BELAY DIAGNOSTICS

Date: 11/11/2024
Order ID: BDX-N0CMHU

1375 W. Fulton Street, Suite 530
Chicago, IL 60607
Email: contact@belaydiagnostics.com
Phone: (331) 320-0155 | Fax: (800) 501-9246

Summit™ + Vantage™ Report

Patient	Specimen	Physician Information
Name: Jane Doe **DOB:** 01/01/1950 **Sex Assigned at Birth:** Female **MRN:** 11xx22xx11 **Diagnosis:** Glioblastoma	**Type:** CSF **Collected:** 11/05/2024 **Received:** 11/06/2024 **Specimen ID:** 22222	**Institution:** Medicine Center **Referring Physician:** Joe Medicine, MD

RESULT SUMMARY

POSITIVE

Clinically Significant Alterations Detected	*IDH1* R132H (Tier 1A)
	TP53 V274D (Tier 2C)
	TP53 R273C (Tier 2C)
	MGMT Promoter Methylation Positive

Actionability Summary	0 therapies
	9 trials

ALTERATION DETAILS

Clinically Significant Genomic Variants

Alteration	Type of Alteration	Classification	Clinical Implications
IDH1 p.R132H c.395G>A VAF: 0.8%	Substitution - Missense	Tier 1A	Potentially Relevant Clinical Trials; Prognostic Implications; Diagnostic Implications
TP53 p.V274D c.821T>A VAF: 2.4%	Substitution - Missense	Tier 2C	Potentially Relevant Clinical Trials; Diagnostic Implications
TP53 p.R273C c.817C>T VAF: 1.4%	Substitution - Missense	Tier 2C	Potentially Relevant Clinical Trials; Diagnostic Implications

Aneuploidy Variants (Chromosome Arm Level Loss or Gain): *None*

MGMT Promoter Methylation

Status	Guidelines	Clinical Implications
Methylated	NCCN	Potentially Relevant Clinical Trials; Prognostic Implications; Diagnostic Implications

Clinical Implications: Associated with drug response = related to drug sensitivity or resistance as described in Drug Response section of this report; Potentially relevant clinical trials = gene is related to a trial in the Clinical Trials section of this report; Prognostic Implications = related to prognosis as described in Prognostic Implications section of this report; Diagnostic Implications = related to diagnosis as described in Diagnostic Implications
VAF: Variant Allelic Frequency

Variants of Unknown Significance (Tier 3): *None*

ACTIONABILITY SUMMARY

FDA / NCCN Therapies for the Patient's Tumor Type (Tier 1A): *None*

FDA / NCCN Therapies with Resistance / Decreased Response (Tier 1A): *None*

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Prognostic Implications per NCCN			
Biomarker	**Prognostic Association**	**Diseases**	**Note**
IDH1 R132H	Favorable	Glioma	For glioma, IDH1 or 2 mutations are associated with a relatively favorable prognosis.
MGMT Promoter Methylation Positive	Favorable	Glioma	MGMT promoter methylation confers a survival advantage in glioblastoma.

