

Practical Approach to Histotyping and Molecular Characterization of Endometrial Carcinoma

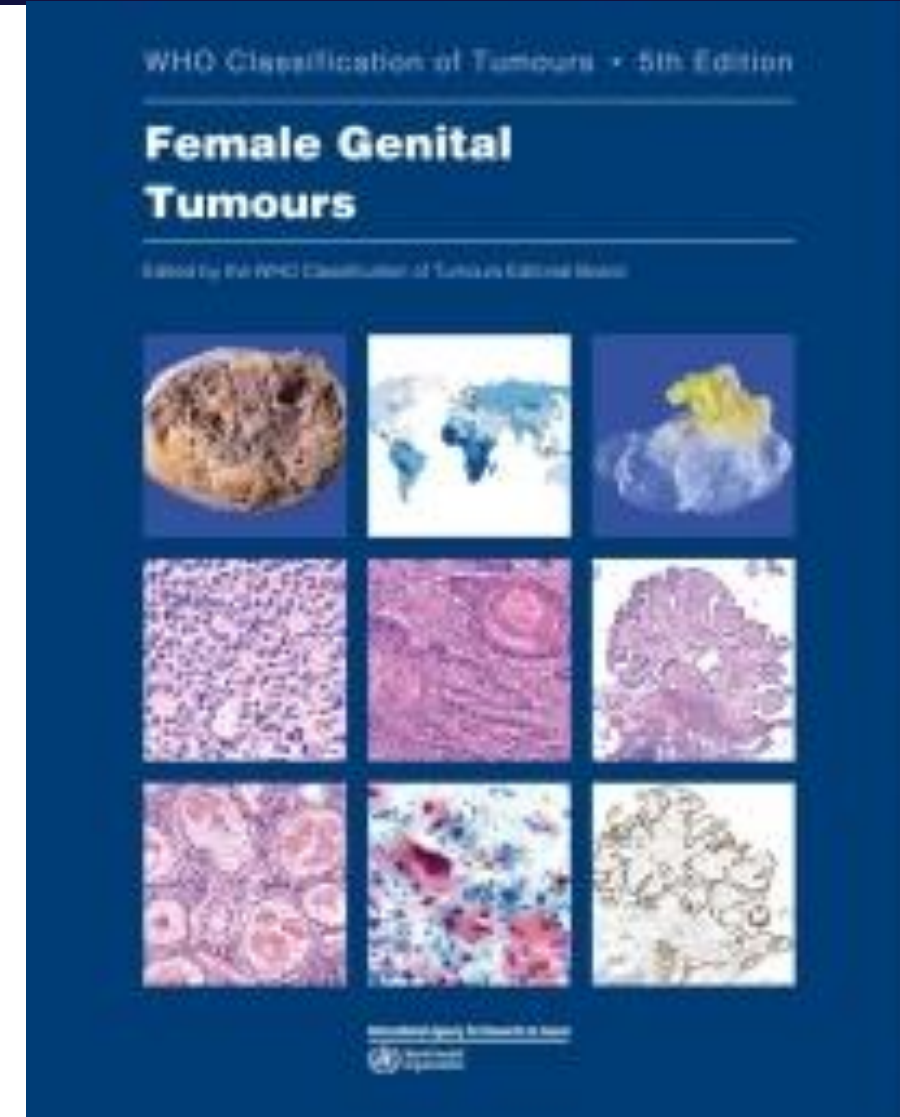
Gulisa Turashvili, MD, PhD

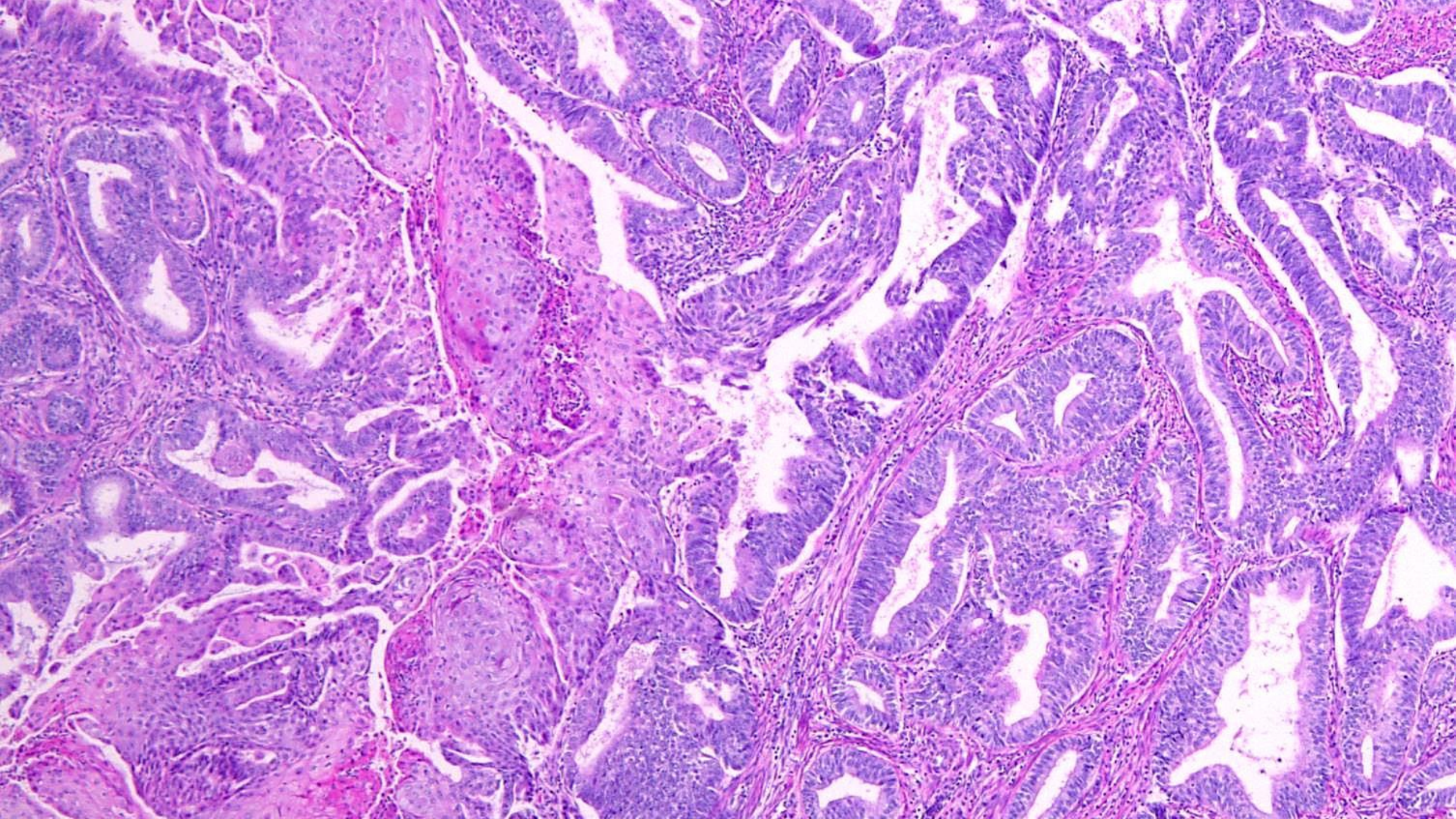
Massachusetts General Hospital
Harvard Medical School, Boston, MA

gturashvili@mgh.harvard.edu

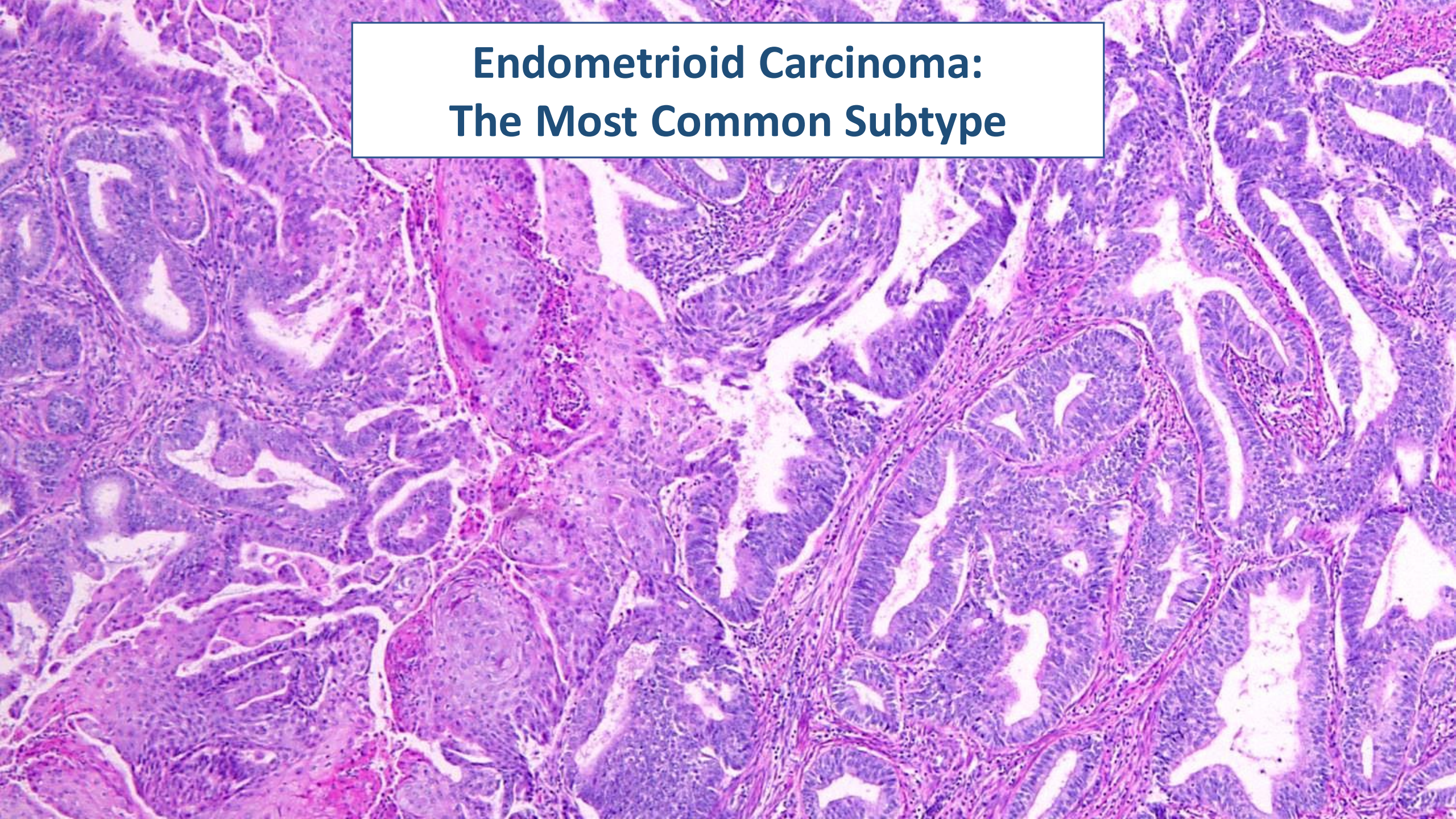
WHO Classification of Endometrial Carcinoma

- ✓ Endometrioid carcinoma***
- ✓ Serous carcinoma
- ✓ Clear cell carcinoma
- ✓ Un-/dedifferentiated carcinoma
- ✓ Carcinosarcoma
- ✓ Mixed carcinoma
- ✓ Other endometrial carcinomas:
 - Mesonephric-like
 - Mucinous carcinoma, gastric (gastrointestinal)-type
 - Squamous cell carcinoma
 - *Other*



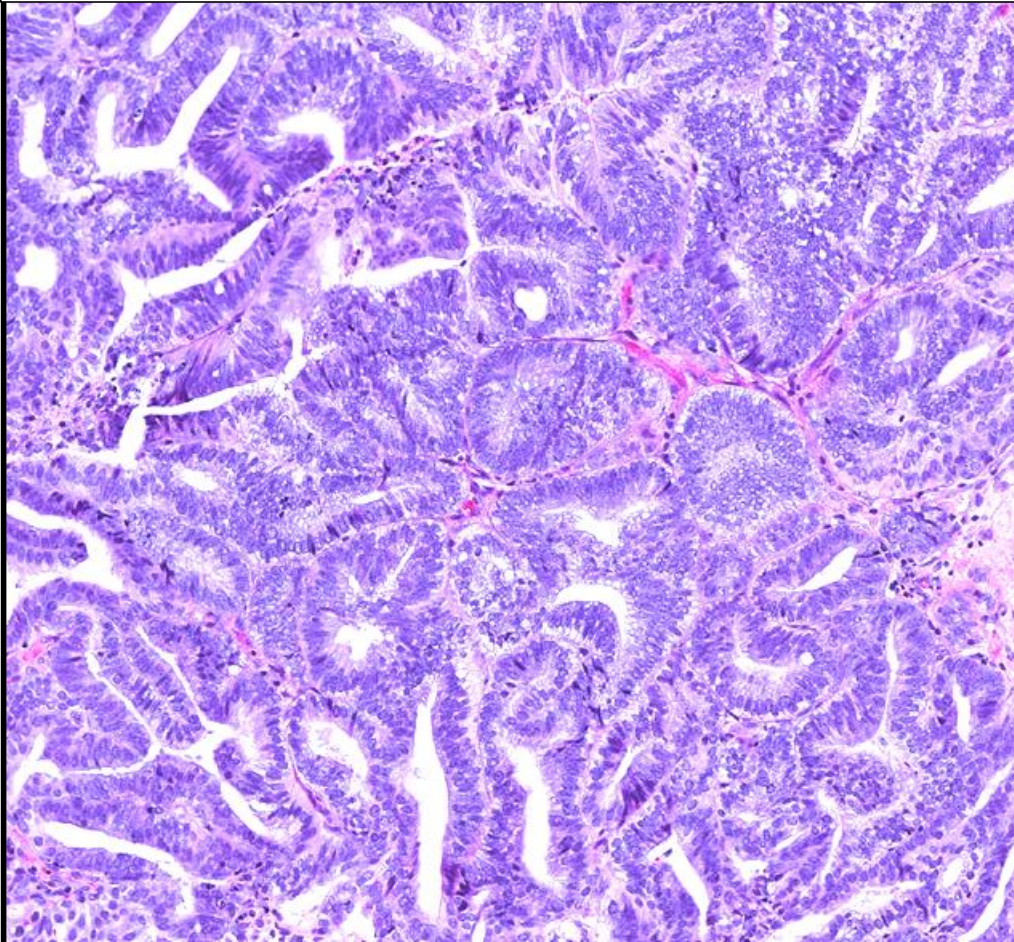


Endometrioid Carcinoma: The Most Common Subtype

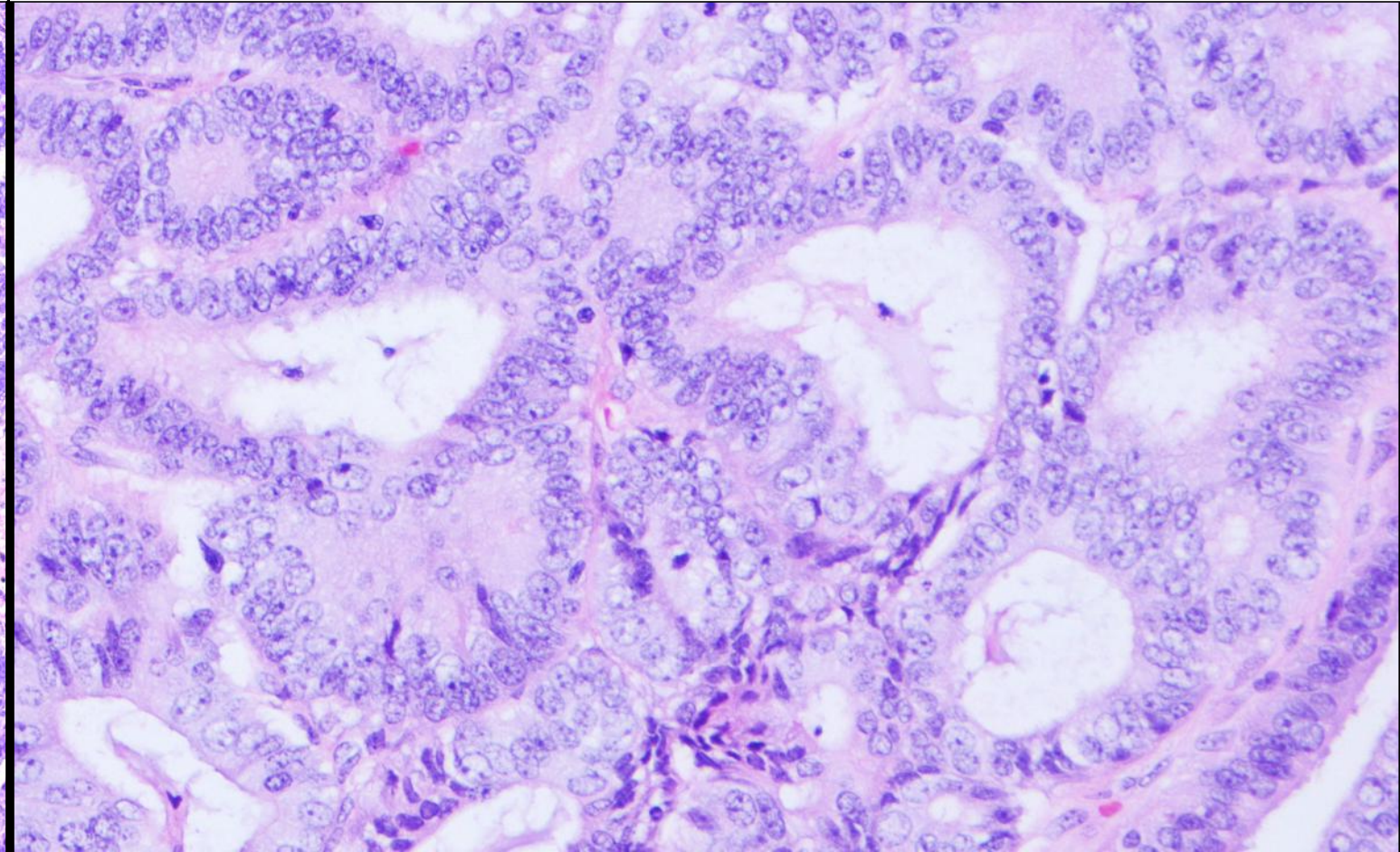


Endometrioid Carcinoma

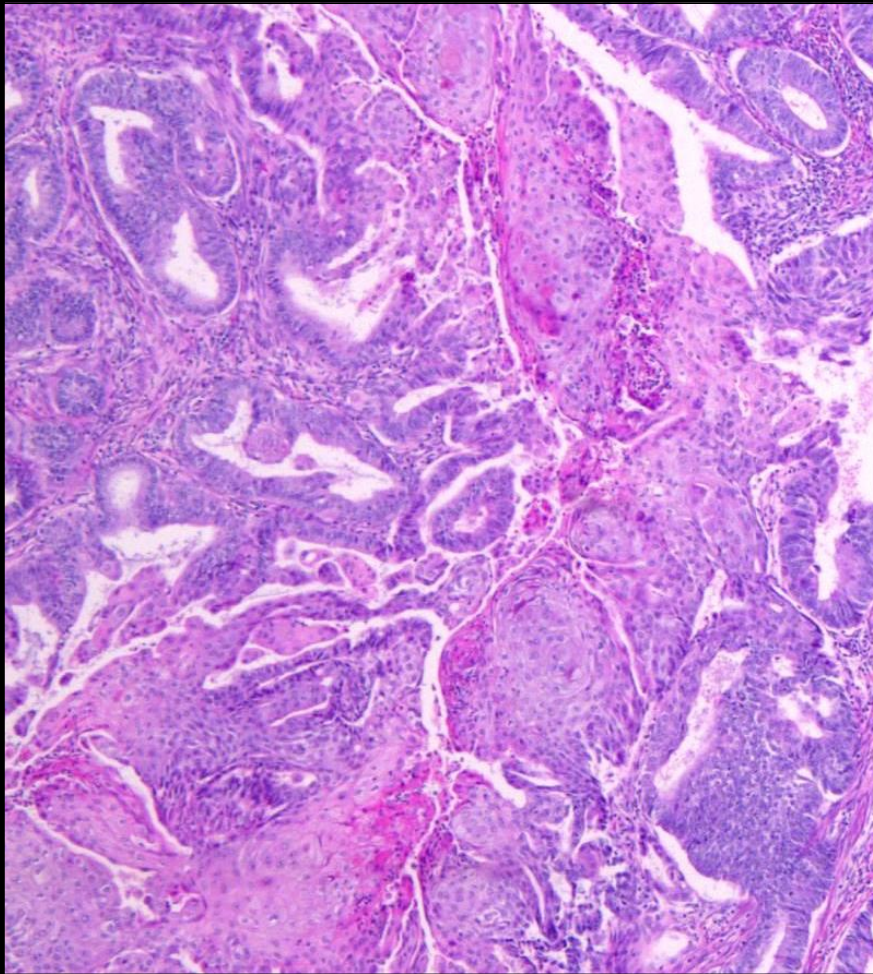
Complex proliferation of endometrioid glands with well-defined luminal borders, no intervening stroma



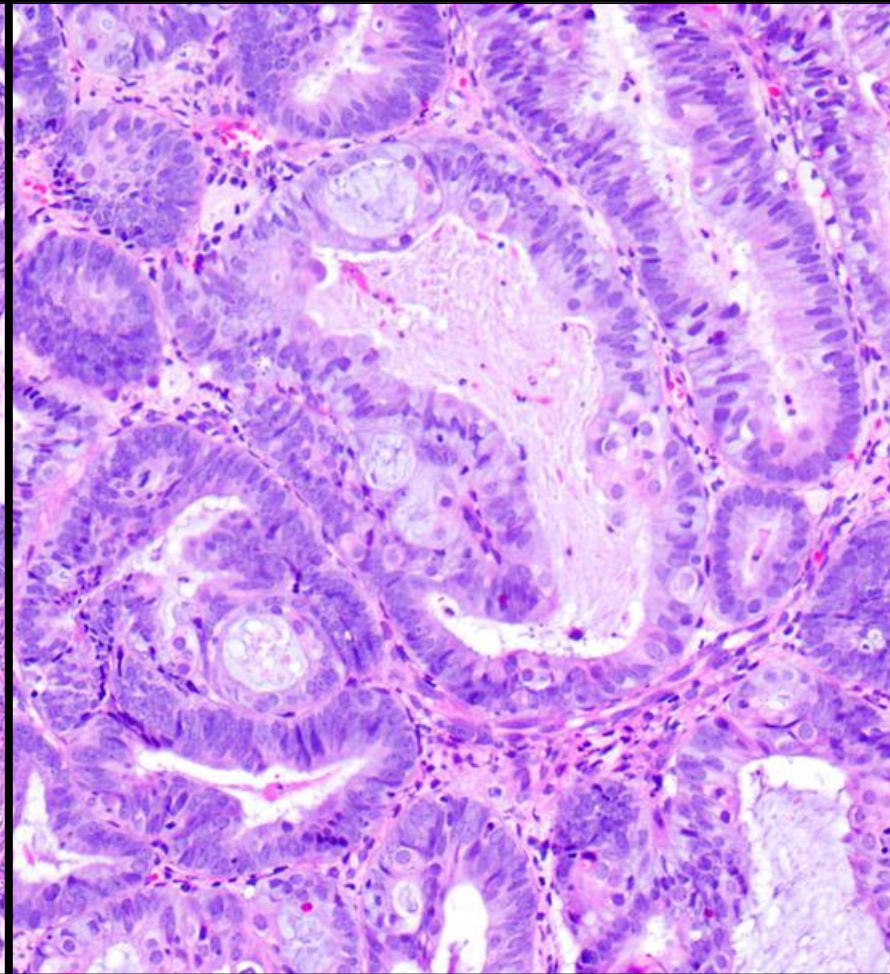
Columnar epithelium with tall nuclei and variable cytoplasm, preserved nuclear polarity, usually mild cytologic atypia and low mitotic activity



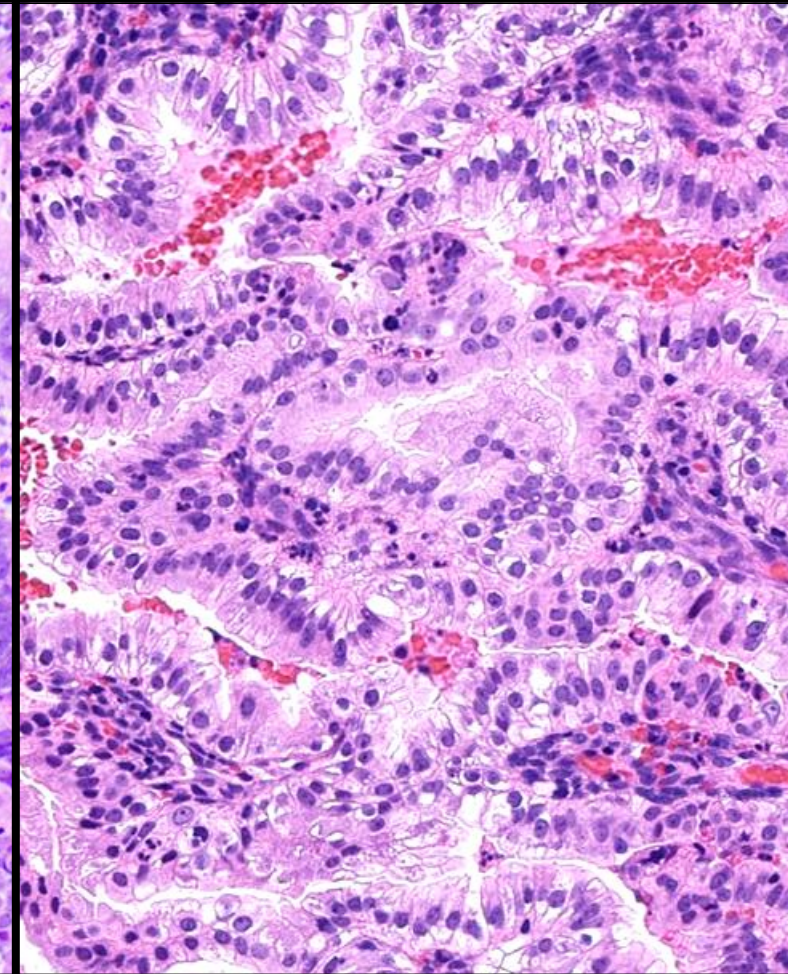
Squamous



Mucinous



Secretory



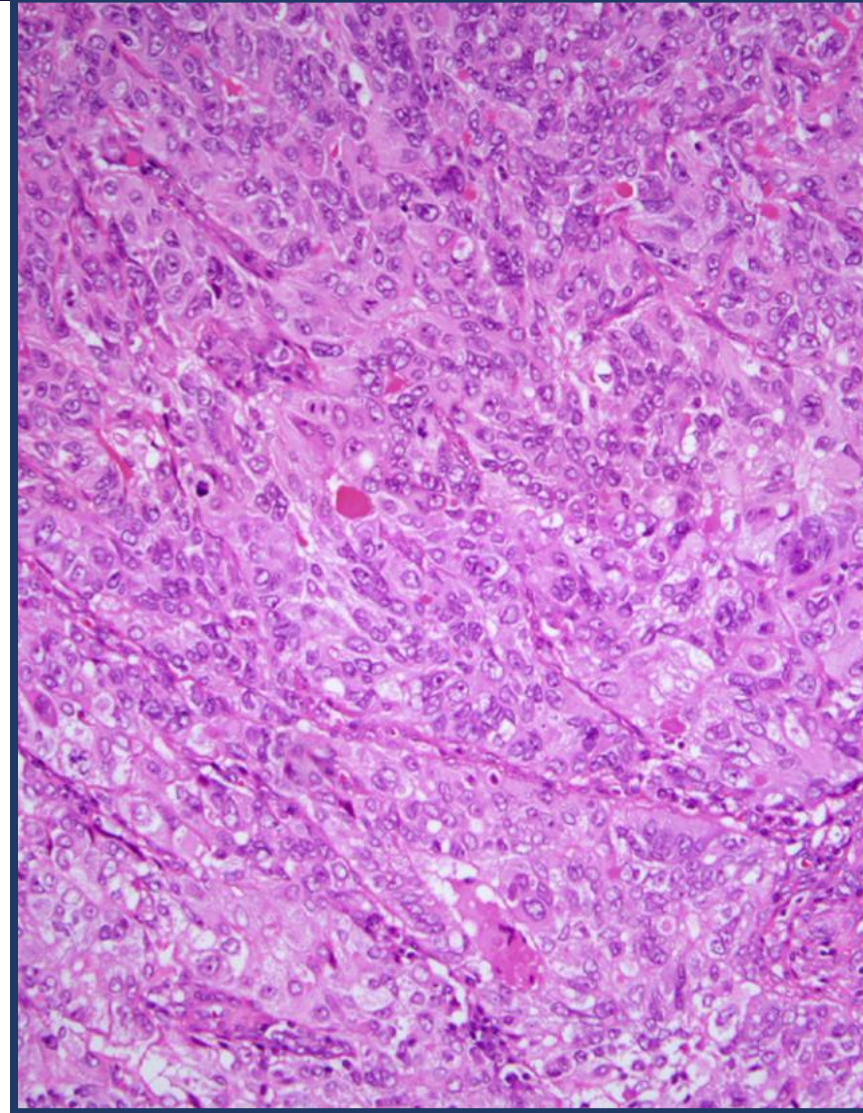
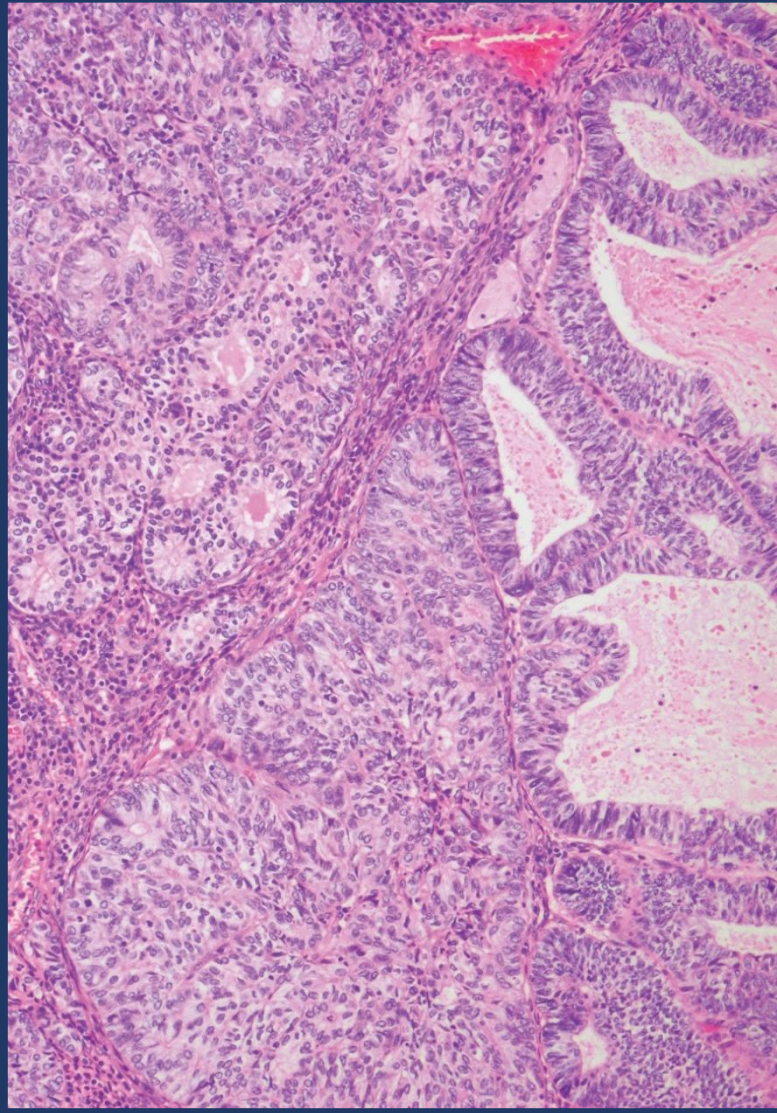
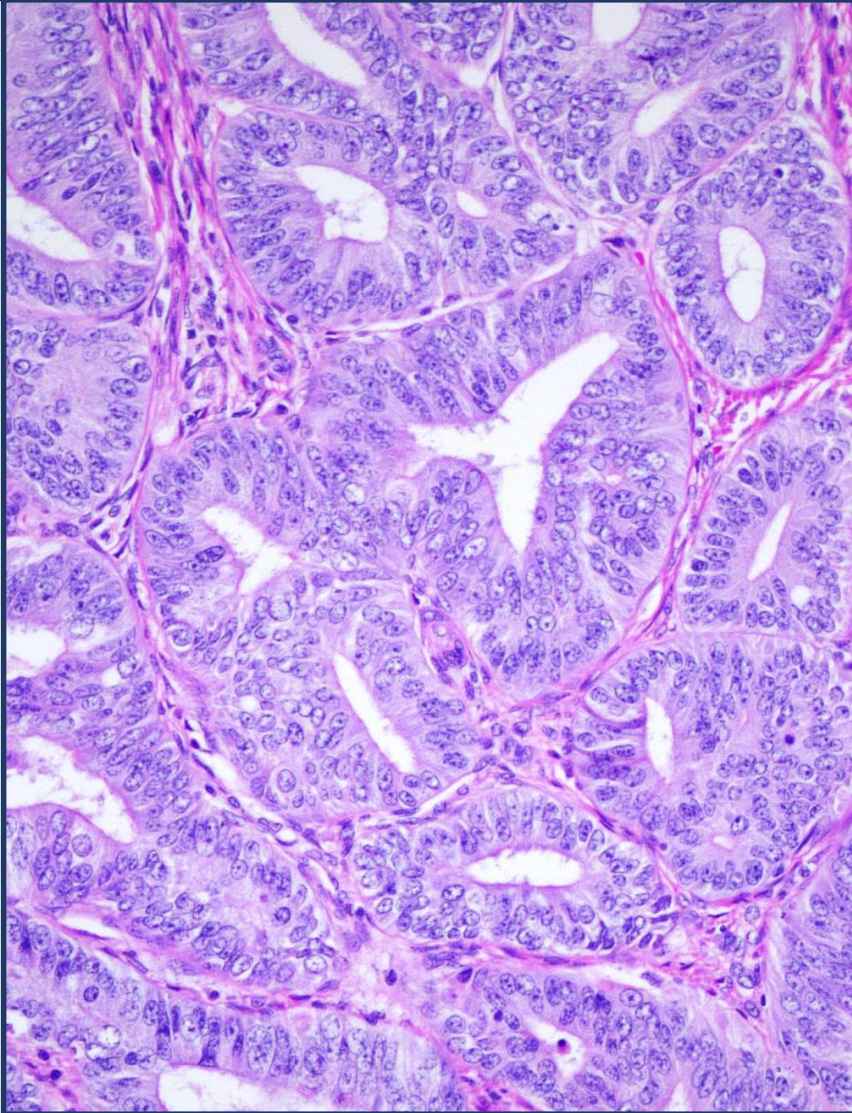
WHO Desirable Criteria: Some Degree of Squamous, Mucinous or Secretory Differentiation

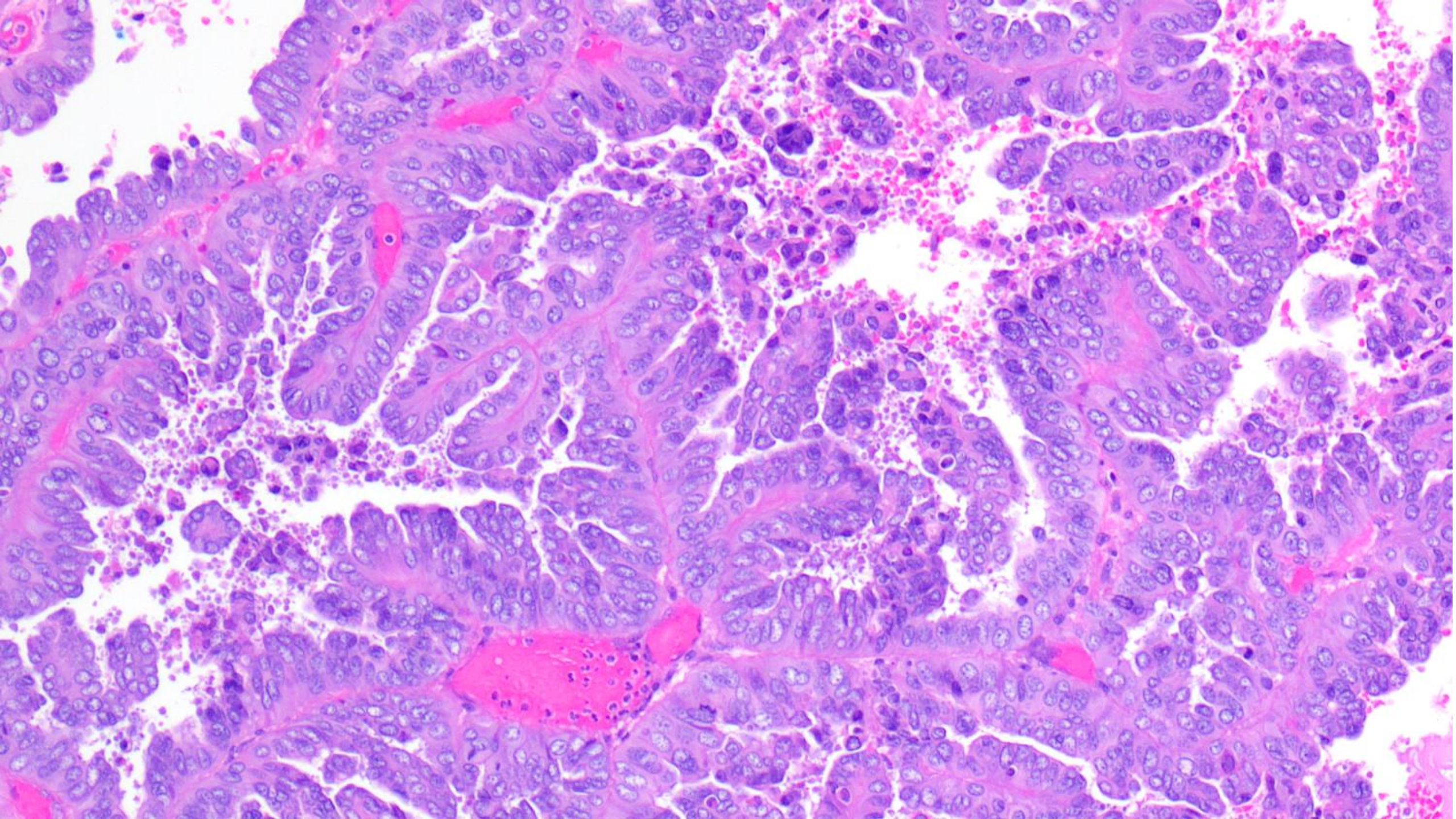
FIGO Grading Based on Non-Squamous Solid Growth:

1 (<5%)

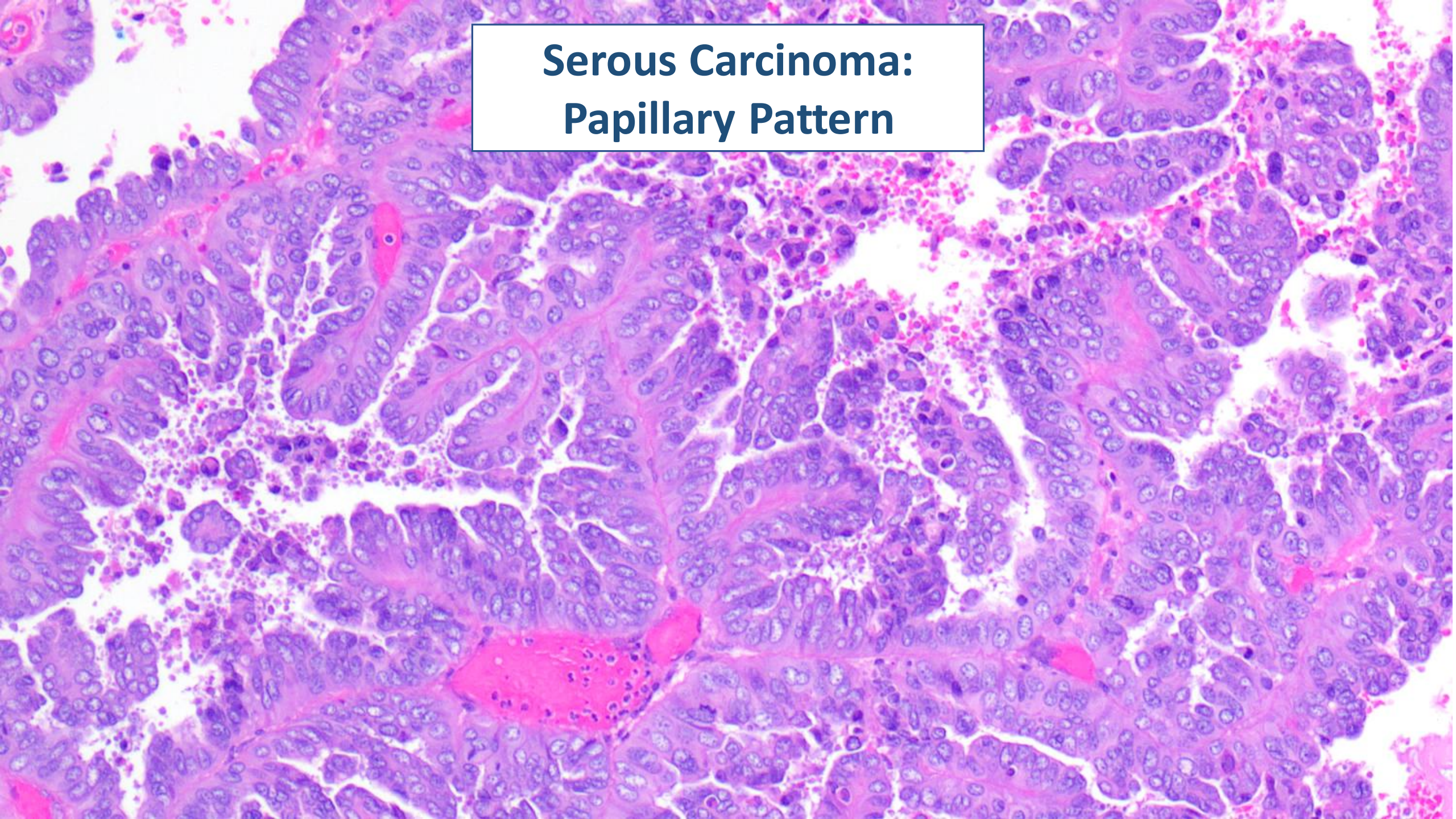
2 (6-50%)

3 (>50%)





Serous Carcinoma: Papillary Pattern

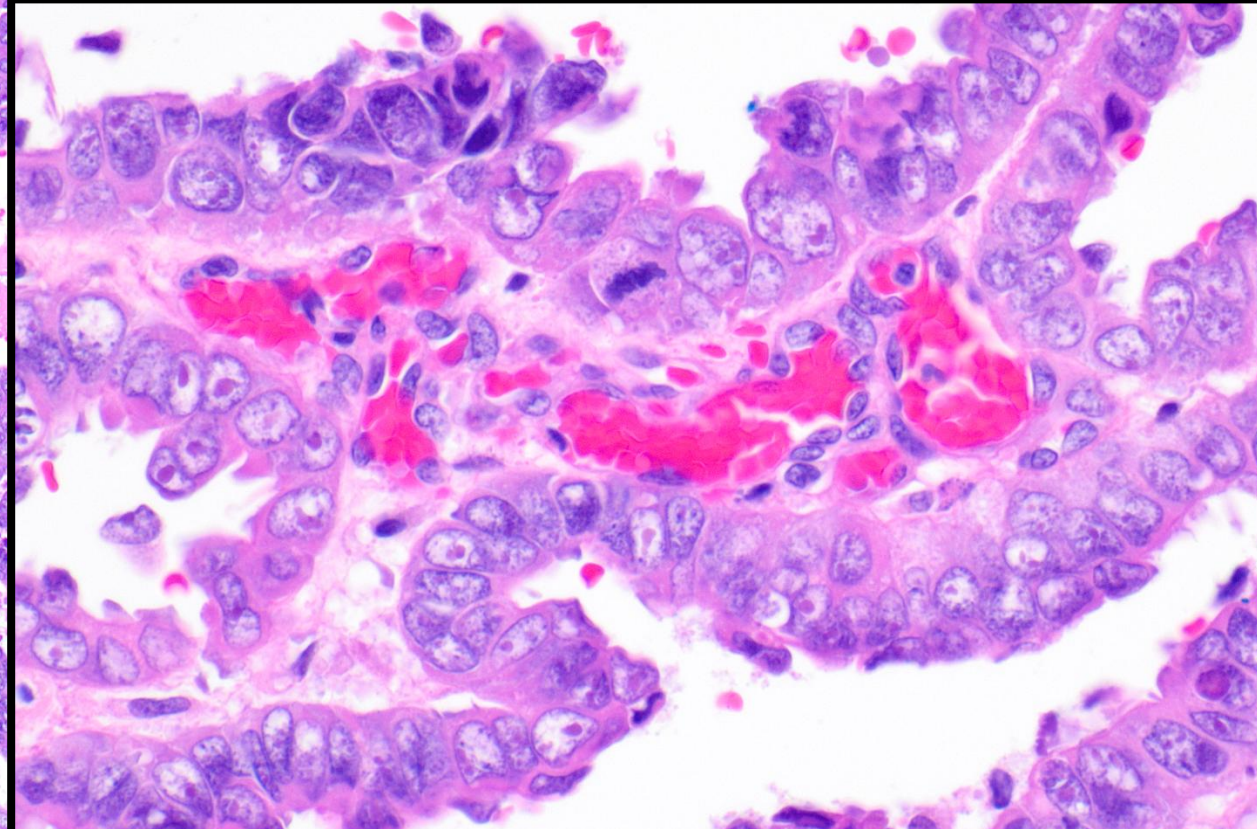
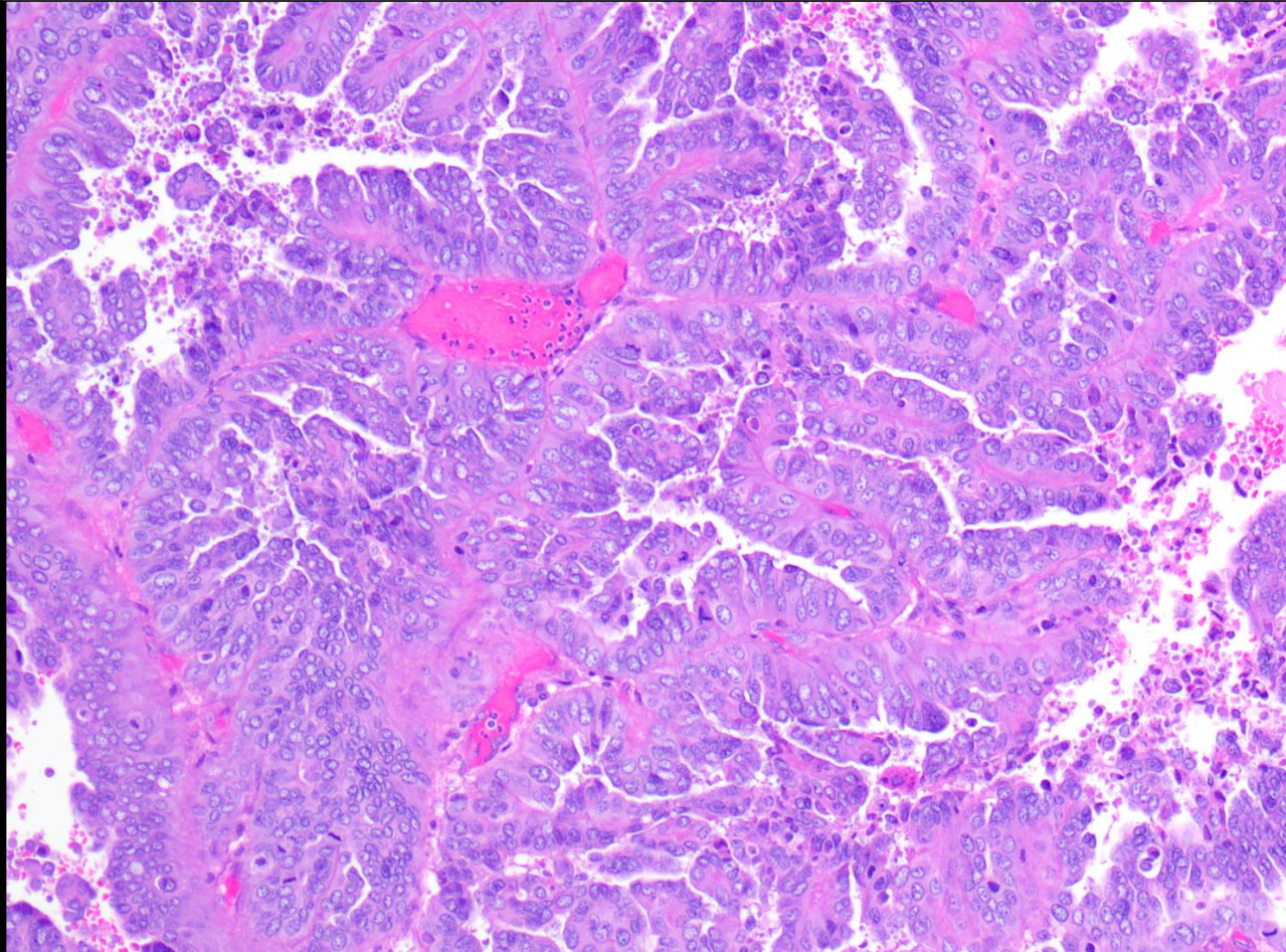


Serous Carcinoma: Papillary Pattern

Glands with papillae, cellular tufts, detachment, budding, loss of nuclear polarity, serrated or slit-like luminal borders

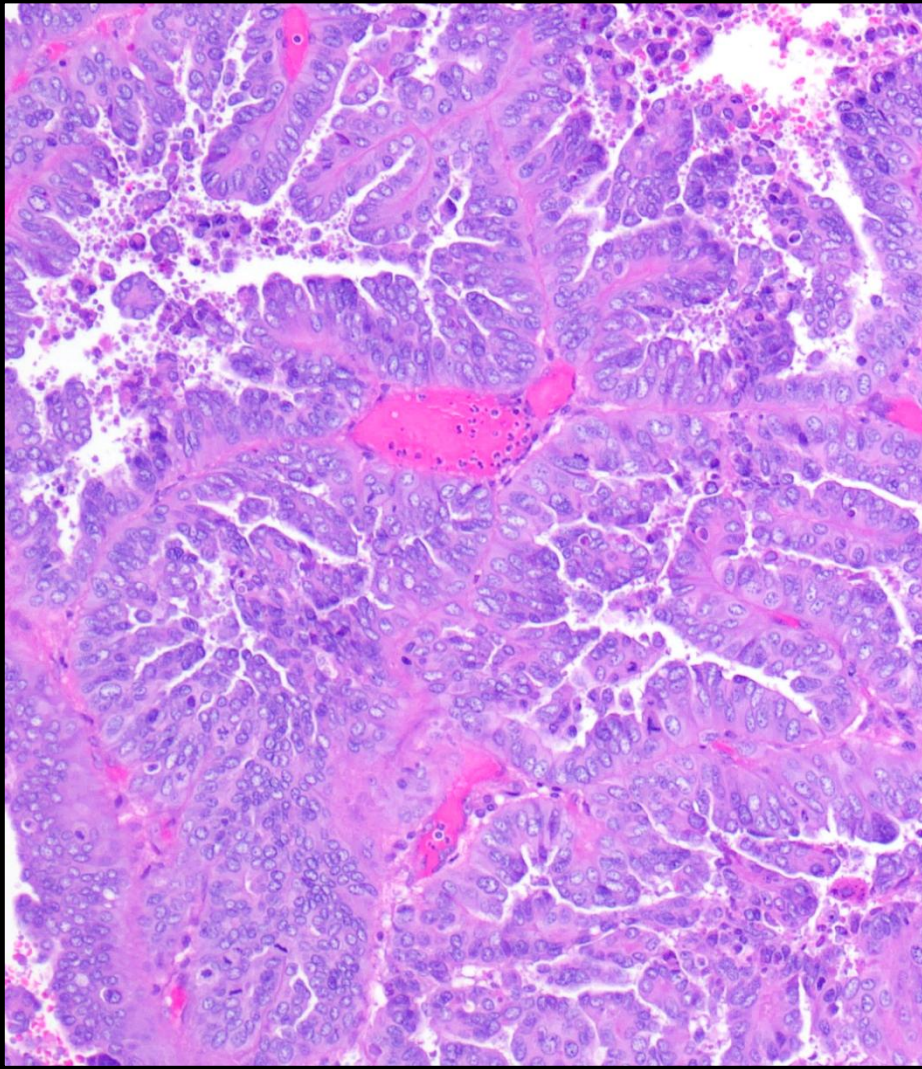
**High-grade
cytologic atypia**

- ✓ High N/C ratio
- ✓ Pleomorphism
- ✓ Hyperchromasia
- ✓ Macronucleoli
- ✓ Mitoses ($\pm$ atypical)

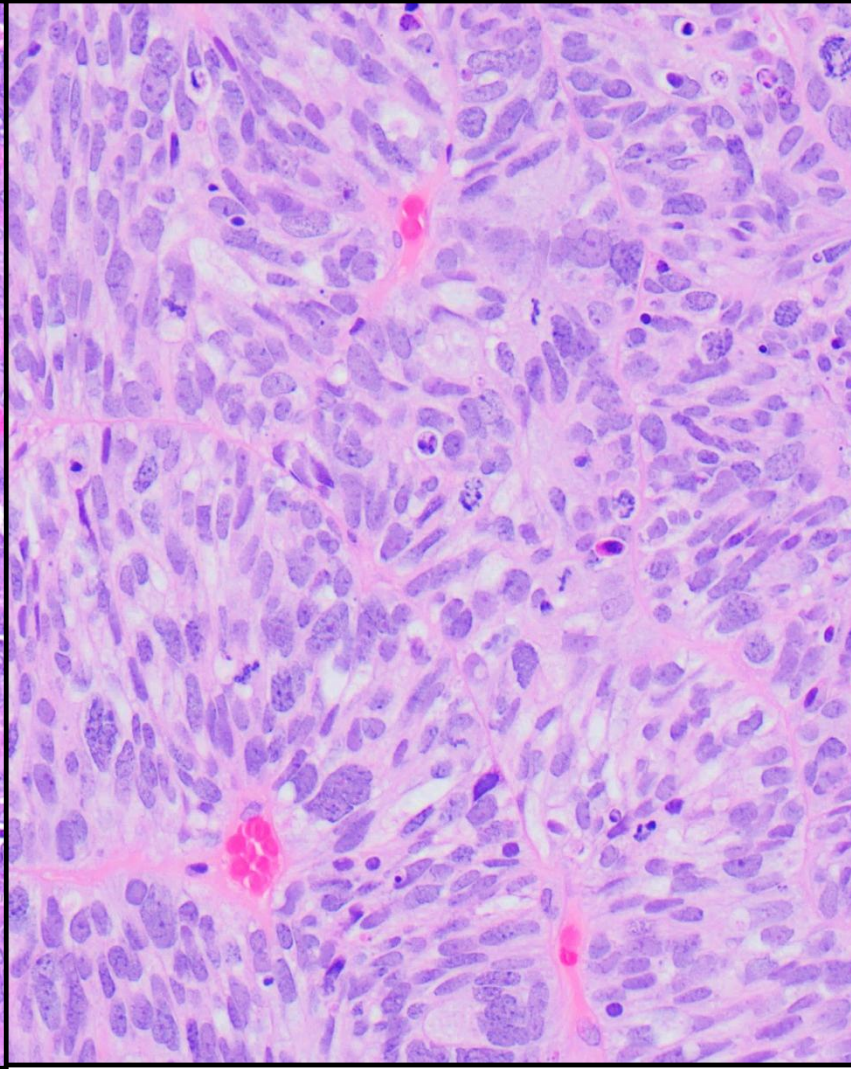


Serous Carcinoma Exhibits Variable Architecture

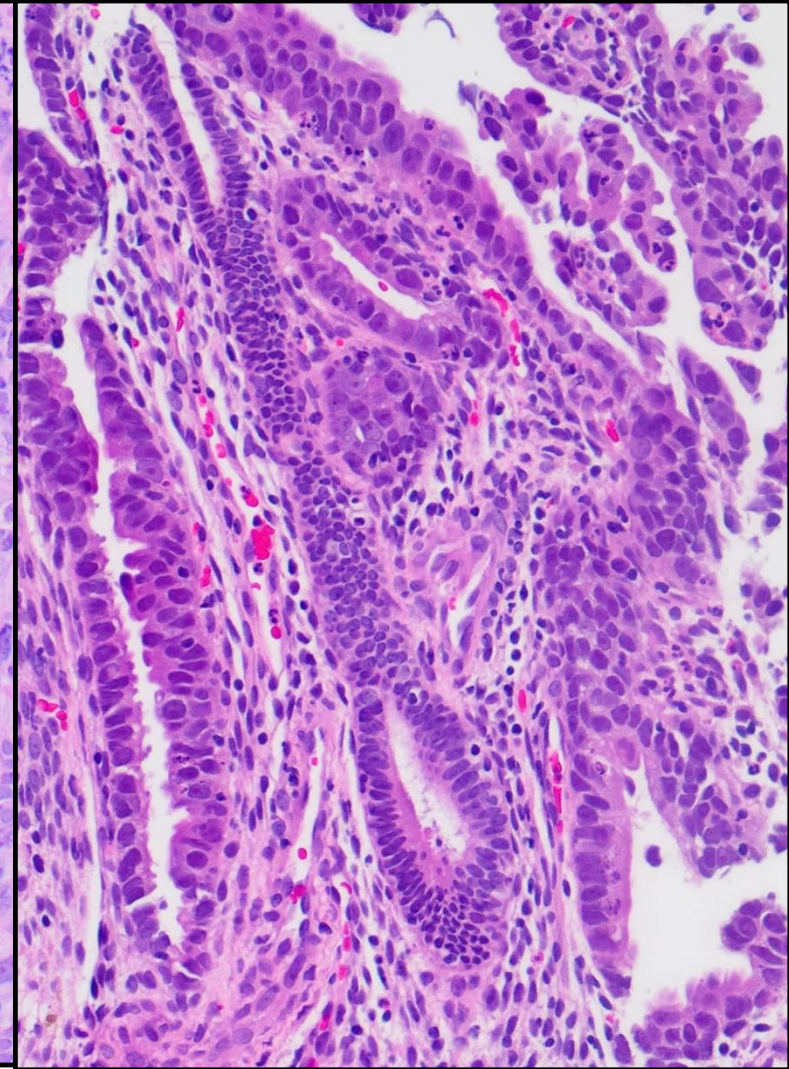
Papillary



Solid

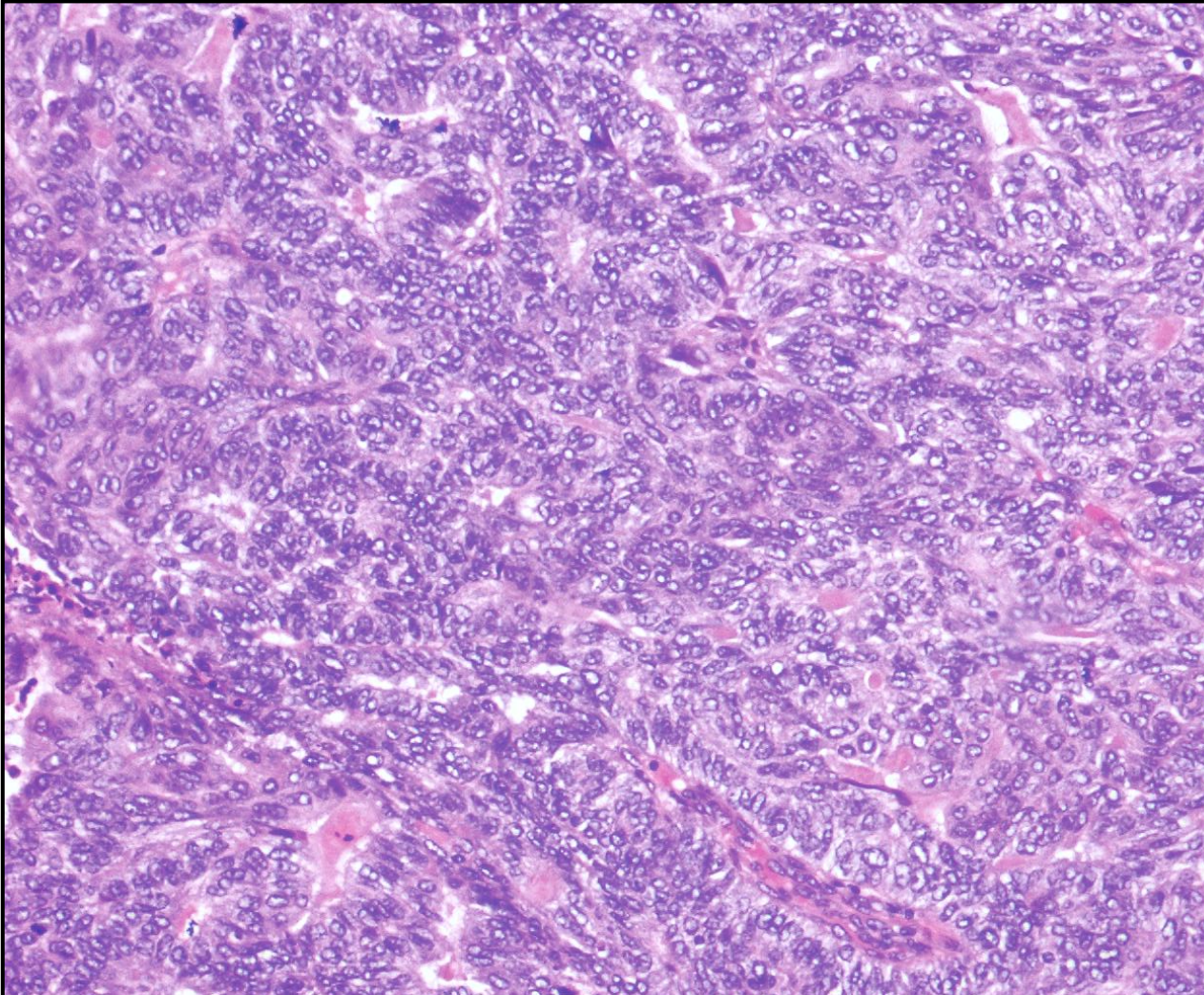


Glandular

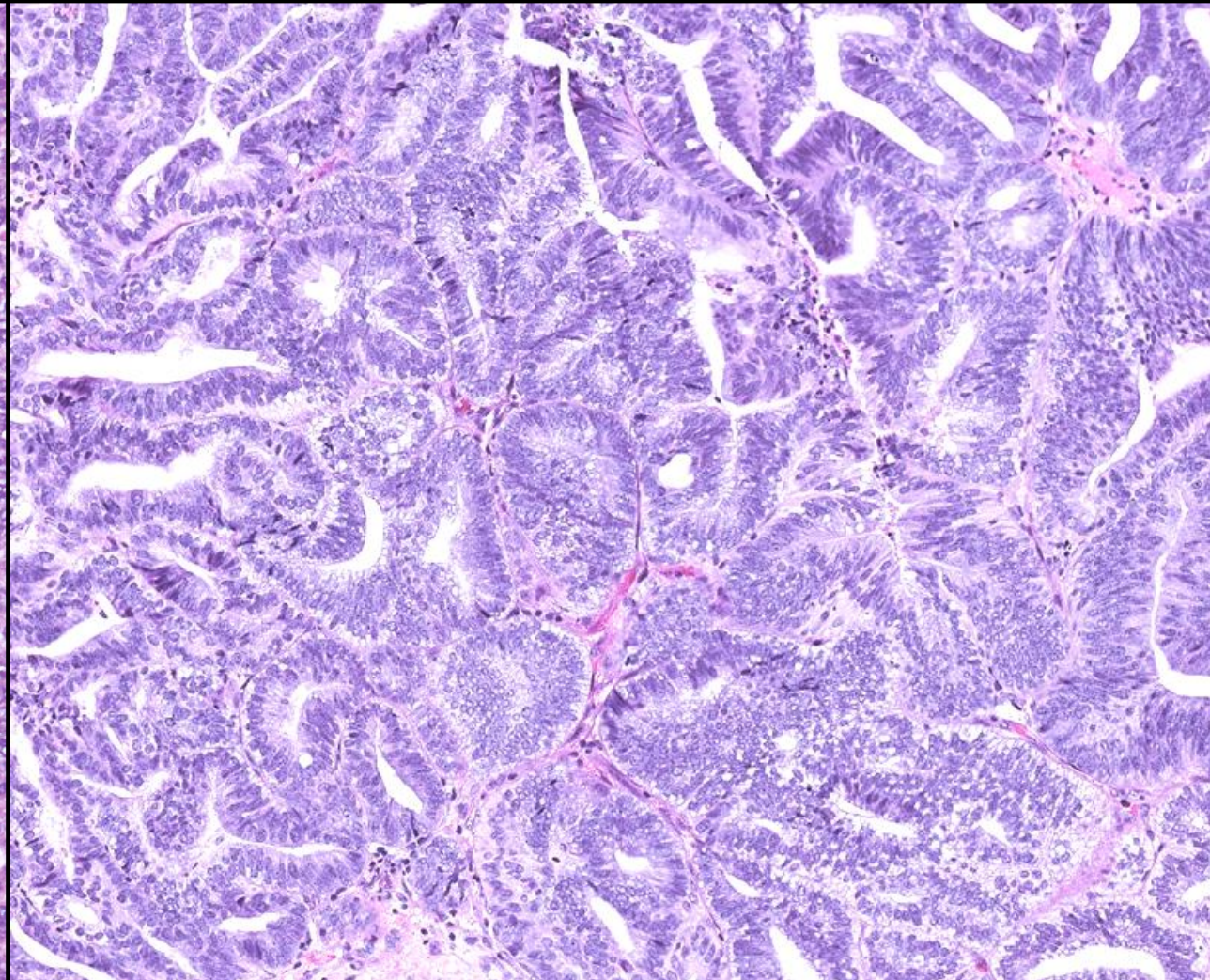


Solid & Glandular Patterns of Serous Carcinoma Should Be Differentiated from Endometrioid Carcinoma

Grade 3

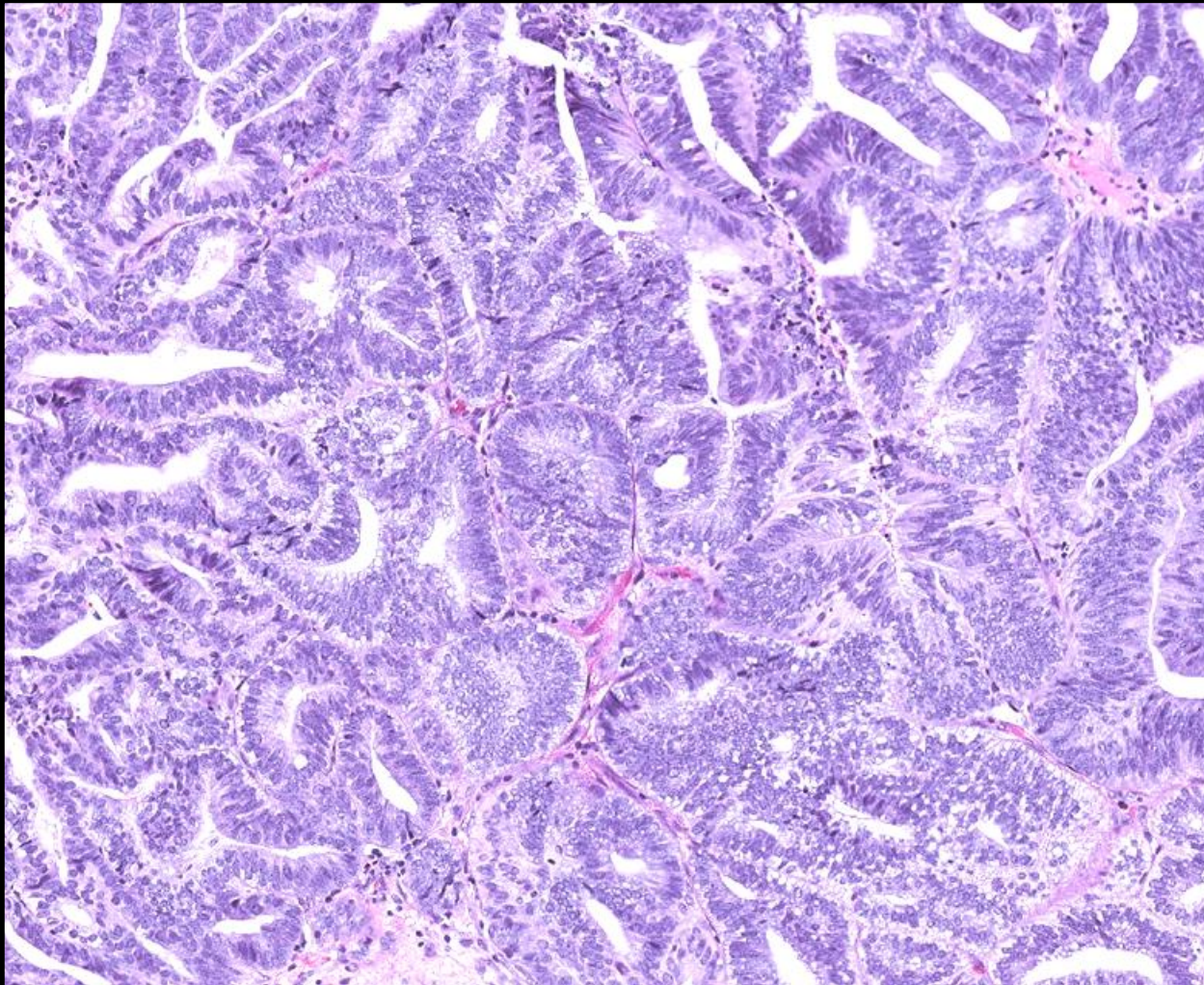


Grade 1

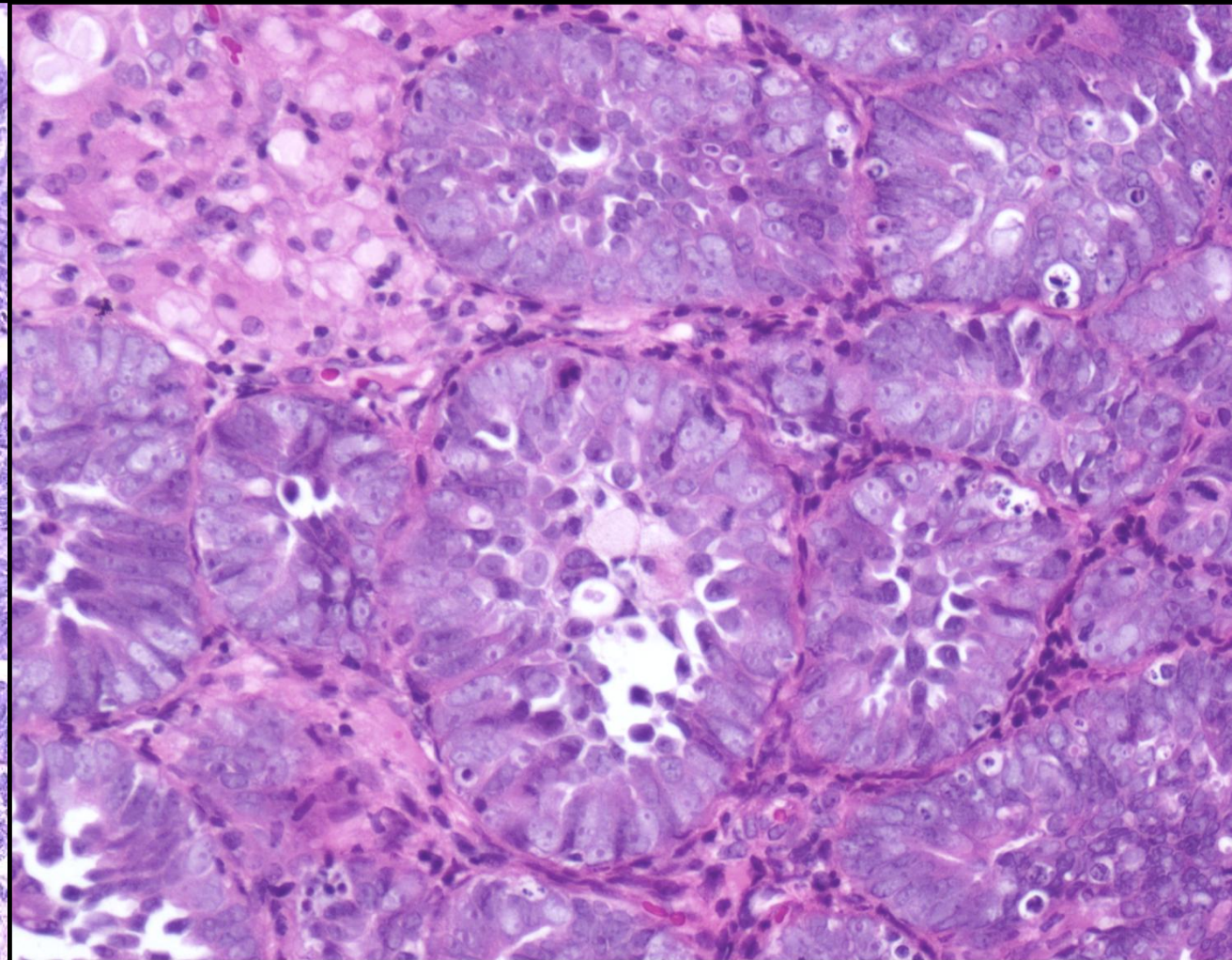


Glandular Serous Carcinoma May Mimic Low-Grade Endometrioid Carcinoma

Grade 1 Endometrioid Carcinoma

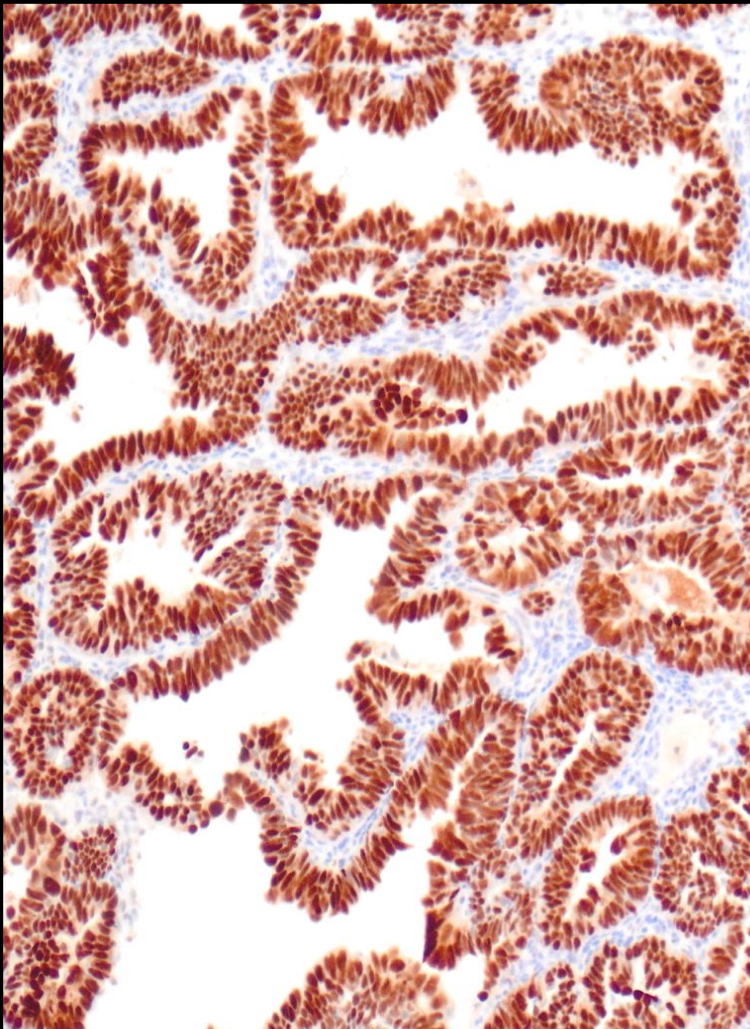


Serous Carcinoma

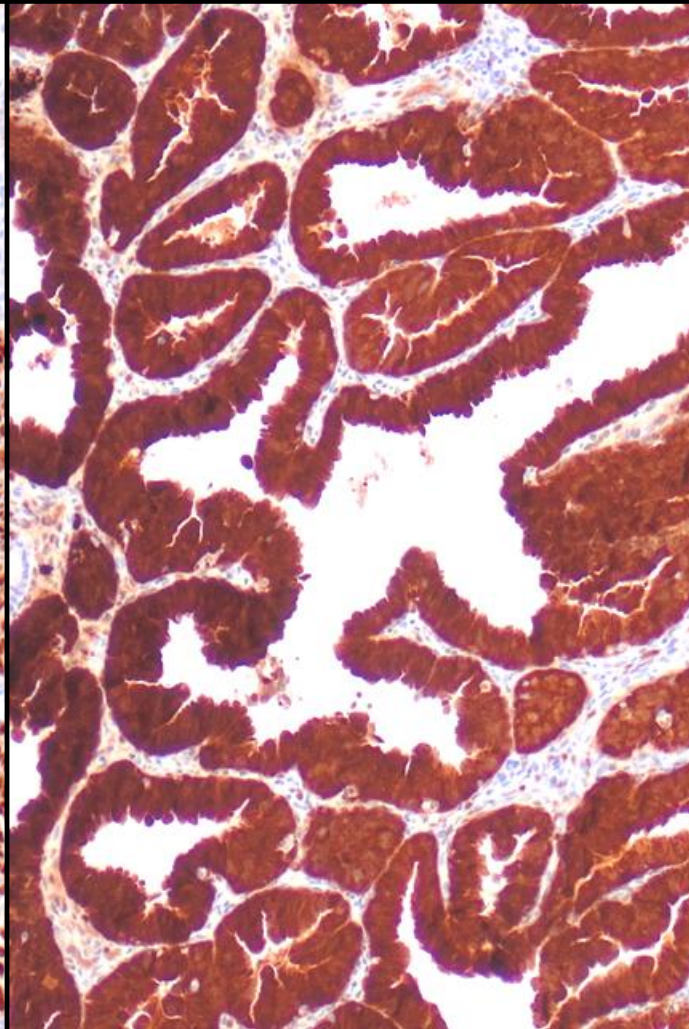


Recognition of Glandular Serous Carcinoma May Require Immunohistochemical Studies

