Molecular Pathways

Inhibition of ALK Signaling for Cancer Therapy

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Abstract

Paradigm shifting advances in cancer can occur after discovering the key oncogenic drivers of the malignant process, understanding their detailed molecular mechanisms, and exploiting this transdisciplinary knowledge therapeutically. A variety of human malignancies have anaplastic lymphoma kinase (ALK) translocations, amplifications, or oncogenic mutations, including anaplastic large cell lymphoma, inflammatory myofibroblastic tumors, non–small cell lung cancer, and neuroblastoma. This finding has focused intense interest in inhibiting ALK signaling as an effective molecular therapy against diseases with ALK-driven pathways. Recent progress in the elucidation of the major canonical signaling pathways postulated to be activated by NPM-ALK signaling has provided insight into which pathways may present a rational therapeutic approach. The identification of the downstream effector pathways controlled by ALK should pave the way for the rational design of ALK-inhibition therapies for the treatment of a subset of human cancers that harbor ALK aberrations. (Clin Cancer Res 2009;15(18):5609–14)

Background

Anaplastic lymphoma kinase (ALK) is an orphan receptor tyrosine kinase first identified as part of the t(2;5) chromosomal translocation associated with most anaplastic large cell lymphomas (ALCL) and a subset of T-cell non-Hodgkin’s lymphomas (1). It has recently become clear that many human cancers activate ALK signaling by creating unique oncogenic fusions of the ALK gene at chromosomal band 2p23 with a variety of partners through chromosomal translocation events (2), resulting in the generation of oncogenic ALK fusion genes and their encoded proteins. These oncogenic fusion proteins lead to constitutive activation of the ALK kinase domain and have been identified in various solid tumors, including inflammatory myofibroblastic tumors (IMT; ref. 3), squamous cell carcinomas (4), and non-small cell lung cancers (NSCLC; ref. 5, 6). It has also recently been discovered that germline mutations in ALK are the major cause of hereditary neuroblastoma (7), and that these mutations can also be somatically acquired (7–10).

The extracellular region of ALK shows significant homology to the leukocyte tyrosine kinase (11), which places ALK in the insulin receptor superfamily of RTKs. The ALK gene encodes a 1,620-amino acid protein that undergoes posttranslational N-linked glycosylation to a fully mature form weighing 220 kDa. ALK expression is restricted to the developing central and peripheral nervous system with a postulated role in participating in the regulation of neuronal differentiation (12). The absence of published structural studies of ALK extracellular and intracellular domains, and the conflicting literature over whether pleiotrophin (13, 14) and/or midkine (15) are ALK ligands, emphasizes that much remains to be learned about ALK structure, function, and signaling. Recently it has become clear that ALK is one of the few oncogenes activated in both hematopoietic and nonhematopoietic malignancies. Although constitutive ALK signaling has been shown in these contexts to induce cell transformation in vitro and in vivo by controlling key cellular processes such as cell-cycle progression, survival, cell migration, and cell shaping, the canonical signaling pathways and cell-type specificities of signaling remain poorly defined. Regardless, it is now clear that ALK represents a tractable target for innovative therapies on the basis of selective inhibition of its tyrosine kinase activity. This review focuses on the normal and cancer-related biology of ALK and the preclinical proof-of-principle for ALK as a therapeutic target.

Role of ALK in Cancer

A variety of mechanisms that lead to aberrant ALK signaling in a variety of human cancers have been characterized, and these include translocations or structural rearrangements, ALK gene amplification, mutations, and overexpression (Fig. 1). Importantly, missense germ-line mutations in the tyrosine kinase domain have been described in patients with hereditary neuroblastoma, with acquired somatic mutations seen in the more common sporadic form of the disease.

ALK Fusion Proteins—Translocations

Translocations are the most common known cause of genomic ALK aberration. Recent data suggest that deregulation
of several genes (Fra 2 on 2p23, HLH protein Id2 on 2p25, and the CSF1 receptor on 5q33.1) located near the ALCL translocation breakpoint occurs before the formation of translocation events, and that aberrant transcriptional activity of certain genomic regions is linked to their propensity to undergo chromosomal translocations (16). In physiological ALK signaling, ligand-induced homo-dimerization of the extracellular domains is hypothesized to bring the tyrosine kinase domains into sufficient proximity to enact trans-phosphorylation and kinase activity. By contrast, translocations resulting in pathogenic fusion partners provide dimerization domains that are ligand independent, leading to unregulated constitutive kinase activity and malignant transformation.

