Clinical and Prognostic Implications of ALK and ROS1 Rearrangements in Never-Smokers with Surgically Resected Lung Adenocarcinoma

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Directed by Professor Byoung Chul Cho

The Master's Thesis submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science

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This certifies that the Master's Thesis of
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<ABSTRACT>

Clinical and Prognostic Implications of *ALK* and *ROS1* Rearrangements in Never-Smokers with Surgically Resected Lung Adenocarcinoma

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(Directed by Professor Byoung Chul Cho)

The aim of this study is to evaluate the prevalence and prognostic significance of anaplastic lymphoma kinase (*ALK*) and c-ros oncogene 1 (*ROS1*) rearrangement in never-smokers with surgically resected lung adenocarcinoma. We enrolled 162 consecutive never-smokers who underwent curative resection for stage IB to IIIA lung adenocarcinoma. We concurrently analyzed mutations in the epidermal growth factor receptor (*EGFR*) and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) genes, and investigated *ALK* rearrangements by fluorescence in situ hybridization assay. *ROS1* rearrangement was also determined in all triple (*EGFR/KRAS/ALK*)-negative tumors. Of 162

never smokers with lung adenocarcinoma, 14 (8.6%) and 5 (3.1%) had *ALK* and *ROS1* rearrangements, respectively. Nineteen of the 74 (25.7%) *EGFR* and *KRAS* mutation-negative patients were fusion-positive (*ALK* or *ROS1* fusion). Fusion-positive patients tended to have shorter median disease-free survival (DFS) than fusion-negative patients (28.0 *vs.* 33.9 months; $p = 0.128$). In multivariate analysis, fusion-positive patients had significantly poorer DFS than fusion-negative patients after adjustment for age, sex, T stage, lymph node metastasis, and adjuvant chemotherapy use ($p = 0.022$; hazard ratio, 2.11; 95% confidence interval, 1.19-4.30). The difference in DFS between fusion-positive and fusion-negative patients was more prominent among patients with stage IB to IIB tumors (20.7 *vs.* 38.2 months; $p = 0.046$). Pleural (38.5% *vs.* 23.9%) and extrathoracic distant (46.2% *vs.* 35.8%) metastasis rates at the first recurrence were higher in fusion-positive patients. This study shows significantly poorer DFS of *ALK* or *ROS1* fusion-positive lung adenocarcinoma in never-smokers after curative surgery.

Key words: anaplastic lymphoma kinase, c-ros oncogene 1, prognosis, never-smokers, lung adenocarcinoma

Clinical and Prognostic Implications of *ALK* and *ROS1* Rearrangements in Never-Smokers with Surgically Resected Lung Adenocarcinoma

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I. INTRODUCTION

Recent advances in the molecular characterization of non-small cell lung cancer (NSCLC) have improved prognosis of patients with personalized therapies on their molecular targets. The discovery of activating mutations within the kinase domain of the epidermal growth factor receptor (*EGFR*) and their association with sensitivity to EGFR-tyrosine kinase inhibitors (TKIs) [1-3] have led to the extensive investigation of genetic alterations in lung adenocarcinoma. A number of mutations in key oncogenes, including Kirsten rat sarcoma 2 viral oncogene homolog (*KRAS*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), *BRAF*, and *HER2* have been identified as important genetic alterations in lung adenocarcinoma.[4,5] In

addition to oncogene mutations, rearrangements of oncogenic kinase proteins, such as anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*), and rearranged during transfection (*RET*) have also been discovered in a number of subsets of lung adenocarcinoma patients.[6-9] *ALK* and *ROS1* rearrangements are present in approximately 3–5% and 1–3% of all NSCLCs, respectively, and have a higher prevalence in young, never-smoker, lung adenocarcinoma patients.[10,11] Previous studies have shown that *ALK* and *ROS1*-rearranged NSCLCs are both highly sensitive to the ALK inhibitor crizotinib,[12,13] and this is probably attributable to the high degree of homology between their kinase domains. It is unknown whether *ALK* and *ROS1*-rearranged NSCLCs share a common oncogenic pathogenesis; however, *ALK* and *ROS1*-rearranged NSCLCs have many clinical and epidemiological features in common. Improved understanding of the clinical properties and prognosis of *ALK* and *ROS1* rearranged lung adenocarcinomas is needed to characterize subsets of NSCLC patients who will benefit from crizotinib treatment.

Recurrence often occurs in NSCLCs after curative surgery even in patients with early-stage disease, and is associated with poor prognosis. A 5-year survival rate of 48.8% for stage IB patients after curative resection has been reported.[14] Pathological findings at surgery, such as lymph node metastasis, histologic grade, and pleural invasion, cannot fully predict the probability of recurrence. Therefore, identification of biomarkers and development of adjuvant targeted molecular therapies are needed to predict recurrences and to reduce

