Anaplastic Lymphoma Kinase (ALK) as a therapeutic target in non-small cell lung cancer

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Abstract

The therapeutic targeting of *anaplastic lymphoma kinase* (*ALK*) has been a burgeoning area of research since 2007 when *ALK* fusions were initially identified in patients with non-small cell lung cancer (NSCLC). The field has rapidly progressed through development of the first-generation ALK inhibitor, crizotinib, to an understanding of mechanisms of acquired resistance to crizotinib, and is currently witnessing an explosion in the development of next-generation ALK inhibitors such as certinib, alectinib, PF-06463922, AP26113, X-396, and TSR-011. As with most targeted therapies, acquired resistance appears to be an inevitable outcome. Current pre-clinical and clinical studies are focused on the development of rational therapeutic strategies, including novel ALK inhibitors as well as rational combination therapies to maximize disease control by delaying or overcoming acquired therapeutic resistance. This review summarizes the existing clinical data and ongoing research pertaining to the clinical application of ALK inhibitors in patients with NSCLC.

Keywords

Anaplastic lymphoma kinase; ALK; non-small cell lung cancer; lung adenocarcinoma; tyrosine kinase inhibitor; TKI; clinical trials; acquired resistance

Introduction

In 1994, the *anaplastic lymphoma kinase* (*ALK*) gene was first discovered as part of the characteristic t(2,5) found in patients with anaplastic large cell lymphoma, which results in a fusion between *nucleophosmin* (*NPM*) and *ALK*[1]. More then a decade later, analogous fusions involving *ALK* and the *echinoderm microtubule associated protein like 4* (*EML4*) were discovered in patients with non-small cell lung cancer (NSCLC)[2]. The *ALK* gene encodes a receptor tyrosine kinase in the insulin receptor family with ill-defined but suspected ligands including midkine, heparin, and pleiotrophin[3–5]. The ALK receptor is

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thought to be activated by ligand-mediated dimerization and transphosphorylation[4]. The expression profile and normal function of ALK in humans is unknown, but in adult mice it is thought to assist in normal functioning of the frontal cortex and hippocampus[6].

Amongst all patients with NSCLC, 3–7% harbor an *ALK* gene rearrangement in their tumor tissue. *ALK* rearrangements are more common in younger patients with little to no smoking history and adenocarcinoma histology[7]. In patients with NSCLC, many different *ALK* gene fusions have been identified, the most common of which is the *EML4-ALK* fusion. All ALK fusion proteins found in patients with NSCLC feature preservation of the ALK tyrosine kinase domain, utilization of a promoter from the N-terminal fusion partner gene, and exploitation of an oligomerization domain in the N-terminal fusion partner to achieve constitutive activation[7] (Figure 1). ALK fusion proteins in lung cancer mediate downstream signaling through several pro-growth and anti-apoptotic pathways in the cell, including the mitogen activated protein kinase (MAPK) pathway, the phosphatidyl-inositol-3-kinase (PI3K)-AKT pathway, and the JAK-STAT pathway. ALK fusion proteins are also known to be clients of the heat shock protein-90 (HSP-90) chaperone which aids in stabilization of the fusion protein[8].

Interestingly, aside from NSCLC, many other different tumor types – in both pediatric and adult malignancies - have been shown to harbor oncogenic *ALK* abnormalities. These include *ALK* amplification and *ALK* point mutations in patients with neuroblastoma, *ALK* point mutations in patients with anaplastic thyroid carcinoma, and *ALK* gene fusions in patients with anaplastic large cell lymphoma, diffuse large B cell lymphoma (DLBCL), inflammatory myofibroblastic tumor (IMT), and others[9]. For the specific details of known *ALK* point mutations and fusions we refer the reader to a recently published review[7].

