Title: Explaining an Atypical Immunohistochemical Pattern Using Paired Tumor/Germline Testing for Lynch syndrome

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Background
An atypical but not uncommon immunohistochemistry (IHC) result, abnormal (absent/equivocal/weak) staining of MLH1 and/or PMS2 in addition to abnormal MSH6 staining, has traditionally been difficult to fully explain with follow-up germline genetic testing. It has been demonstrated that abnormal MSH6 staining can be associated with somatic *MSH6* coding microsatellite (CM) mutations[1]. This study examined the potential of paired tumor/germline testing to explain this atypical IHC pattern.

Methods
Data was analyzed from all paired tumor/germline tests (n=30) ordered from a single commercial laboratory between 2/2017-6/2018 where results from previous IHC testing on colorectal and endometrial tumors showed abnormal staining of MLH1 and/or PMS2 in addition to abnormal MSH6 staining. Germline and somatic findings were assessed to determine whether the results of paired tumor/germline testing explained the atypical IHC staining pattern.

Results
Abnormal staining of MLH1 and/or PMS2 in addition to MSH6 on IHC was seen in 30/1190 (2.5%) of all cases. As shown in Table 1, paired tumor/germline testing fully explained the atypical IHC in 3/30 cases (10%) and explained part but not the entire IHC pattern in 10/30 cases (33%). Paired testing results mirrored previously published data[1] and were concordant with the atypical IHC pattern in 11/30 cases (37%), but did not fully explain abnormal MSH6 staining due to the presence of only a single CM mutation.

Conclusions
Paired tumor/germline testing fully explained IHC results in 10% of cases, while germline testing alone would not have fully explained any cases. Results also support previous research showing that somatic *MSH6* CM mutations can contribute to abnormal MSH6 IHC staining in cases that also demonstrate abnormal MLH1 and/or PMS2 staining. Our data confirms that this atypical IHC pattern can be fully explained by paired tumor/germline testing in some cases, while other cases may be partially or completely unexplained due to possibly erroneous IHC results, potentially undetected germline or somatic alterations, or other causes. Paired tumor/germline testing offers improved clinical utility over germline testing alone in cases with atypical IHC results by increasing the likelihood of providing diagnostic resolution with a complete explanation of the IHC pattern.

Reference(s)
1) Shia J, et al. Secondary mutation in a coding mononucleotide tract in MSH6 causes loss of immunoexpression of MSH6 in colorectal carcinomas with MLH1/PMS2 deficiency. *Mod Pathol.* 2013; 26(1): 131-8.