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

Companion Diagnostic (CDx) Associated Findings

GENOMIC FINDINGS DETECTED	FDA-APPROVED THERAPEUTIC OPTIONS
EGFR L858R	Gilotrif® (Afatinib) Iressa® (Gefitinib) Tagrisso® (Osimertinib) Tarceva® (Erlotinib)

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.

Microsatellite status MS-Stable §
Tumor Mutational Burden 24 Muts/Mb §
ARFRP1 amplification §
ARID1A Y471*
ARID1A Q944*
CDK12 Q1050*
CDKN2A loss §
CDKN2B loss §
EGFR A289V
MTAP loss §
PIK3CA E453K
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

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

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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

INDICATION	BIOMARKER	THERAPY
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Gilotrif® (Afatinib), Iressa® (Gefitinib), Tagrisso® (Osimertinib), or Tarceva® (Erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (Osimertinib)
	ALK rearrangements	Alecensa® (Alectinib), Xalkori® (Crizotinib), or Zykadia® (Ceritinib)
	BRAF V600E	Tafinlar® (Dabrafenib) in combination with Mekinist® (Trametinib)
Melanoma	*BRAF* V600E	Tafinlar® (Dabrafenib) or Zelboraf® (Vemurafenib)
	BRAF V600E and V600K	Mekinist® (Trametinib) or Cotellic® (Cobimetinib) in combination with Zelboraf® (Vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	Herceptin® (Trastuzumab), Kadcyla® (Ado-trastuzumab emtansine), or Perjeta® (Pertuzumab)
	PIK3CA C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations	Piqray® (Alpelisib)
Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (Cetuximab)
	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)	Vectibix® (Panitumumab)
Ovarian cancer	*BRCA1/2* alterations	Lynparza® (Olaparib) or Rubraca® (Rucaparib)

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ABOUT THE TEST FoundationOne®CDx is the first and only FDA-Approved comprehensive companion diagnostic for all solid tumors.

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

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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†
CDK12 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.

14 Therapies with Clinical Benefit
0 Therapies with Lack of Response
37 Clinical Trials

BIOMARKER FINDINGS

Tumor Mutational Burden - 24 Muts/Mb

10 Trials *see p. 18*

Microsatellite status - MS-Stable

THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)		THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
Atezolizumab	1	Avelumab
Durvalumab	1	Cemiplimab
Pembrolizumab	1	
Nivolumab	2A	

No therapies or clinical trials. see Biomarker Findings section

GENOMIC FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
EGFR - A289V, L858R	Afatinib 1	none
	Dacomitinib 1	
	Erlotinib 1	
	Gefitinib 1	
	Osimertinib 1	
10 Trials *see p. 22*		
PIK3CA - E453K, M1043I	none	Alpelisib
		Everolimus
		Temsirolimus
10 Trials *see p. 24*		
ARID1A - Q944*, Y471*	none	none
8 Trials *see p. 20*		

1 *NCCN category*

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.

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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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[1-3] and anti-PD-1 therapies[1-4]. 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[1-2,5-15]. Improved OS of patients with NSCLC treated with pembrolizumab plus chemotherapy relative to chemotherapy only[16], or those treated with nivolumab plus ipilimumab also relative to chemotherapy[17], has been observed across all TMB levels.

FREQUENCY & PROGNOSIS

A large-scale genomic analysis found that unspecified lung non-small cell lung carcinoma (NSCLC), lung adenocarcinoma, and lung squamous cell carcinoma (SCC) samples harbored median TMBs between 6.3 and 9 Muts/Mb, and 12% to 17% of cases had an elevated TMB of greater than 20 Muts/Mb[18]. Lower TMB is observed more commonly in NSCLCs harboring known driver mutations (EGFR, ALK, ROS1, or MET) with the exception of BRAF or KRAS mutations, which are commonly observed in elevated TMB cases[19]. Although some studies have reported a lack of association between smoking and mutational burden in NSCLC[20-21], several other large studies did find a strong association with increased TMB[22-25]. TMB >10 muts/Mb was found to be more frequent in NSCLC metastases compared with primary tumors for both adenocarcinoma (38% vs. 25%) and SCC (41% vs. 35%) subtypes[26]. A large study of Chinese patients with lung adenocarcinoma reported a shorter median OS for tumors with a higher number of mutations in a limited gene set compared with a lower mutation number (48.4 vs. 61.0 months)[20]. Another study of patients with NSCLC correlated elevated TMB with poorer prognosis and significantly associated lower TMB in combination with PD-L1 negative status with longer median survival in patients with lung adenocarcinoma[27]. However, no significant prognostic association of TMB and/or PD-L1 status with survival has been reported in patients with lung SCC[27-28].

