LABORATORY REPORT

Reg. No	:	**30100200078**	Reg. Date	:	15-Jan-2023 11:07
Name	:	**MAKHANLAL SATNALIWALA**	Collected on	:	15-Jan-2023 11:07
Sex/Age	:	**Male / 85 Years**	Approved Date	:	30-Jan-2023 17:38
Ref. By	:		Tele. No	:	9833253102
Location	:	LILAC INSIGHTS PVT. LTD. @ MUMBAI	Dispatch At	:	

Please find detailed report of NGS 546 Gene Oncomine Comprehensive Assay Plus (DNA mutations, CNVs, RNA Fusions, MSI, TMB, HRR) in the following pages.

---------- End Of Report ----------

Neeraj Arora

Dr. Neeraj Arora
M.D (Path), PDF (Mol Haemat),
PDF (Haematopath)
22396

Patient Name	**MAKHANLAL SATNALIWALA**	**Sample Id/LabID**	**30100200078**
Gender	Male	**DOB/AGE**	85 yrs
Ref.By	LILAC INSIGHTS PVT. LTD.	**Date of Sample Collection**	15/01/2023
Sample Type	FFPE BLOCK:H-143-22/B3	**Date of Receipt**	15/01/2023
Tumor Cellularity	60%	**Date of Report**	30/01/2023

NGS 546 Gene Oncomine Comprehensive Assay Plus (DNA mutations, CNVs, RNA Fusions, MSI, TMB, HRR)

Clinical Details:

Known case of Ca Prostate (In Progression)

Result Summary:

Alteration Description	Findings
Mutations (SNVs and Indels)	**PIK3CA, HRAS, KMT2D**
Novel and known fusions identified	**TMPRSS2-ERG**
Copy number variants (CNVs)	**Not Identified**
Loss of Heterozygosity (LOH)	**0%**
Tumor mutational burden (TMB)- Status	**LOW**
TMB Score (Mutation/mb)	**9.49**
Microsatellite instability (MSI) -Status	**MSI STABLE-(MSS)**
MSI Score	**19.15**

Variants Identified:

Table-1:SNV Identified

Gene/Transcript	Locus	Variant/Amino Acid Change	Total Coverage/VAF	Impact on Protein Function	Variant classification	TIER classification
PIK3CA NM_006218.4	Chr3:178952085	c.3140A>G p.His1047Arg	1999X 10.60%	Gain-of-Function	**Pathogenic**	**Tier IIC**
HRAS NM_001130442.2	Chr11:533499	c.404G>A p.Arg135Gln	917X 53.54%	-	**Variant of Uncertain Significance**	**Tier IIC**
KMT2D NM_003482.4	Chr12:49444379	c.2992C>T p.Pro998Ser	935X 48.23%	-	**Variant of Uncertain Significance**	**None**

Table-2:Fusion Identified

Gene/Transcript	Locus	Coverage/VAF	Impact on Protein Function	Variant type	TIER classification
TMPRSS2(2) – ERG(4)	chr21:42870046 – chr21:39817544	25250	Gain-of-function	Fusion	**None**

Variants Description:

PIK3CA:c.3140A>G:p.His1047Arg: Pathogenic: The p.His1047Arg variant (also known as c.3140A>G), was detected in PIK3CA gene on chromosome 3 at position 178952085 with variant allele frequency of 10.60% (represented by 212 reads). This heterozygous mutation is having a total depth of 1999X. It is located at exon 21 of NM_006218.4 transcript and was found to change amino acid, Histidine to Arginine at codon 1047. It leads to Gain-of-Function. It is a hotspot variant. It is represented by rs121913279 in dbSNP and COSM775 in Cosmic database. It is interpreted pathogenic according to ClinVar database[VCV000013652]. It is predicted as deleterious by SIFT, polyphen2 and MutationTaster2 which are an in-silico DNA variant effect prediction tool. This variant was found in the population frequency database like gnomAD and ExAC at global minor allele frequency of 0.0004028% and 0.0008317% respectively.
Note: PIK3CA mutation correlates with poor prostate cancer prognosis.(PMID: 29581176).

