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Crizotinib (PF02341066) as a ALK /MET inhibitor— Special Emphasis as a Therapeutic Drug Against Lung Cancer

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Abstract

There are a number of molecular abnormalities that can occur in normal cells to induce a malignant phenotype. Recently, the receptor tyrosine kinase anaplastic lymphoma kinase (ALK) has been shown to have gain-of-function when partnered with different proteins. As an example, on chromosome 2p, with inversion, there is translocation with generation of EML4-ALK tyrosine kinase in lung cancer. In a phase I trial, EML4-ALK patients were selected to determine the response to a potent small molecule tyrosine kinase inhibitor crizotinib (previously identified as PF02341066). Dramatic durable responses were observed with crizotinib at 250 mg twice a day (orally). Interestingly, crizotinib also has activity against MET receptor tyrosine kinase. We have previously shown that MET can be overexpressed, sometimes mutated, or sometimes amplified in lung cancer. Thus, this review will emphasize the characteristics of crizotinib, and detail the clinical experience.

Introduction

Lung Cancer is the second most common cancer in the United States of America, with an estimated 116,750(15%) new cases among males in 2010, and 105,770(14%) among females. It is however the number one killer of all cancers, with a projected 157,300 deaths in the US in 2010, which is equivalent to 431 deaths per day. Recent advances in molecular biology in lung cancer have lead to the development of novel therapies.

Previous experience has proven that clinical efficacy and improved survival can be achieved through the use of inhibitors directed towards oncogenic receptor tyrosine kinases (RTK) that are mutated or otherwise dysregulated in selected advanced tumors. In consequence, most recent efforts have gone into designing and identifying additional RTK inhibitors that are even more potent and specific.[1] Multiple examples exist of successful therapeutic intervention with inhibitors to these tyrosine kinases. The first successful small molecule tyrosine kinase inhibitor (TKI) was with imatinib, which was targeted against the bcr-abl in chronic myeloid leukemia, and later against c-kit mutated gastrointestinal stromal tumors (GIST). Other tyrosine kinase inhibitor available include erlotinib to treat non-small lung cancer (NSCLC) with mutant epidermal growth factor receptor (EGFR), trastuzumab against

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breast cancers with amplified/elevated HER-2, and sunitinib that targets the von Hippel-Lindau (VHL)-dependent vascular endothelial growth factor (VEGF) pathway in renal cell cancer[2].

As more molecular signatures are identified, we are likely to see an increasing number of highly targeted therapeutics in lung and other cancers. Most recently, EML4-ALK and MET have been identified to be potential targets for lung cancer. A recent advance in molecular therapeutics is the development of crizotinib, a potent inhibitor of EML4-ALK that is highly effective in clinical trials. In addition to its ability to inhibit ALK, it was also shown to suppress c-Met tyrosine kinase activity. Below are described some of the properties of crizotinib, and its functionality against a subset of lung cancer.

Molecular targets Of Lung Cancer

Several molecular genetic abnormalities have been described in NSCLC, including chromosomal aberrations, overexpression of oncogenes, deletion and/ or mutations in tumor suppressor genes and telomerase activity. This has led to the development of a variety of pathway antagonists with potential clinical applications. The three main approaches of pathway-selective anticancer drug development have included antagonism of ligand/receptor interaction, inhibition of the tyrosine kinase catalytic activity, and blockade of the receptor/ effector interaction.

Here we shall be discussing the newly developed Met/ALK inhibitor, crizotinib that is presently undergoing Phase I, II, and III clinical trials.

Anaplastic Lymphoma Kinase (ALK)

