COLLEGE of AMERICAN PATHOLOGISTS
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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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GUIDELINE DEVELOPMENT METHODS

Panel Composition

The College of American Pathologists (CAP) along with its collaborators, the Association for Molecular Pathology (AMP), American Society for Clinical Oncology (ASCO), and Fight Colorectal Cancer convened an expert panel (EP) consisting of members with experience and expertise in the diagnosis and treatment of patients being considered for checkpoint inhibitor therapy to develop evidence-based recommendations for mismatch repair (MMR) and microsatellite instability (MSI) testing. Members include practicing pathologists, clinicians, oncologists, genetic counselor, guideline methodologist, and patient advocates from the from the United States. The CAP approved the appointment of the project chair and panel members. The role of the EP members was to identify key questions, perform a systematic review of the literature, review the evidence base, draft recommendations, and author the manuscript.

An advisory panel (AP) consisting of pathologists, clinicians, and patient advocates was also formed. The role of the AP members was to provide feedback on the key questions for the literature search, vet the draft guideline statements prior to the public comment period, and to review and provide feedback for the manuscript and supplemental digital content (SDC). They did not vote on the recommendations.

Conflict of Interest (COI) Policy

Prior to acceptance on the expert or advisory panel, potential members completed the collaborative conflict of interest (COI) disclosure process, whose policy and form (effective January 2019) require disclosure of material financial interest in, or potential for benefit of significant value from, the guideline's development or its recommendations 24 months prior through 12 months post-publication. The potential members completed the COI disclosure form, listing any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Each potential EP member's disclosures were assessed by a COI review committee and categorized as:

No Relevant Conflicts of Interest**:** Individuals with no relevant COI are approved for full participation including determining the scope and questions to be addressed, reviewing and discussing the evidence, formulating and grading recommendations, voting on recommendations, and writing the document. Research funding that is free of direct or indirect industry funding or control, such as that provided by a government program or a non-profit organization that does not receive industry funding and uses an award mechanism and oversight that is independent of industry, is not regarded to be a conflict of interest. Service on a data and safety monitoring board for such research is also not regarded as a conflict of interest. Finally, industry funded research unrelated to the content of the *Joint Recommendations* is not regarded as a conflict of interest.

Manageable Conflicts of Interest: Individuals with manageable conflicts must disclose their conflicts to the whole guideline panel. They may participate in discussions about the evidence, but must excuse themselves or be recused from decision-making, including formulating, voting on, writing, and grading recommendations related to their COI (ie, recommendations addressing a product of the commercial entity with which they have a relationship or addressing a product of a competitor of the commercial entity with which they have a relationship). COI that require management include:

A. Research funding from an industry grant that is paid to the participant's institution and related to the content of the *Recommendations*;
B. Research funding from a government program or non-profit organization that receives funding from industry with business interests in the content of the *Recommendations*;
C. Participation on a data and safety monitoring board concerned with research that is relevant to the content of the *Recommendations* and is funded by an industry with business interests in the content of the *Recommendations*, or by a government program or non-profit organization that receives funding from industry with business interests in the content of the *Recommendations*.

D. Participation in scientific advisory board or consultant activities that are exclusively scientific in nature (ie, does not involve any activities that could be perceived as promotional) related to the subject matter of the *Recommendations.*
E. Participation in industry-funded research, scientific advisory committees, consulting roles, non-promotional speaking engagements, or expert testimony on matters that are unrelated to the subject matter of the *Recommendations*, but the company involved is known to have business interest in the subject matter;
F. Delivery of non-promotional talks in which the speaker has full control of the content and is either unpaid or paid by a third party that is responsible for ensuring that the event is free of influence of relevant industry (ie, if the event has industry financial support, all planning and content must be free of industry influence, and any payment of expenses and honoraria must occur through a third party, such as the medical society or institution sponsoring the event, or an event manager acceptable to them, rather than directly by a commercial entity with an interest in guideline subject matter or its agent);
G. Professional roles or activities (ie, roles and activities performed as part of an individual's profession, whether reimbursed or not) that place an individual in a position to personally gain or lose depending upon the recommendations.

Disqualifying Conflicts of Interest: Disqualifying conflicts of interest include the following:

A. Any current professional relationship with or investment in a company involved in the manufacture or distribution of MMR and/or MSI assays.
B. A direct financial relationship with a relevant commercial entity that has an interest in the content of the Recommendations, exclusive of the research, data safety monitoring board activities, and scientific advisory board and consultant activities noted above. Such direct financial relationships include the following, whether paid to or held by the individual directly or issued to another entity at the direction of the individual (such as to a panelist's institution):
    i. Payment of wages, consulting fees, honoraria, or other payments (in cash, in stock or stock options, or in kind) by a relevant company as compensation for the individual's services or expertise, exclusive of the research and data safety monitoring board activities noted above. Examples of such services are: participation on scientific advisory committees or consulting that is, in full or in part, promotional in nature; non-CME speaking engagements and inclusion in speaker bureaus where control of material is held by industry; expert testimony on matters related to guideline content provided on behalf of a relevant company or a law firm representing a relevant company; employment by a relevant commercial entity (such as a relevant pharmaceutical or medical device company or a third party payer exclusive of commercial laboratory employment that has financial interests in the content of the Recommendations).
    ii. Investments in relevant companies by the panelist or the panelist's spouse or life partner (exclusive of general mutual funds).
C. A patent or other intellectual property that is relevant to the Recommendations' subject matter and has resulted or could result in payments to the panelist or the panelist's institution.

All panel members were required to disclose conflicts prior to beginning and continuously throughout the project's timeline.

Disclosures of interest judged by the oversight group as manageable conflicts are listed in the manuscript. The Appendix in the manuscript also includes a table of all disclosed interest of the expert panel members during the development of the guideline for complete transparency.

Funding

The CAP provided funding for the administration of the project; no industry funds were used in the development of the guideline.

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Expert Panel Responsibilities

The EP met a total of 8 times during the guideline development process. The EP met in person on September 29, 2019, to prioritize outcomes and finalize the scope and key questions. The EP met again in person on November 2 – 3, 2020 to draft recommendations. The EP met 8 times through teleconference and all additional work was completed via electronic mail.

All EP members participated in the systematic evidence review (SER). Each level of the SER (title-abstract screening, full-text review, and data extraction) was performed in duplicate by two members of the EP or one member of the EP and a methodologist. All EP members and a methodologist performed adjudication of the conflicts.

Project Scope

The EP approved the following scope to develop evidence-based recommendations to address the overarching question "what test best identifies defects in deoxyribonucleic acid (DNA) mismatch repair".

The EP approved the following key questions for the systematic evidence review:

KQ1a. In patients being considered for immune checkpoint inhibitor therapy, does mismatch repair protein loss by immunohistochemistry (IHC) accurately detect defects in DNA mismatch repair?
KQ1b. In patients being considered for immune checkpoint inhibitor therapy, does polymerase chain reaction (PCR)-based microsatellite instability analysis accurately detect defects in DNA mismatch repair?
KQ1c. In patients being considered for immune checkpoint inhibitor therapy, does NGS-based microsatellite instability analysis accurately detect defects in DNA mismatch repair?
KQ1d. Does tumor mutation burden by next generation sequencing (NGS) have adequate performance characteristics to act as a surrogate for PCR and NGS-based microsatellite instability assays?
KQ1e. In patients being considered for immune checkpoint inhibitor therapy, which DNA mismatch repair 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?
KQ3a. What are the diagnostic test characteristics of MMR-IHC when predicting germline Lynch mutations?
KQ3b. What are the diagnostic test characteristics of PCR-based and NGS-based MSI when predicting germline Lynch mutations?

Systematic Evidence Review (SER)

The objective of the SER was to identify articles that provided data to inform the recommended testing for the MMR and MSI for patients being considered for checkpoint inhibitor therapy. If of sufficient quality, findings from this review would provide an evidence-base to support the recommendations of the guideline. The scope of the SER and the key questions (KQs) with the PICO elements (Population, Intervention, Comparator, Outcome(s)) were established by the EP in consultation with the methodologist prior to beginning the literature search.

Detailed key questions including the PICO is included in Supplemental Table 1.

Outcomes Ranking and Selection

According to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, it is important for clinical guideline panels to review a comprehensive list of outcomes.[1] The EP was polled to collect information on which outcomes should be included in the PICO. These outcomes included, but were not limited to, accuracy in diagnosis (specificity, sensitivity, positive and negative predictive values), change in patient management, cost, optimal and adequacy of specimen selection, patient preference, quality of life, rates of adverse reactions, survival rates, test/assay utility, and timely communication to the clinicians.

In consideration of the limited scope and resources, the EP ranked the outcomes used in the PICO. Using the GRADE approach[1] of considering the relative importance of outcomes, the EP was polled to rate each

initially identified outcome in terms of importance for decision making. The EP voted on a scale of 1 – 9: outcomes rated 1-3 were defined as “of limited importance”; outcomes rated 4-6 as “important, but not critical”; and outcomes rated 7-9 were “critical for decision making”. The EP finalized the outcomes after a discussion during the first in-person meeting.

Outcomes of Limited Importance
Note: These outcomes not used for decision making
1. Survival rates
2. Treatment response rates

Important Outcomes
1. Patient experience and quality of life
2. Complication rates based on unnecessary diagnostic procedures (false positive [FP]) and delayed treatment (false negative [FN])
3. Turn-around times

Critical Outcomes
1. Accuracy of diagnosis
2. Diagnostic test accuracy
3. Risk stratification
4. Test utilization

Search and Selection

Bibliographic database searches (Supplemental Figure 1) were completed in Ovid MEDLINE and Elsevier Embase.com on 12/16/2018 using 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 encompassing the publication dates of 1/1/2008 to 12/16/2018. In order to search for additional evidence for MMR and MSI testing, a targeted search was also completed in Ovid MEDLINE and Elsevier Embase.com on 12/16/2018 encompassing the publication dates of 1/1/2008 to 12/16/2018. This search used standardized vocabulary and keyword terms for the following concepts: 1) Lynch syndrome; 2) microsatellite instability, mismatch repair, or tumor mutational burden; and 3) laboratory testing methods. All database searches were limited to English language and human studies. Case reports, commentaries, editorials, and letters were excluded. Supplemental searches were completed for unindexed literature and included a review of ClinicalTrials.gov, Cochrane Library, Guidelines International Network, Trip search engine, University of York Centre for Reviews and Dissemination-CRD Database, National Institute for Health Research-International prospective register of systematic reviews (PROSPERO), and pertinent organizations’ websites using the terms mismatch repair or microsatellite instability or tumor mutation burden or MSI or TMB. EP members were also polled for relevant unpublished data at the onset of the project. The database searches were rerun in February 2020 (Ovid MEDLINE on 2/21/2020 and Embase.com on 2/24/2020) to identify articles published from 12/16/2018 though the date of the search. A second literature refresh was run on 3/30/2021 in the same databases to capture literature published since 2/24/2020. Limits were set based on predetermined inclusion/exclusion criteria. The literature search strategies and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) chart are included as Supplemental Figures 1 and 2.

Selection at all levels was also based on the predetermined inclusion/exclusion criteria.

Included:
1. Study population must consist of either:
    a. Adult and pediatric patients with advanced solid malignancies being considered for checkpoint inhibitor therapy
    b. Adult and pediatric patients with possible Lynch syndrome
2. Studies must evaluate either:
    a. Mismatch repair protein loss by IHC
    b. PCR or NGS-based microsatellite instability

   c. Tumor mutation burden by NGS
3. Studies must include one of the following as primary outcomes:
   a. Diagnostic test characteristics, including diagnostic sensitivity, specificity, positive predictive value, and negative predictive value
   b. Accuracy of MMR defect detection
   c. Patient survival outcomes or treatment response
   d. Germline testing or genetic counseling
4. Studies must be peer-reviewed full-text articles

Excluded:

- Letters
- Commentaries
- Editorials
- Narrative reviews
- Case reports
- Studies in animal models
- *In vitro* studies
- Consensus documents
- Articles not in the English language
- Meeting abstracts
- Less than 30 patients per study arm

Data Extraction & Management

The data elements from an included article/document were extracted by one reviewer into standard data formats and tables developed using the systematic review database software, DistillerSR (Evidence Partners Inc., Ottawa, Canada); a second reviewer confirmed accuracy and completeness. Any discrepancies in data extraction were resolved by discussion between the co-chairs and the methodologist. A bibliographic database was established in EndNote (Thomson Reuters, Carlsbad, CA) to track all literature identified and reviewed during the study.

Risk of Bias and Quality Assessment Methods

An assessment of the quality of the evidence was performed for all retained studies following application of the inclusion and exclusion criteria. Using this method, studies deemed be of low quality would not be excluded from the systematic review, but would be retained, and their methodological strengths and weaknesses discussed where relevant. To define an overall risk of bias rating for each included study, validated study-type specific tools were used to assess the risk of bias, plus additional important quality features were extracted. Specific details for each study type are outlined below.

Prospective Cohort Studies (PCS) and Retrospective Cohort Studies (RCS)

- The following domains were assessed using the Risk of Bias in Non-Randomized Studies – of Intervention (ROBINS-I)[2] tool using low risk, moderate risk, serious risk, critical risk, or unclear:
  1. Confounding
  2. Patient selection (selection bias)
  3. Intervention classification (performance bias)
  4. Deviation from intended intervention (performance bias)
  5. Missing data (reporting bias)
  6. Outcome measurements (detection bias)
  7. Selection of reported outcomes (detection bias)
- Additional assessed items included and were assessed as yes, no, or unclear:
  1. Adequately powered statistical analysis
  2. Reporting of funding sources
  3. Industry funding

Assessing the Strength of Recommendations and Considered Judgement

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The central question that the panel addressed in developing the guideline was: “What test best identifies defects in DNA mismatch repair?”

Development of recommendations required that the panel review the identified evidence and make a series of key judgments:

1. What are the significant findings related to each KQ or outcome? Determine any regulatory requirements and/or evidence that support a specific action.
2. What is the overall certainty of evidence supporting each KQ or outcome? Certainty of evidence is graded as High, Moderate, Low, and Very Low, based on published criteria (Supplemental Table 2). Certainty of evidence is a key element in determining the strength of a recommendation. Supplemental Tables 3 – 5 includes the detailed risk of bias assessment and overall certainty of evidence that supports the KQs and outcomes.
3. What is the strength of each recommendation? The strength of recommendations is designated as Strong or Conditional. There are many methods for determining the strength of a recommendation based on the strength of evidence and the magnitude of net benefit or harm. According to the GRADE approach, the strength of a recommendation demonstrates the extent to which an EP is “confident that the desirable effects of an intervention outweigh undesirable effects”.[3] For each statement, the panel rated each GRADE evidence to decision framework (EtD)[4] domain. With a strong recommendation designation, the EP judgements will mostly be favoring the right or left of the framework and indicate high confidence that the desirable effects of the guidance statement outweigh the undesirable effects. With a conditional recommendation, the EP judgements will be more towards the center of the framework or with a dispersed pattern indicating lower confidence.

Evidence-to-Decision Framework (EtD) Domains

1. Problem Priority
    - Is the problem a priority and is a recommendation needed to address it?
    - Are there consequences that are serious if the problem is not addressed?
2. Benefits and Harms
    - Are the desirable anticipated effects large?
    - Are the undesirable anticipated effects small?
    - Are the desirable effects large relative to undesirable effects?
3. Values and preferences of stakeholders:
    - Is there certainty of how stakeholders (patients, clinicians) value the outcomes?
    - Is there variability on how patients and clinicians value the outcomes?
    - Will there be different decisions from key stakeholders because of the different values placed on the outcomes?
4. Resources Required:
    - If the Recommendation is made, how large are the resource requirements?
5. Health Equity
    - Are there groups or settings that might be disadvantaged in relation to the Recommendation being considered?
    - Are there different baseline conditions across groups or settings that affect the absolute effectiveness of the Recommendation or the importance of the problem for disadvantaged groups or settings?
    - Are there important considerations that should be made when implementing the Recommendation to ensure that inequities are reduced, if possible, and that they are not increased?
6. Feasibility
    - Is the option (or recommendation) feasible to implement?
    - Is the Recommendation sustainable? Are there important barriers that are likely to limit the feasibility of implementing the Recommendation? If yes, do these barriers require consideration when implementing the Recommendation?
7. Acceptability
    - Is the option acceptable to key stakeholders?