HRAS:c.404G>A:p.Arg135Gln: Variant of Uncertain Significance: The p.Arg135Gln variant (also known as c.404G>A), was detected in HRAS gene on chromosome 11 at position 533499 with variant allele frequency of 53.54% (represented by 491 reads). This heterozygous mutation is having a total depth of 917X. It is located at exon 4 of NM_001130442.2 transcript and was found to change amino acid, Arginine to Glutamine at codon 135. It is represented by rs1473091760 in dbSNP database and COSM7609609 in Cosmic database. It is interpreted as uncertain significance according to ClinVar database[VCV001002072]. It is identified as deleterious by SIFT, FATHMM, MutationTaster2, however it is predicted as benign by Polyphen2, which are an in-silico DNA variant effect prediction tool. This variant was found in the population frequency databases like GnomAD exome, with global allele frequency of 0.0012%.

KMT2D:c.2992C>T:p.Pro998Ser: Variant of Uncertain Significance: The p.Pro998Ser variant (also known as c.2992C>T), was detected in KMT2D gene on chromosome 12 at position 49444379 with variant allele frequency of 48.23% (represented by 451 reads). This heterozygous mutation is having a total depth of 935X. It is located at exon 12 of NM_003482.4 transcript and was found to change amino acid, Proline to Serine at codon 998. It is represented by rs143711798 in dbSNP database. It is interpreted uncertain significance according to ClinVar database[VCV000547412]. It is predicted as deleterious by SIFT, polyphen2 and MutationTaster2 which are an in-silico DNA variant effect prediction tool. This variant was not found in the population frequency database like gnomAD, ExAC and 1000G database.

TMPRSS2(2) – ERG(4) Fusion: This fusion was detected between exon 2 of TMPRSS2 and exon 4 of ERG gene located at genomic position 42870046 of chromosome 21 and position 39817544 of chromosome 21 respectively. This fusion is supported by 25250 reads. This fusion is represented as TMPRSS2(2) – ERG(4). It results in Gain-of-Function.
Prostate cancer is a major malignancy in males. TMPRSS2-ERG is a high-frequency fusion gene expressed in prostate cancer and plays a vital role in carcinogenesis. Recent studies showed that TMPRSS2-ERG is a potential predictive biomarker for prostate cancer. However, the predictive value of TMPRSS2-ERG fusion is yet unclear(PMID: 30459527, PMID: 17965219).

Biomarker Descriptions

ERG (ETS transcription factor ERG)

Background: The ERG gene encodes the erythroblast transformation-specific (ETS) transcription factor ERG, which belongs to the ETS family of transcriptional regulators that are involved in embryonic development, cell proliferation, differentiation, angiogenesis, inflammation, and apoptosis[1].

Alterations and prevalence: ERG gene fusions are the most common molecular subtype of prostate cancer and are present in over 30% of cases[2,3,4]. ERG fusions to the androgen-regulated TMPRSS2 promoter occur in over 90% of prostate cancer with ERG rearrangements. The fusion of ERG to EWSR1 is a common abnormality in Ewing sarcoma and is observed in 5% of cases[5].

Biomarker Descriptions (continued)

Potential relevance: Currently, no therapies are approved for ERG aberrations. The t(21;22)(q22;q12) and t(16;21)(p11;q22) translocations resulting in EWSR1-ERG and FUS-ERG fusions, respectively, are useful as ancillary diagnostic markers in Ewing sarcoma/peripheral neuroectodermal tumors[6,7]. TMPRSS2-ERG fusions overexpress ERG and are associated with poor prognosis as well as tumor aggressiveness in prostate cancer[2,8]. Because TMPRSS2 is involved in androgen regulation, TMPRSS2-ERG expression in prostate cancer tends to be positively correlated with androgen receptor (AR) overexpression[9]. Therapies for treating advanced prostate cancer often involve androgen deprivation, which in turn leads to a reduction of TMPRSS2-ERG expression. However, acquired resistance to these therapies restores androgen signaling and TMPRSS2-ERG expression[10].

