COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

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

Early Online Release Publication:
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Outline

- Introduction
- Key questions and results
- Guideline recommendations and good practice statements
- Guideline development process
- Conclusions

Introduction

Introduction

- **US Food and Drug Administration (FDA) approved pembrolizumab immune checkpoint therapy for adult and pediatric patients with unresectable or metastatic MSI-H or dMMR solid tumors who have progressed following prior treatment and who have no satisfactory alternative treatment options**

FDA News Release

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

For Immediate Release

May 23, 2017

Keytruda (pembrolizumab) is indicated for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors that have been identified as having a biomarker referred to as microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR). This indication covers patients with solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options and patients with colorectal cancer that has progressed following treatment with certain chemotherapy drugs.

Fig 1. U.S. Food & Drug Administration News Release. May 23, 2017.

Introduction continued

- **Missing from the FDA announcement is guidance on which method to use evaluate patients for eligibility for treatment with immune checkpoint therapy**
  - **Immunohistochemistry (IHC) for DNA mismatch repair (MMR) proteins**
  - **Polymerase chain reaction (PCR)-based microsatellite instability (MSI) assays**
  - **Next-generation sequencing (NGS)-based MSI analyses, or**
  - **NGS-based assessment of tumor mutation burden (TMB) as a surrogate for underlying mismatch repair**

Introduction continued

- **To help address this uncertainty, the College of American Pathologists convened a workgroup to develop an evidence-based guideline to critically evaluate the different laboratory approaches to measuring MSI and DNA MMR**
- **The panel addressed the overarching question, “what test best identifies defects in DNA mismatch repair?”**

Key questions and results

Key questions

- **KQ1a. In patients being considered for checkpoint inhibitor therapy, does MMR protein loss by IHC, PCR-based MSI analysis, or NGS-based MSI analysis accurately detect defects in DNA MMR?**
- **KQ1b. Does TMB by NGS have adequate performance characteristics to act as a surrogate for PCR and NGS-based MSI assays?**
- **KQ1c. In patients being considered for checkpoint inhibitor therapy, which DNA MMR assay best predicts improved patient outcomes?**
- **KQ2. When comparing MMR-IHC and PCR or NGS-based MSI, does any assay have better performance characteristics in specific cancer types?**
- **KQ3. What are the diagnostic test characteristics of MMR-IHC, PCR-based MSI analysis, and NGS-based MSI analysis when predicting germline Lynch mutations?**

Results

- **Six evidence-based recommendations and three good practice statements are offered to help pathologists and their clinical colleagues in MMR and MSI testing considered for immune checkpoint blockade**
- **More evidence and evidence of higher quality were identified for colorectal cancer and other cancers of the gastrointestinal (GI) tract compared to cancers arising outside the GI tract**

Guideline recommendations

Recommendation 1

For patients with colorectal carcinoma (CRC) being considered for immune checkpoint inhibitor therapy, pathologists should use mismatch repair immunohistochemistry (MMR-IHC) and/or microsatellite instability (MSI) by polymerase chain reaction (PCR) for the detection of DNA mismatch repair defects. Although MMR-IHC or MSI by PCR are preferred, pathologists may use a validated MSI by NGS assay for the detection of DNA mismatch repair defects.

Note: **MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency.**

Strength of Recommendation: Strong

Certainty of Evidence: Moderate**patients**

Recommendation 2

For patients with gastroesophageal and small bowel cancer being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by PCR over MSI by NGS for the detection of DNA mismatch repair defects.

Note: **This recommendation does not include esophageal squamous cell carcinoma.**

Strength of Recommendation: Strong

Certainty of Evidence: Low

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 mismatch repair defects.

Strength of Recommendation: Strong

Certainty of Evidence: Low

Recommendation 4

For patients with cancer types other than CRC, GEA, small bowel, and endometrial being considered for immune checkpoint inhibitor therapy, pathologists should test for DNA mismatch repair, although the optimal approach for the detection of MMR defects has not been established.

