PGD | CancerSELECT® Report

REPORT DATE: 8/17/2017
TUMOR TYPE: NON-SMALL CELL LUNG CANCER
SPECIMEN TYPE: FFPE

PATIENT NAME

John Doe

PATIENT INFORMATION

DATE OF BIRTH	PATIENT GENDER	PGDX NUMBER	MEDICAL RECORD NUMBER	PATIENT PHONE NUMBER
1/1/1960	Male	PGDX12345	Not Provided	000-000-0000

PATIENT EMAIL	INSTITUTION
john@doe.com	Hospital, City

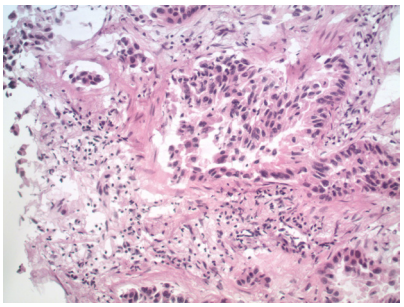
PHYSICIAN	TUMOR SAMPLE RECEIVED	NORMAL SAMPLE RECEIVED
John Smith, MD	8/1/2017	8/1/2017

SAMPLE CHARACTERISTICS

DIAGNOSIS
Non-Small Cell Lung Cancer

PRIMARY TUMOR SITE	HISTOLOGY
Lung	Adenocarcinoma
TUMOR LOCATION	PATHOLOGICAL TUMOR PURITY
Lung	50%
SPECIMEN TYPE	SOURCE OF NORMAL DNA
FFPE	Saliva
TUMOR COLLECTION DATE	SPECIMEN ID
5/30/2017	123456

TUMOR HISTOLOGY



MICROSATELLITE INSTABILITY ANALYSIS

Microsatellite Status	Active Clinical Trial
MSS	No

SEQUENCE MUTATIONS

Gene	Mutation	Consequence	Mutant fraction	Exon	FDA Approved Therapy		Active Clinical Trial
					Same Indication	Other Indication	
KRAS	G12D	Missense	37%	1	No	Yes	Yes
PIK3CA	H1047R	Missense	40%	20	No	Yes	Yes
TP53	E349Nfs*21	Frameshift	26%	9	No	No	Yes

AMPLIFICATIONS OR TRANSLOCATIONS

Gene	Fold Change	Consequence	FDA Approved Therapy		Active Clinical Trial
			Same Indication	Other Indication	
EGFR	18.2	Amplification	Yes	Yes	Yes
EML4-ALK	N/A	Rearrangement	Yes	No	Yes

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Luis A Diaz, MD	21D2039282	8/17/2017

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS

Gene (Mutation)

ALK (EML4-ALK fusion)

Description:
EML4-ALK fusion is an activating mutation. ALK encodes anaplastic lymphoma kinase (Alk), a tyrosine kinase receptor. Activating mutations or translocations involving the ALK gene may predict sensitivity to small molecule Alk kinase inhibitors (Chand et al., 2013; 23104988). The Alk inhibitors crizotinib, ceritinib, and alectinib have been approved by the FDA for ALK-translocation-positive non-small cell lung cancer; additional Alk inhibitors are under investigation in clinical trials (Kwak et al., 2010; 20979469, Socinski et al., 2013; 23553849, Chabner, 2014; 24789171, Ou et al., 2016; 26598747, Gandhi et al., 2015; ASCO 2015, Abstract 8019).

The EML4-ALK rearrangement results in the juxtaposition of the promoter and N-terminal half of Eml4 with the intracellular portion, including the kinase domain, of Alk (Soda et al., 2007; 17625570). This fusion results in the activation of Alk by causing the constitutive oligomerization of the protein via the Eml4 coiled coil domain (Mano, 2008; 19032370). EML4-ALK has been demonstrated to transform fibroblasts and result in the formation of tumors in mice (Soda et al., 2007; 17625570, Soda et al., 2008; 19064915). In addition, this fusion has been reported to confer sensitivity to the Alk inhibitors crizotinib and ceritinib (Shaw et al., 2014; 24670165, Kwak et al., 2010; 20979469, Curran, 2012; 22191798).

ALK mutations have been reported in 4.8% (69/1437) of Non-small cell lung carcinoma (NSCLC) samples analyzed in COSMIC (Sep 2016). ALK mutations have been reported in 4.5% (51/1144) of Non-small cell lung carcinoma (NSCLC) samples (Pan-Lung Cancer (TCGA, Nat Genet 2016), cBioPortal for Cancer Genomics, Sep 2016). In the scientific literature, ALK rearrangements, most commonly the EML4-ALK fusion, have been reported in 1.2-9.0% of NSCLC cases (To et al., 2013; 23625156, Selinger et al., 2013; 23743928, Zhou et al., 2013; 23277484, Fu et al., 2015; 26253541, Doval et al., 2015; 25609979, Song et al., 2016; 27635639, Li et al., 2016; 27614248, Mattsson et al., 2016; 27495736).

ALK was originally identified in anaplastic lymphoma as a fusion partner with the gene product of NPM1; ALK has subsequently been identified as a fusion partner with numerous other genes, including EML4 in lung cancer (Bagci et al., 2012; 22085494). The ALK gene can become oncogenic by a gene rearrangement, copy number gain, or genetic mutation (Bagci et al., 2012; 22085494, Grande et al., 2011; 21474455). Patients with EML4-ALK fusions generally have wild-type EGFR, KRAS, and TP53, and are resistant to Egfr inhibitors, although there have been reports of NSCLC tumors which harbor concomitant EGFR mutations and EML4-ALK translocations (Tiseo et al., 2011; 21168933, Yang et al., 2011; ASCO 2011, Abstract 10517). ALK rearrangements have been associated with younger age, nodal metastasis, higher disease stage, and epithelial-mesenchymal transition marker expression in a study of 80 ALK-rearranged and 213 ALK-negative lung adenocarcinoma cases (Kim et al., 2013; 24194854).

Tumors with ALK activation, by either mutation, fusion, or amplification, may be sensitive to Alk inhibitors. The Alk inhibitor crizotinib (Xalkori) has been approved for the treatment of NSCLC patients whose tumors test positive for ALK rearrangement, on the basis of Phase 2 and Phase 3 studies (Kwak et al., 2010; 20979469, Bang et al., 2010; ASCO 2010, Abstract 3, Camidge et al., 2011; ASCO 2011, Abstract 2501, Curran, 2012; 22191798, Shaw et al., 2013; 23724913). A preclinical study has reported that the activity of Alk harboring point mutations, conferring both ligand-independent and ligand-dependent activity, could be inhibited by crizotinib (Chand et al., 2013; 23104988). The Alk inhibitors ceritinib and alectinib have been FDA approved for the treatment of NSCLC patients with ALK rearrangements who experienced disease progression or were found to be intolerant to crizotinib (Chabner, 2014; 24789171, Shaw et al., 2014; 24670165, Ou et al., 2015; ASCO 2015, Abstract 8008, Ou et al., 2016; 26598747, Gandhi et al., 2015; ASCO 2015, Abstract 8019). In addition, studies of Alk inhibitors and Hsp90 inhibitors are underway for patients with EML4-ALK rearrangements who may have developed resistance to crizotinib (Sang et al., 2013; 23533265, Iragavarapu et al., 2015; 25888090, Normant et al., 2011; 21258415).

FDA Approved Drugs in Current Indication:
Crizotinib, ceritinib, alectinib.

Phase 3 Data for ALK (EML4-ALK fusion):
Two Phase 3 studies comparing crizotinib with chemotherapy in ALK-positive lung carcinoma patients have reported crizotinib to be superior to chemotherapy with improved progression-free survival as both a first-line and second-line therapy (Solomon et al., 2014; 25470694, Shaw et al., 2013; 23724913). The accelerated FDA approval of ceritinib for ALK-positive NSCLC patients who have progressed on or are intolerant to crizotinib was based on the results of a clinical trial in which 163 such patients were treated with ceritinib; an overall response rate of 54.6% and a duration of response of 7.4 months were reported (Dong-Wan et al., 2014; ASCO 2014, Abstract 8003). The J-ALEX Phase 3 study of alectinib versus crizotinib in 207 ALK-positive non-small cell lung cancer patients without prior Alk inhibitor treatment reported a median progression-free survival (PFS) of 10.2 months in the crizotinib arm, while median PFS was not reached in the alectinib arm; adverse events, including grade 3-4 adverse events, were reported to be more frequent in the crizotinib arm as compared with the alectinib arm (Nokihara et al., 2016; ASCO 2016, Abstract 9008).