HRAS (HRas proto-oncogene, GTPase)

Background: The HRAS proto-oncogene encodes a GTPase that functions in signal transduction and is a member of the RAS superfamily which also includes KRAS and NRAS. RAS proteins mediate the transmission of growth signals from the cell surface to the nucleus via the PI3K/AKT/MTOR and RAS/RAF/MEK/ERK pathways that control the regulation of cell division, differentiation, and survival[11,12,13]. RAS proteins require the covalent attachment of a hydrophobic group to their C-terminus (prenylation) for membrane localization and downstream signaling[14]. Whereas KRAS and NRAS are subject to prenylation by farnesyl transferase or geranylgeranyl transferase, HRAS is completely dependent on farnesylation.

Alterations and prevalence: Recurrent mutations in RAS oncogenes cause constitutive activation and are found in 20-30% of cancers. HRAS mutations are observed in 4-10% of pheochromocytoma and paraganglioma, thymoma, bladder, and head and neck cancers[15,16]. The majority of HRAS mutations consist of point mutations at G12, G13, and Q61[15,17,18].

Potential relevance: Currently, no therapies are approved for HRAS aberrations. However, the farnesyl transferase inhibitor, tipifarnib[19,20], was granted fast-track (2019) and breakthrough therapy (2021) designation by the FDA for the treatment of HRAS mutant head and neck squamous cell carcinomas (HNSCC) after disease progression on platinum chemotherapy.

KMT2D (lysine methyltransferase 2D)

Background: The KMT2D gene encodes the lysine methyltransferase 2D protein, a transcriptional coactivator and histone H3 lysine 4 (H3K4) methyltransferase[1]. KMT2D belongs to the SET domain protein methyltransferase superfamily[21]. KMT2D is known to be involved in the regulation of cell differentiation, metabolism, and tumor suppression due to its methyltransferase activity[21]. Mutations or deletions in the enzymatic SET domain of KMT2D are believed to result in loss of function and may contribute to defective enhancer regulation and altered gene expression[21].

Alterations and prevalence: Somatic mutations in KMT2D are predominantly missense or truncating and are observed in 29% of diffuse large B-cell lymphoma (DLBCL), 28% of bladder urothelial carcinoma, 27% of uterine corpus endometrial carcinoma, 22% of lung squamous cell carcinoma, 21% of skin cutaneous melanoma, 17% of stomach adenocarcinoma, 15% of head and neck squamous cell carcinoma, and 14% of cervical squamous cell carcinoma[4,15].

Potential relevance: Currently, no therapies are approved for KMT2D aberrations.

PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha)

Background: The PIK3CA gene encodes the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha of the class I phosphatidylinositol 3-kinase (PI3K) enzyme[22]. PI3K is a heterodimer that contains a p85 regulatory subunit, which couples one of four p110 catalytic subunits to activated tyrosine protein kinases[23,24]. The p110 catalytic subunits include p110α, β, δ, γ and are encoded by genes PIK3CA, PIK3CB, PIK3CD, and PIK3CG, respectively[23]. PI3K catalyzes the conversion of phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2) into phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3) while the phosphatase and tensin homolog (PTEN) catalyzes the reverse reaction[25,26]. The reversible phosphorylation of inositol lipids regulates diverse aspects of cell growth and metabolism[25,26,27,28]. Recurrent somatic alterations in PIK3CA are frequent in cancer and result in the activation of PI3K/AKT/MTOR pathway, which can influence several hallmarks of cancer including cell proliferation, apoptosis, cancer cell metabolism and invasion, and genetic instability[29,30,31].

Alterations and prevalence: Recurrent somatic activating mutations in PIK3CA are common in diverse cancers and are observed in 20-30% of breast, cervical, and uterine cancers and 10-20% of bladder, gastric, head and neck, and colorectal cancers[4,15]. Activating mutations in PIK3CA commonly occur in exons 10 and 21 (previously referred to as exons 9 and 20 due to exon 1 being untranslated)[32,33]. These mutations typically cluster in the exon 10 helical (codons E542/E545) and exon 21 kinase (codon H1047) domains, each having distinct mechanisms of activation[34,35,36]. PIK3CA resides in the 3q26 cytoband, a region frequently amplified

Biomarker Descriptions (continued)

(10-30%) in diverse cancers including squamous carcinomas of the lung, cervix, head and neck, and esophagus, and in serous ovarian and uterine cancers[4,15].

