A Novel, Highly Sensitive Antibody Allows for the Routine Detection of ALK-Rearranged Lung Adenocarcinomas by Standard Immunohistochemistry


Abstract

Objective: Approximately 5% of lung adenocarcinomas harbor an EML4-ALK gene fusion and define a unique tumor group that may be responsive to targeted therapy. However, ALK-rearranged lung adenocarcinomas are difficult to detect by either standard fluorescence in situ hybridization or immunohistochemistry (IHC) assays. In the present study, we used novel antibodies to compare ALK protein expression in genetically defined lung cancers and anaplastic large cell lymphomas.

Experimental Design: We analyzed 174 tumors with one standard and two novel monoclonal antibodies recognizing the ALK protein. Immunostained tissue sections were assessed for the level of tumor-specific ALK expression by objective quantitative image analysis and independently by three pathologists.

Results: ALK protein is invariably and exclusively expressed in ALK-rearranged lung adenocarcinomas but at much lower levels than in the prototypic ALK-rearranged tumor, anaplastic large cell lymphoma, and as a result, is often not detected by conventional IHC. We further validate a novel IHC that shows excellent sensitivity and specificity (100% and 99%, respectively) for the detection of ALK-rearranged lung adenocarcinomas in biopsy specimens, with excellent interobserver agreement between pathologists (κ statistic, 0.94).

Conclusions: Low levels of ALK protein expression is a characteristic feature of ALK-rearranged lung adenocarcinomas. However, a novel, highly sensitive IHC assay reliably detects lung adenocarcinomas with ALK rearrangements and obviates the need for fluorescence in situ hybridization analysis for the majority of cases, and therefore could be routinely applicable in clinical practice to detect lung cancers that may be responsive to ALK inhibitors.

Lung cancer remains the leading cause of cancer death worldwide. In the United States alone, there are >200,000 new cases of lung cancer, resulting in 150,000 deaths/y (1). Despite improvements in the detection and treatment of lung cancer, the overall 5-year survival rate remains at 15% (2). A subset of lung cancers harbors activating mutations in the epidermal growth factor receptor (EGFR) gene

(3, 4). The majority of patients with lung cancers harboring EGFR mutations, but only a small fraction of those without EGFR mutations, can show dramatic responses to drugs that inhibit EGFR kinase activity, resulting in prolonged patient survival (5). Therefore, the identification of critical tyrosine kinases and the development of specific tyrosine kinase inhibitors targeting individual tumors has become a new paradigm in lung cancer treatment (6, 7).

Recently, two groups independently discovered that rare lung adenocarcinomas harbor rearrangements of the anaplastic large cell kinase (ALK) gene that result in the pathologic expression of a fusion protein, most commonly EML4-ALK (8, 9). EML4-ALK shows constitutive kinase activity, and ALK-rearranged lung cancer cell lines are dependent upon ALK kinase activity for growth and survival (8, 10, 11). However, standard tyrosine kinase inhibitors that target EGFR are poor inhibitors of ALK kinase activity, and as such, they have shown no therapeutic benefit to patients with ALK-rearranged lung adenocarcinomas (12). In contrast, a novel tyrosine kinase inhibitor that targets ALK kinase activity has shown dramatic clinical responses among the few patients with ALK-rearranged tumors treated to date (13). These data highlight the importance of
development of tyrosine kinase inhibitors that specifically target ALK enzymatic activity and, in addition, a method to accurately identify patients with ALK-rearranged lung adenocarcinoma, and thus the best candidates for clinical trials (12, 13).

