MMR, MSI testing guideline nears finish line

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December 2021—No single assay can capture all cancer patients with DNA mismatch repair deficiency, and in determining a patient's eligibility for immune checkpoint inhibitor therapy, assays for MMR deficiency, microsatellite instability, and tumor mutation burden should not be considered interchangeable, say the authors of a forthcoming CAP guideline on MMR and MSI testing.

The guideline expert panel considered three tests: immunohistochemistry for the mismatch repair proteins MLH1, MSH2, MSH6, and PMS2; PCR-based MSI analysis; and next-generation-sequencing–based MSI analysis. When guideline proceedings began in early 2018, the FDA had not yet approved high TMB as a biomarker for immune checkpoint inhibitor therapy. Thus, the expert panel also considered whether high TMB can be used as a surrogate for MMR deficiency or high levels of MSI.

Expert and advisory panel members have approved a working final draft of the guideline, which at the end of November was being reviewed by an independent review panel established by the CAP. "We're getting closer to the finish line," Russell Broaddus, MD, PhD, professor in the Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, said in a CAP21 session on the guideline's development. Once approved by the review panel, the guideline will be submitted to the *Archives of Pathology & Laboratory Medicine*.

"The main question we set out to answer with this guideline," Dr. Broaddus said, "is 'what clinical test best identifies defects in DNA mismatch repair?'" The clinical trial that accompanied the FDA's 2017 approval of the checkpoint inhibitors across all cancer types did not distinguish sufficiently between MMR deficiency and high levels of MSI, he said. "And contrary to what many in the field may believe, MMR deficiency is not always synonymous with MSI-high. They may overlap in certain tumor types, primarily colorectal cancer." And while it's standard of care to screen colorectal and endometrial cancer patients for Lynch syndrome with MMR by IHC or PCR-based MSI analysis, MMR deficiency occurs in significant numbers of cancers outside the colon and endometrium.

Dr. Broaddus

In gathering evidence for the guideline, "we were relatively confident there would be sufficient literature for colorectal and endometrial cancer," Dr. Broaddus said. But because they were less certain of the available evidence for other cancer types, they examined the diagnostic characteristics of the tests when predicting germline Lynch mutations, "recognizing this may be an imperfect surrogate for immune checkpoint inhibitors."

The guideline expert panel developed the following draft guideline statements on MMR by IHC, MSI by PCR, and MSI by NGS:

1. In colorectal cancer patients being considered for immune checkpoint blockade therapy, pathologists should use MMR by IHC and/or MSI by PCR for detection of DNA MMR defects. Although MMR by IHC or MSI by PCR are preferred, a validated MSI by NGS assay also may be used (validated against MMR IHC or MSI by PCR and showing equivalency).

"This is a strong recommendation," Dr. Broaddus said. "For colorectal cancer there was by far the most published

evidence to evaluate compared to any other cancer type. Mismatch repair immunohistochemistry, PCR-based MSI analysis, and MSI by next-generation sequencing are nearly comparable methods in the detection of mismatch repair or MSI defects."

2. In gastroesophageal and small bowel cancer patients being considered for immune checkpoint blockade therapy, pathologists should use MMR by IHC and/or MSI by PCR over MSI by NGS. This recommendation does not include esophageal squamous cell carcinoma.

"Again, this is a strong recommendation," he said. Once sufficient evidence has accumulated, it's likely that MSI by NGS will be shown to be comparable to MMR by IHC and PCR-based MSI for these two cancer types.

3. In endometrial cancer patients being considered for immune checkpoint blockade therapy, pathologists should use MMR by IHC over MSI by PCR or NGS to detect DNA MMR defects (strong recommendation).

4. In patients with cancer types other than CRC, GEA, small bowel, and endometrial, pathologists should test for DNA MMR, though the optimal approach for detecting defects has not been established. "The expert panel found that only colorectal cancer, gastroesophageal adenocarcinoma, small bowel cancer, and endometrial cancer had sufficient evidence to formulate a specific recommendation. For all other cancer types, we recommend an assay be performed to detect MSI or mismatch repair defects, but there is no specific guidance as to which assay is best," he said.

PCR- and NGS-based methods for determining MSI perform well for colorectal cancer and other GI cancers, possibly because many of these assays first were optimized for detection of MSI in colorectal cancer, Dr. Broaddus said. But PCR-based MSI approaches have significantly lower performance metrics in cancer types outside the GI tract. Evidence shows that when PCR-based or NGS-based methods are optimized for an individual cancer type, performance metrics improve. "This implies there may not be a universal PCR-based approach, which seems daunting for most clinical labs testing a wide variety of cancer types." Thus, the expert panel concluded that MMR by IHC may be the preferred testing approach for cancer types outside the GI tract.

