GENOMIC TESTING COOPERATIVE

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CLIA # 05D2111917 CAP # 9441574

Liquid Trace Hematology

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

MRN:				Indication for Testing:	C91.00 Acute lymphoblastic leukemia not having achieved remission
Collected Date:		Time:	12:00 AM		
Received Date:		Time:	09:37 AM		
Reported Date:		Time:	05:40 PM		

Detected Genomic Alterations				
AKT1	MTOR	PALB2	KMT2C (2 mutations)	NOTCH2
TMEM127	GALNT12	TRPM4	APC (2 mutations)	NF2
ATRX	BCR	TTV viral RNA: Detected (63 copies)	Autosomal chromosomal structural analysis shows: Small 9p- (homozygous CDKN2A deletion), partial 14q-	T-cell clonality: Detected (TRAV17 / TRBV14, TRGV4)
B cell clonality: Not detected	-			

Results Summary

- **-Mutations in AKT1, MTOR, PALB2, KMT2C (2 mutations), NOTCH2, TMEM127, GALNT12, TRPM4, APC (2 mutations), NF2, ATRX, and BCR genes**
  -Autosomal chromosomal structural analysis shows: Small 9p- (homozygous CDKN2A deletion), partial 14q-
  -T-cell clonality: Detected (TRAV17 / TRBV14, TRGV4)
  -B-cell clonality: Not detected
  -T cell markers: Increased
  -TdT mRNA: Increased
  -GATA3 mRNA: Marked increase
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Detected (63 copies)
  -HLA Genotyping:
  -HLA-A: A*02:11-A*02:11
  -HLA-B: B*44:03-B*44:03

-HLA-C: C*07:06-C*07:06

-These findings are consistent with relapsed T-cell acute lymphoblastic leukemia (T-ALL).

-TTV (Torque teno virus) is considered as a marker of level of immunocompetence in patients with immunological impairment and inflammatory disorders. TTV is regarded as a part of the human virome and, in general, does not cause pathology in immune-competent individuals. However, 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 and monitoring of peripheral blood TTV level may be helpful.

See additional report information at the end of the report.

Heterogeneity
There is an abnormal clone with AKT1, MTOR, PALB2, KMT2C (2 mutations), NOTCH2, TMEM127, GALNT12, TRPM4, APC (2 mutations), NF2, ATRX, and BCR mutations.

Expression	
T cell markers: Increased	TdT mRNA: Increased
GATA3 mRNA: Marked increase	-

Diagnostic Implications	
AKT1, MTOR, PALB2, KMT2C (2 mutations), NOTCH2, TMEM127, GALNT12, TRPM4, APC (2 mutations), NF2, ATRX, BCR	These findings are consistent with relapsed T-cell acute lymphoblastic leukemia (T-ALL).

Therapeutic Implications	
AKT1	PI3K/AKT/MTOR inhibitors
MTOR	MTOR inhibitors
PALB2	PARP inhibitors
NOTCH2	NOTCH inhibitors
APC	WNT, beta-catenin, and COX-2 inhibitors
NF2	AKT/MEK/MTOR inhibitors
ATRX	PARP inhibitors and WEE1 inhibitors

Prognostic Implications	
AKT1, MTOR, PALB2, NOTCH2, APC, NF2, ATRX	Poor
KMT2C, TMEM127, GALNT12, TRPM4, BCR	Unknown

Relevant Genes with NO Alteration
No evidence of mutation in 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 302 genes and cfRNA in greater than 1600 genes implicated in hematologic neoplasms, including leukemia, lymphoma, myeloma, myelodysplastic syndrome, and myeloproliferative neoplasms. Whenever possible, clinical relevance and implications of detected abnormalities are described below. If a gene is not reported, then no somatic mutations were detected. This assay facilitates myelodysplastic syndrome risk assessment as it includes evaluation for mutations and significant chromosomal gains and losses in all of the genes included in the IPSS-M risk calculator: ASXL1, BCOR, BCORL1, CBL, CEBPA, DNMT3A, ETNK1, ETV6, EZH2, FLT3, GATA2, GNB1, IDH1, IDH2, KMT2A (including KMT2A(MLL)-PTD), KRAS, NF1, NPM1, NRAS, PHF6, PPM1D, PRPF8, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, STAG2, TP53, U2AF1, and WT1.

