UCSF Clinical Cancer Genomics Laboratory

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UCSF 500 Cancer Panel Final Report

CCGL No: CCGL-1135

Tumor
Source: Brain, left frontal lobe,
Diagnosis: Diffuse astrocytoma, IDH-mutant, WHO grade II

Normal
Source: Peripheral Blood

Pathogenic or Likely Pathogenic SOMATIC ALTERATIONS

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS	MUTANT ALLELE FREQUENCY
ATRX p.S1236fs	NM_000489	Pathogenic	1198	7%
ATRX p.M860fs	NM_000489	Pathogenic	1492	12%
ATRX p.D774fs	NM_000489	Pathogenic	1394	16%
IDH1 p.R132H	NM_005896	Pathogenic	1376	33%
TP53 p.L252fs	NM_000546	Pathogenic	659	30%
TP53 p.T155P	NM_000546	Pathogenic	709	28%

'Reads' indicate the number of unique DNA molecules sequenced. 'Mutant Allele Frequency' indicates the percentage of the reads with the respective 'Variant' and is affected by the degree of normal cell contamination of the sample and whether the variant is fully clonal or subclonal.

Pathogenic or Likely Pathogenic GERMLINE ALTERATIONS*

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS (Normal/Tumor)	MUTANT ALLELE FREQUENCY (Normal/Tumor)
MUTYH c.1187G>A, p.G396D	NM_001128425	Pathogenic	402/424	54%/50%

*Germline variants are reported if classified as pathogenic or likely pathogenic in ClinVar and confirmed by a CCGL molecular pathologist. For variants not classified in ClinVar, truncating variants in well-established tumor suppressor genes are reported if present in <1% of 1000g or esp6500 datasets. Germline variants are limited to single nucleotide variants and small indels in gene coding regions.

INTERPRETATION

This diffuse astrocytoma demonstrates the p.R132H hotspot mutation in IDH1 and two inactivating mutations in the TP53 tumor suppressor gene at clonal mutant allele frequencies, one missense and the other a frameshift. These mutations are too far apart to phase but are likely to be in occurring in trans and resulting in biallelic inactivation of TP53 in the tumor. Also seen are multiple frameshift mutations in the ATRX tumor suppressor gene, all at subclonal mutant allele frequencies suggesting that they are present within separate subclones of the tumor and arose during tumor progression. A couple somatic variants of unknown significance were also identified that are listed below. These include a missense mutation in the MET gene that localizes within the intracellular kinase domain of the encoded receptor tyrosine kinase. However, this specific variant has not been identified as a somatic variant before in human cancers [COSMIC database, version 81 release], and MET is not known to be a recurrently mutated gene in IDH-mutant diffuse lower-grade gliomas.

The tumor demonstrates a balanced diploid genome without chromosomal gains, losses, or focal amplifications or deletions identified. The tumor is microsatellite stable at the greater than 1,200 microsatellites assayed by this panel.

Together, the genetic profile is that of a diffuse astrocytic neoplasm, IDH-mutant. Diffuse astrocytomas, anaplastic astrocytomas, and secondary glioblastomas arising from lower-grade infiltrating astrocytomas within the cerebral hemispheres of adults are defined by the combination of IDH, ATRX, and TP53 mutations as seen in this tumor [refs.1-3]. Genetic features of an oligodendroglial neoplasm (e.g. chromosomes 1p/19 co-deletion and mutations in TERT promoter, CIC, or FUBP1) are not identified. Genetic features of an IDH-wildtype glioblastoma (e.g. EGFR, PTEN, CDKN2A, NF1, or TERT alterations) are not identified.

Identified in the submitted peripheral blood sample at heterozygous allele frequency is a missense variant in the MUTYH gene. This variant is present at 0.4% frequency in the NHLBI Exome Sequencing Project 6500 dataset and at 0.3% frequency in the ExAC database (dbSNP ID rs36053993). It has been found in individuals affected by MUTYH-associated polyposis and has been recurrently classified in the ClinVar database as Pathogenic by multiple submitters including

Invitae and Ambry Genetics. However, there is no loss of heterozygosity of this variant in the tumor, nor is a second alteration of the MUTYH gene identified in either the germline or tumor. Thus, the contribution of this MUTYH variant to this diffuse astrocytoma is uncertain, and this patient is expected to be a carrier only for MUTYH-associated polyposis (also known as familial adenomatous polyposis type 2, Online Mendelian Inheritance in Man entry #608456), an autosomal recessive disorder caused by homozygous or compound heterozygous inactivating germline mutations in the MUTYH gene on chromosome 1p34. Heterozygous carriers may possibly have an increased risk of colorectal and other cancers. Genetic counseling may be warranted.

