Test Description

The MolQ LiquiTrack® analyze somatic mutations in 56 theranostic genes associated with common cancers.

Patient Demographic

Name: Mr Ashish Kusum Dutta
Sex: Male
Date of Birth/Age: 57 years
Disease: Metastatic Lung Cancer

Clinician

Clinician Name: Dr Amit Verma
Medical Facility: Max Hospital
Pathologist: Not Provided

Specimen

Booking ID: 01012101280060
Specimen Acceptance: Plasma yielded >30ng DNA which is sufficient to proceed further with the test
Sample Type: Blood
Date of Collection: 28-01-2020
Date of Booking: 28-01-2020

CLINICAL SYNOPSIS

Lung cancer under evaluation [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

Clinically relevant actionable mutation was detected in *EGFR* gene of 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
EGFR	*c.2239_2256del (ENST00000275493)	p.Leu747_Ser752del / Exon 19	1623X / 1.9%	Osimertinib/ Gefitinib / Erlotinib / Afatinib/ Dacomitinib	Sensitive to EGFR TKIs	Yes	Oncogene

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
			None	

CLINICAL CORRELATION AND VARIANT INTERPRETATION

EGFR (p.Leu747_Ser752del) (Table 1):

Epidermal Growth Factor Receptor (EGFR) gene encodes a cellular transmembrane receptor tyrosine kinase. The activation of EGFR plays an important role in tumor growth, proliferation and metastasis.

An in-frame deletion (**chr7:g.55242469_55242486del; c.2239_2256del**) that resulted in the amino acid deletion at codon 747 (**p.Leu747_Ser752del**) was detected in the EGFR gene of this subject. This is a gain of function variant and has not been reported in ExAC and 1000 genomes databases.

EGFR exon 19 deletion mutations are observed in 48% of EGFR mutated Non-small-cell lung carcinoma (NSCLC) patients [PMID: 19922469]. Presence of in-frame deletions in exon 19 of EGFR gene are associated with responsiveness to EGFR tyrosine kinase inhibitors: Gefitinib, Erlotinib and Afatinib [PMID: 15118073, PMID:23982599].

The FDA has approved Osimertinib (Tagrisso) as a first-line treatment for patients with NSCLC with EGFR mutations (exon 19 deletions or exon 21 L858R substitution mutations) [https://www.fda.gov/drugs/resources-information-approved-drugs/osimertinib-tagrisso]. This approval was based on the FLAURA trial where 556 treatment-naïve patients with EGFR-positive locally advanced or metastatic NSCLC were randomly assigned to Osimertinib (n=279) or a standard TKI (Erlotinib or Gefitinib; n=277). Frontline Osimertinib treatment reduced the risk of progression or death by 54% versus standard TKI therapy. The median progression-free survival was 10.2 months (95% CI, 9.6-11.1) for standard therapy and 18.9 months (95% CI, 15.2-21.4) with Osimertinib (P<0.0001) [PMID: 33544337].

The FDA has approved Dacomitinib (VIZIMPRO) as a first-line treatment for patients with NSCLC with EGFR mutations (exon 19 deletions or exon 21 L858R substitution mutations) [https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-dacomitinib-metastatic-non-small-cell-lung-cancer]. This approval was based on the phase 3 randomized (ARCHER 1050) trial, which compared Dacomitinib versus Gefitinib as first line therapy for patients with sensitizing EGFR positive metastatic NSCLC (n=452) [PMID: 28958502]. This trial demonstrated a significant improvement in progression-free survival and no improvement in overall response rate or overall survival. The median progression-free survival was 14.7 and 9.2 months for Dacomitinib and Gefitinib respectively (hazard ratio 0.59; 95% CI: 0.47, 0.74; P<0.0001) [PMID: 28958502]. Kindly correlate clinically.

***This particular variant has been detected at paired read support less than 10, which is below the threshold criteria for routine variant reporting. Kindly correlate clinically.**

ADDITIONAL FINDINGS

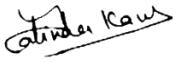
No other variant that warrants to be reported was detected.
The coverage of analyzed genes is given in Appendix 2.

