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ROS1 fusions in Chinese patients with non-small-cell lung cancer

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Background: To determine the prevalence and clinicopathological features of *ROS1* fusions in Chinese patients with non-small-cell lung cancer (NSCLC).

Methods: Formalin-fixed and paraffin-embedded (FFPE) tissue sections from 392 patients with NSCLC were screened for *ROS1* fusions by multiplex RT–PCR and all *ROS1* fusions were validated by direct sequencing. The relationship between *ROS1* fusions and clinicopathological features and the prognostic effect of the *ROS1* fusion status on survival were analyzed.

Results: In this study, 8 of 392 (2.0%) evaluable samples were found to harbor *ROS1* fusions. Of the *ROS1*-positive patients, seven presented with adenocarcinoma, and one with adenosquamous carcinoma. The ratio of female to male and never smoker to smokers in a *ROS1* fusion-positive group was 5:3. There was no statistically significant difference in age, sex, smoking history, histological type and pathological stage between *ROS1* fusion-positive and *ROS1* fusion-negative patients. *ROS1* fusion-negative patients had a significantly longer survival when compared with *ROS1* fusion-positive patients ($P = 0.041$). Lower pathological stage, younger age and *ROS1* fusion-negative status were significantly associated with better prognosis on multivariate analysis.

Conclusions: *ROS1* fusions occurred in ~2.0% of Chinese patients with NSCLC and had no specific clinicopathological feature. *ROS1* fusion-negative patients may have a better survival than *ROS1* fusion-positive patients.

Key words: fusion, non-small-cell lung cancer, oncogenic driver, *ROS1*, rearrangement

introduction

Lung cancer is the most common malignant tumor and the leading cause of cancer death worldwide, with ~1.6 million new cases and ~1.38 million deaths annually [1, 2]. With the identification of oncogenic drivers, small molecular tyrosine kinase inhibitors have been shown to be highly effective in patients with NSCLC expressing corresponding oncogenic drivers [3–7]. Like *EGFR* mutation [3, 4, 8] and *EML4-ALK* fusions [9–11], *ROS1* fusions are shown to be essential for lung cancer development and serve as an effective therapeutic target of crizotinib (PF02341066, Xalkori, Pfizer) in subgroups of populations [12, 13].

ROS1 (also known as *ROS*, *MCF3* or c-ros-1) is a proto-oncogene highly expressed in a variety of tumor cell lines [14], and a member of sevenless subfamily of tyrosine kinase insulin receptor genes. *ROS1* protein is a type I integral membrane protein with tyrosine kinase activity and may serve as a growth or differentiation factor receptor [15]. The *ROS1* rearrangement rendering a constitutively active tyrosine kinase was first discovered in glioblastoma in 1987 [14], then in NSCLC in 2007 [16], and in cholangiocarcinoma and ovarian cancer in 2011 [17, 18]. The patterns of *ROS1* fusion identified in lung cancer to date include *SLC34A2-ROS1*, *CD74-ROS1*, *TPM3-ROS1*, *SDC4-ROS1*, *EZR-ROS1*, *LRIG3-ROS1*, *FIG-ROS1*, *KDELR2-ROS1* and *CCDC6-ROS1*, with overall prevalence of 0.9–2.6% in NSCLC [6, 12, 13, 19–22]. A study found *ROS1* fusion-positive patients tended to be younger never smokers with a histologic diagnosis of adenocarcinoma [6]. However, there is no evidence strong enough to support this finding as far due to a very low frequency of *ROS1* fusions in NSCLC. Additionally, Davies et al. identified *ROS1* fusions in squamous cell carcinoma histology for the first time [13].

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Moreover, only two of five *ROS1* fusion-positive patients were never smokers. Therefore, the definitive clinicopathological features associated with the *ROS1* fusion status remain elusive, although some previous studies have investigated the frequency and clinical features of *ROS1* fusions.

