Client

Gurugram

Pathkind Diagnostics Pvt. Ltd.

Plot No. 55-56, Udhyog Vihar Ph-IV, Gurugram - 122015

Processed By

Pathkind Diagnostics Pvt. Ltd.

Plot No. 55-56, Udhyog Vihar Ph-IV, Gurugram - 122015

Name	: **Mr. SP587**	Billing Date	:	07/07/2023 12:47:51
Age	: 45 Yrs	Sample Collected on	:	10/07/2023 10:01:31
Sex	: Male	Sample Received on	:	10/07/2023 11:02:13
P. ID No.	: P1000100013579	Report Released on	:	
Accession No	: **10002305635**	Barcode No.	:	10002305635
Referring Doctor	: Self			
Referred By	:	Ref no.	:	

Report Status - Preliminary Report

Test Name	Result	Biological Ref. Interval	Unit

MOLECULAR DIAGNOSTICS

ONCOPRO HOMOLOGOUS RECOMBINATION REPAIR	See Attachment		

Sample: EDTA Blood

** End of Report**

ONCOPRO HOMOLOGOUS RECOMBINATION REPAIR - GERMLINE

Clinical Indication :

PERSONAL/FAMILY HISTORY

:

	Proband	Immediate Relatives (Parents, Siblings)	Son/Daughter	Paternal/Maternal Relatives
Relationship		-	-	-
Cancer Type		-	-	-
Age at diagnosis (in years)		-	-	-

GENOMIC HIGHLIGHTS

VUS

Variant of Uncertain clinical Significance was identified

Description of variants classifications based on Published Literature and ACMG Guidelines

Positive	VUS	Negative
Identified variant is known to elevate the cancer risk. Identified variant is known to elevate the cancer risk. Already known as Pathogenic variant Pleas e consult with your doctor to create a screening and management plan and to identify relatives who may need to be tested.	Identified variant is presently not known to increase the cancer risk. The genetic/clinical data is insufficient for us to analyze the mutation or to associate it with any kind of a clinical condition. Patient may or may not develop any associated condition, and its risk cannot be predicted due to inadequate scientific reported evidence.	No clinically relevant variant was identified that is associated with elevated cancer risk. Please consult with your doctor to discuss the surveillance recommendations.

Note: In absence of pedigree information, detailed curation and clinical significance could not be summarized. Please correlate clinically.

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CLINICAL SIGNIFICANCE OF HRR TESTING IN BREAST, OVARIAN, PANCREATIC AND PROSTATE CANCERS

BRCA-mutant cancer cells have abnormal Homologous Recombination (HR) repair of DNA. In these tumors, the Base Excision Repair (BER) pathway is important for cell survival. The Poly (ADP-ribose) polymerase (PARP) enzymes play a key role in BER, and PARP inhibitors (PARPi) are effective in causing cell death in BRCA-mutant cells while sparing normal cells-a concept called synthetic lethality.

Recent clinical trials in BRCA mutant metastatic breast cancer have demonstrated improved outcomes with single agent PARPi's (olaparib and talazoparib) over chemotherapy. Treatment of BRCA-mutant tumors with a PARP inhibitor (PARPi) leads to accumulation of DNA damage resulting in cell cycle arrest and apoptosis. This effect of PARPi's in cells with defects in the HR pathway is an example of synthetic lethality

On January 12, 2018, the Food and Drug Administration granted regular approval to olaparib tablets (Lynparza), a poly (ADP-ribose) polymerase (PARP) inhibitor, for the treatment for patients with deleterious or suspected deleterious germline BRCA-mutated (gBRCAm), HER2-negative metastatic breast cancer who have been treated with chemotherapy either in the neoadjuvant, adjuvant, or metastatic setting.

https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-olaparib-germline- BRCA-mutated-metastatic-breast-cancer

As per a recent study on High Grade Serous Ovarian Carcinomas (HGOAC), those patients with Germline/Somatic HRR mutations had shown increased sensitivity to platinum drugs as compared those without HRR mutations (Pennington et al. 2014). In another study on triple negative breast cancer patients, it was observed that patients with HR deficiency could achieve pCR, as compared to those who were HR non-deficient (Telli et al. 2018). Clinical trial of ARIEL3 studies have shown that PARP inhibitor maintenance is associated with higher PFS benefits in BRCA mutated as well as HR deficient subgroups of recurrent platinum-sensitive high-grade serous or endometrioid ovarian, primary peritoneal, or fallopian tube carcinoma. This study also demonstrated that Rucaparib maintenance treatment significantly improved PFS of HRR deficient patients across all the cancers mentioned above (Coleman et. al. 2017).

https://www.fda.gov/drugs/resources-information-approved-drugs/niraparib-zejula (last viewed on 29th October 2019