Initial therapeutic targeting

The therapeutic efficacy of ALK inhibition in patients with *ALK*-rearranged NSCLC was first demonstrated with crizotinib (PF-02341066) in the PROFILE series of clinical trials (Table 1). Crizotinib is an oral inhibitor of the tyrosine kinases ALK, mesenchymal-epithelial transition factor (MET), and c-ros oncogene 1 (ROS1)[7]. In a phase I clinical trial, crizotinib demonstrated a 60.8% objective response rate (ORR), a median progression free survival (PFS) of 9.7 months, and overall survival (OS) rates of 87.9% at six months and 74.8% at one year in patients with advanced *ALK*-rearranged NSCLC[10]. In this clinical trial, *ALK* rearrangements were identified with fluorescence *in-situ* hybridization (FISH), and the frequencies of specific *ALK* fusion gene types were not reported[10]. In a retrospective analysis using this phase I data comparing OS between 30 patients with *ALK*-rearranged NSCLC treated with crizotinib in the second or third line setting to 23 patients with *ALK*-rearranged NSCLC treated with standard, non-ALK inhibitor therapy in the second line setting, patients who received crizotinib had a significantly longer two-year OS, 55% vs 12% (p=0.004)[11]. The phase II clinical trial assessing crizotinib in patients with *ALK*-rearranged NSCLC who had undergone at least one prior line of therapy showed a similar ORR of 60% and a PFS of 8.1 months[12].

In a subsequent phase III, open label clinical trial involving 347 patients with locally advanced or metastatic *ALK*-rearranged NSCLC who had received one line of prior therapy with a platinum-based regimen, patients treated with crizotinib compared to standard cytotoxic chemotherapy (pemetrexed or docetaxel) had significantly better ORR and PFS, but no demonstrable OS benefit. In this clinical trial, the ORR was 65% in the crizotinib arm compared to 20% in the control arm (p<0.001), median PFS was 7.7 months in the crizotinib arm compared to 3.0 months in the control arm (p<0.001), and median OS was 20.3 months in the crizotinib arm compared to 22.8 months in the control arm (p=0.54). Importantly, the OS data was calculated on an intention-to-treat basis, and 64% of patients randomized to conventional chemotherapy in the second line setting went on to receive crizotinib. The frequencies of specific *ALK* fusion gene types were not reported in this clinical trial[13]. Similar results were obtained when crizotinib was compared to conventional cytotoxic chemotherapy with pemetrexed and either cisplatin or carboplatin in a phase III clinical trial involving 343 patients with treatment-naïve, *ALK*-rearranged, advanced NSCLC. In this cohort, the ORR was 74% in patients treated with crizotinib compared to 45% in patients treated with cytotoxic chemotherapy (p<0.001), and the median PFS was 10.9 months in patients treated with crizotinib compared to 7.0 months in patients treated with cytotoxic chemotherapy (p<0.001). One-year OS was not statistically different, as it was 84% in patients receiving crizotinib and 79% in patients receiving cytotoxic chemotherapy. Similar to the aforementioned second line phase III clinical trial, this first line therapy trial was assessed on an intention-to-treat basis, 70% of patients randomized to first-line cytotoxic chemotherapy eventually received crizotinib, and the frequencies of specific *ALK* fusion gene types were not reported[14].

The time from the initial discovery of *ALK* alterations in patients with NSCLC to Food and Drug Administration (FDA) approval of crizotinib for the treatment of patients with *ALK*-rearranged NSCLC was four years. The impressive example of *ALK*-rearranged NSCLC serves as a paradigm for accelerated approval of molecularly targeted therapies in cancer care.

Mechanisms of acquired resistance

As occurs among patients with *EGFR*-mutant NSCLC treated with EGFR tyrosine kinase inhibitors (TKI's), patients with *ALK*-rearranged NSCLC have been shown to acquire resistance to ALK inhibition with crizotinib by several mechanisms, broadly classified as target modification and 'bypass' signaling (Figure 2). Target modifications include *ALK* gene amplification, present in approximately 9% of tumors resistant to crizotinib[15], as well as point mutations and insertion mutations in the tyrosine kinase domain of the EML4-ALK fusion protein[7,16], which have been observed in approximately one third of patients with crizotinib-resistant tumors[15]. Unlike EGFR T790M 'gatekeeper' mutation, which is the predominant point mutation detected in approximately 50–60% of patients with acquired resistance to first- and second-generation EGFR TKIs, crizotinib resistance mutations appear to span the ALK kinase domain. The analogous gatekeeper mutation, L1196M, has been detected. Other mutations include L1152R, C1156Y, I1171T/N/S, F1174L/C/V, V1180L, G1202R, S1206Y, and G1269A. The clinical frequency of the various ALK kinase domain mutations which confer crizotinib resistance is actively being studied. In addition to

modification of the ALK target, 'bypass' pathway signaling has also been shown to mediate crizotinib resistance in several pre-clinical and clinical studies. This 'bypass' signaling serves to provide an escape mechanism for the tumors to functionally circumvent the inhibited ALK fusion protein and signal to pro-growth downstream pathways through redundant pathways. For example, up-regulation of phosphorylated EGFR was detected in approximately 44% of patients at the time of crizotinib resistance[17]. Up-regulation of insulin-like growth factor receptor 1R (IGF-1R)[18] and Src[19] have also been detected at the time of crizotinib resistance. Finally, genomic amplification of *cKIT* has been detected in patients at the time of crizotinib resistance[17].

