Thoracic Cancer
Open Access
Thoracic Cancer ISSN 1759-7706

ORIGINAL ARTICLE

Ventana immunohistochemistry assay for anaplastic lymphoma kinase gene rearrangement detection in patients with non-small cell lung cancer: A meta-analysis

Wang Zhiwei*, Jiang Yuan*, Yao Yihui*, Hou Xin, Chen Jingtao, Shi Lei & Duan Yongjian

Department of Radiotherapy, 1st Affiliated Hospital of Henan University, Kaifeng, China

Keywords
Anaplastic lymphoma kinase gene; meta-analysis; non-small cell lung cancer; Ventana immunohistochemistry assay.

Correspondence
Duan Yongjian, Department of Radiotherapy, 1st Affiliated Hospital of Henan University, No. 357 Ximen Road, Kaifeng City, Henan Province 475000, China.
Tel: +86 371 2382 2222
Email: w777h7@163.com

*These authors contributed equally to this work.

Received: 26 April 2017;
Accepted: 5 June 2017.

doi: 10.1111/1759-7714.12468

Thoracic Cancer **8** (2017) 471–476

Abstract

Background: The aim of this study was to evaluate the diagnostic value of Ventana immunohistochemistry (IHC) assay for anaplastic lymphoma kinase (ALK) gene rearrangement screening in patients with non-small cell lung cancer (NSCLC).

Methods: Open published studies that reported the diagnostic performance of Ventana IHC assay for ALK gene rearrangement detection in NSCLC patients were extracted from PubMed, Embase, Google scholar, Wanfang, and China National Knowledge Infrastructure. The general information and number of true positive (tp), false positive (fp), false negative (fn), and true negative (tn) cases identified by Ventana IHC assay were extracted. The diagnostic sensitivity, specificity, positive likelihood ratio (+lr), negative likelihood ratio (−lr), diagnostic odds ratio (dor) and the summary receiver operating characteristic (ROC) curve were calculated using Stata 11.0 software.

Results: Ten studies, including 240 ALK positive and 1973 ALK negative NSCLC patients were included in this meta-analysis. The pooled diagnostic sensitivity, specificity, +lr, −lr, and dor were 0.94 (95% confidence interval [CI] 0.85–0.98), 1.00 (95% CI 0.99–1.00), 859.61 (95% CI 60.81–1200.00), 0.06 (95% CI 0.03–0.16), and 1400.00 (95% CI 813.29–23 000.00), respectively. The area under the ROC curve was 0.996 for Ventana IHC assay in detecting ALK gene rearrangement in NSCLC patients.

Conclusion: The sensitivity and specificity of Ventana IHC assay for the detection of ALK gene rearrangement were high, thus Ventana IHC could substitute fluorescence in situ hybridization for the screening of ALK+ NSCLC patients.

Introduction

Clinical epidemiological studies have demonstrated that patients with anaplastic lymphoma kinase-positive (ALK+) non-small cell lung cancer (NSCLC) account for about 5% of the total number of NSCLC patients.[1] ALK-positive patients are generally young and smoke either occasionally or not at all; the most common pathological type is adenocarcinoma, which is more common in women than in men.[2] Crizotinib, a small-molecule inhibitor of echinoderm microtubule associated protein- like 4 (EML4)-ALK fusion gene targets, has been demonstrated by a number of clinical studies to exert good therapeutic effects.[3,4] Several prospective randomized clinical trials have shown that the clinical efficacy of crizotinib treatment is superior to that of conventional chemotherapy.[5–7] National Comprehensive Cancer Network NSCLC clinical practice guidelines suggest that ALK fusion gene detection should initially be conducted for NSCLC patients with suspected ALK gene fusion mutation. If ALK+ is confirmed, crizotinib is recommended as first-line treatment.

