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**Letter to the Editor**

# Identification of *RANBP2–ALK* fusion in *ALK* positive diffuse large B-cell lymphoma

To the Editor

Translocation of the *ALK* gene in malignant lymphoma is unique in *ALK*-positive anaplastic large cell lymphoma (ALCL) but can be observed in a subset of diffuse large B-cell lymphoma (DLBCL). *ALK*-positive large B-cell lymphoma is a rare subtype of lymphoma and is characterized by a monomorphic proliferation of immunoblast-like cells expressing a plasmablastic phenotype; most cases follow an aggressive clinical course with a poor prognosis [1]. Unlike *ALK*-positive ALCL, in which *ALK* is expressed more often in both the nucleus and cytoplasm of tumour cells, most cases of *ALK*-positive large B-cell lymphoma show a granular cytoplasmic staining pattern, and only a few cases of cytoplasmic and nuclear *ALK* staining have been reported [2,3]. Identifying the subcellular localization of *ALK* expression is helpful in predicting the fusion partner gene(s). Nuclear and diffuse cytoplasmic staining of *ALK* is seen in cases with *NPM–ALK* translocation; by contrast, fusion of *ALK*–clathrin (*CLTC*) produces granular cytoplasmic staining.

We have reported previously on three cases of *ALK*-positive DLBCL [4]. The tumour comprised immunoblastic cells with prominent nucleoli (Figure 1A). Two of the cases exhibited granular cytoplasmic staining of *ALK* protein, but one case showed unique nuclear membrane staining with perinuclear dot (Figure 1B). Nuclear membrane staining for *ALK* has been described by Ma et al. [5] in only two cases of an inflammatory myofibroblastic tumour with the fusion of *ALK* to *RANBP2*, which encodes Ran-binding protein 2; nuclear membrane staining for *ALK* has not been reported for *ALK*-positive DLBCL or ALCL.

To confirm the translocation partner gene of *ALK* in *ALK*-positive DLBCL with this peculiar nuclear membrane staining, we performed fluorescence in situ hybridization (FISH) studies using an *ALK* split probe and fusion assays for *RANBP2–ALK* and *CLTC–ALK*. Unstained sections were processed using a Histology FISH Accessory Kit (Dako) and were subjected to hybridization with fluorescence-labelled bacterial artificial chromosome clone probes for *RANBP2* and *ALK* (self-produced probes; *CLTC*: CTD-2001 K22, *RANBP2*: RP11-348G16, and *ALK*: RP11-984I21 and RP11-62B19)(Figure 2A). The sections were then stained with 4,6-diamidino-2-phenylindole and examined with a fluorescence microscope (BX51; Olympus). *ALK* split FISH was positive. *RANBP2–ALK* fusion FISH was positive (Figure 2B). *CLTC–ALK* FISH was negative (Figure 2B).

Additionally, we performed reverse transcriptase polymerase chain reaction (RT-PCR) to confirm *RANBP2–ALK* fusion. Total RNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumour sample using the ReliaPrep™ FFPE Total RNA Miniprep System (Promega, Madison, WI). RT-PCR was conducted using a high-capacity cDNA Reverse Transcription Kit (catalog no. 4368814; Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Then, a 5′ rapid amplification of cDNA ends (RACE) using the SMARTRACE cDNA Amplification Kit (Clontech, Mountain View, CA) was performed according to the manufacturer's instructions, with *ALK*-4169R primer (5′-GGTTGTAGTCGGTCATGATGG-3′) [5]. *ALK–RANBP* fusion was confirmed by using RT-PCR with primers for the *RANBP2*-2442*F* and *RANBP2*-2686F genes combined with direct Sanger sequencing of PCR products. The result revealed fusion between exon 18 of *RANBP2* and exon 20 of *ALK*. The *RANBP2–ALK* cDNA sequence was identical to that of inflammatory myofibroblastic tumours previously reported by Ma et al.[5] (Figure 3).

Because the initial report of *ALK*-positive DLBCL by Delsol [6] et al. in 1997, about 70 cases have been described in the literature. Table 1 presents a summary of the 70 published cases of *ALK*-positive DLBCL. Several partner genes fused to *ALK* have been described in *ALK*-positive DLBCL, and the underlying gene rearrangement has been shown to correlate with the immunohistochemical staining pattern for *ALK* protein. As shown in Table 1, the most common gene rearrangement is between *CLTC* and *ALK* [t(2;17)(p23;q23)], resulting in the *CLTC–ALK* chimeric protein. Additional fusion partner genes such as *SEC31A–ALK* and *SQSTM1–ALK* rearrangement have been identified recently [7–10]. *SEC31A–ALK* fusion shows granular cytoplasmic staining similar to that of *CLTC–ALK* fusion, but *SQSTM1–ALK* fusion shows diffuse cytoplasmic and ill-demarcated spots.

