review

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Crizotinib resistance: implications for therapeutic strategies

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In 2007, a chromosomal rearrangement resulting in a gene fusion leading to expression of a constitutively active anaplastic lymphoma kinase (ALK) fusion protein was identified as an oncogenic driver in non-small-cell lung cancer (NSCLC). ALK rearrangements are detected in 3%–7% of patients with NSCLC and are particularly enriched in younger patients with adenocarcinoma and a never or light smoking history. Fortuitously, crizotinib, a small molecule tyrosine kinase inhibitor initially developed to target cMET, was able to be repurposed for ALK-rearranged (ALK+) NSCLC. Despite dramatic and durable initial responses to crizotinib; however, the vast majority of patients will develop resistance within a few years. Diverse molecular mechanisms underlie resistance to crizotinib. This review will describe the clinical activity of crizotinib, review identified mechanisms of crizotinib resistance, and end with a survey of emerging therapeutic strategies aimed at overcoming crizotinib resistance.

introduction

Over the last decade, advances in molecular genetics have transformed our understanding of the pathogenesis of non-small-cell lung cancer (NSCLC). The discovery of a correlation between activating mutations in the epidermal growth factor receptor (*EGFR*) gene and responsiveness to EGFR-tyrosine kinase inhibitors (TKIs) not only led to establishment of these drugs as standard therapy for this molecular subgroup of patients but also marked the advent of genotype-directed therapies in NSCLC [1, 2]. This discovery subsequently fueled efforts to identify additional oncogenic drivers in NSCLC. In 2007, Soda and colleagues identified an inversion of chromosome 2 resulting in a fusion gene juxtaposing the 5′ end of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene with the 3′ end of the anaplastic lymphoma kinase (*ALK*) gene. This fusion led to expression of a constitutively active novel ALK fusion protein with transforming activity [3].

It is now estimated that 3%–7% of patients with NSCLC will have an ALK rearrangement [4, 5]. The incidence of ALK rearrangements is increased in the subgroup of young patients with adenocarcinoma and a never or light smoking history [6, 7]. Simultaneous with the identification of patients with ALK-rearranged (ALK+) NSCLC, crizotinib, a new small molecule TKI with preclinical activity against ALK, had already entered the clinic and was being studied in a phase I trial, primarily designed for patients with aberrant activation of cMET. Early responses among patients with ALK+ NSCLC enrolled in this trial established ALK as a clinically validated molecular target.

In the years that have elapsed since the adoption of genotype-directed therapies in NSCLC, much has been learned about response and resistance to targeted therapeutics. Experiences with crizotinib resistance have been instrumental in illustrating the complex and dynamic nature of TKI resistance. Improved understanding of the molecular mechanisms underlying resistance has facilitated the development of next-generation TKIs. This review will describe the clinical activity of crizotinib, evaluate the known mechanisms of crizotinib resistance, and highlight established and emerging therapeutic strategies aimed at overcoming crizotinib resistance.

clinical activity of crizotinib

Crizotinib (PF-02341066) is a first-in-class, oral small molecule ATP competitive inhibitor initially developed as a cMET kinase inhibitor which was subsequently found to inhibit ALK and ROS1 kinases during biochemical characterization. This led to the enrollment of patients with ALK+ NSCLC in the original phase I trial investigating crizotinib in patients with various types of advanced cancer (PROFILE 1001). Due to promising clinical activity in two patients with ALK+ NSCLC who enrolled during the dose escalation phase, an expansion cohort was created. Based on an objective response rate (ORR) of 61% in the first 119 assessable patients in this trial, along with an ORR of 50% from the first 136 patients treated with crizotinib in the phase II trial (PROFILE 1005), crizotinib was granted accelerated approval by the FDA on 26 August 2011 [8–11].

Two phase III studies of crizotinib have been conducted. In the phase III trial of crizotinib versus single-agent chemotherapy in the second-line setting (PROFILE 1007), crizotinib significantly improved progression-free survival (PFS) from 3.0 to 7.7 months (hazard ratio [HR] 0.49, $P < 0.001$). ORR was also significantly higher with crizotinib at 65%, compared with 20% with chemotherapy [12]. In the phase III trial comparing upfront crizotinib

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to platinum-based combination chemotherapy (PROFILE 1014), crizotinib significantly improved PFS from 7.0 to 10.9 months (HR 0.45, $P < 0.001$). ORR with crizotinib was 74%, while ORR with chemotherapy was 45% [13]. In both phase III studies, crizotinib was well tolerated and was associated with a significantly greater improvement in quality of life compared with chemotherapy. Based on the positive data from PROFILE 1007, crizotinib was granted full approval by the FDA on 20 November 2013. Crizotinib was initially approved by the EMA as a second-line therapy before recent approval for use in the first-line setting on 24 November 2015. Crizotinib is also approved in many other countries for the treatment of patients with advanced, ALK+ NSCLC.

