175 Technology Drive, Suite 100, Irvine, CA 92618
Tel: 1-866-484-8870 www.genomictestingcooperative.com
CLIA #: 05D2111917 CAP #: 9441547

Solid Tumor Profile Plus

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

MRN:				Indication for Testing:	
Collected Date:	06/18/2020	Time:	11:30 AM		
Received Date:	07/22/2020	Time:	12:17 PM	Tumor Type:	
Reported Date:	08/02/2020	Time:	02:31 PM		

Test Description:

This is a comprehensive molecular profile which uses next generation sequencing (NGS), fragment length analysis and Sanger Sequencing testing to identify molecular abnormalities in DNA of 434 genes and RNA in 1,385 genes with a focus on 55 genes implicated in solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

Detected Genomic Alterations

Level 1 (FDA-Approved)	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Relevance)	Other
No evidence of microsatellite instability	Tumor Mutation Burden-High: 17 Mut/Mb	PTEN, RB1, ERBB4, KMT2C (2 mutations)	CDH1, CBFB, RBBP6, PBRM1 (5 mutations), GATA1, HOXA11, NUP93, RIT1	Chromosomal structural abnormalities including: 1q+, 4q-, 6p+, 7q+, -13, +15, 17p- and others.
-t(5;14)(q35; q32) FGFR4-CEP170B fusion mRNA	-	-t(3;10)(q26;q23) PTEN-TBL1XR1 fusion mRNA	-	-

Tumor Heterogeneity

There is a dominant abnormal clone with CDH1 mutation. The PTEN, CBFB, RBBP6, RB1, PBRM1 (5 mutations), ERBB4, GATA1, HOXA11, KMT2C, NUP93, KMT2C, and RIT1 mutations are detected in subclones.

Diagnostic Implications	
CDH1, PTEN, CBFB, RBBP6, RB1, PBRM1 (5 mutations), ERBB4, GATA1, HOXA11, KMT2C (2 mutations), NUP93, RIT1	These abnormalities are consistent with aggressive neoplasm.

Prognostic Implications	
CDH1	Poor
PTEN	Poor
CBFB	Poor
RBBP6	Poor
RB1	Poor
PBRM1 (5 mutations)	Poor
ERBB4	Poor
GATA1	Poor
HOXA11	Poor
KMT2C (2 mutations)	Poor
NUP93	Poor
RIT1	Poor

FDA-Approved Therapeutics in Other Tumor Types	
Markedly high ERBB2 mRNA	Markedly high FGFR4 mRNA

Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence)	
PTEN	PARP inhibitors, PI3K/AKT inhibitors as well as FRAP/mTOR inhibitors.
RB1	CDK inhibitors, aurora a kinase inhibitors or BCL2 inhibitors as well as cisplatin based therapy
ERBB4	DACOMITINIB
KMT2C (2 mutations)	HDAC Inhibitors

Relevant Genes with NO Alteration		
No evidence of mutation in: KRAS, NRAS, EGFR, BRAF, TP53	No evidence of fusion mRNA involving ALK, RET, ROS1, or NTRK	No evidence of MET14 deletion, EGFR Viii
No evidence of microsatellite instability	Tumor Mutation Burden-High: 17	

Results Summary

- **-Mutations in CDH1, PTEN, CBFB, RBBP6, RB1, PBRM1 (5 mutations), ERBB4, GATA1, HOXA11, KMT2C (2 mutaitons), NUP93, and RIT1 genes**
  -Chromosomal structural abnormalities including: 1q+, 4q-, 6p+, 7q+, -13, +15, 17p- and others.
  -t(3;10)(q26;q23) PTEN-TBL1XR1 fusion mRNA
  -t(5;14)(q35; q32) FGFR4-CEP170B fusion mRNA
  -Markedly high FGFR4 mRNA
  -Markedly high ERBB2 mRNA
  -Tumor mutation burden (TMB): High 17 Mut/Mb
  -No evidence of microsatellite instability

  The presence of high TMB suggests response to immune checkpoint inhibitors

  -The fusin and overexpression of FGFR4 suggests response to FGFR inhibitors.

  -Although we cannot demonstrate ERBB2 gene amplification, the ERBB2 mRNA is markedly high and suggests possible response to anti-HER2 therapy.

  -PTEN mutation and fusion suggests response to PARP inhibitors.

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

Biological relevance of detected Alterations

- RABEP1, also known as U2AF1_HUMAN, U2AF1, U2AF35, U2AFBP. Plays a critical role in both constitutive and enhancer-dependent splicing by mediating protein-protein interactions and protein-RNA interactions required for accurate 3'-splice site selection. Recruits U2 snRNP to the branch point. Directly mediates interactions between U2AF2 and proteins bound to the enhancers and thus may function as a bridge between U2AF2 and the enhancer complex to recruit it to the adjacent intron. Interacts (via RS domain) with PHF5A (via N-terminus) (By similarity). Identified in the spliceosome C complex. Heterodimer with U2AF2. Interacts with ZRANB2.

