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: #
BRAIN TUMOR

GLIOSEQ® RESULTS SUMMARY

Genomic Alterations Identified:

- **EGFRvIII mutation**
- **EGFR amplification**
- **TERT mutation p.C228T**
- **PTEN copy number LOSS**
- **CDKN2A copy number LOSS**
- **RB1 copy number LOSS**
- See Interpretation and Detailed Results

INTERPRETATION
The mutation profile detected in this tumor (IDH wild-type and EGFR/PTEN/CDKN2A/TERT/RB1 alterations) is characteristic of high grade gliomas (WHO Grade IV). The majority of high grade gliomas exhibit a number of genetic alterations affecting major cancer pathways, including RTK/RAS/PI3K signaling pathway (mutations and copy number alterations in EGFR, PTEN, PIK3CA, NF1, RAS, MET) and p53/Rb tumor suppressor pathway (TP53, CDKN2A, CDK6, RB1), and are characterized by unfavorable prognosis.

EGFRvIII
Activating genomic alterations in EGFR are frequent events in glioblastomas, more so in primary (IDH wild type) GBMs. About 50% of EGFR-amplified GBMs harbor the EGFRvIII mutation, which is an inframe deletion of 267 amino acid residues (exon 2-7) in the extracellular domain of EGF receptor. Immune therapy has been reported to be a promising therapeutic option for newly diagnosed glioblastomas with EGFRvIII mutation. Several possible clinical trials are listed below and additional details can be found at https://clinicaltrials.gov.

DETAILED RESULTS

Marker Type	Marker Result			AF/CNR	Class
Gene mutations	TERT	p.C228T	c.1-124C>T	33%	Tier 1/2
Gene fusions	EGFRvIII				
Copy number alterations	EGFR	7p11.2	GAIN	38.7	
	PTEN	10q23.31	LOSS		
	CDKN2A	9p21.3	LOSS		
	RB1	13q14.2	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/)

- **TERT**: NCT03491683 - An Open-Label, Multi-Center Trial of INO-5401 and INO-9012 Delivered by Electroporation (EP) in Combination With REGN2810 in Subjects With Newly-Diagnosed Glioblastoma (GBM)
- **EGFR**: NCT03296696 - Phase 1 Study to Evaluate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of AMG 596 in Subjects With Glioblastoma Expressing Mutant Epidermal Growth Factor Receptor Variant III (EGFRvIII)
- **EGFR**: NCT01454596 - A Phase I/II Study of the Safety and Feasibility of Administering T Cells Expressing Anti-EGFRvIII Chimeric Antigen Receptor to Patients With Malignant Gliomas Expressing EGFRvIII
- **EGFR**: NCT02861898 - Phase I/II Trial of Super-selective Intra-arterial Repeated Infusion of Cetuximab for the Treatment of Newly Diagnosed Glioblastoma
- **EGFR**: NCT02573324 - A Randomized, Placebo Controlled Phase 3 Study of ABT-414 With Concurrent Chemoradiation and Adjuvant Temozolomide in Subjects With Newly Diagnosed Glioblastoma (GBM) With Epidermal Growth Factor Receptor (EGFR) Amplification (Intellance1)
- **PTEN**: NCT02465060 - Molecular Analysis for Therapy Choice (MATCH)

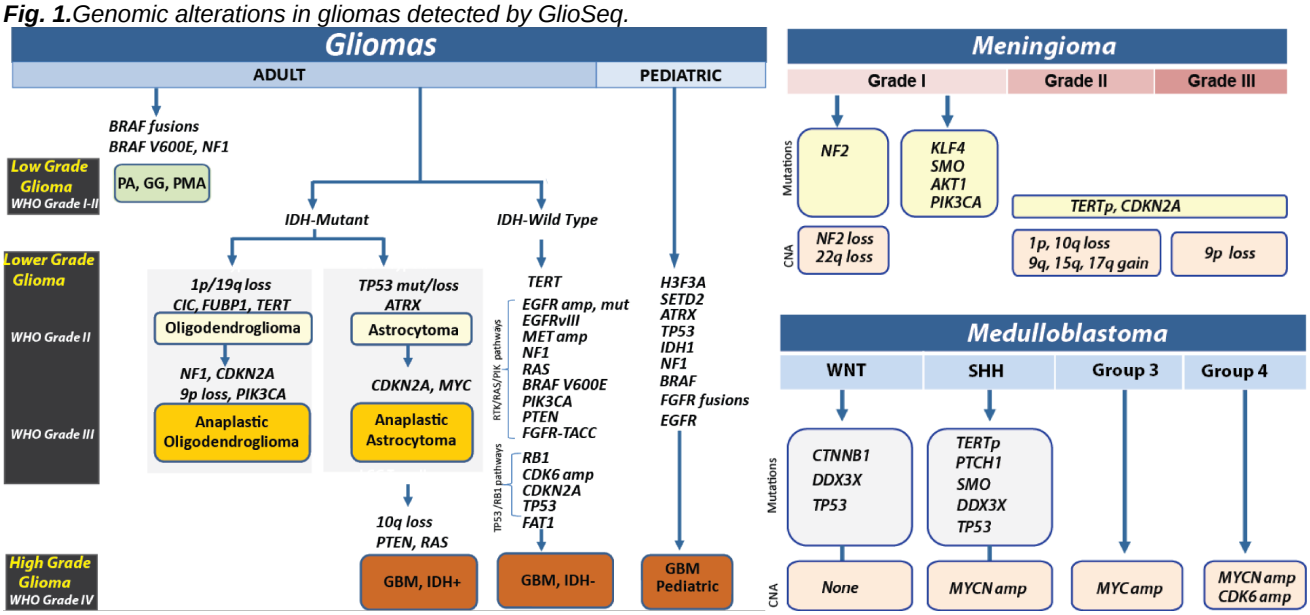
NOTE

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 harbors 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
TERT	NM_198253.2	chr5:1295228G>A

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:

Yuri Nikiforov, MD, PhD

01/18/2019 16:25