ALK (D5F3) CDx: an immunohistochemistry assay to identify ALK-positive NSCLC patients

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Abstract: Screening for anaplastic lymphoma kinase (ALK) rearrangements is a very important process in treatment decision making for advanced non-small-cell lung cancer (NSCLC). Although fluorescent in situ hybridization (FISH) is considered the universally accepted reference standard, it is associated with technical difficulties and high costs that have made global implementation of this assay challenging. Conversely, ALK immunohistochemistry has shown high sensitivity and specificity compared to FISH and other molecular assays and is more cost-effective. In fact, the ALK (D5F3) CDx immunohistochemistry assay was approved by the US Food and Drug Administration as a standalone test for ALK rearrangements in lung cancer in 2015. In this review, we will discuss the overview of ALK rearrangements in NSCLC, various testing methods for ALK rearrangements, and the details of immunohistochemistry for ALK, in particular one with the ALK antibody clone D5F3.

Keywords: anaplastic lymphoma kinase gene ALK, D5F3 antibody, ALK (D5F3) CDx, non-small-cell lung cancer, adenocarcinoma

ALK rearrangements in NSCLC

Molecular targeted therapy has brought a paradigm shift in treatment for advanced non-small-cell lung cancer (NSCLC). It has been shown that NSCLC patients with driver mutations have better progression-free survival (PFS) and overall survival with first- and second-line therapies compared to those with no treatable driver mutations.4 After Lynch et al identified epidermal growth factor receptor (EGFR) mutations in tissue samples from NSCLC patients who had responded to gefitinib,5 EGFR tyrosine kinase inhibitors (TKIs) have become innovative therapeutic agents in the field of lung cancer. As researchers strove to find new driver mutations, fusions of the echinoderm microtubule-associated protein-like four gene (EML4) and the anaplastic lymphoma kinase gene (ALK) in NSCLC patients were first reported in 2007 by Soda et al.3

EML4–ALK fusions are derived from inversions within the short arm of chromosome 2, and several EML4–ALK variants classified by the site of fusion have been reported.4 ALK-rearranged tumors comprise 3%–7% of NSCLCs,1,4,5 and the vast majority harbor EML4–ALK fusions, while rare fusion partners, such as KIF5B, TFG, KLC1, HIP1, ASXL2, ATP6V1B1, PRKAR1A, and SPDYA, have also been reported.6–8 Clinically, ALK rearrangement-positive NSCLCs are typically seen in never or light smokers, of younger age, and harboring wild-type EGFR and KRAS.9–12 Pathologically, most ALK rearrangement-positive NSCLCs exhibit adenocarcinoma histology; solid pattern with signet cells and/or mucinous cribriform pattern are often seen, at least focally, in these tumors.5,10,12,13
Treatment for ALK-rearranged NSCLC

ALK rearrangement-positive NSCLCs are highly sensitive to ALK-TKIs. Shaw et al conducted a Phase III study and showed that crizotinib, a first-generation ALK TKI, had better response rate and longer PFS compared to pemetrexed or docetaxel in previously treated ALK rearrangement-positive NSCLC patients (65% vs 20% and 7.7 vs 3.0 months, respectively).

The PROFILE 1014 Phase III study compared crizotinib with pemetrexed plus carboplatin/cisplatin in treatment-naïve ALK rearrangement-positive lung cancer patients, and again showed better response rate and longer PFS (74% and 45% and 10.9 vs 7.0 months, respectively). Interestingly, patients with ALK variant 1 were more responsive to crizotinib than those with non-variant 1. Alectinib, a second-generation ALK TKI, showed better PFS compared to crizotinib in untreated ALK rearrangement-positive NSCLC in two Phase III studies, the one in a Japanese population (the J-ALEX trial) and the other in a worldwide population (the ALEX trial). Ceritinib, another second-generation ALK TKI, showed longer PFS in treatment-naïve ALK rearrangement-positive NSCLC patients compared to platinum-based chemotherapy, and in patients after development of resistance to crizotinib compared to chemotherapy (the ASCEND-5 trial).

A Phase II study of lorlatinib, a third-generation ALK TKI, resulted in an objective response rate of 59% in ALK or ROS-1 rearrangement-positive NSCLC patients, most of whom had previously been treated with ALK TKIs. Lorlatinib was granted breakthrough therapy status in the United States based on these results.

