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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:	Peripheral Blood
Case #:		Specimen ID:	
Body Site:	PERIPHERAL BLOOD		

Collected Date:		Time:	12:00 AM
Received Date:		Time:	10:26 AM
Reported Date:		Time:	12:28 PM

Detected Genomic Alterations				
SAMD9L (?Germline, VUS)	NSD1	WHSC1	CALR	MAP3K1
KMT2B	NF2	GATA2	PIM1	MAP3K14
SETD2	FGFR2	PMS2	AXIN1	PIK3CA
TSC1 (2 mutations)	PLCG1	RAD21	KRAS (G12R)	ZNF217
AURKC	APC	SMARCA4	PRPF8	INHBA
IKZF1	MSH3	PRKDC	LRP1B	Autosomal chromosomal structural analysis shows low-level: 1q +, -13
B cell clonality: Detected, biclonal (One heavy chain: IGHV3-33 and two light chains: IGKV1D-12, IGLV4-3), predominant kappa	T cell clonality: Not detected			

Results Summary

- **-Low-level somatic mutations in NSD1, WHSC1, CALR, MAP3K1, KMT2B, NF2, GATA2, PIM1, MAP3K14, SETD2, FGFR2, PMS2, AXIN1, PIK3CA, TSC1 (2 mutations), PLCG1, RAD21, KRAS, ZNF217, AURKC, APC, SMARCA4, PRPF8, INHBA, IKZF1, MSH3, PRKDC, and LRP1B genes**
  -Possible germline mutation in SAMD9L gene, heterozygous
  -Increased MAFB mRNA reflecting promoter hijacking [t(4;20)]
  -Autosomal chromosomal structural analysis shows low-level: 1q+, -13
  -No evidence of MYD88 or CXCR4 mutations
  -B cell clonality: Detected, biclonal [One heavy chain: IGHV3-33 and two light chains: IGKV1D-12

(Major), IGLV4-3(Minor)].
-T cell clonality: Not detected
-Plasma cell markers (CD138, BCMA): Increased
-EBV viral RNA: Not detected
-HPV viral RNA: Not detected
-TTV viral RNA: Not detected
-HLA Genotyping:
-HLA-A: A*29:02-A*25:01
-HLA-B: B*44:04-B*18:01
-HLA-C: C*16:01-C*12:03

-These findings are consistent with multiple myeloma with MAFB gene rearrangement and unusually markedly high tumor mutation burden. However, the DNMT3A, PPM1D and TET2 mutations are most likely in myeloid cells, consistent with CHIP (clonal hematopoiesis of indeterminate potential).

-Note: The peripheral blood cfDNA/cfRNA show findings similar to those seen in bone marrow sample, except for the detection of a small clone that is expressing lambda light chain that was not detected in the BM sample.

-The SAMD9L mutation is detected at high level, raising the possibility 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.

See quantitative presentation of mutations and clonality at the end of the report.

Additional mutations detected: TET2 (p.Ser1583Leu, 1.84%), RAD51C (p.Gln344Ter, 1.61%), KMT2B (p.Ser1195Phe, 1.29%), PPM1D (p.Gln47Glu, 1.22%), ARID2 (c.4774-5C>A, 1.03%), STAT3 (p.Glu616del, 0.82%), TET2 (p.Gln690Ter, 0.57%), EGLN1 (p.Met202Ile, 0.52%), ARID1A (p.Gln515Ter, 0.46%), PPM1D (p.Phe101SerfsTer64, 0.45%), DNMT3A (c.1474+2dupT, 0.45%), AURKA (p.Thr384Ala, 0.43%), GNAS (p.Arg844His, 0.4%), ERBB3 (p.Asp1275Asn, 0.34%), FBXW7 (p.Ser601Cys, 0.33%), ROS1 (p.Val1223Ile, 0.29%), KMT2B (p.Arg2649Cys, 0.29%), SMC1A (p.Glu394Gln, 0.27%), KMT2C (p.Pro386Ser, 0.26%), CALR (p.Glu370Ter, 0.18%), NFE2 (p.Ile235Phe, 0.16%), MAFB (RNA, p.Arg222Leu, 50.48%), CXCL8 (RNA, p.Glu31Ter, 30.3%)

Heterogeneity

There is an abnormal low-level clone with NSD1, WHSC1, CALR, MAP3K1, KMT2B, NF2, GATA2, PIM1, MAP3K14, SETD2, FGFR2, PMS2, AXIN1, PIK3CA, TSC1 (2 mutations), PLCG1, RAD21, KRAS, ZNF217, AURKC, APC, SMARCA4, PRPF8, INHBA, IKZF1, MSH3, PRKDC, and LRP1B mutations. The SAMD9L mutation is detected at high level, possible germline abnormality.