clinical relapses on crizotinib

Patients with ALK+ NSCLC most often present with advanced disease involving multiple sites, particularly lymph nodes, pleural and pericardial surfaces, the brain, and liver [14]. Despite dramatic and typically durable responses, the vast majority of patients treated with crizotinib will develop disease progression. Most relapses occur within the first year of treatment, although prolonged responses lasting over 6 years can rarely be seen. For the majority of patients, disease progression after treatment with crizotinib will similarly involve multiple sites [10]. In a smaller proportion of patients, oligoprogression, or progression limited to a few metastatic sites, has been described. The following sections will review two patterns of progression that have emerged with increased experience with treating patients with crizotinib (Figure 1), and briefly discuss some early strategies that have been successful in addressing these unique patterns of treatment failure.

central nervous system only relapses

Brain metastases are commonly present at diagnosis of ALK+ NSCLC and at the time of disease progression on crizotinib. In fact, brain metastases were present at baseline in 26% of patients enrolled on PROFILE 1014 [13]. Similarly, in one single-institution study, brain metastases were present in 23.8% and 58.4% of patients at the time of diagnosis and at 3 years despite treatment with crizotinib [15]. In patients with treated brain metastases enrolled on PROFILE 1014, there was a significant improvement in the intracranial disease control rate (DCR) and intracranial PFS in those treated with crizotinib compared with those treated with chemotherapy [16]. Unfortunately, despite significantly improved disease control with crizotinib compared with chemotherapy, central nervous system (CNS) progression is frequently observed [17, 18]. In a retrospective pooled analysis from the PROFILE 1005 and 1007 trials, median time to intracranial progression among patients with asymptomatic untreated brain metastases was 7 months compared with a 12.5-month median time to systemic progression [19]. In this pooled analysis, in patients with known brain metastases, the CNS was a site of new lesions or progression of non-target lesions in 70% of patients while on treatment with