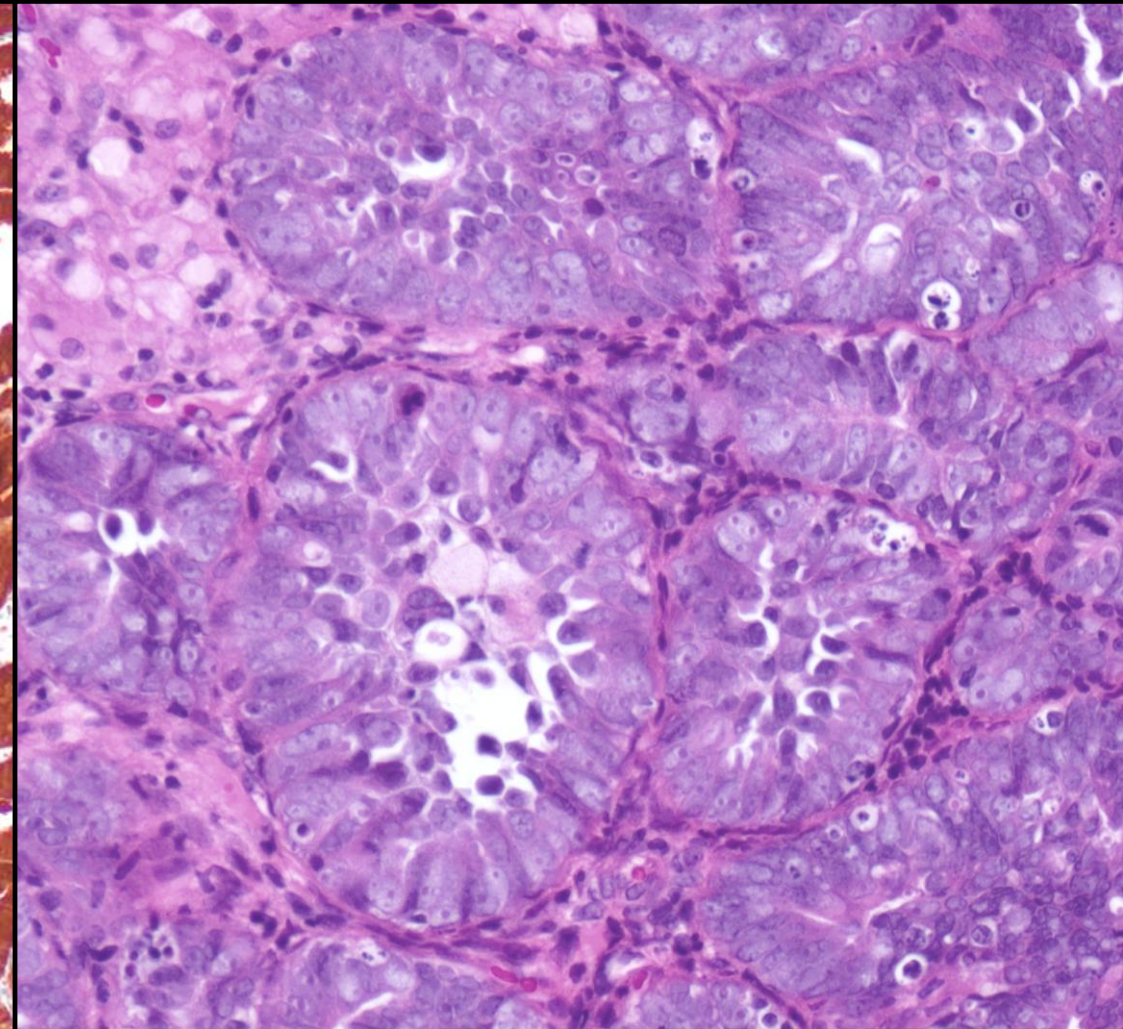
p53



p16

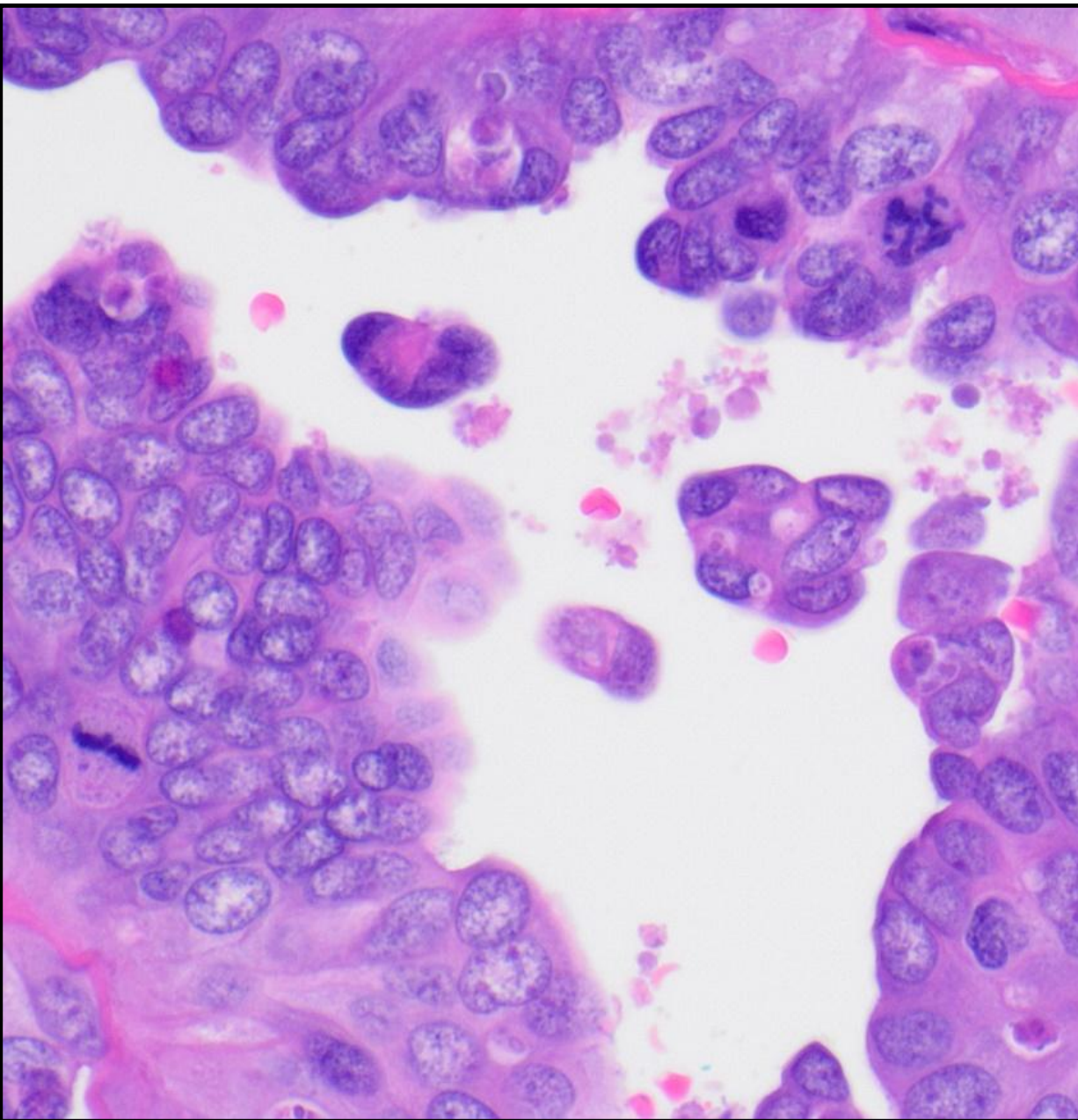


Serous Carcinoma

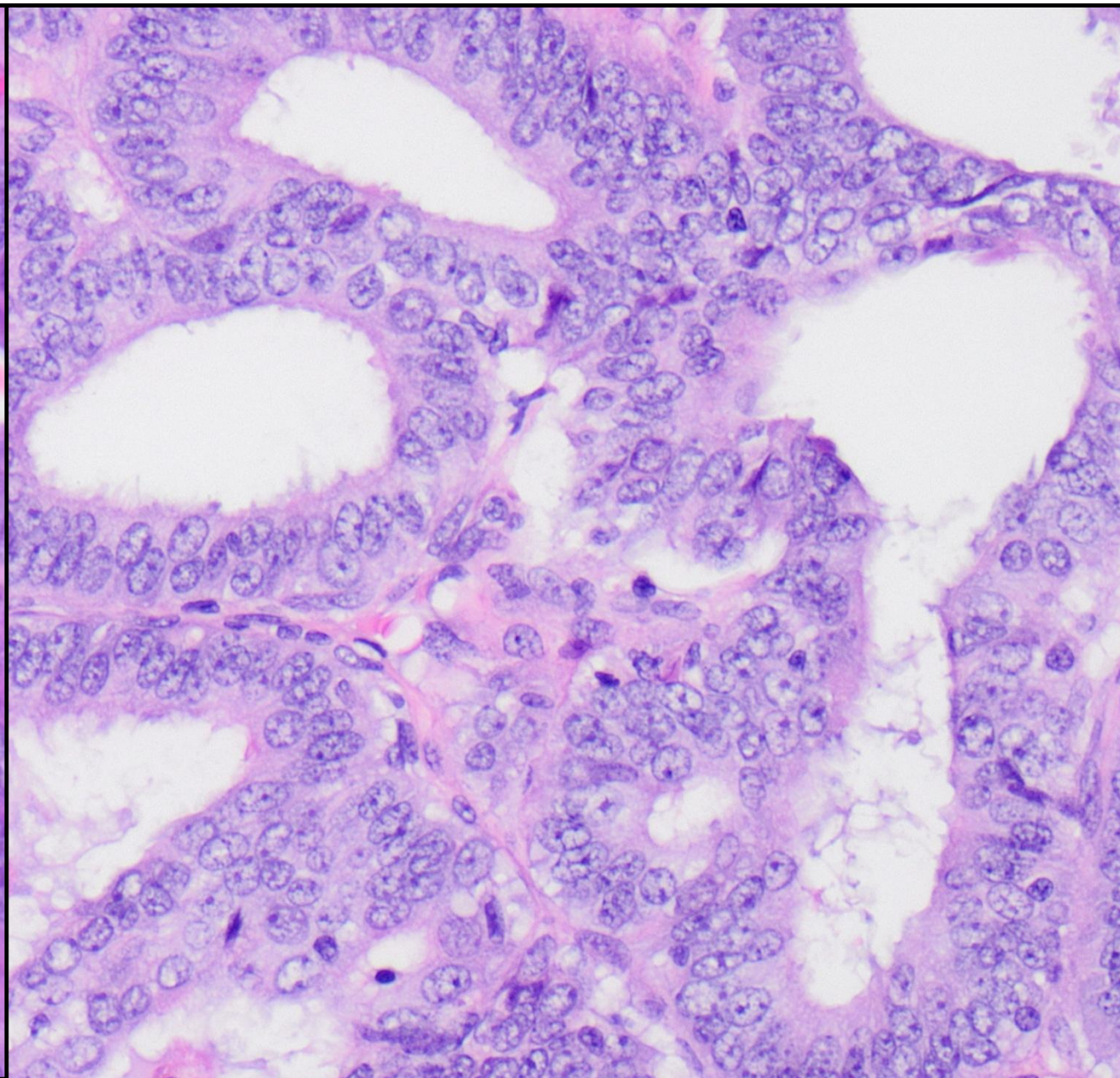


	Glandular Serous	Endometrioid
Architecture vs cytology	Discordant: Low-grade architecture, high-grade atypia	Concordant: Low-grade architecture & atypia
<i>Secondary features</i>		
Nuclear polarity	Lost	Preserved
Cell detachment, budding, tufting	Present	Absent
Luminal borders	Slit-like	Well-defined
Squamous/mucinous/secretory features	Absent	Usually present
Background	Atrophy, polyp	Atypical hyperplasia

Glandular Serous

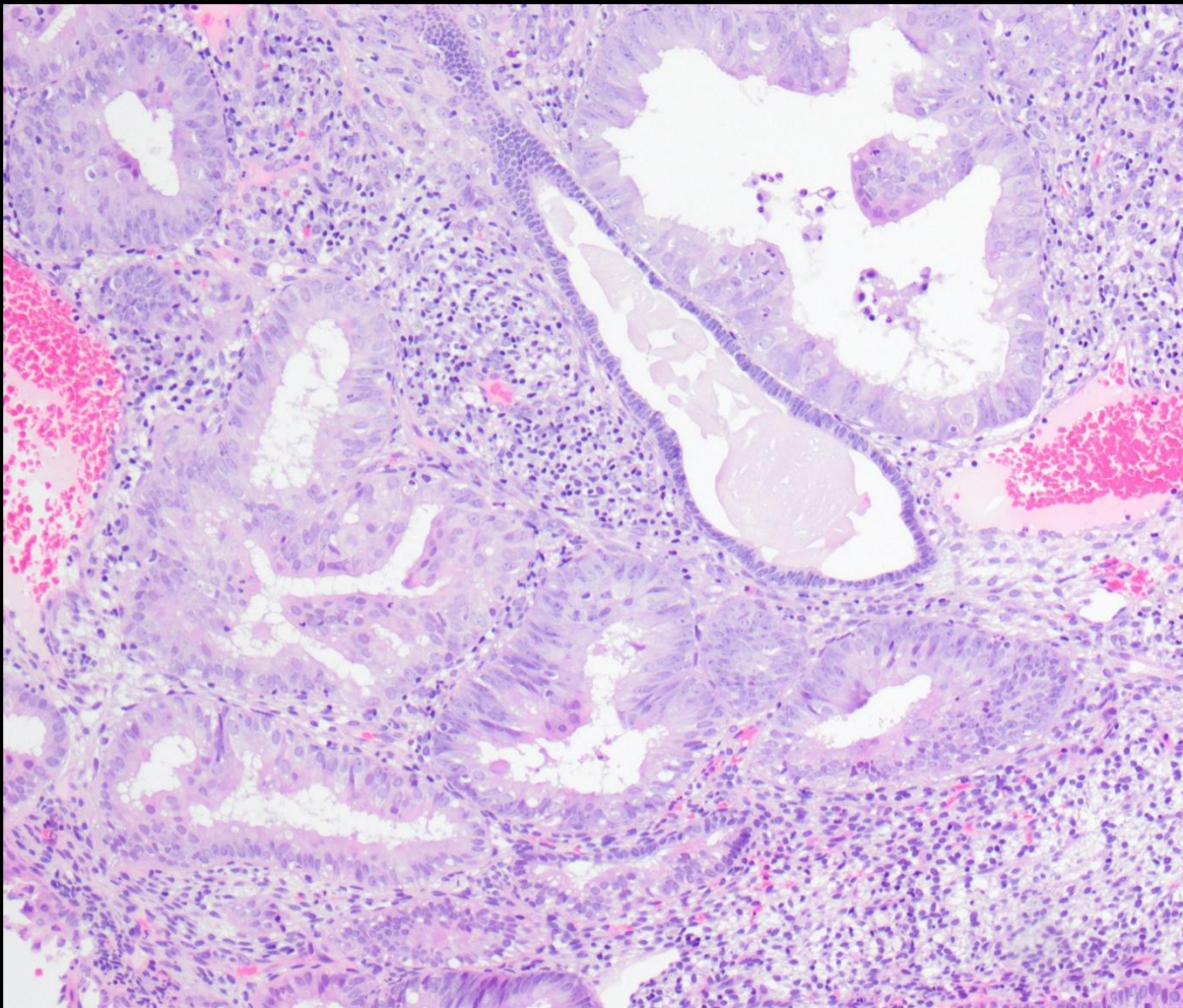


Endometrioid

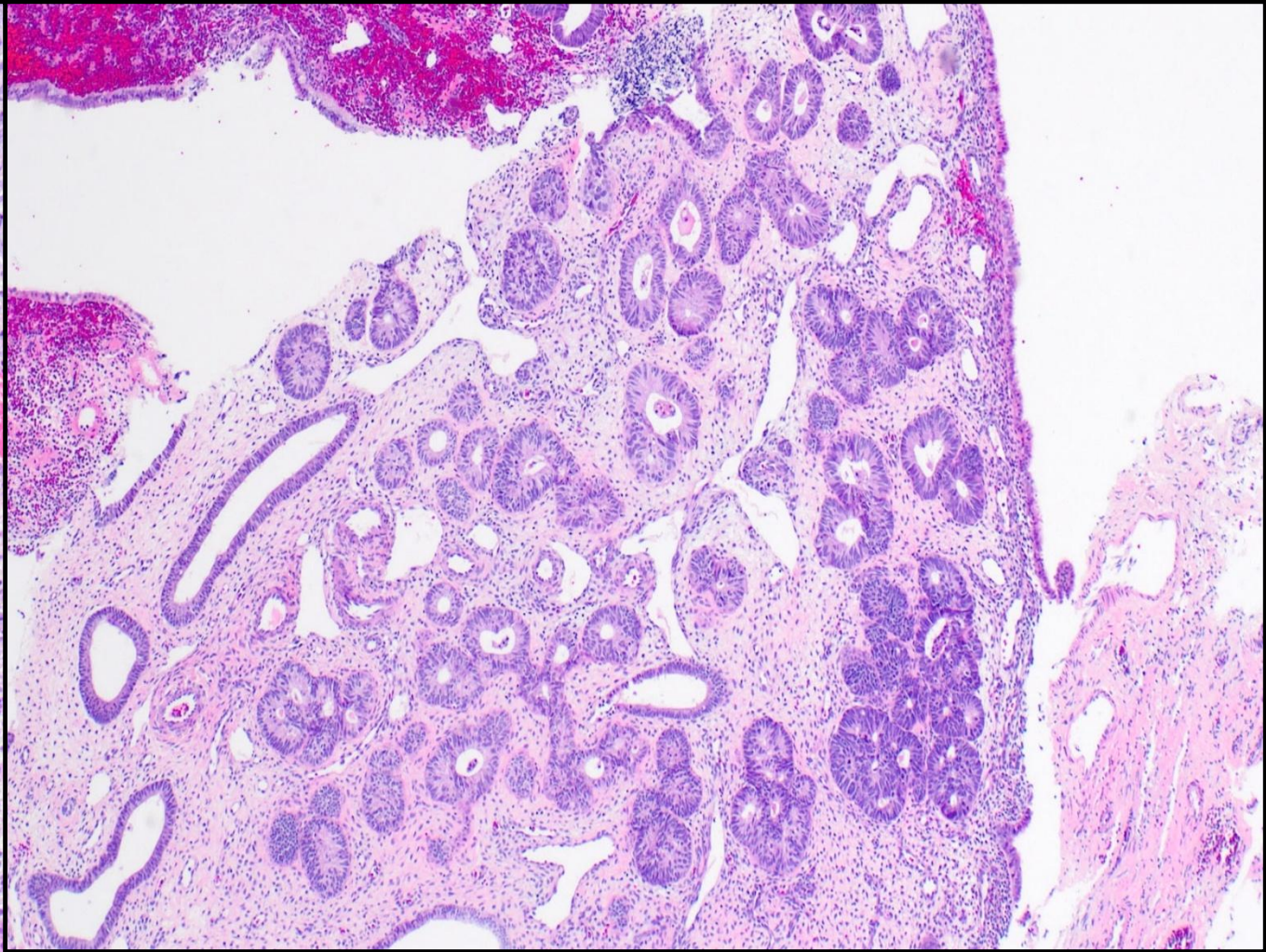


Glandular Serous Carcinoma May Mimic Atypical Hyperplasia

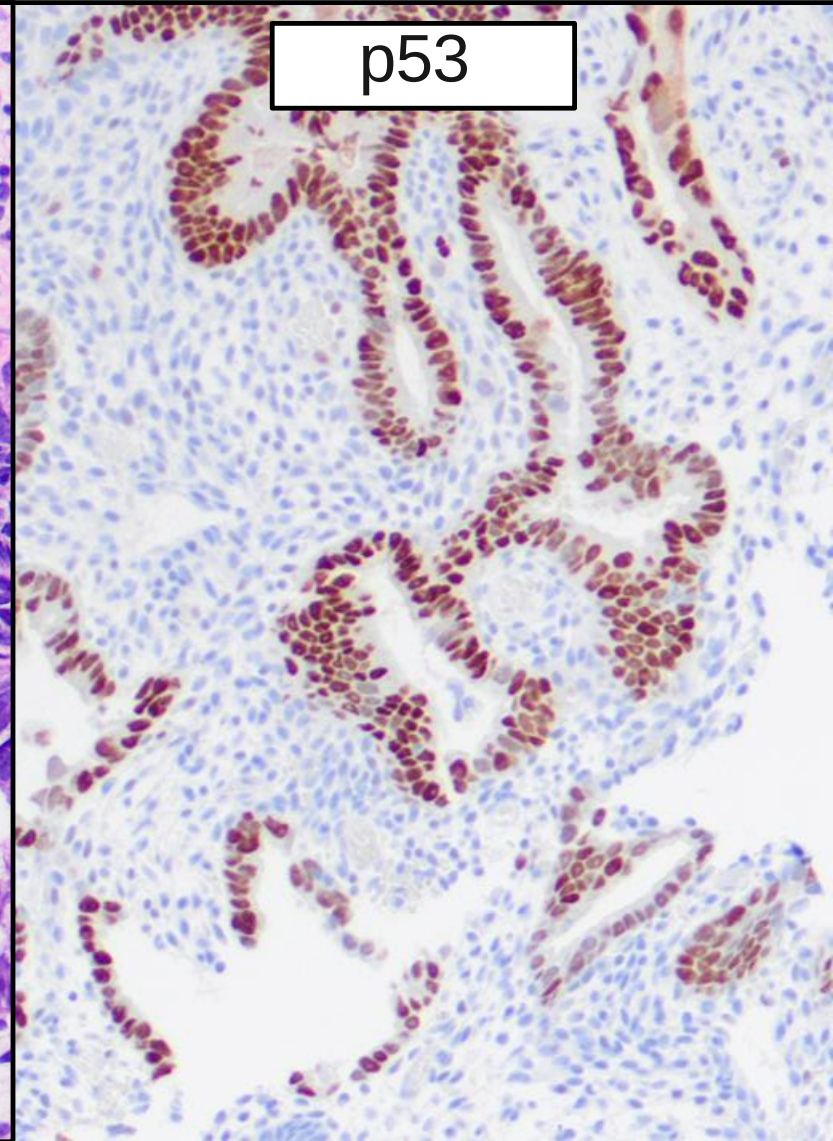
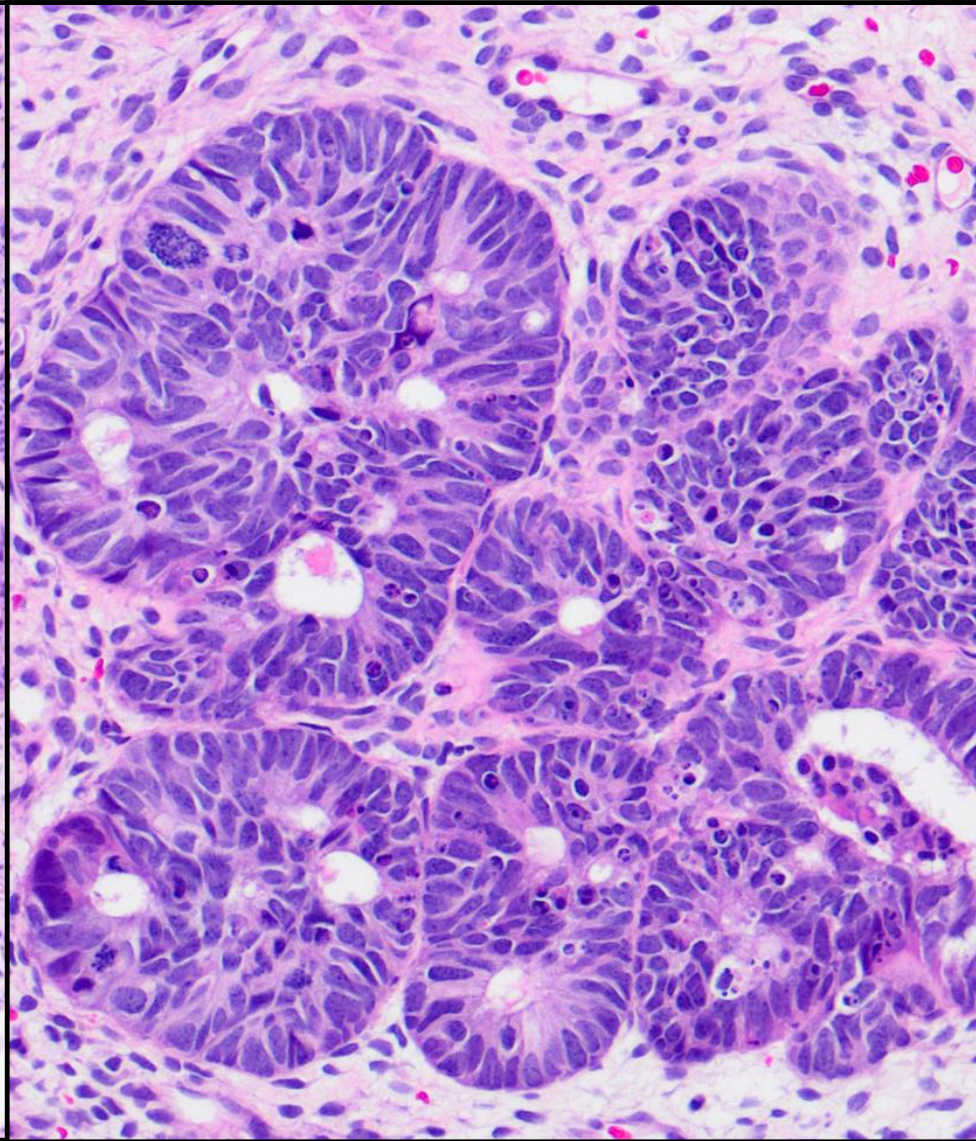
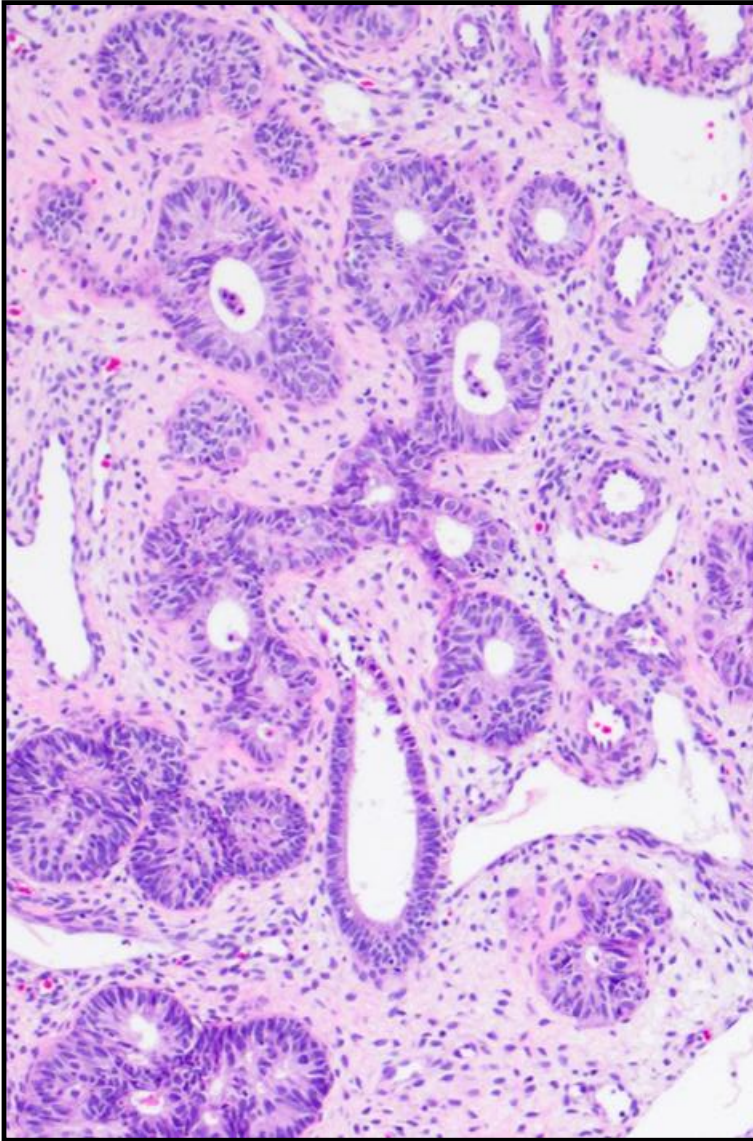
Atypical Hyperplasia



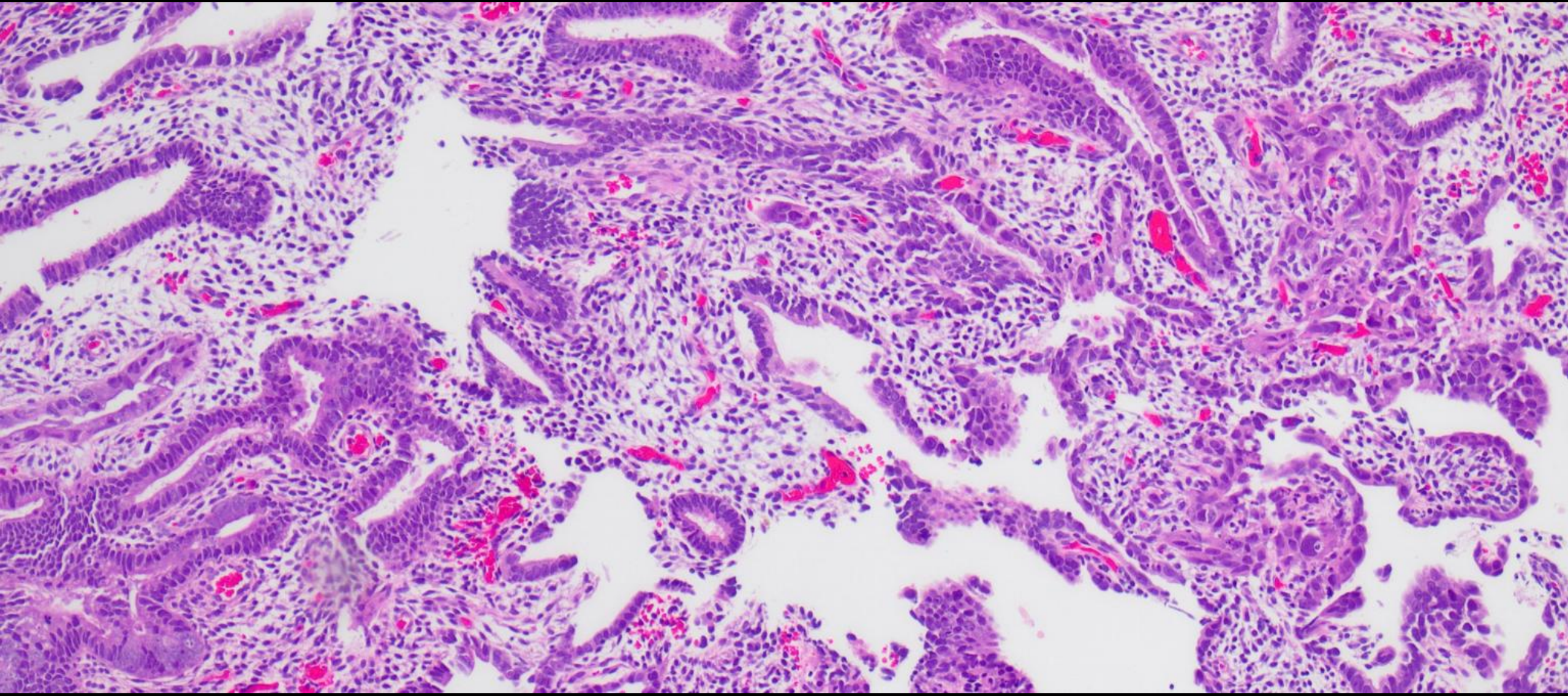
Serous Carcinoma

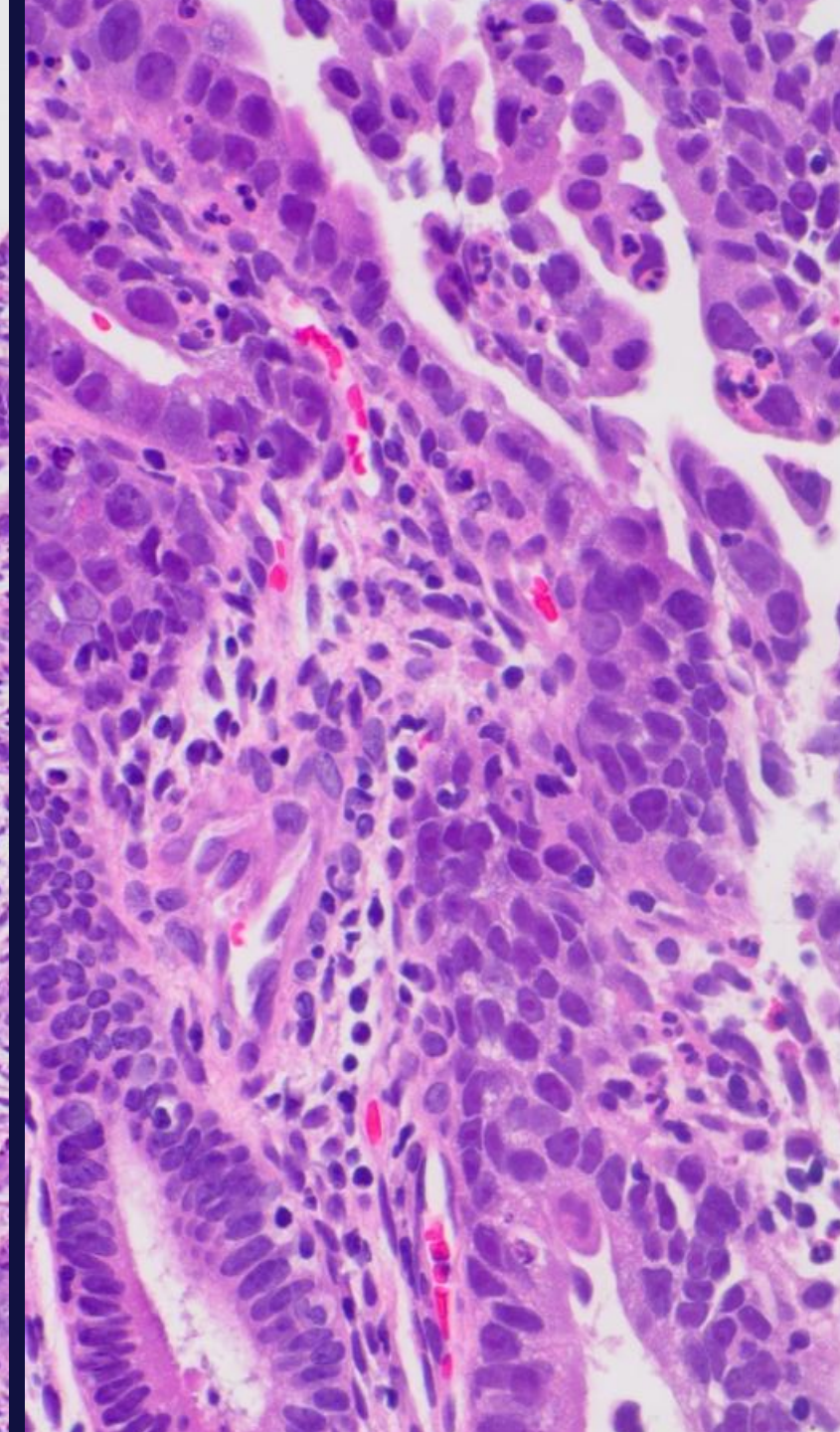
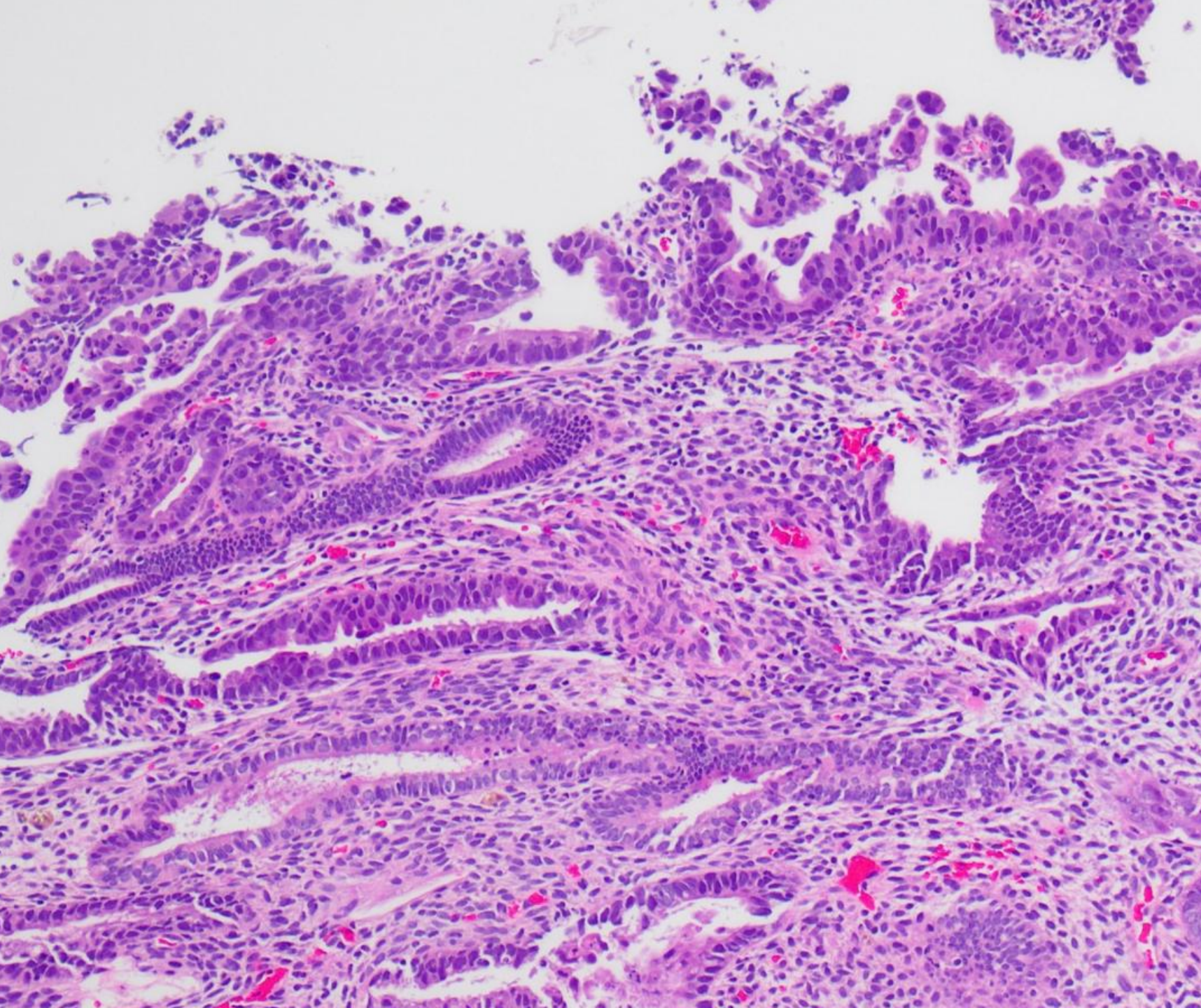


Glandular Serous Carcinoma May Mimic Atypical Hyperplasia

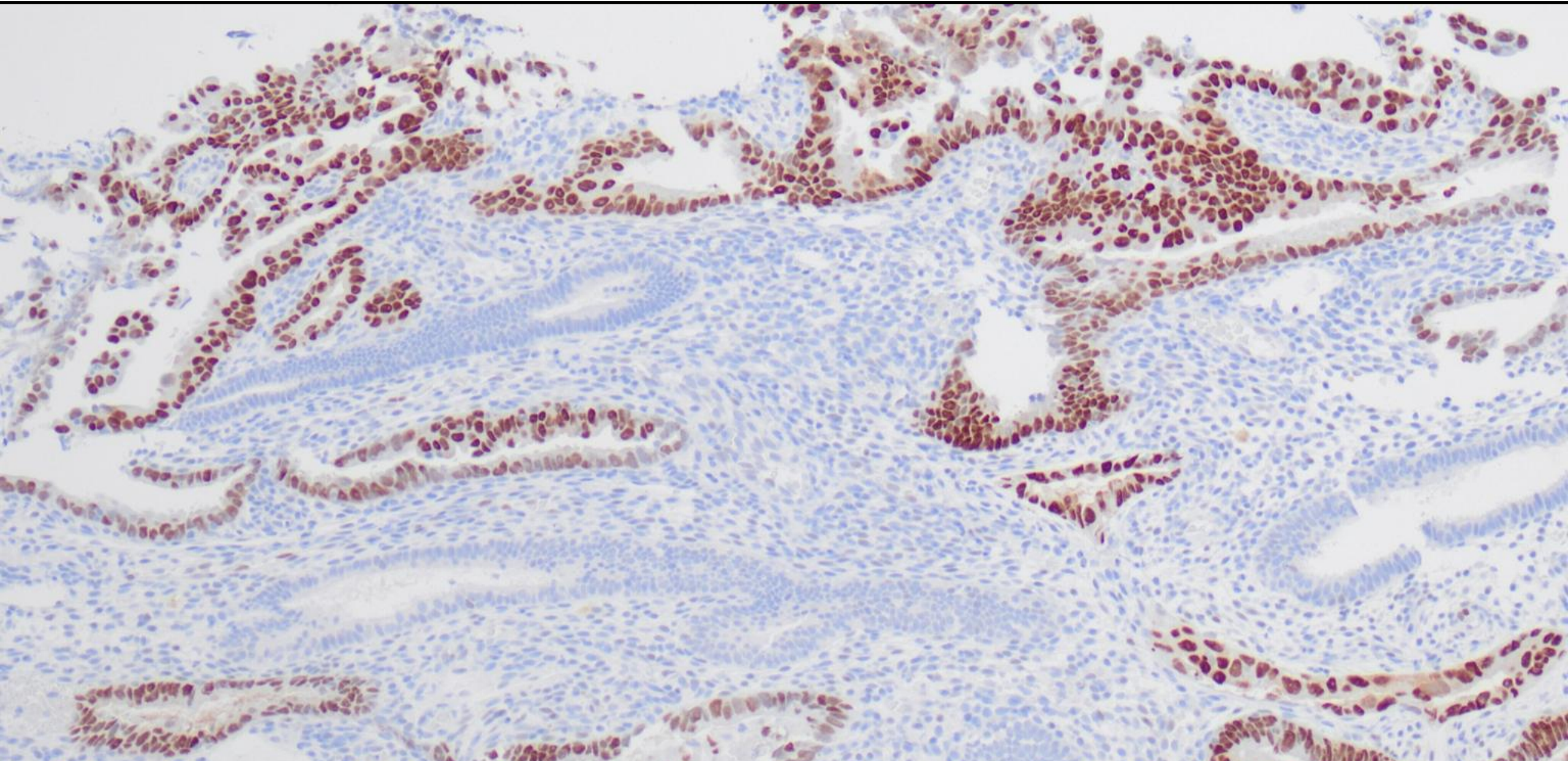


Glandular Serous Carcinoma May Be Missed Due to Lack of Significant Architectural Complexity

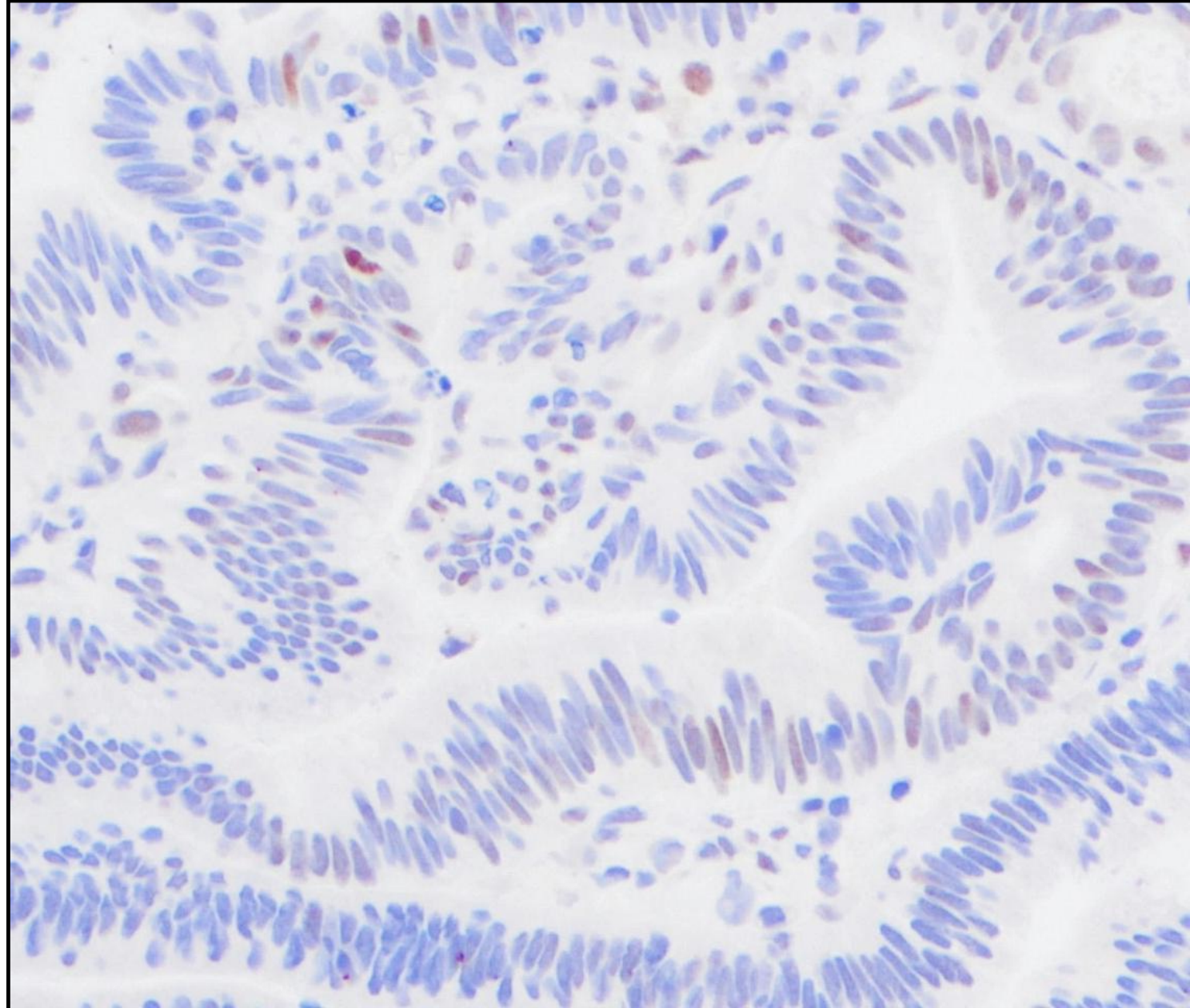
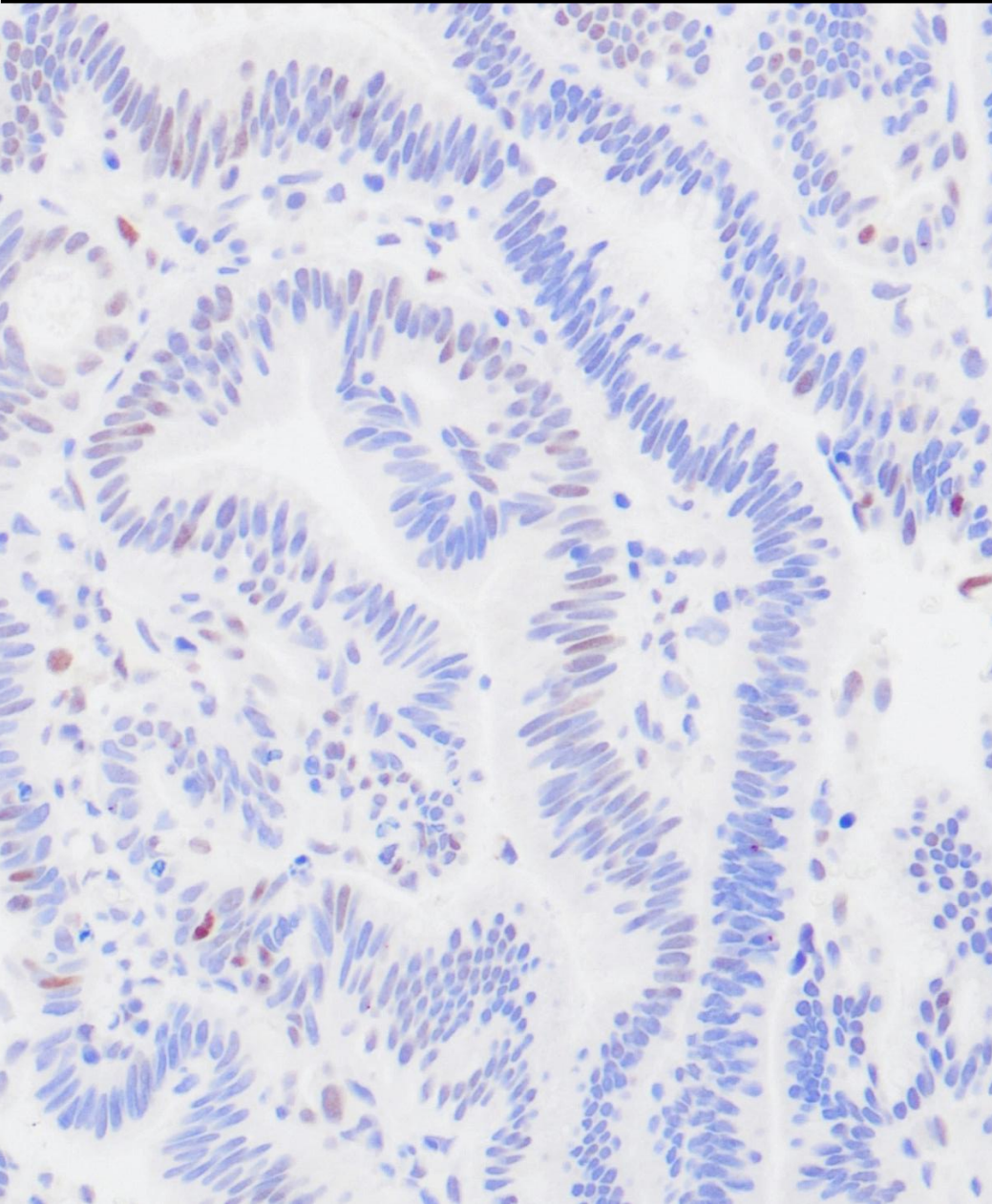




p53: Abnormal / Mutated Overexpression

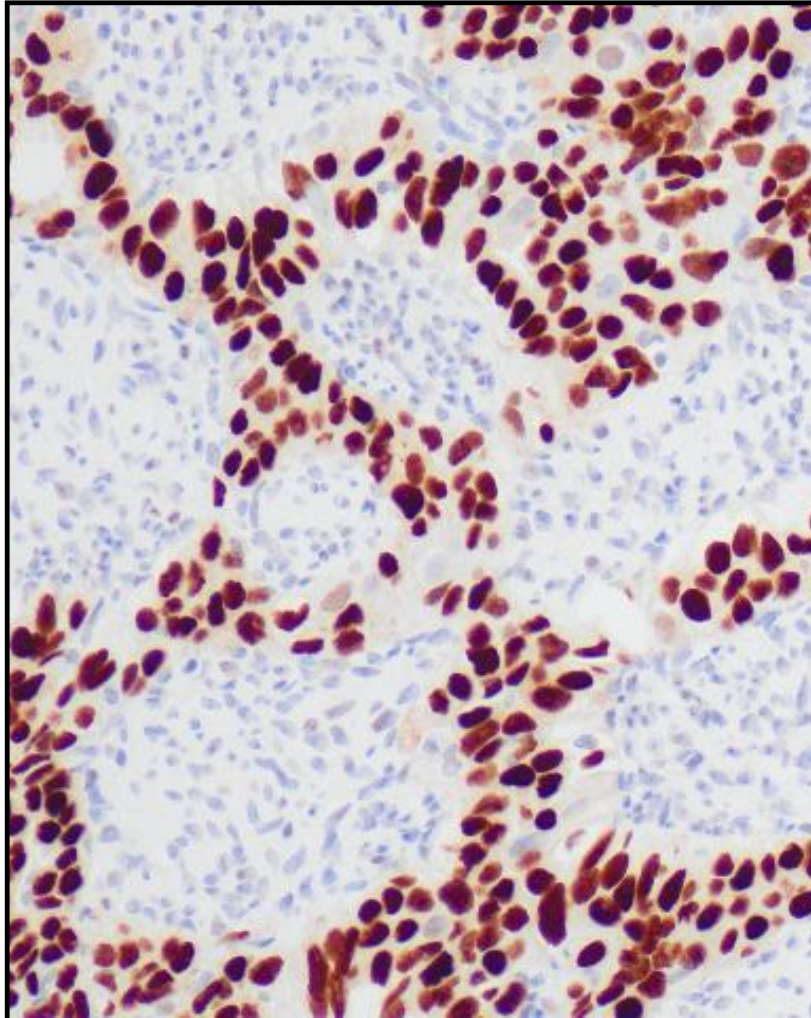


p53: Wild-Type expression

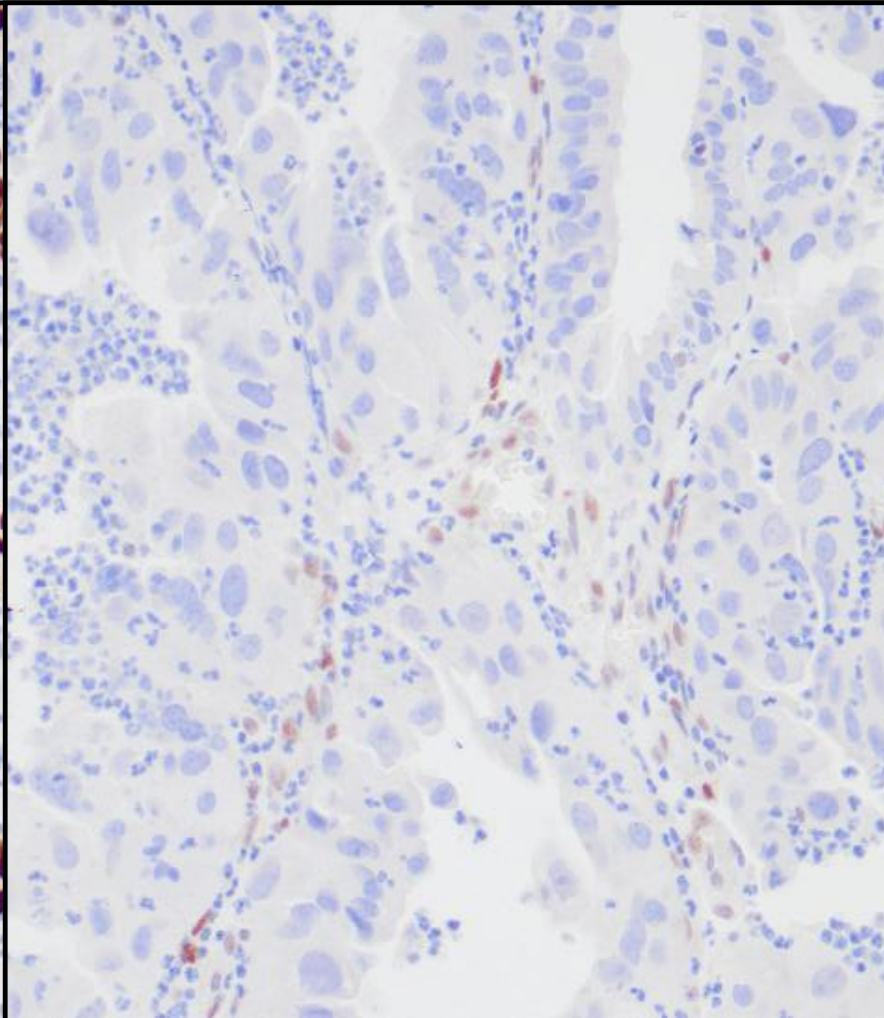


Abnormal p53 Expression Patterns

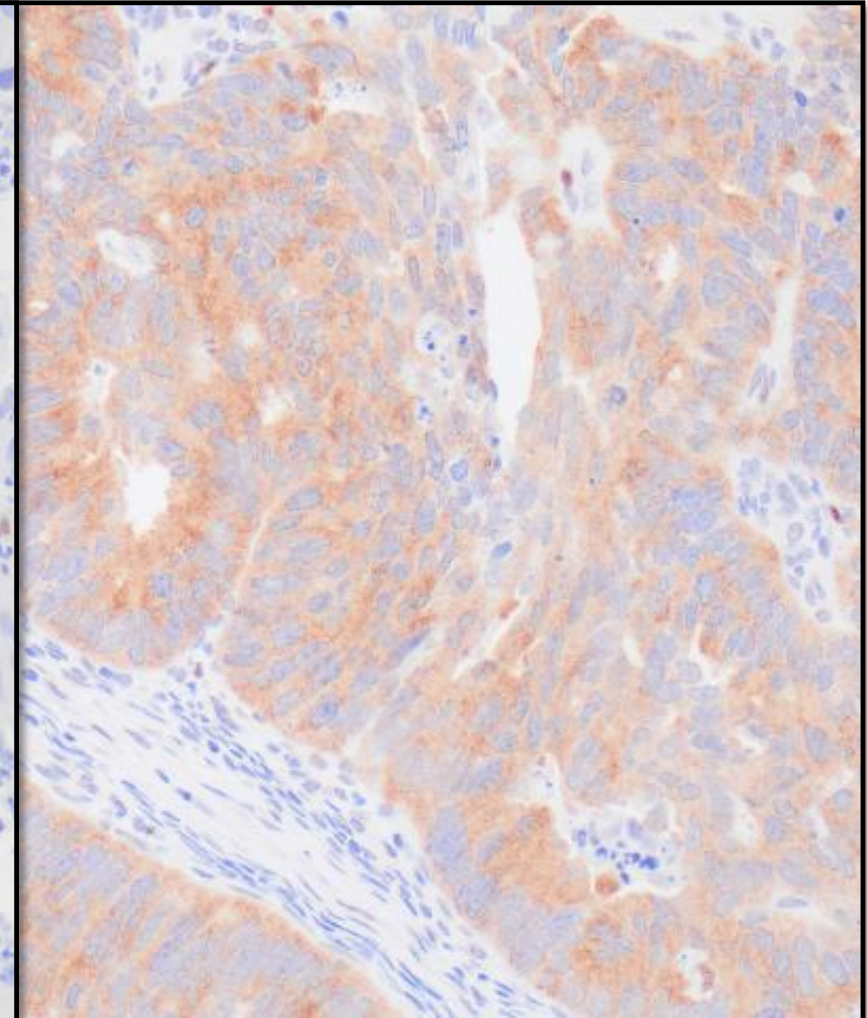
**Overexpression
(missense)**



**Null-type
(loss of function)**



**Cytoplasmic
(loss of function)**

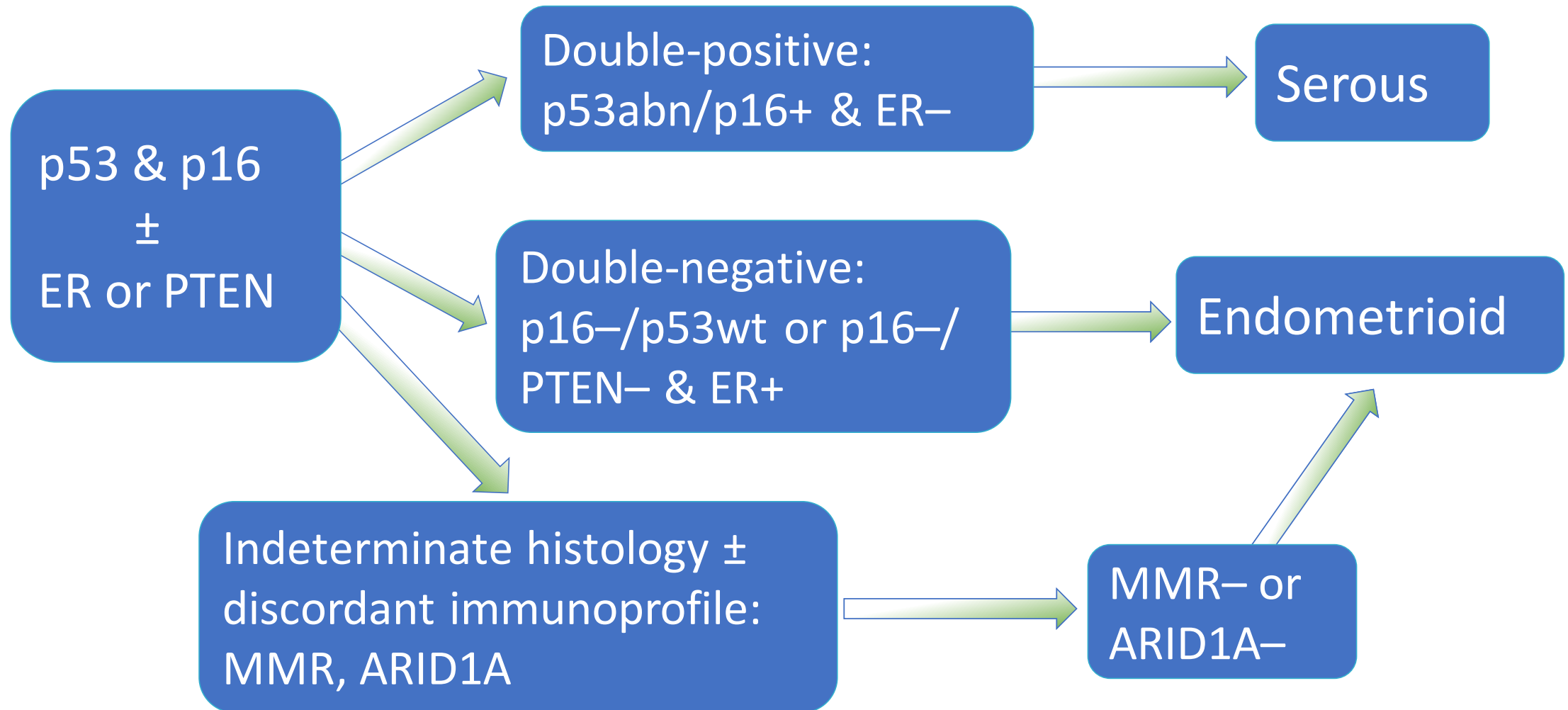


Immunohistochemical Studies

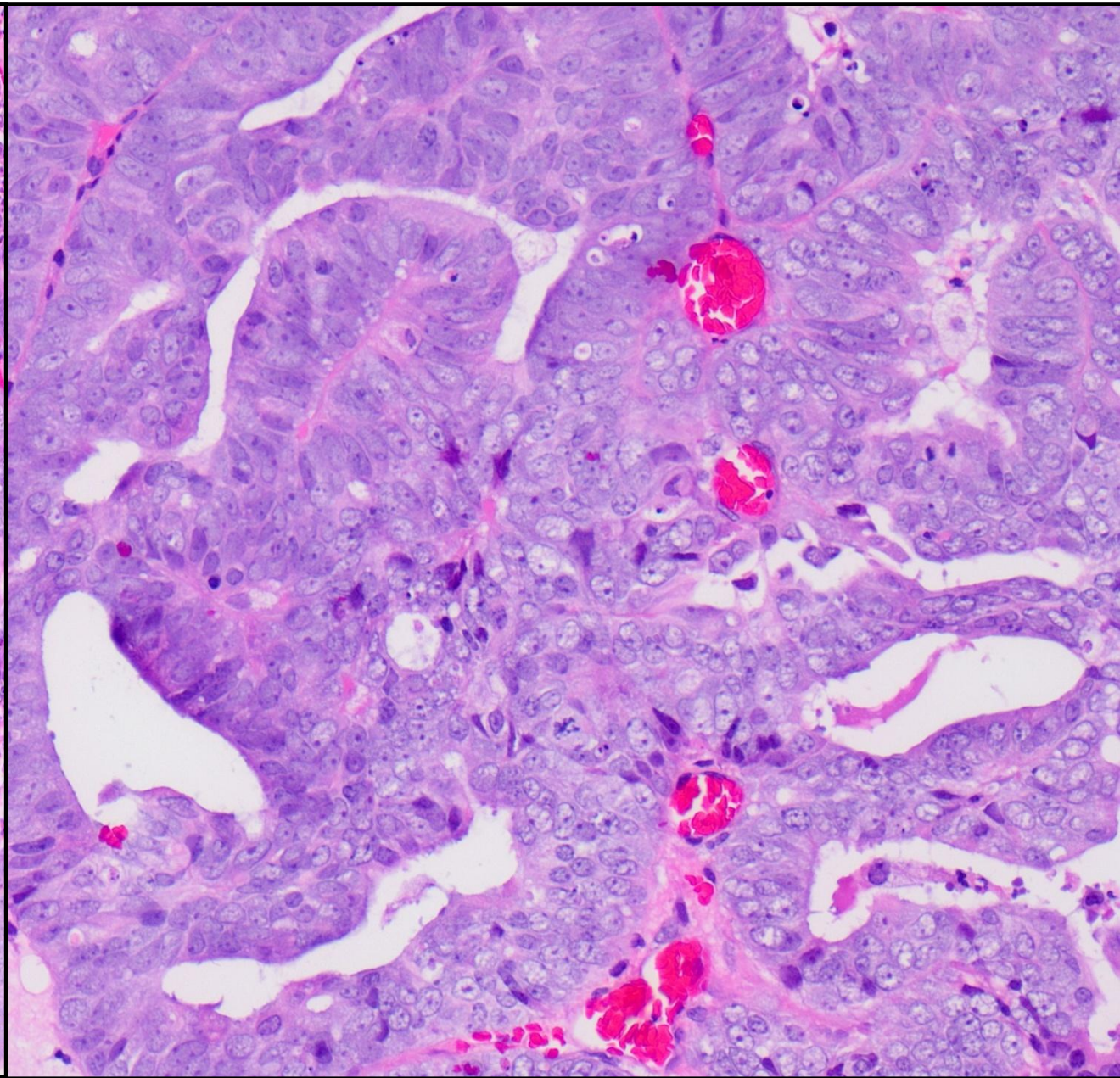
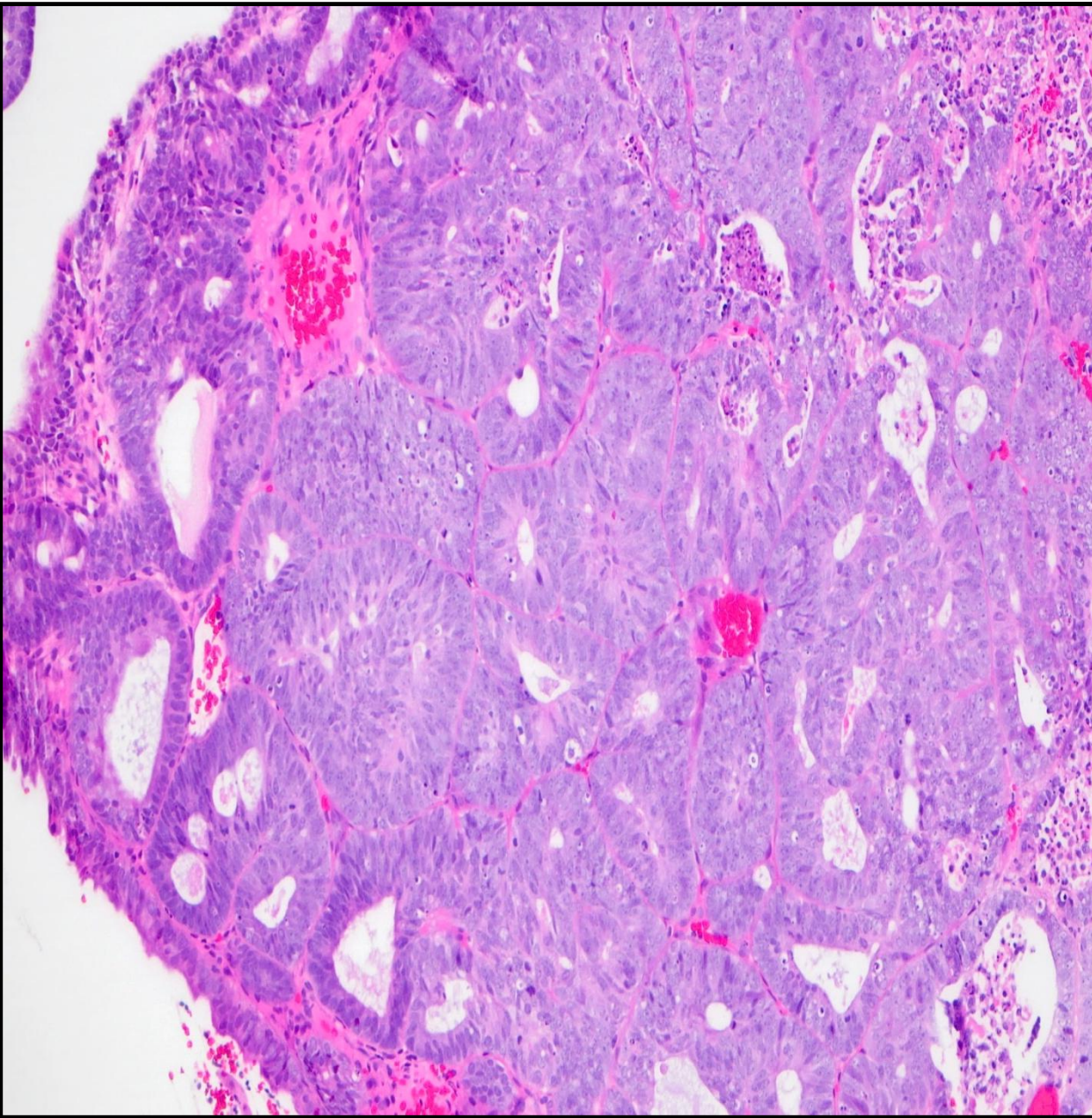
p53 & p16
±
ER or
PTEN

	Serous Carcinoma	Endometrioid Carcinoma
p53	Abnormal	Wild-type (usually)
p16	+ (Block-like)	—
ER	±	+
PTEN	+	±
<i>MMR</i>	+	±
<i>ARID1A</i>	+	±

Endometrioid VS Serous Carcinoma

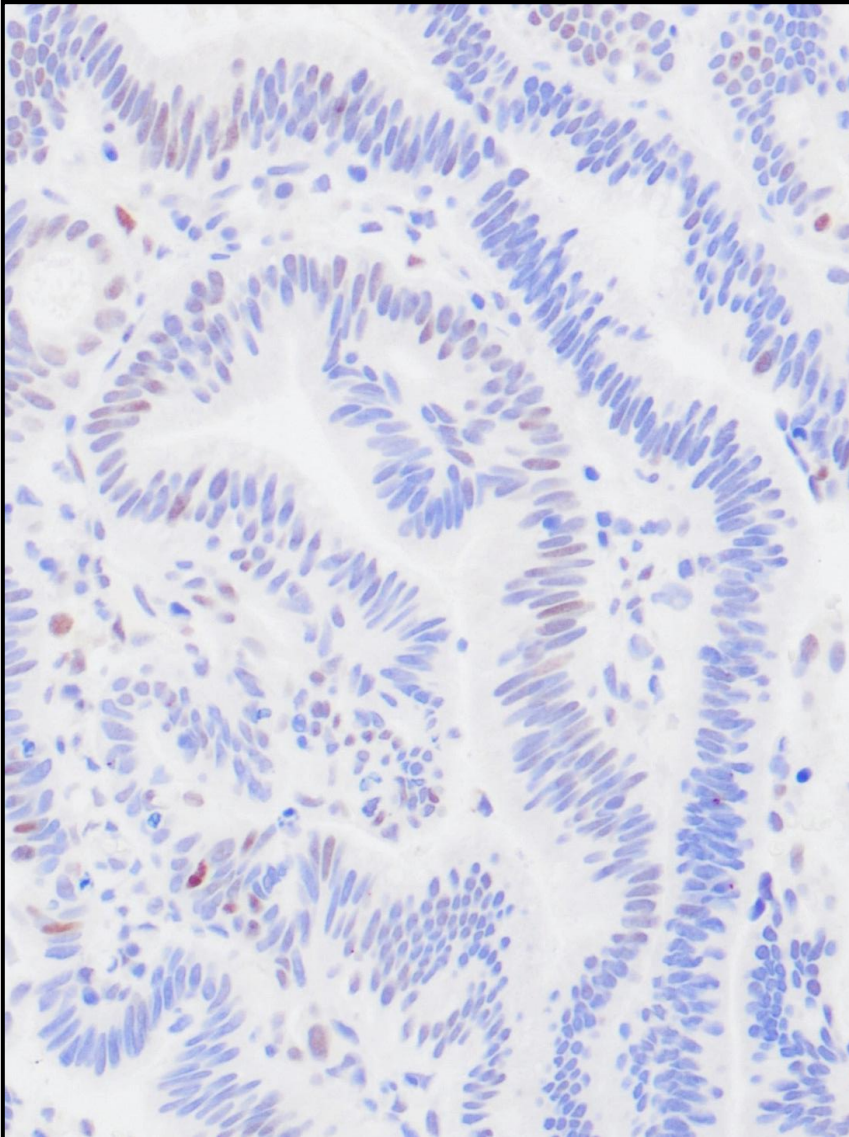


Serous VS Endometrioid Carcinoma

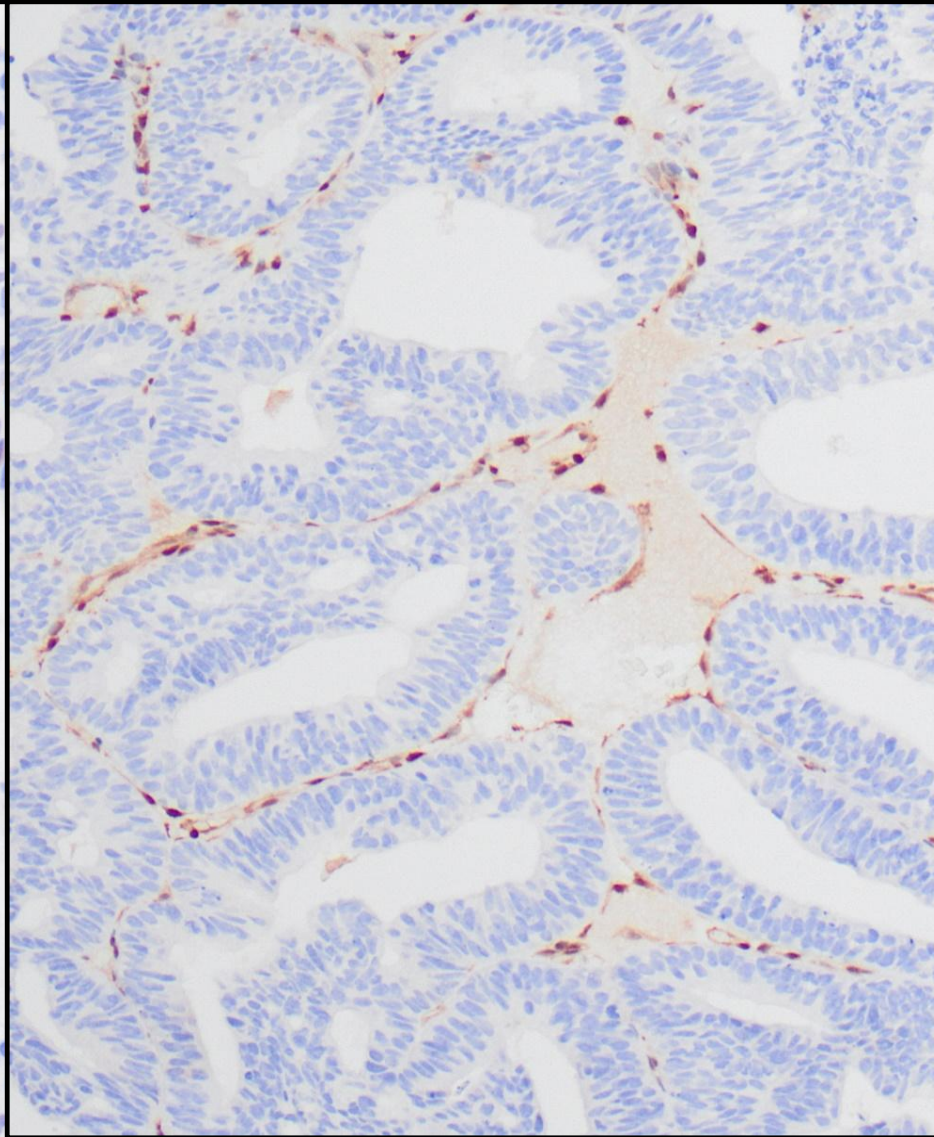


Immunoprofile Supports Endometrioid Carcinoma

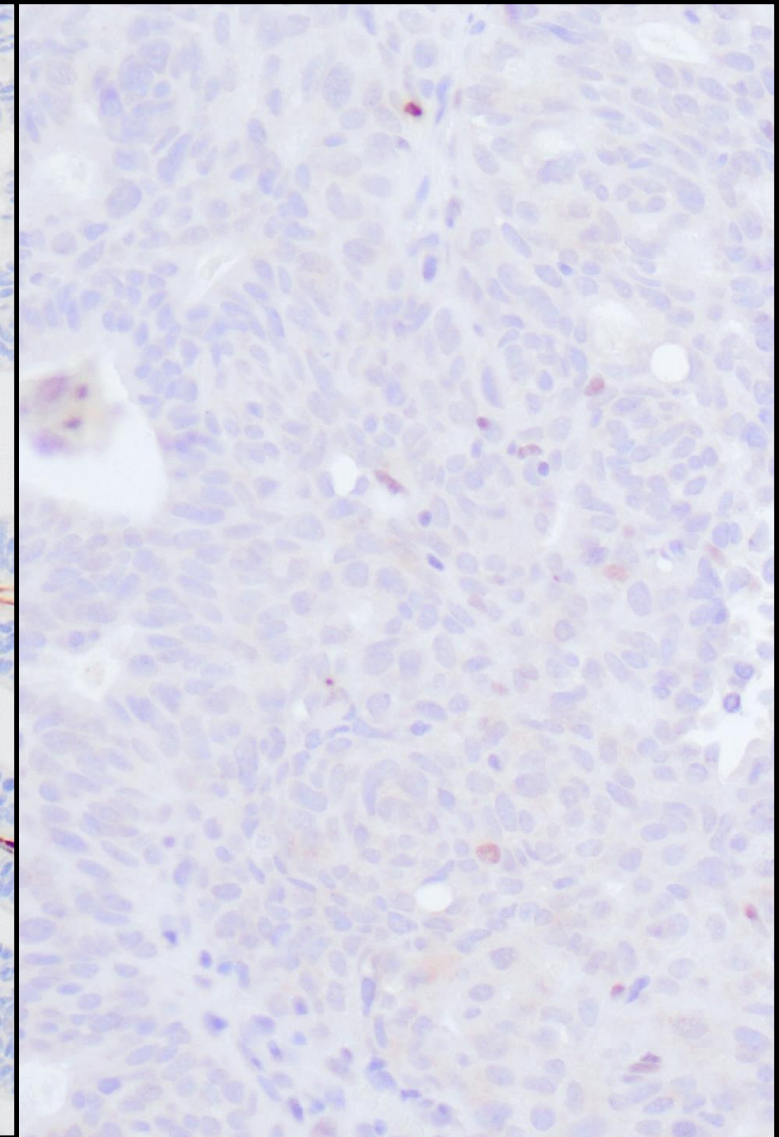
p53: Wild-Type

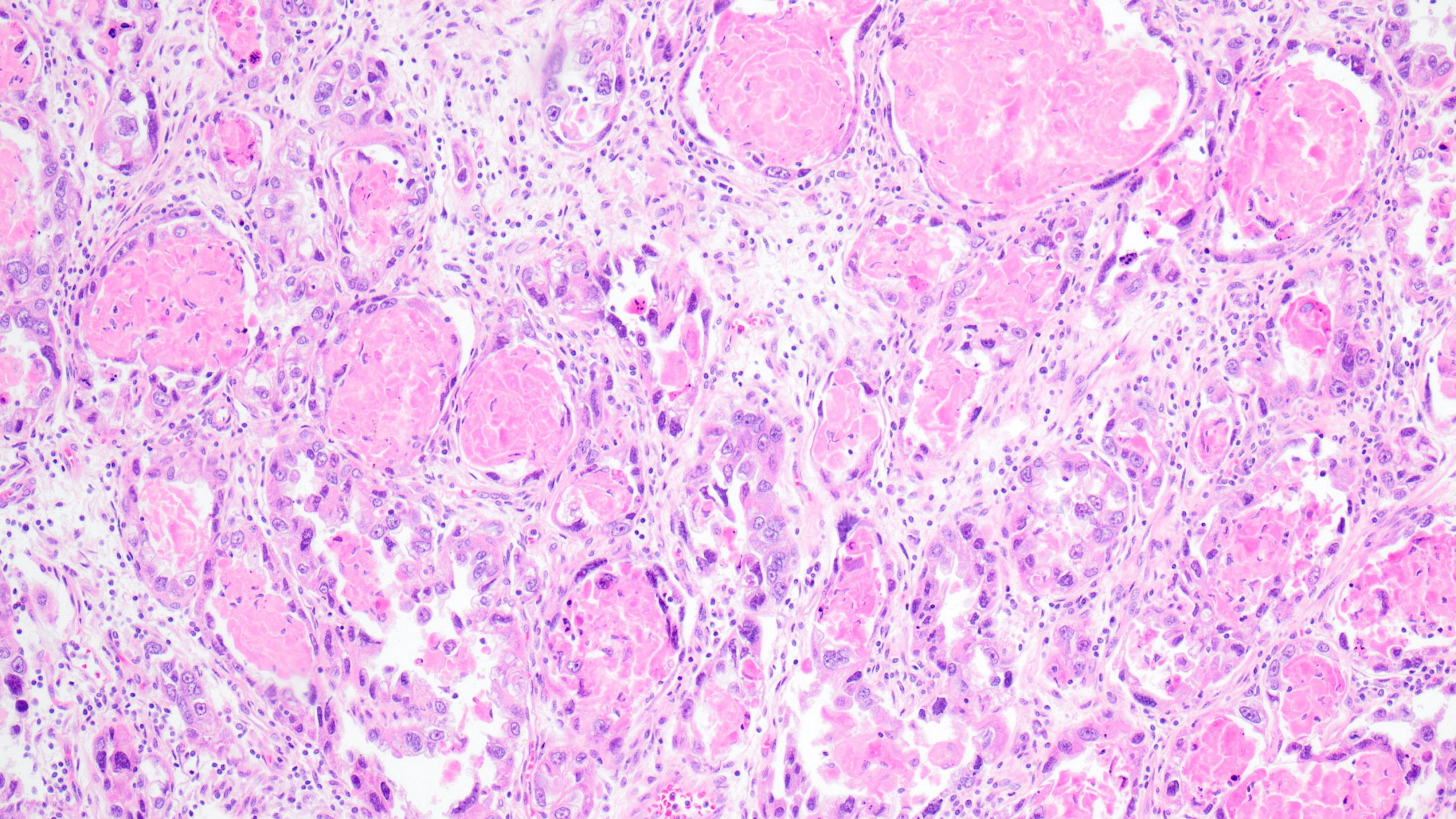


PTEN: Lost

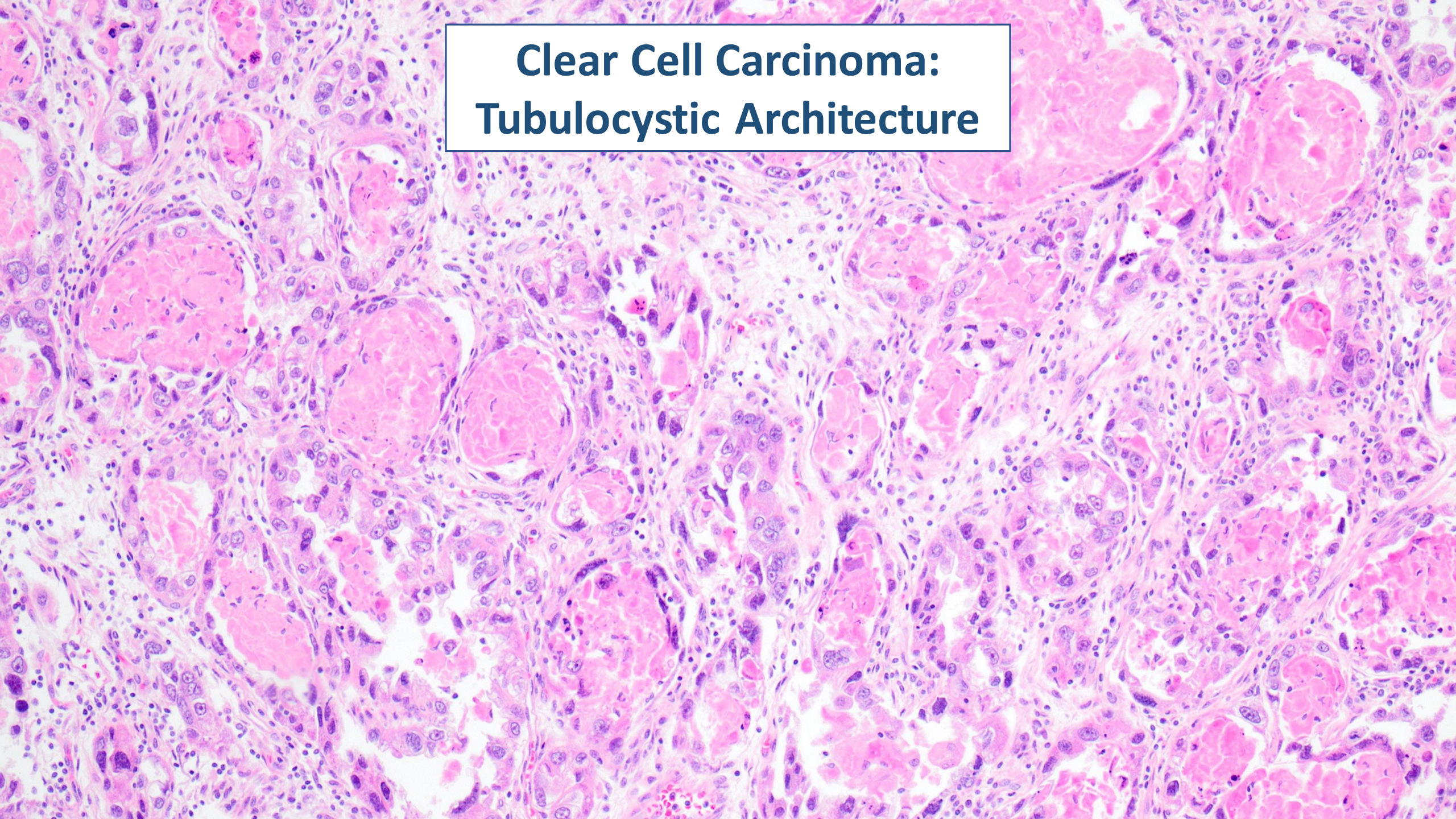


MSH2/6: Lost





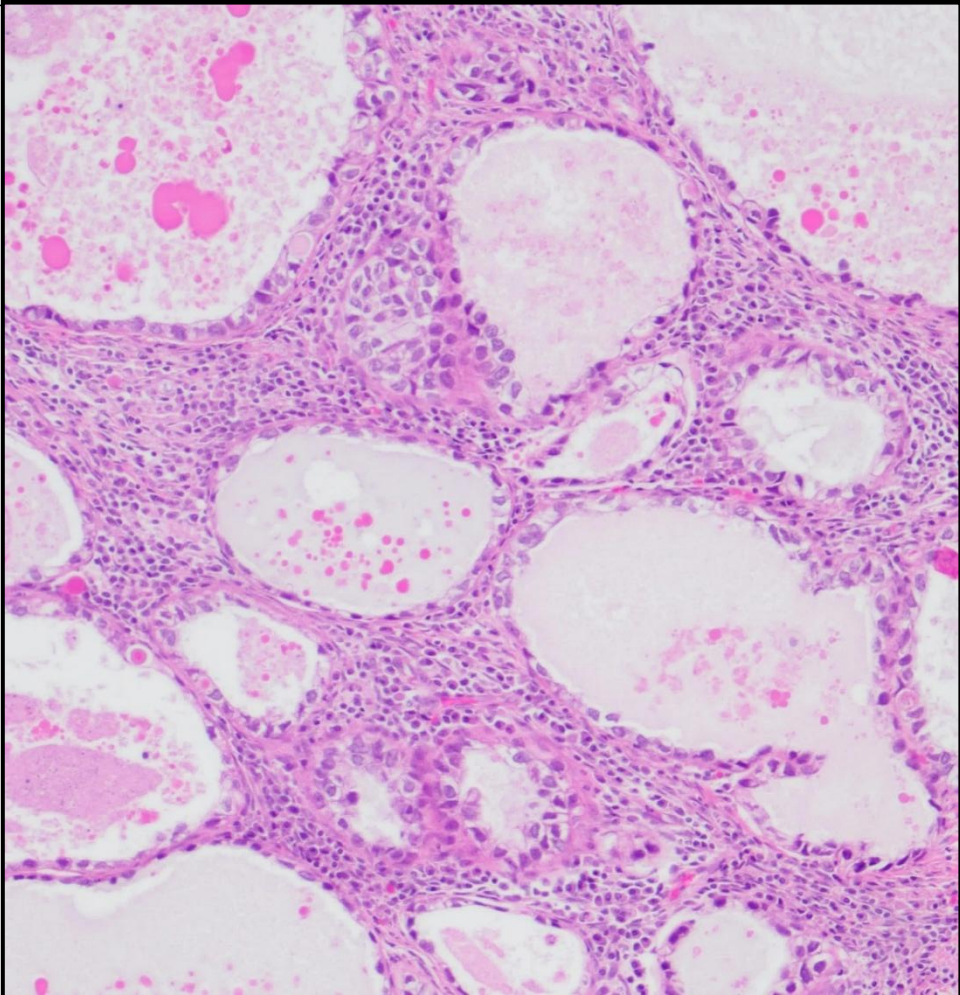
Clear Cell Carcinoma: Tubulocystic Architecture



