Current Status of Targeted Therapy for Anaplastic Lymphoma Kinase–Rearranged Non–Small Cell Lung Cancer

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The identification of chromosomal rearrangements involving the anaplastic lymphoma kinase (*ALK*) gene in ~3–5% of non–small cell lung cancer (NSCLC) tissues and the demonstration that the first-in-class ALK tyrosine kinase inhibitor, crizotinib, can effectively target these tumors represent a significant advance in the evolution of personalized medicine for NSCLC. Single-arm studies demonstrating rapid and durable responses in the majority of ALK-positive NSCLC patients treated with crizotinib have been followed by a randomized phase III clinical trial in which superiority of crizotinib over chemotherapy was seen in previously treated ALK-positive NSCLC patients. However, despite the initial responses, most patients develop acquired resistance to crizotinib. Several novel therapeutic approaches targeting ALK-positive NSCLC are currently under evaluation in clinical trials, including second-generation ALK inhibitors, such as LDK378, CH5424802 (RO5424802802), and AP26113, and heat shock protein 90 inhibitors.

Anaplastic lymphoma kinase (*ALK*) was originally identified in 1994 as the tyrosine kinase component of a novel fusion gene that results from a chromosomal translocation in a rare human lymphoma, anaplastic large-cell lymphoma.[1] Activation of *ALK* by gene rearrangements or mutations has subsequently been described in other tumors, including a subset of non–small cell lung cancer (NSCLC).[2,3] NSCLC tissues harboring *ALK* gene rearrangements have the characteristics of "oncogene-addicted tumors," as evidenced by the dramatic and durable responses observed to the first-in-class ALK tyrosine kinase inhibitor, crizotinib. Crizotinib is now established as the standard of care for the management of ALK-positive NSCLC. In this review, we describe the biology and clinical features of ALK-positive NSCLC, outline the therapeutic efforts undertaken to target ALK with crizotinib, detail the mechanisms of acquired resistance to crizotinib, and finally discuss novel strategies to target ALK using second-generation ALK inhibitors and heat shock protein 90 (HSP90) inhibitors.

BIOLOGY OF ALK AND ALK FUSIONS

The *ALK* gene is located on chromosome 2p and encodes a 1,620 amino acid receptor tyrosine kinase in the insulin receptor superfamily. ALK is most closely related to leukocyte tyrosine kinase, c-ros oncogene 1 (ROS1), insulin-like growth factor-1 receptor, and the insulin receptor. Like other classic receptor tyrosine kinases, the native ALK protein consists of an extracellular ligand-binding domain, a short transmembrane region, and an intracellular tyrosine kinase domain.

The normal physiological role of ALK is poorly understood.[4] Activation is believed to occur through ligand-induced dimerization. ALK ligands have been identified in *Drosophila melanogaster* (Jelly-belly or Jeb)[5,6] and in Caenorhabditis *elegans* (HEN-1),[7] but no Jeb/HEN-1-like ligand has yet been identified in vertebrates. The growth factors pleiotrophin and midkine have been proposed as ligands for mammalian ALK and other receptor tyrosine kinases, but their role in activating ALK is controversial.[4] The expression pattern of ALK suggests an important role in the development of the nervous system. However, *ALK*-knockout mice are viable and exhibit no apparent developmental or tissue abnormalities. More extensive analyses of these mice have revealed only age-related increases in hippocampal progenitor cells and changes in behavioral testing.[8]

Oncogenic activation of ALK occurs in a variety of human malignancies. In most cases, ALK is aberrantly activated due to chromosomal rearrangement, either an intrachromosomal inversion or an interchromosomal translocation. First discovered in anaplastic large-cell lymphoma,[1] *ALK* gene rearrangements have since been found in inflammatory myofibroblastic

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tumors,[9] NSCLC,[2,3] renal cell carcinomas,[10] and other solid tumors.[11,12] In addition to rearrangements, activating point mutations in the *ALK* tyrosine kinase domain have been reported in pediatric neuroblastoma[13–16] and anaplastic thyroid cancer.[17]

In general, chromosomal rearrangements of *ALK* lead to fusion of the tyrosine kinase domain of ALK with a 5′-end partner, such as echinoderm microtubule-associated protein-like 4 (EML4) in NSCLC[2] or nucleophosmin (NPM)[1] in anaplastic large-cell lymphoma. The break point within *ALK* is highly conserved across all *ALK* fusion genes, residing in the intron immediately upstream of the exons encoding the kinase domain. Because the portion of *ALK* involved in the rearrangement does not include the transmembrane domain, the resulting fusion protein is relocated from the cell membrane to the cytoplasm. The 5′-end partners typically contain coiled-coil or leucine zipper domains that enable oligomerization of the fusion protein, leading to ligand-independent activation of the ALK tyrosine kinase. This in turn leads to constitutive activation of downstream signaling pathways, such as the Ras/MAPK, PI3K/AKT, and JAK/STAT pathways (**Figure 1**).

In cell line experiments and genetically engineered mouse models, ALK fusion proteins function as potent oncogenic drivers. For example, transgenic mice expressing *EML4–ALK* in alveolar type 2 pneumocytes develop multiple adenocarcinomas throughout the lungs. These ALK-driven lung tumors are responsive to ALK inhibition and regress on treatment with a small-molecule tyrosine kinase inhibitor of ALK.[18] This finding illustrates the phenomenon of "oncogene addiction," whereby cancer cells become dependent on or addicted to the oncogenic driver and hence are highly sensitive to inhibition of the oncogene.[19] At the cellular level, ALK fusions such as EML4–ALK become the sole regulator of critical downstream signaling pathways. Inhibition of ALK suppresses these pathways, leading to growth arrest and cell death.[20]

To date, nearly 20 different ALK fusion partners have been identified across the diverse tumor types harboring *ALK* rearrangements, including 5 in NSCLC (EML4), kinesin family member 5B, kinesin light chain 1, transforming growth factor, and striatin.[21,22] In NSCLC, EML4 is the most common ALK fusion partner, and multiple *EML4–ALK* variants have been identified.[21] All of these variants contain essentially the same portion of the *ALK* gene fused to different truncations of the *EML4* gene. Whether the different fusions or variants possess different biological properties is uncertain. *In vitro* studies comparing three *EML4–ALK* variants suggest that there may be differences in sensitivity to small-molecule ALK inhibition, possibly related to differences in fusion protein stability.[23] However, in the clinic, no correlation between *EML4–ALK* variant and objective response to crizotinib has been observed.[24]

ALK IN NSCLC

ALK gene rearrangements were first identified in NSCLC by two independent groups in 2007.[2,3] They are present in ~3–5% of NSCLC, with wide variations in the reported frequency depending on the criteria used for identifying tumors as positive for *ALK* rearrangement and the population evaluated.[25] ALK-positive NSCLCs are typically adenocarcinomas, are associated with characteristic morphological features, such as a solid signet ring cell pattern or mucinous cribriform pattern,[26–28] and commonly express both thyroid transcription factor-1 and p63.[27] ALK-positive NSCLC is associated with a younger age of onset, with a median age of ~50 years in ALK-positive NSCLC,[29–31] in contrast to the median age of ~70 years in all patients with NSCLC. Patients with *ALK* rearrangements are more likely to be light or never-smokers.[24,32–34] Of significance, however, *ALK* rearrangements have been detected in smokers in some studies and rarely in patients with squamous cell carcinoma. *ALK* rearrangements are almost always mutually exclusive of epidermal growth factor receptor (*EGFR*) or *KRAS* mutations,[35] although uncommon exceptions to this rule have also been reported.[36] Because many of the clinical and pathologic features of ALK-positive NSCLC overlap with changes in other molecular entities, e.g., *EGFR* mutations and *ROS1* and Rearranged during transfection (*RET*) gene rearrangements, molecular characterization is required to accurately identify this population.

