# Solid Tumor Profile Plus

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

| | | | | | |
|---|---|---|---|---|---|
| MRN: | | | | Indication for Testing: | |
| Collected Date: | 05/14/2015 | Time: | 12:00 AM | Tumor Type: | |
| Received Date: | 08/21/2019 | Time: | 10:37 AM | | |
| Reported Date: | 09/02/2019 | Time: | 09:49 AM | | |

## 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 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 |
| PIK3CA | -FGFR2-TACC2 fusion and FGFR2 gene amplification; -Tumor Mutation Burden (TMB): High (above upper quartile). | -CREBBP, TP53, MAP2K4. -There is no evidence of microsatellite instability or HER2 amplification | EPHA7 | Multiple chromosomal structural abnormalities involving gene amplification of SETBP1 and LYST |

| Tumor Heterogeneity |
|---|
| There is a dominant abnormal clone with PIK3CA, CREBBP, TP53, EPHA7, and MAP2K4 mutations. |

| Expression | |
|---|---|
| High expression of FGFR2 mRNA | Expression of fusion FGFR2-TACC2 mRNA resulting from interstitial deletion on 10q26.13 |

| Diagnostic Implications | |
|---|---|
| PIK3CA, CREBBP, TP53, EPHA7, MAP2K4; High expression of FGFR1 and FGFR2 mRNA; Tumor Mutation Burden (high) | These abnormalities are consistent with aggressive neoplasm. |

| Prognostic Implications | |
|---|---|
| PIK3CA | Poor |
| CREBBP | Poor |
| TP53 | Poor |
| EPHA7 | Unknown |
| MAP2K4 | Poor |
| High expression of FGFR1 and FGFR2 mRNA | Favorable |
| Tumor Mutation Burden (high) | Poor |

| FDA-Approved Therapeutics | |
|---|---|
| PIK3CA | ALPELISIB |

| FDA-Approved Therapeutics in Other Tumor Types | |
|---|---|
| FGFR1 abnormalities | ERDAFITINIB |

| Relevant Alteration Associated with Resistance |
|---|
| PIK3CA mutations may predict resistance to anti-RTK therapy, including cetuximab, anti-EGFR TKIs, and trastuzumab and lapatinib. |
| TP53 mutation is associated with resistance to therapy. |

| Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence) | |
|---|---|
| PIK3CA | PI3K, AKT, MTOR inhibitors (Alpelisib) |
| FGFR1 abnormalities | FGFR inhibitors (Erdafitinib) |
| CREBBP | Inhibitors targeting EP300 |
| TP53 | Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy |
| MAP2k4 | MEK inhibitors |
| TMB (high) | immunotherapy with anti-PD-1 or anti-PD-L1 |

| Relevant Genes with NO Alteration | | |
|---|---|---|
| No evidence of fusion mRNA involving ALK, RET, ROS1, or NTRK | No evidence of mutation in: KRAS, NRAS, EGFR, BRAF | No evidence of MET14 deletion, EGFR Viii |
| There is no evidence of microsatellite instability. | - | - |

## Results Summary

- **-Expression of fusion FGFR2-TACC2, t(10;10)**
  **-Amplification and High expression of FGFR2 mRNA**
  **-Mutations in PIK3CA, CREBBP, TP53, EPHA7, and MAP2K4 genes.**
  **-Multiple structural chromosomal abnormalities involving amplification of SETBP1, LYST and other Genes**
  **-High Tumor Mutation Burden (TMB): 20 (above upper quartile).**

  -The presence of PIK3CA mutation suggests sensitivity to the dual PI3K/mTOR inhibitors (Alpelisib).

  -The abnormalities in FGFR2 gene suggest sensitivity to FGFR inhibition (Erdafitinib).

  -The high TMB suggests possible response to immunotherapy with anti-PD-1 or anti-PD-L1.

  -CREBBP mutations suggests possible response to inhibitors targeting EP300.

  -TP53 mutation is considered an adverse prognostic marker and is associated with higher rate of metastasis and resistance to therapy. Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins may have a therapeutic value in cancers with TP53 mutation.

  -MAP2K4 mutation suggests possible response to MEK inhibitors

  -There is no evidence of microsatellite instability.