- CDH1 (E-cadherin), a tumor suppressor involved in cell adhesion, is altered by mutation or deletion in various cancer typess, most frequently in breast and esophagogastric cancers.
  CDH1, also known as E-cadherin, is a calcium-dependent transmembrane glycoprotein that is mainly expressed in epithelial cells and functions in cell-cell adhesion, signaling cascades and epithelial-to-mesenchymal transition (EMT) (PMID: 18726070). The extracellular portion of E-cadherin facilitates homophilic cell-to-cell adhesion by binding to cadherins on adjacent cells, while the intracellular domain is tethered to the actin cytoskeleton through interactions with catenins and functions to activate signaling cascades that play a role in the EMT (PMID:7885471, 2788574). The transcription factor SNAIL, a key regulator of the EMT during embryonic development, represses expression of the E-cadherin gene in tumor cell lines (PMID: 10655586, 10655587). Lack of E-cadherin function/expression enables cancer progression by altering cellular morphology, decreasing cellular adhesion, and increasing cellular motility (PMID: 10439038, 2070412, 9515965). Along with point mutations and loss of heterozygosity (LOH), epigenetic silencing by hypermethylation of the CDH1 promoter has been associated with the loss of E-cadherin gene expression during cancer progression (PMID: 7543680). Individuals with a germline CDH1 mutation have an increased risk of developing diffuse gastric cancer and breast cancer (PMID: 11729114). Loss of E-cadherin has also been demonstrated in a variety of sporadic cancer types including gastric cancer, colorectal cancer, and esophageal cancer (PMID: 11313896, 22716209, 21373750).

- PTEN is a multi-functional tumor suppressor that is very commonly lost in human cancer. Observed in prostate cancer, glioblastoma, endometrial, lung and breast cancer to varying degrees. Up to 70% of prostate cancer patients have been observed to have loss of expression of the gene. It is a part of the PI3K/AKT/mTOR pathway and mTOR inhibitors have been relatively ineffective in treating patients with PTEN loss. New appoaches using microRNAs are currently being investigated.SUMMARY
  PTEN, a lipid and protein phosphatase, is one of the most frequently mutated genes in cancer.
  PTEN encodes a tumor suppressor that is one of the most frequently mutated genes in human cancer (PMID: 9072974, 9090379, 22473468). PTEN has several physiological functions, most notably operating as a phosphatase that converts phosphatidylinositol (3,4,5)-triphosphate (PIP3) to phosphatidylinositol (4,5)-triphosphate (PIP2) at the cell membrane (PMID: 18767981). Impairment of PTEN function through multiple mechanisms, including through non-synonymous mutations, results in PIP3 accumulation and constitutive activation of catabolic downstream AKT/mTOR signaling. PTEN inactivation therefore promotes cell growth, proliferation and survival (PMID: 12040186). Additionally, nuclear PTEN is thought to regulate RAD51 expression, and in this way is also associated with homologous recombination and

repair of DNA strand breaks (PMID: 17218262, 23888040). Thus, loss of PTEN may also lead to greater genomic instability and provide a setting for the accumulation of other deleterious mutations. PTEN is frequently mutated in many types of human cancers (PMID: 15254063). Germline loss-of-function PTEN mutations occur in approximately 80% of patients with the cancer predisposition syndrome Cowden disease, which is associated with high-penetrance breast and thyroid cancer (PMID: 9467011, 24136893, 21430697).

- CBFB has been shown to be mutated in estrogen receptor positive breast cancer, although the significance of many of these mutations is yet to be determined. The CBFB-MYH11 fusion is a commonly accepted marker of favorable prognosis in acute myeloid leukemia.SUMMARY
  CBFB, a protein involved in transcriptional activation, is altered by chromosomal rearrangement in a subset of acute myeloid leukemia.
  The CBFB (core-binding factor, beta subunit) gene encodes a heterodimeric transcription factor component, that together with a core-binding factor alpha component, RUNX1/2/3 (Runt related transcription factor) proteins, forms a transcription factor complex (PMID: 8929538). CBFB is a non-DNA binding subunit that functions to enhance the DNA binding of the CBF alpha component. The CBF complex targets specific genes for activation or repression and also recruits activating or repressive cofactors such as p300 and HDACs (Histone deacetylases) (PMID: 23148227, 21059642). CBFB complexed with RUNX1 regulates important steps in hematopoiesis through processes such as cell cycle progression, differentiation and development (PMID: 11561154). With RUNX2, CBFB regulates skeletal development (PMID: 12434152, 24798493). Inversion of chromosome 16 can result in a CBFB-MYH11 (Myosin heavy chain 11) fusion gene and is associated with the M4 type of acute myeloid leukemia, and often with associated eosinophilia (PMID: 23160462). This fusion protein disrupts the CBF complex and results in a block in hematopoiesis (PMID: 20007544). CBFB may also influence solid tumor development, as mutations have been identified in breast and cervical cancer samples (PMID: 22722202, 24390348).

