Liquid Biopsy

Test Description

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

Patient Demographic

Name: Ms Sapna Mandal
Sex: Female
Date of Birth/Age: 54 years
Disease: Non-small Cell Lung Cancer

Clinician

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

Specimen

Booking ID: 012204220159
Specimen Acceptance: Plasma yielded >30ng DNA which is sufficient to proceed further with the test
Sample Type: Blood
Date of Collection: 25-04-2022
Date of Booking: 25-04-2022

CLINICAL SYNOPSIS

Known case of non-small cell lung cancer, under Osimertinib [as per the clinical details provided in the Test Requisition Form]. She has been evaluated for mutations in the 56 genes listed in Appendix 2.

RESULTS

No clinically relevant mutation was detected in this subject

TABLE 1: GENOMIC ALTERATIONS THAT CAN BE TARGETED WITH APPROVED DRUGS IN THE SUBJECT'S TUMOR TYPE

Gene	CDS Variant Details	Amino acid Change/ Exon No.	Overall Depth/ Mutant Allele Percentage	FDA Approved Drugs Against Variant	Drug Response	Hot Spot Mutation	Function of the Gene in Cancer
			None				

TABLE 2: NON-DRUGGABLE/DRUGGABLE CLINICALLY SIGNIFICANT GENOMIC ALTERATIONS INDICATED IN OTHER TUMORS

Gene	CDS Variant Details	Amino acid Change/ Exon No.	Overall Depth/ Mutant Allele Percentage	Impact on Protein Function	Function of the Gene in Cancer	Pathways in Which the Gene Functions
			None			

TABLE 3: VARIANTS OF UNKNOWN SIGNIFICANCE

Gene	CDS Variant Details	Amino acid Change/ Exon No.	Overall Depth/ Mutant Allele Percentage	Function of the Gene in Cancer
USH2A	c.7390C>T (ENST00000366943.2)	p.pro2464Ser / Exon 39	1132X/ 45.4%	-

CLINICAL CORRELATION AND VARIANT INTERPRETATION

No clinically relevant mutation was detected in this subject.

ADDITIONAL FINDINGS

VARIANT OF UNKNOWN SIGNIFICANCE (VUS) IN GENES RELEVANT IN CANCER

Table 3 provides a list of VUS in genes known to function as oncogene or tumor suppressor or relevant in epigenetic regulation in human cancers. These variants specifically detected in this tumor have not been characterized sufficiently in biochemical assays and therefore their impact in this cancer remains speculative.

USH2A (p.Pro2464Ser)

A missense variation in exon 39 of the *USH2A* gene (**chr1:g.216074158G>A; Depth: 1132x**) that results in the amino acid substitution of Serine for Proline at codon 2464 (**p.Pro2464Ser; ENST00000366943.2**) was detected. The p.Pro2464Ser variant has not been reported in the 1000 genomes and gnomAD database. The observed variation lies in Fibronectin type III domain of the USH2A protein. The *in-silico* predictions of the variant are probably damaging by PolyPhen-2 (HumDiv), damaging by SIFT, damaging by LRT.
However, the clinical significance of this variant has not been well documented in the medical literature. Hence, this variant is reported as variant of unknown significance. Kindly correlate clinically.

The list of analyzed genes is given in Appendix 2.

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.

DISCLAIMER

Reference laboratory in-house validation data[15] has shown a false positives and false negatives in the range of 3-4% with this test. Therefore, it is recommended to correlate these findings with tissue testing results wherever applicable.

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 key 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.

The scope of this test is to assess the mutation of tumor somatic mutations from liquid biopsy: circulating tumor DNA, from the patient's plasma as the source of tumor genetic material. Liquid biopsy is an investigational / screening test. Unlike traditional biopsy which is an invasive procedure, liquid biopsy is non-invasive as it requires only a peripheral blood draw 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

ctDNA isolated from plasma was used to perform UMI-based target enrichment and sequencing using a custom capture kit. The libraries were sequenced 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 UMI corrected .clc pipeline and also 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 reference laboratory in-house annotation pipeline (VariMAT). Gene annotation of the variants was performed using VeP program[5] against the Ensembl release 90 human gene Model[6]. Clinically relevant mutations were annotated using published literature, databases and reference laboratory in-house propriety databases. The common variants were filtered for reporting based on the presence in various population databases (1000G, ExAC, EVS, 1000Japanese, dbSNP, UK10K, MedVarDb[7-12]. Reportable mutations are prioritized and prepared based AMP-ASCO-CAP guidelines[13] based on annotation metrics from OncoMD[14], 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 were determined by Reference Laboratory. It has not been cleared or approved by the FDA. As per ASCO guidelines this test has to be considered as investigatory test only[16].

DISCLAIMER

- This test is not a diagnostic test. It is a screening test and can be used as a treatment monitoring tool, which could help in assessment of treatment response and early recurrence.
- A false negative result due to the presence of mutations at low mutant allele fraction below the limit of detection (LOD) of this assay cannot be ruled out.
- A false negative result could also be due to inherent biology of the tumor, that it did not release enough mutant cell free DNA in circulation. In such cases, it is recommended to perform reflex testing on fresh tissue biopsy in order to decipher the mutation status of different genes.
- The classification of variants of unknown significance can change over time. Please contact MolQ laboratory at a later date for any change.
- Intronic variants are not assessed using this method.
- Large deletions, copy number variations and rearrangements cannot be assessed using this method. TREATMENT DECISIONS BASED ON THESE MUTATIONS MAY BE TAKEN IN CORRELATION WITH OTHER CLINICAL AND PATHOLOGICAL INFORMATION.
- All the genes in the panel have not been clinically validated, hence this report should be considered for research use only (RUO).

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APPENDIX 2: LIST OF ANALYZED GENES

AFF2	*BRCA2*	*ERBB3*	*IDH1*	*MAP2K1*	*NF2*	*PTEN*	*STK11*
AKT1	*CCND1*	*ERBB4*	*IDH2*	*MET*	*NOTCH1*	*RB1*	*SUFU*
ALK	*CDH1*	*ESR1*	*INPP4B*	*MLH1*	*NRAS*	*RET*	*TET2*
APC	*CDKN2A*	*FBXW7*	*JAK2*	*MSH2*	*NTRK1*	*ROS1*	*TP53*
AR	*CIC*	*FGFR1*	*KIT*	*MSH6*	*PDGFRA*	*SMAD4*	*TRAF7*
ATM	*CTNNB1*	*FGFR2*	*KLF4*	*MTOR*	*PIK3CA*	*SMARCA4*	*USH2A*
ATRX	*DDX3X*	*FUBP1*	*KMT2C*	*MUTYH*	*PMS2*	*SMARCB1*	*VEGFA*
BRAF	*EGFR*	*H3-3A*	*KMT2D*	*MYD88*	*PPARG*	*SMARCE1*	*VEGFB*
BRCA1	*ERBB2*	*HRAS*	*KRAS*	*MYO3A*	*PTCH1*	*SMO*	*VEGFC*