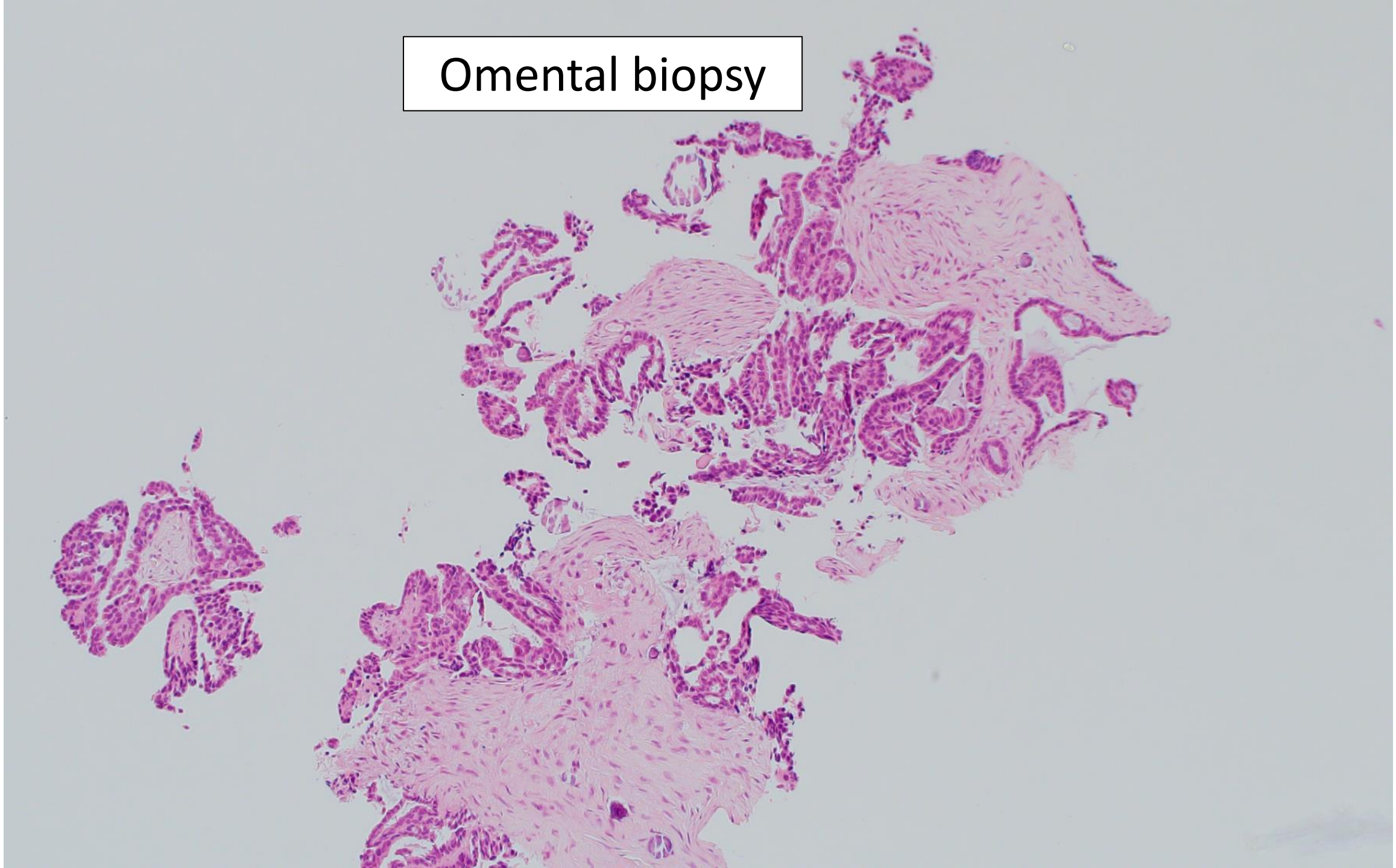


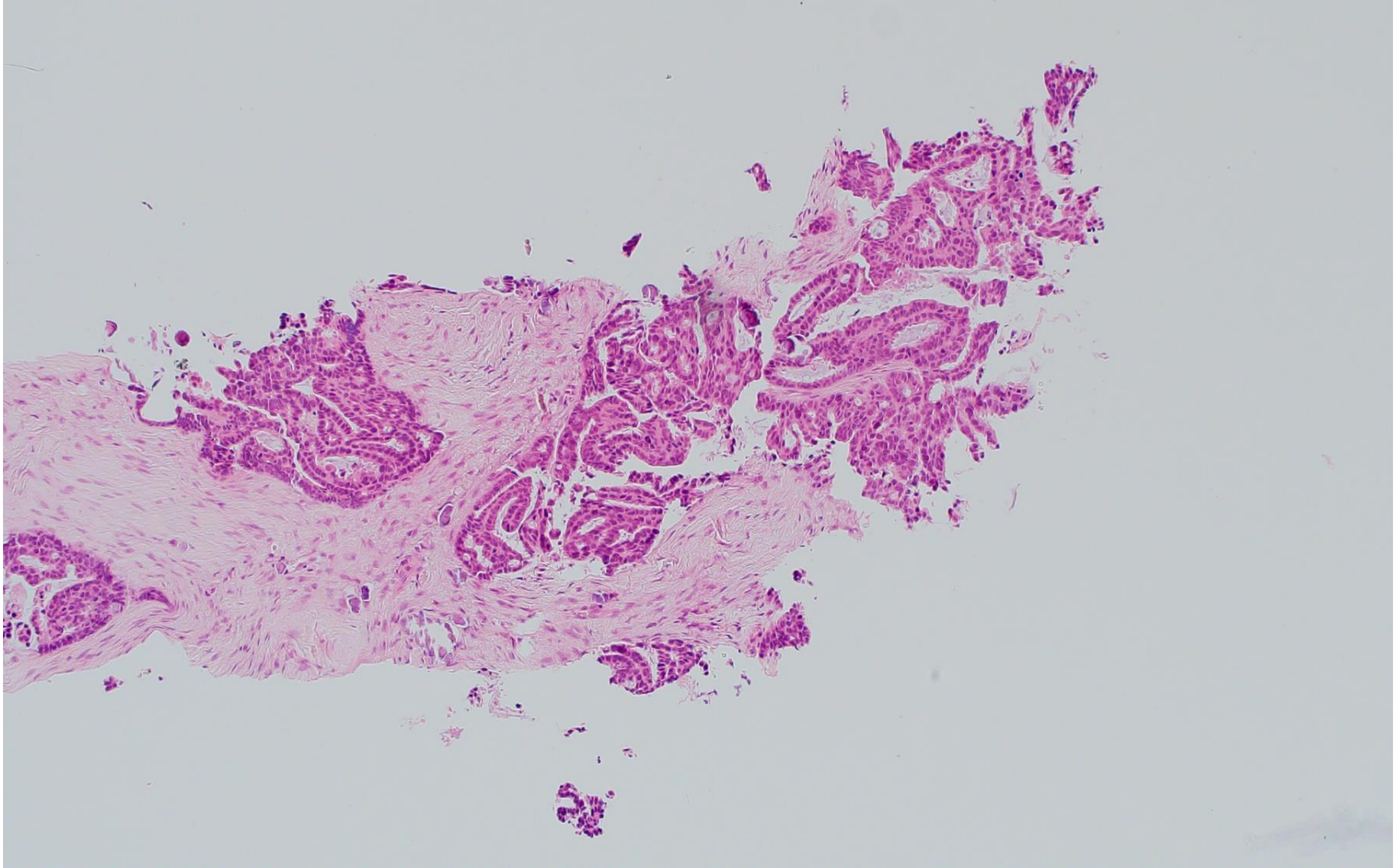


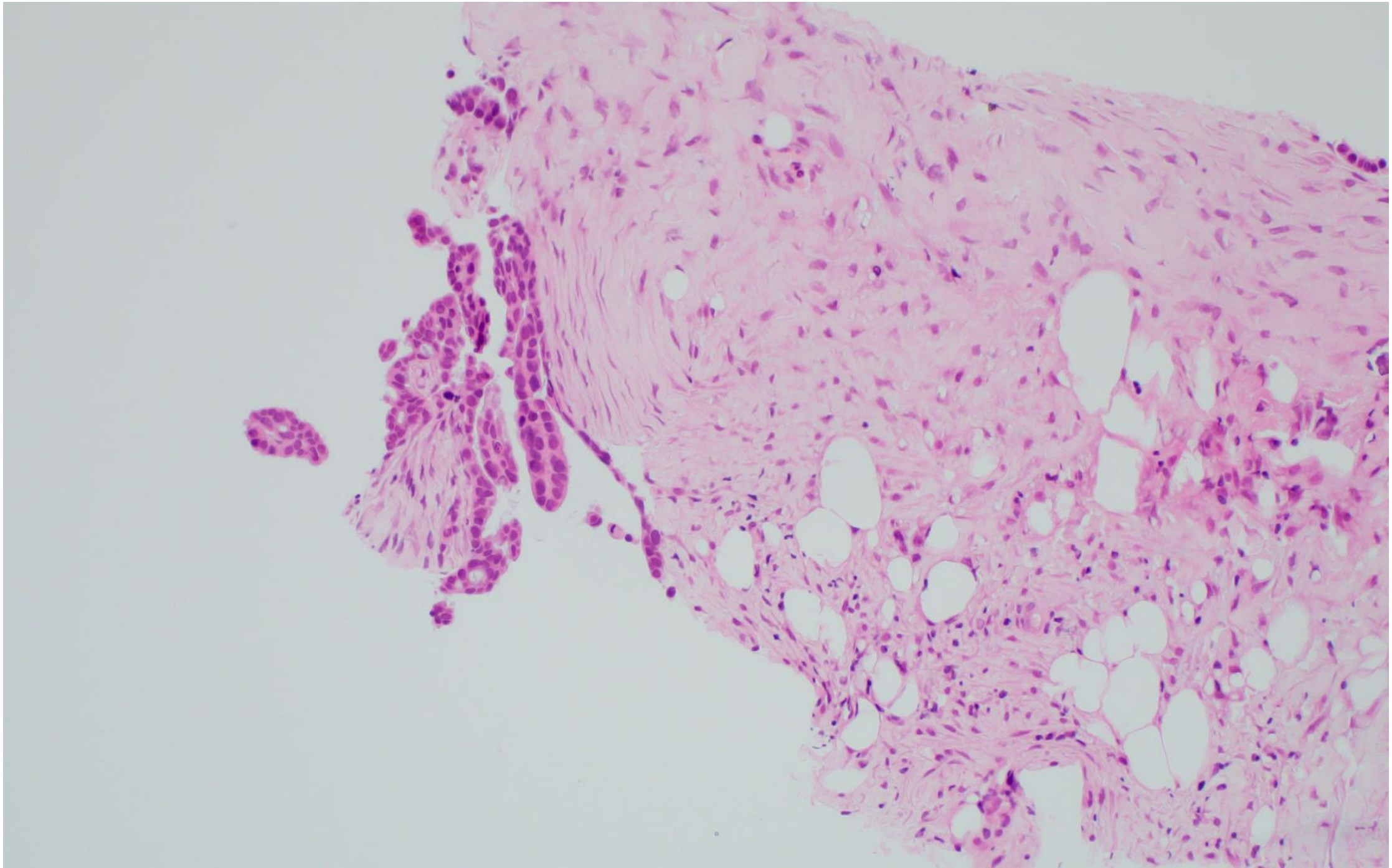
Left ovary