Expression

Plasma cell markers (CD138, BCMA): Increased

Diagnostic Implications	
SAMD9L, NSD1, WHSC1, CALR, MAP3K1, KMT2B, NF2, GATA2, PIM1, MAP3K14, SETD2, FGFR2, PMS2, AXIN1, PIK3CA, TSC1 (2 mutations), PLCG1, RAD21, KRAS, ZNF217, AURKC, APC, SMARCA4, PRPF8, INHBA, IKZF1, MSH3, PRKDC, LRP1B	-These findings are consistent with multiple myeloma. -The SAMD9L mutation is likely a germline variant.

Therapeutic Implications	
MAP3K1	MEK inhibitors
NF2	AKT/MEK/MTOR inhibitors
SETD2	SETD2 mutation suggests response to WEE1 inhibitors
FGFR2	FGFR inhibitors
PMS2	PARP inhibitors
AXIN1	WNT inhibitors
PIK3CA	PI3K, AKT, MTOR inhibitors
TSC1	MTOR inhibitors
KRAS	MEK inhibitors
APC	WNT, beta-catenin, and COX-2 inhibitors
SMARCA4	HDAC inhibitors
PRKDC	PI3K/AKT, PARP inhibitors

Prognostic Implications	
MAP3K1, NF2, SETD2, FGFR2, PMS2, AXIN1, PIK3CA, TSC1, KRAS, APC, SMARCA4, PRKDC	Poor
NSD1, WHSC1, CALR, KMT2B, GATA2, PIM1, MAP3K14, PLCG1, RAD21, ZNF217, AURKC, PRPF8, INHBA, IKZF1, MSH3, LRP1B	Unknown

Relevant Genes with NO Alteration
No evidence of mutation in: NOTCH, SF3B1, TP53, 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

- SAMD9L. This gene encodes a cytoplasmic protein that acts as a tumor suppressor but also plays a key role in cell proliferation and the innate immune response to viral infection. The encoded protein contains an N-terminal sterile alpha motif domain. Naturally occurring mutations in this gene are associated with myeloid disorders such as juvenile myelomonocytic leukemia, acute myeloid leukemia, and myelodysplastic syndrome. Naturally occurring mutations are also associated with hepatitis-B related hepatocellular carcinoma, normophosphatemic familial tumoral calcinosis, and ataxia-pancytopenia syndrome. [provided by RefSeq, Apr 2017]

- NSD1. This gene encodes a protein containing a SET domain, 2 LXXLL motifs, 3 nuclear translocation signals (NLSs), 4 plant homeodomain (PHD) finger regions, and a proline-rich region. The encoded protein enhances androgen receptor (AR) transactivation, and this enhancement can be increased further in the presence of other androgen receptor associated coregulators. This protein may act as a nucleus-localized, basic transcriptional factor and also as a bifunctional transcriptional regulator. Mutations of this gene have been associated with Sotos syndrome and Weaver syndrome. One version of childhood acute myeloid leukemia is the result of a cryptic translocation with the breakpoints occurring within nuclear receptor-binding Su-var, enhancer of zeste, and trithorax domain protein 1 on chromosome 5 and nucleoporin, 98-kd on chromosome 11. Multiple transcript variants encoding distinct isoforms have been identified for this gene. [provided by RefSeq, Sep 2018]

- NSD2. This gene encodes a protein that contains four domains present in other developmental proteins: a PWWP domain, an HMG box, a SET domain, and a PHD-type zinc finger. It is expressed ubiquitously in early development. Wolf-Hirschhorn syndrome (WHS) is a malformation syndrome associated with a hemizygous deletion of the distal short arm of chromosome 4. This gene maps to the 165 kb WHS critical region and has also been involved in the chromosomal translocation t(4;14)(p16.3;q32.3) in multiple myelomas. Alternative splicing of this gene results in multiple transcript variants encoding different isoforms. Some transcript variants are nonsense-mediated mRNA (NMD) decay candidates, hence not represented as reference sequences. [provided by RefSeq, Jul 2008]

