Original Article

Comparative Analyses of Overall Survival in Patients With Anaplastic Lymphoma Kinase-Positive and Matched Wild-Type Advanced Nonsmall Cell Lung Cancer

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BACKGROUND: The purpose of this study was to investigate the overall survival (OS) of patients with advanced *ALK*-positive nonsmall cell lung cancer (NSCLC) who were managed in the pre-ALK inhibitor era and to compare their survival with that of a matched case cohort of *ALK* wild-type (WT) patients. **METHODS:** Data from 1166 patients who had stage IIIB/IV NSCLC with nonsquamous histology were collected from the NSCLC database of Seoul National University Hospital between 2003 and 2009. *ALK* fluorescence in situ hybridization (FISH) was used to analyze 262 patients who either had the WT epidermal growth factor receptor (*EGFR*) or were nonresponders to previous EGFR tyrosine kinase inhibitor (TKI) therapy. Overall survival (OS) was compared between 3 groups: 1) *ALK*-positive patients, 2) *EGFR* mutation-positive patients, and 3) *ALK*-WT/*EGFR*-WT patients. Progression-free survival (PFS) after first-line chemotherapy and EGFR TKIs also was analyzed. **RESULTS:** Twenty-three patients were *ALK*-positive according to FISH analysis and did not receive ALK inhibitors during follow-up. The median OS for *ALK*-positive patients, *EGFR* mutation-positive patients, and WT/WT patients was 12.2 months, 29.6 months, and 19.3 months, respectively (vs *EGFR* mutation-positive patients, $P = .001$; vs WT/WT, $P = .127$). The PFS after first-line chemotherapy for the 3 groups was not different. However, the PFS for patients who received EGFR TKIs was shorter in *ALK*-positive patients compared with the other 2 groups (vs *EGFR* mutation-positive patients, $P < .001$; vs WT/WT, $P < .021$). **CONCLUSIONS:** In the pre-ALK inhibitor era, *ALK*-positive patients experienced the shortest survival, although it did not differ statistically from that of WT/WT patients. Although their responses to platinum-based chemotherapy were not different from comparator groups, *ALK*-positive patients were even more resistant to EGFR TKI treatment than WT/WT patients. ***Cancer* 2012;118:3579-86.** © *2011 American Cancer Society.*

KEYWORDS: anaplastic lymphoma kinase, epidermal growth factor receptor, nonsmall cell lung carcinoma, overall survival, tyrosine kinase inhibitor.

INTRODUCTION

It has become evident that nonsmall cell lung cancer (NSCLC) has distinct genetic alterations that are crucial for tumorigenesis. These molecular changes, called driver mutations, allowed a new way to classify lung cancer into clinically relevant subgroups.[1-3] One of these groups is the echinioderm microtubule-associated protein-like 4 (*EML4*)-anaplastic lymphoma kinase (*ALK*) gene translocation *EML4-ALK*, which was identified in 2007.[4,5] A small inversion within chromosome 2p results in the formation of a fusion gene comprising portions of the *EML4* gene and the *ALK* gene.[6] The product of this fusion gene works as a driver for proliferation in lung cancer cells that harbor this translocation, demonstrating the

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Presented in part at the metastatic/nonsmall cell lung cancer poster discussion session of 47th Annual Meeting of the American Society of Clinical Oncology; June 3-7, 2011; Chicago, IL.

DOI: 10.1002/cncr.26668, **Received:** August 25, 2011; **Revised:** October 3, 2011; **Accepted:** October 5, 2011, **Published online** November 15, 2011 in Wiley Online Library (wileyonlinelibrary.com)

phenomenon of oncogene addiction.[7,8] Less than 3 years after identification of the *EML4-ALK* translocation, a phase 1 trial of crizotinib (PF-02341066; Pfizer, New York, NY), an orally active ALK and MET dual inhibitor, resulted in a significant response in patients who had the *EML4-ALK* translocation. In a pretreated patient population that generally has a 10% response rate to conventional chemotherapy, treatment with crizotinib yielded an overall response rate (ORR) of 55% and an estimated 6-month progression-free survival (PFS) rate of 72%.[9,10] Furthermore, the mechanism of resistance to crizotinib was identified at the same time.[11]

