Personalized Therapeutic Report

Test Description

It is an integrated report of multiplatform testing including comprehensive genomic profiling (170 gene panel) and tumor mutation burden (TMB) using next generation sequencing (NGS), microsatellite instability (MSI) using molecular beacon probe-based multiplex polymerase chain reaction (7 biomarkers) and PD-L1 expression using immunohistochemistry.

Patient Demographic

Name: Kusum Lata
Sex: Female
Date of Birth/Age: 50 years
Disease: Metastatic Adenoid Cystic Carcinoma of Lung

Clinician

Clinician Name: Dr Archit Pandit
Medical Facility: Max Hospital
Pathologist: Not Provided

Specimen

Booking ID: 011910260187
Site: Lung mass
Sample Type: FFPE blocks (12); S-13743/15 - A to L
Date of Collection: 26-10-2019
Date of Booking: 26-10-2019

CLINICAL SYNOPSIS

Ms Kusum Lata is a known case of adenoid cystic carcinoma, parotid gland [as per the histopathology report dated 21-10-2015]. The tumor was identifiable in the blocks [S-13743/15 – C, D, F & G] and it was adequate for further analysis.
Note: This sample has failed at library QC level for RNA and hence, the RNA fusion analysis could not be performed. The scope of the analysis is limited to SNVs and indels. The same has been conveyed to the clinician via email.

RECOMMENDATION & REPORT INTERPRETATION

Clinically relevant alteration was identified in *TSC2* gene. *S*ensitivity to mTOR inhibitor Everolimus may be predicted.

Disclaimer: *Report interpretation & recommendation(s) should not be considered as final; and should be used at the discretion of the treating Physician or the molecular tumor board. The report interpretation & recommendation(s) does not bear any medical, legal, ethical & moral responsibilities, and liabilities.*

BIOMARKERS

Targeted Therapy

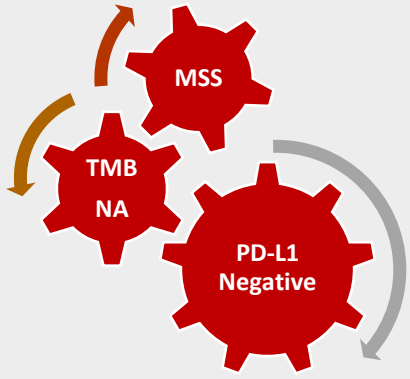
Genomic Findings

Clinically Significant

TSC2 ***p.Lys1415ArgfsTer61***, Exon 34

Variant of Unknown Significance
SLX4 ***p.Gln1386Ter***, Exon 12
CDK12 ***p.Ala1253(=)***, Intron 13
JAK3 ***p.Arg911Cys***, Exon 20
EP300 ***p.Pro1114Ser***, Intron 18

Immunotherapy



TMB **NA**
Microsatellite Stable **(MSS)**
PD-L1 **Negative**

Personalized Therapeutic Report

TABLE 1: GENOMIC ALTERATIONS THAT CAN BE TARGETED WITH APPROVED DRUGS IN THE SUBJECT'S TUMOR TYPE

Gene (Transcript) #	Location	Variant	Overall Depth	Mutant Allele Percentage	Function of the Gene in Cancer
		None			

TABLE 2: NON-DRUGGABLE/DRUGGABLE CLINICALLY SIGNIFICANT GENOMIC ALTERATIONS INDICATED IN OTHER TUMORS

Gene (Transcript) #	Location	Variant	Overall Depth	Mutant Allele Percentage	Function of the Gene in Cancer
TSC2 (ENST00000219476.3)	Exon 34	c.4243del (p.Lys1415Argfs)	932X	10.5%	Tumor Suppressor

