



Contact:
 2340 Sutter Street, Room S151
 San Francisco, CA 94115
 Tel: (415) 502-3252
 Fax: (415) 502-2773
 Email: ccgl@ucsf.edu

Executive Director:
 Boris C. Bastian, MD
Medical Director:
 James P. Grenert, MD, PhD
Associate Directors:
 Courtney Onodera, PhD
 Jessica Van Ziffle, PhD
 Iwei Yeh, MD, PhD

UCSF500 Gene Panel Final Report

CCGL No: CCGL-3205

Tumor

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

Normal

Source: Buccal Cells

Pathogenic or Likely Pathogenic SOMATIC ALTERATIONS

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS	MUTANT ALLELE FREQUENCY
IDH1 p.R132H	NM_005896.2	Pathogenic	709	8%
TP53 heterozygous (one copy) deletion of 5' coding exons	NM_000546	Pathogenic	N/A	heterozygous

'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 ALTERATIONS IN THE NORMAL SAMPLE*

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS (Normal/Tumor)	MUTANT ALLELE FREQUENCY (Normal/Tumor)
TP53 c.743G>A, p.R248Q	NM_000546.5	Pathogenic	347/224	55%/44%

*Alterations in the normal sample are reported for cancer-related genes if classified as pathogenic or likely pathogenic in ClinVar and confirmed by a CCGL molecular pathologist. For variants not classified in ClinVar, truncating or splice-site variants in well-established tumor suppressor genes are reported if present in <1% of 1000g or esp6500 datasets. Alterations in the normal sample are limited to single nucleotide variants and small indels in gene coding regions. Carrier status is not reported for variants not strongly related to cancer.

INTERPRETATION

As previously reported, the submitted buccal swab sample contains a pathogenic missense mutation in the TP53 tumor suppressor gene, which is present at a heterozygous allele frequency of approximately 50% consistent with being present in the germline. This sequence change replaces arginine with glutamine at codon 248 of the encoded p53 protein. The arginine residue is highly conserved and there is a small physicochemical difference between arginine and glutamine. This variant (dbSNP ID rs11540652) is extremely rare in human populations (0% frequency in the 1000genomes dataset, 0.008% in ESP6500, 0.006% in ExAC). This variant has been recurrently classified as Pathogenic in the ClinVar database by multiple submitters including Invitae, Ambry Genetics, and GeneDx. This variant has been reported in multiple individuals and families affected with Li-Fraumeni syndrome (PMID: 1565143, 17606709, 21601526, 7887414, 21305319). Experimental studies have shown that this missense change severely affects the functional activity of the p53 protein, abolishing its DNA-binding and transcriptional transactivation activities (PMID: 21343334, 17606709, 20128691). The tumor demonstrates a focal deletion event on chromosome 17p13 that encompasses the 5' coding exons of the TP53 gene, which is likely causing inactivation of the remaining wildtype allele. Together, these findings are considered diagnostic of Li-Fraumeni syndrome, a tumor predisposition syndrome with significantly increased risk of multiple malignancies including osteosarcoma and diffuse gliomas (Online Mendelian Inheritance in Man, entry #151623). Genetic counseling of this child and his family is recommended.

Somatic alterations in this diffuse astrocytoma centered in the cerebral hemispheres include the p.R132H hotspot mutation in the IDH1 oncogene and focal heterozygous (one copy) deletion of the 5' coding exons of the TP53 tumor suppressor gene.

The somatic mutation burden of this astrocytoma is very low, with less than 1 somatic mutation per Mb based on this assay. Only 4 of the 1,015 assessed microsatellites (<1%) demonstrate instability, consistent with a microsatellite stable tumor.

No chromosomal gains or losses are seen.

Together, the genetic profile is that of a diffuse astrocytic neoplasm, IDH-mutant, arising in the setting of Li-Fraumeni syndrome. Diffuse astrocytomas, anaplastic astrocytomas, and secondary glioblastomas arising from progression of lower-grade infiltrating astrocytomas within the cerebral hemispheres of adults are genetically defined by the combination of IDH and TP53 mutations, as seen in this tumor [refs. 1-3]. In adults with IDH-diffuse astrocytic neoplasms, there is very frequently accompanying ATRX inactivation associated with alternative lengthening of telomeres [refs. 1-3]. However, IDH-mutant diffuse astrocytomas in children and teenagers often lack this accompanying ATRX mutation, presumably due to the lesser number of cell divisions that have taken place in the tumor-

initiating cell and the absence of selection for telomere maintenance mechanism [refs. 4-5].

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. 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.
3. Suzuki H, et al. Mutational landscape and clonal architecture in grade II and III gliomas. *Nature Genetics* 47: 458-468, 2015.
4. Ferris SP, ..., Solomon DA. IDH1 mutation can be present in diffuse astrocytomas and giant cell glioblastomas of young children under 10 years of age. *Acta Neuropathologica* 132: 153-155, 2016.
5. Lee J, Putnam AR, Chesier SH, ..., Solomon DA. Oligodendrogliomas, IDH-mutant and 1p/19q-codeleted, arising during teenage years often lack TERT promoter mutation that is typical of their adult counterparts. *Acta Neuropathologica Communications* 6: 95, 2018.

SOMATIC ALTERATIONS OF UNKNOWN SIGNIFICANCE*				
VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS	MUTANT ALLELE FREQUENCY
No somatic variants of uncertain significance identified.				

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

TEST METHODOLOGY:

The UCSF500 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 by the UCSF Genomic Sequencing Services Lab at Institute for Human Genetics CLIA laboratory (San Francisco, CA). 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. Microsatellite instability analysis is performed with MSIsensor (Niu B et al, 2014). MSI-High cases have greater than 30% of sites unstable and Microsatellite stable (MSS) cases have less than 20% of sites unstable, corresponding to the MSI-High or MSS categories of the revised Bethesda guidelines (Umar A et al, 2004). Cases that have 20-30% of sites unstable are equivocal between MSS and MSI-High.

REFERENCES

1. Niu B, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics*. 2014 Apr 1;30(7):1015-6. PubMed PMID: 24371154.
2. Umar A, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst*. 2004 Feb 18;96(4):261-8. PubMed PMID: 14970275.

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 dependent on the specific rearrangement. If the pre-test probability of a structural rearrangement is high and the test is negative, an orthogonal testing method should be considered.

Sensitivity and positive predictive value 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 and sequencing tumor samples also sequenced at reference laboratories. For samples with at least 25% tumor, $\geq 200\times$ coverage for the tumor sample, and $\geq 100\times$ 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%. Sequencing of target intervals is performed to high depth, with greater than 250x mean target coverage. Less than 0.5% of the exonic footprint of targeted genes performs with a mean of $<100\times$ coverage with reduced sensitivity. These regions are not recurrently mutated in cancer and are available on request.

Microsatellite instability is assessed using MSIsensor analysis of hundreds of mononucleotide and dinucleotide repeats. In a cohort of 42 validation cases, there was 100% concordance between results of MSIsensor and MSI-High and MSS classification of microsatellite instability by multiplex PCR (Promega).

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	ATRAX	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	DYNC111	EBF1	EDNRB	EGFR	EGR1	EIF1AX	ELF3
EP300	EPCAM	EPHA2	EPHA3	EPHA5	EPHA7	EPHB1	EPOR	ERBB2	ERBB3	ERBB4
ERCC1	ERCC2	ERG	ERRF1	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	SOC3S1	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
ZFH3	ZFH4	ZMYM3	ZNF217	ZNF703	ZRSR2					

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