Lynch Syndrome/Hereditary Nonpolyposis Colorectal Cancer

Indications for Ordering

Microsatellite instability (MSI) and/or immunohistochemistry (IHC) tumor analyses
- Universal screening for Lynch syndrome (LS) in all individuals with newly diagnosed colorectal cancer (CRC) (NCCN, 2016)
- Screening for LS in individuals with endometrial cancer (ASCO, 2015)

Germline mismatch repair (MMR) gene testing
- Order following abnormal screening test result
- Confirms diagnosis of LS

Test Description

Mismatch repair by IHC
- Determines MMR protein expression (MLH1, MSH2, MSH6, and PMS2) in tumor tissue

MSI testing
- Genotyping of 5 mononucleotide repeats (BAT-25, BAT-26, MONO-27, NR-21, and NR-24)
- Allele size within tumor sample is compared to allele size within normal tissue from the same individual

BRAF codon 600 mutation
- Pyrosequencing at codon 600

MLH1 promoter methylation
- Uses bisulfite conversion followed by real-time polymerase chain reaction (PCR)

MMR gene pathogenic germline variants
- PCR amplification followed by bidirectional sequencing of coding regions and intron/exon boundaries of MLH1, MSH2, MSH6, and PMS2
- Multiplex ligation-dependent probe amplification (MLPA) for large MLH1, MSH2, MSH6, and PMS2 exonic deletions/duplications, 10 Mb inversion of MSH2 exons 1-7, and exon 9 deletions in EPCAM

Tests to Consider

Screening studies (requires pathological tissue)

Mismatch Repair by Immunohistochemistry 0049302
- First-line screening test for LS
- Highly recommended prior to ordering germline MMR gene testing
  - Directs subsequent genetic diagnostic testing

Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR 0051740
- First-line screening test for LS
- Directs subsequent genetic diagnostic testing for LS

Mismatch Repair by Immunohistochemistry with Reflex to BRAF Codon 600 Mutation and MLH1 Promoter Methylation 2002327
- Preferred screening test for LS in individuals with CRC
- If MLH1 IHC is abnormal, evaluations of BRAF codon 600 and, possibly, MLH1 methylation are performed
- Definitive diagnosis of LS requires additional targeted MMR germline molecular studies
- Do not use in endometrial cancer

BRAF Codon 600 Mutation Detection with Reflex to MLH1 Promoter Methylation 0051750
- Recommended reflex test to differentiate between LS and sporadic CRC in tumors showing loss of MLH1
- If no BRAF variant is detected, MLH1 promoter methylation is evaluated

Mismatch Repair by Immunohistochemistry with Reflex to MLH1 Promoter Methylation 2005270
- Preferred reflex screening test for LS in non-CRC tumors (eg, endometrial carcinoma)
- If MLH1 expression is lost, MLH1 methylation is performed

MLH1 Promoter Methylation, Paraffin 2002499
- Recommended test to distinguish between LS and sporadic non-CRC tumors with loss of MLH1

Diagnostic germline genetic studies (peripheral blood)
- Germline genetic testing is available for all 4 MMR genes known to cause LS

HNPCC/Lynch Syndrome (MLH1) Sequencing and Deletion/Duplication 0051650
- Detect germline MLH1 variants
- Use in MMR-deficient carcinoma with suggestive IHC results (loss of MLH1 and PMS2 proteins), negative for the BRAF codon 600 pathogenic variant, and with normal MLH1 promoter methylation studies
**HNPCC/Lynch Syndrome (MSH2) Sequencing and Deletion/Duplication 0051654**
- Detect germline MSH2 variants
- Use in MMR-deficient carcinoma with suggestive IHC results (loss of MSH2 and MSH6 proteins)
- Includes evaluation of EPCAM exon 9 deletions and 10 Mb inversion of MSH2 exons 1-7

**HNPCC/Lynch Syndrome (MSH6) Sequencing and Deletion/Duplication 0051656**
- Detect germline MSH6 variants
- Use in MMR-deficient carcinoma with suggestive IHC results (isolated loss of MSH6 protein)

**HNPCC/Lynch Syndrome (PMS2) Sequencing and Deletion/Duplication 0051737**
- Detect germline PMS2 variants
- Use in MMR-deficient carcinoma with suggestive IHC results (isolated loss of PMS2 protein)

**Gastrointestinal Hereditary Cancer Panel, Sequencing and Deletion/Duplication, 16 Genes 2013449**
- Confirm a diagnosis of hereditary gastrointestinal (GI) cancer in individuals with a personal or family history of GI cancer and/or polyposis
- Includes all 4 MMR genes known to cause LS

**HNPCC/Lynch Syndrome Deletion/Duplication 2001728**
- Order if sequencing studies have been performed previously at another laboratory
- Order if there is a known familial deletion or duplication
- Both sequencing and deletion/duplication testing are necessary to detect all pathogenic variants in MMR genes

**Familial Mutation, Targeted Sequencing 2001961**
- Useful when a pathogenic familial variant identifiable by sequencing is known

**Disease Overview**

**Incidence**
- Accounts for 2-4% of CRC and ~1-2% of endometrial cancers (NCCN, 2016; Chadwick, 2001)
- Most common hereditary CRC syndrome
- Most common cause of hereditary endometrial cancer

**Risk estimates**
- The following lifetime risk estimates apply to individuals with MLH1 and MSH2 pathogenic variants; risks for variants in MSH6 or PMS2 may be lower (NCCN, 2016)
  - CRC – 52-82%
  - Endometrial – 25-60%
  - Ovarian – 4-24%
  - Gastric – 6-13%
  - Urinary tract – 1-7%
  - Brain/central nervous system – 1-3%
  - Hepatobiliary tract – 1-4%
  - Small bowel – 3-6%
  - Pancreatic – 1-6% (MLH1 and MSH2 only)

**Genetics**

**Genes** – MLH1, MSH2, MSH6, and PMS2

**Inheritance** – autosomal dominant

**Test Interpretation**

**Microsatellite Instability (MSI), HNPCC/Lynch Syndrome, by PCR**

**Sensitivity/specificity**
- Clinical sensitivity – 90% (NCCN, 2016)
- Analytical sensitivity/specificity – >99%

**Results**
- High MSI (MSI-H)
  - MMR deficiency, either sporadic or LS
- Indeterminate MSI (MSI-I)
  - Instability in even 1 mononucleotide repeat can be associated with LS
  - Follow-up IHC studies are recommended
- Microsatellite stable (MSS)
  - LS unlikely

**Limitations**
- 15% of sporadic CRCs are also MSI-H (NCCN, 2016)
- Preoperative chemoradiation of rectal cancer
  - May complicate IHC interpretation and/or decrease tumor mass
  - May make MSI testing difficult
  - Evaluation of pretreatment biopsies will avoid this limitation
- Screens for LS only and does not evaluate other hereditary causes of CRC or endometrial cancer

**Mismatch Repair by IHC**

**Clinical sensitivity** – 90% (NCCN, 2016)

**Results**
- Normal – MMR proteins are normally expressed
  - MMR deficiency is unlikely
  - LS unlikely
Sensitivity/specificity

Germline genetic studies (MLH1, MSH2, MSH6, or PMS2)

Sensitivity/specifcity

Results

Limitations

References