REVIEW

Development of anaplastic lymphoma kinase (ALK) inhibitors and molecular diagnosis in *ALK* rearrangement-positive lung cancer

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Abstract: The fusion of echinoderm microtubule-associated protein-like 4 with anaplastic lymphoma kinase (*ALK*) was identified as a transforming gene for lung cancer in 2007. This genetic rearrangement accounts for 2%–5% of non-small-cell lung cancer (NSCLC) cases, occurring predominantly in younger individuals with adenocarcinoma who are never- or light smokers. A small-molecule tyrosine-kinase inhibitor of ALK, crizotinib, was rapidly approved by the US Food and Drug Administration on the basis of its pronounced clinical activity in patients with *ALK* rearrangement-positive NSCLC. Next-generation ALK inhibitors, such as alectinib, LDK378, and AP26113, are also being developed in ongoing clinical trials. In addition, the improvement and validation of methods for the detection of *ALK* rearrangement in NSCLC patients will be key to the optimal clinical use of ALK inhibitors. We here summarize recent progress in the development of new ALK inhibitors and in the molecular diagnosis of *ALK* rearrangement-positive NSCLC.

Keywords: ALK, rearrangement, NSCLC, ALK inhibitor, targeted therapy, diagnosis

Background

Lung cancer is the leading cause of cancer deaths worldwide. Non-small-cell lung cancer (NSCLC) accounts for 85% of lung cancer cases, and has usually achieved an advanced stage by the time of diagnosis.[1] Cytotoxic chemotherapy has been the mainstay of treatment for metastatic NSCLC, but its efficacy has plateaued in recent years. Further improvement in the clinical outcome of individuals with NSCLC will thus depend on the development of new treatment strategies, such as molecularly targeted therapies. In 2004, the identification of activating mutations of the epidermal growth-factor receptor (*EGFR*) gene in a subset of NSCLC patients led to a change in treatment of the disease.[2,3] Treatment of patients with NSCLC positive for *EGFR* mutations with such EGFR tyrosine-kinase inhibitors (TKIs) as gefitinib and erlotinib was found to have a high response rate and to result in both prolonged progression-free survival (PFS) and improved quality of life compared with cytotoxic chemotherapy.[4,5] The discovery of *EGFR* mutations and the efficacy of EGFR TKIs in selected patients thus opened a new era of personalized treatment for NSCLC.

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase whose gene was initially identified in a subset of individuals with anaplastic large-cell lymphoma. A reciprocal translocation between chromosomes 2 and 5 apparent in such patients[6] was found to result in the formation of a fusion gene comprising the 5′ portion of the nucleophosmin gene and the 3′ portion of *ALK* encoding the kinase domain.[7] In 2007, a fusion gene formed by *ALK* and the echinoderm microtubule-associated

protein-like 4 (*EML4*) gene was identified in the tumor of a 62-year-old Japanese man with adenocarcinoma of the lung, and was shown to possess pronounced oncogenic activity.[8] This genetic rearrangement has since been found to occur in 2%–5% of NSCLC patients, predominantly in those with adenocarcinoma who are of younger age and never- or light smokers.[9,10]

The *EML4–ALK* fusion oncogene arises from a small inversion within the short arm of chromosome 2 that joins the 5′ region of *EML4* (encoding the NH_2-terminal portion of EML4, including its coiled-coil domain) to the 3′ region of *ALK* (encoding the COOH-terminal portion of ALK, including the tyrosine-kinase domain). It exists in multiple variants that encode the same intracellular tyrosine-kinase domain of ALK but different truncations of EML4.[11,12] The most common variants are variant 1 (detected in 33% of patients), in which exon 13 of *EML4* is fused to exon 20 of *ALK* (E13;A20), and variant 3a/b (detected in 29% of patients), in which exon 6 of *EML4* is fused to exon 20 of *ALK* (E6a/b;A20).[12] Two other rare fusion partners of *ALK* (tyrosine-kinase receptor-fused gene and kinesin family member 5B) in addition to *EML4* have also been identified in individuals with NSCLC.

All of these ALK fusion proteins undergo ligand-independent dimerization mediated by the coiled-coil domain of the fusion partner, resulting in constitutive activation of the ALK tyrosine kinase.[13,14] Such phosphorylation-mediated activation of the ALK fusion proteins results in activation of downstream signaling pathways – including the JAK–STAT, MEK–ERK, and PI3K–AKT pathways – that contribute to oncogenicity.[15–17] TKIs that target the kinase activity of ALK (ALK TKIs) have been found to have pronounced antiproliferative and proapoptotic effects in *EML4–ALK*-positive lung cancer cells.[14,18]

