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MONOGRAPH

# CRIZOTINIB

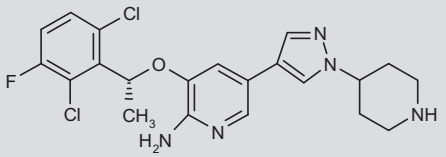
Prop INN; USAN

*ALK/Met inhibitor*
*Oncolytic*

PF-02341066
PF-2341066

3-[1(*R*)-(2,6-Dichloro-3-fluorophenyl)ethoxy]-5-[1-(4-piperidinyl)-1*H*-pyrazol-4-yl]pyridin-2-amine

InChI: 1S/C21H22Cl2FN5O/c1-12(19-16(22)2-3-17(24)20(19)23)30-18-8-13(9-27-21(18)25)14-10-28-29(11-14)15-4-6-26-7-5-15/h2-3,8-12,15,26H,4-7H2,1H3,(H2,25,27)/t12-/m1/s1




$C_{21}H_{22}Cl_2FN_5O$

Mol wt: 450.337

CAS: 877399-52-5

CAS: 877399-53-6 (monoacetate)

EN: 421155


## SUMMARY

*There are a number of molecular abnormalities that can occur in normal cells to induce a malignant phenotype. Recently, the tyrosine kinase receptor 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 fusion protein EML4-ALK in lung cancer. In a phase I trial, EML4-ALK-positive patients were selected to determine the response to a potent, small-molecule tyrosine kinase inhibitor, crizotinib (PF-02341066). Marked durable responses were observed with crizotinib 250 mg p.o. twice a day. Interestingly, crizotinib also has activity against tyrosine-protein kinase Met. 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.*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## SYNTHESIS*

Crizotinib can be prepared by several different ways:

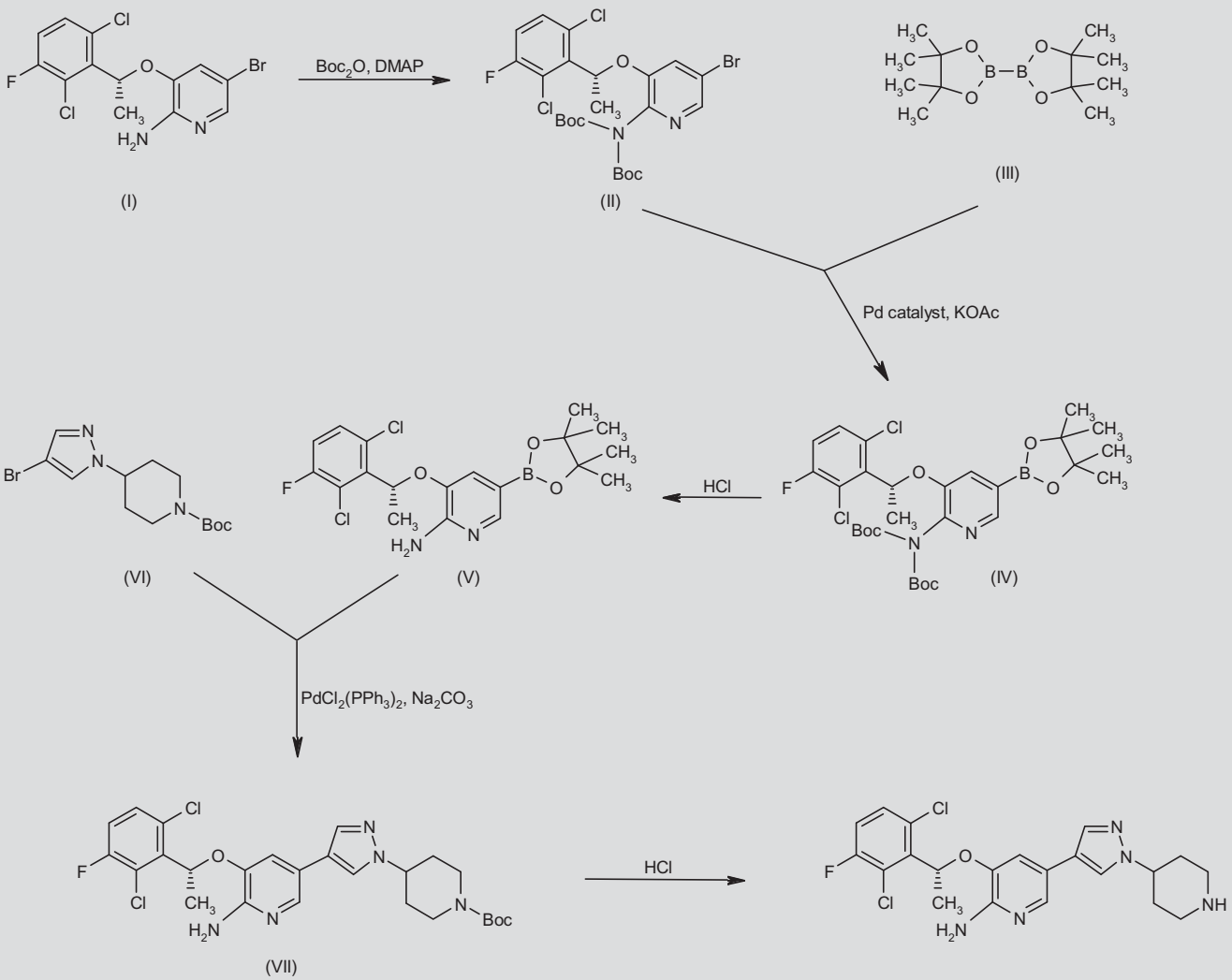
Reaction of 5-bromo-3-[1(*R*)-(2,6-dichloro-3-fluorophenyl)ethyl]-pyridin-2-amine (I) with $Boc_2O$ by means of DMAP in DMF gives the protected aniline (II), which is condensed with bis(pinacolato)-diboron (III) by means of a Pd catalyst and KOAc in DMSO to yield the *N*-protected boronic ester (IV). Deprotection of the amino group of compound (IV) by means of HCl in dioxane/dichloromethane affords the boronic ester (V), which is condensed with 1-(1-Boc-piperidin-4-yl)-4-bromo-1*H*-pyrazole (VI) by means of $PdCl_2(PPh_3)_2$ and $Na_2CO_3$ in hot DME/water to provide the adduct (VII). Finally, this compound is *N*-deprotected by means of HCl in dioxane/dichloromethane, as before (1, 2). Scheme 1.