TABLE 3: VARIANTS OF UNKNOWN SIGNIFICANCE

Gene (Transcript) #	Location	Variant	Overall Depth	Mutant Allele Percentage	Function of the Gene in Cancer
SLX4 (ENST00000294008.3)	Exon 12	c.4156C>T (p.Gln1386Te)	626X	9.4%	DNA repair
CDK12 (ENST00000447079.4)	Exon 13	c.3759A>T (p.Ala1253(=))	959X	31.8%	Tumor Suppressor
JAK3 (ENST00000458235.1)	Exon 20	c.2731C>T (p.Arg911Cys)	446X	7.2%	Oncogene
EP300 (ENST00000263253.7)	Exon 18	c.3340C>T (p.Pro1114Ser)	254X	11.8%	Tumor Suppressor

CLINICAL CORRELATION AND VARIANT INTERPRETATION

TSC2 **p.Lys1415ArgfsTer61** **c.4243del** **Tumor Suppressor**
Overall depth: 932X **Mutant Allele Percentage: 10.5%**

Tuberous sclerosis 2 (*TSC2*) is a gene that encodes a protein tuberin that acts as a tumor suppressor as well as an activator of GTPases. Mutations in this gene lead to tuberous sclerosis and Lymphangioleiomyomatosis. A frameshift deletion (**chr16:g.2134466del: c.4243del**) that resulted in a stop codon and premature truncation of the protein, 61 amino acids downstream of codon 1415 (**p.Lys1415ArgfsTer61**) was detected in the *TSC2* gene of this subject. This is a loss of function mutation. This variant has not been reported in ExAC and 1000 genomes databases.

Somatic mutations in *TSC1* and *TSC2* and their frequency in solid tumors is not been well documented. In a clinical study on 85 patients of different solid tumors including lung, breast, CRC, kidney and liver showed a mutation frequency of 57.6% in *TSC2*[1]. *TSC1/TSC2* mutations in HCC cell lines and xenograft models, have shown increased sensitivity to mTOR inhibition by Rapamycin[2]. In another study on 39 patients with five different tumor types (13 with gastric cancer, 15 with renal cell

carcinoma, 2 with thyroid cancer, 2 with head and neck cancer, and 7 with sarcoma), 4 patients had *TSC1/TSC2* gene mutation[3]. *TSC1/TSC2/MTOR* mutations were key components in determining Everolimus sensitivity. The incidence of these mutations was 31.8% (7/22) in patients with clinical benefit as compared with 0% in those with non-clinical benefit (P=0.012)[3]. *TSC1/TSC2* mutations in HCC cell lines and xenograft models, have shown increased sensitivity to mTOR inhibition by Rapamycin[4]. Clinical significance of TSC2 gene mutations has not been very well documented in the medical literature for the tumor type of the subject under investigation. Please correlate clinically.

ADDITIONAL FINDINGS

Table 3 provides a list of VUS in genes known to function as oncogene or tumor suppressor or relevant in epigenetic regulation in human cancers. These variants specifically detected in this tumor have not been characterized sufficiently in biochemical assays and therefore their impact in this cancer remains speculative.

Note:

- The *SLX4* gene variant ***p.Gln1386Ter*** is not reported in ExAC and 1000 genomes databases. Although it is a loss of function variant, the clinical significance of this variant is not well documented.
- The *CDK12* gene variant ***p.Ala1253(=)*** is not reported in ExAC and 1000 genomes databases.
- The *JAK3* gene variant ***p.Arg911Cys*** is not reported in ExAC and 1000 genomes databases. It is predicted to be damaging SIFT and possibly damaging by Polyphen2 functional prediction tools.
- The *EP300* gene variant ***p.Pro1114Ser*** is reported in ExAC with MAF of 0.0008% and it is not reported in 1000 genomes database. It is predicted to be damaging SIFT, LRT and probably damaging by Polyphen2 functional prediction tools.

However, the functional impact and clinical significance of these variants in the tumor type of the subject under investigation are not well documented in medical literature. Hence, these variants have been included in the Table 3 as VUS. Please correlate clinically.

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.