Detecting MSI in endometrial cancers may be more difficult than in colorectal cancers, Dr. Broaddus said. In an early study of eight families with known *MLH1* or *MSH2* mutations, microsatellite stable was seen in only 11 percent of colon cancers but in 23 percent of endometrial cancers (Kuismanen SA, et al. *Am J Pathol.* 2002;160[6]:1953–1958). Two other studies highlight another problem with MSI analysis in endometrial cancer. The chromatogram of a representative MSI-high colorectal cancer demonstrating loss of MMR proteins by IHC compared with the normal control shows that "for each microsatellite there are a lot more peaks in the tumor compared to the normal," which makes detection of MSI in colon cancers typically straightforward. But in a representative endometrial cancer also demonstrating loss of MMR proteins by IHC, each microsatellite in the tumor has far fewer additional peaks in the tumor than in the normal tissue (Wang Y, et al. *J Mol Diagn.* 2017;19[1]:57–64; Wu X, et al. *Mod Pathol.* 2019;32[5]:650–658). "This minimal microsatellite shift is quite easy for an operator to miss," he said. "By most standard PCR-based MSI assays, we may be missing subtle shifts in the microsatellites, thus classifying some tumors as MSI-low and microsatellite stable when they should be MSI-high."

A study comparing MSI methods in prostate cancer "continues the theme from endometrial cancer," Dr. Broaddus said, "that the traditional [PCR-based] five-marker panel for MSI detection that many clinical laboratories use may not be optimal for cancer types outside the GI tract." In the study, Hempelmann, et al., identified 29 MMR-deficient and 62 MMR-intact prostate cancers by targeted sequencing of DNA MMR genes using Large-Panel NGS, MSIplus (an 18-marker NGS panel), and the traditional PCR-based five-marker panel. MSI-PCR was associated with the most false-negatives, while both NGS approaches were associated with better detection of true MSI-high cases (Hempelmann JA, et al. *J Immunother Cancer.* 2018;6[1]:29).

But a 2019 study, he said, "highlights a possible pitfall of the NGS-based approach for assessing microsatellite instability" (Trabucco SE, et al. *J Mol Diagn.* 2019;21[6]:1053–1066). Most NGS-based approaches employ a scoring system that includes an indeterminate range in which the MSI call is more uncertain. As tumor purity decreases, the MSI score decreases, and it becomes more difficult to distinguish MSI-intermediate from MSI-high. "Many of the

cancer types we test by NGS-based approaches fall into the 20 to 40 percent tumor purity range," he said of his own experience. In that range, mutations can be identified accurately by NGS, "but it becomes increasingly difficult to classify tumors as MSI-high or microsatellite stable, and thus we may get more tumors in the microsatellite-indeterminate range."

The expert panel encountered methodological problems in the literature on NGS-based approaches to MSI detection, Dr. Broaddus said. In one study of 100 cancers, 73 of which were colorectal, the authors reported 98 percent concordance between MSI-NGS and MSI-PCR. But 15 of the 100 tumors had inconclusive results by MSI-NGS. "Thus, the true concordance is nowhere close to 98 percent."

In another example, a study reported in *JAMA Oncology* summarizes "the seemingly impressive data for a different approach for detecting MSI-high" using NGS, Dr. Broaddus said, but the expert panel found, by "digging deeply into the methodology," important information: In 313 of the 1,346 patients for whom the NGS assay was performed, MSI could not be accurately assessed, and for another 28 patients, MSI results were indeterminate. For 25 percent of the patients, then, there were no MSI results. "This was a common finding," he said of several studies that reported results for MSI by NGS, and "a real limitation to using this approach broadly."

Draft guideline statement No. 5 indicates that for all cancer patients being considered for immune checkpoint blockade therapy, tumor mutation burden should not be used as a surrogate for the detection of DNA MMR defects. "This is a strong recommendation," Dr. Broaddus said.

A meta-analysis of the correlation between high TMB and objective response rate with immune checkpoint blockade therapy in 27 tumor types demonstrated broadly that the higher the TMB, the more likely the patient will respond to the therapy (Yarchoan M, et al. *N Engl J Med.* 2017;377[25]:2500–2501). "I have encountered numerous pathologists and oncologists who believe high tumor mutation burden is synonymous with microsatellite instability-high, and this may be because of the known findings that high levels of microsatellite instability are commonly associated with larger numbers of tumor mutations."