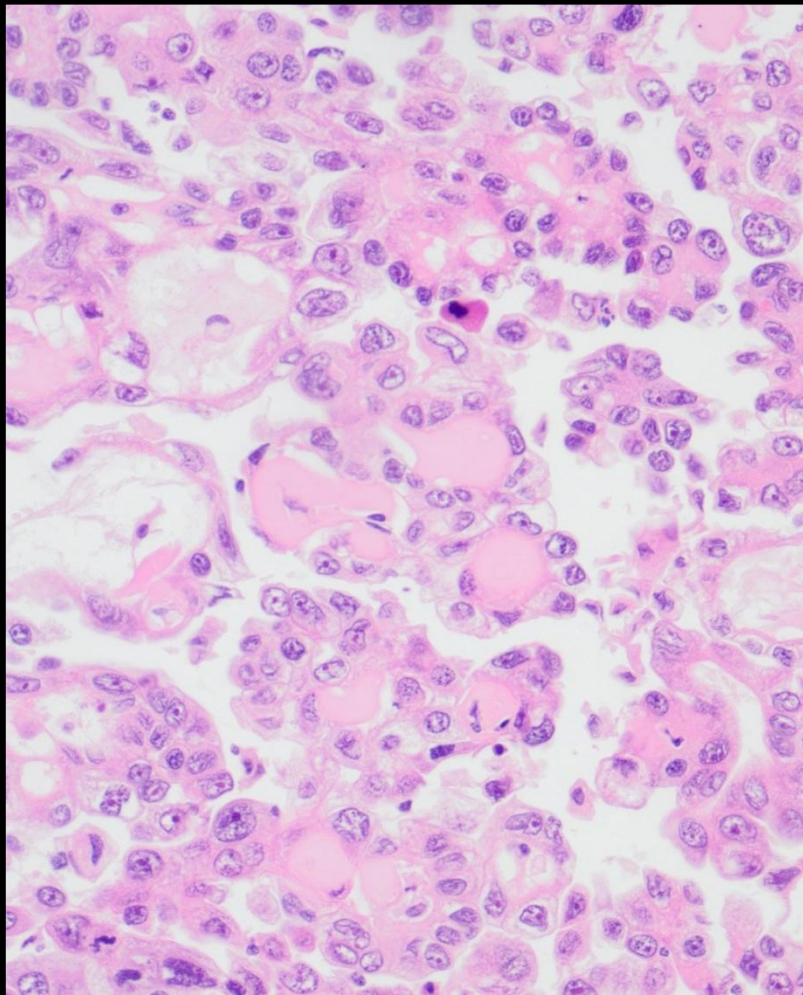
Clear Cell Carcinoma

**Clear to eosinophilic cytoplasm, hobnail cells,
usually no marked pleomorphism or brisk mitoses**

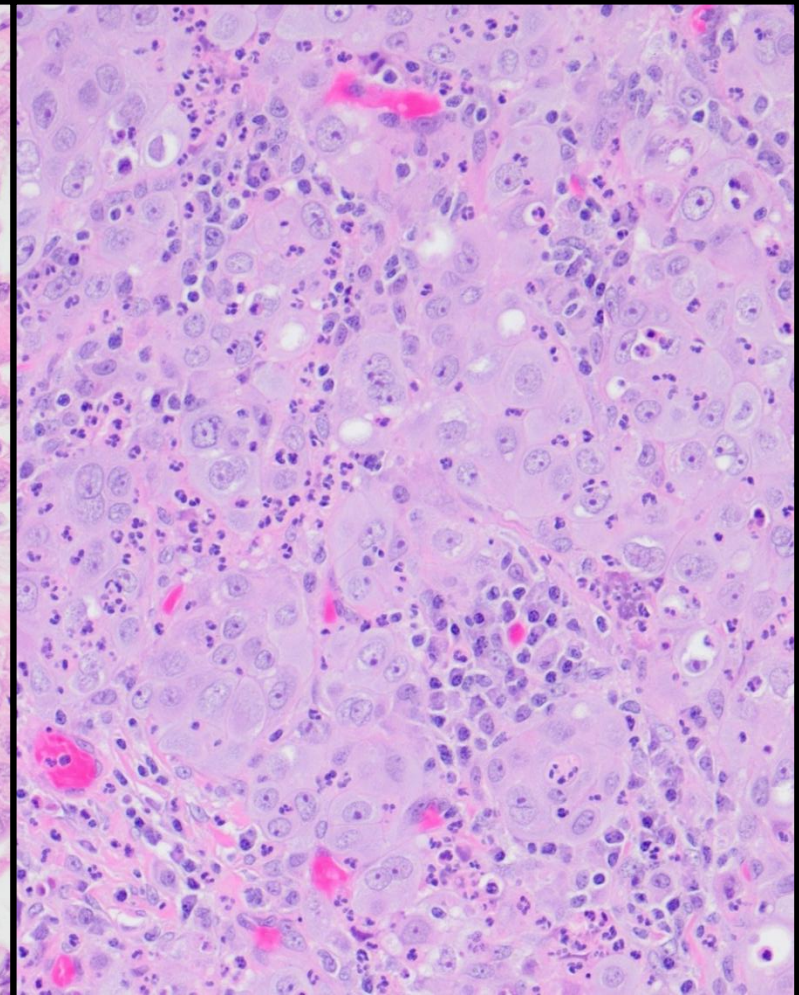
Tubulocystic



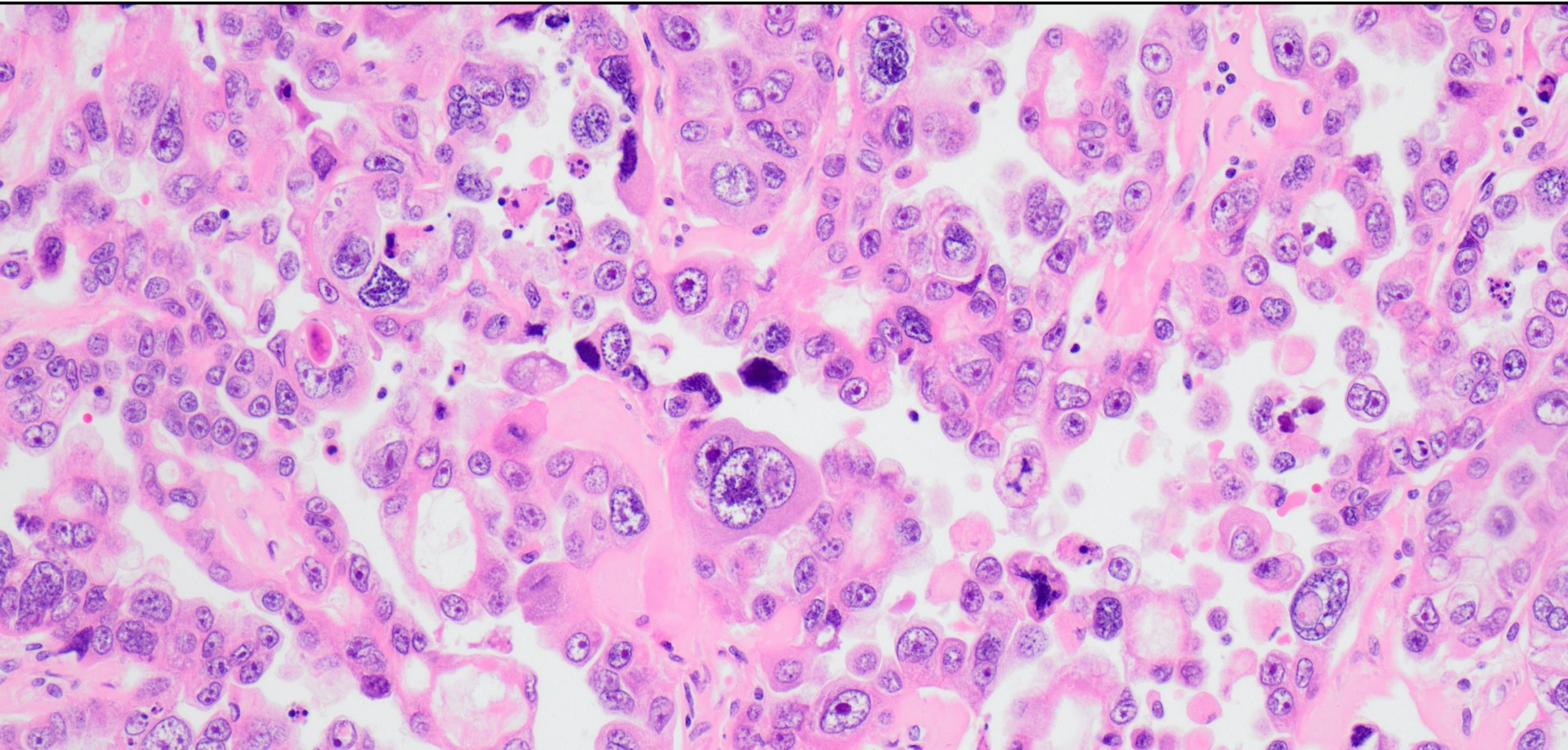
Papillary



Solid



Marked Atypia May Be Seen in Some Tumors

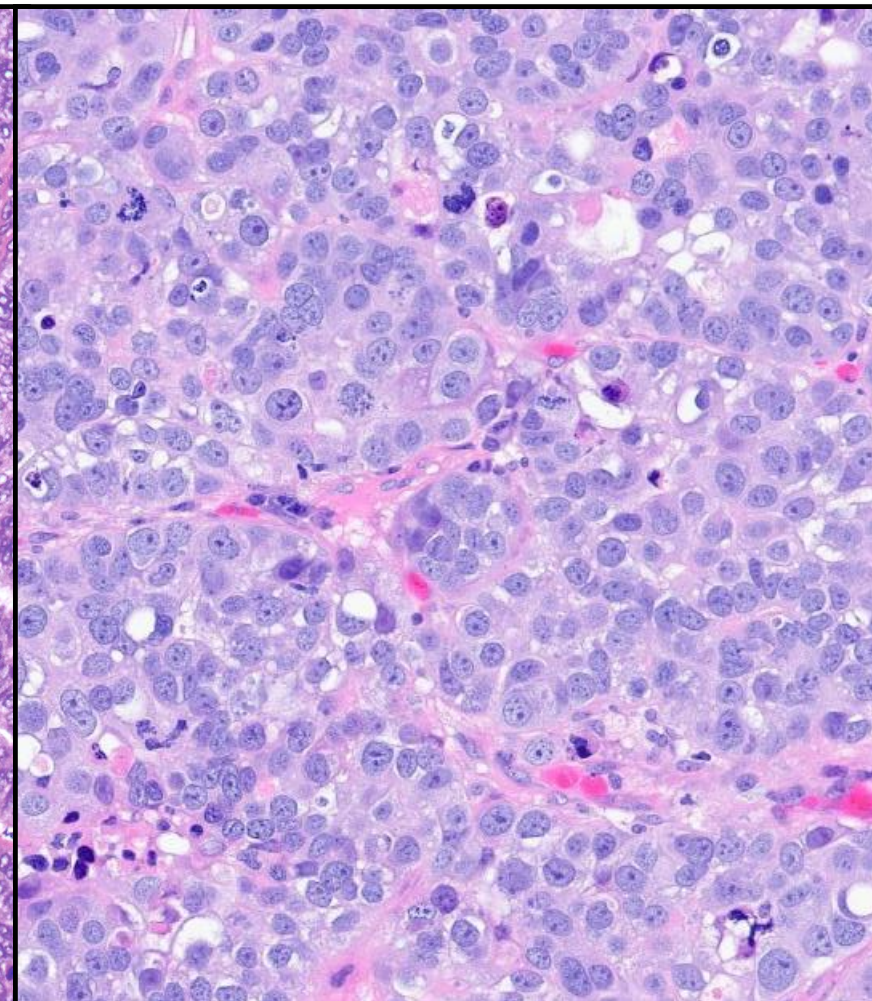
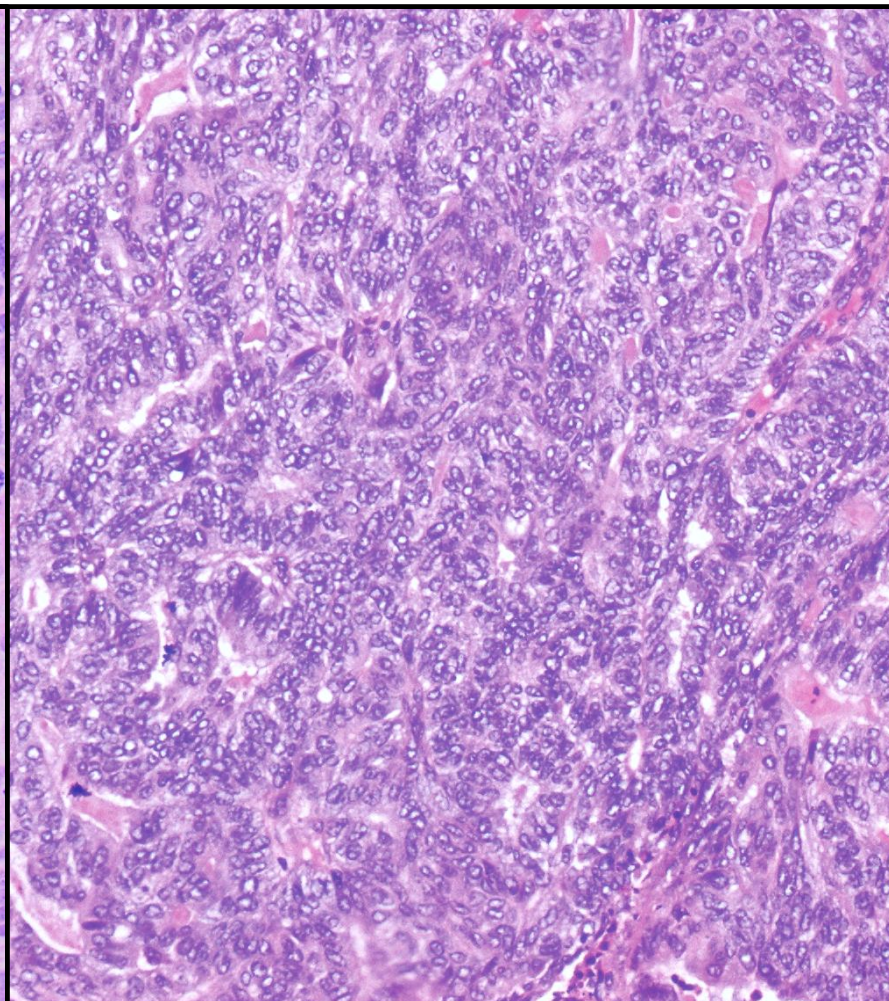
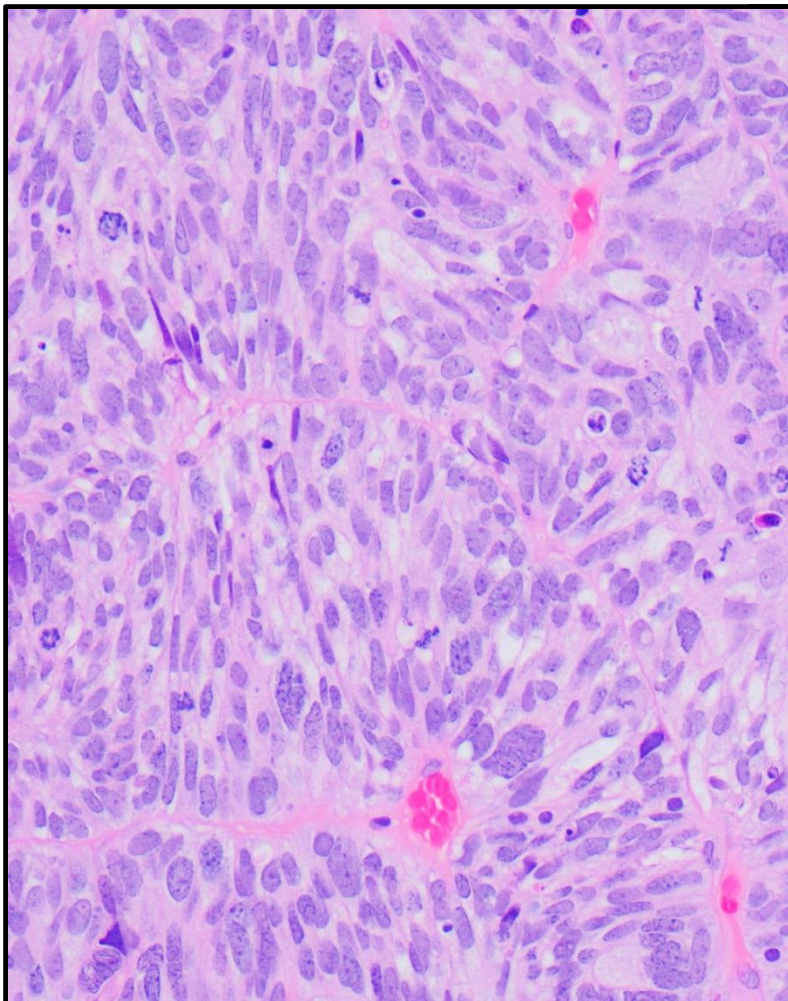


Differential Diagnosis for High-Grade Endometrial Carcinoma with Solid Architecture

Serous

Grade 3 endometrioid

Clear cell



Serous

- ✓ Solid growth
- ✓ **±Papillary or glandular areas**
- ✓ Cell detachment, budding, tufting
- ✓ Slit-like spaces
- ✓ Marked atypia
- ✓ Brisk mitoses
- ✓ **±Apoptotic bodies**
- ✓ **±Atrophy, polyp**

Grade 3 Endometrioid

- ✓ >50% solid non-squamous growth
- ✓ **±Gland formation**
- ✓ **±Squamous/mucinous/secretory features**
- ✓ Moderate atypia
- ✓ **±Atypical hyperplasia**

Clear Cell

- ✓ Papillary, tubulocystic, solid architecture
- ✓ Polygonal cells with clear or eosinophilic cytoplasm, hobnail & flat cells
- ✓ Variable atypia
- ✓ Low mitoses

✓ No squamous/mucinous/secretory features

Serous

Grade 3 Endometrioid

Clear Cell

Poor Interobserver Agreement!

Discordance rates range from 20% to 37%

PMID: 23807777

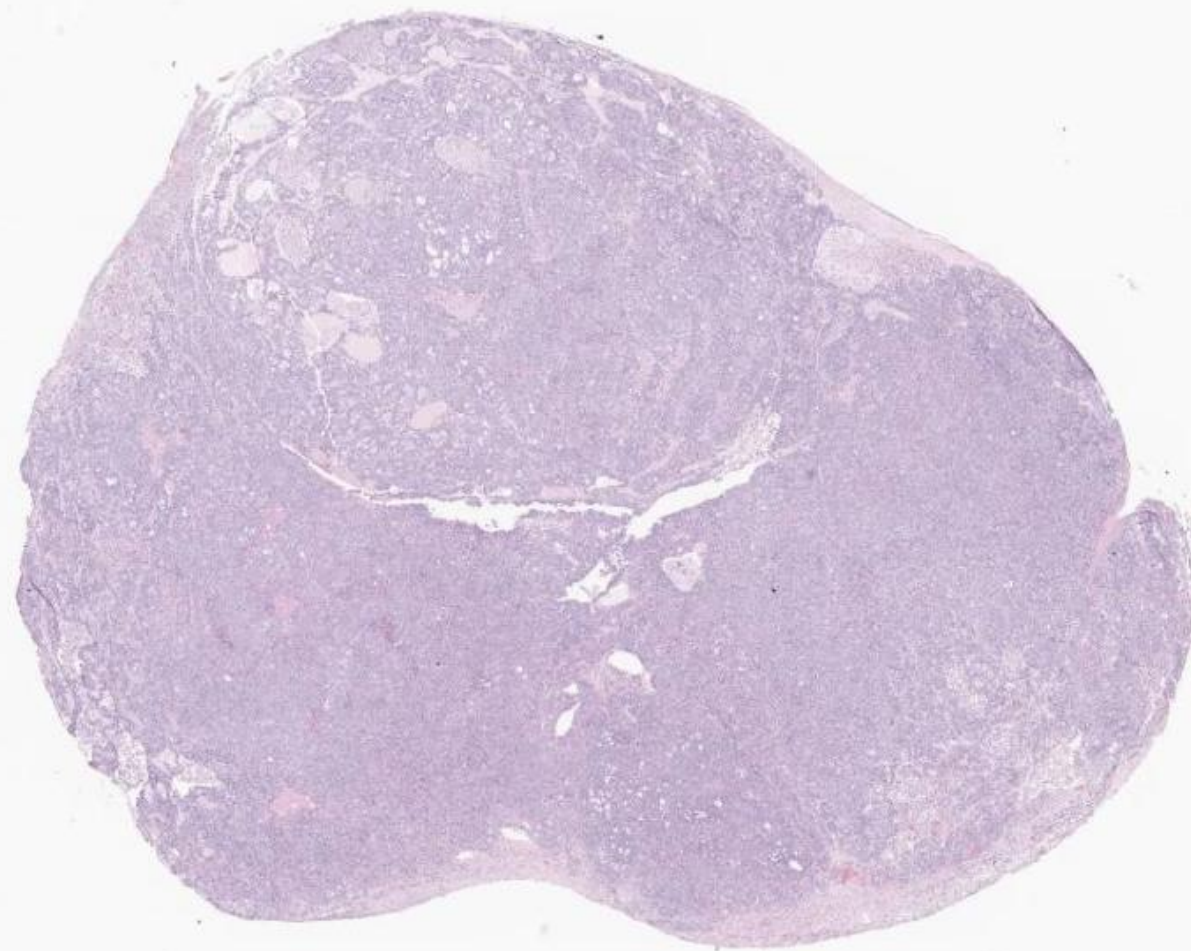
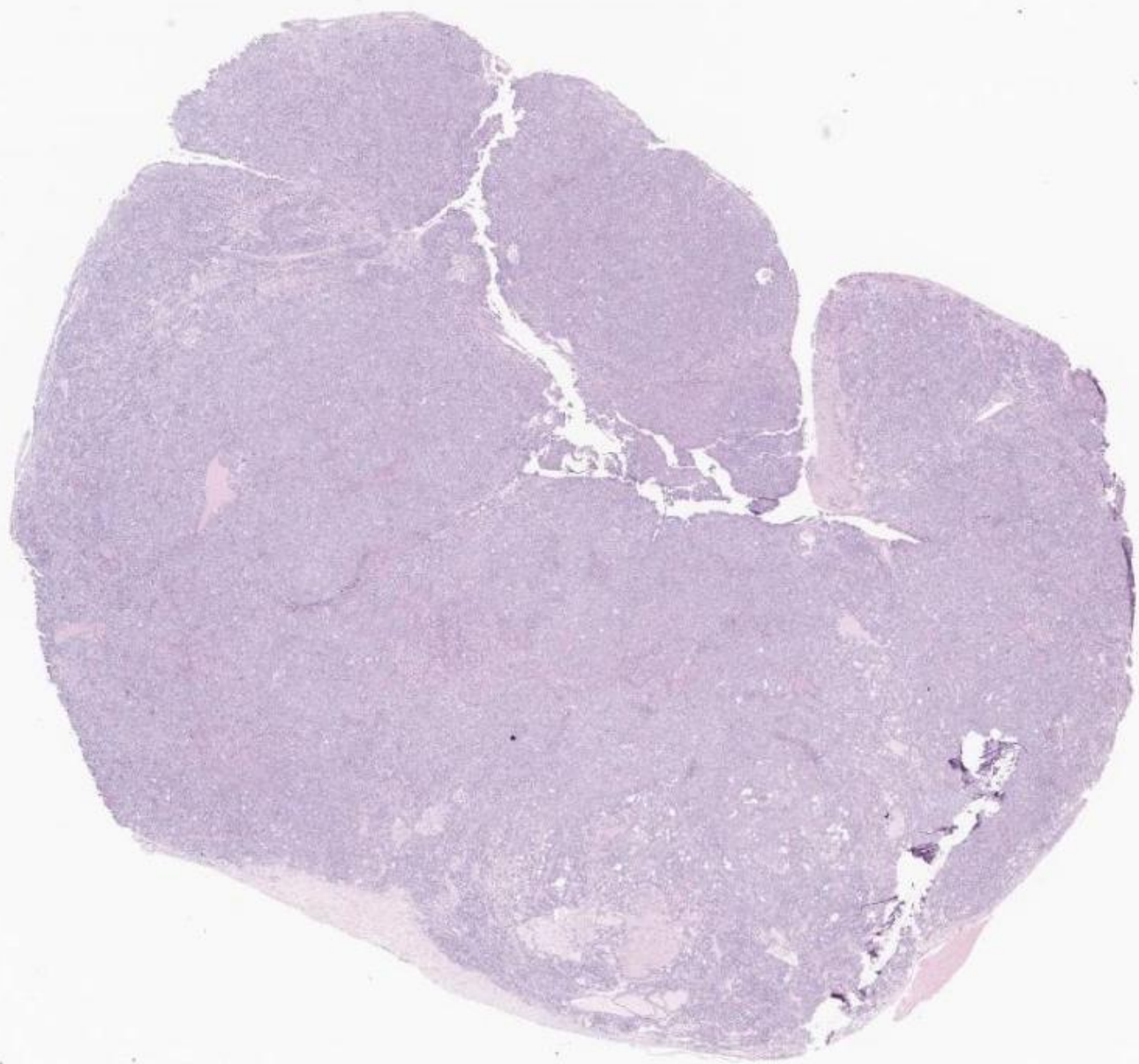
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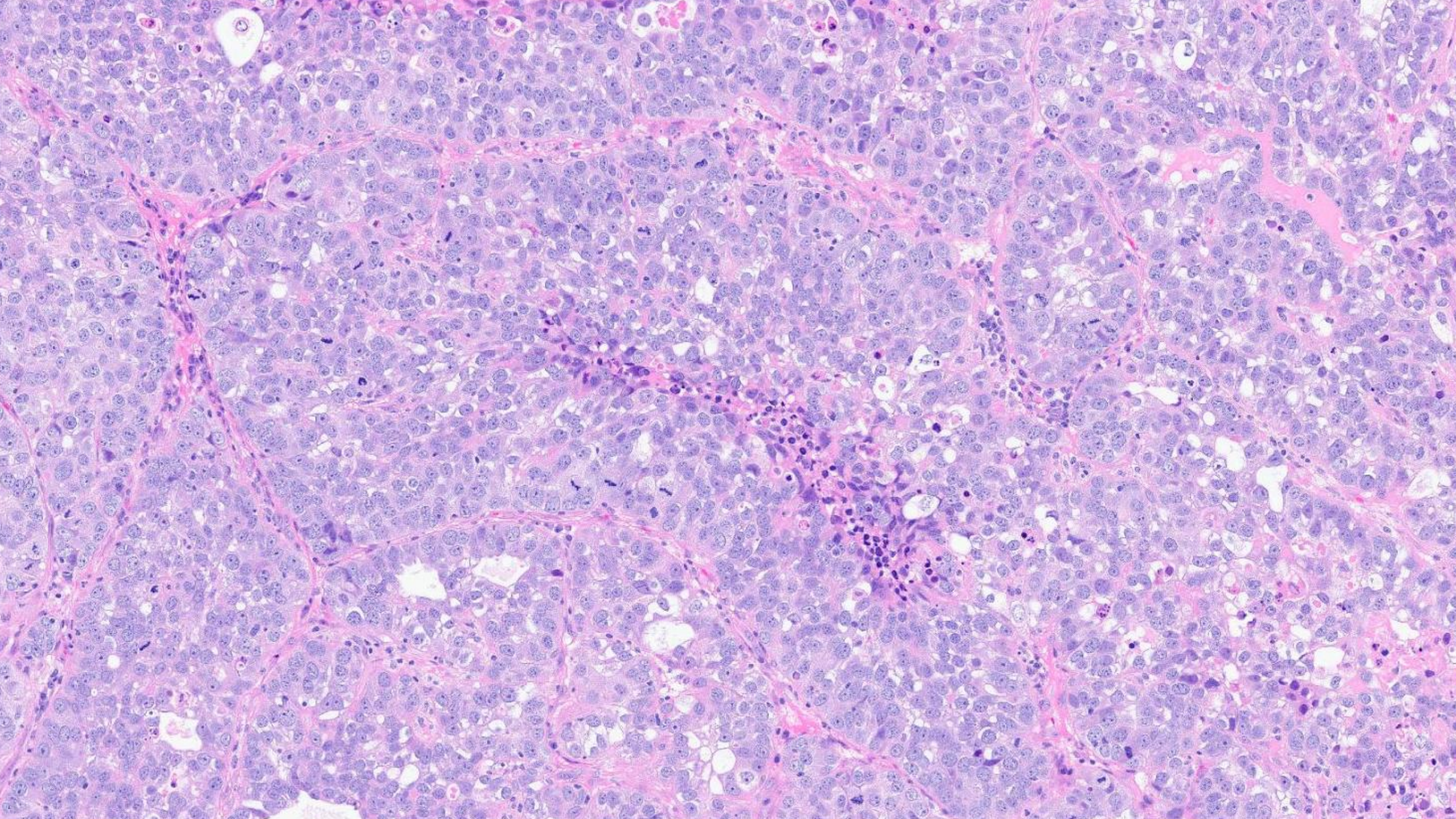
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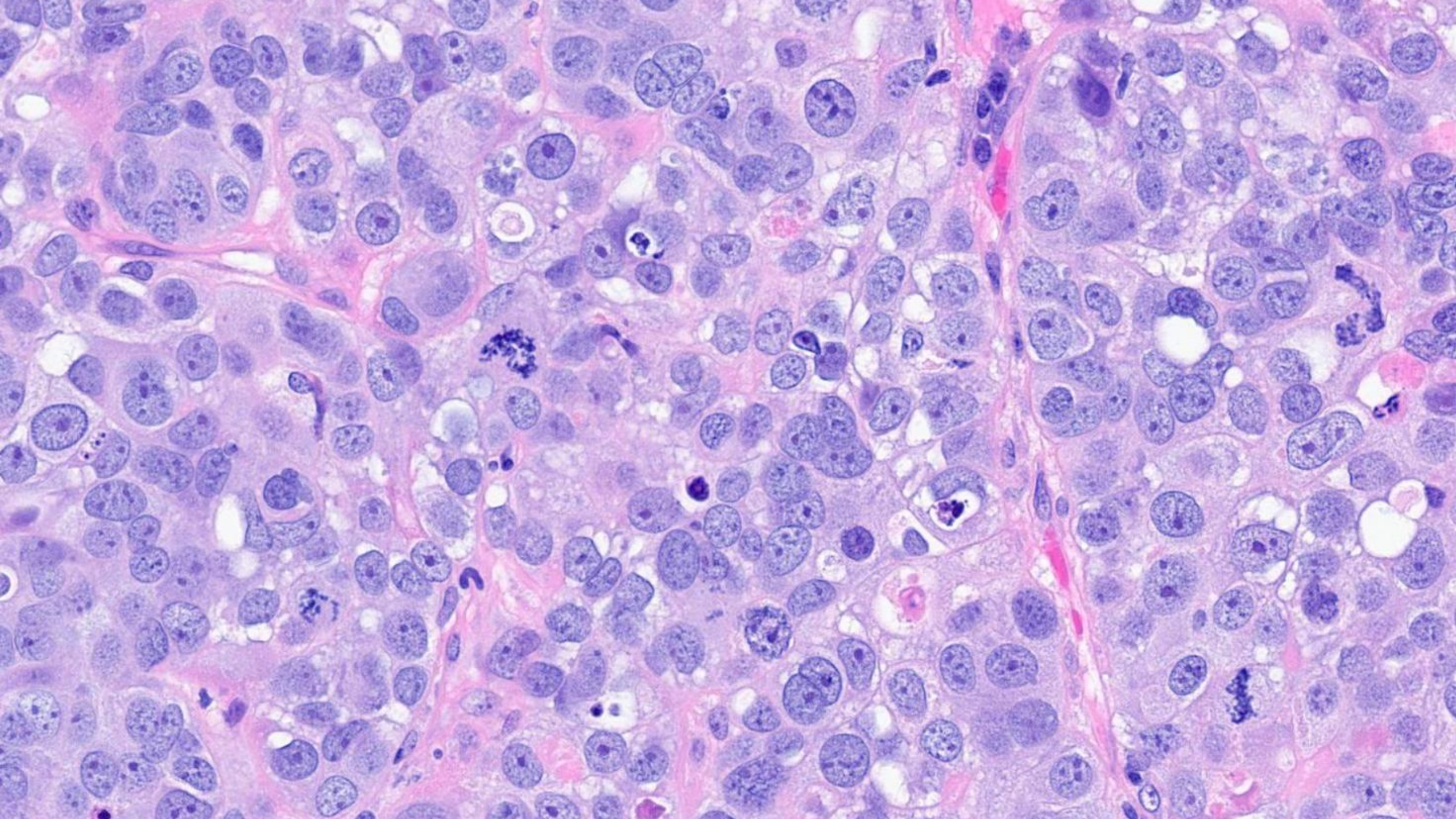
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Immunohistochemical Studies

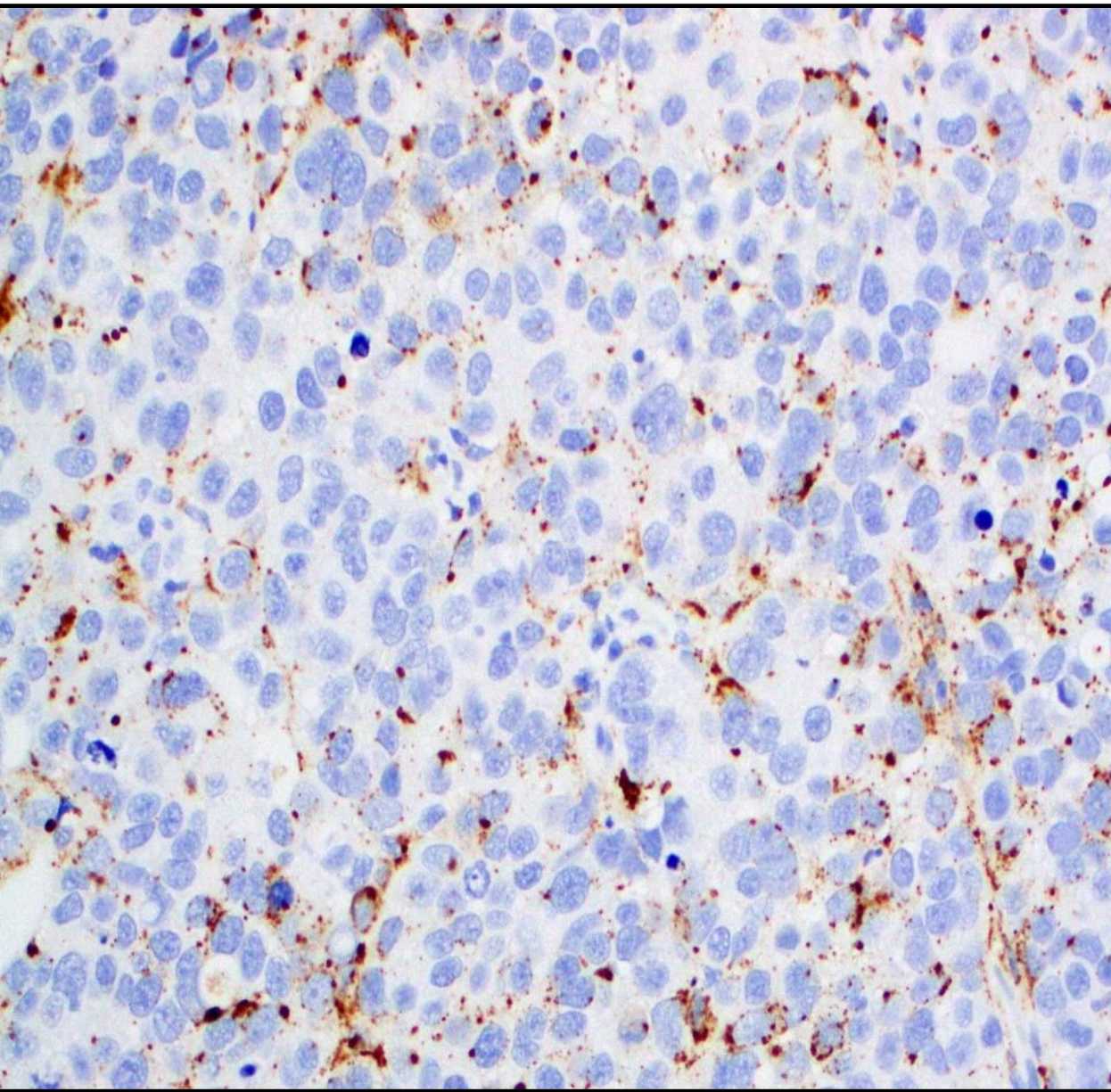
	Serous Carcinoma	Endometrioid Carcinoma	Clear Cell Carcinoma
p53	Abnormal	Abnormal ~30%	Abnormal ~30%
p16	+ (block-like)	—	—
ER/PR	±	+	—
PTEN	+	±	±
MMR	+	±	±
ARID1A	+	±	±



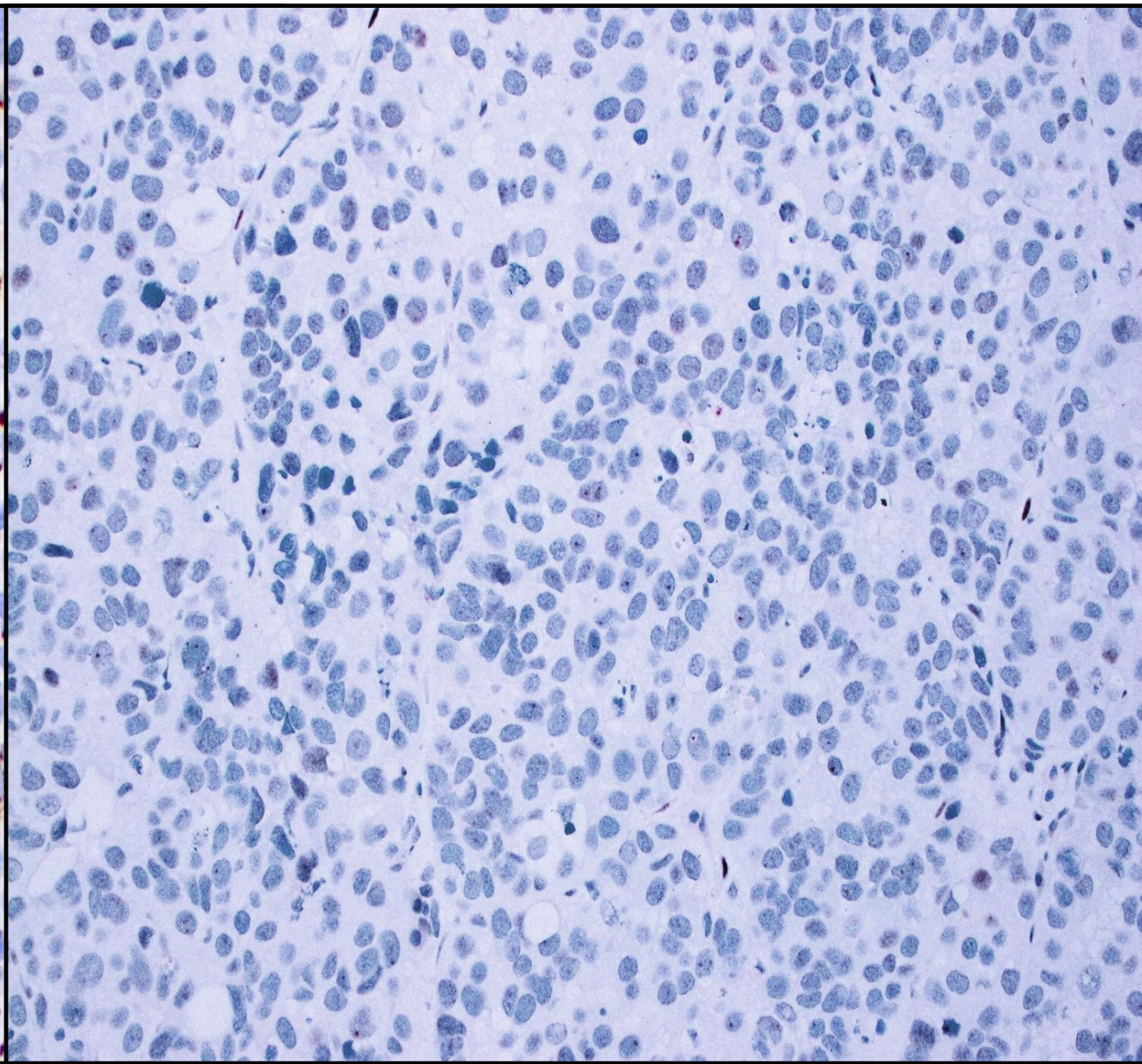




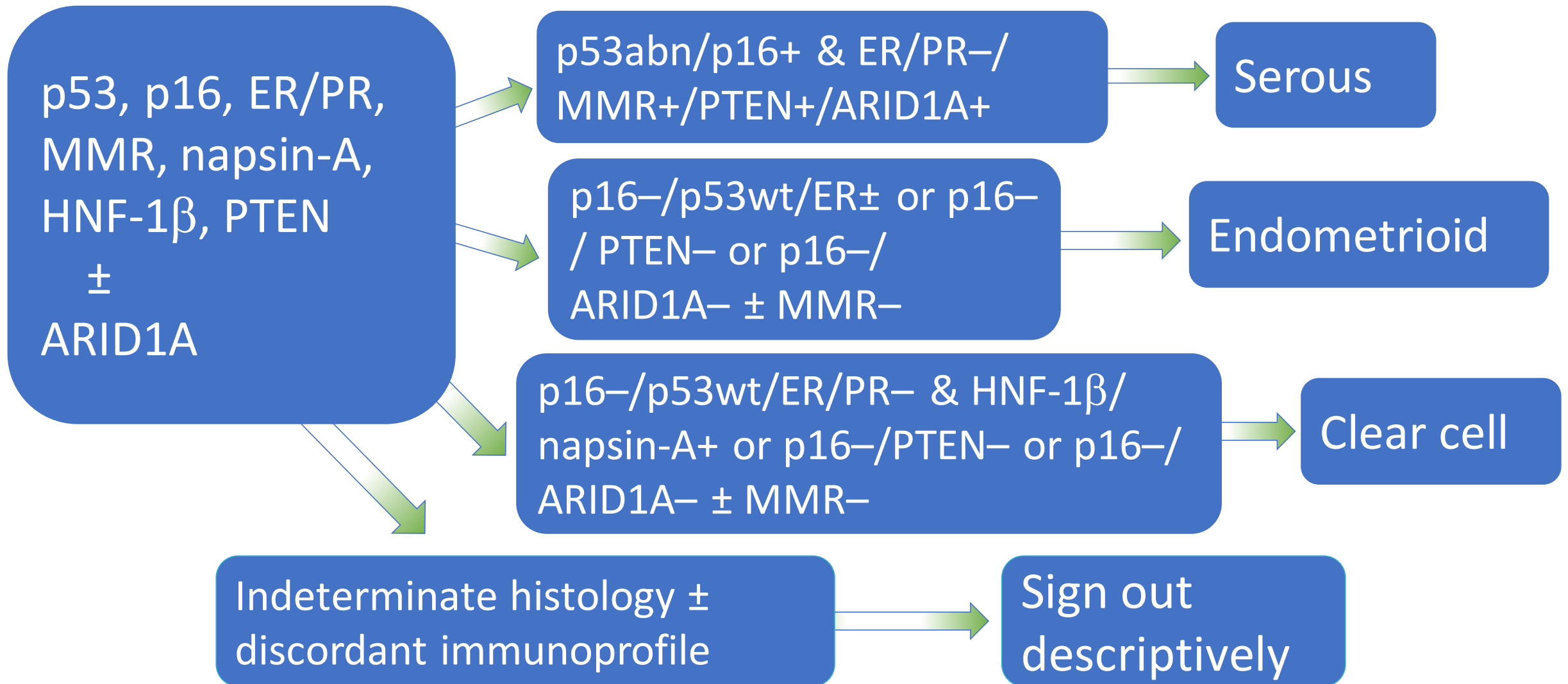
Napsin-A

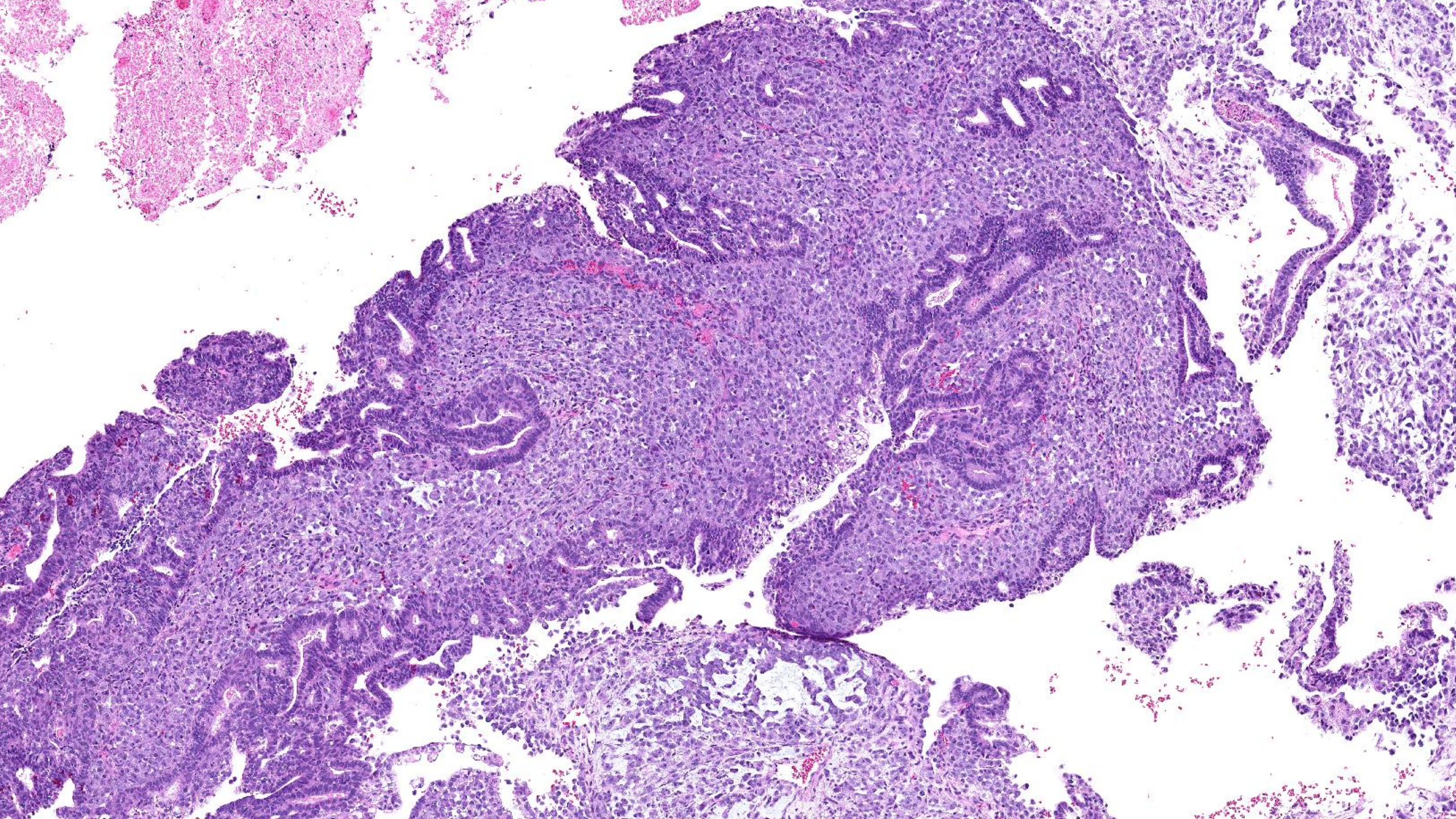


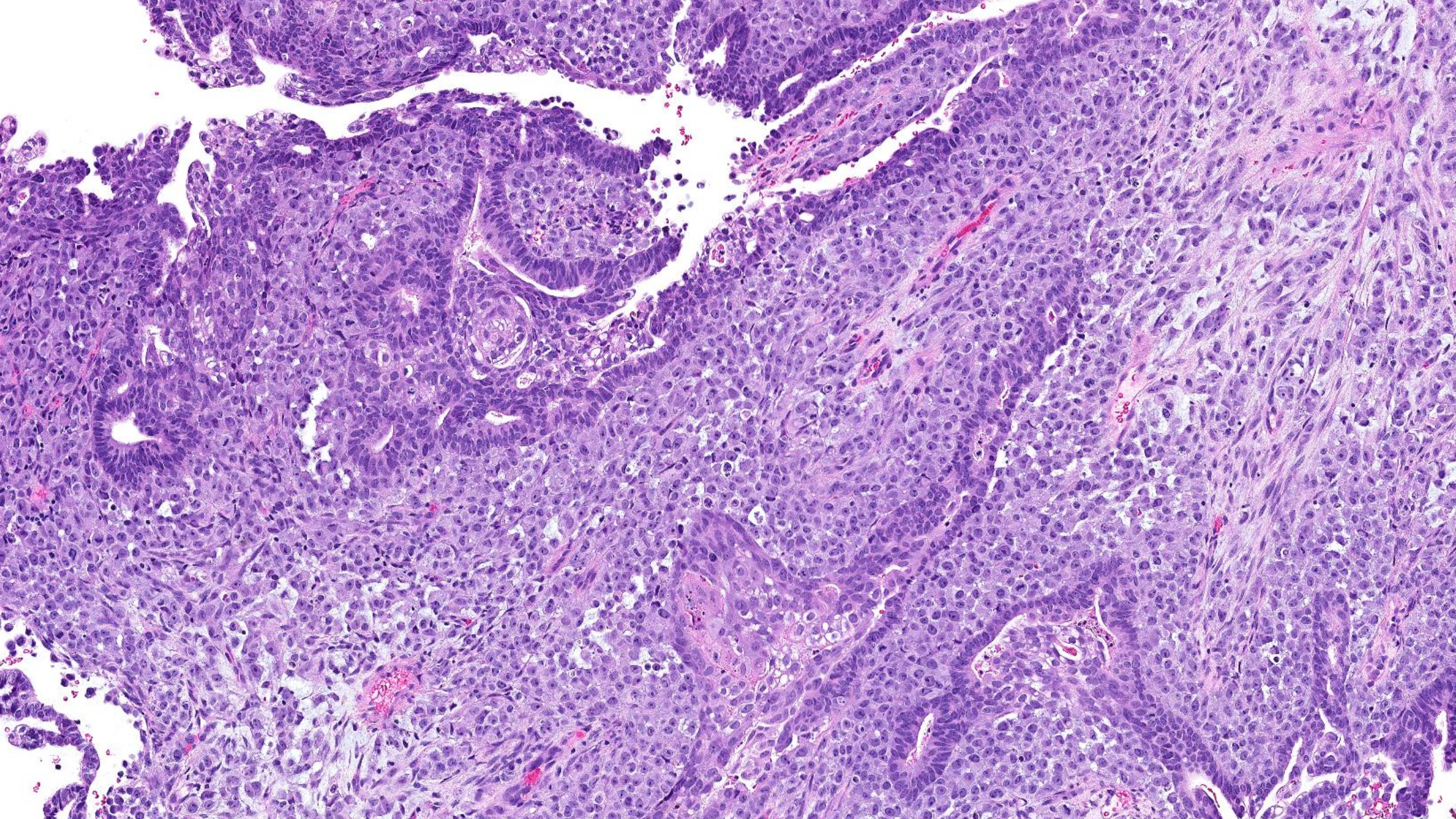
ER

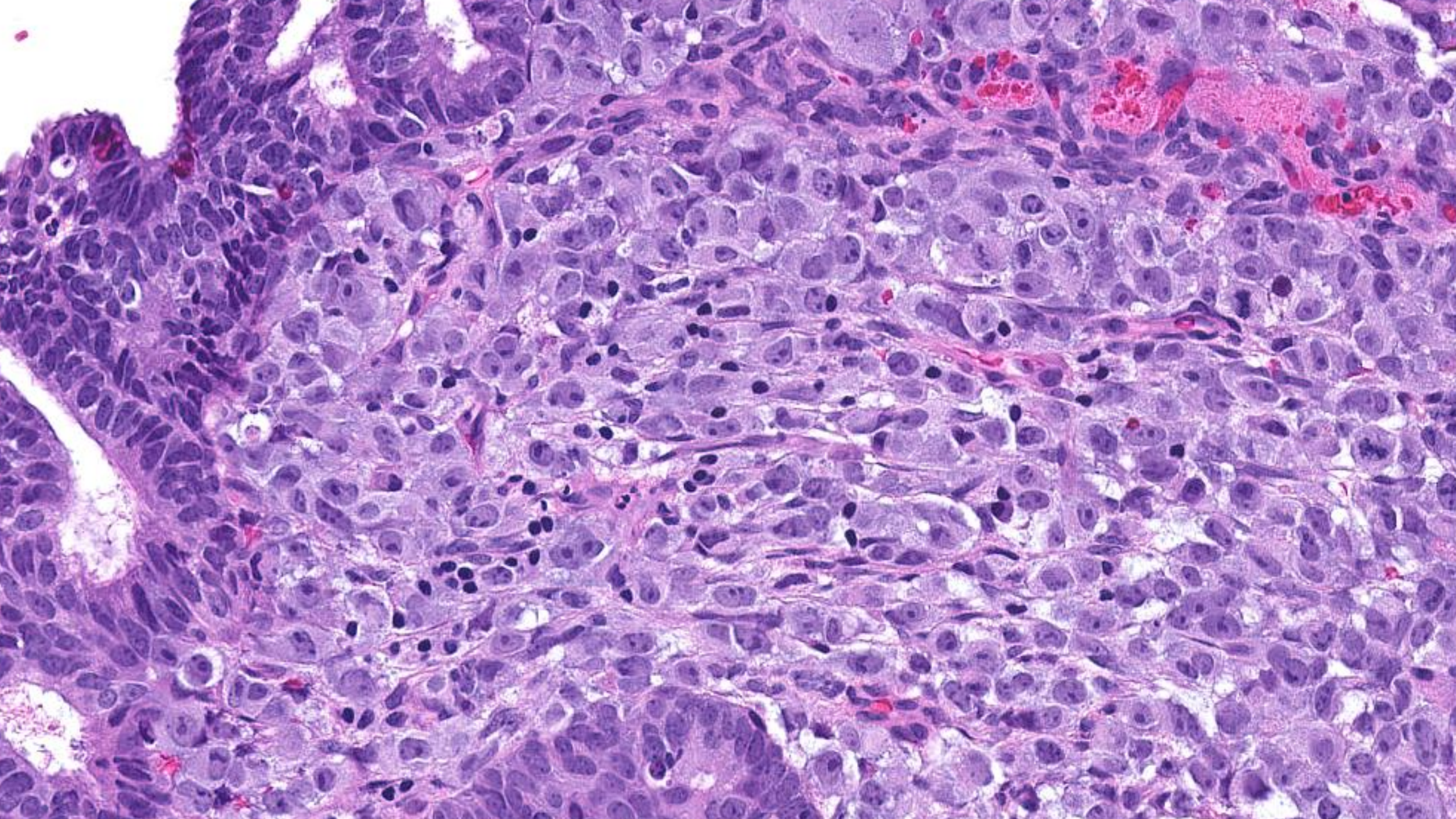


Endometrioid VS Serous VS Clear Cell Carcinoma

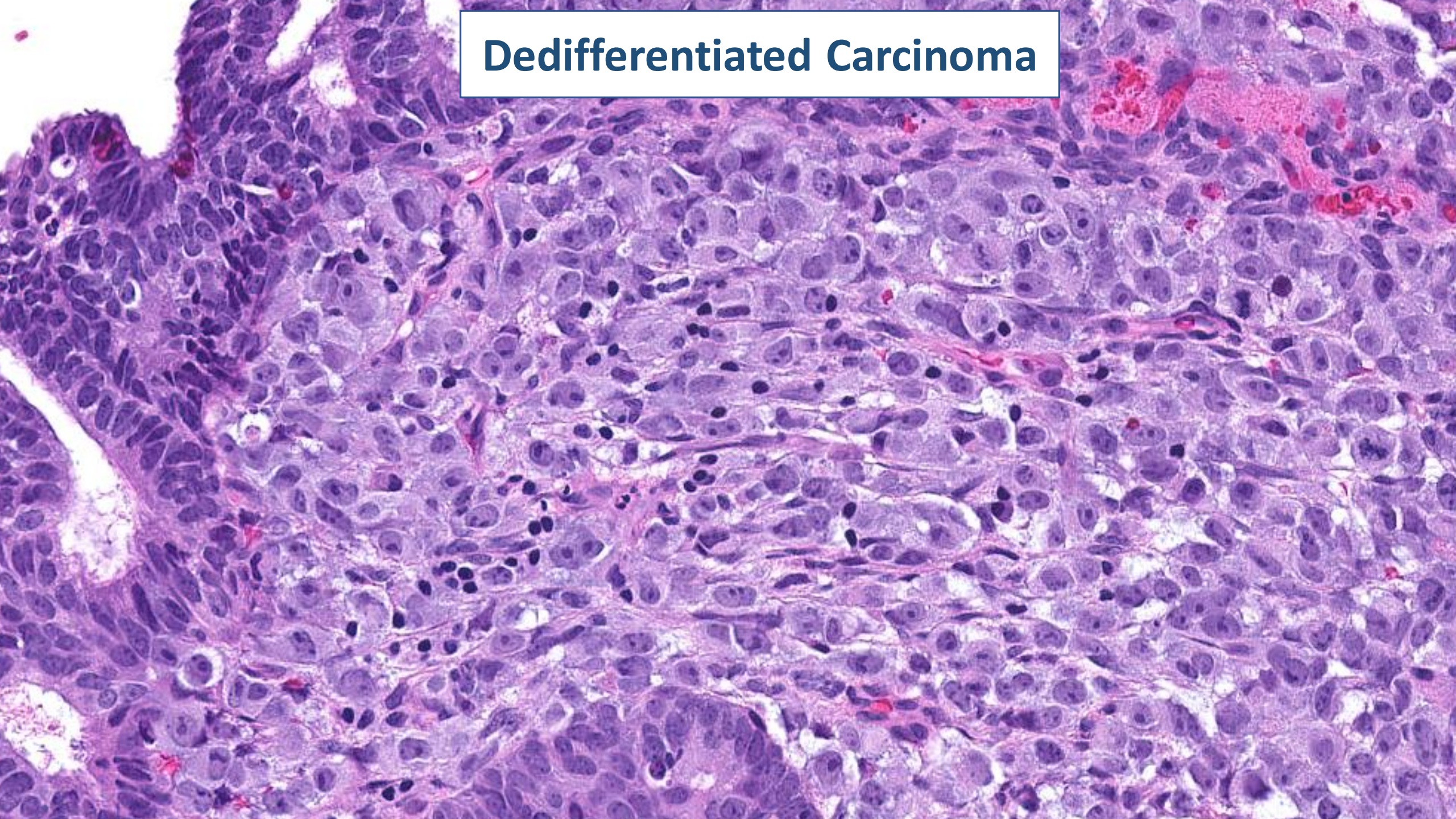




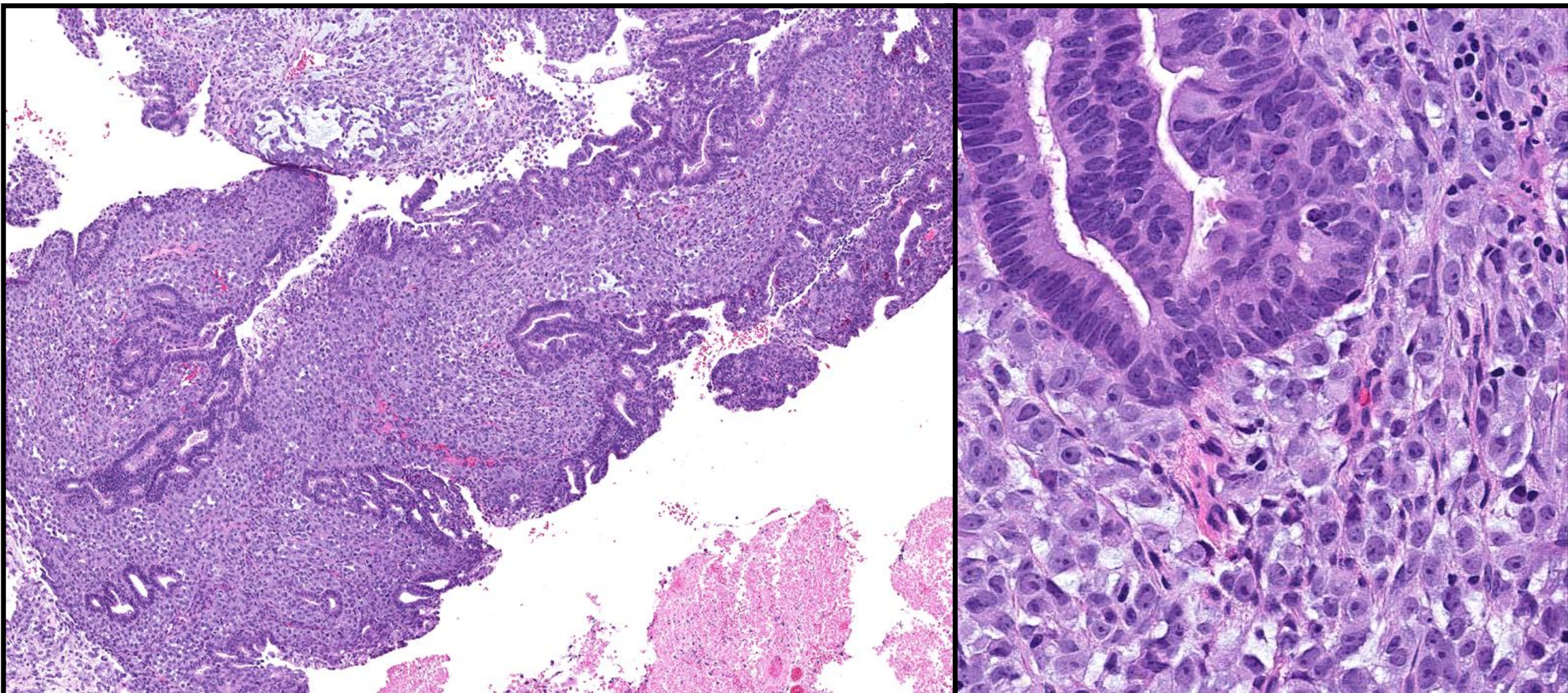




Dedifferentiated Carcinoma

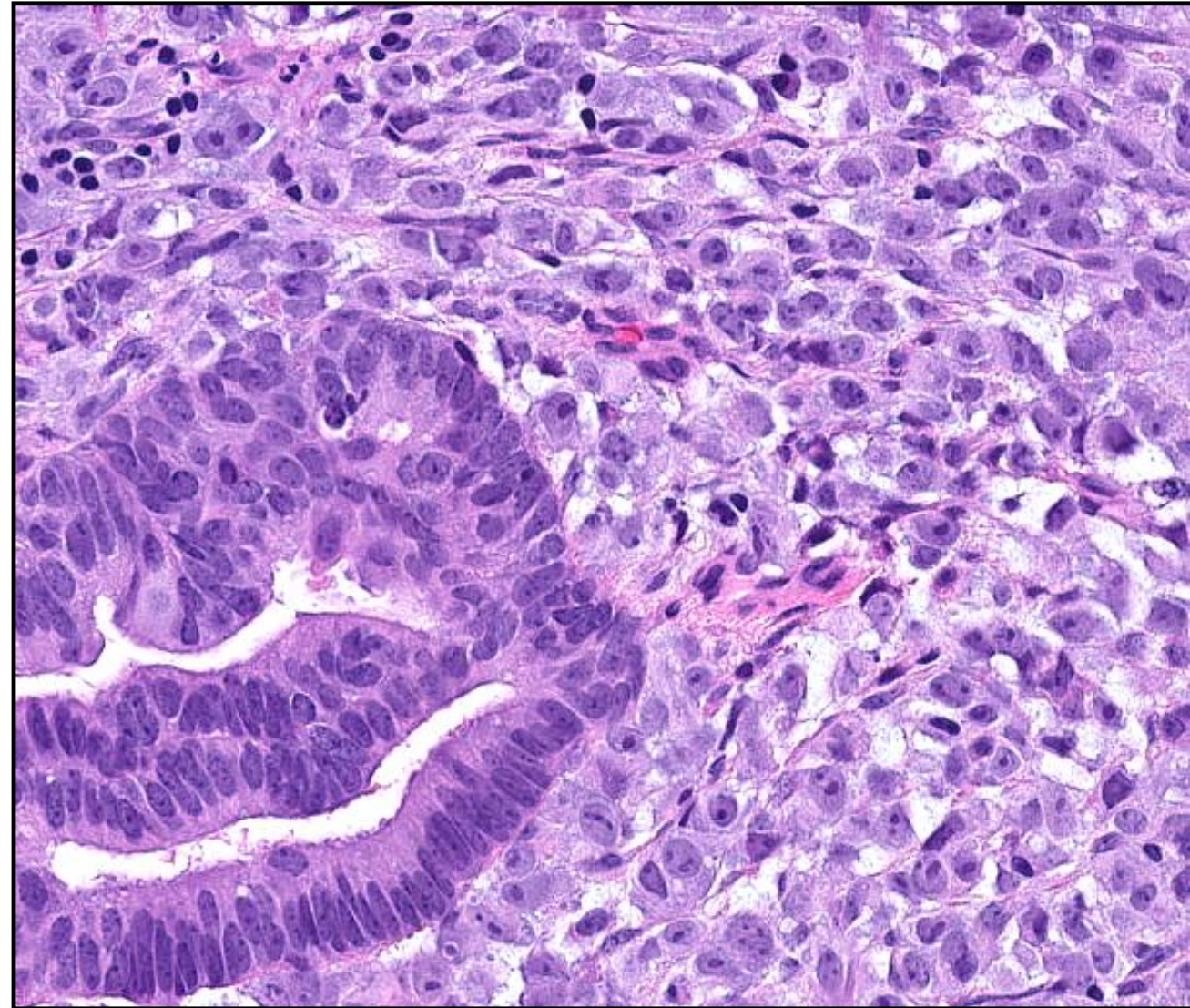


Dedifferentiated Carcinoma Comprises an Undifferentiated Carcinoma & a Second Component of (Usually) Low-grade Endometrioid Carcinoma

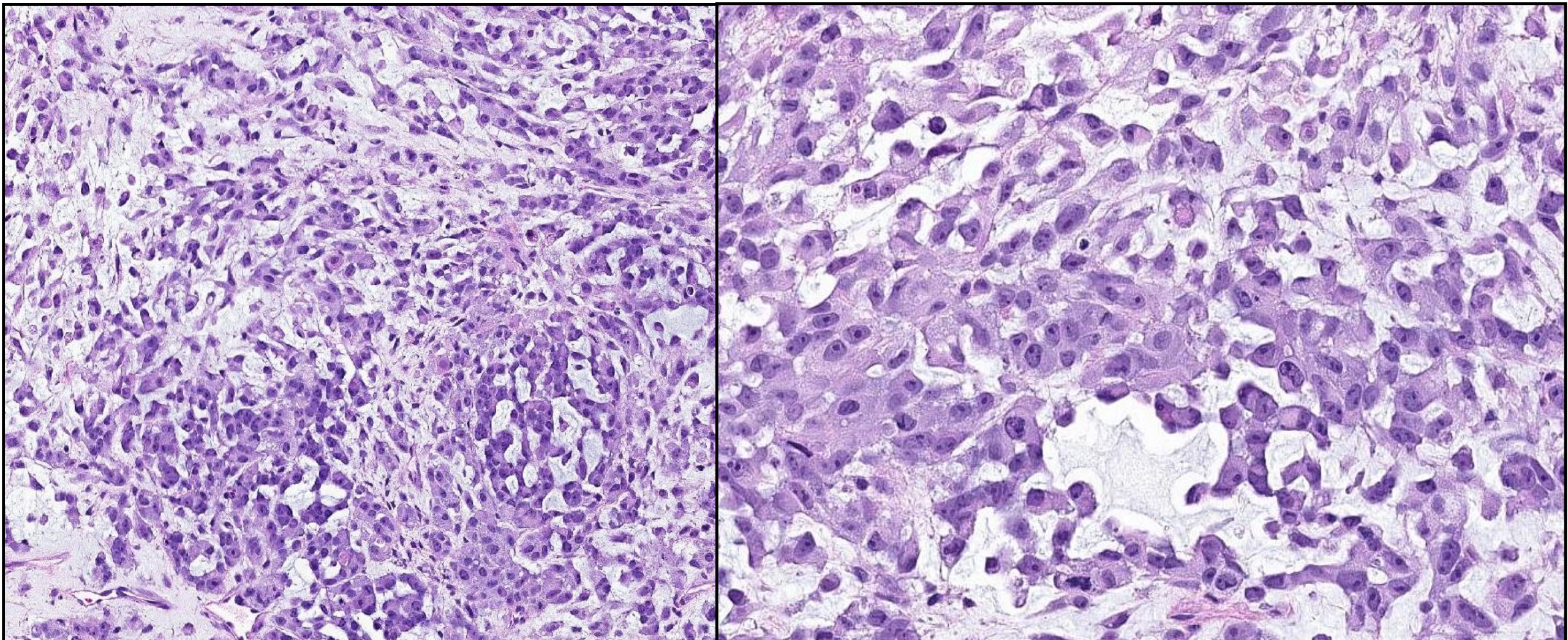


Undifferentiated Carcinoma & Low-Grade Endometrioid Carcinoma Are Spatially Distinct

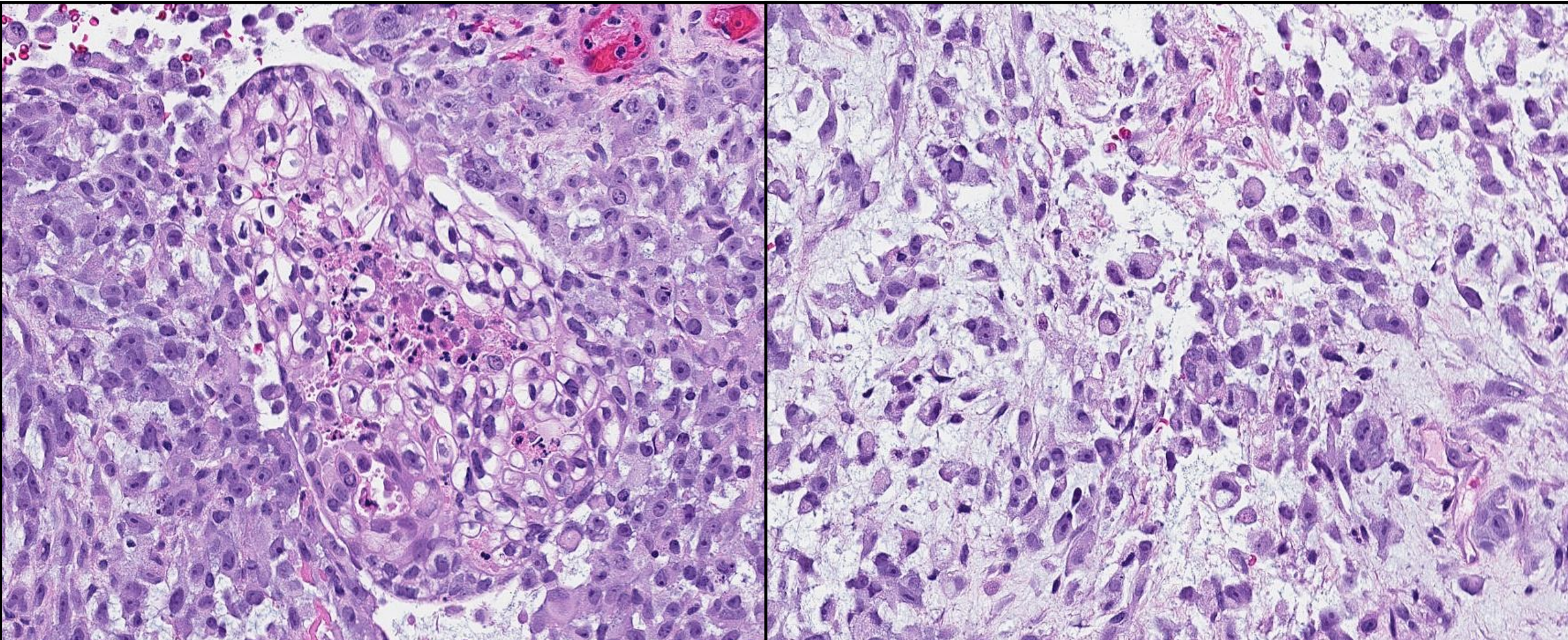
- ✓ Undifferentiated carcinoma:
 - Diffuse/patternless architecture
 - Monomorphic dyscohesive cells
- ✓ Even small undifferentiated component may portend aggressive behavior



**Focal Alveolar, Nested, Myxoid, Vaguely Corded,
Trabecular Patterns, Abrupt Squamous Differentiation
or Rhabdoid Morphology May be Seen**



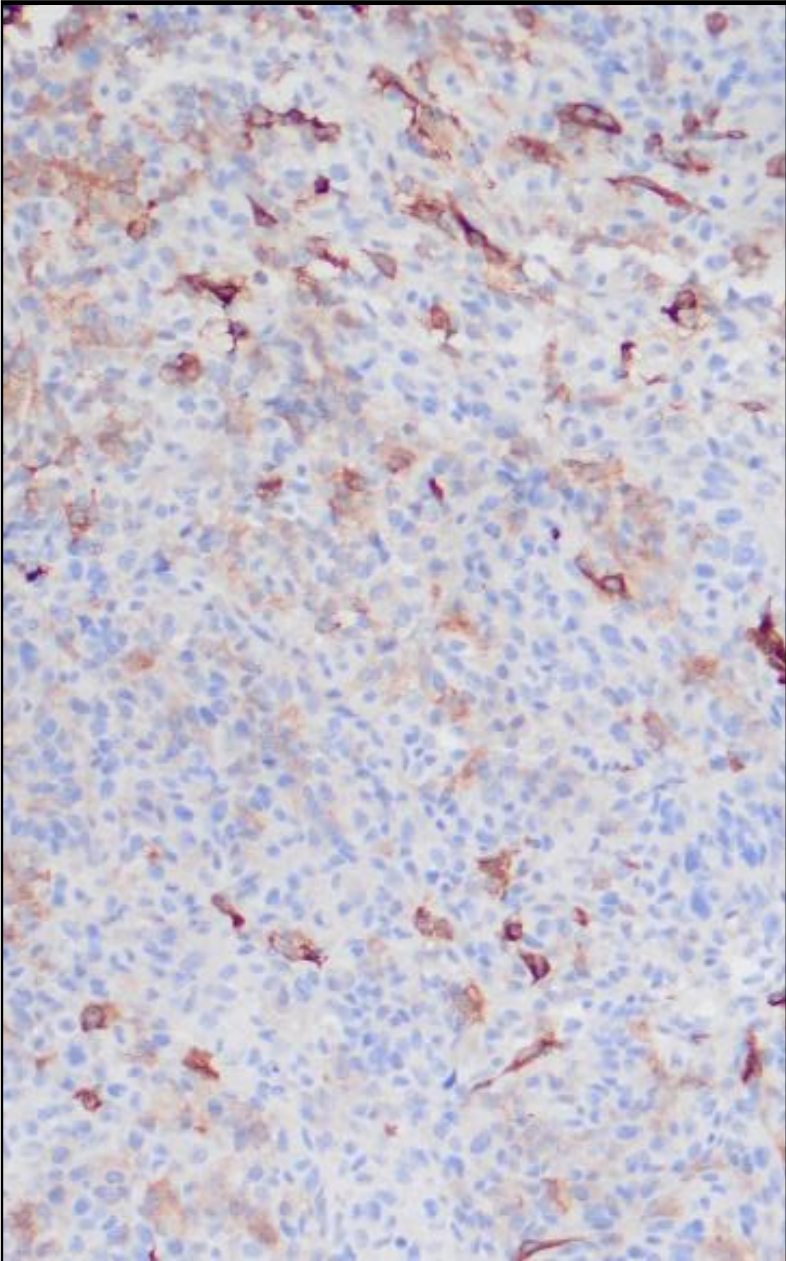
Focal Alveolar, Nested, Myxoid, Vaguely Corded, Trabecular Patterns, Abrupt Squamous Differentiation or Rhabdoid Morphology May be Seen



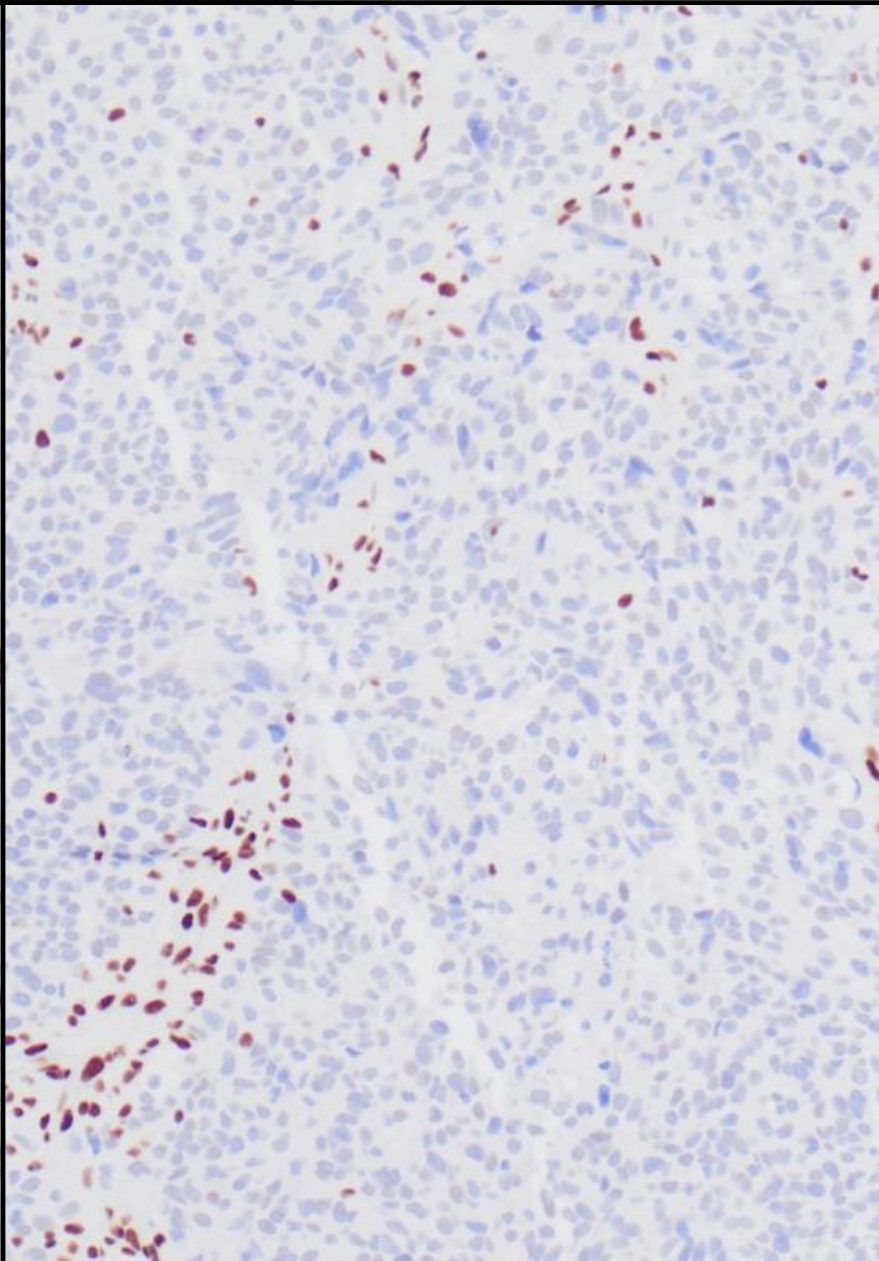
Dedifferentiated Carcinoma Is Characterized by MMR Deficiency & SWI/SNF Complex Deficiency