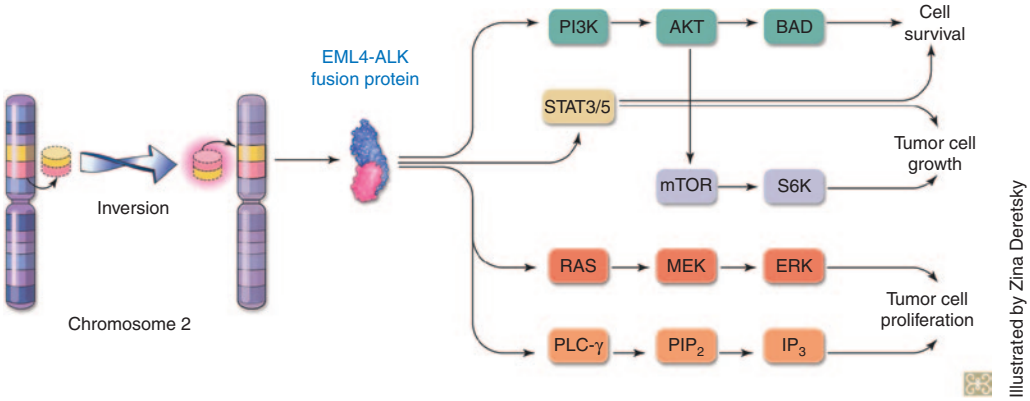

Figure 1 ALK activation and downstream signaling in ALK-rearranged NSCLC. EML4–ALK fusions are due to small inversions within chromosome 2p (left). These fusions lead to aberrant expression of ALK and constitutive activation of the ALK tyrosine kinase and downstream signaling pathways (right). The end result is uncontrolled proliferation and survival of cancer cells. ALK, anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein-like 4; NSCLC, non–small cell lung cancer.

The prognosis and natural history of *ALK* rearrangements in NSCLC have been explored retrospectively. In early-stage disease, the prognostic effect of *ALK* rearrangements is unclear, with reports of both improved and worsened survival in patients with resected ALK-positive NSCLC.[37,38] In the advanced disease setting, ALK-positive patients have been reported to have a higher propensity for pericardial and pleural disease than triple-negative patients (*EGFR*, *KRAS*, *ALK* wild type).[39] Although brain metastases have been reported in ~30% of ALK-positive patients enrolled in clinical trials of crizotinib, the lifetime incidence may be as high as 50% based on a retrospective study of ALK-positive patients.[29] Whether ALK-positive patients have a greater propensity for brain metastases as compared with other genotypes is not known.

In general, the response of ALK-positive patients to platinum-doublet chemotherapy does not appear to be different as compared with patients of other genotypes.[31] Interestingly, however, *ALK*-rearranged tumors demonstrate relatively high response rates to single-agent pemetrexed—with an objective response rate (ORR) of 29% observed in a phase III study in ALK-positive patients,[31] as compared with RRs of ~10% in unselected NSCLC patients.[40] Although some retrospective studies have suggested improved progression-free survival (PFS) in ALK-positive patients treated with pemetrexed,[41] more modest results have been seen in other series[42] and in the recently reported phase III study.[31] As expected, EGFR tyrosine kinase inhibitor therapy is ineffective in ALK-positive patients.[32,43,44] Most significantly, ALK positivity seems to be associated with an improved prognosis, including probable beneficial effects on survival, in the context of treatment with ALK inhibitors.[29]

DETECTION OF ALK IN NSCLC

ALK rearrangements in NSCLC can be detected in clinical samples using several techniques, primarily fluorescence *in situ* hybridization (FISH), reverse transcriptase PCR, and immunohistochemistry (IHC), each with advantages and limitations.[45]

FISH using break-apart probes has come to represent the gold standard because it was clinically validated in trials with crizotinib and is approved by the US Food and Drug Administration (FDA) for this indication. The FISH assay uses the Vysis *ALK* break-apart probes (Abbott Molecular) and involves labeling the 5′ and 3′ ends of the *ALK* gene with differently colored fluorescent probes (typically, red and green). Rearrangements result in a split appearance of the signal (~70% of cases) or loss of the 5′ signal (~30%) in at least 15% of cells counted.[45] FISH can be performed on formalin-fixed paraffin-embedded tissue and is able to detect fusions independently of knowledge of the ALK fusion partner. However, FISH is expensive, requires laboratories with expertise because it can be technically challenging, requires fluorescence microscopy, and may miss rare rearrangements. More recently, methods that use bright-field microscopy obviating the need for fluorescence, namely, chromogenic *in situ* hybridization, have been developed.[46]

Reverse transcriptase PCR is a highly sensitive technique for detection of mRNA for *ALK* that can define both the fusion partner and the variant. Although it has historically required extraction of RNA from frozen tissue, the technique can now be applied to limited tissue samples such as those from clinical biopsies.

Finally, IHC is a readily available, rapid, and cheap technique that may have advantages over other techniques for screening of clinical samples for *ALK* rearrangements. The basis for this test is that ALK is not normally expressed in lung tumors or lung tissue. In the setting of an *ALK* rearrangement, aberrant cytoplasmic expression of the ALK fusion protein can be detected by ALK-specific antibodies. Three major antibodies are available (ALK1 (Dako), D5F3 (Cell Signaling), and 5A4 (Novocastra)); these are typically used with various enhanced detection systems, given the relatively low cytoplasmic expression of ALK in NSCLC. Numerous studies have demonstrated that IHC for ALK performs with excellent sensitivity and specificity for detecting ALK in clinical samples.[34,46–54] As a result, various algorithms have been proposed to use IHC either as a complement to FISH (i.e., FISH confirmation of ALK IHC–positive cases) or as a single test. Validation of these algorithms in larger-scale concordance studies and implementation of standardized protocols and quality control measures will be critical before their routine use in the clinic.

In addition to FISH, PCR, and IHC performed on tumor tissue, newer techniques, including detection of *ALK* gene rearrangements in circulating tumor cells[55] and next-generation sequencing,[56] have also been described. The latter approach has the potential advantage of permitting simultaneous assessment of multiple potentially actionable markers. Ultimately, the technique used in each clinical situation will be dependent on available resources and expertise, as well as the suitability of tissue.