Note: **Assays must be adequately validated for the specific cancer type being tested with careful consideration of performance characteristics of MMR-IHC and MSI by NGS or PCR for the detection of DNA mismatch repair defects.**

Strength of Recommendation: Conditional

Certainty of Evidence: Low

Discussion for recommendations 1 - 4

- **MMR-IHC, MSI-PCR, and MSI-NGS has comparable performance metrics in CRC patients**
  - **MMR-IHC and MSI-PCR are the preferred screening methods**
  - **NGS-based assays require more tissue as the DNA input requirements are typically 500 ng to 1 ug**
  - **Biopsies for MMR-IHC and MSI-PCR testing may yield limited tissues required for NGS**

Discussion for recommendations 1 - 4 continued

- **MMR-IHC can identify the most probable gene defect while NGS may not be able to accurately identify (MSI-L) tumors that have loss of MMR protein by IHC**
- **MMR-IHC and MSI-PCR can typically be performed in a day, whereas NGS typically takes several weeks to complete**
- **NGS may have increased TAT due to specialized laboratory staff expertise needed, as most samples are sent out to reference laboratories**

Rationale for recommendations 1 - 4

- **MSI – Colorectal vs Endometrial Cancers**
  - **44 colorectal cancers and 57 endometrial cancers from 8 families with known MLH1 or MSH2 mutations**
  - **MSS: EC 23%; CRC 11%**
  - **Amongst the MSI-High tumors, EC had fewer microsatellites affected**

Kuismanen et al. *Am J Pathol. 2002;*160(6):1953-1958.

MSI profiles for colorectal cancer and endometrial cancer are distinct

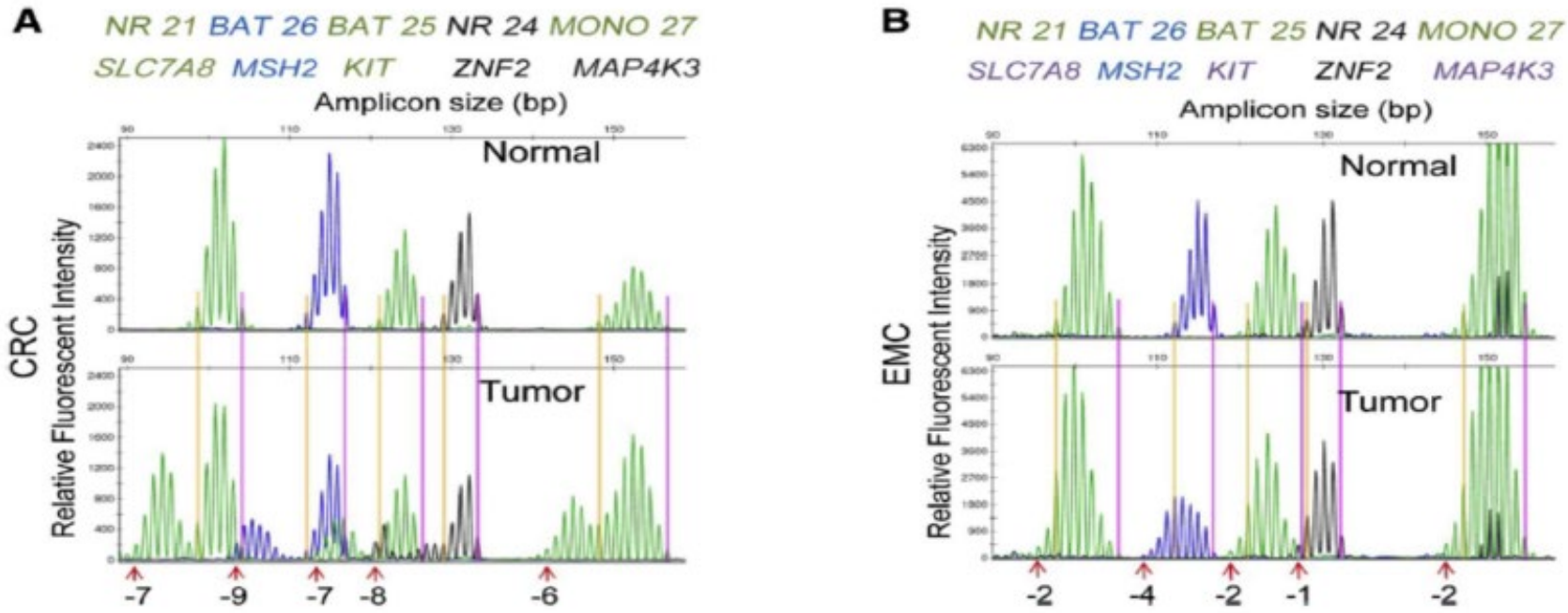
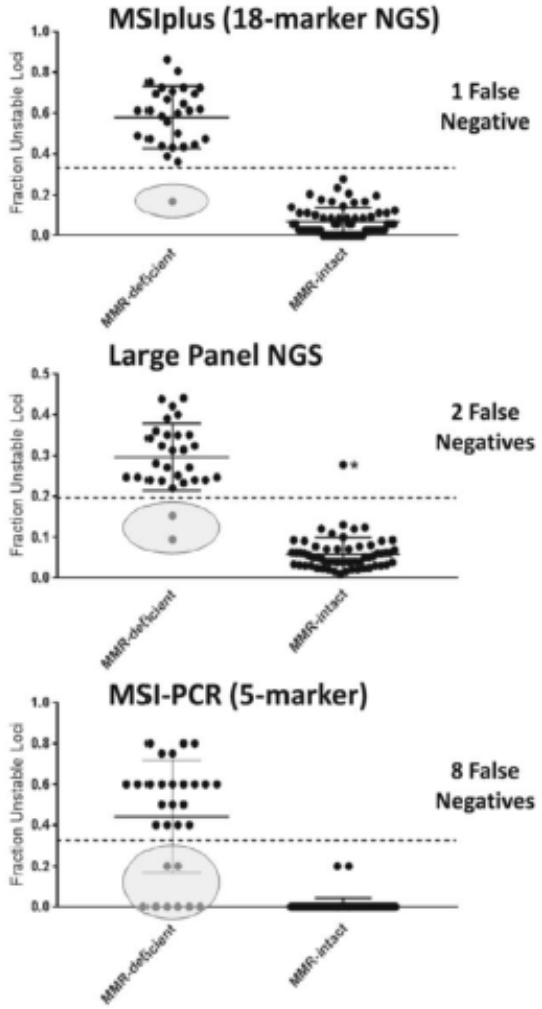