REFERENCES

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2. Ho DWH, Chan LK, Chiu YT, Xu IMJ, Poon RTP, Cheung TT, Tang CN, Tang VWL, Lo ILO, Lam PWY, Yau DTW, Li MX, Wong CM, Ng IOL. TSC1/2 mutations define a molecular subset of HCC with aggressive behaviour and treatment implication. Gut. 2017 Aug;66(8):1496-1506. doi: 10.1136/gutjnl-2016-312734. Epub 2016 Dec 14.
3. Lim, Sun Min, et al. "Next-generation sequencing reveals somatic mutations that confer exceptional response to everolimus." Oncotarget 7.9 (2016): 10547.
4. Ho, Daniel WH, et al. "TSC1/2 mutations define a molecular subset of HCC with aggressive behaviour and treatment implication." Gut 66.8 (2017): 1496-1506.

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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 170 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.

Method

Tumor genomic DNA and RNA isolated from FFPE tissue was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced with a panel mean coverage depth of **4852X and 16229X** in DNA and RNA library respectively, 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 VariMAT annotation pipeline. 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 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[7-12]. Gene fusions at the RNA level were assessed using multiple fusion detection programs to arrive at the consensus on predicted fusions. The fusions are confirmed based on the number of spanning reads and/or split reads supporting the finding[13-17]. Reportable mutations are prioritized and prepared based AMP-ASCO-CAP guidelines[18] based on annotation metrics from OncoMD[19], 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 determined by Reference lab".

**In this case, the panel average sequencing depth of the library was less than 250X, which is slightly below the QC pass criteria for our routine clinical reporting. Nevertheless, with the best interest of identifying any significant variants, the analysis was performed on this data and reported. Please correlate clinically.

DISCLAIMER

- The classification of variants of unknown significance can change over time. Please contact MolQ laboratory at a later date for any change.
- The scope of this assay limits to SNPs, Short Indels (in DNA) and gene fusions and splice variants (in RNA)
- Intronic variants are not assessed using this method.
- Large deletions of more than 20 bp or copy number variations / rearrangements cannot be assessed using this method.
- This panel is intended to screen for complete coding region of the genes enlisted below in the appendix 2.
- The mutations have not been validated by Sanger sequencing.
- This NGS panel is not intended to report germline variants.
- This NGS test used does not allow definitive differentiation between germline and somatic variants
- TREATMENT DECISIONS BASED ON THESE MUTATIONS MAY BE TAKEN IN CORRELATION WITH OTHER CLINICAL AND PATHOLOGICAL INFORMATION.
- A false negative result for any variant below the LOD, i.e., 5% for SNVs and 10% for short indels, cannot be ruled out.

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APPENDIX 2: GENE LIST OF THE 170 GENES PANEL

SNVs and Short Indels (<25bp) (from DNA)									
AKT1	*BRIP1*	*CREBBP*	*FANCI*	*FGFR2*	*JAK3*	*MSH3*	*PALB2*	*RAD51D*	*TSC1*
AKT2	*BTK*	*CSF1R*	*FANCL*	*FGFR3*	*KDR*	*MSH6*	*PDGFRA*	*RAD54L*	*TSC2*
AKT3	*CARD11*	*CTNNB1*	*FBXW7*	*FGFR4*	*KIT*	*MTOR*	*PDGFRB*	*RB1*	*VHL*
ALK	*CCND1*	*DDR2*	*FGF1*	*FLT1*	*KMT2A(MLL)*	*MUTYH*	*PIK3CA*	*RET*	*XRCC2*
APC	*CCND2*	*DNMT3A*	*FGF2*	*FLT3*	*KRAS*	*MYC*	*PIK3CB*	*RICTOR*	
AR	*CCNE1*	*EGFR*	*FGF3*	*FOXL2*	*MAP2K1*	*MYCL1*	*PIK3CD*	*ROS1*	
ARID1A	*CD79A*	*EP300*	*FGF4*	*GEN1*	*MAP2K2*	*MYCN*	*PIK3CG*	*RPS6KB1*	
ATM	*CD79B*	*ERBB2*	*FGF5*	*GNA11*	*MCL1*	*MYD88*	*PIK3R1*	*SLX4*	
ATR	*CDH1*	*ERBB3*	*FGF6*	*GNAQ*	*MDM2*	*NBN*	*PMS2*	*SMAD4*	
BAP1	*CDK12*	*ERBB4*	*FGF7*	*GNAS*	*MDM4*	*NF1*	*PPP2R2A*	*SMARCB1*	
BARD1	*CDK4*	*ERCC1*	*FGF8*	*HNF1A*	*MET*	*NOTCH1*	*PTCH1*	*SMO*	
BCL2	*CDK6*	*ERCC2*	*FGF9*	*HRAS*	*MLH1*	*NOTCH2*	*PTEN*	*SRC*	
BCL6	*CDKN2A*	*ERG*	*FGF10*	*IDH1*	*MLLT3*	*NOTCH3*	*PTPN11*	*STK11*	
BRAF	*CEBPA*	*ESR1*	*FGF14*	*IDH2*	*MPL*	*NPM1*	*RAD51*	*TERT*	
BRCA1	*CHEK1*	*EZH2*	*FGF23*	*INPP4B*	*MRE11A*	*NRAS*	*RAD51B*	*TET2*	
BRCA2	*CHEK2*	*FAM175A*	*FGFR1*	*JAK2*	*MSH2*	*NRG1*	*RAD51C*	*TP53*	

