EML4-ALK in non-small-cell lung cancer: the breathtaking progress from benchtop to Phase III clinical trial

EML4-ALK is a fusion oncogene found in approximately 5% of patients with non-small-cell lung cancer. It was first discovered in 2007, and is found predominantly in those who never smoke or light smokers (≤10 pack/year tobacco history), younger patients and those with adenocarcinoma histology. It occurs almost exclusively in patients without mutations in *K-ras* or *EGFR*. Completed Phase I and II trials suggest significant clinical activity of an ALK kinase inhibitor, PF-02341066 (crizotinib), in patients with advanced non-small-cell lung cancer who harbor an *ALK* translocation. A Phase III evaluation of crizotinib versus chemotherapy in the second-line setting is ongoing.

KEYWORDS: EML4-ALK ■ lung cancer ■ oncogene ■ targeted therapy

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In the past 5 years, the treatment of advanced non-small-cell lung cancer (NSCLC) has evolved. What was originally believed to be a homogenous disease that was uniformly treated with cytotoxic chemotherapy is now recognized as being composed of distinct molecular subtypes that correspond to specific clinical phenotypes.

In particular, the development of oral tyrosine kinase inhibitors (TKIs) against the EGF receptor (EGFR) have been an important milestone. The subsequent dual recognition that EGFR TKIs are only beneficial for patients whose tumors have activating mutations in the *EGFR*, and that these mutations are more likely to occur in light or never smokers with adenocarcinoma histology culminated in the Iressa Pan-Asia Study (IPASS) study [1]. This study in East Asian patients with the aforementioned clinical characteristics and advanced disease demonstrated a superior progression-free survival (PFS) for gefitinib over carboplatin/paclitaxel chemotherapy. Similar PFS benefits were seen in two Japanese studies that randomized advanced NSCLC patients with known *EGFR* mutations to gefitnib or chemotherapy (cisplatin/docetaxel [2] and carboplatin/paclitaxel [3], respectively).

An important footnote to the IPASS study is that, out of approximately a third of patients in the trial whose tumors were analyzed, only 59.7% harbored *EGFR* mutations (including 4.2% who had a T790M exon 20 mutation, which is known to confer resistance to anti-EGFR therapies). This frequency implies that, even in a population enriched by clinical parameters for the likelihood of an EGFR mutation, up to 40% of patients have tumors that are driven by some other oncogene(s).

One such potential oncogene is the fusion product *EML4-ALK*. In this article, we summarize the preclinical data for *EML4-ALK* and its potential role in carcinogenesis, the clinical characteristics of the subset of NSCLC patients in which it has been identified and the rapid evaluation of a targeted agent against the ALK tyrosine kinase, which is now undergoing Phase III testing.

Preclinical data

ALK gene

The *ALK* gene was originally identified as a fusion partner of various genes in two relatively rare malignancies, anaplastic T-cell lymphoma and inflammatory myofibroblastic tumors [4,5]. In each case, the fusion point of *ALK* is conserved so that the entire intracellular kinase domain of the ALK protein is fused to its partner. Activity of this ALK fusion protein has been demonstrated to be essential to the proliferation of lymphoma cells [6]. The *ALK* gene has also been demonstrated to be overamplified or to contain gain-of-function mutations that play a primary role in the oncogenesis of neuroblastoma, the most common pediatric solid tumor [7].

EML4-ALK fusion gene

The *EML4-ALK* fusion gene consists of *EML4* at the amino-terminal portion and the intracellular domain of the *ALK* gene at the C-terminal portion. It was first identified in 2007 by Soda and

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colleagues, who generated a retroviral cDNA library from the resected lung adenocarcinoma specimen of a 62-year-old smoker [8]. Mouse fibroblasts were then infected with recombinant retroviruses containing these cDNA fragments, yielding numerous transformed foci, from which the cDNA insert was recovered. One of these cDNAs comprised the *EML4-ALK* fusion product. As both genes are found in close proximity on chromosome 2p, the chimeric product arises from a chromosomal inversion.

Soda *et al.* then demonstrated that the *EML4-ALK* fusion gene was required for the transforming activity, and that fibroblasts containing the *EML4-ALK* cDNA were capable of forming tumors when injected into nude mice. More recently, they confirmed the oncogenic activity of the EML4-ALK kinase by developing a transgenic mouse that expressed *EML4-ALK* in lung alveolar epithelial cells [9]. All of the transgenic mice developed hundreds of adencarcinomas in both lungs several weeks after birth.

