MYPG | CancerSelect™ Report

REPORT DATE: 6/29/2015
TUMOR TYPE: NON-SMALL CELL LUNG CANCER

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	6/1/2014	6/18/2014

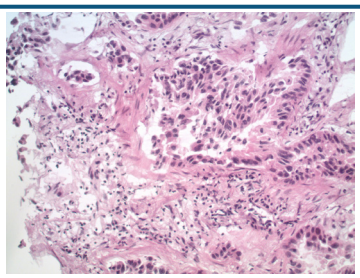
TEST INFORMATION AND SEQUENCING CHARACTERISTICS

TEST PERFORMED	NUMBER OF GENES SEQUENCED	BASES IN TARGET GENES
CancerSelect	88	478,861
SEQUENCED BASES (TUMOR)	NUMBER OF SEQUENCES AT EACH BASE (TUMOR)	NUMBER OF DISTINCT SEQUENCES AT EACH BASE (TUMOR)
1,667,418,600	1737	685
SEQUENCED BASES (NORMAL)	NUMBER OF SEQUENCES AT EACH BASE (NORMAL)	NUMBER OF DISTINCT SEQUENCES AT EACH BASE (NORMAL)
822,428,100	827	495

SAMPLE CHARACTERISTICS

TUMOR TYPE	
Non-small cell lung cancer	
TUMOR LOCATION	PATHOLOGICAL TUMOR PURITY
Cerebellum	50%
SPECIMEN TYPE	SOURCE OF NORMAL DNA
FFPE	Saliva
TUMOR COLLECTION DATE	SPECIMEN ID
1/30/2014	123456

TUMOR HISTOLOGY



MICROSATELLITE ANALYSIS

MSS - Microsatellite Stable (0 of 5 markers positive for MSI)

SEQUENCE MUTATIONS

Gene	Mutation	Consequence	Mutant fraction	FDA Approved	Active Clinical Trial
KRAS	G12D	Missense	13%	No	Yes
PIK3CA	H1047R	Missense	24%	No	Yes
TP53	E349Nfs*21	Frameshift	26%	No	Yes

AMPLIFICATIONS OR TRANSLOCATIONS

Gene	Fold	Consequence	FDA Approved	Active Clinical Trial
EGFR	18.2	Amplification	Yes	Yes
EML4-ALK	N/A	Rearrangement	Yes	Yes

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Luis A. Diaz, M.D. Chief Medical Officer	21D2039282	6/29/2015

CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH SOMATIC ALTERATIONS

Gene (Mutation)

KRAS (G12D)

Description:
KRAS-G12D is an activating mutation. Activating mutations in KRAS result in activation of downstream pathways, including the Raf/MEK/ERK pathway (Nakano et al., 1984; 6320174, Pylayeva-Gupta et al., 2011; 21993244). The MEK inhibitor trametinib has been approved for use in BRAF V600-mutant melanoma. Trametinib and other MEK inhibitors, alone or in combination therapy, are in clinical trials, as are multiple other approaches to targeting K-Ras signaling (Flaherty et al., 2012; 22663011, Jänne et al., 2013; 23200175, Britten, 2013; 23443307).

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 demonstrated to induce tumorigenesis in mouse models (O'Hagan and Heyer, 2011; 21779503, Johnson et al., 2001; 11323676).

KRAS mutations have been reported in 16.6% (4167/25008) of non-small cell lung carcinoma (NSCLC) samples, including 18.8% (2311/12288) of lung adenocarcinoma cases, and 4.6% (127/2770) of lung squamous cell carcinoma samples (COSMIC, Dec 2014). In the Lung Adenocarcinoma, and the Lung Squamous Cell Carcinoma TCGA datasets, KRAS mutations have been found in 26.2% (60/229), and in 1.1% (2/178) of cases, respectively (cBioPortal for Cancer Genomics, Dec 2014). Literature reports have identified KRAS mutations in 8-28% of NSCLC samples and specifically in 32-38% of lung adenocarcinoma samples (Maus et al., 2014; 24331409, Gainor et al., 2013; 23729361, Shigematsu et al., 2005; 15741570, Villaruz et al., 2013; 23526491, Yip et al., 2013; 23392229, Ragusa et al., 2014; 23357969). The incidence of KRAS mutations in lung adenocarcinoma differs significantly between former or current smokers and never-smokers; one study cited a KRAS mutation frequency of 4% in never-smokers compared to 43% in former or current smokers (Paik et al., 2012; 22605530).

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). Activating KRAS mutations can result in constitutive activation of the Ras/Raf/MEK/ERK and PI3K/Akt pathways (Nakano et al., 1984; 6320174, Pylayeva-Gupta et al., 2011; 21993244). KRAS mutations have been found to be mutually exclusive with ALK rearrangements and EGFR mutations in NSCLC (Gainor et al., 2013; 23729361, Shigematsu et al., 2005; 15741570, Shigematsu et al., 2005; 15753357). However, case studies of NSCLC patients harboring ALK mutations or EML4-ALK fusions have reported the emergence of KRAS mutations upon acquired resistance to crizotinib, demonstrating a role for KRAS in crizotinib resistance in NSCLC (Doebele et al., 2012; 22235099, Rossing et al., 2013; 24279718).

