Anaplastic Lymphoma Kinase as a Cancer Target in Pediatric Malignancies

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Abstract

In this era of more rational therapies, substantial efforts are being made to identify optimal targets. The discovery of translocations involving the anaplastic lymphoma kinase (ALK) receptor tyrosine kinase in a subset of non–small cell lung cancers has become a paradigm for precision medicine. Notably, ALK was initially discovered as the fusion gene in anaplastic large cell non-Hodgkin lymphoma, a disease predominantly of childhood. The discovery of activating kinase domain mutations of the full-length ALK receptor as the major cause of hereditary neuroblastoma, and that somatically acquired mutations and amplification events often drive the malignant process in a subset of sporadic tumors, has established ALK as a tractable molecular target across histologically diverse tumors in which ALK is a critical mediator of oncogenesis. We are now uncovering the reexpression of this developmentally regulated protein in a broader subset of pediatric cancers, providing therapeutic targeting opportunities for diseases with shared molecular etiology. This review focuses on the role of ALK in pediatric malignancies, alongside the prospects and challenges associated with the development of effective ALK-inhibition strategies.

Clinical Translocation-Driven Pediatric Cancers

Anaplastic large cell lymphoma

The ALK gene was first discovered in 1994 as a fusion oncogene with nucleophosmin (NPM) in a subset of anaplastic large cell lymphomas (ALCL), a malignancy predominantly of childhood, from a translocation involving the ALK gene on chromosome 2p (3). Since then, multiple fusion partners forming ALK chimeric proteins have been identified in this disease, and ALK fusions have also been reported in diffuse large B-cell lymphomas (8). ALCLs represent a well-recognized and distinct subgroup of non-Hodgkin lymphomas that account for 10% to 15% of all childhood lymphomas (9, 10), and are characterized by the expression of the membrane receptor CD30, a member of the nerve growth factor/TNF receptor family (11). The vast majority of pediatric cases harbor the characteristic ALK gene translocation (83% vs. 31% in adult ALCL) with overexpression of the ALK protein, and it is possible that variant ALK fusion transcripts may identify biologically and clinically distinct subgroups of ALCL (12, 13). These fusion proteins can all be recognized immunohistochemically on fixed tissue as well as by molecular and cytogenetic techniques. Interestingly, primary cutaneous ALCL is ALK negative and thought to be an unrelated disease entity. Children with advanced-stage ALCL have not fared well, with disease-free survival rates in the range of 50% to 80% using chemotherapeutic regimens varying in duration and intensity (10, 14).
Because ALK is not expressed in most normal cells, the possibility exists that the ALK protein would be the target of an immune response, which could explain the paradox that ALK-positive ALCL is highly aggressive historically but has a better prognosis than other large cell lymphomas. The possibility that ALK proteins are immunogenic was investigated by Pulford and colleagues, and circulating antibodies against NPM-ALK protein were found to be present in all ALK-positive ALCL patients studied while several patients also had antibodies recognizing normal ALK protein (15). These data suggest that the presence of anti-ALK antibodies may be relevant to the more favorable prognosis of ALK-positive ALCL.

Although numerous clinical studies have used a wide range of chemotherapeutic strategies, no intervention has been able to improve on the approximate failure rate of 25% to 30% that exists regardless of treatment strategy. In addition, disease progression while receiving chemotherapy portends a very poor prognosis, with only 25% expected to survive even with aggressive salvage therapy, including allogeneic transplant (16, 17). Quite remarkably, the Children's Oncology Group (COG) phase I trial of single-agent crizotinib revealed marked antidrug activity in 8 of 9 patients with relapsed ALK-translocated ALCL at a range of doses (18). Crizotinib was well tolerated without evidence of cumulative toxic effects. Nine patients with ALK-translocated ALCL were enrolled, all of whom received previous mult-agent chemotherapy, and 7 had a complete and sustained response with crizotinib monotherapy. As we await the results from the phase II trial and an update on the status of durability of response in the ALCL patients treated on the phase I study, this robust activity has set the stage for the current COG pilot phase II trial that will determine the toxicity and efficacy of integrating crizotinib with standard chemotherapy in newly diagnosed ALCL patients (NCT01979536) with the goal of preventing relapse, especially on-therapy progression, and reducing toxicity in order to have a significant impact on overall survival.

