BRIEF REPORT

Reliability Assurance of Detection of *EML4-ALK* Rearrangement in Non–Small Cell Lung Cancer: The Results of Proficiency Testing in China

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Received 12 January 2016; revised 3 March 2016; accepted 15 March 2016
Available online - 14 March 2016

ABSTRACT

Introduction: Currently, several approaches are being used to detect echinoderm microtubule associated protein like 4 gene (*EML4*)–anaplastic lymphoma receptor tyrosine kinase gene (*ALK*) rearrangement, but the performance of laboratories in China is unknown. To evaluate the proficiency of different laboratories in detecting *EML4-ALK* rearrangement, we organized a proficiency test (PT).

Methods: We prepared formalin-fixed, paraffin-embedded samples derived from the xenograft tumor tissue of three non–small cell lung cancer cell lines with different *EML4-ALK* rearrangements and used PTs to evaluate the detection performance of laboratories in China.

Results: We received results from 94 laboratories that used different methods. Of the participants, 75.53% correctly identified all samples in the PT panel. Among the errors made by participants, false-negative errors were likely to occur. According to the methodology applied, 82.86%, 76.67%, 77.78%, and 66.67% of laboratories using reverse transcriptase polymerase chain reaction, fluorescence in situ hybridization, next-generation sequencing, and immunohistochemical analysis, respectively, could analyze all the samples correctly. Moreover, we have found that the laboratories' genotyping capacity is high, especially for variant 3.

Conclusion: Our PT survey revealed that the performance and methodological problems of laboratories must be addressed to further increase the reproducibility and accuracy of detection of *EML4-ALK* rearrangement to ensure reliable results for selection of appropriate patients.

Keywords: *EML4-ALK* rearrangement; Proficiency testing; Non–small cell lung cancer; Xenograft

Introduction

Echinoderm microtubule associated protein like 4 gene (*EML4*)–anaplastic lymphoma receptor tyrosine kinase gene (*ALK*) rearrangement is observed in approximately 5% of non–small cell lung cancer cells. The effective use of crizotinib critically depends on the accurate detection of *ALK* rearrangement. Currently, several approaches, including immunohistochemical (IHC) analysis, fluorescent in situ hybridization (FISH), and reverse transcriptase polymerase chain reaction (RT-PCR), have been developed to detect *EML4-ALK* rearrangement. More recently, next-generation sequencing (NGS) has been gradually applied to the detection of *EML4-ALK* rearrangement.[1,2] For optimal detection of *EML4-ALK* rearrangements in non–small cell lung cancer, the external quality assessment or proficiency test (PT) becomes one of the most important measures to evaluate the proficiency of detection of *EML4-ALK* rearrangement.

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Disclosure: The authors declare no conflict of interest.

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ISSN: 1556-0864
http://dx.doi.org/10.1016/j.jtho.2016.03.004

To date, several nationwide round robin tests have been conducted to assess the performance of *EML4-ALK* FISH and IHC testing.[3–6] But the accuracy of *EML4-ALK* testing in China was unknown, especially for molecular testing. Thus, to ensure the reliability of such *EML4-ALK* rearrangement testing, the National Center for Clinical Laboratories organized a PT of *EML4-ALK* detection in China for the first time.

Materials and Methods

As cell lines used for test samples, we selected H3122 with variant 1 fusion type. We selected H3122 with variant 1 fusion type, H2228 with the variant 3a/b fusion type, and H1299 cells (which are negative for *EML4-ALK* rearrangement). Once the number of cells reached 1×10^7, cells were injected into nude mice. Female nu/nu nude mice between 21 and 28 days of age were purchased from Vitalriver (Beijing, People's Republic of China) for xenograft production. After 6 to 8 weeks, the size of the xenograft tumors had progressed to approximately 500 mm^3 and they were surgically removed immediately. After tissue fixation and handing, formalin-fixed, paraffin-embedded (FFPE) samples were evaluated using three different methods: RT-PCR, FISH, and IHC analysis, which were widely used in the routine work.

