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Minireview

ALK Inhibition for Non-Small Cell Lung Cancer: From Discovery to Therapy in Record Time

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It was only 3 years ago that an acquired translocation of *EML4* with *ALK* leading to the expression of an EML4-ALK oncoprotein in non-small cell lung cancer (NSCLC) was reported. Tumor cells expressing EML4-ALK are "addicted" to its continued function. Now, crizotinib, an oral ALK inhibitor, is demonstrated to provide dramatic clinical benefit with little toxicity in patients having such advanced NSCLC, and a mechanism of clinical resistance to crizotinib is identified. Such therapy "targeted" at oncogenic proteins provides "personalized" medicine and prompts genome-wide mutation analysis of human tumors to find other therapeutic targets.

An Amazing, Rapid Success Story of Translational Cancer Research

In 2007, rearrangements of the anaplastic lymphoma kinase (ALK) gene in non-small cell lung cancer (NSCLC) were reported (Soda et al., 2007). Less than 3 years later, studies of ALK inhibition yielding dramatic response rates in patients with advanced NSCLC containing *ALK*-rearrangements and determination of the mechanism of resistance to the ALK-targeted therapy were reported in the *New England Journal of Medicine* (Choi et al., 2010; Kwak et al., 2010). Together, these reports represent a stunning and rapid translation of preclinical molecular findings into the clinic. In a pretreated patient population that generally has a 10% response rate to conventional chemotherapy, treatment with the oral ALK inhibitor crizotinib yielded an overall response rate of 55% and an estimated 6 month, progression-free survival rate of 72%. In addition, the mechanism of resistance was associated with mutations in the ALK kinase domain, providing genetic evidence that ALK was indeed the target of the "targeted" therapy. During this brief period, translational research provided insights into ALK biology, clinicopathologic features of the target population, development of a clinical diagnostic test, drug development, and identification of resistance mechanisms. By contrast, analogous development for other druggable kinases, such as breakpoint cluster region-Abelson (BCR-ABL) in chronic myeloid leukemia and epidermal growth factor receptor (EGFR) mutations in NSCLC, unfolded over decades (see Table 1). This rapid clinical development of ALK-targeted therapy was greatly accelerated, in part by previous experience with clinical development of other tyrosine kinase inhibitors (TKIs) and in part by the fact that crizotinib (PF-02341066, Pfizer), which was developed initially as a MET inhibitor but was soon realized to also be an ALK inhibitor, was developed before the *EML4-ALK* translocation was identified in NSCLC (Christensen et al., 2007; Zou et al., 2007).

EML4-ALK is the latest of tumor-associated genetic changes providing a very specific therapeutic target along with a genetic diagnostic test to "personalize" this therapy. Such success has led to a number of international consortium approaches for genome-wide tumor sequencing in lung and other cancers to identify all of the somatically acquired genetic (and epigenetic) changes in an individual tumor that could represent new diagnostic and therapeutic targets (Ciccarelli, 2010; Ding et al., 2008; Forbes et al., 2010; Kan et al., 2010; Lee et al., 2010; Pleasance et al., 2010; Syed et al., 2010; Thomas et al., 2007; Weir et al., 2007). It has also led to academic consortia, such as the Cancer Target Discovery and Development Network (CTD^2N) of the National Cancer Institute (NCI), to integrate genomics and new chemical compound screening for development of patient-based therapeutics (Schreiber et al., 2010). From this information, we hope to identify additional therapeutic targets as well as a method for "personalizing" their application for lung and other cancer patients (Janku et al., 2010).

Integration of Discoveries of Genetic Abnormalities, Preclinical and Early Clinical Studies

ALK encodes a tyrosine kinase normally expressed only in certain neuronal cells. The *ALK* gene was originally identified through cloning of the t(2;5)(p23;q35) translocation found in a subset of anaplastic large cell lymphomas (Morris et al., 1994). In a rare subset of NSCLCs, interstitial deletion and inversion within chromosome 2p result in fusion of the N-terminal portion of the protein encoded by the echinoderm microtubule-associated protein-like 4 (EML4) gene with the intracellular signaling portion of the ALK receptor tyrosine kinase (Soda et al., 2007). While genetic alterations involving *ALK* have been identified in other malignancies, thus far, the *EML4-ALK* fusion gene appears unique to NSCLC. A number of *EML4-ALK* variants have been identified in NSCLCs, all of which appear to confer gain-of-function properties (Choi et al., 2008). Equivalent to *EGFR* mutations, *EML4-ALK* fusions result in constitutive tyrosine kinase activity, dependence of the cancer cell on activated downstream mitogenic pathways, and exquisite sensitivity to ALK inhibition, and thus represents another case of "oncogene addiction" (Weinstein and Joe, 2008).