- RBBP6, also known as RBBP6_HUMAN, RBBP6, P2PR, PACT, RBQ1. E3 ubiquitin-protein ligase which promotes ubiquitination of YBX1, leading to its degradation by the proteasome (PubMed:18851979). May play a role as a scaffold protein to promote the assembly of the p53/TP53-MDM2 complex, resulting in increase of MDM2-mediated ubiquitination and degradation of p53/TP53; may function as negative regulator of p53/TP53, leading to both apoptosis and cell growth (By similarity). Regulates DNA-replication and the stability of chromosomal common fragile sites (CFSs) in a ZBTB38- and MCM10-dependent manner. Controls ZBTB38 protein stability and abundance via ubiquitination and proteasomal degradation, and ZBTB38 in turn negatively regulates the expression of MCM10 which plays an important role in DNA-replication (PubMed:24726359). Interacts with p53/TP53 and RB1 (By similarity). Interacts also with MDM2 and YBX1. Interacts with NEK6. Interacts with ZBTB38.

- RB1, a regulator of the cell cycle, is inactivated by mutation, deletion or allelic loss in various cancer types, including retinoblastoma and lung cancer.
  RB1, also known as RB, is involved in the cell-cycle checkpoint and in its active form inhibits the transition from G1 to S phase of the cell cycle until the cell is ready to divide. RB is active in its unphosphorylated form where it binds to E2F family of transcription factors, which together with the E2F Dimerization Partner (E2F-DP), inhibits the transcription of S-phase promoting factors by recruiting histone deacetylases (HDACs) and induce heterochromatin formation (PMID: 1655277). At the end of G1, cyclin-dependent kinases (CDKs) phosphorylate RB to pRB which leads to its dissociation from the E2F-DP complex, thereby allowing entry into S-phase. RB remains phosphorylated until the end of mitosis at which point it is dephosphorylated by protein phosphatase 1 (PP1) to activate the G1-S-phase checkpoint (PMID: 20694007). In addition to its role in G1 cell cycle arrest, RB1 has also been shown to play a role in safeguarding genome stability and mediating apoptosis, senescence and differentiation in response to various stimuli (PMID: 22293180). As a result of its role in these essential cellular functions, loss of function of RB1 not only leads to unregulated cell division and growth but also to the abrogation of multiple mechanisms that safeguard against cellular transformation and tumorigenesis. Loss-of-function and deletions of RB1 have been associated with many human cancers including lung, breast, prostate and bladder cancers, and concomitant loss of RB1 and p53 are thought to constitute a tumor-initiating event (PMID: 12204530). Homozygous loss or inactivation of the RB1 gene is a hallmark of retinoblastoma (PMID: 22293180), and germline mutations in RB1 and are at an increased risk of retinoblastoma as well as other cancer types (22205104).

- PBRM1 encodes a tumor suppressor and component of the SWI/SNF chromatin-remodeling complex. Inactivating mutations of PBRM1 are frequently found in renal carcinoma.
  The PBRM1 gene encodes the protein BAF180, which is a component of the nucleosome-remodeling complex switching defective/sucrose non-fermenting (SWI/SNF) (PMID: 21248752). Nucleosomes are histone octamers around which DNA is wrapped in order to regulate its exposure to transcription factors and RNA polymerases (PMID: 23113498). Remodeling complexes such as the SWI/SNF family serve to loosen, reposition and break DNA/histone contacts ultimately rendering the DNA accessible to modulation (PMID: 21654818). BAF180 contains 6 bromodomains, which bind to lysine residues in histone tails (PMID:19084573, PMID:22435813). Aberrations of each individual bromodomain are sufficient to disrupt the protein's function as a tumor suppressor (PMID:22435813, PMID:24613357). Specifically, one study demonstrated that BAF180 is among the key components required for p53-dependent cellular senescence (PMID:20660729). Additional work focusing on breast cancer cell lines identified BAF180 as a critical promoter in the induction of p21 activity, which functions as a key component of cell cycle regulatory functions (PMID:18339845). PBRM1 truncation mutants can no longer bind and remodel the p21 locus leading to cell cycle defects and aberrant cell proliferation (PMID: 18339845, 22949125). Loss of PBRM1 activity is also associated with chromosomal instability due to its inability to promote cohesion (PMID: 24613357).

- ALDH2, also known as ALDH2_HUMAN, ALDH2, ALDM. Homotetramer.