In the center of this rapid advance of translational research, there has been an early understanding of the clinical and pathologic characteristics of patients with the *EML4-ALK* translocation.[12] Prevalence of the *EML4-ALK* translocation in unselected patients with NSCLC ranges from 3% to 5%.[13-16] The *EML4-ALK* translocation is highly correlated with younger age and never-smoking or light-smoking history.[15,17] The pathologic features of *ALK*-positive tumors also are distinct. Almost all of them are adenocarcinomas; signet-ring cell histology and acinar pattern were commonly identified.[13,18-20] Recent studies have proposed using these clinicopathologic characteristics as screening strategies to enrich for the likelihood of *ALK*-positive tumors.[3,21-23]

The *EML4-ALK* translocation is now a clear positive predictive marker of ALK-inhibitor therapy.[9,24] However, the prognostic value of *ALK* translocation is not fully understood. Previous studies tried to analyze overall survival (OS) in patients with the *EML4-ALK* translocation, but the clinical significance was in question because of small numbers of events in enrolled patients and confounding from the administration of crizotinib in the *ALK*-positive group.[12,23] Therefore, we present the current study, in which we compare *ALK*-positive patients and matched *ALK*-WT patients who were treated in the pre-ALK inhibitor era. Informed consent was obtained from the patients and/or surrogates who participated in this study. This protocol was reviewed and approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB No. H-1008-035-326).

MATERIALS AND METHODS

Study Population

In total, 1166 patients who had stage IIIB/IV NSCLC with nonsquamous histology were collected from the NSCLC database of Seoul National University Hospital, Seoul, Korea, between January 1, 2003 and August 31, 2009. To enrich for *ALK*-positive patients, we excluded patients who harbored *EGFR* mutations, because the *EML4-ALK* translocation is rarely coincident with *EGFR* mutation.[14,25] Among the patients with unknown *EGFR* mutation status, we excluded patients who achieved an objective response with gefitinib or erlotinib, using this as a proxy for the likelihood of an *EGFR* mutation-positive patient (Fig. 1).[12] Patients who had insufficient tissue for pathologic examination or whose tissue produced an inconclusive result in *EML4-ALK* fluorescence in situ hybridization (FISH) also were excluded. Therefore, in total, 262 patients with examinable tissue were enrolled. Patients who had received crizotinib were not included in the analysis.

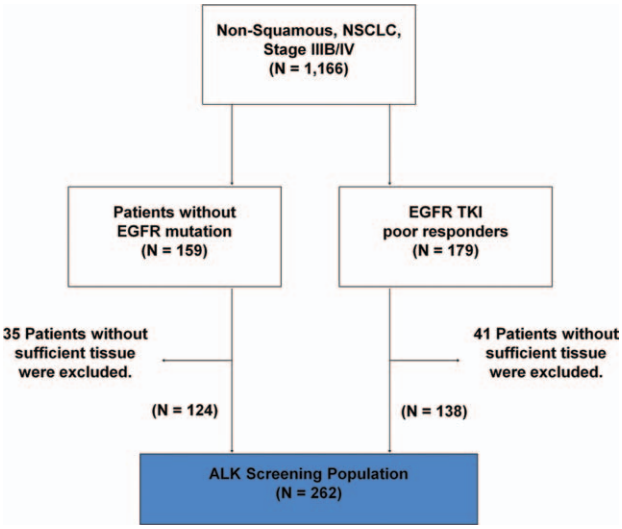

Figure 1. This is a Consolidated Standards of Reporting Trials (CONSORT) diagram illustrating the sample-enrichment strategy. NSCLC indicates nonsmall cell lung carcinoma; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase.

Data Collection

The medical records of enrolled patients were reviewed to collect demographic, clinical, and pathologic information. We examined chemotherapeutic regimens, responses, sites of metastases, and clinical outcomes. We also recorded the *EGFR* mutation status of patients, which had been determined using a direct sequencing method of *EGFR* exons 18 to 21. Radiologic responses were evaluated according to version 1.0 of Response Evaluation Criteria in Solid Tumors.[26] OS was calculated from the date metastatic disease was diagnosed to the date of death. PFS was measured from the first day of chemotherapy until radiologic or clinical disease progression.

