available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Current perspective

The biology and treatment of *EML4-ALK* non-small cell lung cancer

Takaaki Sasaki [a,b], *Scott J. Rodig* [c], *Lucian R. Chirieac* [c], *Pasi A. Jänne* [a,b,d,*]

[a] *Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, MA, USA*
[b] *Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA*
[c] *Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA*
[d] *Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA*

ARTICLE INFO

Article history:
Received 26 March 2010
Accepted 1 April 2010
Available online 24 April 2010

Keywords:
Non-small cell lung carcinoma
Translocation
Kinase inhibitor
Clinical trial

ABSTRACT

The fusion between echinoderm microtubule-associated protein-like 4 (EML4) and anaplastic lymphoma kinase (ALK) has recently been identified in a subset of non-small cell lung cancers (NSCLCs). *EML4-ALK* is most often detected in never smokers with lung cancer and has unique pathologic features. *EML4-ALK* is oncogenic both *in vitro* and *in vivo* and ALK kinase inhibitors are quite effective in pre-clinical model systems. More recently ALK inhibitors have entered clinical development and remarkably clinical efficacy has been observed in NSCLC patients harbouring *EML4-ALK* translocations. This review will focus on the biology, clinical characteristics, diagnosis and treatment of *EML4-ALK* NSCLC.

1. Introduction

Non-small cell lung cancer (NSCLC) is a major cause of death worldwide, with most of the patients being diagnosed with disease in advanced stage, when treatment is only palliative.[1] Chemotherapy represents the standard of care for patients with advanced disease but conventional cytotoxic agents has reached a plateau in terms of efficacy in the last few years, encouraging the investigation of new compounds which target proteins that are selectively expressed and/or that undergo genomic alterations in cancer cells.[2] In the past several years an increase in the molecular understanding of lung cancer has led to a change in the treatment of the disease. This is highlighted by somatic mutations in *EGFR* where treatment with an EGFR kinase inhibitor (gefitinib) in *EGFR* mutant NSCLC patients leads to a superior response rate, a prolonged progression free survival and an improved quality of life compared to cytotoxic chemotherapy.[3]

The fusion of the anaplastic lymphoma kinase (ALK) with the echinoderm microtubule-associated protein-like 4 (EML4) was identified in 2007 in Japanese non-small cell lung cancers (NSCLCs).[4] Additional studies, mostly involving East Asian patients, have reported that between 3% and 13% of lung tumours harbour *EML4-ALK* fusions.[4–11] By extrapolation this would suggest that approximately 5% of all NSCLC cases contain an *EML4-ALK* translocation, equivalent to over 70,000 patients diagnosed annually worldwide.

Since the ALK tyrosine kinase activity is necessary for its transforming activity and oncogenicity, several ALK kinase inhibitors have been identified and are being evaluated in

* *Corresponding author at:* Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, D820, 44 Binney Street, Boston, MA 02115, USA. Tel.: +1 617 632 6076; fax: +1 617 582 7683.
E-mail address: pjanne@partners.org (P.A. Jänne).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2010.04.002

pre-clinical models *in vitro* and *in vivo* as potential clinical therapies.[7,12,13] ALK inhibitors lead to apoptosis *in vitro* and tumour shrinkage *in vivo* thus demonstrating the phenomenon of 'oncogene addiction'.[7] This is further confirmed by the dramatic clinical studies to date. In the phase I trial of PF-02341066, a remarkable 60% radiographic response rate has been observed specifically in *EML4-ALK* NSCLC patients.[14] This is a remarkably short period of time from the initial identification of the *EML4-ALK* translocation as an oncogene to validation as a clinical target in NSCLC.

In this review, we highlight the clinical, biological and molecular feature of *EML4-ALK* NSCLC patients and discuss the use of ALK inhibitors as therapies for this patient population.

2. Clinical and molecular features of *EML4-ALK* NSCLC

EML4-ALK NSCLC occurs most commonly in a unique clinical subgroup of NSCLC patients. These patients share many of the clinical features of NSCLC patients likely to harbour *EGFR* mutations.[10,15] However, for the most part, apart from rare exceptions, *EML4-ALK* and *EGFR* mutations are mutually exclusive.[6,7,10,12] *EML4-ALK* translocations tend to occur in younger patients and those with more advanced NSCLC while this relationship has not been reported for *EGFR* mutant NSCLC.[6,11]

2.1. *Smoking history*

Initially, the *EML4-ALK* fusion gene was identified in a smoker with lung cancer; however, the accumulating evidence reveals that this genetic alterations is much more common in never/former light (often defined as $\leqslant$10 pack years and quit $\geqslant$1 year ago) smokers with NSCLC.[4,7,10] As shown in Fig. 1A and Table 1, among the NSCLC patients that were never or former light smokers 9.4% of the tumours contained *EML4-ALK* translocations while the frequency was only 2.9% in current smokers ($p < 0.0001$).[4–11] In this clinical population, never or former light smokers, *EGFR* mutations still account for the vast majority of patients while a minority contain either *KRAS* or *ERBB2* mutations (Fig. 1B).[16–28] Of note, genetic alterations have been identified in approximately 25% of never/former light smokers (Fig. 1B).