In a small population of patients with NSCLC, the fusion of the echinoderm microtubule-associated protein-like 4 (EML4) gene with the signaling portion of the anaplastic lymphoma kinase (ALK) gene, resulting in EML4-ALK is believed to be a driver of oncogenesis. An inversion on the short arm of chromosome 2 (Inv (2) (p21p23)) that joins exons 1-13 of EML4 to exons 20-29 of ALK leads to the formation of the EML4-ALK fusion oncogene. The resulting chimeric protein, EML4-ALK, contains an N-terminus derived from EML4 and a C-terminus containing the entire intracellular tyrosine kinase domain of ALK. This EML4-ALK translocation was initially identified in 2007 in a Japanese patient with NSCLC[3] The oncogenic activity of the fusion gene was demonstrated when transgenic mouse lines that expressed EML4-ALK specifically in lung alveolar epithelial cells were all found to develop hundreds of adenocarcinoma nodules in both lungs within a few weeks after birth.[4] EML4-ALK induction of oncogenesis is mediated by the ligand-independent dimerization and/or oligomerization of ALK, resulting in constitutive kinase activity. In vivo treatment of EML4-ALK transgenic mice with oral small molecule inhibitor of the kinase activity of ALK resulted in tumor regression.

About 7% of patients with NSCLC have an EML4-ALK translocation[5]. Although multiple variants exist, all encode fusion between the same cytoplasmic portion of ALK but contain different truncation of EML4. Various isoforms of this fusion gene has been reported, with each variant comprised of segments from either exon 6, 13, 20 or exon 18 of the 5' EML4 fused to the same 3' ALK kinase domains. Fusion of ALK with other partners has also been

described in lung cancer. Examples include KIF5B-ALK[6] and TFG (TRK-fused gene) - ALK[7]

Patients with the EML4-ALK translocation are usually never or former light smokers (often defined as ≤10 pack years and quit ≥ 1 year ago) relatively younger at age of onset, and of adenocarcinoma histology. A study reported the incidence among non smokers to be 8.5%, while ever smoker was found to be 0.8%. The same study found that the fusion gene was not identified in any of the squamous cell lung cancer tissue screened.[8] Other studies have found the fusion gene to be seen only in adenocarcinoma indicating that the EML4-ALK fusion gene may be related to the oncogenes that have been mutated in some non-smokers' lung adenocarcinoma.[9-11]. However new upcoming data suggests that it may be present in any histology.

Patients with the EML4-ALK fusion gene share most of the clinical features of NSCLC patients harboring the EGFR mutations, but apart from rare exceptions EML4-ALK and EGFR mutations are mutually exclusive.[3, 9]. EML4-ALK mutations are also mutually exclusives with mutations in v-Ki-ras2/Kirsten rat sarcoma viral oncogene homolog (KRAS) and Human Epidermal growth factor Receptor 2 (ERBB2) genes.[9] EML4- ALK-rearranged NSCLC has unique histopathologic characteristics. The majority of tumors (56%) have a solid pattern of growth and a significant (≥ 10%) component of signet ring cells[12]. This pattern is a well-recognized variant of adenocarcinoma of the stomach, colon and breast, but rarely observed in lung adenocarcinoma.

ALK fusion oncogene has also been implicated in anaplastic large cell lymphoma (ALCL) and other lymphomas.[13] In Anaplastic large cell lymphoma, the translocation is at (2;5) (p23;q35), and this creates a fusion gene composed of nucleophosmin (NPM) and ALK. This NPM-ALK chimeric gene encodes a constitutively activated tyrosine kinase that has been shown to be a potent oncogene. Clincopathologic studies have shown that ALK expression in ALCL is associated with improved 5-year survival rates as compared with ALCL lacking ALK expression. ALK gene rearrangements have also been seen in patients with inflammatory myofibroblastic tumor (IMT). The rearrangement involves the ALK locus on chromosome 2p23[14]. The incidence ALK positivity in IMT has been reported to be as high as 35%[15].

Diagnosis of ALK rearranged Gene

ALK-rearrangements in a subset of ALCLs have been recognized for over 15 years, and a variety of diagnostic techniques currently employed in clinical practice have already been validated as sensitive and specific for detecting the genetic lesions characteristic of this tumor type. Immunohistochemical studies using antibody against ALK1, and FISH (fluorescence in situ hybridization) for ALK gene rearrangement t(2;5), are the standard of care tests and are both equally effective[16].

However there is currently no standard method for detecting EML4-ALK in NSCLC. Several methods including polymerase chain reaction (PCR), immunohistochemistry (IHC) and FISH are currently being evaluated.