Testing for ALK rearrangements is most frequently done during the pathologic evaluation of anaplastic large cell lymphoma (ALCL), a tumor of T-cell origin, and most generally involves one of three methodologies: karyotyping (g-banding) of tumor metaphase spreads, fluorescence in situ hybridization (FISH) with probes flanking the ALK locus, and immunohistochemical staining of tumor biopsy tissue with antibodies specific for the ALK protein (14). All three methodologies are highly specific for detecting ALK rearrangements in ALCL and extensively validated (15). However, each of these methods confronts significant challenges when applied to the detection of ALK rearrangements in lung adenocarcinoma (16). Unlike ALCL, ALK rearrangements in lung adenocarcinoma frequently involve an intrachromosomal deletion of genetic material 5′ of the ALK locus not visible by routine karyotyping analysis. Although FISH analysis is able to resolve the defect, the altered probe hybridization patterns are subtle and have led to false negative results (16). Finally, we have reported that lung adenocarcinomas known to harbor an ALK rearrangement by FISH analysis do not always have detectable ALK protein expression when tested by conventional or tyramide-amplified IHC. This finding, coupled with reports that tumors can express EML4-ALK transcripts in the absence of ALK protein, raises questions about whether ALK protein expression is invariably expressed by tumors harboring an EML4-ALK fusion and whether IHC is a reliable diagnostic test for the diagnosis of ALK-rearranged lung adenocarcinoma (16, 17).

Here we compare ALK protein expression in a large cohort of genetically defined tumors by IHC with the use of novel, highly sensitive antibodies and show that the ALK protein is universally expressed by ALK-rearranged lung adenocarcinomas. We also show that ALK protein expression in ALK-rearranged lung adenocarcinomas is much lower than in ALK-rearranged ALCLs and, as a result, is often not detected by conventional IHC. This novel immunohistochemical test shows very high sensitivity and specificity for detecting ALK-rearranged lung adenocarcinomas when evaluated by experienced pathologists, and therefore will serve as a robust diagnostic tool in routine surgical pathology practice.

Materials and Methods

Case selection

Optimization studies. We examined 19 cases of ALCL and 2 cases of ALK-rearranged inflammatory myofibroblastic tumor to test and optimize two newly developed monoclonal antibodies (mAb) specific for the ALK protein in formalin-fixed paraffin embedded (FFPE) specimens.

Lung adenocarcinoma studies. We examined 153 cases of lung adenocarcinoma, 22 ALK rearranged, including 8 previously analyzed (16), and 131 ALK germline. All cases consisting of FFPE tissue biopsies or resection specimens were evaluated with the use of standard pathology methods as described (18), and classified according to standard pathology practice (14, 19). Cases primarily originated at the authors’ primary institutions (Brigham and Women’s Hospital, Dana-Farber Cancer Institute, Beth Israel-Deaconess Medical Center, and Massachusetts General Hospital, Boston, MA); 13 cases of the ALK-rearranged lung adenocarcinomas were samples from outside institutions sent as referrals. Among the lung adenocarcinomas, 116 of 153 cases were part of a tissue microarray described previously (16).

Immunohistochemistry

Immunohistochemical staining was done on 4-μm thick FFPE tissue sections. Briefly, slides were deparaffinized and pretreated with 1 mmol/L EDTA (pH 8.0; Zymed, San Francisco, CA) and heat-mediated antigen retrieval solution in a steam pressure cooker (Decloaking Chamber, Biocare Medical, Walnut Creek, CA). All further steps were done at room temperature in a hydrated chamber. Endogenous peroxidase activity was quenched with Peroxidase Block (Dako USA, Carpinteria, CA) for 5 min, and slides were preincubated in 20% normal goat serum in 50 mmol/L Tris-HCl (pH 7.4). Mouse monoclonal anti-human CD246 (clone ALK1; Dako USA) was applied either at 1:50 dilution in Dako diluent (for ALCLs) or at 1:2 dilution in Dako diluent (for lung adenocarcinomas and inflammatory myofibroblastic tumors) overnight. The slides were then washed in Tris-HCl and detected with horseradish peroxidase-conjugated anti-mouse EnVision+ kit (Dako). Rabbit monoclonal anti-human CD246 (clones D5F3 and D9E4; Cell Signaling Technology, Danvers, MA) was applied at either 1:500 (for ALCLs) or 1:100 dilution.
ALK Protein Expression in ALK-Rearranged Lung Cancer

Published OnlineFirst February 23, 2010; DOI: 10.1158/1078-0432.CCR-09-2845

dilution (for lung adenocarcinomas and inflammatory myofibroblastic tumors) in Dako diluent overnight. The slides were then washed in Tris-HCl and detected with horseradish peroxidase-conjugated anti-rabbit EnVision+ kit (Dako). All slides were counterstained with hematoxylin.

