Prognostic and Therapeutic Biomarkers in Gynecologic Pathology

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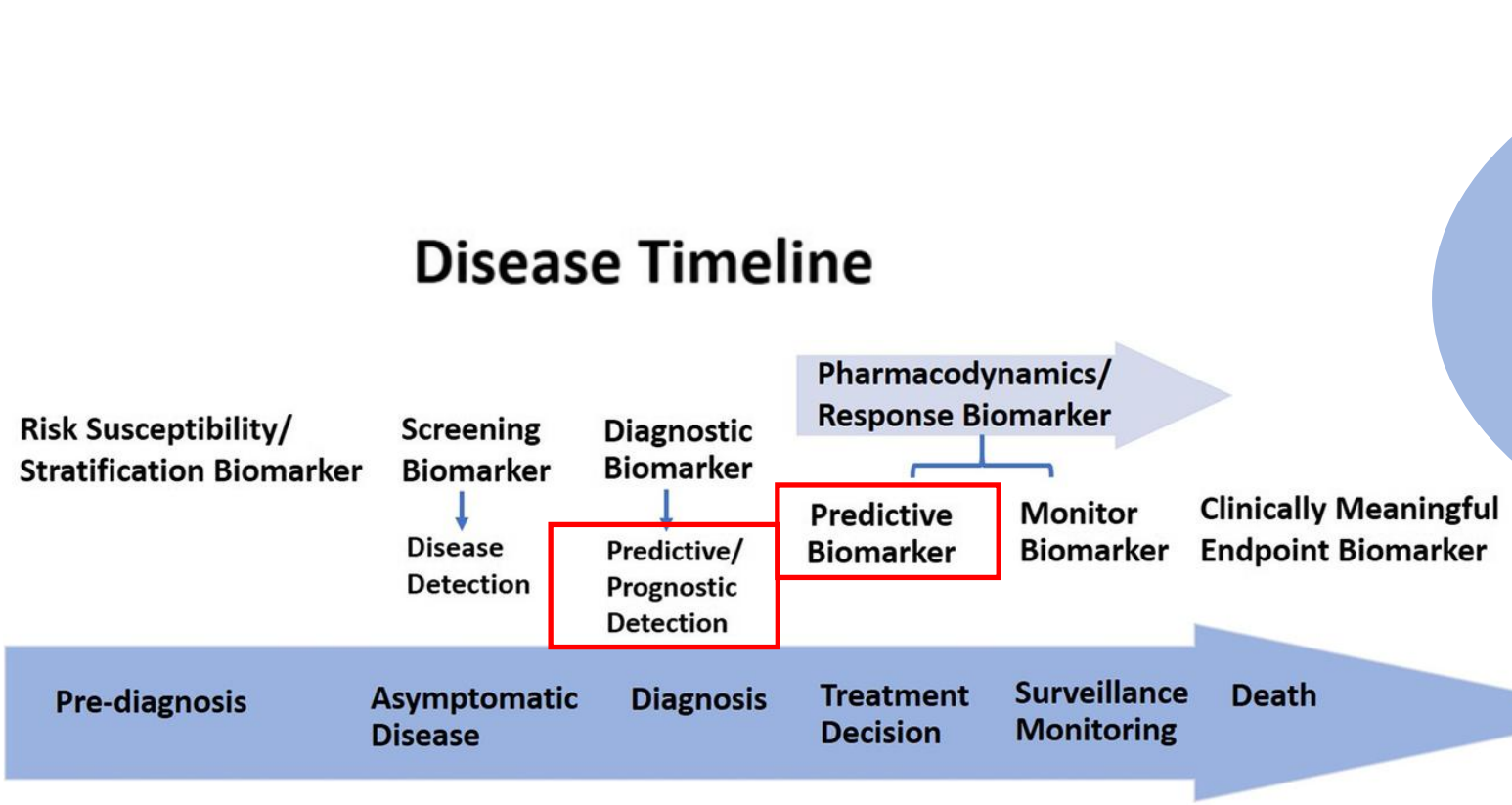
Biomarker Categories Help to Place Continuum Order in the Progression of Disease



PROGNOSTIC MARKER

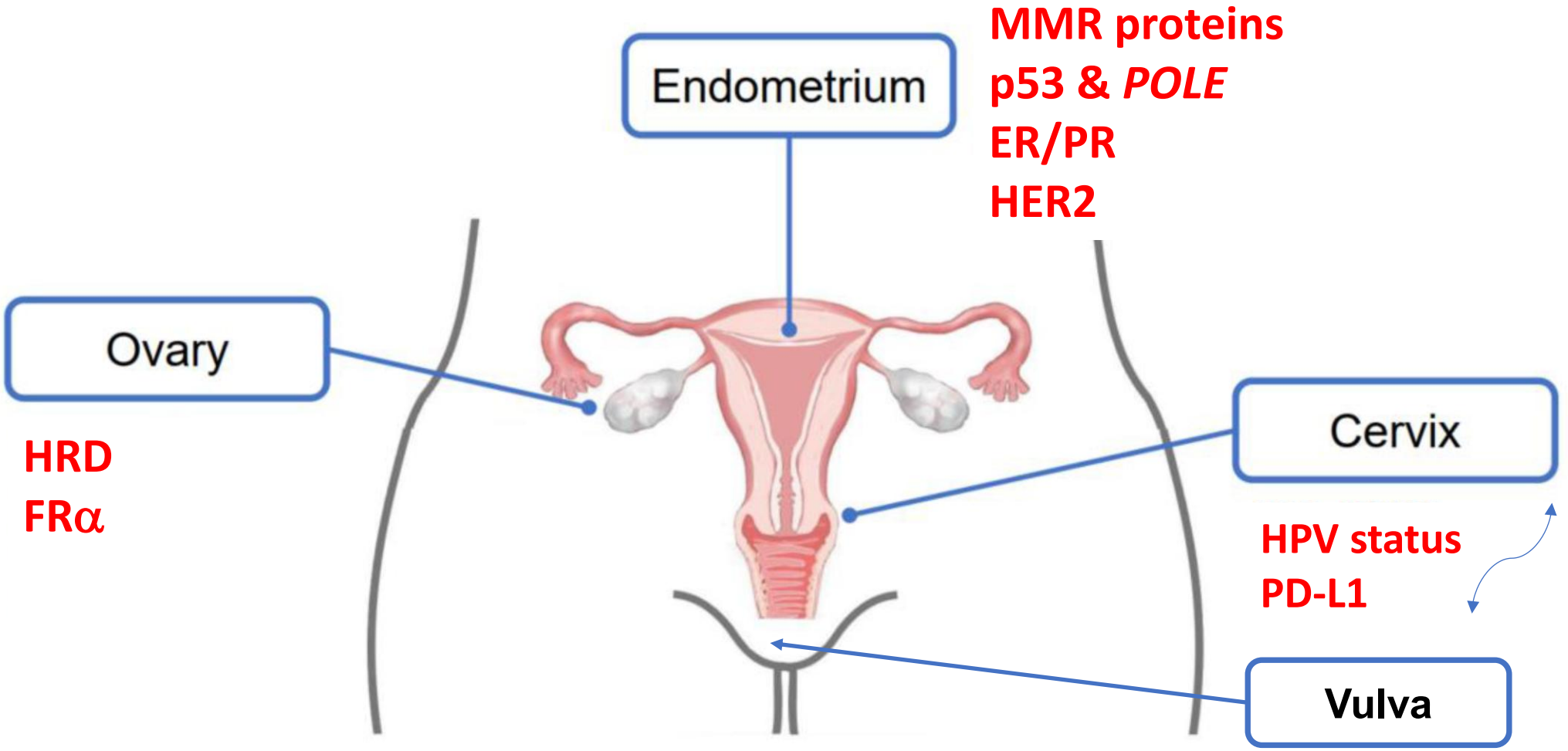
A measurement that is associated with clinical outcome in the absence of therapy or with the application of a standard therapy

PREDICTIVE MARKER

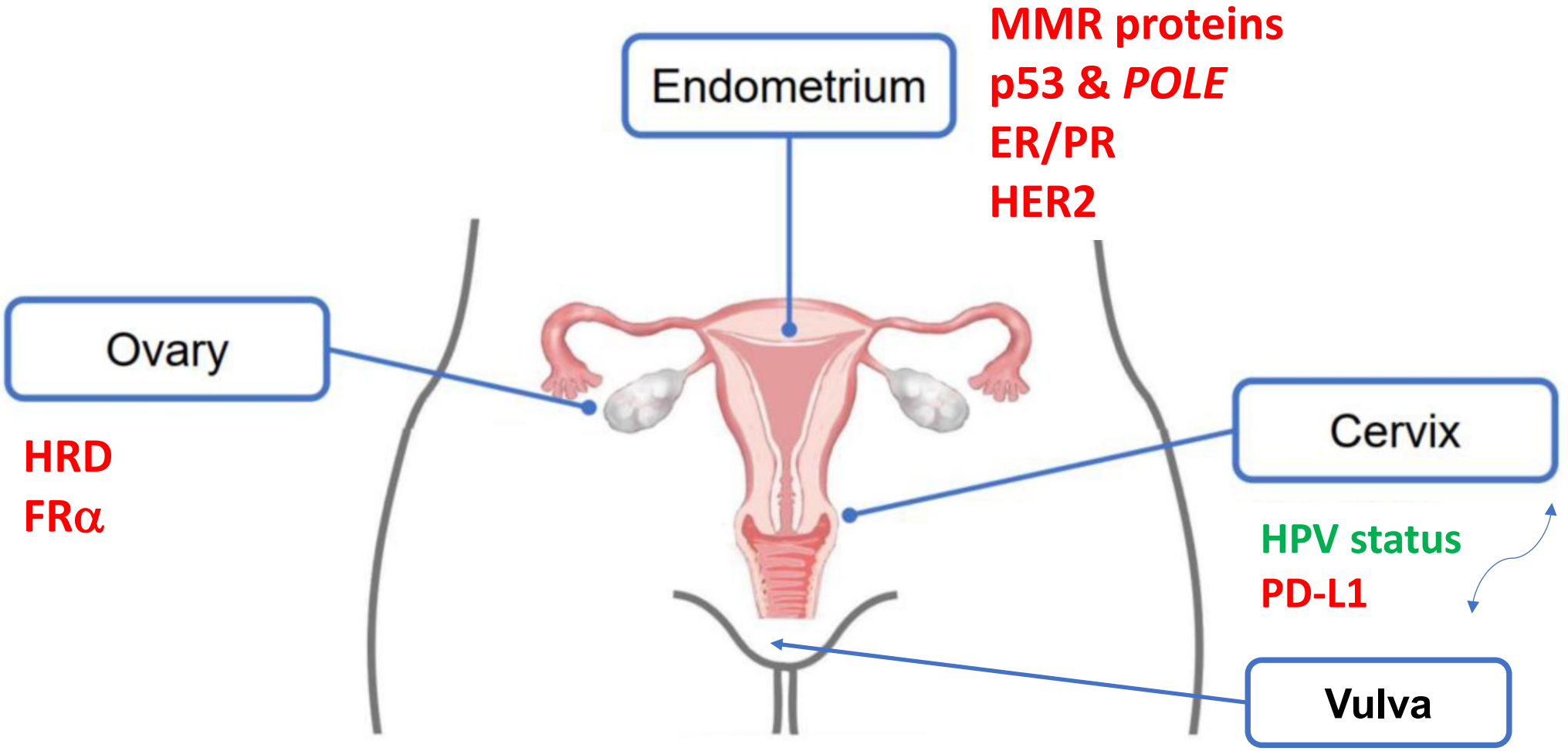
A measurement that is associated with response or lack of response to a particular therapy

BEST (Biomarkers, EndpointS, and other Tools) Resource, FDA-NIH Biomarker Working Group; PMID: 36991494, 17409968

Prognostic and Therapeutic Markers in Gynecologic Pathology



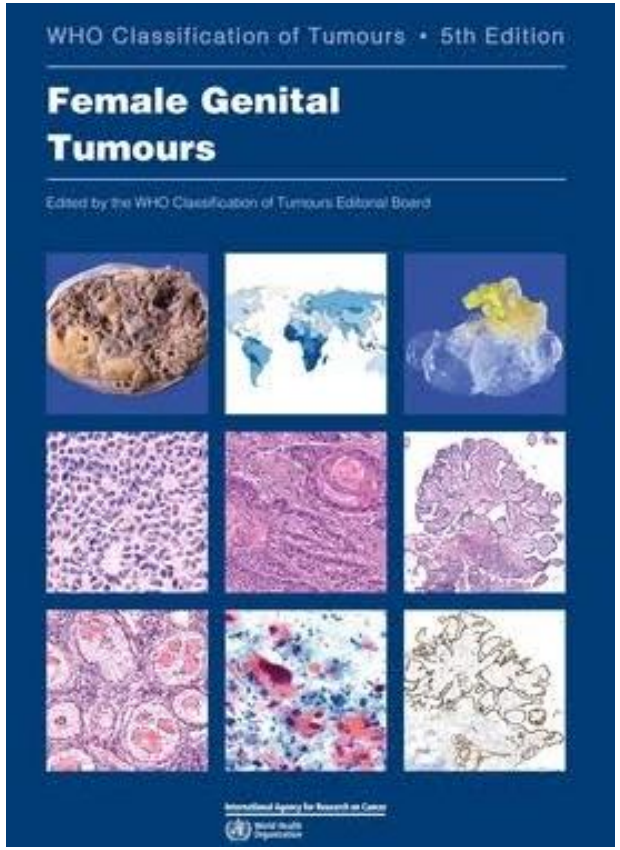
Prognostic and Therapeutic Markers in Gynecologic Pathology



Cervical Carcinomas and Precursors, and Vulvar Squamous Cell Carcinoma (SCC) and Precursors Are Classified Based on Their Association with Human Papillomavirus (HPV)

Cervix:

- **Adenocarcinomas:**
  - Adenocarcinoma in situ, HPV-associated
  - Adenocarcinoma in situ, HPV-independent
  - Adenocarcinoma, HPV-associated
  - Adenocarcinoma, HPV-independent:
    - ✓ Gastric, Clear cell, Mesonephric
  - Other
- ***Squamous lesions:***
  - *Squamous intraepithelial lesions, HPV-associated*
  - *SCC, HPV-associated*
  - *SCC, HPV-independent*
  - *SCC NOS*



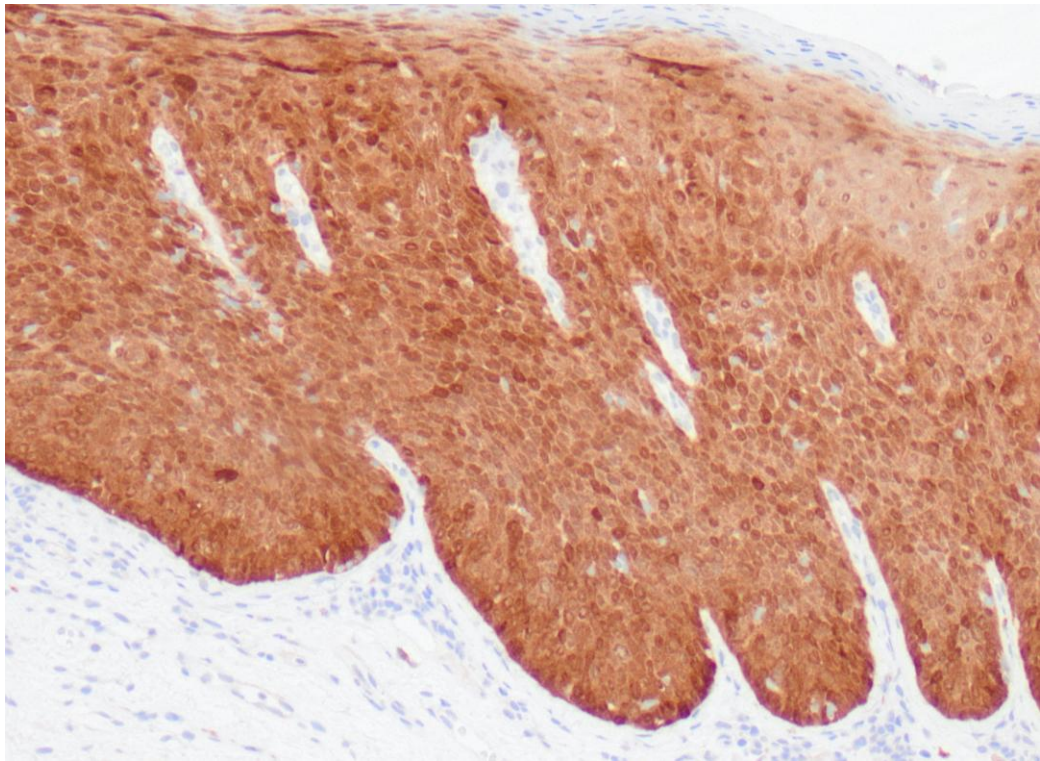
Vulva:

- Squamous intraepithelial lesions, HPV-associated
- Vulvar intraepithelial neoplasia, HPV-independent
- SCC, HPV-associated
- SCC, HPV-independent
- SCC NOS
- Basal cell carcinoma

HPV Status is Determined by p16 Immunohistochemistry (Surrogate) or Molecular Methods, e.g. RNA *In Situ* Hybridization (ISH) or Polymerase Chain Reaction (PCR)

- **The Lower Anogenital Squamous Terminology (LAST) recommendations for p16 immunohistochemistry:**
  - ✓ Morphologic differential diagnosis between precancer (HSIL) and a benign mimic
  - ✓ Morphologic diagnosis is between LSIL and HSIL / CIN 2
  - ✓ Adjudication tool for disagreement in morphologic interpretation where the differential diagnosis includes HSIL

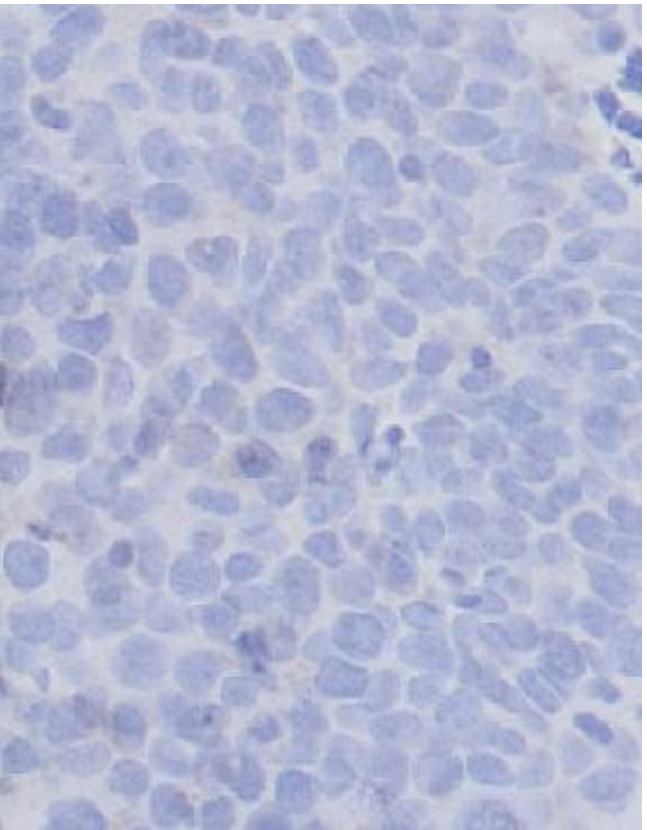
Positive / "Block-like" Expression is Defined as Diffuse, Strong Nuclear or Nuclear-Cytoplasmic Staining Involving ≥1/3 of the Epithelial Thickness



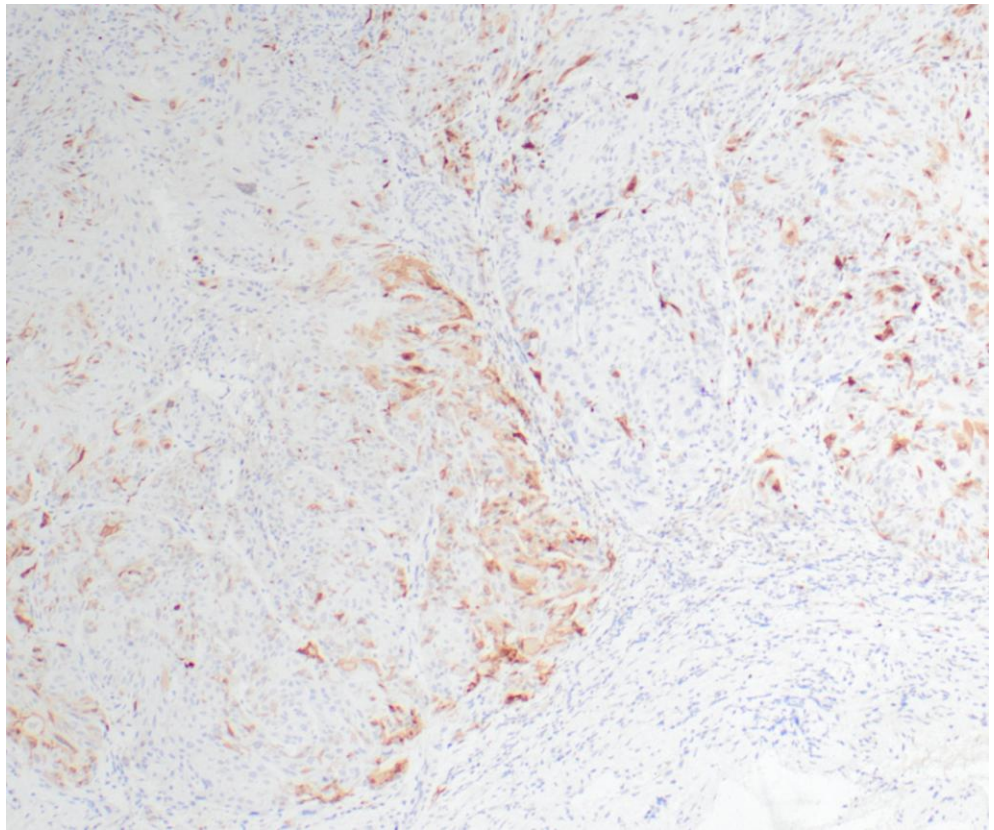
HPV Status is Determined by p16 Immunohistochemistry (Surrogate Marker) or Molecular Methods, e.g. RNA *In Situ* Hybridization (ISH) or Polymerase Chain Reaction (PCR)

Negative p16

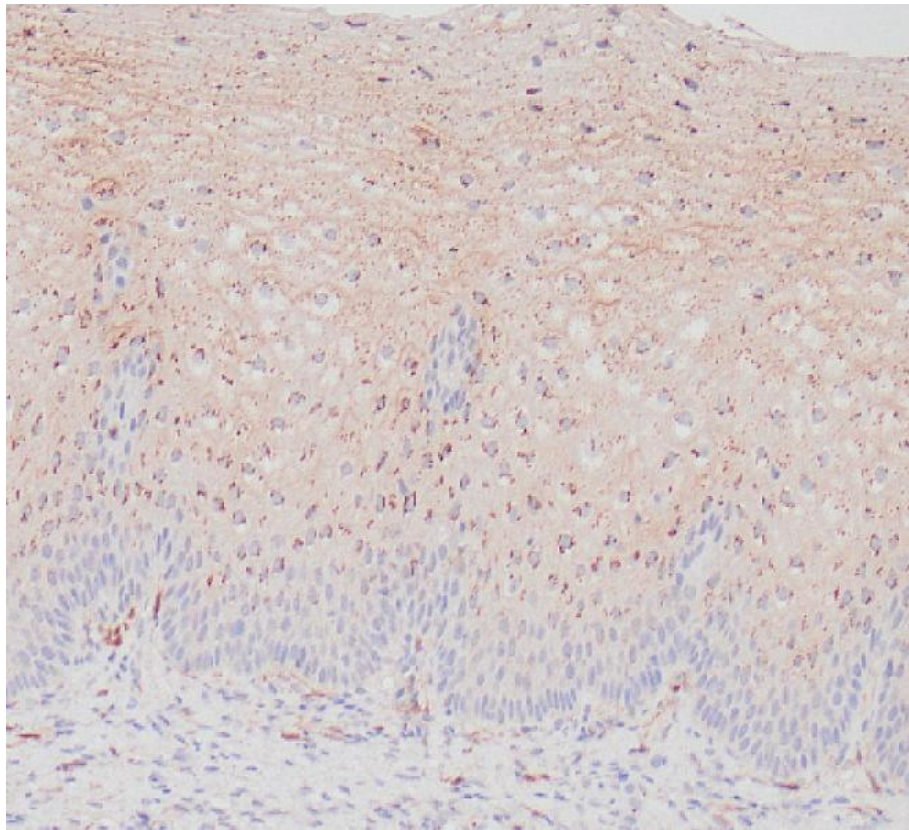
No staining



Focal/patchy staining

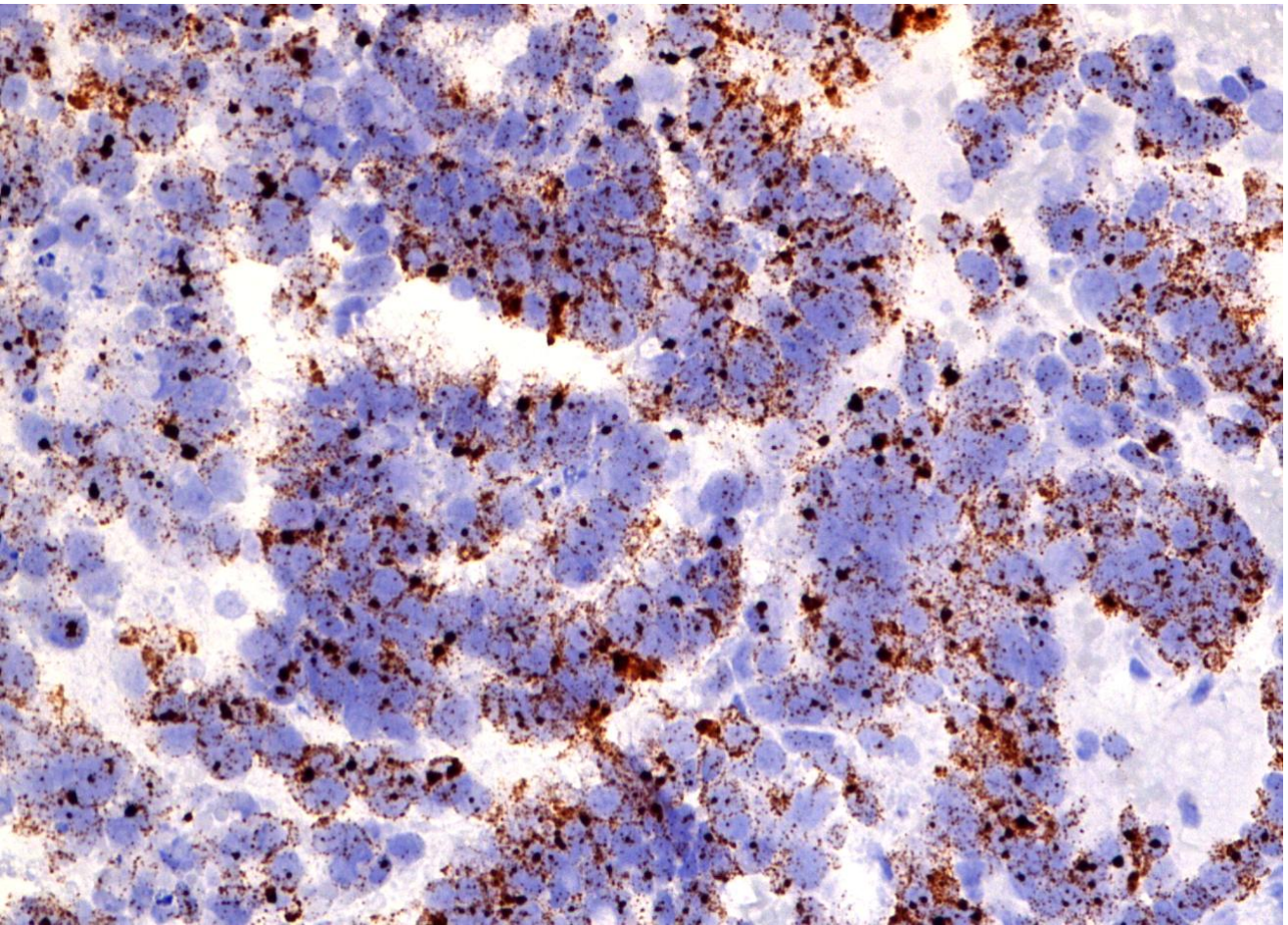


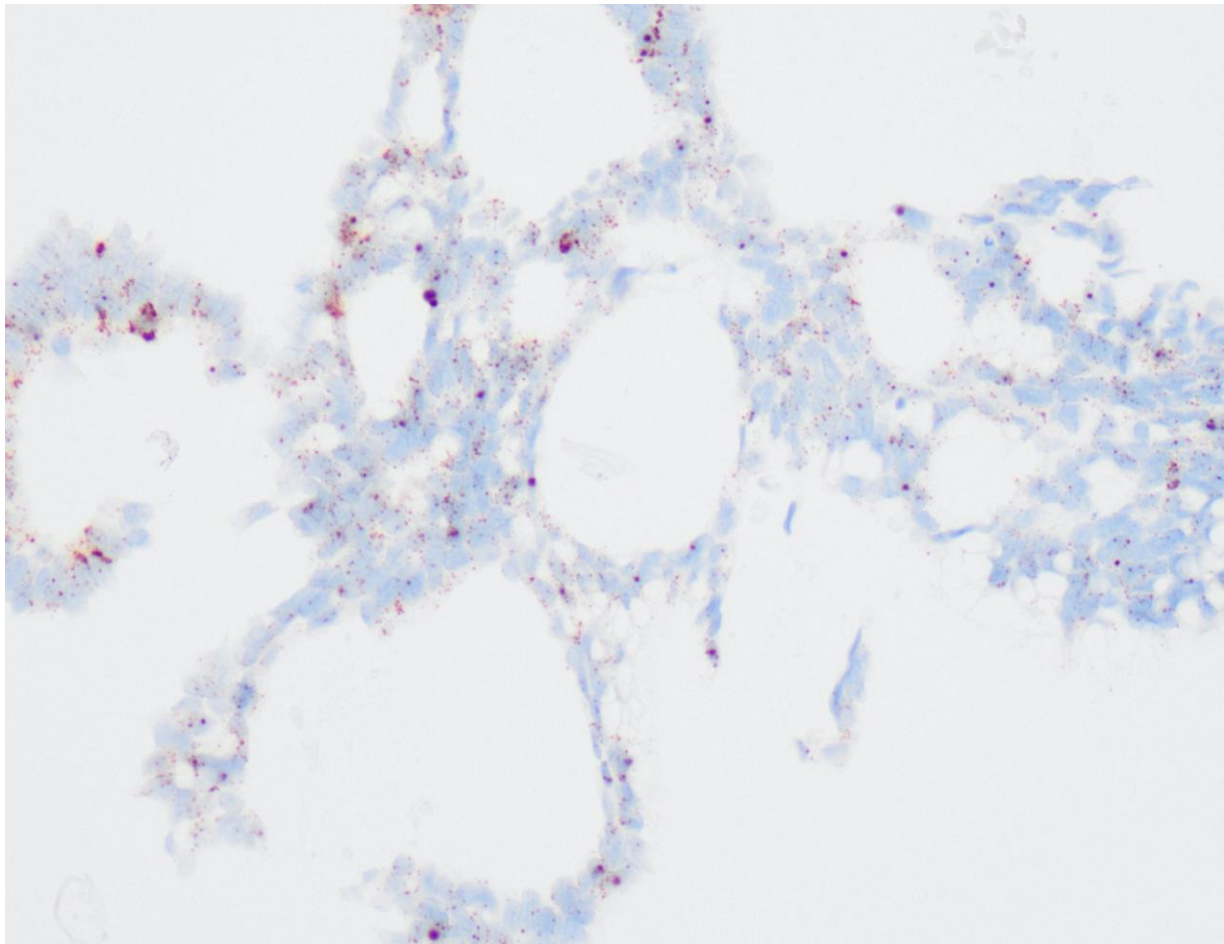
Cytoplasmic only staining



HPV Status is Determined by p16 Immunohistochemistry (Surrogate Marker) or Molecular Methods, e.g. RNA *In Situ* Hybridization (ISH) or Polymerase Chain Reaction (PCR)

Variable Nuclear and Cytoplasmic Staining





RNA HPV ISH Is the Most Sensitive and Specific Method

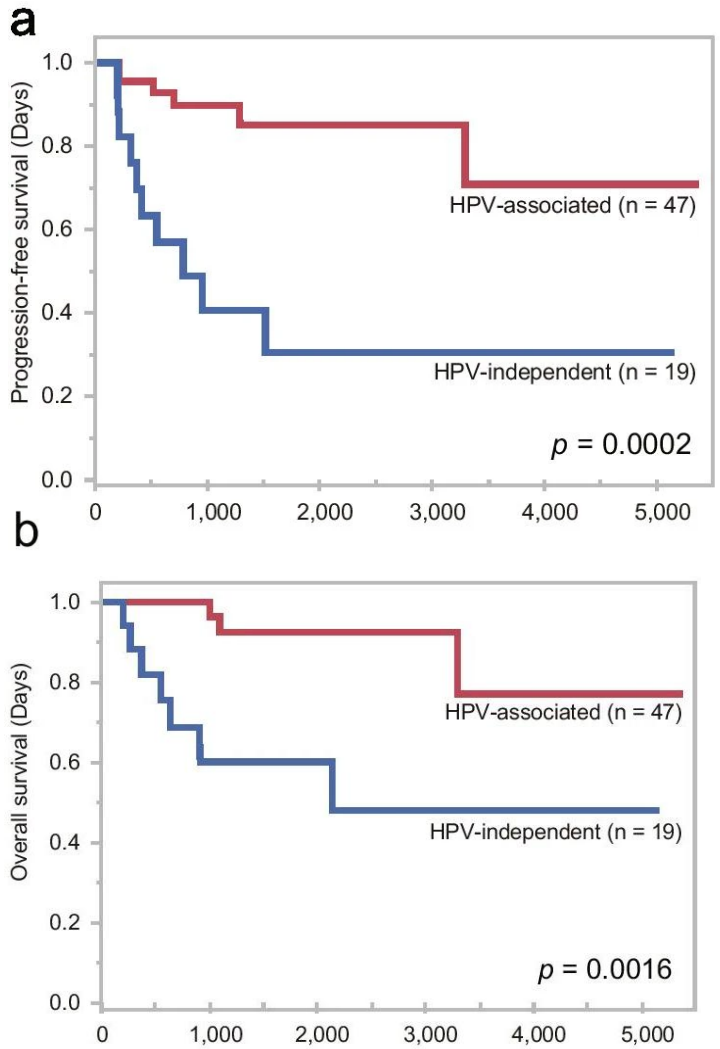
- **Pitfalls with p16 Immunohistochemistry:**
  - LSIL and HPV-independent carcinomas may show diffuse block-like p16 expression
  - Up to a third of HSIL and some HPV-associated carcinomas may be p16 negative

ASSAY METHOD	SENSITIVITY	SPECIFICITY	PPV	NPV
p16 immunohistochemistry	97%	82%	80%	97%
HR-HPV DNA ISH	94%	91%	89%	95%
PCR	91%	87%	83%	93%
RNA HR-HPV ISH	**97%**	**93%**	**91%**	**98%**

PMID: 23412100

HPV-Negative Cervical Adenocarcinomas Are More Aggressive, Larger and Occur in Older Patients

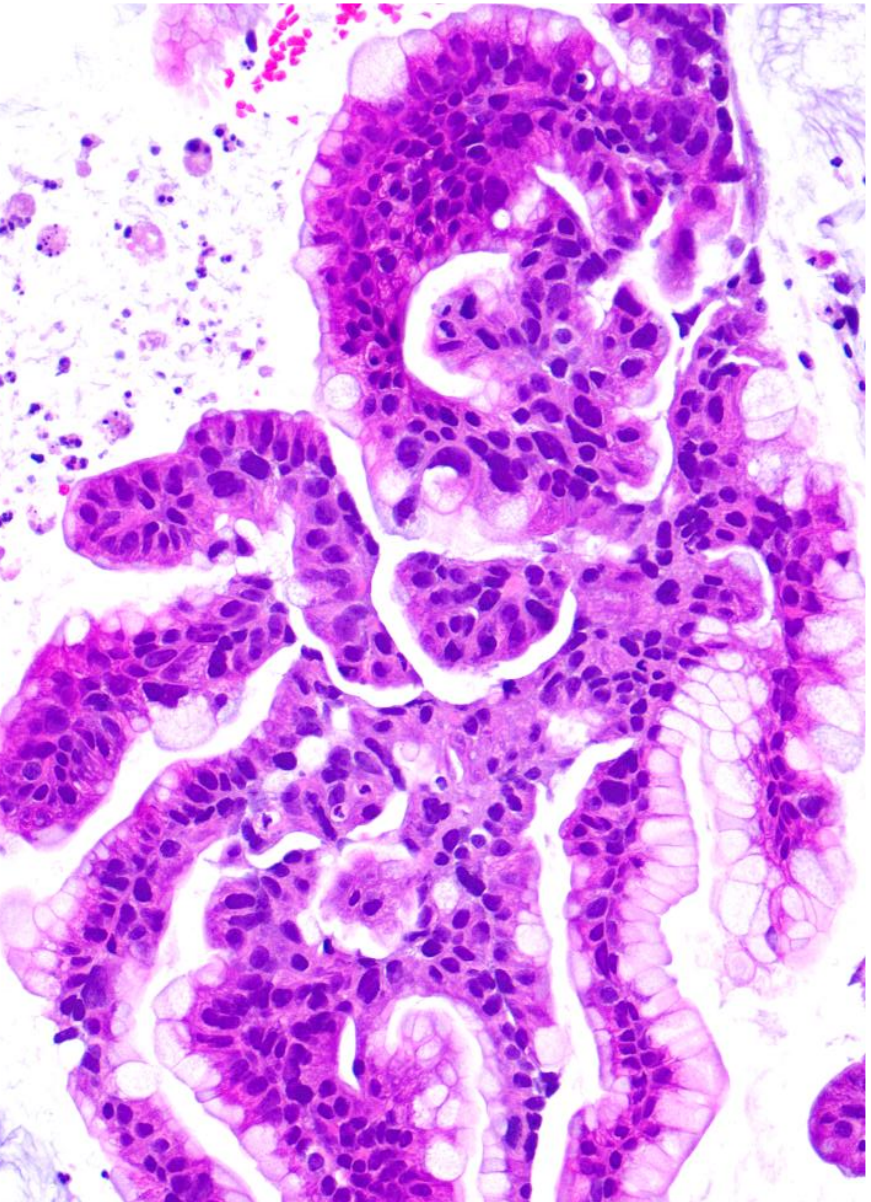
- **Progression-free and overall survival:**
  - ✓Worse than HPV-associated carcinomas
- **Patient age:**
  - ✓55 (43-67) vs 42 (36-52) years
- **Tumor size:**
  - ✓3.8 (21-45) vs 21 (10-35) cm



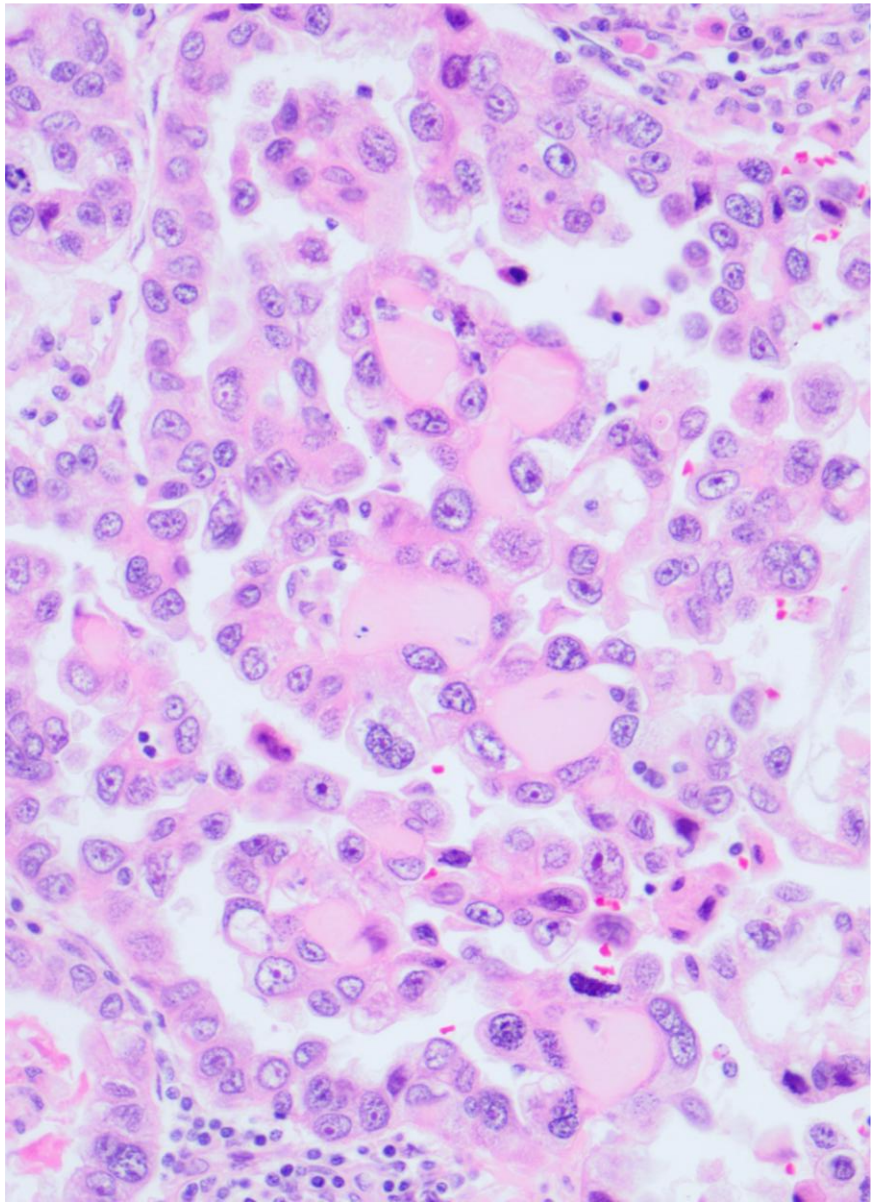
PMID: 29135516, 33570863, 30679193, 30720532, 34581850