- Are there key stakeholders that would not accept the distribution of the benefits, harms, or costs?
- Are there key stakeholders that would not accept the costs or undesirable effects in the short term for desirable effects (benefits) in the future?

Supplemental Table 6 provides a summary of the EP judgments within the EtD framework for each recommendation statement.

Statements not supported by evidence (ie, evidence was missing or insufficient to permit a conclusion to be reached) and made based on consensus expert opinion will be included as Good Practice Statements.[5]

Articulation of Recommendations

In order to articulate statements that were clearly written and easy to implement, the EP followed GLIDES (Guidelines Into Decision Support) and accompanying BridgeWiz software (Yale University, New Haven, CT) guidance on the wording of recommendations.[6] Statements should clearly address "who is doing what to whom", meaning the "actor" is defined within the statement to perform a specific action or intervention to a patient or population. The GLIDES program prioritizes the use of active voice because using the passive voice may lack the clarity and transparency of the statement. However, in some situations, the person responsible for ensuring guidance is implemented is dependent on the organization of the clinic and/or laboratory. To ensure clarity of guidance in these situations, the EP may use passive language to emphasize the recommended action. The guideline uses a two-tier system to rate the strength of recommendations (Table 2 in the manuscript). Supplemental Table 2 summarizes the certainty of evidence and considered judgement, as well as obligatory language that was used for each of the recommendation types.

Peer Review

An open comment period was held from February 19, 2020–March 13, 2020, on the CAP web site (www.cap.org). Six draft statements, demographic questions, and questions to assess feasibility were posted for peer review. An announcement was sent to the following societies deemed to have interest:

Medical societies:

- American Association for Cancer Research (AACR)
- American Society for Clinical Oncology (ASCO)
- American Society for Clinical Pathology (ASCP)
- American Society of Cytotechnologists
- Arthur Purdy Stout Society (APSS)
- Association for Molecular Pathology (AMP)
- Association of Community Cancer Centers (ACCC)
- Association of Pathology Chairs (APC)
- Canadian Association of Pathologists (CAP-APC)
- Canadian Association of Medical Oncology
- European Society for Medical Oncology (ESMO)
- European Society of Pathology (ESP)
- Japanese Society of Medical Oncologists (JSMO)
- National Comprehensive Cancer Network (NCCN)
- National Cancer Institute (NCI)
- Quality Initiative in Interpretive Pathology (QIIP)
- Royal College of Pathologists
- Society to Improve Diagnoses in Medicine (SIDM)
- United States & Canadian Academy of Pathology (USCAP)

Patient advocacy groups:

- American Cancer Society
- Canadian Partnership Against Cancer
- Cancer Leadership Council
- Cancer Research and Prevention Foundation

- Fight Colorectal Cancer
- Partnership Against Cancer
- Union for International Cancer Control

Government and other stakeholders:

- Centers for Medicare & Medicaid Services (CMS)
- Centers for Disease Control and Prevention (CDC)
- European Medical Agency
- US Food and Drug Administration (FDA)
- Veteran's Affairs (VA) and Department of Defense (DOD)

"Agree as written", "Agree with suggested modifications", "Disagree", and "Does not pertain to my area of expertise or practice" responses were captured for each draft recommendation statement. The website received over 350 written comments. Five draft statements achieved more than 90% agreement and 1 draft statement received below the 80% agreement threshold. All draft recommendation statements have agreements that range between 77.9% - 98.3%. Volunteer EP members were assigned draft recommendation statements for which members reviewed the comments and provide suggestions to the entire panel to: keep original draft language, edit with minor changes for clarity, or edit with major changes. After consideration of the comments, a total of 6 final recommendations were included in the guideline: One 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. Resolution of all changes was obtained by majority consensus of the panel using nominal group technique (discussion during teleconference webinars, email discussion, and multiple edited recommendations) amongst the panel members. The final recommendations were approved by the EP with a formal vote.

Document Review and Approval

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. Once approved, the collaborating organizations' names were added to the guideline title as official collaborators.

Dissemination Plans

The CAP plans to host an MMR and MSI Testing in for Checkpoint Inhibitor Therapy resource webpage which will include a link to the manuscript and supplement; a summary of the recommendations, a teaching PowerPoint (Microsoft Corporation, Redmond, WA), a frequently asked question (FAQ) document, and an infographic along with other additional tools such as webinar recordings as applicable. The guideline will be promoted and presented at various society meetings.

Quality Assessment Results

A total of 80 studies identified by the systematic review informed the recommendations. Although data was extracted from 103 studies, 15 studies contained insufficient detail to inform statements, and eight studies reported on outcomes that were not relevant to this guideline (Supplemental Figure 2). The body of evidence was comprised of 17 prospective cohort studies and 63 retrospective cohort studies. The risk of bias assessment of the prospective cohort studies is detailed in Supplemental Table 3, while assessment for the retrospective cohort studies can be found in Supplemental Table 4. The GRADE certainty of evidence for each outcome informing a recommendation and the overall certainty ranking for the statement is presented in Supplemental Table 5.

Overall, the body of evidence included in this clinical practice guideline represents a methodologically rigorous and representative summary of the available evidence. The aggregate risk of bias for outcomes used to inform statements ranged from serious to extremely serious and certainty of effect was assessed as moderate and low. In general, included evidence was limited by the predominance of retrospective studies, confirmatory testing in only a subset of samples, differences in testing panels, and a paucity of data for cancers arising in locations outside of colorectal, GEA, small bowel, and endometrial.

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Recommendation Statements

Statement 1. For patients with CRC being considered for checkpoint 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.
Note: MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency.
Strong Recommendation.

The certainty of evidence to support this guideline statement is *moderate* for MMR-IHC and MSI-PCR, and *low* for MSI-NGS.

The evidence for this statement comprised a total of 37 studies that evaluated the ability of MMR-IHC or MSI-PCR to detect DNA mismatch repair defects and eight studies that evaluated the ability of MSI by NGS to detect DNA mismatch repair defects. To evaluate the diagnostic test characteristics of MMR-IHC, seven studies defined MSI-PCR as the reference standard,[7-13] and seven studies used verification of germline mutation to define a true positive.[14-20] An additional nine studies reported on the concordant DNA mismatch repair defect status between MMR-IHC and germline testing.[15, 17, 18, 20-25] To evaluate the diagnostic test characteristics of MSI-PCR, four studies defined MMR-IHC as the reference standard,[26-29] two studies used sequencing as a reference standard,[27, 30] and four studies used germline mutation testing to verify MSI-PCR status.[14, 16, 19, 20] Three additional studies reported on concordance of DNA mismatch repair defect detection between MSI-PCR and germline testing.[31-33] Concordance of DNA mismatch repair defect status between MMR-IHC and MSI-PCR was evaluated in 22 studies, with five studies defining MMR-IHC as the reference standard[26, 28, 34-36] and 17 studies using MSI-PCR.[7-9, 12, 13, 16, 19, 20, 24, 25, 33, 37-42] Supplemental Table 7 summarizes mismatch repair defect concordance using MMR-IHC and MSI-PCR. Although 22 studies reported on concordance, only 14 studies reported raw mismatch repair status for both assays and concordance as a percent agreement and were deemed appropriate for inclusion in the summary table. The certainty of evidence for diagnostic test characteristics of both MMR-IHC and MSI-PCR was assessed as moderate. This assessment was based on serious risk of bias across studies informing both testing methods; however, evidence was not downgraded for any other domain in either. It is worth noting that for studies using germline testing as reference standard, generally only dMMR or MSI tumors underwent testing. This resulted in a false zero false negatives included in the calculation for sensitivity and could mean that sensitivity is overestimated in these studies. As this was only a subset of included studies, the limitation was noted, but evidence was not downgraded. MMR-IHC and MSI-PCR status concordance was also ranked as a critical outcome. Again, studies informing this outcome were limited by a serious risk of bias, but evidence was not further downgraded (Supplemental Table 5).

For studies evaluating MSI using NGS, five studies reported on NGS diagnostic test characteristics using MSI-PCR as the reference standard,[35, 43-46] three studies defined MMR-IHC as the reference standard,[44, 46, 47] and one study verified NGS status using a single-molecule molecular inversion probes (smMIP) NGS assay against a genome-wide microsatellite instability NGS (mSINGS) assay .[30] Two additional studies reported on the concordance between MSI using NGS and MMR-IHC status.[35, 48] Of the included eight studies, two used a prospective design and six were retrospective. The six studies that reported on MSI-NGS diagnostic test characteristics were limited by an aggregate very serious risk of bias plus evidence was further downgraded for inconsistency of results across the studies resulting in a very low certainty of evidence. The two studies that reported on the MMR MSI status concordance between MSI-NGS and MMR-IHC were limited by a serious risk of bias but evidence was not further downgraded for any domain leading to a moderate certainty of evidence (Supplemental Table 5).

Based on the available evidence, EP members concluded that the use of MMR-IHC and MSI-PCR for DNA mismatch repair detection in CRC patients was very accurate and carried large benefits and only small harms. Guidance for the use of MMR-IHC or MSI-PCR was deemed to be acceptable and feasible to implement. Refer to Supplemental Tables 3-5 for a summary of the risk of bias assessment for all included studies and the certainty of evidence assessment for all outcomes informing the statement,

Supplemental Table 6 summarizes the Evidence to Decision (EtD) framework. The EP did have longer discussions when considering resource requirements and a distinction was made between cost of resources to run the test and charge applied to recipients. When both were included, the resource requirements were considered negligible. The EP also used the EtD framework when considering inclusion of MSI by NGS in the recommendation. Accuracy of NGS for DNA mismatch repair defects was considered as accurate in CRC patients, while both benefits and harms were defined as moderate. The EP concluded that the benefits probably outweighed the harms; however, the use of NGS carries a moderate 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.

Statement 2. For patients with gastroesophageal and small bowel cancer being considered for immune checkpoint 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.
Strong Recommendation.

The certainty of evidence to support this guideline statement is *low*.

The evidence base for this statement includes one prospective cohort study[49] and five retrospectively designed studies.[50-54] Two studies reported on the diagnostic test characteristics of MMR-IHC using MSI-PCR in gastroesophageal carcinoma patients,[51, 52] three studies reported on the DNA mismatch repair defect status between MMR-IHC and MSI-PCR in gastroesophageal adenocarcinoma (GEA) patients,[50, 51, 54] one study reported on the concordance of MMR-IHC and MSI-PCR in duodenal carcinoma patients,[53] and the final study reported on the concordance of MSI-NGS and MMR-IHC in upper gastrointestinal (GI) cancers.[49] The certainty of evidence was low for both MMR-IHC diagnostic test characteristics and for the status concordance between MSI-NGS and MMR-IHC. In the first outcome, the aggregate risk of bias was very serious, but evidence was not further downgraded. The latter outcome was only supported by one study with a very serious risk of bias. The final outcome used to support this statement was status concordance between MMR-IHC and MSI-PCR (Supplemental Table 8). The certainty of evidence for this outcome was very low based on extremely serious risk of bias across the four included retrospective studies. Additionally, inconsistency of results was noted for these studies, but evidence was not further downgraded as the inconsistency was likely a consequence of difference in reference standards across the studies (Supplemental Table 5).

Based on the available evidence, EP members concluded that detection of DNA mismatch repair defects in gastroesophageal and small bowel carcinoma patients by MMR-IHC and MSI by PCR was very accurate. After discussions, EP members defined the benefits of both modalities as large and the harms as small and concluded that the benefits outweighed the harms. 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 this recommendation would results in moderate savings and would probably increase health equity. Refer to Supplemental Tables 3-5 for a summary of the risk of bias assessment for all included studies and the certainty of evidence assessment for all outcomes informing the statement, Supplemental Table 6 summarizes the EtD framework.

Statement 3. For patients with endometrial cancer being considered for immune checkpoint therapy, pathologists should use MMR-IHC over MSI by PCR or NGS for the detection of DNA mismatch repair defects. *Strong Recommendation.*

The certainty of evidence to support this guideline statement is *low*.

The evidence informing this statement is comprised of two prospectively designed studies[55, 56] and 15 retrospectively designed studies[27, 30, 44, 48, 57-67] all evaluating DNA mismatch repair defect detection in endometrial carcinoma patients. To evaluate the diagnostic test characteristic of MMR-IHC, two studies defined MSI-PCR as the reference standard,[61, 63] one study used verification of MMR status with germline testing.[64] To evaluate the diagnostic test characteristics of MSI by PCR, two studies used MMR-IHC as

the reference standard,[27, 62] and two validated the MSI status using sequencing.[27, 30] Finally, to evaluate the diagnostic test characteristics of MSI by NGS, one study defined MMR-IHC as the reference standard,[57] one used MSI by PCR,[44] and one used NGS of the tumor.[30] Additional studies reported on the concordance of DNA mismatch repair status between MMR-IHC and MSI by PCR,[55, 56, 58-60, 62, 63, 66, 67] MMR-IHC and germline mutations,[64, 65] MSI by PCR and germline mutations,[65] and MSI by NGS and MMR-IHC.[48, 57] Supplemental Table 9 summarizes mismatch repair defect concordance using MMR-IHC and MSI-PCR. Although nine studies reported on concordance, only six studies reported raw mismatch repair status for both assays and concordance as a percent agreement and were deemed appropriate for inclusion in the summary table. The certainty of evidence for diagnostic test characteristics of MMR-IHC, MSI-PCR, and MSI-NGS were all assessed as low. This assessment was based on very serious risk of bias across studies informing both testing methods; however, evidence was not downgraded for any other domain in either. For MSI-PCR there was also inconsistency noted in the studies but was likely a consequence of different mononucleotide, dinucleotide, and single gene panels being used in the studies and evidence was not downgraded. MMR MSI status concordance across the testing methods were also ranked as a critical outcome. Concordance for MMR-IHC and MSI-PCR was assessed as low based on a serious risk of bias and serious inconsistency, while concordance for MSI-NGS and MMR-IHC was also assessed as low but based on very serious risk of bias and no further downgrading (Supplemental Table 5).

Based on the available evidence, EP members concluded that DNA mismatch repair defect detection was very accurate by MMR-IHC and less accurate using MSI by PCR or MSI by NGS. The benefits of testing with MMR-IHC were considered to be large while the harms were defined as small and thus EP members concluded that the benefits outweighed the harms. Based on the cost of resources to conduct IHC and the associated costs of interpretation, resource requirements for MMR-IHC were considered to be negligible when compared to the other assay options. Based on discussion among the EP members, this guidance is expected to be acceptable to key stakeholders and feasible to implement. Refer to Supplemental Tables 3-5 for a summary of the risk of bias assessment for all included studies and the certainty of evidence assessment for all outcomes informing the statement, Supplemental Table 6 summarizes the EtD framework.

Statement 4. For patients with cancer types other than CRC, GEA, small bowel, and endometrial being considered for immune checkpoint 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. *Conditional Recommendation.*

The certainty of evidence to support this guideline statement is *very low*.

