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:	MOL23-
Body Site:	NOT SPECIFIED		

MRN:				Indication for Testing:	Acute leukemia of unspecified cell type not having achieved remission (C95.00)
Collected Date:		Time:			
Received Date:		Time:			
Reported Date:		Time:			

Detected Genomic Alterations				
STAG2	t(12;21)(p13;q22); ETV6-RUNX1	No detectable autosomal chromosomal structural gain or loss	B cell clonality: Detected (IGHV3-13 / IGLV2-8)	T cell clonality: Not detected

Results Summary

- **-Low-level mutation in STAG2 gene**
  -t(12;21)(p13;q22); ETV6-RUNX1 fusion mRNA
  -No detectable autosomal chromosomal structural gain or loss
  -B cell clonality: Detected (IGHV3-13 / IGLV2-8)
  -T cell clonality: Not detected
  -B cell markers: Increased with abnormal pattern
  -Marked increase in CD10, TdT and KI67 mRNA
  -Low CD34 and CD117 mRNA
  -Increased ERG, FLT3, LEF1, MYB, TCL1A mRNA
  -EBV viral RNA: Not detected
  -HPV viral RNA : Not detected
  -HLA Genotyping:
  -HLA-A: A*02:05-A*33:01
  -HLA-B: B*57:03-B*44:03
  -HLA-C: C*07:01-C*02:02

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

Heterogeneity
There is an abnormal low-level clone with STAG2 mutation.

Expression	
B cell markers: Increased with abnormal pattern	Marked increase in CD10, TdT and KI67 mRNA
Low CD34 and CD117 mRNA	Increased ERG, FLT3, LEF1, MYB, TCL1A mRNA

Diagnostic Implications	
STAG2, ETV6/RUNX1	These findings are consistent with B-cell acute lymphoblastic leukemia (B-ALL)

Therapeutic Implications	
STAG2	PARP inhibitors

Prognostic Implications	
STAG2	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 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

- STAG2. The protein encoded by this gene is a subunit of the cohesin complex, which regulates the separation of sister chromatids during cell division. Targeted inactivation of this gene results in chromatid cohesion defects and aneuploidy, suggesting that genetic disruption of cohesin is a cause of aneuploidy in human cancer. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Sep 2013]

Drug Information

Rituximab

Rituximab is a monoclonal antibody that targets the CD20 antigen, which is expressed on the surface of pre-B and mature B-lymphocytes. After binding to CD20, rituximab mediates B-cell lysis (or breakdown). The possible mechanisms of cell lysis include complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC).

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

Ibrutinib

Ibrutinib is a small molecule that acts as an irreversible potent inhibitor of Burton's 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 Waldenstrm 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

A BCL-2 inhibitor indicated for the treatment of patients with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL), with or without 17p deletion, who have received at least one prior therapy. Venetoclax induces rapid and potent onset apoptosis of CLL cells, powerful enough to act within 24h and to lead to tumor lysis syndrome. Selective targeting of BCL2 with venetoclax has demonstrated a manageable safety profile and has been shown to induce significant response in patients with relapsed CLL (chronic lymphocytic leukemia) or SLL (small lymphocytic leukemia), including patients with poor prognostic features.

Talazoparib

Talazoparib is a poly(ADP-ribose) Polymerase 1, 2 (PARP 1;2 inhibitor).

Talazoparib was approved by the FDA for use in germline BRCA mutated, HER2 negative, locally advanced or metastatic breast cancer on October 16, 2018 under the trade name Talzenna. Talazoparib prevents PARP-mediated repair of DNA damage in cancer cells, allowing accumulation of damage and PARP-DNA complexes. Repair related errors by error prone secondary repair pathways may also contribute to the cytotoxicity of Talazoparib. Talazoparib is indicated for the treatment of deleterious or suspected deleterious germline BRCA mutated, HER2 negative locally advanced or metastatic breast cancer in adults.

Niraparib

Niraparib (ZEJULA) is an inhibitor of poly (ADP-ribose) polymerase (PARP) with potential antineoplastic activity. PARP Inhibitor MK4827 inhibits PARP activity, enhancing the accumulation of DNA strand breaks and promoting genomic instability and apoptosis. The PARP family of proteins detect and repair single strand DNA breaks by the base-excision repair (BER) pathway. The specific PARP family member target for PARP inhibitor MK4827 is unknown. (NCI Thesaurus)

ZEJULA is a poly(ADP-ribose) polymerase (PARP) inhibitor indicated for the maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy.

Olaparib

Olaparib (Lynparza) is an antineoplastic agent, Poly(ADP-ribose) Polymerase 1;2;3 inhibitor. (PARP 1;2;3 inhibitor).

