25371 Commercentre Drive, Lake Forest, CA 92630
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CLIA # 05D2111917 CAP # 9441574

Liquid Trace Solid Tumor

Patient Name:		Ordering Physician:	
Date of Birth:		Physician ID:	
Gender (M/F):		Accession #:	
Client:		Specimen Type:	
Case #:		Specimen ID:	
Body Site:	CSF		

MRN:				Indication for Testing:	G93.9 Disorder of brain, unspecified; H55.09 Other forms of nystagmus
Collected Date:		Time:	12:19 PM		
Received Date:		Time:	12:19 PM	Tumor Type:	Brain
Reported Date:		Time:	06:47 PM		

Detected Genomic Alterations

Level 1 (FDA-Approved)	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Evidence)	Other
-	Tumor Mutation Burden Low: 6 Mut/Mb	TP53, MYD88, EP300 (2 mutations)	-B-cell clonality: Detected (IgHV 3-7/IgLV 2-8) -T-cell clonality : Not detected	Autosomal chromosomes show 3p+, 5q-, 6q-, 8q+(MYC amplification), +11, 15q-, 17p-, 18q +(BCL2 amplification), and +21.

Results Summary

- **-Mutations in TP53, MYD88, and EP300 (2 mutations) genes**
  -Tumor Mutation Burden Low: 0 Mut/Mb
  -No evidence of IDH1/2, H3 K27M and PTEN mutations
  -No evidence of EGFR gene amplification
  -No evidence of CDKN2A/B gene deletion
  -No evidence of 1p/19q Co-deletion or combined +7/-10 chromosomal changes
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*02:01-A*02:01
  -HLA-B: B*07:02-B*07:02
  -HLA-C: C*03:03-C*07:02
  -Autosomal chromosomes show 3p+, 5q-, 6q-, 8q+(MYC amplification), +11, 15q-, 17p-, 18q+(BCL2 amplification), and +21.
  -B-cell clonality: Detected (IgHV 3-7/IgLV 2-8)
  -T-cell clonality : Not detected
  -Increased B-cell markers

-Increased MYC and BCL2 mRNA

-These findings are consistent with CNS highly aggressive diffuse large B-cell lymphoma with bi-alleic TP53 abnormalities and amplification and overexpression of BCL2 and MYC genes.

See additional report information at the end of the report.

Tumor Heterogeneity
There are dominant abnormal clones with TP53 and MYD88 mutations. The (2) EP300 mutations are detected in subclones.

Expression	
Increased B-cell markers	Increased MYC and BCL2 mRNA

Diagnostic Implications	
TP53, MYD88, EP300 (2 mutations)	These findings are consistent with aggressive CNS diffuse B-cell lymphoma (see results summary).

Relevant Alteration Associated with Resistance
TP53 mutation is associated with resistance to therapy.

Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence)	
TP53	Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy
MYD88	BTK inhibitors
EP300	Bromodomain Extra-Terminal (BET) inhibitors

Relevant Genes with NO Alteration		
No evidence of mutation in KRAS, NRAS, EGFR, BRAF, or BRCA 1/2	No specific mutation in DPYD gene, associated with enzymatic deficiency	No evidence of METex14 skipping or EGFRvIII

Test Description:

This is a comprehensive molecular profile which uses next generation sequencing (NGS) to identify molecular abnormalities, including single nucleotide variants (SNVs), insertions/deletions (indels), copy number variants (CNVs), tumor mutation burden (TMB), fusions, B- and T-cell clonality, and viruses (HPV, EBV, and TTV), in cell-free (cf) DNA of 302 genes and cfRNA in greater than 1600 genes implicated in solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Biological relevance of detected Alterations

- TP53. This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons from identical transcript variants (PMIDs: 12032546, 20937277). [provided by RefSeq, Dec 2016]

- MYD88. This gene encodes a cytosolic adapter protein that plays a central role in the innate and adaptive immune response. This protein functions as an essential signal transducer in the interleukin-1 and Toll-like receptor signaling pathways. These pathways regulate that activation of numerous proinflammatory genes. The encoded protein consists of an N-terminal death domain and a C-terminal Toll-interleukin1 receptor domain. Patients with defects in this gene have an increased susceptibility to pyogenic bacterial infections. Alternate splicing results in multiple transcript variants. [provided by RefSeq, Feb 2010]