## Biological relevance of detected Alterations

- PIK3CA is the most recurrently mutated gene in breast cancer, and has been found to important in a number of cancer types. An integral part of the PI3K pathway, PIK3CA has long been described as an oncogene, with two main hotspots for activating mutations, the 542/545 region of the helical domain, and the 1047 region of the kinase domain. PIK3CA, and its interaction with the AKT and mTOR pathways, is the subject of an immense amount of research and development, and PI3K inhibition has seen some limited success in recent clinical trials. While monotherapies seem to be limited in their potential, there is a recent interest in pursuing PI3K inhibition as part of a combination therapy regiment with inhibition partners including TKI's, MEK inhibitors, PARP inhibitors, and in breast cancer, aromatase inhibitors.SUMMARY
  PIK3CA, the catalytic subunit of PI3-kinase, is frequently mutated in a diverse range of cancers including breast, endometrial and cervical cancers.
  BACKGROUND
  Phosphatidylinositol-3-kinase (PI3K) is comprised of a regulatory subunit (p85) as well as a catalytic subunit (p110) and it is the catalytic subunit that is encoded by the PIK3CA gene. PIK3CA is among the most commonly mutated genes in cancer and aberrant activation of PI3K is a transforming event (PMID: 17376864). Multiple receptor tyrosine kinases, including EGFR, ERBB2 (HER2), RET, MET, and VEGFR, among others, convert extracellular cues into intracellular signals and recruit PI3K to the plasma membrane via scaffold proteins such as IRS1 or by activating RAS. Upon stimulation, PI3K-110 converts its lipid substrate PIP2 (phosphatidylinositol - 4,5 - bisphosphate) to PIP3 (phosphatidylinositol - 3,4,5 - bisphosphate), which activates several signaling cascades, including the well-characterized AKT-mTOR pathway. Once activated, AKT-mTOR downstream signaling promotes cell survival, proliferation, growth and motility (PMID:16341083). Adding to this complexity, exposure to some PI3K/mTOR pathway targeted drugs relieves cancer cells of self-regulatory properties inherent in the PI3K-AKT-mTOR pathway thereby promoting tumor resistance to these agents (PMID: 22576208).

- CREBBP, a tumor suppressor and transcriptional co-activator, is frequently inactivated in hematologic malignancies.
  BACKGROUND
  CREBBP (CREB binding protein) is a transcriptional co-activator with intrinsic histone acetyltransferase (HAT) activity; it is closely homologous to the co-activator EP300 (PMID: 8004670, 18273021). As a co-factor, CREBBP binds to DNA binding proteins where it functions as a scaffold to recruit a range of transcription complex components (PMID: 9215639, 9445474). CREBBP itself can recruit the basal transcriptional machinery, and through its HAT activity acetylate lysine tails to modify chromatin into a more open conformation for active transcription (PMID: 8576192,8967953). The HAT activity of CREBBP is also active on non-histone proteins, including tumor suppressors such as p53 and tissue-specific transcription factors such as GATA1 (PMID: 9830059, 9859997). In leukemias, CREBBP can be disrupted by translocations that fuse the HAT domain with the MOZ/KAT6A (lysine acetyltransferase 6a) protein t(8;16) (PMID: 9447825). Somatic mutations of CREBBP have been found in leukemia, lymphoma and solid tumors including small-cell lung cancer, squamous carcinoma and bladder cancer (PMID: 24670651, 21796119, 21390130, 25151357, 21390130). Most CREBBP mutations are truncating and commonly co-occur with loss of the wildtype allele, suggesting that CREBBP is a tumor suppressor. Inherited mutations can result in the Rubinstein-Taybi syndrome with stereotypical facial and digit abnormalities along with neurological deficits (PMID:7630403). CREBBP-mutated tumors are dependent on EP300 activity and inhibitors targeting EP300 are efficacious in CREBBP-mutated cell line and mouse models (PMID: 26603525).

- 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).

- EPHA7, a receptor tyrosine kinase, is altered by mutation or deletion in various cancer types, most frequently in skin cancers.
  BACKGROUND
  EPHA7 (ephrin receptor A7) is a part of the subfamily of the protein-tyrosine kinase family and preferentially binds glycosylphosphatidylinositol (GPI)-anchored ligands. EPHA7 is composed of one tyrosine kinase domain and an extracellular domain that contains a ligand-binding domain, a cysteine-rich domain, and two fibronectin type III repeats (PMID 10197531, 9883737). EPHA7 structurally resembles other members of the Eph family and is associated with promoting cellular transformation, invasion, and proliferation through the JAK2 signaling pathway (PMID: 24003208, 20179713, 22862837). Differential expression levels of EPHA7 and diverse roles in carcinogenesis have been shown in various cancer types (PMID: 26160986). Studies have shown overexpression of EPHA7 in glioblastoma, breast, and gallbladder adenocarcinoma, which promotes cell proliferation and migration through the FGFR1 signaling pathway and is correlated with poor prognosis (PMID 18790757, 15147954, 18366728). Other studies have shown downregulation of EPHA7 by hypermethylation in gastric, colorectal, esophageal squamous cell and prostate cancers, as well as in follicular lymphomas; and its association with tumor progression (PMID 17669470, 16007213, 26160986, 18821581, 29022918, 22036564).

