ALK Inhibitors in Non–Small Cell Lung Cancer: Crizotinib and Beyond

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Keywords
Alectinib, anaplastic lymphoma kinase (ALK), ceritinib, crizotinib, non–small cell lung cancer (NSCLC)

Abstract: The treatment of patients with advanced non–small cell lung cancer (NSCLC) harboring chromosomal rearrangements of anaplastic lymphoma kinase (*ALK*) has been revolutionized by the development of crizotinib, a small molecule inhibitor of the tyrosine kinases ALK, ROS1, and MET. Resistance to crizotinib invariably develops, however, through a variety of mechanisms. In the last few years, a flurry of new and more potent ALK inhibitors has emerged for the treatment of ALK-positive NSCLC, including ceritinib (LDK378), alectinib (RO5424802/CH5424802), AP26113, ASP3026, TSR-011, PF-06463922, RXDX-101, X-396, and CEP-37440. Cancers harboring *ALK* rearrangements may also be susceptible to treatment with heat shock protein 90 inhibitors. This review focuses on the pharmacologic and clinical properties of these compounds, either as monotherapies or in combination with other drugs. With so many ALK inhibitors in development, the challenges of how these agents should be studied and ultimately prescribed are also discussed.

Introduction

Non–small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths, both worldwide and in the United States. Most patients who have NSCLC present with advanced or incurable disease, and cytotoxic chemotherapy generally results in low response rates and only modest improvements in overall survival (OS). Groundbreaking research on the molecular drivers of NSCLC has led to major treatment advances over the past decade, starting with the discovery in 2004 that activating mutations in the epidermal growth factor receptor (*EGFR*) gene are the basis of dramatic responses to the EGFR tyrosine kinase inhibitors (TKIs) gefitinib (Iressa, AstraZeneca) and erlotinib (Tarceva, Genentech/Astellas).[1-3]

Just 3 years later, investigators in Japan identified anaplastic lymphoma kinase (ALK) as another potential target in NSCLC. In a small subset of NSCLC tumors, a chromosomal inversion event leads to fusion of a portion of the *ALK* gene with the echinoderm microtubule–associated protein-like 4 (*EML4*) gene. The resulting EML4-ALK fusion protein is constitutively activated

Table 1. ALK Inhibitors in Clinical Development

Drug Name(s)	Pharmaceutical Company
Crizotinib, PF-02341066 (Xalkori)[a]	Pfizer
Ceritinib, LDK378 (Zykadia)[a]	Novartis
Alectinib, RO5424802/CH5424802	Roche
AP26113	Ariad
ASP3026	Astellas Pharma
TSR-011	Tesaro
PF-06463922	Pfizer
RXDX-101 (formerly NMS-E628)	Ignyta
X-396	Xcovery
CEP-37440	Teva

[a] FDA-approved.

and transforming, leading to a state of oncogene addiction.[4] EML4-ALK fusion and other *ALK* rearrangements occur in 3% to 7% of patients with NSCLC (herein referred to as "ALK-positive" lung cancer) and are associated with younger age, never smoking or light smoking history, and adenocarcinoma histology.[4,5] Patients who have advanced ALK-positive NSCLC are highly responsive to the ALK inhibitor crizotinib (Xalkori, Pfizer), with an objective response rate (ORR) of approximately 60% and a median progression-free survival (PFS) of 8 to 10 months.[6,7]

Enthusiasm for crizotinib has been tempered, however, by the emergence of drug resistance. Most patients with ALK-positive lung cancer who respond to crizotinib undergo a relapse within a few years after starting therapy.[8,9] In particular, the central nervous system (CNS) is one of the most common sites of relapse in patients with ALK-positive NSCLC, and CNS disease can prove refractory to standard therapies.[10] In light of these limitations with crizotinib, many novel ALK inhibitors that have greater potency and different kinase selectivity compared with crizotinib are currently in development (Table 1). Additionally, heat shock protein 90 (Hsp90) inhibitors have emerged as potentially active agents in the treatment of ALK-positive lung cancers, and these are being tested alone and in combination with ALK TKIs. This review provides an update on each of the TKIs and Hsp90 inhibitors in clinical development for ALK-positive NSCLC (Table 2), focusing on drug potency, selectivity, and side effects (Table 3).

Crizotinib

Crizotinib in ALK-Positive Non–Small Cell Lung Cancer

The impact of crizotinib on the clinical course of patients with ALK-positive NSCLC was quickly appreciated after the results of the PROFILE 1001 study were published in 2010.[6] In this open-label, phase 1 study, 82 patients who had ALK-positive NSCLC were treated with crizotinib. An ORR of 57% was noted, and stable disease was observed in an additional 33% of patients. Crizotinib was generally well tolerated, with mild gastrointestinal symptoms as the most commonly reported adverse events.[6] The OS rates in this cohort of 82 patients at 1 and 2 years were 74% and 54%, respectively.[11] Updated results from the phase 1 study of 149 patients showed an ORR of 60.8%, with a median PFS of 9.7 months.[9] Similarly, the ongoing phase 2 study of crizotinib (PROFILE 1005) demonstrated a response rate of 59.8% and a median PFS of 8.1 months.[12] On the basis of the response rates in the phase 1 and phase 2 studies, the US Food and Drug Administration granted accelerated approval to crizotinib in 2011.

