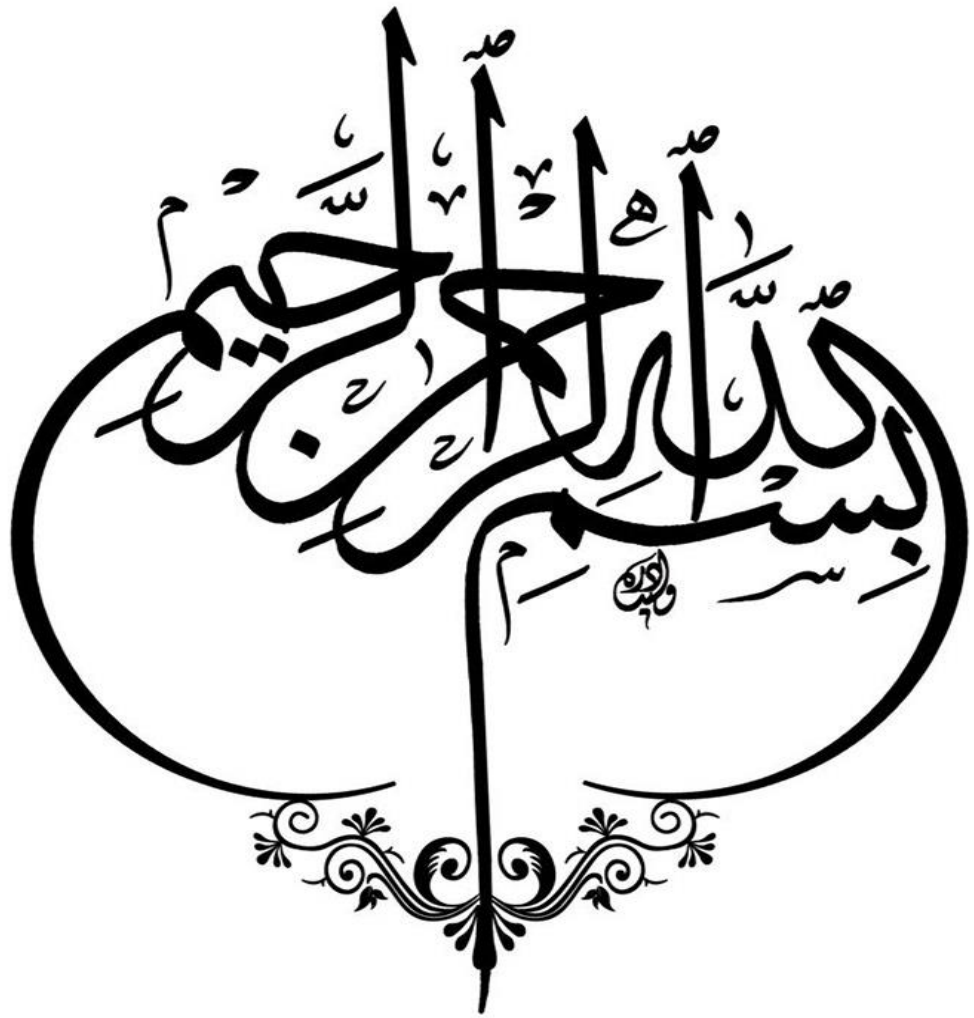

بسم الله الرحمن الرحيم

Diagnostic Immunohistochemistry in Gynecologic Pathology A Morphology-Driven Approach Endometrium-part 1

Fereshteh Ameli
MD, FRCPA
Associate Professor
Department of Pathology
Tehran University of Medical Sciences

Learning Objectives

- Objectives
  - Review practical applications of IHC in endometrial pathology
  - Develop a morphology-based diagnostic approach
  - Understand the role of molecular classification in daily practice
  - Review predictive biomarkers relevant to patient management

Key Message:
Morphology remains the foundation of diagnosis.
Immunohistochemistry should answer a specific diagnostic, prognostic, or predictive question.

WHY DO WE ORDER IHC IN ENDOMETRIAL CARCINOMA?

- **Practical Questions Faced During Sign-Out**
  - EIN versus benign mimics?
  - Is this really an endometrial carcinoma?
  - Which histologic subtype is present?
  - Could this represent metastatic disease?
  - Does the tumor belong to a specific molecular subgroup (Molecular Classification)?
  - Are there therapeutic implications (Predictive Biomarkers)?

- **Role of IHC**
  - Histotype assignment
  - Differential diagnosis
  - Molecular classification
  - Lynch syndrome screening
  - Selection for targeted therapy

Modern endometrial pathology integrates:
Morphology + Immunohistochemistry + Molecular Testing

A PRACTICAL FRAMEWORK FOR IHC SELECTION

- Before Ordering Any Immunostain, Ask:
  - What is my differential diagnosis?
  - Which entities need to be separated?
  - Which marker is most likely to answer that question?
  - How will the result change my diagnosis?

- COMMON CAUSES OF DIAGNOSTIC ERROR
  - Overuse of Immunohistochemistry
  - Large unfocused antibody panels
  - Ignoring morphologic clues
  - Overinterpretation of focal staining
  - Failure to recognize aberrant expression patterns
  - Lack of clinicopathologic correlation

Endometrial Intraepithelial Neoplasia (AH / EIN)

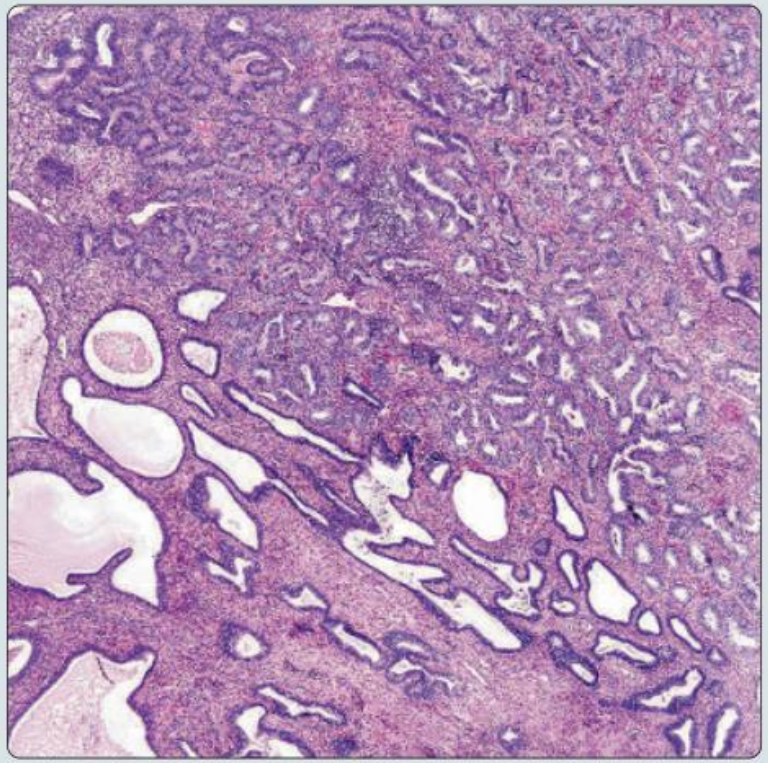
- **Why Is It Important?**
  - AH/EIN is the precursor lesion of most endometrioid endometrial carcinomas.
  - Characterized by abnormal architecture and cytology distinct from surrounding endometrium and prone to malignant transformation
- Accurate diagnosis:
  - Prevents overtreatment
  - Prevents underdiagnosis
  - Identifies patients at risk for carcinoma

Endometrial Intraepithelial Neoplasia (AH / EIN)

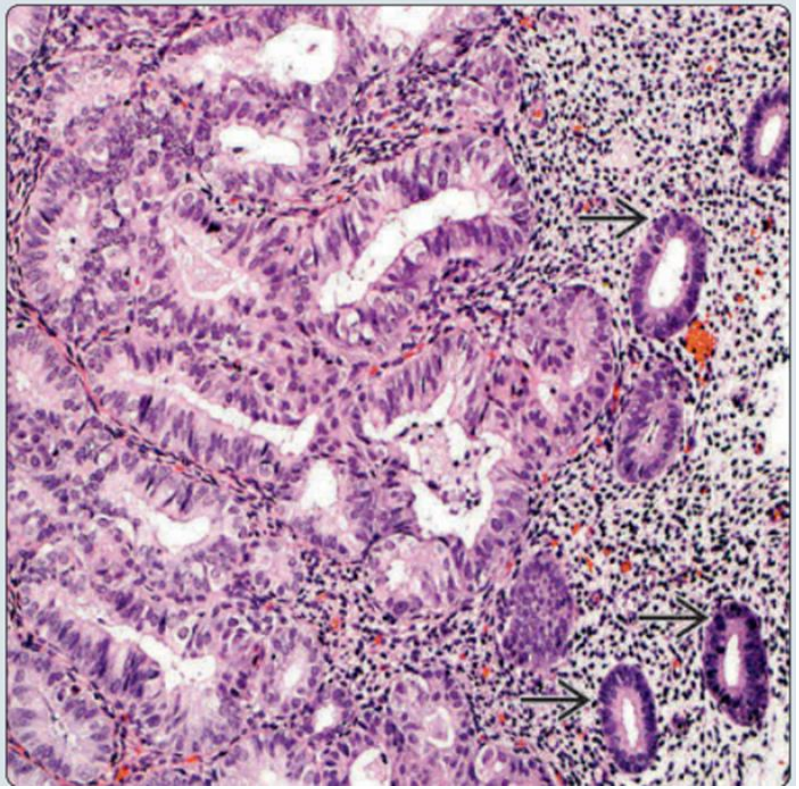
- **Morphologic Criteria for EIN**
  - Gland crowding
  - Reduced stromal volume
  - Cytologic alteration
  - Lesion >1 mm
  - Nonendometrioid differentiation (metaplasia) common: Squamous (typically morules), mucinous, tubal, secretory
  - Exclusion of benign mimics and carcinoma
- AH / EIN
  - Prior terminologies (simple and complex) are no longer included

Endometrial Intraepithelial Neoplasia (AH / EIN)

Atypical Endometrial Hyperplasia/Endometrial Intraepithelial Neoplasia in Endometrial Polyp

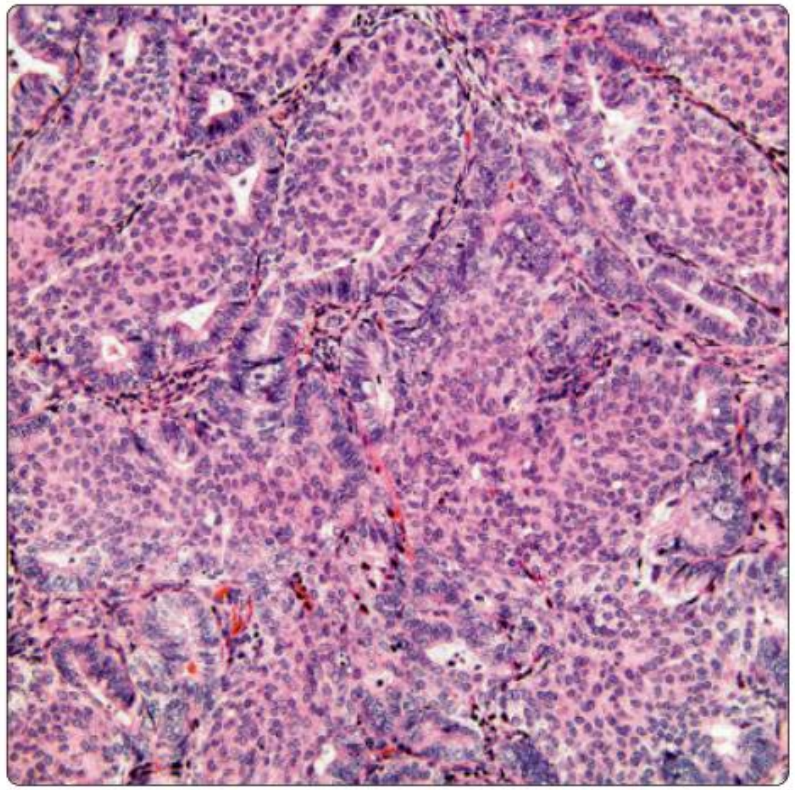


Gland Crowding With Morphology Distinct From Surrounding Endometrial Glands

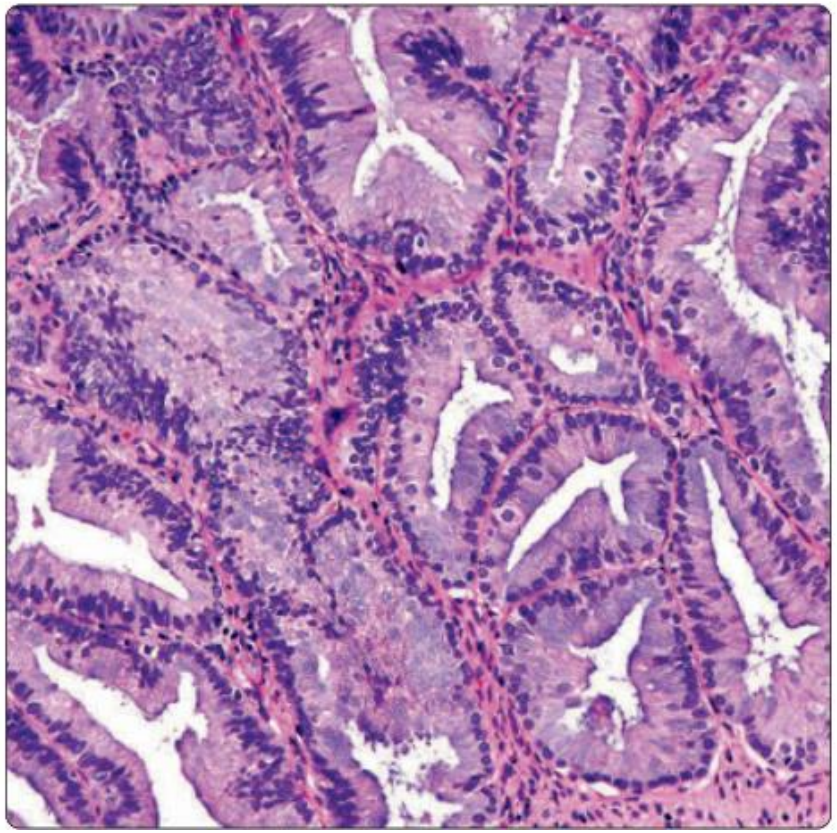


Endometrial Intraepithelial Neoplasia (AH / EIN)