In contrast to the sensitivity of IHC in detecting other ALK fusion genes, such as NPMALK, IHC mediated identification of EML4-ALK has been difficult, probably due to the low expression level of the protein[17]. The low expression level of the EML4-ALK protein is as a result of weak transcriptional activity of the promoter-enhancer region of the EML4 that drives expression of EML4-ALK compared with that of the NPM promoter. Other methods have been developed to overcome this limitation, one of which is the development of an intercalated antibody-enhancing polymer (iAEP) method by Takeuchi et al[6].

Commercially, EML4-ALK is detected through FISH and PCR-based assays.

c-MET

c-Met is a proto-oncogene implicated in the etiology of lung cancer. It is structurally distinct from other RTKs. The protein product of c-Met is a tyrosine kinase receptor for hepatocyte growth factor (HGF)[18], and is it's only known high affinity receptor. c-Met is overexpressed in a variety of tumors including lung cancer, and is usually present in higher pathologic tumor stage and is associated with a worse outcome. Recent studies suggest that the signal pathway between HGF and its receptor c-Met plays an important role in oncogenesis. HGF-receptor gene is located on chromosome 7q21-q31 and is also known as scatter factor. It is 120 kb in length with 21 exons and 20 introns. The protein comprises of a 50 kD extracellular alpha chain and a 140 kD transmembrane beta chain which are linked by disulfide bonds. It contains the following domains: a large seven-blade propeller (Sema domain), PSI (as in Plexins, Semaphorins, Integrins), four IPT repeats (as in Immunoglobulins, Plexins, Transcription factors), TM (transmembrane), JM (juxtamembrae) and TK (tyrosine kinase)[19][20]. Several distinct mechanisms including amplification, translocation or mutation of c-Met may underlie the uncontrolled c-Met activation frequently seen in lung cancer.

Unlike EGFR mutations that are usually somatic in nature, majority of c-MET mutations are germline in nature. c-Met mutations have been analyzed extensively, and is also said to differ based on ethnicity. A recent trial performed by Krishnaswamy et al, amplified the individual exons of semaphorin, juxtamembrane, and tyrosine kinase domains of c-Met using tissue genomic DNA from 141 Asians, 76 Caucasians, and 66 African American lung cancer patients by PCR, with the mutations being analyzed by polymerase chain reaction (PCR). Nine nucleotide substitutions leading to c-Met mutations were detected with six of them involving nonsynonymous amino acid changes. Four of the nonsynonymous substitutions were also detected in the adjacent normal tissue consistent with a germline origin. All the nonsynonymous mutations were clustered in the semaphoring domain except R988C of the juxtamembrane domain. N375S was the most frequently seen nonsynonymous amino acid substitution and occurred at a higher frequency in East Asians compared with Caucasians, and was not seen in African Americans[21].

c-Met is selectively expressed in several normal epithelial tissues. High levels of c-Met mRNA have been found in liver, gastrointestinal tract, thyroid and kidney. The tissue distribution of the Met/HGF receptor indicates that this molecule is involved in growth control of epithelial cells other than hepatocytes and suggests that its increased expression may confer a growth advantage to neoplastic cells[22]. Activation of HGF/MET signaling

has multifunctional effects on mammalian cells, including stimulation of cellular proliferation, promotion of cell movement, invasion into extracellular matrix (ECM), and epithelial morphogenesis. It also plays an important role in angiogenesis, tumorgenesis and tissue regeneration. Studies have suggested that patients with NSCLC and c-Met overexpression have poorer outcomes after complete resection.[23]

Mutations of the MET gene has been implicated in various malignancies including NSCLC[24], small cell lung cancer[25], hereditary papillary renal cell cancer[26], gastric cancer[27], childhood hepatocellular carcinoma[28] and metastases of head and neck cancer[29].

c-Met over expression has also been implicated in TKI resistance in EGFR positive lung cancer cells. A recent study utilizing gefitinib-sensitive lung cancer cell lines found that these cell lines developed resistance to gefitinib as a result of focal amplification of the c-Met proto-oncogene. Inhibition of c-Met signaling in these cells restored their sensitivity to gefitinib. The mechanism of action of this resistance was thought to be through the amplification of c-Met driven ERBB3 (HER3)-dependent activation of PI3K, a pathway thought to be specific to EGFR/ERBB family receptors[30].