Mitsunobu coupling between 1(*S*)-(2,6-dichloro-3-fluorophenyl) ethanol (VIII) and 3-hydroxy-2-nitropyridine (IX) in the presence of DIAD and $PPh_3$ affords ether (X), which by subsequent nitro group reduction using iron and HCl in EtOH yields the aminopyridine (XI). Bromination of compound (XI) by treatment with *N*-bromosuccinimide provides the bromopyridine (I), which is then submitted to palladium-catalyzed coupling with the pyrazolyl boronate (XII) to give adduct (VII). Finally, the *N*-Boc protecting group is removed by treatment with HCl (3). Scheme 2.

## BACKGROUND

Lung cancer is the second most common cancer in the U.S., 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 U.S. in 2010, which is equivalent to 431 deaths per day. Recent advances in molecular biology in lung cancer have led to the development of novel therapies.

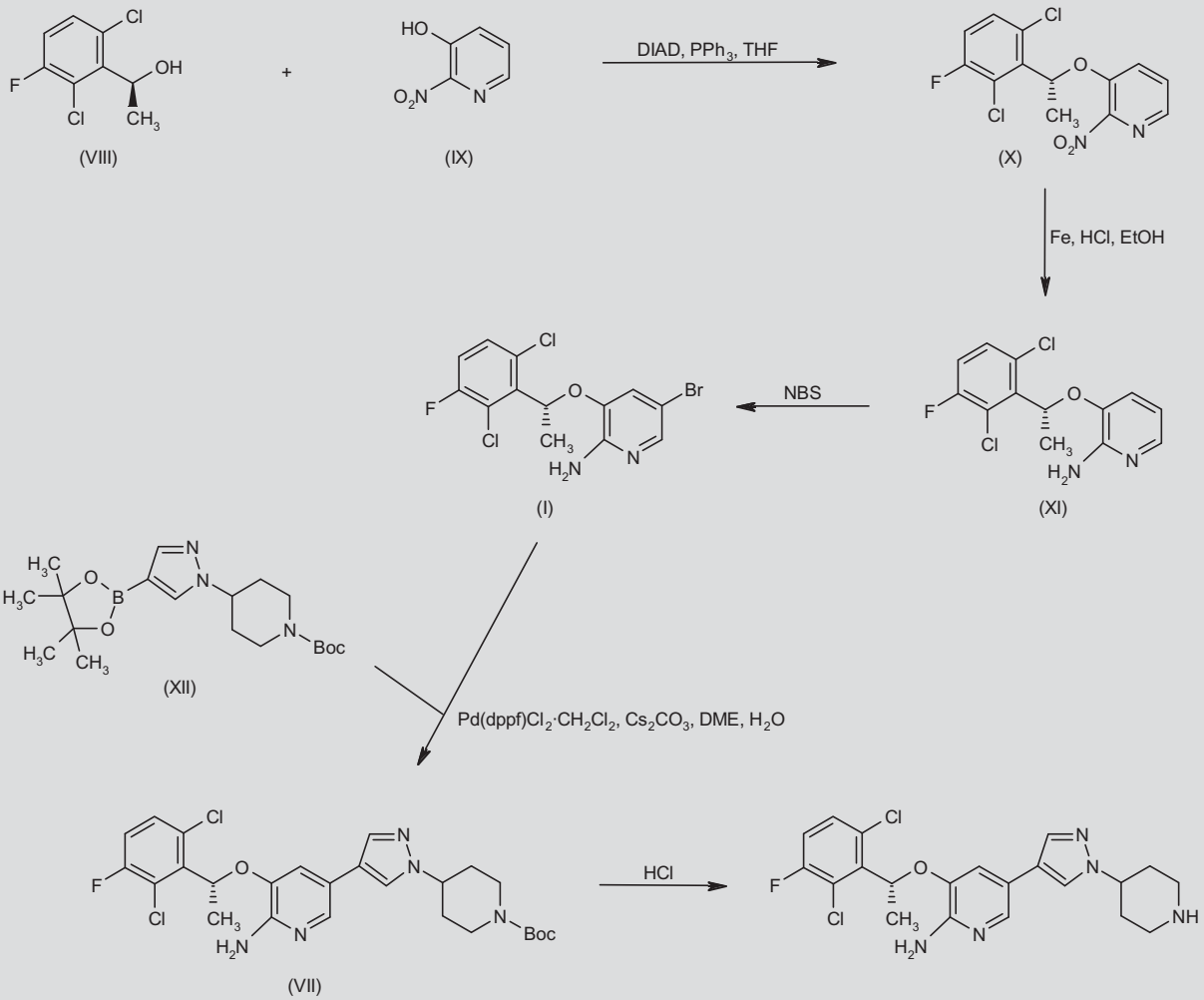
**Scheme 1.** Synthesis of Crizotinib




Previous experience has proven that clinical efficacy and improved survival can be achieved through the use of inhibitors directed towards oncogenic receptor tyrosine kinases (RTKs) 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 (4). Multiple examples exist of successful therapeutic interventions with inhibitors of these tyrosine kinases. The first successful small-molecule tyrosine kinase inhibitor (TKI) was imatinib, which was targeted against BCR/ABL in chronic myeloid leukemia (CML), and later against c-Kit-mutated gastrointestinal stromal tumors (GISTs). Other tyrosine kinase inhibitors include erlotinib for the treatment of non-small lung cancer (NSCLC) with mutant epidermal growth factor receptor (EGFR), trastuzumab for breast cancer with amplified/elevated HER2, and sunitinib, targeting the von Hippel-Lindau (VHL)-dependent vascular endothelial growth factor (VEGF) pathway in renal cell cancer (5).

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 as

**Scheme 2.** Synthesis of Crizotinib




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.

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 tyrosine kinase catalytic activity and blockade of the receptor/effector interaction. Here we shall discuss the newly developed Met/ALK inhibitor crizotinib, which is presently undergoing phase I, II and III clinical trials.

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 results in fusion protein EML4/ALK as 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 contains an *N*-terminus derived from EMAP-4 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 (6). The oncogenic activity of the fusion gene was demonstrated when transgenic murine cell lines that expressed *EML4-ALK* specifically in lung alveolar epithelial cells were found to develop hundreds of adenocarcinoma nodules in both lungs within a few weeks after birth (7). 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 an 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 (8). Although multiple variants exist, all encode a fusion between the same cytoplasmic portion of *ALK* but contain different truncations of *EML4*. Various isoforms of this fusion gene have 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* (9) and *TFG-ALK* (10).