Approximately 70 to 80% of ALK-positive ALCL express the nucleophosmin-ALK (NPM-ALK) fusion protein derived from the t(2;5)(p23;q35) translocation, and about the same frequency of ALCLs stain positive for ALK by immunohistochemistry (17, 18). Within the truncated nucleophosmin protein the essential domain required for transformation is a coiled-coil oligomerization domain. Site-directed mutagenesis of the dimerization domain abrogates NPM-ALK-transforming activity suggesting that proximity of the ALK kinase domains is a prerequisite to facilitate trans-phosphorylation of the partner kinase (19). The truncated NPM also encodes a nucleolar localization domain that explains why in contrast to other ALK fusions, NPM-ALK can be detected in the cytoplasm, nucleus, and nucleolus (2), whereas most fusion products are largely cytoplasmic. The remaining 20% of translocations fuse ALK to other partners that commonly encode coiled-coil oligomerization domains such as Tyrosine Receptor Kinase-fused gene (TFG),...
Tropomyosin 3 (TPM3), and Tropomyosin 4 (TPM4). There are also several nonnuclear ALK fusion chimeras that induce transformation in vitro. The clathrin heavy chain-like gene (CLTCL), for example, encodes a clathrin family member that coalesces to produce cytoplasmic vesicles of CLTCL-ALK bringing the ALK tyrosine kinase domains into close proximity (20). Although almost all ALK fusions retain the same portion of ALK because of translocation events that result in loss of the entire extracellular portion of normal ALK as well as the transmembrane segment, they show slight differences in pathway activation, likely because of differences in their subcellular compartmentalization and/or cell-type. NPM-ALK transgenic mouse models have shown T-cell transformation as well as B-cell lymphomas (21) and may be useful in the evaluation of new therapeutic agents for these diseases.

The deregulated expression of full length ALK and ALK fusions has recently been observed in several nonlymphoid neoplasms. Interest in pharmacological inhibition of ALK has most recently been spurred by the discovery of transforming echnoderm microtubule associated protein like 4-anaplastic lymphoma kinase (EML4-ALK) fusions in a subset of NSCLCs (5), and a recent report of a novel fusion transcript, kinesin family member 5B-anaplastic lymphoma kinase (KIF5B-ALK), in this disease (22). Dimerization is enabled as the truncated EML4 protein retains a coiled-coil oligomerization domain in a manner similar to NPM-ALK. Epidemiologic characterization of EML4-ALK translocations is ongoing but it seems to be a rare aberration, most common in Asian nonsmokers or light-smokers with the adenocarcinoma subtype of NSCLC, forming a distinct subgroup from patients harboring EGFR, KRAS, or NXX2-1 aberrations (23–26). The initial frequency of EML4-ALK fusion transcripts was approximately 6.7% in Japanese NSCLC tumor samples (5), but subsequent studies showed lower frequencies of 3% or less (24, 25, 27). Direct demonstration of EML4-ALK as a dominant oncogenic driver in NSCLC includes transformation of mouse 3T3 fibroblastic cells with forced overexpression of human EML4-ALK and de novo lung adenocarcinoma formation in mice engineered to express EML4-ALK targeted to the lung alveolar epithelial compartment (28).

IMT is a rare tumor of mesenchymal origin that arises in multiple tissues, possesses metastatic potential (29), and is only minimally responsive to conventional chemotherapy or radiation therapy. IMT is associated with various ALK translocations that overlap with those found in ALCCL, including TPM3 and TPM4 (30), CARS (31), and CLTCL (32), but not to date with fusion partners seen in NSCLC. Immunohistochemical ALK reactivity occurs in 56% of cases and is restricted to the cytoplasm consistent with the observation that NPM-ALK translocations do not occur in this disease. A subset of esophageal cancers show the TPM4-ALK fusion protein, but frequency and significance of this has not been fully defined (33).