A second concern related to a potential therapeutic ceiling with crizotinib treatment for *ALK*-rearranged NSCLC is its central nervous system (CNS) penetration. While it has been noted that crizotinib's cerebrospinal fluid (CSF) concentration is low compared to plasma (0.26% that of the plasma crizotinib concentration)[20], the brain parenchymal concentrations of crizotinib are unknown. It has also been observed that in patients with *ALK*-rearranged NSCLC treated with crizotinib intracranial metastases are a common site of disease progression. However, it is not clear whether this represents primary or acquired resistance to crizotinib because the mechanisms underlying CNS progression in patients treated with crizotinib are unknown[21]. Targeting the CNS as a sanctuary site in patients with *ALK*-rearranged NSCLC is a key aspect of therapy with second-generation ALK inhibitors, and comparisons of the efficacy of these newer agents with crizotinib for CNS disease are ongoing.

Therapeutic strategies to overcome acquired resistance

Multiple second-generation ALK inhibitors are currently in clinical trials, both in the setting of patients with acquired resistance to crizotinib and as first-line therapies.

Ceritinib (LDK378) is a second-generation oral ALK inhibitor, which has shown efficacy when used to treat patients with locally advanced or metastatic *ALK*-rearranged NSCLC, including those whose tumors have acquired resistance to crizotinib. In a phase I clinical trial, among 114 patients with *ALK*-rearranged NSCLC who received at least 400 milligrams (mg) of ceritinib daily, the ORR was 58% and the PFS was 7.0 months. Among 86 patients who had previously been treated with crizotinib, the ORR remained 56%, indicating that ceritinib therapy was able to overcome acquired resistance to crizotinib in a substantial proportion of patients. Specifically, among seven patients in whom crizotinib resistance via *ALK* point mutations in the tyrosine kinase domain or *ALK* gene amplification were identified before treatment with ceritinib, six (86%) experienced an objective response[22]. In preliminary results from two larger clinical trials (ASCEND 2 and ASCEND 3; NCT01685060 and NCT01685138, respectively), among 140 patients previously treated with platinum based chemotherapy who had progressed through crizotinib, the ORR was 54% and the PFS was 5.7 months[23]. In 124 patients with *ALK*-rearranged NSCLC who were ALK inhibitor naïve, the ORR was 79% and the PFS was 11.1 months[24].

Alectinib (CH5424802/RO5424802) is a second-generation oral ALK inhibitor that has also shown efficacy in patients with *ALK*-rearranged NSCLC. In a phase I clinical trial involving 44 patients with *ALK*-rearranged NSCLC who developed resistance to crizotinib or were intolerant of crizotinib, the ORR was 55%[25]. In a phase I/II clinical trial of alectinib in 46 patients with *ALK*-rearranged, advanced NSCLC with no prior ALK inhibitor therapy, the ORR was 93.5% and the PFS at the most recent update at the American Society of Clinical Oncology (ASCO) meeting in 2015 is expected to be greater than 29 months[25,26]. Furthermore, there has been suggestion that alectinib may have improved therapeutic efficacy for CNS metastases compared to crizotinib and ceritinib, as it achieves notably higher CSF levels and has induced CNS disease responses in tumors resistant to crizotinib and ceritinib[27–29]. There were also two phase II clinical trials presented at ASCO 2015, showing an ORR of 48–49% in patients with *ALK*-rearranged locally advanced or metastatic NSCLC who had progressed through crizotinib (NCT01801111 and NP28761)[30–31].

PF-06463922 is an oral inhibitor of ALK and ROS1 that has been shown to have antitumor effects in both ALK-rearranged and ROS1-rearranged pre-clinical models[32]. PF-06463922 is currently in a phase I/II clinical trial, preliminary results of which were presented at ASCO 2015. Among 18 patients with *ALK* or *ROS1*-rearranged advanced NSCLC the ORR was 40%, and five of the six patients with documented responses to PF-06463922 had progressed through prior ALK TKI therapy (NCT01970865)[33].