EML4-ALK variants & other fusion partners

While the fusion point of the *ALK* gene is conserved, *EML4* is variously truncated so that different *EML4-ALK* variants have been noted. At least nine variants have been identified, most of which are oncogenic [10]. The most common are E13;A20 and E6a/b:A20, termed variants 1 and 3, respectively (the nomenclature refers to the fusion point between the exons in EML4 [E] and ALK [A]). They have been identified in 38 and 32% of *EML4-ALK*-positive NSCLC patients, respectively. At this time, the clinical significance of the different variants is not known.

In addition to *EML4*, there appear to be other potential fusion partners in *ALK*-positive NSCLC. Two novel fusion genes of *ALK* with *TFG* and *KIF5B* have also been described [11,12].

Detection of *EML4-ALK* fusion products in clinical samples

Currently, three different methodologies have been employed to identify *EML4-ALK*-positive tumor samples: reverse transcriptase (RT)-PCR-based techniques, immunohistochemical staining and FISH. A thorough discussion of the merits and disadvantages of each of these methodologies is beyond the scope of this article.

Briefly, RT-PCR screening may have higher sensitivity than the other methods. However, it may be impractical for large-scale clinical trials and clinical practice because most patients' tumor samples are preserved as paraffin-embedded tumor tissue. RNA extracted from these samples is relatively more difficult to perform PCR on than RNA from fresh–frozen tissue. In addition, any PCR screening must include validated primers for all the possible *ALK* fusion oncogenes, both with *EML4* and with other novel genes.

Immunohistochemical staining has the advantage of being a routine procedure that can be performed in pathology laboratories worldwide on paraffin-embedded tissue. However, the sensitivity and specificity of this method is unclear. At least one study failed to detect any ALK positivity in six tumor samples that were known to be *EML4-ALK* positive, by RT-PCR, or in an additional 662 unselected paraffin-embedded samples [13]. This may be caused by very low levels of expression of the EML4-ALK protein.

Finally, FISH techniques involve the use of commercially available probes. One probe is upstream of the *ALK* gene, while the other probe is downstream of the gene. In the absence of a translocation, both probes are in close physical proximity and yield a merged green–orange fluorescent signal when hybridized against normal nuclei. Any translocation or rearrangement results in a splitting of this signal. This methodology is not able to distinguish between the different *EML4-ALK* variants based on the different *EML4* gene breakpoints or even the actual fusion partner with *ALK*. Current clinical trials of an ALK inhibitor utilizes this methodology to identify *EML4-ALK*-positive patients by defining positivity as 15% or more split nuclei [14].

Preclinical data for ALK inhibition

Koivunen *et al.* demonstrated that an ALK kinase inhibitor, TAE684, was able to inhibit the growth of an EML4-ALK-positive NSCLC cell line, H3122, and also caused complete inhibition of phosphorylated ALK and downstream kinases, including Akt and ERK1/2 [15]. TAE684 also inhibited the growth of the H3122 cells when implanted into nude mice. Similarly, treatment of transgenic mice expressing *EML4-ALK* in their lung tissue with an oral ALK inhibitor resulted in a significant reduction in tumor burden compared with control mice [9]. In another study, treatment of transgenic mice harboring the *EML4-ALK* translocation with TAE684 resulted in improved survival compared with carboplatin/paclitaxel chemotherapy [16].

Clinical characteristics

Smoking history

Although the *EML4-ALK* gene was first identified in a smoker, the subgroup of NSCLC patients who harbor this oncogene has very similar characteristics to those with *EGFR* mutations (i.e., never or light smokers with a ≤10 pack/year tobacco history).

To date, nine separate studies have analyzed more than 1400 patients' tumor samples by RT-PCR [9,10,13,15,17–21]. A tenth study by Inamura *et al.* of 149 patients [22] exists but it is not clear if these patients represent a subset of their larger study of 253 patients [17]. These data are shown in Table 1.

While some of the smaller studies indicate a higher incidence of *EML4-ALK* translocations in smokers [13,18], summation of all the patients in the nine studies suggests that they are found in approximately 10% of light or never smokers versus 2.3% of heavy smokers. The variation between these studies is probably caused by the small numbers of heterogenous patients and also because the study by Shaw *et al.* selected for patients with clinical characteristics associated with *EGFR* mutations, thereby also enriching for the number of *EML4-ALK*-positive tumors [20].