Fusions and Splice Variants (from RNA)									
ABL1	*BRAF*	*EML4*	*ETV4*	*FGFR4*	*KIF5B*	*MYC*	*NTRK2*	*PIK3CA*	*TMPRSS2*
AKT3	*BRCA1*	*ERBB2*	*ETV5*	*FLI1*	*KIT*	*NOTCH1*	*NTRK3*	*PPARG*	
ALK	*BRCA2*	*ERG*	*EWSR1*	*FLT1*	*KMT2A(MLL)*	*NOTCH2*	*PAX3*	*RAF1*	
AR	*CDK4*	*ESR1*	*FGFR1*	*FLT3*	*MET*	*NOTCH3*	*PAX7*	*RET*	
AXL	*CSF1R*	*ETS1*	*FGFR2*	*JAK2*	*MLLT3*	*NRG1*	*PDGFRA*	*ROS1*	
BCL2	*EGFR*	*ETV1*	*FGFR3*	*KDR*	*MSH2*	*NTRK1*	*PDGFRB*	*RPS6KB1*	

iMSI Rapid™ Assay

MSI testing is used for Hereditary Cancer screening (Hereditary Non-Polyposis Colorectal Cancer -HNPCC or Lynch syndrome);
As a biomarker (Prognostic and predictive biomarker for the response of Immunotherapy)

Result — Microsatellite - Stable (MSS)

BIOMARKER FINDINGS

ACVR2A	No mutation detected
BTBD7	No mutation detected
DIDO1	No mutation detected
MRE11	No mutation detected
RYR3	No mutation detected
SEC13A	No mutation detected
SULF2	No mutation detected

INTERPRETATION

*MSS	<2 of the 7 markers demonstrate instability
#MSI-H	≥2 of the 7 markers demonstrate instability

*Microsatellite stable

Microsatellite Instability-High

For valid batch test results specific controls are being run with every batch.

METHODOLOGY

Multiplex detection of seven mononucleotide repeats using molecular beacon probe-based polymerase chain reaction followed by high resolution melt-curve analysis. The assay uses seven novel biomarkers *ACVR2A*, *BTBD7*, *DIDO1*, *MRE11*, *RYR3*, *SEC31A* and *SULF2* as this set of biomarkers is stable over different cancer types and ethnicities and show high performance than other known assays like *Bethesda Panel.* This test is carried out on Idylla platform using the MSI/1.0 Cartridge based kit which is CE IVD approved.