Confluent Morular Metaplasia May Cause Concern for Adenocarcinoma

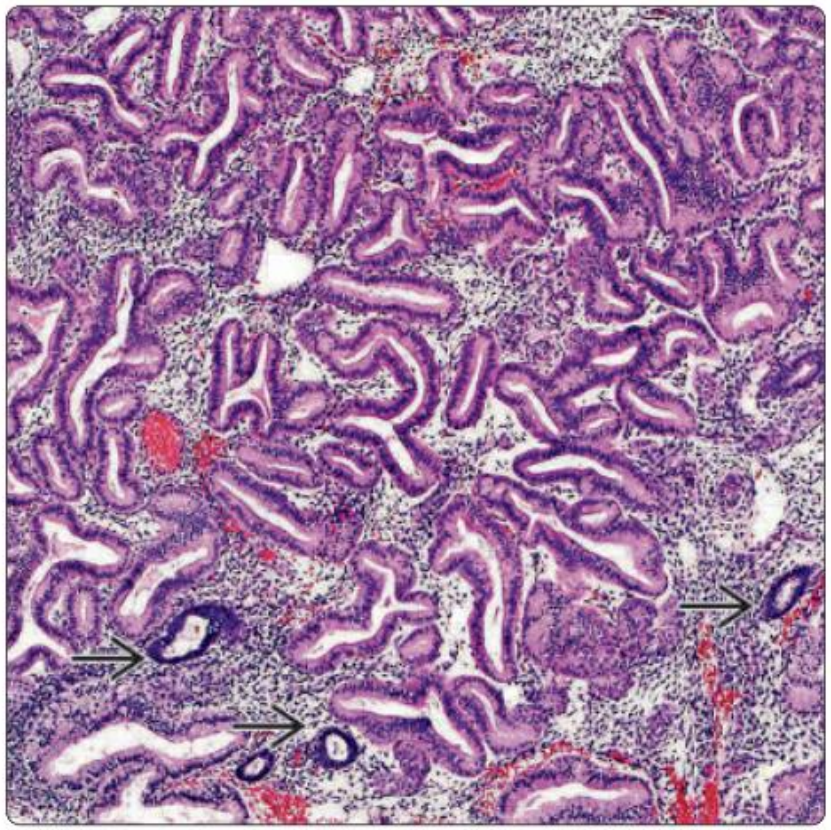


Mucinous and Eosinophilic Metaplasias

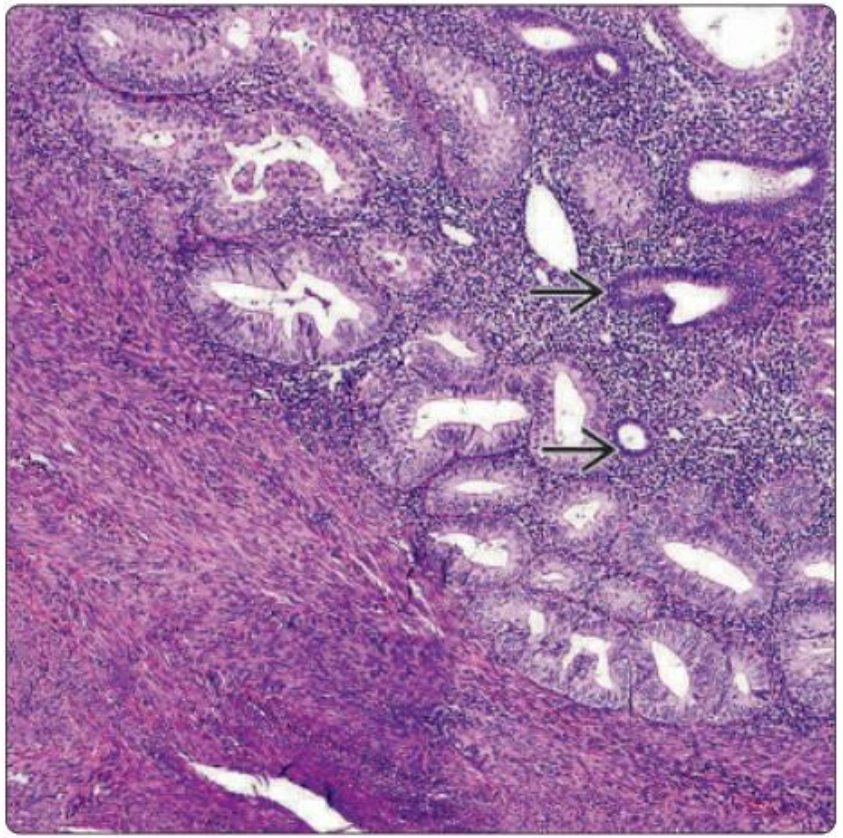


Endometrial Intraepithelial Neoplasia (AH / EIN)

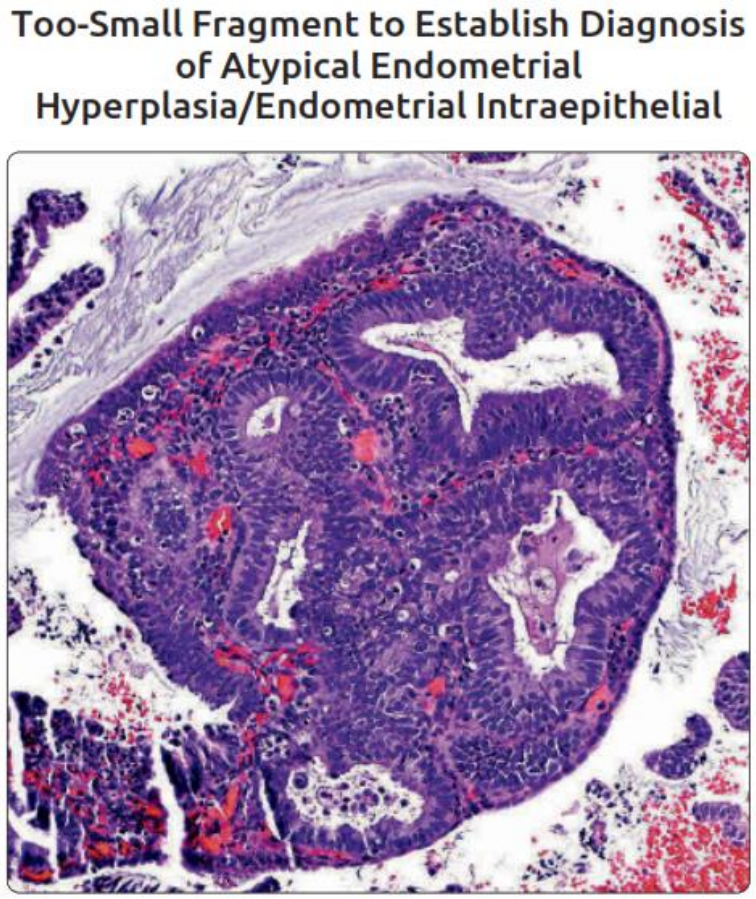
Secretory Change



Involvement of Adenomyosis



Endometrial Intraepithelial Neoplasia (AH / EIN)



- If focus is not diagnostic for EIN (< 1 mm or close to but does not meet 50% threshold for gland crowding), followup sampling indicated as "gland crowding" associated with subsequent EIN (20%)

Endometrial Intraepithelial Neoplasia (AH / EIN)

- TOP DIFFERENTIAL DIAGNOSES
  - Technical artifacts
  - Endometrial polyp
  - Atypical polypoid adenomyoma
  - Endometrial hyperplasia without atypia
  - Endometrioid adenocarcinoma
  - HPV-associated endocervical adenocarcinoma insitu("colonization of endometrial glands")
- AH / EIN should not have
  - Extensive cribriforming, confluent glands
  - Labyrinthine intraluminal connections
  - Villoglandular pattern
  - Areas of purely solid epithelium
  - Stromal alteration suggesting invasion - desmoplasia

Endometrial Intraepithelial Neoplasia (AH / EIN)

- ANCILLARY TESTS
  - Immunohistochemistry
    - ER, PR positive
    - p16 negative
    - ± PAX2 and PTEN loss
    - p53 wild-type pattern
  - Genetic Testing
    - PTEN 44% inactivated; PAX2 71% inactivated
    - Combined PTEN and PAX2 mutations (30%)

Endometrial Intraepithelial Neoplasia (AH / EIN)

- Role of IHC:
  - IHC may support morphology in difficult cases.
- Useful Markers and Limitations
  - Frequently Used Markers
    - PAX2
    - PTEN
    - ARID1A
    - β-catenin

Typical Findings

- PAX2:

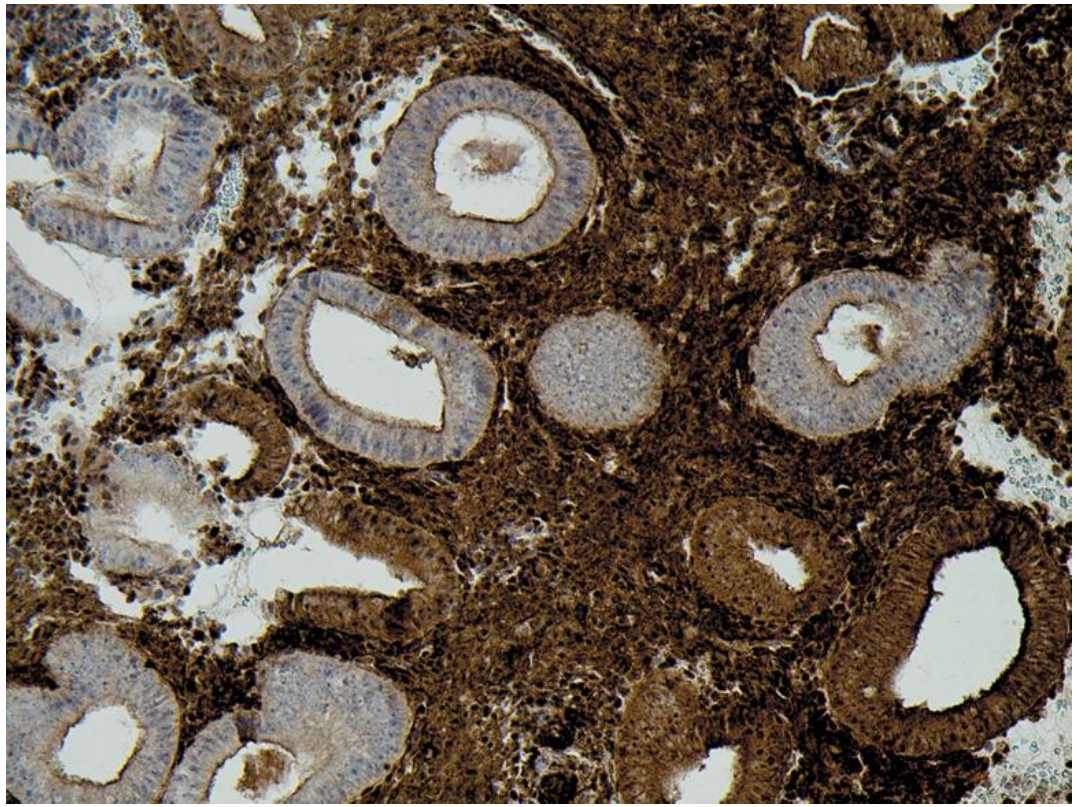
  Loss in many EIN lesions
- PTEN:

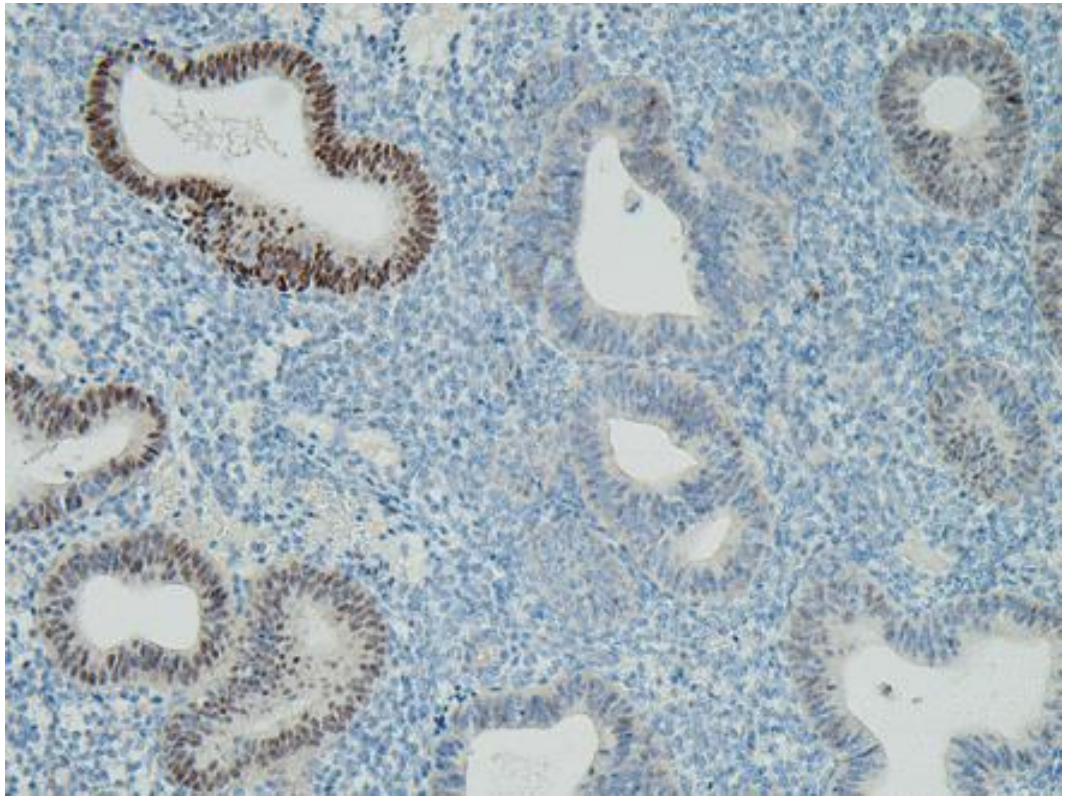
  Loss in a subset of EIN lesions
- ARID1A:

  Loss may occur early in carcinogenesis
- β-catenin:

  Nuclear expression in selected lesions

Endometrial Intraepithelial Neoplasia (AH / EIN)





(PAX2)-null glands were found to be unstained in contrast to the surrounding normal brown-stained glands

Endometrial Intraepithelial Neoplasia (AH / EIN)

- PTEN and PAX2 IHC stains, while helpful in understanding pathogenesis of endometrial neoplasia, should not be used as routine diagnostic markers, as 1/2 of EINs retain PTEN and 1/3 retain PAX2 expression
- Loss of expression supports a clonal neoplastic process.
- Retention of expression does not exclude EIN
- A crowded glandular focus showing loss of PAX2 plus PTEN loss and/or nuclear β-catenin is much more convincing as a true neoplastic clone than a single isolated abnormality
- Demonstration of **clonality through multiple concordant abnormalities**

Endometrial Intraepithelial Neoplasia (AH / EIN)

- PTEN loss by itself should not be considered diagnostic of EIN because PTEN-null glands may occur in histologically benign endometrium.
- Interpretation of PAX2 and PTEN should always be performed in the context of morphology and the distribution pattern of the abnormal glands.
- **Important Limitation**
  - No single immunohistochemical marker can reliably distinguish EIN from carcinoma.
  - Morphology remains essential.

