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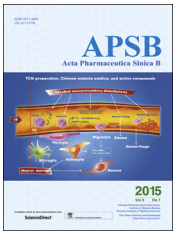

REVIEW

Alectinib: a novel second generation anaplastic lymphoma kinase (ALK) inhibitor for overcoming clinically-acquired resistance

Zilan Song, Meining Wang, Ao Zhang*

CAS Key Laboratory of Receptor Research, Synthetic Organic & Medicinal Chemistry Laboratory, Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences, Shanghai 201203, China

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Abstract The development of inhibitors for the tyrosine anaplastic lymphoma kinase (ALK) has advanced rapidly, driven by biology and medicinal chemistry. The first generation ALK inhibitor crizotinib was granted US FDA approval with only four years of preclinical and clinical testing. Although this drug offers significant clinical benefit to the ALK-positive patients, resistance has been developed through a variety of mechanisms. In addition to ceritinib, alectinib is another second-generation ALK inhibitor launched in 2014 in Japan. This drug has a unique chemical structure bearing a 5*H*-benzo[*b*]carbazol-11(6*H*)-one structural scaffold with an IC_{50} value of 1.9 nmol/L, and is highly potent against ALK bearing the gatekeeper mutation L1196M with an IC_{50} of 1.56 nmol/L. In the clinic, alectinib is highly efficacious in treatment of ALK-positive non-small cell lung cancer (NSCLC), and retains potency to combat crizotinib-resistant ALK mutations L1196M, F1174L, R1275Q and C1156Y.

*Corresponding author. Tel./fax: +86 21 50806035.
E-mail address: aozhang@simm.ac.cn (Ao Zhang).

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1. EML4-ALK—an emerging oncogenic drug target

Anaplastic lymphoma kinase (ALK), a member of the insulin receptor superfamily, was originally identified in 1994 in anaplastic large-cell lymphoma (ALCL) cell lines as a tyrosine kinase component of a chromosomal translocation[1], which fuses the entire nucleophosmin (NPM) gene on chromosome 5 to the 3′-position of the ALK gene. Subsequently, more than two dozen additional gene rearrangements or mutations have been disclosed as different oncogenic forms of ALK across diverse tumor types, including the echinoderm microtubule-associated protein-like 4 (EML4)-ALK in non-small cell lung cancer (NSCLC). The fusion gene of *EML4-ALK*, containing the N-terminal half of *EML4* and the intracellular catalytic domain of *ALK*, was identified in 2007 by two independent groups[2,3]. Although the occurrence of this fusion protein was found in only 5% of NSCLCs, the large number of NSCLC patients makes *EML4-ALK* the most prevalent *ALK* gene rearrangement, accounting for over 11,000 new occurrences per year in the USA[4].

The landmark discovery of *EML4-ALK* as an oncogene was reported by Soda et al.[3] in 2007, who found that mouse 3T3 fibroblasts forced to express this human fusion tyrosine kinase generated transformed foci in culture and subcutaneous tumors in nude mice. Clinically, the patient population harboring epidermal growth factor receptor (*EGFR*) mutations was found to have no overlap with the population harboring the *EML4-ALK* fusion gene, suggesting that *EML4-ALK*-positive cancer represents a distinct subclass of NSCLC. Therefore, targeting EML4-ALK may provide a new strategy to treat the subclass of NSCLC patients, for whom current treatments are poorly effective[5,6].

2. First-generation ALK inhibitor and its acquired resistance

Crizotinib, a 3,5-disubstituted 2-aminopyridine (PF-2341066) developed by Pfizer as a c-Met inhibitor initially, was identified as a potent ALK inhibitor and advanced rapidly in the clinic[7,8]. It received fast-track FDA approval in 2011 as the first generation ALK inhibitor (Xalkori™) for the treatment of advanced non-small cell lung cancer (NSCLC) harboring *ALK* rearrangements. In clinical studies, crizotinib showed an objective response rate (ORR) of 60% and a median progression-free survival (PFS) of 9.7 months[9]. However, the early success of this drug was shadowed by disease relapses in the majority of crizotinib-treated patients within one year, under various mechanisms including *ALK* fusion gene amplification, secondary ALK kinase domain mutations, activation of bypass signaling pathways (EGFR, c-Kit), and others.

The development of secondary kinase mutations clustering around the ATP binding site of the EML4-ALK rearrangement is likely the most important mechanism underling the resistance to crizotinib[10–12]. L1196M and C1156Y were the earliest two secondary ALK mutations conferring resistance to crizotinib identified clinically. The L1196M mutation corresponds to the 'gatekeeper' mutation, whereas the C1156Y mutation is located in the αC-helix near the upper edge of the ATP-binding site. The former mutation is analogous to the mutation T315I in ABL and the mutation T790M in EGFR, where the original amino acid moiety was replaced with a bulky one to interfere with the binding of an inhibitor. However, unlike the well-established T790M mutation that accounts for the majority of the secondary EGFR mutations of the acquired resistance, the resistance to crizotinib is much more complicated, and a large number of other mutations with a similar frequency of occurrence is found later in different resistant patients, including G1269A, L1152R, G1202R, F1174C, I1171T, S1206Y and the T1151 insertion (1156T-ins). These secondary mutations account for roughly one-third of the acquired resistance of currently discovered crizotinib-resistant patients. Therefore, development of new generation ALK inhibitors to combat the acquired resistance to crizotinib treatment is of urgent need[13,14].