Other lesser described chromosomal aberrations exists in lung cancer. Of note is the newly described ROS translocation in non-small lung cancer. A global survey of phosphotyrosine signaling by Rikova et al, detected a fusion of ROS to the transmembrane solute carrier protein SLC34A2. The N-terminal region of SLC34A2, ending just after the first transmembrane region is fused N-terminal to the transmembrane region of ROS producing a truncated fusion protein with two transmembrane domains. The SLC34A2-ROS fusion protein expresses both Fused in Glioblastoma (FIG) and ROS gene[7, 31]. Other forms of this fusion protein were also observed. Cell lines expressing FIG-ROS were found to be inhibited by an ALK inhibitor (TAE684). This is most likely due to the fact that ROS kinase shares high sequence homology with ALK[31], and indicates another potential area of targeted therapy.

Crizotinib (PF-02341066)

Crizotinib (Pfizer, PF-02341066 [(R)-3-[1-(2, 6-dichloro-3-fluoro-phenyl)-ethoxy]-5-(1-piperidin-4-yl-1*H*-pyrazol-4-yl)-pyridin-2-ylamine) is a potent, orally bioavailable, ATP competitive small molecule inhibitor of the catalytic activity of c-Met and ALK kinases. Previous selective small molecule inhibitors of c-Met (e.g. PHA-665752) that showed cytoreductive anti tumor activity in vivo[32] were not viable clinical agents due to poor pharmaceutical properties and oral bioavailability

Preclinical studies with Crizotinib

Crizotinib potently inhibits c-Met phosphorylation and signal transduction, as well as c-Met dependent oncogenic phenotypes of tumor cells and endothelial cells in vitro and showed antitumor efficacy in tumor models at well tolerated doses in vivo. The underlying mechanism of the antitumor activity of crizotinib may be due to its direct suppression of tumor cell growth or survival, as well as its potent antiangiogenic effect[33].

Crizotinib was used to inhibit c-Met in a variety of functional assays and tumor models. It was found to be a potent ATP-competitive inhibitor of c-Met kinase, and inhibited c-Met phosphorylation across a panel of cell lines. It inhibited a variety of diverse mutant variants of c-Met in cellular assays, including those located at the ATP binding pocket (V1092I and H1094R), P-loop (M1250T), and juxtamembrane domain (R988C and T1010I). In contrast, crizotinib was less potent against the Y1230C and Y1235D mutant variants of c-Met located near the kinase domain activation loop. This indicates that crizotinib activity is dependent on the location of the mutation in the active site, which has unique implications in molecular modeling of c-Met inhibitory activity, indicating that PF-2341066 is active in certain mutations identified in papillary renal carcinoma, head and neck cancer and lung cancer, at that particular mutations (e.g. activation loop) should be taken into account during patient selection[34].

During these preclinical trails, crizotinib was selective for c-Met and ALK compared with a panel of > 120 diverse tyrosine and serine-threonine kinases, and was found to be nearly 20 fold selective for ALK and c-Met compared with other kinases evaluated. In another study, crizotinib was found to be effective on ALK positive ALCL cell lines. It potently inhibited NPM-ALK phosphorylation in Karpas299 or SU-DHL-1 ALCL cells (mean IC_{50} value, 24nmol/L). It also effectively inhibited cell proliferation, that was associated with G_1-S-phase cell cycle arrest and induction of apoptosis in ALK-positive ALCL cells (IC_{50} values, ~ 30 nmol/L) but not ALK negative lymphoma cells. The induction of apoptosis was confirmed using terminal deoxyribonucleotide transferase-mediated nick-end labeling and Annexin V staining (IC_{50} values, 25-50 nmol/L). The study showed that oral administration of PF-2341066 to severe combined immunodeficient Beige mice bearing Karpas299 ALCL tumor xenografts resulted in dose-dependant antitumor efficacy with complete regression of all tumors at a 100mg/kg/day dose within 15 days of initial drug administration. A strong correlation was observed between antitumor response and inhibition of NPM-ALK phosphorylation, and induction of apoptosis in tumor tissue. In addition, inhibition of NPM-ALK phosphorylation and function, lead to inhibition in key NPM-ALK signaling mediators including phospholipase C-γ, signal transducers and activators of transcription 3, extracellular signal regulated kinases, and Akt (a serine/threonine protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, cell proliferation, apoptosis, transcription and cell migration).[34]

Early Clinical trials

Crizotinib has been found to be active in NSCLC with the EML4-ALK gene rearrangements, NPM-ALK positive anaplastic lymphoma and ALK-positive non-Hodgkin's lymphoma or inflammatory myofibroblastic tumor (IMT). It has been studied in large clinical trials on patients with EML4-ALK positive NSCLC.