The mouse mAb ALK1 was raised against a peptide encoded by amino acids 1359 to 1460 of the human ALK protein (Pulford et al., 1997). This peptide spans the c-terminal portion of the tyrosine kinase domain of ALK, and is preserved in NPM-ALK, EML4-ALK, and all other known pathologic ALK fusions. The rabbit mAbs D5F3 and D9E4 were raised against a peptide derived from the c-terminal portion of the human ALK downstream of the kinase domain, and preserved in NPM-ALK, EML4-ALK, and all other known pathologic ALK fusions.

Fluorescence in situ hybridization

FISH was done on FFPE tumor tissues with the use of a break-apart probe specific to the ALK locus (Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Molecular, Abbott Park, IL) according to the manufacturer’s instructions on all cases of lung adenocarcinoma. FISH-positive cases were defined as ≥15% split signals in tumor cells as previously described (12).

Image analysis

Whole-section slides stained with D5F3 antibody or ALK1 antibody (n = 56 and n = 51, respectively) were scanned at ×200 magnification with the use of an Aperio ScanScope XT workstation (Aperio Technology, Inc., Vista, CA). Images were visualized and annotated with the ImageScope software (version 10.0.35.1800, Aperio Technology), and analyzed with the use of a standard analysis algorithm (color deconvolution v9.0, Aperio Technology). Briefly, one pathologist (S.J.R.) selected a minimum of three regions consisting of tumor cells with the most intense staining. These regions were then subjected to the deconvolution program, which separates brown (3,3′-diaminobenzidine) from blue (hematoxylin) tissue staining. Based on a single baseline absorbance determined as the threshold for positive staining by a pathologist (and which was applied to all analyzed slides), the software calculated a score that was defined as the average absorbance of the positively stained area multiplied by the percentage of the area staining above the baseline. The threshold for positive staining with objective analysis was determined as the minimum image analysis score necessary to ensure perfect specificity of the assay (to exclude any false positive cases). For both D5F3 and ALK1 antibodies, a score of ≥1.0 was considered positive in the ALCIs. In the lung adenocarcinomas, a score ≥1.0 was considered positive for the D5F3 antibody, and a score >2.7 was considered positive for the ALK1 antibody.

Study of interobserver variation and statistical analysis

Three independent pathologists (M.M-K., L.R.C., and S.J.R.) blindly reviewed each stained slide, and semiquantitatively graded the intensity of staining (0-3) and assessed the percentage of immunoreactive tumor cells in 10% increments. Before the analysis, each pathologist reviewed two to three lung adenocarcinomas of known ALK status stained with each antibody. This allowed each pathologist to assess the level of nonspecific or background staining characteristic of the individual reagents.

Positive ALK protein expression as determined by any individual pathologist was defined as tumor-specific staining of any intensity over background staining in ≥10% of the tumor cells. The correlation between the individual blinded pathologist’s scoring was done with the use of the Fleiss k statistic. For sensitivity and specificity calculations, an individual case was deemed positive for ALK protein expression if two of three pathologists scored the case as positive. Sensitivity, specificity, positive predictive value, and negative predictive value for detecting ALK-rearranged cases were calculated with the use of GraphPad Prism (version 5.02 for Windows, GraphPad Software, San Diego, CA).