- MAP2K4, a tumor suppressor and intracellular kinase, is altered in various cancer types.
  BACKGROUND
  MAP2K4 is a dual specificity kinase that directly phosphorylates and activates JNK (c-Jun N-terminal kinase) and p38 MAP kinase (PMID: 7716521). MAP2K4 itself is phosphorylated and activated by one of several upstream MAP kinase kinase kinases (MAPKKK). The activation of JNK and p38 MAP kinase pathways occurs in response to a variety of environmental stressors, such as DNA damage, hypoxia, heat shock, ionizing radiation, as well as inflammatory cytokines and growth factors (PMID: 17496914,19629069). Activation of these signaling pathways leads to altered transcriptional activity of downstream effector molecules such as c-Jun, p53, ELK1, ATF2 and several other transcription factors involved in apoptosis, cell survival, growth and differentiation (PMID: 17496914,19629069). Inactivating mutations in MAP2K4 have been identified in a variety of tumor cell lines and human cancers, suggesting that MAP2K4 functions primarily as a tumor suppressor (PMID: 9331070, 9622070, 17496914, 22522925). Loss of heterozygosity of the 17p chromosomal region, where MAP2K4 is located in close proximity to TP53, is observed at a high frequency in many cancers (PMID: 16721048, 16627982, 19603523). In addition, MAP2K4 has been found to act as a suppressor of metastasis in prostate and ovarian cell lines (PMID: 10554023, 12438272).

# Drug Information

## 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.

## ERDAFITINIB

Erdafitinib is subsequently an oral selective pan-FGFR kinase inhibitor that binds to and inhibits the enzymatic activity of expressed FGFR1, FGFR2, FGFR3, and FGFR4. Erdafitinib demonstrates inhibition of FGFR phosphorylation and signaling as well as decreased cell viability in cell lines expressing FGFR genetic alterations, including point mutations, amplifications, and fusions. Erdafitinib demonstrated antitumor activity in FGFR-expressing cell lines and xenograft models derived from tumor types.

## IPATASERTIB

Ipatasertib is an orally administered, ATP-competitive, selective AKT inhibitor. AKT is a key component of the PI3K/AKT pathway. This pathway is dysregulated in many malignancies, often through acquisition of activating mutations in AKT and phosphatidylinositol 3-kinase (PI3K), loss of the tumor suppressor phosphatase and tensin homolog (PTEN), or amplification of AKT and PI3K.

## ATEZOLIZUMAB (TECENTRIQ)

Atezolizumab is a monoclonal antibody that binds to PD-L1 and blocks its interactions with both PD-1 and B7.1 receptors. This prevents PD-L1/PD-1 mediated inhibition of the immune response, including activation of the anti-tumor immune response without inducing antibody-dependent cellular cytotoxicity.

Atezolizumab is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who: 1) have disease progression during or following platinum-containing chemotherapy, and 2) have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy.

Currently the only antibody against PD-L1 and available as the product Tecentriq (FDA), atezolizumab is indicated for the treatment of locally advanced or metastatic urothelial carcinoma.

## PEMBROLIZUMAB (KEYTRUDA)

Pembrolizumab is a highly selective IgG4-kappa humanized monoclonal antibody against PD-1 receptor. It was generated by grafting the variable sequences of a very high-affinity mouse antihuman PD-1 antibody onto a human IgG4-kappa isotype with the containing a stabilizing S228P Fc mutation.

## FULVESTRANT (FASLODEX)

Fulvestrant is a drug treatment of hormone receptor (HR)-positive metastatic breast cancer in post-menopausal women with disease progression following anti-estrogen therapy. It is an estrogen receptor antagonist with no agonist effects, which works both by down-regulating and by degrading the estrogen receptor. While it is used as monotherapy for the treatment of breast cancers, it is also used in combination with alpelisib for the treatment of HR-positive, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated, advanced or metastatic breast cancer.

Fulvestrant competitively and reversibly binds to estrogen receptors present in cancer cells and achieves its anti-estrogen effects through two separate mechanisms. First, fulvestrant binds to the receptors and downregulates them so that estrogen is no longer able to bind to these receptors. Second, fulvestrant degrades the estrogen receptors to which it is bound. Both of these mechanisms inhibit the growth of tamoxifen-resistant as well as estrogen-sensitive human breast cancer cell lines.

