The F1CDx assay will be performed at Foundation Medicine, Inc. Rubraca (rucaparib) maintenance therapy in accordance with the RUBRACA product label. Rubraca (rucaparib) maintenance therapy in accordance with the associated with improved progression-free survival (PFS) from tBRCA-positive and/or LOH high) in ovarian cancer patients is (LOH) from FFPE ovarian tumor tissue. Positive homologous therapeutic product.

Additionally, F1CDx is intended to provide tumor mutation profiling in vitro diagnostic device for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The F1CDx assay is the first FDA-approved broad companion diagnostic for solid tumors.

Microsatellite Instability (MSI) results, confirmatory testing using a validated orthogonal method should be performed.

Other alterations & biomarkers identified

Results reported in this section are not prescriptive or conclusive for labeled use of any specific therapeutic product. See professional services section for additional information.

Microsatellite status MS-Stable §
Tumor Mutational Burden 5 Muts/Mb §
CDK4 amplification §
ESR1 Y537S

§ Refer to appendix for limitation statements related to detection of any copy number alterations, gene rearrangements, BRCA1/2 alterations, LOH, MSI, or TMB results in this section.

Please refer to appendix for Explanation of Clinical Significance Classification and for variants of unknown significance (VUS).

<table>
<thead>
<tr>
<th>Genomic Findings Detected</th>
<th>FDA-Approved Therapeutic Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA E542K</td>
<td>Piqray® (Alpelisib)</td>
</tr>
</tbody>
</table>

For Microsatellite Instability (MSI) results, confirmatory testing using a validated orthogonal method should be performed.

Table 1: Companion Diagnostic Indications

<table>
<thead>
<tr>
<th>Indication</th>
<th>Genomic Alteration</th>
<th>Therapeutic Option</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-small cell lung cancer (NSCLC)</td>
<td>EGFR exon 19 deletions and EGFR exon 21 L858R alterations</td>
<td>Gloit® (Afatinib), Iressa® (Geftinib), Tagrisso® (Osimertinib), or Tarceva® (Erlotinib)</td>
</tr>
<tr>
<td></td>
<td>EGFR exon 20 G719S alterations</td>
<td>Tagrisso® (Osimertinib)</td>
</tr>
<tr>
<td></td>
<td>ALK rearrangements</td>
<td>Alecensa® (Alcetinib), Xalkori® (Crizotinib), or Zyrdar® (Ceritinib)</td>
</tr>
<tr>
<td></td>
<td>BRAF V600E</td>
<td>Tafinlar® (Dabrafenib) in combination with Mekinist® (Trametinib)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>BRAF V600E</td>
<td>Tafinlar® (Dabrafenib) or Zelboraf® (Vemurafenib)</td>
</tr>
<tr>
<td></td>
<td>BRAF V600E and V600K</td>
<td>Mekinist® (Trametinib) or Cotellic® (Cibimetinib) in combination with ZeBora® (Vemurafenib)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>ERBB2 (HER2) amplification</td>
<td>HERceptin® (Trastuzumab), Kadcyla® (Ado-trastuzumab-emtansine), or Perjeta® (Pertuzumab)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>KRAS wild-type (absence of mutations in codons 12 and 13)</td>
<td>Erbitux® (Cetuximab)</td>
</tr>
<tr>
<td></td>
<td>KRAS wild-type (absence of mutations in exons 2, 3, and 4)</td>
<td>Vectibix® (Panitumumab)</td>
</tr>
<tr>
<td></td>
<td>KRAS wild-type (absence of mutations in exons 2, 3, and 4) and NRAS wild-type (absence of mutations in exons 2, 3, and 4)</td>
<td>Vectibix® (Panitumumab)</td>
</tr>
<tr>
<td></td>
<td>Ovarian cancer</td>
<td>BRCA1/2 alterations</td>
</tr>
</tbody>
</table>

Note: The intended use (IU) statement and claims made on this sample report may not be up to date. For the latest version of the FoundationOne CDx claims and IU, please see the current label: www.foundationmedicine.com/1cdx
Biomarker Findings