- EP300. This gene encodes the adenovirus E1A-associated cellular p300 transcriptional co-activator protein. It functions as histone acetyltransferase that regulates transcription via chromatin remodeling and is important in the processes of cell proliferation and differentiation. It mediates cAMP-gene regulation by binding specifically to phosphorylated CREB protein. This gene has also been identified as a co-activator of HIF1A (hypoxia-inducible factor 1 alpha), and thus plays a role in the stimulation of hypoxia-induced genes such as VEGF. Defects in this gene are a cause of Rubinstein-Taybi syndrome and may also play a role in epithelial cancer. [provided by RefSeq, Jul 2008]

Drug Information

APR-246

APR-246 is a first-in-class agent targeting mutant p53. In vitro and in vivo preclinical models have demonstrated that APR-246 has excellent efficacy in OC (both adenocarcinoma and squamous cell carcinoma) and potently synergises with chemotherapies used in the treatment of OC, restoring sensitivity to chemotherapy-resistant tumours. An initial phase I clinical trial has shown APR-246 to be safe in humans and early results from a currently running phase Ib/II trial of APR-246 with carboplatin and liposomal doxorubicin in ovarian cancer have been promising. Together, these data provide a strong rationale for investigating the efficacy of APR-246 in OC.

APR-246 has been used in trials studying the treatment of Prostatic Neoplasms, Hematologic Neoplasms, and Platinum Sensitive Recurrent High-grade Serous Ovarian Cancer With Mutated p53.

APR-246 is an analogue of PRIMA-1, which modifies the core domain of mutant p53, resulting in restoration of wild-type p53 conformation and reactivation of normal p53 function, leading to increased cell cycle arrest and tumor cell death (PMID: 20498645).

Zanubrutinib

Zanubrutinib, sold under the brand name Brukinsa, is a medication for the treatment of mantle cell lymphoma and Waldenström macroglobulinemia. It was approved for medical use in the United States in November 2019. Zanubrutinib is classified as a Bruton tyrosine kinase inhibitor. Zanubrutinib is an immunomodulating agent that decreases the survival of malignant B cells. Zanubrutinib inhibits BTK by forming a covalent bond with cysteine 481 residue in the adenosine triphosphate (ATP)–binding pocket of BTK, which is the enzyme's active site.

Ibrutinib

Ibrutinib is a small molecule that acts as an irreversible potent inhibitor of Bruton tyrosine kinase (BTK). It is designated as a targeted covalent drug and it presents a very promising activity in B cell malignancies. Ibrutinib forms a covalent bond with a cysteine residue in the active site of BTK (Cys481), leading to its inhibition. The inhibition of BTK plays a role in the B-cell receptor signaling and thus, the presence of ibrutinib prevents the phosphorylation of downstream substrates such as PLC-gamma.

Ibrutinib was developed by Pharmacyclics Inc and in November 2013 was FDA-approved for the treatment of mantle cell lymphoma. Later, in February 2014, ibrutinib was approved for the treatment of chronic lymphocytic leukemia and it is also indicated for the treatment of patients with Waldenstrom Macroglobulinemia. Ibrutinib has also been approved by the EMA for the treatment of chronic lymphocytic leukemia and mantle cell lymphoma. Ibrutinib was approved for use in chronic graft versus host disease in August 2017.

Ibrutinib is indicated for:
-treatment of mantle cell lymphoma who have received at least one prior therapy.
-treatment of chronic lymphocytic leukemia (CLL) who have at least one prior therapy.
-treatment of chronic lymphocytic leukemia (CLL) with 17p deletion.
-treatment of patients with Waldenstrom Macroglobulinemia (WM).

Birabresib

Birabresib (OTX015 or MK-8628) is a potent BET bromodomian inhibitor, which targets the BET bromodomain proteins 2, 3, and 4 (BRD2/3/4). BRDs 2, 3, and 4 are considered potential cancer targets because of their pivotal role in regulating the transcription of growth-promoting genes and cell cycle regulators. OTX015 is the first BRD2/3/4 inhibitor to enter clinical trials. Upon administration, birabresib binds to the acetylated lysine recognition motifs on the bromodomain of BET proteins, thereby preventing the interaction between the BET proteins and acetylated histone peptides. This disrupts chromatin remodeling and gene expression.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://clinicaltrials.gov/study/NCT05226494	Recruiting	A Phase 1 Trial to Evaluate the Safety and Tolerability of Fb-PMT in Patients With Recurrent Glioblastoma	Glioma	fb-PMT	Smilow Cancer Hospital, New Haven, Connecticut 06511
https://clinicaltrials.gov/study/NCT03213002	Recruiting	Phase I/II Study of Oral Capecitabine and Temozolomide (CAPTEM) for Newly Diagnosed Glioblastoma (GBM)	Glioma	Capecitabine, Temozolomide	Lenox Hill Brain Tumor Center, New York, New York 10075
https://clinicaltrials.gov/study/NCT05188508	Recruiting	A Phase II Study of Pembrolizumab, Olaparib, and Temozolomide in Patients with Glioma	Glioma	Pembrolizumab, Olaparib and Temozolomide	BAPTIST ALLIANCE - MCI (Data Collection Only), Miami, Florida 33143 Lehigh Valley Health Network (Data Collection Only), Allentown, Pennsylvania 18103 Memorial Sloan Kettering Basking Ridge (Limited Protocol Activities), Basking Ridge, New Jersey 07920
https://clinicaltrials.gov/study/NCT06011109	Recruiting	A Pilot Study of APG-157 With Bevacizumab for Patients With Recurrent High-Grade Glioma	Glioma	APG-157	University of Nebraska Medical Center, Omaha, Nebraska 68198 Mayo Clinic, Rochester, Minnesota 55905