Detection of ALK rearrangements in lung cancer

Fluorescent in situ hybridization (FISH) is considered as the universally accepted reference standard for detection of ALK rearrangements, and the Vysis LSI ALK Break Apart FISH Probe Kit (Abbott Molecular Inc., Des Plaines, IL, USA) was approved by the US Food and Drug Administration (FDA) as the first screening method for ALK rearrangements in lung cancer. The Vysis LSI ALK Break Apart FISH Probe Kit consists of two probes, Vysis LSI 3′-ALK (Orange) and Vysis LSI 5′-ALK (Green). In the normal condition (without rearrangements), two signals (red/green) appear to be overlapped or fused leading to a yellow signal due to their proximity. However, under the 2p23 ALK rearrangement, the red and green signals are apart with some distance (two or more times the diameter of the largest signal) or red-only signals may be seen when the nonfunctioning 5′ side of ALK gene is eliminated upon rearrangement. To minimize technical bias, a two-step assessment strategy with two independent reviewers is recommended. The first reviewer scores 50 tumor cells. If the split pattern and/or isolated 3′ (red) pattern are seen in <10% of the examined tumor cells, the tissue sample is considered negative for an ALK rearrangement; a rate greater than 50% is considered positive; and a rate of 10%–50% is considered equivocal. In the latter situation, a second independent reviewer evaluates an additional 50 tumor cells, and a final rate of tumor cells with the positive signal patterns is calculated based on the sum of the first and second scores. The specimen is then classified based on the final rate with the cutoff of 15%. However, there are several preanalytical and analytical issues that may result in false negative or false positive interpretation of FISH. First, inadequate fixation and storage could cause false negative results. Second, ALK (2p23.2) is located close to EML4 (2p21) on the same chromosome arm; thus, the split signals in NSCLC with an EML4–ALK fusion could be erroneously interpreted as fused signals leading to false negative results. Third, normal cells could be interpreted as tumor cells in the dark field and dilute the rate of positive cells resulting in false negative results. Fourth, the rate of tumor cells with break-apart or isolated red signals falls within the range of 10%–20% in approximately 5%–10% of NSCLCs. Such equivocal counts represent one of the major sources of false positive or false negative results and discordant results between the observers. Therefore, an external quality assessment is extremely important for ALK FISH testing. In addition, small biopsy specimens, including transbronchial lung biopsy, endobronchial ultrasound-guided transbronchial needle aspiration, or computed tomography-guided transthoracic needle biopsy, may not provide enough tumor cells for FISH analysis because at least 50 more tumor cells need to be evaluated.

Real-time polymerase chain reaction (RT-PCR) is another method used for diagnosis of ALK rearrangement-positive NSCLC. Takeuchi et al showed that RT-PCR had 100% sensitivity and specificity for EML4–ALK rearrangement-positive NSCLC diagnosed by FISH. Several studies revealed high concordance between RT-PCR and FISH/immunohistochemistry (IHC), with 94%–100% sensitivity and specificity. However, high-quality RNA is required for RT-PCR, and formalin-fixed paraffin-embedded (FFPE) specimens are usually inappropriate for RT-PCR. In addition, we need to know exact fusion partners to design primers for RT-PCR; thus, ALK rearrangements with unknown/novel partners will not be captured by this method.
Next-generation sequencing (NGS) is an emerging technology that allows examination of millions or billions of DNA strands in parallel. NGS can examine a large panel of driver mutations simultaneously, and requires a smaller volume of specimen compared to sequential analyzing for driver mutations such as EGFR, ALK, ROS1, RET, and BRAF. There were two main types of NGS, DNA-based NGS and hybrid capture-based NGS. DNA-based NGS could assess for already known and designed breakpoints. A recent study from Europe showed the sensitivity and specificity of DNA-based NGS using the Oncomine Solid Tumor Fusion Transcript Kit (Thermo Fisher Scientific, Waltham, MA, USA) for ALK rearrangement-positive NSCLC diagnosed by FISH and IHC as 85% and 79%, respectively. On the contrary, hybrid capture-based NGS could analyze most breakpoints, even if they are unknown. Drilon et al performed hybrid-captured NGS on lung adenocarcinomas from patients with a ≤15 pack-year smoking history and without 11 major driver mutations and fusions including EGFR, ALK, and ROS1 by conventional (non-NGS) molecular testing. They were successful in detecting SOCS5-ALK and HIP1-ALK, and concluded that hybrid capture-based NGS was comprehensive and efficient. However, turnaround time for NGS is typically 2 weeks or longer and that may be too long for patients with advanced NSCLC to wait. Needless to say, NGS is much more expensive than other methods at this time.

