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The frequency and impact of *ROS1* rearrangement on clinical outcomes in never smokers with lung adenocarcinoma

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Background: To determine the frequency and predictive impact of *ROS1* rearrangements on treatment outcomes in never-smoking patients with lung adenocarcinoma.
Patients and methods: We concurrently analyzed *ROS1* and *ALK* rearrangements and mutations in the epidermal growth factor receptor (*EGFR*), and *KRAS* in 208 never smokers with lung adenocarcinoma. *ROS1* and *ALK* rearrangements were identified by fluorescent *in situ* hybridization.
Results: Of 208 tumors screened, 7 (3.4%) were *ROS1* rearranged, and 15 (7.2%) were *ALK*-rearranged. *CD74-ROS1* fusions were identified in two patients using reverse transcriptase–polymerase chain reaction. The frequency of *ROS1* rearrangement was 5.7% (6 of 105) among *EGFR/KRAS/ALK*-negative patients. Patients with *ROS1* rearrangement had a higher objective response rate (ORR; 60.0% versus 8.5%; $P = 0.01$) and a longer median progression-free survival (PFS; not reached versus 3.3 months; $P = 0.008$) to pemetrexed than those without *ROS1/ALK* rearrangement. The PFS

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to EGFR-tyrosine kinase inhibitors in patients harboring *ROS1* rearrangement was shorter than those without *ROS1/ALK* rearrangement (2.5 versus 7.8 months; $P = 0.01$).

Conclusions: The frequency of *ROS1* rearrangements in clinically selected patients is higher than that reported for unselected patients, suggesting that *ROS1* rearrangement is a druggable target in East-Asian never smokers with lung adenocarcinoma. Given the different treatment outcomes to conventional therapies and availability of ROS1 inhibitors, identification of *ROS1* rearrangement can lead to successful treatment in *ROS1*-rearranged lung adenocarcinomas.

Key words: lung adenocarcinoma, never smoker, outcome, *ROS1*

introduction

Lung cancer in never smokers (LCINS) is regarded as a distinct disease entity with a distinct molecular subclassification [1]. Notably, actionable mutations in epidermal growth factor receptor (*EGFR*), *KRAS*, and anaplastic lymphoma kinase (*ALK*)-rearrangement are three major recurrent oncogenic alterations in LCINS [1–3]. Other genetic aberrations in LCINS include *HER2* mutations and *KIB5B-RET* fusion, although these mutations are known to occur with low frequencies [4].

Recently, *ROS1* rearrangement has emerged as a new molecular subtype in non-small-cell lung cancer (NSCLC), and now comprises a distinct molecular classification of NSCLC. *ROS1* rearrangement in NSCLC was discovered in 2007 by Rikova, who identified two *ROS1* fusion products (*SLC34A2-ROS1* and *CD74-ROS1)* in a cell line and a tumor with high ROS1 phospho-peptides [5]. *ROS1* rearrangements result in the formation of fusion proteins having constitutive tyrosine-kinase activity through the dimerization of *ROS1* fusion partners which subsequently stimulates downstream signaling, resulting in enhanced cell growth, proliferation, and decreased apoptosis.

In an unselected NSCLC population, the frequency of *ROS1* rearrangement ranged from 0.9% to 1.7% [3, 6, 7]. Recently, Bergethon et al. [3] reported a strong association between *ROS1* rearrangements and never smoking. However, because most studies investigated predominantly smokers, the frequency of *ROS1* rearrangements in East-Asian never smokers remains unknown. Furthermore, whether patients with *ROS1*-rearranged NSCLC share similar outcomes to other genetically defined subsets, particularly in the metastatic setting, also is unknown.

Preclinical and clinical data have demonstrated that *ROS1*-positive tumors are sensitive to crizotinib [3]. This indicates that *ROS1*-positive NSCLC represents a novel patient subset that may derive clinical benefit from *ROS1* inhibition [8]. Preliminary data from a phase I trial of crizotinib (NCT00585195) in the *ROS1*-positive NSCLC expansion-cohort demonstrated an overall response rate (ORR) of 57%. However, the sensitivity of *ROS1*-positive NSCLC to cytotoxic chemotherapy or EGFR-tyrosine kinase inhibitors (TKIs) in patients with NSCLC with *ROS1* fusions has not been evaluated.

