Crizotinib as a personalized alternative for targeted anaplastic lymphoma kinase rearrangement in previously treated patients with non-small-cell lung cancer

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Abstract: Crizotinib, the first clinically designed and synthesized as a tyrosine kinase inhibitor targeting mesenchymal–epithelial transition factor, indicating marked anticancer activity in patients with advanced, anaplastic lymphoma kinase-positive non-small-cell lung cancer, was approved by the US Food and Drug Administration in 2011. In this review, we focus on the efficacy of crizotinib compared with chemotherapy in advanced anaplastic lymphoma kinase-positive lung cancer and present the role of crizotinib as a personalized alternative in previously treated patients with non-small-cell lung cancer.

Keywords: crizotinib, anaplastic lymphoma kinase rearrangement, non-small-cell lung cancer

Introduction

Lung cancer is the most common cancer and the leading cause of tumor-related death worldwide, ∼85%–90% of which are characterized as non-small-cell lung cancer (NSCLC). For the treatments of lung cancer, the traditional methods include surgery, radiotherapy, and chemotherapy. The majority of patients (68%) with early stage NSCLC undergo surgery, and 16% patients also receive chemotherapy or radiation therapy. A total of 18% of patients with advanced-stage NSCLC are treated with chemotherapy alone, 15% radiation therapy alone, and 33% a combination therapy. However, in recent years, targeted drugs shift the traditional treatment mode of NSCLC. This paradigm was first established with the discovery of epidermal growth factor receptor (EGFR), and EGFR tyrosine kinase inhibitors (TKIs) are excellent examples of personalized alternative. Anaplastic lymphoma kinase (ALK), a member of the insulin receptor family of receptor tyrosine kinases (RTKs), as a fusion oncogene with nucleophosmin, was first identified in anaplastic large-cell lymphomas on chromosome 2p23. ALK encodes a 1,620-amino acid transmembrane protein, including an extracellular ligand-binding domain, a transmembrane domain, and an intracellular kinase catalytic region. Echinoderm microtubule-associated protein-like 4 (EML4) is an intracellular protein of 120 kDa, a member of echinoderm microtubule-associated protein family and microtubule stabilizing protein, and plays the role in the formation of microtubules. EML4-ALK fusion gene is a new gene mutation, which is closely related to the growth and proliferation of tumor cells. Soda et al identified the transforming EML4-ALK fusion gene in 6.7% of patients with NSCLC, resulting from a small inversion within the short arm of chromosome 2p (2p21 and 2p23), which produces a fusion protein consisting...
of the amino-terminal protein of EML4 and the intracellular region tyrosine kinase ALK. It is the most common ALK fusion gene in patients with NSCLC, and the clinical characteristics of these individuals are significantly distinct from EGFR-positive patients. One of the distinctive clinicopathological features is more prevalent in the fluorescence in situ hybridization (FISH)-positive ALK rearrangement patients with the history of never smoked or a light smoking (<10 pack-years), and the other includes younger age at diagnosis and adenocarcinoma histologic analyses associated with ALK-positive lung cancers. EML4-ALK-positive lung adenocarcinomas were less-differentiated grade and acinar-predominant structure observed by histology. Furthermore, EML4-ALK expression was mutually exclusive with EGFR and KRAS mutations in sufficient tissues. ALK gene rearrangements or the resulting fusion proteins in NSCLC can be detected in tumor specimens using FISH, reverse transcriptase polymerase chain reaction, and immunohistochemistry. NSCLC tissues harboring ALK gene rearrangements are representing 3%–5% and define a distinct molecular subgroup of the tumor; a total of >60,000 new cases with ALK-positive are projected to occur in NSCLC annually.

Crizotinib (PF-02341066, trade name Xalkori; Pfizer Inc., New York, NY, USA), the first clinically designed and synthesized as a TKI targeting mesenchymal–epithelial transition factor (c-Met), also called MET and hepatocyte growth factor receptor, indicating marked anticancer activity in patients with advanced, ALK-positive NSCLC, was approved by the US Food and Drug Administration in 2011. In this article, the structure, mechanism, pharmacokinetics, and pharmacogenetics of crizotinib are reviewed. We have also summarized the efficacy of crizotinib compared with chemotherapy in advanced ALK-positive lung cancer and presented the role of crizotinib as personalized alternative in previously treated patients with NSCLC.

