# ALK FISH and IHC

## *You Cannot Have One without the Other*

*Yasushi Yatabe, MD, PhD*

Two years have passed since crizotinib, the first anaplastic lymphoma kinase (ALK) inhibitor, was approved based on the results of a phase II study (profile 1005).[1] Now, results of a phase III study of crizotinib versus chemotherapy are also available, which show that the inhibitor significantly prolongs PFS as compared with standard single-agent chemotherapy in patients previously treated with first-line platinum-based chemotherapy.[1] Recently, it was shown that this agent is also superior to standard first-line pemetrexed-plus-platinum chemotherapy.[2] In addition to crizotinib, so-called second-generation ALK inhibitors have been developed and are being used in clinical practice in some countries, based on high efficacy shown in phase I/II studies.[3,4] Accordingly, treatment with an ALK inhibitor is now recognized as a standard care regimen, whereas it has also become increasingly important to appropriately select patients with the alteration. Break-apart fluorescent in-situ hybridization (FISH) is currently the only diagnostic tool approved by the FDA; however, the low incidence of ALK rearrangement (about 4% in non–small-cell lung cancer [NSCLC]) requires a more rapid and cost-efficient method for screening. Many countries have adopted immunohistochemistry (IHC) screening followed by FISH confirmation.[5] Figure 1 shows the diagnostic algorithm of ALK rearrangement used by the Japanese Lung Cancer Society[6] and this workflow has been recommended in the molecular testing guidelines provided by CAP/IASLC/AMP.[7]

For effective screening, high sensitivity is essential and ALK IHC is considered to meet that requirement, as a very high concordance between IHC and FISH results have been reported. However, recent large-scaled studies have also found more than a few cases with discordant results between IHC and FISH (Table 1). Blackhall et al.[8] recently reported 52 cases with discrepant results in simultaneous examinations of ALK using IHC and FISH in a matched cohort of 240 NSCLC cases. In their study, eight of the samples with discrepant results were subjected to reverse transcription-polymerase chain reaction, of which six were concordant with FISH results, whereas IHC+/FISH- cases were positive and IHC-/FISH+ cases were negative for detection of the fusion transcript. Pfizer Japan conducted a prereimbursement program,[9] in which ALK testing and crizotinib were provided for free to compensate for ethical conflicts with patients diagnosed as ALK positive during the 3 months between drug approval and price listing in the national health care reimbursement system. Among the 2337 tumors simultaneously examined with IHC and FISH, 48 showed discrepant results, including 12 classified as IHC+/FISH- and 36 as IHC-/FISH+. Re-analysis of these tumors by the Biomarker Committee of the Japanese Lung Cancer Society revealed that all tissue-available tumors with IHC+/FISH- results were incorrectly diagnosed, whereas 21 of 29 IHC-/FISH+ results were not overturned. Notably, progressive disease was shown in 9 of 15 of the patients with discrepant results who were treated with crizotinib. Cabillic et al.[10] also simultaneously examined 3244 consecutive NSCLC specimens with IHC and FISH, and found 19 IHC+/FISH- and 36 IHC-/FISH+ cases. In contrast

Department of Pathology and Molecular Diagnostics, Aichi Cancer Center, Nagoya Japan.
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Address for correspondence: Yasushi Yatabe, MD, PhD, Chief, Department of Pathology and Molecular Diagnostics, Aichi Cancer Center, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. Email: yyatabe@aichi-cc.jp
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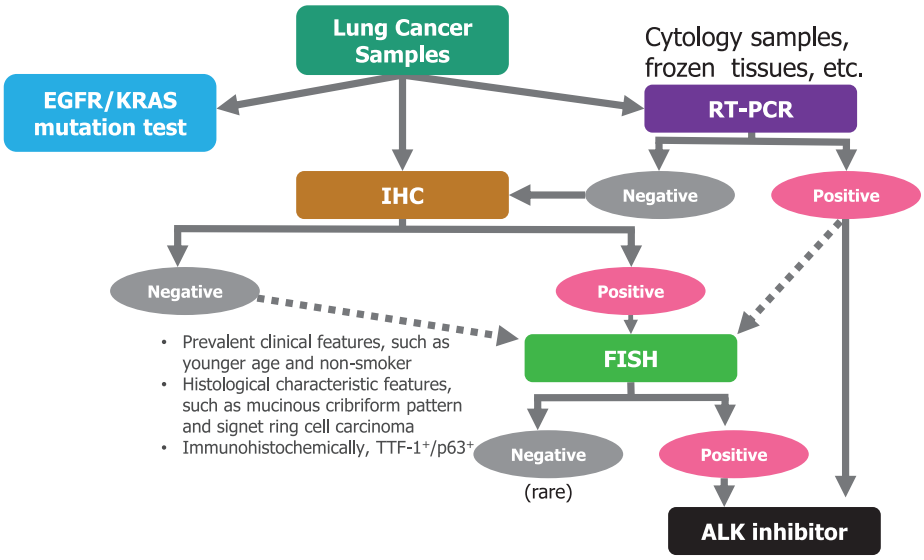