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PARP inhibitor is also approved as maintenance treatment of adult patients with deleterious or suspected deleterious gBRCAm metastatic pancreatic adenocarcinoma whose disease has not progressed on at least 16 weeks of a first-line platinum-based chemotherapy regimen.

https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-olaparib-gbrcam-metastatic-pancreatic-adenocarcinoma

PARP inhibitor is also approved in treatment of adult patients with deleterious or suspected deleterious germline or somatic homologous recombination repair (HRR) gene-mutated metastatic castration- resistant prostate cancer (mCRPC) who have progressed following prior treatment with enzalutamide or abiraterone.

https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-olaparib-hrr-gene- mutated-metastatic-castration-resistant-prostate-cancer

STATUS OF HRR GENES

The ability to adequately repair DNA double-strand breaks (DSBs) relies on HR repair, which reconstructs damaged DNA by copying the respective undamaged strand from the homologous sister chromatid. A complex set of proteins are required to interact within this process, including the gene products of BRCA1/2 and several others (Bartl T. et al., 2020; Zhou P. et al., 2020).

Any dysfunctional protein involved may impair the ability to adequately mend DSBs, thereby inducing a phenotypical cell behavior termed HR deficiency (HRD) or "BRCAness." HRD can modify the tumor immune microenvironment by increasing the number of tumor-infiltrating lymphocytes (TILs), indicating that HRD might be a biomarker for the immunotherapy response (Ratner ES et al., 2020).

Poly (ADP-ribose) polymerase (PARP) inhibitors take advantage of HRR (Homologous recombination-related) deficiency to kill tumor cells based on the concept of synthetic lethality. An interaction between PARP inhibition and BRCA mutations is the best example of the synthetic lethality (Jang A. et al., 2020).

Several PARP inhibitors (PARPis) have been successful in various malignancies with HRR gene mutations including BRCA1/2, especially in breast cancer and ovarian cancer.

Below is a table enlisting HRR genes and the status of mutations in this patient:

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Gene name	Gene Coverage	Detected Mutation
ATM	100.00%	Not detected
BARD1	100.00%	Not detected
BRCA1	100.00%	Not detected
BRCA2	100.00%	Not detected
BRIP1	100.00%	Not detected
CDK12	100.00%	Not detected
CHEK1	100.00%	Not detected
CHEK2	**100.00%**	**VUS**
FANCL	100.00%	Not detected
PALB2	100.00%	Not detected
PPP2R2A	100.00%	Not detected
RAD51B	99.67%	Not detected
RAD51C	100.00%	Not detected
RAD51D	100.00%	Not detected
RAD54L	100.00%	Not detected

For all the HRR genes analyzed in the above-mentioned table, variants were classified as:

- Pathogenic/ Likely pathogenic' - variants for the gene were deleterious or disease-causing
- VUS' - variants for the gene were of uncertain significance
- Not detected' - variants for the gene were not detected in the patient

Note: This test only mentions the status of the HRR genes in our panel. The results mentioned are not an equivalent of the HRD status and/or positive Genomic Instability Score.

VARIANT DETAILS

Gene	Genomic alteration	Protein, Mutant allelic burden (%)	Clinical Significance	Mode of inheritance	Zygosity	Reference
CHEK2	chr22:29121077T>C c. 609A>G [ENST00000382580.2]	p. Ile203Met (48%)	VUS	Autosomal dominant	Heterozygous	rs575910805, VCV000128077.34

Contd...6

RELEVANT GENE DETAILS

CHEK2-Checkpoint kinase 2

Checkpoint kinase 2 (CHEK2) is a gene that encodes a protein that functions as a regulator of the cell cycle as well as a tumor suppressor. The protein is activated in the presence of DNA damage in order to prevent entry into mitosis. Missense mutations, nonsense mutations, silent mutations, and frameshift deletions and insertions are observed in cancers such as cancers of the central nervous system, endometrial cancer, and intestinal cancer. CHEK2 is altered in 1.54% of all cancers with an alteration in 1.44% of ovarian carcinoma patients.

The variant p.Ile203Met has a total depth of 429X and an alternative depth of 206X. The variant is classified as VUS in ClinVar with 16 submissions including hereditary cancer-predisposing syndrome. The isoleucine at codon 203 has been replaced by methionine, an amino acid with similar properties and this amino acid position is well conserved among primates. The In-silico prediction tools like SIFT, polyphen and MutationTaster predict this variant to be deleterious, possibly damaging and benign respectively.This variant is not present in ALFA population database and present in in 1000Genome, GnomAD and ExAC with allelic frequencies of 0.0004, 0.000119 and 0.000165 respectively. The identified variant lies in the 5th exon of the gene and is not present in any functional domain. In vivo functional studies in yeast showed that this variant has partial to no impact on response to DNA damage (Roeb W et al ., 2012, Delimitsou A et al ., 2019). This variant has been reported among several patients affected with breast cancer (Maxwell KN et al., 2016 ; Mohamad S et al., 2015; Dufault MR et al ., 2004) .Germline mutation in the CHEK2 gene is associated with breast and ovarian cancer (Kleiblova P et al. 2019). Due to lack of clinical and functional evidence, this variant is classified as a Variant of Uncertain Significance (VUS) and must be clinically correlated with other findings for any further management.