- ErbB4 (HER4) is one of the four members in the EGFR subfamily of receptor tyrosine kinases. Ligands include EGF, epiregulin, betacellulin and the neuregulins (Sundvall et. al.). Of these, NRG3 and NRG4 exclusively bind HER4 (Hynes et. al.).
  Mutations in ERBB4 have been identified in various cancer types including melanoma, lung adenocarcinoma and medulloblastoma. A therapeutic value of these aberrations still remains unknown (Arteaga et. al.).SUMMARY

ERBB4, a receptor tyrosine kinase, is altered at low to moderate frequencies in various cancer types, most frequently in melanoma and lung cancer.
ERBB4 (also HER4) is a transmembrane receptor that is a member of the ERBB family of receptor tyrosine kinases, including EGFR, ERBB2, and ERBB3 (PMID: 14504474, 9208852, 22427524, 25492965). Binding of ERBB4 by its ligands, including neuregulins (NRG1-4), betacellulin (BTC), HB-EGF or epiregulin (EPR), activates the receptor and initiates downstream signaling pathways including the canonical MAPK and PI3K/AKT/mTOR signaling cascades. ERBB4 forms active kinase dimers with other ERBB isoforms, including the preferential binding partner ERRB2 (PMID: 9130710, 10220407,19208461, 11252954). Somatic activating mutations in ERBB4 have been identified in melanoma (PMID: 22817889, 22842228, 24755198), lung adenocarcinoma (PMID: 18948947), gastric (PMID: 25079317, 25583476), and colorectal cancers (PMID: 22895193); however, the role of ERBB4 in cancer is not well established. Evidence in breast cancer cell lines suggests that ERBB4 is not required for mediating tumorigenesis in ERBB2-positive breast cancer but rather for mediating resistance to ERBB2 inhibitors (PMID: 25590338). Conversely, a subset of literature points to a growth-suppressive role for ERBB4 in breast cancer (PMID: 17120616, 24791013, 16832345, 20603612). The prognostic value of ERBB4 expression in cancer is still debatable, as reports have documented both better and worse outcomes in these tumors; however, this may be due to the lack of discrimination between ERBB4 variants (PMID: 25492965, 18454307).

- BACKGROUND
  GATA1 is a transcription factor that functions as a master regulator of hematopoietic differentiation (PMID: 1987478). GATA1 activates the expression of many important genes involved in erythroid and megakaryocyte development (PMID:7568185, 7823932), including the beta-globin gene and erythropoietin receptor (PMID:1924329, 1660143). Appropriate expression of GATA1 in hematopoietic progenitor cells is critical for the maturation of red blood cells, megakaryocytes, mast cells and eosinophils (PMID: 15659348). Loss of GATA1 expression in murine models suppresses the production of red blood cells (PMID: 8901585), highlighting the importance of GATA1 expression in erythroid development. Germline mutations in GATA1 are associated with anemia, thrombocytopenia and porphyria (PMID: 10700180, 11675338, 16783379, 17148589). Inherited GATA1 mutations have been implicated in Diamond Blackfan anemia due to the reduced translation of GATA1 protein (PMID: 24952648). GATA1 mutations observed in Down Syndrome patients are associated with acute megakaryocytic leukemia development and transient myeloid disorders (PMID: 12172547, 12747884). Reduced expression of GATA1 corresponds with the development of hematopoietic malignancies due to inadequate production of erythroid cell types (PMID: 12149188). Recurrent GATA1 rearrangements are found in patients with acute basophilic leukemia (PMID: 21474671).
  GATA1, a transcription factor involved in red blood cell and platelet development, is altered in several hematologic malignancies including transient leukemia and acute megakaryoblastic leukemia.

- HOXA11, also known as HXA11_HUMAN, HOXA11, HOX1I. Sequence-specific transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis.

- KMT2C, a tumor suppressor and histone methyltransferase, is altered in various solid and hematologic malignancies.
  KMT2C encodes the histone methyltransferase MLL3, an epigenetic modulator that methylates lysine residue 4 on the tail of histone H3 (H3K4). Methylation of H3K4 leads to increased genome accessibility, recruitment of transcriptional complexes, and activation of gene expression (PMID: 25998713). MLL3 specifically binds histones at enhancer regions, leading to increased expression of a wide range of target genes and regulation of enhancer RNA synthesis (PMID: 24081332, 23166019, 28483418, 27926873). MLL3 is ubiquitously expressed and its function is crucial for normal embryonal development and cell proliferation (PMID: 11718452, 17021013). Genetic deletion of the region containing KMT2C is the most common chromosomal abnormality in acute myeloid leukemia (PMID: 25794446, 11891048, 22234698, 25030029), and KMT2C is mutated in various types of cancer (PMID: 25537518, 25303977, 25151357, 28801450).

