Targeting Oncogenic ALK: A Promising Strategy for Cancer Treatment

Enrique Grande¹, María-Victoria Bolós², and Edurne Arriola³

Abstract

Recently, the anaplastic lymphoma kinase (ALK) has been found to be altered in several solid and hematologic tumors. Novel drugs targeting this tyrosine kinase receptor are under development, and early clinical trials are showing promising activity in non–small cell lung cancer patients with ALK+ tumors. Here, we review the structure and function of the ALK receptor, the mechanisms associated with its deregulation in cancer, methods for ALK detection in tumor samples, its potential as a new marker for candidate patient selection for tailored therapy, and novel drugs under development that target ALK. Mol Cancer Ther; 10(4); 569–79. ©2011 AACR.

Introduction

Traditionally, demonstration of clinical activity has been shown by direct comparison between standard care (control) and an experimental treatment in randomized clinical trials conducted in nonmolecularly selected populations. Deeper knowledge of underlying molecular processes of tumor development has changed this classical concept toward rationally designed trials in subsets of patients carrying specific molecular alterations. Personalized or tailored therapy has been applied in cancer treatment for more than 3 decades as, for example, in the administration of antiestrogen drugs in breast cancer (1). In recent years, the discovery of a variety of molecular and genetic alterations in different malignancies leading to oncogenesis has provided insight into the complexity of tumorigenesis and the opportunity to target specific markers with the objective of improving patient outcomes (2). For example, imatinib has dramatically improved the prognosis of chronic myeloid leukemia and gastrointestinal stromal tumors via its targeted blocking of Bcr-Abl abnormal fusion protein and of c-KIT tyrosine kinase (TK) domains, respectively (3).

In current clinical practice, personalized cancer therapy is well established for a number of predictive biomarkers. HER-2 overexpression, K-RAS and epidermal growth factor receptor (EGFR) mutations, and methyl guanine methyl transferase (MGMT) methylation are routinely measured to help select the most appropriate treatment for cancer patient subtypes (4).

The anaplastic lymphoma kinase (ALK) TK receptor (TKR) has emerged recently as a potentially relevant biomarker and therapeutic target in solid and hematologic tumors. Due to the similarity between the spelling of ALK and ALK-1 that could lead to misunderstanding, we need to highlight that ALK, the subject of this review, is quite different from the activin receptor-like kinase 1 (ALK-1), which is an orphan type I receptor of the TGF-beta receptor family involved in angiogenesis (5).

A variety of alterations in the ALK gene such as mutations, overexpression, amplification, translocations, or other structural rearrangements have been implicated in human cancer tumorigenesis (see Table 1; refs. 6–32).

Here, we review the potential role that ALK may have in solid tumor origin, the methodology to detect the different molecular alterations, and the new targeted agents being developed.

ALK Receptor Structure and Function

The ALK TK receptor gene is located at 2p23.2 (19) and belongs to the insulin receptor superfamily. The structure of this single chain transmembrane receptor consists of an extracellular domain containing an N-terminal signal peptide sequence, together with the binding sites for the activating ligands of ALK, pleiotrophin, and midkine (33–35). Pleiotrophin and midkine have a similar distribution to ALK, mainly in the nervous system during fetal development followed by downregulation at birth. These ligands display neurotrophic functions on receptor binding (36). However, their role as ligands for ALK has not

References

(References are not included in the natural text provided.)
been shown definitively because they are known to bind to other receptors as well, and the exact mechanisms of interaction between the soluble ligands and the ALK receptor in some models have not been unequivocally confirmed (37).

The next domains are the transmembrane and juxtamembrane region; the latter contains a binding site for phosphotyrosine-dependent interaction with the insulin receptor substrate-1. The final section has an intracellular TK domain with 3 phosphorylation sites (Y1278, Y1282, and Y1283), followed by the C-terminal domain with interaction sites for phospholipase C-gamma and Src homology 2 domain containing SHC (38). Structural analysis of the catalytic domain of ALK reveals the importance of the phosphorylation of the Y1278 on kinase activity, as well as a partially inactive kinase conformation distinct from that observed in inactive insulin-like growth factor receptor (IGFIR) and insulin receptor structures (39).

Theoretically, ligand binding stimulates receptor homodimerization, which enables trans-phosphorylation and protein kinase activation.