Fig 2. Reprinted from *J Mol Diagn,* Vol.19(1), Wang Y, Shi C, Eisenberg R, Vnencak-Jones CL, Differences in microsatellite instability profiles between endometrioid and colorectal cancers: A potential cause for false-negative results*?* p. 58. Copyright 2017, with permission from Elsevier.

Comparison of MSI methods in prostate cancer



MSIplus = 5 Promega MSI markers + 13 more MSI markers

MMR intact vs MMR deficient determined by NGS sequencing of MMR genes

Performance Characteristics of MSIplus, large-panel NGS, and MSI-PCR in Prostate Cancer

Assay	Sensitivity [95% CI]	Specificity [95% CI]
MSI Plus	96.6% (80.4 – 99.8%)	100% (92.7 – 100%)
Large panel NGS	93.1% (75.8 – 98.8%)	98.4% (90.2 – 99.9%)
MSI-PCR	72.4% (52.5 – 86.6%)	100% (92.7 – 100%)

Fig 3. Hempelmann et al. *J Immunother Cancer.* 2018;6(1):29.

Recommendation 5

For all cancer patients being considered for immune checkpoint inhibitor therapy based upon defective mismatch repair, pathologists should NOT use TMB as a surrogate for the detection of DNA mismatch repair 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 mismatch repair deficiency.

Strength of Recommendation: Strong

Certainty of Evidence: Low

Rationale for recommendation 5

- **The evaluated studies show that although there is a relationship between MSI-H and TMB-H, the heterogeneity for individual neoplasms is such that TMB-H cannot be used as a surrogate measure of MSI-H**
- **Increased TMB observed in dMMR neoplasms, a subset of extremely elevated TMB values was associated with other etiology (eg, POLE exonuclease-domain mutations in CRC)**
- **One study evaluating MSI and TMB status using a NGS platform across a wide variety of cancer types, compared against MMR-IHC or MSI-PCR, noted that 30% of MSI-H cases were TMB-low (<17 mutations /MB)**

Rationale for recommendation 5 continued

- **There was 95% concordance between elevated TMB and MSI-H status in CRCs**
- **Only 57% of MSI-H endometrial cancers were TMB-High (TMB-H), with discrepant rates of agreement also observed in ovarian (24%), neuroendocrine (57%), and cervical (33%) cancers.**
- **In melanoma, squamous cell carcinoma, and lung carcinoma, high TMB is common but MSI-H is very uncommon.**

Vanderwalde et al. *Cancer Med*. 2018;7(3):746-756.
Chalmers et al. *Genome Med.* 2017;9(1):34.

