# Immunotherapy Biomarkers in the Gynecologic Tract:

## MMR, PD-L1, and TMB


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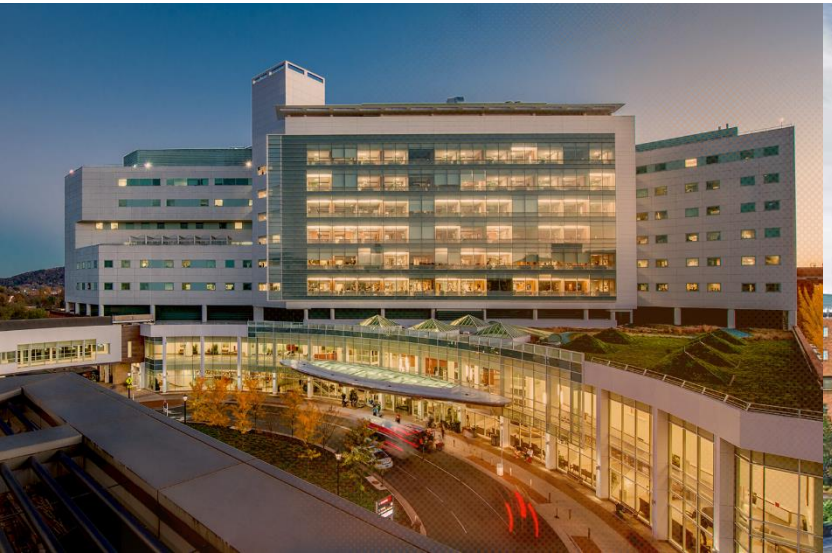
Practical Management Pearls Gynecologic CPG Webinar, September 7th 2023
Society for Immunotherapy in Gynecologic Cancer



# Outline

- We will review FDA-approved biomarkers for immunotherapy in the Gynecologic Tract, with attention to recently published STIC guidance for their utilization.

  - Mismatch Repair (MMR) Immunohistochemistry (IHC)
  - Microsatellite Instability (MSI)
  - PD-L1 Immunohistochemistry (IHC)
  - Tumor Mutation Burden (TMB)

**Table 1** Biomarkers for ICI patient selection in gynecologic cancer (adapted from Mills *et al*, *Modern Pathology*)[4]

| Biomarker | Supporting trial(s) | Initial FDA approval year | FDA-approved agent(s) | Tumor type | Treatment setting | Definition of positivity |
|---|---|---|---|---|---|---|
| dMMR/MSI-H | KEYNOTE-016<br>KEYNOTE-164<br>KEYNOTE-012<br>KEYNOTE-028<br>KEYNOTE-158 | 2017 | Pembrolizumab | Any solid tumor | Unresectable or metastatic tumors with disease progression following prior treatment and no satisfactory alternative treatment options | **dMMR**: Total loss of MMR protein expression in tumor nuclei by IHC*<br>**MSI-H**: Instability at multiple sites by NGS or PCR† |
| | KEYNOTE-158 | 2022 | Pembrolizumab | Endometrial cancer | Advanced tumors with disease progression following prior systemic therapy in any setting that are not candidates for curative surgery or radiation | |
| dMMR | GARNET | 2021 | Dostarlimab | Endometrial cancer | Advanced or recurrent tumors with disease progression on or after prior treatment with a platinum-containing regimen | Total loss of MMR protein expression in tumor nuclei by IHC* |
| | | 2021 | | Any solid tumor | Advanced or recurrent tumors with disease progression on or after prior treatment and no satisfactory alternative treatment options | |
| PD-L1 | KEYNOTE-158 | 2018 | Pembrolizumab | Cervical cancer | Recurrent or metastatic tumors with disease progression on or after chemotherapy | CPS≥1‡ |
| | KEYNOTE-826 | 2021 | Pembrolizumab+platinum-based chemotherapy±bevacizumab | Cervical cancer | Persistent, recurrent, or metastatic tumors | |
| TMB-H | KEYNOTE-158 | 2020 | Pembrolizumab | Any solid tumor | Unresectable or metastatic tumors with disease progression following prior treatment and no satisfactory alternative treatment options | ≥10 mut/Mb by NGS§ |

*In 2021, the FDA approved the VENTANA MMR IHC assay as a companion diagnostic for determining MMR status for treatment with dostarlimab. Companion diagnostic status for assessing MMR status across solid tumors for treatment with pembrolizumab was granted in 2022.
†In 2022 the FoundationOne CDx assay was approved as a companion diagnostic for assaying MSI-H tumors for use with pembrolizumab.
‡In 2018 the FDA granted companion diagnostic status to the PD-L1 IHC 22C3 pharmDx assay for determining PD-L1-positive cervical tumors for use with pembrolizumab.
§In 2020, the FDA approved the FoundationOne CDx assay as a companion diagnostic for determining TMB-H tumors for use with pembrolizumab.
CPS, combined positive score; dMMR, mismatch repair deficiency; FDA, US Food and Drug Administration; ICI, immune checkpoint inhibitor; IHC, immunohistochemistry; MMR, mismatch repair; MSI-H, high microsatellite instability; Mut/Mb, mutations/Megabase; NGS, next-generation sequencing; PD-L1, programmed death-ligand 1; TMB-H, high tumor mutational burden.

# MMRd as an immunotherapeutic biomarker

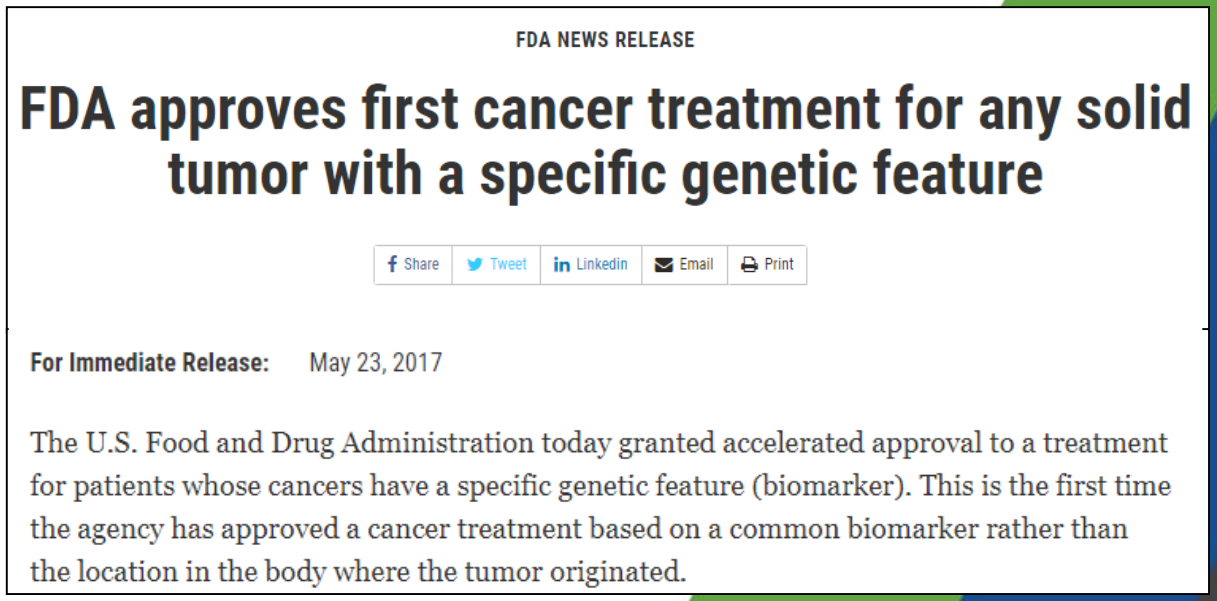
- In their groundbreaking 2017 *Science* article, Le et al. showed that 53% of MMR-deficient/MSI-high tumors respond to pembrolizumab, including 21% with complete response.

- In May 2017 the FDA approved pembrolizumab in any solid tumor with this molecular signature.