In mammals, in the adult, normal ALK expression seems to be restricted to central and peripheral nervous systems (34). In line with this finding, ALK knockout mice present an absence of significant phenotype abnormalities, except for increased hippocampal neurogenesis (40).

**Mechanisms of ALK Deregulation in Cancer**

**ALK mutations**

Point mutations have been found in 6 to 8% of primary neuroblastomas (Table 1; refs. 16, 17, 41). Germ-line mutations have been identified in families with more than 1 sibling with neuroblastoma (16, 18, 41). Somatic mutations with wild-type ALK in matched constitutional DNAs have also been described in nonfamilial neuroblastoma cases. These mutations are located mainly in the TK domain; the most frequent being the gain-of-function mutations F1174L and R1275Q. These mutations are associated with increased expression, phosphorylation, and kinase activity of the ALK protein. Further, they have been shown to have Ba/F3 cell-transforming capacity. In some cases, these mutations coexist with an increased copy number of the ALK gene (16). Interestingly, these mutations (particularly the F1174L) are predictive of response (as indicated by increased apoptosis and inhibition of growth) to short hairpin ALK-specific knockdown and TK ALK inhibitors (TAE684 and PF-12341066). Notably, protein expression levels in ALK mutant neuroblastoma models

<table>
<thead>
<tr>
<th><strong>Tumor type</strong></th>
<th><strong>ALK alteration</strong></th>
<th><strong>Incidence</strong></th>
<th><strong>Reference</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>EML4-ALK</td>
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</tr>
<tr>
<td>Colorectal cancer</td>
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<td>0–2.4%</td>
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<td>IMT</td>
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<td>10–15</td>
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<td></td>
<td>TPM4-ALK</td>
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<tr>
<td></td>
<td>CARS-ALK</td>
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<td>RANBP2</td>
<td>Rare</td>
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</tr>
<tr>
<td></td>
<td>CTL/C-ALK</td>
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<td></td>
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<td>SEC31L1</td>
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<td>Neuroblastoma</td>
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<tr>
<td></td>
<td>TFG-ALK</td>
<td>Rare</td>
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Abbreviations: ALK, anaplastic lymphoma kinase; IMT, inflammatory myofibroblastic tumor; ALCL, anaplastic large cell lymphoma; DLBCL, diffuse large B-cell lymphoma.
do not directly correlate with sensitivity to ALK inhibitors. It seems that this finding could be explained by the existence of a higher turnover rate of the ALK protein in cells with constitutively activated ALK (17).

**ALK amplifications**

An increased copy number of ALK has also been described in neuroblastoma cell lines and tumors (Table 1; refs. 16, 42), which can coexist with ALK gene mutation. In this disease, amplification, as well as mutation of ALK, has been associated with MYCN amplification, the most frequent amplicon in neuroblastoma defining a high-risk subgroup of patients that may benefit from ALK selective inhibition (see below; refs. 16, 17).

In addition, we and others (43, 44) have described ALK gene amplification in non–small cell lung cancer (NSCLC) specimens. Our study showed a relatively high frequency of copy number of mainly low level gains (60%), and amplification (10%). The pattern of amplification in NSCLC is characterized by, in the majority of cases, a small percentage of cells within the tumor harboring this amplification. However, we found some cases with >40% of cells with ALK amplification. This amplification (or copy number gain) was not associated with protein expression in our series of patients (with the immunohistochemical assay we used). The clinical implications of these alterations remain to be elucidated.

**ALK translocations**

The best characterized alterations of ALK associated with cancer are gene rearrangements; these have been observed in hematologic as well as in nonhematologic malignancies (Table 1). The role of ALK in cancer was first identified as part of the NPM-ALK gene fusion involved in the pathogenesis of a subset of anaplastic large cell lymphoma (ALCL; refs. 19, 45, 46). Since then, multiple fusion partners forming ALK chimeric proteins in this disease have been identified (Table 1). ALK rearrangements have also been reported in other lymphomas, such as diffuse large B-cell lymphomas (DLBCL; see Table 1; ref. 47). In solid tumors, ALK translocations were first described in inflammatory myofibroblastic tumors (IMT; 15, 10–13, 48). More recently, the novel fusion first described in inflammatory myofibroblastic tumors such as diffuse large B-cell lymphomas (DLBCL; see Table 1; ref. 47) to promote cell cycle progression, survival, and proliferation (Fig. 1). Activation of the phospholipase C-γ is also thought to contribute to NPM-ALK–mediated transformation (63).