2.2. *Outcomes with current NSCLC therapies*

Limited data exist to date on the efficacy of currently available therapies in patients with *EML4-ALK* NSCLC. In a study by Shaw and colleagues, 12 patients with ALK genomic alterations were treated with platinum-based chemotherapy. The response rate, time to progression and overall survivals were similar to NSCLC patients harbouring *EGFR* mutations or those that were wild for both *EML4-ALK* and *EGFR*.[10] In contrast, patients with *EML4-ALK* did not benefit from EGFR tyrosine kinase based therapy; their outcome was similar to patients that lacked *EGFR* mutations.[10] These findings are also mirrored in pre-clinical studies where erlotinib is ineffective in a murine model harbouring of *EML4-ALK* NSCLC.[7]

2.3. *Morphologic profile of ALK-rearranged NSCLC*

A variety of histological features are reported to be associated with ALK-rearranged lung adenocarcinomas including acinar (ranging from well-differentiated tubulopapillary and cribriform patterns) to mostly signet-ring cell nests with mucin production.[6,10,29,30] Other histological types such as squamous cell carcinoma and mucoepidermoid carcinoma also rarely contain *EML4-ALK* translocations.[4,10] The acinar pattern is mostly reported to be associated with ALK-rearranged lung adenocarcinomas in Asian populations,[6,30] whereas the signet-ring cell histology was reported mostly in the Western patients.[10,29] The majority of Western patients showed tumour cells with a solid or sheet-like pattern easily distinguishable from the acinar, papillary or bronchioloalveolar patterns. Occasionally, a predominantly acinar pattern and

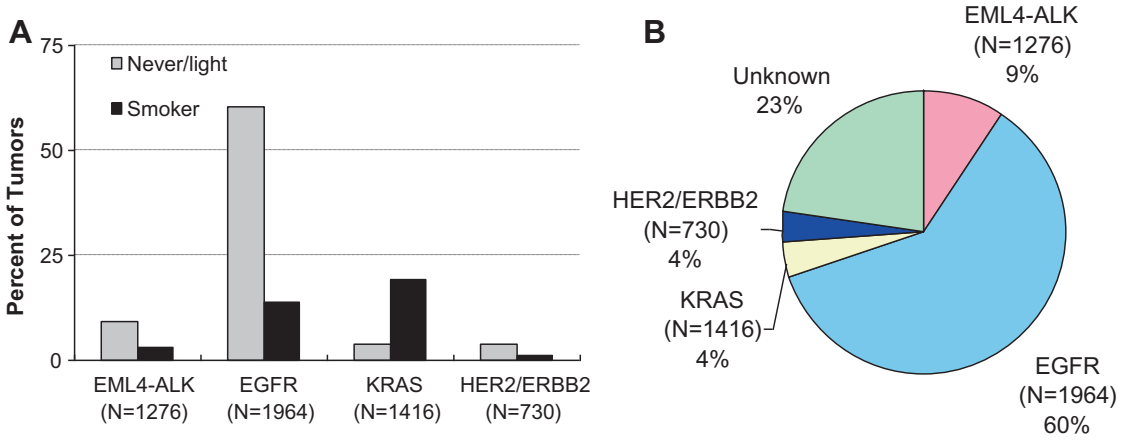

Fig. 1 – Frequency of somatic genetic changes in NSCLC. (A) *EML4-ALK* translocations, *EGFR*, *KRAS* and *ERBB2* mutation frequencies broken down by smoking history. (B) Frequency of somatic mutations in never or former light ($\leqslant$10 pack years; quit $\geqslant$1 year ago) smokers. Data obtained from.[16–28] Of note the somewhat higher *EGFR* mutation frequency is likely a reflection of the predominance of studies from East Asian countries. The *EGFR* mutation frequency in Caucasian never/former light smokers is ~35%.[21]

Table 1 – Frequency of *EML4-ALK* translocations broken down based on smoking history.

	No. of smokers	No. of never smokers	EML4-ALK+ (smokers) (%)	EML4-ALK+ (never smokers) (%)	*p*-Value	Reference
Soda et al.	24	9	8.3	11.1	1.0	[4]
Inamura et al.	84	65	2.4	4.6	0.65	[5]
Inamura et al.	147	105	3.4	5.7	0.53	[6]
Koivunen et al.	184	69	1.1	8.5	<.01	[7]
Shinmura et al.	41	22	4.9	0	0.54	[8]
Martelli et al.	101	16	7.9	6.3	1.0	[9]
Wong et al.	125	141	0.8	8.5	<.01	[11]
Shaw et al.	56	85	0	22.4	<.0001	[10]
Total	762	514	2.9	9.4	<.0001	