Structure, mechanism, and pharmacokinetics

Structure of crizotinib

Crizotinib as multitargeted TKI is a small molecule (molecular weight =450 Da), oral, highly selective and potent competitive inhibitor of ALK with additional MET, c-ros oncogene (ROS1), and recepteur d’origine nantais kinase inhibitory property. Molecular formula of crizotinib is C$_{21}$H$_{22}$Cl$_{2}$FN$_{5}$O, and the chemical formula is (R)-3-[1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-[1-(piperidin-4-yl)-1H-pyrazol-4-yl] pyridine-2-amine, which is shown in Figure 1.

Mechanism

In biochemical and cellular screens for kinase selectivity, crizotinib was shown to be selective for c-Met and ALK with high potency and specificity across a panel of >120 diverse
A chromosomal inversion on chromosome 2p leads to an aberrant EML4-ALK fusion oncogene in NSCLC. Thus, ALK tyrosine kinase is constitutively activated, leading to uncontrolled cell growth and proliferation through activation of phosphoinositide 3-kinase and mitogen-activated protein kinase. Apoptosis in EML4-ALK NSCLC cell lines and tumor shrinkage in murine models were observed when ALK kinase activity was inhibited via small-molecule ALK kinase inhibitors. Crizotinib potently inhibited cell proliferation, which was associated with G1-S-phase cell cycle arrest and induction of apoptosis in ALK-positive anaplastic large-cell lymphomas but not ALK-negative lymphoma cells. Crizotinib dose dependently inhibited the phosphorylation of c-Met and ALK and effectively inhibited downstream effector functions in vitro and in vivo.

**Pharmacokinetics and pharmacogenetics**
Crizotinib was determined orally as a capsule, and clinical studies indicated 250 mg twice daily (bid) as the maximal tolerated dose in 167 patients with cancer. Peak plasma crizotinib concentrations were achieved 4–6 hours after absorption of a single dose of 250 mg. After repeated dosing at 250 mg bid, steady-state concentrations were reached within 15 days. Bioavailability was 43% (range: 32%–66%) and crizotinib exposure was influenced by food only to a minor degree. Age, sex, race, or body weight appeared to have no effects on the single-dose crizotinib. Crizotinib treated in ALK-positive NSCLC patients was similar to patients with other cancer types of pharmacokinetic parameters. Mean values for crizotinib peak plasma concentrations (C_{max}) and area under the plasma concentration–time curve were greater in Asian patients than in non-Asian patients. Metabolization was executed primarily by cytochrome P450 enzymes, resulting in time-dependent inhibition of cytochrome P450 3A4 under extensive hepatic metabolism. Furthermore, crizotinib was also modulated by other drugs interacting with this cytochrome oxidase. The lifetime incidence of central nervous system (CNS) disease in patients with advanced ALK-positive NSCLC approaches 50%. However, crizotinib and the other small-molecule TKIs, including imatinib, erlotinib, and gefitinib, had the low penetration of the cerebrospinal fluid and may require alternative dosing schemes or increased dose adjustments. Local ablative therapy such as radiotherapy or surgery could be a strategy for systemic cancer control with continuation of crizotinib. PF-06463922 (developed by Pfizer), which is a novel multitargeted ALK and ROS1 TKI, a low-efflux substrate from cell lines overexpressing P-glycoprotein, was designed to increase potential CNS penetration. PF-06463922 exhibited superior potency against brain metastases compared with crizotinib and alectinib; a Phase I and II clinical trials of PF-06463922 is currently under way, and this drug can be effective in ALK-rearranged NSCLC patients with CNS disease in the crizotinib naïve or resistance.

**Personalized alternative for targeted ALK-positive in previously treated patients with NSCLC**
The efficacy of crizotinib in the treatment of NSCLC has been investigated in several clinical trials, including various Phase I, II, and III studies on register (Table 1).