HPV-independent Endocervical Adenocarcinomas

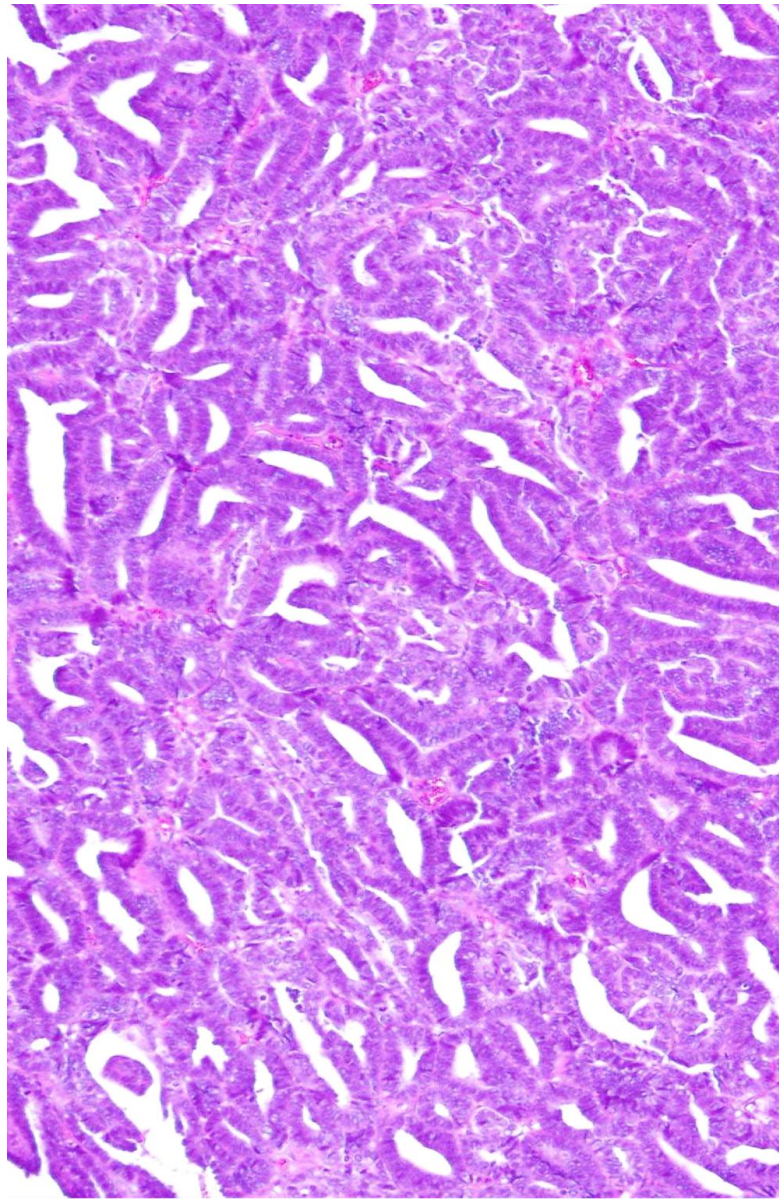
Gastric-type



Clear cell

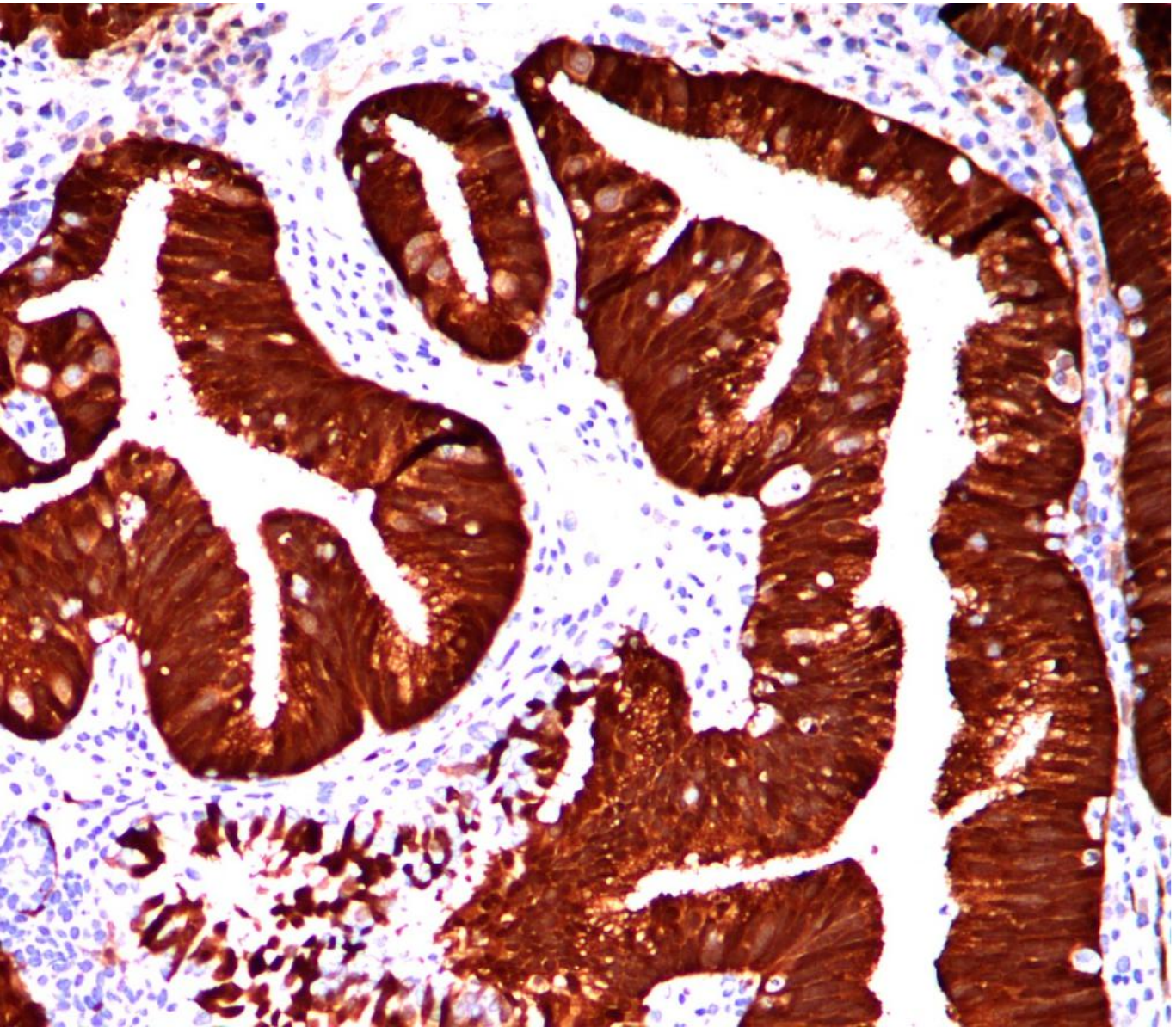


Mesonephric

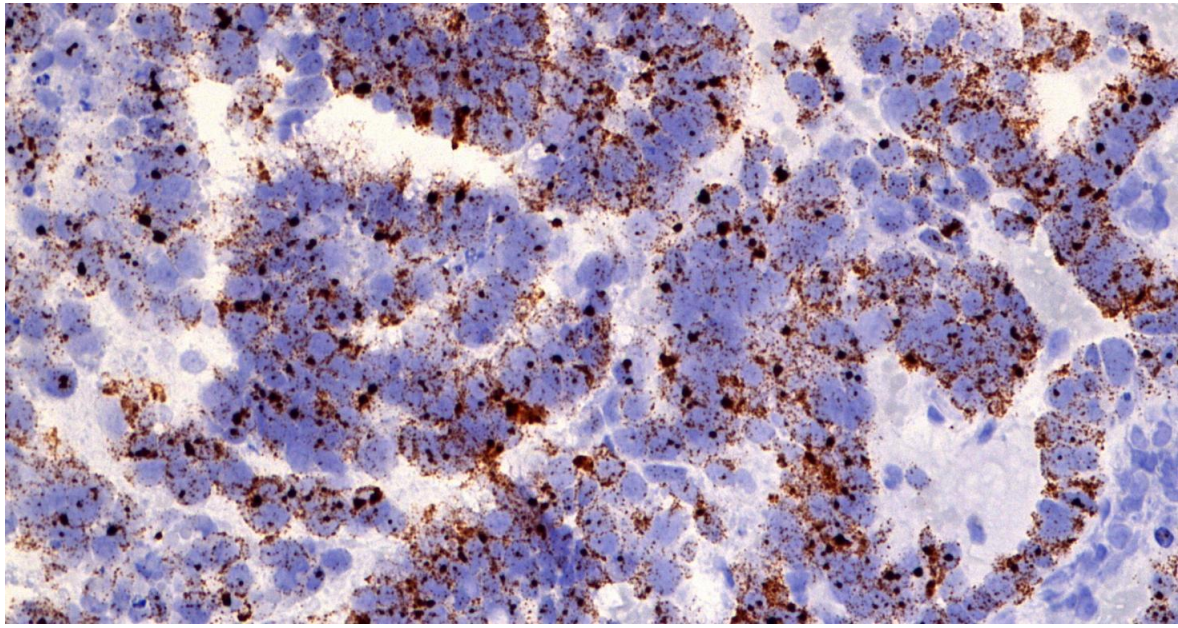


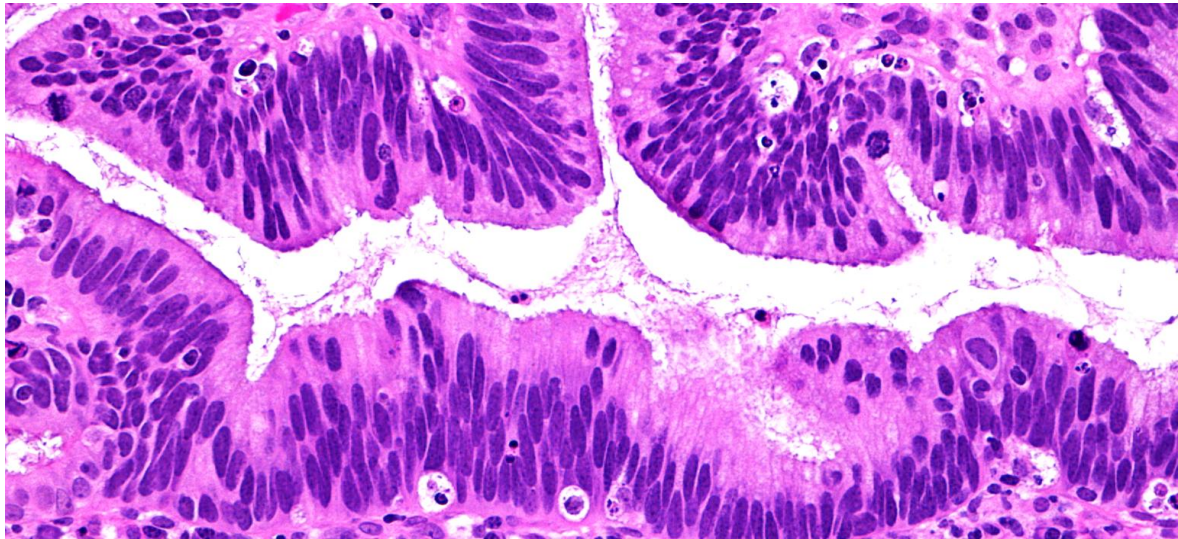
HPV-associated Endocervical Adenocarcinoma

Diffuse block-like expression of p16



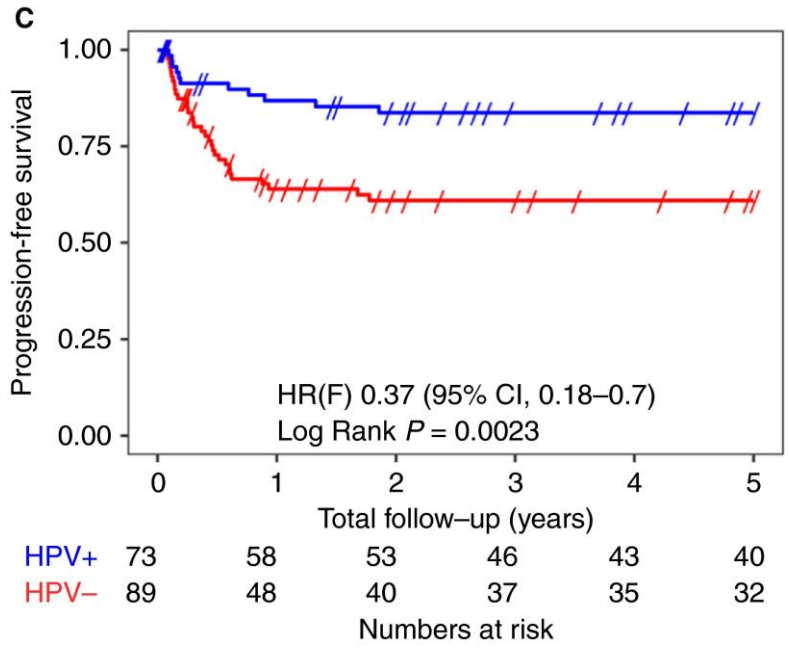
HPV RNA ISH

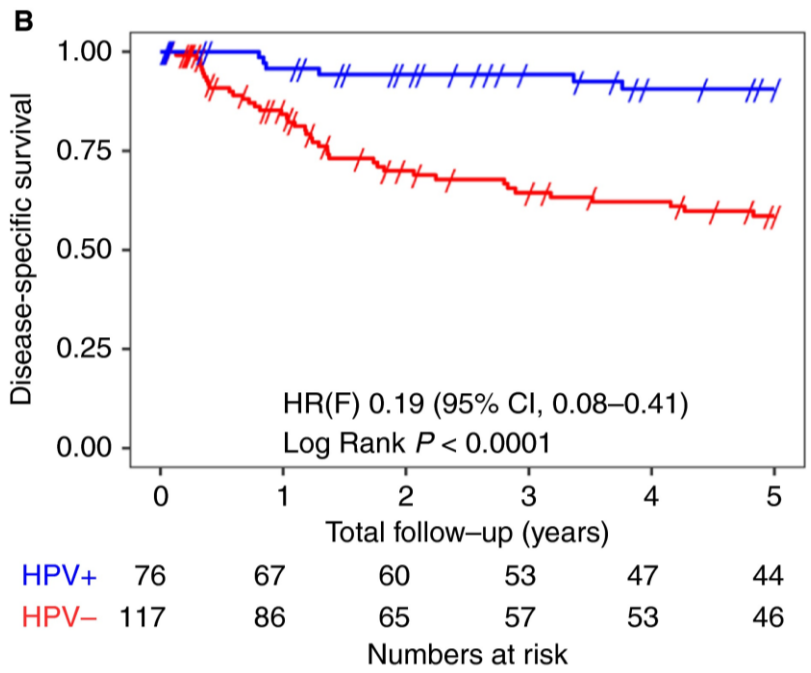




HPV Status Is an Independent Prognostic Factor for Progression-Free and Disease-Specific Survival in Vulvar SCC

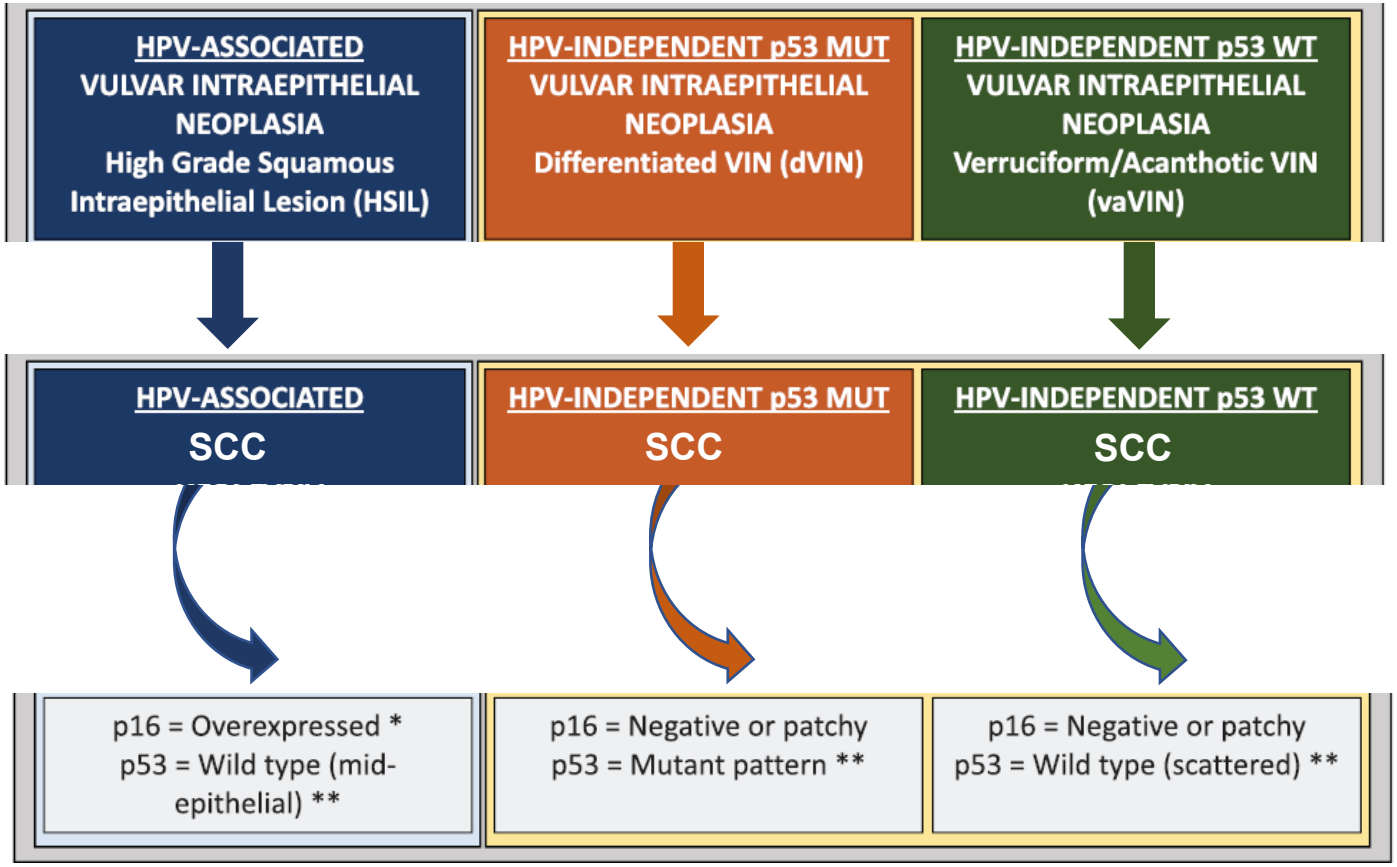
- **HPV-negative SCC:**
  - ✓Worse prognosis compared with HPV-positive SCC





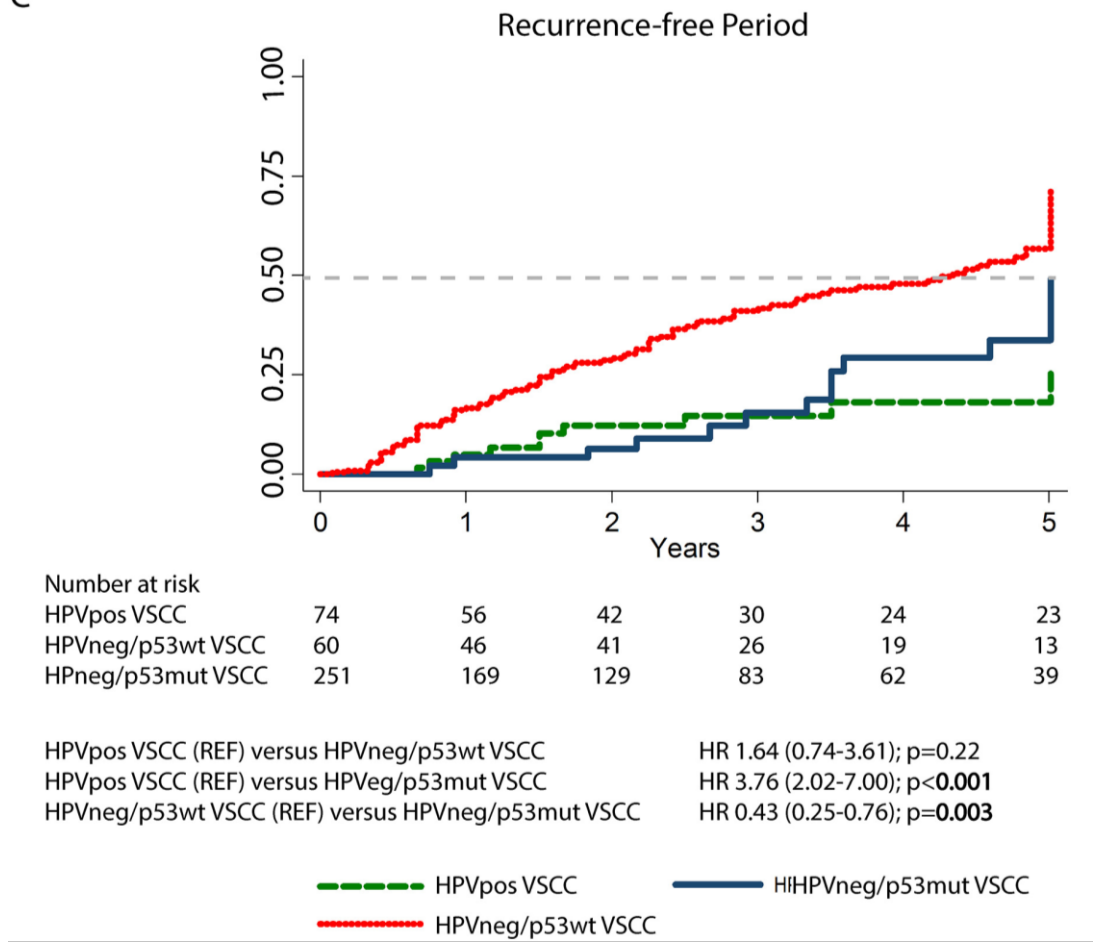
PMID: 28257152, 28319571, 26855143

p16/HPV and p53 Status Allows Separation of Vulvar Precursors and SCCs Into 3 Clinically Distinct Subtypes



PIK3CA, HRAS, NOTCH1

C



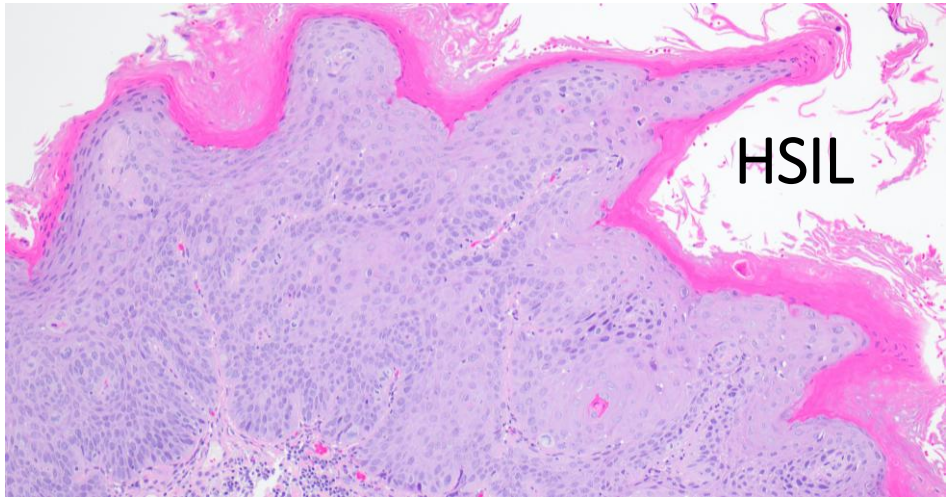
Modified from PMID: 35437330; 32280343

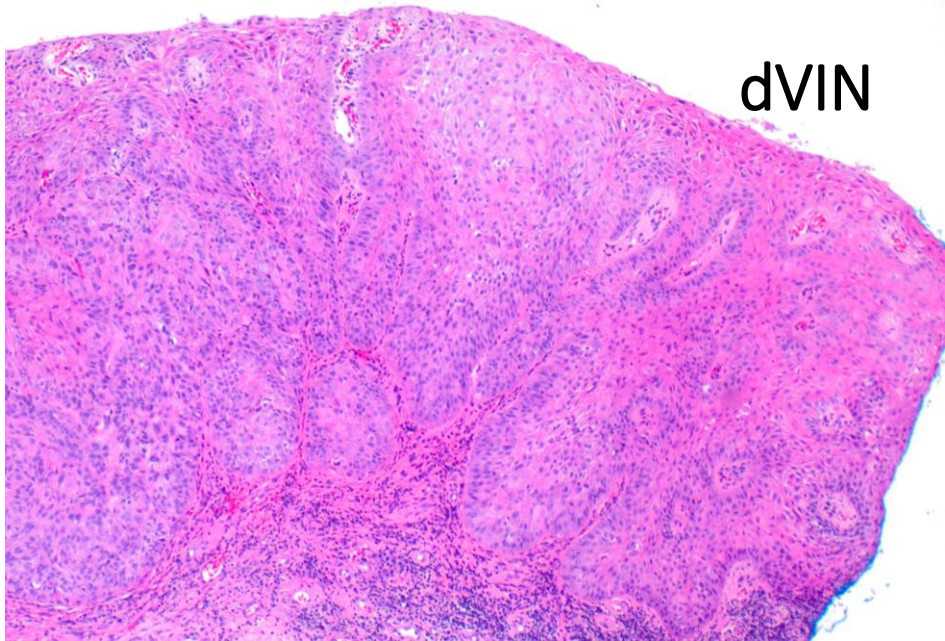
p16/HPV and p53 Status Allow Separation of Vulvar Squamous Precursors and SCCs Into 3 Clinically Distinct Subtypes

VULVAR SQUAMOUS INTRAEPITHELIAL NEOPLASIA

HPV-ASSOCIATED VULVAR INTRAEPITHELIAL NEOPLASIA High Grade Squamous Intraepithelial Lesion (HSIL)	HPV-INDEPENDENT p53 MUT VULVAR INTRAEPITHELIAL NEOPLASIA Differentiated VIN (dVIN)	HPV-INDEPENDENT p53 WT VULVAR INTRAEPITHELIAL NEOPLASIA Verruciform/Acanthotic VIN (vaVIN)
● Atypia extending to upper layers ● Large hyperchromatic nuclei ● Loss of polarity and organization ● Loss of maturation with basaloid appearance & brisk mitotic activity with extension beyond basal layers	● Atypia often confined to the basal layer ● Large nuclei with open chromatin ● Prominent nucleoli ● Retained but abnormal maturation with elongated and/or fused rete ridges, acantholysis & dyskeratotic cells	● No cytologic atypia ● Verruciform acanthosis with flat (VAAD) or exophytic growth pattern (DEVIL) ● Retained but altered maturation with hypogranulosis & cytoplasmic pallor (VAAD/DEVIL) or hypergranulosis (vLSC)
p16 = Overexpressed * p53 = Wild type (mid-epithelial) **	p16 = Negative or patchy p53 = Mutant pattern **	p16 = Negative or patchy p53 = Wild type (scattered) **

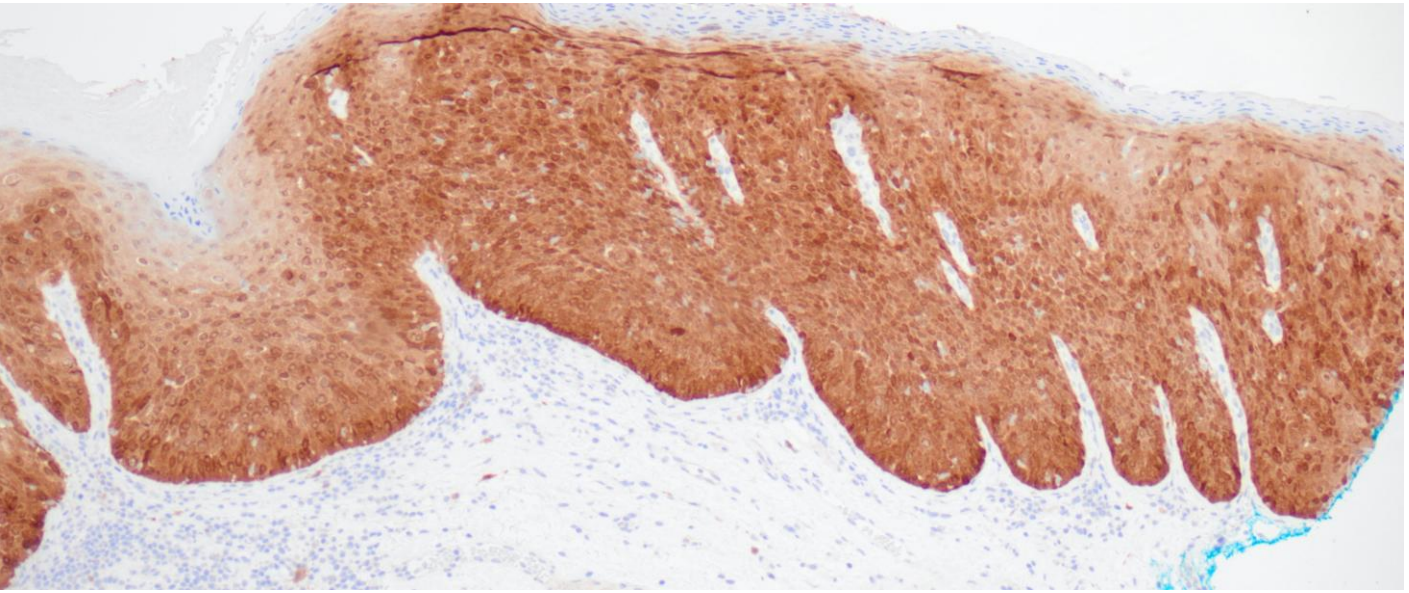
PMID: 35437330



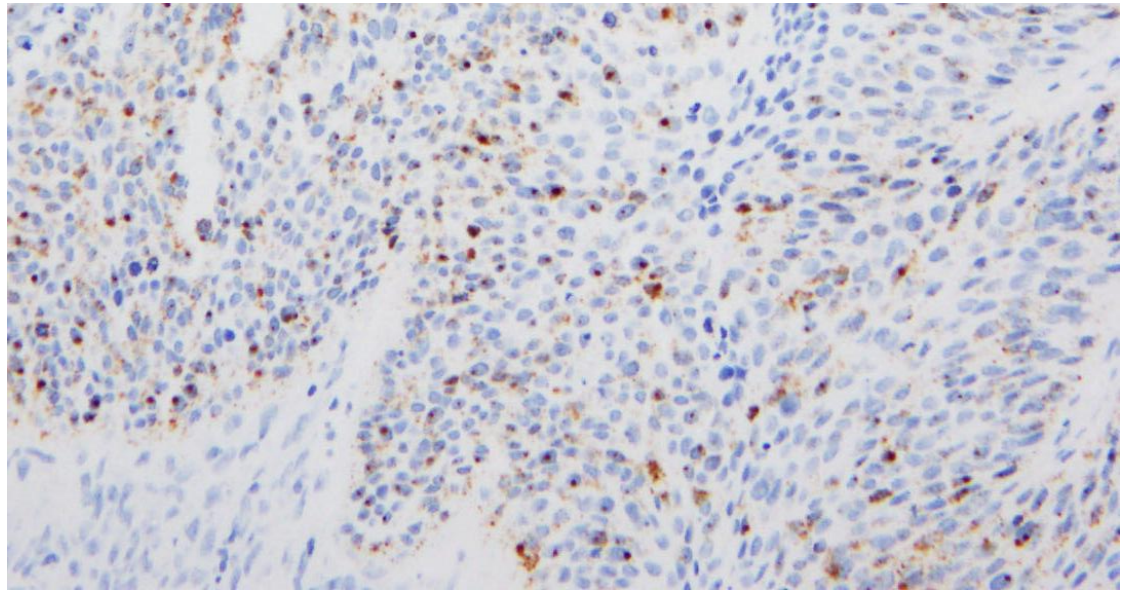


HSIL (HPV-Associated)

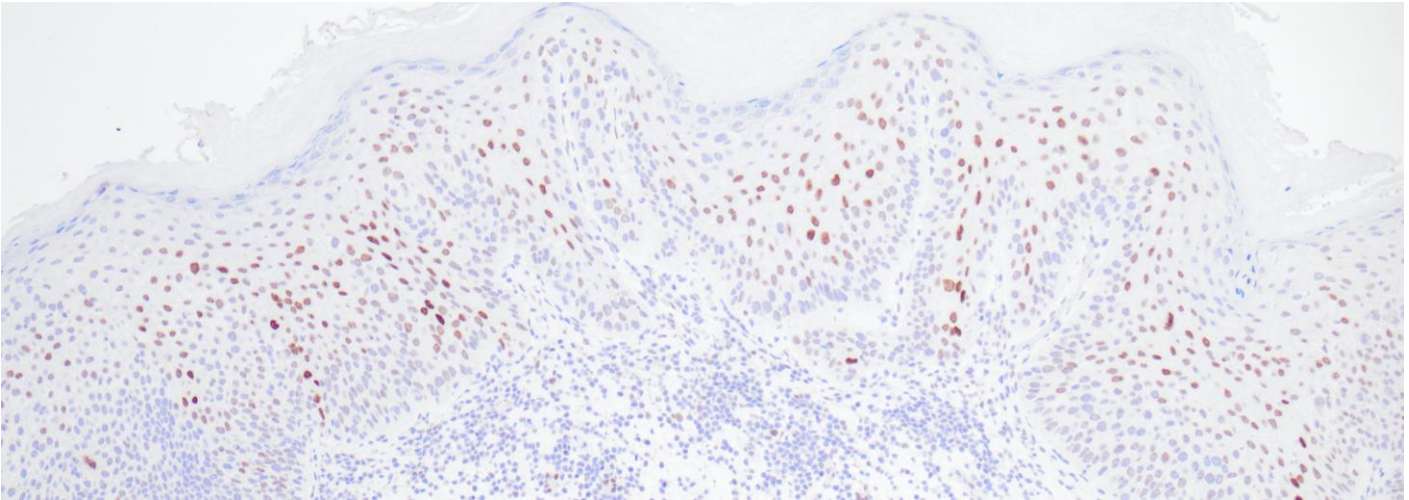
Diffuse block-like expression of p16

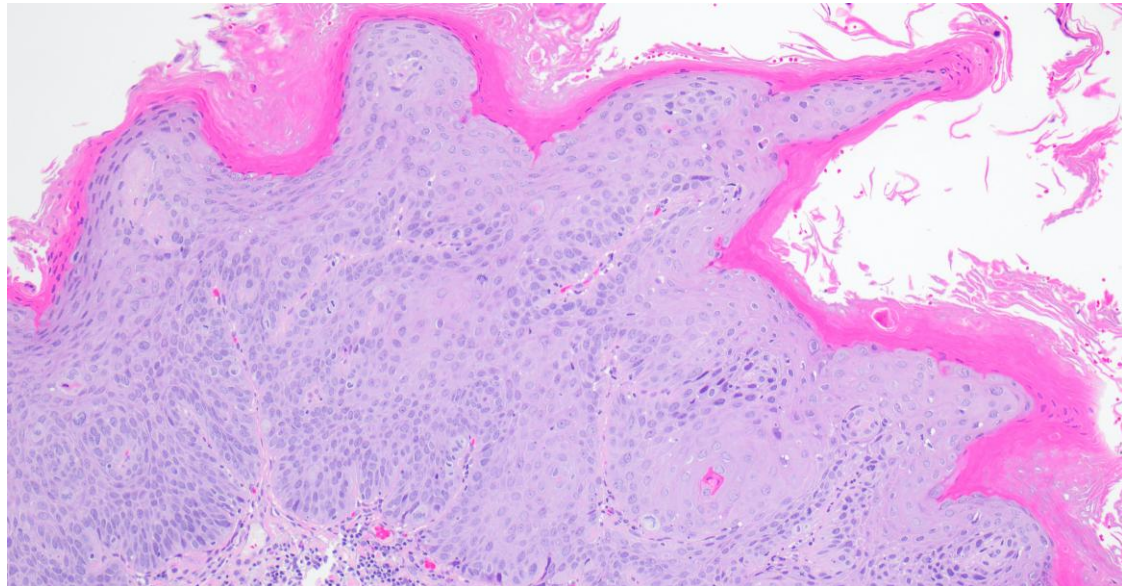


HPV RNA ISH



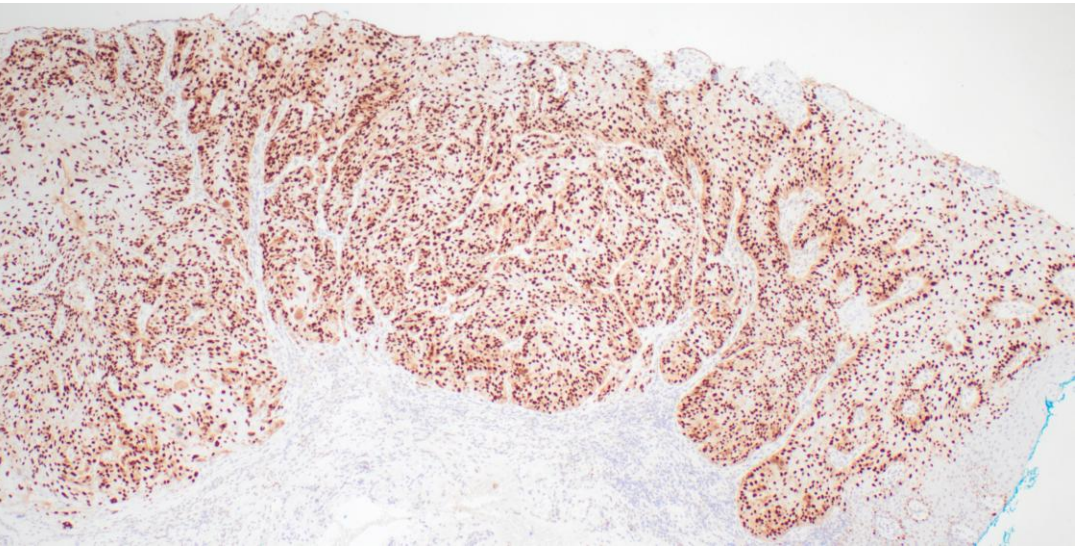
Wild-type expression of p53

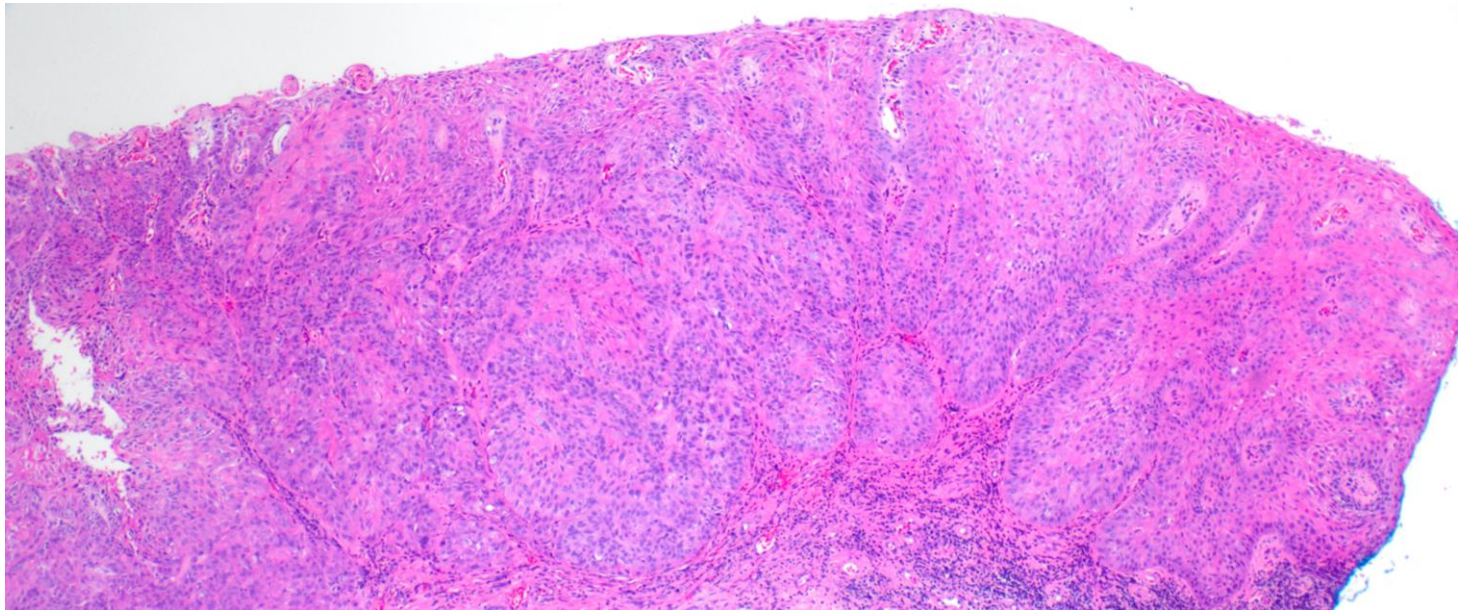




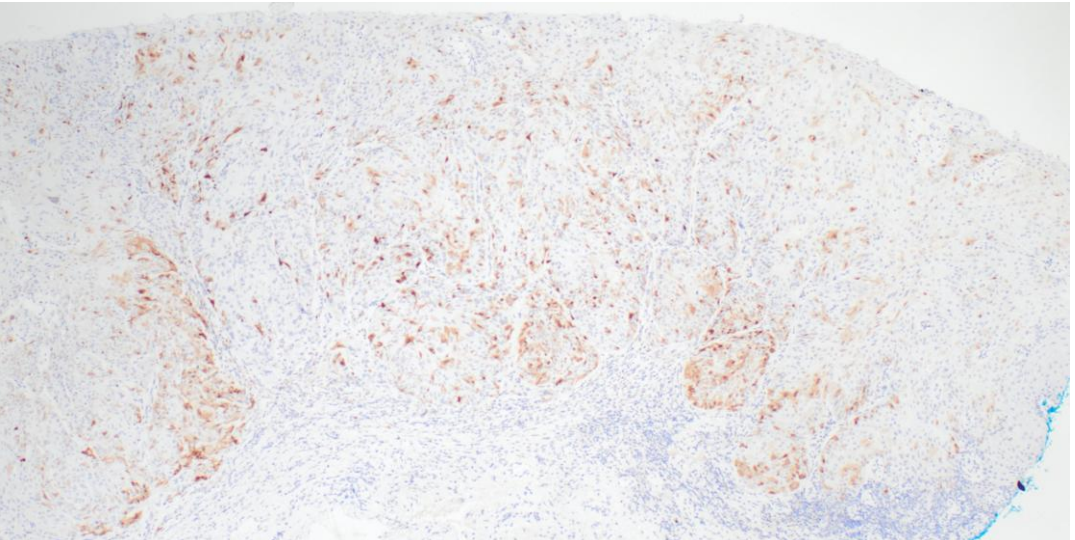
dVIN (HPV-Independent)

