Strand® Homologous Recombination Repair Test Report

CAP ACCREDITED COLLEGE of AMERICAN PATHOLOGISTS #8750941O1

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Patient Name	:	**D. M. A. D. B. Dissanayaka**	Test	:	**Homologous Recombination Repair Test**
Gender	:	**Male**	Referring Center	:	**General Hospital Kandy**
Age	:	**53 Years**	Referred by	:	**Dr. Senaka Kandegedara**
MRN #	:	**NA**	Sample Collected	:	**25-May-2024**
Sample ID	:	**STRAN-2024-51652**	Sample Received	:	**29-May-2024**
Specimen	:	**FFPE Block**	Report Generated	:	**18-Jun-2024**

Indications for Test

Colorectal carcinoma

Results

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

Mutation Status of other HRR genes: **Negative** for deleterious or likely deleterious mutation

HRR status: **Negative**

Test Details

The Homologous Recombination Repair Test measures the presence of deleterious/likely deleterious mutation in BRCA1/2 and 12 other genes associated with homologous recombination repair.

Note

The recommendations of this test with respect to HRR status pertain to the following cancers: ovarian, breast, pancreatic and prostate cancers. According to the provided pathology report this sample is from a colorectal cancer. Clinical correlation recommended.

Interpretation Summary

No deleterious/likely deleterious mutation was detected in BRCA1/2 or any of the other HRR genes tested.

This sample is categorized as HRR negative and PARP inhibitor therapy is not indicated 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

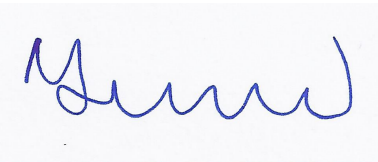
For each test gene, the fraction of the gene covered by less than 100 reads is indicated below.

Gene	% of Coding Region under covered	Gene	% of Coding Region under covered
CHEK2	0.45 %	*RAD51B*	1.09 %

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 Homologous Recombination Repair (HRR) test is a Next Generation Sequencing Test (NGS) that evaluates 14 genes including BRCA1/2 which are associated with Homologous Recombination Repair (HRR). 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 genes associated with homologous recombination repair are likely to show better response to PARP inhibitors.

Genes Evaluated: 14 genes

ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, RAD54L

Methodology

A commercial kit was used to extract genomic DNA from FFPE tissue (Qiagen, USA). The DNA was quantified using the Qubit fluorescent assay (Life Technologies, USA), and the quality was determined using Alu PCR. The adapter ligated library was created using 25ng of DNA (Kapa Biosystems, Roche,USA). The library was quantified with the Qubit fluorescent assay (Life Technologies, USA) and its quality was evaluated with the Tapestation 4200. (Agilent Technologies, USA). Up to ten uniquely indexed libraries were pooled for capture, and hybridization was performed using custom DNA probes (IDT, USA). A commercial kit was used for post-hybridization steps (IDT,USA). The captured libraries were pooled and sequenced on an Illumina sequencing platform to produce FASTQ files.

Analysis

The reads from the FASTQ files were aligned against the whole genome build hg19_hsd37d5 using StrandNGS™ v3.3.5 (http://www.strand-ngs.com). Five base pairs from the 3' end of the reads were trimmed with quality below 20. Reads which had length less than 25 bp after trimming were not considered for alignment. A maximum of 5 matches of alignment score at least 90% with a gap percentage of 45 were computed.

Split Alignment is performed and trimming is performed if the portion of the read goes beyond 5'end of its mate. Reads that failed QC (quality control), reads with average quality less than 20, reads with ambiguous characters and reads which are duplicates, unaligned, unknown, mate missing and translocated reads were all filtered out. The reads were realigned using the local realignment tool in StrandNGS™. Reads with alignment score less than 95% and partially aligned reads were all filtered out and split reads were realigned.

The StrandNGS™ Low frequency variant caller was used to detect variants at locations in the target regions covered by a minimum of 10 reads with at least 3 variant reads. Reference locations and spill over at locations with homopolymer stretch greater than 7 bps are ignored for variant detection. Split and partially aligned reads were ignored while calling the variants. Variants with a decibel score of at least 50 were reported. Variants with a Strand Bias ≥50% and with a Total Reads ≥100, and supporting reads ≤5% were filtered out. Variants were then imported into StrandOmics®.

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 a 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 hundreds of global scientific publications.

StrandOmics® v6.26.1: StrandOmics® (https://clinical.strandomics.com) is a clinical genomics interpretation and reporting platform from Strand Life Sciences. The StrandOmics® Variant Annotation engine includes algorithms to identify variant impact from both public content (ClinVar, HPO, dbSNP, 1000 Genomes, Exome Variant Server and COSMIC and dbNSFP) and proprietary content on genes, diseases, and therapeutic impact of somatic variants. 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 identified variants to be carried into template-driven reports efficiently. StrandOmics® has been used for interpretation and reporting 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 Homologous Recombination Repair Test is a Laboratory Developed Test (LDT) that was developed by Strand Life Sciences. The Limit of Detection (LOD) for this test is 5% variant allele frequency (VAF) at a coverage of 200X.

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

Homologous Recombination Repair Test is a laboratory developed test/procedure that was validated by Strand Life Sciences Pvt. Ltd. It has not been cleared or approved by the US Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. 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