MOLQ LABORATORY

Personalized Therapeutic Report- Focused

Test Description

It is an integrated report of multiplatform testing including comprehensive genomic profiling (56 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.

Clinician

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

Patient Demographic

Name: Vishnu Raj Atreya
Sex: Male
Date of Birth/Age: 77 years
Disease: Carcinoma Gall Bladder

Specimen

Booking ID: 011911080209
Site: Peritoneal Nodule
Sample Type: FFPE block (1), SB 4353 /19
Date of Collection: 08-11-2019
Date of Booking: 08-11-2019

CLINICAL SYNOPSIS

Clinical impression of carcinoma gall bladder. Peritoneal nodule (FS) biopsy suggestive of metastatic adenocarcinoma with mucinous differentiation [as per the histopathology report dated 06-11-2019]. The tumor was identifiable in the block [SB/4353B/19] and it was adequate for further analysis.

RECOMMENDATION & REPORT INTERPRETATION

Clinically actionable alteration in *PTEN* suggests loss of function which may predict response to mTOR inhibitors (Everolimus).

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

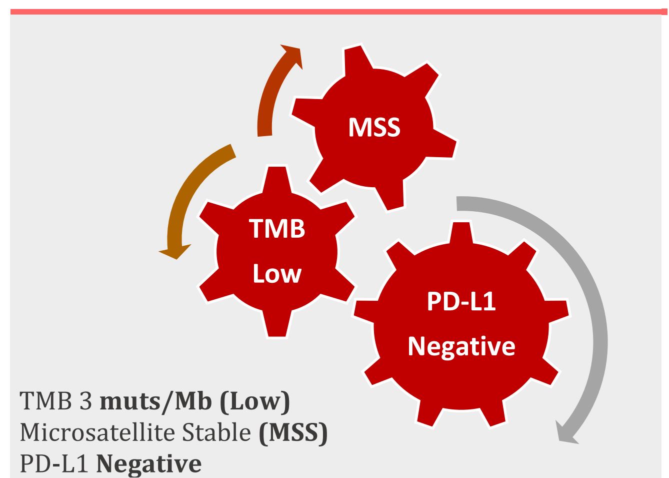
Genomic Findings

Clinically Significant

PTEN ***p.Cys136Tyr***, Exon 5

Variant of Unknown Significance
SMAD4 ***p.Val506Gly,*** Exon 12

Immunotherapy



TMB 3 **muts/Mb (Low)**
Microsatellite Stable **(MSS)**
PD-L1 **Negative**

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
PTEN (ENST00000371953.3)	Exon 5	c.407G>A (p.Cys136Tyr)	33655X	1.7%	Tumor Suppressor

TABLE 3: VARIANTS OF UNKNOWN SIGNIFICANCE

Gene (Transcript) #	Location	Variant	Overall Depth	Mutant Allele Percentage	Function of the Gene in Cancer
SMAD4 (ENST00000342988.3)	Exon 12	c.1517T>G (p.Val506Gly)	16236X	8.1%	Tumor Suppressor

CLINICAL CORRELATION AND VARIANT INTERPRETATION

PTEN **p.Cys136Tyr** **c.407G>A** **Tumor Suppressor**
Overall depth: 33655X **Mutant Allele Percentage: 1.7%**

This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded by this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. *PTEN* is a multi-functional tumor suppressor that is very commonly lost in human cancer. Observed in prostate cancer, glioblastoma, endometrial, lung and breast cancer to varying degrees. Up to 70% of prostate cancer patients have been observed to have loss of expression of the gene. It is a part of the PI3K/AKT/mTOR pathway and mTOR inhibitors have been relatively ineffective in treating patients with PTEN loss. A missense mutation (**chr10:g.89692923G>A; c.407G>A**) that resulted in an amino acid change at codon 136 (**p.Cys136Tyr**) was detected in the *PTEN* gene of this subject. This is a loss of function mutation. This variant has not been reported in ExAC and 1000 genomes databases. In Clinvar database, this variant of germline origin is reported in hamartoma tumor syndrome condition [VCV000189406.2]. However, the clinical significance of this mutation in carcinoma gall bladder has not been reported in medical literature.

In a study on 38 patients with primary small cell carcinoma of the esophagus (PSCCE), *PTEN* mutations presented in 14 (36.84%) out of 38 patients[1]. In order to investigate the role of PTEN in modulating the response to MAPK or PI3K pathway inhibition, panels of thirty tumor cell lines of different histological origin (melanoma, n=7; breast cancer, n=6; non-small cell lung cancer, n=6; colorectal cancer, n=8; pancreatic adenocarcinoma, n=2; glioblastoma, n=1) were analyzed for *PTEN* gene status[2]. Neither PTEN mutational status appeared to significantly influence response to Trametinib (P=0.15) or Everolimus (P=0.79). Overall, PTEN-loss was significantly associated with a synergistic interaction between Trametinib and Everolimus (P<0.0001). It was reported that combination of Trametinib and Everolimus had a significantly greater tumor growth inhibitory effect, as compared to individual treatment (P=0.02)[2].