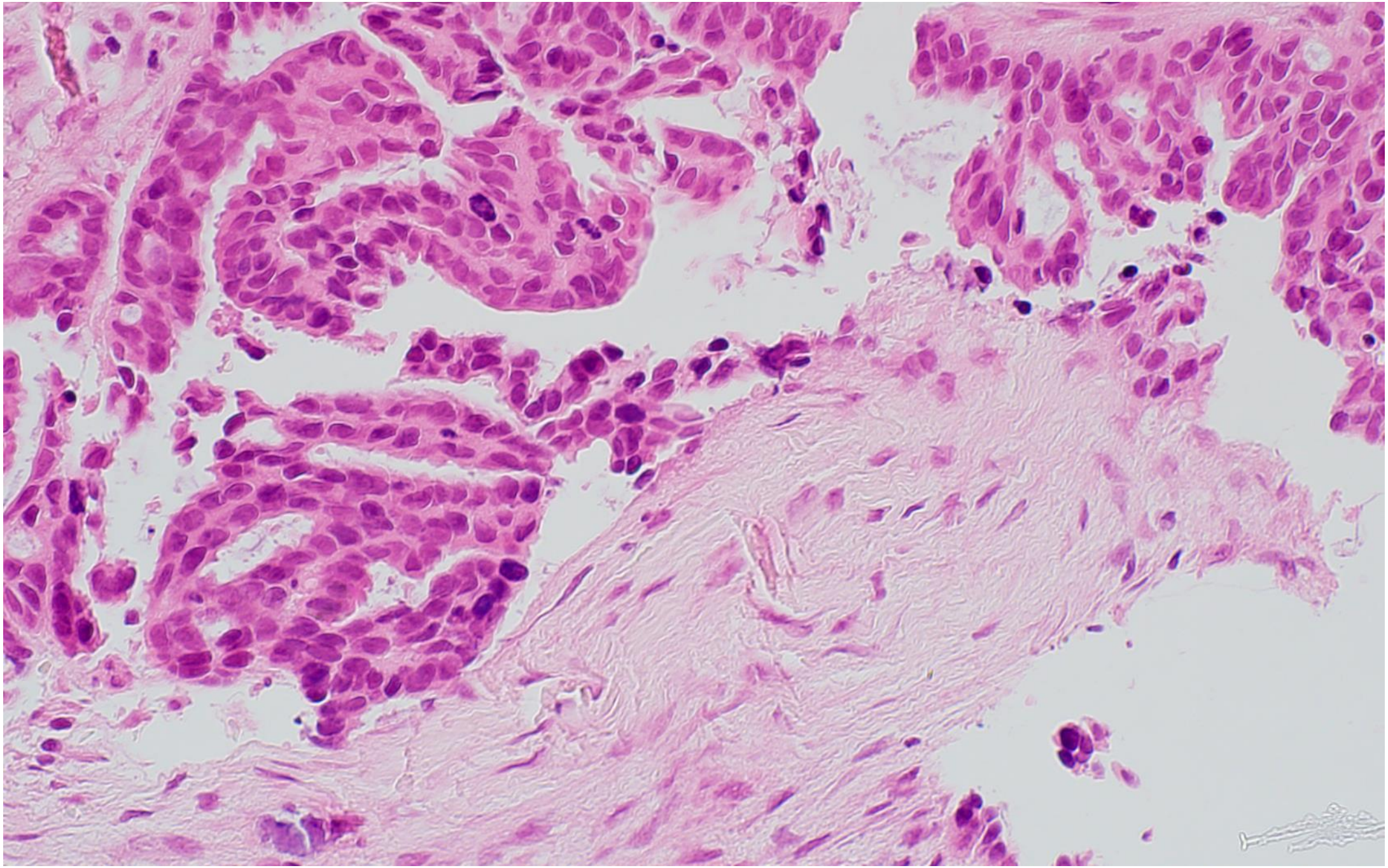
Case 2

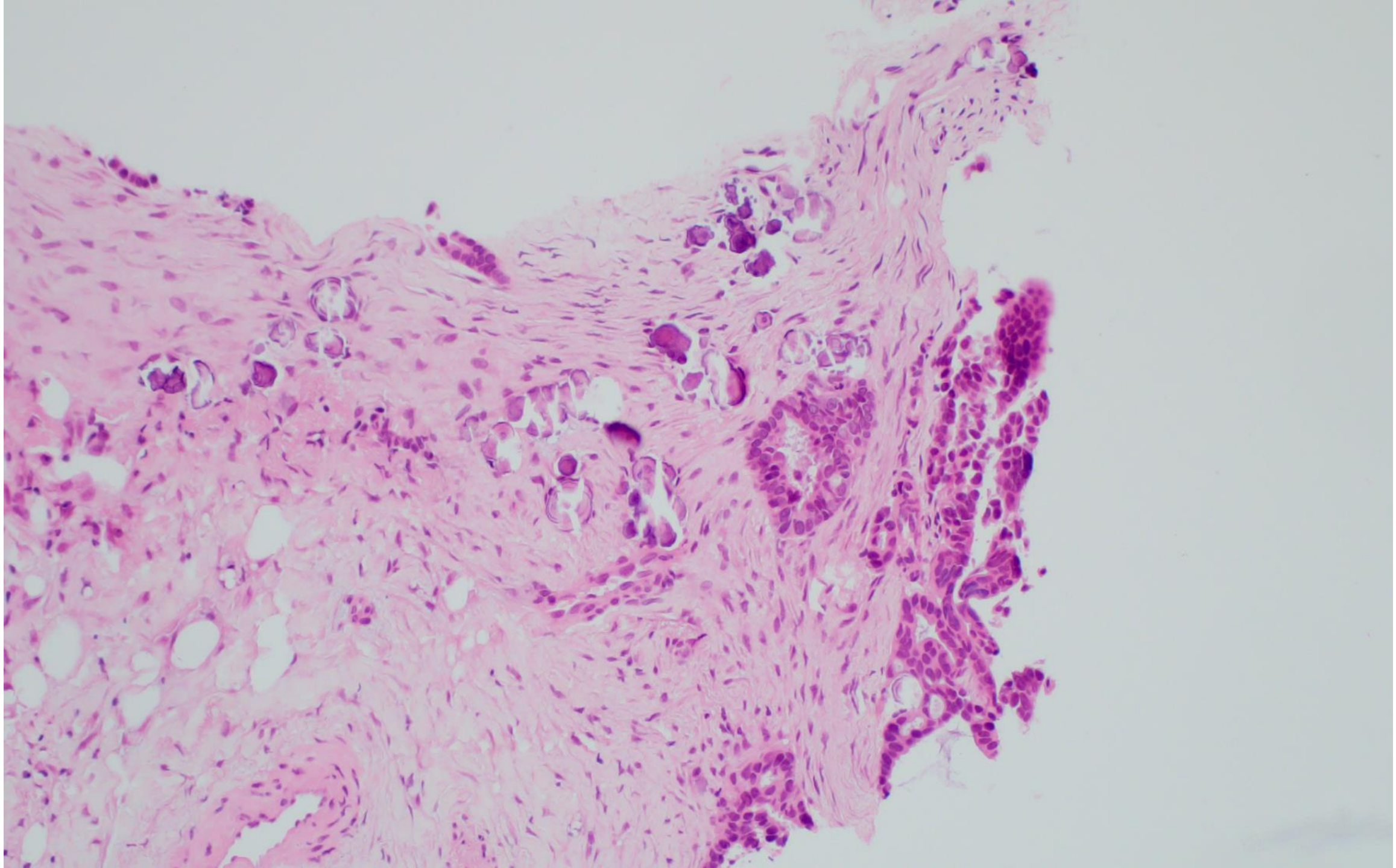
- 57-year old female
- Stage IV ovarian carcinoma – diagnosed in April 2021