Results

D5F3 and D9E4 antibodies detect ALK protein expression in formalin-fixed tumor samples. We first sought to determine whether two novel rabbit mAbs, clones D5F3 and D9E4, are able to detect ALK protein expression in FFPE specimens. We optimized standard immunohistochemical staining with the use of known cytogenetically defined types of tumors: an ALK-rearranged ALCL, an ALK-germline ALCL, and an ALK-rearranged inflammatory myofibroblastic tumor (Fig. 1). We confirmed that both D5F3 and D9E4 antibodies showed robust tumor-specific staining in ALK-rearranged tumors and essentially no tissue staining in ALK-germline tumors with the use of a wide range of antibody dilutions, antigen retrieval methods, and secondary antibody detection methods (data not shown). The optimal staining conditions were chosen based on the specifics of ALK expression in each tumor type. Whereas ALK protein expression in the ALK-rearranged ALCL was relatively easy to detect (Figs. 1A and B, and 2), lower titers of antibody were used to conserve reagent and minimize any background staining (Fig. 1D and E). In contrast, in the two ALK-rearranged inflammatory myofibroblastic tumors that we tested, we found lower levels of ALK protein that necessitated the highest concentration of D5F3 and D9E4 antibodies possible without an increase in nonspecific background staining (Fig. 1 and data not shown). For these cases we also determined the maximum concentration of a standard IHC reagent, the mAb ALK1, that we could use (16). At these highest concentrations, we found that IHC with the use of either D5F3 or D9E4 resulted in more intense staining of tumor cells (Fig. 1G and H) than IHC with the use of ALK1.

5 www.graphpad.com
antibody (Fig. 1I); however, the staining intensity with D9E4 was slightly lower than that with D5F3. We thus decided to use the D5F3 antibody in the subsequent evaluation.

**Correlation between D5F3 and ALK1 antibodies in detecting ALK protein in ALCL.** IHC with the use of the antibody ALK1 has been extensively tested against genetically defined groups of ALCLs and shown to be an effective surrogate for genetic testing (15). We therefore compared IHC staining with the use of one of our novel antibodies, D5F3, with that using ALK1 antibody on a group of 19 ALCLs. The staining patterns were objectively analyzed by image analysis (Fig. 2A) and blindly interpreted by three pathologists. By image analysis, the cases were separable into two groups: 11 cases with staining intensity scores ≥1.0 and deemed positive for ALK protein, and 8 cases with intensity scores <1.0 and deemed negative for ALK protein. Importantly, with this threshold for staining, all cases deemed positive for ALK protein with the use of the ALK1 antibody were also deemed positive with the use of the D5F3 antibody, indicating a perfect correlation. The same cases were independently and blindly read by three pathologists. With the use of both the ALK1 and D5F3 antibodies, and evaluating the ALK protein expression, there was perfect agreement between individual pathologists and with the objective analysis done by digital scanning (data not shown). Cytogenetic/FISH analysis and IHC analysis were done on the tumor samples in 9 of 19 cases and in 12 of 19 cases, respectively, at the time of the original diagnosis. The staining results with the use of the D5F3 or ALK1 antibody in the current analysis were in complete concordance with those prior studies (data not shown). The ALK-rearranged tumor with the lowest staining intensity was categorized as negative for ALK protein expression (Case 11, Fig. 2A) and the ALK-germline tumor with the highest staining intensity was categorized as negative for ALK protein expression (Case 12, Fig. 2A) by both standard microscopy and digital scanning, and were easily distinguishable visually (Fig. 2B). We conclude that IHC staining with D5F3 antibody is specific to ALK protein expression and is comparable with IHC staining with ALK1 antibody for establishing the diagnosis of ALK-positive ALCL.

