Crizotinib for the treatment of non-small-cell lung cancer with ALK gene rearrangements


Approximately 2–5% of non-small-cell lung cancers (NSCLC) contain rearrangements in the ALK gene. These tumors most frequently occur in younger, never or light smokers with adenocarcinoma and are typically independent of EGFR or KRAS mutations. Crizotinib is a small molecule inhibitor of the ALK tyrosine kinase that has demonstrated clinical activity in advanced ALK rearranged NSCLC patients with a response rate of 51–61% in a Phase I and II study with an estimated median progression-free survival of approximately 10 months. On the basis of this data the US FDA approved crizotinib for the treatment of ALK rearranged NSCLC in August 2011. In this article we review the clinical and pathologic features associated with ALK gene rearrangements in NSCLC, diagnostic assays for ALK gene rearrangements, the safety and efficacy of crizotinib, and outline remaining challenges in this population including the problem of acquired resistance.

Keywords: ALK • crizotinib • gene rearrangement • non-small-cell lung cancer • targeted therapy

Identification and subsequent targeting of critical oncogenic kinases, as exemplified by BCR-Abl positive chronic myeloid leukemia treated with imatinib or Her2 amplified breast cancer treated with trastuzumab, has proven to be a highly effective therapeutic strategy that represents a paradigm for personalized therapy for cancer. In non-small-cell lung cancer (NSCLC), a malignancy that results in more cancer deaths worldwide than any other [1], the importance of identifying the subset of patients with EGFR mutations [2–4] and the efficacy of treating these patients with EGFR tyrosine kinase inhibitors (TKIs) compared with standard chemotherapy has been demonstrated by no fewer than six randomized trials [5–10]. The recent identification of rearrangements in the ALK gene in a subset of patients with NSCLC has been rapidly followed by a first-in-class clinical trial with the oral ALK TKI crizotinib leading to approval of crizotinib for ALK rearranged NSCLC in August 2011. Here we review the clinicopathological features of ALK gene rearrangements in NSCLC, clinical data with crizotinib the first approved ALK inhibitor, and outline current challenges in the identification and treatment of this patient population.

ALK
ALK was originally identified as a novel tyrosine kinase involved in a chromosomal translocation, t(2;5), which occurs in anaplastic large-cell lymphoma (ALCL). This translocation results in the juxtaposition of part of the NPM gene on chromosome 5q35 with the ALK gene on chromosome 2p23 and results in a fusion protein NPM–ALK that contains the amino terminus of NPM fused to the carboxy terminus of ALK [11,12]. The fusion results in abnormal nuclear expression and constitutive activity of the ALK kinase, driving proliferation and enhancing...
Figure 1. (A) Immunohistochemistry using the Novocastra 5A4 clone ALK antibody showing a strong cytoplasmic staining for ALK in lung adenocarcinoma (x20). (B) Fluorescence in situ hybridization using the Vysis ALK Dual Colour Break-Apart probe showing a rearrangement of the ALK gene (x200). 

EML4–ALK & NSCLC

In 2007 two groups, using different approaches, independently identified the presence of ALK gene rearrangements in a subset of patients with NSCLC [28,29]. Soda and colleagues screened DNA extracted from a tumor specimen from a 62-year-old Japanese light smoker with lung adenocarcinoma for transforming activity using a retroviral-mediated complementary DNA expression system [28]. They identified a cDNA that proved to contain a novel fusion gene involving EML4 and the kinase-containing portion of ALK that arose as a result of an inversion on chromosome two. Independently, Rioko and colleagues used a global phosphoproteomic approach to analyse the tyrosine kinase activity in NSCLC cell lines and tissue specimens, and also identified the EML4–ALK fusion [29].

