Crizotinib for ALK-Rearranged Non–Small Cell Lung Cancer: A New Targeted Therapy for a New Target

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Abstract

Crizotinib (PF02341066, Xalkori; Pfizer) was recently approved by the U.S. Food and Drug Administration for treatment of ALK-positive non–small cell lung cancer (NSCLC) as defined by a jointly approved diagnostic test using a break-apart fluorescence in situ hybridization assay. The approval was based on dramatic response rates in ALK-positive NSCLC patients of 54% to 61% in phase I and II trials. To date, the overall disease control rates in these trials are close to 90%. Progression-free survival approaches 10 months. This review focuses on the ALK-inhibitory activity of crizotinib in preclinical and clinical trials that led to approval, as well as the diagnostic methods to classify patients with ALK-positive NSCLC. Although these patients represent a small subset of all patients with NSCLC, the rapid time course from identification of this unique target to an approved targeted therapy with striking benefit serves as a paradigm for the development of targeted therapeutics in an era of personalized medicine. Clin Cancer Res; 18(14); 3737–42. ©2012 AACR.

Introduction

Non–small cell lung cancer (NSCLC) is the most common cause of cancer-related deaths in both men and women in the United States. Most patients present with advanced disease, and standard chemotherapeutic agents have marginal effects on survival. However, select populations of patients with NSCLC carry somatic genomic activating mutations that, when present, seem to singularly drive the growth of the cancer. The most frequent examples of these are mutations in the epidermal growth factor receptor (EGFR), which are present in ~15% of all patients with NSCLC in the United States. EGFR-targeted tyrosine kinase inhibitors (TKI) show markedly greater response rates and progression-free survival than first-line chemotherapy in EGFR-mutant patients (1–3).

Anaplastic Lymphoma Kinase Rearrangement in NSCLC

Anaplastic lymphoma kinase (ALK) rearrangements were first identified as a fusion to a portion of the nucleophosmin (NPM) gene in 60% of anaplastic large cell lymphoma, a small subset of non-Hodgkin lymphomas.

ALK rearrangements in NSCLC were identified in late 2007, primarily as fusions to echinoderm microtubule-like protein 4 [EML4 (4, 5)]. These fusion proteins are found in ~3% to 7% of patients with NSCLC overall but have been identified primarily in lung adenocarcinomas and more frequently in younger patients and in never or light smokers (4, 6, 7). Like EGFR, KRAS, ERBB2, and BRAF, they are mostly found exclusive of other known activating mutations (8, 9).

Although the most frequent fusion partner is EML4, several different breakpoints of EML4 have been described (4, 10, 11). In addition, other, rare fusion partners have also been described, such as TFG and KIF5B (12). Most fusions link to ALK in frame with exon 20, and the resulting protein carries a coiled-coil basic domain from the upstream fusion partner, which may facilitate activation of the ALK tyrosine kinase by promoting dimerization (4).

Most of the identified EML4-ALK fusion proteins have been shown to be oncogenic both in vitro and in vivo (4, 10). EML4-ALK variant 1 expressed in mouse fibroblasts generates tumors in nude mice and results in multiple adenocarcinomas when expressed in the lungs of transgenic mice (4, 13, 14). Although the detailed mechanism of oncogenic activation by ALK is not fully characterized, overexpression in cell lines results in activation of both STAT3 and extracellular signal-regulated kinase (ERK) (15). Studies of crizotinib and TAE684, another small-molecule ALK inhibitor, have shown downregulation of both AKT and ERK phosphorylation (16, 17). One study showed that TAE684 results in apoptosis mediated by BIM upregulation that is ERK dependent, and survivin downregulation that is STAT3 dependent (15).

Crizotinib

Crizotinib is a potent and specific small-molecule inhibitor of both ALK and c-MET tyrosine kinases. It is a 3-benzyloxy-2-aminopyridine series inhibitor that was derived from a first-generation c-MET inhibitor,
PHA-66752, using the cocrystal structure of this inhibitor with c-MET to further optimize active-site binding (18). In a screen for kinase selectivity, crizotinib inhibited autophosphorylation of both c-MET and ALK with high potency and specificity across a panel of >120 kinases (18). Crizotinib binds in a bidentate manner at the hinge region of c-MET and competes with ATP binding in both kinases [a crystal structure of crizotinib binding in the inactive conformation of ALK has been reported as well (PDB ID: 2XP2)].