The evidence base informing this statement includes one prospectively designed study,[68] one study with both prospective and retrospective arms,[39] and 11 studies with a retrospective design.[30, 44, 69-77] Of these 13 studies, MMR-IHC was evaluated in renal cell carcinoma[72] and across multiple cancer types,[39, 68, 71] MSI by PCR was evaluated in prostate cancer,[30] breast cancer,[73] and across multiple cancer types,[78] and MSI by NGS was evaluated in prostate cancer[30, 69, 74] and across cancer types.[30, 44, 75-77] The certainty of evidence for the diagnostic test characteristics of the testing methods varied. While both MSI-PCR and MSI-NGS were assessed as low certainty based on very serious risk of bias across studies, MMR-IHC was assessed as very low certainty. This was a consequence of very serious risk of bias again, but also majority of these studies were large mixed population studies that included mostly CRC patients without subgroup analyses for the lesser represented cancer types. This has potentially resulted in an overestimate of effect in the non-CRC patient populations and evidence was downgraded. MMR MSI status concordance across the testing methods was assessed as very low certainty for MMR-IHC and MSI-PCR, low for MSI-NGS and MMR-IHC, and very low for MSI-NGS and MSI-PCR (Supplemental Table 6).

Based on the available evidence, EP members were unable to determine which assay would most accurately detect DNA mismatch repair defects in carcinoma patients not covered by recommendations 1 through 3. Although an assay could not be recommended, the benefits of testing for defects to determine

which patients should be eligible for checkpoint therapy, were considered to be moderate and the harms small, leading to the conclusion that the benefits probably outweigh the harms. Based on the fact this recommendation promotes testing but without direction on how, the EP members expect this statement to probably be acceptable to key stakeholders and probably feasible to implement. Refer to Supplemental Tables 3-5 for a summary of the risk of bias assessment for all included studies and the certainty of evidence assessment for all outcomes informing the statement, Supplemental Table 6 summarizes the EtD framework.

Statement 5. For all cancer patients being considered for immune checkpoint 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. *Strong Recommendation.*

The certainty of evidence to support this guideline statement is *low*.

The evidence for this statement comprised a total of five studies that evaluated the use of TMB as a surrogate for DNA mismatch repair defects in CRC,[44, 79, 80] gastroesophageal cancer,[81] endometrial carcinoma,[44] and glioma.[82] Of these five studies, one used a prospective design[79] and the other four used a retrospective design.[44, 80-82] Critical outcomes informing this statement included TMB diagnostic test characteristics and MMR MSI status concordance for TMB when compared with MMR-IHC and MSI-NGS. The certainty of evidence for all three outcomes was low based on very serious risk of bias in each outcome but no further downgraded for any domain.

Based on the available evidence, EP members concluded that TMB use as a surrogate for mismatch repair deficiency was inaccurate and would carry small benefits and moderate harms, leading to the harms outweighing the benefits. 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 draft a strong recommendation against the use of TMB. This guidance is expected to be acceptable to key stakeholders and feasible to implement. Refer to Supplemental Tables 3-5 for a summary of the risk of bias assessment for all included studies and the certainty of evidence assessment for all outcomes informing the statement, Supplemental Table 6 summarizes the EtD framework.

Statement 6. For cancer patients being considered for immune checkpoint therapy, if an MMR deficiency consistent with Lynch Syndrome is identified in the tumor, pathologists should communicate this finding with the treating physician. *Strong Recommendation.*

The certainty of evidence to support this guideline statement is *low*.

The evidence informing this statement is comprised on six prospectively designed studies and 22 retrospectively designed studies. Studies reported on the concordance between mismatch repair deficiency status using MMR-IHC[15, 17, 20-25, 64, 65] or MSI by PCR[19, 20, 23-25, 28, 31-33, 56, 62, 65, 67] 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.[16, 19, 24, 32, 38, 41, 55, 56, 60, 65, 83-87] There were no studies identified that evaluated NGS. All identified studies enrolled patients with colorectal carcinoma or endometrial carcinoma. The certainty of evidence for both MMR-IHC and MSI-PCR status concordance when compared when Lynch Syndrome detection by germline testing was low. For MMR-IHC, the studies were limited by a serious risk of bias and evidence was further downgraded for inconsistency. For MSI-PCR, the aggregate risk of bias was very serious but evidence was not further downgraded for any domain (Supplemental Table 5). It is also worth noting that for both MMR-IHC and MSI-PCR, a majority of the identified studies only performed germline testing in dMMR 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.

Based on the available evidence, EP members concluded that communication of the potential for Lynch Syndrome would carry large benefits and only small harms, and thus benefits of this communication

would outweigh the harms. The EP also concluded that this guidance would increase health equity and would be feasible to implement. Based on the potential perceived burden to pathologists, the EP expect that this guidance will be probably acceptable to key stakeholders. Refer to Supplemental Tables 3-5 for a summary of the risk of bias assessment for all included studies and the certainty of evidence assessment for all outcomes informing the statement, Supplemental Table 6 summarizes the EtD framework.

Good Practice Statements

According to the GRADE approach, good practice statements (GPS) are recommendations panels may consider important but are not appropriate to be formally rated for certainty of evidence.[5] In addition to the set of key questions formulated *a priori* for the SER, the EP decided to draft GPSs, which reflect expert consensus opinions supported by a limited number of studies and data that were not formally included in the evidence-base nor systematically rated and assessed for quality. The EP wanted to address the following:

- Discordant results
- Indeterminate results
- Subclonal loss

The EP co-chairs followed a framework to review the questions for the good practice statements (Figure 3). A targeted literature search was performed based on these questions. The EP co-chairs reviewed the available literature and incorporated data collected in a pre-guideline development practice survey to arrive at the GPSs.

- Discordant results: In the event of discordant results, pathologists should interpret any definitive evidence of MMR deficiency by IHC or MSI by NGS/PCR as a positive result for patients to be eligible for checkpoint inhibitor therapy. Discordant results should be reviewed to ensure that the discordance is not due to an interpretive error.
- Indeterminate results: In the event of an indeterminate result in any method, pathologists should perform an alternative technique or repeat the same assay using a different tumor block. Laboratories should have a robust peer review process for indeterminate cases.
- 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.

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Supplemental Digital Content

Supplemental Table 1. Key Questions and PICO Elements

<table>
<tr><td colspan="3">KQ1a. In patients being considered for checkpoint inhibitor therapy, does mismatch repair protein loss by IHC accurately detect defects in DNA mismatch repair?</td></tr>
<tr><td colspan="3">Population</td></tr>
<tr><td colspan="3">Patients with advanced solid malignancies being considered for checkpoint inhibitor therapy
Note: Specimens may be from primary tumor or metastatic tumor</td></tr>
<tr><td>Intervention</td><td>Comparator</td><td>Outcomes</td></tr>
<tr><td>MLH1 IHC
MSH2 IHC
MSH6 IHC
PMS2 IHC</td><td>• MSI analysis
• Studies may be single arm
• Standard will be defined by the study</td><td><u>Critical</u>
• Diagnostic test characteristics – sensitivity, specificity, PPV, NPV
• Accuracy of MMR defect detection
• Tissue concordance</td></tr>
<tr><td colspan="3">KQ1b. In patients being considered for checkpoint inhibitor therapy, does PCR-based microsatellite instability analysis accurately detect defects in DNA mismatch repair?</td></tr>
<tr><td colspan="3">Population</td></tr>
<tr><td colspan="3">Patients with advanced solid malignancies being considered for checkpoint inhibitor therapy
Note: Specimens may be from primary tumor or metastatic tumor</td></tr>
<tr><td>Intervention</td><td>Comparator</td><td>Outcomes</td></tr>
<tr><td>PCR-based MSI
MLH1 methylation assays</td><td>• MMR-IHC</td><td><u>Critical</u>
• Diagnostic test characteristics – sensitivity, specificity, PPV, NPV
• Accuracy of MMR defect detection
• Tissue concordance</td></tr>
<tr><td colspan="3">KQ1c. In patients being considered for checkpoint inhibitor therapy, does NGS-based microsatellite instability analysis accurately detect defects in DNA mismatch repair?</td></tr>
<tr><td colspan="3">Population</td></tr>
<tr><td colspan="3">Patients with advanced solid malignancies being considered for checkpoint inhibitor therapy
Note: Specimens may be from primary tumor or metastatic tumor</td></tr>
<tr><td>Intervention</td><td>Comparator</td><td>Outcomes</td></tr>
<tr><td>NGS-based MSI</td><td>• MMR-IHC
• PCR-based MSI</td><td><u>Critical</u>
• Diagnostic test characteristics – sensitivity, specificity, PPV, NPV
• Accuracy of MMR defect detection</td></tr>
<tr><td colspan="3">KQ1d. Does tumor mutation burden by NGS have adequate performance characteristics to act as a surrogate for PCR- and NGS-based microsatellite instability assays?</td></tr>
<tr><td colspan="3">Population:</td></tr>
<tr><td colspan="3">Specimens from patients with advanced solid malignancies being considered for checkpoint inhibitor therapy
Note: Specimens may be from primary tumor or metastatic tumor</td></tr>
<tr><td>Intervention</td><td>Comparator</td><td>Outcomes</td></tr>
<tr><td>Tumor mutation burden by NGS</td><td>• PCR-based MSI
• NGS-based MSI</td><td><u>Critical</u>
• Diagnostic test characteristics – sensitivity, specificity, PPV, NPV
• Accuracy of MMR defect detection
• Tissue concordance</td></tr>
<tr><td colspan="3">KQ1e. In patients being considered for checkpoint inhibitor therapy, which DNA mismatch repair assay best predicts improved patient outcomes?</td></tr>
<tr><td colspan="3">Population</td></tr>
<tr><td colspan="3">Specimens from patients with advanced solid malignancies being considered for checkpoint inhibitor therapy
Note: Specimens may be from primary tumor or metastatic tumor</td></tr>
<tr><td>Intervention</td><td>Comparator</td><td>Outcomes</td></tr>
<tr><td>MMR-IHC
PCR-based MSI</td><td>• Any other assay</td><td><u>Critical</u></td></tr>
</table>

NGS-based MSI	• Studies may be single arm	• Treatment response Important • Survival rates (OS, PFS, RFS) • Germline testing/genetic counseling
KQ2. When comparing MMR-IHC and PCR- or NGS-based MSI, does any assay have better performance characteristics in specific cancer types?		
Population		
Patients with advanced solid malignancies being considered for checkpoint inhibitor therapy Subgroups: Colorectal cancer, endometrial cancer, upper urinary tract carcinoma, urothelial, duodenal adenocarcinoma, gastrointestinal cancers, patients with other types/forms of cancer *Note:* Specimens may be from primary tumor or metastatic tumor		
Intervention	**Comparator**	**Outcomes**
MMR-IHC PCR-based MSI NGS-based MSI *MLH1* methylation assays	• MMR-IHC • MSI analysis • *MLH1* methylation analysis • NGS mutation burden • Clinical follow-up	Critical • Diagnostic test characteristics – sensitivity, specificity, PPV, NPV • Tissue concordance
KQ3a. What are the diagnostic test characteristics of MMR-IHC when predicting germline Lynch mutations?		
Population		
Patients with possible Lynch syndrome Subgroups: Colon cancer patients, endometrial cancer patients, patients with other types/forms of cancer *Note:* Specimens may be from primary tumor or metastatic tumor		
Intervention	**Comparator**	**Outcomes**
MLH1 IHC MSH2 IHC	• Gold standard: presence of germline Lynch mutation in MLH1, MSH2, MSH6, PMS2, EPCAM • MSI analysis	Critical • Diagnostic test characteristics – sensitivity, specificity, PPV, NPV o Specificity must be based on somatic testing • Accuracy of MMR defect detection (surrogates: survival rates, treatment response)
KQ3b. What are the diagnostic test characteristics of PCR-based and NGS-based MSI when predicting germline Lynch mutations?		
Population		
Patients with possible Lynch syndrome Subgroups: Colon cancer patients, endometrial cancer patients, patients with other types/forms of cancer *Note:* Specimens may be from primary tumor or metastatic tumor		
Intervention	**Comparator**	**Outcomes**
PCR-based MSI NGS-based MSI	• Gold standard: presence of Lynch mutation in MLH1, MSH2, MSH6, PMS2, EPCAM • MLH1, MSH2, MSH6, PMS2 IHC	Critical • Diagnostic test characteristics – sensitivity, specificity, PPV, NPV • Accuracy of MMR defect detection (surrogates: survival rates, treatment response)
Other Overarching Criteria		
Patient Population	Adult and pediatric patients with advanced solid malignancies being considered for checkpoint inhibitor therapy or possible Lynch syndrome	
Setting	Academic and community laboratory settings	
Minimum Sample Size	30 patients per study arm	

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Supplemental Digital Content

Search Dates	KQs 1, 2: 2008 – 2018 KQ 3: 2000 – 2018
Included Study Types	• Guidelines • Systematic reviews with and without meta-analysis • Randomized controlled trials • Observational studies with prospective or retrospective design ○ Comparative and single arm • Case-control studies
Study Types to Exclude • Letters, commentaries, editorials • Case reports • Narrative reviews • *In vitro* and animal model studies • Non-English	

Abbreviations: DNA, deoxyribonucleic acid; EPCAM, Epithelial Cell Adhesion Molecule; FN, false negative; FP, false positive; IHC, immunohistochemistry; KQ, key questions; MLH1, MutL Homolog 1; MSH2, MutS Homolog 2; MMR, mismatch repair; MSH6, MutS Homolog 6; MSI, microsatellite instability; NGS, next generation sequencing; NPV, negative predictive value; OS, overall survival; PCR, polymerase chain reaction; PFS, progression free survival; PICO, population, intervention, comparator, outcomes; PMS2, PMS1 Homolog 2, Mismatch Repair System Component; PPV, positive predictive value; QoL, quality of life; RFS, recurrence free survival

Supplemental Table 2: Certainty of Evidence

Designation	Description
High	There is high confidence that available evidence reflects true effect. Further research is very unlikely to change the confidence in the estimate of effect. Included studies will be of high or intermediate quality.
Moderate	There is moderate confidence that available evidence reflects true effect. Further research is likely to have an important impact on the confidence in estimate of effect and may change the estimate. Included studies will be of intermediate or low quality.
Low	There is limited confidence in the estimate of effect. The true effect may be substantially different from the estimate of the effect. Included studies will be of low quality.
Very Low	There is very little confidence in the estimate of effect. The true effect is likely to be substantially different from the estimate of effect. Any estimate of effect is very uncertain. Included studies will be of low or very low quality.

Data derived from Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group Materials.[3]

Supplemental Table 3. Risk of Bias Assessment of Included Prospective Cohort Studies

Study	ROBINS-I Assessment								Additional Quality Features		
	Confounding	Patient selection	Intervention classification	Deviation from intended intervention	Missing data	Outcome measurements	Selection of reported outcomes	Overall Risk of Bias	Adequately powered	Reported funding sources	Industry funded
Latham et al,[68] 2018	MR	MR	LR	LR	MR	LR	LR	MR	NS	Y	N
Dong et al,[57] 2018	MR	MR	LR	LR	LR	MR	MR	MR	NS	N	U
Middha et al,[48] 2017	MR	SR	LR	LR	MR	SR	MR	SR	NS	Y	N
Fabrizio et al,[79] 2018	MR	SR	LR	LR	MR	MR	MR	SR	Y	Y	Y
Berardinelli et al,[26] 2018	MR	SR	LR	MR	LR	LR	LR	SR	Y	Y	N
Nowak et al,[46] 2017	MR	SR	LR	MR	MR	LR	LR	SR	NS	N	U
Egoavil et al,[55] 2013	MR	LR	LR	MR	MR	LR	MR	MR	Y	Y	N
Bonnet et al,[24] 2012	MR	MR	LR	MR	MR	LR	LR	MR	NS	Y	N
Leenen et al,[56] 2012	MR	MR	LR	LR	MR	LR	LR	MR	NS	Y	N
Van Lier et al,[86] 2012	MR	MR	LR	LR	MR	MR	LR	MR	NS	Y	N
Canard et al,[16] 2012	MR	MR	LR	LR	MR	SR	MR	SR	NS	N	U
Yoon et al,[13] 2011	MR	SR	LR	LR	LR	MR	LR	SR	NS	Y	N
Mojtahed et al,[39] 2011	MR	SR	LR	LR	MR	SR	MR	SR	NS	N	U
Jin et al,[33] 2008	SR	MR	LR	LR	LR	MR	LR	SR	NS	N	U
Jensen et al,[42] 2008	MR	SR	LR	LR	MR	MR	LR	SR	NS	N	U
Abida et al,[74] 201	MR	MR	MR	MR	SR	SR	MR	SR	NS	Y	N
Christakis et al,[49] 2019	MR	MR	LR	LR	MR	MR	LR	MR	NS	Y	N

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; N, no; NS, no statistical analysis: ROBINS-I, Risk of Bias in Non-Randomized Studies – of Intervention; SR, serious risk; U, unclear; Y, yes.

