Molecular Pathways

Targeting Anaplastic Lymphoma Kinase in Lung Cancer

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Abstract

Several decades of cancer research have revealed a pivotal role for tyrosine kinases as key regulators of signaling pathways controlling cell growth and differentiation. Derepression of tyrosine kinase-mediated signaling occurs frequently in cancer and is believed to drive the initiation and progression of disease. Chromosomal rearrangements involving the tyrosine kinase anaplastic lymphoma kinase (ALK) occur in a variety of human malignancies including non-small cell lung cancer (NSCLC), anaplastic large cell lymphomas, and inflammatory myofibroblastic tumors. The aberrant activation of ALK signaling leads to “oncogene addiction” and marked sensitivity to ALK inhibitors such as crizotinib (PF-02341066). This review will focus on ALK rearrangements in NSCLC, starting with the discovery of the EML4-ALK fusion oncogene, and culminating in the recent validation of ALK as a therapeutic target in patients with ALK-rearranged NSCLC. Current efforts seek to expand the role of ALK kinase inhibition in lung and other cancers, and to address the molecular basis for the development of resistance.
Background
The last decade has witnessed tremendous advances in the treatment of patients with cancer. Chief among these is the discovery and successful development of new, targeted cancer therapies. These therapies are highly effective in genetically-defined subsets of patients, ie patients whose tumors harbor specific genetic abnormalities. Examples of targeted therapies include imatinib for chronic myelogenous leukemia, trastuzumab and lapatinib for HER2-amplified breast cancer, and erlotinib, a tyrosine kinase inhibitor (TKI) targeting epidermal growth factor receptor (EGFR), for EGFR-mutant non-small cell lung cancer (NSCLC). Unfortunately, however, the majority of human cancers are not susceptible to molecularly targeted agents. As an example, in the case of NSCLC, only 10% of Caucasian patients harbor an activating EGFR mutation and are thus sensitive to erlotinib; in the remaining 90% of patients, EGFR is wild-type, and erlotinib is minimally effective. In lung and other solid tumors, there is clearly an urgent need to identify new therapeutic targets and to expand the role of novel targeted agents, many of which have now entered clinical trials. This review centers on an exciting new example of successful targeted therapy in NSCLC, specifically lung cancers harboring ALK fusion oncogenes.

The EML4-ALK fusion oncogene represents one of the newest molecular targets in NSCLC. EML4-ALK was first identified in 2007 by Soda et al. who screened a cDNA library derived from the tumor of a 62 year-old Japanese male patient with adenocarcinoma of the lung (1). This fusion arises from an inversion on the short arm of chromosome 2 (Inv(2)(p21p23)) that joins exons 1-13 of EML4 (echinoderm microtubule associated protein-like 4) to exons 20-29 of ALK (Fig.1)(1). The resulting chimeric protein, EML4-ALK, contains an N-terminus derived from EML4 and a C-terminus containing the entire intracellular tyrosine kinase domain of ALK. Since the initial discovery of this fusion, multiple other variants of EML-ALK have been reported, all of which encode the same cytoplasmic portion of ALK but contain different truncations of EML4 (2) (3) (4) (5) (6). In addition, fusions of ALK with other partners including TFG (TRK-fused gene) (7) and KIF5B (8) have also been described in lung cancer, but appear to much less common than EML4-ALK.
Translocations involving ALK have been identified in several other cancers, including anaplastic large cell lymphomas (ALCL), inflammatory myofibroblastic tumors (IMT), and neuroblastomas (9). In all cases, including EML4-ALK, the fusion partner has been shown to mediate ligand-independent dimerization of ALK, resulting in constitutive kinase activity. In cell culture systems, EML4-ALK possesses potent oncogenic activity (1). In transgenic mouse models, lung-specific expression of EML4-ALK leads to the development of numerous lung adenocarcinomas (10). Cancer cell lines harboring the EML4-ALK translocation can be effectively inhibited by small molecule inhibitors targeting ALK (4). Treatment of the EML4-ALK transgenic mice with ALK inhibitors also results in tumor regression (10). Taken together, these results support the notion that ALK-driven lung cancers are dependent upon or “addicted” to the fusion oncogene.

