Int J Clin Exp Med 2017;10(8):12782-12789
www.ijcem.com /ISSN:1940-5901/IJCEM0049479

Original Article

The diagnostic and follow-up role of radiological examination in advanced lung adenocarcinoma with anaplastic lymphoma kinase gene rearrangement

Li Zhang[1], Ning Wu[1,2], Meng Li[1], Jianming Ying[3], Han Ouyang[1], Chenghuan Yang[1], Lv Lv[1]

Departments of [1]Diagnostic Radiology, [3]Pathology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; [2]PET-CT Center, Department of Diagnostic Radiology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Received December 10, 2016; Accepted April 22, 2017; Epub August 15, 2017; Published August 30, 2017

Abstract: This study aimed to determine the imaging characteristics of patients with advanced lung adenocarcinoma who harbor anaplastic lymphoma kinase gene mutation (ALK+) and to explore the follow-up value of radiological examination of these patients. Forty patients with stage IV lung adenocarcinoma were enrolled, including 20 ALK+ patients (study group) and 20 patients with no ALK mutation (ALK-) (control group). In the study group, 20 patients were treated with crizotinib or chemotherapy randomly. All patients underwent serial enhanced neck-thoracic computed tomography (CT), abdominal-pelvic CT, and enhanced brain magnetic resonance imaging (MRI) during treatment. Compared to ALK- patients, ALK+ patients were significantly more likely to have advanced N stage disease, a larger short diameter of metastasizing lymph nodes (ND), and a higher ratio of ND/TD (long diameter of the primary tumor) (P = 0.002, 0.044 and 0.002, respectively). A cut-off value of ND/TD ≥ 0.84 was useful for identifying ALK+ patients, with a specificity of 100% and a sensitivity of 50%. Brain metastasis, as detected by brain MRI, was the most common cause of progression in patients treated with crizotinib. Thus, the ND/TD ratio may be of value in selecting candidates for ALK mutation screening, and enhanced MRI of the brain is necessary during crizotinib treatment of ALK+ patients.

Keywords: Adenocarcinoma of lung, anaplastic lymphoma kinase, diagnosis, follow-up, radiology

Introduction

Lung cancer is the most common cause of cancer-related death worldwide. The prognosis is poor, especially in patients with advanced-stage disease, and many Chinese patients have an initial diagnosis of advanced-stage lung cancer. Lung cancer is considered a heterogeneous disease with regard to not only histological subtype but also molecular features [1, 2]. Accurate diagnosis of lung cancer subtypes is determined on both a histological and a genetic level, which is critical to providing personalized precision therapy on the basis of driver oncogenes.

The most important and well-studied oncogene as a therapeutic target is epidermal growth factor receptor (EGFR), as patients positive for EGFR mutations show better response rates and prolonged progression-free survival upon treatment with EGFR tyrosine kinase inhibitors (EGFR-TKIs) compared to those treated with standard platinum-based chemotherapy [3, 4]. Anaplastic lymphoma kinase (ALK) is another emerging oncogene [5, 6] that is attracting increasing attention. Lung cancer with ALK rearrangement (ALK+), which is commonly reported as an echinoderm microtubule-associated protein-like 4 (EML4)-ALK translocation, is a subgroup that exhibits a striking response to specific targeted drugs such as crizotinib, an oral small-molecule inhibitor of ALK [7, 8]. Crizotinib was approved by the US FDA in 2011 for the treatment of advanced or metastatic non-small cell lung cancers that are ALK+ and was approved by the China Food and Drug Administration (CFDA) in 2013 [9, 10]. Although ALK+ lung cancer only occurs in approximately 4-5% of patients with non-small cell lung cancer (NSCLC) [5, 11-13], there are approximately 35,000 new ALK+ lung cancer cases per year in

China, which is a sizeable population of lung cancer patients.