**Pharmacokinetics and pharmacodynamics**

Crizotinib blocks c-MET phosphorylation and c-MET–dependent cell growth and invasion in a variety of cell lines at an IC50 of 5 to 20 nmol/L (18, 19). The IC50 of crizotinib in blocking cell proliferation and apoptosis in ALK-rearranged lymphoma cell lines is 25 to 50 nmol/L (18, 20). The IC50 of crizotinib in NSCLC lines carrying an EML4-ALK fusion is significantly smaller, ranging from 250 to 340 nmol/L (16, 21). This is notably less potent than other ALK inhibitors in development (17).

In a pharmacokinetic/pharmacodynamic modeling study from Pfizer, crizotinib was projected to reach adequate plasma levels to inhibit ALK phosphorylation and achieve an antitumor effect. Specifically, in xenograft models using either H3122 cells (NSCLC line with EML4-ALK fusion) or Karpas299 cells (ALCL line with NPM-ALK fusion), ALK inhibition was seen at an EC50 of 233 and 660 nmol/L, respectively, and tumor growth inhibition was observed at 255 and 875 nmol/L, suggesting that ≥50% ALK inhibition is required for antitumor efficacy (22). On the basis of clinical pharmacokinetic data, >70% ALK inhibition was projected at the now U.S. Food and Drug Administration (FDA)–approved dose of 250 mg twice daily.

In a phase I clinical study of crizotinib, patients treated at the recommended phase II dose were noted to reach a Cmax of crizotinib at a median Tmax of 4 hours, with a terminal half-life of 42 hours after a single dose (23). Steady-state levels were reached within 15 days, with nonlinear pharmacokinetics (decreasing mean value of clearance with multiple dosing). This is believed to be due to autoinhibition of CYP3A after reaching steady state. Because of these observations, the phase I study has been amended to evaluate once-daily dosing, and to date, safety has been observed at doses up to 650 mg daily (K. Wilner, Pfizer, unpublished data). Of note, the Cmax and area under the curve (AUC) for crizotinib in a population of Asian patients were ~2.5-fold greater than those in non-Asian patients, but without increased toxicities.

**Safety**

Crizotinib was well tolerated overall, with rare grade 3/4 toxicities that included pneumonitis (1.6% across phase I and II studies) that usually occurred within 2 months of initiating the drug (24). Two deaths attributed to treatment-related pneumonitis were reported in the phase II study (25). Grade 3/4 alanine aminotransferase elevation (4% in the phase I study and 7% in stage II) was also noted, resulting in 1 treatment discontinuation. Overall, elevated transaminases were attributable to the drug in 19% of the patients.

More common treatment-related toxicities included visual disturbances, which occurred in 62% of the patients, but all were grade 1 or 2 effects. These were primarily described as light trails (following a moving lighted object) or light flashes or brief image persistence (i.e., following camera flashbulb use). Flipped dark–light registration in high-contrast images was also reported. No ophthalmologic abnormalities were noted in the limited examinations that were performed.

Other frequent treatment-related toxicities that were primarily grade 1 or 2 included nausea (53%), diarrhea (43%), vomiting (40%), constipation (27%), edema (28%), fatigue (20%), anorexia (19%), dizziness (16%), neuropathy (13%), and dysgeusia (12%). Rash was noted in 10% of the patients, and reflux or other esophageal symptoms were observed in 11%. After a food-effect evaluation was done, yielding findings of only a 14% reduction in Cmax and AUC after a high-fat meal, patients were allowed to take the drug in a nonfasting state, which resulted in a substantial reduction of nausea and vomiting.

**Clinical efficacy**

Preclinical data suggested the potential efficacy of crizotinib against both MET and ALK; however, it was the dramatic results seen in ALK-positive patients with NSCLC that served as the impetus for accelerated FDA approval. Following responses seen in 2 ALK-positive patients during dose escalation, the phase I study of crizotinib in solid tumors was amended to include an expanded cohort of ALK-positive patients with lung cancer, into which 119 patients have been enrolled. The overall response rate among these patients is 61% (26), with an overall clinical benefit rate (complete response + partial response + stable disease) of 88%. In patients who were chemotherapy naïve (n = 14), the response rate was 68%. Preliminary median survival was 10 months. These striking data served as the basis for rapid initiation of both a phase II trial of crizotinib for patients who have relapsed following first-line chemotherapy and a phase III randomized trial of crizotinib versus second-line therapy. As reported to date, in the phase II trial, the response rate in the population of 76 patients evaluable for response was 54%, with a 91% disease control rate [partial response + stable disease (25)].

