Int J Clin Exp Pathol 2016;9(7):7634-7644
www.ijcep.com /ISSN:1936-2625/IJCEP0028293

Case Report

Long-lasting response to crizotinib in NSCLC harboring EML4-ALK fusion gene and an EGFR mutation: a case report and review of the literature

Wei-Wei Ning[1*], Cheng Chen[1*], Feng Guo[2], Dong-Mei Gu[3], Ze-Yi Liu[1,4], Yuan-Yuan Zeng[1,4], Jian-Jie Zhu[1,4], Yuan-Yuan Guo[1], Xiao-Hui Zhang[1], Nan Zhang[1], Hui Zeng[1], Wen-Wen Du[1], Jian-An Huang[1,4]

[1]Department of Respiratory Medicine, The First Affiliated Hospital of Soochow University, Suzhou, China; [2]Central Laboratory, The First Affiliated Hospital of Soochow University, Suzhou, China; [3]Department of Pathology, The First Affiliated Hospital of Soochow University, Suzhou, China; [4]Institute of Respiratory Disease, Soochow University, Suzhou, China. [*]Equal contributors.

Received March 15, 2016; Accepted May 27, 2016; Epub July 1, 2016; Published July 15, 2016

Abstract: Lung cancer is the leading cause of cancer-related deaths worldwide. In recent years, molecular targeted therapy has been the most popular and promising development in non-small-cell lung carcinoma (NSCLC). The *EML4-ALK* fusion gene has defined a new molecular subtype of NSCLC, and this was generally thought to be mutually exclusive to other somatic mutations. However, some researchers have described rare cases of co-alterations of *ALK* and *EGFR* and/or *KRAS*. In this article, we report a patient with lung adenocarcinoma harboring the *EML4-ALK* fusion gene and an *EGFR* activating mutation in exon 19, who had shown no clinical response to EGFR-tyrosine kinase inhibitors (TKIs) but obtained a durable complete remission in the lungs and a partial remission of brain metastases with crizotinib treatment. To our knowledge, no previous article has reported a case of NSCLC with an *EGFR* mutation and *ALK* rearrangement that achieved long-lasting responses to crizotinib in both the lungs and brain.

Keywords: Non-small-cell lung carcinoma, echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase, epidermal growth factor receptor, crizotinib

Introduction

Most cancer-related deaths worldwide are due to lung cancer, around 80%-85% of which are non-small-cell lung carcinoma (NSCLC). As most NSCLC patients are diagnosed at an advanced stage, the median 5-year survival rate with traditional therapeutic approaches (surgery, radiotherapy and chemotherapy) is only about 10%. In recent years, molecular targeted therapy has been the most popular and promising development in NSCLC, especially in patients with lung adenocarcinoma. Among the relevant driver genes, the *EML4-ALK* (echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase) fusion gene has been identified as a new molecular target in NSCLC. Although *EML4-ALK* fusion was once regarded as mutually exclusive with *EGFR* (epidermal growth factor receptor) mutations and *KRAS* (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) mutations [1-3], an increasing number of cases of coexisting *EML4-ALK* fusion and *EGFR* mutations, and even triple mutations (*EML4-ALK* fusion gene coupled with *EGFR* and *KRAS* mutations) have been reported in patients with NSCLC in recent years [4].

The co-occurrence of *EGFR* and *EML4-ALK* fusion gene mutations has been described as a rare molecular event in NSCLC. Whereas the *EGFR* mutation rate in East Asian patients with NSCLC has been reported to be around 30%-35%, it is only 10% in the Caucasian population [5]. For *ALK* rearrangement, the incidence in NSCLC patients has been reported to be 5% [1, 6-8], and in the East Asian population 3%-11% [2, 8, 9]. In a recent study, Gainor et al. [3] identified 301 (17.88%) *EGFR* mutations and 75 (4.46%) *ALK* rearrangements in a group of 1683 Western patients with NSCLC, but no concomitant occurrences were observed. In a study

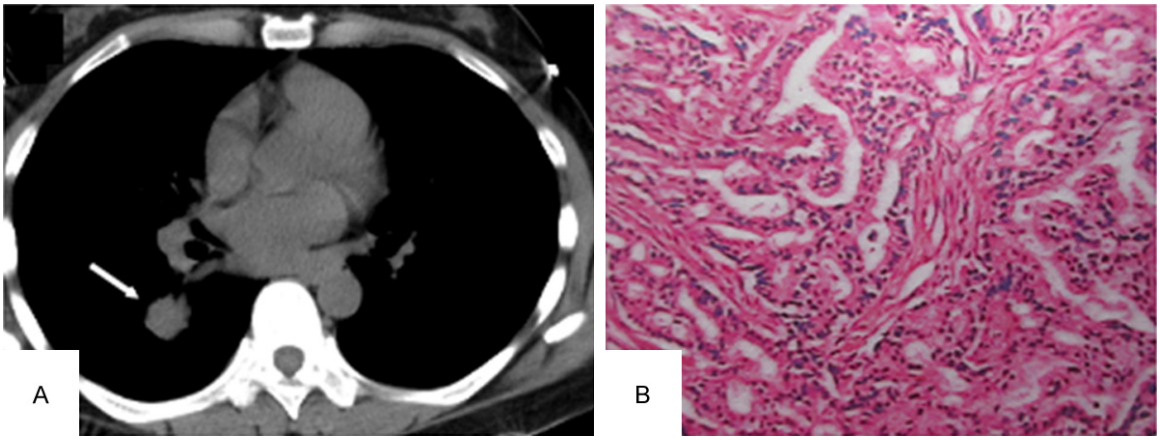

Figure 1. A. Preoperative computed tomography (CT) of the lung revealed a solitary nodule in the right lower lobe. B. Moderately differentiated pulmonary adenocarcinoma in tumor tissue obtained during surgery (HE, ×100).