NSCLC-related mortality. Several studies have reported that adjuvant EGFR-TKI therapy (erlotinib and gefitinib) improves disease-free survival (DFS) in NSCLC with *EGFR* mutations after surgical resection.[15,16] However, the prognostic implication of *ALK* or *ROS1* rearranged NSCLC after curative surgery and the role of crizotinib in adjuvant therapy have not been established. Although an initial report indicated that *ALK*-rearranged NSCLC tends to have a higher stage than NSCLC with other genotypes,[10] *ALK* rearrangement did not affect overall survival (OS) in metastatic or locally advanced NSCLC.[10,17,18] However, several studies have suggested that *ALK*-rearranged tumors possess aggressive features on radiologic imaging and circulating tumor cell analysis.[18-20] Yang et al. reported that *ALK* rearrangement was significantly associated with poor DFS in never-smoker patients with surgically resected lung adenocarcinoma.[21] Zhou et al. also found that *ALK* rearrangement was significantly associated with poor prognosis in a stage IIIA subgroup of lung adenocarcinoma patients.[22] However, the prognostic significance of *ALK* rearrangement in surgically resected lung adenocarcinoma remains inconclusive.[21-23] Furthermore, little research has been done to evaluate the prognostic implication of *ROS1* rearrangement in lung adenocarcinoma. In this study, we comprehensively evaluated the prognostic significance of *ALK* or *ROS1* fusion (fusion-positivity) on DFS and OS in never-smokers with surgically resected stage IB to IIIA lung adenocarcinoma.

II. MATERIALS AND METHODS

1. Study population and data collection

We analyzed consecutive stage IB to IIIA never-smoker lung adenocarcinoma patients who underwent curative surgery between April 2005 and November 2012 at Severance Hospital (Seoul, Korea). During this period, 837 lung adenocarcinoma patients received curative surgery for primary lung adenocarcinoma. Pathologic staging of tumors according to the seventh American Joint Committee on Cancer (AJCC) and precise smoking history at the time of diagnosis were reviewed in all patients. Only patients with never-smoker status, which was defined as a lifetime smoking dose of less than 100 cigarettes, were selected. Among the selected patients, only stage IB to IIIA patients were enrolled and stage IA and IIIB/IV patients were excluded. Therefore, 162 consecutive patients with stage IB to IIIA lung adenocarcinoma with available clinical and survival data were included in the study after agreement of participation in the molecular genetic analysis (Fig. 1). Patient clinical characteristics including age at diagnosis, date of recurrence, date of death, and treatment modalities were retrospectively reviewed by electronic medical chart review. The Institutional Review Board (IRB) of Severance Hospital approved this study, and all patients signed written informed consents for genetic analysis.

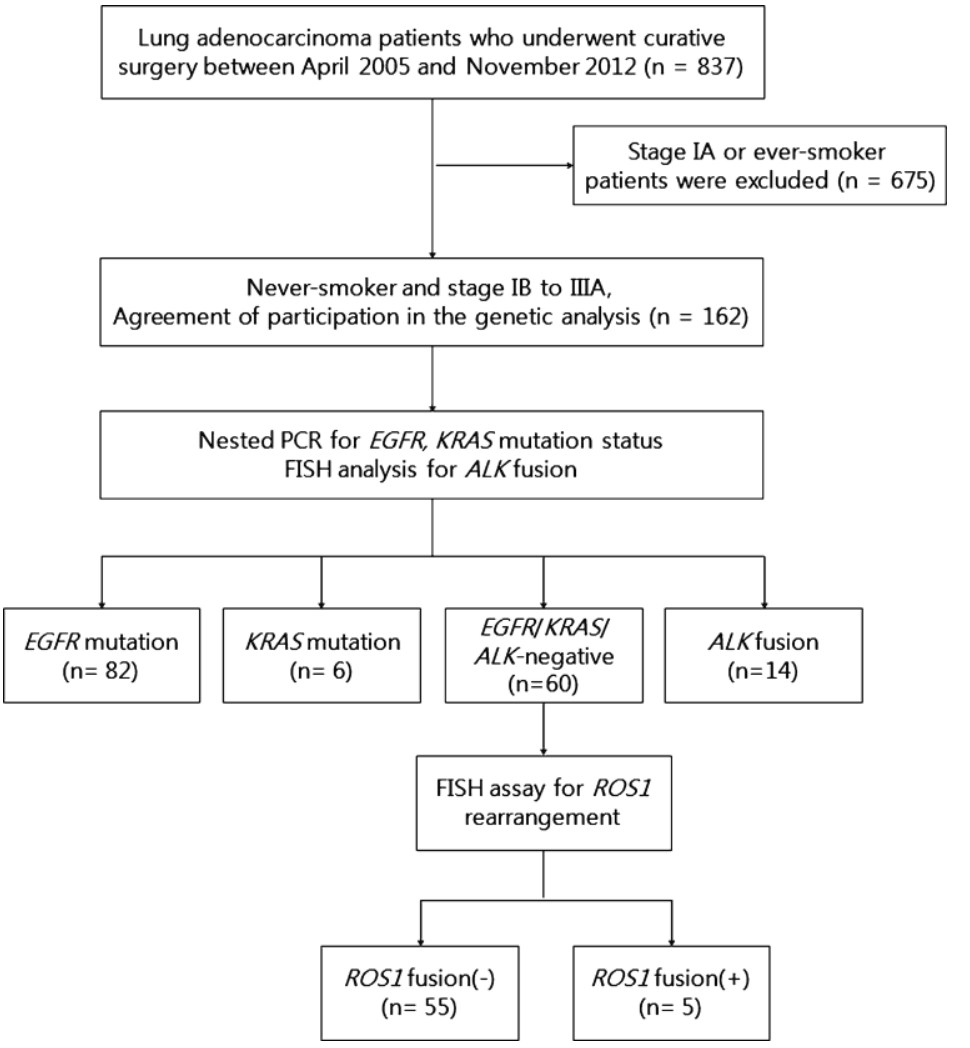

Figure 1. CONSORT diagram.

