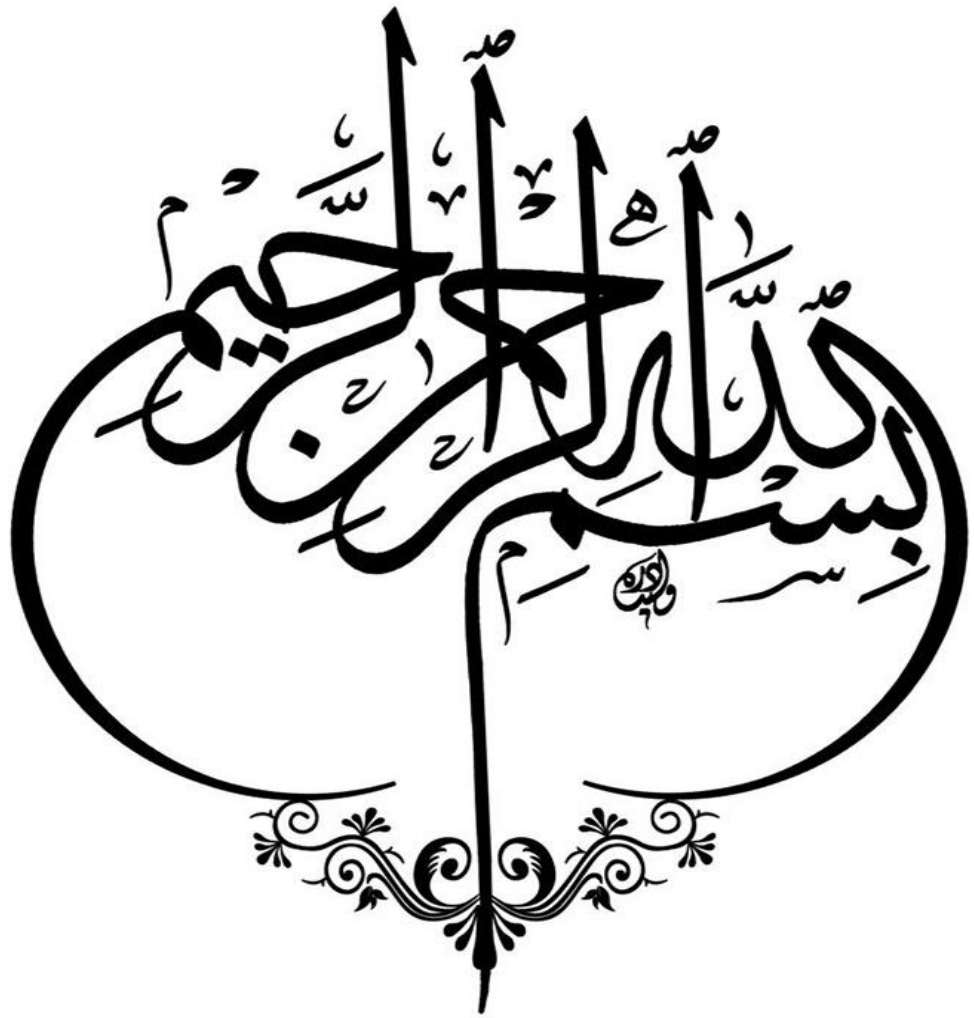

بسم الله الرحمن الرحيم

Diagnostic Immunohistochemistry in Gynecologic Pathology
A Morphology-Driven Approach
Endometrium-part 2

Dr 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

Section II

The Cancer Genome Atlas (TCGA)

Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE)

The Cancer Genome Atlas (TCGA)

Why was molecular classification needed?

- Histologic classification alone cannot fully predict tumor behavior.
- Morphologically similar endometrial carcinomas may demonstrate markedly different clinical outcomes.
- Molecular classification improves:
  - Prognostic stratification
  - Therapeutic decision-making
  - Precision medicine

The Cancer Genome Atlas (TCGA)

Integrated Genomic Characterization

- TCGA performed comprehensive molecular profiling using:
  - Whole-exome sequencing
  - Copy-number analysis
  - DNA methylation profiling
  - Microsatellite instability (MSI) analysis
  - mRNA expression profiling
  - Reverse-phase protein array (RPPA)
- **Integration of these data identified four molecular subtypes of endometrial carcinoma.**

Translation of TCGA into Clinical Practice

- **Surrogate Molecular Classification (ProMisE)**
  - Routine genomic profiling is not feasible in daily practice.
- Surrogate biomarkers are used instead:
  - **POLE sequencing** → POLE-ultramutated
  - **MMR immunohistochemistry** → MMR-deficient
  - **p53 immunohistochemistry** → Copy-number high
  - **No abnormality detected** → NSMP (Copy-number low)
- This surrogate approach has been validated and incorporated into WHO classification and international guidelines.

Stepwise Molecular Classification Algorithm

- **Recommended Testing Sequence**
- **POLE mutation analysis**
  - Pathogenic mutation → **POLE-ultramutated**
- **MMR immunohistochemistry**
  - Deficient expression → **MMR-deficient**
- **p53 immunohistochemistry**
  - Abnormal expression → **Copy-number high**
- **If all tests are negative**
  - **NSMP (Copy-number low)**

TCGA Molecular Subtypes

Molecular Subtype	Frequency	Prognosis
POLE-ultramutated	7–12%	Excellent
MMR-deficient	25–30%	Intermediate
NSMP (Copy-number low)	35–45%	Intermediate
p53-abnormal (Copy-number high)	20–25%	Poor

Clinical Significance of Molecular Classification

- Molecular classification provides:
  - Improved prognostic stratification
  - Risk-adapted treatment planning
  - Identification of Lynch syndrome
  - Selection of patients for immunotherapy
  - Guidance for targeted therapies
  - Standardized molecular reporting

Relationship Between Histology and Molecular Classification

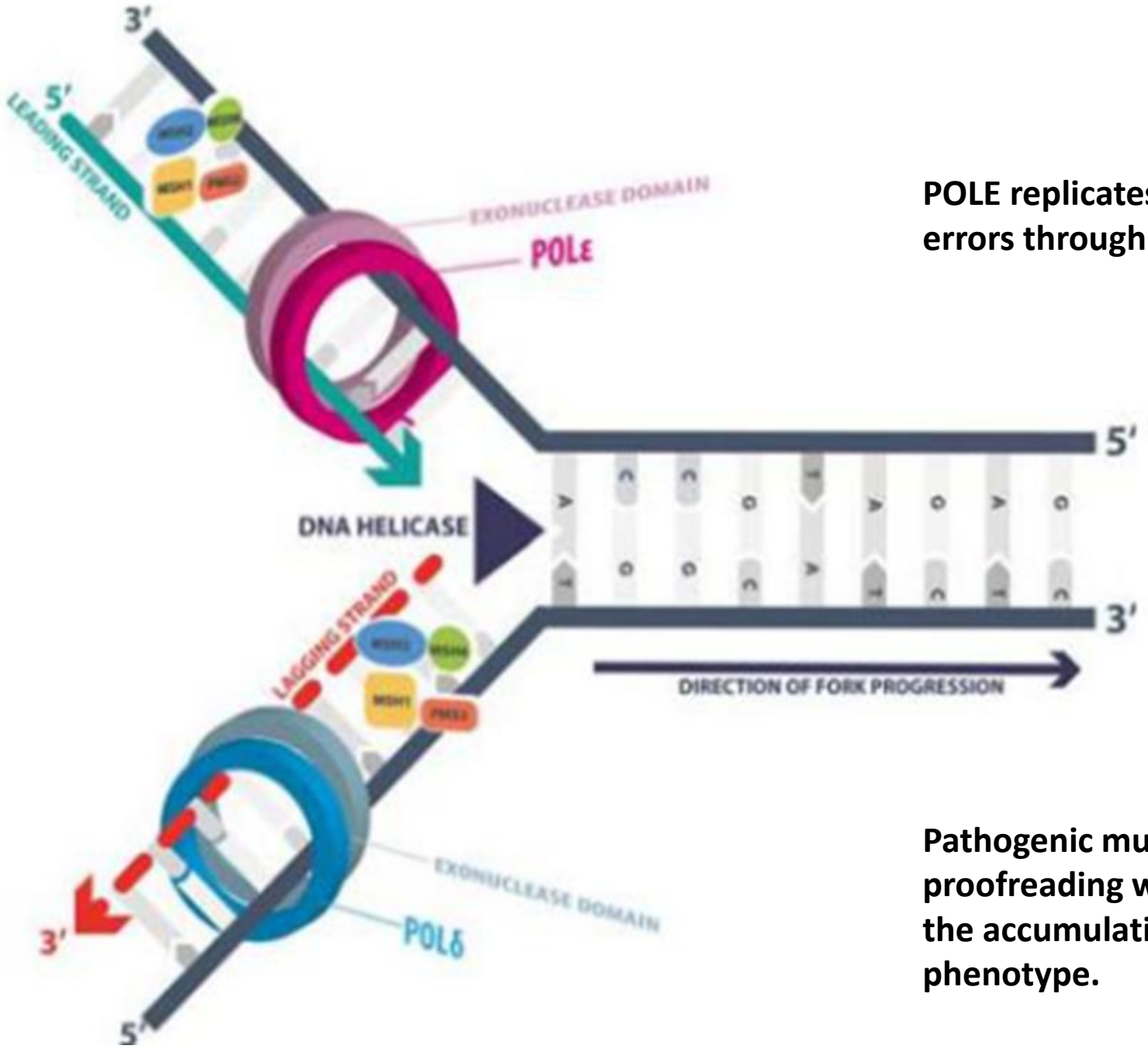
- Molecular subtype is **independent of histologic subtype**.
- The same histologic subtype may belong to different molecular groups.
- High-grade morphology does not always indicate poor prognosis.
- Molecular classification complements, rather than replaces, histopathologic evaluation.

Multiple-Classifier Tumors

- A subset of endometrial carcinomas shows abnormalities in more than one molecular classifier.
- Integrated interpretation is required.
- **POLE mutation supersedes other molecular classifiers.**
- Interpretation of **MMRd/p53-abnormal** tumors should integrate morphology and molecular findings.

POLE-ultramutated

- **Biology of POLE**
- **POLE** encodes the catalytic subunit of **DNA polymerase epsilon (Pol ε)**.
- DNA polymerase ε is responsible for **leading-strand DNA replication**.
- The **3′→5′ exonuclease domain** provides proofreading activity by correcting replication errors.
- Pathogenic mutations within the exonuclease domain impair proofreading function.
- Failure of proofreading results in accumulation of somatic mutations and an **ultramutated phenotype**.
- POLE-mutated tumors exhibit the **highest tumor mutational burden (TMB)** among endometrial carcinomas.



POLE replicates the leading strand and corrects replication errors through its proofreading exonuclease domain.

Pathogenic mutations in the exonuclease domain impair proofreading without abolishing DNA synthesis, resulting in the accumulation of somatic mutations and an ultramutated phenotype.

Molecular Features of POLE-Ultramutated Tumors

- Characterized by **pathogenic exonuclease domain (EDM) mutations**.
- Extremely high tumor mutational burden (>100 mutations/Mb).
- Increased production of **neoantigens**.
- Prominent activation of the host immune response.
- Dense **CD8+ tumor-infiltrating lymphocytes (TILs)** are commonly observed.
- Despite extensive genomic alterations, chromosomal instability is uncommon.

POLE-ultramutated-Clinicopathologic Features

- Frequency: **Approximately 7–12%** of endometrial carcinomas.
- Most commonly associated with **endometrioid histology**.
- Frequently presents as **FIGO grade 3** tumors.
- May exhibit:
  - Marked nuclear atypia
  - High mitotic activity
  - Tumor giant cells
  - Prominent lymphocytic infiltrates
- Most patients present with **early-stage disease (FIGO stage I–II)**.

POLE-ultramutated-Clinical Significance

- Associated with the **best prognosis** among all TCGA molecular subtypes.
- Very low recurrence rate despite high-grade morphology.
- Excellent disease-specific and overall survival.
- High-grade histology alone should **not** determine treatment intensity.
- Molecular classification may support **de-escalation of adjuvant therapy** in selected patients.

POLE-ultramutated-Indications for POLE Testing

- POLE mutation analysis is particularly recommended in:
  - High-grade endometrioid endometrial carcinoma.
  - Tumors with ambiguous morphology.
  - Tumors with abnormal p53 expression.
  - Cases considered for molecular risk stratification.
  - Selected low-grade tumors when molecular classification may alter clinical management.

POLE-ultramutated-Multiple-Classifier POLE Tumors

- A subset of endometrial carcinomas harbors more than one molecular alteration.
- Examples:
  - **POLE-mutated + p53-abnormal**
  - **POLE-mutated + MMR-deficient**
- **Interpretation**
- **POLE mutation supersedes all other molecular classifiers.**
- These tumors are classified as **POLE-ultramutated**.
- Prognosis follows the **POLE subtype**, not the accompanying molecular alteration.

POLE-ultramutated

- **Pitfalls in POLE Interpretation**
  - **Pathogenic vs Non-Pathogenic POLE Variants**
    - Not all POLE variants are pathogenic.
    - Only **pathogenic exonuclease domain mutations** define the POLE molecular subtype.
    - Variants of uncertain significance (**VUS**) should **not** be classified as POLE-ultramutated.
    - POLE mutation analysis should be interpreted using validated pathogenic variant databases.

POLE-ultramutated

- **Pathogenic POLE Mutations**
- **Exonuclease Domain Hotspot Mutations**
- The vast majority of pathogenic POLE mutations occur within the **exonuclease domain (amino acids 268–471).**
- **Common pathogenic hotspot mutations**
  - **P286R**
  - **V411L**
  - S297F
  - A456P
  - S459F
- These hotspot mutations are responsible for the ultramutated phenotype and define the POLE molecular subtype.

POLE-ultramutated

- **Do NOT classify as POLE-ultramutated**
  - Variants outside the exonuclease domain
  - Variants of uncertain significance (VUS)
  - Benign polymorphisms
  - Non-pathogenic missense variants
- Classification should follow validated pathogenic variant interpretation systems.

POLE-ultramutated

- **Why Do POLE Tumors Have an Excellent Prognosis?**
- **Proposed Biological Mechanism**
  - Pathogenic POLE mutations lead to:
    - Extremely high tumor mutational burden (TMB)
    - Increased neoantigen production
    - Enhanced $CD8^+$ T-cell infiltration
    - Strong anti-tumor immune response
    - Although these tumors accumulate numerous mutations, immune surveillance limits tumor progression and recurrence.