TARGETING ALK-POSITIVE NSCLC WITH CRIZOTINIB

Crizotinib (Xalkori, PF-02341066; Pfizer) is a selective adenosine triphosphate–competitive small-molecule oral inhibitor of ALK, c-MET/hepatocyte growth factor receptor, and ROS1 receptor tyrosine kinases and their oncogenic variants (e.g., c-MET/hepatocyte growth factor receptor mutations and ALK or ROS1 fusion proteins).[24,57,58] The efficacy of crizotinib in patients with ALK-positive advanced NSCLC has been demonstrated in two multicenter, multinational, single-arm studies of crizotinib (250 mg b.i.d.) and a recently completed open-label, randomized, multicenter, multinational, phase III study of crizotinib vs. standard-of-care chemotherapy (pemetrexed or docetaxel).

The first of the two single-arm studies enrolled 119 previously treated and untreated patients who received crizotinib for a median duration of 32 weeks. There were 2 complete responses and 69 partial responses for an ORR of 61%, with a median duration of response of 48 weeks.[24] These results were recently updated to include 143 patients with ALK-positive NSCLC.[30] The ORR was maintained at 61%, with a median duration of response of 49 weeks (**Figure 2**). The ORR was independent of age, sex, and performance status. Median PFS was estimated to be 9.7 months.

The second of the two single-arm studies enrolled 136 previously treated patients who received crizotinib for a median

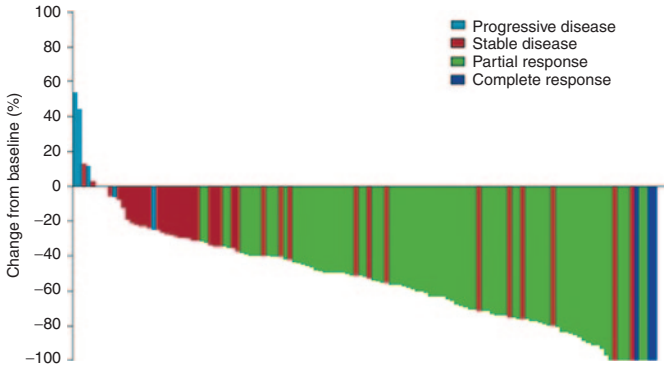

Figure 2 Waterfall plot of best percentage change in target lesions from baseline for patients with ALK-positive NSCLC treated in the phase I study of crizotinib. ALK, anaplastic lymphoma kinase; NSCLC, non–small cell lung cancer. From ref. 30.

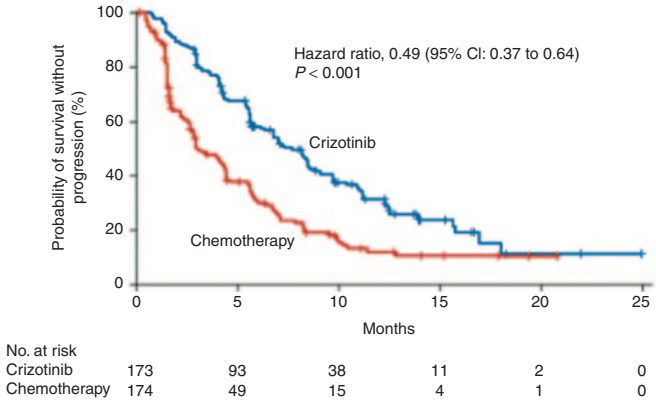

Figure 3 Kaplan–Meier estimates of progression-free survival in the phase III study of crizotinib vs. chemotherapy (pemetrexed or docetaxel). The median progression-free survival was 7.7 months with crizotinib as compared with 3.0 months with chemotherapy (hazard ratio: 0.49 (95% confidence interval: 0.37–0.64); $P < 0.001$). Reprinted with permission from ref. 31.

duration of 22 weeks. There were 1 complete response and 67 partial responses for an ORR of 50%, with a median duration of response of 47 weeks.[59] The results of this study were updated for the first 261 patients with ALK-positive NSCLC who enrolled into the study.[60] The median duration of treatment for these patients was 48 weeks. The ORR was 60%, with a median duration of response of 46 weeks. The median PFS was estimated to be 8.1 months. These two studies formed the basis for the accelerated approval of crizotinib by the FDA in August 2011 for the treatment of advanced ALK-positive NSCLC.

The phase III study of crizotinib vs. chemotherapy met its primary objective by demonstrating that in patients previously treated with first-line platinum-based chemotherapy, crizotinib significantly prolonged PFS as compared with standard, single-agent chemotherapy, as assessed by independent radiology review.[31] In the primary PFS analysis, crizotinib more than doubled the median PFS as compared with chemotherapy, with a median PFS of 7.7 months for 173 patients randomized to crizotinib and 3.0 months for 174 patients randomized to chemotherapy (**Figure 3**). The hazard ratio comparing crizotinib with chemotherapy was 0.487 (95% confidence interval: 0.371–0.638) with a one-sided *P* value of <0.0001 (stratified log-rank test).[31] Median PFS with crizotinib was also superior to either type of chemotherapy, with median PFS rates of 4.2 and 2.6 months for pemetrexed and docetaxel, respectively. Consistent with the PFS results, crizotinib more than tripled the response rate, with an ORR of 65% in the crizotinib group vs. 19% in the chemotherapy group. By type of chemotherapy, the ORR was 29% for pemetrexed and 7% for docetaxel chemotherapy; differences in ORRs between crizotinib and each type of chemotherapy were statistically significant. A randomized controlled study of crizotinib vs. chemotherapy in patients with advanced ALK-positive NSCLC in the first-line treatment setting has recently completed accrual (NCT01154140).

In addition to ALK-positive NSCLC, complete and partial responses have been observed in a variety of other advanced solid tumors treated with crizotinib, including ROS1-positive NSCLC,[61] c-MET-amplified glioblastoma multiforme,[62] c-MET-amplified gastroesophageal carcinoma,[63] ALK-positive inflammatory myofibroblastic tumor,[64] ALK-positive diffuse large B-cell lymphoma,[65] pediatric and adult ALK-positive anaplastic large- cell lymphoma,[65,66] and pediatric ALK-positive neuroblastoma.[66]

In the phase I, II, and III studies of crizotinib, the most commonly reported treatment-related adverse events of any severity and grade were vision disorder, nausea, diarrhea, vomiting, edema, constipation, and elevated levels of the transaminases.[30,31,59,60] The most common grade 3 or 4 crizotinib-related adverse events were neutropenia and elevated transaminase levels.[30,31,59,60] Drug-induced pneumonitis has been reported with crizotinib. To date, across all studies of crizotinib, the estimated incidence of grade 3 or higher treatment-related pneumonitis was ~1–5%, similar to that observed with EGFR tyrosine kinase inhibitors.[31] In addition, in small retrospective studies, other adverse events associated with crizotinib have been described, including asymptomatic bradycardia[67] and decreased total testosterone in male patients.[68]

The pharmacokinetics of crizotinib was evaluated in 167 cancer patients.[69] After oral administration of a single 250-mg dose, crizotinib peak concentration was achieved at a median time to peak concentration of 4 h and then declined in a multiexponential manner with a median half-life of 42 h. Following repeat dosing at 250 mg b.i.d., steady-state concentrations were reached within 15 days. Steady-state concentrations of crizotinib exceeded the target efficacious concentrations for ALK, ROS1, or c-MET inhibition. Crizotinib appeared to exhibit nonlinear pharmacokinetics, reflected by a decrease in clearance observed with multiple dosing.