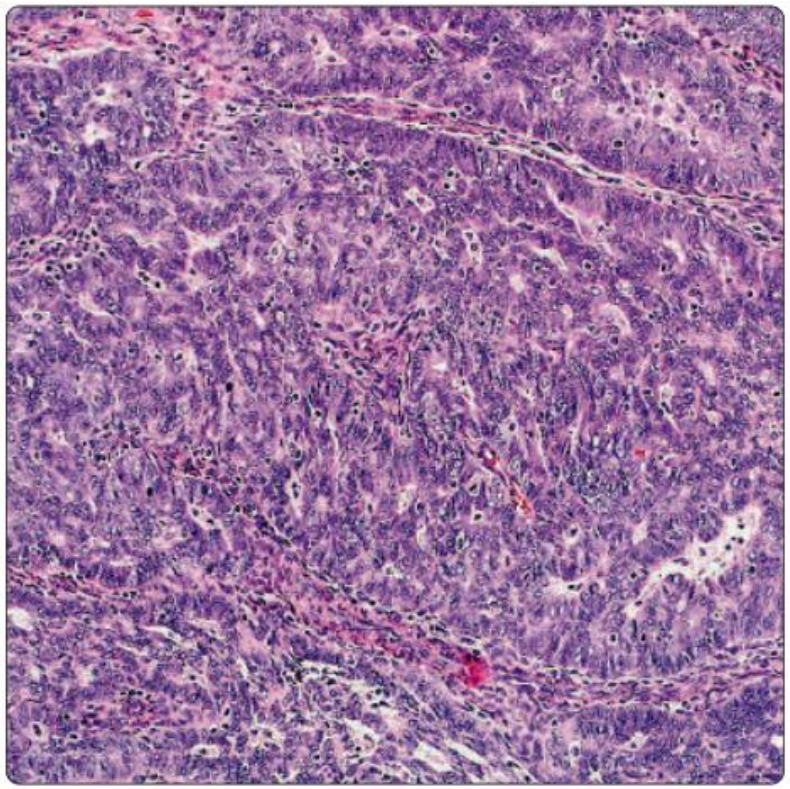
EIN VERSUS ENDOMETRIOID CARCINOMA

- Morphologic Clues Favoring Carcinoma
  - Confluent glandular growth
  - Cribriform architecture
  - Solid growth
  - Labyrinthine glands
  - Stromal exclusion
  - Destructive invasion

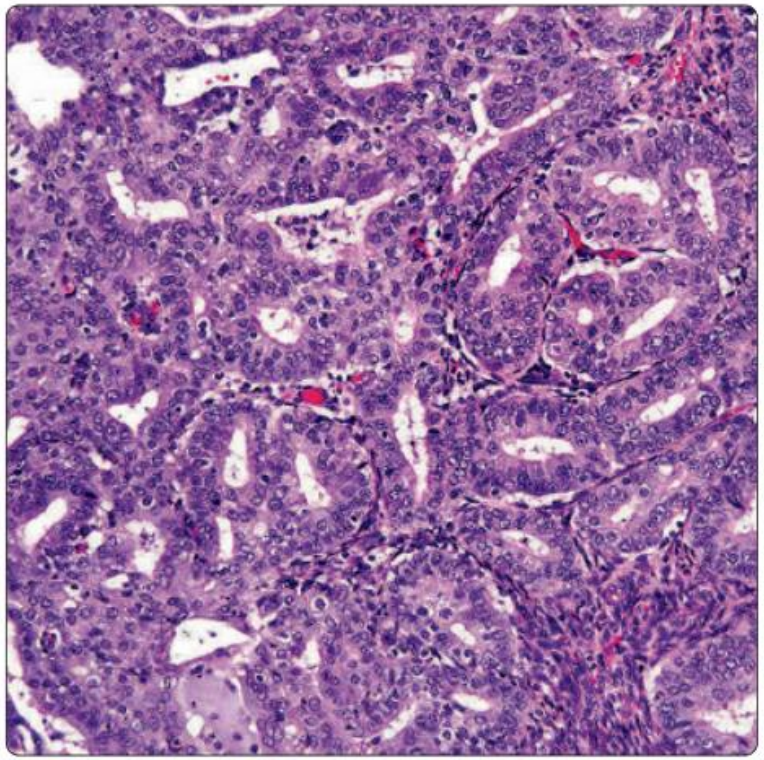
- Loss of expression supports a clonal neoplastic process.
- However
  - similar abnormalities may occur in EIN
  - similar abnormalities may occur in carcinoma
- Key Point
  - Diagnosis remains primarily morphology-based.
  - No immunohistochemical marker can reliably distinguish EIN from endometrioid carcinoma.

EIN VERSUS ENDOMETRIOID CARCINOMA

Well-Differentiated Endometrioid Adenocarcinoma With Cribriforming



Well-Differentiated Endometrioid Adenocarcinoma With Back-To-Back Glands



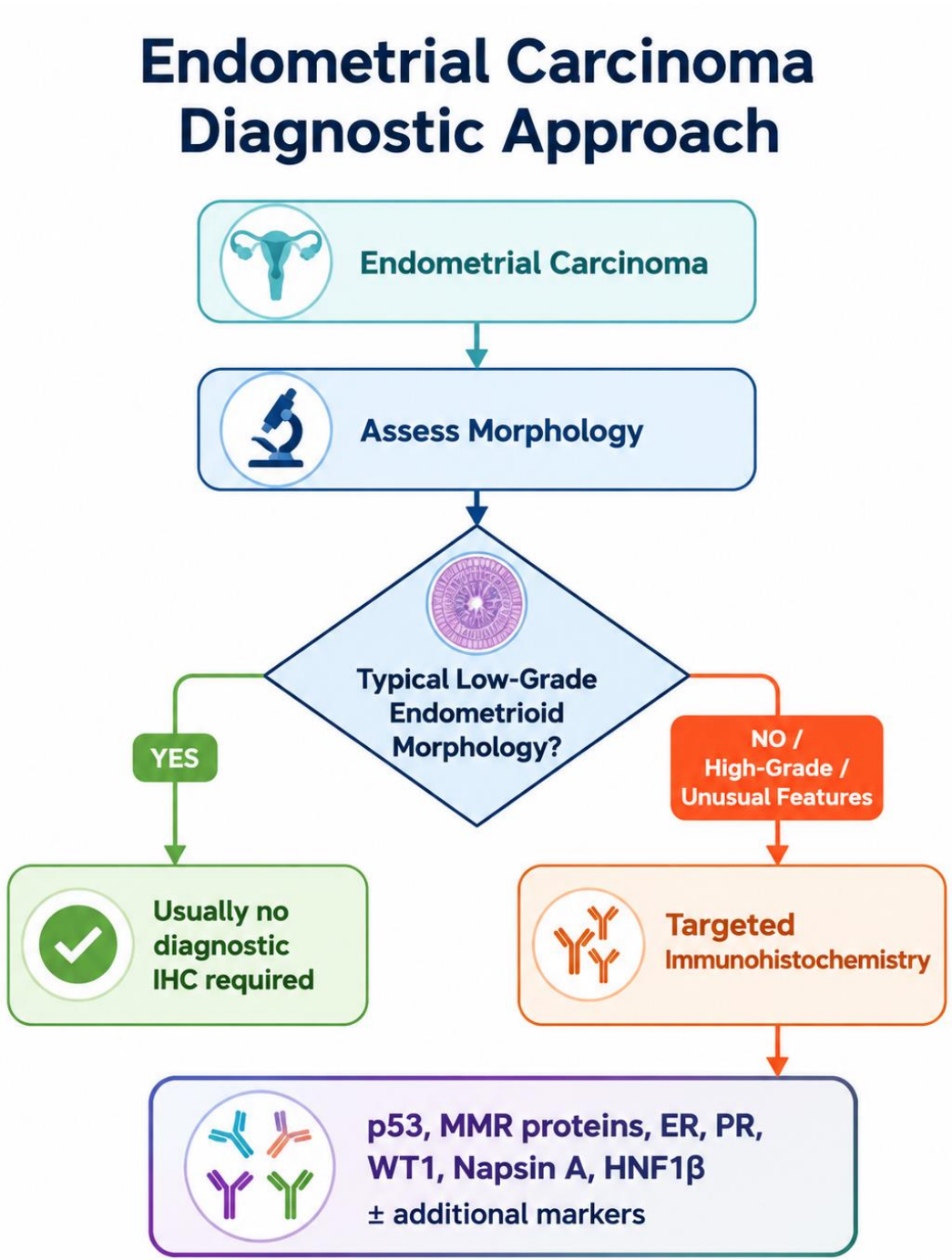
ENDOMETRIOID ENDOMETRIAL CARCINOMA

- Key Facts
  - Most common subtype of endometrial carcinoma
  - Frequently associated with estrogen exposure
  - Usually ER and PR positive
  - Often presents at early stage
- Typical Morphology
  - Back-to-back glands
  - Cribriform architecture
  - Squamous differentiation
  - Variable cytologic atypia
- Role of IHC
  - Most low-grade tumors are diagnosed based on morphology alone.

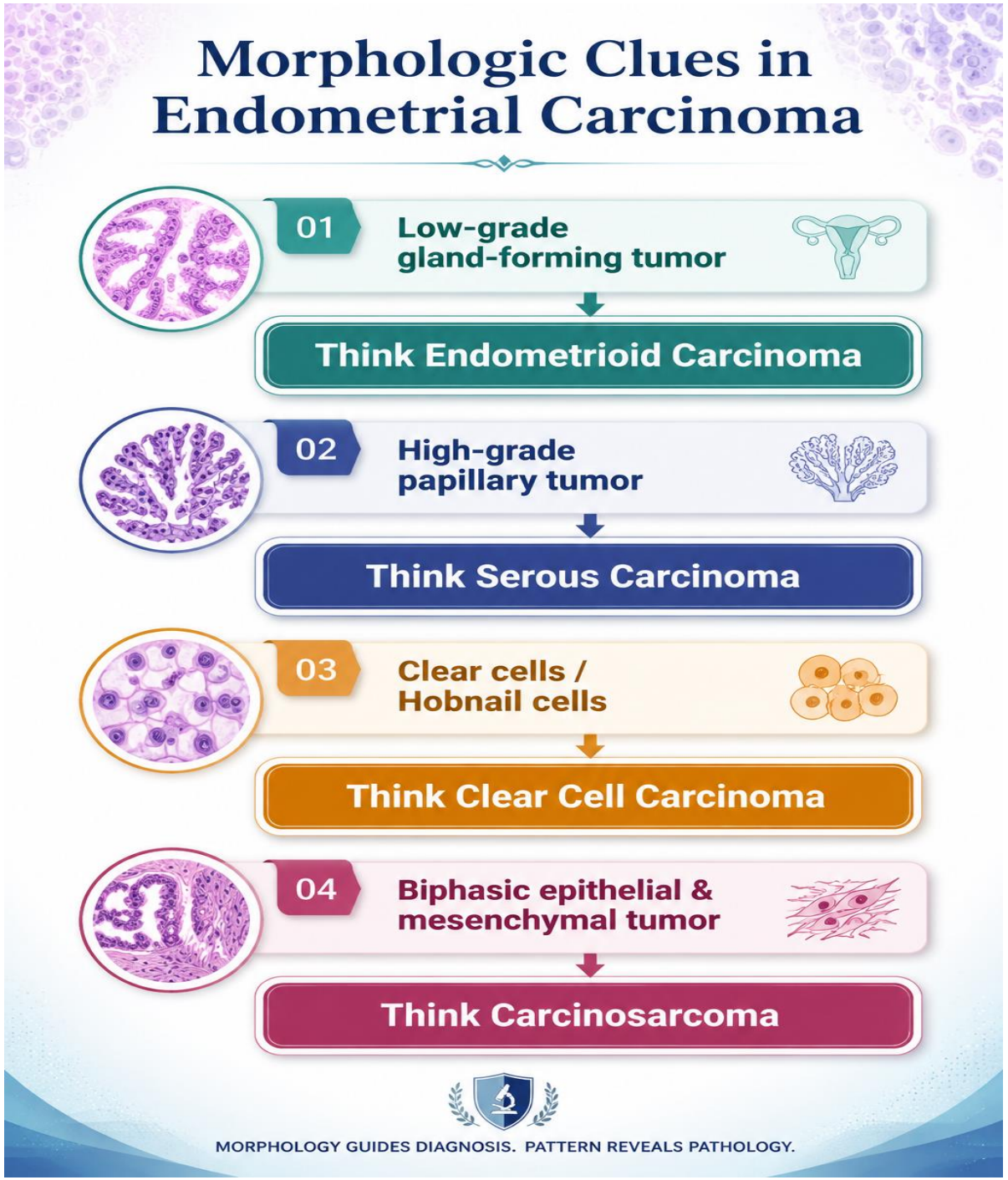
WHEN DO WE ORDER IHC IN ENDOMETRIOID CARCINOMA?

- Diagnostic Scenarios
  - Endometrioid versus Serous Carcinoma
  - Endometrioid versus Clear Cell Carcinoma
  - Endometrial versus Endocervical Adenocarcinoma
  - Primary Tumor versus Metastasis

- The immunohistochemical panel should be selected according to the differential diagnosis.



The goal is not to stain every case.
The goal is to answer a diagnostic question.



MORPHOLOGY FIRST: AN APPROACH TO ENDOMETRIAL CARCINOMA

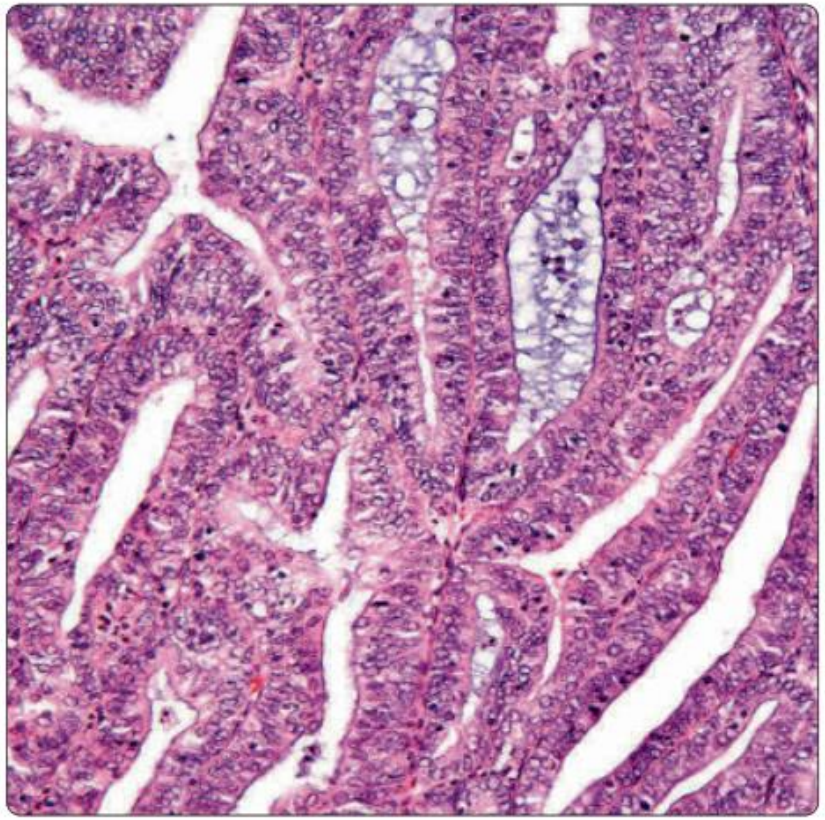
- Principle
  - Morphology generates the differential diagnosis.
  - IHC refines or confirms the diagnosis.