Diagnostic Implications per WHO		
Biomarker	**Diseases**	**Note**
IDH1 R132H	Central Nervous System Neoplasm	Among central nervous system neoplasms, an IDH1 codon 132 missense is an essential diagnostic criterion for astrocytoma, IDH-mutant and oligodendroglioma, IDH-mutant and 1p/19q-codeleted. In the case of diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype, absence of IDH1 mutation is an essential diagnostic criterion. In the case of IDH-wildtype glioblastoma, absence of immunoreactivity for IDH1 p.R132H is sufficient (i.e. without further sequencing) to diagnose IDH-wildtype glioblastoma in a patient aged ≥ 55 years at diagnosis who has a histologically classic glioblastoma not located in midline structures and no history of a pre-existing lower-grade glioma (PMID: 27157931). Patients aged < 55 years, or patients with a history of lower-grade glioma and/or whose tumors show immunohistochemical loss of nuclear ATRX expression, negative IDH1 p.R132H immunostaining should be followed by DNA sequencing for less common IDH1 or IDH2 mutations. Angiocentric gliomas lack mutations in IDH1 (PMID: 22445362). Chordoid gliomas have lacked accompanying pathogenic alterations in genes characteristic of other brain tumor entities (e.g. IDH1, IDH2, H3-3A, H3C2 [HIST1H3B], FGFR1, BRAF, NF1, CDKN2A, TP53). IDH mutation (either IDH1 p.R132 or IDH2 p.R172) is not compatible with the diagnosis of ganglioglioma. No mutations in IDH1 have been reported to date in diffuse leptomeningeal glioneuronal tumors. In the case of pituicytoma, granular cell tumor of the sellar region, and spindle cell oncocytoma, IDH1 p.R132H mutation is absent (PMID: 23887161).
MGMT Promoter Methylation Positive	Central Nervous System Neoplasm	Among central nervous system neoplasms, MGMT promoter methylation is detectable in the majority of oligodendrogliomas (PMID:15455350). The MGMT gene encodes a DNA repair protein (PMID:24071851) and is transcriptionally silenced by promoter methylation in approximately 40-50% of IDH-wildtype glioblastomas (PMID:24120142; PMID:10029064; PMID:15758010; PMID:22294349). Although H3 G34-mutant diffuse hemispheric gliomas show widespread DNA hypomethylation, MGMT is often methylated (PMID:23079654; PMID:28966033; PMID:30101054).
TP53 V274D, *TP53* R273C	Central Nervous System Neoplasm	Among central nervous system neoplasms, detection of TP53 mutations is an essential diagnostic criterion in the diagnosis of medulloblastoma, SHH-activated and TP53-mutant. TP53 mutations are reported in 10-15% of SHH-activated medulloblastomas, over half of which are germline. TP53 mutations are also desirable in the diagnoses of choroid plexus carcinoma (CPC) and astrocytoma, IDH-mutant. About 50% of CPCs carry TP53 mutations, whereas most IDH-mutant astrocytomas show widespread (> 50%) p53 expression (PMID:25040820).

CLINICAL TRIALS / INVESTIGATIONAL THERAPIES

***IDH1* R132H**		
Therapy	**Clinical Trial**	**Location/Sponsor**
nivolumab	NCT03718767 (Phase 2) Nivolumab in Patients With IDH-Mutant Gliomas With and Without Hypermutator Phenotype	Bethesda, Maryland National Cancer Institute (NCI) ncinobreferrals@mail.nih.gov
pembrolizumab + olaparib + temozolomide	NCT05188508 (Phase 2) Pembrolizumab, Olaparib, and Temozolomide for People With Glioma	Hartford, Connecticut Memorial Sloan Kettering Cancer Center schaffl@mskcc.org
retifanlimab + tretinoin	NCT05345002 (Phase 2) All-Trans Retinoic Acid (ATRA) Plus PD-1 Inhibition in Recurrent IDH-Mutant Glioma	Philadelphia, Pennsylvania Stephen Bagley, MD, MSCE
talazoparib	NCT04550494 (Phase 2) Measuring the Effects of Talazoparib in Patients With Advanced Cancer and DNA Repair Variations	Gainesville, Florida National Cancer Institute (NCI)
talazoparib	NCT04692662 (Phase 2) Talazoparib, an Oral PARP Inhibitor, in Patients With Advanced Solid Tumors and Aberrations in Genes Involved in DNA Damage Response	Bethesda, Maryland National Cancer Institute (NCI) zlottjh@mail.nih.gov

***MGMT* Promoter Methylation Positive**		
Therapy	**Clinical Trial**	**Location/Sponsor**
tuvusertib + temozolomide	NCT05691491 (Phase 1/Phase 2) Testing the Combination of the Anti-Cancer Drugs Temozolomide and M1774 to Evaluate Their Safety and Effectiveness	La Jolla, California National Cancer Institute (NCI)

