Clinicopathologic Characteristics and Outcomes of Patients with Anaplastic Lymphoma Kinase-Positive Advanced Pulmonary Adenocarcinoma

Suggestion for an Effective Screening Strategy for These Tumors

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Introduction: The purpose of this study was to analyze the clinicopathologic characteristics and outcomes of patients with anaplastic lymphoma kinase (*ALK*)-positive advanced pulmonary adenocarcinoma and to devise an effective screening strategy to identify such patients.

Methods: We screened advanced pulmonary adenocarcinoma patients to identify *ALK*-positive cases. The presence of *ALK* rearrangements was confirmed by fluorescence in situ hybridization.

Results: Of the 221 screened patients, 45 demonstrated *ALK* rearrangements, and these individuals were younger than the *ALK*-negative patients ($p < 0.001$). The proportion of never smokers and light smokers was found not to differ according to the *ALK* status ($p = 0.537$). Epidermal growth factor receptor (*EGFR*) mutations and *ALK* rearrangements were found to be mutually exclusive. Thyroid-transcription factor-1 (TTF-1) expression was observed in all *ALK*-positive tumors for which immunohistochemistry data were available. The objective response rate and progression-free survival to first-line platinum-based chemotherapy showed no significant differences between *ALK*-positive and *ALK*-negative patients. On the other hand, no patient with *ALK*-positive tumors achieved objective tumor responses to EGFR tyrosine kinase inhibitors (TKIs). *ALK* rearrangements were not found among individuals who had *EGFR* mutations, an objective response to a previous EGFR TKI treatment or TTF-1-negative tumors.

Conclusion: The clinical outcomes of platinum-based chemotherapy were found not to differ according to the *ALK* status. Both smokers and never/light smokers should be candidates for *ALK* screening. We suggest that the exclusion of patients with activating *EGFR* mutations, an objective response to previous EGFR TKIs, or TTF-1-negative tumors from ALK screening could be an effective enrichment strategy for *ALK*-positive cases.

Key Words: Lung, Adenocarcinoma, *ALK*, *EGFR*, TTF-1.

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Oncogenic addiction refers to the phenomenon of tumor cell dependence on an activated oncogene for their survival and proliferation and provides an explanation for the rapid regression of some tumors after targeted therapies.[1] The identification of an oncogenic addiction pathway is, therefore, not only of great interest for cancer researchers but also of great importance for improving treatment outcomes for patients with cancer. The translocation of the anaplastic lymphoma kinase (*ALK*) gene represents a recently discovered oncogenic addiction pathway in lung cancer. The fusion of *ALK* with echinoderm microtubule-associated protein-like-4 was first identified in 2007 in patients with non-small cell lung cancer (NSCLC).[2] This echinoderm microtubule-associated protein-like-4-*ALK* gene fusion is created by an inv(2)(p21p23) in lung cancer cells, and this fusion demonstrates oncogenic potential in a mouse model.[3] Fusion between kinesin family member 5B (*KIF5B*) and *ALK* has also been identified in NSCLC, indicating that the *ALK* rearrangements itself may represent a key pathogenic mechanism for this cancer.[4]

Pharmacologic approaches to targeting *ALK* rearrangements in patients with NSCLC have been successful thus far, and encouraging results were observed recently in a phase I trial of *ALK* inhibitor, crizotinib (PF-02341066; Pfizer, New York, NY).[5] This sponsor-initiated trial involved 82 patients and reported an overall response rate (RR) of 57%. Based on

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these results, crizotinib should benefit a significant number of patients with NSCLC with *ALK* rearrangements in the near future.[6] To effectively screen and identify patients with NSCLC with *ALK* rearrangements, the specific characteristics of *ALK*-positive tumors need to be adequately defined. Several studies have reported that patients with *ALK* rearrangements tend to be younger and to have never smoked.[7,8] Pathologically, *ALK*-positive tumors are predominately adenocarcinomas and contain signet ring cells.[9] Furthermore, *ALK* rearrangements and activating epidermal growth factor receptor (*EGFR*) mutations are mutually exclusive.[10,11] Although these findings have yielded valuable preliminary data on *ALK*-positive tumors, they did not provide precise information on the clinicopathologic characteristics of *ALK*-positive advanced NSCLCs. Most previous reports have analyzed surgical cases in the main. Moreover, there is little statistical power associated with previous studies of *ALK* rearrangements as the cohorts examined were small.

