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

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• ***Context.***—**The US Food and Drug Administration (FDA) approved immune checkpoint inhibitor therapy for patients with advanced solid tumors that have DNA mismatch repair defects or high levels of microsatellite instability; however, the FDA provided no guidance on which specific clinical assays should be used to determine mismatch repair status.**

Objective.—**To develop an evidence-based guideline to identify the optimal clinical laboratory test to identify defects in DNA mismatch repair in patients with solid tumor malignancies who are being considered for immune checkpoint inhibitor therapy.**

Design.—**The College of American Pathologists convened an expert panel to perform a systematic review of the literature and develop recommendations. Using the National Academy of Medicine–endorsed Grading of Recommendations Assessment, Development and Evaluation approach, the recommendations were derived from available evidence, strength of that evidence, open comment feedback, and expert panel consensus. Mismatch repair immunohistochemistry, microsatellite instability derived from both polymerase chain reaction and next-generation sequencing, and tumor mutation burden derived from large panel next-generation sequencing were within scope.**

Results.—**Six recommendations and 3 good practice statements were developed. More evidence and evidence of higher quality were identified for colorectal cancer and other cancers of the gastrointestinal (GI) tract than for cancers arising outside the GI tract.**

Conclusions.—**An optimal assay depends on cancer type. For most cancer types outside of the GI tract and the endometrium, there was insufficient published evidence to recommend a specific clinical assay. Absent published evidence, immunohistochemistry is an acceptable approach readily available in most clinical laboratories.**

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Clinical laboratory assays for detection of high levels of tumor microsatellite instability (MSI-High) or loss of DNA mismatch repair (MMR) protein expression by immunohistochemistry (IHC) are familiar to many pathologists owing to their importance in routine screening for hereditary colorectal cancer (CRC) or endometrial cancer secondary to Lynch syndrome. In recent years, these same assays have become increasingly important in the treatment approach to many advanced solid tumor malignancies as well. Programmed death receptor-1 (PD-1) blockade is emerging as an effective treatment option for patients with advanced MSI-High (MSI-H) and/or mismatch repair–deficient (dMMR) cancers.[1–4] These treatment approaches

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are especially exciting, as there have been early hints of durable response and even curative potential for at least some patients.[1,5] These findings contributed to the US Food and Drug Administration (FDA) decision on May 23, 2017, to approve pembrolizumab immune checkpoint inhibitor therapy for adult and pediatric patients with unresectable or metastatic MSI-H or dMMR solid tumors whose disease has progressed after prior treatment and who have no satisfactory alternative treatment options. This announcement was especially significant, as it represented the first FDA tissue/site agnostic approval of an oncology drug.

Absent in the original FDA announcement is whether pathologists should use IHC for DNA MMR proteins, polymerase chain reaction (PCR)–based MSI assays, next-generation sequencing (NGS)–based MSI analyses, or NGS-based assessment of tumor mutation burden (TMB) as a surrogate for underlying MMR to evaluate patients for eligibility for treatment with immune checkpoint inhibitor therapy. This may be due, in part, to unclear laboratory methodology used by the clinical trials that contributed to the FDA announcement. 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.

METHODS

This evidence-based guideline was developed following the standards of the National Academy of Medicine.[6] A detailed description of the methods and the systematic review (including panel composition, quality assessment, and complete analysis of the evidence; Supplemental Tables 1 through 6) used to create this guideline can be found in the supplemental digital content (SDC at https://meridian.allenpress.com/aplm in the October 2022 table of contents).

Panel Composition

The College of American Pathologists (CAP) in collaboration with the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), and patient advocacy group Fight Colorectal Cancer convened a multidisciplinary expert and advisory panel to develop the guideline. The CAP approved the appointment of the members. Detailed information about the panel composition can be found in the SDC.

Conflict of Interest Policy

In accordance with the CAP conflict of interest policy (in effect January 2018), expert and advisory panel members disclosed all financial interests from 24 months before appointment through the time of guideline publication. Individuals were instructed to disclose any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Complete disclosures of the expert panel (EP) members are listed in the Appendix. Most of the EP members (9 of 13 members) were assessed as having no relevant conflicts of interest. The CAP provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement, except for the contracted methodologist. Please see the SDC for full details on the conflict of interest policy.

OBJECTIVE

The panel addressed the overarching question, "What test best identifies defects in DNA mismatch repair?" This led to the following key questions:

KQ1a. In patients being considered for immune 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 immune 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?

See the SDC for a detailed description of the key questions and additional scope questions (Supplemental Table 1).

Outcomes of Interest

The primary outcomes of interest included diagnostic test characteristics (sensitivity, specificity, positive predictive value, negative predictive value), accuracy of MMR defect detection, tissue concordance, treatment response rates, and survival rates. The EP provided possible outcomes within the scope of the guideline and prioritized the outcomes to be included in the guideline. See the SDC for a detailed description of outcomes of interest. This guideline's target audience includes general pathologists, molecular pathologists, genetic counselors, geneticists, and oncologists.

Literature Search and Collection

A comprehensive search for relevant evidence was completed by the CAP's medical librarian using the bibliographic databases Ovid MEDLINE and Elsevier Embase.com on December 16, 2018. The database searches used standardized vocabulary and keywords for the following concepts derived from the key questions: (1) MSI, MMR, or TMB, (2) laboratory testing methods, and (3) checkpoint inhibitors, encompassing the publication dates January 1, 2008, to December 16, 2018. A targeted search was completed in the same databases that included standardized vocabulary and keywords for the concepts: (1) Lynch syndrome, (2) MSI, MMR, or TMB, and (3) laboratory testing methods, also completed on December 16, 2018, with publication date limits set to January 1, 2000, through December 16, 2018. All database searches were limited to English language, and the Cochrane search filter for humans was applied.[7] Case reports, commentaries, editorials, and letters were excluded. Database searches were supplemented with a search for unindexed literature, and EP members were polled for relevant unpublished data at the onset of the project. The database literature searches were rerun in February 2020 (Ovid MEDLINE: February 21, 2020; Embase.com: February 24, 2020) to identify articles published from December 16, 2018, through the date of the search. A second literature refresh was run in the same databases on March 30, 2021, to capture literature published from February 24, 2020, through March 30, 2021. Detailed information regarding the literature search, including unindexed literature sources and database search terms used (Supplemental Figures 1 and 2), is available in the SDC.

Table 1. Grades for Strength of Recommendations[a]		
Designation	**Recommendation**	**Evidence to Decision Judgment**
Strong Recommendation	Recommend for or against a particular practice (Can include "must" or "should")	Supported by assessment with the GRADE EtD framework showing EP consensus of judgments directed to the far right or far left poles of the framework
Conditional Recommendation	Recommend for or against a particular practice (Can include "should" or "may")	Supported by assessment with the GRADE EtD framework showing EP consensus of judgments directed toward the center of the framework or with a dispersed pattern

Abbreviations: EP, expert panel; EtD, evidence to decision framework; GRADE, Grading of Recommendations Assessment, Development and Evaluation.

[a] Data derived from GRADE Working Group materials.[114]

Inclusion Criteria

Studies were selected for inclusion in the systematic review of evidence if they met the following criteria: (1) the study included human patients; (2) the study population consisted of adult or pediatric patients with advanced solid malignancies, being considered for immune checkpoint inhibitor therapy, and adult and pediatric patients with possible Lynch syndrome; (3) the study was published in English; (4) the study compared, prospectively or retrospectively, laboratory testing methodologies for MMR and MSI; (5) the study addressed one of the key questions; (6) the study included measurable data such as diagnostic test characteristics, accuracy of MMR defect detection, survival outcomes or treatment response, germline testing or genetic counseling; (7) the studies must be peer-reviewed full-text articles. Detailed information about the inclusion criteria is available in the SDC.

Exclusion Criteria

Articles were excluded from the systematic review if they were meeting abstracts; noncomparative or qualitative studies, including editorials, commentaries, and letters; animal studies; full-text articles not available in English; and studies that did not address at least one of the key questions with outcomes of interest as agreed upon. Detailed information about the exclusion criteria is available in the SDC.

Certainty of Evidence

The included studies underwent a risk of bias assessment and the certainty of evidence underpinning each recommendation was assessed for an overall certainty of effect. Refer to the SDC for definitions of the certainty of evidence (Supplemental Table 2), the individual study risk of bias assessment (Supplemental Tables 3 and 4), and the aggregate certainty of evidence assessment (Supplemental Table 5).

