ORIGINAL ARTICLE

ALK Protein Analysis by IHC Staining after Recent Regulatory Changes: A Comparison of Two Widely Used Approaches, Revision of the Literature, and a New Testing Algorithm

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ABSTRACT

Introduction: Recent regulatory changes have allowed the diagnostic use of immunohistochemical (IHC) analysis for the identification of patients with non–small cell lung cancer who are eligible for treatment with anaplastic lymphoma receptor tyrosine kinase (ALK) inhibitors. The U.S. Food and Drug Administration has approved the VENTANA ALK (D5F3) CDx Assay (Ventana Medical Systems, Tucson, AZ) as companion diagnostics, and the Italian Medicines Agency has recognized IHC analysis as a diagnostic test indicating an algorithm for patient selection.

Methods: On the basis of the new regulations, we compared two commonly used IHC assays on 1031 lung adenocarcinomas: the VENTANA ALK (D5F3) CDx Assay with the OptiView Amplification Kit (Ventana Medical Systems) and a standard IHC test with the clone 5A4 (Novocastra, Leica Biosystems, Newcastle Upon Tyne, United Kingdom) along with their interpretative algorithms. Fluorescence in situ hybridization (FISH) was performed in all cases. Next-generation sequencing was performed in FISH/IHC analysis–discordant samples.

Results: FISH gave positive results in 33 (3.2%) cases. When FISH was used as a reference, the VENTANA ALK (D5F3) CDx assay had a sensitivity of 90.9% ± 2.6%, a specificity of 99.8% ± 0.6%, and positive and negative predictive values of 93.8% ± 2.1% and 99.7% ± 0.6%, respectively. The clone 5A4–based IHC test showed a sensitivity of 90.9% ± 2.6%, a specificity of 98.3% ± 1.3%, and positive and negative predictive values of 63.8% ± 4.2% and 99.7% ± 0.6%, respectively. Five cases with IHC analysis/FISH-discordant results in our series were analyzed together with those previously reported in the literature. Overall, data from 35 patients indicate a response rate to ALK inhibitors in 100% of FISH-negative/IHC analysis–positive cases (seven of seven) and 46% of FISH-positive/IHC analysis–negative cases (13 of 28), respectively.

Conclusions: Our results confirm the difficulty in managing an IHC test without amplification in the absence of confirmatory FISH analysis, as well as the possibility of performing a direct diagnosis in approximately 90% of patients by the VENTANA ALK (D5F3) CDx Assay. On the basis of the recent regulatory changes, the data that have emerged from the literature, and the results of the present study, a new algorithm for ALK assessment in non–small cell lung cancer has been devised.

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Drs. Marchetti, Di Lorito, and Pace equally contributed to this study.

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Introduction

Anaplastic lymphoma receptor tyrosine kinase gene (*ALK*) rearrangements have been described in 3% to 5% of cases of non–small cell lung cancer (NSCLC), and their identification is mandatory to select patients for treatment with anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitors.[1,2]

Different technologies are available to assess *ALK* gene rearrangements. Fluorescence in situ hybridization (FISH) is the accepted standard because it has been used as a reference method in clinical trials; however, it is an expensive, time-consuming, and labor-intensive assay. In addition, result interpretation is often operator dependent.[3,4] An alternative diagnostic method based on the detection of ALK fusion protein expression is immunohistochemical (IHC) analysis. This method is widely used in pathology laboratories, faster, cheaper, and particularly useful in patients with advanced-stage carcinoma, for whom small biopsy specimens with a limited number of neoplastic cells are often available.[5,6]

Different monoclonal antibodies for the detection of ALK protein expression are commercially available, including the clone ALK1 (Dako, Glostrup, Denmark), the clone 5A4 (Novocastra, Leica Biosystems, Newcastle Upon Tyne, United Kingdom), and the clone D5F3 (Cell Signaling Technology, Danvers, MA). At the moment, the clones D5F3 and 5A4 are the most widely used for the detection of ALK expression in patients with NSCLC.[5,7,8]

The clone 5A4 has been utilized on different platforms, including the BOND-MAX immunostainer (Leica Microsystems, Wetzlar, Germany). In this case, a scoring system based on four levels of ALK expression (0, 1+, 2+, and 3+) has been adopted in most previous reports.[4,7,9,10] In a large multicenter study, 1+ tumors were found to be positive by FISH analysis in 4% of cases and 2+ tumors were found in 60% of cases.[11] Therefore, 1+ or 2+ samples should be considered equivocal and should be validated by FISH. This leads to a marked cost increase and delayed medical reports.

A D5F3-based immunoassay, the VENTANA ALK (D5F3) CDx Assay (Ventana Medical Systems, Tucson, AZ), has been developed and standardized on the automated immunostaining platform BenchMark XT (Ventana) combined with the OptiView Amplification Kit (Ventana). The interpretation of results is based on a dichotomic algorithm described in the product data sheet. Cases are defined as positive or negative according to the presence or absence of a specific immunoreaction in tumor cells.[12]

In June 2015, the U.S. Food and Drug Administration (FDA) approved the VENTANA ALK (D5F3) CDx Assay as a companion diagnostic to aid in the identification of patients eligible for treatment with the ALK inhibitor crizotinib.[13] The Italian Medicines Agency (AIFA), in line with the FDA, has recognized IHC analysis as a diagnostic test, suggesting an algorithm for patients selection that is based on a definitive IHC testing result (positive or negative) regardless of the antibody used. Equivocal cases must be confirmed by FISH (Supplementary Fig. 1, Supplementary Digital Content 1).[14]

On the basis of the new recommendation for the IHC analysis of ALK in NSCLC, we decided to compare two commonly used IHC assays on a large series of lung adenocarcinomas: the ALK (D5F3) CDx Assay on the BenchMark XT platform with the Optiview Amplification Kit along with its related interpretative algorithm and an assay based on the use of the clone 5A4 on the BOND-MAX platform with its own algorithm. The main objective of this study was to compare the performances of these two diagnostic approaches for the selection of patients to be enrolled for treatment with anti-ALK drugs.

Materials and Methods

Tumor Samples

The study was conducted on a retrospective series of 1031 lung adenocarcinoma samples obtained from as many patients as underwent a radical resection of a primary NSCLC at the Department of Thoracic Surgery, University of Chieti (Chieti, Italy). Tumor samples were fixed in formalin, embedded in paraffin, and histologically classified as adenocarcinomas on the basis of hematoxylin and eosin and IHC staining according to the WHO classification of lung tumors.[15] Representative tumor areas were identified and tissue microarrays (TMAs) were built using two large (2-mm-diameter) cores for each case. Informed consent was obtained from all patients under study. The study was approved by the local human investigations committee and was conducted in accordance with the precepts of the Helsinki Declaration.

ALK IHC Analysis

TMA samples were cut to a thickness of 4 μm and stained using two different ALK IHC assays: the Novocastra mouse monoclonal antibody p80 ALK (Clone 5A4, Leica Biosystems, Newcastle Upon Tyne, United Kingdom) and the Ventana anti-ALK rabbit monoclonal primary antibody (Clone D5F3, Cell Signaling Technology).