**ALK IHC in lung cancer**

Because of technical difficulties and/or higher costs of FISH, RT-PCR, and NGS, IHC is increasingly used to detect ALK rearrangements. There are four ALK antibody clones that have been evaluated for NSCLC. They are ALK1, 5A4, D5F3, and anti-ALK(1A4). The clone ALK1 (Dako, Carpinteria, CA, USA) that recognizes the c-terminal of ALK tyrosine kinase does not have enough sensitivity to detect often weak ALK protein expression secondary to ALK rearrangements in NSCLC. The sensitivity and specificity of IHC with the clone ALK1 (1:2) and EnVison+ detection system (Dako) in detecting ALK rearrangement-positive NSCLC diagnosed by FISH were 67% and 97%, respectively. The 1A4 anti-ALK antibody (Origene Technologies Inc., Rockville, MD, USA), a recombinant protein that recognizes amino acids 426–528 of the 680 NPM-ALK protein, has been shown to have great sensitivity (100%), but low specificity (70%) (although no details in the IHC protocol were provided). Thus, screening with the anti-ALK antibody may result in a high false positive rate.

Conversely, IHC with the clone 5A4 or D5F3 has good sensitivity and specificity for ALK rearrangements in NSCLC and can be used as a screening method. Paik et al and Kim et al used the clone 5A4 (1:30; Novocasta, Newcastle, UK) and iVIEW detection system (Ventana Medical Systems Inc., Tucson, AZ, USA) for ALK IHC with four-tiered scoring system (0–3+), and reported 100% and 100% sensitivity, and 96% and 98% specificity, respectively, with >2+ as positive. Similarly, the clone 5A4 produced by Abcam (Cambridge, UK) has shown sensitivity and specificity of 100% for ALK rearrangement-positive NSCLC with FISH as the gold standard. In this study, to increase the sensitivity of detection, the intercalated antibody enhanced polymer (iAEP) as well as EnVision FLEX+ detection system (Dako) were used for IHC with the antibody clone (1:100). However, the performance of this antibody (clone 5A4; Abcam) may not be optimal in detecting ALK rearrangements. For instance, in the study with 3,244 consecutive NSCLC cases analyzed at two independent French centers, Cabillic et al reported many (55/150) discordant cases between FISH and IHC using the antibody (5A4, 1:50; Abcam, Cambridge, UK) and ultraView Detection Kit (Ventana Medical Systems Inc.).

Overall, several studies have reported 95%–100% sensitivity and specificity of the clone 5A4, in particular the Novocasta/Leica antibody, for ALK rearrangement-positive NSCLC with FISH as the gold standard. Subsequently, IHC with the clone 5A4 and iAEP (Histofine ALK iAEP Kit; Nichirei Biosciences Inc., Tokyo, Japan) was approved in Japan as a companion diagnostic test for alectinib. It is important to note, however, that sensitivity and specificity of ALK IHC may differ depending on the cutoff applied when an intensity score or H scoring (opposed to a binary system) is used for the analysis. This issue is elucidated by European Thoracic Oncology Platform Landscape Project that assessed ALK IHC in 1,281 stage I–III adenocarcinomas completely resected at 16 institutions. In the study, the clone 5A4 (no dilution mentioned, Novocasta; Leica Biosystems, Buffalo Gove, IL, USA) with Novolink detection system (Leica Biosystems) was used, and each case was evaluated with both intensity score (0–3+ in any number of cells stained) and H scoring (range: 0–300). When any intensity was considered positive, 6.2% of the study cohort exhibited ALK protein expression. ALK FISH was examined in the ALK IHC positive and matched ALK IHC negative cases (1:2 ratio) and showed that only 35.0% of the samples with any positivity were FISH positive, while the sensitivity of the FISH testing.
increased to 81.3% in those with 2+ or 3+ intensity, with the corresponding specificity of 99.0%. In the selected cohort, the positive FISH rates were 0% in those with intensity score 0, 4.2% in intensity score 1+, 60% in intensity score 2+, 90.9% in intensity score 3+, 5.6% in those with H score <120 and 96.2% in H score >120.