The most common histological subtype of ALK+ lung cancer is adenocarcinoma. Personalized targeted therapy for ALK+ tumors has become a revolutionary strategy for treating advanced lung adenocarcinoma, which is commonly observed upon initial diagnosis in Chinese patients, and it is vital to select and identify patients with this ALK mutation to ensure that appropriate treatment is instituted. Fluorescent in situ hybridization (FISH) analysis is the standard method for determining a patient's ALK mutation status. However, FISH is invasive, expensive, time-consuming, and not universally available. Because of the relatively low frequency of ALK rearrangement, another useful screening method for determine how to best identify the ALK+ population is needed. Medical imaging, especially computed tomography (CT), is an essential non-invasive procedure for diagnosing and staging lung cancer and for assessing treatment effectiveness, though it is seldom related to a specific oncogene. During our clinical work, we found that ALK+ advanced lung adenocarcinoma maybe correlated with certain imaging characteristics. The present study aimed to determine the imaging characteristics that would be helpful for predicting ALK+ advanced lung adenocarcinoma.

Material and methods

All clinical studies were conducted at Cancer Hospital, Chinese Academy of Medical Sciences. The study was approved by the Ethics in Research Committee of Cancer Hospital, Chinese Academy of Medical Sciences. Patient informed consent was waived for this retrospective study, and the methods were performed in accordance with approved guidelines.

Patient enrollment/study population

Patients with advanced lung adenocarcinoma (stage IV) whose tumor specimens were examined for ALK mutation status to identify potential candidates for crizotinib treatment at our institution between 2011 and 2014 were included. Patients were eligible for inclusion if they were 18 years of age or older, if they had histologically confirmed advanced (stage IV) lung adenocarcinoma, and if their tumor specimens were examined for ALK mutation status to identify potential candidates for crizotinib treatment. Based on an initial screen, 20 patients satisfied the inclusion criteria. A cohort of 20 patients with advanced (stage IV) lung adenocarcinoma and no ALK rearrangement (ALK-) were randomly selected from our institution and used as a control group. All patients had more than two metastasis-involved organs, were classified as stage M_1 according to the 2015 World Health Organization (WHO) system, and had enhanced CT (neck-thoracic and abdominal-pelvic) and enhanced MRI (brain) to confirm the diagnosis of stage IV lung adenocarcinoma.

Histopathology and detection of ALK rearrangement

All histological and oncogene mutation analyses were performed on biopsy specimens. For histological evaluation, tumors were classified on the basis of standard WHO criteria [14]. Molecular analysis of patients' ALK status was performed on formalin-fixed paraffin-embedded (FFPE) tumor samples by means of FISH with the use of an ALK break-apart (or split-signal) probe, as previously described [15]. ALK FISH was considered positive when more than 15% of the tumor cells showed split red and green signals and/or single red signals; otherwise, the specimen was classified as ALK FISH negative. A sample was defined as ALK+ only when the FISH result was positive.

CT/MRI image acquisition and interpretation

All patients enrolled in this study underwent evaluable pretreatment neck-thoracic and abdominal-pelvic enhanced CT as well as enhanced brain MRI of either the primary tumor or dominant metastasis. The following CT equipment was used: a Toshiba Aquilion 64-MDCT (pitch, 0.828; thickness, 5 mm; 120 kV; 380 mA; scan time, 0.5 s per rotation; reconstruction thickness, 1 mm; and spacing, 0.8 mm) and a GE LightSpeed 64-VCT (pitch, 0.984; thickness, 5 mm; 120 kV; 450 mA; scan time, 0.5 s per rotation; reconstruction thickness, 1.25 mm; and spacing, 0.8 mm). Contrast-enhanced scanning was performed 30-35 seconds after intravenous injection of iodine contrast (300 mg/ml). Brain MR images were obtained with 1.5 or 3.0 T GE MRI scanners and included axial T_2WI/FLAIR (TR, 8000

Table 1. Clinical characteristics of enrolled patients with different ALK mutation

Patient characteristics	ALK+	ALK-	*P* value
Number	20	20	
Age (y)	49 (24-82)	53 (39-81)	0.398
Sex			
Male	8	9	0.749
Female	12	11	
Treatment			
Crizotinib	10	0	
Chemotherapy	10	20	
T stage			0.020
T1	7	0	
T2	6	13	
T3	5	4	
T4	2	3	
N stage			0.002
N1	1	3	
N2	1	7	
N3	18	5	
M stage			
M0	0	0	
M1a	0	0	
M1b	20	20	

Note: ALK+, patients with anaplastic lymphoma kinase gene mutation; ALK-, patients with no anaplastic lymphoma kinase gene mutation.

ms; TE, 130 ms; NEX, 1; slice thickness, 5 mm and slice space, 1 mm) and an axial and sagittal enhancement scan with intravenous injection of Gd-DTPA contrast (TR, 420 ms; TE, 20 ms; NEX, 1; slice thickness, 5 mm and slice space, 1 mm).