**Mutations and Genomic Amplification**

Expression of ALK has been reported in a large fraction of human-derived neuroblastoma cell lines (34), and our group and others had previously identified ALK as a candidate neuroblastoma oncogene through somatically acquired amplification of the genomic locus (35, 36). Recently, both germline (7) and somatic (7–10) mutations that activate ALK have been discovered in neuroblastoma, an often lethal embryonal malignancy that contributes 15% to the overall childhood cancer mortality rate (37). Heritable gain-of-function mutations in ALK are the cause of most hereditary neuroblastoma cases; one of the few pediatric tumors to be initiated by gain-of-function mutations. To date, heritable mutations have been restricted to the kinase domain of ALK, and it is presumed, but not yet proven, that the second hit is somatic gain or amplification of the mutant allele, similar to what is seen at the MET locus in hereditary renal papillary cancer (38). Although the number of families studied remains relatively small, it is apparent that the R1275Q mutation is most common, and it seems that disease penetrance may be attributable to mutation type as rarer mutations, such as G1128A seen in one large pedigree, was associated with very low penetrance compared with the near complete penetrance seen with the R1275Q mutation. Both mutations fall within the kinase activation loop in a region strongly associated with activating mutations seen in other oncogenic kinases (7). This fact compounds the already controversial topic of the role for genetic screening in this disease. Because there are noninvasive methods to screen for neuroblastoma (urinary catecholamine metabolites have relatively high sensitivity and specificity for detecting disease), we currently recommend genetic testing of the proband in any case in which there is a first or second degree relative with neuroblastoma, or the proband has multifocal disease consistent with hereditary predisposition. Because neuroblastoma often occurs in families with other young children or plans for future children, there is often parental interest in testing even when there is no objective evidence for hereditary predisposition. As we define the frequency of occult germline mutations and further understand the role of ALK in the initiation of neuroblastomas without a family history, screening recommendations can be solidified.

ALK activation by mutation and/or amplification is functionally relevant in models of high-risk neuroblastoma, and thus might offer a tractable therapeutic target. We and others have shown that ALK is expressed in the majority of human neuroblastoma-derived cell lines, and that ALK expression is significantly higher in neuroblastoma cells harboring ALK mutations (39). Analysis of protein lysates from these lines showed constitutive phosphorylation in each of the cell lines harboring mutations, with weak phosphostaining in a smaller subset of wild-type cell lines, suggesting that ALK activation can occur by mechanisms other than mutation within the kinase domain. Transient siRNA knockdown directed against ALK in neuroblastoma cell lines showed significant inhibition of growth restricted to cells with evidence for ALK activation. Baf3 cells expressing the two most common mutations showed cytotoxicity to NVP-TAE684, a small molecular competitive inhibitor of ATP binding in the ALK kinase domain (40). NVP-TAE684 has been shown to block the growth of ALCCL-derived and ALK-dependent cell lines with IC_{50} values between 2 and 10 nM; however, due to its toxicity profile, it is now only used as a tool compound (40). More recently, pharmacologic inhibition with a highly specific small molecule inhibitor of ALK currently in phase 1 clinical trials (PF-02341066, see Clinical Translational Advances) showed potent antitumor activity in preclinical models of neuroblastoma both in vitro and in vivo, in a mutation-specific manner. Although these data are still emerging, it is somewhat clear that ALK phosphorylation status is a good biomarker for sensitivity to pharmacologic inhibition, but there seem to be mutation-specific differences in sensitivity. Solving the crystal structure of the ALK kinase domain, and cocystalization with
pharmacologic inhibitors, will be necessary to understand if some mutations confer resistance, and to understand how resistance mutations may arise under selective pressure. In addition, defining the downstream signaling pathways that mediate the effects of ALK activity in the context of a neuroblastic cell will also be critical, especially for the development of rational combination therapies. Work is ongoing to characterize the full spectrum of germline and somatic DNA alterations leading to ALK activation to develop strategies for future clinical trials on the basis of inhibiting ALK-mediated signaling in neuroblastoma.