CANCER BIOMARKERS

Le *et al.*, *Science* **357**, 409–413 (2017) 28 July 2017

**Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade**



FDA NEWS RELEASE

**FDA approves first cancer treatment for any solid tumor with a specific genetic feature**

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**For Immediate Release:** May 23, 2017

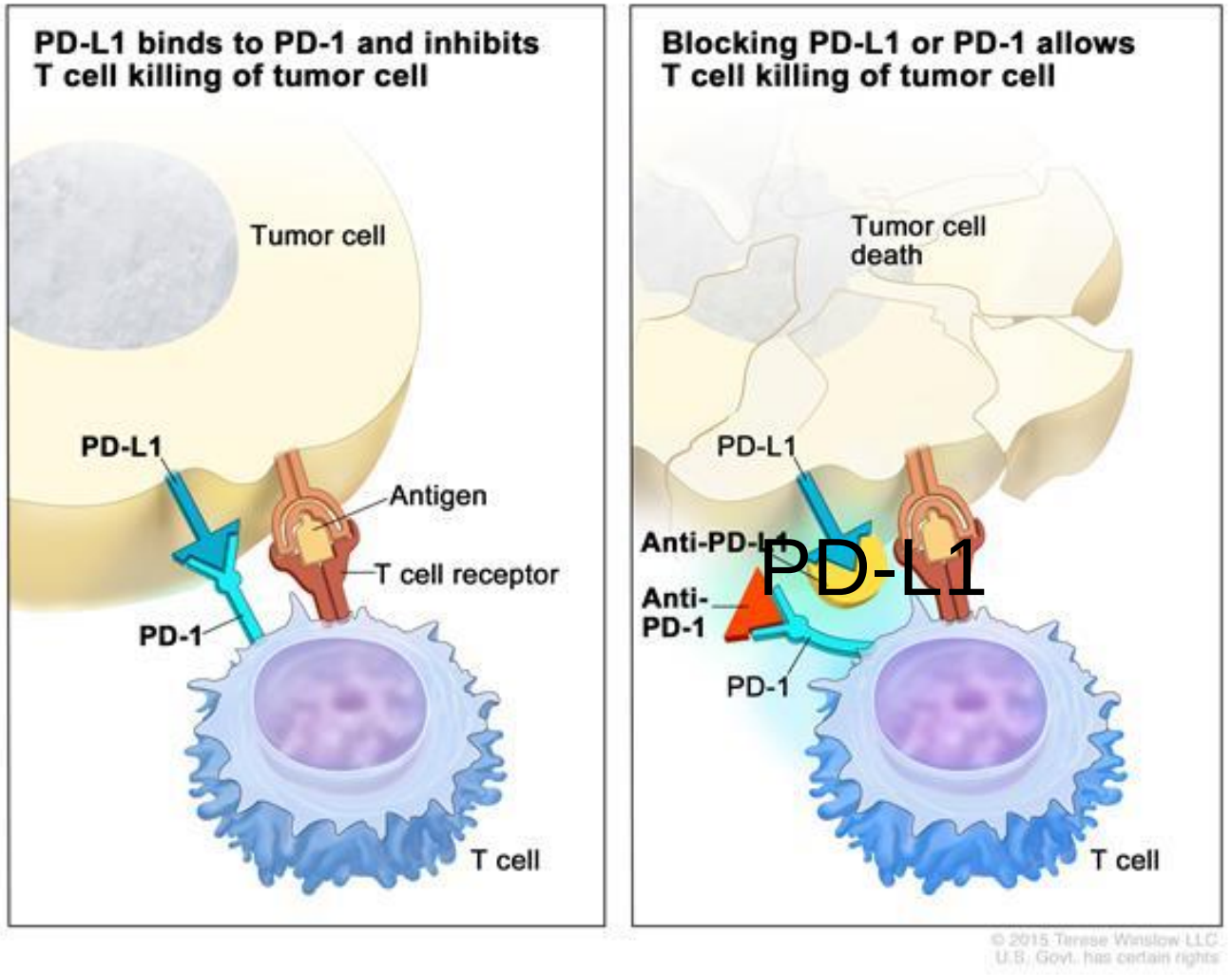
The U.S. Food and Drug Administration today granted accelerated approval to a treatment for patients whose cancers have a specific genetic feature (biomarker). This is the first time the agency has approved a cancer treatment based on a common biomarker rather than the location in the body where the tumor originated.

# MMRd & Immunotherapy

- Immune checkpoints such as PD-1 put the brakes on the adaptive immune response to prevent perpetual activation following inflammatory stimulation.
  - The PD-1/PD-L1 interaction promotes immune tolerance.

- Checkpoint ligands such as PD-L1 can be co-opted by tumor cells as a “cloaking device” to evade immune attack.

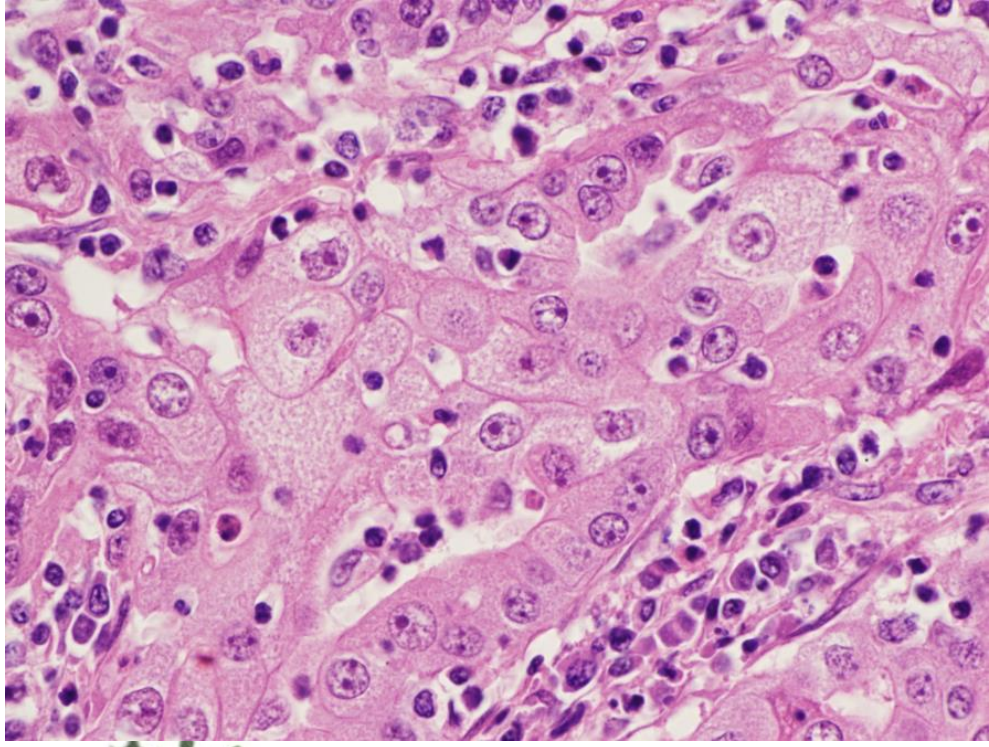
- Blocking these inhibitory checkpoints (or their ligands) “takes off the cloak” and allows cytotoxic T cells to recognize and attack tumor.




https://www.cancer.gov/publications/dictionaries/cancer-terms/def/immune-checkpoint-inhibitor

# MMRd & Immunotherapy

- For the immunotherapy to work, immune cells must be able to recognize and respond to the tumor.
- Immune recognition requires that the tumor be different from normal.
- More neoantigens (e.g. high neoantigen load) means more immune recognition.
- MMR deficiency is a mechanism of neoantigen production because the tumors mutate so rapidly.



# What's the optimal test for MMR deficiency?

NEWS · 09 APRIL 2018

nature
International journal of science

## Cutting-edge cancer drug hobbled by diagnostic test confusion

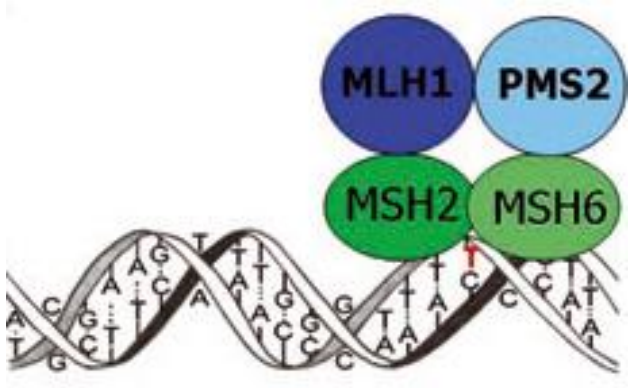
*Physicians struggle to identify which patients are likely to respond to a recently approved therapy.*

A landmark cancer drug approved last year seemed to herald a long-anticipated change in the treatment of some tumours: with medicines selected on the basis of molecular markers, rather than the tissue in which the cancer first took root.

But the three kinds of tests commonly used to look for the DNA damage that arises from that defect can produce conflicting results, says Heather Hampel, a genetic counsellor at Ohio State University in Columbus. One relies on PCR, a process that amplifies specific regions of the genome; a second looks for certain proteins; and a third relies on DNA sequencing. "Which is the best? Is any positive on any test sufficient?" Hampel says. "Does that mean you should try them all? No one wants to miss a patient who might benefit from pembrolizumab."

# MMR Immunohistochemistry

- **MSH2**, **MSH6**, **MLH1**, and **PMS2** protein expression evaluated in tumor cell nuclei.
- Background lymphocytes & stroma serve as positive internal controls.
- Report as “Intact” vs. “Deficient.”