Table 1. The Shortening Interval between Target Discovery and Effective New Cancer Treatments

Target	Year Target Discovered	Disease(s) and Proportions	Estimated Total # Pts Annually (US)	Drug(s)	Clinical Outcomes	Outcomes from Conventional Chemotherapy	Year Mutation-Targeted Treatment Documented
BCR-ABL	1960	CML (100%)	5,000	Imatinib Dasatinib Nilotinib	RR 90% 5y PFS 80% 5y OS 90%	RR 35% 5y OS 70%	2001
EGFR	1978	*EGFR* mutated NSCLC (10% of NSCLC)	17,000	Erlotinib Gefitinib	RR 75% Median PFS 11 mos Median OS 31 mos	RR 30% Median PFS 5 mos Median OS 24 mos	2004
KIT	1998	GIST	6,000	Imatinib	RR 55% Median PFS 27 mos Median OS 58 mos	RR 5% Median OS 20 mos	2002
BRAF	2002	V600E *BRAF* mutated melanoma (50% of melanoma)	34,000	PLX4032	RR 77% Median PFS 7 mos OS not yet determined	RR 10-20% PFS 1.5 mos OS 8 mos.	2010
ALK	2007	*EML4-ALK* NSCLC (5% of NSCLC)	8,500	Crizotinib	RR 55% 6 month PFS 70% OS not yet determined	RR 25% Median PFS 4-6 mos Median OS 12 mos	2010

ALK, anaplastic lymphoma kinase; CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; GIST, gastrointestinal stromal tumor; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; RR, response rate; mos, months.

ALK preclinical drug development, elucidation of the target population, and early phase clinical development proceeded together rapidly. In transgenic mice expressing EML4-ALK in lung epithelial cells, numerous bilateral lung adenocarcinomas develop shortly after birth, supporting the oncogenic nature of this fusion protein (Soda et al., 2008). Administration of a specific inhibitor of ALK tyrosine kinase activity resulted in rapid eradication of these nodules. In 2008, a phase I clinical trial was initiated, followed in 2009 by reports of cases with dramatic clinical benefit of ALK-targeted therapy among patients with ALK-positive NSCLC (Kwak et al., 2009), which in 2010, led to the opening of a phase 3 registration trial of crizotinib in ALK-positive patients.

Clinicopathologic Features of EML4-ALK NSCLCs

Central to this remarkable progress has been an early understanding of the clinicopathologic features of *EML4-ALK* NSCLC, which represents ~5% of all NSCLCs (Kwak et al., 2010; Rodig et al., 2009; Shaw et al., 2009). In general, patients with NSCLCs harboring *ALK* rearrangements tend to be younger and have little (<10 pack-years) to no smoking history. Almost all cases have been adenocarcinomas, predominantly signet-ring cell type with abundant intracellular mucin. While this histologic pattern is well recognized in gastrointestinal and breast adenocarcinomas, it is rarely observed in lung cancer. *EML4-ALK* rearrangements appear to be mutually exclusive of *EGFR* and *KRAS* mutations, and, by contrast to *EGFR* mutant NSCLCs, there does not appear to be an association with patient ethnicity, gender, or differential outcomes with combination platinum-based chemotherapy.

