

## Solid Tumor Profile Plus

Patient Name:	<input type="text"/>	Ordering Physician:	<input type="text"/>
Date of Birth:	<input type="text"/>	Accession #:	<input type="text"/>
Gender (M/F):	F	Specimen Type:	<input type="text"/>
Client:	<input type="text"/>	Specimen ID:	<input type="text"/>
Case #:	<input type="text"/>		
Body Site:	Breast		

MRN:	<input type="text"/>	Indication for Testing:	<input type="text"/>
Collected Date:	05/14/2015	Time:	12:00 AM
Received Date:	08/21/2019	Time:	10:37 AM
Reported Date:	09/02/2019	Time:	09:49 AM
		Tumor Type:	<input type="text"/>

### 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.
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### 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
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### FDA-Approved Therapeutics in Other Tumor Types

FGFR1 abnormalities	ERDAFITINIB
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### 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 regimen 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 p53 deficient cell lines. In vivo, AZD1775 is well tolerated and shows enhancement of anti-tumor efficacy by gemcitabine, carboplatin, cisplatin, 5-fluorouracil (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
<a href="https://ClinicalTrials.gov/show/NCT03959891">https://ClinicalTrials.gov/show/NCT03959891</a>	Recruiting	AKT Inhibitor, Ipatasertib, With Endocrine and CDK 4/6 Inhibitor for Patients With Metastatic Breast Cancer (TAKTIC)	Breast Cancer	Ipatasertib Fulvestrant Aromatase Inhibitor Palbociclib	Massachusetts General Hospital Cancer Center, Boston, Massachusetts, United States
<a href="https://ClinicalTrials.gov/show/NCT03285412">https://ClinicalTrials.gov/show/NCT03285412</a>	Recruiting	CDK 4/6 Inhibitor, LEE011 (Ribociclib), in Combination With Adjuvant Endocrine Therapy at Varying Duration for ER-positive Breast Cancer	Breast Cancer	Ribociclib Endocrine therapy	Massachusetts General Hospital, Boston, Massachusetts, United States Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States Mass General/North Shore Cancer Center, Danvers, Massachusetts, United States
<a href="https://ClinicalTrials.gov/show/NCT02953860">https://ClinicalTrials.gov/show/NCT02953860</a>	Recruiting	Fulvestrant Plus Enzalutamide in ER +/-Her2- Advanced Breast Cancer	Breast Cancer	Fulvestrant with Enzalutamide	University of Colorado, Aurora, Colorado, United States Lone Tree Medical Center, Lone Tree, Colorado, United States West Cancer Center, Germantown, Tennessee, United States

<a href="https://ClinicalTrials.gov/show/NCT03789110">https://ClinicalTrials.gov/show/NCT03789110</a>	Recruiting	NIMBUS: Nivolumab Plus Ipilimumab in Metastatic Hypermutated HER2-negative Breast Cancer	Breast Cancer	Nivolumab Ipilimumab	Dana-Farber Cancer Institute, Boston, Massachusetts, United States
<a href="https://ClinicalTrials.gov/show/NCT02776917">https://ClinicalTrials.gov/show/NCT02776917</a>	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
<a href="https://ClinicalTrials.gov/show/NCT02623972">https://ClinicalTrials.gov/show/NCT02623972</a>	Recruiting	A Phase 2 Study of Eribulin Followed by AC as Preoperative Therapy for HER2-negative Inflammatory Breast Cancer	Inflammatory Breast Cancer	Eribulin Adriamycin Cyclophosphamide	Brigham and Womens Hospital, Boston, Massachusetts, United States Dana-Farber Cancer Institute, Boston, Massachusetts, United States
<a href="https://ClinicalTrials.gov/show/NCT02955394">https://ClinicalTrials.gov/show/NCT02955394</a>	Recruiting	Preoperative Fulvestrant With or Without Enzalutamide in ER+/Her2- Breast Cancer	Breast Cancer	Enzalutamide Fulvestrant	University of Colorado, Aurora, Colorado, United States Memorial Sloan Kettering Cancer Center, New York, New York, United States West Cancer Center, Germantown, Tennessee, United States
<a href="https://ClinicalTrials.gov/show/NCT04072952">https://ClinicalTrials.gov/show/NCT04072952</a>	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 Norwalk Hospital, Norwalk, Connecticut, United States Moffitt Cancer Center, Tampa, Florida, United States (and 1 more sites)
<a href="https://ClinicalTrials.gov/show/NCT02632045">https://ClinicalTrials.gov/show/NCT02632045</a>	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 Fulvestrant Placebo	University of Alabama at Birmingham (UAB), Birmingham, Alabama, United States Northwestern Medical Hospital, Chicago, Illinois, United States Albert Einstein University / Montefiore Medical Center, Bronx, New York, United States (and 8 more sites)
<a href="https://ClinicalTrials.gov/show/NCT02738866">https://ClinicalTrials.gov/show/NCT02738866</a>	Recruiting	Palbociclib With Fulvestrant for Metastatic Breast Cancer After Treatment With Palbociclib and an	Metastatic Breast Cancer	Palbociclib 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 Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, United States (and 2 more sites)
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## Detailed Results