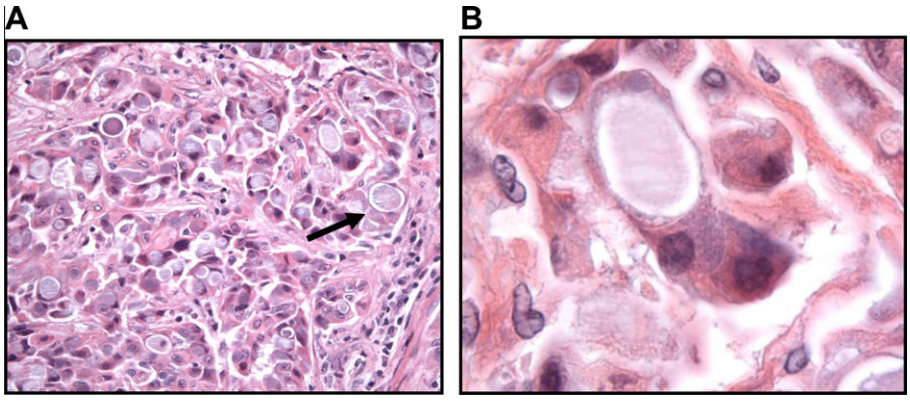

Fig. 2 – Pathologic characteristics of *EML4-ALK* NSCLC. *EML4-ALK* NSCLC demonstrates a signet cell features (arrow). (A) 40× magnification, (B) 1000× magnification.

bronchioloalveolar patterns could also be seen.[29] Even more striking were the cytologic features of tumours harbouring ALK-rearrangements. *ALK*-rearranged tumours showed at least focally tumour cells with abundant intracellular mucin and small, marginalised nuclei (Fig. 2A). In majority of cases, cells with abundant intracellular mucin comprised >10% of the overall tumour cellularity.[29] This distinct cytologic characteristic, unusual for lung carcinoma, is reminiscent of the 'signet-ring' cells more commonly seen in gastric, colonic and breast adenocarcinomas (Fig. 2B). The majority of ALK-rearranged tumours (>60%) demonstrate a solid growth pattern with >10% signet-ring cells. In contrast only a small minority of *EML4-ALK* wild type tumours, including those with *EGFR* mutations, demonstrate a solid growth pattern with >10% signet-ring cells.

2.4. *Variants of EML4-ALK and non-EML4 translocation partners*

The inversion on chromosome 2 p, leading to the formation of the *EML4-ALK* fusion oncogene, is most commonly found in lung cancer patients and lung cancer cell lines. A few reports have also identified *EML4-ALK* in other cancers including breast and colorectal cancers.[31] The chromosomal inversion does not always occur in the same location and multiple *EML4-ALK* variants have been identified (Fig. 3A). All involve the intracellular tyrosine kinase domain of ALK beginning at the portion encoded by exon 20. EML4, however, is variably truncated and gives rise to various variants of *EML4-ALK*. The amino-terminal coiled-coil domain within EML4 is necessary and sufficient for the transforming activity of *EML4-ALK*, probably through dimerisation of the fusion proteins and hence is contained in all the variants (Fig. 3A).[4] At least 11 variants have been reported to date and most of them are oncogenic as assayed in NIH-3T3 cells or in Ba/F3 cells.[4–11,30,32–36] The most common variants were E13;A20 (the nomenclature refers to the exons in EML4 (E) that are fused to ALK (A)) and E6a/b;A20, which are also referred to as variants 1 and 3a/b, respectively. These two are the most common variants of *EML4-ALK* and have been detected in 33% and 29% of NSCLC patients, respectively (Fig. 3B). The NSCLC cell lines, H3122 and DFCI032, contain the E13;A20 variant while H2228 contains the E6a/b;A20 variant.[7] The clinical significance, if any, of the different variants is currently not defined.

In ALK translocated NSCLC, *EML4* does not appear to be the exclusive fusion partner with *ALK*. Two other fusions have been described, *TFG* and *KIF5B*, and both were identified as an ALK-fusion partner from NSCLC tumour samples[30,37] these two proteins also fuse with intracellular domain of ALK. Intriguingly *TFG-ALK* has also been described in anaplastic large cell lymphoma.[38] The presence of these non-EML4 fusion partners for ALK has implications for the method used for clinical detection of ALK translocated NSCLC.