References:
1. The Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. New England Journal of Medicine 372: 2481-2498, 2015.
2. Suzuki H, et al. Mutational landscape and clonal architecture in grade II and III gliomas. Nature Genetics 47: 458-468, 2015.
3. Eckel-Passow JE, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. New England Journal of Medicine 372: 2499-2508, 2015.

SOMATIC ALTERATIONS OF UNKNOWN SIGNIFICANCE*

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS	MUTANT ALLELE FREQUENCY
FRS2 p.N283S	NM_001278351	VUS	2161	18%
MET p.A1261G	NM_001127500	VUS	1613	17%

*The above variants have not yet been adequately characterized and are therefore classified as variants of unknown significance.

TEST METHODOLOGY:

The UCSF 500 Cancer Gene Test uses capture-based next-generation sequencing to target and analyze the coding regions of 479 cancer genes, as well as select introns of 47 genes (gene list on last page of this report). Genomic DNA was extracted from both tumor and normal tissue for library preparation. Target enrichment was performed by hybrid capture using custom oligonucleotides. Sequencing of captured libraries was performed on an Illumina HiSeq 2500. Sequence reads are de-duplicated to allow for accurate allele frequency determination and copy number calling. The analysis uses open source or licensed software for alignment to the human reference sequence UCSC build hg19 (NCBI build 37) and variant calling.

TEST LIMITATIONS:

This assay is designed to detect single nucleotide variants, small to medium insertion/deletions (indels), and copy number changes. Large insertions/deletions and gene rearrangements may also be detected by the assay; however, the sensitivity of detection of structural rearrangements is variable for different genes. If the pre-test probability of a structural rearrangement is high and the test is negative, an orthogonal testing method should be considered.

Specificity and sensitivity of this test to detect single nucleotide variants (SNVs) and small indels (≤ 5 bp) was determined by sequencing well characterized HapMap DNA samples from the Coriell Cell Repositories and comparing the genotypes produced by our assay with those from Illumina Platinum Genomes as the gold standard. For samples with at least 25% tumor, ≥ 200x coverage for the tumor sample, and ≥ 100x coverage for the normal sample, the sensitivity of the test for fully clonal SNVs and small indels is >98% and the positive predictive value for fully clonal SNVs and small indels is >99%. Sensitivity for detection of copy number changes is >98% for samples with high tumor content. Sensitivity for detection of NPM1, FLT3, and EGFR exons 19 and 20 insertions and deletions is 95%.

CLIA NOTE:

This test was developed and its performance characteristics determined by the UCSF Clinical Cancer Genomics Laboratory. It has not been cleared or approved by the U.S. Food and Drug administration. The Clinical Cancer Genomics Laboratory is certified by the Clinical Laboratory Improvement Act of 1988 (CLIA certified) and as such is allowed to perform high complexity clinical testing.

