Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618
Tel: 1-866-484-8870 www.genomictestingcooperative.com
CLIA #: 05D2111917 CAP #: 9441547

Hematology Profile

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

MRN:				Indication for Testing:	Anemia, unspecified (D64.9)
Collected Date:		Time:			
Received Date:		Time:			
Reported Date:		Time:			

Detected Genomic Alterations				
DNMT3A	TET2	No detectable autosomal chromosomal structural gain or loss		

Results Summary

- **-Low-level mutations in DNMT3A and TET2 genes.**
  -No detectable autosomal chromosomal structural gain or loss.

  -Low level mutations can be seen in aplastic anemia and in normal individuals above the age of 50, consistent with CHIP (clonal hematopoiesis of indeterminate potential) or CCUS (clonal cytopenia of undetermined significance). This abnormality is not diagnostic for hematologic neoplasm. However, individuals with this abnormality are considered at higher risk of developing hematologic neoplasms as well as at higher risk (4-fold) of having a cardiovascular event. Correlation with clinical and other laboratory data is recommended.

  -The sample is not tested for gene fusions (chromosomal translocations) or expression profiling. To evaluate B- and T-cell clonality, IgVH mutation status in CLL, fusion mRNA (PML-RARA, BCR-ABL...), molecular immunophenotyping, and lymphoid neoplasm classifications, we recommend ordering RNA and DNA testing (HemePLUS).

Heterogeneity
There are abnormal low-level clones with DNMT3A and TET2 mutations.

Diagnostic Implications	
DNMT3A, TET2	These findings at such a low level can be seen in CHIP (see results summary)

Therapeutic Implications	
DNMT3A	DNA methyltransferase inhibitors
TET2	DNA methyltransferase inhibitors

Prognostic Implications	
DNMT3A	Poor
TET2	Neutral

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), Sanger Sequencing and fragment length analysis testing to identify molecular abnormalities (including SNVs, INDELS, and CNVs) in 284 genes implicated in hematologic neoplasms, including leukemia, lymphoma, and MDS. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Biological relevance of detected Alterations

- 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]

- 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]

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
DNMT3A	NP_783328.1:p.Tyr536LeufsTer10	NM_175629.2:c.1605dupC	-/X	-/C	frameshift_variant	4.79	1023	0
TET2	0	NM_001127208.2:c.3954+2T>G	0	0	splice_donor_variant	3.49	372	0

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes DNA for abnormalities in 284 genes that are reported to be altered in various types of hematologic neoplasms. Nucleic acid can be isolated from fresh cells, peripheral blood cells, bone marrow, body fluid, or paraffin-embedded tissue. For optimal results, neoplastic cells should be >30% of the analyzed cells. Decalcified specimens have not been validated. For fresh bone marrow specimens with the clinical indication of myeloma, enrichment for CD138-positive cells may be performed using immunomagnetic positive selection and both the CD138-positive and CD138-negative cell fractions extracted for NGS testing and the findings integrated within the final report. 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 sequencing method has a sensitivity of 1% for detecting single nucleotide variants (SNVs) and small (<60 bp) insertions/ deletions (indels). Significant gene amplification and deletion (copy number variants) are also reported. In addition, fragment length analysis is performed for CALR, FLT3, and NPM1 to enhance the detection of large indels and has a sensitivity of 2%-5% for detecting CALR, FLT3-ITD, and NPM1 indels in wildtype background. For cases with indication of acute myeloid leukemia, preliminary FLT3-ITD results based on fragment analysis will be reported. Performance of the assay may vary dependent 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.

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. This poor coverage is mainly due to high GC content with inherent problem in obtaining adequate coverage. 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/hematology-profile/ *(click the DNA tab)*

Tested genes

Genes Tested for Abnormalities in Coding Sequence												
ABL1	BCL2	CBL	CDKN2C	DICER1	FAS	IDH2	KMT2A	MEF2B	NSD1	PPM1D	SETD2	TERT
AKT1	BCL2L1	CBLB	CEBPA	DNMT3A	FBXW7	IGF1R	KMT2B	MPL	PALB2	PPP2R1A	SF3B1	TET2
AKT2	BCL6	CBLC	CHEK1	EP300	FLT3	IKZF1	KMT2C	MRE11A	PAX5	PTCH1	SMAD2	TGFBR2
AKT3	BCOR	CCND1	CHEK2	ERG	GATA1	IKZF3	KMT2D	MTOR	PBRM1	PTEN	SMAD4	TP53
ALK	BCORL1	CCND3	CIC	ETV6	GATA2	IRF4	KRAS	MUTYH	PDGFRA	PTPN11	SMARCA4	TSC1
AMER1	BCR	CD274	CREBBP	EZH2	GATA3	JAK1	MAP2K1	MYC	PDGFRB	RAD21	SMARCB1	TSC2
APC	BIRC3	CD79A	CRLF2	FAM175A	GEN1	JAK2	MAP2K2	MYD88	PHF6	RAD50	SMC1A	TSHR
ARID1A	BLM	CD79B	CSF1R	FAM46C	GNAQ	JAK3	MAP2K4	NFE2	PIK3CA	RAD51	SMO	U2AF1
ARID1B	BRAF	CDH1	CSF3R	FANCA	GNAS	KAT6A	MAP3K1	NFKBIA	PIK3R1	RB1	SOCS1	UBA1
ARID2	BRCA1	CDK12	CTNNA1	FANCC	H3F3A	KDM5C	MAP3K14	NOTCH1	PIK3R2	RHOA	SRC	WT1
ASXL1	BRCA2	CDK4	CTNNB1	FANCD2	HNF1A	KDM6A	MAPK1	NOTCH2	PIM1	RNF43	SRSF2	ZNF217
ATM	BTK	CDK6	CUX1	FANCE	HOXB13	KDR	MCL1	NOTCH3	PLCG1	RUNX1	STAG2	ZRSR2
ATRX	CALR	CDKN2A	CXCR4	FANCF	HSP90AA1	KEAP1	MDM2	NPM1	POLD1	SDHB	STAT3	-
B2M	CARD11	CDKN2B	DDR2	FANCG	IDH1	KIT	MDM4	NRAS	POLE	SETBP1	STK11	-

Electronic Signature

Sally Agersborg, MD

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.