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SAMPLE REPORT

Ordered By Contact ID:1815167 Org ID:1 Last, First, MD, PhD, FACMG Ambry **Additional Authorized Recipient:** Last, Doctor MD	**Normal Specimen** Accession #: **00-108883** Type: Blood EDTA (Purple top) Specimen ID: Collected: 04/27/2020 Received: 04/28/2020 **Tumor Specimen** Accession #: **00-108884** Specimen Type: Tissue block Specimen Site: Colon Primary Tumor Site: Colon Tumor Type: Invasive adenocarcinoma Tumor Block ID: 1234-2A Collected: 04/23/2020 Received: 04/28/2020	Patient Name: **Last, First** AP2 Order #: 834415 DOB: 01/01/1990 Gender: M MRN #: Indication: Internal Testing

TumorNext®-Lynch+CancerNext®: Lynch Syndrome Paired Germline and Tumor Analyses plus Analyses of 31 Additional Genes Associated with Hereditary Cancer

OVERALL SUMMARY

This individual's germline results are consistent with a diagnosis of Lynch syndrome. See below for additional information.

SEQUENCING AND DELETION/DUPLICATION RESULTS

GERMLINE ORIGIN

Gene	Variant	Classification/Effect
MSH2	**c.943-1G>T**	**Pathogenic Mutation**

Germline Genes Analyzed: *MLH1, MSH2, MSH6, PMS2, APC, ATM, BARD1, BMPR1A, BRIP1, CDH1, CDKN2A, CHEK2, DICER1, MUTYH, NBN, PALB2, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53, CDK4, NF1, BRCA1, BRCA2, MSH3, NTHL1, RECQL, SMARCA4, AXIN2* (sequencing and deletion/duplication); *POLD1, POLE, HOXB13* (sequencing only); *EPCAM, GREM1* (deletion/duplication only) .

SOMATIC ORIGIN

Gene	Variant	Classification/Effect
MSH2	**p.Y121***	**Pathogenic Mutation**

Somatic Genes Analyzed: *MLH1, MSH2, MSH6, PMS2* (sequencing and deletion/duplication); *EPCAM* (deletion/duplication only) .

ADDITIONAL TUMOR RESULTS

Tumor Test	Result	FDA Approved Therapies
MLH1 Promoter Hypermethylation	Absent	None
Microsatellite Instability	**MSI-High**	**Pembrolizumab**

INTERPRETATION

- This individual is heterozygous for the **c.943-1G>T** pathogenic mutation in the *MSH2* gene.
  - This result is consistent with a diagnosis of **hereditary non-polyposis colorectal cancer (HNPCC)/Lynch syndrome.**
  - **Risk estimate**: lifetime risks of 52-82% for colorectal cancer, 25-60% for endometrial cancer, 6-13% for stomach cancer, 4-12% for ovarian cancer, and increased risk for prostate cancer
  - The expression and severity of disease for this individual cannot be predicted.
  - Genetic testing for pathogenic mutations in family members can be helpful in identifying at-risk individuals.

- The p.Y121* pathogenic mutation in the *MSH2* gene was identified to be of somatic origin in the tumor of this individual.
- Analysis of the *MLH1* promoter of the tumor of this individual demonstrated the absence of *MLH1* promoter hypermethylation.
- Microsatellite analysis of the tumor of this individual demonstrated high microsatellite instability (MSI-H).
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

Therapeutic Interpretation

- Pembrolizumab therapy may be indicated for this individual. In addition, there may be other therapies which are approved by the FDA, depending on tumor histology (https://www.fda.gov/Drugs/default.htm).

This test is designed to detect and report the alterations listed under the "Results Reports" section of the assay information pages; however, variants other than the ones listed above may also have been detected. If interested, these can be released upon request. Accuracy and interpretation of results may be impacted for a number of reasons, including but not limited to: tumor heterogeneity, prior tumor exposure to chemoradiation, discrepant tumor blocks used for somatic and IHC analyses, error from external IHC analysis, or other unknown specimen processing related variables. This test may detect multiple somatic copy gains or losses associated with the chromosomal instability (CIN) pathway (Muller M et al. Virchows Arch. 2016 Jun; 469:125-134; Pino M and Chung D. Gastroenterology. 2010 Jun; 138(6): 2059–2072).

