



Biomarkers in Colorectal Cancer: BRAF Testing as a Follow-up to Microsatellite Instability in the Exclusion of Lynch Syndrome for Clinicians

SYNOPSIS AND RELEVANCE

This module promotes using an algorithmic approach for evaluating colorectal cancers (CRC) that lack MLH1 and PMS2 protein expression by immunohistochemistry (IHC) or demonstrate microsatellite instability (MSI) by polymerase chain reaction (PCR) testing. A subset of these tumors will test positive for the *BRAF* V600E mutation. This finding is associated with sporadic methylation of *MLH1*, which correlates with sporadic microsatellite instability (MSI). In this subset of patients, it is not recommended to perform germline mutation analysis to exclude Lynch syndrome. Furthermore, it should be noted that *BRAF* V600E mutation analysis may be performed in other contexts and alone is not useful for excluding Lynch syndrome.

Establishing and adhering to algorithms for evaluating CRC can:

1. Ensure that the proper tests and methodologies are used to evaluate patients with CRC.
2. Optimize the utilization of IHC markers and molecular diagnostic tests in CRC.
3. Impact patient care by ensuring that the most clinically useful tests are used to evaluate and guide therapy in patients with CRC.

INSIGHTS

1. While absent MSH6 protein expression by IHC is characteristic of Lynch syndrome, only a small subset of CRC tumors with absent MLH1 protein by IHC will harbor a germline mutation in a mismatch repair protein (ie., germline sequencing for Lynch syndrome is not usually indicated).
2. CRC tumors with absent MLH1 protein by IHC are usually associated with sporadic *MLH1* hypermethylation and the *BRAF* V600E mutation has been frequently detected in these sporadic MSI cases. The *BRAF* V600E mutation is not associated with Lynch syndrome.
3. *MLH1* hypermethylation assays are not recommended as an initial strategy to distinguish between sporadic MSI CRC and Lynch syndrome because *MLH1* hypermethylation can be seen in up to 15% of Lynch syndrome patients as a secondary finding, however many laboratories find value in combining *MLH1* hypermethylation assays with *BRAF* V600E mutation analysis.
4. *BRAF* V600E mutation analysis either alone or in combination with *MLH1* hypermethylation analysis may be more cost-effective than germline sequencing tests for detecting Lynch Syndrome-associated mutations. Laboratories should consider using these tests prior to germline analysis of MMR system genes when a CRC has abnormal *MLH1* IHC.

BACKGROUND

Lynch syndrome (hereditary nonpolyposis colorectal cancer or HNPCC) is a form of hereditary cancer syndrome based on inherited defects in mismatch repair genes causing a phenotype called *microsatellite instability*. Importantly, 15% of colorectal cancers will have microsatellite instability but only a minority of these will be due to Lynch syndrome; others are caused by “sporadic” events. Testing for microsatellite instability has become routine for many institutions upon colon cancer tissue diagnosis for a variety of reasons; for example: prognosis, prediction of chemotherapeutic and immunotherapeutic response and screening for Lynch syndrome. There is sometimes confusion around the ‘next steps’ to take if testing demonstrates microsatellite instability and the treating or ordering physician wants to specifically exclude Lynch syndrome.

REFERENCE

Sepulveda AR, Hamilton SR, Allegra CJ, et al. Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. *Arch Pathol Lab Med*. 2017;141(5):625-657. <https://doi.org/10.5858/arpa.2016-0554-CP>