At present, the methods for ALK fusion gene detection are mainly fluorescence in situ hybridization (FISH), PCR, and Ventana immunohistochemistry (IHC).[8–10] Clinically, FISH is the gold standard for the determination of ALK gene fusion. Ventana IHC can be performed

 © 2017 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

on an automated instrument for batch detection, with enhanced detection and assessment of results.[11] It is more advantageous than conventional IHC. Numerous studies have evaluated the clinical value of Ventana IHC for detecting the ALK fusion gene in NSCLC patients. However, given the small sample size of previous studies and low statistical efficiency, some limitations in its clinical application exist. In this study, we analyzed the clinical research on Ventana IHC for detecting the ALK fusion gene in NSCLC patients, as well as exploring its clinical application value.

Methods

Publication search and inclusion/exclusion criteria

Open published studies that reported the diagnostic performance of Ventana IHC assay for ALK gene rearrangement in NSCLC patients were extracted from PubMed, Embase, Google scholar, Wanfang, and China National Knowledge Infrastructure electronic databases. The search terms used were: non-small cell lung cancer (NSCLC), cancer, tumor,

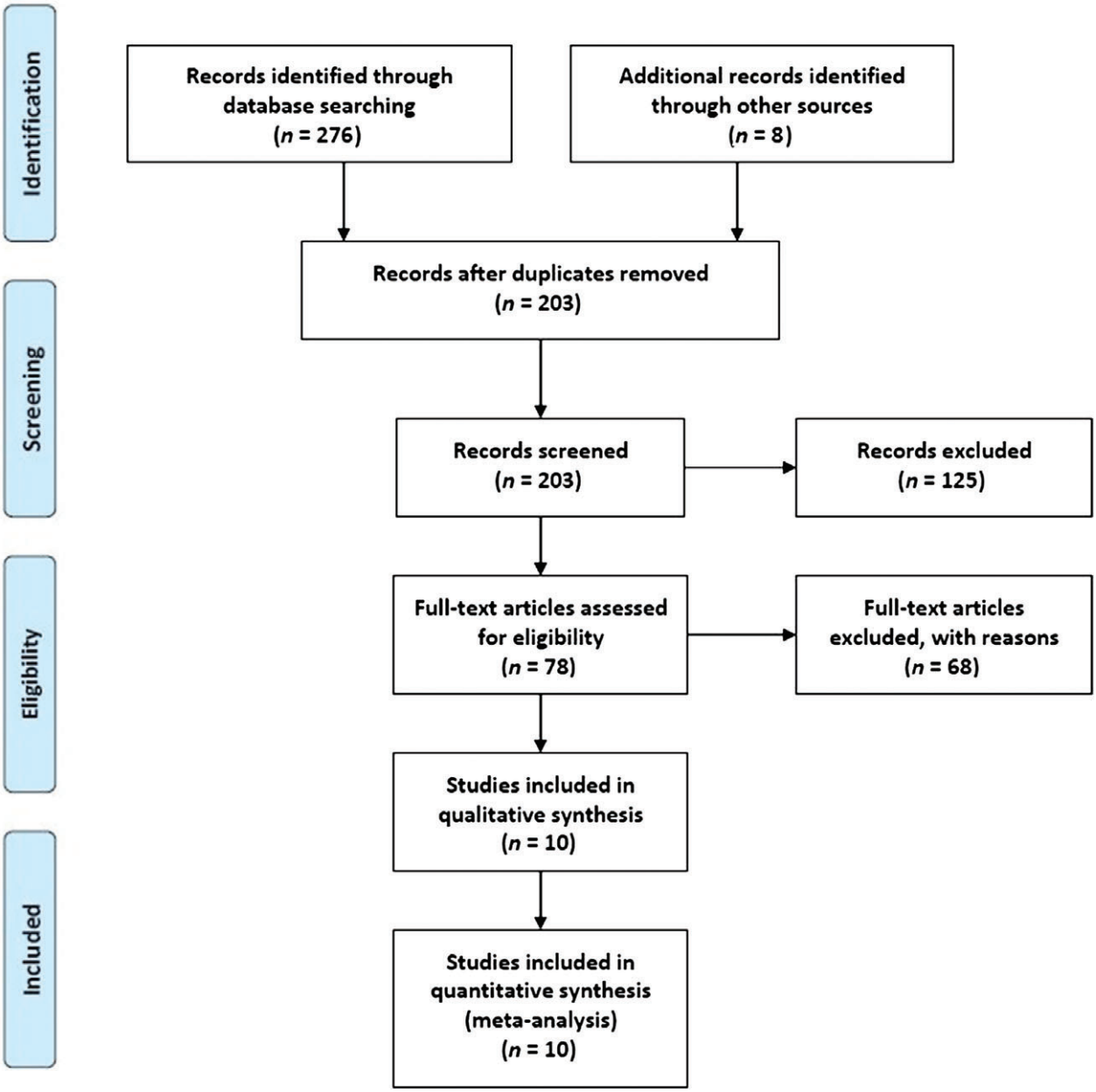

Figure 1 Flow-chart of the publication search.

