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Fishing for Precision in ALK-Rearranged Non-Small-Cell Lung Cancers

Xiuning Le[1], Mary Linton Peters[1], Paul A VanderLaan[2], Daniel B Costa[1] and Deepa Rangachari[1]*

[1]Department of Medicine, Division of Hematology/Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
[2]Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Abstract

Fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) represent the current standard for identification of therapeutically relevant anaplastic lymphoma kinase (ALK) rearrangements in advanced non-small-cell lung cancers (NSCLCs). Use of tyrosine kinase inhibitors (TKIs) targeting the oncogenic ALK fusion protein is approved for use in advanced ALK-rearranged NSCLCs and with benefits both with regards to clinical outcomes and quality of life when compared with traditional palliative cytotoxic therapies. We report here 2 cases in which initial standard of care ALK testing by FISH yielded false positive results, leading to delays in determination of optimal systemic therapy.

Keywords: Non-small cell lung cancer; NSCLC; ALK; Crizotinib, FISH; Targeted therapy

Introduction

Roughly 3-7% of non-small-cell lung cancers (NSCLCs) harbor anaplastic lymphoma kinase (*ALK*) rearrangements that are responsive to the tyrosine kinase inhibitor (TKI) crizotinib. Since August 2011, the Vysis *ALK* break apart FISH probe (Abbott Molecular, Inc.), had been the sole United States (US) Food and Drug Administration (FDA)-approved method for detection of *ALK* rearrangements in NSCLC, with the cutoff for *ALK* positivity defined as ≥15% of cells. However, as of June 2015, the VENTANA *ALK* (D5F3) CDx IHC assay (Ventana Medical Systems, Inc.) has been additionally approved. False negative and discordant *ALK* FISH results have been reported [1,2]. Here, we report patients who received false positive results on *ALK* FISH, leading to delay of optimal systemic therapy. *ALK* FISH testing in both instances was performed as a send out test by a certified commercial vendor utilizing the FDA-approved companion assay (Vysis ALK Break Apart FISH Kit, Abbott Molecular).

Case 1

An 83 year-old gentleman with a prior <10 pack/years tobacco history presented with metastatic lung adenocarcinoma (LAC) with malignant pleural effusion (Figure 1A). Single gene assays were completed on formalin-fixed, paraffin-embedded (FFPE) cell blocks created from the pleural fluid cytologic specimen and showed 16% of cells with an *ALK* rearrangement by FISH; *EGFR, KRAS,* and *ROS1* were unaffected. The patient was considered for a clinical trial (NCT02075840); however, central confirmation of tumor *ALK* status by IHC showed no evidence of an *ALK* rearrangement (IHC score was 0). Crizotinib 250 mg twice daily was started. Follow-up one month later demonstrated clinical and radiographic disease progression (Figure 1B). Right pleural biopsy and targeted next generation sequencing (SNaPshot NGS and fusion gene assay, Massachusetts General Hospital) demonstrated an *EGFR* exon 19 deletion, with no evidence of *ALK* gene fusion. Crizotinib was discontinued, and erlotinib at a dose of 150 mg daily was started. This resulted in a partial radiographic response with improvement in clinical symptoms that has been sustained for 6 months (Figure 1C).

Case 2

A 64 year-old woman with a prior 10 pack/years tobacco history presented with metastatic LAC with malignant pleural effusion (Figure 2A). Sequential single gene assays were completed on FFPE cell blocks created from the pleural fluid cytologic specimen. The tumor was found to harbor both *ALK* rearrangement by FISH in >90% of cells and concurrent *EGFR* exon 19 mutation. Crizotinib 250 mg twice daily was started. However, symptomatic and radiographic progression following six weeks of therapy was noted (Figure 2B). Repeat testing on the same cytologic specimen again showed an *ALK* rearrangement by FISH; however, NGS showed the *EGFR* exon 19 mutation only. Crizotinib was discontinued, and erlotinib 150 mg daily was started. Eight weeks later, imaging showed a partial response that has been sustained for more than 9 months (Figure 2C).