Herein, we determined the frequency of *ROS1* rearrangement with the identification of fusion partners in the largest-ever cohort of East-Asian never smokers with NSCLC. We also examined the clinicopathological characteristics and treatment outcomes of patients with lung adenocarcinomas with *ROS1* rearrangement.

materials and methods

study population and data collection

This study was conducted in a cohort of histologically confirmed never smokers with lung adenocarcinoma at Severance Hospital, Seoul, Korea, between January 2005 and February 2012. The criteria used for patient selection included: (i) availability of tumor tissue, (ii) smoking-history, (iii) genetic data (*EGFR* and *KRAS* mutation and *ALK* rearrangement), and (iv) survival data. Tumor histology was classified using the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification of Lung Adenocarcinoma. Never smokers were defined as those with a lifetime smoking dose of <100 cigarettes. A total of 208 consecutive never smokers with histologically confirmed lung adenocarcinoma were enrolled.

A predesigned data collection format was used to review the patients' medical records for evaluation of clinicopathological characteristics and survival outcomes. For patients with metastatic disease, we examined treatment regimens, ORR, and survival outcomes. Clinical responses were classified using the Response Evaluation Criteria in Solid Tumor (RECIST version 1.1). Progression-free survival (PFS) was measured from the first day of treatment to tumor progression or death, while overall survival (OS) was measured from the date of diagnosis of metastatic disease until the date of death. Patients were censored on 26 October 2012, if alive and progression free. Patients without a known date of death were censored at the time of last follow-up. This study was approved by the Institutional Review Board of Severance Hospital. All patients signed a written informed consent for genetic analysis.

ROS1 rearrangements

To identify *ROS1* rearrangements, fluorescent *in situ* hybridization (FISH) assays were carried out on formalin-fixed and paraffin-embedded (FFPE) tumors by using a break-apart probe to *ROS1* (Break-Apart Rearrangement Probe; Abbott Molecular®) according to manufacturer's instructions. FISH-analyses were interpreted by two experienced evaluators (HSS and JKP) who were blinded to the clinical and genetic data. At least 100 nuclei per case were evaluated. FISH positivity for *ROS1* rearrangement was defined as >15% of tumor cells with a split signal. FISH-positive tumors were confirmed by immunohistochemistry (IHC) using antibody against ROS1. FFPE tissues were sectioned at a thickness of 4 μm and stained using Ventana automated immunostainner BenchMark XT. The slides were dried at 60°C for 1 h and deparaffinized using EZ Prep at 75°C for 4 min. Cell conditioning was carried out using CC1 solution at 100°C for 64 min. ROS1 antibody (rabbit monoclonal, clone D4D6, Cell Signaling Technology) was diluted to 1:50, treated, and incubated at 37°C for 32 min. Signals were detected using OptiView DAB IHC Detection Kit (Ventana Medical Systems). Counterstaining was carried out with Hematoxylin I for 4 min at room temperature.

To identify the known fusion partners of *ROS1*, reverse transcriptase–polymerase chain reaction (RT-PCR) was carried out using the SuperScript

III First-Strand Synthesis System (Invitrogen) with a previously published *ROS1* primer located in exon 32, exon 34, exon 35, and exon 36 [9]. PCR products positive for the ROS1 fusions were excised from the agarose gel, purified (Wizard SV Gel and PCR Clean-Up Kit; Promega), and then sequenced.

thymidylate synthase IHC and scoring

Thymidylate synthase (TS) antibody (rabbit polyclonal, clone H-265, Santa Cruz Biotechnology) was diluted to 1:50, treated, and incubated at 37°C for 32 min. For TS, a semiquantitative scoring was used, and the results were represented as intensity multiplied by proportion.