Assessing the Strength of Recommendations

Development of recommendations required that the panel review the identified evidence and make a series of key judgments, using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Evidence to Decision (EtD) Framework.[8] See Table 1 for the definitions of strength of recommendation. Supplemental Table 6 found in the SDC provides a summary of the key judgments the panel considered, including the benefits and harms of each guideline statement using the GRADE EtD framework.[9]

RESULTS

Summary of Evidence

A total of 6642 studies met the eligibility requirements for screening. Based on review of these titles and abstracts, 427 articles met the inclusion criteria and continued to full-text review. A total of 103 articles were included for data extraction and qualitative analysis. Excluded articles were available as discussion or background references. Additional information about the systematic review is available in the SDC, including a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) table outlining details of the systematic review. Refer to the write-up for each recommendation for specific details about supporting evidence.

The panel convened 8 times (6 times by teleconference and 2 face-to-face meetings) to develop the scope, draft recommendations, review and respond to solicited feedback, and assess the certainty of evidence that supports the final recommendations. A nominal group technique was used for consensus decision-making to encourage unique input with balanced participation among group members. An open comment period was posted on the CAP Web site (www.cap.org) from February 19 to March 13, 2020, during which the draft recommendation statements were posted for public feedback. Refer to the SDC for more details. The EP approved the final recommendations with a unanimous vote.

An independent review panel, masked to the EP and vetted through the conflict of interest process, recommended approval by the CAP Council on Scientific Affairs. The manuscript was also approved by the Association for Molecular Pathology and Fight Colorectal Cancer. The final recommendations are summarized in Table 2.

Metrics for the evidence supporting the 6 recommendations are summarized in Tables 3 through 5. Complete GRADE evidence profiles, EtD frameworks, and PCR and IHC concordance tables are included in the SDC. As with most CAP evidence-based guidelines, the certainty of evidence is lowered by the presence of relatively few prospective studies. Recommendations 1 (CRC) and 6 (Lynch syndrome) had by far the most publications supporting the evidence framework. While the number of studies supporting Recommendation 4 (other cancer types) is comparable to that for Recommendation 3 (endometrial cancer), note that many different cancer types are represented in Recommendation 4. For a number of these references, multiple cancer types were represented in the studies. While such studies may have had a relatively large aggregate number of patients examined, the number from any one cancer type was relatively small.

Table 2. Summary of Guideline Statements	
Guideline Statement	**Strength of Recommendation**
1. For patients with CRC being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by 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	Strong Recommendation
Note: MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency	
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	Strong Recommendation
Note: This recommendation does not include esophageal squamous cell carcinoma	
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	Strong 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 mismatch repair defects has not been established	Conditional Recommendation
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	
5. For all cancer patients being considered for immune checkpoint inhibitor therapy based on 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	Strong Recommendation
6. For cancer patients being considered for immune checkpoint inhibitor therapy, if a mismatch repair deficiency consistent with Lynch syndrome is identified in the tumor, pathologists should communicate this finding to the treating physician	Strong Recommendation

Abbreviations: CRC, colorectal cancer; GEA, gastroesophageal adenocarcinoma; MMR-IHC, mismatch repair immunohistochemistry; MSI, microsatellite instability; NGS, next-generation sequencing; PCR, polymerase chain reaction; TMB, tumor mutation burden.

The EtD framework for each of the 6 recommendations is summarized in Table 5. For Recommendations 1, 2, 3, and 6, the EtD framework metrics were quite comparable. When paired with the magnitude of evidence supporting the recommended intervention, the EP members concluded that the strength of each of these recommendations was strong. Recommendation 5 (TMB) also had a strong recommendation designation, but against the use of TMB as a surrogate for measuring MMR or MSI. This was based on the paucity of evidence evaluating TMB coupled with metrics in the EtD (Table 5) shifting to the far left. The relatively high cost of determining TMB and its lower accessibility in clinical laboratories were significant factors contributing to this left shift. Recommendation 4 (other cancer types) was determined to be of conditional strength, based in part on the smaller number of patients for the many different individual cancer types represented in the studies. The low numbers contributed to a higher level of uncertainty among the panel members regarding the variability of results of different testing approaches on different cancer types.

Recommendation Statements

The discussion for recommendation statements follows guideline statement No. 4.

1. Strong Recommendation.—For patients with CRC who are being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by PCR for the detection of DNA MMR defects. Although MMR-IHC or MSI by PCR are preferred, pathologists may use a validated MSI by NGS assay for the detection of DNA MMR defects.

Note: MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency.[10] (Certainty of Evidence: Moderate).

The evidence for this statement included a total of 37 studies that evaluated the ability of MMR-IHC or MSI-PCR to detect DNA MMR defects and 8 studies that evaluated the ability of MSI by NGS to detect DNA MMR defects. To evaluate the diagnostic test characteristics of MMR-IHC, 7 studies defined MSI-PCR as the reference standard,[11–17] and 7 studies used verification of germline mutation to define a true positive.[18–24] Nine studies reported on the concordant DNA MMR defect status between MMR-IHC and germline testing.[19,21,22,24–29] To evaluate the diagnostic test characteristics of MSI-PCR, 4 studies defined MMR-IHC as the reference standard,[30–33] 2 studies used NGS for MSI detection tumor as a reference standard,[31,34] and 4 studies used germline mutation testing to verify MSI-PCR status.[18,20,23,24] Three additional studies reported on concordance of DNA MMR defect detection between MSI-PCR and germline testing.[35–37] Concordance of DNA MMR defect status between MMR-IHC and MSI-PCR was evaluated in 22 studies, with 5 studies defining MMR-IHC as the reference standard[30,32,38–40] and 17 studies using MSI-PCR.* For studies evaluating MSI using NGS, 5 studies used MSI-PCR as the reference standard[39,47–50]; 3 studies defined MMR-IHC as the reference standard[48,50,51]; and 1 study verified NGS status, using a single-molecule molecular inversion probe (smMIP) NGS assay against a genome-wide microsatellite instability NGS (mSINGS) assay.[34] Two studies reported on the concordance between MSI, using NGS and MMR-IHC status.[39,52]

The EP members concluded that the use of MMR-IHC and MSI-PCR for DNA MMR detection in patients with CRC was both very accurate and carried large benefits, and created only small harms. MMR-IHC is sensitive and

* *References 11–13, 16, 17, 20, 23, 24, 28, 29, 37, 41–46.*

Table 3. Recommendation Certainty of Evidence

Recommendation	No. PCS Studies	No. RCS Studies	Outcomes Used (Importance Ranking)	COE Grade[b]
1 - CRC	IHC/PCR: 7 NGS: 2	IHC/PCR: 30 NGS: 5	Diagnostic test characteristics (Critical) Status concordance[a] (Critical) Status concordance with germline testing (Important)	Moderate[c] Low[d]
2 - GEA and SI	IHC/PCR: 0 NGS: 1	IHC/PCR: 5 NGS: 0	Diagnostic test characteristics (Critical) Status concordance[a] (Critical)	Low[e]
3 - EC	4	13	Diagnostic test characteristics (Critical) Status concordance[a] (Critical) Status concordance with germline testing (Important)	Low[f]
4 - Other cancer	3	9	Diagnostic test characteristics (Critical) Status concordance[a] (Critical)	Very low[g]
5 - TMB	1	4	Diagnostic test characteristics (Critical) Status concordance[a] (Critical) Association with MMR-IHC status (Important)	Low[h]
6 - LS	6	22	Status concordance with germline testing (Critical) Association between LS prevalence and MSI status (Important)	Low[i]

Abbreviations: COE, certainty of evidence; CRC, colorectal carcinoma; dMMR, mismatch repair deficient; EC, endometrial carcinoma; GEA, gastroesophageal; GRADE, Grading of Recommendations Assessment, Development and Evaluation; IHC, immunohistochemistry; LS, Lynch syndrome; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, MSI-high; NGS, next-generation sequencing; PCR, polymerase chain reaction; PCS, prospective cohort study; RCS, retrospective cohort study; SI, small intestine; TMB, tumor mutation burden.

[a] Status concordance between any 2 testing assays of interest (MMR-IHC, MSI-PCR, MSI-NGS).

[b] Refer to supplemental digital content for a complete risk of bias assessment for individual studies (Supplemental Tables 3 and 4) and aggregate GRADE profile for each statement (Supplemental Table 5).

[c] Aggregate risk of bias for studies reporting on the critical outcomes of interest was serious for each outcome, but evidence was not further downgraded for any domain. For status concordance when compared with germline, aggregate risk of bias for MMR-IHC and MSI-PCR was serious, and evidence was further downgraded for inconsistency for both.

[d] Aggregate risk of bias for NGS studies reporting on outcomes of interest was serious for concordance with MMR-IHC and very serious for diagnostic test characteristics. MSI-NGS diagnostic test characteristic outcome evidence was further downgraded for inconsistency.

