

Patient		Accession #: MGP19-###
DOB/Age/Sex		Client Accession #:
Client Identifier	Client	
Collection Date		
Accession Date	Requesting Physician	
Reported Date	Ordering Physician	
	T	
	F	

Specimen Type: Paraffin, Block:#
RIGHT SUBOCCIPITAL TUMOR

GLIOSEQ[®] RESULTS SUMMARY

Genomic Alterations Identified:

- **H3F3A mutation p.K28M**
- **TP53 mutation p.E258K**
- **TP53 copy number LOSS**
- See Interpretation and Detailed Results

INTERPRETATION

The mutation profile detected in this tumor (IDH wild-type, H3F3A, TP53 mutation) is typical of diffuse midline glioma H3 K28M-mutant, which corresponds to WHO grade IV and confers a poor prognosis. Mutation in H3F3A, which encodes the replication-independent histone 3 variant H3.3, was detected at p.K28M position (known in the literature as K27M). This mutation is commonly found in diffuse midline glioma of children and in a small subset of adult patients, with the most common locations being brain stem, thalamus and spinal cord (1,2). In addition, H3F3A mutated high-grade gliomas commonly harbor concurrent alterations in TP53.

H3F3A

H3F3A, which encodes the replication-independent histone 3 variant H3.3, is mutated in many pediatric gliomas. However, more recently it has been reported in diffuse midline gliomas (brainstem and thalamic gliomas) of adults <50 years of age. H3F3A K28M (K27M in published literature) was the only mutation type reported in these tumors and the presence of this mutation in brainstem gliomas was associated with poorer prognosis than in thalamic gliomas. H3F3A K28M mutation has been shown to often overlap with TP53 mutations.

DETAILED RESULTS

Marker Type	Marker Result			AF/CNR	Class
Gene mutations	TP53	p.E258K	c.772G>A	73%	Tier 1/2
	H3F3A	p.K28M	c.83A>T	39%	Tier 1/2
Gene fusions	Negative				
Copy number alterations	TP53	17p13.1	LOSS		

AF=Variant Allele Frequency, Tier 1/2=Variants of Clinical or Potential Clinical Significance, VUS=Variants of Uncertain Significance, Estimated copies of gene=copy number ratio (CNR) x 2

CLINICAL TRIAL INFORMATION (details available at <http://molecularmatch.com/>)

- **TP53:** NCT01849146 - Phase I Study of AZD1775 (MK-1775) With Radiation and Temozolomide in Patients With Newly Diagnosed Glioblastoma and Evaluation of Intratumoral Drug Distribution in Patients With Recurrent Glioblastoma
- **TP53:** NCT02942264 - Phase I Trial of TG02 Plus Dose-Dense or Metronomic Temozolomide Followed by Randomized Phase II Trial of TG02 Plus Temozolomide Versus Temozolomide Alone in Adults With Recurrent Anaplastic Astrocytoma and Glioblastoma