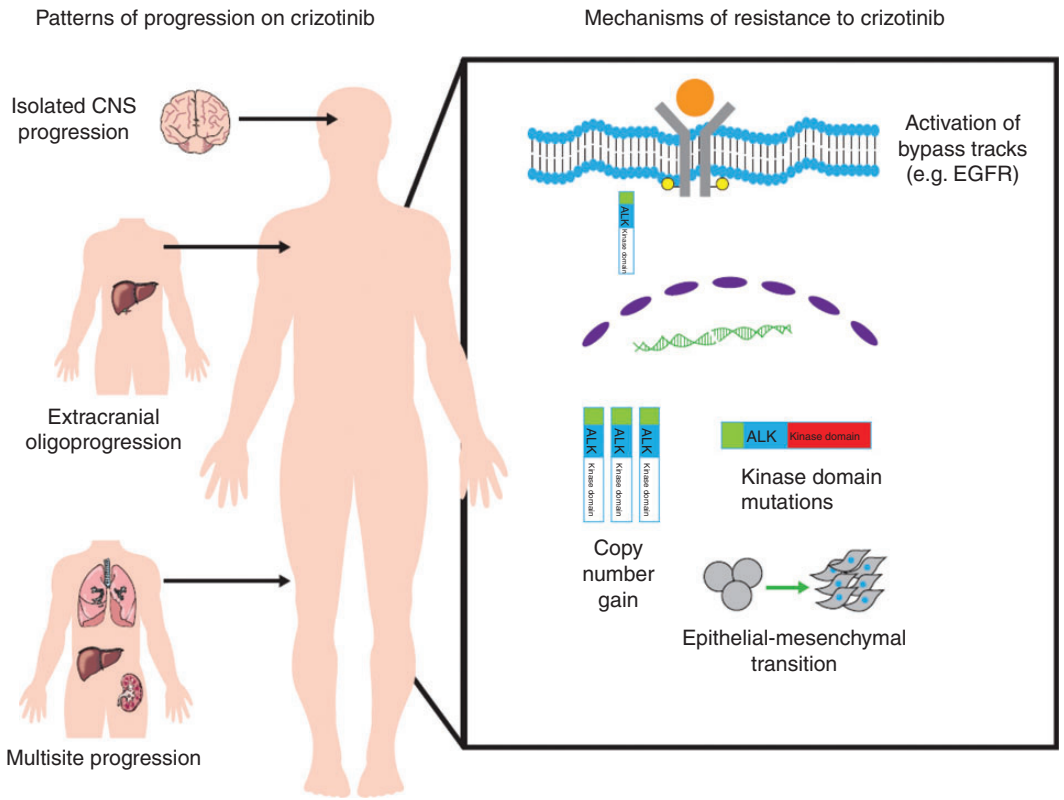

Figure 1. Diverse mechanisms of resistance leading to systemic relapse can emerge in the setting of selective pressure exerted by crizotinib. Identified mechanisms of resistance are depicted on the right. Different patterns are seen during progression on crizotinib (depicted on the left). Progression typically involves multiple sites. Patients with ALK+ non-small-cell lung cancer who are treated with crizotinib are prone to central nervous system relapse, particularly isolated central nervous system relapse. A subgroup of patients will have oligoprogression, or relapse involving only limited sites.

crizotinib. Notably, 20% of those without brain metastases at study enrollment developed brain metastases on crizotinib.

The predisposition toward CNS relapse as an initial site of failure has been largely attributed to pharmacokinetic shortcomings of crizotinib. In particular, crizotinib is a known substrate of P-glycoprotein, a drug efflux pump that limits accumulation of the drug in the CNS [20, 21]. In several studies, resuming crizotinib after local ablative therapies for brain metastases has been shown to be a feasible and effective strategy for ongoing extracranial disease control [22]. In the phase I PROFILE 1001 trial, of the 10 patients who continued crizotinib beyond CNS progression, the duration of treatment after progression ranged from 82 to >591 days [10]. Similarly, the median duration of treatment with crizotinib after progression for the 34 patients with CNS progression treated on PROFILE 1005 and 1007 was 19.3 weeks, with a range from 3.1 to 63.6 weeks. Of note, 27 of the 34 patients received local CNS therapies after progression before resuming treatment with crizotinib [19].

oligoprogression at extracranial sites

Although most patients who develop disease progression after treatment with crizotinib will progress at multiple sites, some patients will have discordant progression limited to a few sites. In these situations, brief interruption of crizotinib for local ablative therapy has been rationalised as an approach to eradicate emerging resistant clones before dissemination and to capitalise on continued ALK dependence at responding sites. Experience with this strategy has been limited to small, single-institution studies. While the rationale for this approach is compelling, randomised trials are indicated to determine whether such maneuvers truly impact the biological course of the disease.

In a single-institution study which included 14 patients with ALK+ NSCLC who had intracranial or extracranial oligoprogression on crizotinib, interruption of crizotinib for local ablative therapy to extracranial sites followed by continuation of crizotinib beyond progression was associated with an additional 7.0 months of extracranial PFS [23]. A follow-up study from the same group looking exclusively at 14 patients who developed extracranial-only oligoprogression on crizotinib treated with repeat local ablative therapies reported a 6- and 12-month actuarial local lesional control rate of 100% and 86% following local ablative therapies, respectively [24]. In those considered suitable for repeat local ablative therapy at progression, crizotinib was continued more than a year on average beyond initial diagnosis of extracranial progression. Experiences with treatment of oligoprogressive ALK+ NSCLC not only highlight the heterogeneity of the disease but also lend support for a multidisciplinary management approach in select patients.

mechanisms of resistance to crizotinib

The vast majority of patients with ALK+ NSCLC will initially respond to first-line treatment with crizotinib. In fact, in PROFILE 1014, the phase III trial comparing upfront crizotinib to chemotherapy, the DCR with crizotinib was 91% [13]. The mechanisms underlying innate or *de novo* lack of response to therapy, often referred to as intrinsic resistance, are not well understood. In those with initial response, the median duration of response to upfront treatment is 11.3 months [13]. This review will focus on mechanisms underlying acquired resistance (Figure 1) or progression that occurs after initial disease stabilization or shrinkage with treatment.

secondary resistance mutations in ALK

Initial insight into mechanisms of resistance to crizotinib was provided when Choi and colleagues identified two independently acquired ALK kinase domain mutations, L1196M and C1156Y, through deep sequencing of pre-treatment and post-progression biopsies in a patient who developed resistance after 5 months of crizotinib [25]. Structural modeling and comparison with other receptor tyrosine kinases revealed that the L1196M mutation most likely corresponds to a gatekeeper residue, or a residue located in the ATP-binding pocket of a protein kinase that when mutated causes a change in the structure of the kinase that prevents TKI binding. Since the initial report, at least eight additional secondary resistance mutations involving the kinase domain have been identified from efforts using patient samples at progression, patient-derived cell lines, and ALK cell lines made resistant *in vitro* (Figure 2). These include G1269A, F1174, 1151Tins, L1152R, S1206Y, I1171T, G1202R, and D1203N [26–29].

