LABORATORY REPORT

Reg. No	:	**30100200184**	Reg. Date	:	31-Jan-2023 10:16
Name	:	**JOYDEV DUTTA**	Collected on	:	31-Jan-2023 10:16
Sex/Age	:	**Male / 53 Years**	Approved Date	:	17-Feb-2023 11:06
Ref. By	:		Tele. No	:	
Location	:	LILAC INSIGHTS PVT. LTD. @ MUMBAI	Dispatch At	:	

Please find detailed report of NGS Oncomine Tumor Mutation Load assay in the following pages.

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Dr. Ekta Jajodia
M.D (Path.),PDF (Molecular Hematology, CMC, Vellore),Consultant Pathologist
20799

Patient Details

Patient Name	**JOYDEV DUTTA**	**Sample Id/LabID**	**30100200184**
Gender	Male	**DOB/AGE**	53 yrs
Ref.By	LILAC INSIGHTS PVT. LTD.	**Date of Sample Collection**	31/01/2023
Sample Type	FFPE BLOCK:HT/20/9108	**Date of Receipt**	31/01/2023
Tumor Cellularity	50%	**Date of Report**	16/02/2023

NGS Oncomine Tumor Mutation Load Assay

Clinical Details:

Squamous cell carcinoma poorly differentiated, at Diagnosis.

RESULT

Tumor Mutational Burden (Mutations/Mb): 7.08

Average Coverage:1003.0

TMB classification (based on specified parameters): LOW

Deamination score: 5 (QC: PASS; observed (5) < threshold (100.0))

Background:

Immunotherapy, is a type of treatment given to stimulate or remove inhibition of the immune system can help combat a cancer. Although PD-L1 is used as a biomarker for immunotherapy, but this assay alone remain insufficient as some patients that are negative for PD-L1 expression revealed response to immunotherapy. Since no single biomarker is fully predictive, 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 and neoantigen load vary considerably within and across tumour types, with melanomas, lung cancers, and bladder cancers being among those with the highest TMB compared with other tumour types.High TMB can be associated with microsatellite instability (MSI) high as it is assumed that TMB is caused by defects in mismatch repair (MMR) genes that are in turn use to derive microsatellite instability (MSI).

Comments:

These findings should be correlated with other clinical and laboratory tests for a definite conclusive interpretation.

Methodology

Nucleic acid (DNA) was extracted from FFPE tissue sample, using standard Qiagen nucleic acid isolation kits.Briefly, 20ng of DNA was amplified using Oncomine Tumor Mutation Load Assay (Thermo Fisher Scientific) kit as per instruction manual and sequencing was performed using Ion S5 platform (Thermo Fisher Scientic) as per user manual. Quality of the Ion S5 XL run was assessed with the Ion Torrent Suite 5.16.1 (Thermo Fisher Scientific). Reads were aligned to hg19 for variant calling and TMB calculation using the Ion Reporter 5.18 (Thermo Fisher Scientific).Oncomine Tumor Mutation Load Assay facilitates simultaneous assessment of tumor mutational burden and variant profiling in one run. It covers 1.65 Mb of genomic region including 1.2 Mb of exonic region across 409 oncogenes relevant across major cancer types with depth of coverage >500X.

After variant calling, filter is applied to select variants to be included in TMB calculation (mutations/Mb) which are as follows:
1) Only non-germline mutations (SNV and small INDELs) called from exonic region are considered. It filters germline mutation based on information provided in 1,000 genomes, 5,000 exomes, and ExAC databases, nonsynonymous variants are accurately called and assessed for TMB (mutations/Mb). 2)Type of nonsynonymous variants considered are missense, frameshiftDeletion, frameshiftInsertion, nonframeshiftDeletion, nonframeshiftInsertion and nonsense. 3)Minimum depth of base coverage was considered as 60x. 4)Minimum alternate allele frequency of 5%. 5)Deamination value should be below 100.

TMB score of <10 is considered low, >10 & <15 is considered intermediate and >15 is considered high.