3. Diagnosis of *EML4-ALK* NSCLC

ALK-rearrangements in a subset of anaplastic large cell lymphomas (ALCLs) have been recognised for over 15 years and

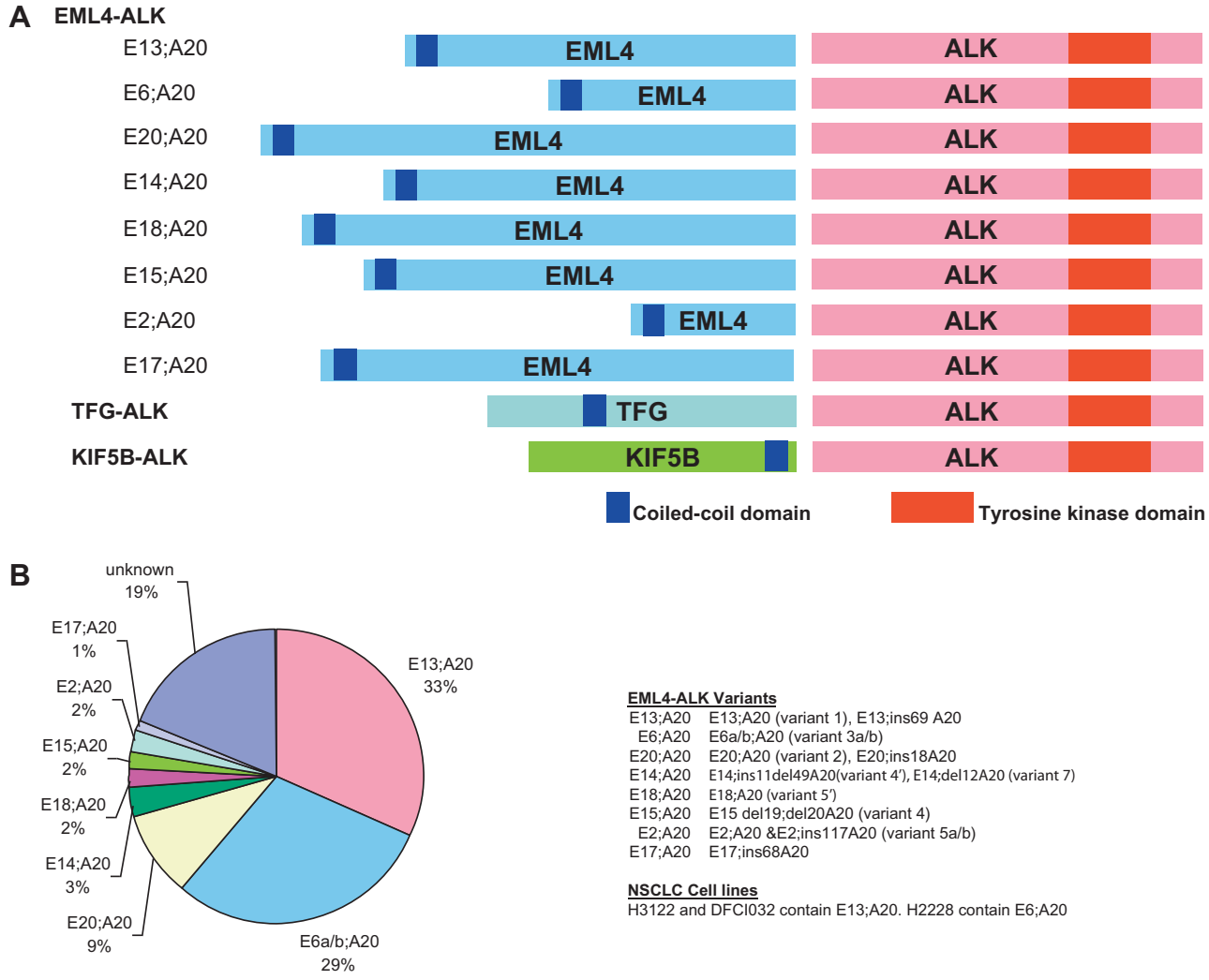

Fig. 3 – Different variants of *EML4-ALK* and non-*EML4* fusion partners. (A) Different variants of *EML4-ALK* are depicted. The nomenclature refers to the exon in *EML4* translocated to the exon in *ALK*. (B) Frequency of different *EML4-ALK* variants. The most common variants are E13;A20 (variant 1) and E6a/b; A20 (variant 3). Data obtained from.[4–11,30,32–36] Of note not all studies list the specific *EML4-ALK* variant.

a variety of diagnostic techniques, currently employed in clinical practice, have been validated as sensitive and specific for detecting the genetic lesions characteristic of this tumour type.[39] However, there is currently no standard method for detecting *EML4-ALK* NSCLC. Several methods including polymerase chain reaction (PCR), immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) are currently being evaluated.

