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CORRESPONDENCE

Interpretation of mismatch repair protein expression using obsolete criteria results in discrepancies with microsatellite instability and mutational testing results. Comment on Hechtman et al. Mod Pathol 2020; 33:871–879

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To the Editor:

Re: Hechtman JF, Rana S, Middha S et al. Retained mismatch repair (MMR) protein expression occurs in ~6% of microsatellite instability (MSI)-high cancers and is associated with missense mutations in MMR genes. Mod Pathol (2020) 33:871–879.

We write to express our concerns about the recent paper, noted above, in which MSI status, as determined by NGS, was compared to MMR immunostaining.

Accurate interpretation of the expression of MMR proteins is of vital importance in current clinical practice, as a screening test for Lynch Syndrome [1, 2], to identify cases that may benefit from immunotherapy [3] and also for diagnosis of the molecular class of endometrial carcinoma [4, 5], with implications for surgical and nonsurgical treatment [6]. In comparison to MSI, MMR IHC is the more accessible test for pathologists worldwide [7]. This study elucidates the mechanisms of demonstrable MMR protein expression in cases with (largely missense) gene mutations and MSI-H. While these are excellent data, the authors miss some important points and the vital opportunity to emphasize increasingly recognized potential pitfalls in interpretation of MMR IHC.

While the methods for determination of MSI are well described, the MMR immunostaining protocols are not provided.

With regards to interpretation of MMR IHC, the authors define MMR deficient status as "complete loss of nuclear expression of MMR protein(s) within the tumor as per prior studies". The interpretation of MMR IHC is more subtle and complex than a simplistic "all gone = loss", and "any expression = retained" approach [8–11]. In the past there has been a lack of correct and clear guidance for interpretation of MMR IHC and it is important to exploit every possible opportunity to promote the correct approach. Normal MMR IHC staining consists of staining throughout the tumor that is clearly stronger in intensity than that of the internal control, ideally carried out on well-fixed biopsy tissue; any deviation from this *potentially* constitutes an abnormal pattern. This progress in the interpretation of MMR IHC is well documented in the literature, but not taken into account by the authors. They are, therefore, comparing MSI status to MMR immunostaining interpreted according to criteria that are now obsolete.

For the 6% cases stated in the title as showing "retained MMR expression", a variety of examples are illustrated which highlight the inadequacy of the commonly used "retained expression" threshold for MMR IHC interpretation. Dot-like nuclear staining, heterogeneous/subclonal staining, and weak or focal staining (manuscript figure 1) are all examples of "abnormal" expression that should warrant additional workup when encountered in the evaluation of MMR IHC, which is reinforced by the abnormal genetic findings in this study.

This study supports what is well documented in the literature regarding the discordance between MMR IHC and MSI, but misses one side of the message: the absolute concordance of MMR IHC and MSI is stated to be 96% but the lack of concordance is bidirectional [2, 9]. Just as IHC would miss a certain percentage of MSI-H, especially if incorrectly interpreted, MSI using conventional tests would also miss a similar proportion of MMRd cases that are picked up by IHC; this is especially the case for MSH6 defects, which often

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show MSS or MSI-L status on conventional MSI testing. While this may not be the case if MSI is tested on an NGS platform where MMR genes are sequenced, a test such as MSK-IMPACT is totally impracticable in most healthcare systems at this time.

The use of any adjunct technique must be in an expert fashion for it to be meaningful. MMR IHC is no exception and should be reported with due regard to pitfalls and appropriate quality assurance. A minor point is that two of the eight MSI-H endometrial carcinomas included in the study had pathogenic mutations in *POLE*; the multiple mutations observed in the MMR genes and the MSI status are attributable to the underlying *POLE* exonuclease domain mutation, and normal/subclonal loss of protein expression is not surprising in these cases.

We refer readers to guidelines on interpretation of MMR immunostains (Interpretation and Reporting Terminology for Mismatch Repair Protein Immunohistochemistry in Endometrial Cancer) with numerous photomicrographs illustrating the situations described above, produced by the British Association of Gynecological Pathologists and posted on their website [12].

Sincerely

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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