 © 2017 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd

anaplastic lymphoma kinase (ALK), fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), and polymerase chain reaction (PCR). The search was performed by two reviewers who cross-checked each other's work. The references of included studies were also screened in order to locate suitable publications. The publication inclusion criteria were: (i) prospective or retrospective studies related to Ventana IHC assay for ALK gene rearrangement; (ii) patients were pathologically or cytologically confirmed with NSCLC; (iii) the gold diagnostic reference was FISH; and (iv) the number of true positive (tp), false positive (fp), false negative (fn), and true negative (tn) cases could be extracted from the original studies. The exclusion criteria were: (i) case reports and review studies; (ii) NSCLC diagnosis was not confirmed by pathology or cytology; (iii) duplicated publications; and (iv) inadequate data to calculate tp, fp, fn and tn.

Data extraction

Two reviewers read the full text and independently extracted the data using a standardized data extraction sheet. General information, such as first and corresponding author names, the year the paper was published, and the study design (retrospective or prospective), were compiled. The extracted data included tp, fp, fn, and tn cases identified by Ventana IHC assay. All information and data was cross-checked and reviewed by the corresponding author.

Statistical analysis

Data analysis was performed using STATA version 11.0 (Stata Corp., College Station, TX, USA). The statistical heterogeneity across the included studies was analyzed by I^2 test. $I^2 \geq 50\%$ indicated significant statistical heterogeneity.

Table 1 Characteristics of the included studies

Study	Year	Country	No.	Tp	Fp	Fn	Tn	Study design
Ying *et al.*[12]	2013	China	196	63	2	0	131	Retrospective
Guo *et al.*[16]	2014	China	404	29	0	0	375	Retrospective
Hutarew *et al.*[15]	2014	Austria	303	14	0	0	289	Retrospective
Liang *et al.*[17]	2014	China	65	3	0	2	60	Retrospective
Ali *et al.*[13]	2014	Italy	523	18	0	2	503	Retrospective
Wynes *et al.*[14]	2014	United States	98	39	2	4	53	Retrospective
Meng *et al.*[20]	2015	China	172	11	0	1	160	Retrospective
Ilie *et al.*[19]	2015	France	176	21	2	5	148	Prospective
Wang *et al.*[11]	2015	China	58	6	0	2	50	Retrospective
Gruber *et al.*[18]	2015	Germany	218	19	0	1	198	Retrospective

Fn, false negative; Fp, false positive; Tn, true negative; Tp, true positive.

