**Companion Diagnostic (CDx) Associated Findings**

<table>
<thead>
<tr>
<th>GENOMIC FINDINGS DETECTED</th>
<th>FDA-APPROVED THERAPEUTIC OPTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR L858R</strong></td>
<td>Gilotrif® (Afatinib)</td>
</tr>
<tr>
<td></td>
<td>Iressa® (Gefitinib)</td>
</tr>
<tr>
<td></td>
<td>Tagrisso® (Osimertinib)</td>
</tr>
<tr>
<td></td>
<td>Tarceva® (Erlotinib)</td>
</tr>
</tbody>
</table>

For 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.

<table>
<thead>
<tr>
<th>Microsatellite status</th>
<th>CDKN2A loss $</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-Stable</td>
<td></td>
</tr>
</tbody>
</table>

| Tumor Mutational Burden | CDKN2B loss $  |
| 24 Muts/Mb              |                |

| ARFRP1 amplification   | EGFRA289V       |
| ARID1A Y471*           |                 |
| ARID1A Q944*           | MTAP loss $     |
| CDK12 Q1050*           | PIK3CA E453K    |
| CDKN2A loss §          | PIK3CA M1043I   |

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).

**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/f1cdx](http://www.foundationmedicine.com/f1cdx)
FoundationOne®CDx (F1CDx) is a next generation sequencing based 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 test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

The test is also used for detection of genomic loss of heterozygosity (LOH) from FFPE ovarian tumor tissue. Positive homologous recombination deficiency (HRD) status (F1CDx HRD defined as tBRCA-positive and/or LOH high) in ovarian cancer patients is associated with improved progression-free survival (PFS) from Rubraca (rucaparib) maintenance therapy in accordance with the RUBRACA product label.

The F1CDx assay will be performed at Foundation Medicine, Inc. sites located in Cambridge, MA and Morrisville, NC.

**TABLE 1: COMPANION DIAGNOSTIC INDICATIONS**

<table>
<thead>
<tr>
<th>INDICATION</th>
<th>BIOMARKER</th>
<th>THERAPY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-small cell lung cancer</td>
<td>EGFR exon 19 deletions and EGFR exon 21 L858R alterations</td>
<td>Gilotrif® (Afatinib), Iressa® (Gefitinib), Tagrisso® (Osimertinib), or Tarceva® (Erlotinib)</td>
</tr>
<tr>
<td></td>
<td>EGFR exon 20 G719M alterations</td>
<td>Tagrisso® (Osimertinib)</td>
</tr>
<tr>
<td></td>
<td>ALK rearrangements</td>
<td>Alcendia® (Alectinib), Xalkori® (Crizotinib), or Zykadia® (Ceritinib)</td>
</tr>
<tr>
<td></td>
<td>BRAF V600</td>
<td>Tafinlar® (Dabrafenib) in combination with Mekinist® (Trametinib)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>BRAF V600</td>
<td>Tafinlar® (Dabrafenib) or Zeolbor® (Vemurafenib)</td>
</tr>
<tr>
<td></td>
<td>BRAF V600 and V600K</td>
<td>Mekinist® (Trametinib) or Cobit-cell® (Cobimetinib) in combination with Zeolbor® (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>Ovarian cancer</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>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>Lynparza® (Olaparib) or Rubraca® (rucaparib)</td>
</tr>
</tbody>
</table>

**ABOUT THE TEST** FoundationOne®CDx is the first FDA-approved broad companion diagnostic for solid tumors.

Electronically signed by Claire Edgerly, M.D. | Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 2202027531
Shakti Ramkisson, M.D., Ph.D., M.M. Sc, Laboratory Director CLIA: 3402044309
Foundation Medicine, Inc | 1.888.988.3639

**Sample Preparation:** 150 Second St., 1st Floor, Cambridge, MA 02141 - CLIA: 2202027531
**Sample Analysis:** 150 Second St., 1st Floor, Cambridge, MA 02141 - CLIA: 2202027531
**Post-Sequencing Analysis:** 150 Second St., 1st Floor, Cambridge, MA 02141 - CLIA: 2202027531

FDA APPROVED CLAIMS - PAGE 2 of 2
About the Test

Interpretive content on this page and subsequent pages is provided as a professional service, and is not reviewed or approved by the FDA.

