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REVIEW ARTICLE

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Drug resistance in anaplastic lymphoma kinase-rearranged lung cancer

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The anaplastic lymphoma kinase *(ALK)* gene encodes a receptor tyrosine kinase, and many kinds of *ALK* fusion genes have been found in a variety of carcinomas. There is almost no detectable expression of ALK in adults. However, through *ALK* gene rearrangement, the resultant ALK fusion protein is aberrantly overexpressed and dimerized through the oligomerization domains, such as the coiled-coil domain, in the fusion partner that induces abnormal constitutive activation of ALK tyrosine kinase. This results in dysregulated cell proliferation. *ALK* gene rearrangement has been observed in 3%-5% of non-small-cell lung cancers, and multiple ALK inhibitors have been developed for the treatment of ALK-positive lung cancer. Among those inhibitors, in Japan, 3 (4 in the USA) ALK tyrosine kinase inhibitors (TKIs) have been approved and are currently used in clinics. All of the currently approved ALK-TKIs have been shown to induce marked tumor regression in *ALK*-rearranged non-small-cell lung cancer; however, tumors inevitably relapse because of acquired resistance within a few years. This review focuses on ALK-TKIs, their resistance mechanisms, and the potential therapeutic strategies to overcome resistance.

KEYWORDS
ALK, fusion gene, mutation, resistance, tyrosine kinase inhibitor

1 | INTRODUCTION

The *ALK* gene encodes a single transmembrane tyrosine kinase, which is widely conserved from *Caenorhabditis elegans* to *Homo sapiens*. *ALK* was first identified as the fusion gene nucleophosmin (*NPM*) *1-ALK* in ALCL in 1994.[1] After the discovery of ALK, its function was investigated, but its detailed characteristics were largely unknown until recently. In 2007, a novel *ALK* fusion gene, *EML4-ALK*, was discovered in lung cancers as a strong driver oncogene.[2] Subsequently, research on ALK in the context of cancer and the development of ALK inhibitors has received increased attention. In addition to the *ALK* fusion gene, ALK point mutation-mediated constitutive activation of *ALK* has been discovered in neuroblastoma and thyroid cancers.[3-6] To date, a number of *ALK* fusion oncogenes and ALK point mutations have been discovered in various types of cancer. Because aberrant constitutive activation of ALK tyrosine kinase induces dysregulated cell growth, which results in tumor development, many ALK-TKIs have been developed and tested in clinical trials, mainly for the treatment of *ALK*-rearranged NSCLC. Subsequently, three ALK-TKIs have been approved in Japan (4 in the USA) and used clinically, and several ALK inhibitors are under development or clinical evaluation. The ALK-TKIs have often shown marked tumor shrinkage in ALK-rearranged cancer patients and induce prolonged clinical responses; however, the tumors inevitably relapse because of acquired resistance mediated by multiple mechanisms. To overcome this resistance, it is important to identify the

Abbreviations: ABCB1, ATP binding cassette subfamily B member 1; ALCL, anaplastic large-cell lymphoma; ALK, anaplastic lymphoma kinase; CNS, central nervous system; CSF, cerebrospinal fluid; EGFR, epidermal growth factor receptor; EML4, echinoderm microtubule-associated protein-like 4; EMT, epithelial-mesenchymal transition; IGF1R, insulin-like growth factor 1 receptor; IHC, immunohistochemical; NB, neuroblastoma; NGS, next-generation sequencing; NSCLC, non-small-cell lung cancer; ORR, overall response rate; PFS, progression-free survival; SCLC, small-cell lung cancer.

molecular mechanisms underlying resistance and develop effective therapeutic strategies corresponding to each resistance mechanism. This review focuses on the molecular mechanisms of acquired resistance to ALK-TKIs in *ALK*-rearranged NSCLC and discusses the potential therapeutic strategies for overcoming resistance.

2 | CHARACTERISTICS OF ALK

2.1 | Function of ALK in normal cells and cancer cells

ALK was first discovered as a fusion oncogene, *NPM1-ALK* in ALCL, which is an aggressive $CD30^+$ T-cell lymphoma.[1] Mathew et al[7] mapped the mouse homolog of *ALK* to chromosome 17 and confirmed that human *ALK* was located on the short arm of chromosome 2. Anaplastic lymphoma kinase shows greatest sequence similarity to the insulin receptor subfamily of kinases and encodes a single-pass transmembrane receptor tyrosine kinase. Pleiotrophin, midkine, and heparin have been reported as potential ligands of ALK for inducing its activation.[8-10] However, as different results have been reported by other groups, the actual ligand remains to be identified. Finally, FAM150A and FAM150B were discovered by 2 different groups in 2015 and reported as genuine ligands of ALK.[11,12] Expression of ALK protein in human adults has been shown only in a few cell types in the CNS and a few organs. Additionally, ALK has been shown to be expressed in the thalamus, hypothalamus, midbrain, and dorsal root ganglion in a mouse model during development.[13,14] However, *ALK* knockout mice develop without obvious defects and have shown no differences in life spans and only mild behavioral phenotypes.[15,16]

Several types of *ALK* gene rearrangements (*ALK* fusion genes) have been found in many types of cancers. More than 10 years after the first identification of the *ALK* fusion gene, *NPM-ALK*, in ALCL, the *EML4-ALK* fusion oncogene was found in NSCLC. Until then, it had been believed that fusion oncogene-driven cancer was relatively rare in solid tumors. However, 3%-5% of NSCLC tumors have the *ALK* fusion gene. *ALK* rearrangement has been found in many types of cancer, and various fusion partners have been identified. The common characteristics of *ALK* fusion genes are that: (i) the ALK fusion protein is expressed constitutively through the active promoter of the fusion partner; and (ii) the fusion partner protein harbors the conserved oligomerization domain, such as the coiled-coil domain, which enables constitutive activation of ALK through dimerization or oligomerization. *ALK*-rearranged cancers are highly susceptible to aberrant growth signaling exerted from the constitutively activated ALK tyrosine kinase. Thus, inhibition exerted by the ALK-TKI markedly inhibits the tumor growth of *ALK*-rearranged cancer cells. In addition, transgenic mice expressing EML4-ALK in the lung under a surfactant protein C promoter, which is exclusively expressed in type II alveolar epithelial cells, developed hundreds of adenocarcinoma nodules in both lungs within a few weeks of birth.[17] An EML4-ALK lung cancer mouse model has also been easily generated by inhalation of adenoviruses of the Crispr-Cas9 system targeting the intron of *EML4* and *ALK*.[18]

In NB, which is a secondary frequent childhood cancer, various point mutations in the ALK kinase domain have been identified. Most of those point mutations have been shown to cause constitutive activation of ALK tyrosine kinase. Bresler et al[19] examined the genetic abnormalities of >1500 NB patients by NGS and found that 8% of NB patients had point mutations in the ALK kinase domain, such as F1174L (the phenylalanine residue at 1174 changes to leucine) or R1275Q.

