PGDx | PlasmaSELECT™ Report

REPORT DATE: 3/21/2017
DIAGNOSIS: NON-SMALL CELL LUNG CANCER
SPECIMEN TYPE: PLASMA

PATIENT NAME

John Doe

PATIENT INFORMATION

DATE OF BIRTH	PATIENT GENDER	PGDX NUMBER	PATIENT PHONE NUMBER
1/1/1960	Male	PGDX12345	000-000-0000

PATIENT EMAIL	INSTITUTION
john@doe.com	Hospital, City

PHYSICIAN	PLASMA SAMPLE RECEIVED
John Smith, MD	8/1/2016

SAMPLE CHARACTERISTICS

DIAGNOSIS	HISTOLOGY
Non-Small Cell Lung Cancer	Adenocarcinoma

SPECIMEN TYPE	PLASMA COLLECTION DATE
Plasma	7/30/2016

TEST INFORMATION AND SEQUENCING CHARACTERISTICS

TEST PERFORMED	NUMBER OF GENES SEQUENCED	BASES IN TARGET REGION
PlasmaSELECT	64	328,656

TOTAL PLASMA VOLUME	TOTAL DNA YIELD	TOTAL GENOME EQUIVALENTS	GENOME EQUIVALENTS PER ML OF PLASMA
10 ml	150 ng	45,455	4,545

Microsatellite Instability Analysis

MSI-H Detected	Active Clinical Trial
No	No

SEQUENCE MUTATIONS

Gene	Mutation	Consequence	Mutant fraction	Exon	FDA Approved Therapy		Active Clinical Trial
					Same Indication	Other Indication	
EGFR	L747_E749del	In-frame deletion	6.32%	19	Yes	No	Yes
MET	3082+2T>C	Splice site	5.11%	14	No	Yes	Yes
TP53	R273H	Missense	9.60%	7	No	No	Yes

AMPLIFICATIONS OR TRANSLOCATIONS

Gene	Fold Change	Consequence	FDA Approved Therapy		Active Clinical Trial
			Same Indication	Other Indication	
EGFR	1.9	Amplification	Yes	Yes	Yes
EML4-ALK	N/A	Rearrangement	Yes	No	Yes

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ELECTRONICALLY SIGNED BY	CLIA NUMBER	DATE
Luis A. Diaz, M.D.	21D2039282	3/21/2017

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., 2017; 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 (Kim 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 reported response rates of 72% (51/71) and 100% (8/8) in crizotinib-resistant and crizotinib-naive ALK-rearranged NSCLC patients respectively, with a median duration of response of 11.2 months and a median progression-free survival time of 13.2 months. In patients with measurable CNS metastases, 53% (8/15) showed a brain response (Langer et al., 2016; ASCO 2016, Abstract 9057). 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).

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, M.D.	21D2039282	3/21/2017

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

EGFR (L747_E749del)

Description:
EGFR-L747_E749del is an activating mutation. 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).

EGFR L747_E749del is an in frame deletion in exon 19 that occurs in the tyrosine kinase domain of the Egfr protein (UniProt, Integrative Genomics Viewer, v.2.3). Exon 19 kinase domain deletions similar to the one seen here, such as L747_A750, have been shown to activate the tyrosine kinase activity of Egfr and confer sensitivity to Egfr tyrosine kinase inhibitors such as erlotinib and gefitinib (Lynch et al., 2004; 15118073, Paez et al., 2004; 15118125, Pao et al., 2004; 15329413). This alteration has been reported to have transforming activity as a single mutation, and one study also reported that the combination of EGFR L747_E749del and A750P led to ligand-independent activation of Egfr and downstream activation of Stat3 and Akt activity; this complex mutation had transforming ability and conferred sensitivity to erlotinib and gefitinib (Schnidar et al., 2009; 19190345, Greulich et al., 2005; 16187797).