Potential relevance: The PI3K inhibitor, alpelisib[37], is FDA approved (2019) in combination with fulvestrant for the treatment of patients with PIK3CA-mutated, hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, advanced or metastatic breast cancer. Additionally, a phase Ib study of alpelisib with letrozole in patients with metastatic estrogen receptor (ER)-positive breast cancer, the clinical benefit rate, defined as lack of disease progression ≥ 6 months, was 44% (7/16) in PIK3CA-mutated tumors and 20% (2/20) in PIK3CA wild-type tumors[38]. Specifically, exon 20 H1047R mutations were associated with more durable clinical responses in comparison to exon 9 E545K mutations[38]. However, alpelisib did not improve response when administered with letrozole in patients with ER+ early breast cancer with PIK3CA mutations[39]. Case studies with MTOR inhibitors sirolimus and temsirolimus report isolated cases of clinical response in PIK3CA mutated refractory cancers[40,41].

Comments:

Note: PIK3CA mutation correlates with poor prostate cancer prognosis and causes prostate cancer in mice. Moreover, PIK3CA mutation and PTEN loss coexist in prostate cancer and can cooperate in vivo to accelerate tumorigenesis and facilitate CRPC.(PMID: 29581176).

Prostate cancer is a major malignancy in males. TMPRSS2-ERG is a high-frequency fusion gene expressed in prostate cancer and plays a vital role in carcinogenesis. Recent studies showed that TMPRSS2-ERG is a potential predictive biomarker for prostate cancer. However, the predictive value of TMPRSS2-ERG fusion is yet unclear.(PMID: 30459527)

Benign and likely benign variants identified are not reported. These findings should be correlated with other clinical and laboratory tests for a definite conclusive interpretation.

Homologous Recombination Repair genes:

Homologous recombination repair (HRR) pathway genes play vital role in maintaining genome stability and tumor suppression. Alterations in HRR gene can lead to genome instability which in turn may increase the risk of developing tumors. Thus knowing the alterations in HRR genes can act as potential biomarker to decide the personalized therapy to be undertaken.
Note: From the total of 46 HRR genes (including BRCA1 and BRCA2) covered in Oncomine Comprehensive Assay Plus, No alterations Identified.

HRR Details

Gene/Genomic Alteration	Finding
LOH percentage	***0.0%***

Homologous recombination repair (HRR) genes were defined from published evidence in relevant therapies, clinical guidelines, as well as clinical trials, and include - BRCA1, BRCA2, ATM, BARD1, BLM, BRIP1, CDK12, CHEK1, CHEK2, FANCL, NBN, PALB2, POLD1, POLE, PPP2R2A, RAD51B, RAD51C, RAD51D, and RAD54L.

Tumor Mutation Burden:

TMB result in this sample is 9.49 mut/mb, considered as LOW.

Tumor mutational burden (TMB), which is the measured as the total number of somatic mutation per Mb of a tumour genome, is a promising emerging biomarker for cancer immunotherapy across many cancer types. For calculating TMB, only non-synonymous changes are considered whereas synonymous and germline variants are discarded, as it is assumed that these variants are not likely to be directly involved in creating neoantigen. The higher number of mutations in a tumor (TMB of 10 mut/Mb or greater (called TMB-high) can also be associated with a greater probability of response to drugs called immune checkpoint inhibitors that help activate the immune system to better recognize cancer cells and thus provide survival benefit. **TMB score of <10 is considered low, >10 & <15 is considered intermediate and >15 is considered high.**

Microsatellite Instability(MSI):

MSI Results for this sample is: MSI-STABLE(MSS), MSI Score: 19.15

During cell division, the number and length of microsatellite is maintained in normal cells with the help of mismatch repair (MMR) system. However, loss in function of member of the DNA mismatch repair gene can lead to alteration in repeated bases in microsatellites during cell division known as Microsatellite Instability (MSI).Based on the frequency of MSI, it can be categorized into microsatellite stability (MSS), and high microsatellite instability - MSI-H.MSI score of <25 is considered MSS and >25 is considered high(MSI-H).Here in this panel around 76 markers are used for MSI calculation.