2.2 | Detection of ALK-positive cancer

To detect ALK-positive ALCL, IHC analysis using anti-ALK antibodies (such as clone 5A4), which recognize the tyrosine kinase domain of ALK, has been used. The expression of the ALK fusion protein is much lower in *ALK*-rearranged lung cancer than in ALK-positive ALCL. Initially, ALK-rearranged lung cancer was mainly screened for by using FISH, which can detect *ALK* gene split by using 2 different fluorescent probes set on both sides of the break point of the *ALK* gene. In addition, multiplex RT-PCR-based screening has also been used to detect known *ALK* fusion genes. Major *ALK* fusion genes include *EML4-ALK* variant 1 (E13:A20; fusion at exon 13 of *EML4* and exon 20 of *ALK*), variant 3 (E6:A20), and variant 2 (E20:A20), but a number of fusion partners and patterns have been found, which makes it difficult to set the multiplex PCR primer to not miss rare *ALK* fusion genes. After the establishment of highly sensitive IHC staining methods of ALK,[20] IHC and FISH are now widely used to screen *ALK*-rearranged NSCLC. Currently, NGS can be used to detect *ALK* fusion genes by sequencing the intron between exons 19 and 20 because most *ALK* gene rearrangements occur in this intron of *ALK*, with rare exceptions.[21,22]

2.3 | Drugs for targeting ALK

A number of ALK-TKIs have been developed and evaluated in clinical trials; three ALK-TKIs, crizotinib, alectinib, and ceritinib, have been approved in many countries, including the USA, the EU, and Japan, and brigatinib has been approved in the USA. The following ALK inhibitors are clinically available or currently under clinical evaluation.

2.3.1 | Crizotinib

Initially, crizotinib was developed as a cMET receptor TKI. However, soon after the discovery of *ALK* fusion gene-positive NSCLC, a phase I clinical trial of crizotinib for treatment of *ALK*-rearranged NSCLC was started, in August 2008, because crizotinib can potently inhibit ALK in addition to cMET.[23] In the phase I clinical trial, 149 *ALK*-rearranged NSCLC patients were screened by *ALK* split FISH and treated with crizotinib. The ORR and PFS were reported to be 60.8% and 9.7 months, respectively.[24] Similar ORR and PFS have been observed in the subsequent phase II clinical trial.[25] Based on the results of phase I and II clinical trials, the US FDA granted accelerated approval to crizotinib for the treatment of *ALK*- rearranged

NSCLC in 2011, and crizotinib was also approved in Japan in 2012. In the phase III clinical trials, the PFS was longer and ORR was higher for crizotinib than for chemotherapy.[26,27] Crizotinib has been recommended as a first-line treatment of *ALK*-rearranged NSCLC. Although crizotinib often shows marked tumor shrinkage in *ALK*-rearranged NSCLC patients, crizotinib reportedly has limited potential against tumors metastasized to the brain because of poor penetration into the CSF. Indeed, disease progression has often been seen in the brain. One report showed that the crizotinib concentration in the CSF was 0.26% of that in the plasma.[28] A study using ABC transporter *ABCB1* (*abcb1a* and *abcb1b*) knockout mice reported that the crizotinib concentration in the CSF was low, mainly because crizotinib was pumped out with ABCB1.[29] In addition, crizotinib is highly active against ROS1, which has a high degree of homology with ALK at the ATP binding site. The *ROS1* fusion oncogene has been observed in approximately 1% of NSCLC cancer patients.[30]

2.3.2 | Alectinib

Alectinib was developed as a selective ALK inhibitor, and the IC_{50} of ALK has been shown to be 2 nmol/L, which is lower than that of crizotinib.[31] In addition, alectinib has been shown to be active against several crizotinib-resistant mutations, such as L1196M or G1269A mutations, in ALK in vitro and in vivo xenograft models.[32] A phase I/II clinical trial of alectinib against ALK-TKI treatment-naïve *ALK*-rearranged NSCLC patients was carried out in Japan. The recommended dose of alectinib was determined to be 300 mg twice daily. The ORR was quite high (93.5%), and the PFS was reported to be over 3 years.[33] Another phase I/II clinical trial of alectinib for ALK-rearranged NSCLC patients who showed progression after crizotinib treatment was undertaken mainly in the USA. The results showed that the recommended dose was 600 mg twice a day, which is double that decided on after the trial in Japan. Even in patients with crizotinib treatment history, an ORR of 52% and a PFS of 8.2 months were observed.[34] As alectinib is not a substrate of ABCB1 and undergoes relatively little pumping out through the blood brain barrier, high CSF penetration was reported. Accordingly, alectinib showed activity in brain metastases.[35] Two phase III clinical trials of alectinib compared with crizotinib in treatment-naïve *ALK*-rearranged NSCLC patients were carried out in Japan (J-ALEX) and globally (ALEX). In these trials, the recommended dose of alectinib was 300 mg twice daily in J-ALEX, and 600 mg twice daily in ALEX. The PFS was longer for alectinib than for crizotinib (25.7 months for alectinib vs 10.4 months for crizotinib in J-ALEX, and 25.7 months for alectinib vs 11.1 months for crizotinib in ALEX).[36,37]