THE ALK PATHWAY

ALK is a highly conserved, receptor tyrosine kinase (RTK) first discovered over 15 yrs ago as a fusion with nucleophosmin (NPM) in ALCL(11). Like other RTKs, ALK has three structural domains: an extracellular ligand-binding domain, a transmembrane region, and an intracellular tyrosine kinase domain. By homology, ALK is most similar to leukocyte tyrosine kinase (LTK), and both belong to the insulin-receptor superfamily. Under physiologic conditions, binding of ligand induces homodimerization of ALK, leading to trans-phosphorylation and kinase activation. In ALK translocations, the 5’ fusion partners provide dimerization domains, enabling ligand-independent activation of the kinase. In addition, unlike native ALK which localizes to the plasma membrane, the majority of ALK fusion proteins localize to the cytoplasm. This difference in cellular localization may also contribute to deregulated ALK activation.

In mammals, the precise function of ALK is poorly understood (12). Based on its expression pattern in the mouse, ALK is believed to play a role in the development and function of the nervous system. However, ALK knockout mice are completely viable and appear grossly normal (13).
Subsequent studies using independently generated ALK knockout mice have reported an increase in hippocampal progenitor proliferation and an increase in dopamine levels within the basal cortex (14). In the adult, ALK expression is weak and restricted primarily to the central nervous system. While the ligand for ALK is known in Drosophila melanogaster (Jelly Belly), no homolog of this ligand has been identified in vertebrates. Putative ALK ligands include pleiotrophin (PTN) and midkine (MK), both of which are small, heparin-binding growth factors implicated in neuron development as well as neurodegenerative diseases (12). Recent work suggests that PTN may also activate ALK indirectly by binding to and inactivating the receptor protein tyrosine phosphatase Z1 (15). Whether there are other ALK ligands or other mechanisms of ALK activation remains to be determined.

The key downstream effectors of ALK are better understood than the upstream activators, and include the Ras/Mek/Erk, PI3K/Akt, and JAK3-STAT3 signaling pathways (Fig. 1)(reviewed in (16)). These pathways have been most extensively studied in the context of ALCL and NPM-ALK-mediated transformation. In general, the Ras/Mek/Erk pathway is important for driving cell proliferation, while the PI3K/Akt and JAK3-STAT3 pathways are important for cell survival and cytoskeletal changes. While different ALK fusions may differentially activate downstream signalling pathways, EML4-ALK, like NPM-ALK, signals through Erk and PI3K. Pharmacologic inhibition of EML4-ALK using TKis leads to downregulation of Ras/Mek/Erk and PI3K/Akt and apoptosis (4), consistent with the notion that activation of these two pathways is critical for EML4-ALK-mediated transformation. Furthermore, in models of acquired ALK TKI resistance, both Ras/Mek/Erk and PI3K/Akt pathways are re-activated despite the continued presence of the TKI. Potential mechanisms of resistance that lead to re-activation of canonical signalling pathways are discussed below.

CLINICOPATHOLOGIC FEATURES

EML4-ALK is associated with several key pathologic and demographic features. One of the most striking features of EML4-ALK-positive lung cancer
is young age of onset. In the largest study of EML4-ALK positive NSCLC to date, patients harboring this translocation were significantly younger than non-ALK positive patients, with a median age of 54 years compared with 64 (22). Among the 47 patients with EML4-ALK, 8 were under 40 years old. Several other studies of EML4-ALK in NSCLC patients have also noted a trend toward younger median age (6) (17) (23). Interestingly, other cancers known to harbor ALK rearrangements, such as ALCLs and IMTs, are also associated with younger age, and are in fact most common in children and young adults.

The presence of EML4-ALK in NSCLC is also strongly associated with never or light smoking history. In the first report of EML4-ALK in NSCLC, the chromosomal inversion was detected in five patients, two of whom were noted to have a smoking history (1). In several follow-up studies, EML4-ALK was variably detected in both smokers and non-smokers, suggesting a lack of association between smoking history and presence of EML4-ALK (18). However, a number of more recent studies suggest that EML4-ALK is in fact strongly associated with never or light smoking history (23) (4) (24) (6) (17). In the study mentioned above, only 4 of 47 (9%) ALK positive patients had a >10 pack-year smoking history (22). Conversely, among the screened patients with >10 pack-year smoking history, only 5 of 232 (2.1%) were found to have NSCLC harboring ALK rearrangements (A. Shaw, unpublished data).