In this study, we screened patients with advanced pulmonary adenocarcinoma for *ALK* rearrangements. Our present report represents the largest series comparing *ALK*-positive and *ALK*-negative advanced NSCLCs yet studied. We analyzed the clinicopathologic characteristics and treatment outcomes of our patient subjects, including their previous responses to platinum-based chemotherapy and EGFR tyrosine kinase inhibitors (TKIs) treatment. In addition, we suggested an effective future screening strategy to more readily identify *ALK*-positive cases.

PATIENTS AND METHODS

Study Population

Patients with advanced pulmonary adenocarcinoma whose tumor specimens underwent examination for *ALK* status to find potential candidates of crizotinib treatment at Seoul National University Hospital Oncology Clinic between January 2008 and April 2010 were included in this study. Each subject had a biopsy-proven pulmonary adenocarcinoma, and these cancers were either metastatic or recurrent at the time of *ALK* screening. Patient demographics and clinicopathologic characteristics, including smoking history, histologic subtype, *EGFR* mutations status, thyroid-transcription factor-1 (TTF-1) protein expression status, RR, and progression-free survival (PFS) from previous chemotherapy, were obtained from medical records. Patients who had smoked more than 100 cigarettes in their lifetime were defined as smokers. Among smokers, patients who had smoked $\leq$10 pack-years cigarettes were defined as light smokers. For exsmokers (smoking cessation for at least 6 months), patients who did not smoke for at least 10 years were defined as remote smokers. The other exsmokers were defined as recent exsmokers. Tumor histology and subtype were classified according to the World Health Organization criteria.[12] Activating *EGFR* mutations included the deletion of exon 19 and a L858R point mutation in exon 21.[13,14] Responses were classified as complete response, partial response, stable disease, or progressive disease according to RECIST version 1.0.[15] The PFS was calculated from the initiation of chemotherapy to the documentation of disease progression or death from any cause. The study protocol was reviewed and approved by the institutional review board of Seoul National University Hospital (H-1005-08-320) and adhered to the recommendations of the Declaration of Helsinki for biomedical research involving human subjects.

ALK Testing

Immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH) was used to screen tumor samples for *ALK* status. *ALK* was classified as negative when either the IHC or FISH result was negative. *ALK* FISH was performed in all the cases that were IHC positive. *ALK* was defined as positive only when the FISH result was positive.

ALK IHC was performed using a bond-max automated immunostainer (Leica Microsystems, Milton Keynes, UK). Paraffin sections (3 μm) were evaluated by immunohistochemical staining according to standard protocols. Each paraffin section was dewaxed and subjected to antigen retrieval. The use of antibodies was performed in accordance with the manufacturer's instructions. For antigen retrieval, the slides were heated for 20 minutes at 100°C in Epitope Retrieval Solution, pH 9.0 (Leica Microsystems). The slides were then incubated with a mouse monoclonal antibody for *ALK* (Novocastra, Clone 5A4, Newcastle Upon Tyne, UK). Antibody binding was detected using the Bond Polymer Refine Detection kit (Leica Microsystems). Mayer's hematoxylin was used as the counterstain. Various normal and cancer tissue microarray blocks were included as positive and negative controls. Tumor staining was assessed by two trained pathologists (D.H.C. and W.-H.K.) who were unaware of patient's clinical data. ALK IHC was considered positive if cytoplasmic staining was detected in 10% or more tumor cells.

For *ALK* FISH, 4-μm-thick sections were deparaffinized, dehydrated, immersed in 0.2 N HCl, and incubated in 1 M NaSCN for 30 minutes at 80°C. Sections were then immersed in pepsin solution. Dual-probe hybridization for *ALK* was performed using the LSI *ALK* break-apart probe set (Vysis, Downers Grove, IL). The probe mixture was applied to the slides, which were then incubated in a humidified atmosphere with HYBrite (Vysis) at 77°C for 5 minutes to simultaneously denature the probe and target DNA. An additional 16 hours at 37°C was required for hybridization. Next, the slides were immersed in 0.3% NP-40/0.4× saline sodium citrate for 5 minutes at room temperature, followed by 0.3% NP-40/0.4× saline sodium citrate for 5 minutes at 72°C. The nuclei were counterstained with 4′,6-diamidino-2-phenylindole. *ALK* FISH was considered positive when more than 15% of 50 or more analyzed cells showed splitting apart of the fluorescent probes flanking the *ALK* locus.

Statistical Analysis

The variables included for analysis in this study were age, gender, smoking history, histologic profile, *EGFR* mutations status, TTF-1 protein expression status, *ALK* positivity, RR, and PFS after chemotherapy. Statistical analysis of 2×2 contingency tables of categorical variables was performed using the Pearson's χ^2 test or Fisher's exact test, as appropriate. The *t* test was performed to compare continuous variables between two groups. The median durations of PFS