25371 Commercentre Dr, Lake Forest, CA 92630
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CLIA # 05D2111917 CAP # 9441574

Liquid Trace Solid Tumor

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

MRN:				Indication for Testing:	C56.3 Malignant neoplasm of bilateral ovaries C79.31 Secondary malignant neoplasm of brain
Collected Date:		Time:	12:00 AM		
Received Date:		Time:	10:46 AM	Tumor Type:	ovarian
Reported Date:		Time:	03:51 PM	Stage:	metastasis

Detected Genomic Alterations

Level 1 (FDA-Approved)	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Evidence)	Other
BRCA1 (?Germline)	-Tumor Mutation Burden Low: 5 Mut/Mb -Chromosomal abnormalities suggest homologous recombination deficiency (HRD) positivity	TP53, NF1, CDK12, RB1, CSF3R	TSHR, ACVR1B, ABCB1 (?Germline, VUS), TPR	Autosomal chromosomes show numerous abnormalities including: +1, 2p-, 3q+,-4, 5p+, 5q-, 6p+, 6q-, 7p+, 8p-, 10q-, 11p-, 11q+, 12q-, 13q-, 15q-,16p-, 17p-, 17q+, 18q-, 19p- and others.

Results Summary

- **-Somatic mutations in TP53, NF1, TSHR, ACVR1B, CDK12, RB1, CSF3R, and TPR genes**
  -Possible germline mutations in BRCA1 and ABCB1 genes, heterozygous
  --Chromosomal abnormalities suggest homologous recombination deficiency (HRD) positivity
  -Tumor Mutation Burden Low: 5 Mut/Mb
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*01:01-A*24:02
  -HLA-B: B*18:01-B*13:02
  -HLA-C: C*07:01-C*06:02
  -Autosomal chromosomes show numerous abnormalities including: +1, 2p-, 3q+,-4, 5p+, 5q-, 6p+, 6q-, 7p+, 8p-, 10q-, 11p-, 11q+, 12q-, 13q-, 15q-,16p-, 17p-, 17q+, 18q-, 19p- and others.

-Increased ERBB2 and ESR1 mRNA
-Markedly high FOLR1 mRNA

-These findings suggest are consistent with circulating solid tumor DNA/RNA, likely of ovarian origin with markedly high tumor burden.

-Markedly high FOLR1 expression suggests response to anti-FOLR1 therap (ELAHERE) assuming it crossesblood-brain barrier.

-BRCA1 mutation and HRD suggest response to PARP inhibitors.

-NF1 mutation suggests possible response to MAP2K (MEK) inhibitor in combination with mTOR inhibitor.

-RB1 mutation suggests possible response to aurora A kinase inhibitors or BCL2 inhibitors as well as cisplatin-based therapy.

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

-The BRCA1 mutation is detected at high level raising the possibility of a germline abnormality. This mutation has been reported as a germline pathogenic abnormality associated with predisposition to cancer.

-The ABCB1 mutation is detected at high levels, raising the possibility of germline abnormality. This gene encodes for P-glycoprotein that is a membrane transporter and responsible for increasing efflux and multidrug resistance.

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

Tumor Heterogeneity

There is a dominant abnormal clone with TP53 and NF1 mutations. The TSHR and ACVR1B mutations are detected in subclones. There are abnormal low-level clones with CDK12, RB1, CSF3R, and TPR mutations. The BRCA1 and ABCB1 mutations are detected at high level, possible germline abnormalities.

Expression

Increased ERBB2 and ESR1 mRNA	Markedly high FOLR1 mRNA

Diagnostic Implications

BRCA1, TP53, NF1, TSHR, ACVR1B, CDK12, RB1, CSF3R, ABCB1, TPR	-These findings suggest the presence of circulating solid tumor DNA/RNA (see results summary). -The BRCA1 and ABCB1 mutation is likely a germline variant.

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

FDA-Approved Therapeutics in Other Tumor Types	
NF1	Selumetinib

Relevant Alteration Associated with Resistance
TP53 mutation is associated with resistance to therapy.

Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence)	
TP53	Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy
NF1	PI3K/AKT/MTOR, RAF/MEK inhibitors
CDK12	CDK4/6 inhibitors
RB1	Aurora A kinase inhibitors or BCL2 inhibitors as well as cisplatin-based therapy
CSF3R	CSF3R inhibitors

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]

- 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]

- NF1. This gene product appears to function as a negative regulator of the ras signal transduction pathway. Mutations in this gene have been linked to neurofibromatosis type 1, juvenile myelomonocytic leukemia and Watson syndrome. The mRNA for this gene is subject to RNA editing (CGA>UGA->Arg1306Term) resulting in premature translation termination. Alternatively spliced transcript variants encoding different isoforms have also been described for this gene. [provided by RefSeq, Jul 2008]

- TSHR. The protein encoded by this gene is a membrane protein and a major controller of thyroid cell metabolism. The encoded protein is a receptor for thyrothropin and thyrostimulin, and its activity is mediated by adenylate cyclase. Defects in this gene are a cause of several types of hyperthyroidism. Three transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Dec 2008]

- ACVR1B. This gene encodes an activin A type IB receptor. Activins are dimeric growth and differentiation factors which belong to the transforming growth factor-beta (TGF-beta) superfamily of structurally related signaling proteins. Activins signal through a heteromeric complex of receptor serine kinases which include at least two type I and two type II receptors. This protein is a type I receptor which is essential for signaling. Mutations in this gene are associated with pituitary tumors. Alternate splicing results in multiple transcript variants. [provided by RefSeq, Jun 2010]

- CDK12. Enables RNA polymerase II CTD heptapeptide repeat kinase activity and cyclin binding activity. Involved in phosphorylation of RNA polymerase II C-terminal domain; protein autophosphorylation; and regulation of MAP kinase activity. Located in nuclear speck. Part of cyclin K-CDK12 complex. [provided by Alliance of Genome Resources, Apr 2022]

- RB1. The protein encoded by this gene is a negative regulator of the cell cycle and was the first tumor suppressor gene found. The encoded protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure. The active, hypophosphorylated form of the protein binds transcription factor E2F1. Defects in this gene are a cause of childhood cancer retinoblastoma (RB), bladder cancer, and osteogenic sarcoma. [provided by RefSeq, Jul 2008]

- CSF3R. The protein encoded by this gene is the receptor for colony stimulating factor 3, a cytokine that controls the production, differentiation, and function of granulocytes. The encoded protein, which is a member of the family of cytokine receptors, may also function in some cell surface adhesion or recognition processes. Alternatively spliced transcript variants have been described. Mutations in this gene are a cause of Kostmann syndrome, also known as severe congenital neutropenia. [provided by RefSeq, Aug 2010]

- ABCB1. The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the MDR/TAP subfamily. Members of the MDR/TAP subfamily are involved in multidrug resistance. The protein encoded by this gene is an ATP-dependent drug efflux pump for xenobiotic compounds with broad substrate specificity. It is responsible for decreased drug accumulation in multidrug-resistant cells and often mediates the development of resistance to anticancer drugs. This protein also functions as a transporter in the blood-brain barrier. Mutations in this gene are associated with colchicine resistance and Inflammatory bowel disease 13. Alternative splicing and the use of alternative promoters results in multiple transcript variants. [provided by RefSeq, Feb 2017]

- TPR. This gene encodes a large coiled-coil protein that forms intranuclear filaments attached to the inner surface of nuclear pore complexes (NPCs). The protein directly interacts with several components of the NPC. It is required for the nuclear export of mRNAs and some proteins. Oncogenic fusions of the 5' end of this gene with several different kinase genes occur in some neoplasias. [provided by RefSeq, Jul 2008]

Drug Information

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

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).

Alpelisib

Alpelisib is an orally bioavailable phosphatidylinositol 3-kinase (PI3K) inhibitor with potential antineoplastic activity. Alpelisib specifically inhibits PIK3 in the PI3K/AKT kinase (or protein kinase B) signaling pathway, thereby inhibiting the activation of the PI3K signaling pathway. This may result in inhibition of tumor cell growth and survival in susceptible tumor cell populations. Activation of the PI3K signaling pathway is frequently associated with tumorigenesis. Dysregulated PI3K signaling may contribute to tumor resistance to a variety of antineoplastic agents.

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.

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.

Palbociclib

Palbociclib is an investigational selective, small-molecule inhibitor of CDK4 and CDK6. CDK4 and CDK6 along with their regulatory partner cyclin D1 play a key role in regulating the G1- to S-phase cell-cycle transition via regulation of phosphorylation of the retinoblastoma (Rb) protein. Inhibition of these proteins leads to reduced phosphorylation of Rb, inhibition of downstream signalling, and increased tumor growth arrest.

Palbociclib is indicated in combination with letrozole for the treatment of postmenopausal women with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer as initial endocrine-based therapy for their metastatic disease.

Abemaciclib

Abemaciclib is an antitumor agent and dual inhibitor of cyclin-dependent kinases 4 (CDK4) and 6 (CDK6) that are involved in the cell cycle and promotion of cancer cell growth in case of unregulated activity. On September 28, 2017, FDA granted approval of Abemaciclib treatment under the market name Verzenio for the treatment of HR-positive and HER2-negative advanced or metastatic breast cancer that has progressed after unsuccessful endocrine therapy. Unlike other CDK inhibitors such as Palbociclib and Ribociclib, Abemaciclib exhibits greater selectivity for CDK4 compared to CDK6.

