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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:	C17.0 Malignant neoplasm of duodenum
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				
MET	DDR2	PIK3CA	LRP1B	APC (2 mutations)
KRAS (G12V)	ERBB4	MAP2K1	Chromosomal structural analysis shows 5p+, 8q+, 10p+, 13q+, and +20	-

Heterogeneity
There are abnormal clones with MET, DDR2, PIK3CA, LRP1B, APC (2 mutations), KRAS (G12V), ERBB4, and MAP2K1 mutations.

Diagnostic Implications	
MET, DDR2, PIK3CA, LRP1B, APC (2 mutations), KRAS (G12V), ERBB4, MAP2K1	These abnormalities are consistent with the presence of circulating solid tumor DNA with high tumor burden

Therapeutic Implications	
MET	ALK/MET inhibitors
PIK3CA	PI3K, AKT, MTOR inhibitors
APC	WNT, beta-catenin, COX-2 inhibitors

KRAS (G12V)	HSP-90 inhibitor, MEK inhibitors
MAP2K1	MEK inhibitors

Prognostic Implications	
MET	Poor
PIK3CA	Poor
APC (2 mutations)	Poor
KRAS (G12V)	Poor
ERBB4	Poor
MAP2K1	Poor

Relevant Genes with NO Alteration
No evidence of mutation in: NRAS, EGFR, BRAF, TP53

Results Summary

- **-Mutations in MET, DDR2, PIK3CA, LRP1B, APC (2 mutations), KRAS (G12V), ERBB4, and MAP2K1 genes**
  -Chromosomal structural analysis shows 5p+, 8q+, 10p+, 13q+, and +20

  -These abnormalities are consistent with circulating solid tumor DNA with high tumor burden.

  -KRAS (G12V) mutation suggests resistance to anti-EGFR therapy and to Sotorasib, but possible response to ERK/MEK inhibitors (Selumetinib, Trametinib, Binimetinib, Vemurafenib, Cobimetinib..).

  -MET mutation suggests response to response to MET inhibitors (tepotinib, capmatinib..).

  -DDR2 mutation suggests response to multi-targeted kinase inhibitor dasatinib.

  -PIK3CA mutation suggests response to PI3K/mTOR inhibitors.

  -APC mutations activate beta-catenin and WNT signaling pathway and suggest possible response to WNT and COX-2 inhibitors.

  -ERBB4 mutation suggests possible response to HER2small molecule kinase inhibitors. (lapatinib, erlotinib, gefitinib, afatinib, neratinib).

  -MAP2K1 mutation suggests response to MEK inhibitors (Selumetinib, Trametinib, Binimetinib, Vemurafenib, Cobimetinib..).

Biological relevance of detected Alterations

- Also known as MET_HUMAN, MET. Receptor tyrosine kinase that transduces signals from the extracellular matrix into the cytoplasm by binding to hepatocyte growth factor/HGF ligand. Regulates many physiological processes including proliferation, scattering, morphogenesis and survival. Ligand binding at the cell surface induces autophosphorylation of MET on its intracellular domain that provides docking sites for downstream signaling molecules. Following activation by ligand, interacts with the PI3-kinase subunit PIK3R1, PLCG1, SRC, GRB2, STAT3 or the adapter GAB1. Recruitment of these downstream effectors by MET leads to the activation of several signaling cascades including the RAS-ERK, PI3 kinase-AKT, or PLCgamma-PKC. The RAS-ERK activation is associated with the morphogenetic effects while PI3K/AKT coordinates prosurvival effects. During embryonic development, MET signaling plays a role in gastrulation, development and migration of muscles and neuronal precursors, angiogenesis and kidney formation. In adults, participates in wound healing as well as organ regeneration and tissue remodeling. Promotes also differentiation and proliferation of hematopoietic cells. May regulate cortical bone osteogenesis (By similarity)., (Microbial infection) Acts as a receptor for Listeria monocytogenes internalin InlB, mediating entry of the pathogen into cells. Heterodimer made of an alpha chain (50 kDa) and a beta chain (145 kDa) which are disulfide linked. Binds PLXNB1. Interacts when phosphorylated with downstream effectors including STAT3, PIK3R1, SRC, PCLG1, GRB2 and GAB1. Interacts with SPSB1, SPSB2 and SPSB4 (By similarity). Interacts with INPP5D/SHIP1. When phosphorylated at Tyr-1356, interacts with INPPL1/SHIP2. Interacts with RANBP9 and RANBP10, as well as SPSB1, SPSB2, SPSB3 and SPSB4. SPSB1 binding occurs in the presence and in the absence of HGF, however HGF treatment has a positive effect on this interaction. Interacts with MUC20; prevents interaction with GRB2 and suppresses hepatocyte growth factor-induced cell proliferation. Interacts with GRB10. Interacts with PTPN1 and PTPN2. Interacts with LECT2; this interaction may have an antagonistic effect on receptor activation (PubMed:27334921). Interacts with HSP90AA1 and HSP90AB1; the interaction suppresses MET kinase activity (PubMed:26517842).

