GENOMIC® TESTING COOPERATIVE

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CLIA #: 05D2111917 CAP #: 9441574

Liquid Trace Solid Tumor

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

Collected Date:		Indication for Testing:	
Received Date:		Tumor Type:	
Reported Date:		Stage:	

Detected Genomic Alterations

Level 1 (FDA-Approved)	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Evidence)	Other
BRCA1	Tumor Mutation Burden Intermediate: 8 Mut/Mb	KMT2A, TP53, RET, ESR1, EP300	BLM, LRP1B, CUX1, BCR, KMT2D	Autosomal chromosomal structural analysis shows numerous changes: 1q+, -4, 5p=, 5q-, 8q+, 9p-, 10p+, 12q-, 16q+, 17p-, -18, 22q- and others

Results Summary

- **-Mutations in BRCA1, KMT2A, TP53, RET, BLM, LRP1B, CUX1, BCR, ESR1, EP300, and KMT2D genes**
  -Tumor Mutation Burden Intermediate: 8 Mut/Mb
  -Increased CA15-3, CA125, CK, CEA, PAX8, TP53, FOLR1 mRNA
  -Autosomal chromosomal structural analysis shows numerous changes: 1q+, -4, 5p=, 5q-, 8q+, 9p-, 10p+, 12q-, 16q+, 17p-, -18, 22q- and others
  -Numerous chromosomal changes imply Homologous recombination deficiency (HRD)
  -No evidence of PALB2 mutations
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected

  -The findings suggest the presence of circulating solid tumor DNA/RNA of possibly ovarian cancer primary.

  -Homologous recombination deficiency (HRD) suggests response to platinum-based chemotherapy and PARP inhibitors.

  -Intermediate TMB suggests response to immune checkpoint inhibitors.

-BRCA1 mutation suggests response to PARP inhibitors.

-KMT2A mutation suggests response to HDAC inhibitors.

-RET mutation suggests response to RET tyrosine kinase inhibitor (alectinib, sunitinib, sorafenib, vandetanib, alectinib hydrochloride, amuvatinib, motesanib).

-EP300 gene mutation suggests possible response to BET (Bromodomain Extra-Terminal) inhibitors.

-TP53 mutation suggests possible response to eprenetapopt (APR-246), Aurora kinase A and Wee1 inhibitors.

See additional report information at the end of the report.

Tumor Heterogeneity
There is a dominant abnormal clone with BRCA1 and KMT2A mutations. The TP53, RET, BLM, LRP1B, CUX1, BCR, ESR1, EP300, and KMT2D mutations are detected in subclones.

Expression
Increased CA15-3, CA125, CK, CEA, PAX8, TP53, FOLR1 mRNA

Diagnostic Implications	
BRCA1, KMT2A, TP53, RET, BLM, LRP1B, CUX1, BCR, ESR1, EP300, KMT2D	The findings suggest the presence of circulating solid tumor DNA/RNA of possibly ovarian cancer primary (see results summary).

FDA-Approved Therapeutics	
BRCA1	Niraparib, Olaparib, Olaparib + Bevacizumab, Rucaparib

Relevant Alteration Associated with Resistance
TP53 mutation is associated with resistance to therapy.
ESR1 mutations may confer acquired resistance to estrogen deprivation therapies

Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence)	
BRCA1	PARP inhibitors
KMT2A	HDAC Inhibitors
TP53	Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy
RET	RET inhibitors
EP300	Bromodomain Extra-Terminal (BET) inhibitors

TMB-Intermediate	Immunotherapy with checkpoint inhibitor

Relevant Genes with NO Alteration		
No evidence of mutation in KRAS, NRAS, EGFR, BRAF, or BRCA2	No specific mutation in DPYD gene, associated with enzymatic deficiency	No evidence of METex14 skipping or EGFRvIII

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), tumor mutation burden (TMB), 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 solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Biological relevance of detected Alterations

