CHAPTER NINETEEN

Anaplastic Lymphoma Kinase Inhibitors for the Treatment of ALK-Positive Cancers

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Contents

1. INTRODUCTION

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase belonging to the insulin receptor superfamily. *ALK* was originally identified as a part of the fusion oncogene nucleophosmin *(NPM)-ALK* resulting from a *t*(2;5) chromosomal translocation in anaplastic large cell lymphomas (ALCL).[1] The fusion gene *NPM-ALK* is detected in approximately 75% of all *ALK*-positive ALCL and is implicated in the pathogenesis of ALCL.[2] The function of ALK in normal human tissues is unclear but it appears to play a role in physiological development and function of the nervous system.[3] Importantly, *ALK* knockout mice indicated no overt developmental, anatomical, or locomotor deficiencies; therefore, inhibition of ALK does not lead to serious disorders.

The discovery of echinoderm microtubule-associated protein-like 4 *(EML4)-ALK* fusion gene in nonsmall cell lung cancer (NSCLC) led to ALK emerging as a novel drug target for cancer therapy.[4] This was the first

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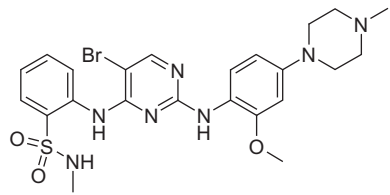
report of an oncogenic fusion gene in a solid tumor. Thus, just as breakpoint cluster region-Abelson *(BCR-ABL)* is a key oncogenic factor in chronic myeloid leukemias (CML),[5] *EML4-ALK* is the key oncogenic factor in *EML4-ALK*-positive NSCLC.[6] *EML4-ALK* has been detected in approximately 5% of NSCLC patients and is mutually exclusive for known oncogenic mutated endothelial growth factor receptor *(EGFR)* and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog *(K-RAS)*, which are identified as oncogenic drivers in NSCLC.[7] One study revealed that *EML4-ALK*-positive patients had a lower response rate to platinum-based chemotherapy than patients who have an EGFR mutation.[8] Further, 2,4-pyrimidinediamine ALK inhibitor **1** inhibits the growth of EML4-ALK tumors in transgenic mice.



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Other fusion genes of ALK have been identified not only in ALCL and NSCLC but also in inflammatory myofibroblastic tumors[9] and diffuse large B-cell lymphomas.[10] Furthermore, in childhood neuroblastoma, it was found that genetic mutations R1275Q and F1174L were activated, and the ALK gene was amplified.[11]

Thus, ALK is an attractive drug target for the treatment of various *ALK*-positive cancers in both blood and solid tumors, and a number of ALK inhibitors have been developed. Carrying out structural studies facilitated rational drug design efforts, and consequently seven crystal structures of human ALK in complex with small molecule inhibitors have been published thus far. Five inhibitors, namely crizotinib **2** (PDB ID 2XP2),[12] CH5424802 **3** (PDB ID 3AOX),[13] NVP-TAE684 **4** (PDB ID 2XB7),[14] PHA-E429 **5** (PDB ID 2XBA), and piperidine carboxamide **6** (PDB ID 4DCE),[15] have been designed to achieve target selectivity (Fig. 19.1). They are all ATP-competitive inhibitors and contain hinge-binding moieties that compete with the adenine base of ATP in order to interact with the ATP-binding site, commonly referred to as the hinge region. These inhibitors form one to three hydrogen bonds with the hinge region (marked by dotted lines), where the number of hydrogen bonds presumably affects their kinase selectivity.[16]

