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SAMPLE REPORT

Ordered By Contact ID:1815168 Org ID:1 Last, First, MD, PhD, FACMG Ambry **Additional Authorized Recipient:** Last, Doctor MD	**Normal Specimen** Accession #: **00-108875** Type: Blood EDTA (Purple top) Specimen ID: Collected: 04/27/2020 Received: 04/28/2020 **Tumor Specimen** Accession #: **00-108876** Specimen Type: Tissue block Specimen Site: Right ovary Primary Tumor Site: Right ovary Tumor Type: High grade serous carincoma Tumor Block ID: 1234-2A Collected: 04/27/2020 Received: 04/28/2020	Patient Name: **Last, First** AP2 Order #: 834411 DOB: 01/01/1987 Gender: F MRN #: Indication: Internal Testing

TumorNext®-HRD+CancerNext®: Paired Germline and Tumor Analyses of 11 Genes Associated with Homologous Recombination Repair plus Germline Analyses of 26 Additional Genes Associated with Hereditary Cancer

OVERALL SUMMARY

No pathogenic mutations or variants of unknown significance of germline origin were identified. An alteration of somatic origin was detected in this individual's tumor. See below for additional information.

SEQUENCING AND DELETION/DUPLICATION RESULTS

GERMLINE ORIGIN

NO VARIANTS DETECTED

Germline Genes Analyzed: *ATM, BARD1, BRIP1, CHEK2, MRE11A, NBN, PALB2, RAD51C, RAD51D, BRCA1, BRCA2, APC, BMPR1A, CDH1, CDKN2A, DICER1, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53, CDK4, NF1, 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	FDA Approved Therapies
BRCA1	**p.S1503***	**Pathogenic Mutation**	**Rucaparib, Olaparib**

Somatic Genes Analyzed: *ATM, BARD1, BRIP1, CHEK2, MRE11A, NBN, PALB2, RAD51C, RAD51D, BRCA1, BRCA2* (sequencing only) .

INTERPRETATION

Germline Interpretation

- No germline mutations or variants of unknown significance were detected.
- **Risk Estimate:** low likelihood of germline variants in the genes analyzed contributing to this individual's clinical history.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

Somatic Interpretation

- The p.S1503* pathogenic mutation in the *BRCA1* gene was detected in this individual's tumor.

THERAPEUTIC INFORMATION

***BRCA1* and *BRCA2* Therapeutic Information**

PARP inhibitors have been approved by the FDA for certain indications; please refer to the Prescribing Information for Olapirib and Rucaprib on https://www.fda.gov for full details.

- **Olaparib:** This PARP inhibitor has been approved by the FDA
  - For the treatment of adult patients with advanced ovarian cancer with germline *BRCA1* or *BRCA2* mutations who have received previous treatment with at least three lines of chemotherapy.
  - For the maintenance treatment of adult patients with advanced epithelial ovarian, Fallopian tube or primary peritoneal cancer with germline and/or somatic *BRCA1* or *BRCA2* mutations who are in complete or partial response to first-line platinum-based chemotherapy. (For the full indications please refer to https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208558s001lbl.pdf).
- **Rucaparib:** This PARP inhibitor has been approved by the FDA for use in advanced ovarian cancer patients with germline and/or somatic *BRCA1* or *BRCA2* mutations who have received previous treatment with at least two lines of chemotherapy. (For the full indications please refer to https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/209115s003lbl.pdf)

Only FDA approved PARP inhibitor therapies are reported. The therapies listed in this report may not be suitable for this particular patient and the effect of PARP inhibitor therapies in this individual remains unknown. Decisions regarding patient management and treatment, including therapy selection, must be based on the medical judgment of the treating physician. This test should be interpreted in context with other clinical findings, including patient's personal and family history, external testing results, and standard of care of the medical community.

GENE AND ALTERATION INFORMATION

***BRCA1* Additional Information**

Somatic Alteration Information

The **p.S1503*** pathogenic mutation (also known as c.4508C>A), located in coding exon 13 of the *BRCA1* gene, results from a C to A substitution at nucleotide position 4508. This changes the amino acid from a serine to a stop codon within coding exon 13. This pathogenic mutation has been reported in the literature in breast and breast/ovarian cancer families (Liede A et al. *Am. J. Hum. Genet.* 2002 Sep; 71(3):595-606; van der Hout AH et al. *Hum. Mutat.* 2006 Jul; 27(7):654-66; Rashid M et al. *Int J Cancer.* 2006 Dec 15;119(12):2832-9). Of note, this alteration is also designated as 4627C>A in published literature. 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.

