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 Hematology

Patient Name:		Ordering Physician:	M.D.
Date of Birth:		Physician ID:	
Gender (M/F):		Accession #:	
Client:		Specimen Type:	Peripheral Blood
		Specimen ID:	
Case #:	NGSXX-XXXXXX		
Body Site:	PERIPHERAL BLOOD		

MRN:				Indication for Testing:	C90.00 Multiple myeloma not having achieved remission
Collected Date:		Time:			
Received Date:		Time:			
Reported Date:		Time:			

Detected Genomic Alterations				
KMT2B	No detectable autosomal chromosomal structural gain or loss	B-cell clonality: Detected (IgH V3-21/IgK V2-24)	T cell clonality: Not detected	CCND1 mRNA: Increased, consistent with promoter hijacking

Results Summary

- **-Low-level mutation in KMT2B gene**
  -No detectable autosomal chromosomal structural gain or loss
  -No evidence of MYD88 & CXCR4 mutations
  -B-cell clonality: Detected (IgH V3-21/IgK V2-24)
  -T cell clonality: Not detected
  -Plasma cell markers (CD138, BCMA): Mildly increased
  -CCND1 mRNA: Increased, consistent with promoter hijacking
  -B cell markers: Not increased with normal pattern
  -Blast markers (CD34, CD117 and TdT): Low level expression
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*26:01-A*26:01
  -HLA-B: B*41:02-B*38:01
  -HLA-C: C*17:03-C*12:03

  -These findings are consistent with low-level plasma cell dyscrasia with t(11;14).

  -Significant reduction (10-fold) in the detected clonal immunoglobulin RNA is noted as compared with previous sample (NGSXX-XXXXXX, dated MM/DD/YYYY).

-There is no evidence of a clinically relevant myeloid neoplasm.

Heterogeneity
There is an abnormal low-level clone with KMT2B mutation.

Expression	
Plasma cell markers (CD138, BCMA): Mildly increased	CCND1 mRNA: Increased, consistent with promoter hijacking
B cell markers: Not increased with normal pattern	Blast markers (CD34, CD117 and TdT): Low level expression

Diagnostic Implications	
KMT2B	This finding is consistent with plasma cell dyscrasia

Prognostic Implications	
KMT2B	Unknown

Relevant Genes with NO Alteration
No evidence of mutation in 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 284 genes and cfRNA in greater than 1600 genes associated with hematologic neoplasms, including leukemia, lymphoma, myeloma, myelodysplastic syndrome and myeloproliferative neoplasms. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Biological relevance of detected Alterations

- KMT2B. This gene encodes a protein which contains multiple domains including a CXXC zinc finger, three PHD zinc fingers, two FY-rich domains, and a SET (suppressor of variegation, enhancer of zeste, and trithorax) domain. The SET domain is a conserved C-terminal domain that characterizes proteins of the MLL (mixed-lineage leukemia) family. This gene is ubiquitously expressed in adult tissues. It is also amplified in solid tumor cell lines, and may be involved in human cancer. Two alternatively spliced transcript variants encoding distinct isoforms have been reported for this gene, however, the full length nature of the shorter transcript is not known. [provided by RefSeq, Jul 2008]

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
KMT2B	NP_055542.1:p.Ser2086Leu	NM_014727.1:c.6257C>T	S/L	tCg/tTg	missense_variant	3.54	705	0

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA for abnormalities in 284 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 assay 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 contains 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)*

The table below contains a partial list of the tested RNA genes (Fusions/Expression). For a complete list, 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	CTNNB1	ETV6	FLT4	IDH2	MAP2K1	MYCL	PDGFRB	RAD50	SOCS1	TSHR

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

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.