Strand® Somatic *BRCA* Test Report

Patient Name	:	**W. M. A. A. B. Walisundara**	Test	:	**Somatic BRCA**
Gender	:	**Female**	Referring Center	:	**General Hospital Kandy Srilanka**
Age	:	**43 Years**	Referred by	:	**Dr. Roshan Gunarathne**
MRN #	:	**NA**	Sample Collected	:	**15-Jun-2024**
Sample ID	:	**STRAN-2024-55944**	Sample Received	:	**06-Aug-2024**
Specimen	:	**FFPE Block**	Report Generated	:	**06-Sep-2024**

Indications for Test

Ovarian carcinoma

Results

BRCA1/2 Mutation Status: **Negative** for deleterious or likely deleterious mutation in BRCA1

Test Details

The Somatic BRCA test measures the presence of deleterious/likely deleterious mutation in BRCA1/2.

Note

This is a qualified report. The data obtained post sequencing was insufficient for comprehensive detection of alterations. While BRCA1 was well covered and no mutation was detected in BRCA1, BRCA2 was found to be poorly covered and the mutational status of BRCA2 could not be determined. Repeat testing with a different block is recommended.

Interpretation Summary

No deleterious/likely deleterious mutation was detected in BRCA1. The mutation status of BRCA2 is undetermined.

This sample is categorized as BRCA1 negative and as BRCA2 mutation status is undetermined, PARP inhibitor therapy recommendation is undetermined in this case.

Recommendations

Cancers with impaired homologous recombination repair (HRR) are sensitive to PARP inhibitors due to synthetic lethality [1-3]. PARP inhibitors are approved for BRCA1/2 altered ovarian, breast, prostate and pancreatic cancers and HRR deficient prostate cancer. A deleterious or likely deleterious mutation in any of the HRR genes may be informative about response to PARP inhibitors.

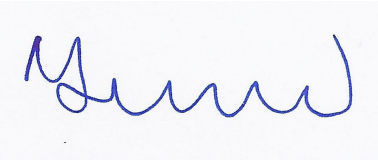
Limitations of Gene Coverage

For each test gene, all gene target regions were adequately covered by greater than 200 reads.

References

1. Lord CJ *et al.* 2017. PARP inhibitors: Synthetic lethality in the clinic. *Science* **355** (6330):1152-1158 [PMID: 28302823].
2. Fong PC *et al.* 2009. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N. Engl. J. Med.* **361** (2):123-34 [PMID: 19553641].
3. Pilié PG *et al.* 2019. PARP Inhibitors: Extending Benefit Beyond BRCA-Mutant Cancers. *Clin Cancer Res* **25** (13):3759-3771 [PMID: 30760478].

Signatures



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Supplementary Information

Test Description

The Somatic BRCA test is a Next Generation Sequencing Test (NGS) that evaluates BRCA1/2. DNA is extracted from FFPE tumor samples. Sequencing is carried out using Illumina NGS platforms. The data is analysed using StrandNGS™ and StrandOmics® softwares to identify clinically significant variants. Tumors with mutations in BRCA1/2 are likely to show better response to PARP inhibitors.

Genes Evaluated: 2 genes

BRCA1, BRCA2

Methodology

Sample preparation: Genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor samples or fresh tumor tissue using our internally standardized extraction method. Quantification of DNA was performed using Qubit, and 50 ng of amplifiable DNA was selected for library preparation following the vendor's recommendations.

Assay Methods: Our methodology utilizes SomaticAdvantage74 (SA74), a targeted hybrid-capture-based next-generation sequencing assay designed to identify various mutation classes, including single-nucleotide variants (SNVs), multinucleotide variants (<3bp) and small insertions (1-18bp)/deletions (1-27bp).

Library Preparation: DNA extracted from FFPE tissue using our standardized method was processed into DNA libraries. Regions of interest were hybridized to biotinylated probes using the xGen Hybridization and Wash Kit from Integrated DNA Technologies. Subsequently, the probes, along with the hybridized DNA, were selectively captured using streptavidin-coated beads and eluted to enrich the library pool. Libraries were then normalized using a simple bead-based protocol, pooled, and sequenced on an Illumina NovaSeq instrument.

Analysis

The sequence data is processed using the analysis pipeline and performs the following analysis steps in the Local App and then writes analysis output files to the folder specified.