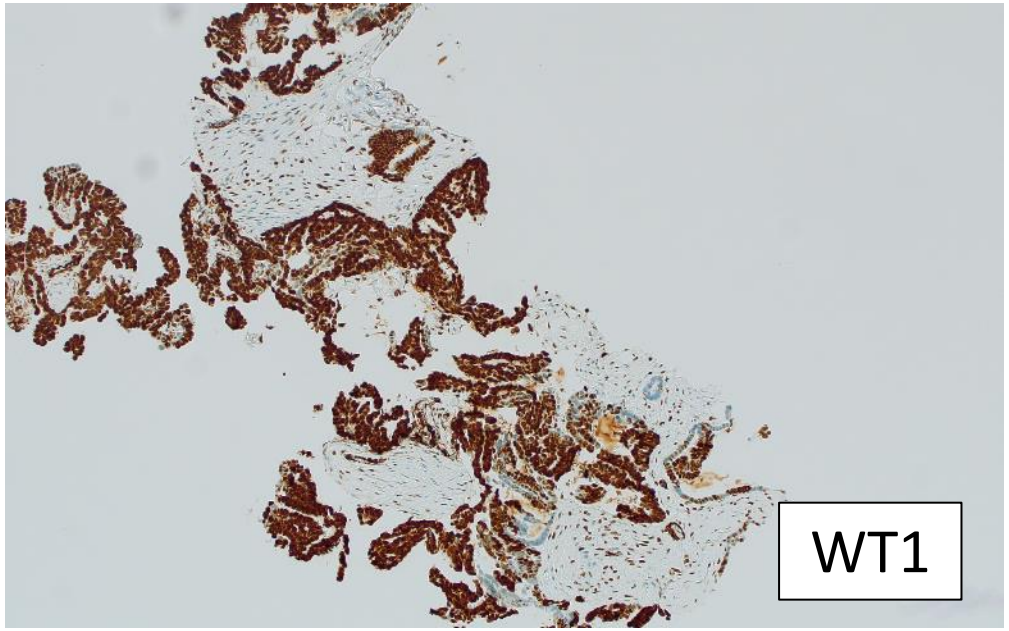

Omental biopsy

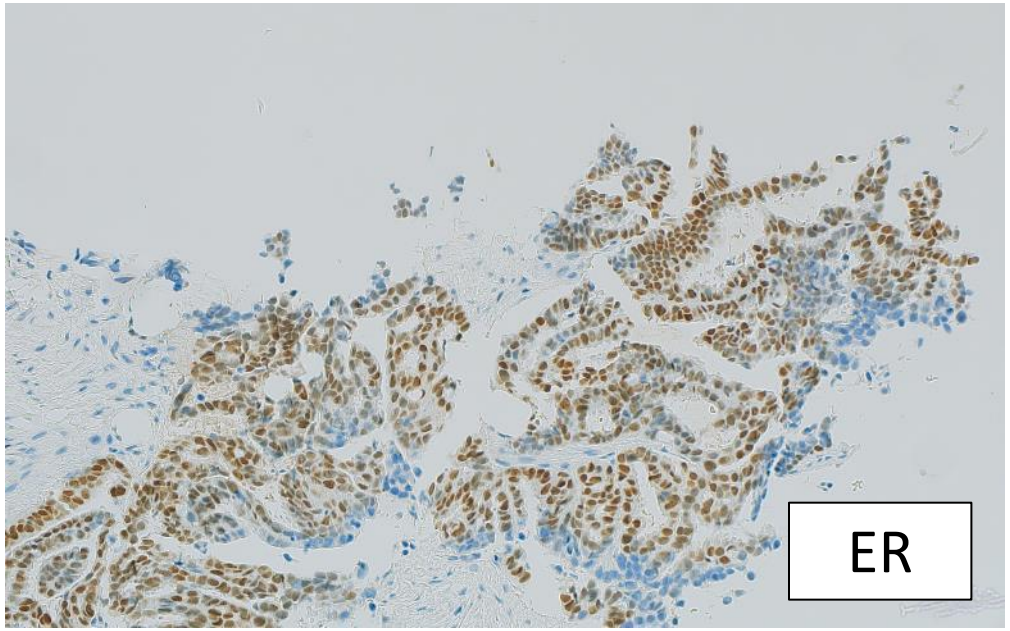


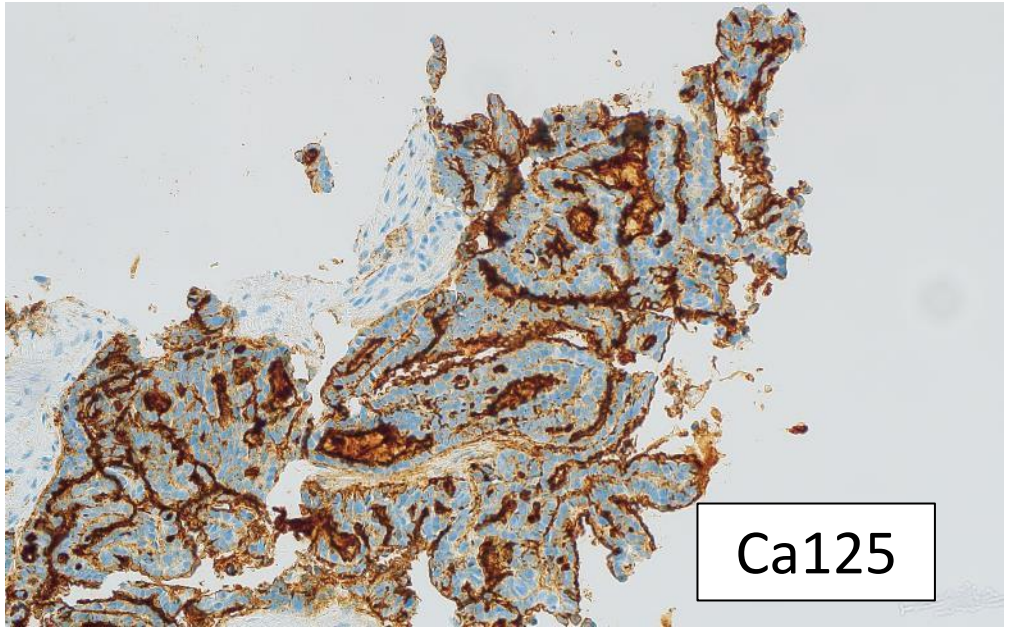


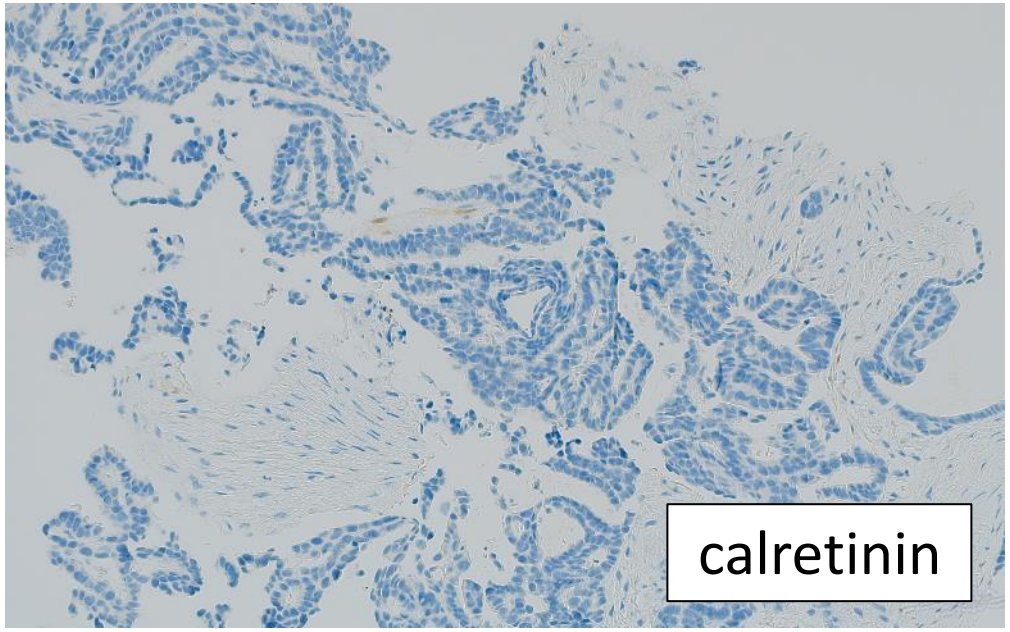


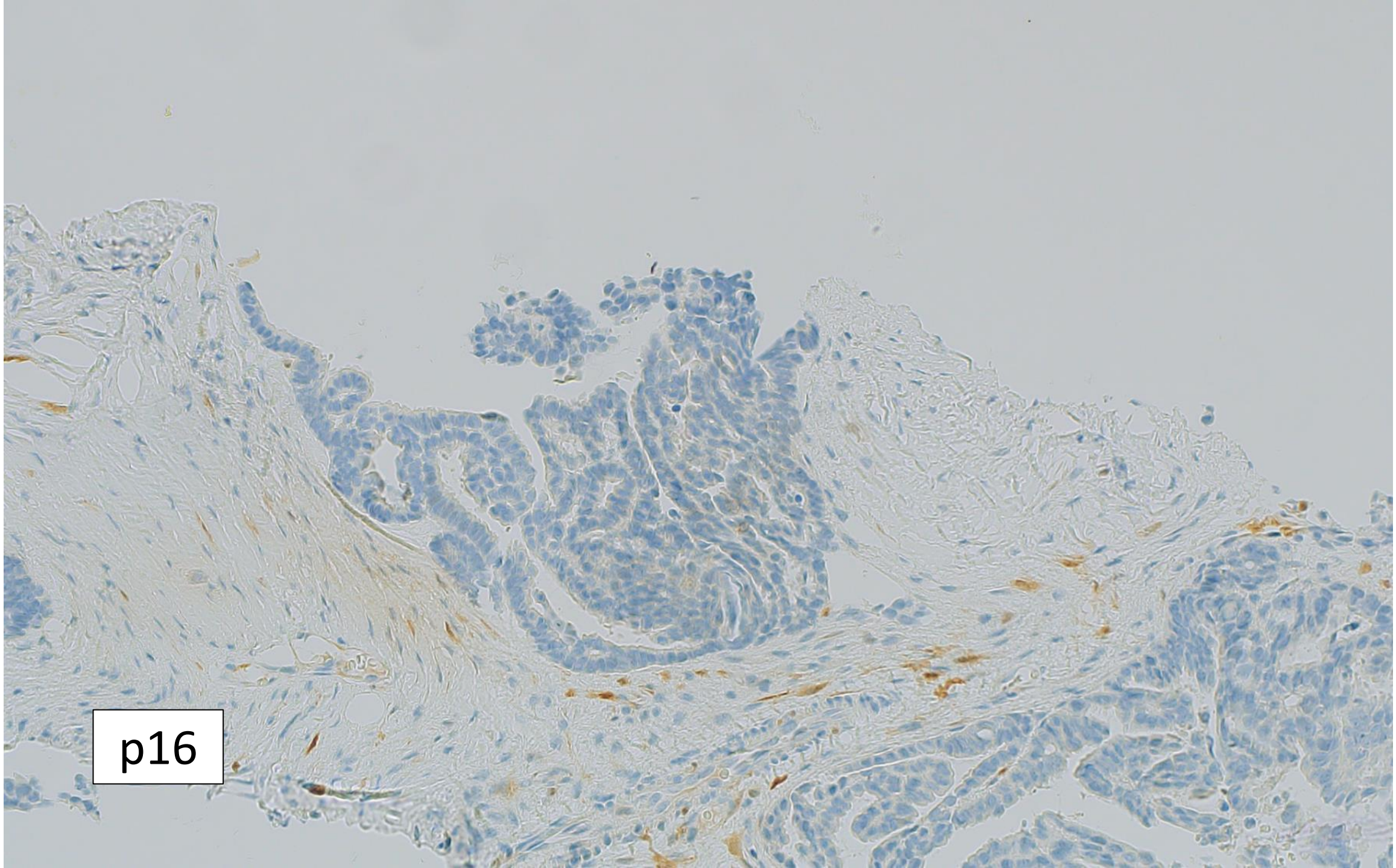