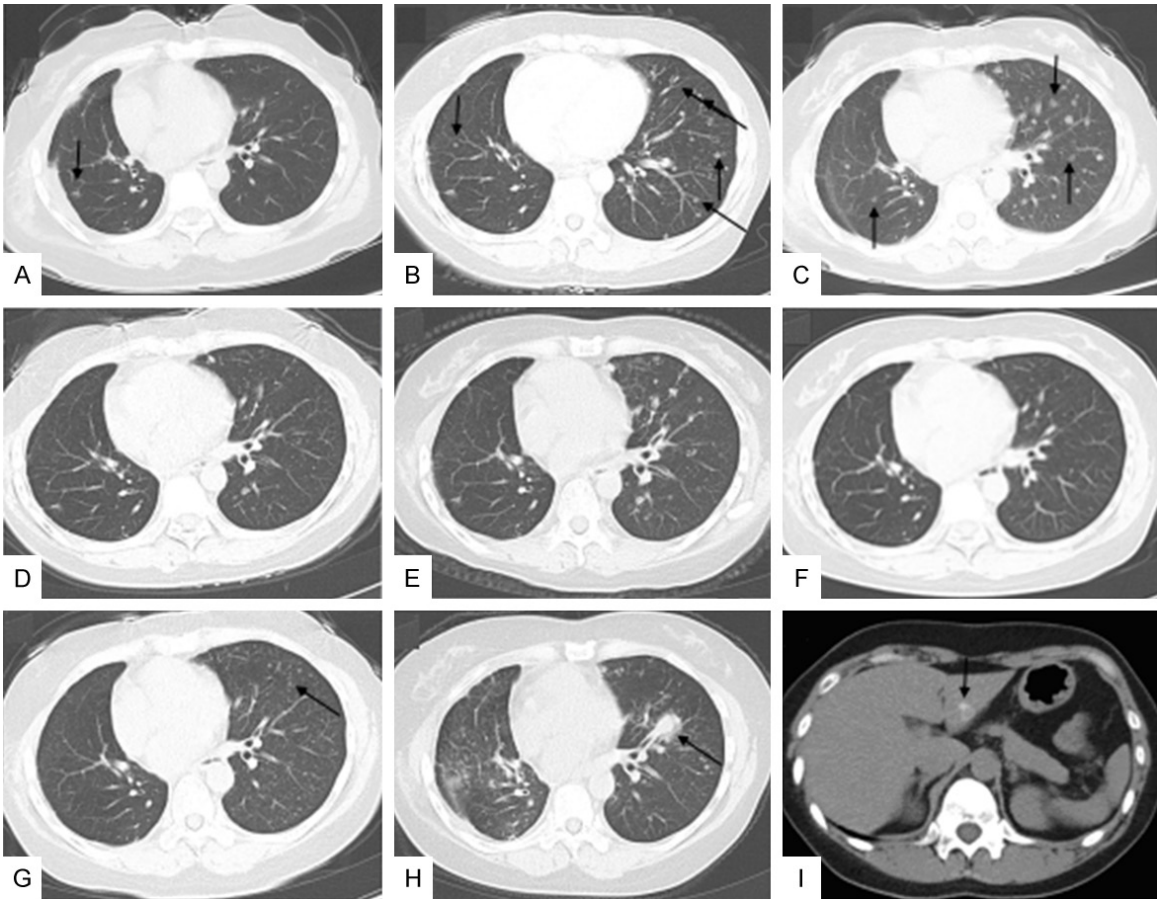

Figure 2. Computed tomography (CT) of the chest and abdomen after different therapies. A. A new nodule in the right lower lobe and right pleural effusion after postoperative radiotherapy and chemotherapy (August 2011). B. Multiple metastases in the lungs (June 2012). C. Further metastatic nodules in the lungs after treatment with erlotinib for 1 month (July 2012). D. Fewer metastatic nodules in the lungs during chemotherapy administration (cisplatin and pemetrexed) [November 2012]. E. More metastases in the lungs 4 months after sequential therapy with docetaxel and afatinib (May 2013). F. No significant metastases in both lungs after crizotinib treatment for 8 months (January 2014). G, H. New metastases in the lungs after crizotinib treatment for 14 months and 20 months, respectively (July 2014, January 2015). I. A metastasis appeared in the left lobe of the liver (October 2014).