Biological relevance of detected Alterations

- AKT1. Also referred to as protein kinase B, is a known oncogene. AKT activation relies on the PI3K pathway, and is recognized as a critical node in the pathway. The E17 hotspot is the most characterized of AKT1 mutations, and has been shown to result in activation of the protein. Mutations in AKT1 have also been shown to confer resistance to allosteric kinase inhibitors in vitro.This gene encodes one of the three members of the human AKT serine-threonine protein kinase family which are often referred to as protein kinase B alpha, beta, and gamma. These highly similar AKT proteins all have an N-terminal pleckstrin homology domain, a serine/threonine-specific kinase domain and a C-terminal regulatory domain. These proteins are phosphorylated by phosphoinositide 3-kinase (PI3K). AKT/PI3K forms a key component of many signalling pathways that involve the binding of membrane-bound ligands such as receptor tyrosine kinases, G-protein coupled receptors, and integrin-linked kinase. These AKT proteins therefore regulate a wide variety of cellular functions including cell proliferation, survival, metabolism, and angiogenesis in both normal and malignant cells. AKT proteins are recruited to the cell membrane by phosphatidylinositol 3,4,5-trisphosphate (PIP3) after phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) by PI3K. Subsequent phosphorylation of both threonine residue 308 and serine residue 473 is required for full activation of the AKT1 protein encoded by this gene. Phosphorylation of additional residues also occurs, for example, in response to insulin growth factor-1 and epidermal growth factor. Protein phosphatases act as negative regulators of AKT proteins by dephosphorylating AKT or PIP3. The PI3K/AKT signalling pathway is crucial for tumor cell survival. Survival factors can suppress apoptosis in a transcription-independent manner by activating AKT1 which then phosphorylates and inactivates components of the apoptotic machinery. AKT proteins also participate in the mammalian target of rapamycin (mTOR) signalling pathway which controls the assembly of the eukaryotic translation initiation factor 4F (eIF4E) complex and this pathway, in addition to responding to extracellular signals from growth factors and cytokines, is disregulated in many cancers. Mutations in this gene are associated with multiple types of cancer and excessive tissue growth including Proteus syndrome and Cowden syndrome 6, and breast, colorectal, and ovarian cancers. Multiple alternatively spliced transcript variants have been found for this gene. [provided by RefSeq, Jul 2020]

- MTOR. The protein encoded by this gene belongs to a family of phosphatidylinositol kinase-related kinases. These kinases mediate cellular responses to stresses such as DNA damage and nutrient deprivation. This kinase is a component of two distinct complexes, mTORC1, which controls protein synthesis, cell growth and proliferation, and mTORC2, which is a regulator of the actin cytoskeleton, and promotes cell survival and cell cycle progression. This protein acts as the target for the cell-cycle arrest and immunosuppressive effects of the FKBP12-rapamycin complex. Inhibitors of mTOR are used in organ transplants as immunosuppressants, and are being evaluated for their therapeutic potential in SARS-CoV-2 infections. Mutations in this gene are associated with Smith-Kingsmore syndrome and somatic focal cortical dysplasia type II. The ANGPTL7 gene is located in an intron of this gene. [provided by RefSeq, Aug 2020]

- PALB2. This gene encodes a protein that may function in tumor suppression. This protein binds to and colocalizes with the breast cancer 2 early onset protein (BRCA2) in nuclear foci and likely permits the stable intranuclear localization and accumulation of BRCA2. [provided by RefSeq, Jul 2008]