- NUP93 encodes subunit of the nucleoporin complex that controls the transport of molecules across the nuclear envelope.
  NUP93 is a subunit of the nuclear pore complex that is essential for the exchange of macromolecules across the nuclear envelope (PMID: 24572986). NUP93 activity promotes and maintains the correct assembly of the nuclear pore complex (PMID: 15229283, 22171326). Functional studies have suggested that NUP93 plays a role in gene regulation by tethering chromatin at superenhancer sites to generate the necessary structural environment for transcriptional repression or activation (PMID: 26341556, 27807035). Expression of NUP93, along with other members of the nucleoporin complex, is increased in cardiac tissue of patients with heart failure and decreased in the thymus of patients with Down syndrome (PMID: 23152829, 21856934). While NUP93 copy number alterations are observed in a variety of solid cancers (cBioPortal, MSKCC, Nov. 2017), NUP93 mutations in human cancers are not common. However, a recent analysis identified the NUP93 E14K mutation as a hotspot mutation with unknown function in multiple cancers (PMID: 26619011).

- RIT1 encodes a RAS-related GTPase that plays a role in neuronal development and differentiation. Activating mutations in RIT1 are frequent in Noonan syndrome and are found in a variety of cancers, including lung adenocarcinoma, hepatocellular carcinoma and myeloid malignancies.
  RIT1 encodes a RAS-related small GTP-binding protein that belongs to the RAS subfamily of small GTPases (PMID: 8824319, 10545207). These proteins function as binary molecular switches; in response to external stimuli, they exchange GDP with GTP, thereby triggering several intracellular signaling networks. RIT1 is widely expressed in adult and embryonic tissue, including primary neurons and the developing brain. It plays a central role in the induction of neuronal differentiation through activation of both the BRAF/ERK and the p38 MAP kinase signaling pathways (PMID: 15632082, 11914372). Additionally, RIT1 plays a role in regulating axonal versus dendritic growth, and it contributes to IFN-induced dendritic retraction (PMID: 17460085, 12668729, 18957053). RIT1 has been identified as a regulator of a p38/MAPK-dependent cascade that functions as a prosurvival mechanism in response to oxidative stress (PMID: 21737674, 21444726, 23123784). RIT1 gain-of-function mutations are associated with Noonan syndrome (PMID: 25049390, 24939608, 23791108); activating mutations in the Switch II domain of RIT1 have been observed in lung adenocarcinoma and myeloid malignancies (PMID: 25079552, 24469055, 23765226). The transforming potential of RIT1 is associated with the activation of a p38-dependent signaling pathway (PMID: 11821041, 15831491).

Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
https://ClinicalTrials.gov/show/NCT03272334	Recruiting	Her2-BATS and Pembrolizumab in Metastatic Breast Cancer	Metastatic Breast Cancer	HER2 BATs with Pembrolizumab	University of Virginia, Charlottesville, Virginia, United States
https://ClinicalTrials.gov/show/NCT02738866	Recruiting	Palbociclib With Fulvestrant for Metastatic Breast Cancer After Treatment With Palbociclib and an Aromatase Inhibitor	Metastatic Breast Cancer	Palbociclib Fulvestrant	Kimmel Cancer Center at Johns Hopkins at Sibley Memorial Hospital, Washington, District of Columbia, United States Anne Arundel Health System Research Institute, Inc., Annapolis, Maryland, United States Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, United States (and 2 more sites
https://ClinicalTrials.gov/show/NCT02913430	Recruiting	Phase II Treatment of Metastatic Breast Cancer With Fulvestrant Plus Palbociclib or Tamoxifen Plus Palbociclib	Metastatic Breast Cancer	Fulvestrant Tamoxifen Palbociclib	Magee-Womens Hospital UPMC, Pittsburgh, Pennsylvania, United States
https://ClinicalTrials.gov/show/NCT03326102	Recruiting	Oral Paclitaxel Efficacy Safety and PK in Recurrent and Metastatic Breast Cancer	Metastatic Breast Cancer	DHP107 IV Paclitaxel	California Research Institute (CRI), Los Angeles, California, United States University of California San Francisco (UCSF), San Francisco, California, United States Boca Raton Regional Hospital (BRRH), Boca Raton, Florida, United States (and 2 more sites
https://ClinicalTrials.gov/show/NCT04024436	Recruiting	A Study of TAS-120 in Patients With Metastatic Breast Cancer	Metastatic Breast Cancer	TAS-120 Fulvestrant	UT Southwestern, Dallas, Texas, United States MD Anderson, Houston, Texas, United States
https://ClinicalTrials.gov/show/NCT03787303	Recruiting	Study of Euthyroid Hypothyroxinemia in Metastatic Breast Carcinoma	Metastatic Breast Cancer	Triiodothyronine (T3)	Aultman Medical Group Hematology and Oncology, Canton, Ohio, United States
https://ClinicalTrials.g	Recruiting	MEN1611 With	Metastatic Breast	MEN1611	Holy Cross Hospital