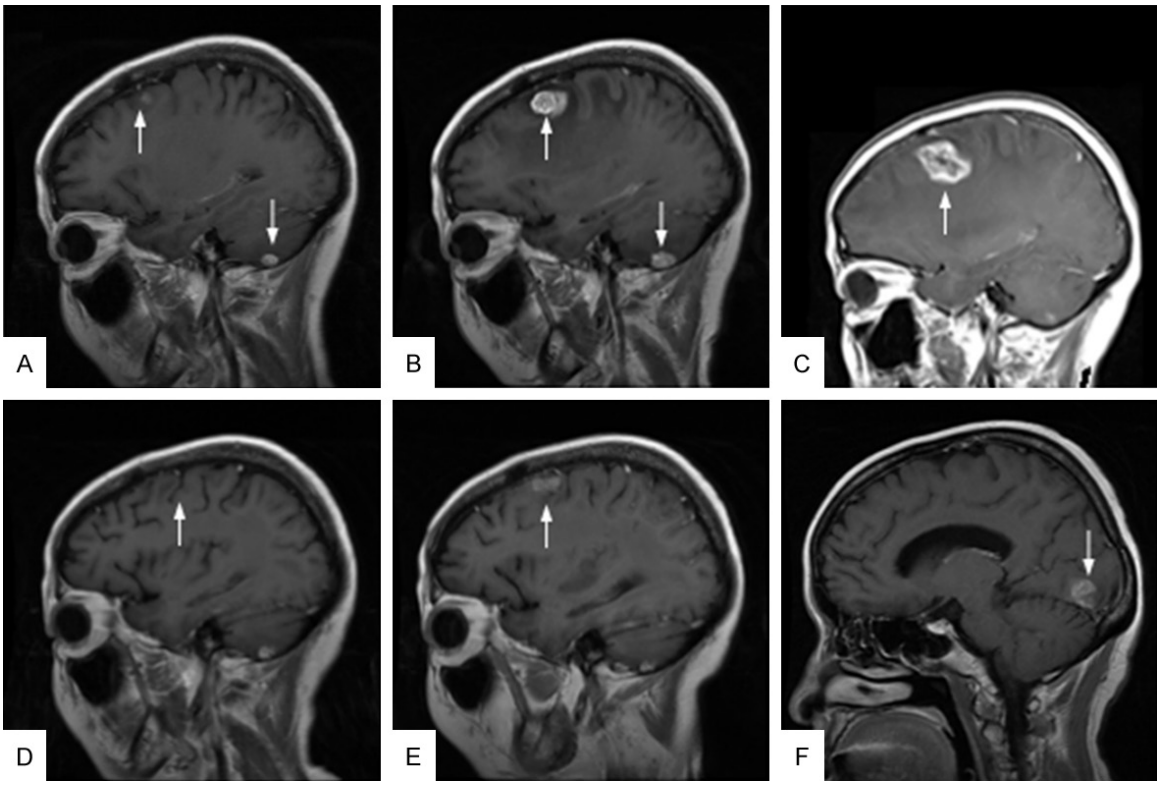

Figure 3. Magnetic resonance imaging (MRI) of the brain after different therapies. A. Metastases appeared in the left frontal lobe and cerebellum after postoperative radiotherapy and chemotherapy (February 2012). B. Enlargement of both metastatic sites of the brain during chemotherapy administration (cisplatin and pemetrexed) [November 2012]. C. Enlargement of the metastasis in the frontal lobe 4 months after sequential therapy with docetaxel and afatinib (May 2013). D. The brain lesions decreased dramatically after crizotinib treatment for 8 months (January 2014). E, F. Enlargement in the frontal lobe of brain after crizotinib treatment for 10 months, with a new metastasis appearing in the occipital lobe (March 2014).

in Chinese patients, Yang et al. [10] identified 336 (33.70%) *EGFR* mutations and 70 (7.02%) *ALK* rearrangements in a group of 997 NSCLC patients, and 13 of these (1.30%) had coexisting mutations.

In the following case report, we describe a patient with lung adenocarcinoma harboring the *EML4-ALK* fusion gene and an *EGFR* mutation in exon 19, who did not respond to EGFR-tyrosine kinase inhibitor (TKI) therapy but achieved a complete response in the lungs and partial remission of brain metastases with crizotinib treatment.

Case report

A 52-year-old Asian woman who had never smoked was referred to our hospital in December 2010 due to the discovery of a nodule in the right lower lobe field on a chest x-ray. A physical examination revealed no noticeable abnormalities. Computed tomography (CT) revealed a solitary nodule in the right lower lobe (**Figure 1A**), but no signs of a related metastasis were detected by ultrasonography of the abdomen, magnetic resonance imaging (MRI) of the brain, and a bone scan. Following exclusion of surgical contraindications, the patient underwent a right lower lobectomy together with a pulmonary hilar lymphadenectomy and a mediastinal lymphadenectomy. The postoperative pathological diagnosis of the neoplasm specimen was moderately differentiated adenocarcinoma (**Figure 1B**), with visceral pleura involvement and metastases of the hilar lymph nodes and mediastinal lymph nodes in groups of 2, 3, 4, 6, 10 and 11. A diagnosis of right lower lobe adenocarcinoma pT2N2M0 stage IIIA was therefore made. Four cycles of combined platinum and gemcitabine chemotherapy were administered at 3-week intervals, followed by radiotherapy for the N2 lymph node metastasis. However,