EGFR mutations have been reported in 30% (23583/79212) of Non-small cell lung carcinoma (NSCLC) samples analyzed in COSMIC (Sep 2016). EGFR mutations have been reported in 10% (117/1144) of Non-small cell lung carcinoma (NSCLC) samples (Pan-Lung Cancer (TCGA, Nat Genet 2016), cBioPortal for Cancer Genomics, Sep 2016). EGFR mutations have been reported in 14-49% of NSCLC cases, and found to be more common in East Asian patients as compared with other ethnicities (Vallee et al., 2013; 23934203, Rizzo et al., 2016; 25956936, Arrieta et al., 2015; 26358312, Zhou et al., 2016; 27039821, Lee et al., 2016; 26992209).

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).

The presence of a sensitizing EGFR mutation in a tumor is the strongest biological predictor of sensitivity to an Egfr tyrosine kinase inhibitor (TKI). Compared with conventional chemotherapy, Egfr TKIs have been shown to improve progression-free survival in non-small cell lung cancer patients whose tumors harbor EGFR mutations (Mok et al., 2009; 19692680, Rosell et al., 2009; 19692684, Tsao et al., 2005; 16014883, Rosell et al., 2012; 22285168). 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). 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:
Afatinib. Erlotinib. Gefitinib.

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CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 3 Data for EGFR (L747_E749del):
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). 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). 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). 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).

Phase 2 Data for EGFR (L747_E749del):
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).

Phase 1 Data for EGFR (L747_E749del):
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).

Preclinical Data for EGFR (L747_E749del):
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).

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CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

FDA Approved Therapies in Other Indications:
None.

MET (exon 14 splice site)

Description:
MET-exon 14 splice site is predicted to be an activating mutation. Aberrant activation of Met in cancer can occur through MET gene mutation or amplification, or excessive/inappropriate signaling via the Met receptor's ligand, HGF (Lennerz et al., 2011; 22042947). Cabozantinib, which targets Met and other kinases, has been FDA approved in metastatic medullary thyroid cancer and advanced renal cell carcinoma (Hart and De Boer, 2013; 23319867, Choueiri et al., 2015; 26406150). Crizotinib, a kinase inhibitor that has been FDA approved for treatment of EML4-ALK-positive and ROS-1-altered non-small cell lung cancer, also targets Met (Shaw et al., 2013; 23724913, Mazières et al., 2015; 25667280, Solomon et al., 2014; 25470694). Met-specific inhibitors are also in clinical development (Bendell et al., 2013; 23810377, Borin et al., 2015; 25542267).

The MET splice site alteration reported here occurs near the splice junction of transcribed exon 14 (Integrative Genomics Viewer, v.2.3). Mutations that disrupt the exon 14 splice donor or splice acceptor site have been reported to result in skipping of exon 14, which results in the deletion of part of the juxtamembrane domain. The loss of exon 14 has been reported to result in the stabilization and activation of Met, due in part to loss of interaction with the ubiquitin ligase Cbl (Kong-Beltran et al., 2006; 16397241, Frampton et al., 2015; 25971938, Awad et al., 2016; 26729443). Therefore, this alteration is expected to be activating. Additionally, studies have described that patients with tumors harboring alterations resulting in exon 14 skipping show clinical responses to treatment with Met inhibitors, including crizotinib (Jenkins et al., 2015; 25769807, Liu et al., 2016; 26215952, Frampton et al., 2015; 25971938).

MET mutations have been reported in 3.0% (34/1144) of Non-small cell lung carcinoma (NSCLC) samples (Pan-Lung Cancer (TCGA, Nat Genet 2016), cBioPortal for Cancer Genomics, Sep 2016). MET mutations have been reported in 1.8% (66/3600) of Non-small cell lung carcinoma (NSCLC) samples analyzed in COSMIC (Sep 2016). MET mutation has been reported in 1.7-3.8% of non-small cell lung carcinoma (NSCLC) cases in the scientific literature, including several studies specifically examining MET exon 14 mutations (Okuda et al., 2008; 19037978, Quinn et al., 2015; 25634010, Ludovini et al., 2012; 22302407, Liu et al., 2016; 27257131, Tong et al., 2016; 26847053, Schrock et al., 2016; 27343443, Awad et al., 2016; 26729443).