It should be noted that mutations in the *EGFR* remain the most common in this group of light or never smokers. However, of the 78 patients whose tumor samples had *EML4-ALK* translocations and also underwent analysis of *EGFR* and *K-ras* mutation status, only one (1.3%) had a coexisting *K-ras* mutation and one had a deletion in exon 19 of the *EGFR* gene, suggesting that these mutations are virtually mutually exclusive.

Other demographic factors

In addition to smoking history, *EML4-ALK*-positive tumors may occur in younger patients than *EML4-ALK*-negative tumors do. In their study, Inamura *et al.* noted that these tumors occurred at a median age of 56 versus 64 years for patients with *EML4-ALK* negative tumors, respectively [17]. A total of 36% of *EML4-ALK*-positive tumors occurred in patients younger than 50 years-old versus 5% of *EML4-ALK*-negative tumor patients. This association with a younger age at diagnosis for patients with *EML4-ALK*-positive tumors was also noted by Wong *et al.* (only for adenocarcinoma histology) [19] and by Shaw and colleagues [20].

The data also indicate that there does not appear to be a significant difference in the incidence of *EML4-ALK* translocations by ethnicity (0–19.2% of Asians vs 6.3–22.4% of Caucasians/non-Asians).

Tumor histology

As with *EGFR* mutations, *EML4-ALK* translocations are noted almost entirely in tumors with pure adenocarcinoma histology or a component of

Table 1. Incidence of *EML4-ALK* translocation in smokers versus light or never smokers.

Study	Ethnicity	Detection method	*EML4-ALK* +ve smokers	*EML4-ALK* +ve light/never smokers	Ref.
Soda *et al.*	East Asian	RT-PCR	8.3% (2/24)	11.1% (1/9)	[8]
Koivunen *et al.*	45% Caucasian 55% East Asian	RT-PCR	1.1% (2/184)	5.8% (4/69)	[15]
			75% (6/8)†		
Shinmura *et al.*	East Asian	RT-PCR	4.9% (2/41)	0% (0/22)	[18]
Inamura *et al.*	East Asian	RT-PCR	0.8% (1/133)	8.4% (10/119)‡	[17]
Martelli *et al.*	Caucasian	RT-PCR	7.9% (8/101)	6.3% (1/16)	[13]
	82% Caucasian 18% Asian	IHC	0%		
Wong *et al.*	East Asian	RT-PCR	0.8% (1/125)	8.5% (12/141)	[19]
		IHC (of +ve PCR samples)	100% (1/1)	91.7% (11/12)	
Shaw *et al.*	94% non-Asian 6% Asian	RT-PCR	0% (0/56)	22.4% (19/85, all non-Asian)	[20]
Takahashi *et al.*	East Asian	RT-PCR	0.8% (1/119)§	4.3% (4/92)§	[21]
Zhang *et al.*	East Asian	RACE-PCR	3.9% (2/51)	19.2% (10/52)	[10]
Total			2.3% (19/834)	10.1% (61/605)	

†*Although this study identified eight of 305 tumor samples with* EML4-ALK *translocations, smoking history was only available for six of the positive samples.*
‡*Light or never smokers were defined based on the smoking index (SI) – the product of the number of cigarettes/day multiplied by the duration in years – as having an SI <400 while smokers had an SI ≥400. By contrast, other studies defined light-smokers as having a ≤10 pack-year history, which is equivalent to an SI ≤200 (assuming 20 cigarettes/pack).*
§*Patients were categorized as being nonsmokers or smokers. The* EML4-ALK*-positive 'smoker' only had a 0.25 pack-year tobacco history.*
+ve: Positive; IHC: Immunohistochemistry; RACE: Rapid amplification of cDNA ends; RT: Reverse transcriptase.

adenocarcinoma. Of the 170 *EML4-ALK*-positive tumors detected to date [8,10,13,15,17–21,23,24], 150 (88%) were pure adenocarcinomas, nine (5%) were squamous cell carcinomas, four (2%) were bronchioalveolar carcinomas, three (2%) were adenosquamous carcinomas, one (1%) was a mucoepidermoid carcinoma and one (1%) was a poorly differentiated cancer (believed to be either a mucoepidermoid carcinoma or an adenosquamous cancer).

