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CLIA #: 05D2111917 CAP #: 9441547

Liquid Biopsy, Solid Tumor

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

MRN:	[redacted]			Indication for Testing:	C34.81 Malignant neoplasm of overlapping sites of right bronchus and lung
Collected Date:	[redacted]	Time:	[redacted]		
Received Date:	[redacted]	Time:	[redacted]		
Reported Date:	[redacted]	Time:	[redacted]		

Test Description:

This is a comprehensive molecular profile which uses next generation sequencing (NGS) testing performed on cell-free DNA (cfDNA) to identify molecular abnormalities in 275 genes implicated in solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Detected Genomic Alterations				
SMC1A	TP53	LRP1B	NFE2L2	BRIP1
SMARCA4	FGFR1	WT1	KIT (exon1)	Chromosomal structural analysis shows 1p-, 2p-, 2q+, 3p-, 4p+, -6, 7p- (distal), 7P +(proximal), 8q +(MYC amplification), 10q-, 13q-, 15q-, 16q-, 17p-, 17q+ and others.

Heterogeneity
There are abnormal clones with SMC1A, TP53, LRP1B, NFE2L2, BRIP1, SMARCA4, FGFR1, WT1, and KIT mutations.

Diagnostic Implications	
SMC1A, TP53, LRP1B, NFE2L2, BRIP1, SMARCA4, FGFR1, WT1, KIT	These abnormalities are consistent with circulating tumor DNA with markedly high tumor load.

Therapeutic Implications	
TP53	Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy
NFE2L2	Bardoxolone Methyl (NF-?B inhibitor)
BRIP1	PARP inhibitors
SMARCA4	HDAC inhibitors
FGFR1	FGFR1 inhibitors (Pazopanib, Erdafitinib, Vandetanib, Nintedanib)
WT1	Sensitive to hypomethylating agents (Azacitidine)
KIT	The detected KIT mutation is outside the kinase domain and the effects of conventional KIT inhibitors on this particular mutation is unknown.

Prognostic Implications	
SMC1A	Poor
TP53	Poor
LRP1B	Poor
NFE2L2	Poor
BRIP1	Poor
SMARCA4	Poor
FGFR1	Poor
WT1	Poor
KIT	Poor

Relevant Genes with NO Alteration
No evidence of mutation in: KRAS, NRAS, EGFR, BRAF

Results Summary

- **-Mutations in SMC1A, TP53, LRP1B, NFE2L2, BRIP1, SMARCA4, FGFR1, WT1, and KIT(exon1) genes -Chromosomal structural analysis shows 1p-, 2p-, 2q+, 3p-, 4p+, -6, 7p-(distal), 7P+(proximal), 8q +(MYC amplification), 10q-, 13q-, 15q-, 16q-, 17p-, 17q+ and others.**

  -These findings are consistent with circulating tumor DNA with markedly high tumor load.

  -NFE2L2 mutation suggests response to Dimethyl Fumarate.

  -FGFR1 mutation suggests response to FGFR1 inhibitor (Pemigatinib).

  -BRIP1 mutation suggests response to PARP inhibitors.

  -SMARCA4 mutation suggests possible response to CDK/4/6, HDAC and PARP inhibitors.

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

-MYC abnormality suggests response to histone deacetylases, histone methyltransferases, histone demethylases, DNA methyltransferases, and bromodomain and extra-terminal motif (BET) bromodomains.

-The detected KIT mutation is outside the kinase domain and the effects of conventional KIT inhibitors on this particular mutation is unknown.