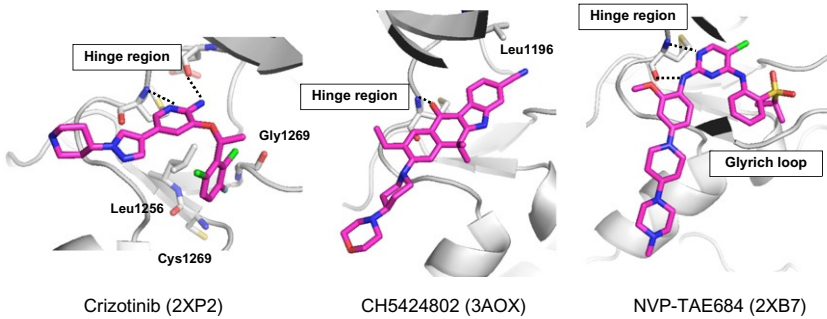
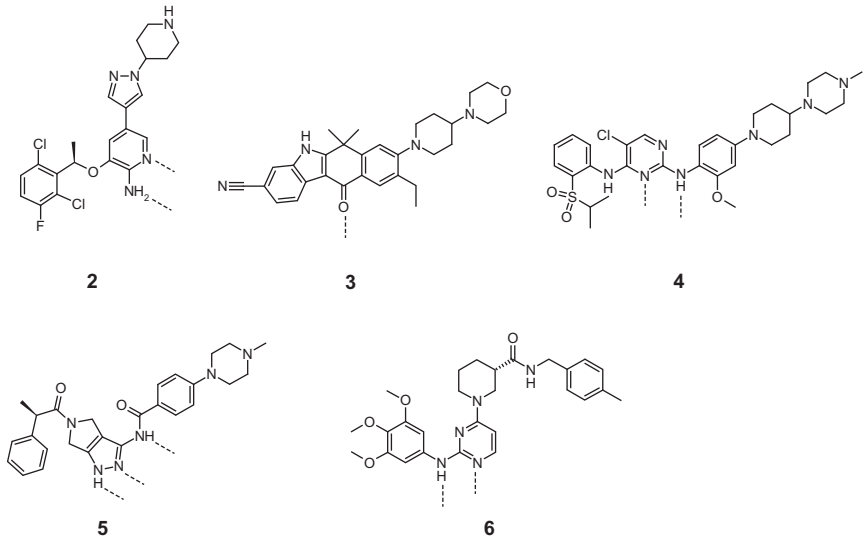


Figure 19.1 Binding modes of representative ALK inhibitors.



2. CRIZOTINIB (XALKORI®)

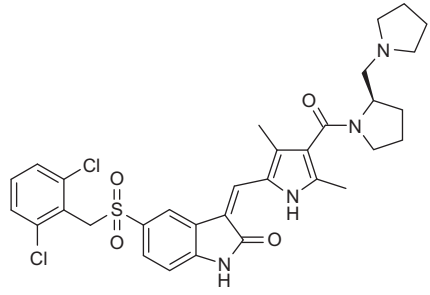
Crizotinib (PF-02341066) by Pfizer is the first marketed ALK inhibitor, which was originally designed in a c-Met inhibitor program. Pfizer's researchers screened kinase inhibitors, 3-substituted indoline-2-ones, to identify a potent and selective c-Met inhibitor PHA-665752 (**7**). In order to improve physicochemical properties of **7**, they re-engineered the core scaffold by structure-based drug design (SBDD) and produced the novel 5-aryl-3-benzyloxy-2-aminopyridine core. Further, optimization of the substituents led to crizotinib as a potent c-Met inhibitor, which also demonstrated potent

inhibitory activity against the ALK enzyme and an *NPM-ALK*-positive cell line, with IC_{50} values of < 1.0 and 20 nM, respectively.

One of the remarkable structural characteristics of crizotinib is an L-shaped conformation in the binding state (Fig. 19.1), which is driven by a methyl group attached to the chiral benzylic position. The hinge-binding motif of crizotinib, 2-aminopyridine, is located above Leu1256 and forms two hydrogen bonds with the hinge region, with the attached 2,6-dichloro-3-fluorophenyl moiety bending down into a hydrophobic binding pocket formed by amino acid residues including Cys1255, Leu1256, and Gly1269. The hydrophobic binding pocket of ALK and c-Met (PDB ID 2WGJ) are formed by a unique activation loop, which seems to contribute to the selectivity observed with ALK inhibitors.

When the *EML4-ALK* fusion oncogene was reported, Pfizer was conducting a Phase I study of crizotinib for c-Met-targeted cancer therapy. In a new clinical study, they immediately tested the same inhibitor for the treatment of *EML4-ALK*-positive NSCLC. The Phase I clinical trial of crizotinib for *EML4-ALK*-positive NSCLC patients demonstrated an overall response rate of 57%, and a 72% probability of progression-free survival (PFS) for 6 months.[17] The U.S. Food and Drug Administration approved crizotinib for the treatment of patients with locally advanced or metastatic *ALK*-positive NSCLC in August 2011.[18] Thus, time between discovery of the novel drug target and approval was only 4 years, which is surprisingly short.