Two independent radiologists evaluated the main tumor in each patient and determined the tumor size, type, margins, lymph node metastasis, and distant metastasis site (lung or lymphangitic, pleural or pericardial, brain, liver, adrenal gland, bone). Decisions regarding the CT findings were reached by consensus. The following CT features were assessed: the size and location of thoracic lymphadenopathy; the size, contour, consistency and location of the primary tumor; the presence/absence of pleural effusion, pleural metastasis or lymphangitic carcinomatosis; and the presence and location of metastasis. Clinical TNM stages were classified according to the 2015 WHO system.

Treatment and imaging follow-up

Of the 20 study patients with ALK+ advanced lung adenocarcinoma, 10 were randomly treated with crizotinib and the other 10 with standard chemotherapy. To evaluate the curative effect, follow-up enhanced neck-thoracic CT, abdominal-pelvic CT and enhanced brain MRI studies were performed at 6-week intervals according to the treatment period. RECIST 1.1 (Response Evaluation Criteria in Solid Tumors) was adopted for curative evaluation [16]. The progression-free survival (PFS) of each patient was monitored.

Statistical analysis

All values are reported as the mean ± standard deviation for continuous variables or as frequencies or percentages for categorical variables. To explore discriminating factors of imaging between the two groups, we used the X^2 test, Fisher's exact test, Student's t-test or the Wilcoxon rank-sum test. PFS curves were calculated using the Kaplan-Meier method. A probability (*P*) value of $P < 0.05$ indicated statistically significant differences. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software (SPSS, Inc., an IBM Company, Chicago, IL, USA).

Results

Clinical characteristics

The mean overall age of the patients at the time of diagnosis was 51 years (range, 24-82 years). The mean age of the patients in the ALK+ group was 49 years (range, 24-82 years), and that in the ALK- group was 53 years (range, 39-81 years). The detailed clinical and pathological characteristics of the study and control group patients are summarized in **Table 1**.

CT characteristics of ALK+ advanced lung adenocarcinoma

The CT features of advanced lung adenocarcinoma according to ALK mutation status are listed in **Table 2**. Compared with the ALK- patients, the ALK+ patients were significantly more likely to have an advanced N stage ($P = 0.002$). The average short diameter of metastasizing lymph nodes (ND) for the ALK+ patients was significantly higher than that for the ALK-

Table 2. CT characteristics of enrolled patients with different ALK mutation

CT characteristics	ALK+	ALK-	*P* value
Type			0.091
Mass (> 30 mm)	11	16	
Nodule (< 30 mm)	9	4	
CT density			0.147
Solid	20	18	
Part solid	0	2	
GGO	0	0	
Location			0.327
Central	9	6	
Peripheral	11	14	
TD (mm)	33.90 ± 21.48	46.95 ± 21.27	0.061
ND (mm)	20.10 ± 7.93	15.40 ± 6.25	0.044
TD/ND	2.00 ± 1.68	3.53 ± 2.19	0.018
ND/TD	0.83 ± 0.54	0.38 ± 0.20	0.002
Pleura metastasis			0.749
Negative	8	9	
Positive	12	11	
Lung metastasis			0.342
Negative	12	9	
Positive	8	11	
Lymphangitic metastasis			0.077
Negative	12	17	
Positive	8	3	
Liver metastasis			0.677
Negative	17	16	
Positive	3	4	
Bone metastasis			0.752
Negative	10	11	
Positive	10	9	
Brain metastasis			0.292
Negative	17	19	
Positive	3	1	
Adrenal gland metastasis			0.077
Negative	15	19	
Positive	5	1	