Patient

Disease: Lung adenocarcinoma
Name
Date of Birth
Sex
Medical Record #
Physician
Ordering Physician
Medical Facility
Additional Recipient
Medical Facility ID
Pathologist
Specimen
Specimen Site
Specimen ID
Specimen Type
Date of Collection
Specimen Received

Biomarker Findings

Tumor Mutational Burden - 24 Muts/Mb

Microsatellite status - MS-Stable

Genomic Findings

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

EGFR A289V, L858R
PIK3CA E453K, M1043I
ARID1A Q944*, Y471*
ARFRP1 amplification - equivocal†
CDX2 Q1050*
CDKN2A/B loss
MTAP loss

7 Disease relevant genes with no reportable alterations: ALK, BRAF, ERBB2, KRAS, MET, RET, ROS1

† See About the Test in appendix for details.

Therapies with Clinical Benefit

Atezolizumab
Durvalumab
Pembrolizumab
Nivolumab

Therapies with Lack of Response

No therapies or clinical trials. see Biomarker Findings section

Sample Preparation: 150 Second St., 1st Floor, Cambridge, MA 02141 - CLIA: 22D2027531
Sample Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141 - CLIA: 22D2027531
Post-Sequencing Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141 - CLIA: 22D2027531
**GENOMIC FINDINGS**

**EGFR** - A289V, L858R

10 Trials see p. 22

**PIK3CA** - E453K, M1043I

10 Trials see p. 24

**ARID1A** - Q944*, Y471*

8 Trials see p. 20

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**THERAPIES WITH CLINICAL BENEFIT**

(**IN PATIENT’S TUMOR TYPE**)

<table>
<thead>
<tr>
<th>Therapeutic Agent</th>
<th>NCCN Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afatinib</td>
<td>1</td>
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<tr>
<td>Dacomitinib</td>
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</tr>
<tr>
<td>Erlotinib</td>
<td>1</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>1</td>
</tr>
<tr>
<td>Osimertinib</td>
<td>1</td>
</tr>
</tbody>
</table>

**THERAPIES WITH CLINICAL BENEFIT**

(****IN OTHER TUMOR TYPE**)

<table>
<thead>
<tr>
<th>Therapeutic Agent</th>
<th>NCCN Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpelisib</td>
<td></td>
</tr>
<tr>
<td>Everolimus</td>
<td></td>
</tr>
<tr>
<td>Temsirolimus</td>
<td></td>
</tr>
</tbody>
</table>

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**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.

**ARFRP1** - amplification - equivocal ------- p. 6

**CDKN2A/B** - loss ------- p. 7

**CDK12** - Q1050* ------- p. 7

**MTAP** - loss ------- p. 8

---

**NOTE** 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.

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.

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**PROFESSIONAL SERVICES - PAGE 2 of 25**
**Biomarker: Tumor Mutational Burden**

**Result:** 24 Muts/Mb

**Potential Treatment Strategies:**
On the basis of clinical evidence in solid tumors, increased TMB may be associated with greater sensitivity to immunotherapeutic agents, including anti-PD-L1 and anti-PD-1 therapies. Multiple clinical trials of PD-1- or PD-L1-targeting immune checkpoint inhibitors in NSCLC have reported that patients with tumors harboring TMB ≥10 Muts/Mb derive greater clinical benefit from these therapies than those with TMB <10 Muts/Mb; similarly, higher efficacy of anti-PD-1 or anti-PD-L1 immunotherapy for treatment of patients with NSCLC, compared with the use of chemotherapy, has been observed more significantly in cases of TMB ≥10 Muts/Mb. Improved OS of patients with NSCLC treated with pembrolizumab plus chemotherapy relative to chemotherapy only, or those treated with nivolumab plus ipilimumab also relative to chemotherapy, has been observed across all TMB levels.

**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). This sample harbors a TMB level that may be associated with sensitivity to PD-1- or PD-L1-targeting immune checkpoint inhibitors, alone or in combination with other agents.

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**Biomarker: Microsatellite status**

**Result:** MS-Stable

**Potential Treatment Strategies:**
On the basis of clinical evidence, MSS tumors are significantly less likely than MSI-H tumors to respond to anti-PD-1 immune checkpoint inhibitors, including approved therapies nivolumab and pembrolizumab. In a retrospective analysis of 36 NSCLC patients with solid tumors treated with pembrolizumab, 3% were MSI-H and experienced a significantly higher ORR compared with non-MSI-H cases (70% vs. 12%, p=0.001). 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. MSS status indicates MMR proficiency and typically correlates with intact expression of all MMR family proteins.
### POTENTIAL TREATMENT STRATEGIES

