MEDGENOME

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DNA TEST REPORT

SOMATIC MUTATION PANEL ANALYSIS REPORT			
Subject Information		**Sample Information**	
Patient Name:	**XXXXX**	Order ID/Sample ID:	**XXX/12345**
Gender:	**Male**	Collection Date & Time:	**NA**
Age:	**43 years**	Receipt Date & Time:	**11th January, 2013, 10:00 AM**
Father-Mother:	**NA**	Report Date & Time:	**9tht Feb, 2013 4:00 PM**
Sample Type:	**FFPE Tumor tissue Blood (2.5ml)**	Requested by:	
Clinical Indication (If any):	**Metastatic carcinoma of bladder**		

The raw sequencing fields (including the fastq as well as sequence alignment results) can be provided upon request from the referring physician.

Analysis:	Tumor DNA Sequencing

The genes sequenced and their coverage details are listed in Appendix 1.

Table 1	Genomic alterations that can be targeted with approved drugs								
Sl. No.	**Gene**	**CDS variant#**	**Amino acid variant**	**Impact on Protein Function**	**Approved drugs**	**Drug response**	**Function of the gene in cancer**	**Hot spot mutation**	**Classification of variant**
NONE									

Table 2	Non-druggable, but clinically significant alterations								
Sl. No	**Gene**	**CDS variant#**	**Amino acid variant**	**Impact on Protein Function**	**Approved drugs**	**Drug response**	**Function of the gene in cancer**	**Hot spot mutation**	**Classification of variant**
1.	***PTEN***	**c.596delT (ENST00000371953)**	**p.Met199SerfsTer22**	**Yes**	**NA**	**NA**	**TSG**	**No**	**Pathogenic**
2.	***STK11***	**c.541delA (ENST00000326873)**	**p.Asn181ThrfsTer106**	**Yes**	**NA**	**NA**	**TSG**	**No**	**Pathogenic**

TSG- Tumor Suppressor Gene

Annotation is performed against GRCh37/hg19 version of human gene.

 # cDNA base is reverse compliment of genomic base in case on negative strand.

Test Details & Interpretation

Background

The next-generation sequencing based multi-gene analysis for cancer predisposition genes and tumor mutations will enable effective risk management and treatment by doctors. Thus, it allows us to sequence and identify variants associated with multiple genes with implications in cancer. 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 with the appropriate drug, based on the mutational profile.

Test

DNA isolated from tumor tissue and paired normal (blood) samples were used to perform enrichment and sequencing using tumor gene panel kit. The tumor tissue and the paired normal (blood) panels were covered at an average sequencing depth of 166X and 739X respectively. The sequences obtained were aligned to human reference genome (GRCh37/hg19) using BWA program [2, 3] and analyzed using Picard and GATK-Lite toolkits [4, 5]. Somatic variants unique to the tumor tissue were identified using Strelka somatic caller and annotated using our in-house annotation pipeline. Clinically relevant mutations were annotated using published variants in literature and a set of well-curated databases including ClinVar, SwissVar, OMIM, GWAS, OncoMD and COSMIC [6-11]. Only non-synonymous variants found in the sample were used for clinical interpretation.

Interpretation

***PTEN* p.Met199SerfsTer22** (Table 2)**:**
A single nucleotide deletion **(chr10:89711978delT)** that results in a frame shift and subsequent termination of the protein 22 amino acids downstream to codon 199 **(p.Met199SerfsTer22)** was detected in the *PTEN* gene of this subject. This deletion result in the production of a truncated PTEN protein which is functionally deficient. The *PTEN* gene encodes a ubiquitously expressed tumor suppressor protein that acts as the major cellular suppressor of PI3K signalling and AKT activation in cell proliferation. Loss of function variations in *PTEN* have been characterized in 50% of human gallbladder carcinoma (12). Targeted agents acting at the level of PI3K or downstream may be most effective for treatment of PTEN-deficient cancers. Several such drugs are currently in clinical trials for the treatment of patients with PTEN-deficient cancers (table 4). PTEN deficiency is also associated with resistance to RTK inhibitors *in vitro* (13).

***STK11* p.Asn181ThrfsTer106** (Table 2)**:**
A single nucleotide deletion **(chr19:1220448delA)** that results in a frame shift and subsequent termination of the protein 106 amino acids downstream to codon 181 **(p.Asn181ThrfsTer106)** was detected in the *STK11*gene of this subject. This deletion also results in the production of a truncated, functionally deficient protein. Homozygous deletions or somatic sequence mutations coupled with loss of heterozygosity in the *STK11* gene, have been identified in sporadic pancreatic and biliary adenocarcinomas (14). *STK11* acts as a tumor suppressor by negatively regulating the mTOR pathway. Hence, *STK11* loss of function results in increased mTOR signaling suggesting that therapies targeting mTOR (eg. mTOR inhibitors like FDA approved Everolimus and Temsirolimus) may be relevant for *STK11* deficient tumors (15,16). A patient with pancreatic cancer and an *STK11* mutation experienced a partial response to the mTOR inhibitor Everolimus (18). Loss of *STK11* also leads to activation of

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the downstream kinase Src, suggesting that inhibitors such as dasatinib or bosutinib may be relevant for the treatment of *STK11*-deficient tumors (19).

Variants of unknown significance (VUS) in genes relevant in cancer in human cancer. The variants specifically detected in this tumor have not been characterized.
Table 3 (Appendix 2) provides a list of VUS in genes known to function as oncogenes or tumor suppressor genes tly in biochemical assays and therefore their impact in this cancer remains speculative.