- CALR. Calreticulin is a highly conserved chaperone protein which resides primarily in the endoplasmic reticulum, and is involved in a variety of cellular processes, among them, cell adhesion. Additionally, it functions in protein folding quality control and calcium homeostasis. Calreticulin is also found in the nucleus, suggesting that it may have a role in transcription regulation. Systemic lupus erythematosus is associated with increased autoantibody titers against calreticulin. Recurrent mutations in calreticulin have been linked to various neoplasms, including the myeloproliferative type.[provided by RefSeq, May 2020]

- MAP3K1. The protein encoded by this gene is a serine/threonine kinase and is part of some signal transduction cascades, including the ERK and JNK kinase pathways as well as the NF-kappa-B pathway. The encoded protein is activated by autophosphorylation and requires magnesium as a cofactor in phosphorylating other proteins. This protein has E3 ligase activity conferred by a plant homeodomain (PHD) in its N-terminus and phospho-kinase activity conferred by a kinase domain in its C-terminus. [provided by RefSeq, Mar 2012]

- KMT2B. This gene encodes a protein which contains multiple domains including a CXXC zinc finger, three PHD zinc fingers, two FY-rich domains, and a SET (suppressor of variegation, enhancer of zeste, and trithorax) domain. The SET domain is a conserved C-terminal domain that characterizes proteins of the MLL (mixed-lineage leukemia) family. This gene is ubiquitously expressed in adult tissues. It is also amplified in solid tumor cell lines, and may be involved in human cancer. Two alternatively spliced transcript variants encoding distinct isoforms have been reported for this gene, however, the full length nature of the shorter transcript is not known. [provided by RefSeq, Jul 2008]

- 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]

- GATA2. This gene encodes a member of the GATA family of zinc-finger transcription factors that are named for the consensus nucleotide sequence they bind in the promoter regions of target genes. The encoded protein plays an essential role in regulating transcription of genes involved in the development and proliferation of hematopoietic and endocrine cell lineages. Alternative splicing results in multiple transcript variants.[provided by RefSeq, Mar 2009]

- PIM1. The protein encoded by this gene belongs to the Ser/Thr protein kinase family, and PIM subfamily. This gene is expressed primarily in B-lymphoid and myeloid cell lines, and is overexpressed in hematopoietic malignancies and in prostate cancer. It plays a role in signal transduction in blood cells, contributing to both cell proliferation and survival, and thus provides a selective advantage in tumorigenesis. Both the human and orthologous mouse genes have been reported to encode two isoforms (with preferential cellular localization) resulting from the use of alternative in-frame translation initiation codons, the upstream non-AUG (CUG) and downstream AUG codons (PMIDs:16186805, 1825810).[provided by RefSeq, Aug 2011]

- MAP3K14. This gene encodes mitogen-activated protein kinase kinase kinase 14, which is a serine/threonine protein-kinase. This kinase binds to TRAF2 and stimulates NF-kappaB activity. It shares sequence similarity with several other MAPKK kinases. It participates in an NF-kappaB-inducing signalling cascade common to receptors of the tumour-necrosis/nerve-growth factor (TNF/NGF) family and to the interleukin-1 type-I receptor. [provided by RefSeq, Jul 2008]

- SETD2. Huntington disease (HD), a neurodegenerative disorder characterized by loss of striatal neurons, is caused by an expansion of a polyglutamine tract in the HD protein huntingtin. This gene encodes a protein belonging to a class of huntingtin interacting proteins characterized by WW motifs. This protein is a histone methyltransferase that is specific for lysine-36 of histone H3, and methylation of this residue is associated with active chromatin. This protein also contains a novel transcriptional activation domain and has been found associated with hyperphosphorylated RNA polymerase II. [provided by RefSeq, Aug 2008]