2.3.3 | Ceritinib

Ceritinib has a very low IC_{50} of 150 pM to ALK in vitro; that is, 20- to 30-fold lower than that of crizotinib. Similar to alectinib, ceritinib is also active against several crizotinib-resistant mutations, such as L1196M and G1269A in ALK.[38,39] Phase I clinical trials for *ALK*-rearranged NSCLC patients with and without prior crizotinib treatment showed an ORR of 58% and a PFS of 7.0 months.[40] It was reported that the response was seen even in CNS metastasis cases.[41] A phase III clinical trial comparing ceritinib with chemotherapy in advanced *ALK*-rearranged lung cancer with (ASCEND-5) or without prior crizotinib and chemotherapy (ASCEND-4) showed the superiority of ceritinib over chemotherapy. The median PFS in the ASCEND-4 trial was 16.6 months for ceritinib vs 8.1 months for chemotherapy, and even in patients previously treated with both chemotherapy and crizotinib, the median PFS was 5.4 months for ceritinib and 1.6 months for chemotherapy.[42,43] In addition, ceritinib treatment has been shown to be affected by low-fat meals: it was shown that a lower dose of 450 mg once a day with a low-fat meal gave similar exposure and a more favorable gastrointestinal safety profile than 750 mg once a day in the fasting state in *ALK*-rearranged NSCLC (ASCEND-8).[44] Moreover, ceritinib has been shown to be active against *ROS1*-rearranged NSCLC patients.[45]

2.3.4 | Brigatinib

Brigatinib was first reported as a selective ALK-TKI with a subnanomolar IC_{50} against ALK in vitro and was also shown to be active against crizotinib-resistant L1196M gatekeeper mutation.[46] In the phase I/II clinical trial of brigatinib, 71 crizotinib refractory *ALK*-rearranged lung cancer patients were treated with brigatinib, and the ORR was 62% and the PFS was 13 months. Serious treatment-emergent adverse events were observed in some patients, and 6% of the patients died during treatment or within 31 days of the last dose of brigatinib. Thus, severe adverse events occurred, so the phase II recommended dose was set at 90 mg once daily and 180 mg once daily with a 7-day lead-in at 90 mg.[47] In the follow-up phase II study, the ORR was 45% in a group treated with 90 mg brigatinib once daily and 54% in patients treated with 180 mg once daily with a 7-day lead-in at 90 mg, and the PFS periods for the treatment groups were 9.2 and 12.9 months, respectively. Severe (grade $\geq$3) treatment-emergent adverse events were seen in 3% of the patients, and those pulmonary toxicities tended to be associated with older patients within shorter intervals (<7 days) between the last crizotinib and the first brigatinib. No such severe pulmonary adverse events were seen after dose escalation to 180 mg after a 7-day lead in at 90 mg.[48]

Brigatinib has been shown to be active against almost all of the crizotinib-, alectinib-, or ceritinib-resistant mutations, including the G1202R mutation in *ALK*, which is highly resistant to crizotinib, alectinib, or ceritinib. However, from an analysis of a few brigatinib-resistant cases, G1202R point mutations were also seen.[49] In 2017, brigatinib was approved in the USA for the treatment of *ALK*-rearranged NSCLC patients with crizotinib-refractory or -intolerable disease. Of note, it was reported that brigatinib has some activity against *ROS1*-rearranged cancer, and EGFR-C797S/T790M/activating mutation mediated osimertinib resistance by combining with anti-EGFR antibody.[47,50]

2.3.5 | Lorlatinib

Lorlatinib is the first macrocyclic ALK/ROS1-TKI designed from crizotinib. Lorlatinib has been shown to be active against almost all *ALK* mutants, including the G1202R mutant, and resistant to first- and second-generation ALK-TKIs in vitro and in vivo. In addition, lorlatinib effectively penetrates into the CSF because it is not a substrate of p-glycoprotein. In the experimental model, lorlatinib induced marked tumor shrinkage in an intracranially injected brain metastasized model. The results of a phase I study showed that the objective response rate was 46% for 41 patients, and the recommended dose for the phase II study was 100 mg once daily. The analysis of the CSF concentration in the clinical evaluation showed a ratio of lorlatinib in CSF to serum of >0.6, and a response was observed in intracranial metastasized tumor.[51] Currently, a phase I/II study is ongoing, and a phase III study comparing lorlatinib with crizotinib has been started.

2.3.6 | Other ALK inhibitors

To date, several ALK-TKIs have been developed and are currently being evaluated in multiple clinical trials. Entrectinib is a potent inhibitor of neurotrophic receptor tyrosine kinases (NTRKs), ROS1, and ALK and is being evaluated in a clinical trial. From the results of a phase I/II study, entrectinib gave a response rate of 57% for *ALK*-rearranged tumors and 85% for *ROS1*-rearranged cancers. Central nervous system metastasized tumors also showed responses to entrectinib in the trial.[52] Ensartinib (X-396) is an orally available small molecule ALK-TKI that potently inhibits multiple crizotinib-resistant mutants.[53] In a phase I/II clinical trial of ensartinib, the response rate was 55% in *ALK*-positive lung cancer patients. Additionally, intracranial activity was observed in all of the patients with brain metastasis. Phase III clinical trials are currently ongoing. In addition, other ALK inhibitors, ASP-3026,[54] TSR-011, CEP-37440,[55] and PLB1003 have been developed or are currently under clinical evaluation.

3 | MECHANISMS OF ALK-TKI RESISTANCE

3.1 | Mutation or gene amplification of ALK-mediated resistance

In patients with *ALK*-rearranged lung cancer, when crizotinib was used as the initial treatment of ALK-TKI, >60% of the patients experienced partial response or complete response, and the PFS of crizotinib was approximately 10 months, which suggests that recurrence due to acquired resistance occurs within 1 year in >50% of cases. In 2010, ALK-L1196M (the ALK-1196th amino acid leucine is converted to methionine) and C1156Y mutations were found in a patient refractory to crizotinib by analyzing the cells in pleural effusion; those ALK mutants conferred resistance to crizotinib.[56] Subsequently, various mutations, such as G1269A, I1151T-ins, G1202R, S1206Y, and I1171T, have been reported as crizotinib-resistant mutations in addition to the above two, and the L1196M gatekeeper mutation, which is in the equivalent position of the EGFR-TKI-resistant gatekeeper mutation EGFR-T790M, has been most frequently observed in crizotinib-resistant patients.[49,57-59] However, the resistant mutation patterns in Japanese patients and their population frequency have not been reported.