The *RANBP2* gene, located in chromosomal region 2q13, encodes a 358-kDa multidomain nuclear pore protein that functions in the terminal steps of nuclear export and in the initial steps of nuclear import [5]. The leucine zipper of *RANBP2* has been predicted to mediate

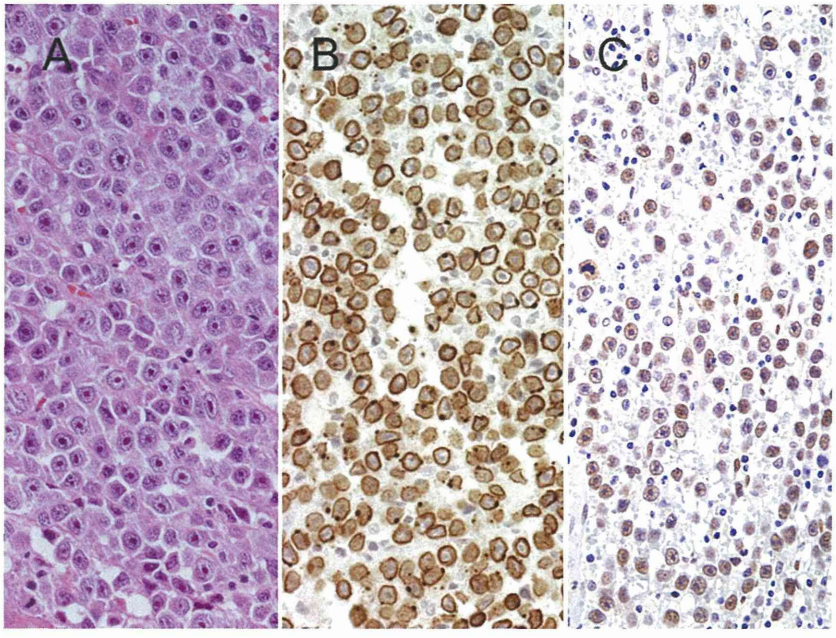


**Figure 1.** (A) Immunoblastic or plasmablastic tumour cells of *ALK* positive large B-cell lymphoma; (B) Immunohistochemical stain for *ALK* showing unique nuclear membrane staining with perinuclear dot; (C) Immunohistochemical stain for pSTAT3 revealed strong positive staining in the nucleus of all tumour cells