GENE AND ALTERATION INFORMATION

***MSH2* Additional Information**

The **c.943-1G>T** intronic pathogenic mutation results from a G to T substitution one nucleotide upstream from coding exon 6 of the *MSH2* gene. Two other alterations at the same nucleotide position, (c.943-1G>C and c.943-1G>A), have been reported in probands with Lynch syndrome (Mangold E et al. *Int J Cancer.* 2005 Sep 20;116(5):692-702; Pistorius SR et al. *Int J Colorectal Dis.* 2000 Nov;15(5-6):225-63). RNA studies have demonstrated that this alteration results in abnormal splicing in the set of samples tested (Ambry internal data). In addition to the clinical data presented in the literature, alterations that disrupt the canonical splice site are expected to cause aberrant splicing, resulting in an abnormal protein or a transcript that is subject to nonsense-mediated mRNA decay. As such, this alteration is classified as a disease-causing mutation.

The **p.Y121*** pathogenic mutation (also known as c.363T>A), located in coding exon 2 of the *MSH2* gene, results from a T to A substitution at nucleotide position 363. This changes the amino acid from a tyrosine to a stop codon within coding exon 2. A different substitution (c.363T>G) resulting in the same premature stop codon was identified both in an individual meeting Amsterdam I criteria whose colon tumor demonstrated loss of MSH2 protein expression (Casey G et al. *JAMA* 2005 Feb;293:799-809) and in two related individuals with urothelial cancers demonstrating microsatellite instability or absence of MSH2 protein expression by IHC (Skeldon SC et al. *Eur. Urol.* 2013 Feb;63:379-85). In addition to the clinical data presented in the literature, this alteration is expected to result in loss of function by premature protein truncation or nonsense-mediated mRNA decay. As such, this alteration is interpreted as a disease-causing mutation.

The *MSH2* gene (NM_000251.1) encodes MutS homolog 2 protein (Msh2). This protein binds the Msh6 protein to form the MutSalpha complex which is involved in genome maintenance as part of the DNA mismatch repair (MMR) pathway. Tumors that harbor inactivating mutations in *MSH2* may demonstrate microsatellite instability and/or loss of MSH2 and/or MSH6 protein expression on immunohistochemistry. Germline mutations in *MSH2* cause hereditary non-polyposis colorectal cancer (HNPCC)/Lynch syndrome, an autosomal dominant cancer predisposition syndrome estimated to account for at least 2% of all colon cancers and up to 2% of all endometrial cancers diagnosed (Hampel H et al. *Cancer Res.* 2006 Aug 1;66(15):7810-7). HNPCC is associated with a significantly increased risk for colon cancer (52-82% lifetime risk), uterine/endometrial cancer (25-60% lifetime risk in females), stomach cancer (6-13% lifetime risk), and ovarian cancer (4-12% lifetime risk in females). Risks for cancer of the small intestine, hepatobiliary tract, upper urinary tract, central nervous system, and sebaceous glands are also elevated in individuals with a germline *MSH2* mutation compared to the general population (Aarnio M et al. *Int J Cancer.* 1999 Apr 12;81(2):214-8; Hampel H et al. *Gastroenterology.* 2005 Aug;129(2):415-21; Watson P et al. *Int J Cancer.* 2008 Jul 15;123(2):444-9; Stoffel E et al. *Gastroenterology.* 2009 Nov;137(5):1621-7; Capelle LG et al. *Gastroenterology.* 2010 Feb;138(2):487-92). In addition, it has been estimated that germline mutations in mismatch repair genes confer approximately a 2-fold risk for prostate cancer (Raymond VM et al. *J. Clin. Oncol.* 2013 May;31(14):1713-8; Ryan S et al. *Cancer Epidemiol. Biomarkers Prev.* 2014 Mar;23(3):437-49). Biallelic germline mutations in the *MSH2* gene are known to cause constitutional mismatch repair deficiency (CMMR-D) syndrome, a rare autosomal recessive disorder characterized by café au lait macules as well as increased risk for hematologic malignancies, brain tumors, and early onset Lynch syndrome associated cancers. Parents who each carry a *MSH2* mutation have a 25% chance for a child with CMMR-D in every pregnancy.