POLE-ultramutated

- **Diagnostic Pearls**
  - High-grade morphology does **not** exclude excellent prognosis.
  - POLE mutation supersedes p53 and MMR abnormalities.
  - Most POLE tumors present at an early stage.
  - POLE status may support treatment de-escalation.
- **Diagnostic Pitfalls**
  - Do not overinterpret Variants of uncertain significance.
  - Morphology alone cannot predict POLE mutation.
  - POLE testing should be interpreted together with histology, MMR, and p53 results.

POLE-ultramutated- Take-Home Messages

- POLE mutations affect the **proofreading function** of DNA polymerase ε.
- POLE-ultramutated tumors have the **highest tumor mutational burden**.
- They frequently demonstrate **abundant CD8+ tumor-infiltrating lymphocytes**.
- Most cases are **high-grade endometrioid carcinomas**.
- Despite aggressive morphology, prognosis is **excellent**.
- POLE mutation is associated with a **very low recurrence rate**.
- POLE status may influence **adjuvant treatment decisions**.
- **POLE mutation overrides p53 abnormalities and MMR deficiency** in multiple-classifier tumors.
- Only **pathogenic exonuclease domain mutations** qualify for the POLE molecular subtype.

پاک‌ترین قلب را

کسانی دارند

که از ذوق دیگران

ذوق می کُشند

Mismatch Repair (MMR) System

- **DNA Mismatch Repair**
- The mismatch repair (MMR) system maintains genomic stability by correcting DNA replication errors.
- MMR proteins repair:
  - Base-base mismatches
  - Small insertion-deletion loops (indels)
- Defective MMR results in:
  - Microsatellite instability (MSI)
  - Hypermutated phenotype
  - Increased tumor mutational burden
- MMR deficiency is one of the four TCGA molecular subtypes of endometrial carcinoma.

Mismatch Repair (MMR) System

- **The MMR Proteins**
- **Mismatch Repair Heterodimers**

MutS Complex

MSH2

MSH6

Function:

Recognizes DNA mismatches

MutL Complex

MLH1

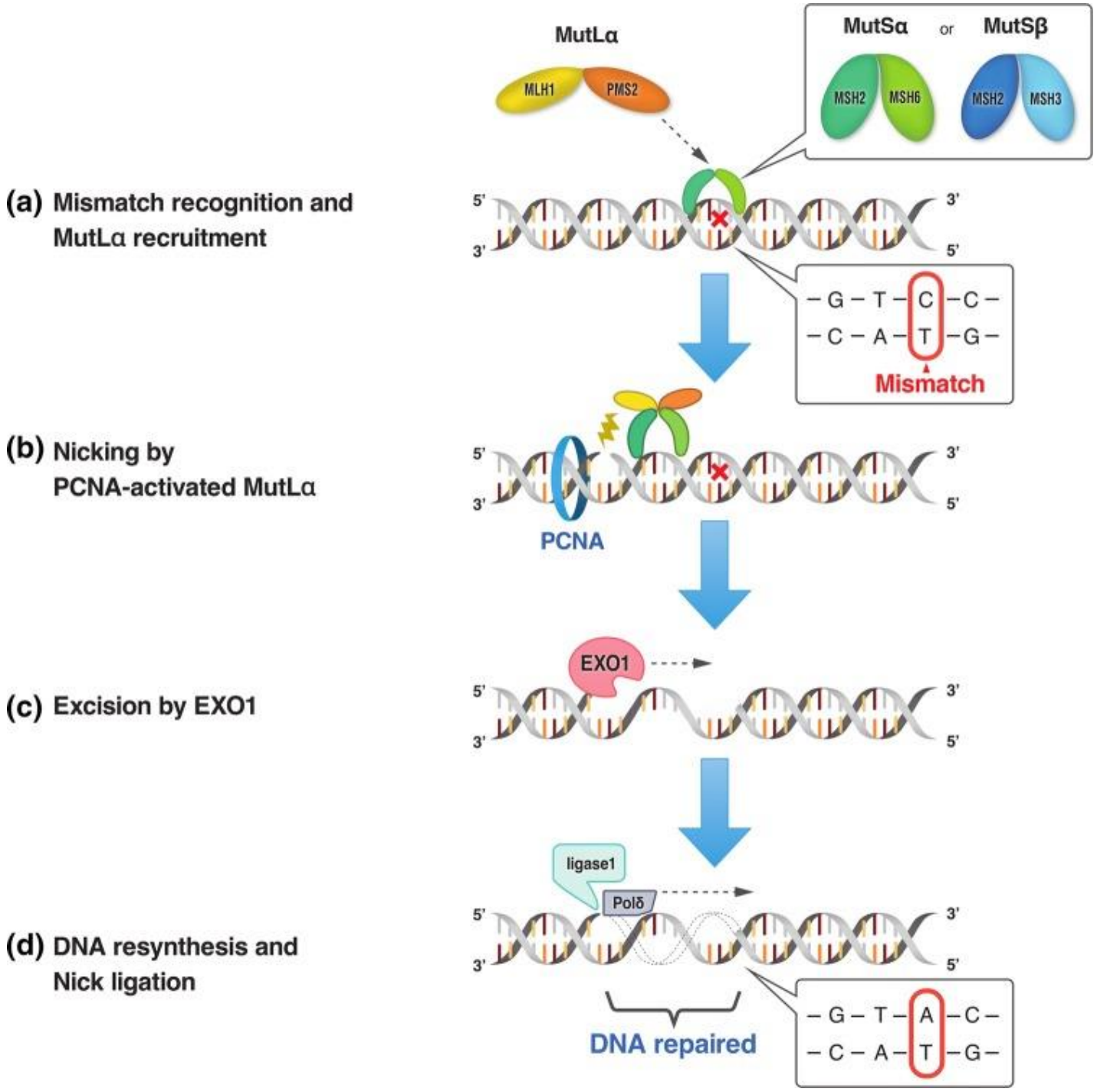
PMS2

Function:

Coordinates DNA repair after mismatch recognition

- **Functional Heterodimers:** MLH1 ↔ PMS2 MSH2 ↔ MSH6
- Loss of the dominant partner results in degradation of its binding partner.

Schematic diagram of the DNA mismatch repair (MMR) pathway.



TWO TEAMS, ONE MISSION – DEPENDENT PAIRS

DRIVER TEAM (SEARCH) = MutSα

Heterodimer:
MSH2 + MSH6

Finds the error (mismatch)

DEPENDENT PAIR

If DRIVER (MSH2) is lost →
PASSENGER (MSH6) is also lost.

REPAIR TEAM (FIX) = MutLα

Heterodimer:
MLH1 + PMS2

Coordinates and implements repair

DEPENDENT PAIR

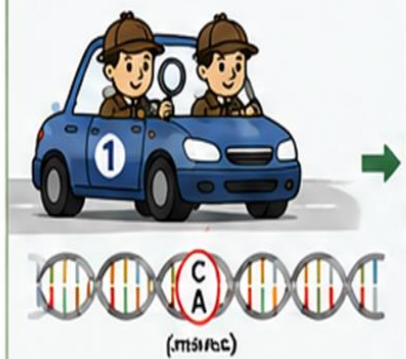
If REPAIR LEADER (MLH1) is lost →
PASSENGER (PMS2) is also lost.

BOTH TEAMS WORK TOGETHER.
If one team (driver) is missing → entire mission fails.

THE MMR MISSION: FIND → FIX → FINISH

1 FIND THE ERROR

Driver team (MSH2–MSH6) searches the DNA.



They detect the mismatch.

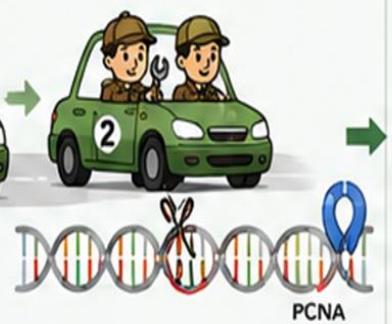
2 CALL FOR HELP

Driver team calls the repair team.

They recruit the mechanics (MLH1–PMS2).

3 MAKE A NICK

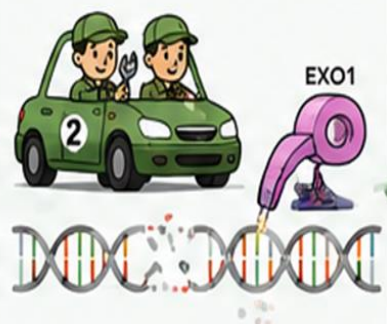
Repair team arrives and makes a cut near the mismatch.



PCNA helps position the cut.

4 REMOVE WRONG PART

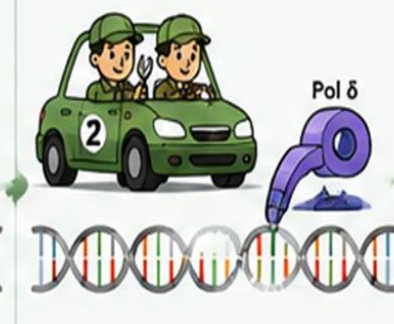
EXO1 removes the wrong section.



The incorrect part is excised.

5 REBUILD CORRECTLY

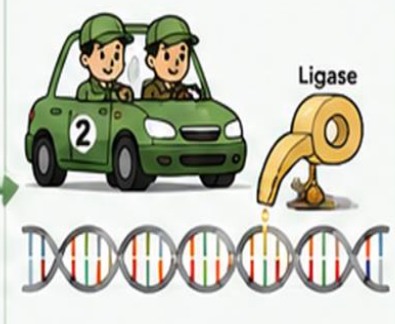
DNA polymerase δ fills in the correct sequence.



New correct DNA is synthesized.

6 SEAL AND FINISH

DNA ligase seals the DNA.



The DNA is sealed. Mission complete!

KEY TAKEAWAY

- ✓ **DRIVER TEAM (MSH2–MSH6) = SEARCH TEAM**
  Finds the error (mismatch).
- ✓ **REPAIR TEAM (MLH1–PMS2) = MECHANICS TEAM**
  Coordinates and fixes the error.

- ☑ If DRIVER is lost → both DRIVER proteins are lost.
- ☑ If REPAIR LEADER is lost → only that passenger is lost.

= Driver Team (Search) = MutSα
MSH2 + MSH6

= Repair Team (Fix) = MutLα
MLH1 + PMS2

MISSION FAILURE SCENARIOS

If MSH2 is lost (the DRIVER):

MSH2 and MSH6 both are lost.

If MLH1 is lost (the LEADER):

Only PMS2 is lost.

Mechanisms of MMR Deficiency

- Three major mechanisms cause MMR deficiency:

Sporadic
MLH1 promoter hypermethylation
Most common mechanism
Usually associated with concurrent MLH1/PMS2 loss

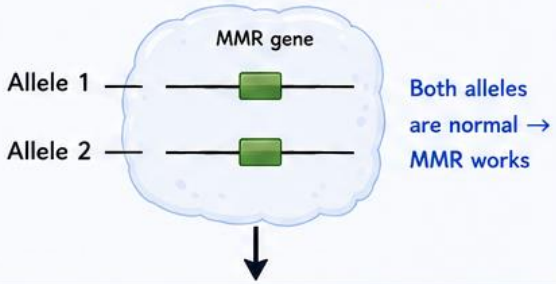
Hereditary
Lynch syndrome
Germline pathogenic variants in:
- MLH1
- MSH2
- MSH6
- PMS2

Double somatic mutations
Two acquired mutations affecting the same MMR gene
Not hereditary

MMR Deficiency: Three Genetic Routes

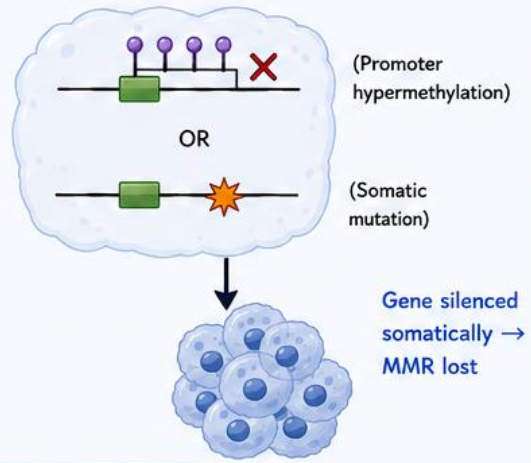
1 Sporadic Somatic Mutation

Normal (MMR Proficient)



Tumor (MMR Deficient)

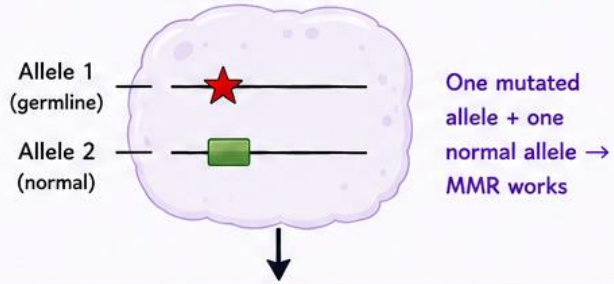
Promoter hypermethylation or somatic mutation silences the gene



No family history (occurs in tumor only)

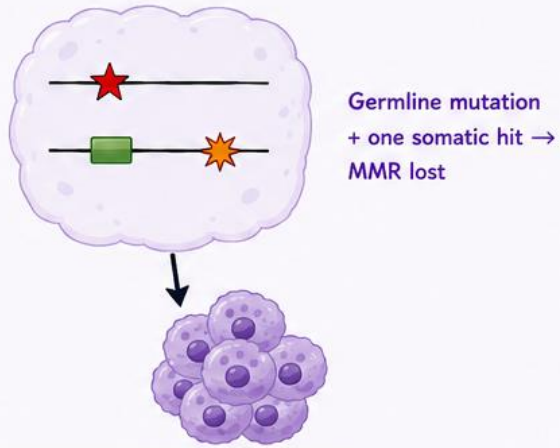
2 Germline Mutation (Lynch Syndrome)

Normal (Carriers: one mutated allele)



Tumor (MMR Deficient)

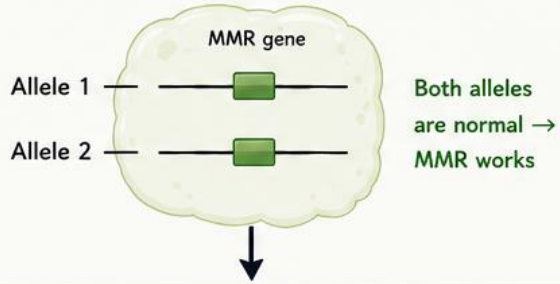
One somatic mutation hits the remaining normal allele



Often family history (Lynch Syndrome)
Autosomal dominant inheritance

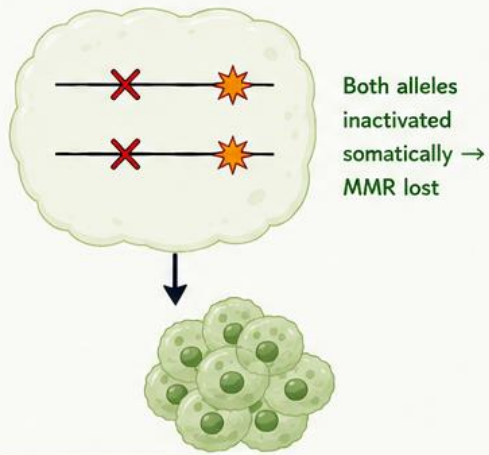
3 Double Somatic Mutations

Normal (MMR Proficient)



Tumor (MMR Deficient)

Two different somatic mutations inactivate both alleles



No family history (occurs in tumor only)

Normal allele (functional)
Germline mutation (present in all cells)
Somatic mutation (acquired in tumor cells only)
Loss of function (gene inactivated)
Promoter hypermethylation
→ Leads to loss of MMR and microsatellite instability (MSI)

Lynch Syndrome

- Autosomal dominant hereditary cancer syndrome
- Caused by germline pathogenic variants in:
  - MLH1
  - MSH2
  - MSH6
  - PMS2
- Increases lifetime risk of:
  - Endometrial carcinoma
  - Colorectal carcinoma
  - Ovarian carcinoma
  - Other Lynch-associated malignancies
- Universal MMR screening is recommended for all endometrial carcinomas.