Note: ALK+, patients with anaplastic lymphoma kinase gene mutation; ALK-, patients with no anaplastic lymphoma kinase gene mutation; TD, long diameter of primary tumor; ND, short diameter of metastasizing lymph nodes.

patients (20.10 ± 7.93 mm vs. 15.40 ± 6.25 mm, $P = 0.044$). The average long diameter of the primary tumors (TD) for the ALK+ patients was smaller than that for the ALK- patients, though with no significant difference (33.90 ± 21.48 mm vs. 46.95 ± 21.27 mm, $P = 0.061$). In addition, the difference in ND/TD ratios of the two groups was statistically significant (0.83 vs. 0.38, $P < 0.001$) (**Table 2**; **Figure 1**).

Diagnostic role of CT in ALK+ advanced lung adenocarcinoma

Receiver operating characteristic (ROC) analysis was conducted to predict ALK+ advanced lung adenocarcinoma, and the area under the ROC curve (AUC) for the ND/TD ratio as a predictive marker for ALK mutation status was 0.785 ($P = 0.002$; 95% confidence interval [*CI*], 0.64-0.93). The optimal cutoff value of ND/TD for identifying ALK+ advanced lung adenocarcinoma was greater than or equal to 0.84 (sensitivity, 50.0%; specificity, 100.0%), and the positive and negative predictive values for ALK+ status were 100% and 66.7%, respectively (**Figure 2**).

Role of follow-up imaging for ALK+ advanced lung adenocarcinoma

In the study group, all 20 patients were followed up until May 26, 2016, with a median follow-up time of 20.5 weeks (range, 6-104 weeks). The median time to recurrence for the ALK+ patients treated with crizotinib or treated with chemotherapy was 60 weeks (range, 12-104 weeks) and 18 weeks (range, 6-54 weeks), respectively. At the time of the last follow-up, progression had occurred in 90% of the ALK+ patients receiving crizotinib (9/10). In the patients with new lesions from disease progression (PD), the brain was the most common site of metastasis, occurring in 88.9% (8/9) of these patients.

Discussion

Recently, it has been shown that for patients with ALK+ NSCLC, crizotinib leads to improved outcomes when compared with conventional chemotherapy. In our study, the PFS of ALK+ advanced lung adenocarcinoma patients treated with crizotinib was 60 weeks, which is longer than that of patients treated with standardized chemotherapy, whose PFS was 18 weeks. The