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***TP53* R273C + *TP53* V274D**		
Therapy	**Clinical Trial**	**Location/Sponsor**
ART6043 + niraparib; ART6043 + olaparib; ART6043	**NCT05898399** (Phase 1/Phase 2) Study of ART6043 in Advanced/Metastatic Solid Tumors Patients	Grand Rapids, Michigan Artios Pharma Ltd info@artios.com
ATRN-119	**NCT04905914** (Phase 1/Phase 2) Study Of ATRN-119 In Patients With Advanced Solid Tumors	New Haven, Connecticut Aprea Therapeutics crystal.miller@aprea.com
niraparib	**NCT04992013** (Phase 2) Niraparib in Tumors Metastatic to the CNS	Boston, Massachusetts Massachusetts General Hospital pbrastianos@mgh.harvard.edu

TIER 1A THERAPY DETAILS

None

TEST DETAILS

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SNVs and Indels (32 genes, 112 amplicons)			
AKT1 NM_001014432.1	*ERBB2* NM_004448.2	*GNAS* NM_000516.4	*NRAS* NM_002524.4
APC NM_000038.5	*ERBB3* NM_001982.3	*H3-3A* NM_002107.4	*PIK3CA* NM_006218.2
BRAF NM_004333.4	*ERCC2* NM_000400.3	*HRAS* NM_005343.2	*PTEN* NM_000314.4
CD79B NM_000626.2	*FBXW7* NM_033632.3	*IDH1* NM_005896.2	*RAF1* NM_002880.3
CDH1 NM_004360.3	*FGFR2* NM_022970.3	*IDH2* NM_002168.2	*SMAD4* NM_005359.5
CDKN2A NM_000077.4	*FGFR3* NM_000142.4	*KRAS* NM_004985.3	*TERT* NM_198253.2
CTNNB1 NM_001904.3	*FUS* NM_004960.3	*MYD88* NM_002468.4	*TP53* NM_000546.5
EGFR NM_005228.3	*GATA3* NM_001002295.1	*NFE2L2* NM_006164.4	*VHL* NM_000551.3

Aneuploidy (chromosome arm level loss and gain)									
chr1p	chr3p	chr5p	chr7p	chr9p	chr11p	chr13q	chr16q	chr18q	chr20q
chr1q	chr3q	chr5q	chr7q	chr9q	chr11q	chr14q	chr17p	chr19p	chr21q
chr2p	chr4p	chr6p	chr8p	chr10p	chr12p	chr15q	chr17q	chr19q	chr22q
chr2q	chr4q	chr6q	chr8q	chr10q	chr12q	chr16p	chr18p	chr20p	

Methods and Limitations

The Summit™ next-generation sequencing (NGS) test investigates tumor DNA (tDNA) extracted from cerebrospinal fluid (CSF) for clinically relevant single/multi nucleotide variants (SNVs, MNVs, indels) and aneuploidy events associated with primary and metastatic central nervous system (CNS) cancers. Methodology involves targeted duplex sequencing of 32 key genes (SNVs, MNVs and Indels) and low pass whole genome sequencing (>0.1x) for the detection of chromosomal arm level loss or gain, aneuploidy (PMID: 37014860). Post target enrichment libraries, generated from 20-40ng of tDNA, are sequenced on the Illumina NovaSeq XPlus, generating 100 bp paired-end sequence reads. The LOD (limit of detection) for SNVs, MNVs and Indels was determined as 0.3% variant allelic fraction (VAF). Variants (mutations and aneuploidy) are called against the human genome build reference hg19 using the Summit™ Genome Analytics (SGA) pipeline (SNVs, MNVs, and Indels - version 0.5.1 and aneuploidy - version 0.4.1), developed at Belay Diagnostics.