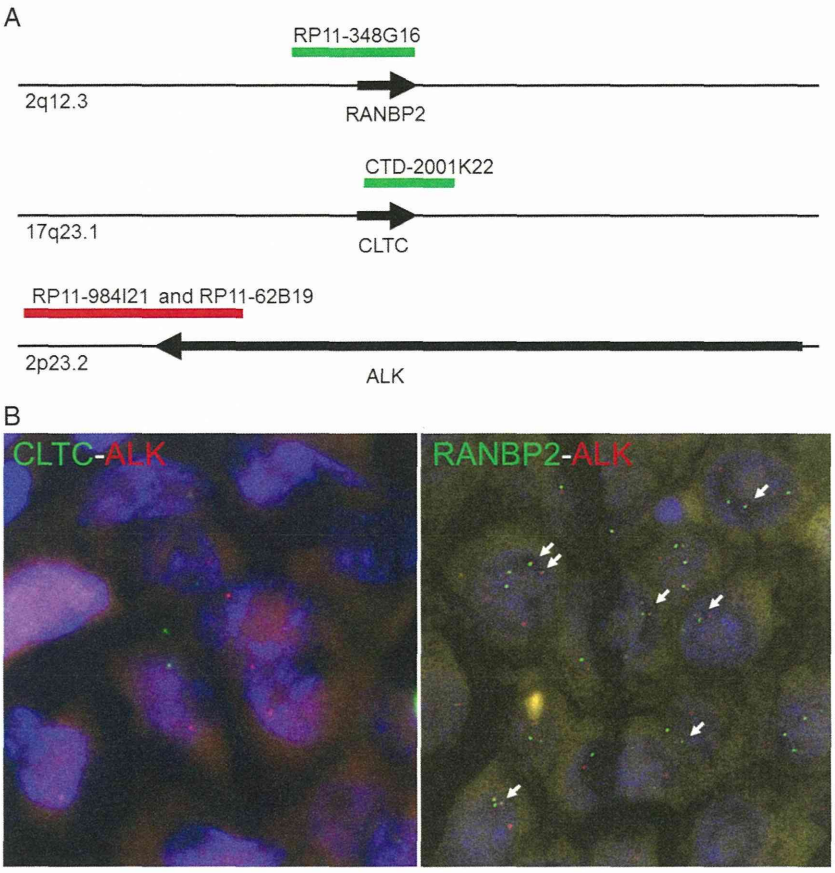


**Figure 2.** Fluorescence in situ hybridization study to confirm fusion partner gene. (A) Probe map. (B) Fusion assays for *CLTC–ALK* (left) and *RANBP2–ALK* (right). Fusion signals of *ALK* (red) and *RANBP2* (green) were frequently found (arrow). *CLTC–ALK* fusion was not seen




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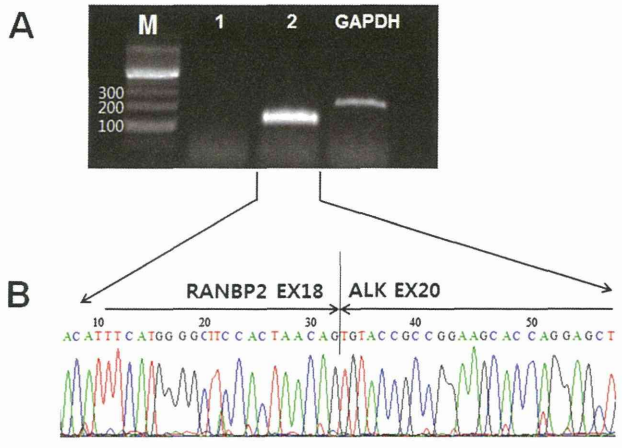


**Figure 3.** Real-time polymerase chain reaction (RT-PCR) to confirm *RANBP2–ALK* fusion (A) Fusion chimera was detected in RT-PCR using primers for exon 18 of *RANBP2* and exon 20 of *ALK* (lane 2), but not in RT-PCR using primers for exon 16 for *RANBP2* and exon 20 for *ALK* (lane 1), GAPDH was used for control (lane 3). (B) Sanger sequencing of RT-PCR product confirmed identical junction sequence to that of *RANBP2–ALK* fusion gene reported in inflammatory myofibroblastic tumour[5]

homooligomerization of *RANBP2–ALK*, thus activating *ALK* kinase catalytic function [5]. Activation of *ALK* leads to the activation of different downstream signalling pathway, one of the most relevant being the STAT3 pathway [11,12]. STAT3 activation has been identified in *ALK*-positive neoplasm with various *ALK* fusion protein including ALCL with *NPM–ALK* fusion, *ALK*-positive DLBCL with *CLTC-ALK* fusion or *SQSTM1-ALK* fusion [8,13]. To confirm the STAT3 activation status, we carried out the immunohistochemical stain using phopho-STAT3 monoclonal antibody (Catalog # 9134, Cell Signaling Technology, Inc., MA, U.S.A.). STAT3 was ubiquitously hyperphosphorylated in all tumour cells (Figure 1C )

In summary, we report the first example of RANBP2–*ALK* gene fusion identified in *ALK*-positive DLBCL with a unique nuclear membrane staining of *ALK* protein. The breakpoint is identical to that of *RANBP2–ALK* gene fusion reported in inflammatory myofibroblastic tumour [5]. The function of this fusion seems to be similar to that of other *ALK* fusions involving STAT-3 signalling pathway.