3.1. *PCR-based identification of EML4-ALK*

Reverse transcriptase (RT)-PCR is a potentially rapid diagnostic method for identifying *ALK* translocated NSCLCs. A theoretical advantage of this technique is its extreme sensitivity for detecting mutant transcript and the presence of any amplification product implies an *ALK* rearrangement. However in practice, the technique faces several challenges. First, the RT-PCR analysis must be multiplexed. As mentioned above there are at least 11 variant *EML4-ALK* fusions, and non-*EML4* translocation partners, therefore any PCR-based strategy must incorporate validated primer pairs for all known *ALK* fusions. Second, the vast majority of patient biopsy specimens from lung cancer patients are stored as formalin-fixed paraffin embedded (FFPE) tissues. RNA extracted from FFPE is highly degraded and, in general, more difficult to PCR relative to non-fixed, fresh-frozen tissue. Third, there is published evidence indicating that RT-PCR based detection of *EML4-ALK* can yield positive results in the absence of detectable *ALK*-rearrangements in both tumour, and non-tumour tissues.[9] Although the interpretation of these findings is still open to debate, it suggests a propensity for false positive results. Despite these disadvantages, there are advocates for using RT-PCR based screening methods.[32] However, this method may be difficult to implement in a routine clinical diagnostic laboratory.

3.2. *FISH-based methods for identification of EML4-ALK*

More specific detection of *ALK*-rearrangements can be achieved by the fluorescence in situ hybridisation (FISH) of

probes flanking the *ALK* breakpoint with tumour cell nuclei.[10] A big advantage of FISH is that a commercially available probe set, developed for the diagnosis of *ALK*-rearranged ALCLs, is applicable for the diagnosis of *ALK*-rearranged lung adenocarcinomas. The test employs one probe 5′ of the *ALK* locus and one probe within the *ALK* gene, which when hybridised against normal nuclei, yield a merged (green–orange fluorescent) signal that is easily visualised microscopically. However, when the probe set is hybridised against nuclei with a rearrangement involving the 5′ portion of the *ALK* locus the result is a 'split' (green and orange fluorescent) signal (Fig. 4). In theory, any inter-chromosomal or intra-chromosomal lesion involving *ALK* (including cancers harbouring non-*EML4* fusion partners) will be detected by this test. However a cautionary finding is that the 'split' signal characteristic of an *EML4-ALK* fusion can be subtle, due to the loss and inversion of only a small amount of genetic material on chromosome 2. Also the 5′ probe occasionally fails to hybridise, presumably due a loss of the target locus in the tumour. These patterns contrast the widely split, dual hybridisation pattern found in tumours with an inter-chromosomal rearrangement of the *ALK* locus such as *ALK*-rearranged ALCLs. An additional complicating factor with FISH is the destruction of tissue morphology when formalin-fixed, paraffin embedded (FFPE) biopsy specimens are analysed in this manner. Thus, although FISH is a sensitive and specific means to detect *ALK*-rearrangements in lung adenocarcinoma, it is not infallible. In fact, in a recent study, an *ALK*-rearranged lung adenocarcinoma by immunohistochemical testing for ALK protein expression that was, initially, mistakenly diagnosed as *ALK* wild type by prior FISH analysis.[29] Furthermore, unlike PCR, FISH cannot distinguish between the different *EML4-ALK* fusion variants. It is currently not clear whether there are any functional or therapeutic differences amongst the different variants to warrant more specific knowledge. FISH is the diagnostic method used as an eligibility criterion in the current clinical trials of PF-02341066. Current studies use $\geqslant$15% split nuclei as indicative of an *ALK* rearrangement.[40] However, the therapeutic implications, if any, of the frequency of split signals and the number of nuclei evaluated remains to be determined.

3.3. *IHC detection methods for EML4-ALK*

Immunohistochemical (IHC) analysis of FFPE tissue specimens remains a mainstay of routine surgical pathology practice. The major advantage of this approach is an ability to assay for tumour-specific antigen expression without loss of the cytologic and architectural features that distinguish normal from pathologic tissue. Several antibodies specific for the human ALK protein have been developed and a few validated in IHC tests that are widely used to diagnose *ALK*-rearranged ALCLs today.[39] The sensitivity and specificity of the