Issues with TMB

- **Gold standard based on whole exome sequencing (not practical for routine clinical use)**
- **Likely can use larger NGS panels(200-300 genes or 1 megabase)**

Features	WES	MSK-IMPACT (MSKCC	FoundationOne CDx (FMI)
Genes	~22K	468	324
Size	~30 Mbp	1.22 Mbp	0.8 Mbp
Germline filtering	Blood	Blood	Databanks (dbSNP, ExAC, FMI internal) algorithm
TMB	Somatic, coding mutations (Nonsynonymous)/exome	Somatic, coding mutations (Nonsynonymous)/Mbp	Somatic, coding mutations (nonsynonymous + indels + synonymous)/Mbp

Allgäuer et al. *Transl Lung Cancer Res.* 2018;7(6): 703-715.

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.

Strength of Recommendation: Strong

Certainty of Evidence: Low

Rationale

- **Tumor dMMR or MSI-H without evidence of MLH1 gene promoter methylation is potentially consistent with Lynch syndrome and should trigger consideration for genetic counseling and germline testing if indicated**

- MLH1 IHC loss, absent *MLH1* gene methylation
- MSH2/PMS2/MSH6 IHC loss
- MSI-High, absent *MLH1* gene methylation

Rationale continued

- **Communication of important pathology findings may be more readily operationalized in hospital-based settings where pathologists and other types of physicians interact regularly**
- **Communication should be done irrespective of practice setting**
- **Systems should already be in place for the tumors most frequently associated with Lynch syndrome—colorectal carcinoma and endometrial carcinoma—and that dMMR is far less common in other tumor types**

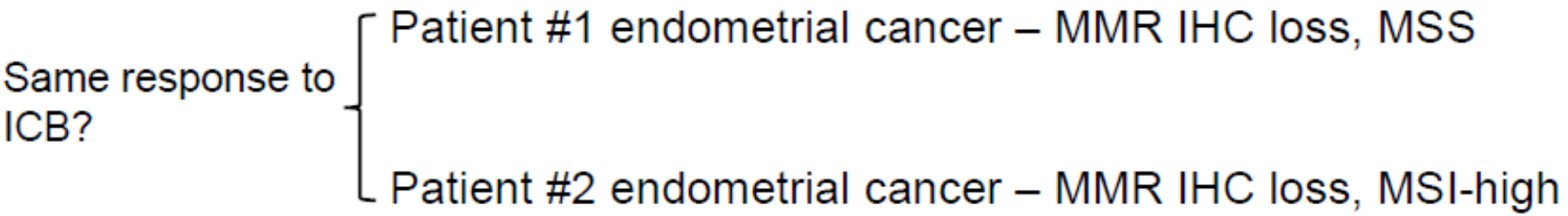
Good practice statements

Good practice statements (GPSs)

- **High level of certainty that the recommended action will do more good than harm, but has little direct evidence**
- **Not evidence-based**

Good practice statements (GPSs) continued

- **Discordant results: In the event of discordant results, pathologists should interpret any evidence of MMR deficiency by IHC or MSI by NGS/PCR as a positive result for patients to be eligible for immune checkpoint therapy. Discordant results should be reviewed to ensure that the discordance is not due to an interpretive error.**



Discordant results

MSI-low endometrial carcinoma (A, H&E) that was shown to have immunohistochemical loss of a DNA MMR protein. The carcinoma has intact nuclear expression of MLH1 (B), PMS2 (C), and MSH2 (D). The tumor demonstrates loss of MSH6 nuclear expression (E). Subsequently, a deleterious MSH6 germline mutation was identified in this patient.