RESISTANCE TO CRIZOTINIB

Despite often marked initial responses to crizotinib, the majority of patients relapse during the first year of treatment due to the development of resistance.[30,31] This type of resistance, termed acquired resistance, has been observed in other oncogene addiction paradigms, such as *EGFR*-mutant NSCLC treated with

EGFR inhibitors and chronic myelogenous leukemia treated with ABL inhibitors.

Mechanisms of acquired crizotinib resistance can be classified into two general categories (**Figure 4**). The first category involves genetic alteration of the target, i.e., *ALK*, by mutation or gene amplification. In the first report of acquired resistance to crizotinib, deep sequencing of a pleural fluid specimen from a patient relapsing during crizotinib treatment revealed two nonoverlapping mutations in the *ALK* tyrosine kinase domain, L1196M and C1156Y, each of which independently confers resistance to crizotinib *in vitro*.[70] Of note, the L1196M substitution represents the "gatekeeper" mutation, which is believed to cause steric hindrance of drug binding and is analogous to the gatekeeper mutations in EGFR and ABL kinase domains. In subsequent studies, numerous other resistance mutations in the *ALK* kinase domain have been identified from patient samples, including G1269A, 1151Tins, L1152R, G1202R, and S1206Y.[20,71–74] *In vitro*, these secondary mutations appear to confer different degrees of resistance to crizotinib.[20] In addition, amplification of the *ALK* fusion gene has also been identified as a bona fide resistance mechanism both *in vitro* and *in vivo*.[20,71] Overall, approximately one-third of patients relapse during crizotinib treatment due to an *ALK* resistance mutation and/or amplification of the *ALK* fusion gene.

The secondary category of resistance mechanisms involves activation of alternative signaling pathways that can bypass ALK (**Figure 2**). For example, upregulation of EGFR signaling has been observed in ALK-positive cell lines made resistant to crizotinib *in vitro*.[20,72] Consistent with these preclinical findings, nearly one-half of ALK-positive tumors demonstrate immunohistochemical evidence of EGFR activation at the time of crizotinib resistance.[20] CKIT, also known as mast/stem cell growth factor receptor (SCFR) or CD117, has also been identified as a potential bypass track in crizotinib-resistant patients.[20] In 2 of 13 crizotinib-resistant tumors, *CKIT* gene amplification was identified by FISH. In cell line studies, activation of either EGFR or CKIT is capable of conferring resistance to crizotinib. Other potential bypass mechanisms that have been proposed include the acquisition of activating mutations in *EGFR* and *KRAS*.[71,74] Of note, multiple different mechanisms of resistance may be present within the same patient,[20,74] suggesting the need for combination therapies to induce the most durable remissions in patients with crizotinib-resistant disease.

In the clinic, relapses during crizotinib treatment commonly involve the central nervous system (CNS). In one study, the CNS was the first site of disease progression in 13 of 28 ALK-positive patients (46%) treated with crizotinib.[75] The exposure of crizotinib in the CNS is uncertain; however, in one patient who developed CNS disease during crizotinib treatment, the level of drug in the cerebrospinal fluid was extremely low relative to plasma drug levels, suggesting poor penetration into the CNS.[76] This finding raises the possibility that a pharmacokinetic issue, namely, inadequate drug exposure in a sanctuary site, could underlie the high rate of CNS relapse in ALK-positive patients. Many of the next-generation ALK inhibitors (discussed below) are being developed in part to address this potential liability of crizotinib. Whether *ALK* gene alterations or bypass signaling may also contribute to resistance in the CNS is unknown.

SECOND-GENERATION ALK INHIBITORS

LDK378

LDK378 (Novartis Pharmaceuticals) is a novel, potent, and selective small-molecule tyrosine kinase inhibitor targeting

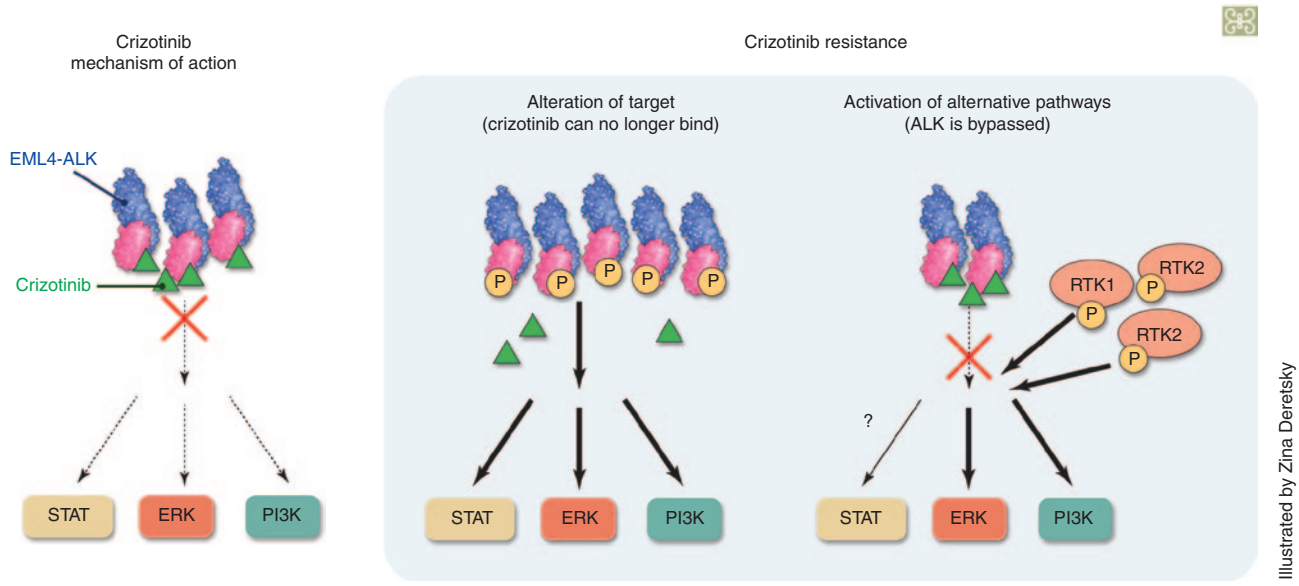

Figure 4 Mechanisms of crizotinib resistance. The left panel depicts crizotinib-sensitive cancer cells expressing EML4–ALK. Crizotinib binds and inactivates the ALK fusion, disrupting downstream signaling. The right panel depicts two general classes of crizotinib resistance: one mediated by genetic mutation of the target so that crizotinib can no longer bind to the active site, and the other mediated by activation of alternative pathways (or bypass tracks) that can engage downstream signaling pathways even when ALK is inhibited. ALK, anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein-like 4; RTK, receptor tyrosine kinase.