It was also noteworthy that crizotinib was concurrently approved with a fluorescence *in situ* hybridization (FISH) diagnostic test, the Vysis® ALK Break-Apart FISH Probe Kit (Abbott Molecular, Inc.) to help identify the minor population of patients with the ALK fusion gene (ca. 5%) from the total NSCLC patients. Other diagnostic modalities such as immunohistochemistry and reverse transcriptase-polymerase chain reaction are also being explored in order to establish a reliable and cost-effective *ALK*-positive NSCLC screening.[19]



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3. ACQUIRED CRIZOTINIB RESISTANCE

One of the main issues of tyrosine kinase inhibitors is acquired drug resistance. This means that although many patients with *ALK*-positive NSCLC obtain clinical benefit from crizotinib treatment, the effect is relatively short-lasting and most patients treated with crizotinib develop acquired resistance eventually. In a Phase I clinical trial of crizotinib, the median duration of response is 48 weeks and PFS is 10 months, although median overall survival has not been reached as of the data cut off.[20]

A secondary point mutation is a well-known mechanism of acquired drug resistance against kinase inhibitors. A so-called gatekeeper of kinases, the amino acid residue adjacent to the hinge region, and point mutations of the gatekeeper often diminish the inhibitory activity of kinase inhibitors. T315I mutation in BCR-ABL[21] and T790M in EGFR[22] are typical examples of gatekeeper mutations. The gatekeeper of ALK is Leu1196, and L1196M mutation not only hampers crizotinib from interacting with the kinase domain but also enhances catalytic efficiency.[23]

In addition to the mutation L1196M, various types of secondary mutations within the kinase domain from *ALK*-positive NSCLC tumors have been identified from patients who were treated with crizotinib. Cys1156 and Lys1152 are located far from the ATP-contact surface, and these residues are unlikely to interact with crizotinib directly. Therefore, C1156Y and L1152R mutations appear to induce resistance by modifying the kinetics of ALK's conformation change.[24] The presence of a secondary mutation, G1269A, reduces the binding affinity of crizotinib by sterically hindering the binding event.[25] In addition, G1202R, S1206Y, and 1151Tins mutations were recently identified in crizotinib-resistant patients.[26] Gly1202 and Ser1206 residues are located at the solvent front of the ATP-binding pocket, and the mutation of these amino acids may also lead to steric hindrance of the crizotinib binding event. The T1151 insertion located far from the crizotinib contact surface is speculated to change the affinity of ALK for ATP. Amplification of the ALK fusion gene, aberrant activation of other oncogenic drivers by K-RAS mutation, amplification of KIT, and increased autophosphorylation of EGFR are other mechanisms of crizotinib resistance. In a subset of patients, multiple resistance mechanisms develop simultaneously, creating a formidable problem.

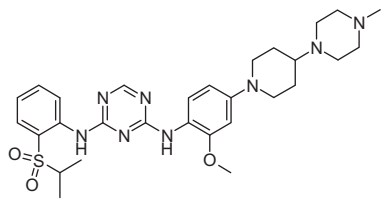
4. CLINICAL CANDIDATES

Four ALK inhibitors, namely LDK378 (Phase I),[27] AP26113 (Phase I/II),[28] ASP3026 (Phase I, **8**)[29], and CH5424802 (Phase I/II, **3**), are in clinical trials for the treatment of tumors. Only the structures of CH5424802 and ASP3026 have been disclosed.

LDK378, created by Novartis, is a selective ALK inhibitor with weak inhibitory activity against c-Met. In the *EML4-ALK*-positive cell line, NCI-H2228 mouse xenograft model, oral administration of LDK378 resulted in tumor regression, and after 14 days of treatment with LDK378, tumor regrowth did not occur for the monitored period of 4 months. LDK378 also demonstrated *in vivo* efficacy against the crizotinib-resistant mouse model (developed by treating the NCI-H2228 xenograft model with crizotinib).