ASSAY INFORMATION

General Information: Genetic mutations can be present in an individual's constitutional DNA (germline) or arise within a tumor (somatic). Microsatellite instability (MSI) and/or loss of expression of one or more mismatch repair (MMR) proteins on immunohistochemistry (IHC) are seen more commonly in tumors associated with Lynch syndrome, but these results can also be caused by biallelic somatic inactivation of an MMR gene. Allelic inactivation can be due to somatic mutations, including deletions, and/or copy neutral loss of heterozygosity (CN-LOH) where the normal, or wildtype, allele is lost or replaced with a duplicated copy of the mutated allele. Somatic hypermethylation of the *MLH1* promoter is the most common reason for tumor MSI and MLH1 and/or PMS2 deficiency on IHC. Approximately 60-70% of tumors with somatic *MLH1* promoter hypermethylation carry the V600E somatic mutation in the *BRAF* gene. While there have been rare cases reported in individuals with Lynch syndrome, finding somatic *MLH1* promoter hypermethylation in a tumor generally indicates that the tumor was sporadic rather than due to a germline MMR mutation. The *BRAF* V600E mutation, along with somatic mutations at certain codons in the *KRAS* and *NRAS* genes, have been found to correspond to poorer responses of colorectal tumors to EGFR-targeted therapy.

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen(s) using standardized methodology and quantified. For FFPE section, one tissue section is first cut and stained with hematoxylin and eosin (H&E). The H&E slide is examined by a pathologist to determine tissue quantity/quality and neoplastic cellularity (20% minimum). Sequence enrichment of the germline and tumor sample for the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and Next-Generation sequencing. The bioinformatics pipeline performs paired analysis of sequence data from both tumor and germline specimens to differentiate variants of somatic origin from germline origin. Optimized variant calling filters require a read coverage depth of >100X for tumor and > 20X for matched control blood DNA. For molecular analysis of variants of germline origin only, additional Sanger sequencing is performed for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Suspect variant calls of germline origin other than those classified as "likely benign" or "benign" detected on the paired analysis are verified by Sanger sequencing. Germline variants in regions complicated by pseudogene interference, variant calls not satisfying depth of coverage and variant allele frequency quality thresholds, and potentially homozygous variants identified on the hereditary cancer panel are verified by Sanger sequencing. The germline *BRCA2* Portuguese founder mutation, c.156_157insAlu (also known as 384insAlu), and the *MSH2* coding exons 1-7 inversion are detected by next generation sequencing and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis. Germline gross deletion/duplication analysis for the sequenced genes (excluding *HOXB13, POLD1, POLE*, and *PMS2*) is performed using a custom pipeline based on NGS data and/or targeted chromosomal microarray with confirmatory MLPA when applicable. Germline gross deletion/duplication analysis of *PMS2* is performed using MLPA kit P008-B1. If a deletion is detected in exons 13, 14, or 15 of *PMS2*, double stranded sequencing of the appropriate exon(s) of the pseudogene *PMS2CL* will be performed to determine if the deletion is located in the *PMS2* gene or pseudogene. Whole gene copy number variants (CNV) and copy neutral loss-of-heterozygosity (CN-LOH) status are determined for the tumor specimen using a custom bioinformatics pipeline from NGS data and/or Affymetrix OncoScan® array when applicable. Sequence analysis is based on the following NCBI reference sequences: *APC*- NM_000038.5 & NM_001127511.2, *ATM*- NM_000051.3, *AXIN2*- NM_004655.3, *BARD1*- NM_000465.2, *BMPR1A*- NM_004329.2, *BRCA1*- NM_007294.3, *BRCA2*- NM_000059.3, *BRIP1*- NM_032043.2, *CDH1*- NM_004360.3, *CDK4*- NM_000075.3, *CDKN2A*- NM_000077.4 and NM_058195.3 (p14ARF), *CHEK2*- NM_007194.3, *DICER1*-NM_177438.2, *HOXB13*- NM_006361.5, *MLH1*- NM_000249.3, *MSH2*- NM_000251.1, *MSH3*- NM_002439.3, *MSH6*- NM_000179.2, *MUTYH*- NM_001128425.1, *NBN*- NM_002485.4, *NF1*- NM_000267.3, *NTHL1*- NM_002528.5, *PALB2*- NM_024675.3, *PMS2*- NM_000535.5, *POLD1*-NM_002691.2, *POLE*-NM_006231.2, *PTEN*- NM_000314.4, *RAD51C*- NM_058216.1, *RAD51D*- NM_002878.3, *RECQL*- NM_002907.3, *SMAD4*- NM_005359.5, *SMARCA4*- NM_001128849.1, *STK11*- NM_000455.4, *TP53*- NM_000546.4. Sequence analysis is based on the following NCBI reference sequences: *KRAS* (NM_004985.3), *NRAS* (NM_002524.4), and *BRAF* (NM_004333.4).

