Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618
Tel: 1-866-484-8870 www.genomictestingcooperative.com
CLIA #: 05D2111917 CAP #: 9441547

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		

Ethnicity:		Indication for Testing:	D49.6 Neoplasm of unspecified behavior of brain
MRN:			
Collected Date:		Tumor Type:	Brain
Received Date:			
Reported Date:			

Detected Genomic Alterations				
Level 1 (FDA-Approved)	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Evidence)	Other
IDH2 R140G	-	DNMT3A (2 mutations)	-	No definitive autosomal chromosomal structural gain or loss

Results Summary

- **-Mutations in DNMT3A (2 mutations) and IDH2 genes**
  -No definitive autosomal chromosomal structural gain or loss

  -These findings suggest the presence of a neoplastic DNA, most suggestive of low grade glioma. Correlation with clinical and other laboratory data is recommended.

  -IDH2 mutation suggests response to IDH2 inhibitors.

  -An unusual variant in BRCA2 (Lys3326Ter) gene is detected. However, this variant has been reported to be benign and does not lead to increased predisposition to cancer.

Tumor Heterogeneity
There is an abnormal low-level clone with DNMT3A (2 mutations) and IDH2 mutations.

Expression

Diagnostic Implications	
DNMT3A (2 mutations), IDH2	These findings suggest the presence of circulating neoplastic tumor DNA (see results summary).

FDA-Approved Therapeutics in Other Tumor Types	
IDH2	Enasidenib

Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence)	
DNMT3A	DNA methyltransferase inhibitors
IDH2	IDH2 inhibitors

Relevant Genes with NO Alteration
No evidence of mutation in KRAS, NRAS, EGFR, BRAF, TP53, or BRCA1

Test Description:

This is a comprehensive molecular profile of cell-free DNA (cfDNA) and cell-free RNA (cfRNA), which uses next generation sequencing (NGS) to identify molecular abnormalities (including SNVs, INDELS, CNVs, Fusions, EBV and HPV) in DNA of 284 genes and RNA in 1600 genes associated with solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Biological relevance of detected Alterations

- DNMT3A. CpG methylation is an epigenetic modification that is important for embryonic development, imprinting, and X-chromosome inactivation. Studies in mice have demonstrated that DNA methylation is required for mammalian development. This gene encodes a DNA methyltransferase that is thought to function in de novo methylation, rather than maintenance methylation. The protein localizes to the cytoplasm and nucleus and its expression is developmentally regulated. [provided by RefSeq, Mar 2016]

- IDH2 mutations have been observed in a number of cancer types, including sarcomas, hematologic malignancies, colon cancer and brain cancer. Mutations in the two isocitrate dehydrogenase enzymes involved in cytoplasmic (IDH1) and mitochondrial (IDH2) conversion of alpha-ketoglutarate to D-2-hydroxyglutarate have been described as mutually exclusive in many of these cancer types. The most frequent mutations involve R132 (IDH1) and R172 (IDH2) involve the active site and result in neomorphic enzyme activity. Although IDH2 (R172) mutations are associated with poorer overall prognosis in AML patients, its utility as a prognostic marker in MDS is still under debate. Additionally, IDH2 (R140) has been associated with improved overall survival in AML. IDH2 mutations have been associated with improved prognosis in gliomas.Isocitrate dehydrogenases catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. These enzymes belong to two distinct subclasses, one of which utilizes NAD(+) as the electron acceptor and the other NADP(+). Five isocitrate dehydrogenases have been reported: three NAD(+)-dependent isocitrate dehydrogenases, which localize to the mitochondrial matrix, and two NADP(+)-dependent isocitrate dehydrogenases, one of which is mitochondrial and the other predominantly cytosolic. Each NADP(+)-dependent isozyme is a homodimer. The protein encoded by this gene is the NADP(+)-dependent isocitrate dehydrogenase found in the mitochondria. It plays a role in intermediary metabolism and energy production. This protein may tightly associate or interact with the pyruvate dehydrogenase complex. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Feb 2014]

Drug Information

Enasidenib

Enasidenib is an orally available treatment for the treatment of adult patients with relapsed or refractory acute myeloid leukemia (AML) with specific mutations in the isocitrate dehydrogenase 2 (IDH2) gene.

Enasidenib is a selective inhibitor of IDH2, a mitochondria-localized enzyme involved in diverse cellular processes, including adaptation to hypoxia, histone demethylation and DNA modification. Enasidenib primarily targets the mutant IDH2 variants R140Q, R172S, and R172K with higher potency than the wild type enzyme form. Inhibition of the enzyme leads to decreased levels of 2-hydroxyglutarate (2-HG) and promotion of proper differentiation and clonal proliferation of cells of the myeloid lineage.

Enasidenib was approved by U.S. Food and Drug Administration on August 1, 2017.

Detailed Results

Single Nucleotide Variant (SNV) and Insertions-Deletions (INDELS)								
Gene Name	Hgvsp	Hgvsc	Aminoacids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on Protein
DNMT3A	NP_783328.1:p.Val763Ile	NM_175629.2:c.2287G>A	V/I	Gtt/Att	missense_variant	3.75	80	deleterious (0.05)
DNMT3A	NP_783328.1:p.Phe772Ile	NM_175629.2:c.2314T>A	F/I	Ttt/Att	missense_variant	3.61	83	deleterious (0.01)
IDH2	NP_002159.2:p.Arg140Gln	NM_002168.2:c.419G>A	R/Q	cGg/cAg	missense_variant	3.12	64	deleterious - low confidence (0)

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA for abnormalities in 284 genes that are reported to be altered in various types of hematologic neoplasms. Nucleic acid is isolated from plasma. Testing is performed using parallel sequencing of the coding DNA of the listed genes. This includes sequencing of all the exons as well as 50 nucleotides at the 5' and 3' ends of each coding exon. Using UMI, our sequencing method has a typical sensitivity of less than 0.1% for detecting common specific mutations and 0.1% for other mutations. 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. Performance of the assay may vary depending on the quantity and quality of nucleic acid, sample preparation and sample age. The assay is designed to detect significant gene amplification and deletion in addition to various single nucleotide variations (SNV) and indels. Indels greater than 80bp may not be detected.
In addition to cfDNA analysis, targeted cfRNA NGS analysis is performed. This is a next generation sequencing (NGS) test that analyzes targeted cfRNA on 1,501 genes associated with hematologic neoplasms. It is based on hybrid capture of targeted cfRNA. Duplicates are excluded for levels measurements. While the major focus of the analysis is the detection of fusion mRNA, mutations in the expressed cfRNA of the analyzed genes are also analyzed and reported.
All detected fusion transcripts are reported. 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 expression level of most of these genes is not characterized at this time, only few specific genes (MYC, BCL2, CD274, CD19, CD22, CD79A, CD79B) will be commented on. .The sensitivity of this assay in detecting fusion mRNA is between 1% and 5%. The Universal Human Reference (UHR) RNA is also used as control.

The table below contains 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)

The table below contains a partial list of the tested RNA genes (Fusions/Expression). For a complete list, please go to:

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Tested genes

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

Electronic Signature

The test (sample processing, sequencing and data generation) was performed at Genomic Testing Cooperative, LCA, Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618. Medical Director Maher Albitar, M.D. Analysis of the data was performed by Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618. 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.