- Also known as DDR2_HUMAN, DDR2, NTRKR3, TKT, TYRO10. Tyrosine kinase involved in the regulation of tissues remodeling (PubMed:30449416). It functions as cell surface receptor for fibrillar collagen and regulates cell differentiation, remodeling of the extracellular matrix, cell migration and cell proliferation. Required for normal bone development. Regulates osteoblast differentiation and chondrocyte maturation via a signaling pathway that involves MAP kinases and leads to the activation of the transcription factor RUNX2. Regulates remodeling of the extracellular matrix by up-regulation of the collagenases MMP1, MMP2 and MMP13, and thereby facilitates cell migration and tumor cell invasion. Promotes fibroblast migration and proliferation, and thereby contributes to cutaneous wound healing. Binds hydroxyproline-rich sequence motifs in fibrillar, glycosylated collagen, such as the GQOGVMGFO motif, where O stands for hydroxyproline. Interacts with SRC. Interacts (tyrosine phosphorylated) with SHC1.

- Also known as PK3CA_HUMAN, PIK3CA. Phosphoinositide-3-kinase (PI3K) phosphorylates phosphatidylinositol (PI) and its phosphorylated derivatives at position 3 of the inositol ring to produce 3-phosphoinositides (PubMed:15135396, PubMed:23936502, PubMed:28676499). Uses ATP and PtdIns(4,5)P2 (phosphatidylinositol 4,5-bisphosphate) to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3) (PubMed:15135396, PubMed:28676499). PIP3 plays a key role by recruiting PH domain-containing proteins to the membrane, including AKT1 and PDPK1, activating signaling cascades involved in cell growth, survival, proliferation, motility and morphology. Participates in cellular signaling in response to various growth factors. Involved in the activation of AKT1 upon stimulation by receptor tyrosine kinases ligands such as EGF, insulin, IGF1, VEGFA and PDGF. Involved in signaling via insulin-receptor substrate (IRS) proteins. Essential in endothelial cell migratior during vascular development through VEGFA signaling, possibly by regulating RhoA activity. Required for lymphatic vasculature development, possibly by binding to RAS and by activation by EGF and FGF2, but not by PDGF. Regulates invadopodia formation through the PDPK1-AKT1 pathway. Participates in cardiomyogenesis in embryonic stem cells through a AKT1 pathway. Participates in vasculogenesis in embryonic stem cells through PDK1 and protein kinase C pathway. In addition to its lipid kinase activity, it displays a serine-protein kinase activity that results in the autophosphorylation of the p85alpha regulatory subunit as well as phosphorylation of other proteins such as 4EBP1, H-Ras, the IL-3 beta c receptor and possibly others (PubMed:23936502, PubMed:28676499). Plays a role in the positive regulation of phagocytosis and pinocytosis (By similarity). Heterodimer of a catalytic subunit PIK3CA and a p85 regulatory subunit (PIK3R1, PIK3R2 or PIK3R3) (PubMed:26593112). Interacts with IRS1 in nuclear extracts (By similarity). Interacts with RUFY3 (By similarity). Interacts with RASD2 (By similarity). Interacts with APPL1. Interacts with HRAS and KRAS (By similarity). Interaction with HRAS/KRAS is required for PI3K pathway signaling and cell proliferation stimulated by EGF and FGF2 (By similarity). Interacts with FAM83B; activates the PI3K/AKT signaling cascade (PubMed:23676467).