Patients with the *EML4-ALK* translocation are usually nonsmokers 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 nonsmokers to be 8.5%, while in smokers it 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 (11). Other studies have found the fusion gene only in adenocarcinoma, indicating that the *EML4-ALK* fusion gene may be related to the oncogenes that have been mutated in some nonsmokers' lung adenocarcinoma (12-14). However, new upcoming data suggest 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 *EGFR* mutations, but apart from rare exceptions, *EML4-ALK* and *EGFR* mutations are mutually exclusive (6, 12). *EML4-ALK* mutations are also mutually exclusive with mutations in the v-Ki-ras2/Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and *ERBB2* genes (12). *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 (15). This pattern is a well-recognized variant of adenocarcinoma of the stomach, colon and breast, but rarely observed in lung adenocarcinoma.

The *EML4-ALK* fusion oncogene has also been implicated in anaplastic large cell lymphoma (ALCL) and other lymphomas (16). 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. Clinicopathologic 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 tumors (IMTs). The rearrangement involves the *ALK* locus on chromosome 2p23 (17). The incidence of *ALK* positivity in IMT has been reported to be as high as 35% (18).

*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 (IHC) studies using an antibody against ALK-1 and FISH (fluorescence in situ hybridization) for *ALK* gene rearrangement t(2;5) are the standard-of-care tests and are both equally effective (19).

However, there is currently no standard method for detecting *EML4-ALK* in NSCLC. Several methods, including polymerase chain reaction (PCR), IHC and FISH are currently being evaluated. In contrast to the sensitivity of IHC in detecting other *ALK* fusion genes, such as *NPM-ALK*, IHC-mediated identification of *EML4-ALK* has been difficult, probably due to the low expression level of the protein (20). The low expression level of the EML4/ALK protein is a result of weak transcriptional activity of the promoter–enhancer region of the *EML4* gene 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 an intercalated antibody-enhancing polymer (iAEP) method designed by Takeuchi et al. (9). Commercially, *EML4-ALK* is detected through FISH and PCR-based assays.

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) (21), and is its 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 stages and 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. The *MET* gene is located on chromosome 7q21-q31 and is also known as scatter factor receptor. It is 120 kb in length, with 21 exons and 20 introns. The protein is comprised 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 (juxtamembrane) and TK (tyrosine kinase) (22, 23).

Several distinct mechanisms, including amplification, translocation or mutation of *MET,* may underlie the uncontrolled c-Met activation frequently seen in lung cancer. Unlike *EGFR* mutations, which are usually somatic in nature, the majority of *MET* mutations are germ line in nature. *MET* mutations have been analyzed extensively and are 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 Asian, 76 Caucasian and 66 African American lung cancer patients by PCR, with the mutations being analyzed by PCR. Nine nucleotide substitutions leading to *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 germ line origin. All the nonsynonymous mutations were clustered in the semaphoring domain, except R988C in 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 (24).

c-Met is selectively expressed in several normal epithelial tissues. High levels of c-Met mRNA have been found in the liver, gastrointestinal tract, thyroid and kidney. The tissue distribution of the c-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 (25). Activation of c-Met/HGF 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, tumorigenesis and tissue regeneration. Studies have suggested that patients with NSCLC and c-Met overexpression have poorer outcomes after complete resection (26).

Mutations of the *MET* gene have been implicated in various malignancies, including NSCLC (27), small cell lung cancer (28), hereditary papillary renal cell cancer (29), gastric cancer (30), childhood hepatocellular carcinoma (31) and metastatic head and neck cancer (32).

c-Met overexpression 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 phosphatidyl-inositol 3-kinase (PI3K), a pathway thought to be specific for the EGFR/erbB family receptors (33).