Up to date, there were still some problems in the clinical application of fluorescence *in situ* hybridization (FISH), including high costs, difficulty in interpretation and expertise required, although FISH is the unique diagnostic approach approved to screen for *ALK* rearrangement in NSCLC by FDA. Besides that, immunohistochemistry (IHC) is a cheap and widely used assay for fusion proteins; however, there has been no preferred antibody for identification of *ROS1* fusion proteins to date. A large study Lungscape project conducted in Europe screened for *ALK* rearrangement using IHC and demonstrated a prevalence of *ALK* rearrangement of 7.3% [23]. Although a high concordance between *ALK* IHC 3+ and FISH-positive results has been demonstrated, FISH sensitivity was only 36.7% in *ALK* IHC 1+, 2+ and 3+ samples (data present in the 37th ESMO). In addition to FISH and IHC, multiplex RT–PCR is an alternative method used to identify gene fusion in NSCLC. A previous Chinese study [24] detected two *ROS1* fusion-positive cases from 202 East Asian never smokers with lung adenocaricnoma (LADC) using RT–PCR and sequencing, with a prevalence of 1%. However, the study just designed primers for *CD74-ROS1* and *SLC34A2-ROS1* at that time, so it is very possible that some *ROS1*-positive patients with other fusion patterns were missed. The RT–PCR assay is highly sensitive, relatively cheap and easily widespread, and can determine specific fusion partners, although it cannot identify unknown variants. We identified eight *ROS1* fusion-positive cases from 392 NSCLC patients using multiplex RT–PCR, with a prevalence of 2.0%. We characterized all *ROS1* fusion-positive cases to define the most likely population to benefit from specific targeted therapy.

methods

patients and tissue specimens

Formalin-fixed and paraffin-embedded (FFPE) tissue sections were collected from patients with histologically confirmed primary NSCLC who underwent resection or biopsy at the Shanghai Pulmonary Hospital from 2003 to 2010. Pathological diagnosis and staging was carried out according to the 2004 World Health Organization (WHO) classification and the tumor-node-metastasis staging system of the International Association for the Study of Lung Cancer (version 7). All FFPE tissue sections were reviewed by pathologists for confirmation of histology and assessment of tumor content. All clinical data were obtained from inpatient/outpatient medical records. Patients were classified into three groups according to the smoking status: never (0 pack year), light (<10 pack years), and smokers (≥10 pack years, including former smokers and current smokers). Survival analysis was carried out in patients who received at least one follow-up phone call or visit. All enrolled patients met the following inclusion criteria: written informed consent; aged ≥18 years; NSCLC histologically confirmed; sufficient FFPE tissue available for *ROS1* fusion screening and validation; the demographic data, including age, gender, smoking status, histological type and disease stage, were available for analysis. Patients did not receive pre-operative systemic or radiation therapy. This study was approved by the institutional review boards of the Shanghai Pulmonary Hospital.

RNA extraction and reverse transcription

Total RNA was extracted from 3 to 4 sections of 3 μm-thick FFPE tissue using a RNeasy FFPE kit (QIAGEN, Cat. no.73504). Then, total RNA was subject to reverse transcription using an AmoyDx® *ROS1* fusion gene detection kit (Amoy Diagnostics Co., Ltd, Xiamen, China). Total amount of RNA should be within 0.1–5 μg. Reverse transcription conditions were as follows: 42°C, 1 h; 95°C, 5 min. The resulting complementary DNA (cDNA) solutions are used for a multiplex RT–PCR.

multiplex RT–PCR and direct sequencing

To rapidly and high-efficiently identify *ROS1* fusions using small amounts of RNA extracted from archival FFPE sections, we used a multiplex RT–PCR to screen for these fusions in this study. The patterns of *ROS1* fusions screened in this study are shown in Table 1. All multiplex RT–PCRs were carried out using a Stratagene Mx3000P real-time PCR system (Strategene, CA) with an AmoyDx® *ROS1* fusion gene detection kit (Amoy Diagnostics Co., Ltd, Xiamen, China). An internal reference gene (β-actin) and *ROS1*-rearranged DNA were used as control. The PCR conditions were as follows: one cycle of 95°C for 5 min; 15 cycles of denaturation at 95°C for 25 s, annealing at 64°C for 20 s and elongation at 72°C for 20 s; 31 cycles of 93°C for 25 s, 60°C for 35 s (data collection) and 72°C for 20 s. All fusion-positive samples were validated using direct sequencing. The sequence primers used in this study are shown in supplementary Table S1, available at *Annals of Oncology* online. The PCR conditions for sequence primers were as follows: one cycle of 95°C for 5 min, followed by 50 cycles of denaturation at 90°C for 30 s, annealing at 50°C for 30 s and elongation at 72°C for 40 s, and the last cycle was extended by a 7 min elongation at 72°C.