- Also known as LRP1B_HUMAN, LRP1B, LRPDIT. Potential cell surface proteins that bind and internalize ligands in the process of receptor-mediated endocytosis. Binds LRPAP1, PLAU, PLAT and SERPINE1; binding is followed by internalization and degradation of the ligands.

- Also known as APC_HUMAN, APC, DP2.5. Tumor suppressor. Promotes rapid degradation of CTNNB1 and participates in Wnt signaling as a negative regulator. APC activity is correlated with its phosphorylation state. Activates the GEF activity of SPATA13 and ARHGEF4. Plays a role in hepatocyte growth factor (HGF)-induced cell migration. Required for MMP9 up-regulation via the JNK signaling pathway in colorectal tumor cells. Acts as a mediator of ERBB2-dependent stabilization of microtubules at the cell cortex. It is required for the localization of MACF1 to the cell membrane and this localization of MACF1 is critical for its function in microtubule stabilization. Forms homooligomers and heterooligomers with APC2. Interacts with DIAPH1 and DIAPH2 (By similarity). Interacts with PDZ domains of DLG1 and DLG3. Associates with catenins. Binds axin. Interacts with ARHGEF4 (via N-terminus). Interacts with MAPRE1 (via C-terminus); probably required for APC targeting to the growing microtubule plus ends. Interacts with MAPRE2 and MAPRE3 (via C-terminus). Found in a complex consisting of ARHGEF4, APC and CTNNB1. Interacts with SCRIB; may mediate APC targeting to adherens junctions of epithelial cells. Interacts with SPATA13 (via N-terminus and SH3 domain). Interacts with ASAP1 (via SH3 domain). Found in a complex composed of MACF1, APC, AXIN1, CTNNB1 and GSK3B (By similarity). Interacts at the cell membrane with AMER1 and AMER2 (via ARM repeats). Interacts with KHDRBS1. The complex composed, at least, of APC, CTNNB1 and GSK3B interacts with JPT1; the interaction requires the inactive form of GSK3B (phosphorylated at 'Ser-9') (PubMed:25169422).

- Also known as RASK_HUMAN, KRAS, KRAS2, RASK2. Ras proteins bind GDP/GTP and possess intrinsic GTPase activity (PubMed:20949621). Plays an important role in the regulation of cell proliferation (PubMed:23698361, PubMed:22711838). Plays a role in promoting oncogenic events by inducing transcriptional silencing of tumor suppressor genes (TSGs) in colorectal cancer (CRC) cells in a ZNF304-dependent manner (PubMed:24623306). Interacts with PHLPP. Interacts (active GTP-bound form preferentially) with RGS14 (By similarity). Interacts (when farnesylated) with PDE6D; this promotes dissociation from the cell membrane (PubMed:23698361). Interacts with SOS1 (PubMed:22431598). Interacts (when farnesylated) with GPR31 (PubMed:28619714).