Crizotinib was compared with single-agent chemotherapy (pemetrexed [Alimta, Lilly] or docetaxel) in an open-label, phase 3 trial (PROFILE 1007) of patients with ALK-positive NSCLC who had disease progression after previously receiving platinum-based chemotherapy.[7] Compared with chemotherapy, crizotinib was associated with a significantly longer median PFS (7.7 vs 3.0 months; hazard ratio [HR], 0.49; $P<.001$) and a higher response rate (65% vs 20%; $P<.001$). Patients in the crizotinib group reported greater improvements in their global quality of life and better mitigation of their lung cancer–related symptoms than did patients in the chemotherapy group. Adverse effects that were more common in the crizotinib group included visual disturbances, gastrointestinal symptoms, and elevated aminotransferase levels; patients in the chemotherapy group experienced more fatigue, alopecia, and dyspnea.[7] In this study, there was no difference in OS between the 2 groups (20.3 months with crizotinib vs 22.8 months with chemotherapy; HR, 1.02; $P=.54$), likely owing to crossover of the majority of patients from chemotherapy to crizotinib.[7] However, in a retrospective analysis comparing 30 patients who had crizotinib-treated ALK-positive NSCLC with 23 ALK-positive controls who had never received crizotinib, OS was significantly higher at 1 year (70% vs 44%) and 2 years (55% vs 12%) in the patients who received crizotinib.[11] Crizotinib is also being compared with chemotherapy in the first-line setting in the PROFILE 1014 study, with a primary endpoint of PFS; the results of this study have not yet been reported.

Efficacy of Crizotinib in Other Tumor Types With ALK Genomic Aberrations

ALK rearrangement or mutation is a dominant oncogenic driver in several tumor types other than NSCLC, and crizotinib appears to be active in these cancers as well. Roughly 50% of inflammatory myofibroblastic tumors (IMTs) harbor *ALK* rearrangements,[13] and several

Table 2. Clinical Trials of Drugs for ALK-Positive Non–Small Cell Lung Cancer

Drug Name(s)	Clinicaltrials.gov Identifier	Phase	Description/Comments
Tyrosine kinase inhibitors			
Crizotinib, PF-02341066 (Xalkori)	NCT00585195	1	PROFILE 1001, advanced malignancies
	NCT00932451	2	PROFILE 1005, in ALK+ NSCLC
	NCT02034981	2	Tumors with alterations in ALK, ROS1, RON, or MET
	NCT00932893	3	PROFILE 1007, in ALK+ NSCLC; crizotinib vs pemetrexed (Alimta, Lilly) or docetaxel in second-line setting
	NCT01154140	3	PROFILE 1014, in ALK+ nonsquamous lung cancer; crizotinib vs platinum and pemetrexed in first-line setting
Ceritinib, LDK378 (Zykadia)	NCT01283516	1	ALK+ tumors
	NCT01685138	2	ALK+ NSCLC, crizotinib-naive patients
	NCT01685060	2	ALK+ NSCLC, patients previously treated with chemotherapy and crizotinib
	NCT01828099	3	Ceritinib vs standard chemotherapy in previously untreated ALK+ NSCLC
	NCT01828112	3	Ceritinib vs standard chemotherapy in ALK+ NSCLC previously treated with chemotherapy and crizotinib
	NCT01964157	2	ROS1+ NSCLC, previously treated with chemotherapy
Alectinib, RO5424802/CH5424802	NCT01588028	1	ALK+ NSCLC, previously treated with crizotinib
	NCT01871805	2	ALK+ NSCLC, previously treated with chemotherapy and crizotinib
	NCT01801111	1/2	ALK+ NSCLC, previously treated with crizotinib
AP26113	NCT01449461	1/2	ALK+ NSCLC
ASP3026	NCT01401504	1	Advanced solid tumors
	NCT01284192	1	Solid tumors and B-cell lymphoma
TSR-011	NCT02048488	1/2a	ALK+ or TRK+ solid tumors and lymphomas
PF-06463922	NCT01970865	1/2	ALK+ or ROS1+ NSCLC
RXDX-101, NMS-E628	N/A	1	Trial open in Italy, to be opened in the United States within the year
X-396	NCT01625234	1	Advanced solid tumors
CEP-37440	NCT01922752	1	Advanced solid tumors
Heat shock protein 90 inhibitors			
AUY922	NCT01752400	2	ALK+ NSCLC, resistant to prior ALK TKI
Ganetespib + crizotinib	NCT01579994	1/2	ALK+ lung cancer, crizotinib-naive
AT13387 + crizotinib	NCT01712217	1/2	ALK+ NSCLC, crizotinib-resistant
AUY922 + ceritinib	NCT01772797	1b	ALK+ NSCLC, resistant to ALK TKI
Combination with immunotherapy			
Ipilimumab (Yervoy, Bristol-Myers Squibb) + crizotinib (or erlotinib [Tarceva, Genentech/Astellas])	NCT01998126	1	Ipilimumab + crizotinib, in ALK+ NSCLC Ipilimumab + erlotinib, in EGFR-mutant NSCLC

ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; NSCLC, non–small cell lung cancer; TKI, tyrosine kinase inhibitor.

patients with *ALK*-rearranged IMTs have achieved partial responses to crizotinib.[14,15] Dramatic and durable responses to crizotinib have also been observed in adult and pediatric patients with heavily pretreated, chemotherapy-refractory, ALK-positive anaplastic large cell lymphoma.[15-17] Activating mutations within the *ALK* tyrosine kinase domain have been detected in approximately 10% of cases of neuroblastoma; the most commonly described amino acid substitutions are R1275Q and F1174L.[18] Both in preclinical models and in phase 1 clinical trials of

Table 3. Characteristics of ALK Inhibitors

Drug	Maximum Dose	Targets Other Than ALK	Toxicities	References
Crizotinib, PF-02341066 (Xalkori)	250 mg twice daily	c-MET ROS1	Vision disorder, diarrhea, nausea, vomiting, constipation, aminotransferase elevation, edema, upper respiratory infection, dysgeusia, dizziness	6,7,9,11,12,14, 15,17,27,35
Ceritinib, LDK378 (Zykadia)	750 mg daily	IGF-1R INSR STK22D	Diarrhea, vomiting, nausea, dehydration, ALT elevation, hypophosphatemia	49-52
Alectinib, RO5424802/ CH5424802	300 mg twice daily	LTK GAK	Dysgeusia, abnormal liver function tests, increased serum creatinine, rash, gastrointestinal side effects, decreased neutrophil count, increased serum creatine phosphokinase, stomatitis, myalgias	53-57
AP26113	180 mg daily	ROS1	Fatigue, nausea, diarrhea, headache, cough, decreased appetite, muscle spasms, pain in extremity, peripheral edema, vomiting, early onset of pulmonary symptoms	58-60,94-96
ASP3026	525 mg daily	ROS1 ACK	Constipation, vomiting, diarrhea, nausea, abdominal pain, ALT/AST elevation, rash	61,62
TSR-011	TBD	TRK-A TRK-B TRK-C	Dysesthesia, QTc prolongation, anorexia, peripheral neuropathy, fatigue, asthenia	64
PF-06463922	TBD	ROS1	NA	68-70
RXDX-101, NMS-E628	TBD	ROS1 TRK-A TRK-B TRK-C	Paresthesias, nausea, dysguesia, diarrhea	71-74
X-396	TBD	MET	Rash, fatigue, nausea, vomiting, edema	75,76
CEP-28122	N/A	RSK2 RSK3 RSK4	Not in clinical development	77
CEP-37440	TBD	N/A	NA	NA

ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not available; TBD, to be determined.

neuroblastoma, crizotinib has been shown to be an effective inhibitor in cases with the R1275Q mutation, but not the F1174L mutation[15,19]; this finding is consistent with the fact that F1174L has also been described as an acquired mutation that confers resistance to crizotinib in *ALK*-rearranged IMTs.[20] Genomic aberrations in *ALK* have also been described in other cancer types, including renal cell carcinoma,[21] rhabdomyosarcoma,[22] thyroid carcinoma,[23] colorectal cancer,[24] spitzoid melanomas,[25] and others, but the use of ALK inhibitors in these patient populations has not been described.

Efficacy of Crizotinib in Non–Small Cell Lung Cancer With MET or ROS1 Abnormalities

In addition to being an inhibitor of ALK, crizotinib is a potent inhibitor of the tyrosine kinases MET[26] and ROS1,[27] and these findings have translated into clinical benefit for patients who have NSCLC with genomic aberrations in these kinases. In patients who have lung cancer with de novo genomic *MET* amplification and no *ALK* rearrangements, crizotinib has resulted in rapid and durable responses.[28,29] Short-term responses to crizotinib in *MET*-amplified esophagogastric adenocarcinomas[30] and glioblastoma[31] have also been reported. Furthermore, a subset of *EGFR*-mutant NSCLC tumors that have become resistant to EGFR TKIs demonstrate genomic amplification of the *MET* locus as a mechanism of acquired resistance.[32,33] In preclinical models of *MET*-amplified, *EGFR*-mutant tumors resistant to EGFR TKIs, the addition of crizotinib results in regression of mouse xenografts.[34] This finding is the basis for the phase 1 clinical trial combining the irreversible EGFR/HER2/HER4 inhibitor dacomitinib with crizotinib in patients who have NSCLC resistant to EGFR TKIs (NCT01121575).

