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Hallmarks of Anaplastic Lymphoma Kinase Inhibitors with Its Quick Emergence of Drug Resistance

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Abstract

Anaplastic lymphoma kinase (ALK) is one of the most popular targets for anticancer therapies. In the past decade, the use of anaplastic lymphoma tyrosine kinase inhibitors (ALK-TKIs), including crizotinib and ceritinib, has been a reliable and standard options for patients with lung cancer, particularly for patients with nonsmall cell lung carcinoma. ALK-targeted therapies initially benefit the patients, yet, resistance eventually occurs. Therefore, resistance mechanisms of ALK-TKIs and the solutions have become a formidable challenge in the development of ALK inhibitors. In this review, based on the knowledge of reported ALK inhibitors, we illustrated the crystal structures of ALK, summarized the resistance mechanisms of ALK-targeted drugs, and proposed potential therapeutic strategies to prevent or overcome the resistance.

Keywords
- ALK inhibitors
- drug resistance
- NSCLC

Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Over the past few decades, the identification of the small-molecule inhibitors that shut down cell signaling pathways perpetually activated by cancer-specific mutated kinases is one of the greatest success stories in the "War on Cancer."[1] Among them, the most representative is the discovery of anaplastic lymphoma kinase (ALK) inhibitors including crizotinib and ceritinib that have benefited tens of thousands of patients with nonsmall cell lung cancer

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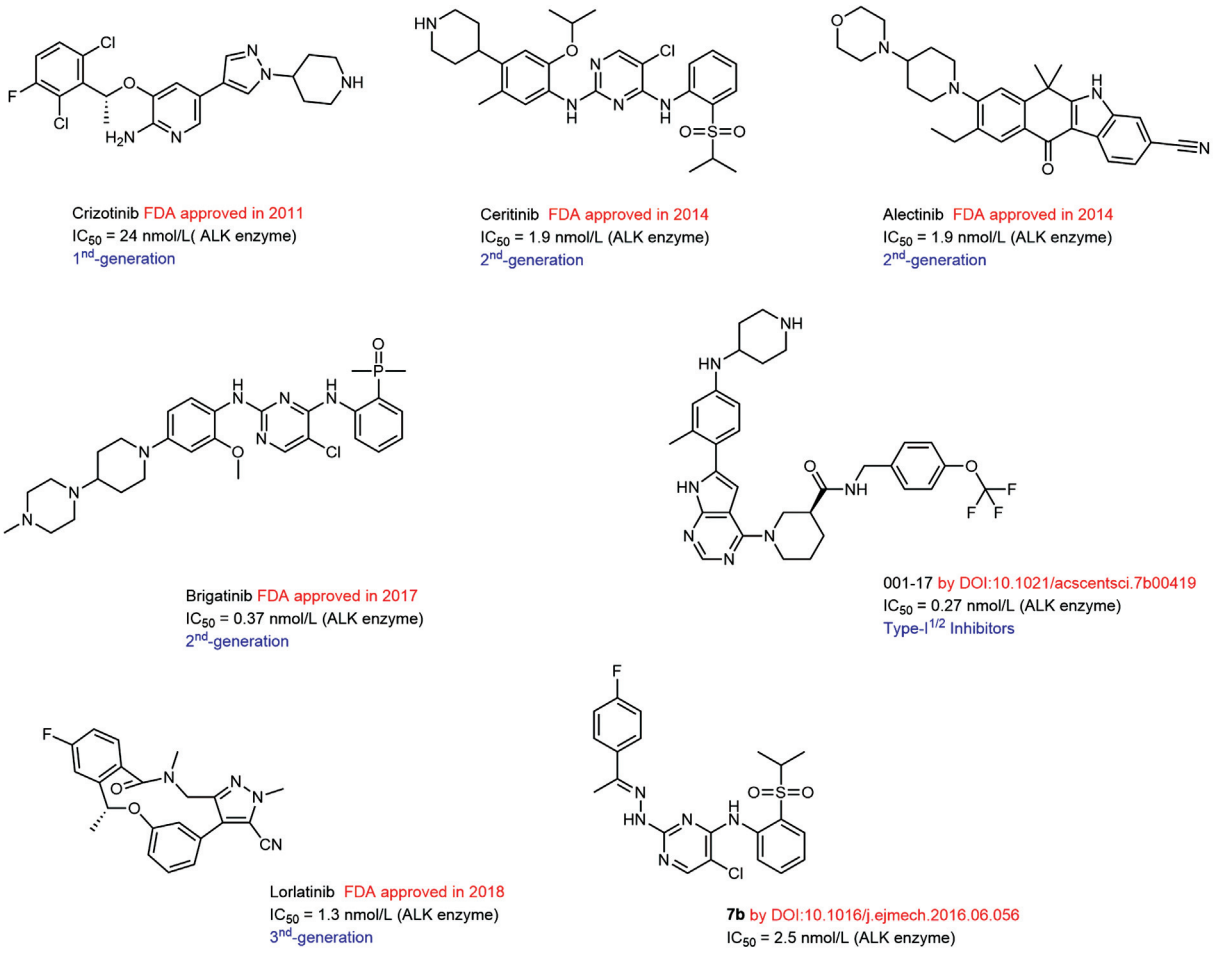

Fig. 1 Some recent development of ALK inhibitors. ALK, anaplastic lymphoma kinase.

(NSCLC) (►**Fig. 1**). The clinical use of crizotinib (Xalkori) as a prescription medicine against ALK$^+$ or ROS1$^+$ metastatic NSCLC has generated a great success. However, the emergence of drug resistance appears lately, and appropriately one-third of crizotinib-resistant patients develop point mutations within the ALK kinase domain after 1 to 2 years of treatment with the drug.[2] Initially, the most frequent gatekeeper mutation L1196M and mutation C1156Y were found in the kinase domain of EML4-ALK,[3] followed by various other resistance mutations, such as F1174L, K1062M, G1269A, G1202R, S1206Y, L1152R, and insertion mutation 1151Tins.[4] The second generation of ALK inhibitor ceritinib (LDK-378) can effectively inhibit several crizotinib-resistant mutations (e.g., L1196M and G1269A), but fails to overcome some other resistant ALK mutants, including G1202R and F1174C.[5] Inspired, lorlatinib, approved by the Food and Drug Administration (FDA) in 2018, is a third-generation macrocyclic ALK inhibitor for ALK/ROS1 cancer therapy. It is a second-line treatment for patients with advanced ALK-positive NSCLC,[6] and becomes a first-line treatment for the disease in March 2021.[7]

Originally, ALK was discovered in 1994 in anaplastic large-cell lymphomas (ALCLs) as a part of nucleophosmin (NPM)–ALK fusion protein.[8] It is a transmembrane receptor tyrosine kinase, which belongs to the member of the insulin receptor superfamily. It consists of an extracellular ligand-binding domain, a transmembrane domain, a juxtamembrane domain, an intracellular kinase domain, and a C-terminal tail. A complete picture of ALK signaling can be pieced together through the study of multiple forms of activated ALK (fusion proteins, cancer-associated mutants, and amplifications), albeit with certain challenges.[1] Interestingly, the identification of ALK fused to NPM in ALCL enabled the first roles of ALK as the fusion protein in the field of oncology.[8] ALK plays an important role in many tumor types, such as NSCLC, ALCL, inflammatory myofibroblastic tumor (IMT), and more. This makes ALK an attractive target for cancer treatment.[9] However, the efficacy of targeting ALK using ALK inhibitors, such as crizotinib mentioned above, is always limited by the quick emergence of drug resistance.[10,11] The emergence of drug resistance has prompted the discovery of a new generation of ALK inhibitors.