Supplemental Table 4. Risk of Bias Assessment of Included Retrospective Cohort Studies

Study	ROBINS-I Assessment								Additional Quality Features		
	Confounding	Patient selection	Intervention classification	Deviation from intended intervention	Missing data	Outcome measurements	Selection of reported outcomes	Overall Risk of Bias	Adequately powered	Reported funding sources	Industry funded
Signoroni et al,[14] 2018	MR	CR	LR	LR	LR	LR	LR	CR	Y	Y	N
Jang et al,[7] 2018	MR	CR	LR	LR	LR	MR	LR	CR	NS	Y	N
Wang et al,[43] 2018	MR	CR	LR	LR	LR	MR	LR	CR	NS	N	U
Papke et al,[47] 2018	MR	SR	LR	LR	LR	LR	LR	SR	NS	N	U
Waalkes et al,[30] 2018	MR	CR	LR	LR	MR	LR	LR	CR	NS	Y	N
Takehara et al,[27] 2018	MR	CR	LR	LR	MR	SR	LR	CR	NS	Y	N
Vanderwalde et al,[44] 2018	MR	CR	LR	LR	MR	LR	MR	CR	NS	Y	Y
Gray et al,[45] 2018	MR	CR	LR	LR	LR	LR	MR	CR	NS	N	U
Salem et al,[81] 2018	MR	CR	LR	LR	MR	LR	MR	CR	NS	Y	Y
Hempelmann et al,[69] 2018	MR	CR	LR	MR	MR	LR	LR	CR	NS	Y	N
Alpert et al,[34] 2018	MR	CR	LR	SR	SR	LR	MR	CR	NS	Y	N
Zhu et al,[35] 2018	MR	CR	MR	LR	MR	LR	MR	CR	NS	Y	N
Brennan et al,[21] 2017	SR	CR	LR	CR	MR	LR	LR	CR	NS	Y	N
Yan et al,[8] 2016	MR	CR	LR	MR	MR	MR	LR	CR	Y	Y	N
Yuan et al,[9] 2015	MR	CR	LR	LR	LR	LR	LR	CR	Y	Y	N
Haraldsdottir et al,[15] 2017	MR	CR	LR	MR	MR	LR	LR	CR	NS	Y	N
Zheng et al,[28] 2018	MR	CR	LR	LR	LR	LR	LR	CR	Y	Y	N
Haruma et al,[58] 2018	MR	CR	LR	LR	MR	LR	LR	CR	Y	Y	N
Goodfellow et al,[83] 2015	MR	SR	LR	MR	MR	MR	LR	CR	NS	N	U
Stadler et al,[80] 2016	MR	CR	LR	MR	MR	MR	LR	CR	NS	Y	N
Hodges et al,[82] 2017	MR	CR	LR	MR	MR	MR	LR	CR	NS	Y	N
Bacher et al,[29] 2015	MR	CR	LR	MR	MR	LR	SR	CR	Y	Y	N
Mathiak et al,[50] 2017	MR	CR	LR	LR	MR	SR	LR	SR	NS	N	U
Stelloo et al,[59] 2017	MR	CR	LR	LR	LR	LR	MR	CR	NS	Y	N
Bruegl et al,[60] 2017	MR	CR	LR	MR	MR	LR	LR	CR	NS	Y	N
Buchanan et al,[84] 2017	MR	CR	LR	MR	MR	LR	LR	CR	NS	Y	N
Wang et al,[61] 2017	MR	CR	LR	LR	MR	SR	LR	CR	Y	Y	N
Libera et al,[62] 2017	MR	CR	LR	LR	MR	SR	LR	CR	NS	Y	N
Siddique et al,[10] 2016	MR	CR	LR	LR	MR	LR	LR	CR	NS	Y	N
Batur et al,[11] 2016	MR	CR	LR	LR	MR	MR	LR	CR	Y	N	U
Rosty et al,[22] 2016	MR	CR	LR	LR	LR	LR	LR	CR	NS	Y	N
Bae et al,[51] 2015	MR	CR	LR	LR	LR	MR	LR	CR	NS	Y	N

McConechy et al,[63] 2015	MR	CR	LR	MR	MR	MR	LR	CR	N	Y	N
Salipante et al,[70] 2014	MR	CR	LR	MR	MR	MR	LR	CR	NS	Y	N
Buchanan et al,[64] 2014	MR	CR	LR	LR	MR	SR	MR	CR	NS	Y	N
De Lellis et al,[23] 2013	MR	CR	LR	MR	MR	MR	LR	CR	NS	Y	N
Rodriguez-Hernandez et al,[88] 2013	MR	CR	LR	LR	MR	LR	MR	CR	NS	Y	N
Jensen et al,[37] 2013	MR	CR	LR	LR	MR	MR	LR	CR	N	Y	N
Moline et al,[65] 2013	SR	CR	LR	MR	SR	MR	LR	CR	NS	Y	N
Benmoussa et al,[36] 2012	MR	CR	LR	LR	MR	LR	MR	CR	NS	N	U
Peterson et al,[66] 2012	MR	CR	LR	LR	MR	LR	LR	CR	NS	N	U
Schofield et al,[85] 2012	MR	CR	LR	MR	MR	LR	LR	CR	NS	N	U
Bartley et al,[71] 2012	MR	CR	LR	MR	MR	MR	LR	CR	NS	Y	N
Perez Carbonell et al,[38] 2012	MR	CR	LR	MR	LR	MR	LR	CR	NS	Y	N
Warrier et al,[17] 2011	MR	CR	LR	LR	MR	MR	LR	CR	NS	Y	N
Kim et al,[12] 2011	MR	CR	LR	MR	MR	SR	SR	CR	NS	Y	N
Limburg et al,[18] 2011	MR	CR	LR	LR	LR	MR	LR	CR	NS	N	U
Giraldez et al,[25] 2010	MR	CR	LR	LR	MR	MR	LR	CR	NS	Y	Y
Altavilla et al,[72] 2010	MR	CR	LR	LR	MR	MR	MR	CR	NS	Y	N
Chang et al,[19] 2010	SR	CR	LR	MR	SR	CR	LR	CR	NS	Y	N
Gu et al,[52] 2009	MR	CR	LR	LR	SR	LR	LR	CR	NS	Y	N
Berginc et al,[31] 2009	MR	CR	LR	LR	MR	MR	LR	CR	NS	N	U
Bertagnolli et al,[40] 2009	MR	CR	SR	LR	LR	LR	LR	CR	NS	Y	Y
Ruemmele et al,[53] 2009	MR	CR	LR	LR	SR	MR	LR	CR	NS	N	U
Seo et al,[54] 2009	MR	CR	LR	LR	LR	MR	LR	CR	NS	N	U
Schofield et al,[32] 2009	SR	CR	LR	LR	MR	MR	LR	CR	NS	Y	N
Hampel et al,[20] 2008	SR	CR	LR	MR	MR	MR	LR	CR	NS	Y	N
Ramsoekh et al,[41] 2008	MR	CR	LR	MR	MR	LR	LR	CR	NS	N	U
Balmana et al,[87] 2008	MR	CR	LR	LR	MR	MR	LR	CR	NS	Y	N
Yoon et al,[67] 2008	MR	CR	LR	LR	SR	MR	MR	CR	NS	Y	N
Fusco et al,[73] 2018	MR	CR	LR	LR	MR	MR	LR	CR	NS	Y	N
Hechtman et al,[75] 2019	MR	CR	LR	LR	LR	LR	MR	CR	NS	Y	N
Pabla et al,[76] 2019	MR	CR	LR	LR	SR	SR	SR	CR	NS	Y	Y
Trabucco et al,[77] 2019	MR	CR	LR	MR	SR	MR	LR	CR	NS	Y	Y

Abbreviations: CR, critical risk; LR, low risk; MR, moderate risk; N, no; NS, no statistical analysis; ROBINS-I, Risk of Bias in Non-Randomized Studies – of Intervention; SR, serious risk; U, unclear; Y, yes.

Supplemental Table 5. GRADE Certainty of Evidence Assessment

Number of Studies and Design	Aggregate Risk of Bias	Inconsistency	Indirectness	Imprecision	Other [A]	Certainty of Evidence Grade for Outcome	Overall Certainty of Evidence Grade for Statement
STATEMENT 1 – COLORECTAL CANCER							
MMR-IHC diagnostic test characteristics (critical importance [B])							IHC and PCR: Moderate NGS: Low
2 PCS, 8 RCS	Serious	Not serious	Not serious	Not serious	Confounding [C]	Moderate	
MSI-IHC status concordance with germline mutation testing (important outcome [B])							
1 PCS, 8 RCS	Serious	Serious	Not serious	Not serious	None	Low	
MSI-PCR diagnostic test characteristics (critical importance [B])							
2 PCS, 7 RCS	Serious	Not serious	Not serious	Not serious	Confounding [C]	Moderate	
MSI-PCR status concordance with germline mutation testing (important outcome [B])							
1 PCS, 2 RCS	Serious	Serious	Not serious	Not serious	None	Low	
MMR-IHC and MSI-PCR status concordance (critical importance [B])							
7 PCS, 15 RCS	Serious	Not serious	Not serious	Not serious	None	Moderate	
MSI-NGS diagnostic test characteristics (critical importance [B])							
1 PCS, 5 RCS	Very Serious	Serious	Not serious	Not serious	None	Very Low	
MSI-NGS and MMR-IHC status concordance (critical importance [B])							
1 PCS, 1 RCS	Serious	Not serious	Not serious	Not serious	None	Moderate	
STATEMENT 2 – GASTROESOPHAGEAL AND SMALL BOWL CANCER							
MMR-IHC diagnostic test characteristics (critical importance [B])							Low
2 RCS	Very Serious	Not serious	Not serious	Not serious	None	Low	
MMR-IHC and MSI-PCR status concordance (critical importance [B])							
4 RCS	Extremely Serious	Not serious [D]	Not serious	Not serious	None	Very Low	
MSI-NGS and MMR-IHC status concordance (critical importance [B])							
1 PCS	Very Serious	NA	NA	NA	Concordance only reported for small subset of samples	Low	
STATEMENT 3 – ENDOMETRIAL CANCER							
MMR-IHC diagnostic test characteristics (critical importance [B])							Low
3 RCS	Very Serious	Not serious	Not serious	Not serious	None	Low	
MMR-IHC status concordance with germline mutation testing (important outcome [B])							
2 RCS	Extremely Serious	Serious	Not serious	Not serious	None	Very Low	
MSI-PCR diagnostic test characteristics (critical importance [B])							
3 RCS	Very Serious	Not serious [E]	Not serious	Not serious	None	Low	
MSI-PCR status concordance with germline mutation testing (important outcome [B])							
1 RCS	Extremely Serious	NA	NA	NA	NA	Very Low	
MMR-IHC and MSI-PCR status concordance (critical importance [B])							

Number of Studies and Design	Aggregate Risk of Bias	Inconsistency	Indirectness	Imprecision	Other [A]	Certainty of Evidence Grade for Outcome	Overall Certainty of Evidence Grade for Statement
2 PCS, 7 RCS	Serious	Serious	Not serious	Not serious	None	Low	
MSI-NGS diagnostic test characteristics (critical importance [B])							
1 PCS, 2 RCS	Very Serious	Not serious	Not serious	Not serious	None	Low	
MSI-NGS and MMR-IHC status concordance (critical importance [B])							
2 PCS	Very Serious	Not serious	Not serious	Not serious	None	Low	
STATEMENT 4 – OTHER FORMS OF CANCER							
MMR-IHC diagnostic test characteristics (critical importance [B])							Low
2 PCS, 2 RCS	Very Serious	Not serious	Not serious	Not serios	Overestimate of effect in non-CRC populations [F]	Very Low	
MSI-PCR diagnostic test characteristics (critical importance [B])							
1 RCS	Very Serious	NA	NA	NA	NA	Low	
MMR-IHC and MSI-PCR status concordance (critical importance [B])							
2 RCS	Very serious	Not serious	Not serious	Not serious	Overestimate of effect in non-CRC populations [F]	Very Low	
MSI-NGS diagnostic test characteristics (critical importance [B])							
5 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
MSI-NGS and MMR-IHC status concordance (critical importance [B])							
1 PCS, 1 RCS	Very serious	Not serious	Not serious	Not serious	None	Low	
MSI-NGS and MSI-PCR status concordance (critical importance [B])							
1 RCS	Extremely Serious	NA	NA	NA	Overestimate of effect in non-CRC populations [F]	Very Low	
STATEMENT 5 – ROLE OF TUMOR MUTATION BURDEN							
TMB diagnostic test characteristics (critical importance [B])							Low
1 RCS	Very Serious	NA	NA	NA	NA	Low	
TMB and MMR-IHC status concordance (critical importance [B])							
2 RCS	Very Serious	Not serious	Not serious	Not serious	None	Low	
TMB and MSI-NGS status concordance (critical importance [B])							
1 PCS, 2 RCS	Very Serious	Not serious	Not serious	Not serious	None	Low	
STATEMENT 6 – FURTHER EVALUATION FOR LYNCH SYNDROME							
MMR-IHC status concordance with Lynch Syndrome detection by germline testing (critical importance [B])							Low
1 PCS, 9 RCS	Serious	Serious	Not serious	Not serious	Confounding [G]	Low	
MSI-PCR status concordance with Lynch Syndrome detection by germline testing (critical importance [B])							
3 PCS, 10 RCS	Very serious	Not serious	Not serious	Not serious	Confounding [G]	Low	
Association between MMR-MSI status and Lynch Syndrome prevalence (important outcome [B])							
5 PCS, 10 RCS	Serious	Not serious	Serious	Not serious	None	Low	

Abbreviations: GRADE, The Grading of Recommendations Assessment, Development and Evaluation; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; NA, not applicale based on one study included for the outcome; NGS, next generation sequencing; PCR, polyermase chain reaction; PCS, prospective cohort study; RCS, retrospective cohort study; TMB, tumor mutation burden.

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Footnotes

A. Other category includes assessment for detection of publication bias, large effect, and confounding.
B. Outcomes were rated *a priori* as critical or important for decision making.
C. For studies using germline testing as the reference standard, generally only dMMR/MSI tumors underwent testing, resulting in 0 FN and perhaps an overestimate of sensitivity. As this was only a subset of included studies, this limitation is noted, but the evidence was not downgraded.
D. Inconsistency noted but believed to be a consequence of differences in reference standards across the studies and evidence was not downgraded.
E. Although inconsistency was noted, the identified studies used different mononucleotide, dinucleotide, and single gene panels and this was believed to be the source of the inconsistency.
F. Identified large mixed population studies included mostly CRC patients, leading to an overestimate of effect in other patient populations and evidence was downgraded.
G. Most of the identified studies only performed germline testing in dMMR 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.

Supplemental Table 6. Evidence-to-Decision Framework

Recommendation 1. For patients with CRC being considered for checkpoint 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.
Note: MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency.