Microsatellite status - MS-Stable

Tumor Mutational Burden - 5 Muts/Mb

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

CDK4 amplification

ESR1 Y537S

PIK3CA E542K

PTEN T319fs*1

FGFR2 amplification

TP53 splice site 559+1G>A

3 Disease relevant genes with no reportable alterations: BRCA1, BRCA2, ERBB2

8 Therapies with Clinical Benefit

35 Clinical Trials

3 Therapies with Lack of Response

BIOMARKER FINDINGS

Microsatellite status - MS-Stable

Tumor Mutational Burden - 5 Muts/Mb

ACTIONABILITY

No therapies or clinical trials. see Biomarker Findings section

No therapies or clinical trials. see Biomarker Findings section
### GENOMIC FINDINGS

**CDK4 - amplification**
- 10 Trials [see p. 17](#)

**ESR1 - Y537S**
- 10 Trials [see p. 19](#)

**PIK3CA - E542K**
- 10 Trials [see p. 23](#)

**PTEN - T319fs**
- 10 Trials [see p. 25](#)

**FGFR2 - amplification**
- 9 Trials [see p. 21](#)

### THERAPIES WITH CLINICAL BENEFIT

<table>
<thead>
<tr>
<th>GENOMIC ALTERATION</th>
<th>THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)</th>
<th>THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CDK4</strong></td>
<td>Palbociclib</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>Ribociclib</td>
<td>none</td>
</tr>
<tr>
<td><strong>ESR1</strong></td>
<td>Fulvestrant</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>Anastrozole</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Exemestane</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Letrozole</td>
<td>None</td>
</tr>
<tr>
<td><strong>PIK3CA</strong></td>
<td>Alpelisib</td>
<td>Temsirolimus</td>
</tr>
<tr>
<td></td>
<td>Everolimus</td>
<td>Temsirolimus</td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td>Everolimus</td>
<td>Temsirolimus</td>
</tr>
<tr>
<td><strong>FGFR2</strong></td>
<td>none</td>
<td>Erdafitinib</td>
</tr>
<tr>
<td></td>
<td>Pazopanib</td>
<td></td>
</tr>
</tbody>
</table>

1. Patient may be resistant to indicated therapy

### GENOMIC FINDINGS WITH NO REPORTABLE THERAPEUTIC OR CLINICAL TRIAL OPTIONS

For more information regarding biological and clinical significance, including prognostic, diagnostic, germline, and potential chemosensitivity implications, see the Genomic Findings section.

**TP53 - splice site 559+1G>A**

<table>
<thead>
<tr>
<th>NOTE</th>
<th>Genomic alterations detected may be associated with activity of certain FDA-approved drugs; however, the agents listed in this report may have varied clinical evidence in the patient's tumor type.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither the therapeutic agents nor the trials identified are ranked in order of potential or predicted efficacy for this patient, nor are they ranked in order of level of evidence for this patient's tumor type.</td>
<td></td>
</tr>
</tbody>
</table>
Microsatellite status

RESULT
MS-Stable

FREQUENCY & PROGNOSIS
No MSI was observed in two large scale analyses of breast cancer samples. However, in Lynch syndrome-related breast cancer, MSI has been reported in 51-85% of cases. A prospective study observed increased MSI following chemotherapy treatment, and MSI is associated with incidence of secondary tumors.

FINDING SUMMARY
Microsatellite instability (MSI) is a condition of genetic hypermutability that generates excessive amounts of short insertion/deletion mutations in the genome; it generally occurs at microsatellite DNA sequences and is caused by a deficiency in DNA mismatch repair (MMR) in the tumor. Defective MMR and consequent MSI occur as a result of genetic or epigenetic inactivation of one of the MMR pathway proteins, primarily MLH1, MSH2, MSH6, or PMS2. This sample is microsatellite-stable (MSS), equivalent to the clinical definition of an MSS tumor: one with mutations in none of the tested microsatellite markers.

Tumor Mutational Burden

RESULT
5 Muts/Mb

FREQUENCY & PROGNOSIS
Breast carcinoma harbors a median TMB of 3.8 muts/Mb, and 3.1% of cases have high TMB (>20 muts/Mb). The Breast Invasive Carcinoma TCGA analysis reported an average (non-silent) mutation load of 0.84 muts/Mb for luminal A tumors, 1.38 muts/Mb for luminal B tumors, 2.05 muts/Mb for HER2-enriched tumors, and 1.68 muts/Mb for basal-like tumors. In breast cancer, TMB is significantly higher in recurrent versus primary tumors and CDH1-mutated versus CDH1-wildtype tumors.

