

Test Description

It is an integrated report of multiplatform testing including comprehensive genomic profiling (170 gene panel) and tumor mutation burden (TMB) using next generation sequencing (NGS), microsatellite instability (MSI) using molecular beacon probe-based multiplex polymerase chain reaction (7 biomarkers) and PD-L1 expression using immunohistochemistry.

Patient Demographic

Name: Manju Jain
Sex: Female
Date of Birth/Age: 59 years
Disease: Poorly differentiated adenocarcinoma of fallopian tube/ ovary

Clinician

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

Specimen

Booking ID: 011902230322
Site: Ovary
Sample Type: FFPE block (1), 8641/17, C
Date of Collection: 25-02-2019
Date of Booking: 27-02-2019

CLINICAL SYNOPSIS

Patient is diagnosed case of poorly differentiated adenocarcinoma fallopian tube/ovary since July 2017. Two lines of chemotherapy. Short progression free interval. The tumor was identifiable in the blocks [8641/17, C] and it was adequate for further analysis.

RECOMMENDATION & REPORT INTERPRETATION

Target therapy against PARP (PARP inhibitors) can be used in combination with chemotherapy or immunotherapy or alone.

Genetic counselling is recommended for HBOC and Li-Fraumeni syndrome for observed potential germline BRCA2/ TP53 gene mutations.

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

Targeted Therapy

Genomic Findings

Pathogenic- Driver

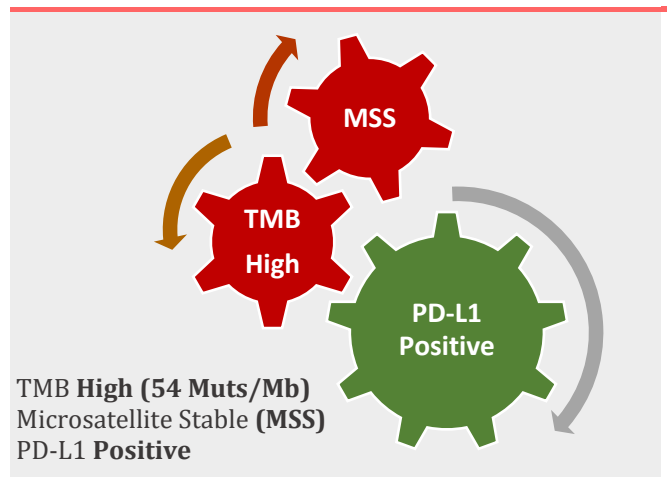
BRCA2 p.Glu2981Lys, Exon 22

TP53 p.Arg248Trp, Exon 7

Variant of Unknown Significance

ERRB2 p.Gln24LysfsTer8, Exon 1

Immunotherapy



CLINICAL CORRELATION AND VARIANT INTERPRETATION

BRCA2 p.Glu2981Lys Overall depth: 1367X	c.8941G>A (ENST00000544455.1) Mutant Allele Percentage: 3.1%	Tumor Suppressor Gene
ERBB2 p.Gln24LysfsTer8 Overall depth: 1040X	c.70del (ENST00000269571.5) Mutant Allele Percentage: 3.2%	Oncogene
TP53 p.Arg248Trp Overall depth: 1640X	c.742C>T (ENST0000 0269305.4) Mutant Allele Percentage: 39.1%	Tumor Suppressor Gene (Gain of function)

ERBB2 gene p.Gln24LysfsTer8 and *BRCA2* p.Glu2981Lys gene variants specifically detected in this tumor have not been characterized sufficiently. These variants are detected with significant allele burden. The variants Gln24LysfsTer8 and Glu2981Lys are not reported in ExAC and 1000 genomes databases whereas the variant Glu2981Lys is predicted to be damaging and possibly damaging in functional prediction tools SIFT and Polyphen2 respectively.

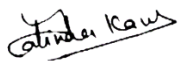
The missense hot spot mutations (HSMs) at codons R273, R248 and R175 are the most common oncogenic *p53* mutations that comprise 19.7% of all *p53* mutations in HGS EOC^{1,2}. These three *TP53* mutations convert *p53* from a tumor suppressor to an oncogene².