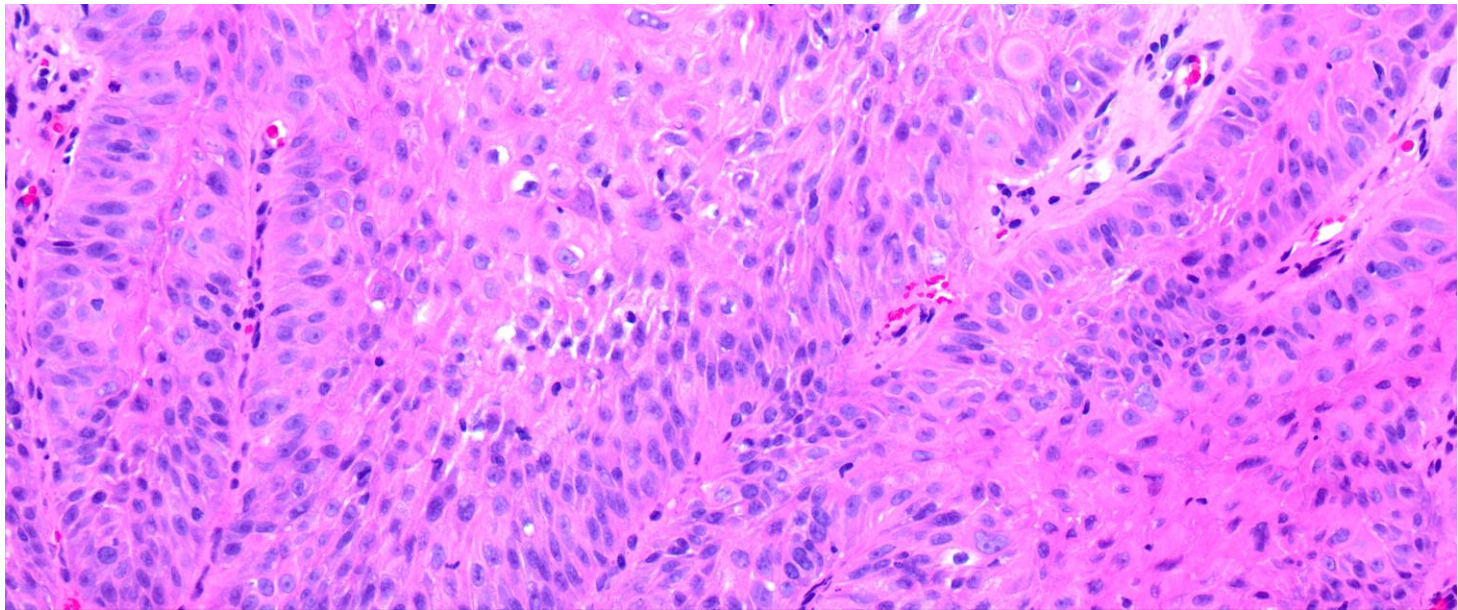
Abnormal overexpression of p53





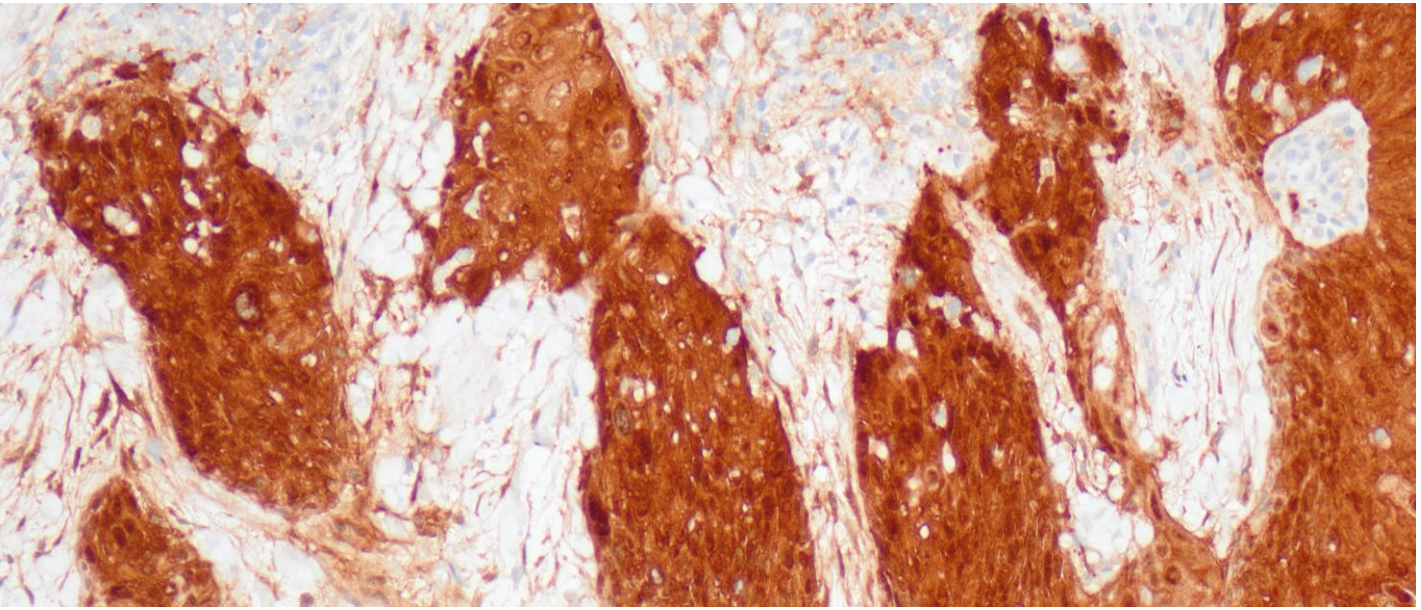
Focal expression of p16



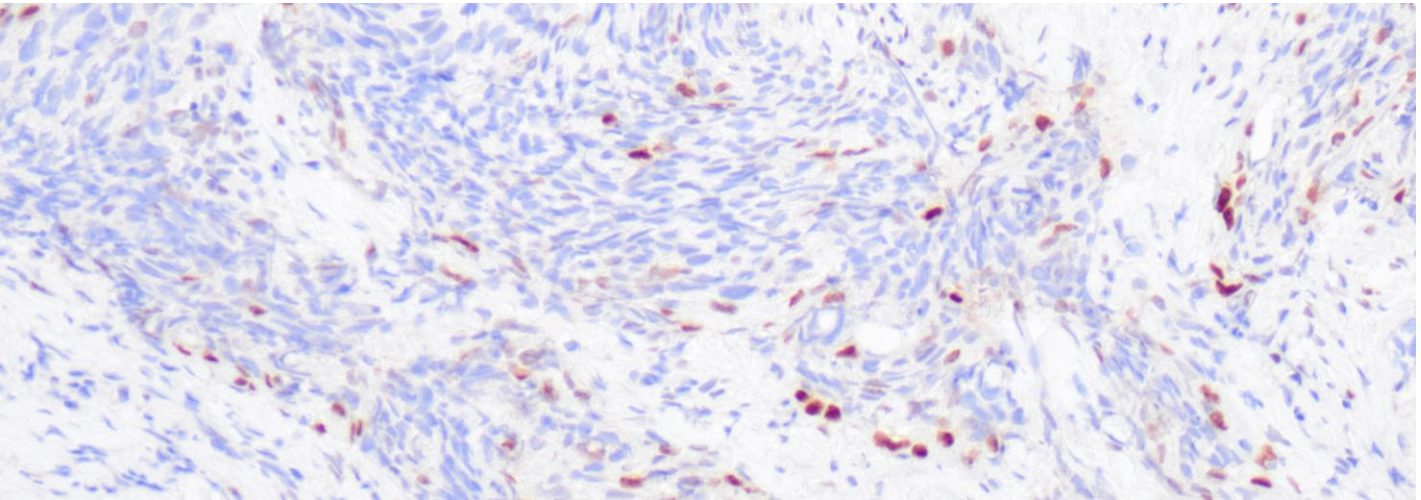


HPV-Associated SCC

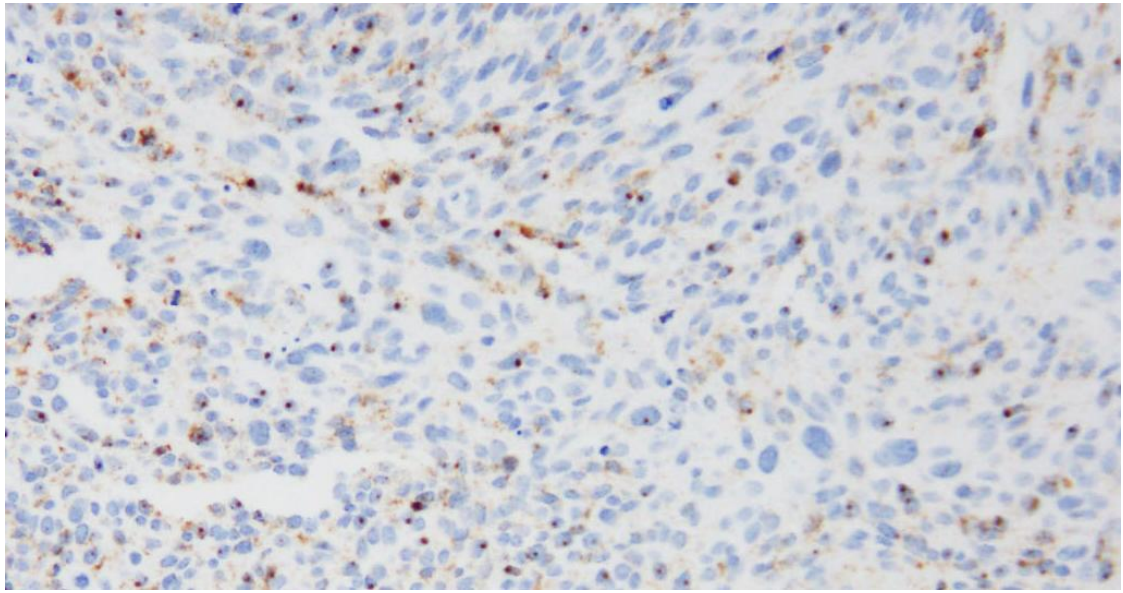
Diffuse “block-like” expression of p16

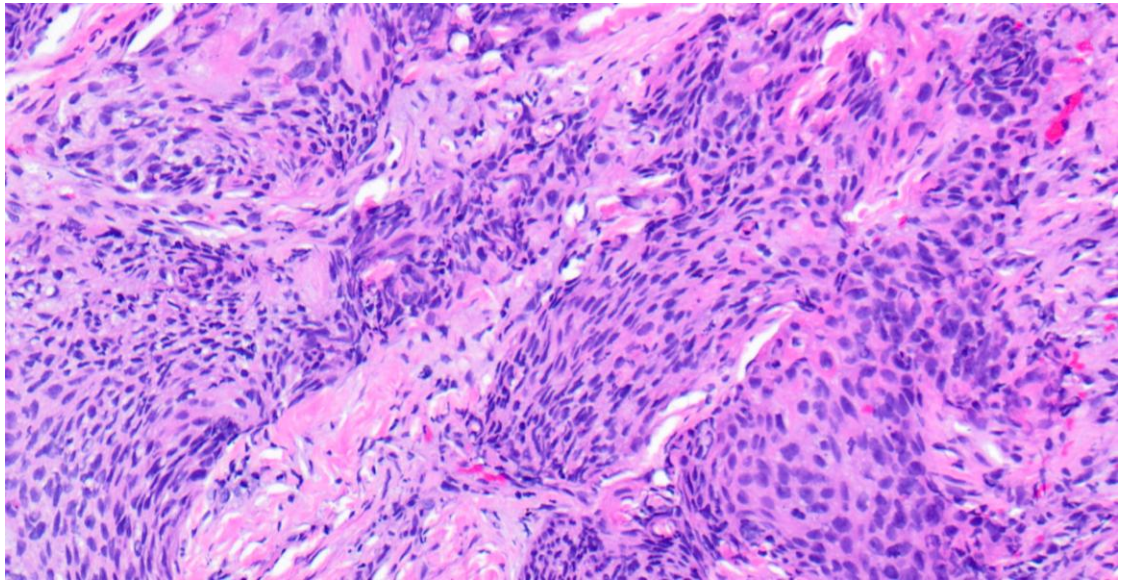


Wild-type expression of p53



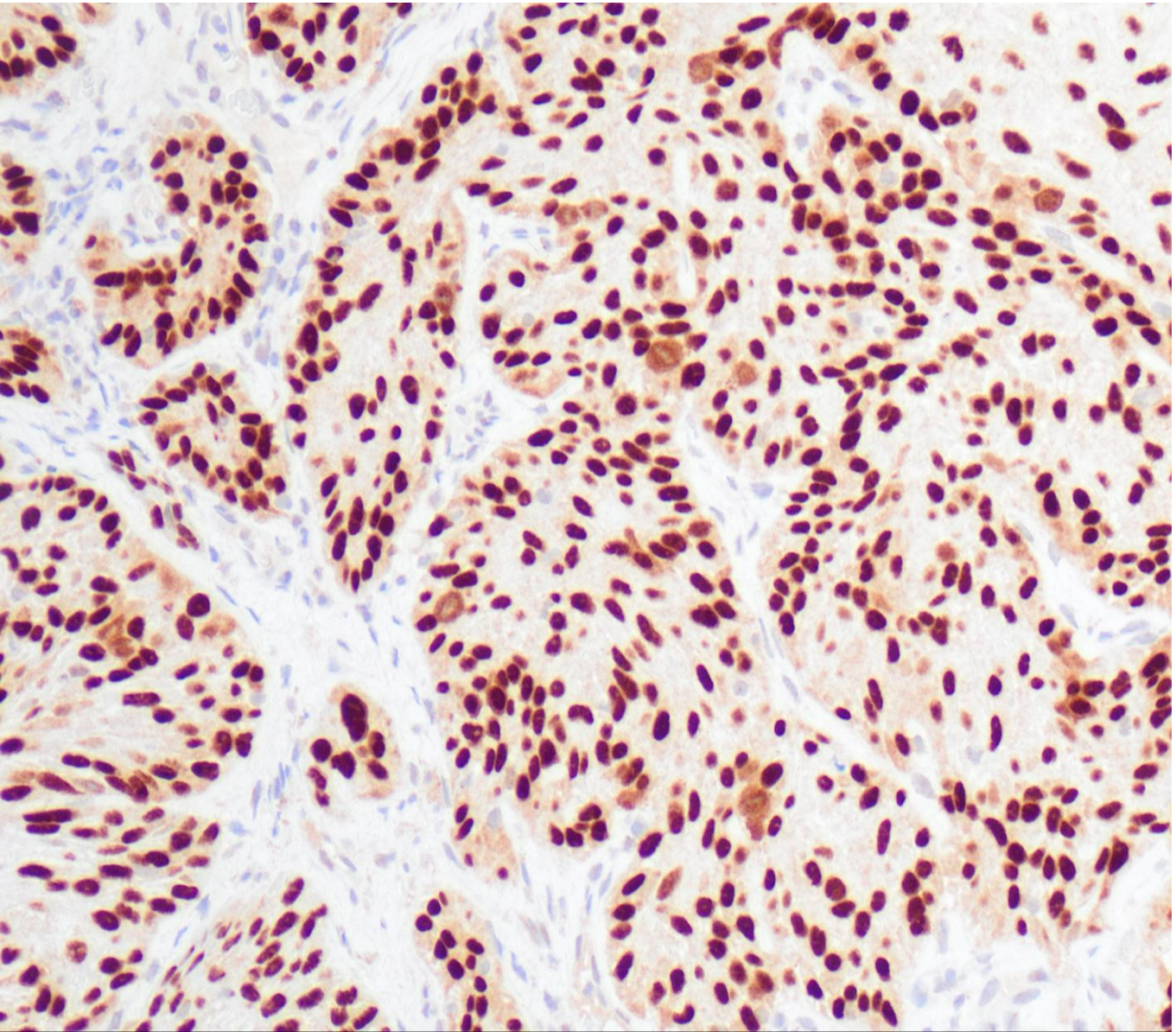
HPV RNA ISH



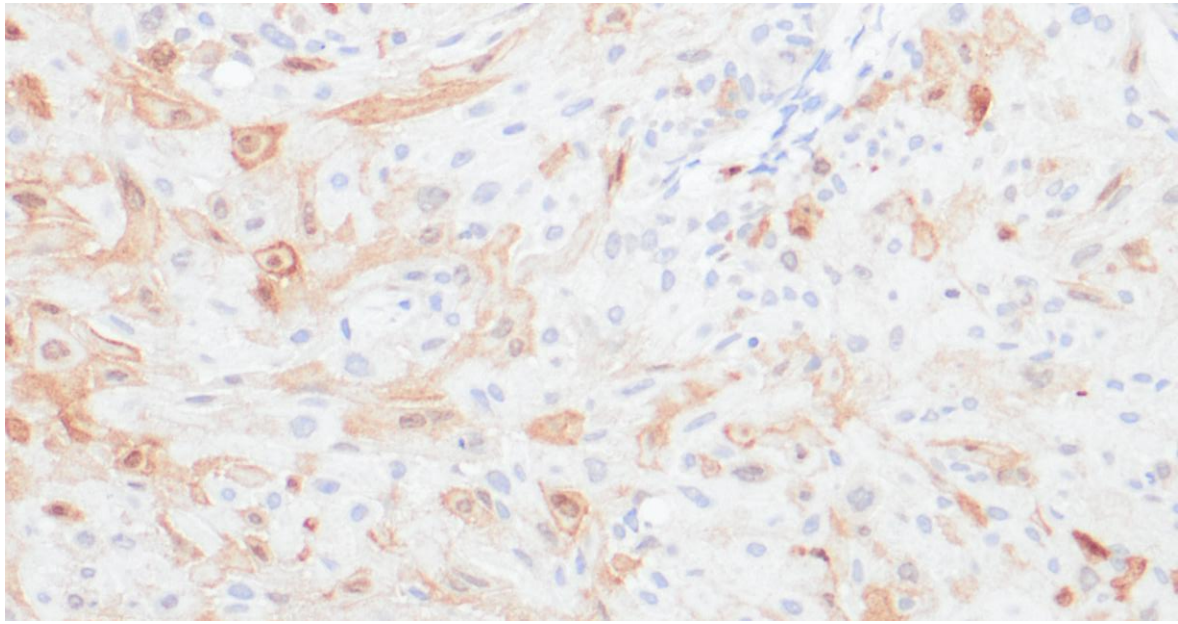


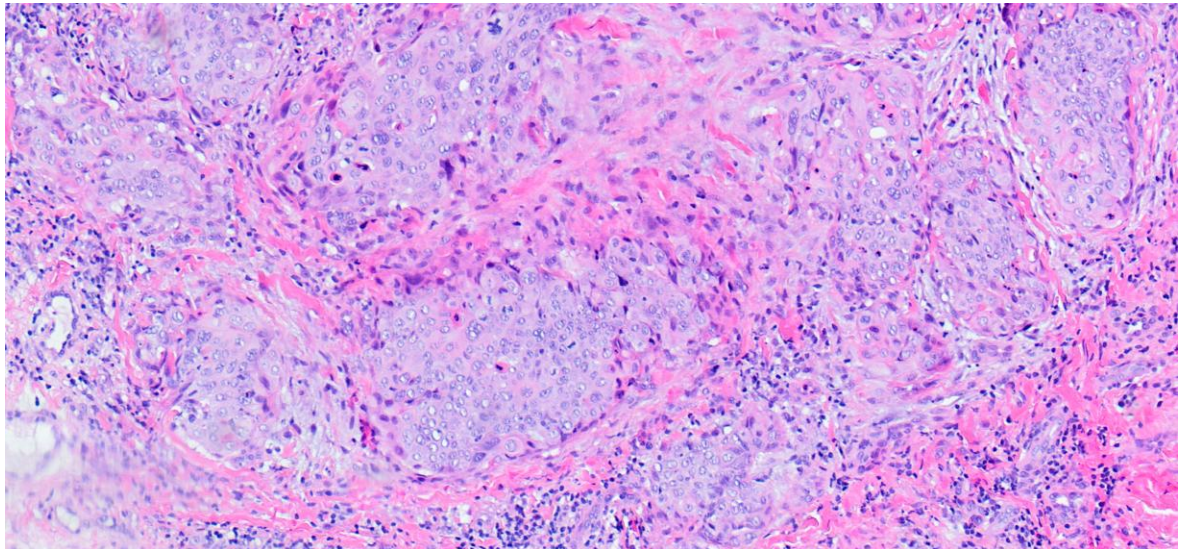
HPV-Independent SCC

Abnormal overexpression of p53

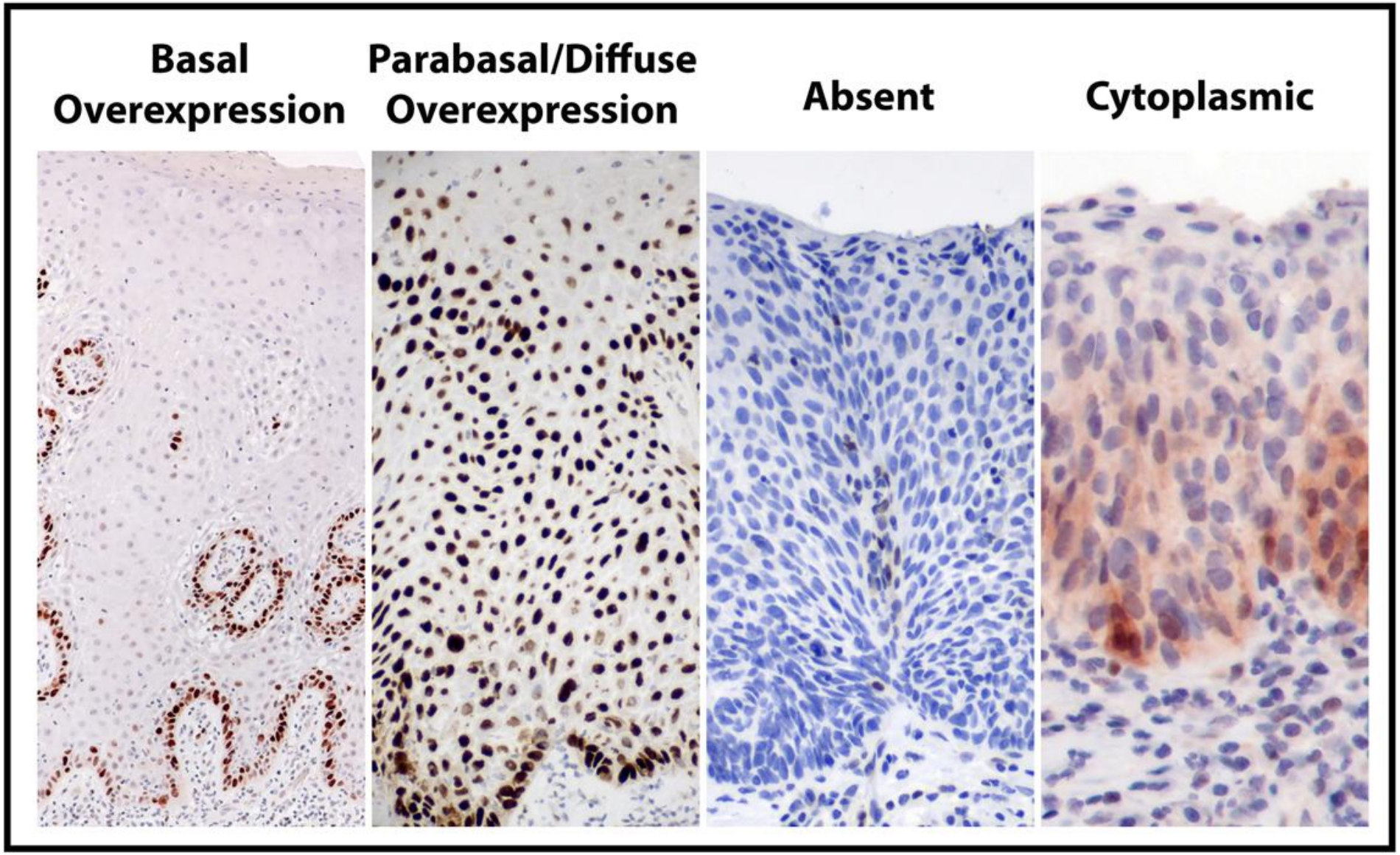


Focal expression of p16





Summary of Mutant p53 Patterns in the Vulva: VIN



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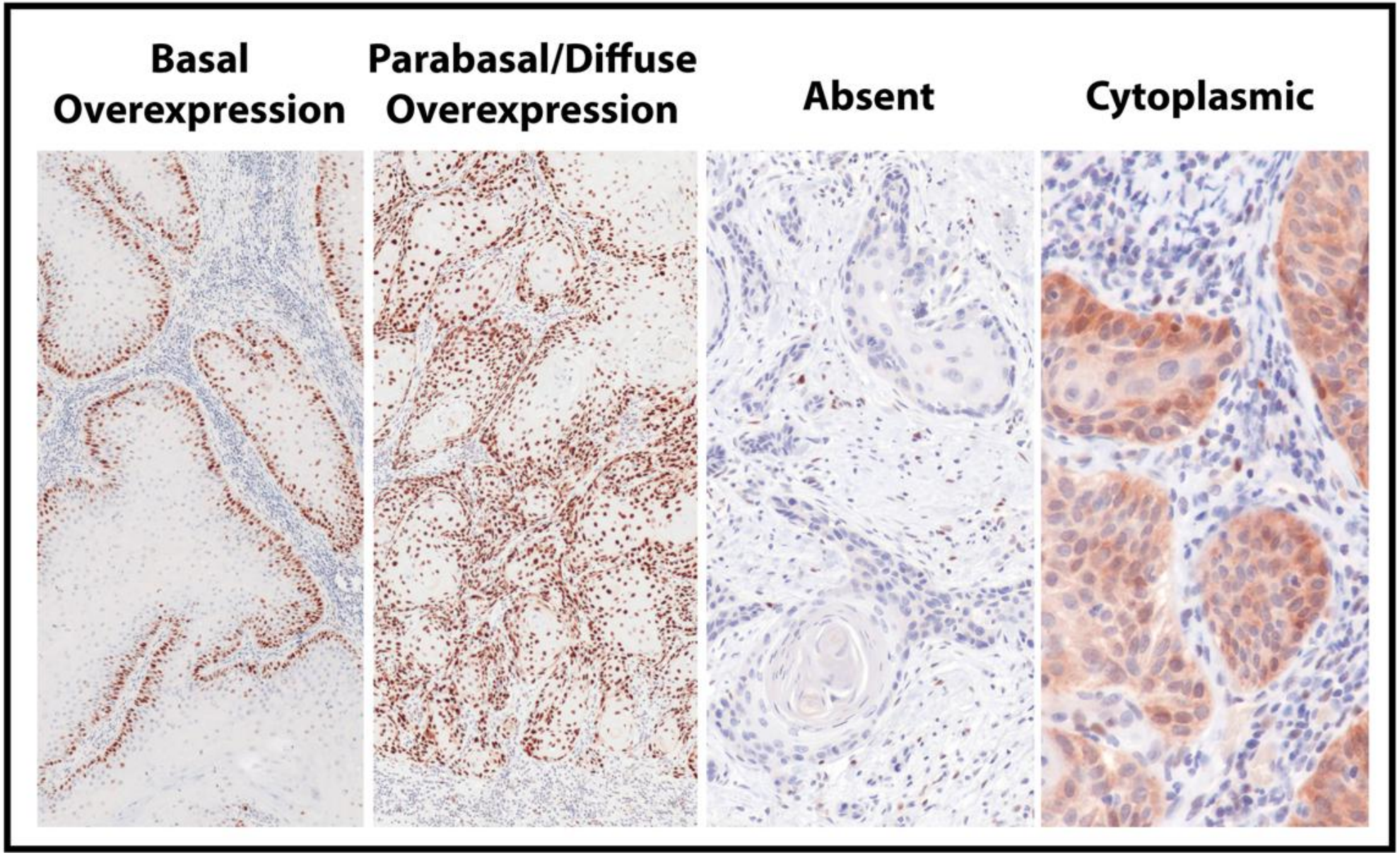
Summary of Mutant p53 Patterns in the Vulva: SCC

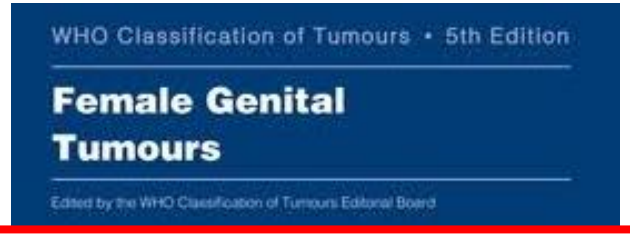


PMID: 32203095

Cervical Carcinomas and Precursors, and Vulvar SCC and Precursors Are Classified Based on Their Association with HPV

Cervix:

- **Adenocarcinomas:**
  - Adenocarcinoma in situ, HPV-associated
  - Adenocarcinoma in situ, HPV-independent

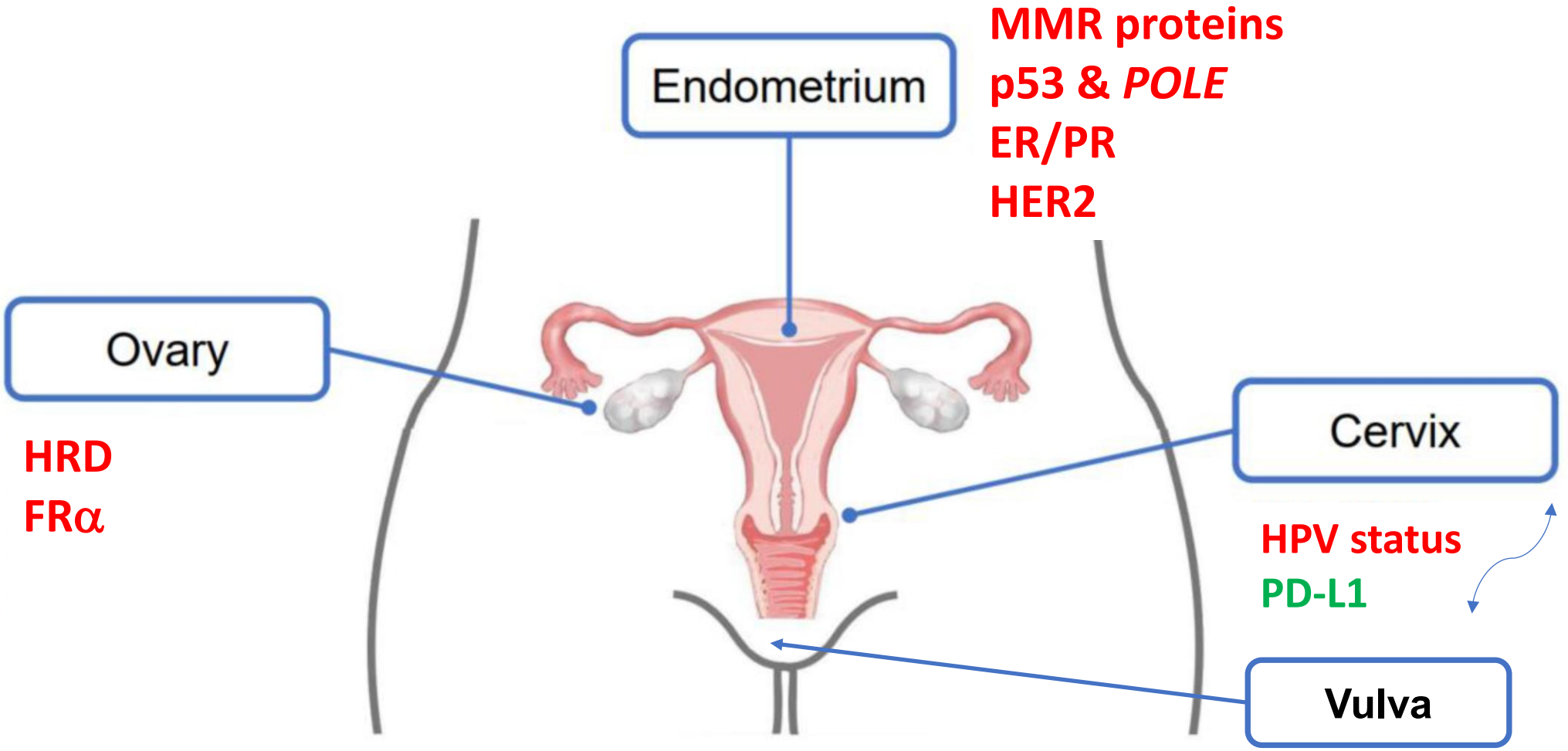


Vulva:

- Squamous intraepithelial lesions, HPV-associated

- ✓ **Determine HPV status by p16 immunohistochemistry and/or molecular testing in all vulvar, cervical (and vaginal) SCCs**
- ✓ **Perform p16 and p53 immunohistochemistry in SCCs and intraepithelial squamous cell proliferations:**
  - ➢ **Accurate pathogenetic classification and prognostication**
  - ➢ **May impact treatment decisions**

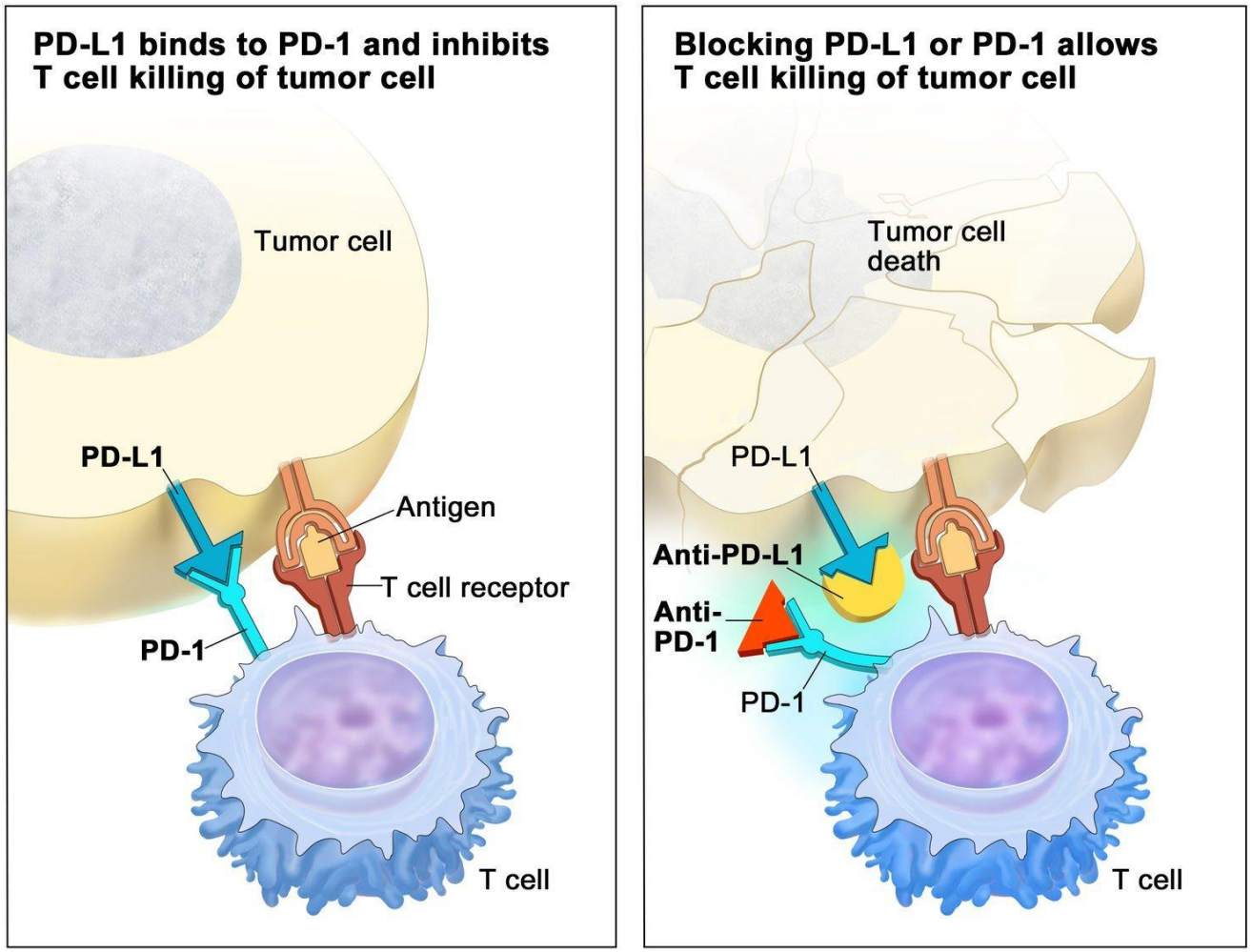
Prognostic and Therapeutic Markers in Gynecologic Pathology



Cervical and Endometrial Cancers Can Be Treated with Immune Checkpoint Inhibitors

- Immune checkpoints (e.g. binding PD-L1 to PD-1) prevent an immune response from attacking neoplastic cells
- Blocking immune checkpoints allows cytotoxic T cells to recognize and attack neoplastic cells
- Based on KEYNOTE-158 (NCT02628067) trial, in 2018 the FDA approved pembrolizumab for PD-L1+ recurrent or metastatic cervical cancer with disease progression on or after chemotherapy

Immune Checkpoints



PMID: 31682550, 29095678, 30943124

https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors

Current FDA-Approved Indications

First-line Combination Therapy for Advanced Cervical Cancer

KEYTRUDA, in combination with chemotherapy, with or without bevacizumab, is indicated for the treatment of patients with persistent, recurrent, or metastatic cervical cancer whose tumors express programmed death ligand 1 (PD-L1) [combined positive score (CPS) ≥1] as determined by an FDA-approved test.

Cut Point

CPS ≥1

Second-line Monotherapy for Advanced Cervical Cancer

KEYTRUDA, as a single agent, is indicated for the treatment of patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy whose tumors express programmed death ligand 1 (PD-L1) [combined positive score (CPS) ≥1] as determined by an FDA-approved test.

Cut Point

CPS ≥1

NEW FDA-Approved Indication

KEYTRUDA® (pembrolizumab) and KEYTRUDA QLEX™ (pembrolizumab and berahyaluronidase alfa-pmph), Plus Paclitaxel ± Bevacizumab, Approved for Certain Adults with PD-L1+ (CPS ≥1) Platinum-Resistant Ovarian Carcinoma as Second or Third Line Treatment

February 11, 2026 6:45 am EST

- **Phase 3 KEYNOTE-B96 trial (ENGOT-ov65):**
  - ✓ KEYTRUDA + paclitaxel ± bevacizumab improved PFS by 28% (HR=0.72, 95% CI, 0.58-0.89; p=0.0014) and OS by 24% (HR=0.76, 95% CI, 0.61-0.94; p=0.0053) in patients with platinum-resistant recurrent ovarian cancer with CPS ≥1

PD-L1 Expression Is Determined by Combined Positive Score (CPS) Using the FDA-approved 22C3 pharmDx Kit (Agilent Technologies, Santa Clara, CA) on the Dako Autostainer (Dako, Carpinteria, CA)

$$\text{CPS} = \frac{\text{\# of PD-L1–positive cells}}{\text{Tumor cells, lymphocytes, macrophages}} \div \text{Total \# of PD-L1–positive + PD-L1–negative tumor cells} \times 100$$

*Max. CPS 100

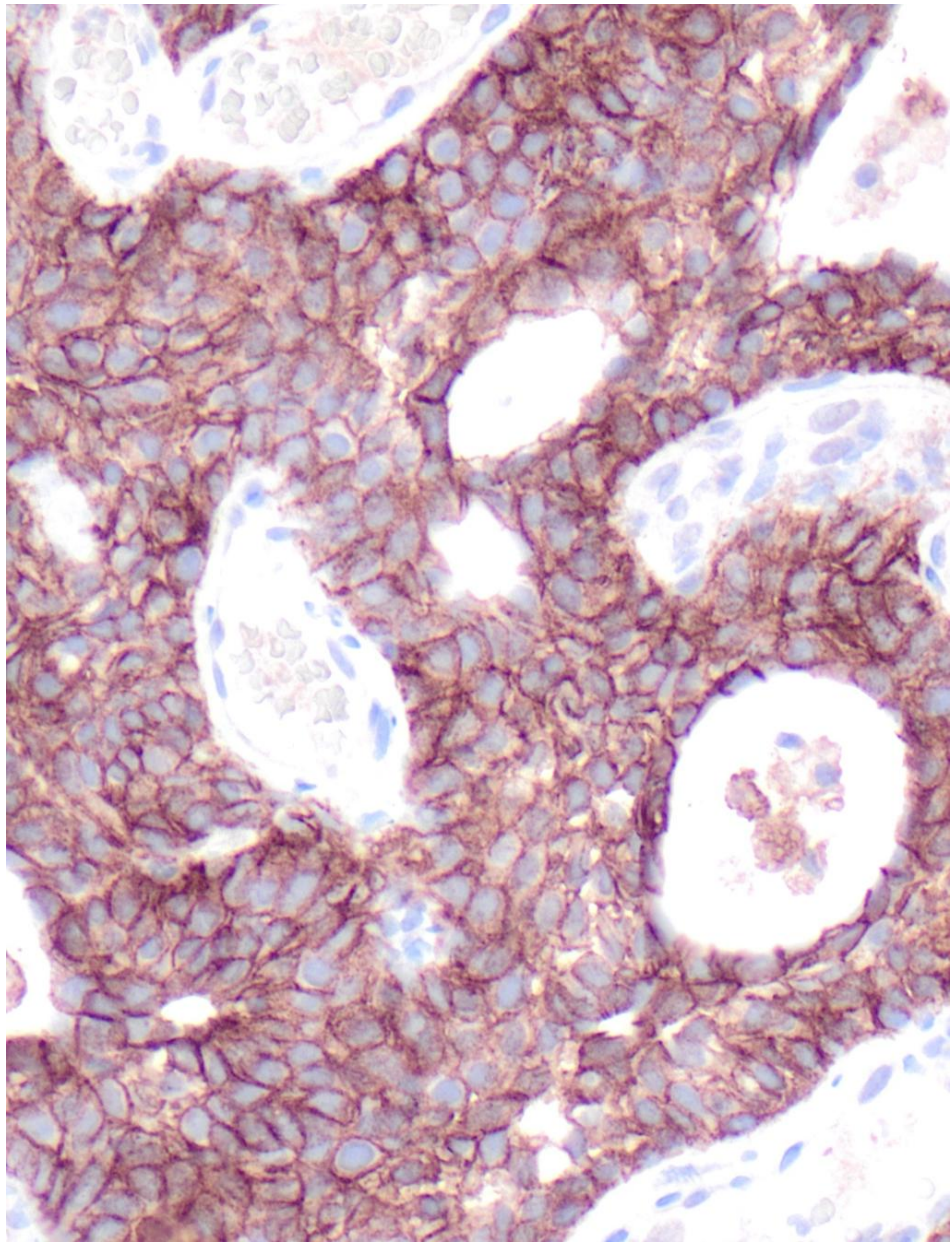
Cut point for PD-L1 expression: ≥1

- ✓ 82% of cervical cancers are PD-L1-positive:
  - Complete response in 2.6%, partial response in 11.7%

PMID 31924334, 28870938; https://www.keytrudahcp.com/biomarker-testing/pd-l1

Guidelines for Assessing the CPS

- Specimen adequacy:
  - ✓ Minimum 100 viable tumor cells in a tissue section
- Average in the entire tumor rather than the hot spots
- Assess at 20x to capture focal expression
- Count tumor cells and immune cells (lymphocytes and macrophages):
  - ✓ Count only if associated with tumor
  - ✓ Either intra- or peritumoral
  - ✓ Lymphoid aggregates OK
- Exclude stromal cells, plasma cells, and neutrophils
- Subcellular localization for positive staining:
  - ✓ Membranous in tumor cells
  - ✓ Membranous or cytoplasmic in immune cells



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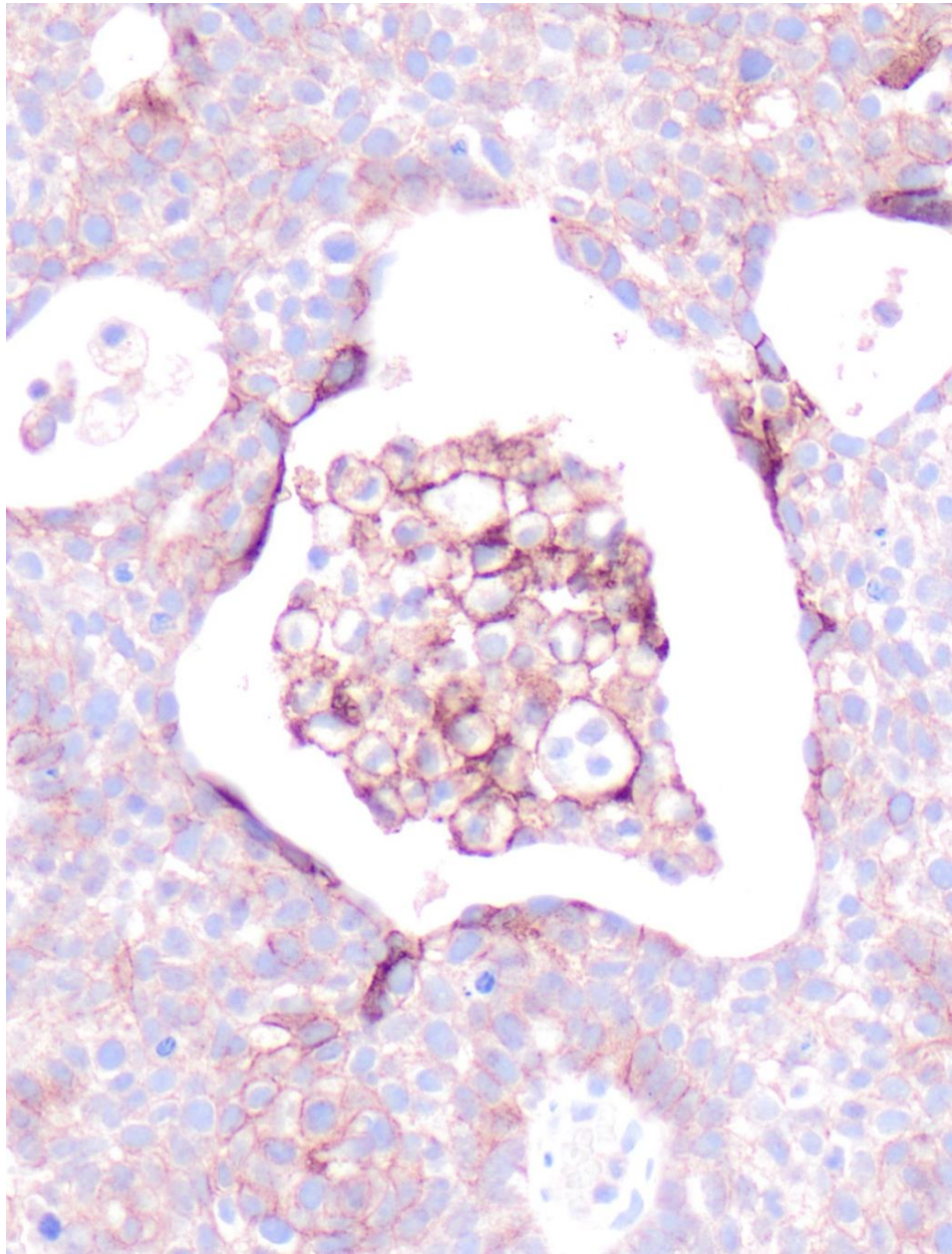
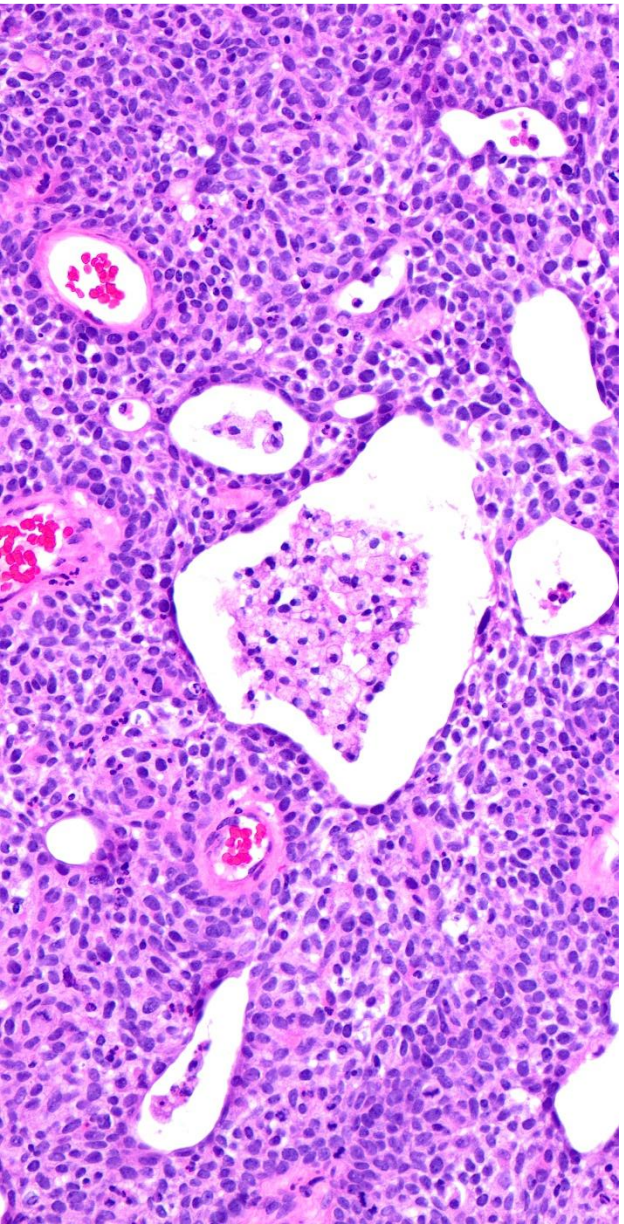
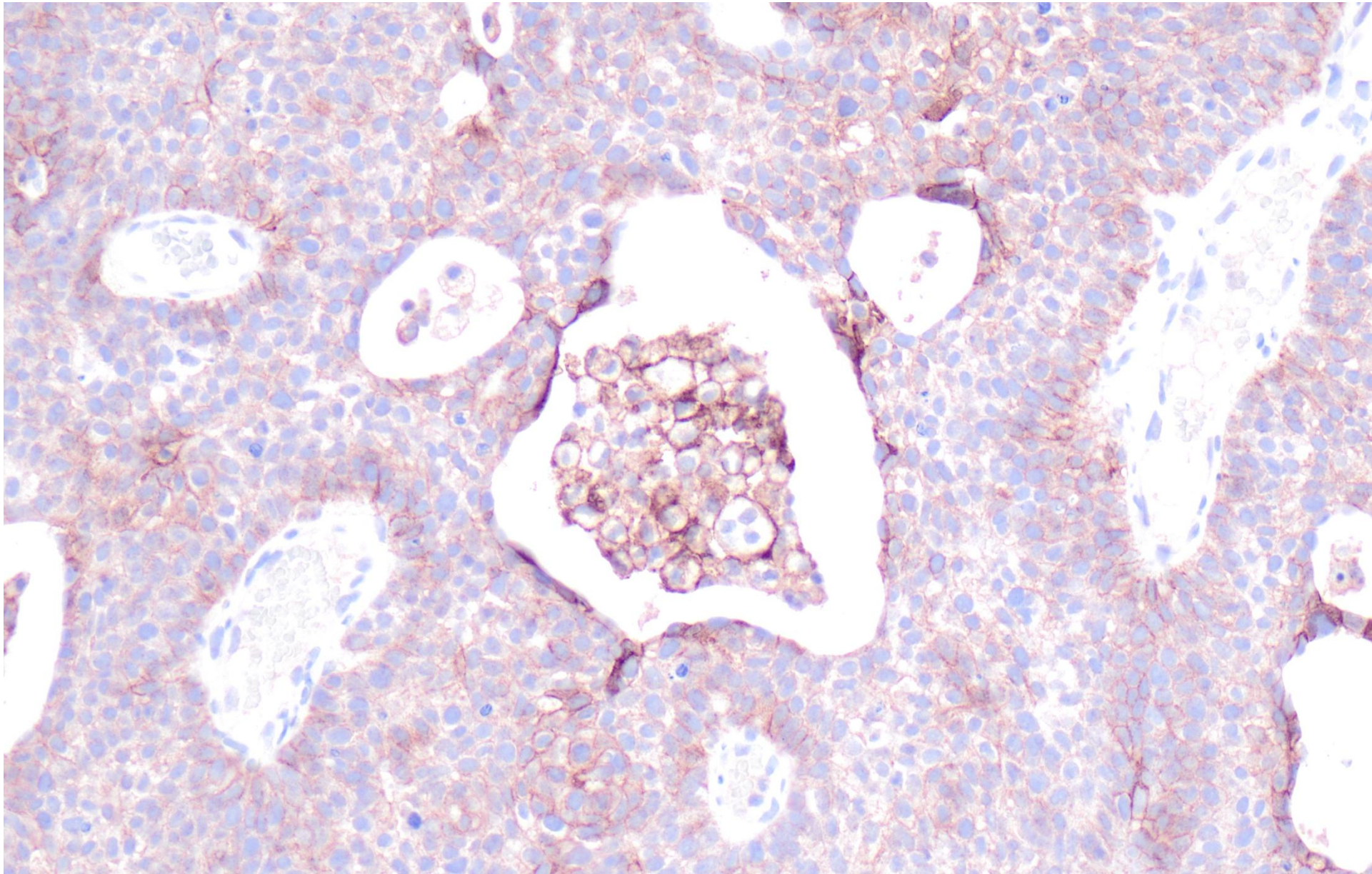
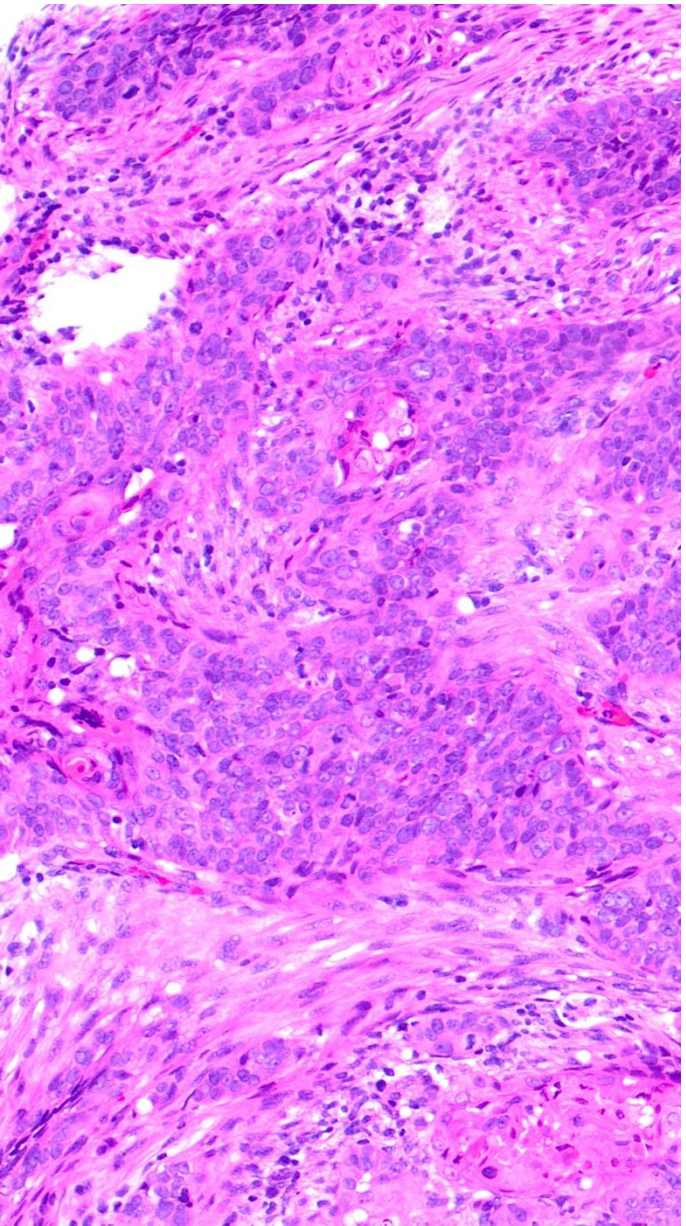