Table 1. Clinicopathologic Characteristics of Matched Cohorts

Characteristic	No. of Patients (%)			*P*	
	ALK Positive, *N* = 23	*EGFR* Mutation Positive, *N* = 46	WT/WT, *N* = 46	*ALK* vs *EGFR*	*ALK* vs WT/WT
Age at diagnosis: Mean±SD [median], y	47.4±11.4 [47.8]	49.6±6.0 [51.1]	50.9±8.1 [52.0]	.383[a]	.140
Sex					
Men	9 (39.1)	17 (37)	19 (41.3)	.861[b]	.862
Women	14 (60.9)	29 (63)	27 (58.7)		
Smoking history					
Never or light-smoker	18 (78.3)	37 (80.4)	34 (73.9)	.832	.693
Heavy smoker[c]	5 (21.7)	9 (19.6)	12 (26.1)		
Pathology					
Adenocarcinoma	16 (69.6)	41 (89.1)	37 (80.4)	.043	.313
Nonsmall cell carcinoma, NOS	7 (30.4)	5 (10.9)	9 (19.6)		
Stage					
IIIB	0 (0)	1 (2.2)	0 (0)	.476	
IV	23 (100)	45 (97.8)	46 (100)		
ECOG PS					
0	7 (30.4)	12 (26.1)	12 (26.1)		
1	13 (56.5)	26 (56.5)	27 (58.7)		
2	3 (13.1)	7 (15.2)	6 (13)		
3	0 (0)	1 (2.2)	1 (2.2)		
4	0 (0)	0 (0)	0 (0)		
First-line cytotoxic chemotherapy					
Total	21 (91.3)	34 (73.9)	37 (80.4)		
Gemcitabine/cisplatin	6	14	12		
Paclitaxel/carboplatin	3	12	18		
Others	12	8	7		
EGFR TKI, any line					
Total	17 (73.9)	42 (91.3)	27 (58.7)		
Gefitinib	14	31	12		
Erlotinib	3	11	15		
Pemetrexed, any line	12 (52.2)	20 (43.5)	22 (47.8)		

Abbreviations: ALK, anaplastic lymphoma kinase; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; NOS, not otherwise specified; SD, standard deviation; TKI, tyrosine kinase inhibitor.
[a] *T* test.
[b] Chi-square test.
[c] Heavy smoker means smoker who have smoked ≥10 pack years.

Pathologic Examination and Anaplastic Lymphoma Kinase Testing

In total, 262 patients with testable formalin-fixed, paraffin-embedded tissue were included in this study. Two hundred six samples were biopsied by percutaneous technique, and 56 samples were surgically resected. All histologic diagnoses were reviewed based on the latest World Health Organization classification.[27]

ALK FISH analysis was performed using a dual-color break-apart probe (Abbott Molecular, Abbott Park, Ill), which hybridizes the 2p23 band (red signal) and the *ALK* gene breakpoint (green signal). All procedures were conducted according to the manufacturer's instructions. Three micron-sectioned, formalin-fixed, paraffin-embedded tissues were deparaffinized, dehydrated, immersed in 0.2 N HCl, and incubated in 1 M NaSCN at 80°C for 30 minutes. A pepsin solution was added to treated sections; then, dual-probe hybridization for *ALK* was performed. After application of the probe mixture, the slides were treated with protease and then incubated in a humidified atmosphere with HYBrite (Abbott Molecular) at 77°C for 5 minutes for denaturation. Subsequently, the slides were incubated at 37°C for 16 hours for hybridization. Slides were then immersed in 0.3% NP-40 (Abbott Molecular)/0.4 × saline sodium citrate (SSC) for 5 minutes at room temperature, followed by 0.3% NP-40 of 0.4 × SSC for 5

Table 2. Distribution of Metastasis Sites

Site of Metastasis	At the Initial Evaluation				Including Sites of Disease Progression			
	ALK Positive, *N* = 23	*EGFR* Mutation Positive, *N* = 46	WT/WT, *N* = 46	*P*[a]	*ALK* Positive, *N* = 23	*EGFR* Mutation Positive, *N* = 46	WT/WT, *N* = 46	*P*[a]
Lung to lung	14	32	25	.322	16	33	33	.979
Liver	2	9	3	.136	8	16	6	.034
Adrenal	1	3	5	.581	5	4	6	.317
Bone	8	16	17	.972	12	23	26	.818
CNS	6	15	9	.363	7	31	14	<.001
Pleural effusion	4	8	6	.821	7	12	16	.663
Pericardial effusion	2	2	1	.457	3	3	4	.663

Abbreviations: ALK, anaplastic lymphoma kinase; CNS, central nervous system; EGFR, epidermal growth factor receptor; WT, wild type.
[a] Chi-square test.

minutes at 72°C. For the counterstaining of nuclei, 4,6-diamidino-2-phenylindole was used. FISH was regarded as positive when break-apart signals or 5′-deletions were observed in >15% of ≥50 tumor cells. All specimens from the FISH assay were examined by 1 trained pathologist (H.S.P.) in a blinded manner.