A missense variation in exon 7 of *TP53* gene (**chr17:7577539: G>A; c.742C>T**) that results in an amino acid substitution at codon 248 (**p.Arg248Trp**) was detected in the *TP53* gene of this subject. This is a loss of function mutation in *TP53*. The ExAC MAF for this variant is 0.0008% and has not been reported in 1000 genomes database.

In a study conducted on 82 advanced-stage high-grade pelvic serous carcinoma (HGPs), more than 90% of patients with high-grade serous ovarian carcinoma shows mutation in *TP53*³ and presence of *TP53* mutation has been demonstrated as a potential biomarker for monitoring treatment response using liquid biopsy as a tool⁴. Pathogenic *TP53* mutations are verified as the earliest events in tumor evolution, followed by deletions or loss of heterozygosity (LOH) of chromosomes carrying *TP53*, *BRCA1*, and *BRCA2*, in ovarian carcinogenesis. Ovarian cancer patients harboring different mutated *TP53* show different types of chemotherapy resistance and survival outcomes⁵. One study compared overall survival of ovarian cancer patients with different *TP53* hot spot mutations (HSMs) by analyzing the TCGA database (TCGA HGS EOC patients (n= 316)) showed that R248G confers chemoresistance while R273H demonstrated resistance to paclitaxel⁶. Another study investigated the association of *TP53* K351N mutation with drug-resistance on tumor samples obtained from 153 patients with advanced EOC who received platinum-based chemotherapy. This study showed that *TP53* K351N mutation is associated with induction of platinum resistance after neoadjuvant chemotherapy (NACT) and is an independent factor for shorter disease-free survival in multivariate analysis⁷. However, the prognostic significance of the variant identified in the subject under investigation is not well established in medical literature. Kindly correlate clinically.

REFERENCES

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6. Seagle BLL *et al*. TP53 hot spot mutations in ovarian cancer: Selective resistance to microtubule stabilizers in vitro and differential survival outcomes from The Cancer Genome Atlas. *Gynecol Oncol*. 2015; Jul; 138(1): 159–164.
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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 **1876X and 14462X** 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 (in RNA)
- 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	

iMSI Rapid™ Assay

MSI testing is used for Hereditary Cancer screening (Hereditary Non-Polyposis Colorectal Cancer -HNPCC or Lynch syndrome);
As a biomarker (Prognostic and predictive biomarker for the response of Immunotherapy)

Result

Microsatellite - Stable (MSS)

BIOMARKER FINDINGS

<i>ACVR2A</i>	No mutation detected
<i>BTBD7</i>	No mutation detected
<i>DIDO1</i>	No mutation detected
<i>MRE11</i>	No mutation detected
<i>RYR3</i>	No mutation detected
<i>SEC13A</i>	No mutation detected
<i>SULF2</i>	No mutation detected

INTERPRETATION

*MSS <2 of the 7 markers demonstrate instability
#MSI-H ≥2 of the 7 markers demonstrate instability

*Microsatellite stable
Microsatellite Instability-High

For valid batch test results specific controls are being run with every batch.

METHODOLOGY

Multiplex detection of seven mononucleotide repeats using molecular beacon probe-based polymerase chain reaction followed by high resolution melt-curve analysis. The assay uses seven novel biomarkers *ACVR2A*, *BTBD7*, *DIDO1*, *MRE11*, *RYR3*, *SEC31A* and *SULF2* as this set of biomarkers is stable over different cancer types and ethnicities and show high performance than other known assays like *Bethesda Panel*. This test is carried out on Idylla platform using the MSI/1.0 Cartridge based kit which is CE IVD approved.

REFERENCES

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Zhao et al. (2018) ASCO Abstract #e15654

Programmed Death Ligand 1 (PD-L1) Immunohistochemistry

Test Description

This test is useful for identification of neoplasms expressing programmed cell death 1-ligand 1 (clone SP263). PD-L1 also known as B7 homolog 1 (B7-H1) or CD274, is a transmembrane protein involved in the regulation of cell-mediated immune responses through interaction with the receptor programmed death protein-1 (PD-1). PD-L1 has been identified as both a prognostic and theranostic marker in a variety of neoplasms. Overexpression of PD-L1 has been observed in carcinomas of the urinary bladder, lung, thymus, colon, pancreas, ovary, breast, kidney, and in melanoma and glioblastoma.