Crizotinib is also a potent inhibitor of ROS1, a tyrosine kinase closely related to ALK, and dramatic clinical responses to crizotinib have been observed.[27,35] As in ALK-positive NSCLC, resistance to crizotinib in *ROS1*-

rearranged NSCLC has been reported. In one case, a patient with ROS1-positive NSCLC developed a G2032R substitution in the ROS1 kinase domain, which is analogous to the G1202R mutation previously described in crizotinib-resistant ALK-positive NSCLC.[36,37] In another case, a patient with ROS1-positive NSCLC developed resistance to crizotinib, but no mutations in the *ROS1* kinase domain were identified.[38]

Limitations of Crizotinib

Central Nervous System Relapse. Although there are individual case reports of patients with ALK-positive NSCLC and brain metastases having a CNS response to crizotinib,[39] a significant limitation of crizotinib appears to be poor activity in the CNS. Numerous reports have highlighted the ineffectiveness of crizotinib at controlling disease in the CNS.[40,41] In a retrospective analysis of pooled data from the PROFILE 1005 and PROFILE 1007 studies, the intracranial ORR to crizotinib in patients with ALK-positive NSCLC and previously treated or untreated brain metastases was only 7%, although the 12-week intracranial disease control rate (percentage of complete responses + partial responses + stable disease) was approximately 60%.[42] Further, among the 146 patients with ALK-positive NSCLC from the crizotinib phase 1 and phase 2 trials (PROFILE 1001 and PROFILE 1005) in whom progressive disease developed while they were taking crizotinib, the brain was the most common site of cancer recurrence in a single organ. In many of these patients with brain-only recurrence, it was possible to maintain systemic cancer control with continued administration of crizotinib once their CNS disease had been treated with radiation or surgery.[10] The high rate of CNS relapse in patients treated with crizotinib is likely due to poor blood-brain barrier penetration of crizotinib; in one patient with ALK-positive NSCLC on crizotinib who had a relapse only in the CNS, the ratio of the cerebrospinal fluid concentration of crizotinib to the plasma concentration was found to be just 0.0026, a very low value.[43]

Resistance to Crizotinib. For patients who have ALK-positive NSCLC, the median PFS with crizotinib is 8 to 10 months.[9] Multiple mechanisms of crizotinib resistance have been described in ALK-positive NSCLC (Table 4). To date, there are 69 reported cases of patients with crizotinib-resistant ALK-positive NSCLC who underwent additional biopsies at the time of crizotinib resistance. Secondary mutations in the *ALK* kinase domain were detected in 20 patients (29%), most commonly L1196M (the "gatekeeper" mutation) and G1269A. Amplification of the rearranged *ALK* locus was detected in 6 patients (9%). In a few patients, activation of alternative receptor tyrosine kinases, such as EGFR and KIT, was observed, and in the remaining patients, the mechanism of resistance was not known.[8,20,37,44-46] Several potential approaches that could delay or overcome crizotinib resistance include alternative TKI dosing schedules, next-generation TKIs, Hsp90 inhibitors, and combinations of ALK TKIs with other drugs[47]; these strategies are discussed below. There is a relative paucity of data on combining ALK TKIs with cytotoxic chemotherapy, monoclonal antibodies, proapoptotic small molecules, immunotherapies, or other TKIs, and therefore this review does not focus on these approaches.

Table 4. Mechanisms of Crizotinib Resistance in Samples of Patients With ALK-Positive Non–Small Cell Lung Cancer

Resistance Mechanisms	Patients N=69 (percent)
Secondary mutations in ALK kinase domain	20 (29)
L1196M	9
G1269A	7[a]
C1156Y	2
S1206Y	1
1151Tins	1
L1152R	1
G1202R	1
Amplification of rearranged ALK locus	6[b] (9)
No secondary ALK mutation or amplification	44 (64)

[a] Two cases occurred in the setting of another secondary mutation in the ALK kinase domain (1151Tins in one patient, L1196M in another patient).

[b] One case of amplification occurred in setting of an ALK kinase domain mutation (G1269A).

Sources: Choi YL et al,[8] Katayama R et al,[37] Doebele RC et al,[44] Sasaki T et al,[45] Gainor JF et al,[46] Kim S et al,[97] Huang D et al,[98] Costa DB et al.[99]

Next-Generation ALK Tyrosine Kinase Inhibitors

Ceritinib

Ceritinib (Zykadia, Novartis; formerly called LDK378) is a recently FDA-approved potent ALK inhibitor derived from the Novartis lead compound TAE684.[48] It has an IC_{50} of 200 pM in ALK enzymatic assays and an IC_{50} of approximately 25 nM in various cell-based assays. In rat xenograft models with the H2228 NSCLC EML4-ALK cell line, the daily administration of ceritinib resulted in complete tumor regression. In ALK-positive cell line models, ceritinib was able to effectively inhibit ALK harboring the crizotinib-resistant mutations L1196M, G1269A, I1171T, and S1206Y, but it was ineffective at inhibiting ALK containing the G1202R and F1174C mutations.[49] Among a panel of 46 other tested kinases, ceritinib showed strong activity only against IGF-1R (IC_{50}=8 nM), INSR (IC_{50}=7 nM), and STK22D (IC_{50}=23 nM); therefore, its selectivity for ALK is 40-, 35-, and 115-fold higher than its selectivity for these other kinases, respectively.[50,51]