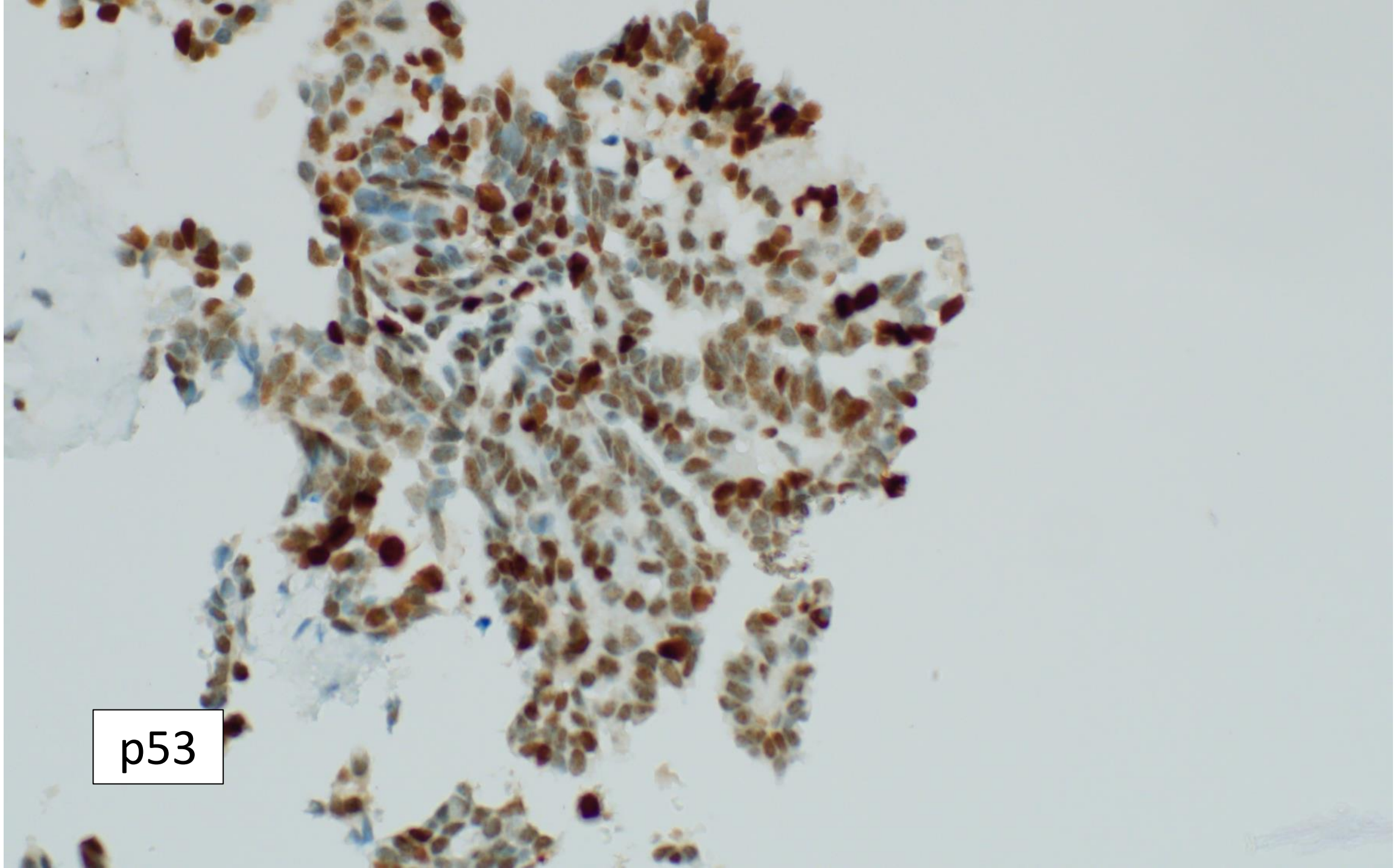

WT1


ER


Ca125


calretinin

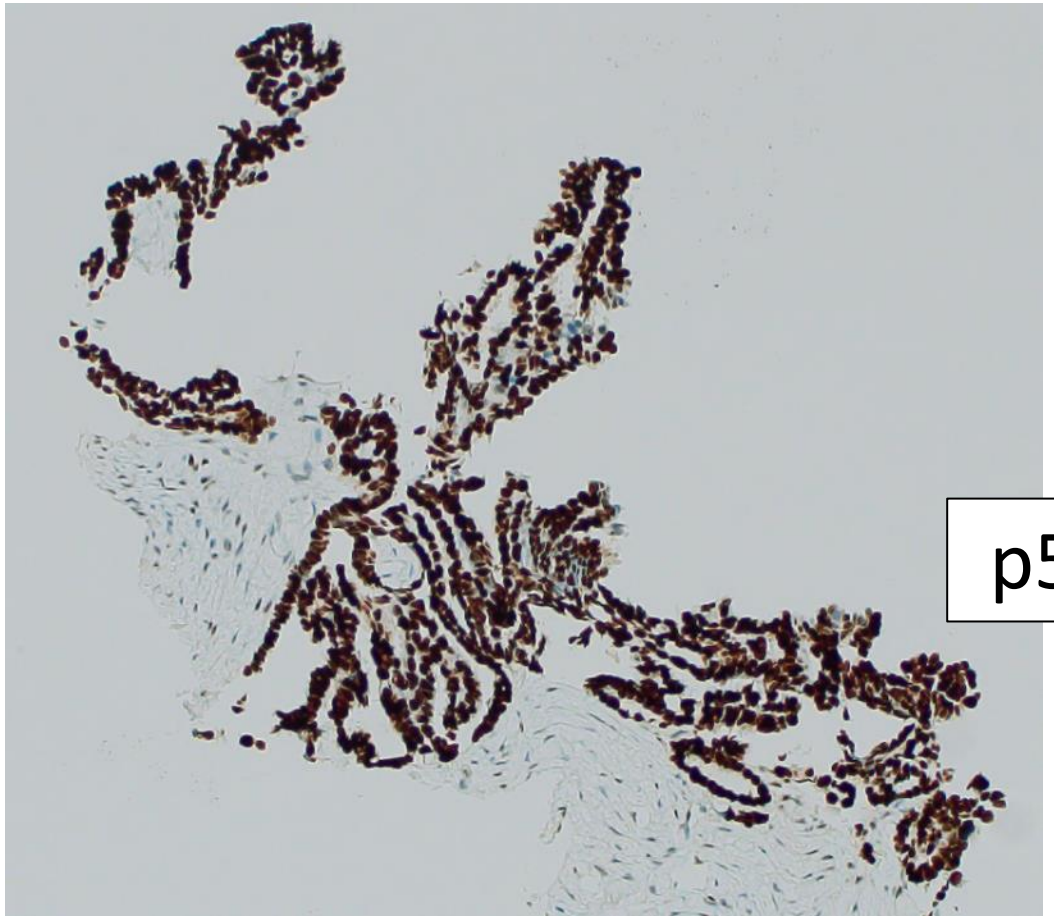

p16


p53

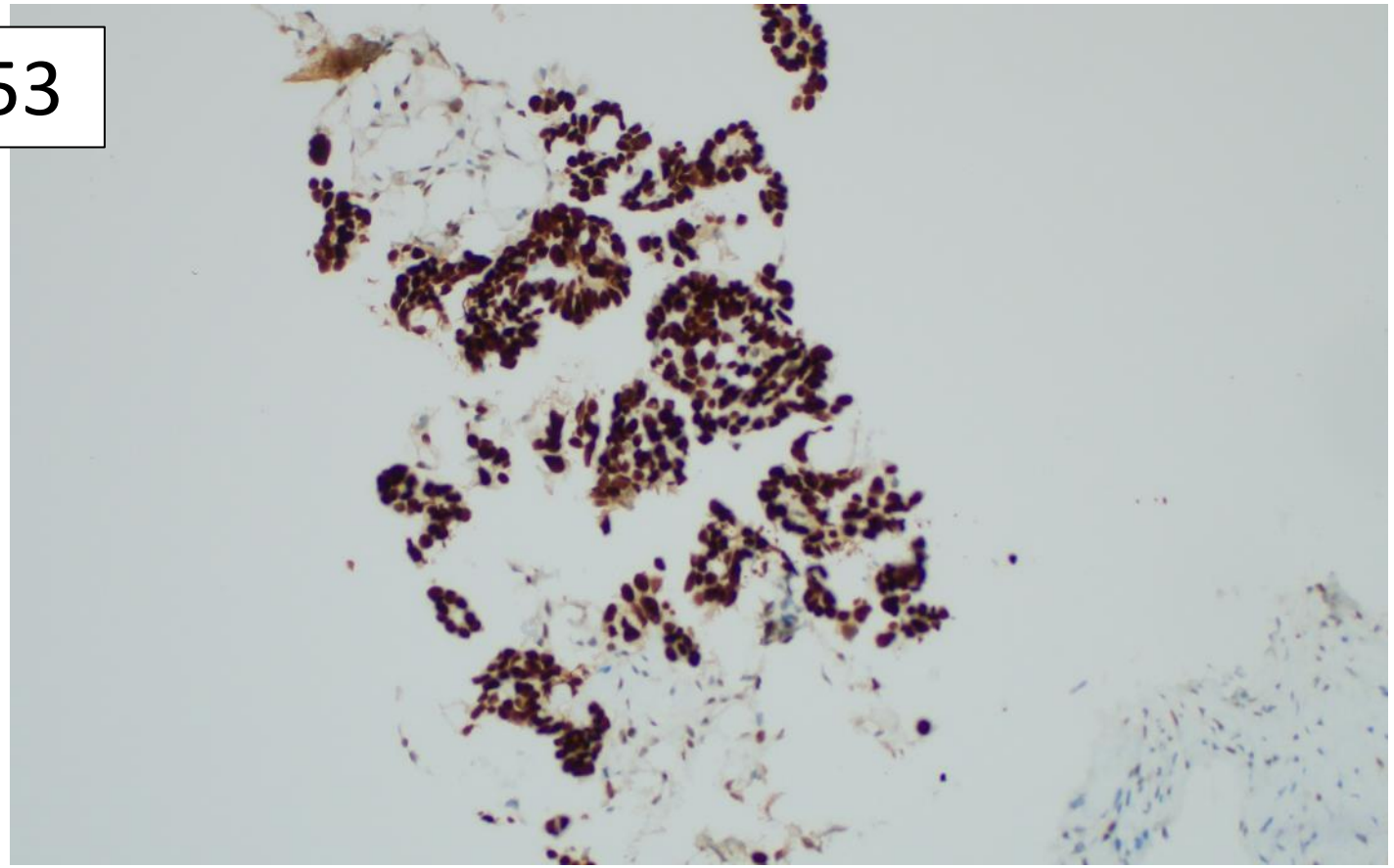
Poll question 3

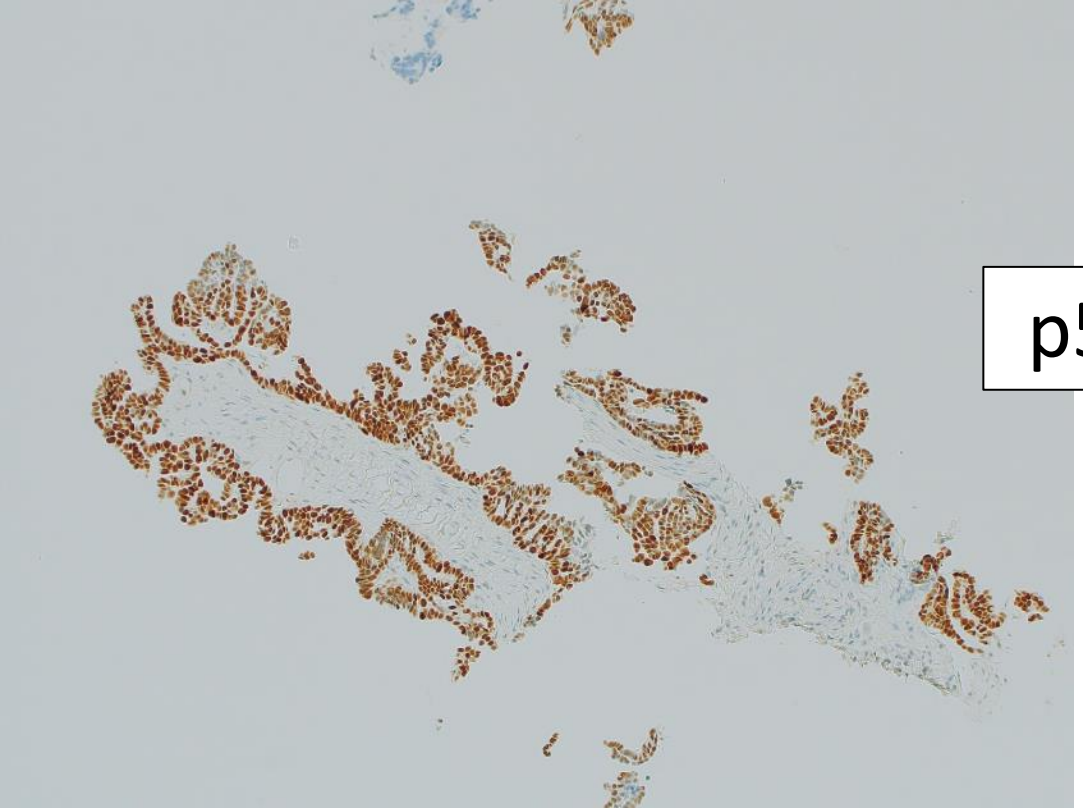
Diagnosis?