**Inflammatory myofibroblastic tumor**

In solid tumors, ALK fusions were first reported in inflammatory myofibroblastic tumors (IMT; ref. 4), a rare mesenchymal tumor that can occur at any age but has a predilection for children and adolescents (19). These soft tissue tumors most commonly involve the lung, abdomen/pelvis, and retroperitoneum and the mainstay of curative therapy is complete surgical resection. Systemic therapy options such as cytotoxic chemotherapy or anti-inflammatory agents are limited for patients with unresectable, recurrent, or metastatic disease. Approximately 50% of IMTs express the ALK protein by IHC (20, 21) as a result of numerous structural rearrangements involving the ALK gene that have been described (4, 5, 22, 23). Objective and sustained responses to crizotinib have been reported in children with IMTs (18), supporting the dependence of ALK-rearranged tumors on ALK signaling and demonstrating the significance of identifying this target. Notably, recent targeted next-generation sequencing (NGS) efforts have defined this disease as one that is largely a kinase-fusion driven malignancy. Lovly and colleagues identified novel ALK gene fusions in only samples that tested negative for ALK expression by IHC, as well as ALK fusions with various 5′ gene fusion partners and ALK fusions with noncanonical fusion breakpoints (24). Because these patients are likely to benefit from ALK inhibition strategies, it is now essential to incorporate these atypical but recurrent fusions into diagnostic sequencing platforms, and to move away from IHC alone for identification of ALK-driven IMTs.

**Pediatric Cancers Harboring ALK Mutations: Neuroblastoma**

Despite major enhancements in treatment approaches over the past several decades, neuroblastoma remains a leading cause of childhood cancer deaths and survivors are left with long-term side effects (25). The discovery of germline missense point mutations in the intact ALK oncogene as the genetic etiology of familial neuroblastoma (26, 27), and of somatically acquired mutations that predict for inferior outcome in patients with the most aggressive form of this disease (28), has positioned ALK as the only mutated oncogene tractable for targeted therapy in this disease. ALK mutations are identified in 8% of neuroblastoma patients and span the entire spectrum of disease, including stage IV disease, congenital cases (<30 days old), and adolescents/young adults (28). Within the high-risk subset of patients, ALK aberrations are found in 14% (10% mutations, 4% amplification), and the presence of an ALK aberration is a biomarker of inferior outcome (28). These findings provide an opportunity to integrate ALK-targeted therapy for a subgroup of patients with a poor prognosis whose tumors harbor a molecular vulnerability that can be targeted therapeutically. However, despite our increasing knowledge of how to treat ALK-rearranged lung cancer and other ALK-rearranged malignancies, inhibition of full-length mutated ALK remains a therapeutic challenge. Interestingly, the most frequently identified secondary mutations in ALK-fusion positive NSCLC at time of acquired crizotinib resistance are L1196M (rarely identified in NB) and G1269A (Table 1; ref. 29). These observations intimate that certain molecular contexts enable differential sensitivity to direct ALK kinase inhibition over others, suggesting a context-dependent role for ALK in driving response and mechanisms of resistance.

Our work, and that of others, has been successful in positioning ALK as the first tractable oncogene for targeted therapy in neuroblastoma, revealing that activating mutations are oncogenic drivers in some cases (26, 27, 30, 31). In developing approaches to act on this knowledge, discriminating nonactivating from activating mutations is extremely important, and identifying nonactivating mutations is one of the most central unmet challenges in progress toward personalized medicine (32). Preclinical data suggest that not all clinically observed ALK mutations are functionally relevant as ALK activators (Table 1), and further, that some ALK-activating mutations also cause primary resistance to direct ALK kinase inhibition with crizotinib (28). We find that one out of 11 patients with an ALK mutation harbors a “silent” mutation (Table 1). This highlights the importance of bringing mechanistic considerations to bear that can distinguish “silent” mutations in ALK from those that are activating, so as to determine which next-generation ALK inhibitor has the broadest applicability across mutants, and so as to accurately distinguish the patients that are good candidates for ALK-targeted therapy. In the case of EGFR in lung cancer, mutations have now been found at approximately 70% of sites in the kinase (33). Determining which sites are not relevant and which are surprisingly common is as important as identifying those that are functional. Moreover, identifying which ALK variants are crizotinib resistant and which are not (among those with activating mutations) has immediate clinical relevance as we begin to use...
Table 1. Spectrum and frequency of ALK mutations identified in diagnostic neuroblastoma tumors

