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CLIA #: 05D2111917 CAP #: 9441547

# Liquid Trace Solid Tumor

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

| | | | |
|---|---|---|---|
| Collected Date: | | Indication for Testing: | Carcinoma of Pancreas |
| Received Date: | | Stage: | Metastasis |
| Reported Date: | | | |

## Detected Genomic Alterations

| Level 1 (FDA-Approved) | Level 2 (Standard of Care) | Level 3 (Clinical Evidence) | Level 4 (Biological Evidence) | Other |
|---|---|---|---|---|
| No evidence of BRCA1/2 or PALB2 mutations | - | KRAS (A146V) | TET2, KMT2C, CTCF | No detectable autosomal chromosomal structural gain or loss |

## Results Summary

- **-Low level mutations in KMT2C, TET2, CTCF, and KRAS genes**
  **-No detectable autosomal chromosomal structural gain or loss**
  **-No evidence of BRCA1/2 or PALB2 mutations**
  **-EBV viral RNA: Not detected**
  **-HPV viral RNA: Not detected**

  -These findings suggest the presence of circulating solid tumor DNA/RNA. The TET2 mutation is most likely in myeloid cells, consistent with CHIP (clonal hematopoiesis of indeterminate potential).

  -KRAS (A146V) mutation suggests resistance to Sotorasib, but possible response to ERK/MEK inhibitors (Selumetinib, Trametinib, Binimetinib, Vemurafenib, Cobimetinib..).

## Tumor Heterogeneity

There is an abnormal low-level clone with KMT2C, TET2, CTCF, and KRAS mutations.

## Diagnostic Implications

| | |
|---|---|
| KMT2C, TET2, CTCF, KRAS | These findings suggest the presence of circulating solid tumor DNA/RNA (see result summary). |

| Relevant Alteration Associated with Resistance |
|---|
| KRAS mutations suggest resistance to targeted anti-EGFR therapy. |

| Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence) | |
|---|---|
| TET2 | DNA methyltransferase inhibitors |
| KRAS | ERK/MEK inhibitors |

| Relevant Genes with NO Alteration | | |
|---|---|---|
| No evidence of mutation in: NRAS, EGFR, BRAF, TP53 | No evidence of: FGFR1, FGFR2, FGFR3, FGFR4, NTRK, ALK, ROS1, RET Fusion | No evidence of MET14 deletion, EGFR Viii |

## Test Description:

This is a comprehensive molecular profile of cell-free DNA (cfDNA) and cell-free RNA (cfRNA), which uses next generation sequencing (NGS) to identify molecular abnormalities (including SNVs, INDELS, CNVs, Fusions, EBV and HPV) in DNA of 284 genes and RNA in 1501 genes associated with solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

## Biological relevance of detected Alterations

- KMT2C. This gene is a member of the myeloid/lymphoid or mixed-lineage leukemia (MLL) family and encodes a nuclear protein with an AT hook DNA-binding domain, a DHHC-type zinc finger, six PHD-type zinc fingers, a SET domain, a post-SET domain and a RING-type zinc finger. This protein is a member of the ASC-2/NCOA6 complex (ASCOM), which possesses histone methylation activity and is involved in transcriptional coactivation. [provided by RefSeq, Jul 2008]

- TET2. The protein encoded by this gene is a methylcytosine dioxygenase that catalyzes the conversion of methylcytosine to 5-hydroxymethylcytosine. The encoded protein is involved in myelopoiesis, and defects in this gene have been associated with several myeloproliferative disorders. Two variants encoding different isoforms have been found for this gene. [provided by RefSeq, Mar 2011]

- CTCF. This gene is a member of the BORIS + CTCF gene family and encodes a transcriptional regulator protein with 11 highly conserved zinc finger (ZF) domains. This nuclear protein is able to use different combinations of the ZF domains to bind different DNA target sequences and proteins. Depending upon the context of the site, the protein can bind a histone acetyltransferase (HAT)-containing complex and function as a transcriptional activator or bind a histone deacetylase (HDAC)-containing complex and function as a transcriptional repressor. If the protein is bound to a transcriptional insulator element, it can block communication between enhancers and upstream promoters, thereby regulating imprinted expression. Mutations in this gene have been associated with invasive breast cancers, prostate cancers, and Wilms' tumors. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jul 2010]

- KRAS. This gene, a Kirsten ras oncogene homolog from the mammalian ras gene family, encodes a protein that is a member of the small GTPase superfamily. A single amino acid substitution is responsible for an activating mutation. The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma, mucinous adenoma, ductal carcinoma of the pancreas and colorectal carcinoma. Alternative splicing leads to variants encoding two isoforms that differ in the C-terminal region. [provided by RefSeq, Jul 2008]