Detailed Results

Single Nucleotide Variant (SNV) and Insertions-Deletions (INDELS)								
Gene name	Hgvsp	Hgvsc	Amino acids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
TP53	NP_000537.3:p.Tyr205Cys	NM_000546.5:c.614A>G	Y/C	tAt/tGt	missense_variant	74.45	775	deleterious
MYD88	NP_002459.2:p.Leu265Pro	NM_002468.4:c.794T>C	L/P	cTg/cCg	missense_variant	73.4	3139	deleterious
EP300	NP_001420.2:p.Tyr1446Asn	NM_001429.3:c.4336T>A	Y/N	Tat/Aat	missense_variant	3.22	1244	deleterious
EP300	NP_001420.2:p.Ser1557Ile	NM_001429.3:c.4670G>T	S/I	aGc/aTc	missense_variant	2.17	1336	deleterious

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA in 302 genes and cfRNA in >1600 genes for abnormalities that are reported to be altered in various types of solid tumors. For cases with detectable circulating solid tumor DNA, tumor mutation burden (TMB) is reported. The assay also detects several viruses that are important in oncology, including EBV, HPV and TTV. TTV (torque teno virus) was first discovered in a patient with non-A-E hepatitis and is now regarded as a part of the human virome. In general, TTV does not cause pathology in immunocompetent individuals. TTV is considered as a marker of immune competence in patients with immunological impairment and inflammatory disorders. High TTV load is associated with increased risk of infection. In patients with organ transplant, low TTV load is associated with an increased risk of rejection.

Nucleic acid is isolated from peripheral blood plasma or CSF. When CSF sample is submitted, RNA sequencing is performed on the CSF cell pellet instead of cfRNA due to degradation. Performance of the assays may vary depending on the quantity and quality of nucleic acid, sample preparation and sample age. Testing is performed using massive parallel sequencing of the coding DNA of the listed genes. This includes sequencing of all the exons as well as approximately 50 nucleotides at the 5' and 3' ends of each coding exon to detect splice site abnormalities. The TERT promoter region, including the hotspots at -124 and -146 bp, is also covered. Our cfDNA sequencing method has a sensitivity of 0.1% for detecting hot spot mutations, 0.5% for detecting single nucleotide variants (SNVs) and 1% for small (<60 bp) insertions/ deletions (indels). Known hot spots in specific genes such as IDH1/2, NRAS, and KRAS are reported at levels of 0.01% and higher when both cfRNA and cfDNA results are combined. Significant gene amplification and deletion (copy number variants) are also reported. TMB is calculated and cut-off points were determined based on comparison with tissue samples obtained from the same patient. Using cut-off of 6 mut/Mb, 17% of cases called as negative by cfDNA are false negative (FN). However, cases with ≤3 show only 6% FN. Intermediate cases (TMB between 6 and 9 mut/Mb) show 51% false positivity. Positive cases (TMB ≥9 Mut/Mb) show only 7% false positive. Cases without circulating solid tumor DNA are reported as "unable to evaluate" for TMB. Targeted RNA NGS is performed by hybrid capture and duplicates are excluded for levels measurements. The Universal Human Reference (UHR) RNA is used as control. All detected fusion transcripts are reported. While the major focus of the RNA analysis is the detection of fusion mRNA, mutations in the expressed RNA of the analyzed genes, HLA class I genotyping, and Epstein-Barr virus (EBV), human papillomavirus (HPV) and torque teno virus (TTV) viral RNA are also analyzed and reported. B- and T-cell clonality will be reported, if clonal or clinically relevant. The sensitivity of this assay in detecting fusion mRNA is between 5% and 10%. This test specifically covers translocations that lead to the expression of fusion RNA. Translocations that lead to deregulation of expression can be addressed by this test if compared to the expression proper normal control. Since the clinical relevance of the RNA expression level of most of the genes is not characterized at this time, only a few specific genes will be commented on when abnormalities are detected. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated.