- BRCA1 mutations in the germline have become a hallmark for hereditary breast and ovarian cancers. Variants that have been demonstrated to reduce the function of the protein have been shown to increase the risk for these cancers, as well as prostate and pancreatic cancer. These findings have been the impetus for the increased popularity of genetic testing of healthy individuals to assess risk. Recent studies in ovarian cancer have also demonstrated that BRCA mutation status can predict treatment response. A number of trials assessing BRCA mutation status have shown an improved response to platinum agents, and more recently has led to the FDA-approval of PARP inhibitors for BRCA-positive ovarian cancers. These studies have resulted in the Society of Gynecologic Oncology to recommend germline BRCA testing in all patients with a diagnosis of ovarian cancer.This gene encodes a 190 kD nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumor suppressor. The BRCA1 gene contains 22 exons spanning about 110 kb of DNA. The encoded protein combines with other tumor suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex (BASC). This gene product associates with RNA polymerase II, and through the C-terminal domain, also interacts with histone deacetylase complexes. This protein thus plays a role in transcription, DNA repair of double-stranded breaks, and recombination. Mutations in this gene are responsible for approximately 40% of inherited breast cancers and more than 80% of inherited breast and ovarian cancers. Alternative splicing plays a role in modulating the subcellular localization and physiological function of this gene. Many alternatively spliced transcript variants, some of which are disease-associated mutations, have been described for this gene, but the full-length natures of only some of these variants has been described. A related pseudogene, which is also located on chromosome 17, has been identified. [provided by RefSeq, May 2020]

- KMT2A. This gene encodes a transcriptional coactivator that plays an essential role in regulating gene expression during early development and hematopoiesis. The encoded protein contains multiple conserved functional domains. One of these domains, the SET domain, is responsible for its histone H3 lysine 4 (H3K4) methyltransferase activity which mediates chromatin modifications associated with epigenetic transcriptional activation. This protein is processed by the enzyme Taspase 1 into two fragments, MLL-C and MLL-N. These fragments reassociate and further assemble into different multiprotein complexes that regulate the transcription of specific target genes, including many of the HOX genes. Multiple chromosomal translocations involving this gene are the cause of certain acute lymphoid leukemias and acute myeloid leukemias. Alternate splicing results in multiple transcript variants.[provided by RefSeq, Oct 2010]

- TP53. This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons from identical transcript variants (PMIDs: 12032546, 20937277). [provided by RefSeq, Dec 2016]

- RET. This gene encodes a transmembrane receptor and member of the tyrosine protein kinase family of proteins. Binding of ligands such as GDNF (glial cell-line derived neurotrophic factor) and other related proteins to the encoded receptor stimulates receptor dimerization and activation of downstream signaling pathways that play a role in cell differentiation, growth, migration and survival. The encoded receptor is important in development of the nervous system, and the development of organs and tissues derived from the neural crest. This proto-oncogene can undergo oncogenic activation through both cytogenetic rearrangement and activating point mutations. Mutations in this gene are associated with Hirschsprung disease and central hypoventilation syndrome and have been identified in patients with renal agenesis. [provided by RefSeq, Sep 2017]

- BLM. The Bloom syndrome is an autosomal recessive disorder characterized by growth deficiency, microcephaly and immunodeficiency among others. It is caused by homozygous or compound heterozygous mutation in the gene encoding DNA helicase RecQ protein on chromosome

15q26. This Bloom-associated helicase unwinds a variety of DNA substrates including Holliday junction, and is involved in several pathways contributing to the maintenance of genome stability. Identification of pathogenic Bloom variants is required for heterozygote testing in at-risk families. [provided by RefSeq, May 2020]

- 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]

- CUX1. The protein encoded by this gene is a member of the homeodomain family of DNA binding proteins. It may regulate gene expression, morphogenesis, and differentiation and it may also play a role in the cell cycle progession. Several alternatively spliced transcript variants encoding different isoforms have been identified.[provided by RefSeq, Feb 2011]

- 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]

- ESR1. This gene encodes an estrogen receptor and ligand-activated transcription factor. The canonical protein contains an N-terminal ligand-independent transactivation domain, a central DNA binding domain, a hinge domain, and a C-terminal ligand-dependent transactivation domain. The protein localizes to the nucleus where it may form either a homodimer or a heterodimer with estrogen receptor 2. The protein encoded by this gene regulates the transcription of many estrogen-inducible genes that play a role in growth, metabolism, sexual development, gestation, and other reproductive functions and is expressed in many non-reproductive tissues. The receptor encoded by this gene plays a key role in breast cancer, endometrial cancer, and osteoporosis. This gene is reported to have dozens of transcript variants due to the use of alternate promoters and alternative splicing, however, the full-length nature of many of these variants remain uncertain. [RefSeq, Jul 2020]

