Report Index

OncoRx Analysis Report

Patient & Sample details

Patient Name	*Mr. Vijay Kumar Bante*	***Age/Gender***	*45/M*
Sample ID	KHHSPTGPONC116	**Report Date**	18/09/2024
Sample Type	FFPE Tissue	**Biopsy number**	NH/348A/24
Referred Doctor	Dr. Jahananada	**UHID**	--
Cancer Type	Lung Adenocarcinoma		

Sequencing details

Sequencing	Somatic Testing
WGS/WES/Targeted Seq	Hybrid capture- Targeted Sequencing
Encoding	Illumina 1.9 / Novoseq6000
Total data generated	7.8 Gbp
Mean Depth	2009x
Alignment Rate	99.76%

METHODOLOGY OF THE TEST

A targeted Hybrid capture-based technology was performed to enable profiling cancer specific loci using The Next Generation Sequence (NGS) analyzer – Illumina's NovaSeq 6000. DNA was extracted from Formalin-Fixed Paraffin-Embedded (FFPE) tissue, quantified, fragmented and the library is constructed using Agilent Sureselect XT HS2 Library Preparation kit. Targeted Genes were captured using Sureselect XT HS2 target Enrichment kit and the library is sequenced at a high depth.

The raw data was checked for quality by fastQC and aligned to human reference genome GRCh38 considering the targeted regions from the panel. Various well validated tools such as FASTQC, Trimmomatic & Cutadapt, BOWTIE2, SAM Tools (with various utilities), IGV, PICARDS along with a few In-house programs are used to process, visualize and analyze the raw data. GATK is employed for structural variant identification and validation. The variants were annotated and filtered using the in-house and GoldenHelix analysis workflow implementing the AMP guidelines for interpretation of sequence variants. This includes comparison against the gnomAD population catalog of variants in 123,136 exomes, the 1000 Genomes Project Consortium's publication of 2,500 genomes, the NCBI ClinVar database of clinical assertions on variant's pathogenicity and multiple lines of computational evidence on conservation and functional impact. The test results are then carefully reviewed and manually curated by our team of highly trained and experienced individuals. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

Variant Assessment Process

The following databases and algorithms are used to annotate and evaluate the impact of the variant in the context of human disease: 1000 genomes, gnomAD, ClinVar, OMIM, dbSNP, NCBI RefSeq Genes, ExAC Gene Constraints, VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Analysis was reported using HGVS nomenclature (www.hgvs.org/mutnomen) as implemented by the VarSeq transcript annotation algorithm. The reported transcript matches that used most frequently by the clinical labs submitting to ClinVar.

SUMMARY OF GENOMIC BIOMARKER FINDINGS

Tumors' genetic makeup was analyzed by targeted genomic profiling of 53 genes.

POTENTIAL TREATMENT OPTIONS

I. **Response to FDA-approved targeted therapy drugs: Negative.**

II. **Rearrangements:** No clinically relevant rearrangements in the RET, NTRK genes which are associated with targeted therapies for Lung Adenocarcinoma.

Unlikely to benefit from TRK inhibitors such as Larotrectinib or Entrectinib.

I. Genomic findings with Evidence of Clinical Significance

Genomic Finding	Result	FDA-Approved Therapies in Patient's Tumor Type	Therapies of Potential Significance	Resistance
--	--	--	--	--

II. Negative Genomic findings

Biomarkers	Result	Associated Therapy; (Level of Evidence)	Efficacy	Potential Clinical Trials
NTRK FUSIONS	Negative	Larotrectinib + Entrectinib **(1A)**	**Ineffective**	-
RET FUSIONS	Negative	Selpercatinib **(1A)**	**Ineffective**	-
ALK Mutations	Negative	--	--	--
ROS1 Mutations	Negative	--	--	--

II. Other Somatic Variants

Genomic Finding	Variant Allele Frequency; Variant Allele Depth	Impact	Location	Classification	Evidence (Therapeutic/ Diagnostic And Prognostic)
FBXW7 Ser668Valfs*39	4.26%; 610x	Loss Of Function	Exon 14	Likely oncogenic	--
APC Ser1465Argfs*9	4.00%, 250x	Loss Of Function	Exon 16	Oncogenic	--
EGFR Thr363Ile	5.52%; 290x	Activating Mutation	Exon 9	Likely oncogenic	--
EGFR Glu746_Ala750del	47.13%; 157x	Activating Mutation	Exon 19	Oncogenic (Possibly Germline)	--
CDH1 Lys653Ter	4.95%; 101x	Loss Of Function	Exon 13	Likely oncogenic	