**Downstream Signaling Pathways Activated by Oncogenic ALK**

The activated signaling pathways implicated in tumorigenesis induced by ALK remain poorly characterized. In ALCL, in which the greater amount of research has been conducted to date, a variety of intracellular signaling cascades has been implicated in the oncogenic transformation induced by aberrant ALK (Fig. 1; refs. 63–66).

NPM-ALK activates many different pathways that are closely interconnected and overlapping. Oncogenic fusion protein promotes the activation of, mainly, 3 key signaling pathways: (1) Janus kinase 3 (JAK3)-STAT3 intracellular pathway (Fig. 1; ref. 67); (2) phosphoinositide 3-kinase (PI3K)–Akt pathway (Fig. 1; ref. 63); (3) PI3K-AKT, because of the amplification of SHH, and this results in ligand-independent dimerization and, thus, constitutive activation of the kinase. The oncogenic role of ALK chimeric proteins has also been shown by preclinical studies and mouse models with forced expression of ALK (46, 53–56).

Of note is that, in NSCLC, the ALK translocation seems to define a subgroup of patients with specific clinical, pathologic, and molecular characteristics. The alteration is more frequent in younger patients, who are non– or light-smokers, with adenocarcinoma histology with presence of signet ring–type cells, EGFR, and/or K Ras wild-type tumors (57–60), together with non-c-MET copy number increase (61); however, a significant number of cases not fitting into this subgroup have also been described (44, 62).

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NIPA, a SCF-type E3 ligase, has been cloned in a complex with NPM-ALK (70) and has been suggested to be involved in NPM-ALK–mediated cell cycle progression. NPM-ALK promotes inactivation of NIPA, which prevents cyclin B1 degradation, therefore, allowing cell cycle progression (71).
play a key role in the pathogenesis of EML4-ALK tumors (72).

Detection Methods for Altered ALK

PCR

One key issue in the detection of altered ALK is the method that best defines ALK status, in a clinical setting. The first studies published describing ALK translocations in lung cancer used reverse transcriptase PCR (RT-PCR; refs. 31, 57, 60, 62, 73). This technique requires good quality (nondegraded) RNA, which is sometimes difficult to obtain from formalin-fixed paraffin-embedded stored tissue samples, which is the usual sample material acquired for molecular diagnosis. Further, RT-PCR is not available in all pathology departments in hospital-based settings. One relevant challenge with this technique is that fusions between ALK and its partner genes happen at different break points, and several variables have been described to date. As such, several different PCR primers need to be generated to be able to detect all known fusion transcripts (9). Danenberg and colleagues have recently reported a functional real-time PCR method able to detect 9 different EML4-ALK fusion gene variants from formalin-fixed paraffin-embedded tumor samples, either as one-by-one individual variants or a variant in any one assay (74); however, this technique might still miss some of the less frequent transcripts.

In situ hybridization methods

More recently, FISH with break-apart probes has been reported as a specific method for screening ALK

Figure 1. Oncogenic NPM-ALK signaling pathway. A schematic of ALK TKR. The mechanisms associated with deregulation are in solid lines. Hematologic tumors are shown in the figure’s upper part. The hypothetical downstream signaling pathways and cellular responses subsequent to ALK constitutive activation in tumors are shown in the figure’s lower part.
translocations in lung cancer specimens (see Fig. 2A; refs. 43, 44, 59). The probes consist of an orange-red telomeric signal and a green centromeric signal. In normal cells, signals present as overlapping orange-red and green (yellowish) signals. In tumors with gene rearrangement, cases are considered typically rearranged when green and orange-red signals are separated (at least by 3 times the signal diameter) and atypically rearranged when a single orange or green signal is observed (Fig. 2C). One potential problem, particularly with the EML4-ALK translocation, is that the split signals can be subtle due to the loss of a small amount of chromosomal material at the 2p location. Another limitation of FISH is that it cannot distinguish between the different rearranged variants of ALK. Although no data exist on the implication of these variants in differential prognosis, or prediction of response to treatment, these differences do seem to exist but would not be detected by FISH. However, additional information about copy number changes can be obtained by this technique (Fig. 2D). This information is of interest in other tumor types such as neuroblastoma, but its role remains to be defined in other tumors such as NSCLC. Finally, FISH is the method currently being used in clinical trials evaluating the efficacy of ALK inhibitors (e.g., PF-02341066) in patients with tumors harboring ALK translocations. Results from these trials will provide, hopefully, definitive answers about the value of FISH in extensive screening for ALK translocations. However, one conflicting issue is the arbitrary cut-off (15% cells harboring the translocation) that has been set as the definition of a translocated case in this trial (75). There is no reported biological rationale for this established cut-off, and this might need to change when future data on ALK become available.