- 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]

- NOTCH2. This gene encodes a member of the Notch family. Members of this Type 1 transmembrane protein family share structural characteristics including an extracellular domain consisting of multiple epidermal growth factor-like (EGF) repeats, and an intracellular domain consisting of multiple, different domain types. Notch family members play a role in a variety of developmental processes by controlling cell fate decisions. The Notch signaling network is an evolutionarily conserved intercellular signaling pathway which regulates interactions between physically adjacent cells. In Drosophilia, notch interaction with its cell-bound ligands (delta, serrate) establishes an intercellular signaling pathway that plays a key role in development. Homologues of the notch-ligands have also been identified in human, but precise interactions between these ligands and the human notch homologues remain to be determined. This protein is cleaved in the trans-Golgi network, and presented on the cell surface as a heterodimer. This protein functions as a receptor for membrane bound ligands, and may play a role in vascular, renal and hepatic development. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jan 2011]

- TMEM127. This gene encodes a transmembrane protein with four predicted transmembrane domains. The protein is associated with a subpopulation of vesicular organelles corresponding to early endosomal structures, with the Golgi, and with lysosomes, and may participate in protein trafficking between these structures. Mutations in this gene and several other genes cause pheochromocytomas. Alternatively spliced transcript variants encoding the same protein have been identified. [provided by RefSeq, Aug 2022]

- GALNT12. This gene encodes a member of a family of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases, which catalyze the transfer of N-acetylgalactosamine (GalNAc) from UDP-GalNAc to a serine or threonine residue on a polypeptide acceptor in the initial step of O-linked protein glycosylation. Mutations in this gene are associated with an increased susceptibility to colorectal cancer. [RefSeq, Mar 2011]

- TRPM4 (Transient Receptor Potential Cation Channel Subfamily M Member 4) is a Protein Coding gene. Diseases associated with TRPM4 include Progressive Familial Heart Block, Type Ib and Erythrokeratodermia Variabilis Et Progressiva 6. Among its related pathways are Sensory perception of taste and Ion channel transport. Gene Ontology (GO) annotations related to this gene include calmodulin binding and calcium channel activity. An important paralog of this gene is TRPM5.

- APC. This gene encodes a tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway. It is also involved in other processes including cell migration and adhesion, transcriptional activation, and apoptosis. Defects in this gene cause familial adenomatous polyposis (FAP), an autosomal dominant pre-malignant disease that usually progresses to malignancy. Mutations in the APC gene have been found to occur in most colorectal cancers. Disease-associated mutations tend to be clustered in a small region designated the mutation cluster region (MCR) and result in a truncated protein product. [provided by RefSeq, Dec 2019]

- NF2. This gene encodes a protein that is similar to some members of the ERM (ezrin, radixin, moesin) family of proteins that are thought to link cytoskeletal components with proteins in the cell membrane. This gene product has been shown to interact with cell-surface proteins, proteins involved in cytoskeletal dynamics and proteins involved in regulating ion transport. This gene is expressed at high levels during embryonic development; in adults, significant expression is found in Schwann cells, meningeal cells, lens and nerve. Mutations in this gene are associated with neurofibromatosis type II which is characterized by nervous system and skin tumors and ocular abnormalities. Two predominant isoforms and a number of minor isoforms are produced by alternatively spliced transcripts. [provided by RefSeq, Jul 2008]

- ATRX. The protein encoded by this gene contains an ATPase/helicase domain, and thus it belongs to the SWI/SNF family of chromatin remodeling proteins. This protein is found to undergo cell cycle-dependent phosphorylation, which regulates its nuclear matrix and chromatin association, and suggests its involvement in the gene regulation at interphase and chromosomal segregation in mitosis. Mutations in this gene are associated with X-linked syndromes exhibiting cognitive disabilities as well as alpha-thalassemia (ATRX) syndrome. These mutations have been shown to cause diverse changes in the pattern of DNA methylation, which may provide a link between chromatin remodeling, DNA methylation, and gene expression in developmental processes. Multiple alternatively spliced transcript variants encoding distinct isoforms have been reported. [provided by RefSeq, Jul 2017]