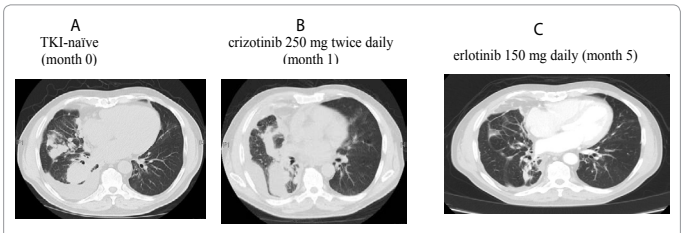

Figure 1: Computerized tomography (CT) images of the chest for patient 1 over the course of treatment. (**A**) At the time of diagnosis. (**B**) After one month of crizotinib therapy. **C.**) Five months after erlotinib therapy. Single gene assay (SGA) and next generation sequencing (NGS) results are displayed Patient no. 1, tumor SGA: *EGFR* wild-type, *ALK* FISH positive (nuc ish(ALKx2~3)(5'ALK sep 3'ALKx1)[7/100]/ (3'ALKx2,5'ALKx1)(3'ALK con 5'ALKx1)[4/100]/ (3'ALKx3,5'ALKx2)(3'ALK con 5'ALKx1)[5/100]) tumor NGS: *EGFR*-delE746_R748+A750P, no *ALK* rearrangement.

Discussion

With the dramatic responses and tolerability observed with the use of crizotinib in *ALK*-rearranged NSCLC, it has become imperative to perform timely and accurate molecular testing. Currently, both FISH and IHC serve as FDA-approved options for testing. Nevertheless, there have been an increasing number of reports highlighting false negative results using FISH, on the order of 5-15% [3]. False negative results

***Corresponding author:** Deepa Rangachari, Division of Hematology/Oncology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Shapiro 9 Boston, MA 02215, United states, Tel: 617-667-1580; Fax: 617-667-2518; E-mail: drangach@bidmc.harvard.edu

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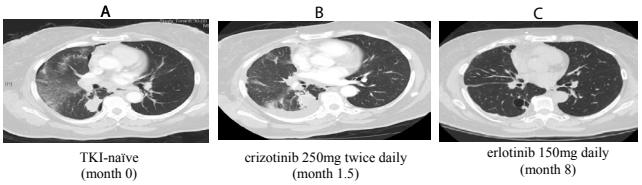

Figure 2: Computerized tomography (CT) images of the chest for patient 2 over the course of treatment. (**A)** At the time of diagnosis. (**B)** After six weeks of crizotinib therapy. **C.)** Eight months after erlotinib therapy. Single gene assay (SGA) and next generation sequencing (NGS) results are displayed. Patient no. 2: tumor SGA: *EGFR*-delE746_T751insl; *ALK* FISH positive (nuc ish(3'ALKx2~3,5'ALKx1~2)(3'ALK con 5'ALKx1~2)[46/50]) tumor NGS: *EGFR*-delE746_T751insl, no *ALK* rearrangement.

may occur due to variant forms of *EML4-ALK* rearrangements and/or RNA editing abnormalities resulting in intron changes that cannot be detected by FISH [4]. Several groups have published on the comparative accuracy of FISH, IHC, and NGS; however, the sensitivity, specificity, and cost of these modalities have not been directly compared in large prospective trials to date [3-6]. We report here two cases with clinically false positive FISH results. In both cases, initiation of crizotinib resulted in rapid disease progression and warranted further interrogation by NGS. Interestingly, both tumors were found to harbor *EGFR* mutations and with brisk and ongoing responses to erlotinib. The practice of precision medicine is incumbent upon use of assays which detect drug targets with optimal accuracy. As NGS becomes increasingly accessible and cost effective and as the spectrum of actionable therapeutic targets continues to expand, NGS may replace other modalities for optimally pairing patients with best therapies especially in cases where there is a paradoxical/unexpected response to therapy that is inconsistent with the predictive biomarker assayed.

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