Abbreviations: *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma 2 viral oncogene homolog; *ALK*, anaplastic lymphoma kinase; *ROS1*, c-ros oncogene 1; FISH, fluorescence in situ hybridization

2. Molecular profiling of lung adenocarcinoma: *EGFR*, *KRAS*, *ALK*, and *ROS1* Analysis

Comprehensive molecular profiling of *EGFR*, *KRAS*, and *ALK* was performed in all surgically resected tumors. *ROS1* rearrangement was only evaluated in triple (*EGFR*/*KRAS*/*ALK*)-negative patients (n = 60). Nucleotide sequencing of *EGFR* (exons 18 to 21) and *KRAS* (codons 12/13 of exon 2) kinase domains was performed using nested polymerase chain reaction (PCR) amplification of the individual exons. Fluorescent in situ hybridization (FISH) assays were performed to detect *ALK* and *ROS1* rearrangements in formalin-fixed, paraffin-embedded tumors as described previously.[17,24] Specific break apart FISH probes for *ALK* (Vysis LSI *ALK* Dual Color Break Apart Rearrangement Probe; Abbott Molecular, Abbott Park, IL, USA) and *ROS1* (Break Apart Rearrangement Probe; Abbott Molecular) were used in the FISH assays according to the manufacturer's instructions. At least 100 nuclei per case were evaluated, and FISH positivity for *ALK* and *ROS1* rearrangement was defined as >15% of tumor cells with a split signal.

3. Analysis of survival outcomes and first recurrence sites

DFS and OS were calculated from the date of curative operation to the date of the event of interest: tumor recurrence, death, or final follow-up date. All

patients were followed up until the date of death or June 15, 2013. For patients who experienced recurrence during the follow-up period, imaging studies at the time of first recurrence were reviewed and information on the first recurrence site were collected. The first recurrence sites were categorized according to their locations (intrathoracic, extrathoracic distant, and lymph node), and the proportion of each recurrence site to the total number of recurrences was determined. Progression-free survival (PFS) on EGFR-TKIs was reviewed in all patients who received EGFR-TKIs (gefitinib or erlotinib) after recurrence. PFS was calculated from the date of the first EGFR-TKI treatment to the date of tumor progression, death, or drug cessation (unacceptable toxicity or refusal). Data on patients alive on June 15, 2013 were censored.

4. Statistical analysis

Data were analyzed using SPSS for Windows version 20.0 (SPSS Inc., Chicago, IL, USA). The chi-squared test, Fisher's exact test, or *t*-test was used to compare the baseline characteristics of patients according to tumor genotype. Kaplan-Meier curves were used to compare DFS, PFS and OS. Survival outcome differences between genotypes were compared using the log-rank test. Cox regression modeling was used to calculate hazard ratios (HRs) for the risk of recurrence or death after curative surgery according to genotype. Age, sex, T stage, lymph node metastasis, and adjuvant therapy use were included as

independent variables in the Cox regression model. All statistical tests were two-tailed, and a *p*-value of <0.05 was considered statistically significant.

III. RESULTS

1. Patient characteristics

Patient baseline characteristics are summarized in Table 1. The study population consisted of 25 male (15.4%) and 137 female (84.6%) never-smoker patients, with a median age of 60 years (range, 42–75 years). The majority of patients received adjuvant chemotherapy, which consisted of platinum-based chemotherapy (with vinorelbine or taxane) or uracil-tegafur chemotherapy. The median follow-up duration was 3.4 years (range, 3.0–3.8 years).

2. Prevalence and baseline characteristics of the molecular genotypes

Of the 162 patients, 14 (8.6%) were *ALK* fusion-positive and 5 (3.1%) were *ROS1* fusion-positive. *EGFR* and *KRAS* mutations were detected in 82 (50.6%) and 6 (3.7%) patients, respectively. Genetic alterations in *ALK*, *EGFR*, and *KRAS* were mutually exclusive. Of the 60 triple (*EGFR*/*KRAS*/*ALK*)-negative patients, *ROS1* fusion positivity was noted in 5 (8.3%) patients. In addition, fusion positivity (*ALK* or *ROS1* fusion) was present in 19 of 74 (25.7%) patients

TABLE 1. Baseline Characteristics of the Patients According to Molecular Genotype