At the histologic level, the vast majority of lung tumors harboring EML4-ALK are adenocarcinomas. However, EML4-ALK-positive cases are significantly more likely than EGFR mutant or WT/WT tumors to have a solid pattern with abundant signet ring cells (21) (25). Signet ring cells are frequently found in gastric cancers, and rarely in cancers of other organs such as the lung. Several small case series suggest that signet ring cells may be associated with an aggressive clinical course and a poor prognosis. Whether the presence of signet ring cells in EML4-ALK mutant lung cancer has biological or clinical significance remains to be determined. Of note, not all studies of EML4-ALK in NSCLC have reported an association with signet ring cells (20, 23). This discrepancy may reflect differences in pathologic interpretation, differences in stage of disease, or ethnic differences in patients with EML4-ALK positive lung cancer.
ALK rearrangements appear to be largely mutually exclusive with EGFR or KRAS mutations (26) (23) (24) (27) (17). While the overall frequency of EML4-ALK in the general NSCLC population is low, knowledge of the clinicopathologic features enables enrichment for this genetically defined subset. In one study in which patients were selected for genetic screening based on clinical features commonly associated with EGFR mutation, including never/light smoking status and adenocarcinoma histology, 13% were found to harbor EML4-ALK (24). Within the group of never or light smokers in this study, the frequency of EML4-ALK was 22%; among never or light smokers without EGFR mutation, the frequency of EML4-ALK was 33%. These findings suggest that in NSCLC patients with clinical characteristics associated with EGFR mutation but with negative EGFR testing, as many as 1 in 3 may harbor EML4-ALK (24).

Clinical-Translational Advances

**Crizotinib.** Significant effort has been directed towards the development of therapeutically useful ALK inhibitors. In preclinical studies, several ALK inhibitors have shown activity against NPM-ALK and EML4-ALK containing cell lines (4, 28) (29) (1) (10). TAE684, a small molecule ALK inhibitor, inhibits the growth of and induces apoptosis in the EML4-ALK-containing cell line H3122, and causes regression of xenografts in vivo (4). Another small molecule tyrosine kinase inhibitor (TKI) crizotinib (PF02341066), originally developed as an inhibitor of mesenchymal epithelial transition growth factor (c-MET), was found to also be a very potent inhibitor of ALK. Crizotinib inhibits ALK phosphorylation and signal transduction, with associated G1-S phase cell cycle arrest and induction of apoptosis in NPM-ALK–positive ALCL cells in vitro and in vivo (28).

The first ALK-targeted therapy tested in the clinic is crizotinib. An international, multicenter phase 1 trial has recently been conducted to investigate the safety, PK/PD, and antitumor activity of crizotinib in patients with advanced cancer (30). This trial was designed to include a dose-escalation phase, followed by a dose-expansion phase at the maximum tolerated dose (MTD) in patients with MET amplification or ALK
rearrangement. Of note, this trial was already enrolling patients in the dose-escalation phase when EML4-ALK in NSCLC was first reported in August 2007. Two patients with NSCLC harboring EML4-ALK were treated with crizotinib during dose escalation and demonstrated dramatic improvement in their symptoms. This observation led to large-scale prospective screening of NSCLC patients and recruitment of those with ALK-positive NSCLC into an expanded molecular cohort at the MTD of 250 mg twice daily (30).

The clinical activity of crizotinib in ALK-positive NSCLC has recently been published as well as updated at ESMO in October 2010 (30, 31). Results were reported for one-hundred thirteen patients, all of whom had ALK-positive NSCLC as demonstrated by FISH performed in the molecular pathology laboratory at MGH. The majority of these patients had adenocarcinoma histology, and 73% were neversmokers. Of note, 93% of patients had received one or more lines of therapy, and 30% had received more than 3 prior lines. Among 105 evaluable patients, the objective response rate (ORR) was 56%. The ORR was independent of number of prior treatments, gender, age, and Eastern Cooperative Oncology Group (ECOG) performance status. In a number of cases, patients reported symptomatic improvement within one to two weeks, reminiscent of the effect of erlotinib in patients with EGFR-mutant lung cancer. Radiologic responses were similarly rapid and often noted at the time of the first or second set of restaging scans. Among 113 evaluable patients, median progression-free survival (PFS) was 9.2 months (31). To date, the longest duration of response has been >24 months, suggesting that patients can experience prolonged clinical benefit. The impact of crizotinib on overall survival remains to be determined; however, based on the >9 month PFS in a heavily pre-treated population of NSCLC patients, the impact on overall survival is likely to be substantial.