ENDOMETRIOID ENDOMETRIAL CARCINOMA

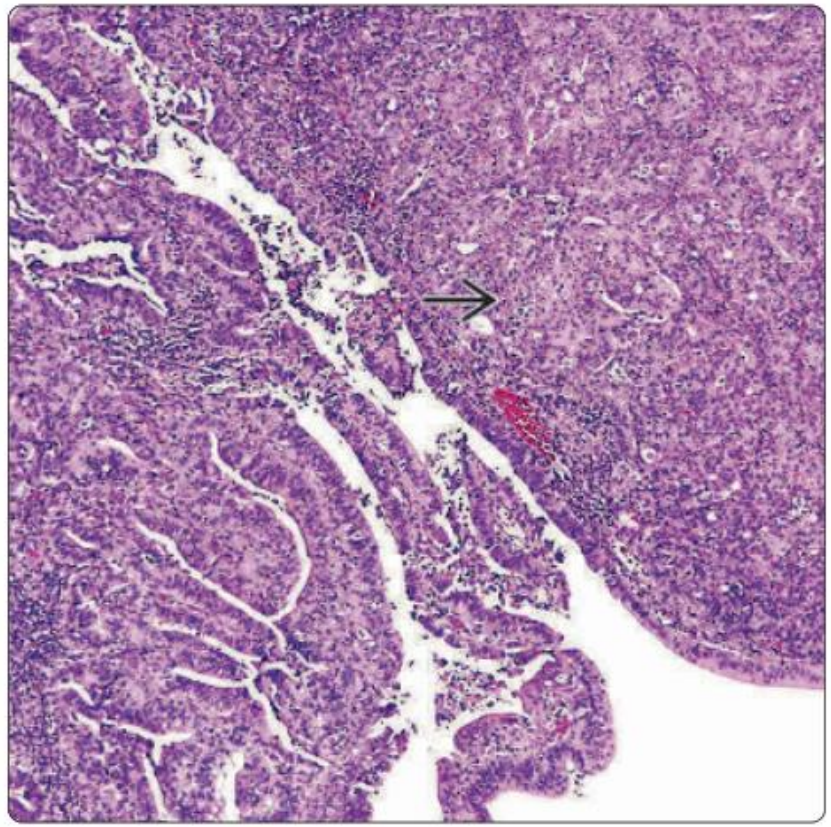
- Key Facts
  - Most common subtype of endometrial carcinoma
  - Usually low grade
  - Frequently hormone receptor positive
  - Generally associated with favorable prognosis
- Typical Morphology
  - Back-to-back glands
  - Cribriform architecture
  - Stratified columnar epithelium
  - Cytologic atypia
  - Squamous differentiation may be present

ENDOMETRIOID ENDOMETRIAL CARCINOMA

Grade 1 (< 5% Solid Growth)



Grade 2 (5-50% Solid Growth)



APPROACH TO HIGH-GRADE ENDOMETRIAL CARCINOMA

- Major Diagnostic Considerations
- High-grade Endometrioid Carcinoma
- Serous Carcinoma
- Clear Cell Carcinoma
- Udifferentiated/Dedifferentiated Carcinoma
- Carcinosarcoma
- Mesonephric-like Adenocarcinoma

- Initial Practical Panel

  P53; MMR proteins; ER; PR
- Additional Markers As Needed

  WT1; Napsin A; HNF1β; PAX8,GATA3,TTF1
- The next step is histotype assignment before molecular classification.

HIGH-GRADE ENDOMETRIOID VS SEROUS CARCINOMA

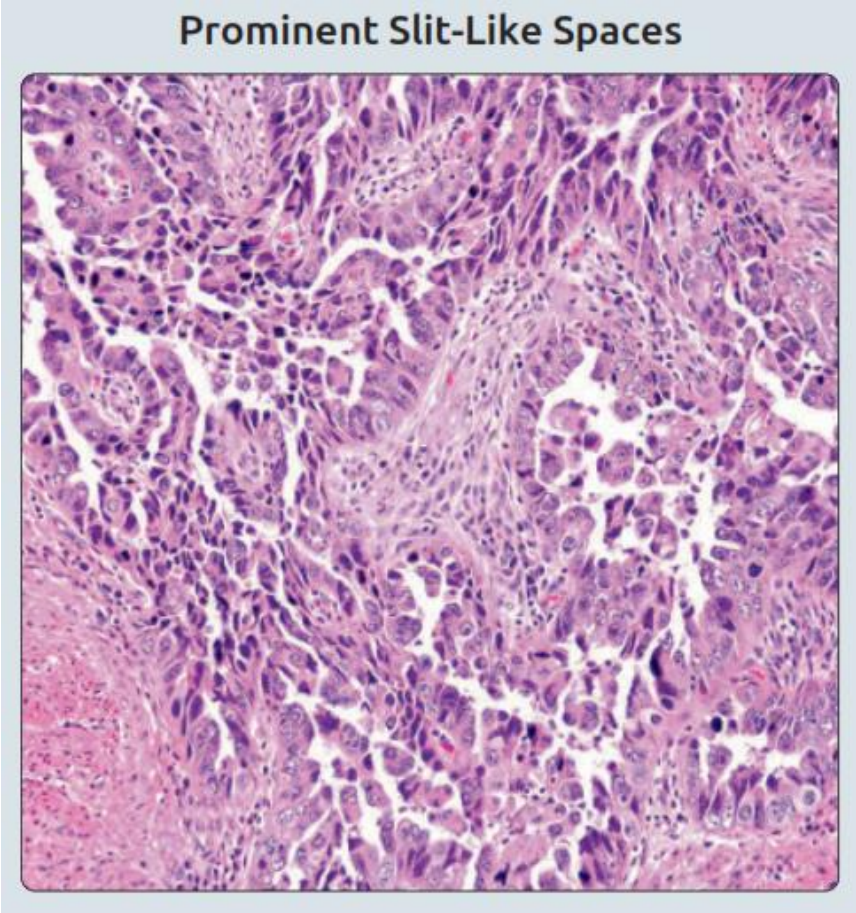
- Features Favoring Endometrioid Carcinoma
  - Residual gland formation
  - Squamous differentiation
  - Mucinous differentiation
  - ER/PR positivity
  - MMR deficiency
  - PTEN and ARID1A loss
- Features Favoring Serous Carcinoma
  - Marked nuclear atypia
  - Loss of nuclear polarity
  - Non-cohesive tumor cells
  - Papillary or micropapillary architecture
  - Diffuse p16 expression
  - Aberrant p53 expression

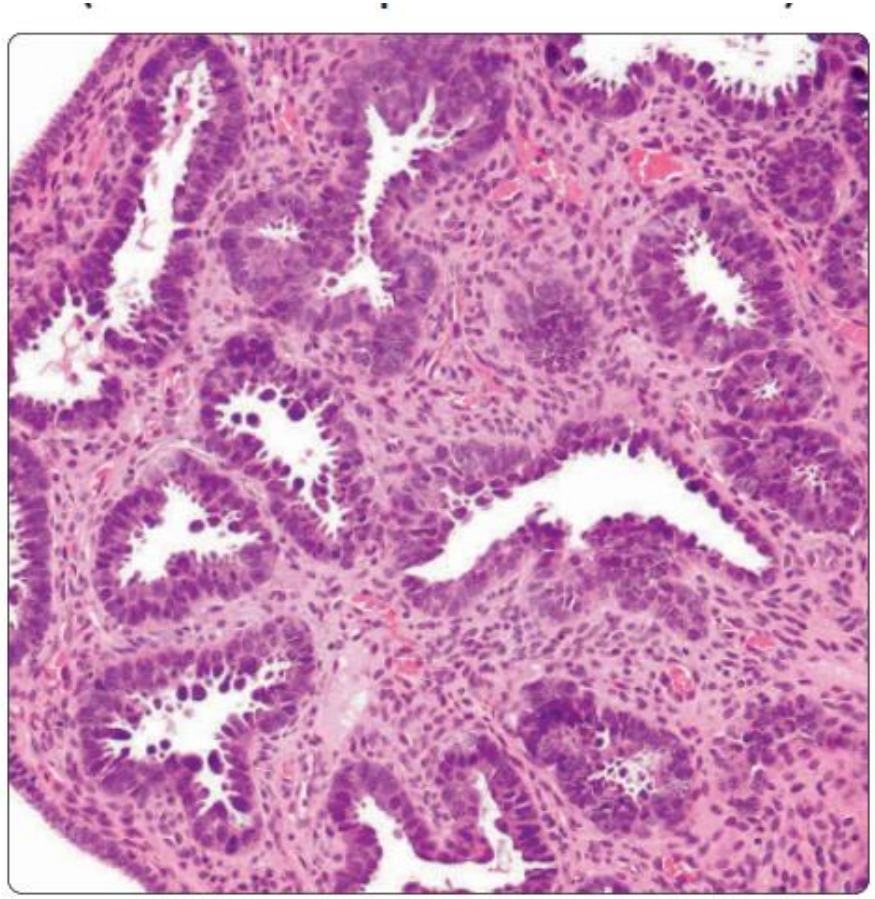
Diagnostic Pearl
High-grade cytology out of proportion to architecture strongly favors serous carcinoma.

UTERINE SEROUS CARCINOMA

- Key Morphologic Features
  - Complex papillary architecture
  - Micropapillae
  - Slit-like spaces
  - Marked nuclear pleomorphism
  - High-grade nuclear features that do not correlate with glandular architecture
  - Frequent mitoses
  - Frequent lymphovascular invasion
- Characteristic Immunophenotype
  - p53-abnormal
  - Diffuse p16 positivity
  - IMP3 positive
  - HMGA2 positive
  - Ki-67 usually >75%
  - ER and PR: variable
  - WT1:
    - Negative or focal in most cases
    - Diffuse positivity suggests tubo-ovarian origin

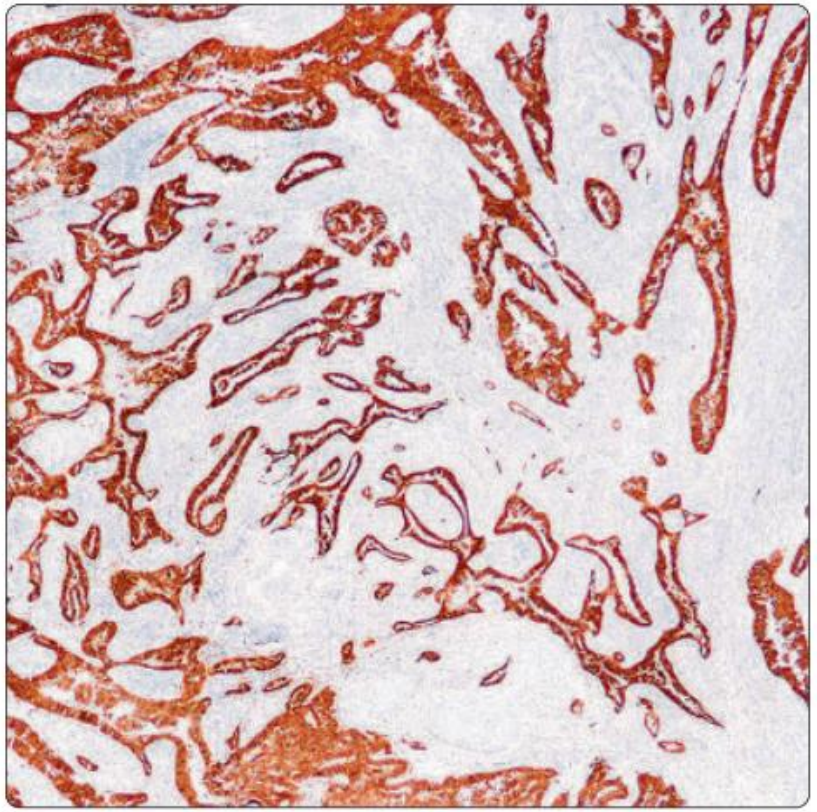
UTERINE SEROUS CARCINOMA



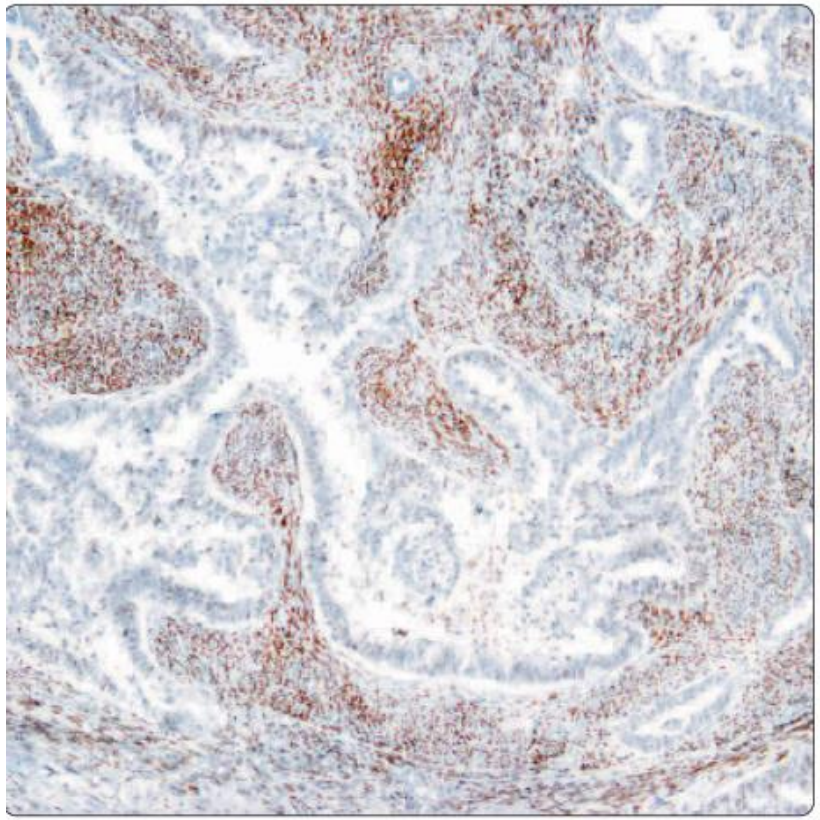


UTERINE SEROUS CARCINOMA

Diffuse and Strong p16 Positivity



Weak ER Positivity

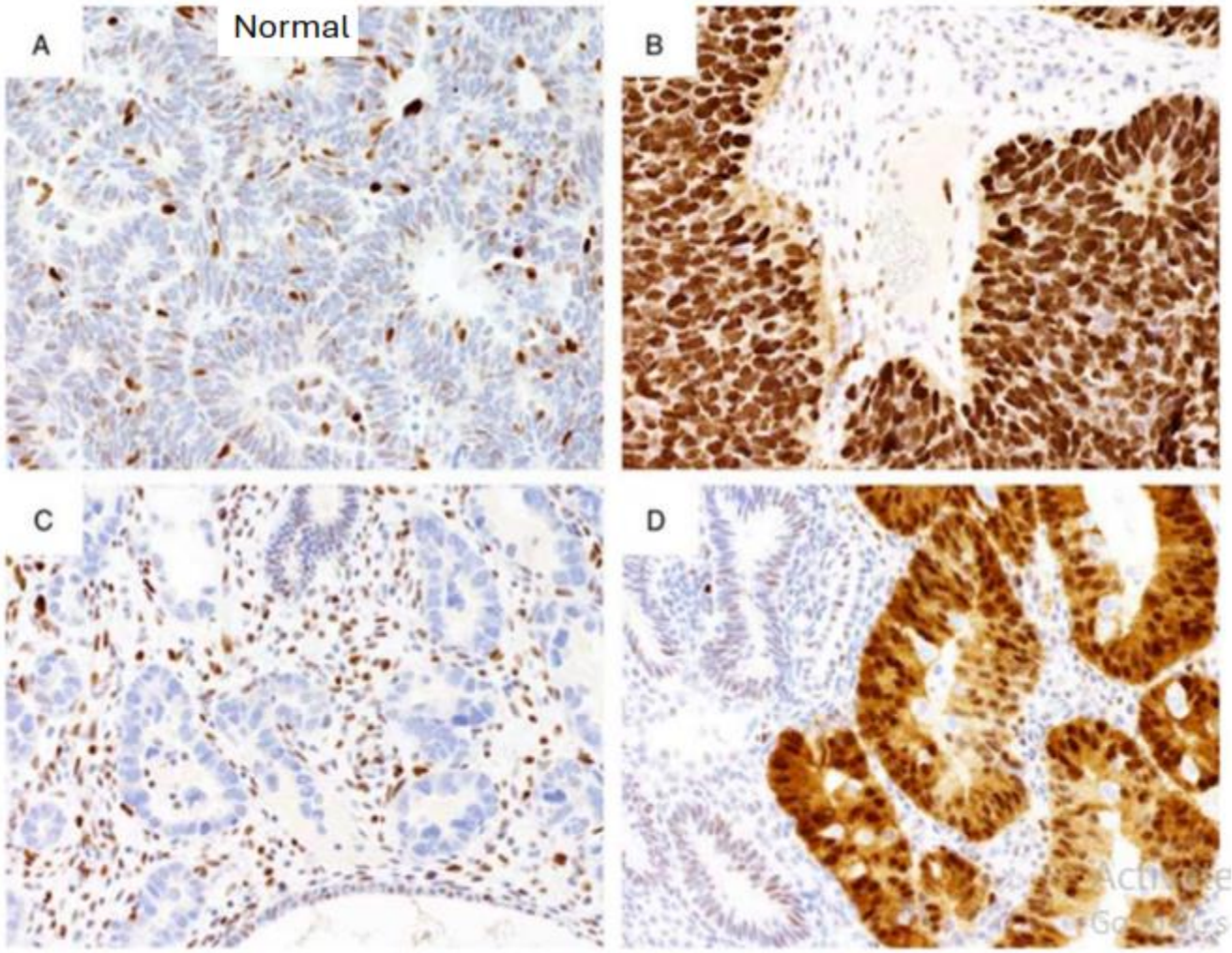


INTERPRETATION OF p53 IN ENDOMETRIAL CARCINOMA

- **Wild-Type Pattern**
  - Variable staining intensity
  - Heterogeneous distribution