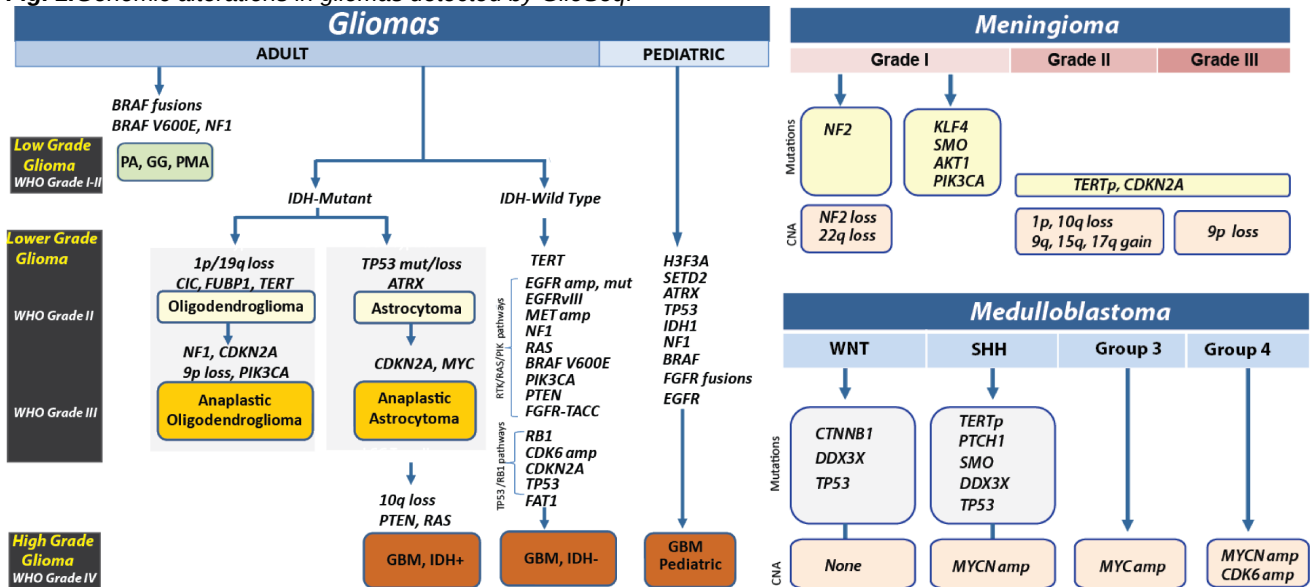
NOTE

The quantity and quality of isolated DNA and RNA were sufficient for the analysis. Amplification of controls was acceptable.

BACKGROUND

Molecular markers are used to support and enhance the diagnosis, prognosis and treatment of adult and pediatric CNS tumors. The GlioSeq test identifies genetic alterations that are relevant to different CNS tumors subtypes and grades (1). Low grade gliomas (WHO Grade I-II) , e.g. pilocytic astrocytoma, pilomyxoid astrocytoma, ganglioglioma, and pleomorphic xanthoastrocytoma often harbor mutations or gene fusions in BRAF (2,3). Neurofibromatosis type 1 associated pilocytic astrocytoma characteristically harbor mutations and/or loss in NF1 gene, resulting in bi-allelic inactivation of the gene (4). Diffusely infiltrative Gliomas (WHO grade II-III) were recently classified into three glioma subtypes based on histopathologic features, molecular alterations, and clinical behavior (5). Lower Grade Glioma (LGG) Type 1 or oligodendrogliomas harbor IDH mutations, 1p/19q co-deletion, TERT promoter mutation and alterations in CIC, and FUBP1 genes. LGG Type 2 or infiltrating astrocytoma of adults harbor IDH, TP53, and ATRX mutations. Both can progress to a higher grade glioma by acquiring additional genomic alterations in the RTK-RAS-PI3K pathway genes. LGG Type 3 do not harbor IDH mutations, but have genetic alterations similar to high grade gliomas (WHO grade IV) and considered to be a precursor to secondary GBMs (5,6). Primary GBMs are IDH wild-type and harbor a number of genetic alterations that lead to dysregulation of critical signaling pathways including i) receptor tyrosine kinase (RTK)/RAS/PI(3)K pathway via amplification and mutation in EGFR, PIK3CA, RAS, NF1, EGFR, and MET and FGFR fusions, ii) TP53 and RB1 pathways via inactivation mutation/loss of TP53, CDKN2A, and RB1 genes, and iii) TERT promoter mutations (6). Pediatric gliomas are unique, featuring mutations and other genetic alterations in H3F3A, SETD2, ATRX, NF1, and BRAF. IDH mutations are rare and usually restricted to adolescent patients. They also can harbor fusions involving BRAF and FGFR genes (7,8).

Fig. 1. Genomic alterations in gliomas detected by GlioSeq.



Medulloblastomas have been recently classified into four groups (WNT (wingless), SHH (sonic hedgehog), Group 3, and Group 4) based on molecular profiling and clinical outcome. Mutations in CTNNB1 or DDX3X can help to identify Wnt pathway medulloblastomas that tend to have a much better prognosis. Tumors with PTCH, SMO, and TERT promoter alterations characterize the Shh class, and have intermediate prognosis between Wnt and group 3/4 tumors. In contrast, MYC or MYCN/CDK6 amplification are characteristic of group 3 and 4 medulloblastomas, respectively, and are far more likely to metastasize and have a poor prognosis even with therapy (7,9).

Finally, inactivation of NF2 via mutation or loss of 22q is the most common early genetic alteration in meningiomas and multiple

copy number alterations including 1p,10q, and 9p (CDKN2A) loss and TERT promoter mutations are seen in higher grade meningiomas. Recurrent mutations in KLF4, AKT1, and SMO genes are often present in NF2-negative sporadic meningiomas (10,11). These and other genetic alterations can serve as diagnostic, prognostic, and predictive biomarkers for tumor classification, patient risk stratification, and targeted therapies.

