GENOMIC TESTING COOPERATIVE®
25371 Commercentre Drive, Lake Forest, CA 92630
Tel: 1-866-484-8870 | genomictestingcooperative.com
CLIA # 05D2111917 CAP # 9441574

Liquid Trace Hematology

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:	Acute lymphoblastic leukemia not having achieved remission (C91.00)
Collected Date:		Time:	12:00 AM		
Received Date:		Time:	11:08 AM	Tumor Type:	ALL
Reported Date:		Time:	04:23 PM		

Detected Genomic Alterations				
No evidence of mutations	No detectable autosomal chromosomal structural gain or loss	Low level t(4;11)(q21;q23) AFF1::KMT2A fusion mRNA	CD28::CD247 (CD3Z) CAR-T construct mRNA	B cell clonality: Detected, heavy chain only (IGHV3-43)
T cell clonality: Not detected	-			

Results Summary

- **-Low level t(4;11)(q21;q23) AFF1::KMT2A fusion mRNA**
  -CD28::CD247 (CD3Z) CAR-T construct mRNA
  -No evidence of mutations
  -No detectable autosomal chromosomal structural gain or loss
  -B cell clonality: Detected, heavy chain only (IGHV3-43)
  -T cell clonality: Not detected
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*32:01-A*66:02
  -HLA-B: B*58:01-B*81:01
  -HLA-C: C*08:04-C*07:18
  -Extremely low B-cell markers
  -Increased T-cell markers

  -These findings are consistent with the presence of extremely low-level B-acute lymphoblastic leukemia (B-ALL) with AFF1::KMT2A fusion and co-presence of CAR-T cells.

  -The ratio of CAR-T RNA to AFF1::KMT2A RNA is 40:1.

  -KMT2A gene fusion suggests response to Menin inhibitors (revumenib).

Expression	
Extremely low B-cell markers	Increased T-cell markers

Relevant Genes with NO Alteration
No evidence of mutation in JAK2, MPL, CALR, FLT3, NPM1, IDH1, or IDH2

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), 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 hematologic neoplasms, including leukemia, lymphoma, myeloma, myelodysplastic syndrome, and myeloproliferative neoplasms. Whenever possible, clinical relevance and implications of detected abnormalities are described below. If a gene is not reported, then no somatic mutations were detected. This assay facilitates myelodysplastic syndrome risk assessment as it includes evaluation for mutations and significant chromosomal gains and losses in all of the genes included in the IPSS-M risk calculator: ASXL1, BCOR, BCORL1, CBL, CEBPA, DNMT3A, ETNK1, ETV6, EZH2, FLT3, GATA2, GNB1, IDH1, IDH2, KMT2A (including KMT2A(MLL)-PTD), KRAS, NF1, NPM1, NRAS, PHF6, PPM1D, PRPF8, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, STAG2, TP53, U2AF1, and WT1.

Methodology and Test Background

This is a next generation sequencing (NGS) test that involves separate analysis of DNA and RNA panels for abnormalities that are reported in various types of hematologic neoplasms. The DNA panel is composed of 302 genes, and the RNA panel is composed of >1600 genes. The DNA and RNA components of this assay were developed, validated, and set up as separate workflows, with independent extraction, library preparation, sequencing, and analysis pipelines. The NGS 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. 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, B- and T-cell clonality, HLA class I genotyping,

and Epstein-Barr virus (EBV), human papillomavirus (HPV) and torque teno virus (TTV) viral RNA are also analyzed and reported. 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.

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.

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-hematologic-malignancies/ (click the DNA tab)

