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CLIA #: 05D2111917 CAP #: 9441547

Liquid Trace Hematology

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:	C83.10 Mantle cell lymphoma, unspecified site
Collected Date:		Time:			
Received Date:		Time:			
Reported Date:		Time:			

Detected Genomic Alterations				
RNF43 (?Germline, VUS)	ATM	KMT2D	BIRC3	KMT2C
LRP1B	EBV viral RNA: Detected (High level)	Autosomal chromosomal structural analysis shows: Proximal 1p-, partial 11q-	B cell clonality: Detected, light chain only (IGKV1D-33)	T cell clonality: Not detected
CCND1 mRNA: Increased, suggestive of promoter hijacking	-			

Results Summary

- **-Low-level mutations in ATM, KMT2D, BIRC3, KMT2C, and LRP1B genes**
  -Possible germline mutation in RNF43 gene, heterozygous
  -Autosomal chromosomal structural analysis shows: Proximal 1p-, partial 11q-
  -B cell clonality: Detected, light chain only (IGKV1D-33)
  -T cell clonality: Not detected
  -B cell markers: Mildly increased with normal pattern
  -CCND1 mRNA: Increased, suggestive of promoter hijacking
  -CD5 mRNA: Slightly increased
  -KI67 mRNA: Increased
  -EBV viral RNA: Detected (High level)
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*11:303-A*68:01
  -HLA-B: B*58:02-B*40:02
  -HLA-C: C*15:02-C*06:02

-These findings are consistent with low-level circulating mantle cell lymphoma (MCL) DNA/RNA.

-The RNF43 mutation is detected at high level, suggestive of a germline mutation. This mutation leads to early termination (loss of function). However, there is no data on its clinical relevance and should be classified as of "uncertain significance" at this time.

Heterogeneity
-Low-level mutations in KMT2D, BIRC3, KMT2C, and LRP1B genes. -RNF43 mutation is detected at a high level, likely germline variant.

Expression	
B cell markers: Increased with normal pattern	CCND1 mRNA: Increased, suggestive of promoter hijacking
CD5 mRNA: Slightly increased	KI67 mRNA: Increased

Diagnostic Implications	
RNF43, ATM, KMT2D, BIRC3, KMT2C, LRP1B	-These findings are consistent with mantle cell lymphoma (MCL). -RNF43 mutation is likely germline variant.

Therapeutic Implications	
RNF43	Porcupine inhibitors
ATM	PARP inhibitors

Prognostic Implications	
RNF43	Poor
ATM	Poor
KMT2D	Unknown
BIRC3	Unknown
KMT2C	Unknown
LRP1B	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) to identify molecular abnormalities, including single nucleotide variants (SNVs), insertions/deletions (indels), copy number variants (CNVs), fusions, B- and T-cell clonality, and viruses (HPV, EBV, and TTV), in cell-free (cf) DNA of 284 genes and cfRNA 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

- RNF43. The protein encoded by this gene is a RING-type E3 ubiquitin ligase and is predicted to contain a transmembrane domain, a protease-associated domain, an ectodomain, and a cytoplasmic RING domain. This protein is thought to negatively regulate Wnt signaling, and expression of this gene results in an increase in ubiquitination of frizzled receptors, an alteration in their subcellular distribution, resulting in reduced surface levels of these receptors. Mutations in this gene have been reported in multiple tumor cells, including colorectal and endometrial cancers. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Mar 2015]

- 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]

- KMT2D. The protein encoded by this gene is a histone methyltransferase that methylates the Lys-4 position of histone H3. The encoded proteir is part of a large protein complex called ASCOM, which has been shown to be a transcriptional regulator of the beta-globin and estrogen receptor genes. Mutations in this gene have been shown to be a cause of Kabuki syndrome. [provided by RefSeq, Oct 2010]

- BIRC3. This gene encodes a member of the IAP family of proteins that inhibit apoptosis by binding to tumor necrosis factor receptor-associated factors TRAF1 and TRAF2, probably by interfering with activation of ICE-like proteases. The encoded protein inhibits apoptosis induced by serum deprivation but does not affect apoptosis resulting from exposure to menadione, a potent inducer of free radicals. It contains 3 baculovirus IAP repeats and a ring finger domain. Transcript variants encoding the same isoform have been identified. [provided by RefSeq, Aug 2011]

- KMT2C. This gene is a member of the myeloid/lymphoid or mixed-lineage leukemia (MLL) family and encodes a nuclear protein with an AT hook DNA-binding domain, a DHHC-type zinc finger, six PHD-type zinc fingers, a SET domain, a post-SET domain and a RING-type zinc finger. This protein is a member of the ASC-2/NCOA6 complex (ASCOM), which possesses histone methylation activity and is involved in transcriptional coactivation. [provided by RefSeq, Jul 2008]

- 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]

