TEST REQUESTED

Max NGS CNS/Glioma Molecular Panel

METHOD USED

Next Generation Sequencing (NGS)- *IDH1, IDH2, PTEN, BRAF, TP53* mutation
FISH- 1p19q Co-deletion
RT-PCR- MGMT Promoter Methylation

CLINICAL INFORMATION

As per Immunohistochemistry impression, there is Glioblastoma, IDH-1 wild type, WHO Grade 4, recurrent in biopsy of Right frontal glioma.
As per clinical data, there is h/o Left hemiplegia seizure, k/c/o right frontal astrocytoma- 2021, Recurrent right frontal astrocytoma

SAMPLE INFORMATION

FFPE Block (Block No.: S-6373/23-B, Tumor Content: ~85%-90%)

TARGETED GENES

METHOD – NEXT GENERATION SEQUENCING

IDH1	*IDH2*	*PTEN*	*BRAF*	*TP53*
NOT DETECTED	NOT DETECTED	NOT DETECTED	NOT DETECTED	NOT DETECTED

METHOD - FISH

1p19q Co-deletion
DETECTED

METHOD – RT-PCR

MGMT Promoter Methylation
DETECTED

SUMMARY

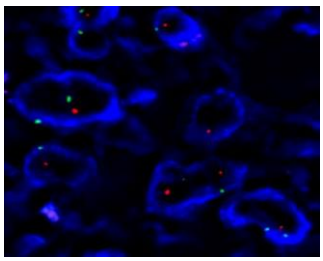
- DNA sequencing did not identify any variant in the genes mentioned in the panel.
- FISH test identified **1p19q Co-deletion** in the block provided.
- RT-PCR identified **MGMT Promoter methylation** in the block provided.

MOLECULAR AND BIO-MARKER DIRECTED THERAPY (AS PER NCCN GUIDELINES**)**		
Therapy	**Tested Markers**	**Predicted Response**
Lomustine	IDH1, IDH2, 1p19q co deletion	Good response
Carmustine	IDH1, IDH2	-
Temozolomide	IDH1, IDH2, 1p19q co-deletion, MGMT Promoter Methylation	Therapeutic guidelines not available for 1p19q co-deletion; Good response for MGMT Promoter Methylation
Bevacizumab	1p19q co-deletion, IDH1, IDH2, BRAF	Therapeutic guidelines not available

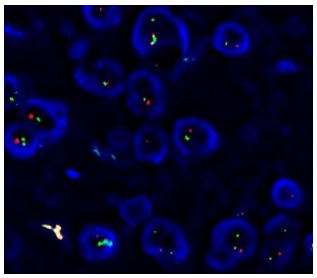
PREDICTED RESPONSE OF THERAPY	
Green	Good response
Red	Contraindicated response
Orange	Limited response
Blue	Therapeutic guidelines not available

RESULTS OF FLUORESCENCE IN-SITU HYBRIDIZATION (FISH)

Zytovision LSI 1p36 Spectrum orange/LSI 1q25 Spectrum Green	
Number of cells scored	100
Number of 1p signals	125
Number of 1q signals	163
Ratio 1p/1q	0.76
Result	**Positive**



Zytovision LSI 19q13 Spectrum orange/LSI 19p13 Spectrum Green	
Number of cells scored	100
Number of 19p signals	158
Number of 19q signals	123
Ratio 19q/19p	0.77
Result	**Positive**



Interpretation: The tumor cells are **POSITIVE** for 1p/19q Co-deletion.

Comments: The probes for 1p/1q and 19q/19p were enumerated in 100 nuclei. A normal ratio is approximately 1.0. Any ratio <0.80 is considered with deletion of the region of interest. The presence of 1p deletion and combined 1p and 19q deletion supports a diagnosis of oligodendroglioma may indicate that the patient may respond to chemotherapy and radiation therapy. Combined 1p and 19q loss is infrequent in gliomas of astrocytic origin. The presence of gain of chromosome 19 and of the 19 q-arm supports a diagnosis of high-grade astrocytoma (glioblastoma multiforme). A negative result does not exclude a diagnosis of oligodendroglioma or high-grade astrocytoma.

Reference: Jenkins et al., Am J Clin Oncol 24:506−8, 2001 This test was validated and its performance characteristics determined by Cytogenetics Laboratory, Max hospital. Since only a portion of the tumor was tested, it is possible that this result may not represent the entire tumor population.

RESULTS OF RT-PCR for MGMT PROMOTER METHYLATION

Methylation	Interpretation
DETECTED	**POSITIVE**

COMMENTS

As per NCCN Guidelines, MGMT promoter methylation is an essential part of molecular diagnostics for all high-grade gliomas (grade 3 and 4). MGMT ((O-6-methylguanine-DNA methyltransferase) is a DNA repair enzyme that reverses the DNA damage caused by alkylating agents, resulting in tumor resistance to TMZ and nitrosourea-based systemic therapy. Methylation of the MGMT promoter silences MGMT, making the tumor more sensitive to treatment with alkylating agents. The MGMT gene has a TATA-less, CAAT-less promoter containing a CpG island. Expression of the MGMT gene is heavily regulated by methylationdependent epigenetic silencing. Methylation of cytosines in CpG dinucleotides is a covalent modification catalyzed by DNA methyltransferases. Methylation occurs primarily in CpG islands, which are short (typically 300 to 3000 bp) stretches of CG-rich DNA, found primarily in promoter regions. Tumor growth and survival can become enhanced by resultant methylation and inactivation of tumor suppressor genes, DNA repair genes, and proapoptotic genes. MGMT promoter methylation confers a survival advantage in glioblastoma and is used for risk stratification in clinical trials. Patients with glioblastoma that is not MGMT promoter methylated derive less benefit from treatment with TMZ compared to those whose tumors are methylated. So, MGMT Promoter Methylation is used as a prognostic indicator to treatment with alkylating agents.

