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

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

- 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

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 variants detected
  - 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 (Peltomaki, 2003; Berends, 2002; Senter, 2008)
  - *MLH1* – 45-50%
  - *MSH2* – 40%
  - *MSH6* – 5-10%
  - *PMS2* – <5%
- Analytical sensitivity/specificity – 99%

Results

- Positive – 1 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 and deep intronic variants
  - Sequence variants and large deletion/duplications in genes other than *MLH1, MSH2, MSH6, PMS2*, and large deletions in *EPCAM* exon 9
  - Large gene inversions, other than the *MSH2* 10 Mb exons 1-7 inversion
  - Causes of hereditary CRC or endometrial cancer other than LS
- Diagnostic errors can occur due to rare sequence variations

References

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