Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618
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CLIA #: 05D2111917 CAP #: 9441547

Liquid Trace Hematology

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

MRN:				Indication for Testing:	C82.90 Follicular lymphoma, unspecified, unspecified site
Collected Date:	/2022	Time:	12:00 AM		
Received Date:	/2022	Time:	11:22 AM		
Reported Date:	/2022	Time:	01:24 PM		

Detected Genomic Alterations

AURKC (?germline)	DNMT3A (4mutations)	TP53	CREBBP	EPHA5
ATM	RET	EBV-4 detected (RNA)	No evidence of chromosomal structural abnormalities.	

Results Summary

- **-Mutations in AURKC (?germline), DNMT3A (4 mutations), TP53, CREBBP, EPHA5, ATM, and RET genes**
  -High mRNA Expression of EBV-4
  -No evidence of expression in HPV
  -No definitive evidence of fusion abnormality
  -No evidence of chromosomal abnormalities

  -The mutations in ATM, CREBBP and RET are consistent with low level lymphoma DNA circulatiion. The mutations in DNMT3A gene are most consistent with CHIP (clonal hematopoiesis of indeterminate potential.

  -The AURKC mutation is detected at high level, most suggestive of a germline mutation. This mutation leads to early termination (loss of function). However, there is no data on its clinical relevance and should be classified as of "uncertain significance" at this time.

Heterogeneity

-The AURKC mutation is detected at high level, most suggestive of a germline mutation.
-There are abnormal low-level clones with DNMT3A (4 mutations), TP53, CREBBP, EPHA5, ATM, and RET mutations.

Expression	
High mRNA Expression: EBV-4	No significant increase in B-cell markers
No definitive evidence of fusion abnormality	

Diagnostic Implications	
DNMT3A (4 mutations), TP53, CREBBP, EPHA5, ATM, RET	These findings suggest extremely low-level B-cell lymphoma (see results summary)

Therapeutic Implications	
DNMT3A	DNA methyltransferase inhibitors
TP53	Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy
CREBBP	Bromodomain and Extra-Terminal motif (BET) inhibitors
EPHA5	EPHA5 inhibitors
ATM	PARP inhibitors
RET	RET inhibitors

Prognostic Implications	
AURKC (?germline)	Unknown
DNMT3A	Poor
TP53	Poor
CREBBP	Poor
EPHA5	Poor
ATM	Poor
RET	Poor

Relevant Genes with NO Alteration
No evidence of mutation in: NOTCH, SF3B1, TP53, MYD88

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, EBV and HPV) in DNA of 284 genes and RNA in 1501 genes associated with hematologic neoplasms, including leukemia, lymphoma, myeloma, and MDS. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Biological relevance of detected Alterations

- AURKC. This gene encodes a member of the Aurora subfamily of serine/threonine protein kinases. The encoded protein is a chromosomal passenger protein that forms complexes with Aurora-B and inner centromere proteins and may play a role in organizing microtubules in relation to centrosome/spindle function during mitosis. This gene is overexpressed in several cancer cell lines, suggesting an involvement in oncogenic signal transduction. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jul 2008]

- 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]

- 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]

- CREBBP. This gene is ubiquitously expressed and is involved in the transcriptional coactivation of many different transcription factors. First isolated as a nuclear protein that binds to cAMP-response element binding protein (CREB), this gene is now known to play critical roles in embryonic development, growth control, and homeostasis by coupling chromatin remodeling to transcription factor recognition. The protein encoded by this gene has intrinsic histone acetyltransferase activity and also acts as a scaffold to stabilize additional protein interactions with the transcription complex. This protein acetylates both histone and non-histone proteins. This protein shares regions of very high sequence similarity with protein p300 in its bromodomain, cysteine-histidine-rich regions, and histone acetyltransferase domain. Mutations in this gene cause Rubinstein-Taybi syndrome (RTS). Chromosomal translocations involving this gene have been associated with acute myeloid leukemia. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Feb 2009]