- FGFR2. The protein encoded by this gene is a member of the fibroblast growth factor receptor family, where amino acid sequence is highly conserved between members and throughout evolution. FGFR family members differ from one another in their ligand affinities and tissue distribution. A full-length representative protein consists of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain. The extracellular portion of the protein interacts with fibroblast growth factors, setting in motion a cascade of downstream signals, ultimately influencing mitogenesis and differentiation. This particular family member is a high-affinity receptor for acidic, basic and/or keratinocyte growth factor, depending on the isoform. Mutations in this gene are associated with Crouzon syndrome, Pfeiffer syndrome, Craniosynostosis, Apert syndrome, Jackson-Weiss syndrome, Beare-Stevenson cutis gyrata syndrome, Saethre-Chotzen syndrome, and syndromic craniosynostosis. Multiple alternatively spliced transcript variants encoding different isoforms have been noted for this gene. [provided by RefSeq, Jan 2009]

- PMS2. The protein encoded by this gene is a key component of the mismatch repair system that functions to correct DNA mismatches and small insertions and deletions that can occur during DNA replication and homologous recombination. This protein forms heterodimers with the gene product of the mutL homolog 1 (MLH1) gene to form the MutL-alpha heterodimer. The MutL-alpha heterodimer possesses an endonucleolytic activity that is activated following recognition of mismatches and insertion/deletion loops by the MutS-alpha and MutS-beta heterodimers, and is necessary for removal of the mismatched DNA. There is a DQHA(X)2E(X)4E motif found at the C-terminus of the protein encoded by this gene that forms part of the active site of the nuclease. Mutations in this gene have been associated with hereditary nonpolyposis colorectal cancer (HNPCC; also known as Lynch syndrome) and Turcot syndrome. [provided by RefSeq, Apr 2016]

- AXIN1. This gene encodes a cytoplasmic protein which contains a regulation of G-protein signaling (RGS) domain and a dishevelled and axin (DIX) domain. The encoded protein interacts with adenomatosis polyposis coli, catenin beta-1, glycogen synthase kinase 3 beta, protein phosphate 2, and itself. This protein functions as a negative regulator of the wingless-type MMTV integration site family, member 1 (WNT) signaling pathway and can induce apoptosis. The crystal structure of a portion of this protein, alone and in a complex with other proteins, has been resolved. Mutations in this gene have been associated with hepatocellular carcinoma, hepatoblastomas, ovarian endometriod adenocarcinomas, and medullablastomas. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jan 2016]

- PIK3CA. Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers. A pseudogene of this gene has been defined on chromosome 22. [provided by RefSeq, Apr 2016]

- TSC1. This gene is a tumor suppressor gene that encodes the growth inhibitory protein hamartin. The encoded protein interacts with and stabilizes the GTPase activating protein tuberin. This hamartin-tuberin complex negatively regulates mammalian target of rapamycin complex 1 (mTORC1) signalling which is a major regulator of anabolic cell growth. This protein also functions as a co-chaperone for Hsp90 that inhibits its ATPase activity. This protein functions as a facilitator of Hsp90-mediated folding of kinase and non-kinase clients, including Tsc2 and thereby preventing their ubiquitination and proteasomal degradation. Mutations in this gene have been associated with tuberous sclerosis. [provided by RefSeq, Apr 2018]

- PLCG1. The protein encoded by this gene catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate. This reaction uses calcium as a cofactor and plays an important role in the intracellular transduction of receptor-mediated

tyrosine kinase activators. For example, when activated by SRC, the encoded protein causes the Ras guanine nucleotide exchange factor RasGRP1 to translocate to the Golgi, where it activates Ras. Also, this protein has been shown to be a major substrate for heparin-binding growth factor 1 (acidic fibroblast growth factor)-activated tyrosine kinase. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jul 2008]

- RAD21. The protein encoded by this gene is highly similar to the gene product of Schizosaccharomyces pombe rad21, a gene involved in the repair of DNA double-strand breaks, as well as in chromatid cohesion during mitosis. This protein is a nuclear phospho-protein, which becomes hyperphosphorylated in cell cycle M phase. The highly regulated association of this protein with mitotic chromatin specifically at the centromere region suggests its role in sister chromatid cohesion in mitotic cells. [provided by RefSeq, Jul 2008]

- KRAS. This gene, a Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily. A single amino acid substitution is responsible for an activating mutation. The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma. Alternative splicing leads to variants encoding two isoforms that differ in the C-terminal region. [provided by RefSeq, Jul 2008]