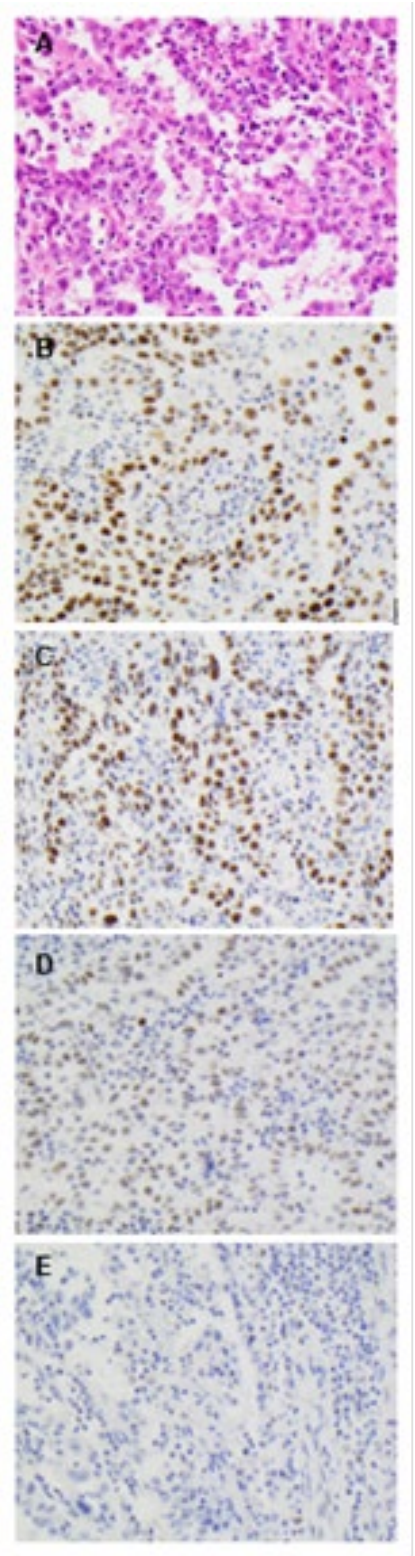


Fig 4. Discordant results. (Mills, A. 2022)

Good practice statements (GPSs) continued

- **Indeterminate results: In the event of indeterminate result in any method, pathologists should perform an alternative technique or repeat on a different tumor block. Laboratories should have a robust peer review process for indeterminate cases.**

Indeterminate results

Colorectal adenocarcinoma bulky metastasis to the liver, initially with indeterminate immunohistochemistry results for MLH1 (A). Note that tumor cell nuclei have loss of MLH1 expression, but there is also lack of nuclear expression of MLH1 in adjacent stromal cells. MLH1 immunohistochemistry was repeated using a different block of the metastasis (B), this time yielding definitive strongly and diffusely positive intact nuclear expression of MLH1.