In a recently reported phase 1 study of patients with advanced cancers harboring genomic ALK alterations, ceritinib was shown to be highly active in ALK-positive NSCLC, both in the crizotinib-naive and crizotinib-treated settings. ALK positivity was established with either local or central *ALK* fluorescence in situ hybridization (FISH) testing. The ORR for 114 patients with NSCLC who received ceritinib at a dose of 400 mg per day or higher was 58%, with a median PFS of 7.0 months. The response rate for 80 patients who had previously been treated with crizotinib was 56%, while the response rate among patients who were crizotinib-naive was 62%. Responses were also seen in untreated CNS lesions in patients with crizotinib-resistant disease. Common adverse events included nausea (82%), diarrhea (75%), vomiting (65%), fatigue (47%), and elevated transaminases. The most frequent grade 3 or 4 adverse events were increased alanine aminotransferase (ALT, 21%), increased aspartate aminotransferase (AST, 11%), diarrhea (7%), and elevated lipase (7%); these toxicities were reversible after ceritinib was stopped. In 6% of patients, ceritinib was discontinued because of adverse events.[52]

To define molecular correlates of response, 19 patients with crizotinib-resistant disease underwent a second tumor biopsy before the initiation of ceritinib. All of these tumors still demonstrated *ALK* rearrangement by FISH. Of these 19 resistant samples, 7 had either genomic amplification of the *ALK* locus or an acquired resistance mutation within *ALK*; responses to ceritinib were observed in 5 of 7 of these patients. In the other 12 patients, no additional genetic alterations in *ALK* (other than the original rearrangement) were observed; responses to ceritinib were seen in 7 of these patients. Taken together, these findings suggest that ceritinib is highly active in the majority of patients with ALK-positive NSCLC who relapse on crizotinib, including patients with and without acquired resistance mutations.[52]

Like those treated with crizotinib, patients treated with ceritinib invariably relapse because of the emergence of resistance. To date, little is known about the mechanisms of resistance to next-generation ALK inhibitors such as ceritinib. In one small study, 11 patients who relapsed on ceritinib underwent biopsy of a resistant site. Of the 11 patients, 5 were found to have acquired new mutations in the *ALK* kinase domain, either at position G1202 or at position F1174. These same kinase domain mutations conferred resistance to ceritinib in vitro as well.[49] The fact that the more commonly occurring crizotinib-resistant *ALK* mutations (L1196M, G1269A; Table 4) were not observed in ceritinib-resistant samples suggests that different patterns of resistance will emerge depending on the selective pressure of the TKI for resistant tumor subclones.

Two phase 2 studies of ceritinib in patients who had ALK-positive NSCLC previously treated with chemotherapy have recently been completed: one for crizotinib-naive patients (NCT01685138) and the other for patients previously treated with crizotinib (NCT01685060). Two phase 3 trials with ceritinib are currently ongoing. One compares ceritinib with standard chemotherapy in patients who have previously untreated ALK-positive NSCLC (NCT01828099), and the other compares ceritinib with standard chemotherapy in patients who have ALK-positive NSCLC previously treated with chemotherapy and crizotinib (NCT01828112). Results of these studies have not yet been reported.

Alectinib

Alectinib (RO5424802/CH5424802), which is being developed by Roche, was designed to be a more selective and potent ALK inhibitor than crizotinib; in addition to activity against ALK, it has activity against the kinases LTK and GAK, but it is not active against INSR, IGF-1R, MET, or ROS1.[53-55] Preclinical studies have demonstrated that alectinib is active against the crizotinib-resistant *ALK* mutations L1196M, C1156Y, and F1174L,[54] suggesting that alectinib may be effective in patients who have become resistant to crizotinib through these mechanisms. The activating mutations F1174L and R1275Q, which are found in neuroblastoma, are susceptible to inhibition by alectinib,[54] indicating a potential clinical use for this compound in neuroblastoma.

The activity of alectinib was first studied in a multicenter Japanese phase 1/2 trial of patients with ALK-positive NSCLC who had not previously received crizotinib or other ALK inhibitors. Patients were identified as having ALK-positive NSCLC based on positive immunohistochemical staining for ALK expression, followed by *ALK* FISH for confirmation. Among 46 patients who were treated with the recommended phase 2 dose of 300 mg twice daily, the ORR was 93.5%. Median PFS has not yet been reached. The most common side effects of alectinib at this dose included predominantly grade 1 or 2 dysgeusia, elevated AST, increased bilirubin, decreased neutrophil count, increased creatinine, increased creatine phosphokinase (CPK), myalgias, gastrointestinal symptoms, and rash. The only grade 3 adverse event that occurred at this dose was decreased neutrophil count, and no grade 4 toxicities were observed.[55,56]

Like ceritinib, alectinib appears to be active in the majority of patients with crizotinib-resistant ALK-positive NSCLC. A phase 1 study of alectinib has been conducted in the United States, with doses ranging from 300 to 900 mg twice daily. This study enrolled 47 patients who had ALK-positive NSCLC previously treated with crizotinib (but not with other ALK inhibitors). Among 44 evaluable patients, the unconfirmed response rate to alectinib was 54.5%. Preliminary results also suggest that alectinib has antitumor activity in the

CNS in patients with disease refractory to crizotinib.[57] Common adverse events included fatigue (30%), myalgia, peripheral edema, CPK elevation, nausea, increased ALT, photosensitivity, constipation, and rash. Grade 3 or 4 adverse events were uncommon and included headache, neutropenia, fluid retention/peripheral edema, increased γ-glutamyltransferase, and hypophosphatemia.[57] An ongoing phase 2 study of alectinib is open for patients who have ALK-positive NSCLC previously treated with crizotinib (NCT01871805).