- ZNF217. Enables DNA-binding transcription repressor activity, RNA polymerase II-specific and RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. Involved in negative regulation of transcription by RNA polymerase II. Located in mitochondrion and nuclear speck. Part of histone deacetylase complex. [provided by Alliance of Genome Resources, Apr 2022]

- AURKC. This gene encodes a member of the Aurora subfamily of serine/threonine protein kinases. The encoded protein is a chromosomal passenger protein that forms complexes with Aurora-B and inner centromere proteins and may play a role in organizing microtubules in relation to centrosome/spindle function during mitosis. This gene is overexpressed in several cancer cell lines, suggesting an involvement in oncogenic signal transduction. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jul 2008]

- 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]

- SMARCA4. The protein encoded by this gene is a member of the SWI/SNF family of proteins and is similar to the brahma protein of Drosophila. Members of this family have helicase and ATPase activities and are thought to regulate transcription of certain genes by altering the chromatin structure around those genes. The encoded protein is part of the large ATP-dependent chromatin remodeling complex SNF/SWI, which is required for transcriptional activation of genes normally repressed by chromatin. In addition, this protein can bind BRCA1, as well as regulate the expression of the tumorigenic protein CD44. Mutations in this gene cause rhabdoid tumor predisposition syndrome type 2. Multiple transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, May 2012]

- PRPF8. Pre-mRNA splicing occurs in 2 sequential transesterification steps. The protein encoded by this gene is a component of both U2- and U12-dependent spliceosomes, and found to be essential for the catalytic step II in pre-mRNA splicing process. It contains several WD repeats, which function in protein-protein interactions. This protein has a sequence similarity to yeast Prp8 protein. This gene is a candidate gene for autosomal dominant retinitis pigmentosa. [provided by RefSeq, Jul 2008]

- INHBA. This gene encodes a member of the TGF-beta (transforming growth factor-beta) superfamily of proteins. The encoded preproprotein is proteolytically processed to generate a subunit of the dimeric activin and inhibin protein complexes. These complexes activate and inhibit, respectively, follicle stimulating hormone secretion from the pituitary gland. The encoded protein also plays a role in eye, tooth and testis development. Elevated expression of this gene may be associated with cancer cachexia in human patients. [provided by RefSeq, Aug 2016]

- IKZF1. This gene encodes a transcription factor that belongs to the family of zinc-finger DNA-binding proteins associated with chromatin remodeling. The expression of this protein is restricted to the fetal and adult hemo-lymphopoietic system, and it functions as a regulator of lymphocyte differentiation. Several alternatively spliced transcript variants encoding different isoforms have been described for this gene. Most isoforms share a common C-terminal domain, which contains two zinc finger motifs that are required for hetero- or homo-dimerization, and for interactions with other proteins. The isoforms, however, differ in the number of N-terminal zinc finger motifs that bind DNA and in nuclear localization signal presence, resulting in members with and without DNA-binding properties. Only a few isoforms contain the requisite three or more N-terminal zinc motifs that confer high affinity binding to a specific core DNA sequence element in the promoters of target genes. The non-DNA-binding isoforms are largely found in the cytoplasm, and are thought to function as dominant-negative factors. Overexpression of some dominant-negative isoforms have been associated with B-cell malignancies, such as acute lymphoblastic leukemia (ALL). [provided by RefSeq, May 2014]

- MSH3. The protein encoded by this gene forms a heterodimer with MSH2 to form MutS beta, part of the post-replicative DNA mismatch repair system. MutS beta initiates mismatch repair by binding to a mismatch and then forming a complex with MutL alpha heterodimer. This gene contains a polymorphic 9 bp tandem repeat sequence in the first exon. The repeat is present 6 times in the reference genome sequence and 3-7 repeats have been reported. Defects in this gene are a cause of susceptibility to endometrial cancer. [provided by RefSeq, Mar 2011]

- PRKDC. This gene encodes the catalytic subunit of the DNA-dependent protein kinase (DNA-PK). It functions with the Ku70/Ku80 heterodimer protein in DNA double strand break repair and recombination. The protein encoded is a member of the PI3/PI4-kinase family.[provided by RefSeq, Jul 2010]