**Table 1.** Summary of *ALK* positive diffuse large B-cell lymphomas published in the literatures

| Authors | Years | No. of patients | *ALK* partner gene | Detection technique | Immunohistochemical stain for *ALK* |
|---|---|---|---|---|---|
| Delsol [6] | 1997 | 7 | ND | RT-PCR | — |
| Onciu [3] | 2003 | 2 | *NPM* (2) | RT-PCR | Nuclear and cytoplasmic |
| Adam [2] | 2003 | 1 | *NPM* (1) | RT-PCR | Nuclear and cytoplasmic |
| Gascoyne [13] | 2003 | 6 | *CLTC* (6) | FISH and RT-PCR | Granular cytoplasmic |
| Chikatsu [14] | 2003 | 1 | *CLTC* | RT-PCR | Granular cytoplasmic |
| De Paepe [15] | 2003 | 3 | *CLTC* (3) | FISH and RT-PCR | Granular cytoplasmic |
| McManus [16] | 2004 | 1 | *CLTC* | RT-PCR | Granular cytoplasmic |
| Gesk [17] | 2005 | 3 | *CLTC* | FISH | Granular cytoplasmic |
| Rudzski [18] | 2005 | 2 | *NPM* (2) | FISH | Nuclear and cytoplasmic |
| Isimbaldi [19] | 2006 | 1 | *CLTC* | RT-PCR | Granular cytoplasmic |
| Bubala [20] | 2006 | 1 | *CLTC* | FISH | Granular cytoplasmic |
| Reichard [21] | 2007 | 4 | suggestive of *CLTC* (1/4) | FISH | Granular cytoplasmic |
| Lee [4] | 2008 | 3 | suggestive of *CLTC* (2/3) | FISH | Granular cytoplasmic |
| Momose [22] | 2009 | 2 | *CLTC* (2) | RT-PCR | Granular cytoplasmic |
| Van Roosbroeck [10] | 2010 | 2 | *SEC31A* (1/2) and *NPM* (1/2) | FISH, RT-PCR and RACE PCR | Granular cytoplasmic (1/2) and nuclear and cytoplasmic (1/2) |
| Laurent [23] | 2010 | 38[a] | *CLTC* (9/38) | FISH and RT-PCR | Granular cytoplasmic |
| Takeuchi [9] | 2011 | 1 | *SQSTM1* | FISH and RT-PCR | Diffuse cytoplasmic and with fewer and finer granules |
| Bedwell [7] | 2011 | 1 | *SEC31A* | FISH and RT-PCR | Granular cytoplasmic |
| Holtan [24] | 2011 | 1 | *CLTC* | FISH | Granular cytoplasmic |
| d'Amore [8] | 2013 | 1 | *SQSTM1* | FISH and RACE PCR | Diffuse cytoplasmic and ill-demarcated spots |
| This study | 2013 | 1 | *RANBP2* | FISH | Nuclear membrane |

ND, not detected; IHC, immunohistochemical; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; RACE, rapid amplification of cDNA ends.
[a]Twelve of 38 cases were previously published.




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## Conflict of interest

The authors have no competing interest.

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# Activity of EGFR-tyrosine kinase and ALK inhibitors for EML4--ALK-rearranged non--small--cell lung cancer harbored coexisting EGFR mutation

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# Activity of EGFR-tyrosine kinase and ALK inhibitors for *EML4–ALK*-rearranged non–small–cell lung cancer harbored coexisting *EGFR* mutation

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# Abstract

## Background

The *EML4–ALK* (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) fusion oncogene represents a novel molecular target in a small subset of non–small–cell lung cancers (NSCLCs). The *EML4–ALK* fusion gene occurs generally in NSCLC without mutations in epidermal growth factor receptor *(EGFR)* and *KRAS*.

## Case presentation

We report that a case of *EML4–ALK*-positive NSCLC with *EGFR* mutation had a response of stable disease to both an EGFR tyrosine kinase inhibitor (EGFR-TKI) and ALK inhibitor.

**Conclusions**

We described the first clinical report of a patient with *EML4–ALK*-positive NSCLC with *EGFR* mutation that had a response of stable disease to both single-agent EGFR-TKI and ALK inhibitor. *EML4–ALK* translocation may be associated with resistance to EGFR-TKI, and EGFR signaling may contribute to resistance to ALK inhibitor in *EML4–ALK*-positive NSCLC.

## Keywords

EML4–ALK, *EGFR* mutation, Lung cancer

## Background

The *EML4–ALK* (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) fusion oncogene was recently identified as a novel genetic alteration in non-small-cell lung cancer (NSCLC) [1]. *EML4–ALK* fusions have been detected in 2 to 7% of NSCLC patients. Patients harboring *ALK* rearrangements tend to be never and light smokers, have a history of adenocarcinoma, and be younger in age [1-6]. In general, the *EML4–ALK* fusion oncogene existed exclusively in NSCLC patients without the epidermal growth factor receptor (*EGFR*) gene mutation [1,7,8].