- Pattern is informative:
  - Loss of **MSH2**/**MSH6**:

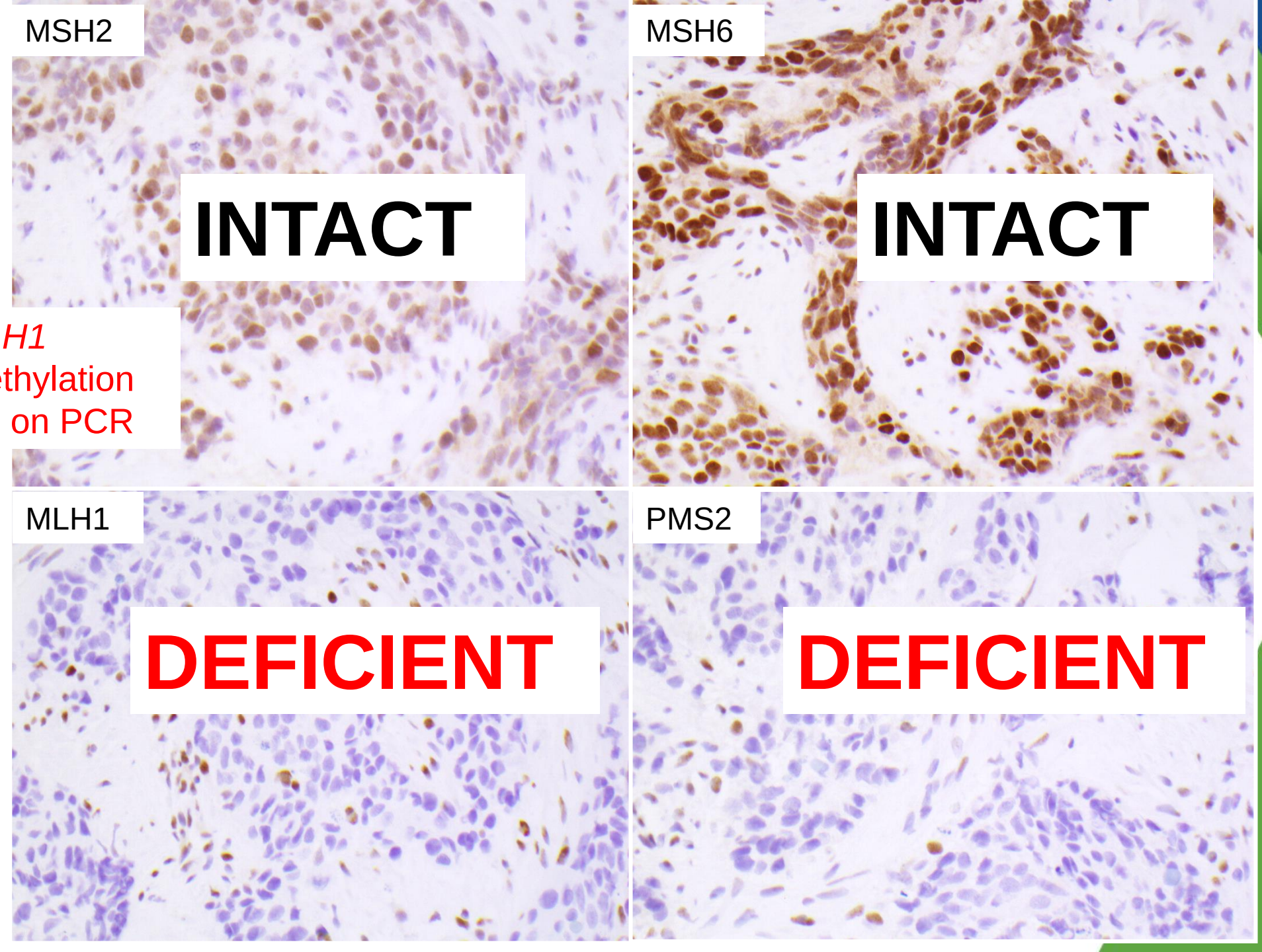
    Defect in ***MSH2*** ***rarely *EPCAM, MSH6***
  - Loss of **MSH6** alone:

    Defect in ***MSH6***
  - Loss of **MLH1**/**PMS2**:

    Defect in **MLH1**
  - Loss of **PMS2** alone:

    Defect in ***PMS2*** **gene*** ***rarely MLH1**

*Accounts for the majority of cases; most often due to sporadic methylation*




*MLH1* hypermethylation detected on PCR

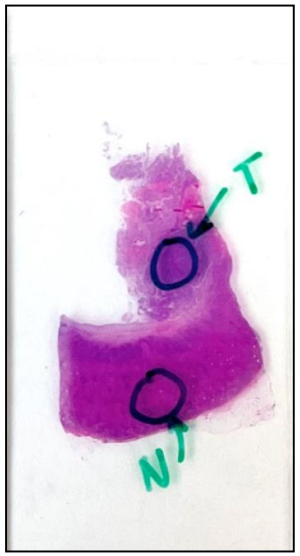
# PCR-based Microsatellite Instability (MSI) Testing

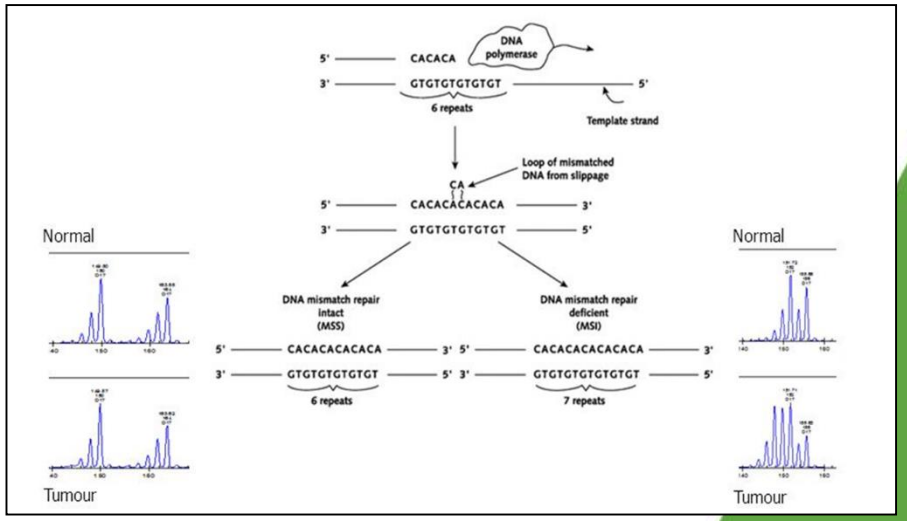
- Microsatellites are repetitive sequences which are vulnerable to replicative error without a functioning MMR system.

- MSI testing compares normal to tumor & assesses replicative errors in these sequences.

- PCR-based MSI tests assess at least 5 microsatellite markers (BAT25, BAT26, D2S123, D5S346, & D17S250)
  - If ≥2 of the 5 loci are unstable= MSI-High
  - If only 1 unstable= MSI-low.
  - These assays were initially developed for colorectal carcinoma, and are optimized for that tumor type.

- High-level MSI (MSI-H) serves as a proxy for germline *or* sporadic impairments to MMR.

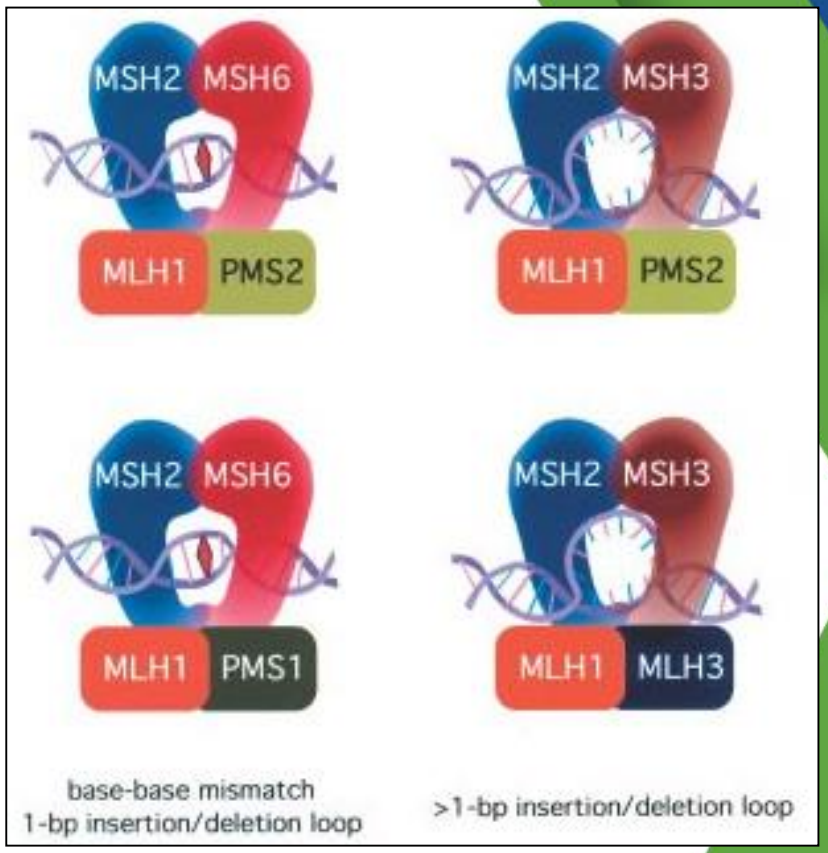


# MSI testing in the endometrium: *Limitations*

- Although MSI testing has proven a robust test for MMR defects in colorectal cancer, it has lower sensitivity in endometrial carcinoma.
  - Sensitivity for different assays ranges from 58-75%

- Why? When compared to colorectal cancers, endometrial cancers show a higher proportion of *MSH6* mutations and minimal microsatellite shifts.
  - MSH2-MSH6 heterodimers repair single base-pair mismatches and dinucleotide insertion-deletion loops.
  - MSH2-MSH3 heterodimers are specialized for larger insertion-deletion loops.