- EP300. This gene encodes the adenovirus E1A-associated cellular p300 transcriptional co-activator protein. It functions as histone acetyltransferase that regulates transcription via chromatin remodeling and is important in the processes of cell proliferation and differentiation. It mediates cAMP-gene regulation by binding specifically to phosphorylated CREB protein. This gene has also been identified as a co-activator of HIF1A (hypoxia-inducible factor 1 alpha), and thus plays a role in the stimulation of hypoxia-induced genes such as VEGF. Defects in this gene are a cause of Rubinstein-Taybi syndrome and may also play a role in epithelial cancer. [provided by RefSeq, Jul 2008]

- 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]

Drug Information

Pembrolizumab (Keytruda)

Pembrolizumab is a highly selective IgG4-kappa humanized monoclonal antibody against PD-1 receptor. It was generated by grafting the variable sequences of a very high-affinity mouse antihuman PD-1 antibody onto a human IgG4-kappa isotype with the containing a stabilizing S228P Fc mutation.

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)

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.

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.

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

Vorinostat

Vorinostat is a member of a larger class of compounds that inhibit histone deacetylases (HDAC). Histone deacetylase inhibitors (HDI) have a broad spectrum of epigenetic activities.
Clinical trials including vorinostat in treatment of advanced non-small-cell lung carcinoma (NSCLC) showed improved response rates and increased median progression free survival and overall survival.

APR-246

APR-246 is a first-in-class agent targeting mutant p53. In vitro and in vivo preclinical models have demonstrated that APR-246 has excellent efficacy in OC (both adenocarcinoma and squamous cell carcinoma) and potently synergises with chemotherapies used in the treatment of OC, restoring sensitivity to chemotherapy-resistant tumours. An initial phase I clinical trial has shown APR-246 to be safe in humans and early results from a currently running phase Ib/II trial of APR-246 with carboplatin and liposomal doxorubicin in ovarian cancer have been promising. Together, these data provide a strong rationale for investigating the efficacy of APR-246 in OC.

APR-246 has been used in trials studying the treatment of Prostatic Neoplasms, Hematologic Neoplasms, and Platinum Sensitive Recurrent High-grade Serous Ovarian Cancer With Mutated p53.

APR-246 is an analogue of PRIMA-1, which modifies the core domain of mutant p53, resulting in restoration of wild-type p53 conformation and reactivation of normal p53 function, leading to increased cell cycle arrest and tumor cell death (PMID: 20498645).

Sunitinib

Sunitinib is a small-molecule multi-targeted receptor tyrosine kinase (RTK) inhibitor.

Sunitinib was evaluated for its inhibitory activity against a variety of kinases (>80 kinases) and was identified as an inhibitor of platelet-derived growth factor receptors (PDGFRa and PDGFRb), vascular endothelial growth factor receptors (VEGFR1, VEGFR2 and VEGFR3), stem cell factor receptor (KIT), Fms-like tyrosine kinase-3 (FLT3), colony stimulating factor receptor Type 1 (CSF-1R), and the glial cell-line derived neurotrophic factor receptor (RET). Sunitinib inhibition of the activity of these RTKs has been demonstrated in biochemical and cellular assays, and inhibition of function has been demonstrated in cell proliferation assays. The primary metabolite exhibits similar potency compared to sunitinib in biochemical and cellular assays.

Sunitinib is an oral, small-molecule, multi-targeted receptor tyrosine kinase (RTK) inhibitor that was approved by the FDA on January 26, 2006.

Sorafenib

Sorafenib is a small molecular inhibitor of Raf kinase, PDGF (platelet-derived growth factor), VEGF receptor 2 & 3 kinases and c Kit the receptor for Stem cell factor. A growing number of drugs target most of these pathways. The originality of Sorafenib lays in its simultaneous targeting of the Raf/Mek/Erk pathway.

Sorafenib interacts with multiple intracellular (CRAF, BRAF and mutant BRAF) and cell surface kinases (KIT, FLT3, VEGFR-2, VEGFR-3, and PDGFRB). Several of these kinases are thought to be involved in angiogenesis, thus sorafenib reduces blood flow to the tumor. Sorafenib is unique in targeting the Raf/Mek/Erk pathway. By inhibiting these kinases, genetic transcription involving cell proliferation and angiogenesis is inhibited.

Sorafenib is indicated for the treatment of unresectable hepatocellular carcinoma and advanced renal cell carcinoma.