Similarly, mutations resistant to ceritinib and alectinib, second-generation ALK-TKIs, and the next-generation ALK inhibitors brigatinib and lorlatinib have also been reported during the analysis of clinical samples resistant to each TKI. Alectinib and Ceritinib have previously been approved for crizotinib-refractory or -intolerability patients, and they have been recently approved for treatment-naïve ALK-positive lung cancer patients. G1202R, F1174C/L/V, and G1123S mutations have been found to be ceritinib-resistant in an analysis of patients treated with ceritinib after crizotinib.[38] G1202R, V1180L, and I1171T/N/S mutations have been reported as alectinib-resistant mechanisms.[60] Among these secondary mutations, the G1202R mutation is resistant to all three of these ALK inhibitors, but the F1174C/L/V ceritinib-resistant mutations are sensitive to alectinib; additionally, the I1171T/N/S and V1180L mutations, which confer alectinib resistance, are sensitive to ceritinib.[38,49,60] In contrast, brigatinib and lorlatinib have also been shown to be effective for various ALK-TKI-resistant mutations, including L1196M (Figure 1A).[46,61] However, it has been shown that at least 2 mutations occur within the ALK kinase region, which makes them resistant. Regarding brigatinib, E1210K + S1206C, and E1210K + D1203N mutation has been found in patients with brigatinib resistance.[49] For lorlatinib, the C1156Y + L1198F mutation in ALK has been found to confer resistance in lorlatinib-resistant patients. Interestingly, an L1198F double mutation has also been shown to be resensitized to crizotinib as the phenylalanine substitution at L1198 does not clash with crizotinib and in fact moves slightly closer to the inhibitor (Figures 1B and 2).[62]

3.2 | Bypass pathway or other mechanism-mediated resistance

In the crizotinib-resistant cases in which the resistance mechanisms were examined, secondary resistance mutation or *ALK* fusion gene amplification was observed in only one-third of the cases. From the analysis of the remaining cases and the cell line models, it has been reported that amplification of the *cKIT* gene with increased expression of stem cell factor (SCF) as its ligand, activation of EGFR was found to be the mechanism of resistance to crizotinib (Figure 3).[57] The concomitant mutation of KRAS or EGFR has also been reported, but with low frequency.[59,63] Activation of IGF1R has been found in crizotinib-resistant cases. Furthermore, in studies using cultured cell lines and cell lines derived from resistant patients,[64] it has been reported that resistance due to activation of Src is observed relatively frequently, but the detailed molecular mechanism underlying activation of Src is not yet clear.[65] As crizotinib is a potent inhibitor of cMET with an even lower IC_{50} than that for ALK, and was originally developed as a cMET inhibitor, cMET overactivation, such as *MET* gene amplification-mediated resistance, has not been observed.[66-68]

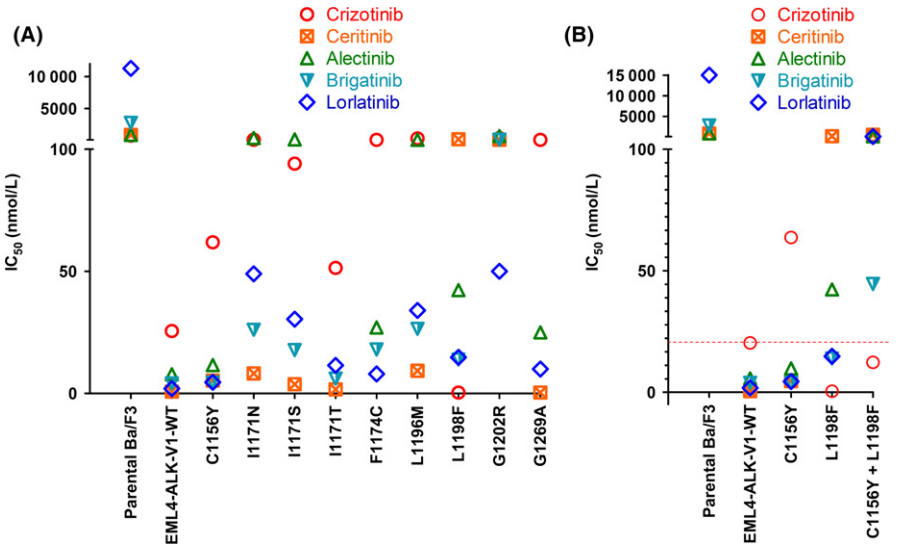

FIGURE 1 Half maximal inhibitory concentration values of the indicated Ba/F3 cells (*EML4-ALK* WT or major resistant mutants) are shown in dot plots. A, IC_{50} data were obtained from Gainor et al[49] B, The L1198F double mutant confers resistance to lorlatinib but is extremely sensitive to crizotinib. IC_{50} data were obtained from Shaw et al[62]

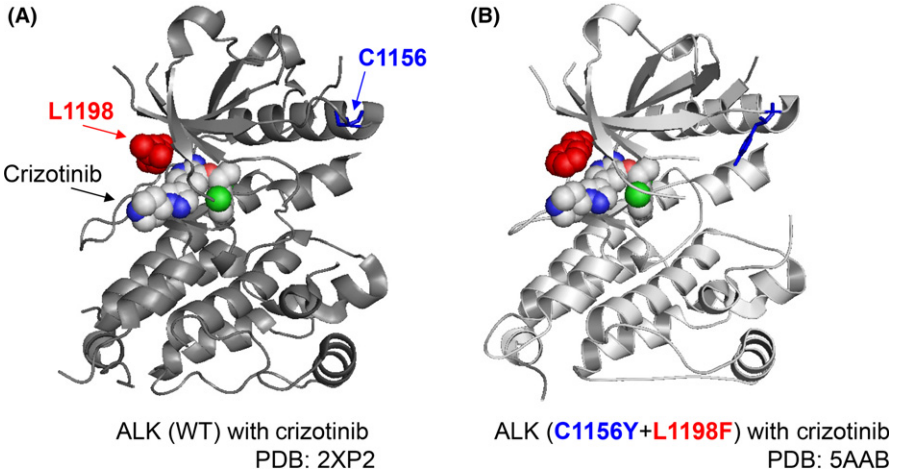

FIGURE 2 Structure of anaplastic lymphoma kinase (ALK) (WT or crizotinib resensitized mutant C1156Y + L1198F) is depicted using the indicated crystal structure analysis data (Protein Data Bank: 2XP2 [left] and 5AAB [right])