Based on our validation study, the following exonic regions of the genes listed below are not covered appropriately <100 X coverage and sequencing by NGS may not be reliable in these regions. The poor coverage is primarily due to the inherent difficulty in obtaining adequate sequencing coverage in regions with high GC content. No well-characterized hotspots are present in these regions. RAD51 NM_133487 chr15:40994004-40994124, BRCA1 NM_007300 chr17:41231351-41231416, FUBP1 NM_003902 chr1:78435609-78435699, CBLB NM_170662 chr3:105420938-105421303, TERT NM_198253 chr5:1295183-1295250, ARID1B NM_017519 chr6:157098715-157100605, CUX1 NM_001202543 chr7:101740644-101740781, KMT2C NM_170606 chr7:151891314-151891346 and 151935792-151935911, GALNT12 NM_024642 chr9:101569952-101570351, ATM NM_000051 chr11:108164040-108164204, CDK17 NM_001170464 chr12:96679880-96679926, RB1 NM_000321 chr13:48954189-48954220, SETBP1 NM_015559 chr18:42643044-42643692, KMT2B NM_014727 chr19:36208921-36209283, AR NM_000044 chrX:66764889-66766604, STAG2 NM_001042749 chrX:123200025-123200112.

DEX Z-Code Z00WY is approved by MolDx for use in assessing DNA SNVs/Indels only.

The table below may contain a partial list of the tested DNA genes. For a complete list, please go to:
https://genomictestingcooperative.com/genomic-tests/liquid-trace-solid-tumor/ (click the DNA tab)

For a complete list of tested RNA genes (Fusions/Expression), please go to:
https://genomictestingcooperative.com/genomic-tests/liquid-trace-solid-tumor/ (click the RNA tab)

Tested genes

Genes Tested for Abnormalities in Coding Sequence															
ABL1	ATRX	BRAF	CDK12	CUX1	EPHA5	FGF4	GNAQ	IL7R	MAP2K1	MSH3	NPM1	PIM1	RAD21	SDHB	SRSF2	TRAF3
ABRAXAS1	AURKA	BRCA1	CDK4	CXCR4	ERBB2	FGF6	GNAS	INHBA	MAP2K2	MSH6	NRAS	PLCG1	RAD50	SDHC	STAG2	TSC1
ACVR1B	AURKB	BRCA2	CDK6	CYLD	ERBB3	FGFR1	GNB1	IRF4	MAP2K4	MTOR	NSD1	PMS1	RAD51	SDHD	STAT3	TSC2
AKT1	AURKC	BRIP1	CDKN1B	DAXX	ERBB4	FGFR2	GREM1	JAK1	MAP3K1	MUTYH	NSD2 (WHSC1)	PMS2	RAD51C	SETBP1	STAT5B	TSHR
AKT2	AXIN1	BTK	CDKN2A	DDR2	ERG	FGFR3	GRIN2A	JAK2	MAP3K14	MYC	NTHL1	POLD1	RAD51D	SETD2	STK11	U2AF1
AKT3	AXIN2	CALR	CDKN2B	DDX41	ESR1	FGFR4	H3-3A (H3F3A)	JAK3	MAPK1	MYCL	NTRK1	POLE	RAF1	SF3B1	SUFU	U2AF2
ALK	B2M	CARD11	CDKN2C	DICER1	ETNK1	FH	H3C2	KAT6A	MCL1	MYCN	NTRK2	POT1	RB1	SMAD2	SUZ12	UBA1
AMER1	BAP1	CBL	CEBPA	DNM2	ETV6	FLCN	HGF	KDM5C	MDM2	MYD88	NTRK3	PPM1D	RET	SMAD4	TAL1	VHL
ANKRD26	BARD1	CBLB	CHEK1	DNMT3A	EXO1	FLT3	HNF1A	KDM6A	MDM4	NBN	PAK3	PPP2R1A	RHEB	SMARCA4	TCF3	WT1
APC	BCL2	CBLC	CHEK2	DOT1L	EZH2	FLT4	HOXB13	KDR	MED12	NF1	PALB2	PRDM1	RHOA	SMARCB1	TENT5C (FAM46C)	XPO1
AR	BCL2L1	CCND1	CIC	EED	FANCA	FOXL2	HRAS	KEAP1	MEF2B	NF2	PAX5	PRKAR1A	RIT1	SMC1A	TERC	XRCC2
ARAF	BCL6	CCND3	CREBBP	EGFR	FANCC	FUBP1	HSP90AA1	KIT	MEN1	NFE2	PBRM1	PRKDC	RNF43	SMC3	TERT	XRCC3
ARID1A	BCOR	CCNE1	CRLF2	EGLN1	FANCD2	GALNT12	ID3	KMT2A	MET	NFE2L2	PDGFRA	PRPF8	ROS1	SMO	TET2	ZNF217
ARID1B	BCORL1	CD274	CSF1R	ELANE	FANCE	GATA1	IDH1	KMT2B	MITF	NFKBIA	PDGFRB	PRSS1	RUNX1	SOCS1	TGFBR2	ZRSR2
ARID2	BCR	CD79A	CSF3R	EP300	FANCF	GATA2	IDH2	KMT2C	MLH1	NKX2-1	PHF6	PTCH1	SAMD9	SOX2	TMEM127	-
ASXL1	BIRC3	CD79B	CTCF	EPAS1	FANCG	GATA3	IGF1R	KMT2D	MPL	NOTCH1	PIK3CA	PTEN	SAMD9L	SOX9	TNFAIP3	-
ATM	BLM	CDC73	CTNNA1	EPCAM	FAS	GEN1	IKZF1	KRAS	MRE11	NOTCH2	PIK3R1	PTPN11	SDHA	SPOP	TNFRSF14	-
ATR	BMPR1A	CDH1	CTNNB1	EPHA3	FBXW7	GNA11	IKZF3	LRP1B	MSH2	NOTCH3	PIK3R2	RAC1	SDHAF2	SRC	TP53	-