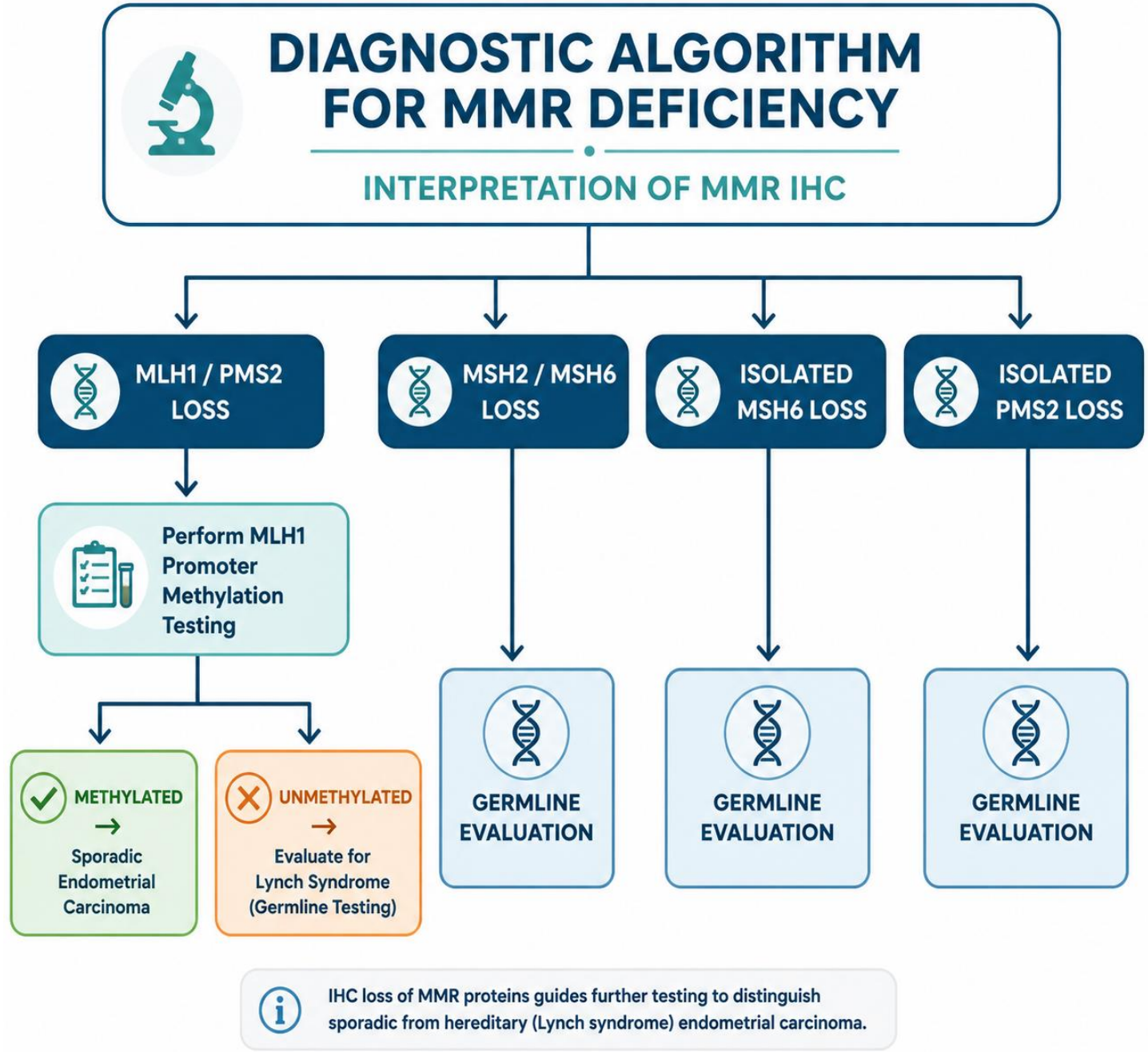
Mismatch Repair (MMR) System

- **Double Somatic MMR Mutations**
  - Caused by two acquired somatic mutations affecting the same MMR gene.
  - Produces MMR-deficient tumors without germline mutations.
  - Mimics Lynch syndrome by immunohistochemistry.
  - Germline testing is negative.
  - Family members are not at increased hereditary risk.
  - Molecular tumor sequencing is required for confirmation.

Mismatch Repair (MMR) System

IHC Pattern	Most Likely Alteration
MLH1− / PMS2−	MLH1 inactivation (hypermethylation or mutation)
PMS2 loss only	PMS2 mutation
MSH2− / MSH6−	MSH2 mutation
MSH6 loss only	MSH6 mutation

Interpretation should always be performed in conjunction with an adequate internal positive control.


DIAGNOSTIC ALGORITHM FOR MMR DEFICIENCY
INTERPRETATION OF MMR IHC
MLH1 / PMS2 LOSS
MSH2 / MSH6 LOSS
ISOLATED MSH6 LOSS
ISOLATED PMS2 LOSS
Perform MLH1 Promoter Methylation Testing
METHYLATED
Sporadic Endometrial Carcinoma
UNMETHYLATED
Evaluate for Lynch Syndrome (Germline Testing)
GERMLINE EVALUATION
GERMLINE EVALUATION
GERMLINE EVALUATION
IHC loss of MMR proteins guides further testing to distinguish sporadic from hereditary (Lynch syndrome) endometrial carcinoma.

Mismatch Repair (MMR) System-Subclonal MMR Loss

- **Subclonal loss is defined as:**
  - Complete loss of one or more MMR proteins in a **distinct subpopulation** of tumor cells
  - Adjacent tumor cells retain normal MMR expression
- **Clinical Significance**
  - Reflects intratumoral heterogeneity
  - May represent tumor evolution
  - Should be reported separately
  - May require MSI-PCR or molecular testing if immunotherapy is being considered

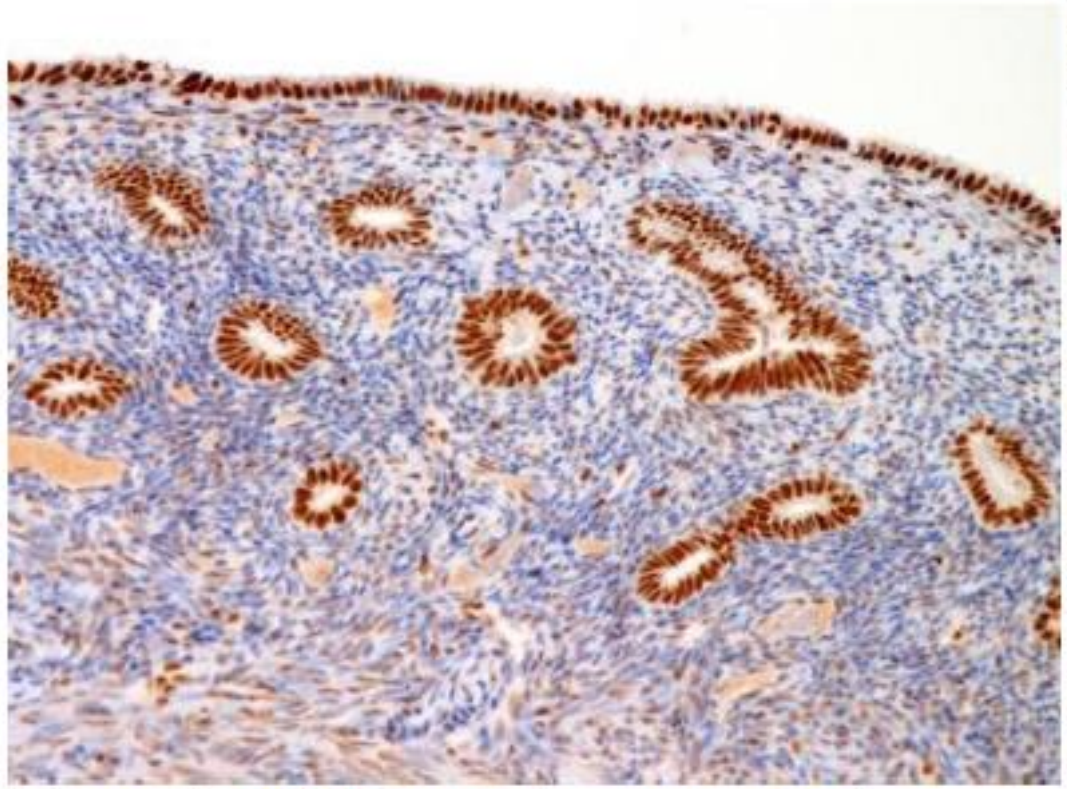


Figure 1a. MMR protein expression in normal inactive endometrium (PMS2).

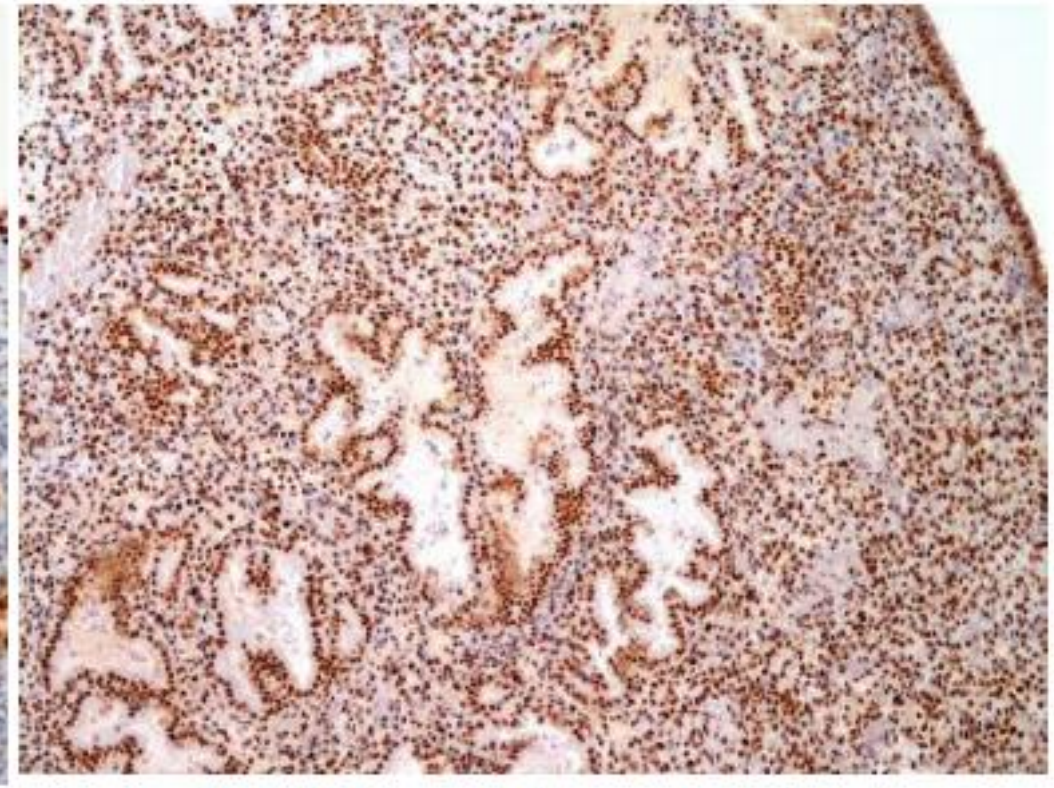


Figure 1b. MMR protein expression in secretory endometrium (MSH2).

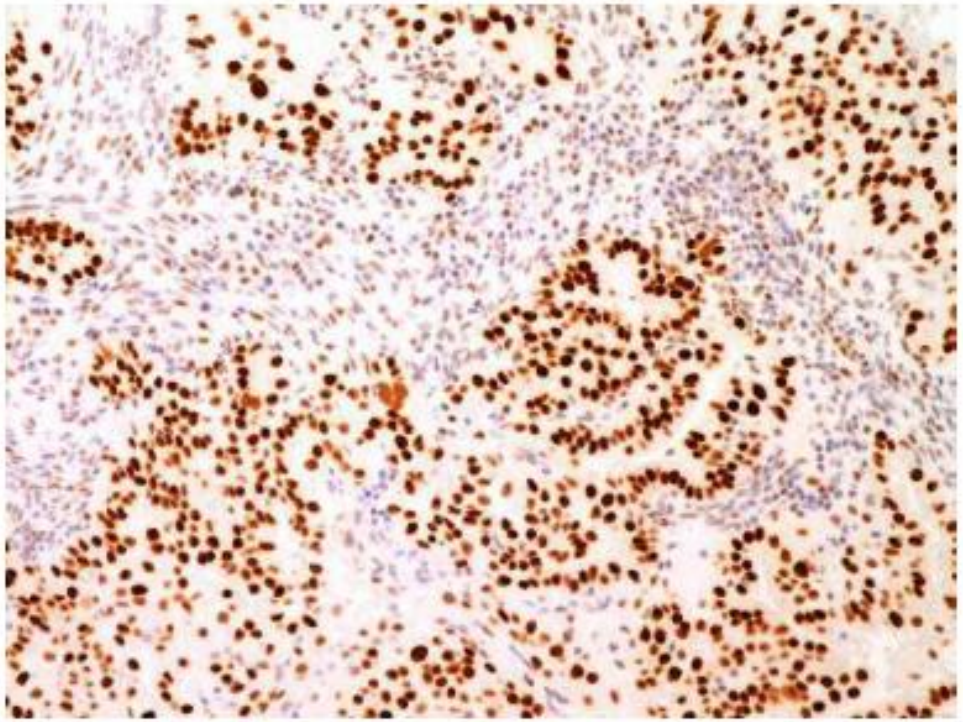


Figure 2a. MMR protein expression in endometrial carcinoma (PMS2).