Bellacosa et al. Cell Death & Differentiation 2001


- Dedeurwaerdere F et al. Comparison of microsatellite instability detection by immunohistochemistry and molecular techniques in colorectal and endometrial cancer. *Scientific reports* 18;11(1) 2021
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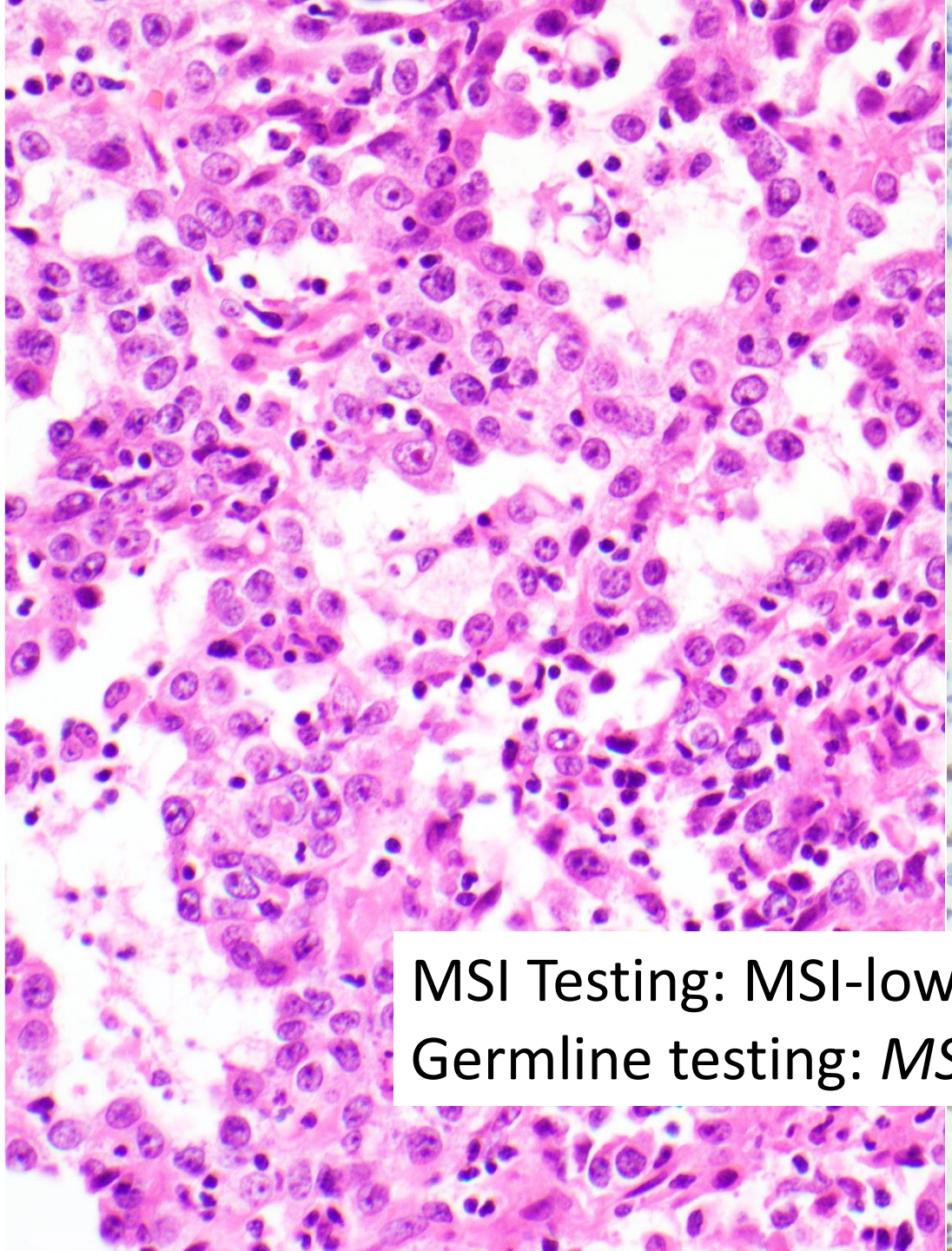
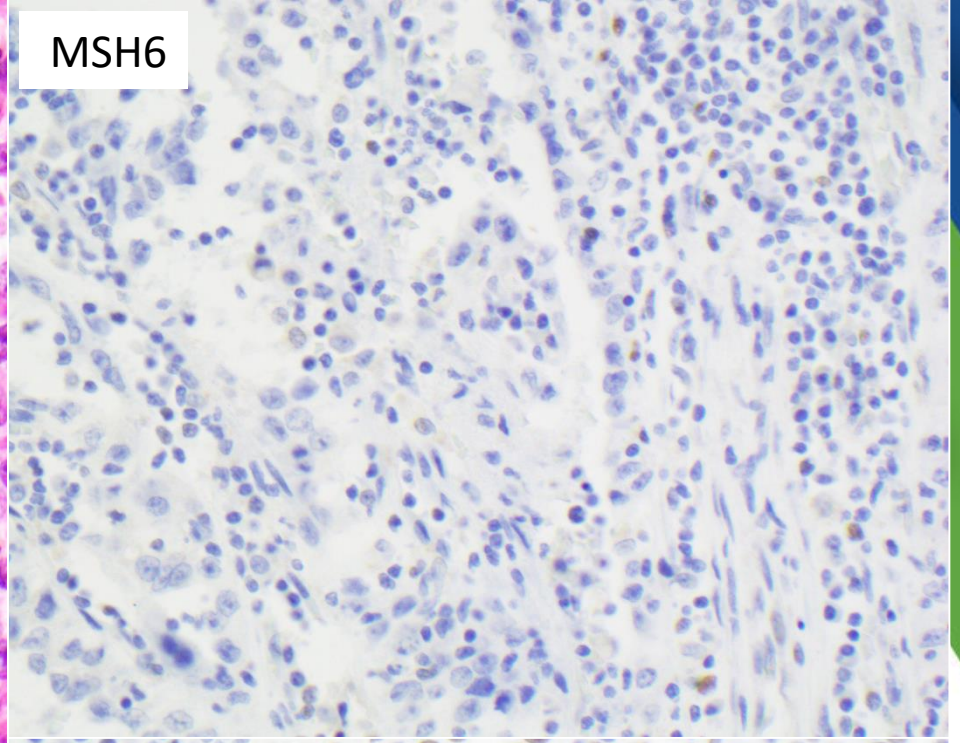
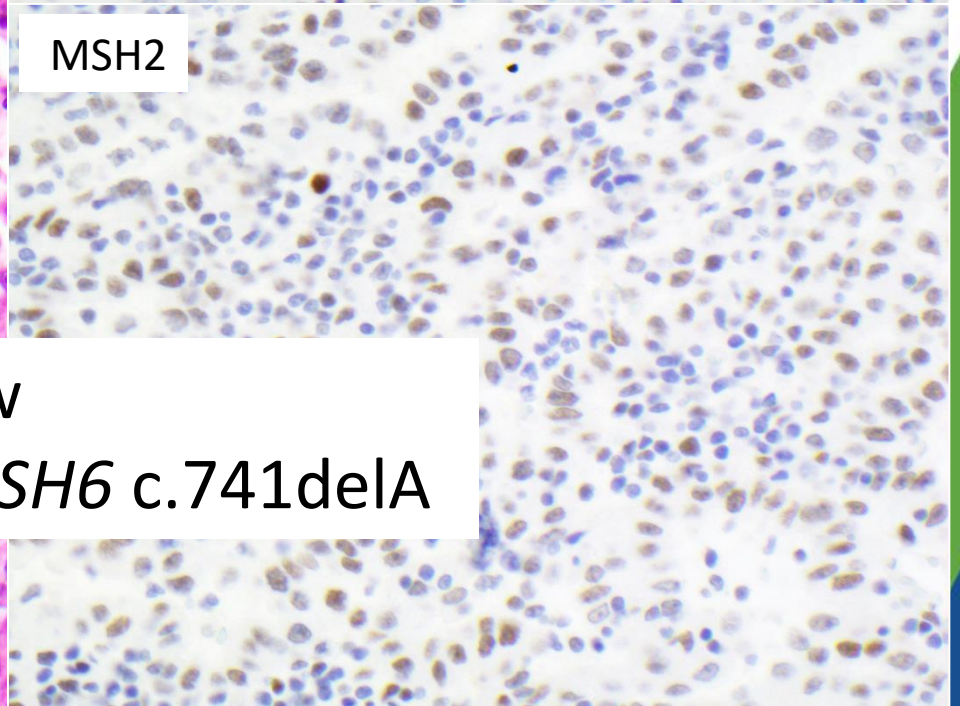





MSI Testing: MSI-low

Germline testing: *MSH6* c.741delA

# *What about broad NGS Panels?*

- Some institutions have access to large somatic tumor testing panels testing 100s of genes, which can include MMR genes.

- Benefits:
  - Can simultaneously provide information about other genes of interest.
  - Like MMR IHC and MSI, can detect germline and somatic pathogenic variants
  - Easy in settings where such testing is routine.