ALK inhibitors such as crizotinib are clinically effective in NSCLC patients harboring ALK rearrangements [9]. Crizotinib produced a high response rate and prolonged median progression-free survival among patients with ALK-positive NSCLC [9]. Crizotinib was recently approved by the US Food and Drug Administration and Japanese Ministry of Health, Labour and Welfare for the treatment of patients with advanced, ALK-rearranged NSCLC.

In this paper, we report a patient with NSCLC with concomitant ALK rearrangement and *EGFR* mutation that had a response of stable disease to both an EGFR tyrosine kinase inhibitor (EGFR-TKI) and ALK inhibitor.

## Case presentation

In December 2009, a 55-year-old female who had never smoked was noted to have left lung opacity on a routine chest X-ray. No significant previous medical history was reported. Computed tomography (CT) scan of the chest revealed a 1.5 × 1.5 cm nodular lesion in the left upper lobe and hilar lymph node metastasis. Transthoracic needle biopsy histology revealed adenocarcinoma, and the histopathological subtype of the specimen was papillary adenocarcinoma with signet-ring cell components (Figure 1A-1C). The specimen was positive for periodic acid–Schiff (PAS) (Figure 1C). On immunohistochemical staining, the tumor cells were positive for thyroid transcription factor-1 (TTF-1) (Figure 1D). Laboratory findings were within normal range, except for the carcinoembryonic antigen (CEA) level of 158.0 ng/mL (normal range, 0 to 4.3 ng/mL) in the serum. She had multiple dorsal vertebra metastases (cT1N1M1b, stage IV).

**Figure 1 Histology of the primary tumour**: (**A**) and (**B**) shows a papillary adenocarcinoma (hematoxylin and eosin 200× magnification), (**C**) a mucin stain shows positive for both signet-ring and papillary morphology (PAS, 400× magnification). (**D**) immunohistochemical analysis of lung adenocarcinoma specimens with *EML4-ALK* fusion using a monoclonal anti-TTF-1 antibody (200× magnification).

Analysis for *EGFR* gene mutation was performed using a cytological specimen by means of the peptide nucleic acid–locked nucleic acid (PNA-LNA) polymerase-chain-reaction (PCR) clamp method as described previously [10,11]. The specimen showed a deletion in exon 19 (L747-A750del T751S). We collected mRNA from the same tumor specimens using Pinpoint Slide RNA Isolation System in order to clarify whether there was *EML4–ALK* (echinoderm microtubule-associated protein-like 4 gene and the anaplastic lymphoma kinase gene) fusion gene in each tumor. Reverse transcription polymerase-chain-reaction (RT-PCR) followed by direct sequencing confirmed the presence of *EML4–ALK* variant 2 [1] (Figure 2). In addition, *EML4–ALK* was identified by using fluorescent in situ hybridization (FISH) for *ALK* rearrangements (Figure 3B) and was confirmed by immunohistochemistry for ALK expression in tumor [2] (Figure 3A).

**Figure 2 The sequence of the junction between *EML4* exon 20 and *ALK* exon 20.**

**Figure 3 Diagnosis of an EML4-ALK-positive non-small cell lung cancer.** (A) Immunostaining for ALK protein expression in tumor cells. (**B**) The results of a break-apart FISH assay of tumor cells from a patient with rearrangement of the gene encoding *ALK*.

A platinum doublet was chosen as first line therapy according to existing treatment protocol in 2009. Four cycles of combination chemotherapy comprising cisplatin and pemetrexed was administered at 3-week intervals. She was judged as having a stable disease. After 7 months, spinal magnetic resonance imaging (MRI) revealed progression of the dorsal vertebra lesions. Therefore, EGFR-TKI was chosen as a 2nd-line therapy. She received gefitinib therapy at 250 mg/day administered orally for 2 months. CT imaging of the chest showed that the pulmonary nodule was not growing after gefitinib therapy, and the tumor marker levels had not changed. However, spinal MRI demonstrated growing dorsal vertebra metastases 2 months after the start of gefitinib therapy. The carcinoembryonic antigen (CEA) level increased from 117 ng/ml to 250 ng/ml. Therefore, the patient was judged as having progressive disease. After local radiation therapy with a total of 30 Gy for dorsal metastases, a second EGFR-TKI was chosen given the stable primary disease. She received another EGFR-TKI, erlotinib (150 mg/day), as 3$^{rd}$-line therapy. After being progression-free for 3 months, spinal MRI revealed a growing thoracic vertebra metastasis. She received 4$^{th}$-line treatment with 2 cycles of docetaxel (DTX). However, her disease progressed 6 months later. Finally, she received a targeted inhibitor of ALK. The patient initially had SD associated with a temporary decrease in the CEA level from 743 ng/ml to 520 ng/ml, but her disease progressed after 4 months of therapy. The ALK inhibitor treatment was ceased and full supportive care was given. All lines of therapy were well tolerated.