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 2 Data for ALK (EML4-ALK fusion):
A Phase 2 trial of Hsp90 inhibitor ganetespib in NSCLC patients with either EGFR, KRAS or neither mutation reported progression-free survival rates of 13.3%, 5.9% or 19.7%, respectively at 16 weeks; 4/99 patients in the wild-type group achieved a partial response, and all four patients were subsequently found to harbor an ALK gene rearrangement (Socinski et al., 2013; 23553849). Alectinib has received accelerated FDA approval in crizotinib-resistant ALK-rearranged NSCLC patients based on the results of two Phase 2 studies (2016; 26739884). A Phase 2 trial of alecitinib in 138 patients with ALK-rearranged metastatic NSCLC reported, at a median follow up of 30 weeks, an overall response rate of 49.2% and a disease control rate of 79.5%. In the 96 patients previously treated with chemotherapy and crizotinib and the 34 patients with CNS disease, the overall response rates were 43.8% and 55.9%, respectively, and the disease control rates were 78.1% and 55.9%, respectively; five patients with CNS disease showed complete responses (Ou et al., 2015; ASCO 2015, Abstract 8008, Ou et al., 2016; 26598747). A Phase 2 study of alectinib in 83 patients with ALK-positive non-small-cell lung cancer who had progressed after crizotinib treatment reported confirmed partial response in 33/69 of patients at the time of the primary analysis, for an objective response of 48%; treatment was reported to be well-tolerated, with a predominance of grade 1 or 2 adverse events (Shaw et al., 2016; 26708155). Preliminary results from a Phase 2 trial of brigatinib in 222 ALK-rearranged NSCLC patients refractory to crizotinib reported overall response rates of 46% and 54% and median progression-free survival of 8.8 and 11.1 months, respectively, depending on the dosing regimen. Additionally, the safety profile was reported to be acceptable (Kim et al., 2016; ASCO 2016, Abstract 9007). An ongoing Phase 1/2 trial of brigatinib in 137 patients with advanced malignancies, including 79 ALK-rearranged NSCLC cases, reported a response rate of 72% (52/72) in evaluable NSCLC patients, specifically 69% (45/65) and 100% (7/7) of those previously treated with crizotinib and crizotinib-naive, respectively, with a median duration of response of 49 weeks, and a median progression-free survival time of 56 weeks. In patients with measurable CNS metastases, 50% (6/12) showed a brain response, and 31% (8/26) of those with nonmeasurable lesions showed disappearance of all lesions (Camidge et al., 2015; ASCO 2015, Abstract 8062). Preliminary results from a Phase 1/2 trial of X-396 in ALK-rearranged NSCLC patients reported partial responses in 63% (19/30) and stable disease in 7% (2/30) of evaluable patients, including partial responses in 88% (7/8) and 83% (10/12) of crizotinib naive and previously treated patients, respectively, with a median duration of response ranging from 24-128 weeks (Horn et al., 2016; ASCO 2016, Abstract 9056).

Phase 1 Data for ALK (EML4-ALK fusion):
A Phase 1 clinical trial of crizotinib in pediatric solid tumors reported objective responses in 14/79 patients, including nine complete responses and five partial responses; response was enriched in patients with activating alterations in ALK (Mossé et al., 2013; 23598171). The ASCEND-1 Phase 1 trial, which evaluated ceritinib in advanced NSCLC, reported overall response rates of 72% (60/83) and 56% (92/163) in ALK inhibitor-naive and ALK inhibitor-pretreated patients, respectively, with intracranial disease control in 79% (15/19) of ALK inhibitor-naive and in 65% (49/75) of ALK inhibitor-pretreated patients with confirmed brain metastases and post-baseline tumor assessment; 48% (117/246) of all patients experienced serious adverse events (Kim et al., 2016; 26973324). A Phase 1 study of ceritinib treatment in 114 advanced or metastatic non-small cell lung carcinoma patients harboring genetic alterations in ALK reported an overall response rate of 58%, which included one complete response and 65 patients with partial response. This study also reported an overall response rate in 56% (45/80) of the patients who had previously received crizotinib. Responses to LDK378 were reported in patients with or without various resistance mutations in ALK (Shaw et al., 2014; 24670165). Multiple case studies have reported that failure of alectinib treatment in NSCLC harboring ALK rearrangements is associated with transformation of disease into SCLC (Miyamoto et al., 2016; 26613679, Fujita et al., 2016; 26751586, Takegawa et al., 2016; 26811347). An ongoing Phase 1/2 trial of TSR-011 in patients with advanced cancers has enrolled at least 23 subjects, including ten NSCLC patients. Preliminary results include partial responses in three of five NSCLC patients with ALK-rearrangements (Weiss et al., 2014; ASCO 2014, Abstract e190005).

Preclinical Data for ALK (EML4-ALK fusion):
Preclinical work using X-396 has shown that it has potent inhibitory activity against cells harboring EML4-ALK alterations or other ALK mutations that underlie crizotinib resistance (Lovly et al., 2011; 21613408). Preclinical work suggests efficacy of the Alk inhibitor alectinib in ALK-driven tumor models, including NSCLC cells expressing the EML4-ALK fusion, anaplastic large-cell lymphoma cells expressing the NPM-ALK fusion, and cells expressing the resistance mutation ALK L1196M (Sakamoto et al., 2011; 21575866). A preclinical study reported that the combination of an Alk inhibitor with a MEK inhibitor enhanced apoptosis in EML4-ALK positive NSCLC cells as compared with Alk inhibitor treatment alone (Tanizaki et al., 2012; 22240786).

FDA Approved Therapies in Other Indications:
None.

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Luis A Diaz, MD	21D2039282	8/17/2017

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

KRAS (G12D)

Description:
KRAS-G12D is an activating mutation. KRAS encodes the signaling protein K-Ras, a member of the Ras family; activating KRAS alterations may result in activation of downstream signaling pathways, including the Raf/MEK/ERK pathway (Pylayeva-Gupta et al., 2011; 21993244, Nakano et al., 1984; 6320174). Several MEK inhibitors are under clinical investigation, including the FDA-approved therapies trametinib and cobimetinib, and may be relevant for tumors harboring K-Ras activation (Flaherty et al., 2012; 22663011, Larkin et al., 2014; 25265494, Britten, 2013; 23443307, Jänne et al., 2013; 23200175).

The G12D mutation lies within the first "G box" domain of the K-Ras protein, one of several conserved regions responsible for GTP binding and hydrolysis; disruption of this region creates a protein that is defective for GTP hydrolysis and is therefore constitutively active (McCoy et al., 1984; 6092920, Motojima et al., 1993; 8439212, Colicelli, 2004; 15367757). KRAS G12D has been shown to be an oncogenic mutation, inducing tumor formation and metastasis in mice (O'Hagan and Heyer, 2011; 21779503, Johnson et al., 2001; 11323676, Rachagani et al., 2011; 21364589, Jackson et al., 2001; 11751630).

KRAS mutations have been reported in 18% (5795/31409) of Non-small cell lung carcinoma (NSCLC) samples analyzed in COSMIC (Sep 2016). KRAS mutations have been reported in 19% (222/1144) of Non-small cell lung carcinoma (NSCLC) samples (Pan-Lung Cancer (TCGA, Nat Genet 2016), cBioPortal for Cancer Genomics, Sep 2016). KRAS mutations have been reported in 8-30% of NSCLC samples analyzed in scientific studies, with mutations reported more frequently in adenocarcinoma as compared with squamous cell carcinoma samples (Arrieta et al., 2015; 25634006, Maus et al., 2014; 24331409, Gainor et al., 2013; 23729361, Chatziandreou et al., 2015; 26208325, Shigematsu et al., 2005; 15741570, Giannini et al., 2016; 27373829, Barlesi et al., 2016; 26777916).

The KRAS gene is one of the most commonly mutated genes in human malignancies, with high incidences in pancreatic, colorectal, and lung cancers (Farber et al., 2011; 22016105, Feldmann et al., 2007; 17520196, Han et al., 2011; 22011285). KRAS mutations have been associated with smoking and adenocarcinoma histology in studies of NSCLC, and are generally mutually exclusive with EGFR mutations and ALK rearrangements (Gainor et al., 2013; 23729361, Lee et al., 2016; 26992209, Chatziandreou et al., 2015; 26208325, Paik et al., 2012; 22605530, Sholl et al., 2015; 25738220).

Many of the current attempts to target K-Ras are directed against its downstream signaling pathways, Raf/MEK/ERK and PI3K/Akt/mTOR (Yeh et al., 2009; 19372556, Britten, 2013; 23443307). The MEK inhibitors trametinib and cobimetinib (in combination with vemurafenib) have been FDA-approved for BRAF V600E- and V600K-mutant melanoma and are under investigation in clinical trials (Flaherty et al., 2012; 22663011, Larkin et al., 2014; 25265494). A novel clinical approach for KRAS-positive tumors, based on synthetic lethal interactions that occur in the presence of a KRAS mutation and either diminished Cdk4 activity or diminished Bcl-2/Bcl-xL activity, is a treatment combination of MEK inhibition and either Cdk4/6 inhibition or Bcl-2/Bcl-xL inhibition (Mao et al., 2014; 24496383, Puyol et al., 2010; 20609353, Tan et al., 2013; 23475955, Corcoran et al., 2013; 23245996).