In the two largest published case series of patients with available post-progression biopsy samples amenable to molecular characterization, secondary resistance mutations were reported to occur in between 22% and 36% of patients [26, 29]. In the series by Doebele and colleagues, 11 patients underwent repeat biopsy at disease progression. Four patients were noted to have secondary ALK kinase domain mutations. Notably, in the four cases, there was not sufficient tissue in the pre-treatment biopsy to determine whether the mutations were present before treatment. In the series of 18 patients who underwent biopsy after relapse on crizotinib reported by Katayama and colleagues, four patients were found to have secondary ALK kinase domain mutations. Sequencing of the pre-treatment biopsies in three of these patients determined that the resistance mutations were not detectable before treatment. For those mutations where the biochemical impact has been elucidated, resistance is thought to be mediated by either increasing catalytic activity of ALK or by diminishing affinity of crizotinib for mutant ALK, primarily through steric hindrance [30].

ALK copy number alterations

In a cell line model of crizotinib resistance, resistance to intermediate doses of crizotinib was mediated by amplification of *EML4-ALK* [31]. Although these cells later developed the L1196M mutation as they became progressively more resistant, highly sensitive PCR assays were used to verify that the initial amplification event occurred before acquisition of a resistance mutation, suggesting a stepwise progression. Two subsequent studies using patient biopsies at progression have verified that *ALK* copy number gain can serve as a mechanism of resistance to crizotinib [26, 29]. While copy number gain in some cases coexisted with acquisition of secondary resistance mutations, in at least one case copy number gain alone was sufficient to confer resistance [29]. In all of these cases, resistance arose through selective copy number gain or amplification of *ALK* rather than polysomy.

bypass tracks

Approximately two-thirds of patients who develop resistance to crizotinib will not have identifiable secondary resistance mutations

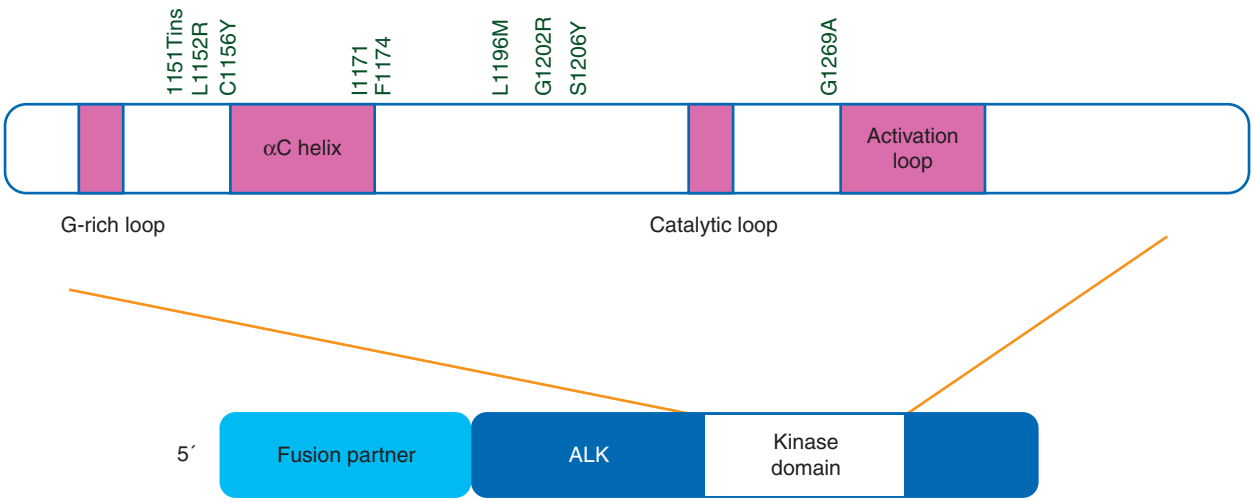

Figure 2. The anaplastic lymphoma (ALK) receptor tyrosine kinase comprises an extracellular ligand-binding domain (residues 19–1038), transmembrane domain (residues 1039–1059), and intracellular domain (residues 1060–1620). The tyrosine kinase domain is located in the cytoplasm and spans residues 1116–1392. The kinase domain includes a glycine rich (G-rich) loop (residues 1123–1128), an αC helix (residues 1157–1173), a catalytic loop (residues 1246–1251), and an activation loop (residues 1271–1288). The identified acquired secondary ALK kinase mutations conferring resistance to crizotinib are located between the G-rich loop and the activation loop. Structural modeling of these mutations suggests that crizotinib resistance is caused by increased catalytic activity of ALK or diminished affinity of crizotinib for mutant ALK.