- BCR. A reciprocal translocation between chromosomes 22 and 9 produces the Philadelphia chromosome, which is often found in patients with chronic myelogenous leukemia. The chromosome 22 breakpoint for this translocation is located within the BCR gene. The translocation produces a fusion protein which is encoded by sequence from both BCR and ABL, the gene at the chromosome 9 breakpoint. Although the BCR-ABL fusion protein has been extensively studied, the function of the normal BCR gene product is not clear. The unregulated tyrosine kinase activity of BCR-ABL1 contributes to the immortality of leukaemic cells. The BCR protein has serine/threonine kinase activity and is a GTPase-activating protein for p21rac and other kinases. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jan 2020]

Drug Information

Capivasertib

Capivasertib (AZD5363) is a novel pyrrolopyrimidine derivative, and an orally available inhibitor of the serine/threonine protein kinase AKT (protein kinase B) with potential antineoplastic activity. Capivasertib binds to and inhibits all AKT isoforms. Inhibition of AKT prevents the phosphorylation of AKT substrates that mediate cellular processes, such as cell division, apoptosis, and glucose and fatty acid metabolism. A wide range of solid and hematological malignancies show dysregulated PI3K/AKT/mTOR signaling due to mutations in multiple signaling components. By targeting AKT, the key node in the PIK3/AKT signaling network, this agent may be used as monotherapy or combination therapy for a variety of human cancers. Capivasertib has been investigated for the treatment of Metastatic Breast Cancer.

Ipatasertib

Ipatasertib is an orally administered, ATP-competitive, selective AKT inhibitor. AKT is a key component of the PI3K/AKT pathway. This pathway is dysregulated in many malignancies, often through acquisition of activating mutations in AKT and phosphatidylinositol 3-kinase (PI3K), loss of the tumor suppressor phosphatase and tensin homolog (PTEN), or amplification of AKT and PI3K.

Everolimus

Everolimus is a PI3K/Akt/mTOR pathway inhitior. The PI3K/Akt/mTOR plays a cruitial role in trastuzumab resistance, dysregulating the HER2 downstream signal. The mTOR inhibitor everolimus inhibits the mTOR/S6K signal, and therefore improves fluorouracil-induced apoptosis in gastric cancer cells with HER2 amplification. A concordant therapy using HER2-targeted agents and everolimus might lead to an improvement in therapy of HER2-positive gastric cancer.

Temsirolimus

Temsirolimus is an inhibitor of mTOR (mammalian target of rapamycin). Temsirolimus binds to an intracellular protein (FKBP-12), and the protein-drug complex inhibits the activity of mTOR that controls cell division. Inhibition of mTOR activity resulted in a G1 growth arrest in treated tumor cells. When mTOR was inhibited, its ability to phosphorylate p70S6k and S6 ribosomal protein, which are downstream of mTOR in the PI3 kinase/AKT pathway was blocked. In in vitro studies using renal cell carcinoma cell lines, temsirolimus inhibited the activity of mTOR and resulted in reduced levels of the hypoxia-inducible factors HIF-1 and HIF-2 alpha, and the vascular endothelial growth factor.

Temsirolimus is indicated for the treatment of renal cell carcinoma (RCC). Also investigated for use/treatment in breast cancer, lymphoma (unspecified), rheumatoid arthritis, and multiple myeloma.

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)

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.

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.

Brontictuzumab

Brontictuzumab is a humanized monoclonal antibody directed against the Notch-1 receptor with potential antineoplastic activity. Upon administration, brontictuzumab binds to Notch-1 on the cell surface, thereby inhibiting Notch-mediated signaling and tumor cell proliferation. Notch 1, a type 1 transmembrane protein belonging to the Notch family, functions as a receptor for membrane bound ligands and has various roles during development; dysregulated Notch signaling is associated with increased cell growth and chemoresistance in cancers.