Biological relevance of detected Alterations

- SMC1A, an ATPase that functions as a subunit of the cohesin complex, is recurrently mutated in Cornelia de Lange syndromes, hematologic malignancies, and solid tumors.
  BACKGROUND
  SMC1A (also SMC1L1) is an ATPase that is a member of the SMC family of proteins. SMC1A functions as a subunit of the cohesin complex that aligns and stabilizes sister chromatids during metaphase (PMID: 24854081). Cohesion between sister chromatids is initiated during DNA replication and must be maintained throughout mitosis or meiosis to ensure proper chromosome-spindle attachments (PMID: 26903600). The cohesin ring that encircles sister chromatids is comprised of two large structural proteins, SMC1A and SMC3, and this ring opens and closes through the binding of alpha-kleisin subunits to the RAD21 and STAG adapter proteins (PMID: 24854081, 22885700). The cohesin complex also functions to maintain chromatin looping structures or 3D arrangements of DNA that allow for regulatory control of gene expression (PMID: 19468298). SMC1A localizes to chromatin sites bound by the insulator protein CTCF, which inhibits tissue-specific enhancer-promoter interactions (PMID: 19468298, 23704192). Germline mutations in SMC1A have been identified in patients with cohesinopathies, including Cornelia de Lange syndrome, leading to a spectrum of developmental defects (PMID: 17221863, 17273969, 18996922). Somatic mutations and deletions in SMC1A have been identified in acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), bladder cancers, and additional hematopoietic malignancies, among others (PMID: 23955599, 24121791, 24335498, 25080505, 24056718, 18299561, 20514443). Mutations in SMC1A are predicted to be loss-of-function and impact the association of SMC proteins with chromatin (PMID: 18996922). Alterations in SMC1A are also predicted to be initiating events in acute myeloid leukemia (PMID: 22932223).

- TP53 mutations are universal across cancer types. The loss of a tumor suppressor is most often through large deleterious events, such as frameshift mutations, or premature stop codons. In TP53 however, many of the observed mutations in cancer are found to be single nucleotide missense variants. These variants are broadly distributed throughout the gene, but with the majority localizing in the DNA binding domain. There is no single hotspot in the DNA binding domain, but a majority of mutations occur in amino acid positions 175, 245, 248, 273, and 282 (NM_000546) (Olivier et al., 2010). To fulfill its proper biological function four TP53 polypeptides must form a tetramer which functions as a transcription factor, therefore even if one out of four polypeptides has inactivating mutation it may lead to dominant negative phenotype of variable degree. While a large proportion of cancer genomics research is focused on somatic variants, TP53 is also of note in the germline. Germline TP53 mutations are the hallmark of Li-Fraumeni syndrome, and many (both germline and somatic) variants have been found to have a prognostic impact on patient outcomes. The significance of many polymorphisms for susceptibility and prognosis of disease is still very much up for debate.SUMMARY
  TP53, a tumor suppressor in the DNA damage pathway, is the most frequently mutated gene in cancer.
  BACKGROUND
  TP53 encodes the p53 tumor suppressor protein, a transcription factor that responds to cellular stresses, including DNA damage and oncogenic activation, by inducing downstream anti-tumor responses such as DNA repair and apoptosis (PMID: 11099028). TP53 is the most commonly mutated gene in human cancers, and germline mutations occur in the cancer predisposition syndrome Li-Fraumeni (PMID: 22713868, 21765642). The p53 protein consists of an N-terminal transactivation domain, a central DNA-binding domain, an oligomerization domain and a C-terminal regulatory domain (PMID: 22713868).

- LRP1B is a putative tumor suppressor and a member of the low-density lipoprotein (LDL) receptor family. The LDL receptor family have roles related to clearance of extracellular ligand and are proposed to be involved in extracellular signal transduction. silencing and down-expression of LRP1B as been observed in renal cell carcinoma and thyroid cancer. Further Deletion of LRP1B has been associated with chemotherapy resistance in high-grade serous cancers.