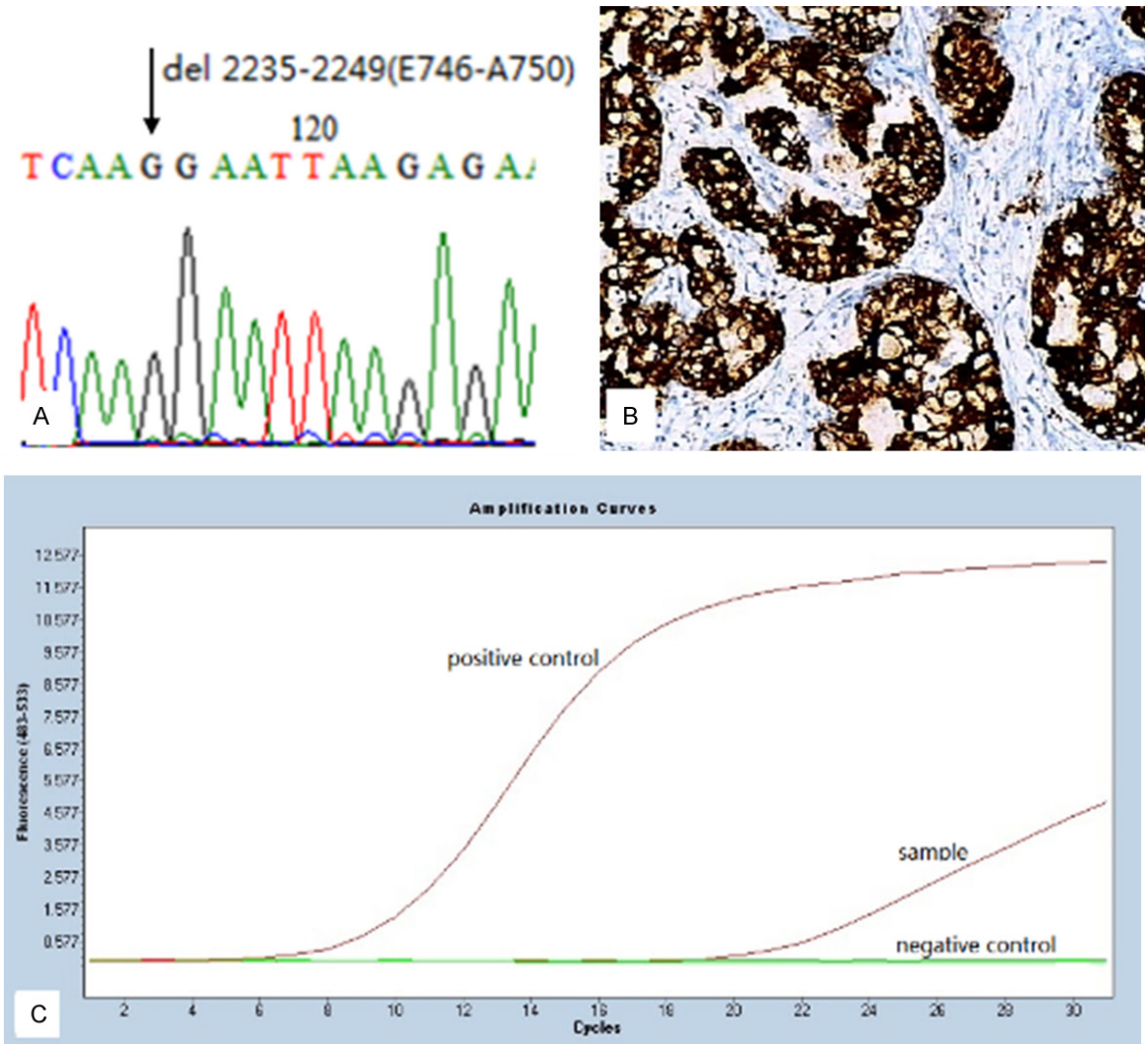

Figure 4. Tests for EGFR mutation (A) and EML4-ALK fusion gene (B, C). (A) A direct sequencing method showed EGFR del E746-A750 in exon 19. (B) ALK immunohistochemistry (IHC) showed that tumor cells were positive for ALK (D5F3) [×200]. (C) EML4-ALK fusion gene was positive by Cycleave probe RT-PCR.

CT imaging of the chest revealed a new suspicious nodule in the right lower lobe and right pleural effusion (**Figure 2A**), and MRI revealed metastases in the brain (**Figure 3A**) at 3 and 9 months after radiotherapy and chemotherapy, respectively. She then received palliative radiation of the brain.

The patient was found to have significant disease progression in June 2012 when follow-up examinations revealed multiple metastases in her lungs (**Figure 2B**) and enlargement of the right supraclavicular and infraclavicular lymph nodes, with a stable lesion in the brain. A genotyping test using tumor tissue obtained during surgery was performed via a direct sequencing method, which revealed *EGFR* del E746-A750 in exon 19 (**Figure 4A**). The patient was then started on treatment with erlotinib 150 mg daily (early June 2012). However, after just 1 month of erlotinib treatment, further metastases were found in her lungs (**Figure 2C**) and there were multiple lesions in the bones, but the brain foci were still stable. Erlotinib treatment was therefore abandoned and the patient was switched to a combined cisplatin and pemetrexed chemotherapy regimen for 4 cycles, followed by single-agent pemetrexed therapy for another 4 cycles. This achieved a partial response in the lungs (**Figure 2D**) but the disease in the brain progressed during this time (**Figure 3B**). The patient subsequently

Table 1. Summary of data from studies of NSCLC patients harboring *EGFR* mutations or *ALK* rearrangement

Reference [No. of patients]	Histology/stage	Distant metastases	Sample (before/after treatment)	*EGFR* mutation, *ALK*+	Treatment [outcome]		
					Chemotherapy	EGFR-TKIs	Crizotinib
Patients who received both EGFR-TKI and ALK inhibitor therapy:							
Present study [n = 1]	AC/IIIA	Brain, bone, lymph nodes, pleura	Tumor tissue (before)	del exon 19, FISH+/IHC+	Pt+Gem (1st line) [PR] Cis+Pem (3rd line) [PR] Pem (maintenance) [PR] Doce (4th line) [SD]	Erlotinib (2nd line) [PD] Afatinib (4th line) [SD]	5th line [CR]
Yang et al. [10] [n = 4]	AC/IIIA			del exon 19, FISH+/IHC+		Erlotinib (1st line) [PD]	3rd line [PR]
	AC/IV			exon 20 insertion, FISH+/IHC+		Afatinib (1st line) [PR]	[PD]
	AC/IV			del exon 19, FISH+/RT-PCR+/IHC+		Erlotinib (1st line) [PR]	[SD]
	AC/IV			del exon 19, FISH+/RT-PCR+/IHC+		Gefitinib (3rd line) [PD]	[PR]
Miyanaga et al. [19] [n = 1]	AC/IV	Bones	Tumor tissue (before)	del exon 19, FISH+/RT-PCR+/IHC+	Cis+Pem (1st line) [SD] Doce (4th line) [PR]	Gefitinib (2nd line) [SD] Erlotinib (3rd line) [SD]	5th line [SD]
Chen et al. [21] [n = 1]	AC/IV	Lymph nodes	Tumor tissue (before) Lymph node (after 2nd line)	del exon 19, ARMS-/RT-PCR- del exon 19, FISH+/RT-PCR+	Cis+Gem (1st line) [Intolerant]	Erlotinib (2nd line) [SD]	3rd line [CR]
Chiari et al. [26] [n = 1]	AC/IV	Pleura, brain, bones	Pleura (before) Tumor tissue (after 5th line)	L858R exon 21, FISH-/IHC+ Wild-type, FISH+/IHC+	Cis+Gem (1st line) [SD] Carbo+Pem (3rd line) [SD] Doce+Gem (5th line) [SD] Pem (6th line) [PD]	Gefitinib (2nd line) [PR] Erlotinib/afatinib (4th line) [PD]	7th line [PR]
Baldi et al. [27] [n = 1]	AC/IV		Tumor tissue (before and after 2nd line)	L858R exon 21, FISH+/IHC+	Cis+Pem (1st line) [SD] Pem (maintenance) [PD]	Erlotinib (2nd line) [SD]	3rd line [PR]
Lee et al. [28] [n = 1]	AC/IV	Brain, bones, lymph nodes	Tumor tissue (before)	del exon 19, FISH+/IHC+		Gefitinib (1st line) [PD]	2nd line [PR]
Patients who received EGFR-TKI or ALK inhibitor therapy:							
Yang et al. [10] [n = 7]	AC/IV			del exon 19, FISH+/RT-PCR+/IHC+		Gefitinib (1st line) [PR]	
	AC/IV			L858R exon 21, FISH+/RT-PCR+/IHC+		Gefitinib (1st line) [PR]	
	AC/IV			L858R exon 21, FISH+/RT-PCR+/IHC+		Erlotinib (1st line) [PR]	
	AC/IV			L858R exon 21, FISH+/RT-PCR+		Erlotinib (1st line) [PR]	
	AC/IV			del exon 19, FISH+/RT-PCR+/IHC+		Erlotinib (1st line) [PR]	
	AC/IV			del exon 19, FISH+/RT-PCR+/IHC+		Afatinib (1st line) [SD]	
	AC/IV			L858R exon 21, FISH+		Gefitinib (1st line) [PR]	
Sasaki et al. [13] [n = 1]	NOS			L858R exon 21, FISH+/IHC- del exon 19, FISH+/IHC-		Erlotinib [PR] Erlotinib [PR]	
Kuo et al. [14] [n = 1]	AC/IV	Pleura, brain, bones	Tumor tissue (before)	del exon 19, RT-PCR+		Gefitinib (1st line) [PR]	
Tiseo et al. [15] [n = 1]	SqAC/IV	Bones, liver, lymph nodes	Tumor tissue (after 1st line) Lmph node (after 1st line)	del exon 19, FISH- del exon 19, FISH+	Cis+Gem (1st line) [PR]	Erlotinib (2nd line) [PD]	
Popat et al. [16] [n = 1]	AC/IIIA		Tumor tissue (before)	del exon 19, FISH+/IHC-	Carbo+Vin (1st line) [PR]	Erlotinib (2nd line) [CR]	