Ribociclib

Ribociclib inhibits both CDK4 and CDK6. An orally available cyclin-dependent kinase (CDK) inhibitor targeting cyclin D1/CDK4 and cyclin D3/CDK6 cell cycle pathway, with potential antineoplastic activity. CDK4/6 inhibitor LEE011 specifically inhibits CDK4 and 6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation. Inhibition of Rb phosphorylation prevents CDK-mediated G1-S phase transition, thereby arresting the cell cycle in the G1 phase, suppressing DNA synthesis and inhibiting cancer cell growth. Overexpression of CDK4/6, as seen in certain types of cancer, causes cell cycle deregulation.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://clinicaltrials.gov/study/NCT05592626	Recruiting	A Phase 1/2, First-in-Human, Open-Label, Dose Escalation and Expansion Study of STAR0602, a Selective T Cell Receptor (TCR) Targeting, Bifunctional Antibody-fusion Molecule, in Subjects With Unresectable, Locally Advanced, or Metastatic Solid Tumors That Are Antigen-rich (START-001)	Urogenital Neoplasms	STAR0602	AdventHealth Celebration, Celebration, Florida 34747 National Institutes of Health, Bethesda, Maryland 20892 Memorial Sloan-Kettering Cancer Center, New York, New York 10065
https://clinicaltrials.gov/study/NCT03412877	Recruiting	A Phase II Study Using the Administration of Autologous T-Cells Genetically Engineered to Express T-Cell Receptors Reactive Against Neoantigens in Patients With Metastatic Cancer	Genitourinary Cancers	Cyclophosphamide, Fludarabine, Aldesleukin, Individual Patient TCR-Transduced PBL, Pembrolizumab (KEYTRUDA)	National Institutes of Health Clinical Center, Bethesda, Maryland 20892

https://clinicaltrials.gov/study/NCT04235777	Recruiting	A Phase I Study of Bintrafusp Alfa (M7824) and NHS-IL12 (M9241) Alone and in Combination With Stereotactic Body Radiation Therapy (SBRT) in Adults With Metastatic Non-Prostate Genitourinary Malignancies	Genitourinary Cancers	M7824, M9241, Stereotactic body radiation therapy (SBRT)	National Institutes of Health Clinical Center, Bethesda, Maryland 20892
https://clinicaltrials.gov/study/NCT04703920	Recruiting	A Phase 1 Dose-Escalation Trial of Talazoparib in Combination With Belinostat for Metastatic Breast Cancer, Metastatic Castration Resistant Prostate Cancer, and Metastatic Ovarian Cancer	Metastatic Ovarian Carcinoma	Talazoparib, Belinostat	University of Michigan Rogel Cancer Center, Ann Arbor, Michigan 48109

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_009231.2:p.Cys61Gly	NM_007300.3:c.181T>G	C/G	Tgt/Ggt	missense_variant	94.37	657	deleterious (0)
TP53	NP_000537.3:p.Phe134Leu	NM_000546.5:c.402T>G	F/L	ttT/ttG	missense_variant	86.87	1150	deleterious (0)
NF1	NP_001035957.1:p.Ser1355Ter	NM_001042492.2:c.4064C>G	S/*	tCa/tGa	stop_gained	83.58	737	0
TSHR	NP_000360.2:p.Val435CysfsTer46	NM_000369.2:c.1302dupT	-/X	-/T	frameshift_variant	49.3	3059	0
ACVR1B	NP_064733.3:p.Arg415Ser	NM_020328.3:c.1245G>T	R/S	agG/agT	missense_variant	45.69	1812	deleterious (0)
CDK12	NP_057591.2:p.Ser614Pro	NM_016507.2:c.1840T>C	S/P	Tct/Cct	missense_variant	3.6	1834	0
RB1	NP_000312.2:p.Asn295IlefsTer6	NM_000321.2:c.884delA	K/X	Aaa/aa	frameshift_variant	1.33	1131	0
CSF3R	NP_724781.1:p.Pro123Thr	NM_156039.3:c.367C>A	P/T	Cca/Aca	missense_variant	1.12	2044	deleterious (0)
ABCB1 (RNA)	NP_000918.2:p.Asn21Asp	NM_000927.4:c.61A>G	N/D	Aat/Gat	missense_variant	63.33	30	tolerated (0.56)
TPR (RNA)	NP_003283.2:p.Leu1166Ter	NM_003292.2:c.3497T>G	L/*	tTa/tGa	stop_gained	22.54	936	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. 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.

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)

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

Reference

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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.

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