FDA Approved Drugs in Current Indication:
None.

Phase 3 Data for KRAS (G12D):
None.

Phase 2 Data for KRAS (G12D):
A Phase 2 clinical trial evaluating the response to sorafenib treatment in 57 NSCLC patients with KRAS mutations reported 52.6% of patients with partial response or stable disease at six weeks (Dingemans et al., 2013; 23224737). The Phase 2 BATTLE trial reported better response to sorafenib than to the other three regimens under study in NSCLC patients with KRAS-mutant tumors, although this result has yet to be corroborated in larger studies (Kim et al., 2011; 22586319). A randomized Phase 2 trial in 129 KRAS-mutant NSCLC patients treated with either trametinib or docetaxel reported a similar response rate and progression free survival between the two regimens. In the trametinib cohort, 12% (10/86) of patients achieved a partial response, with a 12 week median progression free survival, and in the docetaxel cohort, 12% (5/43) of patients achieved a partial response, with an 11 week median progression free survival (Blumenschein et al., 2015; 25722381). A randomized Phase 2 trial of docetaxel with or without the MEK inhibitor selumetinib (AZD6244) in KRAS-mutant NSCLC found that the addition of selumetinib resulted in an improvement in progression-free survival and response rate (Jänne et al., 2013; 23200175, Jänne et al., 2015; 26125448). A Phase 2 study of the MEK inhibitor PD0325901 in previously-treated NSCLC patients did not report any objective responses, but did report stable disease of up to ten months in 20.6% (7/34) of patients (Haura et al., 2010; 20332327). A randomized Phase 2 trial of the combination of selumetinib and erlotinib in 79 NSCLC patients reported no significant improvement in objective response rate or progression free survival with combination treatment as compared with either monotherapy; in addition, KRAS mutational status had no impact on treatment outcomes (Carter et al., 2016; 26802155).

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 1 Data for KRAS (G12D):
A Phase 1 study of binimetinib in Japanese solid tumor patients has reported a lack of partial or complete responses, but stable disease in 14/21 (67%) patients; the therapy was well tolerated (Watanabe et al., 2016; 27071922). Phase 1 studies of trametinib in combination with docetaxel, pemetrexed, or buparlisib have reported stable disease in 46%, 59%, and 53% of NSCLC patients, respectively; KRAS mutation status was not found to significantly affect these rates (Gandara et al., 2013; ASCO 2013, Abstract 8028, Kelly et al., 2013; ASCO 2013, Abstract 8027, Bedard et al., 2015; 25500057). A Phase 1 multicenter trial of refametinib in 53 patients with advanced cancer reported suppression of ERK phosphorylation and stable disease in 11 patients for four or more courses of therapy (Weekes et al., 2013; 23434733). A Phase 1 study of refametinib in combination with sorafenib in 54 patients with advanced solid tumors reported, in 38 non-hepatocellular carcinoma (non-HCC) patients evaluable for response, a partial response lasting approximately one year in a colorectal carcinoma patient and stable disease in 63.2% (24/38) of patients; treatment was associated with a reduction in ERK activation in five of six non-HCC biopsied cases (Adjei et al., 2016; 26644411). A Phase 1 study of cobimetinib in combination with pictilisib in patients with advanced solid tumors reported partial responses in 6.5% (3/46) of patients (a melanoma, pancreatic carcinoma, and endometrioid carcinoma patient, all harboring mutant KRAS) and stable disease for at least five months in 10.9% (5/46) of evaluable patients (LoRusso et al., 2012; ASCO 2012, Abstract 2566). A Phase 1 dose expansion trial of cobimetinib in 97 patients with advanced solid tumors reported one complete and six partial responses, with all responses occurring in melanoma patients; BRAF V600E was reported to be present in 6/7 patients who responded (Rosen et al., 2016; 27424159).

Preclinical Data for KRAS (G12D):
A preclinical study of cobimetinib in NSCLC xenograft models reported that tumor growth inhibition did not correlate with KRAS status; pictilisib increased the efficacy of cobimetinib in an NSCLC xenograft model harboring a KRAS mutation (Hoeflich et al., 2012; 22084396). Preclinical studies in human cell line and genetic mouse models of NSCLC harboring coincident KRAS mutation and loss of STK11 reported that treatment with phenformin, a mitochondrial inhibitor, resulted in significant cell and tumor growth inhibition. Specifically, combined treatment with phenformin and the mTOR inhibitor INK128 showed efficacy in lung adenocarcinoma tumors, whereas lung squamous cell carcinoma tumors were found to be resistant to both single agent phenformin and combination therapy due to activation of Akt; combined treatment of lung squamous cell carcinoma cell lines with INK128 and the Akt inhibitor MK-2206 showed increased efficacy as compared with either single agent (Shackelford et al., 2013; 23352126, Momcilovic et al., 2015; 26574479). A preclinical study reported that treatment of NSCLC cell lines with binimetinib inhibited cell growth and induced cell cycle arrest, apoptosis, and autophagy; binimetinib also showed synergistic effects on cell growth when combined with either the PI3K inhibitor buparlisib or the autophagy inhibitor chloroquine (Yao et al., 2015; 25937299).

FDA Approved Therapies in Other Indications:
Trametinib, Cobimetinib

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CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

PIK3CA (H1047R)

Description:
PIK3CA-H1047R is an activating mutation. PIK3CA encodes the protein 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 (Samuels et al., 2005; 15950905, Engelman, 2009; 19629070). Alterations that activate the PI3K/Akt/mTOR pathway may predict sensitivity to PI3K or Akt inhibitors, which are under investigation in clinical trials, or to mTOR inhibitors, which are approved in some tumor types and in clinical trials (Janku et al., 2011; 21216929, Massacesi et al., 2013; 23551097).

H1047R is a common hotspot mutation in PIK3CA. The H1047R mutation, located in the kinase domain of the protein, leads to constitutive activation of the protein, conferring oncogenic potential on the cells (Kang et al., 2005; 15647370). Experiments in cancer cell culture and animal models have demonstrated that the most common PIK3CA mutations, E542K and E545K (exon 9, located in the helical domain) and H1047R (exon 20, located in the kinase domain), all lead to oncogenic transformation (Bader et al., 2006; 16432179, Isakoff et al., 2005; 16322248).

PIK3CA mutations have been reported in 3.6% (345/9462) of Non-small cell lung carcinoma (NSCLC) samples analyzed in COSMIC (Sep 2016). PIK3CA mutations have been reported in 8.2% (94/1144) of Non-small cell lung carcinoma (NSCLC) samples (Pan-Lung Cancer (TCGA, Nat Genet 2016), cBioPortal for Cancer Genomics, Sep 2016). In the scientific literature, PIK3CA mutation has been found in 1-4% of NSCLC cases, including in 1-4% of lung adenocarcinoma and 4-9% of lung squamous cell carcinoma cases (Wang et al., 2014; 24533074, Scheffler et al., 2015; 25473901, Barlesi et al., 2016; 26777916, Song et al., 2016; 27342566).

PIK3CA mutations are not mutually exclusive with EGFR or KRAS or BRAF mutations, and are associated with increased PI3K signaling and increased activation of Akt (Yamamoto et al., 2008; 18757405, Janku et al., 2011; 21829508). PIK3CA mutations have been associated with activation of PI3K/Akt signaling and colony formation in NSCLC cell lines, and the PIK3CA H1047R activating mutation has been shown to drive tumorigenesis in combination with BRAF V600E in a mouse model of NSCLC (Yamamoto et al., 2008; 18757405, Trejo et al., 2013; 24019382). PIK3CA mutations and amplification have been reported to occur more frequently in lung squamous cell carcinoma as compared with lung adenocarcinoma in several studies, although two mutation studies did not find a statistically significant difference (Scheffler et al., 2015; 25473901, Wang et al., 2014; 24533074, An et al., 2012; 22768234, Spoerke et al., 2012; 23136191, Stjernström et al., 2014; 24500884, Ji et al., 2011; 21507233, Xu et al., 2012; 22430133).

Activating PIK3CA mutations may predict sensitivity to PI3K/Akt/mTOR pathway inhibitors, although data have been mixed in clinical trials (Janku et al., 2011; 21216929, Loi et al., 2013; 23301057, Mackay et al., 2014; 24166148, Deming et al., 2013; 23593290, Massacesi et al., 2013; 23551097). Inhibitors of PI3K and Akt, alone or in combination with other therapies, are currently in clinical trials. Several inhibitors designed to target both the mTORC1/Raptor and mTORC2/Rictor complexes are being tested in early phase clinical trials for advanced solid tumors (Grunt and Mariani, 2013; 23215720). The mTOR inhibitors everolimus and temsirolimus, which have been approved by the FDA in some tumor types, as well as other mTOR inhibitors, are being tested in clinical trials in a variety of solid tumors. Preclinical studies in PIK3CA-mutated NSCLC cell lines have reported sensitivity to PI3K and mTOR inhibitors, including pictilisib and PF-04691502, with one study reporting that PIK3CA-mutant cells were more sensitive to pictilisib as compared with PIK3CA wild-type cells (Spoerke et al., 2012; 23136191, Yuan et al., 2011; 21750219, Zou et al., 2012; 22101421).