Met protein activation or overexpression promotes angiogenesis, resistance to apoptosis, proliferation, and invasion of cancer cells (Appleman, 2011; 22042966, Jung et al., 2012; 22553051, Gherardi et al., 2012; 22270953, Takeuchi et al., 2003; 12684423). Met protein expression in NSCLC has been associated with a predisposition to the development of brain metastases (Benedettini et al., 2010; 20489150, Breindel et al., 2013; 23794705).

Increased Met expression, possibly as a result of MET mutation or amplification, may lead to enhanced Met activation and may therefore confer sensitivity to Met inhibitors (Cecchi et al., 2010; 20303741, Lennerz et al., 2011; 22042947). Crizotinib and cabozantinib target multiple kinases, including Met, and have been FDA-approved for certain indications (Traynor, 2013; 23292257, Hart and De Boer, 2013; 23319867, Choueiri et al., 2015; 26406150, Mazières et al., 2015; 25667280, Solomon et al., 2014; 25470694, Shaw et al., 2013; 23724913). Met-specific inhibitors are in clinical development, including onartuzumab, a monoclonal antibody targeting Met (Bendell et al., 2013; 23810377, Borin et al., 2015; 25542267). Tivantinib is a small molecule inhibitor originally thought to target Met, but has more recently been reported to act as a microtubule depolymerizer; caution has been expressed in interpreting clinical trial results of tivantinib in tumors harboring MET alteration (Adjei et al., 2011; 21632449, Calles et al., 2015; 25226813, Basilico et al., 2013; 23532890). Additionally, studies have described that patients with tumors harboring alterations resulting in exon 14 skipping show clinical responses to treatment with Met inhibitors, including crizotinib (Jenkins et al., 2015; 25769807, Liu et al., 2016; 26215952, Frampton et al., 2015; 25971938).

FDA Approved Drugs in Current Indication:
None.

Phase 3 Data for MET (exon 14 splice site):
A Phase 3 trial of tivantinib in combination with erlotinib versus erlotinib alone in 1048 patients with advanced nonsquamous NSCLC reported no differences in overall survival, and increased median progression-free survival with combination treatment; the trial was terminated at interim analysis (Scagliotti et al., 2015; 26169611).

Phase 2 Data for MET (exon 14 splice site):
A Phase 2 trial of tivantinib in combination with erlotinib as compared to erlotinib alone in 167 Egfr TKI-naive NSCLC patients reported no significant difference in progression-free survival between the two arms, and objective responses in 10% and 7% of patients, respectively; MET amplification was reported in 37% of patients in the study, including one patient who displayed an objective response to the combination therapy (Sequist et al., 2011; 21768463). A Phase 2 study of tivantinib in combination with erlotinib in EGFR mutation-positive NSCLC with acquired resistance to EGFR tyrosine kinase inhibitors reported an overall response rate of 6.7% (3/45), with three patients achieving partial response; longer median progression-free survival and overall survival were observed in cases with high Met or Hgf expression (Azuma et al., 2016; 27843623). A Phase 2 randomized discontinuation trial of cabozantinib in metastatic NSCLC has reported a 10% response rate by RECIST, though a 64% rate of overall tumor regression in heavily pretreated patients, with a safety profile similar to that of other TKI inhibitors (Hellerstedt, 2012; ASCO 2012, Abstract 7514). A Phase 2 randomized trial of cabozantinib, erlotinib, or the combination in 111 EGFR wild-type NSCLC patients reported significantly improved median progression-free survival in the cabozantinib and combination groups as compared with the erlotinib group (4.3, 4.7, and 1.8 months, respectively), as well as longer median overall survival; Met expression was detected in 85% of cases and not was not correlated with progression-free survival (Neal et al., 2016; 27825638).