Pralsetinib

Pralsetinib is a RET receptor tyrosine kinase inhibitor for the treatment of metastatic RET-driven non-small cell lung cancer. Enhanced RET (Rearranged during transfection) oncogene expression is a hallmark of many cancers. Pralsetinib (BLU-667) and Selpercatinib (LOXO-292) represent the first generation of specific RET RTK inhibitors for the treatment of RET-driven cancers.

Pralsetinib exerts an anti-tumour effect through specific inhibition of the rearranged during transfection (RET) tyrosine kinase, including multiple distinct oncogenic RET fusions, mutated RET kinase domains harbouring gatekeeper mutations, and in RET kinases with a variety of activating single point mutations.

Selpercatinib

Selpercatinib is a kinase inhibitor with enhanced specificity for RET tyrosine kinase receptors (RTKs) over other RTK classes. Enhanced RET (Rearranged during transfection) oncogene expression is a hallmark of many cancers. Selpercatinib (LOXO-292) and pralsetinib (BLU-667) represent the first generation of specific RET RTK inhibitors for the treatment of RET-driven cancers

Birabresib

Birabresib (OTX015 or MK-8628) is a potent BET bromodomian inhibitor, which targets the BET bromodomain proteins 2, 3, and 4 (BRD2/3/4). BRDs 2, 3, and 4 are considered potential cancer targets because of their pivotal role in regulating the transcription of growth-promoting genes and cell cycle regulators. OTX015 is the first BRD2/3/4 inhibitor to enter clinical trials. Upon administration, birabresib binds to the acetylated lysine recognition motifs on the bromodomain of BET proteins, thereby preventing the interaction between the BET proteins and acetylated histone peptides. This disrupts chromatin remodeling and gene expression.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://clinicaltrials.gov/study/NCT06315491	Recruiting	A Randomized Phase 2 Study of CBX 12 in Subjects With Platinum Resistant or Refractory Ovarian Cancer	Ovarian Cancer	CBX-12	Multicare Institute For Research & Innovation, Tacoma, Washington 98405 Oncology Associate or Oregon, Eugene, Oregon 97401 Usc Norris Comprehensive Cancer Center, Los Angeles, California 90033
https://clinicaltrials.gov/study/NCT05377996	Recruiting	A Phase 1, First-in-human, Dose Escalation and Expansion, Multicenter Study of XMT-1660 in Participants With Solid Tumors	Ovarian Cancer	XMT-1660	Summit Cancer Centers, Spokane, Washington 99208 UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, California 94158 Huntsman Cancer Institute, Salt Lake City, Utah 84112

https://clinicaltrials.gov/study/NCT04590326	Recruiting	Phase 1/2 Study of REGN5668 (MUC16xCD28, a Costimulatory Bispecific) Administered in Combination With Cemiplimab or REGN4018 (MUC16xCD3)	Ovarian Cancer	REGN5668, Cemiplimab, REGN4018, Sarilumab	Seattle Cancer Care Alliance at South Lake Union - G3630, Seattle, Washington 98109 City of Hope Comprehensive Cancer Center, Duarte, California 91010-3012 The City of Hope Orange County Lennar Foundation Cancer Center, Irvine, California 92618
https://clinicaltrials.gov/study/NCT06242470	Recruiting	A Phase 1/1b First-in-Human, Open Label, Dose Escalation and Cohort Expansion Study of MGC026 in Participants With Advanced Solid Tumors	Ovarian Cancer	MGC026 Dose Escalation, MGC026 Dose for Expansion	Providence Cancer Institute, Portland, Oregon 97213 START Mountain Region, West Valley City, Utah 84119 The Angeles Clinic and Research Institute, Los Angeles, California 90025

