Liquid Biopsy

Test Description

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

Patient Demographic

Name: Mr. Jagdish Vainjani
Sex: Male
Date of Birth/Age: 73 years
Disease: Non-small cell lung carcinoma

Clinician

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

Specimen

Booking ID: 012304220089
Specimen Acceptance: Plasma yielded ≥30ng DNA which is sufficient to proceed further with the test
Sample Type: Blood
Date of Collection:22-04-2023
Date of Booking: 22-04-2023

CLINICAL SYNOPSIS

Known case of non-small cell lung carcinoma on Osimertinib [as per the clinical details provided in the Test Requisition Form]. He has been evaluated for mutations in the 117 genes listed in Appendix 2.

RESULTS

No clinically relevant variant was detected in this subject

AMP Classification^	CDS Variant Details	Interpretation	Treatment Recommendation	Treatment Response$
			No significant variant detected	

^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.

No other biomarkers that warrant to be reported was detected

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

Liquid Biopsy

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

Jatinder Kaur, PhD
Head, Molecular Biology & Genomics

Dr. Gulshan Yadav, MD
Head, Pathology

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

REFERENCES

1. Li, H, and R, Durbin. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 26.5: (2010): 589- Li H. and Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 2009. 25(14): 1754-1760.
2. Meyer L.R, et al. The UCSC Genome Browser database: extensions and updates 2013. Nucleic Acids Res., 2013. 41(D1): D64-69.
3. Kendig K, et al. Sentieon DNASeq Variant Calling Workflow Demonstrates Strong Computational Performance and Accuracy. Front Genet., 2019, 10:736.
4. Li M.M. et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017. 19 (1): 4-23.
5. Veldore, VH, et al. Validation of liquid biopsy: plasma cell-free DNA testing in clinical management of advanced non-small cell lung cancer. Lung Cancer: Targets and Therapy. 2018:9 1–11.

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*	