

## Liquid Trace Hematology

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

MRN:		Indication for Testing:	C91.10 Chronic lymphocytic leukemia of B-cell type not having achieved remission
Collected Date:		Time:	12:00 AM
Received Date:		Time:	10:29 AM
Reported Date:		Time:	01:26 PM

Detected Genomic Alterations				
GEN1 (?Germline, VUS)	KMT2C (2 mutations)	DNMT3A	CUX1	FLT4
RAD21	CREBBP	VHL	CMKLR1 (?Germline, VUS)	ABCG2 (?Germline)
TTV viral RNA: Detected (87 copies)	No detectable autosomal chromosomal structural gain or loss	B-cell clonality: Detected at a very low level, light chain only (IgLV8-61)	T-cell clonality: Not detected	

### Results Summary

- **-Low-level somatic mutations in KMT2C (2 mutations), DNMT3A, CUX1, FLT4, RAD21, CREBBP, and VHL genes.**
- **-Possible germline mutations in GEN1, CMKLR1, and ABCG2 genes, heterozygous**
- **-No detectable autosomal chromosomal structural gain or loss.**
- **-EBV viral RNA: Not detected.**
- **-HPV viral RNA: Not detected.**
- **-TTV viral RNA: Detected (87 copies).**
- **-HLA Genotyping:**
  - HLA-A: A\*02:01-A\*66:01
  - HLA-B: B\*44:02-B\*41:02
  - HLA-C: B\*44:02-B\*41:02

-These findings are consistent with residual low level B-cell chronic lymphocytic leukemia (B-cell CLL).

-The GEN1 and CMKLR1 mutations are detected at high levels, raising the possibility of germline mutations. These mutations lead to early termination (loss of function). However, there is no data on their clinical relevance and should be classified as of "uncertain significance" at this time.

-The ABCG2 mutation is detected at high level raising the possibility of a germline abnormality. This

gene is involved in membrane transport and possibly associated with drug response.

**See quantitative presentation of mutations at the end of the report.**

### Heterogeneity

There are abnormal low-level clones with KMT2C (2 mutations), DNMT3A, CUX1, FLT4, RAD21, CREBBP, and VHL mutations.  
 The GEN1, CMKLR1, and ABCG2 mutations are detected at high levels, possible germline abnormalities.

### Diagnostic Implications

GEN1, KMT2C (2 mutations), DNMT3A, CUX1, FLT4, RAD21, CREBBP, VHL, CMKLR1, ABCG2	-These findings are consistent with residual low level B-cell chronic lymphocytic leukemia (B-cell CLL). -The GEN1, CMKLR1, and ABCG2 mutations are likely germline variants.
--	--

### Therapeutic Implications

DNMT3A	DNA methyltransferase inhibitors
FLT4	mTOR, angiogenic, KDR inhibitors
CREBBP	Bromodomain and Extra-Terminal motif (BET) inhibitors
VHL	Hypoxia-inducible factor 2a (HIF-2a) inhibitor and anti-VEGFA antibodies