- ✓ **Loss of MMR expression (MLH1/PMS2) in ~45-70%**
- ✓ **SWI/SNF complex proteins:**
 - **SMARCA4/BRG-1 loss (~35%)**
 - **SMARCB1/INI-1 loss (~10%)**
 - **ARID1A/B loss (~25%)**
- ✓ **Loss of pan-CK, CK18, EMA (~80%), e-cadherin (~50%)**
- ✓ **Negative PAX8 (80-90%), ER/PR (70-90%)**
- ✓ **Abnormal p53 expression ~15-30%**
- ✓ **Neuroendocrine marker expression (<10% of tumor cells)**

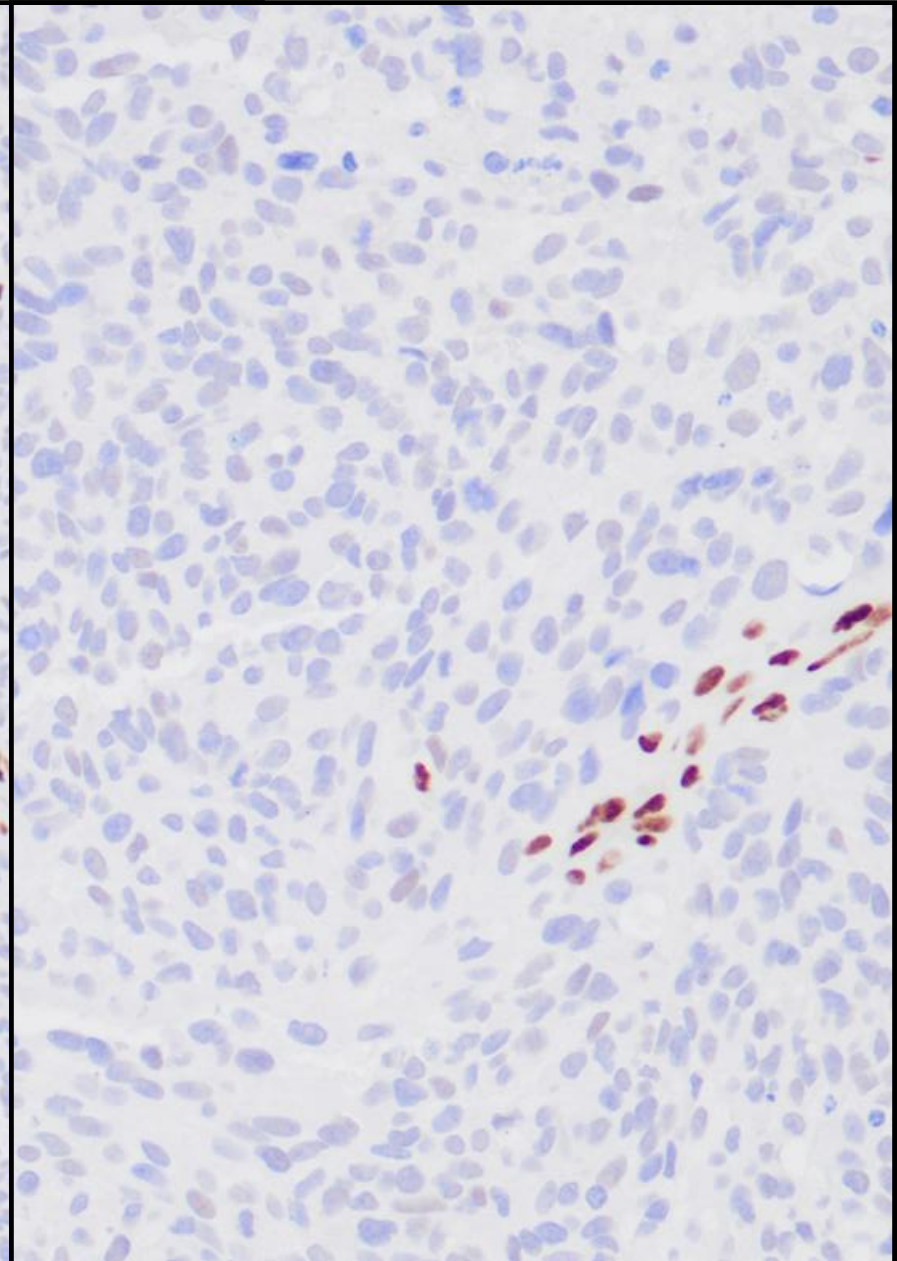
CK18



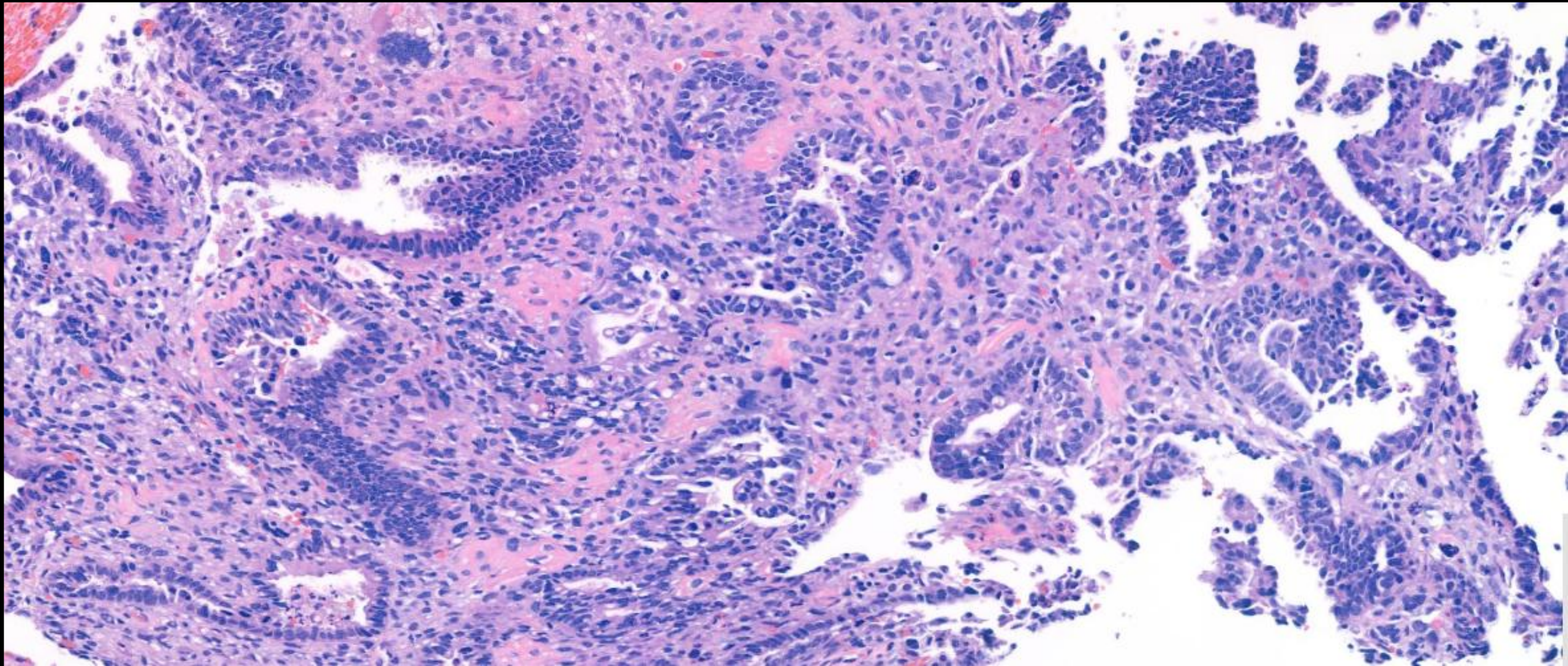
MLH1/PMS2



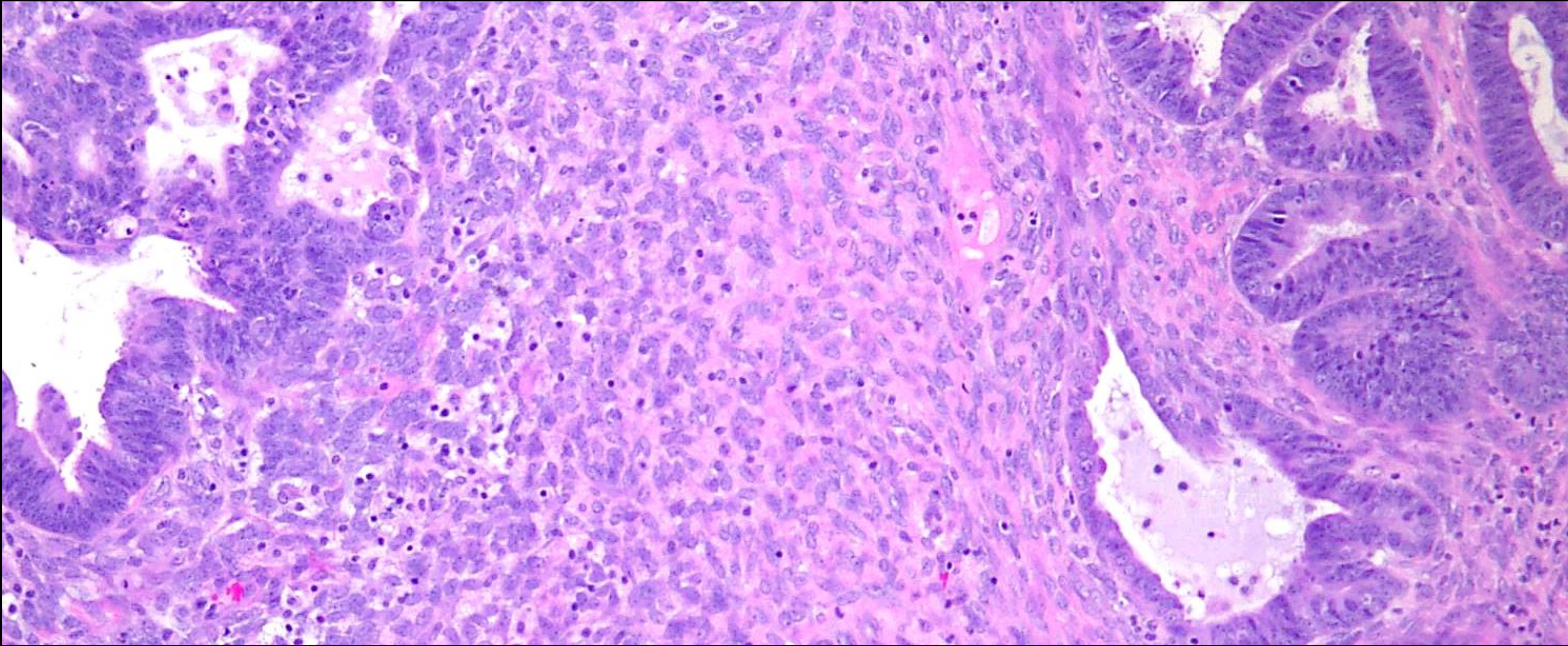
BRG-1



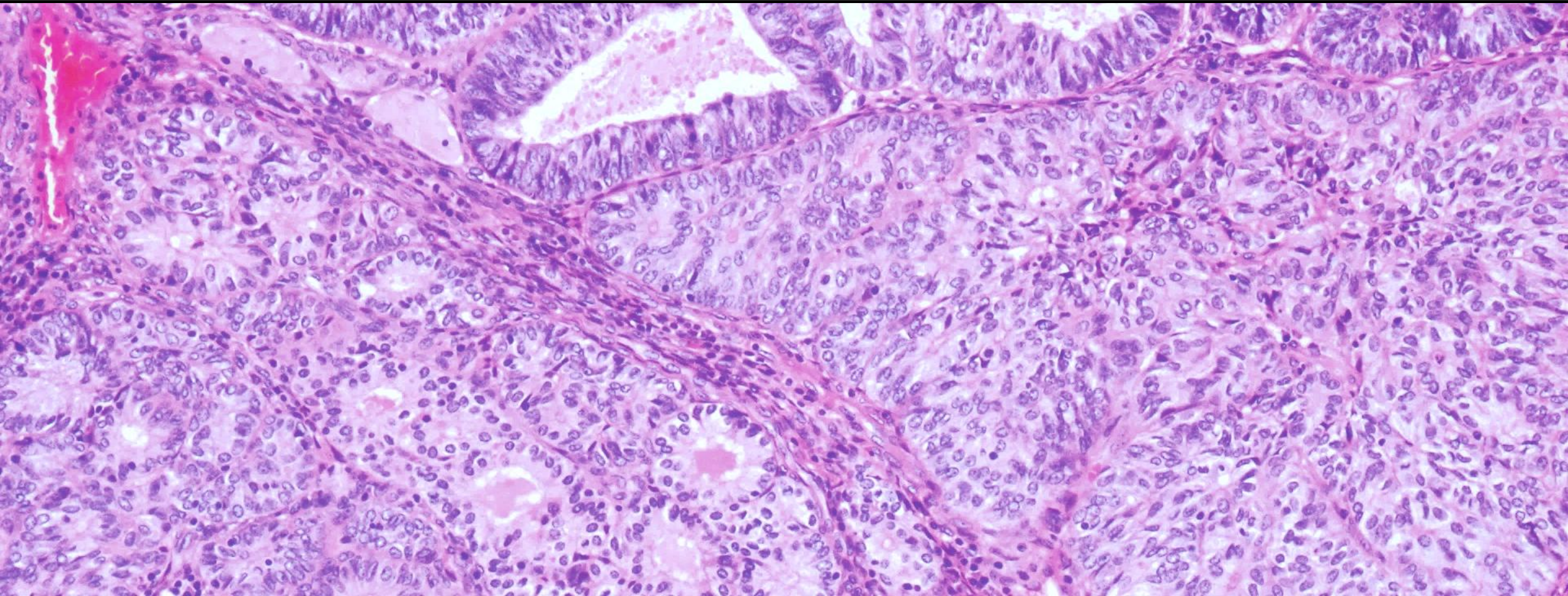
Dedifferentiated Carcinoma Should Be Differentiated from Carcinosarcoma (Spatially Distinct High-Grade Carcinomatous & Sarcomatous Components)



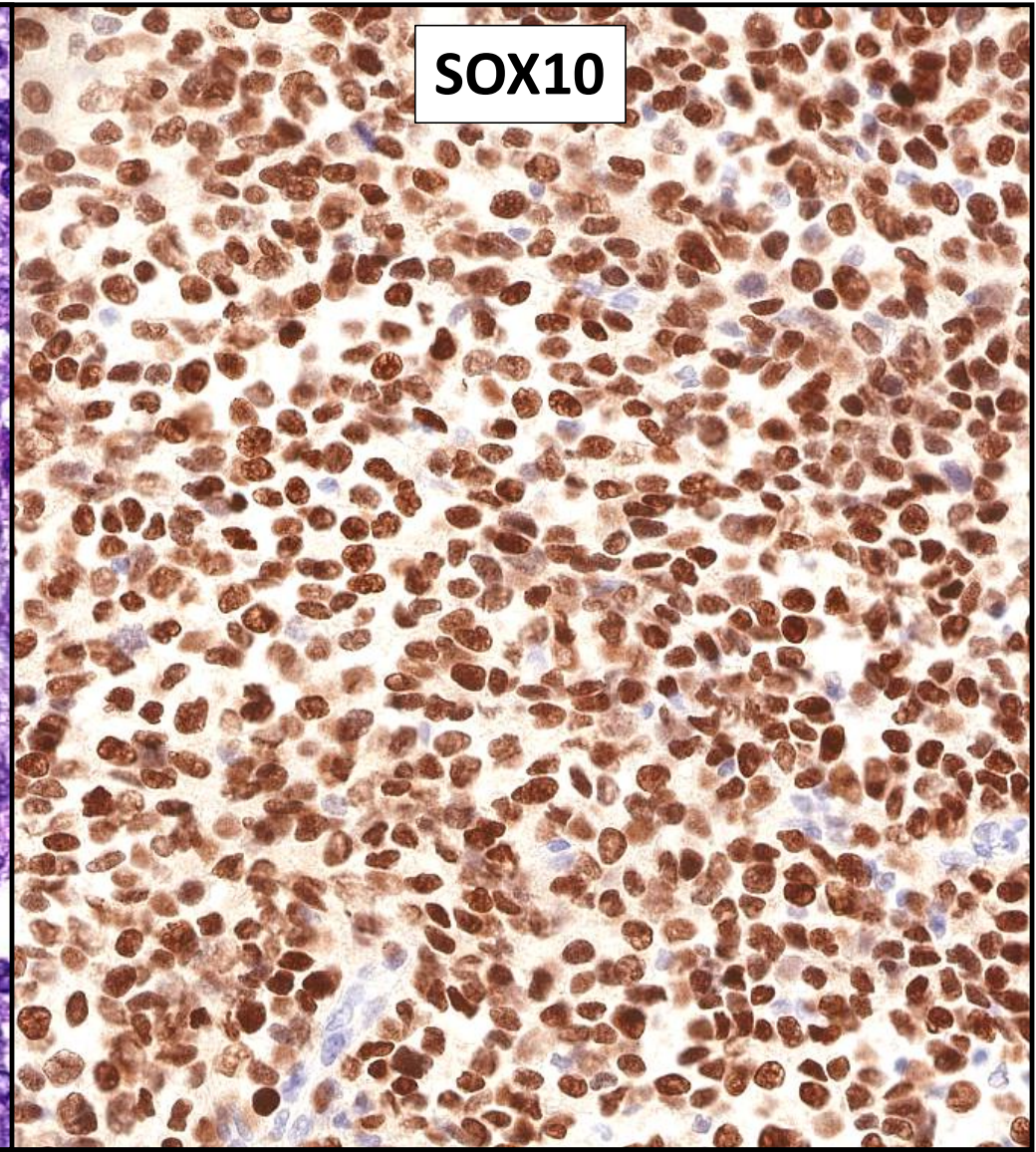
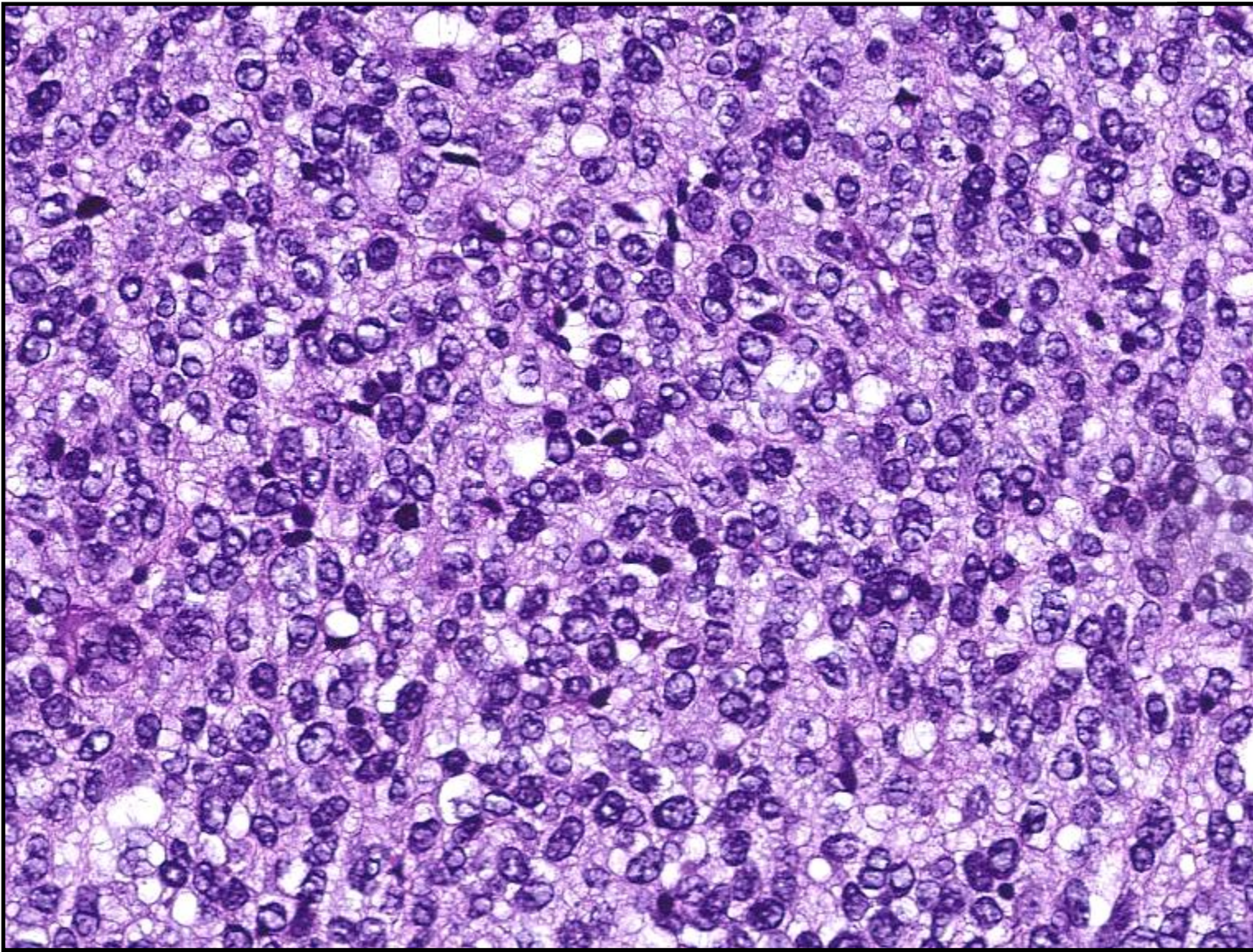
Dedifferentiated Carcinoma Should Be Differentiated from Corded & Hyalinized Endometrioid Carcinoma (Usually Non Spatially Distinct, Bland Cytology)



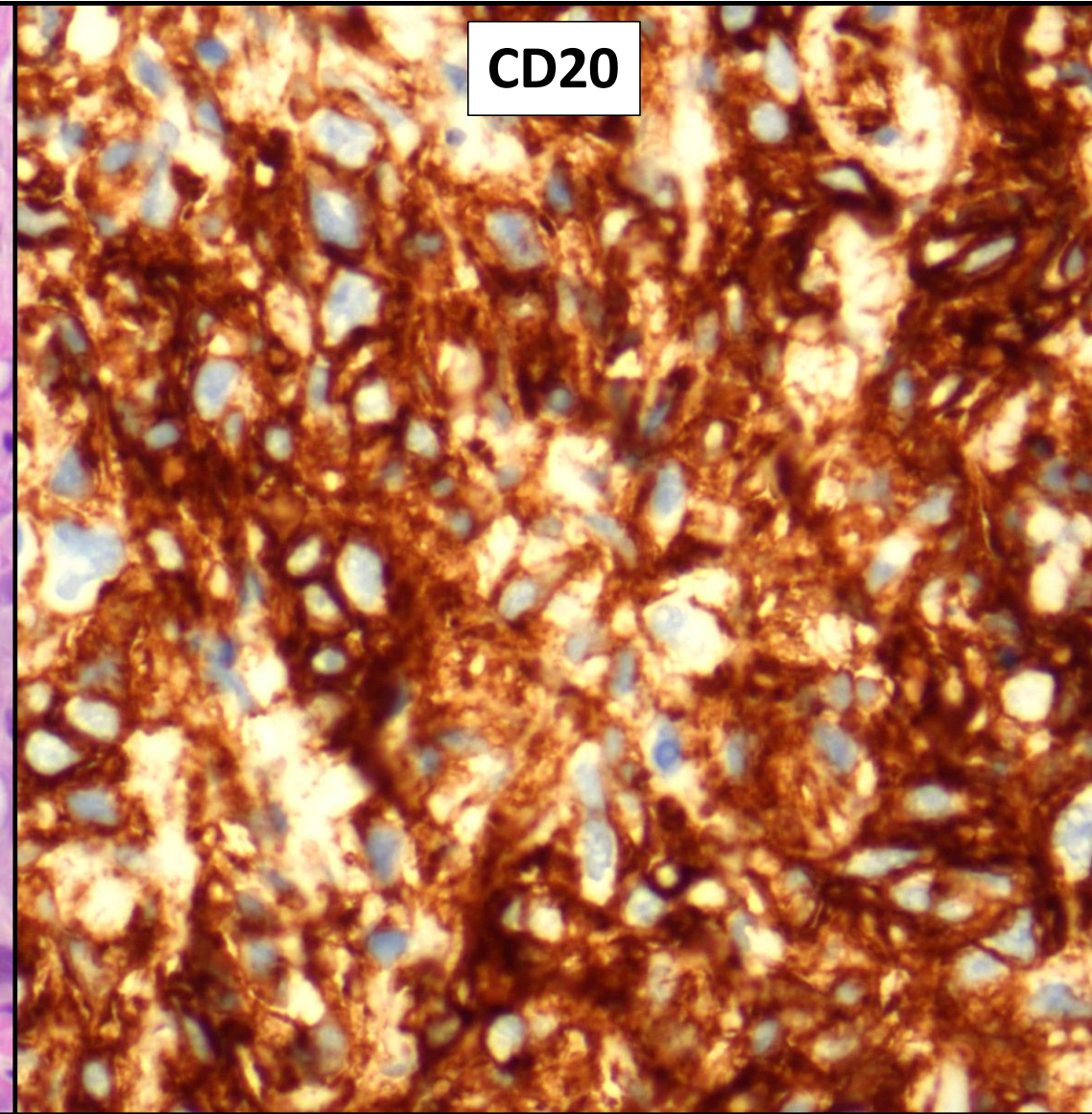
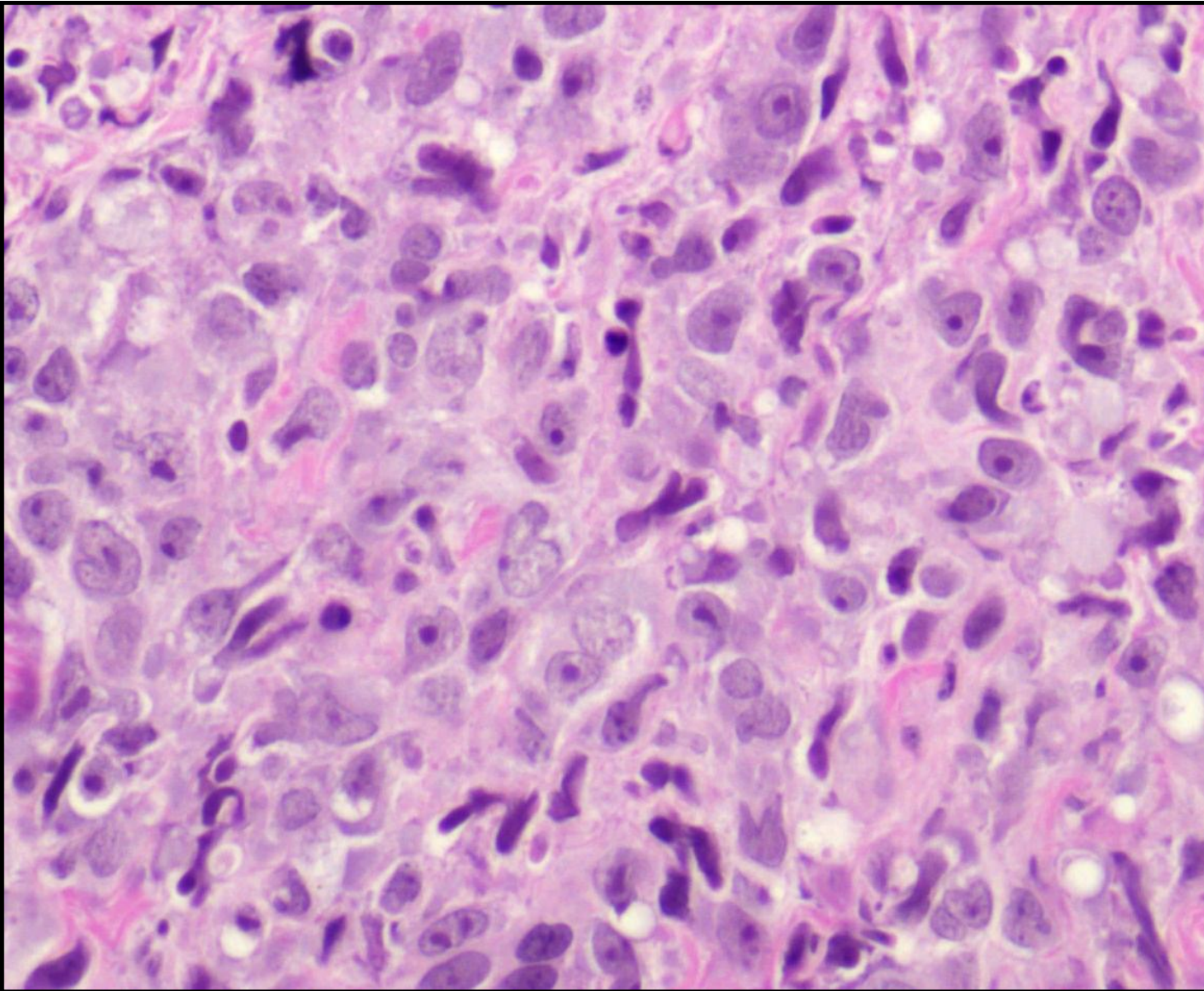
**Dedifferentiated Carcinoma Should Be Differentiated
from Grade 2-3 Endometrioid Carcinoma
(Non Spatially Distinct Non-Squamous Solid Growth,
Positive for Keratins, ER, PAX8)**



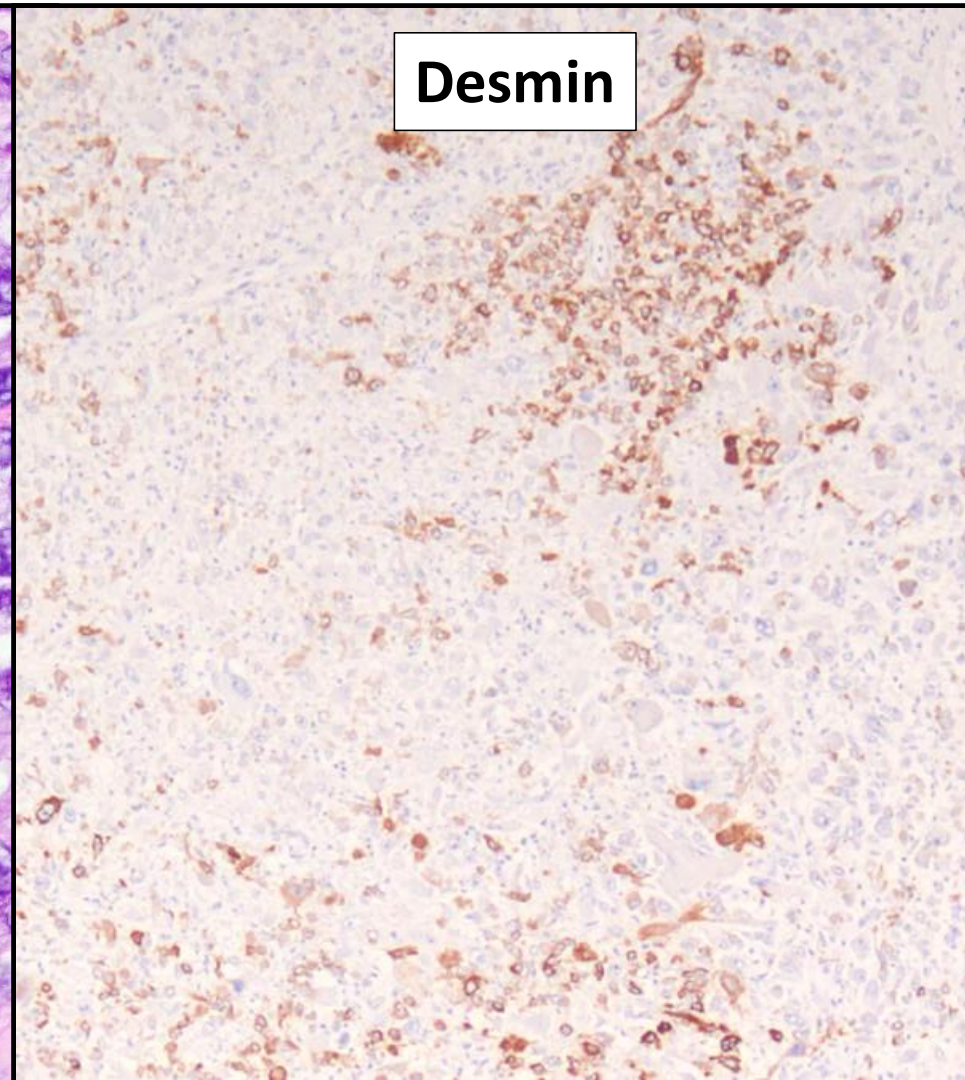
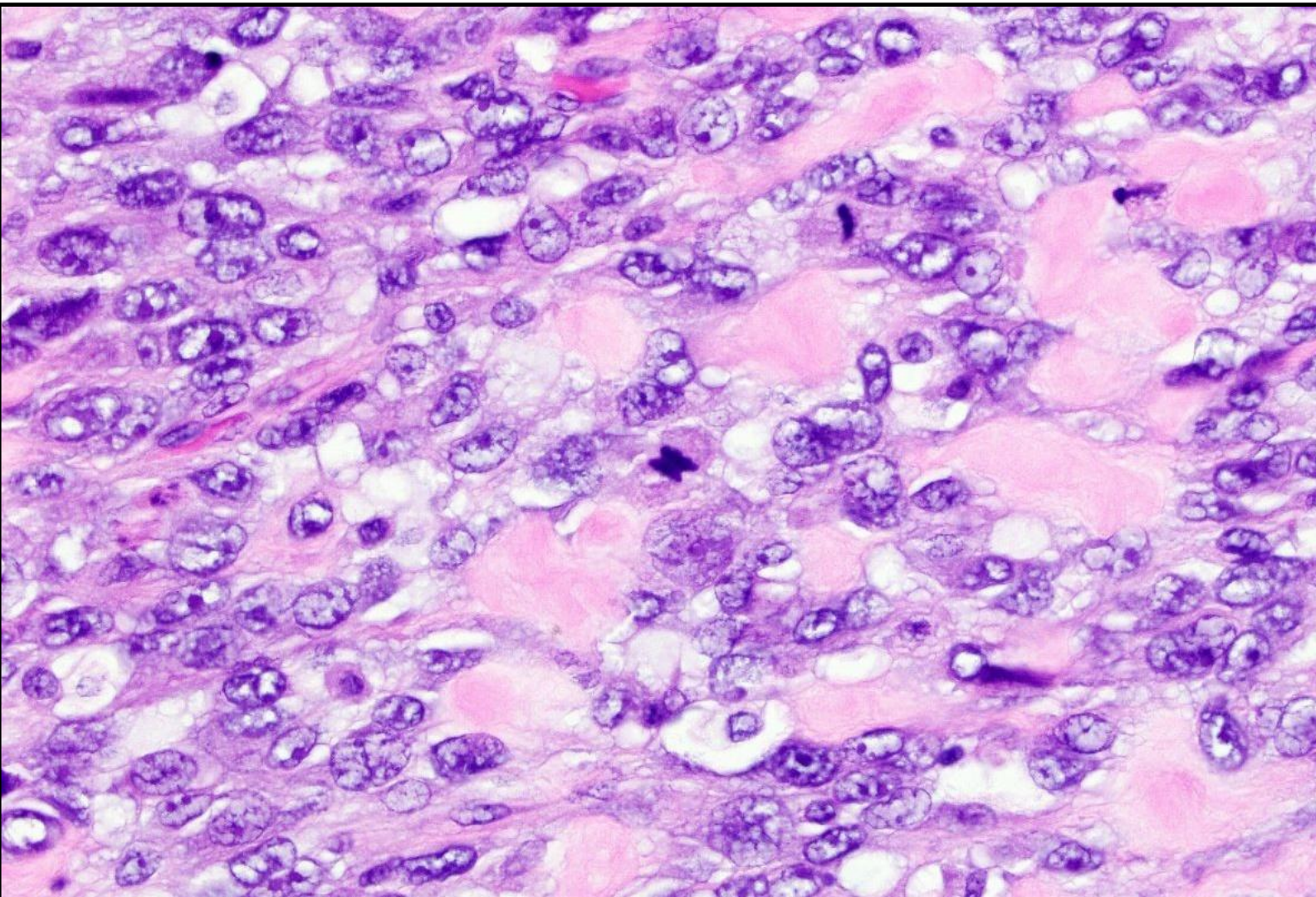
Dedifferentiated Carcinoma Should Be Differentiated from Melanoma (Requires Immunohistochemistry)

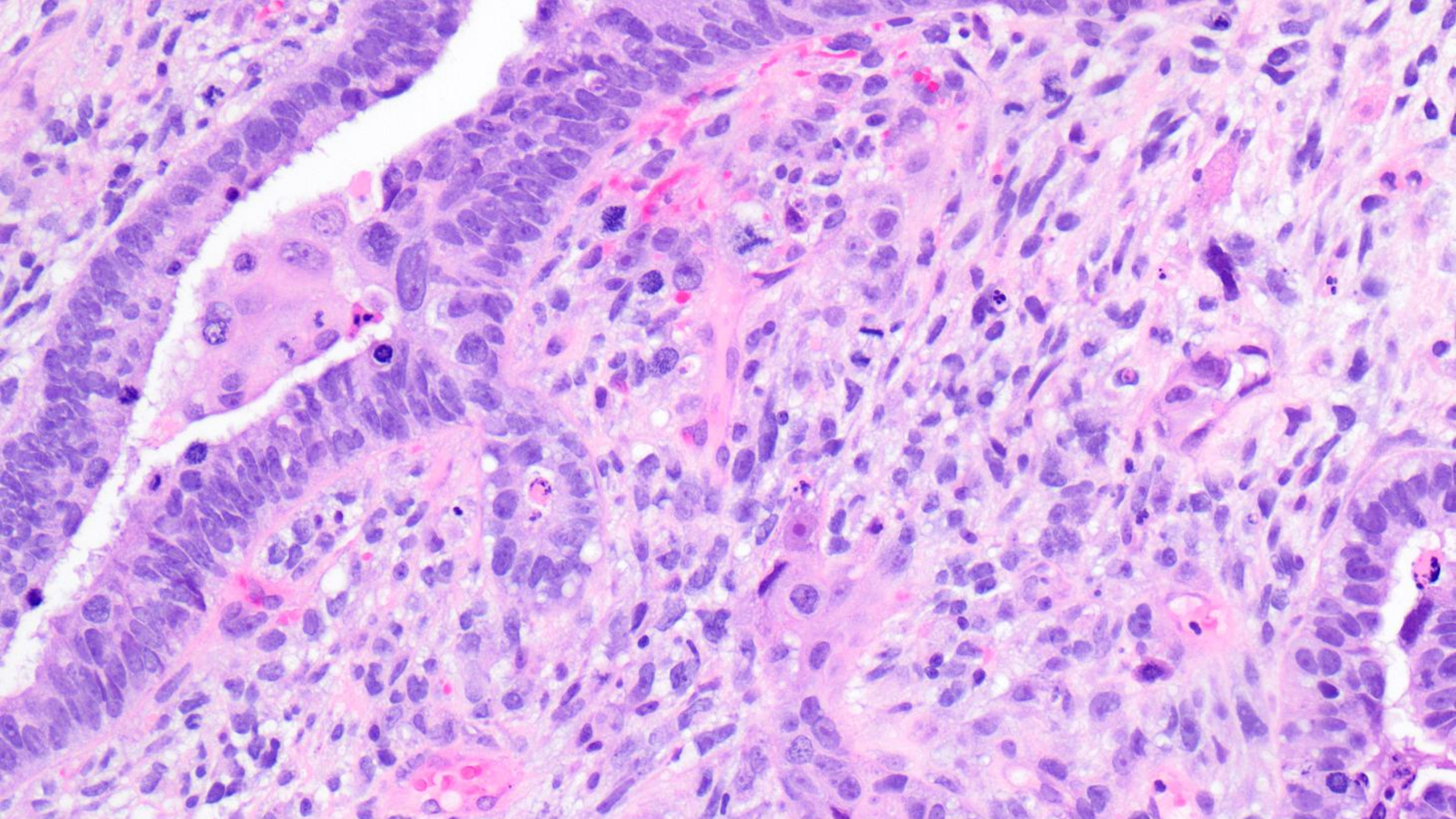


Dedifferentiated Carcinoma Should Be Differentiated from Lymphoma (Requires Immunohistochemistry)

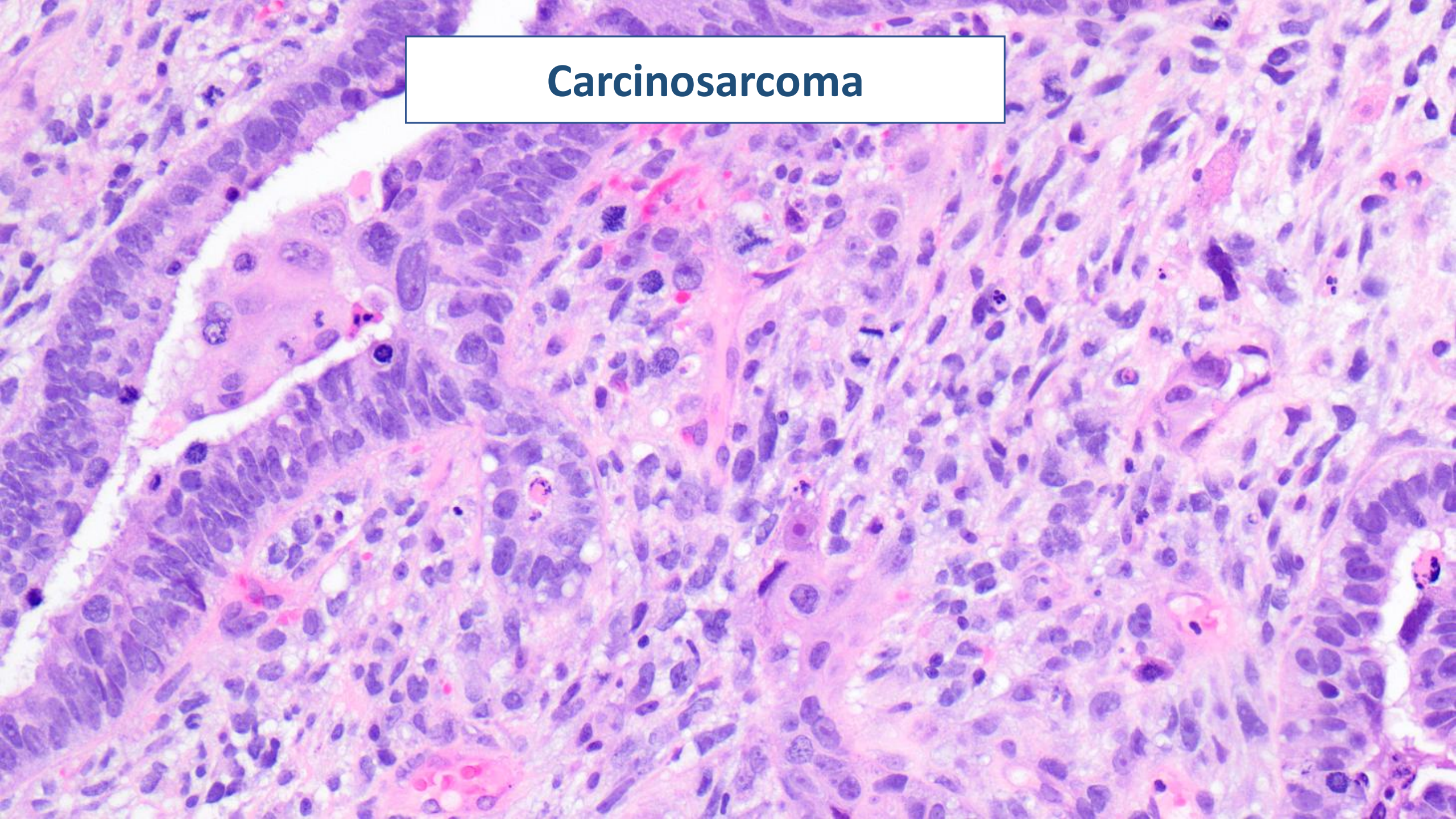


Dedifferentiated Carcinoma Should Be Differentiated from Sarcoma, e.g. Epithelioid Leiomyosarcoma (Requires Immunohistochemistry)



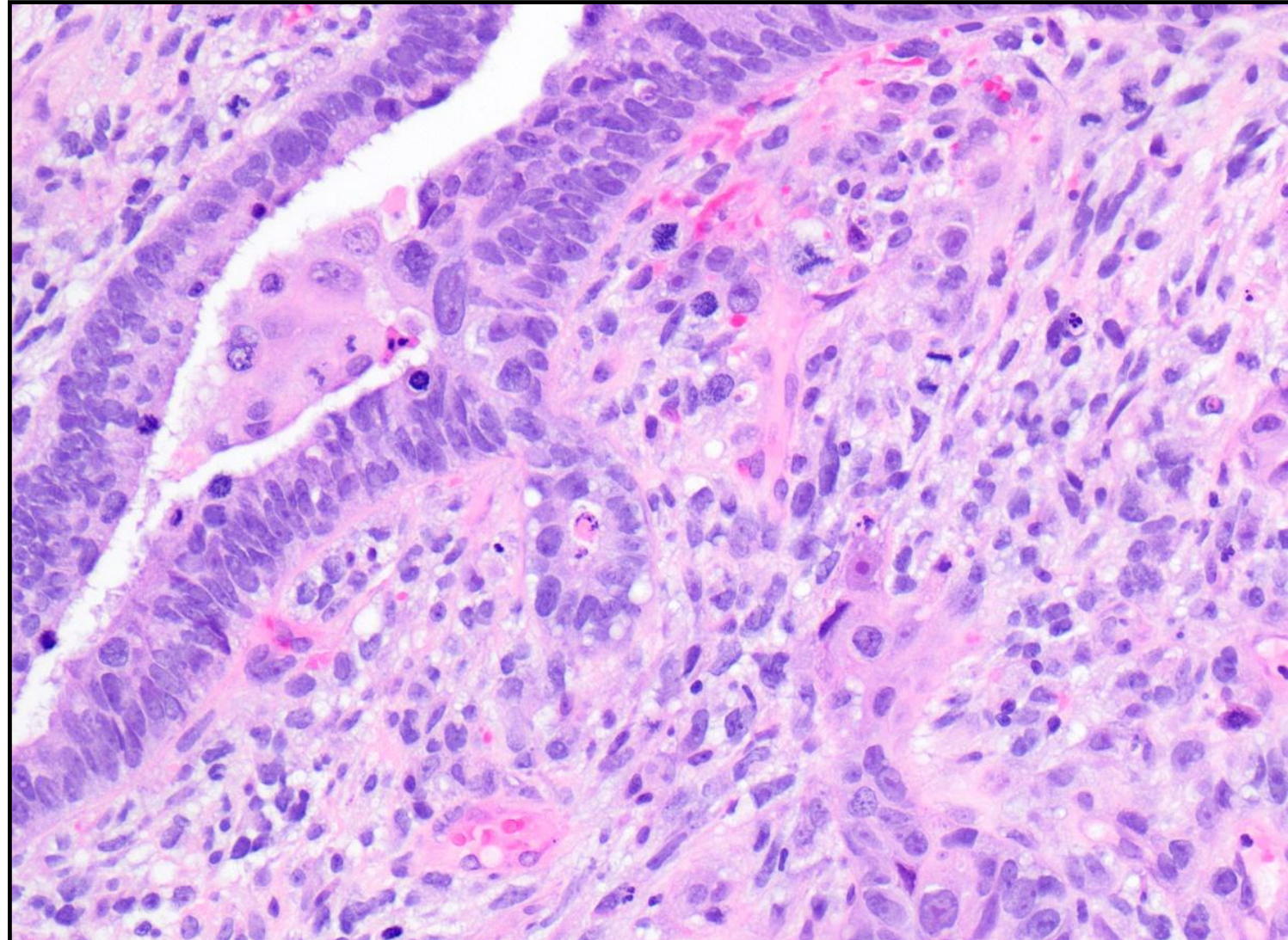


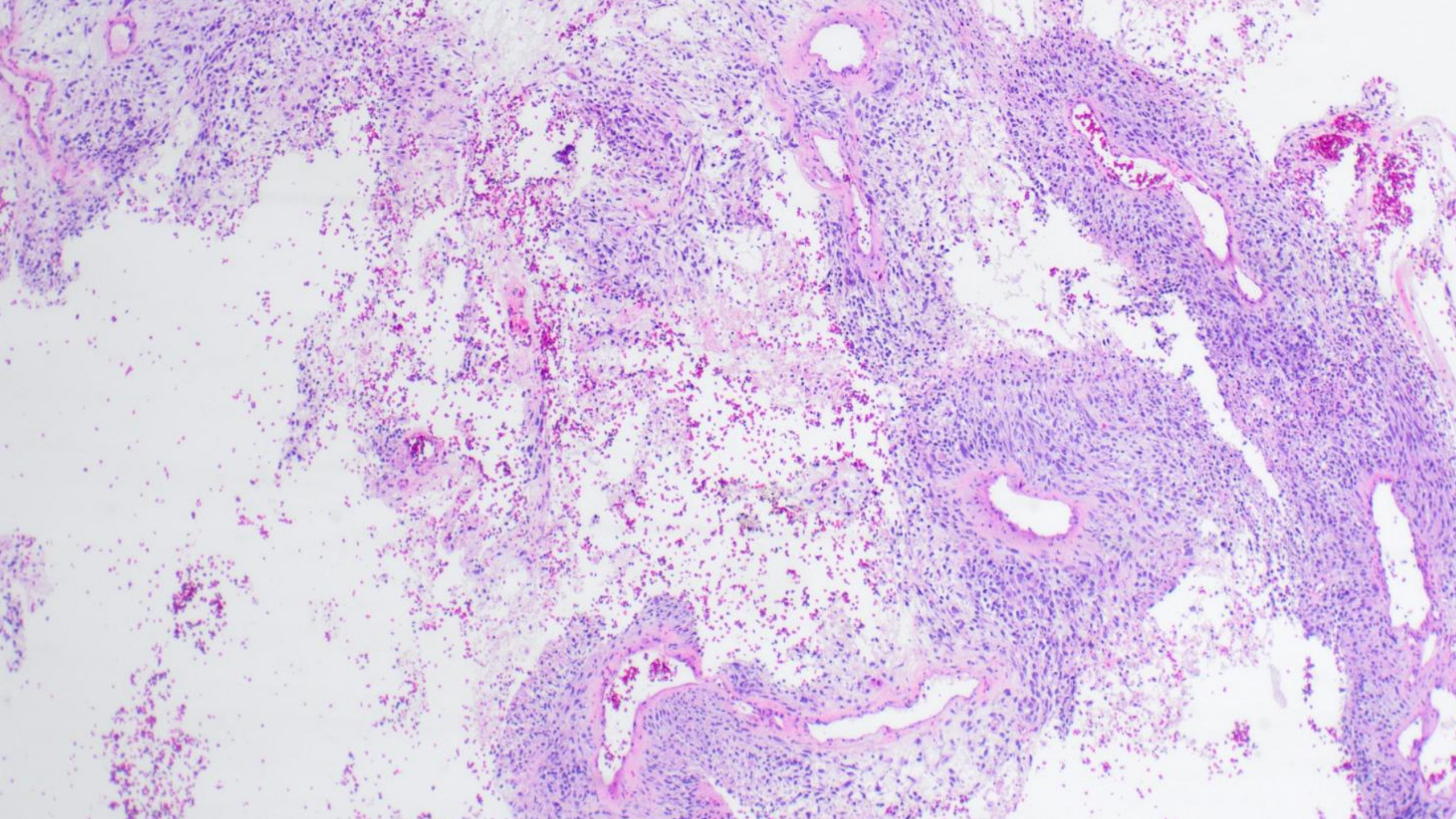
Carcinosarcoma

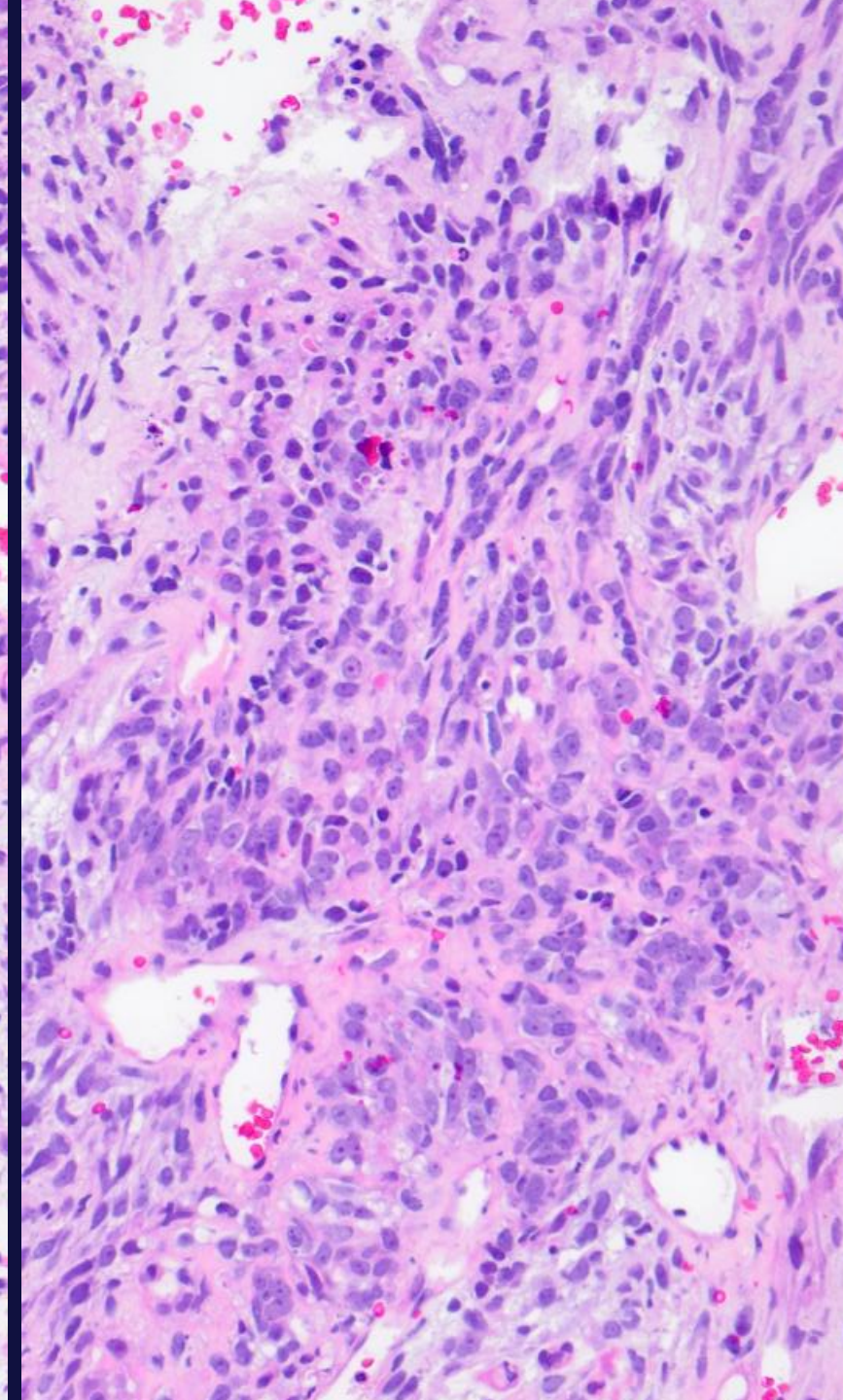
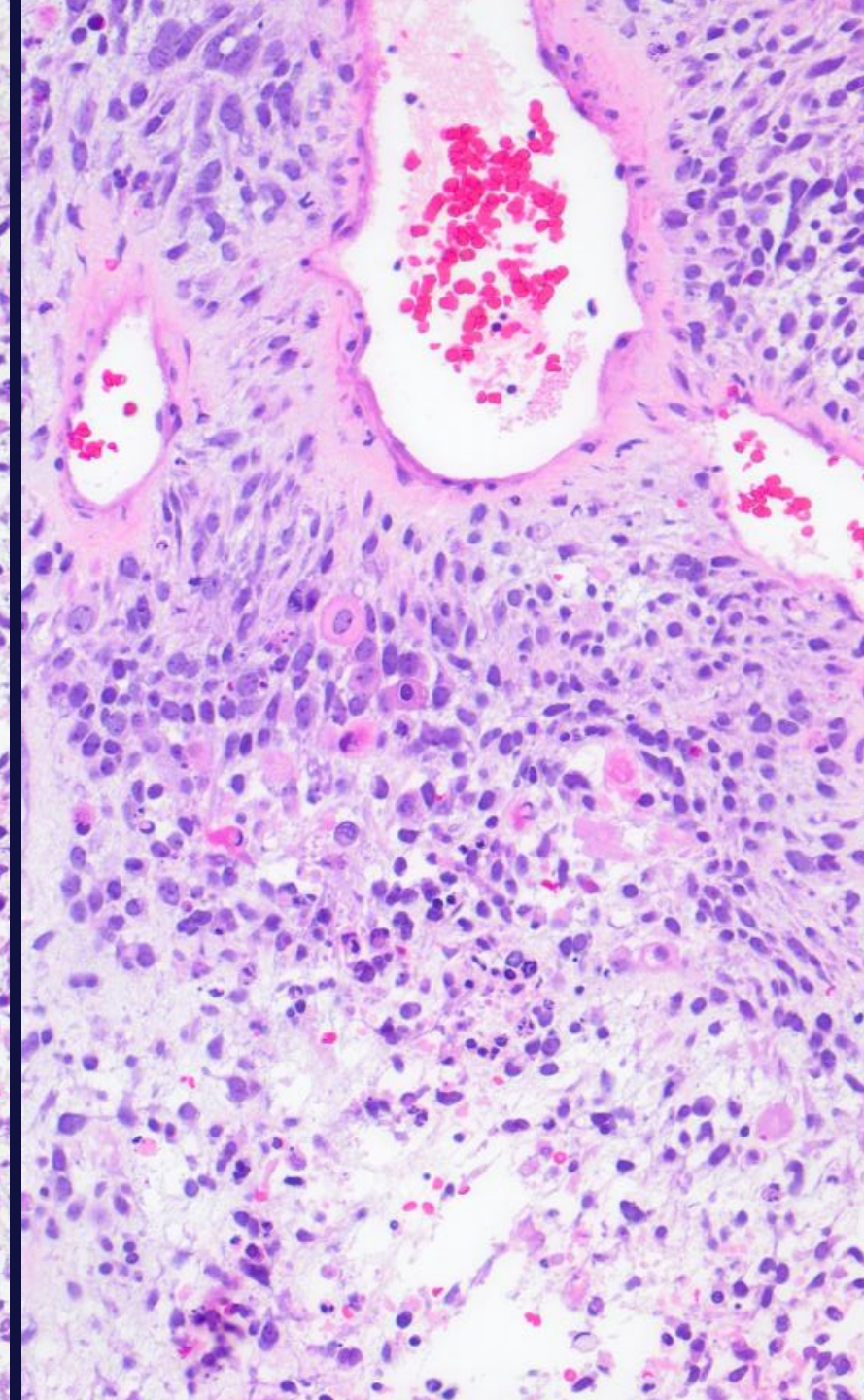
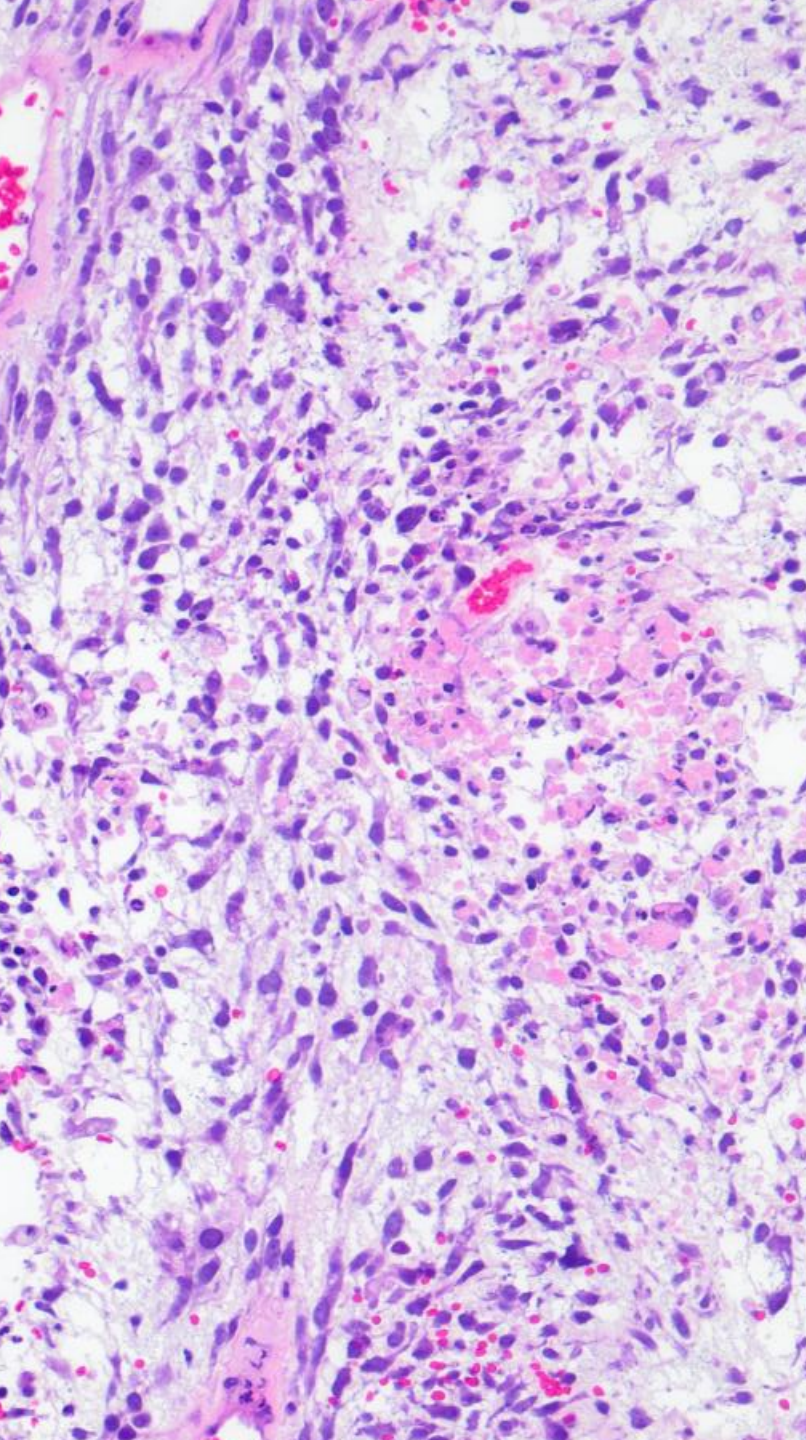


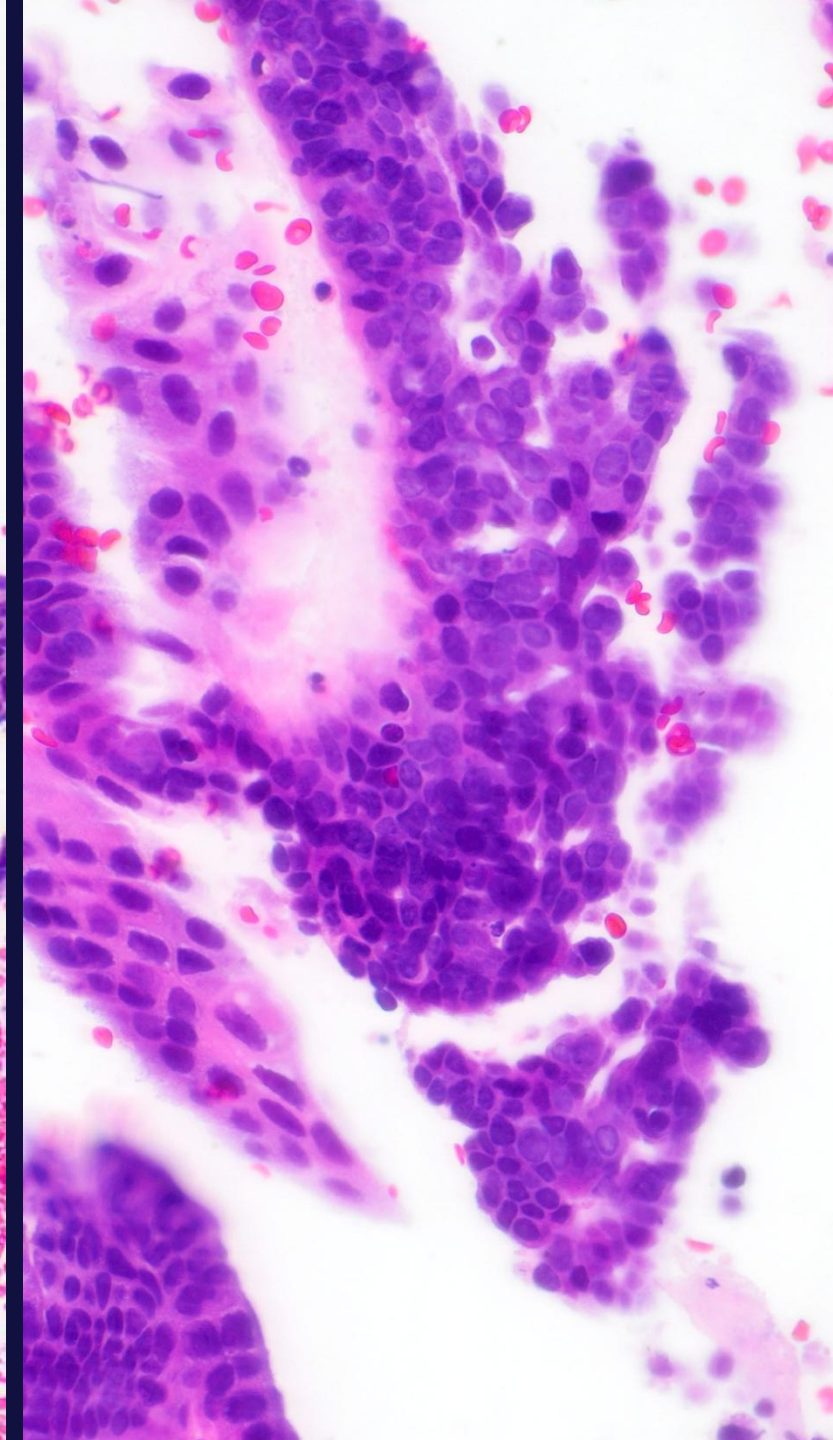
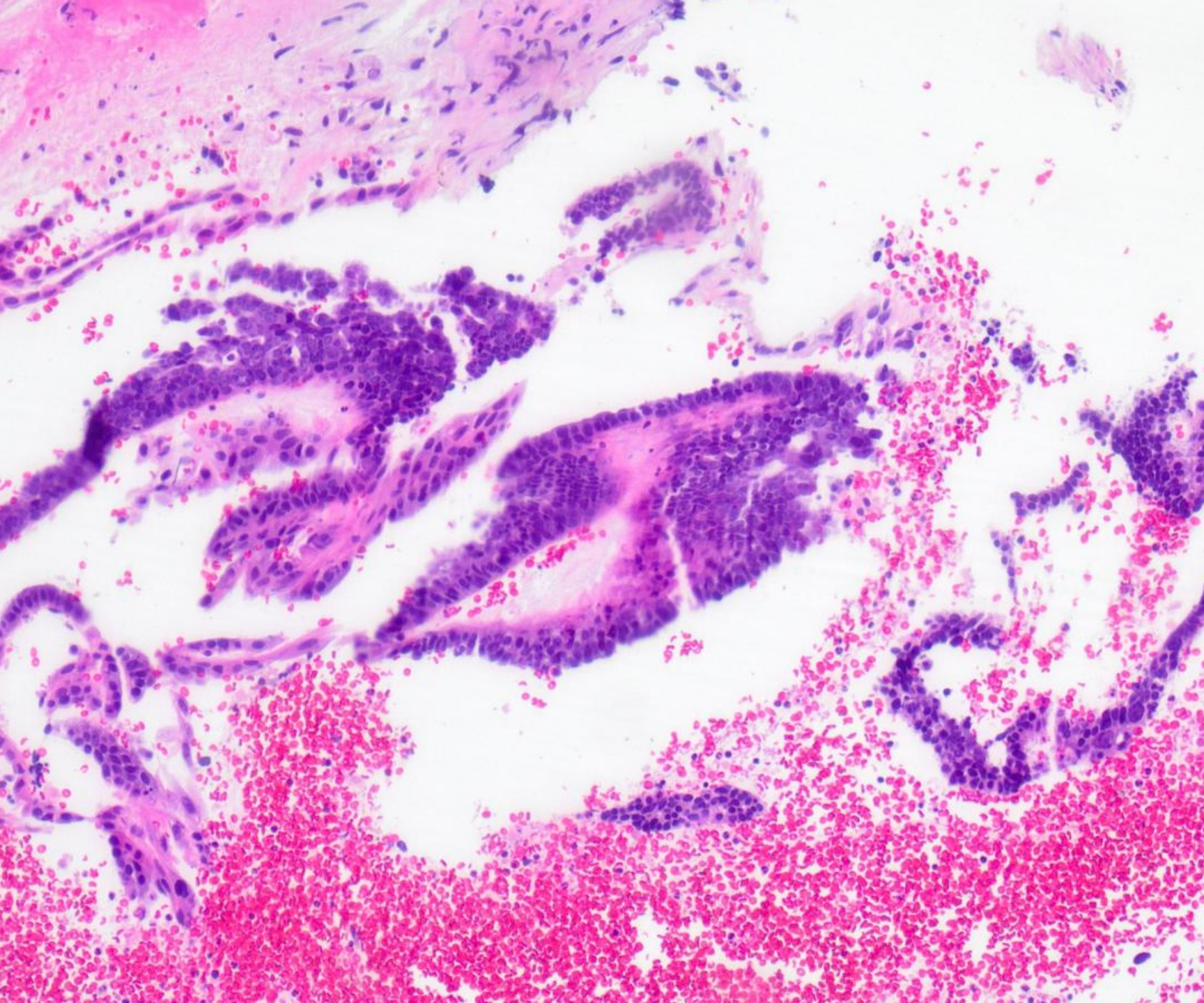
Carcinosarcoma Is Composed of Spatially Distinct High-Grade Epithelial & Sarcomatous Components

- ✓ **Epithelial component:**
 - Often solid growth
 - Variable differentiation
 - Marked atypia
- ✓ **Sarcomatous component:**
 - ≥ 1 mm (arbitrary)
 - $\pm$ Heterologous differentiation

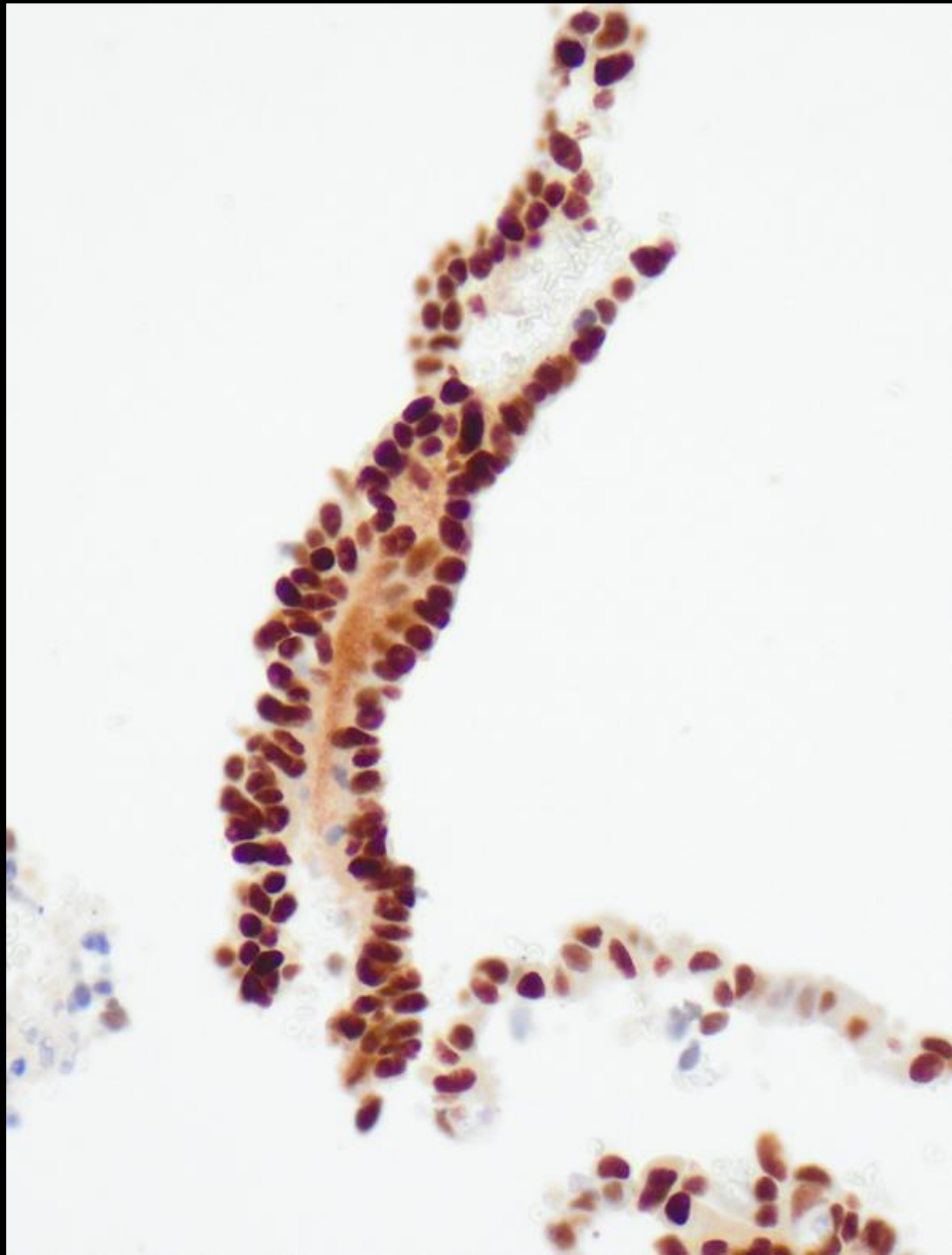
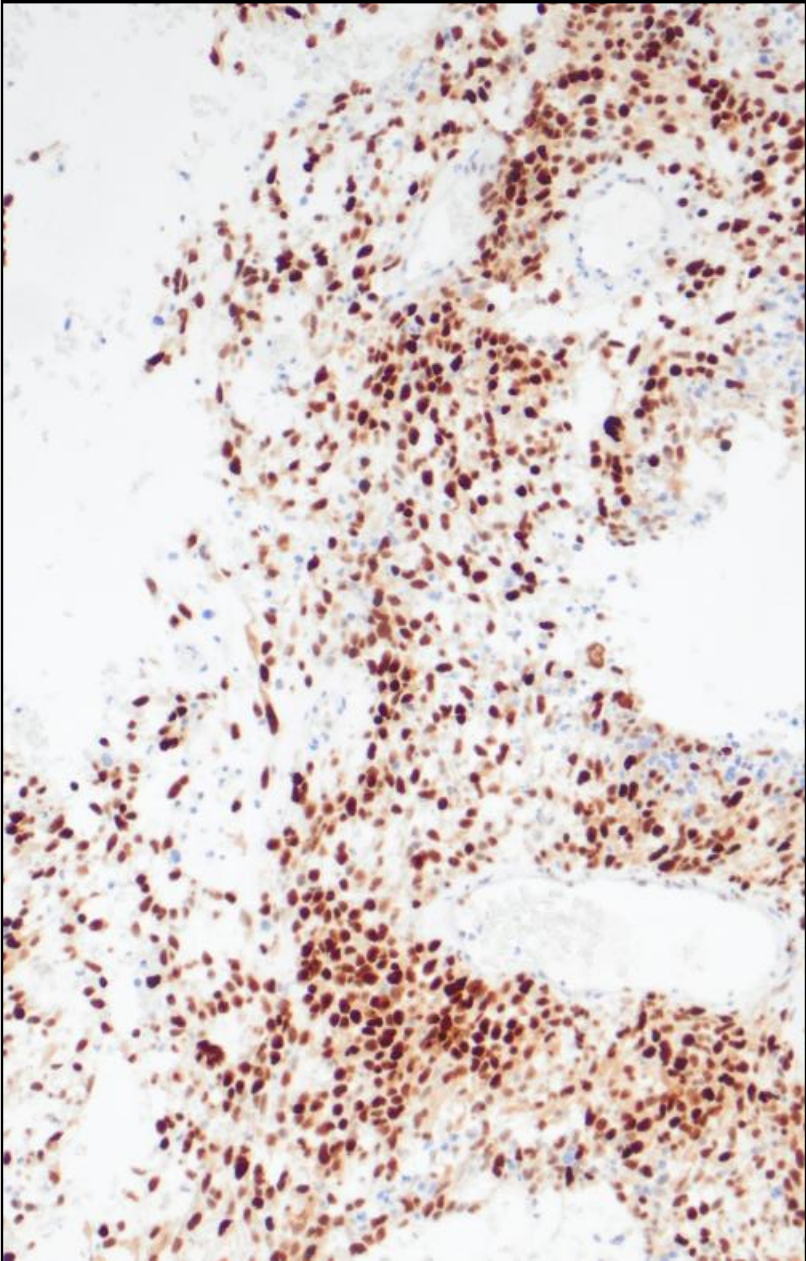