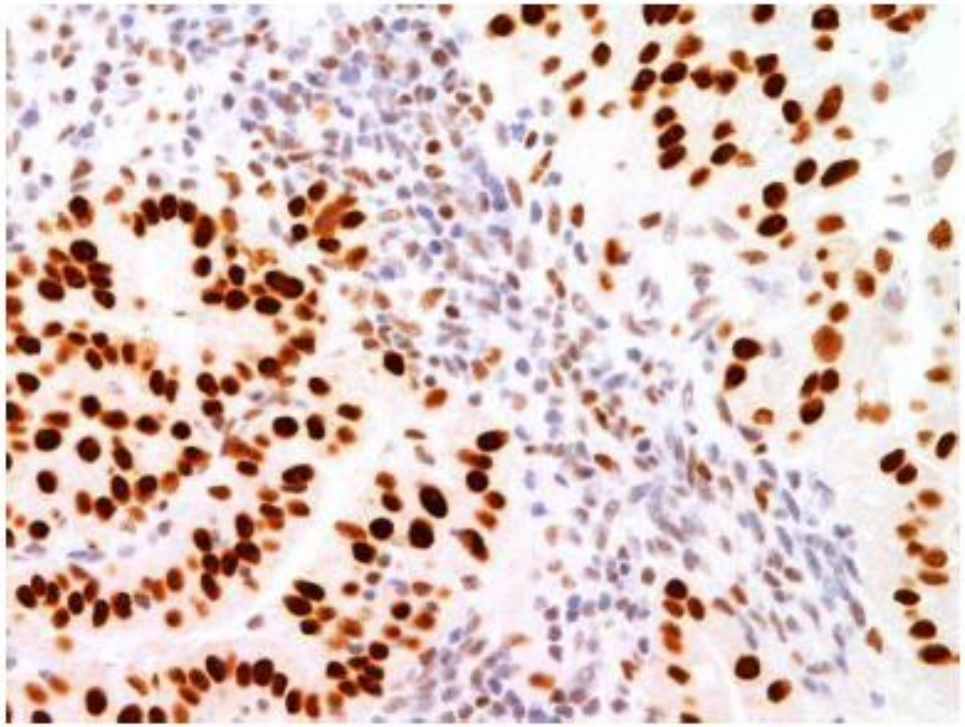


Figure 2b. Note the stronger intensity in tumour cells as compared to stromal cells (same area as Figure 2a).

In cancer cells, generally characterized by higher proliferation rates relative to normal tissue, the staining intensity is typically strong, and generally higher than that seen in the background stroma, normal glands or inflammatory cells that serve as an internal control.

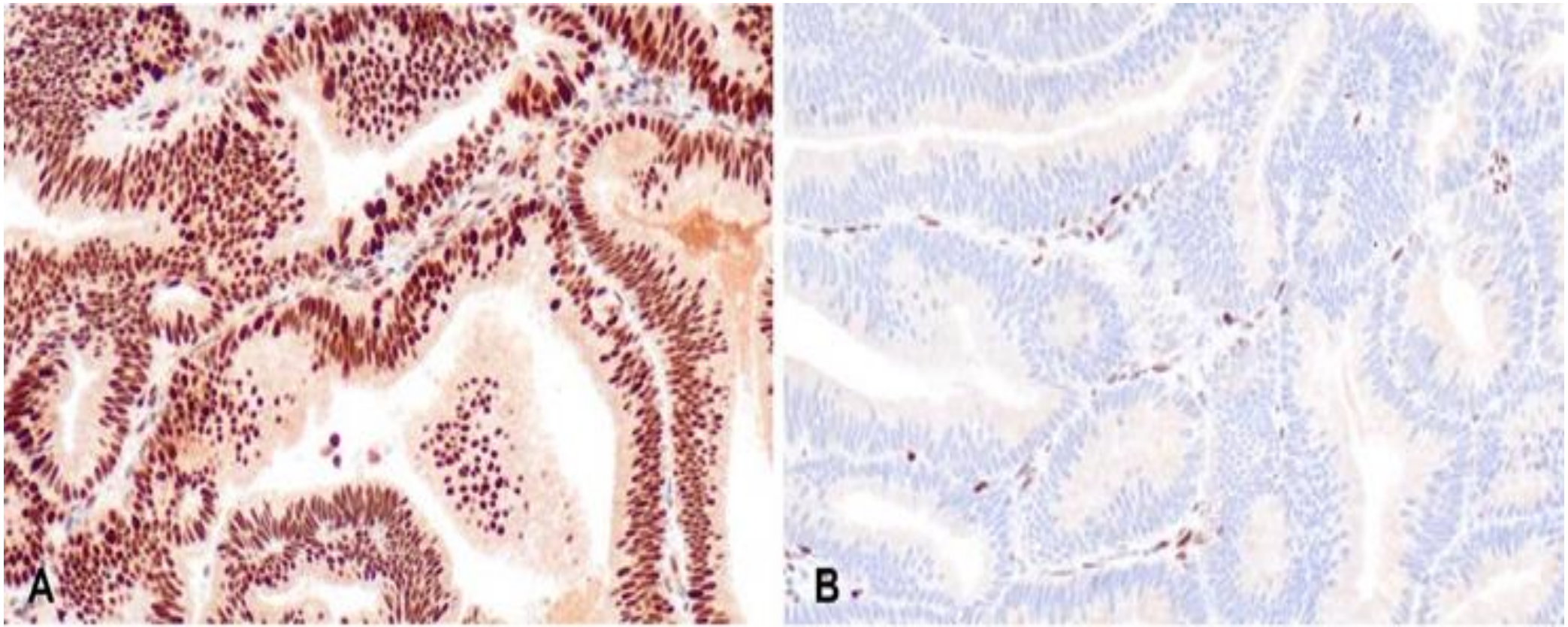


Figure . Mismatch repair immunohistochemistry in endometrial carcinoma. **A,** Intact nuclear expression of **PMS2**. **B,** Loss of (**MSH6**) expression in tumor cells with satisfactory internal positive control

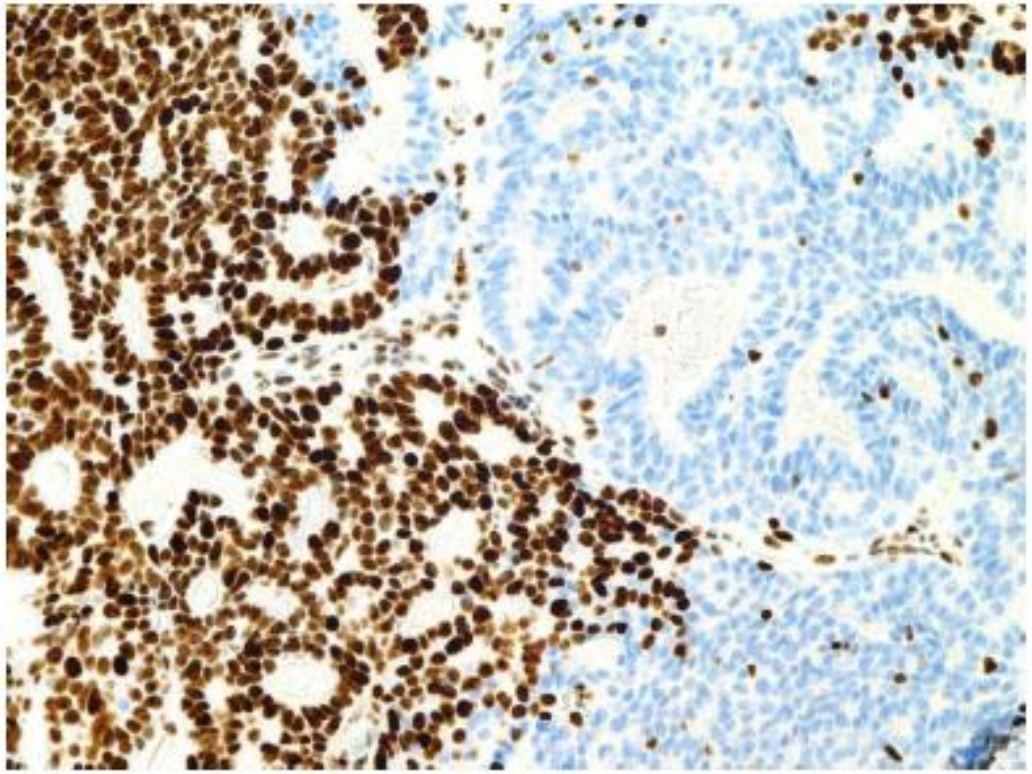


Figure 9a. Subclonal expression of MSH6 in part of this endometrial carcinoma (left) coexisting with an area of negative staining (right). Note the positive staining of stromal cells among the negative tumour cells.

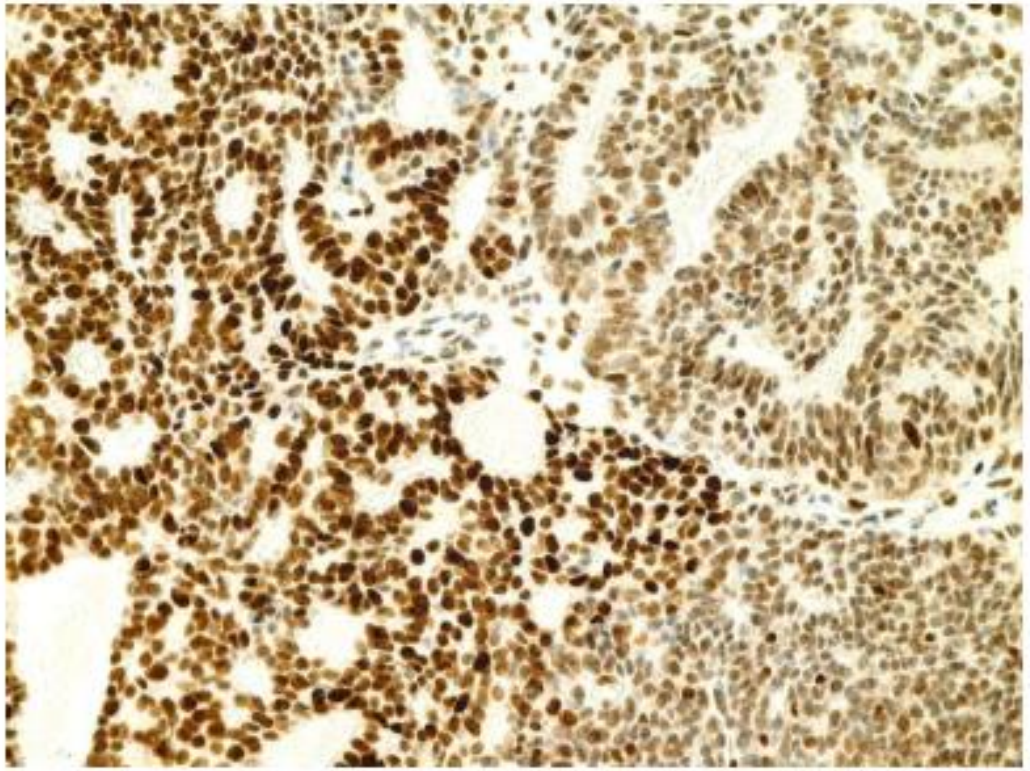


Figure 9b. Expression of MSH2 in the same tumour area as Figure 9a.

An arbitrary cut-off of 10% is suggested to avoid reporting this pattern in cases where it is extremely focal and of unlikely clinical significance

MMR Immunohistochemistry Pitfalls

- **Technical Pitfalls**
  - Inadequate fixation
  - Delayed fixation
  - Poor antigen preservation
  - Weak staining
  - Absence of internal control
- **Interpretation Pitfalls**
  - Patchy staining ≠ Subclonal loss
  - Cytoplasmic staining is **not** considered retained expression
  - Nucleolar staining should not be interpreted as positive
  - Internal control is mandatory before interpreting loss of expression

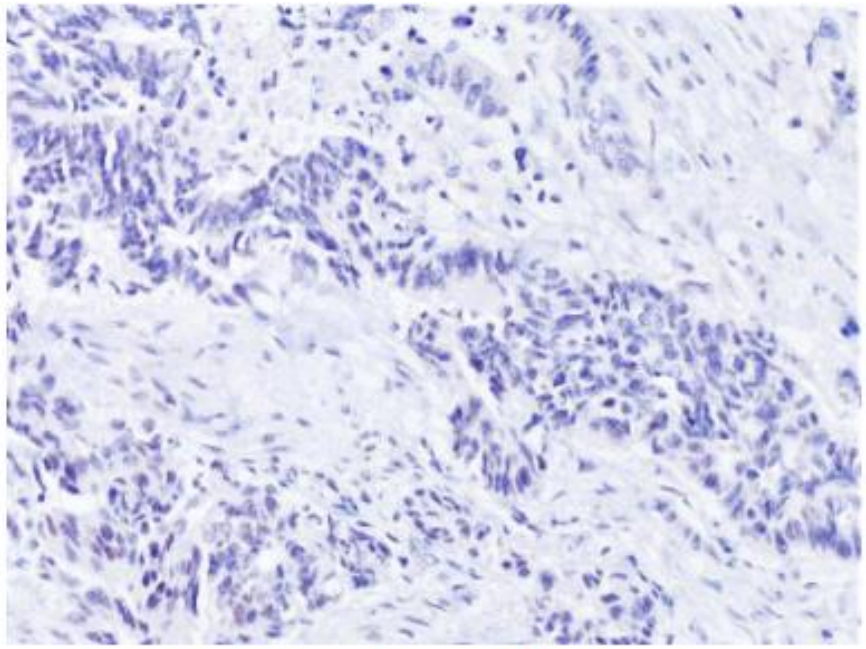


Figure 4c. In this area (PMS2 for same case of endometrial carcinoma as Figure 4a), there is complete absence of staining for PMS2 in both tumour cells and stromal cells. This area is non-interpretable for the lack of valid internal controls, likely representing a poorly fixed area with antigen degradation.