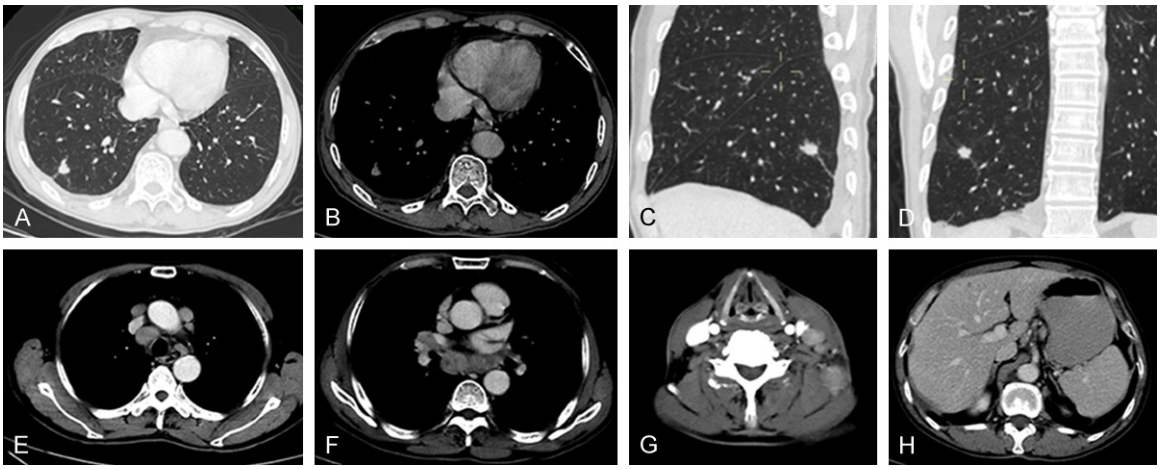

Figure 1. Male, 59 years old, adenocarcinoma in the inferior lobe of the right lung. CT (A-D) showed the primary tumor with a long diameter of 10 mm; CT (E-H) showed neck, mediastinal, and abdominal lymph nodal metastases with the largest lymph nodal short diameter of 24 mm. The ND/TD is 2.4. Molecular pathological results after cervical lymph node biopsy showed ALK rearrangement.

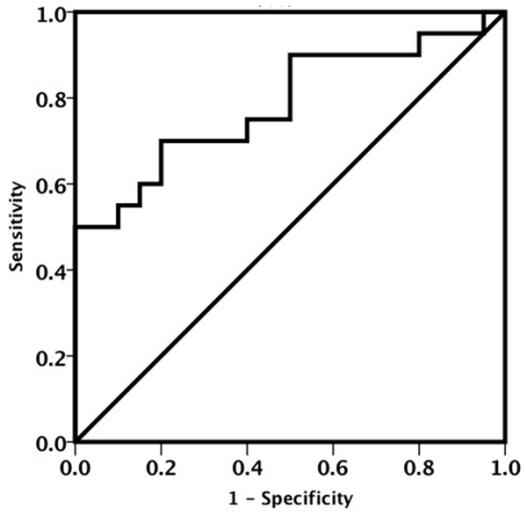

Figure 2. Receiver operating characteristic (ROC) curve for predicting advanced lung adenocarcinoma with ALK rearrangements; the area under the ROC curve (AUC) for ND/TD is 0.785.

clinical benefit gained by using ALK-targeted therapies has led to a transition in current clinical practice based on determination of a patient's ALK rearrangement status [17].

There are three primary methods for ALK mutation detection. FISH is currently the only approved standard technique for assessing ALK mutation status, even though this technique has a relatively high cost and a long testing time and is not universally available [18, 19]. Immunohistochemistry (IHC) has been intensely investigated as a screening tool for detecting ALK mutation status because it is affordable for most patients and is easier to implement. However, the specificity of IHC for ALK mutation testing is not as robust as that of FISH, and sensitivity varies with the specific antibodies used [20]. Although reverse transcription-polymerase chain reaction (RT-PCR) is a highly sensitive test for detecting ALK mutation, high-quality RNA is required, which is often difficult to obtain. In our study, we used FISH as a standard technique for assessing ALK mutation status.

ALK rearrangement-positive lung cancer is reported to be more common in younger patients, men, and non-smokers or light smokers [21-23]. However, in our study, there was no statistically significant difference between the study group and the control group when analyzing these demographics. The reason for this finding may be the relatively small sample size and the extremely advanced stage of the population selected, which may have resulted in outcome bias. Although there are several reported clinical characteristics of ALK+ NSCLC patients, these characteristics have a limited role in optimizing patient selection to screen for ALK mutation status.

In the current study, we provide a more comprehensive radiological overview of ALK+ advanced lung adenocarcinoma. The results demonstrate that compared to ALK- advanced lung adeno-

carcinomas, ALK+ advanced lung adenocarcinomas are more likely to be associated with larger volume lymphadenopathy compared with the primary tumor as well as more advanced and multifocal lymphadenopathy, such as extensive mediastinal, supraclavicular, or axillary lymphadenopathy, upon CT evaluation. However, in our study, ALK+ advanced adenocarcinoma showed no tendency toward other lymphangitic spread, such as lymphangitic carcinomatosis, compared to the control group. In the correct clinical context, these radiological presentations could help to identify patients with ALK rearrangements. It is important to note that these CT characteristics of ALK+ advanced lung adenocarcinoma can mimic the CT imaging characteristics of small cell lung cancer (SCLC) or even lymphomas.

