

## ***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 hereditary 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.<sup>1</sup>

The *MLH1* gene, located at 3p21.3, encodes a protein that plays an essential role in DNA mismatch repair<sup>2</sup>. 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<sup>3</sup>. The *MLH1* gene is frequently mutated and thus inactive in Lynch syndrome.<sup>4</sup> 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.<sup>5</sup>

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**, or Email: [karen.weck@unchealth.unc.edu](mailto:karen.weck@unchealth.unc.edu)

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