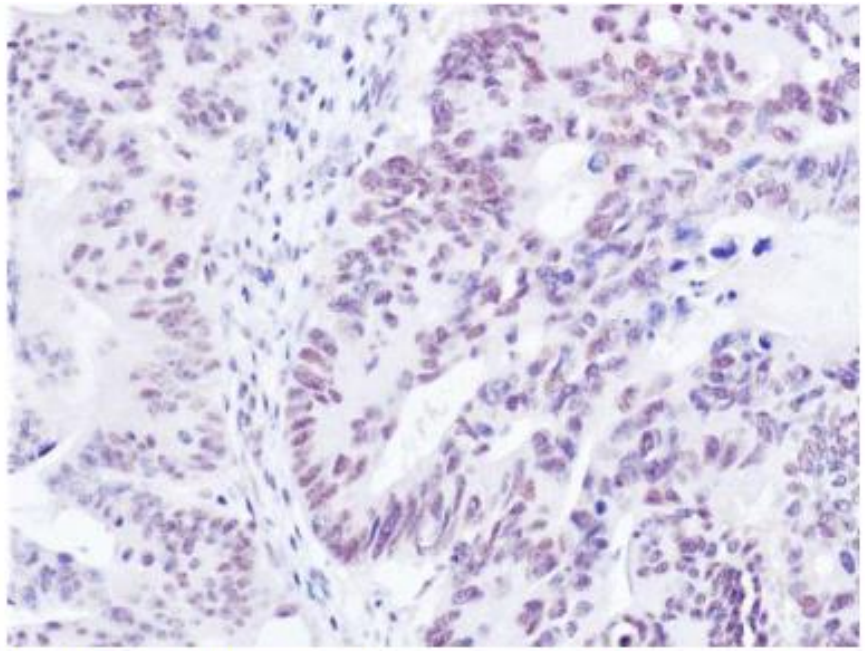
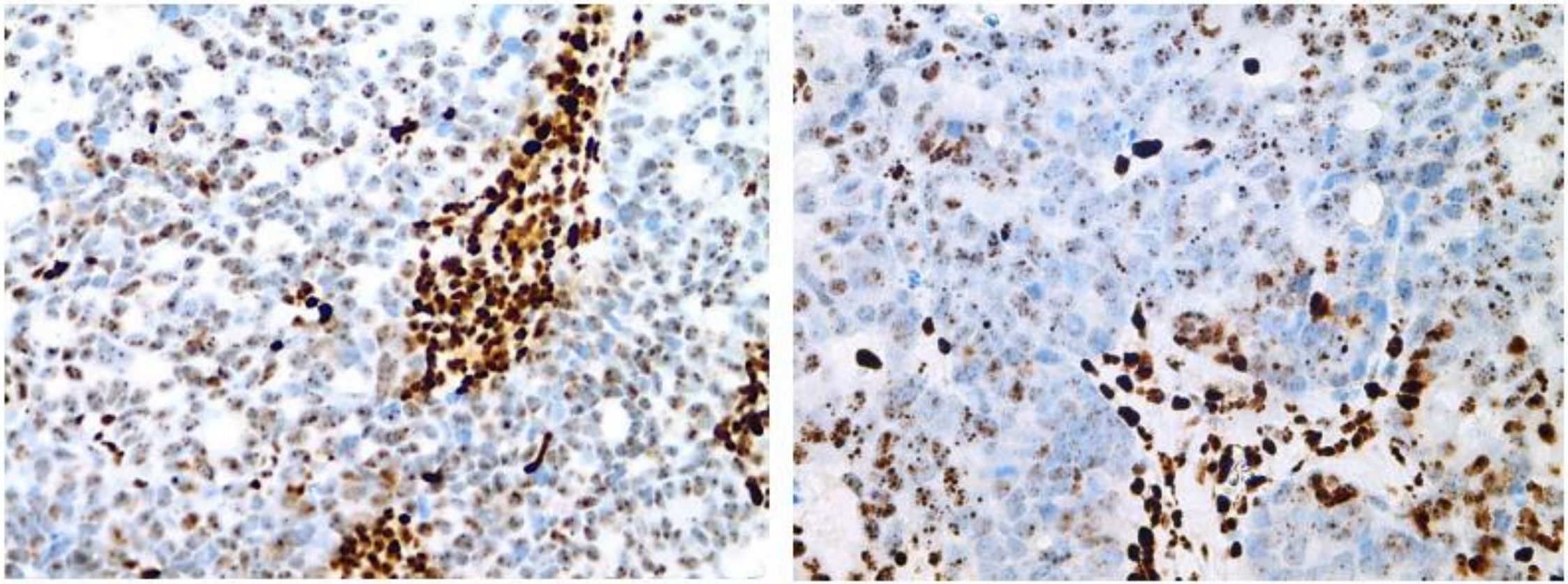
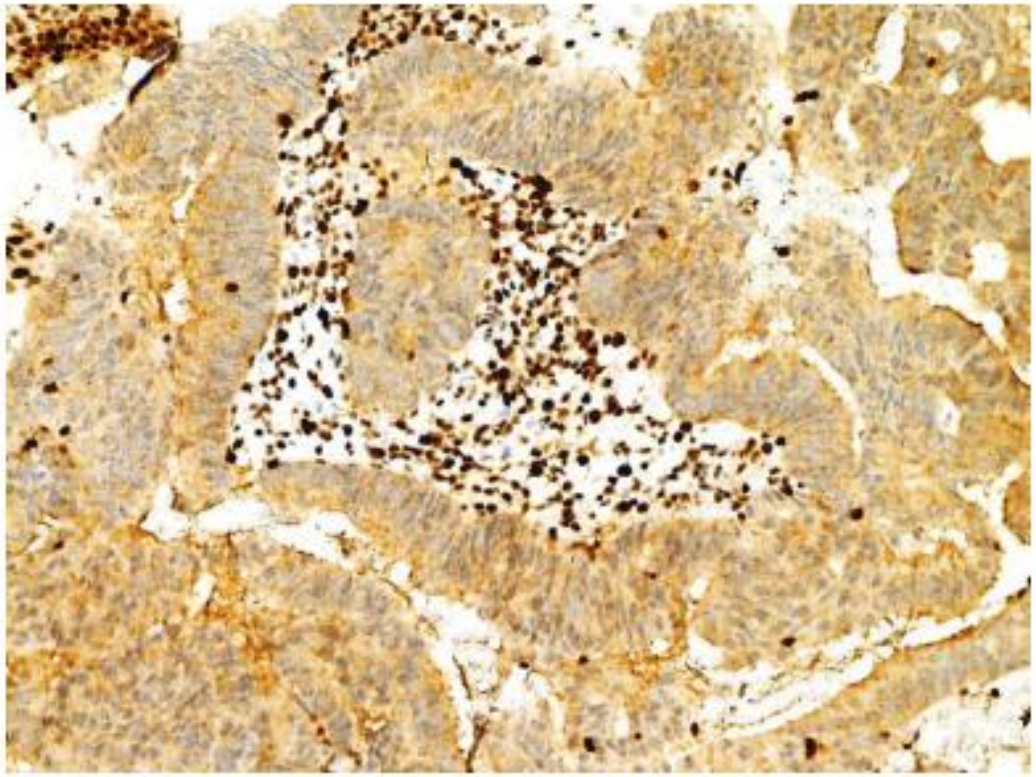
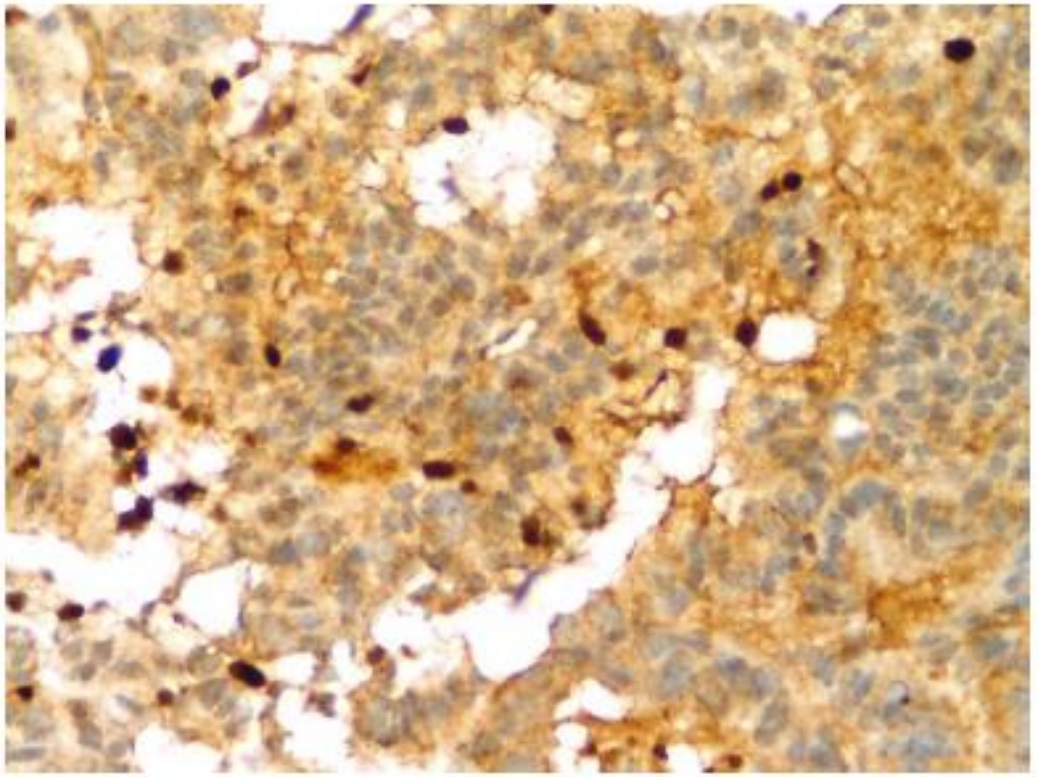


Figure 4d. In another area (PMS2 for same case of endometrial carcinoma as Figure 4a), there is weak to moderate nuclear staining in the tumour cells, together with scanty faint staining in the stromal cells as internal controls. This is interpreted as retained expression. The patchy staining in this case probably represents an artifact related to suboptimal fixation instead of genuine subclonal expression.



Punctate, granular or dot-like staining pattern is most often observed with the MLH1 M1 clone; if there is concurrent PMS2 loss, this pattern most likely represents MLH1 deficiency and should not be reported as isolated PMS2 loss.





Cytoplasmic/membranous staining does not constitute normal expression and should be reported as abnormal.

Microsatellite Instability (MSI)

- **Relationship Between MMR and MSI**
  - Defective MMR results in accumulation of replication errors within microsatellite regions.
  - Most MMR-deficient tumors demonstrate **MSI-High (MSI-H)**.
  - MMR immunohistochemistry is the preferred first-line screening method.
  - MSI-PCR is reserved for selected situations.
- **Indications for MSI-PCR**
  - Equivocal MMR IHC
  - Discordant morphology and IHC
  - Subclonal MMR loss
  - Selected cases for therapeutic decision-making

Clinical Significance of MMR Deficiency

- MMR status provides important information regarding:
  - Molecular classification
  - Lynch syndrome screening
  - Prognosis
  - Immunotherapy eligibility
- Patients with **MMR-deficient/MSI-H** tumors may benefit from:
  - PD-1 inhibitors
  - Immune checkpoint inhibitor therapy

Mismatch Repair (MMR) System

- **Reporting Recommendations**
- The pathology report should include:
  - MMR proteins evaluated
  - Expression pattern of each protein
- Overall interpretation:
  - MMR proficient (pMMR)
  - MMR deficient (dMMR)
- Identification of the deficient protein(s)
- Presence of subclonal loss (if applicable)
- Recommendation for MLH1 promoter methylation testing when MLH1/PMS2 loss is identified

Mismatch Repair (MMR) System-Take-Home Messages

- MMR deficiency is one of the four TCGA molecular subtypes.
- Universal MMR testing is recommended in endometrial carcinoma.
- MLH1/PMS2 loss should be followed by **MLH1 promoter methylation testing**.
- In endometrial carcinoma, MLH1 promoter hypermethylation is usually BRAF-independent, unlike colorectal carcinoma.
- MSH2/MSH6 and isolated PMS2/MSH6 loss suggest possible Lynch syndrome.
- Subclonal loss should be reported separately.
- MMR IHC is preferred over MSI-PCR for routine screening.
- MMR status is essential for molecular classification, Lynch syndrome screening, and immunotherapy selection.


فروتنی ،
زیباترین لباسِ دانایی است.

p53-Abnormal Endometrial Carcinoma

- The **p53-abnormal (p53abn)** subtype corresponds to the **copy-number high** molecular group identified by TCGA.
- **Key Molecular Features**
  - TP53 mutation
  - Extensive chromosomal instability (copy-number alterations)
  - Low mutational burden compared with POLE
  - Aggressive biological behavior
  - Poor clinical outcome

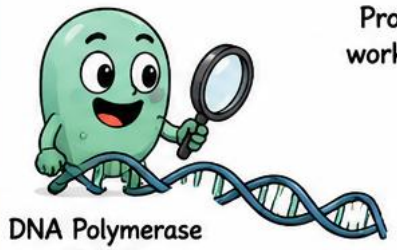
p53-Abnormal Endometrial Carcinoma

Biology of p53

- **TP53 Tumor Suppressor Gene**
- The p53 protein plays a critical role in maintaining genomic stability by regulating:
  - DNA damage response
  - Cell-cycle arrest
  - DNA repair
  - Cellular senescence
  - Apoptosis
- Loss of normal p53 function permits accumulation of genomic abnormalities and tumor progression.

POLE vs p53-abnormal (Copy-number High) Endometrial Carcinoma

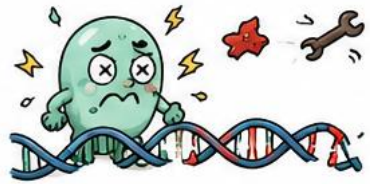
POLE-ULTRAMUTATED



DNA Polymerase
(POLE)

Proofreading
works normally

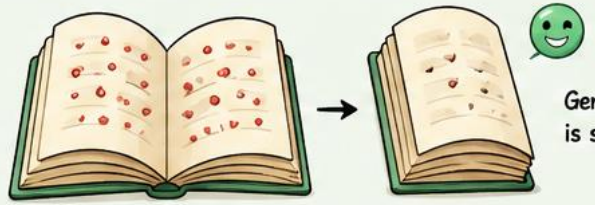
POLE mutation



Proofreading fails →
many mistakes (point mutations)

What happens?

