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# UCSF500 Gene Panel Final Report

CCGL No: CCGL-3619

**Tumor**
*Source:* Brain, left frontal, *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 c.6110+3_6110+6delAAGT | NM_000489.3 | Pathogenic | 326 | 31% |
| ATRX p.E886fs | NM_000489.3 | Pathogenic | 532 | 34% |
| ATRX p.I360fs | NM_000489.3 | Pathogenic | 495 | 11% |
| IDH1 p.R132H | NM_005896.2 | Pathogenic | 1105 | 42% |
| NF1 p.T1273fs | NM_001042492.2 | Pathogenic | 1209 | 3% |
| NOTCH1 p.S990fs | NM_017617.3 | Pathogenic | 197 | 11% |
| SPRED1 p.V309fs | NM_152594.2 | Pathogenic | 1162 | 14% |
| TP53 p.Y163C | NM_000546.5 | Pathogenic | 397 | 83% |

'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) |
|---|---|---|---|---|
| IDH1 p.R132H | NM_005896.2 | Pathogenic | 786 peripheral blood, 754 buccal swab, 1105 tumor | 6% peripheral blood, 3% buccal swab, 42% tumor |

*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

In this individual with a clinical diagnosis of Ollier disease and a personal history of multiple benign bone tumor resections (likely enchondromas but pathology has not been reviewed at UCSF Medical Center for any of these lesions), there are IDH1 p.R132H mutant alleles detected at 6% frequency in the peripheral blood sample, as well as at 3% frequency in a buccal swab sample that was also sequenced on the UCSF500 NGS Panel (collection date 7/18/2019, library prep ID CGP-10513, no separate report issued). This genetic result supports that this patient does indeed have Ollier disease due to constitutional or post-zygotic mosaicism for the p.R132H activating mutation in the IDH1 oncogene. Ollier disease (also known as multiple enchondromatosis syndrome or Maffucci syndrome when accompanied by hemangiomata, Online Mendelian Inheritance in Man %166000) is a tumor predisposition syndrome caused by constitutional mosaicism for an activating mutation at codon p.R132 of the IDH1 gene or codon p.R172 of the IDH2 gene and is characterized by multiple enchondromas, soft tissue hemangiomas, and diffuse astrocytomas [refs. 1-3]. The post-zygotic IDH1 p.R132H mutation in this patient is at a clonal allele frequency of 42% in the diffuse astrocytoma, indicating that this tumor arose from a precursor cell affected by the mosaicism. Genetic counseling is recommended.

This diffuse astrocytoma centered in the cerebral hemispheres demonstrates the p.R132H hotspot mutation in the IDH1 oncogene, a damaging missense mutation in the TP53 tumor suppressor gene with loss of the remaining wildtype allele, and multiple truncating mutations in the ATRX tumor suppressor

gene. Additionally seen at subclonal allele frequencies are a truncating frameshift mutation in the NF1 tumor suppressor gene, a truncating frameshift mutation in the SPRED1 tumor suppressor gene, and a truncating frameshift mutation in the NOTCH1 tumor suppressor gene.

The somatic mutation burden is low overall, with less than 3 somatic mutations per Mb based on this assay. Only 4 of the 1,129 assessed microsatellites (<1%) demonstrate instability, consistent with a microsatellite stable tumor.

Chromosomal copy number analysis reveals losses of distal 10q and distal 11p. There is also copy-neutral loss of heterozygosity involving chromosome 17p (containing TP53). No focal amplifications or deep deletions are seen.

Together, the genetic profile is that of a diffuse astrocytic neoplasm, IDH-mutant, arising in the setting of Ollier disease. Diffuse astrocytomas, anaplastic astrocytomas, and secondary glioblastomas arising from lower-grade infiltrating astrocytomas within the cerebral hemispheres of younger adults are genetically defined by the combination of IDH, TP53, and ATRX mutations, as seen in this tumor [refs. 4-6]. The additional genetic changes that drive anaplastic/malignant transformation in IDH-mutant diffuse astrocytic neoplasms are just beginning to be elucidated, but studies to date have indicated that alterations causing activation of the Ras-Raf-MAP kinase signaling are one potential pathway [refs. 7-8]. The additional subclonal NF1 and SPRED1 inactivating mutations are expected to be causing MAP kinase pathway activation in a subset of the tumor cells and may likely correspond with the focally increased mitotic activity in this diffuse astrocytoma. This tumor lacks the CDKN2A homozygous deletion that has been associated with worse prognosis when present in IDH-mutant diffuse astrocytic neoplasms [ref. 9].

References:
1. Pansuriya TC, et al. Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. Nature Genetics 43: 1256-1261, 2011.
2. Amary MF, et al. Ollier disease and Maffucci syndrome are caused by somatic mosaic mutations of IDH1 and IDH2. Nature Genetics 43: 1262-1265, 2011.
3. Bonnet C, et al. Characteristics of gliomas in patients with somatic IDH mosaicism. Acta Neuropathologica Communications 4: 31, 2016.
4. The Cancer Genome Atlas Research Network. Comprehensive integrative genomic analysis of diffuse lower-grade gliomas. New England Journal of Medicine 372: 2481-2498, 2015.
5. 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.
6. Suzuki H, et al. Mutational landscape and clonal architecture in grade II and III gliomas. Nature Genetics 47: 458-468, 2015.
7. Bai H, et al. Integrated genomic characterization of IDH1-mutant glioma malignant progression. Nature Genetics 48: 59-66, 2016.
8. Johnson BE, et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science 343: 189-193, 2014.
9. Shirahata M, et al. Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. Acta Neuropathologica 136: 153-166, 2018.

**SOMATIC ALTERATIONS OF UNKNOWN SIGNIFICANCE***

| VARIANT | TRANSCRIPT ID | CLASSIFICATION | READS | MUTANT ALLELE FREQUENCY |
|---|---|---|---|---|
| IGF2R p.Q1135* | NM_000876.2 | VUS | 1142 | 21% |

*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, ≥ 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%. 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 <100x 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 | 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 | | | | | |