Crizotinib
The first clinically available ALK TKI

The structure of crizotinib is shown in Figure 1. Crizotinib is an oral and potent small-molecule ALK TKI that was initially designed as an inhibitor of the tyrosine kinase MET. Crizotinib competes with adenosine triphosphate for binding to the tyrosine kinase pocket of ALK and thereby inhibits its tyrosine-kinase activity, leading to inhibition of downstream signaling and to anticancer effects. Crizotinib exerts proapoptotic activity, with a median effective concentration
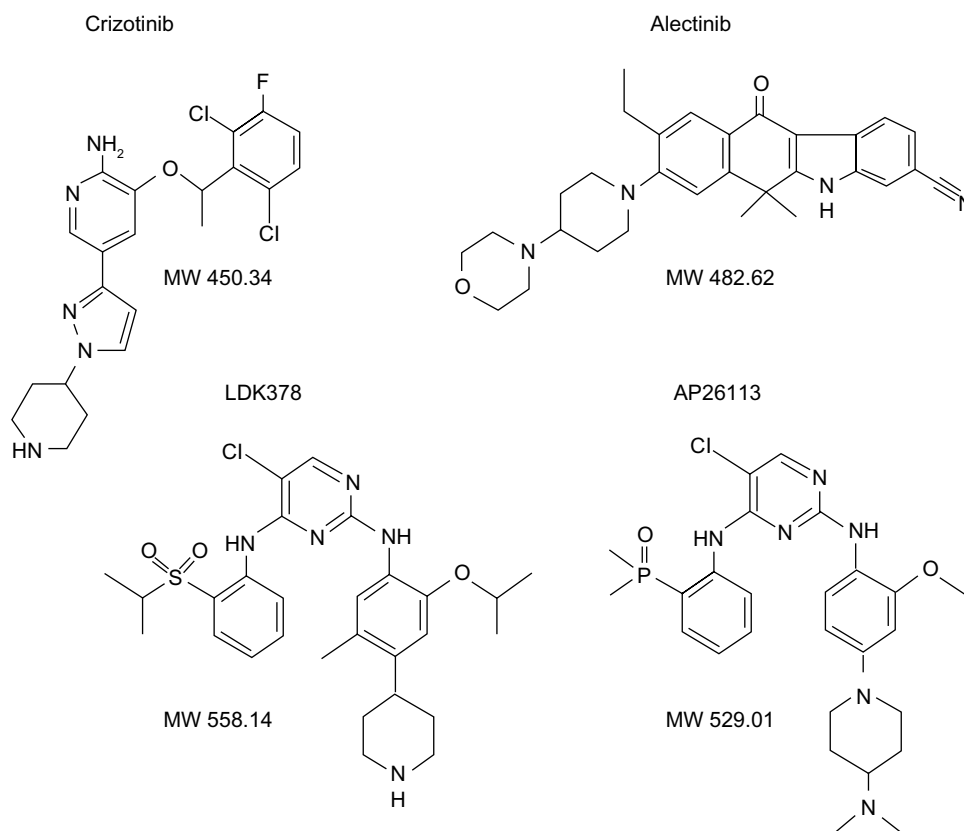

Figure 1 Chemical structures and molecular weight (MW) of crizotinib, alectinib, LDK378, and AP26113.

in the 5–25 nM range in vitro for cells with activated ALK or MET receptor tyrosine kinases.[19,20]

Crizotinib was the first ALK TKI introduced into clinical trials. A dose-escalation component of a Phase I trial (Profile 1001, NCT00585195) identified 250 mg twice daily (bid) as the recommended Phase II dose for crizotinib.[21] Fatigue was the dose-limiting toxicity (DLT), occurring at grade 3 in two of the six patients treated with crizotinib at 300 mg bid. On the basis of promising results apparent in two patients with *ALK* rearrangement-positive NSCLC enrolled during the dose-escalation component, the protocol was amended to expand the cohort of such patients in the second part of this Phase I trial. A total of 149 *ALK* rearrangement-positive patients was thus enrolled, and 143 of these individuals were evaluated. The patients received crizotinib orally at 250 mg bid. The objective response rate (ORR) was 61%, independent of age, sex, performance status, or number of prior treatment regimens, and the median PFS was 9.7 months.[22] On the basis of its pronounced clinical activity, crizotinib was approved by the US Food and Drug Administration (FDA) in August 2011.

In a subsequent randomized Phase III trial (Profile 1007, NCT00932893), 347 patients with *ALK* rearrangement-positive advanced NSCLC who had previously undergone platinum-based chemotherapy were randomly assigned to receive crizotinib (250 mg bid) or standard chemotherapy with either pemetrexed or docetaxel.[23] Crizotinib treatment yielded a significantly better ORR (65% versus 20%, $P<0.001$) and longer PFS (hazard ratio 0.49, 95% confidence interval 0.37–0.64; $P<0.001$) compared with pemetrexed or docetaxel, whereas there was no significant difference in overall survival between the two treatment groups (hazard ratio 1.02, 95% confidence interval 0.68–1.54), as a result of crossover to the comparator treatment.[23,24] Another randomized Phase III trial (Profile 1014, NCT01154140), designed to test the efficacy of crizotinib versus standard chemotherapy (pemetrexed–cisplatin or pemetrexed–carboplatin) as a first-line treatment for patients with *ALK*-rearranged NSCLC, is ongoing (Figure 2).[25]