- Also known as ERBB4_HUMAN, ERBB4, HER4. Tyrosine-protein kinase that plays an essential role as cell surface receptor for neuregulins and EGF family members and regulates development of the heart, the central nervous system and the mammary gland, gene transcription, cell proliferation, differentiation, migration and apoptosis. Required for normal cardiac muscle differentiation during embryonic development, and for postnatal cardiomyocyte proliferation. Required for normal development of the embryonic central nervous system, especially for normal neural crest cell migration and normal axon guidance. Required for mammary gland differentiation, induction of milk proteins and lactation. Acts as cell-surface receptor for the neuregulins NRG1, NRG2, NRG3 and NRG4 and the EGF family members BTC, EREG and HBEGF. Ligand binding triggers receptor dimerization and autophosphorylation at specific tyrosine residues that then serve as binding sites for scaffold proteins and effectors. Ligand specificity and signaling is modulated by alternative splicing, proteolytic processing, and by the formation of heterodimers with other ERBB family members, thereby creating multiple combinations of intracellular phosphotyrosines that trigger ligand- and context-specific cellular responses. Mediates phosphorylation of SHC1 and activation of the MAP kinases MAPK1/ERK2 and MAPK3/ERK1. Isoform JM-A CYT-1 and isoform JM-B CYT-1 phosphorylate PIK3R1, leading to the activation of phosphatidylinositol 3-kinase and AKT1 and protect cells against apoptosis. Isoform JM-A CYT-1 and isoform JM-B CYT-1 mediate reorganization of the actin cytoskeleton and promote cell migration in response to NRG1. Isoform JM-A CYT-2 and isoform JM-B CYT-2 lack the phosphotyrosine that mediates interaction with PIK3R1, and hence do not phosphorylate PIK3R1, do not protect cells against apoptosis, and do not promote reorganization of the actin cytoskeleton and cell migration. Proteolytic processing of isoform JM-A CYT-1 and isoform JM-A CYT-2 gives rise to the corresponding soluble intracellular domains (4ICD) that translocate to the nucleus, promote nuclear import of STAT5A, activation of STAT5A, mammary epithelium differentiation, cell proliferation and activation of gene expression. The ERBB4 soluble intracellular domains (4ICD) colocalize with STAT5A at the CSN2 promoter to regulate transcription of milk proteins during lactation. The ERBB4 soluble intracellular domains can also translocate to mitochondria and promote apoptosis. Monomer in the absence of bound ligand. Homodimer or heterodimer with another ERBB family member upon ligand binding, thus forming heterotetramers. Interacts with EGFR and ERBB2. Interacts with CBFA2T3 (By similarity). Interacts with DLG2 (via its PDZ domain), DLG3 (via its PDZ domain), DLG4 (via its PDZ domain) and SNTB2 (via its PDZ domain). Interacts with MUC1. Interacts (via its PPxy motifs) with WWOX. Interacts (via the PPxY motif 3 of isoform JM-A CYT-2) with YAP1 (via the WW domain 1 of isoform 1). Interacts (isoform JM-A CYT-1 and isoform JM-B CYT-1) with WWP1. Interacts (via its intracellular domain) with TRIM28. Interacts (via the intracellular domains of both CYT-1 and CYT-2 isoforms) with KAP1; the interaction does not phosphorylate KAP1 but represses ERBB4-mediated transcriptional activity. Interacts with PRPU, DDX23, MATR3, RBM15, ILF3, KAP1, U5S1, U2SURP, ITCH, HNRNPU, AP2A1, NULC, LEO1, WWP2, IGHG1, HXK1, GRB7 AND SRRT. Interacts (phosphorylated isoform JM-A CYT-1 and isoform JM-B CYT-1) with PIK3R1. Interacts with SHC1. Interacts with GRB2. Interacts (soluble intracellular domain) with STAT5A. Interacts (soluble intracellular domain) with BCL2. Interacts (phosphorylated) with STAT1.