[e] Aggregate risk of bias for studies reporting on the outcomes of interest using MMR-IHC and MSI-PCR was very serious and extremely serious, based predominantly on identification of only retrospective studies. Additionally, there was inconsistency in reported status concordance across the studies; however, inconsistency was believed to be a consequence of differences in reference standards across the studies and evidence was not downgraded. The one study that compared status concordance for MSI-NGS and MMR-IHC carried a very serious risk of bias and reported concordance in a small subset of the study population.

[f] Aggregate risk of bias for studies reporting on the outcomes of interest ranged from serious through extremely serious. There was inconsistency noted in both IHC and PCR status concordance and in PCR diagnostic test characteristics outcomes. For status concordance, evidence was downgraded for inconsistency, but for PCR diagnostic test characteristics, the identified studies used different mononucleotide, dinucleotide, and single gene panels and this was believed to be the source of the inconsistency.

[g] Aggregate risk of bias for studies reporting on the outcomes of interest was very serious and extremely serious. Additionally, the identified large mixed population studies included mostly patients with CRC, leading to an overestimate of effect in other patient populations and evidence was downgraded.

[h] Aggregate risk of bias for studies reporting on the outcomes of interest was very serious, but evidence was not further downgraded for any domain for any outcome.

[i] Aggregate risk of bias for studies reporting on the outcomes of interest was serious and very serious. Outcome evidence was further downgraded for inconsistency when evaluating MMR-IHC or for indirectness when evaluating the association between Lynch syndrome prevalence and MSI status. Additionally, most of the identified studies comparing MMR-IHC and MSI-PCR status with germline testing only performed germline testing in dMMR/MSI-H cases, thus eliminating false negatives and perhaps overestimating sensitivity. Although this has been noted, evidence was not downgraded, as a study design with all patients tested for germline mutation would not be feasible.

specific for predicting MSI status in colorectal tumors. IHC is readily available in most clinical laboratories, is relatively inexpensive to perform, and does not require a significant amount of tumor tissue. MSI-PCR is slightly more technically challenging to perform and requires matched nonneoplastic tissue, with a slightly higher cost, and does not identify the specific gene that may be responsible for the MMR deficiency. Guidance for the use of MMR-IHC or MSI-PCR was deemed to be acceptable and feasible to implement. Refer to the SDC Supplemental Table 5 for the certainty of evidence assessment for this statement, Supplemental Table 6 for a complete summary of the EtD framework, and Supplemental Table 7 for the summary of the concordance data. Although limited by the number of studies that investigated MSI-NGS, the accuracy of MSI-NGS was considered comparable to that of MMR-IHC and MSI-PCR in patients with CRC, while both benefits and harms were judged to be moderate by the EP. The certainty of evidence for MSI-NGS was low (Table 3). NGS-based MSI testing can have several advantages over conventional capillary electrophoresis MSI-PCR methods. MSI-NGS can simultaneously interrogate mutations of other genes across cancer types; does not require matched nonneoplastic tissue; and interpretation is streamlined and semi-automated, which is likely to reduce interobserver and interlaboratory variation. The use of NGS does carry a higher cost when compared with IHC or PCR. The EP members concluded that inclusion of NGS as an option when validated would be acceptable to key stakeholders and feasible to implement especially if NGS tumor testing is being performed in a larger panel to identify other actionable therapeutic targets.

2. Strong Recommendation.—For patients with gastroesophageal and small bowel cancer who are 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 MMR defects.

Table 4. Number of Studies by Outcome

Test	Rec 1 CRC	Rec 2 GEA and SI	Rec 3 EC	Rec 4 Other Cancer	Rec 5 TMB	Rec 6 LS
MMR-IHC diagnostic test characteristics	2 PCS 8 RCS	2 RCS	3 RCS	2 PCS 2 RCS	0	0
MMR-IHC status concordance with germline testing	1 PCS 8 RCS	0	2 RCS	0	0	1 PCS 9 RCS
MSI-PCR diagnostic test characteristics	2 PCS 7 RCS	0	3 RCS	1 RCS	0	0
MSI-PCR status concordance with germline testing	1 PCS 2 RCS	0	1 RCS	0	0	3 PCS 10 RCS
MMR-IHC and MSI-PCR status concordance	6 PCS 16 RCS	4 RCS	2 PCS 7 RCS	2 RCS	0	0
MSI-NGS diagnostic test characteristics	1 PCS 5 RCS	0	1 PCS 2 RCS	5 RCS	0	0
MSI-NGS and MMR-IHC status concordance	1 PCS 1 RCS	1 PCS	2 PCS	1 PCS 1 RCS	0	0
MSI-NGS and MSI-PCR status concordance	0	0	0	1 RCS	0	0
TMB diagnostic test characteristics	0	0	0	0	1 RCS	0
TMB and MMR-IHC status concordance	0	0	0	0	2 RCS	0
TMB and MSI-NGS status concordance	0	0	0	0	1 PCS 2 RCS	0
Association between LS prevalence and MMR MSI status	0	0	0	0	0	5 PCS 10 RCS

Abbreviations: CRC, colorectal carcinoma; EC, endometrial carcinoma; GEA, gastroesophageal; IHC, immunohistochemistry; LS, Lynch syndrome; MMR, mismatch repair; MSI, microsatellite instability; NGS, next-generation sequencing; PCR, polymerase chain reaction; PCS, prospective cohort study; RCS, retrospective cohort study; Rec, recommendation; SI, small intestine; TMB, tumor mutation burden.

Note: This recommendation does not include esophageal squamous cell carcinoma. (Certainty of Evidence: Low).

The evidence base for this statement includes 1 prospective cohort study[53] and 5 retrospectively designed studies.[54–58] Two studies reported on the diagnostic test characteristics of MMR-IHC, using MSI-PCR in patients with gastroesophageal carcinoma[55,56]; 3 studies reported on the DNA MMR defect status between MMR-IHC and MSI-PCR in patients with gastroesophageal adenocarcinoma[54,55,58]; 1 study reported on the concordance of MMR-IHC and MSI-PCR in patients with duodenal carcinoma[57]; and the final study reported on the concordance of MSI-NGS and MMR-IHC in upper gastrointestinal (GI) cancers.[53] EP members concluded that detection of DNA MMR defects in patients with gastroesophageal and small bowel carcinoma by MMR-IHC and MSI by PCR was very accurate. After discussions, the EP members defined the benefits of both modalities as large and the harms as small. It is expected that this guidance will be acceptable to key stakeholders and feasible to implement. Discussions around resource requirements were focused on the assay costs as well as the cost to interpret the results; however, when compared with NGS, the EP concluded that this recommendation would result in moderate savings and would probably increase health equity. Refer to the SDC Supplemental Table 5 for the certainty of evidence assessment for this statement, Supplemental Table 6 for a complete summary of the EtD framework, and Supplemental Table 8 for the summary of the concordance data.

3. Strong Recommendation.—For patients with endometrial cancer who are being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC over MSI by PCR or NGS for the detection of DNA MMR defects. (Certainty of Evidence: Low).

The evidence informing this statement includes 2 prospectively designed studies[59,60] and 15 retrospectively designed studies,[31,34,48,52,61–71] all evaluating DNA MMR defect detection in patients with endometrial carcinoma. To evaluate the diagnostic test characteristics of MMR-IHC, 2 studies defined MSI-PCR as the reference standard,[65,67] and 1 study used germline testing to verify MMR tumor status.[68] To evaluate the diagnostic test characteristics of MSI by PCR, 2 studies used MMR-IHC as the reference standard,[31,66] and 2 validated the MSI status by using tumor sequencing.[31,34] Finally, to evaluate the diagnostic test characteristics of MSI by NGS, 1 study defined MMR-IHC as the reference standard,[61] 1 used MSI by PCR,[48] and another used NGS of the tumor.[34] Additional studies reported on the concordance of DNA MMR status between MMR-IHC and MSI by PCR,† MMR-IHC and germline mutations,[68,69] MSI by PCR and germline mutations,[69] and MSI by NGS and MMR-IHC.[52,61]

EP members concluded that DNA MMR defect detection using MMR-IHC was very accurate, but less accurate when using MSI by PCR or MSI by NGS. The benefits of testing with MMR-IHC were considered large, while the harms were defined as small. Resource requirements for MMR-IHC were considered negligible when compared to the other assay options. This guidance is expected to be acceptable to key stakeholders and feasible to implement. Refer to the SDC Supplemental Table 5 for the certainty of evidence assessment for this statement, Supplemental Table 6 for a complete summary of the EtD framework, and Supplemental Table 9 for the summary of the concordance data.