ALK rearrangements, *EGFR*, and *KRAS* mutation analysis

To identify *ALK* rearrangements, FISH studies were carried out on FFPE tumors by using a break-apart probe to *ALK* (Vysis LSI ALK Dual Color, Break-Apart Rearrangement Probe; Abbott Molecular) [2]. Nucleotide sequencing of the kinase domain of *EGFR* (exons 18 to 21) and *KRAS* exon 2 (codons 12/13) was carried out using nested PCR-amplification of the individual exons [2]. Details of these methods have been described previously [2].

cell culture and cell viability assay

The human NSCLC cell lines, A549, H460, H1299, HCC827, and H2228, were purchased from the American Type Culture Collection and PC-9 and H3122 were kind gifts from Drs K. Hayata (Tokyo Medical College) and John D. Minna (University of Texas Southwestern). After cells were exposed to drugs for 72 h, 0.5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution was added to the well. The optical density of the MTT formazan product was read at 565 nm on a microplate reader.

statistical analysis

Significant differences in variables according to each genotype were tested using the χ^2 test, Fisher's exact test, or *t*-test, as appropriate. The Kaplan–Meier method was used to estimate PFS and OS, and the differences according to genotype were compared using the log-rank test. Adjusted hazard ratios (AHRs) for the risk of progression or death in response to treatment according to genotype were calculated using a Cox-regression model that included age, gender, and genetic alteration as independent variables. All *P*-values were based on a two-tailed hypothesis.

results

clinicopathologic characteristics of patients with *ROS1* rearrangement

We screened *ROS1* rearrangements in 208 never smokers with lung adenocarcinoma. *ROS1*-positive lung adenocarcinoma was detected in 7 of 208 samples (3.4%). There was no significant difference in baseline characteristics between *ROS1*-positive and -negative cases. The median age of *ROS1* positive was numerically younger than that of *ROS1* negative without statistical significance, probably due to the fact that the majorities in our study were elderly women (Table 1). Although *ROS1* rearrangement was mutually exclusive from *ALK* rearrangement and *KRAS* mutation, one of seven *ROS1*-positive

Table 1. Demographic and clinical characteristics of patients with *ROS1*-positive lung adenocarcinoma

Variables	No (%)				*P*-value (*ROS1* versus *WT/WT*)	*P*-value (*ALK* versus *WT/WT*)
	All patients (*n* = 208)	*ROS1* positive (*n* = 7)	*ALK* positive (*n* = 15)	*WT/WT* (*n* = 186)		
Age (years)					0.80	0.95
Median	58	55	58	58		
Range	30–78	30–68	34–78	33–77		
Sex					0.69	0.58
Male	32 (15.4)	1 (14.3)	2 (13.3)	29 (15.6)		
Female	176 (84.6)	6 (85.7)	13 (86.7)	157 (84.4)		
Stage[a]					0.82	0.92
I	41 (19.7)	2 (28.6)	3 (20.0)	36 (19.4)		
II	26 (12.5)	0 (0)	3 (20.0)	23 (12.4)		
IIIA	38 (18.3)	1 (14.3)	3 (20.0)	34 (18.3)		
IIIB	17 (8.2)	1 (14.3)	1 (6.7)	15 (8.1)		
IV	86 (41.3)	3 (42.9)	5 (33.3)	78 (41.9)		
Type of mutation						
EGFR	83 (39.9)	1 (14.3)	–	82 (44.1)	0.24	<0.001
Exon19 deletion	51 (24.5)	–	–	51 (27.4)		
Exon21 L858R	28 (13.5)	1 (14.3)	–	27(14.5)		
Others[b]	4 (1.9)	–	–	4 (2.2)		
KRAS	5 (2.3)	–	–	5 (2.6)	0.83	0.68
Gly12Asp (GGT→GAT)	3 (1.4)	–	–	3 (1.6)		
Gly12Ser (GGT →AGT)	2 (0.9)	–	–	2 (1.0)		

[a]Clinical stage at the time of initial diagnosis was determined according to the 6th American Joint Commission on Cancer guideline.
[b]These four patients had double mutations in exon 19 (del 2235–2249)/exon 20 (T790M), exon 20 (T790M)/exon 21 (L858R), exon 20 (A871G)/exon 21 (L858R), and exon 21 (L858R/Leu833Val).

patients had a concurrent *EGFR* mutation (exon21 L858R) (supplementary Table S1, available at *Annals of Oncology* online). The frequency of *ROS1* rearrangement was 5.7% (6/105) among *EGFR/KRAS/ALK*-negative patients. When we carried out IHC on the FISH-positive tumors, all *ROS1*-FISH-positive cases also were *ROS1* positive in IHC (supplementary Figure S1, available at *Annals of Oncology* online).

