

Test Description

It is an integrated report of multiplatform testing including comprehensive genomic profiling (170 gene panel) using next generation sequencing (NGS).

Patient Demographic

Name: Santosh Mor
Sex: Female
Date of Birth/Age: 51 years
Disease: Breast Cancer

Clinician

Clinician Name: Dr Amish Vora
Medical Facility: HOPE Clinic
Pathologist: Not Provided

Specimen

Booking ID: 011907120112
Site: Breast
Sample Type: FFPE block (3), 18093819 A, B, C
Date of Collection: 12-07-2019
Date of Booking: 12-07-2019

CLINICAL SYNOPSIS

Estrogen receptor (ER)+, Progesterone positive (PR)+ breast cancer [as per the histopathology report dated 26-12-2018]. The tumor was identified in the blocks [18093819 A, B, C] and was adequate for further analysis.

RECOMMENDATION & REPORT INTERPRETATION

Based on **PALB2** alterations, further germline testing is recommended for possible hereditary breast cancer syndrome. PARP-inhibitors and platinumums may have potential clinical utility.

Disclaimer: Report interpretation & recommendation(s) should not be considered as final; and should be used at the discretion of the treating Physician or the molecular tumor board. The report interpretation & recommendation(s) does not bear any medical, legal, ethical & moral responsibilities, and liabilities.

BIOMARKERS

Genomic Findings

PALB2 p.Leu961Pro, Exon 9

TP53 p.Tyr236His, Exon 7

Variant of Unknown Significance

PALB2 p.Lys967_Val969delinsMet, Exon 9

CLINICAL CORRELATION AND VARIANT INTERPRETATION

PALB2 p.Leu961Pro Overall depth: 504X	c.2882T>C (ENST00000261584.4) Mutant Allele Percentage: 28.6%	DNA repair
TP53 p.Tyr236His Overall depth: 498X	c.706T>C (ENST00000269305.4) Mutant Allele Percentage: 74.4%	Tumor Suppressor Gene
PALB2 p.Lys967_Val969delinsMet Overall depth: 498X	c.2900_2905del (261584.4) Mutant Allele Percentage: 59.8%	DNA repair

These variants specifically detected in this tumor have not been characterized sufficiently in biochemical assays and therefore

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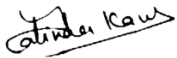
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their impact in this cancer remains speculative.

The *PALB2* gene variants p.Lys967_Val969delinsMet and p.Leu961Pro are not reported in ExAC and 1000 genomes databases. The p.Leu961Pro variant is predicted to be damaging by SIFT and probably damaging by Polyphen2 functional prediction tools. The *TP53* gene variant p.Tyr236His is not reported in ExAC and 1000 genomes databases. This variant is predicted to be damaging by SIFT and probably damaging by Polyphen2 functional prediction tools. However, the functional impact and clinical significance of these variants in the tumor tissue of the subject are not well documented in medical literature.

RECOMMENDATIONS

Correlation of the genetic findings with the clinical condition of the patient is required to arrive at accurate diagnosis, prognosis or for therapeutic decisions.



Jatinder Kaur, PhD
Head, Molecular Biology & Genomics



Dr. Gulshan Yadav, MD
Head, Pathology

APPENDIX 1: TEST METHODOLOGY

Background

The next-generation sequencing based multi-gene analysis, allows us to sequence and identify variants associated with multiple genes with diagnostic, prognostic and therapeutic implications in different cancer types. This tumor somatic panel in investigation, has been designed to screen for somatic mutations in 170 cancer related genes associated with tumorigenesis, prognostication and predictive value for chemotherapy and targeted therapy drugs in different tumor types. Targeted sequencing represents a cost-effective approach with the ability to detect specific variants causing protein-coding changes in individual human genomes. These multi-gene, affordable tests will enable personalized treatment by matching the patient's tumor with the appropriate drug, based on the mutational findings.

Method

Tumor genomic DNA and RNA isolated from FFPE tissue was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced with a panel mean coverage depth of **2394X and 12784X** in DNA and RNA library respectively, on Illumina sequencing platform. The sequences obtained were aligned to human reference genome (GRCh37/hg19) using BWA program^{1,2}. Somatic mutations were identified using LoFreq (version 2) variant caller^{3,4}. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. The mutations were annotated using VariMAT annotation pipeline. Gene annotation of the variants was performed using VeP program⁵ against the Ensembl release 90 human gene Model⁶. Clinically relevant mutations were annotated using published literature, databases and in-house propriety databases. The common variants were filtered for reporting based on the presence in various population databases (1000G, ExAC, EVS, 1000Japanese, dbSNP, UK10K⁷⁻¹²). Gene fusions at the RNA level were assessed using multiple fusion detection programs to arrive at the consensus on predicted fusions. The fusions are confirmed based on the number of spanning reads and/or split reads supporting the finding¹³⁻¹⁷. Reportable mutations are prioritized and prepared based AMP-ASCO-CAP guidelines¹⁸ based on annotation metrics from OncoMD¹⁹, reference lab's curated somatic database which includes somatic mutations from TCGA. Possibility of false negative or false positive below the limit of detection of this assay cannot be ruled out.