**ALK is universally expressed at lower levels in ALK-rearranged lung adenocarcinomas than in ALCL.** We previously reported that among 10 cases of ALK-rearranged lung adenocarcinomas stained by conventional IHC with the ALK1 antibody, we could detect ALK protein in only 4 cases (16). These findings suggested that ALK protein expression may be lower in many cases of ALK-rearranged lung adenocarcinoma relative to ALCL and that conventional IHC analysis may not be a useful surrogate for FISH analysis. Alternatively, ALK protein may not be expressed in all cases of ALK-rearranged lung adenocarcinomas. To determine whether IHC with D5F3 better detects ALK expression in lung adenocarcinoma, we compared 22 ALK-rearranged cases with 131 ALK-germline cases. ALK-rearranged cases included those positive for the transcription factor TTF1 (16 cases;
89%) and negative for TTF1 (2 cases; 11%). In all cases the ALK status of the tumor was determined by FISH analysis. Because our preliminary data indicated that low-level ALK expression may be difficult to detect in lung adenocarcinoma, we increased the titers of D5F3 and ALK1 antibodies in our IHC reactions to maximize the sensitivity without sacrificing the specificity of the assay (see Materials and Methods). Furthermore, we did not find that alternative antigen retrieval methods improved the sensitivity of the assay (data not shown). In 17 of 19 ALK-rearranged lung adenocarcinomas for which a direct comparison can be made, the D5F3 antibody showed more intense staining of the tumor cells than did the ALK1 antibody (Fig. 3A). For the vast majority of ALK-rearranged lung adenocarcinomas, the relative amount of staining with D5F3 was, objectively, much higher than that found with ALK1 (6.6 ± 4.7 absorbance × % positive staining versus 2.9 ± 2.6 absorbance × % positive staining; \( P < 0.001 \)); in the 2 cases in which D5F3 antibody showed lower staining than ALK1 antibody, the relative difference was modest (Fig. 3A). Importantly, the objective intensity score of
D5F3 staining in ALK-rearranged lung adenocarcinomas was lower than that in ALK-rearranged ALCLs in 31 of 33 cases (compare Fig. 2A with Fig. 3A; D5F3 staining). Given that we used five times more D5F3 antibody per IHC reaction (but the same retrieval and secondary reagents) to test the lung adenocarcinomas than the amount we used to test the ALCLs (decreasing the dilution of the antibody from 1:500 to 1:100), we conclude...
that ALK protein expression is always lower in ALK-rearranged lung adenocarcinomas relative to ALK-rearranged ALCLs.

**D5F3 antibody, but not ALK1 antibody, is a useful surrogate for genetic testing in the diagnosis of ALK-rearranged lung adenocarcinoma.** Both visual examination (Table 1) and objective quantitation of IHC staining (Fig. 3B and C) confirm that IHC with D5F3 antibody is superior to that with ALK1 antibody in assessing ALK expression in lung adenocarcinomas. A major difference between the antibodies is the level of nonspecific staining of tumor and non-neoplastic tissues at high concentrations of ALK1 antibody. In fact, when the intensity of nonspecific staining is objectively calculated and the threshold that will ensure 100% specificity is established (Fig. 3B, dotted horizontal line), only 6 of 19 (32%) ALK-rearranged lung adenocarcinomas are identified. Objective analysis of cases stained with D5F3 antibody show very little nonspecific or background staining at the antibody titer necessary to detect ALK expression in lung adenocarcinoma at 100% sensitivity and specificity for the 37 cases (Fig. 3C, dotted horizontal line). Similarly, an evaluation of 153 cases of lung adenocarcinoma (22 ALK rearranged, 131 ALK germline) by three pathologists blinded to the genetic status of the tumors at the time of examination showed reproducibly high sensitivity and specificity for the identification of ALK-rearranged tumors in the clinical setting (sensitivity, 100%; specificity, 99%; Table 1).

**Weak staining with D5F3 antibody suggests the presence of an ALK-rearranged lung adenocarcinoma.** Visual inspection of select ALK-rearranged and ALK-germline adenocarcinomas stained with D5F3 reveals that the expression of the ALK protein may be faint, or detectable only in a subset of the tumor cells. Overall, approximately one third of ALK-rearranged lung adenocarcinomas show robust staining of the tumor cells for ALK protein with D5F3 antibody (Fig. 4A and D, representing Cases 1 and 2, respectively, from Fig. 3C). Another one third of ALK-rearranged lung adenocarcinomas show moderate but distinct tumor cell staining (not shown), and the final one third shows weak and sometimes focal staining for ALK protein with D5F3 antibody (Fig. 4G). Importantly, ALK-germline lung adenocarcinomas show no staining with D5F3 antibody under the conditions we describe here (Fig. 4I).