## Drug Information

### Binimetinib (Mektovi)

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 (Cotellic)

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 (Mekinist)

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 URL | Status | Title | Disease | Drug | Sites |
|---|---|---|---|---|---|
| https://ClinicalTrials.gov/show/NCT05052723 | Recruiting | Cabozantinib and Pembrolizumab in Metastatic Pancreas | Metastatic Pancreatic Cancer | Cabozantinib<br>Pembrolizumab | University of Kentucky Markey Cancer Center, Lexington, Kentucky, United States |
| https://ClinicalTrials.gov/show/NCT05102721 | Recruiting | Trial of Immunotherapy With Avelumab and Pepinemab As Second Line For Patients With Metastatic Pancreatic Adenocarcinoma | Metastatic Pancreatic Cancer | Avelumab and Pepinemab | University of Rochester Medical Center, Rochester, New York, United States |
| https://ClinicalTrials.gov/show/NCT02340117 | Recruiting | Study of Combined SGT-53 Plus Gemcitabine/Nab-Paclitaxel for Metastatic Pancreatic Cancer | Metastatic Pancreatic Cancer | SGT-53<br>nab-paclitaxel<br>Gemcitabine | Mary Crowley Cancer Research Center, Dallas, Texas, United States |
| https://ClinicalTrials.gov/show/NCT01954992 | Recruiting | Glufosfamide Versus 5-FU in Second Line Metastatic Pancreatic Cancer | Metastatic Pancreatic Cancer | Glufosfamide<br>Fluorouracil | Comprehensive Cancer Care Medical Group, Inc., Corona, California, United States<br>Hao Wei Zhang MD, Inc., Los Angeles, California, United States<br>Innovative Clinical Research Institute, Whittier, California, United States |

## Detailed Results

### Single Nucleotide Variant (SNV) and Insertions-Deletions (INDELS)

| Gene Name | Hgvsp | Hgvsc | Aminoacids | Codons | Consequence | Allele frequency | Read depth | Predicted effect on protein |
|---|---|---|---|---|---|---|---|---|
| KMT2C | NP_733751.2:p.Cys394Tyr | NM_170606.2:c.1181G>A | C/Y | tGc/tAc | missense_variant | 4.89 | 2576 | 0 |
| TET2 | NP_001120680.1:p.His1382Arg | NM_001127208.2:c.4145A>G | H/R | cAc/cGc | missense_variant | 2.72 | 956 | deleterious (0) |
| CTCF | NP_006556.1:p.Thr374Ile | NM_006565.3:c.1121C>T | T/I | aCt/aTt | missense_variant | 1.56 | 768 | deleterious (0) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| KRAS | NP_203524.1:p.Ala146Val | NM_033360.2:c.437C>T | A/V | gCa/gTa | missense_variant | 0.43 | 1172 | deleterious (0.02) |

## Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA for abnormalities in 284 genes that are reported to be altered in various types of hematologic neoplasms. Nucleic acid is isolated from plasma. Testing is performed using 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. Using UMI, our sequencing method has a typical sensitivity of less than 0.1% for detecting common specific mutations and 0.1% for other mutations. 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. Performance of the assay may vary depending 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. Indels greater than 80bp may not be detected.
In addition to cfDNA analysis, targeted cfRNA NGS analysis is performed. This is a next generation sequencing (NGS) test that analyzes targeted cfRNA on 1,501 genes associated with hematologic neoplasms. It is based on hybrid capture of targeted cfRNA. Duplicates are excluded for levels measurements. While the major focus of the analysis is the detection of fusion mRNA, mutations in the expressed cfRNA of the analyzed genes are also analyzed and reported.
All detected 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. Since the clinical relevance of the expression level of most of these genes is not characterized at this time, only few specific genes (MYC, BCL2, CD274, CD19, CD22, CD79A, CD79B) will be commented on. .The sensitivity of this assay in detecting fusion mRNA is between 1% and 5%. The Universal Human Reference (UHR) RNA is also used as control.

The table below contains 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)