- **The classification of variants of unknown significance can change over time. Please contact MedGenome at a later date for any change.**
- **Intronic variants are not assessed using this method.**
- **Large deletions or copy number variations cannot be assessed using this method.**
- **Certain genes may not be covered completely and few mutations could be missed.**
- **Only the genes that are 100% covered (Appendix-1) are included in this analysis.**

References:

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APPENDIX-1

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
ELF3	99.03	*HIST1H2BD*	100.00	*SIN3A*	100.00
PPOX	100.00	*EPHB6*	100.00	*CTCF*	100.00
CDKN2C	100.00	*PIK3CG*	99.51	*CDH1*	98.46
NRAS	100.00	*NFE2L3*	100.00	*CBFB*	100.00
RPL5	100.00	*KMT2C*	100.00	*CTD-2012K14.6*	100.00
ARID1A	100.00	*NDUFB2*	100.00	*CDK12*	100.00
RPL22	91.58	*HGF*	100.00	*WRAP53*	100.00
MTOR	100.00	*BRAF*	100.00	*MAP2K4*	99.20
B4GALT3	100.00	*EZH2*	100.00	*BRCA1*	99.38
PCBP1-AS1	100.00	*EGFR*	100.00	*MIR142*	100.00
ACVR2A	100.00	*RAD21-AS1*	100.00	*SOX9*	100.00
IDH1	100.00	*EGR3*	100.00	*CTD-2535L24.2*	100.00
ERBB4	100.00	*RP11-459E5.1*	100.00	*NCOR1*	98.62
SF3B1	100.00	*SOX17*	100.00	*AXIN2*	100.00
DNMT3A	98.79	*EPPK1*	100.00	*BZRAP1-AS1*	100.00
IDH1-AS1	100.00	*UTP23*	100.00	*SPOP*	100.00
PCBP1	100.00	*RAD21*	100.00	*VEZF1*	99.45
NFE2L2	100.00	*RP11-145E5.5*	100.00	*NF1*	100.00
TGFBR2	100.00	*CDKN2A*	100.00	*TP53*	98.74
TBL1XR1	100.00	*NOTCH1*	100.00	*SMAD2*	100.00
ATR	100.00	*TLR4*	89.52	*SETBP1*	100.00
PBRM1	100.00	*PTEN*	100.00	*RP11-729L2.2*	100.00
POLQ	100.00	*GATA3*	100.00	*RP11-456K23.1*	100.00
PIK3CA	99.49	*FGFR2*	100.00	*SMAD4*	100.00
VHL	86.66	*SMC3*	100.00	*PPP2R1A*	100.00
CTNNB1	100.00	*ARID5B*	100.00	*KMT2B*	100.00
BAP1	100.00	*ATM*	100.00	*PRX*	100.00
GNL3	100.00	*ORAOV1*	100.00	*KEAP1*	100.00
AK095242	100.00	*C11ORF65*	100.00	*ARHGAP35*	100.00
AK311005	100.00	*MALAT1*	100.00	*CEBPA*	100.00
RP11-641D5.2	100.00	*WT1*	100.00	*TSHZ3*	100.00
MECOM	100.00	*MAPK8IP1*	100.00	*ERCC2*	100.00
EPHA3	98.61	*CCND1*	100.00	*STK11*	100.00
EIF4A2	100.00	*LRRK2*	100.00	*FOXA2*	100.00
SETD2	100.00	*NAV3*	100.00	*ASXL1*	99.43
FBXW7	100.00	*H3F3C*	100.00	*AL354993.1*	99.07
TET2	99.67	*LYRM5*	100.00	*TSHZ2*	100.00
KIT	100.00	*ACVR1B*	100.00	*RUNX1*	100.00
FGFR3	100.00	*PTPN11*	99.86	*U2AF1*	100.00
CRIPAK	100.00	*CDKN1B*	100.00	*EP300*	100.00
FIP1L1	100.00	*TBX3*	100.00	*CHEK2*	100.00

Gene	Percentage of coding region covered	Gene	Percentage of coding region covered	Gene	Percentage of coding region covered
PDGFRA	100.00	*KMT2D*	100.00	*TAF1*	95.81
NSD1	100.00	*KRAS*	100.00	*RP1-315G1.3*	100.00
PIK3R1	99.36	*RB1*	100.00	*ATRX*	100.00
LIFR	100.00	*BRCA2*	100.00	*AR*	99.84
LIFR-AS1	100.00	*FLT3*	100.00	*SMC1A*	87.67
MAP3K1	100.00	*AKT1*	100.00	*KDM5C*	97.70
APC	100.00	*AJUBA*	100.00	*PHF6*	100.00
CTC-554D6.1	100.00	*RP11-298I3.5*	100.00	*USP9X*	100.00
NPM1	100.00	*FOXA1*	100.00	*KDM6A*	100.00
CDKN1A	100.00	*IDH2*	100.00	*STAG2*	100.00
HIST1H1C	100.00				

APPENDIX -2

Table 3	Variants of unknown significance in oncogenes and tumor suppressor genes

Sl. No.	Gene	CDS variant	Amino acid variant	Function of the gene in cancer
1.	*KMT2D*	c.3379_3383delGGGAT (ENST00000301067)	p.Gly1127Ter	TSG

TSG- Tumor Suppressor Gene

Table 4	Approved/ Investigational drugs against variants in oncogenes and tumor suppressor genes of this subject

Sl. No.	Gene	Drug (Generic name)	Approved in patient's cancer	Approved in other cancer
1.	***PTEN***	Temsirolimus, Everolimus (Approved)	No	Renal cell carcinoma
		Ridaforolimus (under clinical development)	-	-
2.	***STK11***	Temsirolimus, Everolimus (Approved)	No	Renal cell carcinoma
		Several under clinical development (Ref.13)	-	-

TSG- Tumor Suppressor Gene