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 inhibitor trametinib has been FDA approved for the treatment of unresectable or metastatic BRAF V600E or V600K mutant melanoma, and it is currently being studied in clinical trials in other solid tumors (Flaherty et al., 2012; 22663011). Multiple clinical trials of trametinib and other MEK inhibitors, alone or in combination with other agents, are in progress, and multiple other strategies are being developed preclinically. 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 for *KRAS* (G12D):
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). The combination of erlotinib and the Met inhibitor tivantinib (ARQ 197) in a Phase 2 study also resulted in better outcomes for NSCLC patients whose tumors bore KRAS mutations, compared to treatment with erlotinib alone (Sequist et al., 2011; 21768463). A randomized Phase 2 trial of docetaxel with or without the MEK inhibitor selumetinib (AZD6244) in KRAS-mutant non-small cell lung cancer found that the addition of selumetinib resulted in an improvement in progression-free survival and response rate (Jänne et al., 2013; 23200175). 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 10 months in 20.6% (7/34) of patients (Haura et al., 2010; 20332327).

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CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH SOMATIC ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 1 Data for *KRAS* (G12D):
A Phase 1 dose-escalation study of MEK162 in pretreated patients with advanced solid tumors has reported partial response in 6% (1/17) of patients and stable disease in 53% (9/17) of patients; the authors report that MEK162 displayed an acceptable safety profile and induced pharmacodynamics changes suggestive of MEK inhibition (Bendell et al., 2011; AACR-NCI-EORTC 2011, Abstract B243). A Phase 1 study of cobimetinib in combination with the PI3K inhibitor GDC-0941 in patients with advanced solid tumors reported partial responses and stable disease for at least five months in 3/46 (a melanoma patient with mutated BRAF, a pancreatic carcinoma patient with mutated BRAF, and a endometrioid carcinoma patient with mutated KRAS) and 5/46 evaluable patients, respectively (LoRusso et al., 2012; ASCO 2012, Abstract 2566). A Phase 1 study of cobimetinib in 13 patients with advanced solid tumors reported that treatment was well tolerated; one patient with progressive NSCLC exhibited ongoing stable disease of at least seven months (Rosen et al., 2008; ASCO 2008, Abstract 14585). A Phase 1 multicenter trial of the MAPK inhibitor refametinib (BAY86-9766) in patients with advanced cancer reports suppression of ERK phosphorylation and stable disease in 11 patients for four or more courses of therapy (Weekes et al., 2013; 23434733). A Phase 1b trial of the combination of copanlisib (BAY 80-6946), a pan-PI3K inhibitor, and refametinib (BAY 86-9766), a MEK inhibitor, in patients with advanced cancer has reported a partial response in one endometrial cancer patient and stable disease in nine patients (Ramesh et al.,; ASCO 2014, Abstract 2588). 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 effect these rates (Gandara et al., 2013; ASCO 2013, Abstract 8028, Kelly et al., 2013; ASCO 2013, Abstract 8027, Bedard et al., 2014; 25500057).

Preclinical Data for *KRAS* (G12D):
A preclinical study of GDC-0973 in NSCLC xenograft models reported that tumor growth inhibition did not correlate with KRAS status; the class I PI3K inhibitor GDC-0941 increased the efficacy of GDC-0973 in an NSCLC xenograft model harboring a KRAS mutation (Hoeflich et al., 2012; 22084396). Coincident KRAS mutation and loss of the tumor suppressor STK11 in a preclinical model of NSCLC was correlated with response to phenformin, a mitochondrial inhibitor and metformin analogue (Shackelford et al., 2013; 23352126). In NSCLC cell lines containing KRAS or BRAF mutations, a combination of sorafenib and paclitaxel showed antitumor activity (Zhang et al., 2012; 22433711).

FDA Approved Therapies in Other Indication for *KRAS* (G12D):
Trametinib

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CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH SOMATIC 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 for other solid tumors (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 mutation has been reported in 3.6% (310/8514) of non-small cell lung carcinoma (NSCLC) samples analyzed in COSMIC, specifically in 3.9% (153/3908) of lung adenocarcinoma and 6.3% (70/1112) of lung squamous cell carcinoma samples (Jan 2015). In the Lung Adenocarcinoma TCGA dataset, PIK3CA mutations were found in 4.8% (11/229) of cases, while in the Lung Squamous Cell Carcinoma TCGA dataset, PIK3CA mutations were observed in 15.2% (27/178) of cases (cBioPortal for Cancer Genomics, Jan 2015). In the scientific literature, PIK3CA mutation has been found in 3-5% of NSCLC cases, including in 2.7-4.2% of lung adenocarcinoma and 3.9-8.9% of lung squamous cell carcinoma cases (An et al., 2012; 22768234, Stjernström et al., 2014; 24500884, Yip et al., 2013; 23392229, Wang et al., 2014; 24533074, Scheffler et al., 2014; 25473901, Xu et al., 2012; 22430133).

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 (Stjernström et al., 2014; 24500884, Scheffler et al., 2014; 25473901, Xu et al., 2012; 22430133, Wang et al., 2014; 24533074, An et al., 2012; 22768234, Ji et al., 2011; 21507233, Spoerke et al., 2012; 23136191).