ALK Structure

Virtually, ALK fusion proteins share many standard features (►**Fig. 2**): (1) the transcription of the chimeric protein is

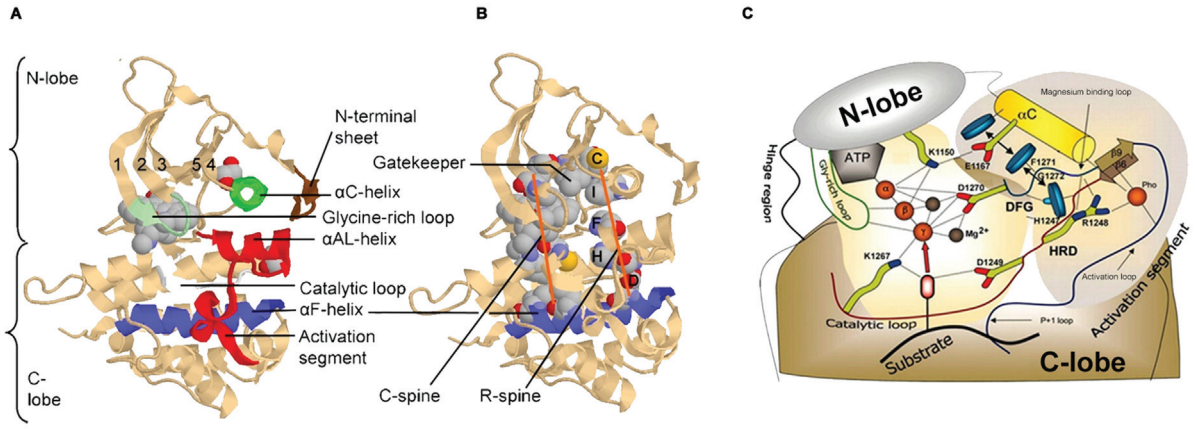

Fig. 2 Diagram of inferred interactions between human anaplastic lymphoma kinase (ALK) catalytic core residues, ATP, and a protein substrate. (**A**) Ribbon diagram of human ALK. The small lobe is dominated by a five-stranded antiparallel β-sheet, which is represented by number 1–5 in N-lobe. (**B**). Orange lines denote the residues (space-filling models) that constitute the catalytic and regulatory spines. (**C**) Two-dimensional diagram of the inferred interactions. Catalytically important residues that are in contact with ATP and the protein substrate occur within the light khaki background. Secondary structures and residues that are involved in regulation of catalytic activity occur within the gray background. Hydrophobic interactions between the HRD motif (the first D of K/D/D), the DFG motif (the second D of K/D/D), and the αC-helix are shown by the double arrows, while polar contacts are shown by dashed lines. Pho is the phosphate attached to Tyr1283. (Adapted from Roskoski 2013[14] copyright Pharmacological Research.)

driven by an ectopic/partner promoter; (2) the localization of these proteins is largely determined by the N-terminus partner region; and (3) the presence of an oligomerization domain by the ALK partner protein, which induces autophosphorylation and activation of the ALK kinase domain.[12] The N-terminal region of human ALK (h-ALK) comprises two MAM domains (amino acids 264–427 and 480–626), a low-density lipoprotein class A (LDLa) domain (amino acids 453–471), and a glycine-rich (G-rich) region (amino acids 816–940). A transmembrane-spanning segment connects the extracellular region with the protein tyrosine kinase domain (amino acids 1116–1383)-containing intracellular region. The signal peptide (amino acids 1–16), the glycine-rich domain (amino acids 63–334), and the kinase domain (amino acids 510–777) are located in the intracellular C-terminal region of the protein. The 2;5 chromosomal translocation is frequently associated with ALCLs. The translocation creates a fusion gene consisting of ALK and NPM, and the 3′ half of ALK derived from chromosome 2 is fused to the 5′ portion of NPM from chromosome 5.[13]

The ALK extracellular region contains a unique combination of domains among the RTKs, exhibiting an N-terminal signal peptide, followed by two MAM (meprin, A5 protein, and receptor protein tyrosine phosphatase mu) domains, and this is an LDLa motif and a sizable glycine-rich region proximal to the membrane.[14]

ALK Signaling

ALK signaling is a part of an extended family of proteins that control aspects of cell growth, differentiation, antiapoptotic signal, and development.[12] Similar to the great majority of typical and oncogenic tyrosine kinases, ALK fusions activate many different pathways that are strictly interconnected and overlapping, including the Ras/Raf/MEK/ERK1/2 pathway, the JAK/STAT pathway, the PI3K/Akt (PKB) pathway, and the PLC-γ pathway (►**Fig. 3**).[2,4,14]

In addition, Akt, a protein serine/threonine kinase, binds phosphatidylinositol bisphosphate or trisphosphate with high affinity, which is also known as protein kinase B (PKB) and has some contact with ALK. The Ras–ERK pathway, JAK3–STAT3 pathway, and the PI3K–Akt pathway have many points of interaction to mediate the effects of ALK activity.[14–17] The Ras–ERK pathway is essential for ALCL proliferation, whereas the JAK3–STAT3 pathway and the PI3K–Akt pathway are vital primarily for cell survival and phenotypic changes.[18]

Downstream Regulation of ALK Signaling in Cancers

ALK and its mutants, F1174L and K1062M, were found stably expressed in NIH3T3 cells, and ►**Fig. 4** shows that the downstream molecules of ALK signaling, including AKT, mammalian target of rapamycin (mTOR), sonic hedgehog, JUNB, CRKL–C3G (also known as RAPGEF1)–RAP1 GTPase, and mitogen activated protein kinase (MAPK) signaling cascades, affected cell growth, transformation, and antiapoptotic signaling.[19]