DNA Data Analysis: The sequenced reads from the DNA of the sample were processed on STRAND® NGS v3.3.5 (http://www.strand-ngs.com) using the pipeline 'SA74_DNA_Somatic_Pipeline_v1'. This pipeline aligns the raw reads from the FASTQ files against the whole genome build hg19_hsd37d5, applies various quality filters on the read data, and finally calls SNVs and InDels.

The called variants were then imported into StrandOmics where the variants were annotated and prioritized by automated pipelines. The StrandOmics user interface was then used for identifying the variants of interest and for reporting these variants. All variants reported were verified to have good quality using the STRAND® NGS genome browser.

The Quality Control step outputs the Run QC, DNA Sample QC and RNA Sample QC which are used to perform the sample QC based on the internal thresholds.

A VCF file was created including small variants and uploaded into StrandOmics® for further interpretation and reporting. Annotation and prioritization of variants was done by automated pipelines in StrandOmics®. The StrandOmics® user interface was then used for identifying variants of interest and for reporting these variants. All variants reported were verified to have good raw read quality using the StrandNGS™ genome browser. Variants were then assessed for clinical significance and deleterious/likely deleterious variants were included in the report.

StrandNGS™ v3.3.5: StrandNGS™ (http://www.strand-ngs.com) is an NGS analysis platform from Strand Life Sciences. It comprises algorithms for alignment, variant calling, exon deletion/duplication analysis, and structural variant calling. A built in genome browser enables inspection of read level data. Several QC steps enable inspection of read quality. StrandNGS™ has been cited in at least 200 publications.

StrandOmics® v6.27.2: StrandOmics® is a clinical genomics interpretation and reporting platform from Strand Life Sciences. The StrandOmics® Variant Annotation engine includes algorithms to identify variant impact on gene using both public content (ClinVar, HPO, links to dbSNP, 1000 Genomes, Exome Variant Server, and five in-silico predictors - FATHMM, LRT, Mutation Assessor, Mutation Taster, and SIFT) and proprietary content (curated variant records). The interpretation interface in StrandOmics® allows quick filtering and evaluation of variants along with capture of justification for inclusion/exclusion. The reporting interface in StrandOmics® enables included variants to be carried into template-driven reports efficiently. StrandOmics® has already been used for interpretation of thousands of clinical cases. Omics Data version 2.3.0

Data Versions: Data Annotations are updated periodically. Data version or date of download used for annotations are as mentioned: Human Genome (hg19), NCBI RefSeq (Annotation Release 105), NCBI RefSeq genes - curated subset (Apr 2018), NCBI Gene (June 2018), ClinVar (June 2023), UniProt (June 2023), GWAS (Mar 2015), dbSNP (v156), Exome Variant Server (Jan 2017), ExAC (v0.3.1), 1000 Genomes(Jan 2017), dbNSFP (v2.9.3), HPO (Aug 2023)

Performance Characteristics

The Somatic BRCA Test is a Laboratory Developed Test (LDT) that was developed by Strand Life Sciences. This test has a limit of detection of 5% for SNVs and Indels with >99.9% sensitivity i.e., any SNV or Indel present in the sample with an allele frequency of at least 5% can be detected with >99.9% probability. On the other hand, the detection of SNVs and Indels has a specificity of >99.99%, i..e., any SNV or Indel detected by the test is likely to be a true variant with a probability of >99.99% even if the observed allele frequency is less than 5%.

Limitations and Disclaimer

A histopathological review of samples is conducted and the samples are deemed acceptable for testing if they meet our sample acceptance criteria of a minimum 20% tumor content. Lower tumor cell concentration (less than 20%) or severely degraded samples may cause a false negative mutation result

This clinical report should be carefully assessed by the treating physician and further interpreted along with clinical, histopathological findings, contraindications and guidelines before deciding the course of therapy. Treatment decisions are the responsibility of the clinician. It is recommended that the most recent block is used for testing as the mutation profile may change in response to treatment and hence differ at different sampling points.

Compliance Statement

Somatic BRCA Test is a laboratory developed test/procedure that was validated by Strand Life Sciences Pvt. Ltd. This report has been prepared by individual(s) with appropriate genetics training and certification in medical/laboratory genetics or in molecular genetic pathology. Recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, American College of Medical Genetics and Genomics (ACMG) and College of American Pathologists for interpretation and reporting of sequence variants in cancer are followed [1,2].

Supplementary Information - References

1 . Li MM *et al.* 2017. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn.* **19** (1):4-23 [PMID: 27993330].

2 . Richards S *et al.* 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* **17** (5):405-424 [PMID: 25741868].

End of report