Brigatinib (AP26113) is an oral ALK inhibitor currently in a phase I/II clinical trial. At the most recent interval reporting of this trial at ASCO 2015, 79 patients with advanced, *ALK*-rearranged NSCLC had been treated with an ORR of 72% and median PFS of 14 months (NCT01449461)[34].

X-396 is an oral ALK inhibitor also currently in a phase I/II clinical trial. At the most recent interval reporting of this trial in 2014, among 17 patients with *ALK*-rearranged NSCLC the ORR was 59%, and the ORR among the 13 patients previously treated with crizotinib was 54% (NCT01625234)[35].

TSR-011 is an oral ALK and tropomyosin receptor kinase (TRK) inhibitor that is being evaluated in patients with *ALK*-rearranged tumors, including NSCLC, in a phase I trial. The first data was reported at ASCO 2015, and at an optimal dose regimen of 120mg per day, three of three patients achieved an objective response (NCT02048488)[36].

Resistance to second-generation agents

Resistance to second-generation ALK inhibitors is an active area of investigation. Mechanisms of resistance to crizotinib and second-generation ALK inhibitors show overlap as well as novel mechanisms outlined in Figure 2. Similar to crizotinib resistance mechanisms but with less well-defined frequencies, resistance to second-generation ALK inhibitors has been observed to occur through *ALK* point mutations and alternative signaling pathways including EGFR, Src, MAP2K1, MEK, and MET activation[37–38].

Sequential ALK inhibition and combination strategies

One of the most pressing questions facing the application of ALK inhibitors in patients with *ALK*-rearranged NSCLC is the optimal sequential or combination therapy regimen to utilize in order to maximize disease control and survival. In an early assessment of sequential ALK inhibitor therapy, a retrospective analysis has shown that among patients treated sequentially with crizotinib followed by ceritinib after progression on crizotinib the median combined PFS is 17.4 months and OS is 49.4 months[29]. In this analysis, there was no difference in PFS with certinib therapy based on the presence or absence of a known crizotinib-resistance-mediating *ALK* mutation[29]. Prospective trials evaluating the sequence of investigational ALK TKIs versus crizotinib are being planned.

To maximize disease control and patient survival, heterogeneous resistance mechanisms, such as the simultaneous existence of ALK point mutations and alternative pathway activation, must be addressed. Acknowledging the need to simultaneously inhibit multiple oncogenic checkpoints in the same patient, several combination strategies including vertical pathway inhibition, horizontal pathway inhibition, heat shock protein-90 (HSP-90) inhibition, ALK inhibition combined with immunotherapy, and ALK inhibition combined with cytotoxic chemotherapy have been proposed. Vertical pathway inhibition refers to inhibiting multiple components within the same oncogenic pathway, such as combined MEK and ALK inhibition. Horizontal inhibition, in contrast, refers to inhibiting multiple different oncogenic pathways simultaneously, such as combining ALK inhibition with Src, IGF-1R, EGFR, or cKIT inhibition.

The combination of ALK inhibition with immunotherapy is an active area of investigation, stimulated in part by an analogous finding that *EGFR*-mutant NSCLC utilizes activation of the programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) pathway to evade destruction by a patient's immune system[39]. This treatment strategy is being assessed through a phase I clinical trial of combination ceritinib and the PD-1 inhibitor nivolumab (NCT02393625).

Finally, based on data suggesting that patients with *ALK*-rearranged NSCLC are sensitive to pemetrexed-based cytotoxic chemotherapy[40], there is an ongoing clinical trial comparing the combination of pemetrexed and crizotinib to pemetrexed alone in patients with *ALK*-rearranged NSCLC that has progressed through crizotinib monotherapy (NCT02134912).