† *References 59, 60, 62–64, 66, 67, 70, 71.*

Table 5. Summary of GRADE Evidence-to-Decision Framework[a]					
Criteria	**Favors the Comparison**		**Neutral**	**Favors the Intervention**	
Problem	No	Probably no		Probably yes	Yes
					R1, R2, R3, R4, R5, R6
Test accuracy	Very inaccurate	Inaccurate		Accurate	Very accurate
		R5		R4	R1, R2, R3
Desirable effects	Trivial	Small		Moderate	Large
		R5		R4	R1, R2, R3, R6
Undesirable effects	Large	Moderate		Small	Trivial
		R5		R1, R2, R3, R4, R6	
Certainty of effects	Very low	Low		Moderate	High
	R4	R2, R3, R5, R6		R1	
Values	Important uncertainty of variability	Possibly important uncertainty of variability		Probably no important uncertainty of variability	No important uncertainty of variability
	R4, R5	R2, R3, R6		R1	
Balance of effects	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the intervention
	R5			R4	R1, R2, R3, R6
Resources required	Large cost	Moderate cost	Negligible costs and savings	Moderate savings	Large savings
	R5	R4	R1, R2, R6	R2	
Equity	Reduced	Probably reduced	Probably no impact	Probably increased	Increased
	R5		R1, R3	R2, R4	
Acceptability	No	Probably no		Probably yes	Yes
	R5			R4, R6	R1, R2, R3
Feasibility	No	Probably no		Probably yes	Yes
	R5			R4	R1, R2, R3, R6

Abbreviations: GRADE, Grading of Recommendations Assessment, Development and Evaluation; R1, Recommendation statement 1; R2, Recommendation statement 2; R3, Recommendation statement 3; R4, Recommendation statement 4; R5, Recommendation statement 5; R6, Recommendation statement 6.

[a] "Test accuracy domains" does not apply to Recommendation 6. For a detailed description of the GRADE evidence-to-decision framework, please see the supplemental digital content.

4. Conditional Recommendation.—For patients with cancer types other than CRC, gastroesophageal adenocarcinoma, small bowel, and endometrial, who are being considered for immune checkpoint inhibitor therapy, pathologists should test for DNA MMR, 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 MMR defects. (Certainty of Evidence: Low).

The evidence base informing this statement includes 1 prospective study,[72] 1 study with both prospective and retrospective arms,[43] and 11 studies with a retrospective design.[34,48,73–81] Of these 13 studies, MMR-IHC was evaluated in renal cell carcinoma[76] and across multiple cancer types[43,72,75]; MSI by PCR was evaluated in prostate cancer,[34] breast cancer,[77] and across multiple cancer types[75]; and MSI by NGS was evaluated in prostate cancer[34,73,78] and across multiple cancer types.[34,48,79–81] For the studies including large populations of patients with different cancer types, most of these patients had CRC but outcomes were calculated for the entire cohort, which potentially led to an overestimate of effect in the patients with other cancer types. Evidence was further downgraded for this confounding domain.

Because of the paucity of evidence for cancer types outside the GI tract or endometrium, EP members were unable to determine which specific assay would most accurately detect DNA MMR defects in patients with carcinoma not covered by recommendations 1 through 3. However, the benefits of testing to identify patients potentially eligible for immune checkpoint inhibitor therapy were considered moderate and the harms small. Therefore, this recommendation promotes testing but without specific direction on how. Absent sufficient specific publications on-point, pathologists are advised to rely on admittedly imperfect surrogates of assay utility. Such surrogates include cost, availability, and amount of tissue required. Considering such imperfect criteria, the EP favors performing MMR-IHC to evaluate for MMR defects in these other cancer types. EP members expect this statement to probably be acceptable to key stakeholders and feasible to implement. Refer to Supplemental Table 5 in the SDC for the certainty of evidence assessment for this statement, and Supplemental Table 6 for a complete summary of the EtD framework.

Discussion Supporting Guideline Statements 1 to 4

For patients with CRC, the EP found that MMR-IHC, MSI-PCR, and MSI-NGS had comparable performance

metrics (Table 5). MMR-IHC and MSI-PCR may be the preferred screening methods, as NGS-based tumor profiling assays tend to require significantly more tissue because the DNA input requirements are typically 500 ng to 1 μg. Biopsy samples, typically the standard specimen type for CRCs submitted for MMR-IHC and MSI-PCR, can pose a challenge for NGS assays as tissue may be limited.[49] MMR-IHC also has the advantage of identifying the most probable gene defect. NGS may also not be able to accurately identify MSI-Low (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. Specialized laboratory staff expertise is also an issue, as a minority of hospitals can currently perform NGS in their clinical laboratory, necessitating sending the specimens to reference laboratories, therefore causing greater turnaround time delays and possible increased expense.

A large study including 645 upper GI tract carcinomas found 100% concordance between MSI-NGS and MMR-IHC, with only 1 indeterminate case. Twenty of 23 cases classified to have MSI had available tumor tissue for IHC. Nineteen of 20 (95%) showed loss of expression of at least 1 MMR protein, including 14 with loss of MLH1 and PMS2; 2 with loss of PMS2; 2 with loss of MSH2 and MSH6; and 1 with loss of MSH6. The final case showed an unusual IHC pattern, with nuclear staining of 5% of tumor nuclei for all 4 MMR proteins and was interpreted as indeterminate.[53] In a more recent study,[82] 100 small intestinal adenocarcinomas were examined by MMR-IHC and MSI-NGS. Twenty-six percent (26 of 100) demonstrated IHC loss of an MMR protein, primarily MLH1 and PMS2, secondary to *MLH1* methylation. Nearly all of these were MSI-H when assessed by an NGS assay. However, 1 case was indeterminate for MSI, and 5 others did not have NGS data available for unspecified reasons. Additional studies may help to increase the certainty of evidence supporting Recommendation 2.

MMR deficiency is known to confer different molecular phenotypes in endometrial cancers compared to CRCs, so tissue-based testing of patients with endometrial cancer can be more problematic. Endometrial cancers and colon cancers from the same patient can exhibit different levels of MSI, and *MSH6* mutation carriers are more likely to develop endometrial cancers than those with MSI-L tumors.[75] The prevalence of MSI-L or microsatellite stable (MSS) status in endometrial cancers with MSH6 deficiency can range from 29% to 50%.[83,84] IHC testing in endometrial cancers reliably detects loss of MSH6 protein expression, whereas MSI-PCR and MSI-NGS can be less sensitive in detecting MSI-L tumors. Many of the studies examining the performance of MSI-NGS in endometrial cancer arbitrarily classified MSI-L tumors as MSS[34,47–49,52,61,74] and did not include IHC testing for comparison. In the studies that included MSI-L endometrial cases, NGS failed to correctly identify the MSI-L status[61,74] in all but 1 study that identified 1 "MSH-6 equivocal" endometrial case as MSI-H; 2 MSI-H cases were missed in this same study.[52]

Another study of 259 endometrial cancers used NGS and classified samples as MMR deficient (n = 48), proficient (n = 199), or indeterminate (n = 12).[61] Sequencing findings were concordant with loss of expression of at least 1 MMR protein in 47 of 48 cases (98%) classified as MMR deficient and retained expression of all 4 proteins in 190 of 199 cases (95%) classified as MMR proficient. Of the 12 cases classified as indeterminate, 7 (58%) demonstrated MMR protein loss. The authors hypothesized that of the 9 cases predicted to be proficient by sequencing with loss of at least 1 MMR protein expression by IHC, the discordance may have been due to several factors. Two of the 9 cancers exhibited isolated loss of MSH6 expression. Three of the 9 cancers showed low variant allele fractions of less than 10% for pathogenic somatic mutations, consistent with specimens with low tumor purity. No definitive explanation for discordance between MSI-NGS and MMR-IHC could be identified for the remaining 4 endometrial cancers.

Another study compared MSI-NGS to MSI-PCR, with the NGS platform able to correctly classify all endometrial carcinomas except for 1 tumor that was falsely diagnosed as MSS by NGS and PCR, which was confirmed to harbor double somatic mutations in the MMR gene *MSH6* and had loss of MMR gene expression by IHC.[47] In this same study, 6 other endometrial cancers were incorrectly designated as MSS by PCR. Four of these demonstrated loss of MMR protein by IHC and had double somatic pathogenic mutations in the corresponding MMR genes by targeted sequencing. One MSS endometrial cancer had equivocal IHC results but demonstrated homozygous pathogenic mutations in *MSH6*. One MSS endometrial tumor carried only a single somatic mutation in *MSH2* and was associated with IHC loss of MSH2 and MSH6.