- Low grade serous carcinoma
- High grade serous carcinoma

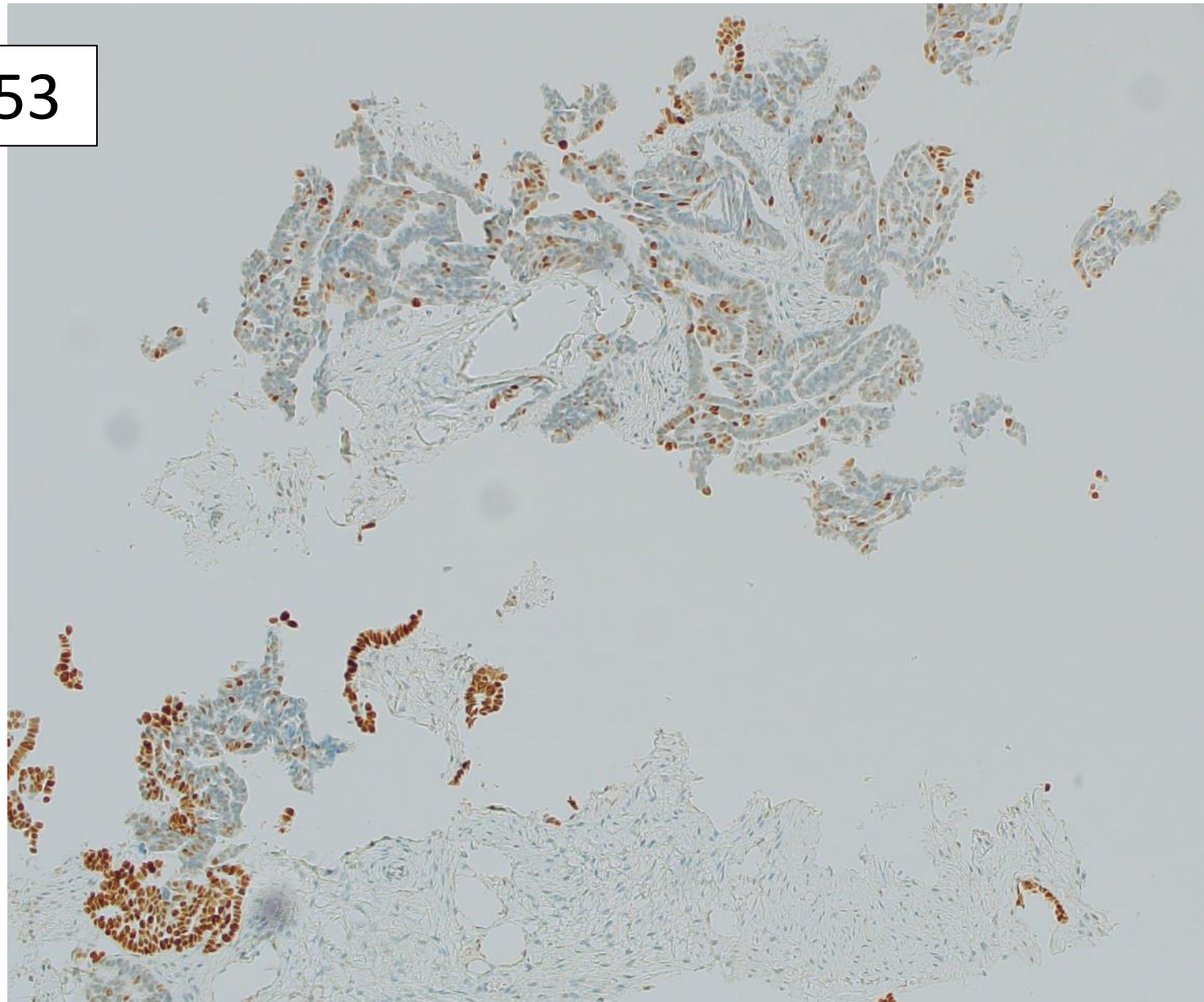


p53

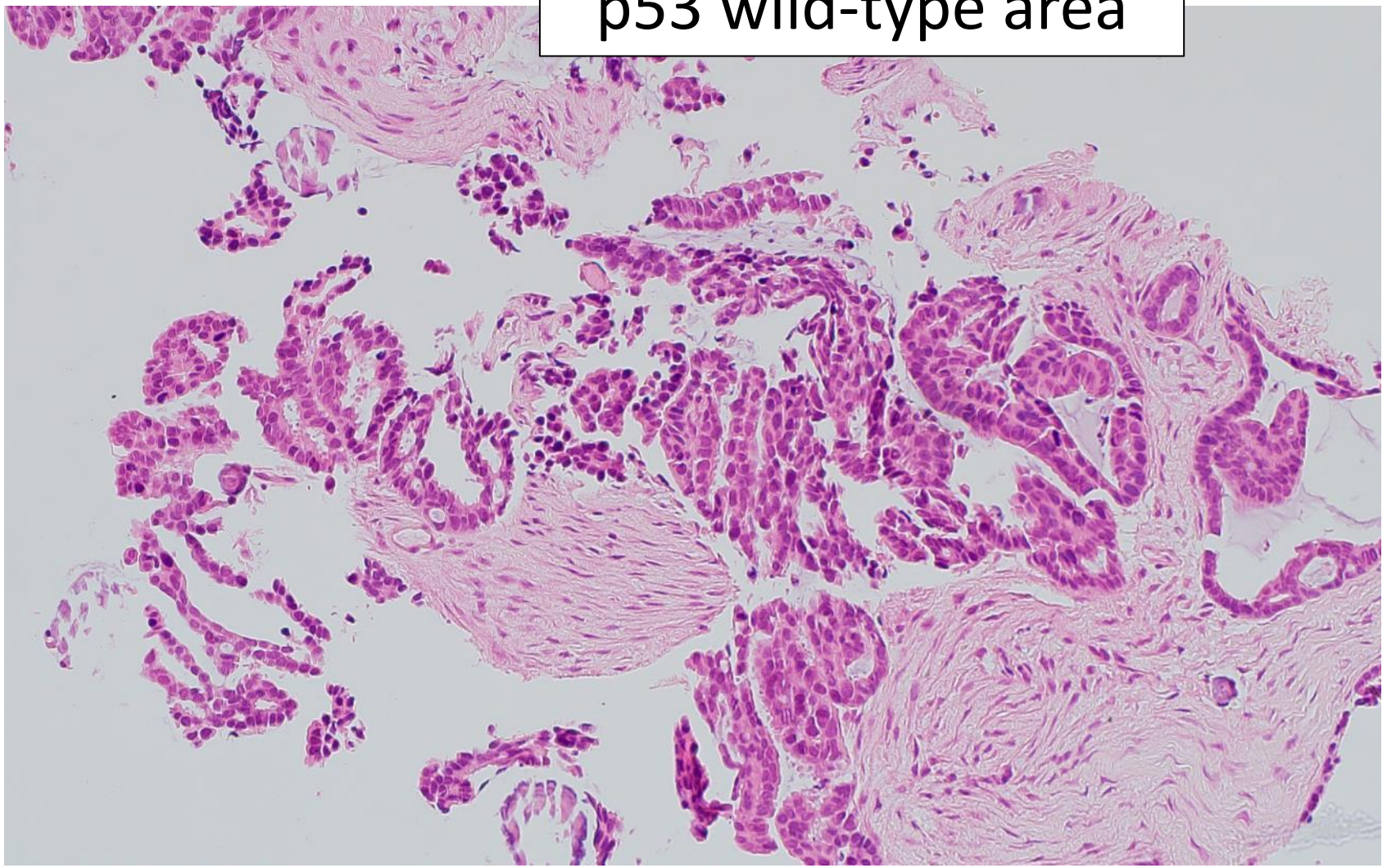




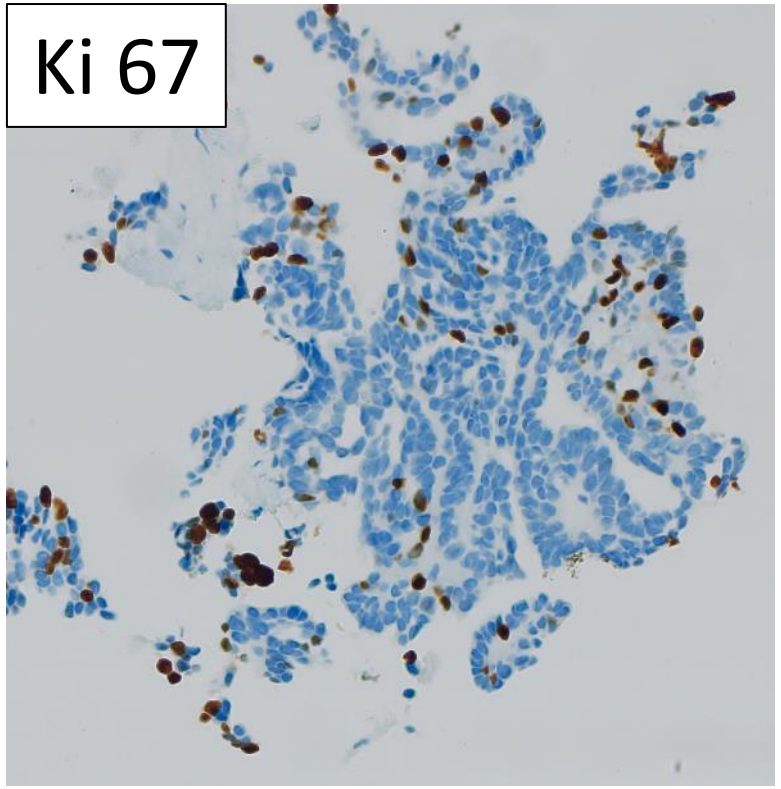
p53



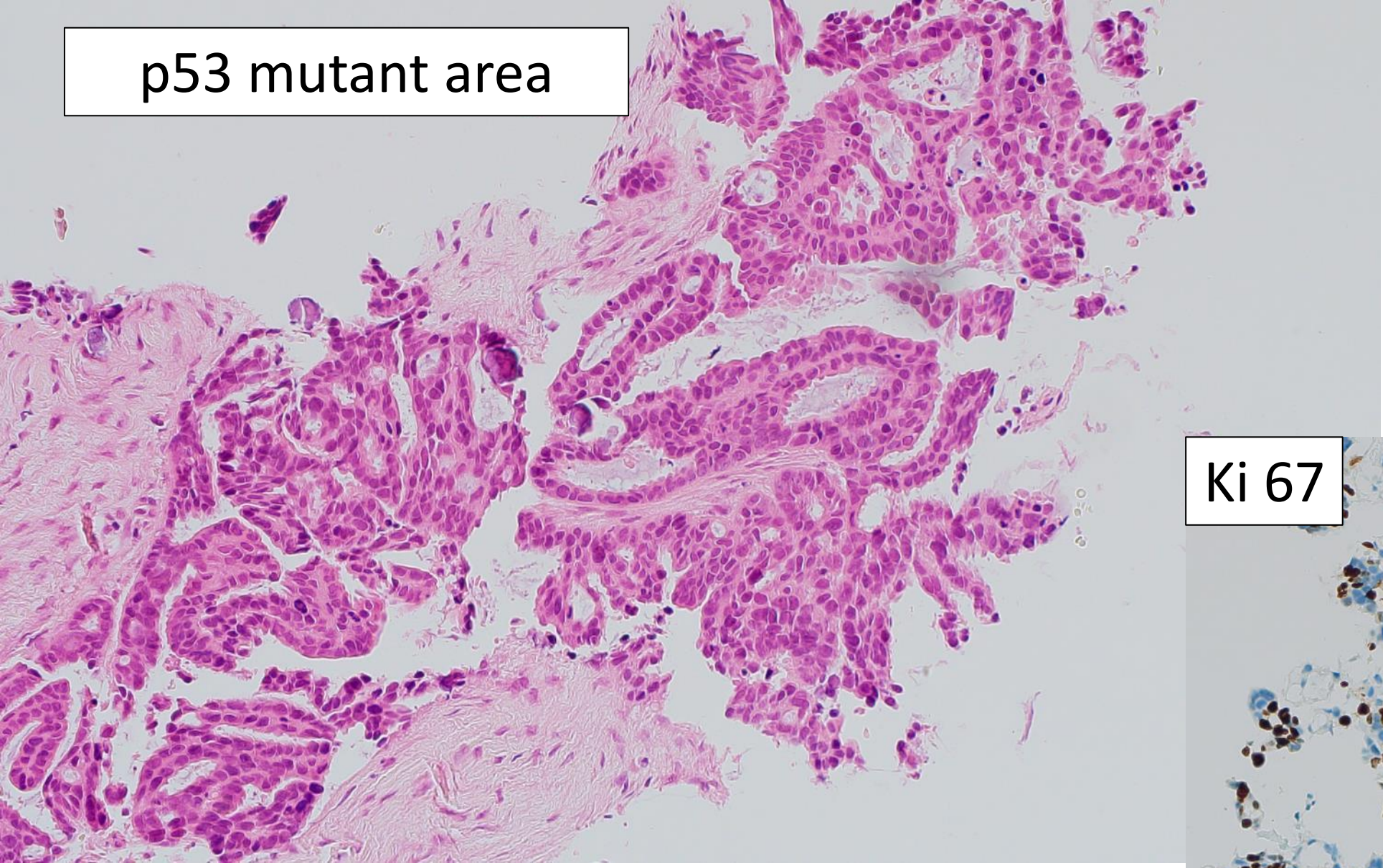
p53 wild-type area



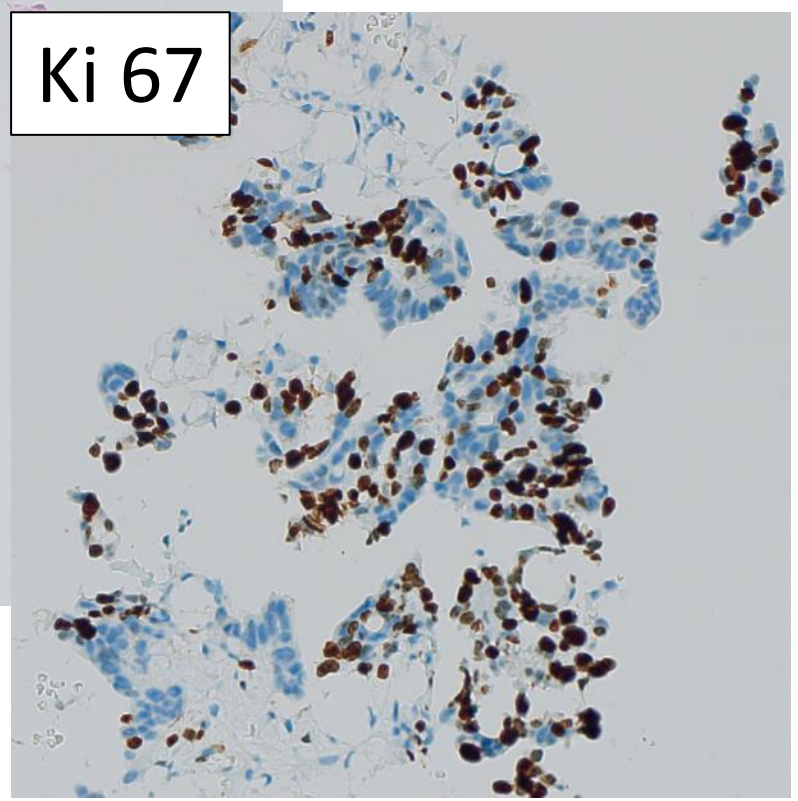
Ki 67



p53 mutant area



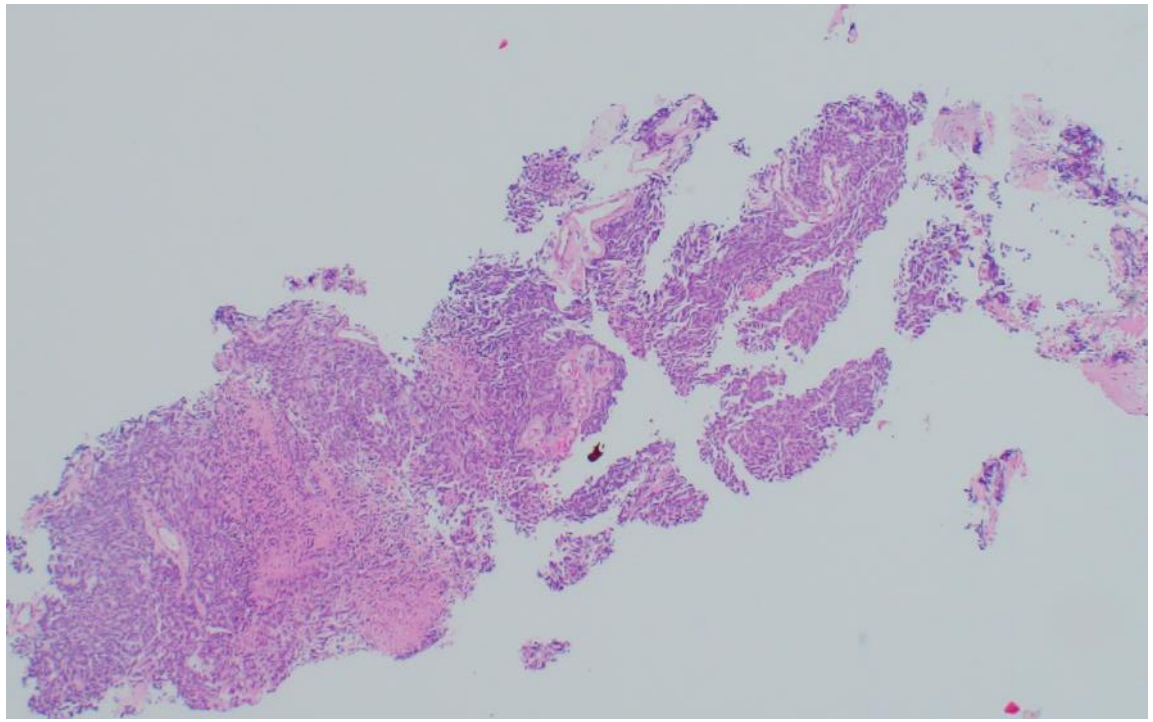
Ki 67

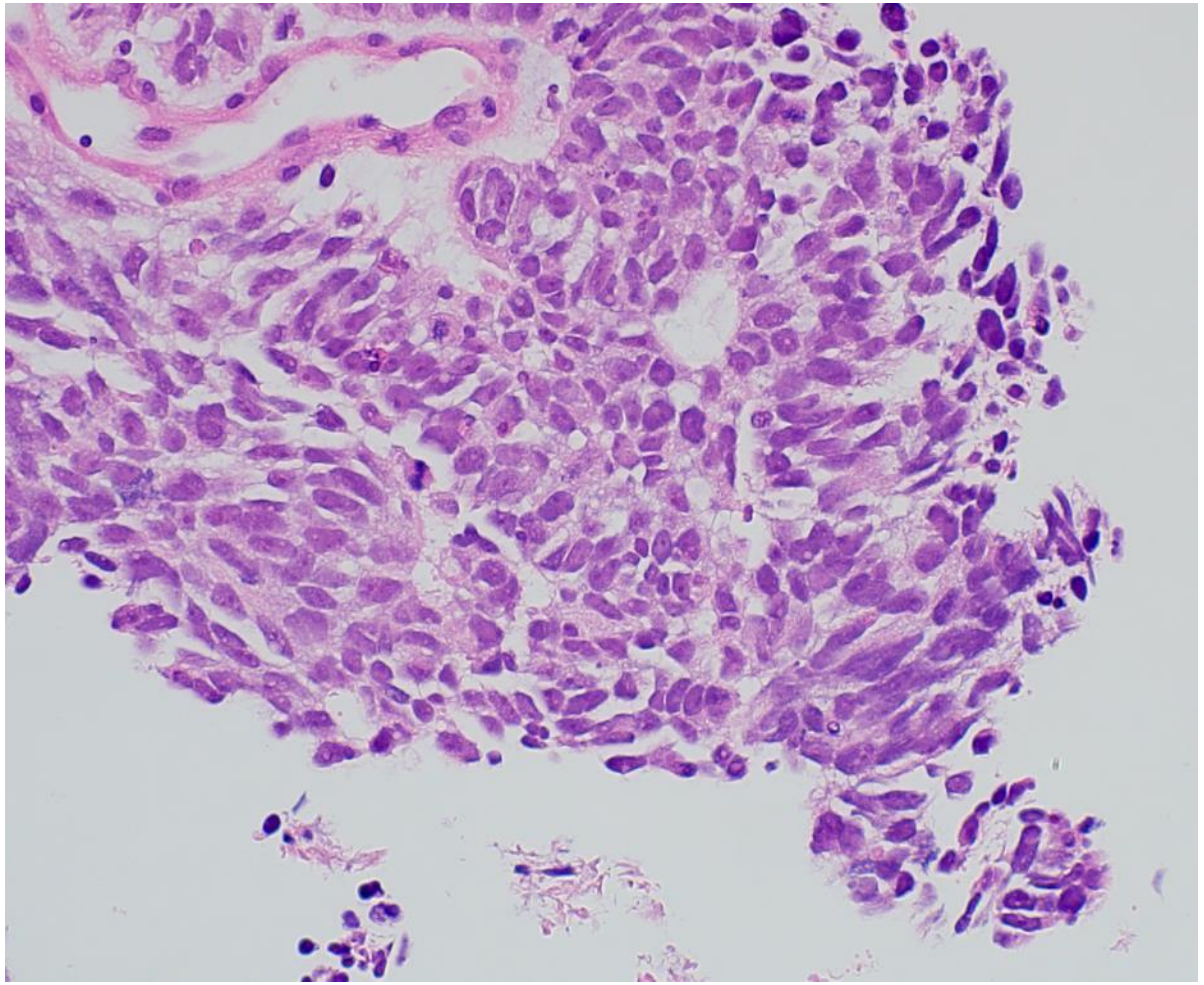