- 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

Binimetinib

Binimetinib is an orally available inhibitor of mitogen-activated protein kinase kinase 1 and 2 (MEK1/2) with potential antineoplastic activity. Binimetinib, noncompetitive with ATP, binds to and inhibits the activity of MEK1/2. Inhibition of MEK1/2 prevents the activation of MEK1/2-dependent effector proteins and transcription factors, which may result in the inhibition of growth factor-mediated cell signaling. This may eventually lead to an inhibition of tumor cell proliferation and an inhibition in production of various inflammatory cytokines including interleukin-1, -6 and tumor necrosis factor. MEK1/2 are dual-specificity threonine/tyrosine kinases that play key roles in the activation of the RAS/RAF/MEK/ERK pathway and are often upregulated in a variety of tumor cell types.

Cobimetinib

Cobimetinib is a reversible inhibitor of mitogen-activated protein kinase 1 (MAPK)/extracellular signal regulated kinase 1 (MEK1) and MEK2. MEK inhibitor Cobimetinib specifically binds to and inhibits the catalytic activity of MEK1, resulting in inhibition of extracellular signal-related kinase 2 (ERK2) phosphorylation and activation and decreased tumor cell proliferation. Cobimetinib targets kinase activity in the RAS/RAF/MEK/ERK pathway.

Selumetinib

Selumetinib is a MEK inhibitor that targets PDGFR, KIT, VEGFR, FLT3, RET, CSF1R. It is an orally bioavailable small molecule with potential antineoplastic activity. Selumetinib inhibits mitogenactivated protein kinase kinases (MEK or MAPK/ERK kinases) 1 and 2, which may prevent the activation of MEK1/2-dependent effector proteins and transcription factors, and so may inhibit cellular proliferation in MEK-overexpressing tumor cells. MEK 1 and 2 are dual-specificity kinases that are essential mediators in the activation of the RAS/RAF/MEK/ERK pathway, are often upregulated in various tumor cell types, and are drivers of diverse cellular activities, including cellular proliferation.

Trametinib

Trametinib is an orally bioavailable inhibitor of mitogen-activated protein kinase kinase (MEK MAPK/ERK kinase) with potential antineoplastic activity. Trametinib specifically binds to and inhibits MEK 1 and 2, resulting in an inhibition of growth factor-mediated cell signaling and cellular proliferation in various cancers. MEK 1 and 2, dual specificity threonine/tyrosine kinases often upregulated in various cancer cell types, play a key role in the activation of the RAS/RAF/MEK/ERK signaling pathway that regulates cell growth.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://clinicaltrials.gov/study/NCT05434689	Recruiting	Phase Ib/II Trial Of Iberdomide-Combinations In Patients With Positive Minimal Residual Disease (>10-5) After Autologous Hematopoietic Cell Transplantation In The Upfront Management Of Patients With Multiple Myeloma	Multiple Myeloma	Iberdomide, Daratumumab, Dexamethasone, Carfilzomib	University of Alabama at Birmingham, Birmingham, Alabama 35233 Duke University, Durham, North Carolina 27710 Ohio State University Medical College, Columbus, Ohio 43210

https://clinicaltrials.gov/study/NCT05090566	Recruiting	A PHASE 1B/2, OPEN LABEL UMBRELLA STUDY OF ELRANATAMAB (PF-06863135), A B-CELL MATURATION ANTIGEN (BCMA) CD3 BISPECIFIC ANTIBODY, IN COMBINATION WITH OTHER ANTI-CANCER TREATMENTS IN PARTICIPANTS WITH MULTIPLE MYELOMA	Multiple Myeloma	Elranatamab + Nirogacestat, Elranatamab + lenalidomide + dexamethasone	Banner Gateway Medical Center, Gilbert, Arizona 85234 Banner MD Anderson Cancer Center, Gilbert, Arizona 85234 University of Arkansas for Medical Sciences - Winthrop P. Rockefeller Cancer Institute, Little Rock, Arkansas 72205
https://clinicaltrials.gov/study/NCT04091126	Recruiting	A Phase 1, Randomized, Dose and Schedule Evaluation Study to Investigate the Safety, Pharmacokinetics, Pharmacodynamics and Clinical Activity of Belantamab Mafodotin Administered in Combination With Standard of Care in Participants With Newly Diagnosed Multiple Myeloma	Multiple Myeloma	Belantamab mafodotin, Bortezomib, Lenalidomide, Dexamethasone	GSK Investigational Site, Yuma, Arizona 85364 GSK Investigational Site, Westwood, Kansas 66205 GSK Investigational Site, Charlotte, North Carolina 28204