### Expression

ALK expression is of uncertain pathogenic significance in several human cancers. The detection of ALK mRNA in diverse human tumor-derived cell lines (41), as well as the detection of higher ALK expression in tumor compared with neighboring normal tissue (15), likely reflects physiological expression rather than seminiferous pathogenic events (41). ALK has previously been shown to be upregulated and functionally relevant in glioblastoma, and recent work has shown that ribozyme-mediated knockdown of ALK results in abolishment of tumor growth in a xenograft model (42). This offers potentially novel therapeutic options for an otherwise lethal brain tumor. Immunohistochemical detection of ALK protein overexpression is pathognomonic for ALCL, and recent work suggests that ALK immunohistochemistry may have utility as a screening tool or surrogate marker for EML4-ALK fusion gene-positive NSCLC tumors (43). Current work is focused on developing reliable techniques for phospho-ALK staining in routine clinical specimens, and this may be critically important to properly evaluate future ALK inhibitor clinical trials.

### Differential Activation of Downstream Signaling Pathways

The critical pathways involved in transformation because of dysregulated ALK signaling are best characterized by translocations that juxtapose ALK to dimerization partners (Fig. 1; ref. 2). The constitutive activation of ALK fusion proteins leads to cellular transformation through a complex signaling network. Among the potential combinations of proteins phosphorylated by ALK kinase activity, it has been postulated that the most important effects involve activation of STAT3 (44, 45), AKT/Pi3K (46), and Ras/ERK (47) pathways, which control cell proliferation, survival, and cell cycling. These pathways are typically upregulated in transformed cell lines (48), and pharmacological inhibition of ALK leading to apoptosis abrogates phosphorylation of these key signal-transducing proteins in ALCL models (49, 50). Inhibition of downstream signal transduction proteins, such as STAT3, has been shown to prevent NPM-ALK induced transformation in vivo (45). Recent work has shown that the sonic hedgehog signaling pathway (SHH/GLI1) is also activated in ALK-positive ALCLs (51). SHH/GLI1 activation is the result of SHH gene amplification and is further mediated by NPM-ALK through activation of Pi3K/AKT and stabilization of GLI1 protein (51). Inhibition of this pathway induces apoptosis and cell cycle arrest, suggesting that inhibition of SHH/GLI1 signaling in combination with blocking NPM-ALK and/or the Pi3K/AKT pathway may present a rational therapeutic approach in ALK+ ALCL.

Different ALK aberrations produce diverse pathogenic signaling anomalies through a combination of differentially activating common signal transduction pathways and unique pathogenic mechanisms. Different ALK fusion proteins transfected into NIH3T3 cells produced clones with differential signaling and phenotypic differences beyond those that could be explained by the variable kinase activity of each fusion. TPM3-ALK cells showed the most AKT phosphorylation and migratory capacity in vitro, but in vivo tumorigenicity was less than that observed in NPM-ALK cells. In contrast, ATIC phosphorylation enhances enzymatic activity of NPM-ALK (52), and ATIC-ALK cells cause STAT3 phosphorylation with only modest invasion and in vivo tumorigenicity (48). TPM3-ALK invasive capacity may partly be mediated by a unique pathogenic mechanism. TPM interacts with endogenous tropomyosin in the cytoskeleton, which is hypothesized to alter cytoskeletal organization and led to greater motility and metastatic potential (29). It has been postulated that the STAT family is unlikely to play a key role of the pathogenesis of EML4-ALK (53), even though STAT3 anti-sense oligonucleotides suppress the in vivo transforming activity of NPM-ALK (45). Tissue context and signaling differences may explain why some ALCL and IMT share some fusion partners, but display no overlap with NSCLC. Additional signaling and phenotypic variation will likely be discovered when the full spectrum of mutated ALK and pathogenically overexpressed wild-type (via genomic amplification) proteins are studied. These signaling variations could arise because of multiple mechanisms including: variable subcellular localizations of activated ALK, altered sequences of tyrosine auto-phosphorylation, altered kinase substrate specificity, tissue context, autocrine or paracrine ligand effects, and by breakpoints disrupting the original loci in which truncated genes are translocated from.

