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).¹ 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.² 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.³ 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 2p23.³ ALK encodes a 1,620-amino acid transmembrane protein, including an extracellular ligand-binding domain, a transmembrane domain, and an intracellular kinase catalytic region.⁴ 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.⁵,⁶ 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 ligand-binding domain of EML4 and the intracellular kinase domain of ALK. 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.

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of the amino-terminal protein of \textit{EML4} and the intracellular region tyrosine kinase \textit{ALK}. It is the most common \textit{ALK} fusion gene in patients with NSCLC, and the clinical characteristics of these individuals are significantly distinct from \textit{EGFR}-positive patients.

One of the distinctive clinicopathological features is more prevalent in the fluorescence in situ hybridization (FISH)-positive \textit{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 \textit{ALK}-positive lung cancers. \textit{EML4-ALK}-positive lung adenocarcinomas were less-differentiated grade and acinar-predominant structure observed by histology. Furthermore, \textit{EML4-ALK} expression was mutually exclusive with \textit{EGFR} and \textit{KRAS} mutations in sufficient tissues.\textit{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 \textit{ALK} gene rearrangements are representing 3%-5% and define a distinct molecular subgroup of the tumor; a total of >60,000 new cases with \textit{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 \textit{MET} and hepatocyte growth factor receptor, indicating marked anticancer activity in patients with advanced, \textit{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 \textit{ALK}-positive lung cancer and presented the role of crizotinib as personalized alternative in previously treated patients with NSCLC.

Structure, mechanism, and pharmacokinetics

\textbf{Structure of crizotinib}

Crizotinib as multitargeted TKI is a small molecule (molecular weight =450 Da), oral, highly selective and potent competitive inhibitor of \textit{ALK} with additional \textit{c-Met}, \textit{c-ros} oncogene (\textit{ROS1}), and \textit{recepteur d’origine nantais} kinase inhibitory property. Molecular formula of crizotinib is \textit{C}\textsubscript{21}\textit{H}\textsubscript{22}\textit{Cl}\textsubscript{2}\textit{FN}\textsubscript{5}\textit{O}, and the chemical formula is \((\text{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.

\textbf{Mechanism}

In biochemical and cellular screens for kinase selectivity, crizotinib was shown to be selective for \textit{c-Met} and \textit{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 (Cmax) 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.

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 5% 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>
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<tbody>
<tr>
<td>Kwak et al⁷</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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<td>Camidge et al¹⁷</td>
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<tr>
<td>Crinò et al⁰</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>Shaw et al¹²</td>
<td>Phase III</td>
<td>PROFILE 1007</td>
<td>Second line</td>
<td>347</td>
<td>7.7</td>
<td>65</td>
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<td>Solomon et al⁴⁴</td>
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<td>Crizotinib vs pemetrexed or docetaxel</td>
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<td>Crizotinib vs pemetrex/cisplatin or carboplatin</td>
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Abbreviations: PFS, progression-free survival; ORR, objective response rate; ID, identification.
The efficacy of crizotinib compared with standard chemotherapy in previously treated patients with NSCLC

<table>
<thead>
<tr>
<th>Efficacy parameter</th>
<th>Crizotinib (N=173)</th>
<th>Pemetrexed or docetaxel (N=174)</th>
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</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>
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<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>
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<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>
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**Abbreviations:** NSCLC, non-small-cell lung cancer; PFS, progression-free survival; CI, confidence interval; ORR, objective response rate.

Guo et al. 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.\textsuperscript{9,40,41,44}

### Resistance to crizotinib

Despite the excellent efficacy, the majority of patients relapse during the first year of treatment and become resistant to crizotinib.\textsuperscript{15} Mechanisms of the acquired crizotinib resistance can be divided into two main classes.\textsuperscript{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.\textsuperscript{55} Choi et al\textsuperscript{49} reported the second 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 mutation was identified in a patient with acquired resistance.\textsuperscript{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.\textsuperscript{15,52} Katayama et al\textsuperscript{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.\textsuperscript{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.\textsuperscript{55,56} Ceritinib has demonstrated antitumor activity in both crizotinib-naive and crizotinib-refractory ALK-rearranged NSCLC patients.\textsuperscript{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.\textsuperscript{31,32} 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.\textsuperscript{31,32,58} 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.\textsuperscript{58,59} Currently, a clinical study (NCT01588028) assessing the activity of alectinib in patients who failed to respond to crizotinib-based treatment is ongoing.\textsuperscript{59} 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.\textsuperscript{31} Brigatinib has been shown to be effective for intracranial metastasis.\textsuperscript{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), TSR-011 (Tesaro, Inc., Waltham, MA, USA), and CEP-37440 (TEVA, Petah Tikva, Israel), are in various stages of clinical development for ALK-positive cancers.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{60} Immunotherapy through inhibition of programmed death 1 has demonstrated efficacy in treating advanced NSCLC.\textsuperscript{31,61} Ongoing trials will further define the utility of Hsp90 inhibitors in NSCLC.\textsuperscript{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 therapeutics strategies should be implemented and developed to overcome crizotinib resistance in the future.

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Disclosure

The authors report no conflicts of interest in this work.

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