Case-Case Matching and Statistical Analysis

To control for known prognostic variables in lung cancer survival, each *ALK*-positive patient was matched to 2 *EGFR* mutation-positive patients and 2 WT/WT patients. All patients in the matched cohort also were restricted to nonsquamous histology. Matching variables were age at diagnosis, sex, disease stage, and smoking status. The cutoff point for survival analysis was January 13, 2011.

Statistical analyses of categorical variables were performed using Pearson chi-square tests or Fisher exact tests, as appropriate. The *t* test was used to compare continuous variables between groups. The median durations of OS and PFS were calculated using the Kaplan-Meier method. Comparisons between groups were done using the log-rank test. Multivariate analysis was performed using a Cox proportional hazards model. Two-sided *P* values < .05 were considered statistically significant. All analyses were performed using the PASW Statistics software package (version 18.0; SPSS Inc. Chicago, Ill).

RESULTS

Clinicopathologic Characteristics

Among 262 examined tumors, we identified 23 *ALK*-positive cases by FISH. One *ALK*-positive case was matched to 2 *EGFR* mutation-positive patients and to 2 WT/WT patients, as mentioned above (Table 1). All 3 groups included patients with stage IV disease or recurrent tumors except for 1 *EGFR* mutation-positive patient who had stage IIIB disease. Consequently, a total of 115 patients (23 *ALK*-positive, 46 *EGFR* mutation-positive, and 46 WT/WT patients) were included in the survival analysis. On pathologic examination, the *ALK*-positive group included more unspecified nonsmall cell carcinomas (30%) than the *EGFR* mutation-positive and WT/WT groups (11% and 20%, respectively). Three *ALK*-positive patients (13%) and 1 WT/WT patient (2%) had signet ring cell carcinoma. In terms of metastatic site, 30% of both *ALK*-positive and WT/WT patients had central nervous system (CNS) metastases proven in radiologic or cerebrospinal fluid cytopathologic examinations during treatment. However, *EGFR* mutation-positive patients had a higher rate of CNS metastasis (63%; $P < .001$) (Table 2). Fewer liver metastases were observed in the WT/WT group ($P = .035$).

Treatment Responses and Survival Analyses

We examined patient responses to and clinical outcomes after chemotherapy and EGFR TKI treatment documented in the medical records (Tables 3 and 4). Among 115 patients, 92 patients (80%) received cytotoxic chemotherapy as first-line treatment. All of those patients received a platinum-based doublet regimen, except for 2 patients who received gemcitabine/vinorelbine and docetaxel. Various doublet combinations were identified; the most common regimen was paclitaxel/carboplatin, which was received by 33 patients, followed by gemcitabine/cisplatin (32 patients). Response rates to cytotoxic chemotherapy did not differ between the 3 groups (*ALK*-positive patients, 27%; *EGFR* mutation-positive patients, 32%; WT/WT patients, 35%). Although PFS for *EGFR* mutation-positive patients (4.93 months) was longer than for the other 2 groups (*ALK*-positive patients, 3.87 months; WT/WT patients, 3.73 months) (Fig. 2), the difference was not statistically significant (Table 4). In total, 73