Summary of Judgements – IHC and PCR

Criteria	*Favors the comparison*		*Neutral*	*Favors the intervention*	
Problem	No	Probably no		Probably yes	**Yes +**
Test Accuracy	Very inaccurate	Inaccurate		Accurate	**Very accurate +**
Desirable Effects	Trivial	Small		Moderate	**Large +**
Undesirable Effects	Large	Moderate		**Small +**	Trivial
Certainty of Effects	Very low	Low		**Moderate +**	Large
Values	Important certainty or variability	**Possibly important uncertainty or variability +**		Probably no important uncertainty of variability	No important uncertainty of variability
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 +**
Resources Required	Large costs	Moderate costs	**Negligible costs and savings +**	Moderate savings	Large savings
Equity	Reduced	Probably reduced	**Probably no impact +**	Probably increased	Increased
Acceptability	No	Probably no		Probably yes	**Yes +**
Feasibility	No	Probably no		Probably yes	**Yes +**

Summary of Judgements - NGS

Criteria	*Favors the comparison*		*Neutral*	*Favors the intervention*	
Problem	No	Probably no		Probably yes	**Yes +**

Test Accuracy	Very inaccurate	Inaccurate		**Accurate +**	Very accurate
Desirable Effects	Trivial	Small		**Moderate +**	Large
Undesirable Effects	Large	**Moderate +**		Small	Trivial
Certainty of Effects	Very low	**Low +**		Moderate	Large
Values	Important certainty or variability	**Possibly important uncertainty or variability +**		Probably no important uncertainty of variability	No important uncertainty of variability
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
Resources Required	Large costs	**Moderate costs +**	Negligible costs and savings	Moderate savings	Large savings
Equity	Reduced	Probably reduced	**Probably no impact +**	Probably increased	Increased
Acceptability	No	Probably no		Probably yes	**Yes +**
Feasibility	No	Probably no		Probably yes	**Yes +**

Recommendation 2. For patients with gastroesophageal and small bowel cancer being considered for immune checkpoint 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.

Summary of Judgements – GEA and small bowel

Criteria	*Favors the comparison*		*Neutral*	*Favors the intervention*	
Problem	No	Probably no		Probably yes	**Yes +**
Test Accuracy	Very inaccurate	Inaccurate		Accurate	**Very accurate +**
Desirable Effects	Trivial	Small		Moderate	**Large +**
Undesirable Effects	Large	Moderate		**Small +**	Trivial
Certainty of Effects	Very low	**Low +**		Moderate	Large

Values	Important certainty or variability	**Possibly important uncertainty or variability +**		Probably no important uncertainty of variability	No important uncertainty of variability
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 +**
Resources Required	Large costs	Moderate costs	Negligible costs and savings	**Moderate savings +**	Large savings
Equity	Reduced	Probably reduced	Probably no impact	**Probably increased +**	Increased
Acceptability	No	Probably no		Probably yes	**Yes +**
Feasibility	No	Probably no		Probably yes	**Yes +**

Recommendation 3. For patients with endometrial cancer being considered for immune checkpoint therapy, pathologists should use MMR-IHC over MSI by PCR or NGS for the detection of DNA mismatch repair defects.

Summary of Judgements - Endometrial

Criteria	*Favors the comparison*		*Neutral*	*Favors the intervention*	
Problem	No	Probably no		Probably yes	**Yes +**
Test Accuracy	Very inaccurate	Inaccurate		Accurate	**Very accurate +**
Desirable Effects	Trivial	Small		Moderate	**Large +**
Undesirable Effects	Large	Moderate		**Small +**	Trivial
Certainty of Effects	Very low	**Low +**		Moderate	Large
Values	Important certainty or variability	**Possibly important uncertainty or variability +**		Probably no important uncertainty of variability	No important uncertainty of variability
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 +**

Resources Required	Large costs	Moderate costs	**Negligible costs and savings +**	Moderate savings	Large savings
Equity	Reduced	Probably reduced	**Probably no impact +**	Probably increased	Increased
Acceptability	No	Probably no		Probably yes	**Yes +**
Feasibility	No	Probably no		Probably yes	**Yes +**

Recommendation 4. For patients with cancer types other than CRC, GEA, small bowel, and endometrial being considered for immune checkpoint 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 for the detection of mismatch repair defects.

Summary of Judgements - All other cancer types

Criteria	*Favors the comparison*		*Neutral*	*Favors the intervention*	
Problem	No	Probably no		Probably yes	**Yes +**
Test Accuracy	Very inaccurate	Inaccurate		**Accurate +**	Very accurate
Desirable Effects	Trivial	Small		**Moderate +**	Large
Undesirable Effects	Large	Moderate		**Small +**	Trivial
Certainty of Effects	**Very low +**	Low		Moderate	Large
Values	**Important certainty or variability +**	Possibly important uncertainty or variability		Probably no important uncertainty of variability	No important uncertainty of variability
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
Resources Required	Large costs	**Moderate costs +**	Negligible costs and savings	Moderate savings	Large savings
Equity	Reduced	Probably reduced	Probably no impact	**Probably increased +**	Increased
Acceptability	No	Probably no		**Probably yes**	Yes

				+	
Feasibility	No	Probably no		**Probably yes** **+**	Yes

Recommendation 5. For all cancer patients being considered for immune checkpoint 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.

Summary of Judgements - TMB

Criteria	*Favors the comparison*		*Neutral*	*Favors the intervention*	
Problem	No	Probably no		Probably yes	**Yes** **+**
Test Accuracy	Very inaccurate	**Inaccurate** **+**		Accurate	Very accurate
Desirable Effects	Trivial	**Small** **+**		Moderate	Large
Undesirable Effects	Large	**Moderate** **+**		Small	Trivial
Certainty of Effects	Very low	**Low** **+**		Moderate	Large
Values	**Important certainty or variability** **+**	Possibly important uncertainty or variability		Probably no important uncertainty of variability	No important uncertainty of variability
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
Resources Required	**Large costs** **+**	Moderate costs	Negligible costs and savings	Moderate savings	Large savings
Equity	**Reduced** **+**	Probably reduced	Probably no impact	Probably increased	Increased
Acceptability	**No** **+**	Probably no		Probably yes	Yes
Feasibility	**No** **+**	Probably no		Probably yes	Yes

Recommendation 6. For cancer patients being considered for immune checkpoint therapy, if an MMR deficiency consistent with Lynch Syndrome is identified in the tumor, pathologists should communicate this finding with the treating physician.

Summary of Judgements - Lynch syndrome evaluation

Criteria	*Favors the comparison*		*Neutral*	*Favors the intervention*	
Problem	No	Probably no		Probably yes	**Yes**

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					+
Desirable Effects	Trivial	Small		Moderate	**Large +**
Undesirable Effects	Large	Moderate		**Small +**	Trivial
Certainty of Effects	Very low	**Low +**		Moderate	Large
Values	Important certainty or variability	**Possibly important uncertainty or variability +**		Probably no important uncertainty of variability	No important uncertainty of variability
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 +**
Resources Required	Large costs	Moderate costs	**Negligible costs and savings +**	Moderate savings	Large savings
Equity	Reduced	Probably reduced	Probably no impact	Probably increased	**Increased +**
Acceptability	No	Probably no		**Probably yes +**	Yes
Feasibility	No	Probably no		Probably yes	**Yes +**

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

Supplemental Table 7. Mismatch Repair Defect Concordance using MMR IHC and MSI PCR in Colorectal Cancer Patients

Study, Study Design	Sample Size	IHC Panel	PCR Panel	Concordance	
				pMMR and MSS	dMMR and MSI
Zheng et al,[28] 2018 RCS	n=245	MLH1, MSH2, MSH6, PMS2[A]	1. NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250) 2. BAT25, BAT26, NR21, NR24, MONO27	Concordance, NCI and IHC: 99.1% o pMMR: n=217/245 o MSS: n=216/245 o MSI-L: n=2/245 Concordance, PCR panel #2 and IHC: 98.6% o pMMR: n=217/245 o MSS: n=221/245 o MSI-L: n=1/245	Concordance, NCI and IHC: 89.3% o dMMR: n=28/245 o MSI-H: n=27/245 Concordance, PCR panel #2 and IHC: 71.4% o dMMR: n=28/245 o MSI-H: n=23/245
Benmoussa et al,[36] 2012 RCS	n=70	MLH1, MSH2, MSH6[A]	BAT25, BAT26, NR24, CAT25	Concordance: 100% o pMMR: n=60/70 o MSS: n=60/70	Concordance: 100% o dMMR: n=10/70 o MSI-H: n=10/70
Mojtahed et al,[39] 2011 PCS	n=323 (CRC subgroup)	MLH1, MSH2, MSH6, PMS2	1. NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250); n=117[B] 2. BAT25, BAT26, NR21, NR24, MONO27; n=315[B]	Concordance: 100% (n=206/206) o pMMR: n=264/323 o MSS: n=214/304 o MSI-L: n=12/304	Concordance: 79.6% (n=39/49) o dMMR: n=59/323 o MSI-H: n=78/304
Giraldez et al,[25] 2010 RCS	n=140	MLH1, MSH2, MSH6, PMS2[A]	BAT25, BAT26, NR21, NR24, MONO27	Concordance: not reported o pMMR: n=120/140 o MSS: n=124/139	Concordance: 75.0% o dMMR: n=20/140 o MSI-H: n=15/139
Jang et al,[7] 2018 RCS	n=166	MLH1, MSH2, MSH6, PMS2	NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250)[B]	Concordance: not reported o pMMR: n=91/166 o MSS: n=90/166	Concordance: 96.1% o dMMR: n=75/166 o MSI-H: n=76/166
Jensen et al,[37] 2013 RCS	n=208	MLH1	NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250)[B]	Concordance: not reported o pMMR: n=180/208 o MSS: n=179/208	Concordance: 96.6% o dMLH1: n=28/208 o MSI pos: n=29/208
Bonnet et al,[24] 2012 PCS	n=307	MLH1, MSH2, MSH6	1. NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250); n=275[B] 2. BAT25, BAT26, NR21, NR24, MONO27; n=43[B]	Concordance: 88.3% o pMMR: n=257/307 o MSS: n=228/275 o MSI-L: n=7/275	Concordance: 82.6% o dMMR: n=46/307 o MSI-H: n=40/307
Canard et al,[16] 2012 PCS	n=1040	MLH1, MSH2	NR21, NR22, NR24, BAT25, BAT26[B]	Concordance: 99.7% o pMMR: n=938/1040 o MSS: n=942/1040	Concordance: 93.1% o dMMR: n=102/1040 o MSI pos: n=98/1040
Perez-Carbonell	n=2093	MLH1, MSH2, MSH6, PMS2	BAT26, NR24[B]	Concordance: not reported o pMMR: n=1740/1895 o MSS: n=1753/1905	Concordance: 83.6% o dMMR: n=155/1895 o MSI-L/H: n=152/1905

Supplemental Table 7. Mismatch Repair Defect Concordance using MMR IHC and MSI PCR in Colorectal Cancer Patients

et al,[38] 2012 RCS					
Kim et al,[12] 2011 RCS	n=197	MLH1, MSH2	NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250)[B]	Concordance: 94.1% o pMMR: n=174/197 o MSS/MSI-L: n=118/123	Concordance: 100% o dMMR: n=23/197 o MSI-H: n=5/123
Yoon et al,[13] 2011 PCS	n=2028	MLH1, MSH2	NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250)[B]	Concordance: 98.9% o pMMR: n=1821/2028 o MSS: n=1765/2028	Concordance: 63.0% o dMMR: n=207/2028 o MSI-H: n=203/2028
Bertagnolli et al,[40] 2009 PCS	n=1264	MLH1, MSH2	BAT25, BAT26, D17S250, D5S346, ACTC, D18S55, BAT40, D10S197, BAT34c4, MycL[B]	Concordance: 99.3% o pMMR: n=677/783 o MSS/MSI-L: n=709/846	Concordance: 85.0% o dMMR: n=106/783 o MSI-H: 137/846
Hampel et al,[20] 2008 RCS	n=500	MLH1, MSH2, MSH6, PMS2	Not reported[B]	Concordance: not reported o pMMR: n=412/483 o MSS: n=387/500 o MSI-L: n=34/500	Concordance: 78.9% o dMMR: n=71/483 o MSI-H: n=64/500
Jensen et al,[42] 2008 PCS	n=262	MLH1, MSH2, MSH6	NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250)[B]	Concordance: not reported o pMMR: n=223/262 o MSS: n=214/262 o MSI-L: n=9/262	Concordance: 94.9% (100% following repeat PCR) o dMMR: n=39/262 o MSI-H: n=37/262

Abbreviations: ACTC, dinucleotide marker; BAT, Big Adenine Tract ;CRC, colorectal cancer; D10S197, dinucleotide repeat on chromosome 10 marker; D2S123, dinucleotide repeat on chromosome 2 marker; D5S346, dinucleotide repeat on chromosome 5 marker; D17S250, dinucleotide repeat on chromosome 17 marker; D18S55, dinucleotide repeat on chromosome 18 marker; dMMR, deficient mismatch repair; IHC, immunohistochemistry; MLH1, mutL homolog 1; MONO27, loci coordinates for PCR panel; MSH2, mutS homolog 2; MSH6, mutS homolog 6; MSI, microsatellite instability; MSI-H, high microsatellite instability; MSI-L, low microsatellite instability; MSS, microsatellite stable; MycL, MYCL Proto-Oncogene, BHLH Transcription Factor; NCI, National Cancer Institute; NR21, loci coordinates for PCR panel; NR24, loci coordinates for PCR panel; PCR, polymerase chain reaction; PCS, prospective cohort study; pMMR, proficient mismatch repair; PMS2, PMS1 homolog 2; pos, positive; RCS, retrospective cohort study.

Footnotes

A. IHC panel defined as the reference standard.

B. PCR panel defined as the reference standard.

Supplemental Table 8. Mismatch Repair Defect Concordance using MMR IHC and MSI PCR in Gastroesophageal and Small Bowel Cancer Patients

Study, Study Design	Sample Size	IHC Panel	PCR Panel	Concordance	
				pMMR and MSS	dMMR and MSI
Duodenal Carcinoma					
Ruemmele et al,[53] 2009 RCS	n=170 (carcinoma subgroup)	MLH1, MSH2, MSH6	BAT25, BAT26, D17S250, D2S123, D5S346, D18S61, BAT40[A]	Concordance: 96.8% o pMMR: not reported o MSS: n=124/144 o MSI-L: n=5/144	Concordance: 73.3% o dMMR: not reported o MSI-H: n=15/144
Gastric Carcinoma					
Mathiak et al,[50] 2019 RCS	n=452	MLH1, MSH2, MSH6, PMS2	BAT25, BAT26, NR21, NR24, NR27[A]	Concordance: not reported o pMMR: n not reported o MSS: n not reported	Concordance: 88.2% o dMMR: n=30/452 o MSI-H: n=34/158
Bae et al,[51] 2015 RCS	n=464	MLH1, MSH2	Not reported[A]	Concordance: 98.5% o pMMR: n=275/464 o MSS: n=261/464	Concordance: 91.1% o dMMR: n=189/464 o MSI-H: n=203/464
Seo et al,[54] 2009 RCS	n=328	MLH1, MSH2[B]	NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250)	Discordance: 3.4% (n=10/292) Concordance: 96.6% (not reported, 100% minus discordance) o pMMR: n=292/328 o MSS: not reported	Discordance: 52.8% (n=19/36) Concordance: 47.2% (not reported, 100% minus discordance) o dMMR: n=36/328 o MSI-H: not reported

Abbreviations: ACTC, dinucleotide marker; BAT, Big Adenine Tract ;CRC, colorectal cancer; D10S197, dinucleotide repeat on chromosome 10 marker; D2S123, dinucleotide repeat on chromosome 2 marker; D5S346, dinucleotide repeat on chromosome 5 marker; D17S250, dinucleotide repeat on chromosome 17 marker; D18S55, dinucleotide repeat on chromosome 18 marker; dMMR, deficient mismatch repair; IHC, immunohistochemistry; MLH1, mutL homolog 1; MONO27, loci coordinates for PCR panel; MSH2, mutS homolog 2; MSH6, mutS homolog 6; MSI, microsatellite instability; MSI-H, high microsatellite instability; MSI-L, low microsatellite instability; MSS, microsatellite stable; NCI, National Cancer Institute; NR21, loci coordinates for PCR panel; NR24, loci coordinates for PCR panel; PCR, polymerase chain reaction; PCS, prospective cohort study; pMMR, proficient mismatch repair; PMS2, PMS1 homolog 2; pos, positive; RCS, retrospective cohort study.