- NFE2L2 encodes a transcription factor that regulates critical antioxidant and stress-responsive genes. Aberrant activation of NFE2L2 results in increased proliferation, apoptosis resistance, and resistance to drugs and radiotherapy.
  BACKGROUND
  NFE2L2 (Nuclear factor-erythroid 2-related factor 2), also known as NRF2, is a transcription factor that is important in activating antioxidant proteins to protect against certain environmental and oxidative stresses (PMID: 16968214). Under normal cellular conditions, the interaction of NRF2 with KEAP1 retains the protein in the cytoplasm and promotes its proteasomal degradation via ubiquitination (PMID: 9887101). Upon sensing stress signals, KEAP1 undergoes a conformational change, preventing it from interacting with NRF2 and allowing NRF2 to translocate to the nucleus and drive the expression of specific genes (PMID:12359864). NRF2 can have a protective role in cancer formation from certain chemical carcinogens (PMID: 11248092). However, chronic activation of NRF2 can also support the development of chemo- and radio-

resistance (PMID: 24142871). Tumor-associated NRF2 activation can result from inactivation of KEAP1 through mutation, loss of heterozygosity or epigenetic silencing (PMID: 19321346). NRF2 activation can also arise directly from mutations in NFE2L2 in the KEAP1-binding domains (PMID: 18757741, 19967722).

- BRIP1 (BACH1 and FANCJ) is a member of the RecQ DEAH helicase family. DEAH helicases participate in pre-messenger RNA splicing and ribosome biogenesis (PMID: 20168331). This family of genes includes those that have been implicated in heritable human diseases, including BLM, WRN and RECQL4 (PMID: 24606147). Specifically, BRIP1 interacts with the BRCT motif-containing domain of BRCA1 (PMID: 17033622). In the HCC1937 cell line that produces BRCA1 with a truncated C-terminal, BRIP1 failed to co-immunoprecipitate with BRCA1, suggesting this domain is important for interaction with BRIP1 (PMID: 11301010). In the same study, two intact BRCT repeat units on BRCA1 were shown to be necessary for the BRCA1 and BRIP1 interaction; a mutation at K52R on BRIP1 may control the interaction of the two proteins. BRIP1 has been shown to be mutated in hereditary breast (PMID: 11301010, 17033622), ovarian (PMID: 21964575) and prostate (PMID: 19127258) cancers as well as the Fanconi anemia (FA), a disorder caused by genetic defects in a number of proteins involved in DNA repair that is characterized by bone marrow failure, sensitivity to DNA cross-linking agents and the development of cancer, often acute myelogenous leukemia (AML) (PMID: 24348213, 16493006). Cell lines that are deficient in BRIP1 are sensitive to mitomycin C, a crosslinking agent (PMID: 16153896).
  SUMMARY
  BRIP1 is a tumor suppressor involved in DNA repair. Germline mutations of BRIP1 are associated with Fanconi anemia and predispose to certain cancers.

- SMARCA4, a tumor suppressor involved in chromatin remodeling, is recurrently altered in small cell carcinoma of the ovaries, hypercalcemic type (SCCOHT).
  BACKGROUND
  SMARCA4 is an ATP-dependent helicase that is a catalytic subunit of the SWI/SNF chromatin remodeling complex (PMID: 21654818). This complex plays a role in altering chromatin structure, a process that is necessary for various cellular functions, including transcription, DNA synthesis and DNA repair (reviewed in PMID: 25387058). Secondary to ARID1A, SMARCA4 is the most frequently mutated gene among the SWI/SNF subunits and is significantly altered in malignant rhabdoid tumors, lymphoma, medulloblastoma, lung and ovarian cancer (PMID: 23644491, 25060813, 23143597). Mutations in the SMARCA4 gene result in loss of function, suggesting its tumor suppressor properties. Germline SMARCA4 mutations predispose to pediatric atypical teratoid/rhabdoid tumors (AT/RT) (PMID: 20137775, 25060813) and small cell carcinoma of the ovaries, hypercalcemic type (SCCOHT) (PMID: 24658002, 24658004). Almost all SCCOHT cases have mutations in the SMARCA4 gene. In the majority of cases this is the only mutation present, and thus thought to be a driver mutation for this disease (PMID: 24658002, 24658004, 24658001).