ALK. The synthesis and preclinical characterization of LDK378 have been recently reported.[77] LDK378 was synthesized based on the structure of TAE684,[78] a previously described ALK inhibitor with the potential for reactive adduct formation. Using a rational design strategy, novel derivatives that did not form reactive adducts and that potently inhibited ALK were synthesized. One of these was designated compound 15b or LDK378.[77]

In biochemical and cellular assays, LDK378 is both highly potent and selective against ALK. At the enzymatic level, LDK378 inhibits ALK with a half-maximal inhibitory concentration (IC_{50}) value of 200 pmol/l. Only three other kinases in a panel of 30 showed IC_{50} values <100 nmol/l: insulin-like growth factor-1 receptor (8 nmol/l), InsR (7 nmol/l), and STK22D (23 nmol/l). At the cellular level, LDK378 inhibits EML4–ALK with an IC_{50} value of 2.2 nmol/l. No other kinases in a panel of 18 were inhibited by LDK378 at levels <100 nmol/l.[77]

LDK378 has potent antitumor activity in two different rat xenograft models, including one generated from the EML4–ALK-positive H2228 lung cancer cell line and one generated from Karpas299 cells harboring the *NPM–ALK* fusion. In the H2228 model, LDK378 treatment induced dose-dependent tumor regression, with complete regression observed at a dose of 25 mg/kg. LDK378 was noted to be well tolerated at all doses tested.[77]

On the basis of these preclinical data, an international, multicenter phase I study of LDK378 was conducted, and preliminary results were recently presented.[79] LDK378 has been associated with a high response rate in patients with advanced ALK-positive NSCLC, including those with crizotinib-resistant disease. On the basis of the phase I results, LDK378 was granted Breakthrough Therapy designation by the FDA in March 2013. This new designation is intended to expedite development and review of promising new therapies by offering not only fast-track program features but also more intensive guidance from the FDA. The first regulatory filing of LDK378 is anticipated in early 2014.

Two confirmatory phase II studies of LDK378 are currently under way, one for crizotinib-resistant patients (NCT01685060) and one for crizotinib-naive patients (NCT01685138) with advanced, ALK-positive NSCLC. In addition, two ongoing phase III NSCLC studies are comparing LDK378 with standard chemotherapy, one on ALK-positive patients previously treated with platinum-based chemotherapy and crizotinib (NCT01828112), and one on previously untreated, ALK-positive patients (NCT01828099).

CH5424802

CH5424802 (RO 5424802; Chugai Pharmaceuticals and Roche Pharmaceuticals) is a highly potent (IC_{50} = 1.9 nmol/l), selective, orally available benzo[*b*]carbazole derivative ALK inhibitor with demonstrated activity against both ALK and ALK tyrosine kinase domain mutations, including the L1196M gatekeeper mutation and other mutations, e.g., F1174L and R1275Q.[80,81]

A phase I/II study of CH5424802 conducted in Japanese patients with ALK-positive NSCLC has been reported.[82] Patients were required to be positive by both FISH and either IHC or reverse transcriptase PCR for study entry. In the phase I portion of the study, no dose limiting toxicities (DLTs) were observed at the maximally administered dose of 300 mg twice daily, which was chosen as the recommended phase II dose. Forty-three of the 46 patients (93.5%) enrolled in the phase II portion of the study had an independently assessed objective response (2 patients with a complete response and 41 patients with a partial response). Responses occurred early, with 30/46 (65%) achieving partial response within 3 weeks and 40/46 (87%) within 6 weeks. Median PFS had not been reached at the time of the report. Responses were seen in brain metastases in

Table 1 ALK inhibitors currently in the clinic or in clinical development

Drug	Company	Activity against L1196M ALK mutation	Other kinases inhibited	Status	Ongoing studies	Reference
Crizotinib	Pfizer	No	ROS1	Approved	Phase III	Camidge *et al.*,[30] Shaw *et al.*[31]
			c-MET			
LDK378	Novartis	Yes	IGF-1R	Investigational (Breakthrough Therapy designation)	Phase I, phase II, and phase III	Masilje *et al.*,[77] Shaw *et al.*[79]
			ROS1			
CH5424802/ RO5424802	Chugai/Roche	Yes	ROS1	Investigational	Phase I/II	Sakamoto *et al.*,[80] Kinoshita *et al.*,[81] Seto *et al.*[82]
AP26113	Ariad	Yes	EGFR (including T790M)	Investigational	Phase I/II	Zhang *et al.*,[83] Rivera *et al.*,[84] Camidge *et al.*[85]
			ROS1			
ASP3026	Astellas	Yes	ROS1	Investigational	Phase I	Kuromito *et al.*,[87] Patnaik *et al.*[88]
X-396	Xcovery	Yes	Yes	Investigational	Phase I	Lovly *et al.*[86]
TSR-011	Tesaro	Yes	Unknown	Investigational	Phase I	Wilcoxen *et al.*[85]

ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; IGF-1R, insulin-like growth factor-1 receptor; ROS1, c-ros oncogene 1.

three patients. The drug was well tolerated, with the most common adverse events being grade 1 dysgeusia (30%), transient increased aspartate aminotransferase (28%), increased bilirubin (30%), and rash (28%). A phase II study with this compound is ongoing (NCT01871805).

AP26113

AP26113 (Ariad Pharmaceuticals) is a novel inhibitor of ALK (IC_{50}: 5–11 nmol/l) with activity against the ALK tyrosine kinase gatekeeper mutation L1196M (IC_{50}: 15–45 nmol/l) and other *ALK* resistance mutations.[83] This compound is also active against ROS1 and, to a lesser extent, mutant *EGFR* harboring the gatekeeper T790M mutation.[84] In an ongoing phase I/II study (NCT01449461), the recommended phase II dose was determined to be 180 mg once daily, and antitumor activity was seen in patients with advanced, ALK-positive NSCLC.[85] Of the 24 ALK-positive NSCLC patients enrolled in the study, 15 (63%) demonstrated objective responses (1 complete response and 14 partial responses), including 12 of 16 (75%) with crizotinib-resistant tumors. Significantly, four of five patients had objective responses in CNS metastases. The most common treatment-related adverse events were nausea (33%), fatigue (22%), and diarrhea (20%). A confirmatory phase II study of AP26113 is planned.

Several other ALK inhibitors are in clinical development, including X-396 (Xcovery)[86] (NCT01625234), ASP 3026 (Astellas Pharma)[87,88] (NCT01284192), and TSR-011 (Tesaro)[89] (summarized in **Table 1**).