In terms of adenocarcinoma histology, subtypes include papillary, acinar and cribiform patterns. In a series of 20 North American patients, there appeared to be an association between an unusual signet cell subtype (comprising >10% intracellular mucin pools) and *EML4-ALK* positivity [25]. A clear association between any of these subtypes and an increased incidence of *EML4-ALK* positivity will require validation in larger patient cohorts.

Response to standard therapy

While there is a relative paucity of data, Shaw and colleagues evaluated the response of 19 *EML4-ALK*-positive patients to the EGFR TKIs and to cytotoxic chemotherapy [20]. As would be expected, there were no clinical responses to erlotinib and the best response was stable disease in 40% of patients. Patients treated with platinum-based chemotherapy (with or without targeted therapies, such as the antiangiogenic agent bevacizumab) appeared to have a comparable response and time to progression as patients with *EGFR* mutations and those without an identifiable *EML4-ALK* translocation or *EGFR* mutation. Although underpowered, an analysis of Chinese patients by Zhang *et al.* demonstrated a nonsignificant trend toward improved survival following surgery for *EML4-ALK*-positive versus *EML4-ALK*-negative patients (median overall survival not reached versus 50.5 months, hazard ratio 0.54, p = 0.15) [10].

Clinical trials

Results of a Phase I/II study of an oral ALK inhibitor, PF-02341066 (crizotinib), have recently been published [22]. This study recruited patients with advanced NSCLC who were found to have an *EML4-ALK* translocation by FISH. Approximately 1500 patients from the USA, Australia and Korea were screened to identify 82 patients who could be evaluated for response and toxicity. Significant demographic data included the fact that 56% of patients were Caucasian and 35% were Asian. A total of 96% of patients had adenocarcinoma histology and most never smoked or smoked ten or less packs/year (76 and 18%, respectively). Although the protocol placed no restrictions on the tumor histologies that were eligible, tumor samples that were screened were increasingly adenocarcinomas, as the clinicopathologic correlates of *ALK*-positive tumors became established.

In the Phase I component (which began enrollment in 2006), the maximum tolerated dose was defined as 250 mg twice daily, with the dose-limiting toxicity being fatigue. Additional patients were then treated on the expansion cohort phase. In the 82 patients who were evaluated, the major toxicities (all grade 1/2) comprised nausea/vomiting (54 and 44%, respectively), diarrhea (44%) and constipation (24%). A total of 41% of patients reported visual abnormalities, these were noted as streaking effects when ambient light conditions changed. The only notable grade 3/4 toxicity was elevation in liver enzymes, observed in approximately 6% of patients.

In terms of response, 46 out of 82 patients had a partial response while one patient had a complete response, at a response rate of 57%. An additional 27 patients (33%) had stable disease, while the disease-control rate at 8 weeks was 87%. Given that this study was not designed to evaluate PFS, the median PFS in this heterogenous, relatively heavily pretreated population was 6.4 months (95% CI: 5.5–7.2). In a separate case report, tumor cells from a patient who experienced a partial response to crizotinib followed by rapid progressive disease 5 months later were analyzed [26]. Two distinct point mutations were identified within the kinase region of the *EML4-ALK* gene, conferring resistance to crizotinib in *in vitro* testing. It was not known if these mutations were present prior to or developed during therapy.

Based on these promising data, a Phase III trial is currently ongoing [101]. This study plans to randomize 318 *ALK*-positive NSCLC patients with prior progression on first-line platinum-based chemotherapy to pemetrexed or docetaxel versus crizotinib. A single-arm Phase II study of crizotinib is recruiting 250 patients who are not eligible for the Phase III trial (owing to more extensive prior therapy) or who have experienced progression on the chemotherapy arm of the Phase III trial [101].

Conclusion

If the Phase III trial of crizotinib is successful, this medication will emerge as a new option for the approximately 5% of patients with advanced NSCLC who harbor an *ALK* translocation.

While this may not necessarily seem like a large number, when considering the global incidence of lung cancer, this means that 80,000 patients may benefit from this therapy (whether the cost of this drug would be affordable to citizens of developing countries, where lung cancer rates are disproportionately high, is another matter) [27].

In particular, light or never smokers may especially stand to benefit. If up to 40% of such patients do not have a detectable *EGFR* mutation and if approximately 10% of this population has a mutually exclusive *ALK* translocation, this would imply that a quarter of nonsmokers with advanced NSCLC without an *EGFR* mutation would benefit from ALK inhibition.