The potential for false negatives in screening for ALK-rearranged lung adenocarcinomas by IHC. In our hands, the IHC test we describe here shows very high sensitivity and specificity for detecting ALK-rearranged lung adenocarcinomas and as such can act as a surrogate for genetic testing. However, the results of one case illustrate that a false negative result can occur. Case 20 (Fig. 3C) showed positive staining for ALK protein objectively and by the pathologists' interpretation of the IHC (Table 1; Fig. 5A). Over the course of this patient study, three more biopsy samples were obtained, of which one showed positive staining (Fig. 5B), one showed equivocal staining (Fig. 5C), and one showed no staining despite repeated testing (Fig. 5D). We considered the possibility that the third biopsy sample without detectable ALK expression did not harbor an ALK rearrangement; however, FISH analysis of this specimen confirmed the presence of the genetic abnormality (Fig. 5D, inset), and reverse transcriptase-PCR (RT-PCR) analysis also confirmed the expression of the EML4-ALK transcript (Supplementary Table S1). We therefore conclude that the IHC result is false negative.

**Correlation among IHC, FISH, and RT-PCR analysis.** In addition to IHC and FISH analysis, RT-PCR analysis has been suggested as a potential tool to detect ALK rearrangements in lung adenocarcinoma (20, 21). We had sufficient tissue to do RT-PCR analysis on 10 cases of ALK-rearranged lung adenocarcinoma in an assay designed to detect the three major EML4-ALK variant fusions: V1, V2, and V3 (21). We obtained a positive RT-PCR result in 9 of the 10 cases, including 6 cases with an EML4-ALK V1 fusion and 3 cases with an EML4-ALK V3 fusion. All of these cases were considered positive for an ALK rearrangement by FISH analysis and for ALK protein expression by IHC with the D5F3 antibody (Supplementary Table S1). We found one case to be positive for ALK rearrangement by FISH analysis and for ALK protein expression by IHC, but negative by RT-PCR (Supplementary Table S1). Our failure to detect an ALK fusion transcript in this single case might be due to the presence of an EML4-ALK fusion variant not assayed for by our RT-PCR test or an ALK fusion with a gene other than EML4 (21). Thus, within this limited sample set, we have found that a sensitive IHC-based assay can show benefit over RT-PCR–based analyses.

### Table 1. Interpretation of IHC staining on lung adenocarcinomas by three pathologists

<table>
<thead>
<tr>
<th></th>
<th>Lung Adenocarcinoma (n = 153)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D5F3 antibody</td>
</tr>
<tr>
<td>Sensitivity (%)*</td>
<td>100</td>
</tr>
<tr>
<td>Specificity (%)*</td>
<td>99</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>96</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>100</td>
</tr>
<tr>
<td>( \kappa ) statistic</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*Of the pathologists' IHC interpretation as positive staining in predicting an ALK rearrangement

www.aacrjournals.org

Clin Cancer Res; 16(5) March 1, 2010 1567
Discussion

Until recently, chromosomal abnormalities leading to pathologic ALK protein expression had been described only in ALCLs, inflammatory myofibroblastic tumors, and rare diffuse large B-cell lymphomas (14, 22–25). The identification of ALK rearrangements in ALCL has traditionally been through karyotypic analysis of metaphase spreads, or FISH analysis of mitotic or interphase nuclei with the use of probes flanking the ALK locus (14). However, with the advent of mAbs that recognize the ALK protein, IHC analysis of tumor tissues has become a highly sensitive and cost-effective surrogate for genetic testing (26–28).

The ALK locus is now recognized to be pathologically dysregulated in ~5% of lung adenocarcinomas (8, 9, 16, 29). Most commonly, the genetic lesion consists of an intrachromosomal deletion and inversion event resulting in an EML4-ALK fusion that cannot be detected by conventional karyotypic analysis (8–10). Therefore, the diagnosis of ALK-rearranged lung adenocarcinoma requires IHC-based, FISH-based, or RT-PCR-based analysis of biopsy tissue.