RNA Fusions/Expression

Fusion/Expression													
ABL1	BCL6	CD274 (PD-L1)	EGFR	EWSR1	FLI1	IKZF3	MAP3K1	NRG1	NUP98	PML	RET	SS18	THADA
AKT3	BRAF	CIC	ERG	FGFR1	FOXO1	JAK2	MECOM	NTRK1	PAX8	PPARG	RHOA	STAT6	TMPRSS2
ALK	CAMTA1	CREB1	ETS1	FGFR2	FUS	KIAA1549	MYB	NTRK2	PDGFRA	PRKACA	ROS1	TAL1	YAP1
AR	CBFB	CREBBP	ETV1	FGFR3	GLI1	KMT2A	MYC	NTRK3	PDGFRB	RAF1	RUNX1	TCF3	YWHAE
BCL2	CCND1	ERBB2	ETV6	FIP1L1	HMGA2	MAML2	NOTCH1	NUP214	PICALM	RARA	RUNX1T1	TFG	ZFTA

Reference

1. Clinical immunotherapy in glioma: current concepts, challenges, and future perspectives. Liu J, Peng J, Jiang J, Liu Y. Front Immunol. 2024 Nov 1;15:1476436. doi: 10.3389/fimmu.2024.1476436. eCollection 2024. PMID: 39555054.

2. Targeting Innate Immunity in Glioma Therapy. Gillard AG, Shin DH, Hampton LA, Lopez-Rivas A, Parthasarathy A, Fueyo J, Gomez-Manzano C. Int J Mol Sci. 2024 Jan 12;25(2):947. doi: 10.3390/ijms25020947. PMID: 38256021.

3. Immunotherapy: a promising approach for glioma treatment. Yasinjan F, Xing Y, Geng H, Guo R, Yang L, Liu Z, Wang H. Front Immunol. 2023 Sep 7;14:1255611. doi: 10.3389/fimmu.2023.1255611. eCollection 2023. PMID: 37744349.

4. Primary brain tumours in adults. van den Bent MJ, Geurts M, French PJ, Smits M, Capper D, Bromberg JEC, Chang SM. Lancet. 2023 Oct 28;402 (10412):1564-1579. doi: 10.1016/S0140-6736(23)01054-1. Epub 2023 Sep 19. PMID: 37738997.

Electronic Signature

Maher Albitar, M.D.

The test (sample processing, sequencing and data generation) was performed at Genomic Testing Cooperative, LCA, 25371 Commercentre Drive Lake Forest, CA 92630. Medical Director Maher Albitar, M.D. Analysis of the data was performed by Genomic Testing Cooperative, LCA, 25371 Commercentre Drive, Lake Forest, CA 92630. Medical Director: Maher Albitar, M.D.

The test was developed and its performance characteristics have been determined by Genomic Testing Cooperative, LCA. This test has not been approved by the FDA. The FDA has determined such clearance or approval is not necessary. This laboratory is CLIA certified to perform high complexity clinical testing.

Additional Report Information

Mutations Load (mol/mL)