Table 3. Treatment Responses by Molecular Subtypes

Variable	*ALK* Positive, *N* = 23		*EGFR* Mutation Positive, *N* = 46		WT/WT, *N* = 46		*P*[a]	
	No.	%	No.	%	No.	%	*ALK* vs *EGFR*	*ALK* vs WT/WT
Best response to first-line cytotoxic chemotherapy								
Total	21	91.3	34	73.9	37	80.4		
CR	0	0	0	0	0	0		
PR	6	28.6	11	32.4	13	35.1		
SD	8	38	12	35.3	15	40.5		
PD	6	28.6	11	32.4	9	24.4		
Unevaluable	1	4.8	0	0	0	0		
Best response to EGFR TKI								
Total	10[b]	21.7	42	91.3	27	58.7		
CR	0	0	3	7.1	0	0		
PR	0	0	31	73.8	4	14.8		
SD	2	20	6	14.3	7	25.9		
PD	8	80	2	4.8	16	59.3		
Unevaluable	0	0	0	0	0	0		
Response rate, %								
Chemotherapy		28.6		32.4		35.1	.857	.695
EGFR TKI		0		80.9		14.8	<.001	.096

Abbreviations: ALK, anaplastic lymphoma kinase; CR, complete response; EGFR, epidermal growth factor receptor; PD, progressive disease; PR, partial response; SD, stable disease; TKI, tyrosine kinase inhibitor; WT, wild type.
[a] Chi-square test.
[b] Excludes patients that were enrolled due to previous non-response to EGFR TKIs.

Table 4. Results of Survival Analysis by Molecular Subtypes

Survival Variable	*ALK* Positive, *N* = 23	*EGFR* Mutation Positive, *N* = 46	WT/WT, *N* = 46
OS			
No. of patients	23	46	46
Median OS (95% CI), mo	12.23 (6.60-17.87)	29.63 (24.73-34.53)	19.33 (9.11-29.55)
P vs ALK-positive[a]		.001	.127
PFS after first-line chemotherapy			
No. of patients	21	34	37
Median (95% CI), mo	3.87 (0.43-7.31)	4.93 (4.40-5.46)	3.73 (2.32-5.14)
P vs ALK-positive[a]		.825	.474
PFS after EGFR TKI therapy			
No. of patients	10[b]	42	27
Median (95% CI), mo	1.37 (1.07-1.67)	9.80 (4.94-14.66)	2.07 (0.15-3.99)
P vs ALK-positive[a]		<.001	.037

Abbreviations: ALK, anaplastic lymphoma kinase; CI, confidence interval; EGFR, epidermal growth factor receptor; OS, overall survival; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; WT, wild type.
[a] Log-rank P values were derived from a comparison of Kaplan-Meier estimates between patients who had ALK-positive tumors versus patients who had other tumor types.
[b] Excludes patients who were enrolled because of a previous nonresponse to EGFR TKIs.

patients (63%) received subsequent cytotoxic chemotherapy. The proportion of patients who received second-line chemotherapy was well balanced between the groups (*ALK*-positive patients, 70%; *EGFR* mutation-positive patients, 59%; WT/WT patients, 65%,). Pemetrexed[32] was the most commonly used agent as second-line therapy

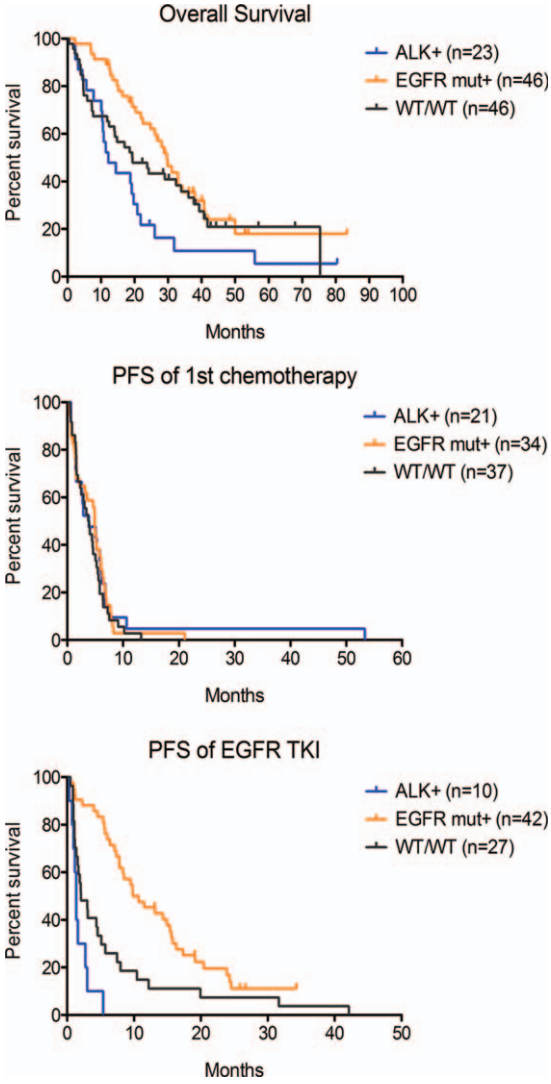

Figure 2. Kaplan-Meier estimates of (*Top*) overall survival, (*Middle*) progression-free survival (PFS) after first-line chemotherapy, (C) progression-free survival of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) therapy among study patients. ALK indicates anaplastic lymphoma kinase; mut+, mutation positive; WT, wild type.