**FIGURE 1.** Diagnostic algorithm for ALK rearrangement from guidance for ALK gene testing in lung cancer patients, presented by the Japanese Lung Cancer Society. ALK, anaplastic lymphoma kinase.

**TABLE 1.** Studies of more than 500 Tumors in which Simultaneous ALK Testing for both IHC and FISH was Performed

| | ETOP | Japan | Cabillic et al. | Ali et al. |
|---|---|---|---|---|
| **Patients** | **Resected Stage I–III Tumors** | **EGFR-Negative, Crizotinib Ready** | **Advanced Disease** | **Consecutive NSCLC** |
| **No.** | **1281 (240)** | **2337** | **2714** | **522** |
| FISH method | Vysis ALK break-apart FISH | Vysis ALK break-apart FISH | Vysis ALK break-apart FISH, and Dako split probe | Vysis ALK break-apart FISH |
| IHC Clone | 5A4 | 5A4 | 5A4 | D5F3 |
| IHC detection system | Novolink, Leica Biosystems | Envision Flex+, Dako and iAEP, Nichirei | UltraView and OptiView systems, Ventana | OptiView and OptiView amplification system, Ventana |
| Discrepant IHC/FISH | 52 (21%) | 48 (2%) | 55 (2%) | 2 (0.3%) |
| FISH-/IHC+ | 52 | 12 | 19 | 0 |
| FISH+/IHC- | 0 | 36 | 36 | 2 |
| Sensitivity[a] | 35% | 86% | 68% | 90% |
| Specificity[a] | 100% | 99% | 99% | 100% |
| Positive prediction value[a] | 100% | 95% | 81% | 100% |
| Negative prediction value[a] | 25% | 98% | 99% | 99% |

[a]Expectation of IHC power for FISH standard.

EGFR, epidermal growth factor receptor; ETOP, European Thoracic Oncology Platform; NSCLC, non–small-cell lung cancer; IHC, immunohistochemistry; ALK, anaplastic lymphoma kinase; FISH, fluorescent in-situ hybridization.

to the Japanese study, only one case showed progressive disease. Also, in a recent study by Ali et al.,[11] two IHC-/FISH+ cases were identified in examinations of 523 NSCLCs subjected to simultaneous IHC and FISH analyses. MassARRAY and reverse transcription polymerase chain reaction assays were used to examine two of the cases with discordant results, which revealed a negative fusion transcript. Taken together, these results show that not a few cases with IHC/FISH discrepant results exist in clinical practice.

As for discrepancies between IHC and FISH, some possible causes have been proposed (Table 2). Intracellular and extracellular mucin can cause false-negative and false-positive results, respectively, in IHC analysis. Just as in normal ganglion cells, neuroendocrine tumors including small-cell lung cancer express ALK without ALK alterations. In terms of FISH, atypical FISH signals, such as a 3′-5′-3′ red doublet pattern, and compressed z-stacked images for vertically split signals may give false-negative results. In addition, it has been revealed that specimens with discordant IHC/FISH results commonly harbor a borderline percentage of break-apart signals. Currently, ALK FISH is considered to be positive when split and/or single red signals are detected in more than 15% of the tumor cells. Ilie et al.[12] reported that in both IHC-/FISH+ and IHC+/FISH- discrepant cases, the percentage of split signals ranged from 10% to 20%. In the present issue of *Journal of Thoracic Oncology*, Martin et al.[13] reported that borderline cases were associated with patterns of split signals. They measured the distances of the

 

**TABLE 2.** Possible Mechanisms Related to False-Positive and False-Negative Results

| | False-Positive | False-Negative |
|---|---|---|
| IHC | Nonspecific staining (particularly to mucin)<br>High-grade neuroendocrine tumor | Mucin-rich cells (signet ring cells)<br>Technical issues (poor fixation, insensitive detection method, etc.) |
| FISH | Tissue sectioning[a] | Compressed z-stacked images<br>Atypical signal profile |

[a]In sectioning paraffin-blocks, tumor cell nuclei in the tissues are always sliced into several sections, because section thickness is approximately 4 μm in contrast to 20 μm or more of the tumor nuclei. Therefore, it could be possible to make FISH signals on the sectioning surface separated, resulting in imitated break-apart signals.