Crizotinib has been shown to be extremely well tolerated. In the update of the phase 1 trial, the most common treatment-related adverse events (AEs) were grade 1 to 2 gastrointestinal toxicities, including nausea, vomiting, and diarrhea. Visual disturbances were also common but all grade 1, with no evidence of ocular pathology in any patient. Peripheral edema has been observed in 20% of patients and has generally responded well to conservative
measures or diuretic therapy. Twelve-percent of patients did develop drug-induced transaminitis, including 4 with grade 3 and 1 with grade 4 ALT elevation. Some, but not all, of these patients were able to resume crizotinib at a lower dose without recurrent hepatotoxicity. Overall, crizotinib appears to have an excellent safety profile.

The marked activity of crizotinib observed in this phase 1 study has led to a phase 3 registration trial comparing crizotinib to standard, single-agent chemotherapy in metastatic, EML4-ALK-positive NSCLC (PROFILE 1007, Clinical Trials.gov Identifier NCT00932893). All patients must have advanced NSCLC harboring ALK rearrangements, as demonstrated by FISH analysis performed at a central laboratory. This trial is also restricted to patients who have received only one prior line of chemotherapy, and that chemotherapy must have been a platinum combination. The primary endpoint of this study is PFS. This study opened in the US in January 2010 and is slated to open at a total of 179 sites worldwide in order to reach its goal accrual of 318.

In addition to this phase 3 trial, there is also a companion, single-arm phase 2 trial of crizotinib (PROFILE 1005, Clinical Trials.gov Identifier NCT00932451). As with PROFILE 1007, all patients must have ALK FISH testing performed in a central laboratory. Eligible patients include those who received standard chemotherapy on PROFILE 1007 and discontinued treatment due to RECIST-defined disease progression. This trial in effect serves as a mechanism by which PROFILE 1007 patients can cross over into the crizotinib arm. Patients who have received more than 1 prior line of chemotherapy and are therefore ineligible for PROFILE 1007 may also be eligible for PROFILE 1005. The primary endpoint of this study is ORR. Of note, at the present time, previously untreated, ALK-positive patients are not eligible for treatment with crizotinib. However, a first-line trial comparing crizotinib with a standard platinum/pemetrexed combination in ALK-positive NSCLC will be opening in early 2011 (Clinical Trials.gov Identifier NCT01154140).

**IPI-504 and other hsp90 inhibitors.** IPI-504 (retaspimycin hydrochloride) is a potent and selective heat shock protein 90 (hsp90) chaperone inhibitor. In a phase 2 trial of IPI-504 in patients with advanced NSCLC who were previously...
treated with an EGFR TKI, the ORR among 78 patients was 7% (32). Retrospective molecular analysis led to the serendipitous discovery of ALK rearrangements in two of the five patients who achieved a partial response (PR). A third patient with ALK positive NSCLC demonstrated stable disease (24% reduction in tumor burden). All three ALK positive patients were crizotinib-naïve and received IPI-504 for approximately 7 months. Subsequent studies in the laboratory have confirmed the sensitivity of cancer cell lines harboring ALK fusions to hsp90 inhibition (32) (33). These preliminary findings are now undergoing validation in a study of IPI-504 in NSCLC harboring ALK rearrangements, as well as within ongoing trials of other novel hsp90 inhibitors. Whether hsp90 inhibitors will show activity in crizotinib-resistant patients is unknown, but there is the theoretical possibility of cross-resistance which may limit the utility of these agents in ALK-rearranged NSCLC.

Other treatments. In a small retrospective study, patients with tumors harboring either EML4-ALK, EGFR, or neither genetic alteration (WT/WT) were compared in terms of response rate, time to progression (TTP) and overall survival (24). Among metastatic patients who received any platinum-based combination, EML4-ALK positive patients showed similar response rates and TTP as WT/WT (or nonEGFR) patients. In contrast to EGFR patients, EML4-ALK patients did not appear to respond to EGFR TKIs such as erlotinib. Within the EML4-ALK cohort, there were no clinical responses to EGFR TKIs, and the median time to progression was only 5 months. These findings are consistent with preclinical studies showing that the EML4-ALK-containing NSCLC cell line H3122 is resistant to erlotinib (4), and suggest that ALK-positive patients do not benefit from treatment with EGFR TKIs.