- FGFR1 is a member of the Fibroblast Growth Factor family, comprising of 4 receptors and 18 Ligands.
  FGFR1 signalling downstream functions mainly via PI3K and MAPK pathways (Turner et. al.).
  Several ways of involvement of FGFR1 in cancer have been proposed: auto- and paracrine activation, amplification and overexpression (Marshall et. al, Weiss et. al., Cheng et. al.).
  Especially amplification of FGFR1 in lung cancer is an emerging treatment target with clinical studies currently ongoing (e.g. NCT01004224). However, FGFR1 amplification does not always correlate with protein expression and predictive biomarkers still remain to be defined in clinic (von Mässenhausen et. al.).
  Mutation of FGFR1 seems to be less common, but has been described in glioblastoma, pilocytic astrocytomas and Ewing's sarcoma (Rand et. al., Jones et. al., Agelopoulos et. al.).SUMMARY
  FGFR1, a receptor tyrosine kinase, is altered by mutation, chromosomal rearrangement or amplification in various cancer types including lung and breast cancers.
  BACKGROUND
  FGFR1 is a receptor tyrosine kinase that is a member of the fibroblast growth factor receptor (FGFR) family. Binding of FGF ligands to FGFR1 results in the rapid dimerization and activation of downstream signaling pathways including the PI3K/AKT and MAPK pathways (PMID: 16597617). FGFR1 is widely expressed and is necessary for a variety of cellular functions such as embryonic development, skeletogenesis, mitogenesis and differentiation. Cell-type specific FGFR1 regulation is dependent on tissue distribution and ligand availability (PMID: 16597617). Germline mutations in FGFR1 are associated with congenic disorders that present with physical malformations, mental retardation and neurologic deficits (PMID: 23812909). Amplifying or activating mutations in FGFR1 occur in varying frequency in multiple cancers including those of the lung, breast, prostate, head and neck and esophagus (PMID: 21160078, 20179196, 14614009, 16807070, 12147242). In metastatic renal cell carcinoma, FGF signaling mediates acquired treatment resistance from VEGF-directed therapies (PMID: 24387233). Currently, a number of small molecule inhibitors of the FGFR proteins are in use, with the major difference among them being their specificity to FGFR versus other receptor tyrosine kinases (RTKs) (PMID: PMID: 23696246, 24265351).

- WT1 is a tumor suppressor gene associated with the development of Wilms' Tumor, from which it was named. Mutations in exon 7 and 9 of WT1 have been recurrently identified in acute myeloid leukemia and associated with poorer prognosis and chemotherapy resistance.SUMMARY
  WT1, a transcription factor, is overexpressed in various cancer types including leukemias.
  BACKGROUND
  WT1 (Wilms tumor 1 gene) is a transcription factor expressed in a tissue-specific manner throughout development (PMID: 20013787, 17524167, 17361230, 12835718). WT1 has been implicated in the protein stabilization of TP53 and regulates the expression of several target genes include MYC and BCL2, which are important for cellular growth and metabolism (PMID: 7585606, 8389468). In hematopoietic cells, WT1 interacts with the epigenetic proteins TET2 and TET3 that regulate hydroxymethylation of DNA, an epigenetic modification of DNA that may also serve as a methylation state intermediate (PMID: 25482556). Loss of WT1 expression results in depletion of global 5-hydroxymethylation

levels (PMID: 25482556), implicating WT1 in the regulation of DNA methylation. WT1 was initially discovered as a tumor suppressor in Wilms tumor (PMID: 2163761, 9090524); however, WT1 loss only contributes to the pathogenesis of a fraction of Wilms' tumors (PMID: 16110318). Somatic WT1 mutations have been identified in patients with acute myeloid leukemia (AML) and are predicted to be loss-of-function, leading to decreased DNA binding activity (PMID: 25482556). Importantly, TET and IDH family mutations are mutually exclusive with WT1 mutations in AML patients, suggesting that WT1 functions as a regulator of DNA methylation (PMID: 25482556). Patients with WT1 mutations may be increasingly sensitive to hypomethylating agents, such as azacytidine, due to the role of WT1 in the regulation of methylation (PMID: 27252512). WT1 is overexpressed in a large percentage of patients with myeloid and lymphoid leukemias (PMID: 27252512, 16461320, 15084694). Vaccines that target overexpression of WT1 are currently in clinical development (PMID: 23486779, 26389576).