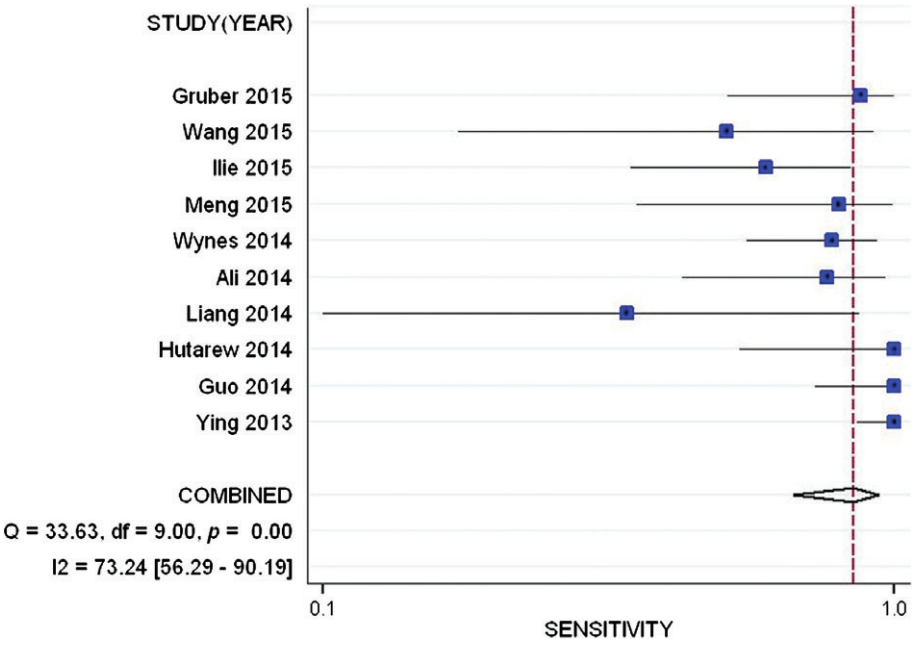

Figure 2 Forest plot of diagnostic sensitivity of Ventana-immunohistochemistry assay in detection of anaplastic lymphoma kinase gene rear rangement.

 © 2017 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd

The data was pooled by random or fixed effect model according to the statistical heterogeneity. Begg's funnel plot and Egger's line regression test were used to assess publication bias.

Results

General characteristics of included studies

Ten diagnostic studies including 240 ALK positive and 1973 ALK negative NSCLC patients were included in this meta-analysis according to the inclusion and exclusion criteria. The search strategy is demonstrated in Figure 1. Two hundred and eighty-four publications were initially identified. After removing the duplicated studies, 203 studies were included for further assessment and the full text was assessed in 78. Finally, 10 studies were included for quantitative synthesis.[11–20] Five of the studies were performed in a Chinese population, while the remaining five trials were performed in Austria, France, Germany, Italy, and the United States. One study was prospectively designed, while the other nine were retrospectively designed. The characteristics of the included studies are shown in Table 1.

Statistical heterogeneity evaluation

The statistical heterogeneity across the studies was evaluated by I^2 test. Significant statistical heterogeneity was found in the effect size of sensitivity (I^2 = 73.24%), specificity (I^2 = 76.84%), and negative likelihood ratio (–lr, 62.20%). However, there was no statistical heterogeneity for positive likelihood ratio (+lr, 28.90%) and diagnostic odds ratio (dor, 43.40%).

Meta-analysis

The sensitivity, specificity, and –lr were pooled by random effect model because of significant statistical heterogeneity. The pooled sensitivity, specificity and –lr were 0.94 (95% confidence interval [CI] 0.85–0.98) (Fig 2), 1.00 (95% CI 0.99–1.00) (Fig 3), and 0.06 (95% CI 0.03–0.16), respectively. The pooled +lr and dor were 859.61 (95% CI 60.81–1200.00) and 1400.00 (95% CI 813.29–23 000.00), respectively, by fixed effect model because of a lack of significant statistical heterogeneity. The area under the receiver operating characteristic (ROC) curve was 0.996 for Ventana IHC assay in detection of ALK gene rearrangement in NSCLC patients (Fig 4).

Publication bias

Publication bias was evaluated by Begg's funnel plot and Egger's line regression test. The funnel plot was asymmetrical at the bottom and the line regression test also indicated significant publication bias (t = 3.19; $P < 0.05$) (Fig 5).

Discussion

Echinoderm microtubule associated protein-like 4 (EML4)-ALK was first reported by Soda *et al.* in 2007 as a driving gene for NSCLC.[21] This gene is transposed from the central granule of the short arm of human chromosome 2, namely,