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 in solid tumors. 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. Metformin, an approved type 2 diabetes drug that has been associated with decreased cancer risk and has demonstrated in vitro anti-cancer activity and indirect inhibition of mTOR activity (via AMPK activation) in preclinical studies, is being evaluated in early phase clinical trials for solid tumors (Viollet et al., 2012; 22117616, Zakikhani et al., 2010; 20135346, Wang et al., 2014; 24676803, Sinnett-Smith et al., 2013; 23159620, Karnevi et al., 2013; 23663483). 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 for *PIK3CA* (H1047R):
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). A Phase 2 trial of everolimus as a monotherapy in NSCLC has reported modest activity, with 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 has reported that the combination was well tolerated, but had modest efficacy with partial response and stable disease reported in 7% (2/28) and 54% (15/28) of patients, respectively, and a 6-month progression-free survival rate of 5% (Ramalingam et al., 2013; 23407561).

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 4 months, and grade 3/4 adverse events were reported in 65% (73/113) of all study patients (Bedard et al., 2014; 25500057). A Phase 1 study of 60 advanced cancer patients reported a partial response in 1/13 NSCLC patients treated with MK-2206 (Molife et al., 2014; 24387695). 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).

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Gene (Mutation)

Preclinical Data for *PIK3CA* (H1047R):
A preclinical study has reported that the combination of everolimus and BKM120 had a synergistic effect in non-small cell lung carcinoma cells and xenograft models (Ren et al., 2012; 22781393). Preclinical studies have reported MK-2206 treatment leads to growth inhibition in NSCLC cell lines, and has synergistic anti-tumor effects in combination with other therapies, such as AZD6244 or erlotinib (Meng et al., 2010; 21124782, Hirai et al., 2010; 20571069).

FDA Approved Therapies in Other Indication for *PIK3CA* (H1047R):
Temsirolimus, Everolimus

TP53 (E349fs*21)

Description:
TP53 is a tumor suppressor; loss or mutation of TP53 may result in genomic instability and excessive cell proliferation (Levine, 1997; 9039259). 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 (Hirai et al., 2010; 20107315, Bridges et al., 2011; 21799033). Additional p53-targeted approaches under clinical investigation, which may be relevant in the context of certain TP53 alterations, include kevetrin and ALT-801 (Kumar et al., 2012; AACR 2012, Abstract 2874, Fishman et al., 2011; 21994418). However, as the effect of this truncation on p53 function is unknown, the relevance of any therapeutic approaches is also unknown.

TP53 E349fs*21 is expected to effectively truncate the p53 protein. The resulting predicted protein would lack a portion of the C-terminal regulatory domain (Joerger and Fersht, 2008; 18410249). The C-terminal regulatory domain has been shown to be required for DNA binding and transcriptional activation by p53 (Kim et al., 2012; 22178617). However, as the majority of the protein and the C-terminal regulatory domain are retained, the functional effect of this truncation is unknown (PubMed, Mar 2015).

TP53 is one of the most commonly mutated genes in lung cancer; scientific studies have reported TP53 mutations to be present in 29-42% of non-small cell lung cancer (NSCLC) cases (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). TP53 mutations have been detected in 32.6% (2048/6291) of NSCLC samples analyzed in COSMIC, including 34.9% (727/2082) of lung adenocarcinoma cases and 42.6% (602/1412) of lung squamous cell carcinoma cases (Jan 2015). Additionally, TP53 mutations have been reported in 52% (119/229) and 67% (120/178) of sequenced tumors in the Lung Adenocarcinoma TCGA dataset and the Lung Squamous Cell Carcinoma TCGA dataset, respectively (cBioPortal for Cancer Genomics, Jan 2015).

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). TP53 alterations are believed to be early events in NSCLC, preceding lymph node metastasis (Chang et al., 2011; 20811949).

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. Chk1 inhibitors in combination with chemotherapy are also under investigation in clinical trials. Kevetrin (thioureidobutyronitrile) is a novel molecule currently under clinical investigation, which has been reported to have anti-tumorigenic effects in preclinical models. It has been suggested to act upon several tumor-associated pathways, with its activities including activation of wild-type p53 and selective destabilization of mutant p53 (cellceutix.com) (Kumar et al., 2012; AACR 2012, Abstract 2874). ALT-801 is a fusion of IL-2 with a T-cell receptor domain that recognizes a p53-derived peptide in the context of a specific HLA haplotype; this molecule is expected to target cells that express p53 containing the peptide epitope (aa 264-272), and would not be relevant in the context of TP53 alterations that are not expressed (Fishman et al., 2011; 21994418). In the case of this uncharacterized variant, the relevance of any available therapeutic approaches is unknown.

FDA Approved Drugs in Current Indication for *TP53* (E349fs*21):
None.

Phase 3 Data for *TP53* (E349fs*21fs):
None.

Phase 2 Data for *TP53* (E349fs*21):
None.

Phase 1 Data for *TP53* (E349fs*21):
A Phase 1 clinical trial of ALT-801 in patients with the relevant haplotype and p53-positive solid tumors concluded that the drug is safe and elicits an immunologic response (Fishman et al., 2011; 21994418). A Phase 1 trial reported that the Wee1 inhibitor MK-1775 in combination with chemotherapy in patients with advanced solid tumors has some anti-tumor activity (Schellens et al., 2009; ASCO 2009, Abstract 3510, J. et al., 2011; ASCO 2011, Abstract 3068). A Phase 1 trial of MK-1775 in 18 patients with refractory solid tumors reported a partial response in a head and neck cancer patient. Two dose-limiting toxicities occurred at dose level 3 (Do et al., 2014; ASCO 2014, Abstract 2503).