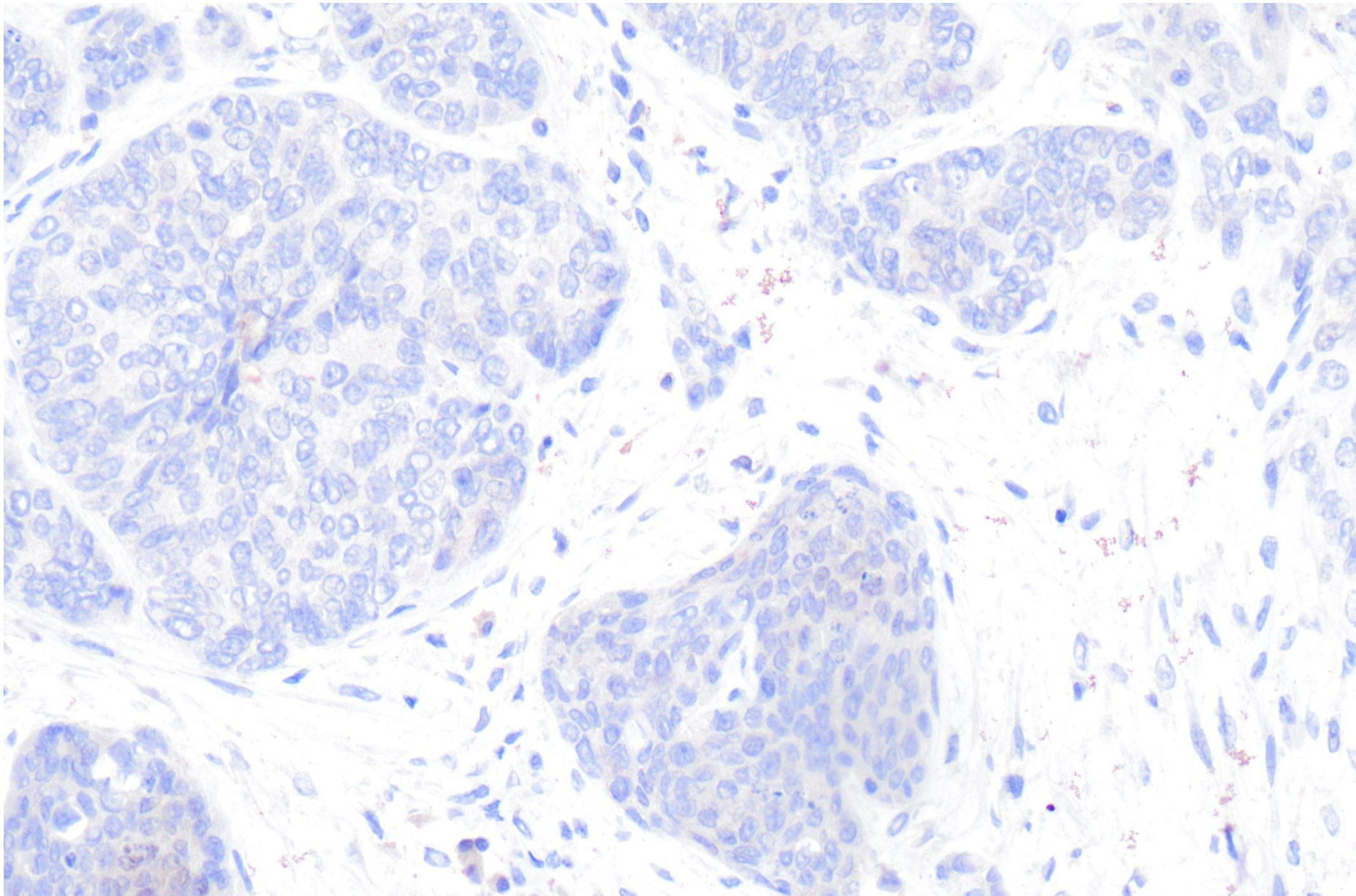
Uniform Staining in Neoplastic and Immune Cells, CPS=100



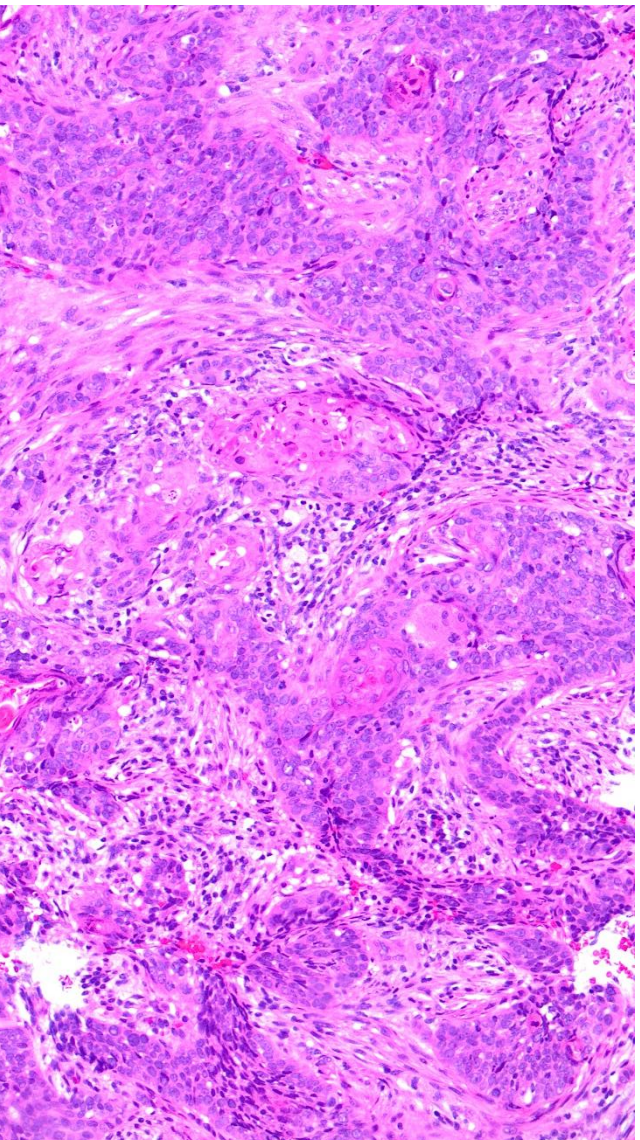


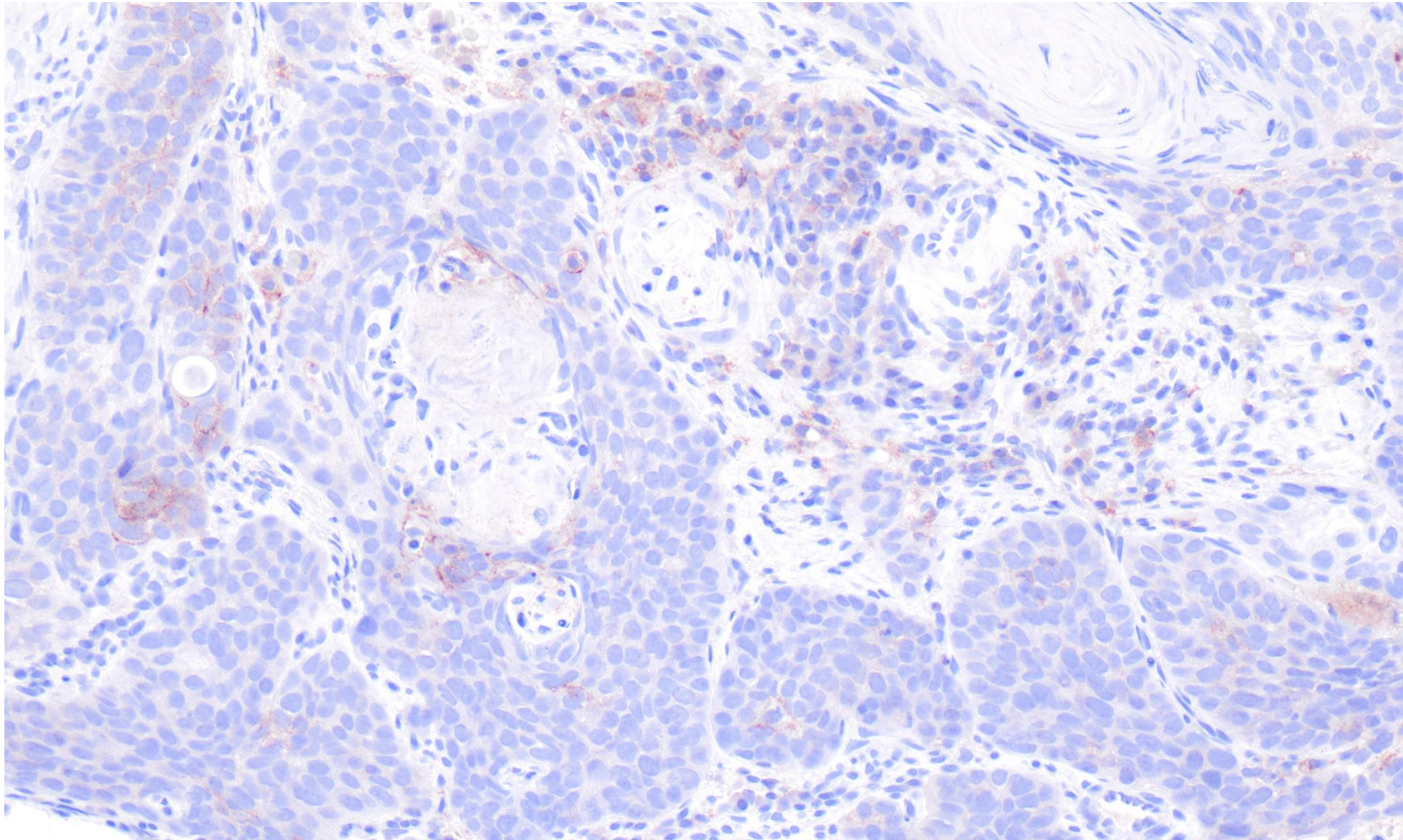
No Staining in Neoplastic or Immune Cells, CPS=0



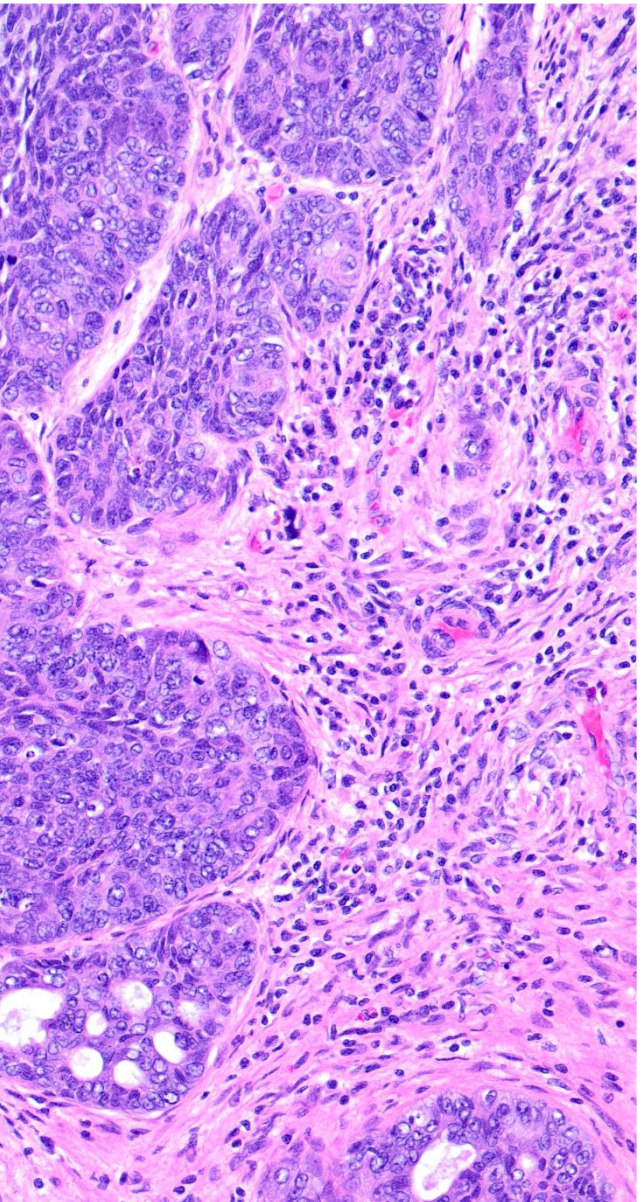


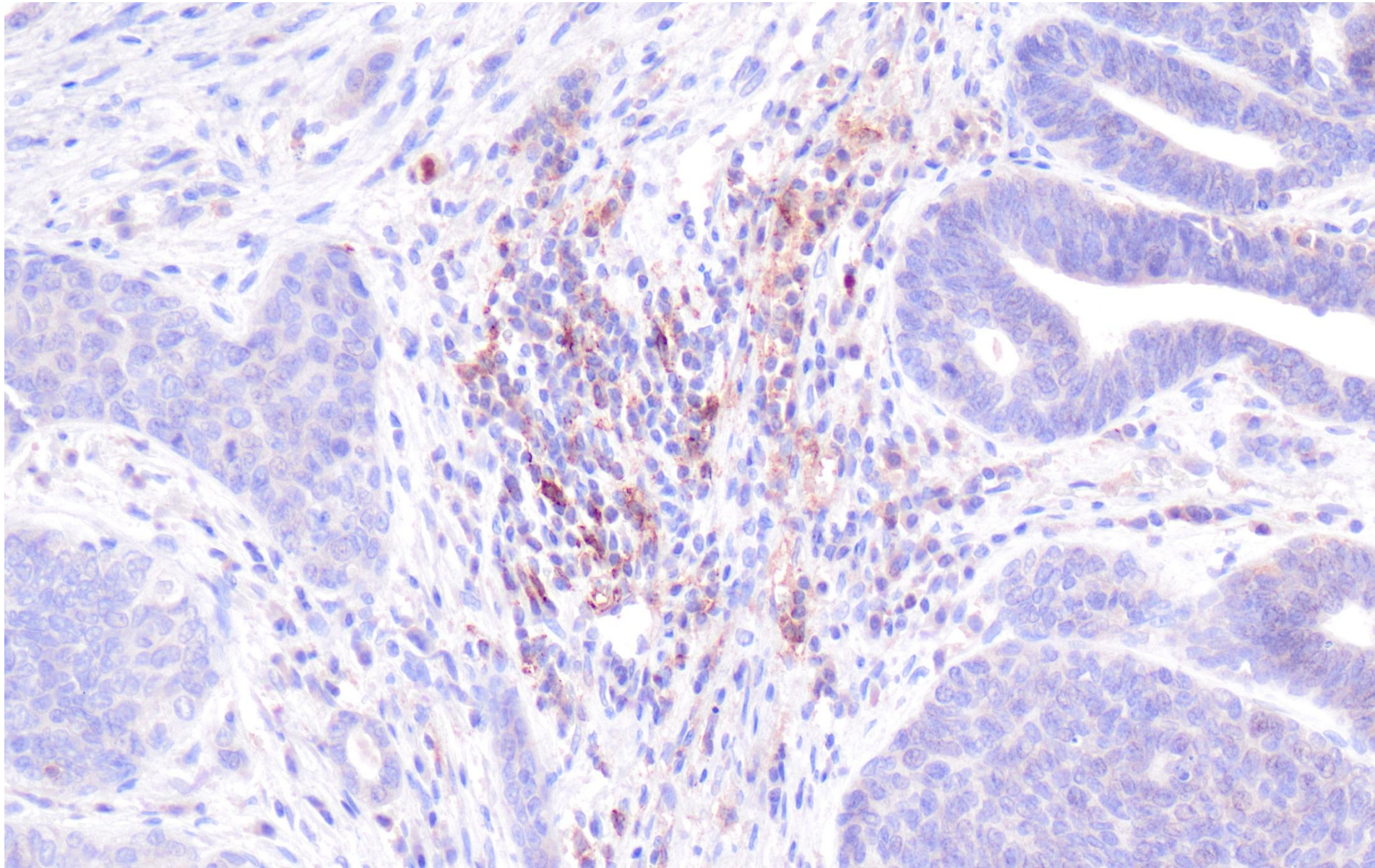
Heterogeneous / Patchy Staining in Neoplastic and Immune Cells Throughout the Tumor, Average CPS=15 (5+10+15+30=60, 60/4)





Patchy Staining in Immune Cells, Average CPS=35





PD-L1 Testing May Also Be Requested on Vulvar SCC or Endometrial Carcinoma

- 10-50% of vulvar cancers express PD-L1
- No FDA approval for immunotherapy
- Response in case reports but no large studies

- **NCCN guidelines:**
  - ✓ Pembrolizumab is a 2nd line, useful in certain circumstances option for TMB-high, PD-L1+ or MMR-deficient/MSI-high advanced or recurrent/metastatic vulvar cancer

PMID: 30016784, 26913631

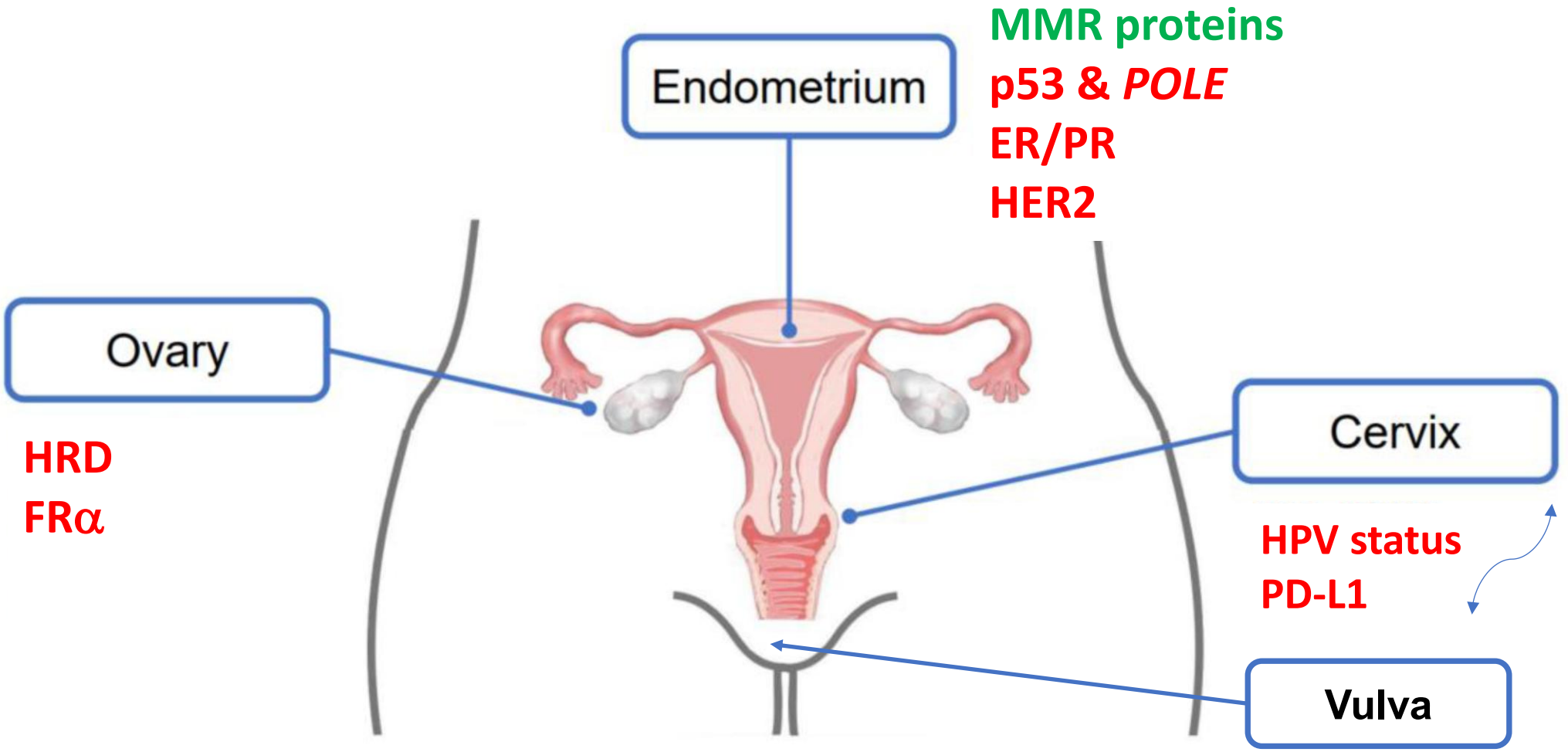
PD-L1 Testing May Also Be Requested on Vulvar SCC or Endometrial Carcinoma

- PD-1 and PD-L1 expressed in ~80%
- ↑PD-L1 expression in MMR-deficient vs MMR-proficient tumors and Lynch syndrome associated tumors vs MLH1 promoter methylated tumors:
  - ✓ Related to ↑neoantigen production and ↑immunogenicity
- PD-L1 testing not required

- FDA approved indications for immunotherapy:
  - ✓ MMR-deficient/MSI-high tumors, i.e. eligibility determined based on MMR/MSI status
  - ✓ Advanced endometrial cancer (irrespective of MMR or PD-L1 status)

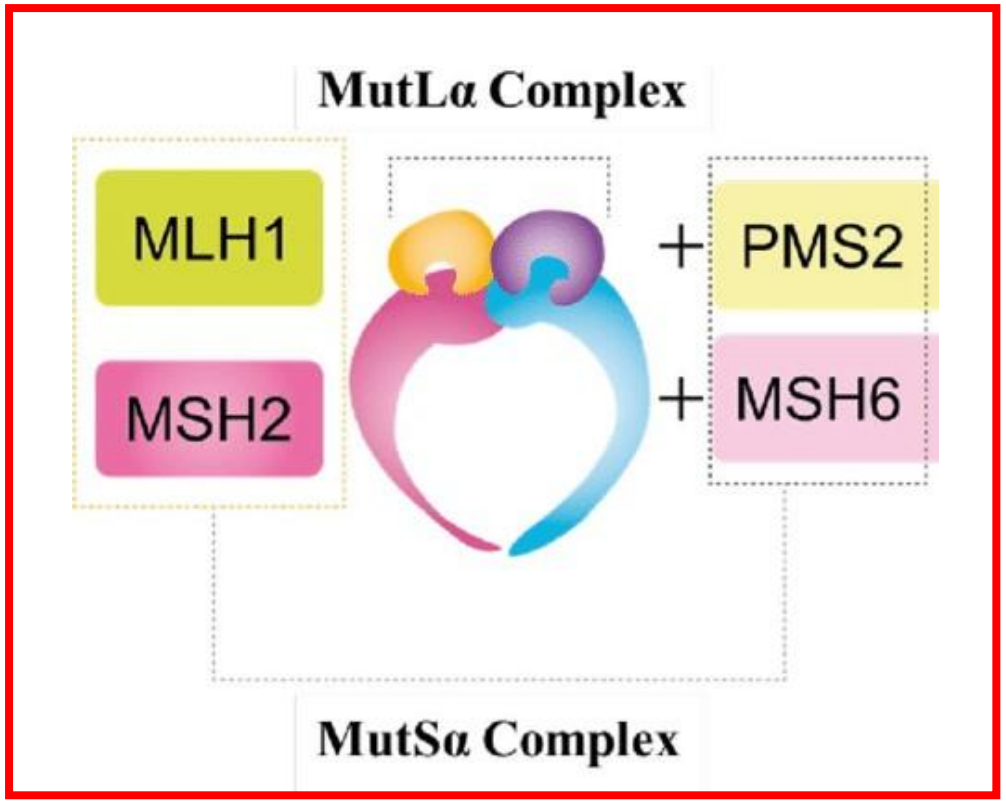
PMID: 30016784, 27984238, 26181000

Prognostic and Therapeutic Markers in Gynecologic Pathology



MMR Proteins Repair Erroneous Insertions, Deletions and Mismatched Bases During DNA Replication, Recombination, and DNA Damage

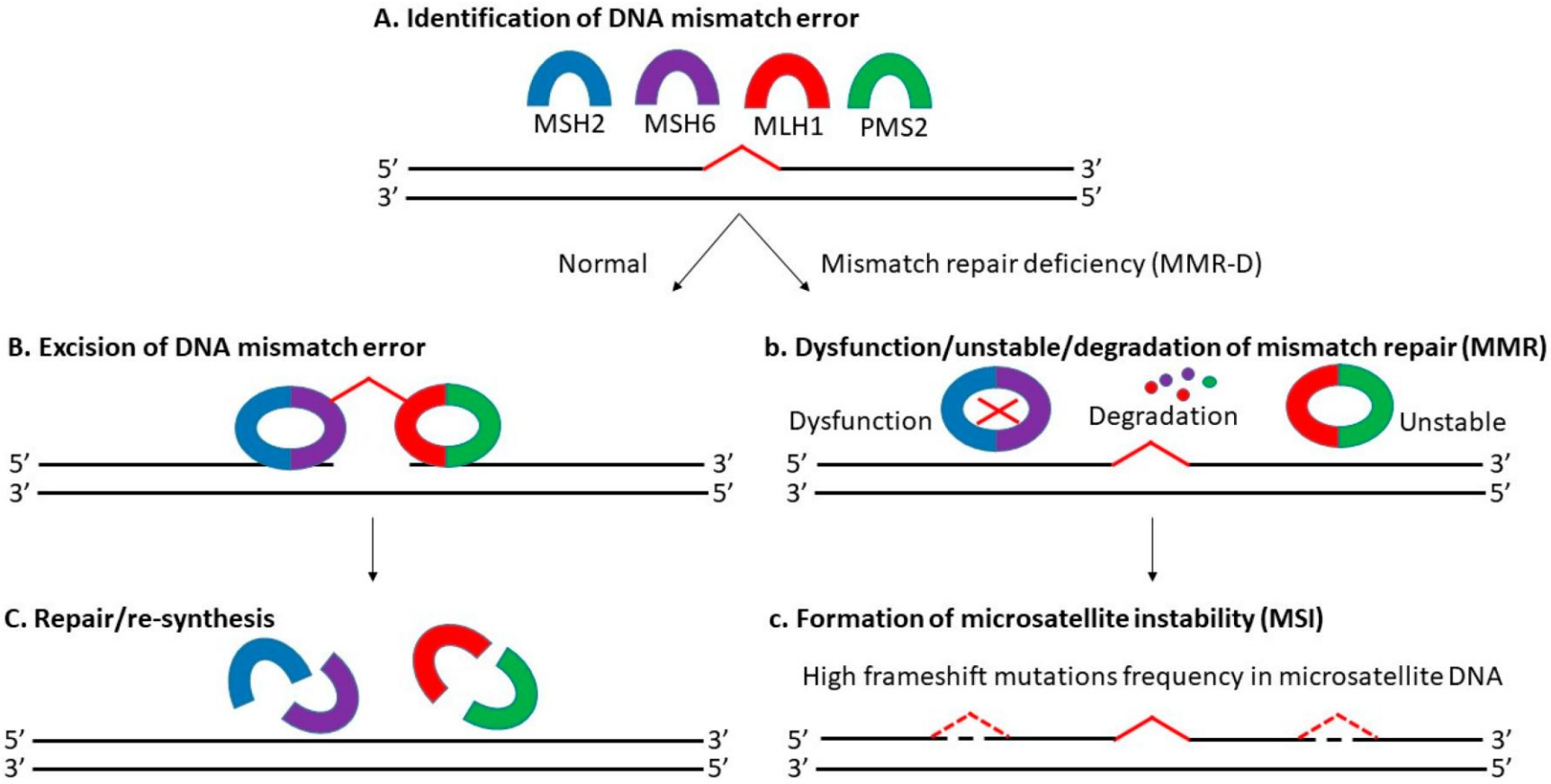
- **MMR proteins work in dimers**
- PMS2 and MSH6 proteins are unstable when not paired:
  - MLH1 loss ⇒ MLH1 and PMS2 loss
  - MSH2 loss ⇒ MSH2 and MSH6



PMID: 34750021, 31286289, 33803882

Microsatellite Instability (MSI) Is a Manifestation of Defective MMR

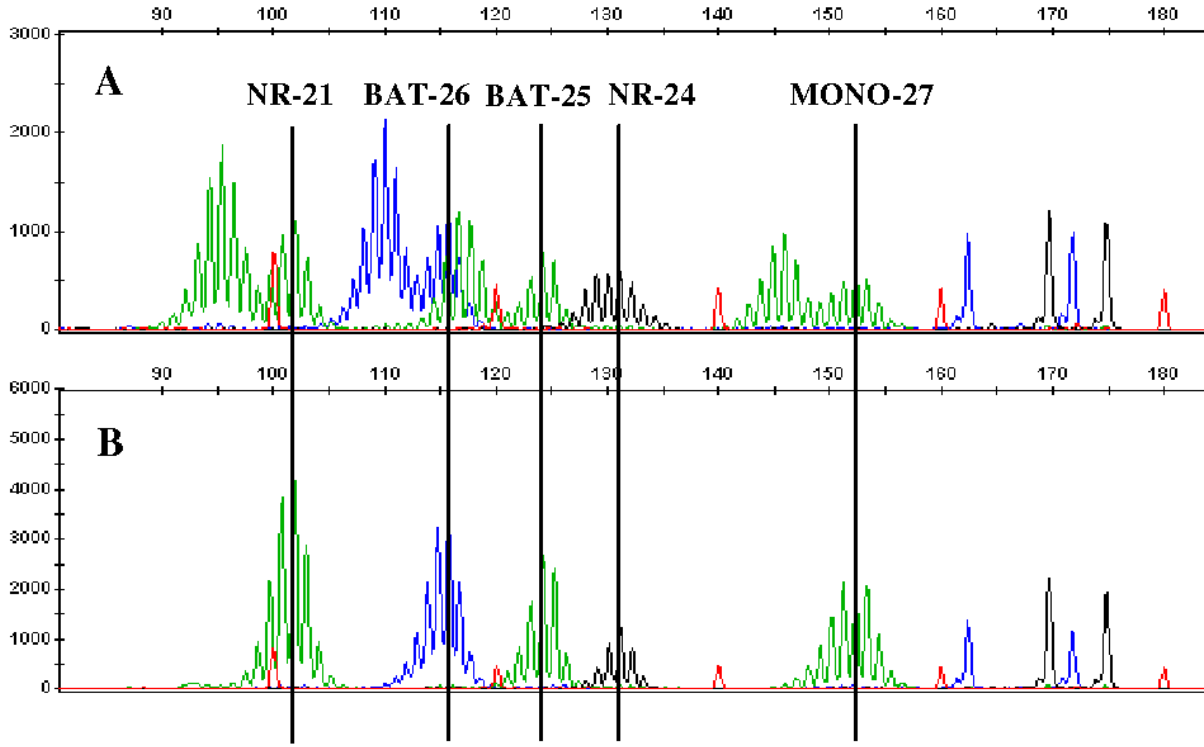
- High MSI (MSI-H) = sporadic or germline MMR defect
- Microsatellites are repetitive sequences susceptible to replicative errors



PMID: 34750021, 31286289, 33803882

MMR Status May Be Determined by 3 Methods that Have Comparable Performance Metrics

- **MSI assay by PCR:**
  - ✓ ↓Microsatellites in MSI-H and ↑*MSH6* mutations in endometrial tumors ⇒ ↓sensitivity than in colorectal cancer
  - ✓ 90% concordant with IHC
- **Next-generation sequencing (NGS):**
  - ✓ ↑TAT
  - ✓ May miss some MMR-deficient tumors
  - ✓ Biopsy may not yield enough DNA
- **MMR IHC:**
  - ✓ Widely available
  - ✓ Can identify the most probable gene defect
  - ✓ Can test biopsies and hysterectomies



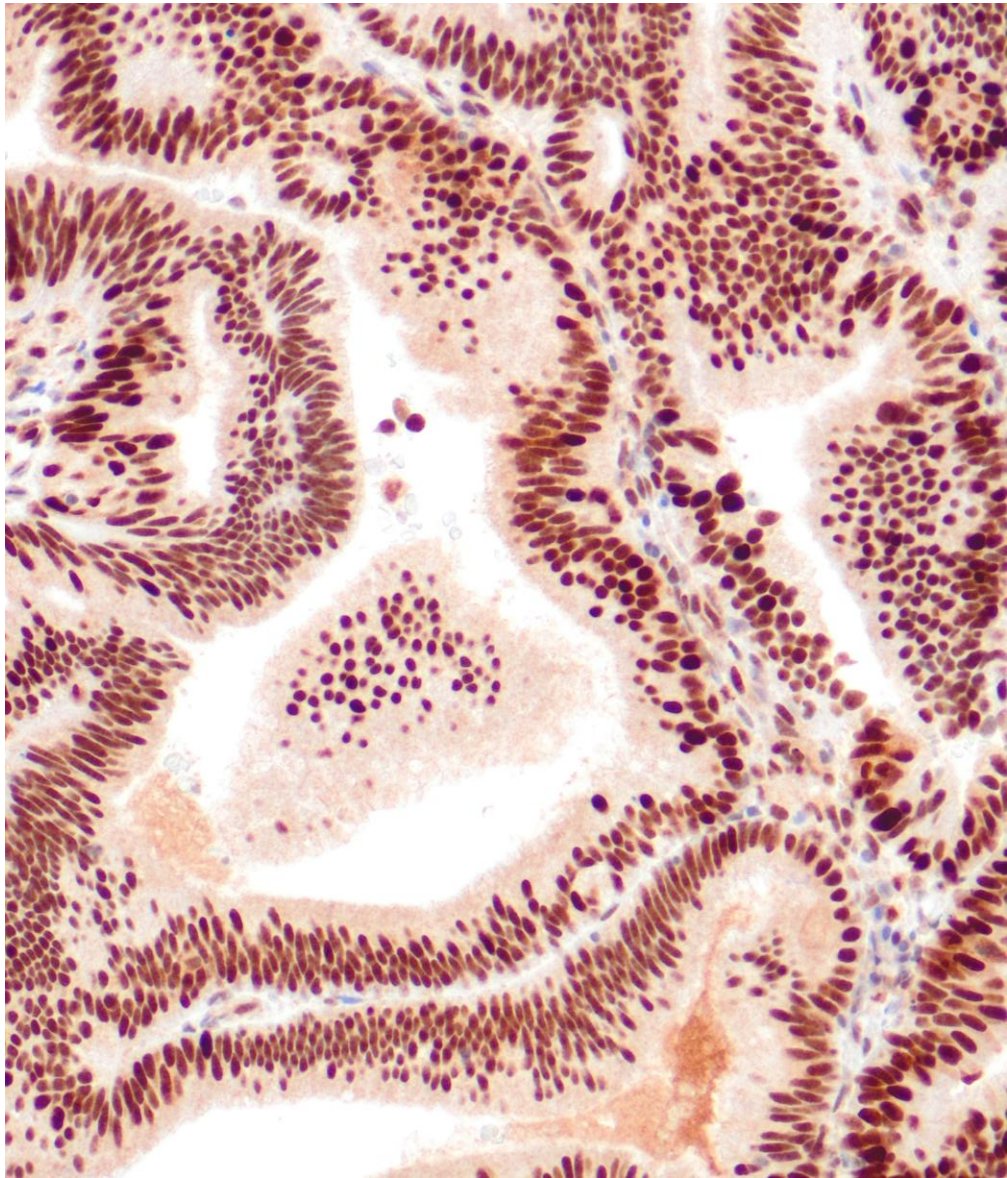
PMID: 12057899, 34312297, 34312297

MMR Protein Expression by Immunohistochemistry Is Evaluated in Tumor Cell Nuclei

- **4-protein panel:** MLH1/PMS2, MSH2/MSH6
- **2-protein panel:** PMS2, MSH6

- **Intact nuclear expression:**
  - ✓ Intensity ≥ internal positive control (stromal cells, lymphocytes)

- **Loss of nuclear expression ⇒ MMR-deficiency:**
  - ✓ Internal positive control must be positive



PMID: 34750021, 36104536

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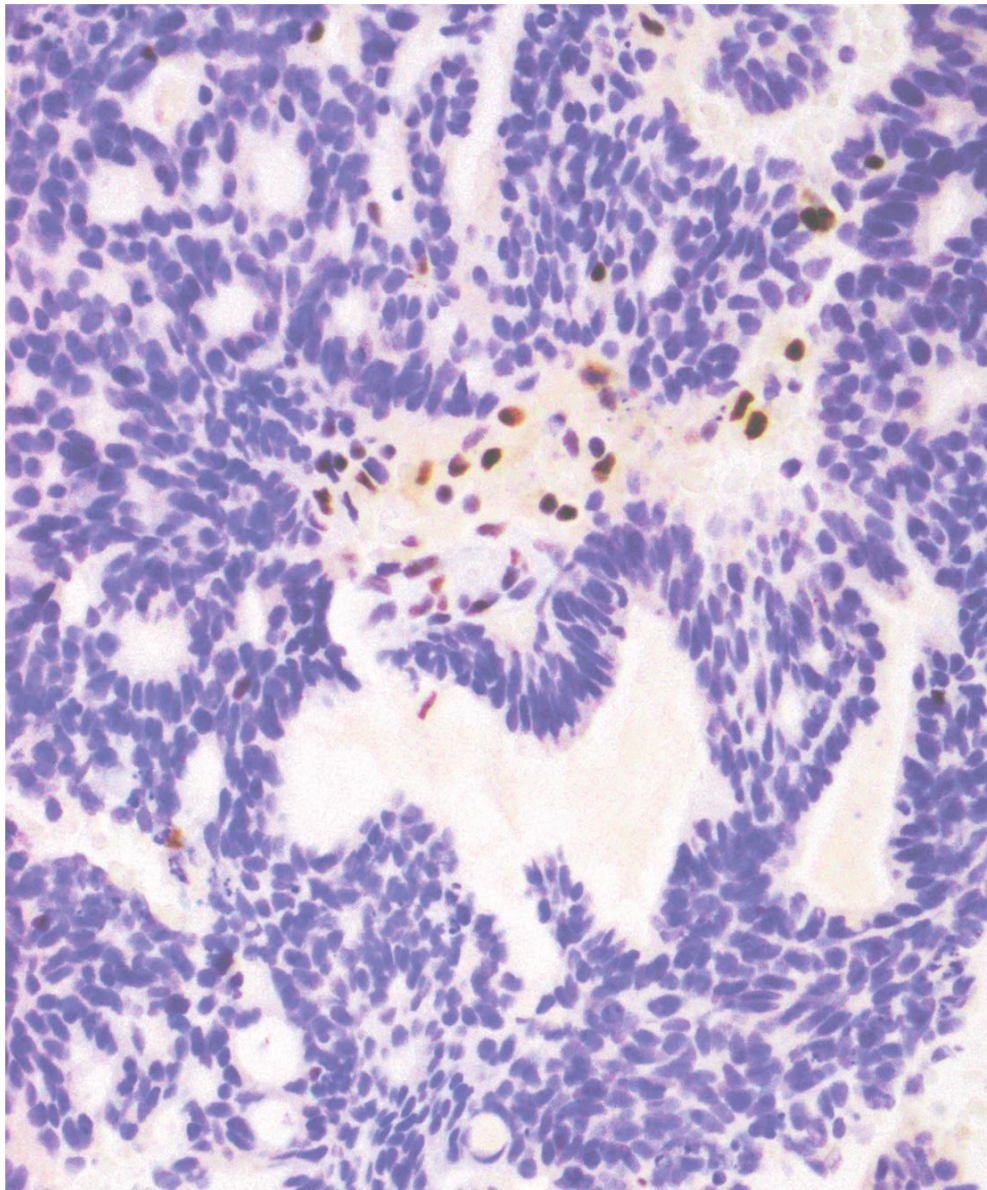
- **4-protein panel:** MLH1/PMS2, MSH2/MSH6
- **2-protein panel:** PMS2, MSH6

- **Intact nuclear expression:**
  - ✓ Intensity ≥ internal positive control (endometrial stromal cells, lymphocytes)

- **Loss of nuclear expression ⇒ MMR-deficiency:**
  - ✓ Internal positive control must be positive



PMID: 34750021, 36104536

MMR Testing Is Performed for Several Indications

- **Eligibility for immune checkpoint inhibitor therapy:**
  - ✓ MMRd tumors have high neoantigen burden which recruits T cells and mediates response to immune checkpoint inhibitors
- **Lynch syndrome screening:**
  - ✓ All endometrial carcinomas, including carcinosarcomas
  - ✓ Extrauterine endometriosis-associated carcinomas (endometrioid, clear cell, de-/undifferentiated)
  - ✓ Guides follow-up (genetic counseling, germline evaluation)
- **Molecular classification:**
  - ✓ Identified MSI-high (MMRd) subtype

PMID: 34750021, 31286289, 27186853, 36866757

COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy

SUMMARY OF RECOMMENDATIONS

Guideline Statement	Strength of Recommendation
3. For patients with endometrial cancer being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC over MSI by PCR or NGS for the detection of DNA MMR defects.	Strong Recommendation
5. For all cancer patients being considered for immune checkpoint inhibitor therapy based upon defective MMR, pathologists should **NOT** use TMB as a surrogate for the detection of DNA MMR defects. If a tumor is identified as TMB-high, pathologists may perform IHC and/or MSI by PCR to determine if high TMB is secondary to MMR deficiency.	Strong Recommendation
6. For cancer patients being considered for immune checkpoint inhibitor therapy, if an MMR deficiency consistent with Lynch Syndrome is identified in the tumor, pathologists should communicate this finding with the treating physician.	Strong Recommendation