	Total (*n* = 162,%)	***EGFR* mutation (*n*=82,%)**	***KRAS mutation* (*n*=6,%)**	***ALK* fusion (*n*=14,%)**	***ROS1* fusion (*n*=5,%)**	**Wild-type[a] (*n*=55,%)**	***p*-value[b] Fusion-positive[d] *vs.* fusion-negative**	***p*-value[b] Fusion-positive *vs. EGFR* mutation**	***p*-value[b] Fusion-positive *vs.* Wild-type**
Age (years)									
<60	80 (49.4)	44 (53.7)	4 (67.0)	6 (42.9)	4 (80.0)	22 (40.0)	0.763	0.936	0.338
≥60	82 (50.6)	38 (46.3)	2 (33.0)	8 (57.1)	1 (20.0)	33 (60.0)			
Sex									
Male	25 (15.4)	10 (12.2)	2 (33.0)	3 (21.4)	2 (40.0)	8 (14.5)	0.178	0.151	0.480
Female	137 (84.6)	72 (87.8)	4 (67.0)	11 (78.6)	3 (60.0)	47 (85.5)			
Stage[c]									
IB	68 (42.0)	34 (41.5)	2 (33.3)	6 (42.9)	2 (40.0)	24 (43.6)	0.805	0.185	0.920
IIA	26 (16.0)	14 (17.1)	3 (50.0)	3 (21.4)	0 (0.0)	6 (10.9)			
IIB	29 (17.9)	17 (20.7)	1 (16.7)	2 (14.3)	0 (0.0)	9 (16.4)			
IIIA	39 (24.1)	17 (20.7)	0 (0.0)	3 (21.4)	3 (60.0)	16 (29.1)			
T stage									
T1	12 (7.4)	6 (7.3)	0 (0.0)	3 (21.4)	1 (20.0)	2 (3.6)	0.051	0.351	0.042
T2	128 (79.0)	65 (79.3)	5 (83.0)	10 (71.4)	4 (80.0)	44 (80.0)			
T3–T4	22 (13.6)	11 (13.4)	1 (17.0)	1 (7.1)	0 (0.0)	9 (16.4)			
N stage									
N0	94 (58.0)	46 (56.1)	5 (83.0)	7 (50.0)	3 (60.0)	33 (60.0)	0.612	0.803	0.575
N1–N3	68 (42.0)	36 (43.9)	1 (17.0)	7 (50.0)	2 (40.0)	22 (40.0)			
Adjuvant chemotherapy									
None	42 (25.9)	22 (26.8)	1 (16.7)	1 (7.1)	0 (0.0)	18 (32.7)	0.027[e]	0.065[e]	0.030[e]
Platinum-based	118 (72.8)	60 (73.2)	5 (83.3)	13 (92.9)	5 (100.0)	35 (63.6)			
UFT	2 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.6)			
Lymphovascular invasion									
No	134 (82.3)	66 (80.5)	6 (100.0)	12 (85.7)	4 (80.0)	46 (83.6)	1.000	1.000	1.000
Yes	28 (17.3)	16 (19.5)	0 (0.0)	2 (14.3)	1 (20.0)	9 (16.4)			
Visceral pleural invasion									
No	71 (43.8)	25 (30.5)	2 (33.3)	7 (50.0)	3 (60.0)	34 (61.8)	0.410	0.068	0.482
Yes	91 (56.2)	57 (69.5)	4 (66.7)	7 (50.0)	2 (40.0)	21 (38.2)			

Abbreviations: *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; *ROS1*, c-ros oncogene 1; UFT, tegafur-uracil

[a] *EGFR*/*KRAS*/*ALK*/*ROS1*-negative patients.

[b] *p*-values were calculated using the chi-squared test or Fisher's exact test.

[c] Pathologic staging of tumors according to the seventh American Joint Committee on Cancer (AJCC)

[d] *ALK* or *ROS1* fusion-positive patients based on fluorescence in situ hybridization assay.

[e] *p*-value was calculated by comparing patients with platinum-based or UFT adjuvant chemotherapy *vs*. patients without adjuvant chemotherapy.

who were negative for *EGFR* and *KRAS* mutations. *ALK* or *ROS1* fusion patients had a lower T stage than wild-type (*EGFR*/*KRAS*/*ALK*/*ROS1*-negative) patients, whereas lymph node stage was not different between these patients. Age, sex, AJCC stage, lymphovascular invasion, and visceral pleural invasion were not significantly different among fusion-positive, *EGFR* mutation-positive and wild-type patients (Table 1).

3. Prognostic significance of *ALK* or *ROS1* fusion on patient survival after curative surgery

DFS after surgery was analyzed to define the prognostic impact of *ALK* or *ROS1* fusion in lung adenocarcinoma. The median DFS and OS of all patients were 33.3 months (range, 27.1–39.5 months) and 77.4 months (range, 72.5–92.2 months), respectively, and the 3-year DFS rate was 46.3%. In univariate analysis, pathologic T stage and lymph node metastasis were significantly associated with poor DFS, whereas sex, age, lymphovascular invasion, visceral

pleural invasion, and adjuvant chemotherapy use were not significant prognostic factors for DFS (Table 2). Fusion-positive (*ALK* or *ROS1* fusion) patients tended to have poorer DFS than fusion-negative patients; however, this trend was not statistically significant (median DFS, 28.0 *vs.* 33.9 months; $p = 0.124$; HR, 1.59; Fig. 2A). The 3-year DFS rate was 32.7% in fusion-positive patients and 47.0% in fusion-negative patients. DFS was also not significantly different according to *EGFR* mutation status (28.9 months for wild-type *EGFR vs.* 34.1 months for *EGFR* mutation-positive; $p = 0.379$*)*. However, multivariate analysis by Cox-proportional hazard modeling showed that DFS was significantly poorer in fusion-positive patients than in fusion-negative patients after adjustments for age, sex, T stage, lymph node metastasis, and adjuvant chemotherapy ($p = 0.023$; HR, 2.11; 95% confidence interval, 1.11–3.99*;* Table 2). In order to define a prognostic role of fusion genes in a more homogenous group of patients, we selected stage IB to IIB patients ($n = 123$), and fusion-positive patients had significantly poorer DFS than fusion-negative patients in this subgroup (20.7 *vs.* 38.2 months; $p = 0.046$; HR, 2.04 Fig. 2C). By using pair-wise comparison of all genotypes, *ALK* fusion-positive patients showed a shorter DFS than those with *EGFR* mutations (20.7 *vs.* 33.9 months, $p = 0.061$, Fig. 2D), and those with wild-type (median 20.7 months *vs.* median not reached, $p = 0.018$) in the stage IB to IIB subgroup. On the contrary, DFS was unaffected by fusion status in the stage IIIA subgroup ($p = 0.878$), but this result should be cautiously interpreted because of a small sample size. OS after surgery was not

different between fusion-positive and fusion-negative patients (p = 0.720; Fig. 3A), and wild-type patients had a significantly poorer OS than those with *EGFR* mutations in pair-wise comparison of all genotypes (p = 0.020, Fig. 3B).