References:

1.Nikiforova, MN *Neuro Oncol* 2016 18 379-87 3, 2.Schindler, G *Acta Neuropathol* 2011 121 397-405 3, 3.Lee, EQ *J Clin Oncol* 2016 34 e87-9 10, 4.Gutmann, DH *Genome Res* 2013 23 431-9 3, 5.Brat, DJ *N Engl J Med* 2015 372 2481-98 26, 6.Aldape, K *Acta Neuropathol* 2015 129 6 829-48, 7.Gajjar, A *J Clin Oncol* 2015 33 2986-98 27, 8.Korshunov, A *Acta Neuropathol* 2015 129 669-78 5, 9.Gajjar, AJ *Nat Rev Clin Oncol* 2014 11 714-22 12, 10.Clark, VE *Science* 2013 339 1077-80 6123, 11.Suva, ML *Curr Opin Neurol* 2013 26 681-7 6

METHODOLOGY

For surgical specimens, manual microdissection of tumor target is performed from unstained slides under the microscope with H&E guidance. Genomic DNA and RNA are isolated from FFPE tissue specimens using standard laboratory procedure. GlioSeq next generation sequencing analysis is performed to detect base substitutions (SNVs) and small insertions/deletions in targeted regions of 30 key brain tumors genes, for copy number changes in 24 genes, and 14 types of structural alterations involving BRAF, EGFR (EGFRvIII) and FGFR3 genes. The Torrent Suite Software v5.8 is used for data analysis. The analytical sensitivity is 3-5% allele frequency (AF) for detection of SNVs and indels (<40bp) and 1-5% for detection of fusions, and 30-40% for detection of copy number alterations. The minimal required sequencing depth is 300x. If 300x coverage not achieved for the TERT gene, bidirectional Sanger sequencing analysis is performed. Other genetic regions that did not meet minimal sequencing coverage requirements are specified in the report as failed. Gene expression of PGK, HPRT1, GUSB genes is used to control the quality of the tested specimen. Copy Number Ratio (CNR) is established for chromosomal gains to estimate the level of gene amplification. GRCh37 human reference genome (GCA_000001405.1) and HGVS variant nomenclature was used for analysis and reporting.

GENES ASSAYED BY GLIOSEQ

Substitutions and indels:

AKT1	ATRX	BRAF	CDK6	CDKN2A	CIC	CTNNB1	DDX3X
EGFR	FUBP1	H3F3A	HRAS	IDH1	IDH2	KLF4	KRAS
MET	MYC	MYCN	NF1	NF2	NRAS	PIK3CA	PTCH1
PTEN	RB1	SETD2	SMO	TERT	TP53		

Copy number alterations:

ATRX	CDK6	CDKN2A	CIC	CTNNB1	DDX3X	EGFR	FUBP1
HRAS	IDH2	KRAS	MET	MYC	MYCN	NF1	NF2
NRAS	PIK3CA	PTCH1	PTEN	RB1	SETD2	SMO	TP53

Gene fusions:

EGFRvIII KIAA1549/BRAF FAM131B/BRAF FGFR3/TACC3

ADDITIONAL DETAILS OF DNA SEQUENCE VARIANTS

Gene	Transcript	Genomic Position
TP53	NM_000546.5	chr17:7577509C>T
H3F3A	NM_002107	chr1:226252135A>T

LOW COVERAGE HOTSPOTS OBSERVED IN THE FOLLOWING GENES

NONE

GROSS DESCRIPTION

1 part(s) labeled with patient name and identifiers received.

Sample 1: 10 unstained and 1 stained slide received and labeled ##.

DISCLAIMER

GlioSeq was developed and its performance characteristics determined by the UPMC Molecular and Genomic Pathology laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research only. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. GlioSeq test does not sequence genes in their entirety and mutations outside of mutation hotspots, some insertions and deletions may not be detected. This test does not provide information on germline or somatic status of detected mutations. Copy number ratio (CNR) provides only estimated level of gene amplification. Certain sample characteristics may result in reduced sensitivity, including sample heterogeneity, low sample quality, and other causes. The information in this report must be used in conjunction with all relevant clinical information and does not intend to substitute clinical judgment. Decisions on patient care must be based on the independent clinical judgment of the treating physician. A treating physician's decision should not be based solely on this or any other single tests or the information in this report.

Electronically signed out by:

Simion Chiose, MD

01/31/2019 12:12