GENOMIC® TESTING COOPERATIVE

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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:	CSF
Case #:		Specimen ID:	
Body Site:			

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

Detected Genomic Alterations				
Level 1 (FDA-Approved)	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Evidence)	Other
Tumor Mutation Burden High: 9 Mut/Mb	-	PTCH1 (2 mutations), SMO (6 mutations), CCNE1, NF1, ATR, DDX3X	BCOR, MYCN, TERT, SDHA, KAT6B	Autosomal chromosomal structural analysis shows 3q+

Results Summary

- **-Mutations in PTCH1 (2 mutations), BCOR, MYCN, TERT, SMO (6 mutations), SDHA, CCNE1, NF1, ATR, KAT6B, and DDX3X genes**
  -Tumor Mutation Burden High: 9 Mut/Mb
  -Autosomal chromosomal structural analysis shows 3q+
  -No evidence of IDH1/2, H3 K27M, PTEN and TP53 mutations
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -TTV viral RNA: Not detected
  -HLA Genotyping:
  -HLA-A: A*03:01-A*25:01
  -HLA-B: B*07:02-B*18:01
  -HLA-C: C*07:02-C*12:03

  -The findings suggest the presence of solid tumor DNA/RNA of possibly medulloblastoma, SHH activated and TP53 wildtype.

  -High TMB suggests response to immune checkpoint inhibitors.

  -PTCH1 mutations suggest possible response to hedgehog inhibitors (vismodegib and Erivedge)

  -CCNE1 mutation suggest possible response to CDK4/6 inhibitors.

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

inhibitor.

-ATR mutation suggest response to PARP inhibitors.

See additional report information at the end of the report.

Tumor Heterogeneity
There are dominant abnormal clones with PTCH1 (2 mutations), BCOR, MYCN, and TERT mutations. The SMO (6 mutations), SDHA, CCNE1, NF1, ATR, KAT6B, and DDX3X mutations are detected in subclones.

Diagnostic Implications	
PTCH1 (2 mutations), BCOR, MYCN, TERT, SMO (6 mutations), SDHA, CCNE1, NF1, ATR, KAT6B, DDX3X	The findings suggest the presence of solid tumor DNA/RNA of possibly medulloblastoma, SHH activated and TP53 wildtype (see results summary).

FDA-Approved Therapeutics	
TMB-High	Pembrolizumab

FDA-Approved Therapeutics in Other Tumor Types	
PTCH1	Sonidegib
NF1	Selumetinib
ATR	Talazoparib + Enzalutamide

Relevant Alteration Associated with Resistance
DDX3X may mediate resistance to EGFR tyrosine kinase inhibitors

Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence)	
PTCH1	Hedgehog inhibitors
SMO	Hedgehog inhibitors
CCNE1	CDK2 inhibitor
NF1	PI3K/AKT/MTOR, RAF/MEK inhibitors
ATR	PARP Inhibitors
DDX3X	DDX3X inhibitors
TMB-High	Immunotherapy with checkpoint inhibitor

Relevant Genes with NO Alteration		
No evidence of mutation in KRAS, NRAS, EGFR, BRAF, TP53, or BRCA 1/2	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

- PTCH1. This gene encodes a member of the patched family of proteins and a component of the hedgehog signaling pathway. Hedgehog signaling is important in embryonic development and tumorigenesis. The encoded protein is the receptor for the secreted hedgehog ligands, which include sonic hedgehog, indian hedgehog and desert hedgehog. Following binding by one of the hedgehog ligands, the encoded protein is trafficked away from the primary cilium, relieving inhibition of the G-protein-coupled receptor smoothened, which results in activation of downstream signaling. Mutations of this gene have been associated with basal cell nevus syndrome and holoprosencephaly. [provided by RefSeq, Aug 2017]

- BCOR. The protein encoded by this gene was identified as an interacting corepressor of BCL6, a POZ/zinc finger transcription repressor that is required for germinal center formation and may influence apoptosis. This protein selectively interacts with the POZ domain of BCL6, but not with eight other POZ proteins. Specific class I and II histone deacetylases (HDACs) have been shown to interact with this protein, which suggests a possible link between the two classes of HDACs. Several transcript variants encoding different isoforms have been found for this gene. A pseudogene of this gene is found on chromosome Y.[provided by RefSeq, Jun 2010]

- MYCN. This gene is a member of the MYC family and encodes a protein with a basic helix-loop-helix (bHLH) domain. This protein is located in the nucleus and must dimerize with another bHLH protein in order to bind DNA. Amplification of this gene is associated with a variety of tumors, most notably neuroblastomas. Multiple alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jun 2014]