ov/show/NCT03767335		Trastuzumab (+/- Fulvestrant) in Metastatic Breast Cancer	Cancer	Trastuzumab Fulvestrant	Inc., Fort Lauderdale, Florida, United States Detroit Clinical Research Center, Farmington Hills, Michigan, United States Washington University, Saint Louis, Missouri, United States (and 2 more sites
https://ClinicalTrials.gov/show/NCT03939897	Recruiting	Testing the Addition of Copanlisib to Usual Treatment (Fulvestrant and Abemaciclib) in Metastatic Breast Cancer - Dose-Finding Study	Metastatic Breast Cancer	Abemaciclib Copanlisib Hydrochloride Fulvestrant	University of Kentucky/Markey Cancer Center, Lexington, Kentucky, United States Siteman Cancer Center at West County Hospital, Creve Coeur, Missouri, United States Washington University School of Medicine, Saint Louis, Missouri, United States (and 2 more sites
https://ClinicalTrials.gov/show/NCT02595320	Recruiting	Capecitabine in Metastatic Breast and GI Cancers	Metastatic Breast Cancer	Capecitabine	University of Kansas Cancer Center - CRC, Fairway, Kansas, United States St. Catherine Hospital - Central Care Cancer Center, Garden City, Kansas, United States Heartland Cancer Center - Central Care Cancer Center, Great Bend, Kansas, United States (and 2 more sites
https://ClinicalTrials.gov/show/NCT03237572	Recruiting	Focused Ultrasound and Pembrolizumab in Metastatic Breast Cancer	Metastatic Breast Cancer	Pembrolizumab High-intensity focused ultrasound (HIFU)	University of Virginia, Charlottesville, Virginia, United States
https://ClinicalTrials.gov/show/NCT03344536	Recruiting	A Study of Debio 1347 Plus Fulvestrant in Patients With Metastatic Breast Cancer	Metastatic Breast Cancer	Fulvestrant Debio 1347	Memorioal Sloan Kettering Cancer Center, New York, New York, United States
https://ClinicalTrials.gov/show/NCT04039230	Recruiting	Study to Evaluate Sacituzumab Govitecan in Combination With Talazoparib in Patients With Metastatic Breast Cancer.	Metastatic Breast Cancer	Talazoparib Sacituzumab Govitecan	Massachusetts General Hospital Cancer Center, Boston, Massachusetts, United States
https://ClinicalTrials.gov/show/NCT02824575	Recruiting	Rebastinib Plus Antitubulin Therapy With Paclitaxel or Eribulin in Metastatic Breast Cancer	Breast Cancer	Rebastinib Paclitaxel Eribulin Mesylate	Montefiore Medical Center, Bronx, New York, United States

Detailed Results

Single Nucleotide Variant (SNV)

Gene name	Hgvsp	Hgvsc	Aminoacids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
RABEP1	NP_004694.2:p.Asn538Ser	NM_004703.4:c.1613A>G	N/S	aAt/aGt	missense_variant	80.64	155	deleterious (0.01)
CDH1	NP_004351.1:p.Cys60TrpfsTer34	NM_004360.3:c.179dupG	C/WX	tgc/tGgc	frameshift_variant	75.38	65	0
PTEN	NP_000305.3:p.Tyr16_Glu18delinsTer	NM_000314.4:c.48_53delTCAAGA	YQE/*	tATCAAGag/tag	"stop_gained,inframe_deletion"	51.67	120	0
CBFB	NP_074036.1:p.Lys20Ter	NM_022845.2:c.58A>T	K/*	Aag/Tag	stop_gained	49.4	83	0
RBBP6	NP_008841.2:p.Gln910ArgfsTer40	NM_006910.4:c.2729delA	Q/X	cAg/cg	frameshift_variant	38.14	118	0
RB1	NP_000312.2:p.Asn522IlefsTer2	NM_000321.2:c.1561delC	L/X	Ctt/tt	frameshift_variant	29.52	105	0
PBRM1	NP_060783.3:p.Glu374Lys	NM_018313.4:c.1120G>A	E/K	Gaa/Aaa	missense_variant	27.78	108	deleterious (0.01)
ALDH2	NP_000681.2:p.Arg11Cys	NM_000690.3:c.31C>T	R/C	Cgc/Tgc	missense_variant	24.59	61	deleterious (0.04)
PBRM1	NP_060783.3:p.Glu367Ter	NM_018313.4:c.1099G>T	E/*	Gaa/Taa	stop_gained	23.23	99	0
PBRM1	NP_060783.3:p.Met499Ile	NM_018313.4:c.1497G>A	M/I	atG/atA	missense_variant	23.08	52	tolerated (0.68)
ERBB4	NP_005226.1:p.Asn253Ser	NM_005235.2:c.758A>G	N/S	aAt/aGt	missense_variant	22.35	170	deleterious (0.01)
GATA1	NP_002040.1:p.Gly192Arg	NM_002049.3:c.574G>A	G/R	Gga/Aga	missense_variant	15.73	89	deleterious (0.02)
PBRM1	NP_060783.3:p.Glu441Gln	NM_018313.4:c.1321G>C	E/Q	Gaa/Caa	missense_variant	15.7	121	deleterious (0.04)
PBRM1	NP_060783.3:p.Glu443Lys	NM_018313.4:c.1327G>A	E/K	Gaa/Aaa	missense_variant	15.7	121	tolerated (0.57)
HOXA11	NP_005514.1:p.Met13Thr	NM_005523.5:c.38T>C	M/T	aTg/aCg	missense_variant	14.29	98	deleterious (0)
KMT2C	NP_733751.2:p.Gln2513Ter	NM_170606.2:c.7537C>T	Q/*	Cag/Tag	stop_gained	13.09	168	0
NUP93	NP_055484.3:p.Leu548Phe	NM_014669.4:c.1642C>T	L/F	Ctc/Ttc	missense_variant	11.19	143	deleterious (0.01)
KMT2C	NP_733751.2:p.Gln2903Glu	NM_170606.2:c.8707C>G	Q/E	Caa/Gaa	missense_variant	10.14	138	0
RIT1	NP_001243750.1:p.Asp121His	NM_001256821.1:c.361G>C	D/H	Gat/Cat	missense_variant	9.69	258	deleterious (0.01)