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
SAMD9L	NP_689916.2:p.Arg406Ter	NM_152703.2:c.1216C>T	R/*	Cga/Tga	stop_gained	49.46	1207	0
NSD1	NP_071900.2:p.Glu1136Gln	NM_022455.4:c.3406G>C	E/Q	Gag/Cag	missense_variant	6.24	2115	tolerated - low confidence (0.14)
WHSC1	NP_001035889.1:p.Ile117Met	NM_001042424.2:c.351C>G	I/M	atC/atG	missense_variant	5.98	2072	tolerated (0.15)
CALR	NP_004334.1:p.Glu363Ter	NM_004343.3:c.1087G>T	E/*	Gag/Tag	stop_gained	5.79	5218	0
MAP3K1	NP_005912.1:p.Ser258Leu	NM_005921.1:c.773C>T	S/L	tCa/tTa	missense_variant	5.7	2051	deleterious - low confidence (0.01)
KMT2B	NP_055542.1:p.Arg706Ter	NM_014727.1:c.2116C>T	R/*	Cga/Tga	stop_gained	5.57	2728	0
NF2	NP_000259.1:p.His242Tyr	NM_000268.3:c.724C>T	H/Y	Cac/Tac	missense_variant	4.98	1666	deleterious (0.01)
GATA2	NP_116027.2:p.Ser425Leu	NM_032638.4:c.1274C>T	S/L	tCa/tTa	missense_variant	4.92	2155	tolerated (0.06)
PIM1	NP_001230115.1:p.Ser40Leu	NM_001243186.1:c.119C>T	S/L	tCg/tTg	missense_variant	4.91	3073	0
MAP3K14	NP_003945.2:p.Ser604Phe	NM_003954.3:c.1811C>T	S/F	tCt/tTt	missense_variant	4.8	1042	0
SETD2	NP_054878.5:p.Ser1660Ter	NM_014159.6:c.4979C>A	S/*	tCa/tAa	stop_gained	4.73	1227	0

FGFR2	NP_075259.4:p.Tyr567Asp	NM_022970.3:c.1699T>G	Y/D	Tat/Gat	missense_variant	4.26	1879	deleterious (0)
PMS2	NP_000526.1:p.Ser456Phe	NM_000535.5:c.1367C>T	S/F	tCt/tTt	missense_variant	4.06	2092	tolerated (0.2)
AXIN1	NP_003493.1:p.Leu150Val	NM_003502.3:c.448C>G	L/V	Ctt/Gtt	missense_variant	3.91	1894	tolerated - low confidence (0.21)
PIK3CA	0	NM_006218.2:c.2666+8G>A	0	0	splice_region_variant,intron_variant	3.9	1231	0
TSC1	NP_000359.1:p.Leu512Val	NM_000368.4:c.1534C>G	L/V	Ctc/Gtc	missense_variant	3.73	2146	tolerated (0.07)
PLCG1	NP_002651.2:p.Ser18Leu	NM_002660.2:c.53C>T	S/L	tCg/tTg	missense_variant	3.71	1348	tolerated (0.54)
RAD21	NP_006256.1:p.Gln492Ter	NM_006265.2:c.1474C>T	Q/*	Cag/Tag	stop_gained	3.52	1419	0
KRAS	NP_203524.1:p.Gly12Arg	NM_033360.2:c.34G>C	G/R	Ggt/Cgt	missense_variant	3.47	952	deleterious (0.03)
ZNF217	NP_006517.1:p.Gln584Ter	NM_006526.2:c.1750C>T	Q/*	Cag/Tag	stop_gained	3.38	1333	0
TSC1	NP_000359.1:p.Ser526Cys	NM_000368.4:c.1577C>G	S/C	tCt/tGt	missense_variant	3.29	2217	tolerated (0.07)
AURKC	NP_001015878.1:p.Gln306His	NM_001015878.1:c.918G>C	Q/H	caG/caC	missense_variant	3.19	1882	tolerated - low confidence (0.13)
APC	NP_000029.2:p.Ser1222Leu	NM_000038.5:c.3665C>T	S/L	tCa/tTa	missense_variant	3.18	2168	0
SMARCA4	NP_001122321.1:p.Glu1242Lys	NM_001128849.1:c.3724G>A	E/K	Gag/Aag	missense_variant	3.01	1896	0
PRPF8	NP_006436.3:p.Glu2053Gln	NM_006445.3:c.6157G>C	E/Q	Gag/Cag	missense_variant	2.97	3234	tolerated (0.13)
INHBA	NP_002183.1:p.Glu208Lys	NM_002192.2:c.622G>A	E/K	Gaa/Aaa	missense_variant	2.94	1700	deleterious (0)
IKZF1	NP_006051.1_dupl12.1:p.Pro397Ala	NM_006060.4_dupl12.1:c.1187C>G	P/A	Ccg/Gcg	missense_variant	2.83	1801	0
MSH3	NP_002430.3:p.Asp708Asn	NM_002439.4:c.2122G>A	D/N	Gac/Aac	missense_variant	2.74	1166	tolerated (0.18)
PRKDC	NP_008835.5:p.Ser2976Leu	NM_006904.6:c.8927C>T	S/L	tCa/tTa	missense_variant	2.31	1040	0
LRP1B	NP_061027.2:p.Glu2542Lys	NM_018557.2:c.7624G>A	E/K	Gaa/Aaa	missense_variant	2.2	1275	0

