Liquid Biopsy

Test Description

The MolQ Liquid Biospy analyze somatic mutations in key theranostic genes associated with common cancers.

Patient Demographic

Name: Ms. Sunita Garg
Sex: Female
Date of Birth/Age: 47 years
Disease: Breast Carcinoma Triple Negative

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Dr AV Institute of Personalized Cancer Therapy and Research
Pathologist: Not Provided

Specimen

Booking ID: 012308290158
Specimen Acceptance: Plasma yielded ≥30ng DNA which is sufficient to proceed further with the test
Sample Type: Blood
Date of Collection: 29-08-2023
Date of Booking: 29-08-2023

CLINICAL SYNOPSIS

Known case of metastatic triple negative breast cancer (TNBC) [as per the clinical details provided in the Test Requisition Form]. She has been evaluated for mutations in the 117 genes listed in Appendix 2.

RESULTS

Clinically relevant variant(s) detected

TP53 p.Arg248Trp (MISSENSE)

AMP Classification^	CDS Variant Details	Interpretation	Treatment Recommendation	Treatment Response$
Tier II	c.742C>T (ENST00000269305.9)	Oncogenic	NA	Diagnostic

Gene	CDS Variant Details/ Nucleotide Change	Amino acid Change/ Exon No.	Variant Allele Depth/Total Depth	Variant Allele Frequency	Population MAF	*In-silico* Predictions	Function of the Gene
TP53	c.742C>T chr17:g.7674221G>A (ENST00000269305.9)	p.Arg248Trp / Exon 7	720/6404x	11.24%	NA (1000G); 0(gnomAD)	D(SIFT); D(LRT); PrD(Polyphen2)	Tumor Suppressor Gene

ARID1A p.Val719CysfsTer23 (FRAMESHIFT-DEL)

AMP Classification^	CDS Variant Details	Interpretation	Treatment Recommendation	Treatment Response$
Tier II	c.2154del (ENST00000324856.13)	Oncogenic	NA	NA

Gene	CDS Variant Details/ Nucleotide Change	Amino acid Change/ Exon No.	Variant Allele Depth/ Total Depth	Variant Allele Frequency	Population MAF	*In-silico* Predictions	Function of the Gene
ARID1A	c.2154del chr1:g.26761089del (ENST00000324856.13)	p.Val719CysfsTer23 / Exon 5	316/5917x	5.34%	NA (1000G); NA(gnomAD)	NA(SIFT); NA(LRT); NA(Polyphen2)	Tumor Suppressor Gene

^Refer to Glossary section for the classification criteria details.
$Drug Approvals are based on US-FDA Guidelines. Kindly refer to local guidelines if required.

ADDITIONAL BIOMARKERS DETECTED

This section provides information about variants that do not have any therapeutic value. However, these variants may or may not have a likely oncogenic effect.

MUTYH p.Leu411Arg

Gene	CDS Variant Details/ Nucleotide Change	Amino acid Change/ Exon No.	Alternate Allele Depth (x)	Allele Burden	Population MAF	*In-silico* Predictions	Function of the Gene
MUTYH	c.1232T>G chr1:g.45331427A>C (ENST00000456914 .7)	p.Leu411Arg / Exon 13	66x	1.06%	0 (1000G); 0(gnomAD)	T(SIFT); N(LRT); BN(Polyphen2)	Tumor Suppressor Gene

CLINICAL CORRELATION AND VARIANT INTERPRETATION

TP53 p.Arg248Trp

Gene Summary*: TP53* encodes the p53 tumor suppressor protein, a transcription factor that responds to cellular stresses, including DNA damage and oncogenic activation, by inducing downstream anti-tumor responses such as DNA repair and apoptosis[1]. *TP53* is the most commonly mutated gene in human cancers, and germline mutations occur in the cancer predisposition syndrome Li-Fraumeni[2].

Clinical and Therapeutic Relevance: In a study on 2433 primary breast tumors, 35.4% of patients harbored *TP53* mutations and *TP53* mutations were associated with higher grade in both ER+ (P<0.001) and ER− (P<0.001) tumors. *TP53* mutations were associated with worse outcome in ER+ (P=0.0001), but not in ER− disease. A recent study on 47 patients with breast cancer treated with Abemaciclib reported that patients with tumors harboring oncogenic *TP53* gene mutations were resistant to treatment[3]. In another study, 83 patients with metastatic breast cancer (HR+/HER2-) on CDK 4/6 inhibitor-based therapy harboring *TP53* mutations has shown shorter median progression-free survival as compared to those patients who did not harbor *TP53* mutations in their tumor[4]. In a study on *TP53* mutations in 145 patients with advanced cancers, it was identified that incidence of liver metastases was 69.2% vs. 43% (P=0.002) in mutated *TP53* and wild type I, respectively. The best progression-free survival on standard systemic therapy was significantly longer with Bevacizumab-containing regimens as compared to non-Bevacizumab containing regimen in patients with mutated *TP53* (median 11.0, n=22 vs. 4.0 months, n=35, P<0.0001)[5].