PMID: 35920830

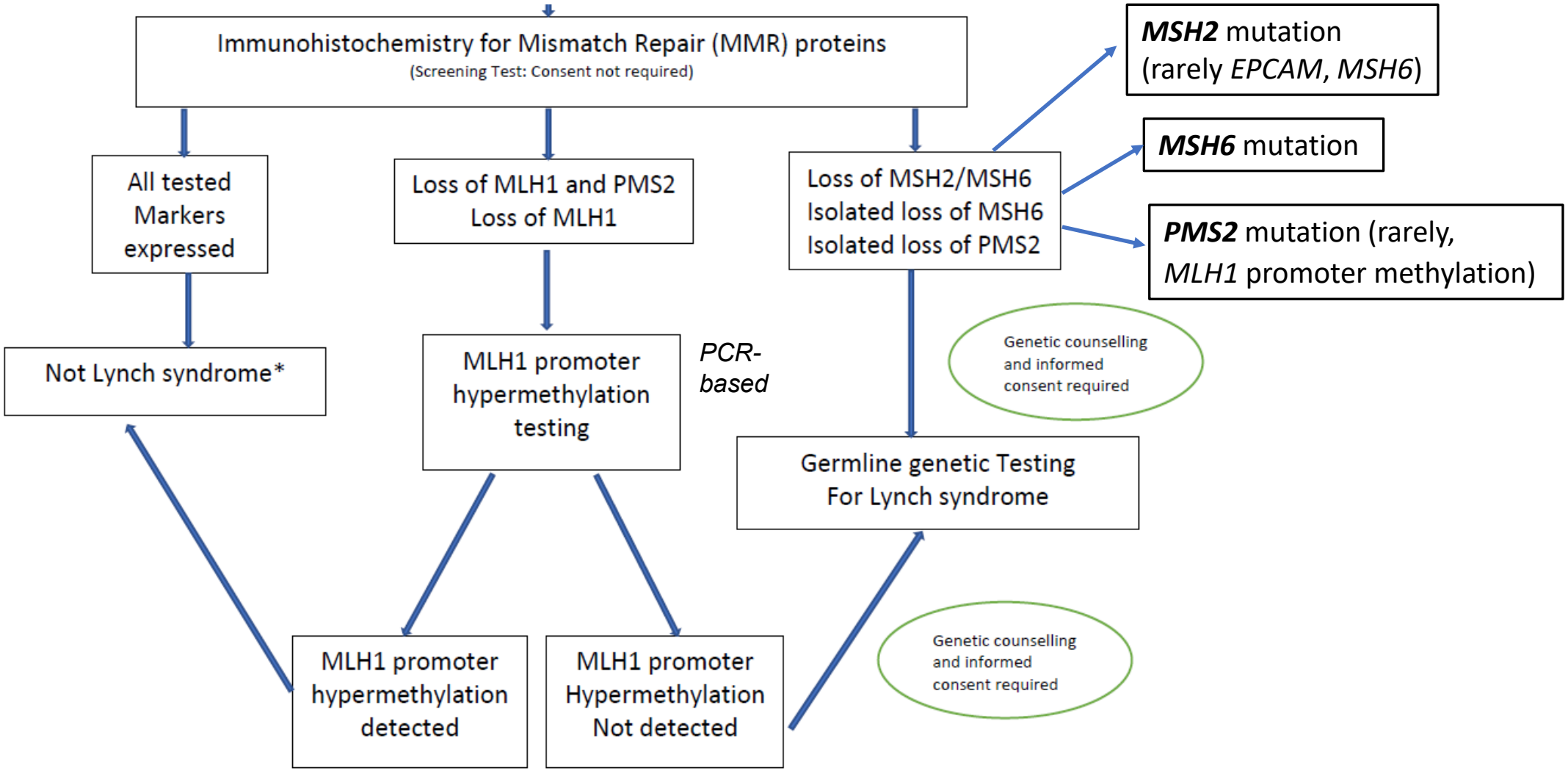
MMR-Deficiency Is Significantly More Common Than Lynch Syndrome

- **Prevalence of MMR-deficiency:**
  - ✓ 20-30% of endometrial carcinomas
  - ✓ 8-14% of ovarian endometriosis-associated carcinomas

- **Lynch syndrome:**
  - ✓ 5-6% of endometrial cancers (~2.5% germline-confirmed)
  - ✓ 1% of ovarian cancers

PMID: 34750021, 31286289

Lynch Syndrome Screening

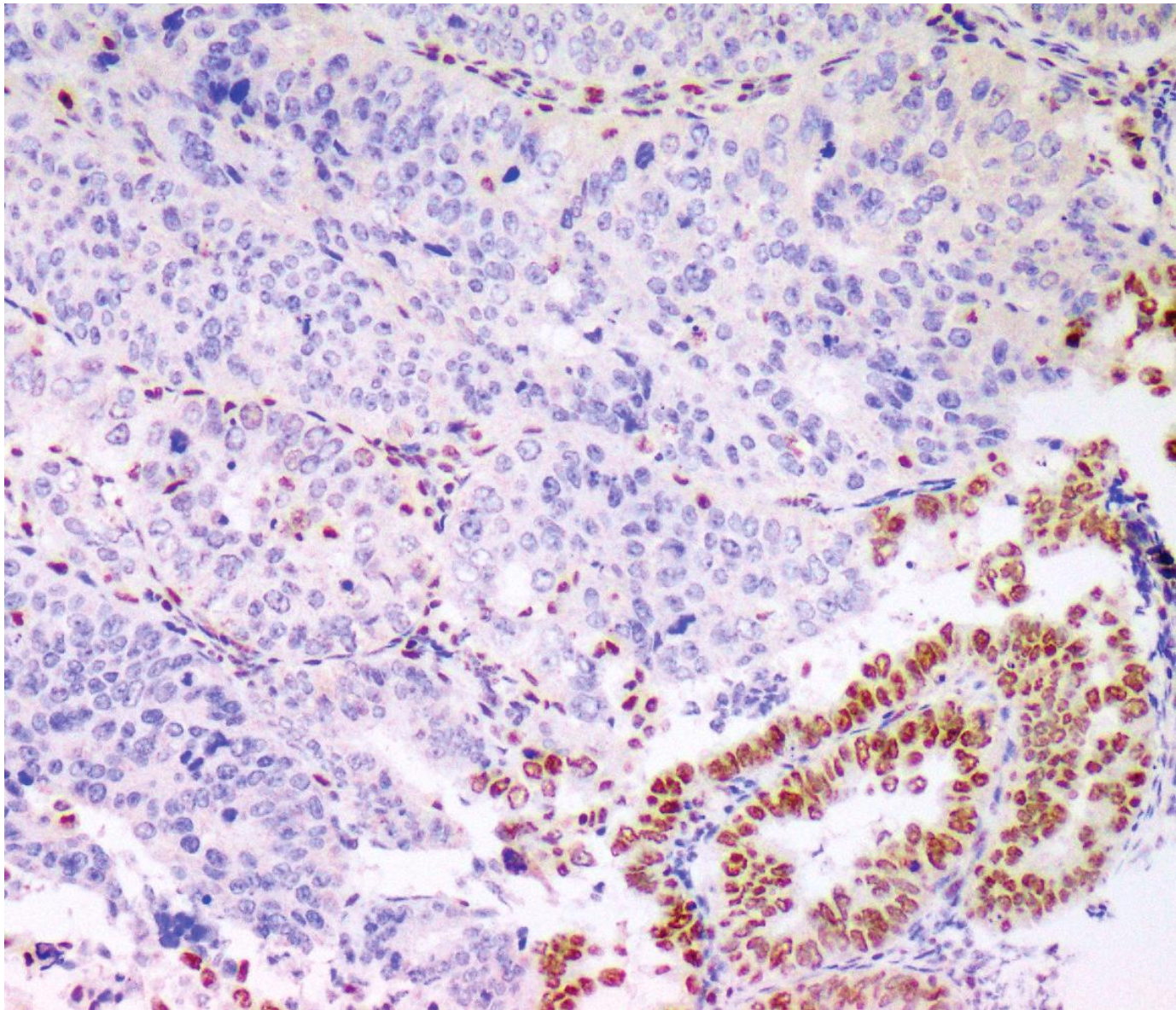


*Rarely, germline epimutation of *MLH1* promoter (PMID: 33934415)

Modified from https://www.thebagp.org/resources/?wpdmc=bagp-guidance-document

Unusual Staining Patterns for MMR Proteins

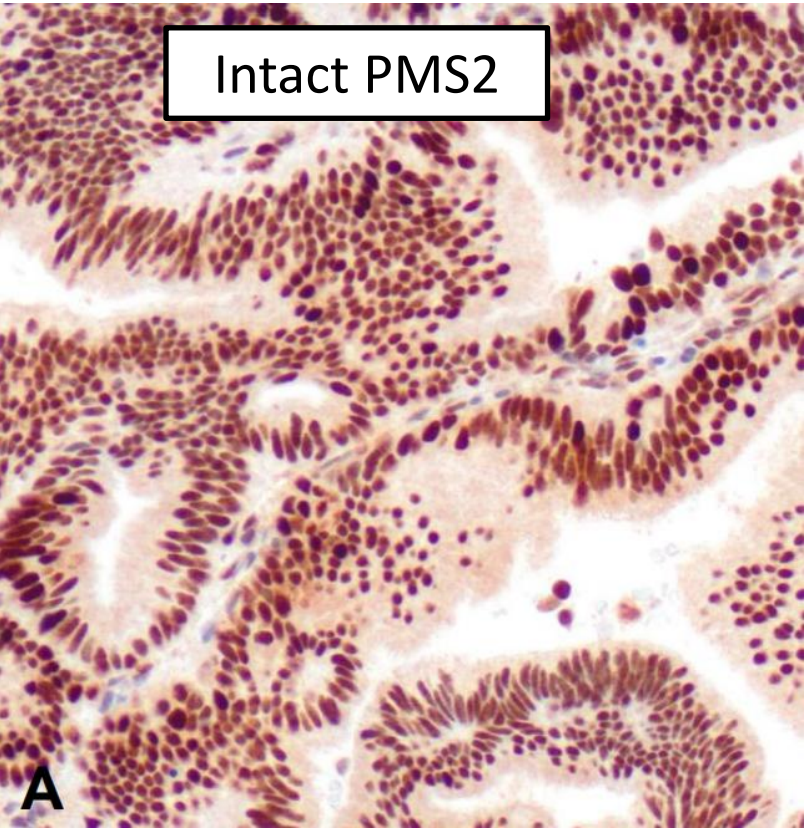
- **Subclonal loss of expression:**
  - ✓Rare
  - ✓Most often involves MLH1/PMS2
  - ✓Subclonal methylation of *MLH1* promoter
  - ✓Lynch-syndrome associated tumors
  - ✓*POLE*-mutated tumors
  - ✓Consider MSI testing of the area with loss of expression to determine eligibility for immunotherapy trials

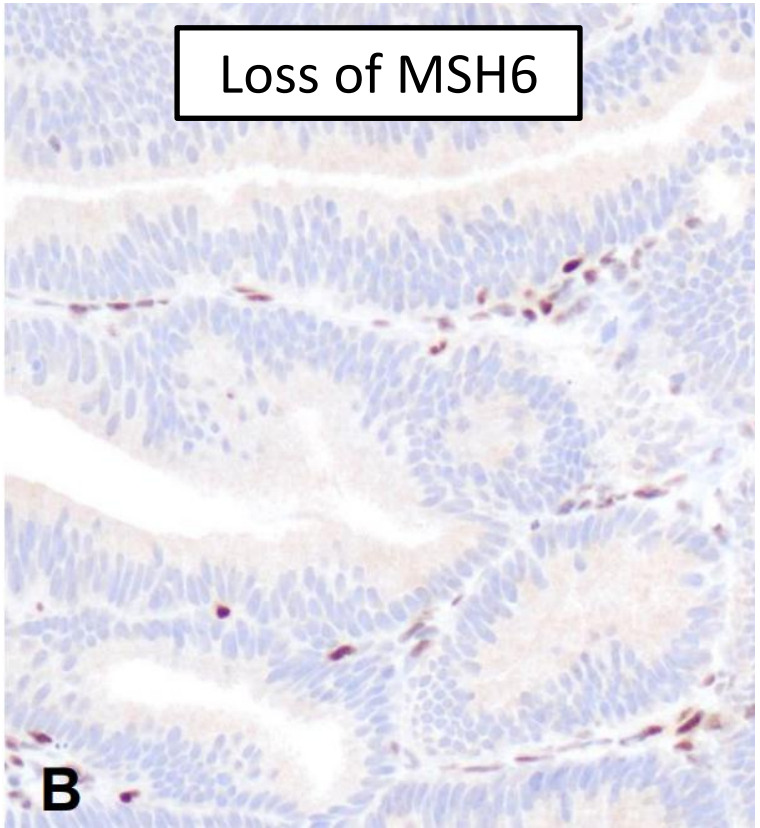


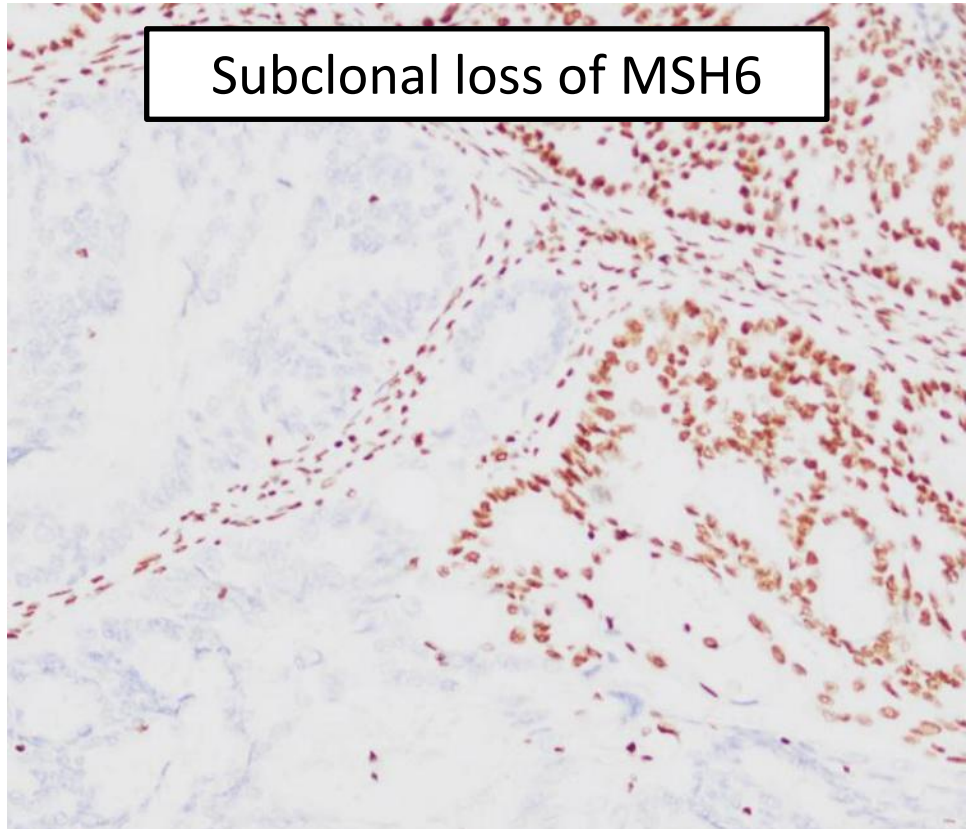
PMID: 36866757, 35920830

Table 5. Reporting Results of Mismatch Repair Status by Immunohistochemistry *PMID: 40956279*

Result	Criteria
Intact expression	Nuclear staining with similar or stronger intensity compared with the background internal control cells (endometrial stromal cells, smooth muscle cells, nonneoplastic epithelial cells, inflammatory cells)
Abnormal expression patterns	
Loss of expression	Absence of nuclear staining in tumor cells with satisfactory internal positive control
Subclonal loss of expression	Discrete areas of tumor with complete loss of nuclear expression adjacent to tumor cells with retained expression (no minimum percentage of cells with loss of expression required) and with satisfactory internal positive control

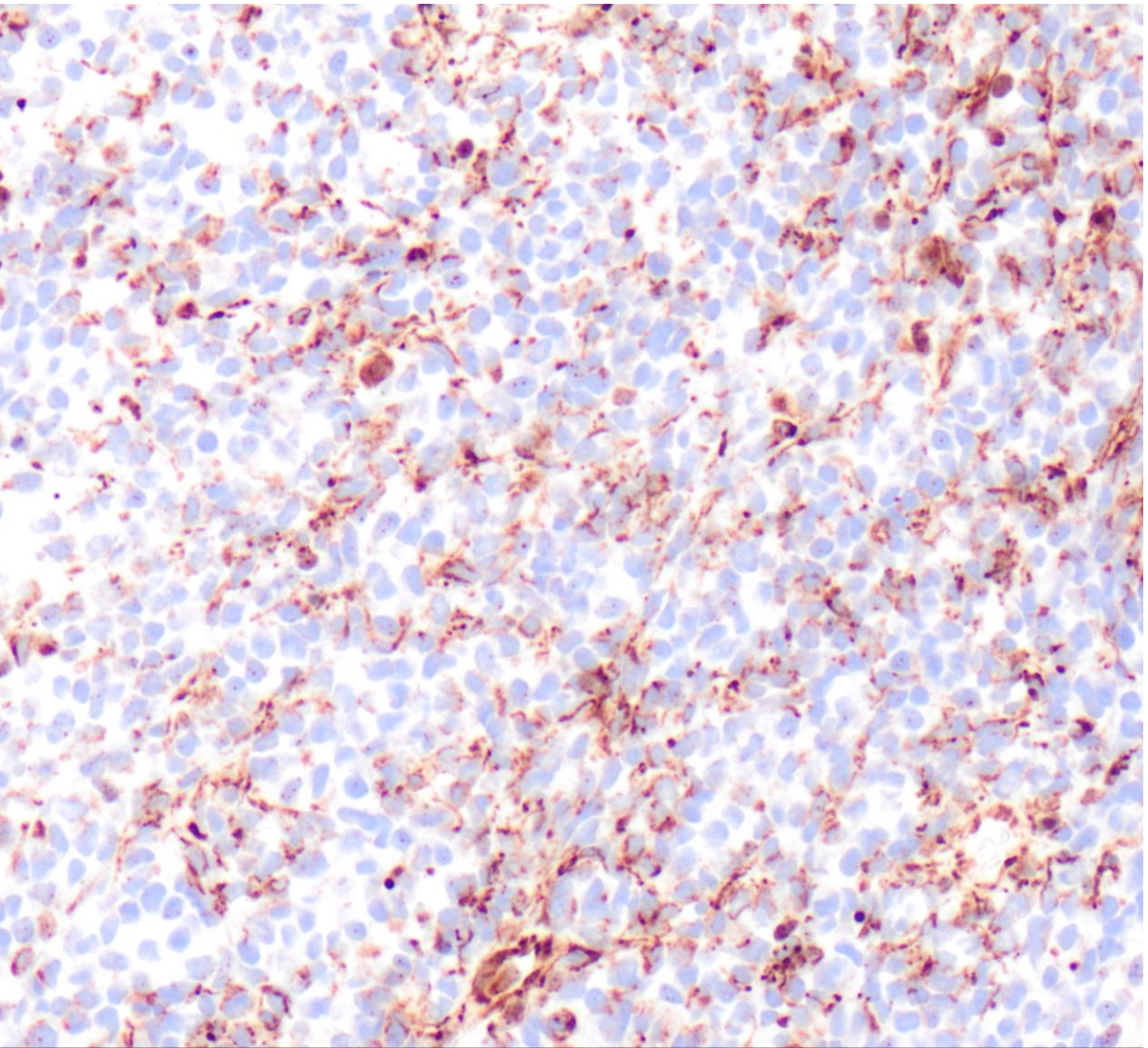




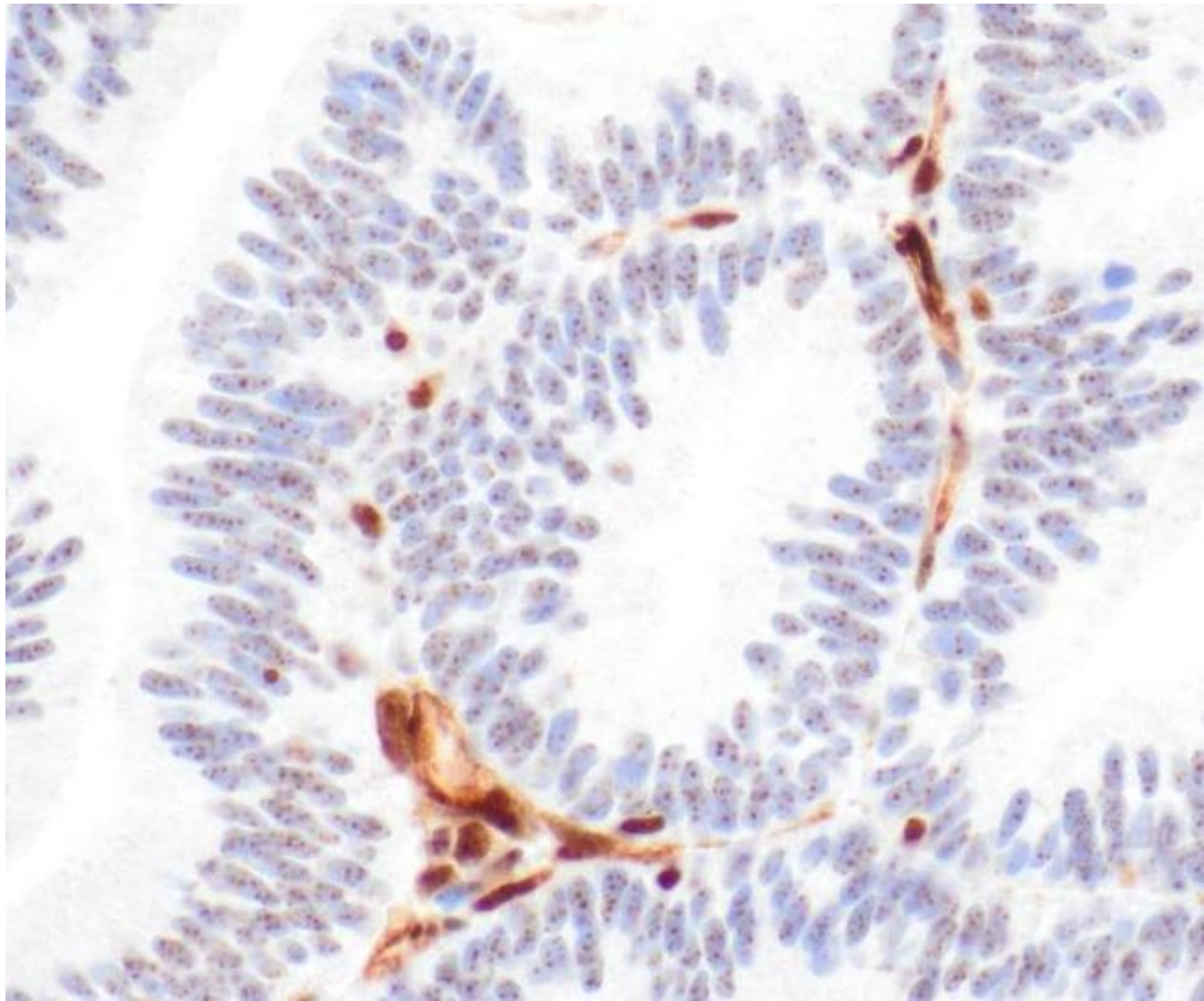


Unusual Staining Patterns for MMR Proteins

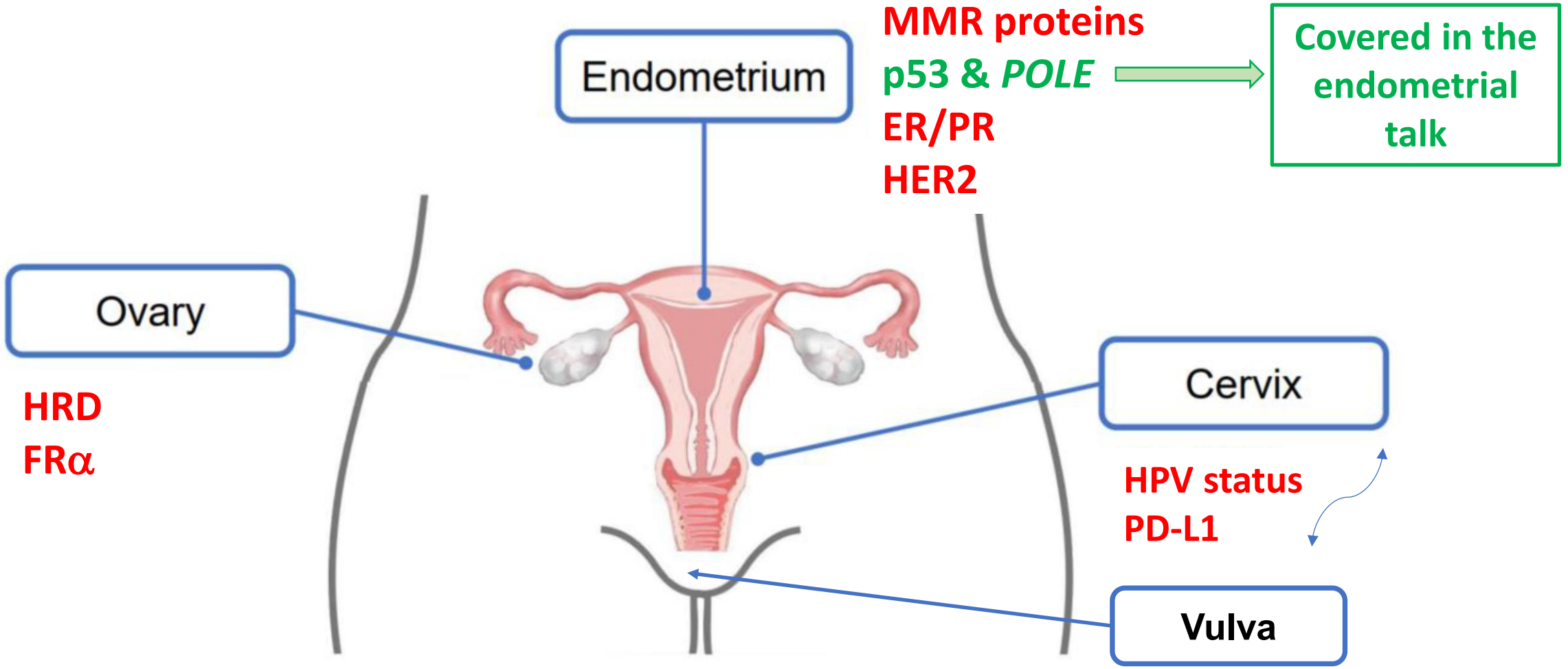
Cytoplasmic staining (PMS2)



Punctate staining (MLH1)



Prognostic and Therapeutic Markers in Gynecologic Pathology



TP53 Is a Tumor Suppressor Gene

- p53 protein regulates proliferation and apoptosis, and maintains genome stability (Guardian of the Genome)

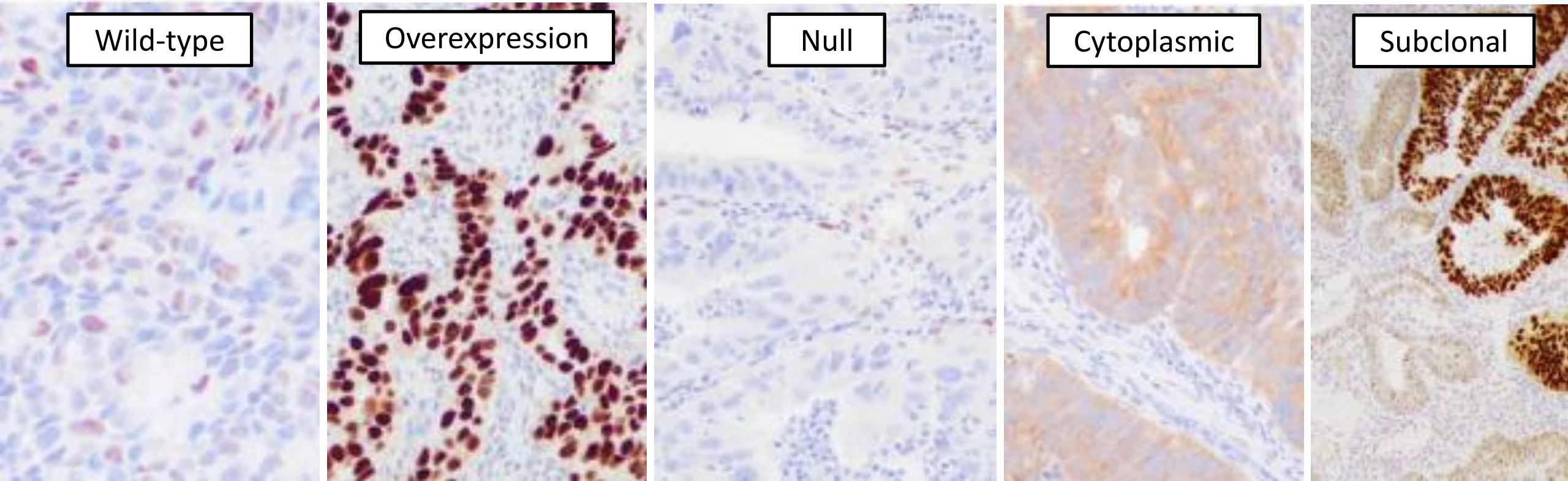
- **Indications for p53 testing:**
  - ✓ ***Tumor subtyping:*** Abnormal expression in most serous carcinomas and carcinosarcomas, ~30% of endometrioid and clear cell carcinomas
  - ✓ ***Molecular classification:*** Identifies copy number high/p53-abnormal subtype

- p53 IHC is accurate for identifying *TP53* mutations:
  - ✓ ~95% sensitivity in endometrial and ovarian carcinomas

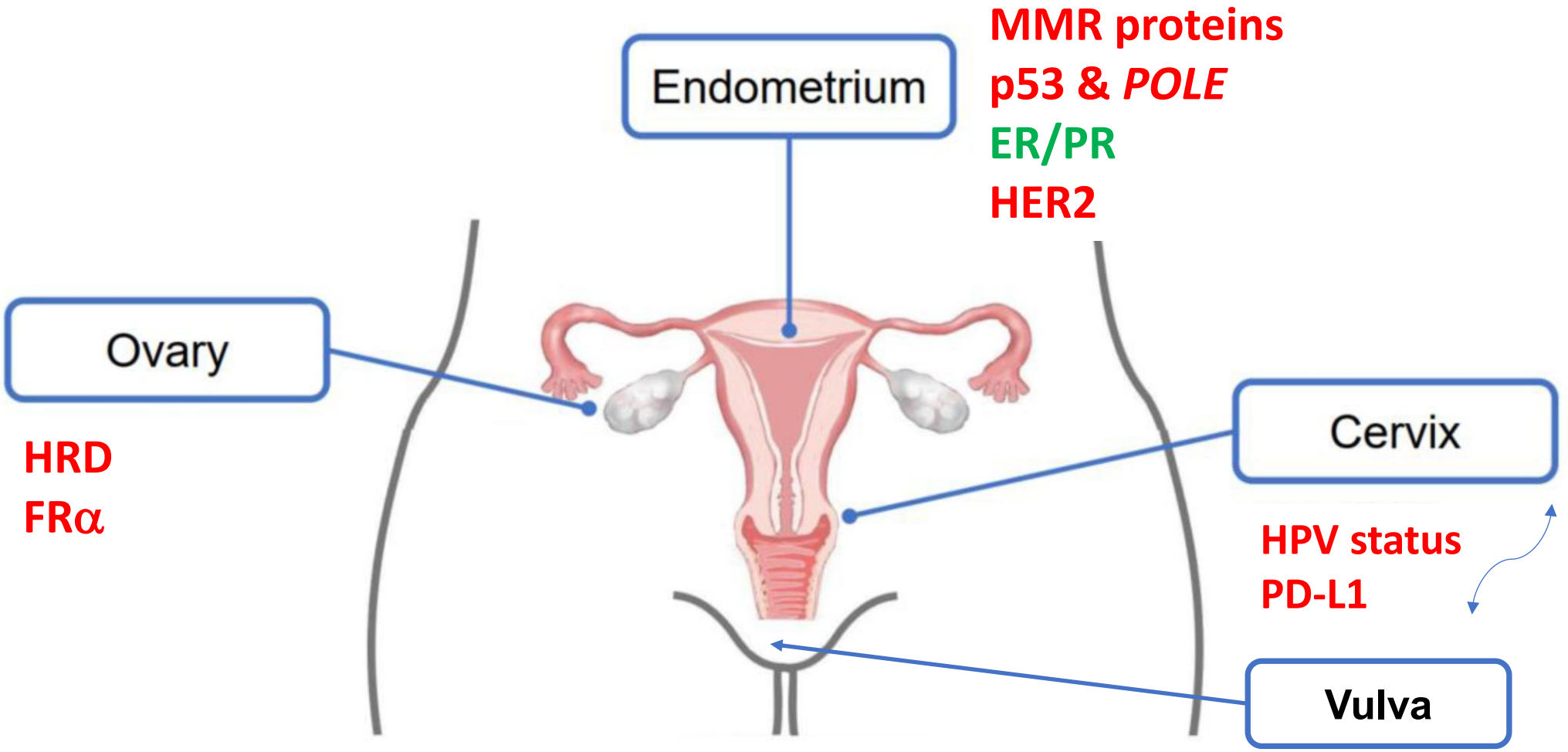
PMID: 29517499, 29517499, 36948887

Table 6. Reporting Results of p53 Status by Immunohistochemistry PMID: 40956279

Result	Criteria
Wild-type expression	Nuclear staining of varying intensity admixed with negative nuclei
Abnormal (mutated) expression patterns	
Abnormal expression (overexpression)	Diffuse, strong nuclear positivity in at least 80% of tumor cells
Abnormal expression (null type)	Complete absence of nuclear and cytoplasmic reactivity in tumor cells (with satisfactory internal positive control)
Abnormal expression (cytoplasmic)	Cytoplasmic staining that may be accompanied by nuclear reactivity
Subclonal abnormal expression	Abnormal expression (any of the above) in a subset of tumor cells



Prognostic and Therapeutic Markers in Gynecologic Pathology



ER and PR Are Nuclear Hormone Receptor Transcription Factors

- **Indications for ER/PR testing:**
  - ✓ ***Tumor subtyping:*** Usually negative in clear cell, gastric-type and mesonephric-like carcinomas; positive in endometrioid carcinomas, variable in serous carcinomas, etc
  - ✓ ***Prognostic/predictive:*** Direct targets (antiestrogens, progestins) or indirect effectors (aromatase inhibitors) in treatment of low-grade endometrioid carcinoma and atypical hyperplasia
  - ✓ ***Molecular classification:*** Risk stratification of NSMP (no specific molecular profile) subtype
- **ER/PR IHC:**
  - ✓ 1% cut-off for positivity
  - ✓ Variable cut-offs for prognostic/predictive value (10%)

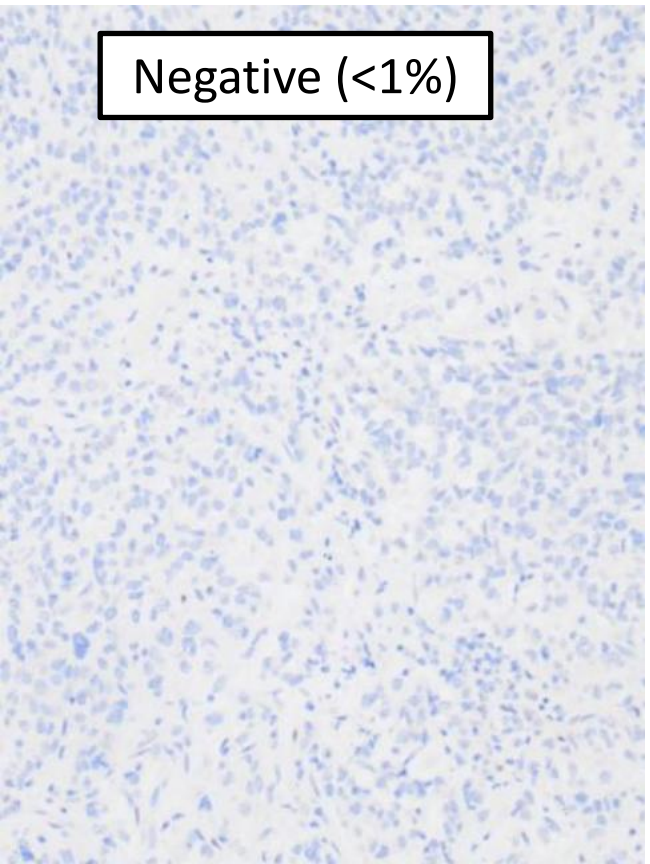
PMID: 36788084, 27006490, 36690721, 33338506, 39515079

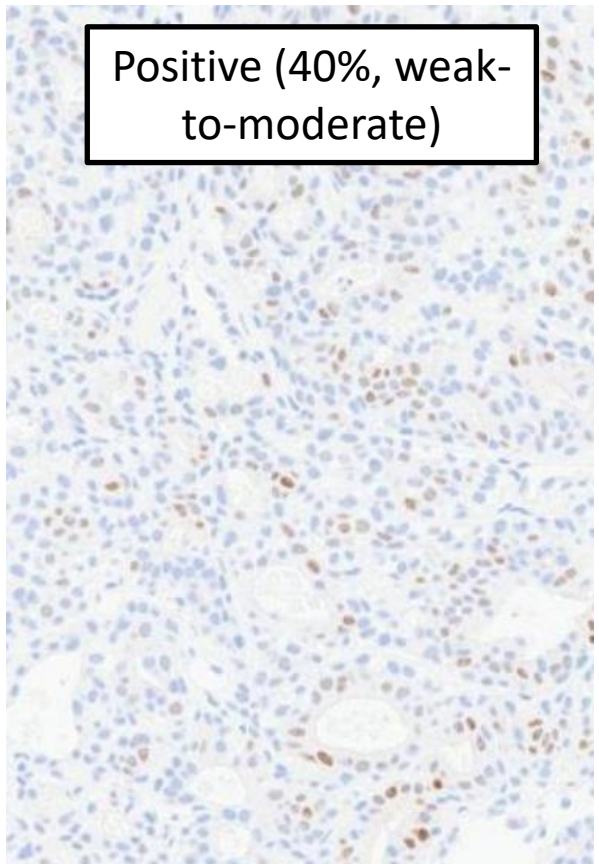
Table 1. Reporting Results of Estrogen Receptor and Progesterone Receptor Testing by Immunohistochemistry (Modified Reporting Format)[a]

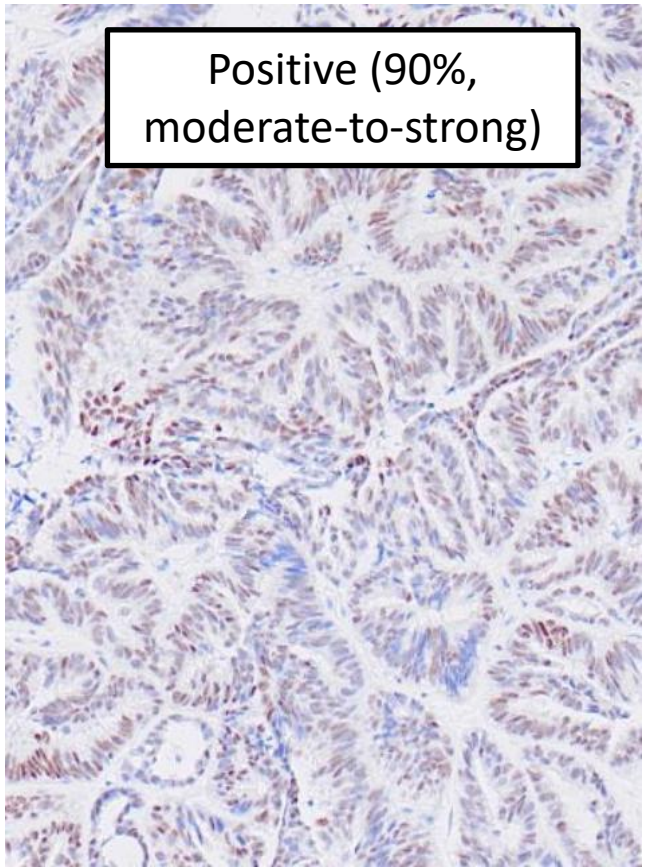
Result	Criteria	Comments
Positive	Immunoreactive tumor cells present (≥1%) showing nuclear staining	The percentage of immunoreactive cells may be determined by visual estimation or quantitation. Quantitation should be provided by reporting the percentage of positive cells in the entire section. If there is significant regional variation, that should also be reported.
Negative	Less than 1% immunoreactive tumor cells present	

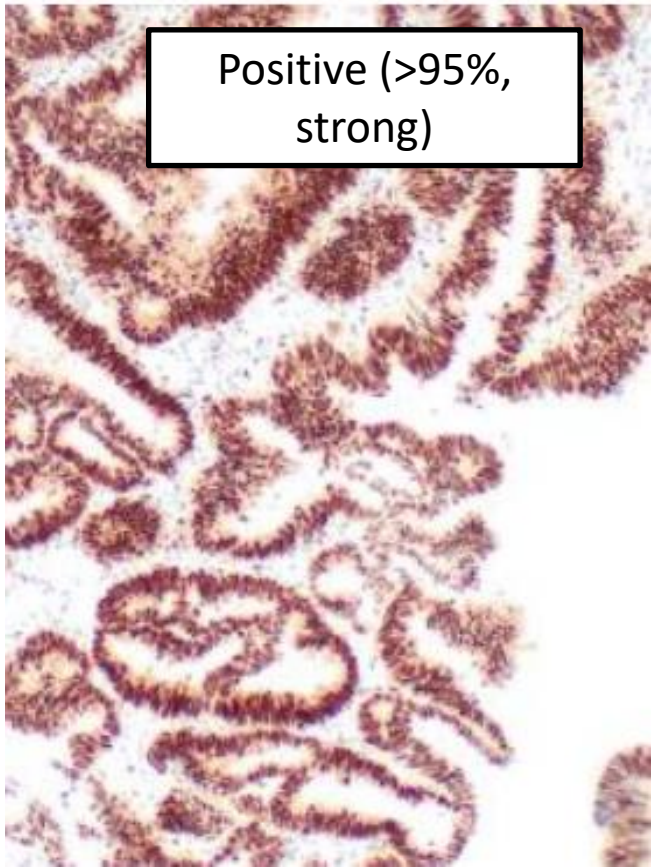
PMID: 40956279

[a] Modified reporting format suggested for gynecologic cancers as opposed to the current American Society of Clinical Oncology/College of American Pathologists guideline for breast cancer.