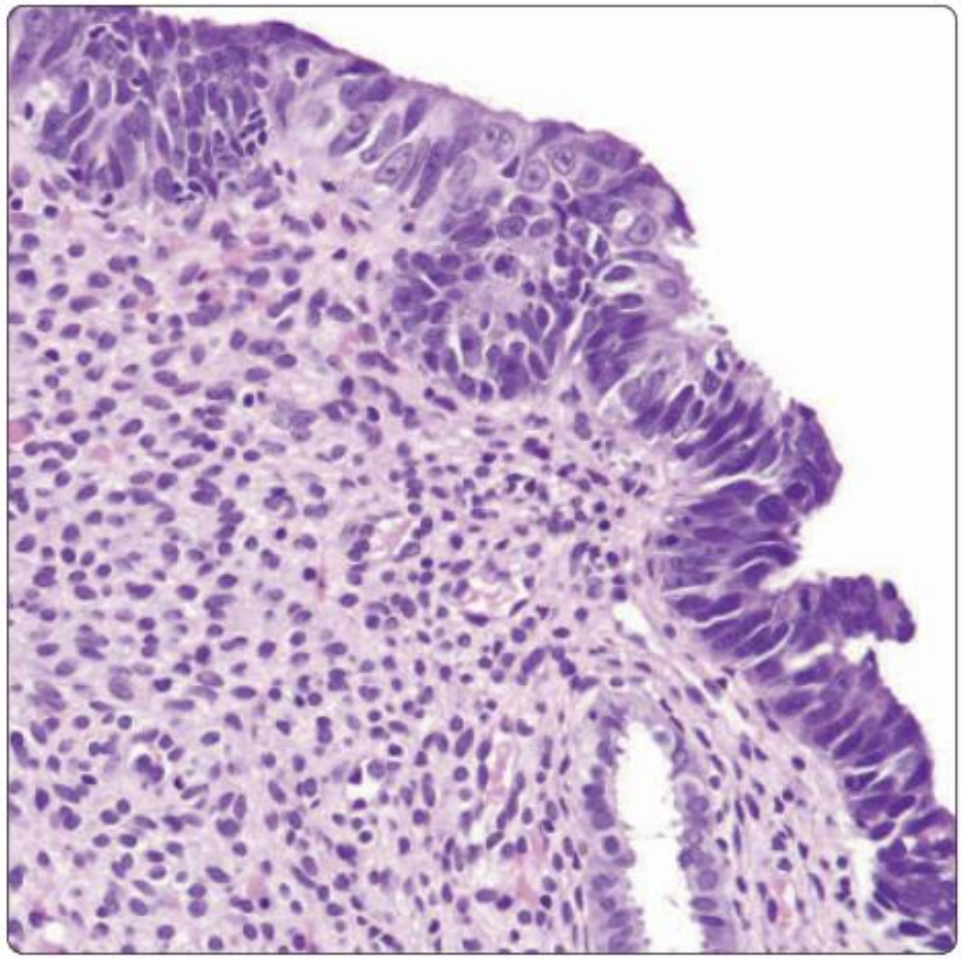
- **Mutation-Type Patterns**
- **Overexpression Pattern**
  - Strong nuclear staining in >80% of tumor cells
- **Null Pattern**
  - Complete absence of staining
  - Internal control required
- **Cytoplasmic Pattern**
  - Aberrant cytoplasmic staining
- **Subclonal Pattern**
  - Distinct abnormal and wild-type populations
- All abnormal patterns are classified as p53-abnormal.

INTERPRETATION OF p53 IN ENDOMETRIAL CARCINOMA

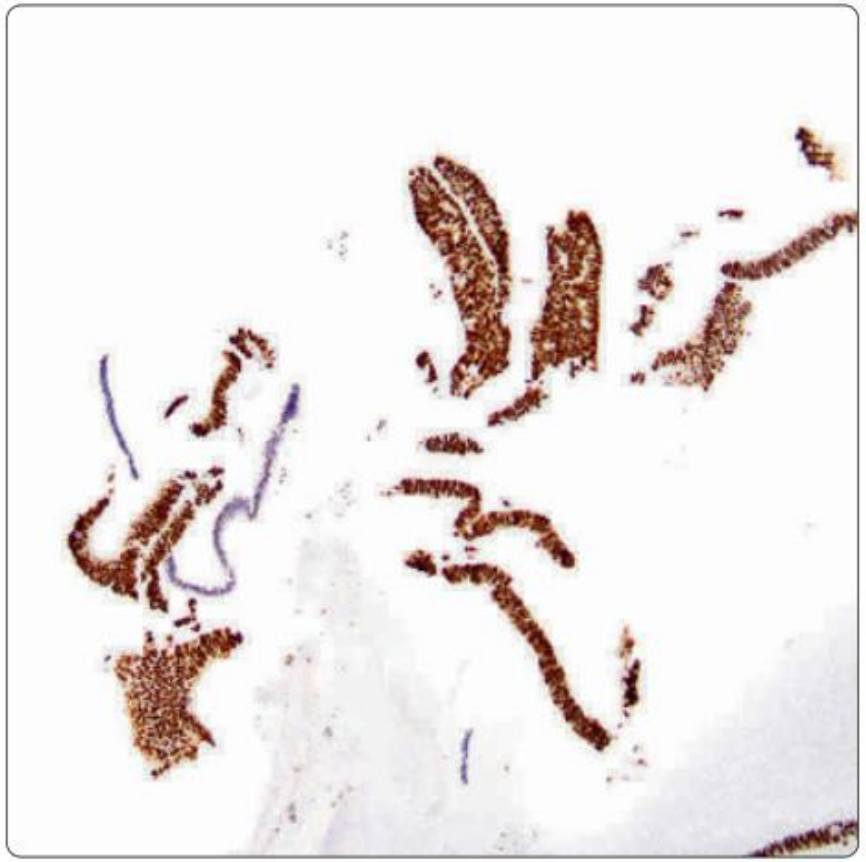


UTERINE SEROUS CARCINOMA

Limited Surface Involvement



Diffuse and Strong p53 Positivity in Atypical Strips in Biopsy



PRACTICAL PEARLS IN THE DIAGNOSIS OF SEROUS CARCINOMA

- Pitfall #1

Diffuse p16 expression is not specific for serous carcinoma.

  - Typical endometrioid carcinoma:
    - Patchy p16 expression
  - However:
    - Diffuse p16 expression may occur in 2–5% of low-grade and up to 20% of high-grade endometrioid carcinomas

- Pitfall #2
- Aberrant p53 expression is not synonymous with serous carcinoma.
  - Approximately 30% of grade 3 endometrioid carcinomas show abnormal p53 staining
- POLE-mutated endometrioid carcinomas may demonstrate:
  - Aberrant p53 expression
  - Marked nuclear atypia
  - Tumor giant cells
  - Prominent tumor-infiltrating lymphocytes
- When classic serous morphology is absent, consider POLE testing before classifying the tumor as serous carcinoma.

PRACTICAL PEARLS IN THE DIAGNOSIS OF SEROUS CARCINOMA

- Pitfall #3
- When making diagnosis of serous carcinoma, always exclude component of carcinosarcoma.
  - Carefully search for a sarcomatous component
  - Extensive sampling may be required
- Identification of a mesenchymal component changes tumor classification and clinical management

- Key Message
  - Neither diffuse p16 nor abnormal p53 alone is diagnostic of serous carcinoma.
  - Morphologic correlation remains essential.

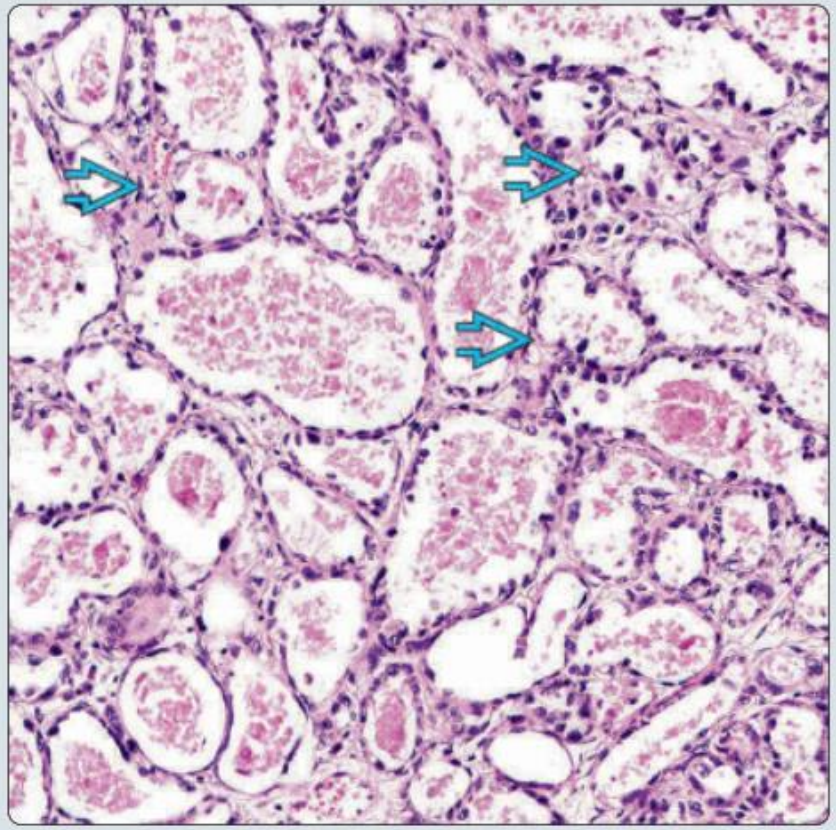
CLEAR CELL CARCINOMA

- Key Morphologic Features
  - Tubulocystic architecture
  - Papillary architecture
  - Solid growth
  - Hyalinized stromal cores
  - Hobnail cells
  - Clear or eosinophilic cytoplasm
- Diagnostic Challenges
- May mimic:
  - Serous carcinoma
  - High-grade endometrioid carcinoma
  - Mesonephric-like adenocarcinoma

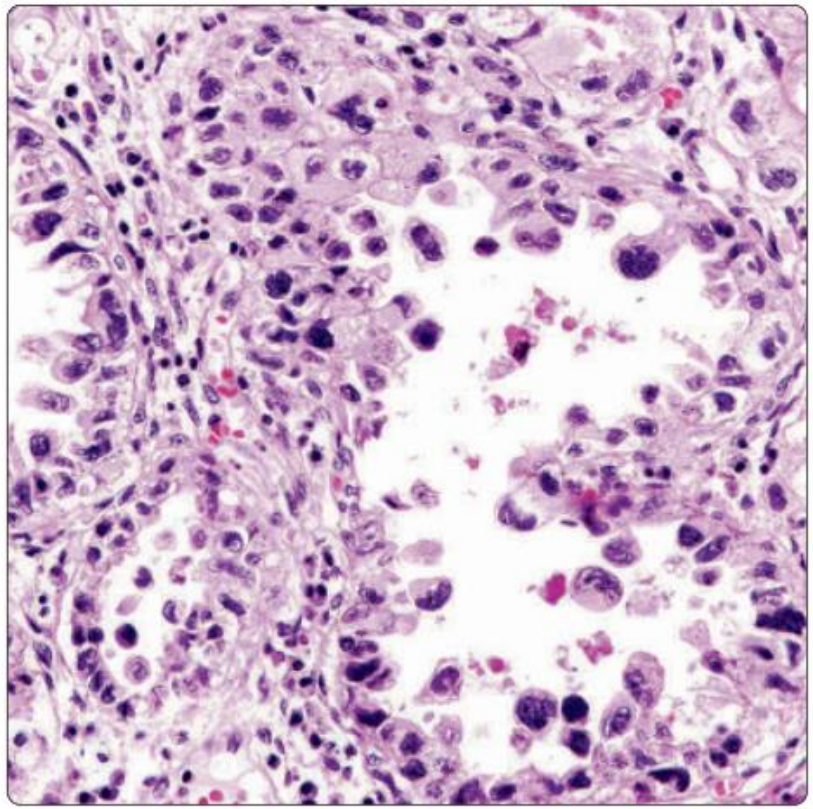
Morphology remains the most important diagnostic tool.