Drug Information

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/NCT05976763	Recruiting	Testing Continuous Versus Intermittent Treatment With the Study Drug Zanubrutinib for Older Patients With Previously Untreated Mantle Cell Lymphoma	Mantle Cell Lymphoma	Zanubrutinib Rituximab Patient Observation Bone Marrow Biopsy Fludeoxyglucose F-18 Positron Emission Tomography Computed Tomography Magnetic Resonance Imaging Esophagogastroduodenoscopy Colonoscopy Biospecimen Collection Questionnaire Administration	City of Hope Comprehensive Cancer Center, Duarte, California, United States City of Hope at Irvine Lennar, Irvine, California, United States Helen F Graham Cancer Center, Newark, Delaware, United States
https://classic.clinicaltrials.gov/show/NCT05025423	Recruiting	Phase II Trial of Venetoclax and Rituximab as Initial Therapy in Older Patients With Mantle Cell Lymphoma	Mantle Cell Lymphoma	Venetoclax Oral Tablet [Venclexta]	Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, United States
https://classic.clinicaltrials.gov/show/NCT04189757	Recruiting	Acalabrutinib for the Treatment of Ibrutinib-Intolerant Mantle Cell Lymphoma	Mantle Cell Lymphoma	Acalabrutinib	M D Anderson Cancer Center, Houston, Texas, United States

https://classic.clinicaltrials.gov/show/NCT06192888	Recruiting	A Study of Glofitamab and Lenalidomide in People With Mantle Cell Lymphoma	Mantle Cell Lymphoma	Glofitamab Obinutuzumab Lenalidomide	Dana Farber Cancer Institute (Data Collection and Specimen Analysis), Boston, Massachusetts, United States Mayo Clinic (Data Collection Only), Rochester, Minnesota, United States Washington University (Data Collection Only), Saint Louis, Missouri, United States
https://classic.clinicaltrials.gov/show/NCT05788289	Recruiting	A Study of Tafasitamab and Lenalidomide in People With Mantle Cell Lymphoma	Mantle Cell Lymphoma	Tafasitamab Lenalidomide	Memorial Sloan Kettering Basking Ridge (All protocol activities), Basking Ridge, New Jersey, United States Memorial Sloan Kettering Monmouth (All protocol activities), Middletown, New Jersey, United States Memorial Sloan Kettering Bergen (All protocol activities), Montvale, New Jersey, 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
RNF43	NP_060233.3:p.Pro660SerfsTer87	NM_017763.4:c.1976dupG	G/GX	ggt/ggGt	frameshift_variant	49.1	2224	0
ATM	NP_000042.3:p.Asp2448Gly	NM_000051.3:c.7343A>G	D/G	gAt/gGt	missense_variant	11.0	1654	tolerated (0.06)
KMT2D	NP_003473.3:p.Cys1507Ter	NM_003482.3:c.4521C>A	C/*	tgC/tgA	stop_gained	9.07	2526	0
BIRC3	NP_001156.1:p.Arg600del	NM_001165.4:c.1798_1800delCGT	VR/V	gtTCGt/gtt	inframe_deletion	8.5	882	0
KMT2C	NP_733751.2:p.Tyr308Phe	NM_170606.2:c.923A>T	Y/F	tAt/tTt	missense_variant	1.7	1886	0
LRP1B	NP_061027.2:p.Trp608Cys	NM_018557.2:c.1824G>T	W/C	tgG/tgT	missense_variant	0.35	1127	0

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA for abnormalities in 284 genes and cfRNA 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 peripheral blood plasma. Performance of the assay may vary depending on the quantity and quality of nucleic acid, sample preparation and sample age. 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 cfDNA sequencing method has a sensitivity of 0.1% for detecting hot spot mutations, 0.5% for detecting single nucleotide variants (SNVs) and 1% for small (<60 bp) insertions/ deletions (indels). Known hot spots in specific genes such as IDH1/2, NRAS, and KRAS are reported at levels of 0.01% and higher when both cfRNA and cfDNA results are combined. Significant gene amplification and deletion (copy number variants) are also 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. The sensitivity of this assay in detecting fusion mRNA is between 5% and 10%. 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 RNA expression level of most of the genes is not characterized at this time, only a few specific genes will be commented on when abnormalities are detected.