- c-KIT activation has been shown to have oncogenic activity in gastrointestinal stromal tumors (GISTs), melanomas, lung cancer, and other tumor types. The targeted therapeutics nilotinib and sunitinib have shown efficacy in treating KIT overactive patients, and are in late-stage trials in melanoma and GIST. KIT overactivity can be the result of many genomic events from genomic amplification to overexpression to missense mutations. Missense mutations have been shown to be key players in mediating clinical response and acquired resistance in patients being treated with these targeted therapeutics.SUMMARY
  KIT, a receptor tyrosine kinase, is recurrently mutated in gastrointestinal stromal tumors.
  BACKGROUND
  The proto-oncogene KIT encodes a type 3 transmembrane receptor tyrosine kinase. The receptor is activated through dimerization and autophosphorylation upon binding by its ligand, stem cell factor (SCF) also known as mast cell growth factor (MGF) (PMID: 9438854). KIT activation results in increased intracellular signaling through several pathways including PI3K, MAPK and STAT, ultimately leading to cell proliferation and survival (PMID: 17546049, 11896121, 22089421). For patients with wildtype gastrointestinal stromal tumors (GIST; no KIT or PDGFRA mutations), NCCN recommends testing for germline succinate dehydrogenase (SDH) mutations. About 10-15% of GISTs are wildtype; thus, the absence of a mutation does not exclude the diagnosis of GIST. In patients without KIT mutations, a subset of those with advanced GISTs benefit from imatinib (0-45% of patients) (NCCN Soft Tissue Sarcoma v.2.2017). Activating KIT mutations occur in 80 - 90% of GISTs and are distributed over multiple exons with different frequencies (exons 11 (66.1%), exon 9 (13%), exon 13 (1.2%), and exon 17 (0.6%)) (PMID:15365079, 17268243, 11719439). There are at least eight small molecule tyrosine kinase inhibitors (TKIs) targeting KIT that have been approved by the US Food and Drug Administration with the efficacy of each TKI strongly depending on the location of the activating KIT mutation (PMID: 2427414, 18955458,19164557).

Drug Information

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).

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.

ROMIDEPSIN

Romidepsin is an HDAC inhibitor.

-Many HDAC inhibitors are potential treatments for cancer through the ability to epigenetically restore normal expression of tumor suppressor genes, which may result in cell cycle arrest, differentiation, and apoptosis.

PAZOPANIB

Pazopanib (Votrient) is a potent and selective multi-targeted receptor tyrosine kinase inhibitor that blocks tumor growth and inhibits angiogenesis. It is a second-generation multitargeted tyrosine kinase inhibitor against vascular endothelial growth factor receptor-1, -2, and -3, platelet-derived growth factor receptor-alpha, platelet-derived growth factor receptor-beta, and c-kit. It has been approved for treatment of advanced renal cell cancer and advanced soft tissue sarcoma (in patients previously treated with chemotherapy) sarcoma by numerous regulatory administrations worldwide (FDA approved on October 19, 2009).

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://ClinicalTrials.gov/show/NCT04656652	Recruiting	Study of DS-1062a Versus Docetaxel in Previously Treated Advanced or Metastatic Non-small Cell Lung Cancer Without Actionable Genomic Alterations (TROPION-LUNG01)	Non-small Cell Lung Cancer	DS-1062a Docetaxel	Ironwood Cancer and Research Center, Chandler, Arizona, United States St. Joseph Heritage Healthcare, Anaheim, California, United States The Oncology Institute of Hope and Innovation, Glendale, California, United States (and 2 more sites Loma Linda University Cancer Institute, Loma Linda, California, United States
https://ClinicalTrials.gov/show/NCT03800134	Recruiting	A Study of Neoadjuvant/Adjuvant Durvalumab for the Treatment of Patients With Resectable Non-small Cell Lung Cancer	Non-Small Cell Lung Cancer	Durvalumab Placebo Carboplatin/Paclitaxel Cisplatin/Gemcitabine Pemetrexed/Cisplatin Pemetrexed/Carboplatin	Research Site, Phoenix, Arizona, United States Research Site, Duarte, California, United States Research Site, Orange, California, United States (and 2 more sites Research Site, Boca Raton, Florida, United States

