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

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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 5H-benzo[b]carbazol-11(6H)-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.

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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, 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. 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.

The landmark discovery of EML4-ALK as an oncogene was reported by Soda et al. 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.

2. **First-generation ALK inhibitor and its acquired resistance**

Crizotinib, a 3,5-disubstituted 2-aminoypyridine (PF-2341066) developed by Pfizer as a c-Met inhibitor initially, was identified as a potent ALK inhibitor and advanced rapidly in the clinic. It received fast-track FDA approval in 2011 as the first generation ALK inhibitor and its acquired resistance to crizotinib has been characterized by secondary mutations (ALK gatekeeper mutation L1196M with an IC50 of 1.56 nmol/L). It inhibited ALK with an IC50 value of 1.9 nmol/L and showed higher selectivity for ALK than for a number of other serine/threonine kinases. More importantly, crizotinib also inhibited the ALK gatekeeper mutation L1196M-driven tumors.

Since 2010, clinical trials with crizotinib were started in ALK positive patients with locally advanced or metastatic NSCLC in the US. In a multicentre, single-arm, open-label, phase I dose escalation trial, 24 patients were treated at doses of 200–300 mg twice daily, and no dose-limiting toxicities (DLTs) or adverse events of grade 4 were observed. In the phase II setting with crizotinib dosed 1196M-driven tumors.

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. Alectinib is a unique second generation ALK inhibitor bearing a 5H-benzol[b]carbazol-11(6H)-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(6H)-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 Eo region of the ATP binding site to improve the kinase potency, selectivity and the pharmacokinetic properties, led to the compound alectinib. It inhibited ALK with an IC50 value of 1.9 mmol/L and showed higher selectivity for ALK than for a number of other serine/threonine kinases. More importantly, alectinib also inhibited the ALK gatekeeper mutation L1196M with an IC50 of 1.56 mmol/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 IC50 values of 3, 4.5 and 53 mmol/L, respectively. 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.

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...
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 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.

The Japanese Ministry of Health, Labor and Welfare (JMHLW) granted alectinib Orphan Drug designation in 2013, and Chugai led 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 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.

Figure 1 Chemical structures of approved ALK inhibitors.

Figure 2 ALK is on the high-speed train.
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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References

1. Morris SW, Kirstein MN, Valentine MB, Dittmer KG, Shapiro DN, Saltman DL, et al. Fusion of a kinase gene, ALK, to a nuclear protein gene, NPM, in non-Hodgkin’s lymphoma. Science 1994;263:1281–4.

2. Rikova K, Guo AL, Zeng QF, Possemato A, Yu J, Haack H, et al. Global survey of phosphoryryosine signaling identifies oncogenic kinases in lung cancer. Cell 2007;131:1190–203.

3. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561–6.

4. Roskoski Jr. R. Anaplastic lymphoma kinase (ALK): structure, oncogenic activation, and pharmacological inhibition. Pharmacol Res 2013;68:68–94.

5. Shaw AT, Solomon B. Targeting anaplastic lymphoma kinase in lung cancer. Clin Cancer Res 2011;17:2081–6.

6. Gridelli C, Peters S, Sgambato A, Casaluce F, Adjei AA, Ciardiello F. ALK inhibitors in the treatment of advanced NSCLC. Cancer Treat Rev 2014;40:300–6.

7. Cui JJ, Tran-Dubé M, Shen H, Nambu M, Kung PP, Pairish M, et al. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal—epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). J Med Chem 2011;54:6342–63.

8. Cui JJ, McTigue M, Kania R, Edwards MP. Case history: Xalkori™ (crizotinib), a potent and selective dual inhibitor of mesenchymal epithelial transition (MET) and anaplastic lymphoma kinase (ALK) for cancer treatment. Ann Rep Med Chem 2013;48:421–32.

9. Shaw AT, Yeap BY, Solomon BJ, Riely GJ, Gainor J, Engelman JA, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. Lancet Oncol 2011;12:1004–12.

10. Doebele RC, Pilling AB, Aisner DL, Kutateladze TG, Le AT, Weickhardt AJ, et al. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res 2012;18:1472–82.

11. Kinoshita K, Oikawa N, Tsukuda T. Anaplastic lymphoma kinase inhibitors for the treatment of ALK-positive cancers. Ann Rep Med Chem 2012;47:281–93.

12. Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med 2010;363:1734–9.

13. Ramalingam S, Khuri FP. Second generation ALK inhibitors: filling the non ‘MET’ gap. Cancer Disc 2014;4:634–6.

14. Wang WC, Shiao HY, Lee CC, Fung KS, Hsieh HP. Anaplastic lymphoma kinase (ALK) inhibitors: a review of design and discovery. Med Chem Commun 2014;5:1266–79.

15. Awad MM, Shaw AT. ALK inhibitors in non-small cell lung cancer: crizotinib and beyond. Clin Adv Hematol Oncol 2014;12:429–39.

16. Marsilje TH, Wei P, Chen B, Lu W, Uno T, Jin Y, 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-isoproxy-5-methyl-4-(piperidin-4-yl)phenyl)-N4-(2-(isoproxyphenyl)phenyl)pyrimidim-2, 4-diamine (LDK378) currently in phase 1 and phase 2 clinical trials. J Med Chem 2013;56:5675–90.

17. Shaw AT, Kim DW, Mehrara B, Tan DS, Felip E, Chow LQ, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. N Engl J Med 2014;370:1189–97.

18. Latif M, Saeed A, Kim SH. Journey of the ALK-inhibitor CH5424802 to phase II clinical trial. Arch Pharm Res 2013;36:1051–4.

19. Kinoshita K, Kobayashi T, Asoh K, Furuiichi N, Ito T, Kawada H, et al. 9-Substituted 6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b] carbazoles as highly selective and potent anaplastic lymphoma kinase inhibitors. J Med Chem 2011;54:6286–94.

20. Sakamoto H, Tsukaguchi T, Hiroshima S, Kodama T, Kobayashi T, Fukami TA, et al. CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. Cancer Cell 2011;19:679–90.

21. Seto T, Kiura K, Nishio M, Nakagawa K, Maemondo M, Inoue A, 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;14:590–8.