Gene Information

The breast cancer 1 (*BRCA1*, OMIM *113705, NM_007294.3) tumor suppressor gene, located at 17q21.31, encodes the 1863 amino acid BRCA1 protein. BRCA1 plays an integral role in genomic stability/maintenance, DNA repair, and cell cycle control, and pathogenic germline or somatic mutations in *BRCA1* can lead to tumorigenesis through homologous recombination deficiency (Savage et al. Cancer Res. 2014 May 15;74(10):2773-2784). Pathogenic germline mutations in the *BRCA1* gene are associated with significantly increased lifetime risks for breast and ovarian cancers in women. Early studies estimated a female breast cancer risk of 87% by age 70 for female *BRCA1* germline mutation carriers; however, more recent studies suggest a risk of 57-65% by age 70. Pathogenic germline *BRCA1* mutations are also associated with a contralateral female breast cancer risk of 43.4% within 10 years of initial breast cancer diagnosis with no intervention. The risk for ovarian cancer, including primary peritoneal and fallopian tube cancer, by age 70 in women with germline *BRCA1* mutations is estimated to be 39-40%. Male germline *BRCA1* mutation carriers have a cumulative breast cancer risk of 1.2% by age 70 and an increased risk for prostate cancer (RR=1.82 by age 65). In addition, both men and women have an increased risk for melanoma and pancreatic cancer compared to the general population, although the exact risks have not yet been well defined. In addition, published evidence suggests that both germline and somatic mutations in the *BRCA1* gene may predict sensitivity to chemotherapy agents that induce DNA damage as well as to poly(ADP-ribose) polymerase (PARP) inhibitors.

ASSAY INFORMATION

General Information: Ovarian cancer is the fifth most common female cancer, with up to 25% being attributed to inherited/germline mutations in cancer predisposition genes. Genes associated with increased ovarian cancer risk include (but are not limited to): *ATM*, *BARD1*, *BRCA1, BRCA2, BRIP1*, *CHEK2*, *NBN*, *PALB2*, *RAD51C*, *RAD51D*. Additionally, germline and somatic mutations in *BRCA1* and *BRCA2* lead to homologous recombination repair deficiency (HRD) and can predict a patient's increased sensitivity and response to treatments, including platinum-based chemotherapy and PARP inhibitors (Kim G et al. *Clin Cancer Res.* 2015 Oct 1;21(19):4257-61; Rose S. *Cancer Discov.* 2017 Feb;7(2):120-121; Tan DS & Kaye SB. *Am Soc Clin Oncol Educ Book*.2015:114-21).

Methodology: Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen(s) using standardized methodology and quantified. For FFPE section, one thin (5 micron) 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 (NGS). 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. 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, *MRE11A*-NM_005591.3, *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.

Analytical Range: The **TumorNext®-HRD+CancerNext®** Test targets detection of germline and somatic variants in genes in the homologous recombination repair pathway and other hereditary cancer syndromes. Germline sequencing analysis is performed for 35 genes (*APC, ATM, AXIN2 BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, MRE11A, NBN, NF1, NTHL1, PALB2, POLD1, POLE, PMS2, 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 alterations affecting p.G84 are analyzed and reported. For *POLD1* and *POLE*, only missense variants 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* exon 1 repeat region is excluded from analysis. For the germline specimen, gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions of all sequenced genes (excluding *HOXB13, POLD1, and POLE*), *GREM1*, and *EPCAM*. 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 11 genes in the homologous recombination repair pathway (*ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, MRE11A, NBN, PALB2, RAD51C,* and *RAD51D)* and has the same analytical range as the germline analysis.

Result Reports:

- **Germline Sequencing and Del/dup Analysis and Tumor Sequencing:** Alterations reported to be of germline origin are presumed present in the tumor 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 germline 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 germline 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 germline VUS carrier status. Note, intronic VUSs are always reported out to 5 basepairs from the splice junction when detected.
- 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.
- Additional variants of somatic origin may have also been detected and can be released upon request.

- **Therapeutic Information:** Only those FDA approved PARP inhibitor therapies are reported, which are indicated in epithelial ovarian cancer, and this list is not comprehensive Not all FDA-approved therapies may be reported.

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.
5. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP. Accessed Jan 2012).
6. ESEfinder [Internet]. Smith PJ, et al. (2006) *Hum Mol Genet.* 15(16):2490-2508 and Cartegni L, et al. *Nucleic Acid Research.* 2003;31(13):3568-3571. http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home.
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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.
12. Feng BJ. PERCH: A Unified Framework for Disease Gene Prioritization. *Hum Mutat.* 2017 Mar;38(3):243-251.
13. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: http://exac.broadinstitute.org.
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, and deletion/duplication 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 germline 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 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.