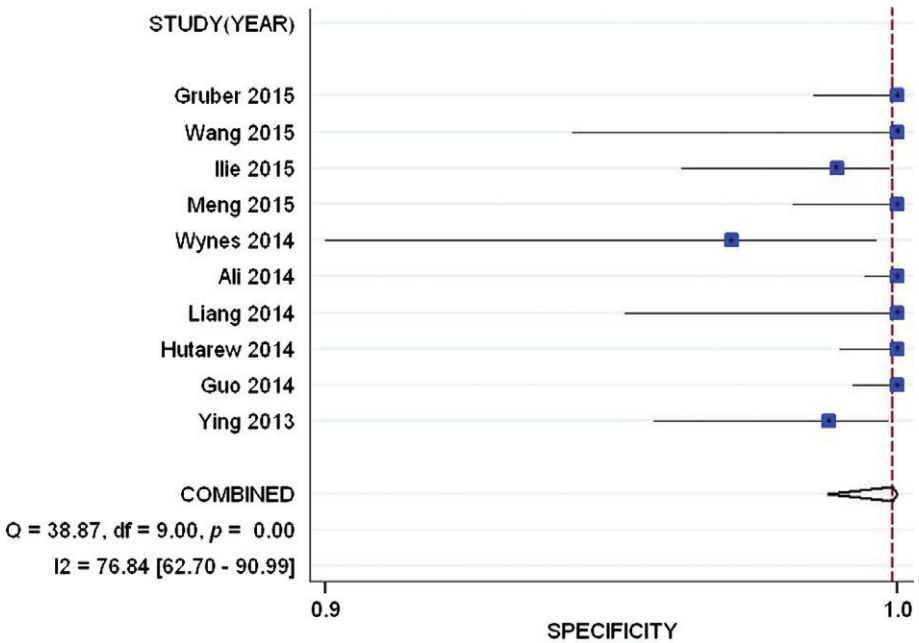

Figure 3 Forest plot of diagnostic specificity of Ventana- immunohistochemistry assay in detection of anaplastic lymphoma kinase gene rearrangement.

 © 2017 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd

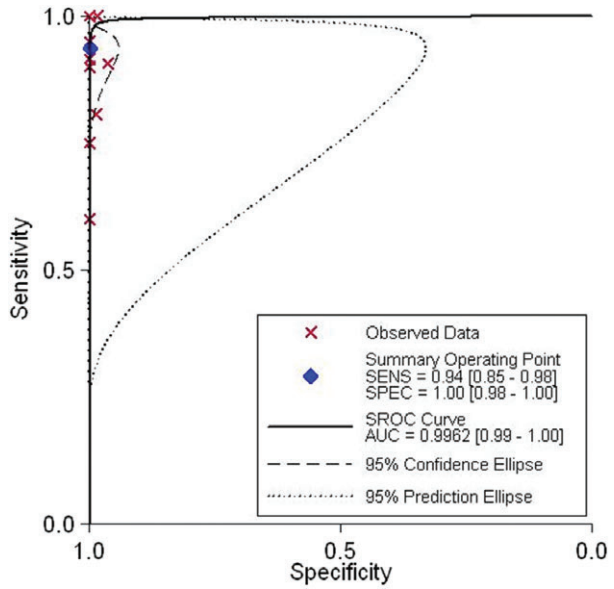

Figure 4 Summary receiver operating characteristic curve (SROC) plot with best-fitting asymmetric curve of Ventana- immunohistochemistry assay in detection of anaplastic lymphoma kinase gene rearrangement. AUC, area under the curve; SENS, sensitivity; SPEC, specificity.

inv. (2)(p21p23), thereby causing the fusion of EML4 (2p21) and ALK(2p23). After fusion, sustained activation of ALK kinase and the downstream signaling pathway occurs, and NSCLC cells are stimulated to divide and proliferate. Crizotinib, a small-molecule inhibitor of EML4-ALK fusion gene targets, exerts good therapeutic effects. A phase I/II clinical study (PROFILE 1001, Clinical Trials.gov, NCT00585195) found that crizotinib improves the total response rate (57%, including 1 case of complete response) and progression-free survival (9.7 months, 95% CI 7.7–12.8).[22] A phase III clinical trial (PRO-FILE 1014 Clinical Trials.gov, NCT01154140) comparing the clinical efficacy of crizotinib and conventional chemotherapy showed that crizotinib has a significant advantage (progression-free survival 10.9 vs. 7.0 months, hazard ratio 0.45, 95% CI 0.35–0.60; $P < 0.001$) for ALK+ NSCLC.[7] Therefore, the National Comprehensive Cancer Network NSCLC clinical practice guidelines suggest that ALK fusion gene detection should first be conducted in NSCLC patients with suspected ALK gene fusion mutation. If the result is ALK+, crizotinib is the first treatment choice.