These results led to the accelerated FDA approval of crizotinib for ALK-positive patients with NSCLC in record time—the timeframe from discovery of the target in late 2007 to FDA approval of a targeted therapy in August 2011 was <4 years (Fig. 1). The approval was granted while both the phase I and phase II trials were ongoing. In addition, a phase III trial comparing crizotinib with standard second-line therapy is still accruing, and a more recent phase III trial comparing crizotinib with first-line platinum doublet chemotherapy has been launched as well. Of importance, the FDA approval was not linked to any line of therapy, and given the commercial availability of crizotinib, accrual to
these large phase III trials, especially the first-line trial, may be slowed.

The approval of the drug also makes a prospective evaluation of the overall survival benefit of crizotinib challenging. Although EGFR-directed TKIs produce markedly better results than first-line chemotherapy in terms of progression-free survival for EGFR-mutated patients, the overall survival is not statistically different because most patients are likely to receive an EGFR TKI during some point of their therapy (1–3), and the same is likely to be true of crizotinib. A retrospective study examining the outcomes of 36 ALK-positive patients who were not treated with crizotinib compared with 56 patients who were treated in the phase I expanded cohort of crizotinib showed that patients treated in the second or third line showed improved overall survival compared with those treated with other therapies [HR 0.36; 95% confidence interval (CI), 0.17–0.75; \( P = 0.004 \) (27)].

**Future clinical directions**

Although much of the attention on crizotinib has focused on its activity against ALK-positive NSCLC, equally dramatic responses have been seen against ALK-positive lymphoma (28) as well as MET-amplified gastroesophageal cancers and NSCLC (29, 30). An independent study of crizotinib in ALK-positive lymphoma is now ongoing (ClinicalTrials.gov, identifier NCT00939770). In addition, in vitro studies identified a ROS1-rearranged cell line as being sensitive to TAE684, another ALK inhibitor (16), and this alteration has been identified in clinical specimens as well (5). An effort to look for ROS1 translocations (using a break-apart FISH probe similar to that employed for ALK) showed ROS1 rearrangements in 18 of 1,073 of NSCLC patients screened (31). One of these patients was treated in the expanded cohort of the phase I study of crizotinib and achieved a dramatic and sustained response similar to those observed in ALK-positive patients. These observations suggest a broader role for crizotinib as an effective therapy against multiple targets in multiple tumor types.

**ALK Diagnostics**

Although the phase I trial of crizotinib allowed several different local laboratory determinations of ALK rearrangement for approval of eligibility, the phase II trial mandated central testing for ALK rearrangement using the now FDA-approved Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular). The test, which uses a red and a green probe flanking the ALK translocation breakpoint, is considered positive if \( \geq 15\% \) of at minimum 50 nuclei evaluated have a split or isolated red signal that results from the intervening fusion of a translocated protein fragment such as EML4. Although this approach was clinically validated in the sense that it was the test used in crizotinib trials, in comparison with other methods, it is associated with several practical challenges. FISH requires specialized equipment and expertise, and it can cost more than $1,000 per test. In addition, the interpretation is sometimes not clear-cut depending on how close the signals are to each other. Multiple alternative methods of evaluation have been and are being studied, but each has potential drawbacks (Table 1).
Chromogenic in situ hybridization (CISH) offers a lower-cost alternative to FISH and allows simultaneous histologic evaluation. A study that used CISH for detection of ALK rearrangement showed a high concordance with FISH [95% (32)]. However, CISH does not get around the problem of a sometimes subtle or difficult-to-interpret hybridization pattern.

Reverse transcriptase PCR (RT-PCR) is an alternative diagnostic method that uses multiplexed primer pairs to amplify known possible ALK fusions. This test has been shown to be sensitive and specific for detecting and identifying particular ALK fusions but will by definition miss as-yet-uncharacterized fusion partners (10). In addition, the success of the test depends on the quality of the sample and whether the RNA is degraded. The test has been optimized on formalin-fixed paraffin-embedded (FFPE) specimens in addition to fresh tissue, but the quality of the former is more variable.