AP26113, created by Ariad, is approximately fivefold more potent *in vitro* than crizotinib: IC_{50} values of AP26113 and crizotinib are 0.62 and 3.6 nM, respectively. AP26113 demonstrated *in vivo* antitumor efficacy in the crizotinib-resistant EML4-ALK mutant mouse xenograft models.[30]

ASP3026 (**8**), created by Astellas, is an analog of NVP-TAE684 (**4**), which was generated by Novartis.[31] The only structural difference between these compounds is a hinge-binding motif (1,3,5-triazine vs. 5-chloropyrimidine, respectively). In the NCI-H2228 mouse xenograft model, oral administration of ASP3026 resulted in tumor regression.



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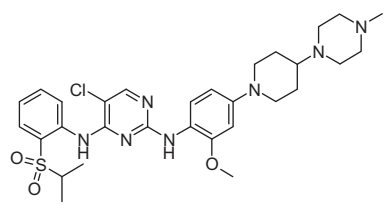

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A highly selective ALK inhibitor created by Chugai, CH5424802 (**3**), is designed by SBDD from a high-throughput screening hit compound.[32] CH5424802 showed potent inhibitory activity against ALK (IC_{50} of 1.9 nM) and the *NPM-ALK*-positive cell line, KARPAS-299 (IC_{50} of 3.0 nM), but demonstrated weak or no inhibition against 24 other protein kinases. In an NCI-H2228 mouse xenograft model, once-daily oral administration of CH5424802 for 11 days resulted in dose-dependent tumor

growth inhibition and tumor regression (168% tumor growth inhibition at 20 mg/kg/day). Further, tumor regrowth did not occur throughout the following 4-week drug-free period. In a Ba/F3 cell growth assay, CH5424802 demonstrated cell growth inhibition against L1196M and C1156Y, which are both reported crizotinib mutants. In addition, in a mouse model bearing Ba/F3 EML4-ALK harboring L1196M, oral administration of CH5424802 showed tumor growth inhibition at 60 mg/kg once daily for 8 days. CH5424802's high ALK selectivity is a result of steric repulsion between the 9-ethyl group of CH5424802 and non-ALK kinases, as ALK has a wide-open surface at the entrance of its ATP-binding pocket.[33]

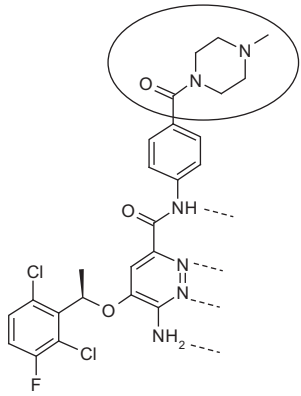
5. PRECLINICAL CANDIDATES

NVP-TAE684 (**4**) was identified before *EML4-ALK* was discovered. The compound was developed to treat ALCL by targeting NPM-ALK and identified in a cellular assay that screened for compounds with selective cytotoxicity against *NPM-ALK*-transformed Ba/F3 cells. The compound showed good antiproliferative activity against Ba/F3 NPM-ALK cells (IC_{50} of 3 nM) and very weak activity against Ba/F3 cells that had constitutively activated tyrosine kinases (IC_{50} values of >500 nM).

Interestingly, comparison of NVP-TAE684 to crizotinib in the two *EML4-ALK*-positive NSCLC cell lines, NCI-H2228 and NCI-H3122, revealed that NVP-TAE684 was a more potent inhibitor than crizotinib in the NSCLC models.[34] In the NCI-H2228 xenograft model, NVP-TAE684 showed complete tumor regression within a week (at 10 mg/kg), whereas crizotinib had no effect on the tumor growth at the same dose. In the NCI-H3122 xenograft mouse model, treatment with NVP-TAE684 at 50 mg/kg resulted in tumor regression, whereas treatment with crizotinib at the same dose demonstrated marginal tumor growth inhibition.