Detailed Results

Single Nucleotide Variant (SNV)

Gene name	Hgvsp	Hgvsc	Aminoacids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
SMC1A	p. Glue693Leu	2677_2679 GAG>TTA	E/L	Gag/Tag	missense_variant	36.06	183	0
TP53	NP_000537.3:p. Arg248Trp	NM_000546.5:c. 742C>T	R/W	Cgg/Tgg	missense_variant	33.9	118	deleterious (0)
LRP1B	NP_061027.2:p.Ile2969Asn	NM_018557.2:c. 8906T>A	I/N	aTt/aAt	missense_variant	23.44	209	0
NFE2L2	NP_006155.2:p. Glu79Lys	NM_006164.4:c. 235G>A	E/K	Gag/Aag	missense_variant	22.29	157	deleterious (0)
BRIP1	NP_114432.2:p.	NM_032043.2:c.	N/I	aAt/aTt	"missense_variant,s	21.43	112	deleterious

	Asn447Ile	1340A>T			plice_region_variant "			(0.01)
SMARCA4	NP_001122321.1:p.Val134Ala	NM_001128849.1:c.401T>C	V/A	gTt/gCt	missense_variant	21.25	287	0
FGFR1	NP_001167538.1:p.Gln584Leu	NM_001174067.1:c.1751A>T	Q/L	cAg/cTg	missense_variant	16.56	157	deleterious (0)
WT1	NP_077744.3:p.Asn77Lys	NM_024426.4:c.231C>A	N/K	aaC/aaA	missense_variant	14.66	266	deleterious - low confidence (0)
KIT	NP_000213.1:p.Phe10Ser	NM_000222.2:c.29T>C	F/S	tTt/tCt	missense_variant	3.9	128	deleterious (0.04)