FDA Approved Drugs in Current Indication:
None.

Phase 3 Data for PIK3CA (H1047R):
None.

Phase 2 Data for PIK3CA (H1047R):
A Phase 2 clinical trial of temsirolimus as a single agent in previously untreated NSCLC patients reported clinical benefit in 35% of patients, including partial response in 8% (4/52) and stable disease in 27% (14/52) of patients; however, grade 3 or 4 adverse events were reported in 63% (33/52) of patients and the study did not meet its pre-specified primary objective for efficacy. In this study, clinical response was not correlated with expression of p70s6 kinase, phospho-p70s6 kinase, Akt, phospho-Akt, or Pten (Reungwetwattana et al., 2012; 22722792). The Phase 2 BASALT-1 study evaluated the efficacy of single agent buparlisib in 63 patients with PI3K-activated, metastatic NSCLC. The 12-week progression-free survival rates were 23.3% and 20% in the squamous and nonsquamous NSCLC groups, respectively; the second phase of this study was not conducted due to failure to meet its primary endpoint of a 12-week progression-free survival rate of at least 50% (Vansteenkiste et al., 2015; 26098748). Results from the Phase 1b/2 BASALT-3 trial evaluating the efficacy of buparlisib combined with docetaxel in 27 patients with previously treated squamous NSCLC reported overall response rates of 6% and 18% in patients receiving 80 mg or 100 mg of burparlisib, respectively. The median progression-free survival was reported to be 2.8 months at both doses with all patients discontinuing treatment, predominately due to disease progression (Adjei et al., 2016; ASCO 2016, Abstract e20522). A Phase 2 multi-arm study in patients with advanced thoracic malignancies, including NSCLC, SCLC, and thymic malignancies, treated patients based on genomic characterization. In four NSCLC patients with alterations in PTEN, AKT1, or PIK3CA, no clinical responses were reported with MK-2206 treatment (Lopez-Chavez et al., 2015; 25667274). A Phase 2 trial of everolimus as a monotherapy in 85 NSCLC patients reported an overall response rate of 5% and an overall disease control rate of 47%; 25% of patients experienced pneumonitis as an adverse effect. In this study, expression of p-Akt was predictive of decreased progression-free survival (Soria et al., 2009; 19549709). A Phase 2 study of everolimus in combination with docetaxel as second or third-line therapy in unselected NSCLC patients reported partial response and stable disease reported in 7% (2/28) and 54% (15/28) of patients, respectively, and a six-month progression-free survival rate of 5% (Ramalingam et al., 2013; 23407561).

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 1 Data for PIK3CA (H1047R):
A Phase 1b trial of buparlisib and trametinib in patients with advanced solid cancer reported stable disease in 53% (9/17) of NSCLC cases, and a partial response in one NSCLC patient with a KRAS mutation; median progression-free survival for NSCLC was four months, and grade 3/4 adverse events were reported in 65% (73/113) of all study patients (Bedard et al., 2015; 25500057). A Phase 1 study in 14 evaluable NSCLC patients that had previously failed chemotherapy and Egfr tyrosine kinase therapy reported two patients with minor responses and symptomatic improvement for eight and 27 weeks following treatment with MK-2206 in combination with gefitinib (Lin et al., 2014; ASCO 2014, Abstract e19013). A Phase 1b study of pictilisib, paclitaxel, and carboplatin with or without bevacizumab in patients with advanced NSCLC reported partial response in 44% (8/18) of patients, including a pathologic complete response in a lung squamous cell carcinoma patient treated with pictilisib and chemotherapy (Besse et al., 2011; ASCO 2011, Abstract 3044). A Phase 1 study analyzing pictilisib in combination with carboplatin, paclitaxel, and bevacizumab in non-squamous NSCLC reported partial responses in 42.9% (3/7) of patients; dose limiting toxicities included grade 3 febrile neutropenia and erythema multiforme (Yamamoto et al., 2016; 27565810).

Preclinical Data for PIK3CA (H1047R):
N/A: Preclinical data are not presented when higher level data are available.

FDA Approved Therapies in Other Indications:
Temsirolimus, Everolimus

TP53 (E349Nfs*21)

Description:
TP53-E349fs*21 is an inactivating mutation. TP53 is a tumor suppressor that encodes the p53 protein; alterations in TP53 may result in a loss of p53 function, yet an increase in the expression and stability of the mutant p53 protein in the nucleus, sometimes leading to oncogenic effects, including genomic instability and excessive cell proliferation (Levine, 1997; 9039259, Wang et al., 2005; 15625370, Koga et al., 2001; 11400116, Kato et al., 2003; 12826609, Houben et al., 2011; 21760960, Olivier et al., 2009; 18802452). At present, there are no approved therapies targeting TP53 alterations, despite their high prevalence in cancer. Therapeutic approaches under investigation include gene therapy for TP53 and (dendritic cell-based) TP53 vaccines (Schuler et al., 2014; 24583792, Vermeij et al., 2011; 21541192, Saito et al., 2014; 24982341). Tumors with TP53 mutations may be sensitive to the Wee1 inhibitor MK-1775, and clinical trials are currently underway for patients with solid tumors and hematologic malignancies (Hirai et al., 2010; 20107315, Bridges et al., 2011; 21799033). Aurora kinase A inhibitors are another therapeutic approach under investigation for TP53-mutated cancers (Vilgelm et al., 2015; 25398437, Li et al., 2015; 25512615, Katayama and Sen, 2011; 21761334, Tentler et al., 2015; 25758253, Kalous et al., 2013; 24091768).

The alteration reported here is expected to effectively truncate the p53 protein within the tetramerization domain; this truncation is expected to result in the loss of a portion of the tetramerization domain and the entire C-terminal regulatory domain (Joerger and Fersht, 2008; 18410249). The tetramerization domain is thought to be critical to normal p53 function (Kamada et al., 2011; 20978130, Kato et al., 2003; 12826609). In addition, the C-terminal regulatory domain has been shown to be required for DNA binding and transcriptional activation by p53 (Kim et al., 2012; 22178617). Therefore, this alteration is predicted to be inactivating.

TP53 mutations have been reported in 36% (2168/5983) of Non-small cell lung carcinoma (NSCLC) samples analyzed in COSMIC (Sep 2016). TP53 mutations have been reported in 68% (775/1144) of Non-small cell lung carcinoma (NSCLC) samples (Pan-Lung Cancer (TCGA, Nat Genet 2016), cBioPortal for Cancer Genomics, Sep 2016). TP53 is one of the most commonly mutated genes in lung cancer; scientific studies have reported TP53 mutations in 29-42% of non-small cell lung carcinoma (NSCLC) cases, with a higher incidence cited in tumors of the squamous cell carcinoma subtype as compared with the adenocarcinoma subtype (Mogi and Kuwano, 2011; 21331359, Tekpli et al., 2013; 23011884, Vignot et al., 2013; 23630207, Ma et al., 2014; 24495481, Maeng et al., 2013; 24222160, Molina-Vila et al., 2014; 24696321, Mattioni et al., 2015; 25884692, Kim et al., 2014; 24323028).

Loss of tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers (Brown et al., 2009; 19935675). Carriers of a germline mutation in TP53 have Li-Fraumeni Syndrome, an inherited cancer syndrome resulting in multiple tumors in early adulthood, including breast cancer, brain tumors, and leukemias (Malkin et al., 1990; 1978757, Srivastava et al., 1991; 2259385, Santibáñez-Koref et al., 1991; 1683921). Expression of p53 in normal cells is low; however, TP53 alterations, including those that result in loss of p53 tumor suppressor function, may lead to stabilization and increased expression of p53, particularly in the nucleus, and several studies have shown that it may have oncogenic gain-of-function effects (Wang et al., 2005; 15625370, Koga et al., 2001; 11400116, Kato et al., 2003; 12826609, Houben et al., 2011; 21760960, Olivier et al., 2009; 18802452). TP53 alterations are believed to be early events in NSCLC, preceding lymph node metastasis (Chang et al., 2011; 20811949). TP53 mutation and expression of p53 have been correlated with the lung squamous cell carcinoma subtype, and p53 expression in lung squamous cell carcinoma has also been associated with disease stage and higher grade tumors (Mattioni et al., 2015; 25884692, Bircan et al., 2010; 20349288, Kim et al., 2014; 24323028).