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Gene (Mutation)

Preclinical Data for *TP53* (E349fs*21):
The Wee1 inhibitor MK-1775 has been reported to synergize with radiation in clonogenic assays in non-small cell lung cancer (NSCLC) cell lines with defective p53, but not in NSCLC cell lines with wild-type p53, and to enhance tumor growth delay in combination with radiation, as compared with radiation alone, in a p53-defective NSCLC xenograft model (Bridges et al., 2011; 21799033). Preclinical studies of kevetrin in lung adenocarcinoma cell lines and xenografts have reported that this compound shows anti-tumor activity (Banu et al., 2009; AACR 2009, Abstract C49, Kumar et al., 2012; AACR 2012, Abstract 2874).

FDA Approved Therapies in Other Indication for *TP53* (E349fs*21):
None.

EGFR (Amplification)

Description:
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).

In the Lung Adenocarcinoma and Lung Squamous Cell Carcinoma TCGA datasets, putative high-level amplification of EGFR has been reported in 5.6% (29/515) and 7.0% (35/501) of cases, respectively (cBioPortal for Cancer Genomics, Jan 2015). Amplification of EGFR has been reported in 7-10% of non-small cell lung carcinoma (NSCLC) samples in the scientific literature (Park et al., 2012; 22207554, Grob et al., 2013; 23238037, Liang et al., 2010; 20637128, Zhang et al., 2014; 24452282). However, some studies have found higher incidences of EGFR amplification in NSCLC, citing it in 35-48% of samples (Russell et al., 2014; 24300726, Liang et al., 2010; 20637128, Tochigi et al., 2011; 21502435). One study has reported positive EGFR mRNA expression in the peripheral blood of 69% (29/42) of 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 specimens (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).

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 have been found to be mutually exclusive with ALK rearrangements and KRAS mutations in NSCLC (Gainor et al., 2013; 23729361, Shigematsu et al., 2005; 15741570, Shigematsu et al., 2005; 15753357). However, case studies of NSCLC patients harboring ALK mutations or EML4-ALK fusions have reported the emergence of EGFR mutations upon acquired resistance to crizotinib, demonstrating a role for EGFR in crizotinib resistance in NSCLC (Doebele et al., 2012; 22235099, Rossing et al., 2013; 24279718).

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). 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 and afatinib have been approved by the FDA for the treatment of EGFR mutant non-small cell lung cancer; gefitinib has been approved in Europe and Asia for this indication (Shepherd et al., 2005; 16014882, Rosell et al., 2012; 22285168, Sequist et al., 2013; 23816960). 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). The dual Egfr/Her2 inhibitor lapatinib has been approved for use in metastatic breast cancer (Geyer et al., 2006; 17192538, Cameron et al., 2010; 20736298). Anti-Egfr monoclonal antibodies are also approved in some indications, including cetuximab, which is an approved therapy for HNSCC and colorectal cancer, and panitumumab, which is approved in colorectal cancer (Cunningham et al., 2004; 15269313, Vermorken et al., 2008; 18784101, Van Cutsem et al., 2007; 17470858). Based on accumulated evidence, the American Society for Clinical Oncology (ASCO) has issued a Provisional Clinical Opinion recommending EGFR mutational analysis for NSCLC patients to predict benefit from Egfr TKIs (Keedy et al., 2011; 21482992).

FDA Approved Drugs in Current Indication for *EGFR* (Amplification):
Erlotinib.

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CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH SOMATIC ALTERATIONS (CONTINUED)

Gene (Mutation)

Phase 3 Data for *EGFR* (Amplification):
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). The Phase 3 OPTIMAL trial compared erlotinib to standard chemotherapy (gemcitabine plus carboplatin) as first-line treatment in stage 3 and 4 NSCLC patients harboring an exon 19 deletion or exon 21 L858R mutation. In 82 evaluable patients in the erlotinib arm and 72 in the chemotherapy arm, median progression-free survival was 13.1 and 4.6 months, respectively; grades 3 and 4 adverse effects were more prevalent in the chemotherapy arm as compared with the erlotinib arm, with neutropenia and thrombocytopenia reported in 42% (30/72) and 40% (29/72) of chemotherapy-treated patients, respectively (Zhou et al., 2011; 21783417). The approval of afatinib for first-line therapy of NSCLC patients with EGFR exon 19 or 21 mutations was 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). The approval of erlotinib in NSCLC is based on a Phase 3 randomized trial demonstrating prolonged overall survival for unselected NSCLC patients treated with erlotinib compared to standard chemotherapy; in this study of 731 stage 3B or 4 NSCLC patients, the response rate of those receiving erlotinib was 8.9%, as compared with less than 1% in the placebo group, with a median duration of response rate of 7.9 and 3.7 months, and overall survival rates of 6.7 and 4.7 months reported, respectively (Shepherd et al., 2005; 16014882). The FDA approval of gefitinib was revoked based on negative results in unselected NSCLC patients, although data from a number of randomized clinical trials in the setting of EGFR mutation have shown a significant improvement in progression-free survival compared with combination chemotherapy in chemo-naive patients (Mok et al., 2009; 19692680, Yu et al., 2014; 24755888, Douillard et al., 2014; 24263064). Analysis of the Phase 3 IPASS study revealed that EGFR amplification alone did not predict improved response to gefitinib, although the combination of EGFR amplification and mutation was associated with lengthened progression-free survival on gefitinib (Fukuoka et al., 2011; 21670455). At present, gefitinib's availability in the U.S. is limited to those patients who are benefitting from or have previously benefited from gefitinib. Gefitinib has been reported to inhibit bone resorption in NSCLC patients and reduced bone fractures in these patients (Okano and Nishio, 2008; 18379035).

