New Strategies for Treatment of ALK-Rearranged Non–Small Cell Lung Cancers

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Abstract
The identification of oncogenic alterations in subsets of patients with non–small cell lung cancer (NSCLC) is transforming clinical care. Genomic rearrangements in anaplastic lymphoma kinase (ALK) are detected in 3% to 7% of patients with NSCLC. The ALK tyrosine kinase inhibitor crizotinib has demonstrated clinical efficacy in ALK-rearranged NSCLC patients and was recently approved by the U.S. Food and Drug Administration. Crizotinib is currently under additional phase III clinical development as both initial and second-line therapy for advanced ALK-rearranged NSCLC. However, new challenges in the diagnosis and treatment of this subset of NSCLC have emerged, including the need to determine the most effective means of diagnosing ALK-rearranged NSCLC and the emergence of acquired drug resistance to crizotinib. In this review, we discuss current strategies for treatment and diagnosis, as well as the current knowledge about mechanisms of acquired resistance to crizotinib. Finally, we discuss the strategies that are underway to clinically overcome acquired drug resistance. Clin Cancer Res; 17(23); 7213–8. ©2011 AACR.

Introduction
Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that was initially identified as a result of a translocation in anaplastic large cell lymphoma (ALCL) in 1998 (1). ALK plays a role during development, and it is not expressed in most, if any, adult tissues. In 2007, a fusion of ALK with echinoderm microtubule–associated protein-like 4 (EML4) was first identified in a patient with non–small cell lung cancer (NSCLC; ref. 2). This occurs due to a chromosomal inversion on chromosome 2p, resulting in the formation of the EML4-ALK fusion oncogene (2). The chromosomal inversion does not always occur in the same location, and multiple EML4-ALK variants have been identified (3). All of these variants involve the intracellular tyrosine kinase domain of ALK, beginning at the portion encoded by exon 20. EML4, however, is variably truncated and gives rise to variants of EML4-ALK (3). The most common variants are E13;A20 [the nomenclature refers to the exons in EML4 (E) that are fused to ALK (A)] and E6a/b;A20, which are referred to as variants 1 and 3a/b, respectively. At least 9 variants have been reported to date, and rare non-EML4 fusion partners have also been described (herein collectively referred to as ALK-rearranged NSCLC; ref. 3).

The EML4-ALK fusion protein is oncogenic both in vitro and in vivo (2, 4, 5). EML4-ALK fusions result in protein dimerization and constitutive activation of ALK kinase activity and of critical downstream signaling proteins involved in cell survival and proliferation (Fig. 1A; refs. 2 and 6). Inhibition of ALK kinase activity via small-molecule ALK kinase inhibitors leads to apoptosis in EML4-ALK NSCLC cell lines and to tumor shrinkage in murine models of EML4-ALK NSCLC (4, 5, 7).

Lung cancers harboring ALK rearrangements represent a unique subpopulation of lung cancer patients. The frequency of ALK rearrangements ranges from 3% to 7% in unselected NSCLC patients (3, 7, 8). Similar to epidermal growth factor receptor (EGFR) mutations, the frequency of this genetic alteration is higher in NSCLC patients with adenocarcinomas and in patients who have never smoked or are light cigarette smokers (defined as ≤10 pack years). ALK rearrangements also tend to be mutually exclusive with EGFR and KRAS mutations and to have a lower frequency of p53 mutations (7, 8). Most tumors with ALK rearrangements are positive for thyroid transcription factor 1. A variety of histologic features associated with ALK-rearranged adenocarcinomas have been reported, including those with acinar, papillary, cribriform, mucin producing (intra- and extracytoplasmic), and signet-ring patterns (9).

Current Diagnosis and Treatment Strategies for ALK-Rearranged NSCLC

Clinical diagnosis of ALK-rearranged NSCLC
Detection of ALK rearrangements can be challenging, and several methods are currently being evaluated, including fluorescence in situ hybridization (FISH), reverse transcriptase PCR (RT-PCR), and immunohistochemistry. Each
technique is associated with specific strengths and weaknesses for the screening of samples for ALK rearrangements. Given the recent approval of crizotinib by the U.S. Food and Drug Administration (FDA), an evaluation for an ALK rearrangement should take place early in the treatment course. Routine molecular diagnostics need to include evaluations for both EGFR mutations and ALK rearrangements because these 2 genetically defined subsets of lung cancer require different therapies.

