BELAY DIAGNOSTICS

Date: 03/17/2025
Order ID: SumPos-TL-B1

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

Summit™ Report

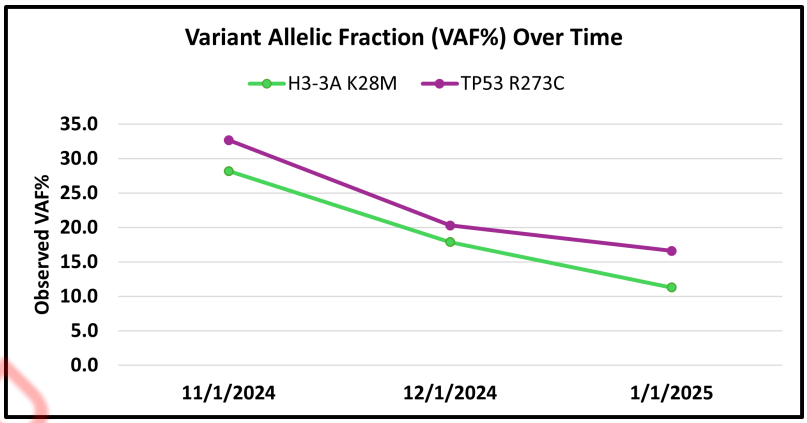
Patient	Specimen	Physician Information
Name: John Smith **DOB:**01/01/1990 **Sex Assigned at Birth:** Male **MRN:**11xx22xx33 **Diagnosis:**Glioma	**Type:**CSF **Collected:**01/01/2025 **Received:**01/02/2025 **Specimen ID:**SumPos-TL-B1	**Institution:**Belay Diagnostics **Referring Physician:**Provider Test

RESULT SUMMARY

POSITIVE

Clinically Significant Alterations Detected	*H3-3A* K28M (Tier 1A)
	TP53 R273C (Tier 1B)

Actionability Summary	2 therapeutic responses
	8 trials



Comments
While no aneuploidy of clinical significance was detected, a high level of chromosomal loss and gain was observed in this sample.

ALTERATION DETAILS

Clinically Significant Genomic Variants			
Alteration	**Type of Alteration**	**Classification**	**Clinical Implications**
H3-3A p.K28M c.83A>T VAF: 11.3%	Substitution - Missense	Tier 1A	Potentially Relevant Clinical Trials; Prognostic Implications
TP53 p.R273C c.817C>T VAF: 16.6%	Substitution - Missense	Tier 1B	Associated with Drug Response; Potentially Relevant Clinical Trials; Diagnostic Implications

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

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)			
chr10q Loss	chr18q Loss	chr3p Gain	chr5q Loss
chr15q Loss	chr20p Loss	chr3q Gain	

ACTIONABILITY SUMMARY

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

For Test Purposes Only

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

Summit™ Report

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

FDA / NCCN Therapies for Other Tumor Types (Tier 2C)

Biomarker	Therapies	Indications
PTEN Loss	capivasertib + fulvestrant	Breast Carcinoma
PTEN Loss + *TP53* R273C	cabazitaxel + carboplatin	Prostate Adenocarcinoma, Prostate Neuroendocrine Carcinoma

Prognostic Implications per NCCN

Biomarker	Prognostic Association	Diseases	Note
H3-3A K28M	Unfavorable	Glioma	K27M/K28M gliomas typically do not have MGMT promoter methylation, and the mutation is an adverse prognostic marker in children and adults.

Diagnostic Implications per WHO

Biomarker	Diseases	Note
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

***H3-3A* K28M**

Therapy	Clinical Trial	Location/Sponsor
ONC201 + panobinostat + radiation therapy; ONC201 + paxalisib + radiation therapy	NCT05009992 (Phase 2) Combination Therapy for the Treatment of Diffuse Midline Gliomas	Birmingham, Alabama University of California, San Francisco PNOC022@ucsf.edu
ONC201 + placebo; ONC201	NCT05580562 (Phase 3) ONC201 in H3 K27M-mutant Diffuse Glioma Following Radiotherapy (the ACTION Study)	Phoenix, Arizona Chimerix clinicaltrials@chimerix.com
bevacizumab + APG-157	NCT06011109 (Phase 1/Phase 2) Treatment of Patients With Recurrent High-Grade Glioma With APG-157 and Bevacizumab	Rochester, Minnesota Aveta Biomics, Inc. nshonka@unmc.edu
nivolumab	NCT03173950 (Phase 2) Immune Checkpoint Inhibitor Nivolumab in People With Recurrent Select Rare CNS Cancers	Chicago, Illinois National Cancer Institute (NCI) NCINOBReferrals@mail.nih.gov