Immunohistochemistry

Immunohistochemical assays have been used as detection methods for ALK overexpression (58–60, 76). Normal adult tissue, except for neural tissue, does not express ALK, and, therefore, expression of this protein would identify cases that harbor genetic aberrations responsible for this increased expression and, probably, gain of function of the expressed protein product. Antibodies used for ALK detection in the published studies to date have yielded widely varying results. For example, antibodies used for ALK protein detection in ALCL do not yield good results in NSCLC harboring the translocation, as was shown by the data of Rodig and colleagues (58). In their work, ALK rearrangement was identified by FISH in 95% of cases and immunohistochemistry (IHC) with and without tyramide amplification in 80% and 40% of cases, respectively. However, neither FISH nor IHC alone detected all cases with ALK rearrangement on initial screening. A more recent study (76) comparing 3 antibodies for ALK detection observed that a novel rabbit monoclonal antibody (D5F3 from Cell Signaling Technology) showed excellent sensitivity and specificity.
(100% and 99%, respectively) for the detection of ALK rearrangement in NSCLC cases. In our experience, this antibody was able to detect the EML4-ALK rearranged tumors, with 100% correlation between IHC and FISH assays (44). The advantage of IHC is the broad experience of pathologists, as well as its availability in routine hospital settings. Further, its correlation with pathologic status and levels of expression may have significance as well; however, these aspects need to be refined.

IHC can also provide information on the possible fusion partner of ALK. Depending on the function and localization of the ALK partner, the derived fusion protein will be detected in different subcellular sites. For instance, the NPM-ALK chimeric protein is typically detected in the nucleus, nucleolus, and cytoplasm (19), whereas the majority of other ALK partners (TPM3, TPM4, TFG, ATIC) are expressed in the cytoplasm (10, 11, 21, 23). Different patterns are discerned, such as localization on the periphery of the nucleus of the RANBP2-ALK fusion protein (13), resulting from the association of the fusion protein with wild-type RANBP2 (a nuclear pore protein), and a cell membrane–associated pattern of moesin (MSN)–ALK owing to the association of wild-type MSN and other membrane proteins (25).

Further efforts are warranted to determine the optimal method for routine diagnostic detection of ALK fusions in cancer.

### ALK Inhibitors under Clinical Development

Currently, several new drugs against oncogenic ALK are under development; most are at the preclinical trial

<table>
<thead>
<tr>
<th>Compound</th>
<th>Biochemical structure</th>
<th>IC_{50} (nmol/L)</th>
<th>Phase of development</th>
<th>Other targets</th>
<th>References</th>
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<tbody>
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<td>Phase III</td>
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<td>IGF-1R, Aurora kinase-2</td>
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</tbody>
</table>

Abbreviation: IGF-1R, insulin-like growth factor receptor-1; IR, insulin receptor.
stage. Table 2 summarizes some of the more promising drugs (68, 77–91).

**Crizotinib**

Although initially designed against the hepatocyte growth factor receptor (c-Met), crizotinib (PF-2341066) is a potent, ATP-competitive, small molecule inhibitor of ALK phosphorylation and its oncogenic variants, available in oral formulation (68). As has occurred in the past with other anticancer agents, such as sorafenib and imatinib, when crizotinib was evaluated across a structurally diverse cross-section of different tyrosine and serine-threonine kinases, it exhibited a high degree of selectivity at pharmacologically relevant concentrations. It was efficacious not only for c-Met (half maximal inhibitory concentration; IC_{50}, 11 nmol/L) but also for other targets, such as ALK, which was inhibited at pharmacologically relevant concentrations (IC_{50}, 24 nmol/L) in lymphoma cell lines expressing the NPM-ALK oncogenic fusion protein.