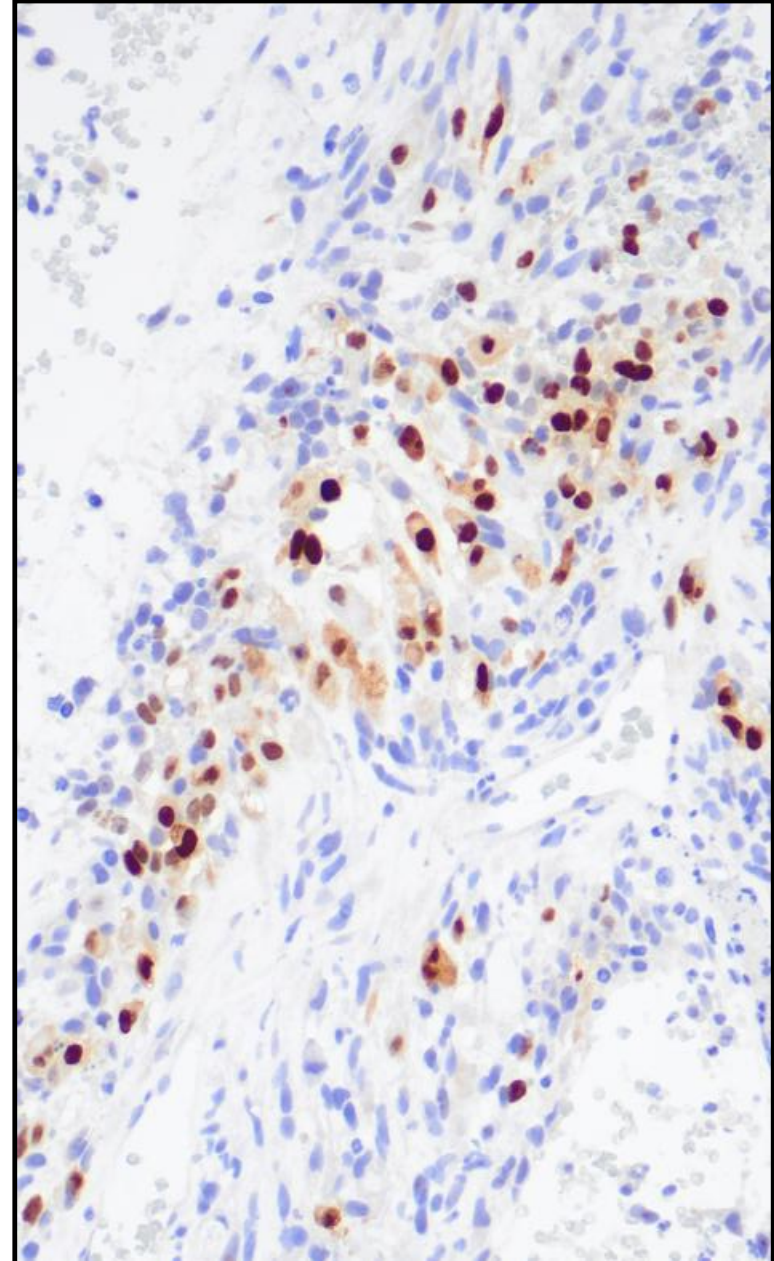




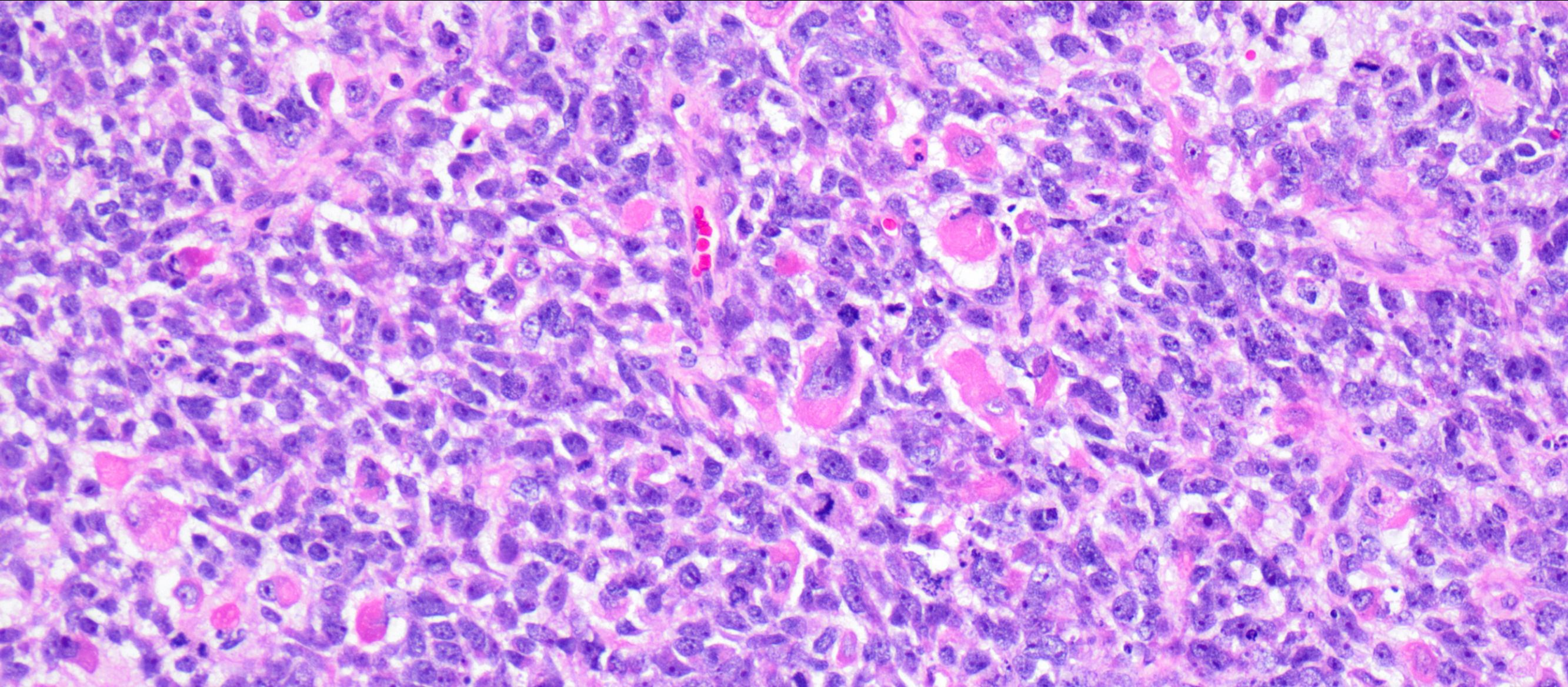
p53



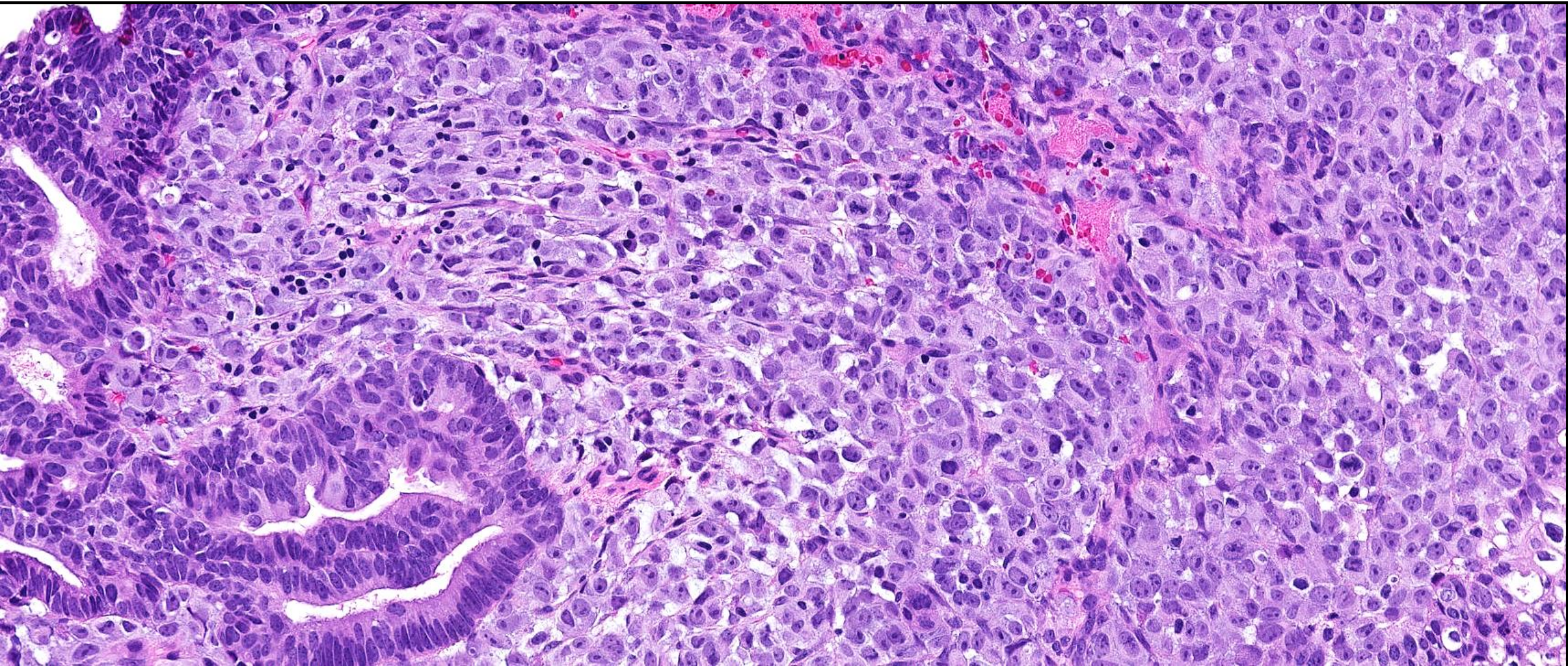
Myogenin



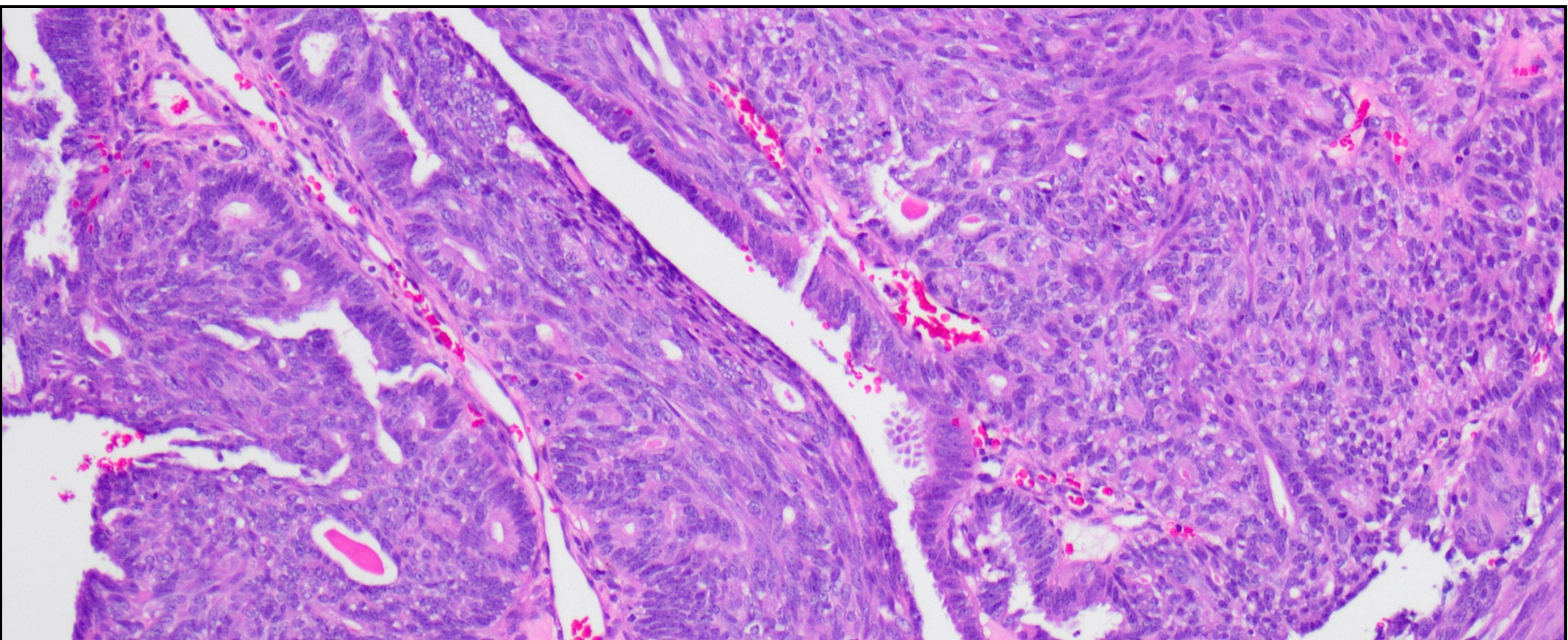
The Most Common Heterologous Element Is Rhabdomyosarcoma



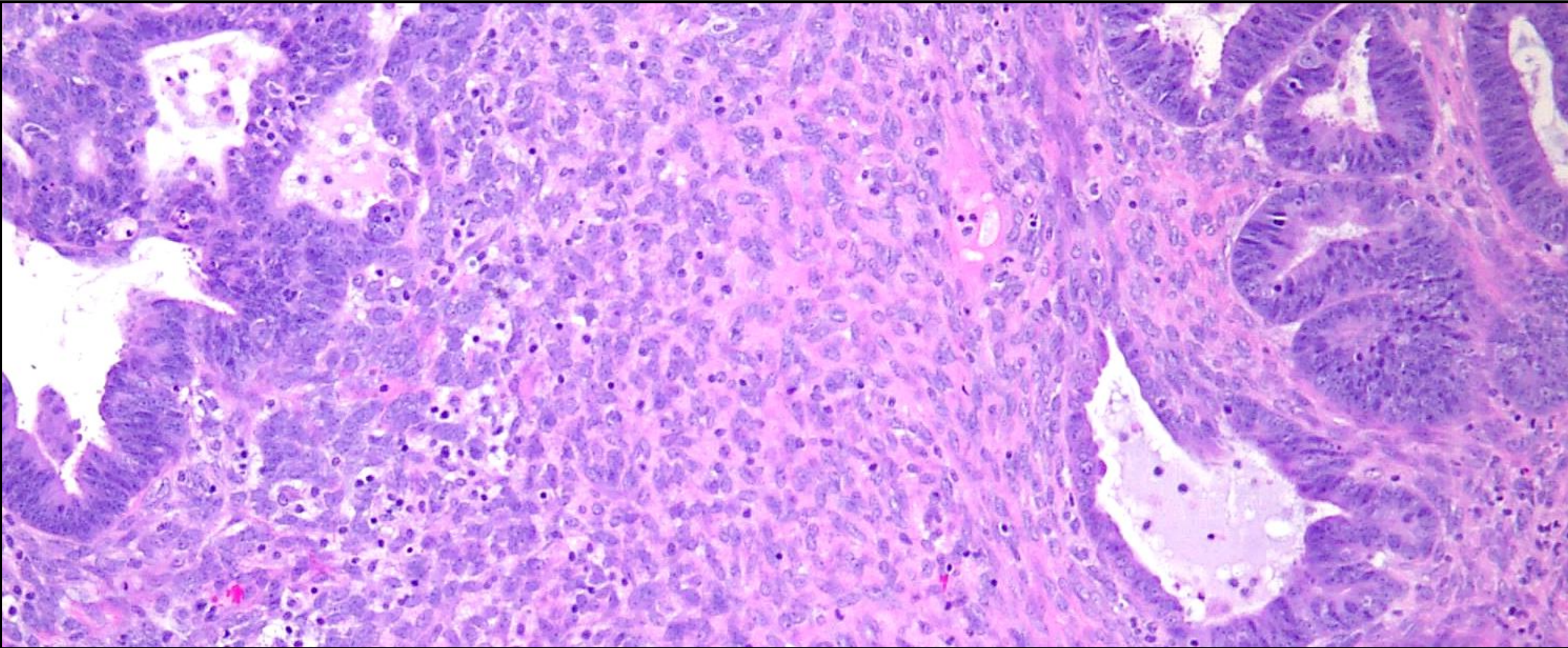
Carcinosarcoma Should Be Differentiated from Dedifferentiated Carcinoma (Undifferentiated Carcinoma & Low-Grade Endometrioid Carcinoma, Spatially Distinct)



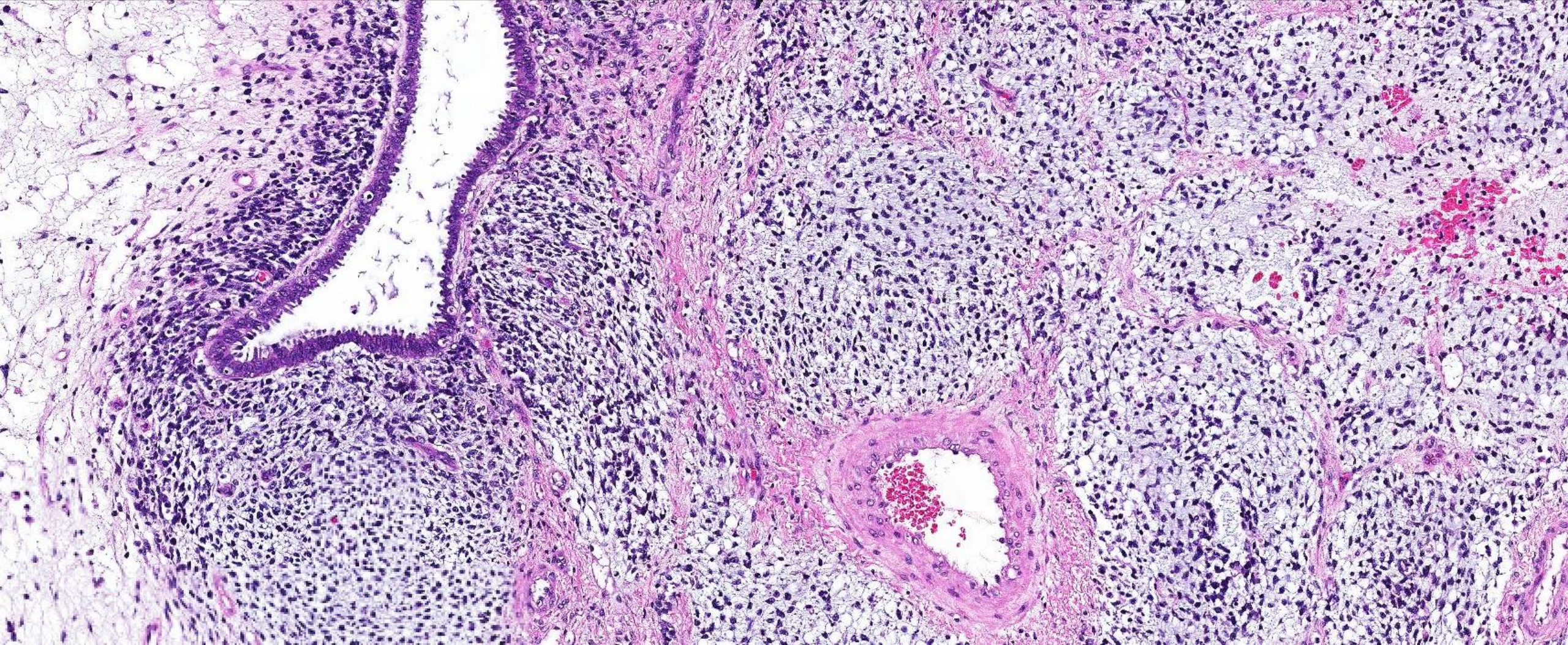
Carcinosarcoma Should Be Differentiated from Endometrioid Carcinoma with Spindling (Non Spatially Distinct, Bland Cytology)



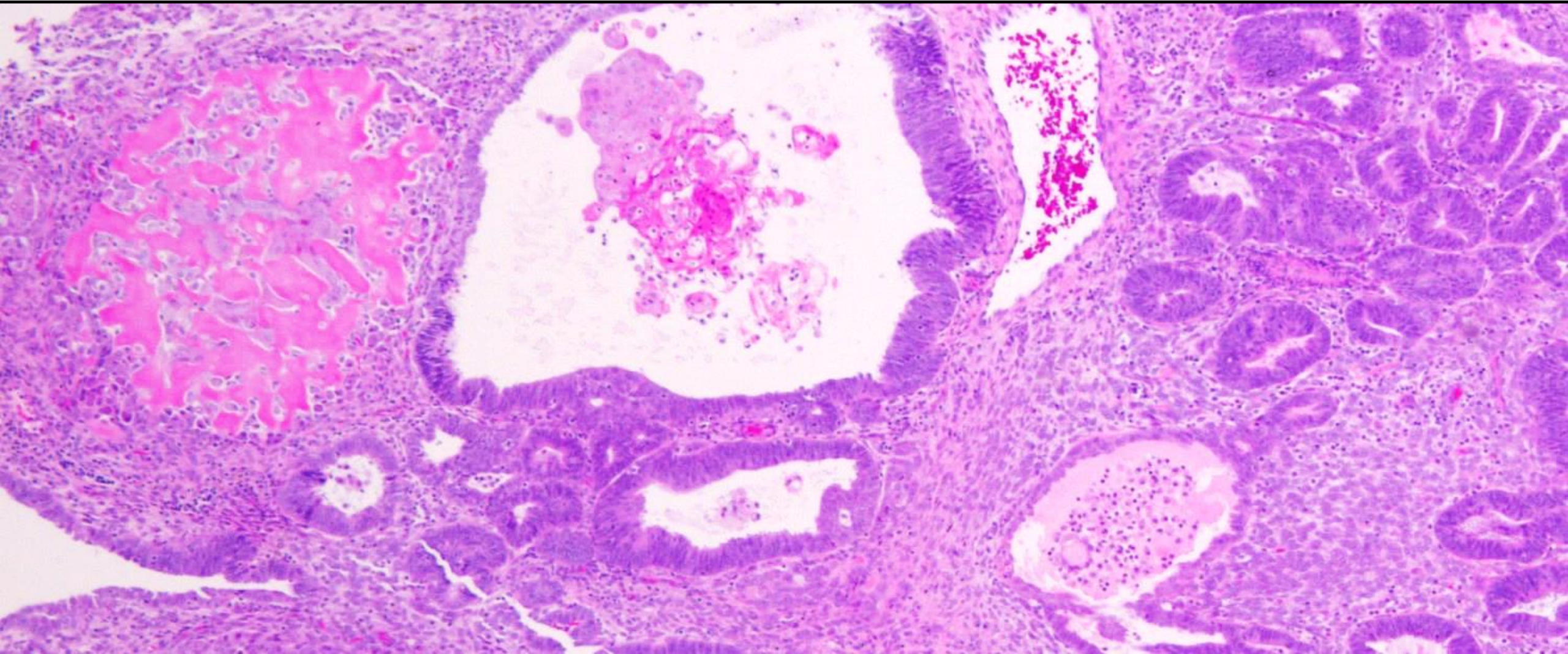
**Carcinosarcoma Should Be Differentiated from
Corded & Hyalinized Endometrioid Carcinoma
(Usually Non Spatially Distinct, Bland Cytology)**



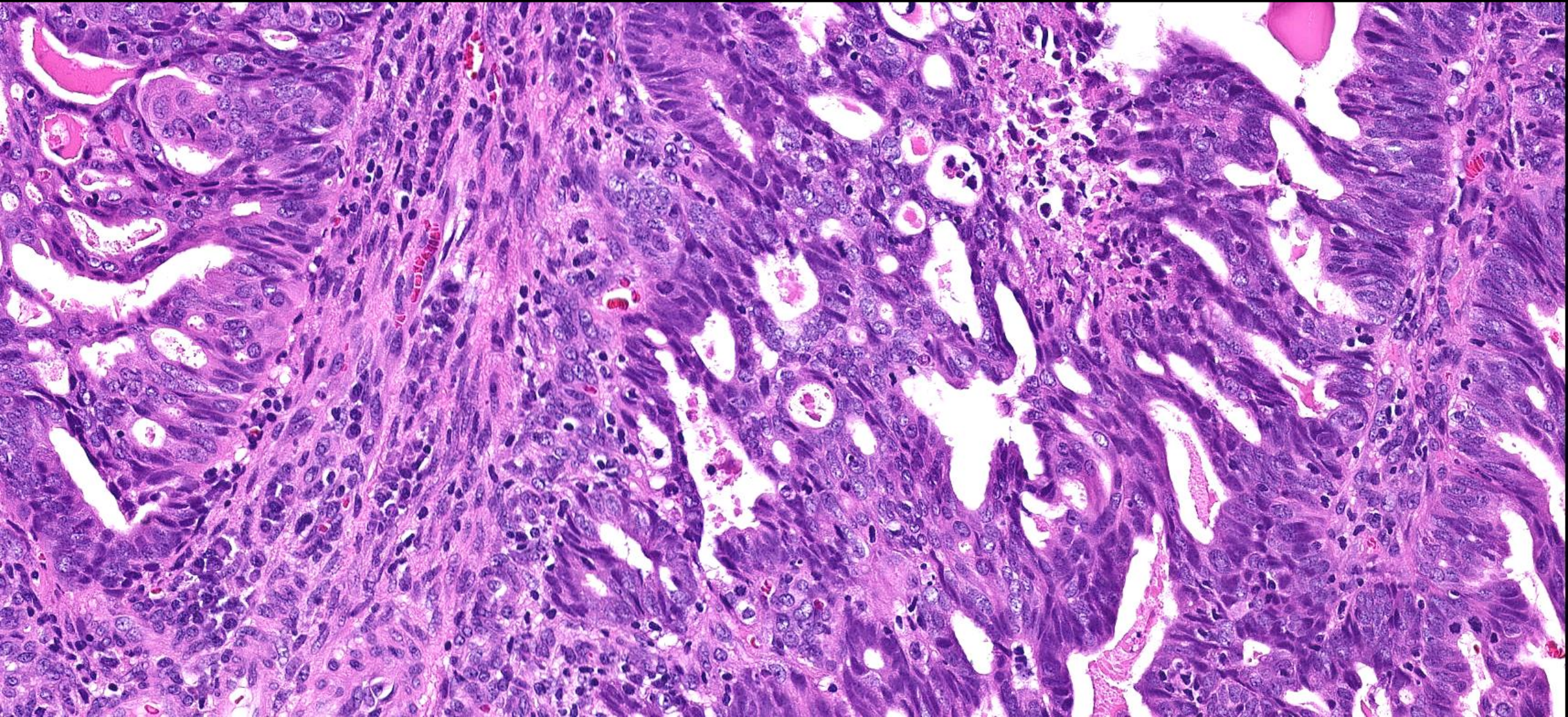
Carcinosarcoma Should Be Differentiated from Adenosarcoma with High-Grade Sarcomatous Overgrowth (Benign Epithelial & High-Grade Sarcomatous Components)



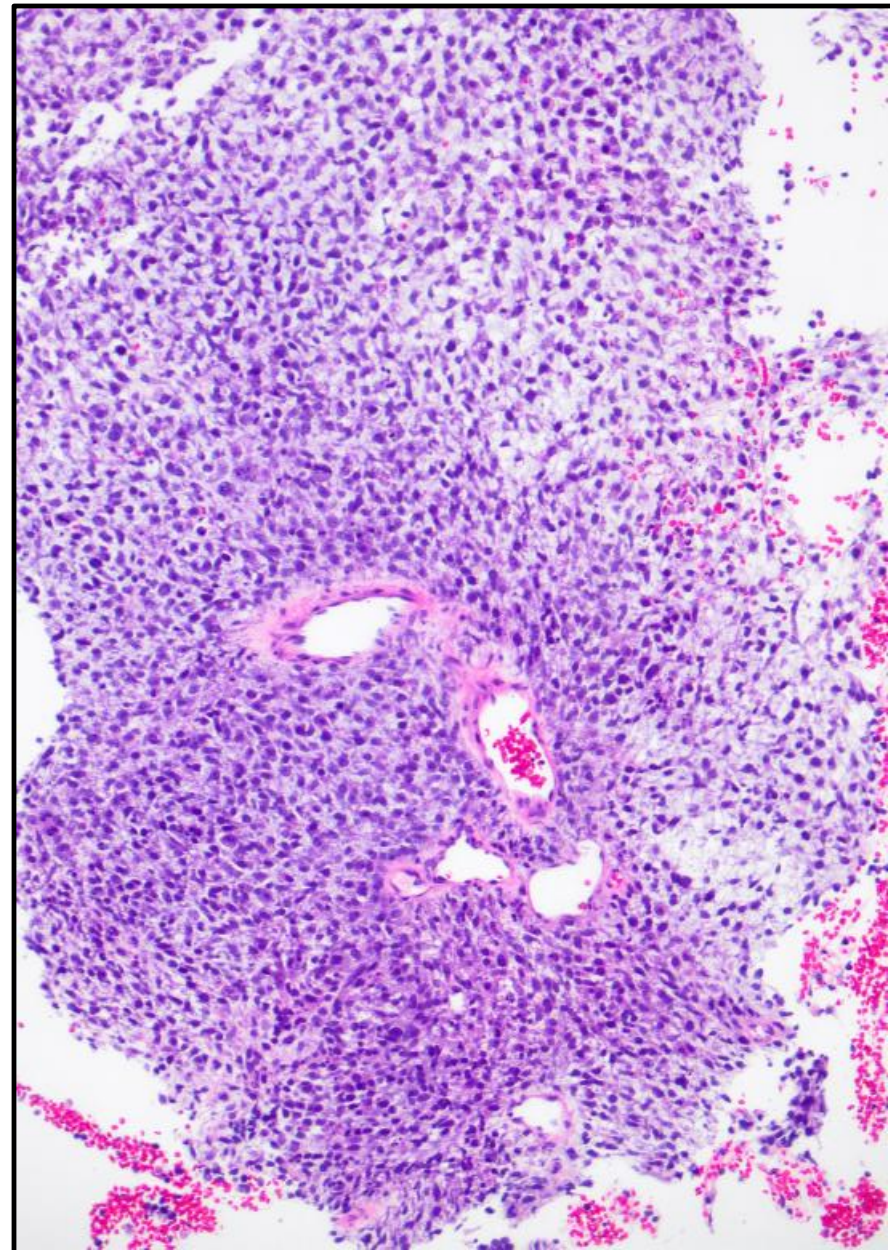
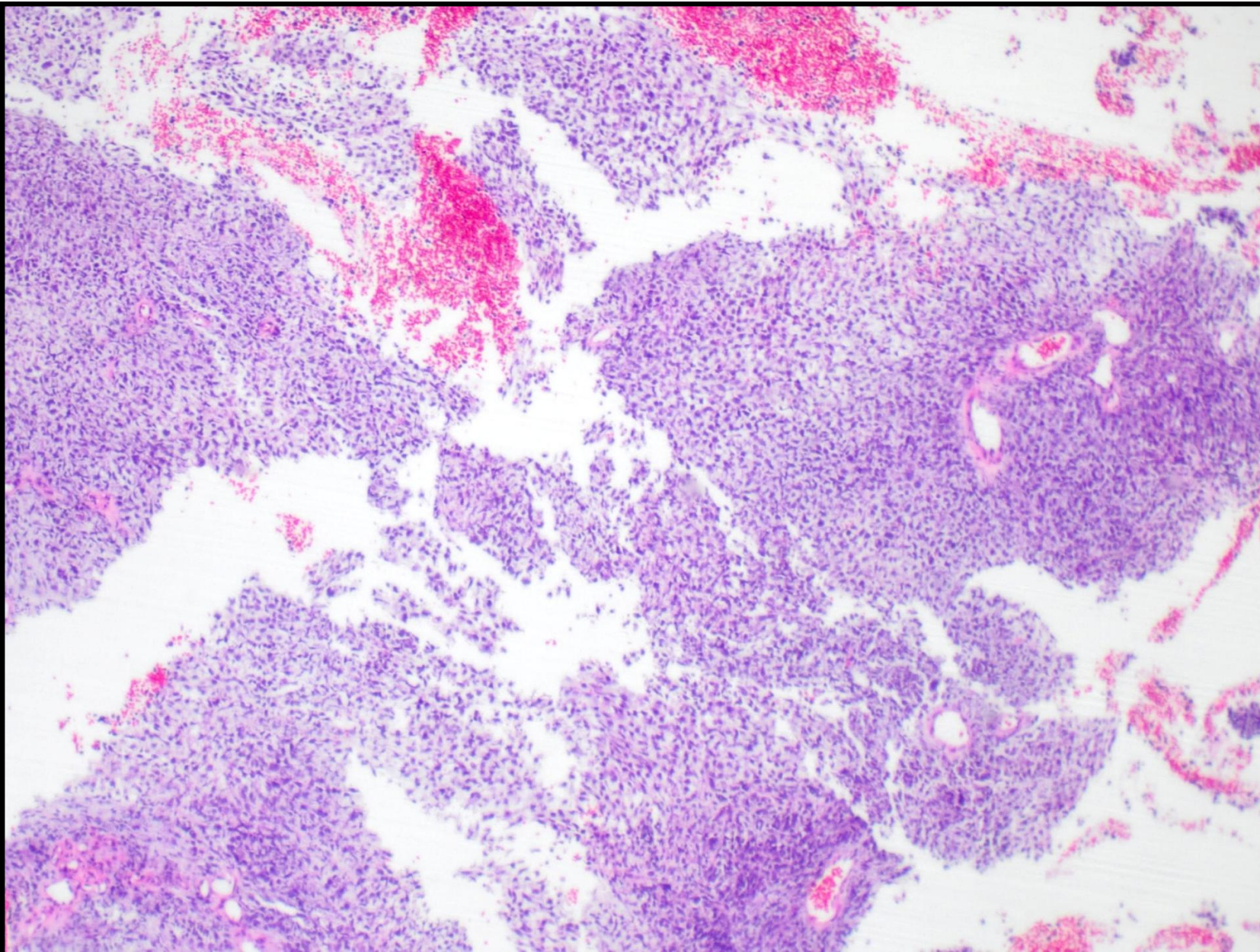
Carcinosarcoma with Heterologous Differentiation Should Be Differentiated from Benign Mesenchymal Elements, e.g. Metaplastic Bone (Bland Cytology)



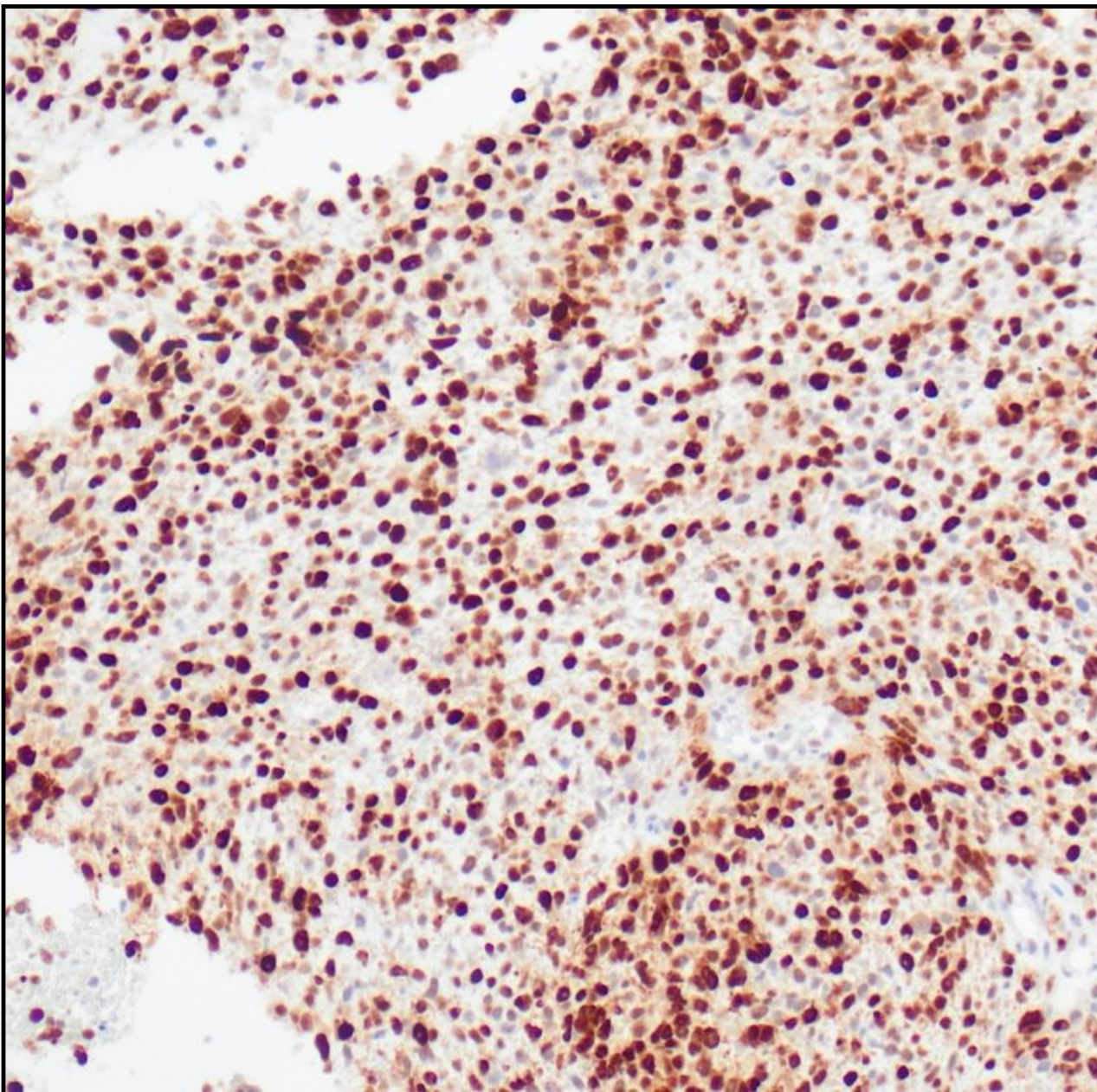
Carcinosarcoma Should Be Differentiated from Desmoplastic Stromal Reaction (Bland Cytology)



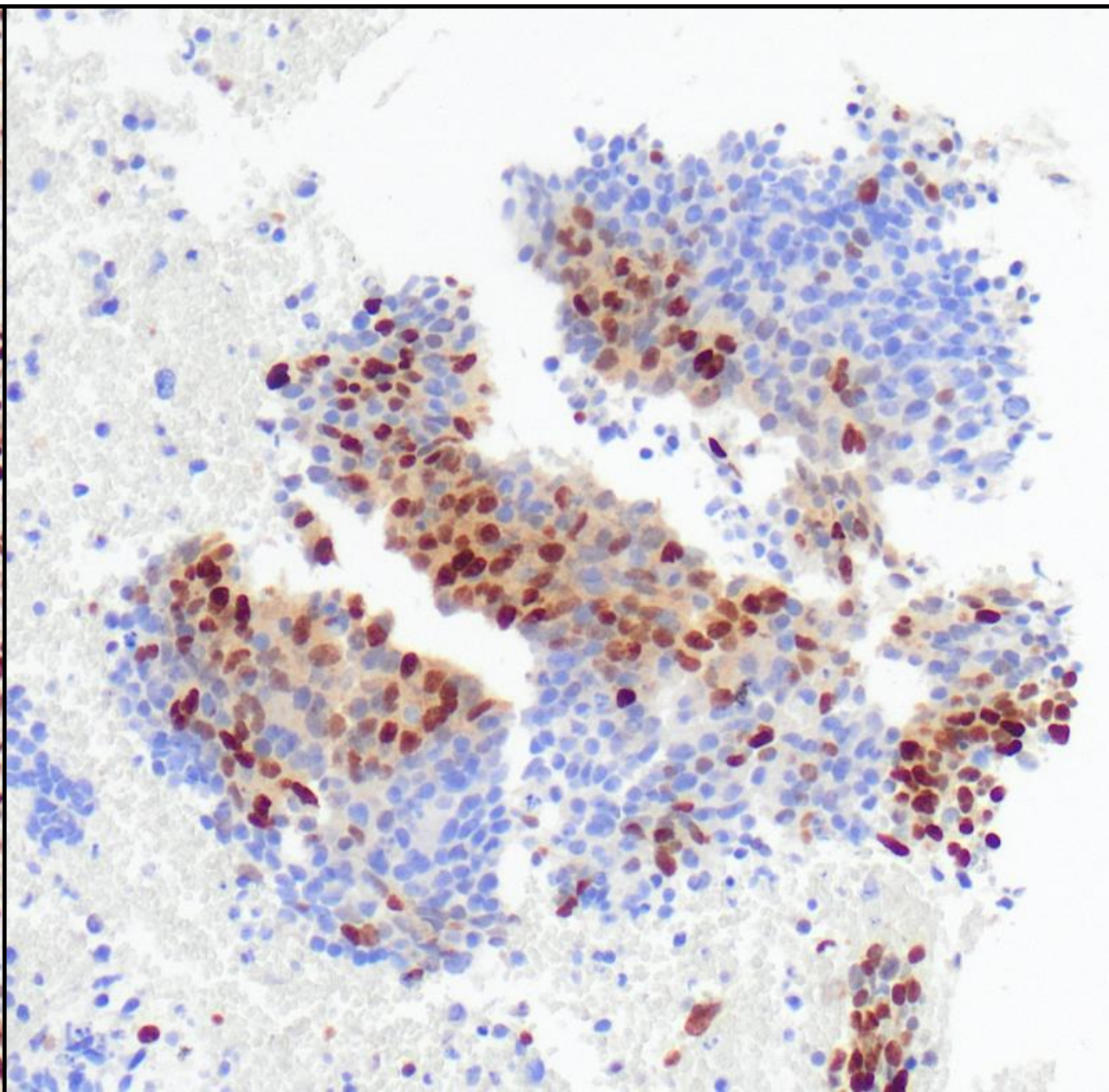
Fragments of High-Grade Sarcoma Only



p53

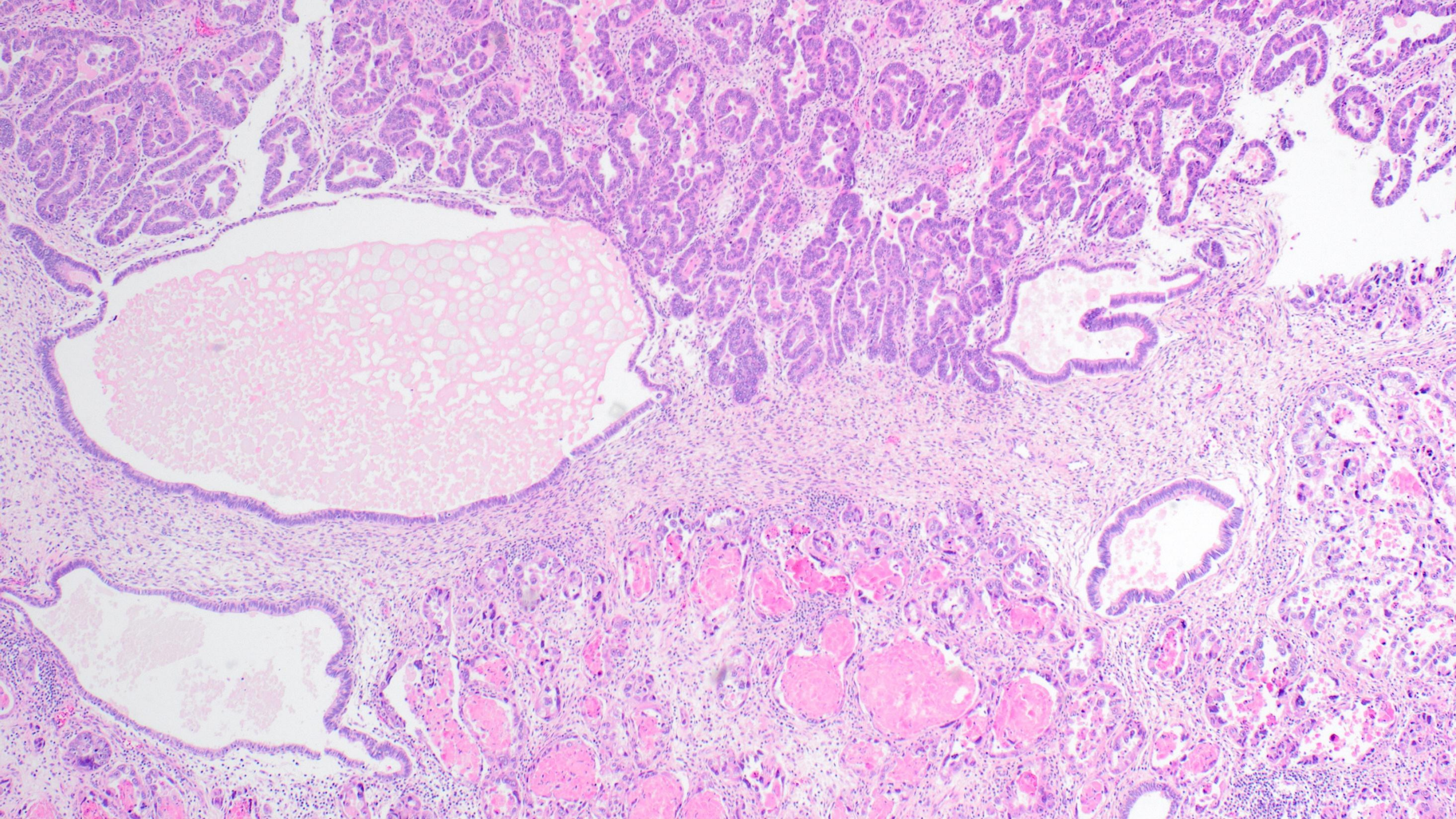


Myogenin

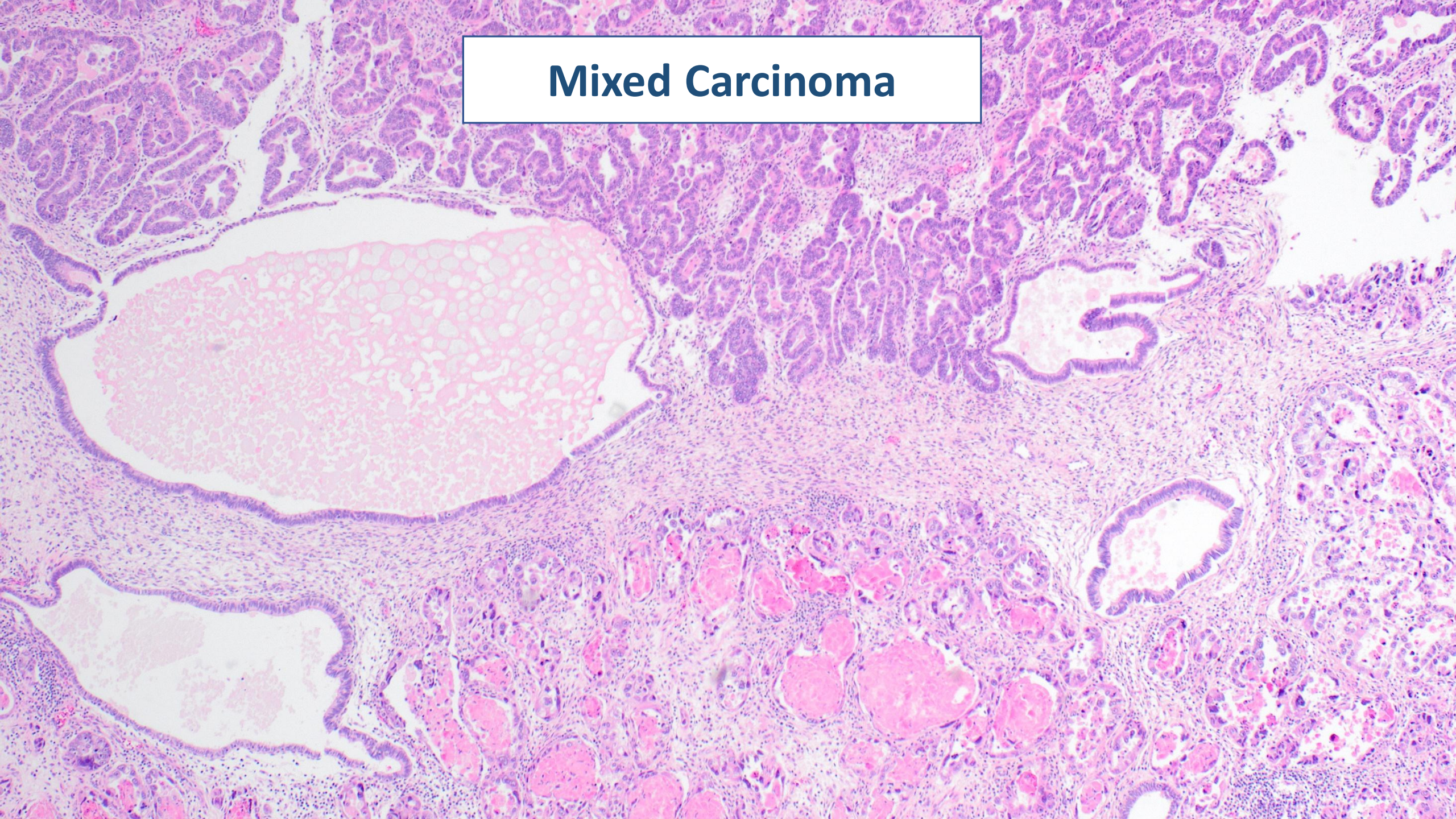


**If no epithelial component is present,
suggest a differential diagnosis:**

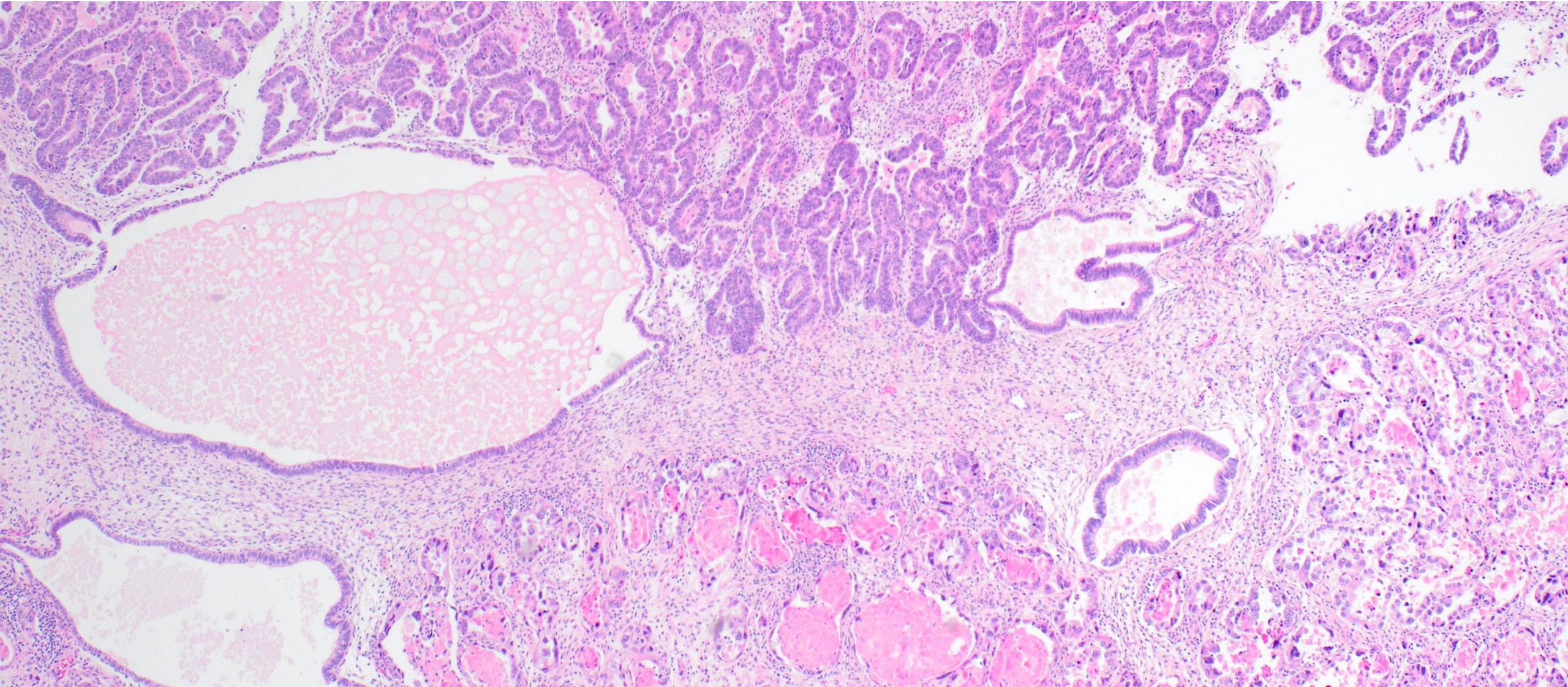
- **Carcinosarcoma (older patients)**
- **Adenosarcoma with high-grade sarcomatous overgrowth**
- **Pure rhabdomyosarcoma (younger patients)**

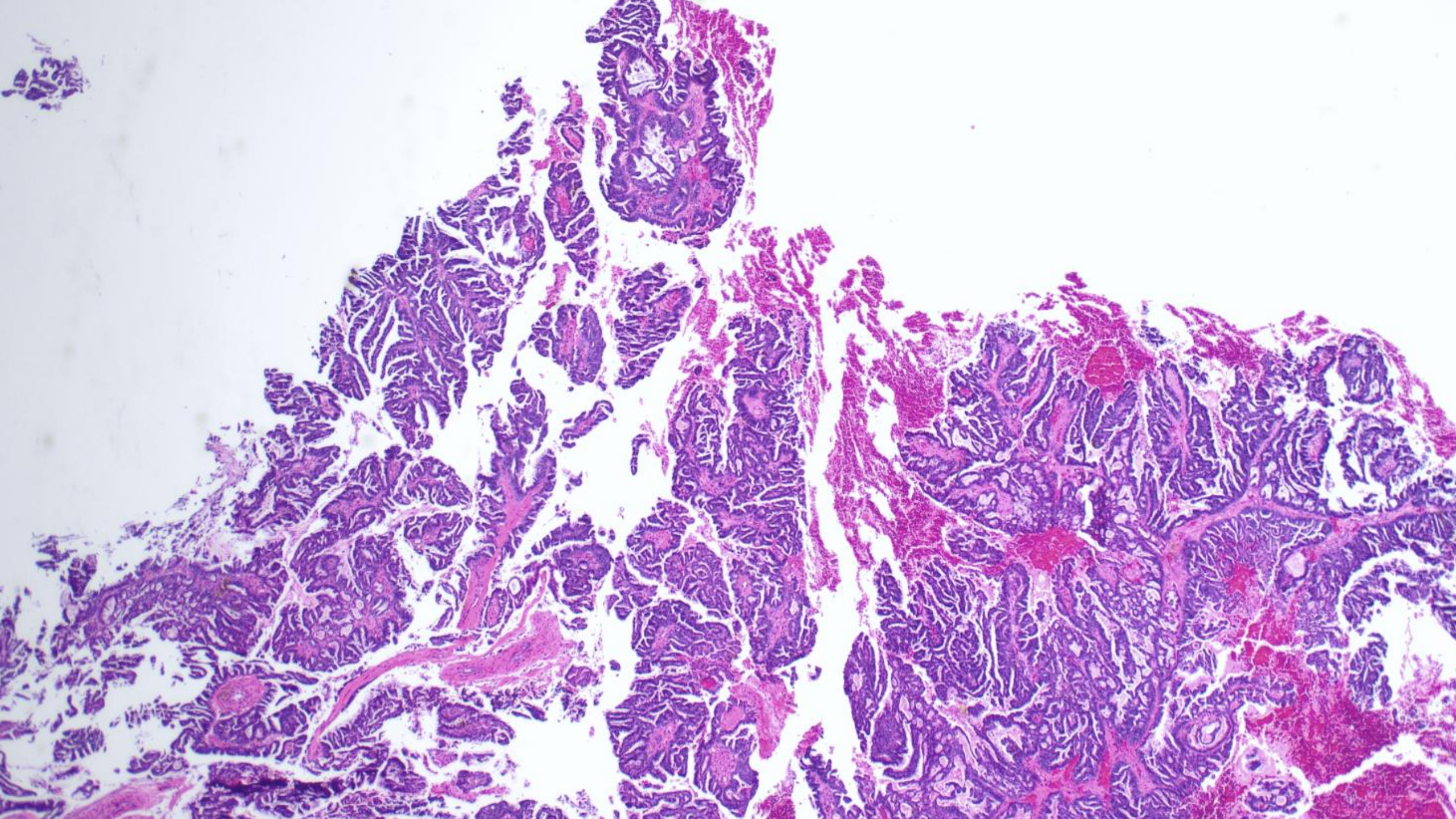


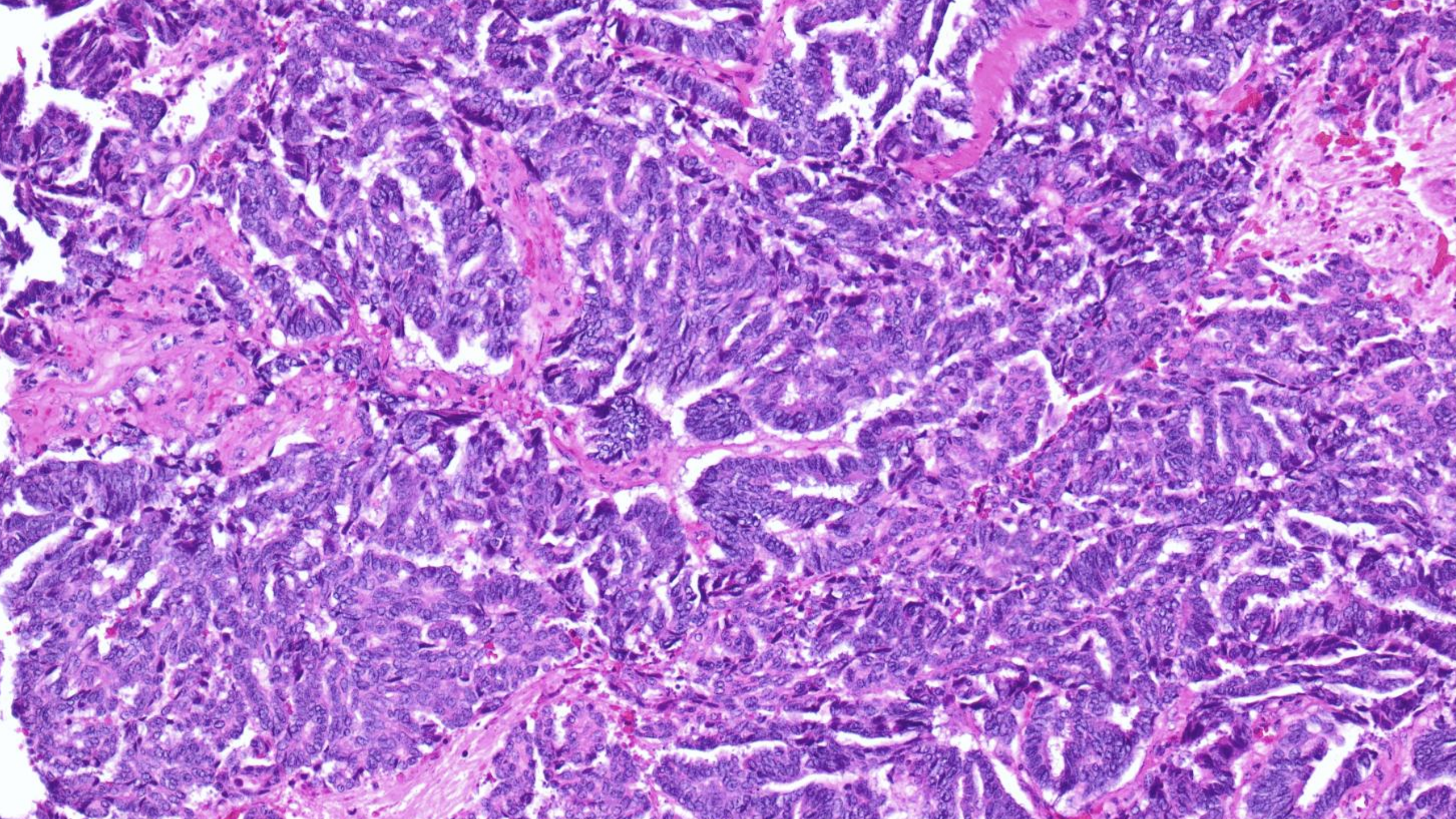
Mixed Carcinoma

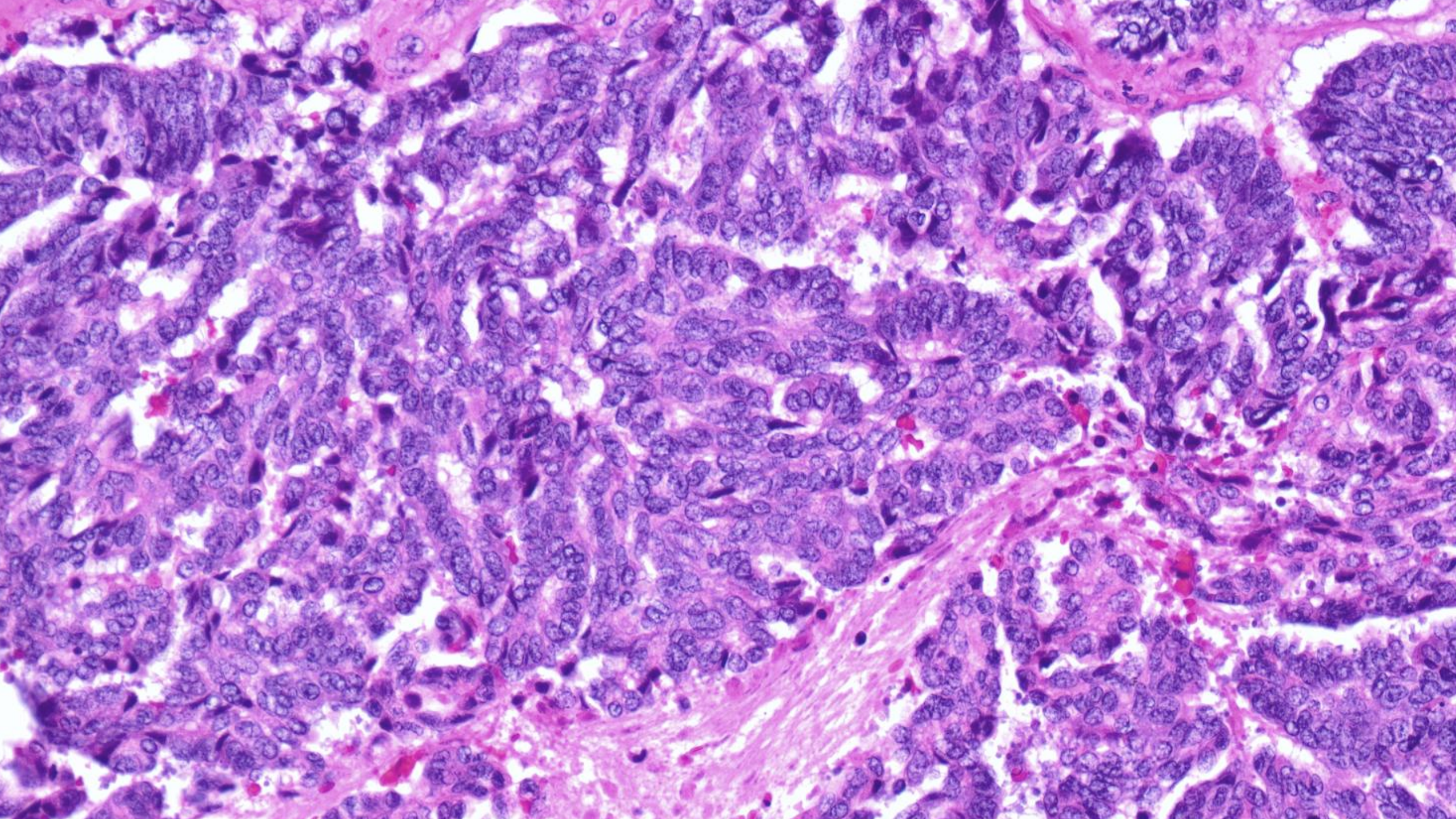


Mixed Carcinoma Is Composed of ≥ 2 Spatially Distinct Tumor Subtypes, with ≥ 1 Being Serous or Clear Cell Carcinoma (+Mesonephric-Like)

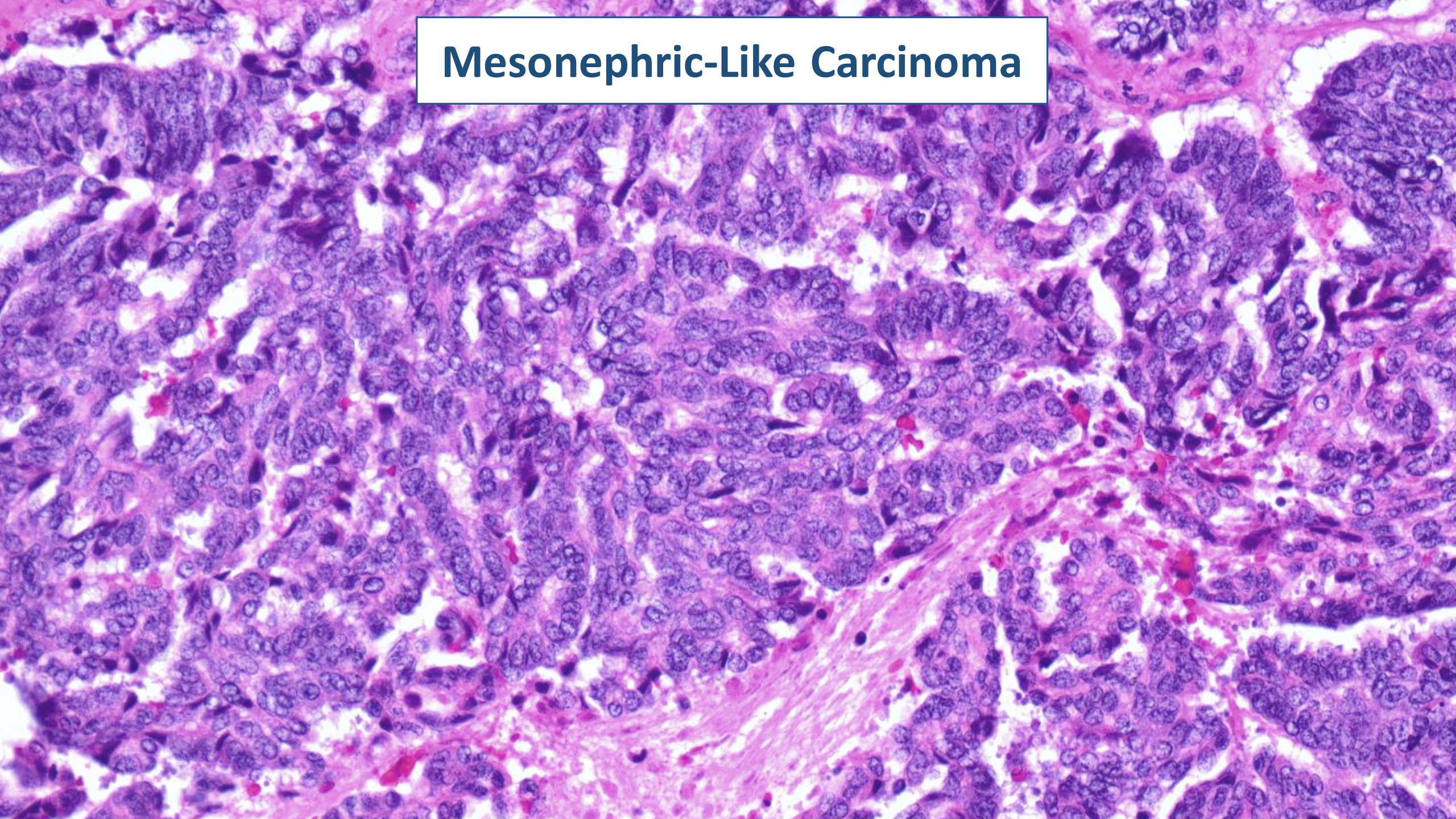




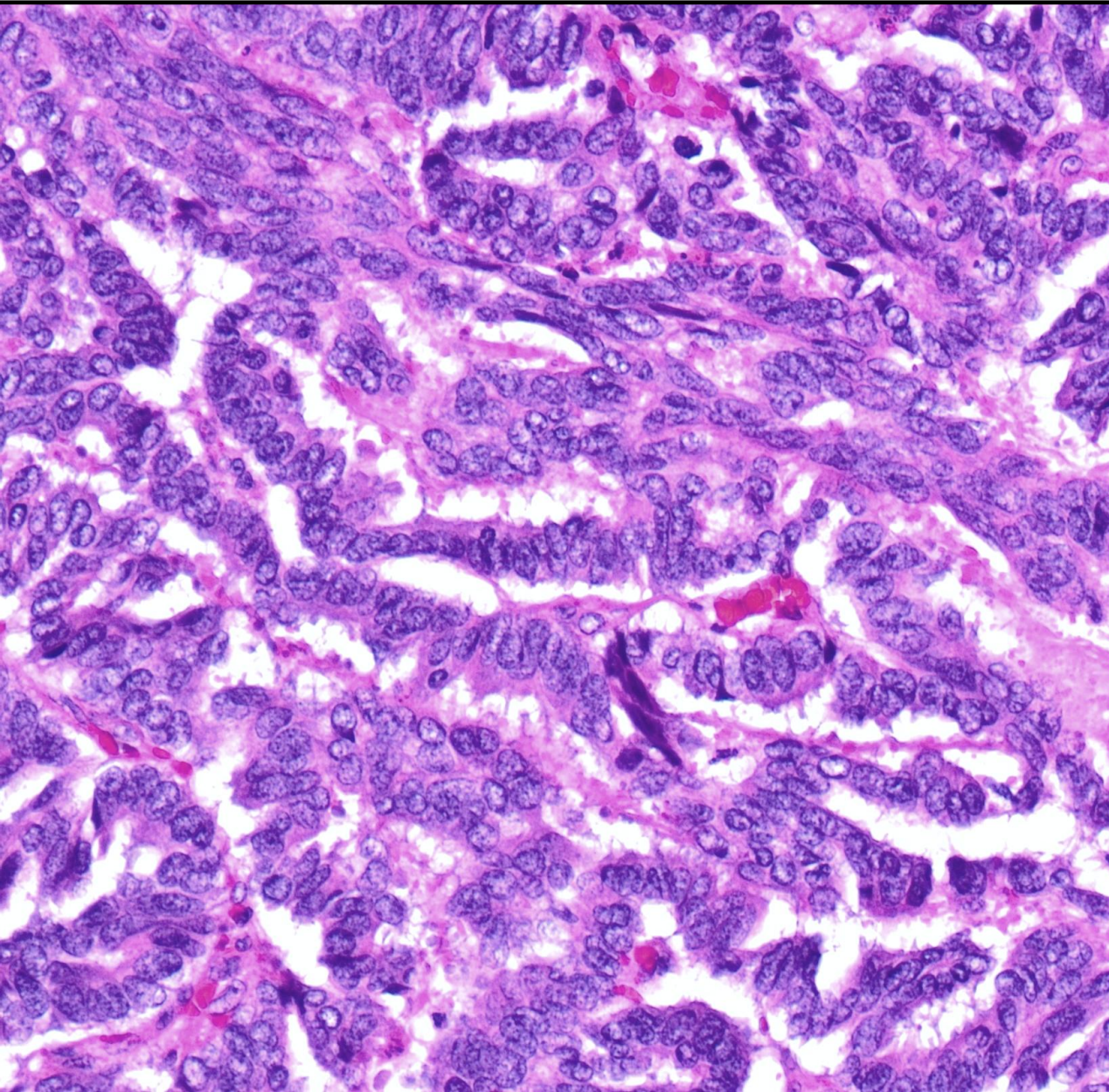




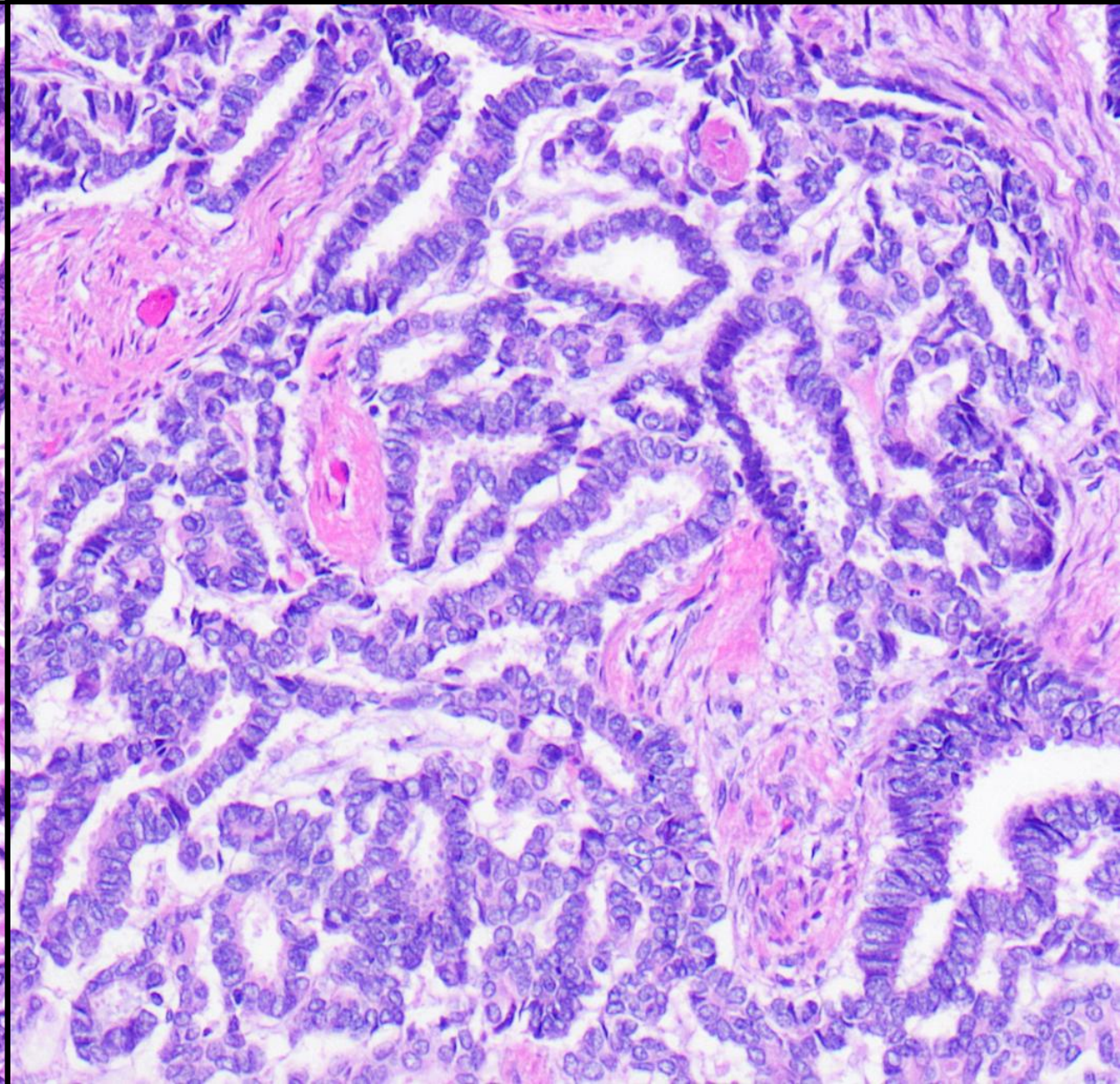
Mesonephric-Like Carcinoma

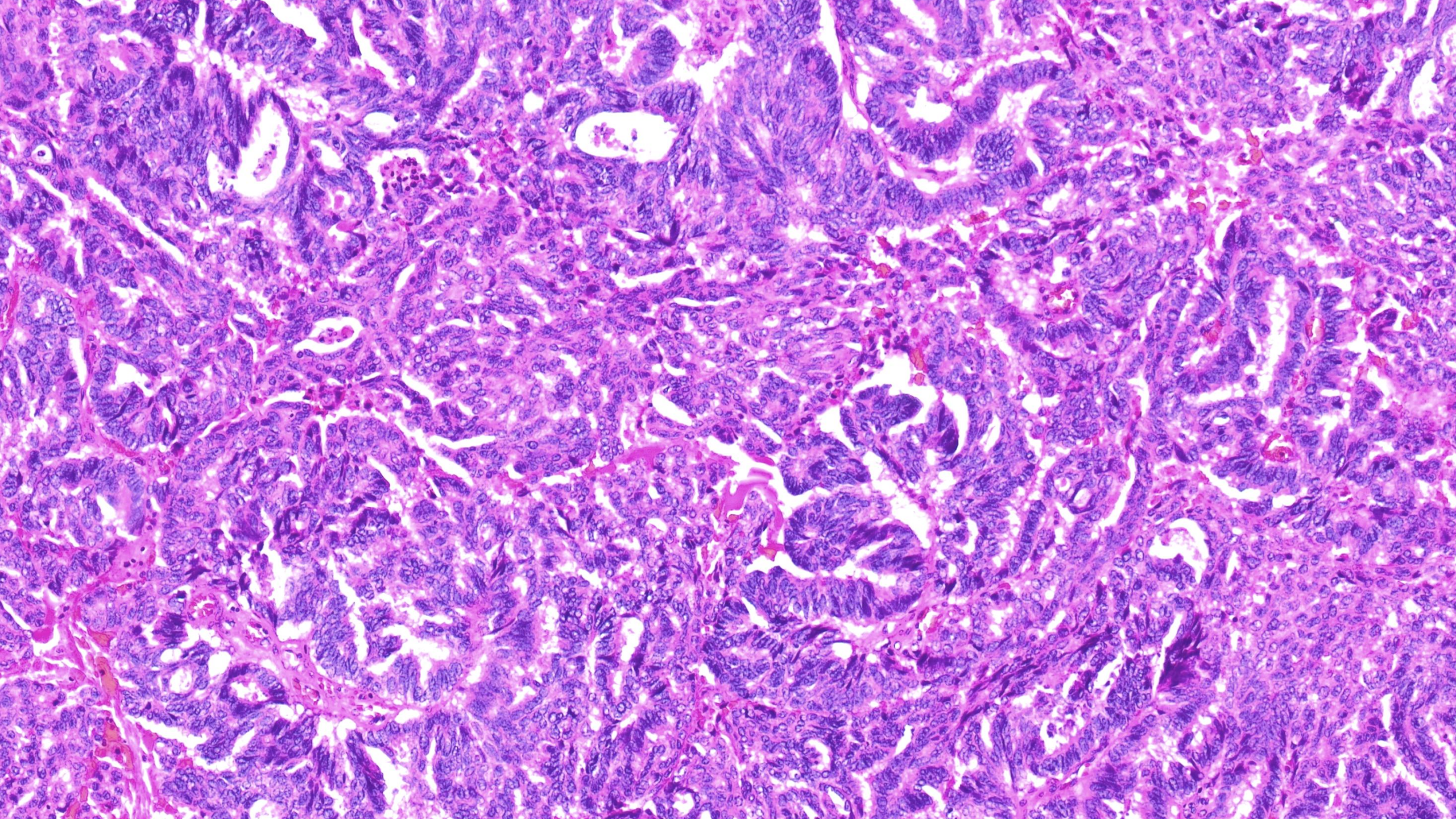


Glandular & Tubular Structures

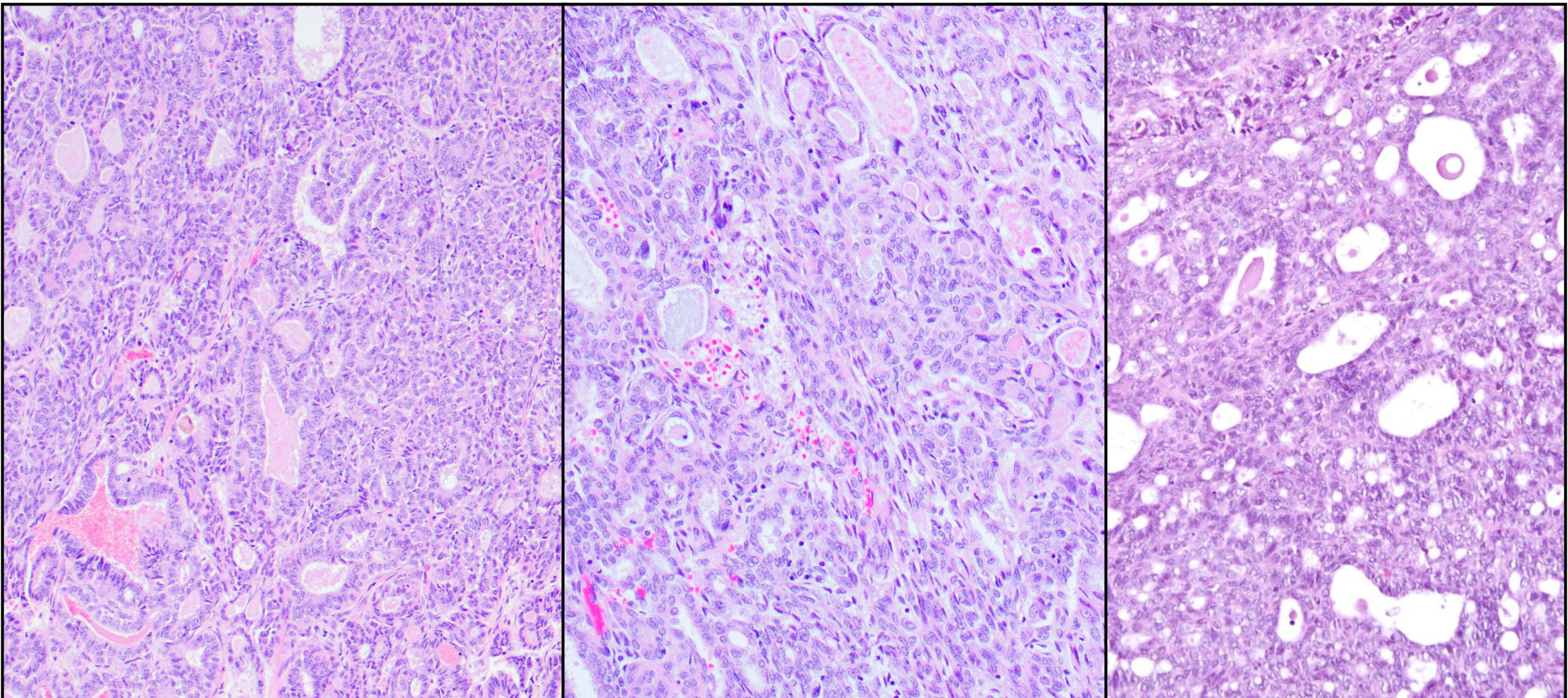


Ductal Structures



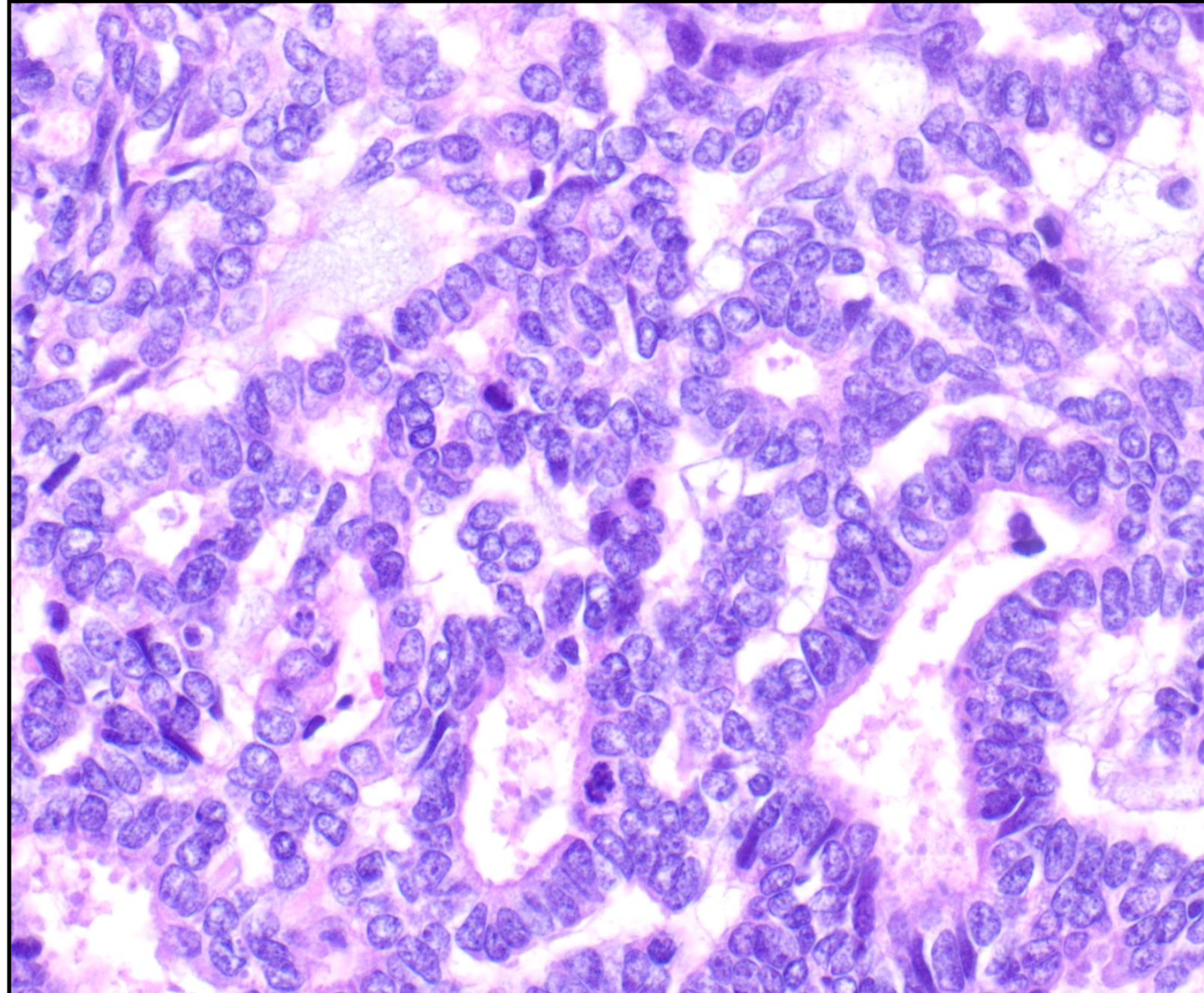


Solid, Spindled, Sieve-Like, Papillary or Corded Growth May Be Seen $\pm$ Colloid-Like Intraluminal Material