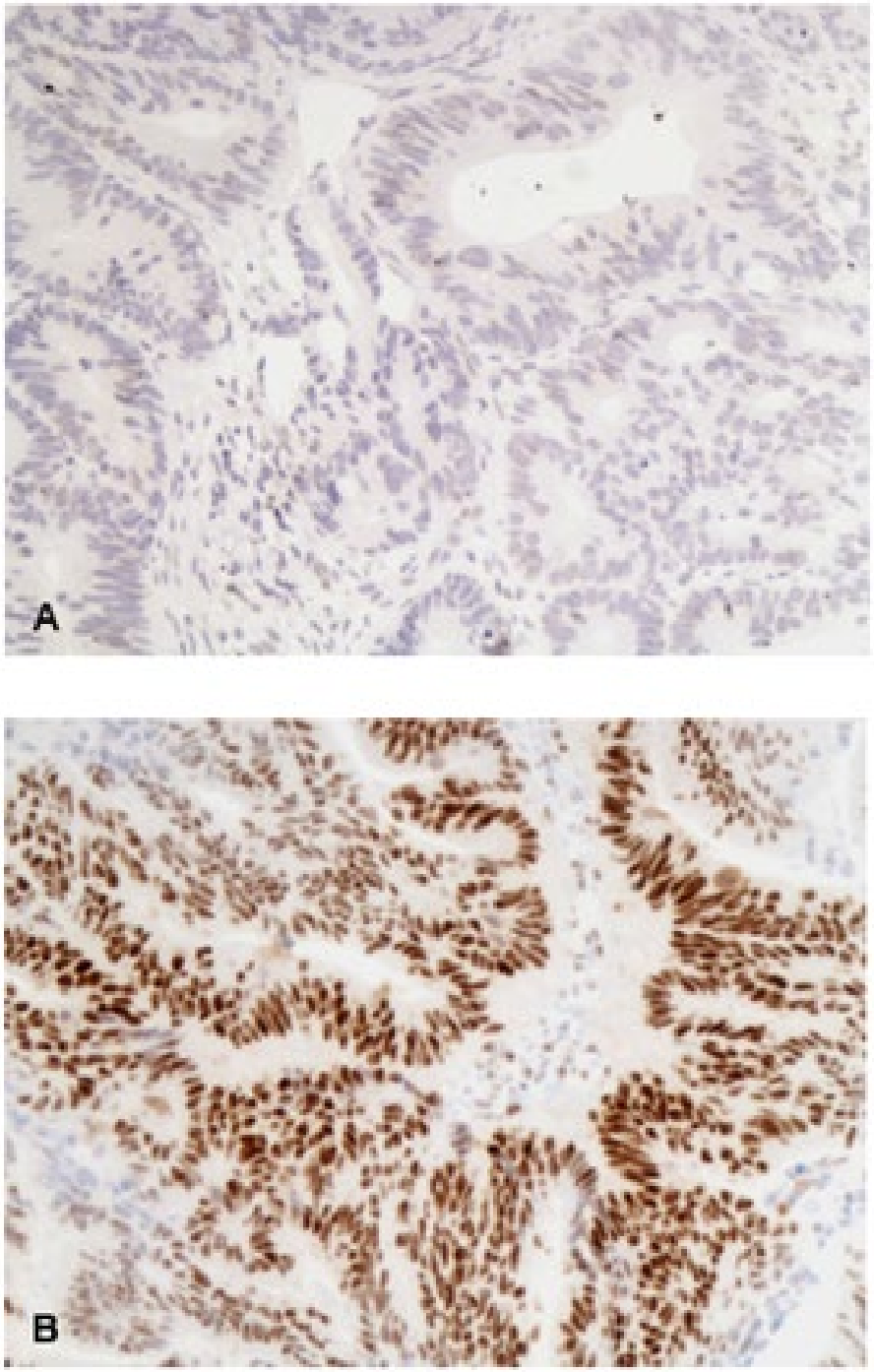
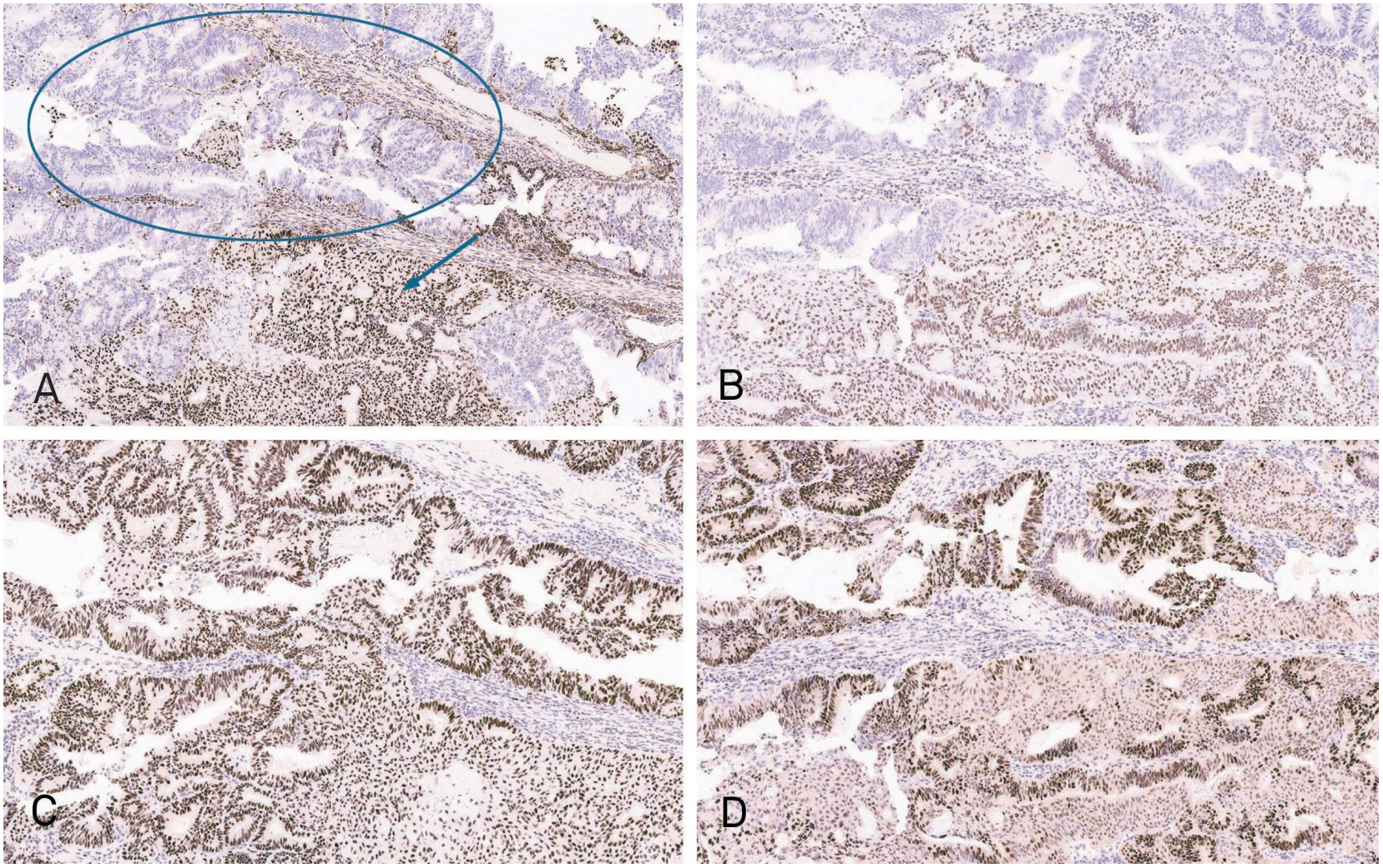