ALK Variants and Drug Resistance

Point mutations and insert mutations have become an epidemic of drug resistance problems. Crizotinib-resistant acquired secondary mutations of ALK have been identified in patients with ALK-positive NSCLC who developed disease progression.[4] EML4-ALK, for example, is an oncoprotein found in 4 to 5% of NSCLC. This fusion gene with C1156Y mutant and L1196M mutant developed independently in subclones of the tumor and conferred marked resistance to two different ALK

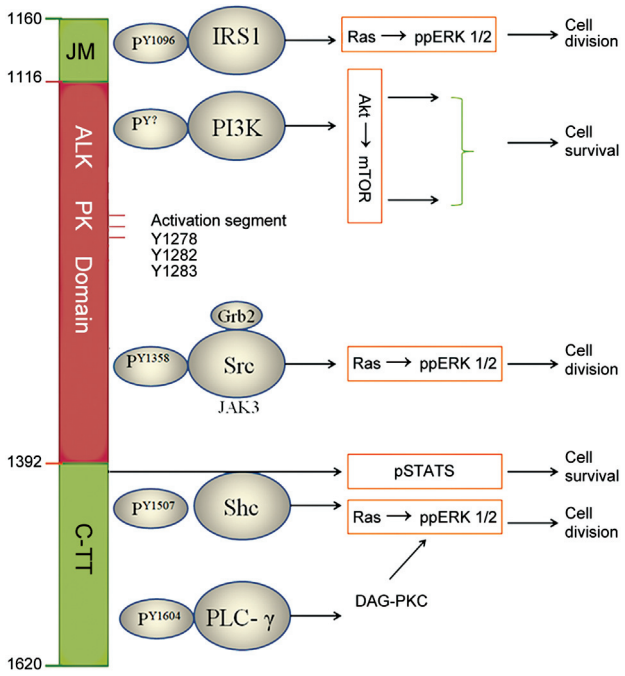

Fig. 3 ALK fusion-protein signaling pathways. Selected phosphotyrosine (pY) residues, their interacting proteins, and the relative location of the activation segment phosphorylation sites are indicated on residues corresponding to the intracellular portion of physiological ALK, which have their counterpart in the ALK fusion proteins. The numbers correspond to native human ALK amino acid residues, even though most experiments on ALK signal transduction have been performed with the NPM-ALK fusion protein. The broken arrows indicate that several steps are involved in the signaling process. DAG activates PKC. ALK, anaplastic lymphoma kinase; C-TT, C-terminal tail; DAG, diacylglycerol; ERK, extracellular-signal-regulated protein kinases; JAK Janus-activated kinase, JM, juxtamembrane; IRS1, insulin receptor substrate 1; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PK, protein kinase; PKC protein kinase C; PLC-γ, phospholipase C-γ; ppERK, bisphospho ERK; pSTAT3, phosphorylated STAT3; STAT, signal transducer and activator of transcription.

inhibitors.[3] L1196M and C1156Y are most frequent gatekeeper mutations of ALK in NSCLC patients during the relapse phase of treatment with crizotinib (Xalkori), and after that, various resistance mutations were identified, including F1174L, G1269A, G1202R, S1206Y, L1152R, R1275Q, and insertion mutation 1151Tins.[1,3,20,21] Among patients treated with the second-generation anaplastic lymphoma tyrosine kinase inhibitors (ALK-TKIs), the incidence of acquired mutations increases to 50 to 70%, with G1202R being as the most common mutation. Resistance mutations to other ALK-TKIs include G1202R/I1171N (alectinib), D1203N/E1210K (brigatinib),[22] and G1202R/F1174V/T1151K/T1151R (ceritinib).[23]

Mechanisms of acquired resistance of ALK-TKIs include ALK gene alterations, such as ALK point mutations, fusion gene copy number gain, and activation of bypass signaling through activation of other oncogenes (►**Fig. 5**).[3,11,21,24] Specific mutations (point mutations, amplification mutations, and insertion mutations) will be discussed in the following sections.

Point Mutations

Point mutations are also commonly regarded as a leading cause of drug resistance, especially in NSCLC. As the name suggests, point mutations are substitutions of one residue with another. Some point variants and cancer-associated mutations in human ALK are listed in ►**Table 1**. The most important main five-point mutations (►**Table 2**) represent that the residues Cys1156, Leu1152, Leu1196, Gly1202, Gly1269, Ser1206, Fhe1174 in C1156Y, L1152Y, L1196M, G1202R, G1269A, S1206Y, and F1174L point mutations will be replaced by tyrosine, tyrosine, methionine, arginine, alanine, tyrosine, and leucine, respectively.[25]

L1196M is the most frequent gatekeeper mutation of ALK, which is analogous to T790M in epidermal growth factor receptor (EGFR) and T315I in ABL.[37,38] To overcome crizotinib resistance to ALK L1196M, pharmacologists have designed some new second-generation ALK inhibitors, but they were unsuccessful until ceritinib was approved in 2014.[39] In addition, inhibitors of **7b** and 001–17, designed by some researchers based on target-based drug design, showed good anti-L1196M resistance mutations (►**Fig. 6**).[40,41] Their resistance to L1196M mutation may be attributed to the improved hydrophobic interactions of the inhibitors with key residues in ALK (Leu1122, Met1199, Leu 1122, Phe1271, and Lys1150), suggesting that the ensemble docking, based on multiple protein structures and target-based drug design, may be essential in the discovery of new generation of ALK-TKIs.

In recent research of crizotinib-resistant mutants of EML4-ALK, Ni et al found that F1174 is at the loop C-terminal to the α-helix C and forms a hydrophobic patch with its neighboring residues including F1271 of the DFG motif.[42] F1174L may stabilize an active conformation that is more oncogenic and less favored for crizotinib binding. F1174L mutation has been identified as an acquired secondary resistance mechanism to crizotinib and diminished crizotinib-mediated inhibition of ALK signaling and blocked apoptosis owing to the increase of adenosine triphosphate-binding affinity.[20,43] Similarly, 001–17 also induces dramatic conformational transition and stabilizes unique DFG-shifted loop conformation, enabling persistent sensitivity to different genetic mutations in ALK.