HSP90 INHIBITORS

Another class of drugs with activity in preclinical models of ALK-driven cancers and in clinical trials on ALK-positive NSCLCs comprises the HSP90 inhibitors. Both NPM–ALK[90] and EML–ALK have been shown to be client proteins for HSP90.[23,91–94] Treatment of EML4–ALK-harboring cells with HSP90 inhibitors, such as 17-AAG, 17-DMAG, or ganetespib (STA-9090; Synta), reduces protein levels of the ALK fusion, resulting in cell death *in vitro*, and leads to tumor regression in *in vivo* models. Consistent with these preclinical findings, antitumor activity has been observed in single-arm phase II studies with several HSP90 inhibitors. Three NSCLC patients with *ALK* rearrangements were enrolled in a phase II study with IPI-504 (Infinity Pharmaceuticals), and two partial responses and one case of prolonged stable disease were noted.[94] In a phase II study with ganetespib,[95] four of eight patients with *ALK* gene rearrangements had partial responses and three had stable disease. An objective response to ganetespib was also reported in one patient with crizotinib-resistant ALK-positive NSCLC.[93] AUY922 (Novartis) showed clinical activity as a single agent in patients with crizotinib-treated and crizotinib-naive ALK-positive NSCLC. Objective responses were seen in 6 of 21 (29%) patients enrolled in the ALK-positive NSCLC cohort; four of the six responders were crizotinib naive, whereas two of the six responders had received previous treatment with crizotinib.[96]

Furthermore, given preclinical studies indicating synergy between ALK inhibitors and HSP90 inhibitors,[23,91–93] several combination studies have been initiated including combinations of crizotinib with ganetespib (NCT01579994), crizotinib with AT13387 (NCT01712217), and LDK378 in combination with AUY922 (NCT01772797).

CONCLUSION

The identification of *ALK* gene rearrangements in NSCLC and their subsequent validation as therapeutic targets in NSCLC through clinical trials with crizotinib is a significant advance in what has been termed precision cancer medicine. Noteworthy is the short time line from the original identification of *ALK* gene rearrangements in NSCLC (2007) to the FDA approval of crizotinib for this indication (2011). Testing of NSCLC for *ALK* gene rearrangements and treatment of those patients with ALK-positive tumors is now established as standard of care. Despite excellent initial responses to therapy, however, most patients will go on to develop acquired resistance, both to crizotinib and to second-generation ALK inhibitors. Elucidating mechanisms of acquired resistance to ALK inhibitors and developing therapeutic strategies that may overcome resistance represent priorities in the field. There are now a rapidly expanding range of novel, highly potent ALK inhibitors and HSP90 inhibitors with demonstrated activity in this setting. Key questions include how to choose between these drugs, how to determine optimal sequencing or schedules for treatment, and how to best develop combinations that will both minimize toxicity and achieve the goal of long-term disease control in patients with ALK-positive NSCLC.

CONFLICT OF INTEREST

A.T.S. has done consulting for Pfizer, Novartis, Chugai, Ariad, and Daiichi Sankyo. K.D.W. is an employee of Pfizer. B.S. has done consulting for Pfizer and Novartis.

1. Morris, S.W. *et al*. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* **263**, 1281–1284 (1994).
2. Soda, M. *et al*. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* **448**, 561–566 (2007).
3. Rikova, K. *et al*. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* **131**, 1190–1203 (2007).
4. Chiarle, R., Voena, C., Ambrogio, C., Piva, R. & Inghirami, G. The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nat. Rev. Cancer* **8**, 11–23 (2008).
5. Englund, C. *et al*. Jeb signals through the Alk receptor tyrosine kinase to drive visceral muscle fusion. *Nature* **425**, 512–516 (2003).
6. Lee, H.H., Norris, A., Weiss, J.B. & Frasch, M. Jelly belly protein activates the receptor tyrosine kinase Alk to specify visceral muscle pioneers. *Nature* **425**, 507–512 (2003).
7. Reiner, D.J., Ailion, M., Thomas, J.H. & Meyer, B.J. *C. elegans* anaplastic lymphoma kinase ortholog SCD-2 controls dauer formation by modulating TGF-beta signaling. *Curr. Biol.* **18**, 1101–1109 (2008).
8. Bilsland, J.G. *et al*. Behavioral and neurochemical alterations in mice deficient in anaplastic lymphoma kinase suggest therapeutic potential for psychiatric indications. *Neuropsychopharmacology* **33**, 685–700 (2008).
9. Griffin, C.A., Hawkins, A.L., Dvorak, C., Henkle, C., Ellingham, T. & Perlman, E.J. Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors. *Cancer Res*. **59**, 2776–2780 (1999).
10. Debelenko, L.V. *et al*. Renal cell carcinoma with novel VCL-ALK fusion: new representative of ALK-associated tumor spectrum. *Mod. Pathol*. **24**, 430–442 (2011).
11. Lipson, D. *et al*. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. *Nat. Med*. **18**, 382–384 (2012).
12. Lin, E. *et al*. Exon array profiling detects EML4-ALK fusion in breast, colorectal, and non-small cell lung cancers. *Mol. Cancer Res*. **7**, 1466–1476 (2009).