ARID1A p.Val719CysfsTer23

Gene Summary: The *ARID1A* gene provides instructions for making a protein that forms one piece (subunit) of several different SWI/SNF protein complexes. SWI/SNF complexes regulate gene activity (expression) by a process known as chromatin remodeling. Variants in the *ARID1A* gene have been found in many types of cancer, including cancers of the ovaries and lining of the uterus (endometrium) in women and cancers of the kidney, stomach, bladder, lung, breast, and brain.

Clinical and Therapeutic Relevance: Two different clinical studies reported a spectrum of mutations in *ARID1A* in different cancer types-pancreas, breast, colon, stomach, lung, prostate, brain, and blood (leukemias). These studies showed that decreased transcript levels of *ARID1A* are associated with poor prognosis in breast cancers[6,7]. In a study of 476 cases of breast cancer, patients with luminal A type disease with low ARID1A expression had significantly shorter disease-free and overall survival rates than patients with high ARID1A expression (p=0.022 and p=0.018, respectively)[8]. Clinical significance of this particular variant for the tumor type of the subject under investigation is not well documented in the literature.

CLINICAL TRIALS

The following trials are potentially best suited for your patient's indication, considering all reported treatment recommendations. See https://clinicaltrials.gov (clinical trials from NCT) or https://trialsearch.who.int (clinical trials from other registries) for more information.

Clinical trials in total: 0 **Trial countries**: IN-India, US-United States

S. No.	Title	Phase and ID	Intervention	Disease	Age & Sex
	No Clinical Trials				

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APPENDIX 1: TEST METHODOLOGY

BACKGROUND

This next-generation sequencing based assay covered complete coding regions of all guideline recommended actionable genes. The scope of this test is to assess cancer causing genomic alterations (SNVs, Indels) in 117 tumor agnostic genes in the circulating free DNA (cfDNA) isolated from blood plasma (liquid biopsy).

cfDNA comprises circulating tumor DNA (ctDNA) present in blood plasma that is shed from tumor tissue and is the source of tumor genetic material. Unlike traditional biopsy, liquid biopsy is noninvasive as it requires only a peripheral blood draw in Streck tube from the cancer patient. This test has several advantages over the traditional treatment management protocols in oncology including - (a) real-time treatment monitoring to evaluate the drug response in cancer patients, (b) early detection of acquired resistance mutations to targeted therapy, (c) detection of recurrence at early stages before significant accumulation of tumor cell mass, (d) identification of tumor heterogeneity arising due to multiple clones and hence the disease progression.

METHOD

cfDNA isolated from plasma was used to perform UMI-based target enrichment and sequencing using a custom capture kit. The QC passed libraries are sequenced to a minimum depth >20000X (pre-UMI) on validated Illumina sequencing platform and compressed to >2000X (post-UMI) for variant analysis. The sequences obtained were aligned to human reference genome (GRCh38/hg38) using BWA program[1,2]. Somatic mutations were identified using UMI corrected Sention pipeline [PMID: 31481971][3]. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. The mutations were annotated using reference laboratory in-house annotation pipeline (VariMAT). Reportable mutations are prioritized, classified and reported based AMP-ASCO-CAP guidelines[4].

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 are not reported.

This test was developed, and its performance characteristics were determined by Reference Laboratory.

- Analytical performance: A minimum of 30ng cfDNA isolated from plasma is considered as an acceptable criterion for proceeding with this testing.
- Analytical validation of this test in reference laboratory has shown sensitivity, and specificity of 100% at Limit of Detection at 0.25% VAF.
- Limit of Detection (LOD): Limit of Detection (LOD): The Limit of detection of the assay for somatic mutations is 0.25% for SNVs and short INDELs[5].