Recently concluded early phase clinical trials demonstrated the efficacy of crizotinib. Approximately 1500 patients with NSCLC cancer were screened for the ALK fusion gene, and 82 patients were identified that were eligible for the trial. Most of these patients had been treated, and almost all patients had adenocarcinoma histology, and were never or former smokers. The patients were enrolled in an expanded cohort study instituted after

phase 1 dose escalation had established a recommended crizotinib dose of 250 mg orally twice daily in 28-day cycles. The result of the trial was very impressive, with almost all the patients that harbored EML4-ALK translocation having some tumor shrinkage. The mean duration of treatment was 6.4 months, with the overall response rate being 57% (47 of 82 patients with 46 confirmed partial responses and 1 confirmed complete response); 27 patients (33%) had stable disease with the disease control rate (DCR) at 8 weeks being 87%. Response duration varied from 1 to 15 months. A total of 63 of 82 patients (77%) are continuing to receive crizotinib (after the time of data cutoff), and the estimated probability of 6 months progression free survival was 72% with no median for the study reached[35] (Table I)

A recent article described the dramatic radiographic and clinical response to crizotinib observed by a 32-year-old Chinese female patient with metastatic lung cancer harboring the EML4-ALK mutation. The patient was a life long non-smoker, who had initially presented with persistent cough, and radiographic studies had revealed a right hilar mass. Biopsy had revealed lung adenocarcinoma with initial molecular studies showing EGFR and KRAS wild type. Staging work up had revealed metastasis to the brain and liver. She underwent stereotatic radiosurgery of her brain metastasis and then underwent treatment with six cycles of cisplatin/pemetrexed/bevacizumab combination therapy and achieved a partial response, and was then continued on bevacizumab maintenance. She then went on a treatment holiday, but progressed after three months and so was started on erlotinib. She progressed on erlotonib, and so enrolled in a clinical trial utilizing docetaxel in combination with a novel vascular disrupting agent. She progressed after her first cycle and was then enrolled in the phase 1 trial of crizotinib, after FISH analysis had demonstrated that her tumor had the ALK gene rearrangement. Prior to initiation of crizotinib, she had been experiencing persistent cough, daily low grade fevers, anorexia, and right neck pain secondary to tumor invasion of the right subscapalaris muscles. After three days of being on crizotonib, her low-grade fevers and right subscapalaris pain had resolved and there was a significant decrease in her cough. A computed tomography (CT)/18F-Fluorodeoxyglucose positron emission tomography (18-FDG PET) fusion performed on the 14 day of therapy revealed a 70.5% decrease in maximum standard uptake value activity, with a corresponding 36.4% decrease in total tumor measurements by RECIST (response evaluation criteria in solid tumors). She continued to demonstrate remarkable response, with the 8 week CT scan showing a further 47.5% decrease in her tumor measurement from her baseline CT scan[36]

Crizotinib has also been found to be active in patients with ALK positive ALCL. A recent article described the effect of Crizotinib in two patients with ALK positive ALCL who had progressive disease after multiple therapies and still responded to Crizotinib. The first patient was a 26-year-old female who had received seven cycles of cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP-15), with a partial response at 1 month. She then was treated with standard salvage combination chemotherapy regimens in an attempt to proceed to an autologous bone marrow transplantation. Relapse however occurred within 2 to 3 weeks after each regimen. She was experiencing fever, cervical and inguinal lymphadenopathy with positive results on PET and CT scan. Bone marrow aspiration showed 3% of cells with ALK rearrangement. With initiation of crizotinib at a dose of 250mg orally twice a day, her fever disappeared within 48 hours, palpable adenopathy