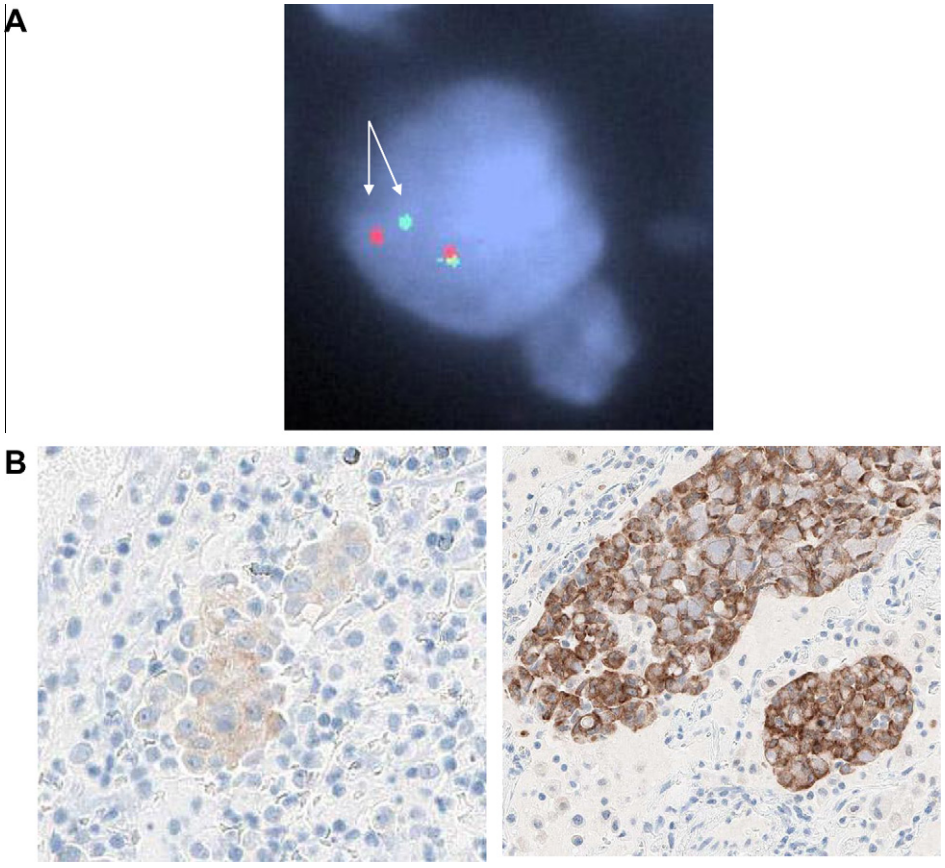

Fig. 4 – Diagnostic methods for *EML4-ALK* NSCLC. (A) FISH analysis using the *ALK* split apart probe. The arrows depict the split signals indicative of a chromosome 2 inversion. The tumour is heterozygous – the signals remain together on the other allele. (B) IHC analysis for ALK using the D5F3 (Cell Signalling Technology[41]) antibody. Shown are examples of low (left) and high (right) ALK expressing tumours.

IHC test are such that genetic testing is considered unnecessary and redundant when it is employed during the routine evaluation of an ALCL of unknown *ALK* status. However the IHC tests used to diagnose *ALK*-rearranged ALCLs in clinical laboratories worldwide are inadequate for the detection of the majority of *ALK*-rearranged lung adenocarcinomas. This is due to the lower level of ALK expression in *ALK*-rearranged NSCLCs compared with *ALK*-rearranged ALCLs. In an initial survey of 10 FISH-confirmed, *ALK*-rearranged lung adenocarcinomas, Rodig and colleagues found that only four stained positively for ALK protein expression by the standard clinical test.[29] They and others have been able to increase the number of positive staining cases using an additional, non-traditional amplification step in the immunostaining protocol.[29,30] However, not all cases stained positive even by this method. These findings initially raised the question of whether a subset of *ALK*-rearranged lung adenocarcinomas fail to express the ALK protein, or whether the levels of ALK protein expression in these cases were simply too low to be detected using standard reagents.

More recently Mino-Kenudson and colleagues reported on an IHC test based on novel antibodies with increased sensitivity and specificity for detecting ALK protein expression in FFPE (Fig. 4B).[41] Using this test, they detected ALK protein expression in all 22 *ALK*-rearranged lung adenocarcinomas tested, and found no expression in 131 *ALK* wild type lung adenocarcinomas. ALK protein expression was detected in all *ALK*-rearranged lung adenocarcinomas to be substantially lower than the expression in *ALK*-rearranged ALCLs, and in 13 of 22 cases (59%), ALK protein expression could only be detected using our novel, highly sensitive IHC test (Fig. 4B).[41] These findings support that ALK expression is restricted to lung cancers that harbour *ALK*-rearrangements. Furthermore they open up the possibility of being able to diagnose such cancers using routine IHC-based methods which, unlike FISH, is available in every pathology laboratory. This may allow pathologist a means to rapidly screen for patients harbouring an *ALK* translocation who may be candidates for ALK targeted therapies. A caveat however is that tissue staining for ALK, even with the most sensitive of IHC tests, may be weak and focal in the biopsy sections (Fig. 4B), in which case confirmatory FISH studies should be considered.

4. ALK-targeted therapy in NSCLC

4.1. *Pre-clinical studies*

The initial studies reporting on the discovery of *EML4-ALK* raised the possibility that inhibiting the kinase activity of ALK may be an effective clinical therapy.[4] Furthermore, transgenic mice expressing *EML4-ALK* in the lung epithelium develop numerous lung adenocarcinomas demonstrating the oncogenic nature of this fusion gene.[12] Pre-clinical studies demonstrate that *EML4-ALK* NSCLC cell lines undergo downregulation of critical survival signalling pathways and apoptosis when treated with an ALK kinase inhibitor.[7,13] This is analogous to what has been observed with EGFR inhibitors in *EGFR* mutant NSCLC.[42] Similarly, ALK inhibitors have been evaluated *in vivo*, both in xenograft models generated from *EML4-ALK* NSCLC cell lines, and lead to effective tumour regressions of established tumours.[7,12] Currently, only one agent targeting ALK, PF-02341066 initially designed as an inhibitor of MET, is in clinical use although others have been examined in pre-clinical model systems.[7,12,43] This orally bioavailable small molecule inhibitor has been shown to inhibit the growth of ALK translocated cancer cell lines including *EML4-ALK* NSCLC.[13,43]