### Clinical Translational Advances

**Small molecule tyrosine kinase inhibitors.** Kinases are critical components of cellular signal transduction cascades, and are key effectors of cell proliferation and differentiation. There now exists clear precedent in clinical oncology that robust antitumor activity can be obtained with inhibitors directed toward oncogenic tyrosine kinases that are genetically dysregulated (54, 55), and these lessons are readily applicable to ALK kinase inhibition. Imatinib showed that off-target kinase inhibition could be rationally exploited because of its efficacy in Kit+ gastrointestinal stromal tumors (56). This is significant, as the first ALK inhibitor to enter phase 1 clinical trials, PF-02341066, was initially designed to inhibit c-Met, but was also found to have significant activity against ALK (50). This orally bioavailable small molecule inhibitor caused complete regression of NPM-ALK xenografts at pharmacologically relevant doses, with a strong correlation between antitumor response and abrogation of phosphorylation of ALK (50). PF-02341066 is currently the only available ALK small-molecule inhibitor in clinical trials; however, recognition of the variety of malignancies in which ALK plays a causative role has prompted broad developmental efforts in this area. Point mutations in the kinase domain of other tyrosine kinases such as BCR-ABL, KIT, and EGFR impair drug binding as the major mechanism of acquired resistance to kinase inhibitors. To begin to address this, investigators have generated several ALK mutants in the kinase domain and evaluated their kinase activity and sensitivity to inhibition by two ALK inhibitors
ALK Inhibition Therapy

Developed from different chemical series (57). Binding of these inhibitors to wild-type and mutant ALK was analyzed using homology models, and showed that some of the mutants are resistant to select small molecule inhibitors. It remains to be seen whether newer inhibitors designed with greater specificity against ALK will eventually prove superior to multitkine inhibitor.

Future Developments

ALK is one of the few examples of a receptor tyrosine kinase implicated in the oncogenesis of both hematopoietic and non-hematopoietic malignancies. Although the importance of autocrine-paracrine growth loops involving normal ALK in tumorigenesis is not yet fully elucidated, there are compelling data to support the hypothesis that tumors with ALK aberrations exhibit oncogene addiction, with inhibition of aberrant ALK signaling resulting in marked antitumor efficacy in preclinical models. Additional studies are required to determine whether tumors that express full-length ALK that is activated by its ligand exhibit the same or at least partial dependence upon ALK growth signals.

There is strong rationale and significant enthusiasm toward ALK inhibition as a targeted therapy against tumors harboring oncogenic fusions or activating mutations, and the results of the first clinical trial testing the PF-02341066 dual c-Met/ALK inhibitor has shown clinical activity in highly refractory patients with tumors harboring ALK translocations, leading to ongoing efforts to select patients for these trials based on ALK translocation status. Defining the epidemiology of ALK aberrations in each cancer that harbors ALK pathway activation seems essential to properly design and interpret clinical trials of ALK inhibitors. Furthermore, as potency will likely be variable on the basis of the mechanism for pathway activation, and the emergence of resistance mechanisms almost assured, co-development of small molecular and monoclonal antibody inhibitors of ALK activation seems prudent. A pediatric trial of PF-02341066 will begin enrollment of subjects in 2009, and plans are currently being made for how to properly integrate ALK inhibition therapy into frontline chemotherapeutic regimens. If the preclinical models do indeed predict what will be observed in the clinic, it is clear that for cancers that can usurp ALK signaling as a mechanism of oncogenicity, ALK pathway status must be assayed for at the time of diagnosis or relapse.

Cautious extrapolation from preclinical models and inhibitor-kinase co-crystalization studies will help us to define biomarkers predictive of therapeutic response. Besides the use of selective ALK inhibitors, it may be crucial to develop strategies to inhibit multiple targets involved in the ALK-signaling pathway. siRNA screens of the druggable genome in combination with ALK inhibitors, and preclinical testing for synergy and antagonism with existing chemotherapy backbones will be important to maximize efficacy. Lastly, the development of monoclonal antibodies against ALK should be pursued, a precedent set by the development of monoclonal antibodies against members of the EGF family, and an approach that may prove to be particularly useful for tumors with ALK gene amplification.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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