Footnotes

A. PCR panel defined as the reference standard.
B. IHC panel defined as the reference standard.

Supplemental Table 9. Mismatch Repair Defect Concordance using MMR IHC and MSI PCR in Endometrial Cancer Patients

Study, Study Design	Sample Size	IHC Panel	PCR Panel	Concordance	
				pMMR and MSS	dMMR and MSI
Haruma et al,[58] 2018 RCS	n=138	MLH1, MSH2, MSH6, PMS2	BAT26, NR21, NR27, CAT25[A]	Concordance: 97.9% o pMMR: n=97/138 o MSS: n=95/138	Concordance: 92.7% o dMMR: n=41/138 o MSI pos: n=38/138
Bruegl et al,[60] 2017 RCS	n=213	MLH1, MSH2, MSH6, PMS2	NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250), TGFBR2[A]	Concordance: 96.5%; 95%CI, 92.0-98.8% o pMMR: n=146/203 o MSS: n=143/199	Concordance: 89.1%; 95%CI, 77.8-95.9% o dMMR: n=57/203 o MSI-H: n=50/199
Egoavil et al,[55] 2013 PCS	n=173	MLH1, MSH2, MSH6, PMS2[B]	BAT25, BAT26, NR21, NR24, NR27	Discordance: 1.7% Concordance: 98.3% (not reported, 100% minus discordance) o pMMR: n=112/173 o MSS: n=126/173	Discordance: 8.1% Concordance: 91.9% (not reported, 100% minus discordance) o dMMR: n=61/173 o MSI pos: n=47/173
Peterson et al,[66] 2012 RCS	n=96	MLH1, MSH2, MSH6, PMS2	BAT25, BAT26, BAT40, BAT24c4, ACTC, MYCL, D17S250, D5S346, D18S55, D10S197[A]	Concordance: 91.5% o pMMR: n=71/95 o MSS: n=61/94 o MSI-L: n=4/94	Concordance: 82.1% (23/28) o dMMR: n=24/95 o MSI-H: n=29/94
Leenen et al,[56] 2012 PCS	n=179	MLH1, MSH2, MSH6, PMS2	BAT25, BAT26, NR21, NR24, MONO27[A]	Concordance: 100% o pMMR: n=137/179 o MSS: n=137/179	Concordance: 100% o dMLH1: n=32/179 o dMSH2/MSH6/PMS2: n=11/179 o MSI-H: 42/179
Yoon et al,[67] 2008 RCS	n=113	MLH1, MSH2, MSH6	NCI panel (BAT25, BAT26, D2S123, D5S346, D17S250)[A]	Concordance: not reported o pMMR: n=87/113 o MSS: n=63/113 o MSI-L: n=21/113	Concordance: 55.2% o dMMR: n=26/113 o MSI-H: n=29/113

Abbreviations: ACTC, dinucleotide marker; BAT, Big Adenine Tract ;CAT25, T25mononucleotide repeat of the Caspase 2 gene; CRC, colorectal cancer; D10S197, dinucleotide repeat on chromosome 10 marker; D2S123, dinucleotide repeat on chromosome 2 marker; D5S346, dinucleotide repeat on chromosome 5 marker; D17S250, dinucleotide repeat on chromosome 17 marker; D18S55, dinucleotide repeat on chromosome 18 marker; dMMR, deficient mismatch repair; IHC, immunohistochemistry; MLH1, mutL homolog 1; MONO27, loci coordinates for PCR panel; MSH2, mutS homolog 2; MSH6, mutS homolog 6; MSI, microsatellite instability; MSI-H, high microsatellite instability; MSI-L, low microsatellite instability; MSS, microsatellite stable; MycL, MYCL Proto-Oncogene, BHLH Transcription Factor; NCI, National Cancer Institute; NR21, loci coordinates for PCR panel; NR24, loci coordinates for PCR panel; PCR, polymerase chain reaction; PCS, prospective cohort study; pMMR, proficient mismatch repair; PMS2, PMS1 homolog 2; pos, positive; RCS, retrospective cohort study; TGFBR2, transforming growth factor, beta receptor II.

Footnotes

A. PCR panel defined as the reference standard.
B. IHC panel defined as the reference standard.

MMR and MSI Testing in Patients being Considered for Checkpoint Inhibitor Therapy
Supplemental Digital Content

Supplemental Figure 1: Database Search Strings

Ovid Search Run on 12/16/2018

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily <1946 to December 14, 2018> Search Strategy:

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1 Microsatellite Instability/ (2675)
2 DNA Mismatch Repair/ (2351)
3 exp MutL Proteins/ (3461)
4 exp MutS Proteins/ (3341)
5 Epithelial Cell Adhesion Molecule/ (1444)
6 ((MLH1 or MSH2 or MSH6 or PMS2 or BAT?25 or BAT?26) adj5 (gene$ or protein? or expression or methylation or
hypermethylation)).tw,kf. (3274)
7 (microsatellite$ adj3 (stability or stable or unstable or instability or ultramutat$ or hypermutat$)).tw,kf. (7462)
8 (MSI or MSI-H or MSI-L or "replication error phenotype?").tw,kf. (6107)
9 (d?MMR or MMR?D or "mismatch repair$").tw,kf. (9281)
10 (EPCAM or TACSTD1 or "epithelial cell adhesion molecule?").tw,kf. (2688)
11 ("tumor mutation$ burden" or "tumour mutation$ burden").tw,kf. (228)
12 or/1-11 (22000)
13 (neoplas$ or tumor$ or tumour$ or carcino$ or cancer$ or oncogen$ or adenocarcinoma$ or sarcoma$ or lymphoma$ or
malignan$ or metastatic or metastas?s).kf,tw. (3257548)
14 ("checkpoint inhibitor$" or immunotherap$ or "immun$ checkpoint").tw,kf. (77134)
15 13 or 14 (3284047)
16 exp Colorectal Neoplasms, Hereditary Nonpolyposis/ (4255)
17 (Lynch or Turcot or "Hereditary Nonpolyposis" or HNPCC or "Muir Torre").ti,ab,kf. (6340)
18 ("hereditary colon cancer" or "inherited colon cancer").tw,kf. (197)
19 or/16-18 (7333)
20 immunohistochemistry/ (282708)
21 Immunoenzyme techniques/ (67620)
22 exp polymerase chain reaction/ (434083)
23 High-Throughput Nucleotide Sequencing/ (22934)
24 whole genome sequencing/ (1556)
25 whole exome sequencing/ (883)
26 Multilocus sequence typing/ (4858)
27 sequence analysis/ (8827)
28 sequence analysis, RNA/ (14115)
29 DNA mutational analysis/ (57059)
30 Sequence analysis, DNA/ (149788)
31 genetic testing/mt (8336)
32 ("sequence analys?s" or "massively parallel sequenc$").tw,kf. (70017)
33 (immunohistochem$ or immunocytochem$ or immunoenzyme$).tw,kf. (389533)
34 ("next gen$" or NGS or "whole genome$" or "whole exome$" or sequencing).tw,kf. (280454)
35 (PCR or (polymerase adj3 chain)).tw,kf. (576287)
36 ("test$ assay$" or "test$ method$").tw,kf. (14657)
37 "Bethesda panel".tw,kf. (34)
38 or/20-37 (1611989)
39 Nivolumab/ or (nivolumab or opdivo).rn,tw,kf. (2854)
40 Ipilimumab/ or (Ipilimumab or Yervoy).tw,kf. (2672)
41 "companion diagnostic$".tw,kf. (782)
42 "checkpoint inhibitor$".tw,kf. (4915)
43 Immunotherapy/ or immunotherapy.tw,kf. (83733)
44 Pembrolizumab/ or (pembrolizumab or Keytruda).rn,tw,kf. (2057)
45 Cemiplimab/ or (cemiplimab or Libtayo).rn,tw,kf. (9)
46 antibodies, monoclonal, humanized/ (32786)
47 (atezolizumab or Tecentriq or Avelumab or Bavencio or Durvalumab or Imfinzi).rn,tw,kf. (728)
48 Antineoplastic agents, immunological/ (1433)
49 "immune?modulatory".tw,kf. (24)
50 "humanized monoclonal antibodies".tw,kf. (252)
51 or/39-50 (121255)

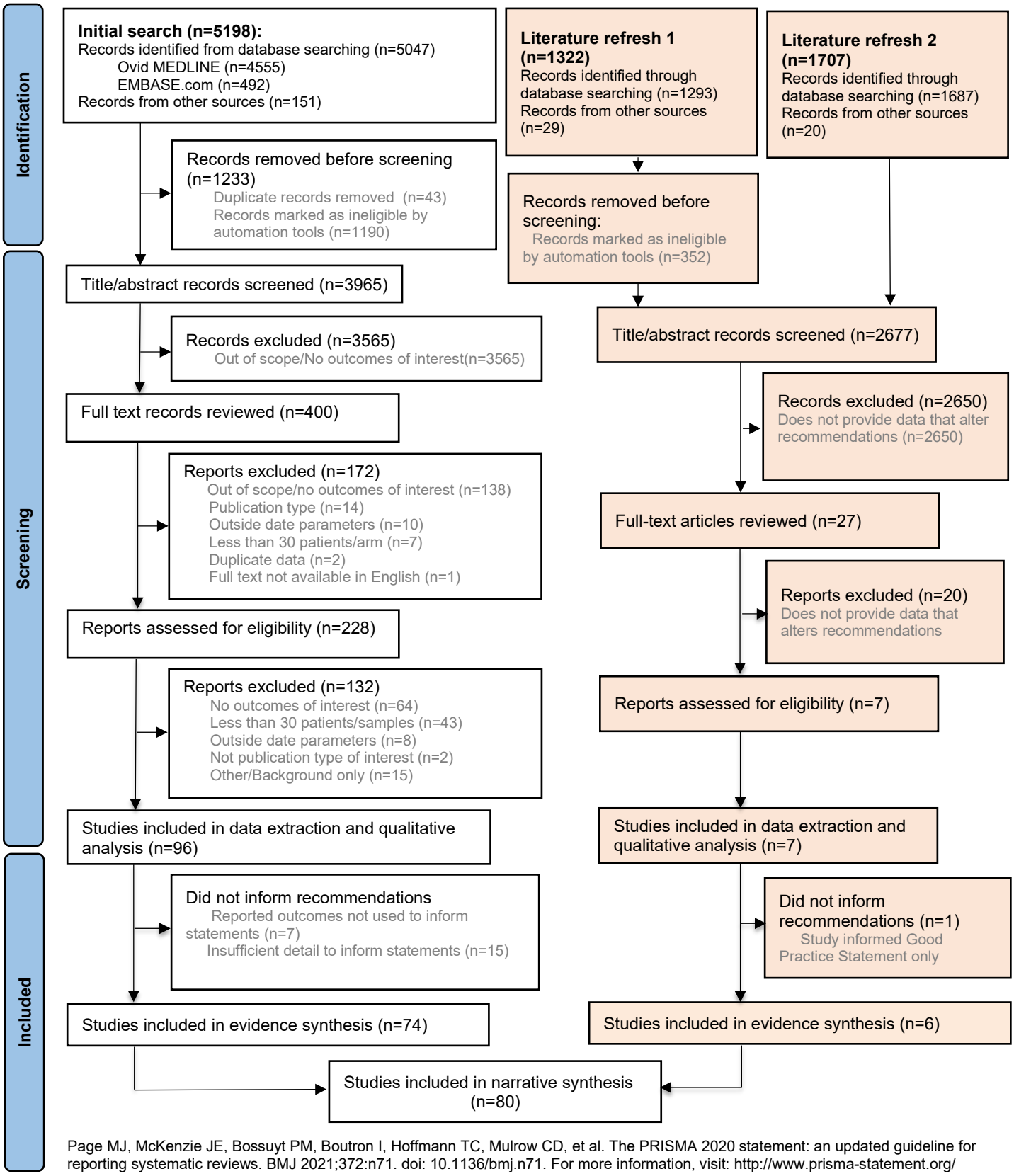
52 12 and 15 and 38 (7568)
53 limit 52 to (english language and yr="2008 -Current") (4347)
54 12 and 19 and 38 (2054)
55 limit 54 to (english language and yr="2000 -Current") (1707)
56 53 or 55 (5011)
57 animals/ not humans/ (4492345)
58 56 not 57 (4933)
59 ("cell line$" or "cell culture$" or xenograft or mouse or mice or murine or rat or dog or cat or porcine or fish or animal).ti. (1317145)
60 58 not 59 (4851)
61 limit 60 to (case reports or comment or editorial or letter) (353)
62 clinical study/ or comparative study/ or exp consensus development conference/ or evaluation studies/ or meta-analysis/ or multicenter study/ or systematic review/ or validation studies/ (2352393)
63 61 not 62 (347)
64 60 not 63 (4504)
65 12 and 15 and 51 (781)
66 limit 65 to (english language and yr="2008 -Current") (645)
67 12 and 19 and 51 (60)
68 limit 67 to (english language and yr="2000 -Current") (54)
69 66 or 68 (646)
70 69 not 57 (640)
71 70 not 59 (628)
72 limit 71 to (case reports or comment or editorial or letter) (38)
73 72 not 62 (38)
74 71 not 73 (590)
75 74 or 64 (4871)
76 remove duplicates from 75 (4860)
77 review/ or review literature as topic/ (2468000)
78 76 not 77 (4394)
79 76 and 77 (466)
80 (systematic or data or evidence or rationale or cohort).tw,kf. (5009886)
81 79 not 80 (306)
82 76 not 81 (4554)

Embase Search String Run on 12/16/2018

1 'microsatellite instability'/de
2 'mismatch repair'/de
3 'protein mutl'/de
4 'mutl protein homolog 1'/de
5 'protein muts'/de
6 'epithelial cell adhesion molecule'/de
7 ((mlh1 OR msh2 OR msh6 OR pms2 OR 'bat 25' OR 'bat 26') NEXT/5 (gene OR genes OR protein OR proteins OR expression OR methylation OR hypermethylation)):ti,ab,kw
8 (microsatellite NEXT/3 (instability OR stable OR unstable OR instability OR ultramutated OR hypermutated OR hypermutation OR ultramutation)):ti,ab,kw
9 msi:ti,ab,kw OR 'msi h':ti,ab,kw OR 'msi l':ti,ab,kw OR 'replication error phenotype':ti,ab,kw OR 'replication error phenotypes':ti,ab,kw
10 'd mmr':ti,ab,kw OR dmmr:ti,ab,kw OR 'mmr d':ti,ab,kw OR 'mismatch repair':ti,ab,kw OR epcam:ti,ab,kw OR tacstd1:ti,ab,kw OR 'epithelial cell adhesion molecule':ti,ab,kw OR 'epithelial cell adhesion molecules':ti,ab,kw
11 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10
12 neoplas*:ti,ab,kw OR tumo?r*:ti,ab,kw OR carcino*:ti,ab,kw OR cancer*:ti,ab,kw OR oncogen*:ti,ab,kw OR adenocarcinoma*:ti,ab,kw OR sarcoma*:ti,ab,kw OR lymphoma*:ti,ab,kw OR malignan*:ti,ab,kw OR metastatic:ti,ab,kw OR metastas?s:ti,ab,kw
13 'checkpoint inhibitor':ti,ab,kw OR 'checkpoint inhibitors':ti,ab,kw OR immunotherap*:ti,ab,kw OR 'immune checkpoint':ti,ab,kw
14 #12 OR #13
15 'hereditary nonpolyposis colorectal cancer'/de
16 lynch:ti,ab,kw OR turcot:ti,ab,kw OR 'hereditary nonpolyposis':ti,ab,kw OR hnpcc:ti,ab,kw OR 'muir torre':ti,ab,kw OR 'hereditary colon cancer':ti,ab,kw OR 'inherited colon cancer':ti,ab,kw
17 #15 OR #16
18 'immunohistochemistry'/de