Sulindac

Sulindac is a COX-1/COX-2 inhibitor. It is being evaluated as a potential cancer chemo-preventive and therapeutic drug in clinical trials for a variety of malignancies.

Celecoxib

Celecoxib is a selective cyclooxygenase-2 (COX-2) inhibitor, is a nonsteroidal anti-inflammatory drug (NSAID). It is used to manage symptoms of various types of arthritis pain and in familial adenomatous polyposis (FAP) to reduce precancerous polyps in the colon. It is marketed by Pfizer under the brand name Celebrex, and was initially granted FDA approval in 1998. Interestingly, selective COX-2 inhibitors (especially celecoxib), have been evaluated as potential cancer chemopreventive and therapeutic drugs in clinical trials for a variety of malignancies.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://clinicaltrials.gov/study/NCT06934382	Recruiting	A Phase 1 Study of Allogeneic Anti-CD7 CAR-T Cells (BEAM-201) in Relapsed/Refractory T-cell Acute Lymphoblastic Leukemia (T-ALL) or T-cell Lymphoblastic Lymphoma (T-LLy)	T-Cell Acute Lymphoblastic Leukemia/Lymphoma	Allogeneic anti-CD7 CAR-T cells (BEAM-201)	Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104
https://clinicaltrials.gov/study/NCT04972942	Recruiting	Phase I Trial of Targeted Immunotherapy with Daratumumab Following Myeloablative TBI-Based Conditioning and AlloHCT in Children, Adolescents and Young Adults with High Risk T-Cell Acute Lymphoblastic Leukemia and Lymphoma (ALLO-T-DART)	T-cell Acute Lymphoblastic Leukemia	Daratumumab	Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104 Boston Children's Hospital, Boston, Massachusetts 02115 Children's National Medical Center, Washington D.C., District of Columbia 20010

https://clinicaltrials.gov/study/NCT04848064	Recruiting	A Pilot Phase I Trial of IL-21 Expanded, Off the Shelf, Third-Party Natural Killer (NK) Cells in Combination With Mogamulizumab in Patients With Cutaneous T-Cell Lymphomas or Adult T-Cell Leukemia/Lymphomas	T-cell Acute Lymphoblastic Leukemia	Cyclophosphamide, Fludarabine, Mogamulizumab, Natural Killer Cell Therapy	Ohio State University Comprehensive Cancer Center, Columbus, Ohio 43210