Because of the high incidence of EGFR mutations in lung adenocarcinoma in the eastern Asian population, a screening method for the detection of EGFR mutations has been established and commercially applied in clinical practice. However, detection of EML4-ALK gene translocation in lung cancer patients remains rare and is considered impractical because of the low incidence and because of financial and technical limitations. The novelty of our study is that we identified a new method for predicting high-risk ALK+ lung adenocarcinoma populations using CT imaging. Our study suggests that the new radio-parameter of the ND/TD ratio may be a potential marker for selecting candidates for ALK mutation screening. Concerning pathologically confirmed advanced lung adenocarcinoma patients, when CT imaging shows a relatively small primary tumor volume with a relatively large lymphadenopathy volume-that is, when the ratio of ND/TD is high (especially ≥ 0.84)-these patients can be classified as high risk and are strongly recommended to undergo ALK testing, as they may greatly benefit from crizotinib treatment.

In our study, none of the primary tumor features observed by CT correlated with the presence of ALK rearrangement when compared to ALK- lesions. All primary tumors in the study group were solid, whereas the control group consisted of 18 solid cases and 2 partially solid cases. No significant difference was detected between the two groups in this regard. In a previous study, when correlating molecular genetic results with imaging findings, EGFR mutations correlated positively with the ground-glass opacity (GGO) ratio and ALK rearrangement with solid nodules in thin-section CT scans of lung adenocarcinomas. However, this difference may be negligible in instances of advanced adenocarcinoma. There was also no statistically significant difference between the study group and the control group with regard to distant metastasis.

In our study, brain metastasis as detected by enhanced MRI was the most common cause of progression in patients treated with crizotinib. Most cytotoxic systemic chemotherapies and some TKIs appear to inefficiently cross the intact blood-brain barrier. Enhanced MRI of the brain is the best method for diagnosing brain metastasis, and brain MRI is necessary during crizotinib treatment because brain metastasis is the most common cause of disease progression. Measurement of crizotinib levels in plasma and cerebrospinal fluid (CSF) have indicated poor penetration of the drug into the brain, thus potentially hindering the efficacy of crizotinib for metastatic brain tumors [24].

Our study had several limitations. First, the cohort was relatively small, which could be overcome by performing larger prospective multi-institutional studies. Second, as we only investigated highly advanced ALK+ lung adenocarcinoma, the results are not applicable to all ALK+ lung adenocarcinomas. Third, to investigate the CT features of ALK+ lung adenocarcinoma, we should further analyze lung cancers with mutations in other commonly mutated oncogenes such as EGFR and K-RAS. Indeed, lung adenocarcinomas with K-RAS mutations are prevalent in mucinous adenocarcinoma and have invasive behaviors based on our clinical experience.

In summary, ALK+ advanced lung adenocarcinoma commonly appears as solid masses on CT images and is more likely to be associated with advanced lymph node metastasis and large volume lymphadenopathy than a primary tumor. An ND/TD ratio greater than or equal to 0.84 may be of value in recommending ALK-rearrangement testing for patients with advanced lung adenocarcinoma. Enhanced MRI of the brain is also necessary during treatment with crizotinib because brain metastasis

is the most common cause of disease progression.

Acknowledgements

This work was supported by the National High-Technology Research and Development Program (Ministry of Science and Technology of the People's Republic of China, 2014AA020602), the Major Project of Beijing Municipal Science and Technology Commission of the People's Republic of China (D141100000214006), the National Natural Science Foundation of China (81601494), and the PUMC Youth Fund and Fundamental Research Funds for the Central Universities (3332016030).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ning Wu, Department of Diagnostic Radiology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; PET-CT Center, Department of Diagnostic Radiology, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. Tel: +86 (010) 8778 7523; Fax: +86 (010) 6771 3359; E-mail: cjr.wuning@vip.163.com

References

[1] Kerr KM. Pulmonary adenocarcinomas: classification and reporting. Histopathology 2009; 54: 12-27.

[2] Travis WD. Reporting lung cancer pathology specimens. Impact of the anticipated 7th edition TNM classification based on recommendations of the IASLC staging committee. Histopathology 2009; 54: 3-11.

[3] Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J and Haber DA. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129-2139.

[4] Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE and Meyerson M. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004; 304: 1497-1500.

