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 four 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)

Hereditary Gastrointestinal Cancer Panel, Sequencing and Deletion/Duplication 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 four 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 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
Sensitivity/specif.

• Abnormal – MMR protein expression is abnormal
  o Loss of expression of one or more proteins is highly predictive of MMR deficiency
  o 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
  o 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
  o Absence of both MSH2 and MSH6
    ▪ MSH2 germline pathogenic variant likely
    ▪ Consider MSH2 germline testing
  o 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

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

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

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

References
• Chen JM. The 10-Mb paracentric inversion of chromosome arm 2p in activating MSH2 and causing hereditary nonpolyposis colorectal cancer: re-annotation and mutational mechanisms. Genes Chromosomes Cancer. 2008;47(6):543-545

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