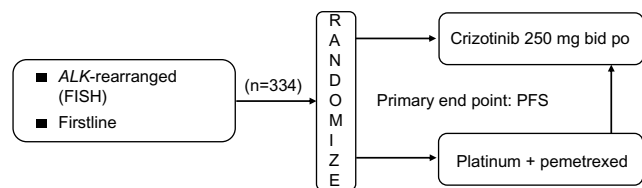Profile 1014 (first line, crizotinib versus chemotherapy)

Figure 2 Ongoing Phase III study (Profile 1014) of crizotinib for the treatment of *ALK* rearrangement-positive non-small-cell lung cancer.
Abbreviations: ALK, anaplastic lymphoma kinase; FISH, fluorescence in situ hybridization; bid, twice daily; po, oral administration; PFS, progression-free survival.

Most adverse events of crizotinib treatment appear to be mild (grade 1 or 2), with those that occur most frequently being visual effects, nausea, diarrhea, constipation, vomiting, and peripheral edema. Three warning adverse events – interstitial lung disease (ILD), hepatotoxicity, and prolongation of the QT interval – have been identified. Life-threatening or fatal treatment-related ILD was found to occur in 1.6% of patients.[26] It remains unclear whether the risk factors for EGFR TKI-associated ILD, such as male sex, a history of smoking, and coincidence of interstitial pneumonia, also apply to crizotinib-associated ILD. It is thus important that patients treated with crizotinib be monitored for pulmonary symptoms and radiographic findings indicative of ILD, and the drug should be discontinued immediately on such a diagnosis. Elevated serum aminotransferase levels of grade 3 or 4 were detected in ~7% of crizotinib-treated patients, with such elevation usually being asymptomatic and reversible on discontinuation of crizotinib. Although crizotinib-induced hepatotoxicity with a fatal outcome has been reported in <1% of treated patients, routine evaluation of liver function, including measurement of aminotransferase and bilirubin levels, should be performed.

Mechanisms of crizotinib resistance

Although treatment with crizotinib has a pronounced clinical benefit for patients with *ALK* rearrangement-positive NSCLC, such individuals inevitably develop drug resistance. Several mechanisms of crizotinib resistance have been described to date, including secondary mutation or copy-number gain of *ALK*,[27,28] inadequate drug delivery, and activation of alternative signaling pathways, such as those mediated by EGFR or KIT (Figure 3).[29–31]

Two secondary mutations of *ALK* associated with crizotinib resistance – L1196M and C1156Y – were first detected in the same patient, who relapsed after achieving a partial response to the drug.[32] The L1196M substitution occurs at the gatekeeper position of ALK (a position that controls the binding of nucleotides and TKIs), and corresponds to the T790M substitution in EGFR and the T315I substitution in the Bcr–Abl fusion protein, both of which confer resistance to corresponding TKIs. Multiple additional mutations in the ALK kinase domain have since been identified in patients who develop resistance to crizotinib.[28,33] In contrast, T790M accounts for the vast majority of secondary mutations of EGFR that confer resistance to EGFR TKIs (Figure 3).[34,35]