FUTURE DIRECTIONS
In conclusion, EML4-ALK defines a new molecular subset of NSCLC with distinct clinical and pathologic features. The patients most likely to harbor EML4-ALK are the young, never or light smokers with adenocarcinoma. Recently published results from a phase 1 study demonstrate that the ALK inhibitor crizotinib is highly active in patients whose tumors harbor EML4-ALK.
A phase 3 study is currently underway to test whether crizotinib is superior to standard chemotherapy in the second-line setting. However, results from this phase 3 trial will not be available for several more years. Based on the impressive ORR of 56% observed in the phase 1 as well as the median PFS of 9.2 months, both of which far exceed standard chemotherapy comparators, it is likely that the FDA will grant accelerated approval for crizotinib in ALK positive NSCLC. As such, in the US, crizotinib may become the new standard of care for this molecularly-defined group of patients before the phase 3 trial has even completed accrual.

Additional studies with crizotinib are now ongoing or under active development. Crizotinib will be tested in another phase 3 trial head-to-head with 1st line chemotherapy, ala the IPASS trial (34). In this study (ClinicalTrials.gov Identifier NCT01154140), newly diagnosed patients with advanced, ALK positive NSCLC will be randomized to receive either crizotinib or a platinum/pemetrexed combination. The primary endpoint will be PFS. This trial will open worldwide in early 2011 and will overlap with the ongoing PROFILE 1007/1005 trials. In NSCLC, crizotinib may also undergo testing in combination with standard chemotherapies in order to evaluate how best to integrate ALK inhibitor therapy into standard treatment regimens. One reasonable combination to pursue may be crizotinib and pemetrexed, as there is anecdotal data suggesting that ALK positive patients may derive prolonged clinical benefit from single agent pemetrexed (A. Shaw, unpublished data).

Crizotinib is also being tested in patients with other malignancies known to harbor ALK rearrangements, including ALCLs, IMTs as well as neuroblastomas (ClinicalTrials.gov identifier NCT00939770 and NCT01121588). Significant and prolonged activity has already been observed for one patient with ALK-rearranged IMT who received crizotinib (35). Finally, the original phase 1 study remains open for patients with MET-amplified cancers as well as for rare patients with ALK positive NSCLC who do not meet eligibility for the PROFILE trials.

While there is little doubt that crizotinib represents another targeted therapy success in lung cancer, it is sobering to recognize that patients with ALK positive NSCLC do relapse on crizotinib due to acquired TKI resistance. In addition, several patients on the phase 1 trial demonstrated progression at
first evaluation (30), raising the possibility of intrinsic TKI resistance in a small minority of patients. Until recently, the molecular mechanisms underlying resistance to crizotinib were unknown. However, one potential mechanism has now been defined in a Japanese patient with ALK positive NSCLC who relapsed after 5 months of crizotinib therapy. Sequencing of the ALK TK domain revealed the presence of two de novo mutations, C1156Y and L1196M, each of which confers resistance to crizotinib (36). Both of these mutations were previously discovered in an in vitro ENU mutagenesis screen using EML4-ALK-expressing BaF3 cells (37). The frequency of this resistance mechanism has not yet been examined, but it is unlikely to represent the sole mechanism of resistance. Of potential promise, AP26113, a more potent ALK TKI compared with crizotinib, retains activity in cell lines with various ALK mutations, including the gatekeeper mutation L1196M (37).

There are other small molecule ALK inhibitors in various stages of development, and some of these will be entering the clinic in 2011. Whether AP26113 or other ALK TKIs are safe and active in patients who have developed crizotinib resistance due to secondary mutation in ALK is unknown. Hsp90 inhibitors could also have a role in treating crizotinib-resistant patients if the mechanism of resistance involves a mutation within the TK domain of ALK and the tumors remain oncogene-addicted. These early findings represent an essential first step in defining mechanisms of resistance in order to develop therapeutic strategies aimed at overcoming acquired TKI resistance.
References


Figure Legend

Figure 1. Schematic representation of ALK fusion oncogenes and important downstream signaling pathways. The EML4-ALK fusion oncogene results from a chromosomal inversion involving chromosome 2p (left). The EML4-ALK fusion protein is aberrantly expressed and activates canonical signalling pathways, including Ras/Mek/Erk and PI3K/Akt cascades. The STAT3 signaling pathway has a central role in NPM-ALK-mediated transformation, but the importance of STAT3 activation in EML4-ALK-positive NSCLC is unknown.
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