- EPHA5. This gene belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family. EPH and EPH-related receptors have been implicated in mediating developmental events, particularly in the nervous system. Receptors in the EPH subfamily typically have a single kinase domain and an extracellular region containing a Cys-rich domain and 2 fibronectin type III repeats. The ephrin receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. Alternatively spliced transcript variants encoding different isoforms have been described. [provided by RefSeq, Aug 2013]

- ATM. The protein encoded by this gene belongs to the PI3/PI4-kinase family. This protein is an important cell cycle checkpoint kinase that phosphorylates; thus, it functions as a regulator of a wide variety of downstream proteins, including tumor suppressor proteins p53 and BRCA1, checkpoint kinase CHK2, checkpoint proteins RAD17 and RAD9, and DNA repair protein NBS1. This protein and the closely related kinase ATR are thought to be master controllers of cell cycle checkpoint signaling pathways that are required for cell response to DNA damage and for genome stability. Mutations in this gene are associated with ataxia telangiectasia, an autosomal recessive disorder. [provided by RefSeq, Aug 2010]

- RET. This gene encodes a transmembrane receptor and member of the tyrosine protein kinase family of proteins. Binding of ligands such as GDNF (glial cell-line derived neurotrophic factor) and other related proteins to the encoded receptor stimulates receptor dimerization and activation of downstream signaling pathways that play a role in cell differentiation, growth, migration and survival. The encoded receptor is important in development of the nervous system, and the development of organs and tissues derived from the neural crest. This proto-oncogene can undergo oncogenic activation through both cytogenetic rearrangement and activating point mutations. Mutations in this gene are associated with Hirschsprung disease and central hypoventilation syndrome and have been identified in patients with renal agenesis. [provided by RefSeq, Sep 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
AURKC	NP_001015878.1:p.Ser32HisfsTer8	NM_001015878.1:c.94_101delAGCCCAGC	TAQ/X	aCAGCCCAG/a	frameshift_variant	46.83	1343	0
DNMT3A	NP_783328.1:p.Asp618ThrfsTer33	NM_175629.2:c.1852delG	D/X	Gac/ac	"frameshift_variant, splice_region_variant"	3.64	1180	0
DNMT3A	NP_783328.1:p.Cys562Tyr	NM_175629.2:c.1685G>A	C/Y	tGt/tAt	missense_variant	2.14	1400	deleterious (0.04)
TP53	NP_000537.3:p.Ile195Thr	NM_000546.5:c.584T>C	I/T	aTc/aCc	missense_variant	2.0	1548	deleterious (0)

CREBBP	NP_004371.2:p.Ser2328Leu	NM_004380.2:c.6983C>T	S/L	tCg/tTg	missense_variant	1.46	2125	0
DNMT3A	NP_783328.1:p.Ser770Leu	NM_175629.2:c.2309C>T	S/L	tCg/tTg	missense_variant	1.42	2185	deleterious (0.01)
EPHA5	NP_004430.4:p.Ser182Arg	NM_004439.5:c.546C>G	S/R	agC/agG	missense_variant	1.26	1990	deleterious (0)
ATM	NP_000042.3:p.Pro2699Leu	NM_000051.3:c.8096C>T	P/L	cCa/cTa	missense_variant	0.84	1312	deleterious (0)
DNMT3A	NP_783328.1:p.Pro307Arg	NM_175629.2:c.920C>G	P/R	cCa/cGa	missense_variant	0.78	1796	deleterious (0)
RET	NP_066124.1:p.Ala1019ArgfsTer9	NM_020975.4:c.3054delT	L/X	cTt/ct	frameshift_variant	0.48	1457	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 0.001% 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 0.001% 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. 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%. .Various sensitivity control are used. 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: tumor/https://genomictestingcooperative.com/genomic-tests/liquid-trace-hematologic-malignancies/ (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: tumor/https://genomictestingcooperative.com/genomic-tests/liquid-trace-hematologic-malignancies/ (click the RNA tab)