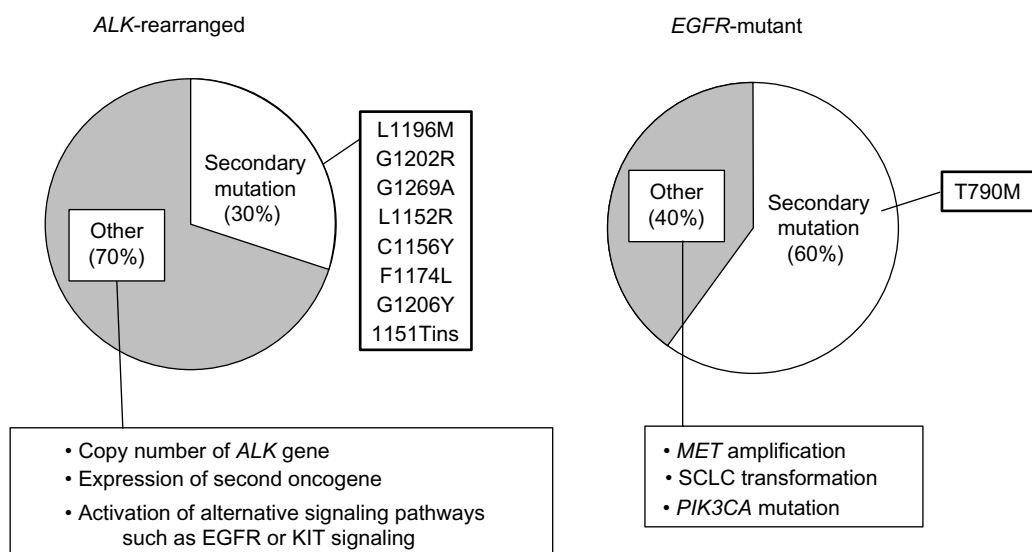ALK-rearranged   EGFR-mutant

Figure 3 Comparison of tyrosine-kinase inhibitor resistance mechanisms for ALK rearrangement-positive and EGFR mutation-positive non-small-cell lung cancer (NSCLC). Only 30% of cases of acquired crizotinib resistance in patients with ALK-rearranged NSCLC are attributable to various secondary mutations of ALK, with the remaining 70% of such cases being due to other mechanisms. In contrast, 60% of cases of acquired resistance to EGFR-tyrosine-kinase inhibitors in patients with EGFR mutation-positive NSCLC are caused by secondary mutation of EGFR, almost exclusively T790M, whereas only 40% of such cases are due to other resistance mechanisms.
Abbreviations: ALK, anaplastic lymphoma kinase; EGFR, epidermal growth-factor receptor; SCLC, small-cell lung cancer.

Although the relative contributions of the different mechanisms to crizotinib resistance remain unclear because of the small numbers of patients examined, biopsy of tumors performed after the onset of acquired resistance has suggested that secondary mutations in the ALK kinase domain account for only ~30% of such cases of resistance.[27,30] This situation also differs from that for EGFR mutation-positive NSCLC, for which the T790M substitution has been detected in up to 60% of tumors with acquired resistance to EGFR TKIs (Figure 3).[36] Repeated biopsy and molecular analysis of relapsed tumors will be required in clinical trials of treatment strategies designed to overcome acquired resistance.

Clinical development of other ALK TKIs

Several new ALK TKIs are currently under development (Table 1).

Alectinib (CH5424802)

Alectinib (Chugai Pharmaceutical, Tokyo, Japan) is a potent and selective ALK inhibitor with a median inhibitory concentration for ALK activity of 1.9 nM, and with little or no inhibitory activity for other protein kinases examined (Figure 1).[37] The specific potency of alectinib for ALK inhibition appears to be related to its one-hinge hydrogen bond, whereas other ALK inhibitors, including crizotinib,

Table 1 Selected clinical trials of anaplastic lymphoma kinase (ALK) inhibitors for the treatment of ALK rearrangement-positive non-small-cell lung cancer

Drug	Phase	Compared drug	Treatment setting	Status	Clinical trial number
Crizotinib	III	PEM or DOC	Second line	Published[23]	NCT00932893
	III	Platinum + PEM	First line	Ongoing*,[25]	NCT01154140
Alectinib	I/II		ALK inhibitor-naive	Published[38]	AF-001JP
	III	Crizotinib	ALK inhibitor-naive	Ongoing[39]	JapicCTI-132316
LDK378	III	Platinum + PEM	First line	Ongoing[45]	NCT01828099
	III	PEM or DOC	Both platinum and crizotinib failure, third line	Ongoing[44]	NCT01828112
AP26113	I/II		ALK inhibitor-naive or failure	Ongoing[47]	NCT01449461
ASP-3026	I		ALK inhibitor-naive or failure	Ongoing[64]	NCT01401504
X-396	I		ALK inhibitor-naive or failure	Ongoing[65]	NCT01625234
CEP-37440	I		ALK inhibitor-naive or failure	Ongoing[66]	NCT01922752

Note: *Patient accrual completed.
Abbreviations: PEM, pemetrexed; DOC, docetaxel.

form two- or three-hinge hydrogen bonds. In contrast to crizotinib, alectinib also shows substantial inhibitory activity against the L1196M mutant of ALK, apparently because it is able to maintain an efficient (CH/π) interaction with position 1196 even after the substitution of methionine for leucine.

A Phase I/II first-in-human study (AF-001JP) performed with previously treated and crizotinib-naive patients with *ALK* rearrangement-positive advanced NSCLC was performed in Japan.[38] The participants were deemed to be *ALK* fusion gene-positive if a positive result was obtained either by reverse-transcription polymerase chain reaction (RT-PCR) analysis or by both immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). In the Phase I portion of the study, 24 patients received alectinib with a dose escalation from 20 to 300 mg bid, with the latter being determined as the highest planned dose on the basis of the available safety information for the additive formulation in Japan. Given that DLTs were not observed, the maximum tolerated dose (MTD) was not identified in this study. The highest planned dose (300 mg bid) was thus judged to be acceptable as the recommended dose for the 46 patients enrolled in the Phase II portion of the trial. Of these 46 patients, 43 individuals (93.5%) achieved an objective response, and 44 (95.7%) achieved disease control. The median PFS had not been determined by the time of publication. This excellent clinical activity was associated with mostly mild adverse events, with those of grade 3 being detected in only 17 (37.0%) patients and those of grade 4 or death in none. The most frequently reported treatment-related adverse events were dysgeusia and liver dysfunction, both of which were of grade 1 or 2 in almost all cases. The characteristic adverse events of crizotinib treatment, including visual effects and gastrointestinal disorders (diarrhea, vomiting, and nausea), occurred at a low rate in this study of alectinib. Application for approval of alectinib in Japan was submitted on October 7, 2013.