At present, there are no approved therapies targeting TP53 alterations, despite their high prevalence in cancer. Therapeutic approaches under investigation include gene therapy for TP53 and (dendritic cell-based) TP53 vaccines (Schuler et al., 2014; 24583792, Vermeij et al., 2011; 21541192, Saito et al., 2014; 24982341). Inhibition of components of the DNA damage checkpoint, including Checkpoint Kinase 1 (Chk1) and Wee1, has been reported to enhance the activity of DNA-damaging agents in preclinical cancer models with deficiency of p53 function (Ma et al., 2011; 21087899, Hirai et al., 2010; 20107315, Bridges et al., 2011; 21799033). Clinical trials of the Wee1 inhibitor MK-1775 are currently underway for patients with solid tumors and hematologic malignancies. Studies have reported Aurora kinase A to be activated in cells harboring TP53 mutation, and Aurora kinase A and B inhibitors have been reported to activate wild-type p53 in cellular assays; thus, tumors retaining a wild-type TP53 allele may benefit from Aurora kinase inhibitors (Vilgelm et al., 2015; 25398437, Li et al., 2015; 25512615, Katayama and Sen, 2011; 21761334, Tentler et al., 2015; 25758253, Gully et al., 2012; 22611192, Marxer et al., 2014; 23955083).

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CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

FDA Approved Drugs in Current Indication:
None.

Phase 3 Data for TP53 (E349Nfs*21):
None.

Phase 2 Data for TP53 (E349Nfs*21):
None.

Phase 1 Data for TP53 (E349Nfs*21):
In a Phase 1 study of ENMD-2076 in 67 patients with advanced cancer, partial responses were seen in two patients with platinum refractory ovarian carcinoma. Therapy was well-tolerated overall, with hypertension, nausea/vomiting, and fatigue being the most common adverse events (Diamond et al., 2011; 21131552). A Phase 1 trial of MK-1775 in 21 evaluable patients with refractory solid tumors, including seven patients with documented BRCA1/2 mutations, reported confirmed partial responses in one head and neck cancer and one ovarian cancer patient, both harboring BRCA1 mutations; however, no responses were seen in any of five patients with confirmed TP53 mutations (Do et al., 2015; 25964244). A Phase 1/2 trial of alisertib treatment in solid tumors included 23 evaluable NSCLC patients. The overall response rate was 4% in NSCLC patients and the median progression-free survival was 3.1 months, but the study of alisertib in NSCLC patients did not proceed to stage 2 (Melichar et al., 2013; ASCO 2013, Abstract 605, Melichar et al., 2015; 25728526). A Phase 1 trial of SGT-53 in 11 patients with refractory cancer reported that the gene therapy complex was well tolerated with stable disease achieved in seven patients at six weeks and a median survival of 340 days; in addition, one tumor which was previously classified as inoperable was able to be resected (Senzer et al., 2013; 23609015). A Phase 1 trial of SGT-53 in combination with docetaxel in 14 patients with advanced cancer has reported three partial responses and two stable diseases per RECIST; this combination was well tolerated (Pirollo et al., 2016; 27357628).

Preclinical Data for TP53 (E349Nfs*21):
A preclinical study reported that ENMD-2076 reduced cell proliferation in a NSCLC cell line in vitro (Fletcher et al., 2011; 21177375). The Wee1 inhibitor MK-1775 has been reported to synergize with radiation in clonogenic assays in NSCLC cell lines with mutant, but not wild-type, TP53, and to enhance tumor growth delay in combination with radiation, as compared with radiation alone, in a TP53-defective NSCLC xenograft model (Bridges et al., 2011; 21799033). A preclinical study reported that MK-1775 treatment led to tumor regression in a NSCLC mouse xenograft model (Guertin et al., 2013; 23699655).

FDA Approved Therapies in Other Indications:
None.

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

EGFR (Amplification)

Description:
EGFR-amplification is an activating alteration. EGFR activating mutations or amplification may predict sensitivity to Egfr-targeted therapies, including inhibitors of multiple ErbB family members, and several have received FDA approval in some tumor types (Mok et al., 2009; 19692680, Rosell et al., 2009; 19692684, Tsao et al., 2005; 16014883). Egfr activation or overexpression may also lead to activation of the PI3K and MAPK pathway and may confer sensitivity to PI3K and MAPK pathway inhibitors (Bancroft et al., 2002; 11992543).

High-level EGFR gene amplification has been correlated with elevated Egfr protein expression, as measured by immunohistochemistry, although this correlation is not consistent for low-level gene amplification (Hemmings et al., 2009; 19404848, Liang et al., 2010; 20637128, Yang et al., 2012; 22490401, Bhargava et al., 2005; 15920544, Miyai et al., 2010; 20608935).

Putative high-level amplification of EGFR has been reported in 6.0% (69/1144) of Non-small cell lung carcinoma (NSCLC) cases (Pan-Lung Cancer (TCGA, Nat Genet 2016), cBioPortal for Cancer Genomics, Sep 2016). Amplification of EGFR has been reported in 6.4-10% of non-small cell lung carcinoma (NSCLC) samples in several large studies (Park et al., 2012; 22207554, Grob et al., 2013; 23238037, Liang et al., 2010; 20637128, Zhang et al., 2014; 24452282, Schrock et al., 2016; 27343443). However, smaller studies of less than 100 samples have detected higher incidences of EGFR amplification in NSCLC, citing it in 35-64% of cases, with one study reporting EGFR amplification in 72% (16/22) and 64% (16/25) of adenocarcinoma and squamous cell carcinoma samples, respectively (Russell et al., 2014; 24300726, Liang et al., 2010; 20637128, Tochigi et al., 2011; 21502435, Oakley and Chiosea, 2011; 21587084, Jia et al., 2015; 26400330). One study reported positive EGFR mRNA expression in the peripheral blood of 69% (29/42) of non-small cell lung carcinoma (NSCLC) patients, as compared with 12.5% (5/40) of control patients without lung cancer (Zhang et al., 2014; 24396405). Egfr expression has been reported in 19-69% of NSCLC cases (Ludovini et al., 2013; 23314677, Dobashi et al., 2011; 21040950, Traynor et al., 2013; 23628526, Watzka et al., 2010; 20353893, Liang et al., 2010; 20637128, Grob et al., 2013; 23238037, Park et al., 2012; 22207554, Hao et al., 2015; 26648997).

The presence of an EGFR abnormality (mutation, amplification, or overexpression) can result in an overabundance or overactivity of Egfr protein, which can lead to excessive proliferation (Ciardiello and Tortora, 2008; 18337605). EGFR mutations in NSCLC have been reported to occur more frequently in women, never-smokers, and in patients with adenocarcinoma histology (Rizzo et al., 2016; 25956936, Lee et al., 2015; 26359571, Naderi et al., 2015; 26362141, Zhou et al., 2016; 27039821, Lee et al., 2016; 26992209).

EGFR amplification or increased copy number have been reported to be associated with increased sensitivity to Egfr targeted therapies in studies of lung cancer, whereas studies in colorectal cancer (CRC) patients have been mixed; efficacy in patients with CRC is dependent on the absence of KRAS and NRAS mutations (Tsao et al., 2005; 16014883, Bell et al., 2005; 16204011, Hirsch et al., 2005; 15998906, Ålgars et al., 2011; 21694725, Sartore-Bianchi et al., 2007; 17664472, Yang et al., 2012; 22897982). The Egfr TKIs erlotinib, afatinib, and gefitinib have been approved by the FDA for the treatment of EGFR mutant non-small cell lung cancer (NSCLC) (Shepherd et al., 2005; 16014882, Rosell et al., 2012; 22285168, Sequist et al., 2013; 23816960, Douillard et al., 2014; 24263064, Mok et al., 2009; 19692680). Erlotinib, in combination with gemcitabine, has also been approved by the FDA for the treatment of locally advanced, unresectable, or metastatic pancreatic cancer (Moore et al., 2007; 17452677, Senderowicz et al., 2007; 18247017). Anti-Egfr monoclonal antibodies are also approved in some indications, including cetuximab, which is an approved therapy for HNSCC and colorectal cancer, panitumumab, which is approved in colorectal cancer, and necitumumab, which has received approval for the treatment of advanced squamous NSCLC (Cunningham et al., 2004; 15269313, Vermorken et al., 2008; 18784101, Van Cutsem et al., 2007; 17470858, Thatcher et al., 2015; 26045340). For NSCLC patients with metastatic disease and tumors harboring a sensitizing EGFR mutation, the NCCN guidelines (v.2.2016) suggest treating with erlotinib, afatinib, or gefitinib if the alteration is discovered prior to first-line chemotherapy or interrupting/completing current therapy and treating with erlotinib, afatinib, or gefitinib if the alteration is discovered during first-line chemotherapy.

FDA Approved Drugs in Current Indication:
Necitumumab (lung squamous cell carcinoma).