Currently, several laboratories rely on FISH analysis of mitotic or interphase tumor nuclei, and identify a “split” hybridization signal to establish the presence of an ALK rearrangement (12). However, FISH is unlikely to be a preferred method for screening lung adenocarcinomas in routine surgical pathology practice because (a) the break-apart signal pattern resulting from the intrachromosomal deletion and inversion event in the setting of polysomy typical of lung cancer is subtle and easily missed (i.e., compare
IHC remains a preferred technique for screening and diagnosis in routine surgical pathology practice, and could prove to be a fast and cost-effective method to identify non-small cell lung cancer patients for clinical studies of ALK inhibitors. We, and others, have shown that IHC-based screening can be used to identify lung adenocarcinomas harboring known or novel ALK rearrangements (16, 21, 31). In an analysis of 10 cases of ALK-rearranged lung adenocarcinoma, we previously reported that a conventional diagnostic IHC test to detect ALK rearrangements in ALCL detected ALK protein expression in only 4 of 10 cases of ALK-rearranged lung adenocarcinoma (16). We were able to increase the sensitivity of the test and provide evidence of ALK protein expression in four of the remaining eight cases by including a nontraditional tyramide-based amplification step in our protocol. Others have published similar results with the use of a different, nontraditional amplification technique (21). However, it remained unresolved whether those cases with an ALK rearrangement detected by FISH but negative for ALK expression by IHC express the mutant protein at all (32).

With the use of 22 ALK-rearranged lung adenocarcinomas, among the largest collections reported to date, and 19 ALCLs, we compared ALK protein expression by the quantitative IHC analysis with the use of one well-described antibody and two novel antibodies. All three antibodies recognize epitopes within the ALK protein that is preserved in all known ALK fusions. We find that tumor-specific ALK protein expression in ALK-rearranged lung adenocarcinomas is much lower than that in ALK-rearranged ALCLs and
that this low level of protein expression necessitates higher titers of antibody for IHC-based detection. Furthermore, we found that IHC with the novel antibody D5F3 shows much greater sensitivity than that with the antibody ALK1 in detecting ALK-rearranged lung adenocarcinomas by either objective image analysis or by three pathologists’ individual interpretations. Most importantly, the interpretation of this novel IHC test is highly reproducible among pathologists and shows complete concordance with genetic data. We acknowledge that in addition to ALK1, D5F3, and D9E4, there are other commercially available antibodies that can detect ALK protein expression in ALCL (21). However, due to the limited tissue available from ALK-rearranged lung adenocarcinomas, we could not extend our study with additional antibodies. Similarly, we were unable to do a correlative study between EML4-ALK transcript expression and ALK protein expression as detected with our novel antibodies. As ALK-rearranged lung adenocarcinomas become more easily identified and larger collections are assembled, these additional comparisons can be made.

It has become clear that ALK-rearranged lung adenocarcinomas are a rare tumor with unique clinicopathologic characteristics. Patients with ALK-rearranged lung adenocarcinomas are unresponsive to tyrosine kinase inhibitors that target EGFR; however, a novel tyrosine kinase inhibitor that targets ALK has shown dramatic clinical responses in an ongoing clinical trial (12, 13). Therefore, the accurate and timely identification of patients with ALK-rearranged lung adenocarcinomas is likely to be of therapeutic importance. We believe that IHC with the use of the novel rabbit mAb D5F3 is a preferred method for identifying ALK-rearranged lung adenocarcinomas in routine clinical practice.  

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Herbert Haack and Katie Cossby of Cell Signaling Technology for providing the D5F3 and D9E4 antibodies. Tyler Caron for expert technical assistance, and Brittany MacFarland for secretarial assistance.

Grant Support

National Cancer Institute Specialized Programs of Research Excellence in Lung Cancer grant CA09578, Department of Defense grant W81XWH06-1-0303, and NIH grant RO1CA136851 (P.A. Jänne and B.E. Johnson).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.


References


Clinical Cancer Research

A Novel, Highly Sensitive Antibody Allows for the Routine Detection of ALK-Rearranged Lung Adenocarcinomas by Standard Immunohistochemistry


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-09-2845

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2010/03/01/1078-0432.CCR-09-2845.DC1

Cited articles
This article cites 29 articles, 13 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/16/5/1561.full#ref-list-1

Citing articles
This article has been cited by 46 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/16/5/1561.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/16/5/1561.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.