Loss of Heterozygosity (LOH):

LOH % for this sample is - 0%

LOH % is a measure of specific type of mutation which leads to loss of one normal copy of gene (heterozygosity) or simultaneous duplication of the remaining allele (copy neutral LOH) or group of genes. LOH in certain types of genes like tumor suppressor genes can lead to instability of genome. This assay can detect LOH at gene and sample level both. LOH is a very common event in the process of oncogenesis and thus can be utilized as biomarker of cancer risk or the prediction of clinical outcomes in certain types of tumors. This assay detect LOH for a subset of 20 genes from total 46 HRR genes.

Relevant Biomarkers

Tier	Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
IIC	*PIK3CA p.(H1047R) c.3140A>G* Allele Frequency: 10.61%	None	**alpelisib + hormone therapy** [1, 2]	24
IIC	*HRAS p.(R135Q) c.404G>A* Allele Frequency: 53.54%	None	None	14

Public data sources included in relevant therapies: FDA1, NCCN, **EMA**2, ESMO
Tier Reference: Li 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 Jan;19(1):4-23.

Methodology

Nucleic acid (DNA/RNA) was extracted from FFPE tissue sample, using standard Qiagen nucleic acid isolation kits. Briefly, 20ng of DNA/RNA was amplified using Oncomine Comprehensive Assay plus kit as per instruction manual and sequencing was performed using Ion S5 platform as per user manual. The sequencing reads QC, mapping on hg19 human reference genome, variant calling (SNVs, small InDels, CNVs) and annotation was carried out with IonReporter™ (IR) Software 5.18.2.0. Latter uses RefSeq database was used for the identification and characterization of genes-associated variants. The annotation for variants was derived using various diseases databases like dbSNP, ClinVar. The population frequency information from 1000 genomes, ExAC, GnomAD and ESP was used for the elimination of common variants/polymorphism.For prediction of the possible impact of coding non-synonymous SNVs on the structure and function of protein, PolyPhen-2 and SIFT score was used. Further Oncomine Reporter software was used for annotating variants with a curated list of relevant labels, guidelines, and global clinical trials. Oncomine Comprehensive plus assay will analyze across >500 genes(SNVs,Indels,CNVs,Fusions), plus key immuno-oncology research biomarkers like tumor mutational burden (TMB), microsatellite instability (MSI), and Homologous Recombination Repair genes (HRR).

Run QC statistics
Sample is sequenced at Average base coverage depth of 1,661. The Target base coverage at 500X is 91.52%.

Variant Classification

- **Pathogenic**: The pathogenic variant are the one which is believed to account for the symptoms. It increases an individual's susceptibility or predisposition to a certain disease or disorder. This mutation is always included in results section of report.
- **Likely Pathogenic**: The likely pathogenic variant are the one which most likely have harmful effect but, there is insufficient evidence that a variant is the definite cause for symptoms. This mutation is always included in results section of report
- **Variant of Uncertain Significance**: The Variant of Uncertain Significance (VUS) are the one which have limited and/or conflicting evidence regarding pathogenicity. Its exact effect on gene function is not known. With more information available over time, a VUS may be reclassified as likely pathogenic or likely benign. This mutation is always included in results section of report.
- **Likely benign**: The likely benign variants are the one which are most likely not associated with disease risk. However, additional evidence is needed to confirm this assertion. This mutation is not included in report
- **Benign**: The benign variants are the one which are represented by alteration in gene compare to wild-type allele but it is not associated with disease risk. This mutation is not included in report.

Evidence-based variant Categorization

Tier I	Variants with strong clinical significance	Level A evidence	FDA-approved therapy included in professional guidelines
		Level B evidence	Well-powered studies with consensus from leaders in the field
Tier II	Variants with potential clinical significance	Level C evidence	FDA-approved therapies for different tumor types or investigational therapies. Multiple small published studies with some consensus
		Level D evidence	Preclinical trials or few case reports without consensus.
Tier III	Variants of unknown clinical significance		Not observed at significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases. No convincing published evidence of cancer association