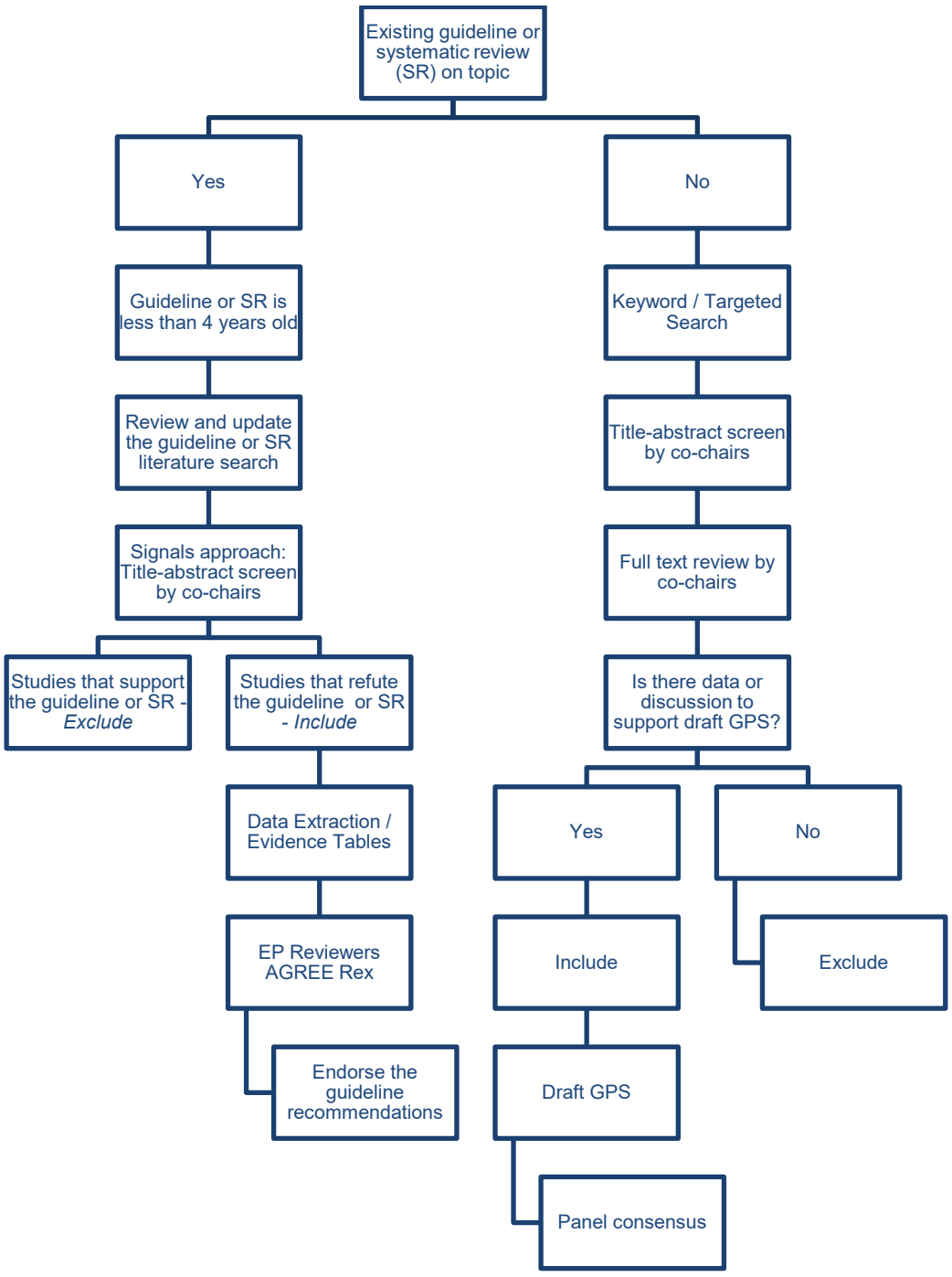
19 'enzyme immunoassay'/de
20 'polymerase chain reaction'/exp
21 'high throughput sequencing'/de
22 'whole genome sequencing'/de
23 'whole exome sequencing'/de
24 'multilocus sequence typing'/de
25 'sequence analysis'/de
26 'rna sequence'/de
27 'dna mutational analysis'/de
28 'dna sequence'/de
29 'sequence analysis':ti,ab,kw OR 'sequence analyses':ti,ab,kw OR 'massively parallel sequencing':ti,ab,kw
30 immunohistochemical OR immunohistochemistry OR immunocytochemical OR immunocytochemistry OR imm unoenzyme OR immunoperoxidase OR ipx:ti,ab,kw
31 'test assay':ti,ab,kw OR 'testing assay':ti,ab,kw OR 'test assays':ti,ab,kw OR 'testing assays':ti,ab,kw OR 'test method':ti,ab,kw OR 'testing method':ti,ab,kw OR 'test methods':ti,ab,kw OR 'testing methods':ti,ab,kw
32 'bethesda panel':ti,ab,kw
33 #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32
34 'nivolumab'/de
35 'nivolumab':ti,ab,kw
36 'ipilimumab'/de
37 'ipilimumab' OR yervoy:ti,ab,kw
38 'companion diagnostic' OR 'companion diagnostics':ti,ab,kw
39 'checkpoint inhibitor' OR 'checkpoint inhibitors':ti,ab,kw
40 'immunotherapy'/de
41 'immunomodulating agent'/de
42 'immunomodulating agent' OR immunotherapy:ti,ab,kw
43 'pembrolizumab'/de
44 'pembrolizumab' OR keytruda:ti,ab,kw
45 'cemiplimab'/de
46 'cemiplimab' OR libtayo:ti,ab,kw
47 'monoclonal antibody'/de
48 'atezolizumab'/de
49 'atezolizumab' OR tecentriq OR avelumab OR bavencio OR durvalumab OR imfinzi:ti,ab,kw
50 'immune modulatory':ti,ab,kw
51 'humanized monoclonal antibodies':ti,ab,kw
52 #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 OR #43 OR #44 OR #45 OR #46 OR #47 OR #48 OR #49 OR #50 OR #51
53 #11 AND #14 AND #33
54 #11 AND #14 AND #52
55 #53 OR #54
56 (#53 OR #54) AND [1-1-2008]/sd NOT [13-12-2018]/sd
57 #11 AND #17 AND #33
58 59) #11 AND #17 AND #52
59 #57 OR #58
60 (#57 OR #58) AND [1-1-2008]/sd NOT [13-12-2018]/sd
61 #56 OR #60
62 (#56 OR #60) AND [English]/lim
63 #62 NOT ([animals]/lim NOT [humans]/lim)
64 #63 AND [medline]/lim
65 #63 NOT #64
66 #63 NOT #64 AND ([conference abstract]/lim OR [editorial]/lim OR [letter]/lim)
67 #65 NOT #66
68 'cell line':ti OR 'cell lines':ti OR 'cell culture':ti OR 'cell cultures':ti OR xenograft:ti OR mouse:ti OR mice:ti OR murine:ti OR rat:ti OR dog:ti OR cat:ti OR porcine:ti OR fish:ti OR animal:ti
69 #67 NOT #68
70 #69 AND [review]/lim
71 systematic:ti,ab,kw OR data:ti,ab,kw OR rationale:ti,ab,kw OR evidence:ti,ab,kw OR cohort:ti,ab,kw
72 #70 NOT #71
73 #69 NOT #72

Supplemental Figure 2: Literature Review Flow Diagram



Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

Supplemental Figure 3. Good Practice Statements (GPS) Literature Review Strategy



References

1. Schuenemann H, Brozek J, Guyatt G, Oxman A; The GRADE Working Group. GRADE handbook for grading quality of evidence and strength of recommendations. Updated October 2013. Accessed August 3, 2021. https://gdt.gradepro.org/app/handbook/handbook.html
2. Sterne JA, Hernan MA, Reeves BC, et al. ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. *BMJ*. 2016;355:i4919. doi:10.1136/bmj.i4919
3. Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction-grade evidence profiles and summary of findings tables. *J Clin Epidemiol*. 2011;64(4):383-394. doi:10.1016/j.jclinepi.2010.04.026
4. Alonso-Coello P, Schunemann HJ, Moberg J, et al. GRADE Evidence to Decision (EtD) frameworks: A systematic and transparent approach to making well informed healthcare choices. 1: Introduction. *BMJ*. 2016;353:i2016. doi:10.1136/bmj.i2016
5. Guyatt GH, Alonso-Coello P, Schunemann HJ, et al. Guideline panels should seldom make good practice statements: Guidance from the GRADE working group. *J Clin Epidemiol*. 2016;80:3-7. doi:10.1016/j.jclinepi.2016.07.006
6. Shiffman RN, Michel G, Rosenfeld RM, Davidson C. Building better guidelines with BRIDGE-Wiz: Development and evaluation of a software assistant to promote clarity, transparency, and implementability. *J Am Med Inform Assoc*. 2012;19(1):94-101. doi:10.1136/amiajnl-2011-000172
7. Jang M, Kwon Y, Kim H, et al. Microsatellite instability test using peptide nucleic acid probe-mediated melting point analysis: A comparison study. *BMC Cancer*. 2018;18(1):1218. doi:10.1186/s12885-018-5127-6
8. Yan WY, Hu J, Xie L, et al. Prediction of biological behavior and prognosis of colorectal cancer patients by tumor MSI/MMR in the Chinese population. *Onco Targets Ther*. 2016;9:7415-7424. doi:10.2147/OTT.S117089
9. Yuan L, Chi Y, Chen W, et al. Immunohistochemistry and microsatellite instability analysis in molecular subtyping of colorectal carcinoma based on mismatch repair competency. *Int J Clin Exp Med*. 2015;8(11):20988-1000.
10. Siddique S, Tariq K, Rafiq S, et al. Sporadic early onset colorectal cancer in Pakistan: A case-control analysis of microsatellite instability. *Asian Pac J Cancer Prev*. 2016;17(5):2587-2592.
11. Batur S, Vuralli Bakkaloglu D, Kepil N, Erdamar S. Microsatellite instability and B-type Raf proto-oncogene mutation in colorectal cancer: Clinicopathological characteristics and effects on survival. *Bosn J Basic Med Sci*. 2016;16(4):254-260. doi:10.17305/bjbms.2016.1238
12. Kim JE, Hong YS, Ryu MH, et al. Association between deficient mismatch repair system and efficacy to irinotecan-containing chemotherapy in metastatic colon cancer. *Cancer Sci*. 2011;102(9):1706-1711. doi:10.1111/j.1349-7006.2011.02009.x
13. Yoon YS, Yu CS, Kim TW, et al. Mismatch repair status in sporadic colorectal cancer: Immunohistochemistry and microsatellite instability analyses. *J Gastroenterol Hepatol*. 2011;26(12):1733-1739. doi:10.1111/j.1440-1746.2011.06784.x
14. Signoroni S, Tibiletti MG, Ricci MT, et al. Performance of tumor testing for Lynch syndrome identification in patients with colorectal cancer: A retrospective single-center study. *Tumori*. 2019;105(1):76-83. doi:10.1177/0300891618792460
15. Haraldsdottir S, Rafnar T, Frankel WL, et al. Comprehensive population-wide analysis of Lynch syndrome in Iceland reveals founder mutations in MSH6 and PMS2. *Nat Commun*. 2017;8:14755. doi:10.1038/ncomms14755
16. Canard G, Lefevre JH, Colas C, et al. Screening for Lynch syndrome in colorectal cancer: Are we doing enough? *Ann Surg Oncol*. 2012;19(3):809-816. doi:10.1245/s10434-011-2014-7
17. Warrier SK, Trainer AH, Lynch AC, et al. Preoperative diagnosis of Lynch syndrome with DNA mismatch repair immunohistochemistry on a diagnostic biopsy. *Dis Colon Rectum*. 2011;54(12):1480-1487. doi:10.1097/DCR.0b013e318231db1f
18. Limburg PJ, Harmsen WS, Chen HH, et al. Prevalence of alterations in DNA mismatch repair genes in patients with young-onset colorectal cancer. *Clin Gastroenterol Hepatol*. 2011;9(6):497-502. doi:10.1016/j.cgh.2010.10.021
19. Chang SC, Lin PC, Yang SH, Wang HS, Liang WY, Lin JK. Taiwan hospital-based detection of Lynch syndrome distinguishes 2 types of microsatellite instabilities in colorectal cancers. *Surgery*. 2010;147(5):720-728. doi:10.1016/j.surg.2009.10.069

20. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J Clin Oncol*. 2008;26(35):5783-5788. doi:10.1200/JCO.2008.17.5950

21. Brennan B, Hemmings CT, Clark I, Yip D, Fadia M, Taupin DR. Universal molecular screening does not effectively detect Lynch syndrome in clinical practice. *Therap Adv Gastroenterol*. 2017;10(4):361-371. doi:10.1177/1756283X17690990

22. Rosty C, Clendenning M, Walsh MD, et al. Germline mutations in PMS2 and MLH1 in individuals with solitary loss of PMS2 expression in colorectal carcinomas from the Colon Cancer Family Registry Cohort. *BMJ Open*. 2016;6(2):e010293. doi:10.1136/bmjopen-2015-010293

23. De Lellis L, Aceto GM, Curia MC, et al. Integrative analysis of hereditary nonpolyposis colorectal cancer: The contribution of allele-specific expression and other assays to diagnostic algorithms. *PLoS One*. 2013;8(11):e81194. doi:10.1371/journal.pone.0081194

24. Bonnet D, Selves J, Toulas C, et al. Simplified identification of Lynch syndrome: A prospective, multicenter study. *Dig Liver Dis*. 2012;44(6):515-522. doi:10.1016/j.dld.2011.12.020

25. Giraldez MD, Balaguer F, Bujanda L, et al. MSH6 and MUTYH deficiency is a frequent event in early-onset colorectal cancer. *Clin Cancer Res*. 2010;16(22):5402-5413. doi:10.1158/1078-0432.CCR-10-1491

26. Berardinelli GN, Scapulatempo-Neto C, Duraes R, Antonio de Oliveira M, Guimaraes D, Reis RM. Advantage of HSP110 (T17) marker inclusion for microsatellite instability (MSI) detection in colorectal cancer patients. *Oncotarget*. 2018;9(47):28691-28701. doi:10.18632/oncotarget.25611

27. Takehara Y, Nagasaka T, Nyuya A, et al. Accuracy of four mononucleotide-repeat markers for the identification of DNA mismatch-repair deficiency in solid tumors. *J Transl Med*. 2018;16(1):5. doi:10.1186/s12967-017-1376-4

28. Zheng J, Huang B, Nie X, Zhu Y, Han N, Li Y. The clinicopathological features and prognosis of tumor MSI in East Asian colorectal cancer patients using NCI panel. *Fut Oncol*. 2018;14(14):1355-1364. doi:10.2217/fon-2017-0662

29. Bacher JW, Sievers CK, Albrecht DM, et al. Improved detection of microsatellite instability in early colorectal lesions. *PLoS One*. 2015;10(8):e0132727. doi:10.1371/journal.pone.0132727