While a number of studies have demonstrated that NGS can accurately establish MSI status for CRCs, the broad application of such algorithms to other solid tumor types requires additional research. With the current evidence, it is difficult to assess MMR-IHC, MSI-PCR, and MSI-NGS in many cancer types owing to the low numbers of individual tumor types with MMR deficiency represented in these studies. One study examined IHC, MSI-PCR, and MSI-NGS in 26 cancer types. NGS missed 14 cases in comparison to IHC in 1986 matched cases (NGS with an 87.1% sensitivity, and specificity of 99.6%), but was better than MSI-PCR in 2189 matched cases for CRC (100% sensitivity and 99.9% specificity), but not in other cancers (95.8% sensitivity and 99.9% specificity). The authors hypothesized that because the MSI-PCR test was developed for CRC, MSI-NGS discrepancies in non-CRC cancer types may have been due to other loci being involved in these cancer types, which are not measured by the MSI-PCR method.[48] They further hypothesized that for IHC expression, there may be a subset of dMMR cases with relatively low microsatellite alterations, which are identified as MSS by NGS, that have lower rates of response to PD-1 inhibition than do dMMR cases that are MSI-H.[48]

IHC has been found to be effective in evaluating MMR status in prostate cancer, even in limited biopsy specimens.[85] Three studies examining MSI-NGS in comparison to IHC and MSI-PCR in prostate cancer seemed to find that IHC was the optimal testing strategy. Hempelmann et al[73] examined 2 NGS platforms (18 and >60 markers, respectively), compared to MSI-PCR, with a few cases of IHC performed on autopsy tissue. In a set of 91 prostate tumors with known MMR status (29-deficient and 62-intact mismatch-repair), the smaller NGS panel had a sensitivity of 96.6% (28 of 29) and a specificity of 100% (62 of 62), the larger NGS panel had a sensitivity of 93.1% (27 of 29) and a specificity of 98.4% (61 of 62), and MSI-PCR had a sensitivity of 72.4% (21 of 29) and a specificity of 100% (62 of 62). The authors concluded that the MSI-PCR algorithm that had been developed and validated for colon cancer had inferior sensitivity when applied to prostate

cancer and that NGS performs more robustly.[73] They also examined 21 prostate cancers with corresponding IHC data (7 MMR-deficient, 14 MMR-intact). The results of the small-panel NGS, large-panel NGS, and MSI-PCR were concordant for these 21 samples (7 of 7 MMR-deficient were MSI-positive; 14 of 14 MMR-intact were MSI-negative). The IHC results were consistent with the findings of the other approaches in all but 2 of the 21 cases. One case had isolated loss of MSH6 by IHC, and another had isolated loss of PMS2 by IHC.[73]

One study validated smMIP-based NGS with MSI-PCR in colorectal, prostate, and endometrial cancers.[34] Of the 144 colorectal, prostate, and endometrial cancers tested, 1 MSI-H endometrial tumor was falsely diagnosed as MSS by smMIP-based NGS, with 100% diagnostic sensitivity and 100% diagnostic specificity for both colorectal and prostate tumors, whereas diagnostic sensitivity of 95.8% and diagnostic specificity of 100% were obtained for endometrial cancers. Two prostate tumors had somatic pathogenic variants in MMR genes by sequencing but were incorrectly classified as MSS by PCR.

Another group prospectively analyzed 1551 tumors from 1346 patients with prostate cancer via targeted NGS (MSIsensor) and IHC in select cases.[78] Among the 1033 patients (67%) who had adequate tumor quality for MSI sensor analysis, 32 patients (3.1%) with prostate cancer had dMMR by IHC. Twenty-three dMMR prostate tumors had high MSIsensor scores, and 9 had indeterminate scores with evidence of dMMR. Seven of the 32 MMR patients (21.9%) had pathogenic variants in a Lynch syndrome–associated gene. Three hundred eighty-four prostate tumors (24.8%) with quality scores sufficiently high for mutational calling had insufficient sequence coverage or tumor purity for MSIsensor analysis. Nine of 313 patients (2.9%) with inadequate tissue quality for MSIsensor analysis had high TMB and/or a somatic mutation in an MMR-associated gene. Two patients were found to have loss of MMR proteins on IHC analysis, consistent with dMMR.

Upper tract urothelial carcinoma (UTUC) is known to be the third most common cancer arising in patients with Lynch syndrome, following gastrointestinal malignancies and endometrial cancer.[86] From the few articles examining UTUC, it appears that sporadic MMR deficiency due to *MLH1* gene methylation is exceptionally uncommon.[87,88] There are insufficient data to recommend any specific method of testing for MMR deficiency in UTUC.

Studies that analyzed other tumor types, such as variants of thyroid, stomach, cervix, melanoma, lung, glioblastoma and hematopoietic malignancies, examined too few cases with dMMR/MSI-H to make any reliable conclusions regarding choice of tissue test.[48,80,81,89,90] From the systematic review of the literature, it appears that validation of the PCR-based assays for individual tumor types is necessary. There is no literature to suggest that such individualized validation is necessary for IHC, therefore IHC may be the most feasible choice.

5. Strong Recommendations.—For all cancer patients being considered for immune checkpoint inhibitor therapy, based on defective MMR, pathologists should NOT use TMB as a surrogate for the detection of DNA MMR defects. If a tumor is identified as TMB-High (TMB-H), pathologists may perform IHC and/or MSI by PCR to determine if high TMB is secondary to MMR deficiency. (Certainty of Evidence: Low).

The evidence for this statement included a total of 5 studies that evaluated the use of TMB as a surrogate for DNA MMR defects in CRC,[48,91,92] gastroesophageal cancer,[93] endometrial carcinoma,[48] and glioma (Table 3).[94] From the available evidence, EP members concluded that TMB use as a surrogate for dMMR was inaccurate and would carry small benefits and moderate harms. The EP also concluded that the use of TMB would carry large costs and reduce health equity. When the low certainty of evidence was paired with the other domains of the EtD framework, the EP decided to strongly recommend against the use of TMB as a surrogate for MMR deficiency testing. This guidance is expected to be acceptable to key stakeholders and feasible to implement. Refer to Supplemental Table 5 in the SDC for the certainty of evidence assessment for this statement, and Supplemental Table 6 for a complete summary of the EtD framework.

While some studies suggested that increased TMB is often observed in dMMR neoplasms, a subset of extremely elevated TMB values was associated with other etiologies, such as *POLE* exonuclease-domain mutations in CRC.[92] One included study evaluating MSI and TMB status using an 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 per megabase).[48] Also, although there was 95% concordance between elevated TMB and MSI-H status in CRCs, only 57% of MSI-H endometrial cancers were 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.[95] The evaluated studies and assessment of the EP indicate that although there is often 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. This assessment was also echoed in comments received during the open comment period.

Although evaluation of TMB as a potential separate biomarker for immunotherapy response was beyond the scope of this guideline, the EP notes that in the summer of 2020, the US FDA approved TMB-H as a pan-cancer biomarker for pembrolizumab therapy, based on the KEYNOTE 158 clinical trial (A Clinical Trial of Pembrolizumab [MK-3475] Evaluating Predictive Biomarkers in Subjects With Advanced Solid Tumors),[96] which demonstrated an objective response rate similar to that observed in patients with non-CRC MSI-H cancer who were enrolled in KEYNOTE 158,[97] although the number of patients in each study was relatively small. The ability of TMB to predict immunotherapy response relative to dMMR has not been extensively evaluated. Limited studies suggest that these biomarkers may have nuanced abilities to predict therapeutic response such that MSI-H and high levels of TMB both appear to predict response to immunotherapy on a continuum, with some low-score MSI-H (as determined by NGS) tumors with lower TMB having lower response rates to immunotherapy.[98,99] In other words, MSI and TMB may not be binary variables with respect to response to immune checkpoint inhibitor therapy. Across different individual studies and assays, "TMB-H" is often variably defined owing to use of different technical methods and variable bioinformatic approaches,[48,95] which complicates comparison of TMB categories and values between studies. Although some data suggest a general concordance of bioinformatic approaches using archival, high-quality data

across pipelines,[100] comparison of methods using clinical materials is likely to be complicated by spatial tumor heterogeneity and tissue selected for testing.[101] The EP recognizes that TMB may be an additional, independent biomarker of immunotherapy response, but important considerations and limitations exist in assessing the utility of TMB, using the wide variety of methods that are currently in clinical use. Once sufficient published data are available, a separate evidence-based guideline may be useful in the evaluation of TMB as a biomarker for the selection of immune checkpoint inhibitor therapy.

6. Strong Recommendation.—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. (Certainty of Evidence: Low).

The evidence informing this statement includes 6 prospectively designed studies and 22 retrospectively designed studies, all examining patients with colorectal carcinoma or endometrial carcinoma (Table 3). Studies reported on the concordance between dMMR status using MMR-IHC[19,21,24–29,68,69] or MSI by PCR‡ and confirmed germline mutation, or the concordance between a Lynch syndrome detection algorithm that included MMR-IHC and/or MSI by PCR and confirmed germline mutation.§ A significant limitation of most of these studies is that germline testing was performed only in dMMR cases, thus eliminating false negatives and perhaps overestimating sensitivity. This limitation was noted, but evidence was not downgraded, as a study design examining for germline mutations in all patients, including those with tumors with intact MMR, would not be feasible.