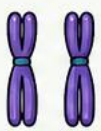
Many small
point mutations
across the genome



Genome structure
is stable

Like a book with many typos,
but pages are in the right order

Mutational burden	Chromosomal instability	Copy-number alterations
Very High	Low	Few



Key Point (POLE)

Proofreading defect → many point mutations,
but genome structure is mostly preserved.

Result: High TMB, better prognosis

VS

p53-ABNORMAL (COPY-NUMBER HIGH)

p53
(Genome Guardian)

Protects genome
(stops damaged cells)

TP53 mutation

Guardian lost →
genome becomes unstable

What happens?

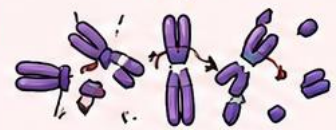
Large-scale
chromosomal
changes
(copy-number
alterations)

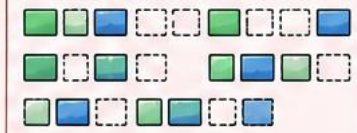
Extra copies
(gains/amplification)

Missing parts
(losses/deletions)

Like a book with pages missing,
duplicated, or out of order

Mutational burden	Chromosomal instability	Copy-number alterations
Lower than POLE	High	Extensive





Key Point (p53-abnormal)

Loss of genome guardian → chromosomal chaos
(copy-number alterations), but fewer point mutations.

Result: Lower TMB than POLE, worse prognosis

Point mutation
(small typo)

Normal copy
(2 copies)

Gain / Amplification
(extra copies)

Loss / Deletion
(missing part)

Chromosome
(normal)

Chromosome
(unstable / broken)

p53 Immunohistochemical Patterns

- **Normal (Wild-type)**
  - Variable nuclear staining
  - Heterogeneous intensity
  - Scattered positive nuclei
  - Internal control preserved
- Interpretation:
  - **Wild-type (normal) expression**

- **Abnormal (Mutation-type)**
  - Overexpression
  - Complete absence (Null pattern)
  - Cytoplasmic staining
- All abnormal staining patterns correlate with TP53 mutation.

p53 Immunohistochemical Patterns-Mutation-Type Pattern

Overexpression Pattern

- **Characteristics:**
  - Strong nuclear staining
  - Diffuse staining
  - ≥80% of tumor nuclei positive
- Mechanism:
  - Usually caused by **missense TP53 mutations**
  - Mutant protein accumulates because of prolonged half-life
- Interpretation:
  - **Abnormal (Mutation-type) expression**

- **Null Pattern**
- **Characteristics:**
  - Complete absence of nuclear staining
  - Internal positive control must be present
- Mechanism:
  - Usually associated with:
  - Nonsense mutations
  - Frameshift mutations
  - Large deletions
- Interpretation:
  - **Abnormal (Mutation-type) expression**

- **Cytoplasmic Pattern**
  - Rare
- **Characteristics:**
  - Diffuse cytoplasmic staining
  - Nuclear staining absent or markedly reduced
- Mechanism:
  - Certain TP53 mutations impair normal nuclear localization.
- Interpretation:
  - **Abnormal (Mutation-type) expression**

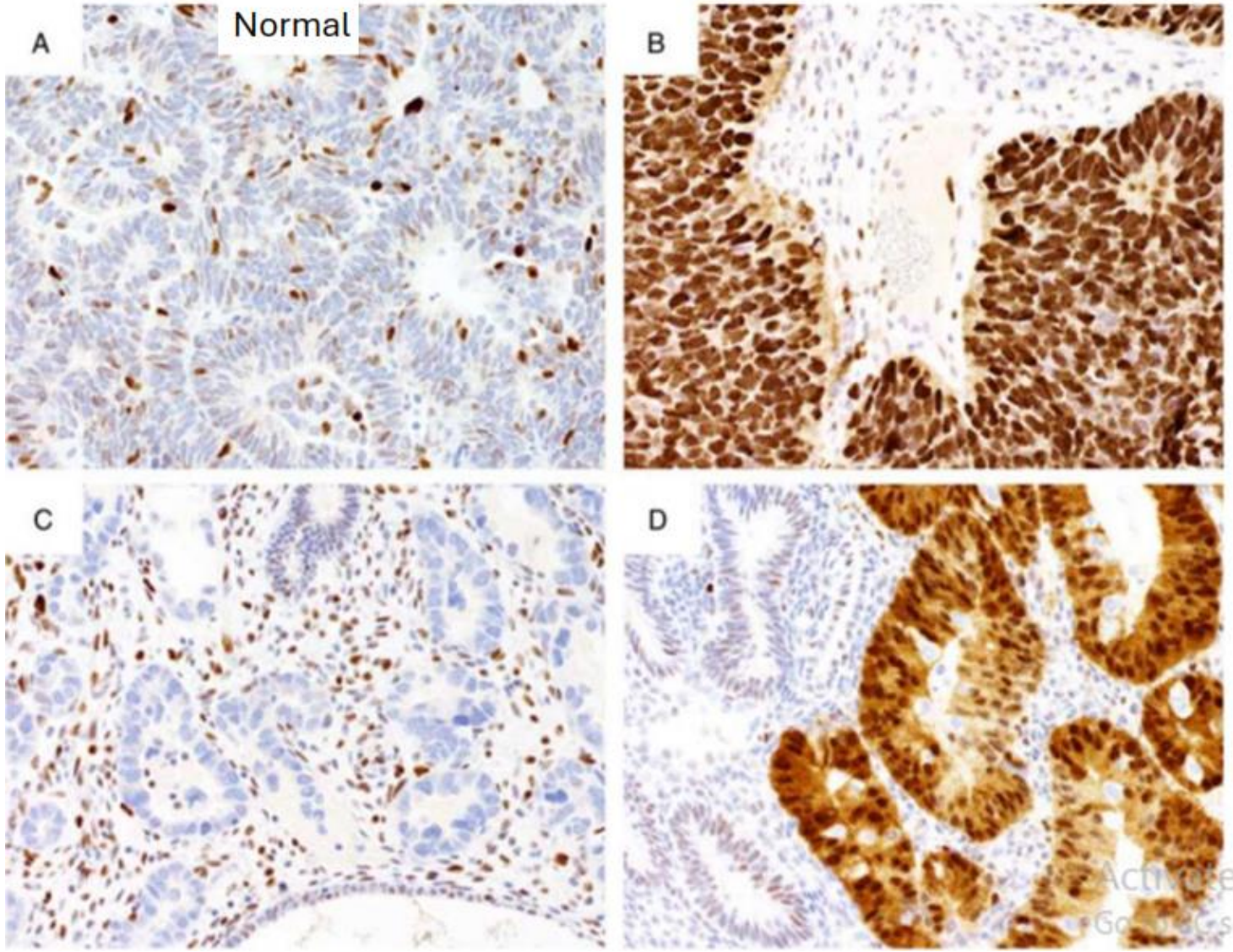
Practical Interpretation of p53

IHC Pattern	Interpretation
Variable heterogeneous nuclear staining	Wild-type
Strong diffuse nuclear staining (≥80%)	Overexpression
Complete absence of staining	Null pattern
Diffuse cytoplasmic staining	Cytoplasmic pattern

Always confirm:

- Adequate internal positive control
- Appropriate tissue fixation
- Correlation with tumor morphology

Copy-Number High (p53-abnormal)



p53-Abnormal Endometrial Carcinoma

Clinicopathologic Features

- Approximately **20–25%** of endometrial carcinomas
- Strongly associated with:
  - Serous carcinoma
  - Carcinosarcoma
  - High-grade endometrioid carcinoma
- Approximately 5% of low-grade endometrioid carcinomas harbor TP53 mutations.
- Frequently presents with:
  - Advanced stage
  - Deep myometrial invasion
  - Lymphovascular space invasion
  - Extrauterine disease

p53-Abnormal Endometrial Carcinoma

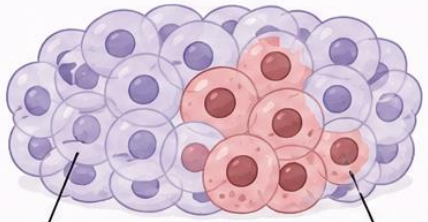
Clinical Significance

- Worst prognosis among all TCGA molecular subtypes
- Highest recurrence rate
- Lowest disease-specific survival
- Frequently requires aggressive multimodal treatment
- Molecular classification influences adjuvant therapy decisions

Subclonal p53 Abnormality

1. Definition

A tumor is made of many cells.
Some cells are different from the others.



Most tumor cells (Clonal population)

Wild-type p53 expression

Normal pattern

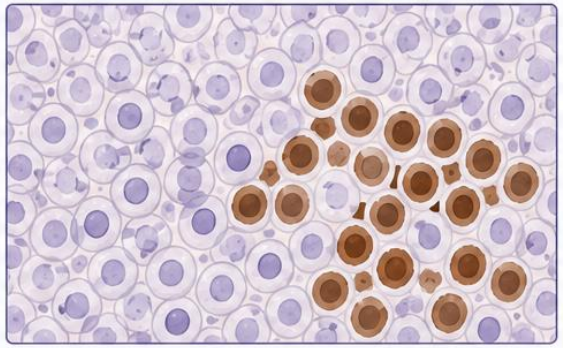
Subpopulation (Subclone)

Mutation-type p53 expression

Abnormal pattern

2. What we see in p53 IHC

In the same tumor,
two different staining patterns are present.



Wild-type pattern
(Normal p53 expression)
Usually weak or scattered staining

Mutation-type pattern
(Abnormal p53 expression)
Strong, diffuse nuclear staining

3. Clinical Significance

Reflects tumor evolution

A small group of cells has changed over time and become different.

May indicate acquisition of secondary TP53 mutations

The mutation happened later in a subpopulation of cells.

Should be specifically reported

Pathologists should mention the presence of subclonal p53 abnormality in the report.

In simple words:

Most tumor cells look normal for p53,
but a small group has an abnormal (mutant) p53 pattern.
→ This means the tumor has a "subclone" with TP53 mutation.

Think of a garden:
Most plants are healthy,
but a few have become different and grown in a new way.

- **Subclonal p53 abnormality alone should not automatically classify the tumor as the p53-abnormal (copy-number high) molecular subtype.**
- **Interpretation should be integrated with morphology, POLE, and MMR status.**

Multiple-Classifier Tumors

- **Definition**
  - Endometrial carcinomas harboring **more than one molecular feature**.
  - Represent approximately **3%–11%** of endometrial carcinomas.
- Examples:
  - POLE-mutated + p53-abnormal
  - POLE-mutated + MMR-deficient
- **Interpretation**
  - POLE mutation overrides the additional molecular abnormalities

- Examples:
  - MMR-deficient + p53-abnormal
- Exhibit **clinicopathologic features intermediate** between:
  - MMR-deficient tumors
  - p53-abnormal tumors
- **Interpretation**
  - MMRd/p53-abnormal tumors require integrated interpretation using molecular and morphologic findings.

Multiple-Classifier Tumors

Molecular Findings	Final Interpretation (CAP 2026)
POLE + p53-abnormal	**POLE-mutated**
POLE + MMRd	**POLE-mutated**
MMRd + p53-abnormal	**Integrated interpretation (intermediate features)**

Pitfalls in p53 Interpretation

- Common pitfalls include:
  - Overcalling focal strong staining as overexpression
  - Interpreting null pattern without an internal control
  - Confusing patchy staining
  - Assuming all serous carcinomas demonstrate abnormal p53
  - Assuming all p53-abnormal tumors are serous carcinomas

Reporting Recommendations

- The pathology report should specify:
- Wild-type expression

or

- Abnormal (Overexpression)

or

- Abnormal (Null pattern)

or

- Abnormal (Cytoplasmic pattern)

Or

- Subclonal abnormal expression

Ambiguous p53 Expression

- **Equivocal p53 Immunostaining**
- Occasionally, p53 immunostaining cannot be confidently classified as:
  - Wild-type
  - Overexpression
  - Null
  - Cytoplasmic
- **Possible causes**
  - Technical artifact
  - Borderline staining pattern
  - Uncommon TP53 variants
- **Recommended approach**
  - Repeat immunohistochemistry on another tissue block.
  - **p53 immunohistochemistry remains the preferred surrogate marker for TP53 status.**
  - Routine TP53 sequencing is **not recommended**.
  - Consider **TP53 sequencing** if interpretation remains uncertain.

Practical Sign-out Pearls

- **Remember**

✓ Wild-type is the **only normal** staining pattern.

✓ Overexpression requires **strong nuclear staining in ≥80% of tumor cells.**

✓ Null pattern cannot be interpreted without **internal positive control.**

✓ Cytoplasmic staining is **abnormal**.

✓ p53-abnormal **does not always indicate serous carcinoma.**

✓ High-grade endometrioid carcinoma may also demonstrate abnormal p53 expression.

✓ POLE mutation supersedes p53 abnormality in multiple-classifier tumors.

✓ Integrate p53 results with **morphology, POLE, and MMR** before assigning the molecular subtype.

زیباترین انسان‌ها،
کسانی اند که
آرامش را به دیگران
هدیه می دهند.

TCGA / ProMisE Molecular Classification
Endometrial Carcinoma

ENDOMETRIAL CARCINOMA

STEP 1
POLE sequencing
(Exonuclease domain)

POLE pathogenic mutation?

YES → **POLE-mutated**
(~7%)
BEST PROGNOSIS

NO

STEP 2
MMR IHC
(MLH1, PMS2, MSH2, MSH6)
or MSI testing

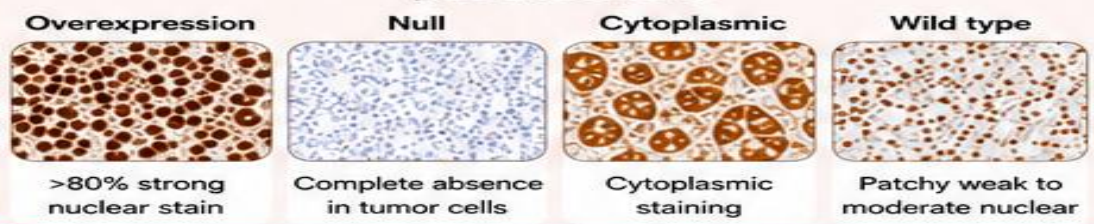
MMR-deficient?
(Loss of any MMR protein or MSI-High)

YES → **MMR-deficient**
(~30%)
INTERMEDIATE PROGNOSIS

NO

STEP 3
p53 IHC
(Interpret pattern)

p53 abnormal?