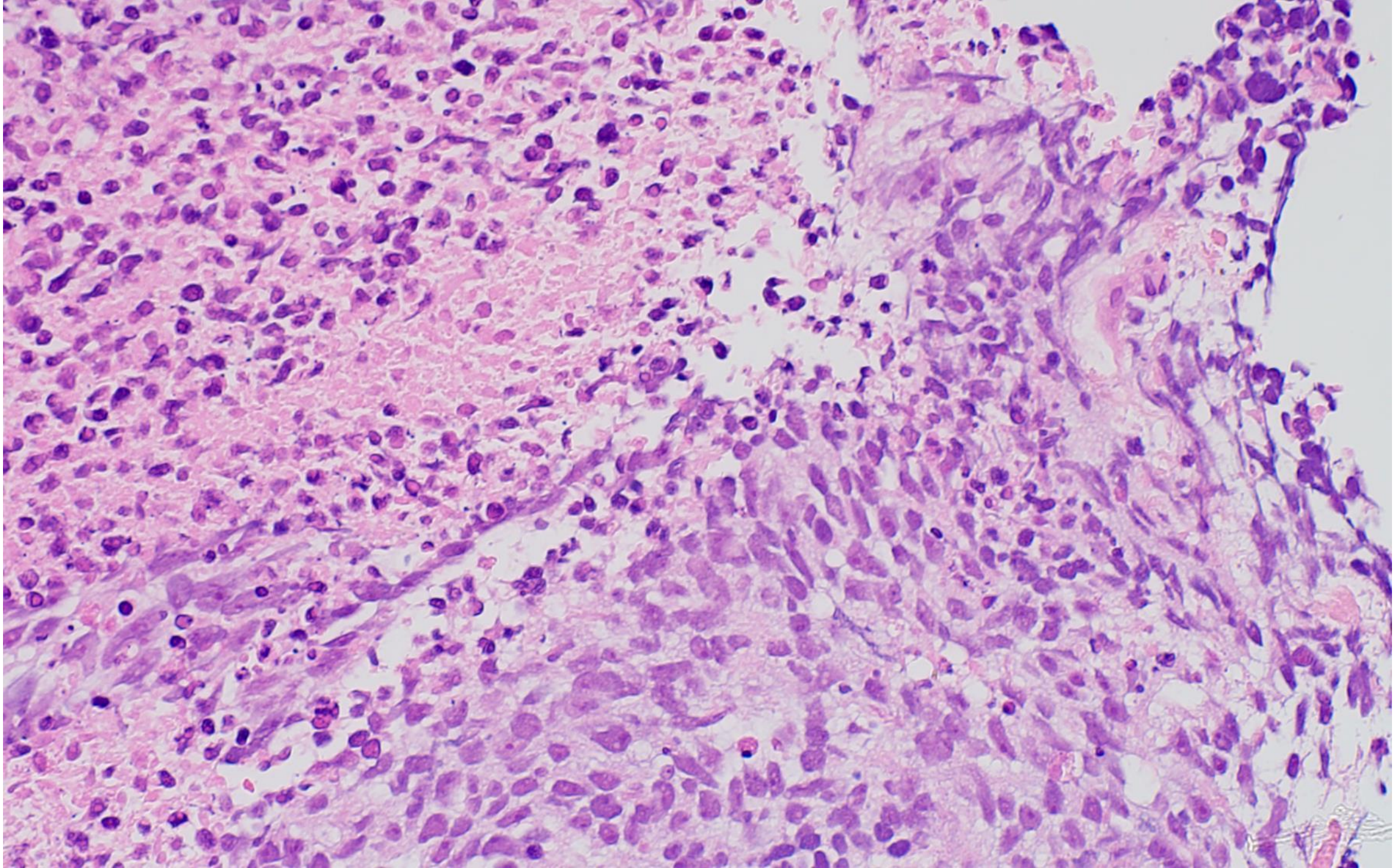
Case 2

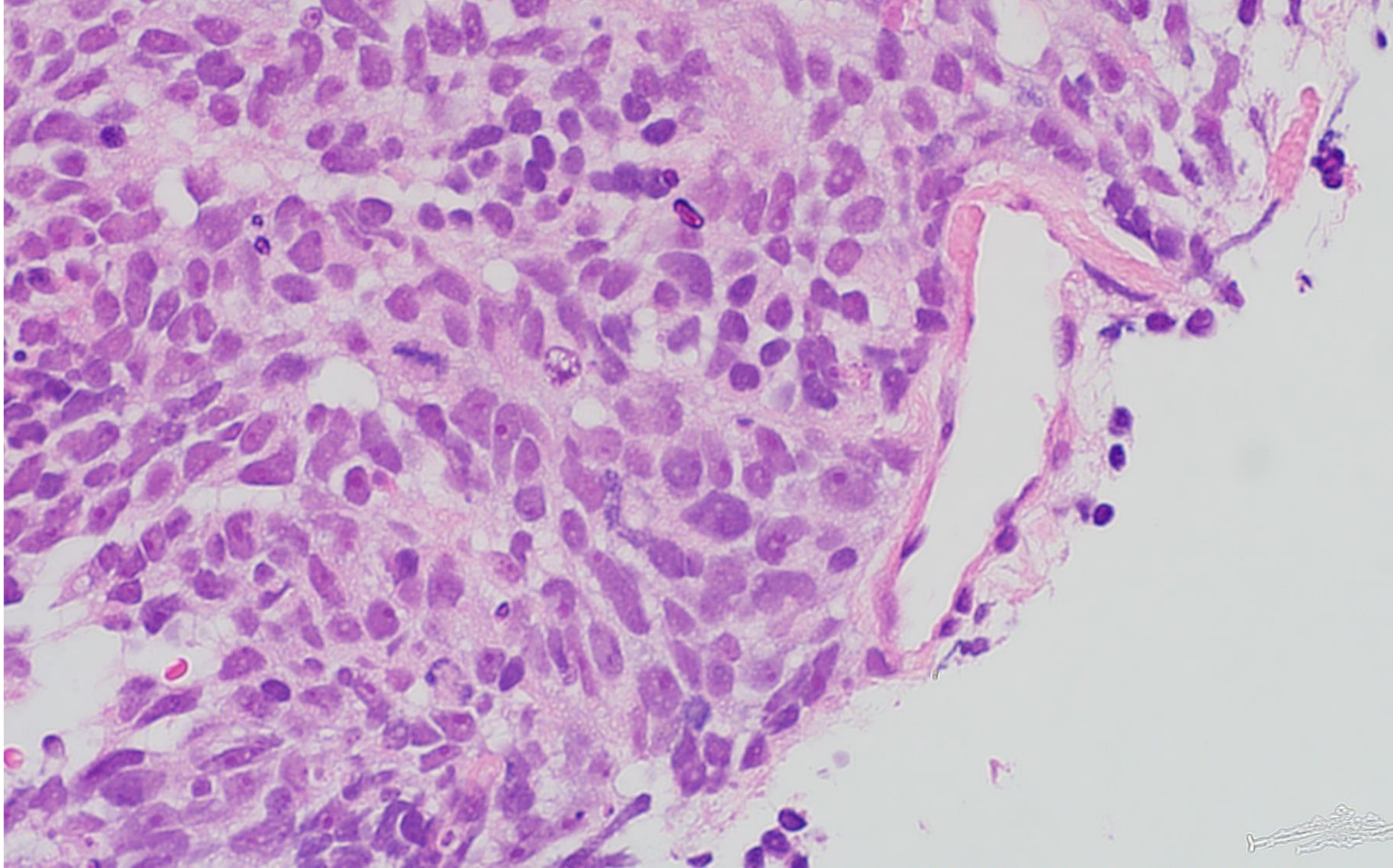
- April 2021: stage IV ovarian malignancy on imaging.
- **Diagnosis: Low grade serous carcinoma, but with extensive, yet subclonal p53 mutation.**
- Neoadjuvant chemotherapy, 3 cycles of Carboplatin/Paclitaxel
- Disease progressed through neoadjuvant therapy with new liver metastases
- Liver biopsy September 2021