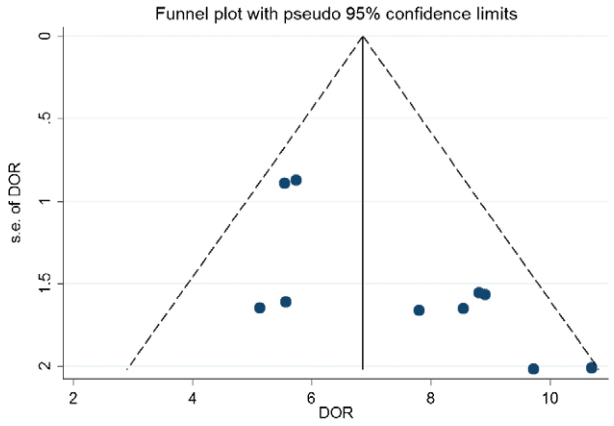

Figure 5 Funnel plot of publication bias of Ventana- immunohistochemistry assay in detection of anaplastic lymphoma kinase gene rearrangement. DOR, diagnostic odds ratio.

Generally, FISH, PCR, IHC, and Ventana IHC are used for ALK fusion gene detection. Ventana IHC can be performed on an automated instrument for batch detection, with enhanced detection and assessment of results. Thus, Ventana IHC is more advantageous than conventional IHC.[13] In this study, we pooled the published clinical studies related to Ventana IHC for the detection of the ALK fusion gene in patients with NSCLC, as well as exploring its clinical application value. The pooled results showed the diagnostic sensitivity, specificity, +lr, –lr, and dor were 0.94, 1.00, 859.61, 0.06, and 1400, respectively. The area under the ROC curve was 0.996 for Ventana IHC assay in detecting ALK gene rearrangement in NSCLC patients. The high sensitivity and specificity of Ventana IHC assay in detecting ALK gene rearrangement could be substituted for FISH to screen ALK+ NSCLC patients. However, there were two major limitations to our study. Firstly, significant statistical heterogeneity existed in the sensitivity, specificity, and –lr effect sizes. Statistical heterogeneity can decrease statistical power. Secondly, obviously publication bias was detected.

In conclusion, Ventana IHC presented high specificity and sensitivity for ALK+ NSCLC detection. To some extent, it may replace FISH for the detection of ALK+ NSCLC.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (No. 81472745).

Disclosure

No authors report any conflict of interest.

References

1 Zhao F, Xu M, Lei H *et al.* Clinicopathological characteristics of patients with non-small-cell lung cancer who harbor EML4-ALK fusion gene: A meta-analysis. *PLoS ONE* 2015; **10**: e0117333.