- Drawbacks:
  - High cost, variably reimbursed and covered by insurance.
  - Failure rate can be high: some studies reporting 10-20% failure rates.
    - Al-Kateb et al. *Mol Oncol* 2014; Goswami et al. *Am J Clin Pathol* 2016
  - Limited data on sensitivity/specificity
    - Several studies showed that somatic results remained discordant with IHC in ~20% of cases
      - Carethers et al. *Gastroenterology* 2014; Haraldsdottir et al. *Gastroenterology* 2013; Elize et al. *Gastroenterology* 2021

## Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy

### Guideline From the College of American Pathologists in Collaboration With the Association for Molecular Pathology and Fight Colorectal Cancer

*Angela N. Bartley, MD; Anne M. Mills, MD; Eric Konnick, MD, MS; Michael Overman, MD; Christina B. Ventura, MPH, MT(ASCP); Lesley Souter, PhD; Carol Colasacco, MLIS, SCT(ASCP); Zsofia K. Stadler, MD; Sarah Kerr, MD; Brooke E. Howitt, MD; Heather Hampel, MS, LGC; Sarah F. Adams, MD; Wenora Johnson, BS; Cristina Magi-Galluzzi, MD, PhD; Antonia R. Sepulveda, MD, PhD; Russell R. Broaddus, MD, PhD*

- Guideline Statement:
  - "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 mismatch repair defects."

- Good Practice Recommendations:
  - **Discordant results**: Interpret any evidence of MMRd by IHC *or* MSI by NGS or PCR as a positive result *after excluding interpretive error.
  - **Indeterminate result:** perform an alternative technique or repeat on a different tumor block.
  - **Clonal loss** by MMR-IHC: perform MSI by PCR specifically in a dissected area of tumor that has IHC loss.

ASCO special articles

## Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy: ASCO Endorsement of College of American Pathologists Guideline

**Praveen Vikas, MD[1]; Hans Messersmith, MPH[2]; Carolyn Compton, MD, PhD[3]; Lynette Sholl, MD[4]; Russell R. Broaddus, MD[5]; Anjee Davis, MPPA[6]; Maria Estevez-Diz, MD, PhD[7]; Rohan Garje, MD[8]; Panagiotis A. Konstantinopoulos, MD[9]; Aliza Leiser, MD[10]; Anne M. Mills, MD[11]; Barbara Norquist, MD[12]; Michael J. Overman, MD[13]; Davendra Sohal, MD[14]; Richard C. Turkington, MD, PhD[15]; and Tyler Johnson, MD[16]**

abstract

**PURPOSE** The College of American Pathologists (CAP) has developed a guideline on testing for mismatch repair (MMR) and microsatellite instability (MSI) for patients considered for immune checkpoint inhibitor therapy. ASCO has a policy and set of procedures for endorsing clinical practice guidelines that have been developed by other professional organizations.

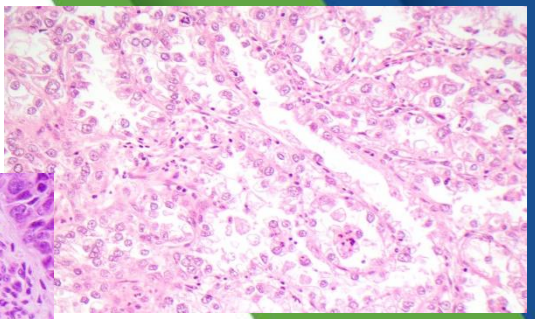
**METHODS** The CAP guideline was reviewed for developmental rigor by methodologists. An ASCO Endorsement Panel subsequently reviewed the content and the recommendations.

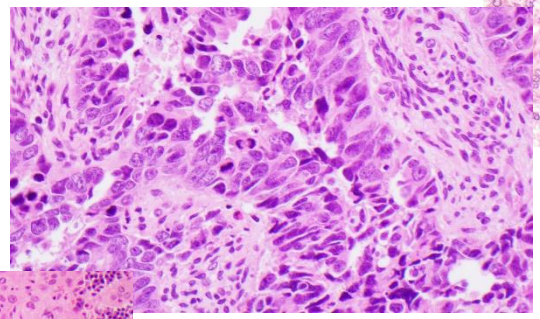
**RESULTS** The ASCO Endorsement Panel determined that the recommendations from the CAP guideline, published on August 3, 2022, are clear, thorough, and based on the most relevant scientific evidence. ASCO endorses *Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy: Guideline From the College of American Pathologists in Collaboration With the Association for Molecular Pathology and Fight Colorectal Cancer.*

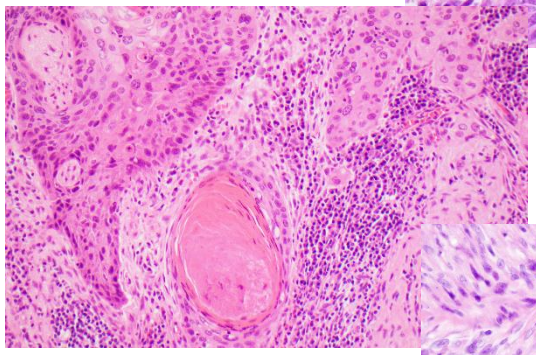
**RECOMMENDATIONS** Within the guideline, MMR immunohistochemistry (IHC), MSI polymerase chain reaction, and MSI next-generation sequencing are all recommended testing options for colorectal cancer, MMR-IHC and MSI-polymerase chain reaction for gastroesophageal and small bowel cancer, and only MMR-IHC for endometrial cancer. No recommendation in favor of any testing method over another could be made for any other cancer. Tumor mutational burden was not recommended as a surrogate for DNA MMR deficiency. If MMR deficiency consistent with Lynch syndrome is detected, it should be communicated to the treating physician.

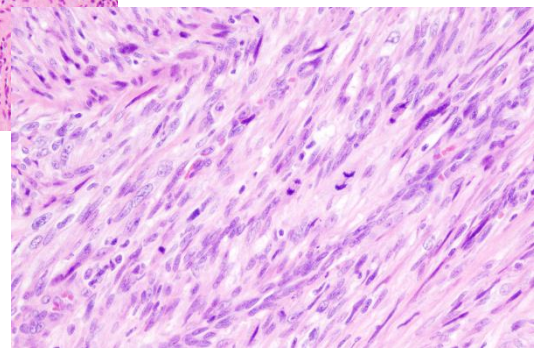
# *How common is MMRd Outside the Endometrium?*

- ~10% of endometrioid & clear cell ovarian carcinomas.
- 1-2% of ovarian serous carcinomas.
- 2-3% of cervical cancers.
- <1% of uterine mesenchymal tumors.







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- Schmoeckel E, Hofmann S, Fromberger D, et al. Comprehensive analysis of PD-L1 expression, HER2 amplification, ALK/EML4 fusion, and mismatch repair deficiency as putative predictive and prognostic factors in ovarian carcinoma. Virchows Archiv 2019;474:599–608.
- Bonneville R, Krook MA, Kautto EA, et al. Landscape of Microsatellite Instability Across 39 Cancer Types. JCO Precision Oncology 2017;:1–15.
- Jensen KC, Mariappan MR, Putcha GV, et al. Microsatellite instability and mismatch repair protein defects in ovarian epithelial neoplasms in patients 50 years of age and younger. The American Journal of Surgical Pathology 2008;32:1029–1037.

Does the lack of MMRd in these tumor types mean that these drugs won't be useful for most gyn cancers?

*Not necessarily!*

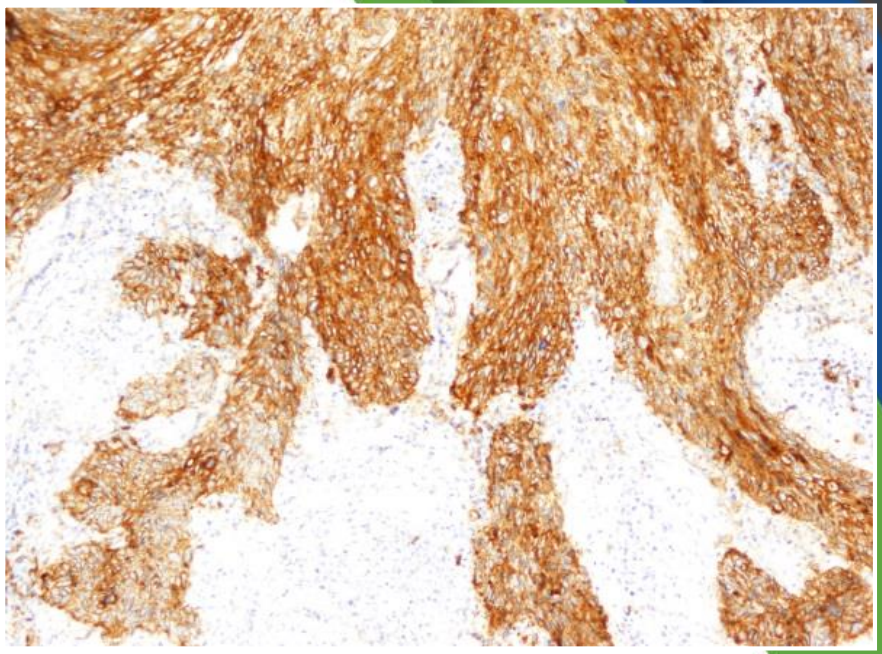
*Anti-PD-1/PD-L1 checkpoint inhibitors first took off in melanoma and non-small cell lung carcinomas, which have extremely low rates of MMR deficiency!*

# FDA Approves Therapy as Treatment in Recurrent or Metastatic Cervical Cancer

06/12/2018

The US Food and Drug Administration (FDA) granted approval for an anti-PD-1 therapy for the treatment of patients with recurrent or metastatic cervical cancer.