Fusion (translocation)

Gene Name	Fusion Reads (%)

Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes DNA for abnormalities in 434 genes that are reported to

be altered in various types of tumors. Nucleic acid is isolated from paraffin-embedded tissue. 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. Our sequencing method has a typical sensitivity of 3% for detecting common specific mutations and 5% for other mutations. Known hot spots in specific genes such as IDH1/2, NRAS, and KRAS are reported at levels of 1% and higher. Performance of the assay may vary dependent on the quantity and quality of nucleic acid, sample preparation and sample age. The assay is designed to detect significant gene amplification and deletion in addition to various single nucleotide variations (SNV) and indels. In addition to DNA analysis, targeted RNA NGS analysis was performed. This analyzes targeted RNA with a focus on 55 genes. It is based on hybrid capture of targeted RNA. Duplicates are excluded for levels measurements. While the major focus of the analysis is the detection of fusion mRNA, mutations in the expressed RNA of the analyzed genes are also analyzed and reported. mRNA expression levels are evaluated, and only significant high expression of specific genes are relatively reported. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated. If requested, detailed expression levels will be provided as a research data and not for clinical use. All detect fusion transcripts are reported. 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. The sensitivity of this assay in detecting fusion mRNA is between 1% and 5%. This assay is not designed to detect minimal residual disease and should be used for diagnosis when neoplastic cells are >10% of the analyzed cells. The Universal Human Reference (UHR) RNA is used as control.