Lynparza is a poly (ADP-ribose) polymerase (PARP) inhibitor indicated for the treatment of adult patients with deleterious or suspected deleterious germline BRCA-mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy. Select patients for therapy based on an FDA-approved companion diagnostic for Lynparza.

Rucaparib

Rucaparib is a potent mammalian poly(ADP-ribose) polymerase 1, 2 and 3 inhibitor with anticancer properties (PARP 1;2;3 inhibitor).

PPAR is an enzyme that plays an essential role in DNA repair by activating response pathways and facilitating repair, and defects in these repair mechanisms have been demonstrated in various malignancies, including cancer. Regulation of repair pathways is critical in promoting necessary cell death. BRCA genes are tumor suppressor genes mediate several cellular processes including DNA replication, transcription regulation, cell cycle checkpoints, apoptosis, chromatin structuring and homologous recombination (HR). Homologous recombination deficiency (HRD), along with PPAR inhibition, is a vulnerability that enhances the cell death pathway when the single mutations alone would permit viability. Ovarian cancer commonly possesses defects in DNA repair pathways such as HRD due to BRCA mutations or otherwise. Rucaparib has shown to induce cytotoxicity in tumor cell lines with deficiencies in BRCA1/2 and other DNA repair genes. Of all the BRCA1/2 mutations in ovarian cancer, most are due to germline

mutations (18%), and approximately 7% represent somatic mutations acquired within the tumor.

Rucaparib is an inhibitor of PARP-1, PARP-2, and PARP-3. Via an inhibitory effect on the PARP enzymatic activity, rucaparib decreases the formation of PARP-DNA complexes resulting in DNA damage, apoptosis, and cell death. It is proposed that PARP inhibition specifically targets tumor cells with preexisting HRD, such as those cells possessing mutations in the BRCA1 or BRCA2 genes.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://classic.clinicaltrials.gov/show/NCT05581030	Recruiting	CalPeg for Newly Diagnosed Acute Lymphoblastic Leukemia (ALL)	Acute Lymphoblastic Leukemia	Hyper CVAD Protocol (Standard of Care Multi-Agent Chemotherapy) Calaspargase Pegol Rituximab	Moffitt Cancer Center, Tampa, Florida, 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/NCT03913559	Recruiting	Inotuzumab Ozogamicin for Children With MRD Positive CD22+ Lymphoblastic Leukemia	Acute Lymphoblastic Leukemia	Inotuzumab ozogamicin Methotrexate Hydrocortisone Cytarabine Diphenhydramine Acetaminophen Methylprednisolone	St. Jude Children's Research Hospital, Memphis, Tennessee, United States
https://classic.clinicaltrials.gov/show/NCT05460533	Recruiting	A Second Infusion (Early Reinfusion) of Tisagenlecleucel in Children and Young Adults With B-Cell Acute Lymphoblastic Leukemia(B-ALL)	Acute Lymphoblastic Leukemia	Tisagenlecleucel	Stanford University (Data Collection Only), Stanford, California, United States Johns Hopkins University (Data Collection Only), Baltimore, Maryland, United States Memorial Sloan Kettering Cancer Center, New York, New York, United States

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
STAG2	NP_001036214.1:p.Ser65Phe	NM_001042749.1:c.194C>T	S/F	tCt/tTt	missense_variant	2.24	803	deleterious (0.04)

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	

Reference

1. Biology of childhood acute lymphoblastic leukemia. Bhojwani D, Yang JJ, Pui CH. Bhojwani D, et al. Pediatr Clin North Am. 2015 Feb;62(1):47-60. doi: 10.1016/j.pcl.2014.09.004. Pediatr Clin North Am. 2015. PMID: 25435111

2. Genomics and pharmacogenomics of pediatric acute lymphoblastic leukemia. Wu C, Li W. Wu C, et al. Crit Rev Oncol Hematol. 2018 Jun;126:100-111. doi: 10.1016/j.critrevonc.2018.04.002. Epub 2018 Apr 10. Crit Rev Oncol Hematol. 2018. PMID: 29759551

3. Pediatric acute lymphoblastic leukemia. Inaba H, Mullighan CG. Inaba H, et al. Haematologica. 2020 Nov 1;105(11):2524-2539. doi: 10.3324/haematol.2020.247031. Haematologica. 2020. PMID: 33054110

4. Treatment and biology of pediatric acute lymphoblastic leukemia. Kato M, Manabe A. Kato M, et al. Pediatr Int. 2018 Jan;60(1):4-12. doi: 10.1111/ped.13457. Pediatr Int. 2018. PMID: 29143423

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.