But many studies show that high TMB is not always associated with MMR defects or MSI-high, he said, noting it appears to have some cancer type specificity. In CRC there is strong concordance between high TMB and MSI-high, but melanoma and non-small cell carcinoma—two cancer types with the highest TMB traditionally—rarely have high levels of MSI (Vanderwalde A, et al. *Cancer Med.* 2018;7[3]:746–756; Chalmers ZR, et al. *Genome Med.* 2017;9[1]:34). "So there is some disconnect in tumor mutation burden and MSI-high."

The final draft guideline statement is as follows: For cancer patients being considered for immune checkpoint blockade therapy, if an MMR deficiency is identified, pathologists should recommend follow-up evaluation for Lynch syndrome. "This is a strong recommendation," Dr. Broaddus said. "For colorectal and endometrial cancer patients, this communication is likely already occurring in the context of standard-of-care mismatch repair immunohistochemistry or MSI screening. However, many different cancer types have been reported in Lynch patients, and oncologists caring for these patients may not be thinking about the possibility of a hereditary cancer syndrome."

Laboratory tests that indicate the possibility of Lynch syndrome are MSH2, PMS2, and MSH6 IHC loss; MLH1 IHC loss that is not associated with *MLH1* gene methylation; and MSI-high with concurrent absence of *MLH1* gene methylation. "It is recommended that the pathologist communicate directly this finding with the patient's oncologist, in addition to the written communication in the pathology report," he said.

The guideline expert panel also developed three draft "good practice" statements; each lacked sufficient evidence to be incorporated as a guideline recommendation. The first addresses discordant results. It says MMR by IHC, MSI-PCR, MSI-NGS, and TMB are not always concordant, especially in cancers outside the GI tract. When results are discordant, it says, make sure discordance is not due to interpretive error.

The clinical significance of true discordance is unclear, Dr. Broaddus said. For example, it is unknown if an endometrial cancer patient with MMR IHC loss and microsatellite stability and another endometrial cancer patient

with MMR IHC loss and MSI-high would have comparable responses to immune checkpoint blockade therapy. “Certainly, some of the data present already in published studies could be mined retrospectively to get this information.”

In two studies the expert panel found that central review detected a substantial number of interpretive errors made initially with MMR IHC (Kim JH, et al. *Cancer Res Treat.* 2020;52[4]:1135–1144; Overman MJ, et al. *Lancet Oncol.* 2017;18[9]:1182–1191).

The second draft good practice statement says when indeterminate results are identified, an orthogonal assay should be performed or the same assay repeated using a different tumor block. It also advises developing a robust peer-review process for such cases.

“For mismatch repair immunohistochemistry,” Dr. Broaddus said, “I have often found that biopsies perform much better when the matching surgical specimen has unclear IHC results. This may be because the surgical specimen has been anoxic too long, wasn’t fixed optimally, or had some problem during the processing cycle.” He cited an example from his practice in which a colon cancer was identified initially as having loss of MLH1. The patient was young, so germline testing was performed, and no *MLH1* mutation was identified. When asked to review it, he noted “the tumor was negative for *MLH1,* but also the adjacent stromal cells did not have convincingly nuclear positive expression of *MLH1*.” When MLH1 IHC was performed again using a different tumor block, the tumor was strongly positive for *MLH1*, “and this patient did not need germline testing for this gene.”

The third draft good practice statement addresses subclonal, or heterogeneous, IHC loss of MMR protein and says it’s uncertain whether these patients respond to immune checkpoint blockade.

“If a more definitive result is needed to place a patient in a clinical trial involving immune checkpoint blockade, it is recommended that a microdissected area of tumor that shows complete loss of mismatch repair protein be analyzed for microsatellite instability by the PCR-based approach, or an NGS-based approach if it’s a GI cancer.”

Dr. Broaddus calls it “distressingly common” for interpretive errors in MMR by IHC to be identified in a study’s central review. In one large study, up to one in five cases identified as MMR deficient by IHC at initial review were found to be MMR intact after central review. “And importantly, this is presumably after the patients were started on immune checkpoint blockade therapy.” Immune checkpoint blockade likely will be a pillar of cancer therapy for years to come, “so this is not going to be a problem that goes away.” A challenge for pathologists, then, is to provide more formal training in interpretation of MMR by IHC. “Ideally, this training should begin in residency and fellowship.”

Another challenge: Immunohistochemistry, PCR-based MSI analysis, NGS-based MSI analysis, and tumor mutation burden as assessed by NGS are not interchangeable assays, he said. In colon cancers the results of each often but do not always overlap, and for cancers outside the GI tract, “this overlap is definitely less frequent.”