3. Alectinib—a novel second generation ALK inhibitor to combat resistance

To date, several structurally distinct small molecules have been developed as second generation ALK inhibitors, including LDK-378 (ceritinib), CH-5424802 (alectinib), PF-06463922, X-396, AP26113 and TSR-011[13–15]. On April 29, 2014, the FDA granted ceritinib accelerated approval for the treatment of patients with ALK-positive metastatic NSCLC who experience disease progression or who are intolerant to crizotinib (Fig. 1) [16,17]. Very recently, the Japanese Ministry of Health, Labor and Welfare (MHLW) approved alectinib for the treatment of people living with NSCLC that is *ALK* fusion gene-positive (ALK^+) (Fig. 1). Although most of the new inhibitors were not originally designed to target the secondary mutations conferring the resistance to crizotinib treatment, they were highly potent against both wild and mutant *ALK* kinases.

Alectinib is a unique second generation ALK inhibitor bearing a 5*H*-benzo[*b*]carbazol-11(6*H*)-one structural scaffold, initially developed by the Japanese company Chugai (a subsidiary of Roche). It originated from the company's high throughput screening program and contains a naphtha-[2,3-*b*]benzofuran-11(6*H*)-one framework. Replacement of the benzofuran fragment with an indole moiety, followed by optimization of the solvent interaction region as well as adjustment of the E_0 region of the ATP binding site to improve the kinase potency, selectivity and the pharmacokinetic properties, led to the compound alectinib (Fig. 1)[18,19]. It inhibited ALK with an IC_{50} value of 1.9 nmol/L and showed higher selectivity for ALK than for a number of other serine/tyrosine kinases. More importantly, alectinib also inhibited the ALK gatekeeper mutation L1196M with an IC_{50} of 1.56 nmol/L. Although the co-crystallization of alectinib with L1196M mutant was not reported, its structure with wild ALK kinase showed that the C3-cyano moiety has a critical role to interact with the kinase by forming H-bonds and CH/π hydrophobic interactions. In the KARPAS-299 (lymphoma), NB-1 (neuroblastoma) and NCI-H2228 (lung cancer) ALK-positive cell lines, alectinib inhibited cell proliferation with IC_{50} values of 3, 4.5 and 53 nmol/L, respectively[19,20]. It is an ATP-competitive ALK inhibitor, and dose-dependently inhibited EML4-ALK positive NCI-H2228 xenograft model at doses ranging from 2 to 20 mg/kg *p.o.*, *q.d.* Significant efficacy was also achieved in the EML4-ALK L1196M-driven tumors[20].

Since 2010, clinical trials with alectinib were started in ALK positive patients with locally advanced or metastatic NSCLC in the US. In a multicentre, single-arm, open-label, phase I–II study in Japan, patients with ALK-rearranged advanced NSCLC were recruited and given alectinib orally twice daily. In the phase I setting, 24 patients were treated at doses of 20–300 mg twice daily, and no dose-limiting toxicities (DLTs) or adverse events of grade 4 were observed. In the phase II setting with alectinib dosed