DISCLAIMER

- **Decisions regarding treatment action plan should not be solely based on these test results. These findings are highly recommended to be correlated with the patient's clinical, pathological, radiological and family history for decisions on diagnosis, prognosis, or therapeutics.**
- The therapy information provided in this report is based on FDA approved drugs data, NCCN guidelines, peer reviewed published literature, standard clinical databases, and strength of biomarker results till date. These therapies may or may not be suitable/beneficial to a particular patient. This clinical report summarizes potentially effective medications, potentially ineffective medications, and medications that may pose a higher risk of adverse reactions by mapping the patient's genetic alterations to the biomedical reference information. The report may also provide prognostic and diagnostic biomarkers detected or shown for the given disease context.
- The identification of a genomic biomarker does not necessarily imply pharmacological effectiveness or ineffectiveness. The medications identified by the treating physician may or may not be suitable for use on a particular patient. Thus, the clinical report does not guarantee that any particular agent will be effective in the treatment of any particular condition. Also, the absence of a treatment option does not determine the effectiveness or predict an ineffective or safety-relevant effect of a medication selected by the treating physician.
- The classification and clinically relevant information for the reported variants is based on peer-reviewed publications, public clinical databases, medical guidelines (NCCN, ASCO, AMP) or other publicly available information and it has been ensured that the information provided is up to date at the time of report generated, however continuous updates may happen in public domains. Also, the classification of variants can change based on the updated literature evidence. Re-analysis of the results can be requested at additional cost.

- This test is performed on the patient's cfDNA sample without a paired blood sample; therefore, it may include variations which may be of germline origin. However, this test is designed and validated for the detection and reporting of somatic genomic variants only and does not discriminate between germline and somatic variants. If clinically warranted, appropriate germline testing and genetic counselling for the patient should be considered for further evaluation.
- Detection of large insertions, deletions, copy number variations, gene rearrangements and deep intronic variations are beyond the scope of this test.
- This test has been validated at the reference laboratory and the limit of detection (LOD) of allele fraction for SNVs and short InDels is 0.25% VAF. However, the report may include, at the discretion of laboratory director, the variants with lower allele burden having strong or potential clinical significance or those have been reported earlier in the patient.
- Variants with <0.1% allele fraction and variants of uncertain significance with <0.25% allele fraction are not routinely reported. However, possibility of false negative or false positive below the limit of detection of this assay cannot be ruled out.
- **Additional case specific disclaimer:** None

AMP-ASCO-CAP CLASSIFICATION CRITERIA

Genetic test results are reported based on the somatic variant classification recommendations of College of American Pathologists (CAP)/American society for Clinical Oncology (ASCO)/Association of Molecular Pathologists (AMP)[4] as described in the table below:

Tier	Criteria
Tier I	Variants of strong clinical significance
Tier II	Variants of potential clinical significance
Tier III	Variants of unknown clinical significance
Tier IV	Benign or likely benign variants

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APPENDIX 2: LIST OF ANALYZED GENES

Complete coding regions of 71 genes are covered (Black font) and hotspot regions of 46 genes (Blue font) are covered in this panel. All genes that are diagnostically, prognostically and therapeutically significant according to the NCCN guidelines in multiple cancer types are completely covered in this test.

ABL1	*BRCA1*	*EGFR*	*FOXL2*	*KIT*	*NF1*	*PTCH1*	*SF3B1*
ABL2	*BRCA2*	*ERBB2*	*GATA3*	*KRAS*	*NF2*	*PTEN*	*SMAD4*
AKT1	*BRIP1*	*ERBB3*	*GNA11*	*MAP2K1*	*NOTCH1*	*PTPN11*	*SMARCB1*
ALK	*C11orf65*	*ERBB4*	*GNAQ*	*MAP2K2*	*NPM1*	*RAD51B*	*SMO*
APC	*CCND1*	*ERCC2*	*GNAS*	*MAPK1*	*NRAS*	*RAD51C*	*SPOP*
AR	*CDH1*	*ESR1*	*HNF1A*	*MET#*	*NTRK1*	*RAD51D*	*SRC*
ARAF	*CDK12*	*EZH2*	*HRAS*	*MLH1*	*NTRK3*	*RAD54L*	*STK11*
ARID1A	*CDK4*	*FANCL*	*IDH1*	*MPL*	*PALB2*	*RAF1*	*TERT*
ARID1B	*CDKN2A*	*FBXW7*	*IDH2*	*MSH2*	*PBRM1*	*RB1*	*TP53*
ATM	*CDX2*	*FGFR1*	*JAK1*	*MSH6*	*PDGFRA*	*RET*	*TSC1*
ATR	*CHEK1*	*FGFR2*	*JAK2*	*MTOR*	*PIK3CA*	*RHEB*	*TSC2*
ATRX	*CHEK2*	*FGFR3*	*JAK3*	*MUTYH*	*PMS2*	*RHOA*	*VHL*
BAP1	*CSF1R*	*FGFR4*	*KDM5C*	*MYC*	*POLD1*	*RIT1*	
BARD1	*CTNNB1*	*FLT3*	*KDM6A*	*MYCN*	*POLE*	*ROS1*	
BRAF	*DDR2*	*FOXA1*	*KEAP1*	*MYD88*	*PPP2R2A*	*SETD2*	

#Exon 14 skipping mutations