EGFR activating mutations may predict sensitivity to EGFR TKIs, including erlotinib, gefitinib, afatinib, dacomitinib, and osimertinib. Third-generation EGFR inhibitors, such as osimertinib, selectively target mutated EGFR, including EGFR T790M. Osimertinib has shown to be sensitive to EGFR tyrosine kinase inhibitors, such as erlotinib, gefitinib, and L858R. Resistance to EGFR inhibition may arise by reactivation of the MAPK pathway, and preclinical evidence suggests that co-targeting EGFR and MAPK signaling may retard the growth and division of acquired resistance to third-generation EGFR inhibitors. Necitumumab is an anti-EGFR antibody that is approved to treat metastatic squamous NSCLC in combination with gemcitabine and cisplatin that has also shown benefit in patients with CRC and melanoma. Irreversible EGFR inhibitors, as well as HSP90 inhibitors, may be appropriate for patients with de novo or acquired resistance to EGFR-targeted therapy. Preclinical studies have reported that EGFR-mutant cells, including cells with exon 20 insertions, are sensitive to HSP90 inhibitors. For patients with EGFR exon 19 deletion/ L858R-positive and T790M-negative NSCLC who had previously progressed on first or second generation EGFR TKIs, a Phase 1 trial evaluating the HER3-targeted antibody U3-1402 reported tumor reduction in 12 patients with 2 confirmed PRs. Consistent with preclinical data demonstrating that the EGFR inhibitor AZD3759 is capable of penetrating the blood-brain barrier and reducing the volume of brain and leptomeningeal metastases, preliminary results from a Phase 1 trial evaluating single-agent AZD3759 reported a reduction in the volume of brain metastases in 40.0% (8/20) of patients with previously treated NSCLC harboring either EGFR L858R or EGFR exon 19 deletion, including 3 confirmed PRs and 3 unconfirmed PRs. In a Phase I/II trial for advanced NSCLC, the brain-penetrant third-generation EGFR TKI lazertinib demonstrated ORRs of 54.3% (69/127) for all evaluable patients and 44.4% (8/18, intracranial) for patients with brain metastases. The reovirus Reolysin targets cells with activated RAS signaling and is in clinical trials in patients with some tumor types. Reolysin has demonstrated mixed clinical efficacy, with the highest rate of response reported for patients with head and neck cancer. The role of EGFR or KRAS mutations as biomarkers for response to Reolysin in NSCLC is unclear. For patients with NSCLC treated with EGFR tyrosine kinase inhibitors, PI3KCA mutation is associated with shorter OS in a meta-analysis (pooled HR of 1.83). Clinical studies of lung cancer have shown that acquired PI3KCA mutations may confer resistance to EGFR inhibitors like osimertinib. The Phase 3 IMPower study showed that the addition of atezolizumab to bevacizumab plus chemotherapy treatment also had clinical efficacy in patients with untreated EGFR-mutated or ALK-rearranged metastatic NSCLC; therefore, the patient’s clinical context should be considered.

### FREQUENCY & PROGNOSIS

EGFR mutation has been reported in 12-36% of lung adenocarcinoma and in 4% of lung squamous cell carcinomas. EGFR protein expression/overexpression has been reported in up to 70% of NSCLC cases. In addition, expression of EGFR protein has been shown to be higher in lung squamous cell carcinoma samples as compared to lung adenocarcinoma. In lung adenocarcinoma, EGFR gene amplification was a predictor of poor disease-free survival in all patients and of poor overall survival in patients with EGFR mutation. Nuclear expression of EGFR in NSCLC has been reported to associate with higher disease stage, shorter progression-free survival, and shorter overall survival. However, EGFR mutations have been reported to predict improved survival in patients with resected Stage 1-3 lung adenocarcinoma or resected Stage 1 NSCLC.

### FINDING SUMMARY

EGFR encodes the epidermal growth factor receptor, which belongs to a class of proteins called receptor tyrosine kinases. In response to signals from the environment, EGFR passes biochemical messages to the cell that stimulate it to grow and divide. EGFR L858 is located in the kinase domain and is encoded by exon 21 mutations at this position including L858R and L858Q have been characterized as activating. Patients with the L858R mutation have been shown to be sensitive to EGFR tyrosine kinase inhibitors, such as erlotinib, gefitinib, and afatinib. Other mutations at this position are predicted to be activating. The EGFR A289V mutation, located in the extracellular domain, has been shown to be activating. Glioblastoma cell lines harboring an EGFR A289V mutation or A289D mutation were shown to be dependent on EGFR kinase activity, and other mutations at this position are also likely activating. In addition, A289V is frequently associated with increased EGFR gene copy number.