AP26113

AP26113 is in development by Ariad. In preclinical studies, AP26113 was found to be a potent inhibitor of wild-type ALK (IC_{50}=21 nM in a Ba/F3 model of EML4-ALK) and maintains reasonable activity against several crizotinib-resistant *ALK* mutants (IC_{50}=26-254 nM). This compound also inhibits ROS1 (IC_{50}=16-41 nM in Ba/F3 models), with a potency similar to that for ALK regardless of the ROS1 upstream fusion partner (including CD74, FIG, SDC4, or EZR).[58]

In an ongoing phase 1/2 study of AP26113, among 16 patients with ALK-positive NSCLC resistant to crizotinib (but who did not receive any other ALK TKIs), 12 patients (75%) responded at doses of AP26113 between 60 and 240 mg daily, with response durations of up to more than 40 weeks. Of 4 TKI-naive patients with ALK-positive NSCLC, 2 patients responded to AP26113, and the other 2 patients had stable disease. Among 3 patients with NSCLC in the trial who had previously received 2 ALK inhibitors (crizotinib and ceritinib), one remains in the study with stable disease, one developed progressive disease, and the third discontinued treatment before follow-up scans. Additionally, 4 of 5 ALK-positive patients who had CNS lesions showed improvements with AP26113 treatment on follow-up magnetic resonance imaging of the brain. AP26113 has also shown clinical activity in a patient with an ALK-positive IMT. The most common adverse events in this trial (all grades) were fatigue (40%), nausea (36%), diarrhea (33%), and headache (18%).[59] In this trial, early-onset pulmonary symptoms (dyspnea, hypoxemia, lung infection) were observed in 9% to 12% of patients, typically on day 1 or 2 after initiation of the medication at a dose of 180 mg daily. Because of this, the recommended phase 2 dosing is 90 mg daily for 1 week, and if no respiratory symptoms arise, the dose is increased to 180 mg daily.[60]

ASP3026

ASP3026, which is being developed by Astellas Pharma, inhibits ALK with an IC_{50} of 3.5 nM in enzymatic assays and an IC_{50} of 64.8 nM in H2228 cells. This agent also displays activity against the neuroblastoma-activating *ALK* mutants F1174L (IC_{50}=10 nM) and R1275Q (IC_{50}=5.4 nM) and against the crizotinib-resistant gatekeeper mutation L1196M. Among a panel of 86 tyrosine kinases, ASP3026 showed the highest selectivity for ROS1 (IC_{50}=8.9 nM) and ACK (IC_{50}=5.8 nM). This compound has antitumor activity in both NCI-H2228 subcutaneous xenograft mouse models and EML4-ALK transgenic mice.[61] Preliminary results of a phase 1 trial of patients with advanced solid tumors (ALK positivity not required) showed that ASP3026 has a favorable safety profile, with a maximum tolerated dose of 525 mg daily. Gastrointestinal symptoms were the most common side effects, but grade 3 rash and elevations of AST and ALT were also observed. The clinical activity of ASP3026 has not yet been reported.[62]

TSR-011

TSR-011, which is being developed by Tesaro, is a potent inhibitor of ALK, with an IC_{50} of approximately 1 nM in various preclinical models.[63] In a phase 1 trial, 65% of the 17 evaluable patients with advanced malignancies (including NSCLC, papillary thyroid cancer, pancreatic cancer, and colorectal cancer) achieved stable disease or a partial response at 8 weeks. Of 3 evaluable patients with ALK-positive NSCLC who had relapsed on crizotinib, 1 patient had a partial response and 2 had stable disease. Dose-limiting toxicities included QTc prolongation and dysesthesia.[64]

Of note, TSR-011 is also a potent inhibitor of TRK-A, TRK-B, and TRK-C (encoded by *NTRK1, NTRK2,* and *NTRK3*, respectively).[64] Rearrangements in *NTRK1* were recently described in a small fraction of patients with lung cancer who did not have other, more commonly found genomic alterations.[65] Because *TRK* rearrangements have also been found in other tumor types, including colon cancer[66] and papillary thyroid cancer,[67] TSR-011 may be a useful targeted agent in a variety of cancer types.

PF-06463922

PF-06463922, which is in development by Pfizer, is a novel, highly potent, selective inhibitor of both ALK and ROS1, and it has strong activity against all known ALK and ROS1 mutants identified in patients with crizotinib-resistant disease. This compound was designed to be a low-efflux substrate from cell lines overexpressing P-glycoprotein in order to increase its potential CNS penetration. Preclinical in vivo rodent models have demonstrated that levels of PF-06463922 in the brain are about 20% to 30% of the levels achieved in plasma. Furthermore, in mice harboring EML4-ALK–driven brain tumors, treatment with PF-06463922 caused brain tumor regression and increased OS, indicating that it may be an excellent therapeutic agent for patients with crizotinib-resistant disease and/or patients with CNS disease in either the crizotinib-naive or crizotinib-resistant setting.[68-70] A phase

1/2 clinical study of PF-06463922 in ALK-positive and ROS1-positive NSCLC is currently under way.