A ceritinib-resistant mechanism other than secondary mutation is involved in an activating mutation of MEK (MAP2K1-K57N), and an MEK inhibitor with ALK-TKI has been found to overcome the resistance. Analysis of cell lines derived from ceritinib-resistant patients has shown that overexpression of p-glycoprotein ABCB1, which is one of the ABC transporters that transport hydrophobic substrates from cells or across the blood brain barrier to protect the brain, conferred resistance to ceritinib and crizotinib but not to alectinib or lorlatinib. Thus, the resistance can be overcome by alectinib or lorlatinib, which have not been shown to be substrates of p-glycoprotein, or by the combination of a p-glycoprotein inhibitor, such as MS209[69] or verapamil[70] with ceritinib. This p-glycoprotein overexpression was found in three out of 13 patients who had been treated with crizotinib or ceritinib.[71] Of note, ceritinib has been shown to actively inhibit IGF1R, so IGF1R activation-mediated resistance, found in crizotinib-refractory cases, might not be observed.[64]

An alectinib-resistant mechanism other than secondary mutation has been shown in cMET activation through *MET* gene amplification or elevation of its ligand hepatocyte growth factor (HGF) from an analysis of patients' samples and cell line models. cMET activation-mediated resistance would be able to be overcome by crizotinib.[66,68] Basically, cMET overactivation can potentially confer resistance to any ALK-TKIs except for crizotinib (Figure 3).

To overcome resistance-mediated bypass pathway activation, combination therapy to inhibit both ALK and the bypass pathways are considered to be necessary. However, no combination therapy has been clinically approved that overcomes bypass pathway-mediated resistance. Thus, further preclinical studies and clinical evaluations are needed to test combination therapies based on resistance mechanisms. In addition, feedback re-activation through the MAPK pathway by decrease of dual specificity phosphatase 6 (DUSP6), a phosphatase that negatively regulates the MAPK pathway, or other mechanisms contribute to the resistance to ALK inhibitors. Indeed, an up-front ALK inhibitor with MEK inhibitor treatment enhanced tumor shrinkage and prolonged initial response in an H3122 tumor-bearing mouse model.[72] Currently, up-front inhibitors of both ALK and MAPK signaling pathways are being evaluated in several clinical trials. In addition, other combination therapies that inhibit distinct pathways, such as ceritinib + CDK4/6 inhibitor, ceritinib + mTOR inhibitor, and alectinib + anti-angiogenesis inhibitor, have been found to be activated or related to ALK-TKI resistance in cancer in vivo or in vitro experiments and have been evaluated in clinical trials.

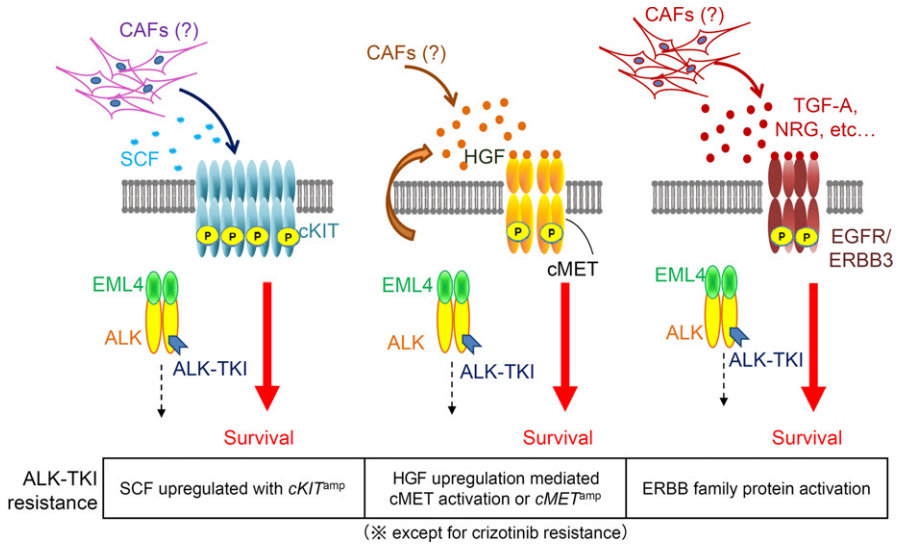

FIGURE 3 Bypass pathway activation-mediated anaplastic lymphoma kinase-tyrosine kinase inhibitor (ALK-TKI) resistance (stromal cell-mediated ligand secretion from tumor- or stromal cell-mediated receptor tyrosine kinase activation). Left panel, *cKIT* gene amplification with stem cell factor (SCF) (a ligand of cKIT) secretion from stromal cell-mediated crizotinib resistance. Middle panel, hepatocyte growth factor (HGF) upregulation led to cMET activation-mediated resistance. HGF was secreted from tumor or stromal cells. Otherwise, cMET gene amplification solely induced the activation of cMET, which resulted in ALK-TKI resistance. Right panel, ERBB receptor, including epidermal growth factor receptor (EGFR) activation-mediated resistance. Various ligands for the ERBB family can induce activation of ERBB receptors and induce resistance. CAF, cancer-associated fibroblast; EML4, echinoderm microtubule-associated protein-like 4; NRG, neuregulin; TGF-A, transforming growth factor-α

In addition, EMT-mediated resistance has also been found in patients' specimens and in cell line models. Gainor et al examined a series of *ALK*-rearranged lung cancer patients and examined cellular EMT status by IHC of vimentin and E-cadherin. They found that 42% of patients' specimens harbored EMT characteristics. Interestingly, approximately half of the EMT-positive specimens also had secondary mutations in the ALK kinase domain; thus, EMT might not be the sole driver of the resistance.[49] In contrast, histological transformation from adenocarcinoma to SCLC has been reported in several ALK-TKI-refractory cases, such as alectinib-resistant cases. Similar to the SCLC transformation, which is well-known in EGFR-TKI-resistant cases, classic cytotoxic chemotherapy is thought to be effective for overcoming resistance.[73,74]