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 1 Data for MET (exon 14 splice site):
Preliminary results from a Phase 1 study of crizotinib in advanced NSCLC patients with MET exon 14 alterations has reported a partial response rate of 67% (10/15), including five confirmed and five unconfirmed partial responses. The median progression-free survival was not determined and treatment-related adverse events were reported in 82% of patients at the time of publication (Drilon et al., 2016; ASCO 2016, Abstract 108). 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). A Phase 1 study of tivantinib in combination with carboplatin and pemetrexed in six patients with malignant pleural mesothelioma and six patients with non-squamous NSCLC reported one complete response, four partial responses, and seven patients with stable disease; grades 3/4 treatment-related adverse events were reported in 50% (6/12) of patients (Zucali et al., 2015; ASCO 2015, Abstract 2549). Preliminary results from a Phase 1 dose-escalation study of merestinib (LY2801653) in 50 patients with advanced cancer reported that nine patients received treatment for more than four months, including one patient with colorectal carcinoma (707 days) and one patient with squamous cervical cancer (566 days); LY2801653 had acceptable toxicity (Hwang et al., 2014; AACR 2014, Abstract CT237).

Preclinical Data for MET (exon 14 splice site):
A preclinical study reported that merestinib (LY2801653, a Met inhibitor) had anti-tumor activity in mouse xenografts of various MET-altered tumors, including a MET-amplified lung adenocarcinoma cell line (Yan et al., 2013; 23275061). A preclinical study demonstrated that combination therapy of Egfr TKIs or cetuximab plus SU11274, a Met-specific TKI, could induce apoptosis in NSCLC cells with high Met expression that were Egfr TKI-resistant (Chen et al., 2013; 23527257). Preclinical studies have reported that single agent treatment with merestinib (LY2801653) can inhibit tumor cell growth in NSCLC tumor cell lines and patient-derived tumor xenograft models (Wu et al., 2013; 23989980, Kawada et al., 2014; 24305878).

FDA Approved Therapies in Other Indications:
Crizotinib, Cabozantinib

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

TP53 (R273H)

Description:
TP53-R273H exhibits altered function compared to wild type. 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). However, as the alteration reported here has been shown to have oncogenic effects, these therapeutic approaches are not expected to be relevant. Some studies suggest that Hsp90 inhibitors may be effective in tumors with oncogenic TP53 alterations (Alexandrova et al., 2015; 26009011, Lin et al., 2008; 17982489).

TP53 R273H occurs at a mutational "hot-spot" within the conserved DNA-binding domain (DBD) region (Joerger and Fersht, 2008; 18410249, Vogelstein and Kinzler, 1994; 8028656, Nishida et al., 1993; 8093350). DBD mutations are thought to result in loss of function via the loss of transactivation of p53-dependent genes (Kato et al., 2003; 12826609). However, several lines of evidence suggest that R273H mutant p53 is not significantly altered with regard to the tumor suppressor functionality of p53 (Sigal and Rotter, 2000; 11156366, Wong et al., 1999; 10411893, Park et al., 1994; 8208536). One study has indicated that TP53 R273H is capable of transactivation in some cancer cell lines but not in others (Park et al., 1994; 8208536). In addition, several functional gains have been ascribed to R273H mutant p53, including Egfr activation, high ROS levels, increased survival, and promotion of invasion and migration via both independently dominant and dominant-negative mechanisms (Wang et al., 2013; 23559009, Kalo et al., 2012; 22899716, Dong et al., 2007; 17636407, Li et al., 2014; 24677579, Quartuccio et al., 2015; 25810107).

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).

Some studies suggest that Hsp90 inhibitors may be effective in tumors with oncogenic TP53 alterations (Alexandrova et al., 2015; 26009011, Lin et al., 2008; 17982489, Li et al., 2011; 21478269).

FDA Approved Drugs in Current Indication:
None.

Phase 3 Data for TP53 (R273H):
None.