identification of *ROS1* fusion partners by RT-PCR

We found *CD74-ROS1* fusions in two patients by RT-PCR and sequencing (supplementary Figure S2, available at *Annals of Oncology* online). No fusion partner was identified in the remaining five tumors. The latest update of *ROS1* fusion partners including our results is illustrated in supplementary Figure S3, available at *Annals of Oncology* online.

treatment outcomes of *ROS1*-rearranged lung adenocarcinoma

Table 2 summarized the treatment outcomes in 162 metastatic lung adenocarcinoma patients who received palliative chemotherapy. Single agent pemetrexed either in the second- or third-line setting was administered in a total of 82 patients (50.6%). The ORR to pemetrexed in *ROS1*-positive patients was higher than that in *WT/WT* (60.0% versus 8.5%; $P = 0.01$). EGFR-TKIs were administered in 120 patients as a second or third line. None had received *ALK* inhibitors previously. None of the patients with *ROS1* rearrangement ($n = 3$) had a clinical response to EGFR-TKIs. Similarly, there was no responder to EGFR-TKIs among patients with *ALK* rearrangement. The ORR to EGFR-TKIs in patients with *ROS1* rearrangement was numerically lower than that in *WT/WT*, although not statistically significant (0% versus 25.7%). These results might be due to a small sample size. With a median follow-up duration of 29.6 months, 82 (39.4%) of 208 patients were still alive at the time of analysis.

ROS1 rearrangement conferred a significantly longer median PFS with pemetrexed than *WT/WT* (not reached versus 3.3 months for *WT/WT*; $P = 0.008$; Figure 1B). However, patients with *ROS1* rearrangement showed significantly shorter median PFS to EGFR-TKIs than *WT/WT* (2.5 versus 7.8 months in *WT/WT*; $P = 0.01$; Figure 1C). Additionally, we compared the treatment outcome of EGFR-TKI in *ROS1*-positive patients with that of triple-negative for *EGFR/ALK/ROS1*. There was no difference in treatment outcome of EGFR-TKI in terms of ORR and PFS.

In a Cox-regression model adjusted for age, gender, *EGFR* mutation, and *ALK* rearrangement, the AHR for the risk of disease progression to pemetrexed was 0.09 ($P = 0.02$) for patients with *ROS1* rearrangement. This suggests that *ROS1*

Table 2. Summary of treatment outcomes by genotype in metastatic lung adenocarcinoma patients who received palliative chemotherapy

Variables	No (%)				*P*-value (*ROS1* versus *WT/WT*)	*P*-value (*ALK* versus *WT/WT*)
	All patients ($n = 162$)	*ROS1* positive[a] ($n = 5$)	*ALK* positive ($n = 13$)	*WT/WT* ($n = 144$)		
Type of treatment						
Platinum-based CT	133 (82.1)	5 (100)	9 (69.2)	119 (82.6)	0.64	0.48
Pemetrexed	82 (50.6)	5 (100)	6 (46.2)	71 (49.3)	0.11	0.58
EGFR-TKI	120 (74.1)	3 (60.0)	8 (61.5)	109 (75.7)	0.22	0.52
Line of platinum-based CT						
First line	133 (100%)	5 (100)	9 (100)	119(100)	–	–
Line of pemetrexed					0.96	0.89
First line	0 (0)	0 (0)	0 (0)	0 (0)		
Second line	33 (40.2)	2 (40.0)	2 (33.3)	29 (40.8)		
Third line and more over	49 (59.8)	3 (60.0)	4 (66.7)	42 (59.2)		
Line of EGFR-TKI					0.67	0.17
First line	4 (3.3)	0 (0)	1 (12.5)	3 (2.8)		
Second line	96 (80.0)	3 (100)	7 (87.5)	86 (78.9)		
Third line	20 (16.7)	0 (0)	0 (0)	20 (18.3)		
Best response to platinum-based CT						
ORR	29 (21.8)	2 (40.0)	0 (0)	27 (22.7)	0.33	0.20
DCR	107 (80.5)	5 (100)	6 (66.7)	96 (80.7)	0.58	0.39
Best response to pemetrexed						
ORR	11 (13.4)	3 (60.0)	2 (33.3)	6 (8.5)	0.01	0.12
DCR	54 (65.9)	5 (100)	4 (66.7)	46 (64.8)	0.16	0.62
Best response to EGFR-TKIs[b]						
ORR	28 (23.5)	0 (0)	0 (0)	28 (25.7)	0.56	0.20
DCR	80 (67.2)	0 (0)	1 (12.5)	79 (72.5)	0.08	0.001

[a]The one patient had *ROS1* rearrangement plus following *EGFR* mutations (exon21 L858R).