statistical analysis

Categorical variables were compared using Fisher's exact test, and continuous variables were compared using the Mann–Whitney *U* test. The survival curve was plotted and median overall survival was calculated using the Kaplan–Meier method. Univariate and multivariate analyses were carried out using the Cox proportional hazard model. Overall survival was defined as the time from the date of resection or biopsy to the date of death. Patients alive or lost to follow-up were censored at the date of last follow-up. A log-rank test was used to compare the survival curves between *ROS1*-positive and *ROS1*-negative groups. The two-sided significance level was set at $P < 0.05$. All data were analyzed using the Statistical Package for the Social Sciences Version 17.0 Software (SPSS).

Table 1. The fusion patterns of *ROS1* screened in this study

Variant No.	Fusion partners for *ROS1*, exon	*ROS1* exon
1	*SLC34A2*, 4	32
2	*SLC34A2*, 13del2046	32
3	*CD74*, 6	32
4	*SDC4*, 2	32
5	*SDC4*, 4	32
6	*SLC34A2*, 4	34
7	*SLC34A2*, 13del2046	34
8	*CD74*, 6	34
9	*SDC4*, 4	34
10	*EZR*, 10	34
11	*TPM3*, 8	35
12	*LRIG3*, 16	35
13	*GOPC*, 8	35

results

patient characteristics

A total of 392 patients with histologically confirmed NSCLC who underwent resection or biopsy at the Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China, from 2003 to 2011 were consecutively enrolled in this study. All patients were of Chinese origin. Of these, 211 patients were male and 181 were female. The median age at diagnosis was 60 years. The number of patients with adenocarcinoma, adenosquamous and squamous cell carcinoma were 231 (58.9%), 37 (9.4%) and 119 (30.4%), respectively. The number of patients with stage I–IV disease were 169 (43.1%), 56 (14.3%), 135 (34.4%) and 32 (8.2%), respectively (shown in Table 2). The *ROS1* fusion status was evaluable in archival FFPE tissue sections from all patients.

ROS1 fusions

Eight out of 392 (2.0%) cases were found positive for the *ROS1* fusions by a multiplex RT–PCR, which were confirmed by direct sequencing. Among them, *SLC34A2-ROS1* fusion was found in four cases, *CD74-ROS1* fusion in three cases and *SDC4-ROS1* fusion in one case. The fusion pattern and clinicopathological features of all *ROS1* fusion-positive patients are shown in Table 3. Notably, a *SLC34A2-ROS1* fusion-positive case was validated to express two variants, S13(del); R32 and S13(del);R34. In the cDNA of these two variants, nucleotide position 568 of *SLC34A2* exon 13 is fused to *ROS1* exons 32 and 34, respectively. The validated sequencing results of *ROS1* fusion-positive cases are shown in Figure 1.

Table 2. Clinicopathological features of 392 patients with NSCLC

Features	All ($n = 392$)	*ROS1* rearrangement		
		Positive ($n = 8$)	Negative ($n = 384$)	*P* value
Age (years)				0.866
Median (range)	60 (27–83)	64 (44–80)	60 (27–83)	
<65	255 (65.1%)	5 (62.5%)	250 (65.1%)	
≥65	137 (34.9%)	3 (37.5%)	134 (34.9%)	
Sex				0.479
Male	211 (53.8%)	3 (37.5%)	208 (54.2%)	
Female	181 (46.2%)	5 (62.5%)	176 (45.8%)	
Smoking				1.0[a]
Never	244 (62.2%)	5 (62.5%)	239 (62.2%)	
Light smoker (<10PY)	6 (1.5%)	0	6 (1.6%)	
Smoker (≥10PY)	142 (36.2%)	3 (37.5%)	139 (36.2%)	
Histology				0.148[b]
Adenocarcinoma	231 (58.9%)	7 (87.5%)	224 (58.3%)	
Adenosquamous	37 (9.4%)	1 (12.5%)	36 (9.4%)	
Squamous cell	119 (30.4%)	0	119 (31.0%)	
Others[c]	5 (1.3%)	0	5 (1.3%)	
Stage				0.475[d]
Ia	34 (8.7%)	1 (12.5%)	33 (8.6%)	
Ib	135 (34.4%)	1 (12.5%)	134 (34.9%)	
IIa	8 (2.0%)	0	8 (2.1%)	
IIb	48 (12.2%)	4 (50.0%)	44 (11.5%)	
IIIa	107 (27.3%)	0	107 (27.9%)	
IIIb	28 (7.1%)	0	28 (7.3%)	
IV	32 (8.2%)	2 (25.0%)	30 (7.8%)	