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

Internal validation data[15] has shown a false positive/negative rate of 3-4% with this test. Also, 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 56 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 target enrichment and sequencing using a custom capture kit. The libraries were sequenced with a panel mean coverage depth of >20000X, 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 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. It is only a treatment monitoring tool, which could help in assessment of treatment response and early recurrence.
- The results of this test cannot be interpreted without the use of other assessment tools, including clinical history, other clinical examinations that includes imaging and laboratory analyses.
- A false negative result due to the presence of mutations with low mutant allele fraction below the limit of detection 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 that a reflex testing on fresh tissue biopsy is performed 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 or copy number variations cannot be assessed using this method.
- THIS TEST IS FOR INVESTIGATIONAL PURPOSE ONLY. TREATMENT DECISIONS BASED ON THESE MUTATIONS MAY BE TAKEN IN CORRELATION WITH OTHER CLINICAL AND PATHOLOGICAL INFORMATION.
- Only the exonic regions designed to detect the hotspots that are 100% covered (Appendix-2) are included in this analysis.

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. Wilm A. et al. LoFreq: A sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Res., 2012. 40(22): 11189-11201.
4. Li H. et al. The Sequence Alignment/Map format and SAM tools. Bioinformatics, 2009. 25(16): 2078-9.
5. McLaren W. et al. The Ensembl Variant Effect Predictor. Genome Biology, 2016. 17(1): 122.
6. Daniel R.Z. et al. Ensembl 2018, Nucleic Acids Res., 2018. 46(D1): D754-D761.
7. Auton A. et al. A global reference for human genetic variation. Nature, 2015. 526(7571): 68-74.
8. Lek M. et al. Analysis of Protein-Coding Genetic Variation in 60,706 Humans. Nature, 2016. 536(7616): 285-291.
9. NHLBI: https://esp.gs.washington.edu/drupal
10. Nagasaki M. et al. Rare Variant Discovery by Deep Whole-Genome Sequencing of 1,070 Japanese Individuals. Nature commun., 2015. 6: 8018.
11. Moayyeri A. et al. The UK Adult Twin Registry (TwinsUK Resource). Twin research and human genetics: the official journal of the International Society for Twin Studies. 2013. 16(1): 144-149.
12. dbSNP: http://www.ncbi.nlm.nih.gov/SNP/
13. 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 AmericanPathologists. J Mol Diagn. 2017. 19 (1): 4-23.
14. Bueno, R, et al. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nature Genetics, 2016. 48(4): 407-416.
15. 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.
16. Merker JD, et al. Circulating Tumor DNA Analysis in Patients with Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. J Clin Oncol. 2018 Mar 5: JCO2017768671.