Multiple breakpoints within the EML4 gene that generate distinct EML4–ALK chimeric variants have been reported of which variant one and three are most frequent (33 and 29% of all cases respectively) [29,30]. In variant one, intron 13 of EML4 is disrupted at a point 3.6 Kb downstream of exon 13 and is inverted to connect to a position 297 base pairs upstream of exon 20 of ALK between exons 13 and 20. Variant three results from a fusion between exon six of EML4 and exon 20 of ALK (E6;A20). The fusion point within ALK is conserved in the resulting chimeric proteins, all of which contains the intracellular portion of ALK and the kinase domain. Other fusion partners of ALK have rarely been described, including TRK-fused gene [31], KIF5B [32] and KLC1 [33].

In cancers harboring the EML4–ALK fusion gene, the activation of kinase activity occurs as a result of aberrant tyrosine phosphorylation of ALK driven by the EML4 promoter and ligand-independent, constitutive dimerization within the EML4 portion of the chimeric protein. This results in transactivation and initiation of signaling through various downstream signaling pathways including the PI3K kinase pathway, the Ras/Raf/MEK/ERK pathway and the JAK3/STAT3 pathway, which are important for cell survival and proliferation [34].

Inhibition of ALK by ALK TKIs or knockdown using RNA interference result in abrogation of downstream signaling and induction of apoptosis through induction of BIM [33–35]. In vivo mouse xenograft models show tumor regression in the EML4-ALK-positive NSCLC cell line H3122 after oral treatment with a small molecule ALK TKI, TAE-684 [35]. Upstream and downstream models of expression of EML4–ALK in alveolar epithelium induces the formation of lung adenocarcinomas that regress after treatment with ALK inhibitors [36,37]. Collectively, these data indicate that cells bearing ALK gene rearrangements are dependent on ALK for survival and proliferation and provide a strong preclinical rationale for targeting ALK in human cancers.

Clinical & pathological features of ALK-positive NSCLC

ALK gene rearrangements are found in approximately 2–5% of all NSCLC [Table 1] with higher frequencies reported in populations selected on the basis of particular clinicopathological features [29,40]. Like EGFR mutations, ALK gene rearrangements are most commonly found in never or light smokers with tumors of adenocarcinoma histology [29,40]. ALK-positive patients tend to be younger with median age typically lower than that of the general NSCLC population. The median age of ALK-positive patients reported in most series and in the Phase I and II clinical trials is between 50 and 60, although a wide age range has been reported [40,43]. Results from this study with crizotinib in unselected ALK-negative NSCLC cohorts is >70 years [34,44–46]. Unlike EGFR mutations, EML4–ALK appears to be found in similar frequencies in Asian and western populations. Historically, almost all ALK-positive tumors are adenocarcinoma with squamous cell differentiation reported in approximately 1% of all positive cases [Table 1] [44,45]. ALK gene rearrangements are frequently found independently of EGFR and KRAS mutations, however instances of coexistent mutations have been described in some series [43–46]. Some authors have reported characteristic morphological features, namely a solid subtype and signet-ring morphology [44], or acinar subtype with extracellular mucin [45]. In the largest series with detailed analysis of morphology the solid, signet-ring cell pattern and the mucinous cribriform pattern were at least focally present in 43 and 56% of ALK-positive cases, with positive staining for both TTF1 and 60, although a wide age range has been reported [40,43]. Positive ALK staining in NSCLC patients who are randomized to either crizotinib or standard chemotherapy (single agent pemetrexed or docetaxel) has recently completed accrual. The other study, Profile 1014 (NCT01154410), is a first-line study in previously untreated ALK-positive advanced NSCLC patients who are randomized to either crizotinib or chemotherapy (platinum (carboplatin or cisplatin) and pemetrexed). The primary end point for both studies is PFS and since crossover is permitted, significant difference between treatment groups on survival is unlikely to be prospectively meaningful. Importantly, in a nonrandomized comparison of survival in ALK-positive patients given crizotinib in the second- or third-line setting survival was significantly longer than ALK-negative patients given any second-line treatment (2-year overall survival 55% vs 12%, hazard ratio 0.36, p = 0.004) [40, indicating...
### Table 1. Studies showing the prevalence of ALK rearrangements in NSCLC.