Clinical Diagnostic Test

Absolutely essential for the clinical application of ALK-targeted therapy is a tumor clinical diagnostic to identify patients most likely to respond. Evidence of *EML4-ALK* in lung tumors has been documented by fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), and reverse transcriptase polymerase chain reaction (RT-PCR). Of these, FISH appears to be the most clinically applicable. *EML4-ALK* FISH employs differently labeled break-apart (split-signal) probes on the 5′ and 3′ ends of the ALK gene. *ALK* rearrangements appear as separate red and green signals, while normal ALK generates a fused (yellow) signal. Using a cut-off of >15% of cells and examining 4+ fields (approximately 60 cells), virtually 100% sensitivity and specificity have been reported (Camidge et al., 2010). ALK IHC is fraught with technical and interpretive challenges, but tyramide amplification appears to improve its yield from 40% to 80%.(Rodig et al., 2009) While RT-PCR is potentially the most sensitive assay, it requires adequate RNA quantity and quality, which are difficult to obtain in routine clinical samples, as well as multiple PCR primers to detect the numerous known fusion transcripts.

Important Role of Multi-Institutional and Multidisciplinary Collaborative Efforts

Given the rarity of *ALK* rearrangements in NSCLC, clinical advancement has required multi-institutional, international, and multi-disciplinary collaboration. To identify 82 ALK-positive patients, Kwak and colleagues screened 1,500 patients over 18 months (Kwak et al., 2010). Patients were treated with crizotinib, a dual inhibitor of ALK and MET tyrosine kinases, 250 mg orally twice daily. All patients tested negative for MET amplification, suggesting that therapeutic effect was achieved via ALK inhibition. Because of neuronal localization of ALK expression, the patients were closely monitored. However, crizotinib was well tolerated, with mild nausea and diarrhea, transient visual disturbances without ophthalmologic findings, and rare elevations in liver chemistries. Radiographic response was assessed every 8 weeks, and crizotinib treatment was associated with many months of disease control in nearly 75% of

patients. Thus, the therapy provided substantial qualitative and quantitative clinical benefit with mild to no toxicities.

Ascertainment of Resistance to ALK-Targeted Therapy Was of Great Importance

Identification of the precise mechanism(s) by which tumors become resistant to targeted therapy has yielded important information and thus needs to be studied in initial clinical trials, acknowledging that such study requires rebiopsying of patient's tumors at the time of relapse. Thus, it was of great interest that accompanying the report of the crizotinib clinical trial, molecular analysis of an EML4-ALK fusion NSCLC that had relapsed on crizotinib therapy was reported by another group (Choi et al., 2010). The resistant tumor had acquired two mutations in the tumor ALK tyrosine kinase domain (C1156Y and L1196M) that appear to be in two subpopulations of tumor cells. In vitro, cells engineered to express either of these mutant ALK fusion proteins were resistant to crizotinib and other ALK inhibitors compared to ALK fusion protein without these changes. The authors presented pictures of the predicted ALK structure, locating the mutations with reference to the crizotinib binding site, and noted that amino acid L1196M corresponds to the imatinib resistance BCR-ABL T315I mutation found in chronic myelogenous leukemias, and gefitinib and erlotinib resistance EGFR T790M mutation found in NSCLCs. These findings strongly suggest L1196M is a "gatekeeper" mutation, which confers resistance to tyrosine kinase inhibitors via altered ATP binding and steric hindrance of drug binding. The mechanism of resistance of C1156, an activating mutation on the N-terminal side of ALK, is less clear.

A lesson learned from resistance to EGFR TKI-targeted therapy in EGFR mutant NSCLCs was that resistance could come from either an *EGFR* mutation or amplification of *MET* (providing a bypass of the blocked pathway), and, thus, there will likely be other mechanisms of crizotinib resistance discovered. In addition, EGFR TKI-resistant clones are often present as minor subpopulations in the original tumor (Ercan et al., 2010; Turke et al., 2010). With knowledge of resistance mutations and new sensitive assays it may be possible to not only type tumors for the first targeted therapy, but also identify, before such therapy, resistant subpopulations that will arise. Thus, the development of targeted therapy mutations in the very protein being targeted provides genetic evidence that ALK was indeed the key target, sets the stage for developing the next generation of drugs to overcome such resistance, and provides powerful molecular probes to monitor emergence of drug resistant populations.