Other lesser described chromosomal aberrations exist in lung cancer. Of note is the newly described proto-oncogene tyrosine-protein kinase ROS translocation in NSCLC. A global survey of phosphotyrosine signaling by Rikova et al. detected a fusion of ROS to the transmembrane solute carrier protein sodium-dependent phosphate transport protein 2B (NaPi-2B; *SLC34A2*). The *N*-terminal region of NaPi-2B 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 NaPi-2B-ROS fusion protein expresses both the fused in glioblastoma (*FIG*) and *ROS* genes (10, 34). Other forms of this fusion protein were also observed. Cell lines expressing *FIG/ROS* were found to be inhibited by an ALK inhibitor (TAE-684). This is most likely due to the fact that ROS kinase shares high sequence homology with ALK (34), and indicates another potential area of targeted therapy.

Crizotinib (PF-02341066) 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 antitumor activity in vivo (35) were not viable clinical agents due to poor pharmaceutical properties and oral bioavailability.

## PRECLINICAL PHARMACOLOGY

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 (36).

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 the activity of crizotinib 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 crizotinib is active against certain mutations identified in papillary renal carcinoma, head and neck cancer, and lung cancer at that particular mutation (e.g., activation loop), and should be taken into account during patient selection (37).

During these preclinical trials, crizotinib was selective for c-Met and ALK compared with a panel of > 120 diverse tyrosine and serine/threonine protein 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 against ALK-positive ALCL cell lines. It potently inhibited NPM-ALK phosphorylation in human lymphoma Karpas 299 or lymphoma SU-DHL-1 ALCL cells (mean $IC_{50}$ = 24 nmol/L). It also effectively inhibited cell proliferation, which was associated with $G_1$/S-phase cell cycle arrest and induction of apoptosis in ALK-positive ALCL cells ($IC_{50}$ ~ 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 ($IC_{50}$ = 25-50 nmol/L). The study showed that oral administration of crizotinib to severe combined immunodeficient Beige mice bearing Karpas 299 ALCL tumor xenografts resulted in dose-dependent antitumor efficacy, with complete regression of all tumors at 100 mg/kg/day 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 led to inhibition of key NPM-ALK signaling mediators, including phospholipase C-γ, signal transducer and activator of transcription STAT3, extracellular signal-regulated kinases (ERKs) 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) (37).

## SAFETY

In a phase I trial crizotinib was generally well tolerated, with recent trials revealing only grade I toxicities and 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 cases of nausea (1%), diarrhea (1%) and vomiting (1%). Liver enzyme 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 four (5%) and five patients (6%), respectively. Only one patient (1%) had grade 4 elevation in ALT. These elevations in liver enzymes were, however, seen to resolve with discontinuation of crizotinib, and four of five patients were able to resume therapy at a reduced dose without recurrence of dose-limiting toxicity (38).

## CLINICAL STUDIES

Crizotinib has been found to be active in NSCLC with *EML4-ALK* gene rearrangements, *NPM-ALK*-positive anaplastic lymphoma and *ALK*-positive non-Hodgkin's lymphoma (NHL) or IMTs. It has been studied in large clinical trials in patients with *EML4-ALK*-positive NSCLC. Presently there are multiple ongoing trials evaluating the efficacy of crizotinib, several of which are listed in Table I. Future trials are listed in Table II.

Recently concluded early-phase clinical trials demonstrated the efficacy of crizotinib. Approximately 1,500 patients with NSCLC were screened for the *EML4-ALK* fusion gene, and 82 patients were identified who were eligible for the trial. Most of these patients had been treated, and almost all patients had adenocarcinoma histology and were nonsmokers or former smokers. The patients were enrolled in an expanded-cohort study instituted after phase I dose escalation had established a recommended crizotinib dose of 250 mg orally twice daily on 28-day cycles. The results of the trial were very impressive, with almost all the patients that harbored an *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 at 8 weeks being 87%. Response dura-