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA for abnormalities in 275 genes that are reported to be altered in various types of solid tumors. Nucleic acid is isolated from plasma. 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 50 nucleotides at the 5' and 3' ends of each coding exon. Analyzed genes are listed below: ABL1 BIRC3 CREBBP EZH2 GNAS KMT2C NF2 PPP2R1A SMC3 XPO1 ACVR1B BLM CRLF2 FAM175A GREM1 KMT2D NFE2L2 PRDM1 SMO XRCC2 AKT1 BRAF CSF1R FAM46C GRIN2A KRAS NFKBIA PRKAR1A SOCS1 XRCC3 AKT2 BRCA1 CSF3R FANCA H3F3A LRP1B NKX2-1 PRKDC SOX2 ZNF217 AKT3 BRCA2 CTCF FANCC HGF MAP2K1 NOTCH1 PRSS1 SOX9 ZRSR2 ALK BRIP1 CTNNA1 FANCD2 HIST1H3B MAP2K2 NOTCH2 PTCH1 SPOP AMER1 BTK CTNNB1 FANCE HNF1A MAP2K4 NOTCH3 PTEN SRC APC CALR CUX1 FANCF HOXB13 MAP3K1 NPM1 PTPN11 SRSF2 AR CARD11 CXCR4 FANCG HRAS MAP3K14 NRAS RAC1 STAG2 ARAF CBL CYLD FAS HSP90AA1 MAPK1 NSD1 RAD21 STAT3 ARID1A CBLB DAXX FBXW7 ID3 MCL1 NTRK1 RAD50 STK11 ARID1B CBLC DDR2 FGF4 IDH1 MDM2 NTRK2 RAD51 SUFU ARID2 CCND1 DICER1 FGF6 IDH2 MDM4 NTRK3 RAF1 SUZ12 ASXL1 CCND3 DNM2 FGFR1 IGF1R MED12 PAK3 RB1 TAL1 ATM CCNE1 DNMT3A FGFR2 IKZF1 MEF2B PALB2 RET TCF3 ATR CD274 DOT1L FGFR3 IKZF3 MEN1 PAX5 RHEB TERT ATRX CD79A EED FGFR4 IL7R MET PBRM1 RHOA TET2 AURKA CD79B EGFR FH INHBA MITF PDGFRA RIT1 TGFBR2 AURKB CDC73 EGLN1 FLCN IRF4 MLH1 PDGFRB RNF43 TNFAIP3 AURKC CDH1 EP300 FLT3 JAK1 MPL PHF6 ROS1 TNFRSF14 AXIN1 CDK12 EPAS1 FLT4 JAK2 MRE11A PIK3CA RUNX1 TP53 AXIN2 CDK4 EPHA3 FOXL2 JAK3 MSH2 PIK3R1 SDHB TRAF3 B2M CDK6 EPHA5 FUBP1 KAT6A MSH6 PIK3R2 SETBP1 TSC1 BAP1 CDKN2A ERBB2 GALNT12 KDM5C MTOR PIM1 SETD2 TSC2 BCL2 CDKN2B ERBB3 GATA1 KDM6A MUTYH PLCG1 SF3B1 TSHR BCL2L1 CDKN2C ERBB4 GATA2 KDR MYC PMS1 SMAD2 U2AF1 BCL6 CEBPA ERG GATA3 KEAP1 MYCL PMS2 SMAD4 U2AF2 BCOR CHEK1 ESR1 GEN1 KIT MYCN POLD1 SMARCA4 VHL BCORL1 CHEK2 ETV6 GNA11 KMT2A MYD88 POLE SMARCB1 WHSC1 BCR CIC EXO1 GNAQ KMT2B NF1 PPM1D SMC1A and WT1. The DNA assay is optimized to be run using 20 ng from cfDNA. Extraction of DNA from plasma is automated. Library for targeted DNA sequencing is based on Single Primer Extension (SPE) chemistry. The DNA sequencing includes all coding exons of 275 genes. Specifically, the test is indicated for: -Molecular profiling of genomic abnormalities (SNV and indels) in DNA from patients with circulating solid tumor DNA. -cfDNA testing is to be used only for detecting abnormalities in solid tumors when biopsy is not obtainable. This test is for in vitro complementary diagnosis and classification. It should not be used as the primary diagnosis of solid tumors or for managing therapy in patients. Our sequencing method has a typical sensitivity of 3% for detecting hot-spots specific mutations and 5% for other mutations. The assay is not designed to detect gene amplification. Based on our validation study, the following regions of the genes listed below are not covered appropriately (<100 X coverage) and sequencing by NGS may not be reliable in these regions. This poor coverage is due to high GC content with inherited problem in obtaining adequate coverage. Region Transcript Exon AA Range Promoter Range TNFRSF14.8 NM_003820 7 232-242 MYCL.117 NM_001033082 1 1-27 AXIN1.1161 NM_003502 1 NC PIK3R2.1897 NM_005027 6 200-272 KMT2B.1928 NM_014727 1 1-121 CD79A.1981 NM_001783 4 167-189 ASXL1.2390 NM_015338 1 1-19 BCR.2530 NM_021574 17 981-1017 TERT.3105 -59 to -72 TERT.3106 -81 to -94 PMS2.3489 NM_001322008 13 710-757 RHEB.3700 NM_005614 1 1-18 Variant calling is based on DRAGEN somatic pipeline v. 3.4.5 using tumor-only analysis against the GRCh37 reference genome.

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, Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618. Medical Director Maher Albitar, M.D. Analysis of the data was performed in part at Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618. 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.