CLEAR CELL CARCINOMA

Tubulocystic Pattern



Prominent Hobnail Cells



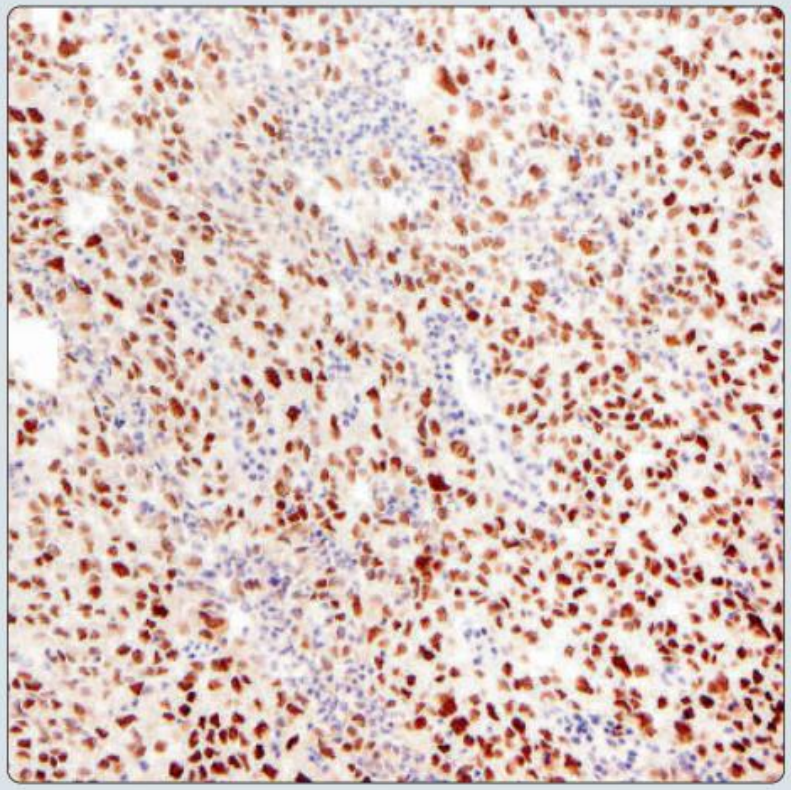
IMMUNOPHENOTYPE OF CLEAR CELL CARCINOMA

- Findings Supporting Clear Cell Carcinoma
  - HNF1β positive
  - Napsin A positive
  - AMACR positive
  - ER/PR negative or only focal
  - PAX8 positive
- Findings That Do NOT Exclude Clear Cell Carcinoma
  - Diffuse p16 expression
  - Abnormal p53 expression (30–40%)
  - WT1 positivity

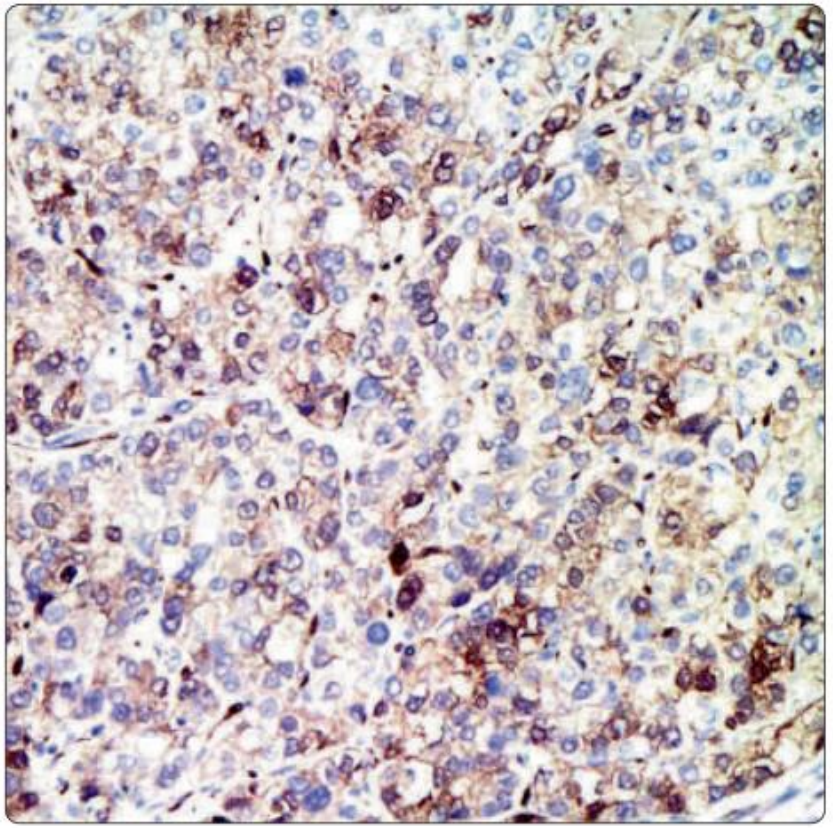
- Clear cell carcinoma is primarily a morphologic diagnosis.
- Napsin A and HNF1β support the diagnosis, but neither marker is entirely specific

IMMUNOPHENOTYPE OF CLEAR CELL CARCINOMA

Diffuse and Strong Nuclear HNF-1-β Positivity

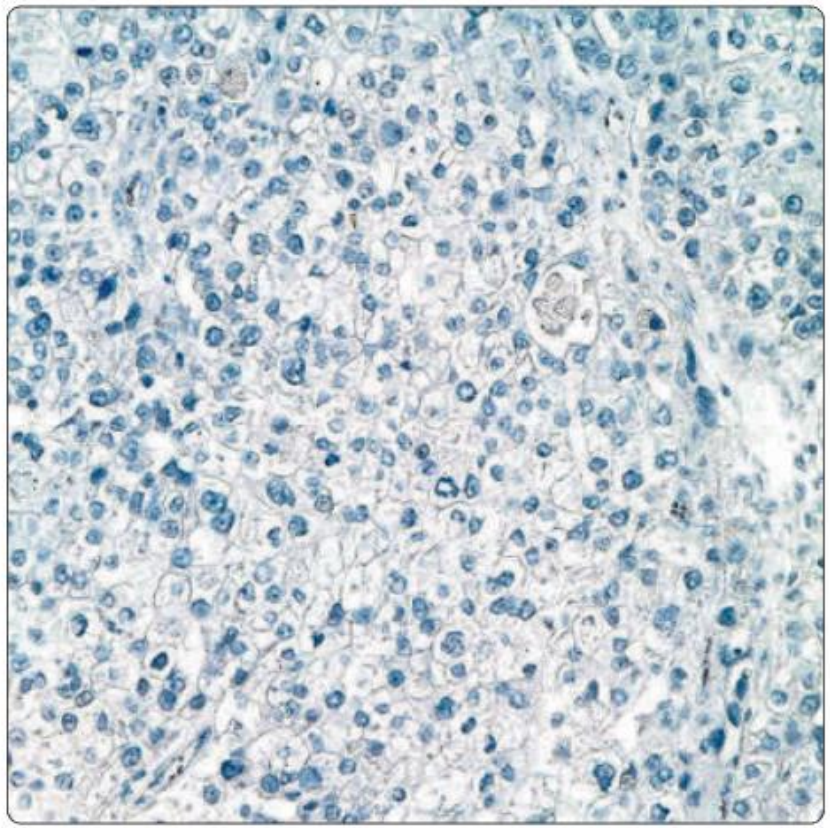


p16 Patchy and Weak Positivity

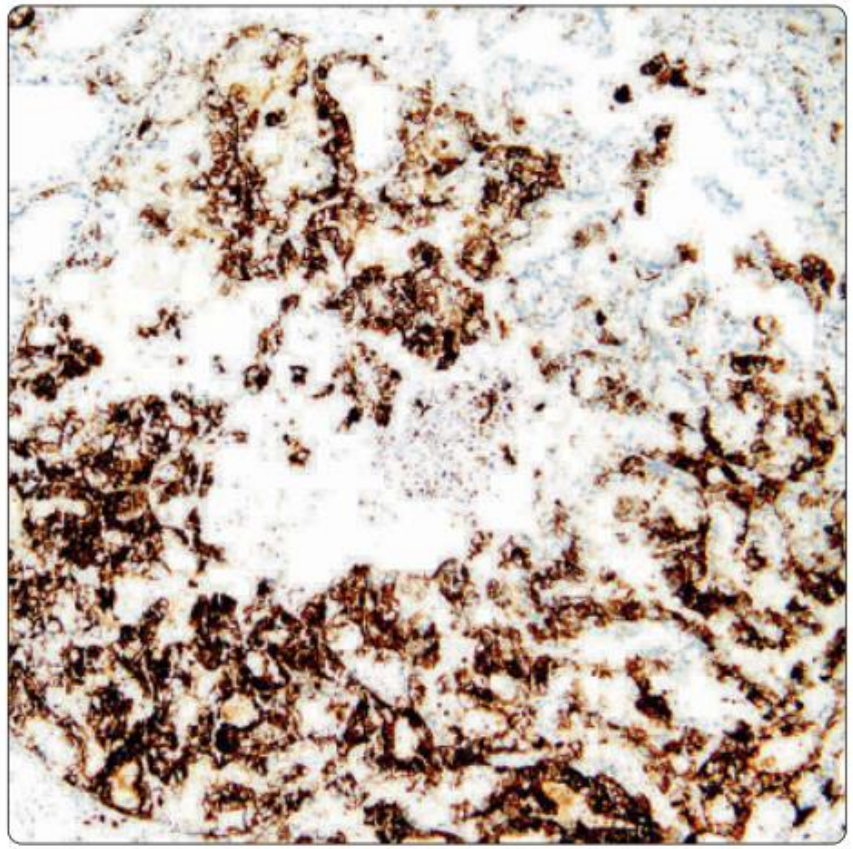


IMMUNOPHENOTYPE OF CLEAR CELL CARCINOMA

Negative ER

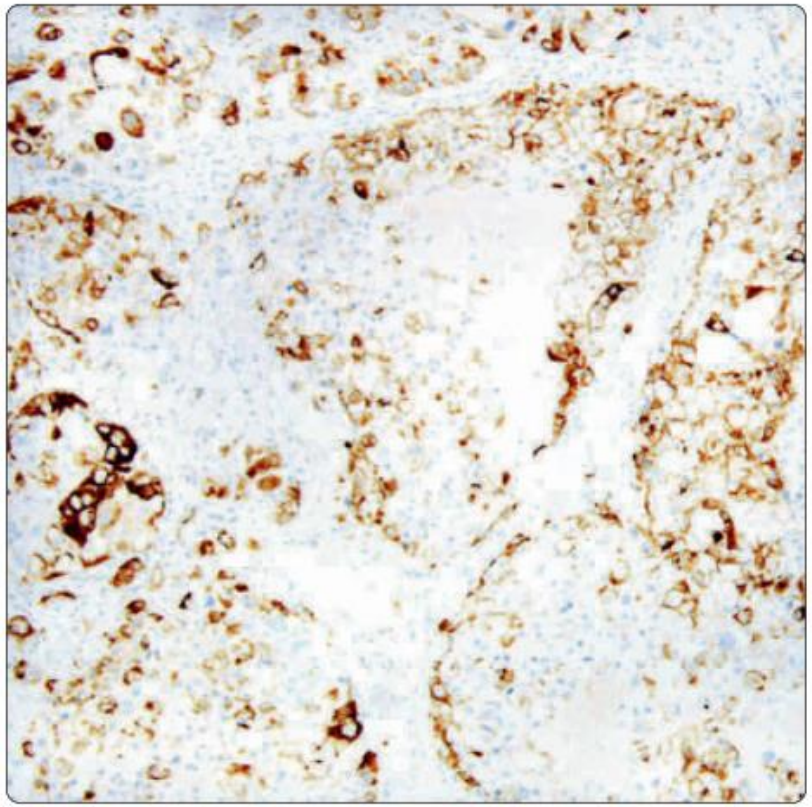


Strong Napsin A Positivity

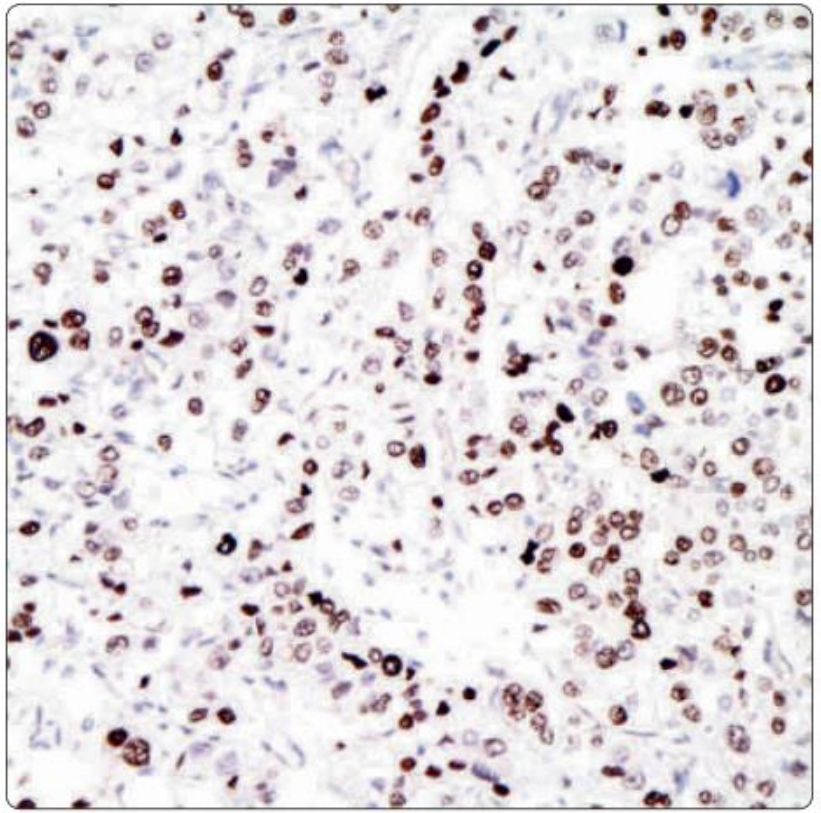


IMMUNOPHENOTYPE OF CLEAR CELL CARCINOMA

Patchy AMACR Positivity



p53 Wild-Type Expression

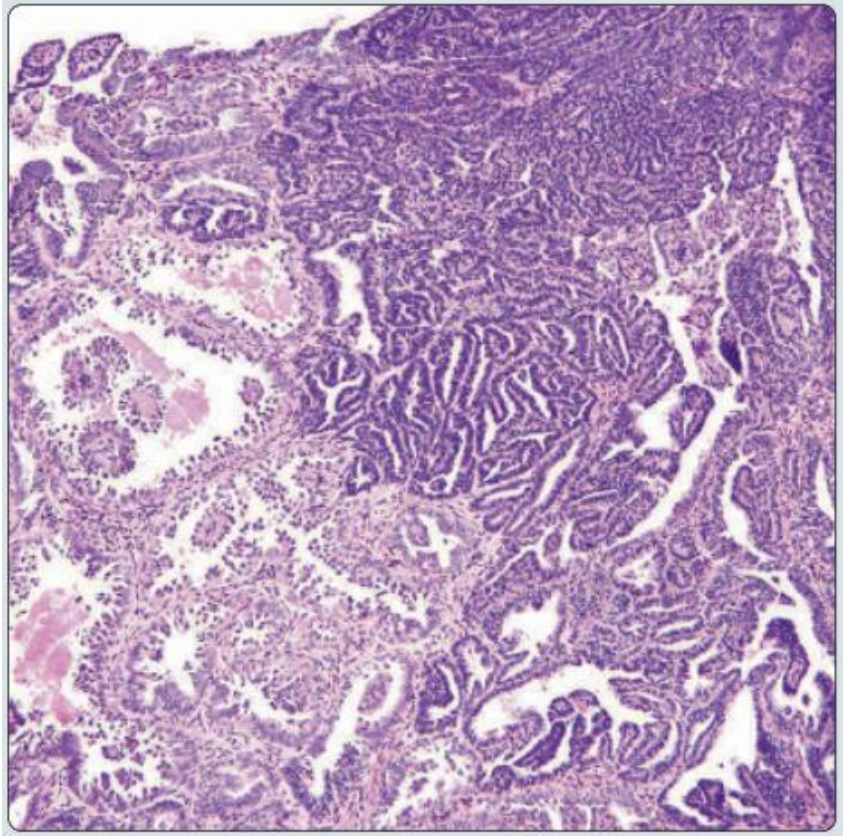


MESONEPHRIC-LIKE ADENOCARCINOMA

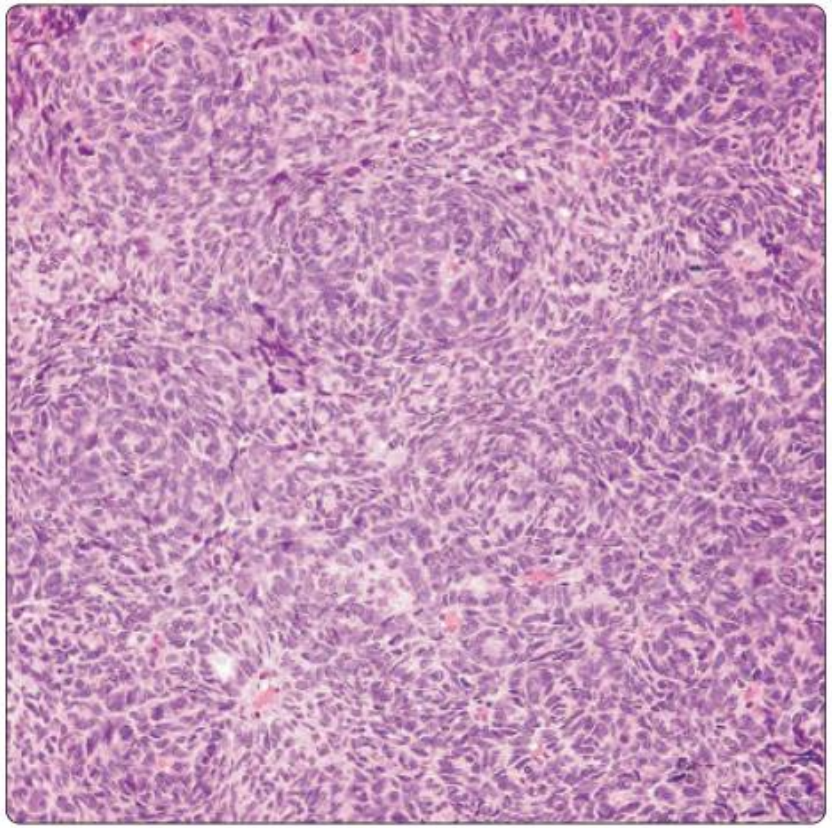
- Key Morphologic Features
  - Tubular architecture
  - Ductal architecture
  - Small angulated glands
  - Intraluminal eosinophilic secretions
  - Lacks squamous and mucinous differentiation
  - Papillary thyroid carcinoma-like nuclei
- Characteristic Immunophenotype
  - GATA3 positive
  - TTF1 positive
  - CD10 positive
- Typically
  - p53 wild type
  - Patchy p16 expression
  - ER negative
  - PR negative