Crizotinib showed antitumor activity by inhibiting c-Met and ALK downstream signaling and by reducing tumor growth in human xenograft models, in which deregulation of c-Met is involved in experimental systems such as GTL-16 human gastric carcinoma, Caki-1 renal cell carcinoma, U87MG human glioblastoma, and NCI-H441 NSCLC (68), as well as in ALCL Karpas299 xenograft models in which NPM-ALK occurs (77). Crizotinib also shows in vitro activity in neuroblastoma cell lines harboring ALK mutations or amplifications. However, little effect has been observed in wild-type cell lines and in lines harboring F1174L mutations, suggesting different patterns of sensitivity depending on the mutation involved.

c-Met is involved in angiogenesis, motility, and invasiveness, and crizotinib also showed its ability to inhibit endothelial cell survival, invasion, and serum-stimulated tubulogenesis in vitro (68).

Crizotinib was the first ALK inhibitor to be tested in the clinical setting. Kwak and colleagues recently reported the dose-escalating phase I trial of crizotinib in solid tumors (78). The recommended dose for phase II studies was 250 mg b.d. Fatigue was the dose-limiting toxicity; grade 3 (on the National Cancer Institute-Common Terminology Criteria) occurred in 2 out of the 6 patients in the 300-mg b.d. treatment cohort. Crizotinib was generally well tolerated, despite nausea (57%), vomiting (41%), diarrhea (36%), visual disturbance (17%), and fatigue (17%) being the principal nonhematologic toxicities. Liver enzymes, particularly ALT, were increased. Plasma clearance half-life was 53 hours at the 250-mg b.d. dose, and the peak plasma concentration occurred 4 hours after a single dose administration. Crizotinib is considered a moderate CYP3A4 inhibitor, and no food-associated effect was observed when administered in fasting or nonfasting conditions. Hence, crizotinib may be taken orally independently of meals. The early activity observed in patients with ALK protein fusions led to widening of the cohort of patients with NSCLC treated with crizotinib. Given as a single agent to 77 evaluable metastatic NSCLC patients in the advanced-stage setting, crizotinib induced 1 complete response and 46 confirmed partial responses, according to the Response Evaluation Criteria in Solid Tumors criteria. The overall clinical benefit (objective responses plus stable disease at 8 weeks) was 87% [95% confidence interval (CI), 77%-93%], and the objective response rate was 57% (95% CI, 48%-68%). The median progression-free survival (PFS) was not achieved by the time of data presentation, but the rate of PFS probability at 6 months was 72% (95% CI, 61%-83%). The median duration of treatment was 5.7 months (78).

Two secondary mutations within the kinase domain of EML4-ALK in tumor cells isolated during the relapse phase of crizotinib treatment from a patient who took part in the above trial were also reported (79).

A phase II, single arm study of crizotinib (the PROFILE 1005 study) in metastatic NSCLC patients with translocation or inversion involving the ALK gene locus is currently recruiting patients (NCT00932451). A phase III study (the PROFILE 1007 study) has also started recently to compare crizotinib versus pemetrexed or docetaxel as single agents in metastatic NSCLC patients with genetic alterations of the ALK gene locus who have had a failed response to a prior platinum-based treatment (NCT00932893). A promising combination of crizotinib with a pan-HER inhibitor (PF-299804) as a single arm trial in erlotinib-resistant patients is underway. Crizotinib is also being tested in other ALK+ tumors such as ALCL (NCT00939770) and neuroblastoma (NCT00939770, NCT01121588, and NCT01182896). A sustained partial response to crizotinib in an IMT patient with ALK-translocated has recently been reported, as compared with no observed activity of the drug in another patient without the ALK translocation (80).