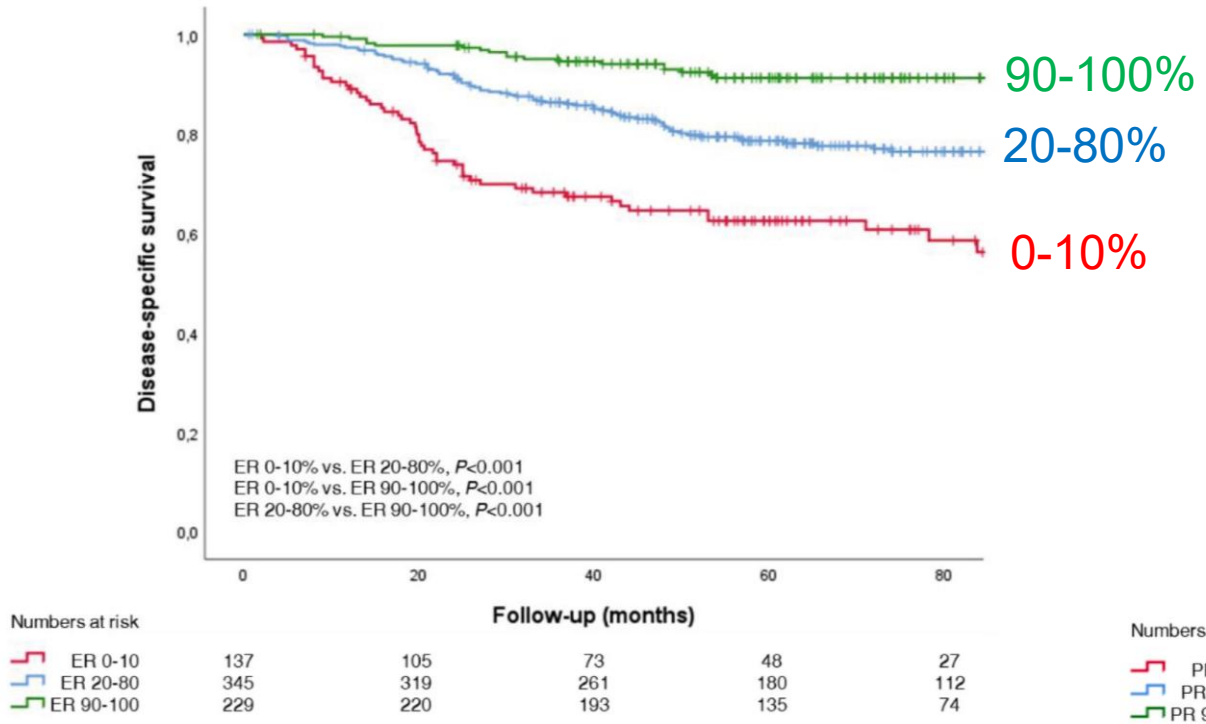




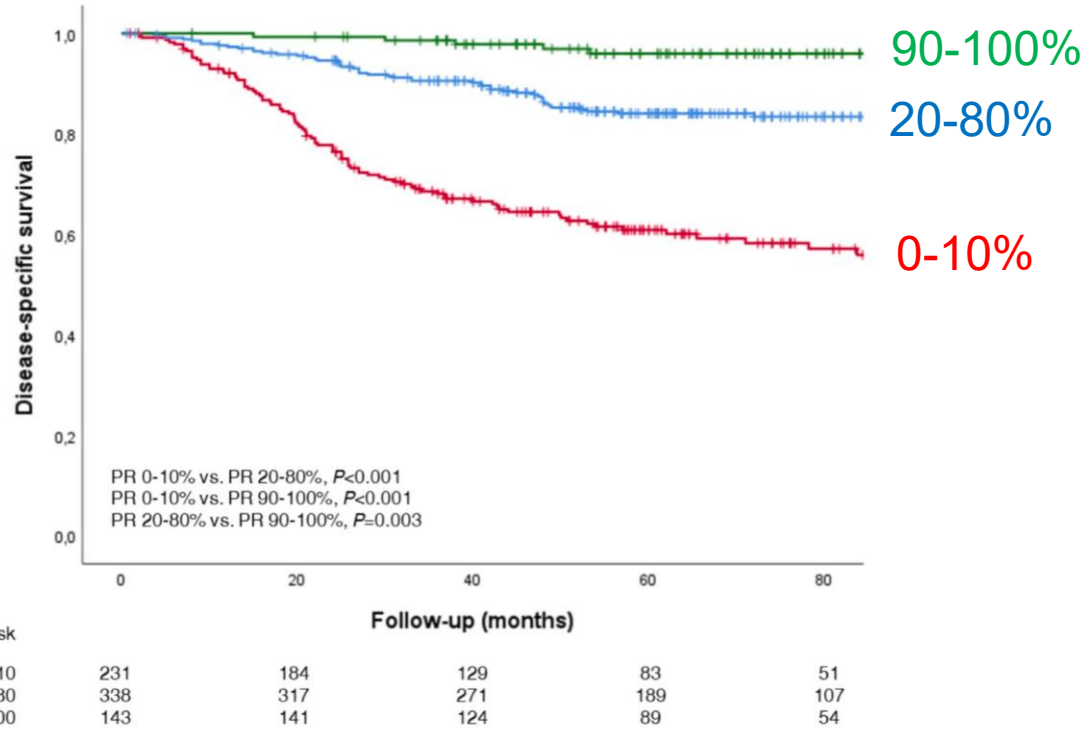


Hormone Receptors Are Prognostic Within the Entire Cohort of Endometrial Cancer Patients

ER IHC



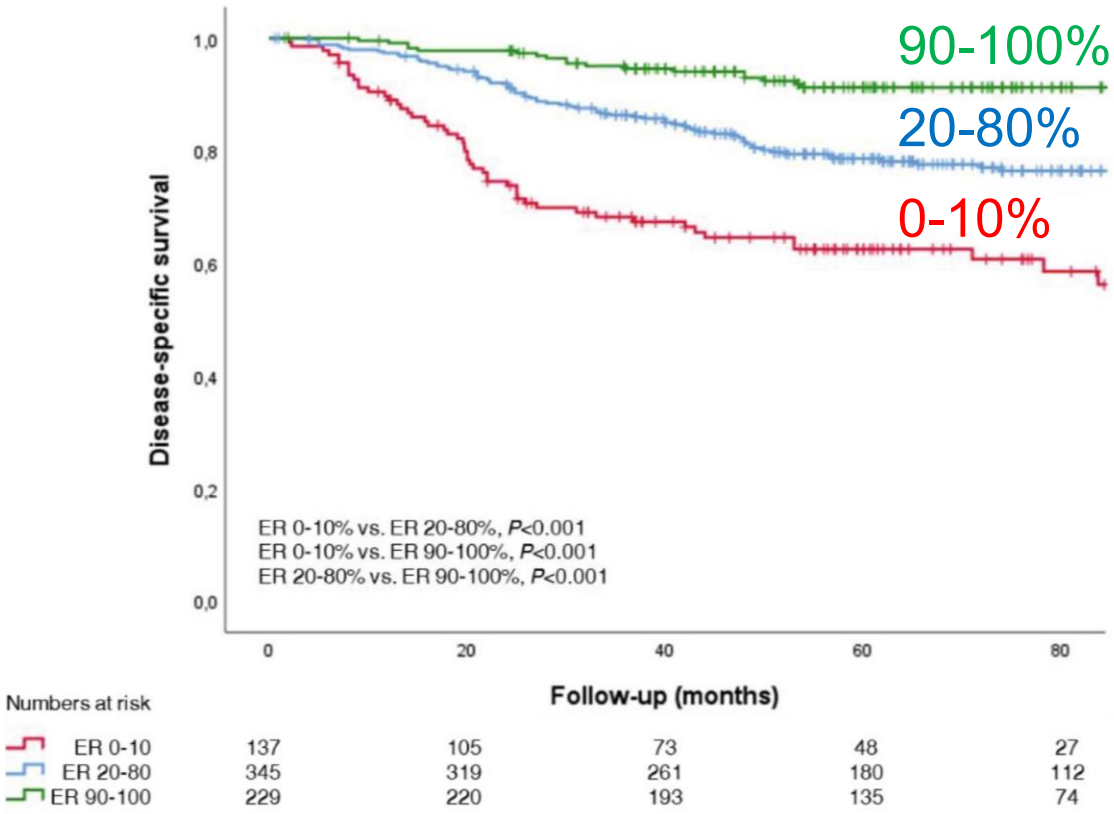
PR IHC



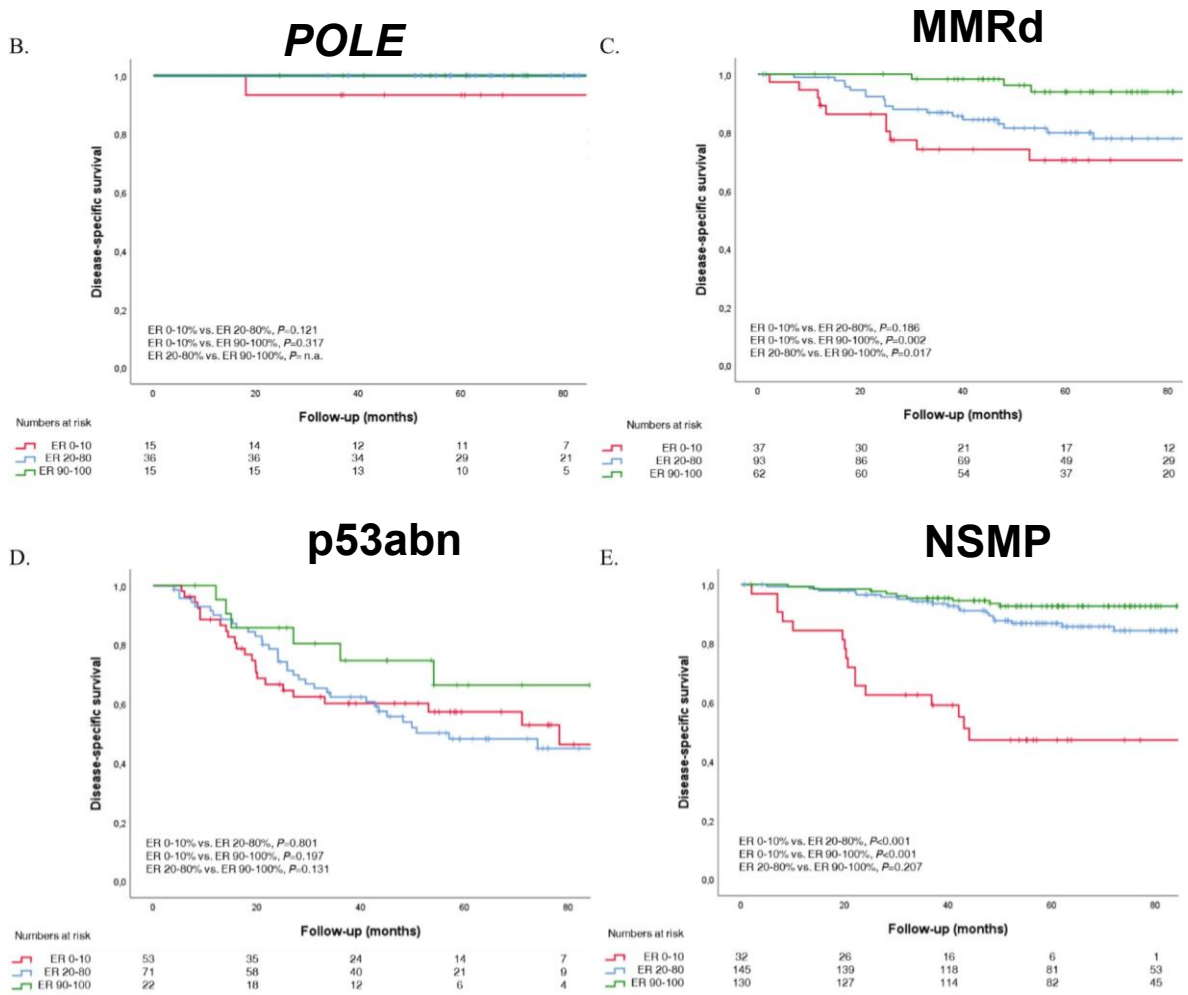
PMID: 39515079

Hormonal Biomarkers Remain Prognostically Relevant Within the Molecular Subgroups in Endometrial Cancer

ER IHC



ER IHC



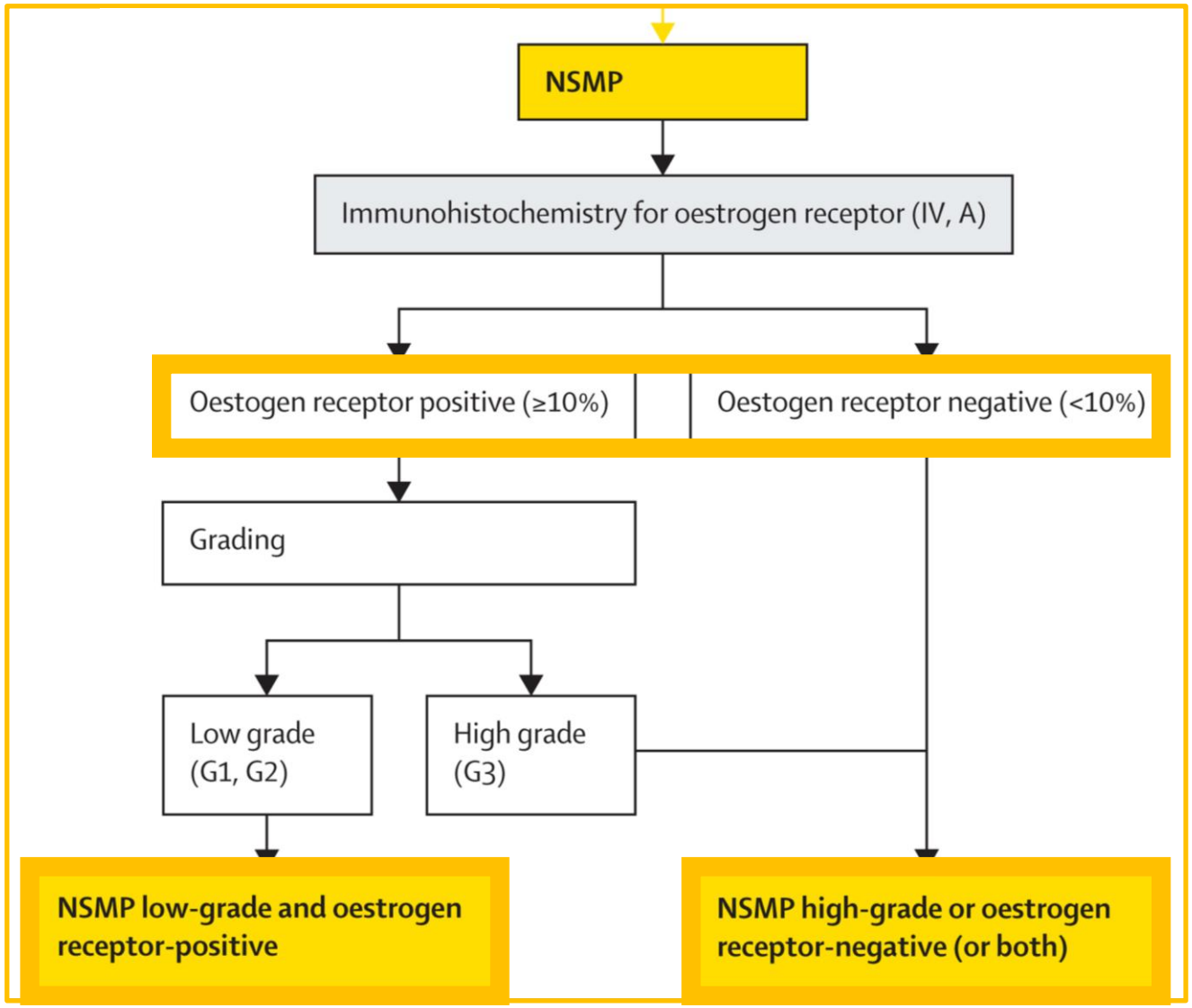
PMID: 39515079

ER/PR IHC: What Cut-off Should You Use for Positivity for Prognostic Purposes?

- 1% (PMID: 27072807, 29085668, 36788084)
- 5% (PMID: 29085668)
- 10% (PMID: 27006490, 36690721)
- Allred score (% and intensity) (PMID: 38890776, 36788084)
- 3-tier: 0-10%, 20-80%, 90-100% (PMID: 33338506, 39515079)

- *CAP recommends using 1% cut-off and specifying percent positive and intensity*
- *Make note of any variation in expression, especially in myoinvasive component*

ESGO-ESTRO-ESP Guidelines (2025 Update)



PMID: 40744042

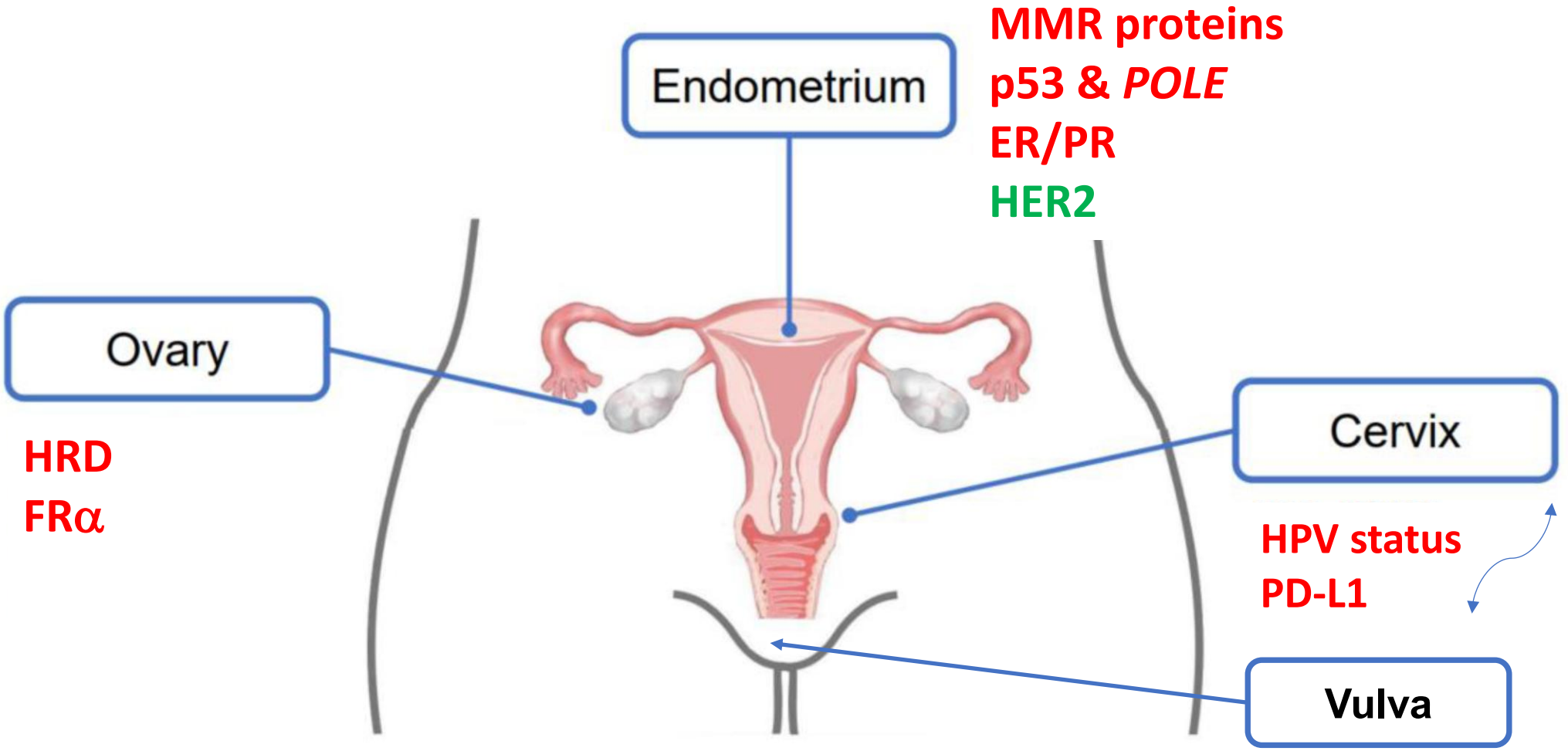
ER/PR IHC: Caveats

- ***Robust prognostic markers if histotyping is not considered***, especially in *NSMP and MMRd* (and some p53-abnormal) subtypes

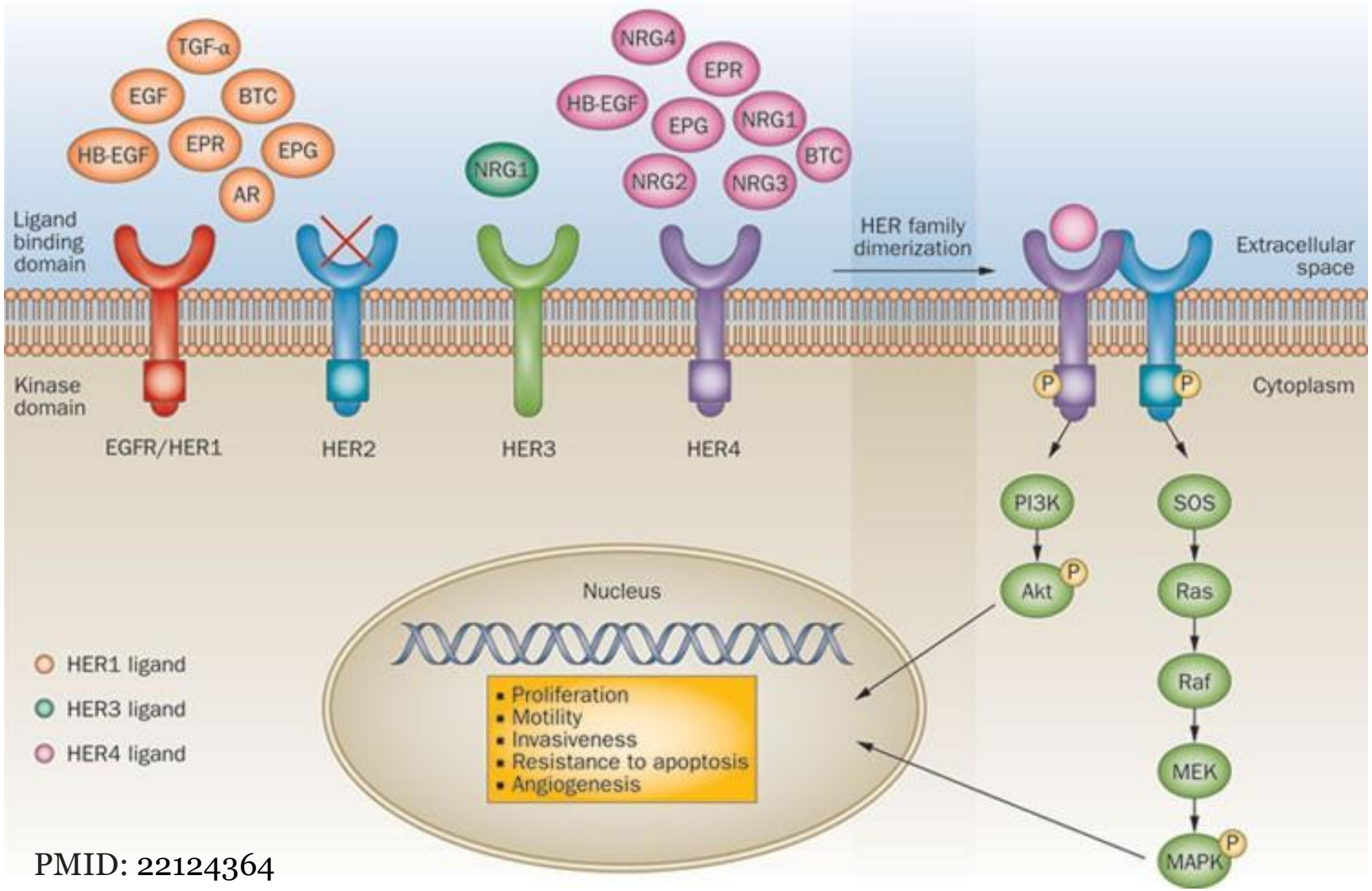
- ER/PR-negative status is ***surrogate marker for high-risk non-endometrioid histotypes*** (clear cell, mesonephric, gastric type, un-/dedifferentiated), which account for a significant percentage of adverse outcomes

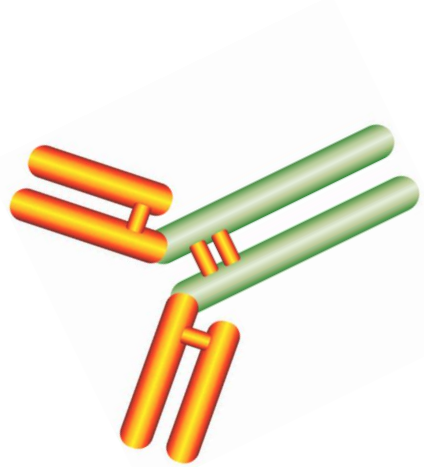
- After careful exclusion of ER-negative non-endometrioid NSMP histotypes, ***ER-negative endometrioid carcinomas show worse outcomes in certain risk groups***

Prognostic and Therapeutic Markers in Gynecologic Pathology



HER2-Directed Therapy in HER2-Overexpressing / Amplified Tumors

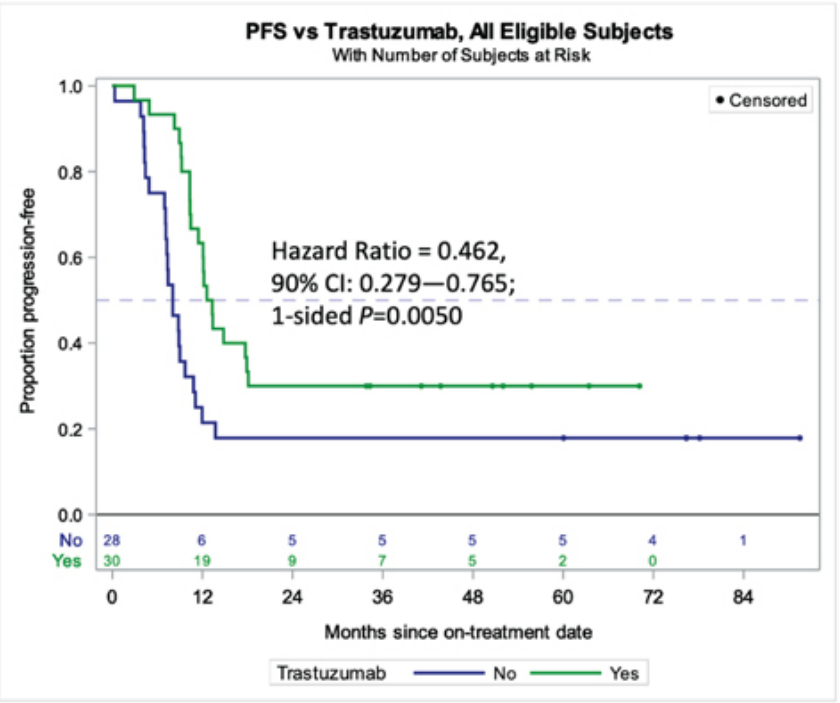


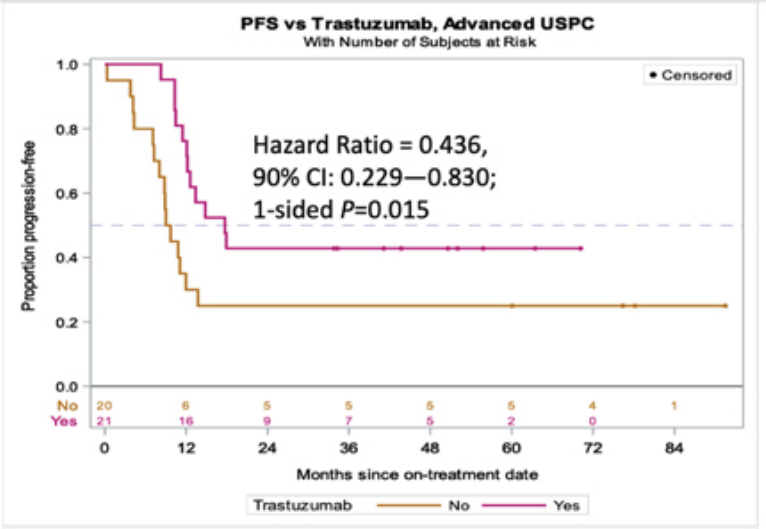


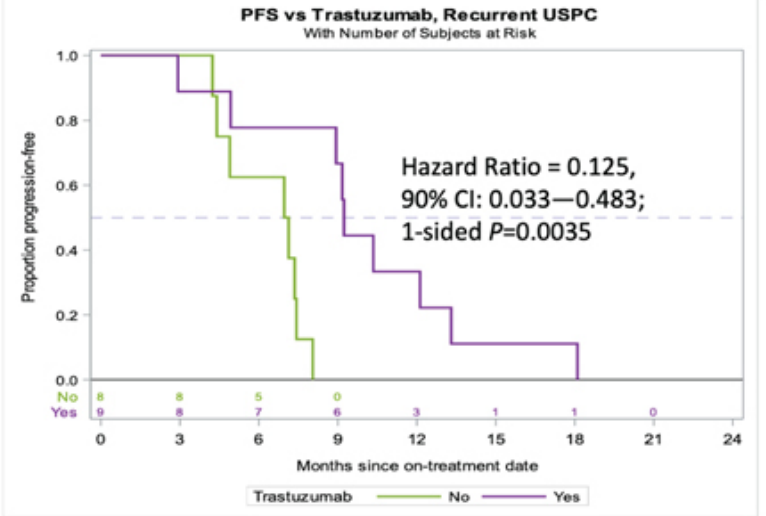
Monoclonal antibody (trastuzumab)

PMID: 22124364

Improved Progression-free (and Overall) Survival Supports the Addition of Trastuzumab to the Treatment of Advanced/Recurrent Serous Carcinoma (Randomized Phase II Clinical Trial NCT01367002)







- Modified breast criteria (gynecologic / endometrial criteria)

PMID: 32601075

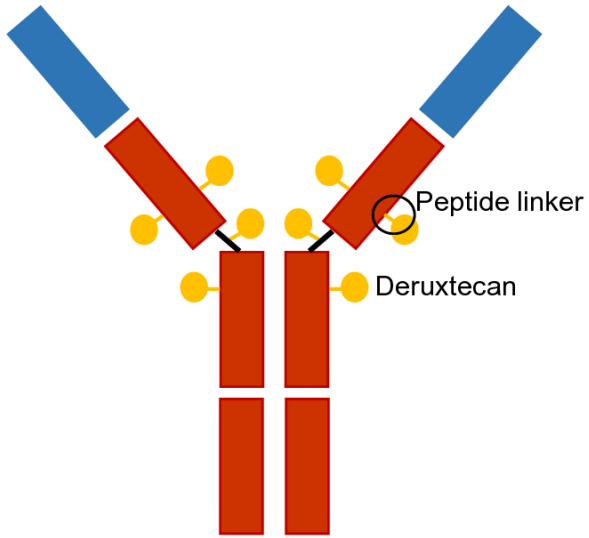
NCCN Guidelines Version 2.2026
Uterine Neoplasms

- Preferred therapy for HER2-positive advanced stage (FIGO III/IV) or recurrent endometrial serous carcinoma and carcinosarcoma:
  - ✓ Carboplatin/paclitaxel/trastuzumab

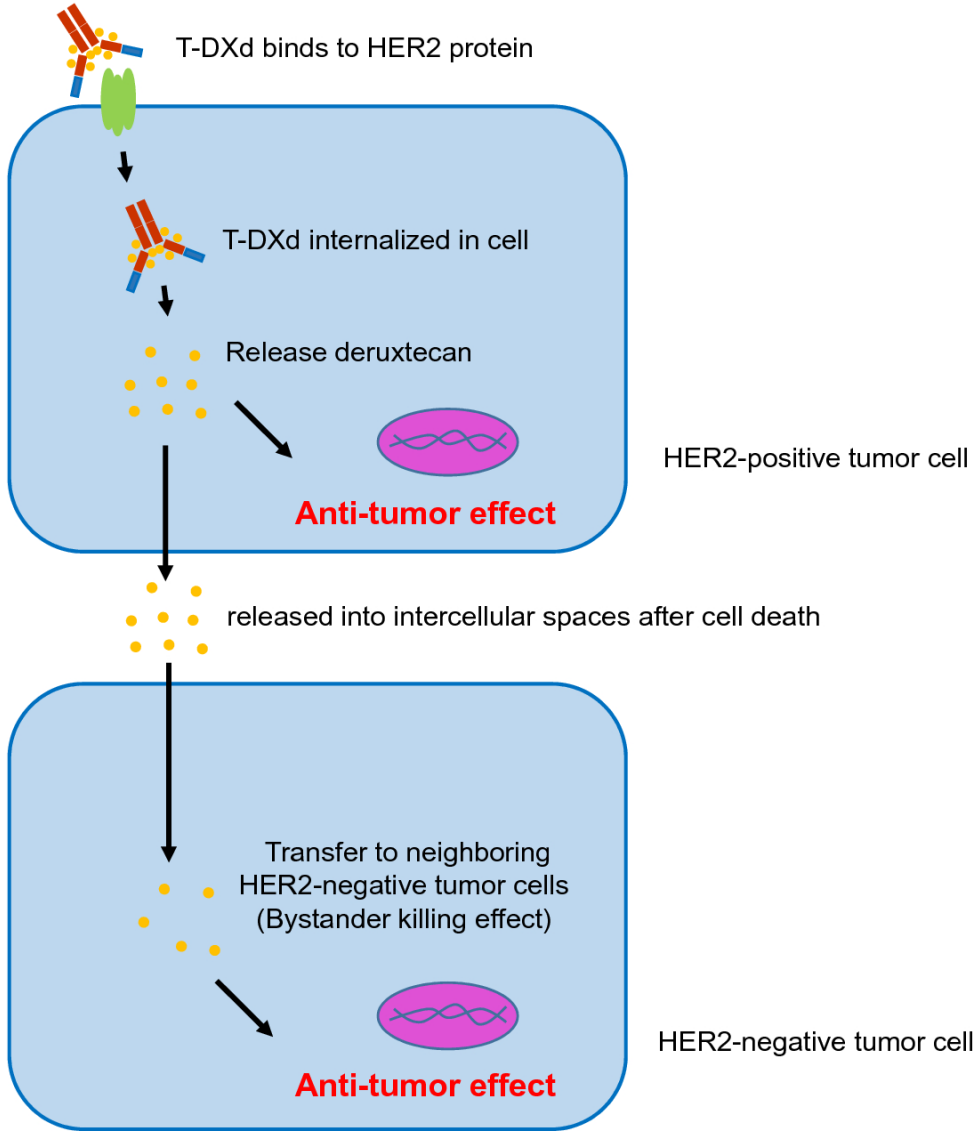
- **HER2 testing recommendations:**
  - ✓ Endometrial serous carcinoma and carcinosarcoma
  - ✓ All p53-abnormal endometrial carcinomas, regardless of histology

PMID: 32601075, 29584549

HER2-directed Therapy: Trastuzumab-Deruxtecan, an Antibody-Drug Conjugate (ADC) Comprising Trastuzumab and a Topoisomerase I Inhibitor Deruxtecan as a Payload with a High Drug-to-Antibody Ratio (8:1)



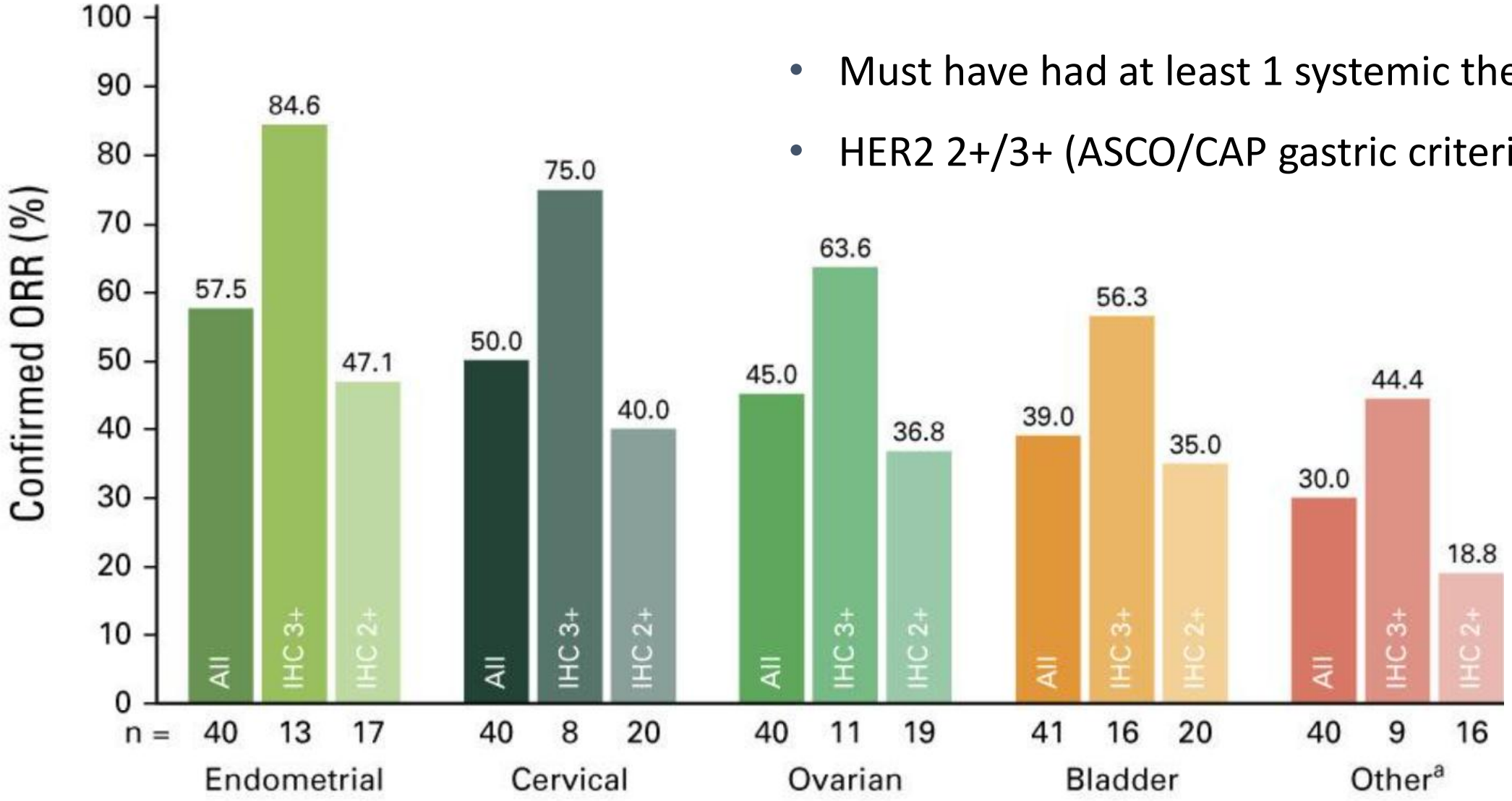
Trastuzumab deruxtecan (T-DXd)



PMID: 33646464

DESTINY-PanTumor02 Phase II Trial: Trastuzumab-Deruxtecan in Solid Tumors

- Must have had at least 1 systemic therapy
- HER2 2+/3+ (ASCO/CAP gastric criteria)

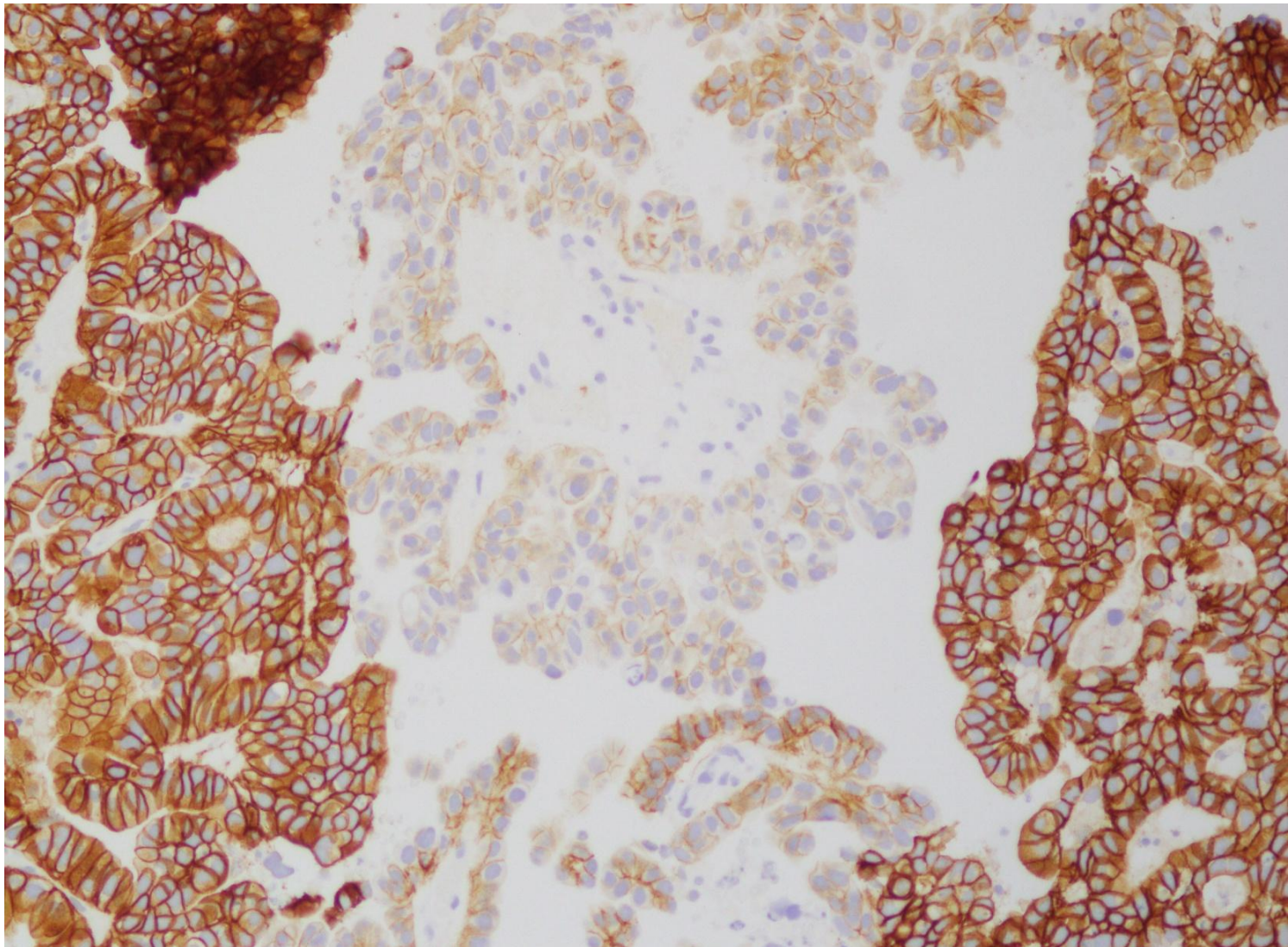


PMID: 37870536

HER2 Protein Overexpression and/or Gene Amplification Can Be Seen in a Subset of Gynecologic Carcinomas

- 25-35% of serous carcinomas
- 16% of carcinosarcomas
- 48% of clear cell carcinomas
- Associated with p53 abnormal expression (96%)

- Frequent intratumoral heterogeneity (53-67%)
- Lateral/basolateral pattern



PMID: 23765245, 31477811, 23765245, 33782344, 33375706, 31550396

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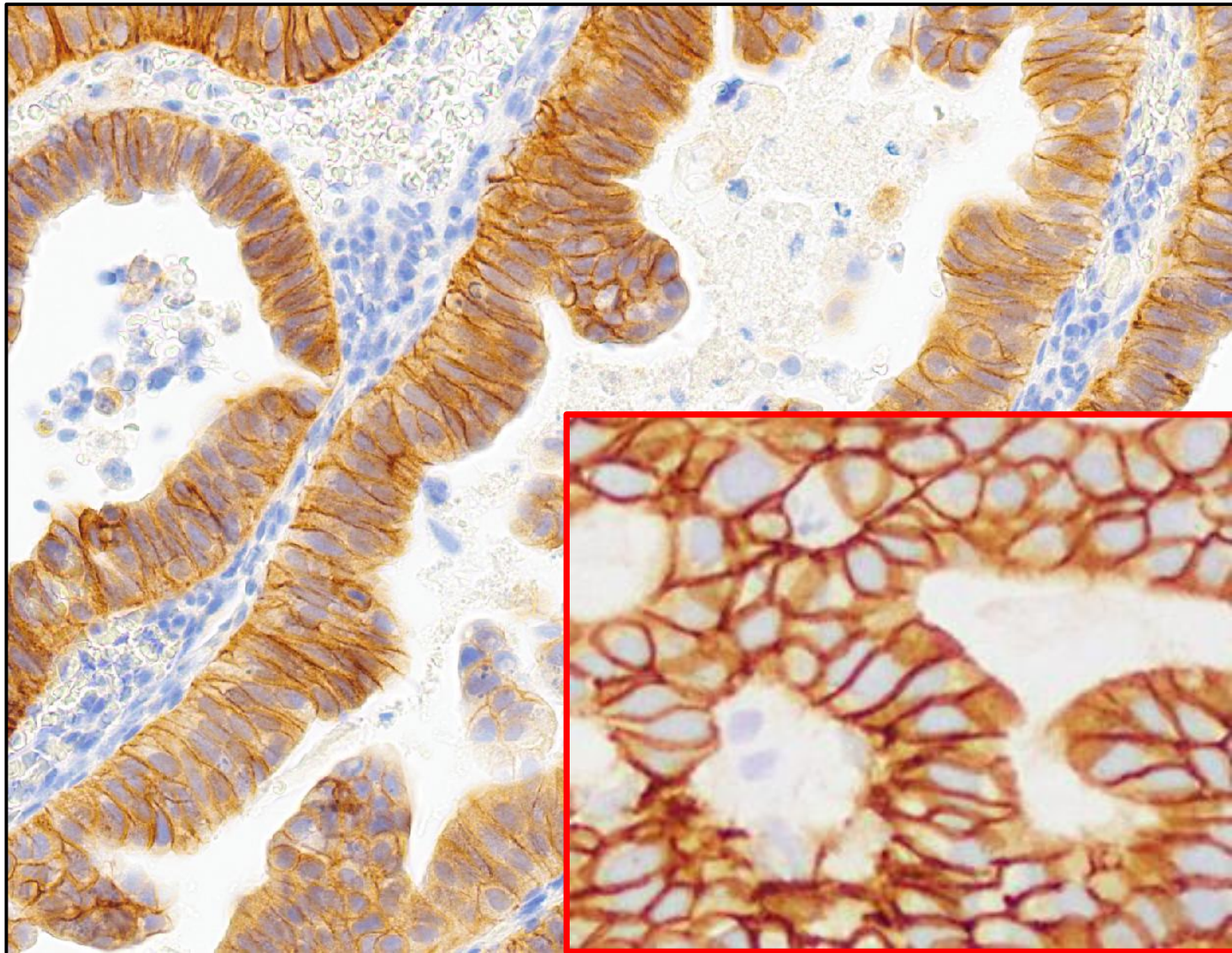


PMID: 23765245, 31477811, 23765245, 33782344, 33375706, 31550396

GYNECOLOGIC / ENDOMETRIAL CRITERIA

COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

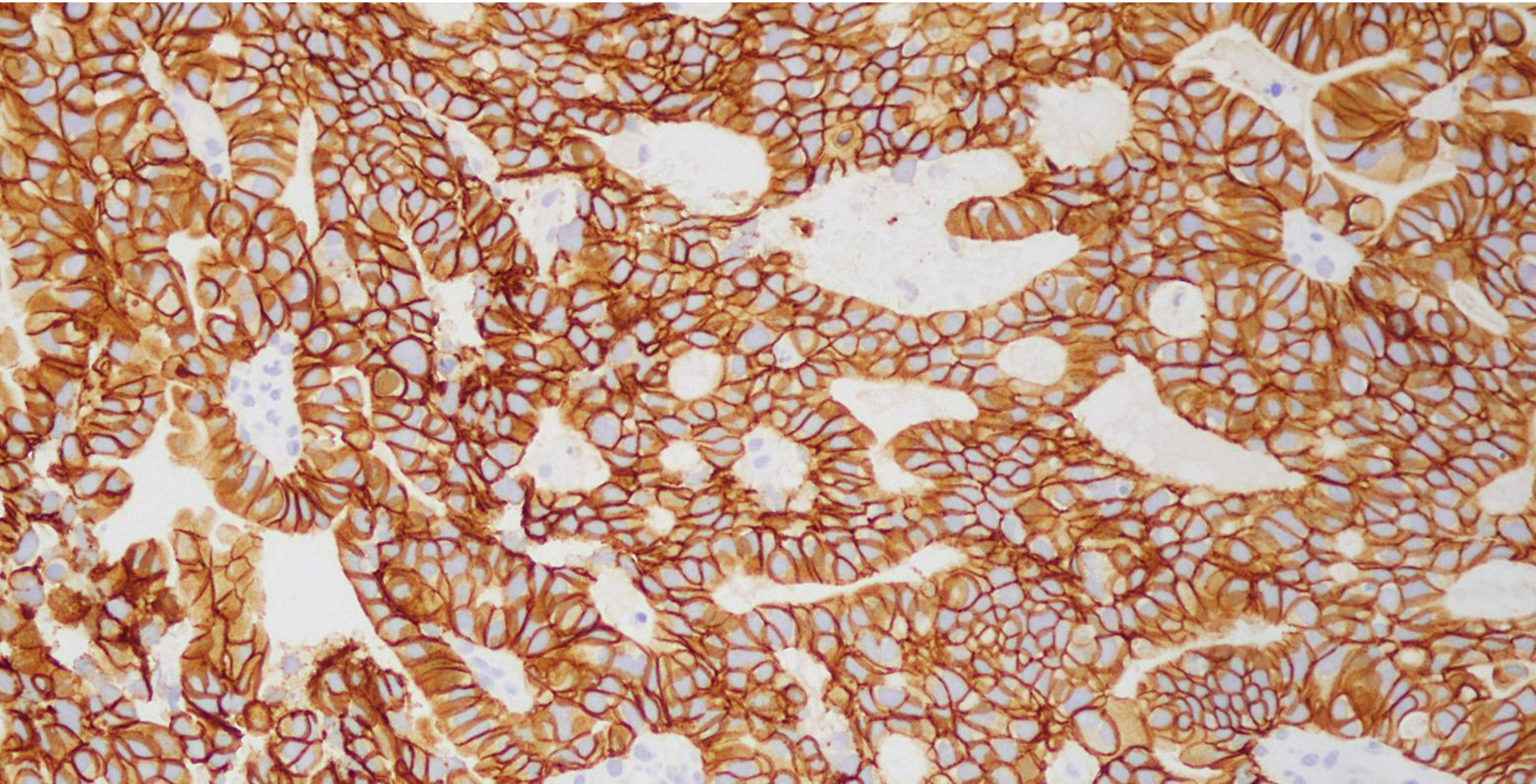
Reporting Results of HER2 Testing by Immunohistochemistry (IHC) for Trastuzumab Use Based on the Enrollment Criteria for the Phase II Clinical Trial NCT01367002

Result	Criteria
Negative (Score 0)	No staining observed
Negative (Score 1+)	Incomplete membrane staining that is faint/barely perceptible in any proportion of cells, ***or*** Weak complete staining in less than 10% of tumor cells
Equivocal (Score 2+)*	Intense complete or **basolateral/lateral** membrane staining in 30% or less tumor cells, ***or*** Weak to moderate staining in greater than or equal to 10% of tumor cells
Positive (Score 3+)	Intense complete or **basolateral/lateral** membrane staining in over 30% of tumor cells

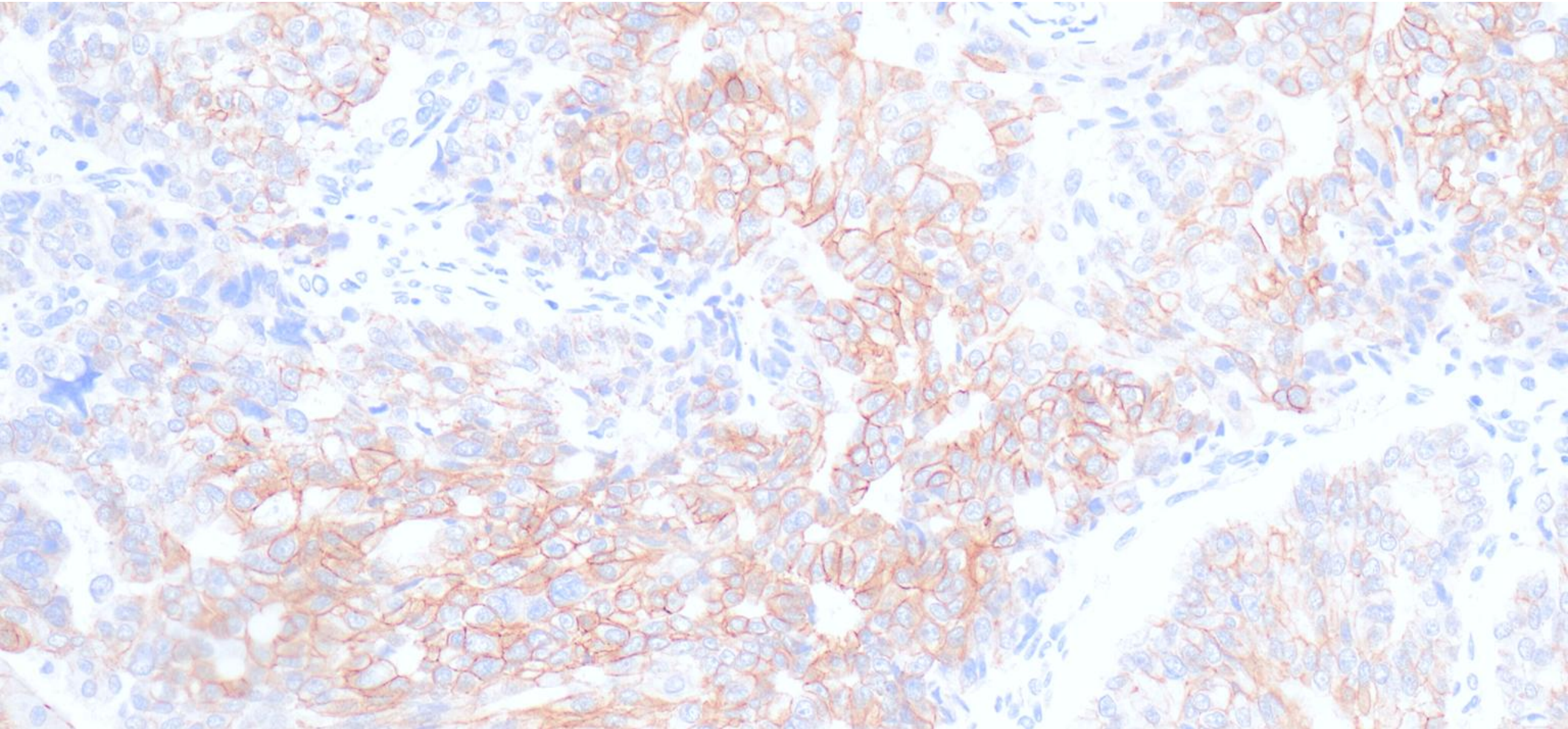
* Must order reflex in situ hybridization test (same specimen).

