GENOMIC TESTING COOPERATIVE®
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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:	PERIPHERAL BLOOD		

MRN:			Indication for Testing:	C90.00 Multiple myeloma not having achieved remission
Collected Date:		Time: 12:00 AM		
Received Date:		Time: 09:16 AM		
Reported Date:		Time: 06:41 PM		

Detected Genomic Alterations				
KDM5C (?Germline, VUS)	PRKDC	STAG2	TP53	ABL1
TET2 (2 mutations)	SMC3 (2 mutations)	CD33 (?Germline, VUS)	No detectable autosomal chromosomal structural gain or loss	B cell clonality: Detected, low-level previously reported clone, light chain only (IgLV 4-3)
T cell clonality: Not detected	-			

Results Summary

- **-Low-level somatic mutations in PRKDC, STAG2, TP53, ABL1, TET2 (2 mutations), and SMC3 (2 mutations) genes**
  -Possible germline mutations in KDM5C and CD33 genes, heterozygous
  -No detectable autosomal chromosomal structural gain or loss
  -B cell clonality: Detected, low-level previously reported clone, light chain only (IgLV 4-3)
  -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*68:01-A*24:03
  -HLA-B: B*39:14-B*40:20
  -HLA-C: C*03:04-C*07:02

  -These findings are consistent with residual multiple myeloma. However, the mutations in STAG2 and TET2 genes are most consistent with CHIP (clonal hematopoiesis of indeterminate potential).

  -The KDM5C and CD33 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.

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

Heterogeneity

There are abnormal low-level clones with PRKDC, STAG2, TP53, ABL1, (2) TET2, and (2) SMC3 mutations. The KDM5C and CD33 mutations are detected at high levels, possible germline abnormalities.

Diagnostic Implications

KDM5C, PRKDC, STAG2, TP53, ABL1, TET2 (2 mutations), SMC3 (2 mutations), CD33	-These findings are consistent with residual multiple myeloma. -The KDM5C and CD33 mutations are likely germline variants.

Therapeutic Implications

PRKDC	PI3K/AKT, PARP inhibitors
STAG2	PARP inhibitors
TP53	Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy
ABL1	BCR::ABL1 tyrosine kinase inhibitors
TET2	DNA methyltransferase inhibitors
SMC3	PARP inhibitors

Prognostic Implications

PRKDC	Poor
STAG2	Poor
TP53	Poor
ABL1	Poor
TET2 (2 mutations)	Poor
SMC3 (2 mutations)	Unknown

Relevant Genes with NO Alteration

No evidence of mutation in NOTCH, SF3B1, 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 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.

Biological relevance of detected Alterations

- KDM5C. This gene is a member of the SMCY homolog family and encodes a protein with one ARID domain, one JmjC domain, one JmjN domain and two PHD-type zinc fingers. The DNA-binding motifs suggest this protein is involved in the regulation of transcription and chromatin remodeling. Mutations in this gene have been associated with X-linked cognitive disability. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Apr 2009]

- PRKDC. This gene encodes the catalytic subunit of the DNA-dependent protein kinase (DNA-PK). It functions with the Ku70/Ku80 heterodimer protein in DNA double strand break repair and recombination. The protein encoded is a member of the PI3/PI4-kinase family.[provided by RefSeq, Jul 2010]

- STAG2. The protein encoded by this gene is a subunit of the cohesin complex, which regulates the separation of sister chromatids during cell division. Targeted inactivation of this gene results in chromatid cohesion defects and aneuploidy, suggesting that genetic disruption of cohesin is a cause of aneuploidy in human cancer. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Sep 2013]

- 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]

- ABL1. This gene is a protooncogene that encodes a protein tyrosine kinase involved in a variety of cellular processes, including cell division, adhesion, differentiation, and response to stress. The activity of the protein is negatively regulated by its SH3 domain, whereby deletion of the region encoding this domain results in an oncogene. The ubiquitously expressed protein has DNA-binding activity that is regulated by CDC2-mediated phosphorylation, suggesting a cell cycle function. This gene has been found fused to a variety of translocation partner genes in various leukemias, most notably the t(9;22) translocation that results in a fusion with the 5' end of the breakpoint cluster region gene (BCR; MIM:151410). Alternative splicing of this gene results in two transcript variants, which contain alternative first exons that are spliced to the remaining common exons. [provided by RefSeq, Aug 2014]

- TET2. The protein encoded by this gene is a methylcytosine dioxygenase that catalyzes the conversion of methylcytosine to 5-hydroxymethylcytosine. The encoded protein is involved in myelopoiesis, and defects in this gene have been associated with several myeloproliferative disorders. Two variants encoding different isoforms have been found for this gene. [provided by RefSeq, Mar 2011]

- SMC3. This gene belongs to the SMC3 subfamily of SMC proteins. The encoded protein occurs in certain cell types as either an intracellular, nuclear protein or a secreted protein. The nuclear form, known as structural maintenance of chromosomes 3, is a component of the multimeric cohesin complex that holds together sister chromatids during mitosis, enabling proper chromosome segregation. Post-translational modification of the encoded protein by the addition of chondroitin sulfate chains gives rise to the secreted proteoglycan bamacan, an abundant basement membrane protein. [provided by RefSeq, Jul 2008]

- CD33. Enables protein phosphatase binding activity and sialic acid binding activity. Involved in several processes, including negative regulation of cytokine production; negative regulation of monocyte activation; and positive regulation of protein tyrosine phosphatase activity. Located in several cellular components, including Golgi apparatus; external side of plasma membrane; and peroxisome. [provided by Alliance of Genome Resources, Apr 2022]

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
KDM5C	NP_004178.2:p.Gly604Ser	NM_004187.3:c.1810G>A	G/S	Ggc/Agc	missense_variant	56.01	507	deleterious (0)
PRKDC	0	NM_006904.6:c.6206+1G>A	0	0	splice_donor_variant	3.53	737	0
STAG2	0	NM_001042749.1:c.2026-2A>C	0	0	splice_acceptor_variant	1.02	294	0
TP53	NP_000537.3:p.Arg249Met	NM_000546.5:c.746G>T	R/M	aGg/aTg	missense_variant	0.84	832	deleterious (0)
ABL1	NP_009297.2:p.Ala225Thr	NM_007313.2:c.673G>A	A/T	Gcc/Acc	missense_variant	0.67	1188	deleterious (0.02)
TET2	NP_001120680.1:p.Glu178_Gln185delinsArgfsTer?	NM_001127208.2:c.531_553delAGAGCTTCAGATTCTGAATGAGCinsA	ELQILNEQ/RX	ccAGAGCTTCAGATTCTGAATGAGCag/ccAag	frameshift_variant	0.5	1189	0
TET2	NP_001120680.1:p.Gln958ThrfsTer14	NM_001127208.2:c.2871dupA	L/LX	tta/ttAa	frameshift_variant	0.36	1114	0
SMC3	NP_005436.1:p.Gly164Asp	NM_005445.3:c.491G>A	G/D	gGt/gAt	missense_variant	0.35	848	deleterious (0)
SMC3	0	NM_005445.3:c.804+1G>A	0	0	splice_donor_variant	0.08	1185	0
CD33 (RNA)	NP_001763.3:p.Gly156ThrfsTer5	NM_001772.3:c.466_469delGGCC	PG/X	cCCGGc/cc	frameshift_variant	29.03	31	0

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	HNF1A	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

Sally Agersborg, MD

The test (sample processing, sequencing and data generation) was performed at Regional Cancer Care Associates Laboratory, Key Genomics 92 Second Street Hackensack, NJ 07601. 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 Regional Cancer Care Associates Laboratory. 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