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 3 Data for EGFR (Amplification):
A meta-analysis of 16 Phase 3 trials including 2962 patients with EGFR-mutant advanced NSCLC evaluated the efficacy of afatinib, erlotinib, and gefitinib. In the overall population, all therapies showed superior outcome as compared with chemotherapy for overall response rate (ORR), disease control rate (DCR), and one year progression-free survival (PFS). In chemotherapy-naive patients, afatinib had improved overall survival (OS) and one year PFS, and erlotinib showed the best DCR. In previously treated patients, gefitinib had enhanced ORR, and erlotinib showed the most improved one- and two-year OS, as compared with gefitinib and second line chemotherapy (Zhang et al., 2016; 26933807). A meta-analysis of six trials including 4675 EGFR-mutant patients reported no significant difference in overall survival, time to progression, or response rate with Egfr TKI monotherapy versus Egfr TKI treatment in combination with chemotherapy as first-line treatment (Yan et al., 2015; 26285137). A Phase 3 randomized study in 1093 stage 4 squamous NSCLC patients reported that treatment with first-line necitumumab in combination with gemcitabine (G) and cisplatin (C) compared with GC alone was associated with an increase in overall survival (11.5 and 9.9 months, respectively) and an increase in median progression free survival (5.7 and 5.5 months, respectively); the objective response rates were similar between the two groups (31% and 29%, respectively), although necitumumab in combination with GC showed an increased disease control rate (82% versus 77%, respectively). An increase in grade 3 or higher adverse events was also reported in the cohort treated with necitumumab plus GC (72%, 388/5387) compared with cohort treated with GC alone (62%, 333/541) (Thatcher et al., 2015; 26045340). A subgroup analysis of 982 stage 4 squamous NSCLC patients reported expression of Egfr in 95% (935/982) of cases; in Egfr-positive patients, the combination of necitumumab and GC resulted in a significantly increased median overall survival time of 11.7 months as compared with 10.0 months with GC alone. No differences in survival between treatments were reported in patients with no Egfr protein expression (Paz-Ares et al., 2016; 27198355, Paz-Ares et al., 2016; 27207107). The approval of afatinib for first-line therapy of NSCLC patients with EGFR exon 19 deletions or the exon 21 L858R mutation is based on a randomized Phase 3 study of 345 patients with EGFR mutations comparing afatinib to chemotherapy with pemetrexed and cisplatin. The median progression-free survival (PFS) for patients treated with afatinib was 11.1 months, compared to 6.9 months for patients treated with chemotherapy. Among patients with exon 19 or 21 mutations, the median PFS for patients treated with afatinib was 13.6 months, compared to 6.9 months for chemotherapy (Sequist et al., 2013; 23816960). Phase 3 studies of afatinib in unselected NSCLC patients previously treated with either erlotinib or gefitinib have reported significantly increased median progression-free survival, but similar overall survival and increased toxicity alone or in combination with chemotherapy, as compared with placebo or single agent chemotherapy (Schuler et al., 2016; 26646759, Miller et al., 2012; 22452896). Afatinib has been FDA-approved for the treatment of lung squamous cell carcinoma (SCC) following progression on platinum-based chemotherapy on the basis of the Phase 3 LUX-Lung 8 trial comparing afatinib with erlotinib as second-line treatment in 795 stage 3b/4 lung SCC patients. Patients treated with afatinib had increased median progression-free survival as compared with erlotinib treatment (2.6 versus 1.9 months), increased median overall survival (7.9 versus 6.8 months), and improved disease control and median duration of objective response. Adverse events were cited in 99% (390/392) and 97% (385/395) of patients in the afatinib and erlotinib groups, respectively, with grades 3/4 adverse events reported in 57% of both groups (Soria et al., 2015; 26156651). The Phase 3 ARCHER 1009 trial of dacomitinib or erlotinib in advanced or metastatic NSCLC patients previously treated with chemotherapy reported an overall median progression-free survival time of 2.6 months in both groups, and 2.6 months in KRAS wild-type patients specifically treated with either drug; serious adverse events were reported in 12% and 9% of those treated with dacomitinib and erlotinib, respectively (Ramalingam et al., 2014; 25439691, Ramalingam et al., 2016; 26768165). In previously untreated NSCLC patients the Phase 3 FLEX study reported that treatment with cetuximab plus chemotherapy resulted in longer overall survival as compared with chemotherapy alone (12.0 vs 9.6 months) specifically in patients with high expression of Egfr (Pirker et al., 2012; 22056021). A meta-analysis of four randomized Phase 2/3 trials that evaluated chemotherapy with, or without, cetuximab as first-line treatment in patients with NSCLC (n=2018) has reported that cetuximab improves median overall survival to 10.3 months from 9.4 months and overall response rate to 32.2% from 24.4% (Pujol et al., 2014; 24332319). Erlotinib was approved by the FDA for unselected NSCLC patients based on a Phase 3 randomized trial demonstrating prolonged overall survival for unselected NSCLC patients treated with erlotinib compared with standard chemotherapy (Shepherd et al., 2005; 16014882). However, FDA approval has been modified to include only NSCLC patients harboring either an exon 19 deletion mutation or the exon 21 L858R mutation based on the results of a double-blind placebo-controlled Phase 3 trial that excluded patients harboring these mutations; this study (NCT01328951) found that in patients without these mutations, erlotinib had no benefit compared with placebo on overall survival of 643 NSCLC patients with no disease progression or unacceptable toxicity during four cycles of platinum-based first-line chemotherapy (FDA). Phase 3 studies of erlotinib compared with standard chemotherapy regimens in Asian (OPTIMAL) and European (EURTAC) populations of NSCLC patients harboring EGFR exon 19 mutations or the exon 21 L858R mutation have reported a progression-free survival of 13.1 and 9.7 months with erlotinib, respectively, and 4.6 and 5.2 months with chemotherapy, respectively (Zhou et al., 2011; 21783417, Rosell et al., 2012; 22285168). The approval of gefitinib for NSCLC patients with EGFR exon 19 deletions or the exon 21 L858R mutation is based on a Phase 4 trial of gefitinib as a first-line treatment in 106 EGFR mutation positive NSCLC patients, including 69 patients harboring an exon 19 deletion, 33 with L858R, and two each with L861Q and G719X mutations. The overall response rate based on investigator assessment was 69.8% (74/106), including two complete and 72 partial responses, and 50% (58/106) by a secondary, central review; the disease control rate was 90.6%, and median progression-free and overall survival times were 9.7 and 19.2 months, respectively. In patients with an exon 19 deletion and the L858R mutation, the overall response rates based on investigator assessment were 72.5% (50/69) and 63.6% (21/33), respectively (Douillard et al., 2014; 24263064, Kazandjian et al., 2016; 26980062). The Phase 3 IPASS study compared gefitinib to carboplatin plus paclitaxel in 1,217 NSCLC patients with adenocarcinoma histology. Progression-free survival at 12 months was 24.9% in the gefitinib group and 6.7% in the carboplatin-paclitaxel group, and the objective response rates were 43% and 32.2%, respectively. In the 261 EGFR mutation positive patients, increased progression-free survival and objective response rates (71.2% and 47.3%, respectively) were reported in the gefitinib group as compared with the chemotherapy group (Mok et al., 2009; 19692680).

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 2 Data for EGFR (Amplification):
A Phase 2 study of erlotinib as first-line treatment in 46 evaluable advanced or metastatic stage 3b/4 Caucasian lung adenocarcinoma patients harboring an EGFR kinase domain mutation reported progression-free survival rates at three and six months of 81% and 72%, respectively, and a median progression-free survival of 11 months; complete remission, partial remission, stable disease, and progressive disease were reported in 2% (1/46), 57% (26/46), 22% (10/46), and 20% (9/46), respectively, for a clinical benefit rate of 81%. The median duration of response was 9.7 months and median overall survival was 23 months, with 17% (8/46) of patients reported to show de novo resistance to erlotinib (De Grève et al., 2016; 27032107). A Phase 2 trial evaluating erlotinib compared to pemetrexed as second-line therapy in 123 lung adenocarcinoma patients with EGFR amplification, but not mutation, reported no significant differences between the two therapy options. A Phase 2 study of neratinib in 167 NSCLC patients has reported limited clinical activity and dose was limited by grade 3 adverse effects. Although none of 48 wild-type EGFR patients showed an objective response, 3% of the EGFR-mutant NSCLC subjects had an objective response. No responses were seen in patients with EGFR T790M mutations, but three partial responses and stable disease were reported in cases with EGFR G719X mutation (Sequist et al., 2010; 20479403). A Phase 2 trial of nimotuzumab in combination with chemotherapy (docetaxel and carboplatin) versus chemotherapy alone in 110 stage 3b/4 NSCLC patients reported an increased overall response rate in the nimotuzumab-treated group as compared with the chemotherapy-treated group (54% and 34.5%, respectively). Complete and partial responses were reported in 3.6% and 50% of nimotuzumab-treated patients, and in 4% and 30.9% in the chemotherapy group, respectively; no significant differences between the groups were observed in median progression-free survival, overall survival, and safety profile (Prabhash et al., 2013; ASCO 2013, Abstract 8053). A Phase 1b/2 study of afatinib and nimotuzumab in 43 evaluable patients with advanced NSCLC and acquired resistance to gefitinib or erlotinib reported median progression-free survival and overall survival of 4.0 and 11.7 months, respectively, and an overall response rate of 23% (10/43) in all evaluable patients and 30% (9/35) in patients harboring EGFR-activating mutations; combination treatment was deemed to have an acceptable toxicity profile (Lee et al., 2016; 26667485).