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
AKT1	NP_001014431.1:p.Glu17Lys	NM_001014431.1:c.49G>A	E/K	Gag/Aag	"missense_variant,splice_region_variant"	33.24	2551	deleterious
MTOR	NP_004949.1:p.Tyr1974His	NM_004958.3:c.5920T>C	Y/H	Tac/Cac	missense_variant	29.95	1993	deleterious
PALB2	NP_078951.2:p.Glu347Ter	NM_024675.3:c.1039G>T	E/*	Gaa/Taa	stop_gained	26.31	1794	0
KMT2C	NP_733751.2:p.Leu2420Val	NM_170606.2:c.7258C>G	L/V	Ctt/Gtt	missense_variant	16.93	2776	tolerated
NOTCH2	NP_001186930.1:p.?	NM_001200001.1:c.3G>C	M/I	atG/atC	start_lost	16.1	1509	tolerated - low confidence
KMT2C	NP_733751.2:p.Val125Ile	NM_170606.2:c.373G>A	V/I	Gta/Ata	missense_variant	15.07	1599	tolerated
TMEM127	NP_001180233.1:p.Thr136Arg	NM_001193304.2:c.407C>G	T/R	aCg/aGg	"missense_variant,splice_region_variant"	9.21	1379	deleterious
GALNT12	NP_078918.3:p.Met518Ile	NM_024642.4:c.1554G>A	M/I	atG/atA	missense_variant	8.26	2626	tolerated
TRPM4	NP_001308211.1:p.Ser459Leu	NM_001321282.1:c.1376C>T	S/L	tCg/tTg	missense_variant	7.83	3282	0
APC	NP_000029.2:p.Ile495Val	NM_000038.5:c.1483A>G	I/V	Att/Gtt	missense_variant	7.64	2540	tolerated
APC	NP_000029.2:p.Asp1871Tyr	NM_000038.5:c.5611G>T	D/Y	Gat/Tat	missense_variant	7.6	1802	deleterious
NF2	NP_000259.1:p.Tyr66His	NM_000268.3:c.196T>C	Y/H	Tac/Cac	missense_variant	4.99	2084	deleterious
ATRX	NP_000480.2:p.Glu2325Gly	NM_000489.3:c.6974A>G	E/G	gAg/gGg	"missense_variant,splice_region_variant"	4.38	1232	0
BCR	NP_004318.3:p.Thr1096Arg	NM_004327.3:c.3287C>G	T/R	aCg/aGg	missense_variant	4.36	1787	deleterious

Methodology and Test Background

This is a next generation sequencing (NGS) test that involves separate analysis of DNA and RNA panels for abnormalities that are reported in various types of hematologic neoplasms. The DNA panel is composed of 302 genes, and the RNA panel is composed of >1600 genes. The DNA and RNA components of this assay were developed, validated, and set up as separate workflows, with independent extraction, library preparation, sequencing, and analysis

pipelines. The NGS 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 or CSF. When CSF sample is submitted, RNA sequencing is performed on the CSF cell pellet instead of cfRNA due to degradation. Performance of the assays 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 may contain 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)

For a complete list of tested RNA genes (Fusions/Expression), 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	CUX1	ETNK1	GALNT12	IL7R	MCL1	NFE2L2	PIM1	RB1	SMO	TRAF3
ABRAXAS1	BAP1	CD274	CXCR4	ETV6	GATA1	INHBA	MDM2	NFKBIA	PLCG1	RET	SOCS1	TSC1
ACVR1B	BARD1	CD79A	CYLD	EXO1	GATA2	IRF4	MDM4	NKX2-1	PMS1	RHEB	SOX2	TSC2
AKT1	BCL2	CD79B	DAXX	EZH2	GATA3	JAK1	MED12	NOTCH1	PMS2	RHOA	SOX9	TSHR