TABLE 2. Univariate and Multivariate Analyses of Prognostic Factors for Disease-Free Survival

Characteristics	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*p*-value[a]	HR	95% CI	*p*-value[a]
Sex						
Male	1			1		
Female	1.04	0.57–1.89	0.899	1.15	0.62–2.14	0.656
Age (years)						
<60	1			1		
≥60	0.75	0.48–1.16	0.191	0.79	0.50–1.25	0.319
Tumor status			0.006			0.001
T1	1			1		
T2	1.33	0.53–3.31	0.545	2.54	0.96–6.75	0.062
T3–4	3.15	1.15–8.63	0.026	6.17	2.08–18.3	0.001
Lymph node status						
Negative	1			1		
Positive	1.67	1.07–2.59	0.023	1.96	1.18–3.27	0.009
Adjuvant chemotherapy						
No	1			1		
Yes	1.574	0.91–2.72	0.105	0.97	0.52–1.82	0.925
ALK or *ROS1* fusion						
Negative	1			1		
Positive	1.59	0.88–2.89	0.128	2.11	1.11–3.99	0.022

Abbreviations: *ALK*, anaplastic lymphoma kinase; *ROS1*, c-ros oncogene 1; HR, hazard ratio; CI, confidence interval.

[a] *p*-values were calculated using Cox-proportional hazard modeling.

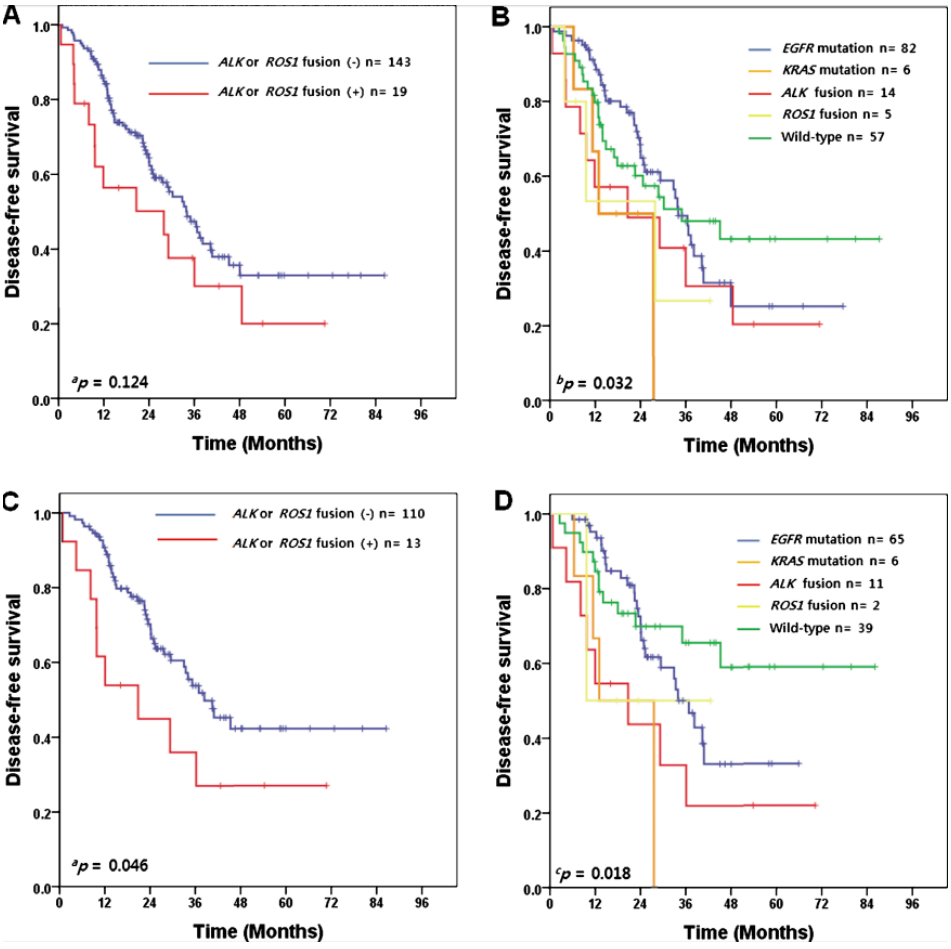

Figure 2. Comparison of disease-free survival after surgery according to genotype. Survival curves for (A) fusion-positive and fusion-negative in all patients, (B) 5 genotypes in all patients, (C) fusion-positive and fusion-negative in the stage IB–IIB patient subgroup, and (D) 5 genotypes in the stage IB–IIB patient subgroup

Abbreviations: *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma 2 viral oncogene homolog; *ALK*, anaplastic lymphoma kinase; *ROS1*, c-ros oncogene 1.