Tanaka et al. [18] [n = 1]	AC/IV	Bones, liver	Tumor tissue (after 2nd line) Tumor tissue* (after 3rd line)	L858R exon 21, NA NA, RT-PCR+/IHC+	Cis+Doce (1st line) [SD] Pem (2nd line) [PR]	Erlotinib (3rd line) [PD]	
Santelmo et al. [20] [n = 1]	AC/IIIA		Lymph node** (before) Tumor tissue (after)	del exon 19, FISH+ del exon 19, FISH+		Gefitinib (neoadjuvant) [PR]	
Jurgens et al. [22] [n = 1]	AC/IV	Bones, lymph node	Tumor tissue (before)	861 exon 21, FISH+	Pem+Carbo+Beva (2nd line) [PR] Pem (maintenance) [PD]	Gefitinib (1st line) [PD]	
Cabillic et al. [23] [n = 4]	AC		Tumor tissue (before)	L858R exon 21, FISH+/IHC-		Gefitinib (1st line)	NA
	NOS		Tumor tissue (before)	L858R exon 21, FISH+/IHC-		Gefitinib (1st line)	NA
	AC		Tumor tissue (before)	L858R exon 21, FISH-/IHC+		Gefitinib (1st line)	NA
	AC		Tumor tissue (before)	L858R+T790M, FISH+/IHC-			NA [SD/PR]
Patients who did not receive targeted therapy:							
Zhang et al. [2] [n = 1]	AC			del exon 19, RT-PCR+			
Yang et al. [10] [n = 2]	AC/IIIA			del exon 19, FISH+/RT-PCR+/IHC+			
	AC/IV			K757R in exon 19, FISH+/RT-PCR+			
Koivunen et al. [11] [n = 1]	AC			del exon 19, FISH+/RT-PCR+			
Sasaki et al. [13] [n = 1]	NOS			A767_V769dupASV exon 19, FISH+/IHC+			
Toyokawa et al. [17] [n = 1]		SCLC+AC		del exon 19, direct sequencing method(+)/IHC+***			
Cabillic et al. [23] [n = 4]	AC		Tumor tissue	L858R exon 21, FISH-/IHC+			
	AC		Tumor tissue	del exon 19, FISH-/IHC+			
	AC		Tumor tissue	del exon 19, FISH-/IHC+			
	AC		Metastasis	del exon 19, IHC+			
Wang et al. [24] [n = 1]	AC			L858R exon 21, FISH+			
Kim et al. [25] [n = 5]	AC/IV			del exon 19, FISH+			
	AC/I			del exon 19, FISH+			
	AC/IV			del exon 19, FISH+			
	SqAC/IV			L858R exon 21, FISH+			
	AC/IV			L858R exon 21, FISH+			
Lee et al. [28] [n = 3]	AC/IIB			del exon 19, FISH+/IHC+			
	AC/IIIA			L718P exon 18, FISH+/IHC+			
	AC/IA			L858R exon 21, FISH+			

*Tumor tissue from re-biopsy. **Mediastinal lymph nodes from EBUS-TBNA. ***Deletion in exon 19 of *EGFR* was detected only in the AC component; only the NSCLC component harbored the *EML4-ALK* fusion gene. AC, adenocarcinoma; *ALK*, anaplastic lymphoma kinase; Beva, bevacizumab; Carbo, carboplatin; Cis, cisplatin; CR, complete response; Doce, docetaxel; EBUS-TBNA, endobronchial ultrasound transbronchial needle aspiration; *EGFR*, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; NA, not available; NOS, not otherwise specified; NSCLC, non-small-cell lung cancer; Pem, pemetrexed; PD, progressive disease; PR, partial response; Pt, platinum; RT-PCR, real-time-polymerase chain reaction; SCLC, small-cell lung carcinoma; SD, stable disease; SqAC, squamous adenocarcinoma; Vin, vinorelbine.

received brain radiotherapy at another hospital. Sequential therapy with docetaxel and afatinib was then given. This achieved stable disease in both metastatic sites for a period of 2 months but disease progression in both lungs, the brain, and bones was evident 2 months later (**Figures 2E**, **3C**). Immunohistochemistry (IHC) testing for *ALK* fusion verified *ALK* expression in the tumor (**Figure 4B**), and Cycleave probe real-time polymerase chain reaction (RT-PCR) methodology confirmed *EML4-ALK* positivity (**Figure 4C**).

In May 2013, the patient was started on treatment with the ALK inhibitor, crizotinib, at a dosage of 250 mg twice a day. Eight months later, no significant metastasis was found in her lungs, and the brain lesions had decreased dramatically (**Figures 2F**, **3D**). There was no evidence of disease progression until the appearance of new metastases in the brain in March 2014 (**Figure 3E**, **3F**) and in the lungs in July 2014 (**Figure 2G**). At a later follow-up, a metastatic mass appeared in her left lung (**Figure 2H**), along with a metastasis in the liver (**Figure 2I**). Ultimately, we discontinued the ALK inhibitor treatment and instituted hospice care for deterioration of the patient's condition.