MESONEPHRIC-LIKE ADENOCARCINOMA

Admixture of Patterns

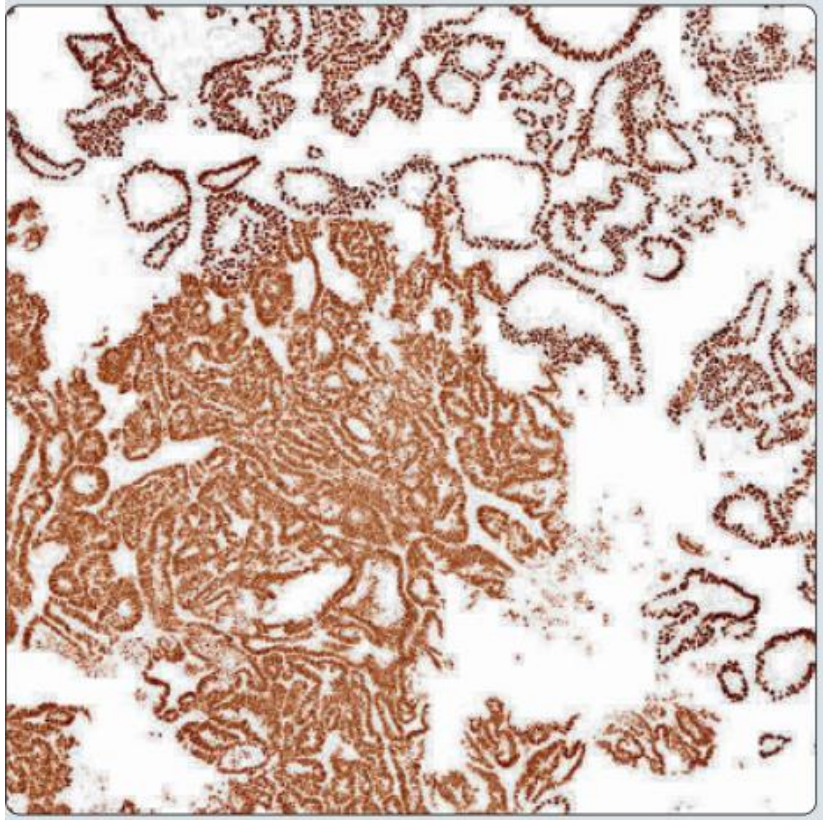


Solid Spindled Growth

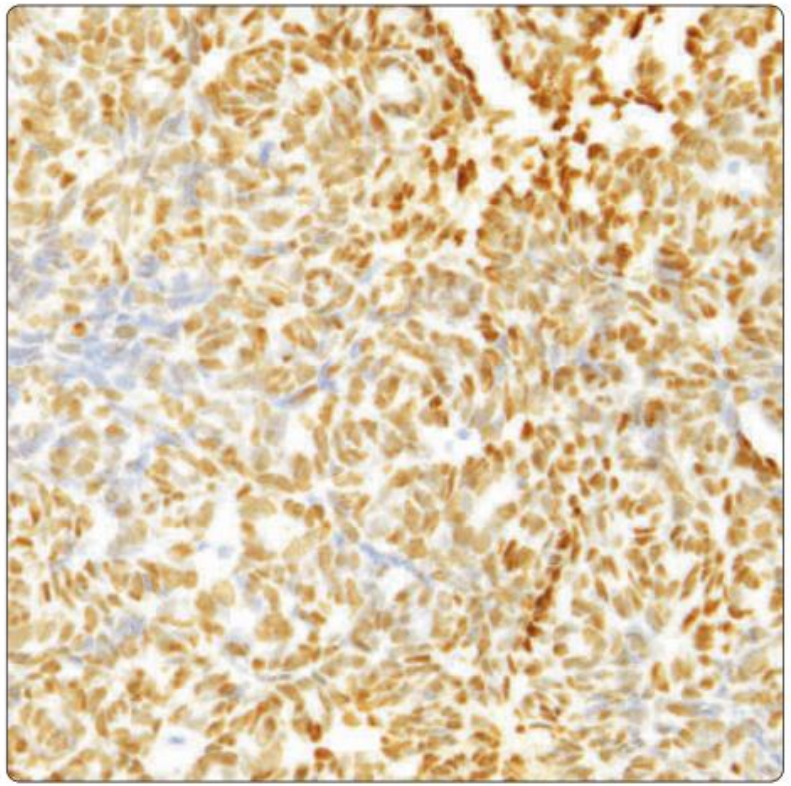


MESONEPHRIC-LIKE ADENOCARCINOMA

TTF-1 Nuclear Positivity



GATA3 Positivity

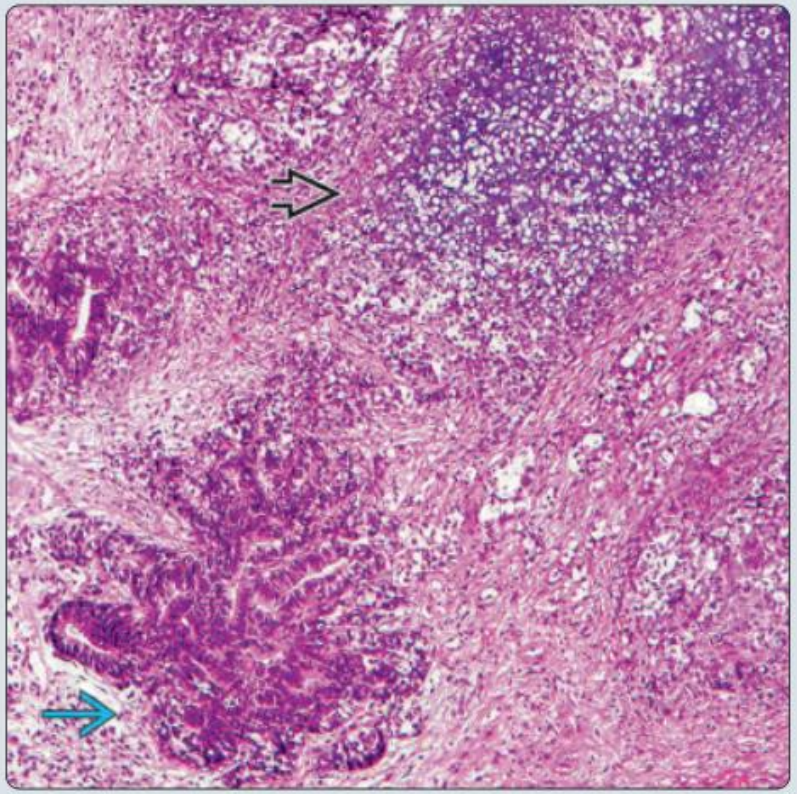


CARCINOSARCOMA

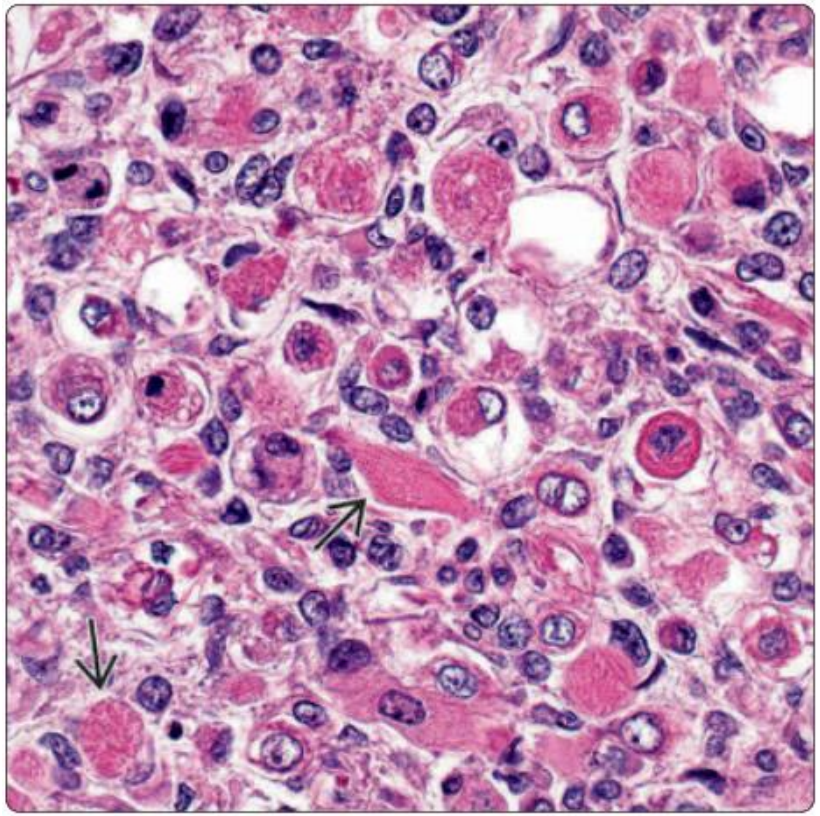
- Key Morphologic Features
- Biphasic Malignancy
  - Carcinomatous component
  - Sarcomatous component
- Diagnostic Message
  - Carcinosarcoma is usually a metaplastic carcinoma rather than a true collision tumor.
  - Both components commonly share the same molecular alterations.
- Common Epithelial Components
  - Serous carcinoma
  - High-grade endometrioid carcinoma
- Common Immunohistochemical Findings
  - Aberrant p53
  - Diffuse p16
  - PAX8 positive
- Practical Point
  - Always search carefully for a sarcomatous component before diagnosing a high-grade endometrial carcinoma.