Characteristic Cytologic Features

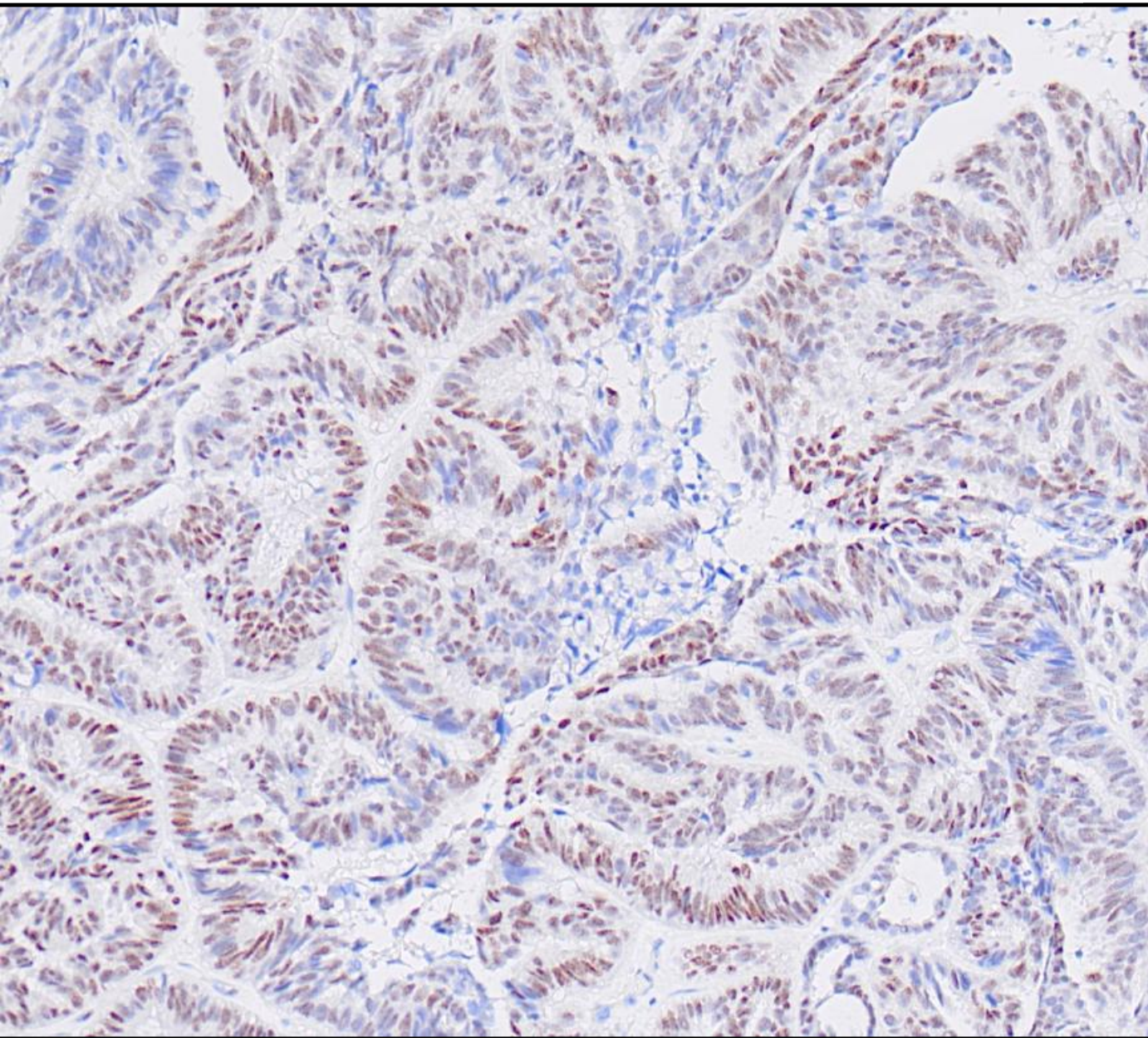
- ✓ Mild to moderate cytologic atypia
- ✓ Monotonous, vesicular, angulated to oval & overlapping nuclei
- ✓ $\pm$ Nuclear grooves
- ✓ Low mitotic activity
- ✓ No mucinous/squamous/secretory or tubal differentiation



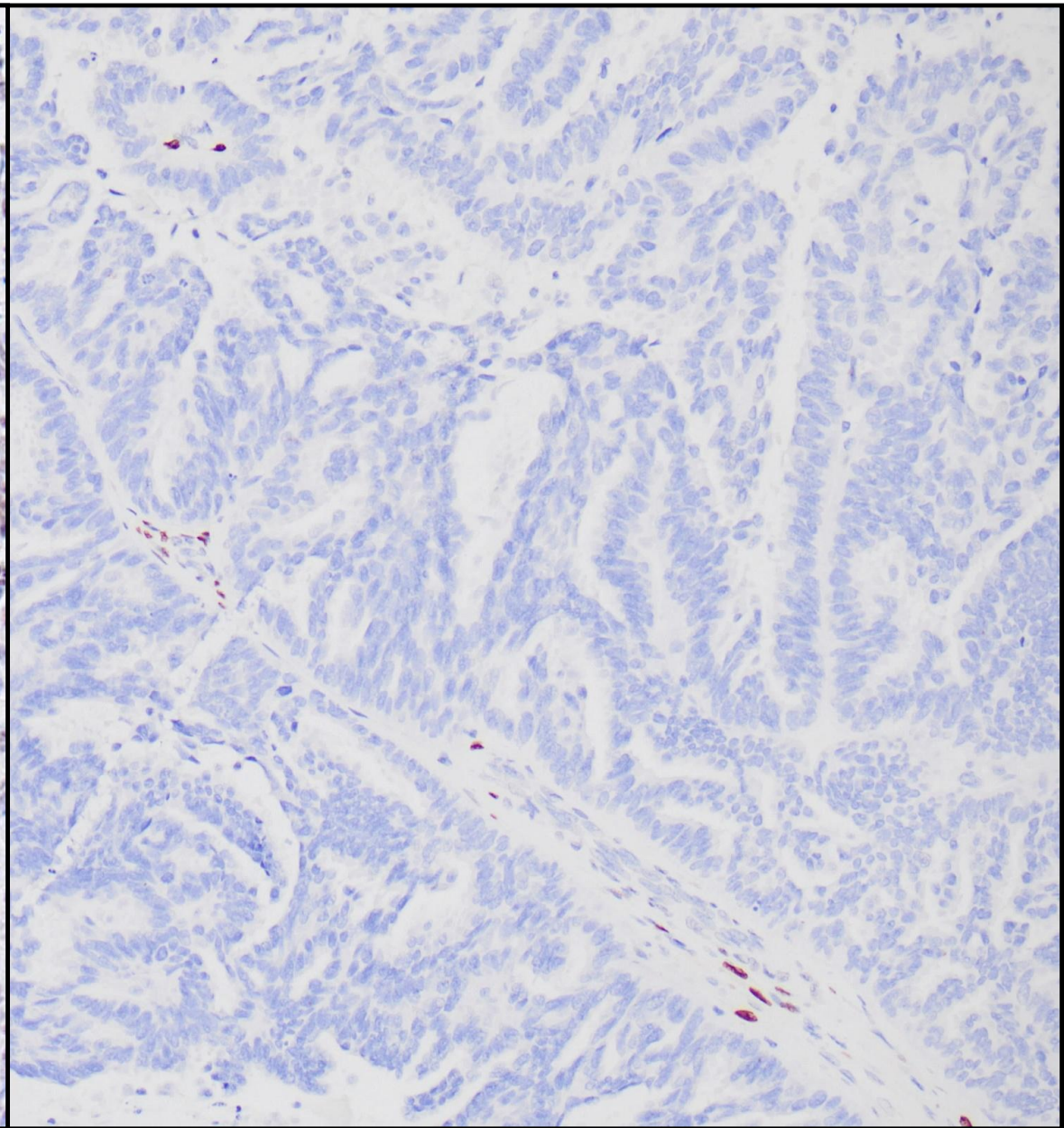
The Main Differential Diagnosis for Mesonephric-Like Carcinoma Is Low-Grade Endometrioid Carcinoma

	Endometrioid	Mesonephric-Like
p53	Wild-type	Wild-type
ER/PR	+	– (or minimal)
TTF-1	–	±
GATA3	–	±
CD10 & Calretinin	–	±
MMR	±	+
PTEN, ARID1A	±	+

GATA3



ER/PR



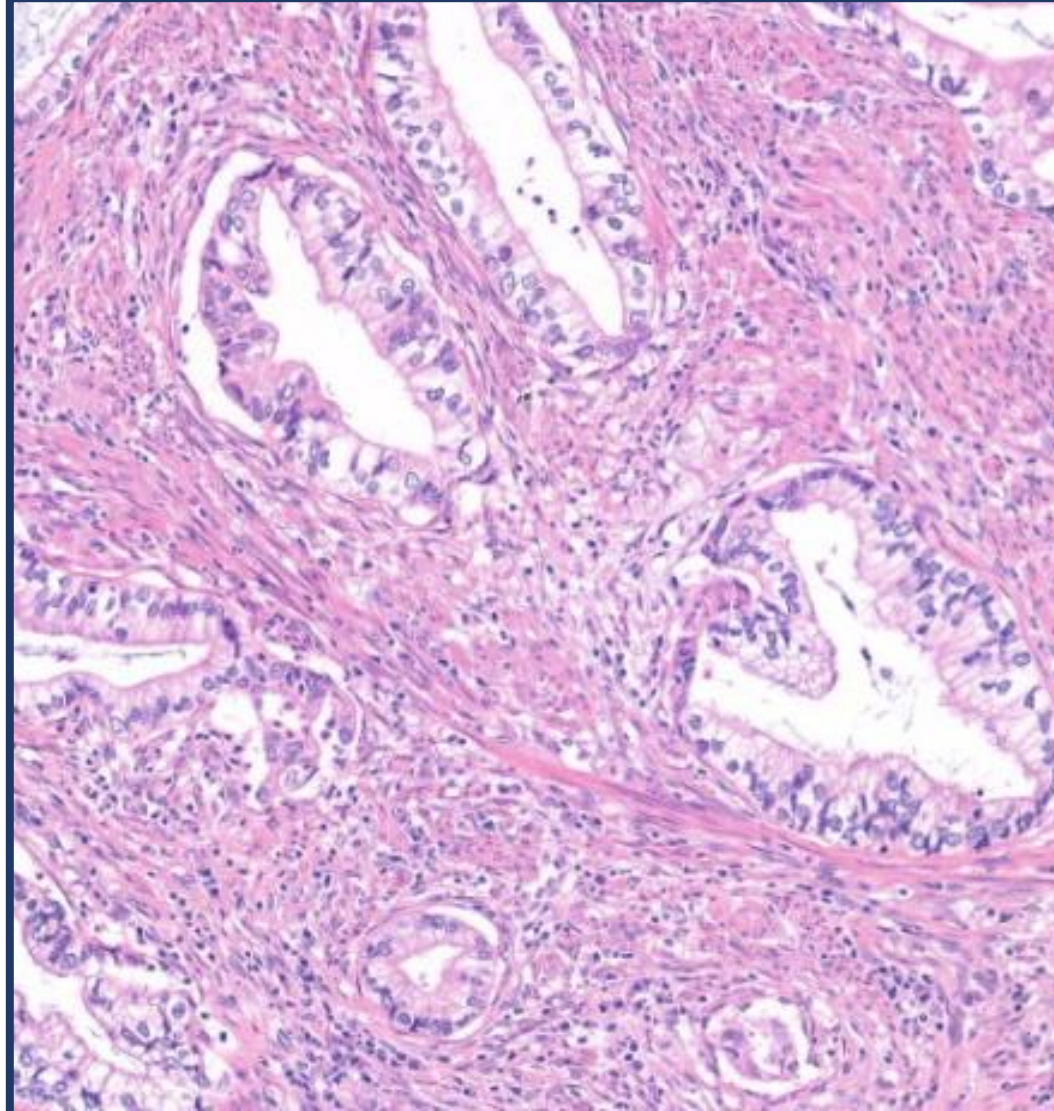
Mesonephric-Like Carcinoma

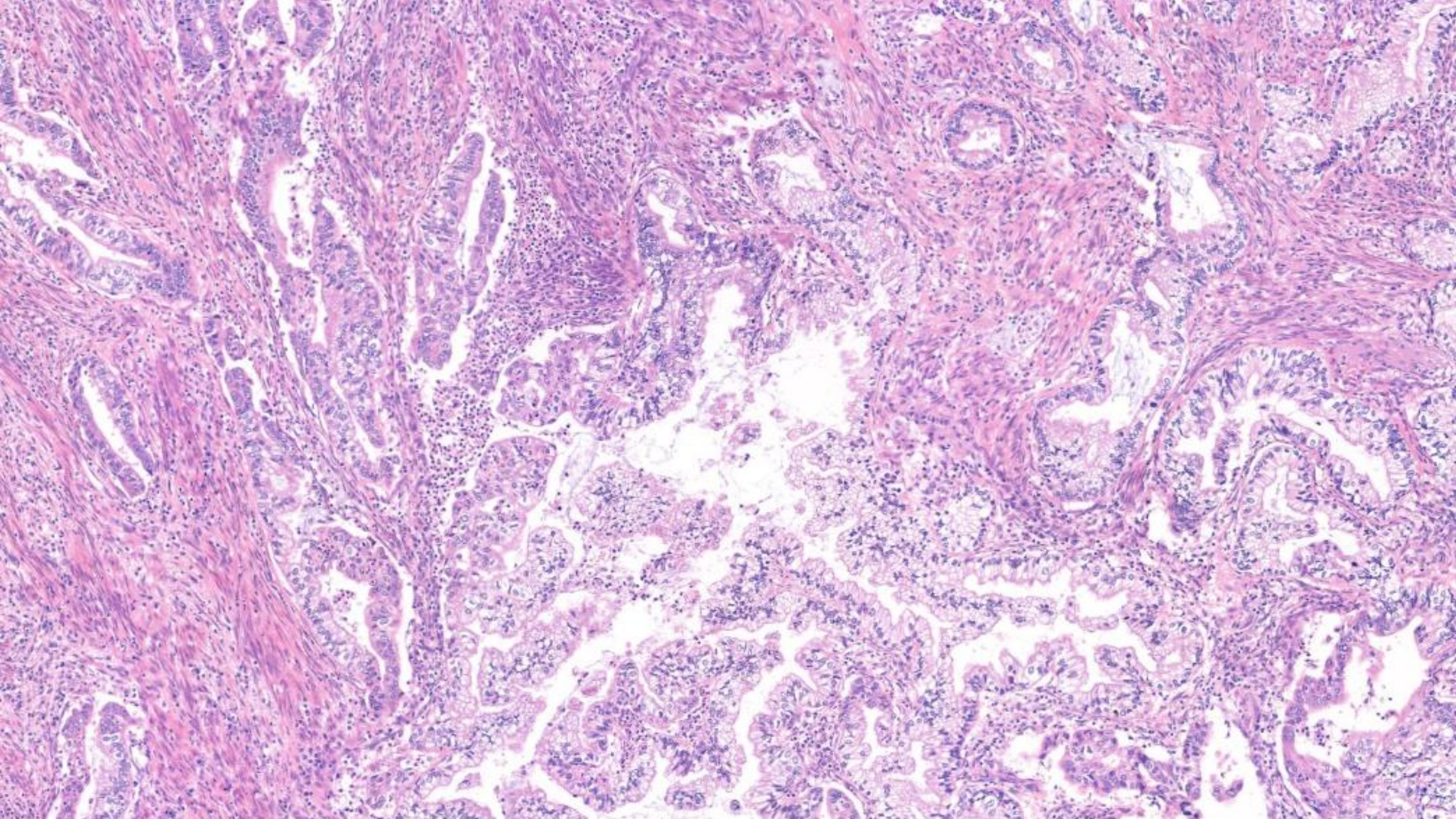
- ✓ <1% of endometrial carcinomas
- ✓ Exclusion of cervical mesonephric carcinoma
- ✓ Aggressive behavior:
 - Median progression free survival 18 months (vs 138 months for endometrioid carcinoma)
 - Distant metastases (pulmonary)
- ✓ *KRAS* mutations (G12V) in most cases
- ✓ *PTEN* or *ARID1A* mutations in a small subset
- ✓ 1q gain

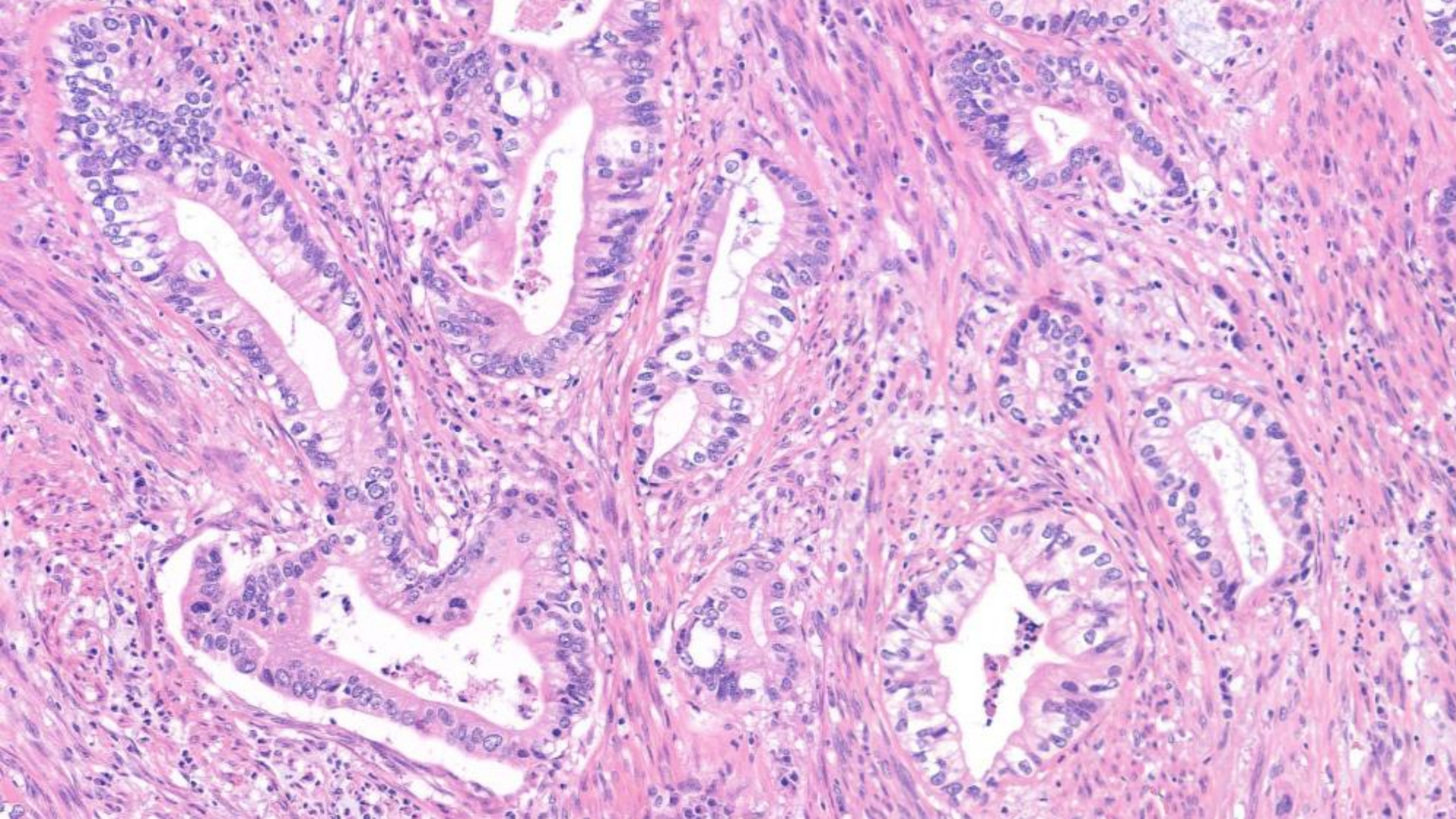
Gastric (Gastrointestinal)-Type Carcinoma Exhibits Gastric/Gastrointestinal Differentiation Without an Endometrioid Component

- ✓ Glandular proliferation with gastric-type (voluminous, pale eosinophilic or clear cytoplasm, distinct cell borders) and/or intestinal-type (goblet cells, Paneth cells) features and marked cytologic and/or architectural atypia
- ✓ Morphologic heterogeneity
- ✓ No typical endometrioid morphology
- ✓ Aggressive clinical behavior

PMID: 20055951, 31567280, 40066786, 26535979, 31567280, 38477341



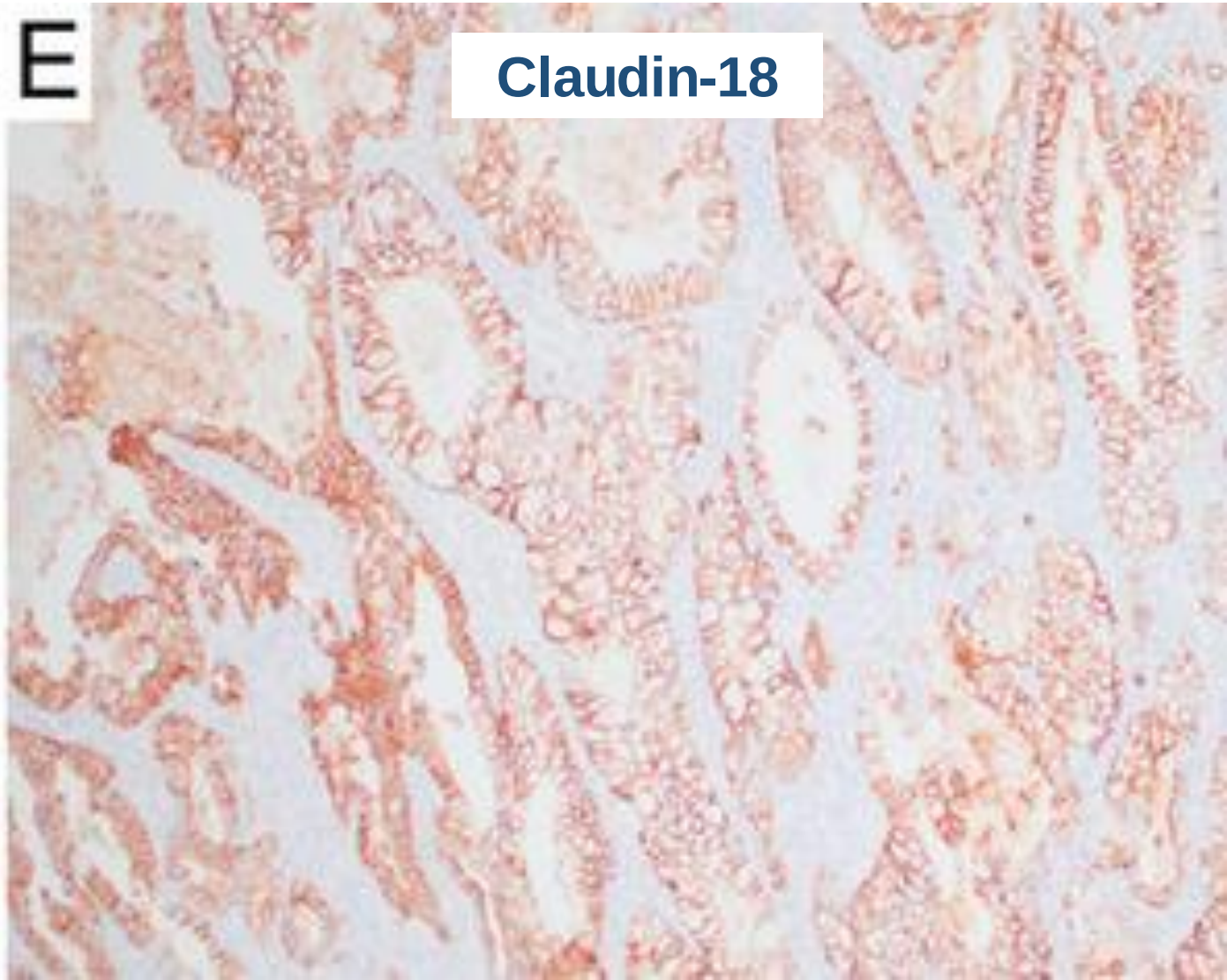




Gastric (Gastrointestinal)-Type Carcinoma Is At Least Focally Positive for Gastrointestinal Markers

- ✓ At least focal CK20, CDX2, MUC6, SATB2, claudin-18
- ✓ No to minimal (<5%) ER
- ✓ Negative PR
- ✓ Positive CK7, PAX8
- ✓ Variable p16
- ✓ Abnormal p53, MMR loss, HER2 positivity in a subset

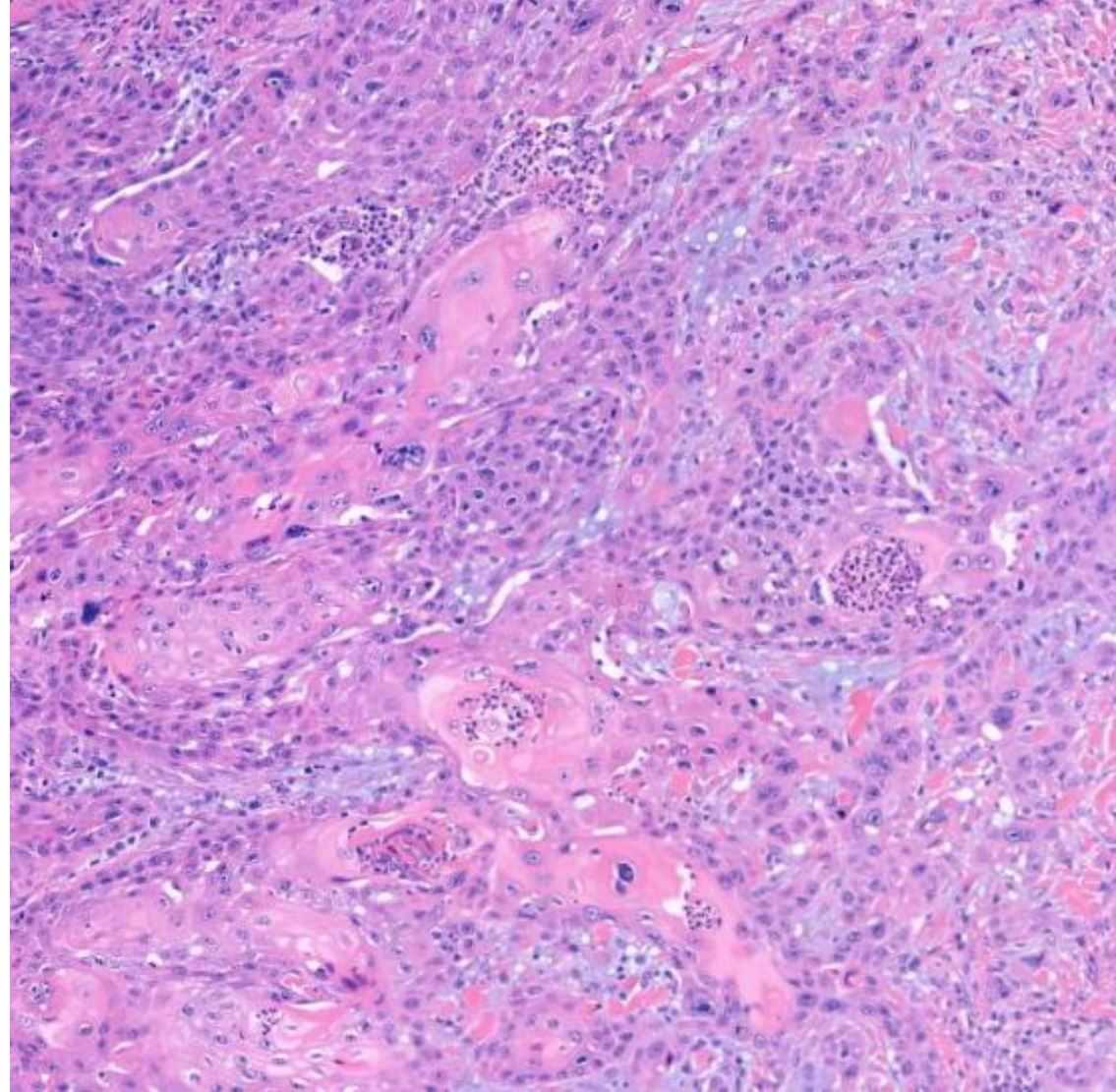
*PMID: 20055951, 31567280,
40066786, 26535979, 31567280*



Squamous Cell Carcinoma Exhibits Squamous Differentiation Without an Endometrioid Component

- ✓ Proliferation of squamous cells with variable cytologic and/or architectural atypia
- ✓ No glandular differentiation:
 - Sample extensively
- ✓ No cervical HPV-associated squamous cell carcinoma or HSIL on complete sampling
- ✓ ±Squamous metaplasia/ichthyosis uteri
- ✓ Often abnormal p53, negative p16, ER/PR
- ✓ Aggressive clinical behavior

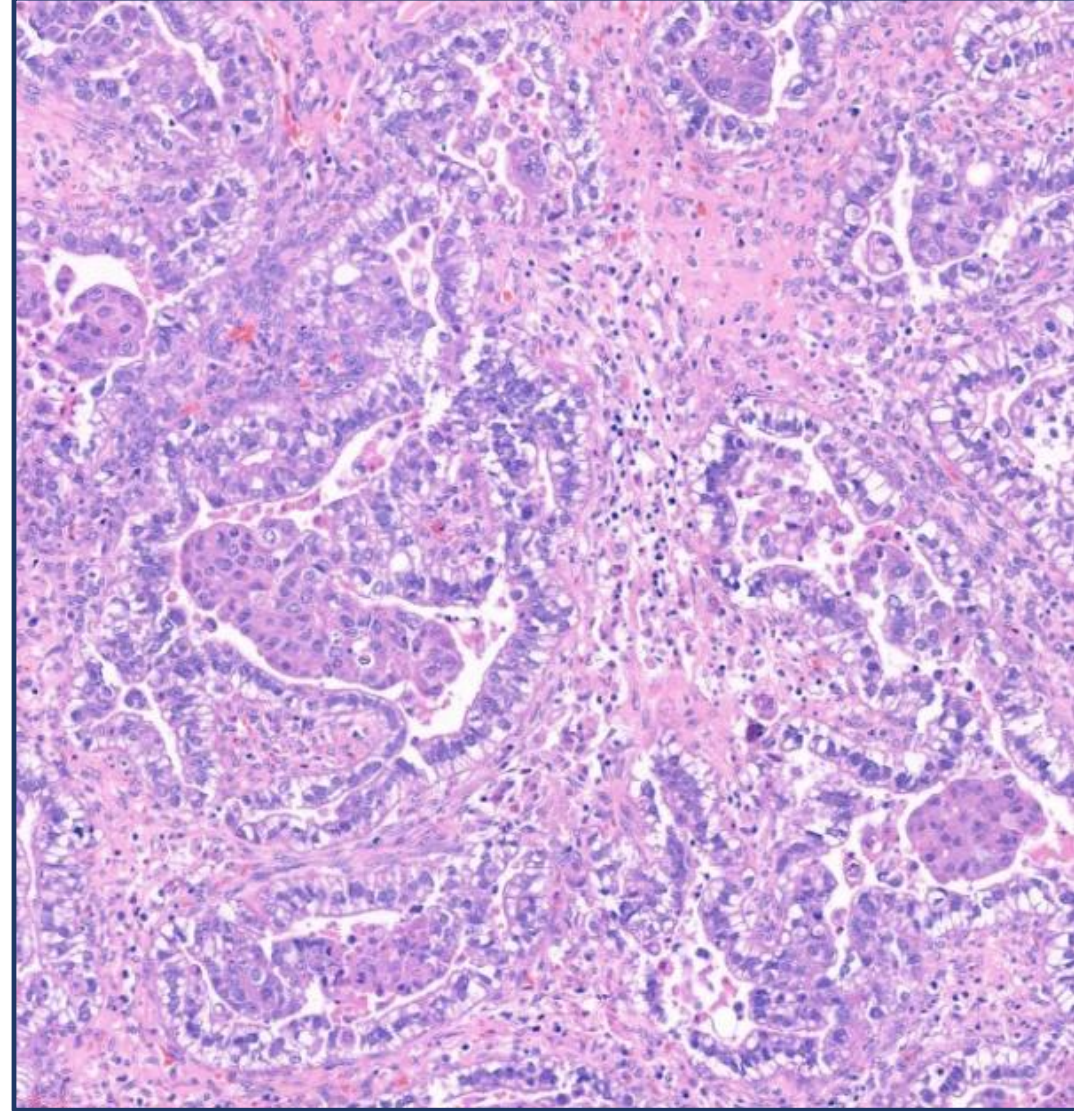
PMID: 24071873, 36069815



Endometrial Carcinoma with Somatically Derived Germ Cell Component

- ✓ Features of yolk sac or other germ cell tumors ± carcinoma of another histotype (endometrioid, serous, clear cell, carcinosarcoma)
- ✓ **Yolk sac (fetal-type adenocarcinoma):**
 - Glands with subnuclear vacuoles and primitive nuclei, cytoplasmic clearing, reticular growth with myxoid stroma, Schiller-Duval bodies, hyaline globules, hepatoid appearance
 - Aggressive behavior

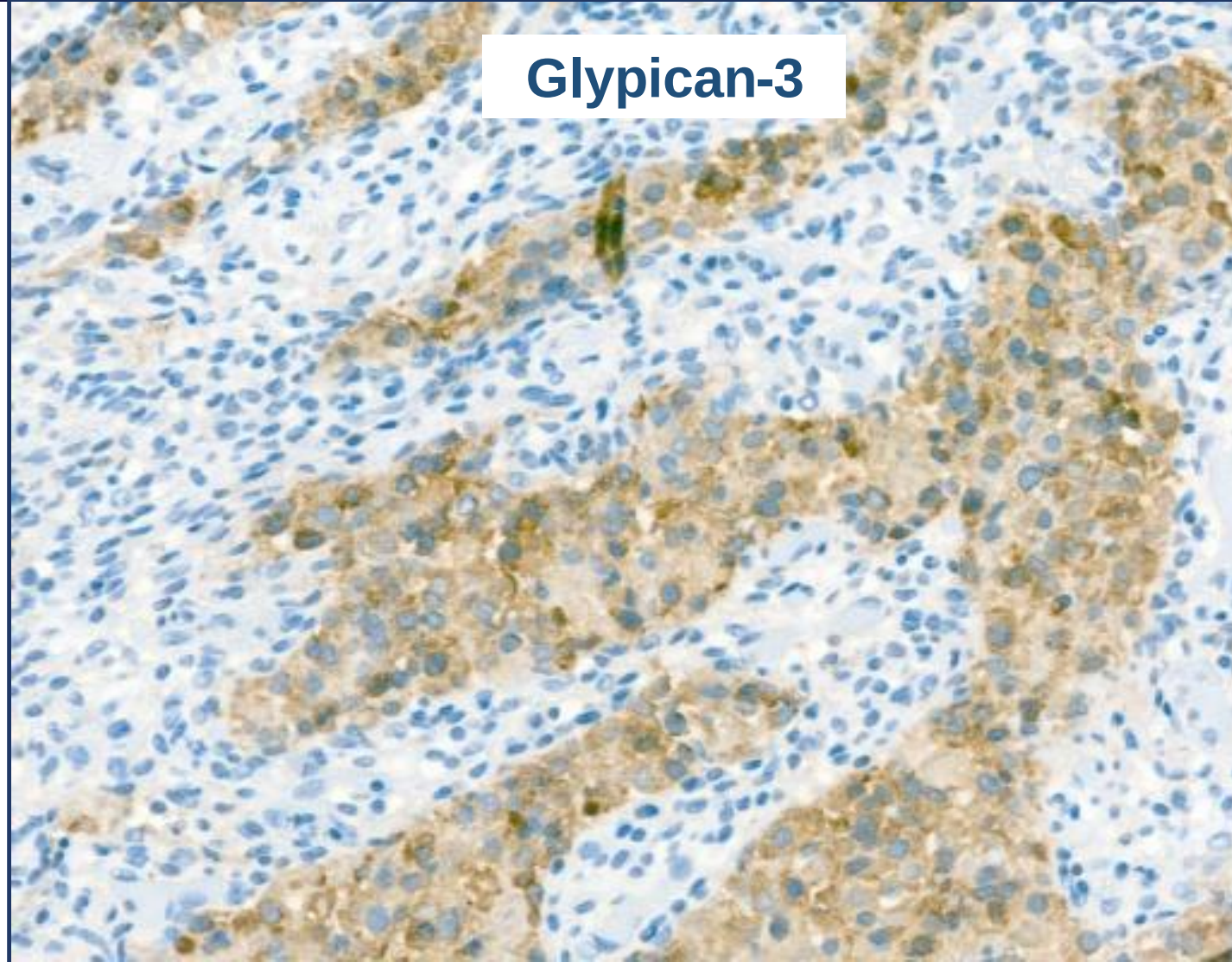
*PMID: 31375771, 32558949, 38651612,
27334714, 31010836, 32404953, 32558949*



Only Perform AFP, Glypican-3 & SALL4 in the Right Morphologic Context

- ✓ Positive AFP, glypican-3, SALL4 (≥ 1 in 70% of tumor cells):
 - May be expressed in other histotypes
- ✓ Positive epithelial markers (EMA, CK7, etc)
- ✓ Positive CDX2, villin
- ✓ Negative PAX8, ER/PR

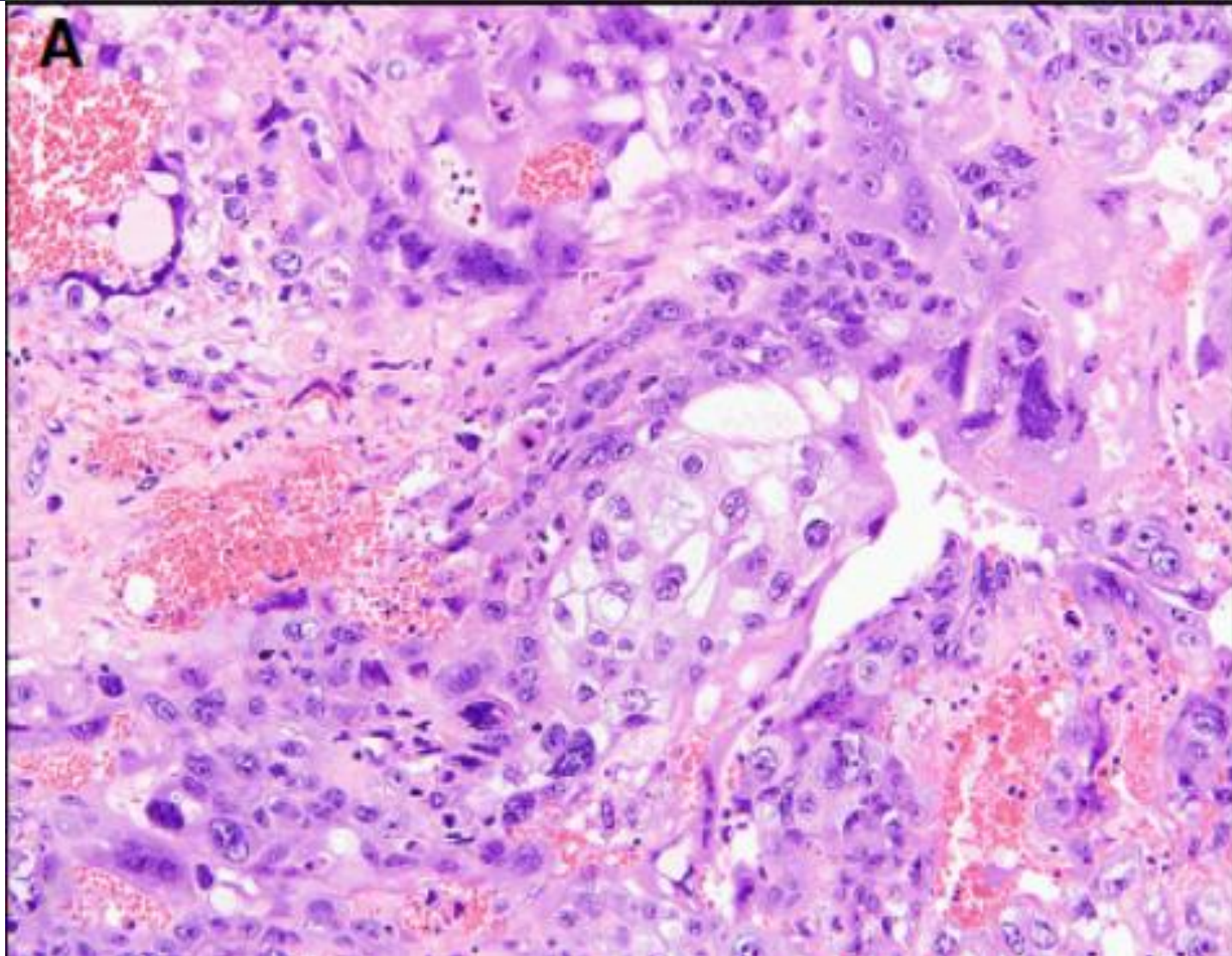
PMID: 31375771, 38651612



Endometrial Carcinoma with Somatically Derived Trophoblastic Component

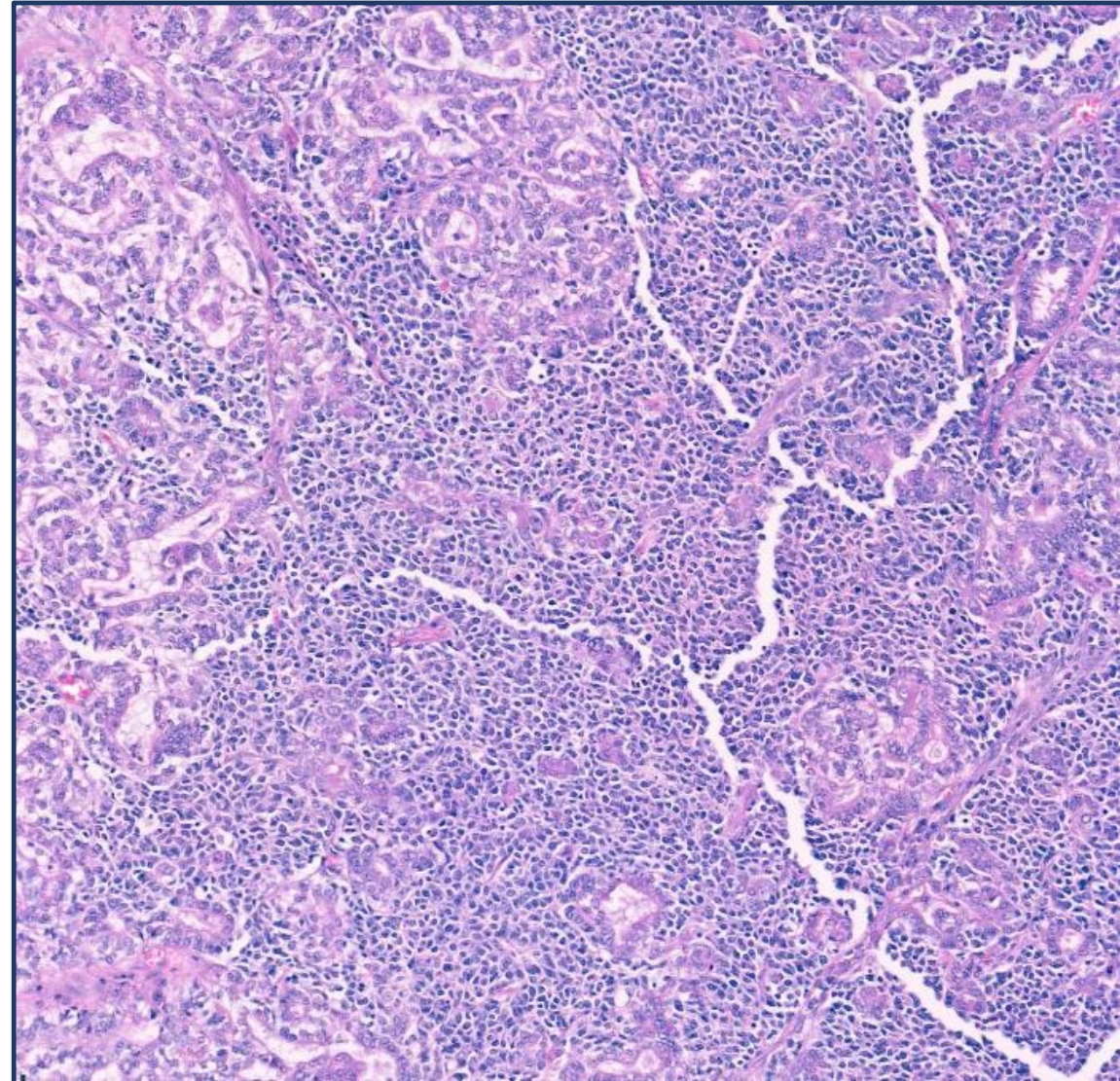
- ✓ Features of choriocarcinoma or other trophoblastic tumors ± carcinoma of another histotype (endometrioid, serous, clear cell, carcinosarcoma)
- ✓ **Choriocarcinoma:**
 - Admixture of cytotrophoblast-like and syncytiotrophoblast-like cells
 - Positive β -hCG and other trophoblastic markers
 - Aggressive behavior

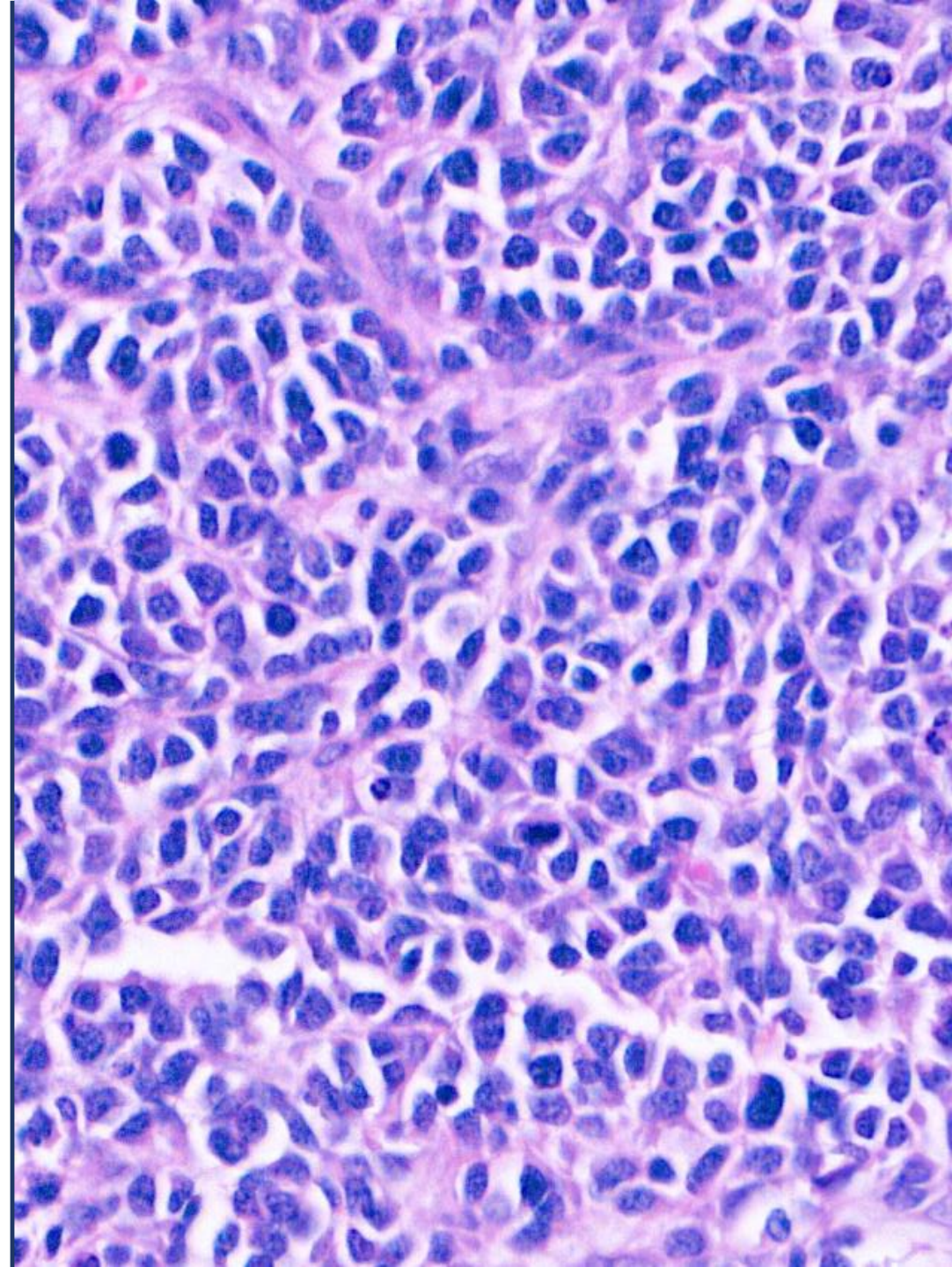
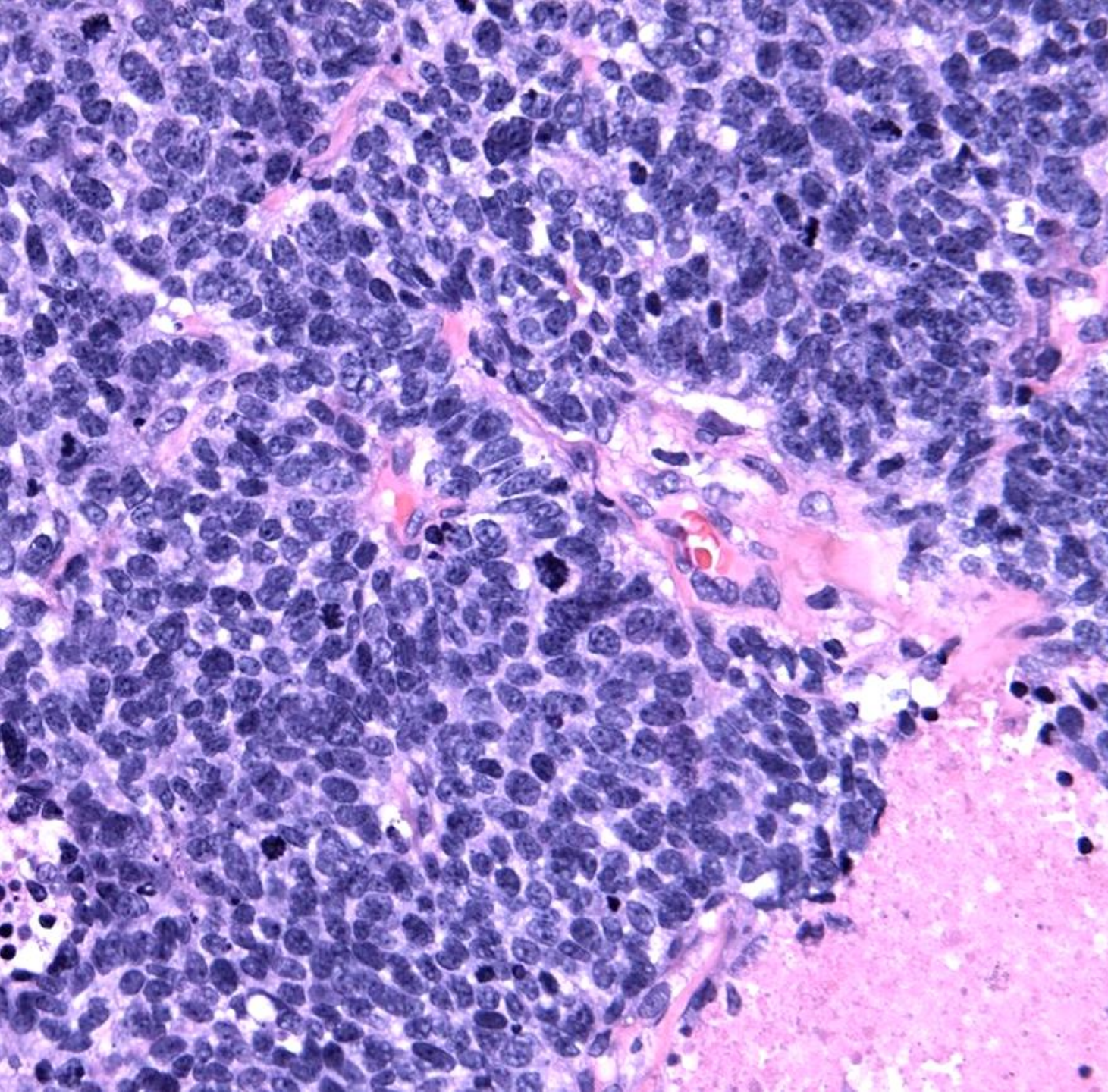
PMID: 32404953, 32558949



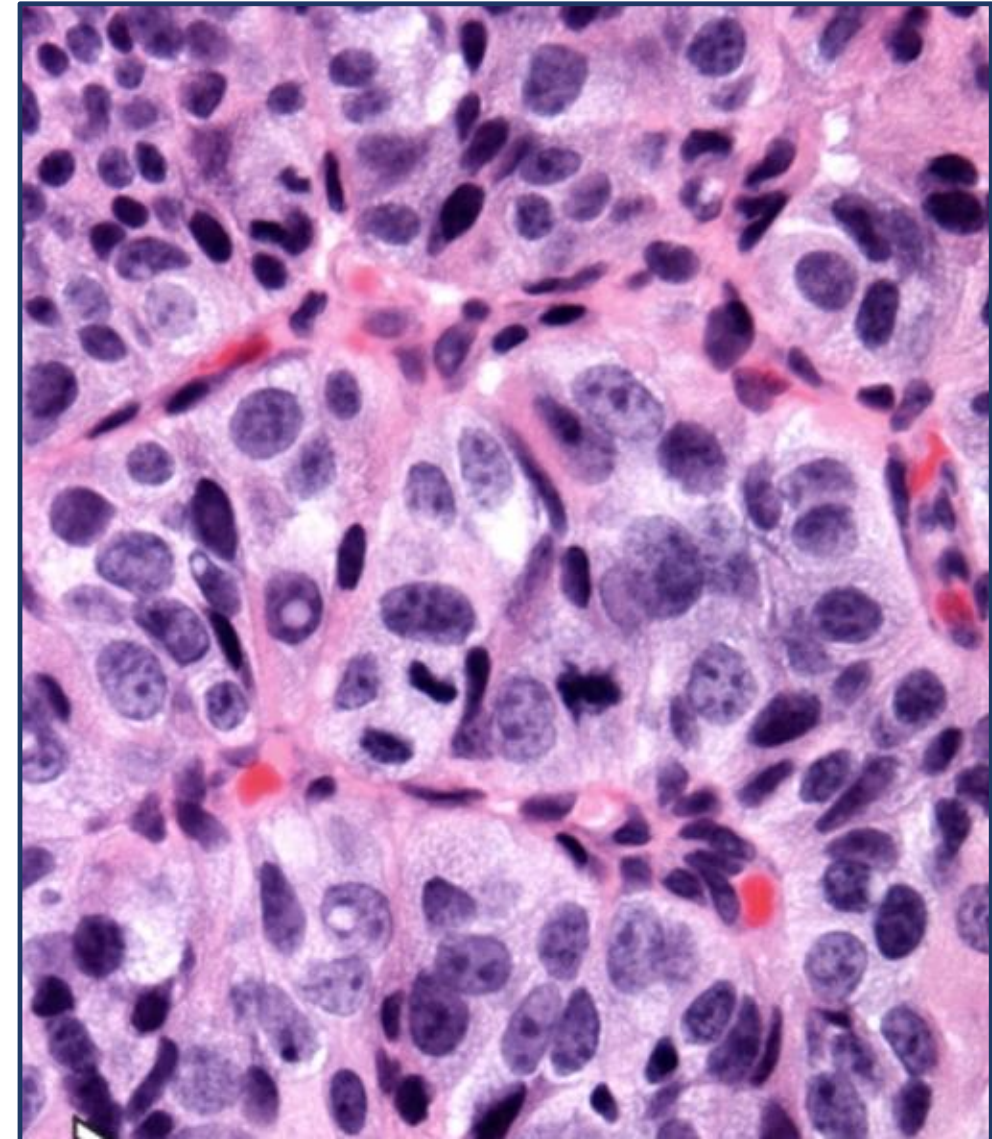
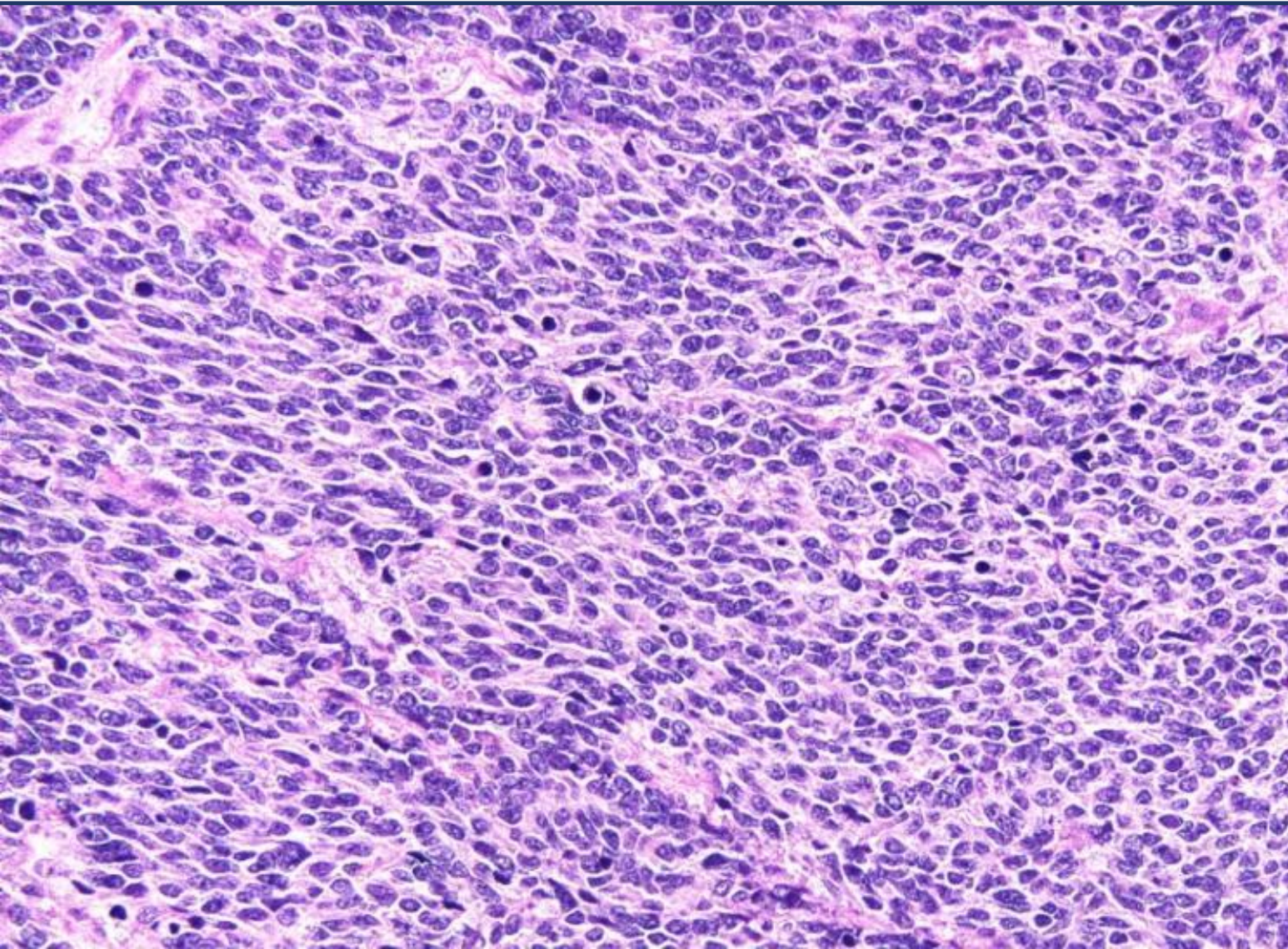
Neuroendocrine Carcinoma Exhibits Neuroendocrine Differentiation with Features of Small Cell or Large Cell Neuroendocrine Carcinoma

- ✓ Usually small cell carcinoma ± conventional histotype (endometrioid, serous, clear cell, carcinosarcoma):
 - Diffuse architecture ± nests and cords
 - Cells with scant cytoplasm, hyperchromatic nuclei with stippled chromatin, molding, brisk mitoses
 - Necrosis
- ✓ Aggressive behavior (5-yr survival 38%)





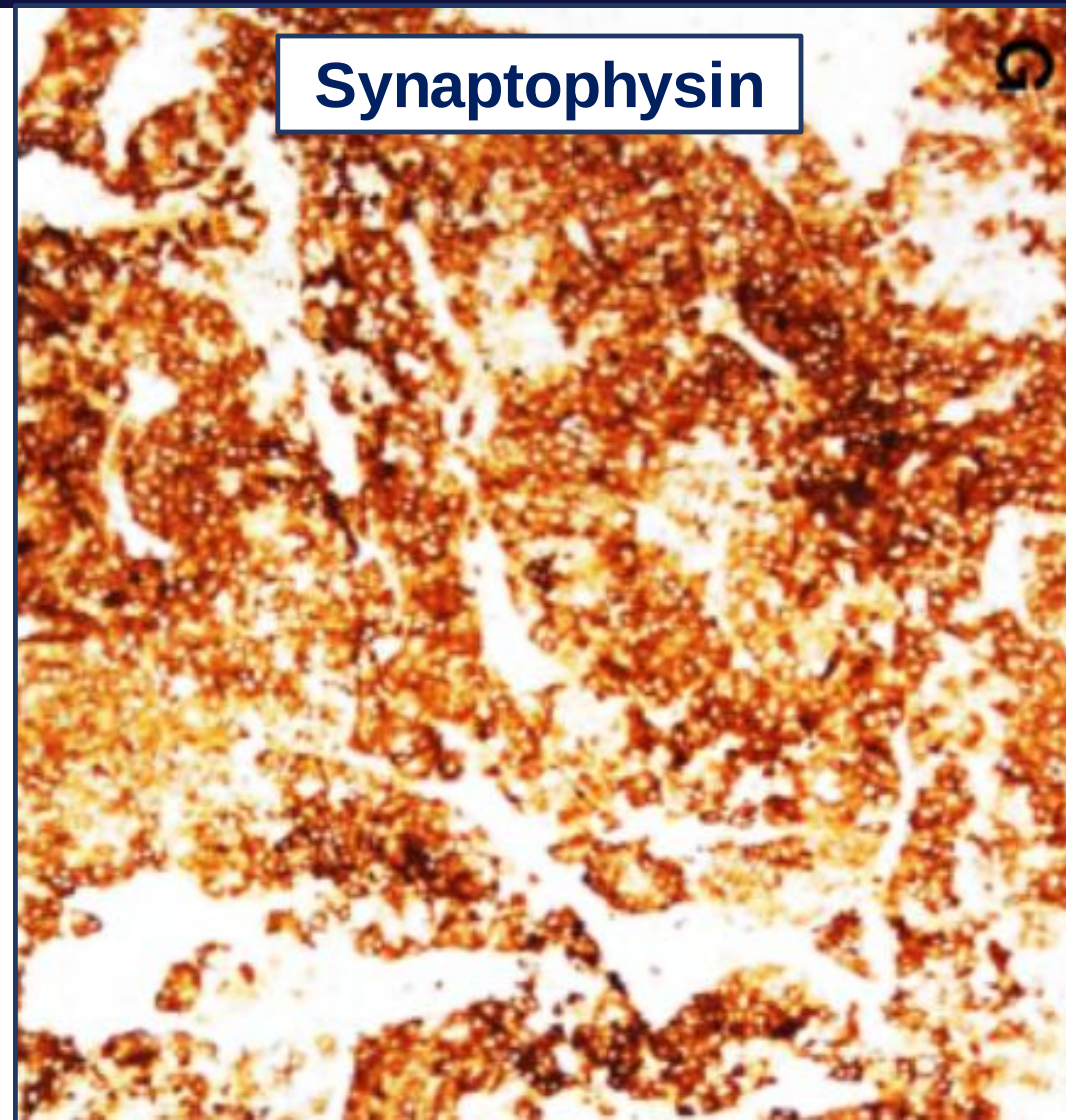
**Large Cell Neuroendocrine Carcinoma (Rare):
Medium/Large, Polygonal Cells with Moderate Eosinophilic /
Amphophilic Cytoplasm & Vesicular Nuclei with Prominent Nucleoli**



Only Perform Neuroendocrine Markers in the Right Morphologic Context

- ✓ IHC confirmation of neuroendocrine differentiation in tumors with morphologic features of neuroendocrine carcinoma:
 - Synaptophysin (sensitive), chromogranin, or INSM1 (specific) in $\geq 10\%$ tumor cells
 - Wide-spectrum and LMW keratins
 - $\pm$ PAX8, CD117, TTF-1, p16, abnormal p53, MMR deficiency, Rb1 loss
 - (Un-/dedifferentiated carcinoma and pilomatrix-like endometrioid carcinoma can be positive!)

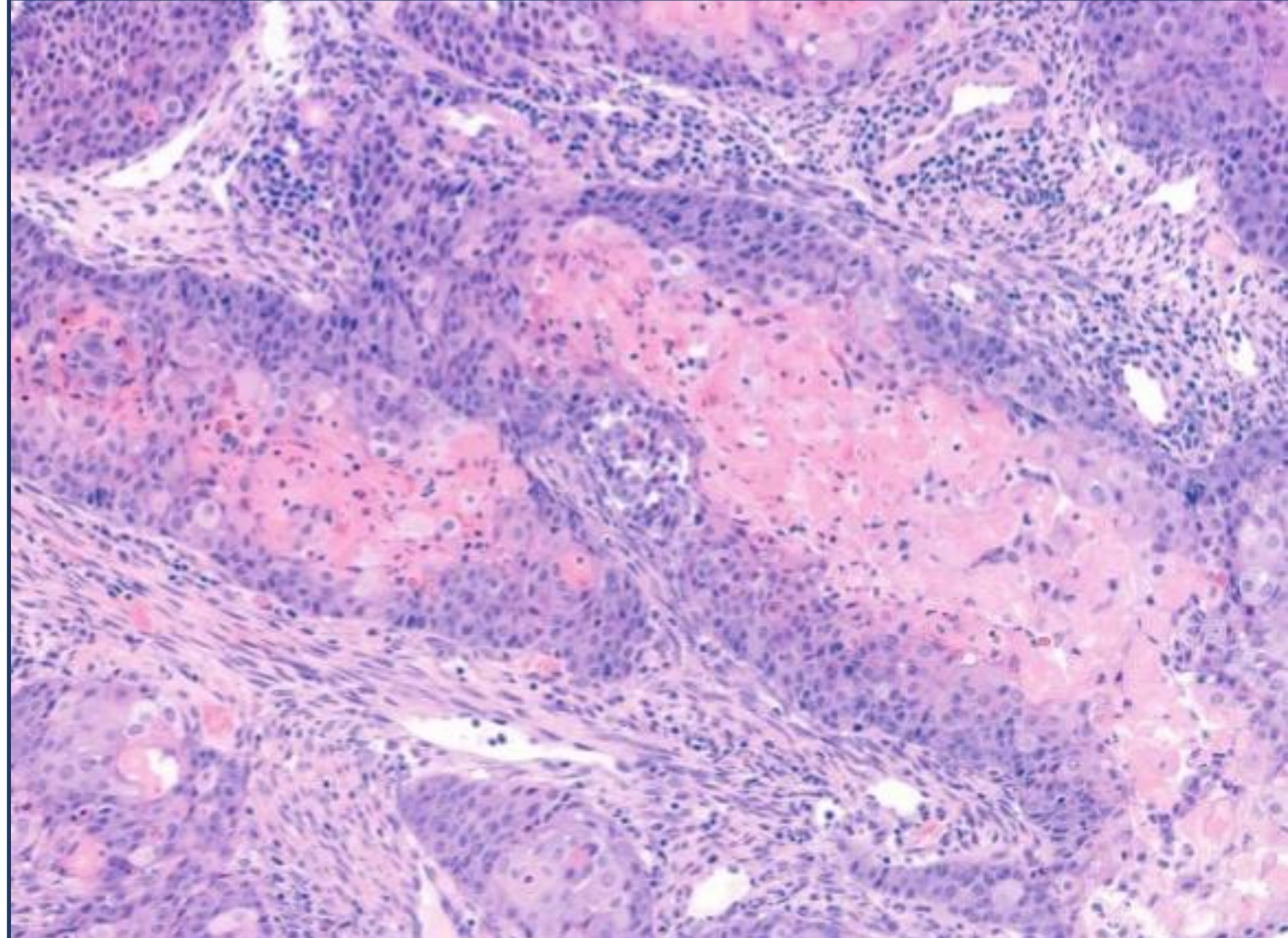
PMID: 33264139, 33323849, 35394077, 26945341, 32773531



Pilomatrix-Like Carcinoma Is a Rare & Aggressive Variant of High-Grade Endometrioid Carcinoma

- ✓ Survival <24 months
- ✓ High-grade basaloid carcinoma with solid and nested architecture, ghost cell keratinization, necrosis
- ✓ Low-grade endometrioid carcinoma component in most cases

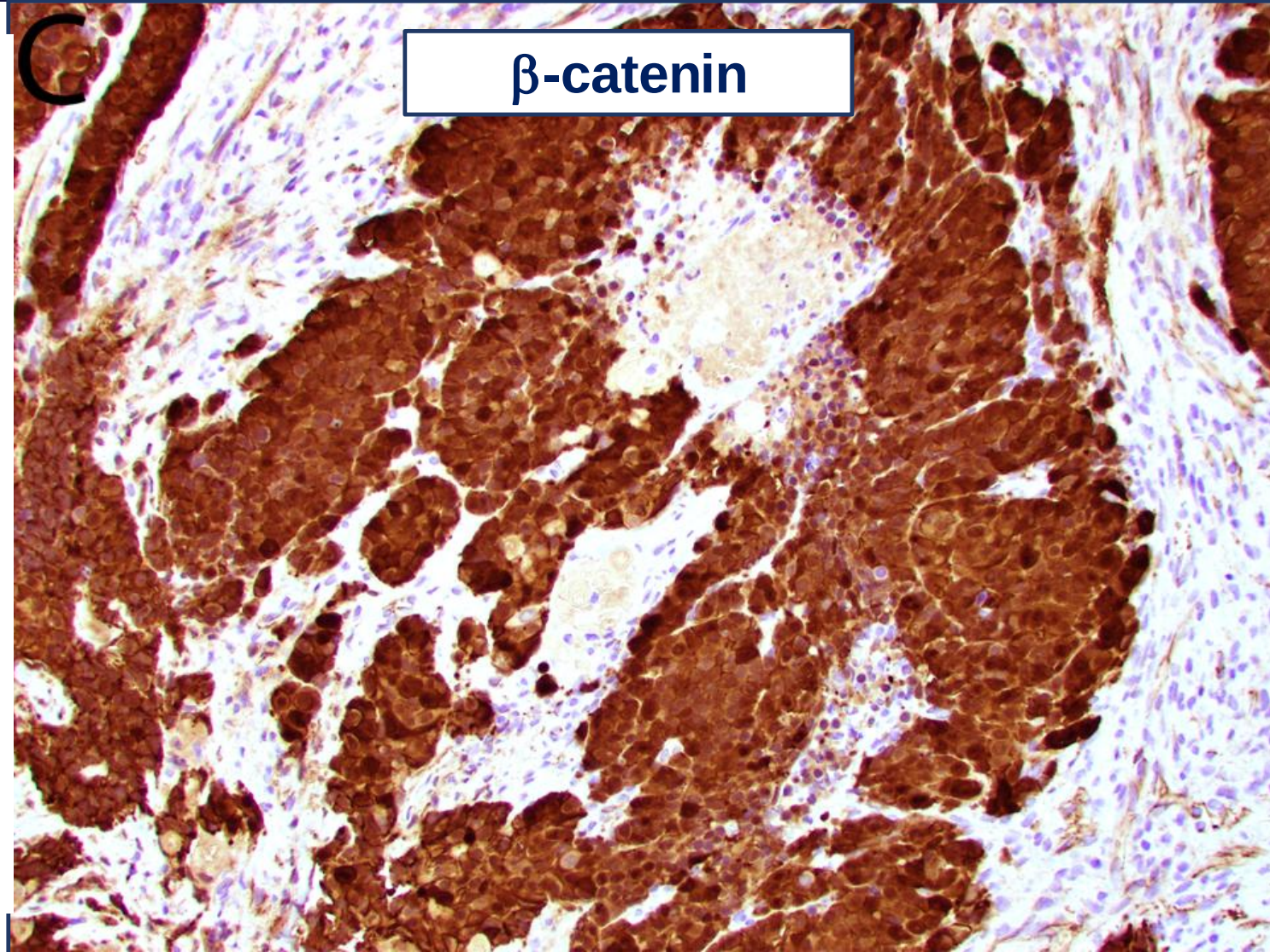
PMID: 33811207, 37192144, 35180769,
40202384, 40886777



Pilomatrix-Like High-Grade Endometrioid Carcinoma

- ✓ Nuclear β -catenin due to *CTNNB1* mutations in most
- ✓ Rare *FGFR4*, *TSC2*, *KRAS* mutations
- ✓ Positive CDX2
- ✓ **Often positive synaptophysin!**
- ✓ Negative PAX8, ER/PR
- ✓ $\pm$ MMR deficiency

PMID 33811207, 37192144, 35180769,
40202384, 40886777



TCGA Subgroups of Endometrial Carcinoma Determined Based on Comprehensive Genomic Characterization

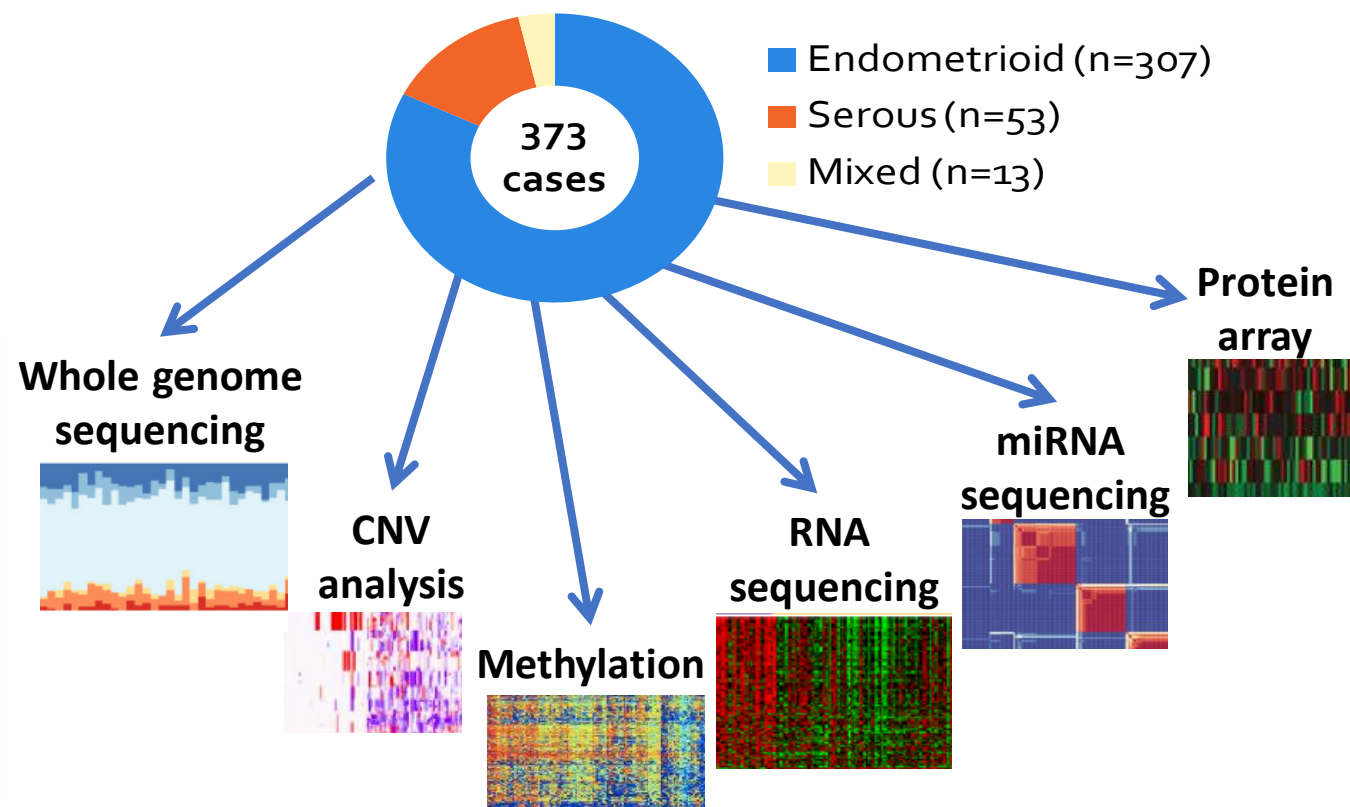
Integrated genomic characterization of endometrial carcinoma

The Cancer Genome Atlas Research Network*

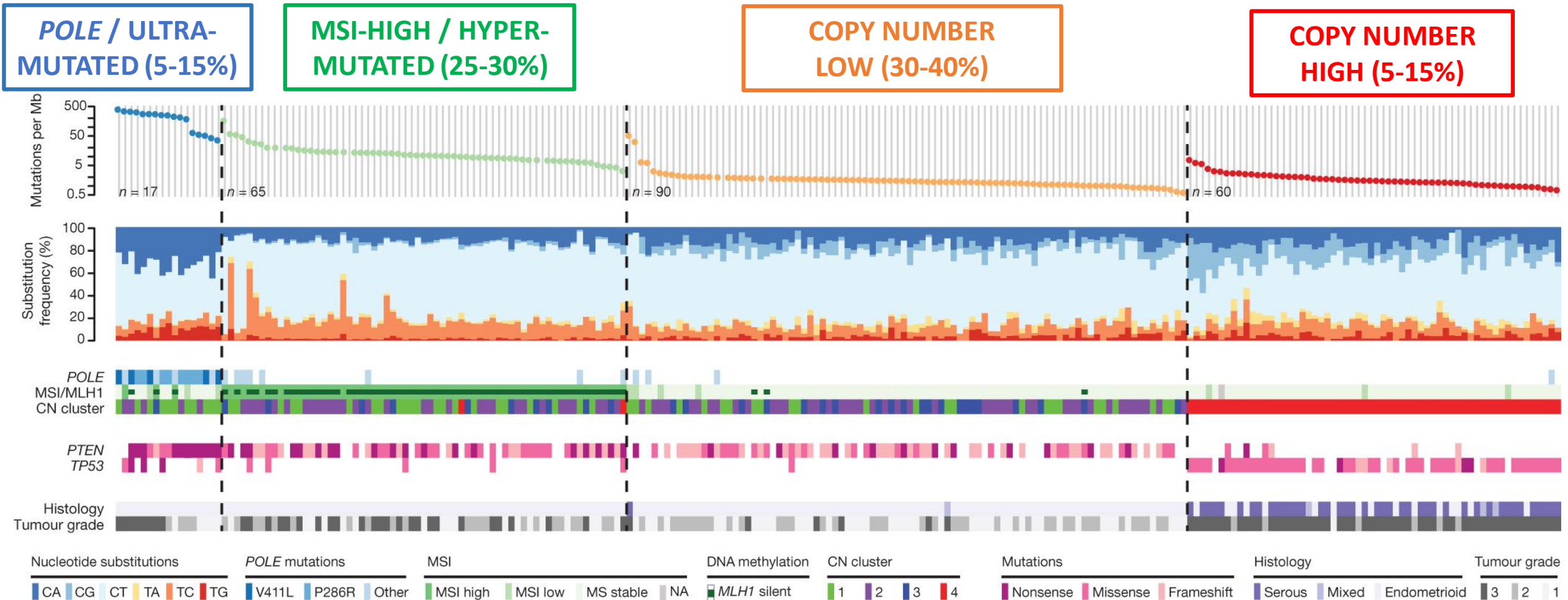
2 MAY 2013 | VOL 497 | NATURE | 73



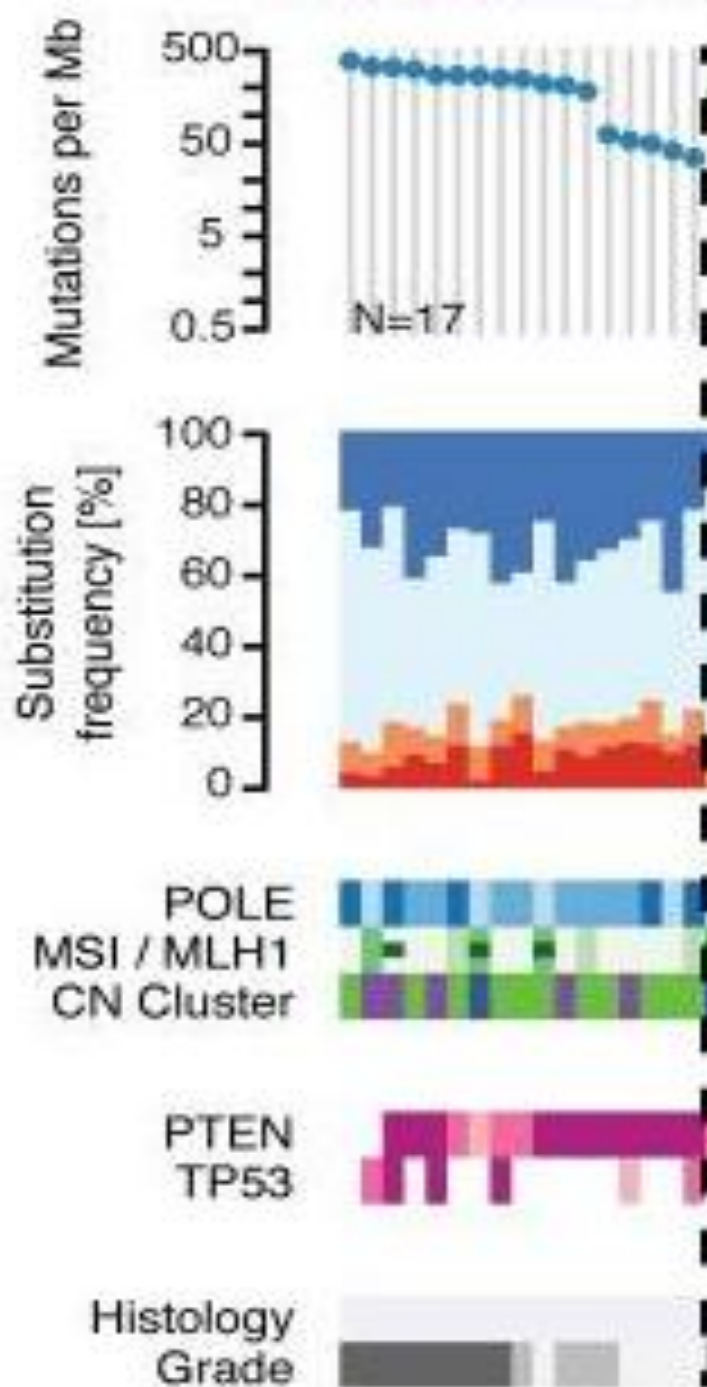
NATIONAL
CANCER
INSTITUTE



TCGA Molecular Classification of Endometrial Carcinoma Reveals 4 Prognostically Distinct Subtypes