***Table I.*** *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 | Open-label, randomized, two-arm phase III study. Patients are given crizotinib 250 mg orally twice daily on a continuous schedule versus either pemetrexed 500 mg/m$^2$ i.v. every 3 weeks or docetaxel 75 mg/m$^2$ by i.v. 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 crizotinib twice daily on days 1-28. Treatment is repeated 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 crizotinib, define and describe toxicities and characterize pharmacokinetics. |
| Phase II 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 crizotinib will be administered orally on a continuous schedule. The planned doses to be evaluated are 200 and 250 mg b.i.d. The dose determined in phase I will be used in phase II. | Determine the maximum tolerated dose and recommended phase II dose for crizotinib in combination with erlotinib (phase I), and progression-free survival of single-agent erlotinib vs. progression-free survival of erlotinib plus crizotinib (phase II). |
| 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 | Nonrandomized trial that will allow patients from a phase III trial who received standard-of-care chemotherapy to receive crizotinib. | 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 (crizotinib) and pan-HER inhibitor (PF-00299804). The starting doses will be 200 mg orally twice a day for crizotinib in tablet form and 30 mg orally once a day for 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 pan-HER inhibitor (PF-00299804) at a dose of 45 mg orally once a day until disease progression, and then with the maximum tolerated combined dose of crizotinib given twice a day. | Overall safety profile of combined crizotinib plus PF-00299804, including adverse events, as defined and graded by the National Cancer Institute CTCAE, and first-cycle dose-limiting toxicity. |

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

***Table II.*** *Future trials involving crizotinib.*

| Official title/ClinicalTrials.gov Identifier | Study 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 eventsinvolving the anaplastic lymphoma kinase gene locus/NCT01121588 | March 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 lungharboring a translocation or inversion event involving the anaplastic lymphoma kinase (ALK) gene locus/NCT01154140 | January 2011 |

tion varied from 1 to 15 months. A total of 63 of 82 patients (77%) continue to receive crizotinib (after the time of data cutoff), and the estimated probability of 6-month progression-free survival was 72%, with no median for the study reached (38).

A recent article described a marked radiographic and clinical response to crizotinib in a 32-year-old Chinese female patient with metastatic lung cancer harboring the *EML4-ALK* mutation. The patient was a lifelong nonsmoker 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 wild-type *EGFR* and *KRAS*. Staging workup had revealed metastasis to the brain and liver. She underwent stereotactic radiosurgery of her brain metastasis and then underwent treatment with six cycles of cisplatin/pemetrexed/bevacizumab combination therapy, achieving a partial response, and was then continued on bevacizumab maintenance. The patient then went on a treatment holiday, but progressed after 3 months and was started on erlotinib. She progressed on erlotonib and was enrolled in a clinical trial utilizing docetaxel in combination with a novel vascular-disrupting agent. She progressed after the first cycle and was then enrolled in the phase I 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 subscapularis muscles. After 3 days on crizotonib, her low-grade fevers and right subscapularis pain had resolved and there was a significant decrease in cough. A computed tomography (CT)/$^{18}$F-fluorodeoxyglucose positron emission tomography (FDG-PET) performed on day 14 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). The patient continued to demonstrate a remarkable response, with the 8-week CT scan showing a further 47.5% decrease in tumor size from baseline CT scan (39).

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 7 cycles of cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP-15), with a partial response at 1 month. She was then treated with standard salvage combination chemotherapy regimens in an attempt to proceed to an autologous bone marrow transplant. However, relapse occurred within 2-3 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 250 mg 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 transplant only yielded a partial response that lasted for a month. He was experiencing fever and axillary and inguinal lymphadenopathy. His PET and CT scans were diffusely positive for the 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 (40).

Less common tumors such as inflammatory myofibroblastic tumor (IMT) have also been found to respond to crizotinib. A recent study by Butrynski et al. (41) described a sustained partial response to crizotinib in a patient with *ALK*-translocated IMT, as compared to no observed activity in another patient without the *ALK* translocation.

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 lead 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 (40). In a similar way, resistance to the EGFR inhibitors gefitinib and erlotinib occurs by additional mutations in the *EGFR* gene acquired during the course of therapy, which change the protein-coding sequence (41).

A recent trial revealed that some patients with *EML4-ALK*-positive NSCLC might become resistant to crizotinib after successful treatment. Two de novo mutations in *EML4-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* (42). Amino acid substitutions at the gatekeeper position of the TKR are thought to be the mechanism for the acquisition of tyrosine kinase inhibitor resistance.

## SOURCES

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## DISCLOSURES

The authors state no conflicts of interest.