<table>
<thead>
<tr>
<th>ALK mutation</th>
<th>Frequency in ALK-mutant tumors</th>
<th>Functional significance</th>
<th>Identified in lung cancer resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1275G/L</td>
<td>45%</td>
<td>Transforming</td>
<td></td>
</tr>
<tr>
<td>F1174L/V/C</td>
<td>30%</td>
<td>Transforming</td>
<td></td>
</tr>
<tr>
<td>F1245V/C</td>
<td>12%</td>
<td>Transforming</td>
<td></td>
</tr>
<tr>
<td>R1060H</td>
<td>0.80%</td>
<td>Not transforming</td>
<td></td>
</tr>
<tr>
<td>M1166R</td>
<td>1.60%</td>
<td>Transforming</td>
<td></td>
</tr>
<tr>
<td>I1171N</td>
<td>0.80%</td>
<td>Transforming</td>
<td></td>
</tr>
<tr>
<td>Y1278S</td>
<td>0.80%</td>
<td>Not transforming</td>
<td></td>
</tr>
<tr>
<td>G1286R</td>
<td>0.80%</td>
<td>Not transforming</td>
<td></td>
</tr>
<tr>
<td>L1240V</td>
<td>0.80%</td>
<td>Transforming</td>
<td></td>
</tr>
<tr>
<td>G128A</td>
<td>0.80%</td>
<td>Transforming</td>
<td></td>
</tr>
<tr>
<td>T1151M</td>
<td>0.80%</td>
<td>Not transforming</td>
<td></td>
</tr>
<tr>
<td>I1183T</td>
<td>0.80%</td>
<td>Not transforming</td>
<td></td>
</tr>
<tr>
<td>G1202R</td>
<td>0.80%</td>
<td>Transforming</td>
<td></td>
</tr>
<tr>
<td>R1192P</td>
<td>0.80%</td>
<td>Transforming</td>
<td></td>
</tr>
<tr>
<td>A1200V</td>
<td>0.80%</td>
<td>Not transforming</td>
<td></td>
</tr>
<tr>
<td>D1270G</td>
<td>0.80%</td>
<td>Not transforming</td>
<td>G1269A, just proximal</td>
</tr>
<tr>
<td>L1204F</td>
<td>0.80%</td>
<td>Not transforming</td>
<td>G1202R, just proximal</td>
</tr>
<tr>
<td>R1231Q</td>
<td>0.80%</td>
<td>Not transforming</td>
<td></td>
</tr>
<tr>
<td>T1331I</td>
<td>0.80%</td>
<td>Not transforming</td>
<td></td>
</tr>
<tr>
<td>D1149H</td>
<td>0.80%</td>
<td>Not transforming</td>
<td></td>
</tr>
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ALK sequence to guide patient treatment. This implies that not all patients harboring an ALK mutation are predicted to respond to crizotinib.

Consistent with this, while marked antitumor activity was observed in pediatric patients with diseases harboring ALK translocations, far fewer objective responses were seen in patients with ALK-mutant neuroblastoma in the COG phase I trial of crizotinib (18). Objective responses were seen in 2 patients with refractory neuroblastoma, both harboring germline ALK R1275Q mutations. In addition, 1 patient with unknown ALK status had a complete response and 5 others had prolonged stable disease, 3 of whom remain on treatment. This uncovers a primary resistance to ALK inhibition in patients who have an appropriate genetic abnormality but fail to respond. We speculate as to which of the newer generation of ALK inhibitors is likely to be most useful for which sets of mutations. Recent work from Friboulet and colleagues suggests that ceritinib would be more useful for I1171 and L1196 mutations (34), and the new PF-06463922 compound may be best for F1174 mutations (34). These findings underscore the need to develop a responder hypothesis for therapeutic stratification of newly diagnosed patients with ALK-mutant neuroblastoma, to discover robust biomarkers of drug sensitivity and mechanisms of resistance to ALK inhibition, and to define circumvention strategies in the clinic in order to maximize patient benefit. In addition, more precise studies of clonal evolution in sequential samples from patients with neuroblastoma have revealed an emergence of ALK mutations at the time of relapse, a finding of utmost clinical importance given the development of ALK inhibition strategies (35).

