UCSF Clinical Cancer Genomics Laboratory

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UCSF 500 Cancer Panel Final Report

CCGL No: CCGL-1044
Date: 06/12/2017

Tumor
Source: Brain, left insular
Diagnosis: Residual/recurrent high grade diffuse glioma

Normal
Source: Peripheral Blood
Collected:

Pathogenic or Likely Pathogenic SOMATIC ALTERATIONS

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS	MUTANT ALLELE FREQUENCY
ATRX c.4214+3A>G	NM_000489	Pathogenic	238	72%
IDH1 p.R132H	NM_005896	Pathogenic	880	37%
TP53 p.V173L	NM_000546	Pathogenic	610	73%
CDKN2A, CDKN2B deep deletion	all	Pathogenic	N/A	N/A
PDGFRA high level amplification and intragenic rearrangement	NM_006206	Pathogenic	~6,000 (>10x)	N/A
PDGFRA p.D284I	NM_006206	Likely Pathogenic	6203	13%
PDGFRA p.G313S	NM_006206	Likely Pathogenic	5807	30%
PDGFRA p.K369N	NM_006206	Likely Pathogenic	4841	28%
PDGFRA p.E372K	NM_006206	Likely Pathogenic	4372	2%

'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)
No pathogenic or likely pathogenic alterations identified.				

*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 residual/recurrent high grade diffuse glioma centered within the cerebral hemispheres demonstrates the p.R132H hotspot mutation in IDH1, a splice site mutation in the ATRX tumor suppressor gene, and an inactivating missense mutation in the TP53 tumor suppressor gene accompanied by loss of the remaining wildtype allele. The splice site mutation in ATRX is at the +3 position of the splice donor, which is not a uniformly conserved nucleotide among all splice sites in the human genome. However, given that IDH-mutant diffuse astrocytic neoplasms are defined by inactivating ATRX mutations and that this tumor demonstrated somatic loss of ATRX expression by immunostaining using an antibody that recognizes an epitope at the C-terminus of the ATRX protein, this mutation is therefore almost certainly disrupting appropriate splicing.

Additionally present is focal homozygous deletion of the CDKN2A and CDKN2B tumor suppressor genes on chromosome 9p21. Also, there is focal high level amplification of the PDGFRA oncogene on chromosome 4q. This amplification of PDGFRA is accompanied by intragenic rearrangement and multiple missense mutations on the amplified alleles. This rearrangement is an intragenic deletion of exons 9-10, with breakpoints identified in intron 8-9 and intron 10-11. While most of the PDGFRA missense mutations are too far apart to phase, the p.K369N and p.E372K variants are present in trans (i.e not on the same allele). One or more of these PDGFRA missense mutations and intragenic deletion are very likely to be causing activation of the amplified PDGFRA alleles, although the p. E372K variant is the only one that has been previously identified in gliomas [COSMIC database v81 release].

Additionally, a few somatic variants of unknown significance are identified that are listed below. However, the somatic mutation burden in this tumor is not suggestive of the hypermutation that is known to occur in a subset of gliomas following treatment with temozolomide [ref. 1].

Chromosomal copy number changes in the tumor include gains of 10p and most of 8, as well as losses of portions of 2q, proximal 4q, distal 7p, distal 9p, 11p, interstitial 13q, 14q, distal 16q, and proximal 21q.

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. 2-4]. Genetic features of an oligodendroglial neoplasm (e.g. chromosomes 1p/19 co-deletion and mutations in TERT promoter, CIC, or FUBP1) are not identified. The presence of CDKN2A homozygous deletion as seen in this tumor is associated with worse outcomes in IDH-mutant diffuse astrocytic neoplasms [ref. 5]. The additional PDGFRA activation seen in this tumor is also suggestive of aggressive clinical behavior beyond the vast majority of IDH-mutant diffuse astrocytic neoplasms that lack genetic alterations involving receptor tyrosine kinases [refs. 2-3].

References:
1. Johnson BE, et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science 343: 189-193, 2014.
2. The Cancer Genome Atlas Research Network. Comprehensive integrative genomic analysis of diffuse lower-grade gliomas. New England Journal of Medicine 372: 2481-2498, 2015.
3. Suzuki H, et al. Mutational landscape and clonal architecture in grade II and III gliomas. Nature Genetics 47: 458-468, 2015.
4. 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.
5. Reis GF, et al. CDKN2A loss is associated with shortened overall survival in lower-grade astrocytomas. Journal of Neuropathology & Experimental Neurology 74: 442-452, 2015.

SOMATIC ALTERATIONS OF UNKNOWN SIGNIFICANCE*

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS	MUTANT ALLELE FREQUENCY
IKZF2 p.C170G	NM_016260	VUS	678	32%
TSHR p.E506K	NM_000369	VUS	822	39%
TSHZ3 p.E235*	NM_020856	VUS	1071	8%

*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					