Phase 2 Data for *EGFR* (Amplification):
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 MK-2206 in combination with erlotinib in advanced non-small cell lung cancer patients who had progressed on erlotinib reported a disease control rate of 47% (16/34) in patients with tumors expressing wild-type EGFR and a response rate of 9% (4/46) in patients with tumors expressing a mutant form of EGFR; median progression-free survival was 4.6 and 4.4 months in patients with wild-type and mutant EGFR, respectively (Lara et al., 2014; ASCO 2014, Abstract 8015). In a Phase 2 trial in patients with advanced or metastatic NSCLC, lapatinib monotherapy did not result in significant tumor reduction (Ross et al., 2010; 20215545). 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 2 study of dacomitinib in 66 NSCLC patients, including 50 subjects with lung adenocarcinoma, has reported 3 partial responses overall in 58 evaluable patients (5%), as well as ten subjects with stable disease of at least 24 weeks. The median progression-free survival was reported to be 12 weeks in 66 subjects and 18 weeks in the 26 patients harboring EGFR mutant-tumors (Reckamp et al., 2014; 24501009). A randomized Phase 2 trial assigned 188 NSCLC patients to receive either dacomitinib (n = 94) or erlotinib (n=94) treatment, and reported that the estimated median progression-free survival was slightly longer in dacomitinib- (2.86 months) compared to erlotinib- (1.91 months) treated patients. The rate of clinical benefit responses, which included complete responses, partial responses, and stable disease of at least 24 weeks, was significantly increased in the dacomitinib arm (29.8%) compared to the erlotinib arm (14.9%). Two patients had grade 4 adverse events and four treatment-related deaths were reported to occur during this study (Ramalingam et al., 2012; 22753918).

Phase 1 Data for *EGFR* (Amplification):
A Phase 1 study of AZD9291 in EGFR mutant NSCLC patients with acquired resistance to Egfr TKIs reported an overall response rate (ORR) of 51% (91/177). In patients with and without the T790M mutation, the ORR was 64% (57/89) and 23% (10/43), respectively, and an overall disease control rate of 96% (85/89) was reported in T790M mutant patients (Janne et al., 2014; ASCO 2014, Abstract 8009). 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). A Phase 1 study of 16 NSCLC patients, including 13 lung adenocarcinoma patients, assessed the combination treatment of gefitinib and nimotuzumab. This study reported the treatment was well tolerated and a recommended dosage was established. Furthermore, partial responses were observed in 25% (4/16) of patients and stable disease was observed in 44% (7/16) of subjects (Kim et al., 2013; 23261229). Phase 1 studies of nimotuzumab in combination with radiotherapy in NSCLC patients who are unsuitable for radical therapy have reported this approach is well tolerated and shows high objective response and disease control rates, although randomized studies are necessary to determine true benefit (Choi et al., 2011; 20451284, Bebb et al., 2011; 20563810). A Phase 1 study of panitumumab in combination with bevacizumab and everolimus in advanced solid tumors showed partial response and stable disease rates of 9.6% (3/31) and 32.3% (10/31), respectively (Vlahovic et al., 2012; 22638798). Another Phase 1 trial of panitumumab as a single agent therapy in various solid tumors reported efficacy of the drug most notably in colorectal cancer, with 13% of patients achieving partial responses and 23% showing stable disease (Weiner et al., 2008; 18223225).

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).

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CLINICALLY ACTIONABLE INFORMATION ASSOCIATED WITH SOMATIC ALTERATIONS (CONTINUED)

Gene (Mutation)

FDA Approved Therapies in Other Indication for *EGFR* (Amplification):
Cetuximab, Lapatinib, Panitumumab, Afatinib, Gefitinib

ALK (EML4-ALK fusion)

Description:
ALK-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 and ceritinib 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).

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 (anaplastic lymphoma kinase) (Soda et al., 2007; 17625570). The EML4-ALK fusion results in activation of Alk by causing the constitutive oligomerization of the protein via the Eml4 coiled coil domain (Mano, 2008; 19032370). The fusion protein has been demonstrated to transform fibroblasts, in a manner dependent upon its kinase function (Soda et al., 2007; 17625570).

ALK mutation has been reported in 4.1% (75/1843) of non-small cell lung carcinoma (NSCLC) samples analyzed in COSMIC, including in 4.6% (53/1142) of lung adenocarcinoma and 2.2% (13/602) of lung squamous cell carcinoma samples (Feb 2015). In the Lung Adenocarcinoma TCGA and Lung Squamous Cell Carcinoma TCGA datasets, ALK mutation has been reported in 8.7% (20/229) and 2.3% (4/178) of sequenced tumors, respectively (cBioPortal for Cancer Genomics, Feb 2015). In the scientific literature, ALK rearrangements, most commonly the EML4-ALK fusion, have been reported in 1.2-6.7% of NSCLC cases (To et al., 2013; 23625156, Selinger et al., 2013; 23743928, Takeuchi et al., 2009; 19383809, Zhou et al., 2013; 23277484).

ALK was originally identified in anaplastic lymphoma as a fusion partner with the gene product of NPM; ALK has subsequently been identified as a fusion protein with numerous other partners, including EML4 in lung cancer (Bagci et al., 2012; 22085494). The ALK gene can become oncogenic by a gene rearrangement, by copy number gain, or by genetic mutations (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).