- TERT. Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG. The enzyme consists of a protein component with reverse transcriptase activity, encoded by this gene, and an RNA component which serves as a template for the telomere repeat. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis. Studies in mouse suggest that telomerase also participates in chromosomal repair, since de novo synthesis of telomere repeats may occur at double-stranded breaks. Alternatively spliced variants encoding different isoforms of telomerase reverse transcriptase have been identified; the full-length sequence of some variants has not been determined. Alternative splicing at this locus is thought to be one mechanism of regulation of telomerase activity. [provided by RefSeq, Jul 2008] In addition, recurring somatic mutations at multiple spots in the proximal promoter (particularly at 124bp and 146bp upstream of the translation start site) are found in tumors of many tissue origins. These mutations are thought to affect binding of Ets family proteins and nuclear factor kappa B and alter secondary structure and long-range interactions, leading to increased promoter activity. [provided by RefSeq, May 2023]

- SMO. The protein encoded by this gene is a G protein-coupled receptor that interacts with the patched protein, a receptor for hedgehog proteins. The encoded protein tranduces signals to other proteins after activation by a hedgehog protein/patched protein complex. [provided by RefSeq, Jul 2010]

- SDHA. This gene encodes a major catalytic subunit of succinate-ubiquinone oxidoreductase, a complex of the mitochondrial respiratory chain. The complex is composed of four nuclear-encoded subunits and is localized in the mitochondrial inner membrane. Mutations in this gene have been associated with a form of mitochondrial respiratory chain deficiency known as Leigh Syndrome. A pseudogene has been identified on chromosome 3q29. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jun 2014]

- CCNE1. The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. This cyclin forms a complex with and functions as a regulatory subunit of CDK2, whose activity is required for cell cycle G1/S transition. This protein accumulates at the G1-S

phase boundary and is degraded as cells progress through S phase. Overexpression of this gene has been observed in many tumors, which results in chromosome instability, and thus may contribute to tumorigenesis. This protein was found to associate with, and be involved in, the phosphorylation of NPAT protein (nuclear protein mapped to the ATM locus), which participates in cell-cycle regulated histone gene expression and plays a critical role in promoting cell-cycle progression in the absence of pRB. [provided by RefSeq, Apr 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]

- ATR. The protein encoded by this gene is a serine/threonine kinase and DNA damage sensor, activating cell cycle checkpoint signaling upon DNA stress. The encoded protein can phosphorylate and activate several proteins involved in the inhibition of DNA replication and mitosis, and can promote DNA repair, recombination, and apoptosis. This protein is also important for fragile site stability and centrosome duplication. Defects in this gene are a cause of Seckel syndrome 1. [provided by RefSeq, Aug 2017]

- KAT6B. The protein encoded by this gene is a histone acetyltransferase and component of the MOZ/MORF protein complex. In addition to its acetyltransferase activity, the encoded protein has transcriptional activation activity in its N-terminal end and transcriptional repression activity in its C-terminal end. This protein is necessary for RUNX2-dependent transcriptional activation and could be involved in brain development. Mutations have been found in patients with genitopatellar syndrome. A translocation of this gene and the CREBBP gene results in acute myeloid leukemias. Three transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Mar 2012]

- DDX3X. The protein encoded by this gene is a member of the large DEAD-box protein family, that is defined by the presence of the conserved Asp-Glu-Ala-Asp (DEAD) motif, and has ATP-dependent RNA helicase activity. This protein has been reported to display a high level of RNA-independent ATPase activity, and unlike most DEAD-box helicases, the ATPase activity is thought to be stimulated by both RNA and DNA. This protein has multiple conserved domains and is thought to play roles in both the nucleus and cytoplasm. Nuclear roles include transcriptional regulation, mRNP assembly, pre-mRNA splicing, and mRNA export. In the cytoplasm, this protein is thought to be involved in translation, cellular signaling, and viral replication. Misregulation of this gene has been implicated in tumorigenesis. This gene has a paralog located in the nonrecombining region of the Y chromosome. Pseudogenes sharing similarity to both this gene and the DDX3Y paralog are found on chromosome 4 and the X chromosome. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Oct 2014]

Drug Information

Pembrolizumab (Keytruda)

Pembrolizumab. A humanized monoclonal immunoglobulin (Ig) G4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1) with potential immune checkpoint inhibitory and antineoplastic activities. Upon administration, pembrolizumab binds to PD-1, an inhibitory signaling receptor expressed on the surface of activated T cells, and blocks the binding to and activation of PD-1 by its ligands, which results in the activation of T-cell-mediated immune responses against tumor cells. The ligands for PD-1 include programmed cell death ligand 1 (PD-L1), overexpressed on certain cancer cells, and programmed cell death ligand 2 (PD-L2), which is primarily expressed on APCs. Activated PD-1 negatively regulates T-cell activation and plays a key role in in tumor evasion from host immunity.y onto a human IgG4-kappa isotype with the containing a stabilizing S228P Fc mutation.