### Prognostic Implications

KMT2C (2 mutations), CUX1, FLT4, RAD21	Unknown
DNMT3A, CREBBP, VHL	Poor

### Relevant Genes with NO Alteration

No evidence of mutation in NOTCH, SF3B1, TP53, or MYD88

## 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 cfrRNA 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.

## Biological relevance of detected Alterations

- **GEN1.** This gene encodes a member of the Rad2/xeroderma pigmentosum group G nuclease family, whose members are characterized by N-terminal and internal xeroderma pigmentosum group G nuclease domains followed by helix-hairpin-helix domains and disordered C-terminal domains. The protein encoded by this gene is involved in resolution of Holliday junctions, which are intermediate four-way structures that covalently link DNA during homologous recombination and double-strand break repair. The protein resolves Holliday junctions by creating dual incisions across the junction to produce nicked duplex products that can be ligated. In addition, this protein has been found to localize to centrosomes where it has been implicated in regulation of centrosome integrity. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jul 2016]
- **KMT2C.** This gene is a member of the myeloid/lymphoid or mixed-lineage leukemia (MLL) family and encodes a nuclear protein with an AT hook DNA-binding domain, a DHHC-type zinc finger, six PHD-type zinc fingers, a SET domain, a post-SET domain and a RING-type zinc finger. This protein is a member of the ASC-2/NCOA6 complex (ASCOM), which possesses histone methylation activity and is involved in transcriptional coactivation. [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]
- **CUX1.** The protein encoded by this gene is a member of the homeodomain family of DNA binding proteins. It may regulate gene expression, morphogenesis, and differentiation and it may also play a role in the cell cycle progression. Several alternatively spliced transcript variants encoding different isoforms have been identified. [provided by RefSeq, Feb 2011]
- **FLT4.** This gene encodes a tyrosine kinase receptor for vascular endothelial growth factors C and D. The protein is thought to be involved in lymphangiogenesis and maintenance of the lymphatic endothelium. Mutations in this gene cause hereditary lymphedema type IA. [provided by RefSeq, Jul 2008]
- **RAD21.** The protein encoded by this gene is highly similar to the gene product of *Schizosaccharomyces pombe* rad21, a gene involved in the repair of DNA double-strand breaks, as well as in chromatid cohesion during mitosis. This protein is a nuclear phospho-protein, which becomes hyperphosphorylated in cell cycle M phase. The highly regulated association of this protein with mitotic chromatin specifically at the centromere region suggests its role in sister chromatid cohesion in mitotic cells. [provided by RefSeq, Jul 2008]
- **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]
- **VHL.** Von Hippel-Lindau syndrome (VHL) is a dominantly inherited familial cancer syndrome predisposing to a variety of malignant and benign tumors. A germline mutation of this gene is the basis of familial inheritance of VHL syndrome. The protein encoded by this gene is a component of the protein complex that includes elongin B, elongin C, and cullin-2, and possesses ubiquitin ligase E3 activity. This protein is involved in the ubiquitination and degradation of hypoxia-inducible-factor (HIF), which is a transcription factor that plays a central role in the

regulation of gene expression by oxygen. RNA polymerase II subunit POLR2G/RPB7 is also reported to be a target of this protein. Alternatively spliced transcript variants encoding distinct isoforms have been observed. [provided by RefSeq, Jul 2008]

- CMKLR1. Enables adipokinetic hormone binding activity and adipokinetic hormone receptor activity. Involved in several processes, including negative regulation of NF-kappaB transcription factor activity; positive regulation of macrophage chemotaxis; and regulation of calcium-mediated signaling. Located in plasma membrane. [provided by Alliance of Genome Resources, Apr 2022]
- ABCG2. The membrane-associated protein encoded by this gene is included in the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the White subfamily. Alternatively referred to as a breast cancer resistance protein, this protein functions as a xenobiotic transporter which may play a major role in multi-drug resistance. It likely serves as a cellular defense mechanism in response to mitoxantrone and anthracycline exposure. Significant expression of this protein has been observed in the placenta, which may suggest a potential role for this molecule in placenta tissue. Multiple transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Apr 2012]

## Detailed Results

Single Nucleotide Variant (SNV) and Insertions-Deletions (INDELS)								
Gene name	Hgvsnp	Hgvsc	Amino acids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
GEN1	NP_872431.3:p.Lys645CysfsTer29	NM_182625.3:c.1933_1936delAAG	IK/X	atAAAG/at	frameshift_variant	51.19	1553	0
KMT2C	NP_733751.2:p.Cys394Tyr	NM_170606.2:c.1181G>A	C/Y	tGc/tAc	missense_variant	4.02	2984	0
KMT2C	NP_733751.2:p.Arg909Gly	NM_170606.2:c.2725A>G	R/G	Agg/Ggg	missense_variant	1.55	1159	0
DNMT3A	0	NM_175629.2:c.1123-1G>C	0	0	splice_acceptor_variant	1.38	1379	0
CUX1	NP_001189472.1:p.Asn1018Lys	NM_001202543.1:c.3054C>G	N/K	aaC/aaG	missense_variant	1.33	1276	tolerated (0.06)
FLT4	NP_891555.2:p.Arg1145Gly	NM_182925.4:c.3433C>G	R/G	Cgc/Ggc	"missense_variant,splice_region_variant"	1.12	984	tolerated (0.08)
RAD21	NP_006256.1:p.Gln119Ter	NM_006265.2:c.355C>T	Q/*	Cag/Tag	stop_gained	0.81	988	0
CREBBP	NP_004371.2:p.Met529Val	NM_004380.2:c.1585A>G	M/V	Atg/Gtg	missense_variant	0.61	978	0
VHL	NP_000542.1:p.Gly29Val	NM_000551.3:c.86_87delGCinsTT	G/V	gGC/gTT	missense_variant	0.31	1634	deleterious - low confidence (0.01)
CMKLR1 (RNA)	NP_001135815.1:p.Pro290LeufsTer22	NM_001142343.1:c.869delC	P/X	cCt/ct	frameshift_variant	42.94	715	0
ABCG2 (RNA)	NP_004818.2:p.Gln141Lys	NM_004827.2:c.421C>A	Q/K	Cag/Aag	missense_variant	42.28	149	tolerated (0.19)

## Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA for abnormalities in 302 genes and cfRNA of >1600 genes for abnormalities that are reported in various types of hematologic neoplasms. 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. 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	B2M	CCNE1	CUX1	ETNK1	GALNT12	IL7R	MCL1	NFE2L2	PIM1	RB1	SMO	TRAF3
ABRAXAS1	BAP1	CD274	CXCR4	ETV6	GATA1	INHBA	MDM2	NFKBIA	PLCG1	RET	SOCS1	TSC1
ACVR1B	BARD1	CD79A	CYLD	EXO1	GATA2	IRF4	MDM4	NKX2-1	PMS1	RHEB	SOX2	TSC2
AKT1	BCL2	CD79B	DAXX	EZH2	GATA3	JAK1	MED12	NOTCH1	PMS2	RHOA	SOX9	TSHR
AKT2	BCL2L1	CDC73	DDR2	FANCA	GEN1	JAK2	MEF2B	NOTCH2	POLD1	RIT1	SPOP	U2AF1
AKT3	BCL6	CDH1	DDX41	FANCC	GNA11	JAK3	MEN1	NOTCH3	POLE	RNF43	SRC	U2AF2
ALK	BCOR	CDK12	DICER1	FANCD2	GNAQ	KAT6A	MET	NPM1	POT1	ROS1	SRSF2	UBA1
AMER1	BCORL1	CDK4	DNM2	FANCE	GNAS	KDM5C	MITF	NRAS	PPM1D	RUNX1	STAG2	VHL
ANKRD26	BCR	CDK6	DNMT3A	FANCF	GNB1	KDM6A	MLH1	NSD1	PPP2R1A	SAMD9	STAT3	WT1
APC	BIRC3	CDKN1B	DOT1L	FANCG	GREM1	KDR	MPL	NSD2 (WHSC1)	PRDM1	SAMD9L	STAT5B	XPO1
AR	BLM	CDKN2A	EED	FAS	GRIN2A	KEAP1	MRE11	NTHL1	PRKAR1A	SDHA	STK11	XRCC2
ARAF	BMPR1A	CDKN2B	EGFR	FBXW7	H3-3A (H3F3A)	KIT	MSH2	NTRK1	PRKDC	SDHAF2	SUFU	XRCC3

ARID1A	BRAF	CDKN2C	EGLN1	FGF4	H3C2 (HIST1H3B)	KMT2A	MSH3	NTRK2	PRPF8	SDHB	SUZ12	ZNF217
ARID1B	BRCA1	CEBPA	ELANE	FGF6	HGF	KMT2B	MSH6	NTRK3	PRSS1	SDHC	TAL1	ZRSR2
ARID2	BRCA2	CHEK1	EP300	FGFR1	HNFB1A	KMT2C	MTOR	PAK3	PTCH1	SDHD	TCF3	-
ASXL1	BRIP1	CHEK2	EPAS1	FGFR2	HOXB13	KMT2D	MUTYH	PALB2	PTEN	SETBP1	TENT5C (FAM46C)	-
ATM	BTK	CIC	EPCAM	FGFR3	HRAS	KRAS	MYC	PAX5	PTPN11	SETD2	TERC	-
ATR	CALR	CREBBP	EPHA3	FGFR4	HSP90AA1	LRP1B	MYCL	PBRM1	RAC1	SF3B1	TERT	-
ATRX	CARD11	CRLF2	EPHA5	FH	ID3	MAP2K1	MYCN	PDGFRA	RAD21	SMAD2	TET2	-
AURKA	CBL	CSF1R	ERBB2	FLCN	IDH1	MAP2K2	MYD88	PDGFRB	RAD50	SMAD4	TGFBR2	-
AURKB	CBLB	CSF3R	ERBB3	FLT3	IDH2	MAP2K4	NBN	PHF6	RAD51	SMARCA4	TMEM127	-
AURKC	CBLC	CTCF	ERBB4	FLT4	IGF1R	MAP3K1	NF1	PIK3CA	RAD51C	SMARCB1	TNFAIP3	-
AXIN1	CCND1	CTNNA1	ERG	FOXL2	IKZF1	MAP3K14	NF2	PIK3R1	RAD51D	SMC1A	TNFRSF14	-
AXIN2	CCND3	CTNNB1	ESR1	FUBP1	IKZF3	MAPK1	NFE2	PIK3R2	RAF1	SMC3	TP53	-

## 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

Arash Mohtashamian, MD

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

