Atypical Negative *ALK* Break-Apart FISH Harboring a Crizotinib-Responsive *ALK* Rearrangement in Non–Small-Cell Lung Cancer

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Anaplastic lymphoma kinase rearranged (*ALK*+) non–small-cell lung cancer demonstrates remarkable sensitivity to crizotinib.[1] Although other methods exist, the dominant method for determining ALK positivity uses the Abbott (Abbott Molecular, Des Plaines, IL) *ALK* break-apart fluorescent in situ hybridization (FISH) assay.[2] The assay has clearly defined positivity criteria but borderline or atypical negative cases occur. Here we describe a case with an atypical negative FISH result that was later confirmed as ALK+ by both immunohistochemistry (IHC) and reverse transcription polymerase chain reaction (RT-PCR) and responded well to crizotinib.

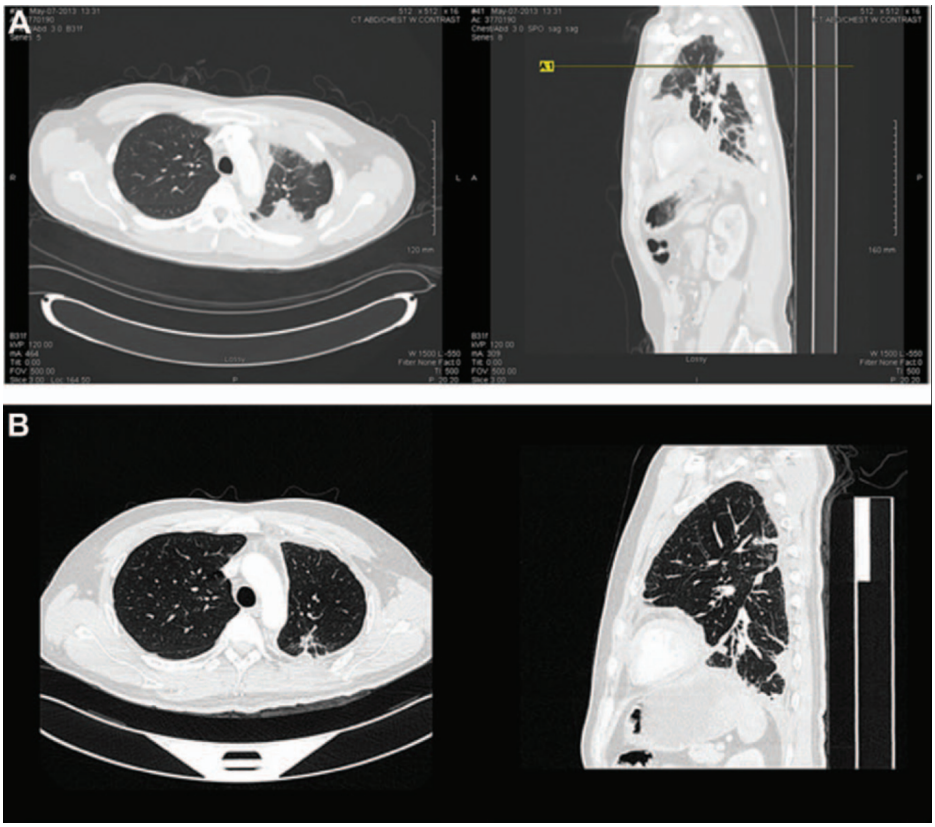

FIGURE 1. *A*, CT scan of thorax before initiation of crizotinib. *B*, CT scan of thorax after 1 month therapy of crizotinib. CT, computed tomography.