AKT2	BCL2L1	CDC73	DDR2	FANCA	GEN1	JAK2	MEF2B	NOTCH2	POLD1	RIT1	SPOP	U2AF1
AKT3	BCL6	CDH1	DDX41	FANCC	GNA11	JAK3	MEN1	NOTCH3	POLE	RNF43	SRC	U2AF2
ALK	BCOR	CDK12	DICER1	FANCD2	GNAQ	KAT6A	MET	NPM1	POT1	ROS1	SRSF2	UBA1
AMER1	BCORL1	CDK4	DNM2	FANCE	GNAS	KDM5C	MITF	NRAS	PPM1D	RUNX1	STAG2	VHL
ANKRD26	BCR	CDK6	DNMT3A	FANCF	GNB1	KDM6A	MLH1	NSD1	PPP2R1A	SAMD9	STAT3	WT1
APC	BIRC3	CDKN1B	DOT1L	FANCG	GREM1	KDR	MPL	NSD2 (WHSC1)	PRDM1	SAMD9L	STAT5B	XPO1
AR	BLM	CDKN2A	EED	FAS	GRIN2A	KEAP1	MRE11	NTHL1	PRKAR1A	SDHA	STK11	XRCC2
ARAF	BMPR1A	CDKN2B	EGFR	FBXW7	H3-3A (H3F3A)	KIT	MSH2	NTRK1	PRKDC	SDHAF2	SUFU	XRCC3
ARID1A	BRAF	CDKN2C	EGLN1	FGF4	H3C2 (HIST1H3B)	KMT2A	MSH3	NTRK2	PRPF8	SDHB	SUZ12	ZNF217
ARID1B	BRCA1	CEBPA	ELANE	FGF6	HGF	KMT2B	MSH6	NTRK3	PRSS1	SDHC	TAL1	ZRSR2
ARID2	BRCA2	CHEK1	EP300	FGFR1	HNF1A	KMT2C	MTOR	PAK3	PTCH1	SDHD	TCF3	-
ASXL1	BRIP1	CHEK2	EPAS1	FGFR2	HOXB13	KMT2D	MUTYH	PALB2	PTEN	SETBP1	TENT5C (FAM46C)	-
ATM	BTK	CIC	EPCAM	FGFR3	HRAS	KRAS	MYC	PAX5	PTPN11	SETD2	TERC	-
ATR	CALR	CREBBP	EPHA3	FGFR4	HSP90AA1	LRP1B	MYCL	PBRM1	RAC1	SF3B1	TERT	-
ATRX	CARD11	CRLF2	EPHA5	FH	ID3	MAP2K1	MYCN	PDGFRA	RAD21	SMAD2	TET2	-
AURKA	CBL	CSF1R	ERBB2	FLCN	IDH1	MAP2K2	MYD88	PDGFRB	RAD50	SMAD4	TGFBR2	-
AURKB	CBLB	CSF3R	ERBB3	FLT3	IDH2	MAP2K4	NBN	PHF6	RAD51	SMARCA4	TMEM127	-
AURKC	CBLC	CTCF	ERBB4	FLT4	IGF1R	MAP3K1	NF1	PIK3CA	RAD51C	SMARCB1	TNFAIP3	-
AXIN1	CCND1	CTNNA1	ERG	FOXL2	IKZF1	MAP3K14	NF2	PIK3R1	RAD51D	SMC1A	TNFRSF14	-
AXIN2	CCND3	CTNNB1	ESR1	FUBP1	IKZF3	MAPK1	NFE2	PIK3R2	RAF1	SMC3	TP53	-

RNA Fusions/Expression

Fusion/Expression																
ABL1	BCL2	CCND1	CREBBP	EGFR	ETV4	FGFR2	FOXO1	IKZF3	MAP3K1	MYH9	NTRK3	PAX5	PDGFRB	PTK2B	ROS1	TAL1
ABL2	BCL6	CD274 (PD-L1)	CRLF2	EPOR	ETV5	FGFR3	FUS	JAK2	MECOM	NOTCH1	NUP214	PBX1	PICALM	RARA	RUNX1	TCF3
AKT3	BRAF	CBL	CSF1R	ERG	ETV6	FIP1L1	GLI1	KMT2A	MRTFA	NTRK1	NUP98	PCM1	PIGA	RET	RUNX1T1	TFG
ALK	CBFB	CIC	DUSP22	ETV1	FGFR1	FLT3	HLF	LYN	MYC	NTRK2	P2RY8	PDGFRA	PML	RHOA	STAT6	TYK2

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Electronic Signature

Maher Albitar, M.D.

The test (sample processing, sequencing and data generation) was performed at Genomic Testing Cooperative, LCA, 25371 Commercentre Drive Lake Forest, CA 92630. Medical Director Maher Albitar, M.D. Analysis of the data was performed by Genomic Testing Cooperative, LCA, 25371 Commercentre Drive, Lake Forest, CA 92630. Medical Director: Maher Albitar, M.D. (CLIA #: 05D2111917 CAP #: 9441574). The signing pathologist is fully responsible for the accuracy and interpretation of results and the release of this report.

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.

Additional Report Information

Mutations Load (mol/mL)