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.[107,108] This will facilitate not only increased cancer screening in patients with Lynch syndrome but also germline testing of potentially impacted family members. EP members concluded that communication of the potential for Lynch syndrome would provide large benefits and create only small harms; this guidance would increase health equity by maximizing the identification of patients and families at risk for a heritable cancer syndrome and would be feasible to implement. Health care disparities may still hinder universal access to genetic counseling and germline testing following the initial communication, as minority patients are less likely to be referred for genetic evaluation or to undergo germline testing for Lynch syndrome.[109] Communication of important pathology findings may be more readily operationalized in hospital-based settings where pathologists and other types of physicians interact regularly. From the pathologists' perspective, however, implementation should be feasible irrespective of practice setting and would not be connected to significant cost. With potential perceived burden to pathologists, the EP expects that this guidance will probably be acceptable to key stakeholders. One factor which limits the potential burden to pathologists is that these communication 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.

Refer to Supplemental Table 5 in the SDC for the certainty of evidence assessment for this statement, and Supplemental Table 6 for a complete summary of the EtD framework.

The EP suggests operationalizing this recommendation by using existing communication mechanisms for dMMR or MSI identified on routine Lynch syndrome screening, with an appreciation for the fact that the role of *MLH1* promoter hypermethylation is less well established in tumors other than colorectal and endometrial carcinoma. Moreover, in patients with a concerning personal or family cancer history, genetic testing may be warranted irrespective of *MLH1* promoter hypermethylation status.[110–112] A sample pathology report text for tumors with different mismatch repair protein loss patterns is provided below. Direct communication with clinicians regarding this result is recommended and should be recorded in the pathology report comment. Alternatively, for MLH1/PMS2-deficient cancers this direct communication may occur after the results of reflexive *MLH1* promoter hypermethylation testing when that test yields a negative result. Pathologists should use good judgement regarding communication strategies for patients at an increased risk for Lynch syndrome. For example, if standard clinical workflows are in place to take action based on universal Lynch syndrome screening results for colon and/or endometrial cancer within the pathologist's health care practice, the written pathology report with standard follow-up recommendations may suffice as adequate communication. For potential patients with Lynch syndrome identified out of the scope of universal screening workflows, direct communication with documentation may be needed to alert treating physicians of an unanticipated hereditary risk.

For tumors with dual MLH1/PMS2 IHC loss:

> *To determine if this patient's tumor is amenable to therapies for mismatch repair-deficient cancers, immunohistochemistry was performed with antibodies to the mismatch repair proteins MLH1, MSH2, MSH6 and PMS2 on histologic sections from block (X). There is loss of expression in tumor cells of MLH1 and PMS2, whereas MSH2 and MSH6 are intact. Appropriate internal control staining is present. While most tumors showing this immunophenotype are sporadic in etiology, further evaluation and germline genetic testing for hereditary risk should be considered in the appropriate clinical context, with close attention to the personal and family history. Dr. (X) communicated this finding to Dr. (Y) on (date).*

For tumors with MSH2/MSH6 loss, or isolated PMS2 loss:

> *To determine if this patient's tumor is amenable to therapies for mismatch repair-deficient cancers, immunohistochemistry was performed with antibodies to the mismatch repair proteins MLH1, MSH2, MSH6 and PMS2 on histologic sections from block (X). There is loss of expression in tumor cells of MSH2 (and/or) MSH6 (or) PMS2, whereas MLH1 is intact. Appropriate internal control staining is present. Tumors showing this immunophenotype are frequently hereditary in etiology and genetic evaluation and testing should be considered in the appropriate clinical context. Dr. (X) communicated this finding to Dr. (Y) on (date).*

‡ *References 23, 24, 28, 29, 32, 35–37, 41, 60, 66, 69, 71.*
§ *References 20, 23, 28, 36, 42, 45, 59, 60, 64, 69, 102–106.*

Table 6. Summary of Good Practice Statements
1. Discordant results: In the event of discordant results, pathologists should interpret any evidence of MMR deficiency by IHC or MSI by NGS or PCR as a positive result for patients to be eligible for immune checkpoint inhibitor therapy. Discordant results should be reviewed to ensure that the discordance is not due to an interpretive error
2. Indeterminate results: In the event of an 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
3. Subclonal loss: In the event of a clonal loss by MMR-IHC, pathologists should perform MSI by PCR specifically in a dissected area of tumor that has IHC loss of MMR protein if the patient is being considered for checkpoint inhibitor clinical trials

Abbreviations: IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; NGS, next-generation sequencing; PCR, polymerase chain reaction.

Good Practice Statements

According to the GRADE approach, good practice statements (GPS) are recommendations that panels may consider important but are not appropriate to be formally rated for certainty of evidence. The EP co-chairs followed a framework to review the questions for the GPS (Supplemental Figure 3). A targeted literature search was performed on the basis of these questions. The EP included 3 GPS (Table 6), which reflect expert consensus opinions supported by a limited number of studies and data that were not formally included in the evidence-base or systematically rated.

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

The EP recognized that the results of MMR-IHC, MSI by PCR, and MSI by NGS are not always concordant, especially for cancers outside the GI tract. In the event of discordant results (Figure 1, A through E), every effort should be made to ensure that the discordance is not due to an interpretive error with repeated testing and use of additional orthogonal testing methods. While the pathologist may interpret any evidence of MMR deficiency as a positive result for patients to be eligible for immune checkpoint inhibitor therapy, discussion of the discordant results with the treating physician is encouraged to allow for optimal treatment selection based on the clinical scenario and the clinician's discretion. Additionally, if a primary method indicates intact MMR in a patient with high clinical index of suspicion for dMMR, consider using a second method.

2. Indeterminate Results.—In the event of an 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.

In the event of an indeterminate result (Figure 2, A and B) in any method, pathologists should perform an orthogonal technique or repeat the same assay on a different tumor block. For example, MMR-IHC sometimes does not work well in larger resection specimens that have had prolonged ischemia times before fixation or are inadequately fixed in formalin. For these cases, MMR-IHC can typically be performed successfully by using the biopsy specimen that preceded the surgery. This approach has been used successfully numerous times when MMR-IHC did not work well in colectomy, hysterectomy, and prostatectomy surgical specimens. Alternatively, if a biopsy specimen is not available, an alternative block from the surgical resection can be used for MMR-IHC. When MMR-IHC is indeterminate, PCR-based approaches may yield more interpretable results. Laboratories should have a robust peer review process for indeterminate cases in which the opinion of a more experienced pathologist or consensus review by a group of pathologists is used.

3. 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 of MMR protein if the patient is being considered for checkpoint inhibitor clinical trials.

The clinical significance of subclonal loss by MMR-IHC (Figure 3, A through D)—where abrupt, complete loss of expression of 1 or more MMR proteins is observed in a discrete area of tumor immediately juxtaposed to tumor showing intact MMR protein expression in the setting of preserved internal control positivity throughout both regions—is uncertain. Therefore, if a more definitive testing result is required to enter a patient into checkpoint inhibitor clinical trial, pathologists should perform MSI assessment by an orthogonal method specifically in a dissected area of tumor that has IHC loss of an MMR protein. The EP notes that many cases of subclonal loss can be interpreted as such without confirmation by an orthogonal method owing to the high correlation with MSI-H, and that secondary review by a pathologist experienced in accurately interpreting such variant patterns may be helpful. It is uncertain whether patients with tumors with subclonal MMR protein loss have response to immune checkpoint inhibitor therapy.