Phase 2 Data for TP53 (R273H):
A Phase 2 study of ganetespib in NSCLC patients reported progression-free survival at 16 weeks in 13.3% (2/15), 5.9% (1/17), and 19.7% (13/66) of cases harboring mutant EGFR, mutant KRAS, or no EGFR or KRAS mutations, respectively; stable disease was reported in 4% (4/98) of cases, all of which harbored ALK rearrangements (Socinski et al., 2013; 23553849). The randomized Phase 2 GALAXY-1 study in previously treated NSCLC patients reported that in 253 evaluable lung adenocarcinoma patients, the combination of ganetespib and docetaxel, compared with docetaxel alone, did not improve progression free survival, and therefore did not meet the primary endpoint of the trial (Ramalingam et al., 2015; 25997818).

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH REPORTED ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 1 Data for TP53 (R273H):
A Phase 1 study of AT13387 in 62 patients with advanced solid tumors reported one partial response in a patient with a gastrointestinal stromal tumor, stable disease in 34% (21/62) of patients, and an acceptable safety profile (Shapiro et al., 2015; 25336693). A Phase 1 study of SNX-5422 in 32 evaluable patients with refractory solid tumors reported one durable complete response in a prostate cancer patient, partial responses in a Her2-positive breast cancer patient and an adrenal gland cancer patient, and three patients with stable disease for greater than or equal to six months (Infante et al., 2014; 25262379). A Phase 1 study of SNX-5422 in 32 patients with refractory solid tumors and lymphomas reported no objective responses, stable disease in 47% (15/32), progressive disease in 53% (17/32), and that the treatment was well tolerated (Rajan et al., 2011; 21908572).

Preclinical Data for TP53 (R273H):
AT13387 has been reported to inhibit NSCLC cell growth in vitro and in a tumor xenograft model (Graham et al., 2012; 22181674).

FDA Approved Therapies in Other Indications:
None.

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).

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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). 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).

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:
Necitumumab, Afatinib, Erlotinib, Gefitinib

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CLINICAL TRIALS SPECIFIC TO MUTATION AND DIAGNOSIS

Gene	Phase	NCT Identifier	Clinical Trial Locations	Clinical Trial Title
ALK	N/A	NCT02473497	PA	Crizotinib (Xalkori) Expanded Access Protocol For The Treatment Of Adult Or Pediatric Patients
ALK	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
ALK	Phase 1	NCT02422589	MI, TX, MI, MO, PD	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 2	NCT02693535	AZ, GA, IL, MI, NE, NC, ND, OK, OR, PA, SD, UT, WA	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	NCT02496663	CA, MA	EGFR Inhibitor AZD9291 and Necitumumab in Treating Patients With EGFR-Positive Stage IV or Recurrent Non-small Cell Lung Cancer Who Have Progressed on a Previous EGFR Tyrosine Kinase Inhibitor
EGFR	Phase 1	NCT02674555	OH	A Study to Investigate the Absorption, Metabolism and Excretion of [14C]ASP8273 in Subjects With Solid Tumors
EGFR	Phase 1/Phase 2	NCT02108964	MA, NY, Ontario, Nordrhein-Westfalen, Korea, Catalunya, Taiwan ROC	A Phase I/II, Multicenter, Open-label Study of EGFRmut-TKI EGF816, Administered Orally in Adult Patients With EGFRmut Solid Malignancies
EGFR	Phase 1/Phase 2	NCT02535338	CA	Erlotinib Hydrochloride and Hsp90 Inhibitor AT13387 in Treating Patients With Recurrent or Metastatic EGFR-Mutant Non-small Cell Lung Cancer
EGFR	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, VT, VA, WA, WV, WI, WY	NCI-MATCH: Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma
EGFR	Phase 2	NCT01746251	CA, MA, NY	Adjuvant Afatinib in Stage I-III NSCLC With EGFR Mutation
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	NCT01553942	MA	Afatinib With CT and RT for EGFR-Mutant NSCLC
EGFR	Phase 2	NCT01822496	AZ, CA, CO, CT, DE, GA, IL, IN, IA, ME, MD, MA, MI, MN, MO, NE, NH, NJ, NY, NC, OH, OK, OR, PA, SC, TX, 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, FL, MO, TN	Study to Assess the Activity of Molecularly Matched Targeted Therapies in Select Tumor Types Based on Genomic Alterations
MET	Phase 1	NCT02511184	AL, CA, FL, GA, MN, NH, OH, WA	Crizotinib Plus Pembrolizumab In Alk-Positive Advanced Non Small Cell Lung Cancer Patients
MET	Phase 2	NCT01970865	CA, CO, District of Columbia, MA, MI, MO, OH, TN, Victoria, Ontario, Madrid, Navarra	A Study Of PF-06463922 An ALK/ROS1 Inhibitor In Patients With Advanced Non Small Cell Lung Cancer With Specific Molecular Alterations
MET	Phase 2	NCT02134912	MT, NE, OR	S1300: Pemetrexed Disodium With or Without Crizotinib in Treating Patients With Stage IV Non-Small Cell Lung Cancer That Has Progressed After Crizotinib