<table>
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<tr>
<th>Author</th>
<th>Total (n)</th>
<th>Histology</th>
<th>Population</th>
<th>Positive (n)</th>
<th>Total (%)</th>
<th>Adenocarcinoma (%)</th>
<th>Test</th>
<th>Median age (years)</th>
<th>Non/light smokers (%)</th>
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<tr>
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<td>75</td>
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<td>NM</td>
<td>Western US/ Swita</td>
<td>16</td>
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<td>305</td>
<td>200 A 88 S 9 AS</td>
<td>US/ Korean</td>
<td>8 A 3.5</td>
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<td>Inamura et al.</td>
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<td>PCR</td>
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<td>109</td>
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<td>Pal et al.</td>
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<td>Salido et al.</td>
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<td>Western US/ European</td>
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<td>Sanders et al.</td>
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<td>Yang et al.</td>
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<td>Wu et al.</td>
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<td>Asian/ Taiwan</td>
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<td>PCR/ FISH</td>
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1. Light smokers as defined by less than 10 pack years smoking history.
2. Selected population.
3. All selected patients were non-smokers.

A: Adenocarcinoma; AS: Adenosquamous histology; FISH: Fluorescent in situ hybridization; IHC: Immunohistochemistry; L: Large-cell carcinoma; NM: Not mentioned; O: Other histology; S: Squamous cell carcinoma; SC: Small-cell carcinoma.
A likely positive impact of crizotinib on the natural history of ALK-positive NSCLC.

**Safety data & major toxicities**

Crizotinib is generally well tolerated with the majority of adverse events reported in the Phase I or II study being either grade 1 or 2 toxicities [55]. The most frequently observed adverse events across the two studies were visual disturbances (59–62%), nausea (49–57%), vomiting (35–43%), diarrhea (42–43%) and edema (26–29%). Nausea, which is usually grade 1 or 2 and may be accompanied by vomiting, can be ameliorated by taking tablets with food. The visual disturbances are typically associated with dark-to-light transitions, are transient (lasting a few seconds or minutes) and have typically been associated with dark-to-light transitions, are transient (lasting a few seconds or minutes) and have been described by patients as either shimmering, flashing or trailing lights (64–75%). Streamers, strings or floaters (56–75%) Overlapping shadows or after-images (51–69%).

They have not been associated with abnormal findings on ophthalmologic examinations [56]. Interestingly, peripheral edema has been reported with MET inhibitors suggesting that this side effect may be related to the MET inhibitory effects of crizotinib. The major serious adverse events noted were uncommon grade 3 or 4 transaminase elevation, with an instance of fatal hepatotoxicity and rare instances of pneumonitis.

**Testing for ALK gene rearrangements**

Given the low frequency of ALK rearrangements in NSCLC a reliable, robust and cost-effective diagnostic assay in clinical specimens is essential to identify patients for appropriate therapy. Three methods that have been used to identify ALK rearrangements in biopsy specimens: FISH with a break-apart probe, reverse transcriptase-PCR (RT-PCR) or immunohistochimistry (IHC), each assay having particular advantages and limitations [43].

FISH using a dual color, break-apart probe (Vysis, Abbott Molecular, IL, USA) is the current gold standard and the only FDA approved test for the identification of ALK gene rearrangement. FISH performed in local laboratories was used as the method to identify patients for the Phase I study with crizotinib and was developed as a companion diagnostic to define eligibility for the Phase II and III studies with crizotinib. The test can be performed on formalin-fixed paraffin-embedded tissue and is positive if >15% of 50 tumor cells have either split red and green signals or alternatively a single (3’) red signal (Figure 1B). An advantage of FISH is that it enables identification of ALK rearrangements irrespective of variants or fusion partner. Limitations of FISH however include the requirement for expertise in interpreting the signal leading to the potential for false negatives due to the presence of novel fusions not covered by the primer repertoire used and for false positives due to contamination. It is also not widely performed in many clinical laboratories.