## ABEMACICLIB (VERZENIO)

Abemaciclib is an antitumor agent and dual inhibitor of cyclin-dependent kinases 4 (CDK4) and 6 (CDK6) that are involved in the cell cycle and promotion of cancer cell growth in case of unregulated activity. On September 28, 2017, FDA granted approval of Abemaciclib treatment under the market name Verzenio for the treatment of HR-positive and HER2-negative advanced or metastatic breast cancer that has progressed after unsuccessful endocrine therapy. Unlike other CDK inhibitors such as Palbociclib and Ribociclib, Abemaciclib exhibits greater selectivity for CDK4 compared to CDK6.

## PALBOCICLIB (IBRANCE)

Palbociclib is an investigational selective, small-molecule inhibitor of CDK4 and CDK6. CDK4 and CDK6 along with their regulatory partner cyclin D1 play a key role in regulating the G1- to S-phase cell-cycle transition via regulation of phosphorylation of the retinoblastoma (Rb) protein. Inhibition of

these proteins leads to reduced phosphorylation of Rb, inhibition of downstream signalling, and increased tumor growth arrest.

Palbociclib is indicated in combination with letrozole for the treatment of postmenopausal women with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer as initial endocrine-based therapy for their metastatic disease.

### RIBOCICLIB (KISQALI)

Ribociclib inhibits both CDK4 and CDK6. An orally available cyclin-dependent kinase (CDK) inhibitor targeting cyclin D1/CDK4 and cyclin D3/CDK6 cell cycle pathway, with potential antineoplastic activity. CDK4/6 inhibitor LEE011 specifically inhibits CDK4 and 6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation. Inhibition of Rb phosphorylation prevents CDK-mediated G1-S phase transition, thereby arresting the cell cycle in the G1 phase, suppressing DNA synthesis and inhibiting cancer cell growth. Overexpression of CDK4/6, as seen in certain types of cancer, causes cell cycle deregulation.

### AZD1775

Wee1 G2 checkpoint serine/threonine protein kinase inhibitor.

AZD1775 is a highly selective, potent, ATP competitive, small molecule inhibitor of Wee1 kinase with an enzyme IC50 of 5.18 nM. In vitro, AZD1775 inhibits Wee1 activity and induces DNA damage as well as G2 checkpoint escape in cell-based assays with an EC50 of about 80 nM. AZD1775 increases cytotoxicity when used in combination with DNA damaging agents, such as gemcitabine, cisplatin, carboplatin and topotecan, in p53deficient cell lines. In vivo, AZD1775 is well tolerated and shows enhancement of anti-tumor efficacy by gemcitabine, carboplatin, cisplatin, 5fluorouracil (5-FU) and capecitabine in nude rat xenograft tumor models. Similarly, in nude mouse xenograft models, AZD1775 treatment results in significant tumor growth inhibition at tolerated doses and also enhances the anti-tumor growth effect of gemcitabine, carboplatin and radiation therapy.

## Potential Clinical Trials

| Trial URL | Status | Title | Disease | Drug | Sites |
|---|---|---|---|---|---|
| https://ClinicalTrials.gov/show/NCT03959891 | Recruiting | AKT Inhibitor, Ipatasertib, With Endocrine and CDK 4/6 Inhibitor for Patients With Metastatic Breast Cancer (TAKTIC) | Breast Cancer | Ipatasertib<br>Fulvestrant<br>Aromatase Inhibitor<br>Palbociclib | Massachusetts General Hospital Cancer Center, Boston, Massachusetts, United States |
| https://ClinicalTrials.gov/show/NCT03285412 | Recruiting | CDK 4/6 Inhibitor, LEE011 (Ribociclib), in Combination With Adjuvant Endocrine Therapy at Varying Duration for ER-positive Breast Cancer | Breast Cancer | Ribociclib<br>Endocrine therapy | Massachusetts General Hospital, Boston, Massachusetts, United States<br>Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States<br>Mass General/North Shore Cancer Center, Danvers, Massachusetts, United States |
| https://ClinicalTrials.gov/show/NCT02953860 | Recruiting | Fulvestrant Plus Enzalutamide in ER +/Her2- Advanced Breast Cancer | Breast Cancer | Fulvestrant with Enzalutamide | University of Colorado, Aurora, Colorado, United States<br>Lone Tree Medical Center, Lone Tree, Colorado, United States<br>West Cancer Center, Germantown, Tennessee, United States |