CONCLUSIONS

Readers may note the evidence-based guideline's emphasis on IHC, particularly for tumor types other than colorectal and gastroesophageal/gastroesophageal junction/small bowel. Large-panel NGS undoubtedly provides more genomic information for cancer patients, sometimes even identifying patients with Lynch syndrome,[82] but amount of data was not the central issue addressed by the guideline EP. The evidence suggests that MSI-NGS is a good assay for patients with CRC and gastroesophageal/gastroesophageal junction/small bowel cancer who are being considered for immune checkpoint inhibitor therapy. However, these same MSI-NGS approaches often fall well short for other cancer types. There is insufficient published evidence to assess their 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. Another shortcoming of several studies examining the MSI-NGS approach is that claims of near 100% concordance with MMR-IHC or MSI-PCR typically did not account for cases excluded because of low specimen tumor cell purity (nearly 25% of cases in one study[113]) or because of inconclusive results (15% from one study[80]). Clearly the limitation of low tumor cell fraction for NGS[78,81] is an important issue for many biopsies or fine-needle aspirates

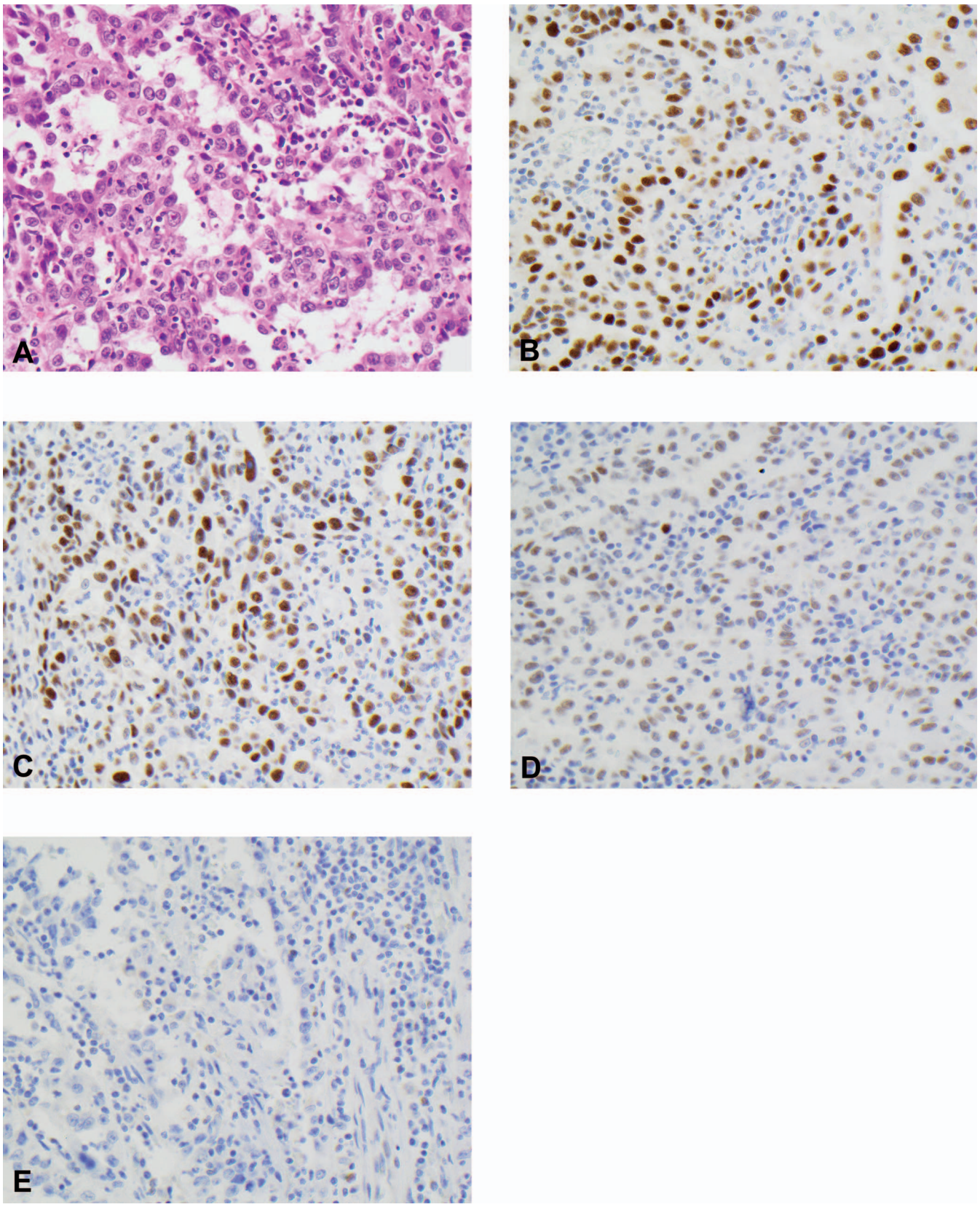

Figure 1. *MSI-low endometrial carcinoma (A) that was shown to have immunohistochemical loss of a DNA MMR protein. The carcinoma has intact nuclear expression of MLH1 (B), PMS2 (C), and MSH2 (D). The tumor demonstrates loss of MSH6 nuclear expression (E). Subsequently, a deleterious* MSH6 *germline mutation was identified in this patient (hematoxylin-eosin, original magnification ×20 [A]; original magnification ×20 [B through E]). Abbreviations: MLH1, mutL homolog 1; MMR, mismatch repair; MSH2, mutS homolog 2; MSH6, mutS homolog 6; MSI, microsatellite instability; PMS2, PMS1 homolog 2.*

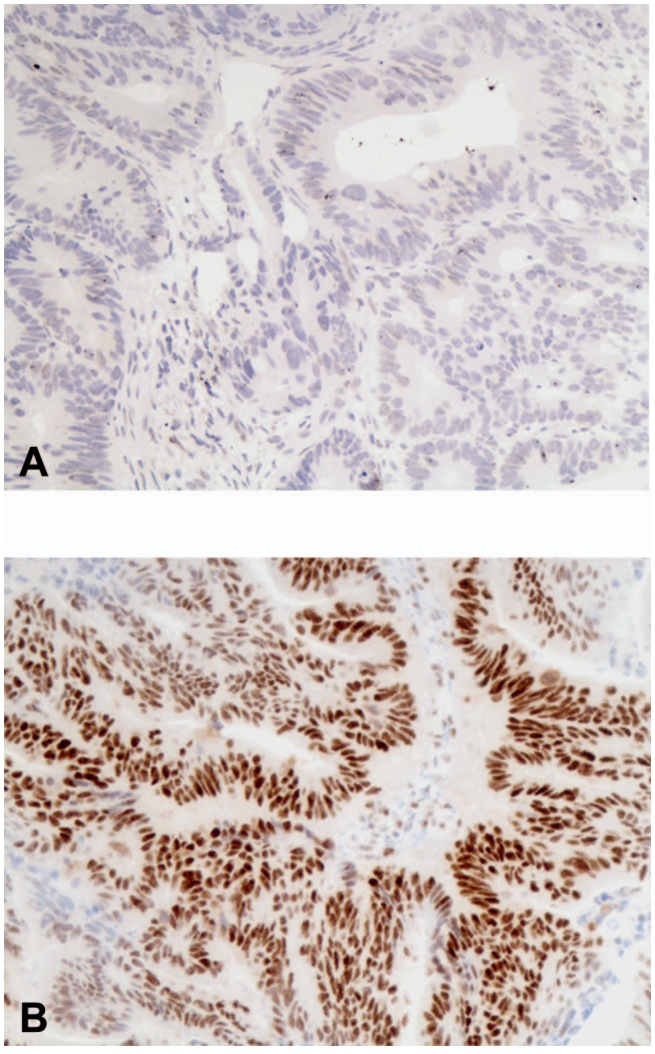

Figure 2. *Colorectal adenocarcinoma bulky metastasis to the liver, initially with indeterminate immunohistochemistry results for MLH1 (A). Note that tumor cell nuclei have loss of MLH1 expression, but there is also lack of nuclear expression of MLH1 in adjacent stromal cells. MLH1 immunohistochemistry was repeated by using a different block of the metastasis (B), this time yielding definitive strongly and diffusely positive intact nuclear expression of MLH1 (original magnification ×20 [A and B]). Abbreviation: MLH1, mutL homolog 1.*

for which the diagnosis of malignancy may be based on the presence of just a few tumor cells. There are published large studies of the utility of MSI-NGS, encompassing thousands of patients with many different cancer types; typically, the MSI data are validated with MMR-IHC or MSI-PCR only in patients with colorectal or endometrial cancer, because dMMR is more common in these cancer types. The critical cross-assay validations are lacking for many other cancer types. These guideline recommendations may run counter, especially regarding NGS assays, to the recommendations reported by other groups, such as the National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO). Here, it is important to recognize that the CAP clinical practice guidelines are based on evidence as endorsed by the National Academy of Medicine, while NCCN guidelines are based on consensus and ESMO guidelines may either be consensus or evidence based.

It should be noted that MMR-IHC is optimal only if the pathologist is competent in the interpretation of MMR-specific protein expression in different types of tumors. Training in the interpretation of MMR-IHC should be encouraged as a part of residency education and reviewed periodically in practice with experts, peers, or as a part of continuing education. It must also be noted that a considerable amount of literature used to build the evidence for the guideline was extracted from Lynch syndrome–based testing, which was not the focus of this guideline and may be an imperfect surrogate for MMR testing for immune checkpoint inhibitor therapy. Interpretation of MMR-IHC testing to identify possible patients with Lynch syndrome is a binary result (intact protein expression versus lost protein expression). The optimal paradigm for immune checkpoint inhibitor therapy may be more complex, involving a combined assessment of MMR and MSI, number of tumor somatic mutations, and number of tumor-infiltrating lymphocytes.