The first-in-man Phase I (PROFILE 1001) crizotinib trial (Funded by Pfizer and others, ClinicalTrials.gov number, NCT00585195), was designed as an open-label, multicenter dose-escalation study. The trial consisted of a subgroup of 82 patients with advanced ALK-positive NSCLC confirmed by FISH. The majority of the patients had received previous treatment. The dose-limiting toxicity was defined at 300 mg bid, in which two patients experienced grade 3 fatigue, and the maximum tolerated dose and recommended Phase II dose were defined at 250 mg bid in 28-day cycles. After the mean duration 6.4 months of

<table>
<thead>
<tr>
<th>Study</th>
<th>Trial</th>
<th>ID</th>
<th>Design</th>
<th>Number</th>
<th>PFS (months)</th>
<th>ORR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwak et al⁹</td>
<td>Phase I</td>
<td>PROFILE 1001</td>
<td>Single arm</td>
<td>149</td>
<td>9.7</td>
<td>60.8</td>
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<tr>
<td>Camidge et al⁷</td>
<td></td>
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<tr>
<td>Crinò et al⁴⁰</td>
<td>Phase II</td>
<td>PROFILE 1005</td>
<td>Single arm</td>
<td>259</td>
<td>8.5</td>
<td>53</td>
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<tr>
<td>Kim et al⁴¹</td>
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<tr>
<td>Shaw et al¹²</td>
<td>Phase III</td>
<td>PROFILE 1007</td>
<td>Second line Crizotinib vs pemetrexed or docetaxel</td>
<td>347</td>
<td>7.7</td>
<td>65</td>
</tr>
<tr>
<td>Solomon et al⁴⁴</td>
<td>Phase III</td>
<td>PROFILE 1014</td>
<td>First line Crizotinib vs pemetrexed/cisplatin or carboplatin</td>
<td>343</td>
<td>10.9</td>
<td>74</td>
</tr>
</tbody>
</table>

**Abbreviations:** PFS, progression-free survival; ORR, objective response rate; ID, identification.
The efficacy of open-label, single-arm Phase II trial with Crizotinib is superior to standard chemotherapy in patients with previously treated NSCLC with ALK rearrangement (Table 2).

<table>
<thead>
<tr>
<th>Efficacy parameter</th>
<th>Crizotinib (N=173)</th>
<th>Pemetrexed or docetaxel (N=174)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS (months) (95% CI)</td>
<td>7.7 (6.0–8.8)</td>
<td>3.0 (2.6–4.3)</td>
</tr>
<tr>
<td>ORR (%) (95% CI)</td>
<td>65 (58–72)</td>
<td>20 (14–26)</td>
</tr>
<tr>
<td>Median duration of response (weeks) (range)</td>
<td>32.1 (2.1–72.4)</td>
<td>24.4 (3.0–43.6)</td>
</tr>
<tr>
<td>Median time to response (weeks) (range)</td>
<td>6.3 (4.4–48.4)</td>
<td>12.6 (5.0–37.1)</td>
</tr>
</tbody>
</table>

Similar impressive clinical activity of crizotinib was observed in an ongoing global Phase II study (PROFILE 1005, NCT00932451) of advanced, ALK-positive NSCLC. The efficacy of open-label, single-arm Phase II trial with crizotinib has been assessed in 136 patients who are ALK-positive. At the latest update, among 901 patients with ALK-positive NSCLC received crizotinib therapy, the ORR of 259 patients evaluable for response was 53%, the CR in one patient and the PR in 67 patients, with median duration of response of 10.8 months and median PFS of 8.5 months. ORR was independent of age, sex, and number of prior metastatic treatment regimens. These studies accelerated approval for crizotinib in ALK-rearranged NSCLC in many countries and granted by the US Food and Drug Administration.