The Vantage™ *MGMT* Promoter Methylation Assay utilizes a quantitative PCR (qPCR) followed by high-resolution melt analysis (HRM) using the EpiMelt *MGMT* kit (MethylDetect) after enzymatic conversion (NEBNext Enzymatic Methyl-seq, New England Biolabs) on a portion of the library generated in the Summit™ workflow. Methylated and unmethylated melting temperature peaks are evaluated using the LightCycler® 480 Software v. 1.5.1 (Roche LifeScience). Qualitative results are reported as “Negative - Unmethylated”, “Positive - Methylated”, or “Indeterminate Results were equivocal”. Specimens with results above the validated 25% methylated control are interpreted as “Positive”. Specimens with results between unmethylated and methylated control are interpreted as “Indeterminate”.

Tertiary analysis is performed using the precision oncology workbench (GenomOncology) based on the joint AMP/ASCO/CAP consensus guidelines for interpretation of sequence variants in cancer (PMID: 27993330). Please reach out to contact@belaydiagnostics.com for additional information or queries.

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Disclaimers

This test was developed, and its performance characteristics determined by Belay Diagnostics, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA as qualified to perform high complexity clinical testing. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). This test may be used for clinical purposes.

This test is performed to only evaluate for somatic (i.e., tumor-specific) mutations within the genes listed and cannot distinguish between germline and somatic alterations with absolute certainty. This test does not establish a diagnosis and should be considered in the context of all other clinical information. Follow-up germline testing using non-neoplastic (normal) tissue should be performed for confirmation of suspected clinically relevant germline alterations. Germline testing should be performed along with genetic counseling. It may be possible for a biomarker variant to be present yet go undetected by our assay either due to the heterogeneous nature of the tissue or the limits of detection of our assay. Therefore, to the extent a particular biomarker variant is not reported, we cannot guarantee that the variant does not exist.

Decisions on patient care must be based on the independent medical judgment of the treating physician, taking into consideration all relevant information about the patient's condition, including patient and family history, physical examinations, information from other diagnostic tests, and patient preferences. A treating physician's decisions should not be based on a single test, such as this test, or the information contained in this report alone. Results of this test should not be used alone for diagnosis or patient care decisions or otherwise replace the judgment of a treating physician and must always be interpreted in the context of all relevant clinical and pathological data.

The information presented in the clinical trials and therapeutic sections of this report is compiled from public sources which are continuously updated. While we endeavor to make this information accurate and complete, we cannot guarantee the accuracy or completeness of this information. This public source information is not ranked in order of potential or predicted efficacy and may not be complete. Specific eligibility criteria should be reviewed as applicable. This information may include associations between a biomarker variant (or lack of a variant) and one or more therapeutic agents with potential clinical benefit (or lack of clinical benefit), including agents that are being studied in clinical research. A finding of a biomarker variant does not necessarily indicate or demonstrate pharmacologic effectiveness (or lack thereof) of any agent or treatment regimen found in public source information. Similarly, a finding of "no biomarker variant" does not necessarily indicate or demonstrate lack of pharmacologic effectiveness (or lack of effectiveness) of any agent or treatment regimen found in public source information. Belay Diagnostics expressly disclaims, and makes no representation of or warranty of, the accuracy or completeness with respect to the publicly available information included herein or reviewed or collected during creation of this report.

Report electronically signed by Honey Reddi, PhD, FACMG

Date: 11/11/24 21:32 PM CST

ACTIONABILITY REFERENCES

FDA: U.S. Food & Drug Administration (fda.gov)

NCCN: National Comprehensive Cancer Network® (NCCN®). Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). © National Comprehensive Cancer Network, Inc. 2024. All rights reserved. The NCCN Guidelines® and illustrations herein may not be reproduced in any form for any purpose without the express written permission of the NCCN. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

WHO: World Health Organization Classification of Tumours online (tumourclassification.iarc.who.int)

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