TumorNext-HRD: Paired Germline and Tumor Analyses of Genes Associated with Hereditary Ovarian 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 (sequencing and deletion/duplication).

SOMATIC ORIGIN

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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.E1308* (c.3922G>T) pathogenic mutation in the BRCA2 gene was detected in this individual's tumor.
This a targeted gene test designed to detect and report the alterations listed under the “Results Reports” section of the assay information pages and is not intended to identify global genomic instability; however, additional variants of somatic origin may have also been detected and can be released upon request.

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.

**THERAPEUTIC INFORMATION**

**BRCA1 and BRCA2 Therapeutic Information**

- **Olaparib:** This PARP inhibitor has been approved by the FDA for the following indications:
  - 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 (Kim G et al. Clin Cancer Res. 2015 Oct 1;21(19):4257-61).
  - 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 (Moore K et al. N Engl J Med. 2018 Dec 27;379(26):2495-2505).

- **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 (Rose S. Cancer Discov. 2017 Feb;7(2):120-121).

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**

**BRCA2 Additional Information**

**Somatic Alteration Information**

The p.E1308* pathogenic mutation (also known as c.3922G>T), located in coding exon 10 of the BRCA2 gene, results from a G to T substitution at nucleotide position 3922. This changes the amino acid from a glutamic acid to a stop codon within coding exon 10. This mutation has been reported in several individuals with personal and/or family histories of breast, ovarian, and/or prostate cancer (Duran M et al. Hum. Mutat. 2003 Apr;21:448; Hall MJ et al. Cancer. 2009 May;115:2222-33; Pritchard CC et al. N. Engl. J. Med. 2016 Aug;375:443-53; Gabaldó Barrios X et al. Fam. Cancer. 2017 Oct;16:477-489; Wen WX et al. J. Med. Genet. 2018 Feb;55:97-103). The p.E1308* mutation has also been reported as a BRCA2 compound heterozygous finding in a child diagnosed with a medulloblastoma at 3.5 years of age and Fanconi Anemia (Offit K et al. J. Natl. Cancer Inst. 2003 Oct;95:1548-51). Of note, this alteration is also designated as 4150G>T 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 2 (BRCA2, OMIM *600185, NM_000059.3) tumor suppressor gene is located at 13q13.1 and encodes the 3418 amino acid BRCA2 protein. BRCA2 is involved in the DNA repair process via homologous recombination and double-strand break repair, and pathogenic germline or somatic mutations in BRCA2 can lead to tumorigenesis through homologous recombination deficiency (Holloman WK. Nat Struct Mol Biol. 2011 Jul;6;18(7):748-54). Pathogenic germline mutations in the BRCA2 gene are associated with significantly increased lifetime risks for breast and ovarian cancers in women. Early studies estimated a female breast cancer risk of 84% by age 70 for female germline BRCA2 mutation carriers; however, more recent studies suggest a risk of 45-49% by age 70. Pathogenic germline BRCA2 mutations are also associated with a contralateral female breast cancer risk of 34.6% 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 BRCA2 mutations is estimated to be 11-18%. Male germline BRCA2 mutation carriers have a cumulative breast cancer risk of over 6% by age 70 and prostate cancer risk of approximately 15% by age 65. In addition, both men and women have an increased risk for melanoma and cancers of the pancreas, gall bladder, bile duct and stomach compared to the general population, although the exact risks have not yet been well defined. Biallelic germline mutations in the BRCA2 gene are known to cause Fanconi anemia type D1 (FA-D1), a rare autosomal recessive disorder affecting multiple body systems. Parents who each carry a germline BRCA2 mutation have a 25% chance for a child with FA-D1 in every pregnancy. 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: \(\text{ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, MRE11A, NBN, PALB2, RAD51C, RAD51D}\). Additionally, germline and somatic mutations in \(\text{BRCA1}\) and \(\text{BRCA2}\) 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. Germline reportable small insertions and deletions, potentially homozygous variants, variants in regions complicated by pseudogene interference, and single nucleotide variant calls not satisfying 100X depth of coverage and 40% het ratio thresholds are verified by Sanger sequencing (Mu W et al. *J Mol Diagn.* 2016 Oct 4). The \(\text{BRCA2}\) Portuguese founder mutation, c.156_157insAlu (also known as 384insAlu) is 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 11 of the genes (excluding \(\text{384insAlu}\)) is 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 11 of the genes (excluding \(\text{384insAlu}\)) is 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 11 of the genes (excluding \(\text{384insAlu}\)) is detected by next generation sequencing and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis.

**Analytical Range:** The TumorNext-HRD Test targets detection of germline and somatic variants in genes in the homologous recombination repair pathway which are associated with hereditary ovarian cancer. Germline and tumor sequencing analysis is performed for 11 genes (\(\text{ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, MRE11A, NBN, PALB2, RAD51C, RAD51D}\)) 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. Germline gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions of all 11 sequenced genes.

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

*Assay Information Continued on Next Page*
Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

7. Exome Variant Server, NHLBI Exome Sequencing Project (ESP) [Internet], Seattle WA. Available from: evs.gs.washington.edu/ EVS.

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 10% or greater, the variant frequency represents the percentage of tumor DNA sequencing reads 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, technical errors, clerical errors, and sequencing errors. Sequencing errors can result from rare genetic variants that interfere with analysis, from high GC content, homopolymer region, or 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.