A Phase III clinical trial (JapicCTI-132316) comparing alectinib with crizotinib in terms of PFS for the treatment of patients with *ALK* rearrangement-positive NSCLC is ongoing in Japan.[39] Major eligibility criteria include advanced or metastatic *ALK*-rearranged NSCLC (identified either by RT-PCR or by both IHC and FISH), no prior treatment with an ALK inhibitor, an Eastern Cooperative Oncology Group performance status of 0–2, and either no previous treatment or one line of prior treatment with chemotherapy (Figure 4).

A dose-finding Phase I study (AF-002JG, NCT01588028) was also performed for alectinib in the US.[40] Key eligibility
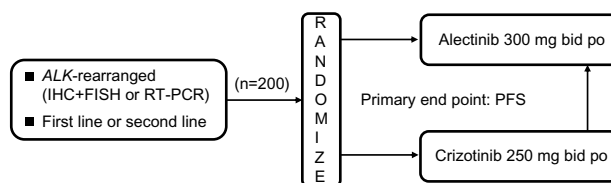
Alectinib versus crizotinib

Figure 4 Ongoing Phase III study (JapicCTI-132316) of alectinib for the treatment of *ALK* rearrangement-positive non-small-cell lung cancer.[39]
Abbreviations: ALK, anaplastic lymphoma kinase; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; RT-PCR, reverse-transcription polymerase chain reaction; PFS, progression-free survival; bid, twice daily; po, oral administration.

criteria for this study included advanced NSCLC with *ALK* rearrangement confirmed by FISH, as well as failed crizotinib treatment. No treatment-related dose reductions were necessary up to a dose of 600 mg bid. Two of seven patients experienced DLTs (headache of grade 3, and neutropenia of grade 3 requiring dose-holding for 7 days) at the dose of 900 mg bid. On the basis of these results, 600 mg bid was determined as the recommended dose of alectinib for a Phase II study in the US. The ORR was 54.5% across all cohorts of the Phase I study, indicating that alectinib possesses significant clinical activity in *ALK* rearrangement-positive patients who are refractory to crizotinib. A global single-arm Phase II study of alectinib in patients with *ALK*-rearranged NSCLC resistant to crizotinib is ongoing (NCT01801111).[41] The FDA granted breakthrough-therapy designation for alectinib on the basis of the NCT01588028 data, with early approval being expected.

LDK378

LDK378 (Novartis, Basel, Switzerland) is also a potent and selective small-molecule ALK inhibitor (Figure 1).[42] In a Phase I study, 59 patients received LDK378 with dose escalation from 50 to 750 mg once daily (qd). DLTs were observed in two of the 14 patients who received the drug at 400 mg qd, in two of the nine patients at 600 mg qd, and in one of the nine patients at 750 mg qd. DLTs included diarrhea, vomiting, nausea, dehydration, and elevated serum aminotransferase levels. The MTD was thus defined as a dose of 750 mg qd. Among 88 evaluable *ALK* rearrangement-positive NSCLC patients who received LDK378 at 400–750 mg qd, the ORR was 70%. In the subset of 64 patients who had experienced crizotinib failure, the ORR was 73%.[43] These results thus suggest that LDK378 may be effective for the treatment of patients with *ALK*-rearranged NSCLC who have developed acquired resistance to crizotinib.

A Phase III clinical trial (NCT01828112) comparing LDK378 with chemotherapy (pemetrexed at 500 mg/m² or
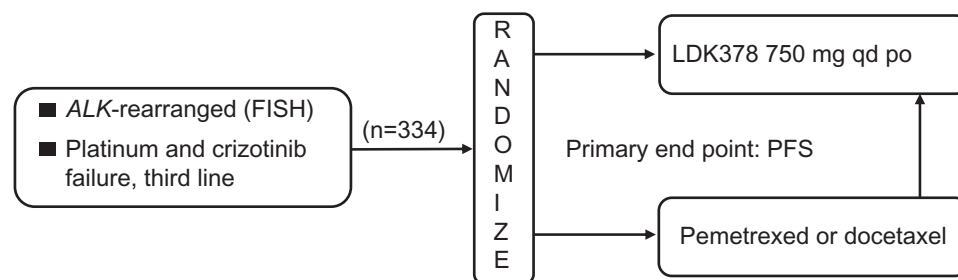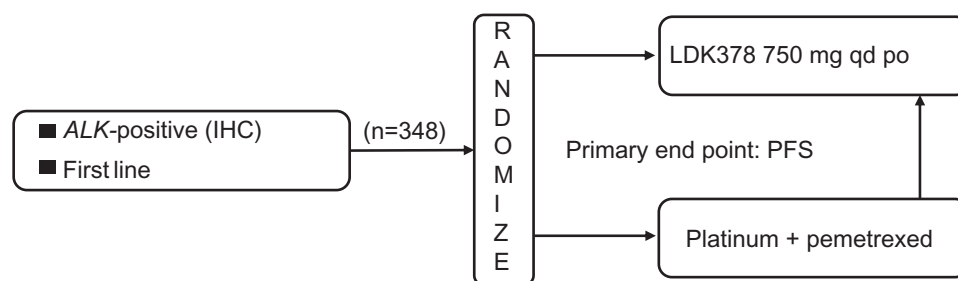
Crizotinib failure, LDK378 versus chemotherapy (NCT01828112)[44]