Conclusions

ALK fusions in lung cancer serve as the paradigm for the rapid and successful translation of a new biomarker from initial discovery to full clinical integration and widespread use of ALK directed targeted therapies for patients whose tumors harbor this alteration. There are several important questions to pursue moving forward in this field: (1) With the on-going clinical development of several new ALK TKIs, how can we systematically study and define the optimal sequence of ALK directed therapies in this cohort of patients? (2) All of the studies to date have examined the efficacy of ALK TKIs in patients with metastatic disease. Will ALK TKIs be efficacious in the adjuvant setting? This question is being addressed in

the ALCHEMIST-ALK clinical trial which will assess the efficacy of crizotinib vs. placebo in patients with *ALK*-rearranged lung cancer following standard of care adjuvant therapy[41]. (3) Will the specific *ALK* gene fusion dictate clinical responses? At present, *ALK* fusions are detected primarily by FISH, which only detects an *ALK* gene rearrangement and cannot detect the actual fusion variant. However, as next generation sequencing based technologies move to the forefront of clinical diagnostics, the field will be able to assess how the various different *ALK* fusion partners and fusion variants effect responses. (4) Finally, as the field of ALK inhibition in patients with NSCLC advances, there is a pressing need to determine the generalizability of findings in NSCLC to other tumor types where *ALK* point mutations, amplification, and rearrangements have been identified. As an example, crizotinib has shown clinical activity in patients with *ALK*-rearranged anaplastic large cell lymphoma and inflammatory myofibroblastic tumors (IMT), and in a minority of patients with neuroblastoma[42–43]. To address this question, one arm of the NCI Molecular Analysis for Therapy Choice Program (NCI-MATCH) is testing crizotinib in patients with *ALK*-rearranged malignancies besides NSCLC[7].

Acknowledgments

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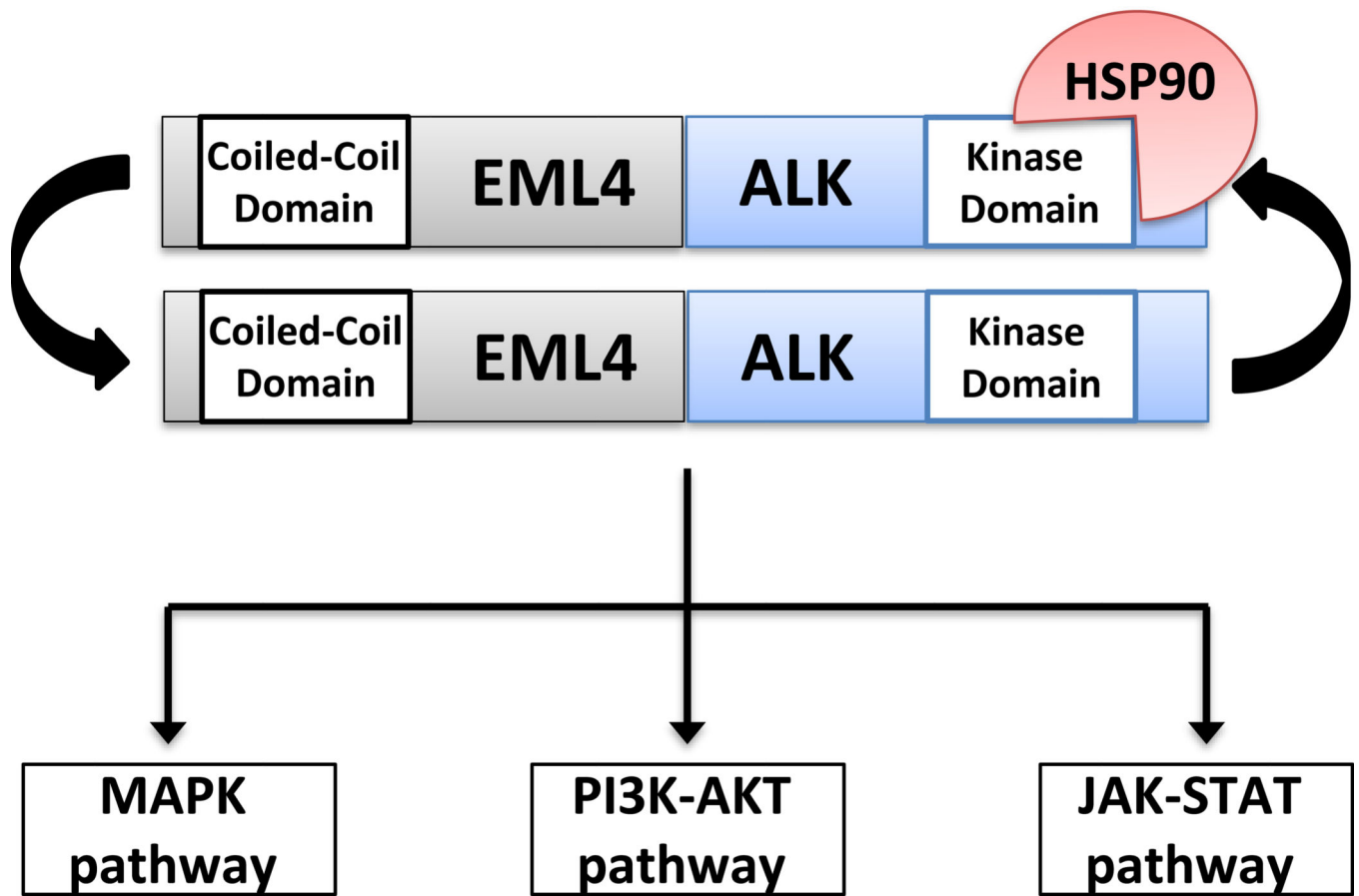
Figure 1. Schematic representation of the EML4-ALK fusion protein