Note: The South Asian allele frequency of the CHEK2 variant in the healthy population is slightly high (24). Kindly correlate clinically.

RESULT INTERPRETATION

Through the process of gene sequencing, certain DNA mutations identified may not have adequate evidence reported in the literature to support the clinical suspicion. Such a variant, with no established clinical significance at the time of testing, is known as a Variant of Uncertain Significance or VUS. There are other factors that could play a role in developing cancer, such as environmental and lifestyle, which cause sporadic mutation.

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TEST DESCRIPTION

Variation (fault/mutation) in certain genes can be inherited, which may sometimes cause cancer. Hereditary Cancer Risk test panel allows to identify specific variations in the genes which are known to be associated to increase risk of cancer as against the normal population. The variations found in the genes could be pathogenic, likely pathogenic or variant of uncertain significance and may attribute to low, moderate or high risk of predisposition cancer.

Hereditary cancer screening test is a gene panel-based screening which enables analysis of all the human protein coding genes associated with specific hereditary cancers and cancer syndromes, in a single comprehensive test. It targets all the genes recommended by ACMG/NCCN/WHO/ESMO guidelines.

ABOUT THE TESTING

HRR test is a gene panel-based screening of 15 HRR genes associated with different cancers collated from published scientific literature.

Homologous Recombination and Repair (HRR) is the ability to adequately repair DNA double-strand breaks (DSBs) relies on HR repair, which reconstructs damaged DNA by copying the respective undamaged strand from the homologous sister chromatid. A complex set of proteins are required to interact within this process, including the gene products of BRCA1/2 and several others (Bartl T. et al., 2020; Zhou P. et al., 2020).

Understanding the genetic testing process and its results require support of a trained genetic counsellor. We suggest the individual to seek genetic counseling prior to consenting for any kind of genetic test to understand the purpose of the test recommended by the clinician and its usefulness to the patient and their family.

Variation (fault/mutation) in certain genes can be inherited, which may sometimes cause cancer. The variations found in the genes could be pathogenic, likely pathogenic or variant of uncertain significance and may attribute to low, moderate or high risk of predisposition to cancer.

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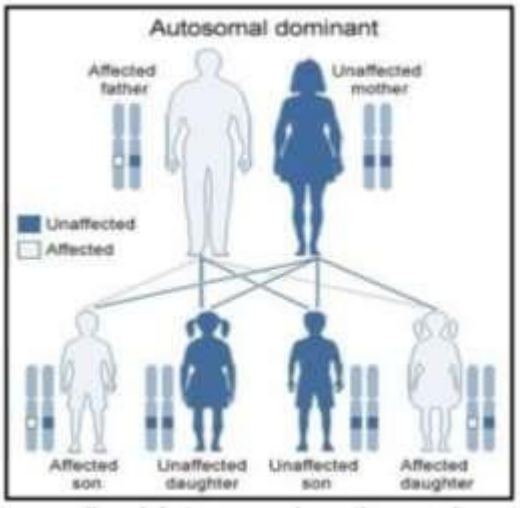

The patient's physician may annually wish to re-analyze the results or recommend re-testing for any variants that may have been newly identified, to associate with the patient's clinical condition. The patient or family members are recommended to consult their physician and approach us for testing services accordingly.

We, strive to ensure that every patient and family members are made accessible to all possible information without breaching the patient's confidentiality.

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Websites

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- NIH- National Cancer Institute - https://www.cancer.gov/
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- https://www.ncbi.nlm.nih.gov/medgen/
- https://www.cancer.net/cancer-types
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- https://www.cancerresearchuk.org/
- http://pfam.xfam.org/
- https://www.uniprot.org/

TEST METHODOLOGY

Genomic DNA is isolated from Whole Blood sample for library preparation and quantified using Qubit Fluorometer, 50 ng is taken for library preparation. The NGS libraries were prepared as per standard procedures for Illumina sequencing. The libraries were sequenced with mean coverage depth >100X.