Table 1. Bypass pathways identified as mediators of crizotinib resistance

Pathway/signal	Mechanism of activation	Potential combinations/ agents[a]	Reference
EGFR	Ligand secretion (EGF, amphiregulin, neuregulin 1)	Crizotinib + dacomitinib Crizotinib + gefitinib Crizotinib + erlotinib	[28, 29, 32, 33]
cKIT	Amplification, ligand secretion (stem cell factor)	Crizotinib + imatinib	[29]
IGF-IR	Ligand secretion (IGF-1)	Crizotinib + linsitinib Ceritinib[b]	[34]
HER2/HER3	Ligand secretion (EGF, neuregulin 1)	Crizotinib + lapatinib	[32, 35]
SRC	The mechanism is unknown. ALK inhibition is speculated to lead to SRC upregulation via release of a negative regulatory signal	Crizotinib + saracatinib Crizotinib + dasatinib	[36]
P2Y purinergic receptors	Activation of protein kinase C	Crizotinib + sotrastaurin	[35]
MAPK	Wild-type KRAS copy number gain, downregulation of DUSP6	Crizotinib + trametinib	[37]

[a]Similar combinations are active in preclinical models and have not been validated in clinical trials.
[b]*Note*: toxicity limits dosing ceritinib in patients at a level necessary to inhibit IGF-1R.

or *ALK* copy number alterations. In these cases, the most commonly implicated mechanism of crizotinib resistance involves aberrant activation of alternate kinases leading to ALK-independent growth (Table 1). Activation of EGFR is the most commonly reported means of bypassing ALK signaling [28, 29]. However, activation of other kinases has also been described. Katayama and colleagues reported two cases of crizotinib resistance mediated by acquired *KIT* amplification [29]. In both cases, *KIT* amplification was accompanied by other resistance mechanisms, specifically EGFR activation and a secondary ALK mutation, supporting the notion that multiple resistance mechanisms can be activated in an individual. In cell line models, treatment with imatinib, a small molecule inhibitor of KIT, was able to overcome resistance. Lovly and colleagues demonstrated IGF-1R activation in crizotinib-resistant tumor samples and the combination of IGF1-R activation and increased IGF-1 ligand levels in cell lines [34]. Combination treatment with crizotinib and monoclonal antibodies against IGF-1R worked synergistically to inhibit growth in cell lines and in mouse models. The second-generation ALK inhibitor, ceritinib, which inhibits ALK and has some activity against IGF-1R, was also able to overcome this resistance in preclinical models [38]. However, in clinical practice, toxicity likely limits dosing ceritinib at a level capable of effectively inhibiting IGF-1R.

Advanced genetic and pharmacologic screening techniques have been instrumental in uncovering additional activated bypass tracks that may mediate crizotinib resistance. Using pharmacologic screens, Crystal and colleagues demonstrated activation of SRC family kinases in six of nine patient-derived resistant cell lines and posited that ALK inhibition may lead to release of a negative regulatory signal for SRC [36]. Regression of tumors in a mouse

xenograft model and attenuated growth of cell lines was seen with the combination of an SRC family kinase inhibitor and an ALK inhibitor. Using an open-reading frame (ORF) library to identify transcripts whose overexpression led to resistance to crizotinib after introduction into an ALK-dependent cell line, Wilson and colleagues identified a potential role for protein kinase C activation via increased expression of P2Y purinergic G-protein-coupled receptors as a mediator of crizotinib resistance [35]. Resistance was able to be reversed by treatment with crizotinib and sotrastaurin, a pan-protein kinase C inhibitor. Protein kinase C activation, however, has yet to be demonstrated in cancers that have developed resistance to crizotinib.

paracrine factors driving resistance

Cancers are dependent on the complex signaling networks between tumor cells and stromal cells in their surrounding microenvironment for promotion of growth and survival. In models of crizotinib resistance, paracrine signaling acts to promote growth of cancer cells by activating downstream signaling cascades in an ALK-independent fashion. As discussed above, experiments by Katayama and colleagues suggest that stromal secretion of stem cell factor can lead to activation of its receptor *KIT* which may promote crizotinib resistance [29]. EGFR ligands, however, are the most commonly implicated paracrine mediators of crizotinib resistance.