UCSF 500 Gene List										
ABL1	ABL2	ACVR1	ACVR1B	AJUBA	AKT1	AKT2	AKT3	ALK	AMER1	APC
APOBEC3G	AR	ARAF	ARFRP1	ARHGAP35	ARID1A	ARID1B	ARID2	ARID5B	ASH2L	ASXL1
ASXL2	ATF1	ATM	ATR	ATRX	AURKA	AURKB	AXIN1	AXIN2	AXL	BAP1
BARD1	BCL2	BCL2A1	BCL2L1	BCL2L12	BCL2L2	BCL6	BCOR	BCORL1	BLM	BRAF
BRCA1	BRCA2	BRD4	BRIP1	BTG1	BTK	C11orf30	CALR	CARD11	CBFB	CBL
CBLB	CCND1	CCND2	CCND3	CCNE1	CD274	CD79A	CD79B	CDC42	CDC73	CDH1
CDK12	CDK4	CDK6	CDK8	CDKN1A	CDKN1B	CDKN2A	CDKN2B	CDKN2C	CEBPA	CHD1
CHD2	CHD4	CHD5	CHEK1	CHEK2	CIC	CLDN18	CNOT3	COL1A1	COL2A1	CRCT1
CREB1	CREBBP	CRKL	CSF1R	CSF3R	CTCF	CTNNA1	CTNNB1	CUL3	CUX1	CXCR4
CYLD	DCC	DDIT3	DDR2	DDX3X	DDX41	DGKH	DICER1	DIS3	DNAJB1	DNMT3A
DOT1L	DUSP2	DUSP4	DUSP6	DYNC1I1	EBF1	EDNRB	EGFR	EGR1	EIF1AX	ELF3
EP300	EPCAM	EPHA2	EPHA3	EPHA5	EPHA7	EPHB1	EPOR	ERBB2	ERBB3	ERBB4
ERCC1	ERCC2	ERG	ERRFI1	ESPL1	ESR1	ESR2	ETS1	ETV6	EWSR1	EZH1
EZH2	FAM46C	FANCA	FANCC	FANCE	FANCF	FANCG	FANCL	FAT1	FAT3	FBXW7
FGF10	FGF14	FGF19	FGF23	FGF3	FGF4	FGF6	FGFR1	FGFR2	FGFR3	FGFR4
FH	FLCN	FLT1	FLT3	FLT4	FOXA1	FOXL2	FOXO1	FOXP1	FRS2	FUBP1
FUS	FYN	GAB2	GATA1	GATA2	GATA3	GLI1	GLI2	GNA11	GNA13	GNAQ
GNAS	GPC3	GPR124	GRIN2A	GRM3	GSK3B	H3F3A	H3F3B	HDAC4	HDAC9	HEY1
HGF	HIF1A	HIST1H3B	HMGA2	HNF1A	HOXB13	HRAS	HSP90AB1	HSPA2	HSPA5	ID3
IDH1	IDH2	IGF1R	IGF2	IGF2R	IKBKE	IKZF1	IKZF2	IKZF3	IL2RB	IL7R
INHBA	INPP4B	IPMK	IRF4	IRS2	JAK1	JAK2	JAK3	JAZF1	KAT6A	KDM5A
KDM5C	KDM6A	KDR	KEAP1	KIT	KLF4	KLHL6	KMT2A	KMT2B	KMT2D	KNSTRN
KRAS	LEF1	LIFR	LRP1B	LZTR1	MALAT1	MAML2	MAP2K1	MAP2K2	MAP2K4	MAP3K1
MAP3K2	MAP3K5	MAP3K7	MAP3K9	MAPK1	MCL1	MDM2	MDM4	MED12	MEF2B	MEN1
MET	MGA	MGMT	MITF	MLH1	MLH3	MPL	MRE11A	MSH2	MSH3	MSH6
MTOR	MUTYH	MYB	MYBL1	MYC	MYCL	MYCN	MYD88	MYH9	NAV3	NBN
NCKAP5	NCOA2	NCOA3	NCOR1	NF1	NF2	NFE2L2	NFKBIA	NFKBIE	NIPBL	NKX2-1
NOTCH1	NOTCH3	NPM1	NRAS	NSD1	NT5C2	NTRK1	NTRK2	NTRK3	NUP93	NUTM1
OR5L1	PAK1	PAK3	PALB2	PARK2	PAX3	PAX5	PAX7	PAX8	PBRM1	PDCD1LG2
PDGFB	PDGFRA	PDGFRB	PDK1	PHF6	PHOX2B	PIK3CA	PIK3CG	PIK3R1	PIK3R2	PLAG1
PLCB4	PMS1	POLD1	POLE	POLQ	POT1	POU3F2	PPM1D	PPP2R1A	PPP6C	PRDM1
PREX2	PRKACA	PRKAG2	PRKAR1A	PRKCA	PRKCH	PRKDC	PTCH1	PTCH2	PTEN	PTK2B
PTPN1	PTPN11	PTPRB	PTPRD	PTPRK	PTPRT	RAC1	RAD21	RAD50	RAD51	RAD51C
RAD51D	RAF1	RARA	RASA1	RASA2	RB1	RBM10	REL	RELA	RET	RHEB
RHOA	RICTOR	RIT1	RNF43	ROBO1	ROS1	RPL10	RPTOR	RRAGC	RRAS	RRAS2
RSPO2	RSPO3	RUNX1	RUNX1T1	SDHB	SDHD	SETBP1	SETD2	SF3B1	SH2B3	SHH
SIN3A	SLIT2	SLITRK6	SMAD2	SMAD3	SMAD4	SMARCA2	SMARCA4	SMARCB1	SMC1A	SMC3
SMO	SNCAIP	SOCS1	SOS1	SOS2	SOX10	SOX2	SOX9	SPEN	SPOP	SPRED1
SPRY1	SPRY2	SPRY4	SPTA1	SRC	SRSF2	SS18	STAG2	STAT3	STAT4	STAT6
STK11	SUFU	SYK	SYNE1	TADA1	TBX3	TCEB1	TCF7L2	TERT	TET2	TFE3
TFEB	TGFBR2	TLR4	TMPRSS2	TNFAIP3	TNFRSF14	TOP1	TOP2A	TP53	TRAF3	TRAF7
TRIM28	TSC1	TSC2	TSHR	TSHZ2	TSHZ3	TSLP	TTYH1	TYK2	U2AF1	USP7
VEGFA	VHL	WHSC1	WISP3	WRN	WT1	XBP1	XPO1	YAP1	YWHAE	ZBTB20
ZFHX3	ZFHX4	ZMYM3	ZNF217	ZNF703	ZRSR2					