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 Plus

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

MRN:				Indication for Testing:	C91.0 Acute lymphoblastic leukemia [ALL]
Collected Date:		Time:			
Received Date:		Time:			
Reported Date:		Time:			

Detected Genomic Alterations

SAMD9 (?Germline, VUS)	JAK2 (R683G)	ETNK1 (?Germline, VUS)	PCM1 (?Germline, VUS)	Autosomal chromosomal structural analysis shows: Small 3p-, small 7p- (IKZF1 deletion), small 9p- (homozygous CDKN2A/B deletion), small 13q- (RB1 deletion)
B and T cell clonality: Not detected	CRLF2 mRNA: Markedly increased, reflecting promoter hijacking			

Results Summary

- **-Somatic mutation in JAK2 (R683G) gene.**
  -Possible germline mutations in SAMD9, ETNK1, and PCM1 genes, heterozygous.
  -Autosomal chromosomal structural analysis shows: Small 3p-, small 7p- (IKZF1 deletion), small 9p- (homozygous CDKN2A/B deletion), small 13q- (RB1 deletion)
  -B and T cell clonality: Not detected
  -CRLF2 mRNA: Markedly increased, reflecting promoter hijacking
  -Blast markers: CD34 and TdT markedly increased; CD117 low level mRNA
  -B cell markers: Increased with abnormal pattern
  -CD10 and FLT3 mRNA: Increased
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*03:368-A*02:11

-HLA-B: B*52:01-B*07:02
-HLA-C: C*07:744-C*15:02

-These findings are consistent with Ph-like B-cell acute lymphoblastic leukemia (Ph-like B-ALL).

-The SAMD9, ETNK1, and PCM1 mutations aare detected at high levels, raising the possibilities 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.

Heterogeneity
There is an abnormal clone with JAK2 mutation. The SAMD9, ETNK1, and PCM1 mutations are detected at a high level, possible germline abnormalities.

Expression	
Blast markers: CD34 and TdT markedly increased; CD117 low level mRNA	B cell markers: Increased with abnormal pattern
CD10 and FLT3 mRNA: Increased	CRLF2 mRNA: Markedly increased, reflecting promoter hijacking

Diagnostic Implications	
SAMD9, JAK2, ETNK1, PCM1	-These findings are consistent with Ph-like acute lymphoblastic leukemia (ALL). -The SAMD9, ETNK1, and PCM1 mutations are likely germline variants.

Therapeutic Implications	
JAK2	JAK inhibitor

Prognostic Implications	
JAK2	Poor

Relevant Genes with NO Alteration
No evidence of mutation in NOTCH, SF3B1, TP53, or MYD88

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

- SAMD9. This gene encodes a sterile alpha motif domain-containing protein. The encoded protein localizes to the cytoplasm and may play a role in regulating cell proliferation and apoptosis. Mutations in this gene are the cause of normophosphatemic familial tumoral calcinosis. Alternate splicing results in multiple transcript variants that encode the same protein.[provided by RefSeq, Jul 2010]

- JAK2. This gene encodes a non-receptor tyrosine kinase that plays a central role in cytokine and growth factor signalling. The primary isoform of this protein has an N-terminal FERM domain that is required for erythropoietin receptor association, an SH2 domain that binds STAT transcription factors, a pseudokinase domain and a C-terminal tyrosine kinase domain. Cytokine binding induces autophosphorylation and activation of this kinase. This kinase then recruits and phosphorylates signal transducer and activator of transcription (STAT) proteins. Growth factors like TGF-beta 1 also induce phosphorylation and activation of this kinase and translocation of downstream STAT proteins to the nucleus where they influence gene transcription. Mutations in this gene are associated with numerous inflammatory diseases and malignancies. This gene is a downstream target of the pleiotropic cytokine IL6 that is produced by B cells, T cells, dendritic cells and macrophages to produce an immune response or inflammation. Disregulation of the IL6/JAK2/STAT3 signalling pathways produces increased cellular proliferation and myeloproliferative neoplasms of hematopoietic stem cells. A nonsynonymous mutation in the pseudokinase domain of this gene disrupts the domains inhibitory effect and results in constitutive tyrosine phosphorylation activity and hypersensitivity to cytokine signalling. This gene and the IL6/JAK2/STAT3 signalling pathway is a therapeutic target for the treatment of excessive inflammatory responses to viral infections. Alternative splicing results in multiple transcript variants encoding distinct isoforms. [provided by RefSeq, Jul 2020]

- ETNK1. This gene encodes an ethanolamine kinase, which functions in the first committed step of the phosphatidylethanolamine synthesis pathway. This cytosolic enzyme is specific for ethanolamine and exhibits negligible kinase activity on choline. Alternative splicing results in multiple transcript variants encoding distinct isoforms. [provided by RefSeq, Jul 2008]

- PCM1. The protein encoded by this gene is a component of centriolar satellites, which are electron dense granules scattered around centrosomes. Inhibition studies show that this protein is essential for the correct localization of several centrosomal proteins, and for anchoring microtubules to the centrosome. Chromosomal aberrations involving this gene are associated with papillary thyroid carcinomas and a variety of hematological malignancies, including atypical chronic myeloid leukemia and T-cell lymphoma. Multiple transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Oct 2015]

Drug Information

Ruxolitinib

Ruxolitinib is a kinase inhibitor that is selective for the Janus Associated Kinases (JAK) 1 and 2. These kinases are responsible for the mediation of cytokine and growth factor signalling which in turn effect immune function and hematopoiesis. The signalling process involves signal transducers and transcription activators (STAT) which modulate gene expression. Patients with myelofibrosis have abnormal JAK1 and JAK2 activity thus ruxolitinib works to regulate this.

