

Screening for *ALK* Rearrangements in Lung Cancer: Time for a New Generation of Diagnostics?

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Disclosures of potential conflicts of interest may be found at the end of this article.

Anaplastic lymphoma kinase (*ALK*) gene rearrangements leading to constitutive expression of oncogenic fusion proteins were initially detected in non-small cell lung cancer (NSCLC) in 2007 [1]. Early studies using reverse transcription polymerase chain reaction and fluorescence in situ hybridization (FISH) suggested that 3%–7% of patients with NSCLC harbor an *ALK* rearrangement [1, 2]. Simultaneous with the discovery of *ALK* rearrangements in NSCLC, crizotinib, a multitargeted tyrosine kinase inhibitor (TKI) with potent activity against *ALK*, was being investigated in a phase I clinical trial. In this trial, as well as the follow-up phase II and III studies, a break-apart *ALK* FISH assay was used as the central laboratory confirmatory test [3]. Based on the efficacy and safety demonstrated in these studies, crizotinib was approved in the United States for advanced *ALK*-positive NSCLC, along with *ALK* FISH as the companion diagnostic test [4–6]. More recently, the FDA approved an *ALK* immunohistochemistry (IHC) companion diagnostic assay based on its ability to accurately identify patients with *ALK*-rearranged NSCLC who benefit from treatment with crizotinib. Current guidelines recommend that all patients with metastatic nonsquamous NSCLC undergo *ALK* testing using an FDA-approved diagnostic test.

In this issue of *The Oncologist*, Ali et al. examine the utility of a third diagnostic modality—next-generation sequencing (NGS)—in detecting *ALK* rearrangements [7]. Among 1,070 cases of NSCLC submitted to Foundation Medicine for genotyping, 47 (4.4%) were found to harbor *ALK* rearrangements. Among these NGS-positive cases, a total of 31 had prior FISH testing results available. In 20 of the 31 cases, NGS and FISH testing were concordant (i.e., both were positive for an *ALK* rearrangement). However, in the remaining 11 cases, only NGS was positive. The majority of these NGS-positive/FISH-negative cases were responsive to crizotinib, highlighting the sensitivity of this NGS assay for detecting *ALK* rearrangements and the potential for false-negative *ALK* FISH results. This study adds to a growing body of work that challenges the position of FISH as the gold standard for detecting *ALK* rearrangements.

Because *ALK* FISH positivity was required for enrollment in registrational trials of crizotinib and the second-generation *ALK* TKIs ceritinib and alectinib, the break-apart *ALK* FISH assay has long been considered the gold standard test. This assay, which involves dual-colored fluorescent probes flanking the highly conserved break point within *ALK*, is considered “positive” if splitting of the red and green signals and/or an isolated 3' red

signal is detected in 15% or more of 50 evaluated tumor nuclei. Although FISH only requires a small amount of tissue, the assay is expensive, labor intensive, and relies on specialized techniques and training for performance and interpretation. The FISH readout can be challenging, as splitting patterns can be subtle, particularly in cases with small intrachromosomal deletions and inversions. As a result, interpretation can vary significantly between laboratories.

Considering the limitations of *ALK* FISH testing, alternative diagnostic assays have been explored, including IHC and NGS. Notably, IHC analysis relies on *ALK* protein expression, which is absent in the majority of normal tissues, including lung, but is induced by rearrangement or gene amplification [8, 9]. Compared with FISH, IHC is faster, cheaper, more accessible, and less operator dependent. Although the initial validation of *ALK* IHC was hampered by relatively low-level expression of *ALK* fusion proteins and variable performance of antibodies, with amplification technology and optimization of antibodies, IHC has emerged as a highly sensitive and specific test for identifying *ALK*-rearranged lung cancers [10–14]. In multiple studies comparing IHC using the D5F3 antibody to a reference standard of FISH, the sensitivity and specificity of IHC has consistently exceeded 90% [11, 13, 14]. The D5F3 antibody-based Ventana automated IHC assay (Ventana Medical Systems, Tucson, AZ, <http://www.ventana.com>) is now an FDA-approved diagnostic for detecting *ALK* rearrangements.