The rapid progress made from the initial identification of the *EML4-ALK* translocation in a patient with NSCLC in 2007 to the current ongoing Phase III evaluation is remarkable. While it is a testament and reaffirmation of the critical importance of translational science and the need for intensive analysis of patient samples to yield important clues regarding future therapeutics, the rapidity of these developments also owes much to two fortuitous coincidences.

The first is that PF-02341066, now crizotinib, was already undergoing Phase I clinical evaluation at the time of the seminal discovery by Soda and colleagues [20]. While crizotinib was initially identified as an inhibitor of another oncogene, c-Met, it was also recognized to be an inhibitor of ALK; at the time it was only implicated in the pathogenesis of a relatively rare variant of lymphoma and soft-tissue sarcomas. Once *ALK* fusion oncogenes were identified in NSCLC, researchers were able to skillfully and quickly pivot to also include that subset of patients in the incipient clinical trial.

Second, and perhaps more importantly, the early clinical activity of crizotinib suggests that we have identified a target that the lung cancer cell is truly dependent on. From our growing understanding of the molecular pathways that drive oncogenesis, it is clear that these pathways involve complex hierarchial interactions and can be multiply redundant. Laboratory and preclinical data cannot guarantee that abrogating the signal of a single empirically chosen target will provide any clinically meaningful benefit, unless this signal is critically required for maintenance of the malignant phenotype, a concept termed 'oncogene addiction' [28].

Rare successes that can legitimately be considered 'home runs' in oncology – and were dependent on the identification and serendipitous inhibition of an 'addictive' target – include the development of imatinib to treat chronic myelogenous leukemia [29] and gastrointestinal stromal tumors [30], and the EGFR TKIs in the treatment of *EGFR* mutated NSCLC [1]. Over the next few years, to much anticipation by patients, physicians and scientists alike, we will learn if crizotinib will have a similar success story.

Future perspective

If the Phase III trial of crizotonib as a second-line therapy in patients with *ALK*-positive NSCLC demonstrates superior efficacy and toxicity over cytotoxic chemotherapy, it will probably become a standard of care. Under these circumstances, it is also likely to be evaluated in the first-line setting against chemotherapy.

From a clinical perspective, this would mean that patients with the same clinical characteristics as those who are thought to harbor *EGFR* mutations (i.e., ≤10 pack-year history, younger age and adenocarcinoma histology) will be routinely and sequentially tested for *EGFR* mutations, followed by *ALK* testing if no *EGFR* mutation is found. In this way, we may be one step closer to the holy grail of offering tailored therapy based on the specific molecular fingerprint of a patient's cancer.

On a more sobering note, a case report identified two distinct mutations in the *EML4-ALK* oncogene kinase domain that conferred resistance to crizotinib in a patient with sudden progression following 5 months of crizotinib therapy. It remains to be seen if the development of such mutations is a rare event, an inevitability or somewhere in between and if, perhaps, second-generation TKIs that target such acquired mutations can be developed.

Finally, the early success of ALK inhibition in NSCLC will hopefully spur further novel translational efforts in this and other cancers. The integration of pertinent correlative analyses into both routine clinical care and clinical trials is clearly of vital importance if we are to identify new targets for drug development, validate biomarkers of response/resistance and, ultimately, improve patient outcomes and minimize toxicity.

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Executive summary

- *EML4-ALK* is a fusion oncogene, discovered in 2007, that is found in approximately 5% of patients with non-small-cell lung cancer. It consists of the intracellular kinase component of the *ALK* gene fused to various breakpoints of the *EML4* gene.
- The *EML4-ALK* oncogene has been demonstrated to have oncogenic properties in transgenic mouse models. Inhibition of ALK kinase activity has also resulted in reduced tumor growth in cell lines and nude mice models.
- *EML4-ALK*-positive non-small-cell lung cancers are found predominantly in light or never smokers (≤10 pack-year tobacco history), younger patients and those with adenocarcinoma histology. It occurs almost exclusively in patients without mutations in *K-ras* or *EGFR*.
- A completed Phase I/II trial suggests significant clinical activity of an ALK kinase inhibitor, PF-02341066 (crizotinib), in patients with advanced non-small-cell lung cancer who harbor an *ALK* translocation. In the recently reported Phase II expansion cohort, the response rate was 57%, while 87 and 72% had disease control (partial responses plus stable disease) at 8 weeks and 6 months, respectively.
- A Phase III evaluation of crizotinib versus chemotherapy in the second-line setting is ongoing.

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