- Also known as MP2K1_HUMAN, MAP2K1, MEK1, PRKMK1. Dual specificity protein kinase which acts as an essential component of the MAP kinase signal transduction pathway. Binding of extracellular ligands such as growth factors, cytokines and hormones to their cell-surface receptors activates RAS and this initiates RAF1 activation. RAF1 then further activates the dual-specificity protein kinases MAP2K1/MEK1 and MAP2K2/MEK2. Both MAP2K1/MEK1 and MAP2K2/MEK2 function specifically in the MAPK/ERK cascade, and catalyze the concomitant phosphorylation of a threonine and a tyrosine residue in a Thr-Glu-Tyr sequence located in the extracellular signal-regulated kinases MAPK3/ERK1 and MAPK1/ERK2, leading to their activation and further transduction of the signal within the MAPK/ERK cascade. Activates BRAF in a KSR1 or KSR2-dependent manner; by binding to KSR1 or KSR2 releases the inhibitory intramolecular interaction between KSR1 or KSR2 protein kinase and N-terminal domains which promotes KSR1 or KSR2-BRAF dimerization and BRAF activation (PubMed:29433126). Depending on the cellular context, this pathway mediates diverse biological functions such as cell growth, adhesion, survival and differentiation, predominantly through the regulation of transcription, metabolism and cytoskeletal rearrangements. One target of the MAPK/ERK cascade is peroxisome proliferator-activated receptor gamma (PPARG), a nuclear receptor that promotes differentiation and apoptosis. MAP2K1/MEK1 has been shown to export PPARG from the nucleus. The MAPK/ERK cascade is also involved in the regulation of endosomal dynamics, including lysosome processing and endosome cycling through the perinuclear recycling compartment (PNRC), as well as in the fragmentation of the Golgi apparatus during mitosis. Found in a complex with at least BRAF, HRAS, MAP2K1, MAPK3/ERK1 and RGS14 (By similarity). Forms a heterodimer with MAP2K2/MEK2 (By similarity). Forms heterodimers with KSR2 which further dimerize to form tetramers (By similarity). Interacts with KSR1 or KSR2 and BRAF; the interaction with KSR1 or KSR2 mediates KSR1-BRAF or KSR2-BRAF dimerization (PubMed:10409742, PubMed:29433126). Interacts with ARBB2, LAMTOR3, MAPK1/ERK2 and RAF1 (By similarity). Interacts with MORG1 (By similarity). Interacts with PPARG (PubMed:17101779). Interacts with isoform 1 of VRK2 (PubMed:20679487). Interacts with SGK1 (PubMed:19447520). Interacts with BIRC6/bruce (PubMed:18329369). Interacts with KAT7; the interaction promotes KAT7 phosphorylation (By similarity). Interacts with RAF1 and NEK10; the interaction is required for ERK1/2-signaling pathway activation in response to UV irradiation (PubMed:20956560). Interacts with TRAF3IP3 (PubMed:26195727).

Drug Information

CRIZOTINIB

Crizotinib an inhibitor of receptor tyrosine kinase for the treatment of non-small cell lung cancer (NSCLC).
Crizotinib is a tyrosine kinase receptor inhibitor. More specifically, it inhibits anaplastic lymphoma kinase (ALK), hepatocyte growth factor receptor (HGFR, c-MET), and Recepteur d'Origine Nantais (RON). Abnormalities in the ALK gene caused by mutations or translocations may lead to expression of oncogenic fusion proteins. In patients with NSCLC, they have the EML4-ALK gene. Crizotinib inhibits ALK tyrosine kinase which

ultimately results in decreased proliferation of cells that carry the genetic mutation and tumour survivability.

CABOZANTINIB (COMETRIQ)

Cabozantinib inhibits specific receptor tyrosine kinases such as VEGFR-1, -2 and -3, KIT, TRKB, FLT3, AXL, RET, MET, and TIE-2.

Cabozantinib suppresses metastasis, angiogenesis, and oncognesis by inhibiting receptor tyrosine kinases.

Cabozantinib is indicated for the treatment of metastatic medullary thyroid cancer and for the treatment of patients with advanced renal cell carcinoma (RCC) who have received prior anti-angiogenic therapy.

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.

CELECOXIB

Celecoxib selectively inhibits cyclo-oxygenase-2 activity (COX-2); COX-2 inhibition may result in apoptosis and a reduction in tumor angiogenesis and metastasis.

Celecoxib is a Nonsteroidal Anti-inflammatory Drug. The mechanism of action of celecoxib is as a Cyclooxygenase Inhibitor. The chemical classification of celecoxib is Nonsteroidal Anti-inflammatory Compounds.

GANETESPIB

Ganetespib (STA-9090), a Nongeldanamycin (Triazolone-containing) HSP90 Inhibitor, structurally unrelated to first-generation ansamycin-family Hsp90 inhibitors, has potent antitumor activity in invitro and invivo models of non-small cell lung cancer. Ganetespib is currently being evaluated in more than 20 different clinical trials. It has not yet received FDA approval.