[b]The patient with concurrent *ROS1* rearrangement and *EGFR* mutation was not included in the response analysis in EGFR-TKI.

WT, wild type; CT, chemotherapy; EGFR, epidermal growth factor receptor; NS, not significant; TKIs, tyrosine kinase inhibitors; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate (CR + PR + SD).

rearrangement is a strong predictive factor for a longer median PFS to pemetrexed. The AHR for the risk of progression to the EGFR-TKIs was 2.40 ($P = 0.14$) for *ROS1* rearrangement, 0.56 ($P = 0.007$) for *EGFR* mutation, and 2.70 ($P = 0.02$) for *ALK* rearrangement (supplementary Table S2, available at *Annals of Oncology* online). These results indicate that *EGFR* mutations are a strong positive predictive factor for a longer median PFS after EGFR-TKI, whereas *ALK* rearrangements have a negative predictive impact. Furthermore, *ROS1* rearrangement may be a negative predictor for a longer PFS to EGFR-TKI, although statistical significance was not reached likely due to sample size limitation.

pemetrexed inhibits ROS1 activity and cell growth *in vitro*

We carried out a cell viability assay in various NSCLC cell lines using pemetrexed, gefitinib, crizotinib, and TAE684. Pemetrexed is highly sensitive, but gefitinib is resistant to *ROS1*-rearranged HCC78 and *ALK*-rearranged H3122. As the TS expression has been reported to be associated with the sensitivity to pemetrexed [10], we examined TS level in various cell lines. TS expression was rarely detected in pemetrexed-sensitive HCC78, H3122, PC9, and HCC827, while pemetrexed-resistant H358 and H1299 showed high TS expression (Figure 2). As shown in supplementary Figure S4, available at *Annals of Oncology* online, patients with *ROS1* or *ALK* rearrangement tend to show lower TS scoring than those with *WT/WT*.

discussion

We reported the frequency and treatment outcomes of *ROS1*-rearranged NSCLC from East-Asian never smokers. The enrichment of never smokers resulted in a higher frequency (3.4%) of *ROS1* rearrangement compared with that in unselected populations (0.9%∼1.7%), further supporting that *ROS1* rearrangements, together with *EGFR* mutations and *ALK* rearrangements, are the genetic alterations that are specific for LCINS [2, 3]. *ROS1* rearrangements were mutually exclusive with three major recurrent oncogenic mutations in LCINS, such as *EGFR* or *KRAS* mutation or *ALK* rearrangement, comprising a unique and nonoverlapping molecular subset of LCINS. The treatment outcome of *ROS1*-rearranged NSCLC was distinct from that of *WT/WT* tumors, but similar to that of *ALK*-rearranged NSCLC, which may suggest the biological similarity of *ROS1*- and *ALK*-rearranged NSCLC. To our knowledge, this is the first and the most comprehensive study reporting the frequency, clinicopathologic features, and treatment response at the same time in *ROS1*-rearranged lung adenocarcinomas from East-Asian never smokers.

Several studies have examined the frequency of *ROS1* rearrangement in NSCLC. Bergethon et al. [3] reported that *ROS1* rearrangements were enriched in Asians and never smokers. However, in the other studies that primarily involved Asian patients and/or never smokers, the frequency of *ROS1* rearrangement has been reported to range from 0.9% to 1.6%, which was similar to that reported in unselected population [6, 7, 11]. Potential explanations for the lower frequency of *ROS1* rearrangement in these studies may exist. In a report by Li et al., the authors examined *ROS1* rearrangement by RT-PCR which limited the detection of fusion partners to *CD74* and *SLC34A2* only. Rimkunas et al. [7] screened *ROS1* rearrangements using IHC assay from Chinese patients with unknown smoking-status. A study by Takeuchi et al. [6] investigated predominantly smokers. Recently, Cai et al. [12] reported that the *ROS1* rearrangements were found in ∼2.0% of Chinese patients and had worse survival outcome compared with *ROS1* negative. Unfortunately, most of the above studies did not concurrently analyze *EGFR* and *KRAS* mutations and *ALK* rearrangements, the three most frequently identified and clinically relevant genetic alterations in LCINS.