3.3 | Challenges for the future

Currently, multiple ALK-TKIs (three in Japan and 4 in the USA) have been approved and are used clinically. In Japan, all three ALK-TKIs have been approved as first-line therapies for ALK-rearranged NSCLC. From the direct comparison between alectinib and crizotinib as first-line therapies, alectinib has been shown to have significantly longer PFS than crizotinib. There are no published data available for the direct comparison of the PFS periods of ceritinib and crizotinib or alectinib, but there are data comparing them with that of chemotherapy. In that study, compared with the chemotherapy-treated group's PFS of 8.1 months, the PFS of ceritinib was 16.6 months, which is longer than that of first-line crizotinib but shorter than that of first-line alectinib. Both ceritinib and alectinib showed significant tumor shrinkage after crizotinib failure; however, it is believed that crizotinib has limited efficacy after alectinib or ceritinib because most crizotinib-resistant mutations are sensitive to alectinib or ceritinib, but not vice versa. In addition, brigatinib or next-generation ALK-TKIs have been shown to be effective for almost all single crizotinib, ceritinib, or alectinib-resistant mutations. Therefore, in the near future, it would be important to select the best sequencing therapy on the basis of resistance mechanisms. As the number of *ALK*-rearranged lung cancer patients is relatively small, each drug has a relatively long PFS period, and a number of ALK-TKIs have been developed recently. Currently, the median overall survival of ALK-TKI-treated patients is >4 years.[75] Therefore, it is almost impossible to decide the order of ALK-TKIs based on overall survival. Thus, it remains very difficult to decide which drug should be used first.

Approximately one-third of ALK-TKI-treated patients acquire resistance to TKIs through bypass pathway activation. Presently, however, there is no clear clinical evidence of the effectiveness of ALK-TKI combination therapy with an inhibitor that targets bypass pathways, such as cMET or cKIT. Thus, further studies are needed to identify bypass pathways and new effective therapeutic strategies to overcome the resistance. In addition, tumor heterogeneity is a difficult hurdle that must be overcome. Multiple resistance mechanisms, such as multiple mutations, are often observed in 1 patient. Moreover, recently collected data from NGS analysis of circulating tumor DNA samples has shown dynamic changes in therapy-persistent/resistant cancers. Thus, further studies are needed in the following areas:

- Elucidation of unidentified resistance mechanisms in approximately 25% of resistant cases.

- Resistance mechanisms and fusion variants (are G1202R mutations only seen in EML4-ALK-variant 3 patients?).
- Sensitive detection methods to identify the resistance mechanisms from blood (and tissues).
- Heterogeneity and dominance of the populations with resistant tumors.
- Drugs to overcome compound-resistant mutations to lorlatinib.
- Up-front combination therapy to effectively eradicate persistent cancer cells.
- Understanding the immune checkpoint in *ALK*-rearranged lung cancer.

In addition, it is also important to fully understand the mechanisms and functions of ALK tyrosine kinase itself and the downstream signaling of ALK, including the substrates of ALK fusion proteins. A recent study showed that some drug-resistant cells have drug-dependent growth characteristics, called "drug addiction," instead of oncogene addiction. In the drug-addicted-resistant cells, TKI removal induced hyperactivation of oncogene signaling, which resulted in induction of apoptosis. Moreover, intermittent kinase inhibitor treatment has been shown to prolong survival in vivo. Indeed, intermittent kinase inhibitor treatment strategies in *BRAF* mutant melanoma have been sought for in vitro and in vivo studies and evaluation in clinical trials. Thus, even in *ALK*-rearranged lung cancer, distinct treatment strategies might enhance the efficacy of ALK-TKIs. The use of ALK-TKIs has dramatically improved the prognosis of *ALK*-rearranged lung cancer, but a cure has not been found. Therefore, further studies are needed to develop better therapies.

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CONFLICTS OF INTEREST

The author has no conflict of interest.

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REFERENCES

1. Morris SW, Kirstein MN, Valentine MB, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science*. 1994;263:1281-1284.
2. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature*. 2007;448:561-566.
3. Chen Y, Takita J, Choi YL, et al. Oncogenic mutations of ALK kinase in neuroblastoma. *Nature*. 2008;455:971-974.
4. Mosse YP, Laudenslager M, Longo L, et al. Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature*. 2008;455:930-935.
5. Murugan AK, Xing M. Anaplastic thyroid cancers harbor novel oncogenic mutations of the ALK gene. *Cancer Res*. 2011;71:4403-4411.
6. Janoueix-Lerosey I, Lequin D, Brugieres L, et al. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature*. 2008;455:967-970.
7. Mathew P, Morris SW, Kane JR, et al. Localization of the murine homolog of the anaplastic lymphoma kinase (ALK) gene on mouse chromosome 17. *Cytogenet Cell Genet*. 1995;70:143-144.
8. Stoica GE, Kuo A, Aigner A, et al. Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. *J Biol Chem*. 2001;276:16772-16779.
9. Stoica GE, Kuo A, Powers C, et al. Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. *J Biol Chem*. 2002;277:35990-35998.
10. Murray PB, Lax I, Reshetnyak A, et al. Heparin is an activating ligand of the orphan receptor tyrosine kinase ALK. *Sci Signal*. 2015;8:ra6.
11. Guan J, Umapathy G, Yamazaki Y, et al. FAM150A and FAM150B are activating ligands for anaplastic lymphoma kinase. *Elife*. 2015;4:e09811.
12. Reshetnyak AV, Murray PB, Shi X, et al. Augmentor alpha and beta (FAM150) are ligands of the receptor tyrosine kinases ALK and LTK: hierarchy and specificity of ligand-receptor interactions. *Proc Natl Acad Sci USA*. 2015;112:15862-15867.
13. Iwahara T, Fujimoto J, Wen D, et al. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. *Oncogene*. 1997;14:439-449.
14. Vernersson E, Khoo NK, Henriksson ML, Roos G, Palmer RH, Hallberg B. Characterization of the expression of the ALK receptor tyrosine kinase in mice. *Gene Expr Patterns*. 2006;6:448-461.
15. Bilsland JG, Wheeldon A, Mead A, et al. Behavioral and neurochemical alterations in mice deficient in anaplastic lymphoma kinase suggest therapeutic potential for psychiatric indications. *Neuropsychopharmacology*. 2008;33:685-700.
16. Weiss JB, Xue C, Benice T, Xue L, Morris SW, Raber J. Anaplastic lymphoma kinase and leukocyte tyrosine kinase: functions and genetic interactions in learning, memory and adult neurogenesis. *Pharmacol Biochem Behav*. 2012;100:566-574.
17. Soda M, Takada S, Takeuchi K, et al. A mouse model for EML4-ALK-positive lung cancer. *Proc Natl Acad Sci USA*. 2008;105:19893-19897.
18. Maddalo D, Manchado E, Concepcion CP, et al. In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. *Nature*. 2014;516:423-427.
19. Bresler SC, Weiser DA, Huwe PJ, et al. ALK mutations confer differential oncogenic activation and sensitivity to ALK inhibition therapy in neuroblastoma. *Cancer Cell*. 2014;26:682-694.
20. Takeuchi K, Choi YL, Togashi Y, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. *Clin Cancer Res*. 2009;15:3143-3149.
21. Anai S, Takeshita M, Ando N, et al. A case of lung adenocarcinoma resistant to crizotinib harboring a novel EML4-ALK variant, exon 6 of EML4 fused to exon 18 of ALK. *J Thorac Oncol*. 2016;11:e126-e128.
22. Zheng Z, Liebers M, Zhelyazkova B, et al. Anchored multiplex PCR for targeted next-generation sequencing. *Nat Med*. 2014;20:1479-1484.

23. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med*. 2010;363:1693-1703.
24. Camidge DR, Bang YJ, Kwak EL, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. *Lancet Oncol*. 2012;13:1011-1019.
25. Blackhall F, Ross Camidge D, Shaw AT, et al. Final results of the large-scale multinational trial PROFILE 1005: efficacy and safety of crizotinib in previously treated patients with advanced/metastatic ALK-positive non-small-cell lung cancer. *ESMO Open*. 2017;2:e000219.
26. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med*. 2014;371:2167-2177.
27. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med*. 2013;368:2385-2394.
28. Costa DB, Kobayashi S, Pandya SS, et al. CSF concentration of the anaplastic lymphoma kinase inhibitor crizotinib. *J Clin Oncol*. 2011;29:e443-e445.
29. Chuan Tang S, Nguyen LN, Sparidans RW, Wagenaar E, Beijnen JH, Schinkel AH. Increased oral availability and brain accumulation of the ALK inhibitor crizotinib by coadministration of the P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) inhibitor elacridar. *Int J Cancer*. 2014;134:1484-1494.
30. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. *Nat Med*. 2012;18:378-381.
31. Sakamoto H, Tsukaguchi T, Hiroshima S, et al. CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. *Cancer Cell*. 2011;19:679-690.
32. Kodama T, Tsukaguchi T, Hasegawa M, et al. Selective ALK inhibitor alectinib (CH5424802/RO5424802) with potent antitumor activity in models of crizotinib resistance, including intracranial metastases AACR annual meeting 2014. 2014; 754.
33. Seto T, Kiura K, Nishio M, et al. CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study. *Lancet Oncol*. 2013;14:590-598.
34. Gadgeel SM, Gandhi L, Riely GJ, et al. Safety and activity of alectinib against systemic disease and brain metastases in patients with crizotinib-resistant ALK-rearranged non-small-cell lung cancer (AF-002JG): results from the dose-finding portion of a phase 1/2 study. *Lancet Oncol*. 2014;15:1119-1128.
35. Gainor JF, Sherman CA, Willoughby K, et al. Alectinib salvages CNS metastases in ALK-positive lung cancer patients previously treated with crizotinib and ceritinib. *J Thorac Oncol*. 2015;10:232-236.
36. Hida T, Nokihara H, Kondo M, et al. Alectinib versus crizotinib in patients with ALK-positive non-small-cell lung cancer (J-ALEX): an open-label, randomised phase 3 trial. *Lancet*. 2017;390:29-39.
37. Peters S, Camidge DR, Shaw AT, et al. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med*. 2017;377:829-838.
38. Friboulet L, Li N, Katayama R, et al. The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer. *Cancer Discov*. 2014;4:662-673.
39. Marsilje TH, Pei W, Chen B, et al. Synthesis, structure-activity relationships, and in vivo efficacy of the novel potent and selective anaplastic lymphoma kinase (ALK) inhibitor 5-chloro-N2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N4-(2-(isopropylsulf onyl) phenyl)pyrimidine-2,4-diamine (LDK378) currently in phase 1 and phase 2 clinical trials. *J Med Chem*. 2013;56:5675-5690.
40. Shaw AT, Kim DW, Mehra R, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med*. 2014;370:1189-1197.
41. Crino L, Ahn MJ, De Marinis F, et al. Multicenter phase II study of whole-body and intracranial activity with ceritinib in patients with ALK-rearranged non-small-cell lung cancer previously treated with chemotherapy and crizotinib: results from ASCEND-2. *J Clin Oncol*. 2016;34:2866-2873.
42. Shaw AT, Kim TM, Crino L, et al. Ceritinib versus chemotherapy in patients with ALK-rearranged non-small-cell lung cancer previously given chemotherapy and crizotinib (ASCEND-5): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol*. 2017;18:874-886.
43. Soria JC, Tan DSW, Chiari R, et al. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet*. 2017;389:917-929.
44. Cho BC, Kim DW, Bearz A, et al. ASCEND-8: a randomized phase 1 study of ceritinib, 450 mg or 600 mg, taken with a low-fat meal versus 750 mg in fasted state in patients with anaplastic lymphoma kinase (ALK)-rearranged metastatic non-small cell lung cancer (NSCLC). *J Thorac Oncol*. 2017;12:1357-1367.
45. Lim SM, Kim HR, Lee JS, et al. Open-label, multicenter, phase II study of ceritinib in patients with non-small-cell lung cancer harboring ROS1 rearrangement. *J Clin Oncol*. 2017;35:2613-2618.
46. Katayama R, Khan TM, Benes C, et al. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. *Proc Natl Acad Sci USA*. 2011;108:7535-7540.
47. Gettinger SN, Bazhenova LA, Langer CJ, et al. Activity and safety of brigatinib in ALK-rearranged non-small-cell lung cancer and other malignancies: a single-arm, open-label, phase 1/2 trial. *Lancet Oncol*. 2016;17:1683-1696.
48. Kim DW, Tiseo M, Ahn MJ, et al. Brigatinib in patients with crizotinib-refractory anaplastic lymphoma kinase-positive non-small-cell lung cancer: a randomized, multicenter phase II trial. *J Clin Oncol*. 2017;35:2490-2498.
49. Gainor JF, Dardaei L, Yoda S, et al. Molecular mechanisms of resistance to first- and second-generation ALK inhibitors in ALK-rearranged lung cancer. *Cancer Discov*. 2016;6:1118-1133.
50. Uchibori K, Inase N, Araki M, et al. Brigatinib combined with anti-EGFR antibody overcomes osimertinib resistance in EGFR-mutated non-small-cell lung cancer. *Nat Commun*. 2017;8:14768.
51. Shaw AT, Felip E, Bauer TM, et al. Lorlatinib in non-small-cell lung cancer with ALK or ROS1 rearrangement: an international, multicentre, open-label, single-arm first-in-man phase 1 trial. *Lancet Oncol*. 2017;18:1590-1599.
52. Drilon A, Siena S, Ou SI, et al. Safety and antitumor activity of the multitargeted pan-TRK, ROS1, and ALK inhibitor entrectinib: combined results from two phase I trials (ALKA-372-001 and STARTRK-1). *Cancer Discov*. 2017;7:400-409.
53. Lovly CM, Heuckmann JM, de Stanchina E, et al. Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinase inhibitors. *Cancer Res*. 2011;71:4920-4931.
54. Mori M, Ueno Y, Konagai S, et al. The selective anaplastic lymphoma receptor tyrosine kinase inhibitor ASP3026 induces tumor regression and prolongs survival in non-small cell lung cancer model mice. *Mol Cancer Ther*. 2014;13:329-340.
55. Ott GR, Cheng M, Learn KS, et al. Discovery of clinical candidate CEP-37440, a selective inhibitor of focal adhesion kinase (FAK) and anaplastic lymphoma kinase (ALK). *J Med Chem*. 2016;59:7478-7496.
56. Choi YL, Soda M, Yamashita Y, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N Engl J Med*. 2010;363:1734-1739.
57. Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. *Sci Transl Med*. 2012;4:120ra17.
58. Lovly CM, Pao W. Escaping ALK inhibition: mechanisms of and strategies to overcome resistance. *Sci Transl Med*. 2012;4:120 ps2.