| | | | | | |
|---|---|---|---|---|---|
| https://ClinicalTrials.gov/show/NCT03789110 | Recruiting | NIMBUS: Nivolumab Plus Ipilimumab in Metastatic Hypermutated HER2-negative Breast Cancer | Breast Cancer | Nivolumab<br>Ipilimumab | Dana-Farber Cancer Institute, Boston, Massachusetts, United States |
| https://ClinicalTrials.gov/show/NCT02776917 | Recruiting | Study of Cirmtuzumab and Paclitaxel for Metastatic or Locally Advanced, Unresectable Breast Cancer | Breast Neoplasms | Cirmtuzumab + Paclitaxel | University of California, San Diego, La Jolla, California, United States |
| https://ClinicalTrials.gov/show/NCT02623972 | Recruiting | A Phase 2 Study of Eribulin Followed by AC as Preoperative Therapy for HER2-negative Inflammatory Breast Cancer | Inflammatory Breast Cancer | Eribulin<br>Adriamycin<br>Cyclophosphamide | Brigham and Womens Hospital, Boston, Massachusetts, United States<br>Dana-Farber Cancer Institute, Boston, Massachusetts, United States |
| https://ClinicalTrials.gov/show/NCT02955394 | Recruiting | Preoperative Fulvestrant With or Without Enzalutamide in ER+/Her2- Breast Cancer | Breast Cancer | Enzalutamide<br>Fulvestrant | University of Colorado, Aurora, Colorado, United States<br>Memorial Sloan Kettering Cancer Center, New York, New York, United States<br>West Cancer Center, Germantown, Tennessee, United States |
| https://ClinicalTrials.gov/show/NCT04072952 | Recruiting | Clinical Trial of ARV-471 in Patients With ER+/HER2- Locally Advanced or Metastatic Breast Cancer | Breast Cancer | ARV-471 | UCLA Medical Center, Santa Monica, California, United States<br>Norwalk Hospital, Norwalk, Connecticut, United States<br>Moffitt Cancer Center, Tampa, Florida, United States<br>(and 1 more sites) |
| https://ClinicalTrials.gov/show/NCT02632045 | Recruiting | Study of Efficacy of Ribociclib After Progression on CDK4/6 Inhibition in Patients With HR+ HER2- Advanced Breast Cancer | Metastatic Breast Cancer | LEE-011<br>Fulvestrant<br>Placebo | University of Alabama at Birmingham (UAB), Birmingham, Alabama, United States<br>Northwestern Medical Hospital, Chicago, Illinois, United States<br>Albert Einstein University / Montefiore Medical Center, Bronx, New York, United States<br>(and 8 more sites) |
| https://ClinicalTrials.gov/show/NCT02738866 | Recruiting | Palbociclib With Fulvestrant for Metastatic Breast Cancer After Treatment With Palbociclib and an | Metastatic Breast Cancer | Palbociclib<br>Fulvestrant | Kimmel Cancer Center at Johns Hopkins at Sibley Memorial Hospital, Washington, District of Columbia, United States |

| | | | | | |
|---|---|---|---|---|---|
| | | Aromatase Inhibitor | | | Anne Arundel Health System Research Institute, Inc., Annapolis, Maryland, United States<br>Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, United States<br>(and 2 more sites) |

## Detailed Results

### Single Nucleotide Variant (SNV)

| Gene name | Hgvsp | Hgvsc | Aminoacids | Codons | Consequence | Allele frequency | Read depth | Predicted effect on protein |
|---|---|---|---|---|---|---|---|---|
| PIK3CA | NP_006209.2:p.Glu545Lys | NM_006218.2:c.1633G>A | E/K | Gag/Aag | missense_variant | 32.41 | 145 | deleterious (0.02) |
| CREBBP | NP_004371.2:p.Gln1970HisfsTer6 | NM_004380.2:c.5909_5910insCCATACCGAGACTGATTCCTCTTGCTTAATGTACAGTATT | Q/HPYRD*FLLLNVQYX | cag/caCCCATACCGAGACTGATTCCTCTTGCTTAATGTACAGTATTg | stop_gained,frameshift_variant | 31.82 | 22 | 0 |
| TP53 | NP_000537.3:p.Gln144Ter | NM_000546.5:c.430C>T | Q/* | Cag/Tag | stop_gained | 29.27 | 41 | 0 |
| EPHA7 | NP_004431.1:p.Glu850ArgfsTer30 | NM_004440.3:c.2546dupT | I/IX | ata/atTa | frameshift_variant | 29.09 | 55 | 0 |
| MAP2K4 | NP_001268364.1:p.Gly9AlafsTer17 | NM_001281435.1:c.25_26insCC | G/AX | ggc/gCCgc | frameshift_variant | 27.27 | 22 | 0 |

### 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 | Solid Tumor Profile Plus | 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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Amended Reason: Added TMB and MSI data.

## Electronic Signature

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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.