CARCINOSARCOMA

Endometrioid Carcinoma and Chondrosarcoma

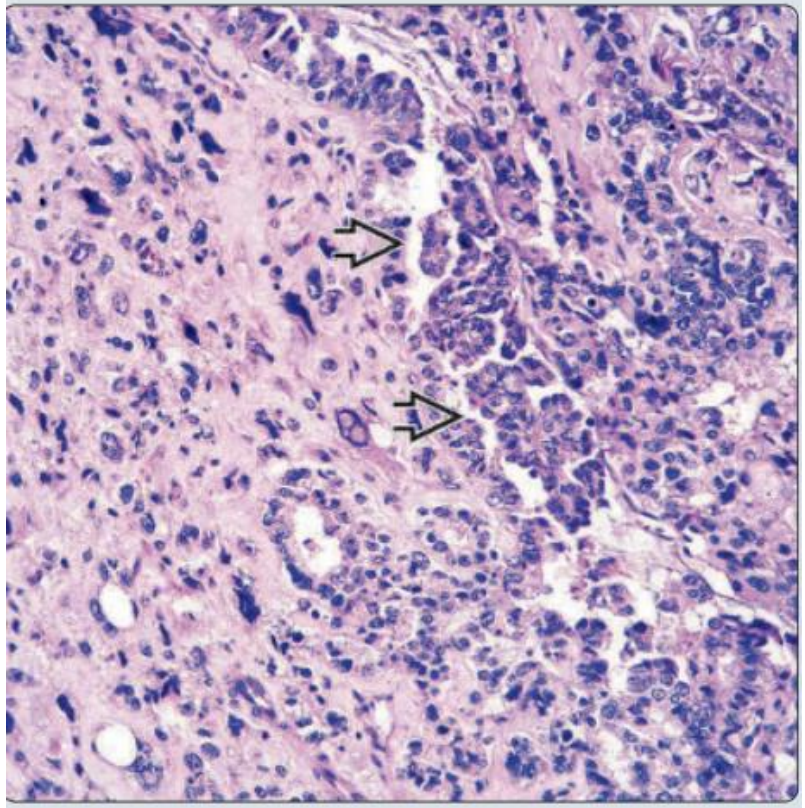


Pleomorphic Rhabdomyosarcoma

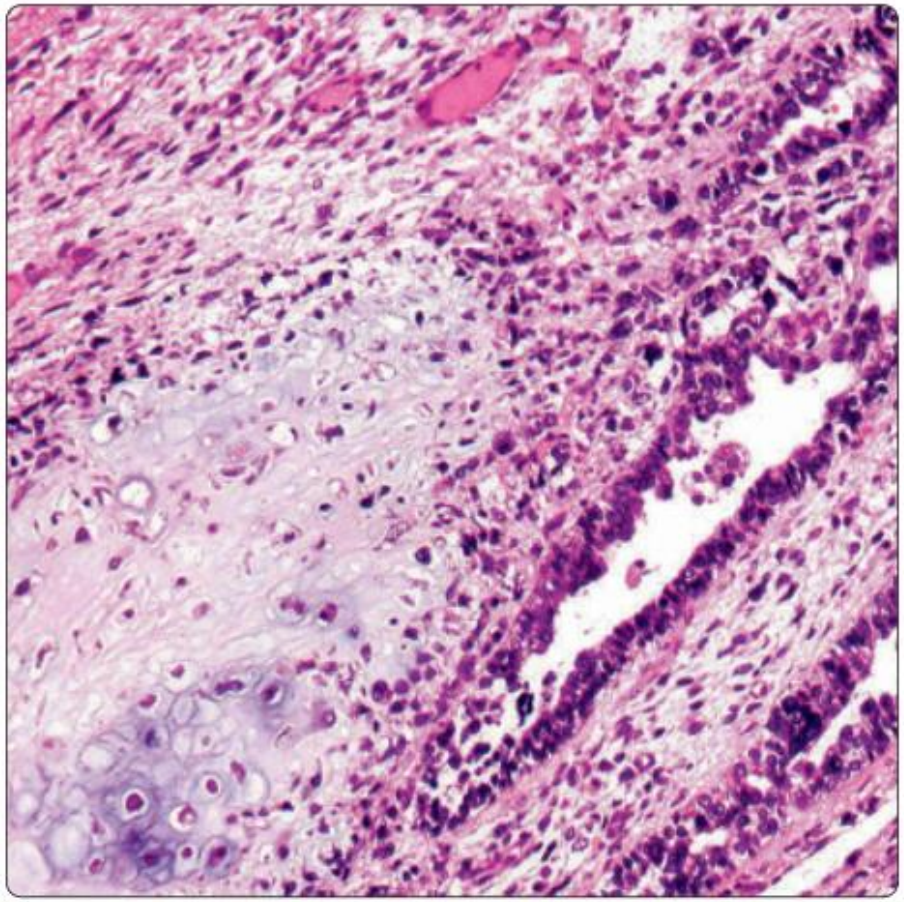


CARCINOSARCOMA

Serous Carcinoma and Homologous Sarcoma

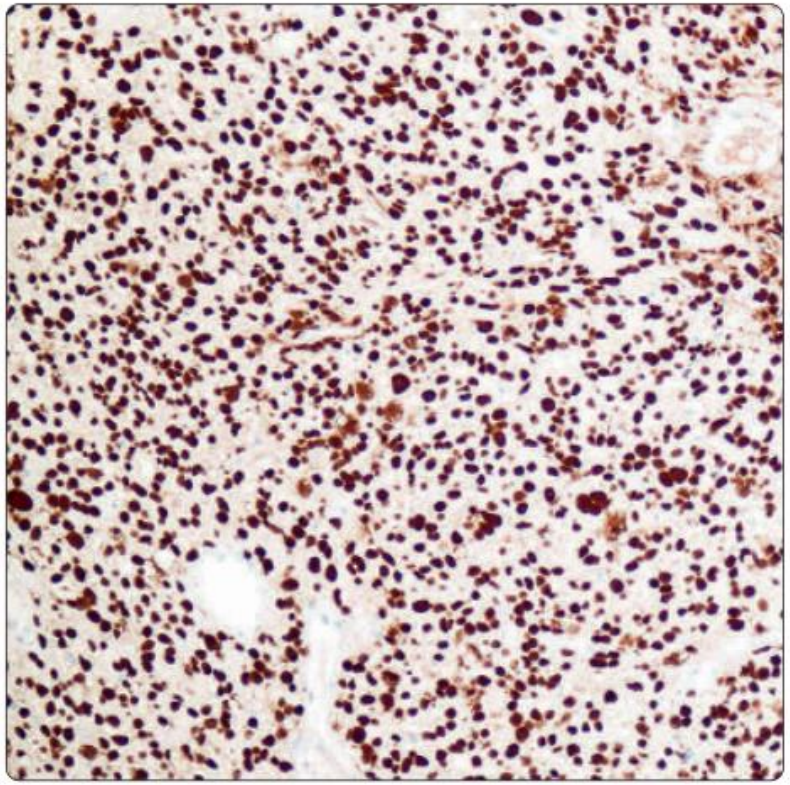


Chondrosarcoma

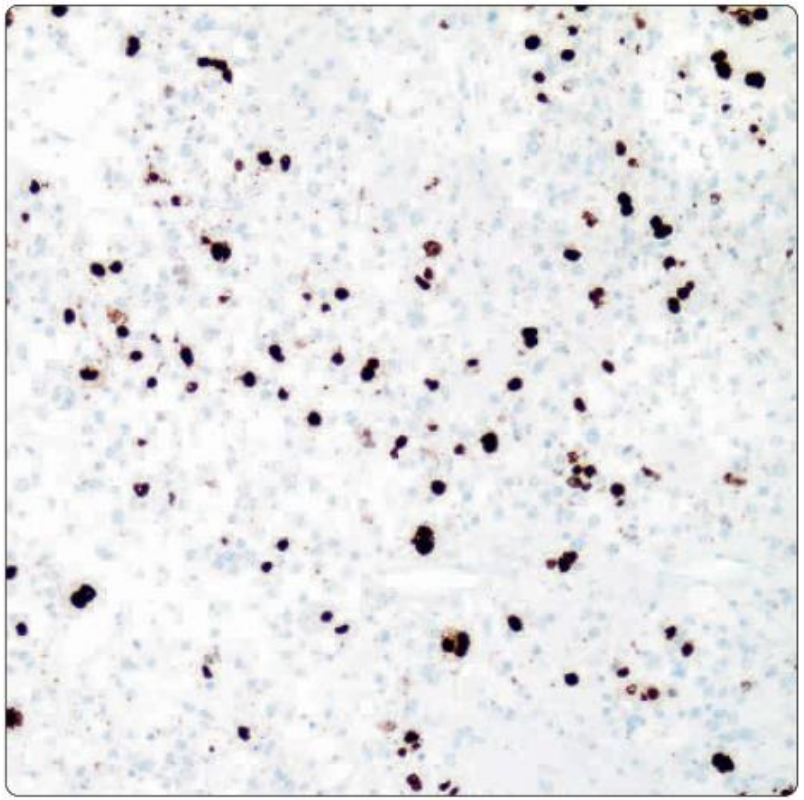


CARCINOSARCOMA

p53 Diffuse Positivity Indicative of *TP53* Mutation



Myogenin (MYF4) Positivity in Rhabdomyoblastic Component



UNDIFFERENTIATED VS DEDIFFERENTIATED CARCINOMA

- Undifferentiated Carcinoma
  - A malignant epithelial neoplasm with no evident line of differentiation.
- Morphology
  - Diffuse solid growth
  - Discohesive tumor cells
  - Marked cytologic atypia
  - Extensive necrosis
  - No gland formation

- Dedifferentiated Carcinoma
- A malignant epithelial neoplasm composed of:
  - An undifferentiated carcinoma component

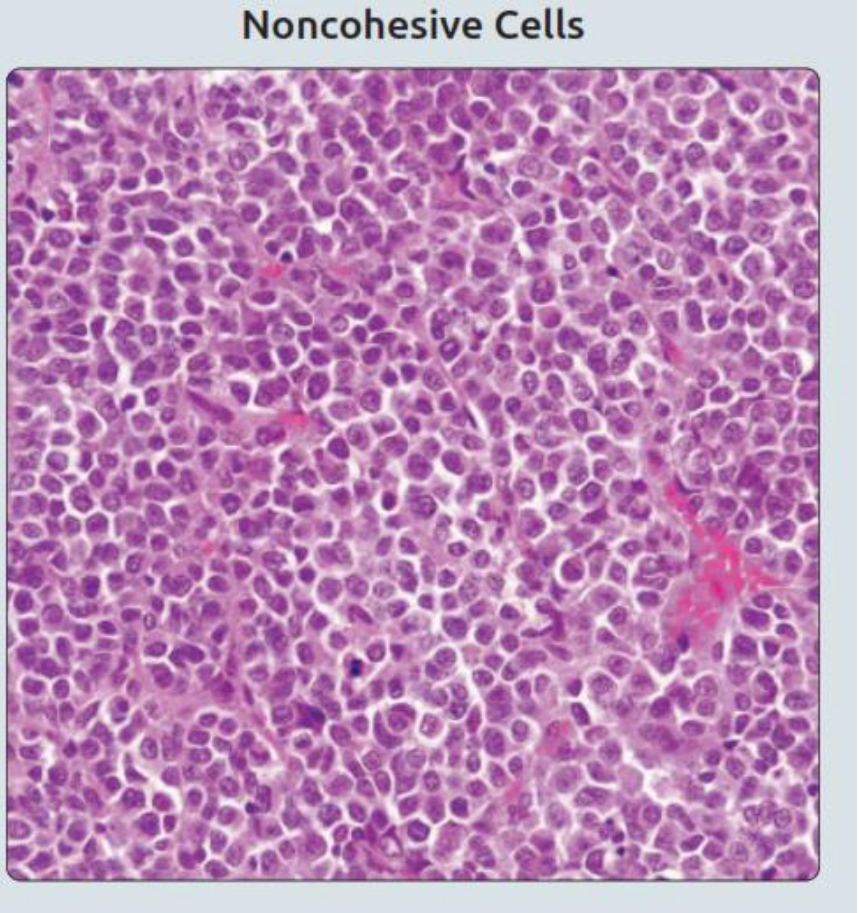
  PLUS
  - A low-grade endometrioid carcinoma component (FIGO grade 1 or 2)

Practical Pearl
If a low-grade endometrioid carcinoma suddenly loses gland formation and becomes a solid discohesive high-grade tumor, consider dedifferentiated carcinoma.

IMMUNOPHENOTYPE OF UNDIFFERENTIATED / DEDIFFERENTIATED CARCINOMA

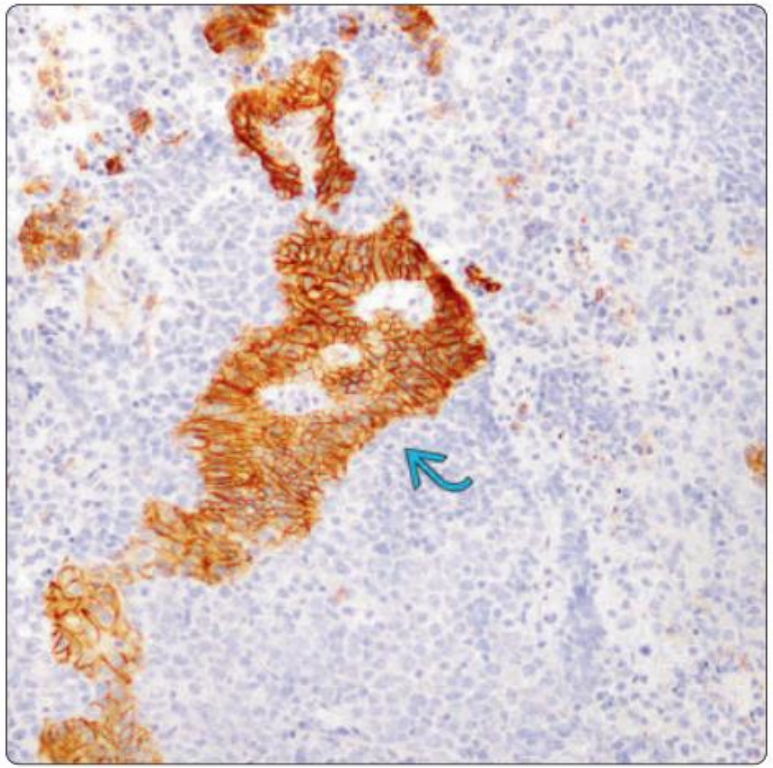
- Undifferentiated Component
  - Cytokeratin absent or only focal positive
  - EMA absent or focal positive
  - PAX8 often lost
  - ER and PR usually negative
  - Claudin-4 absent or only focal
  - Frequently MMR deficient
  - p53 mutant-type expression (~ 50%)
  - p16 (strong and diffuse),

UNDIFFERENTIATED VS DEDIFFERENTIATED CARCINOMA

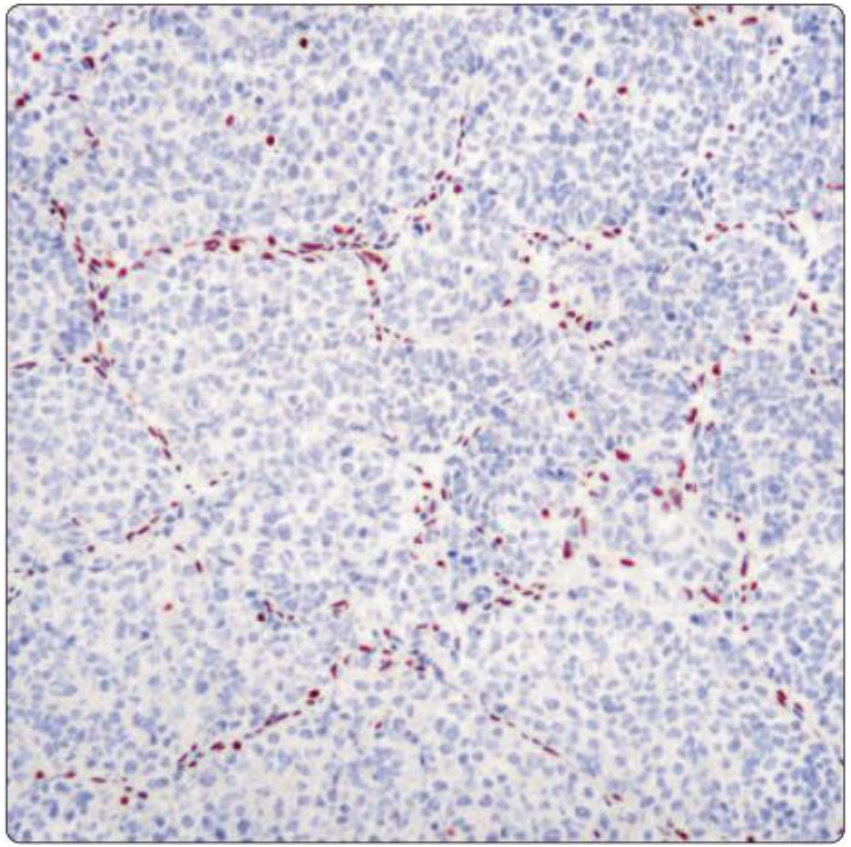


UNDIFFERENTIATED VS DEDIFFERENTIATED CARCINOMA

Loss of E-Cadherin in Dedifferentiated Component



PMS2 Loss



WHEN SHOULD I SUSPECT NEUROENDOCRINE CARCINOMA?

- Morphologic Clues
  - Diffuse solid growth
  - Organoid nesting
  - Trabecular architecture
  - Rosette-like structures
  - Extensive necrosis
  - Brisk mitotic activity
- Small Cell Pattern
  - Nuclear molding
  - Crush artifact
  - Hyperchromatic nuclei
- Large Cell Pattern
  - Prominent nucleoli
  - Abundant cytoplasm
  - Geographic necrosis

Practical Point
Diagnosis begins with morphology, not immunohistochemistry.

IMMUNOHISTOCHEMICAL APPROACH TO NEUROENDOCRINE CARCINOMA

- Neuroendocrine Markers
  - INSM1
  - Synaptophysin
  - Chromogranin
  - CD56
- Additional Findings
  - AE1/AE3 positive
  - CAM5.2 positive
  - CK7 positive
  - p16 frequently positive
  - PAX8 may be positive
  - MMR deficiency in a subset

NEUROENDOCRINE CARCINOMA: DIAGNOSTIC PEARLS AND PITFALLS

- Positivity for neuroendocrine markers not necessary if classic morphologic features of SCNEC
- Tumors lacking NET morphology should not be classified as LCNEC, even in presence of neuroendocrine expression
- INSM1 may be less sensitive but more specific for endometrial neuroendocrine carcinoma

- Large Cell Neuroendocrine Carcinoma (LCNEC)
- Recommended Criteria
- Appropriate morphology

PLUS

- Chromogranin and/or Synaptophysin positivity in >10% of tumor cells
- CD56 Alone Is Not Enough

IHC Markers for High-Grade Endometrial Carcinomas

IHC Markers	Tumor Types					
	Serous	Clear Cell	Endometrioid FIGO 3	Undifferentiated	Mesonephric-like	Carcinosarcoma
p53	Mutant	Mutant	Wild-type (weak/patchy) ~30% mutant	<10% mutant	Wild-type	Mutant
p16	+	+	+	+	+	+
WT1	+	-	-	-	-	+
Napsin A	-	+	-	-	-	-
HNF1β	-	+	-	-	-	-
GATA3	-	-	-	-	+	-
TTF1	-	-	-	-	+	-
ER/PR	+/-	-	+	-	-	+/-
CK	+	+	+	+	+	+
EMA	+	+	+	+	+	+
SWI/SNF	Intact	Intact	Intact	Loss	Intact	Loss


THANK
YOU!