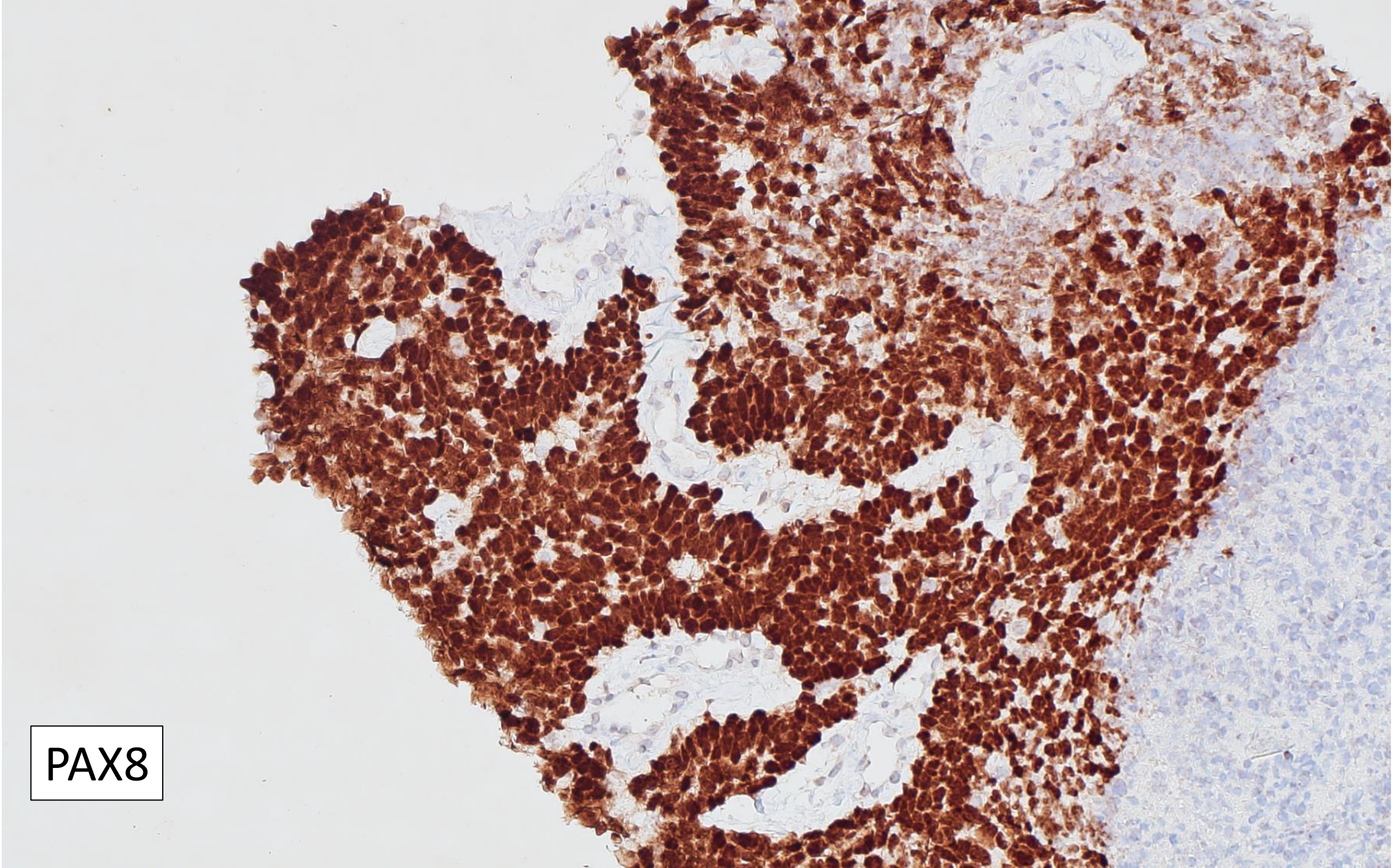
Liver biopsy - Sept 2021

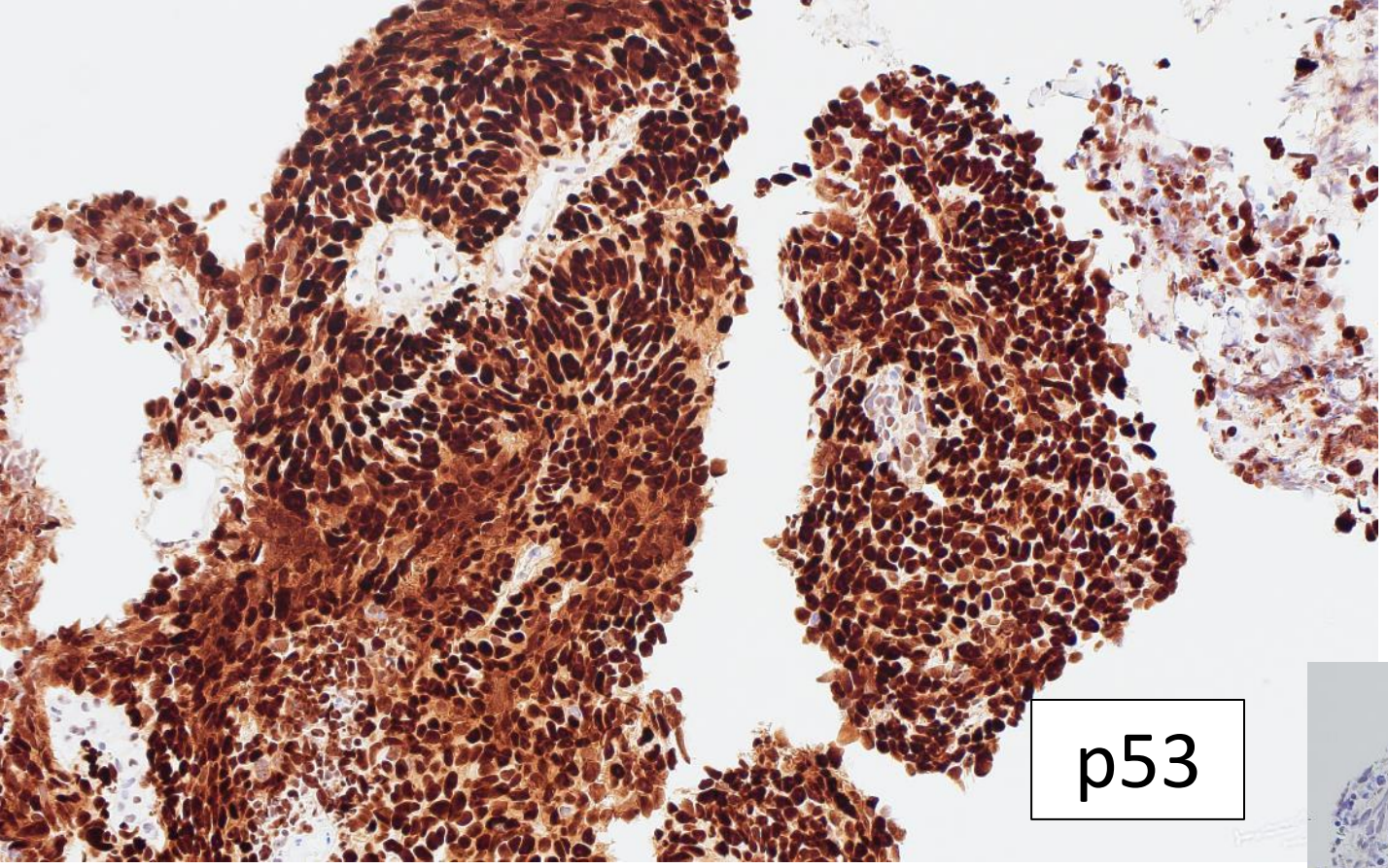


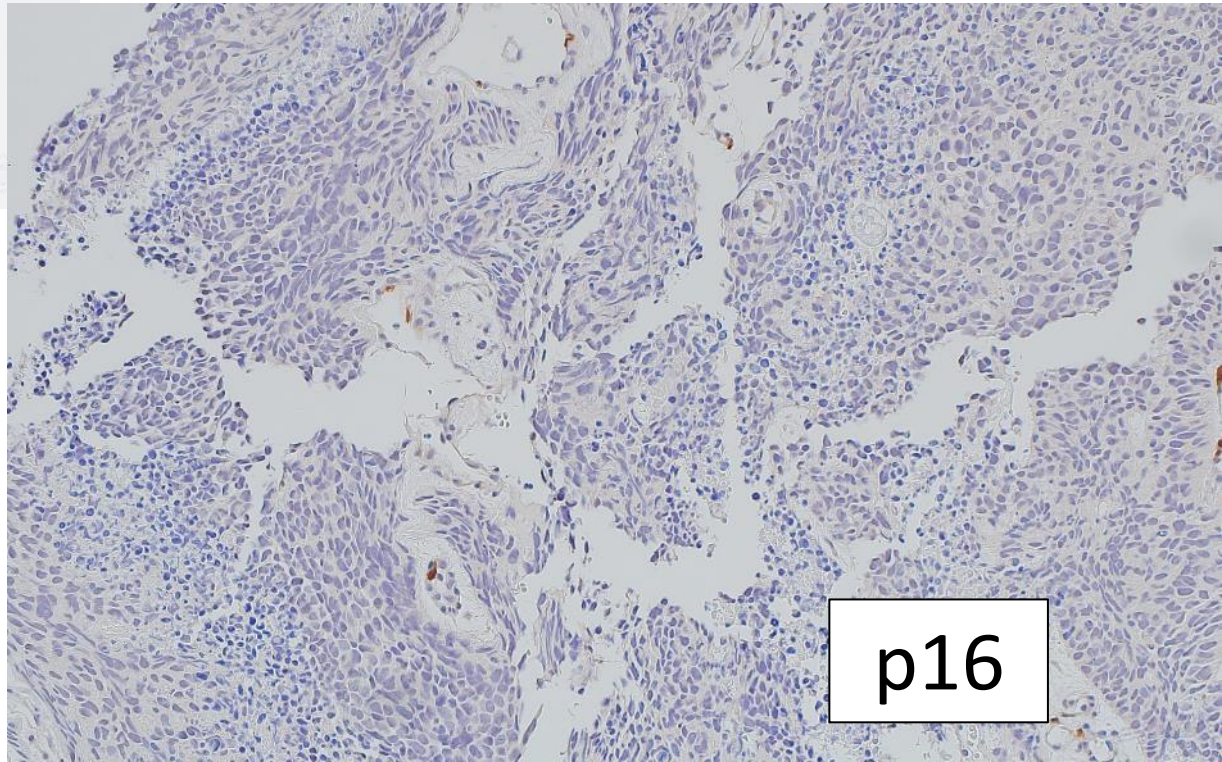


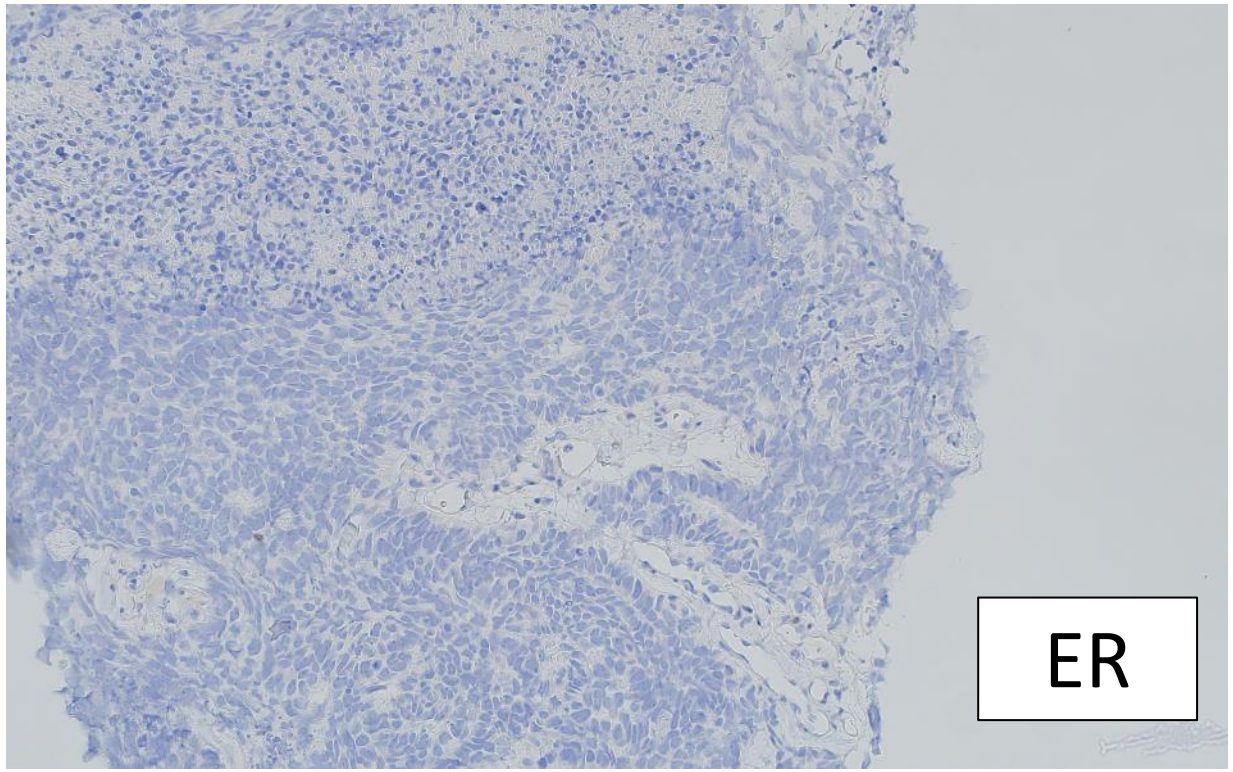


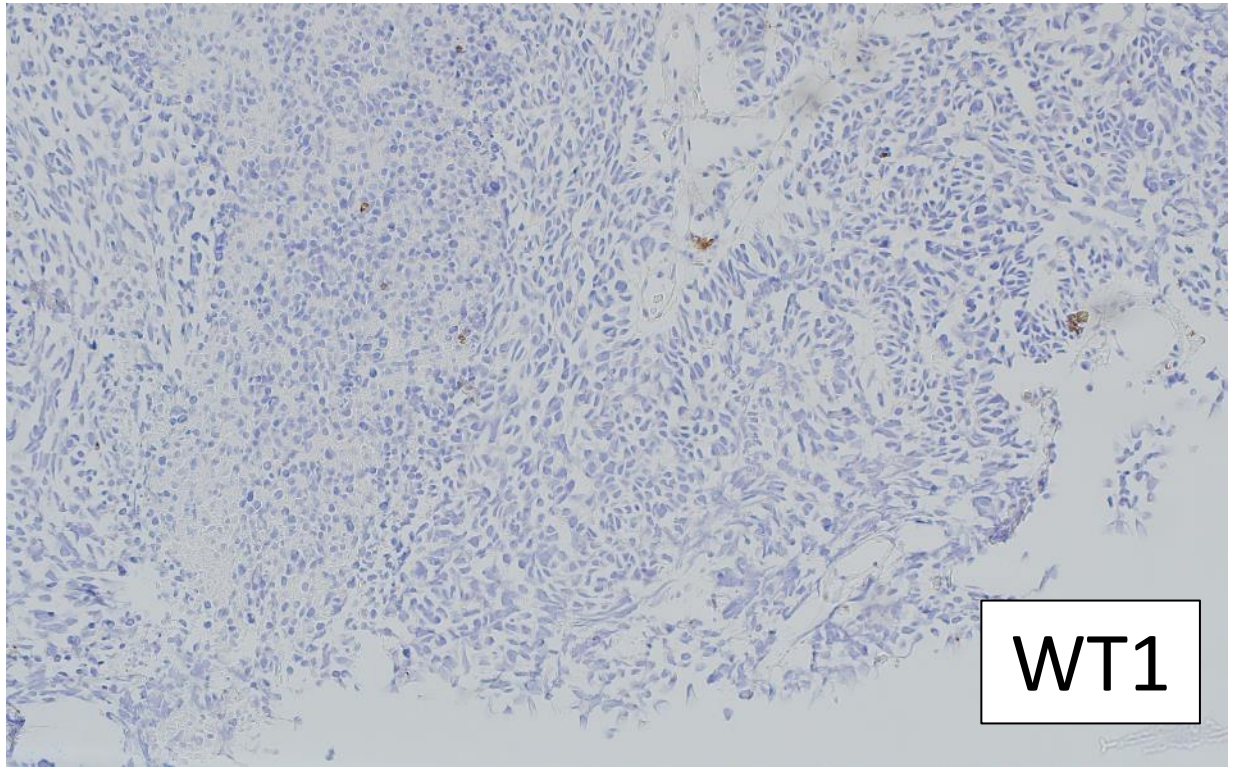


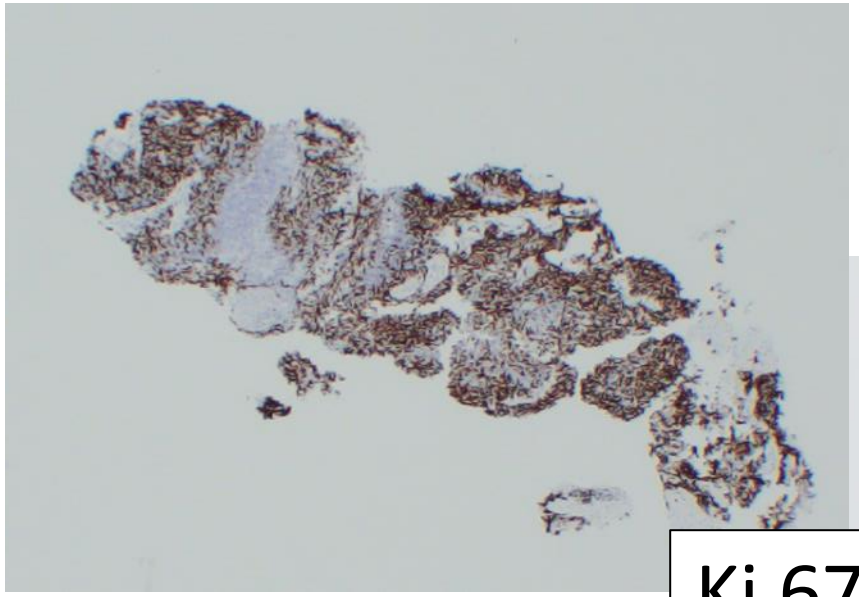

PAX8


p53

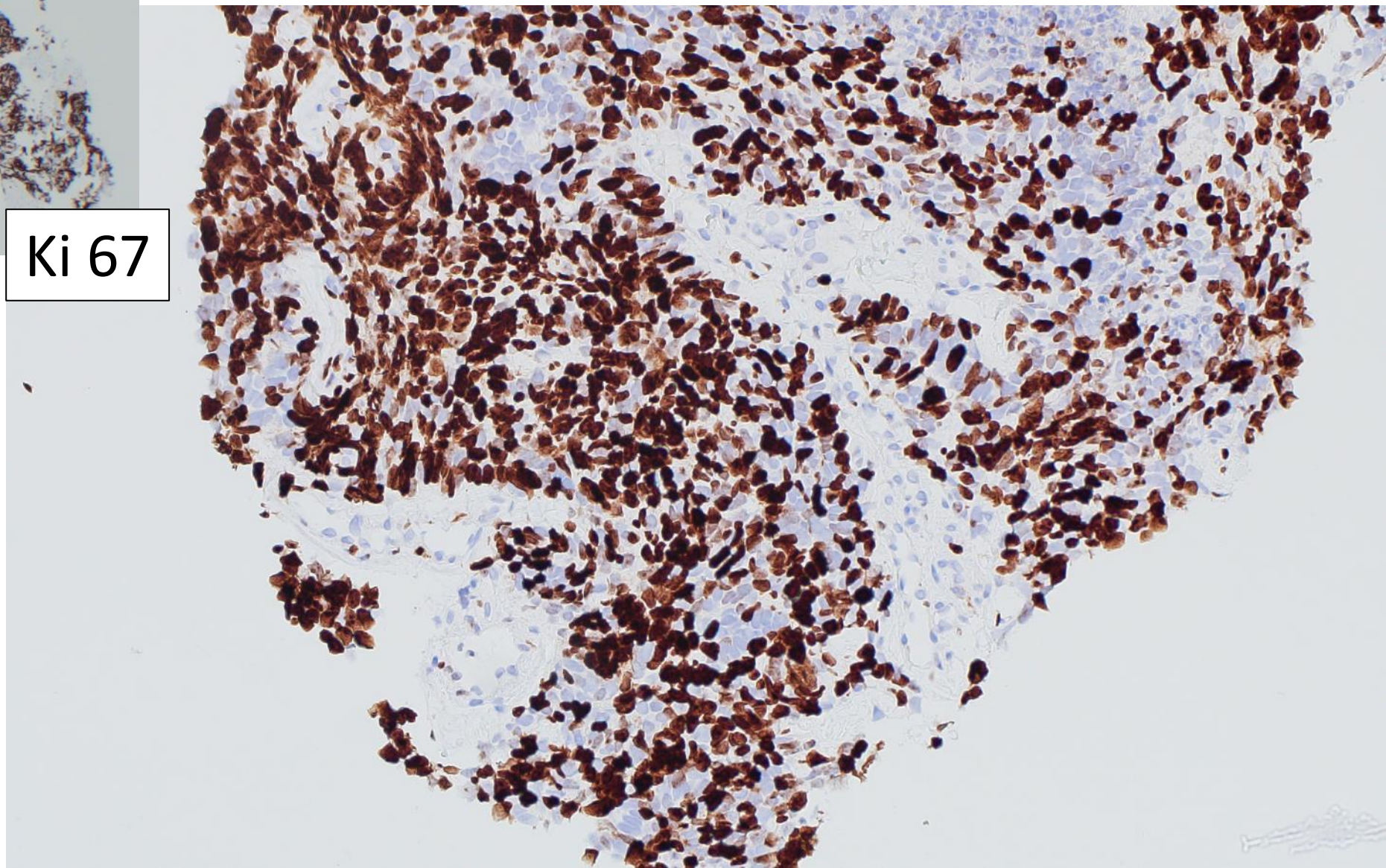

p16


ER


WT1



Ki 67



Case 2

Targeted NGS performed on liver biopsy:

- TP53 pathogenic variant: c.710_718del p.(Met237_Asn239del)
- No mutations in *BRCA1* or *BRCA2* genes
- No mutations in *BRAF*, *NRAS* and *KRAS* genes

Case 2

Follow up:

- September 2021: large pelvic mass, irritating sciatic nerve weekly paclitaxel in the platinum refractory setting.

- 16th October 2021: Died of progressive disease.

Case 2
Diagnosis

High grade transformation of the p53 mutant component of low grade serous carcinoma.

- If previous (diagnostic) biopsy had not been performed, despite some unusual immunophenotypic features, a diagnosis of de novo high grade serous carcinoma would have been made on the liver biopsy

- Careful evaluation of the p53 in the diagnostic biopsy enabled diagnosis of ‘transformation’ from low grade serous carcinoma harbouring (subclonal) mutant *TP53*

