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:	BONE MARROW
Case #:	NGSXX-XXXXXX	Specimen ID:	
Body Site:	Left iliac crest		

MRN:				Indication for Testing:	Other pancytopenia (D61.818)
Collected Date:		Time:			
Received Date:		Time:			
Reported Date:		Time:			

Detected Genomic Alterations

SF3B1	IDH1	SRSF2	TP53	No detectable autosomal chromosomal structural gain or loss

Results Summary

- **-Mutations in SF3B1, IDH1, SRSF2, and TP53 genes.**
  -No detectable autosomal chromosomal structural gain or loss.

  -These findings are consistent with myelodysplastic syndrome with sideroblasts (MDS-RS).

  -Patients with ring sideroblasts/SF3B1 mutation may respond to the FDA approved drug, luspatercept (Reblozyl).

  -IDH1 mutation suggests response to IDH1 inhibitors.

  -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 is a dominant abnormal clone with SF3B1 mutation. The IDH1, SRSF2, and TP53 mutations are detected in subclones.

Diagnostic Implications	
SF3B1, IDH1, SRSF2, TP53	These findings are consistent with myelodysplastic syndrome with sideroblasts (MDS-RS)

Therapeutic Implications	
SF3B1	Spliceosome modifiers
IDH1	IDH1 inhibitors
SRSF2	Spliceosome modifiers
TP53	Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy

Prognostic Implications	
SF3B1	Favorable
IDH1	Neutral
SRSF2	Poor
TP53	Poor

Relevant Genes with NO Alteration
No evidence of mutation in FLT3, NPM1, 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

- SF3B1. This gene encodes subunit 1 of the splicing factor 3b protein complex. Splicing factor 3b, together with splicing factor 3a and a 12S RNA unit, forms the U2 small nuclear ribonucleoproteins complex (U2 snRNP). The splicing factor 3b/3a complex binds pre-mRNA upstream of the intron's branch site in a sequence independent manner and may anchor the U2 snRNP to the pre-mRNA. Splicing factor 3b is also a component of the minor U12-type spliceosome. The carboxy-terminal two-thirds of subunit 1 have 22 non-identical, tandem HEAT repeats that form rod-like, helical structures. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Jul 2008]

- IDH1. Isocitrate dehydrogenases catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. These enzymes belong to two distinct subclasses, one of which utilizes NAD(+) as the electron acceptor and the other NADP(+). Five isocitrate dehydrogenases have been reported: three NAD(+)-dependent isocitrate dehydrogenases, which localize to the mitochondrial matrix, and two NADP(+)-dependent isocitrate dehydrogenases, one of which is mitochondrial and the other predominantly cytosolic. Each NADP(+)-dependent isozyme is a homodimer. The protein encoded by this gene is the NADP(+)-dependent isocitrate dehydrogenase found in the cytoplasm and peroxisomes. It contains the PTS-1 peroxisomal targeting signal sequence. The presence of this enzyme in peroxisomes suggests roles in the regeneration of NADPH for intraperoxisomal reductions, such as the conversion of 2, 4-dienoyl-CoAs to 3-enoyl-CoAs, as well as in peroxisomal reactions that consume 2-oxoglutarate, namely the alpha-hydroxylation of phytanic acid. The cytoplasmic enzyme serves a significant role in cytoplasmic NADPH production. Alternatively spliced transcript variants encoding the same protein have been found for this gene. [provided by RefSeq, Sep 2013]

- SRSF2. The protein encoded by this gene is a member of the serine/arginine (SR)-rich family of pre-mRNA splicing factors, which constitute part of the spliceosome. Each of these factors contains an RNA recognition motif (RRM) for binding RNA and an RS domain for binding other

proteins. The RS domain is rich in serine and arginine residues and facilitates interaction between different SR splicing factors. In addition to being critical for mRNA splicing, the SR proteins have also been shown to be involved in mRNA export from the nucleus and in translation. Two transcript variants encoding the same protein and one non-coding transcript variant have been found for this gene. In addition, a pseudogene of this gene has been found on chromosome 11. [provided by RefSeq, Sep 2010]

- 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]

Drug Information

Luspatercept

Luspatercept-aamt (REBLOZYL) is approved for adult patients with very low- to intermediate-risk myelodysplastic syndromes with ring sideroblasts (MDS-RS) or with myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T).

H3B-8800

H3B-8800 is a spliceosome inhibitor. H3B-8800 preferentially targets cells with spliceosome complexes containing mutant splicing factor 3B1 (SF3B1) protein, modulating intron splicing leading to increased death in cancer cells while having little effect on the viability cells with wild-type SF3B1. Both normal and aberrant mature mRNA are suppressed in mutant and wild-type cells, the selectivity of the lethal effect is thought to be due to the presence of mutant SF3B1 and its implications rather than a change in mechanism or potency of effect on the mutant protein over the wild-type. H3B-8800 was granted orphan drug status by the FDA in August 2017 and is in clinical trials for the treatment of acute myelogenous leukemia and chronic myelomonocytic leukemia.