Genes Assayed

Genes Assayed for the Detection of DNA Sequence Variants

ABL1, ABL2, ACVR1, ACVR2A, AKT1, AKT2, AKT3, ALK, AMER1, APC, AR, ARAF, ARID1A, ARID1B, ARID2, ASXL1, ASXL2, ATM, ATP1A1, ATR, ATRX, AURKA, AURKC, AXIN1, AXIN2, AXL, B2M, BAP1, BCL2, BCL2L12, BCL6, BCOR, BCR, BLM, BMP5, BRAF, BRCA1, BRCA2, BRIP1, BTK, CACNA1D, CALR, CARD11, CASP8, CBL, CCND1, CCND2, CCND3, CCNE1, CD79B, CDC73, CDH1, CDK4, CDK6, CDKN2A, CDKN2C, CHD4, CHEK2, CIC, CREBBP, CSF1R, CTCF, CTNNB1, CUL1, CUL3, CYP2D6, CYSLTR2, DDR2, DDX3X, DGCR8, DICER1, DNMT3A, DPYD, DROSHA, E2F1, EGFR, EIF1AX, EP300, EPAS1, EPHA2, ERBB2, ERBB3, ERBB4, ERCC2, ERCC5, ERRFI1, ESR1, EZH2, FAM135B, FANCM, FBXW7, FGF7, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, FLT4, FOXA1, FOXL2, FOXO1, FUBP1, GATA2, GATA3, GLI1, GNA11, GNA13, GNAQ, GNAS, GPS2, H2BC5, H3-3A, H3-3B, H3C2, HIF1A, HNF1A, HRAS, ID3, IDH1, IDH2, IKBKB, IL6ST, IL7R, IRF4, IRS4, JAK1, JAK2, JAK3, KDM6A, KDR, KEAP1, KIT, KLF4, KLF5, KMT2B, KMT2D, KNSTRN, KRAS, LARP4B, LATS1, MAGOH, MAP2K1, MAP2K2, MAP2K4, MAP2K7, MAP3K4, MAPK1, MAPK8, MAX, MDM4, MECOM, MED12, MEF2B, MEN1, MET, MGA, MITF, MLH3, MPL, MSH3, MSH6, MTOR, MYC, MYCN, MYD88, MYOD1, NBN, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NRAS, NSD2, NT5C2, NTRK1, NTRK2, NTRK3, NUP93, PARP1, PAX5, PBRM1, PCBP1, PDGFRA, PDGFRB, PHF6, PIK3C2B, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PIK3R2, PIM1, PLCG1, PMS2, POLE, PPM1D, PPP2R1A, PPP6C, PRKACA, PTCH1, PTEN, PTPN11, PTPRD, PXDNL, RAC1, RAD50, RAD51, RAF1, RARA, RB1, RET, RGS7, RHEB, RHOA, RICTOR, RIT1, RNF43, ROS1, RPL10, RPL5, RUNX1, RUNX1T1, SDHD, SETBP1, SETD2, SF3B1, SIX1, SIX2, SLCO1B3, SLX4, SMAD2, SMAD4, SMARCA4, SMARCB1, SMC1A, SMO, SNCAIP, SOCS1, SOS1, SOX2, SPOP, SRC, SRSF2, STAG2, STAT3, STAT5B, STAT6, STK11, TAF1, TCF7L2, TERT, TET2, TGFBR1, TGFBR2, TNFAIP3, TOP1, TP53, TPMT, TRRAP, TSC2, TSHR, U2AF1, UGT1A1, USP8, VHL, WAS, WT1, XPO1, XRCC2, ZFHX3, ZNF217, ZNF429