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CLINICAL TRIALS SPECIFIC TO MUTATION AND DIAGNOSIS (CONTINUED)

Gene	Phase	NCT Identifier	Clinical Trial Locations	Clinical Trial Title
MET	Phase 3	NCT02767804	AZ, FL, MI, MO, NY, OR, TN, VA	eXalt3: Study Comparing X-396 (Ensartinib) to Crizotinib in ALK Positive Non-Small Cell Lung Cancer (NSCLC) Patients
MET	Phase 3	NCT02737501	CA, CO, CT, FL, MA, MI, NY, NC, OK, TX, UT, VA, New South Wales, Victoria, Ontario, Cedex 05, Rhone Alpes, Bayern, Seoul, AP, Taichung, Kent, LE2, N7	ALTA-1L Study: A Phase 3 Study of Brigatinib Versus Crizotinib in ALK-positive Advanced Non-Small Cell Lung Cancer Patients
TP53	Phase 1	NCT02898207	MA	Olaparib and Hsp90 Inhibitor AT13387 in Treating Patients With Solid Tumors That Are Metastatic or Cannot Be Removed by Surgery or Recurrent Ovarian, Fallopian Tube, Primary Peritoneal, or Triple-Negative Breast Cancer
TP53	Phase 1	NCT01393509	NY	The First-in-human Phase I Trial of PU-H71 in Patients With Advanced Malignancies
TP53	Phase 1	NCT02503709	MD, MA	Hsp90 Inhibitor AT13387 and CDKI AT7519 in Treating Patients With Solid Tumors That Are Metastatic or Cannot Be Removed by Surgery

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GENES EVALUATED IN TARGETED PLASMA ASSAY (*All coding exons in RefSeq/CCDS transcripts are evaluated)

Sequence Analyses

AKT1	CDK6*	GNAS	NPM1	PTCH1
ALK*	CDKN2A	HRAS	NRAS	PTEN
AR*	CTNNB1	IDH1	NTRK1	RB1
ATM	DNMT3A	IDH2	NTRK2	RET*
BRAF*	EGFR*	JAK2	NTRK3	RNF43
BRCA1	ERBB2*	KIT*	PALB2	ROS1
BRCA2	ESR1	KRAS*	PIK3CA	TERT
CCND1	EZH2	MAP2K1	PIK3CB	TP53*
CCND2	FGFR1	MET	PIK3R1	TSC1
CCND3	FGFR2	MTOR	POLD1	TSC2
CD274*	FGFR3	MYC	POLE	VHL
CDK4*	FLT3	MYCN		