FINDING SUMMARY
Tumor mutational burden (TMB, also known as mutation load) is a measure of the number of somatic protein-coding base substitution and insertion/deletion mutations occurring in a tumor specimen. TMB is affected by a variety of causes, including exposure to mutagens such as ultraviolet light in melanoma and cigarette smoke in lung cancer. Mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes, and microsatellite instability (MSI) are associated with higher TMB. This sample harbors a TMB level associated with lower rates of clinical benefit from treatment with PD-1- or PD-L1 inhibitors.

The content provided as a professional service by Foundation Medicine, Inc., has not been reviewed or approved by the FDA.
Although ESR1 mutations have been reported in malignancies and treatment has also been reported to provide breast cancer (NCCN Guidelines v1.2019). AI (ER+) and/or hormone receptor-positive (HR+) production, are approved to treat ER-positive patients with CDK4-amplified solid tumors in response to therapies that directly target ER-alpha, such as palbociclib. On the basis of a Phase 1b study, PTEN loss of expression may be associated with resistance to combination therapy with CDK4/6 inhibitors such as palbociclib and aromatase inhibitors such as letrozole.

FREQUENCY & PROGNOSIS
Putative high-level amplification of CDK4 occurs in 2% of breast invasive carcinoma cases. CDK4 protein expression has been detected in 70% of breast carcinomas in one study and did not correlate with patient survival.

FINDING SUMMARY
CDK4 encodes the cyclin-dependent kinase 4, which regulates the cell cycle, senescence, and apoptosis. CDK4 and its functional homolog CDK6 are activated by D-type cyclins and promote cell cycle progression by inactivating the tumor suppressor RB. Amplification of the chromosomal region that includes CDK4 has been reported in multiple cancer types, including lung cancer, glioblastoma, and liposarcoma, and has been associated with overexpression of CDK4 protein.

FREQUENCY & PROGNOSIS
The most frequent ESR1 mutations include D538G, Y537S, Y537N, and E380Q, with concurrent ESR1 mutations detected in up to 40% of ER+ breast cancer samples harboring an ESR1 alteration. In the TCGA breast invasive carcinoma datasets, ESR1 amplification was observed in 2 to 3% of cases and ESR1 mutation was observed in fewer than 1% of cases. Rarely identified in patients with localized disease, ESR1 mutations are more frequently detected in metastatic breast cancers. ESR1 mutations may confer sensitivity to the first-generation SERD fulvestrant. In a study of patients with breast cancer treated with fulvestrant as monotherapy or in combination with palbociclib, ESR1 Y537S was the most commonly acquired mutation, suggesting that Y537S may decrease fulvestrant sensitivity.

FINDING SUMMARY
ESR1 encodes estrogen receptor alpha (ER-alpha), one of the major estrogen receptor isoforms in humans. Along with co-activator proteins, the ER complex promotes transcription of genes involved in cell cycle progression and survival. Alterations that occur within the ligand binding domain of ER-alpha, as seen here, result in ligand-independent activation. Emerging clinical evidence suggests that these alterations confer resistance to aromatase inhibitors including anastrozole, letrozole, and exemestane.
PIK3CA