 © 2017 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd

2 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**: 4247–53.
3 Metro G, Tazza M, Matocci R, Chiari R, Crinò L. Optimal management of ALK-positive NSCLC progressing on crizotinib. *Lung Cancer* 2017; **106**: 58–66.
4 Kobayashi T, Fujimoto H, Gabazza EC. Efficacy of crizotinib in ALK fusion variants. *J Thorac Dis* 2016; **8**: E1381–3.
5 Hida T, Nakagawa K, Seto T *et al.* Pharmacologic study (JP28927) of alectinib in Japanese patients with ALK+ non-small-cell lung cancer with or without prior crizotinib therapy. *Cancer Sci* 2016; **107**: 1642–6.
6 Zhao J, Zhang K, Zhang L, Wang H. [Clinical efficacy of crizotinib in advanced ALK positive non-small cell lung cancer.] *Zhongguo Fei Ai Za Zhi* 2015; **18**: 616–20. (In Chinese.)
7 Solomon BJ, Mok T, Kim DW *et al.* First-line crizotinib versus chemotherapy in ALK-positive lung cancer. (Published erratum appears in *N Engl J Med* 2015; 373: 1582.) *N Engl J Med* 2014; **371**: 2167–77.
8 Fu S, Wang F, Shao Q *et al.* Detection of EML4-ALK fusion gene in Chinese non-small cell lung cancer by using a sensitive quantitative real-time reverse transcriptase PCR technique. *Appl Immunohistochem Mol Morphol* 2015; **23**: 245–54.
9 Wen M, Wang X, Sun Y *et al.* Detection of EML4-ALK fusion gene and features associated with EGFR mutations in Chinese patients with non-small-cell lung cancer. *Onco Targets Ther* 2016; **9**: 1989–95.
10 Kobayashi M, Sonobe M, Takahashi T, Yoshizawa A, Kikuchi R, Date H. Detection of ALK fusion in lung cancer using fluorescence in situ hybridization. *Asian Cardiovasc Thorac Ann* 2012; **20**: 426–31.
11 Wang W, Tang Y, Li J, Jiang L, Jiang Y, Su X. Detection of ALK rearrangements in malignant pleural effusion cell blocks from patients with advanced non-small cell lung cancer: A comparison of Ventana immunohistochemistry and fluorescence in situ hybridization. *Cancer Cytopathol* 2015; **123**: 117–22.
12 Ying J, Guo L, Qiu T *et al.* Diagnostic value of a novel fully automated immunochemistry assay for detection of ALK rearrangement in primary lung adenocarcinoma. *Ann Oncol* 2013; **24**: 2589–93.
13 Alì G, Proietti A, Pelliccioni S *et al.* ALK rearrangement in a large series of consecutive non-small cell lung cancers: Comparison between a new immunohistochemical approach and fluorescence in situ hybridization for the screening of patients eligible for crizotinib treatment. *Arch Pathol Lab Med* 2014; **138**: 1449–58.
14 Wynes MW, Sholl LM, Dietel M *et al.* An international interpretation study using the ALK IHC antibody D5F3 and a sensitive detection kit demonstrates high concordance between ALK IHC and ALK FISH and between evaluators. *J Thorac Oncol* 2014; **9**: 631–8.
15 Hutarew G, Hauser-Kronberger C, Strasser F, Llenos IC, Dietze O. Immunohistochemistry as a screening tool for ALK rearrangement in NSCLC: Evaluation of five different ALK antibody clones and ALK FISH. *Histopathology* 2014; **65**: 398–407.
16 Guo L, Liu X, Qiu T, Ling Y, Shan L, Xie Y. [ALK fusion gene assessment by fully automatic immunohistochemistry in non-small cell lung cancer.] *Zhonghua Bing Li Xue Za Zhi* 2014; **43**: 95–8. (In Chinese.)
17 Liang X, Wang M, Zhang J *et al.* [ALK protein expression and gene fusion in bronchoscopic specimens of lung adenocarcinoma.] *Zhonghua Zhong Liu Za Zhi* 2014; **36**: 501–4. (In Chinese.)
18 Gruber K, Kohlhäufl M, Friedel G, Ott G, Kalla C. A novel, highly sensitive ALK antibody 1A4 facilitates effective screening for ALK rearrangements in lung adenocarcinomas by standard immunohistochemistry. (Published erratum appears in *J Thorac Oncol* 2016; 11:606.). *J Thorac Oncol* 2015; **10**: 713–6.
19 Ilie MI, Bence C, Hofman V *et al.* Discrepancies between FISH and immunohistochemistry for assessment of the ALK status are associated with ALK 'borderline'-positive rearrangements or a high copy number: A potential major issue for anti-ALK therapeutic strategies. *Ann Oncol* 2015; **26**: 238–44.
20 Meng H, Gao X, Zhang L, Liu F, Li W. [Assessment of ALK rearrangement in non-small cell lung cancer: Using enhancing immunohistochemical way and fluorescence in situ hybridization.] *Zhongguo Fei Ai Za Zhi* 2015; **18**: 75–9. (In Chinese.)
21 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**: 561–6.
22 Kwak EL, Bang YJ, Camidge DR *et al.* Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. (Published erratum appears in *N Engl J Med* 2011; 364: 588.). *N Engl J Med* 2010; **363**: 1693–703.

 © 2017 The Authors. Thoracic Cancer published by China Lung Oncology Group and John Wiley & Sons Australia, Ltd