For many oncologists, NGS has become the leading platform for molecular testing of advanced cancers. The major advantage of NGS is the potential for multiplex testing (i.e., simultaneous evaluation of multiple genes). However, compared with FISH and IHC, NGS testing requires more tissue, is more expensive, and takes more time for analysis. Because NGS is a new technology for detecting *ALK* rearrangements, the work by Ali et al. is one of only a handful of studies to compare NGS with established diagnostic assays [7]. In their study, NGS appeared to be more sensitive than FISH in detecting *ALK* rearrangements. Indeed, among those cases where both NGS and FISH were performed, 11 (35%) were falsely negative by FISH. Although this rate of discordance is remarkably high, it should be noted that selection bias almost certainly inflated the false-negative rate. *ALK* rearrangements are mutually exclusive with other NSCLC oncogenic drivers, so cases that were identified as *ALK*-positive by FISH were less likely to have been submitted for additional molecular testing. As a result, the cases in this report were likely enriched for those patients with negative *ALK* FISH testing but high clinical suspicion based on

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clinicopathologic features. Based on the authors' report of 1 false-negative case among 45 NSCLC cases tested at 1 major academic center, the false-negative rate of ALK FISH, particularly in experienced hands, may be closer to 5% than 35%.

The performance of NGS compared with ALK IHC is largely unknown. In the study by Ali et al., ALK expression by IHC was not routinely assessed for the 31 cases in which NGS and FISH were performed [7]. Of the two cases for which IHC was reported, concordance with NGS was observed in one. Given the high sensitivity of IHC for detecting *ALK* rearrangements, the decreased operator dependence of IHC compared with FISH, and the ability of IHC to detect expression at the protein level, determining the concordance between IHC and NGS is important. In a retrospective study that assessed ALK status by FISH and IHC in 51 consecutive patients with lung adenocarcinoma, 4 of the 5 cases that were IHC positive/FISH negative were positive for *ALK* rearrangement, using the Foundation Medicine NGS assay [15]. In contrast, the single case that was IHC negative/FISH positive did not have an *ALK* rearrangement by NGS. Moreover, in a recent literature review by Marchetti et al., which reported the response rate to ALK TKIs for 35 patients with discordant ALK FISH and IHC results, the response rate for IHC positive/FISH negative tumors was 100% compared with 46% for IHC negative/FISH positive tumors [11]. Notably, NGS was not performed in the studies included in the review.

Although the small numbers and retrospective nature of these studies preclude drawing definite conclusions, the findings support the notion that detecting ALK protein expression may be more clinically relevant than detecting an alteration at the *ALK* genomic locus. This is particularly important because multiple NGS platforms are available, many of which rely on analysis of genomic DNA. Because it is unclear that fusions detected by FISH and genomic DNA-based NGS platforms will be functional in cases where corresponding IHC is negative, validation studies may be necessary to confirm ALK dependence, particularly in cases with minimal response to ALK TKIs or cases with novel fusions. For example, validation strategies might include techniques similar to those used for characterization in the Ali et al. study of case 3's

novel *PRKAR1A-ALK* fusion—specifically, in vitro modeling to test the effect of expression of the fusion on proliferation of cancer cells, activation of ALK signaling, and sensitivity to ALK TKIs [7].

The study by Ali et al. [7] in this issue illustrates the promise of NGS as an ALK diagnostic. Indeed, NGS may one day become the standard initial test for molecular genotyping of patients with advanced cancers. However, to establish NGS as the new gold standard diagnostic assay for ALK rearrangement, concordance studies comparing NGS, IHC, and FISH, together with clinical outcome data with *ALK* TKIs are needed. For now, the bulk of evidence suggests that IHC may be the most practical initial diagnostic method for *ALK* rearrangements. Alternatively, screening for *ALK* rearrangements may be performed as part of multiplex NGS testing, but because NGS is not yet approved by the FDA, confirmation of ALK positivity using an FDA-approved diagnostic (i.e., IHC or FISH) may be necessary based upon the current indications for ALK inhibitors in the United States.

The significant clinical benefit of ALK TKIs in patients with advanced ALK-positive NSCLC underscores the critical importance of accurately identifying *ALK* rearrangements in patient specimens. False-negative results, or missed identification of the target, would deprive patients of access to life-prolonging targeted therapies. False-positive results would be equally detrimental and direct patients toward ineffective treatments. Although a single test would be ideal, the therapeutic implications of both false-positive and false-negative results suggest that a combination of diagnostics may be necessary in some cases. Because NGS has the potential to improve upon the accuracy of target identification, this new generation of ALK diagnostics is a welcome addition to the current screening repertoire.

DISCLOSURES

Alice T. Shaw: Pfizer, Novartis, Roche, Genentech, Ariad, Taiho, Daiichi, Ignyta, Blueprint Medicine, EMD Serono (C/A). The other author indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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EDITOR'S NOTE: See the related article, "Comprehensive Genomic Profiling Identifies a Subset of Crizotinib-Responsive ALK-Rearranged Non-Small Cell Lung Cancer Not Detected by Fluorescence In Situ Hybridization," by Siraj M. Ali et al. on page 762 of this issue.