GENE DETAILS

FBXW7 Gene Summary:

FBXW7 (*F-box and WD repeat domain containing 7*) is a tumor suppressor that encodes a member of the F-box protein family which function in ubiquitination (PMID: 26416465, 31898225). *FBXW7* is a subunit of the SCF E3 ubiquitin ligase complex that targets proteins for degradation via the ubiquitin-proteasome system (PMID: 30086763, 30791487). Substrates of FBXW7 include oncoproteins involved in cell proliferation, survival, and differentiation, such as c-MYC, JUN, NOTCH1, mTOR, and cyclin-E (PMID: 30086763, 30791487, 34760892). Inactivation of *FBXW7* through mutation, deletion, or hypermethylation is associated with tumor development, proliferation, angiogenesis, and metastasis (PMID: 30086763, 30791487, 35346215, 35515121, 36104351). Somatic alterations in *FBXW7*, including missense mutations that disrupt substrate binding and truncating nonsense or frameshift mutations, have been reported in a variety of cancer types, including endometrial carcinoma, colorectal adenocarcinoma, and T-cell lymphoblastic leukemia (PMID: 30086763, 30791487, COSMIC, cBioPortal).

APC Gene Summary:

APC (*APC regulator of WNT signaling pathway*) is a member of the adenomatous polyposis coli gene family and a tumor suppressor gene that encodes APC (PMID: 31585108, 33105836). APC is a large multifunctional protein linked to numerous cellular processes that include proliferation and migration, cytoskeletal integrity, as well as DNA repair, and chromosomal segregation, and apoptosis (PMID: 31766836, 33105836). APC functions as a negative regulator of the Wnt/ β-catenin signaling pathway. APC loss occurs in numerous cancer types, either through inactivating mutations or promoter hypermethylation (PMID: 33105836, 33127962). This can lead to β-catenin dysregulation and translocation to the nucleus where β-catenin participates in activating expression of various genes that participate in cell proliferation, hyperplasia, and tumorigenesis, particularly in colorectal cancer (PMID: 30792186, 31706287, 33105836, 33597597). Germline mutations in *APC* are associated with familial adenomatous polyposis (FAP), and pose a strong predisposition for colorectal cancer (PMID: 28075483, 35988966). *APC* alterations have been identified most prominently in colorectal cancer, with a lower incidence in skin, prostate, and other cancers, mainly consisting of point mutations, of which a majority are nonsense/truncating substitutions (cBioPortal, COSMIC).

EGFR Gene Summary:

EGFR (*epidermal growth factor receptor*) is a proto-oncogene, that encodes a member of the EGFR/ErbB family of tyrosine kinases which also includes ERBB2 (HER2), ERBB3 (HER3), and ERBB4 (PMID: 18259690, 19208461, 22239438, 32219702).
EGFR is a cell surface transmembrane glycoprotein and is expressed in tissues of epithelial, mesenchymal, and neuronal origin. EGFR has an important role in regulating normal cellular processes including proliferation, differentiation, development, and survival (PMID: 14666659, 29991287, 32124699, 32593400, 35840984). Oncogenic *EGFR* amplification or mutations in the *EGFR* intracellular domain lead to EGFR overexpression and ligand-independent activation of downstream signaling pathways, resulting either directly or indirectly in increased cell proliferation, angiogenesis, invasion, and metastasis (PMID: 25870793, 29991287, 32069320, 35867821). Germline mutations in the *EGFR* kinase domain are associated with a predisposition to lung cancer (PMID: 30225213, 34670806). Somatic *EGFR* alterations have been reported in lung cancer, glioblastoma, and a variety of additional tumor types (cBioPortal, COSMIC). The most common oncogenic EGFR alterations are gene amplification and missense substitutions (cBioPortal, COSMIC).