4.2. *Clinical studies*

PF-02341066 (crizotinib) is an orally bioavailable ALK inhibitor currently under clinical development. The phase I study of this agent started in May 2006. After the discovery of *EML4-ALK*, and that PF-02341066 also inhibits ALK, the dose expansion cohorts in this study included patients with either genomic alterations in *MET* (amplifications and/or mutations) or *ALK*.[14] The initial findings were presented at ASCO 2009 and demonstrated a remarkable 53% response rate (10/19 patients) and a disease control rate (partial response and stable disease) of 79% (15/19).[14] Additional responses continue to be reported. These dramatic findings have led to two subsequent clinical trials of PF-02341066. The first is a randomised phase III trial of PF-02341066 compared with standard second line chemotherapy (pemetrexed or docetaxel) in second line *EML4-ALK* NSCLC. The second is a phase II clinical trial of single agent PF-02341066 in EML4-ALK NSCLC designed for patients not eligible for the phase III trial or patients randomised to chemotherapy who subsequently developed progressive disease. In a remarkably short period of time – from initial discovery to clinical validation – ALK targeted therapies are in advanced clinical development for *EML4-ALK* NSCLC. It is anticipated, that if *EML4-ALK* NSCLC behaves in an analogous manner to *EGFR* mutant NSCLC, that ALK targeted therapies will quickly emerge as the standard systemic therapy for this subset of NSCLC patients.

5. Conclusions

EML4-ALK NSCLC represents a unique subset of NSCLC patients for whom ALK inhibitors may represent a very effective therapeutic strategy. The challenge remains to incorporate and disseminate widespread use of diagnostic testing for *EML4-ALK* to identify this patient subset. As we learn more about *EML4-ALK* NSCLC we will continue to uncover unique biological and molecular features of this patient subset also undoubtedly encounter drug resistance to ALK targeted therapies.

Conflict of interest statement

None declared.

Acknowledgement

This study was supported by the National Institutes of Health R01CA136851 (P.A.J.).