13. Chen, Y. *et al*. Oncogenic mutations of ALK kinase in neuroblastoma. *Nature* **455**, 971–974 (2008).
14. George, R.E. *et al*. Activating mutations in ALK provide a therapeutic target in neuroblastoma. *Nature* **455**, 975–978 (2008).
15. Janoueix-Lerosey, I. *et al*. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature* **455**, 967–970 (2008).
16. Mossé, Y.P. *et al*. Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* **455**, 930–935 (2008).
17. Murugan, A.K. & Xing, M. Anaplastic thyroid cancers harbor novel oncogenic mutations of the ALK gene. *Cancer Res*. **71**, 4403–4411 (2011).
18. Soda, M. *et al*. A mouse model for EML4-ALK-positive lung cancer. *Proc. Natl. Acad. Sci. USA* **105**, 19893–19897 (2008).
19. Sharma, S.V. & Settleman, J. Oncogene addiction: setting the stage for molecularly targeted cancer therapy. *Genes Dev*. **21**, 3214–3231 (2007).
20. Katayama, R. *et al*. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers. *Sci. Transl. Med*. **4**, 120ra17 (2012).
21. Ou, S.H., Bartlett, C.H., Mino-Kenudson, M., Cui, J. & Iafrate, A.J. Crizotinib for the treatment of ALK-rearranged non-small cell lung cancer: a success story to usher in the second decade of molecular targeted therapy in oncology. *Oncologist* **17**, 1351–1375 (2012).
22. Majewski, I.J. *et al*. Identification of recurrent FGFR3 fusion genes in lung cancer through kinome-centred RNA sequencing. *J. Pathol*. **230**, 270–276 (2013).
23. Heuckmann, J.M. *et al*. Differential protein stability and ALK inhibitor sensitivity of EML4-ALK fusion variants. *Clin. Cancer Res*. **18**, 4682–4690 (2012).
24. Kwak, E.L. *et al*. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N. Engl. J. Med*. **363**, 1693–1703 (2010).
25. Solomon, B., Varella-Garcia, M. & Camidge, D.R. ALK gene rearrangements: a new therapeutic target in a molecularly defined subset of non-small cell lung cancer. *J. Thorac. Oncol*. **4**, 1450–1454 (2009).
26. Rodig, S.J. *et al*. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. *Clin. Cancer Res*. **15**, 5216–5223 (2009).
27. Yoshida, A. *et al*. Comprehensive histologic analysis of ALK-rearranged lung carcinomas. *Am. J. Surg. Pathol*. **35**, 1226–1234 (2011).
28. Zhang, Y.G. *et al*. Evaluation of ALK rearrangement in Chinese non-small cell lung cancer using FISH, immunohistochemistry, and real-time quantitative RT- PCR on paraffin-embedded tissues. *PLoS ONE* **8**, e64821 (2013).
29. Shaw, A.T. *et al*. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harbouring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol*. **12**, 1004–1012 (2011).
30. Camidge, D.R. *et al*. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. *Lancet Oncol*. **13**, 1011–1019 (2012).
31. Shaw, A.T. *et al*. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N. Engl. J. Med*. **368**, 2385–2394 (2013).
32. Shaw, A.T. *et al*. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J. Clin. Oncol*. **27**, 4247–4253 (2009).
33. Wong, D.W. *et al*.; University of Hong Kong Lung Cancer Study Group. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. *Cancer* **115**, 1723–1733 (2009).
34. Paik, J.H. *et al*. Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. *J. Thorac. Oncol*. **6**, 466–472 (2011).
35. Gainor, J.F. *et al*. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. *Clin. Cancer Res*. **19**, 4273–4281 (2013).
36. Johnson, B.E., Kris, M.G. & Lynne, D.B. A multicenter effort to identify driver mutations and employ targeted therapy in patients with lung adenocarcinomas: The Lung Cancer Mutation Consortium (LCMC). *J. Clin. Oncol*. **31** (suppl.; abstr. 8019) (2013).
37. Wu, S.G. *et al*. EML4-ALK translocation predicts better outcome in lung adenocarcinoma patients with wild-type EGFR. *J. Thorac. Oncol*. **7**, 98–104 (2012).
38. Yang, P. *et al*. Worse disease-free survival in never-smokers with ALK+ lung adenocarcinoma. *J. Thorac. Oncol*. **7**, 90–97 (2012).
39. Doebele, R.C. *et al*. Oncogene status predicts patterns of metastatic spread in treatment-naive nonsmall cell lung cancer. *Cancer* **118**, 4502–4511 (2012).
40. Hanna, N. *et al*. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J. Clin. Oncol*. **22**, 1589–1597 (2004).
41. Camidge, D.R. *et al*. Anaplastic lymphoma kinase gene rearrangements in non-small cell lung cancer are associated with prolonged progression-free survival on pemetrexed. *J. Thorac. Oncol*. **6**, 774–780 (2011).
42. Shaw, A.T. *et al*. Pemetrexed-based chemotherapy in patients with advanced, ALK-positive non-small cell lung cancer. *Ann. Oncol*. **24**, 59–66 (2013).
43. Lee, J.K. *et al*. Comparative analyses of overall survival in patients with anaplastic lymphoma kinase-positive and matched wild-type advanced nonsmall cell lung cancer. *Cancer* **118**, 3579–3586 (2012).
44. Kim, H.R. *et al*. Distinct clinical features and outcomes in never-smokers with nonsmall cell lung cancer who harbor EGFR or KRAS mutations or ALK rearrangement. *Cancer* **118**, 729–739 (2012).
45. Shaw, A.T., Solomon, B. & Kenudson, M.M. Crizotinib and testing for ALK. *J. Natl. Compr. Canc. Netw*. **9**, 1335–1341 (2011).
46. Kim, H. *et al*. Detection of ALK gene rearrangement in non-small cell lung cancer: a comparison of fluorescence in situ hybridization and chromogenic in situ hybridization with correlation of ALK protein expression. *J. Thorac. Oncol*. **6**, 1359–1366 (2011).
47. Yi, E.S., Chung, J.H., Kulig, K. & Kerr, K.M. Detection of anaplastic lymphoma kinase (ALK) gene rearrangement in non-small cell lung cancer and related issues in ALK inhibitor therapy: a literature review. *Mol. Diagn. Ther*. **16**, 143–150 (2012).
48. Yi, E.S. *et al*. Correlation of IHC and FISH for ALK gene rearrangement in non-small cell lung carcinoma: IHC score algorithm for FISH. *J. Thorac. Oncol*. **6**, 459–465 (2011).
49. Selinger, C.I. *et al*. Testing for ALK rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent in situ hybridization. *Mod. Pathol*. (2013) 2013 Jun 7. doi: 10.1038/modpathol.2013.87. [Epub ahead of print].
50. Sakai, Y. *et al*. Immunohistochemical profiling of ALK fusion gene-positive adenocarcinomas of the lung. *Int. J. Surg. Pathol*. **21**, 476–482 (2013).
51. Park, H.S. *et al*. Immunohistochemical screening for anaplastic lymphoma kinase (ALK) rearrangement in advanced non-small cell lung cancer patients. *Lung Cancer* **77**, 288–292 (2012).
52. Martinez, P. *et al*. Fluorescence in situ hybridization and immunohistochemistry as diagnostic methods for ALK positive non-small cell lung cancer patients. *PLoS ONE* **8**, e52261 (2013).
53. Conklin, C.M., Craddock, K.J., Have, C., Laskin, J., Couture, C. & Ionescu, D.N. Immunohistochemistry is a reliable screening tool for identification of ALK rearrangement in non-small-cell lung carcinoma and is antibody dependent. *J. Thorac. Oncol*. **8**, 45–51 (2013).
54. Boland, J.M. *et al*. Anaplastic lymphoma kinase immunoreactivity correlates with ALK gene rearrangement and transcriptional up-regulation in non-small cell lung carcinomas. *Hum. Pathol*. **40**, 1152–1158 (2009).
55. Pailler, E. *et al*. Detection of circulating tumor cells harboring a unique ALK rearrangement in ALK-positive non-small-cell lung cancer. *J. Clin. Oncol*. **31**, 2273–2281 (2013).
56. Jung, Y. *et al*. Discovery of ALK-PTPN3 gene fusion from human non-small cell lung carcinoma cell line using next generation RNA sequencing. *Genes. Chromosomes Cancer* **51**, 590–597 (2012).
57. Ou, S.H. *et al*. Activity of crizotinib (PF02341066), a dual mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in a non-small cell lung cancer patient with de novo MET amplification. *J. Thorac. Oncol*. **6**, 942–946 (2011).
58. Bergethon, K. *et al*. ROS1 rearrangements define a unique molecular class of lung cancers. *J. Clin. Oncol*. **30**, 863–870 (2012).
59. XALKORI [package insert], Pfizer:US, 2013.
60. Kim, D.K. *et al*. Updated results of a global phase II study with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC). *J. Clin. Oncol*. **30**, (suppl; abstr. 7533) (2012).
61. Ou, S.H. *et al*. Efficacy and safety of crizotinib in patients with advanced ROS1-rearranged non-small cell lung cancer (NSCLC). *J. Clin. Oncol*. **31** (suppl.; abstr. 8032) (2013).
62. Chu, A.S. *et al*. Clinical improvement and rapid radiographic regression induced by a MET inhibitor in a patient with MET-amplified glioblastoma. *J. Clin. Oncol*. **29** (suppl.; abstr. 2072) (2012).
63. Lennerz, J.K. *et al*. MET amplification identifies a small and aggressive subgroup of esophagogastric adenocarcinoma with evidence of responsiveness to crizotinib. *J. Clin. Oncol*. **29**, 4803–4810 (2011).
64. Butrynski, J.E. *et al*. Crizotinib in ALK-rearranged inflammatory myofibroblastic tumor. *N. Engl. J. Med*. **363**, 1727–1733 (2010).
65. Stasia, A. *et al*. Crizotinib obtains durable responses in advanced chemoresistant ALK+ lymphoma patients. *Haematologica* **97** (suppl. 1), 488 (2012).
66. Mossé, Y.P. *et al*. Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: a Children's Oncology Group phase 1 consortium study. *Lancet Oncol*. **14**, 472–480 (2013).
67. Ou, S.H., Tong, W.P., Azada, M., Siwak-Tapp, C., Dy, J. & Stiber, J.A. Heart rate decrease during crizotinib treatment and potential correlation to clinical response. *Cancer* **119**, 1969–1975 (2013).