Genes Assayed for the Detection of Copy Number Variations

ABCB1, ABL1, ABL2, ABRAXAS1, ACVR1B, ACVR2A, ADAMTS12, ADAMTS2, AKT1, AKT2, AKT3, ALK, AMER1, APC, AR, ARAF, ARHGAP35, ARID1A, ARID1B, ARID2, ARID5B, ASXL1, ASXL2, ATM, ATR, ATRX, AURKA, AURKC, AXIN1, AXIN2, AXL, B2M, BAP1, BARD1, BCL2, BCL2L12, BCL6, BCOR, BLM, BMPR2, BRAF, BRCA1, BRCA2, BRIP1, CARD11, CASP8, CBFB, CBL, CCND1, CCND2, CCND3, CCNE1, CD274, CD276, CDC73, CDH1, CDH10, CDK12, CDK4, CDK6, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, CHD4, CHEK1, CHEK2, CIC, CREBBP, CSMD3, CTCF, CTLA4, CTNND2, CUL3, CUL4A, CUL4B, CYLD, CYP2C9, DAXX, DDR1, DDR2, DDX3X, DICER1, DNMT3A, DOCK3, DPYD, DSC1, DSC3, EGFR, EIF1AX, ELF3, EMSY, ENO1, EP300, EPCAM, EPHA2, ERAP1, ERAP2, ERBB2, ERBB3, ERBB4, ERCC2, ERCC4, ERRFI1, ESR1, ETV6, EZH2, FAM135B, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FAT1, FBXW7, FGF19, FGF23, FGF3, FGF4, FGF9, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, FLT4, FOXA1, FUBP1, FYN, GATA2, GATA3, GLI3, GNA13, GNAS, GPS2, H3-3A, H3-3B, HDAC2, HDAC9, HLA-A, HLA-B, HNF1A, IDH2, IGF1R, IKBKB, IL7R, INPP4B, JAK1, JAK2, JAK3, KDM5C, KDM6A, KDR, KEAP1, KIT, KLF5, KMT2A, KMT2B, KMT2C, KMT2D, KRAS, LARP4B, LATS1, LATS2, MAGOH, MAP2K1, MAP2K4, MAP2K7, MAP3K1, MAP3K4, MAPK1, MAPK8, MAX, MCL1, MDM2, MDM4, MECOM, MEF2B, MEN1, MET, MGA, MITF, MLH1, MLH3, MPL, MRE11, MSH2, MSH3, MSH6, MTAP, MTOR, MUTYH, MYC, MYCL, MYCN, MYD88, NBN, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NOTCH3, NOTCH4, NRAS, NTRK1, NTRK3, PALB2, PARP1, PARP2, PARP3, PARP4, PBRM1, PCBP1, PDCD1, PDCD1LG2, PDGFRA, PDGFRB, PDIA3, PGD, PHF6, PIK3C2B, PIK3CA, PIK3CB, PIK3R1, PIK3R2, PIM1, PLCG1, PMS1, PMS2, POLD1, POLE, POT1, PPM1D, PPP2R1A, PPP2R2A, PPP6C, PRDM1, PRDM9, PRKACA, PRKAR1A, PTCH1, PTEN, PTPN11, PTPRT, PXDNL, RAC1, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54L, RAF1, RARA, RASA1, RASA2, RB1, RBM10, RECQL4, RET, RHEB, RICTOR, RIT1, RNASEH2A, RNASEH2B, RNF43, ROS1, RPA1, RPS6KB1, RPTOR, RUNX1, SDHA, SDHB, SDHD, SETBP1, SETD2, SF3B1, SLCO1B3, SLX4, SMAD2, SMAD4, SMARCA4, SMARCB1, SMC1A, SMO, SOX9, SPEN, SPOP, SRC, STAG2, STAT3, STAT6, STK11, SUFU, TAP1, TAP2, TBX3, TCF7L2, TERT, TET2, TGFBR2, TNFAIP3, TNFRSF14, TOP1, TP53, TP63, TPMT, TPP2, TSC1, TSC2, U2AF1, USP8, USP9X, VHL, WT1, XPO1, XRCC2, XRCC3, YAP1, YES1, ZFHX3, ZMYM3, ZNF217, ZNF429, ZRSR2

Genes Assayed for the Detection of Fusions

AKT1, AKT2, AKT3, ALK, AR, BRAF, BRCA1, CDKN2A, EGFR, ERBB2, ERBB4, ERG, ESR1, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, MAP3K8, MET, MTAP, MYB, MYBL1, NOTCH1, NOTCH2, NOTCH3, NRG1, NTRK1, NTRK2, NTRK3, NUTM1, PIK3CA, PIK3CB, PPARG, PRKACA, PRKACB, RAF1, RARA, RELA, RET, ROS1, RSPO2, RSPO3, STAT6, TERT, TFE3, TFEB, YAP1

Genes Assayed with Full Exon Coverage

ABRAXAS1, ACVR1B, ACVR2A, ADAMTS12, ADAMTS2, AMER1, APC, ARHGAP35, ARID1A, ARID1B, ARID2, ARID5B, ASXL1, ASXL2, ATM, ATR, ATRX, AXIN1, AXIN2, B2M, BAP1, BARD1, BCOR, BLM, BMPR2, BRCA1, BRCA2, BRIP1, CALR, CASP8, CBFB, CD274, CD276, CDC73, CDH1, CDH10, CDK12, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, CHEK1, CHEK2, CIC, CIITA, CREBBP, CSMD3, CTCF,