ALK-G1202K mutation may be a novel mechanism of alectinib resistance.[44] G1202R is located in the kinase domain of the ALK protein, and contributes to resistance of the first and second generation of kinase inhibitors. ALK-G1202del confers moderate resistance to second-generation ALK-TKIs. Although many cases have suggested an important role of G1202, the effect of other unknown mutation(s) at G1202 on the available ALK-TKIs remains inconclusive. Notably, lorlatinib has good clinical outcome against the highly resistant G1202R mutation, and is sensitive to three novel compound mutations found in tumor biopsies of patient (F1174L/G1202R, L1196M/D1203N, C1156Y/G1269A, G1202R/S1206Y).[45,46] However, resistance to lorlatinib has emerged in ALK-L1256F, a single mutant, which can be confirmed by some computational simulations.[47]

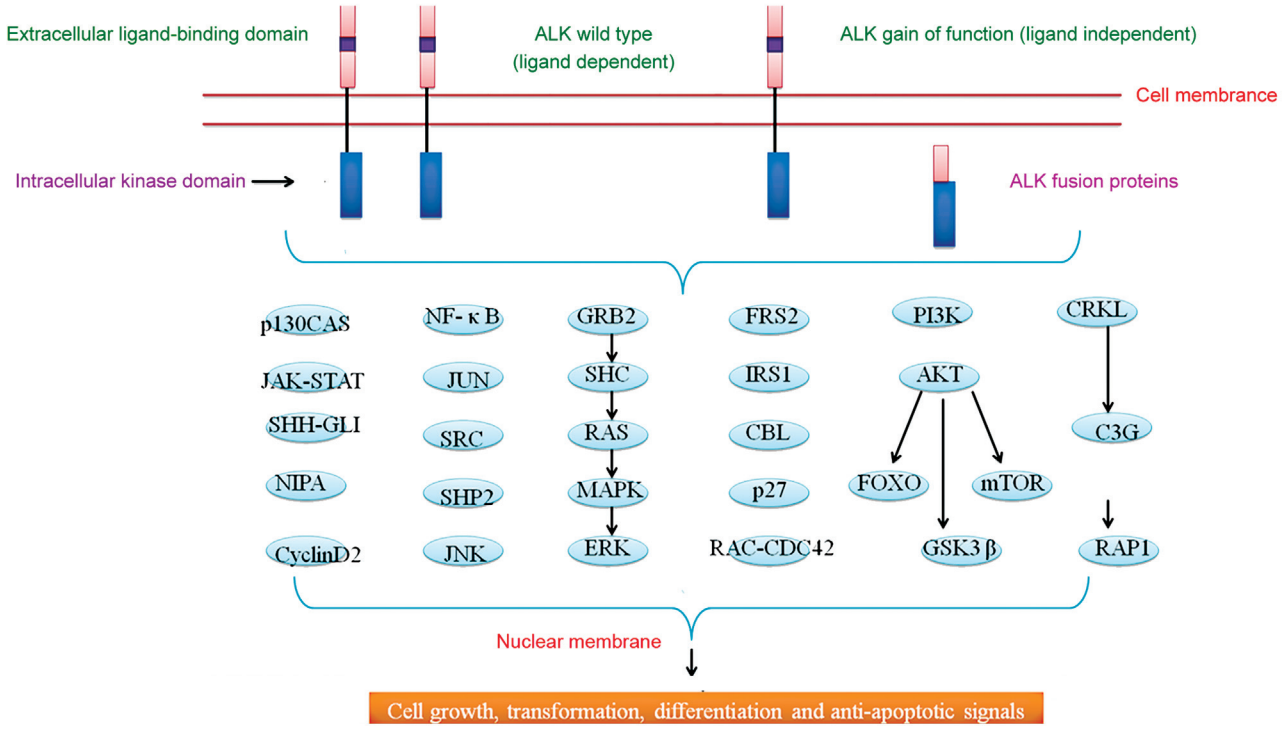

Fig. 4 Signaling downstream of ALK. ALK mediates signaling via the JAK–STAT, JUN, Ras–MAPK, PI3K–mTOR, PLCγ, and RAP1 pathways. ALK, anaplastic lymphoma kinase; CDC42, cell division control protein 42; C/EBPβ, CCAAT/enhancer-binding protein-β; FOXO, forkhead box O; FRS2, fibroblast growth factor receptor substrate 2; GRB2, growth factor receptor-bound protein 2; GSK3β, glycogen synthase kinase 3β; IRS1, insulin receptor substrate 1; JNK, JUN N-terminal kinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; NIPA, nuclear interacting partner of ALK; PLCγ, phospholipase Cγ; SHH, sonic hedgehog.

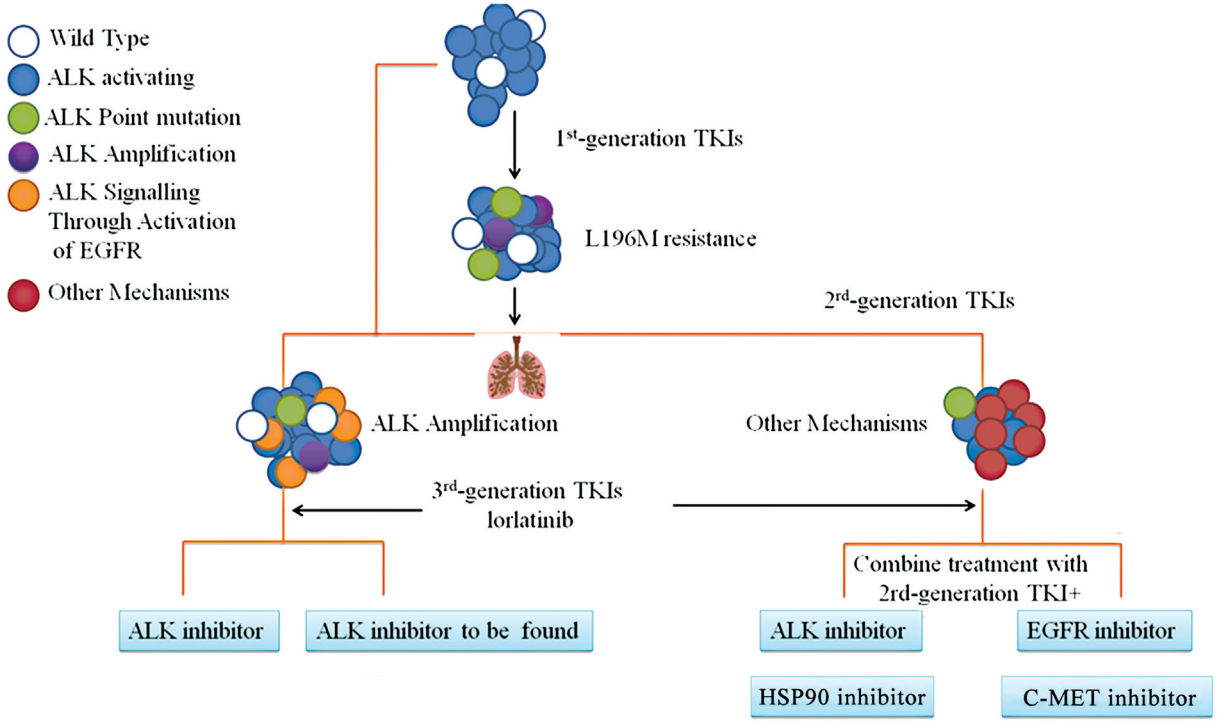

Fig. 5 Intratumoral heterogeneity and paradigm of future treatment of patients with ALK mutant NSCLC. Each colored ball represents a distinct clone with a newly acquired resistance mechanism. ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer.