Pembrolizumab (Keytruda, Merck) is now approved for the treatment of patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy whose tumors express PD-L1 as determined by an FDA approved test.



Cervical squamous cell carcinoma showing strong diffuse PD-L1 expression

This approval is based on the KEYNOTE-158 trial, which enrolled a total of 98 patients. Among those patients, 79% had tumors that expressed PD-L1 with a CPS greater than or equal to 1. For the 77 patients whose tumors expressed PD-L1 with a CPS ≥1, the objective response rate was 14.3 percent with a complete response rate of 2.6 percent and partial response rate of 11.7 percent. Among the 11 responding patients, median DOR was not yet reached and 91 percent experienced a duration of response of six months or longer.

# Summary of Keynote-158 data on PD-L1 and pembrolizumab response in cervical carcinoma

- 98 cervical cancer patients with progressive disease were studied.
  - 92 squamous cell carcinomas, 5 adenocarcinomas, and 1 adenosquamous carcinoma
- 77 ( 79%) were PD-L1-positive at the CPS ≥1 threshold.
- Among the PD-L1-positive tumors, 14.3% responded to pembrolizumab.
  - 2.6% complete responses, 11.7% partial response
- No patients with CPS < 1 responded.

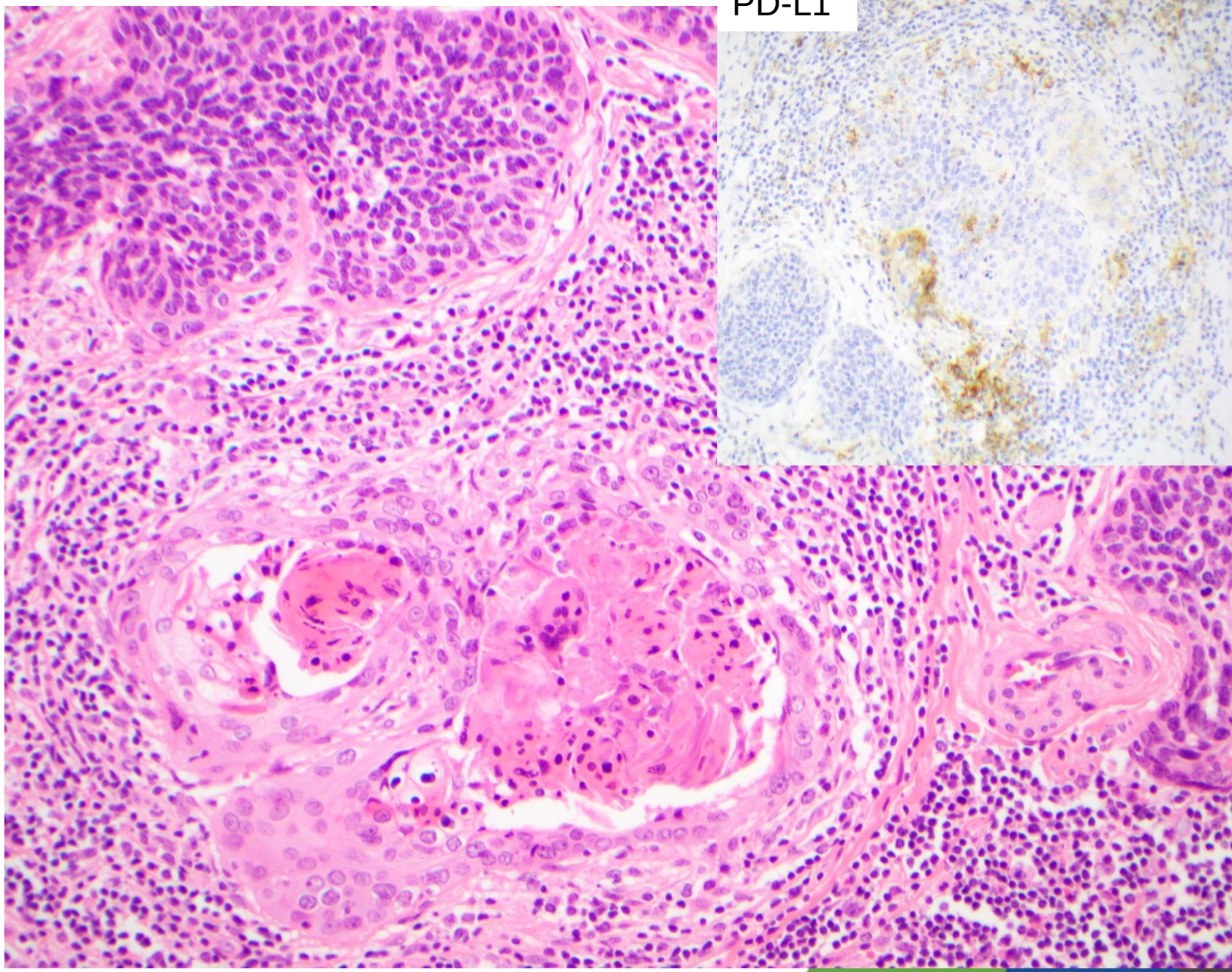
# CPS=Combined Positive Score

$$\frac{\text{\#PD-L1 staining cells (tumor cells, lymphocytes, and macrophages)}}{\text{\# viable tumor cells}} \times 100$$

- Negative=CPS<1
- Positive=CPS≥1

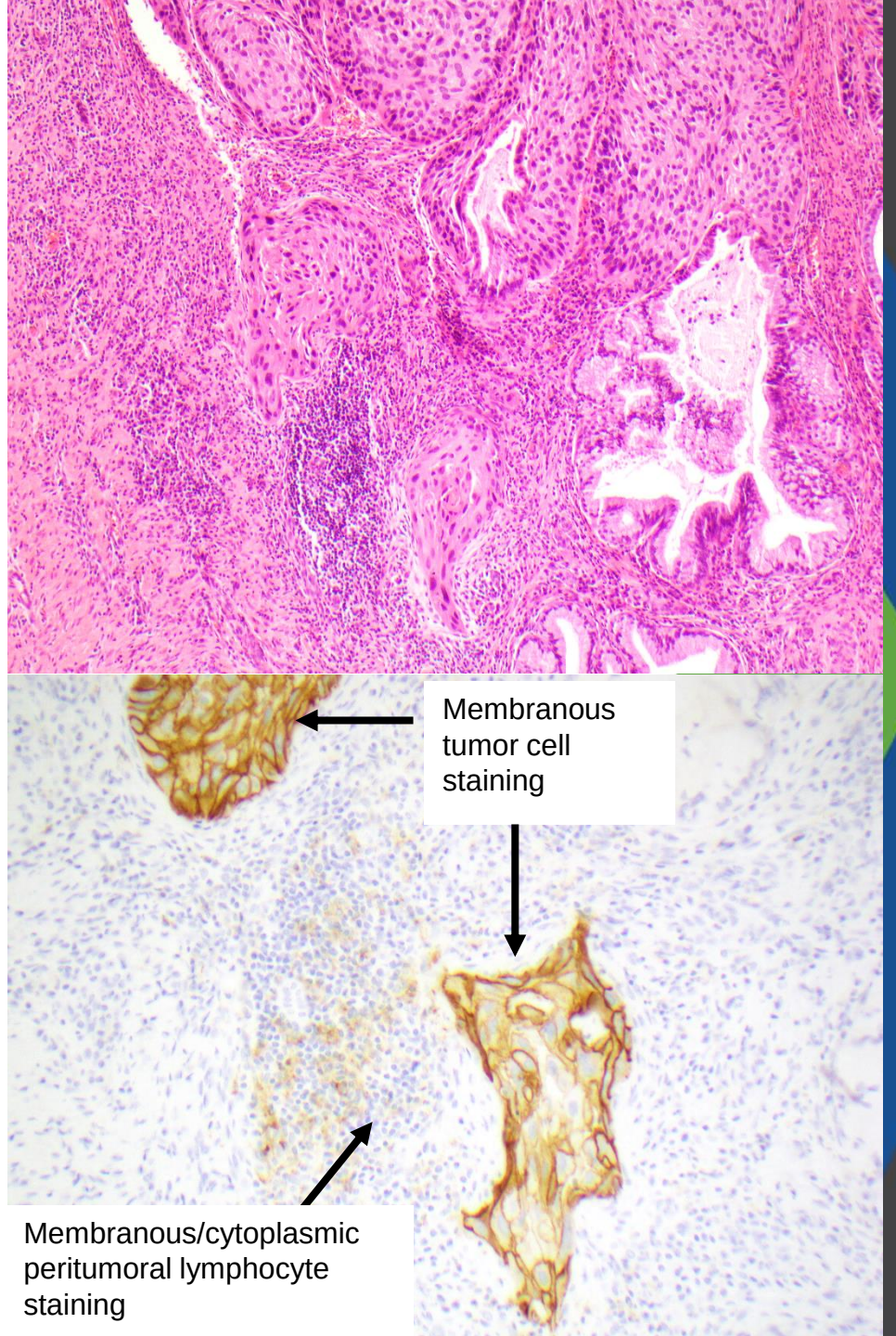
- A 45-year-old woman presents with recurrent cervical squamous cell carcinoma following chemotherapy and radiation.

- Does she qualify for pembrolizumab therapy using the CPS threshold of ≥ 1?