**GSK1838705A**

GSK1838705A is a novel, dual, small molecule inhibitor of the IGF receptor-1 (IGF-1R) and the ALK signaling pathways, with a pharmacologic inhibitory potency of 2.0 nmol/L and 0.5 nmol/L, respectively (81, 82). GSK1838705A has shown antiproliferative activity in a panel of cell lines derived from solid and hematologic tumors. In mice bearing tumor xenografts, GSK1838705A has been shown to delay the growth of IGF-1R-dependent tumors, and to induce complete regression of ALK-dependent tumor models such as Karpas299 and SR-786 ALCLs. Although the agent may block the insulin receptor as a class effect, minimal influence on glucose homeostasis was observed in preclinical studies. To the best of our knowledge, no trial is currently evaluating the efficacy, safety, and recommended dose of GSK1838705A in the clinical setting.

**TAE-684**

TAE-684 is a selective, novel, small molecule inhibitor of ALK that induces a significant reduction of phosphorylation, with an IC_{50} potency <10 nmol/L (83). The
inhibitory activity of TAE-684 is highly selective for ALK-driven cell proliferation; 100- to 1,000-fold higher concentrations are required to inhibit other TKs. TAE-684 has been shown to reduce cell proliferation, kinase activity, and downstream effectors ERK1/2, AKT, STAT3, and STAT5 of cryptic rearrangement SEC31A-ALK and NPM1-ALK protein fusions in 2 in vivo ALCL models (84). Pharmacokinetic parameters of TAE-684 were evaluated in severe combined immunodeficient mice and showed an adequate bioavailability (60 to 70%) following oral dosing. With a protracted half-life of 12 hours, this compound seems to be a promising agent for clinical testing.

CEPT-14083

CEPT-14083 is a small molecule TK inhibitor that has shown activity in an NPM/ALK–carrying T-cell lymphoma in vitro study. Presumably, this compound binds to the hinge region of the kinase in an ATP-competitive manner. It displays a potent activity against ALK in enzymatic assays (IC$_{50}$ = 11 nmol/L; ref. 85). Further, CEP-14083 is also able to inhibit the insulin receptor at a concentration within a nanomolar range. In a preclinical assay, CEP-14083 showed that, via NPM/ALK TK inhibition, it could control the expression of molecules that determine T-cell identity and signaling in lymphoma cells (86). This agent has shown preclinical activity in both cell lines and animal models harboring ALK alterations (86).

AP26113

AP26113 is the lead product of ARIAD Pharmaceuticals Inc. It is a small molecule designed to target ALK. This new agent is a potent, orally formulated ALK inhibitors with selectivity over receptors related to the TK family members IGF-1R and the insulin receptor (87). In vitro, AP26113 shows potency toward inhibiting $p$-ALK in the nanomolar range (IC$_{50}$, 4 to 31 nmol/L). In NSCLC and ALCL animal models, AP26113 induced regression and antitumor activity associated with sustained inhibition of $p$-ALK. Crizotinib and AP26113 have been compared in in vitro and in vivo preclinical studies. AP26113 showed a 10-fold greater potency and a 10-fold broader therapeutic index than crizotinib (88). Further, in an elegant preclinical study conducted by Zhang and colleagues (89), AP26113 has been shown to reverse resistance to crizotinib in G1269S or L1196M (gatekeeper) mutant cell lines. The pharmacokinetic properties of AP26113 of moderate protein binding, single daily dosing, and no inhibition of cytochrome isozymes support the proposed advancement in 2011 of this compound for evaluation in the clinical setting.

NMS-E628

Another biotechnological company, Nerviano Medical Sciences, has also reported promising preclinical data on its lead ALK inhibitor, NMS-E628. This compound displays an IC$_{50}$ of 55 nmol/L against NPM-ALK, with off-target activity over IGF-1R (IC$_{50}$, 263 nmol/L) and aurora-2 kinase (IC$_{50}$, 338 nmol/L; ref. 90). Proliferation profiling on a wide panel of human tumor cell lines showed that this compound selectively blocks proliferation of ALK-dependent cell lines and potently inhibits ALK-dependent signaling. NMS-E628 has been shown to induce tumor regression in NSCLC and ALCL animal models following 10 days of oral b.d. dosing. NMS-E628 has favorable pharmacokinetic and toxicologic properties. A biodistribution analysis in different animal models revealed that it is able to cross the blood-brain barrier (91).

**Future Directions**

The first X-ray crystal structure analysis of the unphosphorylated ALK catalytic domain alone (39), and in complex with 2 ATP competitive inhibitors (92), has been reported recently. The findings provide specific molecular details about ALK regulation and activation. Conformational differences exist with respect to other family members such as IGF-1R and the insulin receptor. Lee and colleagues showed a different auto-inhibition mechanism that maintains the ALK receptor in a state of minimal activity (39).