Ruxolitinib is indicated for the treatment of intermediate or high-risk myelofibrosis, including primary myelofibrosis, post-polycythemia vera (post-PV) myelofibrosis and post-essential thrombocythemia (post-ET) myelofibrosis. [Lexicomp] Myeolofibrosis is the proliferation of abnormal bone marrow stem cells which cause fibrosis (the excessive formation of connective tissue).

Rituximab

Rituximab is a genetically engineered monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. It was originally approved by the U.S. FDA in 1997 as a single agent to treat patients with B-cell Non-Hodgkin Lymphoma (NHL), however, has now been approved for a variety of conditions.

Rituximab is indicated in the following conditions:

-Non–Hodgkin Lymphoma (NHL)
-Chronic Lymphocytic Leukemia (CLL)
-Rheumatoid Arthritis (RA) in combination with methotrexate in adult patients with moderately-to severely-active RA
-Granulomatosis with Polyangiitis (GPA) (Wegener Granulomatosis) and Microscopic Polyangiitis (MPA)
-Moderate to severe Pemphigus Vulgaris (PV) in adult patients
-The biosimilar (approved in November 2018), Truxima, is indicated For the treatment of adult patients with CD20-positive, B-cell non-Hodgkin lymphoma (NHL) to be used as a single agent or in combination with chemotherapy.

Ibrutinib

Ibrutinib is a small molecule that acts as an irreversible potent inhibitor of Burton tyrosine kinase (BTK). It is designated as a targeted covalent drug and it presents a very promising activity in B cell malignancies. Ibrutinib forms a covalent bond with a cysteine residue in the active site of BTK

(Cys481), leading to its inhibition. The inhibition of BTK plays a role in the B-cell receptor signaling and thus, the presence of ibrutinib prevents the phosphorylation of downstream substrates such as PLC-?.

Ibrutinib was developed by Pharmacyclics Inc and in November 2013 was FDA-approved for the treatment of mantle cell lymphoma. Later, in February 2014, ibrutinib was approved for the treatment of chronic lymphocytic leukemia and it is also indicated for the treatment of patients with Waldenström Macroglobulinemia. Ibrutinib has also been approved by the EMA for the treatment of chronic lymphocytic leukemia and mantle cell lymphoma. Ibrutinib was approved for use in chronic graft versus host disease in August 2017.

Ibrutinib is indicated for:
-treatment of mantle cell lymphoma who have received at least one prior therapy.
-treatment of chronic lymphocytic leukemia (CLL) who have at least one prior therapy.
-treatment of chronic lymphocytic leukemia (CLL) with 17p deletion.
-treatment of patients with Waldenstrom Macroglobulinemia (WM).

Venetoclax

Venetoclax is a selective inhibitor of both BCL-2 and BCL-2-like 1 (BCL-X(L)), which has demonstrated clinical efficacy in some BCL-2-dependent hematological cancers. Selective inhibition of BCL-2 by venetoclax, sparing BCL-xL enables therapeutic induction of apoptosis without the negative effect of thrombocytopenia. Venetoclax helps restore the process of apoptosis by binding directly to the BCL-2 protein, displacing pro-apoptotic proteins, leading to mitochondrial outer membrane permeabilization and the activation of caspase enzymes. In nonclinical studies, venetoclax has shown cytotoxic activity in tumor cells that overexpress BCL-2

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://classic.clinicaltrials.gov/show/NCT05386576	Recruiting	A Study of Venetoclax in Combination With Chemotherapy to Treat Newly Diagnosed Acute Lymphoblastic Leukemia (ALL)	Acute Lymphoblastic Leukemia	Venetoclax	Memorial Sloan Kettering Cancer Center, New York, New York, United States
https://classic.clinicaltrials.gov/show/NCT04521231	Recruiting	A Study of Subcutaneous Blinatumomab Administration in Acute Lymphoblastic Leukemia (ALL) Patients	Acute Lymphoblastic Leukemia	Blinatumomab	City of Hope National Medical Center, Duarte, California, United States New York University Langone Health, New York, New York, United States University of Texas MD Anderson Cancer Center, Houston, Texas, United States
https://classic.clinicaltrials.gov/show/NCT05993949	Recruiting	Study of Brexucabtagene Autoleucel Plus Dasatinib in Adults With Acute Lymphoblastic Leukemia	Acute Lymphoblastic Leukemia	Dasatinib	Stanford University, Palo Alto, California, United States
https://classic.clinicaltrials.gov/show/NCT05016947	Recruiting	Venetoclax Plus Inotuzumab for B-ALL	Acute Lymphoblastic Leukemia	Venetoclax Dexamethasone Inotuzumab Ozogamicin	Brigham and Women's Hospital, Boston, Massachusetts, United States Dana Farber Cancer Institute, Boston, Massachusetts, United States

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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
SAMD9	NP_060124.2:p.Tyr1175Ter	NM_017654.3:c.3525T>G	Y/*	taT/taG	stop_gained	49.65	2127	0
JAK2	NP_004963.1:p.Arg683Gly	NM_004972.3:c.2047A>G	R/G	Aga/Gga	missense_variant	40.38	1040	deleterious (0)
ETNK1 (RNA)	NP_061108.2:p.Pro51GlnfsTer32	NM_018638.4:c.152delC	P/X	Ccc/cc	frameshift_variant	44.33	194	0
PCM1 (RNA)	NP_006188.3:p.Glu1353Ter	NM_006197.3:c.4057G>T	E/*	Gag/Tag	stop_gained	18.95	95	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	

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.