Vismodegib

Vismodegib selectively binds to and inhibits the transmembrane protein Smoothened homologue (SMO) to inhibit the Hedgehog signalling pathway.

Mutations of the Hedgehog pathway may results in uncontrolled proliferation of skin basal cells. Vismodegib binds to and inhibits the transmembrane protein Smoothened homologue (SMO) to inhibit the Hedgehog signalling pathway.

Vismodegib inhibits the hedgehog signalling pathway and is indicated for treatment of adult basal cell carcinoma. FDA approved on Jan 30, 2012.

Sonidegib

Sonidegib has been shown to inhibit a transmembrane protein called SMO which plays a role in Hh signal transduction. This has resulted in inhibition of Hh signaling as well as antitumour activity in various animal models.

The hedgehog pathway is involved in many human cancers. Sonidegib effectively inhibits the regulator called smoothened (Smo), preventing the hedgehog pathway from functioning. As a result, tumours that depend on the hedgehog pathway are unable to grow.

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.

Talazoparib

Talazoparib is a poly(ADP-ribose) Polymerase 1, 2 (PARP 1;2 inhibitor). Talazoparib was approved by the FDA for use in germline BRCA mutated, HER2 negative, locally advanced or metastatic breast cancer on October 16, 2018 under the trade name Talzenna. Talazoparib prevents PARP-mediated repair of DNA damage in cancer cells, allowing accumulation of damage and PARP-DNA complexes. Repair related errors by error prone secondary repair pathways may also contribute to the cytotoxicity of Talazoparib. Talazoparib is indicated for the treatment of deleterious or suspected deleterious germline BRCA mutated, HER2 negative locally advanced or metastatic breast cancer in adults

Olaparib

Olaparib (LYNPARZA) is an antineoplastic agent, Poly(ADP-ribose) Polymerase1;2;3 inhibitor. (PARP 1;2;3 inhibitor).

Lynparza is a poly (ADP-ribose) polymerase (PARP) inhibitor indicated for the treatment of adult patients with deleterious or suspected deleterious germline BRCA-mutated(gBRCAm) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy. Select patients for therapy based on an FDA-approved companion diagnostic for Lynparza. (1.1, 2.2)

Niraparib

Niraparib is an inhibitor of poly (ADP-ribose) polymerase (PARP) with potential antineoplastic activity. PARP Inhibitor MK4827 inhibits PARP activity, enhancing the accumulation of DNA strand breaks and promoting genomic instability and apoptosis. The PARP family of proteins detect and repair single strand DNA breaks by the base-excision repair (BER) pathway. The specific PARP family member target for PARP inhibitor MK4827 is unknown. (NCI Thesaurus)

ZEJULA is a poly(ADP-ribose) polymerase (PARP) inhibitor indicated for the maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy.

Rucaparib

Rucaparib is a potent mammalian poly(ADP-ribose) polymerase 1, 2 and 3 inhibitor with anticancer properties (PARP 1;2;3 inhibitor).

PPAR is an enzyme that plays an essential role in DNA repair by activating response pathways and facilitating repair, and defects in these repair mechanisms have been demonstrated in various malignancies, including cancer. Regulation of repair pathways is critical in promoting necessary cell death. BRCA genes are tumor suppressor genes mediate several cellular processes including DNA replication, transcription regulation, cell cycle checkpoints, apoptosis, chromatin structuring and homologous recombination (HR). Homologous recombination deficiency (HRD), along with PPAR inhibition, is a vulnerability that enhances the cell death pathway when the single mutations alone would permit viability. Ovarian cancer commonly possesses defects in DNA repair pathways such as HRD due to BRCA mutations or otherwise. Rucaparib has shown to induce cytotoxicity in tumor cell lines with deficiencies in BRCA1/2 and other DNA repair genes. Of all the BRCA1/2 mutations in ovarian cancer, most are due to germline mutations (18%), and approximately 7% represent somatic mutations acquired within the tumor.