CDH1 Gene Summary:

CDH1 (Cadherin 1) encodes e-cadherin (epithelial cadherin), a tumor suppressor gene of the cadherin superfamily (PMID: 29780167, 28507022). E-cadherin is a calcium-dependent cell adhesion protein and plays an essential role in tissue formation and epithelial cell behavior, including cell-cell adhesion, differentiation, cell signaling and polarity (PMID: 29780167, 28507022). Diminished e-cadherin activity caused by genetic or epigenetic changes contributes to cancer progression via increased cell proliferation, invasion, and metastasis (PMID: 29780167, 29279096, 32301282, 28507022). Germline loss-of-function mutation in *CDH1* is the principal cause of hereditary diffuse gastric cancer (HDGC), an autosomal dominant cancer

syndrome characterized by a high prevalence of diffuse gastric and lobular breast cancer (PMID: 32758476). Somatic alterations in *CDH1* are present in 3.13 % of all cancer (MyCancerGenome), with the highest incidence in the breast and gastric tissue (COSMIC).

APPENDIX: LIST OF BIOMARKERS SCREENED

ABL1	***AKT1***	***ALK***	***APC***	***ATN***	***BRAF***
CDH1	***CDKN2A***	***CTNNB1***	***DDR2***	***DNMT3A***	***EZH2***
EGFR	***ERBB2***	***ERBB4***	***FBXW7***	***FGFR1***	***FGFR2***
FGFR3	***FOXL4***	***GNA11***	***GNAQ***	***GNAS***	***HNF1A***
HRAS	***IDH1***	***IDH2***	***JAK2***	***JAK3***	***KDR***
KIT	***KRAS***	***MAP2K1***	***MET***	***MLH1***	***MSH6***
NOTCH1	***NPM1***	***NRAS***	***PDGFRA***	***PIK3CA***	***PTEN***
PTPN11	***RB1***	***RET***	***ROS1***	***SMAD4***	***SMARCB1***
SMO	***SRC***	***STK11***	***TP53***	***TSC1***	

Intended Use

Tumor is a hub for genetic mutations and hence though the cancers known to date are the same, every tumor is genetically diverse. We decipher the FDA approved molecular biomarkers of the tumors to personalize cancer treatment and increase the survival times and Quality of Life of cancer patients through targeted therapies. One such step forward is OncoRx for precision oncology which identifies molecular targets and recommends tailored therapies for patients with cancer.

OncoRx Condition specific is for prescription use only. The gene targets tested play an important role in the pathogenesis of the disease and aid in patient selection. This test is intended for use as a directional test for drug decision making of approved therapeutic product labeling and helps in choosing the right drug to prevent treatment failure and side effects. It also assists in predicting outcomes of a drug - response and resistance, immunotherapy selection and management.

Test description

This Next-Generation Sequencing (NGS) based invitro diagnostic genomic test targets DNA variants from FFPE tumor tissue sample, will decipher the genomic information including the regulatory regions, SNP, indels, fusions and copy number alterations of targeted cancer genes that are candidate cancer biomarkers with proven evidences from various clinical trials. A few of these cancer genes have potential clinical significance, emerging therapeutic evidences and are being tested at various trials for approved agents and drugs in clinical development.

Test principle

The panel works on a principle of probe hybridization using DNA probes of around 120mer length providing high and specific interactions with target DNA molecules along with - dual molecular barcodes with dual sample indexing for library preparation. The usage of these dual molecular barcodes reduces or eliminates the index hopping and false positives thus making it more reliable.

Precision

High performance standards, regulatory compliance, and quality control are ensured with automated protocols and pipelines. The test results are represented in two parts: companion diagnostic biomarker results with associated therapy indication and other biomarkers with full proof scientific evidences / biomarker results. Accuracy in targeted drug response prediction is an exclusive feature of OncoRx.