resolved by day 7, PET and CT images and bone marrow aspiration performed at day 28 showed complete regression of previous lesions. Complete response has been maintained 6 months after, and she continues on Crizotinib. The second patient is a 20-year-old man who had initially achieved a complete response after 6 cycles of CHOP, but relapsed after a month. Treatment with high dose chemotherapy and autologous bone marrow transplantation only yielded a partial response that lasted for a month. He was experiencing fevers, axillary and inguinal lymphadenopathy. His PET and CT scan was diffusely positive of nodal region and spleen, and his bone marrow aspiration was positive for the ALK rearrangement in 8% of cells. With initiation of Crizotinib, his fever and lymphadenopathy resolved within 8 days. A day 12 PET scan showed regression of all lesions that was sustained on a day 28 PET-CT scan. Finally his bone marrow aspirate was negative for the ALK rearrangement by day 60.[37]

Less common tumors like inflammatory myofibrobalstic tumor (IMT) have also been found to respond to Crizotinib. A recent study by Butrynski et al[38], described a sustained partial response to crizotinib in a patient with ALK-translocated IMT, as compared with no observed activity in another patient without the ALK translocation.

Adverse Reactions

In the phase I trial reported, crizotinib was general well tolerated. With recent trials revealing only Grade I toxicities with rare grade II toxicities, making crizotinib very attractive to patients who will require long-term administration. Grade I clinical toxicities noted during the phase I trial included, nausea (52%), diarrhea (46%), vomiting (43%), change in light/dark accommodation (41%), constipation (22%), peripheral edema (16%), dizziness (15%), decreased appetite (13%) and fatigue (10%)

Only five cases of grade 2 toxicities were reported; two cases of constipation (2%) and single case of nausea (1%), diarrhea (1%) and vomiting (1%).

Liver enzymes elevations were also noted, but were generally grade 1 or 2. Grade 3 elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were observed in 4 patients (5%) and 5 patients (6%) respectively. Only one patient had (1%) had grade 4 elevation in ALT. These elevations on liver enzymes were however seen to resolve with discontinuation of crizotinib, and four out of five patients were able to resume therapy at a reduced dose without recurrence of dose limiting toxic effects[35]

Crizotinib Resistance

It is a well-known fact that resistance to tyrosine kinase inhibitors usually results from acquired mutations within the target kinases. Studies have revealed that mutations in the kinase domain of BCR-ABL leads to imatinib resistance, by either altering amino acids that directly contact imatinib or by preventing BCR-ABL from achieving the inactive conformational state required for imatinib binding[39]. In a similar way, resistance to EGFR inhibitors gefitinib and erlotinib occur by additional mutations in the EGFR gene acquired during the course of therapy, that changes the protein-coding sequence[40].

A recent trial revealed some patients with EML4-ALK-positive NSCLC might become resistant to crizotinib after successful treatment. Two de novo mutations in EMLA-ALK have been implicated in conferring resistance to the drug. In the case-report, a patient who had initially responded to crizotinib and then developed resistance was found to have developed two de novo mutations within the kinase domain of EML4-ALK[41]. Amino acid substitutions at the gatekeeper position of the tyrsosine kinase receptor are thought to be the mechanism for the acquisition of tyrosine kinase-inhibitor resistance.

Ongoing Trials

Presently there are multiple ongoing trials evaluating the efficacy of crizotinib. A few with their objectives and study design are listed in Table II.

Future Trials

Crizotinib is still undergoing clinical trials. Future trials are listed in Table III.

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Table I

Number of prior regimens	Objective Response Rate
0	80 (4/5)
1	52 (14/27)
2	67 (10/15)
3	56 (19/34)

Table II

Ongoing clinical trials for Crizotinib (Targeting the EML4-ALK fusion gene)