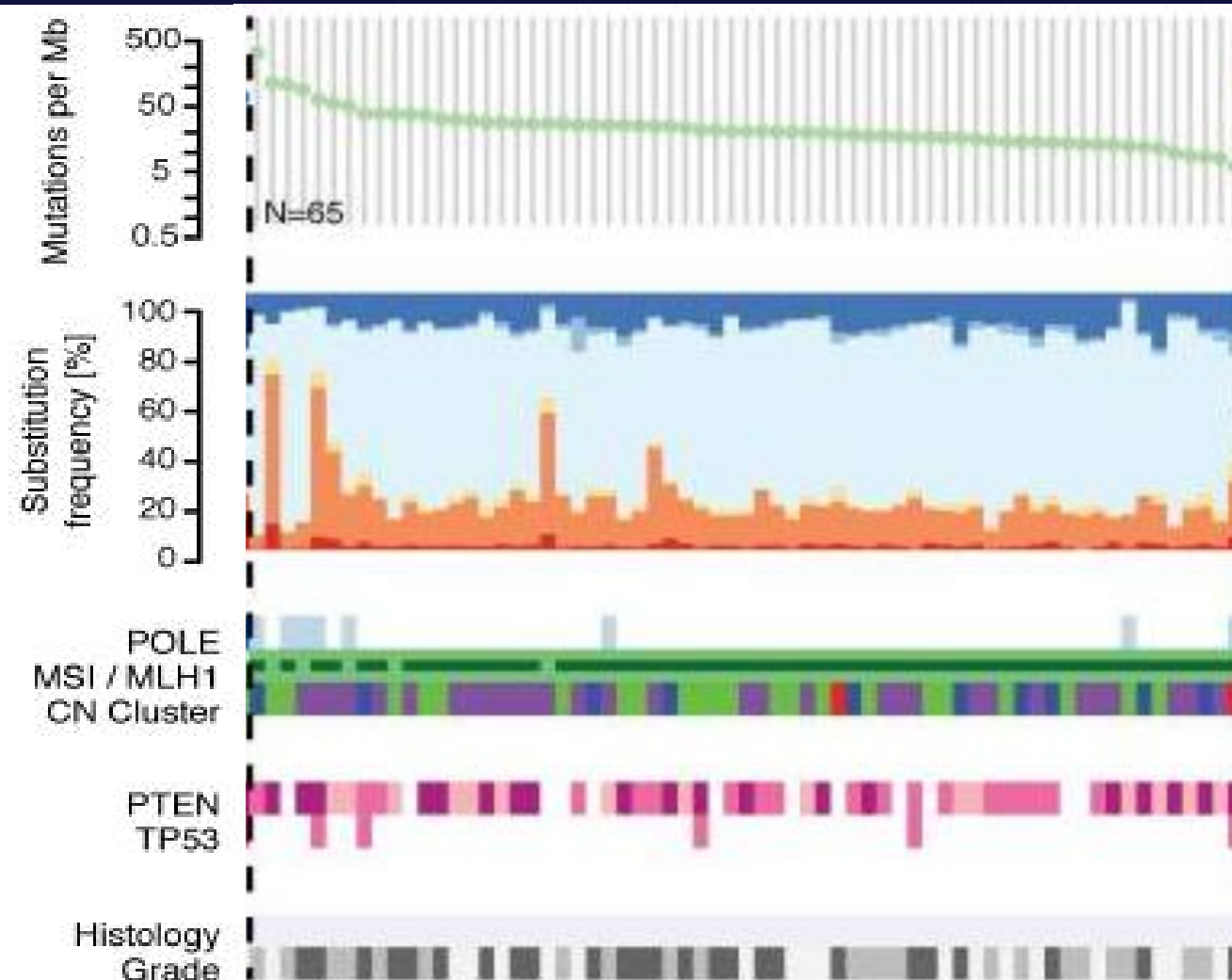


POLE (Ultramutated) Subtype



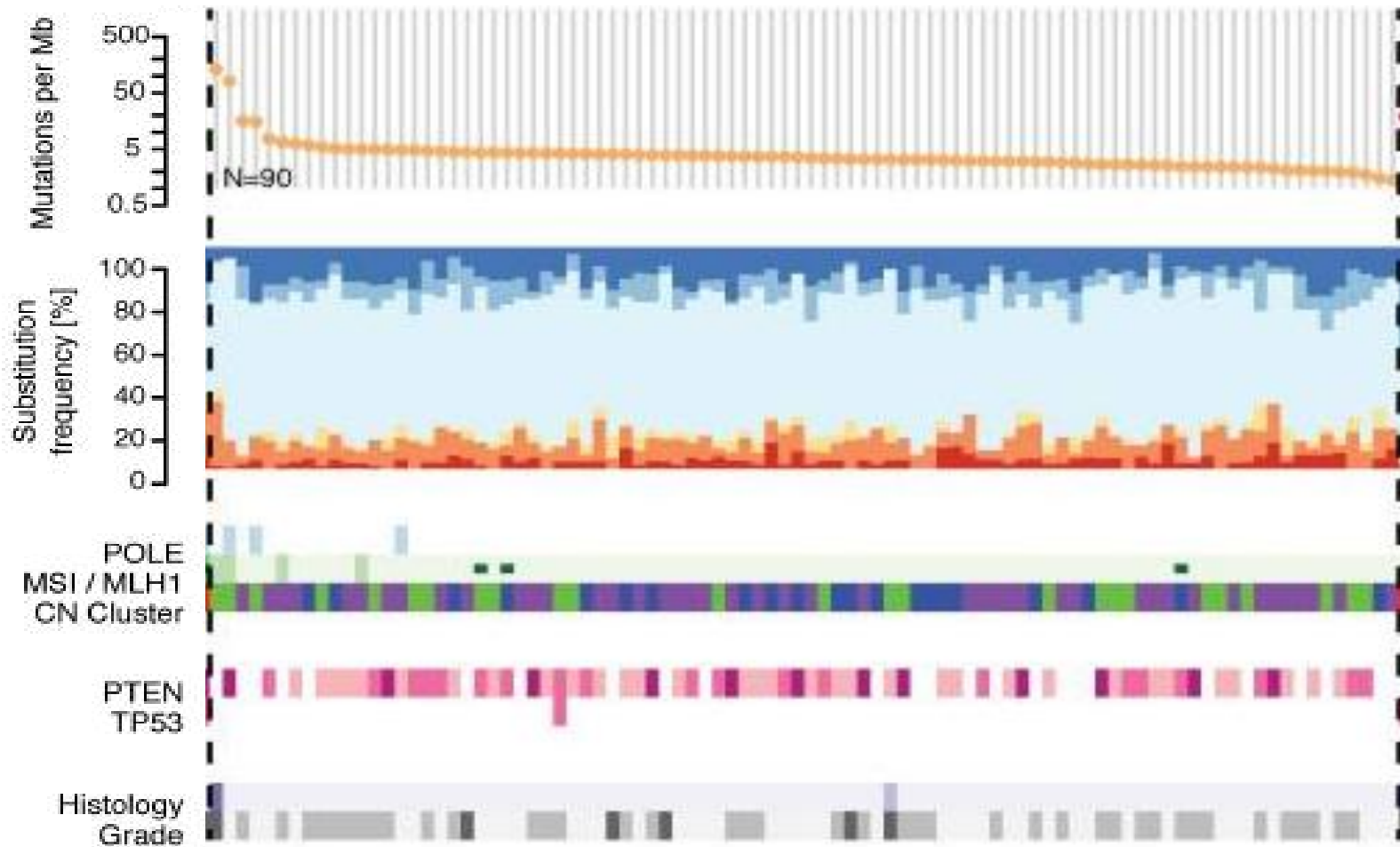
- Mutations in the exonuclease domain of DNA Polymerase Epsilon
- ↑↑Mutation rate
- Unique nucleotide change spectrum (C>A)
- Microsatellite stable
- Few copy number alterations
- Other gene mutations:
 - ✓ *PTEN*
 - ✓ *PIK3CA* & *PIK3R1*
 - ✓ *FBXW7*
 - ✓ *KRAS*
 - ✓ *TP53*

MSI-High (Hypermuted) Subtype



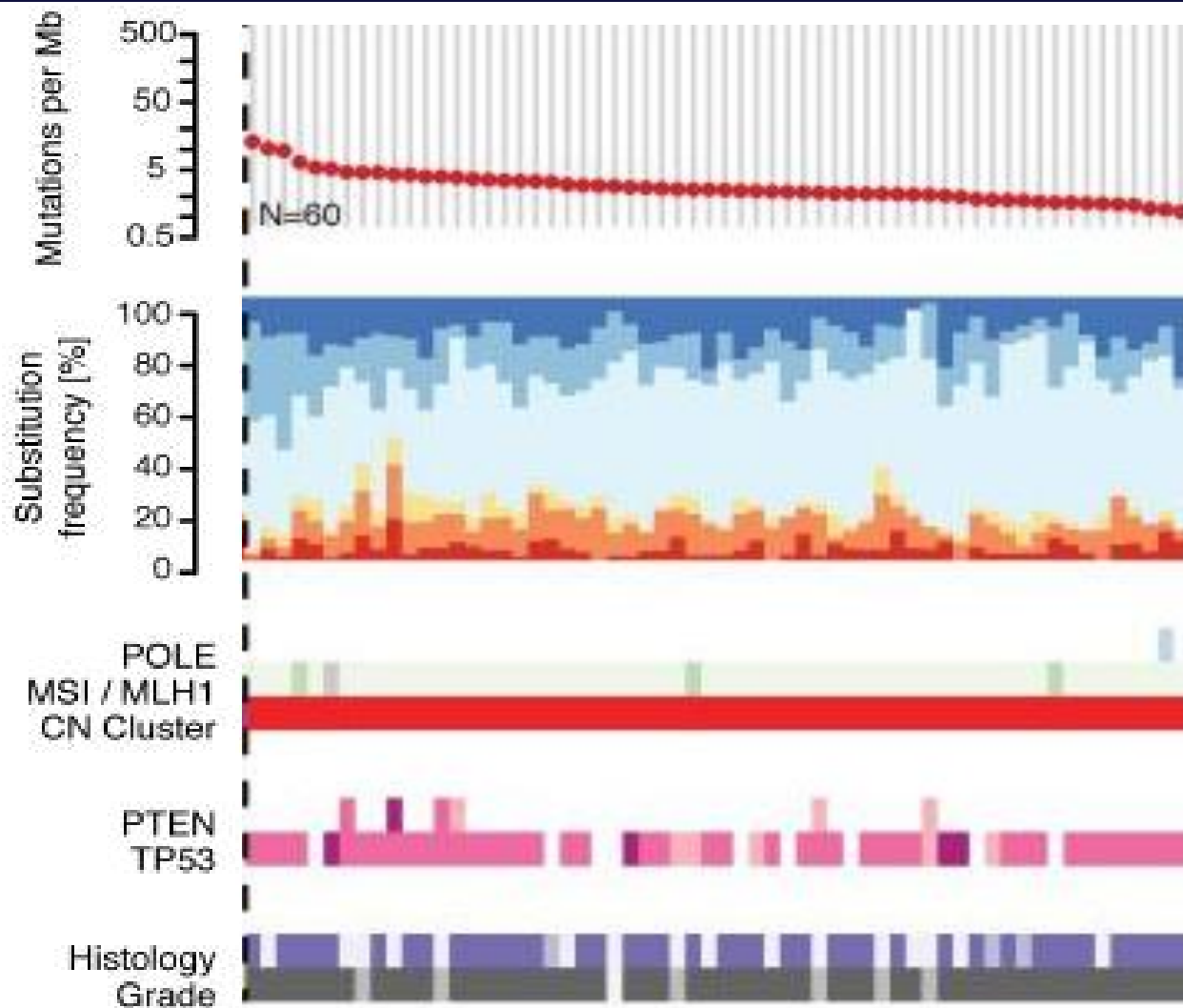
- MSI-high
- Most *MLH1* promoter methylated
- Few copy number alterations
- ↑Mutation rate
- Gene mutations:
 - ✓ *RPL22*
 - ✓ *KRAS*
 - ✓ *CTNNB1*
 - ✓ *FBXW7*
 - ✓ *PPP2R1A*
 - ✓ *TP53*

Copy Number Low Subtype



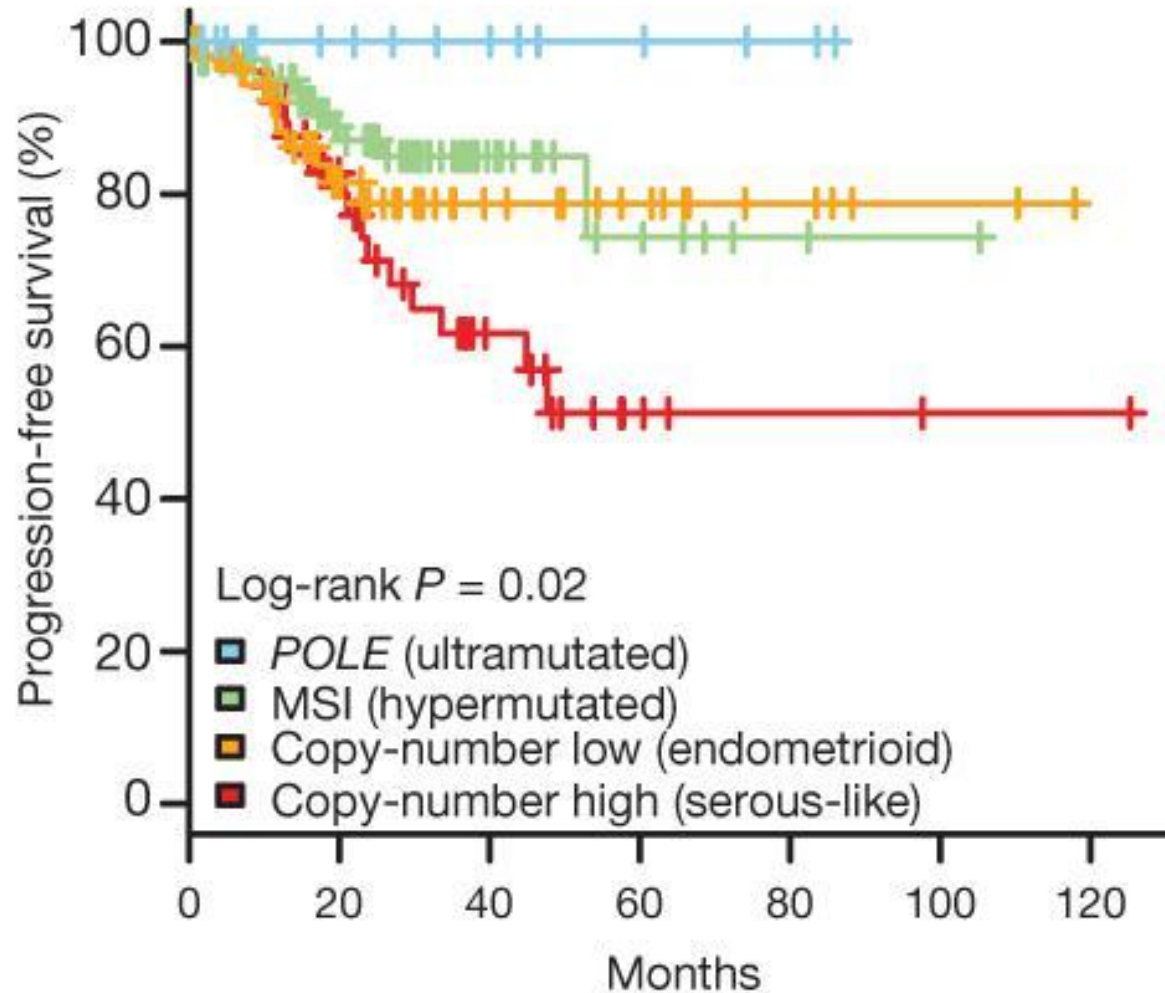
- Microsatellite stable
- Few copy number alterations
- ↓ Mutation rate
- Gene mutation:
 - ✓ *CTNNB1* (52%)

Copy Number High Subtype



- Microsatellite stable
- Many copy number alterations
- ↓Mutation rate
- Gene mutations:
 - ✓ *TP53*
 - ✓ *FBXW7*
 - ✓ *PPP2R1A*

Progression-Free Survival Differs Among the 4 Subtypes of Endometrial Carcinoma



- **Copy number high:** Unfavorable
- **POLE-mutated:** Favorable
- **Copy number low & MSI-high:**
 - ✓ Intermediate prognosis (stage)
 - ✓ *Worse prognosis in CTNNB1-mutated copy number low tumors*
- **POLE-mutated & MSI-high:**
 - ✓ Chemo/radiosensitive, checkpoint blockade (immunotherapy)

Comparison of the 4 Subtypes: Histotype & Molecular Alterations

COPY NUMBER LOW

- No specific molecular profile (NSMP)
- *CTNNB1* (52%)
- Endometrioid, clear cell, carcinosarcoma, undifferentiated, mixed

MSI-HIGH (HYPERMUTATED)

- Most positive for *MLH1* promoter hypermethylation
- *RPL22*, *KRAS*, *CTNNB1*, *FBXW7*, *PPP2R1A*, *TP53*
- Endometrioid, undifferentiated, clear cell, carcinosarcoma

COPY NUMBER HIGH

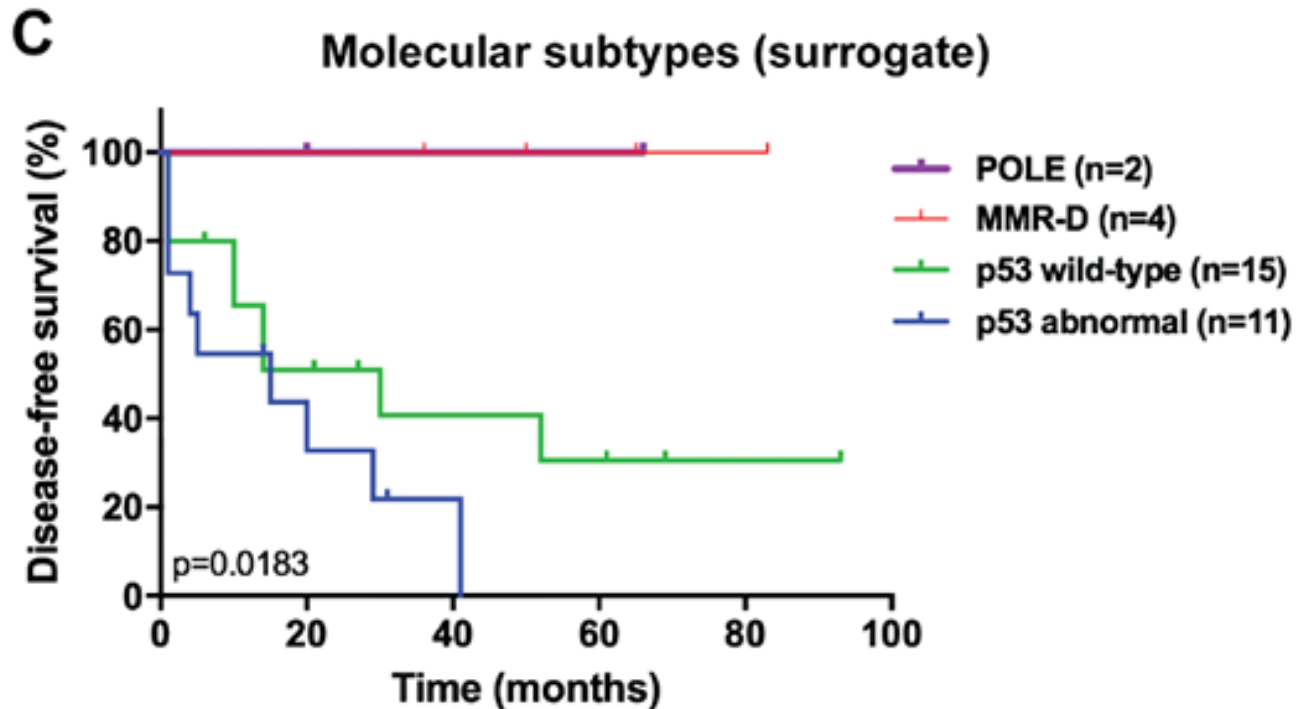
- *TP53*, *FBXW7*, *PPP2R1A*
- Serous, carcinosarcoma, endometrioid, clear cell, undifferentiated, mixed

POLE (ULTRAMUTATED)

- Hotspot mutations in *POLE* exonuclease domain
- *PTEN*, *PIK3CA*, *PIK3R1*, *FBXW7*, *KRAS*, *TP53*
- Endometrioid, clear cell, carcinosarcoma, undifferentiated

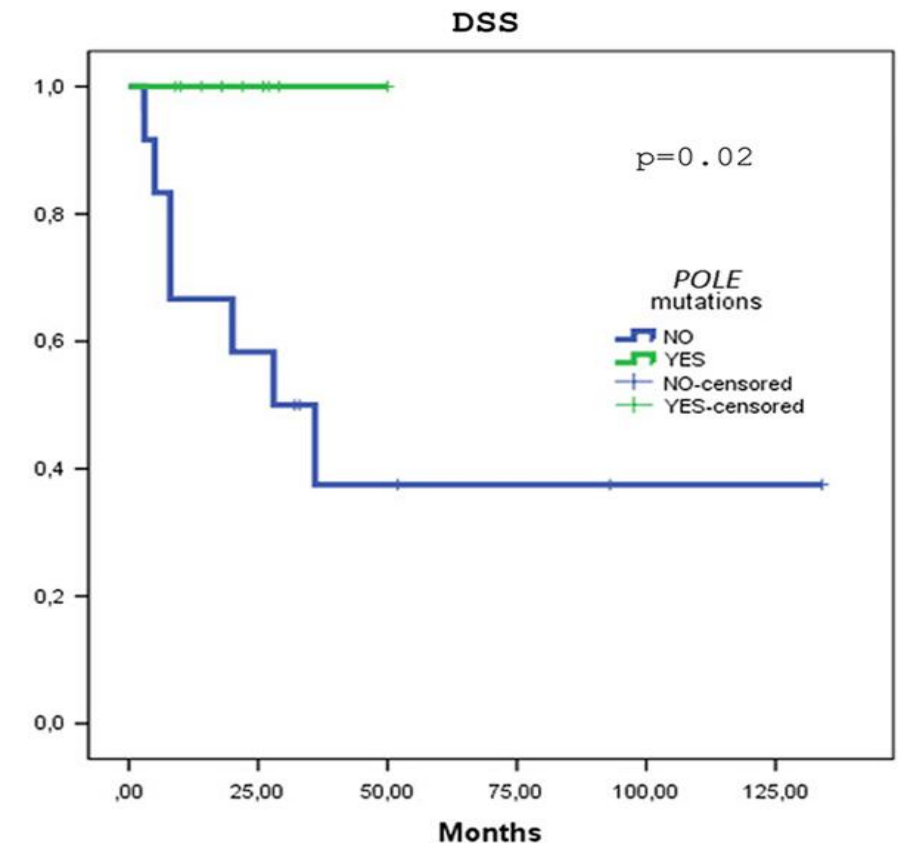
Molecular Subtyping of High-Grade Endometrial Carcinomas Identifies Prognostically Different Groups

Clear Cell Carcinoma



PMID: 28718916

Un/dedifferentiated carcinoma

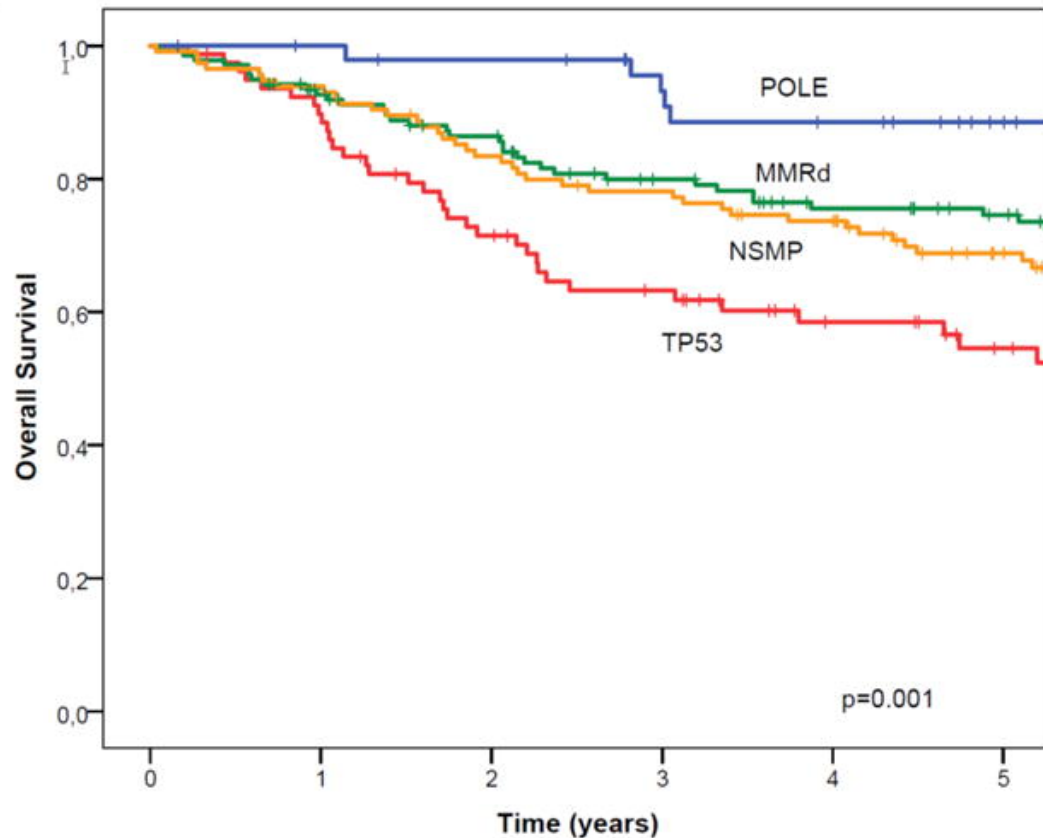


PMID: 28498284

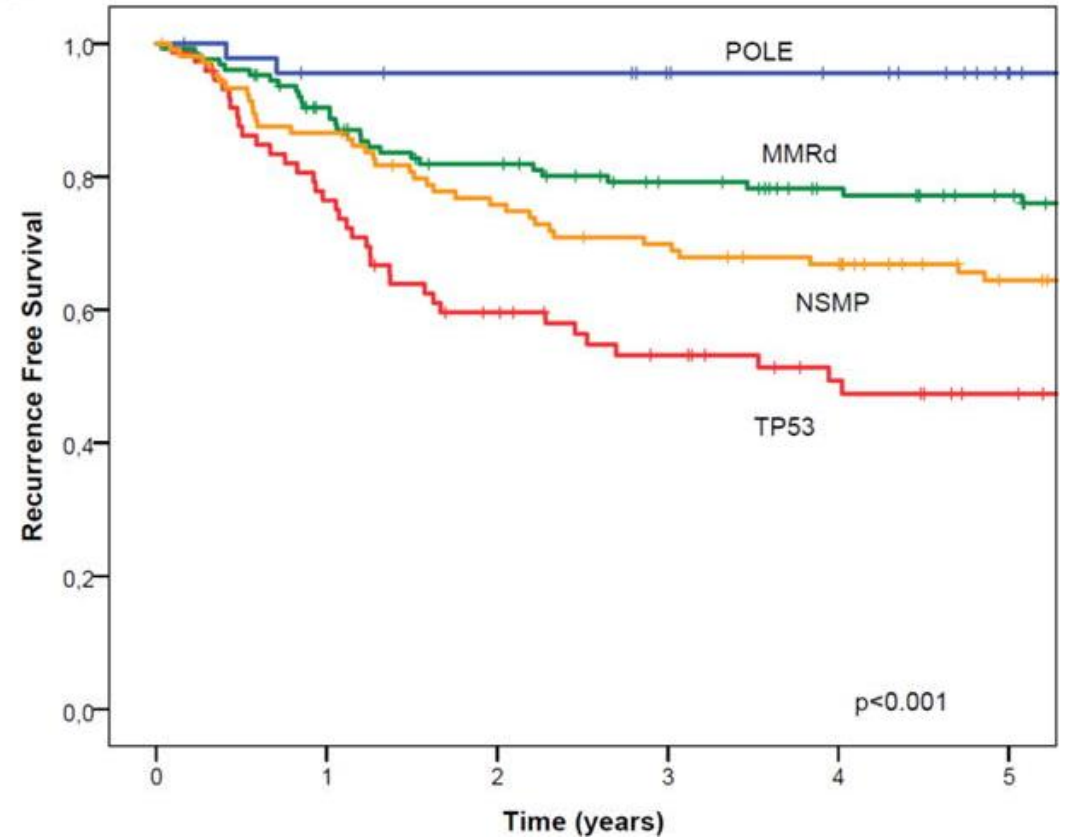
Molecular Subtyping of High-Grade Endometrial Carcinomas Identifies Prognostically Different Groups

Grade 3 Endometrioid Carcinoma

A.

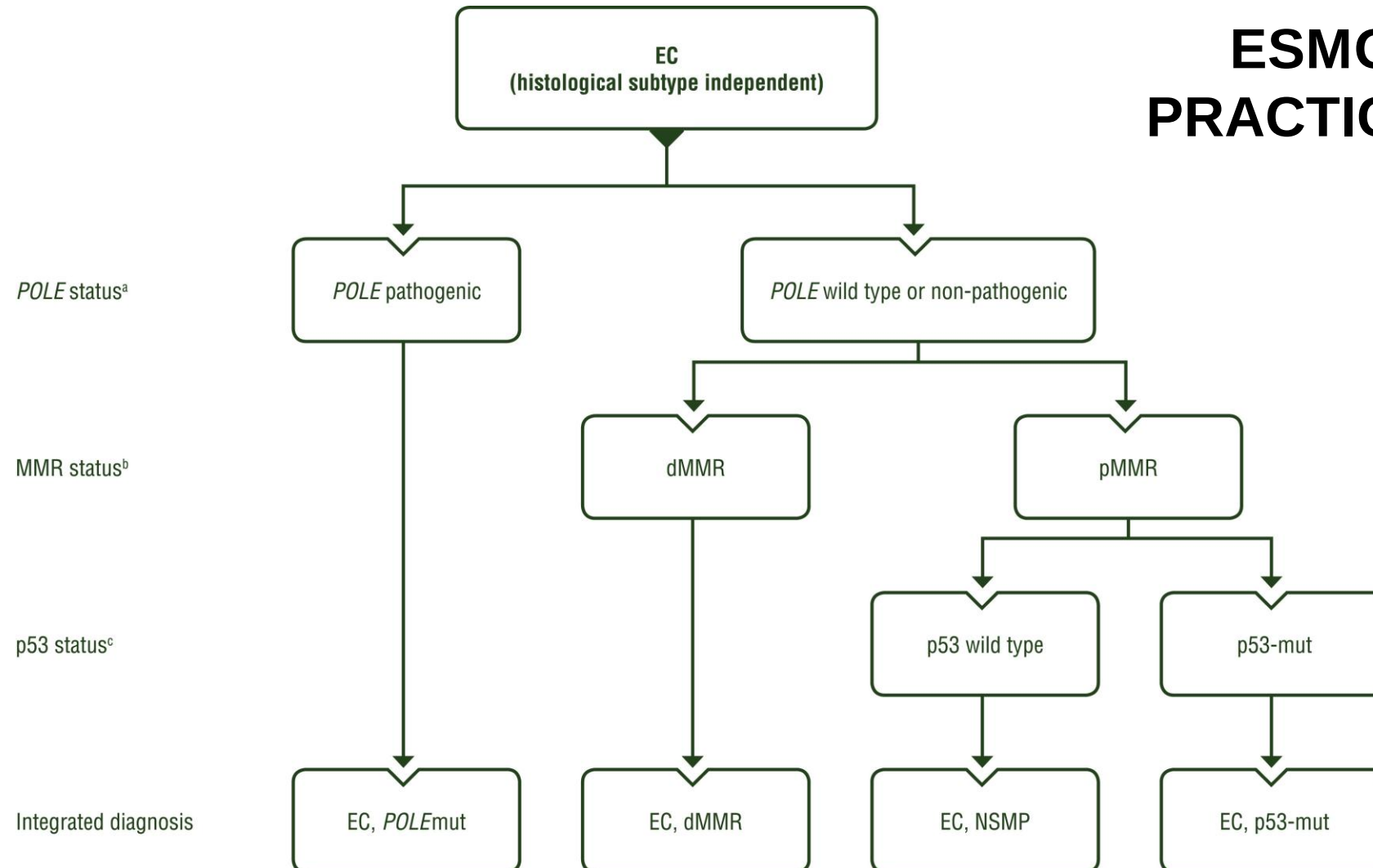


B.



Diagnostic Algorithm for the Integrated Molecular Classification of Endometrial Cancer

ESMO CLINICAL PRACTICE GUIDELINE (2022)



Updated Risk Classification System to Establish the Relative Risk of Recurrence

ESMO CLINICAL PRACTICE GUIDELINE

Table 2. EC risk groups

Risk group	Description ^a
Low risk	Stage IA (G1-G2) with endometrioid type (dMMR ^b and NSMP) and no or focal LVSI Stage I/II <i>POLE</i> mut cancer; for stage III <i>POLE</i> mut cancers ^c
Intermediate risk	Stage IA G3 with endometrioid type (dMMR and NSMP) and no or focal LVSI Stage IA non-endometrioid type (serous, clear-cell, undifferentiated carcinoma, carcinosarcoma, mixed) and/or p53-abn cancers without myometrial invasion and no or focal LVSI Stage IB (G1-G2) with endometrioid type (dMMR and NSMP) and no or focal LVSI Stage II G1 endometrioid type (dMMR and NSMP) and no or focal LVSI
High-intermediate risk	Stage I endometrioid type (dMMR and NSMP) any grade and any depth of invasion with substantial LVSI Stage IB G3 with endometrioid type (dMMR and NSMP) regardless of LVSI Stage II G1 endometrioid type (dMMR and NSMP) with substantial LVSI Stage II G2-G3 endometrioid type (dMMR and NSMP)
High risk	All stages and all histologies with p53-abn and myometrial invasion All stages with serous or undifferentiated carcinoma including carcinosarcoma with myometrial invasion All stage III and IVA with no residual tumour, regardless of histology and regardless of molecular subtype ^b

FIGO 2023 Staging

Stage	Description
Stage I	Confined to the uterine corpus and ovary [‡]
IA	Disease limited to the endometrium OR non-aggressive histological type, i.e., low-grade endometrioid, with invasion of less than half of myometrium with no or focal LVSI OR good prognosis disease
IA1	Non-aggressive histological type limited to an endometrial polyp OR confined to the endometrium
IA2	Non-aggressive histological types involving less than half of the myometrium with no or focal LVSI
IA3	Low-grade endometrioid carcinomas limited to the uterus and ovary [‡]
IB	Non-aggressive histological types with invasion of half or more of the myometrium, and with no or focal LVSI [§]
IC	Aggressive histological types limited to a polyp or confined to the endometrium
Stage II	Invasion of cervical stroma without extrauterine extension OR with substantial LVSI OR aggressive histological types with myometrial invasion
IIA	Invasion of the cervical stroma of non-aggressive histological types
IIB	Substantial LVSI [§] of non-aggressive histological types
IIC	Aggressive histological types with any myometrial involvement
Stage III	Local and/or regional spread of the tumor of any histological subtype
IIIA	Invasion of uterine serosa, adnexa, or both by direct extension or metastasis
IIIA1	Spread to ovary or fallopian tube (except when meeting stage IA3 criteria) [‡]
IIIA2	Involvement of uterine subserosa or spread through the uterine serosa
IIIB	Metastasis or direct spread to the vagina and/or to the parametria or pelvic peritoneum
IIIB1	Metastasis or direct spread to the vagina and/or the parametria
IIIB2	Metastasis to the pelvic peritoneum
IIIC	Metastasis to the pelvic or para-aortic lymph nodes or both [¶]
IIIC1	Metastasis to the pelvic lymph nodes
IIIC1i	Micrometastasis
IIIC1ii	Macrometastasis
IIIC2	Metastasis to para-aortic lymph nodes up to the renal vessels, with or without metastasis to the pelvic lymph nodes
IIIC2i	Micrometastasis
IIIC2ii	Macrometastasis
Stage IV	Spread to the bladder mucosa and/or intestinal mucosa and/or distance metastasis
IVA	Invasion of the bladder mucosa and/or the intestinal/bowel mucosa
IVB	Abdominal peritoneal metastasis beyond the pelvis
IVC	Distant metastasis, including metastasis to any extra-or intra-abdominal lymph nodes above the renal vessels, lungs, liver, brain, or bone

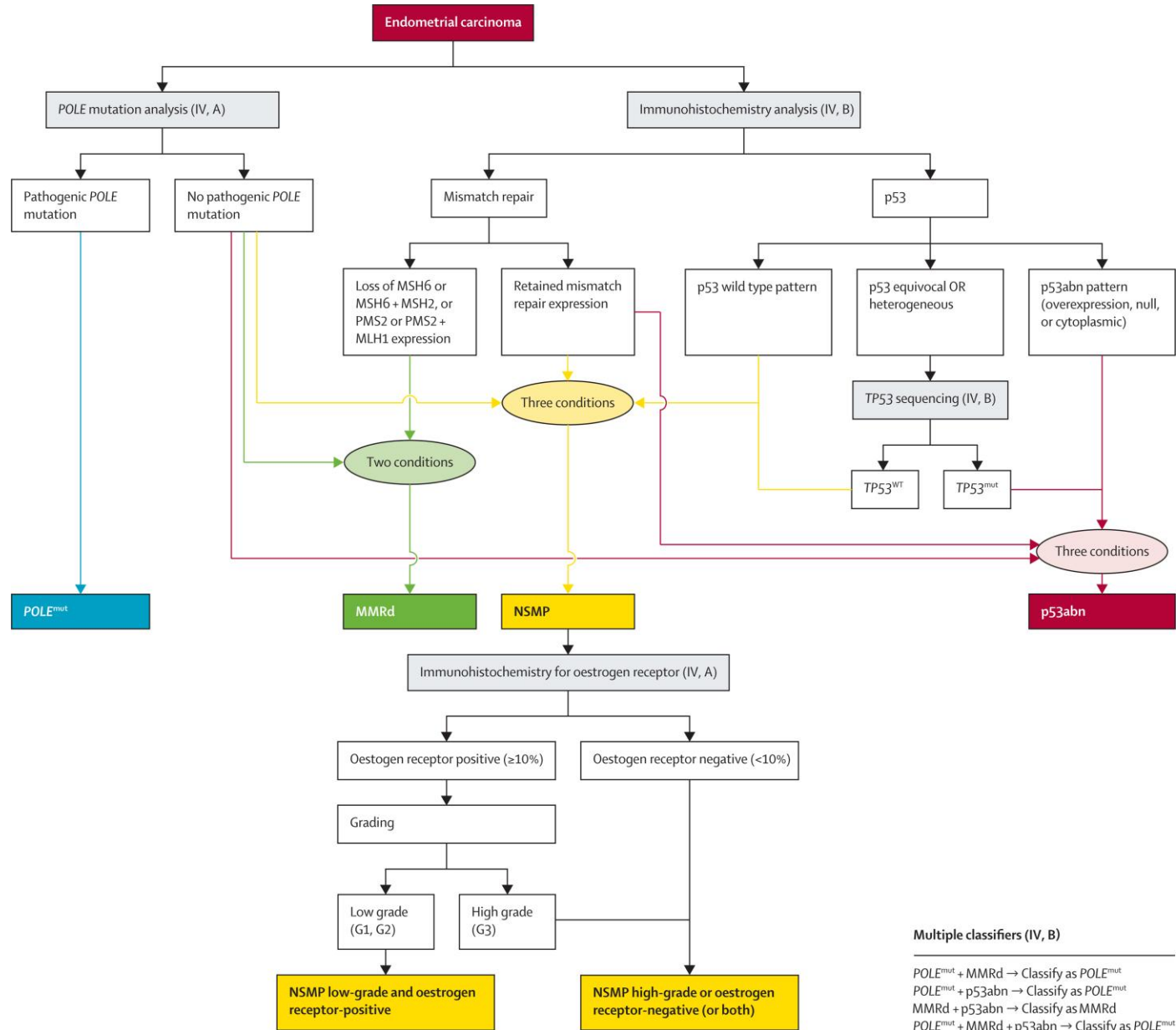
- **Non-aggressive:** Grade 1-2 endometrioid carcinomas.
- **Aggressive:** Grade 3 endometrioid, serous, clear cell, undifferentiated, mixed, mesonephric-like, gastrointestinal mucinous type carcinomas, carcinosarcomas

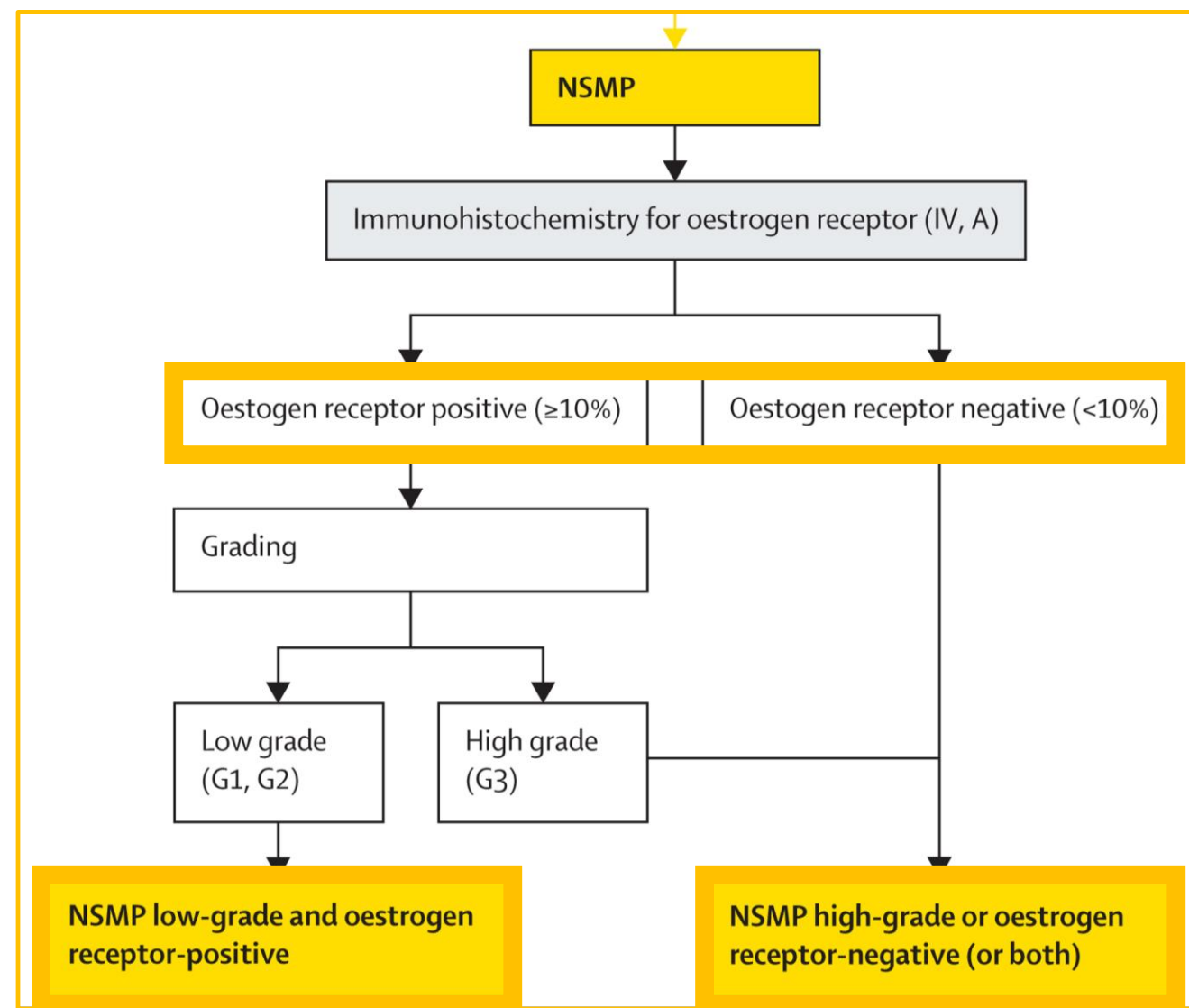
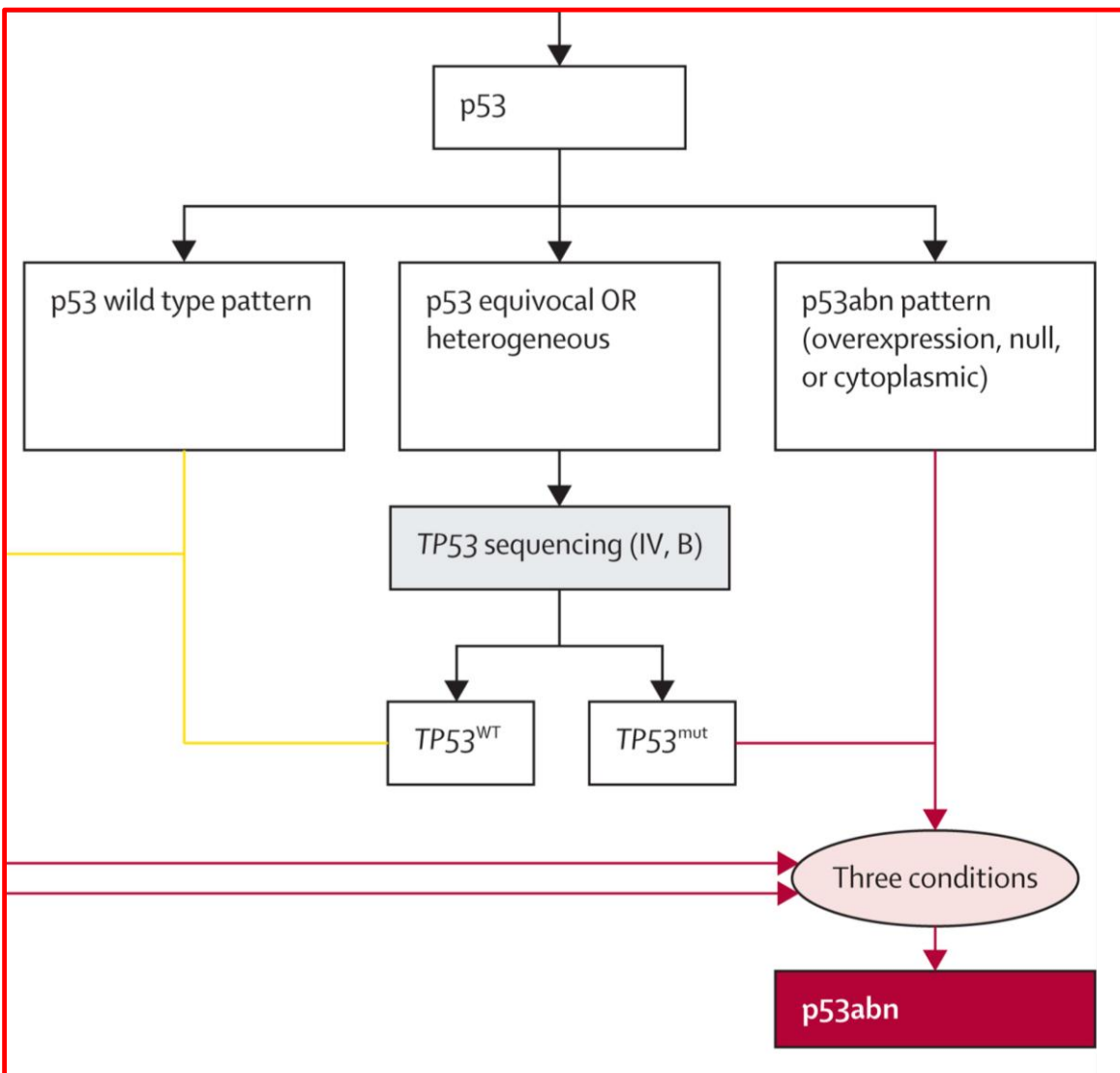
- The performance of complete molecular classification is encouraged in all endometrial cancers.
- If the molecular subtype is known, this is recorded in the FIGO stage by the addition of “m” for molecular classification, and a subscript indicating the specific molecular subtype (e.g. Stage Im_{MMRd}).
- When molecular classification reveals p53abn or *POLE*mut status in Stages I-II, this results in upstaging or downstaging of the disease (IIC_{mp53abn} or IA_{mPOLEmut}).

Table 2. FIGO endometrial cancer stage with molecular classification*

Stage designation	Molecular findings in patients with early endometrial cancer (Stages I and II after surgical staging)
Stage IA _{mPOLEmut}	POLEmut endometrial carcinoma, confined to the uterine corpus or with cervical extension, regardless of the degree of LVSI or histological type
Stage IIC _{mp53abn}	p53abn endometrial carcinoma confined to the uterine corpus with any myometrial invasion, with or without cervical invasion, and regardless of the degree of LVSI or histological type

ESGO-ESTRO-ESP Guidelines (2025 Update)







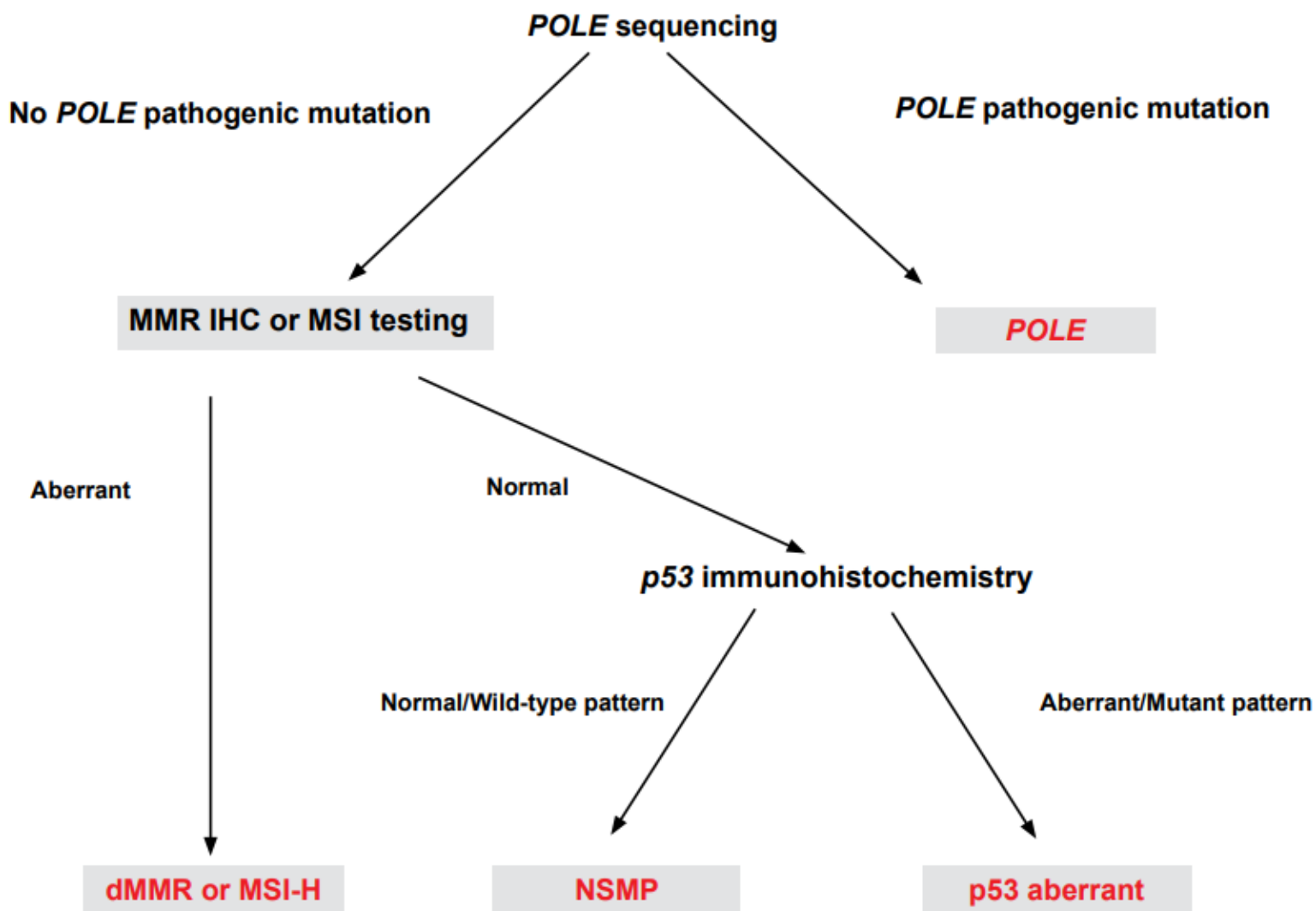
NCCN Guidelines Version 2.2026

Uterine Neoplasms

POLE mutation testing, MMR IHC or MSI testing, and p53 IHC are recommended to complement morphologic assessment regardless of histotype

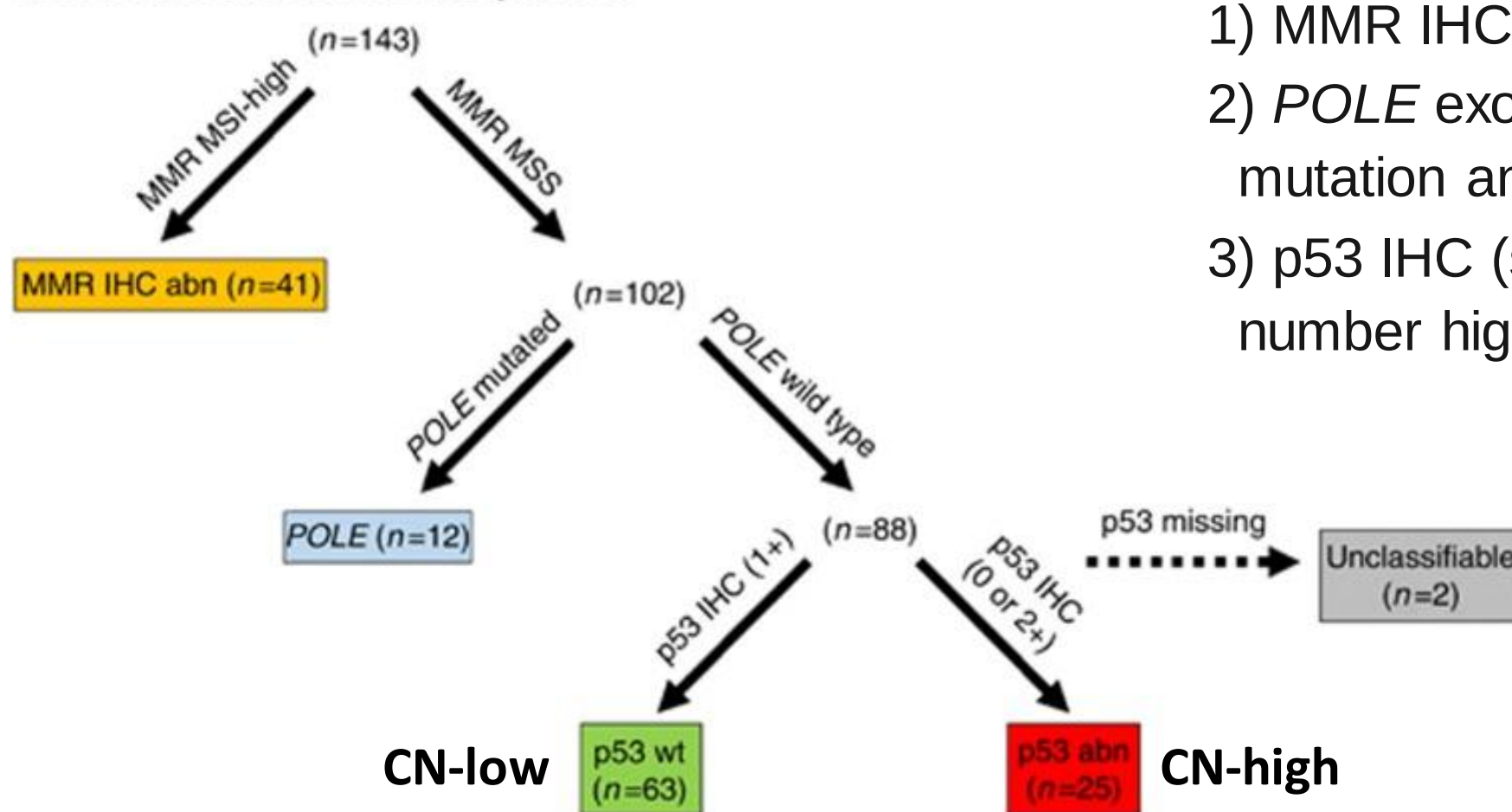
(The decision to use molecular testing/classification depends on the availability of resources and the multidisciplinary team of each center.)

Adapted with permission from PMID: 29439179



Surrogate Panels: **ProMisE** Classifier - Proactive Molecular Risk Classifier for Endometrial Cancer

Model: MMR IHC/*POLE* mut/p53 IHC



- 1) MMR IHC
- 2) *POLE* exonuclease domain mutation analysis
- 3) p53 IHC (surrogate for copy number high status)

PMID: 26172027, 29432521,
36731023, 28061006

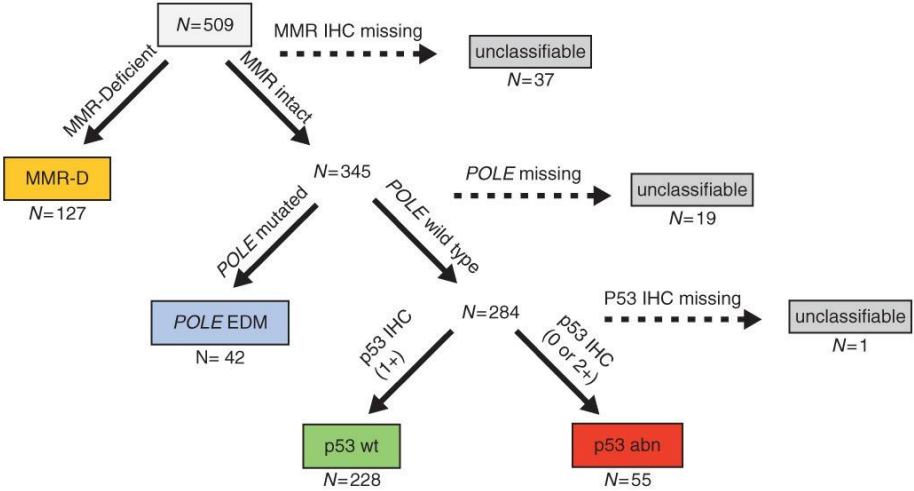
ProMisE has undergone extensive validation

Endorsed by:

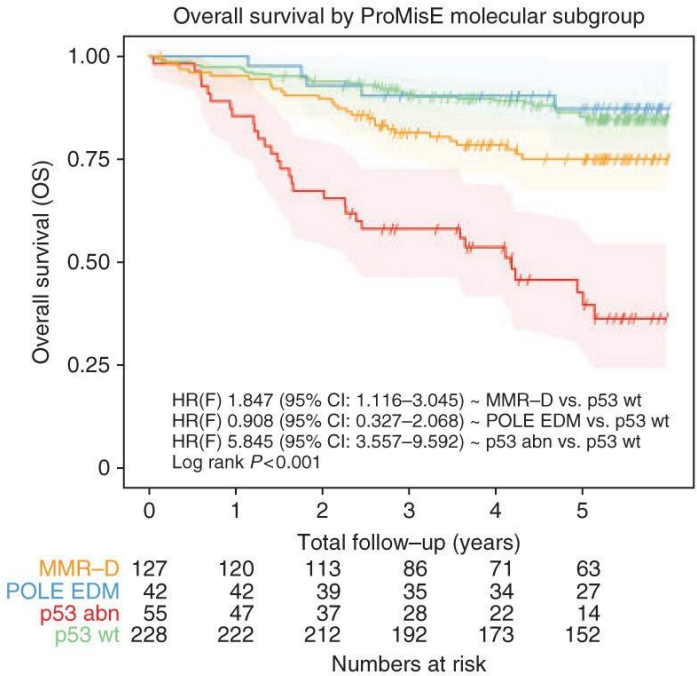
- ✓ ESGO/ESTRO/ESP
- ✓ WHO
- ✓ CAP

PMID: 29432521

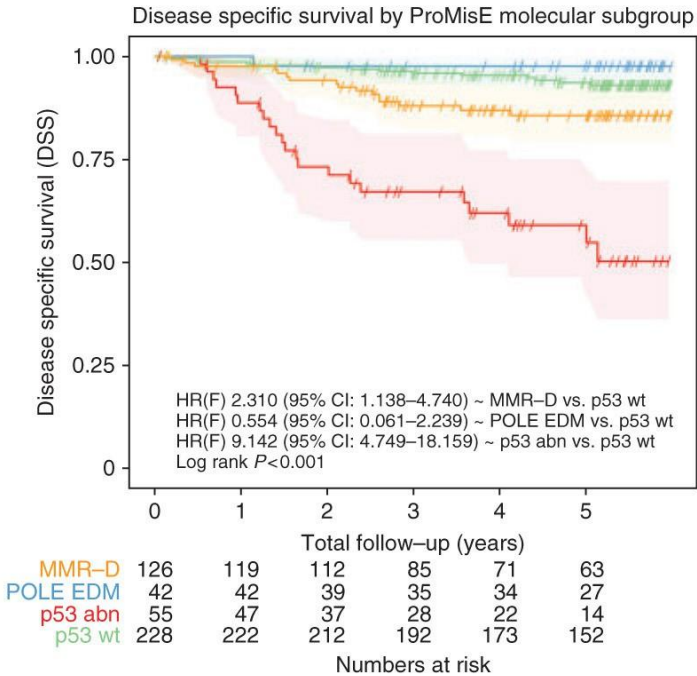
A



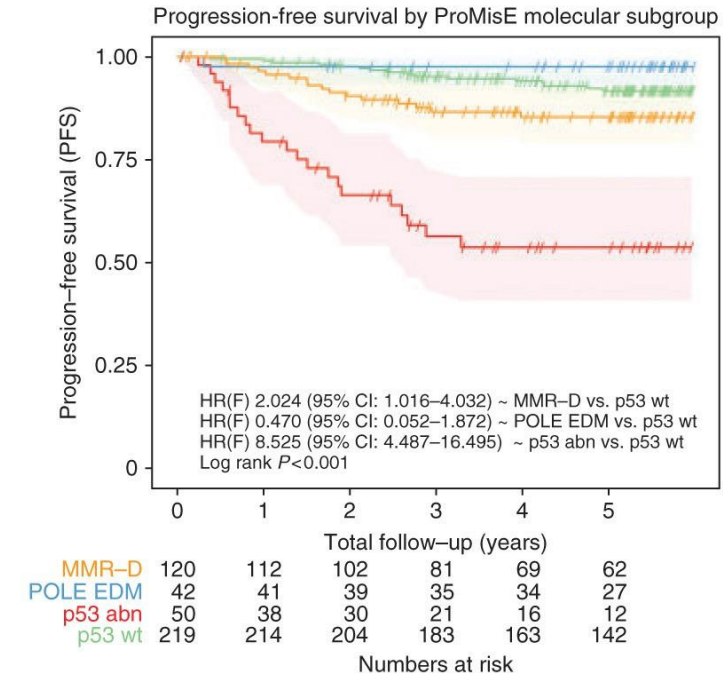
B



C



D



Molecular Classification: Multiple Classifiers

$POLE^{mut} + MMRd \rightarrow$ Classify as $POLE^{mut}$

$POLE^{mut} + p53abn \rightarrow$ Classify as $POLE^{mut}$

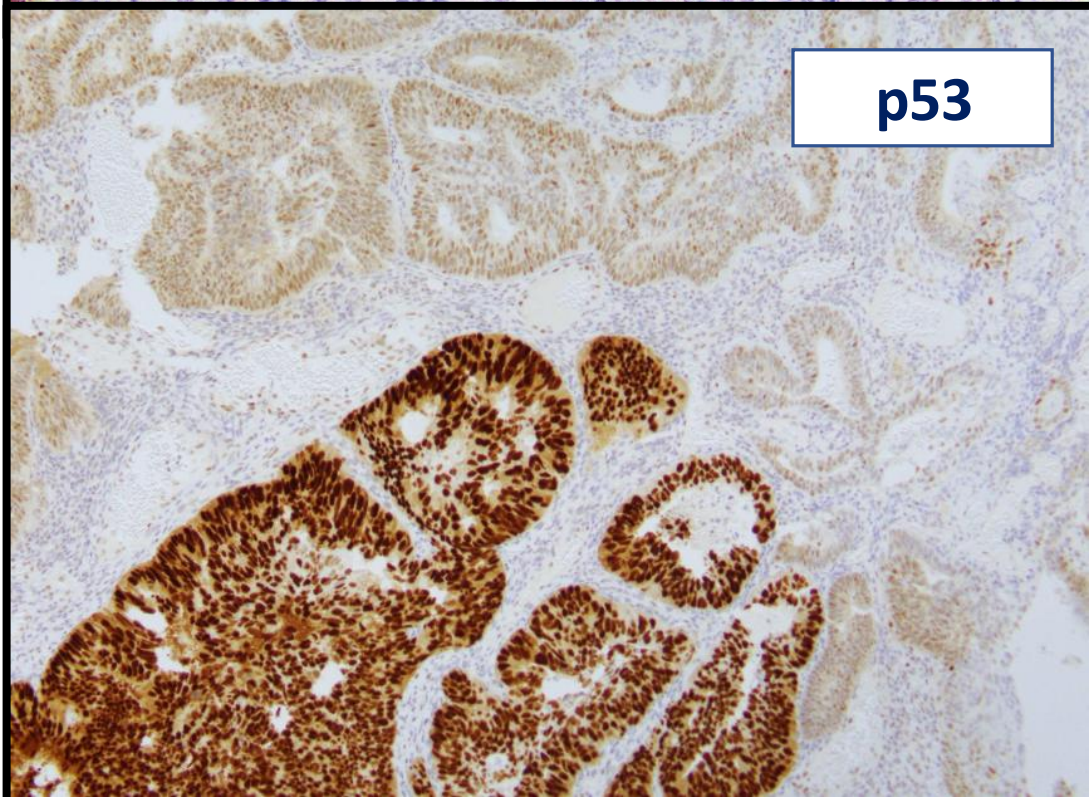
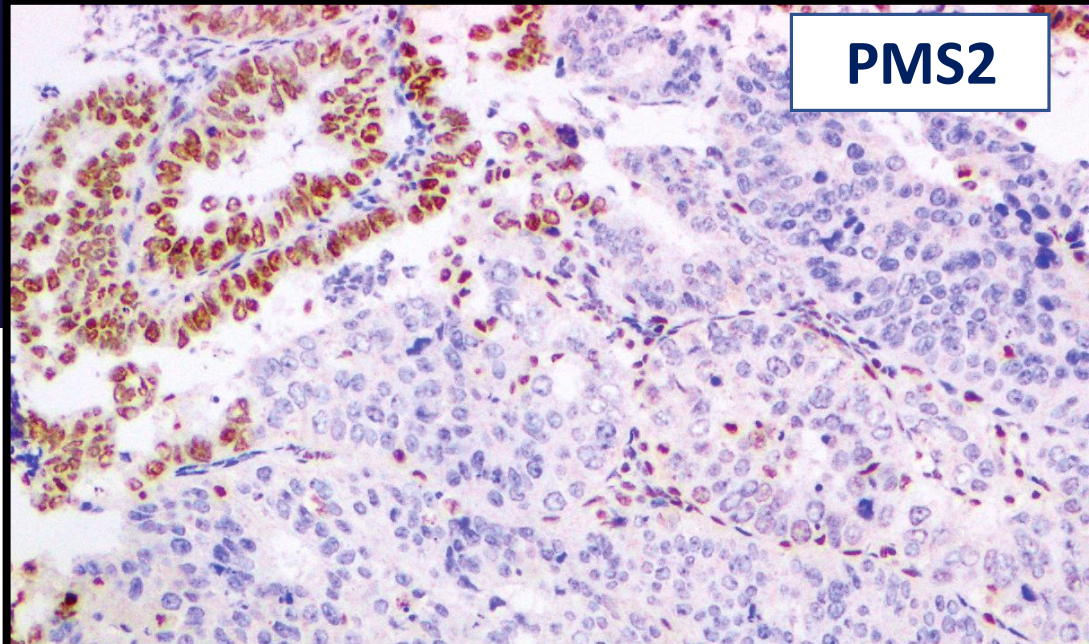
$MMRd + p53abn \rightarrow$ Classify as $MMRd$

$POLE^{mut} + MMRd + p53abn \rightarrow$ Classify as $POLE^{mut}$

- **Subclonal abnormal expression:**

- ✓ $p53 \leftarrow$ MMR-deficiency or $POLE$ mutation
- ✓ $MMR \leftarrow POLE$ mutation

PMID: 36866757, 35920830, 40744042



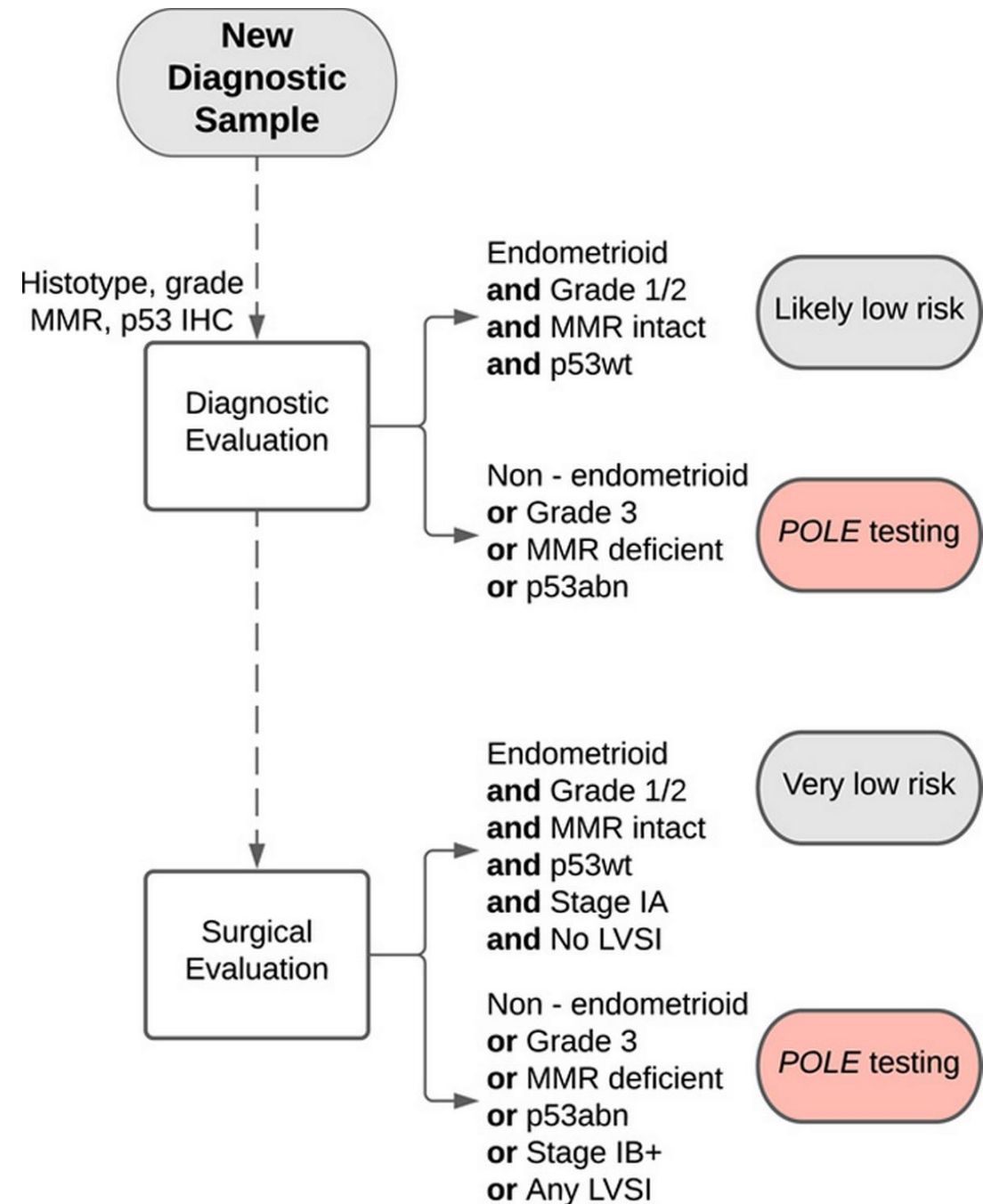
Do We Need to Test Every Patient?