With the screening for *ROS1* rearrangements by using FISH and the enrichment of never smokers, we

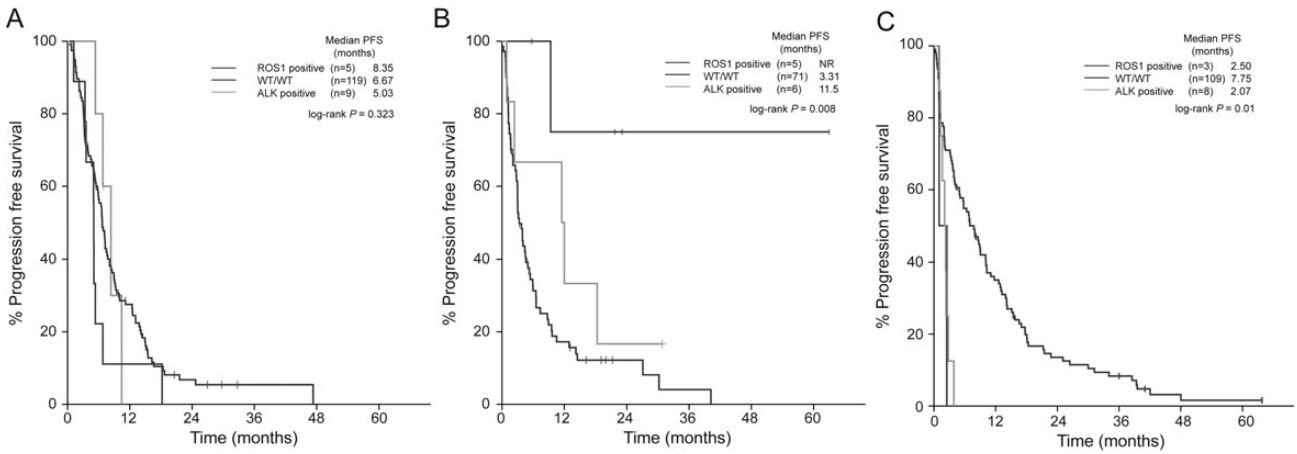
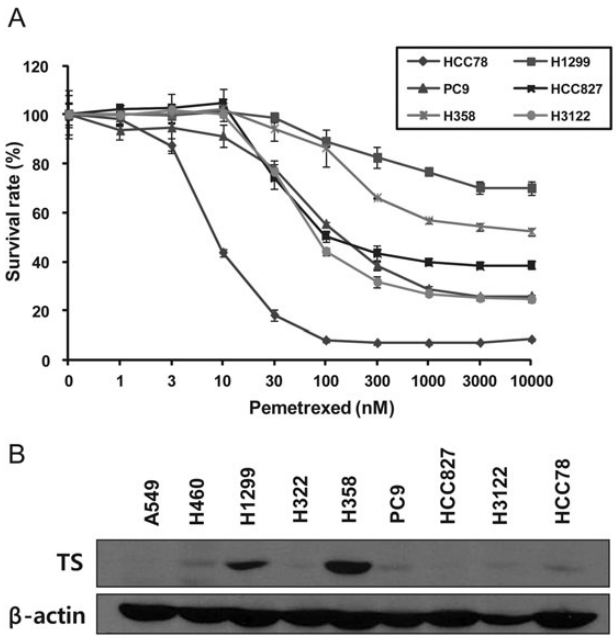


Figure 1. Progression-free survival (PFS) according to *ROS1* rearrangement in lung adenocarcinoma patients who received palliative chemotherapy. (A) PFS in patients treated with platinum-based chemotherapy: patients with *ROS1* rearrangement ($n = 5$), *ALK* rearrangement ($n = 9$), and *WT/WT* ($n = 119$) (B) PFS in patients treated with pemetrexed chemotherapy: patients with *ROS1* rearrangement ($n = 5$), *ALK* rearrangement ($n = 6$), and *WT/WT* ($n = 71$). (C) PFS in patients treated with EGFR-tyrosine kinase inhibitors (TKIs) treatment: patients with *ROS1* rearrangement ($n = 3$), *ALK* rearrangement ($n = 8$), and *WT/WT* ($n = 109$).