IHC with D5F3

The clone D5F3 recognizes the carboxyl terminus of human ALK protein, and many studies have reported excellent performance of the clone D5F3. Mino-Kenudson et al.48 showed a 100% sensitivity and 99% specificity of IHC with the D5F3 (1:100; Cell Signaling Technology, Danvers, MA, USA) and EnVision+ detection system for ALK rearrangement-positive NSCLC diagnosed by FISH. Martinez et al.47 used the clone D5F3 (1:50; Ventana Medical Systems Inc.) combined with ultraView Detection Kit (Ventana Medical Systems Inc.) and reported 83% sensitivity and 100% specificity. Similarly, Minca et al.48 reported 94% sensitivity and 100% specificity of IHC with the D5F3 (1:100) and OptiView Detection Kit (Ventana Medical Systems Inc.) on cell blocks from malignant pleural effusion and reported very high concordance among seven international experts. In this study, each participating institution used a detection system(s) and an autostainer(s) of its choice, and thus, multiple combinations of antibody clone, detection system, and autostainer were applied. They reported comparative ALK protein expression between the clones 5A4 and D5F3, but generally lower expression by the clone ALK-1 leading to the Pearson correlation between 5A4 vs D5F3 and that between 5A4 vs ALK1 of 0.972 and 0.844, respectively. Other studies also showed high concordance between the antibody clones, but some revealed lower sensitivity of ALK1 compared to D5F3 and 5A4 in detecting ALK rearrangements in NSCLC.3,18,69

Diagnostic reproducibility on D5F3 IHC between pathologists has also been well established. The study by Wynes et al.37 reported 95% agreement on ALK protein expression by IHC among seven international experts. In this study, 45 ALK FISH-positive and 55 ALK FISH-negative NSCLC samples were stained with the clone D5F3 (Ventana Medical Systems Inc.) using OptiView Detection Kit and OptiView Amplification Kit on a BenchMark XT autostainer (Ventana Medical Systems Inc.). Similarly, the ALK-Harmonization-Study from Europe using the same D5F3 IHC platform showed high concordance after training of the pathologists.70 Furthermore, in the aforementioned clinical trial study, between-reader agreement rates involving three independent laboratories exceeded 98%.64