An emerging alternative that is potentially a more widely accessible and cost-effective screening methodology is IHC for ALK protein. IHC has shown high sensitivity and specificity for detection of aberrant expression of the NPM–ALK fusion protein in ALCL utilizing the ALK1 antibody (Dako; CA, USA) [44]. As ALK protein is not expressed in normal lung tissue [48], detection in NSCLC specimens should potentially give highly specific results. However, initial IHC studies could not reliably detect EML4–ALK fusions by using the ALK1 antibody presumably due to weak transcriptional activity of 5’ promoter region of EML4 leading to reduced protein levels in comparison with

**Figure 2. Waterfall plot from the Phase I study of crizotinib, showing percent change in tumor burden in 79 previously heavily treated, non-small-cell lung cancer patients.**

One complete and 46 partial responses were seen, giving an overall response rate of 57% (95% CI: 46–68%).

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**Figure 3. Response of an ALK-positive non-small-cell lung cancer patient to ALK inhibition. (A) Baseline CT scan and (B) CT scan 2 months after treatment with oral crizotinib (250-mg twice-daily).**

**Figure 4. Phase III trials of crizotinib.** Second-line (NCT0093893) trial has recently completed accrual and is closed, while estimated completion date for first-line study (NCT01154140) is 31 December 2013. Crossover after progression is allowed in both studies. BID: Twice-daily; NSCLC: Non-small-cell lung cancers.
NP-M–ALK in ALCL [50]. More promising results have been obtained in optimising detection methods leading to the use of ALK antibody (clone S4A; Novocastra, UK, Figure 1A) [31,44,45,67,68]. Recently, a novel and highly sensitive antibody (clone DFS1, Cell Signaling Technology, USA) has also been reported that is under further clinical evaluation [51].

A strategy of initial testing with IHC followed by FISH has been evaluated by some investigators using the ALK5 antibody [48] and with another ALK antibody (clone D5F3; Cell Signaling Technology, USA) [101]. These studies utilized a scoring system based on the intensity of ALK staining with each specimen given a score of 0 to 1, 2, or 3+. In general all ALK IHC negative cases, that is, 0+ staining were consistently negative by FISH and all strongly positive, that is, 3+ cases were ALK FISH positive but variable results were seen in IHC 1 and 2+. The discordance between mild-to-moderate positivity on IHC (1 and 2+) cases and FISH results led to the proposal of a two-tier testing, analogous to Her2 testing in breast cancer, with initial IHC and then FISH for all 2+ [41] or 1 and 2+ specimens [41,43].

Resistance to crizotinib: the next challenge

Although primary resistance to crizotinib is rare, acquired resistance to this drug invariably develops after a median PFS of 10 months – a phenomenon termed ‘acquired resistance’. Analogous to acquired resistance to EGFR TKIs where resistance occurs due to secondary mutations in the EGFR (e.g., T790M) or less commonly through amplification of c-MET, acquired resistance to crizotinib occurs through multiple mechanisms including genetic alterations in ALK itself that directly render the ALK tyrosine kinase resistant to inhibition by crizotinib or through the activation of alternative oncogenic kinases that bypass ALK to restore signalling downstream of ALK.

Modelling of resistance in cell lines in vitro has identified mutations in the ALK tyrosine kinase domain, amplification of ALK, and activation of EGRF as potential mechanisms of resistance [327–329]. Studies in patients undergoing biopsies after progression with crizotinib showed that ALK-positive tumors with various ALK kinase domain occurring in 22–36% of cases evaluated thus far [73–75] the most frequent being the L1196M mutation (a substitution of leucine for methionine at residue 1196 within the ALK kinase domain) [75,76]. This mutation occurs in a so-called gatekeeper residue and results in the substitution of a bulky methionine for a leucine residue within the ALK kinase domain. It is thought to interfere with the affinity of ALK for crizotinib, analogous to the T315I mutation in BCR-ABL. Chronically myelogenous leukemia in the context of acquired resistance to imatinib or nilotinib in chronic myeloid leukemia. In some patients the mechanism of resistance remains unidentified [75,76].