BINIMETINIB

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

COBIMETINIB

Cobimetinib is a reversible inhibitor of mitogen-activated protein kinase 1 (MAPK)/extracellular signal regulated kinase 1 (MEK1) and MEK2.

MEK inhibitor Cobimetinib specifically binds to and inhibits the catalytic activity of MEK1, resulting in inhibition of extracellular signal-related kinase 2 (ERK2) phosphorylation and activation and decreased tumor cell proliferation. Cobimetinib targets kinase activity in the RAS/RAF/MEK/ERK pathway.

TRAMETINIB

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

Potential Clinical Trials

Trial	Status	Title	Disease	Drug	Sites
https://ClinicalTrials.gov/show/NCT036684	Recruiting	Dabrafenib + Trametinib + PDR001	Colorectal Cancer	Dabrafenib Trametinib	Massachusetts General Hosital

31		In Colorectal Cancer		PDR001	Cancer Center, Boston, Massachusetts, United States Dana Farber Cancer Institite, Boston, Massachusetts, United States
https://ClinicalTrials.gov/show/NCT04495621	Recruiting	MEN1611 With Cetuximab in Metastatic Colorectal Cancer (C-PRECISE-01)	Colorectal Cancer	MEN1611 Cetuximab	Phoenix, Phoenix, Arizona, United States The Oncology Institute of Hope and Innovation, Anaheim, California, United States MultiCare Health System Institute for Research and Innovation, Tacoma, Washington, United States
https://ClinicalTrials.gov/show/NCT04457284	Recruiting	Temozolomide, Cisplatin, and Nivolumab in People With Colorectal Cancer	Colorectal Cancer	Temozolomide (TMZ) Cisplatin Nivolumab	Memoral Sloan Kettering Basking Ridge (Limited Protocol Activities), Basking Ridge, New Jersey, United States Memorial Sloan Kettering Monmouth (Limited Protocol Activities), Middletown, New Jersey, United States Memorial Sloan Kettering Bergen (Limited Protocol Activities), Montvale, New Jersey, United States

Detailed Results

Single Nucleotide Variant (SNV)

Gene name	Hgvsp	Hgvsc	Aminoacids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
MET	NP_001120972.1:p.Asp340Gly	NM_001127500.1:c.1019A>G	D/G	gAc/gGc	missense_variant	20.85	235	deleterious (0.02)
DDR2	NP_006173.2:p.Arg839Cys	NM_006182.2:c.2515C>T	R/C	Cgt/Tgt	missense_variant	13.81	210	deleterious (0)
PIK3CA	NP_006209.2:p.Glu545Lys	NM_006218.2:c.1633G>A	E/K	Gag/Aag	missense_variant	10.6	217	deleterious (0.02)
LRP1B	NP_061027.2:p.Lys863Glu	NM_018557.2:c.2587A>G	K/E	Aaa/Gaa	missense_variant	8.24	170	0
APC	NP_000029.2:p.Thr1556AsnfsTer3	NM_000038.5:c.4666dupA	E/EX	gaa/gAaa	frameshift_variant	7.43	148	0
APC	NP_000029.2:p.Phe994Ter	NM_000038.5:c.2969_2978dupATGAAAGTAA	D/DESKX	gat/gATGAAAGTAAat	frameshift_variant	7.32	82	0
KRAS (G12V)	NP_203524.1:p.Gly12Val	NM_033360.2:c.35G>T	G/V	gGt/gTt	missense_variant	6.47	170	deleterious (0)

ERBB4	NP_005226.1:p.Glu1167Asp	NM_005235.2:c.3501G>T	E/D	gaG/gaT	missense_variant	5.43	184	tolerated (0.09)
MAP2K1	NP_002746.1:p.Asp67Asn	NM_002755.3:c.199G>A	D/N	Gac/Aac	missense_variant	3.59	362	tolerated (0.06)

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

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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.