First line, LDK378 versus chemotherapy (NCT01828099)[45]

Figure 5 Ongoing Phase III studies of LDK378 for the treatment of *ALK* rearrangement-positive non-small-cell lung cancer.
Abbreviations: ALK, anaplastic lymphoma kinase; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; qd, once daily; po, oral administration; PFS, progression-free survival.

docetaxel at 75 mg/m^2) for the treatment of *ALK*-rearranged NSCLC patients who have progressed after prior treatment with both crizotinib- and platinum-based chemotherapy is ongoing (Figure 5).[44] In addition, a Phase III clinical trial (NCT01828099) comparing LDK378 with standard first-line chemotherapy (pemetrexed plus either cisplatin or carboplatin) in previously untreated ALK positive NSCLC patients assessed by IHC is also ongoing (Figure 5).[45]

AP26113

AP26113 (Ariad Pharmaceuticals, Inc., Cambridge, MA, USA) is another highly selective small-molecule ALK inhibitor that shows activity against the L1196M mutant (Figure 1).[46] A Phase I/II study of AP26113 (NCT01449461) is ongoing.[47] In the Phase I portion of the study, 44 patients received AP26113 with dose escalation from 30 to 300 mg qd. The most common adverse events were fatigue, nausea, and diarrhea, most of which were of grade 1 or 2. One DLT (increased serum alanine aminotransferase level of grade 3) was observed in one of nine patients treated at a dose of 240 mg, and one DLT (dyspnea of grade 4) was observed in one of two patients at a dose of 300 mg. Although the MTD has not been defined, a recommended Phase II dose was

identified as 180 mg qd on the basis of safety, efficacy, and pharmacokinetic data. In the Phase I portion of the study, 24 patients with *ALK*-rearranged NSCLC were evaluable for response. Fifteen of these patients achieved an ORR of 63%, including 12 of the 16 individuals who had progressed after previous crizotinib therapy (ORR 75%). In addition, four of five patients showed objective responses for metastases in the central nervous system.

Other new ALK TKIs

Other new ALK TKIs, such as ASP-3026 (Astellas Pharma, Tokyo, Japan), NMS-E628 (Nerviano Medical Sciences, Milan, Italy), X-396 (Xcovery, West Palm Beach, FL, USA), CEP-37440 (Teva Pharmaceutical Industries Ltd, Petah Tikva, Israel), TSR-011 (Tesaro, Inc., Waltham, MA, USA), and PF-06463922 (Pfizer, New York, NY, USA), are currently introduced into clinical trials.

Molecular diagnosis of *ALK* rearrangement-positive NSCLC
FISH

Break-apart FISH analysis was applied for detection of *ALK* rearrangement in clinical trials with crizotinib. In this approach, the 5′ and 3′ portions of the *ALK* gene are separately
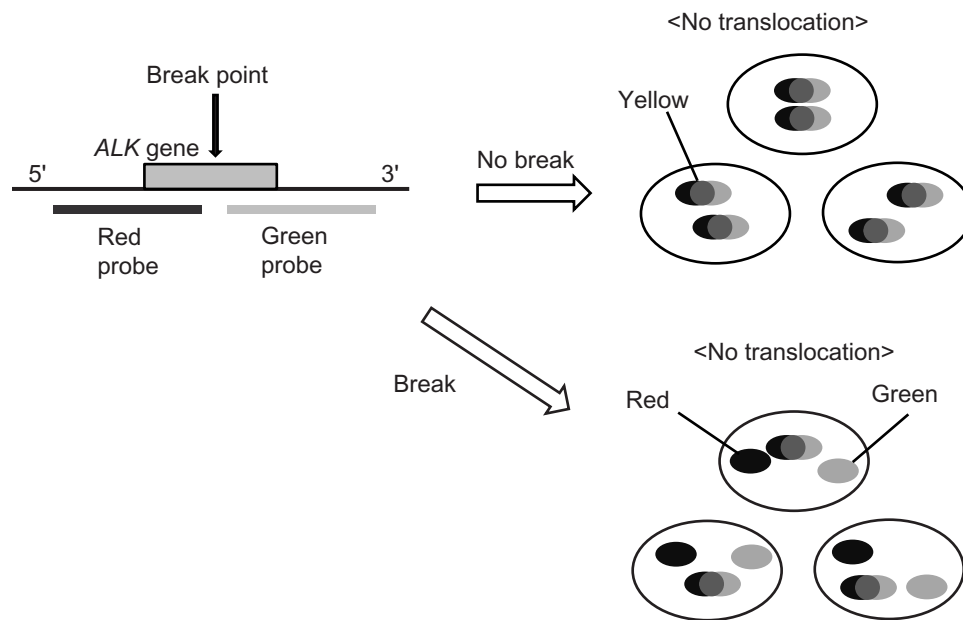
Figure 6 Schematic illustration for break-apart fluorescence in situ hybridization for detecting *ALK* rearrangements.
Abbreviation: ALK, anaplastic lymphoma kinase.