68. Weickhardt, A.J. *et al*. Rapid-onset hypogonadism secondary to crizotinib use in men with metastatic nonsmall cell lung cancer. *Cancer* **118**, 5302–5309 (2012).
69. Li, C., Alvey, C., Bello, A., Wilner, K. & Tan, W. Pharmacokinetics (PK) of crizotinib (PF-02341066) in patients with advanced non-small cell lung cancer (NSCLC) and other solid tumors. *J. Clin. Oncol*. **29** (suppl.; abstr. e13065) (2011).
70. Choi, Y.L. *et al*.; ALK Lung Cancer Study Group. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N. Engl. J. Med*. **363**, 1734–1739 (2010).
71. Doebele, R.C. *et al*. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. *Clin. Cancer Res*. **18**, 1472–1482 (2012).
72. Sasaki, T. *et al*. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. *Cancer Res*. **71**, 6051–6060 (2011).
73. Huang, D. *et al*. Multiplexed deep sequencing analysis of ALK kinase domain identifies resistance mutations in relapsed patients following crizotinib treatment. *Genomics* **102**, 157–162 (2013).
74. Kim, S. *et al*. Heterogeneity of genetic changes associated with acquired crizotinib resistance in ALK-rearranged lung cancer. *J. Thorac. Oncol*. **8**, 415–422 (2013).
75. Weickhardt, A.J. *et al*. Continuation of EGFR/ALK inhibition after local therapy of oligoprogressive disease in EGFR mutant (Mt) and ALK+ non-small cell lung cancer (NSCLC). *J. Clin. Oncol*. **30** (suppl.; abstr. 7526) (2012).
76. Costa, D.B. *et al*. CSF concentration of the anaplastic lymphoma kinase inhibitor crizotinib. *J. Clin. Oncol*. **29**, e443–e445 (2011).
77. Marsilje, T.H. *et al*. Synthesis, Structure-Activity Relationships, and *in Vivo* Efficacy of the Novel Potent and Selective Anaplastic Lymphoma Kinase (ALK) Inhibitor 5-Chloro-N2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (LDK378) Currently in Phase 1 and Phase 2 Clinical Trials. *J. Med. Chem*. (2013).
78. Galkin, A.V. *et al*. Identification of NVP-TAE684, a potent, selective, and efficacious inhibitor of NPM-ALK. *Proc. Natl. Acad. Sci. USA* **104**, 270–275 (2007).
79. Shaw, A.T. & Engelman, J.A. ALK in lung cancer: past, present, and future. *J. Clin. Oncol*. **31**, 1105–1111 (2013).
80. Sakamoto, H. *et al*. CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. *Cancer Cell* **19**, 679–690 (2011).
81. Kinoshita, K. *et al*. Design and synthesis of a highly selective, orally active and potent anaplastic lymphoma kinase inhibitor (CH5424802). *Bioorg. Med. Chem*. **20**, 1271–1280 (2012).
82. Seto, T. *et al*. CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1-2 study. *Lancet Oncol*. **14**, 590–598 (2013).
83. Zhang, S. *et al*. Proceedings: AACR 101st Annual Meeting 2010, 15 April 2010. *Cancer Res*. **70** (8; **70** suppl. 1; abstr. LB-298) (2010).
84. Rivera, V.M. *et al*. AP26113 is a dual ALK/EGFR inhibitor: characterization against EGFR T790M in cell and mouse models of NSCLC Proceedings: AACR 103rd Annual Meeting, AACR 2012, Chicago, USA, 2 April, 2012 (2012).
85. Camidge, D.R. *et al*. First-in-human dose-finding study of the ALK/EGFR inhibitor AP26113 in patients with advanced malignancies: Updated results. *J. Clin. Oncol*. **31** (suppl.; abstr. 8031) (2013).
86. Lovly, C.M. *et al*. Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinase inhibitors. *Cancer Res*. **71**, 4920–4931 (2011).
87. Kuromitsu, S. *et al*. Proceedings of the AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics, San Francisco, CA, 12–16 November 2011. *Mol. Cancer. Ther*. **10** (suppl. 11; abstr. nr A227) (2011).
88. Patnaik, A. *et al*. Pharmacokinetics and safety of an oral ALK inhibitor, ASP3026, observed in a phase I dose escalation trial. *J. Clin. Oncol*. **31** (suppl.; abstr. 2602) (2013).
89. Wilcoxen, K.M. *et al*. Proceedings of AACR, 15 April 2012. *Cancer Res*. **71** (8; suppl. 1; abstr. 1795) (2012).
90. Bonvini, P., Gastaldi, T., Falini, B. & Rosolen, A. Nucleophosmin-anaplastic lymphoma kinase (NPM-ALK), a novel Hsp90-client tyrosine kinase: down-regulation of NPM-ALK expression and tyrosine phosphorylation in ALK(+) CD30(+) lymphoma cells by the Hsp90 antagonist 17-allylamino,17-demethoxygeldanamycin. *Cancer Res*. **62**, 1559–1566 (2002).
91. Katayama, R. *et al*. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. *Proc. Natl. Acad. Sci. USA* **108**, 7535–7540 (2011).
92. Normant, E. *et al*. The Hsp90 inhibitor IPI-504 rapidly lowers EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. *Oncogene* **30**, 2581–2586 (2011).
93. Sang, J. *et al*. Targeted inhibition of the molecular chaperone Hsp90 overcomes ALK inhibitor resistance in non-small cell lung cancer. *Cancer Discov*. **3**, 430–443 (2013).
94. Sequist, L.V. *et al*. Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. *J. Clin. Oncol*. **28**, 4953–4960 (2010).
95. Socinski, M.A. *et al*. A multicenter phase II study of ganetespib monotherapy in patients with genotypically defined advanced non-small cell lung cancer. *Clin. Cancer Res*. **19**, 3068–3077 (2013).
96. Felip, E. *et al*. Phase II activity of the HSP90 inhibitor AUY922 in patients with ALK-rearranged (ALK+) or EGFR-mutated advanced non-small cell lung cancer. *Ann. Oncol*. **23**, ix152; abstr. 4380 (2012).