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
BRCA1	NP_009225.1:p.Thr276AlafsTer14	NM_007294.3:c.815_824dup	G/GAMWX	ggc/ggAGCCATGTGGc	frameshift_variant	87.25	1224	0
KMT2A	NP_001184033.1:p.Arg378Lys	NM_001197104.1:c.1133G>A	R/K	aGg/aAg	missense_variant	83.29	1149	tolerated - low confidence
TP53	NP_000537.3:p.Met246Val	NM_000546.5:c.736A>G	M/V	Atg/Gtg	missense_variant	54.87	1427	deleterious
RET	NP_065681.1:p.Lys907Gln	NM_020630.4:c.2719A>C	K/Q	Aag/Cag	missense_variant	46.95	2837	deleterious
BLM	NP_000048.1:p.Leu1023Phe	NM_000057.2:c.3069G>T	L/F	ttG/ttT	missense_variant	46.87	1628	deleterious
LRP1B	NP_061027.2:p.Gln1997His	NM_018557.2:c.5991A>T	Q/H	caA/caT	missense_variant	33.99	815	deleterious
CUX1	NP_001189472.1:p.Ser1229Asn	NM_001202543.1:c.3686G>A	S/N	aGt/aAt	missense_variant	33.29	3770	deleterious
BCR	NP_004318.3:p.Arg1072His	NM_004327.3:c.3215G>A	R/H	cGc/cAc	missense_variant	21.12	2329	deleterious
ESR1	NP_001315029.1:p.Lys292Arg	NM_001328100.1:c.875A>G	K/R	aAg/aGg	missense_variant	18.2	1522	tolerated
EP300	NP_001420.2:p.Gly762Asp	NM_001429.3:c.2285G>A	G/D	gGc/gAc	missense_variant	15.36	1615	deleterious - low confidence
KMT2D	NP_003473.3:p.His751ValfsTer5	NM_003482.3:c.2250_2254del	HL/X	ccGCACCtg/cctg	frameshift_variant	1.03	4849	0

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA in 302 genes and cfRNA in >1600 genes for abnormalities that are reported to be altered in various types of solid tumors. For cases with detectable circulating

solid tumor DNA, tumor mutation burden (TMB) is reported. 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 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. TMB is calculated and cut-off points were determined based on comparison with tissue samples obtained from the same patient. Using cut-off of 6 mut/Mb, 17% of cases called as negative by cfDNA are false negative (FN). However, cases with ≤3 show only 6% FN. Intermediate cases (TMB between 6 and 9 mut/Mb) show 51% false positivity. Positive cases (TMB ≥9 Mut/Mb) show only 7% false positive. Cases without circulating solid tumor DNA are reported as "unable to evaluate" for TMB. 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, HLA class I genotyping, and Epstein-Barr virus (EBV), human papillomavirus (HPV) and torque teno virus (TTV) viral RNA are also analyzed and reported. B- and T-cell clonality will be reported, if clonal or clinically relevant. 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. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated.

Based on our validation study, the following exonic regions of the genes listed below are not covered appropriately <100 X coverage and sequencing by NGS may not be reliable in these regions. The poor coverage is primarily due to the inherent difficulty in obtaining adequate sequencing coverage in regions with high GC content. No well-characterized hotspots are present in these regions. RAD51 NM_133487 chr15:40994004-40994124, BRCA1 NM_007300 chr17:41231351-41231416, FUBP1 NM_003902 chr1:78435609-78435699, CBLB NM_170662 chr3:105420938-105421303, TERT NM_198253 chr5:1295183-1295250, ARID1B NM_017519 chr6:157098715-157100605, CUX1 NM_001202543 chr7:101740644-101740781, KMT2C NM_170606 chr7:151891314-151891346 and 151935792-151935911, GALNT12 NM_024642 chr9:101569952-101570351, ATM NM_000051 chr11:108164040-108164204, CDK17 NM_001170464 chr12:96679880-96679926, RB1 NM_000321 chr13:48954189-48954220, SETBP1 NM_015559 chr18:42643044-42643692, KMT2B NM_014727 chr19:36208921-36209283, AR NM_000044 chrX:66764889-66766604, STAG2 NM_001042749 chrX:123200025-123200112.

DEX Z-Code Z00WY is approved by MolDx for use in assessing DNA SNVs/Indels only.

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-solid-tumor/ (click the DNA tab)

For a complete list of tested RNA genes (Fusions/Expression), please go to:
https://genomictestingcooperative.com/genomic-tests/liquid-trace-solid-tumor/ (click the RNA tab)