**ALK as an Immunotherapy Target in Childhood Cancers**

Although our understanding of the biology of childhood cancer has advanced substantially, new targeted therapies have not yet significantly improved outcomes or allowed the community to develop less toxic standard therapies. Recent comprehensive genomic analyses have yielded the sobering conclusion that actionable recurrent somatic mutations are rare in pediatric cancers (36, 37), raising the prospect that we need to move beyond small molecules targeting mutant kinases if we want to substantially improve outcomes using precisely developed therapies. We now have the potential to leverage genomic, transcriptomic, and proteomic analyses of childhood cancers to identify cell surface antigens expressed on tumor cells for development of antibody-based immunotherapies. Below are a few examples in which ALK has been found to be reexpressed and that provide the opportunity for immunotherapeutic targeting.

**Neuroblastoma**

It has been firmly established that activation by mutation or amplification renders the intact ALK receptor tyrosine kinase an attractive therapeutic target in neuroblastoma. ALK mRNA expression (38) and native ALK protein expression measured by IHC (39, 40) have been proposed as biomarkers of mutation-independent ALK activation. Although ALK expression levels are significantly higher in tumor cells with activating ALK mutations (point mutations and amplification), no direct correlation has been found between ALK levels and constitutive activation of the ALK protein; hence, validated genetic alterations remain the hallmark that renders neuroblastoma cells susceptible to kinase inhibition. However, neuroblastoma cells almost ubiquitously express ALK on the cell surface, with expression restricted to tumor cells, suggesting that ALK is an optimal tumor antigen for immunotherapy (1, 41). Given the success of therapeutic antibodies that target oncogenic RTKs such as EGFR and HER2/Neu in other cancers, we propose that a similar approach should be developed for specifically targeting ALK in neuroblastoma. The development of ALK-targeted antibodies as a potential therapeutic approach in neuroblastoma may be complicated by the challenges of threshold receptor density, which for ALK varies substantially in this disease, and may require alternative approaches such as antibody drug conjugates (ADC) or chimeric antigen receptor
(CAR)–based therapies to effectively bind to an antigen with selective cell surface expression.

Rhabdomyosarcoma

Rhabdomyosarcomas (RMS) are the most common soft tissue sarcomas of childhood (42) with two main histologic subtypes: embryonal RMS (60%–0%) and alveolar RMS (ARMS; 20%–30%). ARMS is characterized by the t(2;13) translocation (PAX3-FOXO1) in the majority, or a t(1;13) translocation (PAX7-FOXO1), and characteristically has a more aggressive clinical course. Although treatment advances have yielded dramatic improvements in survival, patients with metastatic disease have a poor prognosis (43). Gene-expression profiling studies have identified distinct molecular signatures, with a postulated association between translocation-positive ARMS and ALK overexpression (44). In addition, prior studies have shown that ARMS was more commonly immunopositive for native ALK protein expression than other subtypes (45, 46), with the largest series (n = 116) showing a clear difference in ALK reactivity between alveolar (69%) and other (4%; ref. 47). There have been no reports of recurrent activating point mutations, translocations, or high-level amplifications in these tumors, suggesting that the differential ALK staining is possibly physiologic or from ligand participation, and it is unlikely to render this tumor subtype amenable to therapeutic targeting using small molecules such as crizotinib. Although the role of ALK as an oncogenic therapeutic target remains to be validated in RMS, we can postulate a role for targeting ALK on the cell surface of these tumors using immunotherapeutic approaches.

Other childhood cancers

We have developed a method for identifying tumor antigen candidates by leveraging publicly available microarray gene expression databases of pediatric cancer and normal tissues, and other studies have shown a significant correlation between

![Boxplot of ALK mRNA expression displayed across a set of pediatric tumor microarray studies and one normal microarray study encompassing a variety of tissues. Dashed black line indicates median normal expression. Dashed red line indicates 99th percentile of normal tissue. Points shaded in blue represent patient samples with 2-fold higher levels of ALK expression as compared with 99th percentile of normal tissue level. The microarray data were MAS5 normalized and log2 transformed. Boxplot figure was generated using the ggplot2 package (Wickham, Hadley. Ggplot2. Dordrecht: Springer, 2009. Print). Normalization and analysis were done using the R statistical language and Bioconductor framework. * Accession number GSE 37371.](image-url)
mRNA and protein levels (48). Averaging ALK expression levels from individual tumor samples according to histotype and comparing with average normal tissue expression levels have provided the opportunity to define a broader role for ALK in pediatric cancers (Fig. 1; refs. 49–58).