59. Sasaki T, Koivunen J, Ogino A, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. *Cancer Res*. 2011;71:6051-6060.
60. Katayama R, Friboulet L, Koike S, et al. Two novel ALK mutations mediate acquired resistance to the next-generation ALK inhibitor alectinib. *Clin Cancer Res*. 2014;20:5686-5696.
61. Zou HY, Friboulet L, Kodack DP, et al. PF-06463922, an ALK/ROS1 inhibitor, overcomes resistance to first and second generation ALK inhibitors in preclinical models. *Cancer Cell*. 2015;28:70-81.
62. Shaw AT, Friboulet L, Leshchiner I, et al. Resensitization to crizotinib by the lorlatinib ALK resistance mutation L1198F. *N Engl J Med*. 2016;374:54-61.
63. Doebele RC, Pilling AB, Aisner DL, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. *Clin Cancer Res*. 2012;18:1472-1482.
64. Lovly CM, McDonald NT, Chen H, et al. Rationale for co-targeting IGF-1R and ALK in ALK fusion-positive lung cancer. *Nat Med*. 2014;20:1027-1034.
65. Crystal AS, Shaw AT, Sequist LV, et al. Patient-derived models of acquired resistance can identify effective drug combinations for cancer. *Science*. 2014;346:1480-1486.
66. Isozaki H, Ichihara E, Takigawa N, et al. Non-small cell lung cancer cells acquire resistance to the ALK inhibitor alectinib by activating alternative receptor tyrosine kinases. *Cancer Res*. 2016;76:1506-1516.
67. Kogita A, Togashi Y, Hayashi H, et al. Activated MET acts as a salvage signal after treatment with alectinib, a selective ALK inhibitor, in ALK-positive non-small cell lung cancer. *Int J Oncol*. 2015;46:1025-1030.
68. Tanimoto A, Yamada T, Nanjo S, et al. Receptor ligand-triggered resistance to alectinib and its circumvention by Hsp90 inhibition in EML4-ALK lung cancer cells. *Oncotarget*. 2014;5:4920-4928.
69. Sato W, Fukazawa N, Nakanishi O, et al. Reversal of multidrug resistance by a novel quinoline derivative, MS-209. *Cancer Chemother Pharmacol*. 1995;35:271-277.
70. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y. Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res*. 1981;41:1967-1972.
71. Katayama R, Sakashita T, Yanagitani N, et al. P-glycoprotein mediates ceritinib resistance in anaplastic lymphoma kinase-rearranged non-small cell lung cancer. *EBioMedicine*. 2016;3:54-66.
72. Hrustanovic G, Olivas V, Pazarentzos E, et al. RAS-MAPK dependence underlies a rational polytherapy strategy in EML4-ALK-positive lung cancer. *Nat Med*. 2015;21:1038-1047.
73. Cha YJ, Cho BC, Kim HR, Lee HJ, Shim HS. A case of ALK-rearranged adenocarcinoma with small cell carcinoma-like transformation and resistance to crizotinib. *J Thorac Oncol*. 2016;11:e55-e58.
74. Fujita S, Masago K, Katakami N, Yatabe Y. Transformation to SCLC after treatment with the ALK inhibitor alectinib. *J Thorac Oncol*. 2016;11:e67-e72.
75. Gainor JF, Tan DS, De Pas T, et al. Progression-free and overall survival in ALK-positive NSCLC patients treated with sequential crizotinib and ceritinib. *Clin Cancer Res*. 2015;21:2745-2752.

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