Phase 1 Data for EGFR (Amplification):
A study assessed the efficacy of afatinib in patients with "uncommon EGFR mutations" with metastatic NSCLC progressing after previous treatment with chemotherapy and one line of Egfr TKI treatment. In the 60 enrolled patients, 30 cases of T790M were reported. Median time to treatment failure was 3.8 and 5.1 months in the uncommon and common mutation groups, respectively, with activity noted in patients harboring E709X and T790M mutations, and exon 20 insertions (Heigener et al., 2015; 26354527). A Phase 1b study of the combination of afatinib and cetuximab in 126 EGFR-mutant NSCLC patients previously treated with erlotinib or gefitinib reported an overall response rate of 29% (37/126), including in 32% (23/71) and 25% (13/53) of EGFR T790M-positive and negative patients, respectively. Similar progression-free survival times of 4.8 and 4.6 months for T790M-positive and negative patients were also reported, and serious adverse treatment-related events occurred in 14% of all patients (Janjigian et al., 2014; 25074459). A study of 24 NSCLC patients previously treated with gefitinib, erlotinib, or afatinib who developed resistance assessed the efficacy of bevacizumab in combination with either erlotinib (n=22) or gefitinib (n=2). The response and disease control rates were 13% and 88%, respectively, with three partial responses and 18 patients showing stable disease; median progression-free and overall survival times were 4.1 and 13.5 months, respectively. Increased response rate and disease control rates were also reported in T790M-negative patients as compared with those harboring the T790M mutation (18% versus 0%, 88% versus 86%) (Otsuka et al., 2015; 26349474). A Phase 1 study of the combination therapy of cetuximab, erlotinib, and bevacizumab reported stable disease in 21% (7/34) of NSCLC patients (Falchook et al., 2013; 23435217).

Preclinical Data for EGFR (Amplification):
Preclinical studies suggest that Hsp90 inhibitors may be effective in NSCLC cells that are resistant to Egfr inhibitors (Shimamura et al., 2012; 22806877, Kobayashi et al., 2012; 21767894).

FDA Approved Therapies in Other Indications:
Panitumumab, Necitumumab, Afatinib, Cetuximab, Erlotinib, Gefitinib

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CLINICAL TRIALS SPECIFIC TO MUTATION AND DIAGNOSIS

Gene	Phase	NCT Identifier	Clinical Trial Locations	Clinical Trial Title
ALK	Phase 1	NCT02422589	CA, MI, TX, MI, MO	A Phase I, Multi-center, Open Label, Drug-drug Interaction Study to Assess the Effect of Ceritinib on the Pharmacokinetics of Warfarin and Midazolam in Patients With ALK-positive Advanced Tumors
ALK	Phase 1	NCT02299505	CA, CO, GA, IN, MD, NH, NJ, NY, OK, SC, UT, WA, Alberta, Ontario, GR, Maharashtra, West Bengal, BG, BO, CA, FC, FG, MI, PN, RM, VR, Korea, Russia, Andalucia, Madrid, Navarra, Pais Vasco, Songkla, Greater Manchester, Newcastle	Pharmacokinetic and Safety Study of Lower Doses of Ceritinib Taken With a Low-fat Meal Versus 750 mg of Ceritinib in the Fasted State in Adult Patients With (ALK-positive) Metastatic Non-small Cell Lung Cancer (NSCLC)
ALK	Phase 2	NCT01970865	AR, CA, CO, CT, District of Columbia, FL, MA, MI, MO, NY, OH, PA, TN, New South Wales, Queensland, Victoria, British Columbia, Ontario, Lower Saxony, Aichi, Chiba, Ehime, Hokkaido, Hyogo, Osaka, Shizuoka, Tokyo, Madrid, Navarra	A Study Of PF-06463922 An ALK/ROS1 Inhibitor In Patients With Advanced Non Small Cell Lung Cancer With Specific Molecular Alterations
ALK	Phase 2	NCT02693535	AZ, GA, IL, MI, NC, OK, PA, SD	TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer
ALK	Phase 3	NCT02201992	AL, AK, AZ, AR, CA, CO, CT, DE, District of Columbia, FL, GA, HI, ID, IL, IN, IA, KS, KY, LA, ME, MD, MA, MI, MN, MS, MO, MT, NE, NV, NH, NJ, NM, NY, NC, ND, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VT, VA, WA, WV, WI, WY	Crizotinib in Treating Patients With Stage IB-IIIA Non-small Cell Lung Cancer That Has Been Removed by Surgery and ALK Fusion Mutations (An ALCHEMIST Treatment Trial)
EGFR	Phase 1	NCT02013219	CA, CT, FL, IL, MA, MI, NY, OH, SC	A Phase 1b Study of Atezolizumab in Combination With Erlotinib or Alectinib in Participants With Non-Small Cell Lung Cancer
EGFR	Phase 2	NCT01306045	MD	Molecular Profiling and Targeted Therapy for Advanced Non-Small Cell Lung Cancer, Small Cell Lung Cancer, and Thymic Malignancies
EGFR	Phase 2	NCT01822496	AK, AZ, CA, CO, CT, DE, FL, GA, IL, IN, IA, ME, MD, MA, MI, MN, MO, NE, NH, NJ, NY, NC, OH, OK, OR, PA, SC, TX, WA, WV, WI	Erlotinib Hydrochloride or Crizotinib and Chemoradiation Therapy in Treating Patients With Stage III Non-small Cell Lung Cancer
EGFR	Phase 2	NCT02795156	CO, TN	Study to Assess the Activity of Molecularly Matched Targeted Therapies in Select Tumor Types Based on Genomic Alterations
EGFR	Phase 3	NCT02152631	AR, CA, CO, DE, FL, GA, IL, IN, IA, KS, KY, MD, MA, MI, MO, MT, NE, NH, NY, OH, OK, PA, SC, TN, TX, UT, VT, VA, WA	A Study of Abemaciclib (LY2835219) in Participants With Previously Treated KRAS Mutated Lung Cancer
KRAS	Phase 1	NCT01912625	MN, OH, TX, WI	Trametinib, Combination Chemotherapy, and Radiation Therapy in Treating Patients With Stage III Non-small Cell Lung Cancer That Cannot Be Removed by Surgery
KRAS	Phase 1	NCT01859026	FL	A Phase I/IB Trial of MEK162 in Combination With Erlotinib in NSCLC Harboring KRAS or EGFR Mutation
KRAS	Phase 1/Phase 2	NCT02022982	MA	PALBOCICLIB + PD-0325901 for NSCLC & Solid Tumors
KRAS	Phase 2	NCT02642042	CA, CO, GA, ID, IL, IN, IA, KY, MA, MI, MN, MO, MT, NV, NY, NC, ND, OH, OR, PA, SC, SD, UT, WI, WY	Trametinib and Docetaxel in Treating Patients With Recurrent or Stage IV Non-small Cell Lung Cancer

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CLINICAL TRIALS SPECIFIC TO MUTATION AND DIAGNOSIS (CONTINUED)

Gene	Phase	NCT Identifier	Clinical Trial Locations	Clinical Trial Title
KRAS	Phase 2	NCT02465060	AL, AK, AZ, AR, CA, CO, CT, DE, District of Columbia, FL, GA, HI, ID, IL, IN, IA, KS, KY, LA, ME, MD, MA, MI, MN, MS, MO, MT, NE, NV, NH, NJ, NM, NY, NC, ND, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, WA, WV, WI, WY	NCI-MATCH: Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors or Lymphomas
PIK3CA	Phase 1	NCT01061788	NC	A Trial of AMG 479, Everolimus (RAD001) and Panitumumab in Patients With Advanced Cancer
PIK3CA	Phase 1	NCT02321501	TX	Phase I/Ib Dose Escalation & Biomarker Study of Ceritinib (LDK378) + Everolimus for Locally Advanced or Metastatic Solid Tumors With an Expansion in Non-Small Cell Lung Cancer (NSCLC) Characterized by Abnormalities in Anaplastic Lymphoma Kinase (ALK) Expression
PIK3CA	Phase 1	NCT02079636	AR, CA, IN, NJ, NM, NC, TN	A Study of Abemaciclib (LY2835219) in Combination With Another Anti-cancer Drug in Participants With Lung Cancer (NSCLC)
PIK3CA	Phase 1/Phase 2	NCT01737502	AZ	Sirolimus and Auranofin in Treating Patients With Advanced or Recurrent Non-Small Cell Lung Cancer or Small Cell Lung Cancer
PIK3CA	Phase 2	NCT01248247	CT, TX	BATTLE-2 Program: A Biomarker-Integrated Targeted Therapy Study in Previously Treated Patients With Advanced Non-Small Cell Lung Cancer
TP53	N/A	NCT01827384	CO, MD, MO	Molecular Profiling-Based Targeted Therapy in Treating Patients With Advanced Solid Tumors
TP53	Phase 1	NCT02327169	MA, PA, TX, Gironde, Haute Garonne, Val de Marne, Greater London, Greater Manchester, Oxfordshire	A Phase 1B Study of MLN2480 in Combination With MLN0128 or Alisertib, or Paclitaxel, or Cetuximab, or Irinotecan in Adult Patients With Advanced Nonhematologic Malignancies
TP53	Phase 1	NCT02617277	CO, FL, TN	Safety, Tolerability and Pharmacokinetics of AZD1775 Plus MEDI4736 in Patients With Advanced Solid Tumours
TP53	Phase 1	NCT02719691	CO	Phase I Study of MLN0128 and MLN8237 in Patients With Advanced Solid Tumors and Metastatic Triple-negative Breast Cancer
TP53	Phase 2	NCT02576444	CT, MA	OLAParib COmbinations