[a] *p*-values were calculated using the log-rank test.

[b] A statistically significant difference was observed between *EGFR* mutation and *KRAS* mutation patients. ($p = 0.032$)

[c]A statistically significant difference was observed between *ALK* fusion-positive and wild-Type patients (p = 0.018)

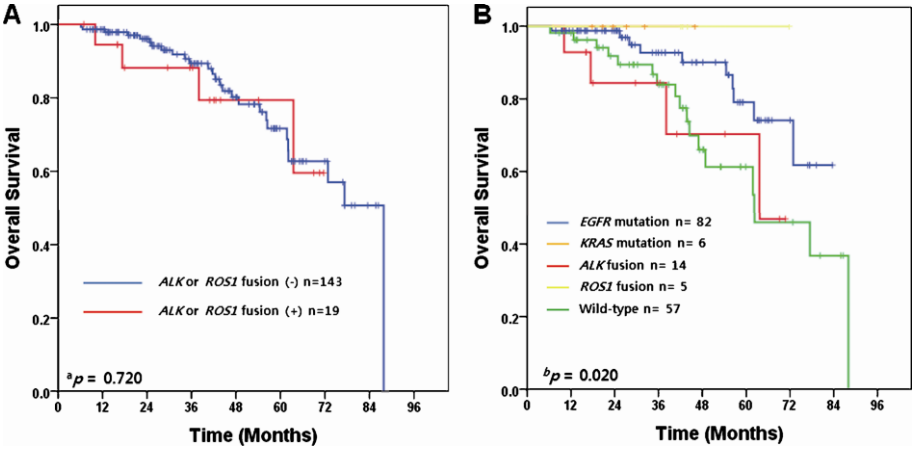

Figure 3. Comparison of overall survival after surgery according to genotype. Kaplan–Meier curves for (A) fusion-positive and fusion-negative, and (B) 5 genotypes in all patients.

Abbreviations: *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma 2 viral oncogene homolog; *ALK*, anaplastic lymphoma kinase; *ROS1*, c-ros oncogene 1.

[a] p-values were calculated using the log-rank test.

[b]A statistically significant difference was observed between *EGFR* mutation patients and wild-type patients. (p = 0.020)

4. Comparison of the first recurrence sites and post-recurrence survival outcomes

Recurrence after curative surgery occurred in 80 patients in this study. For all patients with recurrences, the first recurrence sites on radiologic imaging were compared according to tumor genotype (Table 3). First recurrence sites were not significantly different between fusion-positive and fusion-negative patients; however, pleural metastasis was higher (38.5% *vs.* 23.9%; $p = 0.310$) and contralateral lung metastasis was lower (7.7% *vs.* 35.8%; $p = 0.054$) in fusion-positive patients than in fusion-negative patients. Extrathoracic distant metastasis was higher in fusion-positive patients than in fusion-negative patients patients (46.2% *vs.* 35.8), but this difference was not statistically significant ($p = 0.539$). Thirty-nine patients received EGFR-TKI treatment after recurrence, and fusion-positive patients showed significantly poorer response to EGFR-TKI than those with other genotypes. The median PFS in response to EGFR-TKI treatment was 0.9 months, 10.2 months, and 6.4 months in fusion-positive, *EGFR* mutation-positive, and wild-type patients, respectively ($p = 0.001$). OS from the date of initial diagnosis of recurrence did not differ according to fusion status ($p = 0.727$).

TABLE 3. First Recurrence Sites According to Genotype

	EGFR mutation (*n*=82,%)	*KRAS* mutation (*n*=6, %)	*ALK* fusion (*n*=14,%)	*ROS1* fusion (*n*=5, %)	Wild-type[a] (*n*=55, %)	Fusion-positive (*n*=19, %)	Fusion-negative (*n*=143, %)	*p*- value[b]
No. of recurrences	38	4	10	3	25	13	67	
Intrathoracic metastasis[c]	29 (76.3)	4 (100.0)	7 (60.0)	2 (66.7)	19 (76.0)	9 (69.2)	52 (77.6)	0.496
Lung	22 (57.9)	4 (100.0)	6 (60.0)	1 (33.3)	11 (44.0)	7 (53.8)	37 (55.2)	1.000
Ipsilateral lung	18 (47.4)	3 (75.0)	6 (60.0)	1 (33.3)	9 (36.0)	7 (53.8)	30 (44.8)	0.562
Contralateral lung	16 (42.1)	2 (50.0)	0 (0.0)	1 (33.3)	6 (24.0)	1 (7.7)	24 (35.8)	0.054
Pleural	11 (28.9)	0 (0.0)	5 (50.0)	0 (0.0)	5 (20.0)	5 (38.5)	16 (23.9)	0.310
Lymph node								
Regional	2 (5.3)	0 (0.0)	3 (30.0)	1 (33.3)	8 (32.0)	1 (7.7)	5 (7.5)	0.727
Distant	3 (7.9)	0 (0.0)	1 (10.0)	0 (0.0)	8 (32.0)	4 (30.8)	16 (23.9)	1.000
Extrathoracic distant metastasis[d]	15 (39.5)	0 (0.0)	4 (40.0)	2 (66.7)	9 (36.0)	6 (46.2)	24 (35.8)	0.539
Brain	7 (18.4)	0 (0.0)	2 (20.0)	1 (33.3)	5 (20.0)	3 (23.1)	12 (17.9)	0.702
Liver	2 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.0)	1.000
Bone	5 (13.2)	0 (0.0)	3 (30.0)	0 (0.0)	5 (20.0)	3 (23.1)	10 (14.9)	0.435

Abbreviations: *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; *ROS1*, c-ros oncogene 1.