Immunohistochemistry (IHC) for ALK protein is another alternative test, because there is essentially no ALK expression in normal lung epithelia. However, even within ALK-positive lung tumors, the expression of ALK is relatively low (compared with ALK expression in ALK-rearranged lymphoma), and antibodies that are used routinely for detection of ALK-positive lymphoma, such as the DAKO clone ALK1, showed specificity but relatively low sensitivity for detecting ALK in NSCLC (33). However, increased concentrations of antibody and the use of novel methods to amplify the secondary signal did result in improved sensitivity and higher concordance with FISH (34).

Other antibodies also have improved sensitivity and higher concordance with FISH, including DF3 (developed by Cell Signaling Technology), which showed a sensitivity of 100% and specificity of 99%, and much improved staining compared with the ALK1 antibody (35). Another promising antibody for routine use is the 3A4 antibody from Novoceastra, which showed a sensitivity of 100% and specificity of 96% (36). However, moderate staining (+2) is more variable in determining ALK rearrangement as detected by other methods (36, 37).

Studies comparing all 3 methods (excluding CISH) have not shown a clear superiority for all aspects of any one method (reviewed in ref. 38). Rather, they suggest that ALK testing may one day mirror HER2 testing in breast cancer, with an initial screen by IHC (using an as-yet-to-be-agreed-upon antibody and IHC procedure) with grading of intensity on a scale of +1 to +3, followed by FISH confirmation of moderate staining. RT-PCR may be used to identify specific fusion partners when indicated.

**Crizotinib Resistance**

Additional methods for ALK evaluation will also be required to streamline the detection of resistance mutations. Several resistance mutations have already been identified in patients treated with crizotinib (12, 17, 39). The first report of 2 acquired point mutations, C1156Y and L1196M, was published concurrently with the first report of activity of crizotinib (12). Although some of the identified resistance mutations, such as L1196M and G1269A, are found within the active site and likely interfere with crizotinib binding (L1196M is analogous to resistance mutations found within the active site and likely interfere with crizotinib binding (12). Although some of the identified resistance mutations, such as L1152 and C1156Y) do not have a clear mechanism for causing resistance to crizotinib (40). Studies of acquired resistance in vitro (41, 42) have identified these clinically identified mutations as well as other resistance mutations, all of which have variable resistance to crizotinib in vitro. In the 7 mutations that have been clinically identified to date, the degree of resistance to crizotinib is variable. Some are still sensitive to structurally different ALK inhibitors with greater potency than crizotinib, as well as to Hsp90 inhibitors, which are clinically active against ALK-positive NSCLCs (17).

Unlike EGFR resistance to erlotinib, which occurs by a common mechanism in >50% of cases (i.e., the T790M
gatekeeper mutation). ALK resistance seems to be more variable. Not only have more varied mutations been identified but also in many cases no identified ALK secondary mutation has been identified. EGF activation and ALK copy number gain have been identified as mechanisms of acquired resistance in both patients and in vitro models (17, 31, 39, 40). Clinically, Kit amplification (with concomitant increased expression of the Kit ligand, SCF) and the emergence of independent KRAS-mutant clones have also been identified as mechanisms of resistance to crizotinib (17, 39). The largest series published to date suggests that <25% of resistance occurs via acquisition of a secondary point mutation and >50% of resistance may occur via alternate tyrosine kinase activation. With the use of more potent ALK inhibitors, alternate pathway activation may become an even more important issue with which investigators will have to grapple.

Conclusions

ALK-targeted therapy, as well as the identification of ALK-positive NSCLC tumors, is a rapidly evolving field that has been put on the map by crizotinib.

Although future refinements in diagnostic tests are likely and more potent inhibitors are currently in clinical development, crizotinib and the break-apart FISH test have clearly distinguished a group of NSCLC patients with dramatically improved outcomes compared with the majority of patients. This example of a molecularly defined target and an effective therapeutic strategy mirrors the experience with EGR-mutant NSCLC and EGF-directed TKIs, and, it is hoped, will serve as a model for more examples to come.

Disclosure of Potential Conflicts of Interest

L. Gandhi is a consultant/advisory board member of Chugai Corporation. P.A. Janne is a consultant/advisory board member of Pfizer, Chugai Pharmaceuticals, and Abbott Molecular.

Authors' Contributions

Conception and design: L. Gandhi, P.A. Janne

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