The *EML4-ALK* fusion is formed via an abnormal rearrangement on chromosome 2 which juxtaposes part of the N-terminus of *EML4* to a portion of the C-terminus of *ALK*. The N-terminus of EML4 contains a coiled-coiled domain, which mediates EML4-ALK dimerization. Dimerization has been shown to be critical for EML4-ALK activity (Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007:561–6). The ALK portion of the fusion contains the entire ALK tyrosine kinase domain. EML4-ALK is the most common ALK fusion described in lung cancer to date, however, other ALK fusions partners have also been described. EML4-ALK fusion proteins are clients of the heat shock protein-90 (HSP-90) chaperone, and they signal downstream through the mitogen activated protein kinase (MAPK) pathway, the phosphatidyl-inositol-3-kinase (PI3K)-AKT pathway, and the JAK-STAT pathway to mediate pleiotropic pro-growth and pro-survival effects.
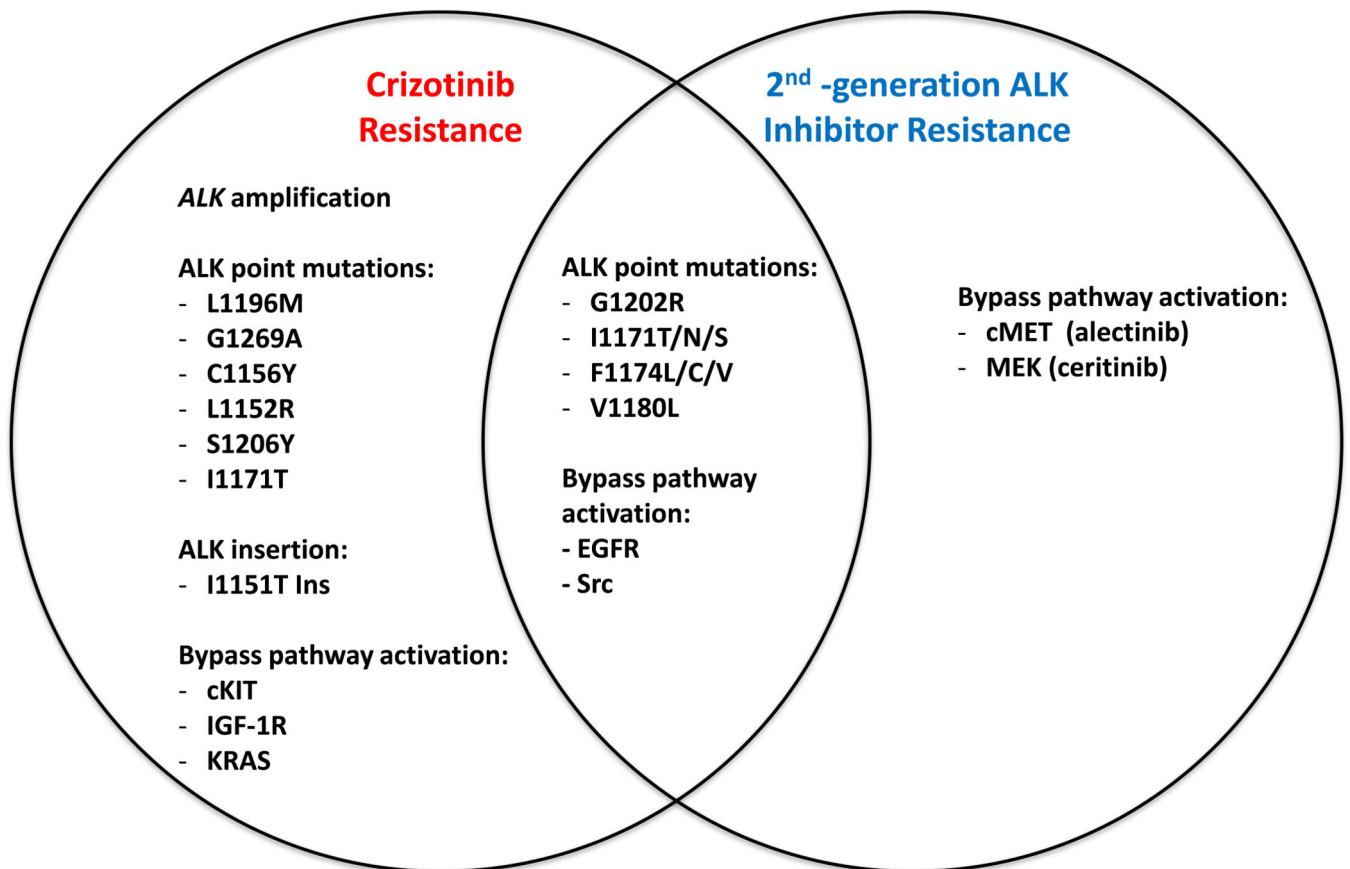
Figure 2. Mechanisms of resistance to crizotinib and second-generation ALK inhibitors both share common strategies and exploit novel routes