# Discussion

We presented a patient with NSCLC with concomitant ALK rearrangement and *EGFR* mutation that had a response of stable disease to both EGFR-TKI and ALK inhibitors. The presence of *EML4–ALK* generally seems to be mutually exclusive of the presence of *EGFR*

or *KRAS* mutations in NSCLC [1,7,8]. Previous reports showed twelve cases of *EML4–ALK*-positive lung cancer with *EGFR* mutation [3,12-17]. Only one patient with harboring *ALK* translocation and EGFR mutation was treated by ALK inhibitor has been reported [17]. Lee et al. reported two ALK-positive and EGFR-mutant NSCLC patient who did not respond to EGFR-TKI but achieved a durable partial response to ALK inhibitor [17]. The present patient was a woman with no history of smoking. Her pathological diagnosis was papillary adenocarcinoma with a signet-ring cell component, which was consistent with the previously reported characteristics of *EML4–ALK*-positive lung adenocarcinoma except for the *EGFR* mutation status [1-6]. It was reported that EGFR-TKI therapy among patients with advanced NSCLC and *EGFR* mutations revealed a response rate of more than 60% and progression-free survival of 9 to 14 months [11,18,19]. In addition, recent reports showed that ALK inhibition in NSCLC patients with the *ALK* rearrangement resulted in tumor shrinkage or stable disease in most patients [9]. Unfortunately, EGFR-TKI treatment was not effective in the tumor regression nor tumor marker level of present patient (disease might be controlled), but treatment with an ALK inhibitor resulted in SD with decreasing tumor markers. Therefore, this case showed that ALK rearrangement might be superior to EGFR mutation for the driver mutation.

It was reported that *EML4–ALK* fusion was associated with resistance to EGFR-TKIs [20]. Patients with NSCLC in the *EML4–ALK* cohort and the wild type cohort showed similar response rates to platinum-based combination chemotherapy and no difference in overall survival [20]. Whereas *EGFR* mutations confer sensitivity to EGFR-TKIs, *EML4–ALK* is strongly associated with resistance to EGFR-TKIs. In a previous case of concomitant *EGFR* mutation and *ALK* translocation, the patient presented the most durable response to an EGFR-TKI and was a case demonstrating no EML4–ALK expression by immunohistochemistry with an *EML4–ALK* rearrangement characterized by an isolated 3_ FISH signal [12]. Our patient presented a concurrent *EML4-ALK* rearrangement and ALK expression by immunohistochemistry; however, EGFR-TKI was not effective.

Among patients with both *EML4–ALK* rearrangement and *EGFR* mutation, *in vitro* studies showed that EGFR signaling can contribute to ALK inhibitor resistance in EML4–ALK NSCLC [14]. In addition, these findings suggested that a cancer cell line that harbors a concurrent *ALK* rearrangement and an *EGFR* mutation would be expected to be resistant to both single agent ALK and EGFR inhibitors [14]. We suggest that the combination of both ALK and EGFR inhibitors as early-line treatment may represent an effective therapy for this subset of NSCLC patients.

# Conclusions

This is the first clinical report of a patient with *EML4–ALK*-positive NSCLC with *EGFR* mutation that had a response of stable disease to both single-agent EGFR-TKI and ALK inhibitor. The *EML4–ALK* fusion gene defines a new molecular subset of NSCLCs with distinct clinical and pathologic features. NSCLCs with *ALK* rearrangement are highly sensitive to ALK inhibition. However, EGFR signaling may contribute to ALK inhibitor resistance in *EML4–ALK* NSCLC. Therefore, we suggest that this provides a translational opportunity whereby laboratory studies should be undertaken to understand the biological link between ALK rearrangement and *EGFR* mutation, with a view to establishing whether there is preclinical justification for using combination therapy for NSCLC with concomitant ALK rearrangement and *EGFR* mutation.

# Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

# Abbreviations

EML4, Echinoderm microtubule-associated protein-like 4; ALK, Anaplastic lymphoma kinase; NSCLC, Non-small cell lung cancer; EGFR, Epidermal growth factor receptor; TKI, Tyrosine kinase inhibitor; CT, Computed tomography; PAS, periodic acid–Schiff; TTF-1, Thyroid transcription factor-1; PNA-LNA, Peptide nucleic acid–locked nucleic acid; PCR, Polymerase chain reaction technique; FISH, Fluorescent in situ hybridization; SD, Stable disease; MRI, Magnetic resonance imaging (MRI); CEA, Carcinoembryonic antigen; RT-PCR, Reverse transcription polymerase chain reaction.

# Competing interests

The authors declare that they have no competing interests.

# Authors' contributions

AM prepared the manuscript and the literature search; RN and MS reviewed and edited the manuscript; HM and AG corrected and revised the manuscript; KS, KK, SK, YM, MS and TS treated and observed the patient; MK and ST performed the histopathological, immunohistochemical examinations; and AY, KH, KT, NY and YI reviewed the manuscript. All authors read and approved of the final manuscript.

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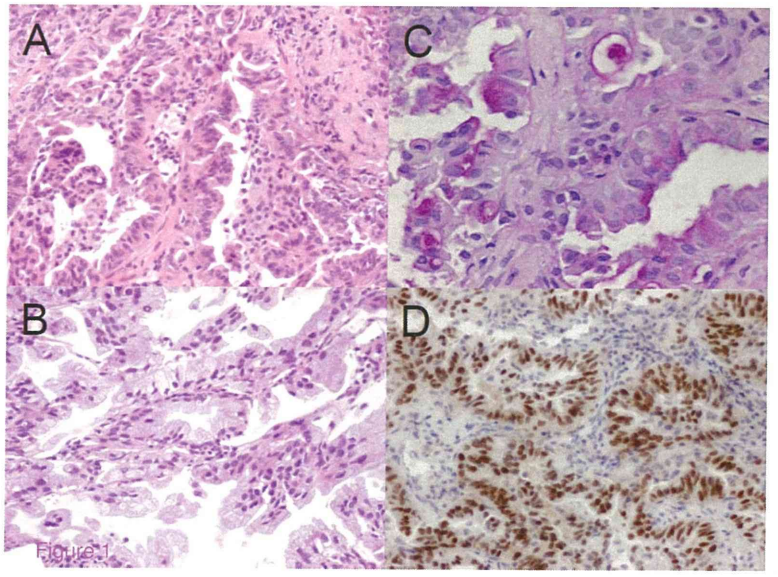
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Figure 1