labeled with red or green fluorescent probes (Figure 6). If the signals of the two probes overlap, resulting in yellow fluorescence, then there is no translocation. If a translocation is present, the two probes are spatially separated, and each is detected as an isolated signal (red or green). Tumors are deemed positive for *ALK* rearrangement if 15% or more of the tumor cells show isolated signals. Such analysis detects *ALK* rearrangement regardless of the *ALK* fusion partner or the specific *EML4–ALK* variant. The break-apart FISH assay is a unique diagnostic approach approved for screening for *ALK* rearrangement in NSCLC by the FDA. FISH has several disadvantages, however. First, it is an expensive and low-throughput method that requires technical expertise. Second, false-negative results sometimes occur because of difficulty in interpretation of separated signals. And third, the use of FISH alone (without IHC or RT-PCR) for screening may give rise to false-positive results. Indeed, in one study, the ORR for crizotinib was only 48% among patients screened with FISH alone, but increased up to 81% among those screened with FISH in combination with IHC or RT-PCR.[48]

IHC

Given that ALK is not expressed in normal lung tissue or in lung cancer negative for *ALK* rearrangement, any level of ALK expression is considered to be abnormal and expected to be the result of *ALK* rearrangement. The abundance of ALK fusion proteins is relatively low, however, and initial attempts to detect such proteins by IHC were disappointing.[49] The

subsequent development of an intercalated antibody-enhanced polymer (iAEP) method for signal enhancement (which incorporates an intercalating antibody between the primary antibody to ALK and the dextran polymer-based detection reagents) resulted in a marked increase in the sensitivity of IHC for the detection of ALK fusion proteins.[50] Several studies have since described the detection of ALK fusion proteins with high sensitivity and specificity by the application of IHC with improved detection methods (such as the iAEP method [Nichirei Biosciences] or EnVision™ FLEX+ [Dako, Glostrup, Denmark]) in combination with antibodies to ALK (ALK1, 5A4, and D5F3) (Table 2).[51–59] The sensitivity of the improved IHC procedures is especially high with the D5F3 or 5A4 antibodies. Given that IHC is a routine methodology in most pathology laboratories, it may be suitable for screening of NSCLC patients for *ALK* rearrangement after appropriate clinical optimization and validation (Figure 7).
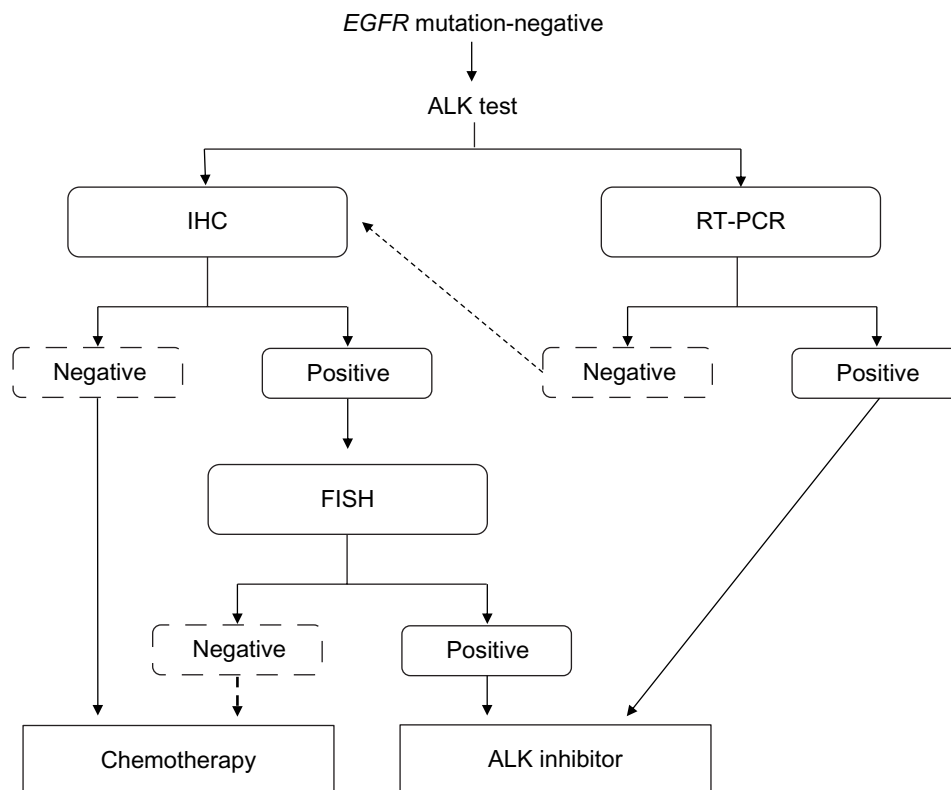
RT-PCR