followed by combined gemcitabine/vinorelbine[18] and docetaxel.[8] PFS after second-line chemotherapy was did not differ between the 3 groups (*ALK*-positive patients, 2.07 months; *EGFR* mutation-positive patients, 1.63 months; WT/WT patients, 2.93 months; $P = .353$).

Eighty-six patients were received an EGFR TKI. Specifically, 57 patients received gefitinib, 29 patients received erlotinib, and 4 patients received both agents. For an appropriate comparison of PFS after EGFR TKI treatment, we excluded *ALK*-positive patients who were enrolled because of their nonresponses to EGFR TKI treatment. Because all 9 *ALK*-positive patients already were preselected on the basis of their nonresponse to EGFR TKI, it was inappropriate to measure their PFS because this was a selection criterion. If they had been included, then it would have biased the group based on nonresponse to TKI. The *EGFR* mutation-positive group had a much higher response rate (81%) than the 2 other groups (*ALK*-positive patients, 0%; WT/WT patients, and 15%). *ALK*-positive patients who received an EGFR TKI had rapid disease progression and did not respond to EGFR TKI treatment. The median PFS for *ALK*-positive patients (1.37 months) was shorter than that for the other 2 groups (*EGFR* mutation-positive patients, 9.80 months; WT/WT patients, 2.07 months; $P = .037$ vs WT/WT) (Fig. 2).

The median OS of *ALK*-positive patients was 12.2 months compared with 29.6 months for *EGFR* mutation-positive patients ($P = .001$) and 19.3 months for WT/WT patients ($P = .127$). In a multivariate analysis that included age, sex, stage, smoking status, and histology with a Cox-proportional hazards model, the calculated hazard ratio was 0.446 for *EGFR* mutation-positive patients and 0.631 for WT/WT patients. Other variables, including histology, did not significantly affect OS. Finally, *ALK*-positive patients had the shortest (albeit statistically nonsignificant) median OS in the pre-ALK inhibitor era. They did not differ in their response to conventional cytotoxic chemotherapy compared with *ALK*-WT patients. However, they were more resistant to EGFR TKI treatment, even compared with WT/WT patients.

DISCUSSION

In this study, we used FISH to identify an historic cohort of ALK inhibitor-naive patients and examined the possible prognostic role of the *EML4-ALK* translocation in the clinical outcomes of patients with NSCLC. We demonstrated that the OS of *ALK*-positive patients did not differ statistically from their WT/WT matched comparators, although their survival was numerically shorter. Shaw et al[12] evaluated survival outcomes in 17 patients with metastatic, *ALK*-positive disease by determining PFS and OS. In that study, *ALK*-positive patients had inferior clinical outcomes compared with *EGFR* mutation-positive patients, resembling the survival of WT/WT patients. However, the number of events was small within the *ALK*-positive patient group, the follow-up duration was relatively short, and there were differences in age

and smoking status between comparator groups in our study. Moreover, 7 of 17 *ALK*-positive patients enrolled in the phase 1 crizotinib clinical trial, which may have, as acknowledged by the author, influenced the OS outcome of the study. To minimize imbalances in potential prognostic clinicopathologic characteristics, we performed a 2:1 case matching of *ALK*-WT patients to *ALK*-positive patients. This matching took into account age at diagnosis, sex, disease stage, and smoking status. The follow-up period of our study was relatively long, with a median follow-up of 26 months. In addition, ALK inhibitor-related effects on survival fundamentally were ruled out in our study. Although our study had the limitations of a single-center, retrospective design and restricted statistical power because of the small sample size, we carefully controlled for confounding factors in our analyses in an effort to present the comparative clinical course of *ALK*-positive patients (treated without ALK inhibitors) and *ALK*-WT patients.