YES → **p53-abnormal**
(~20%)
WORST PROGNOSIS
(Copy-number high)

NO

No POLE mutation + MMR proficient + p53 wild type
NSMP (No Specific Molecular Profile)
(~40–50%)
DIAGNOSIS OF EXCLUSION
INTERMEDIATE PROGNOSIS

TCGA / ProMisE Molecular Classification
Endometrial Carcinoma

TCGA Group	POLE-mutated	MMR-deficient	NSMP	p53-abnormal (Copy-number high)
Key Alteration	POLE mutation	MMR deficiency (MSI-High)	None	TP53 mutation
Genomic Feature	Ultramutated	Microsatellite instability	No specific pattern	High copy-number alterations
Typical Histology	Often high-grade endometrioid	Endometrioid, mixed, clear cell, others	Often endometrioid (any grade)	Serous, high-grade endometrioid, carcinosarcoma, mixed
IHC Clues	Usually ER+	Loss of MMR protein expression	MMR intact, p53 wild type	Abnormal p53, diffuse p16, often HER2+
Prognosis	Best	Intermediate	Intermediate	Worst
Approx. Frequency	~7%	~30%	~40–50%	~20%

No Specific Molecular Profile (NSMP)

- **Definition**
  - NSMP (No Specific Molecular Profile) is defined by the absence of:
  - Pathogenic **POLE** mutation
  - **MMR deficiency**
  - **Abnormal p53** expression
- Also referred to as: **Copy-number Low (CN-Low)**
- Relatively low genomic instability
- Microsatellite stable (MSS)
- p53 wild-type expression
- Intact MMR protein expression
- POLE wild-type
- Common molecular alterations include: **CTNNB1, PIK3CA, PIK3R1** , **PTEN** , **ARID1A** , **KRAS**

Clinicopathologic Features of NSMP

- Most common molecular subgroup
- Approximately **35–45%** of endometrial carcinomas
- Predominantly **endometrioid histology**
- Usually FIGO grade 1 or 2
- Frequently ER and PR positive
- Usually presents at early stage

Clinical Significance of NSMP

- Intermediate prognosis
- Most heterogeneous molecular subgroup
- Clinical outcome is variable
- Additional biomarkers are often required for further risk stratification
- Potential prognostic biomarkers include:
  - **CTNNB1 mutation**
  - **L1CAM overexpression**
  - **ER/PR expression**

Emerging Biomarkers in NSMP

- Several biomarkers may further stratify NSMP tumors:
- **CTNNB1 exon 3 mutation**
  - Associated with increased recurrence risk
- **L1CAM overexpression**
  - Associated with aggressive behavior
- **ER positivity**
  - Better prognosis
  - Potential response to hormonal therapy
- **PR positivity**
  - Favorable prognostic indicator
- These biomarkers are not currently incorporated into the TCGA molecular classification but may refine risk assessment.

Diagnostic Pitfalls of NSMP

- NSMP is a diagnosis of exclusion.
- Before assigning NSMP, exclude:
  - POLE-ultramutated
  - MMR-deficient
  - p53-abnormal
- Remember:
- NSMP is **not** a single biological entity.
- It represents a heterogeneous group of tumors with diverse molecular alterations.
- Histologic grade alone should not be used for molecular classification.

No Specific Molecular Profile (NSMP)

- **Diagnostic Pearls:**
  - NSMP is the most common TCGA molecular subtype.
  - Defined by the absence of POLE mutation, MMR deficiency, and p53 abnormality.
  - Usually associated with endometrioid morphology.
  - Prognosis is intermediate.
  - Represents a biologically heterogeneous group.
  - Additional biomarkers (CTNNB1, L1CAM, ER, PR) may improve risk stratification.
  - NSMP remains an active area of ongoing research.

هیچ دانشی،

جای انسانیت را پر نمی‌کند.

Section III
Predictive Biomarkers

HER2 in Gynecologic Carcinomas

- **Human Epidermal Growth Factor Receptor 2 (HER2)**
- HER2 (ERBB2) is a transmembrane receptor tyrosine kinase.
- HER2 overexpression and/or gene amplification promotes:
  - Cell proliferation
  - Tumor growth
  - Cell survival
- HER2 is an established **predictive biomarker** for anti-HER2 targeted therapy.

When Should HER2 Be Tested?

- **Endometrial Carcinoma**
  - HER2 testing is recommended in:
    - Endometrial serous carcinoma
    - Carcinosarcoma
    - All **p53-abnormal endometrial carcinomas**, regardless of histologic subtype

Clinical Significance of HER2

- HER2 status is essential for selecting patients who may benefit from:
- **Anti-HER2 Therapy**
  - Trastuzumab
  - Trastuzumab deruxtecan (T-DXd)
- HER2 testing directly influences therapeutic decision-making.

HER2 Immunohistochemistry

- **Membranous Staining Pattern**
- Unlike breast carcinoma, Endometrial carcinoma commonly demonstrates:
  - Basolateral staining
  - Lateral staining
  - Incomplete membranous staining
  - Apical sparing
- **Heterogeneous staining is frequently observed.**
- HER2 should be interpreted using the **endometrial cancer–specific scoring algorithm (NCT01367002 criteria).**

HER2 Scoring for Trastuzumab

Score	Interpretation
0 (Negative)	No membranous staining
1+ (Negative)	Faint/incomplete membranous staining in a subset of tumor cells
2+ (Equivocal)	Weak to moderate **complete or basolateral/lateral** membranous staining in **≥10%** of tumor cells.
3+(Positive)	Strong complete or basolateral/lateral staining in **>30%** of tumor cells

Reflex Testing
HER2 IHC 2+
↓
Perform **HER2 ISH/FISH**

HER2 Scoring for Trastuzumab Deruxtecan (T-DXd)

- **Scoring System**
  - Use **gastric-specific HER2 scoring criteria** (DESTINY-PanTumor02).
- **Important Difference**
  - T-DXd uses a different scoring system.
- **Positive**
  - Strong membranous staining in **≥10% of tumor cells**
- **Important**
  - ISH/FISH confirmation is **not required** for T-DXd eligibility.

Intratumoral HER2 Heterogeneity

- HER2 expression may be heterogeneous.
- Characteristics:
  - Variable staining intensity
  - Variable percentage of positive cells
  - Different staining patterns within the same tumor
  - Sampling multiple tumor areas may improve detection.

HER2: Endometrial vs Breast Cancer Scoring

Important Differences

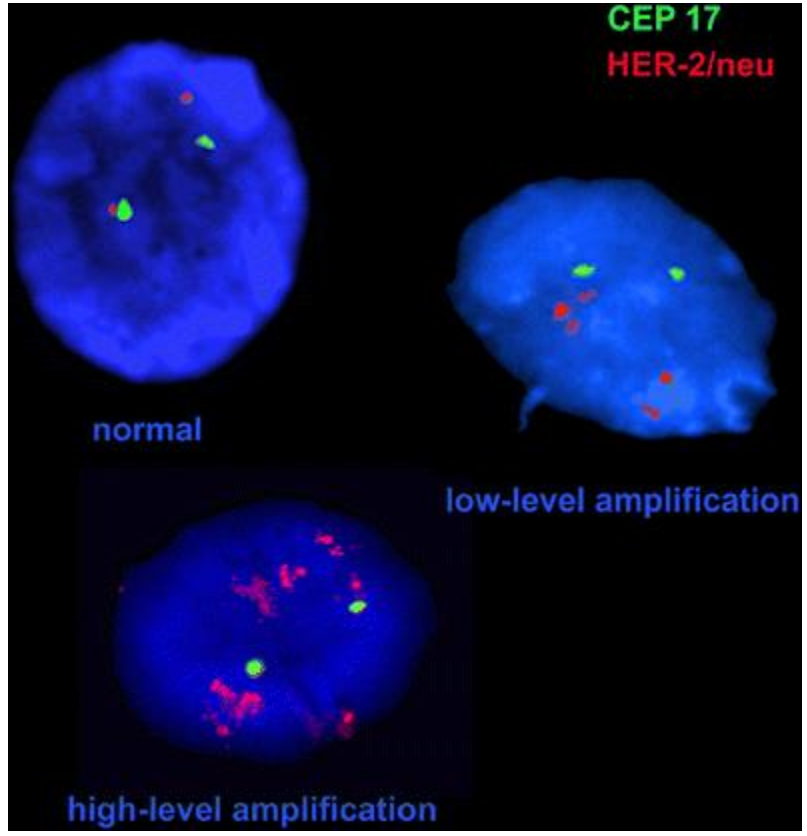
Feature	Endometrial Carcinoma	Breast Carcinoma
Membranous staining	Basolateral or lateral staining is acceptable	Complete circumferential staining required
HER2 heterogeneity	Common	Less common
Scoring criteria	Gynecologic-specific	ASCO/CAP Breast guideline
Interpretation	Do **not** use breast criteria	Breast-specific criteria

Breast HER2 criteria should NOT be applied to endometrial carcinoma.

HER2 Gene Amplification

- **HER2 ISH/FISH**
- Performed only in:
- **IHC Score 2+**
- Positive amplification:
  - HER2/CEP17 ratio ≥2.0

  OR
  - Average HER2 copy number ≥6 signals/cell



HER2 Reporting Recommendations

- The pathology report should include:
  - HER2 IHC score
  - Percentage of positive tumor cells
  - Staining pattern
  - ISH/FISH result (when performed)
  - Final HER2 interpretation

HER2 Testing Pitfalls

- **Common Pitfalls**
  - Applying breast cancer scoring criteria
  - Ignoring basolateral staining
  - Underestimating heterogeneous expression
  - Interpreting cytoplasmic staining as positive
  - Inadequate fixation
  - Small biopsy sampling error

HER2 Immunohistochemistry

- **Practical Pearls**

✓ HER2 is a **predictive biomarker**, not a diagnostic marker.

✓ Endometrial carcinoma should **not** be scored using breast criteria.

✓ Basolateral membranous staining is considered positive.

✓ HER2 heterogeneity is common.

✓ IHC 2+ requires ISH/FISH for **trastuzumab**.

✓ **T-DXd does not require ISH/FISH confirmation.**

✓ HER2 testing is particularly important in **p53-abnormal endometrial carcinomas**.

HER2 Immunohistochemistry

- **Practical Pearls**
  - HER2 testing guides anti-HER2 therapy.
  - Trastuzumab and T-DXd use different interpretation algorithms.
  - Basolateral/lateral staining is characteristic of endometrial carcinoma.
  - HER2 heterogeneity should always be considered.
  - Report IHC score, ISH result (if applicable), and final interpretation.
  - Correct interpretation directly impacts patient management.

HER2 Immunohistochemistry

- **Specimen Selection for HER2 Testing**
- HER2 testing can be performed on:
  - Endometrial biopsy
  - Curettage specimen
  - Hysterectomy specimen
  - Metastatic tumor
- **Recommended specimen**
- Select the specimen with:
  - Highest tumor cellularity
  - Best fixation
  - Best-preserved viable tumor
- **Because HER2 expression may be heterogeneous,**testing more than one specimen may be beneficial in selected cases.

HER2 Heterogeneity

- **Intratumoral Heterogeneity**
  - HER2 expression may vary within the same tumor.
  - Examples include:
    - Different staining intensity
    - Different percentages of positive cells
    - Positive and negative tumor areas within one specimen
- **Clinical Importance**
  - May influence eligibility for anti-HER2 therapy
  - May explain discordant HER2 results between biopsy and hysterectomy specimens

HER2 Practical Sign-out Pearls

- **Before Signing Out**

✓ Confirm adequate fixation.

✓ Evaluate membranous staining only.

✓ Ignore isolated cytoplasmic staining.

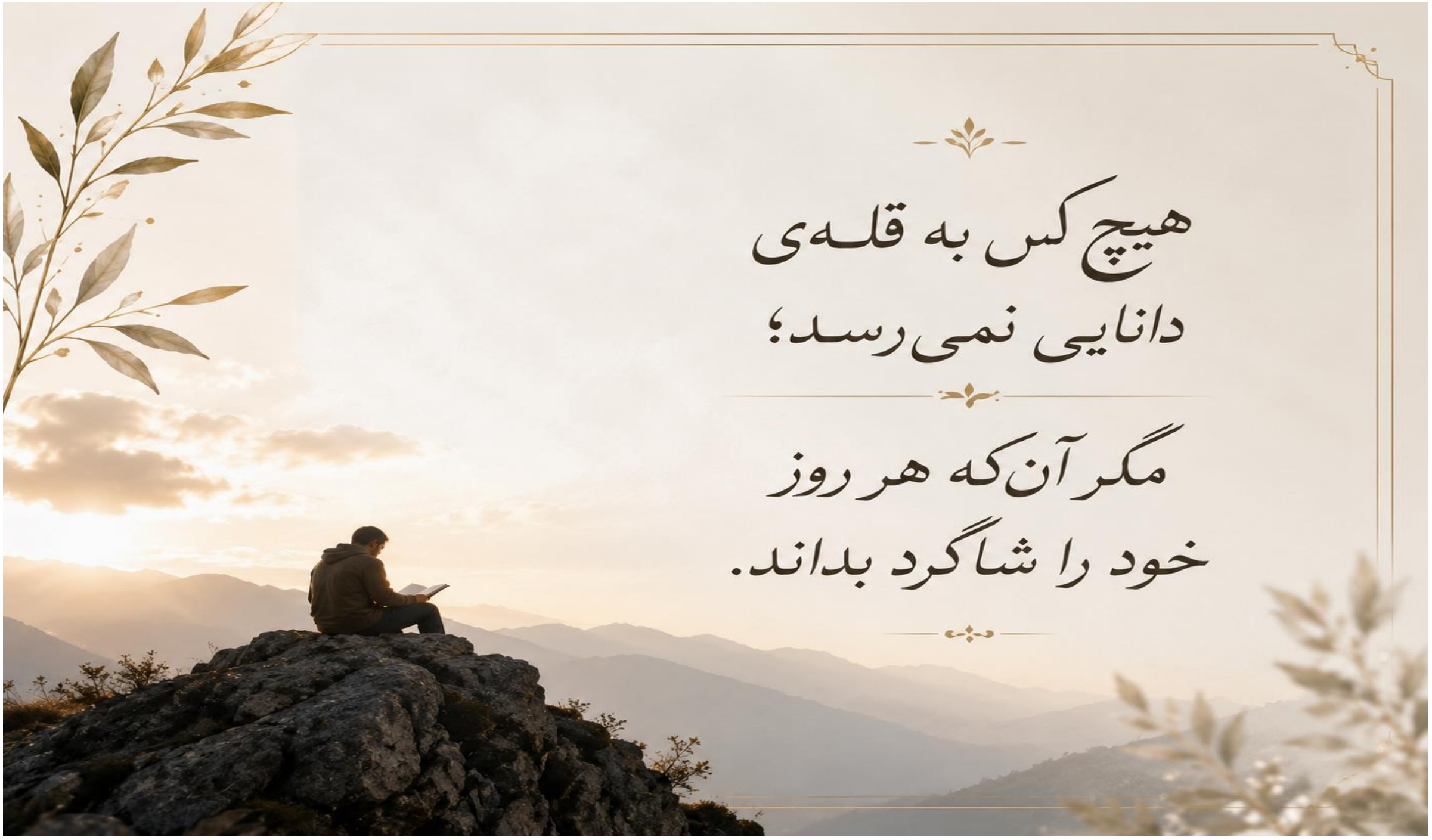
✓ Assess the entire tumor before assigning a score.