Numerous studies have examined the concordance or discordance between MMR by IHC and PCR-based MSI analysis, he noted. In one study of 591 colorectal and endometrial cancers (primarily colorectal) nearly 12 percent of cases identified as MSI-high had intact IHC expression of MMR proteins (Bartley AN, et al. *Cancer Prev Res.* 2012;5[2]:320–327). “Some of these cases, but certainly not all, ended up being patients with *MLH1* germline mutations that resulted in expression of a nonfunctional MLH1 truncated protein.”

In a study of 938 endometrial cancer patients, 13 percent had MSI-high cancers with no evidence of loss of an MMR protein by IHC (Goodfellow PJ, et al. *J Clin Oncol.* 2015;33[36]:4301–4308). “Recall that because mutations in *MSH6* are more common in Lynch-associated endometrial cancer, MSI-low is a more common finding than in colorectal cancer. In colorectal cancer, for the most part, we believe patients with MSI-low tumors can be treated the same as microsatellite stable patients—no evidence of Lynch syndrome and not eligible for immune checkpoint blockade therapy.” But in that study, for 19 of the MSI-low cases that retained MMR protein expression, “we do not have germline sequencing data because of insufficient funding. So who knows how many of the 19 actually had germline mutations in a Lynch gene?”

In a prospective study of 192 endometrial cancer patients, IHC and PCR-based MSI discordances were identified in about three percent of patients, he said. Five patients had microsatellite stable tumors and loss of MMR protein by IHC, and one had an MSI-high result with intact MMR protein by IHC. In three of the MSS cases, the source of discordance was likely heterogeneous expression of MLH1 and PMS2 by IHC (Bruegl AS, et al. *Cancer Prev Res.* 2017;10[2]:108–115).

Testing discordances have been seen in other cancer types also, but the evidence is weaker, he said. "The patient numbers are admittedly much smaller. This represents a problem but also an opportunity for pathologists to provide the published evidence moving forward."

He cited a case of a young patient with Lynch syndrome who had a germline *MSH2* mutation and colon cancer, endometrial cancer, and thyroid anaplastic carcinoma. All three tumor types showed loss of MSH2 by IHC. The colon cancer was MSI-high; the thyroid carcinoma was MSI-low. "There was insufficient endometrial tissue to perform MSI analysis. This again highlights that there can be discordance between immunohistochemistry and MSI results."

The clinical significance of low-level microsatellite instability outside the GI tract is uncertain, Dr. Broaddus said. MSI-low CRCs typically are treated as sporadic microsatellite stable cancers, and "there is some evidence to support this approach." But for endometrial cancer, MSI-low likely has a different meaning. "It is well established that endometrial cancers associated with *MSH6* have a higher incidence of MSI-low."

In one example, a 51-year-old patient with endometrial cancer had intact MMR by IHC and an MSI-low cancer. "Her family history of cancer was modest," with no young relatives with Lynch-like tumors. Her oncologist suspected Lynch syndrome because the tumor was centered in the lower uterine segment, and the oncologist was aware of evidence demonstrating that a high percentage of lower-uterine-segment–based endometrial cancers are associated with Lynch syndrome. The patient "was indeed shown to have a deleterious germline *MSH6* mutation," an MMR defect that would have been missed had IHC been the only screening approach. "The presence of MSI-low by itself is unlikely to classify most cancer types as MMR deficient for the purposes of clinical trials of immune checkpoint inhibitors," Dr. Broaddus said.

Another challenge (he prefers to call them opportunities) moving forward, then, is that the optimal assessment for identifying cancer patients most likely to respond to immune checkpoint blockade therapy may involve multiple modalities. "We have so far only considered these tests in isolation." The best biomarker-of-treatment outcome, he said, might be a combination: high TMB as determined by NGS and MSI-high, or MSI-high and high numbers of tumor-infiltrating lymphocytes, "and there are numerous other combinations you could consider."

In addition, these analyses have been considered previously as having only binary outputs. A tumor assessed by IHC, for instance, is either MMR deficient or intact. TMB is considered high or low, as is MSI, and PD-L1 is considered positive or negative. "Should we contemplate instead that these outputs be considered as continuous variables?" This is an emerging concept, he said, but published evidence indirectly supports it.

Other opportunities for pathologists: The relationship between NGS-based MSI-high and mismatch repair IHC in cancer types outside the GI tract must be better defined, Dr. Broaddus said. And determining the utility of a staged approach: assess PD-L1 IHC first, then MMR IHC, then MSI by NGS. "We would need to gather the evidence for individual cancer types."

If one testing modality does not identify an MMR defect, he said, is there utility in performing additional tests? If NGS doesn't detect MSI-high in a colorectal cancer, for example, is it worthwhile to perform another test, such as MMR IHC, to more definitively determine whether the tumor is microsatellite stable?

"Currently, we have no evidence to support or not support this type of approach."

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