Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618
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CLIA #: 05D2111917 CAP #: 9441547

Hematology Profile Plus

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

MRN:				Indication for Testing:	D46.9 Myelodysplastic syndrome, unspecified
Collected Date:		Time:			
Received Date:		Time:			
Reported Date:		Time:			

Detected Genomic Alterations				
WT1	NRAS	CBL	t(3;12)(q26;p13) ETV6-MECOM fusion mRNA	Autosomal chromosomal structural analysis shows: Monosomy 7 and trisomy 22
B and T cell clonality: Not detected				

Results Summary

- **-Mutations in WT1, NRAS, and CBL genes.**
  -t(3;12)(q26;p13) ETV6-MECOM fusion mRNA
  -Autosomal chromosomal structural analysis shows: Monosomy 7 and trisomy 22
  -Blast markers: CD34 and CD117 increased; TdT low level mRNA
  -FLT3 mRNA: Increased
  -B and 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*02:01-A*23:01
  -HLA-B: B*08:01-B*44:03
  -HLA-C: C*07:02-C*04:456Q

  -These findings are consistent with acute myeloid leukemia (AML) with MECOM rearrangement.

Heterogeneity
There are dominant abnormal clones with WT1 and NRAS mutations. The CBL mutation is detected in a subclone.

Expression	
Blast markers: CD34 and CD117 increased; TdT low level mRNA	FLT3 mRNA: Increased

Diagnostic Implications	
WT1, NRAS, CBL	These findings are consistent with acute myeloid leukemia (AML)

Therapeutic Implications	
WT1	Sensitive to hypomethylating agents (azacitidine)
NRAS	MAPK/MEK inhibitors

Prognostic Implications	
WT1	Poor
NRAS	Poor
CBL	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), Sanger Sequencing and fragment length analysis testing to identify molecular abnormalities , including single nucleotide variants (SNVs), insertions/deletions (indels), copy number variants (CNVs), fusions, B- and T-cell clonality, IgVH mutation analysis and viruses (HPV, EBV, and TTV), in DNA of 284 genes and RNA 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

- WT1. This gene encodes a transcription factor that contains four zinc-finger motifs at the C-terminus and a proline/glutamine-rich DNA-binding domain at the N-terminus. It has an essential role in the normal development of the urogenital system, and it is mutated in a small subset of patients with Wilms tumor. This gene exhibits complex tissue-specific and polymorphic imprinting pattern, with biallelic, and monoallelic expression from the maternal and paternal alleles in different tissues. Multiple transcript variants have been described. In several variants, there is evidence for the use of a non-AUG (CUG) translation initiation codon upstream of, and in-frame with the first AUG. Authors of PMID:7926762 also provide evidence that WT1 mRNA undergoes RNA editing in human and rat, and that this process is tissue-restricted and developmentally regulated. [provided by RefSeq, Mar 2015]

- NRAS. This is an N-ras oncogene encoding a membrane protein that shuttles between the Golgi apparatus and the plasma membrane. This shuttling is regulated through palmitoylation and depalmitoylation by the ZDHHC9-GOLGA7 complex. The encoded protein, which has intrinsic GTPase activity, is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein. Mutations in this gene

have been associated with somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, Noonan syndrome, and juvenile myelomonocytic leukemia. [provided by RefSeq, Jun 2011]

- CBL. This gene is a proto-oncogene that encodes a RING finger E3 ubiquitin ligase. The encoded protein is one of the enzymes required for targeting substrates for degradation by the proteasome. This protein mediates the transfer of ubiquitin from ubiquitin conjugating enzymes (E2) to specific substrates. This protein also contains an N-terminal phosphotyrosine binding domain that allows it to interact with numerous tyrosine-phosphorylated substrates and target them for proteasome degradation. As such it functions as a negative regulator of many signal transduction pathways. This gene has been found to be mutated or translocated in many cancers including acute myeloid leukaemia, and expansion of CGG repeats in the 5' UTR has been associated with Jacobsen syndrome. Mutations in this gene are also the cause of Noonan syndrome-like disorder. [provided by RefSeq, Jul 2016]

Drug Information

Azacitidine

Azacitidine is a pyrimidine analogue that inhibits DNA methyltransferase, impairing DNA methylation. It is also an antimetabolite of cytidine, incorporated primarily into RNA. Azacytidine has been used as an antineoplastic agent.