Discussion

In 2009, Shaw et al. [7] defined ALK-positive lung cancer as a specific molecular subset of NSCLC. *ALK* rearrangement in patients with NSCLC exhibits distinct clinical pathological characteristics, including a younger age, light or no smoking, and adenocarcinoma histology [1, 8, 11, 12]. Generally, *EML4-ALK* fusion gene and other oncogenic drivers such as *EGFR* and *KRAS* mutations are mutually exclusive. However, rare cases of *EGFR* mutations and *EML4-ALK* fusion gene coexisting in NSCLC have been reported in recent years (since 2007). However, statistical tests have not been performed thus far, because of deficiencies in samples and in the available information to evaluate the response rate or survival rate and make qualitative judgments about the clinicopathological features.

At least 47 cases of concurrent *ALK* translocation and *EGFR* mutations have been described in 19 previous reports [2, 10, 11, 13-28]. The available information on these cases are summarized in **Table 1**. Although concomitant *ALK* rearrangement and *EGFR* mutations occurred in all of these cases, the *EGFR* mutations were diverse. All of the patients had coexisting *ALK* rearrangement and single exon *EGFR* mutations, including the patient documented in this report. The characteristics of our patient were young age, non-smoking history, and adenocarcinoma histology, which are similar to those of *ALK*-positive NSCLC patients. After only 1 month of erlotinib treatment, the patient displayed massive disease progression but following sequential treatment with docetaxel and afatinib for 2 months, she then had stable disease (which we think was more likely due to docetaxel than to afatinib).

In patients with advanced NSCLC harboring *EGFR* mutations, it has been found that EGFR-TKI treatment achieves a response rate of more than 60% and progression-free survival of 9 to 14 months [29-31]. However, different response rates with EGFR-TKIs have been reported in patients with coexisting mutations. Some researchers have suggested that *EML4-ALK* fusion may be responsible for primary or acquired resistance to EGFR-TKIs [22, 29-31]. The patient described in this report was found to have disease progression in the lungs after 1 month of erlotinib treatment, and no significant response in the brain. Santelmo et al. [20] and Kuo et al. [14] have both described patients identified as having coexisting mutations before receiving chemotherapy and EGFR-TKI therapy, who acquired resistance to gefitinib after having a good initial response to it. The reason for this phenomenon is currently not clear.

Clinical trials in patients with advanced NSCLC with *ALK* rearrangement have demonstrated greater clinical benefit from crizotinib than from traditional chemotherapy, with higher response rates and longer progression-free survival with second-line treatment with this ALK inhibitor [32]. However, the majority of NSCLC patients with *ALK* rearrangement ultimately acquire resistance to crizotinib, despite showing a rapid and marked response initially. From a literature search, we identified at least 10 patients with coexisting mutations who had received treatment with crizotinib [10, 19, 21, 23, 26-28]. Among these patients, 9 showed a good response, but the remaining patient did not. The patient described in this report responded positively to crizotinib, with a complete remis-

sion in the lungs for 14 months (May 2013 to July 2014) and a partial remission in the brain for 10 months (May 2013 to March 2014).

Currently, there is controversy over the optimal treatment of patients with NSCLC who have both *EGFR* mutations and *ALK* translocation. Among the cases we identified in the literature, most tended to receive EGFR-TKIs initially, and then receive crizotinib after the occurrence of resistance to EGFR-TKIs. It has been suggested the relative phospho-ALK and phospho-EGFR levels in such patients may be predictive factors for the efficacy of EGFR-TKIs and crizotinib [10].

Metastatic involvement of the brain is common in patients with NSCLC, resulting in a heavy symptom and cost burden for patients. Many studies have shown that NSCLC patients with *ALK* rearrangement have a higher risk of brain metastasis than patients with other NSCLC subtypes [33-35]. Other studies have suggested that the poor brain penetration of crizotinib may be an important mechanism of resistance to this drug (i.e., pharmacokinetic rather than genetic resistance), and may result in a limited clinical response [36-38]. However, there have been some reports of a good effect of crizotinib on brain metastasis in *ALK*-positive NSCLC patients [39, 40], as there have been with EGFR-TKIs with low brain permeability, which have sometimes proved effective for NSCLC harboring *EGFR* mutations and brain metastasis. Of note, only 3 patients in the studies we located from our literature search were reported to have brain metastases. In a patient described by Chiari et al. [26], brain metastases appeared after treatment with crizotinib for almost 2 years. Another 2 cases with brain metastases were diagnosed prior to treatment with EGFR-TKIs and/or crizotinib, but no information about the brain responses were provided by the authors. Surprisingly, the patient described in the present report not only displayed a durable complete response in the lungs with crizotinib treatment, but also had a long-lasting partial remission in the brain.

In conclusion, the patient with lung adenocarcinoma harboring *ALK* rearrangement and an *EGFR* mutation in exon 19 described in this report who had progressed on treatment with erlotinib, showed a good response to crizotinib and standard chemotherapy in both the lungs and brain, with a survival time from the initial diagnosis of 4 years. Advanced NSCLCs with such co-alterations could have diverse responses to EGFR-TKIs and crizotinib, as *ALK* rearrangement is a potential mechanism for primary or acquired assistance to EGFR-TKIs. Brain metastases in NSCLC patients with coexisting *EGFR* mutations and *ALK* rearrangement should be further studied to identify the mechanisms underlying different responses to TKIs.

Acknowledgements

We thank the patient with NSCLC described in this report for her participation and cooperation. This work was supported by grants from the Clinical Key Specialty Project of China, the National Natural Science Foundation of China (No. 31270940 to J. Huang; and No. 81201575 to Z. Liu) and the Clinical Medical Center of Suzhou.

Disclosure of conflict of interest

None.

Address correspondence to: Jian-An Huang, Department of Respiratory Medicine, The First Affiliated Hospital of Soochow University, 188 Shizi Street, Suzhou 215006, China. Tel: +86 512-677-80050; Fax: +86 512-652-25636; E-mail: huang_jian_an@163.com

References

[1] Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, Bando M, Ohno S, Ishikawa Y, Aburatani H, Niki T, Sohara Y, Sugiyama Y and Mano H. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007; 448: 561-566.

