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:	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

FANCA	DNMT3A (2 mutations)	IDH1	Autosomal chromosomal structural analysis shows: Small 11q+ (KMT2A-PTD)	B and T cell clonality: Not detected

Results Summary

- **-Mutations in FANCA, DNMT3A (2 mutations), and IDH1 genes**
  -Autosomal chromosomal structural analysis shows: Small 11q+ (KMT2A-PTD)
  -Blast markers: CD34 and CD117 increased; TdT low level mRNA
  -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*24:02
  -HLA-B: B*27:05-B*35:01
  -HLA-C: C*01:02-C*04:456Q

  -These findings are consistent with myelodysplastic syndrome with increased blasts / acute myeloid leukemia (MDS/AML).

  -IDH1 mutation suggests response to IDH1 inhibitors.

Heterogeneity

There is an abnormal clone with FANCA, DNMT3A (2 mutations), and IDH1 mutations.

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

Diagnostic Implications	
FANCA, DNMT3A (2 mutations), IDH1	These findings are consistent with myelodysplastic syndrome with increased blasts / acute myeloid leukemia (MDS/AML)

Therapeutic Implications	
FANCA	DNA cross-linking agents such as diepoxybutane (DEB) and mitomycin C (MMC)
DNMT3A	DNA methyltransferase inhibitors
IDH1	IDH1 inhibitors

Prognostic Implications	
FANCA	Unknown
DNMT3A (2 mutations)	Poor
IDH1	Neutral

Relevant Genes with NO Alteration
No evidence of mutation in FLT3, NPM1, IDH2, MYD88 or CXCR4

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

- FANCA. The Fanconi anemia complementation group (FANC) currently includes FANCA, FANCB, FANCC, FANCD1 (also called BRCA2), FANCD2 FANCE, FANCF, FANCG, FANCI, FANCJ (also called BRIP1), FANCL, FANCM and FANCN (also called PALB2). The previously defined group FANCH is the same as FANCA. Fanconi anemia is a genetically heterogeneous recessive disorder characterized by cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair. The members of the Fanconi anemia complementation group do not share sequence similarity; they are related by their assembly into a common nuclear protein complex. This gene encodes the protein for complementation group A. Alternative splicing results in multiple transcript variants encoding different isoforms. Mutations in this gene are the most common cause of Fanconi anemia. [provided by RefSeq, Jul 2008]

- 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]

- 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]

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 indicated for treatment of patients with the following French-American-British (FAB) myelodysplastic syndrome subtypes: refractory anemia (RA) or refractory anemia with ringed sideroblasts (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).

Decitabine

Decitabine is a cytidine antimetabolite analogue with potential antineoplastic activity. Decitabine incorporates into DNA and inhibits DNA methyltransferase, resulting in hypomethylation of DNA and intra-S-phase arrest of DNA replication.

Decitabine for injection is indicated for treatment of adult patients with myelodysplastic syndromes (MDS) including previously treated and untreated, de novo and secondary MDS of all French-American-British subtypes (refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia) and intermediate-1, intermediate-2, and high-risk International Prognostic Scoring System groups.

Decitabine also has EU approval for acute myeloid leukemia (AML).

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/NCT05184842	Recruiting	Metabolically Optimized, Non-cytotoxic Low Dose Weekly Decitabine/Venetoclax in MDS and AML	Myelodysplastic Syndrome	Venetoclax Decitabine	Montefiore Medical Center, Bronx, New York, United States
https://classic.clinicaltrials.gov/show/NCT03647800	Recruiting	Study of APVO436 in Patients With AML or MDS	Myelodysplastic Syndrome	APVO436	University of California, San Francisco Medical Center, San Francisco, California, United States Colorado Blood Cancer Institute, Denver, Colorado, United States University of Florida College of Medicine, Gainesville, Florida, United States

https://classic.clinicaltrials.gov/show/NCT05168904	Recruiting	A Study to Investigate Fadraciclib (CYC065), in Subjects With Leukemia or Myelodysplastic Syndrome (MDS)	Myelodysplastic Syndrome	fadraciclib	City of Hope, Duarte, California, United States MD Anderson Cancer Center, Houston, Texas, United States
https://classic.clinicaltrials.gov/show/NCT02494167	Recruiting	Administration of Donor Multi TAA-Specific T Cells for AML or MDS (ADSPAM)	Myelodysplastic Syndrome	MultiTAA-specific T cells	Houston Methodist Hospital, Houston, Texas, United States Texas Children's Hospital, 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
FANCA	NP_000126.2:p.Glu288Ter	NM_000135.2:c.862G>T	E/*	Gag/Tag	stop_gained	50.44	1023	0
DNMT3A	NP_783328.1:p.Phe752Leu	NM_175629.2:c.2256C>G	F/L	ttC/ttG	missense_variant	43.22	2050	deleterious (0)
DNMT3A	NP_783328.1:p.Val341Ter	NM_175629.2:c.1021_1022delGT	V/X	GTt/t	frameshift_variant	41.61	1341	0
IDH1	NP_005887.2:p.Arg132Gly	NM_005896.2:c.394C>G	R/G	Cgt/Ggt	missense_variant	40.78	1057	deleterious - low confidence (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. Diagnosis and Treatment of Myelodysplastic Syndromes: A Review. Sekeres MA, Taylor J. Sekeres MA, et al. JAMA. 2022 Sep 6;328(9):872-880. doi: 10.1001/jama.2022.14578. JAMA. 2022. PMID: 36066514

2. Current and emerging strategies for management of myelodysplastic syndromes. Saygin C, Carraway HE. Saygin C, et al. Blood Rev. 2021 Jul;48:100791. doi: 10.1016/j.blre.2020.100791. Epub 2020 Dec 27. Blood Rev. 2021. PMID: 33423844

3. Mutation-Driven Therapy in MDS. Swoboda DM, Sallman DA. Swoboda DM, et al. Curr Hematol Malig Rep. 2019 Dec;14(6):550-560. doi: 10.1007/s11899-019-00554-4. Curr Hematol Malig Rep. 2019. PMID: 31760573

4. 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

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.