The table below contains a partial list of the tested RNA genes (Fusions/Expression). For a complete list, 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 | AURKA | BRCA2 | CDK4 | CXCR4 | ERBB4 | FGF6 | GNAQ | IL7R | LRP1B | MRE11 | NPM1 | PLCG1 | RAD51 | SMARCB1 | TERT | XRCC3 |
| ACVR1B | AURKB | BRIP1 | CDK6 | CYLD | ERG | FGFR1 | GNAS | INHBA | MAP2K1 | MSH2 | NRAS | PMS1 | RAF1 | SMC1A | TET2 | ZNF217 |
| AKT1 | AURKC | BTK | CDKN2A | DAXX | ESR1 | FGFR2 | GREM1 | IRF4 | MAP2K2 | MSH6 | NSD1 | PMS2 | RB1 | SMC3 | TGFBR2 | ZRSR2 |
| AKT2 | AXIN1 | CALR | CDKN2B | DDR2 | ETV6 | FGFR3 | GRIN2A | JAK1 | MAP2K4 | MTOR | NTRK1 | POLD1 | RET | SMO | TNFAIP3 | NFE2 |
| AKT3 | AXIN2 | CARD11 | CDKN2C | DICER1 | EXO1 | FGFR4 | H3-3A | JAK2 | MAP3K1 | MUTYH | NTRK2 | POLE | RHEB | SOCS1 | TNFRSF14 | UBA1 |
| ALK | B2M | CBL | CEBPA | DNM2 | EZH2 | FH | HGF | JAK3 | MAP3K14 | MYC | NTRK3 | PPM1D | RHOA | SOX2 | TP53 | STAT5B |
| AMER1 | PRDM1 | AR | BCL2L1 | CCND1 | CIC | EED | FANCA | FLT4 | HOXB13 | KDM6A | MDM2 | MYD88 | PAX5 | PRKAR1A | ARAF | BCL6 |
| APC | BCL2 | CBLC | CHEK2 | DOT1L | TENT5C | FLT3 | HNF1A | KDM5C | MCL1 | MYCN | PALB2 | PRDM1 | RNF43 | SPOP | TSC1 | ELANE |
| AR | BCL2L1 | CCND1 | CIC | EED | FANCA | FLT4 | HOXB13 | KDM6A | MDM2 | MYD88 | PAX5 | PRKAR1A | ROS1 | SRC | TSC2 | ANKRD26 |
| ARAF | BCL6 | CCND3 | CREBBP | EGFR | FANCC | FOXL2 | HRAS | KDR | MDM4 | NF1 | PBRM1 | PRKDC | RUNX1 | SRSF2 | TSHR | SAMD9L |
| ARID1A | BCOR | CCNE1 | CRLF2 | EGLN1 | FANCD2 | FUBP1 | HSP90AA1 | KEAP1 | MED12 | NF2 | PDGFRA | PRSS1 | SDHB | STAG2 | U2AF1 | SAMD9 |
| ARID1B | BCORL1 | CD274 | CSF1R | EP300 | FANCE | GALNT12 | ID3 | KIT | MEF2B | NFE2L2 | PDGFRB | PTCH1 | SETBP1 | STAT3 | U2AF2 | DDX41 |
| ARID2 | BCR | CD79A | CSF3R | EPAS1 | FANCF | GATA1 | IDH1 | KMT2A | MEN1 | NFKBIA | PHF6 | PTEN | SETD2 | STK11 | VHL | - |
| ASXL1 | BIRC3 | CD79B | CTCF | EPHA3 | FANCG | GATA2 | IDH2 | KMT2B | MET | NKX2-1 | PIK3CA | PTPN11 | SF3B1 | SUFU | NSD2 | - |
| ATM | BLM | CDC73 | CTNNA1 | EPHA5 | FAS | GATA3 | IGF1R | KMT2C | MITF | NOTCH1 | PIK3R1 | RAC1 | SMAD2 | SUZ12 | WT1 | - |
| ATR | BRAF | CDH1 | CTNNB1 | ERBB2 | FBXW7 | GEN1 | IKZF1 | KMT2D | MLH1 | NOTCH2 | PIK3R2 | RAD21 | SMAD4 | TAL1 | XPO1 | - |
| ATRX | BRCA1 | CDK12 | CUX1 | ERBB3 | FGF4 | GNA11 | IKZF3 | KRAS | MPL | NOTCH3 | PIM1 | RAD50 | SMARCA4 | TCF3 | XRCC2 | - |

## Reference

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5. Chemotherapy for pancreatic cancer. Springfeld C, Jger D, Bchler MW, Strobel O, Hackert T, Palmer DH, Neoptolemos JP. Springfeld C, et al. Presse Med. 2019 Mar;48(3 Pt 2):e159-e174. doi: 10.1016/j.lpm.2019.02.025. Epub 2019 Mar 15. Presse Med. 2019. PMID: 30879894

6. Trametinib and Hydroxychloroquine (HCQ) Combination Treatment in KRAS-Mutated Advanced Pancreatic Adenocarcinoma: Detailed Description of Two Cases. Xavier CB, Marchetti KR, Castria TB, Jardim DLF, Fernandes GS. Xavier CB, et al. J Gastrointest Cancer. 2021 Mar;52 (1):374-380. doi: 10.1007/s12029-020-00556-z. Epub 2020 Nov 23. J Gastrointest Cancer. 2021. PMID: 33225411

### Electronic Signature

Ahmad Charifa, 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 by 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.