Recently, the results of two international, randomized Phase III studies of crizotinib were conducted. The first Phase III trial (the second-line trial, PROFILE 1007) compared crizotinib with chemotherapy (single-agent pemetrexed or docetaxel) in 347 patients (173 to crizotinib and 174 to chemotherapy) with advanced ALK-positive NSCLC detected by FISH who have received one prior platinum-based chemotherapy. PFS was the primary end point in the study, with OS as a secondary end point. PFS was prolonged in the crizotinib-treated group, with the median PFS of 7.7 months in the crizotinib group and 3.0 months in the chemotherapy group. The response rate was also obviously higher in patients treated with crizotinib (65%) as compared with chemotherapy (20%). However, there was no significant difference in OS between crizotinib and chemotherapy, and 64% of patients on chemotherapy crossed over to crizotinib at disease progression probably influenced the data. Crizotinib is superior to standard chemotherapy in patients with previously treated NSCLC with ALK rearrangement (Table 2).

The second Phase III trial (The first-line trial, PROFILE 1014) is ongoing, comparing crizotinib with standard chemotherapy (pemetrexed plus either cisplatin or carboplatin) in newly diagnosed 343 patients with ALK-positive advanced NSCLC. PFS of crizotinib and standard chemotherapy was 10.9 months and 7.0 months, respectively. The ORR showed superiority of crizotinib over first-line chemotherapy.

The probability of 1-year survival was 84% with crizotinib and 79% with chemotherapy. In addition, ROS1 (chromosome 6q22) encodes an orphan RTK related to ALK and belongs to the insulin receptor family. Chromosomal rearrangements involving the ROS1 RTK gene have been reported in a subgroup of NSCLC patients, which occurs in 1%–2% of patients with NSCLC. All of the ROS1-positive NSCLCs were remarkably similar to that of ALK-rearranged patients, who have histologic features of adenocarcinoma, a tendency toward higher grade, young age, and never smoked. The preclinical study indicated that crizotinib could inhibit ROS1 activity and cell growth in vitro. In an expansion cohort of the Phase I study (250 mg twice daily) in 50 previously treated patients with ROS1-positive rearrangement advanced NSCLC, the ORR was 72%, including three CRs and 33 PRs. The median duration of response was 17.6 months, and median PFS was 19.2 months, with 25 patients (50%) still in follow-up for progression.

Safety and adverse effect

Crizotinib was generally well tolerated with the majority of adverse events (AEs), the most of which are moderate (grade 1 or 2). The frequently occurring AEs were visual effects (visual impairment, photopsia, blurred vision, vitreous floaters, photophobia, and diplopia), but the visual disorders...
could disappear after discontinuation of crizotinib. Other common AEs included fatigue, decreased appetite, gastrointestinal events (nausea, diarrhea, vomiting, and constipation), peripheral edema, esophageal disorders (dyspepsia, esophagitis, and gastroesophageal reflux), altered taste, neuropathy, dizziness, and rash.9,40,44

Resistance to crizotinib
Despite the excellent efficacy, the majority of patients relapse during the first year of treatment and become resistant to crizotinib.15 Mechanisms of the acquired crizotinib resistance can be divided into two main classes.12 First, the target gene itself can be altered either by mutation or amplification, making tumor cells limit the drug efficacy to inhibit the kinase.35 Choi et al.31 reported the two secondary mutations (C1156Y and L1196M) within the kinase domain of EML4-ALK in tumor cells, L1196M representing gatekeeper mutation that interferes with the binding of crizotinib and EML4-ALK, and the same L1196M gatekeeper mutatation was identified in a patient with acquired resistance.50,51 Second, crizotinib resistance is caused by the activation of alternative signaling pathways or so-called bypass tracks in ALK-positive NSCLCs. As an example, activation of EGFR signaling as a bypass signaling made resistant to crizotinib, suggesting that EGFR and some of its ligands may be upregulated.15,52 Katayama et al.53 also identified aberrant activation of other kinases including marked amplification of KIT and increased autophosphorylation of EGFR in drug-resistant tumors from patients. Finally, ALK-positive NSCLC occurs through somatic kinase domain mutations, ALK gene fusion copy number gain, and emergence of separate oncogenic drivers, which could represent a potential resistance mechanism.54