Several ‘second generation’ ALK inhibitors and Hsp90 inhibitors, which may have activity in crizotinib-resistant ALK-positive NSCLC, have been developed and have entered clinical trials (Table 2). One such compound ‘AP26113’ (ARIAD pharmaceuticals Inc., MA, USA) is a small molecule that has been reported to inhibit both ALK and EGFR. In addition to having potent inhibitory effects on ALK-positive cell lines, AP26113 was active against mutations in ALK that conferred resistance to crizotinib including L1196M [260]. Another compound AFP802 (CH3244208 Chugai) is a benz[b]carbazole derivative and a potent and selective inhibitor of wild-type ALK and also mutated ALK (L1196M and C1156Y) is also under evaluation in Phase I/II clinical trials [77]. Since ALK fusion proteins serve as client proteins for the chaperone protein Hsp90, the use of Hsp90 inhibitors in the Phase I study with crizotinib and was developed as a companion diagnostic for the Phase II and III, studies it represents the gold standard and is the only FDA-approved testing methodology for this purpose. However the relatively high cost [84] and need for expertise in interpretation of the assay provides limitations in widespread implementation. IHC, with antibodies specific for ALK used to identify patients with ALK rearrangements as an alternative methodology that has shown promise in many retrospective studies, with good concordance with FISH in strongly positive or negative cases. Intermediate levels of staining are more problematic and argue for the development of a two-tier testing strategy in which the method of screening and FISH confirmation of equivocal cases has been used in Her2 testing for breast cancer.

Despite excellent responses to initial therapy with crizotinib, patients invariably developed acquired resistance to crizotinib after a median progression free interval of approximately 10 months. Recently insights into the mechanism of resistance have been provided by studies in cell lines and from patient samples. These studies have indicated that in 20–30% of patients the mechanism of resistance is the L1196 mutation occurring in a so called gate-keeper residue within the tyrosine kinase domain of ALK. Other mechanisms of resistance involve bypass mechanisms such as activation of EGFR signaling or amplification of c-KIT. Concerning, even in a single tumor multiple mechanisms of resistance may coexist. In a significant proportion of patients the mechanism of resistance remains to be determined. Currently second-generation ALK inhibitors with activity against secondary mutations in ALK and Hsp90 inhibitors are being evaluated in this population.

The discovery of a molecularly distinct population of NSCLC harboring ALK gene rearrangements in 2007 and the demonstration of a highly effective treatment strategy in crizotinib led to approval of crizotinib for the treatment of ALK-positive NSCLC in 2011. Testing for ALK gene rearrangements is now mandatory for selection of optimal therapy in NSCLC patients [9]. Targeting of ALK gene rearrangements is an excellent example of the potential for rapid translation of research to clinical practice. Furthermore, studies of EGFR inhibitors in EGFR mutant lung cancer provides impetus for the incorporation of ALK inhibitors as alternative strategies in lung cancer such as FGFR1 amplifications in squamous cell carcinoma, ROS1 gene rearrangements

<table>
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<tr>
<th>Table 2. Novel ALK and Hsp90 inhibitors currently in clinical trials in ALK-positive non-small-cell lung cancer</th>
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<td>LAP3112</td>
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<td>LDK378</td>
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<td>IPI-504 (Hsp90)</td>
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58. Grzecinib in ALK-positive non-small-cell lung cancer


59. EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. Oncogene 30(22), 2581–2586 (2011).


62. Grzecinib in ALK-positive non-small-cell lung cancer


63. EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. Oncogene 30(22), 2581–2586 (2011).


66. Grzecinib in ALK-positive non-small-cell lung cancer


67. EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. Oncogene 30(22), 2581–2586 (2011).


70. Grzecinib in ALK-positive non-small-cell lung cancer


71. EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. Oncogene 30(22), 2581–2586 (2011).


74. Grzecinib in ALK-positive non-small-cell lung cancer


75. EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. Oncogene 30(22), 2581–2586 (2011).


78. Grzecinib in ALK-positive non-small-cell lung cancer


79. EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. Oncogene 30(22), 2581–2586 (2011).


82. Grzecinib in ALK-positive non-small-cell lung cancer


83. EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. Oncogene 30(22), 2581–2586 (2011).