RXDX-101

RXDX-101 (formerly called NMS-E628, Nerviano) is an inhibitor of ALK, ROS1, TRK-A, TRK-B, and TRK-C. This compound, which is being developed by Ignyta, induces tumor regression in mouse models of NPM-ALK–driven lymphoma and EML4-ALK–driven NSCLC; it also has activity against the crizotinib-resistant ALK mutants L1196M and C1156Y. RXDX-101 also crosses the blood-brain barrier, inhibits tumor growth, and prolongs survival in mice with intracranially injected NCI-H2228 EML4-ALK cells.[71,72] In vitro and in vivo activity against ROS1-driven cancers has also been reported.[73] Preliminary results of the RXDX-101 phase 1 trial show that the drug is well tolerated, with early evidence of antitumor activity.[74]

X-376 and X-396

Compared with crizotinib, X-376 and X-396 inhibit ALK with approximately 10-fold greater potency in biochemical assays and 3- to 10-fold greater potency in cell-based assays. By contrast, crizotinib is a slightly more potent MET inhibitor than X-376 and X-396 in biochemical assays (IC_{50}=0.51, 0.69, and 0.74 nM for crizotinib, X-376, and X-396, respectively) as well as in cell-based assays with the MKN-45 MET-driven cell line (IC_{50}=51, 150, and 156 nM for crizotinib, X-376, and X-396, respectively). In Ba/F3 models of crizotinib resistance, X-396 was an approximately 10-fold more potent inhibitor than crizotinib of the ALK mutants L1196M and C1156Y.[75] Initial results of a phase 1 study of X-396 showed responses in both crizotinib-naive and crizotinib-reistant ALK-positive NSCLC patients.[76] Both X-376 and X-396 are being developed by Xcovery.

CEP-28122 and CEP-37440

CEP-28122, which is being developed by Teva, is a potent and selective ALK inhibitor (IC_{50}=1.9 nM in enzymatic assays). Against a panel of 259 protein kinases, CEP-28122 also displayed activity against the serine/threonine kinases RSK2, RSK3, and RSK4 (IC_{50} range, 7-19 nM); the degree of inhibition for all other kinases was at least 10-fold weaker than that for ALK. CEP-28122 displayed antitumor activity in the ALK-driven mouse xenograft models with the NCI-H2228 and NCI-H3122 cell lines. Complete tumor regression within 1 to 2 days after the initiation of CEP-28122 was observed in human primary NPM-ALK–positive anaplastic large cell lymphoma mouse grafts.[77] Another Teva compound, CEP-37440, is an inhibitor of ALK as well as focal adhesion kinase (FAK); this drug is in phase 1 development in patients with advanced solid tumors.

Heat Shock Protein 90 Inhibitors

Hsp90 is a protein chaperone involved in regulating the stability of proteins involved in normal cellular functions as well as tumorigenesis.[78] Fusion proteins formed as the result of chromosomal rearrangement are thought to be particularly dependent on Hsp90 for protein folding, transport, and stability,[79] and at least 3 Hsp90 inhibitors have been tested in patients with *ALK* rearrangements: retaspimycin hydrochloride (IPI-504), ganetespib (STA-9090), and AUY922.

Preclinical studies have shown that crizotinib-resistant ALK-positive cell lines are highly sensitive to the Hsp90 inhibitor 17-allylamino-17-demthoxygeldanamycin (17-AAG).[80,81] Retaspimycin hydrochloride (IPI-504), an analogue of 17-AAG, was tested in a phase 1/2 study of patients with NSCLC. Among 76 patients enrolled in the study, the ORR was 7%, but among the 3 patients in the study with *ALK* rearrangements, 2 had a partial response and a third had prolonged stable disease. Although the estimated median PFS for all patients in this study was 2.9 months, the 3 patients with ALK-positive NSCLC received IPI-504 for approximately 7 months.[82] The most common side effects of IPI-504 were grade 1 or 2 fatigue, nausea, and diarrhea; grade 3 or 4 liver function test abnormalities occurred in 12% of patients.[83]

Preclinical work has demonstrated that NSCLC cell lines harboring oncogenic rearrangements of *ALK*, *ROS1*, or *RET* are all sensitive to the Hsp90 inhibitor ganetespib. Of the 99 patients enrolled in a phase 2 study of ganetespib, only 4 patients (4%) achieved a partial response, but all 4 of these patients were ALK-positive and crizotinib-naive, with response durations ranging from 7.4 to 21 months. The most common side effects of ganetespib were diarrhea (82%), fatigue (58%), nausea (41%), and anorexia (37%). Treatment-related deaths occurred in 2 patients (2%): one from cardiac arrest and the other from renal failure.[84]

Preliminary data from a phase 2 study of the Hsp90 inhibitor AUY922 in 121 patients with NSCLC showed partial responses in 6 of 21 patients (29%) with *ALK* rearrangements. Of these 6 responders who had ALK-positive NSCLC, 4 were crizotinib-naive and 2 had previously been treated with crizotinib. The estimated median PFS rate was 42% at 18 weeks in ALK-positive patients. The most frequent adverse events in this study included eye disorders (77%), diarrhea (74%), and nausea (46%).[85]

Hsp90 inhibitors may be challenging to develop in ALK-positive NSCLC, given the established efficacy and safety of crizotinib, ceritinib, and other ALK TKIs. Compared with TKIs, Hsp90 inhibitors appear to have lower response rates and side effects that are less tolerable. Their activity appears to be limited in the setting of crizotinib resistance, and they do not have CNS activity. One potential clinical use of Hsp90 inhibitors in ALK-positive

NSCLC may be for those patients who cannot tolerate TKIs (eg, because of TKI-associated pneumonitis).