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[29-30] and cigarette smoke in lung cancer[5,31], mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes[32-36], and microsatellite instability (MSI)[32,35-36]. 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[1-2,5-15,19,37-45].

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[46-48], including approved therapies nivolumab and pembrolizumab[49]. In a retrospective analysis of 361 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)[50].

FREQUENCY & PROGNOSIS

MSI-H is generally infrequent in NSCLC, reported in fewer than 1% of samples across several large studies[51-56], whereas data on the reported incidence of MSI-H in SCLC has been limited and conflicting[57-60]. The prognostic implications of MSI in NSCLC have not been extensively studied (PubMed, Feb 2020). One study reported MSI-H in lung adenocarcinoma patients with smoking history, and 3 of 4 MSI-H patients examined also had metachronous carcinomas in other organs, although this has not been investigated in large scale studies[51].

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[61]. 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[61-63]. 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[64-66]. MSS status indicates MMR proficiency and typically correlates with intact expression of all MMR family proteins[61,63,65-66].

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GENE
EGFR

ALTERATION
A289V, L858R

TRANSCRIPT NUMBER
NM_005228

CODING SEQUENCE EFFECT
- 866C>T
- 2573T>G

POTENTIAL TREATMENT STRATEGIES

EGFR activating mutations may predict sensitivity to EGFR TKIs, including erlotinib[67], gefitinib[68], afatinib[69], dacomitinib[70], and osimertinib[71]. Third-generation EGFR inhibitors, such as osimertinib, selectively target mutated EGFR, including EGFR T790M[71-72]. Osimertinib achieved an ORR of 61% in T790M-positive cases and 21% in T790M-negative cases[71]. 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 development of acquired resistance to third-generation EGFR inhibitors[73-75]. Necitumumab is an anti-EGFR antibody that is approved to treat metastatic squamous NSCLC in combination with gemcitabine and cisplatin[76-77] that has also shown benefit in patients with CRC and melanoma[78-79]. Irreversible EGFR inhibitors, as well as HSP90 inhibitors, may be appropriate for patients with de novo or acquired resistance to EGFR-targeted therapy[80-83]. Preclinical studies have reported that EGFR-mutant cells[80-82], including cells with exon 20 insertions[84], 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 study evaluating the HER3-targeted antibody U3-1402 reported tumor reduction in 12 patients with 2 confirmed PRs (2/13)[85]. 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[86-87]. In a Phase I/II trial for advanced NSCLC, the brain-penetrant third-generation EGFR TKI lazertinib enabled ORRs of 54.3% (69/127) for all evaluable patients and 44.4% (8/18, intracranial) for patients with brain metastases[88]. The reovirus Reolysin targets cells with activated RAS signaling[89-91] 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[92-100]. The role of EGFR or KRAS mutations as biomarkers for response to Reolysin in NSCLC is unclear[101]. For patients with NSCLC treated with EGFR tyrosine kinase inhibitors, PIK3CA mutation is associated with shorter OS in a meta-analysis (pooled HR of 1.83)[102]. Clinical studies of lung cancer have shown that acquired PIK3CA mutation may confer resistance to EGFR inhibitors like osimertinib[103-104] . 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[105]; therefore, the patient's clinical context should be considered.

FREQUENCY & PROGNOSIS

EGFR mutation has been reported in 12-36% of lung adenocarcinomas[24,106-107] and in 4% of lung squamous cell carcinomas[108]. EGFR protein expression/overexpression has been reported in up to 70% of NSCLC cases[109-114]. In addition, expression of EGFR protein has been shown to be higher in lung squamous cell carcinoma samples as compared to lung adenocarcinoma[115-116]. 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 mutations[117-118]. Nuclear expression of EGFR in NSCLC has been reported to associate with higher disease stage, shorter progression-free survival, and shorter overall survival[119]. However, EGFR mutations have been reported to predict improved survival in patients with resected Stage 1-3 lung adenocarcinoma[120] or resected Stage 1 NSCLC[121].

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[122]. EGFR L858 is located in the kinase domain and is encoded by exon 21; mutations at this position including L858R[123-125] and L858Q[126] 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[123-125], and afatinib[127]. 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[128]. Glioblastoma cell lines harboring an EGFR A289V or A289D mutation were shown to be dependent on EGFR kinase activity[129], and other mutations at this position are also likely activating. In addition, A289V is frequently associated with increased EGFR gene copy number[128].

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