## REFERENCES

1. Cui, J.J., Funk, L.A., Jia, L. et al. (Pfizer, Inc.; Agouron Pharmaceuticals, Inc.). *Enantiomerically pure aminoheteroaryl compounds as protein kinase inhibitors*. EP 1786785, JP 2008510790, US 2006046991, WO 2006021884.
2. Cui, J.J., Funk, L.A., Jia, L. et al. (Pfizer, Inc.). *Pyrazole-substituted aminoheteroaryl compounds as protein kinase inhibitors*. EP 1784396, JP 2008510788, WO 2006021881.
3. Tran-Dube, M., Shen, H., Yamazaki, S. et al. *Lead optimization of 2-amino-3-benzyloxy-5-pyrazol-4-yl-pyridine for the discovery of clinical candidate PF-2341066 as potent and highly selective c-Met inhibitor.* 235th ACS Natl Meet (April 6-10, New Orleans) 2008, Abst MEDI 105.
4. Blume-Jensen, P., Hunter, T. *Oncogenic kinase signalling.* Nature 2001, 411(6835): 355-65.
5. Baselga, J. *Targeting tyrosine kinases in cancer: The second wave.* Science 2006, 312(5777): 1175-8.
6. Soda, M., Choi, Y.L., Enomoto, M. et al. *Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer.* Nature 2007, 448(7153): 561-6.
7. Soda, M., Takada, S., Takeuchi, K. et al. *A mouse model for EML4-ALK-positive lung cancer.* Proc Natl Acad Sci U S A 2008, 105(50): 19893-7.
8. Choi, Y.L., Takeuchi, K., Soda, M. et al. *Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer.* Cancer Res 2008, 68(13): 4971-6.
9. Takeuchi, K., Choi, Y.L., Togashi, Y. et al. *KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer.* Clin Cancer Res 2009, 15(9): 3143-9.
10. Rikova, K., Guo, A., Zeng, Q. et al. *Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer.* Cell 2007, 131(6): 1190-203.
11. Wong, D.W., Leung, E.L., So, K.K. et al. *The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS.* Cancer 2009, 115(8): 1723-33.
12. Takahashi, T., Sonobe, M., Kobayashi, M. et al. *Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene.* Ann Surg Oncol 2010, 17(3): 889-97.
13. Inamura, K., Takeuchi, K., Togashi, Y. et al. *EML4-ALK fusion is linked to histological characteristics in a subset of lung cancers.* J Thorac Oncol 2008, 3(1): 13-7.
14. Sakairi, Y., Nakajima, T., Yasufuku, K. et al. *EML4-ALK fusion gene assessment using metastatic lymph node samples obtained by endobronchial ultrasound-guided transbronchial needle aspiration.* Clin Cancer Res 2010, 16(20): 4938-45.
15. Rodig, S.J., Mino-Kenudson, M., Dacic, S. et al. *Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population.* Clin Cancer Res 2009, 15(16): 5216-23.
16. Drexler, H.G., Gignac, S.M., von Wasielewski, R., Werner, M., Dirks, W.G. *Pathobiology of NPM-ALK and variant fusion genes in anaplastic large cell lymphoma and other lymphomas.* Leukemia 2000, 14(9): 1533-59.
17. Griffin, C.A., Hawkins, A.L., Dvorak, C., Henkle, C., Ellingham, T., Perlman, E.J. *Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors.* Cancer Res 1999, 59(12): 2776-80.
