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:	Chronic lymphocytic leukemia of B-cell type not having achieved remission (C91.10)
Collected Date:		Time:			
Received Date:		Time:		Tumor Type:	CLL
Reported Date:		Time:			

Detected Genomic Alterations				
LRP1B	ATM (2 mutations)	Autosomal chromosomal structural analysis shows: 11q- (ATM deletion)	B cell clonality: Detected, biclonal light chains (IGHV4-39 /Two light chain clones IGLV3-21 / IGLV1-47)	T cell clonality: Not detected

Results Summary

- **-Mutations in LRP1B and ATM (2 mutations) genes**
  -Autosomal chromosomal structural analysis shows: 11q- (ATM deletion)
  -B cell clonality: Detected, biclonal light chains (IGHV4-39 /Two light chain clones IGLV3-21 / IGLV1-47).
  -T cell clonality: Not detected
  -B cell markers: Increased with abnormal pattern
  -CD5 and CD23 mRNA: Increased
  -CCND1 and SOX11 mRNA: Low level
  -BCL2 mRNA: Markedly increased
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*03:01-A*03:01
  -HLA-B: B*49:01-B*35:01
  -HLA-C: C*07:01-C*06:02
  -IgVH mutation status: Unmutated.

  -These findings are consistent with aggressive B-cell chronic lymphocytic leukemia (B-CLL) with biallelic ATM aberrations (deletion and mutation).

  -The findings are similar to the previous specimen (NGSXX-XXXXXX, collected MM/DD/YYYY).

Heterogeneity
There is an abnormal clone with LRP1B and (2) ATM mutations.

Expression	
B cell markers: Increased with abnormal pattern	CD5 and CD23 mRNA: Increased
CCND1 and SOX11 mRNA: Low level	BCL2 mRNA: Markedly increased

Diagnostic Implications	
LRP1B, ATM (2 mutations)	These findings are consistent with B-cell chronic lymphocytic leukemia (B-CLL)

Therapeutic Implications	
ATM	PARP inhibitors

Prognostic Implications	
LRP1B	Unknown
ATM (2 mutations)	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

- LRP1B. This gene encodes a member of the low density lipoprotein (LDL) receptor family. These receptors play a wide variety of roles in normal cell function and development due to their interactions with multiple ligands. Disruption of this gene has been reported in several types of cancer. [provided by RefSeq, Jun 2016]

- ATM. The protein encoded by this gene belongs to the PI3/PI4-kinase family. This protein is an important cell cycle checkpoint kinase that phosphorylates; thus, it functions as a regulator of a wide variety of downstream proteins, including tumor suppressor proteins p53 and BRCA1, checkpoint kinase CHK2, checkpoint proteins RAD17 and RAD9, and DNA repair protein NBS1. This protein and the closely related kinase ATR are thought to be master controllers of cell cycle checkpoint signaling pathways that are required for cell response to DNA damage and for genome stability. Mutations in this gene are associated with ataxia telangiectasia, an autosomal recessive disorder. [provided by RefSeq, Aug 2010]

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

Olaparib

Olaparib (LYNPARZA) is an antineoplastic agent, Poly(ADP-ribose) Polymerase1;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(gBRCAm) 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. (1.1, 2.2)

Niraparib

Niraparib 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)

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/NCT06073821	Recruiting	Study of Sonrotoclax (BGB-11417) Plus Zanubrutinib (BGB-3111) Compared With Venetoclax Plus Obinutuzumab in Participants With Chronic Lymphocytic Leukemia (CLL)	Chronic Lymphocytic Leukemia	Sonrotoclax Zanubrutinib Venetoclax Obinutuzumab	Alaska Oncologyand Hematology, Llc, Anchorage, Alaska, United States Valkyrie Clinical Trials, Los Angeles, California, United States
https://classic.clinicaltrials.gov/show/NCT04215809	Recruiting	Study of APG-2575 as a Single Agent or in Combination With Other Therapeutic Agents for CLL/SLL	Chronic Lymphocytic Leukemia	APG2575	City of Hope, Duarte, California, United States Mayo Clinic, Jacksonville, Florida, United States Dana Farber Cancer Institute, Boston, Massachusetts, United States
https://classic.clinicaltrials.gov/show/NCT05168930	Recruiting	Zanubrutinib and Venetoclax in CLL (ZANU-VEN)	Chronic Lymphocytic Leukemia	Venetoclax Zanubrutinib	New England Cancer Specialists, Scarborough, Maine, United States Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States Dana-Farber Cancer Institute, Boston, Massachusetts, United States
https://classic.clinicaltrials.gov/show/NCT04843904	Recruiting	Safe Accelerated Venetoclax Escalation in CLL	Chronic Lymphocytic Leukemia	Venetoclax Obinutuzumab Rituximab	Dana-Farber Cancer Institute, Boston, Massachusetts, 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
LRP1B	NP_061027.2:p.Cys857Tyr	NM_018557.2:c.2570G>A	C/Y	tGt/tAt	missense_variant	22.75	545	0
ATM	NP_000042.3:p.Tyr2852_Thr2858dup	NM_000051.3:c.8555_8575dupATACGCGCAGTGTAGCTACTT	A/AYTRSVAT	gct/gCTTATACGCGCAGTGTAGCTAct	inframe_insertion	14.98	287	0
ATM	NP_000042.3:p.Trp2300Ter	NM_000051.3:c.6899G>A	W/*	tGg/tAg	stop_gained	0.71	563	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. Chronic lymphocytic leukemia (CLL) treatment: So many choices, such great options. Sharma S, Rai KR. Sharma S, et al. Cancer. 2019 May 1;125(9):1432-1440. doi: 10.1002/cncr.31931. Epub 2019 Feb 26. Cancer. 2019. PMID: 30807655

2. Diagnosis and Treatment of Chronic Lymphocytic Leukemia: A Review. Shadman M. Shadman M. JAMA. 2023 Mar 21;329(11):918-932. doi: 10.1001/jama.2023.1946. JAMA. 2023. PMID: 36943212

3. Initial treatment of CLL: integrating biology and functional status. Jain N, O'Brien S. Jain N, et al. Blood. 2015 Jul 23;126(4):463-70. doi: 10.1182/blood-2015-04-585067. Epub 2015 Jun 11. Blood. 2015. PMID: 26065656

4. Selecting initial therapy in CLL. Ahn IE, Brown JR. Ahn IE, et al. Hematology Am Soc Hematol Educ Program. 2022 Dec 9;2022(1):323-328. doi: 10.1182/hematology.2022000343. Hematology Am Soc Hematol Educ Program. 2022. PMID: 36485152

5. CAR T-cell therapy for CLL: a new addition to our treatment toolbox? Iovino L, Shadman M. Iovino L, et al. Clin Adv Hematol Oncol. 2023 Mar;21 (3):134-141. Clin Adv Hematol Oncol. 2023. PMID: 36867557

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.