When indicated, microsatellite instability analysis is performed using the MSI Analysis System (Promega Inc.). Five microsatellite markers (BAT-25, BAT-26, NR-21, NR-24, MONO-27) are evaluated for sizing shifts in the tumor DNA versus germline DNA.

When indicated, hypermethylation analysis of the *MLH1* promoter region is performed using the methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) kit # ME001-B3 developed by MRC-Holland on genomic DNA isolated from the tumor specimen.

Analytical Range: The **TumorNext®-Lynch+CancerNext®** test targets detection of variants in genes associated with Lynch syndrome and other hereditary cancer syndromes. Germline sequencing analysis is performed for 34 genes (*APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, STK11,* and *TP53*) by either Next-Generation or Sanger sequencing. All coding domains and well into the flanking 5' and 3' ends of all the introns and untranslated regions are analyzed. For *HOXB13*, only variants impacting codon 84 are routinely reported. For *POLD1* and *POLE*, only missense and in-frame indel variants in the exonuclease domains (codons 311-541 and 269-485, respectively) are routinely reported. For *RECQL*, only missense variants in the helicase and RCQ domains (codons 63-592) and exonic truncating variants and are routinely reported. The *MSH3* polyalanine repeat region is excluded from analysis. Germline gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions of sequenced genes (excluding *HOXB13*, *POLD1, and POLE*) plus *EPCAM* and *GREM1.* For *GREM1*, only the status of the 40kb 5'UTR gross duplication is analyzed and reported. For *EPCAM*, only gross deletions encompassing the 3' end of the gene are reported. For *NTHL1*, only full-gene gross deletions and duplications are detected. For *APC*, all promoter 1B gross deletions as well as single nucleotide substitutions within the promoter 1B YY1 binding motif (NM_001127511 c.-196_-186) are analyzed and reported. Analysis of variants of somatic origin is performed for 4 mismatch repair genes

(*MLH1*, *MSH2*, *MSH6* and *PMS2)* and has the same analytical range as the germline analysis. This assay may not be able to differentiate whether somatic variants detected in *PMS2* exons 11 through 15 are in the *PMS2* gene or the *PMS2CL* pseudogene. Somatic copy number analysis of whole gene losses/gains is limited to 5 genes (*MLH1*, *MSH2*, *MSH6*, *PMS2* and *EPCAM*). Copy gains and/or amplifications without evidence of clinical significance are not routinely reported. The sensitivity of CN-LOH detection may be decreased when two alleles are identical by state and is reported as inactivating in the setting of a pathogenic mutation or likely pathogenic variant in the same gene. For orders including *KRAS* and *NRAS*, somatic missense alterations detected in codons 12, 13, 59, 61, 117 and 146 of both genes are analyzed and reported. For orders including *BRAF*, only the somatic p.V600E alteration of the *BRAF* gene is analyzed and reported.