18. Coffin, C.M., Patel, A., Perkins, S., Elenitoba-Johnson, K.S., Perlman, E., Griffin, C.A. *ALK1 and p80 expression and chromosomal rearrangements involving 2p23 in inflammatory myofibroblastic tumor.* Mod Pathol 2001, 14(6): 569-76.
19. Cataldo, K.A., Jalal, S.M., Law, M.E. et al. *Detection of t(2;5) in anaplastic large cell lymphoma: Comparison of immunohistochemical studies, FISH, and RT-PCR in paraffin-embedded tissue.* Am J Surg Pathol 1999, 23(11): 1386-92.
20. Martelli, M.P., Sozzi, G., Hernandez, L. et al. *EML4-ALK rearrangement in non-small cell lung cancer and non-tumor lung tissues.* Am J Pathol 2009, 174(2): 661-70.
21. Bottaro, D.P., Rubin, J.S., Faletto, D.L. et al. *Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product.* Science 1991, 251(4995): 802-4.
22. Cipriani, N.A., Abidoye, O.O., Vokes, E., Salgia, R. *MET as a target for treatment of chest tumors.* Lung Cancer 2009, 63(2): 169-79.
23. Stamos, J., Lazarus, R.A., Yao, X., Kirchhofer, D., Wiesmann, C. *Crystal structure of the HGF beta-chain in complex with the Sema domain of the Met receptor.* EMBO J 2004, 23(12): 2325-35.
24. Krishnaswamy, S., Kanteti, R., Duke-Cohan, J.S. et al. *Ethnic differences and functional analysis of MET mutations in lung cancer.* Clin Cancer Res 2009, 15(18): 5714-23.
25. Di Renzo, M.F., Narsimhan, R.P., Olivero, M. et al. *Expression of the Met/HGF receptor in normal and neoplastic human tissues.* Oncogene 1991, 6(11): 1997-2003.
26. Okuda, K., Sasaki, H., Yakiue, H., Yano, M., Fujii, Y. *Met gene copy number predicts the prognosis for completely resected non-small cell lung cancer.* Cancer Sci 2008, 99(11): 2280-5.
27. Ma, P.C., Jagadeeswaran, R., Jagadeesh, S. et al. *Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer.* Cancer Res 2005, 65(4): 1479-88.
28. Maulik, G., Kijima, T., Ma, P.C. et al. *Modulation of the c-Met/hepatocyte growth factor pathway in small cell lung cancer.* Clin Cancer Res 2002, 8(2): 620-7.
29. Schmidt, L., Duh, F.M., Chen, F., et al. *Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas.* Nat Genet 1997, 16(1): 68-73.
30. Lee, J.H., Han, S.U., Cho, H. et al. *A novel germ line juxtamembrane Met mutation in human gastric cancer.* Oncogene 2000, 19(43): 4947-53.
31. Park, W.S., Dong, S.M., Kim, S.Y. et al. *Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas.* Cancer Res 1999, 59(2): 307-10.
32. Di Renzo, M.F., Olivero, M., Martone, T. et al. *Somatic mutations of the MET oncogene are selected during metastatic spread of human HNSC carcinomas.* Oncogene 2000, 19(12): 1547-55.
33. Engelman, J.A., Zejnullahu, K., Mitsudomi, T. et al. *MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling.* Science 2007, 316(5827): 1039-43.