While the majority of the above studies used FFPE tissue samples (biopsies and resections), two studies specifically looked at the performance of IHC with the clone D5F3 (Ventana Medical Systems Inc.) on cell blocks from malignant pleural effusion and reported very high concordance with FISH.43,44 In addition, comparable expression of ALK protein by D5F3 IHC between samples from primary and
<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Country</th>
<th>Histologic types</th>
<th>Scoring system</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Note for positivity</th>
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<tr>
<td>Mino-Kenudson 2010</td>
<td>153</td>
<td>USA</td>
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<td>Binary</td>
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<td>79</td>
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<td>Binary (cutoff 10%)</td>
<td>83.3</td>
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<td>USA</td>
<td>NSCLC</td>
<td>Binary</td>
<td>100</td>
<td>100</td>
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<td>China</td>
<td>Adenocarcinoma</td>
<td>0–3+</td>
<td>100</td>
<td>95.0</td>
<td>&gt;1+</td>
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<td>Zhou 2014</td>
<td>368</td>
<td>China</td>
<td>Adenocarcinoma</td>
<td>0–3+</td>
<td>100</td>
<td>98.8</td>
<td>&gt;2+, corrected in accordance with the results of RT-PCR</td>
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<td>Shan 2014</td>
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<td>China</td>
<td>Adenocarcinoma</td>
<td>0–2+ based on intensity (0–3+) and extent (0–5+)</td>
<td>100</td>
<td>98.8</td>
<td>&gt;1+, corrected in accordance with the results of RT-PCR</td>
</tr>
<tr>
<td>Le Quesne 2014</td>
<td>15</td>
<td>UK</td>
<td>Adenocarcinoma</td>
<td>Intensity (0–3+) and extent (0–5+)</td>
<td>100</td>
<td>86</td>
<td>Only FISH-positive cases, intensity ≥1 and extent ≥4</td>
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<td>267</td>
<td>Thailand</td>
<td>NSCLC</td>
<td>Binary</td>
<td>80</td>
<td>94.9</td>
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<td>Russia</td>
<td>NSCLC</td>
<td>0–3+</td>
<td>100</td>
<td>100</td>
<td>Only strongly positive staining</td>
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<td>523</td>
<td>Italy</td>
<td>NSCLC</td>
<td>Binary</td>
<td>90</td>
<td>100</td>
<td>&gt;1+</td>
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<td>Conde 2014</td>
<td>103</td>
<td>Spain</td>
<td>NSCLC</td>
<td>0–3+</td>
<td>98</td>
<td>100</td>
<td>RT-PCR for EML4–ALK variants 1–3 was negative in the two FISH+/IHC− cases</td>
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<td>Wang 2014</td>
<td>430</td>
<td>China</td>
<td>Adenocarcinoma</td>
<td>Binary</td>
<td>100</td>
<td>98.2</td>
<td>&gt;2+</td>
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<td>103</td>
<td>USA</td>
<td>NSCLC</td>
<td>Binary</td>
<td>90</td>
<td>95</td>
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<tr>
<td>Pekar-Zlotin 2015</td>
<td>51</td>
<td>Israel</td>
<td>Adenocarcinoma</td>
<td>0–3+</td>
<td>100</td>
<td>97.7</td>
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<td>Rogers 2015</td>
<td>362</td>
<td>Australia</td>
<td>NSCLC</td>
<td>Intensity (0–3+) and extent (0–3+)</td>
<td>100</td>
<td>99.7</td>
<td>H score ≥40, corrected in accordance with the results of NGS</td>
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<td>Lantuejoul 2015</td>
<td>547</td>
<td>France</td>
<td>Adenocarcinoma</td>
<td>0–3+</td>
<td>89</td>
<td>76</td>
<td>≥1 0% of the cells with a 1–3+ intensity, corrected in accordance with the results of RT-PCR</td>
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<td>Savic 2015</td>
<td>72</td>
<td>Switzerland</td>
<td>NSCLC</td>
<td>0–3+</td>
<td>96</td>
<td>100</td>
<td>&gt;3+</td>
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<td>Ilie 2015</td>
<td>176</td>
<td>France</td>
<td>Adenocarcinoma</td>
<td>Binary</td>
<td>81</td>
<td>99</td>
<td>The five FISH+/IHC− cases were FISH borderline positive (15%–20%); three overexpressed c-MET and responded to crizotinib, and two without c-MET expression progressed on crizotinib</td>
</tr>
<tr>
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<td>595</td>
<td>China</td>
<td>Adenocarcinoma</td>
<td>0–3+</td>
<td>75.9</td>
<td>99.8</td>
<td></td>
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<tr>
<td>Thorne-Nuzzo 2017</td>
<td>933</td>
<td>Global</td>
<td>NSCLC</td>
<td>Binary</td>
<td>86.0</td>
<td>96.3</td>
<td>Overall response rate to crizotinib: 86.7% for FISH+/IHC+ and 33.3% for FISH+/IHC− cases (P=0.0083)</td>
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<tr>
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<td>200</td>
<td>India</td>
<td>Adenocarcinoma</td>
<td>Binary</td>
<td>100</td>
<td>90.5</td>
<td>&gt;1+</td>
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<tr>
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<td>India</td>
<td>Adenocarcinoma</td>
<td>Binary</td>
<td>100</td>
<td>94.4</td>
<td></td>
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<tr>
<td>Kheng 2018</td>
<td>304</td>
<td>UK</td>
<td>NSCLC</td>
<td>Binary</td>
<td>100</td>
<td>96.6</td>
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</table>

**Note:** *Studies in which the ALK (D5F3) CDx immunohistochemistry assay or an equivalent assay was used in conjunction with a binary scoring algorithm.