Tested genes

Genes Tested for Abnormalities in Coding Sequence												
ABL1	B2M	CCNE1	CTNNB1	ETV6	FLT4	IDH2	MAP2K1	MYCL	PDGFRB	XPO1	SOCS1	TSHR
ACVR1B	BAP1	CD274	CUX1	EXO1	FOXL2	IGF1R	MAP2K2	MYCN	PHF6	XRCC2	SOX2	U2AF1
AKT1	BCL2	CD79A	CXCR4	EZH2	FUBP1	IKZF1	MAP2K4	MYD88	STK11	XRCC3	SOX9	U2AF2
AKT2	BCL2L1	CD79B	CYLD	ABRAXAS1	GALNT12	IKZF3	MAP3K1	NF1	SUFU	ZNF217	SPOP	VHL
AKT3	BCL6	CDC73	DAXX	TENT5C	GATA1	IL7R	MAP3K14	NF2	SUZ12	ZRSR2	SRC	WHSC1

ALK	BCOR	CDH1	DDR2	FANCA	GATA2	INHBA	MAPK1	NFE2L2	TAL1	NFE2	SRSF2	WT1
AMER1	BCORL1	CDK12	DICER1	FANCC	GATA3	IRF4	MCL1	NFKBIA	TCF3	UBA1	STAG2	XPO1
APC	BCR	CDK4	DNM2	FANCD2	GEN1	JAK1	MDM2	NKX2-1	TERT	STAT5B	STAT3	XRCC2
AR	BIRC3	CDK6	DNMT3A	FANCE	GNA11	JAK2	MDM4	NOTCH1	TET2	ETNK1	STK11	XRCC3
ARAF	BLM	CDKN2A	DOT1L	FANCF	GNAQ	JAK3	MED12	NOTCH2	TGFBR2	ELANE	SUFU	ZNF217
ARID1A	BRAF	CDKN2B	EED	FANCG	GNAS	KAT6A	MEF2B	NOTCH3	TNFAIP3	ANKRD26	SUZ12	ZRSR2
ARID1B	BRCA1	CDKN2C	EGFR	FAS	GREM1	KDM5C	MEN1	NPM1	TNFRSF14	SAMD9L	TAL1	-
ARID2	BRCA2	CEBPA	EGLN1	FBXW7	GRIN2A	KDM6A	MET	NRAS	TP53	SAMD9	TCF3	-
ASXL1	BRIP1	CHEK1	EP300	FGF4	H3-3A	KDR	MITF	NSD1	TRAF3	DDX41	TERT	-
ATM	BTK	CHEK2	EPAS1	FGF6	HGF	KEAP1	MLH1	NTRK1	TSC1	SF3B1	TET2	-
ATR	CALR	CIC	EPHA3	FGFR1	H3C2	KIT	MPL	NTRK2	TSC2	SMAD2	TGFBR2	-
ATRX	CARD11	CREBBP	EPHA5	FGFR2	HNF1A	KMT2A	MRE11	NTRK3	TSHR	SMAD4	TNFAIP3	-
AURKA	CBL	CRLF2	ERBB2	FGFR3	HOXB13	KMT2B	MSH2	PAK3	U2AF1	SMARCA4	TNFRSF14	-
AURKB	CBLB	CSF1R	ERBB3	FGFR4	HRAS	KMT2C	MSH6	PALB2	U2AF2	SMARCB1	TP53	-
AURKC	CBLC	CSF3R	ERBB4	FH	HSP90AA1	KMT2D	MTOR	PAX5	VHL	SMC1A	TRAF3	-
AXIN1	CCND1	CTCF	ERG	FLCN	ID3	KRAS	MUTYH	PBRM1	NSD2	SMC3	TSC1	-
AXIN2	CCND3	CTNNA1	ESR1	FLT3	IDH1	LRP1B	MYC	PDGFRA	WT1	SMO	TSC2	-

Reference

1. Therapy-related myeloid neoplasms. Ganser A, Heuser M. Ganser A, et al. Curr Opin Hematol. 2017 Mar;24(2):152-158. doi: 10.1097/MOH.0000000000000316. Curr Opin Hematol. 2017. PMID: 27930389

Electronic Signature

Maher Albitar, M.D.

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.