impact genetics

MLH1/MSH2/MSH6/PMS2/EPCAM Somatic Tumor MMR

Sequencing and Deletion/Duplication Test

Transparent | Precise testing | Exceptional service

Test Description

Patients with loss or dysfunction of MMR protein(s) detected by immunohistochemistry (IHC) or microsatellite instability (MSI) may harbour somatic (tumor) mismatch repair (MMR) mutations but have a normal germline Lynch MMR result.

For these patients, identification of somatic pathogenic mutations in tumor that are not present in their germline increases confidence that the patient does not have Lynch syndrome.

Genes tested

MLH1, MSH2, MSH6, PMS2, EPCAM.

Sequence analysis

Our lab performs sequence analysis of all coding exons and flanking intronic regions of all 5 genes using Next Generation Sequencing (NGS).

Copy number changes

Multiplex Ligation-dependent Probe Amplification (MLPA) is used to look for whole-exon and multi-exon deletions and duplications in *MLH1*, *MSH2*, *MSH6* and *PMS2* (for *EPCAM*, only a subset of exons are interrogated by MLPA).

Confirmation

All suspicious variants will be confirmed by an alternative sequencing method.

Benefits of Genetic Testing

Reduction of Lynch syndrome risk: Detection of biallelic mutations in MMR gene(s) or *EPCAM* in tumor for patients with abnormal IHC and/or MSI and normal germline testing reduces the likelihood of Lynch Syndrome.

NCCN Guidelines: MMR tumor testing is recommended for patients who have had abnormal MSI and/or IHC and normal germline testing. [*Genetic/Familial High-Risk Assessment: Colorectal. Version 2. 2016.*]

Develop Tailored Surveillance: Patients with no underlying Lynch germline mutation and double MMR somatic hits may require alternative or additional management and surveillance. Testing provides clinicians with additional information to better manage their patients.

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NCCN Guidelines | *Version 2.2016 Lynch Syndrome* (LS-A2 of 3)

Tumor testing results and additional testing strategies

Tumor testing [a]								
IHC				MSI	*BRAF* [b] v600E	*MLH1* Promoter Methylation	Plausible etiologies	Additional testing [d, e]
MLH1	*MSH2*	*MSH6*	*PMS2*					
+	+	+	+	MSS/MSI-Low	N/A	N/A	1) Sporadic cancer 2) Other (not Lynch syndrome) hereditary CRC syndrome	1) None [c]
+	+	+	+	MSI-High	N/A	N/A	1) Germline mutation in any LS gene 2) Sporadic cancer	1) Germline LS genetic testing [f] 2) If germline testing negative, consider somatic MMR genetic testing [h]
N/A	N/A	N/A	N/A	MSI-High	N/A	N/A	1) Sporadic cancer 2) Germline mutation in any LS genes	1) Consider IHC analysis and additional testing depending on IHC results 2) If IHC not performed, consider germline LS genetic testing [f]
--	+	+	--	N/A	N/A	N/A	1) Sporadic cancer 2) Germline mutation *MLH1* or rarely *PMS2*	1) Consider BRAF [b]/methylation studies 2) Germline LS genetic testing [f]
--	+	+	--	N/A	Positive	N/A	1) Sporadic cancer 2) Rarely germline *MLH1* mutation or consititutional *MLH1* epimutation	1) None, unless young age of onset or significant family history; then consider constitutional *MLH1* [g] epimutation testing and/or germline LS genetic testing [f]
--	+	+	--	N/A	Negative	Positive	1) Sporadic cancer 2) Rarely germline *MLH1* mutation or consititutional *MLH1* epimutation	
--	+	+	--	N/A	Negative	Negative	1) Germline mutation *MLH1* or rarely *PMS2* 2) Sporadic cancer	
+	--	--	+	N/A	N/A	N/A	1) Germline mutation *MSH2/EPCAM*; rarely germline mutation in *MSH6* 2) Sporadic cancer	1) Germline LS genetic testing [f] 2) If germline testing negative, consider somatic MMR genetic testing [h]
+	+	+	--	N/A	N/A	N/A	1) Germline mutation *PMS2* 2) Germline mutation *MLH1*	
+	--	+	+	N/A	N/A	N/A	1) Germline mutation *MSH2/EPCAM* 2) Sporadic cancer	
+	+	--	+	N/A	N/A	N/A	1) Germline mutation *MSH6* 2) Germline mutation *MSH2* 3) Sporadic cancer/Treatment effect [i]	1) Germline LS genetic testing [f] 2) If applicable, consider MSI analysis or repeat IHC testing on nontreated tumor [i]
--	+	+	+	N/A	N/A	N/A	1) Germline mutation *MLH1*; possibly sporadic cancer or *PMS2* mutation	3) If germline testing negative, consider somatic MMR genetic testing [h]
--	--	--	--	N/A	N/A	N/A	1) Germline mutation in any LS gene 2) Sporadic cancer	1) Germline LS genetic testing [f] 2) If germline testing of *MLH1* negative, consider BRAF [b]/methylation studies 3) If germline testing negative, consider somatic MMR genetic testing [h]

N/A = Either testing was not done or results may not influence testing strategy, **+** = Normal staining of protein, **--** = Absent staining of protein

a Tumor testing strategies apply to colorectal and endometrial cancers. Limited data exists regarding the efficacy of tumor testing in other LS tumors.

b Testing is not appropriate for tumors other than colorectal cancer.

c If strong family history (i.e. Amsterdam criteria) or additional features of hereditary cancer syndromes (multiple colon polyps) are present, additional testing may be warranted in the probing, or consider tumor testing in another affected family member due to the possibility of a phenocopy.

d Individuals with abnormal MSI and/or IH tutor results and no germline mutation detected in the corresponding genes(s) may still have undetected Lynch Syndrome. At this time, no consensus has been reached as to whether these patients should be managed as LS (LS-4 and LS-5) or managed based on personal/family history (see NCCN Guidelines for Colorectal Cancer Screening – for average risk and for increased risk). Growing evidence suggests that the majority of these individuals with abnormal tumor results and no germline mutation found have double somatic mutations/changes in the MMR genes. Although the efficacy has not yet been proven, genetic testing of the corresponding genes(s) could be performed on tumor DNA to assess for somatic mutations. Individuals found to have double somatic mutations/changes in the MMR genes likely do not have LS and management should be based on personal/family history.

e Prior germline genetic testing, proper pre-test counseling should be done by an individual with expertise in genetics.

f Germline LS genetic testing may include testing of the gene/s that are indicated (see "Plausible Etiologies" for possibilities on LS-A2 of 3) by the abnormal test results, or instead, multi-gene testing that includes *MLH1, MSH2, MSH6, PMS2,* and *EPCAM* concurrently may be performed.

g Evaluation for constitutional *MLH1* epimutation involves *MLH1* promoter hypermethylation studies on blood or other sources of normal tissue.

h Somatic MMR genetic testing of the corresponding genes(s) (see "Plausible Etiologies" for possibilities on LS-A2 of 3) could be performed on tumor DNA to assess for somatic mutations that might explain the abnormal IHC and/or MSI results.

i Absent *MSH6* in rectal tumor tissue may be due to treatment effect (neoadjuvant chemoradiotherapy).

Note: All recommendations are category 2A unless otherwise indicated. **Clinical trials**: NCCN believes that the best management of any cancer patient is in a cinical trial. Participation in clinical trials is especially encouraged.

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