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, PMS2, and MSH6) in tumor tissue

Microsatellite instability (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 PCR

MMR germline mutation
- MLH1, MSH2, PMS2, and MSH6 genes
- PCR amplification followed by sequencing for point mutations and small insertions/deletions in the germline
- Multiplex ligation-dependent probe amplification (MLPA) for large deletions

Tests to Consider

Screening studies (requires pathological tissue)

Mismatch Repair by Immunohistochemistry 0049302
- First-line screening test for LS
- Highly recommended prior to ordering MMR germline mutation analysis gene testing
  - Results direct 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 mutation 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 mutation 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)

HNPP/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 methylation studies
HNPCC/Lynch Syndrome (MSH2) Sequencing and Deletion/Duplication 2001654
- 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

HNPCC/Lynch Syndrome (MSH6) Sequencing and Deletion/Duplication 2001656
- Detect germline MSH6 variants
- Use in MMR-deficient carcinoma with suggestive IHC results (isolated loss of MSH6 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 colorectal cancer syndrome
- Most common 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/CNS - 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 microsatellite instability (MSI-H)
  - MMR deficiency, either sporadic or LS
- Indeterminate microsatellite instability (MSI-I)
  - Instability in even one 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
- Abnormal – MMR protein expression is abnormal
  - Loss of expression of 1 or more proteins is highly predictive of MMR deficiency
- Absence of both MLH1 and PMS2
  - MLH1 germline pathogenic variant is possible
  - Consider MLH1 methylation +/- BRAF V600E studies
    - If methylation and BRAF studies are negative, follow with MLH1 germline genetic testing
- Absence of PMS2 only
  - PMS2 germline pathogenic variant likely
  - Consider PMS2 germline testing
    - If PMS2 testing does not identify a germline pathogenic variant, consider MLH1 germline testing
- Absence of both MSH2 and MSH6
  - MSH2 germline pathogenic variant likely
  - Consider MSH2 germline testing
- Absence of MSH6 only
  - MSH6 germline pathogenic variant likely
  - Consider MSH6 germline testing
    - If MSH6 testing does not identify a germline pathogenic variant, consider MSH2 germline testing

Pancreatic - 1-6%
Small bowel - 3-6%
Hepatobiliary tract - 1-4%
Brain/CNS - 1-3%
Urinary tract - 1-7%
Gastric - 6-13%
Ovarian - 4-24%
Endometrial – 25-60%
CRC - 52-82%
MSH2
MLH1
MSH6
Limitations
• ~10% of individuals with LS will have IHC tests that show normal staining of the MMR proteins
• Because the correlation of MSI with IHC is not 100%, direct testing of MSI by PCR may be helpful
• Screens for LS only and does not evaluate other hereditary causes of CRC or endometrial cancer

BRAF Codon 600 Mutation Detection with Reflex to MLH1 Promoter Methylation

Analytical sensitivity – methylation levels >10% are reported as positive

Results
• BRAF V600E detected
  • Presence in MMR-deficient CRC indicates the tumor is probably sporadic and not associated with LS
  • Further germline testing not typically indicated
• MLH1 promoter methylation detected
  • Presence in an MSI CRC indicates the tumor is probably sporadic and not associated with LS
  • Further germline testing not typically indicated
• No mutations detected
  • In MSI-H tumors with loss of MLH1 protein by IHC, MLH1 germline testing indicated

Germline genetic studies (MLH1, MSH2, MLH6, or PMS2)

Sensitivity/specificity
• Proportion of LS attributed to pathogenic variants in specific MMR gene (Peltomaki, 2003; Berends, 2002; Senter, 2008)
  • MLH1 – 45-50%
  • MSH2 – 40%
  • MSH6 – 5-10%
  • PMS2 – <5%
• Analytical sensitivity/specificity – 99%

Results
• Positive – one pathogenic variant detected
  • Predicted to be causative for LS
• Negative – no pathogenic variants detected
  • Diagnosis of LS unlikely, but not excluded
• Inconclusive – variant detected, but whether it is benign or pathogenic is unknown

Limitations
• Not evaluated
  • Regulatory region pathogenic variants
  • Deep intronic pathogenic variants
  • Causes of hereditary CRC or endometrial cancer other than LS
• Diagnostic errors can occur due to rare sequence variations

References