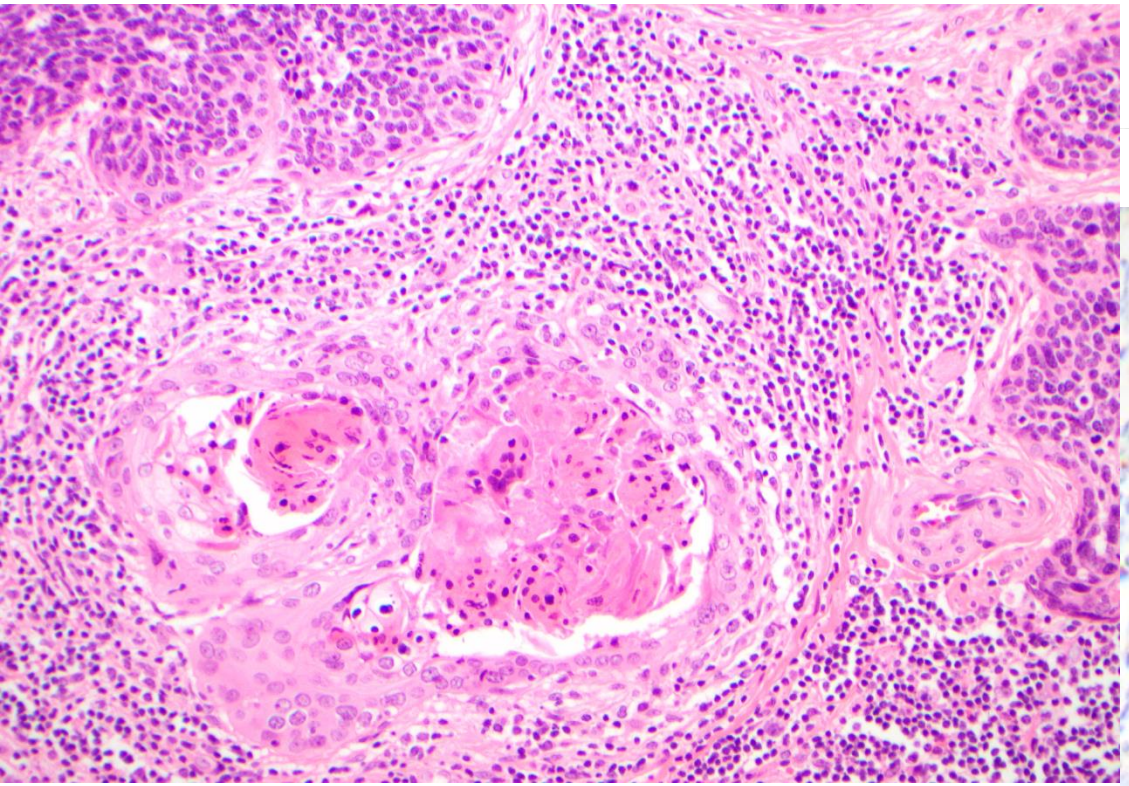


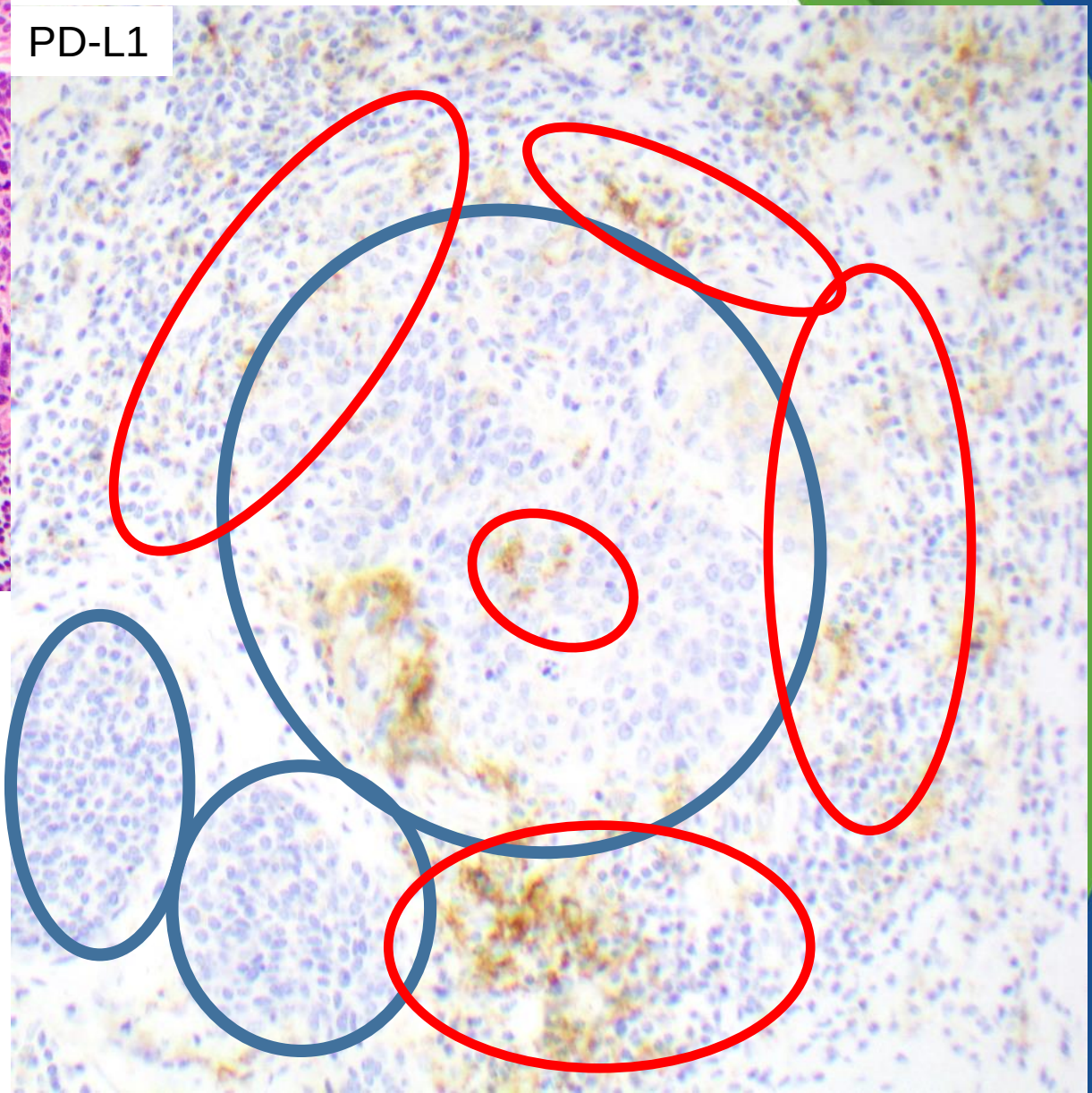
# How to Assess the CPS:

- Any CPS from 1-100 is positive.
  - 100 is the maximum allowable score.
- CPS is averaged across the entire tumor.
  - Don’t just count the hot spots!
- CPS should be assessed at 20x to ensure that even focal positivity is captured.
- Tumor cell staining must be membranous.
- Immune cell staining may be membranous or cytoplasmic.
  - PD-L1+ lymphocytes and macrophages must be associated with response to the tumor.
  - Location can be either intratumoral or peritumoral.



# Returning to our original patient…






Tumor cells: ~400

TILS/TAMS: ~100

CPS= 100/400 X 100

CPS= 25 YES, she qualifies!

# Critical Caveat!

- Even in the setting of PD-L1 positivity, pembrolizumab response rates are low.
  - <3% of patients show complete response
  - <12% show partial response

- The CPS was designed to maximize sensitivity for responders, but did it come at the cost of specificity?

- Are higher PD-L1 expression levels associated with better response rates?
  - We don't know, but in other tumor types this doesn't necessarily seem to be the case.