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA for abnormalities in 302 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 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

Reference

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5. Supportive Care in Multiple Myeloma. Guzdar A, Costello C. Curr Hematol Malig Rep. 2020 Apr;15(2):56-61. doi: 10.1007/s11899-020-00570-9. PMID: 32172361.

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.

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.

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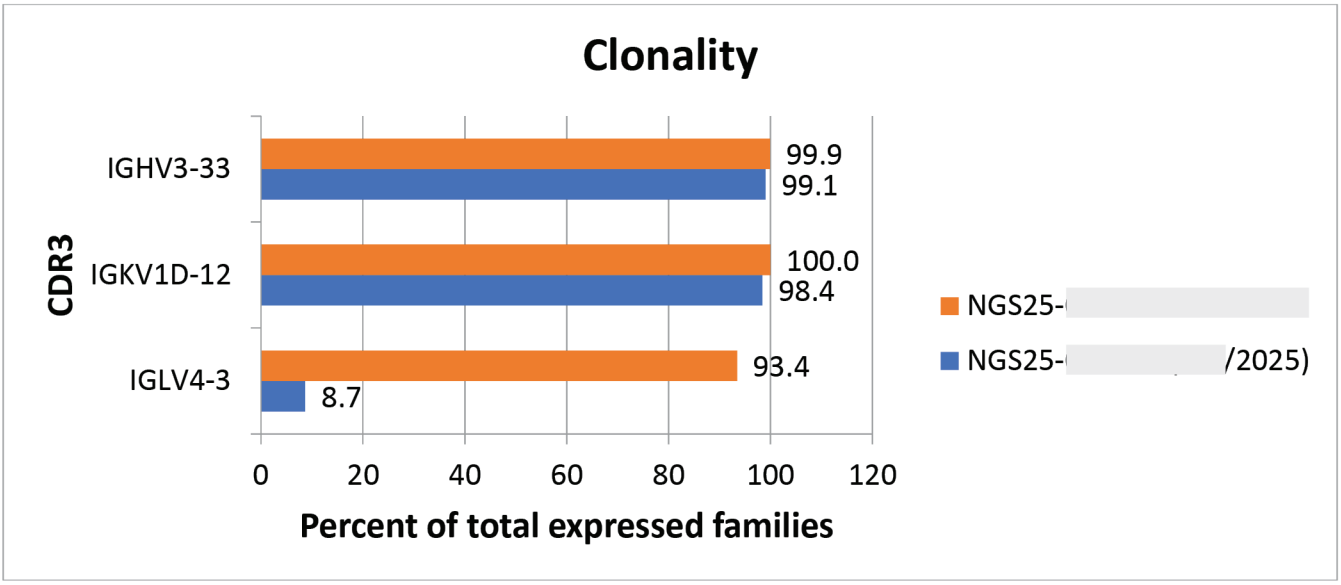
Additional Report Information



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Additional Report Information