Specific ALK tyrosine kinase point mutations have been identified as imparting only resistance to crizotinib (L1196M) or enabling resistance to both crizotinib and second generation ALK inhibitors (G1202R). Similarly, specific bypass signaling pathways have been identified in conjunction with resistance to crizotinib (insulin-like growth factor 1 receptor, IGF-1R), resistance to second generation ALK inhibitors (mesenchymal-epithelial transition factor, MET), or resistance to both crizotinib and second generation ALK inhibitors (epidermal growth factor receptor, EGFR).

Toyokawa G, Seto T, Takenoyama M, et al. Crizotinib can overcome acquired resistance to CH5424802: is amplification of the MET gene a key factor? J Thorac Oncol 2014; 9: e27–8. Crystal AS, Shaw AT, Sequist LV, et al. Patient-derived models of acquired resistance can identify effective drug combinations for cancer. Science 2014; 346: 1480–6.

Table 1

Published clinical trials of ALK inhibitors for patients with *ALK*-rearranged NSCLC

ALK Inhibitor	Reference	N	Phase	Objective Response Rate (%)	Progression Free Survival (months)	Distinguishing Toxicity (frequency, all grades)
Crizotinib	Camidge 2012	143	I	60.8	9.7	Gastrointestinal (40–60%)
Crizotinib	Kim 2012	261	II	60	8.1	Gastrointestinal (35–50%)
Crizotinib	Shaw 2013	347	III	65	7.7	Gastrointestinal (45–60%)
Crizotinib	Solomon 2014	343	III	74	10.9	Gastrointestinal (45–60%)
Ceritinib	Shaw 2014	114	I	58	7.0	Gastrointestinal (65–80%)
Ceritinib	Mok 2015	140	II	54	5.7	Gastrointestinal (60–80%)
Certinib	Felip 2015	124	II	79	11.1	Gastrointestinal (65–80%)
Alectinib	Gadgeel 2014	44	I	55	N/A	Myalgia (17%)
Alectinib	Seto 2013 and Ohe 2015	46	I/II	93.5	>29	Myalgia (13%)
Alectinib	Gandhi 2015	87	II	47.8	N/A	Myopathy (8% grade 3–5)
Alectinib	Ou 2015	138	II	49.2	N/A	Pulmonary (2–4% grade 3–5)
Brigatinib	Camidge 2015	79	I/II	72%	14	Gastrointestinal (35–45%)
X-396	Horn 2014	17	I	59%	N/A	Rash (36%)
TSR-011	Arkenau 2015	46	I/II	100%	N/A	QTc prolongation (22%)