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CORRESPONDENCE

Reply to Singh et al.

Jaclyn F. Hechtman[1] · Robert Soslow[1] · Jinru Shia[1]

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To the Editor:

We write to respond to a letter titled "Interpretation of mismatch repair protein expression using obsolete criteria results in discrepancies with microsatellite instability and mutational testing results [1]. Comment on Hechtman et al. Mod Pathol 2020; 33:871–879." This letter to the editor expresses concerns with the mismatch repair (MMR) immunohistochemistry (IHC) methods utilized in the study [2].

We believe that MMR IHC is indeed a valuable clinical assay, yet like all clinical assays, has limitations. While the letter asserts that the original manuscript misses the limitations of MSI testing, we clarify that the purpose of the manuscript was to assess the sensitivity limitations of MMR IHC, not those of MSI testing by next generation sequencing (NGS). The latter has been documented in separate studies [3], and further studies continue to investigate those limitations as datapoints increase in number. While MMR IHC is indeed currently cheaper and more accessible than comprehensive NGS testing, the use of NGS testing will only increase as technology progresses. Thus, it is important for pathologists to see examples of discrepancies between the two assays. The letter states that the results of our study are well documented in the literature, yet this is the first systematic study of its kind. Documentation of retained MMR IHC in the setting of microsatellite instability-high tumors had been reported as either case reports or case series until the current study, which are generally considered lower quality evidence than larger studies.

The MMR IHC from the cases in the study was performed in a clinical lab with valid internal controls present, meaning each case had lymphocytes and stroma expressing each MMR IHC protein; and the slides were interpreted by trained pathologists. Any retained nuclear MMR IHC was theoretically considered "retained staining," but our study did not include cases with <5% of tumor cells expressing MMR IHC proteins. Several of these discrepant cases had been scored by the original pathologist as 'equivocal' or 'abnormal,' usually in the presence of weak nuclear labeling in 5–10% of cells. In Figure 2 of the study, for example, most cases did not have patchy or focal expression. Focal or weak staining is not an uncommon issue and can be either technical or biological in etiology. Recently, a more specific attempt to define what constitutes 'focal' MMR IHC expression in endometrioid cancer was published in the *American Journal of Surgical Pathology*, and there, the definition used was <5% of tumor cells [4].

The findings in the study have since been verified by another group, which included focal and weak MMR IHC expression in colorectal carcinoma cases from patients with MMR gene missense mutations [5]. This paper indeed asks the question: what is the appropriate cut-off for calling MMR IHC 'focal'? It cites literature calling 'focal' anywhere from 1 up to 10% of tumor cells demonstrating expression [6, 7].

The referenced letter suggests that the study included 'heterogenous/subclonal' staining as retained, but this was not the case. We did include rare cases with 'dot-like' or 'nucleolar' MLH1 staining. The authors of the letter state that this should be interpreted as 'abnormal', and it is acknowledged that this pattern has been previously described as abnormal, although not systematically. Our results add to that literature and they generally confirm that this is an abnormal staining pattern. Interestingly, we found that dot-like MLH1 staining can occur in both MSI-H and MSS cancers, and thus, further study of this uncommon finding is indicated. Loss of PMS2 expression is usually found with nucleolar MLH1 staining, indicating that such questionable staining patterns can usually be adjudicated by the PMS2 results.

The British Association of Gynaecologic Pathologists (BAGP) states that normal MMR IHC staining should be clearly stronger in intensity than that of the internal control [8]. It would be prudent to bear in mind that the internal

✉ Jaclyn F. Hechtman
hechtmaj@mskcc.org

[1] Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

control may vary depending on the presence or absence of more proliferative cellular elements (such as proliferative endometrial glands or activated lymphoid cells). As noted, the BAGP suggests further testing in scenarios with focal or weak nuclear expression, an "abnormal" result according to their criteria. However, United States template guidelines issued by the College of American Pathologists (CAP) recommend reporting MMR IHC as either (1) intact, (2) lost, or (3) cannot be determined [9]. Further, current guidelines from the CAP for the interpretation of MMR IHC in endometrial carcinoma specifically state "Any positive reaction in the nuclei of tumor cells is considered as intact expression (normal), and it is common for intact staining to be somewhat patchy." [10] This statement is supported in the study: there is a wide range of percentage of tumor cells expressing MMR IHC in both MSS and MSI-H tumors as seen in Figure 2 of Hechtman et al [2]. The only discrepancy between the CAP recommendations and our institutional practice occurs in the setting of 1–5% nuclear weak labeling for MSH6 in endometrial carcinoma, which tends to be reported as "equivocal," essentially synonymous with "cannot be determined."

In our opinion, diagnosing focal or weak or atypical MMR IHC expression as 'abnormal' or 'defective' could be detrimental to patient management because it implies MMR-D status when there is a chance that the results could be due to technical issues rather than true MMR-D status. MMR-D status often qualifies a patient for immune checkpoint inhibitor therapy, which can have serious adverse effects, or encourages an oncologist to select against 5-fluoruracil-based therapy in colorectal carcinoma, which may benefit patients who do not truly have MMR-D status. These cases should instead have MMR IHC results reported as 'equivocal' or 'cannot be determined,' which encourages selection of a different assay including DNA-based MSI testing (either stand-alone or through NGS data) and/or specimen. Many centers now use MMR IHC and DNA-based MSI analysis in tandem as MMR IHC alone has been reported to fail to detect up to 11.8% of MSI-H cancers in one study [11].

In summary, we agree with nearly everything in the letter to the editor. In particular, we couldn't agree more with the statement that the interpretation of MMR IHC is more subtle and complex than a simplistic "all gone = loss", and "*any* expression = retained" approach. We acknowledge that in this study, we have considered nucleolar staining as "retained", but we also report that this staining pattern is not restricted to MSI-H carcinomas. Furthermore, this staining pattern should be interpreted alongside that of the partner protein. The differences in the guidelines between the BAGP and the CAP for MMR IHC interpretation in endometrial cancer highlight the fact that more research and literature is needed, followed by more explicit guidelines and suggested testing algorithms on the subject.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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