Alteration: E542K

Transcript Number: NM_006218

Coding Sequence Effect: 1624G>A

Potential Treatment Strategies

Clinical and preclinical data in various tumor types indicate that PIK3CA activating alterations may predict sensitivity to therapies targeting PI3K or AKT\(^{106-107}\). On the basis of clinical benefit for patients with PIK3CA mutations and preclinical evidence, PIK3CA-mutated tumors may also respond to mTOR inhibitors, including everolimus and temsirolimus\(^{108-109}\). In a Phase 1 trial of the dual PI3K/mTOR kinase inhibitor apitolisib, 79\% (11/14) of patients with PIK3CA-mutated advanced solid tumors experienced disease control at the recommended Phase 2 dose (3/14 PRs, 8/14 SDs)\(^{110}\). The addition of everolimus to exemestane for the treatment of hormone-receptor-positive (HR+)/HER2-negative advanced breast cancer has shown clinical benefit regardless of PIK3CA status\(^{111}\). In the BELLE-2 trial for patients with endocrine-resistant HR+ breast cancer, the combination of the pan-PI3K inhibitor buparlisib with fulvestrant resulted in increased PFS (7.0 vs. 3.2 months) and ORR (18% vs. 4\%) compared to placebo with fulvestrant in patients with PIK3CA mutation; no significant improvement in PFS or ORR was observed in patients without PIK3CA mutation\(^{112}\). The pan-PI3K inhibitor buparlisib has shown limited activity as monotherapy against PIK3CA-mutated tumors\(^{113-114}\). PIK3CA-mutated tumors may also respond to therapies targeting PI3K-alpha specifically, as shown in a Phase 2 trial of alpelisib with letrozole in advanced HR+/HER2– breast cancer treated with either taselisib or placebo (18.1%, n=193) or placebo (10.0%, n=80)\(^{115}\). In the phase 3 SOLAR-1 study, the addition of alpelisib to fulvestrant improved PFS (11.0 vs. 5.7 months, HR=0.65) and ORR (26.6 vs. 12.8\%) in PIK3CA-mutated HR+/HER2– breast cancer compared with placebo with fulvestrant\(^{116}\). Combination of alpelisib with letrozole in advanced HR+/HER2– breast cancer achieved an ORR of 25% (4/16) and a DCR of 62% (10/16) in patients with PIK3CA-mutated tumors and an ORR of 10\% (1/10) and a DCR of 70\% (7/10) in patients with PIK3CA-wild-type tumors\(^{117}\). In the phase 3 SANDPIPER study, the addition of taselisib to fulvestrant improved PFS (7.4 vs. 5.4 months, HR=0.76) and ORR (27.3 vs. 11.9\%) in PIK3CA-mutated HR+/HER2– breast cancer compared with placebo with fulvestrant\(^{118}\); additionally, patients with multiple PIK3CA mutations achieved a higher ORR following treatment with taselisib (30.2\%, n=43) as compared with those treated with placebo (8.7\%, n=23) or with patients with single PIK3CA-mutated tumors treated with either taselisib (18.1\%, n=103) or placebo (10.0\%, n=80)\(^{119}\). AKT inhibitors ipatasertib and capivasertib have also been tested in breast cancer. Two Phase 2 studies have reported improved PFS from the addition of either ipatasertib (9.0 vs. 4.9 months, HR=0.44) or capivasertib (9.3 vs. 3.7 months, HR=0.30) to paclitaxel in metastatic triple-negative breast cancer harboring PIK3CA/AKT1/PTEN alterations, compared with paclitaxel and placebo\(^{120}\). Responses to capivasertib were also reported in 20\% (3/15) of patients with PIK3CA-mutated breast cancer in an earlier study\(^{121}\). However, a Phase 1 trial reported no PFS benefit for patients with PIK3CA-mutated, ER+/HER2– metastatic breast cancer from the addition of capivasertib to paclitaxel compared with paclitaxel plus placebo (10.9 vs. 10.8 months)\(^{122}\). Activating mutations in PIK3CA may confer resistance to HER2-targeted therapies; combined inhibition of HER2 and the PI3K pathway may be required in HER2-positive tumors with PIK3CA mutation\(^{123-124}\). In the context of concurrent PIK3CA mutation, PTEN loss or mutation may predict resistance to PIK3CA-alpha-specific inhibitors\(^{125-126}\).

Frequency & Prognosis

Mutations in PIK3CA have been reported in 25-40\% of breast cancer cases\(^{273-278}\). Although double PIK3CA mutations are frequently observed in hormone-receptor-positive, HER2-negative breast cancers, as compared with other receptor subtypes (15.4\% vs. 5.4\%, p=0.004), this did not impact invasive disease-free survival or OS for patients when compared with single PIK3CA mutations by univariate and multivariate analysis in 1 retrospective study\(^{24}\). Mutations in coding exon 20 (H1047R) of PIK3CA have been associated with a better prognosis in breast carcinoma than mutations occurring in coding exon 9 (E542K)\(^{127}\).

Finding Summary

PIK3CA encodes p110-alpha, which is the catalytic subunit of phosphatidylinositol-3-kinase (PI3K). The PI3K pathway is involved in cell signaling that regulates a number of critical cellular functions, including cell growth, proliferation, differentiation, motility, and survival\(^{128-129}\). PIK3CA alterations that have been characterized as activating, such as observed here, are predicted to be oncogenic\(^{130-131}\).