IC50 (μM)	Alimta	Crizotinib	TAE684	Gefitinib
HCC78	0.007	0.7	0.05	>10
H3122	0.077	0.125	0.0008	>10
PC9	0.143	2	0.63	0.0065
HCC827	0.1	3.5	0.9	0.001
H358	>10	2.4	2.1	>10
H1299	>10	1.75	1.1	>10

Figure 2. (A) Dose–response cell survival curves of *ROS1*-rearranged cell line (HCC78) and *ROS1*-negative cell lines in response to pemetrexed (nM); *ROS1*-rearranged cell line (HCC78) showed the high sensitivity to pemetrexed (IC_{50}: 0.007 μM) followed by *ALK*-rearranged H3122 (IC_{50} = 0.077 μM). On the other hand, gefitinib is resistant in HCC78 (IC_{50} > 10 μM) and H3122 cell line (IC_{50} > 10 μM). Crizotinib and TAE864 effectively inhibited the growth of HCC78 and H3122 cell lines. (B) In the western blot assay, TS expression was rarely detected in pemetrexed-sensitive HCC78, H3122, PC9, and HCC827, whereas pemetrexed-resistant H358 and H1299 showed high TS expression.

demonstrated that the frequency of *ROS1* rearrangements was 3.4%, suggesting that *ROS1* rearrangement is a druggable target in East-Asian never smokers with lung adenocarcinoma. Notably, the frequency of *ROS1* rearrangements was 5.7% among *EGFR/KRAS/ALK*-negative patients. Taken together, our data suggest that *ROS1* rearrangements were associated with never-smoking status, especially in patients who are negative for three major oncogenic mutations most frequently identified in LCINS [2].

In our study, *ROS1* rearrangement was associated with a different response and survival outcome after EGFR-TKIs and/or pemetrexed treatment. Patients with *ALK* and *ROS1* rearrangement had poorer outcomes after EGFR-TKIs. Intriguingly, we noted that patients with *ROS1* rearrangement had a significantly better ORR and median PFS on pemetrexed than those without *ROS1* rearrangement. Similarly, previous studies have shown that *ALK*-positive patients had significantly longer PFS on pemetrexed compared with *ALK* negative [13]. It was suggested that *ALK*-positive tumors had low level of TS, leading to high susceptibility to pemetrexed. Our study demonstrated that patients with *ALK* rearrangement tend to show favorable PFS to pemetrexed compared with *WT/WT* even with no statistical significance. The reason for discrepancies with previous data could be explained by following limitations of our study; retrospective data, small number of *ALK* positive, and treatment of pemetrexed as third line and more over. We also discovered low TS level and the highest sensitivity to pemetrexed in *ROS1*-positive HCC78, compared with other cell lines. The similar clinical characteristics might be related with the structural and functional homology between two genotypes. However, the underlying mechanism for the favorable response to pemetrexed is still unclear. Since this was a retrospective study and that the number of *ROS1* positive was small, we think that the sensitivity to pemetrexed of *ROS1*-positive patients should be cautiously interpreted.

To date, eight *ROS1* fusion genes including *CD74-ROS1*, *SLC34A2-ROS1*, *SDC4-ROS1*, *EZR-ROS1*, *FIG-ROS1*, *TPM3-ROS1*, *LRIG3-ROS1*, and *KDELR2-ROS1* have been identified. Among them, *CD74-ROS1* is the most common fusion partner in NSCLC [3, 5–7, 9, 11, 12, 14–16]. In our study, CD74 was found as a fusion partner in two patients. No fusion partner was identified in the other five cases, possibly due to insufficient tissue sample or poor quality of extracted RNA in FFPE. Regarding the *ALK* result in our study, the 7.2% prevalence and mutual exclusiveness with *EGFR* and/or *KRAS* mutation is very similar with recent data [17, 18].

In conclusion, ~3.4% of lung adenocarcinoma from East-Asian never smokers harbors *ROS1* rearrangement. Because of the different treatment outcomes and the existence of ROS1 inhibitors in this molecular subset, the identification of *ROS1* rearrangement before the initiation of treatment should be a routine practice in personalized therapy.

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disclosure

The authors have declared no conflicts of interest.

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