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Tested genes

Genes Tested for Abnormalities in Coding Sequence															
ABL1	ATRX	BRAF	CDK12	CUX1	EPHA5	FGF4	GNAQ	IL7R	MAP2K1	MSH3	NPM1	PIM1	RAD21	SDHB	SRSF2	TRAF3
ABRAXAS1	AURKA	BRCA1	CDK4	CXCR4	ERBB2	FGF6	GNAS	INHBA	MAP2K2	MSH6	NRAS	PLCG1	RAD50	SDHC	STAG2	TSC1
ACVR1B	AURKB	BRCA2	CDK6	CYLD	ERBB3	FGFR1	GNB1	IRF4	MAP2K4	MTOR	NSD1	PMS1	RAD51	SDHD	STAT3	TSC2
AKT1	AURKC	BRIP1	CDKN1B	DAXX	ERBB4	FGFR2	GREM1	JAK1	MAP3K1	MUTYH	NSD2 (WHSC1)	PMS2	RAD51C	SETBP1	STAT5B	TSHR
AKT2	AXIN1	BTK	CDKN2A	DDR2	ERG	FGFR3	GRIN2A	JAK2	MAP3K14	MYC	NTHL1	POLD1	RAD51D	SETD2	STK11	U2AF1
AKT3	AXIN2	CALR	CDKN2B	DDX41	ESR1	FGFR4	H3-3A (H3F3A)	JAK3	MAPK1	MYCL	NTRK1	POLE	RAF1	SF3B1	SUFU	U2AF2
ALK	B2M	CARD11	CDKN2C	DICER1	ETNK1	FH	H3C2	KAT6A	MCL1	MYCN	NTRK2	POT1	RB1	SMAD2	SUZ12	UBA1
AMER1	BAP1	CBL	CEBPA	DNM2	ETV6	FLCN	HGF	KDM5C	MDM2	MYD88	NTRK3	PPM1D	RET	SMAD4	TAL1	VHL
ANKRD26	BARD1	CBLB	CHEK1	DNMT3A	EXO1	FLT3	HNF1A	KDM6A	MDM4	NBN	PAK3	PPP2R1A	RHEB	SMARCA4	TCF3	WT1
APC	BCL2	CBLC	CHEK2	DOT1L	EZH2	FLT4	HOXB13	KDR	MED12	NF1	PALB2	PRDM1	RHOA	SMARCB1	TENT5C (FAM46C)	XPO1
AR	BCL2L1	CCND1	CIC	EED	FANCA	FOXL2	HRAS	KEAP1	MEF2B	NF2	PAX5	PRKAR1A	RIT1	SMC1A	TERC	XRCC2
ARAF	BCL6	CCND3	CREBBP	EGFR	FANCC	FUBP1	HSP90AA1	KIT	MEN1	NFE2	PBRM1	PRKDC	RNF43	SMC3	TERT	XRCC3
ARID1A	BCOR	CCNE1	CRLF2	EGLN1	FANCD2	GALNT12	ID3	KMT2A	MET	NFE2L2	PDGFRA	PRPF8	ROS1	SMO	TET2	ZNF217
ARID1B	BCORL1	CD274	CSF1R	ELANE	FANCE	GATA1	IDH1	KMT2B	MITF	NFKBIA	PDGFRB	PRSS1	RUNX1	SOCS1	TGFBR2	ZRSR2
ARID2	BCR	CD79A	CSF3R	EP300	FANCF	GATA2	IDH2	KMT2C	MLH1	NKX2-1	PHF6	PTCH1	SAMD9	SOX2	TMEM127	-
ASXL1	BIRC3	CD79B	CTCF	EPAS1	FANCG	GATA3	IGF1R	KMT2D	MPL	NOTCH1	PIK3CA	PTEN	SAMD9L	SOX9	TNFAIP3	-
ATM	BLM	CDC73	CTNNA1	EPCAM	FAS	GEN1	IKZF1	KRAS	MRE11	NOTCH2	PIK3R1	PTPN11	SDHA	SPOP	TNFRSF14	-
ATR	BMPR1A	CDH1	CTNNB1	EPHA3	FBXW7	GNA11	IKZF3	LRP1B	MSH2	NOTCH3	PIK3R2	RAC1	SDHAF2	SRC	TP53	-

RNA Fusions/Expression

Fusion/Expression													
ABL1	BCL6	CD274 (PD-L1)	EGFR	EWSR1	FLI1	IKZF3	MAP3K1	NRG1	NUP98	PML	RET	SS18	THADA
AKT3	BRAF	CIC	ERG	FGFR1	FOXO1	JAK2	MECOM	NTRK1	PAX8	PPARG	RHOA	STAT6	TMPRSS2
ALK	CAMTA1	CREB1	ETS1	FGFR2	FUS	KIAA1549	MYB	NTRK2	PDGFRA	PRKACA	ROS1	TAL1	YAP1
AR	CBFB	CREBBP	ETV1	FGFR3	GLI1	KMT2A	MYC	NTRK3	PDGFRB	RAF1	RUNX1	TCF3	YWHAE
BCL2	CCND1	ERBB2	ETV6	FIP1L1	HMGA2	MAML2	NOTCH1	NUP214	PICALM	RARA	RUNX1T1	TFG	ZFTA