Single Nucleotide Variant (SNV)								
Gene name	Hgvsnp	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_5910insCCATACCGAGACTGATTCTTGGCTTAATGTACAGTATT	Q/HPYRD*FLL LNVQYX	cag/caCCCCA TACCGAGA CTGATTCCCT CTTGCTTAA TGTACAGTA TTg	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	XP01
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	GF11	IDH1	KIF23	MDM4	NKX2-1	PIK3CA	QKI	RTKL1	SOCS1	TERT	ZNF703
ARFRP1	BLM	CDH1	CUX1	ERCC4	FGF4	GF11B	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	TGFB2	
ARID1B	BRAF	CDK4	CYLD	ERRF1	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	

## Reference

1. Fulvestrant Plus Vistusertib vs Fulvestrant Plus Everolimus vs Fulvestrant Alone for Women With Hormone Receptor-Positive Metastatic Breast Cancer: The MANTA Phase 2 Randomized Clinical Trial. Schmid P, Zaiss M, Harper-Wynne C, Ferreira M, Dubey S, Chan S, Makris A, Nemsadze G, Brunt AM, Kuemmel S, Ruiz I, Perell A, Kendall A, Brown J, Kristeleit H, Conibear J, Saura C, Grenier J, Mhr K, Schenker M, Sohn J, Lee KS, Shepherd CJ, Oelmann E, Sarker SJ, Prendergast A, Marosics P, Moosa A, Lawrence C, Coetzee C, Mousa K, Corts J. JAMA Oncol. 2019 Aug 29. doi: 10.1001/jamaoncol.2019.2526. [Epub ahead of print] PMID: 31465093