[2] Zhang X, Zhang S, Yang X, Yang J, Zhou Q, Yin L, An S, Lin J, Chen S, Xie Z, Zhu M and Wu YL. Fusion of EML4 and ALK is associated with development of lung adenocarcinomas lacking EGFR and KRAS mutations and is correlated with ALK expression. Mol Cancer 2010; 9: 188.

[3] Gainor JF, Varghese AM, Ou SH, Kabraji S, Awad MM, Katayama R, Pawlak A, Mino-Kenudson M, Yeap BY, Riely GJ, Iafrate AJ, Arcila ME, Ladanyi M, Engelman JA, Dias-Santagata D and Shaw AT. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small

cell lung cancer. Clin Cancer Res 2013; 19: 4273-4281.

[4] Rossing HH, Grauslund M, Urbanska EM, Melchior LC, Rask CK, Costa JC, Skov BG, Sorensen JB and Santoni-Rugiu E. Concomitant occurrence of EGFR (epidermal growth factor receptor) and KRAS (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) mutations in an ALK (anaplastic lymphoma kinase)-positive lung adenocarcinoma patient with acquired resistance to crizotinib: a case report. BMC Res Notes 2013; 6: 489.

[5] Shi Y, Au JS, Thongprasert S, Srinivasan S, Tsai CM, Khoa MT, Heeroma K, Itoh Y, Cornelio G and Yang PC. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). J Thorac Oncol 2014; 9: 154-162.

[6] Inamura K, Takeuchi K, Togashi Y, Hatano S, Ninomiya H, Motoi N, Mun MY, Sakao Y, Okumura S, Nakagawa K, Soda M, Choi YL, Mano H and Ishikawa Y. 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: 508-515.

[7] Shaw AT, Yeap BY, Mino-Kenudson M, Digumarthy SR, Costa DB, Heist RS, Solomon B, Stubbs H, Admane S, McDermott U, Settleman J, Kobayashi S, Mark EJ, Rodig SJ, Chirieac LR, Kwak EL, Lynch TJ and Iafrate AJ. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009; 27: 4247-4253.

[8] Wong DW, Leung EL, So KK, Tam IY, Sihoe AD, Cheng LC, Ho KK, Au JS, Chung LP and Pik Wong M. 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: 1723-1733.

[9] Li H, Pan Y, Li Y, Li C, Wang R, Hu H, Zhang Y, Ye T, Wang L, Shen L, Sun Y and Chen H. Frequency of well-identified oncogenic driver mutations in lung adenocarcinoma of smokers varies with histological subtypes and graduated smoking dose. Lung Cancer 2013; 79: 8-13.

[10] Yang JJ, Zhang XC, Su J, Xu CR, Zhou Q, Tian HX, Xie Z, Chen HJ, Huang YS, Jiang BY, Wang Z, Wang BC, Yang XN, Zhong WZ, Nie Q, Liao RQ, Mok TS and Wu YL. Lung cancers with concomitant EGFR mutations and ALK rearrangements: diverse responses to EGFR-TKI and crizotinib in relation to diverse receptors phosphorylation. Clin Cancer Res 2014; 20: 1383-1392.

[11] Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, Choi HG, Kim J, Chiang D, Thomas R, Lee J, Richards WG, Sugarbaker DJ, Ducko C, Lindeman N, Marcoux JP, Engelman JA, Gray NS, Lee C, Meyerson M and Janne PA. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008; 14: 4275-4283.

[12] Perner S, Wagner PL, Demichelis F, Mehra R, Lafargue CJ, Moss BJ, Arbogast S, Soltermann A, Weder W, Giordano TJ, Beer DG, Rickman DS, Chinnaiyan AM, Moch H and Rubin MA. EML4-ALK fusion lung cancer: a rare acquired event. Neoplasia 2008; 10: 298-302.

[13] Sasaki T, Koivunen J, Ogino A, Yanagita M, Nikiforow S, Zheng W, Lathan C, Marcoux JP, Du J, Okuda K, Capelletti M, Shimamura T, Ercan D, Stumpfova M, Xiao Y, Weremowicz S, Butaney M, Heon S, Wilner K, Christensen JG, Eck MJ, Wong KK, Lindeman N, Gray NS, Rodig SJ and Janne PA. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res 2011; 71: 6051-6060.

[14] Kuo YW, Wu SG, Ho CC and Shih JY. Good response to gefitinib in lung adenocarcinoma harboring coexisting EML4-ALK fusion gene and EGFR mutation. J Thorac Oncol 2010; 5: 2039-2040.

[15] Tiseo M, Gelsomino F, Boggiani D, Bortesi B, Bartolotti M, Bozzetti C, Sammarelli G, Thai E and Ardizzoni A. EGFR and EML4-ALK gene mutations in NSCLC: a case report of erlotinib-resistant patient with both concomitant mutations. Lung Cancer 2011; 71: 241-243.

[16] Popat S, Vieira de Araujo A, Min T, Swansbury J, Dainton M, Wotherspoon A, Lim E, Nicholson AG and O'Brien ME. Lung adenocarcinoma with concurrent exon 19 EGFR mutation and ALK rearrangement responding to erlotinib. J Thorac Oncol 2011; 6: 1962-1963.

[17] Toyokawa G, Taguchi K, Ohba T, Morodomi Y, Takenaka T, Hirai F, Yamaguchi M, Seto T, Takenoyama M, Sugio K and Ichinose Y. First case of combined small-cell lung cancer with adenocarcinoma harboring EML4-ALK fusion and an exon 19 EGFR mutation in each histological component. J Thorac Oncol 2012; 7: e39-41.

[18] Tanaka H, Hayashi A, Morimoto T, Taima K, Tanaka Y, Shimada M, Kurose A, Takanashi S and Okumura K. A case of lung adenocarcinoma harboring EGFR mutation and EML4-ALK fusion gene. BMC Cancer 2012; 12: 558.

[19] Miyanaga A, Shimizu K, Noro R, Seike M, Kitamura K, Kosaihira S, Minegishi Y, Shukuya T, Yoshimura A, Kawamoto M, Tsuchiya S, Hagiwara K, Soda M, Takeuchi K, Yamamoto N, Mano H, Ishikawa Y and Gemma A. Activity of EGFR-tyrosine kinase and ALK inhibitors for EML4-ALK-rearranged non-small-cell lung cancer harbored coexisting EGFR mutation. BMC Cancer 2013; 13: 262.