Work by Yamada and colleagues demonstrated that EGFR ligands produced by endothelial cells reduced ALK cell line sensitivity to crizotinib by reversal of inhibition of ALK-mediated phosphorylation of downstream targets Akt and Erk1/2 [39]. In two separate experiments using cell lines derived from patients with crizotinib-resistant ALK+ NSCLC, increased secretion of the ligands EGF and amphiregulin led to activation of EGFR in the absence of activating mutations or amplification. EGFR activation was identified as the primary mechanism of resistance to crizotinib in both cases [28, 32]. Upregulation of amphiregulin leading to increased phosphorylation of EGFR has also been detected as a mechanism of resistance in cell lines that develop resistance upon chronic exposure to ALK inhibitors [29]. In all of these experiments, concurrent treatment with ALK and EGFR inhibitors was able to attenuate growth of resistant cells.

Ligand activation of the HER2/HER3 axis has also been identified as a potential mechanism of resistance to treatment with crizotinib. In the ORF library screen described previously, Wilson and colleagues found that the ORF that most strongly induced resistance in cell lines, including a patient-derived cell line, was neuregulin-1, an HER3 ligand [35]. Akin to EGFR ligands, neuregulin was shown to reactivate signaling downstream of ALK. Enrichment of HER2 expression in ALK+ lung tumors with acquired resistance to crizotinib compared with crizotinib-naive tumors was confirmed with RNA sequencing. The combination of an ALK inhibitor and lapatinib, an HER2 inhibitor, was able to resensitise cells that developed resistance after exposure to recombinant neuregulin peptide. The role of neuregulin in crizotinib resistance has been confirmed in separate studies using native and patient-derived cell lines [29, 33].

epithelial–mesenchymal transition

Epithelial–mesenchymal transition (EMT), a cellular reprogramming resulting in the morphologic change from an epithelial shape to a more spindled appearance, is associated with enhanced migratory and invasive capacity. ALK+ NSCLC may be more likely to express EMT markers than other molecular subgroups of NSCLC [40]. Kobayashi and colleagues reported a case of a patient who developed sarcomatoid changes in a progressing lesion associated with high-level ALK gene amplification and loss of epithelial markers after 7 months of treatment with crizotinib [41]. Cell line studies have confirmed a potential role for EMT as an independent mechanism of resistance to crizotinib. Kim and colleagues were able to induce reversible EMT in an ALK cell line and correlated this phenotypic change with reversible crizotinib resistance [42]. Hypoxia may promote resistance to crizotinib by upregulating EMT-related genes in ALK+ cell lines [43]. Unfortunately, little is known about the frequency of EMT in ALK+ lung cancer. Moreover, EMT has not been validated in patients as a stand-alone mechanism of crizotinib resistance. As EMT may play an increasingly important role in treatment resistance for patients treated with more potent next-generation ALK inhibitors, additional studies looking at EMT in patient-derived samples are necessary.

therapeutic strategies to overcome resistance to crizotinib

The enthusiasm generated by crizotinib's marked clinical activity in ALK+ NSCLC has been somewhat tempered by the recognition that patients eventually relapse on crizotinib due to acquired resistance. In fact, the original report of secondary resistance mutations leading to crizotinib resistance was published simultaneously with the initial results of the phase I crizotinib trial [25]. As described above, it has since been recognised that diverse mechanisms of resistance can emerge due to the selection pressure exerted by crizotinib. Understanding the fundamental processes underlying these diverse mechanisms of resistance is essential to developing strategies to overcome them.

Since the initial approval of crizotinib in 2011, several other ALK inhibitors have been developed (Table 2). These inhibitors are more potent than crizotinib and are capable of overcoming the gatekeeper L1196M mutation and others, depending on the specific inhibitor. Not surprisingly, with these increases in potency, copy number gain has not been described as a mechanism of resistance to any of these newer ALK inhibitors. The next-generation ALK inhibitors are generally active in crizotinib-resistant patients (Table 3), with an ORR ranging from 48% to 71% and a median PFS ranging from 6.9 to 13.4 months [44, 46–48]. Ceritinib and alectinib, two next-generation ALK inhibitors, have been approved by the FDA for use after progression on crizotinib. Ceritinib has also been approved by the EMA for the same indication. In the international, multicenter Phase I ASCEND-1 trial which enrolled 163 crizotinib-pretreated patients and 83 crizotinib-naive patients, the ORR and median PFS for patients treated with ceritinib in the crizotinib-pretreated group were 56% and 6.9 months, respectively [44]. In two phase II studies of alectinib in crizotinib-resistant patients (NP28761 and NP28673), the ORR and median PFS were ~50% and 8–9 months, respectively [46, 47]. Brigatinib another next-generation ALK inhibitor, received breakthrough therapy designation by the FDA based on an ORR of 71% and median PFS of 13.4 months in crizotinib-pretreated patients [48]. The next-generation agents appear to be particularly