Table 1 ALK point variants and cancer-associated mutations in human ALK

Point mutation	Cancer type	Domain in ALK	Effect on ALK	Refs.
L1152R	NSCLC (EAF)	Between β3 strand and αC helix	GOF	24
K1062M	Neuroblastoma	Juxtamembrane domain	GOF	19
T1087I	Neuroblastoma	Juxtamembrane domain	Ligand-dependent	3,26
D1091N	Neuroblastoma	β1 strand	Ligand-dependent	26,27
A1099T	Neuroblastoma	β2 strand	Ligand-dependent	26,27
G1128A	Neuroblastoma	P loop	GOF	27,28
T1151M	Neuroblastoma	β3 strand	Ligand-dependent	26,29
F1174L	NSCLC (EAF)	End of αC helix	GOF	20
M1166R	Neuroblastoma	αC helix	Ligand-dependent or GOF	26,27
L1196M	NSCLC (EAF)	Gateway mutation	GOF	3,11,21
I1171N	Neuroblastoma	αC helix	GOF	27,28
F1174L/S	Neuroblastoma	End of αC helix	GOF	19,27,29–32
F1174I	Neuroblastoma	End of αC helix	GOF	26,27
G1202R	NSCLC (EAF)	Between β5 strand and αD helix	GOF	21
R1192Q	Neuroblastoma	Between β4 and β5 strands	GOF	27,29
S1206Y	NSCLC (EAF)	In αD helix	GOF	21
A1234T	Neuroblastoma	αE helix	Ligand-dependent	26,29
L1240V	Neuroblastoma	αE helix	Unknown	33
F1174L + L1198P	Experimentally generated (EAF)	αC helix + between β5 strand and αD helix	GOF	34
F1174L/ G1123S/D	Experimentally generated (EAF)	αC helix + between β1 and β2 strands	GOF	34
L1198P	Experimentally generated (EAF)	Between β5 strand and αD helix	GOF	34
G1269S	Experimentally generated (EAF)	−1 to DFG	GOF	34
D1203N	Experimentally generated (EAF)	Between β5 strand and αD helix	GOF	34
Y1278H/G1123S or D	Experimentally generated (EAF)	1278-YRASYY-1283	Not determined	34
L1198F	ATC	Between β5 strand and αD helix	GOF	35
G1201E	ATC	Between β5 strand and αD helix	GOF	35
A1252V	Carcinoma of the endometrium	+3 to HRD	Not a driver	36
C1156Y	IMT (RANBP2–ALK fusion)	Between β3 strand and αC helix	GOF	3

Abbreviations: ALK, anaplastic lymphoma kinase; ATC, anaplastic thyroid cancer; EAF, EML4–ALK fusion; GOF, gain of function; IMT, inflammatory myofibroblastic tumor; NSCLC, non-small-cell lung cancer.

Faced with the endless stream of new ALK mutations, the key challenge lies in a rapid identification of which kinase domain mutations can be classified as drug resistance drivers. Thus, new technologies, such as molecular dynamics simulations, structural bioinformatics methods based on evolutionary analyses, network analysis, and machine learning, have been applied to address this issue.

With computational studies of ALK mutations, some novel point mutations have been revealed. R1192P mutation emerged at the start of the β4 strand of the kinase domain, and increases the k_{cat} in the nonphosphorylated ALK tyrosine kinase domain by 15-fold. R1192P mutation occurs not only in neuroblastic tumors, but also in advanced NSCLC,[48] and might predict sensitivity to alectinib and brigatinib.[49,50]

Insert Mutation

ALK insert mutation 1151Tins is one of the crizotinib-resistant mutations in ALK-positive NSCLC. T1151 position in the protein is shown in ►**Fig. 7**.[21] T1151 insertion is predicted to disrupt a critical hydrogen bond between T1151 and the

Table 2 The location of five-point mutations and one insertion mutation

Kinase	Location
ALK L1196M	Gatekeeper mutation
ALK C1156Y	N-terminal to the αC-helix
ALK F1174L	C-terminal to the αC-helix
ALK G1269A	ATP-binding pocket
ALK R1275Q	Vicinity of the DFG motif
ALK T1151ins	N-terminal to the αC-helix

Abbreviation: ALK, anaplastic lymphoma kinase.

carbonyl backbone of E1129. The location of E1129 on the P loop, adjacent to catalytic Lys1150, suggested that 1151Tins may lead to changes in the affinity of ALK for ATP. To date, ALK insert mutation 1151Tins has been rarely found, and only confers resistance to crizotinib and ceritinib.[5] However, in 2021, Kobayashi et al described a rare case of uterine metastasis in a patient with ALK-rearranged NSCLC.[51] 1151Tins was observed from the tissue of uterine metastasis, and was considered to be a crizotinib- and alectinib-resistant mutation. Besides, some new insert mutations have been discovered. L1196Q insertion is resistant to lorlatinib and can be detected after alectinib and ceritinib therapy.[52] P1094H insertion was acquired following crizotinib and alectinib therapy and was found to induce resistance to ceritinib.[52]

ALK Amplification Mutation

Genetic dissection revealed a hybrid gene (NPM-ALK) at the t (2;5)(p23;q35) chromosomal translocation breakpoint, comprising a fusion of a nucleolar protein gene NPM and a part of a gene coding ALK, a novel tyrosine kinase.[53] In 2007, Soda et al reported a fusion gene containing part of the EML4 gene and ALK gene in NSCLC cells.[54] These hybrid proteins undergo spontaneous dimerization, ultimately leading to a constitutive enzymatic activation of the ALK tyrosine kinase domain and autophosphorylation. Katayama et al revealed that a growing number of fusion copy of ALK gene was