Fig 5. Indeterminate results. (Broaddus, R. 2022)

Good practice statements (GPSs) continued

- **Subclonal loss: In the event of a subclonal loss by MMR-IHC, pathologists should perform MSI by PCR specifically in a dissected area of tumor that has IHC loss MMR protein if the patient is being considered for checkpoint inhibitor clinical trials.**

Subclonal loss



Endometrial endometrioid adenocarcinoma with subclonal immunohistochemical loss of MLH1 (A) and PMS2 (B). Nuclear expression of MSH2 (C) and MSH6 (D) are retained. For MLH1 and PMS2, note foci of tumor with loss of nuclear MLH1 and PMS2 (circle in A) with immediately adjacent stromal cells and tumor (arrow in A) with intact positive expression of MLH1 and PMS2. (Fig 6. Subclonal Loss (Lawson, B. 2022))

Guideline development process

Collaboration

- **The CAP collaborated with the Association for Molecular Pathology (AMP) and Fight Colorectal Cancer. They provided members to participate on the guideline panels and approved the guideline prior to submission to publication**
- **Two oncologists representing the American Society of Clinical Oncology (ASCO) also served on the expert panel.**

Expert panel members

- Russell Broaddus, MD, PhD, FCAP, Chair
- Sarah F. Adams, MD
- Angela Bartley, MD, FCAP
- Heather Hampel, MS, LGC
- Brooke Howitt, MD
- Sarah Kerr, MD
- Eric Konnick, MD, MS, FCAP
- Cristina Magi-Galuzzi, MD, PhD
- Ann M. Mills, MD
- Michael J. Overman, MD – ASCO
- Antonia R. Sepulveda, MD, PhD, FCAP – AMP
- Zsofia K. Stadler, MD – ASCO
- Lesley Souter, PhD, Research Methodology Consultant

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Advisory panel members

- Andrew Bellizzi, MD
- Gregary Bocsi, DO, MS
- Diana M. Cardona, MD
- Rondell P. Graham, MBBS
- Rahul Madhukar Jawale, MD
- Jonathan Loree MD, MS
- Jonathan Nowak MD, PhD
- Jingxin Qiu MD, PhD
- Sinchita Roy Chowdhuri MD, PhD
- Michael T. Tetzlaff MD, PhD

Patient advocates from Fight Colorectal Cancer

- Wendy Lewis
- Wenora Johnson

CAP guideline development process

COLLEGE of AMERICAN PATHOLOGISTS
Laboratory Quality Solutions

Evidence-based Guideline (EBG) Development and Review Process

The Pathology and Laboratory Quality Center for Evidence-based Guidelines (the Center) develops recommendations related to the practice of pathology and laboratory medicine. Through them, we continually improve the quality of diagnostic medicine and patient outcomes.