**Fluorescence in situ hybridization**

In current clinical trials of the ALK kinase inhibitor crizotinib, FISH is used as the diagnostic test for detecting an ALK rearrangement. This test was recently approved by
the FDA. In this assay, the 5' and 3' ends of the ALK gene are differently labeled with red and green fluorescent probes. Under normal circumstances, the 2 probes are close together. However, in the presence of an ALK rearrangement, the red and green signals are farther apart (hence the term "break-apart" probe; ref. 8). Using a cutoff >15% of cells and examining ≥4 fields (~60 cells), investigators have obtained high sensitivity and specificity (10–12). This assay detects ALK rearrangements regardless of the ALK fusion partner or the specific EML4-ALK variant. However, because EML4-ALK results from a chromosomal inversion on chromosome 2p and because the 2 genes are normally only ~12 Mb apart, the split signal can be very subtle and challenging to detect. In addition, because the assay requires specialized technical resources and expertise, it is not available in all pathology laboratories. Furthermore, FISH is a relatively low-throughput assay and costly, and thus, it is not ideal for detecting a relatively infrequent subset of NSCLC patients. Recent studies that compared ALK chromogenic in situ hybridization (CISH) with FISH showed >95% agreement between the 2 methods (13). CISH has potential advantages over FISH because it can be performed without a fluorescence microscope, and the signals are stable over long periods.

**RT-PCR**

As an alternative method of screening and confirming an ALK rearrangement, RT-PCR offers some unique advantages. In the absence of contamination, this assay is very specific, and the resulting PCR product can be sequenced to help define the specific type of translocation that is present (14). It is the only technique capable of defining both the ALK fusion partner and the precise fusion variant. This technique can be applied to samples with limited tissue, such as sputum specimens or material obtained by bronchoscopic biopsies (15). Although RT-PCR is potentially the most sensitive assay, it requires adequate and sufficient quality RNA, which can be difficult to obtain from routine clinical samples such as formalin-fixed, paraffin-embedded tumor specimens. In addition, given the large number of potential EML4-ALK variants, and non-EML4 ALK fusions, several PCR primer sets are necessary to cover all of the potential ALK rearrangements.

**Immunohistochemistry**

Immunohistochemistry is a diagnostic tool available in every clinical pathology laboratory. In addition, immunohistochemistry is cost-effective, can be incorporated into the existing sample flow for pathologists, and is an ideal tool to screen for a subpopulation of patients such as those harboring ALK rearrangements. Furthermore, because ALK is not normally expressed in the lung or in adults, any degree of ALK expression may be a sign of aberrant ALK expression due to an ALK rearrangement. The initial attempts to use ALK antibodies to diagnose NSCLC, as previously done for ALCL, were disappointing because ALK expression is much lower in lung cancer than in ALCL harboring an ALK translocation (9, 16). However, since then, the detection of ALK by immunohistochemistry has improved due to the incorporation of techniques to enhance the immunohistochemistry signal and the development of more sensitive ALK antibodies (9, 16). Methods to amplify the immunohistochemistry signal using existing antibodies include the intercalated antibody-enhanced polymer approach (used with the ALK antibody 5A4) and tyramide amplification (using the ALK antibody ALK1; refs. 9 and 16). For example, the tyramide amplification method improves the ability to detect ALK by immunohistochemistry from 40% to 80% of specimens harboring ALK rearrangements defined by FISH (9). Both techniques have been used successfully as a screening method in surgically resected specimens, as well as in smaller samples obtained by tranbronchial needle biopsies (17). The mouse monoclonal antibody (D5F3) for ALK appears to offer sufficient sensitivity (100%) and specificity (99%) compared with ALK FISH, and it may be suitable for development as a clinical diagnostic tool (11). However, this antibody is not yet commercially available, which limits its use as a screening test. It is anticipated that the optimization of both immunohistochemistry methods and identification of novel ALK antibodies will continue to evolve. Currently, however, immunohistochemistry represents our greatest hope for achieving a widely available, cost-effective, rapid screening tool to identify patients with ALK-rearranged NSCLC.