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Electronic Signature

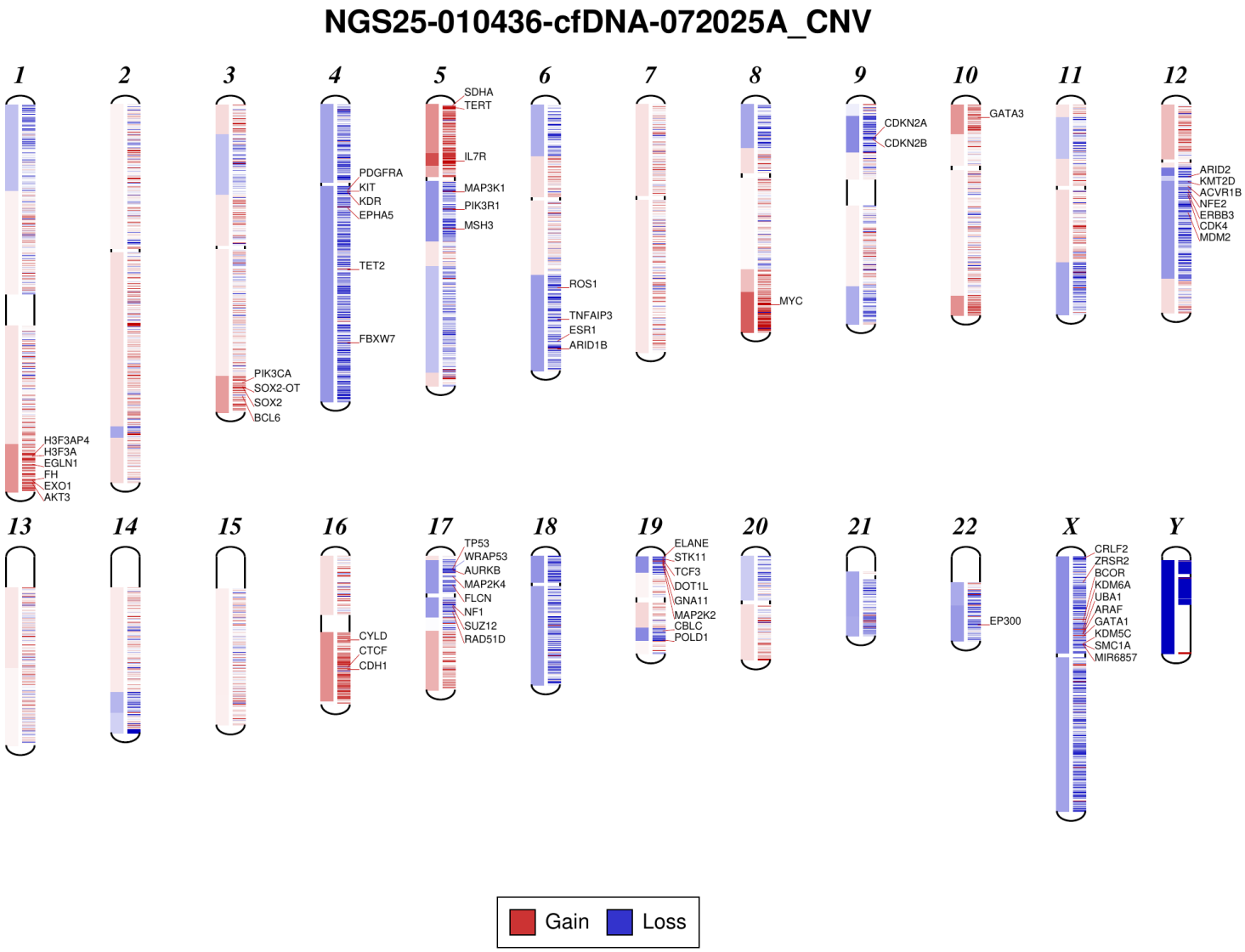
Ahmad Charifa, M.D.

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.

Additional Report Information

Chromosomal Abnormality Graph



Additional Report Information

Mutations Load (mol/mL)