Clinicopathologic, Immunohistochemical, and Molecular Characteristics of Ovarian Serous Carcinoma With Mixed Morphologic Features of High-grade and Low-grade Serous Carcinoma

Shabnam Zarei, MD, Yan Wang, MD,† Sarah M. Jenkins, MS,‡ Jesse S. Voss, BS,§ Sarah E. Kerr, MD,‖ and Debra A. Bell, MD§*

(*Am J Surg Pathol* 2020;44:316–328)

Indeterminate grade serous carcinoma (IGSC)?

A clinically aggressive variant of serous carcinoma.

Different pathogenetic pathway to HGSC and LGSC?

IHC of major ovarian carcinoma histotypes
WHO 2020

Histotype	Proportion of cases showing immunohistochemical expression				
	PAX8	WT1	p53 abnormal	Napsin A	PR
HGSC	95%	97%	94-98%	1%	37-42%
LGSC	87-100%	98-100%	0%	0%	59-60%
Endometrioid Ca	82%	10-14%	14-15%	3-8%	81-85%
Clear cell Ca	95%	1%	11-12%	92%	5-7%
Mucinous Ca	39-47% focal & weak	0-1%	61-66%	0-3%	0-4%

Take home message

‘Morphology remains the mainstay in diagnosis’ WHO 2020

Immunohistochemistry not a stand alone diagnostic modality - it plays a supportive but at times a crucial role in aiding diagnosis.

Use a panel– don’t rely on one marker only, but some markers are better discriminators in certain settings.



Thank you for your attention