Rucaparib is an inhibitor of PARP-1, PARP-2, and PARP-3. Via an inhibitory effect on the PARP enzymatic activity, rucaparib decreases the formation of PARP-DNA complexes resulting in DNA damage, apoptosis, and cell death. It is proposed that PARP inhibition specifically targets tumor cells with preexisting HRD, such as those cells possessing mutations in the BRCA1 or BRCA2 genes.

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://clinicaltrials.gov/study/NCT03904862	Recruiting	PBTC-053: A Pediatric Brain Tumor Consortium Phase I/ II and Surgical Study of CX-4945 in Patients With Recurrent SHH Medulloblastoma	Medulloblastoma	CX 4945	Memorial Sloan-Kettering Cancer Center, New York, New York 10065 Children's National Medical Center, Washington, District of Columbia 20010 Children's Hospital of Pittsburgh of UPMC, Pittsburgh, Pennsylvania 15201
https://clinicaltrials.gov/study/NCT03911388	Recruiting	Phase 1 Trial of Engineered HSV G207 in Children With Recurrent or Refractory Cerebellar Brain Tumors	Medulloblastoma	G207	Children's of Alabama, Birmingham, Alabama 35233 St. Louis Children's Hospital, Saint Louis, Missouri 63110 MD Anderson Cancer Center, Houston, Texas 77030
https://clinicaltrials.gov/study/NCT06466798	Recruiting	Fourth Ventricular Administration of Immune Checkpoint Inhibitor (Nivolumab) and Methotrexate or 5-Azacytidine for Recurrent Medulloblastoma, Ependymoma, and Other CNS Malignancies	Medulloblastoma	Nivolumab, Methotrexate, 5-Azacytidine	The University of Texas Health Science Center at Houston, Houston, Texas 77030

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
PTCH1	0	NM_000264.3:c.1068-1G>A	0	0	splice_acceptor_variant	49.35	1775	0
BCOR	NP_001116857.1:p.Ser185AlafsTer25	NM_001123385.1:c.553_560delAGTTATCT	SYL/X	AGTTATCTg/g	frameshift_variant	47.95	4144	0
PTCH1	NP_000255.2:p.Val1100ThrfsTer7	NM_000264.3:c.3296_3297dupAC	-/X	-/AC	frameshift_variant	42.15	1414	0
MYCN	NP_005369.2:p.Ser375_His386delSerAspSerGluAspSerGluArgArgArgAsnHisinsLeuSerLeuSerProArg	NM_005378.4:c.1124_1161delCTGACTCGGAGGACAGTGAGCGTCGCAGAAACCACinsTGAGCTTGAGCCCCCGA	SDSEDSERRRNH/LSLSPR	tCTGACTCGGAGGACAGTGAGCGTCGCAGAAACCAC/tTGAGCTTGAGCCCCGA	inframe_deletion	40.9	2914	0
TERT	0	NM_198253.2:c.-124C>T	0	0	upstream_gene_variant	33.31	2681	0

SMO	NP_005622.1:p.Asp473Asn	NM_005631.4:c.1417G>A	D/N	Gac/Aac	missense_variant	13.51	3959	deleterious (0.01)
SMO	NP_005622.1:p.Thr241Met	NM_005631.4:c.722C>T	T/M	aCg/aTg	missense_variant	7.09	3764	deleterious (0.04)
SMO	NP_005622.1:p.Val321Met	NM_005631.4:c.961G>A	V/M	Gtg/Atg	missense_variant	5.45	4315	deleterious (0)
SMO	NP_005622.1:p.Thr245Met	NM_005631.4:c.734C>T	T/M	aCg/aTg	missense_variant	2.48	3503	deleterious (0)
SDHA	NP_004159.2:p.Ala454Thr	NM_004168.2:c.1360G>A	A/T	Gca/Aca	missense_variant	2.4	3452	deleterious - low confidence (0.02)
CCNE1	NP_001229.1:p.Asn65_Ala66delinsSerSerProSer	NM_001238.2:c.192_199delAATGinsTCTTCCCCAT	NA/SSPS	AATGca/TCTTCCCCATca	inframe_deletion	2.06	1409	0
SMO	NP_005622.1:p.Gly416Asp	NM_005631.4:c.1247G>A	G/D	gGc/gAc	missense_variant	1.4	4364	deleterious (0.01)
SMO	NP_005622.1:p.Ser387Asn	NM_005631.4:c.1160G>A	S/N	aGt/aAt	missense_variant	0.91	5680	deleterious (0.02)
NF1	NP_001035957.1:p.Gly1406Arg	NM_001042492.2:c.4216G>C	G/R	Gga/Cga	missense_variant	0.5	1796	deleterious (0)
ATR	NP_001175.2:p.Arg1503Leu	NM_001184.3:c.4508G>T	R/L	cGa/cTa	missense_variant	0.25	2764	deleterious (0.01)
KAT6B (RNA)	NP_036462.2:p.Arg622Ter	NM_012330.3:c.1864C>T	R/*	Cga/Tga	stop_gained	15.38	13	0
DDX3X (RNA)	NP_001347.3:p.Ser382Ile	NM_001356.3:c.1145G>T	S/I	aGt/aTt	missense_variant	57.96	1665	deleterious (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.