**Abbreviations:** ALK, anaplastic lymphoma kinase; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; NSCLC, non-small-cell lung cancer; RT-PCR, real-time polymerase chain reaction.
metastatic sites has also been well documented with concordance rates of 94%–100%.71–73

Discordant IHC and FISH results

Several studies have compared the performance of IHC with the clone D5F3 vs FISH in detecting ALK-rearranged NSCLCs. Perlzlotin et al reported the sensitivity and specificity of 100% and 97.7%, respectively, for D5F3 IHC and 42.9% and 97.7%, respectively, for FISH with NGS as the gold standard in 51 lung adenocarcinoma patients.60 More recently, van der Wekken et al looked at the response to crizotinib in 29 stage IV NSCLC patients whose tumors had been shown to have ALK rearrangements by FISH and/or the ALK (D5F3) CDx assay, and reported that all immunohistochemistry-positive (IHC+) patients responded to crizotinib except for three with primary resistance, while no tumor response was observed in 13 FISH-positive (FISH+) but immunohistochemistry-negative (IHC−) patients.74 The results were confirmed in an external cohort of 16 patients. These results are in line with those of the clinical trial study.64 Overall, IHC+/FISH− cases are considered ALK+ and will likely benefit from treatment with crizotinib.75 While the vast majority of IHC−/FISH borderline+ results are attributed to the technical/interpretation difficulty of ALK FISH,28 and are considered ALK−,75 some IHC−/FISH borderline+ results have been reported in NSCLCs with MET overexpression that responded to crizotinib (a MET and ALK inhibitor).26 IHC−/FISH clearly+ results are rare and may be fixation artifacts,31 or there may be no transcription of the ALK fusion gene.75 However, additional NGS-based or treatment response-based clinical observation studies are warranted to formulate a clinically meaningful statement on these rare events.75

ALK IHC as a standalone test for ALK rearrangements in NSCLC

As discussed above, several lines of evidence support the notion that IHC with the clone D5F3, in particular the ALK (D5F3) CDx assay, can be used as a standalone test to select advanced NSCLC patients for treatment with ALK TKIs. Subsequently, the recently updated molecular testing guideline (put forth by the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology) has designated properly validated IHC assays for ALK as an equivalent alternative to ALK FISH.40,76–78 Tissue samples with equivocal results should be tested and confirmed by other methods (FISH, RT-PCR, and/or NGS), however. From a clinical perspective, a recent clinical trial for alectinib required ALK rearrangements confirmed by IHC with the ALK (D5F3) CDx assay,18 while previous clinical trials for crizotinib required ALK FISH positivity.14,15

Pitfalls of ALK IHC

Several pitfalls of ALK IHC, including that with the clone D5F3, should be noted. First, signet ring cells or tumor cells with cytoplasmic mucin, often seen in ALK rearrangement-positive NSCLCs, may be a source of false negative results due to the limited expression in thin and scanty cytoplasm. Therefore, tissue samples with mucin-containing tumor cells require careful interpretation of ALK IHC. Second, false positive staining may be seen in alveolar macrophages, nerve, ganglioni cells, airway epithelial cells, extracellular mucin, and necrotic debris, particularly when strong IHC amplification systems are used.69 False positive cytoplasmic staining in NSCLC, albeit often weaker than true positive expression, has also been identified in association with the clone D5F3 and tyramide amplification system. Third, tumor cells with neuroendocrine differentiation (small cell carcinoma, large cell neuroendocrine carcinoma, and carcinoid tumor) have been reported to show false positive reactivity to ALK IHC,24,79,80 although their expressions are typically heterogeneous or in a checkerboard pattern. Fourth, quality control of staining was found to be important. A study of international quality assessment involving 30 countries showed that about 10% of the slides stained with D5F3 IHC were judged as unacceptable or borderline in quality by pathologists.54 Furthermore, NSCLCs with KIF5B-ALK rearrangements have been reported to show dot-like staining by ALK IHC.5 Thus, it is important to evaluate/confirm samples exhibiting focal and/or equivocal expressions with ALK FISH, RT-PCR, and/or NGS.

Summary

IHC with the ALK antibody clone D5F3, in particular the ALK (D5F3) CDx assay, has been proven to have great sensitivity and specificity for ALK rearrangements in NSCLC, and can be used as a standalone test in practice. Nevertheless, it is important to understand several potential pitfalls of ALK IHC and further evaluate specimens exhibiting focal/equivocal expressions with other ALK testing methods.

Disclosure

M Mino-Kenudson serves as a consultant for Merrimack Pharmaceuticals and H3 Biomedicine. The authors report no other conflicts of interest with this work.
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ALK (D5F3) CDx immunohistochemistry assay