Result Reports:

- **Germline Testing:** Alterations reported to be of germline origin are inferred to be also present in the tumor sample unless otherwise noted on the final report. Alterations in the following classifications are always reported, and are based on the following definition and clinical recommendations:
  - **Pathogenic Mutation:** alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Target testing of at-risk relatives and appropriate changes in medical management for pathogenic mutation carriers recommended. Previously described pathogenic mutations, including intronic mutations at any position, are always reported when detected.
  - **Variant, Likely Pathogenic (VLP):** Alterations with strong evidence in favor of pathogenicity. Targeted testing of at-risk relatives and appropriate changes in medical management for VLP carriers typically recommended. Previously described likely pathogenic variants, including intronic VLPs at any position, are always reported when detected.
  - **Variant, Unknown Significance (VUS):** Alterations with limited and/or conflicting evidence regarding pathogenicity. Familial testing via the Family Studies Program recommended. Medical management to be based personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 5 basepairs from the splice junction when detected.
- **Tumor Testing:**
  - **Lynch genes:** Somatic alterations in the following classifications are always reported for the 5 Lynch genes (*MLH1*, *MSH2*, *MSH6*, *PMS2* and *EPCAM*):
    - **Pathogenic Mutation**
    - **Variant, Likely Pathogenic (VLP)**
    - **Variant, Unknown Significance (VUS)**
  - ***BRAF, KRAS,* and *NRAS***: Targeted variants as described in the 'Analytical Range' section are listed if detected.
  - ***MSI* status*:*** Microsatellite stable is reported when no loci exhibit instability. MSI-low is reported when up to 20% of loci exhibit instability. MSI-high is reported when >20% of loci exhibit instability.
  - ***MLH1* promoter hypermethylation:** The presence or absence of hypermethylation is reported.
- Alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not routinely included on results reports. These include findings classified as "likely benign" and "benign" alterations.

TumorNext®-Lynch+CancerNext® Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

1. Giardiello FM et al. US Multi-Society Task Force on Colorectal Cancer. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-Society Task Force on colorectal cancer. *Gastroenterology.* 2014 Aug;147(2):502-26. PMID: 25043945.
2. Genetic/Familial High-Risk Assessments: Colorectal. version 2.2016 (NCCN Guidelines®) https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf
3. Mensenkamp AR et al. Somatic mutations in *MLH1* and *MSH2* are a frequent cause of mismatch repair deficiency in Lynch syndrome-like tumors. *Gastroenterology.* 2014 Mar;146(3):643-646.e8. PMID: 24333619.
4. Haraldsdottir S et al. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. *Gastroenterology.* 2014 Dec;147(6):1308-1316.e1. PMID: 25194673
5. Pérez-Carbonell L et al. Methylation analysis of *MLH1* improves the selection of patients for genetic testing in Lynch syndrome. *J Mol Diagn.* 2010 Jul;12(4):498-504. PMID: 20489114.
6. Crépin M, et al. Evidence of constitutional MLH1 epimutation associated to transgenerational inheritance of cancer susceptibility. Hum Mutat. 2012 Jan;33(1):180-8. PMID: 21953887.
7. Extended RAS Gene Mutation Testing in Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015 Summary. *J Oncol Pract.* 2016 Feb;12(2):180
8. Salipante SJ et al. Microsatellite instability detection by next generation sequencing. *Clin Chem.* 2014 Sep;60(9):1192-9. PMID: 24987110.
9. Liu T et al. Microsatellite instability as a predictor of a mutation in a DNA mismatch repair gene in familial colorectal cancer. *Genes Chromosomes Cancer.* 2000 Jan;27(1):17-25. PMID: 10564582.

Assay Information Continued on Next Page

ASSAY INFORMATION *(Supplement to Test Results - Continued)*

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature*. 2012;491:56-65.
2. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med.* 2015 May;17(5):405-23.
3. Ambry Genetics Variant Classification Scheme. http://www.ambrygen.com/variant-classification.
4. Berkeley Drosophila Genome Project [Internet]. Reese MG et al. *J Comp Biol.* 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
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7. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/EVS.
8. Grantham R. Amino acid difference formula to help explain protein evolution. *Science.* 1974;185(4151):862-864.
9. HGMD® [Internet]: Stenson PD et al. *Genome Med.* 2009;1(1):13. www.hgmd.cf.ac.uk.
10. Landrum MJ et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
11. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: http://omim.org.
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14. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org.
15. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533
16. Mu W et al. *J Mol Diagn*. 2016 Oct 4. PubMed PMID: 27720647