The predictive role of the *EML4-ALK* translocation in response to EGFR TKI therapy, which has been described in several studies,[12,22,28,29] was affirmed in the current study. *ALK*-positive patients were more resistant to EGFR TKI treatment, even compared with WT/WT patients ($P = .037$). This result also is in concordance with the laboratory data published in 2008, which demonstrated the resistance of an *ALK*-positive lung cancer cell line to erlotinib.[8] Recent studies repeatedly have reported similar data on the resistance of *ALK*-positive tumors to EGFR TKI, and a screening strategy for *ALK* positivity has been proposed based on this characteristic.[12,22,23]

The report by Shaw et al and a previous report from our group also demonstrated an objective response rate to conventional chemotherapy that was numerically smaller in *ALK*-positive patients versus *ALK*-WT patients.[12,22] However, this finding was not statistically significant in those studies, either as a true result or as a function of the limited sample size of *ALK*-positive patients in each study. Larger sample sizes or pooled analyses may address this issue more definitively.

Recent retrospective analyses have indicated that *ALK*-positive patients were more sensitive to pemetrexed compared with *ALK*-WT comparators.[29,30] In the current study, the percentage of pemetrexed exposure in any line of *ALK*-positive, *EGFR* mutation-positive, and WT/WT groups was 52%, 43%, and 48%, respectively. Despite the relatively high use of pemetrexed in the *ALK*-positive patients, this group had the shortest OS estimate.

ALK-positive patients had a lower rate of CNS metastases (30%) during the follow-up period compared with *EGFR* mutation-positive patients (63%), and this rate was identical to that of the WT/WT patients (30%). This result may have been caused by bias, because *EGFR* mutation-positive patients had longer survival compared with the other 2 groups. Six *ALK*-positive patients (26%), 15 *EGFR* mutation-positive patients (33%), and 9 WT/WT patients (20%) had CNS metastases confirmed at their initial staging workup. To compare the rate of CNS metastasis in terms of a survival-related effect, a prospective survey would be helpful.

A significant portion of *ALK*-positive patients (22%) identified in this study had a smoking history, although 3 patients in the heavy-smoker group had 10 pack-year smoking history, which is a borderline value for heavy smoker (defined as a smoking history of $\geq$10 pack-years). Similarly, a recent study by our group in a different patient population also reported a large number of smokers (31%) in the *ALK*-positive group.[22] These finding suggest that smoking status is not appropriate for patient selection in *ALK* testing. Smoking history should be approached and interpreted with caution, because it can vary in different cultural and social contexts. Although we excluded squamous cell histology in selecting our patients, our cohort had a significant portion of nonsmall cell carcinoma, not otherwise specified (NOS). We identified 7 *ALK*-positive patients with NOS histology, including 3 who had an adenocarcinoma immunophenotype that demonstrated expression of thyroid transcription factor-1 and cytokeratin 7. Several previous studies also have reported a small number of *ALK*-positive patients with nonadenocarcinoma histology.[12,29-31] In addition, misclassification in histology can occur in tumors that are difficult to specify; especially in patients who have small specimens harvested by needle biopsy or aspiration.[32] Therefore, we should take care to restrict *ALK* testing in patients with adenocarcinoma histology, because we can miss a small number of *ALK*-positive patients who have large cell, NOS, or other minor histology.

In our current study, *ALK* positivity was suggestive of a poor prognosis (although the finding not statistically significant) and was predictive of poor EGFR TKI outcomes. With the historically dismal survival observed across the unselected NSCLC patient population, this finding may signify an even greater unmet medical need

within the *ALK*-positive subset of patients with NSCLC, and these patients are in need of effective therapy and do not benefit from currently available targeted agents. With the development of molecularly targeted therapy, such as crizotinib, positive *ALK* status may prove to be a positive predictive marker for ALK inhibitor therapy in the near future.

FUNDING SOURCES

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (A110057), and was funded in part by an outcomes research agreement with Pfizer.

CONFLICT OF INTEREST DISCLOSURES

Dong-Wan Kim has acted as a consultant and has received honoraria from Pfizer. Kimary Kulig is an employee of Pfizer. Yung-Jue Bang has acted as a consultant, has received honoraria, and has received research funding from Pfizer.

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