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

Tested genes

Genes Tested for Abnormalities in Coding Sequence												
ABL1	AXIN1	CCND1	CTCF	ERBB3	FH	HSP90AA1	KMT2D	MTOR	PAK3	PTCH1	SMAD2	TERT
ABRAXAS1	AXIN2	CCND3	CTNNA1	ERBB4	FLCN	ID3	KRAS	MUTYH	PALB2	PTEN	SMAD4	TET2
ACVR1B	B2M	CCNE1	CTNNB1	ERG	FLT3	IDH1	LRP1B	MYC	PAX5	PTPN11	SMARCA4	TGFBR2
AKT1	BAP1	CD274	CUX1	ESR1	FLT4	IDH2	MAP2K1	MYCL	PBRM1	RAC1	SMARCB1	TNFAIP3
AKT2	BCL2	CD79A	CXCR4	ETNK1	FOXL2	IGF1R	MAP2K2	MYCN	PDGFRA	RAD21	SMC1A	TNFRSF14
AKT3	BCL2L1	CD79B	CYLD	ETV6	FUBP1	IKZF1	MAP2K4	MYD88	PDGFRB	RAD50	SMC3	TP53
ALK	BCL6	CDC73	DAXX	EXO1	GALNT12	IKZF3	MAP3K1	NF1	PHF6	RAD51	SMO	TRAF3
AMER1	BCOR	CDH1	DDR2	EZH2	GATA1	IL7R	MAP3K14	NF2	PIK3CA	RAF1	SOCS1	TSC1
ANKRD26	BCORL1	CDK12	DDX41	FANCA	GATA2	INHBA	MAPK1	NFE2	PIK3R1	RB1	SOX2	TSC2
APC	BCR	CDK4	DICER1	FANCC	GATA3	IRF4	MCL1	NFE2L2	PIK3R2	RET	SOX9	TSHR
AR	BIRC3	CDK6	DNM2	FANCD2	GEN1	JAK1	MDM2	NFKBIA	PIM1	RHEB	SPOP	U2AF1
ARAF	BLM	CDKN2A	DNMT3A	FANCE	GNA11	JAK2	MDM4	NKX2-1	PLCG1	RHOA	SRC	U2AF2
ARID1A	BRAF	CDKN2B	DOT1L	FANCF	GNAQ	JAK3	MED12	NOTCH1	PMS1	RIT1	SRSF2	UBA1
ARID1B	BRCA1	CDKN2C	EED	FANCG	GNAS	KAT6A	MEF2B	NOTCH2	PMS2	RNF43	STAG2	VHL
ARID2	BRCA2	CEBPA	EGFR	FAS	GREM1	KDM5C	MEN1	NOTCH3	POLD1	ROS1	STAT3	WT1
ASXL1	BRIP1	CHEK1	EGLN1	FBXW7	GRIN2A	KDM6A	MET	NPM1	POLE	RUNX1	STAT5B	XPO1
ATM	BTK	CHEK2	ELANE	FGF4	H3-3A (H3F3A)	KDR	MITF	NRAS	PPM1D	SAMD9	STK11	XRCC2
ATR	CALR	CIC	EP300	FGF6	H3C2 (HIST1H3B)	KEAP1	MLH1	NSD1	PPP2R1A	SAMD9L	SUFU	XRCC3
ATRX	CARD11	CREBBP	EPAS1	FGFR1	HGF	KIT	MPL	NSD2 (WHSC1)	PRDM1	SDHB	SUZ12	ZNF217
AURKA	CBL	CRLF2	EPHA3	FGFR2	HNF1A	KMT2A	MRE11	NTRK1	PRKAR1A	SETBP1	TAL1	ZRSR2
AURKB	CBLB	CSF1R	EPHA5	FGFR3	HOXB13	KMT2B	MSH2	NTRK2	PRKDC	SETD2	TCF3	-
AURKC	CBLC	CSF3R	ERBB2	FGFR4	HRAS	KMT2C	MSH6	NTRK3	PRSS1	SF3B1	TENT5C (FAM46C)	-

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RNA Fusions/Expression

Fusion/Expression															
ABL1	BCL2	CCND1	CREBBP	EGFR	ETV4	FGFR2	FOXO1	IKZF3	MAP3K1	MYH9	NTRK3	PAX5	PDGFRB	PTK2B	ROS1	TAL1
ABL2	BCL6	CD274 (PD-L1)	CRLF2	EPOR	ETV5	FGFR3	FUS	JAK2	MECOM	NOTCH1	NUP214	PBX1	PICALM	RARA	RUNX1	TCF3
AKT3	BRAF	CBL	CSF1R	ERG	ETV6	FIP1L1	GLI1	KMT2A	MRTFA	NTRK1	NUP98	PCM1	PIGA	RET	RUNX1T1	TFG
ALK	CBFB	CIC	DUSP22	ETV1	FGFR1	FLT3	HLF	LYN	MYC	NTRK2	P2RY8	PDGFRA	PML	RHOA	STAT6	TYK2

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. (CLIA #: 05D2111917 CAP #: 9441574)

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.