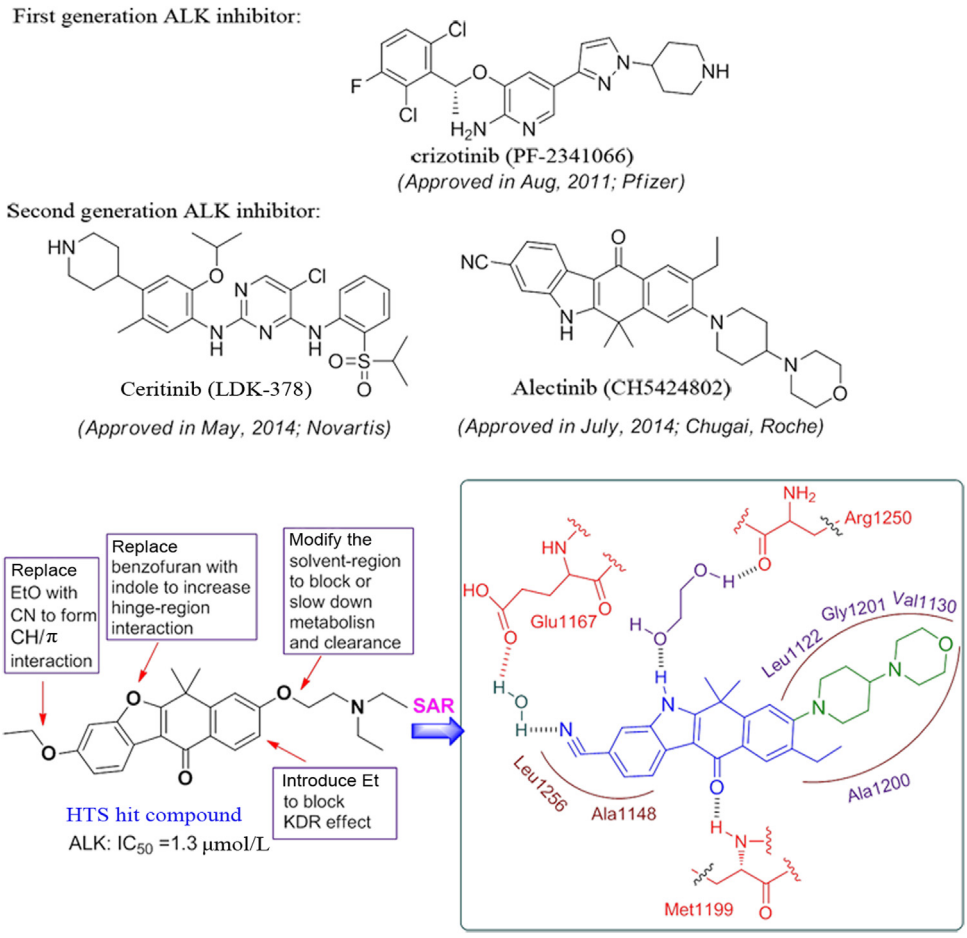

Figure 1 Chemical structures of approved ALK inhibitors.

at 300 mg twice daily, almost 94% of patients achieved an objective response and early reduction in tumor size of at least 30% was noted in most patients within the first 6 weeks. The proportion of patients who achieved an objective response for alectinib is substantially higher than that of crizotinib (60.8% and 53%) in two separate early phase trials. Since 2012, phase I and phase II a studies were conducted in patients who had failed treatment with crizotinib and two dose-limiting toxicities were observed in the 900 mg BID cohort. An overall response rate of 59% was reached with one complete response and 14 confirmed partial responses (PRs). A randomized, active-controlled, open-label, phase III study was initiated in July 2014 in the US, Australia, Europe and many other countries with treatment-naive ALK-positive advanced NSCLC[21].

The Japanese Ministry of Health, Labor and Welfare (JMHLW) granted alectinib Orphan Drug designation in 2013, and Chugai filed an NDA with the JMHLW for ALK fusion gene-positive NSCLC. Alectinib was quickly reviewed by the Japanese Pharmaceutical Affairs and Food Sanitation Council's Second Committee on Drugs and received the NDA's approval within two months. This led to approval of alectinib in September 2014 in Japan for ALK-positive NSCLC.

4. Conclusions and perspectives

The development of inhibitors for ALK has been advanced rapidly through biology, and medicinal chemistry. The first generation

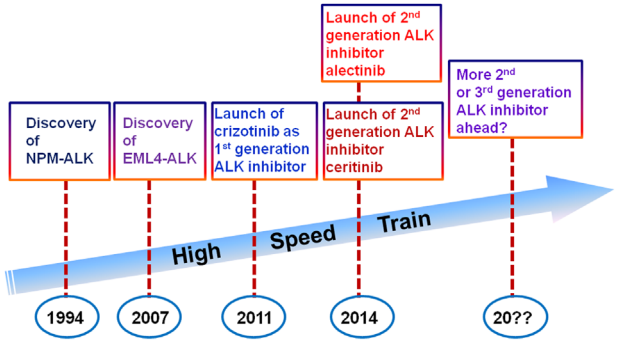

Figure 2 ALK is on the high-speed train.

ALK inhibitor crizotinib received FDA approval with only four years of preclinical and clinical testing since the discovery of the tumor-addicted oncogene *EML4-ALK*. The year of 2014 has been very fruitful with the launch of the two second generation ALK inhibitors ceritinib and alectinib (Fig. 2). In comparison with crizotinib, both ceritinib and alectinib are highly potent and selective against ALK *in vitro* and *in vivo*. Notably, the Novartis drug ceritinib effectively inhibits ALK harboring L1196M, G1269A, I1171T and S1206Y, but is ineffective in G1202R and F1174C, the other two crizotinib-resistant ALK mutations. The newly approved Roche drug alectinib is effective with crizotinib-resistant ALK mutations L1196M, F1174L, R1275Q and C1156Y.

In view of the wide spectrum of ALK mutations identified after crizotinib treatment, more second generation ALK inhibitors with efficacy against other mutations will be needed. Meanwhile, development of new inhibitors with the capacity to penetrate the central nervous system (CNS) also would be important, since many lung cancers will eventually spread to the brain. Although the final outcome of these second generation ALK inhibitors has to wait for the benefit of a larger sample size of patients, more and more inhibitors are already on the way.

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