34. Gu, T.L., Deng, X., Huang, F. et al. *Survery of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma.* PLoS One 2011, 6(1): e15640.

35. Christensen, J.G., Schreck, R., Burrows, J. et al. *A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes in vitro and exhibits cytoreductive antitumor activity in vivo.* Cancer Res 2003, 63(21): 7345-55.

36. Zou, H.Y., Li, Q., Lee, J.H. et al. *An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms.* Cancer Res 2007, 67(9): 4408-17.

37. Christensen, J.G., Zou, H.Y., Arango, M.E. et al. *Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma.* Mol Cancer Ther 2007, 6(12, Pt. 1): 3314-22.

38. Kwak, E.L., Bang, Y.J., Camidge, D.R. et al. *Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer.* N Engl J Med 2010, 363(18): 1693-703.

39. Ou, S.H., Bazhenova, L., Camidge, D.R. et al. *Rapid and dramatic radiographic and clinical response to an ALK inhibitor (crizotinib, PF02341066) in an ALK translocation-positive patient with non-small cell lung cancer.* J Thorac Oncol 2010, 5(12): 2044-6.

40. Gambacorti-Passerini, C., Messa, C., Pogliani, E.M. *Crizotinib in anaplastic large-cell lymphoma.* N Engl J Med 2011, 364(8): 775-6.

41. Butrynski, J.E., D'Adamo, D.R., Hornick, J.L. et al. *Crizotinib in ALK-rearranged inflammatory myofibroblastic tumor.* N Engl J Med 2010, 363(18): 1727-33.

42. Shah, N.P., Nicoll, J.M., Nagar, B., Gorre, M.E., Paquette, R.L., Kuriyan, J., Sawyers, C.L. *Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia.* Cancer Cell 2002, 2(2): 117-25.

43. Hammerman, P.S., Jänne, P.A., Johnson, B.E. *Resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer.* Clin Cancer Res 2009, 15(24): 7502-9.

44. Choi, Y.L., Soda, M., Yamashita, Y. et al. *EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors.* N Engl J Med 2010, 363(18): 1734-9.