Next-generation ALK inhibitors
Ceritinib (Zykadia; Novartis International AG, Basel, Switzerland; formerly called LDK378) is an orally available, potent, small molecule TKI of ALK, and it is effective in preclinical models of ALK-positive NSCLC.55,56 Ceritinib has demonstrated antitumor activity in both crizotinib-naive and crizotinib-refractory ALK-rearranged NSCLC patients.57 In particular, ceritinib increased activity against ALK harboring L1196M, G1269A, I1171T, and S1206Y mutations, but it was ineffective at inhibiting two crizotinib-resistant ALK mutations, G1202R and F1174C.32,57 Alectinib (RO5424802/CH5424802), which is being developed by Roche, is potent, selective, and orally available ALK inhibitor. It was first approved in multicenter, single-arm, open-label, Phase I and II study of Japan.32,58 Based on the results of the study, alectinib could be an effective and safe option for the treatment of ALK-rearranged NSCLC, and it can be used to achieve strong and longlasting inhibitory effects on brain metastases.58,59 Currently, a clinical study (NCT01588028) assessing the activity of alectinib in patients who failed to respond to crizotinib-based treatment is ongoing.58 Brigatinib (previously known as AP26113) is a more potent inhibitor of ALK than crizotinib and has activity against ALK kinase domain mutations that confer resistance to crizotinib.31 Brigatinib has been shown to be effective for intracranial metastasis.59 Other ALK inhibitors, such as PF-06463922 (Pfizer), X-396 (Xcovery, Holding Co LLC, West Palm Beach, FL, USA), ASP3026 (Astellas Pharma Inc., Tokyo, Japan), TS-R-011 (Tesorco, Inc., Waltham, MA, USA), and CEP-37440 (TEVA, Petah Tikva, Israel), are in various stages of clinical development for ALK-positive cancers.31,32 Next-generation ALK inhibitors including ceritinib, alectinib, and brigatinib in patients have seen impressive and durable responses progressing after crizotinib, indicating that these drugs represent effective second-line treatment options.31 Now, a number of effective treatment options raise for patients with ALK-rearranged NSCLC, what sequence to use these ALK inhibitors is yet to be determined.31,32

Heat shock protein 90 inhibitors
Heat shock protein 90 (Hsp90) is a molecular chaperone involved in normal cellular functions as well as tumorigenesis, which has been identified as potential anticancer agents.32,60 Hsp90 inhibitors also showed efficacy in treating ALK-positive NSCLC; compared with TKIs, Hsp90 inhibitors appear to have lower response rates and side effects that are less tolerable.32 However, Hsp90 inhibitors had limited activity against CNS metastatic tumors. The present studies encourage patients to participate in clinical trials to address the best combination or treatment strategy of Hsp90 inhibitors.60 Immunotherapy through inhibition of programmed death 1 has demonstrated efficacy in treating advanced NSCLC.31,61 Ongoing trials will further define the utility of Hsp90 inhibitors in NSCLC.62

Conclusion and future directions for drug development
Crizotinib is a promising antitumor activity for patients with ALK gene rearrangements in NSCLC. The clinical trials showed that crizotinib prolonged PFS, increased response rates, and improved the quality of life in patients. Crizotinib was superior to standard chemotherapy in patients with ALK-positive advanced NSCLC. Crizotinib for targeted ALK rearrangement in previously treated patients with NSCLC brings us one step closer to personalized lung cancer therapy. Though crizotinib
is well benefited for most patients who are ALK positive, some still go on to relapse as a result of the acquired resistance. The multiple therapeutic strategies should be implemented and developed to overcome crizotinib resistance in the future.

Acknowledgments
This work was supported by the National Key Basic Research Program 973 of the People’s Republic of China (number 2010CB732404), the National Nature Science Foundation of the People’s Republic of China (numbers 81170492 and 81370673), and the National High Technology Research and Development Program 863 Projects of the People’s Republic of China (number 2012AA022703).

Disclosure
The authors report no conflicts of interest in this work.

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