## What other FDA-approved biomarkers are out there?

# Tumor Mutational Burden Testing

## FDA approves pembrolizumab for adults and children with TMB-H solid tumors

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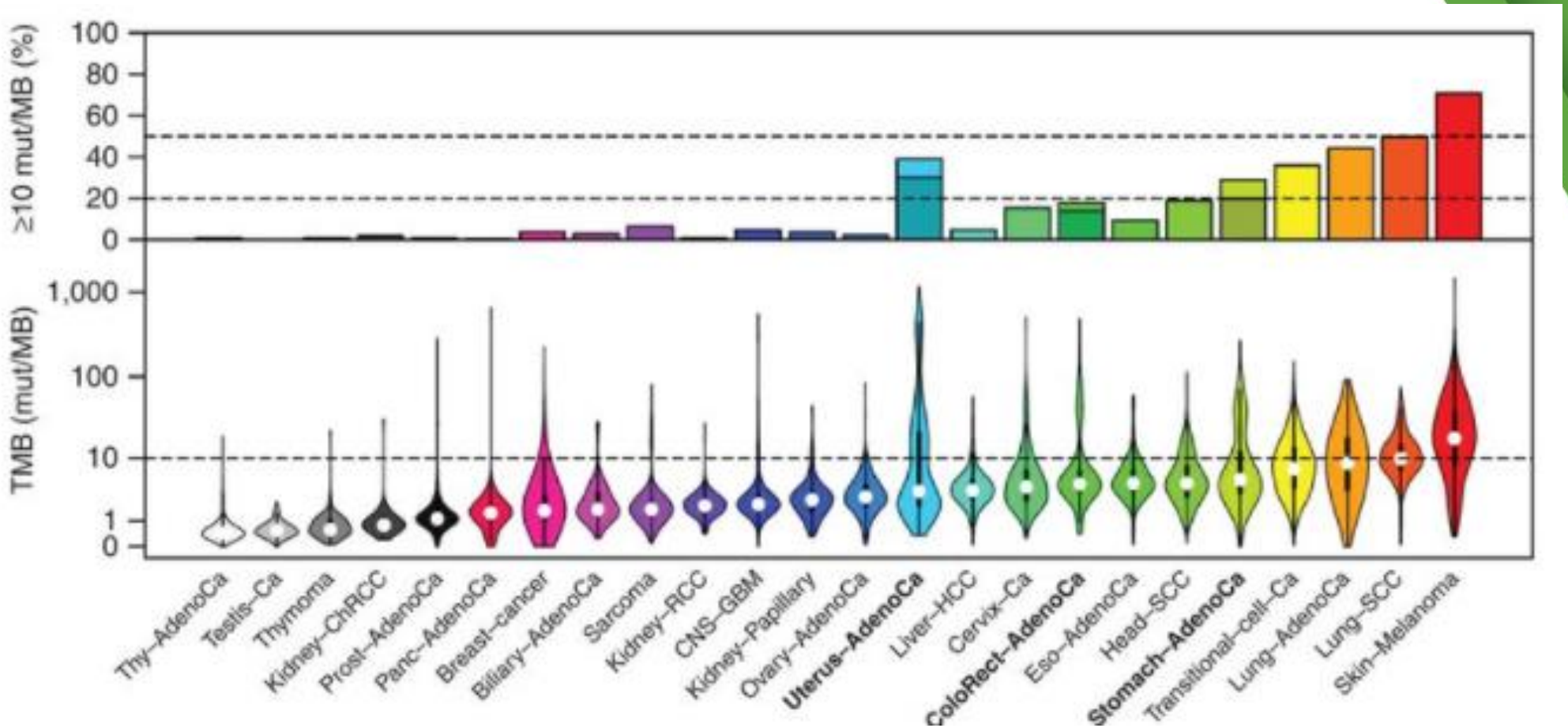
On June 16, 2020, the Food and Drug Administration granted accelerated approval to pembrolizumab (KEYTRUDA, Merck & Co., Inc.) for the treatment of adult and pediatric patients with unresectable or metastatic tumor mutational burden-high (TMB-H) [≥10 mutations/megabase (mut/Mb)] solid tumors, as determined by an FDA-approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options.

Today, the FDA also approved the FoundationOneCDx assay (Foundation Medicine, Inc.) as a companion diagnostic for pembrolizumab.




Sha et al. Tumor Mutational Burden as a Predictive Biomarker in Solid Tumors. *Cancer Discovery* 2020

# Tumor Mutational Burden Testing

- The KEYNOTE-158 study revealed that 29% of patients with TMB-H solid tumors respond to pembrolizumab, including 4% with complete response.
  - Marabelle A, Fakih M, Lopez J, et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. The Lancet Oncology 2020;21:1353–1365.

- Trials in PD-L1-positive endometrial cancer which did not require MMRd/MSI-H showed partial response to pembrolizumab in 13% with stable disease in another 13%.
  - Ott PA, Bang YJ, Berton-Rigaud D, et al. Safety and antitumor activity of pembrolizumab in advanced programmed death ligand 1–positive endometrial cancer: Results from the KEYNOTE-028 study. Journal of Clinical Oncology 2017.

- 14.9% of cervical cancers are TMB-H… roughly parallels response rate… could this be a better marker than PD-L1 or MMR in this tumor type????
  - Shao C, Li G, Huang L, et al. Prevalence of High Tumor Mutational Burden and Association With Survival in Patients With Less Common Solid Tumors. JAMA Netw Open 2020;3:e2025109.

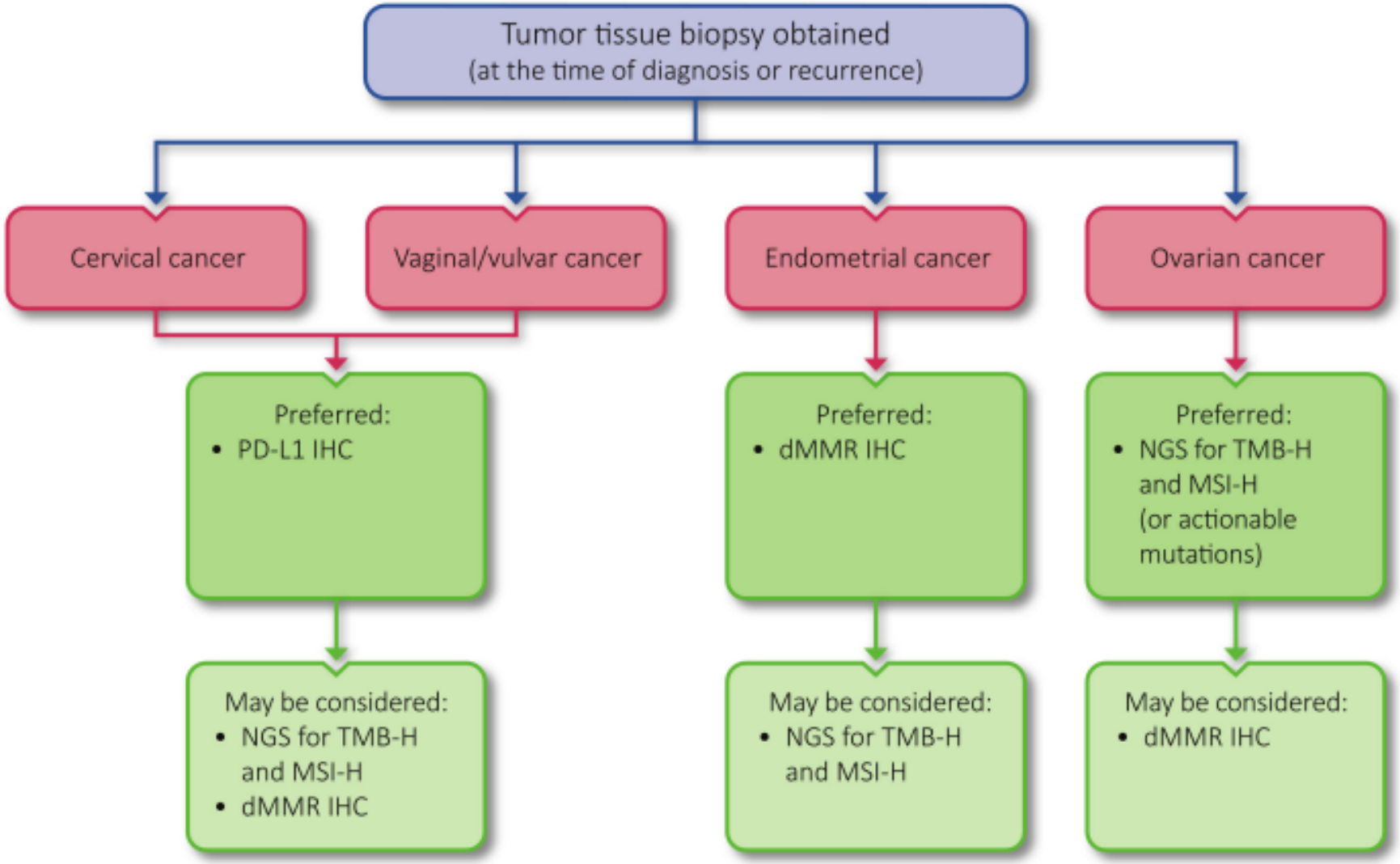



**Figure 1** Immune biomarker testing for gynecologic cancer. The VENTANA MMR IHC assay is the FDA-approved companion diagnostic for determining MMR status for treatment with dostarlimab or pembrolizumab. The FoundationOne CDx is approved as a companion diagnostic for assaying MSI-H and TMB-H status for pembrolizumab treatment. The PD-L1 IHC 22C3 pharmDx assay is approved for measuring PD-L1 expression for the pembrolizumab indication in cervical cancer. dMMR, mismatch repair deficient; IHC, immunohistochemistry; MSI-H, high microsatellite instability; NGS, next-generation sequencing; PD-L1, programmed death-ligand 1; TMB-H, high tumor mutational burden.

# Summary

- The PD-1 checkpoint inhibitors pembrolizumab and dolstarlimab are FDA-approved in solid tumors showing mismatch repair deficiency/high-level microsatellite instability.
  - MMR IHC is the recommended over MSI and NGS as the frontline test in endometrial carcinomas.
  - In ovarian carcinomas, SITC guidelines suggest MSI as first-line test.

- PD-L1 is also FDA-approved as an immunotherapeutic biomarker in cervical carcinomas using the CPS system.
  - The STIC guidelines also recommend this assay for vulvar/vaginal carcinomas.

- Tumor mutational burden (TMB) testing is another FDA-approved gateway for immunotherapy access in solid tumors ("TMB-High"→ ≥10 mutations per megabase).