It is clear from the comprehensive literature review informing this evidence-based guideline that clinical laboratory detection of MMR/MSI is complex and likely a "one size fits all" approach cannot be applied, at least not at this time. While this may be a frustrating message, the pathology and oncology communities should embrace this as an opportunity. We are well equipped to strategically bridge this data gap and provide the published evidence with well-designed studies of different cancer types. To this point, a major limitation of most of the current peer-reviewed literature summarizing checkpoint clinical trials is that MMR-IHC, MSI-PCR, MSI-NGS, and TMB-NGS are often treated as synonymous, interchangeable data points. Therefore, from the current literature it is not possible to determine which assays are associated with best response with immune checkpoint inhibitor therapy. Examination of the peer-reviewed literature clearly revealed that these 4 assays can often yield data that do not overlap. This is especially true for cancer types outside the GI tract. Thus, another opportunity moving forward is to carefully analyze the best responders in the currently published clinical trials. Which specific assay was used for these best responders? Is there a unifying theme?

Further complicating the issue of identifying the cancer patients most likely to have best response to checkpoint inhibitor blockade is that other clinical assays, such as programmed death ligand-1 (PD-L1) IHC and TMB-NGS, are FDA approved for this purpose. While some cancers may have overlapping PD-L1 positivity, MMR defects, and high TMB, many cancers do not.[48,95] It is entirely unclear at this point how all these assays should be used for individual cancer patients. In cancer types in which MMR defects are less common, such as head and neck squamous cell carcinoma, PD-L1 IHC is a logical first step in assessment. However, there is currently no data on the utility, if any, of a stepwise addition of further testing in patients with melanoma and lung cancer who have PD-L1 negative tumors. Similarly, the utility and cost effectiveness of adding a TMB-NGS assay when a patient has a PD-L1–negative, MMR-intact cancer is unknown. Again, this represents excellent opportunities to provide the peer-reviewed data to help bridge these critical knowledge gaps.

Guideline Revision

This guideline will be reviewed every 4 years, or earlier in the event of publication of substantive and high-quality

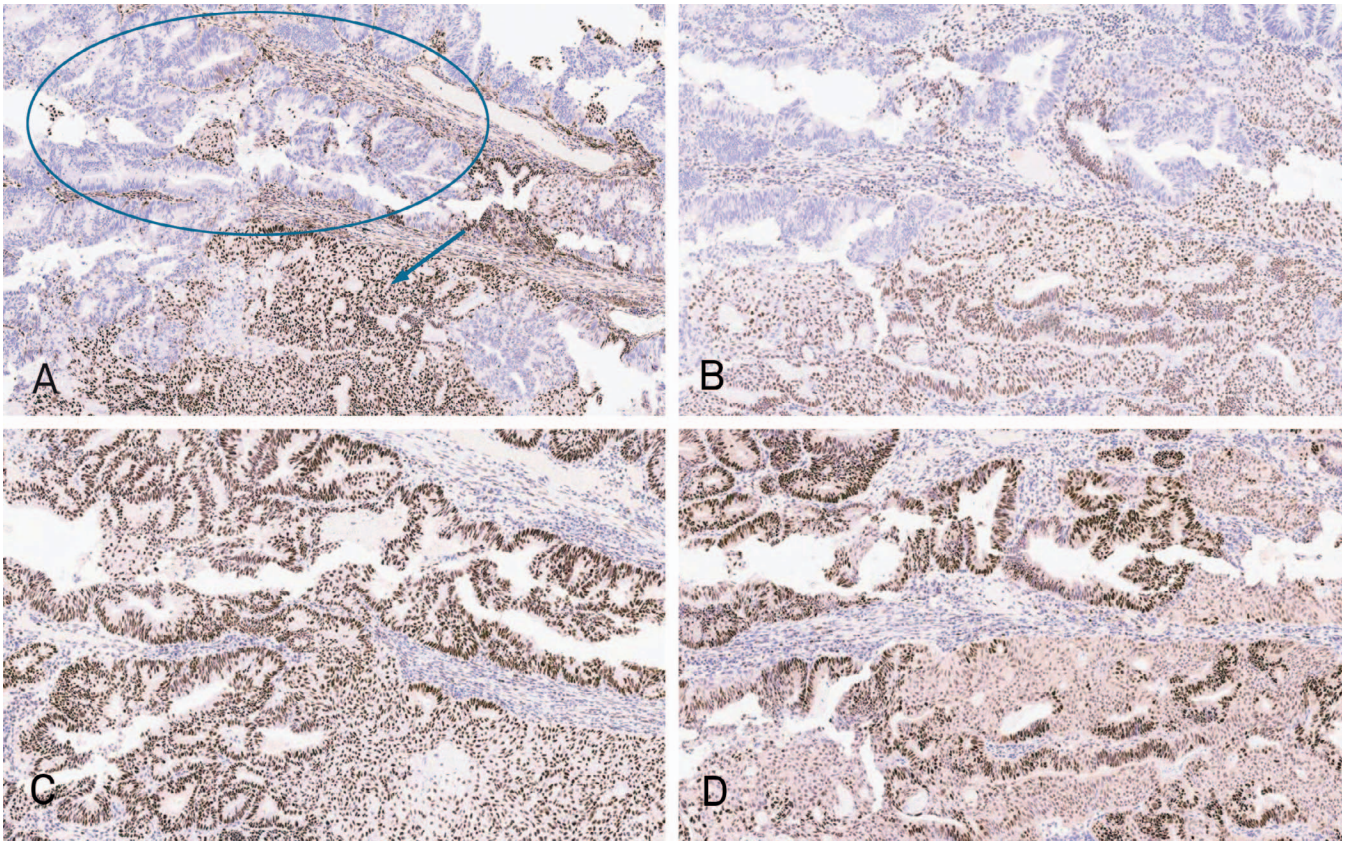

Figure 3. *Endometrial endometrioid adenocarcinoma with subclonal immunohistochemical loss of MLH1 (A) and PMS2 (B). Nuclear expression of MSH2 (C) and MSH6 (D) are retained. For MLH1 and PMS2, note foci of tumor with loss of nuclear MLH1 and PMS2 (circle in A) with immediately adjacent stromal cells and tumor (arrow in A) with intact positive expression of MLH1 and PMS2. Images courtesy of Barrett Lawson, MD, from MD Anderson Cancer Center, Houston, Texas (original magnification ×10 [A through D]). Abbreviations: MLH1, mutL homolog 1; MSH2, mutS homolog 2; MSH6, mutS homolog 6; PMS2, PMS1 homolog 2.*

evidence that could potentially alter the original guideline recommendations. If necessary, the entire panel will reconvene to discuss potential changes. When appropriate, the panel will recommend revision of the guideline to the CAP in collaboration with the AMP, ASCO, and Fight Colorectal Cancer for review and approval.

Disclaimer

The CAP developed the Pathology and Laboratory Quality Center for Evidence-based Guidelines as a forum to create and maintain laboratory practice guidelines (LPGs). Guidelines are intended to assist physicians and patients in clinical decision-making and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time an LPG is developed and when it is published or read. LPGs are not continually updated and may not reflect the most recent evidence. LPGs address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines 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 the patient. Accordingly, adherence to any LPG is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient's individual circumstances and preferences. CAP makes no warranty, express or implied, regarding LPGs and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. CAP assumes 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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APPENDIX

Disclosed Interests and Activities From July 2018 to December 2021

Sarah F. Adams, MD

Research grants: AstraZeneca

Russell R. Broaddus, MD, PhD

Research grants: National Institutes of Health
Speakers' bureau/lecture fees/honoraria: Duke University School of Medicine Department of Pathology, University of California San Francisco Department of Pathology, University of Texas Southwestern Medical School Department of Pathology

Heather Hampel, MS, LGC

Consulting fees or Advisory Board: Genome Medical, InVitae Genetics, Promega
Research grants: Myriad Genetic Laboratories
Stock options/bonds: Genome Medical

Sarah Kerr, MD

Research grants: Abbott Molecular

Eric Konnick, MD, MS

Speakers' bureau/lecture fees/honoraria: Clinical Care Options, LLC, Medscape, Ventana

Michael Overman, MD

Consulting fees or Advisory Board: AbbVie, Acrotech, Bristol Myers Squibb, Biopharma, MedImmune, Merck-Sharp & Dohme Corporation, Novartis, Pfizer, Roche
Research grants: Celgene, Bristol Meyers Squibb, MedImmune, Merck

Antonia R. Sepulveda, MD, PhD

Consulting fees or Advisory Board: Amgen, Bayer Healthcare Pharmaceuticals, Bristol Meyers Squibb, Caris Life Sciences, Merck US, Pfizer

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