MLH1 Promoter Hypermethylation Test to Refine Likelihood of Lynch Syndrome and to Classify Gastric Cancer

DNA pyrosequencing is used to determine promoter methylation status of the *MLH1* gene. MLH1 promoter methylation is a sign of sporadic cancer rather than Lynch syndrome-related cancer. MLH1 status also helps sub classify advanced gastric cancer which impacts clinical trial options.

Biology of the process: Most forms of colorectal adenocarcinoma are sporadic and not predisposed by hereditable gene variants. Approximately 15% of colorectal cancers display microsatellite instability, however only about 10% of those are due to heritable Lynch syndrome (previously called hereditary non-polyposis colorectal cancer (HNPCC)). Lynch syndrome also predisposes to endometrial cancer. Hypermethylation of the promoter region of the *mutL homolog 1 (MLH1)* gene in tumor tissue is a strong indicator that the gene is silenced through epigenetic modifications rather than heritable mutation, thus markedly reducing the likelihood of Lynch syndrome.[1]

The *MLH1* gene, located at 3p21.3, encodes a protein that plays an essential role in DNA mismatch repair[2]. The encoded MLH1 protein combines with PMS2 protein to form a complex that coordinates the activities of other proteins functioning in mismatch repair during DNA replication[3]. The *MLH1* gene is frequently mutated and thus inactive in Lynch syndrome.[4] This gene can be inactivated by a different mechanism in sporadic colorectal cancers, namely via hypermethylation of CpG islands of the *MLH1* promoter. Whether mutated in heritable cancer, or methylated as an acquired defect, MLH1 inactivation causes microsatellite instability and often, but not invariably, loss of *MLH1* protein expression as visualized by pathologist interpretation of immunohistochemical stain for MLH1 protein.[5]

Gastric adenocarcinoma has 4 major molecular subclasses, one of which is characterized by methylation-related MLH1 silencing. This "microsatellite instability" subclass has extensive hypermethylation of many gene promoters and mutation of many genes. In the GastroGenus Gastric Cancer Classifier assay (see separate test information), data on MLH1 silencing as well as EBV status and results of the Solid Tumor Mutation Panel are used to help identify options for clinical trials. MLH1 methylation may qualify patients for experimental therapy with the PD-1 antibody pembrolizumab (in NCT01876511) or PARP inhibitor veliparib (in NCT01264432, clinicaltrials.gov).

Clinical Indications for *MLH1* promoter hypermethylation testing: 1. Patients with colorectal or endometrial carcinoma whose tumor has been confirmed as either MSI-high by microsatellite instability testing or has loss of MLH1 protein in malignant cells by immunohistochemistry. 2. Patients with advanced gastric adenocarcinoma for whom clinical trial options are being explored.

Laboratory testing for *MLH1* promoter hypermethylation: The preferred sample is a paraffin block containing at least 50% malignant cells representing either primary or metastatic colorectal or endometrial adenocarcinoma, or five 10um unstained paraffin sections on plain glass slides plus an H&E stained slide. A copy of the surgical pathology report is requested. This test is ordered reflexively when a colon or endometrial carcinoma is found to be MSI-high or when MLH1 protein is lost or expression status is uncertain in malignant cells. Tumor cells are enriched by macrodissection, and extracted DNA is bisulfite-treated, then PCR-amplified followed by DNA pyrosequencing to identify the extent of promoter methylation of the *MLH1* gene. Results are interpreted by a pathologist in concert with information provided on the surgical pathology report.

References:

1. Funkhouser WK Jr, Lubin IM, Monzon FA, Zehnbauer BA, Evans JP, Ogino S, Nowak JA. Relevance, pathogenesis, and testing algorithm for mismatch repair-defective colorectal carcinomas: a report of the association for molecular pathology. J Mol Diagn 14:91-103, 2012. PMID: 22260991
2. Herman J G, Umar A, Polyak K, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Nat Acad Sci 95: 6870-6875, 1998.
3. Gulley ML: Genomic Assays for Epstein-Barr Virus-Related Gastric Adenocarcinoma. Experimental & Molecular Medicine, 47:e134, 2015. PMID: 25613731

To consult a pathologist about indications for testing or the significance of a result, call the Molecular Genetics Lab at (984) 974-1825, Dr. Weck at (984) 974-1825 or Dr. Gulley at (919) 843-4595. Email: Karen.Weck@unchealth.unc.edu or margaret_gulley@med.unc.edu

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