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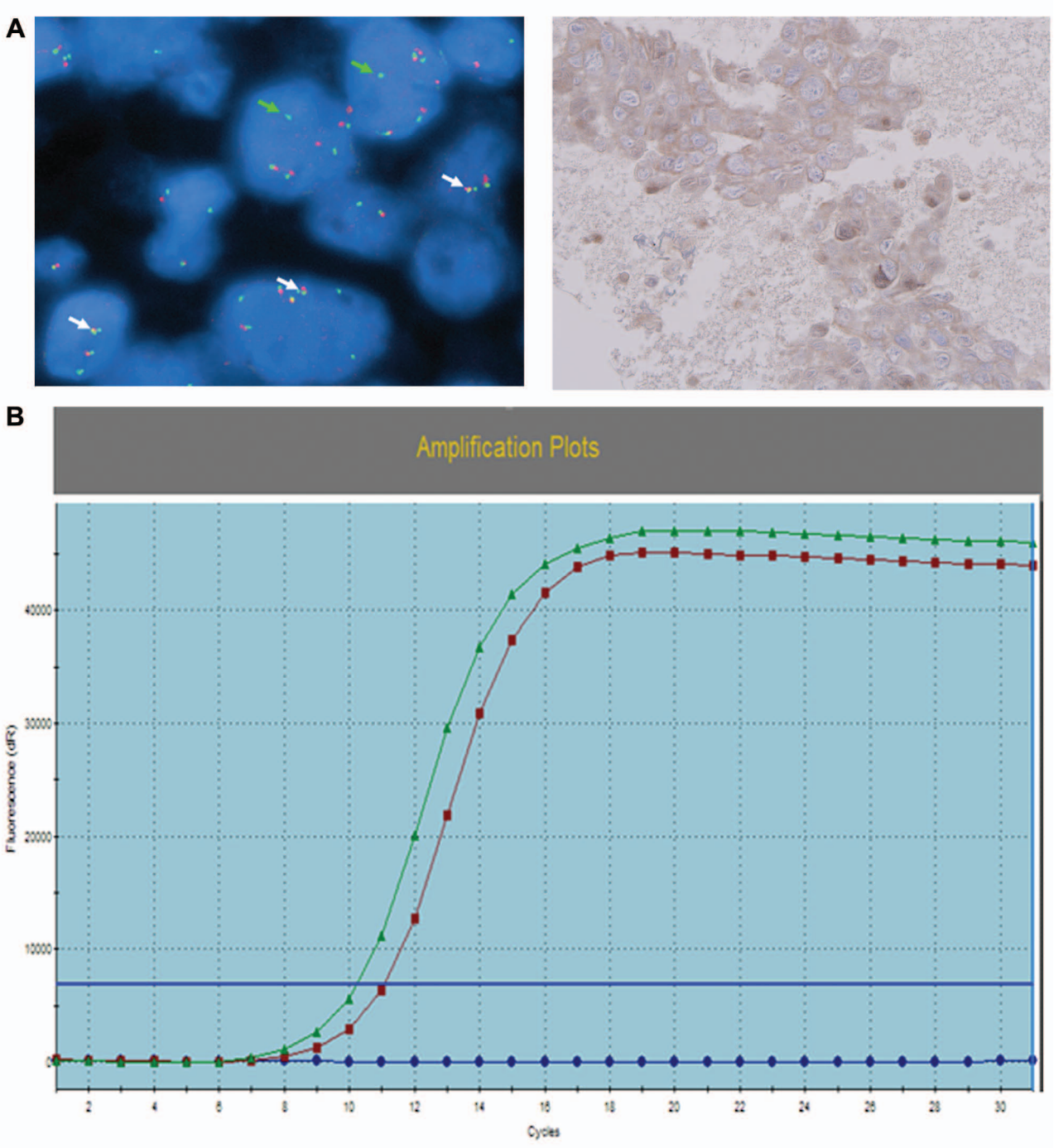

FIGURE 2. *A,* Atypical negative *ALK* FISH result. Specimen hybridized with the Vysis break-apart *ALK* FISH assay showing multiple single 5'*ALK* signals (green arrows) with 3'-–5'*ALK* doublets (white arrows). *B.* Positive IHC result for ALK expression (40×). *C,* Reverse transcription polymerase chain reaction showing positive result for *EML4-ALK* transcript. ALK, Anaplastic lymphoma kinase; FISH, fluorescence in situ hybridization.

CASE PRESENTATION

A 44-year-old Chinese man with a 7 pack-year smoking history was referred to Shanghai Pulmonary Hospital in February 2013 for a left upper lobe lung mass with multiple bilateral intrapulmonary metastases, left pleural effusion, and 2R/4R/10L/11L lymphadenopathy. Pleural fluid cytology revealed adenocarcinoma and Scorpion Amplification Refractory Mutation system (AmoyDx Co., Xiamen, China). showed no detectable epidermal growth factor receptor mutation. He commenced chemotherapy with gemcitabine and cisplatin. However, after a single cycle, his symptoms worsened and imaging confirmed progressive

disease. A second opinion was requested from the University of Colorado and a computed tomography–guided biopsy of the left upper lobe lesion was performed to permit additional molecular testing. In the interim, the patient commenced pemetrexed and nedaplatin. Unfortunately, after two cycles his shortness of breath worsened, with evidence of further progression on his scans (Fig. 1*A*).

Molecular testing on his repeat biopsy specimen revealed no mutations by SNaPshot multiplex PCR testing (Life Technologies, Carlsbad, CA). However, although technically negative, the *ALK* break-apart FISH test showed an atypical negative pattern. Specifically, 68% of cells demonstrated single copies of the 5' *ALK* signal and numerous cells with doublets of the 5' *ALK* signal combined with one 3' *ALK* signal (Fig. 2*A*). Subsequently, confirmatory diagnostic assays demonstrated ALK protein expression by IHC using the D5F3 antibody (Cell Signaling Technology Inc., Danvers, MA; H score = 150; Fig. 2*B*) and the presence of an echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* transcript (E13;A20) by RT-PCR (Fig. 2*C*). The patient then received crizotinib (250 mg twice daily) as third-line therapy in May 2013 with an impressive symptomatic improvement and CT response after 1 month of therapy (Fig. 1*B*). He remains on treatment with crizotinib with no evidence of progression as of September 2013.

DISCUSSION

Isolated 5' *ALK* signals have previously been reported, although, because the 5' probe is not located in the kinase region of *ALK*, these are usually considered ALK negative by FISH.[3,4] Our patient showed multiple single copies of the 5' ALK signal plus additional 5' doublets combined with 3' signals, suggesting the possibility of a complex rearrangement. ALK positivity was confirmed by both IHC and RT-PCR and the patient responded well to crizotinib. A complex rearrangement generating a different atypical negative FISH pattern (3' doublets fused with 5' signals) that also harbored a functional *ALK* fusion was recently described.[5] When FISH is the initial diagnostic assay, consideration should be given to formally defining an atypical negative *ALK* FISH category for additional interrogation with alternative confirmatory assays.[2]

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