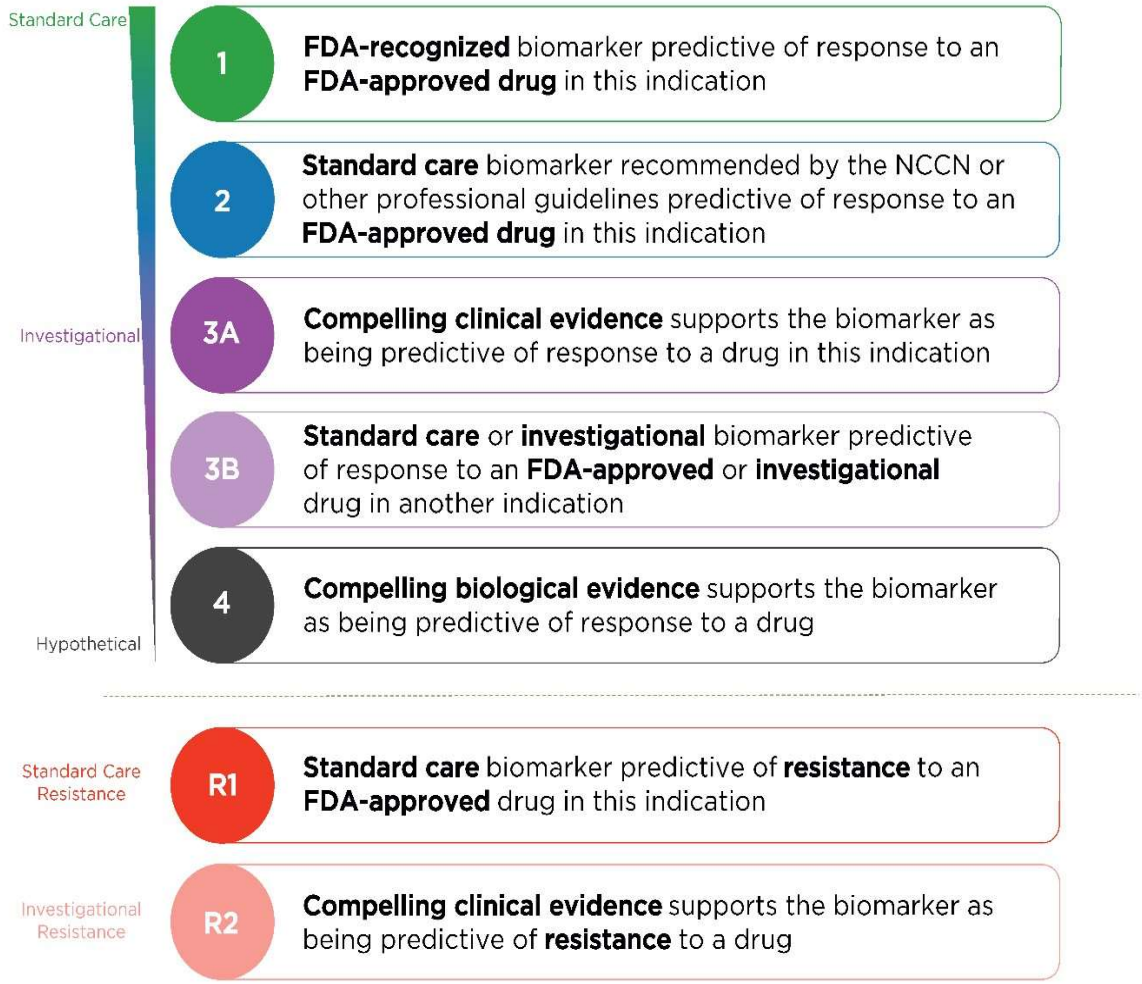
Coverage and Sequence data guidelines

The NGS test is performed with >95% coverage and at a higher depth with Q30 > 90%. Recommended read length of 2 x 150 bps is considered for targeted panel sequencing and paired end reads are considered for analysis.

Sensitivity and specificity

OncoRx pipeline for the targeted panel has been validated for repeatability and reproducibility of alterations with >150 datasets covering various cancers. The raw files for the validation and their related information have been considered from the European Nucleotide Archive (ENA). Repeatability and reproducibility in terms of variant calling has been tested with in-house pipeline and validated commercial pipelines. Across all samples, the pre-sequencing process failure rate is found to be ≤ 1.5%.

OncoKB™ Therapeutic Level of Evidence



Limitations and Disclaimer

The genomic variations reported may include somatic and germline variations; but does not distinguish between the two alterations.

The NGS test is performed with >95% coverage. For any minor alleles or rare alleles, a negative result does not rule out the presence of a mutation due to rare cases of tumor cell purity, intra tumor heterogeneity.

For in vitro research use only. This test must be ordered by a qualified medical professional in accordance with required medical regulations in oncology for patients with solid malignant neoplasms.

Patient care treatment decisions must be based on the self-determining medical judgment of the respective physician. Do consider complete information of the patient such as patient preferences, medical history and family history, physical examination profiles, other lab results in accordance with the standard of care medical practice.

The reported results are for the information of the referring doctor only.

