ALK gene rearrangement testing in non-small cell lung carcinoma: correlation between ultrasensitive IHC and FISH.

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Abstract

Introduction / Background: Anaplastic lymphoma kinase (ALK) gene rearrangements in advanced non-small cell lung carcinomas (NSCLC) are an indicator for targeted therapy with ALK-specific inhibitors, including crizotinib. ALK rearrangement status is commonly assessed by fluorescence in situ hybridization (FISH) using the IVD-class system approved by the US FDA as a companion diagnostic tool for crizotinib-based treatment eligibility (Abbott Molecular, USA). The utility of IHC, a more affordable and accessible method, has been challenged by low protein expression levels of ALK fusion transcripts in NSCLC. Here we assessed a modified ultrasensitive IHC method as an alternative to FISH for detecting ALK rearrangements in a NSCLC case series at our Institution.

Materials and Methods: This study included 318 formalin-fixed paraffin-embedded (FFPE) samples from 296 patients with advanced NSCLC. The standard IHC procedure used for ALK staining was performed using a recently developed rabbit monoclonal antibody to the ALK protein (D5F3, Cell Signaling Technology, Inc., USA). The D5F3 antibody was selected due to its clinical performance in ALK testing. IHC staining was interpreted as positive or negative rather than scored on a semi-quantitative scale. FISH was performed using the AMV ALK Break Apart FISH Probe Kit (Abbott Molecular, USA). A cutoff of 15% positive cells (15% and 18%) was used for both FFPE FISH and ThinPrep FISH.

Results: Of 188 samples negative for ALK rearrangements by FFPE FISH, all were also negative for ALK expression by IHC. Of 33 samples positive by FFPE FISH, 31 were also positive by IHC. Two discordant cases approached the cut-off of 15% positive cells (15% and 18%) and were resolved by ThinPrep FISH as negative, in agreement with the initial IHC result. Overall, after discordance resolution, IHC demonstrated 100% sensitivity and specificity, and perfect agreement with FISH in detecting ALK rearrangements in NSCLC samples.

Conclusions: Ultrasensitive IHC can reliably detect ALK rearrangements resulting from ALK gene rearrangements in NSCLC. The high concordance between IHC and FISH warrants the routine use of IHC as the initial component of an algorithmic approach to detection of ALK rearrangements in NSCLC, followed by reflex FISH confirmation of IHC-positive cases.

Table 1: Concordance between IHC and FISH in detecting ALK status

<table>
<thead>
<tr>
<th>FISH</th>
<th>IHC</th>
<th>Total</th>
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<tbody>
<tr>
<td>Positive</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>210</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>210</td>
</tr>
</tbody>
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Sensitivity 100.0% Specificity 100.0% Agreement 100.0%

References