IHC, immunohistochemistry; FISH, fluorescent in-situ hybridization.

split signals and found them to be continuous with a cut-off value of 15%. Furthermore, the split distances were different between ALK-positive and ALK-negative tumors, with significantly shorter split patterns in ALK-negative and borderline ALK-positive tumors, with borderline tumors defined as having 30% or less ALK rearranged cells. Interestingly, three of those tumors with borderline positivity in FISH did not show a positive reaction with ALK IHC.

As an excellent response can be achieved with molecularly targeted treatment of driver mutations, it is essential to accurately identify eligible patients. Unfortunately, the incidence of tumors with ALK rearrangement is limited to 4% of non–small-cell lung cancer cases, thus selection is difficult. Importantly, results of large cohorts in clinical investigations indicate that some non–small-cell lung cancer cases (0.2–21%) show discordant results between IHC and FISH, implying that a single assay strategy can lead to inadequate selection of patients. Particular attention should be paid to cases with borderline results in FISH analysis, which have been found to be related to discrepant findings, thus adding IHC analysis might be recommended. In turn, IHC findings show similar pitfalls in regard to discrepancies, though few instances have been reported. Detailed studies of IHC discrepant cases are warranted.

## REFERENCES


1. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* 2013;368:2385–2394.
2. Solomon BJ, Mok T, Kim DW, et al.; PROFILE 1014 Investigators. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med* 2014;371:2167–2177.
3. Seto T, Kiura K, Nishio M, et al. CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): A single-arm, open-label, phase 1-2 study. *Lancet Oncol* 2013;14:590–598.
4. Shaw AT, Kim DW, Mehra R, et al. Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med* 2014;370:1189–1197.
5. Tsao SM, Hirsch RF, Yatabe Y. *IASLC Atlas of ALK testing in lung cancer*. Available at: https://www.iaslc.org/publications/iaslc-atlas-alk-testing-lung-cancer. Accessed January 26, 2015.
6. Biomarker Committee tJLCS, Mitsudomi T, Yatabe Y, et al. *Guidance for ALK Gene Testing in Lung Cancer Patients*. Available at: https://www.haigan.gr.jp/uploads/photos/641.pdf. Accessed January 26, 2015.
7. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Thorac Oncol* 2013;8:823–859.
8. Blackhall FH, Peters S, Bubendorf L, et al. Prevalence and clinical outcomes for patients with ALK-positive resected stage I to III adenocarcinoma: Results from the European Thoracic Oncology Platform Lungscape Project. *J Clin Oncol* 2014;32:2780–2787.
9. Biomarker Committee tJLCS. *Second notice of the results on IHC-FISH discordant cases (Japanese)*. Available at: https://www.haigan.gr.jp/uploads/photos/589.pdf. Accessed January 26, 2015.
10. Cabillic F, Gros A, Dugay F, et al. Parallel FISH and immunohistochemical studies of ALK status in 3244 non-small-cell lung cancers reveal major discordances. *J Thorac Oncol* 2014;9:295–306.
11. Ali G, Proietti A, Pelliccioni S, et al. ALK Rearrangement in a large series of consecutive non-small cell lung cancers: Comparison between a new immunohistochemical approach and fluorescent in situ hybridization for the screening of patients eligible for crizotinib treatment. *Arch Pathol Lab Med* 2014;138:1449–1458.
12. Ilie MI, Bence C, Hofman V, et al. Discrepancies between FISH and immunohistochemistry for assessment of the ALK status are associated with ALK 'borderline'-positive rearrangements or a high copy number: A potential major issue for anti-ALK therapeutic strategies. *Ann Oncol* 2015;26:238–244.
13. Martin V, Bernasconi B, Merlo E, et al. ALK testing in lung adenocarcinoma: Technical aspects to improve FISH evaluation in daily practice. *J Thorac Oncol* 2014, in press.