PMID: 29584549, 32601075

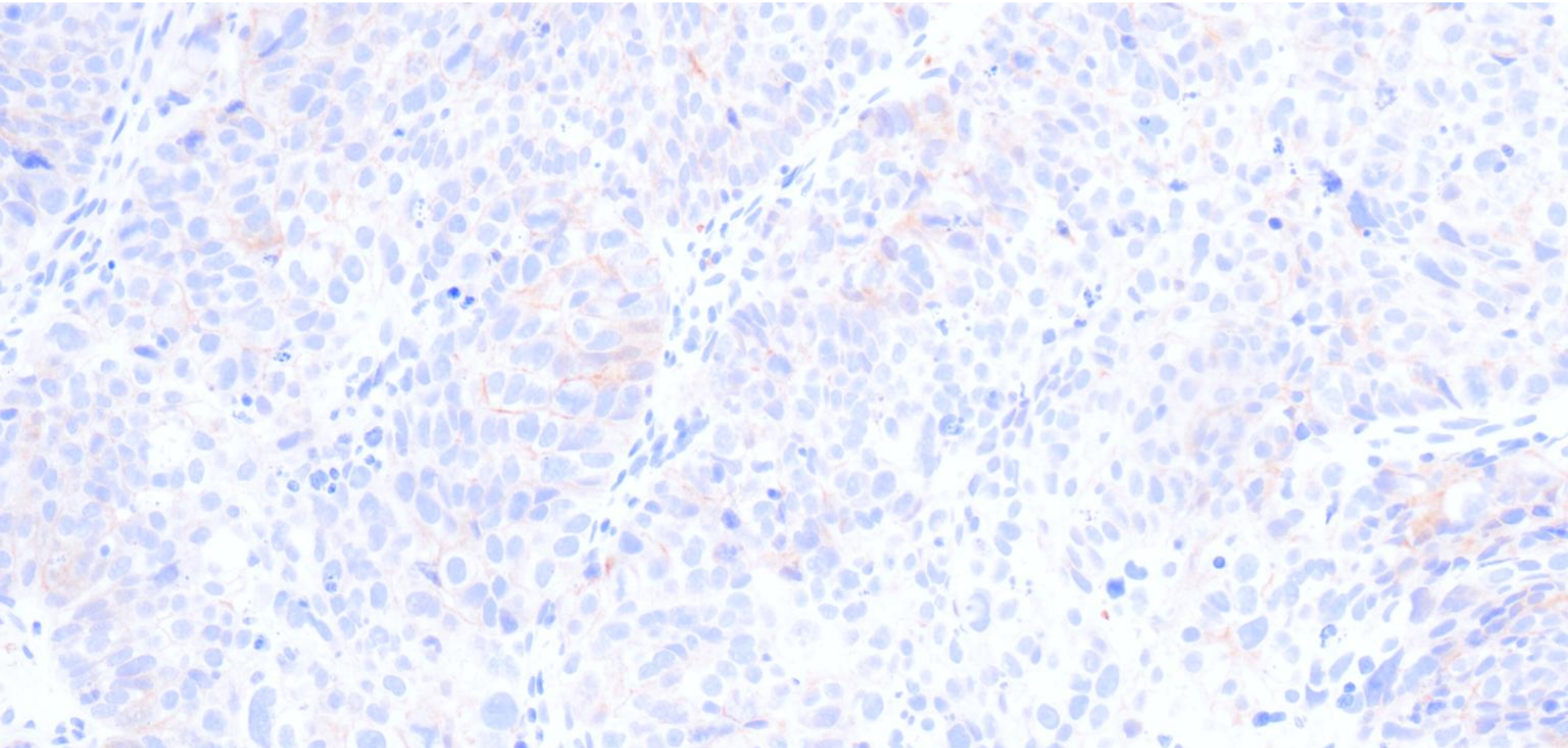
Positive (Score 3+)	Intense complete or **basolateral/lateral** membrane staining in over 30% of tumor cells*



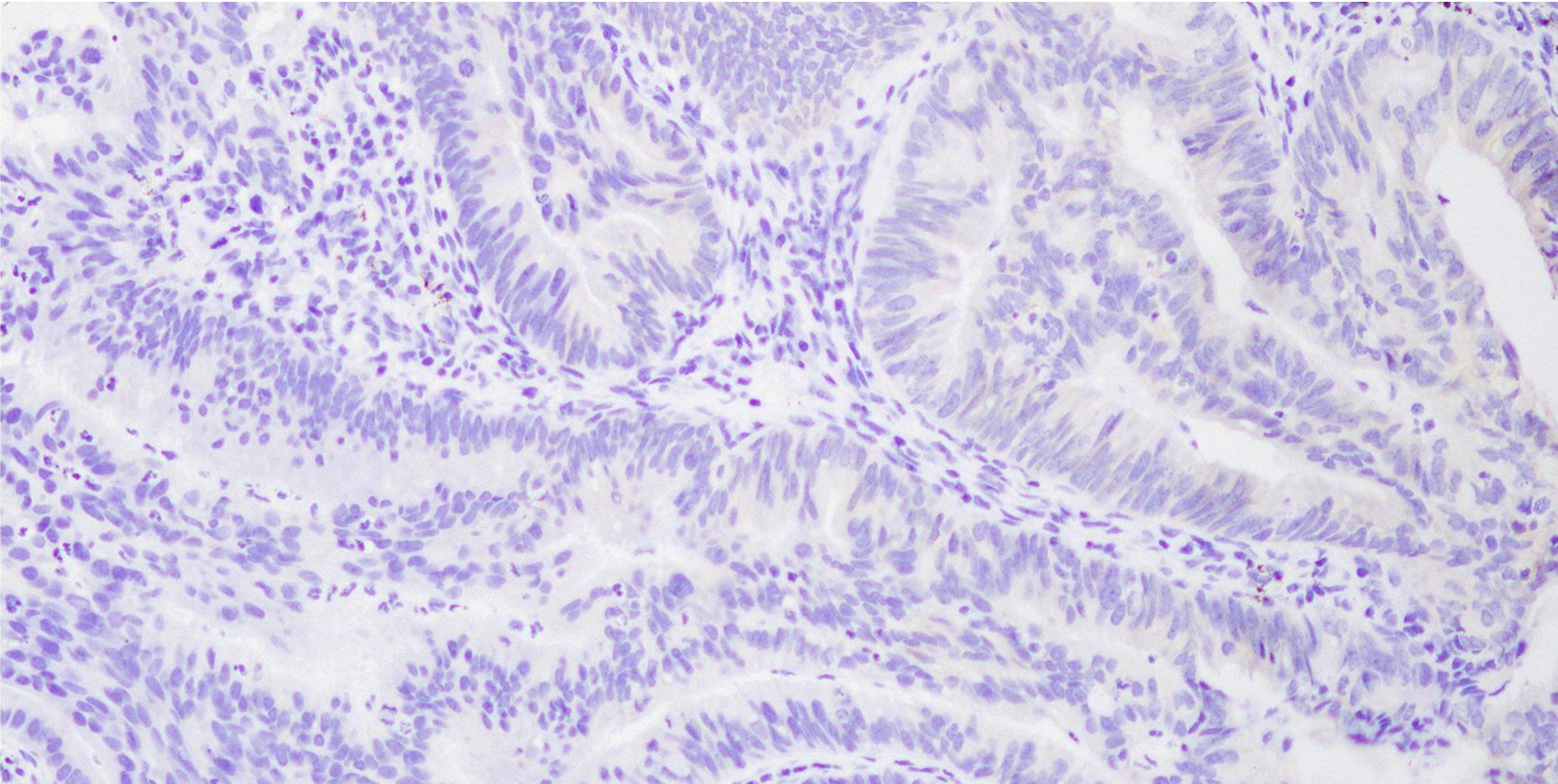
Equivocal (Score 2+)†	Intense complete or **basolateral/lateral** membrane staining in 30% or less tumor cells**or* Weak to moderate staining in greater than or equal to 10% of tumor cells*



Negative (Score 1+)	Incomplete membrane staining that is faint/barely perceptible in any proportion of cells or Weak complete staining in less than 10% of tumor cells*



Negative (Score 0)	No staining observed



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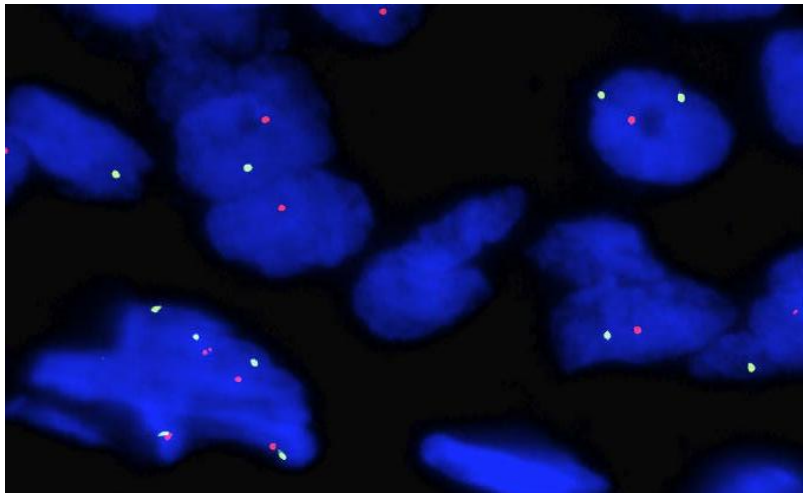
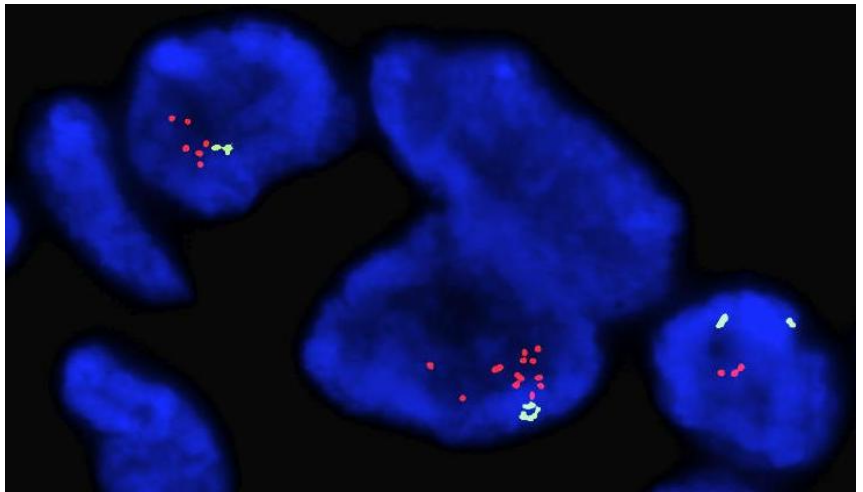
Laboratory Quality Solutions

Table 3. Reporting Results of HER2 Testing by Fluorescent In Situ Hybridization (dual-probe assay)[5]

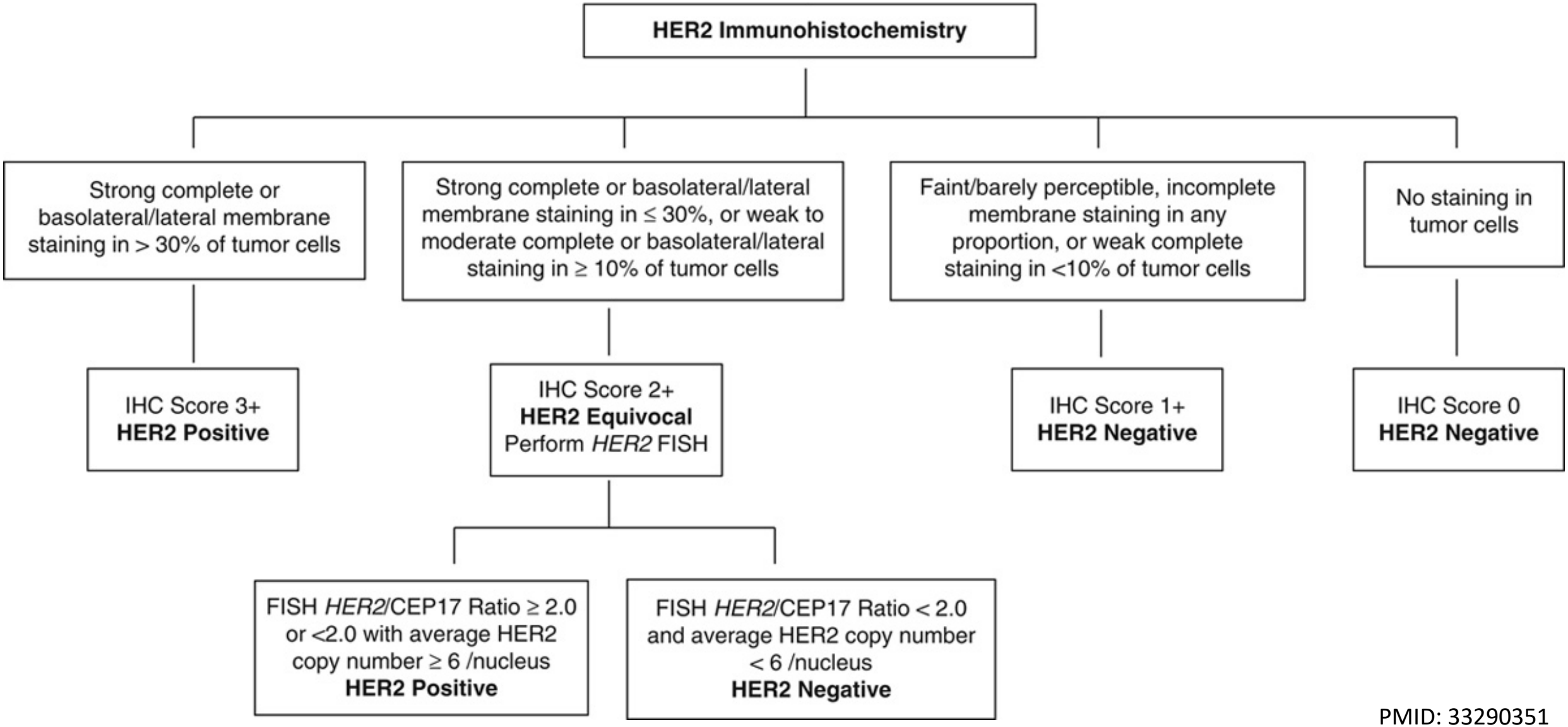
Result	Criteria (dual-probe assay)
Negative	· FISH HER2/CEP17 ratio less than 2.0 and · Average HER2 copy number less than 6 per nucleus
Positive	· FISH HER2/CEP17 ratio greater than or equal to 2.0 or · FISH HER2/CEP17 ratio less than 2.0 with average HER2, copy number equal to or greater than 6 per nucleus





PMID: 33290351

GYNECOLOGIC / ENDOMETRIAL CRITERIA



PMID: 33290351

GASTRIC CRITERIA

COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

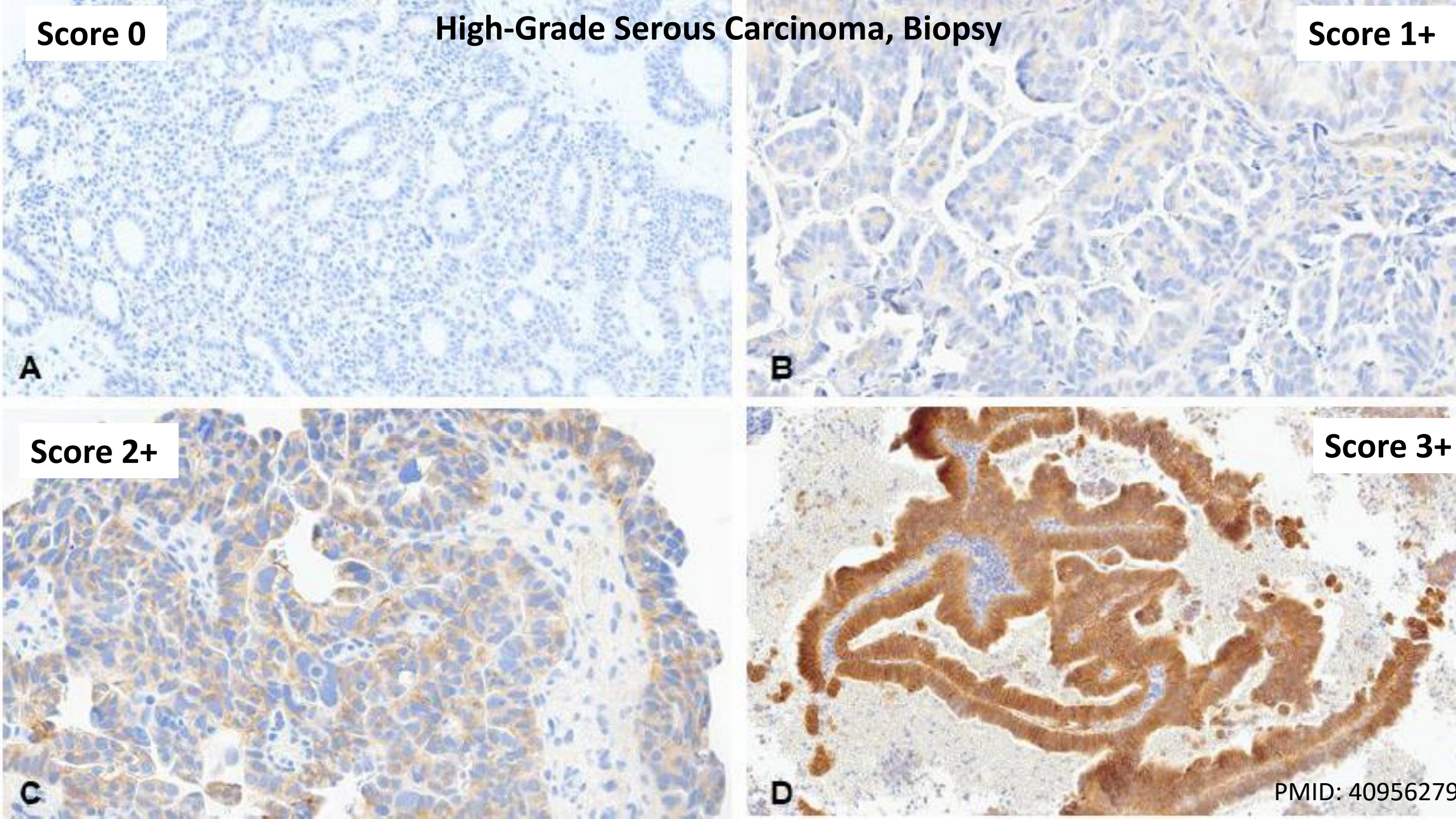
Reporting Results of HER2 Testing by Immunohistochemistry (IHC) for Trastuzumab-Deruxtecan Use Based on the Enrollment Criteria for the DESTINY-PanTumor02 trial (NCT04482309)

Result	Criteria for Surgical Specimens	Criteria for Biopsy Specimens
Negative (Score 0)	No staining or membrane staining in less than 10% of tumor cells	No staining in any tumor cells
Negative (Score 1+)	Faint/barely perceptible incomplete membrane staining in greater than or equal to 10% tumor cells	Tumor cell cluster* with a faint/barely perceptible membrane staining irrespective of percentage of positive tumor cells
Equivocal (Score 2+)	Weak to moderate, complete, basolateral or lateral membrane staining in greater than or equal to 10% of tumor cells	Tumor cell cluster* with a weak to moderate, complete, basolateral or lateral membrane staining irrespective of percentage of positive tumor cells
Positive (Score 3+)	Strong, complete, basolateral or lateral membrane staining in greater than or equal to 10% of tumor cells	Tumor cell cluster* with a strong, complete, basolateral or lateral membrane staining irrespective of percentage of positive tumor cells

*Tumor cell cluster denotes 5 or more tumor cells

FISH not required for 2+ tumors

High-Grade Serous Carcinoma, Biopsy



PMID: 40956279

The Choice of HER2 IHC Scoring Criteria Matters!

- **Variable discordance between GYNECOLOGIC/ENDOMETRIAL and GASTRIC criteria:**
  - ✓ Concordance rates range from 69.8% to 89.2%
  - ✓ HER2-positive tumor by one criteria may be HER2-equivocal (2+) by the other
  - ✓ Important to know which scoring criteria to use
  - ✓ Information on intended therapy best provided by the clinician

PMID: 40135820, 39947031

___ HER2 Status (Note C)

HER2 Scoring System (select all that apply)

___ Based on the enrollment criteria for Trastuzumab in the randomized phase II clinical trial NCT01367002 for endometrial carcinoma

HER2 Status for Trastuzumab Use

 # No staining in tumor cells

___ Negative (score 0) for protein overexpression#

Faint / barely perceptible, incomplete membrane staining in any proportion, or weak complete staining in less than 10% of tumor cells

___ Negative (score 1+) for protein overexpression##

Strong complete or basolateral / lateral membrane staining in less than or equal to 30%, or weak to moderate staining in greater than or equal to 10% of tumor cells

___ Equivocal (score 2+) for protein overexpression###

Strong complete or basolateral / lateral membrane staining in greater than 30% of tumor cells

___ Positive (3+) for protein overexpression####

___ Cannot be determined (explain): _____

__ Based on the enrollment criteria for Trastuzumab-deruxtecan in the DESTINY-PanTumor02 phase II clinical trial (NCT04482309) for endometrial, cervical or ovarian carcinoma

HER2 Status for Trastuzumab-deruxtecan Use

 # Biopsy: No staining in any tumor cells; Surgical specimen: No staining or membrane staining in less than 10% of tumor cells

___ Negative (score 0) for protein overexpression#

Biopsy: Tumor cell cluster (5 or more tumor cells) with a faint / barely perceptible membrane staining irrespective of percentage of positive tumor cells; Surgical specimen: Faint / barely perceptible incomplete membrane staining in greater than or equal to 10% tumor cells

___ Negative (score 1+) for protein overexpression##

Biopsy: Tumor cell cluster (5 or more tumor cells) with a weak to moderate, complete, basolateral or lateral membrane staining irrespective of percentage of positive tumor cells; Surgical specimen: Weak to moderate, complete, basolateral or lateral membrane staining in greater than or equal to 10% of tumor cells

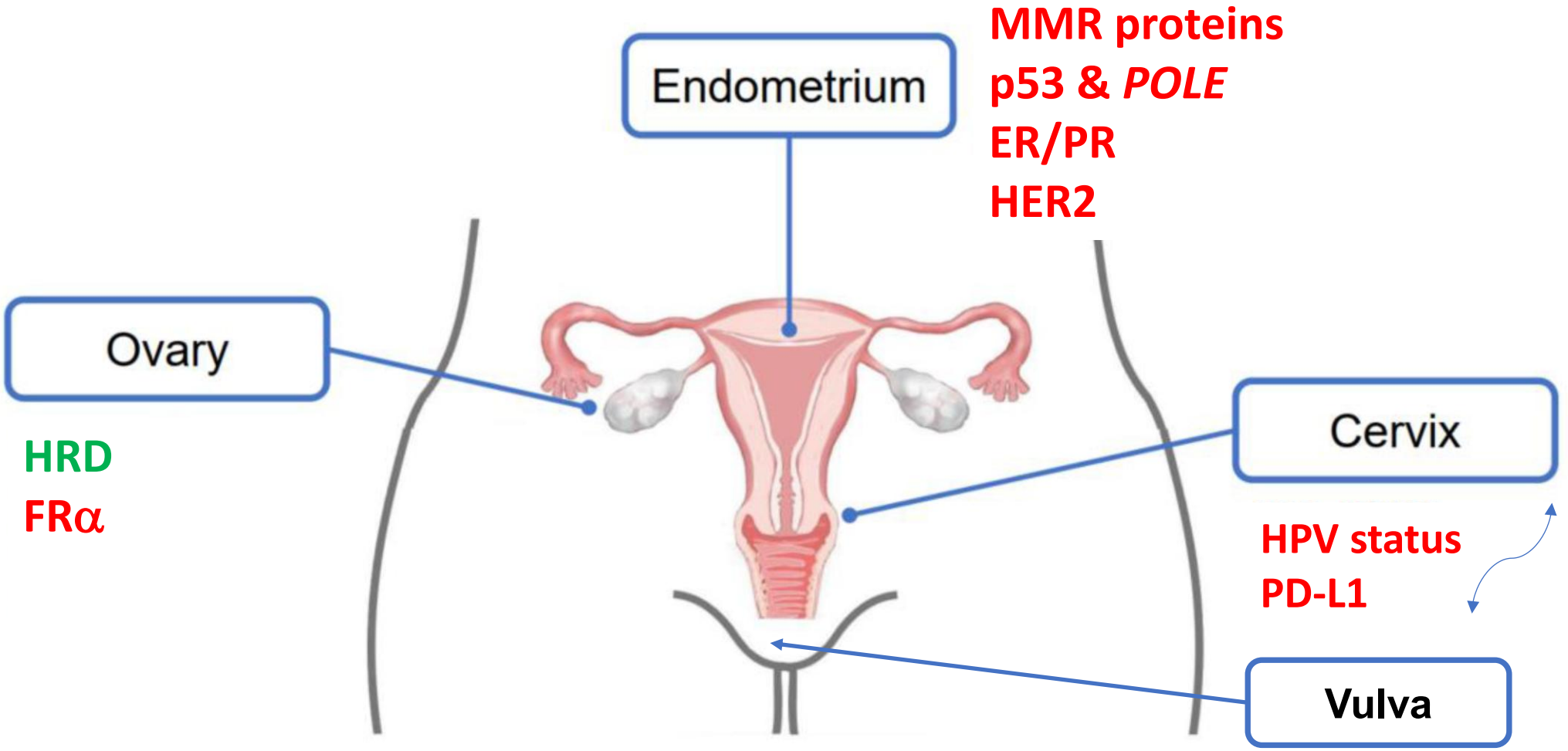
___ Equivocal (score 2+) for protein overexpression###

Biopsy: Tumor cell cluster (5 or more tumor cells) with a strong, complete, basolateral or lateral membrane staining irrespective of percentage of positive tumor cells; Surgical specimen: Strong, complete, basolateral or lateral membrane staining in greater than or equal to 10% of tumor cells

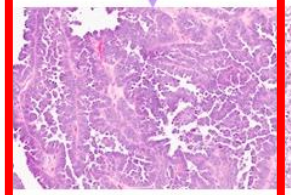
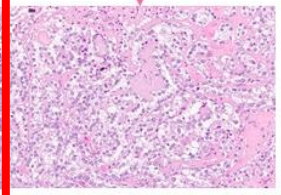
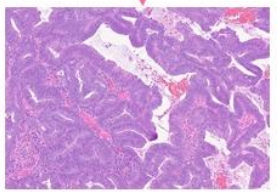
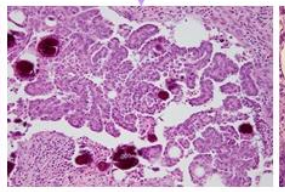
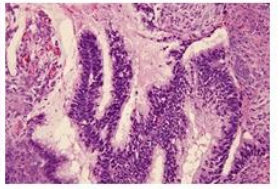
___ Positive (3+) for protein overexpression####

___ Cannot be determined (explain): _____

Prognostic and Therapeutic Markers in Gynecologic Pathology



High-Grade Serous Carcinoma (HGSC)

Origin	Fallopian Tube Epithelium	Endometriosis	Endometriosis	Fallopian Tube Epithelium	?Unknown
					

	High-Grade Serous Carcinoma	**Clear Cell Carcinoma**	**Endometrioid Carcinoma**	**Low-Grade Serous Carcinoma**	**Mucinous Carcinoma**
% of all Ovarian Carcinomas	~70%	~10%	~10%	<5%	<5%
Precursor Lesions	Serous tubal intraepithelial carcinoma (STIC)	Clear Cell Borderline Tumor	Endometrioid Borderline Tumor	Serous Borderline Tumor	Mucinous Borderline Tumor
Inherited Syndromes	BRCA1/2, Hereditary Breast and Ovarian Cancer (HBOC)	Lynch Syndrome	Lynch Syndrome	?	?
Common Mutations and Molecular Aberrations	TP53 BRCA1/2 and HRD Chromosomal instability Aneuploidy (100%)	ARID1A PIK3CA CTNNB1 PPP2R1A MSI	PTEN CTNNB1 ARID1A PPPR2R1A MSI	KRAS BRAF	KRAS HER2 amplification
Potential Molecular Targeted Therapies	PARP inhibitors, immune checkpoint inhibitors	Tyrosine kinase inhibitors	mTOR inhibitors	MEK1/2 inhibitors	Trastuzumab

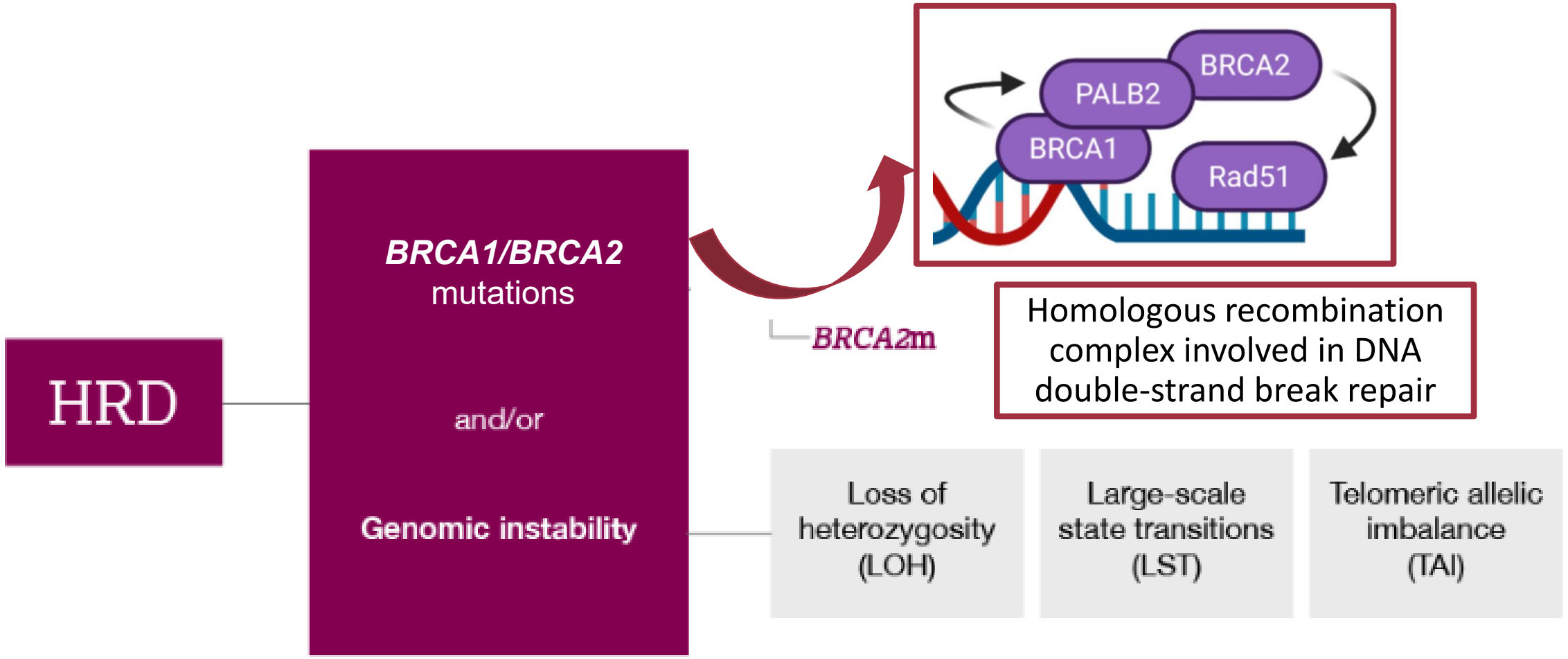
http://www.bccancer.bc.ca/books/ovary-epithelial-carcinoma/histological-classification-of-ovarian-carcinoma

BRCA1/2 (BReast CAncer Genes 1 and 2) Are Tumor Suppressor Genes

Cancer Risk in Mutation Carriers

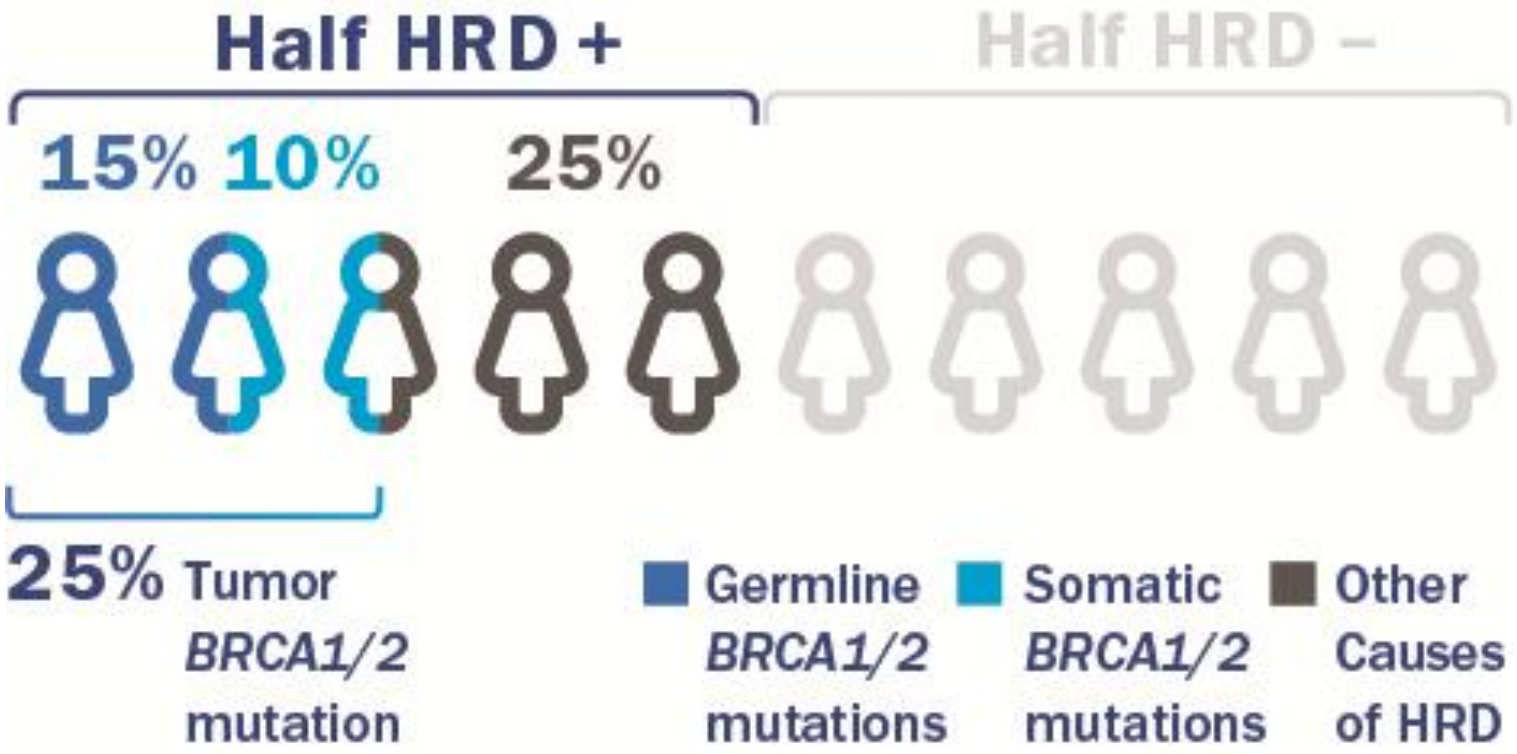
		Mutation Carrier	General Population
Breast cancer	*BRCA1*	50 - 85%	12%
	BRCA2	40 - 70%	
Ovarian cancer	*BRCA1*	50 - 80%	1 - 2%
	BRCA2	24 - 40%	

Homologous Recombination Deficiency (HRD) Is Defined by *BRCA1/2* Mutations or Genomic Instability Markers



PMID: 34711195; Modified from https://www.azprecisionmed.com/tumor-type/ovarian-cancer/hrd-testing.html#interpret-hrd-test-results

~50% of Advanced High-grade Serous Carcinomas Are HRD-Positive



National Comprehensive Cancer Network®

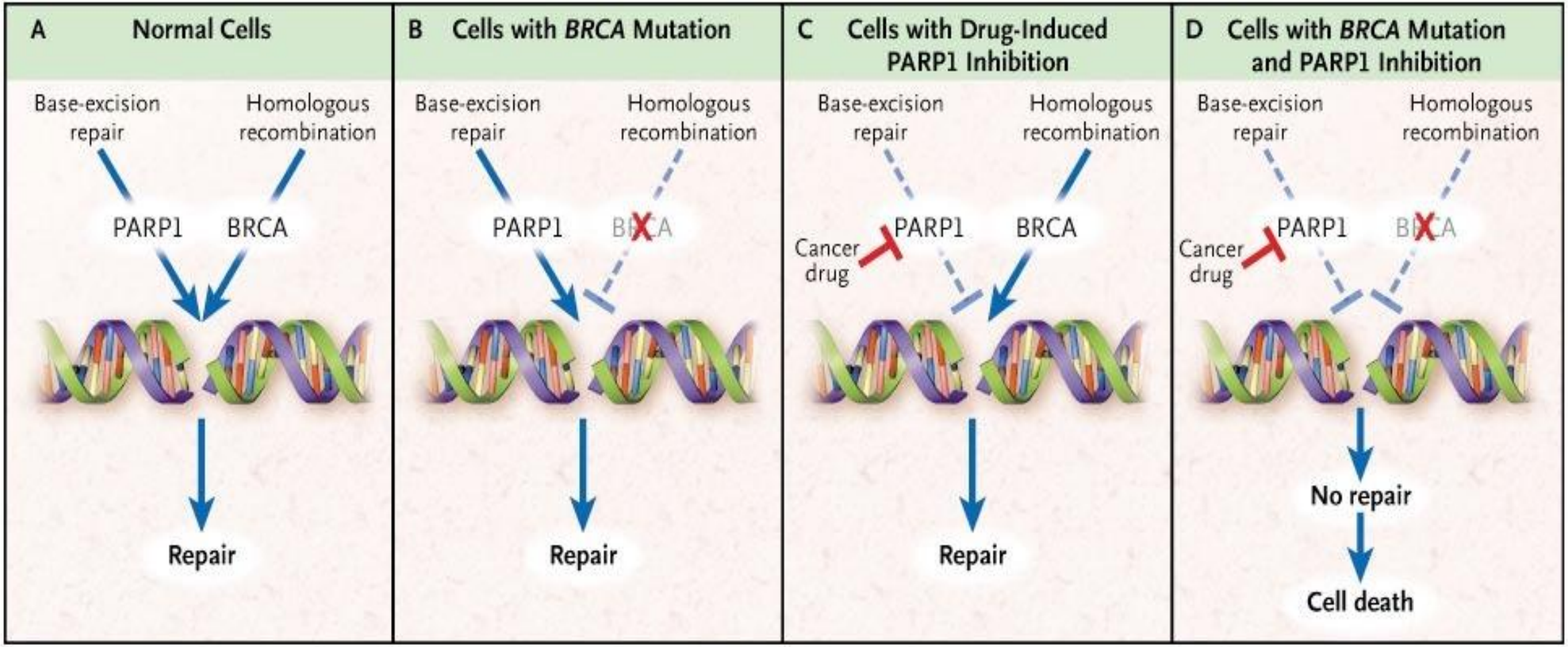
NCCN Guidelines Version 4.2026 Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer

- **HRD testing recommended in all patients with pathologically confirmed epithelial ovarian cancer (high-grade serous carcinoma):**
  - ✓ **Germline – blood sample**
  - ✓ **Somatic – tumor tissue**

- HRD status informs maintenance therapy with PARP inhibitors for patients with stage II-IV disease who are in complete or partial response after 1st line platinum-based chemotherapy

PMID: 31851799, 31562799, 31562800

PARP Inhibitors Work Based on Synthetic Lethality



PARP1, Poly(Adenosine Diphosphate [ADP]-Ribose) Polymerase 1; PMID: 19553640

Response to PARPi After Systemic Therapy in Prospective Phase III Studies in 1st Line Maintenance Setting (Different Methods and Cut-offs Used for HRD)

	SOLO-1 (Olaparib vs. placebo)	PAOLA-1 (Bevacizumab + olaparib vs. bevacizumab + placebo)	PRIMA (Niraparib vs. placebo)	VELIA (Veliparib vs. placebo)
Complete response	82%	73%	69%	Not reported, PFS 35 vs 22 months in control
Partial response	18%	27%	31%	

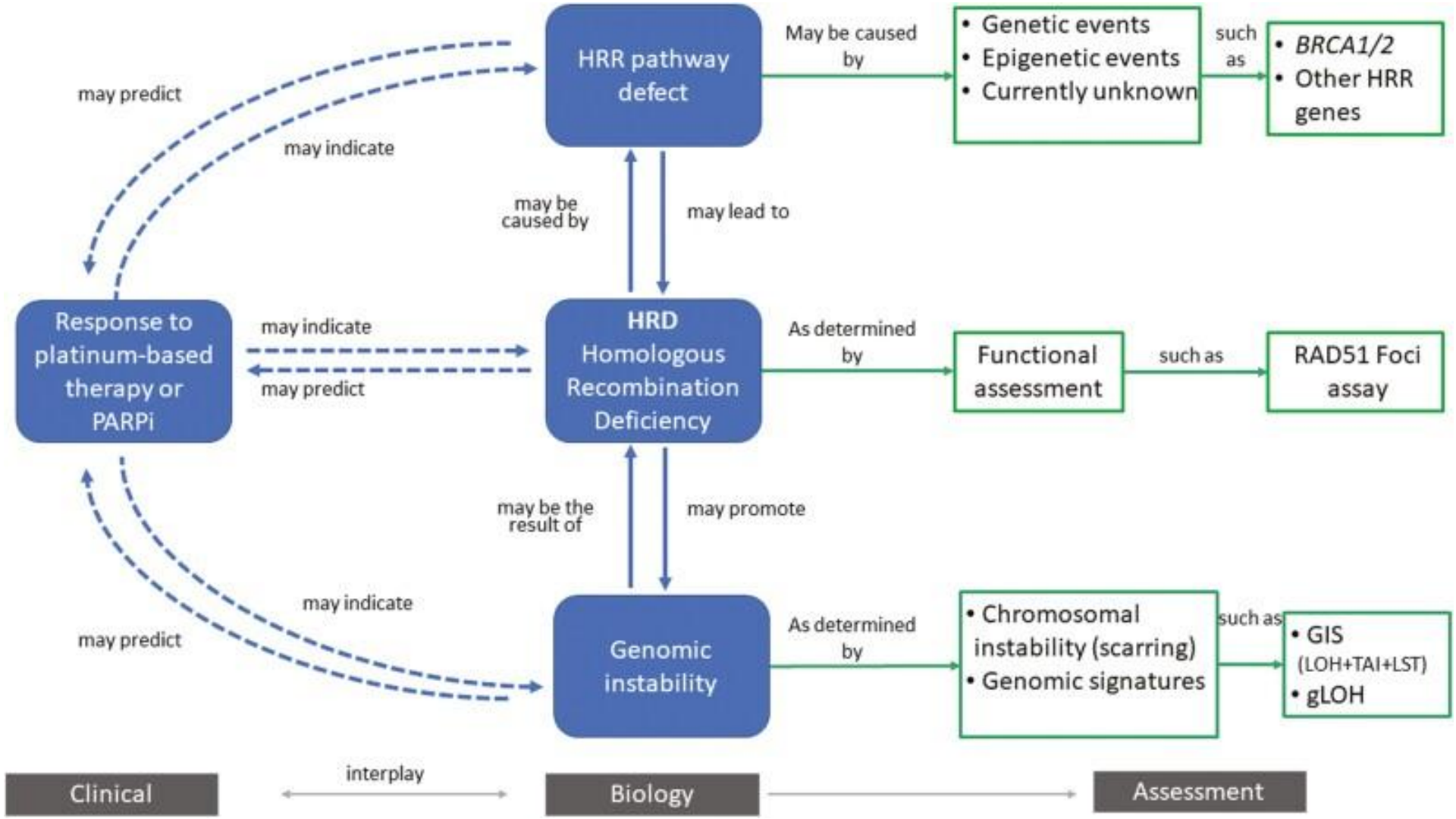
PMID: 30345884, 31851799, 31562799, 31562800

Commercially Available HRD Tests on Tumor Tissue

	Caris Molecular Intelligence	Foundation Medicine FoundationOne CDx	Myriad MyChoice CDx	Tempus Tempus xT
Clinical trials	None	SOLO, ARIEL	PAOLA, PRIMA, SOLO	None
Guidelines	None	ASCO (for *BRCA*)	ASCO, NCCN	None
FDA-approval	No	Yes	Yes	No
HRD markers	*BRCA*m, %LOH	*BRCA*m, %LOH	*BRCA*m, GIS (LOH, TAI, LST)	*BRCA*m ± %LOH
***BRCA1/2* large rearrangements**	No	No	Yes	No

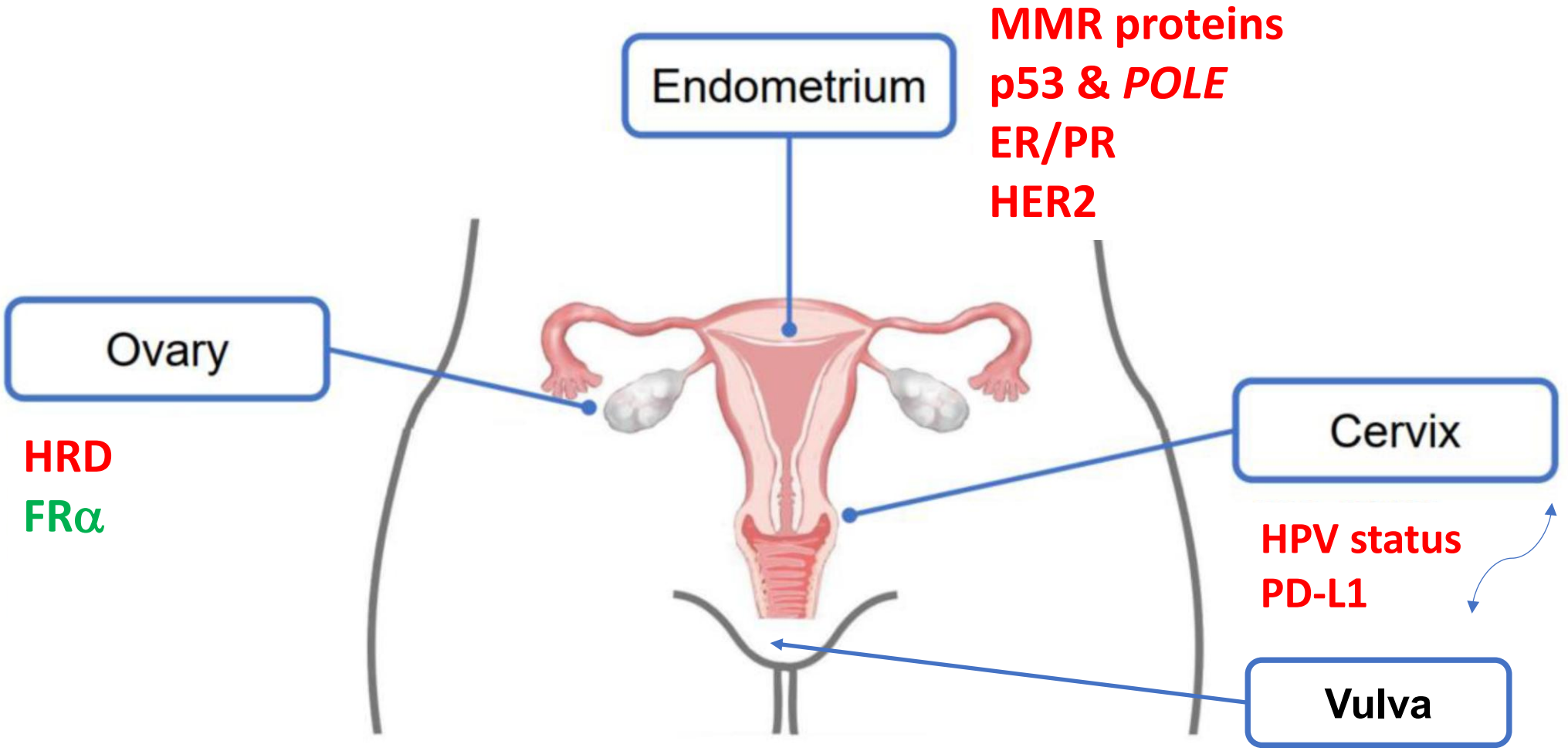
GIS (genomic instability score)

HRD Testing Getting More Complex



PMID: 35274707, 37541687, 36805632

Prognostic and Therapeutic Markers in Gynecologic Pathology



NCCN Guidelines Version 4.2026
Epithelial Ovarian Cancer/Fallopian Tube Cancer/ Primary Peritoneal Cancer

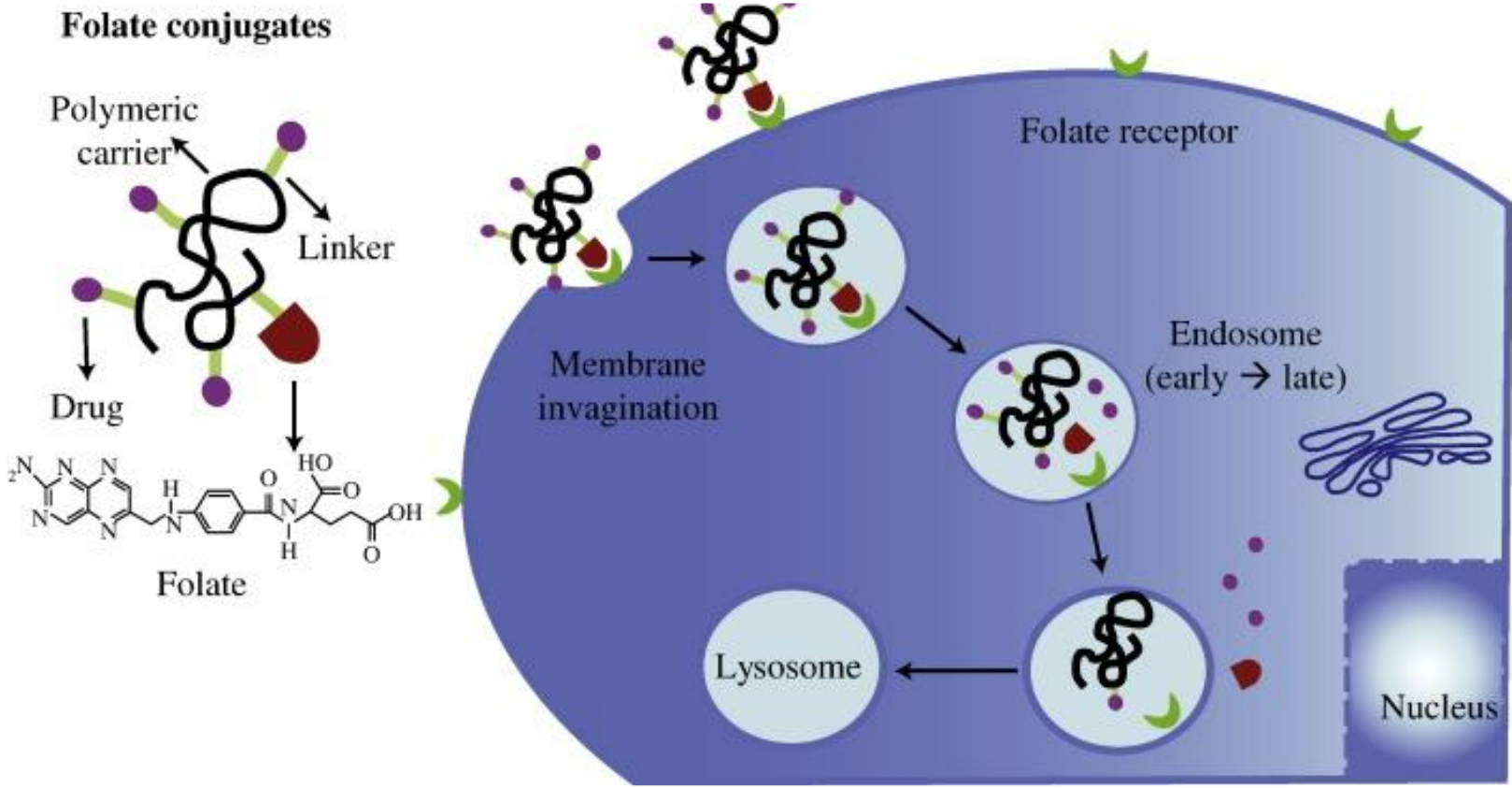
- Most high-grade serous carcinomas are initially platinum-sensitive
- 70-85% will recur within 3 years
- 5-year relative survival rate of ~30% in part due to platinum-resistant disease:
  - ✓ Defined as tumor progression within 6 months of a taxane- or platinum-based regimen

- An antibody-drug conjugate mirvetuximab soravtansine (MIRV) is recommended for folate receptor a (FRa) expressing recurrent platinum-resistant disease:
  - ✓ *Targeted therapy (single agent)*
  - ✓ *Useful in certain circumstances:*
    - With bevacizumab

PMID: 24078660, 36716407, 32081463, 23863359, 23518909, 27941566, 37027520

FRα, a Cell Surface Folate Receptor Mediates Folate Transport Into Epithelial Cells and Required for Basic Biologic Functions

- Limited expression in normal tissues
- Expressed in ovarian, fallopian tube, and endometrial cancers
- Associated with tumor progression and prognosis

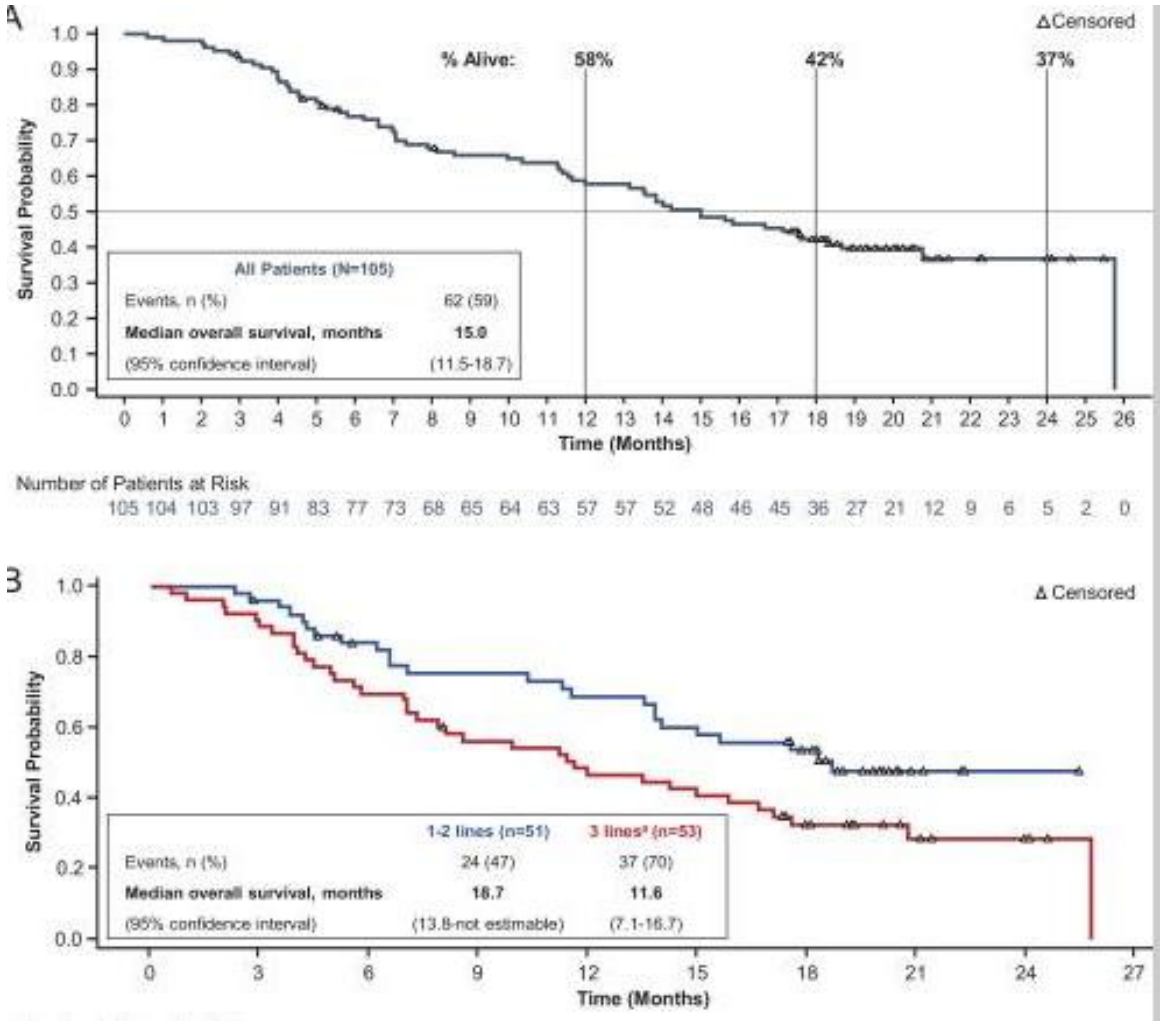


PMID: 23863359

SORAYA, a Single-arm Phase II Study, Showed Objective Response Rate of 32.4% (5 Complete, 29 Partial) to MIRV (ELAHERE) in Patients with Platinum-Resistant Epithelial Ovarian Cancer

- Patients who received MIRV as their first treatment had a moderately higher objective response rate VS patients who did not (34.8% vs 28.2%)

- Results confirmed in a **randomized, phase 3 MIRASOL trial:**
  - ✓ An objective response rate of 42.3% in the MIRV group vs 15.9% in the chemotherapy group
  - ✓ Longer overall survival – median 16.46 vs 12.75 months



PMID: 36716407, 38858103, 38055253

Expression of FRa Is Determined by Ventana FOLR1 RxDx Assay

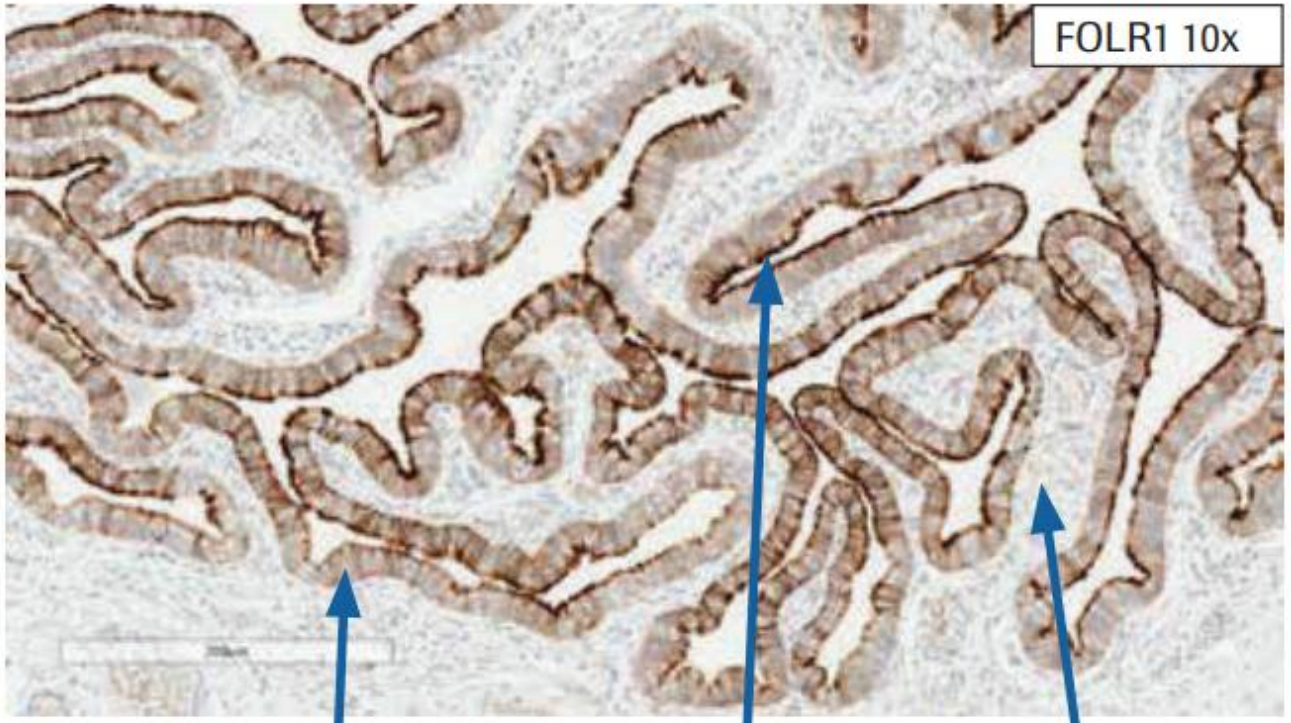
Table 1: VENTANA FOLR1 (FOLR1-2.1) RxDx Assay Scoring Algorithm for EOC

IHC Interpretation	Staining Description
Positive for FOLR1*	≥ 75% of viable tumor cells with moderate (2+) and/or strong (3+) membrane staining
Negative for FOLR1*	< 75% of viable tumor cells with moderate (2+) and/or strong (3+) membrane staining
Not Evaluable	Artifacts making interpretation not possible

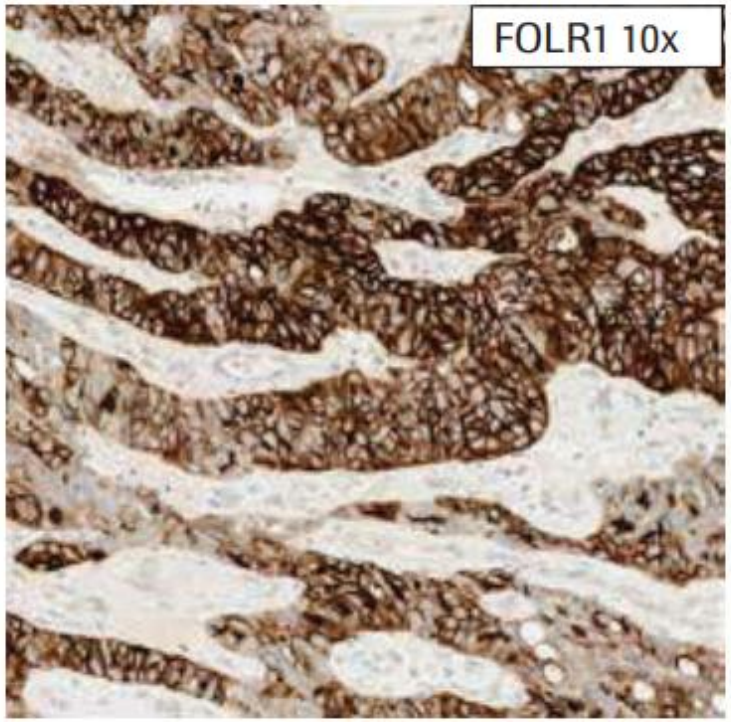
* Re-reading by Additional Pathologists for FOLR1 Scoring

Acceptable Staining of Control Normal Fallopian Tube Tissue

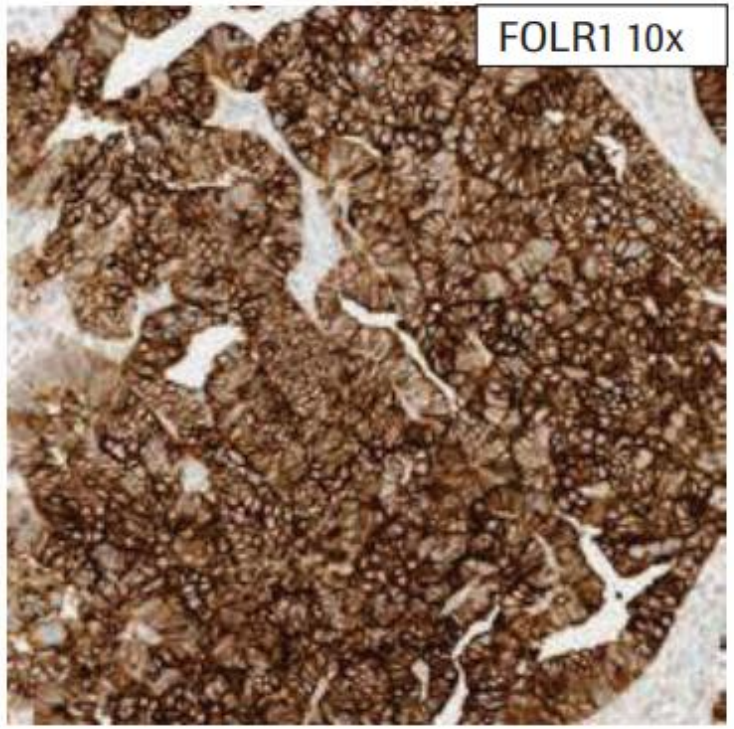
- Normal fallopian tube can be used as a positive and negative control
- FRa is expressed on the luminal surface of the epithelial cells
- Also use a case with moderate circumferential staining as a positive control
- Disregard apical staining of the first layer of the luminal cells when evaluating staining adequacy



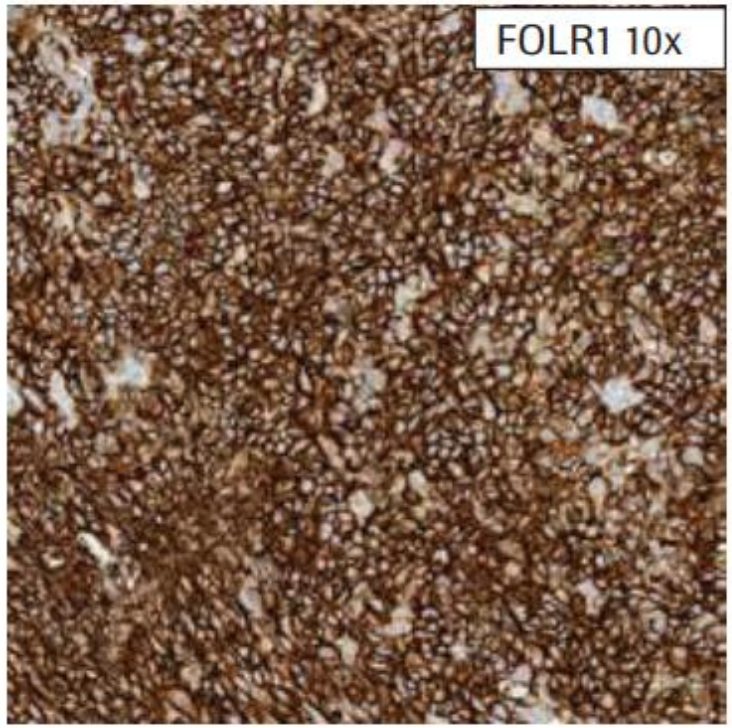
Clinical Diagnosis Positive



Exhibits 95% moderate and strong membrane staining or ≥ 75% moderate or strong membrane staining with complete circumferential pattern*

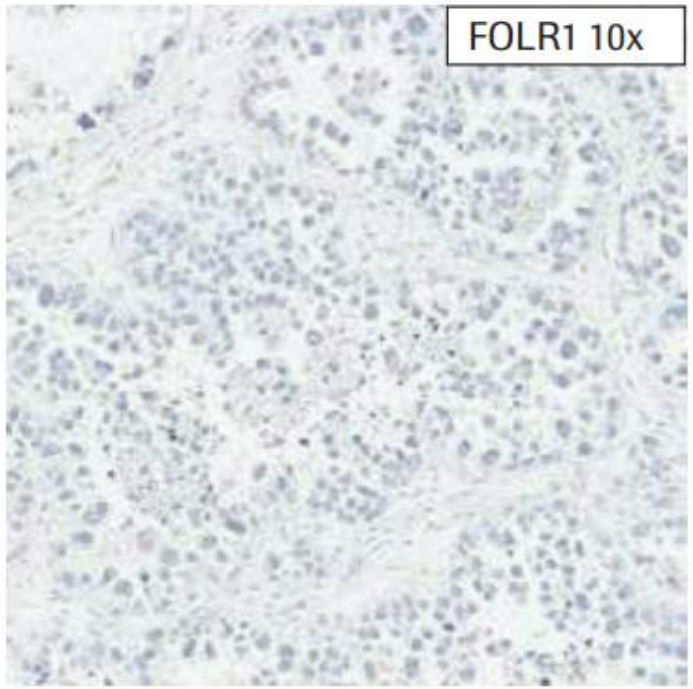


Exhibits 98% moderate and strong membrane staining or ≥ 75% tumor cells membrane staining with complete circumferential pattern*

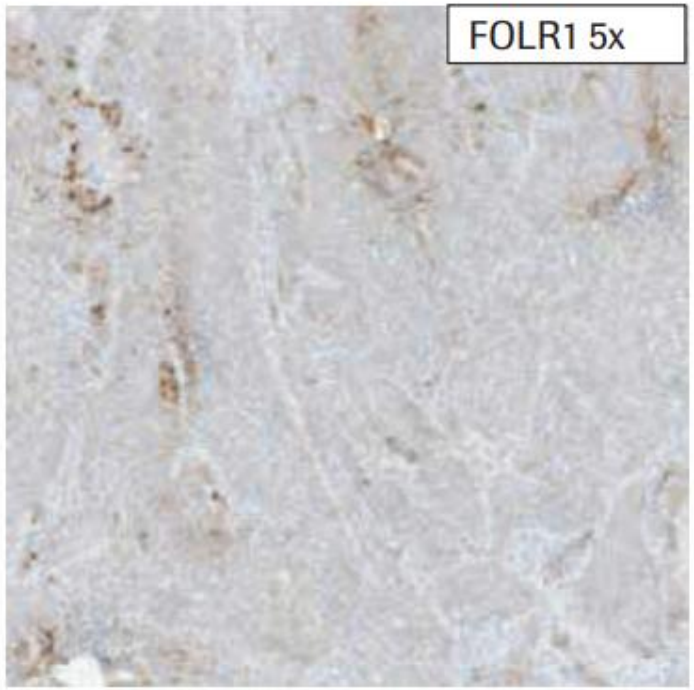


Exhibits 98% moderate and strong membrane staining or ≥ 75% tumor cells membrane staining with complete circumferential pattern*

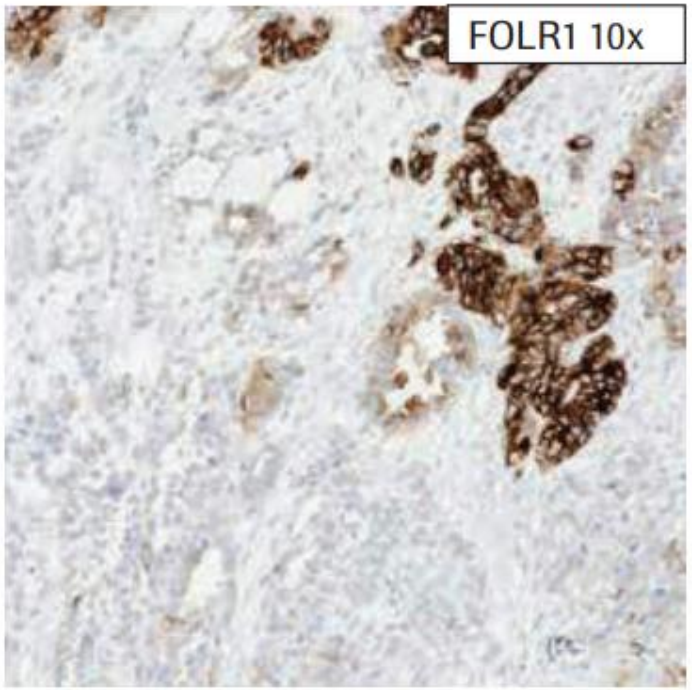
Clinical Diagnosis Negative



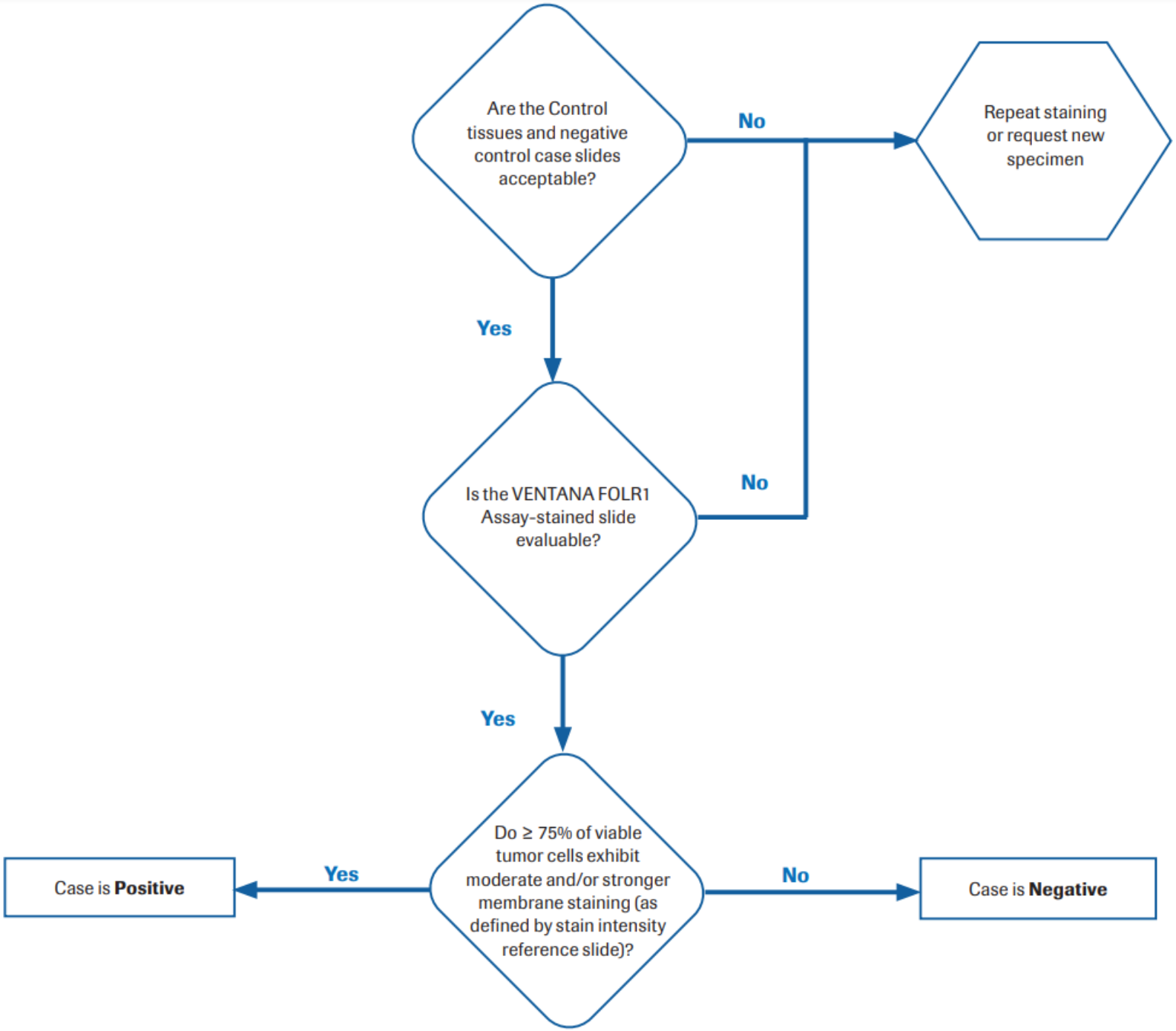
Exhibits no moderate or strong tumor cell membrane staining or < 75% moderate and/or strong tumor cell membrane staining

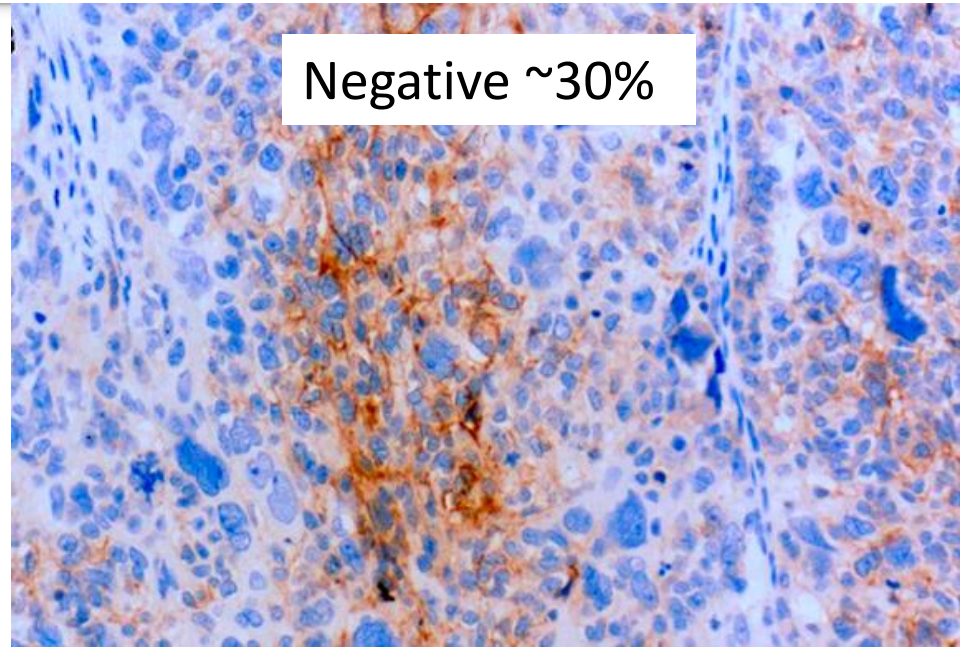


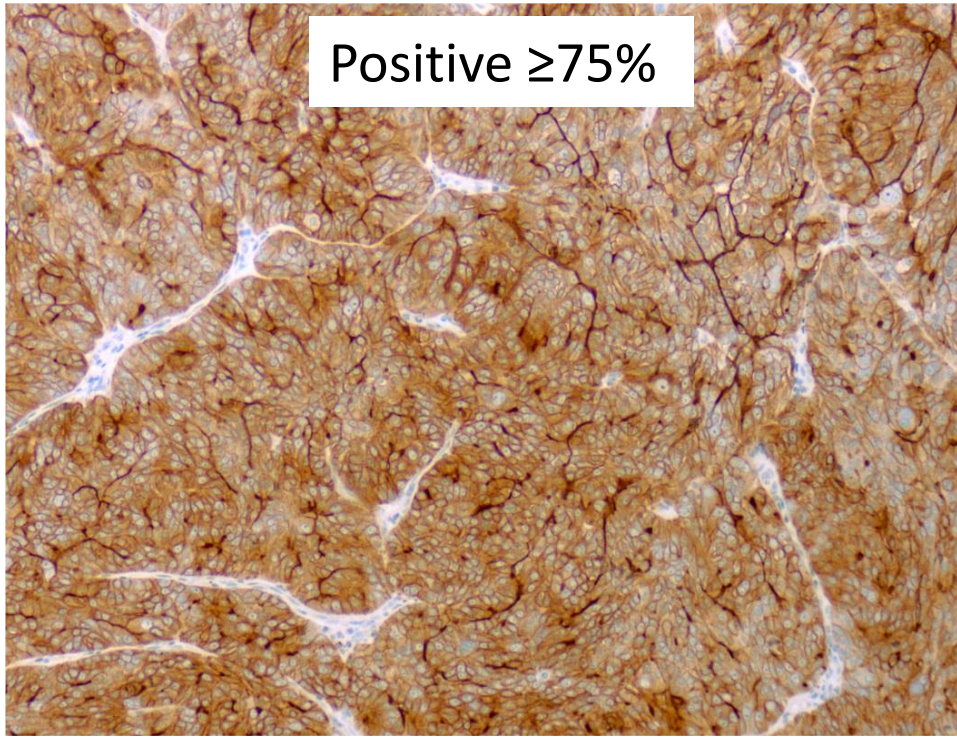
Exhibits 7% moderate and strong membrane staining or < 75% moderate and/or strong tumor cell membrane staining



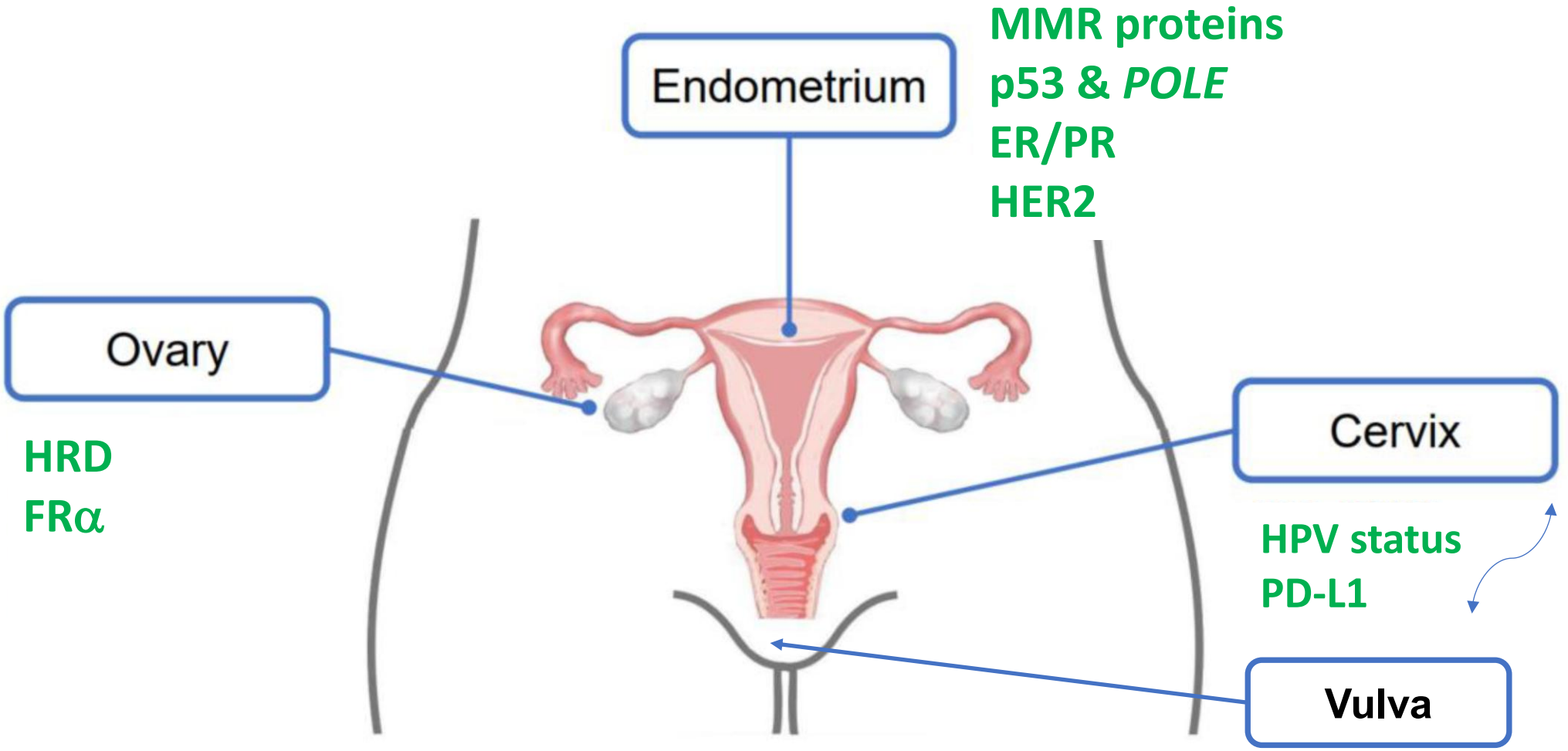
Exhibits 20% moderate and strong membrane staining or < 75% moderate and/or strong tumor cell membrane staining


Are the Control tissues and negative control case slides acceptable?
No
Repeat staining or request new specimen
Yes
Is the VENTANA FOLR1 Assay-stained slide evaluable?
No
Yes
Do ≥ 75% of viable tumor cells exhibit moderate and/or stronger membrane staining (as defined by stain intensity reference slide)?
Yes
Case is **Positive**
No
Case is **Negative**


Negative ~30%


Positive ≥75%

Prognostic and Therapeutic Markers in Gynecologic Pathology



Summary

- Biomarker testing in gynecologic neoplasms has specific indications and prognostic/predictive implications
- Awareness of these indications is essential for correctly applying the test results for optimal patient management
- In particular, awareness of various scoring criteria for HER2 assessment is crucial for optimal HER2 targeted therapy


Thank you!
Questions?