Amplification Analyses

ALK	CCND3	EGFR	FGFR3	MYCN
AR	CD274	ERBB2	KIT	PIK3CA
CCND1	CDK4	FGFR1	MET	ROS1
CCND2	CDK6	FGFR2	MYC	

Rearrangement Analyses

ALK	ETV6	MYC	PDGFRA	RARA
BCR	FGFR1	NTRK1	PDGFRB	RET
BRAF	FGFR2	NTRK2	RAF1	ROS1
EGFR	FGFR3			

Microsatellite Analyses

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

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ADDENDUM

Disclaimer and Limitations of Approach (PlasmaSELECT™64)

In validation studies, sensitivity and specificity were demonstrated to exceed performance acceptance criteria (Table 1). 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 sample and DNA. First, the quantity of DNA obtained can be very low, limiting the amount of DNA molecules that can be successfully analyzed by next generation sequencing. Second, the purity of tumor-derived DNA can be a factor, as a significant portion of the DNA analyzed may be derived from contaminating normal cells. Third, the mutation allele fraction depends on various factors including tumor burden, pre-plasma sample storage time and patient clinical characteristics unrelated to tumor status. These three aspects can reduce the chance of detecting sequence mutations, amplifications, rearrangements, and microsatellite instability.

Table 1. Summary of PlasmaSELECT™64 Performance Metrics

Performance Specification	Mutant Allele Fraction	Sensitivity	Specificity
Sequence Mutations (SBS/Indel)	≥0.50%	99.4%	>99.999%*
Rearrangements	≥0.50%	94.4%	>99%
Microsatellite Instability (MSI)	≥2.0%	>99%	>99%
Amplifications (≥4-fold)	≥20%	97.2%	>99%
Amplifications (≥4-fold)	<20%	*varies depending on level of amplification and tumor content*	

*Per-base specificity provided for sequence mutation analyses [99,359 bases evaluated]

Genetic alterations are defined as clinically significant based on published literature and other evidence. Literature references are not comprehensive, may not be validated, and there may be other studies that relate to the test results. The literature cited here may contain important limitations and qualifications (and should be reviewed by a health care professional in the context of the patient's results.) This test, meant to identify somatic mutations, is not intended to detect the presence or absence of germline mutations. While common germline variants have been removed, the test may detect rare germline alterations as well as mutations occurring in non-tumor cells, including hematopoietic cells. Results presented in this report are intended for use solely by a qualified health care professional. Any diagnosis, counseling, or treatment of the patient should be made by a qualified health care professional in conjunction with other individual patient health information, including clinical presentation, patient history, and other test reports. Information contained within the report is current as of the report date; a qualified health care professional should reassess these data as relevant literature becomes available.

This test result should not be the primary determinant of diagnosing, counseling, or training. 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.

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).

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All positions below use the Human Reference genome hg19

SEQUENCE MUTATION DETAILS

Gene Symbol	Gene Description	Transcript	Genomic Position	Exon	Mutant Fraction	Mutation
EGFR	Epidermal Growth Factor Receptor	CCDS5514.1	Location: Chr7:55242466-55242474 Reference: GAATTAAGA Mutant: .	19	6.32%	L747_E749del
MET	Met Proto-Oncogene	NM_001127500	Location: Chr7:116412045-116412045 Reference: T Mutant: C	14	5.11%	3082+2T>C
TP53	Tumor Protein P53	CCDS11118.1	Location: Chr17:7577120-7577120 Reference: C Mutant: T	7	9.60%	R273H

AMPLIFICATION DETAILS

Gene Symbol	Gene Description	Gene ID	Genomic Position	Fold Change
EGFR	Epidermal Growth Factor Receptor	ENSG00000146648	Chr7:55086724-55275031	1.9

TRANSLOCATION DETAILS

Mutation	Type	Gene Symbols	Transcripts	Gene Descriptions	Approximate Breakpoints
EML4-ALK	Intrachromosomal	EML4	ENST00000480320	Echinoderm Microtubule Associated Protein Like 4	Chr2:42494000
		ALK	ENST00000431873	Anaplastic Lymphoma Receptor Tyrosine Kinase	Chr2:29448000

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