Xcovery reported the discovery of two ALK inhibitors bearing the aminopyridazine scaffold, X-376 (**9**) and X-396 (structure not disclosed).[35] X-376 has the same hydrophobic moiety as that of crizotinib (2,6-dichloro-3-fluoro-phenylethoxy group) but a unique hinge-binding motif (6-aminopyridazine-3-carboxamide). The significant difference between crizotinib and X-376 is in the hydrophilic side chains (circled). The binding model of X-376 with ALK suggests that the compound forms two more hydrogen bonds with ALK than crizotinib, using the pyridazine nitrogen and amide nitrogen (marked by dotted lines), which led a significant increase in affinity of X-376 over crizotinib for ALK. In addition, X-376 and X-396

demonstrated more potent antiproliferative activity against the *EML4-ALK*-positive cell line, H3122, than crizotinib (IC_{50} = 77, 15, and 180 nM, respectively). In the H3122 xenograft mouse model, twice-daily oral administration of either X-376 (50 mg/kg) or X-396 (25 mg/kg) resulted in tumor growth inhibition without body weight loss. The efficacy is comparable to that of crizotinib at 50 mg/kg, bid.



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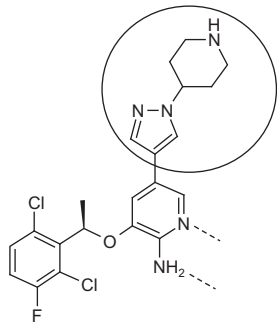

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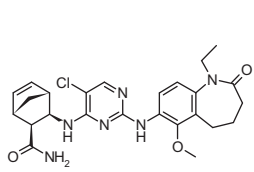
Cephalon recently disclosed three unique inhibitors derived from NVP-TAE684 (**4**). One of the inhibitors (**10**) is a compound bearing a bicyclo [2.2.1]hept-5-ene ring system instead of an aryl moiety.[36] The compound showed potent inhibitory activity against ALK (IC_{50} = 14 nM) and antiproliferative activity against the *NPM-ALK*-positive cell line, KARPAS-299 (IC_{50} = 45 nM). On the other hand, compound **10** had very weak inhibitory activity against the structurally related insulin receptor kinase (InsR) (IC_{50} = 597 nM), achieving > 40-fold selectivity for ALK. Furthermore, in *ALK*-positive ALCL xenografts (SUP-M2) in SCID mice, oral administration of compound **10** resulted in dose-dependent antitumor efficacy (10–55 mg/kg, bid) without overt toxicity. In this study, complete/near-complete tumor regressions were observed at 55 mg/kg, bid.

The second unique inhibitor, **11**, has a 2,7-disubstituted-pyrrolo[2,1-f][1,2,4]triazine scaffold that mimics the bioactive conformation of a diaminopyrimidine inhibitor such as NVP-TAE684 (**4**).[37] Compound **11** also had potent inhibitory activity against ALK (IC_{50} of 10 nM) and KARPAS-299 (IC_{50} of 60 nM). On the other hand, **11** showed more than 100-fold weaker inhibitor activity against InsR than that of ALK. In addition, in the same xenograft models as those used to test compound **10**,

twice-daily oral administration of **11** resulted in 35%, 81%, and 98% tumor growth inhibition at 10, 30, and 55 mg/kg, respectively.

The third compound (**12**) is a macrocyclic ALK inhibitor designed to lock the diaminopyrimidine scaffold into its bioactive conformation.[38] Compound **12** demonstrated strong inhibitory activity against ALK (IC_{50} of 0.51 nM) and cellular activity with inhibition of NPM-ALK phosphorylation ($IC_{50} = 10$ nM). Currently, no *in vivo* data of **12** have been reported.



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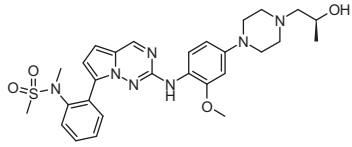

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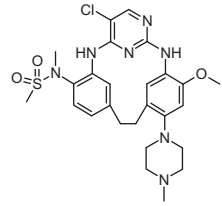

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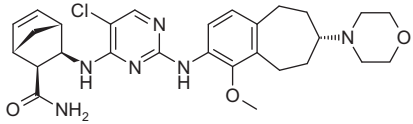
Recently, Cephalon disclosed the structure of CEP-28122 (**13**), which is an analog of compound **10**, but with a difference in the solvent accessible moiety (morpholino group).[39] The *in vitro* profile of CEP-28122 was improved over that for compound **10**,[40] where CEP-28122 showed potent inhibitory activity against ALK ($IC_{50} = 1.9$ nM) and very weak inhibition toward InsR ($IC_{50} = 1257$ nM). In ALK-positive ALCL xenografts (SUP-M2) in SCID mice, CEP-28122 demonstrated dose-dependent antitumor activity (3–30 mg/kg, po, bid) without overt toxicity. Furthermore, in a 4-week antitumor study using the same xenograft model, treatment with CEP-28122 (55 and 100 mg/kg, po, bid) gave complete tumor regression and no tumor regrowth for 60 days after the cessation of treatment. In other *ALK*-positive xenograft models (NCI-H2228, NCI-H3122, and NB-1), CEP-28122 also demonstrated dose-dependent antitumor efficacy (30 and 55 mg/kg, po, bid in each study).