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). The Alk inhibitor ceritinib has been FDA approved for the treatment of NSCLC patients with ALK rearrangements who experienced disease progression or were found to be intolerant to crizotinib, based on the results of a Phase 1 study in both untreated patients and in crizotinib-resistant cases (Chabner, 2014; 24789171, Shaw et al., 2014; 24670165). Clinical trials of crizotinib and ceritinib in solid tumors are ongoing. In addition, clinical trials of second-generation Alk inhibitors and Hsp90 inhibitors are currently underway for patients with EML4-ALK rearrangements who may have developed resistance to crizotinib. 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).

FDA Approved Drugs in Current Indication for *ALK* (EML4-ALK fusion):
Crizotinib, ceritinib.

Phase 3 Data for *ALK* (EML4-ALK fusion):
A Phase 3 open-label international study in 343 patients with ALK-positive nonsquamous NSCLC reported crizotinib treatment to be superior to chemotherapy as a first-line therapy, with a progression-free survival time of 10.9 months with crizotinib and 7.0 months with chemotherapy, and objective response rates of 74% and 45%, respectively (Solomon et al., 2014; 25470694). A Phase 3 trial compared crizotinib treatment with chemotherapy in 347 patients with advanced or metastatic ALK-positive lung cancer who had received one prior platinum-based regimen. This study reports that crizotinib treatment is superior to chemotherapy as measured by the median progression-free survival and response rate (7.7 months and 65% compared to 3.0 months and 20%, respectively) (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 an ongoing 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^).

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). An ongoing Phase 2 study of alectinib in ALK rearranged NSCLC patients who had not received previous ALK inhibitor therapy reported an objective response in 43/46 patients (93.5%), including two complete responses and 41 partial responses (Seto et al., 2013; 23639470). 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).

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Gene (Mutation)

Phase 1 Data for *ALK* (EML4-ALK fusion):
The ASCEND-1 Phase 1 trial, which evaluated ceritinib in advanced NSCLC, reported an overall response rate of 60% (108/180) in all ALK-positive patients (83 Alk-inhibitor naive and 121 Alk-inhibitor pre-treated), and a median progression free survival of seven months for all 180 ALK-positive patients (Dong-Wan et al., 2014; ASCO 2014, Abstract 8003^). 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). An ongoing Phase 1 study of alectinib in ALK rearranged NSCLC patients reported bioequivalence of different drug formulations with good safety; preliminary efficacy findings indicate partial response in 63.3% (19/30) of patients with target lesions and a response rate of 60% (12/20) in crizotinib-resistant patients with target lesions (Nakagawa et al., 2014; ASCO 2014, Abstract 8103). An ongoing Phase 1/2 trial of the Alk/Egfr inhibitor AP26113 in advanced malignancies recently updated the initial findings from the Phase 1 portion indicating that 63% (24/38) of evaluable ALK-positive NSCLC patients that had prior crizotinib exposure have experienced response, including one complete response; median progression-free survival was 47 weeks in 42 evaluable patients with ALK-positive NSCLC. In a subset of patients with untreated or progressing brain metastases, response in the brain was seen in 6/10 patients, as determined by radiological review (Gettinger et al., 2014; ASCO 2014, Abstract 8047). A Phase 1 study of the Alk TKI X-396 in patients with advanced solid tumors reported, in eight ALK-rearranged NSCLC patients assessable for response, responses in both crizotinib-naïve and crizotinib-treated patients; partial response and stable disease were seen in 83% (5/6) and 17% (1/6), respectively, of the six ALK-positive patients who received doses of X-396 greater than or equal to 200 mg (Horn et al., 2014; ASCO 2014, Abstract 8030).

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 Indication for *ALK* (EML4-ALK fusion):
None.