Table 2. Next-generation ALK inhibitors

Next-generation ALK inhibitors	Manufacturer	Approval status	Ongoing trials
Alectinib	Genentech	Approved	Phase 3 study of alectinib versus crizotinib in treatment-naive patients [NCT02075840]
Brigatinib	Ariad	Breakthrough Therapy Designation*	Phase 1/2 study of brigatinib [NCT01449461]
			Phase 2 randomised study testing two doses of brigatinib in patients previously treated with crizotinib (ALTA) [NCT02094573]
			Phase 3 study of brigatinib versus crizotinib in ALK inhibitor-naive patients (ALTA-1L) [NCT02737501]
Ceritinib	Pfizer	Approved[a,b]	Phase 2 study of ceritinib in crizotinib-naive patients [NCT01685138]
			Phase 3 study of ceritinib versus chemotherapy in treatment-naive patients [NCT01828099]
			Phase 3 study of ceritinib versus chemotherapy in patients previously treated with chemotherapy and crizotinib [NCT01828112]
Ensartinib	Xcovery	Investigational	Phase 1/2 study of ensartinib in patients with advanced solid tumors [NCT01625234]
Entrectinib	Ignyta	Investigational	Phase 1/2a study of entrectinib in patients with advanced solid tumors harboring NTRK, ROS1, or ALK alterations [NCT02097810]
			Phase 2 basket study of entrectinib in patients with solid tumors harboring NTRK, ROS1, or ALK rearrangements [NCT02568267]
Lorlatinib	Pfizer	Investigational	Phase 1/2 Study of lorlatinib in patients with ALK or ROS-rearranged lung cancer [NCT01970865]

ALK, anaplastic lymphoma kinase, [a]Approved by the United States Food and Drug Administration, [b]Approved by the European Medicines Agency.

Table 3. Next-generation ALK inhibitor efficacy in crizotinib-resistant patients

	Ceritinib		Alectinib		Brigatinib
	ASCEND-1 [44]	ASCEND-2 [45]	NP28763 [46]	NP28761 [47]	Phase I/II [48]
Number of patients	163	140	138	87	70
Objective response rate (%)	56	38	50	48	71
Disease control rate (%)	74.2	77.1	78.7	80	87
Median PFS (months)	6.9	5.7	8.9	8.1	13.4
Median duration of response (months)	8.3	9.7	11.2	13.5	—

effective in the ALK inhibitor-naive setting with ORR ranging from 66.3% to 100% and median PFS of at least 18 months. A phase III study (NCT02075840) comparing alectinib with crizotinib as first-line treatment is currently underway.

Most patients who develop disease progression on crizotinib will respond to treatment with a next-generation ALK inhibitor, even in the absence of a detectable resistance mutation [49]. These structurally diverse next-generation ALK inhibitors are characterised by variable ability to overcome different secondary resistance mutations. Ceritinib was able to overcome secondary mutations in patients enrolled in the phase I ASCEND trial but additional testing using preclinical *in vitro* and *in vivo* models showed that ceritinib has limited ability to overcome the G1202R and F1174C mutations [50]. Ceritinib, however, is able to overcome distinct resistance mutations that emerge after treatment with alectinib, specifically those involving the I1171 residue [51, 52]. The second-generation ALK inhibitors, as a group, are characterised by an inability to overcome the ALK G1202R mutation [53, 54]. However, the newest next-generation inhibitor lorlatinib (PF-06463922) has shown superior potency compared with other ALK inhibitors against known resistance mutations in preclinical models and is the only known ALK inhibitor to overcome G1202R [55]. Interestingly, a recent case study demonstrated that crizotinib is active against an acquired compound mutation, C1156Y + L1198F, that leads to resistance to next-generation drugs, including lorlatinib [56]. With expansion of the cadre of available ALK inhibitors, it is anticipated that informed sequential selection of ALK inhibitors based on resistance mutation profile will emerge as a standard practice.

The higher potency and improved blood–brain barrier penetration of these newer agents has translated into improved CNS control. In a pooled analysis of the phase II studies of alectinib in crizotinib-resistant ALK+ NSCLC (NP28761 and NP28673), the intracranial ORR and DCR in patients with brain metastases was 64% and 90% in those with measurable CNS disease [57]. In these studies, the median duration of CNS response was ~10–11 months [46, 47]. In a retrospective analysis of 15 assessable patients with measurable CNS disease treated with brigatinib, the intracranial ORR and DCR was reported at 53% and 86%, respectively [48]. Lorlatinib has also been shown to have CNS activity in preclinical and early clinical studies [55, 58]. The initial impressive CNS response seen with these agents may challenge the role of crizotinib as an initial therapeutic strategy in the 26% of patients who present with brain metastasis.