[a] *EGFR/KRAS/ALK/ROS1*-negative patients.

[b] *p*-values were calculated using the chi-squared test or Fisher's exact test comparing fusion-positive patients and fusion-negative patients.

[c] Includes ipsilateral lung, contralateral lung, pleural metastasis, mediastinum, and chest cavity.

[d] Includes distant metastasis outside the thoracic cavity, including brain, bone, liver, kidney, adrenal gland, and other sites.

IV. DISCUSSION

The proportion of never-smokers among lung cancer patients has been gradually increasing, accounting for 20% to 30% of all NSCLC cases.[25,26] Never-smoker lung cancer patients are good candidates for personalized diagnosis and therapy because of their high incidence of targetable genetic alterations. We investigated prevalence and clinical characteristics of *ALK* or *ROS1* fusion-positive lung adenocarcinoma in Asian never-smoker patients, and survival outcome after surgery was compared by genotypes of tumor. We found that *ALK*-positive tumors were present in 8.6% of patients, and 3.1% of patients had *ROS1*-positive tumors in this never-smoker population. These frequencies were comparable to those reported in never-smoker population by previous studies.[17,27] Fusion-positive patients tended to have shorter DFS than fusion-negative patients in univariate analysis. In multivariate analysis, *ALK* or *ROS1* fusion was an independent prognostic factor for DFS after adjustment of age, sex, T stage, lymph node metastasis, and adjuvant chemotherapy use. The difference in DFS between fusion-positive and fusion-negative patients was more prominent among patients with stage IB to IIB tumors. Higher rates of pleural metastasis and extrathoracic distant metastasis at first recurrence were noted in *ALK* or *ROS1* fusion-positive patients, and fusion-positive patients showed poor treatment response on EGFR-TKIs after recurrence. These results

suggested that a considerable proportion of never-smoker patients have *ALK* or *ROS1* fusions, and fusion-positive patients may need specific diagnostic and therapeutic approaches based on their high risk of recurrence and poor prognosis.

Prognosis of *ALK* or *ROS1* fusion-positive patients has been described in several studies; however, the results have been inconsistent. This inconsistency may be attributable to the low incidence of *ALK* or *ROS1* fusion, limited follow-up duration, and influence of various confounding clinical factors, such as baseline tumor stage, patient smoking status, treatment modality, and targeted ALK inhibitor therapy use. We selectively enrolled never-smoker patients in our study. Management of fusion-positive lung adenocarcinoma is a particularly important clinical issue in the never-smoker population based on their high frequency of *ALK* and *ROS1* fusions, and never-smoker patients with *ALK* fusions have been shown to be associated with poor survival outcomes.[21] After exclusion of stage IA patients because of their low probability of recurrence,[22] we evaluated the prognostic value of *ALK* or *ROS1* fusion in consecutive never-smoker patients with stage IB to IIIA lung adenocarcinoma in this study. We found that presence of *ALK* or *ROS1* fusion in tumor was significantly associated with poorer DFS, especially in subgroup with stage IB to IIB patients. However, survival outcome was similar between fusion-positive and fusion-negative patients in the stage IIIA patients.

ALK and *ROS1*-rearranged NSCLCs share similar epidemiological profiles

and are both sensitive to *ALK* inhibitor crizotinib. However, whether *ALK* and *ROS1* fusion-positive lung cancers have a similar oncogenic mechanism and can be classified in a common subgroup is unknown.[11] In the present study, patients with *ALK* or *ROS1* rearrangements were grouped together to clarify the characteristics and prognosis of fusion-positive lung cancer, allowing for better identification of those patients likely to benefit from crizotinib therapy. Rearranged *ALK* and *ROS1* proteins activate diverse oncogenic downstream pathways that are crucial for cell survival and proliferation, such as mitogen-activated protein kinase, Janus kinase/signal transducers and activators of transcription, phosphatidylinositol 3-kinase/Akt, and phospholipase C-γ pathways.[28] Although *ALK* and *ROS1* fusions in NSCLC cells have been shown to have robust transforming potential in a mouse model,[7,8] it is unclear whether *ALK* or *ROS1*-rearranged NSCLCs have a more invasive and aggressive pathogenesis than those with other genotypes. A recent study showed homogenous overexpression of epithelial-mesenchymal transition (EMT) markers in circulating tumor cells, on the contrary to their primary tumor in *ALK*-rearranged NSCLC patients, suggesting that *ALK* rearrangement may promote tumor invasiveness and migration through EMT induction.[19] Radiologic studies also have shown more aggressive features of *ALK*-positive tumors, with a lower ground-glass opacity portion and tumor disappearing rate on computed tomography,[18] and higher glucose uptake on positron emission tomography.[20] Some case studies have reported very late recurrence of *ALK*-

positive tumors even in patients with more than a 10-year DFS, which indicates the durable metastatic potential of *ALK*-rearranged tumors.[29,30] Together, these studies suggest that *ALK*-rearranged tumors are associated with poor prognosis and high risk of recurrence. Our study also showed that *ALK* or *ROS1* fusion was a significant independent predictor of poor DFS.