In analyzing these data, it has become clear that reexpression of the developmentally regulated ALK protein is present across a subset of pediatric tumors, with a percentage of tumor samples showing substantially higher expression than normal tissues (Fig. 1). This distinction is especially important in pediatric cancers where there is the clear concern that targeting receptors that may be present on normal tissues that are crucial to development, even at low levels, could be harmful. Notably, in addition to neuroblastoma and RMS, ALK is differentially expressed at high levels in subsets of medulloblastomas, gliomas, and Ewing sarcomas. The wide distribution of this target provides an opportunity for development of immune targeting strategies that could be safely achieved without loss of host integrity. How a tumor antigen is targeted by the immune system may be as important as the expression level of the antigen on various tissues.

Although antibody-derived therapeutics targeting the ALK receptor at the cell surface is likely to be an approach that will be shared among several pediatric tumors, the safety of targeting ALK in this way is yet to be demonstrated. We have established that there is sufficient differentiation between cancer and normal host tissue using neuroblastoma as a model (1), suggesting that ALK can be readily targeted, though we do not yet have an understanding of the cell surface expression necessary for effective targeting. A large-scale effort will be necessary to definitively credential the role of ALK as a tumor antigen in various pediatric tumors, and to systematically evaluate cell-surface density as one credential the role of ALK as a tumor antigen in various pediatric tumors, with a percentage of tumor samples showing substantially higher expression than normal tissues (Fig. 1). This distinction is especially important in pediatric cancers where there is the clear concern that targeting receptors that may be present on normal tissues that are crucial to development, even at low levels, could be harmful. Notably, in addition to neuroblastoma and RMS, ALK is differentially expressed at high levels in subsets of medulloblastomas, gliomas, and Ewing sarcomas. The wide distribution of this target provides an opportunity for development of immune targeting strategies that could be safely achieved without loss of host integrity. How a tumor antigen is targeted by the immune system may be as important as the expression level of the antigen on various tissues.

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Conclusions and Future Directions

 Childhood cancer represents a rare disease and drug development remains challenging in part because of the limited availability of patient cohort and the limited marketability of drugs even when they are successful. This paradigm is now shifting as we face the opportunity to develop rational targeted therapies for small subsets of patients, and drug trials are beginning to group patients by shared molecular etiology across multiple diseases. A demonstrative example of this is the COG trial of crizotinib that enrolled a molecularly selected cohort of patients with malignancies known to display a dependency on the ALK oncogene (18). Marked antitumor activity was observed in patients with relapsed/refractory ALCL with complete and sustained responses reported, supporting the dependence of ALK-rearranged tumors on ALK signaling. Similarly, robust responses were reported for patients with IMTs, providing an effective and well-tolerated therapeutic strategy for these patients. However, numerous objective responses to crizotinib were not observed in patients with ALK-mutant neuroblastoma. The patients in this trial were heavily pretreated, in the late stages of their disease, and the increased incidence of loss of p53 function after the selective pressure of chemotherapy yields multidrug resistant disease (59). Strikingly, little is known about the genome of neuroblastoma at the time of relapse following intense cytotoxic chemotherapy, in part because patients are rarely subjected to a tumor biopsy. There is some evidence to suggest that the neuroblastoma genome evolves extensively under the selective pressure of chemotherapy, and we hypothesize that targetable somatic mutations are enriched in this population and mediate therapy resistance even in the presence of an activated kinase. Deep-sequencing approaches have provided insight into the clonal evolution of this disease and revealed that ALK mutations may in fact occur as subclones present at low levels, with secondary expansion at time of relapse (35). This provides a strong rationale for performing biopsies and genomic characterization of relapsed neuroblastomas, with the prospect for direct patient benefit.

As high-throughput sequencing approaches generate broad cataloging of genetic variations in cancer, we must be able to distinguish disease-causing or functionally relevant mutations from nonpathogenic passenger variants. This distinction is critical in order to maximize clinical benefit, and false ascription of pathogenicity will result in severe consequences. As the volume of patient sequencing data accumulates, it is critical not only to deliver to the research and clinical community a validated catalog of these variations, but also to correlate these with oncogenic potency, and identify which targeted inhibitors effectively inhibit. The recent discovery and functional validation of oncogenic mutations in the extracellular domain of ALK in leukemia (60) not only broaden the role of this oncogene across histologically diverse tumors, but also present an exciting therapeutic opportunity in rare subsets of patients based on shared molecular etiology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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