Genes Assayed (continued)

Genes Assayed with Full Exon Coverage (continued)

CTLA4, CUL3, CUL4A, CUL4B, CYLD, CYP2C9, CYP2D6, DAXX, DDX3X, DICER1, DNMT3A, DOCK3, DPYD, DSC1, DSC3, ELF3, ENO1, EP300, EPCAM, EPHA2, ERAP1, ERAP2, ERCC2, ERCC4, ERCC5, ERRFI1, ETV6, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FAS, FAT1, FBXW7, FUBP1, GATA3, GNA13, GPS2, HDAC2, HDAC9, HLA-A, HLA-B, HNF1A, ID3, INPP4B, JAK1, JAK2, JAK3, KDM5C, KDM6A, KEAP1, KLHL13, KMT2A, KMT2B, KMT2C, KMT2D, LARP4B, LATS1, LATS2, MAP2K4, MAP2K7, MAP3K1, MAP3K4, MAPK8, MEN1, MGA, MLH1, MLH3, MRE11, MSH2, MSH3, MSH6, MTAP, MTUS2, MUTYH, NBN, NCOR1, NF1, NF2, NOTCH1, NOTCH2, NOTCH3, NOTCH4, PALB2, PARP1, PARP2, PARP3, PARP4, PBRM1, PDCD1, PDCD1LG2, PDIA3, PGD, PHF6, PIK3R1, PMS1, PMS2, POLD1, POLE, POT1, PPM1D, PPP2R2A, PRDM1, PRDM9, PRKAR1A, PSMB10, PSMB8, PSMB9, PTCH1, PTEN, PTPRT, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54L, RASA1, RASA2, RB1, RBM10, RECQL4, RNASEH2A, RNASEH2B, RNASEH2C, RNF43, RPA1, RPL22, RPL5, RUNX1, RUNX1T1, SDHA, SDHB, SDHC, SDHD, SETD2, SLX4, SMAD2, SMAD4, SMARCA4, SMARCB1, SOCS1, SOX9, SPEN, STAG2, STAT1, STK11, SUFU, TAP1, TAP2, TBX3, TCF7L2, TET2, TGFBR2, TMEM132D, TNFAIP3, TNFRSF14, TP53, TP63, TPP2, TSC1, TSC2, UGT1A1, USP9X, VHL, WT1, XRCC2, XRCC3, ZBTB20, ZFHX3, ZMYM3, ZRSR2

Limitations and Disclaimer

1. This test was developed and its performance characteristics determined by Unipath Specialty Laboratory Ltd, Ahmedabad. It has not been cleared or approved by the US Food and Drug Administration and NABL.
2. This NGS test used does not allow definitive differentiation between germline and somatic variants if FFPE is used. However, variants with variant allele frequency at nearly 50% or 100% should be considered Germline mutation. To rule out germ line mutations, repeat analysis using peripheral blood/saliva sample is recommended.
3. False negative results may be due to sampling issues, errors in sample handling, mislabeling, transportation issues, technical limitations of the assay and mutations frequency below the limit of detection of the assay, i.e., 5% for SNVs and 10% for short indels. It is also possible some complex insertion/deletion variants may not be identified.
4. Sanger confirmation of reported mutations is available on request with additional charges.
5. The classification and interpretation of all the variants is carried out based on the current state of scientific knowledge and medical understanding and may change over time with more information available in future.
6. This report should not be considered as medical advice. Results of this test need be interpreted within the context of clinical findings and other relevant clinical and laboratory data and should not be used alone.
7. Likely benign and benign variants are not reported and can be provided upon request.
8. For ruling out large Deletions/duplications/insertions MLPA is advised.

Report Signed by:

DR. SPANDAN CHAUDHARY, Ph.D
(Sr. Scientist, NGS Division)

DR. EKTA JAJODIA, MD (Path.),
PDF (Mol. Hemat),Consultant Pathologist

DR. NEERAJ ARORA, MD ,
PDF (Mol.Hemat), PDF (Haematopath)
Lab Director

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