Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Butrynski JE, D'Adamo DR, Hornick JL, et al. Crizotinib in *ALK*-rearranged inflammatory myofibroblastic tumor. N Engl J Med 2010;363:1727-33.

Supplementary Appendix: Methods

Study Design

The phase I study on which these patients were enrolled was designed by the co-principal investigators, including Drs. Eunice Kwak and Jeffrey Clark, along with members of Pfizer Clinical Development, including Drs. Keith Wilner and James Christensen. Data were gathered by the authors, as well as by Pfizer Clinical Development, and analyzed by the authors. The first draft of the manuscript was written by Drs. Geoffrey Shapiro, James Butrynski, George Demetri and Jason Hornick, with contributions from the other authors, all of whom vouch for the completeness and accuracy of the data gathering and analysis. The decision to submit the manuscript for publication was by all of the authors. Data were considered confidential until the cases were presented at the meeting of the American Society of Clinical Oncology in May 2009.[1]

Immunohistochemistry

ALK immunostaining was performed using monoclonal antibody ALK1 (Dako, Carpinteria, CA) at a dilution of 1:25 following antigen retrieval with a pressure cooker in citrate buffer (pH 6.0). Antibody detection was carried out using the Envision Plus detection system (Dako).

Fluorescence in Situ Hybridization

Fluorescence in Situ Hybridization (FISH) analysis was performed on interphase nuclei isolated from 50-micron sections of formalin-fixed paraffin-embedded tissue (case 1, Brigham and Women's Hospital), or on formalin-fixed 5-micron paraffin sections on charged glass slides (case 2, Memorial Sloan-Kettering Cancer Center), according to standard protocols.[2] *ALK* rearrangements were evaluated using a Vysis LSI ALK Dual Color, Break Apart Rearrangement

Probe (Abbott Molecular, Des Plaines, IL) that contains two differentially labeled probes that flanking the *ALK* gene, located at 2p23. Probes and nuclei were co-denatured simultaneously, followed by hybridization and washing, according to the manufacturer's directions (Abbott Molecular). An intact *ALK* locus is represented as two fused orange/green signals. However, if an *ALK* rearrangement has occurred, one copy of the orange probes is separated from the adjacent green probe.

RT-PCR and DNA sequencing

Total RNA was isolated from tumor snap frozen in liquid nitrogen using Trizol™ (Invitrogen, Carlsbad, CA) and purified using RNeasy™ minielute cleanup kit (Qiagen, Valencia, CA). cDNA was transcribed with Superscript II Reverse Transcriptase (Invitrogen) and used as template for subsequent PCR-based studies. The specific *ALK* rearrangement in patient 1 was determined using PCR primers designed against the known translocations described in IMT (Fig 1D). PCR primers are available upon request. The resulting PCR product was sequenced using the same primers as were used for the amplification.

[1]Kwak EL, Camidge DR, Clark J, et al. Clinical activity observed in a phase I dose escalation trial of an oral c-Met and ALK inhibitor, PF-02341066. J Clin Oncol 2009;27:15S (suppl; abstr 3509).

[2]Weremowicz S, Schofield DE. Preparation of cells from formalin-fixed, paraffin-embedded tissue for use in fluorescence in situ hybridization (FISH) experiments. Curr Protoc Hum Genet 2007; Chapter 8:Unit 8.8.