Our study showed that *ALK* or *ROS1* fusion-positive tumors had higher rates of pleural metastasis and extrathoracic distant metastasis; however, this was not statistically significant. The lack of statistical significance was most likely due to the small sample size. One previous study also reported significantly higher rates of pericardial, pleural, and hepatic metastasis in *ALK*-rearranged metastatic NSCLC, which suggests that *ALK* rearrangement is associated with a more widespread metastatic pattern.[31] Therefore, fusion-positive patients may need more aggressive adjuvant treatment and recurrence surveillance after surgical resection. Furthermore, *ALK* or *ROS1* inhibitors may represent a potential role in adjuvant therapy to overcome the poor prognosis of *ALK* or *ROS1* fusion-positive lung adenocarcinoma patients.

The major strength of this study was that we comprehensively profiled molecular genotypes in a consecutive never-smoker lung adenocarcinoma cohort. We used FISH assay to detect *ALK* and *ROS1* rearrangements, which provided reliable and reproducible results, as shown previously.[24] However, this was a single institution study with a small sample size, and the follow-up duration was not sufficient to compare differences in OS. Moreover, a limited

number of patients with *ROS1*-rearranged tumors were included in the study, and we did not screen for *RET* rearrangement. Therefore, studies with larger sample sizes are needed to further evaluate the prognostic implication of *ROS1* and *RET* rearrangements in lung adenocarcinoma.

V. CONCLUSION

Molecular characterization of surgically resected lung adenocarcinoma is essential in implementing personalized therapy. Our study demonstrated the feasibility of molecular characterization of surgically resected lung adenocarcinoma and a considerable proportion of never-smoker lung adenocarcinoma patients harbored *ALK* or *ROS1* fusion. Presence of *ALK* or *ROS1* fusion was a significant independent prognostic factor for DFS in never-smoker lung adenocarcinoma patients. Our findings suggest that fusion-positive tumors possess highly aggressive features and high risk of recurrence. Clinical development of adjuvant *ALK* or *ROS1*-targeted therapies may be needed to improve survival of fusion-positive lung adenocarcinoma patients after curative surgery.

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ABSTRACT (IN KOREAN)

수술적 절제를 받은 비흡연자, 폐선암 환자에서의 anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*) 전위의 임상적, 예후적 의미

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최근의 연구에 따라 비소세포폐암에서 anaplastic lymphoma kinase (*ALK*) 와 c-ros oncogene 1 (*ROS1*) 전위(rearrangement)가 중요한 유전자 변이로 밝혀지게 되었다. 본 연구는 수술적 절제를 받은 비흡연자 폐선암(lung adenocarcinoma) 환자들에서 *ALK* 와 *ROS1* 전위의 유병율을 조사하고 그 임상적, 예후적 의미를 조사하고자 하였다. 본 연구는 근치적 절제술을 받은 162 명의 연속된 IB 에서 IIIA 병기를 가지는 비흡연자 폐선암환자를 대상으로 하였으며, 모든 환자들의

종양 조직에서 epidermal growth factor receptor (*EGFR*), v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) 유전자 돌연변이를 검사하였고, *ALK* 전위 여부를 fluorescence in situ hybridization assay (FISH) 검사를 통해 조사하였다. 또한 모든 삼중 음성 환자 (*EGFR/KRAS/ALK*-음성) 에서는 *ROS1* 전위 여부도 마찬가지로 FISH 검사를 통해 조사하였다. 본 연구의 162 명의 비흡연자 폐선암 환자에서 *ALK* 전위와 *ROS1* 전위는 각각 14명 (8.6%) 과 5명 (3.1%) 에서 나타났다. *EGFR, KRAS* 가 음성인 74명의 환자들에서 19명 (25.7%)의 환자들이 전위 양성 (*ALK* 혹은 *ROS1* 전위) 으로 나타남이 확인되었다. 전위 양성 환자들은 전위 음성인 화자들에 비해서 더 짧은 중앙 무병생존기간을 나타내는 경향이 있었다 (28.0 개월 *vs.* 33.9 개월; $p = 0.128$; 위험비 1.59). 나이, 성별, T stage, 림프절 전이, 보조항암화학요법 여부를 보정한 다변수 분석에서 전위 양성 환자들은 전위 음성인 환자에 비해서 통계학적으로 유의하게 더 불량한 무병생존기간을 나타냈다. ($p = 0.022$; 위험비 2.11; 95% 신뢰구간 1.19-4.30). 전위 양성 환자와 전위 음성 환자간의 무병생존기간의 차이는 stage IB 에서 IIB 까지의 환자들에서 더 크게 나타났다. (20.7 개월 *vs.* 38.2 개월; $p = 0.046$; 위험비 2.04).

환자들의 전체 생존기간은 *ALK, ROS1* 전위 상태에 따라 다르지는 않았다. 수술 이후 처음 재발한 부위를 비교했을 때, 전위 양성 환자들은 전위 음성 환자들에 비해 더 많은 흉막 전위와 흉곽외 원격전이 소견을 보였다. 결론적으로, 이 연구는 *ALK* 나 *ROS1* 전위 양성인 비흡연자 폐선암 환자들의 근치적 수술 후 무병진행생존기간이 더 불량함을 시사한다.

핵심되는 말 : Anaplastic lymphoma kinase, c-ros oncogene 1, 예후, 비흡연자, 폐선암