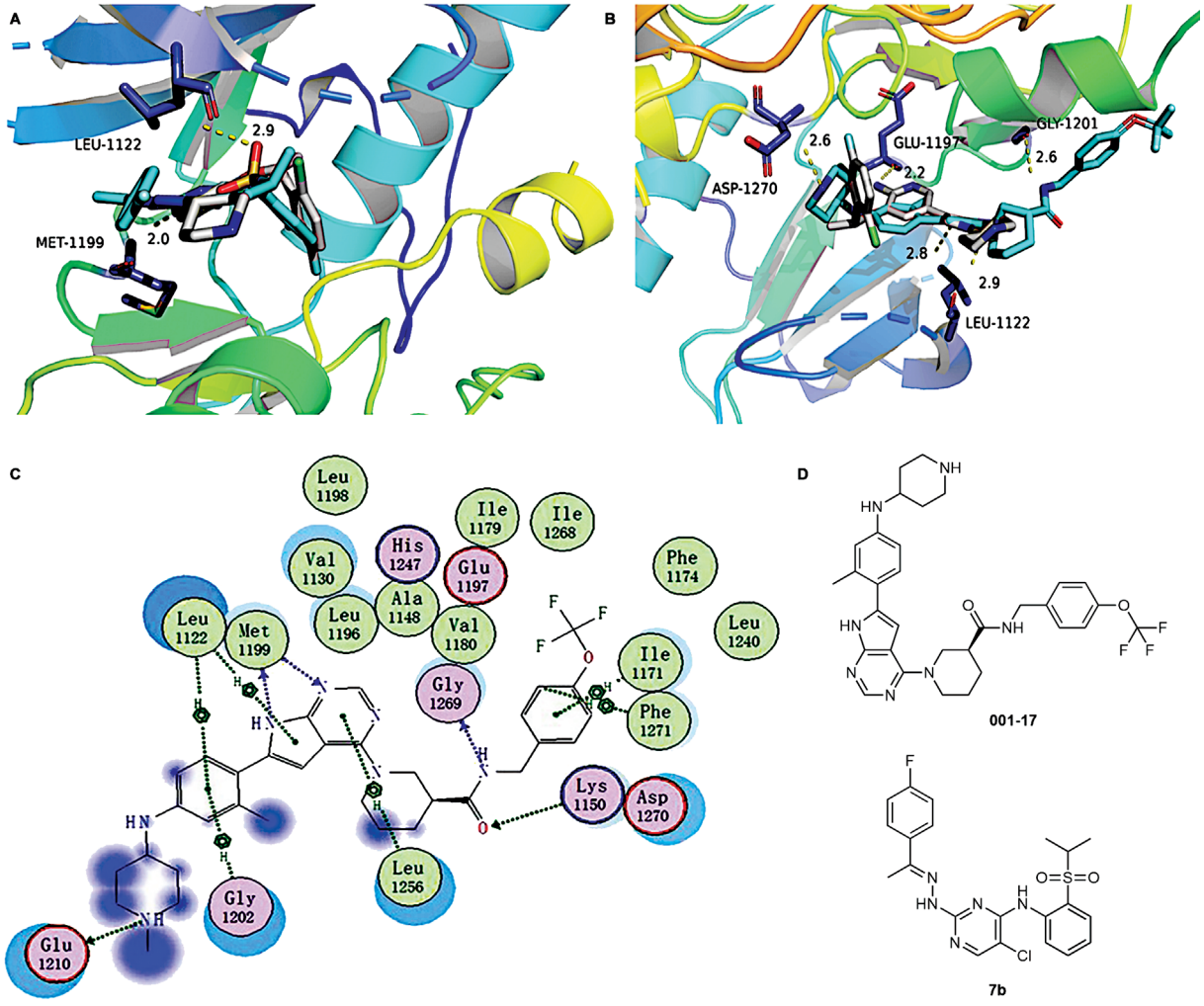

Fig. 6 Docking model of (**A**) compound **7b** (light blue) and (**B**) 001–17 (light blue) in X-ray structure of crizotinib-bound ALK (PDB ID: 2XP2). Critical hinge hydrogen bond interactions are shown in dotted lines (gray). Pymol software was used to generate the picture. (**C**) Two-dimensional schematic diagram of the binding patterns of 001–17. (**D**) Chemical structures of 001–17 and **7b**. ALK, anaplastic lymphoma kinase.

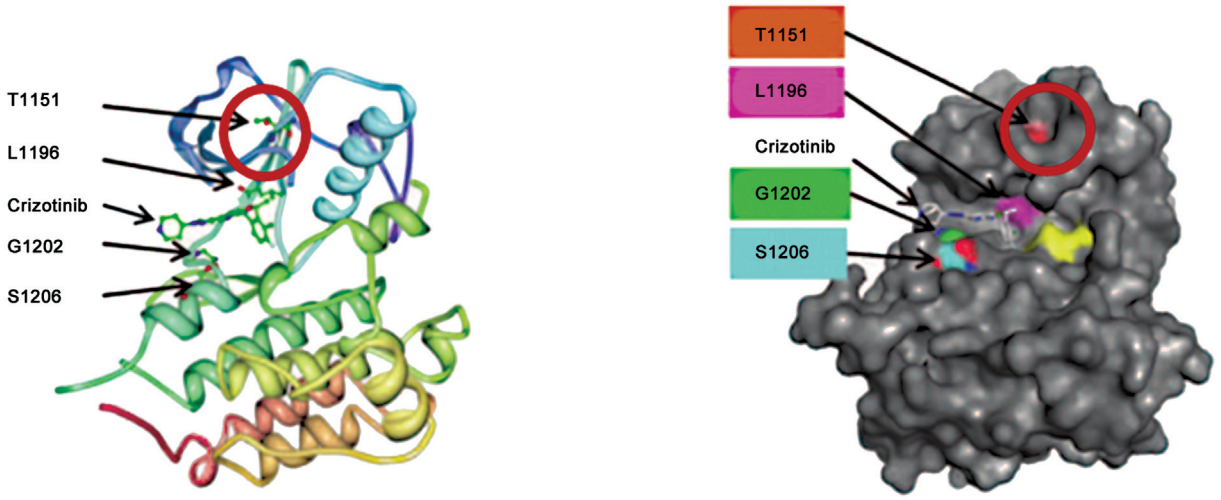

Fig. 7 Position of T1151 in the protein (in red circle). (Adapted from Katayama et al 2012[21] copyright Science Translational Medicine.)

associated with mechanisms of resistance to crizotinib in a cell model of NSCLC.[55] With the increasing adoption of next-generation sequencing, distinct fusion partners identified in ALK-positive NSCLC have expanded to approximately 90 (►**Table 3**). EML4-ALK still accounts for approximately 85% of the fusion variants in ALK-positive NSCLC. Chromosomal rearrangements in ALK gene have been also detected in ALCL, IMT, NSCLC, lung adenocarcinoma, and esophageal squamous cell cancer.

EML4-ALK v1 (E13, A20) and EML4-ALK v3a/b (E6, A20) variants account for 70 to 80% of all EML4-ALK variants, and the third most common variant is EML4-ALK v2, followed by EML4-ALK v5′.[56] Horn et al proposed that most of EML4-ALK variants confer similar level of resistance to individual ALK-TKIs.[57] Against G1202R mutation, lorlatinib and brigatinib show similar potency within the context of EML4-ALK v1.[57] Lorlatinib's potency decreased on the premise of EML4-ALK v3, and this may be caused by differences in stability of intrinsic protein among the variants. EML4-ALK v1 and v3 could form membraneless cytoplasmic granules, which act as a center for organization and activation of downstream signaling pathway components associated with resistance, like RAS.[58] Consequently, ALK-TKI's resistance is multifactorial and the background of fusion variant should be taken into consideration when interpreting ALK resistance mutations.