Mapping ALK-activating mutations associated with neuroblastoma in these crystal structures has been explored by Bossi and colleagues (92). These authors suggest that the mutations might allow easier ATP access to the ATP-binding pocket compared to the wild-type receptor. They also predict that the ATP inhibitors that recognize the active conformation of the kinase domain may be more effective for the treatment of these ALK+ tumors than those that recognize the inactive form.

Binding of ALK inhibitors to ALK wild-type and potential ALK mutants has been analyzed using ALK homology models (93). Lu and colleagues identified potential ALK mutants resistant to small molecule ALK inhibitors (93).

It is still necessary to test the sensitivity of different ALK mutants and fusion proteins to current ALK inhibitors. Crystal structure analysis of the catalytic domain of these oncogenic variants in complexes with current antitarget agents would enable the exploration of the impact of these alterations on the affinity of these ALK inhibitors under development.

Further, although a robust clinical response has already been observed in ALK-positive cancer patients (75), resistances to these ALK inhibitors are expected because of the selection of resistant tumor cells harboring new point mutations, most likely in the ALK kinase domain.

Crystallographic structure analyses by Bossi and colleagues also highlight active-site zones that could be exploited to develop more selective ALK inhibitors (92). Approaches aimed at identifying active ALK mutants that may be associated with treatment resistance would be useful in the rational design of second generation inhibitors. These findings will guarantee the structure-based drug design of the next generation of more effective agents.
Although, NPM-ALK-mediated signaling pathways have been well elucidated and characterized (63, 67, 68), less is known about the downstream pathways mediated by other ALK fusion proteins and the full length ALK receptor. Identification of the key effectors for each oncogenic ALK variant is still needed and would illustrate the potential mechanism of tumorigenesis, while providing potential biomarkers that predict efficacy of, or resistance to, anti-ALK agents.

Conclusions

ALK has emerged as a strong biomarker and therapeutic target for a small, but significant, percentage of cancer patients who may benefit from ALK-targeting agents. ALK alterations are not restricted to a specific tumor type but, instead, seem to be molecular tumorigenic events that appear in the most diverse tumors (now defined as ALKomas; ref. 94) such as NSCLC, ALCL, IMT, neuroblastoma, or other hematologic and solid tumors. In this sense, all tumors bearing ALK mutations, amplification, overexpression, or fusion proteins might potentially benefit from ALK inhibitors, independently of histology or primary site. However, early clinical data with ALK inhibitors, although very promising, should be interpreted with caution, while more detailed information from longer follow-up is eagerly awaited. Further, issues remain, such as the exact definition of ALK positivity, the best method of detection of ALK alterations that are clinically significant, the prognostic significance of these alterations, and whether there should be a preselection of patients for treatment based on the characteristics of molecular testing. It is premature to consider universal determination of ALK alterations in standard clinical practice. However, selection of patients for treatment based on the molecular profile is mandatory if better optimization of resources, drug activity, preemption of unnecessary adverse drug reactions, increased patient compliance, and reductions in time and costs of clinical trials are to be achieved.

Thanks to the efforts of scientific researchers and more rational design of clinical trials, we are closer to being able to stratify patients on the basis of specific genomic features before treatment election, which could lead to more precise tailoring of the treatment. ALK inhibitors offer a new opportunity to treat cancer patients according to the genetic characteristics of their tumors and, ultimately, to improve treatment outcomes.

Disclosure of Potential Conflicts of Interest

M.-V. Bolos is a full-time employee of Pfizer with equity ownership in the company.

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References


Correction: Targeting Oncogenic ALK: A Promising Strategy for Cancer Treatment

In this article (Mol Cancer Ther 2011;10:569–79), which was published in the April 2011 issue of Molecular Cancer Therapeutics (1), the structure of AP26113, as shown in Table 2, was incorrect. The structure of AP26113 has not yet been publicly disclosed. The authors apologize for this error.

Reference
Targeting Oncogenic ALK: A Promising Strategy for Cancer Treatment

Enrique Grande, María-Victoria Bolós and Edurne Arriola


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