Azacitidine for injection is a nucleoside metabolic inhibitor indicated for the treatment of patients with the following French-American-British (FAB) myelodysplastic syndrome (MDS) subtypes: Refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS) (if accompanied by neutropenia or thrombocytopenia or requiring transfusions), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEB-T), and chronic myelomonocytic leukemia (CMMoL).

Binimetinib

Binimetinib is an orally available inhibitor of mitogen-activated protein kinase kinase 1 and 2 (MEK1/2) with potential antineoplastic activity. Binimetinib, noncompetitive with ATP, binds to and inhibits the activity of MEK1/2. Inhibition of MEK1/2 prevents the activation of MEK1/2-dependent effector proteins and transcription factors, which may result in the inhibition of growth factor-mediated cell signaling. This may eventually lead to an inhibition of tumor cell proliferation and an inhibition in production of various inflammatory cytokines including interleukin-1, -6 and tumor necrosis factor. MEK1/2 are dual-specificity threonine/tyrosine kinases that play key roles in the activation of the RAS/RAF/MEK/ERK pathway and are often upregulated in a variety of tumor cell types.

Trametinib

Trametinib is an orally bioavailable inhibitor of mitogen-activated protein kinase kinase (MEK MAPK/ERK kinase) with potential antineoplastic activity. Trametinib specifically binds to and inhibits MEK 1 and 2, resulting in an inhibition of growth factor-mediated cell signaling and cellular proliferation in various cancers. MEK 1 and 2, dual specificity threonine/tyrosine kinases often upregulated in various cancer cell types, play a key role in the activation of the RAS/RAF/MEK/ERK signaling pathway that regulates cell growth.

Cobimetinib

Cobimetinib is a reversible inhibitor of mitogen-activated protein kinase 1 (MAPK)/extracellular signal regulated kinase 1 (MEK1) and MEK2.

MEK inhibitor Cobimetinib specifically binds to and inhibits the catalytic activity of MEK1, resulting in inhibition of extracellular signal-related kinase 2 (ERK2) phosphorylation and activation and decreased tumor cell proliferation. Cobimetinib targets kinase activity in the RAS/RAF/MEK/ERK pathway.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://classic.clinicaltrials.gov/show/NCT03613532	Recruiting	Venetoclax Added to Fludarabine + Busulfan Prior to Transplant and to Maintenance Therapy for AML, MDS, and MDS/MPN	Myelodysplastic Syndrome / Acute Myeloid Leukemia	Venetoclax Fludarabine Busulfan Venetoclax Azacitidine Decitabine/cedazuridine	Dana-Farber Cancer Institute, Boston, Massachusetts, United States
https://classic.clinicaltrials.gov/show/NCT05342584	Recruiting	Venetoclax Plus Intensive Chemotherapy in AML and Advanced MDS	Myelodysplastic Syndrome / Acute Myeloid Leukemia	Venetoclax Oral Tablet Daunorubicin Cytarabine	Montefiore Einstein Cancer Center, Bronx, New York, United States

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 / Acute Myeloid Leukemia	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/NCT01515527	Recruiting	Cladribine Plus Low Dose Cytarabine (LDAC) Alternating With Decitabine in Patients With Acute Myeloid Leukemia (AML) or High-Risk Myelodysplastic Syndrome (MDS)	Myelodysplastic Syndrome / Acute Myeloid Leukemia	Cladribine Cytarabine Decitabine	University of Texas MD Anderson Cancer Center, Houston, Texas, United States