2. Efficacy and safety of palbociclib plus endocrine therapy in North American women with hormone receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer. Gelmon KA, Cristofanilli M, Rugo HS, DeMichele AM, Joy AA, Castrellon A, Sleckman B, Mori A, Theall KP, Lu DR, Huang X, Bananis E, Finn RS, Slamon DJ. *Breast J.* 2019 Aug 25. doi: 10.1111/tbj.13516. [Epub ahead of print] PMID: 31448513
3. First-Line Treatment for Endocrine-Sensitive Bone-Only Metastatic Breast Cancer: Systematic Review and Meta-analysis. Toss A, Venturelli M, Sperduti I, Molinaro E, Isca C, Barbieri E, Piacentini F, Omarini C, Cortesi L, Cascinu S, Moscetti L. *Clin Breast Cancer.* 2019 Jul 24. pii: S1526-8209(19)30418-5. doi: 10.1016/j.clbc.2019.06.011. [Epub ahead of print] Review. PMID: 31427171
4. EMERALD: Phase III trial of elacestrant (RAD1901) vs endocrine therapy for previously treated ER+ advanced breast cancer. Bardia A, Aftimos P, Bihani T, Anderson-Villaluz AT, Jung J, Conlan MG, Kaklamani VG. *Future Oncol* 2019 Aug 20. doi: 10.2217/fo-2019-0370. [Epub ahead of print] PMID: 31426673
5. Neoadjuvant endocrine therapy: A potential strategy for ER-positive breast cancer. Yao LT, Wang MZ, Wang MS, Yu XT, Guo JY, Sun T, Li XY, Xu YY. *World J Clin Cases.* 2019 Aug 6;7(15):1937-1953. doi: 10.12998/wjcc.v7.i15.1937. Review. PMID: 31423426
6. Palbociclib in metastatic breast cancer: current evidence and real-life data. Serra F, Lapidari P, Quaquareni E, Tagliaferri B, Sottotetti F, Palumbo R. *Drugs Context.* 2019 Jul 16;8:212579. doi: 10.7573/dic.212579. eCollection 2019. Review. PMID: 31391852
7. Metastatic breast cancer patients with lung or liver metastases should be distinguished before being treated with fulvestrant. He M, Li JJ, Zuo WJ, Ji L, Jiang YZ, Hu XC, Wang ZH, Shao ZM. *Cancer Med.* 2019 Aug 2. doi: 10.1002/cam4.2453. [Epub ahead of print] PMID: 31373147
8. FGFR1 gene amplification mediates endocrine resistance but retains TORC sensitivity in metastatic hormone receptor positive (HR+) breast cancer. Drago JZ, Formisano L, Juric D, Niemierko A, Servetto A, Wander SA, Spring LM, Vidula N, Younger J, Peppercorn J, Yuen M, Malvarosa G, Sgroi D, Isakoff SJ, Moy B, Ellisen LW, Iafrate AJ, Arteaga CL, Bardia A. *Clin Cancer Res.* 2019 Aug 1. pii: clincanres.0138.2019. doi: 10.1158/1078-0432.CCR-19-0138. [Epub ahead of print] PMID: 31371343
9. Cyclin-dependent kinase 4/6 inhibitors for the management of advanced or metastatic breast cancer in women. Cersosimo RJ. *Am J Health Syst Pharm.* 2019 Aug 1;76(16):1183-1202. doi: 10.1093/ajhp/zxz121. PMID: 31369120
10. Therapeutic Ligands Antagonize Estrogen Receptor Function by Impairing Its Mobility. Guan J, Zhou W, Hafner M, Blake RA, Chalouni C, Chen IP, De Bruyn T, Giltmane JM, Hartman SJ, Heidersbach A, Houtman R, Ingalla E, Kategaya L, Kleinheinz T, Li J, Martin SE, Modrusan Z, Nannini M, Oeh J, Ubhayakar S, Wang X, Wertz IE, Young A, Yu M, Sampath D, Hager JH, Friedman LS, Daemen A, Metcalfe C. *Cell.* 2019 Aug 8;178(4):949-963.e18. doi: 10.1016/j.cell.2019.06.026. Epub 2019 Jul 25. PMID: 31353221
11. Overexpression of TK1 and CDK9 in plasma-derived exosomes is associated with clinical resistance to CDK4/6 inhibitors in metastatic breast cancer patients. Del Re M, Bertolini I, Crucitta S, Fontanelli L, Rofi E, De Angelis C, Diodati L, Cavallero D, Gianfilippo G, Salvadori B, Fogli S, Falcone A, Scatena C, Naccarato AG, Roncella M, Ghilli M, Morganti R, Fontana A, Danesi R. *Breast Cancer Res Treat.* 2019 Jul 26. doi: 10.1007/s10549-019-05365-y. [Epub ahead of print] PMID: 31346846
12. Selective oestrogen receptor degraders in breast cancer: a review and perspectives. Gombos A. *Curr Opin Oncol.* 2019 Sep;31(5):424-429. doi: 10.1097/CCO.0000000000000567. PMID: 31335829
13. Somatic BRCA2 Mutation-Positive Concurrent Accessory Male Breast Cancer (BC) and Non-Small Cell Lung Cancer (NSCLC): Excellent Efficacy of Palbociclib, Fulvestrant and Leuprolide in Platinum-Exposed and Endocrine-Refractory BC Associated with Cyclin D1 and FGFR1 Amplification and of Carboplatin, Paclitaxel and Radiation in NSCLC. Cheng Y, Li N, Eapen A, Parajuli R, Mehta R. *Case Rep Oncol.* 2019 Jun 26;12(2):494-499. doi: 10.1159/000501304. eCollection 2019 May-Aug. PMID: 31320873
14. ESR1 mutations in breast cancer. Dustin D, Gu G, Fuqua SAW. *Cancer.* 2019 Jul 18. doi: 10.1002/cncr.32345. [Epub ahead of print] Review. PMID: 31318440

15. Mitochondrial and ribosomal biogenesis are new hallmarks of stemness, oncometabolism and biomass accumulation in cancer: Mito-stemness and ribo-stemness features. Peiris-Pags M, Ozsvri B, Sotgia F, Lisanti MP. Aging (Albany NY).2019 Jul 16;11(14):4801-4835. doi: 10.18632/aging.102054. PMID: 31311889
16. MONALEESA clinical program: a review of ribociclib use in different clinical settings. Yardley DA. Future Oncol. 2019 Aug;15(23):2673-2686. doi: 10.2217/fon-2019-0130. Epub 2019 Jul 15. PMID: 31305131
17. Multicenter Observational Study of Fulvestrant 500 mg in Postmenopausal Japanese Women with Estrogen Receptor-Positive Advanced or Recurrent Breast Cancer after Prior Endocrine Treatment (SBCCSG29 Study). Kimizuka K, Inoue K, E Nagai S, Saito T, Nakano S, Futsuhara K, Yamada H, Kaneko S, Sakurai T, Hata S, Kurosumi M. J Nippon Med Sch.2019;86(3):165-171. doi: 10.1272/jnms.JNMS.2019\_86-305. PMID: 31292328

Amended Reason: Added TMB and MSI data.

## Electronic Signature

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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.