TP53 (tumor protein p53) is a tumor suppressor gene. TP53 mutations reduced the sensitivity of ALK-TKIs,[59] and[60] patients harboring with both TP53 mutations and EML4-ALK v3 were associated with a worse poor prognosis.[66] Preclinical data indicated that the combination of ALK-TKIs with proteasome inhibitor may be useful in generating TP53-independent apoptosis.

ALK Signaling through Activation of Other Oncogenes

ALK-TKIs are emerging as effective clinical therapies for cancers containing genetic rearrangements in ALK, including NSCLC, IMT, and ALCL. However, the clinical success of this therapeutic approach is uniformly limited by the development of drug resistance. All the different ALK fusion proteins regulate through a multitude of downstream pathways, including activation of MET, EGFR, SRC, and IGF-1R.[108]

EGFR activation is the most common downstream pathway, accounting for approximately 30% of patients with crizotinib resistance. It is mainly achieved by up-regulating the expression of EGFR and its ligands. HER2/3 belongs to the family of HER and EGFR. Some studies have found that HER3 ligand neuroregulatory protein 1 (neuroregulin1, NRG1) is overexpressed in drug-resistant crizotinib cells, which can promote the interaction between HER2 and HER3 and affect the downstream pathway, leading to the drug resistance.[21]

MET activation has been regarded as a bypass pathway in EGFR-mutant NSCLC and has been detected in 5 to 20% of resistance cases.[109] Compared with EGFR-mutant NSCLC, relatively fewer papers have made contribution of aberrant MET activation to resistance in ALK-positive NSCLC. Recently, Molina-Vila and colleagues found MET alterations in 4 out of 12 (33%) fusion-positive patients after progression on TKIs.[110] In addition, Hata and coworkers analyzed more than 200 resistance tissue and plasma specimens and discovered that approximately 15% of tumor biopsies from patients were identified MET amplification and a novel ST7-MET rearrangement has been detected in two cases. Thus, MET amplification can mediate resistance to ALK-TKIs to some extent and suggests that the ALK/ROS1/MET TKI crizotinib may be able to overcome MET-driven resistance.[111,112]

Karaca Atabay et al identified that the loss of PTPN1 and PTPN2, two kinds of protein tyrosine phosphatases, culminate in crizotinib resistance. Downstream signaling analysis showed that the deletion of PTPN1 or PTPN2 would hyperactivate SHP2, the MAPK, and JAK/STAT pathways, and lead to crizotinib resistance. Hence, a combined blockade of SHP2 potentiates the efficacy of ALK inhibitor in antiresistance.[113]

NF2 is a known tumor-suppressing gene that acts as a guardian in the Hippo signaling pathway and approximately 2% of breast cancer patients harbor NF2 mutation.[114] Friboulet and his coworkers knock out NF2 gene in the H3122

Table 3 Recurrent chromosomal translocations and fusion proteins involving ALK gene in human cancers

Fusion protein	Disease	Refs.
NPM-ALK	ALCL	8
ALO17-ALK; two variants	ALCL	60
TFG-ALK; three variants	ALCL	61,62
MSN-ALK	ALCL	63,64
TPM3-ALK	ALCL	65,66
TPM4-ALK	ALCL	67
ATIC-ALK	ALCL	68–70
MYH9-ALK	ALCL	71
CLTC1-ALK	ALCL	72
EML4-ALK; 13 variants	NSCLC	54,73
TFG-ALK	NSCLC	73
TFG-ALK	NSMM	74
KIF5B-ALK	NSCLC	75,76
KLC1-ALK	NSCLC	77
PTPN3-ALK	NSCLC	78
TPM3-ALK	IMT	79
TPM4-ALK	IMT	79
CTLC-ALK	IMT	80,81
ATIC-ALK	IMT	82
CARS-ALK	IMT	60,83
RANBP2-ALK	IMT	84
SEC31L1-ALK	IMT	85
NPM-ALK	DLBCL	86,87
CLTC1-ALK	DLBCL	88
SQSTM1-ALK	DLBCL	89
SEC31A-ALK	DLBCL	90
EML4-ALK	BRCA	91
EML4-ALK	CRC	91
C2orf44-ALK	CRC	92
TPM4-ALK	ESCC	93,94
VCL-ALK	RCC	95
HIP1–ALK	NSCLC	96
SEC31A-ALK	NSCLC	97
CUX1-ALK	NSCLC	98
VKORC1L1-ALK	NSCLC	99
DYSF-ALK	NSCLC	100
ITGAV-ALK	NSCLC	100
TNIP2-ALK	NSCLC/ LUAD	101
ERC1-ALK	NSCLC/ LUAD	102
FBN1-ALK	NSCLC/ LUAD	102
TRIM66-ALK	NSCLC/ LUAD	102
SWAP70	NSCLC/ LUAD	102
WNK3	NSCLC/ LUAD	102
CHRNA7-ALK	NSCLC	103
LIMD1 -ALK	NSCLC	103
TTC271-ALK	NSCLC	103
LINC00327 -ALK	NSCLC	103
SORCS1-ALK	NSCLC	103
LINC00211-ALK	CSF	104
KIF5B-ALK	ALKPH	105
LRRFIP1-ALK	IMT	106
PPP1CB-ALK	CGM	107

Abbreviations: ALCL, anaplastic large cell lymphoma; ALKPH, ALK-positive histiocytosis; BRCA, breast cancer; CGM, congenital glioblastoma; CRC, colorectal cancer; CSF, cerebrospinal fluid; DLBCL, diffuse large B cell lymphoma; ESCC, esophageal squamous cell cancer; IMT, inflammatory myofibroblastic tumor; LUAD, lung adenocarcinoma; NSCLC, non-small cell lung cancer; NSMM, non-secretory multiple myeloma; RCC, renal cell cancer.

cell line and identified that NF2 loss of function, as a novel bypass mechanism of resistance to lorlatinib, was sensitized by mTOR inhibition both *in vitro* and *in vivo*, which offers a novel potential treatment approach for lorlatinib resistance.[115]

Current Solutions to Overcome Drug Resistance

The problem of resistance to ALK inhibitors has become an important obstacle limiting the development of ALK inhibitors. First and foremost, the urgent and essential thing is to develop approaches for rapidly identifying which kinase domain mutations can be classified as cancer drivers and the resistance mechanisms. Due to the continuous research into the mechanism of ALK-TKI resistance, many solutions have been found. The acquired resistance mechanisms of ALK-TKIs have been fully illustrated, and some new strategies to overcome drug resistance are reviewed below. The potent ALK-TKIs that overcome drug resistance are also listed in ►**Fig. 8**.