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/liquid-trace-hematologic-malignancies/ *(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/liquid-trace-hematologic-malignancies/ *(click the RNA tab)*

Tested genes

Genes Tested for Abnormalities in Coding Sequence												
ABL1	B2M	CCNE1	CTNNB1	ETV6	FLT4	IDH2	MAP2K1	MYCL	PDGFRB	RAD50	SOCS1	TSHR
ACVR1B	BAP1	CD274	CUX1	EXO1	FOXL2	IGF1R	MAP2K2	MYCN	PHF6	RAD51	SOX2	U2AF1
AKT1	BCL2	CD79A	CXCR4	EZH2	FUBP1	IKZF1	MAP2K4	MYD88	PIK3CA	RAF1	SOX9	U2AF2
AKT2	BCL2L1	CD79B	CYLD	ABRAXAS1	GALNT12	IKZF3	MAP3K1	NF1	PIK3R1	RB1	SPOP	VHL
AKT3	BCL6	CDC73	DAXX	TENT5C	GATA1	IL7R	MAP3K14	NF2	PIK3R2	RET	SRC	NSD2
ALK	BCOR	CDH1	DDR2	FANCA	GATA2	INHBA	MAPK1	NFE2L2	PIM1	RHEB	SRSF2	WT1
AMER1	BCORL1	CDK12	DICER1	FANCC	GATA3	IRF4	MCL1	NFKBIA	PLCG1	RHOA	STAG2	XPO1
APC	BCR	CDK4	DNM2	FANCD2	GEN1	JAK1	MDM2	NKX2-1	PMS1	RIT1	STAT3	XRCC2
AR	BIRC3	CDK6	DNMT3A	FANCE	GNA11	JAK2	MDM4	NOTCH1	PMS2	RNF43	STK11	XRCC3

ARAF	BLM	CDKN2A	DOT1L	FANCF	GNAQ	JAK3	MED12	NOTCH2	POLD1	ROS1	SUFU	ZNF217
ARID1A	BRAF	CDKN2B	EED	FANCG	GNAS	KAT6A	MEF2B	NOTCH3	POLE	RUNX1	SUZ12	ZRSR2
ARID1B	BRCA1	CDKN2C	EGFR	FAS	GREM1	KDM5C	MEN1	NPM1	PPM1D	SDHB	TAL1	NFE2
ARID2	BRCA2	CEBPA	EGLN1	FBXW7	GRIN2A	KDM6A	MET	NRAS	PPP2R1A	SETBP1	TCF3	UBA1
ASXL1	BRIP1	CHEK1	EP300	FGF4	H3-3A	KDR	MITF	NSD1	PRDM1	SETD2	TERT	STAT5B
ATM	BTK	CHEK2	EPAS1	FGF6	HGF	KEAP1	MLH1	NTRK1	PRKAR1A	SF3B1	TET2	ETNK1
ATR	CALR	CIC	EPHA3	FGFR1	H3C2	KIT	MPL	NTRK2	PRKDC	SMAD2	TGFBR2	ELANE
ATRX	CARD11	CREBBP	EPHA5	FGFR2	HNF1A	KMT2A	MRE11	NTRK3	PRSS1	SMAD4	TNFAIP3	ANKRD26
AURKA	CBL	CRLF2	ERBB2	FGFR3	HOXB13	KMT2B	MSH2	PAK3	PTCH1	SMARCA4	TNFRSF14	SAMD9L
AURKB	CBLB	CSF1R	ERBB3	FGFR4	HRAS	KMT2C	MSH6	PALB2	PTEN	SMARCB1	TP53	SAMD9
AURKC	CBLC	CSF3R	ERBB4	FH	HSP90AA1	KMT2D	MTOR	PAX5	PTPN11	SMC1A	TRAF3	DDX41
AXIN1	CCND1	CTCF	ERG	FLCN	ID3	KRAS	MUTYH	PBRM1	RAC1	SMC3	TSC1	-
AXIN2	CCND3	CTNNA1	ESR1	FLT3	IDH1	LRP1B	MYC	PDGFRA	RAD21	SMO	TSC2	-

Reference

1. Mantle cell lymphoma-Update on molecular biology, prognostication and treatment approaches. Silkenstedt E, Dreyling M. Silkenstedt E, et al. Hematol Oncol. 2023 Jun;41 Suppl 1:36-42. doi: 10.1002/hon.3149. Hematol Oncol. 2023. PMID: 37294961

2. Personalized approaches for treatment-nave mantle cell lymphoma. Qualls D, Kumar A. Qualls D, et al. Expert Rev Hematol. 2023 Feb;16(2):95-107. doi: 10.1080/17474086.2023.2174516. Epub 2023 Feb 9. Expert Rev Hematol. 2023. PMID: 36748785

3. Mantle cell lymphoma in 2022-A comprehensive update on molecular pathogenesis, risk stratification, clinical approach, and current and novel treatments. Jain P, Wang ML. Jain P, et al. Am J Hematol. 2022 May;97(5):638-656. doi: 10.1002/ajh.26523. Am J Hematol. 2022. PMID: 35266562

4. New Directions for Mantle Cell Lymphoma in 2022. Kumar A, Eyre TA, Lewis KL, Thompson MC, Cheah CY. Kumar A, et al. Am Soc Clin Oncol Educ Book. 2022 Apr;42:1-15. doi: 10.1200/EDBK_349509. Am Soc Clin Oncol Educ Book. 2022. PMID: 35561299

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.