Because of the invariable emergence of resistance in patients who have ALK-positive NSCLC treated with crizotinib monotherapy, a number of trials are under way combining an ALK TKI with an Hsp90 inhibitor (see Table 2) in the hope that therapies with nonoverlapping mechanisms of action may be more effective than monotherapies in delaying or overcoming resistance. This approach is supported by preclinical evidence demonstrating synergistic antitumor effects when TKIs are combined with Hsp90 inhibitors in ALK- or MET-driven cancers.[83,86] Crizotinib is being combined with ganetespib in patients who have crizotinib-naive ALK-positive disease (NCT01579994), and 2 clinical trials are open for patients who have crizotinib-resistant NSCLC; in one trial (NCT01712217), the Hsp90 inhibitor AT13387 is being administered alone or in combination with crizotinib, and in another trial (NCT01772797), AUY922 is being combined with ceritinib.

Conclusions

New drugs are urgently needed in the treatment of NSCLC, a prevalent disease with a high mortality rate. Crizotinib has quickly become a promising treatment option for patients with ALK-positive NSCLC, IMT, and anaplastic large cell lymphoma, as well as for patients with cancers harboring aberrations in ROS1. However, the efficacy of crizotinib in these cancers is limited by poor activity in the CNS and the frequent emergence of drug resistance in a relatively short time. Several new TKIs are in various stages of clinical development for ALK-positive cancers, and many of these agents have activity both in the CNS and in cancers that have become resistant to crizotinib. Despite the increased potency and specificity of next-generation ALK TKIs, resistance to these compounds has also been described.[49]

These challenges raise a number of questions for the research community. In what sequence should ALK TKIs be prescribed? Should crizotinib still be prescribed as the first targeted therapy, with next-generation ALK inhibitors reserved for subsequent lines of treatment because they are still able to overcome crizotinib resistance in the majority of cases? On the other hand, would the administration of more potent ALK inhibitors in the first-line setting result in deeper responses and longer response durations, or would the earlier use of more potent ALK TKIs lead to the more rapid development of highly resistant disease? Ongoing clinical trials with ceritinib and alectinib in crizotinib-naive patients may help to address these questions once results are available.

Would the earlier use of brain-penetrable ALK TKIs like PF-06463922 delay or possibly prevent the development of CNS metastases, a common cause of morbidity and mortality in this patient population? Also, if patients become resistant to next-generation ALK TKIs, will they exhibit cross-resistance to all other ALK TKIs? Should patients be switched to a different ALK inhibitor before resistance to their prior treatment emerges? Will combinations of ALK TKIs with other agents (eg, cytotoxic chemotherapy, Hsp90 inhibitors, immunotherapy, other TKIs) be both tolerable and effective in patients with ALK-positive cancers? With so many ALK inhibitors in development, it will be difficult to sort out with randomized clinical trials which drugs should be used and at what times in a patient's treatment course. Mathematical modeling of the growth kinetics of TKI-sensitive and TKI-resistant cancer cells may help us to determine how kinase inhibitors, cytotoxic agents, and other therapies should be dosed and intercalated.[87-89]

Despite the challenges that lie ahead in the treatment of ALK-positive cancers, OS for patients who have NSCLC with targetable mutations is increasing. Although it will be impossible to show OS benefits in clinical trials because of crossover effect, data presented at the 15th World Conference on Lung Cancer in October 2013 demonstrated that patients whose cancers had an oncogenic driver that could be treated with a genotype-directed therapy (eg, EGFR, ALK) had a median OS of 3.5 years, whereas patients whose cancers had an oncogenic driver that could not be treated with genotype-directed therapy (eg, KRAS) lived for 2.4 years ($P<.0001$).[90] With many next-generation ALK inhibitors and combination strategies on the horizon, survival rates in patients with ALK-positive lung cancers will likely continue to improve. However, with these breakthroughs come increasing expenses that place significant financial burdens on health care systems.[91,92] For example, the National Institute for Health and Care Excellence (NICE) denied approval of crizotinib in the United Kingdom because its use was not deemed cost-effective.[93] Improving the efficiency of tumor genotyping and decreasing the costs of drug development and delivery will remain top priorities, so that all patients can have access to these life-prolonging cancer treatments.

Financial Disclosures

Dr Shaw consults for Pfizer, Novartis, Ariad, Chugai, Ignyta, Daiichi-Sankyo, and Genentech.

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