[5] 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.

[6] Horn L and Pao W. EML4-ALK: honing in on a new target in non-small-cell lung cancer. J Clin Oncol 2009; 27: 4232-4235.

[7] Rodig SJ and Shapiro GI. Crizotinib, a small-molecule dual inhibitor of the c-Met and ALK receptor tyrosine kinases. Curr Opin Investig Drugs 2010; 11: 1477-1490.

[8] Casaluce F, Sgambato A, Maione P, Rossi A, Ferrara C, Napolitano A, Palazzolo G, Ciardiello F and Gridelli C. ALK inhibitors: a new targeted therapy in the treatment of advanced NSCLC. Target Oncol 2013; 8: 55-67.

[9] Malik SM, Maher VE, Bijwaard KE, Becker RL, Zhang L, Tang SW, Song P, Liu Q, Marathe A, Gehrke B, Helms W, Hanner D, Justice R and Pazdur R. U.S. Food and drug administration approval: crizotinib for treatment of advanced or metastatic non-small cell lung cancer that is anaplastic lymphoma kinase positive. Clin Cancer Res 2014; 20: 2029-2034.

[10] Kazandjian D, Blumenthal GM, Chen HY, He K, Patel M, Justice R, Keegan P and Pazdur R. FDA approval summary: crizotinib for the treatment of metastatic non-small cell lung cancer with anaplastic lymphoma kinase rearrangements. Oncologist 2014; 19: e5-11.

[11] 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.

[12] 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.

[13] Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Janne PA, Costa DB, Varella-Garcia M, Kim WH, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW and Iafrate AJ. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010; 363: 1693-1703.

[14] Brambilla E, Travis WD, Colby TV, Corrin B and Shimosato Y. The new World Health Organization classification of lung tumours. Eur Respir J 2001; 18: 1059-1068.
[15] Lee HY, Ahn HK, Jeong JY, Kwon MJ, Han JH, Sun JM, Ahn JS, Park K, Choi YL and Ahn MJ. Favorable clinical outcomes of pemetrexed treatment in anaplastic lymphoma kinase positive non-small-cell lung cancer. Lung Cancer 2013; 79: 40-45.
[16] Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D and Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45: 228-247.
[17] Choi CM. Overview of ALK and ROS1 rearranged lung cancer. Tuberc Respir Dis (Seoul) 2013; 75: 236-237.
[18] Camidge DR, Hirsch FR, Varella-Garcia M and Franklin WA. Finding ALK-positive lung cancer: what are we really looking for? J Thorac Oncol 2011; 6: 411-413.
[19] Atherly AJ and Camidge DR. The cost-effectiveness of screening lung cancer patients for targeted drug sensitivity markers. Br J Cancer 2012; 106: 1100-1106.
[20] Conklin CM, Craddock KJ, Have C, Laskin J, Couture C and Ionescu DN. Immunohistochemistry is a reliable screening tool for identification of ALK rearrangement in non-small-cell lung carcinoma and is antibody dependent. J Thorac Oncol 2013; 8: 45-51.
[21] Rodig SJ, Mino-Kenudson M, Dacic S, Yeap BY, Shaw A, Barletta JA, Stubbs H, Law K, Lindeman N, Mark E, Janne PA, Lynch T, Johnson BE, Iafrate AJ and Chirieac LR. Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. Clin Cancer Res 2009; 15: 5216-5223.
[22] Koh Y, Kim DW, Kim TM, Lee SH, Jeon YK, Chung DH, Kim YW, Heo DS, Kim WH and Bang YJ. Clinicopathologic characteristics and outcomes of patients with anaplastic lymphoma kinase-positive advanced pulmonary adenocarcinoma: suggestion for an effective screening strategy for these tumors. J Thorac Oncol 2011; 6: 905-912.
[23] Fukui T, Yatabe Y, Kobayashi Y, Tomizawa K, Ito S, Hatooka S, Matsuo K and Mitsudomi T. Clinicoradiologic characteristics of patients with lung adenocarcinoma harboring EML4-ALK fusion oncogene. Lung Cancer 2012; 77: 319-325.
[24] 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.