REFERENCES

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Programmed Death Ligand 1 (PD-L1) Immunohistochemistry

Test Description

This test is useful for identification of neoplasms expressing programmed cell death 1-ligand 1 (clone SP263). PD-L1 also known as B7 homolog 1 (B7-H1) or CD274, is a transmembrane protein involved in the regulation of cell-mediated immune responses through interaction with the receptor programmed death protein-1 (PD-1). PD-L1 has been identified as both a prognostic and theranostic marker in a variety of neoplasms. Overexpression of PD-L1 has been observed in carcinomas of the urinary bladder, lung, thymus, colon, pancreas, ovary, breast, kidney, and in melanoma and glioblastoma.

Specimen

Sample Type: FFPE block SB 3665A/19
Site: Lung Mass
Pathology ID: MOLQ/IHC-38102019
Disease: Metastatic Adenoid Cystic Carcinoma in Lung

Interpretation

The scoring system is based on type and origin of tumor. If additional interpretation or analysis is needed, send request for Pathology Consultation.

Methodology

Immunostaining for PD-L1 protein was done using Ventana Rabbit Anti-Human PD-L1/CD274 Monoclonal Antibody (Clone SP-263) on Ventana Autostainer.
Positive PD-L1 staining/expression is defined as complete and/or partial, circumferential or linear plasma membrane or cytoplasmic staining at any intensity that can be differentiated from background.

Note

Preclinical studies suggest that positive programmed cell death 1-ligand 1 (PD-L1) immunohistochemistry in tumor cells may predict tumor response to therapy with immune checkpoint inhibitors. This result should not be used as the sole factor in determining treatment, as other factors (eg, tumor mutation burden and microsatellite instability) have also been studied as predictive markers.

References

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Programmed Death Ligand 1 (PD-L1): Negative

Microscopy Evaluation

HE Staining (Figure 1)
Tumor cells: 80%
Immune cells: 15%

Tumor cells positive for PD-L1: 00% (No immunostaining)
Immune cells positive for PD-L1: 00% (No Immunostaining)

PD-L1 IHC- Tumor Cells

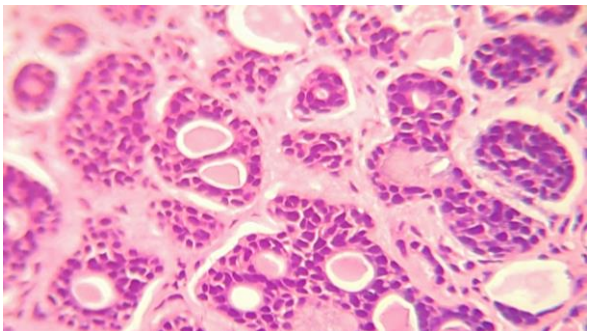

Figure 1

PD-L1 IHC- Tumor Cells

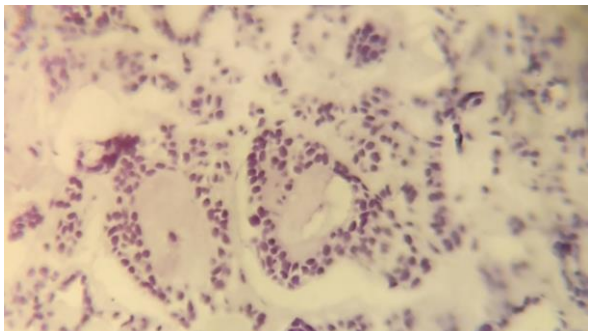

Figure 2

PD-L1 IHC- Immune Cells

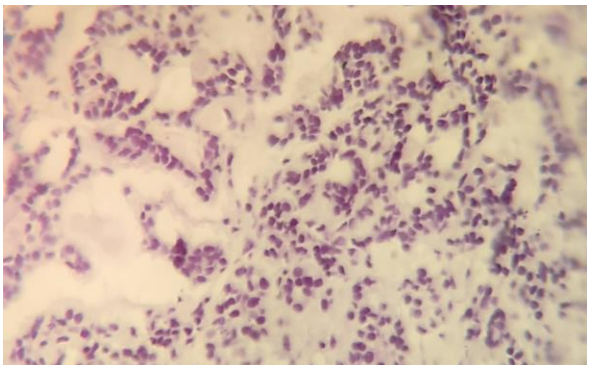

Figure 3