The transcript used for clinical reporting generally represents the canonical transcript (according to Ensembl release 90 human gene model), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.

Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.

"This test was developed, and its performance characteristics determined by Reference lab".

DISCLAIMER

- The classification of variants of unknown significance can change over time. Please contact MolQ laboratory at a later date for any change.
- The scope of this assay limits to SNPs, Short Indels (in DNA) and gene fusions and splice variants (inRNA)
- Intronic variants are not assessed using this method.
- Large deletions of more than 20 bp or copy number variations / rearrangements cannot be assessed using this method.
- This panel is intended to screen for complete coding region of the genes enlisted below in the appendix 2.
- The mutations have not been validated by Sanger sequencing.
- This NGS panel is not intended to report germline variants.
- This NGS test used does not allow definitive differentiation between germline and somatic variants
- TREATMENT DECISIONS BASED ON THESE MUTATIONS MAY BE TAKEN IN CORRELATION WITH OTHER CLINICAL AND PATHOLOGICAL INFORMATION. (For RUO)

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APPENDIX 2: GENE LIST OF THE 170 GENES PANEL

SNVs and Short Indels (<25bp) (from DNA)									
AKT1	BRIP1	CREBBP	FANCI	FGFR2	JAK3	MSH3	PALB2	RAD51D	TSC1
AKT2	BTK	CSF1R	FANCL	FGFR3	KDR	MSH6	PDGFRA	RAD54L	TSC2
AKT3	CARD11	CTNNB1	FBXW7	FGFR4	KIT	MTOR	PDGFRB	RB1	VHL
ALK	CCND1	DDR2	FGF1	FLT1	KMT2A(MLL)	MUTYH	PIK3CA	RET	XRCC2
APC	CCND2	DNMT3A	FGF2	FLT3	KRAS	MYC	PIK3CB	RICTOR	
AR	CCNE1	EGFR	FGF3	FOXL2	MAP2K1	MYCL1	PIK3CD	ROS1	
ARID1A	CD79A	EP300	FGF4	GEN1	MAP2K2	MYCN	PIK3CG	RPS6KB1	
ATM	CD79B	ERBB2	FGF5	GNA11	MCL1	MYD88	PIK3R1	SLX4	
ATR	CDH1	ERBB3	FGF6	GNAQ	MDM2	NBN	PMS2	SMAD4	
BAP1	CDK12	ERBB4	FGF7	GNAS	MDM4	NF1	PPP2R2A	SMARCB1	
BARD1	CDK4	ERCC1	FGF8	HNF1A	MET	NOTCH1	PTCH1	SMO	
BCL2	CDK6	ERCC2	FGF9	HRAS	MLH1	NOTCH2	PTEN	SRC	
BCL6	CDKN2A	ERG	FGF10	IDH1	MLLT3	NOTCH3	PTPN11	STK11	
BRAF	CEBPA	ESR1	FGF14	IDH2	MPL	NPM1	RAD51	TERT	
BRCA1	CHEK1	EZH2	FGF23	INPP4B	MRE11A	NRAS	RAD51B	TET2	
BRCA2	CHEK2	FAM175A	FGFR1	JAK2	MSH2	NRG1	RAD51C	TP53	

Fusions and Splice Variants (from RNA)									
ABL1	BRAF	EML4	ETV4	FGFR4	KIF5B	MYC	NTRK2	PIK3CA	TMPRSS2
AKT3	BRCA1	ERBB2	ETV5	FLI1	KIT	NOTCH1	NTRK3	PPARG	
ALK	BRCA2	ERG	EWSR1	FLT1	KMT2A(MLL)	NOTCH2	PAX3	RAF1	
AR	CDK4	ESR1	FGFR1	FLT3	MET	NOTCH3	PAX7	RET	
AXL	CSF1R	ETS1	FGFR2	JAK2	MLLT3	NRG1	PDGFRA	ROS1	
BCL2	EGFR	ETV1	FGFR3	KDR	MSH2	NTRK1	PDGFRB	RPS6KB1	