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:	
Date of Birth:		Physician ID:	
Gender (M/F):		Accession #:	
Client:		Specimen Type:	BONE MARROW
Case #:		Specimen ID:	
Body Site:	NOT SPECIFIED		

MRN:				Indication for Testing:	C91.00 Acute lymphoblastic leukemia not having achieved remission
Collected Date:		Time:			
Received Date:		Time:			
Reported Date:		Time:			

Detected Genomic Alterations				
EPAS1	t(12;21)(p13;q22); ETV6-RUNX1	Autosomal chromosomal structural analysis shows multiple abnormalities including: +4 (low level), +5 (low level), 11q-, -12, +18	B cell clonality: Detected, heavy chain only (IGHV1-18)	T cell clonality: Detected (TRGV9)

Results Summary

- **-t(12;21)(p13;q22); ETV6-RUNX1 fusion mRNA**
  -Low-level mutation in EPAS1 gene
  -Autosomal chromosomal structural analysis shows multiple abnormalities including: +4 (low level), +5 (low level), 11q-, -12, +18
  -B cell clonality: Detected, heavy chain only (IGHV1-18)
  -T cell clonality: Detected (TRGV9)
  -B cell markers: Increased with abnormal pattern
  -Blast markers (CD34, TdT): Markedly increased
  -CD117 mRNA: Low level
  -Increased FLT3 mRNA
  -T cell markers: Low level
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*03:01-A*30:02
  -HLA-B: B*18:01-B*41:01
  -HLA-C: C*17:01-C*05:01

  -These findings are consistent with acute B lymphoblastic leukaemia/lymphoma (B-ALL/LBL) with ETV6-RUNX1 fusion.

Heterogeneity
There is an abnormal low-level clone with EPAS1 mutation.

Expression	
B cell markers: Increased with abnormal pattern	Blast markers (CD34, TdT): Markedly increased
CD117 mRNA: Low level	Increased FLT3 mRNA
T cell markers: Low level	

Diagnostic Implications	
EPAS1	This finding is consistent with acute B-cell lymphoblastic leukemia/lymphoma (B-ALL/LBL)

Prognostic Implications	
EPAS1	Unknown

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 SNVs, INDELS, CNVs, Fusions, HPV, EBV, and IgVH) in DNA of 284 genes and RNA in 1600 genes associated with hematologic neoplasms, including leukemia, lymphoma, myeloma, and MDS. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Biological relevance of detected Alterations

- EPAS1. This gene encodes a transcription factor involved in the induction of genes regulated by oxygen, which is induced as oxygen levels fall. The encoded protein contains a basic-helix-loop-helix domain protein dimerization domain as well as a domain found in proteins in signal transduction pathways which respond to oxygen levels. Mutations in this gene are associated with erythrocytosis familial type 4. [provided by RefSeq, Nov 2009]

Drug Information

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, it 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 Bruton 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

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
EPAS1	NP_001421.2:p.Pro649Ser	NM_001430.4:c.1945C>T	P/S	Ccc/Tcc	missense_variant	4.08	1054	tolerated (0.14)

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. 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. Fragment length analysis is also performed for CALR, FLT3, and NPM1 to enhance the detection of large indels. Our sequencing method has a sensitivity of 1% for detecting variants. The CALR, FLT3-ITD, and NPM1 fragment analysis assay has a sensitivity of 2%-5% for detecting CALR, FLT3-ITD, and NPM1 in wildtype background. 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.

In addition to DNA analysis, targeted RNA NGS analysis is performed. This is a next generation sequencing (NGS) test

that analyzes targeted RNA on 1,600 genes associated with hematologic neoplasms. It is based on hybrid capture of targeted RNA. Duplicates are excluded for levels measurements. While the major focus of the analysis is the detection of fusion mRNA, mutations in the expressed RNA of the analyzed genes are also analyzed and reported. CRLF2 mRNA levels are reported in acute lymphoblastic leukemia. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated. All detected fusion transcripts are reported. This test specifically covers 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 expression proper normal control. Since the clinical relevance of the expression level of most of these genes is not characterized at this time, only few specific genes (MYC, BCL2, CD274, CD19, CD22, CD79A, CD79B) will be commented on. The sensitivity of this assay in detecting fusion mRNA is between 1% and 5%. For optimal results neoplastic cells should be >30% of the analyzed cells. The Universal Human Reference (UHR) RNA is used as control.

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 inherited 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/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.