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CLINICAL TRIALS SPECIFIC TO MUTATION AND TUMOR TYPE

Gene	Phase	NCT Identifier	Clinical trial title
ALK	N/A	NCT02372448	Multicenter Validation of the Sensitivity of Theranostic ALK Rearrangement Detection by FISH Analysis and Prevalence of Escaping Mutations in Circulating Tumor Cells for the Non-invasive Management of Lung Cancer Patients
ALK	Phase 0	NCT02277457	Personalized Adaptive Radiation Therapy With Individualized Systemic Targeted Therapy (PARTIST) for Locally Advanced, Non-small Cell Lung Cancer With Genomic Driver Mutations
ALK	Phase 1	NCT00585195	A Study Of Oral PF-02341066, A c-Met/Hepatocyte Growth Factor Tyrosine Kinase Inhibitor, In Patients With Advanced Cancer
ALK	Phase 1	NCT01548144	Pazopanib or Pemetrexed and Crizotinib in Advanced Cancer
ALK	Phase 1	NCT01634763	Study of Safety and Preliminary Efficacy for LDK378 in Japanese Patients With Genetic Alterations in Anaplastic Lymphoma Kinase (ALK)
ALK	Phase 1	NCT01742286	Phase I Study of LDK378 in Pediatric, Malignancies With a Genetic Alteration in Anaplastic Lymphoma Kinase (ALK)
ALK	Phase 1	NCT01772797	Phase Ib Study of LDK378 and AUY922 in ALK-rearranged Non-small Cell Lung Cancer
ALK	Phase 1	NCT01970865	A Study Of PF-06463922 An ALK/ROS1 Inhibitor In Patients With Advanced Non Small Cell Lung Cancer With Specific Molecular Alterations
ALK	Phase 1	NCT02259114	A Phase IB Trial With OTX015, a Small Molecule Inhibitor of the Bromodomain and Extra-Terminal (BET) Proteins, in Patients With Selected Advanced Solid Tumors
ALK	Phase 1	NCT02299505	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 1	NCT02321501	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
ALK	Phase 1/Phase 2	NCT01625234	Phase 1/2 Study of X-396, an Oral ALK Inhibitor, in Patients With Advanced Solid Tumors and Non-Small Cell Lung Cancer
ALK	Phase 1/Phase 2	NCT01712217	A Study of AT13387 in Patients With Non-Small Cell Lung Cancer (NSCLC) Alone and in Combination With Crizotinib
ALK	Phase 1/Phase 2	NCT02040870	LDK378 in Adult Chinese Patients With ALK-rearranged (ALK-positive) Advanced Non-small Cell Lung Cancer (NSCLC) Previously Treated With Crizotinib
ALK	Phase 1/Phase 2	NCT02292550	Study of Safety and Efficacy of LEE011 and Ceritinib in Patients With ALK-positive Non-small Cell Lung Cancer.
ALK	Phase 2	NCT01752400	AUY922 for Advanced ALK-positive NSCLC
ALK	Phase 2	NCT01801111	A Study of RO5424802 in Patients With Non-Small Cell Lung Cancer Who Have ALK Mutation and Failed Crizotinib Treatment
ALK	Phase 2	NCT01822496	Erlotinib Hydrochloride or Crizotinib and Chemoradiation Therapy in Treating Patients With Stage III Non-small Cell Lung Cancer
ALK	Phase 2	NCT01871805	A Study of CH5424802/RO5424802 in Patients With ALK-Rearranged Non-Small Cell Lung Cancer
ALK	Phase 2	NCT01922583	AUY922 in Patient With Stage IV NSCLC
ALK	Phase 2	NCT02094573	A Phase 2, Multicenter, Randomized Study of AP26113
ALK	Phase 2	NCT02134912	S1300: Pemetrexed Disodium With or Without Crizotinib in Treating Patients With Stage IV Non-Small Cell Lung Cancer That Has Progressed After Crizotinib
ALK	Phase 2	NCT02314481	Deciphering Antitumour Response and Resistance With INtratumour Heterogeneity
ALK	Phase 2	NCT02336451	A Phase II Study to Evaluate the Efficacy and Safety of Oral Ceritinib in Patients With ALK-positive NSCLC Metastatic to the Brain and/or to Leptomeninges
ALK	Phase 3	NCT01639001	A Study Of Crizotinib Versus Chemotherapy In Previously Untreated ALK Positive East Asian Non-Small Cell Lung Cancer Patients
ALK	Phase 3	NCT01828099	LDK378 Versus Chemotherapy in Previously Untreated Patients With ALK Rearranged Non-small Cell Lung Cancer

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CLINICAL TRIALS SPECIFIC TO MUTATION AND TUMOR TYPE (CONTINUED)