Official Title/ClinicalTrials.gov Identifier	Study Design	Primary Endpoint
A phase III trial of Crizotinib versus standard of care in patients with advanced NSCLC with a specific alteration of the ALK gene/NCT00932893	Phase 3, open-label randomized two-arm study. Patients are given oral crizotinib 250mg twice daily on a continuous schedule versus either pemetrexed 500mg/m^2 intravenously every 3 weeks or docetaxel 75mg/m^2 by intravenous infusion every 3 weeks.	To determine whether Crizotinib prolongs progression free survival versus standard of care chemotherapy in NSCLC patients with an alteration in the ALK gene
A phase I/II Study of MET Tyrosine Kinase Inhibitor PF-02341066 in Children With Relapsed or Refractory Solid Tumors or ALCL/NCT00939770	Phase I dose-escalation study followed by a phase II study. Patients receive oral c-MET tyrosine kinase inhibitor PF-02341066 twice daily on days 1-28. Treatment repeats every 28 days for up to 24 courses in the absence of disease progression or unacceptable toxicity.	Estimate the maximum tolerated dose and recommended phase II dose of MET tyrosine kinase inhibitor PF-02341066, and define and describe the toxicities and characterize the pharmacokinetics.
Phase 1/2, Open-Label, Randomized Study Of The Safety, Efficacy, And Pharmacokinetics Of Erlotinib With Or Without PF-02341066 In Patients With Advanced NSCLC/NCT00965731	Escalating doses of PF-02341066 will be administered orally on a continuous schedule. The planned doses to be evaluated are 200 mg and 250 mg BID. The dose determined in Phase 1 will be used in Phase 2	Determine the maximum tolerated dose and recommended Phase 2 dose for PF-02341066 in combination with erlotinib (phase 1), and progression free survival (PFS) of single agent erlotinib vs progression free survival (PFS) of erlotinib plus PF-02341066 (phase 2).
Phase 2, Open-Label Single Arm Study Of The Efficacy And Safety Of PF-02341066 In Patients With NSCLC Harboring A Translocation Or Inversion Event Involving The ALK Gene/NCT00932451	Non randomized trial that will allow patients from a Phase 3 trial who received standard of care chemotherapy to receive PF-02341066.	Objective response rate, type, incidence, severity, seriousness and relationship to study medication of adverse events and laboratory test abnormalities.
A Phase 1, Open Label, Dose Escalation Study To Evaluate Safety, Pharmacokinetics And Pharmacodynamics Of Combined Oral C-MET/ALK Inhibitor (PF-02341066) And PAN-HER Inhibitor (PF-00299804) In Patients With Advanced NSCLC/ NCT01121575	In Arm 1, patients will be treated with combined c-MET inhibitor (PF-02341066) and panHER inhibitor (PF-00299804). The starting doses will be 200 mg by mouth, twice a day of PF 02341066 in tablet form and 30 mg by mouth once a day of PF 0029804 in tablet form. The dose of each drug in the combination will be escalated or de- escalated until the maximum tolerated combined dose is reached. Patients will then be treated with the maximum tolerated combined dose. In Arm 2, Patients will be treated with single agent panHER inhibitor (PF-00299804) at a dose of 45 mg by mouth once a day, until disease progression and then with the maximum tolerated combined dose of crizotinib (PF-02341066) given twice a day.	Overall safety profile of combined PF 02341066 plus PF 00299804 including adverse events, as defined and graded by the National Cancer Institute CTCAE, and first cycle Dose Limiting Toxicity

Abbreviations: NSCLC, non small cell lung cancer; ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; RECIST, Response Evaluation Criterion in Solid Tumors; CTCAE, Common Terminology Criteria for Adverse Events

* Defined as > 4-fold increase in the ALK signal number as compared to reference signal number on chromosome 2q arm

** The presence of a mutation, duplication, amplification, and/or translocation.

Table III

Future trials involving crizotinib

Official Title/ ClinicalTrials.gov Identifier	Expected Start Date
Phase 1B Open-Label Study Of The Safety And Clinical Activity Of Crizotinib (PF-02341066) In Tumors Except Non Small Cell Lung Cancer With Genetic Events Involving The Anaplastic Lymphoma Kinase Gene Locus/ NCT01121588	January 2011
Phase 3 Randomized Open-Label Study Of The Efficacy And Safety Of Crizotinib Versus Pemetrexed/Cisplatin Or Pemetrexed/Carboplatin In Previously Untreated Patients With Non-squamous Carcinoma Of The Lung Harboring A Translocation Or Inversion Event Involving The Anaplastic Lymphoma Kinase (ALK) Gene Locus/ NCT01154140	December 2010