RT-PCR is a highly sensitive and specific method for the identification of *ALK* rearrangement.[60,61] In addition, unlike FISH or IHC, it can determine both the fusion partner of *ALK* (from among those previously identified) and the *EML4–ALK* variant.[62] RT-PCR requires high-quality ribonucleic acid (RNA) extracted from nonfixed or freshly frozen specimens, however. It is generally difficult to extract suitable RNA from the paraffin-embedded specimens used in daily clinical practice.

Table 2 Summary of the sensitivity and specificity of improved immunohistochemistry (IHC) procedures for the detection of anaplastic lymphoma kinase (ALK) in non-small-cell lung cancer specimens

Reference	FISH-positive cases	Antibody	Cutoff point of IHC score				Detection system
			2+ (including 2+ and 3+ cases)		1+ (including 1+, 2+, and 3+ cases) or IHC score not used		
			Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	
51	43/132	D5F3	97.7	96.6	100	87.4	SignalStain® Boost IHC Detection
52	44/161	D5F3	NE		90.9	99.1	EnVision+
		ALK1			63.6	96.6	
53	63/196	D5F3	NE		100	98.5	OptiView/OptiView Amplification
54	7/594	ALK1	28.6	99.8	100	99.0	EnVision FLEX+
		5A4	85.7	99.8	100	98.1	UltraView/UltraView Amplification
		D5F3	71.4	99.5	100	99.0	Optiview/Optiview Amplification
55	20/351	5A4	100	99.4	100	99.4	Polymer Refine Detection Kit (Leica)
56	15/186	5A4	NE		80.0	99.4	EnVision+
57	25/262	5A4	80.0	99.2	100	98.7	Polymer Refine Detection Kit (Leica)
58	10/101	ALK1	90.0	97.8	100	75.8	ADVANCE (Dako)
59	22/153	ALK1	NE		67.0	97.0	EnVision+
		D5F3			100	99.0	

Notes: SignalStain® Boost IHC Detection (Cell Signaling Technology, Inc., Danvers, MA, USA). EnVision™+ (Dako, Glostrup, Denmark). OptiView/OptiView Amplification (Ventana Medical Systems, Inc., Tucson, AZ, USA). EnVision™ FLEX+ (Dako). UltraView/UltraView Amplification (F. Hoffmann-La Roche Ltd, Basel, Switzerland). Polymer Refine Detection Kit (Leica, Nussloch, Germany). ADVANCE (Dako).
Abbreviations: FISH, fluorescence in situ hybridization; NE, not evaluated.

Figure 7 Proposed algorithm for testing for *ALK* rearrangement in patients with non-small-cell lung cancer.
Abbreviations: ALK, anaplastic lymphoma kinase; IHC, immunohistochemistry; RT-PCR, reverse-transcription polymerase chain reaction; FISH, fluorescence in situ hybridization.

Several new RT-PCR-based methods have recently been developed. MassARRAY is a nucleic acid-analysis platform for the detection of *EML4–ALK* that involves PCR amplification, single-base primer extension, and analysis by MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry.[63] The region of *EML4–ALK* complementary deoxyribonucleic acid containing the fusion point is amplified by PCR, but given that the amplicons are relatively small (70–130 bp), the quality of RNA extracted from paraffin-embedded specimens is sufficient for the analysis. RT-PCR-based assays may thus come to be more convenient and a major tool for detection of *ALK* rearrangement if the use of paraffin-embedded tissue is validated.

Future perspectives

The identification of the *EML4–ALK* fusion gene has accelerated translational research and changed clinical practice for NSCLC, with crizotinib now being in clinical use as an ALK inhibitor for the treatment of patients with *ALK* rearrangement-positive NSCLC. Although crizotinib has an excellent initial therapeutic effect, all treated patients eventually develop resistance to this drug. The development of therapeutic strategies able to overcome crizotinib resistance, including those based on the administration of new ALK inhibitors, is thus warranted. In addition, given the existence of other drivers of NSCLC, such as *EGFR* mutations as well as reactive oxygen species 1 and *RET* fusion genes, it will be important to improve and validate methods for the detection of and screening for these various genetic changes, so that the appropriate drug can be prescribed.

Disclosure

The authors report no conflicts of interest in this work.

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