1	Submit & Select Ideas	The Center Guideline Committee vets all topics submitted via the CAP Center website and recommends approval for those meeting the appropriate criteria to the Council on Scientific Affairs (CSA).
2	Determine Scope & Form Panel	A rigorous and transparent screening is conducted, including conflicts of interest, for the volunteer expert panel who defines the scope and key questions and for the advisory panel.
3	Research & Review Evidence	A systematic review of the literature of the literature using the GRADE approach offers a transparent and sensible method to grading the quality (certainty) of the evidence and strength of recommendations.
4	Draft Recommendations	The expert panel develops draft recommendations based upon the extracted data, the strength of evidence, and the considered judgment process including assessment of benefits to harms.
5	Open Comment Period	The draft recommendations undergo a public peer review during which stakeholder feedback is collected.
6	Complete Recommendations & Draft Manuscript	The expert panel finalizes the recommendations and the guideline manuscript based on updated literature and stakeholder feedback.
7	Review & Approve	The independent review panel, comprised of unconflicted individuals with topic expertise, acts on behalf of the CSA as the CAP approval body.
8	Publish & Implement	The guideline manuscript is submitted for publication to the *Archives of Pathology & Laboratory Medicine* (and partner journals if applicable). The Center develops tools and educational activities to support the adoption and implementation of guideline.
9	Maintain & Monitor	Center EBGs are reviewed every four years (or earlier if evidence becomes available that could potentially alter the original guideline recommendations). Upon review, the guideline will either be reaffirmed, updated, or retired.

Reaffirm	Update	Retire
Confirmation complete guideline is accurate and up to date and then place into step 9	Refresh guideline and start at step 2 of process	Guideline inactive (i.e., no updated systematic review)

Email center@cap.org for questions, comments, or to report concerns including conflict of interest issues.

Literature search

- **Ovid MEDLINE and Embase were searched 12/16/2018**
- **The database searches used standardized vocabulary and keywords for the following concepts derived from the key questions: 1) microsatellite instability, mismatch repair, or tumor mutational burden; 2) laboratory testing methods; and 3) checkpoint inhibitors**
- **Search dates**
  - **1/1/2008 through 12/16/2018**
  - **Literature refresh 2/2020 and 3/2021 to capture literature published after the original search**

Methods

- **This evidence-based guideline was developed following the standards by the National Academy of Medicine**
- **The CAP collaborated with AMP, ASCO, and Fight CRC and convened a multidisciplinary expert and advisory panel to develop the guideline**
- **The panel addressed the overarching question, “What test best identifies defects in DNA mismatch repair?”**

Panel proceedings

- **The expert panel met via conference call/webinar multiple times and twice in-person throughout the development of the guideline to develop the scope, draft recommendations, review and respond to solicited feedback, and assess the certainty of evidence that supports the final recommendations**

Panel proceedings continued

- **The draft recommendations were released to the public for comments February 19 to March 13, 2020**
- **Over 350 comments were received**
- **2 draft recommendations received >90% agree or agree with modifications**
  - **5 draft statements achieved more than 90% agreement**
  - **1 draft statement received below the 80% agreement threshold**
  - **All draft recommendation statements have agreements that range between 77.9% - 98.3%**
  - **1 draft recommendation was maintained with the original language; 4 were revised with minor edits for clarity; and one draft recommendation was edited with a major revision.**

Panel proceedings continued

- **An independent review panel (IRP) was assembled to review and approve the guideline on behalf of the CAP Council on Scientific Affairs.**
- **The IRP was masked to the EP and to each other and were vetted through the COI process.**
- **Collaborating organizations were provided the guideline for approval.**

Conclusions

Conclusions

- **Six evidence-based recommendations and three good practice statements are offered to help pathologists and their clinical colleagues in MMR and MSI testing considered for immune checkpoint blockade.**
  - **MSI-NGS is a good assay for CRC and GEA / GEJ /small bowel cancer patients**
  - **The evidence-based guideline recommends the use of IHC, for tumor types other than CRC and GEA / GEJ /small bowel**

Conclusions continued

- **While NGS panels may provides more genomic information these MSI-NGS approaches often fall short cancer types other than CRC and GEA / GEJ /small bowel cancer**
- **There is insufficient published evidence to assess NGS efficacy in many cancer types. It is possible that to accurately detect MSI-H in these other cancer types, alternative NGS algorithms unique to each individual tumor type need to be developed.**
- **As testing evolves, the guideline will need to be updated.**

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Disclosures

Practice guidelines and consensus statements are intended to assist physicians and patients in clinical decision-making. New evidence may emerge between the time a practice guideline or consensus statement is developed and when it is published or read. Guidelines and statements cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for a patient. Refer to the guideline manuscript for complete details about the recommendations. The CAP and its collaborators make no warranty, express or implied, regarding guidelines and statements and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. The CAP and its collaborators assume no responsibility for any injury or damage to persons or property arising out of or related to any use of this statement or for any errors or omissions.

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