Tested genes

Genes Tested for Abnormalities in Coding Sequence																
ABCS3:AE27B7	AURKB	C15ORF41	CEBPA	DICER1	FANCC	FLT3	GRIN2A	IRF2	LMO1	MSH2	NTRK1	POLE	RANBP2	SETD2	STAT4	TSC2
ABL1	AURKC	CALR	CHD2	DOT1L	FANCD2	FLT4	GRM3	IRF4	LPIN2	MSH6	NTRK2	POT1	RARA	SF3B1	STAT6	TSHR
ABL2	AXIN1	CARD11	CHD4	EED	FANCE	FOXL2	GSK3B	IRS2	LRP1B	MTOR	NTRK3	PPM1D	RB1	SLIT2	STK11	U2AF1
ACD	AXIN2	CBFB	CHEK1	EGFR	FANCF	FOXP1	GSKIP	JAGN1	LYN	MUTYH	NUP93	PPP2R1A	RBBP6	SLX4	SUFU	U2AF2
ACVR1B	AXL	CBL	CHEK2	EGLN1	FANCG	FRS2	H3F3A	JAK1	LYST	MVK	PAK3	PRDM1	RBM10	SMAD2	SUZ12	VEGFA
ADA	B2M	CBLB	CIC	ELANE	FANCI	FUBP1	HAX1	JAK2	LZTR1	MYC	PALB2	PREX2	RBM8A	SMAD3	SYK	VHL
AK2	BAP1	CBLC	CREBBP	EP300	FANCL	G6PC3	HGF	JAK3	MAGI2	MYCL	PARK2	PRKAR1A	RET	SMAD4	TAF1	WAS
AKT1	BARD1	CCND1	CRKL	EPAS1	FANCM	GABRA6	HIST1H3B	JUN	MAP2K1	MYCN	PAX5	PRKCI	RHEB	SMAD9	TAL1	WHSC1
AKT2	BCL2	CCND2	CRLF2	EPCAM	FAS	GALNT12	HNF1A	KAT6A	MAP2K2	MYD88	PBRM1	PRKDC	RHOA	SMAD9L	TBX3	WISP3
AKT3	BCL2L1	CCND3	CSF1R	EPHA3	FAT1	GATA1	HOXA11	KDM5A	MAP2K4	NBN	PDCD1LG2	PRSS1	RICTOR	SMARCA4	TCF3	WT1
ALK	BCL2L2	CCNE1	CSF3R	EPHA5	FBXW7	GATA2	HOXB13	KDM5C	MAP3K1	NF1	PDGFRA	PRSS8	RIT1	SMARCB1	TCIRG1	XPO1
AMER1	BCL6	CD274	CTC1	EPHA7	FGF10	GATA3	HRAS	KDM6A	MAP3K14	NF2	PDGFRB	PSTPIP1	RNF168	SMC1A	TERC	XRCC2
ANKRD26	BCOR	CD79A	CTCF	EPHB1	FGF14	GATA4	HSD3B1	KDR	MAPK1	NFE2L2	PDK1	PTCH1	RNF43	SMC3	TERF1	XRCC3
APC	BCORL1	CD79B	CTNNA1	ERBB2	FGF19	GATA6	HSP90AA1	KEAP1	MCL1	NFKBIA	PHF6	PTEN	ROS1	SMO	TERF2	ZBTB2
AR	BCR	CDAN1	CTNNB1	ERBB3	FGF23	GEN1	ID3	KEL	MDM2	NHP2	PIK3C2B	PTPN11	RPTOR	SNCAIP	TERF2IP	ZNF217
ARAF	BIRC3	CDC73	CUL3	ERBB4	FGF3	GFI1	IDH1	KIF23	MDM4	NKX2-1	PIK3CA	QKI	RTEL1	SOCS1	TERT	ZNF703
ARFRP1	BLM	CDH1	CUX1	ERCC4	FGF4	GFI1B	IDH2	KIT	MED12	NLRP3	PIK3CB	RAB27A	RUNX1	SOX10	TET2	ZRSR2
ARID1A	BMPR1A	CDK12	CXCR4	ERG	FGF6	GID4	IGF1R	KLF1	MEF2B	NME1	PIK3CG	RAC1	RUNX1T1	SOX2	TGFBR2	
ARID1B	BRAF	CDK4	CYLD	ERRFI1	FGFR1	GLI1	IGF2	KLHL6	MEFV	NOP10	PIK3R1	RAD21	SBDS	SOX9	TNFAIP3	
ARID2	BRCA1	CDK6	DAXX	ESR1	FGFR2	GLI2	IKBKE	KLLN	MEN1	NOTCH1	PIK3R2	RAD50	SBF2	SPEN	TNFRSF14	
ASXL1	BRCA2	CDK8	DDR2	ETV6	FGFR3	GNA11	IKZF1	KMT2A	Merged	NOTCH2	PIM1	RAD51	SDHA	SPOP	TNFRSF1A	
ATG2B	BRD4	CDKN1A	DDX11	EXO1	FGFR4	GNA13	IKZF3	KMT2B	MET	NOTCH3	PLCG1	RAD51B	SDHB	SPTA1	TOP1	
ATM	BRIP1	CDKN1B	DDX41	EZH2	FH	GNAQ	IL2RG	KMT2C	MITF	NPM1	PLCG2	RAD51C	SDHC	SRC	TOP2A	
ATR	BTG1	CDKN2A	DKC1	FAM175A	FLCN	GNAS	IL7R	KMT2D	MLH1	NRAS	PMS1	RAD51D	SDHD	SRSF2	TP53	
ATRX	BTK	CDKN2B	DNM2	FAM46C	FLI1	GPR124	INHBA	KRAS	MPL	NROB1	PMS2	RAD54L	SEC23B	STAG2	TRAF3	
AURKA	C11orf30	CDKN2C	DNMT3A	FANCA	FLT1	GREM1	INPP4B	LIG4	MRE11A	NSD1	POLD1	RAF1	SETBP1	STAT3	TSC1	
* Microsatellite markers BAT25, BAT26, D2S123, D5S346, and D17S250 are included.																

RNA Fusions/Expression

Fusion/Expression													
ABL1	BCL2	CBFB	ERG	FGFR2	FOXO1	IKZF3	MAP3K1	NTRK1	NUP98	PICALM	RHOA	SS18	TCF3

AKT3	BCL6	CIC	ETV6	FGFR3	FUS	JAK2	MECOM	NTRK2	PDGFRA	PML	ROS2	STAT6	TFG
ALK	BRAF	CREBBP	EWSR1	FIP1L1	GLI1	KIAA1549	MYC	NTRK3	PDGFRB	RARA	RUNX1	TAFG	YWHAE
BCL1	CAMTA1	EGFR	FGFR1	FLAG1	HMGA2	KMT2A	NOTCH1	NUP214	PD-L1	RET	RUNX1T1	TAL1	

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Electronic Signature

Maher Albitar, M.D.

The Technical Component Processing, Analysis and Professional Component of this test was completed at Genomic Testing Cooperative, LCA, 175 Technology Drive, Suite 100, Irvine, CA 92618. Medical Director: Maher Albitar, M.D..

The performance characteristics of this test have been determined by GTC Laboratories. 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.