Gene	Phase	NCT Identifier	Clinical trial title
ALK	Phase 3	NCT01828112	LDK378 Versus Chemotherapy in ALK Rearranged (ALK Positive) Patients Previously Treated With Chemotherapy (Platinum Doublet) and Crizotinib
ALK	Phase 3	NCT02201992	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)
ALK	Phase 3	NCT02271139	Expanded Access Study of Alectinib for Patients With ALK-Rearranged Non-Small Cell Lung Cancer After Disease Progression on or Intolerance to Prior ALK Tyrosine Kinase Inhibitor Therapy
EGFR	N/A	NCT01393080	Nimotuzumab in Combination With Paclitaxel Liposome and Carboplatin (TP Regimen) for the Advanced NSCLC Patients
EGFR	Phase 1	NCT01320059	Study of 18F-Fluoro-PEG6-IPQA
EGFR	Phase 1	NCT01570296	A Trial of Gefitinib in Combination With BKM120 in Patients With Advanced Non-Small Cell Lung Cancer, With Enrichment for Patients Whose Tumours Harbour Molecular Alterations of PI3K Pathway and Known to Overexpress EGFR
EGFR	Phase 1	NCT01963715	A Phase 1 Study of IMGN289 in Adult Patients With EGFR-positive Solid Tumors
EGFR	Phase 1	NCT02365662	A Study Evaluating Safety and Pharmacokinetics of ABBV-221 in Subjects With Advanced Solid Tumor Types Likely to Exhibit Elevated Levels of Epidermal Growth Factor Receptor
EGFR	Phase 2	NCT00983047	Nimotuzumab Plus Docetaxel in Chemotherapy-Refractory/Resistant Patients With Advanced Non-Small-Cell Lung Cancer
EGFR	Phase 2	NCT02314364	A Trial of Integrating SBRT With Targeted Therapy in Stage IV Oncogene-driven NSCLC
KRAS	Phase 1	NCT01347866	Clinical Study Of PI3K/mTOR Inhibitors In Combination With An Oral MEK Inhibitor Or Irinotecan In Patients With Advanced Cancer
KRAS	Phase 1	NCT01859026	A Phase I/IB Trial of MEK162 in Combination With Erlotinib in NSCLC Harboring KRAS or EGFR Mutation
KRAS	Phase 1	NCT01912625	Trametinib, Combination Chemotherapy, and Radiation Therapy in Treating Patients With Stage II-III Non-Small Cell Lung Cancer That Cannot be Removed by Surgery
KRAS	Phase 1	NCT02015117	Trametinib With or Without Whole Brain Radiation Therapy in Treating Patients With Brain Metastases
KRAS	Phase 1	NCT02185690	A Phase I/Ib Study of MEK162, a MEK Inhibitor, in Combination With Carboplatin and Pemetrexed in Patients With Non-squamous Carcinoma of the Lung
KRAS	Phase 1	NCT02243917	A Phase 1 Study Evaluating CB-5083 in Patients With Advanced Solid Tumors
KRAS	Phase 1	NCT02407509	Phase I Trial of RO5126766
KRAS	Phase 1/Phase 2	NCT02022982	PALBOCICLIB + PD-0325901 for NSCLC & Solid Tumors
KRAS	Phase 1/Phase 2	NCT02039336	Dacomitinib Plus PD-0325901 in Advanced KRAS Mutant Malignancies
KRAS	Phase 1/Phase 2	NCT02079740	Trametinib and Navitoclax in Treating Patients With Advanced or Metastatic Solid Tumors
KRAS	Phase 2	NCT01306045	Molecular Profiling and Targeted Therapy for Advanced Non-Small Cell Lung Cancer, Small Cell Lung Cancer, and Thymic Malignancies
KRAS	Phase 2	NCT01951690	Phase II Study of VS-6063 in Patients With KRAS Mutant Non-Small Cell Lung Cancer
KRAS	Phase 2	NCT02283320	A Study of BIND-014 (Docetaxel Nanoparticles for Injectable Suspension) as Second-line Therapy for Patients With KRAS Positive Non-Small Cell Lung Cancer
KRAS	Phase 3	NCT01933932	Assess Efficacy & Safety of Selumetinib in Combination With Docetaxel in Patients Receiving 2nd Line Treatment for v-Ki-ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS) Positive NSCLC
KRAS	Phase 3	NCT02152631	A Study of LY2835219 in Participants With Previously Treated Lung Cancer
PIK3CA	Phase 1	NCT01928459	Phase 1b Trial of BGJ398/BYL719 in Solid Tumors
PIK3CA	Phase 1	NCT02338622	Trial of Olaparib in Combination With AZD5363 (ComPAKT)
TP53	Phase 1	NCT02042989	MLN9708 and Vorinostat in Patients With Advanced p53 Mutant Malignancies

CLINICAL TRIALS SPECIFIC TO MUTATION AND TUMOR TYPE (CONTINUED)

Gene	Phase	NCT Identifier	Clinical trial title
TP53	Phase 2	NCT02087241	Ph II Trial of Carboplatin and Pemetrexed With or Without AZD1775 for Untreated Lung Cancer

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GENES EVALUATED IN TARGETED CANCER GENE ASSAY

Sequence Analyses

ABL1	ERBB4	GNAQ	MTOR	RET
AKT1	EZH2	GNAS	NF1	ROS1
ALK	FANCA	HNF1A	NF2	SMAD4
APC	FANCC	HRAS	NOTCH1	SMARCB1
ATM	FANCD2	IDH1	NPM1	SMO
BRAF	FANCE	IDH2	NRAS	SRC
BRCA1	FANCF	JAK2	NTRK1	STK11
BRCA2	FANCG	JAK3	PALB2	TERT
BRIP1	FANCL	KDR	PDGFRA	TP53
CDH1	FBXW7	KIT	PDGFRB	TSC1
CDKN2A	FGFR1	KRAS	PIK3CA	TSC2
CSF1R	FGFR2	MET	PMS2	VHL
CTNNB1	FGFR3	MLH1	PTCH1	
DDR2	FLT3	MPL	PTEN	
EGFR	FOXL2	MSH2	PTPN11	
ERBB2	GNA11	MSH6	RB1	

Copy Number Analyses

ALK	ERBB3	FGFR3	MYC	RET
EGFR	FGFR1	KIT	MYCN	
ERBB2	FGFR2	MET	PDGFRA	

Rearrangement Analyses

ABL1	EGFR	ETV6	PDGFRA	ROS1
ALK	ETV1	EWSR1	PDGFRB	TMPRSS2
BCL2	ETV4	MLL	RARA	

Microsatellite Analyses

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

ADDENDUM

Disclaimer and Limitations of Approach

In validation studies, the analytical sensitivity and specificity of the targeted cancer gene assay were > 99% and > 99.9%, respectively. These may be lower for structural alterations and 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.

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.

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).

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. This test, meant to identify somatic mutations, is not intended to detect the presence or absence of germline mutations.

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.

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. 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

SEQUENCE MUTATION DETAILS

Gene Symbol	Gene Description	Transcript	Genomic Position	Mutation
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	CCDS8703.1	chr12:25398284-25398284_C_T	G12D
PIK3CA	phosphoinositide-3-kinase; catalytic; alpha polypeptide	CCDS43171.1	chr3:178952085-178952085_A_G	H1047R
TP53	tumor protein p53	CCDS11118.1	chr17:7573982-7573982_C_	E349Nfs*21

AMPLIFICATION DETAILS

Gene Symbol	Gene Description	Transcript	Genomic Position	Fold
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_001145076	echinoderm microtubule associated protein like 4	chr2:42493496
		ALK	NM_004304	anaplastic lymphoma receptor tyrosine kinase	chr2:29447496

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