Ivosidenib

Ivosidenib is a first in class isocitrate dehydrogenase-1 (IDH1) approved for use by the FDA in acute myeloid leukemia (AML) in July 2018.

Ivosidenib is a reversible inhibitor of IDH1 which is non-competitive with respect to the cofactor NADH. It binds to many different 132-substituted IDH1 mutants as well as the wild type enzyme. It is considered to be a slow-binder of the wild type enzyme and binds to mutant enzymes at lower concentrations, both of which may contribute its selectivity.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://classic.clinicaltrials.gov/show/NCT04730258	Recruiting	A Study of CFI-400945 With or Without Azacitidine in Patients With AML, MDS or CMML	Myelodysplastic Syndrome	CFI-400945 Azacitidine	City of Hope, Duarte, California, United States University of California Davis Comprehensive Cancer Center, Sacramento, California, United States Norton Cancer Institute - Saint Matthews, Louisville, Kentucky, United States

https://classic.clinicaltrials.gov/show/NCT04358393	Recruiting	A Study of APG-115 Alone or Combined With Azacitidine in Patients With AML, CMML, or MDS	Myelodysplastic Syndrome	APG-115 5-azacitidine	Banner MD Anderson Cancer Center, Gilbert, Arizona, United States Rocky Mountain Cancer Centers, Denver, Colorado, United States Duke University, Durham, North Carolina, United States
https://classic.clinicaltrials.gov/show/NCT04256317	Recruiting	A Study of ASTX030 (Cedazuridine in Combination With Azacitidine) in MDS, CMML, or AML	Myelodysplastic Syndrome	Azacitidine ASTX030 (cedazuridine + azacitidine) Cedazuridine	John Theurer Cancer Center / Hackensack University, Hackensack, New Jersey, United States Roswell Park Comprehensive Cancer Center, Buffalo, New York, United States New York University Langone Hospital - Long Island, Mineola, New York, United States
https://classic.clinicaltrials.gov/show/NCT04734990	Recruiting	Seclidemstat and Azacitidine for the Treatment of Myelodysplastic Syndrome or Chronic Myelomonocytic Leukemia	Myelodysplastic Syndrome	Azacitidine Seclidemstat	M D Anderson Cancer Center, Houston, Texas, United States
https://www.clinicaltrials.gov/study/NCT04278768	Recruiting	Dose Escalation/? Expansion Study of CA-4948 as Monotherapy in Patients With AML or MDS	Myelodysplastic Syndrome	CA-4948	Northwestern Memorial Hospital, Chicago, Illinois, United States & Others

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
SF3B1	NP_036565.2:p.Lys666Asn	NM_012433.2:c.1998G>T	K/N	aaG/aaT	missense_variant	27.52	436	deleterious (0)
IDH1	NP_005887.2:p.Arg132His	NM_005896.2:c.395G>A	R/H	cGt/cAt	missense_variant	11.0	627	deleterious - low confidence (0.01)
SRSF2	NP_003007.2:p.Pro95Arg	NM_003016.4:c.284C>G	P/R	cCc/cGc	missense_variant	9.07	1014	tolerated (0.12)
TP53	NP_000537.3:p.Arg175His	NM_000546.5:c.524G>A	R/H	cGc/cAc	missense_variant	1.25	721	tolerated (0.11)

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	-

Reference

1. [MDS & CMML: Diagnostic and classification]. Wagner-Ballon O, Kosmider O. Wagner-Ballon O, et al. Bull Cancer. 2023 Nov;110(11):1106-1115. doi: 10.1016/j.bulcan.2023.02.030. Epub 2023 Jul 13. Bull Cancer. 2023. PMID: 37453834

2. Cutting the cord from myelodysplastic syndromes: chronic myelomonocytic leukemia-specific biology and management strategies. Padron E, Steensma DP. Padron E, et al. Curr Opin Hematol. 2015 Mar;22(2):163-70. doi: 10.1097/MOH.0000000000000112. Curr Opin Hematol. 2015. PMID: 25575034

3. [Chronic myelomonocytic leukemia (CMML): recent advances in molecular pathogenesis and treatment]. Harada Y, Harada H. Harada Y, et al. Rinsho Ketsueki. 2016 Feb;57(2):147-55. doi: 10.11406/rinketsu.57.147. Rinsho Ketsueki. 2016. PMID: 26935632

4. Risk-Adapted, Individualized Treatment Strategies of Myelodysplastic Syndromes (MDS) and Chronic Myelomonocytic Leukemia (CMML). Bewersdorf JP, et al. Cancers (Basel). 2021. PMID: 33807279

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.