***TP53* R273C**

Therapy	Clinical Trial	Location/Sponsor
ART6043 + niraparib; ART6043 + olaparib; ART6043	NCT05898399 (Phase 1/Phase 2) Study of ART6043 in Advanced/Metastatic Solid Tumors Patients	Philadelphia, Pennsylvania 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

For Test Purposes Only

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

Summit™ Report

anti-KRAS and anti-TP53 peripheral blood lymphocytes + aldesleukin + cyclophosphamide + fludarabine; anti-KRAS and anti-TP53 peripheral blood lymphocytes + pembrolizumab + aldesleukin + cyclophosphamide + fludarabine	NCT03412877 (Phase 2) Administration of Autologous T-Cells Genetically Engineered to Express T-Cell Receptors Reactive Against Neoantigens in People With Metastatic Cancer	Bethesda, Maryland National Cancer Institute (NCI) IRC@nih.gov
crizotinib + talazoparib; axitinib + talazoparib; palbociclib + talazoparib	NCT04693468 (Phase 1) Talazoparib and Palbociclib, Axitinib, or Crizotinib for the Treatment of Advanced or Metastatic Solid Tumors, TalaCom Trial	Houston, Texas M.D. Anderson Cancer Center tyap@mdanderson.org

TIER 1A THERAPY DETAILS

None

TEST DETAILS

Summit™ Report

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 0.6.0 and aneuploidy - version 0.5.2), developed at Belay Diagnostics.

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.

Disclaimers

This test was developed, and its performance characteristics determined by Belay Diagnostics Laboratory (CLIA# 14D2302605), which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity testing. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). This test may be used for clinical purposes. However, the results of this test do not establish a diagnosis and 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.

This test is performed only to evaluate for somatic (i.e., tumor-specific) variants within the genes listed and cannot distinguish between germline and somatic alterations with absolute certainty. This test therefore does not report on incidental findings as defined by the American College for Medical Genetics and Genomics (ACMG) (PMID: 37347242). If a germline variant is suspected, follow-up germline testing using non-neoplastic (normal) tissue

For Test Purposes Only

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

Summit™ Report

should be performed by a laboratory permitted to perform germline genetic testing along with genetic counseling. It is possible for a genomic variant to be present yet go undetected by our assay either due to the heterogeneous nature of the specimen or the limits of detection of our assay. Therefore, to the extent a particular genomic variant is not reported, Belay Diagnostics LLC does not guarantee that the variant does not exist in the specimen provided. Likely benign, and benign variants are not reported. For any reported variant of uncertain significance (VUS), if the classification changes, there is no obligation to send out a new report updating this information.

The information presented in the clinical trials and therapeutic sections of this report is compiled from public sources which are continuously updated. While we strive to ensure this information is accurate and complete, we cannot guarantee the accuracy or completeness of this information. This public sourced 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 genomic 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. The finding of a genomic variant does not necessarily indicate or demonstrate pharmacologic effectiveness (or lack thereof) of any agent or treatment regimen found in public source information. Similarly, the finding of "no clinically significant 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.

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)

This report was produced using software licensed by GenomOncology. GenomOncology software is designed to be used in clinical applications solely as a tool to enhance medical utility and improve operational efficiency. The use of GenomOncology software is not a substitute for medical judgment and GenomOncology in no way holds itself out as having or providing independent medical judgment or diagnostic services. GenomOncology is not liable with respect to any treatment or diagnosis made in connection with this report.

For Test Purposes Only