✓ Recognize basolateral/lateral staining as positive.

✓ Remember that HER2 expression may differ between primary and metastatic tumors.

✓ If IHC = 2+, perform reflex ISH/FISH for trastuzumab eligibility.

✓ For T-DXd eligibility, interpret using the gastric algorithm.


هیچ کس به قله‌ی
دانایی نمی‌رسد؛
مگر آن‌که هر روز
خود را شاگرد بداند.

Estrogen Receptor (ER) and Progesterone Receptor (PR)

- **Biology**
- ER and PR are **nuclear hormone receptors**.
- They regulate gene transcription following hormone binding.
- Hormone receptor expression is associated with:
  - Endometrioid differentiation
  - Hormone responsiveness
  - Better clinical outcome
- ER and PR are established **predictive biomarkers** for endocrine therapy in endometrial carcinoma.

When Should ER/PR Be Tested?

- **Clinical Utility**
  - Selection of patients for endocrine therapy
  - Prognostic stratification
  - Supportive information in difficult diagnostic cases

- **Indications for ER/PgR Testing**
  - Stage III–IV endometrioid endometrial carcinoma
  - Recurrent endometrioid endometrial carcinoma

Clinical Significance of ER/PR

- **ER/PR Positive Tumors are associated with:**
  - Endometrioid histology
  - Lower tumor grade
  - Better differentiation
  - Improved prognosis
  - Increased likelihood of response to hormonal therapy
- Loss of ER/PR expression is more frequently observed in:
  - High-grade tumors
  - Non-endometrioid carcinomas
  - Aggressive disease

ER/PR Immunohistochemistry

Interpretation of ER/PgR

- Evaluate:
  - **Nuclear staining only**
- Report:
  - Percentage of positive tumor cells
  - Staining intensity
- **Interpretation:**
  - **Positive:** ≥1% nuclear staining
  - **Negative:** <1% nuclear staining

Prognostic Value of ER

- In **NSMP endometrial carcinoma**:
- A **10% cutoff** showed better prognostic performance than **1%** for recurrence risk.
- Proposed three-tier model:
  - **0–10%:** High risk
  - **20–80%:** Intermediate risk
  - **90–100%:** Low risk
- **However, CAP still recommends reporting ER using the standard ≥1% cutoff until further validation.**

Prognostic Value of PgR

- PgR expression provides additional prognostic information.
- Prognostic value has been demonstrated particularly in:
  - **NSMP**
  - **p53-abnormal** endometrial carcinomas.
- *PgR is discussed as an emerging prognostic biomarker and is not part of the TCGA molecular classification.*

Pre-Analytical Factors

- **Factors Affecting ER/PR Results**
  - Cold ischemia time
  - Delay in fixation
  - Underfixation (<6 hours)
  - Overfixation (>72 hours)
  - Inadequate formalin fixation
  - Decalcification
  - Old unstained slides
  - Proper tissue handling is essential for accurate interpretation.

Internal Controls

- Internal positive controls include:
  - Benign endometrial glands
  - Endometrial stroma
  - Myometrium
- **Important**
  - Internal controls should demonstrate expected nuclear staining before interpreting a tumor as negative.

Pitfalls in ER/PR Interpretation

- **Common Pitfalls**
  - Cytoplasmic staining should **not** be scored.
  - Weak nuclear staining should still be counted if present.
  - Absence of internal control invalidates interpretation.
  - Necrotic or poorly fixed tissue may produce false-negative results.
  - Receptor expression may differ between primary and recurrent tumors.

ER/PR Immunohistochemistry

- **Reporting Recommendations**
- The pathology report should include:
  - Percentage of positive tumor nuclei
  - Staining intensity
  - ER interpretation (Positive/Negative)
  - PR interpretation (Positive/Negative)
- **Example**
  - ER: Positive (85%, strong nuclear staining)
  - PR: Positive (70%, moderate nuclear staining)

ER/PR Immunohistochemistry

- **Practical Sign-out Pearls**

✓ Evaluate **nuclear staining only**.

✓ Positive threshold is **≥1%**.

✓ Always confirm adequate internal controls.

✓ ER/PR testing is primarily indicated to guide **hormonal therapy**.

✓ Loss of hormone receptor expression is associated with more aggressive tumor biology.

✓ If recurrent disease is biopsied, repeat ER/PR testing may be clinically useful because receptor status can change.

دانایی،

آن‌گاه گوهر می‌شود

که در کردار بدرخشد،

نه در گفتار.

Programmed Death-Ligand 1 (PD-L1)

- **Biology**
- PD-L1 (CD274) is an immune checkpoint protein expressed on:
  - Tumor cells
  - Immune cells
- PD-L1 binds to **PD-1** receptors on activated T lymphocytes.
- This interaction suppresses T-cell activation and allows tumor immune evasion.
- Blockade of the PD-1/PD-L1 pathway restores anti-tumor immune responses.

When Should PD-L1 Be Tested?

- **Cervical Carcinoma**
  - PD-L1 testing is recommended in:
    - Recurrent cervical carcinoma
    - Persistent cervical carcinoma
    - Metastatic cervical carcinoma
- **Vulvar Squamous Cell Carcinoma**
  - May be considered in:
    - Advanced disease
    - Recurrent disease
    - Metastatic disease
- **Endometrial Carcinoma**
  - PD-L1 is **not routinely recommended** because treatment selection is primarily based on **MMR/MSI status**.

Programmed Death-Ligand 1 (PD-L1)

- **Clinical Significance**
  - PD-L1 is a **predictive biomarker** used to identify patients who may benefit from:
  - Pembrolizumab
  - Other immune checkpoint inhibitors
- **Primary clinical application**
  - Advanced cervical carcinoma

Programmed Death-Ligand 1 (PD-L1)

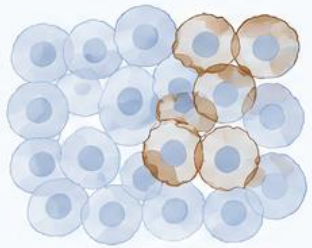
- **PD-L1 Assay**
- **FDA-Approved Companion Diagnostic**
- **22C3 pharmDx**
- **Scoring Method**
  - **Combined Positive Score (CPS)**
  - CPS is the recommended scoring system for cervical carcinoma.

How to Calculate PD-L1 Combined Positive Score (CPS)

$$CPS = \frac{\text{PD-L1 positive tumor cells} + \text{PD-L1 positive lymphocytes} + \text{PD-L1 positive macrophages}}{\text{Total viable tumor cells}} \times 100$$

If the result is > 100, report CPS as 100.

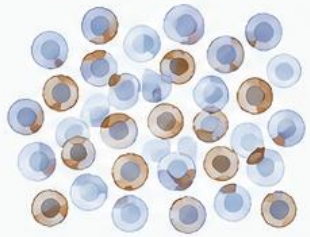
1. PD-L1 positive tumor cells



Count tumor cells with membranous PD-L1 staining (any intensity)

+

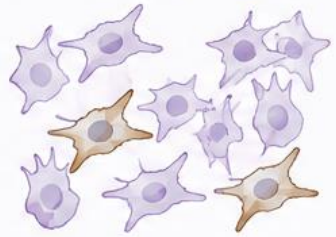
2. PD-L1 positive lymphocytes



Count PD-L1 positive lymphocytes in the tumor (and adjacent) area

+

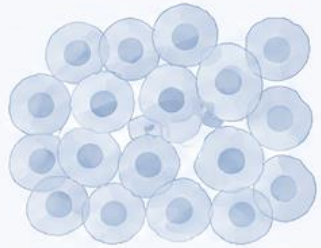
3. PD-L1 positive macrophages



Count PD-L1 positive macrophages in the tumor (and adjacent) area

÷

4. Total viable tumor cells



Count all viable tumor cells (PD-L1 positive and negative)

Example

On the whole slide:

PD-L1 positive tumor cells		PD-L1 positive lymphocytes		PD-L1 positive macrophages		Total viable tumor cells		
	+		+		÷		=	$CPS = \frac{20+15+5}{100} \times 100$
20		15		5		100		= 40 (CPS = 40)

Interpretation (Cervical Carcinoma)

Higher CPS indicates higher PD-L1 expression.

Therapeutic decision (e.g., pembrolizumab) is based on CPS threshold:

- CPS ≥ 1 → Eligible
- CPS ≥ 10 → Stronger likelihood of benefit
- CPS < 1 → Not eligible

Key Points

- Use the FDA-approved assay: 22C3 pharmDx.
- CPS includes PD-L1 positive tumor cells, lymphocytes, and macrophages.
- Report CPS as an integer from 0 to 100.

PD-L1 expression in cervical carcinoma is reported using the Combined Positive Score (CPS), which is expressed as a numeric score (0–100), not as a percentage.

Programmed Death-Ligand 1 (PD-L1)

- **Cells Included in CPS**
- **Count**
  - ✓ Tumor cells
  - ✓ Lymphocytes
  - ✓ Macrophages

- **Do NOT Count**
  - ✗ Plasma cells
  - ✗ Neutrophils
  - ✗ Stromal fibroblasts
  - ✗ Necrotic cells

Requirement
Minimum:
100 viable tumor cells

Programmed Death-Ligand 1 (PD-L1)

- **Interpretation of PD-L1**
- **Positive Result**
  - **CPS ≥1**
  - Eligible for pembrolizumab in the appropriate clinical setting.
- **Negative Result**
  - **CPS <1**

Pitfalls in PD-L1 Interpretation

- **Common Pitfalls**
  - Using TPS instead of CPS
  - Counting plasma cells
  - Counting neutrophils
  - Counting necrotic cells
  - Evaluating only hotspot areas
  - Insufficient viable tumor (<100 cells)
- Evaluate the **entire slide**, not only areas with strongest staining.

Programmed Death-Ligand 1 (PD-L1)

- **Reporting Recommendations**
  - The pathology report should include:
    - Antibody clone (22C3)
    - Scoring method (CPS)
    - CPS value
    - Final interpretation
  - **Example**
    - **PD-L1 (22C3): CPS = 15**
  - Interpretation:
    - **Positive**

Programmed Death-Ligand 1 (PD-L1)

✓ Use **CPS**, not TPS.

✓ Count: Tumor cells , Lymphocytes, Macrophages

✓ Do not count plasma cells or neutrophils.

✓ At least **100 viable tumor cells** are required.

- ✓ Evaluate the **entire slide**.
- ✓ PD-L1 testing is primarily indicated for **advanced cervical carcinoma**.
- ✓ In endometrial carcinoma, **MMR/MSI status is generally more important than PD-L1** for immunotherapy selection.


زیباترین میراثِ
هر مَعلم،
نه سخنانی است که گفته؛
بلکه اندیشه‌هایی است که
در ذهن شاگردانش
جوانه زده است.
اندیشه
دانش
عمل

Folate Receptor Alpha (FOLR1)

- **Clinical Significance**
- **FOLR1** is a cell surface glycoprotein involved in folate transport.
- Overexpressed in many **high-grade serous ovarian carcinomas**.
- Functions as a **predictive biomarker** for targeted therapy.
- Used to identify patients eligible for:
  - **Mirvetuximab soravtansine (ADC)**
- Current FDA-approved indication:
  - **Platinum-resistant ovarian, fallopian tube, and primary peritoneal carcinoma**

FOLR1 Testing

- **Method**
  - Immunohistochemistry (IHC)
- **Positive Expression**
  - Moderate to strong **membranous staining**
  - In **≥75%** of viable tumor cells
- **Practical Points**
  - Evaluate **membranous staining only**
  - Cytoplasmic staining is not scored.
  - Currently **not routinely performed** in many pathology laboratories.
  - Testing should be requested **only when anti-FOLR1 therapy is being considered**.

Predictive Biomarkers in Gynecologic Carcinomas

Biomarker	Primary Indication	Clinical Utility
POLE	Endometrial carcinoma	Molecular classification
MMR/MSI	Endometrial carcinoma	Lynch screening, Immunotherapy
p53	Endometrial carcinoma	Molecular classification
HER2	Serous EC, Carcinosarcoma, p53-abn EC	Trastuzumab / T-DXd
ER / PR	Advanced/Recurrent Endometrioid EC	Hormonal therapy
PD-L1	Recurrent/Metastatic Cervical SCC	Pembrolizumab
FOLR1	Platinum-resistant ovarian carcinoma	Mirvetuximab

Practical Reporting Pearls

- **Before Signing Out**
- ✓ Integrate morphology with molecular findings.
- ✓ Always evaluate internal controls before interpreting IHC.
- ✓ Follow the recommended molecular testing sequence:
- **POLE → MMR → p53**
- ✓ Report subclonal MMR and p53 abnormalities separately.
- ✓ Apply tumor-specific HER2 scoring criteria.
- ✓ Use predictive biomarkers only when clinically indicated.
- ✓ Communicate clinically actionable biomarkers clearly in the final report.

Take-Home Messages

- Molecular classification has transformed the diagnosis and management of endometrial carcinoma.
- **POLE, MMR, p53, and NSMP** represent biologically distinct molecular subtypes.
- Molecular classification complements, rather than replaces, histopathologic evaluation.
- Biomarker testing should be performed according to current CAP/WHO recommendations.
- Accurate interpretation of predictive biomarkers directly influences targeted therapy and patient outcomes.
- Close collaboration between pathologists, molecular laboratories, and clinicians is essential for precision oncology.

باشد که هر آنچه آموختیم،
چراغی برای دیدنِ دقیق‌تر،
اندیشیدنِ عمیق‌تر،
و خدمت کردنِ بهتر باشد.

زیرا ارزشِ دانش،
نه در آن چیزی است که می‌دانیم؛
بلکه در نوری است که با آن،
راهِ زندگی دیگران را روشن می‌کنیم.

سپاس از همراهی شما.