Specimen

Sample Type: FFPE block (1), 8641/17, C
Site: Ovary
Pathology ID: MOLQ/IHC-06022019
Disease: Poorly differentiated adenocarcinoma of fallopian tube/ ovary

Interpretation

The scoring system is based on type and origin of tumor. If additional interpretation or analysis is needed, send request for Pathology Consultation.

Methodology

Immunostaining for PD-L1 protein was done using Ventana Rabbit Anti-Human PD-L1/CD274 Monoclonal Antibody (Clone SP-263) on Ventana Autostainer.

Positive PD-L1 staining/expression is defined as complete and/or partial, circumferential or linear plasma membrane staining at any intensity that can be differentiated from background.

Note

Preclinical studies suggest that positive programmed cell death 1-ligand 1 (PD-L1) immunohistochemistry in tumor cells may predict tumor response to therapy with immune checkpoint inhibitors. This result should not be used as the sole factor in determining treatment, as other factors (eg, tumor mutation burden and microsatellite instability) have also been studied as predictive markers.

References

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Programmed Death Ligand 1 (PD-L1): Positive

Microscopy Evaluation

HE Staining (Figure 1)

Tumor cells: 80%
 Immune cells: 20%

Tumor cells positive for PD-L1 (membrane only): 16%
Immune cells positive for PD-L1: 3%

HE Stained Section

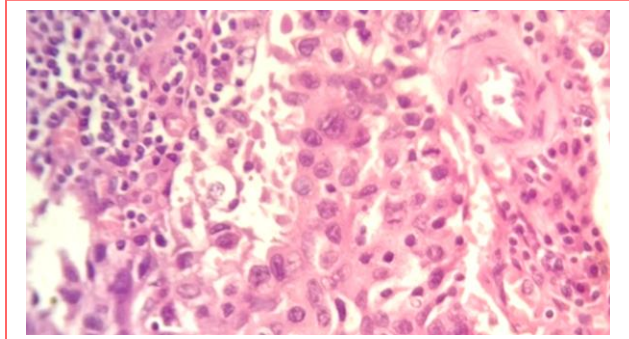


Figure 1

PD-L1 IHC- Tumor Cells

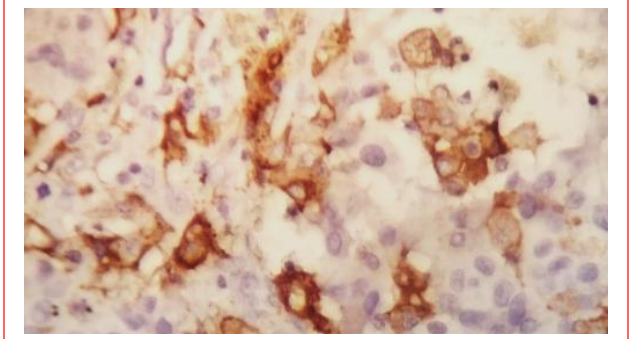


Figure 2

PD-L1 IHC- Immune Cells

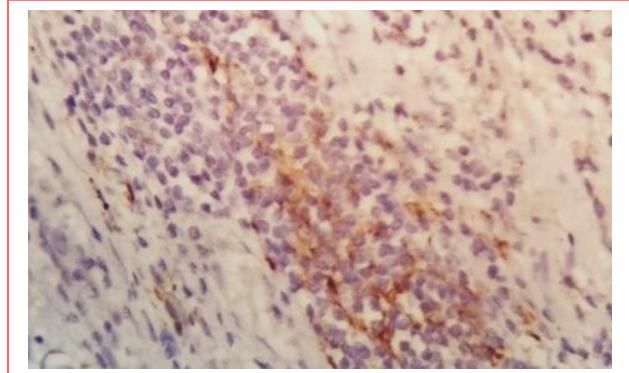


Figure 3