PTEN loss was associated with improved response (assessed by pathological complete response) to neoadjuvant treatment

with the EGFR/HER2 kinase inhibitor Lapatinib followed by Trastuzumab in advanced breast cancers[3]. PTEN inactivation has been shown to be associated with resistance to EGFR-tyrosine kinase inhibitor therapy and lower survival in NSCLC patients[4]. Kindly correlate clinically.

Note: These particular mutations have been detected at a mutant allele percentage below the limit of detection of this assay for the SNPs or missense mutations. Please correlate with other clinical findings.

ADDITIONAL FINDINGS

Variants of unknown significance (VUS) in genes relevant in cancer

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 SMAD4 gene variant **p.Val506Gly** is not reported in ExAC and 1000 genomes databases. It is predicted to be damaging by SIFT & LRT and possibly damaging by probably damaging. However, the clinical significance of this variant is not very well documented in medical literature. Hence, this variant has been classified as variants of unknown significance.

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

1. Zhang, Zhimin, et al. "High-incidence of PTEN mutations in Chinese patients with primary small cell carcinoma of the esophagus." BMC cancer 14.1 (2014): 19.
2. Milella, Michele, et al. "PTEN status is a crucial determinant of the functional outcome of combined MEK and mTOR inhibition in cancer." Scientific reports 7 (2017): 43013.
3. Dave, Bhuvanesh, et al. "Loss of phosphatase and tensin homolog or phosphoinositol-3 kinase activation and response to trastuzumab or lapatinib in human epidermal growth factor receptor 2–overexpressing locally advanced breast cancers." Journal of Clinical Oncology 29.2 (2011): 166.
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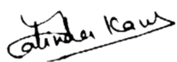

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APPENDIX 1: TEST METHODOLOGY

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 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.

Method

Tumor genomic DNA isolated from FFPE was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced with a panel mean coverage depth of **35311X**, 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 our 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 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]. 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 determined by Reference lab".

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 hotspot mutations only.
- 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 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

APPENDIX 3

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

Biomarker	Finding
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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Zhao et al. (2018) ASCO Abstract #e15654

APPENDIX 4

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 4353/19
Site: Peritoneal Nodule
Pathology ID: MOLQ/IHC-44112019
Disease: Carcinoma Gall Bladder

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: 25%
Immune cells: 10%

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

HE Stained Section

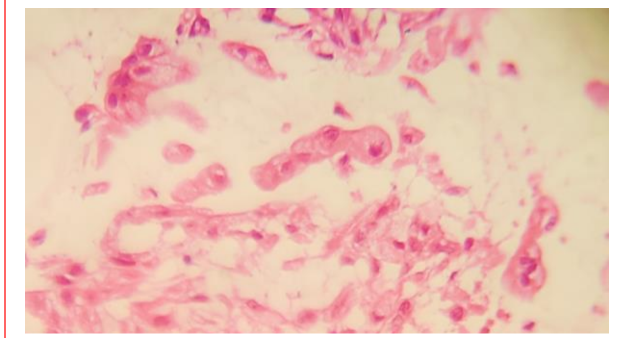

Figure 1

PD-L1 IHC- Tumor Cells

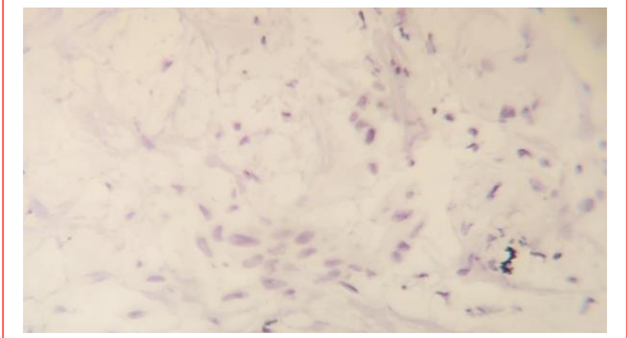

Figure 2

PD-L1 IHC- Immune Cells

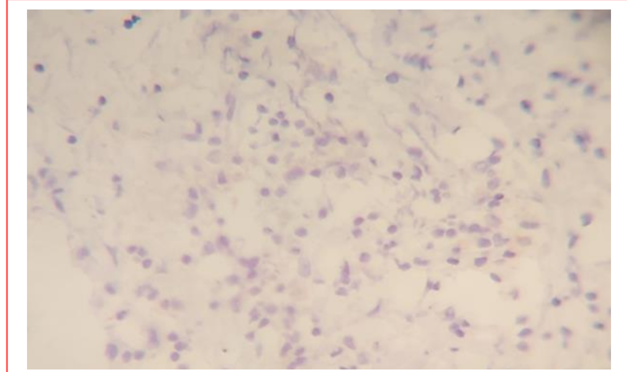

Figure 3