The sequences obtained are aligned to human reference genome (GRCh37/hg19) and variant analysis was performed using set of Bioinformatics Pipeline. Only non- synonymous and splice site variants found in the exome panel consisting of specific set of genes were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

Clinically relevant mutations were annotated using published literature and a set of databases – ClinVar (Landrum et al, 2015.), cbioportal (Cerami et al, 2012; Gao et al, 2013) and dbSNP. Common variants are filtered based on allele frequency in 1000 Genome Phase 3(Auton et al, 2015), ExAC (Karczewski et al. 2016), dbSNP (Sherry et al, 2001), etc. In the absence of a clinically significant reported known variation(s), pathogenicity will be predicted based on in-silico gene prioritization tools: CADD (Rentzsch et al. 2018), SIFT (Ng PC et al, 2003), PolyPhen-2 (Adzhubei et al, 2013) and prioritized for clinical correlation. The identified pathogenic variant will be correlated with observed phenotypic features of the patient and interpreted according to American College of Medical Genetics (ACMG) guidelines.

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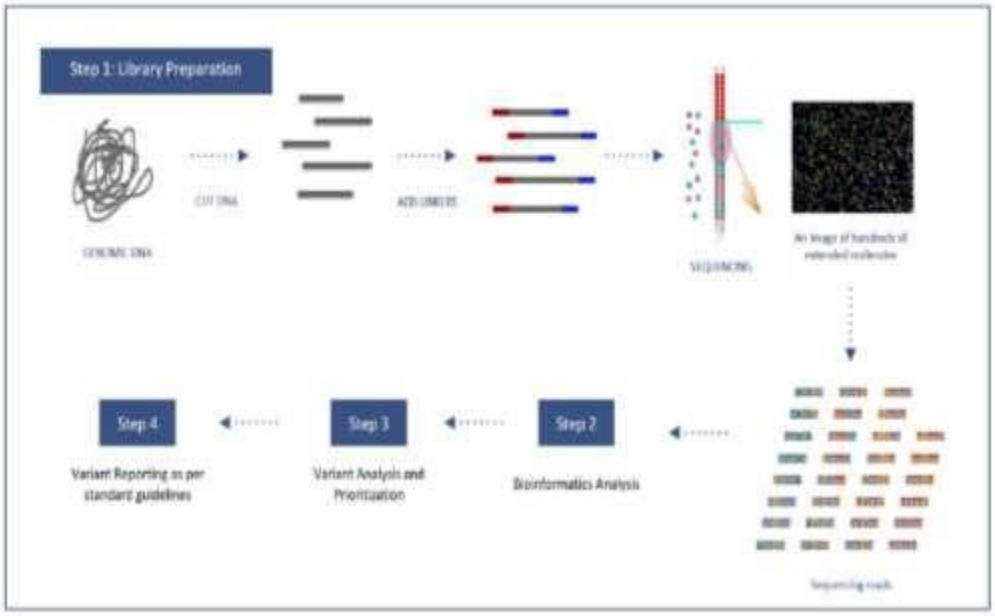

LIMITATIONS AND DISCLAIMER

- This test has been developed, validated and performed, this test has not been cleared or approved by the FDA.
- A comprehensive risk assessment may include other aspects of the patient's personal/family medical history, as well as lifestyle, environment and other factors. This is not included in the scope of this NGS testing.
- We are using the canonical transcript for clinical reporting which is usually the longest coding transcript with strong/multiple supporting clinical evidence. However, in rare cases, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.
- Changes in personal/family history or additional data regarding specific genes/mutations may affect the cancer risk estimates and management recommendations within this report. Personal/family history should be updated with a healthcare provider on a regular basis.
- Certain genes may not be covered completely, and few mutations could be missed. Many factors such as homopolymers, GC-rich regions etc. influence the quality of sequencing and coverage. This may result in an occasional error in sequence reads or lack of detection of a particular genetic alteration.
- As with any laboratory test, there is a small chance that this result may be inaccurate for a preanalytical reasons, such as an error during specimen collection and labeling (incorrect patient identification).
- Large insertions, deletions, duplications, inversions, repeat expansions and complex rearrangements cannot be characterized accurately by NGS as it uses short-read sequencing data. Such structural variants have a much higher false-positive and false-negative rate than seen for SNVs (single nucleotide variant). It is possible that the genomic region where a disease-causing variation exists in the proband was not captured using the current technologies and therefore was not detected.

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- It is possible that a particular genetic abnormality may not be recognized as the underlying cause of the genetic disorder due to incomplete scientific knowledge about the function of all genes in the human genome and the impact of variants on those genes.
- Accurate interpretation of this report is dependent on detailed clinical history of the patient. In the event of unavailability of detailed clinical history, the lab cannot guarantee the accuracy of the interpretation.
- This report is strictly not a medical diagnostic report and shall not be construed as the medical certificate or medical laboratory report or diagnostic report.

Note: Test has been outsourced and performed at PATHKINDR004.