Develop Smaller and More Compact Macrocyclic ALK-TKIs

The current ALK inhibitors on the market share some common characteristics, including large and loose molecular structures, and some motifs near or across the hydrophobic posterior capsule. These characters make them more susceptible to drug-resistant mutations. Thus, new inhibitors with increasingly compact structures have been designed. In 2020, TPX-0131, a macrocyclic molecule, was reported as a next-generation ALK inhibitor (►**Fig. 8**). TPX-0131 is designed to fit within the ATP-binding boundary to inhibit ALK fusion proteins and is more potent than all FDA-approved ALK-TKIs against WT ALK and many types of ALK resistance mutations.[116,117]

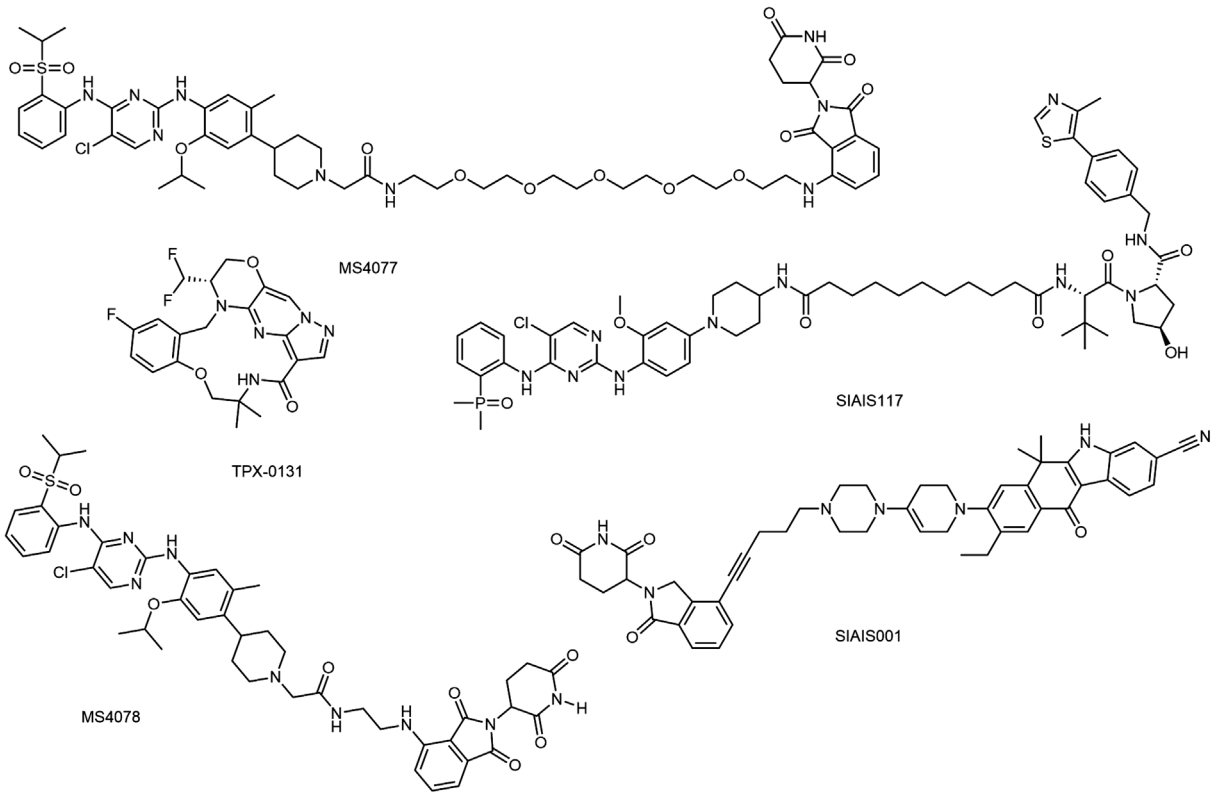

Fig. 8 Some ALK-TKIs designed to overcome drug resistance. ALK-TKI, anaplastic lymphoma tyrosine kinase inhibitor.

Selective Degradation of Mutant Kinase Variants by PROTACs

Proteolysis targeting chimeras (PROTACs), a technology of modulating a protein of interest through degradation, has become one of the most promising cancer therapeutic strategies.[118] PROTACs consist of three parts, a ligand for binding targets, an E3-ubiquitin ligase ligand for hijacking an endogenous E3 ligase, and an optimal linker that connects these two moieties, resulting in the ubiquitination and degradation of the targeted protein via the ubiquitin–proteasome system.[15] PROTACs have been used successfully to selectively degrade ALK protein since 2018. In Jin's laboratory, two ALK degraders, MS4077 and MS4078 (►**Fig. 8**), have been designed to decrease the active oncogenic ALK fusion proteins in SU-DHL-1 lymphoma and NCI-H2228 lung cancer cells, and to mediate ubiquitination and degradation of NPM-ALK and EML4-ALK *in vitro*.[13] However, MS4077 and MS4078 do not significantly improve the antiproliferative effects against ALK mutant lung cancer cells in comparison to ceritinib.

The Jiang group synthesized a series of ALK PROTACs by combining brigatinib and VHL-1 and discovered SIAIS117 as a potential treatment for drug resistance of ALK-TKIs (►**Fig. 8**). This compound shows strong *in vitro* anti-G1202R resistance mutations.[119] In 2021, the Jiang's group also reported SIAIS001, an alectinib-based ALK PROTAC, which can promote G1/S phase arrest and shows much better growth inhibition effects than alectinib (►**Fig. 8**).[120] In light of the rapid development of the PROTACs technology, more and more ALK degraders are being designed and synthesized to anticipate in clinical trials and will be used in clinical practice shortly.

Conclusions and Perspectives

There is no doubt that ALK, a potent carcinogenic driver gene, plays an important role in various types of human cancers. Unfortunately, rapid emergence of the drug resistance could significantly affect the survival of patients treated with ALK-TKIs. Based on the structures of ALK and their variants, as well as the net of ALK-mediating signal transduction, mechanisms of drug resistance, such as point mutations, amplification mutations, activation of bypass signaling, and NF2 loss-of-function mutations, etc., have been discovered.

Based on the crystal structure of the ALK, smaller and more compact macrocyclic ALK-TKIs, including Repotrectinib and TPX-0131, have been designed to positively overcome drug resistance. The PROTAC strategy offers another promising means to overcome the issue of drug resistance. It can be used to degrade ALK driver proteins, and thus evade drug resistance. Although PROTAC-designed ALK inhibitors only have good potency *in vitro*, ALK PROTACs has been considered to have great potential in clinical therapy. Furthermore, computational modeling and machine learning can also be utilized for the discovery and development of novel ALK drugs. To sum up, there is still a long way to go before we can successfully tackle cancer, and there is much more research needed to understand and overcome resistance to ALK-TKIs.

Conflict of Interest

The authors declare no conflict of interest.

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