**Treatment of ALK-rearranged NSCLC**

Because ALK's tyrosine kinase activity is necessary for its transforming activity and oncogenicity, inhibition of this activity by a kinase inhibitor represents a potential therapeutic approach for ALK-rearranged NSCLC (Fig. 1B). Several ALK inhibitors have been identified and are being evaluated in preclinical models both in vitro and in vivo as potential clinical therapies (4, 18–20). In such models, ALK inhibitors lead to apoptosis in vitro and to tumor shrinkage in vivo—a phenomenon known as oncogene addiction (refs. 4, 5, 18, and 21; Fig. 1B). This is further highlighted by recent dramatic clinical studies. Crizotinib (PF-02341066), an oral kinase inhibitor that was initially designed as a mesenchymal–epithelial transition (MET) inhibitor, is a clinically effective ALK inhibitor in NSCLC patients harboring ALK rearrangements (10). In a phase 1 clinical trial with 82 treated patients, the rate of response to crizotinib was 57% (10). The study also highlighted the amount of screening that is necessary to identify patients: the investigators had to screen more than 1,500 patients to identify the 82 patients with ALK rearrangements (10). Because targeted therapies are increasingly focusing on subsets of cancer patients, the screening programs necessary to identify such patients also need to evolve. The ALK example highlights the value of this approach both for drug development and as means of identifying patients who will derive the greatest benefit from crizotinib treatment. Based on its dramatic clinical activity, crizotinib was recently approved by the FDA for ALK-rearranged NSCLC. Its clinical development occurred over a remarkably short period of...
time, from the initial identification of the EML4-ALK translocation as an oncogene in 2007, to validation as a clinical target in NSCLC in 2010, to FDA approval in 2011 (2, 10).

Two randomized phase III clinical trials are currently underway to compare the use of crizotinib with the standard of care (systemic chemotherapy) in patients with advanced ALK-rearranged NSCLC. These include a phase III registration trial testing crizotinib versus second-line therapy (pemetrexed or docetaxel) in ALK-rearranged advanced NSCLC (NCT00932893). Another phase III clinical trial is testing crizotinib versus first-line therapy (pemetrexed/cisplatin or pemetrexed/carboplatin) in treatment-naïve ALK-rearranged patients with advanced NSCLC (PROFILE 1014, NCT01154140). On the basis of the recent FDA approval of crizotinib, the National Comprehensive Cancer Network guidelines already recommend crizotinib as a first-line systemic therapy for patients with ALK-rearranged NSCLC (22).

On the Horizon

Acquired resistance to crizotinib

ALK tyrosine kinase inhibitors are emerging as effective clinical therapies for ALK translocated cancers. However, as has been observed with other targeted therapies, including EGFR kinase inhibitors, their efficacy will ultimately be limited by the development of acquired drug resistance (23). A mechanistic understanding of drug resistance may help investigators develop effective subsequent clinical treatments and/or rational combination-therapy strategies (Fig. 2). The best treatment for patients who develop acquired resistance to crizotinib has not been defined. However, several groups have begun to identify and study crizotinib resistance mechanisms. These mechanisms include secondary mutations in the target of the kinase itself, which abrogate the inhibitory activity of the drug, and activation of alternative signaling pathways that bypass the continued requirement for inhibition of the original target (Fig. 1C and D; refs. 19 and 24–26). The fraction of crizotinib resistance mediated by a secondary mutation compared with activation by an alternative signaling pathway is currently not known.

Secondary mutations in kinases are a common mechanism of acquired drug resistance to kinase inhibitors (27). To date, 4 acquired drug-resistance mutations, all identified in patients with crizotinib-treated NSCLC or inflammatory myofibroblastic tumor, have been reported (Fig. 1C; refs. 24–26). These mutations involve either the gatekeeper residue (L1196M) or residues away from crizotinib binding (L1152R, C1156Y, and F1174L; refs. 24–26). In vitro, cells engineered to express these secondary mutations were shown to be resistant to crizotinib. It is not clear how these secondary mutations actually lead to crizotinib resistance; however, possible explanations include steric hindrance (for the L1196M mutation), promotion of a conformational change that disfavors crizotinib binding, or by an increase in the affinity for ATP (24). Structural and biochemical studies of each of these mutations will be necessary to further our understanding of how they lead to crizotinib resistance. In addition, such studies may provide insight into the potential efficacy of next-generation ALK kinase inhibitors.

Data are also emerging about mechanisms of crizotinib resistance that result from activation of an alternative signaling pathway. A recent study showed that activation of the EGFR signaling pathway can bypass the continued requirement for inhibition of ALK and contribute to ALK inhibitor resistance (26). In some of these models, EGFR is activated by a ligand-mediated process (Fig. 1D). Concurrent inhibition of both EGFR and ALK is therapeutically effective in such resistant models (26). Additional studies are needed to evaluate changes in EGFR signaling from crizotinib-treated
tumor specimens and to determine whether activation of other receptor tyrosine kinases can also contribute to crizotinib resistance.

Understanding the specific mechanism(s) of drug resistance is critical for the selection and evaluation of subsequent therapeutic approaches. Any new therapeutic strategy for patients who have developed acquired resistance to crizotinib must incorporate tumor biopsies as part of early clinical trials. This will be the only way to understand the potential benefits and limitations of a new therapeutic approach.