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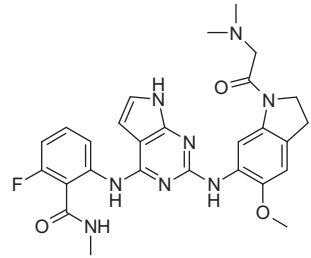

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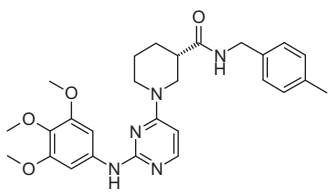
GSK1838705A (**14**) created by GSK has a similar scaffold to NVP-TAE684. The compound has a potent inhibitory activity against ALK, insulin-like growth factor-1 receptor (IGF-1R), and InsR (IC_{50} of 0.5, 2.0, and 1.6 nM, respectively).[41] Despite strong inhibitory activity against IGF-1R and InsR, **14** showed a transient and modest effect on blood glucose levels. Once-daily oral administration of **14** in *ALK*-positive ALCL xenograft mouse models (KARPAS-299 and SR-786) resulted in dose-dependent antitumor effects. Tumor growth inhibition against established KARPAS-299 xenograft was 22% and 93% at 10 and 30 mg/kg/day, respectively. Tumor growth inhibition against established SR-786 xenograft was 63% and 93% at 30 and 60 mg/kg/day, respectively. In addition, excellent oral bioavailability (98%) was observed in a rat pharmacokinetic study.[42]

Amgen reported piperidine carboxamides as ALK inhibitors. High-throughput screening showed that compound **6** had moderate inhibitory activity against ALK ($IC_{50} = 0.174\ \mu M$) with selectivity over IGF-1R ($IC_{50} = 4.61\ \mu M$).[43] X-ray crystallographic analysis of the co-crystal of **6** with ALK (PDB ID 4DCE) revealed that compound **6** has a unique binding interaction resulting from the DFG sequence shift, which is observed in type I 1/2 inhibitors.[44] Type I 1/2 inhibitors recognize DFG-in conformation of a target kinase in the same manner as type I inhibitors and additionally access to the extended hydrophobic pocket created by conformation shift of DFG motif. Utilizing a parallel synthesis approach, rapid SAR development designed to improve ALK selectivity over other kinases resulted in potent ALK inhibitor **15** (ALK, IGF-1R, and KARPAS-299 with IC_{50} values = 31, 7390, and 28 nM, respectively).



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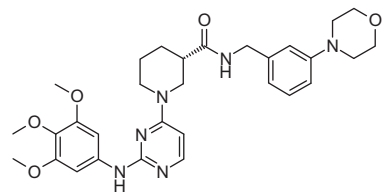

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6. CONCLUSIONS

ALK is one of the most highly competitive drug targets in oncology research. The development of ALK inhibitors was accelerated by the discovery of an oncogenic driver *EML4-ALK*, reminiscent of *BCR-ABL* in CML.

A number of research groups have identified promising drugs, where Pfizer succeeded in launching a potent ALK inhibitor, crizotinib, for treatment of *EML4-ALK*-positive NSCLC. However, acquired drug resistance caused by mutations of ALK has been identified in patients who were treated with crizotinib; therefore, many clinical and preclinical second-generation ALK inhibitors are now required to have efficacy against ALK mutants. The patient population of ALK-related cancers is not large; however, an ALK inhibitor is expected to be critically effective for particular patients who harbor a genetic ALK mutation. Therefore, genetic diagnostics play an important role in ALK-related cancer therapy and the combination of the diagnostic test and crizotinib is one of the most successful milestones in personalized therapy.

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