It should be noted that this test is restricted to a limited number of genes and does not include all intronic and non-coding regions. This report only includes variants that meet a level of evidence threshold for cause or contribute to disease. Certain classes of genomic variants are also not covered using the NGS testing technology, including triplet repeat expansions, or other complex structural rearrangements. More evidence for disease association of genes and causal pathogenic variants are discovered every year, and it is recommended that genetic variants are re-interpreted with updated software and annotations periodically.

In case of ERBB2/ HER2 amplification with copy number 4 (baseline ploidy of tumor +2) and FGFR1 in breast cancer FFPE sample, dual analyte reflex testing with FISH or RNA profiling is recommended for confirmation of amplification.

HER2 overexpression needs to be studied in samples identified with HER2 Copy count 4 (which would be mentioned) for validation and reporting.

Clinical performance of Tagrisso® (osimertinib) in patients with an EGFR exon 20 T790M mutation detected with an allele fraction <5% is ongoing and hence is not an established companion diagnostics biomarker.

Genetic variations at allele frequencies below the established limit of detection may not be detected consistently.

For confirmed germline predisposition of Homologous Recombination Repair (HRR) mutations if any, it is generally recommended to get a re-test with blood sample.

Contraindication

There are no known contraindications.

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This test was developed, and its performance characteristics were determined by GenepoweRx. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. These interpretations are based on ACMG/AMP GATK, CPIC Guidelines. This test can be used for clinical screening and research purposes only. The test is registered in CDSCO, India, as Uppalu-Hyder-TE/M/IVD/008407, Class C of Medical Devices, 2022.

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This report is provided as an information source for clinicians and is not intended it to be considered a substitute for professional medical advice. If you have or suspect you have a medical problem, contact your physician for personalized treatment or therapy. The translation of genomics knowledge and data into the current report requires generalization and continuous expansion of genomic insights from literature. Hence, minor errors are anticipated. This genomic report and the usefulness of the information provided in the report shall not warranty or hold any liability or responsibility for any direct, indirect, incidental, or consequential indemnities arising out of the use of or inability to use the information. This report makes no promises or guarantees that the reported condition/s will develop at any time. Other genetic, environmental, and clinical factors might influence the patients' phenotypic response to the condition. Proper understanding of the risks aids in better management of metabolic conditions with traditional therapies or other treatment options and helps in the prevention/delay of the disease or makes it less harmful.

Genetic testing plays a key role in the diagnosis of the root cause of a disease or condition. It provides excellent guidance in deciding the right medical regimen. But sometimes, a few non-treatable variants are also identified. Not all genetic changes affect health. It is difficult to know whether identified variants are involved in the condition of interest. Sometimes, an identified variant is associated with a different genetic disorder that has not yet been diagnosed (these are called incidental or secondary findings). A finding of biomarker alteration does not necessarily indicate the pharmacologic effectiveness (or lack thereof) of any drug or treatment regimen; a finding of no biomarker alteration does not necessarily indicate the lack of pharmacologic effectiveness (or effectiveness) of any drug or treatment. No Guarantee of Clinical Benefit: This Report makes no promises or guarantees that a drug will be effective in the treatment of disease in any patient. This Report also makes no promises or guarantees that a drug with a potential lack of clinical benefit will, in fact, provide no clinical benefit. It is possible that a pathogenic variant is present in a gene that was not selected for analysis and /or interpretation in cases where insufficient phenotypic information is available. Due to inherent technology limitations and constant upgradation of research and literature, not all bases of the exome can be covered. Accordingly, variants in regions of insufficient coverage may not be identified and/or interpreted. Therefore, it is possible that pathogenic variants are present in one or more of the genes analyzed but have not been detected. The variants not detected by the assay that was performed may impact the phenotype. For in vitro research use only. This test must be ordered by a qualified medical professional in accordance with required medical regulations.

Patient care treatment decisions must be based on the self-determining medical judgment of the respective physician. Do consider complete patient information such as patient preferences, medical history and family history, physical examination profiles, and other lab results per the standard of care medical practice. The reported results are for the information of the referring doctor only. It should be noted that this test is restricted to a limited number of genes and does not include all intronic and non-coding regions. This report only includes variants that meet a level of evidence threshold for cause or contribute to disease. More evidence for disease association of genes and causal pathogenic variants is discovered every year, and it is recommended that genetic variants are re-interpreted with updated software and annotations periodically.