GENES EVALUATED IN TARGETED CANCER GENE ASSAY

Sequence and Copy Number (*) Analysis

ABL1	EP300	HDAC2	MTOR	RAD51
AKT1*	EPHA2	HNF1A	MYC*	RAF1
ALK*	ERBB2*	HRAS	MYCN*	RARA
AR*	ERBB3*	IDH1	MYD88	RB1
ATM	ERBB4	IDH2	NBN	RET*
ATRX	ERCC3	JAK1	NF1	RNF43
AXL*	ERG	JAK2*	NOTCH1	ROS1*
BCL2*	ESR1	JAK3	NPM1	RUNX1*
BRAF*	EZH2	KDR*	NRAS	SDHB
BRCA1	FANCA	KEAP1	NTRK1*	SMAD4
BRCA2	FANCD2	KIT*	NTRK2*	SMARCB1
CCND1*	FANCG	KMT2A	NTRK3*	SMO
CCND2*	FBXW7	KRAS*	PALB2	SRC
CCND3*	FGFR1*	MAP2K1	PDGFRA*	STK11
CDK4*	FGFR2*	MAP2K2	PDGFRB*	TERT
CDK6*	FGFR3*	MEN1	PIK3CA*	TET2
CDKN2A	FGFR4*	MET*	PIK3CB*	TP53
CHEK2	FLT1*	MLH1	PIK3R1	TSC1
CREBBP	FLT3*	MLH3	PMS2	TSC2
CSF1R*	FLT4*	MPL	POLD1	VEGFA*
CTNNB1	FOXL2	MRE11A	POLE	VHL
DDR2	GNA11	MSH2	PTCH1	
DNMT3A	GNAQ	MSH6	PTEN	
EGFR*	GNAS	MST1R*	PTPN11	

Rearrangement Analysis

ALK	BRCA2	ETV5	MYC	RAF1
AXL	CBFB	ETV6	NTRK1	RARA
BCL2	EGFR	EWSR1	NTRK2	RET
BCR	ERG	FGFR1	NTRK3	ROS1
BRAF	ETV1	FGFR2	PDGFRA	TMPRSS2
BRCA1	ETV4	FGFR3	PDGFRB	

Microsatellite Analyses

BAT-25	BAT-26	NR-21	NR-24	MONO-27

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ADDENDUM

Disclaimer and Limitations of Approach

In validation studies, the analytical sensitivity and specificity of the targeted cancer gene assay were demonstrated in accordance with the performance specifications indicated in Table 1. These may be lower for structural alterations and may vary depending on the quality of the specimen. Next generation sequencing approaches may provide incorrect sequence or mutational data due to insufficient coverage in specific regions of the genome, inability to distinguish highly related human sequences, and sequencing errors. The analysis of sequence specific alterations can also be hampered by three aspects related to the tumor DNA. First, the quality of tumor DNA obtained from formalin-fixed samples is generally of poor quality and can result in degraded and damaged DNA. Second, the quantity of DNA obtained can be very low, limiting the amount of DNA molecules that can be successfully analyzed by next generation sequencing. Third, the purity of tumor DNA can be a factor, as a significant portion of the DNA analyzed in the tumor sample may be derived from contaminating normal tissues. These three aspects can reduce the chance of detecting somatic sequence and copy number alterations and rearrangements.

Table 1. Summary of CancerSELECT125 Performance Metrics

Performance Specification for Reported Alterations	Sensitivity	Specificity
Sequence Mutations (≥5% MAF)	99.0%	>99.9999%*
Amplifications (≥4-fold or 8 copies)	>99%	>99%
Rearrangements (≥20% tumor content)	>99%	>99%
MSI-H†	>99%	>99%

*Per-base specificity provided for sequence mutation analyses (979,072 total bases evaluated in the 125 targeted gene panel)

†In validation studies, a classification could not be made for 2% of cases and results were reported as indeterminate.

Sequence mutations, including single base and small insertions/deletions, are evaluated where the allele frequency is ≥2.0% and amplifications are evaluated when the fold-change is ≥4-fold. Specific amplifications are marked as "indeterminate" in situations where there is evidence of amplification ≥3-fold, but a definitive determination cannot be made. Additionally, in certain situations, an MSI classification cannot be made, and results are marked as "indeterminate".

Genetic alterations are defined as clinically significant based on published literature and other evidence. Literature references are not comprehensive and there may be other studies that relate to the test results.

Results presented in this report are intended for use solely by a qualified health care professional. Any diagnosis, counseling, or treatment determination made as a result of data presented in the report should be made by a qualified health care professional in conjunction with other individual patient health information, including clinical presentation and other test reports. Information contained within the report is current as of the report date; a qualified health professional should reassess these data as relevant literature becomes available.

This test was developed and its performance characteristics determined by Personal Genome Diagnostics. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research.

Somatic vs. Germline mutations

Except for BRCA1, BRCA2, PALB2 and ATM, this test is meant to identify only somatic mutations and is not designed to detect the presence or absence of germline mutations. For BRCA1, BRCA2, PALB2 and ATM, the presence of either a somatic or germline change will be included in this report. Our analyses do not determine whether a mutation is somatic or germline, and patients in whom an alteration in these genes is reported may benefit from additional germline testing.

Microsatellite Instability Testing

The microsatellite instability (MSI) phenotype may indicate a deficiency in normal DNA mismatch repair function within the tumor, and may suggest that this individual has an inherited cancer syndrome due to defective DNA mismatch repair (e.g. HNPCC/Lynch syndrome). However, the finding of tumor MSI does not distinguish between somatic and germline alterations leading to MSI. Furthermore, it is also possible that MSI status can be influenced by neoadjuvant chemotherapy, which may lead to a false positive result (Int J Radiat Oncol Biol Phys. 2007 68(5):1584).

Source of Clinical Information

N-of-One, Inc. has provided to PGDx research, analysis and interpretation, on a patient specific basis, of peer-reviewed studies and publically available databases. PGDx uses the information provided in formulating the patient report. This information may include the association between a specific molecular alteration and clinical benefit, or lack thereof, from FDA-approved therapies and therapies under clinical investigation. Additional information from N-of-One is available on its website at www.n-of-one.com.

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All positions below use the Human Reference genome hg19

TEST INFORMATION AND SEQUENCING CHARACTERISTICS

NUMBER OF GENES SEQUENCED	BASES IN TARGET GENES	
125	478,861	
SEQUENCED BASES (TUMOR)	**NUMBER OF SEQUENCES AT EACH BASE (TUMOR)**	**NUMBER OF DISTINCT SEQUENCES AT EACH BASE (TUMOR)**
1,667,418,600	1,737	685
SEQUENCED BASES (NORMAL)	**NUMBER OF SEQUENCES AT EACH BASE (NORMAL)**	**NUMBER OF DISTINCT SEQUENCES AT EACH BASE (NORMAL)**
822,428,100	827	495

SEQUENCE MUTATION DETAILS

Gene Symbol	Gene Description	Transcript	Genomic Position	Exon	Mutant Fraction	Mutation
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog	CCDS8703.1	Location: Chr12:25398284-25398284 Reference: C Mutant: T	1	37%	G12D
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase, Catalytic Subunit Alpha	CCDS43171.1	Location: Chr3:178952085-178952085 Reference: A Mutant: G	20	40%	H1047R
TP53	Tumor Protein P53	CCDS11118.1	Location: Chr17.Fa:7573982-7573982 Reference: C Mutant: .	9	26%	E349Nfs*21

AMPLIFICATION DETAILS

Gene Symbol	Gene Description	Gene ID	Genomic Position	Fold Change
EGFR	Epidermal Growth Factor Receptor	ENSG00000146648	Chr7:55086724-55275031	18.2

TRANSLOCATION DETAILS

Mutation	Type	Gene Symbols	Transcripts	Gene Descriptions	Approximate Breakpoints
EML4-ALK	Inversion	EML4	NM_019063	Echinoderm Microtubule Associated Protein Like 4	Chr2:42523000
		ALK	NM_004304	Anaplastic Lymphoma Receptor Tyrosine Kinase	Chr2:29447000

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