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
WT1	NP_077744.3:p.Ala382GlyfsTer69	NM_024426.4:c.1138_1142dupCGGTC	S/SGX	tcg/tcCGGTCg	frameshift_variant	37.42	962	0
NRAS	NP_002515.1:p.Gly13Arg	NM_002524.4:c.37G>C	G/R	Ggt/Cgt	missense_variant	30.52	629	deleterious (0)
CBL	NP_005179.2:p.Cys404Arg	NM_005188.3:c.1210T>C	C/R	Tgt/Cgt	missense_variant	6.06	775	deleterious (0)

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes DNA of 284 genes and RNA 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 fresh cells, peripheral blood cells, bone marrow, body fluid, or paraffin-embedded tissue. Performance of the assay may vary depending on the quantity and quality of nucleic acid, sample preparation and sample age. 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 DNA

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. 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. In cases of suspected chronic lymphocytic leukemia (CLL), IgVH mutation rate will also be reported. The sensitivity of this assay for detecting fusion mRNA is between 5% and 10%. This test specifically detects 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 proper normal expression control. Since the clinical relevance of the RNA expression level of most of the genes is not well-characterized at this time, only a small subset of the genes may be described based on the suspected disease, including but not limited to MYC, BCL2, CD274, CD19, CD22, CD34, and CD138. CRLF2 mRNA levels are reported in acute lymphoblastic leukemia. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated.

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/gtc-hematology-profile-plus/ *(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/gtc-hematology-profile-plus/ *(click the RNA 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	-

RNA Fusions/Expression

Fusion/Expression															
ABL1	ALK	BRAF	CREBBP	EPOR	ETV5	FGFR2	FOXO1	JAK2	MAP3K1	NOTCH1	NUP214	PCM1	PICALM	RET	RUNX1T1	TCF3
ABL2	BCL1	CBFB	CRLF2	ERG	ETV6	FGFR3	FUS	KMT2A	MECOM	NTRK1	NUP98	PDGFRA	PML	RHOA	SS18	TFG
AKT3	BCL2	CBL	CSF1R	ETV1	EWSR1	FIP1L1	GLI1	KRT18P6	MYC	NTRK2	P2RY8	PDGFRB	PTK2B	ROS2	STAT6	TYK2
ALK	BCL6	CIC	EGFR	ETV4	FGFR1	FLT3	IKZF3	LYN	MYH9	NTRK3	PBX1	PD-L1	RARA	RUNX1	TAL1	

Reference

1. The International Consensus Classification of myelodysplastic syndromes and related entities. Hasserjian RP, Orazi A, Orfao A, Rozman M, Wang SA. Hasserjian RP, et al. Virchows Arch. 2023 Jan;482(1):39-51. doi: 10.1007/s00428-022-03417-1. Virchows Arch. 2023. PMID: 36287260

2. Acute Myeloid Leukemia Arising from Myelodysplastic Syndromes. Kwon A, Weinberg OK. Kwon A, et al. Clin Lab Med. 2023 Dec;43(4):657-667. doi: 10.1016/j.cll.2023.07.001. Epub 2023 Aug 19. Clin Lab Med. 2023. PMID: 37865509

3. Molecular Targeted Therapy and Immunotherapy for Myelodysplastic Syndrome. Lee P, Yim R, Yung Y, Chu HT, Yip PK, Gill H. Lee P, et al. Int J Mol Sci. 2021 Sep 23;22(19):10232. doi: 10.3390/ijms221910232. Int J Mol Sci. 2021. PMID: 34638574

4. The International Consensus Classification of acute myeloid leukemia. Weinberg OK, Porwit A, Orazi A, Hasserjian RP, Foucar K, Duncavage EJ, Arber DA. Weinberg OK, et al. Virchows Arch. 2023 Jan;482(1):27-37. doi: 10.1007/s00428-022-03430-4. Epub 2022 Oct 20. Virchows Arch. 2023. PMID: 36264379

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.