[20] Santelmo C, Ravaioli A, Barzotti E, Papi M, Poggi B, Drudi F, Mangianti M, Salvi M and Crino L. Coexistence of EGFR mutation and ALK trans-

location in NSCLC: literature review and case report of response to gefitinib. Lung Cancer 2013; 81: 294-296.
[21] Chen X, Zhang J, Hu Q, Li X and Zhou C. A case of lung adenocarcinoma harboring exon 19 EGFR deletion and EML4-ALK fusion gene. Lung Cancer 2013; 81: 308-310.
[22] Jurgens J, Engel-Riedel W, Prickartz A, Ludwig C, Schildgen O, Tillmann RL, Stoelben E, Brockmann M and Schildgen V. Combined point mutation in KRAS or EGFR genes and EML4-ALK translocation in lung cancer patients. Future Oncol 2014; 10: 529-532.
[23] Cabillic F, Gros A, Dugay F, Begueret H, Mesturoux L, Chiforeanu DC, Dufrenot L, Jauffret V, Dachary D, Corre R, Lespagnol A, Soler G, Dagher J, Catros V, Le Calve M, Merlio JP and Belaud-Rotureau MA. Parallel FISH and immunohistochemical studies of ALK status in 3244 non-small-cell lung cancers reveal major discordances. J Thorac Oncol 2014; 9: 295-306.
[24] Wang J, Dong Y, Cai Y, Zhou L, Wu S, Liu G, Su D, Li X, Qin N, Nong J, Jia H, Zhang Q, Mu J, Zeng X, Zhang H, Zhang S and Zhang Z. Clinicopathologic characteristics of ALK rearrangements in primary lung adenocarcinoma with identified EGFR and KRAS status. J Cancer Res Clin Oncol 2014; 140: 453-460.
[25] Kim TJ, Park CK, Yeo CD, Park K, Rhee CK, Kim J, Kim SJ, Lee SH, Lee KY and Yoon HK. Simultaneous diagnostic platform of genotyping EGFR, KRAS, and ALK in 510 Korean patients with non-small-cell lung cancer highlights significantly higher ALK rearrangement rate in advanced stage. J Surg Oncol 2014; 110: 245-251.
[26] Chiari R, Duranti S, Ludovini V, Bellezza G, Pireddu A, Minotti V, Bennati C and Crino L. Long-term response to gefitinib and crizotinib in lung adenocarcinoma harboring both epidermal growth factor receptor mutation and EML4-ALK fusion gene. J Clin Oncol 2014; 32: e30-32.
[27] Baldi L, Mengoli MC, Bisagni A, Banzi MC, Boni C and Rossi G. Concomitant EGFR mutation and ALK rearrangement in lung adenocarcinoma is more frequent than expected: report of a case and review of the literature with demonstration of genes alteration into the same tumor cells. Lung Cancer 2014; 86: 291-295.
[28] Lee JK, Kim TM, Koh Y, Lee SH, Kim DW, Jeon YK, Chung DH, Yang SC, Kim YT, Kim YW, Heo DS and Bang YJ. Differential sensitivities to tyrosine kinase inhibitors in NSCLC harboring EGFR mutation and ALK translocation. Lung Cancer 2012; 77: 460-463.
[29] Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S and Nukiwa T. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010; 362: 2380-2388.
[30] Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, Chao TY, Nakagawa K, Chu DT, Saijo N, Duffield EL, Rukazenkov Y, Speake G, Jiang H, Armour AA, To KF, Yang JC and Mok TS. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011; 29: 2866-2874.
[31] Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, Takada M, Yoshioka H, Shibata K, Kudoh S, Shimizu E, Saito H, Toyooka S, Nakagawa K and Fukuoka M. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010; 11: 121-128.
[32] Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D, Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD and Janne PA. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med 2013; 368: 2385-2394.
[33] Paik JH, Choi CM, Kim H, Jang SJ, Choe G, Kim DK, Kim HJ, Yoon H, Lee CT, Jheon S, Choe JY and Chung JH. Clinicopathologic implication of ALK rearrangement in surgically resected lung cancer: a proposal of diagnostic algorithm for ALK-rearranged adenocarcinoma. Lung Cancer 2012; 76: 403-409.
[34] Kang HJ, Lim HJ, Park JS, Cho YJ, Yoon HI, Chung JH, Lee JH and Lee CT. Comparison of clinical characteristics between patients with ALK-positive and EGFR-positive lung adenocarcinoma. Respir Med 2014; 108: 388-394.
[35] Fallet V, Cadranel J, Doubre H, Toper C, Monnet I, Chinet T, Oliviero G, Foulon G, De Cremoux H, Vieira T, Antoine M and Wislez M. Prospective screening for ALK: clinical features and outcome according to ALK status. Eur J Cancer 2014; 50: 1239-1246.
[36] Costa DB, Kobayashi S, Pandya SS, Yeo WL, Shen Z, Tan W and Wilner KD. CSF concentration of the anaplastic lymphoma kinase inhibitor crizotinib. J Clin Oncol 2011; 29: e443-445.
[37] Gainor JF, Ou SH, Logan J, Borges LF and Shaw AT. The central nervous system as a sanctuary

site in ALK-positive non-small-cell lung cancer. J Thorac Oncol 2013; 8: 1570-1573.
[38] Peled N, Zach L, Liran O, Ilouze M, Bunn PA Jr and Hirsch FR. Effective crizotinib schedule for brain metastases in ALK rearrangement metastatic non-small-cell lung cancer. J Thorac Oncol 2013; 8: e112-113.
[39] Kinoshita Y, Koga Y, Sakamoto A and Hidaka K. Long-lasting response to crizotinib in brain metastases due to EML4-ALK-rearranged non-small-cell lung cancer. BMJ Case Rep 2013; 2013.
[40] Kaneda H, Okamoto I and Nakagawa K. Rapid response of brain metastasis to crizotinib in a patient with ALK rearrangement-positive non-small-cell lung cancer. J Thorac Oncol 2013; 8: e32-33.