Panels were prepared by the National Center for Clinical Laboratories from the FFPE samples just described. Each panel comprised samples of two sections of the H1299 xenograft tumor (ALK-01 and ALK-04), samples of two sections of the H3122 xenograft tumor (ALK-02 and ALK-03), and one sample of a section of the H2228 xenograft tumor (ALK-05). Each specimen was cut into 5.76-$cm^2 \times$ 4-μm sections (for FISH and IHC analysis) or 5.76-$cm^2 \times$ 10-μm rolls (for RT-PCR and NGS) that included more than 10^5 target cells. The sections with the same code were derived from the same paraffin block. Because all the methods were qualitative, the different forms and amounts would not affect the results. Participation in this study was opened to all related laboratories in Mainland China in 2015. The results of analysis of the samples that the laboratories tested using their routine procedures were reported as either positive or negative. The percentages of laboratories with completely correct results (perfect), only one wrong result (acceptable), and more than one wrong result (unacceptable) were determined. The sensitivity and specificity of the different assays in the different methodological groups were evaluated. The accuracy of detection of the types of fusion was also evaluated.

Results

Validation of Samples

The results obtained by three evaluation methods were in accordance with the excepted results (Supplementary Fig., Supplementary Digital Content 1; Supplementary Table, Supplementary Digital Content 2; and Supplementary Table, Supplementary Digital Content 3).

Methods and Results of Analysis of the Panel

In total, 94 laboratories submitted their results before the deadline, including 37 hospital pathology departments, 31 other hospital clinical laboratories, and 26 commercial laboratories or reagent manufacturers. The main methodology utilized by participants (Fig. 1) was RT-PCR (70 of 94 [74.47%]), followed by FISH (30 of 94 [31.91%]), IHC analysis (21 of 94 [22.34%]), and NGS (nine of 94 [9.57%]). Of the 94 participants, 28 (29.79%) applied more than one method to detect *EML4-ALK* rearrangement. The combinations RT-PCR plus IHC analysis and RT-PCR plus FISH plus IHC analysis were used most frequently (each used by 28.57% of the participants [8 of 28]), followed by RT-PCR plus FISH (used by 17.86% of the participants [five of 28]) and FISH plus IHC analysis (used by 17.86% [five of 28]). In the RT-PCR, FISH, NGS, and IHC analysis groups, the main kits utilized were the AmoyDx EML4-ALK Fusion Gene Detection Kit (AmoyDX, Xiamen, People's Republic of China) (52 of 70 [74.29%]), Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular, Des Plaines, IL) (23 of 30 [76.67%]), Thermo Fisher Oncomine Solid Tumor Fusion Transcript kit (Thermo Fisher Scientific, Waltham MA) (four of nine [44.44%]), and Ventana ALK (D5F3) CDx Assay (Ventana Medical Systems, Tucson, AZ) (13 of 21 [61.90%]), respectively.

Performance of Participants

In total, 71 participants (75.53%) correctly identified all of the samples in the PT panel (perfect), 12 laboratories (12.77%) made one mistake (acceptable), and 11 laboratories (11.70%) made more than one mistake (unacceptable). Table 1 shows the details of the mistakes made by participants in this survey. Among these errors, false-negative errors (80% [32 of 40]) were the most likely to occur. The most detection errors occurred with sample ALK-02, followed by with ALK-03, which indicates that laboratories might be more likely to give an erroneous result for the detection of variant 1 fusion type.

We also evaluated the performance of the participants according to the methodology they applied (Table 2). Because all these methods were used by more than five participants, the sensitivity and specificity might not be influenced by the results of individual laboratories. The total sensitivities of the RT-PCR group, FISH group, NGS group, and IHC analysis group were 92.38%, 87.78%, 88.89%, and 96.83%, respectively. The total specificities of these groups were 99.29%, 96.67%,