REFERENCES

1. Thun MJ, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. *Carcinogenesis* 2010;**31**(1):100–10.
2. Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002;**346**(2):92–8.
3. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009;**361**(10):947–57.
4. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007;**448**(7153):561–6.
5. Inamura K, Takeuchi K, Togashi Y, et al. EML4-ALK fusion is linked to histological characteristics in a subset of lung cancers. *J Thorac Oncol* 2008;**3**(1):13–7.
6. Inamura K, Takeuchi K, Togashi Y, et al. EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. *Mod Pathol* 2009;**22**(4):508–15.
7. Koivunen JP, Mermel C, Zejnullahu K, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res* 2008;**14**(13):4275–83.
8. Shinmura K, Kageyama S, Tao H, et al. EML4-ALK fusion transcripts, but no NPM-, TPM3-, CLTC-, ATIC-, or TFG-ALK fusion transcripts, in non-small cell lung carcinomas. *Lung Cancer* 2008;**61**(2):163–9.
9. Martelli MP, Sozzi G, Hernandez L, et al. EML4-ALK rearrangement in non-small cell lung cancer and non-tumor lung tissues. *Am J Pathol* 2009;**174**(2):661–70.
10. Shaw AT, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* 2009;**27**(26):4247–53.
11. Wong DW, Leung EL, So KK, et al. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. *Cancer* 2009;**115**(8):1723–33.
12. Soda M, Takada S, Takeuchi K, et al. A mouse model for EML4-ALK-positive lung cancer. *Proc Natl Acad Sci USA* 2008;**105**(50):19893–7.
13. McDermott U, Iafrate AJ, Gray NS, et al. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. *Cancer Res* 2008;**68**(9):3389–95.
14. Kwak EL, Camidge DR, Clark J, et al. Clinical activity observed in a phase I dose escalation trial of an oral c-met and ALK inhibitor, PF-02341066. *J Clin Oncol* 2009;**27**:15s [abstract 3509].
15. Koivunen JP, Kim J, Lee J, et al. Mutations in the LKB1 tumour suppressor are frequently detected in tumours from Caucasian but not Asian lung cancer patients. *Br J Cancer* 2008;**99**(2):245–52.
16. Sun S, Schiller JH, Gazdar AF. Lung cancer in never smokers – a different disease. *Nat Rev Cancer* 2007;**7**(10):778–90.
17. Tomizawa Y, Iijima H, Sunaga N, et al. Clinicopathologic significance of the mutations of the epidermal growth factor receptor gene in patients with non-small cell lung cancer. *Clin Cancer Res* 2005;**11**(19 Pt 1):6816–22.
18. Shih JY, Gow CH, Yu CJ, et al. Epidermal growth factor receptor mutations in needle biopsy/aspiration samples predict response to gefitinib therapy and survival of patients with advanced nonsmall cell lung cancer. *Int J Cancer* 2006;**118**(4):963–9.
19. Hsieh RK, Lim KH, Kuo HT, Tzen CY, Huang MJ. Female sex and bronchioloalveolar pathologic subtype predict EGFR mutations in non-small cell lung cancer. *Chest* 2005;**128**(1):317–21.
20. Tokumo M, Toyooka S, Kiura K, et al. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. *Clin Cancer Res* 2005;**11**(3):1167–73.
21. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;**97**(5):339–46.
22. Tam IY, Chung LP, Suen WS, et al. Distinct epidermal growth factor receptor and KRAS mutation patterns in non-small cell lung cancer patients with different tobacco exposure and clinicopathologic features. *Clin Cancer Res* 2006;**12**(5):1647–53.
23. Le Calvez F, Mukeria A, Hunt JD, et al. TP53 and KRAS mutation load and types in lung cancers in relation to tobacco smoke: distinct patterns in never, former, and current smokers. *Cancer Res* 2005;**65**(12):5076–83.
24. Eberhard DA, Giaccone G, Johnson BE. Biomarkers of response to epidermal growth factor receptor inhibitors in Non-Small-Cell Lung Cancer Working Group: standardization for use in the clinical trial setting. *J Clin Oncol* 2008;**26**(6):983–94.
25. Blons H, Cote JF, Le Corre D, et al. Epidermal growth factor receptor mutation in lung cancer are linked to bronchioloalveolar differentiation. *Am J Surg Pathol* 2006;**30**(10):1309–15.
26. Ahrendt SA, Decker PA, Alawi EA, et al. Cigarette smoking is strongly associated with mutation of the K-ras gene in patients with primary adenocarcinoma of the lung. *Cancer* 2001;**92**(6):1525–30.
27. Shigematsu H, Takahashi T, Nomura M, et al. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res* 2005;**65**(5):1642–6.
28. Mounawar M, Mukeria A, Le Calvez F, et al. Patterns of EGFR, HER2, TP53, and KRAS mutations of p14arf expression in non-small cell lung cancers in relation to smoking history. *Cancer Res* 2007;**67**(12):5667–72.
29. Rodig SJ, Mino-Kenudson M, Dacic S, et al. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. *Clin Cancer Res* 2009;**15**(16):5216–23.
30. Takeuchi K, Choi YL, Togashi Y, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. *Clin Cancer Res* 2009;**15**(9):3143–9.
31. Lin E, Li L, Guan Y, et al. Exon array profiling detects EML4-ALK fusion in breast, colorectal, and non-small cell lung cancers. *Mol Cancer Res* 2009;**7**(9):1466–76.
32. Takeuchi K, Choi YL, Soda M, et al. Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. *Clin Cancer Res* 2008;**14**(20):6618–24.
33. Choi YL, Takeuchi K, Soda M, et al. Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. *Cancer Res* 2008;**68**(13):4971–6.
34. Fukuyoshi Y, Inoue H, Kita Y, et al. EML4-ALK fusion transcript is not found in gastrointestinal and breast cancers. *Br J Cancer* 2008;**98**(9):1536–9.
35. Perner S, Wagner PL, Demichelis F, et al. EML4-ALK fusion lung cancer: a rare acquired event. *Neoplasia* 2008;**10**(3):298–302.
36. Takahashi T, Sonobe M, Kobayashi M, et al. Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. *Ann Surg Oncol* 2010;**17**(3):889–97.
37. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* 2007;**131**(6):1190–203.
38. Hernandez L, Pinyol M, Hernandez S, et al. TRK-fused gene (TFG) is a new partner of ALK in anaplastic large cell lymphoma producing two structurally different TFG-ALK translocations. *Blood* 1999;**94**(9):3265–8.

39. Cataldo KA, Jalal SM, Law ME, et al. Detection of t(2;5) in anaplastic large cell lymphoma: comparison of immunohistochemical studies, FISH, and RT-PCR in paraffin-embedded tissue. *Am J Surg Pathol* 1999;**23**(11):1386–92.
40. Shaw RJ, Paez JG, Curto M, et al. The Nf2 tumor suppressor, merlin, functions in Rac-dependent signaling. *Dev Cell* 2001;**1**(1):63–72.
41. Mino-Kenudson M, Chirieac LR, Law K, et al. A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. *Clin Cancer Res* 2010;**16**(5):1561–71.
42. Tracy S, Mukohara T, Hansen M, et al. Gefitinib induces apoptosis in the EGFRL858R non-small-cell lung cancer cell line H3255. *Cancer Res* 2004;**64**(20):7241–4.
43. Christensen JG, Zou HY, Arango ME, et al. Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. *Mol Cancer Ther* 2007;**6**(12):3314–22.