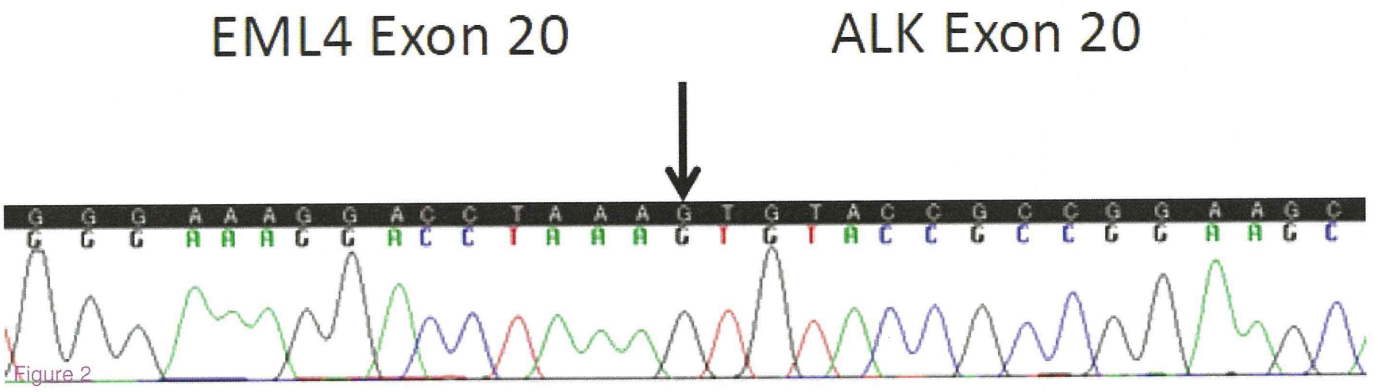


Figure 2

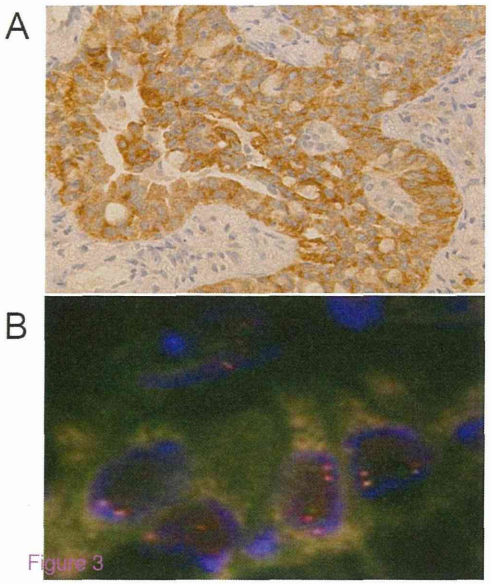


Figure 3

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# A case of synchronous bilateral lung cancers: EML4-ALK positive adenocarcinoma in the right lung and adenocarcinoma in situ (the former bronchioloalveolar carcinoma) in the left lung

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# A case of synchronous bilateral lung cancers: EML4-ALK positive adenocarcinoma in the right lung and adenocarcinoma in situ (the former bronchioloalveolar carcinoma) in the left lung

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## Abstract

### Background

Recently it has been revealed that lung adenocarcinomas with distinct gene mutations or fusions are associated with particular histopathological entities. For example, epidermal growth factor receptor (EGFR) gene mutations are often associated with well differentiated adenocarcinoma of the lung with bronchioloalveolar pattern. On the other hand, Echinoderm

microtubule associated protein-like 4 (EML4)-Anaplastic large cell lymphoma kinase (ALK) fusion gene in a subset of lung adenocarcinoma is related to mucinous cribriform histology.

### Case presentation

Reported herein is a case of synchronous EML4-ALK positive lung adenocarcinoma and adenocarcinoma in situ in the bilateral lungs of a 55-year-old Japanese woman. The woman had EML4-ALK positive lung adenocarcinoma in the right lower lung while adenocarcinoma in situ in the left upper lung, which was EML4-ALK negative.

### Conclusion

To our knowledge, this is the first report of synchronous, bilateral lung adenocarcinomas composed of EML4-ALK positive and negative ones.

## Keywords

EML4-ALK, Lung adenocarcinoma, Adenocarcinoma in situ

## Background

Adenocarcinomas of the lung comprise a group of diseases with heterogeneous clinicopathological characteristics [1,2]. Histopathologically, adenocarcinoma of the lung is composed of a subtype or mixture of subtypes, including adenocarcinoma in situ (the former bronchioloalveolar), papillary, or acinar ones [1,2].

Recent advances in molecular genomic analyses of lung adenocarcinoma specimens have revealed recurrent association of distinct gene mutations or fusions with particular clinicopathological entities [3]. For example, constitutively active mutations of epidermal growth factor receptor (EGFR) gene are often associated with well differentiated adenocarcinoma of the lung showing bronchioloalveolar pattern. Furthermore, using a functional cloning approach with foci-forming assay, Soda et al. [4] discovered Echinoderm-microtubule associated protein-like 4 (EML4)-Anaplastic large cell lymphoma kinase (ALK) fusion gene in a subset of lung adenocarcinoma. EML4-ALK positive lung adenocarcinoma typically occurs in young subjects with non- or low smoking habits [5,6]. Histologically, mucinous cribriform pattern is shown to be frequently associated with EML4-ALK positive lung adenocarcinoma [5-7].

Following this breakthrough, a number of kinase gene fusions have been identified in subsets of adenocarcinoma of the lung, the examples of which include RET or ROS1 fusions [7-9]. Most of these fusion genes contained genes for tyrosine kinases as a fusion partner, which are associated with constitutive (ligand-independent) activities.

Since the activities of these fused kinases and mutated kinases are shown to be tumorigenic in cell culture systems and/or transgenic mice, these kinases are promising candidates for therapeutic targets. In fact, small molecule tyrosine kinase inhibitors have shown dramatic therapeutic effects on subsets of lung adenocarcinoma, once oncogenic tyrosine kinases of