ALK-Targeted Therapy Benefited from Previous Studies of Targeted Agents

The rapid development of ALK inhibition owes much to the experience gained from use of EGFR inhibitors for treating NSCLC. Only after thousands of patients were treated with EGFR TKIs gefitinib or erlotinib were activating *EGFR* mutations—and thus a target population—identified. In fact, many clinical trials in patients whose tumors were not typed for EGFR mutations demonstrated negative and possibly detrimental effects, especially in patients receiving concurrent EGFR TKIs with carboplatin-paclitaxel chemotherapy or concurrent chemo-radiation therapy (Herbst et al., 2005; Kelly et al., 2008). When EGFR TKIs were applied to NSCLCs containing EGFR mutations, clinical benefits became obvious (Janku et al., 2010). These findings led to the design of clinical trials for ALK-targeted therapy.

"Tissue Is the Issue"

Until about 10 years ago, nearly every newly diagnosed patient with lung cancer had an adequate biopsy to allow for clear histologic diagnosis of lung cancer. In efforts to make things "easier" on patients and as a cost-saving measure, invasive diagnostic biopsies have been forsaken for fine needle aspirates and a cytologic diagnosis. Thus, in routine clinical practice, lung cancer diagnoses are made on examination of only a few cells. Clearly, if targeted therapies are going to be used we will need to screen patients' tumors for a large number of mutations for targeting therapy, potentially performing total genomic analyses. This will mean a return to dedicated surgical biopsies and correct handling of tissues (e.g., frozen tissues) to facilitate such analyses. While studies of circulating tumor cells or other easy to obtain biomarkers in blood and body fluids may provide such information, for the next several years it would seem prudent to return to better tumor tissue sampling methods, given that the information has the potential to personalize each patient's treatment and in certain cases provide dramatic clinical benefit. Currently, the NCI's multi-institutional Lung Cancer Mutation Consortium (LCMC) is performing mutational analysis of a large panel of genes including *EML4-ALK* on tumor samples from 1,000 patients with advanced lung adenocarcinoma, and this prospective study will provide a wealth of information on the frequency of such "actionable" mutations.

Targeted Therapy Will Make Clinical Trials Both Easier and More Complex

Clearly, the ability to treat a patient population selected for the EML4-ALK fusion protein mutation greatly facilitated the clinical development of ALK-targeted therapy. However, we point out future complexities that will need to be addressed. While ALK-targeted therapy is clinically beneficial in patients with metastatic NSCLC, it will also have to be tested in randomized trials in early stage (e.g., stages I and II) resected NSCLCs; given the mutation frequency, this will be difficult. One reason for this caution is a recent analysis suggesting a detrimental effect on overall survival when patients with early-stage NSCLC harboring *EGFR* mutations received adjuvant (postoperative) gefitinib. Potent and well-tolerated targeted cancer therapies such as ALK inhibitors are also forcing clinical researchers to face new questions about the design and conduct of clinical trials. For example, in a phase 3 trial of patients with advanced BRAF mutated melanoma designed to fulfill FDA guidelines for drug approval, patients were randomized to PLX4032, a well-tolerated BRAF inhibitor associated with response rates exceeding 75%, and the "standard therapy" with dacarbazine, an alkylating agent with considerable toxicity and, historically, little efficacy. Patients assigned to dacarbazine were not permitted to cross over to experimental therapy, generating an ethical debate recently featured in the *New York Times* (http://www.nytimes.com/2010/09/19/health/research/19trial.html?ref=target_cancer). Because of such dilemmas, ongoing phase 3 trials of crizotinib for ALK-positive NSCLC permitted cross-over to the experimental arm

so that all patients will have a chance to benefit from ALK-targeted therapy.

An Uncommon Mutation in a Common Disease Is Numerically Important

Although *EML4-ALK* is observed in only a small fraction of lung cancers, because lung cancer is such a prevalent disease, there are likely up to 10,000 cases per year of ALK-positive NSCLCs in the United States—more than the total number of cases of several other malignancies (Jemal et al., 2010). Of note, tumor-acquired ALK fusion proteins are found in lymphomas, neuroblastomas, and other malignancies (Kelleher and McDermott, 2010). In fact, a patient with inflammatory fibrous tumor harboring *RANBP2-ALK* fusion responding to crizotinib was reported in the same issue of the *New England Journal of Medicine* (Butrynski et al., 2010). Ongoing efforts to perform genome wide sequencing on large numbers of malignancies will likely increase the total numbers of tumors that will benefit from ALK-targeted therapy.

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