Disclaimer: This test was developed and its performance characteristics were determined by Ambry Genetics Corporation. DNA isolation, NGS and Sanger sequencing, deletion/duplication, microsatellite instability, and *MLH1* promoter hypermethylation analyses are performed at Ambry Genetics 7 Argonaut, Aliso Viejo, CA (CLIA# 05D0981414). Pathology review of stained slides is performed at Ambry Genetics 15 Argonaut, Aliso Viejo, CA (CLIA#: 05D2115857). It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as purely investigational or for research. This test should be interpreted in context with other clinical findings. This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of the relevant medical literature. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. The sequencing and gross deletion duplication portion of this test analyzes the following types of mutations: nucleotide substitutions, small deletions (up to 25 bp), small insertions (up to 10 bp), small indels, gross deletions/duplications, and targeted gross rearrangements known to be associated with somatic tumor development and progression. It is not intended to analyze the following types of mutations: uncharacterized gross rearrangements, deep intronic variations, Alu element insertions, and other unknown abnormalities. The MSI portion of this test analyzes microsatellite instability at 5 loci. The *MLH1* hypermethylation portion analyzes the methylation status of the *MLH1* promoter region. The assay is validated to detect single nucleotide variants (SNVs) and short indels with variant frequency at 5% or greater, the variant frequency represents the percentage of sequencing reads from tumor specimen DNA containing the variants and has not been corrected for the estimated fraction of tumor cells in the specimen. The pattern of mutation types varies with the gene tested and this test detects a high but variable percentage of known and unknown mutations of the classes stated. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable group. This test is designed and validated to be capable of detecting >99% of described abnormalities in the genes and chromosome regions represented on the test (analytical sensitivity). The clinical sensitivity of this test may vary widely according to the specific clinical history, histopathological subtypes of each tumor and tumor heterogeneity. Mutations in other genes or the regions not tested by this test can also play a role in tumorigenesis. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, rare genetic variants that interfere with analysis, germline or somatic mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, active hematologic disease, a history of allogenic bone marrow or peripheral stem cell transplant, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data. In particular, this assay may not be able to determine if somatic variants within exons 11-15 of *PMS2* are located in the *PMS2* gene or the *PMS2CL* pseudogene.

Ambry Genetics®

Understanding Your Genetic Testing

INFORMATION FOR PATIENTS WHO HAVE HAD **TUMOR/GERMLINE** GENETIC TESTING FOR **LYNCH SYNDROME**

5 Things To Know

1	This test is trying to figure out if you do or do not have Lynch syndrome, which may explain the cause of your cancer.
2	Lynch syndrome is a hereditary cancer syndrome that causes a risk for several cancers, including colorectal and endometrial (uterine) cancer. There are 5 genes linked to Lynch syndrome: *MLH1, MSH2, MSH6, PMS2,* and *EPCAM*.
3	Your testing checked for mutations (disease-causing changes in your genes, like a spelling error) both in your blood/saliva AND your cancer tissue.
4	Germline Lynch syndrome gene mutations (if found by testing your blood or saliva) can be passed from parent to child, causing you and potentially your family members to be at an increased risk for certain cancers. People with Lynch syndrome need cancer screening, like colonoscopies, earlier and more often than the average person.
5	Somatic Lynch syndrome gene mutations (if found by testing your tumor) may help to rule out Lynch syndrome or may impact treatment* for your current cancer. Somatic gene mutations cannot impact your family members, as they are not passed from parent to child.

Key differences between germline and tumor testing:

GERMLINE	TUMOR
• Tests genes that look the same in all of the cells that make up your body • Mutations can be found by testing blood (most common), saliva, or other tissue types	• Tests for genetic changes that may only be present in your tumor, but not in the rest of your body • Called somatic mutations and are common in tumors
• Mutations are usually inherited from your parents and can be passed on to your children • Mutations may be found in your family members	• Mutations are not inherited from your parents and cannot be passed on to your children • Mutations are ONLY in your tumor and do not run in your family, so cannot be found in your family members
• Mutations in certain genes can increase your risk for cancer(s) and may impact your cancer screening and/or treatment	• Do not increase your risk for any other cancer(s), but may impact your cancer treatment* and other management

**Tumor (somatic) mutations in the BRAF, KRAS, and NRAS genes, among others, can provide information about how helpful certain medications may/may not be for treating your cancer.*

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