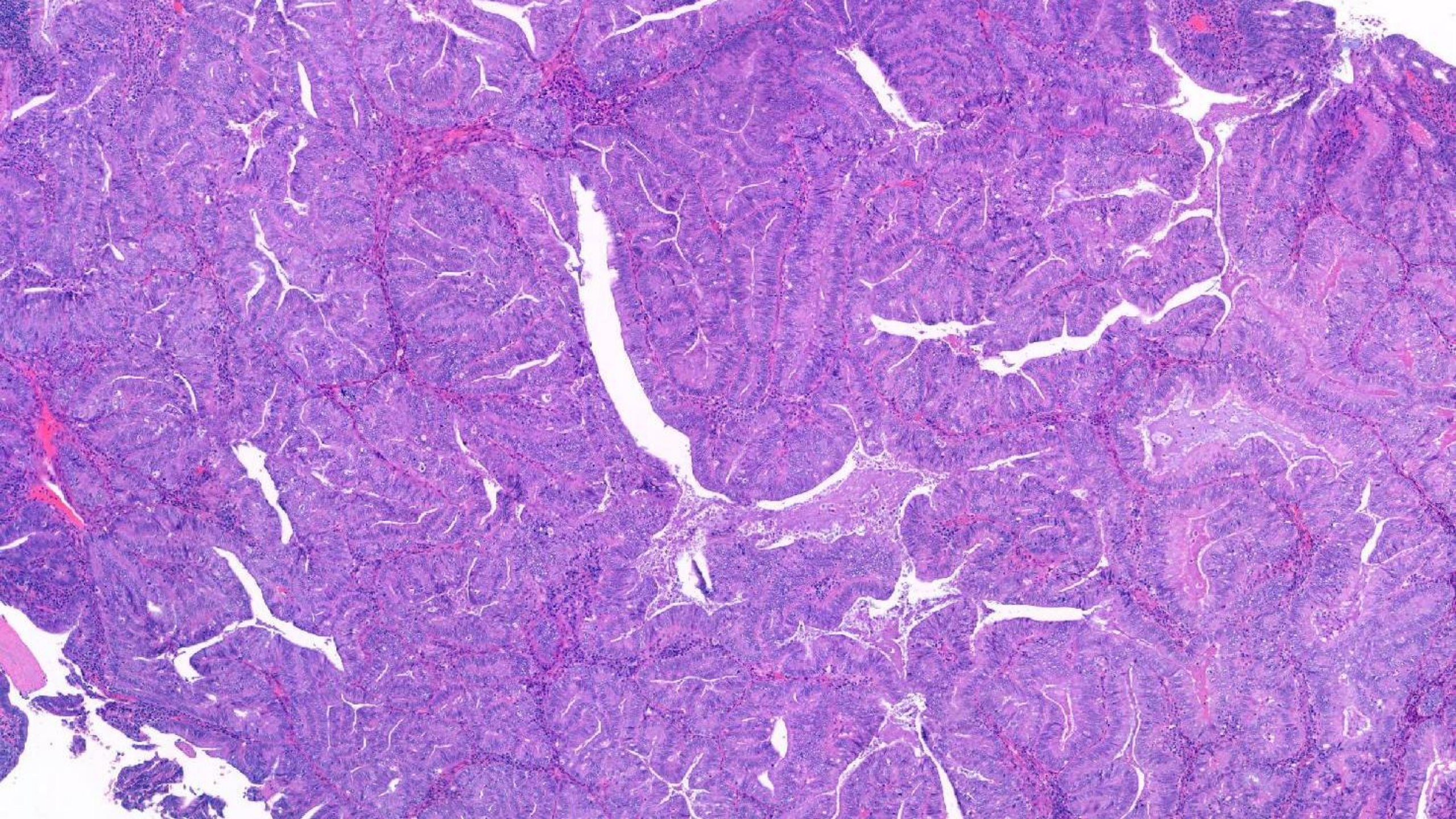
Targeted Molecular Testing

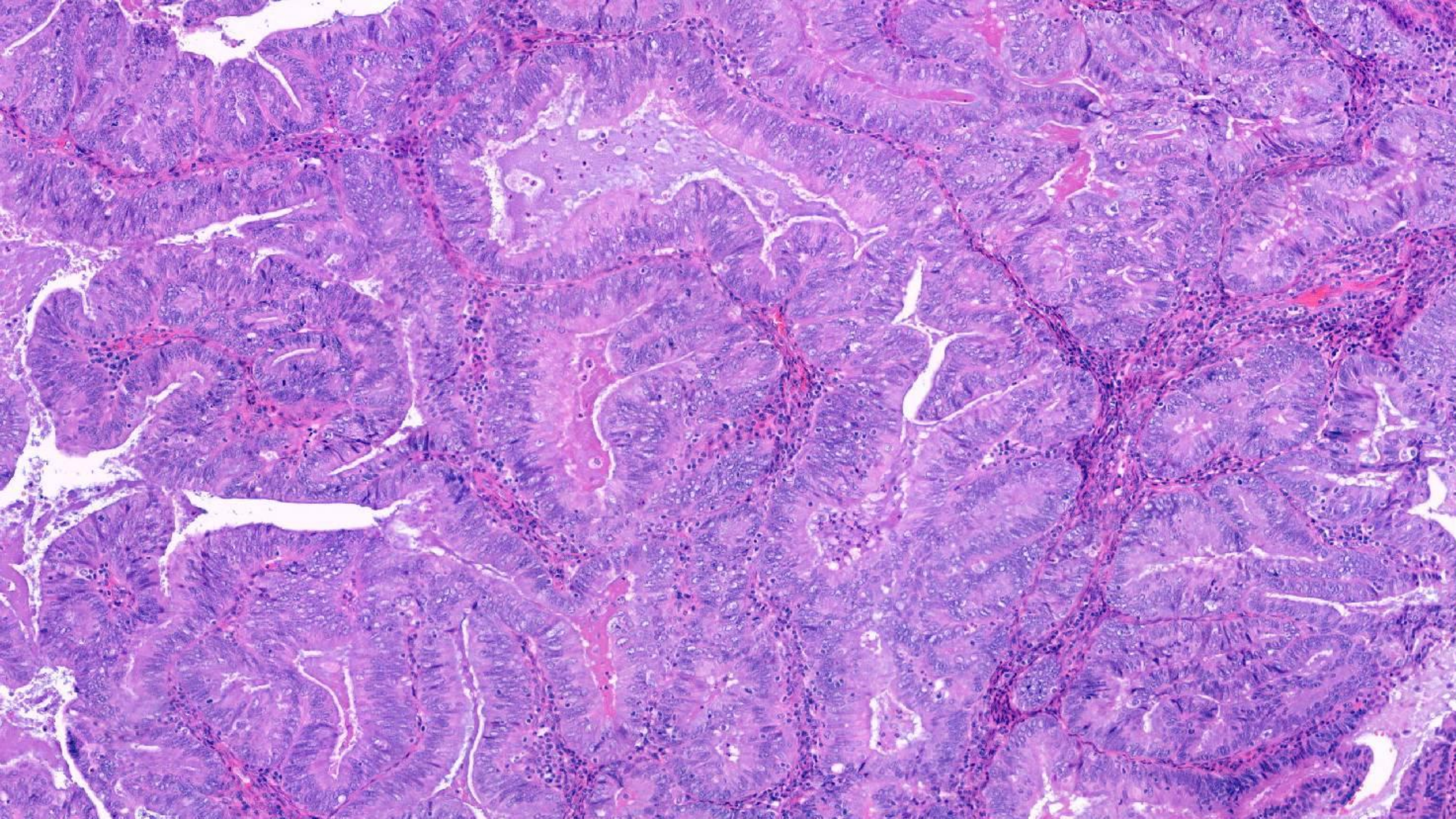
Selected ProMisE

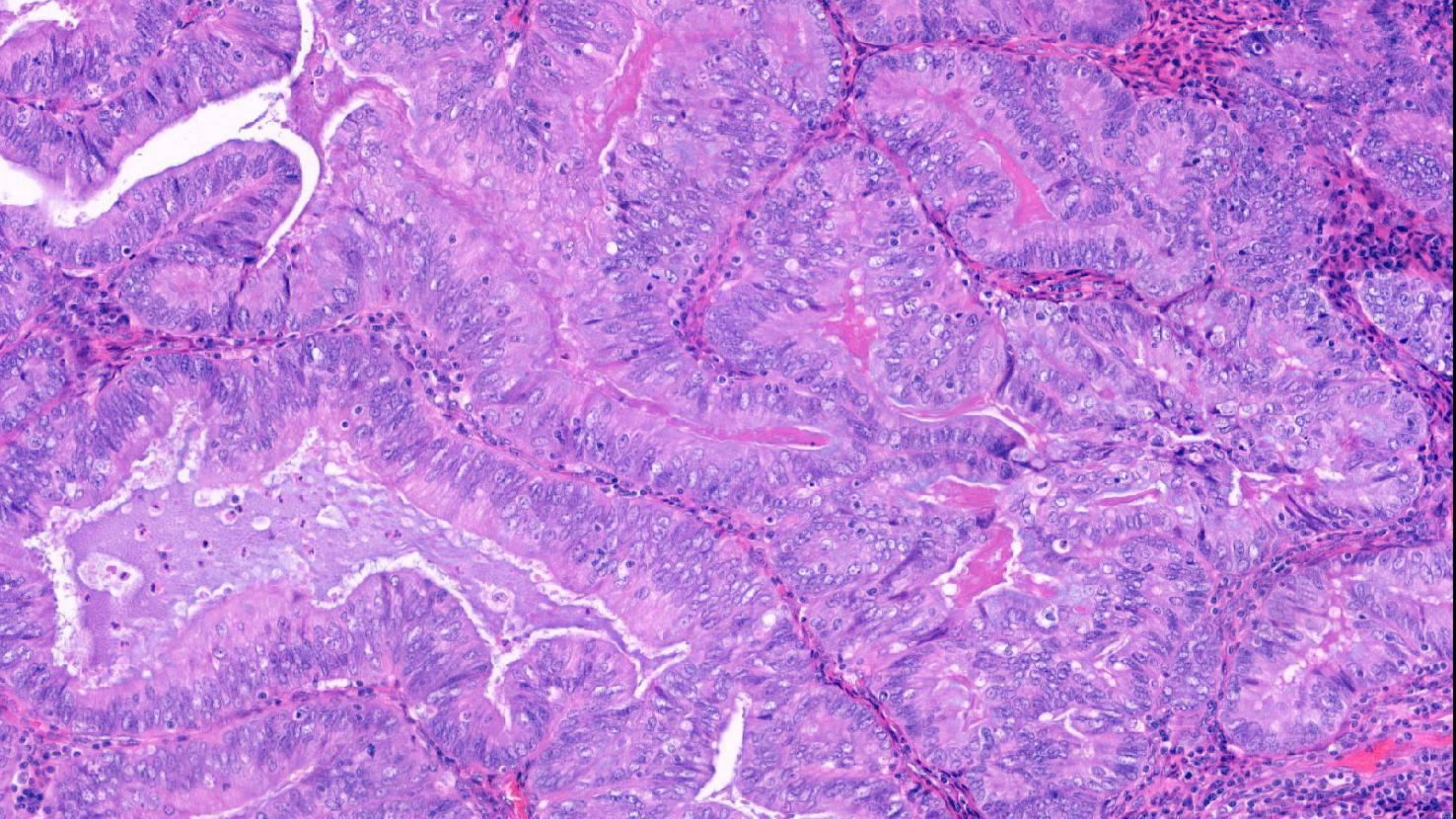
- ✓ The restricted testing protocol requiring the evaluation of histotype, grade, MMR and p53 status
- ✓ Full ProMisE in patients with >stage IA and with any LVSI post-surgically

PMID: 36731023

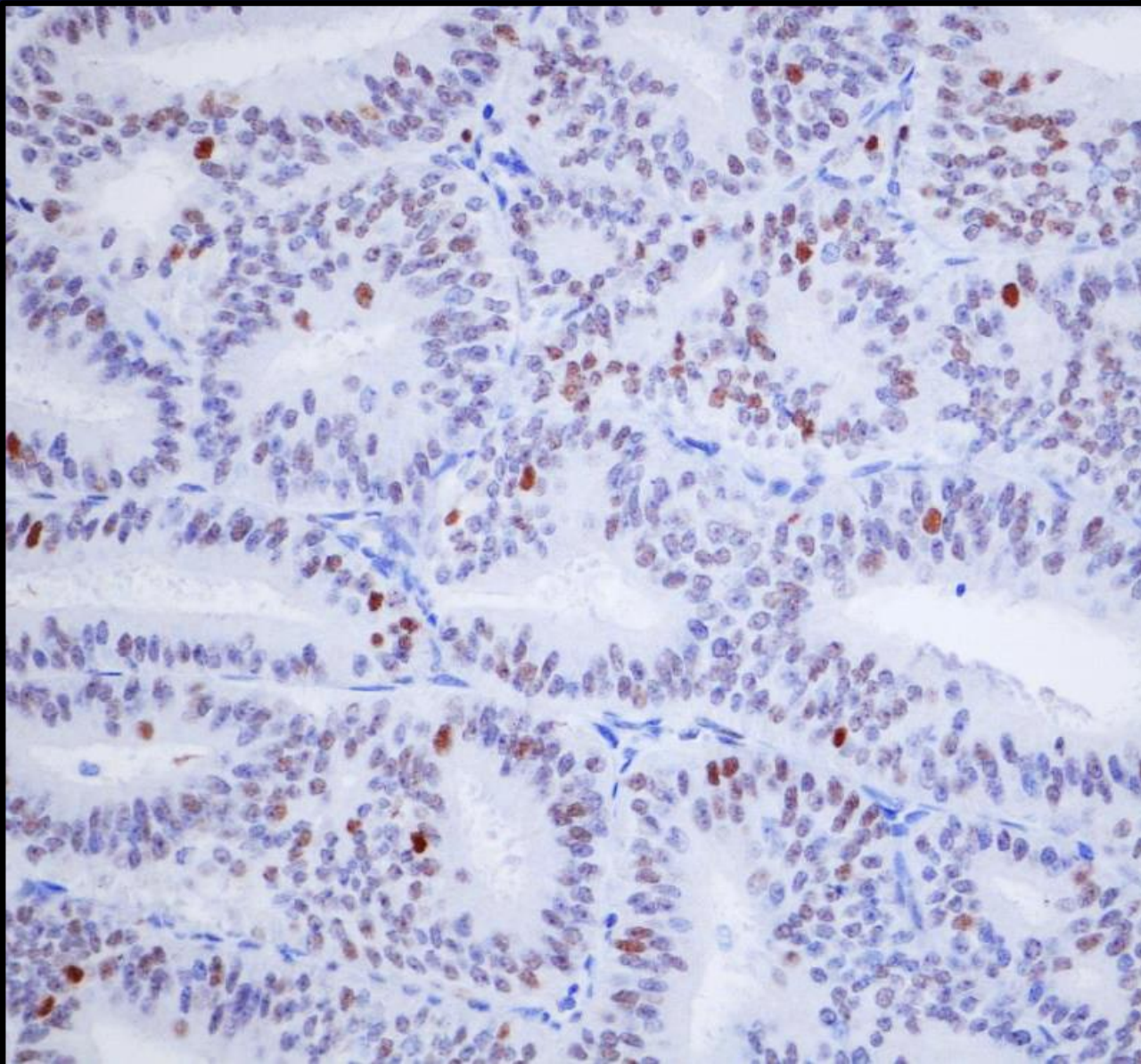




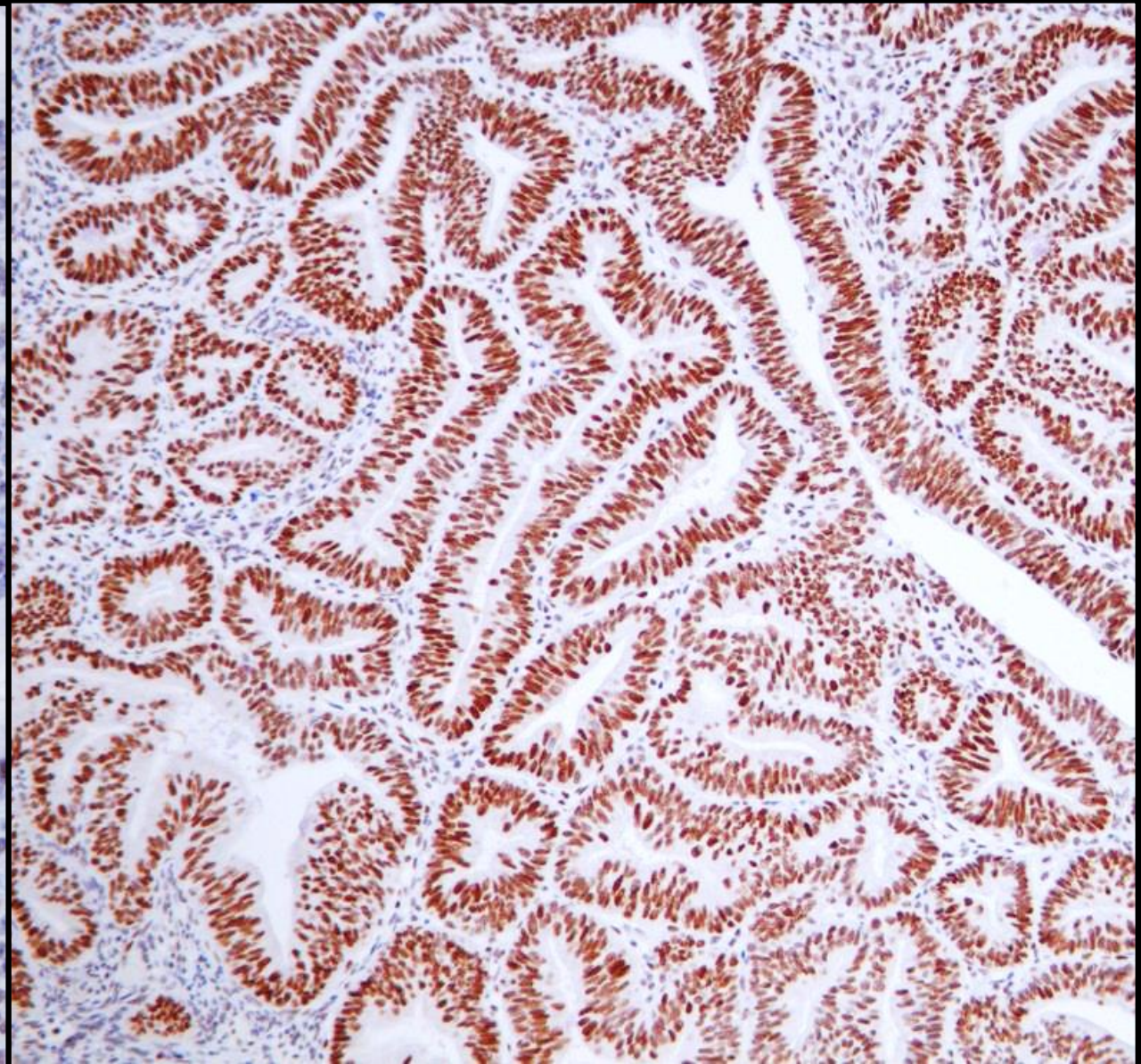




Wild-Type p53



Intact MMR



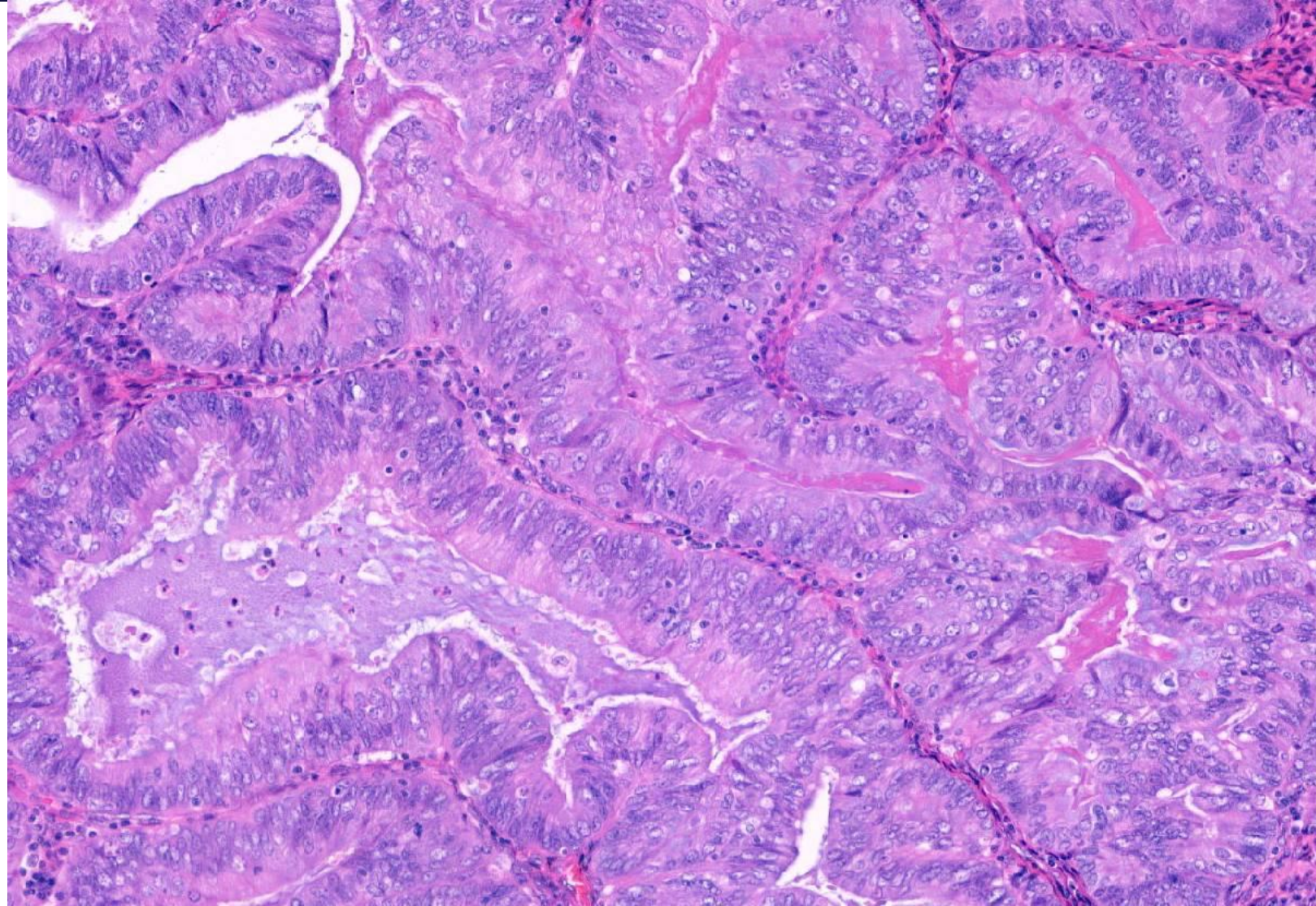
Molecular Subtypes by Histology: Copy Number Low

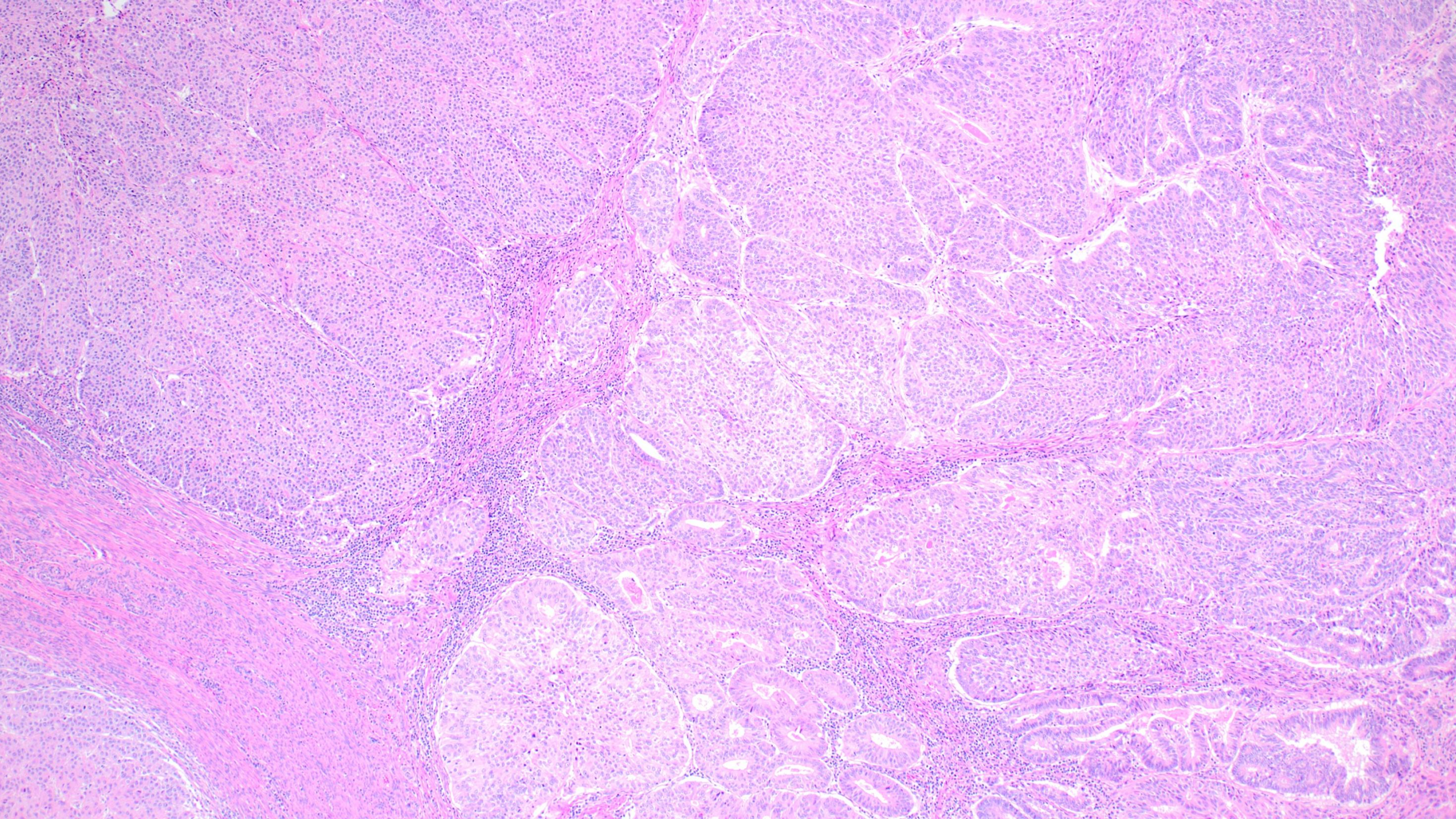
- **Key features:**

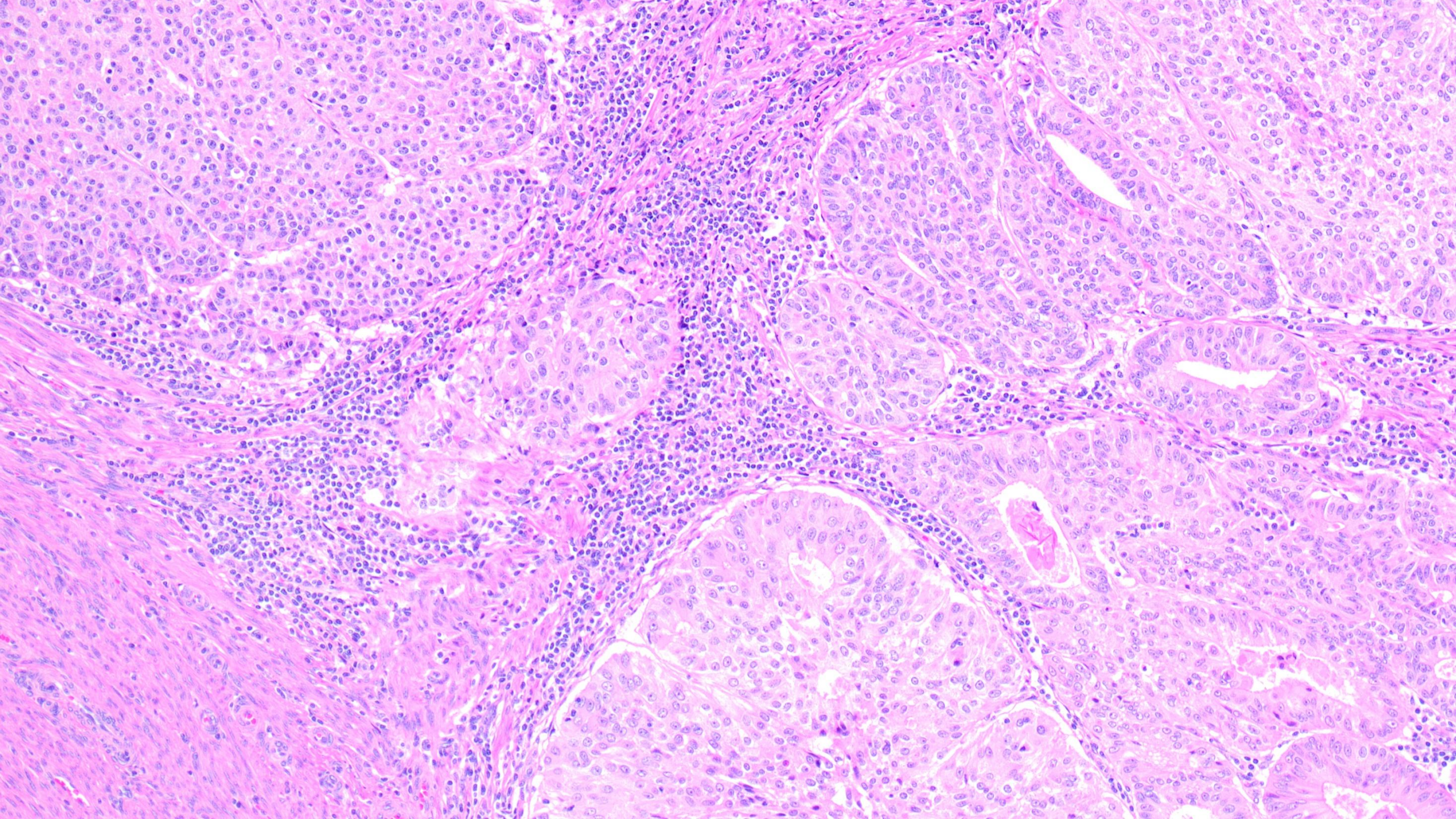
- ✓ P53 wild-type
- ✓ MMR-proficient
- ✓ POLE wild-type

- **Histotypes:**

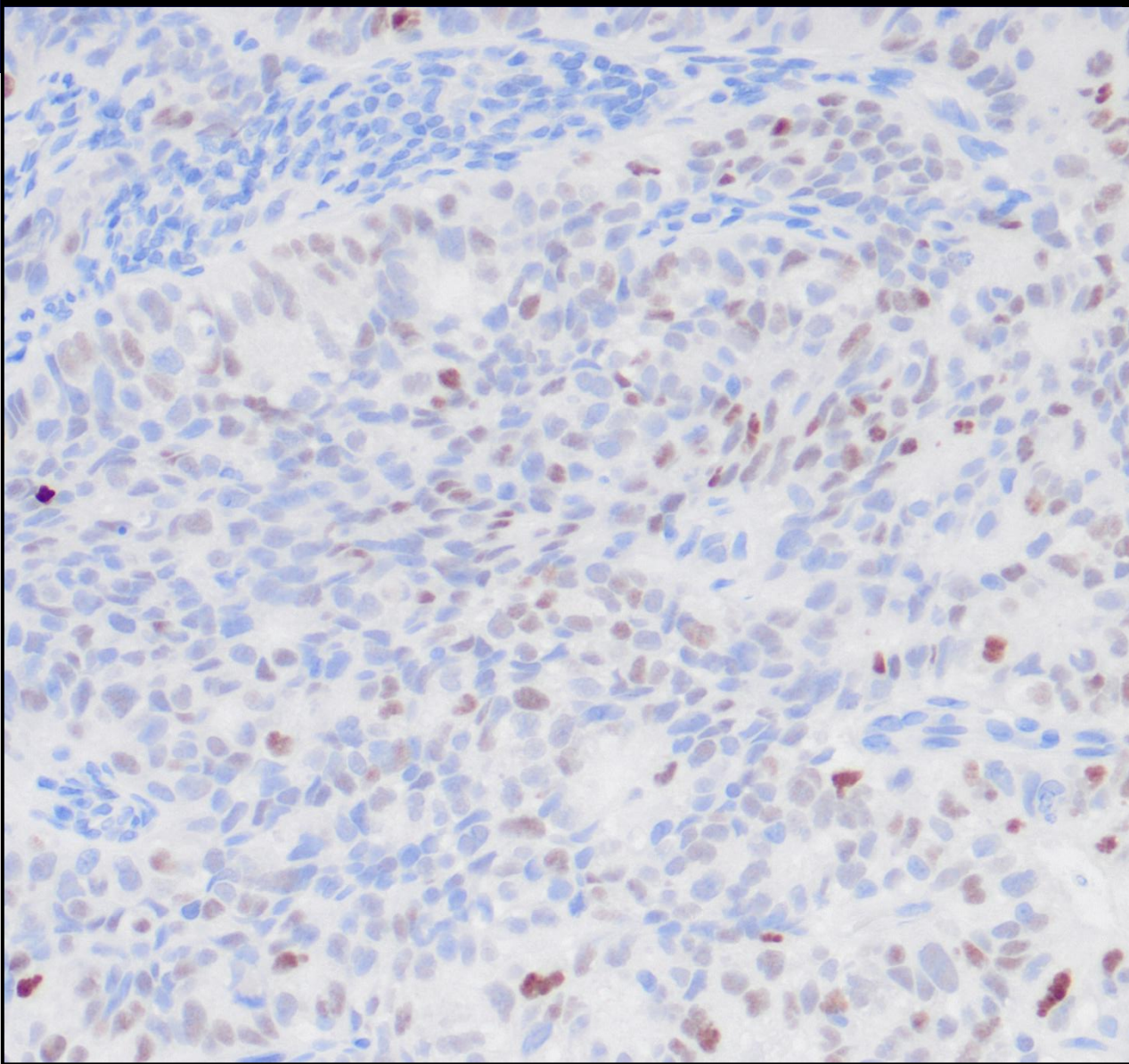
- ✓ Endometrioid
- ✓ Clear cell carcinoma
- ✓ Carcinosarcoma
- ✓ Undifferentiated carcinoma
- ✓ Mixed



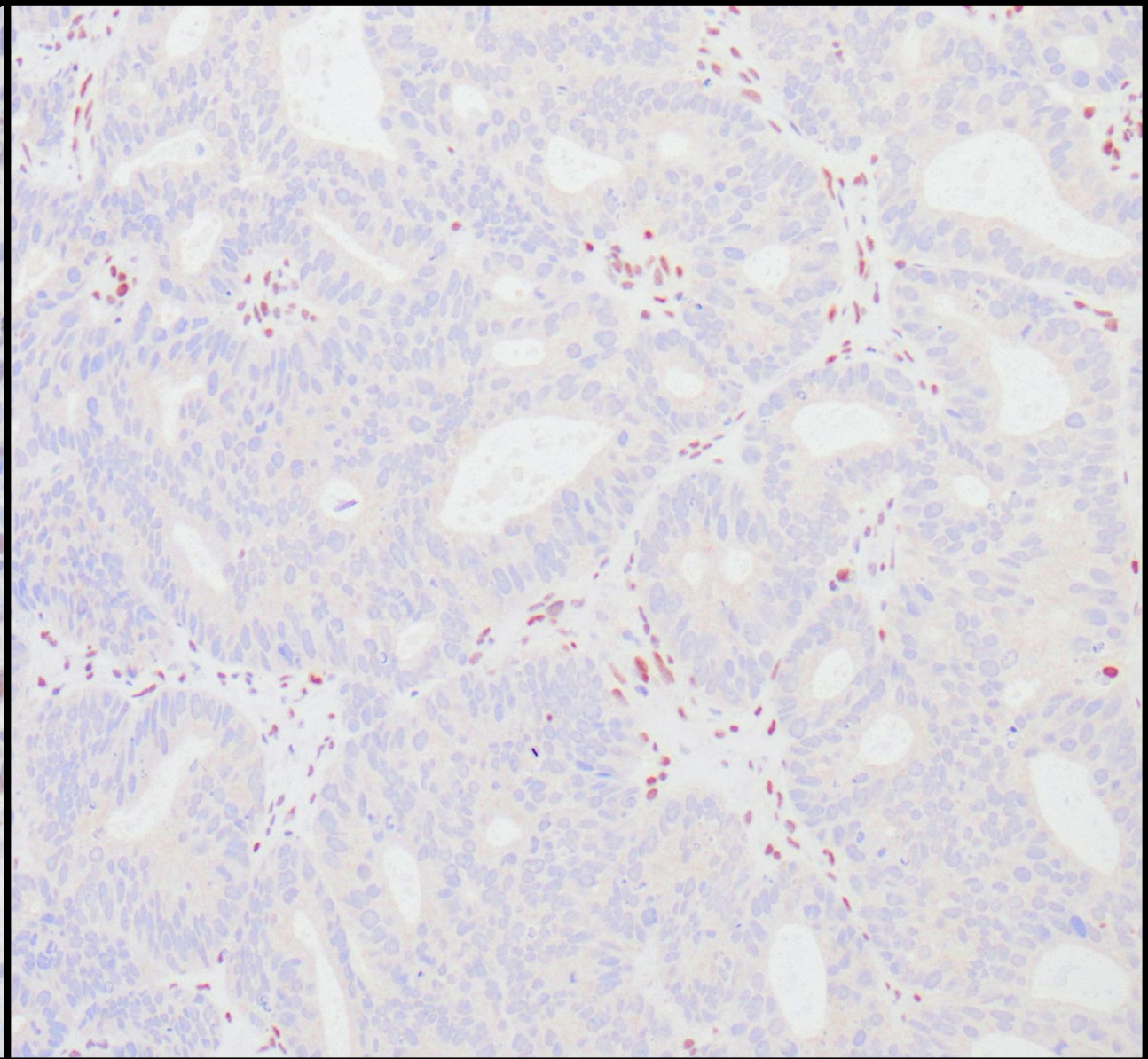




Wild-Type p53



Loss of MLH1/ PMS2



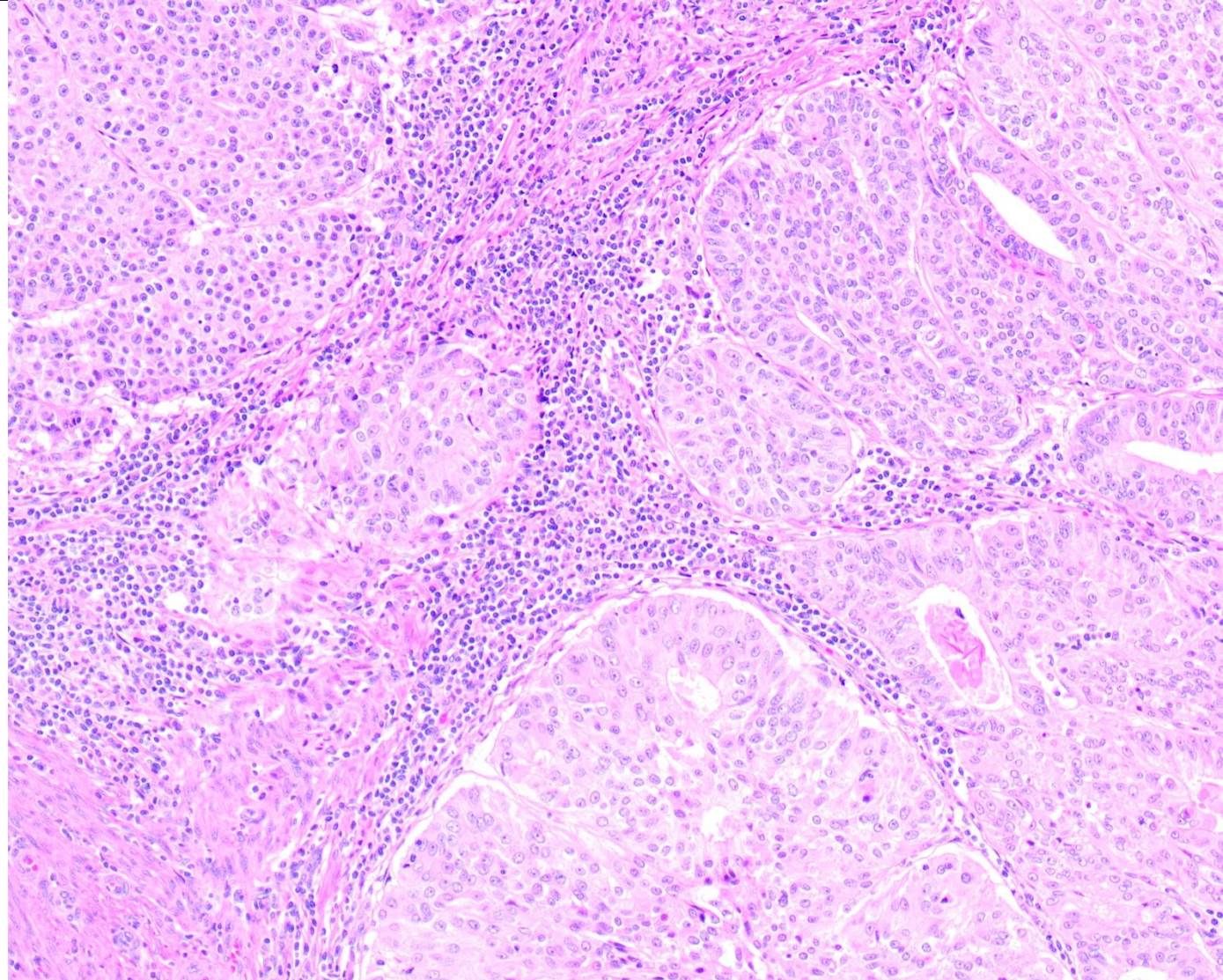
Molecular Subtypes by Histology: MSI-High

- **Key features:**

- ✓ MMR-deficient
- ✓ P53 wild-type
- ✓ *POLE* wild-type

- **Morphology:**

- ✓ Heterogeneity
- ✓ Tumor infiltrating lymphocytes
- ✓ Peritumoral lymphocytes



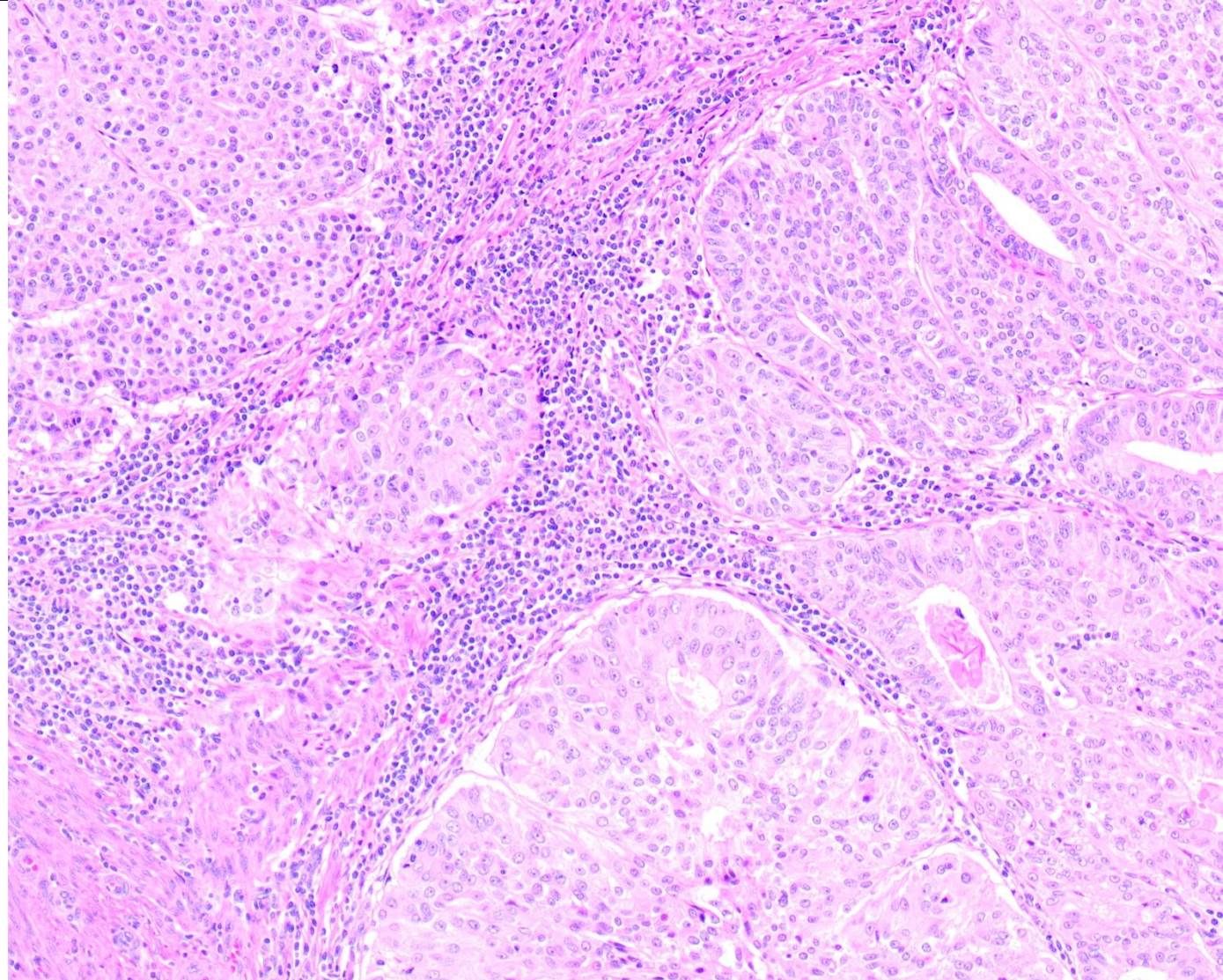
Molecular Subtypes by Histology: MSI-High

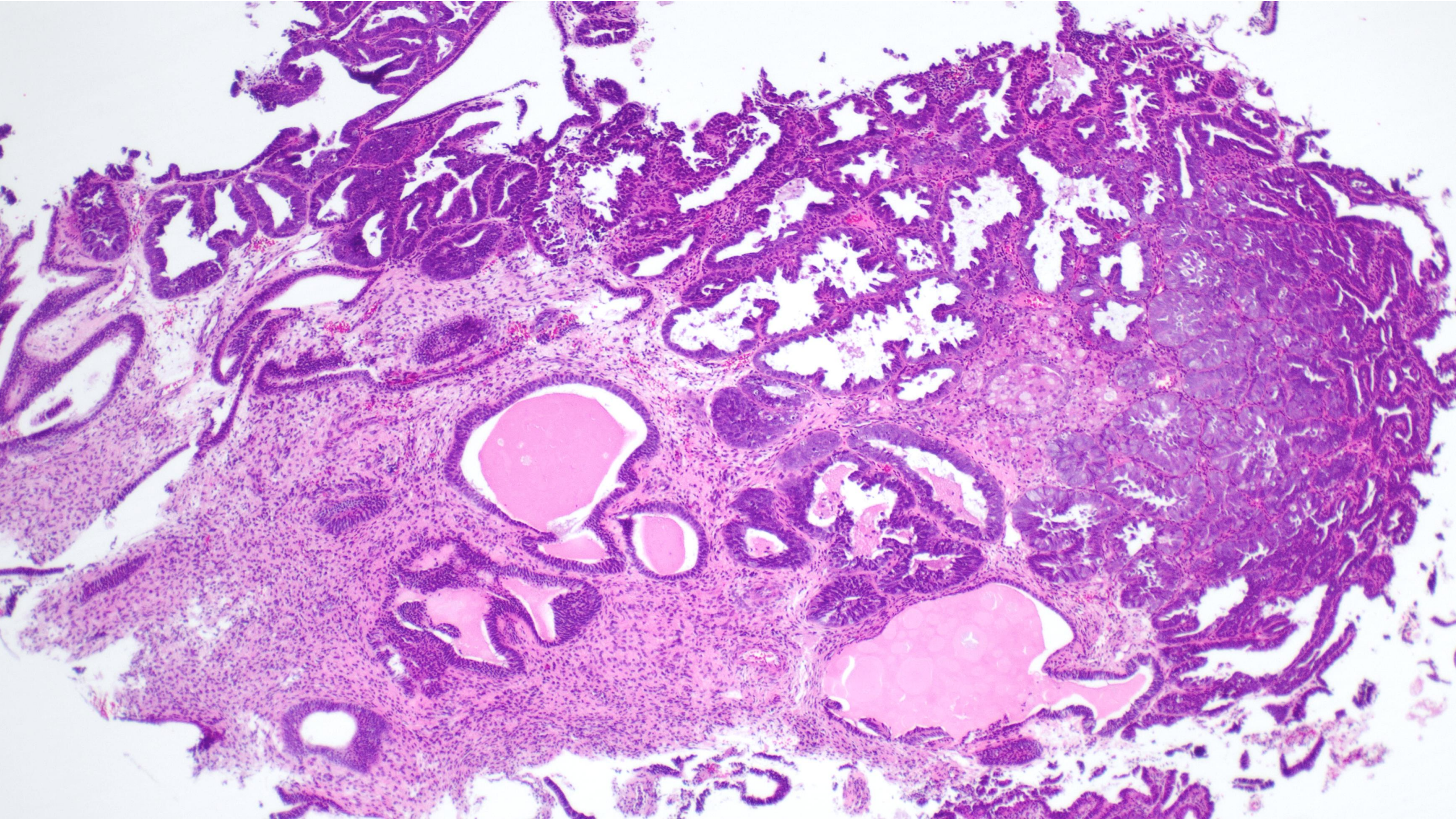
- **Key features:**

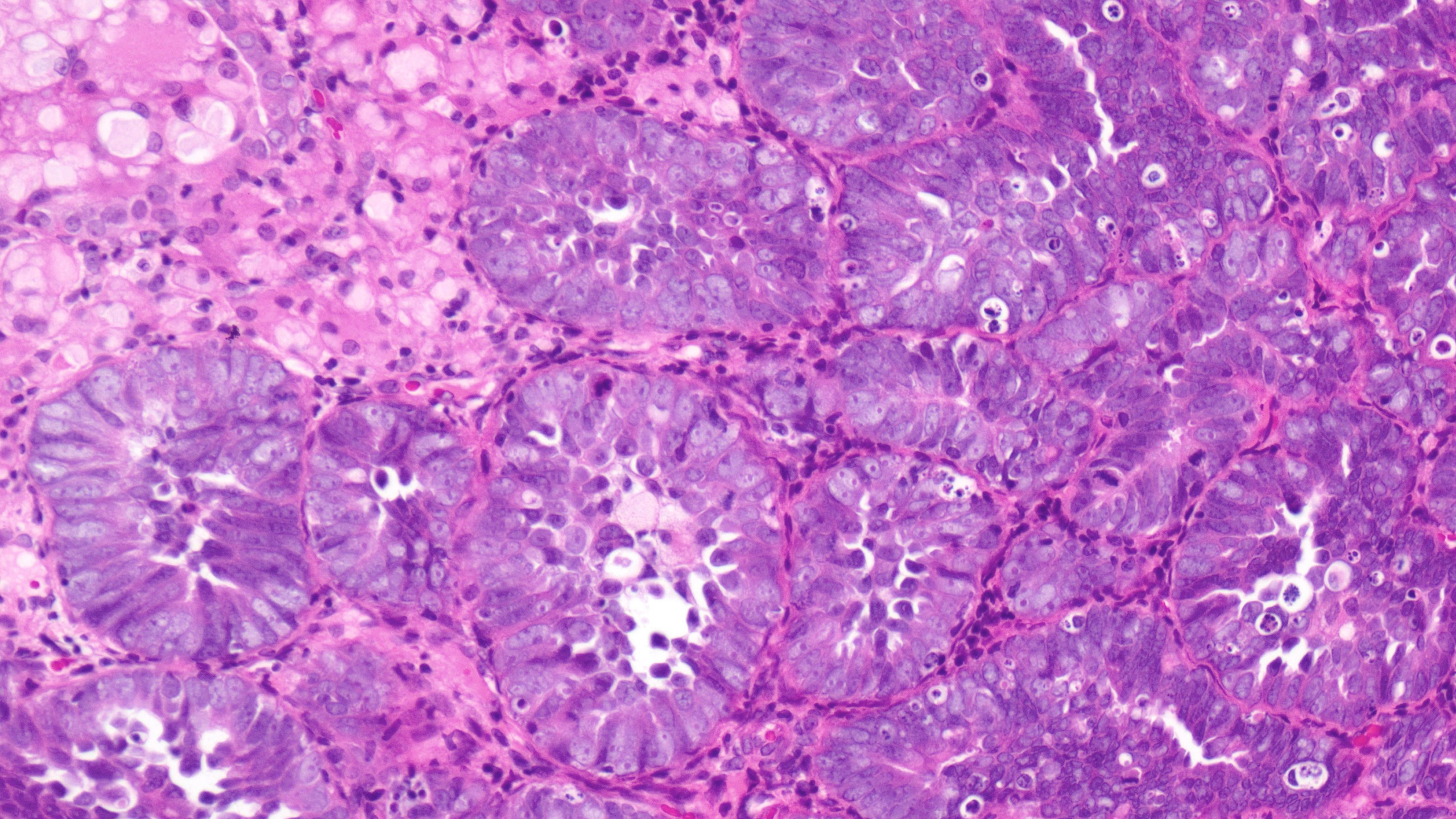
- ✓ MMR-deficient
- ✓ P53 wild-type
- ✓ *POLE* wild-type

- **Histotypes:**

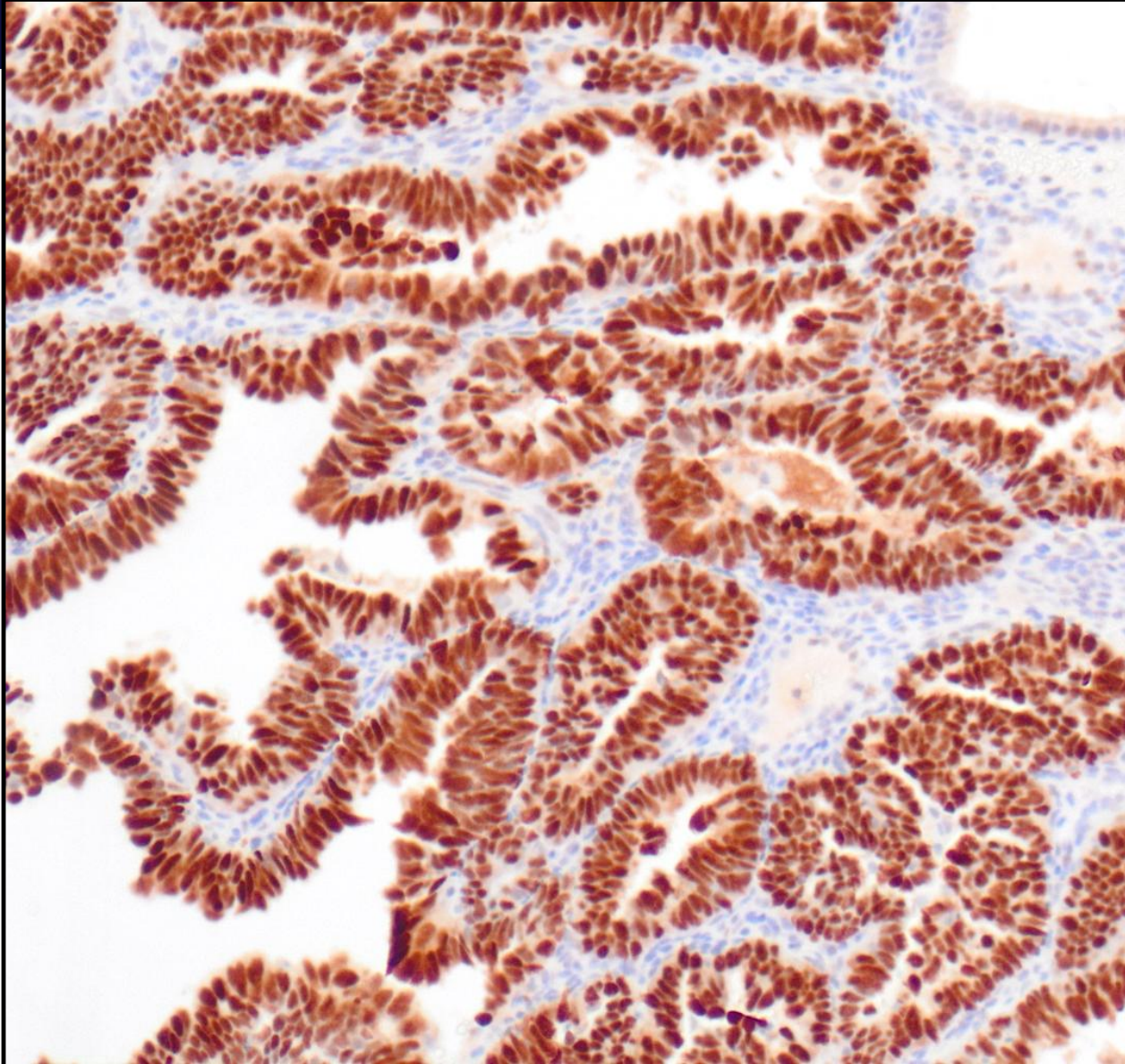
- ✓ Endometrioid carcinoma
- ✓ Undifferentiated carcinoma
- ✓ Clear cell carcinoma
- ✓ Carcinosarcoma



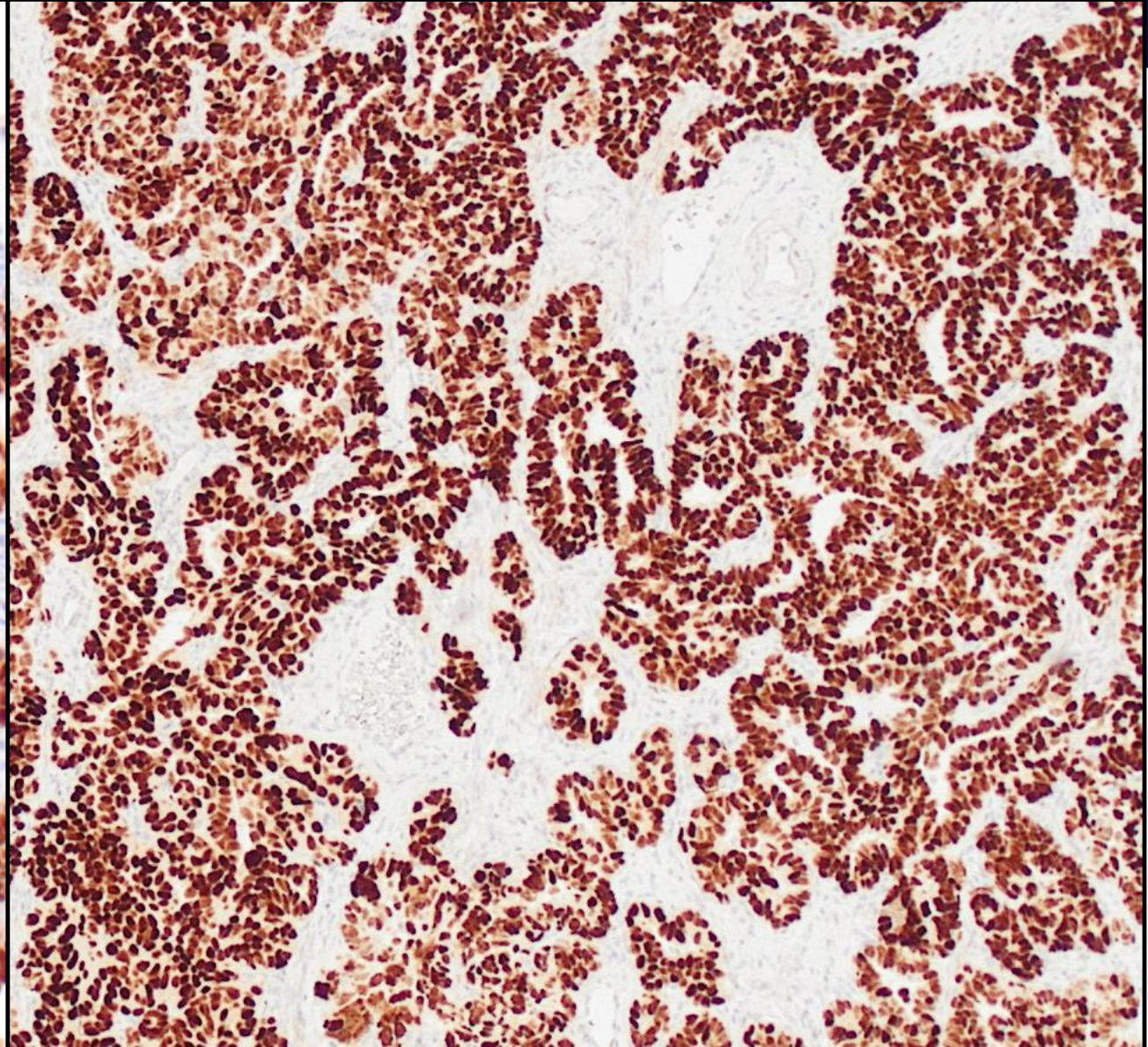




Abnormal p53



Intact MMR



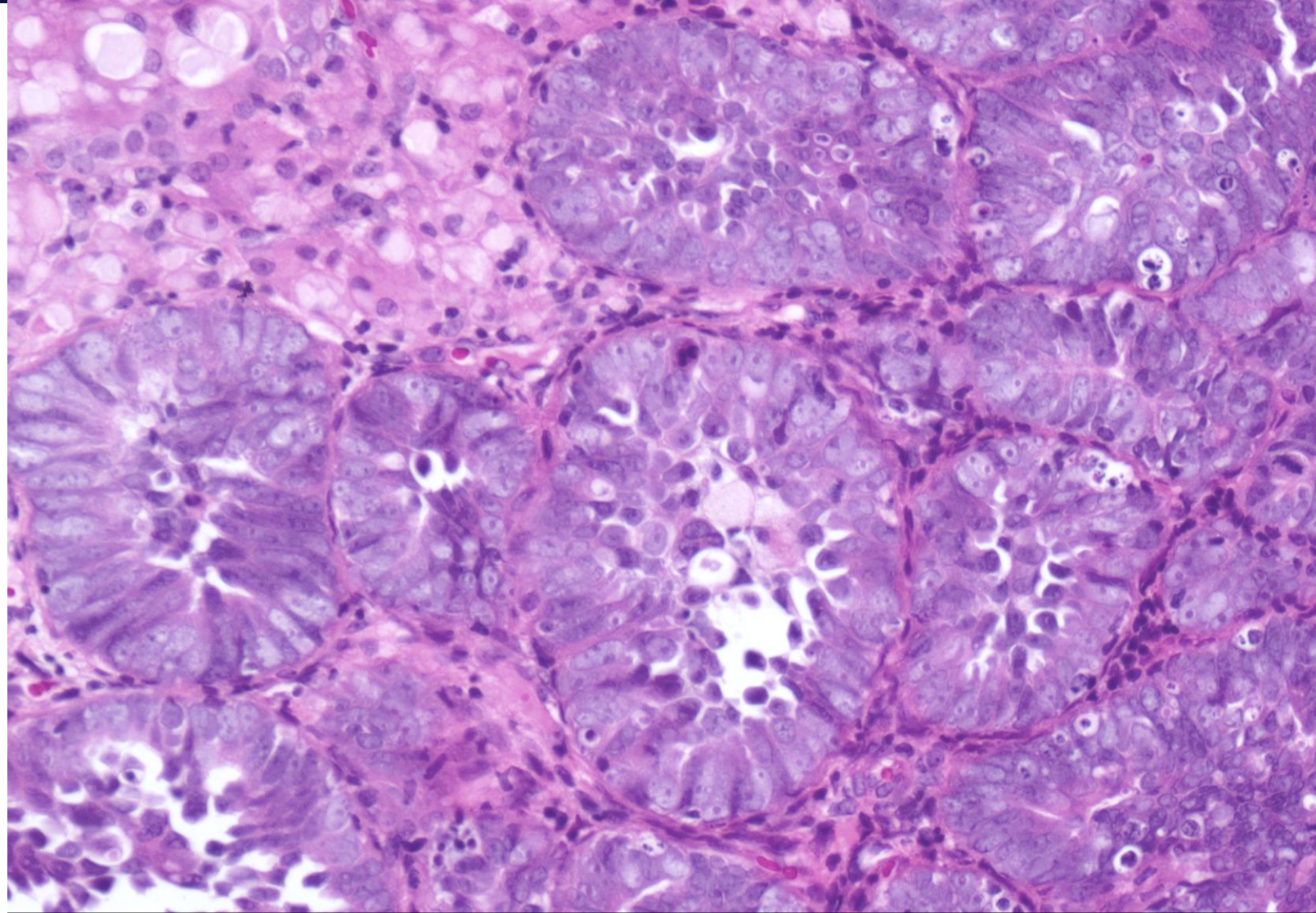
Molecular Subtypes by Histology: Copy Number High

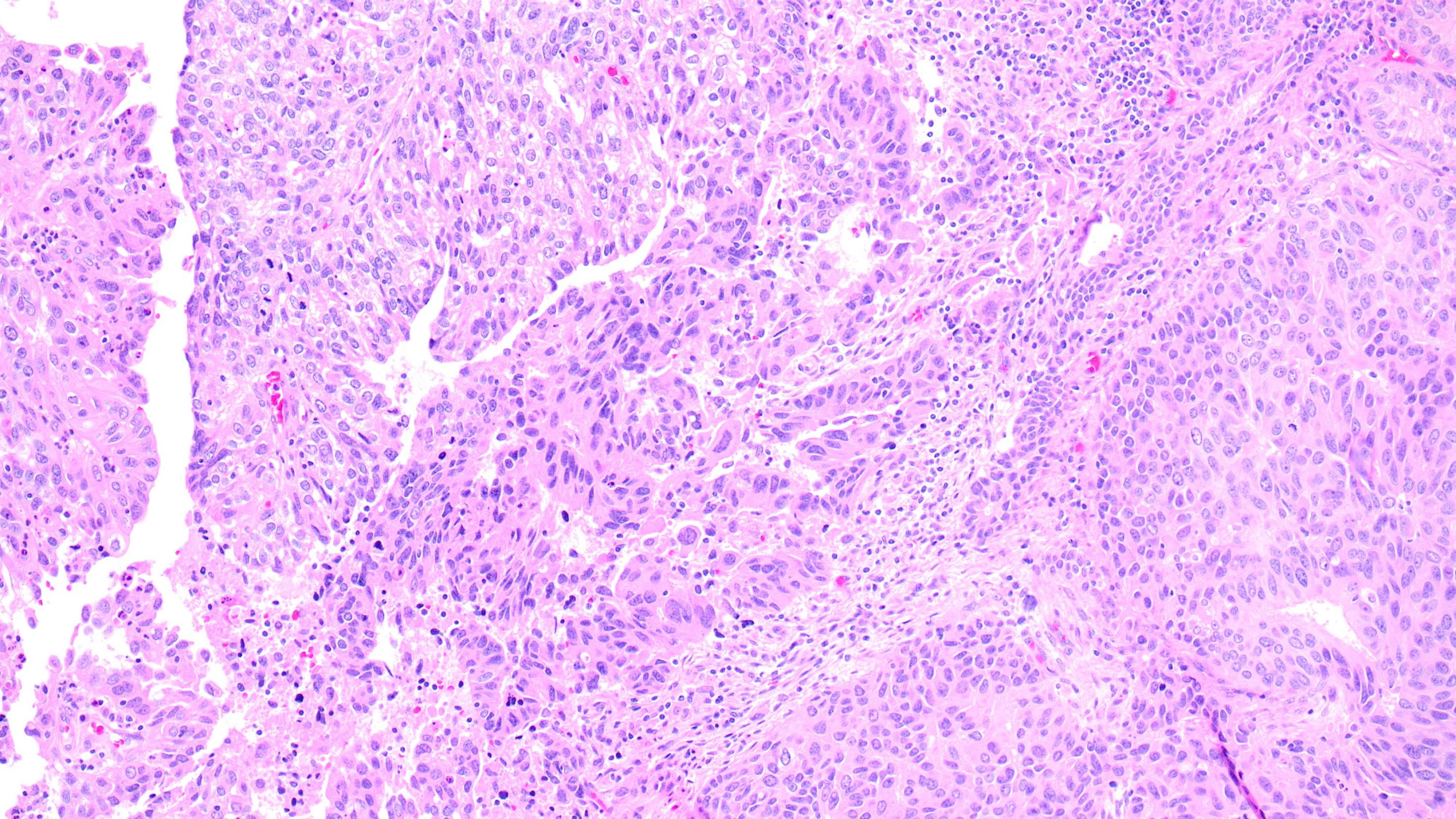
- **Key features:**

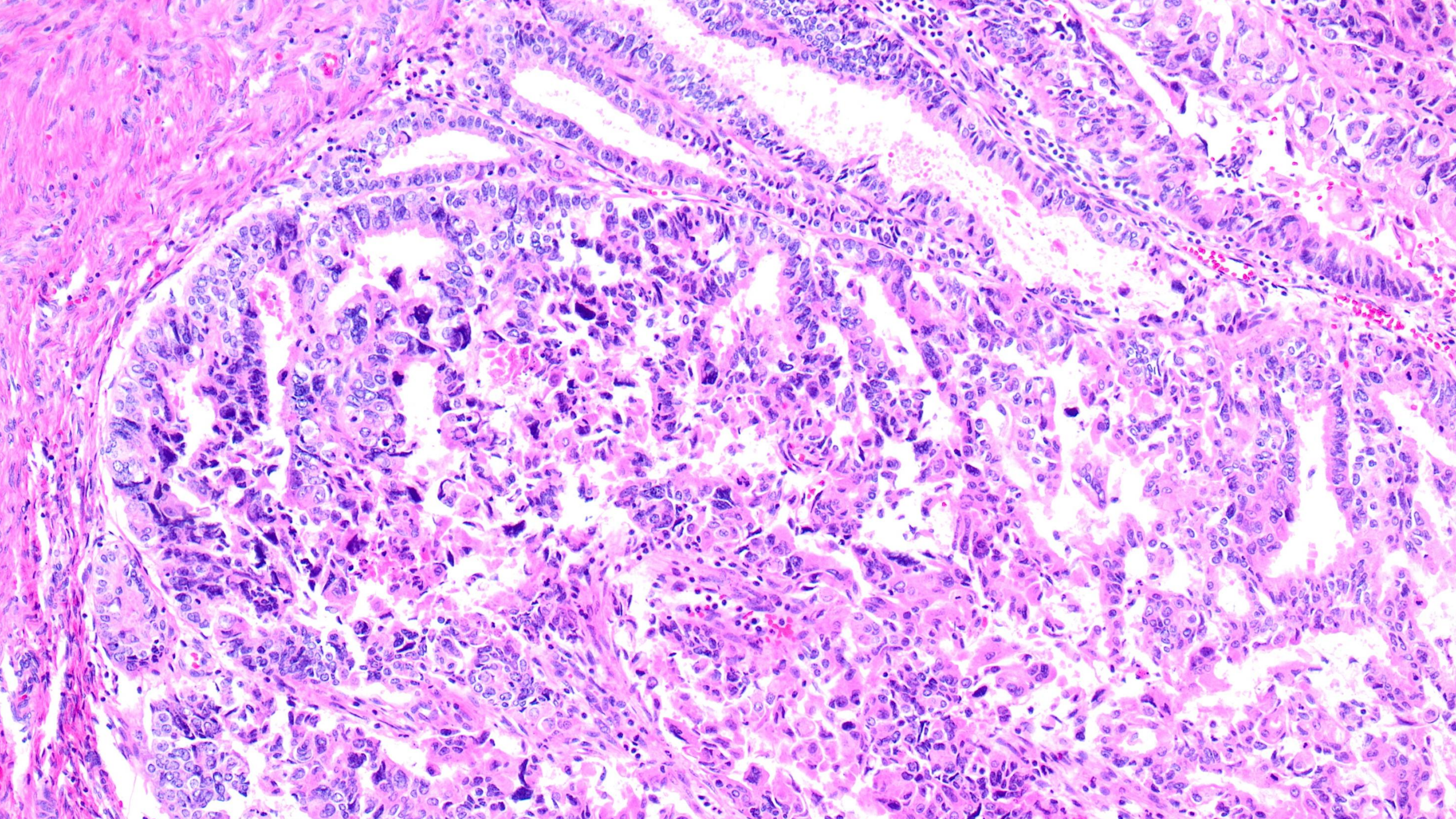
- ✓ P53 abnormal
- ✓ MMR-proficient
- ✓ *POLE* wild-type

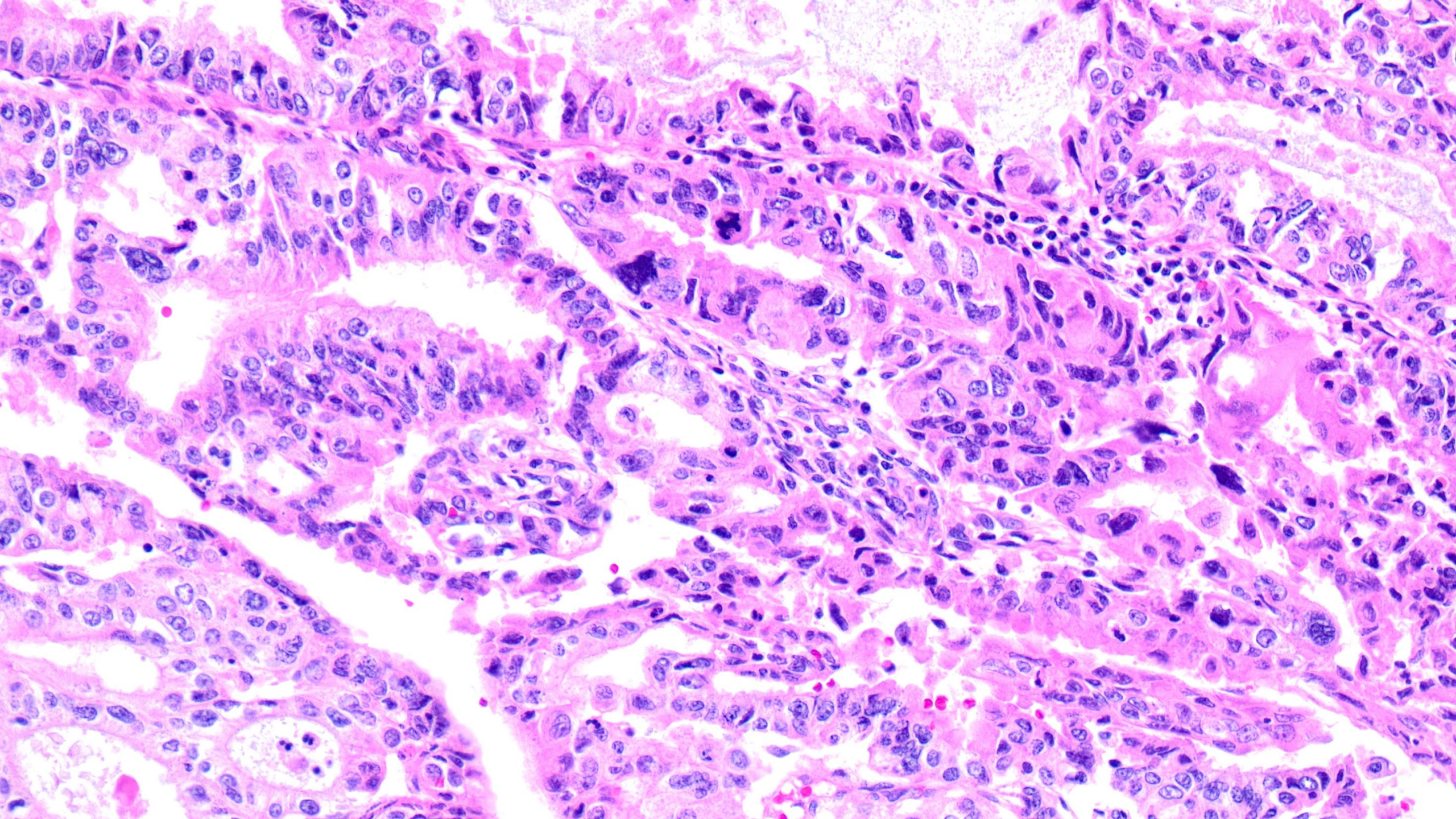
- **Histotypes:**

- ✓ Serous carcinoma
- ✓ Carcinosarcoma
- ✓ Clear cell carcinoma
- ✓ Endometrioid carcinoma
- ✓ Undifferentiated carcinoma
- ✓ Mixed carcinoma









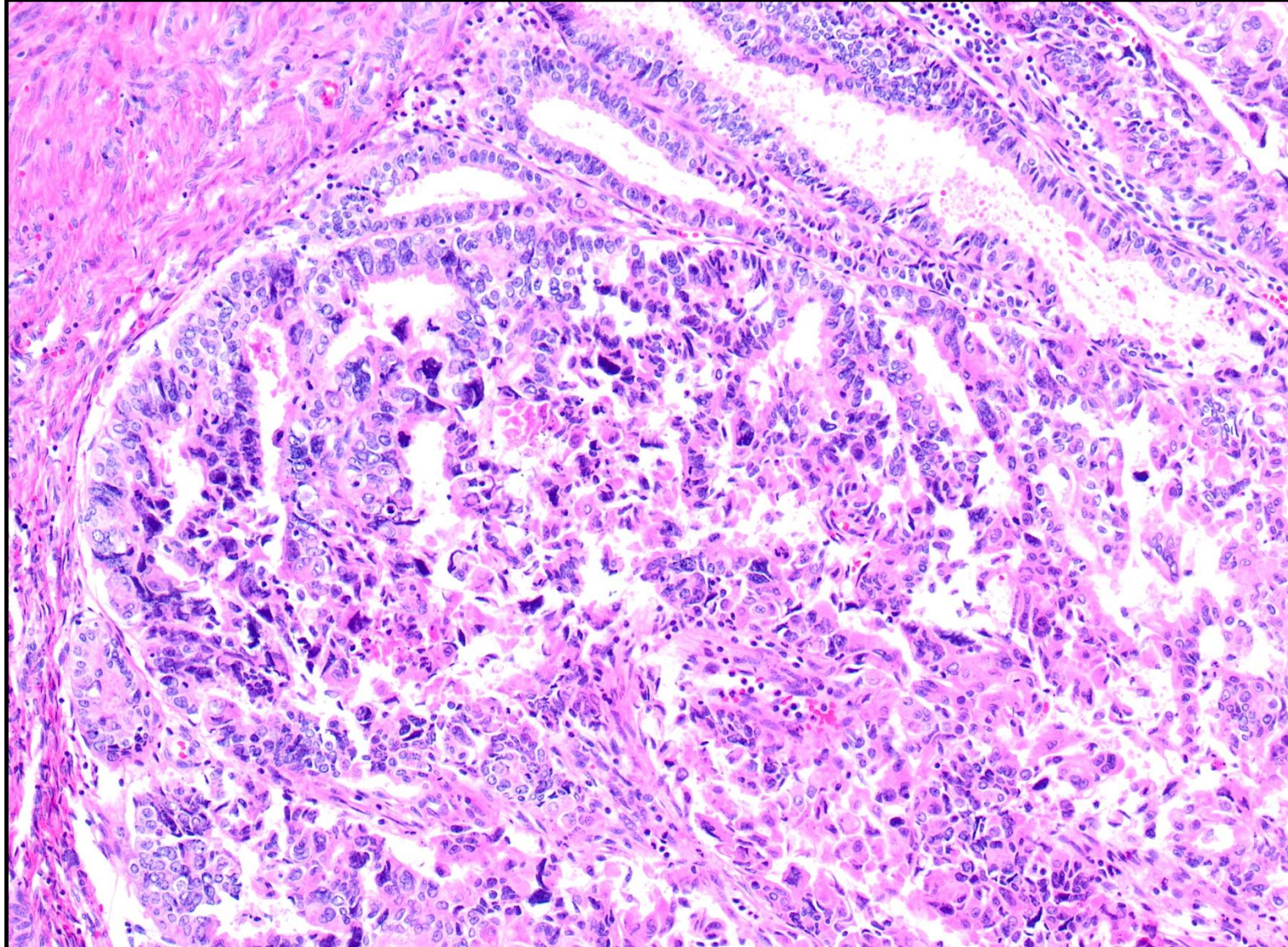
Molecular Subtypes by Histology: *POLE*-Mutated

- **Key features:**

- ✓ *POLE*-mutated
- ✓ P53 wild-type
- ✓ MMR-proficient

- **Morphology:**

- ✓ Heterogeneity
- ✓ “Serous-like” features
- ✓ Often grade 3
- ✓ Patchy bizarre atypia
- ✓ Tumor giant cells
- ✓ Tumor-infiltrating and peritumoral lymphocytes



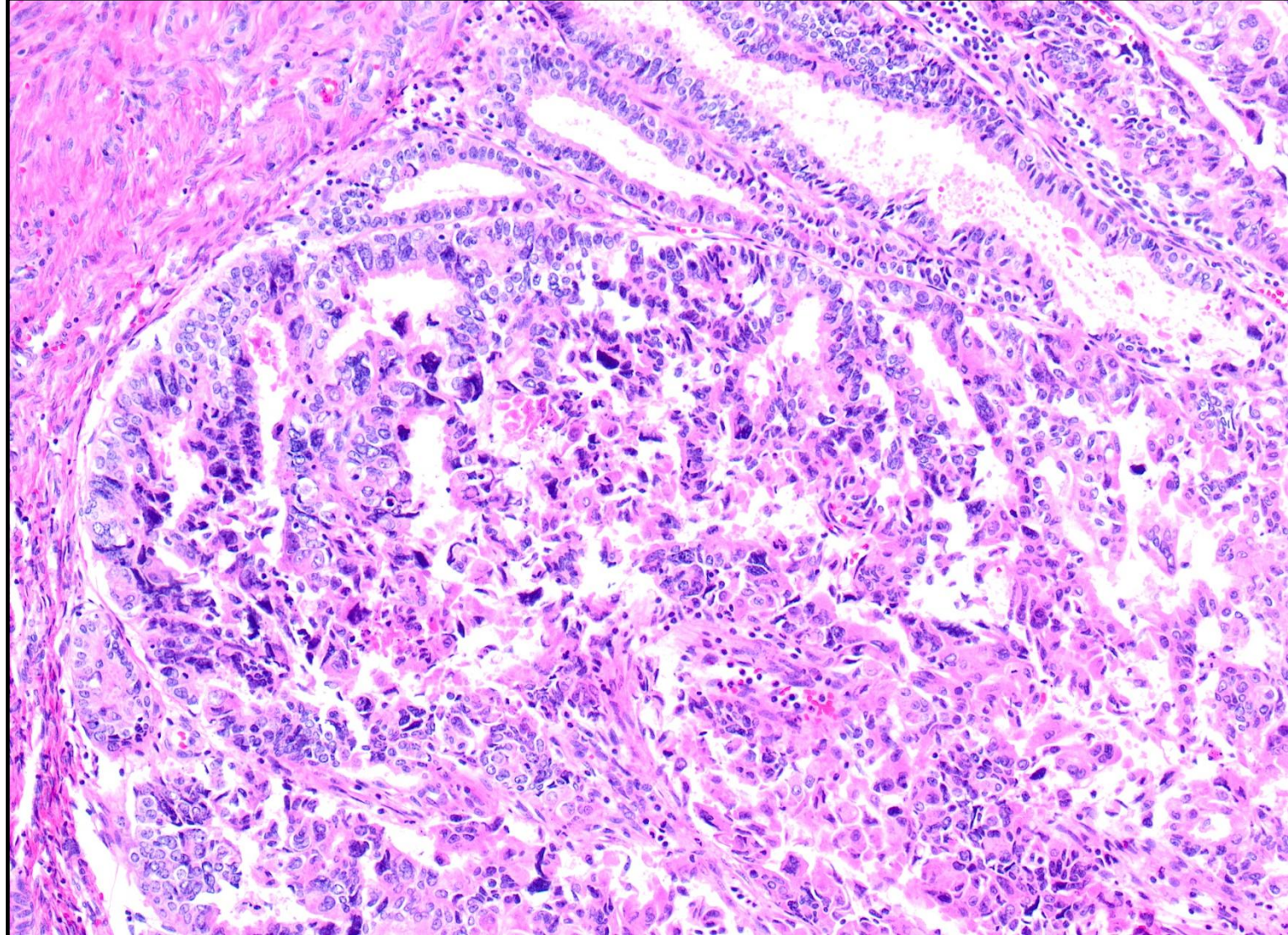
Molecular Subtypes by Histology: *POLE*-Mutated

- **Key features:**

- ✓ *POLE*-mutated
- ✓ P53 wild-type
- ✓ MMR-proficient

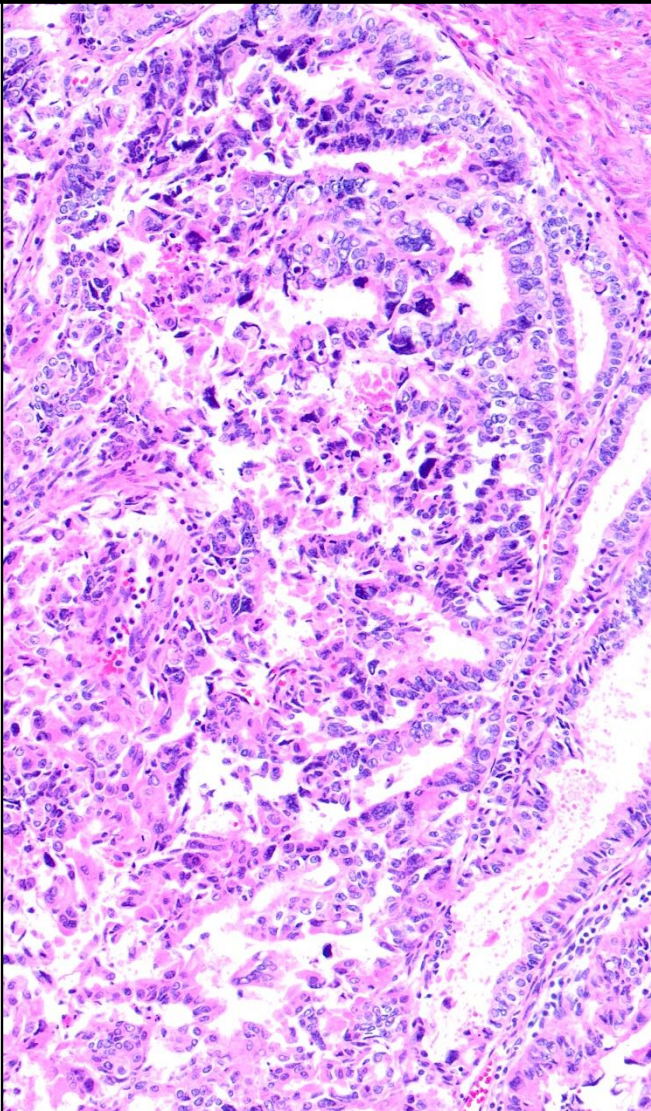
- **Histotypes:**

- ✓ Endometrioid carcinoma
- ✓ Clear cell carcinoma
- ✓ Carcinosarcoma
- ✓ Undifferentiated carcinoma

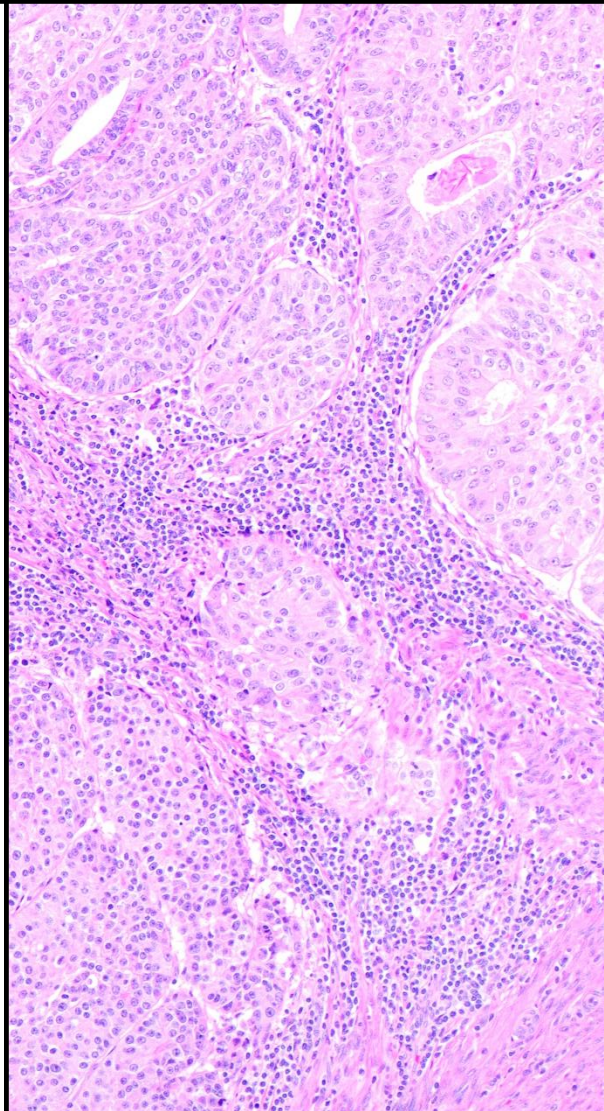


Summary: The 4 Subtypes of Endometrial Carcinoma

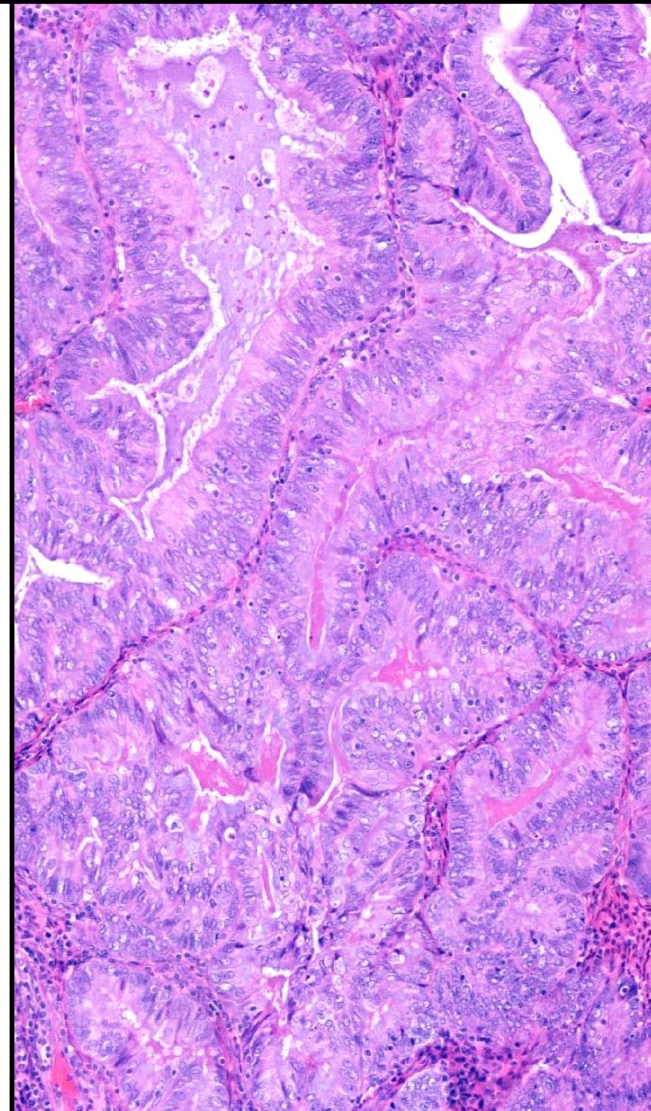
***POLE*-mutated**



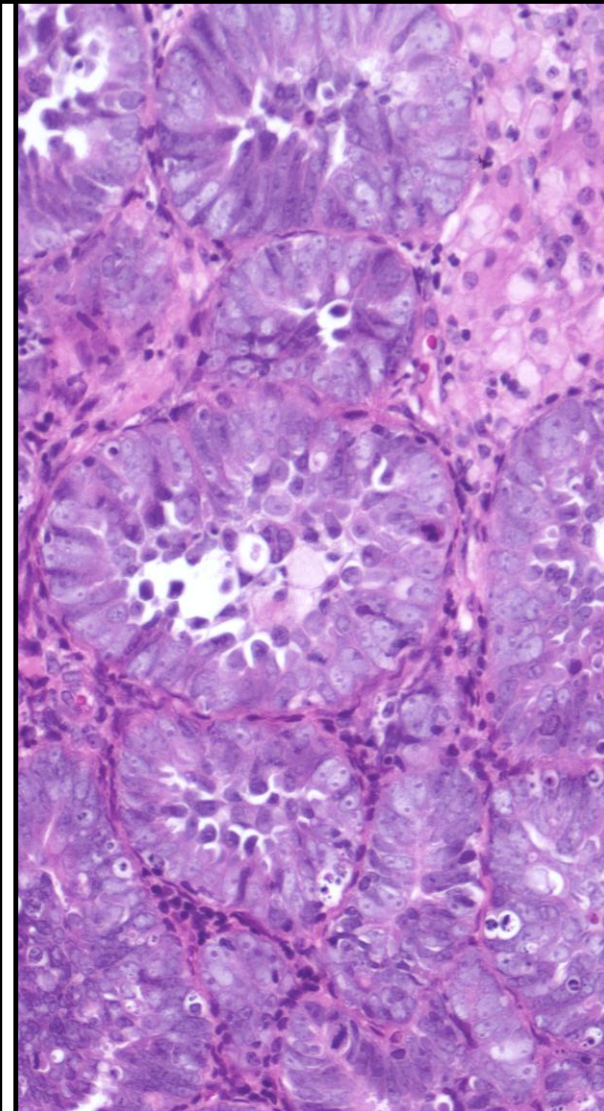
MMRd/MSI-high



NSMP/CN-low



p53abn/CN-high



Summary: The 4 Subtypes of Endometrial Carcinoma

POLE-mutated

MMRd/MSI-high

NSMP/CN-low

p53abn/CN-high

Routine Molecular Subtyping in Practice?

- Ideally all patients
- *POLE* sequencing not universally available
- Partial subtyping (MMR and p53 IHC)
 - ✓ Add ER ± β -catenin
- Selective testing



Thank you!

Questions?