**Treatment of ALK-rearranged tumors with HSP90 inhibitors**

A second class of agents that has demonstrated clinical efficacy in ALK-rearranged NSCLC patients is heat shock protein 90 (HSP90) inhibitors. EML4-ALK associates in complex with multiple cellular chaperones, including HSP90 (5). Inhibitors of HSP90 disrupt this complex, leading to degradation of EML4-ALK and to tumor regression in xenograft and genetically engineered models of EML4-ALK NSCLC (5). In addition, cell lines bearing the crizotinib-resistance mutations (L1196M and F1174L) remain equally sensitive to HSP90 inhibitors compared with those without the secondary mutations (19, 25).

Clinical studies of 2 HSP90 inhibitors, retaspimycin (IPI-504) and ganetespib (STA-9090), have also demonstrated efficacy in ALK NSCLC patients (28, 29). Neither study was specifically designed to evaluate ALK-rearranged patients; both included NSCLC patients with other genotypes (such as EGFR and KRAS mutants). However, a substantial proportion of the ALK-rearranged patients had partial responses, which were not observed in patients with other genotypes (28, 29). Why EML4-ALK is a particularly good HSP90 client protein clinically remains to be determined. Additional clinical trials are underway to further evaluate HSP90 inhibitors in ALK-rearranged NSCLC. Of note, the studies conducted to date predominantly treated crizotinib-naïve patients, and it remains to be determined whether HSP90 inhibitors will also be clinically effective in patients who have developed acquired resistance to crizotinib.

**Next-generation ALK kinase inhibitors and novel therapeutic combinations**

Several new ALK kinase inhibitors have been developed that are entering early clinical development (Table 1). Some of these agents, including CH5424802, AP26113, and X-396, have been tested and have shown preclinical efficacy in models bearing crizotinib-resistance mutations (18–20). Many of these new ALK inhibitors are also more potent inhibitors of ALK than crizotinib, which was originally identified as a MET inhibitor (18, 20). Whether this increase in potency will translate into an increase in clinical efficacy can only be determined from future clinical trials. Investigators are likely to employ 2 drug development strategies: treating patients who have developed acquired resistance to crizotinib and treating patients who are crizotinib-naïve. The former approach assumes that clinical acquired resistance to crizotinib will be mediated by an ALK-dependent process (e.g., secondary mutation). To date, however, only a limited number of studies have attempted to define the fraction of crizotinib resistance mediated by secondary mutations by sequencing ALK from tumors in patients who have developed acquired crizotinib resistance.

Combination treatment strategies may also be effective against crizotinib resistance. At present, there is an ongoing phase I clinical trial of crizotinib and the irreversible EGFR/HER2 inhibitor PF299804 (NCT01121575). This study was originally designed to evaluate the therapeutic benefit of inhibiting MET (crizotinib is a potent MET inhibitor) and EGFR T790M in patients with erlotinib-resistant EGFR-mutant NSCLC (30). However, given the results of recent preclinical studies, which suggest that the combination of crizotinib and PF299804 may represent a rational therapeutic strategy for a subset of patients who develop resistance to crizotinib.

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<tr>
<th>Drug</th>
<th>Manufacturer</th>
<th>Clinical stage</th>
<th>Notes</th>
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<tbody>
<tr>
<td>Crizotinib</td>
<td>Pfizer</td>
<td>Phase III and II for NSCLC</td>
<td>Effective against L1196M, S1206R, and G1269S mutations</td>
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<td>CEP-37440</td>
<td>Cephalon</td>
<td>Preclinical</td>
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<td>AP-26113</td>
<td>Ariad Pharmaceuticals</td>
<td>Preclinical; IND expected 2011</td>
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<tr>
<td>NMS-E628</td>
<td>Nerviano Medical</td>
<td>Preclinical</td>
<td>Effective against L1196M ALK secondary mutations</td>
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<tr>
<td>X-276/396</td>
<td>Xcovery</td>
<td>Preclinical</td>
<td>Effective against L1196M and F1174L ALK secondary mutations</td>
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<tr>
<td>TAE684</td>
<td>Novartis</td>
<td>Not a clinical candidate</td>
<td>Effect against L1196M ALK secondary mutations</td>
</tr>
<tr>
<td>CH5424802</td>
<td>Chugai Pharmaceuticals</td>
<td>Preclinical; phase I trial ongoing in Japan</td>
<td>Effective against L1196M, C1158Y, and F1174L secondary mutations</td>
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<tr>
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<tr>
<td>ASP3026</td>
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crizotinib resistance, this clinical trial may offer a rational treatment strategy.

Disclosure of Potential Conflicts of Interest

P.A. Janne is a consultant for Pfizer and Chugai Pharmaceuticals. T. Sasaki disclosed no potential conflicts of interest.

References


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