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)

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

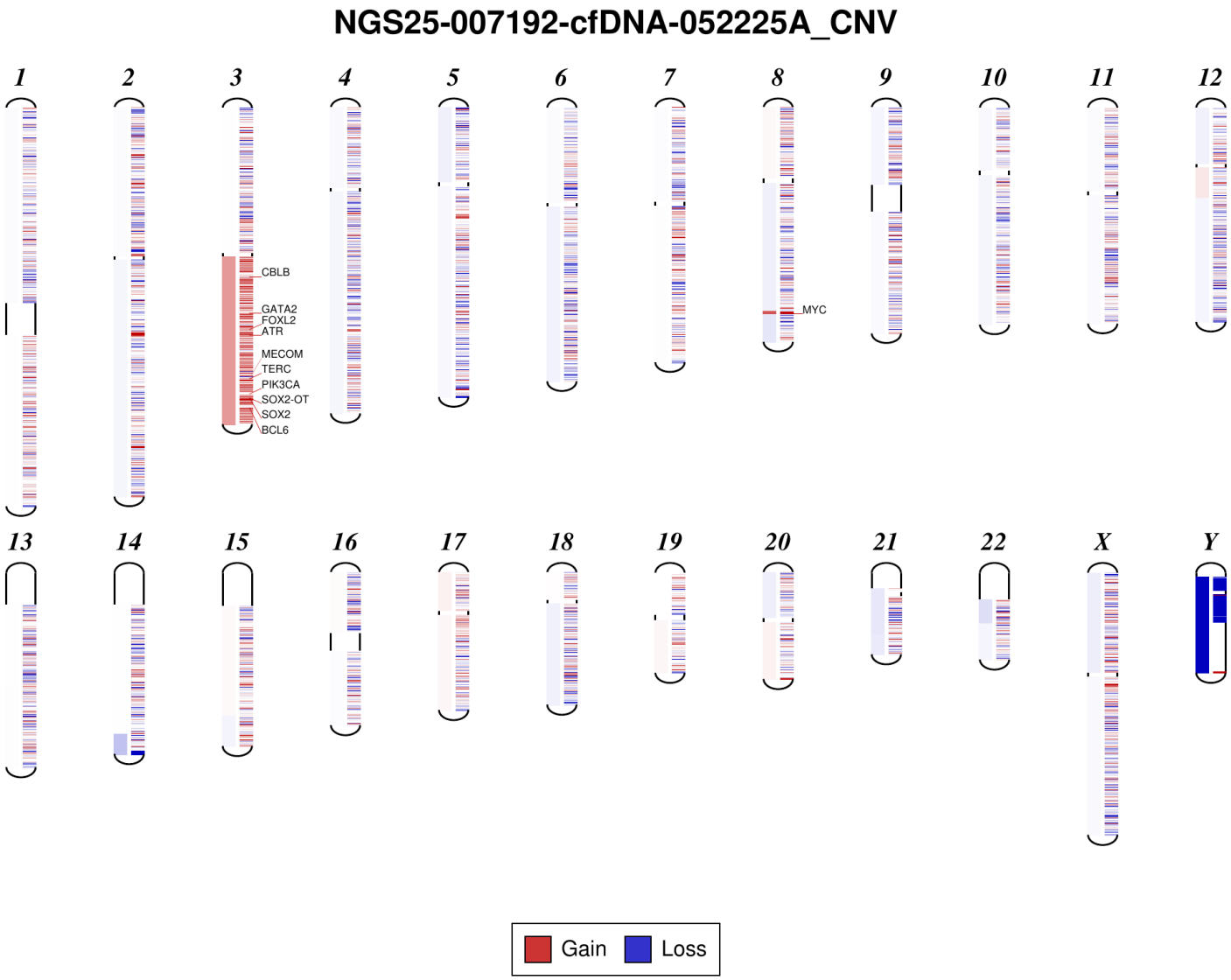
Ahmad Charifa, M.D.

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)