In this panel, MGMT Methylation Detection is a Real-Time amplification test for the detection of MGMT Promoter methylation. It has two-step protocol in which Extracted DNA is first Bisulfite treated; this treated DNA is then amplified by PCR using a pair of specific primers and a specific internal double-dye probe.

RESULT OF NEXT GENERATION SEQUENCING

SAMPLE STATISTICS	
Coverage	100%
Depth	3,786X

VARIANT FINDINGS

Gene	CDS Variant	Amino Acid Change	Exon	Variant Allele Frequency	Coverage	Variant Classsification (AMP)	Pathogenicity (Clinvar/Varsome)
IDH1	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						
IDH2	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						
PTEN	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						
BRAF	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						
TP53	NO CLINICALLY SIGNIFICANT VARIANT(S) WAS FOUND						

EVIDENCE BASED VARIANT CATEGORIZATION: Variant classification (Based on AMP recommendations)

Tier 1 – Variants with strong clinical significance for therapeutic, prognostic and diagnostic for the same tumor type
Tier 2 – Variants with potential clinical significance for therapeutic, prognostic and diagnostic for the different tumor type
Tier 3 – Variants of unknown clinical significance
Tier 4 – Variants deemed benign or likely benign

* Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer (PMID: 27993330)

TEST METHODOLOGY

Background

Multi gene analysis through next generation sequencing allows the identification of variants to understand their prognostic and therapeutic implications in different cancer types, if any. Targeted application of next-generation sequencing (NGS) technology allows detection of specific mutations that can provide treatment opportunities to the patients. This panel with improved primer design and as little as 10 ng of DNA enable researchers to sequence challenging samples such as Formalin fixed, paraffin embedded (FFPE) tissue which exhibit variable quality. Additionally, even degraded samples can be used to generate reliable data using this panel as the primers are designed to produce, on average, 154 bp amplicons. This panel detects co-deletion of 1p19q using FISH and MGMT Promoter Methylation using RT-PCR.

Method - NGS

The Ion Ampliseq Hotspot panel V2 detects SNPs, MNVs and InDels using next generation sequencing. After next generation sequencing, automated analysis was performed with Torrent Suite™ Software version 5.16. Variant annotations were then done using Ion Reporter™ Software version 5.20. Clinically relevant mutations were also checked using published literature and databases.

Method – FISH

1p19q Co-Deletion - Probe description: FISH on FFPE tissue using breakapart probe from Vysis, Abbott Molecular, Inc. This test is approved by U.S Food and Drug Administration. LSI 3-ALK is labeled with Spectrum Orange and LSI 5'-ALK is labeled in Spectrum Green and hybridize to ALK gene at the locus 2p23. This test is designed to detect translocations involving the ALK gene but it does not identify a specific translocation partner. Signals scored in 100 nuclei from invasive or metastatic tumor after confirmation of probe performance by concurrent controls. An abnormal signal pattern seen in 15% or more of the evaluated tumor cells is considered a positive result.

Method – RT-PCR

MGMT Methylation Detection is a Methylation-specific q-PCR assay to detect MGMT Promoter methylation by using allele-specific primers to identify their target. It works by amplifying the epigenetically silenced MGMT promoter regions after bisulfite treatment of DNA to determine the methylation status of the MGMT promoter. β-actin (ACTB) serves as an endogenous internal control (IC) to check sample quality and whole process.

References

- NCCN Guidelines Version 1.2023, Central Nervous System Cancers
- Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, Tsimberidou AM, Vnencak-Jones CL, Wolff DJ, Younes A, Nikiforova MN. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23. PMID: 27993330

Limitations

The accuracy and completeness may vary due to variable information available in different databases. The classification of variants of unknown significance can change over time. Synonymous mutations were not considered while preparing this report. The mutations have not been confirmed using Sanger sequencing and/or alternate technologies. To rule out germ line mutations i.e. variant with variant allele frequency at nearly 50% or 100%, whole blood sample is recommended to process along with tissue sample. No other variant that warrants to be pathogenic was detected. Variations with high minor allele frequencies which are benign/likely benign will be given upon request.

DISCLAIMER

A Negative result implying non-detection of mutation/deletion indicates a Benign/likely Benign polymorphism. A negative test result may also be due to the inherent technical limitations of the assay. Results obtained should be interpreted with consideration of the overall picture obtained from clinical, laboratory, and pathological findings. Rare polymorphisms may lead to false negative or positive results. False negative results may be due to sampling error/errors in sample handling as well as clonal density below the limit of detection. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Identification of a mutation in one or more of these genes does not guarantee activity of the drug in a given indication due to the presence of contraindicated mutation in the gene not covered by the panel.

The accuracy and completeness may vary due to variable information available in different databases. Classification of the variant may change overtime. An updated variant classification may be obtained on request. Insertions and deletions greater than 20bp in size may not be detected by this assay. The scope of this assay limits to SNVs, MNVs and short deletions/duplications. Due to poor quality of FFPE DNA, indeterminate result due to low gene coverage or low variant depth cannot be ruled out. The sensitivity of the assays depends on the quality of the block, and tumor content.

The information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician. Patient care and treatment decisions should only be made by the physician after taking into account all relevant information available including but not limited to the patient's condition, family history, findings upon examination, results of other diagnostic tests, and the current standards of care. This report should only be used as an aid and the physician should employ sound clinical judgment in arriving at any decision for patient care or treatment.

This is a laboratory developed test (LDT). This test was validated and its performance characteristics were determined by Cytogenetics Laboratory, Max Hospital. Since only a portion of the tumor was tested, it is possible that this result may not represent the entire tumor population.

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