30. Waalkes A, Smith N, Penewit K, et al. Accurate pan-cancer molecular diagnosis of microsatellite instability by single-molecule molecular inversion probe capture and high-throughput sequencing. *Clin Chem*. 2018;64(6):950-958. doi:10.1373/clinchem.2017.285981

31. Berginc G, Bracko M, Ravnik-Glavac M, Glavac D. Screening for germline mutations of MLH1, MSH2, MSH6 and PMS2 genes in Slovenian colorectal cancer patients: Implications for a population specific detection strategy of Lynch syndrome. *Fam Cancer*. 2009;8(4):421-429. doi:10.1007/s10689-009-9258-4

32. Schofield L, Watson N, Grieu F, et al. Population-based detection of Lynch syndrome in young colorectal cancer patients using microsatellite instability as the initial test. *Int J Cancer*. 2009;124(5):1097-1102. doi:10.1002/ijc.23863

33. Jin HY, Liu X, Li VK, et al. Detection of mismatch repair gene germline mutation carrier among Chinese population with colorectal cancer. *BMC Cancer*. 2008;8:44. doi:10.1186/1471-2407-8-44

34. Alpert L, Pai RK, Srivastava A, et al. Colorectal carcinomas with isolated loss of PMS2 staining by immunohistochemistry. *Arch Pathol Lab Med*. 2018;142(4):523-528. doi:10.5858/arpa.2017-0156-OA

35. Zhu L, Huang Y, Fang X, et al. A novel and reliable method to detect microsatellite instability in colorectal cancer by next-generation sequencing. *J Mol Diagn*. 2018;20(2):225-231. doi:10.1016/j.jmoldx.2017.11.007

36. Benmoussa A, Badre W, Pedroni M, et al. Clinical and molecular characterization of colorectal cancer in young Moroccan patients. *Turk J Gastroenterol*. 2012;23(6):686-690. doi:10.4318/tjg.2012.0474

37. Jensen LH, Rasmussen AA, Byriel L, et al. Regulation of MLH1 mRNA and protein expression by promoter methylation in primary colorectal cancer: A descriptive and prognostic cancer marker study. *Cell Oncol (Dordr)*. 2013;36(5):411-419. doi:10.1007/s13402-013-0148-2

38. Perez-Carbonell L, Ruiz-Ponte C, Guarinos C, et al. Comparison between universal molecular screening for Lynch syndrome and revised Bethesda guidelines in a large population-based cohort of patients with colorectal cancer. *Gut*. 2012;61(6):865-872. doi:10.1136/gutjnl-2011-300041

39. Mojtahed A, Schrijver I, Ford JM, Longacre TA, Pai RK. A two-antibody mismatch repair protein immunohistochemistry screening approach for colorectal carcinomas, skin sebaceous tumors, and gynecologic tract carcinomas. *Mod Pathol*. 2011;24(7):1004-1014. doi:10.1038/modpathol.2011.55

40. Bertagnolli MM, Niedzwiecki D, Compton CC, et al. Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. *J Clin Oncol*. 2009;27(11):1814-1821. doi:10.1200/JCO.2008.18.2071

41. Ramsoekh D, Wagner A, van Leerdam ME, et al. A high incidence of MSH6 mutations in Amsterdam criteria II-negative families tested in a diagnostic setting. *Gut*. 2008;57(11):1539-1544. doi:10.1136/gut.2008.156695

42. Jensen LH, Lindebjerg J, Byriel L, Kolvraa S, Cruger DG. Strategy in clinical practice for classification of unselected colorectal tumours based on mismatch repair deficiency. *Colorectal Dis*. 2008;10(5):490-497. doi: 10.1111/j.1463-1318.2007.01378.x

43. Wang C, Liang C. MSIpred: A python package for tumor microsatellite instability classification from tumor mutation annotation data using a support vector machine. *Sci Rep*. 2018;8(1):17546. doi:10.1038/s41598-018-35682-z

44. Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. *Cancer Med*. 2018;7(3):746-756. doi:10.1002/cam4.1372

45. Gray PN, Tsai P, Chen D, et al. TumorNext-Lynch-MMR: A comprehensive next generation sequencing assay for the detection of germline and somatic mutations in genes associated with mismatch repair deficiency and lynch syndrome. *Oncotarget*. 2018;9(29):20304-20322. doi:10.18632/oncotarget.24854

46. Nowak JA, Yurgelun MB, Bruce JL, et al. Detection of mismatch repair deficiency and microsatellite instability in colorectal adenocarcinoma by targeted next-generation sequencing. *J Mol Diagn*. 2017;19(1):84-91. doi:10.1016/j.jmoldx.2016.07.010

47. Papke DJ, Jr., Nowak JA, Yurgelun MB, et al. Validation of a targeted next-generation sequencing approach to detect mismatch repair deficiency in colorectal adenocarcinoma. *Mod Pathol*. 2018;31(12):1882-1890. doi:10.1038/s41379-018-0091-x

48. Middha S, Zhang L, Nafa K, et al. Reliable pan-cancer microsatellite instability assessment by using targeted next-generation sequencing data. *JCO Precis Oncol*. 2017:PO.17.00084. doi:10.1200/PO.17.00084

49. Christakis AG, Papke DJ, Nowak JA, et al. Targeted cancer next-generation sequencing as a primary screening tool for microsatellite instability and Lynch syndrome in upper gastrointestinal tract cancers. *Cancer Epidemiol Biomarkers Prev*. 2019;28(7):1246-1251. doi:10.1158/1055-9965.EPI-18-1250

50. Mathiak M, Warneke VS, Behrens HM, et al. Clinicopathologic characteristics of microsatellite instable gastric carcinomas revisited: Urgent need for standardization. *Appl Immunohistochem Molecul Morphol*. 2017;25(1):12-24. doi:10.1097/PAI.0000000000000264

51. Bae YS, Kim H, Noh SH, Kim H. Usefulness of immunohistochemistry for microsatellite instability screening in gastric cancer. *Gut Liver*. 2015;9(5):629-635. doi:10.5009/gnl15133

52. Gu M, Kim D, Bae Y, Choi J, Kim S, Song S. Analysis of microsatellite instability, protein expression and methylation status of hMLH1 and hMSH2 genes in gastric carcinomas. *Hepatogastroenterology*. 2009;56(91-92):899-904.

53. Ruemmele P, Dietmaier W, Terracciano L, et al. Histopathologic features and microsatellite instability of cancers of the papilla of vater and their precursor lesions. *Am J Surg Pathol*. 2009;33(5):691-704. doi:10.1097/PAS.0b013e3181983ef7

54. Seo HM, Chang YS, Joo SH, et al. Clinicopathologic characteristics and outcomes of gastric cancers with the MSI-H phenotype. *J Surg Oncol*. 2009;99(3):143-147. doi:10.1002/jso.21220

55. Egoavil C, Alenda C, Castillejo A, et al. Prevalence of Lynch syndrome among patients with newly diagnosed endometrial cancers. *PLoS One*. 2013;8(11):e79737. doi:10.1371/journal.pone.0079737

56. Leenen CH, van Lier MG, van Doorn HC, et al. Prospective evaluation of molecular screening for Lynch syndrome in patients with endometrial cancer <= 70 years. *Gynecol Oncol*. 2012;125(2):414-420. doi:10.1016/j.ygyno.2012.01.049

57. Dong F, Costigan DC, Howitt BE. Targeted next-generation sequencing in the detection of mismatch repair deficiency in endometrial cancers. *Mod Pathol*. 2019;32(2):252-257. doi:10.1038/s41379-018-0125-4

58. Haruma T, Nagasaka T, Nakamura K, et al. Clinical impact of endometrial cancer stratified by genetic mutational profiles, POLE mutation, and microsatellite instability. *PLoS One*. 2018;13(4):e0195655. doi:10.1371/journal.pone.0195655

59. Stelloo E, Jansen AML, Osse EM, et al. Practical guidance for mismatch repair-deficiency testing in endometrial cancer. *Ann Oncol*. 2017;28(1):96-102. doi:10.1093/annonc/mdw542

60. Bruegl AS, Ring KL, Daniels M, Fellman BM, Urbauer DL, Broaddus RR. Clinical challenges associated with universal screening for Lynch syndrome-associated endometrial cancer. *Cancer Prev Res (Phila)*. 2017;10(2):108-115. doi:10.1158/1940-6207.CAPR-16-0219

61. 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? *J Mol Diagn*. 2017;19(1):57-64. doi:10.1016/j.jmoldx.2016.07.008

62. Libera L, Sahnane N, Carnevali IW, et al. Microsatellite analysis of sporadic and hereditary gynaecological cancer in routine diagnostics. *J Clin Pathol*. 2017;70(9):792-797. doi:10.1136/jclinpath-2017-204348

63. McConechy MK, Talhouk A, Li-Chang HH, et al. Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas. *Gynecol Oncol*. 2015;137(2):306-310. doi:10.1016/j.ygyno.2015.01.541

64. Buchanan DD, Tan YY, Walsh MD, et al. Tumor mismatch repair immunohistochemistry and DNA MLH1 methylation testing of patients with endometrial cancer diagnosed at age younger than 60 years optimizes triage for population-level germline mismatch repair gene mutation testing. *J Clin Oncol*. 2014;32(2):90-100. doi:10.1200/JCO.2013.51.2129

65. Moline J, Mahdi H, Yang B, et al. Implementation of tumor testing for lynch syndrome in endometrial cancers at a large academic medical center. *Gynecol Oncol*. 2013;130(1):121-126. doi:10.1016/j.ygyno.2013.04.022

66. Peterson LM, Kipp BR, Halling KC, et al. Molecular characterization of endometrial cancer: A correlative study assessing microsatellite instability, MLH1 hypermethylation, DNA mismatch repair protein expression, and PTEN, PIK3CA, KRAS, and BRAF mutation analysis. *Int J Gynecol Pathol*. 2012;31(3):195-205. doi:10.1097/PGP.0b013e318231fc51

67. Yoon SN, Ku JL, Shin YK, et al. Hereditary nonpolyposis colorectal cancer in endometrial cancer patients.. *Int J Cancer*. 2008;122(5):1077-1081. doi:10.1002/ijc.22986

68. Latham A, Srinivasan P, Kemel Y et al. Microsatellite instability is associated with the presence of Lynch syndrome pan-cancer. *J Clin Oncol*. 2019;37(4):286-295. doi:10.1200/JCO.18.00283

69. Hempelmann JA, Lockwood CM, Konnick EQ, et al. Microsatellite instability in prostate cancer by PCR or next-generation sequencing. *J Immunother Cancer*. 2018;6(1):29. doi:10.1186/s40425-018-0341-y

70. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. Microsatellite instability detection by next generation sequencing. *Clin Chem*. 2014;60(9):1192-1199. doi:10.1373/clinchem.2014.223677

71. Bartley AN, Luthra R, Saraiya DS, Urbauer DL, Broaddus RR. Identification of cancer patients with Lynch syndrome: Clinically significant discordances and problems in tissue-based mismatch repair testing. *Cancer Prev Res (Phila)*. 2012;5(2):320-327. doi:10.1158/1940-6207.CAPR-11-0288

72. Altavilla G, Fassan M, Busatto G, Orsolan M, Giacomelli L. Microsatellite instability and hMLH1 and hMSH2 expression in renal tumors. *Oncol Rep*. 2010;24(4):927-932. doi:10.3892/or.2010.927

73. Fusco N, Lopez G, Corti C, et al. Mismatch repair protein loss as a prognostic and predictive biomarker in breast cancers regardless of microsatellite instability. *JNCI Cancer Spectr*. 2018;2(4):pky056. doi:10.1093/jncics/pky056

74. Abida W, Cheng ML, Armenia J, et al. Analysis of the prevalence of microsatellite instability in prostate cancer and response to immune checkpoint blockade. *JAMA Oncol*. 2019;5(4):471-478. doi:10.1001/jamaoncol.2018.5801

75. Hechtman JF, Rana S, Middha S, et al. Retained mismatch repair protein expression occurs in approximately 6% of microsatellite instability-high cancers and is associated with missense mutations in mismatch repair genes. *Mod Pathol*. 2019;19:19. doi:10.1038/s41379-019-0414-6

76. Pabla S, Andreas J, Lenzo FL, et al. Development and analytical validation of a next-generation sequencing based microsatellite instability (MSI) assay. *Oncotarget*. 2019;10(50):5181-5193. doi:10.18632/oncotarget.27142

77. Trabucco SE, Gowen K, Maund SL, et al. A novel next-generation sequencing approach to detecting microsatellite instability and pan-tumor characterization of 1000 microsatellite instability-high cases in 67,000 patient samples. *J Mol Diagn*. 2019;21(6):1053-1066. doi:10.1016/j.jmoldx.2019.06.011

78. Rahner N, Brockschmidt FF, Steinke V, et al. Mutation and association analyses of the candidate genes ESR1, ESR2, MAX, PCNA, and CAT2A in patients with unexplained MSH2-deficient tumors. *Fam Cancer*. 2012;11(1):19-26. doi:10.1007/s10689-011-9489-z

79. Fabrizio DA, George TJ, Jr., Dunne RF, et al. Beyond microsatellite testing: Assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. *J Gastrointest Oncol*. 2018;9(4):610-617. doi:10.21037/jgo.2018.05.06

80. Stadler ZK, Battaglin F, Middha S, et al. Reliable detection of mismatch repair deficiency in colorectal cancers using mutational load in next-generation sequencing panels. *J Clin Oncol*. 2016;34(18):2141-2147. doi:10.1200/JCO.2015.65.1067

81. Salem ME, Puccini A, Grothey A, et al. Landscape of tumor mutation load, mismatch repair deficiency, and PD-L1 expression in a large patient cohort of gastrointestinal cancers. *Mol Cancer Res*. 2018;16(5):805-812. doi:10.1158/1541-7786.MCR-17-0735

82. Hodges TR, Ott M, Xiu J, et al. Mutational burden, immune checkpoint expression, and mismatch repair in glioma: Implications for immune checkpoint immunotherapy. *Neuro Oncol*. 2017;19(8):1047-1057. doi:10.1093/neuonc/nox026

83. Goodfellow PJ, Billingsley CC, Lankes HA, et al. Combined microsatellite instability, MLH1 methylation analysis, and immunohistochemistry for Lynch syndrome screening in endometrial cancers from GOG210: An NRG Oncology and Gynecologic Oncology Group study. *J Clin Oncol*. 2015;33(36):4301-4308. doi:10.1200/JCO.2015.63.9518

84. Buchanan DD, Clendenning M, Rosty C, et al. Tumor testing to identify Lynch syndrome in two Australian colorectal cancer cohorts. *J Gastroenterol Hepatol*. 2017;32(2):427-438. doi:10.1111/jgh.13468

85. Schofield L, Grieu F, Goldblatt J, Amanuel B, Iacopetta B. A state-wide population-based program for detection of Lynch syndrome based upon immunohistochemical and molecular testing of colorectal tumours. *Fam Cancer*. 2012;11(1):1-6. doi:10.1007/s10689-011-9494-2

86. van Lier MG, Leenen CH, Wagner A, et al. Yield of routine molecular analyses in colorectal cancer patients <=70 years to detect underlying Lynch syndrome. *J Pathol*. 2012;226(5):764-774. doi:10.1002/path.3963

87. Balmana J, Balaguer F, Castellvi-Bel S, et al. Comparison of predictive models, clinical criteria and molecular tumour screening for the identification of patients with Lynch syndrome in a population-based cohort of colorectal cancer patients. *J Med Genet*. 2008;45(9):557-563. doi:10.1136/jmg.2008.059311

88. Rodriguez-Hernandez I, Garcia JL, Santos-Briz A, et al. Integrated analysis of mismatch repair system in malignant astrocytomas. *PLoS One*. 2013;8(9):e76401. doi:10.1371/journal.pone.0076401