In patients with resistance due to activation of bypass tracks, combination therapy with ALK inhibitors and agents targeting the activated receptor appears to be effective in preclinical studies as

described above. As the bypass tracks converge upon activation of downstream effectors of the MAPK pathway, it is not surprising that the combination of ALK inhibitors and inhibitors of the MAPK pathway shows promising antitumor activity in preclinical studies [37]. Interestingly, some of the more promising ALK inhibitors, alectinib and lorlatinib, derive their potency from greater specificity for ALK and therefore have limited effect on other kinases. It is plausible that with treatment with multiple next-generation ALK inhibitors, escape from growth inhibition via bypass tracks or EMT may become a more prominent resistance mechanism than resistance mutations. Indeed, there have already been reports of MET activation leading to resistance to alectinib [59, 60].

Treatment with other non-TKI agents has also shown promise in patients with ALK+ NSCLC. Inhibition of HSP90, a molecular chaperone that is central to regulation of folding, stability, and function of 'client' proteins including ALK and EGFR, has been adopted as a therapeutic strategy to promote proteosomal degradation and disruption of signals that promote tumor growth and survival. HSP90 inhibitors have been shown to potently suppress growth in preclinical models of acquired resistance in the setting of *ALK* amplification or resistance mutations [31, 61, 62]. The experience with HSP90 inhibitor monotherapy has been mixed. A phase II trial of ganetespib monotherapy in patients with NSCLC noted clinical activity in a small group of crizotinib-naive patients with ALK+ NSCLC [63]. However, in a more recent phase II trial of AUY922 monotherapy in ALK+ patients who had progressed on prior ALK TKIs, no objective responses were seen in six assessable patients. [64]. Although the small number of ALK+ NSCLC patients enrolled on these trials limits drawing firm conclusions, the results suggest that HSP90 inhibition may not represent a viable therapeutic strategy in the TKI-resistant setting.

Recent data from Ota et al. demonstrating upregulation of PD-L1 expression in ALK+ cell lines and NSCLC specimens suggest that checkpoint inhibitors could be a promising strategy in this patient population [65]. However, there is some evidence that despite variable PD-L1 expression in ALK+ lung cancer, treatment with an ALK TKI does not lead to recruitment of CD8+ tumor infiltrating lymphocytes. [66]. Moreover, checkpoint inhibition has been shown to be less effective in never-smokers, a population enriched for ALK rearrangements [67]. This suggests that there may be some limitation with simply combining ALK TKIs with checkpoint inhibitors, and trial designs investigating optimal sequencing of these agents may be necessary. Several trials combining ALK inhibitors and checkpoint inhibitors are underway.

conclusion

The first demonstration of crizotinib's clinical activity in ALK+ lung cancer was a pivotal discovery for a population of patients with an otherwise dismal prognosis. Since the introduction of crizotinib into clinical practice, it has become apparent that the vast majority of patients will invariably relapse due to resistance. As discussed above, there are multiple and diverse resistance mechanisms that underlie relapses on crizotinib. Biopsies at progression have contributed immensely to our understanding of the dynamics of treatment resistance. Relying solely on molecular characterization of tumors derived from diagnostic biopsies provides an insufficient blueprint from which to predict a patient's clinical course. Considering the dynamic evolution of tumors throughout treatment, repeat surveillance of a tumor's molecular signature, particularly in the setting of treatment resistance, is necessary.

Next-generation ALK TKIs have made significant strides toward overcoming some of the limitations of crizotinib, particularly potency and CNS penetration. With these improvements, a shift in the distribution of mechanisms of resistance is anticipated. For example, biopsies at progression on these next-generation agents demonstrate that the spectrum of resistance mutations is narrower, with enrichment for certain mutations like *ALK* G1202R [50]. As only lorlatinib has demonstrated potency against all clinically identified resistance mutations, appropriate sequencing of TKIs will need to be context specific and based on detected mutations. Sequencing strategies must also be flexible rather than linear, as in some cases revisiting previous agents may be the optimal approach [56]. Sequential therapy already has shown promising results, with a multi-institutional series reporting a combined PFS of 17.4 months and impressive median overall survival of 49.4 months with sequential crizotinib and ceritinib [68]. With adoption of next-generation inhibitors, it is expected that resistance will be increasingly driven by ALK-independent processes, including activation of bypass pathways and EMT. It is unclear whether sequential ALK TKIs will be efficacious in patients who develop resistance to these agents who do not have detectable resistance mutations. As such, it is imperative that combination strategies be developed in parallel. Intercalated or intermittent administration of agents may need to be explored when designing combination studies in order to maximize efficacy and minimise toxicity.

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disclosure

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