APPENDIX 2: GENE LIST WITH COVERAGE

S. No.	Gene	Exon Number**	Coverage (%)
1.	*EGFR*	EX 3; EX 7; EX 15; EX 18: G719X; EX 19: DELETIONS; EXON 20: S768I, INSERTIONS, T790M; EX 21: L858R, L861Q	100.00
2.	*KRAS*	EX 2, 3, 4 [CODONS 12, 13, 61, 117, 146]	100.00
3.	*NRAS*	EX 2,3,4 [CODONS 12, 13, 61, 117, 146]	100.00
4.	*HRAS*	EX 2, 3 [CODONS 12, 13, 61]	100.00
5.	*BRAF*	EX 15/L597R/Q/S/V/V600E/K/L/R: K601E: G466V (EX 11)	100.00
6.	*MAP2K1/ MEK1*	EX 2,3,6,7,11 [F53L, I111S, C121S, P124S, P124L, E203K, P264S, N382H, Q56P, K57N, D67N]	100.00
7.	*PIK3CA*	EX 2; EX 5; EX 7; EX 8; EX 10: [E542X,E545X,Q546X,D549N]; EX 14; EX 19; EX 21 [H1047L/R]	100.00
8.	*AKT1*	EX3/E17K	100.00
9.	*PTEN*	EX 1, 2, 3, 4, 6, 7, 8, 9/EX 5: [R130G/*/Q/FS*4,R159S] EX 7: [R233*, K267FS*, P248FS*5], EX 8: [N323FS*2, N323FS*21]	100.00
10.	*KIT*	EX 2, 10, 15; EX 9: A493, Y 494;	100.00
	KIT	EX 11: W557R, V559A/D, V560D, L576P; EX 13: K642E,V654A; EX 14: T670I; EX17: D816H/V, D820X, N822X, Y823D,	100.00
	KIT	EX 18 [A829P]	100.00
11.	*PDGFRA*	EX 12: V561D; EX 14: N659K; EX 15; EX 18 WT	100.00
		EX 18: D846V	100.00
12.	*DDR2*	EX 18: S768R	100.00
13.	*ERBB2*	EXON 20 INSERTIONS (778-780 INS)	100.00
	ERBB2	EX 8(G309A); EX 19(L755S); EX 19 755 TO 759 DELETIONS; EX 19(D769H/Y); EX 20(V777L); EX 21(V842I);	100.00
14.	*IDH1*	EX 4 (R132X)	100.00
15.	*IDH2*	EX 4 (R172X), R140X	100.00
16.	*ALK*	EX 23 AND EX 25/F1174C/R1275Q/Y1278S	100.00
17.	*GNA11*	EX 4, 5 [R183C, Q209L/P]	100.00
18.	*GNAQ*	EX 4, 5 [R183Q, Q209L/P/R]	100.00
19.	*TSC1*	EX 15: E636FS	100.00
20.	*RET*	EX 10, 11(C634W/R/Y), 13,15, 16(M918T)	100.00
21.	*TP53*	ALL EXONS	100.00
22.	*VHL*	EX 1; EX 2; EX 3	100.00
23.	*APC*	EX 16	100.00
24.	*ATM*	EX 8,9,12,17,26,34,35,36,39,50,54,55,56,59,61,63	100.00
25.	*CDH1*	EX 3, 8, 9	100.00
26.	*CDKN2A*	EX 2	100.00
27.	*CSF1R*	EX 7, 22	100.00
28.	*CTNNB1*	EX 3/S37F/Y; S45P/F/Y	100.00
29.	*DNMT3A*	EX 23	100.00
30.	*ERBB4*	EX 3, 4, 6, 7, 8, 9, 15, 23	100.00
31.	*FBXW7*	EX 5, 8, 9, 10, 11	100.00
32.	*FGFR1*	EX 4, 7	100.00
33.	*FGFR2*	EX 5, 7, 10	100.00

34.	*FGFR3*	EX 7, 9, 14, 16, 18	100.00
35.	*FOXL2*	EX 1	100.00
36.	*GNAS*	EX 8 , EX 9	100.00
37.	*HNF1A*	EX 3, 4	100.00
38.	*KDR*	EX 6, 7, 11, 19, 21, 26, 27, 30	100.00
39.	*MET*	EX 2, 11, 14, 16, 19	100.00
40.	*MLH1*	EX 12	100.00
41.	*MSH6*	EX 4	100.00
42.	*PTPN11*	EX 3, 13	100.00
43.	*RB1*	EX 4, 6, 8, 10, 11, 14, 17, 18, 20, 21, 22, 23	100.00
44.	*STK11*	EX 1, 4, 6, 8	100.00
45.	*SMAD4*	EX 3,4,5,6,8,9,10,11,12	100.00
46.	*SMARCB1*	EX 2, 4, 5, 9	100.00
47.	*SMO*	EX 3, 5	100.00
48.	*SRC*	EX 14	100.00
49.	*ABL1*	EX 4, 5, 6, 7	100.00
50.	*EZH2*	EX 16	100.00
51.	*FLT3*	EX 11, 14, 16, 20	100.00
52.	*JAK2*	EX 14, 16	100.00
53.	*JAK3*	EX 10, 13, 14	100.00
54.	*MPL*	EX 10	100.00
55.	*NOTCH1*	EX 26, 27, 34	100.00
56.	*NPM1*	EX 11	100.00