Genes Assayed

Genes Assayed for Tumor Mutational Burden

ABL1, ABL2, ACVR2A, ADAMTS20, ADGRA2, ADGRB3, ADGRL3, AFF1, AFF3, AKAP9, AKT1, AKT2, AKT3, ALK, AMER1, APC, AR, ARID1A, ARID2, ARNT, ASXL1, ATF1, ATM, ATR, ATRX, AURKA, AURKB, AURKC, AXL, BAP1, BCL10, BCL11A, BCL11B, BCL2, BCL2L1, BCL2L2, BCL3, BCL6, BCL9, BCR, BIRC2, BIRC3, BIRC5, BLM, BLNK, BMPR1A, BRAF, BRD3, BRIP1, BTK, BUB1B, CARD11, CBL, CCND1, CCND2, CCNE1, CD79A, CD79B, CDC73, CDH1, CDH11, CDH2, CDH20, CDH5, CDK12, CDK4, CDK6, CDK8, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHEK1, CHEK2, CIC, CKS1B, CMPK1, COL1A1, CRBN, CREB1, CREBBP, CRKL, CRTC1, CSF1R, CSMD3, CTNNA1, CTNNB1, CYLD, CYP2C19, CYP2D6, DAXX, DCC, DDB2, DDIT3, DDR2, DEK, DICER1, DNMT3A, DPYD, DST, EGFR, EML4, EP300, EP400, EPHA3, EPHA7, EPHB1, EPHB4, EPHB6, ERBB2, ERBB3, ERBB4, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERG, ESR1, ETS1, ETV1, ETV4, EXT1, EXT2, EZH2, FANCA, FANCC, FANCD2, FANCF, FANCG, FAS, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FLI1, FLT1, FLT3, FLT4, FN1, FOXL2, FOXO1, FOXO3, FOXP1, FOXP4, FZR1, G6PD, GATA1, GATA2, GATA3, GDNF, GNA11, GNAQ, GNAS, GRM8, GUCY1A2, HCAR1, HIF1A, HLF, HNF1A, HOOK3, HRAS, HSP90AA1, HSP90AB1, ICK, IDH1, IDH2, IGF1R, IGF2, IGF2R, IKBKB, IKBKE, IKZF1, IL2, IL21R, IL6ST, IL7R, ING4, IRF4, IRS2, ITGA10, ITGA9, ITGB2, ITGB3, JAK1, JAK2, JAK3, JUN, KAT6A, KAT6B, KDM5C, KDM6A, KDR, KEAP1, KIT, KLF6, KMT2A, KMT2C, KMT2D, KNL1, KRAS, LAMP1, LCK, LIFR, LPP, LRP1B, LTF, LTK, MAF, MAFB, MAGEA1, MAGI1, MALT1, MAML2, MAP2K1, MAP2K2, MAP2K4, MAP3K7, MAPK1, MAPK8, MARK1, MARK4, MBD1, MCL1, MDM2, MDM4, MEN1, MET, MITF, MLH1, MLLT10, MMP2, MN1, MPL, MRE11, MSH2, MSH6, MTOR, MTR, MTRR, MUC1, MUTYH, MYB, MYC, MYCL, MYCN, MYD88, MYH11, MYH9, NBN, NCOA1, NCOA2, NCOA4, NF1, NF2, NFE2L2, NFKB1, NFKB2, NIN, NKX2-1, NLRP1, NOTCH1, NOTCH2, NOTCH4, NPM1, NRAS, NSD1, NSD2, NTRK1, NTRK3, NUMA1, NUP214, NUP98, PAK3, PALB2, PARP1, PAX3, PAX5, PAX7, PAX8, PBRM1, PBX1, PDE4DIP, PDGFB, PDGFRA, PDGFRB, PER1, PGAP3, PHOX2B, PIK3C2B, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PIK3R2, PIM1, PKHD1, PLAG1, PLCG1, PLEKHG5, PML, PMS1, PMS2, POT1, POU5F1, PPARG, PPP2R1A, PRDM1, PRKAR1A, PRKDC, PSIP1, PTCH1, PTEN, PTGS2, PTPN11, PTPRD, PTPRT, RAD50, RAF1, RALGDS, RARA, RB1, RECQL4, REL, RET, RHOH, RNASEL, RNF2, RNF213, ROS1, RPS6KA2, RRM1, RUNX1, RUNX1T1, SAMD9, SBDS, SDHA, SDHB, SDHC, SDHD, SEPTIN9, SETD2, SF3B1, SGK1, SH2D1A, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SMUG1, SOCS1, SOX11, SOX2, SRC, SSX1, STK11, STK36, SUFU, SYK, SYNE1, TAF1, TAF1L, TAL1, TBX22, TCF12, TCF3, TCF7L1, TCF7L2, TCL1A, TET1, TET2, TFE3, TGFBR2, TGM7, THBS1, TIMP3, TLR4, TLX1, TNFAIP3, TNFRSF14, TNK2, TOP1, TP53, TPR, TRIM24, TRIM33, TRIP11, TRRAP, TSC1, TSC2, TSHR, UBR5, UGT1A1, USP9X, VHL, WAS, WRN, WT1, XPA, XPC, XPO1, XRCC2, ZNF384, ZNF521

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) FFPE samples may harbor artefactual deamination alterations that may impact mutation calling and TMB calculation.
3) TMB threshold for differentiating low, intermediate and high can vary depending upon the cancer type.
4) TMB testing for solid tissue samples, is recommended to be carried out by gene panels with a coverage preferably >1 Mb which is provided by this assay. However result may vary if done using whole genome or whole exome technique.
5) This assay does not detect translocations, gene rearrangements, copy number alterations, microsatellite instability.

6) 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%.
7) Variants at #5% allelic frequency is used for TMB calculation, however in case of samples of low quality that exhibit deamination higher frequency threshold may be considered (#10%).
8) Variants detected below the limit of detection (LOD) i.e < 5% variant allele frequency will not be used for TMB calculation.
9) Large variants (>60 base pairs [bp]) may not be detected.
10) Variants in known pseudogenes, homologous genomic regions, and/or low mappability regions may not be detected.
11) This assay is not intended to detect minimal residual disease.

Report Signed by:

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