[a]Never/light smokers versus smokers.
[b]Adenocarcinoma versus non-adenocarcinoma (adenosquamous, squmaous cell and others).
[c]Others included neuroendocrine tumor and mixed tumor.
[d]Stage I/II versus stage III/IV.
PY, pack year.

clinicopathological features of *ROS1*-positive patients

Of eight *ROS1* fusion-positive patients, seven had adenocarcinoma, and one had adenosquamous carcinoma histology. Seven of 231 patients with LADC were positive for *ROS1* fusions, accounting for 3.03%. *ROS1* fusions appeared to be more common in adenocarcinoma histology. The median age at diagnosis was 64 years. The ratio of female to male and never smoker to smokers in a *ROS1* fusion-positive group was 5:3. There was no statistically significant difference in age ($P = 0.866$), sex ($P = 0.479$), smoking history ($P = 1.0$), histological type ($P = 0.148$) and pathological stage ($P = 0.475$) between *ROS1* fusion-positive and *ROS1* fusion-negative patients.

clinical outcomes

Survival analyses were carried out in 384 (98.0%, 384 out of 392) patients who received at least one follow-up phone call or visit, with the longest follow-up time of 108 months. For patients available for survival analysis, a total of 202 (52.6%, 202 out of 384) death events occurred during the follow-up period, including 196 (52.1%, 196 out of 376) in *ROS1*-negative patients and 6 (75%, 6 out of 8) in *ROS1*-positive patients. The median overall survival for *ROS1* fusion-negative and *ROS1* fusion-positive patients were 53.9 (95%CI, 43.3–64.6 months) and 32.2 (95% CI, 13.3–51.2 months) months, respectively. *ROS1*-negative patients had a significantly longer overall survival than *ROS1*-positive patients, with a *P* value of 0.041. The survival curve is shown in Figure 2. Univariate analysis identified age <65 years ($P = 0.011$), female ($P = 0.023$), low pathological stage ($P < 0.001$) and *ROS1* fusion-negative status ($P = 0.047$) as being significantly associated with longer survival. Additionally, never/light-smoking history showed a strong trend toward better overall survival, without reaching statistical significance ($P = 0.072$). Multivariate analysis identified low pathological stage ($P < 0.001$) as being the strongest independent prognostic factor for better survival. Other independent factors affecting survival were age <65 years ($P = 0.004$) and *ROS1* fusion-negative status ($P = 0.01$) (Table 4).

discussion

In this study, we identified eight patients with NSCLC positive for *ROS1* fusions by multiplex RT–PCR using primers for 13 known variants, with the prevalence of 2.0%. Of these *ROS1* fusion-positive patients, seven had adenocarcinoma and one had adenosquamous carcinoma histology. Analysis indicated

Table 3. Clinicopathological characteristics of eight patients with *ROS1* fusion-positive NSCLC

Pt No.	Age (yr)	Sex	Smoking	Stage	Histology	Fusion Patterns	Survival status
1	65	Female	Never	IIb	Adenocarcinoma	*CD74-ROS1* (C6;R34)	Dead
2	44	Female	Never	IV	Adenocarcinoma	*CD74-ROS1* (C6;R32)	Dead
3	49	Female	Never	Ia	Adenocarcinoma	*CD74-ROS1* (C6;R34)	Alive
4	64	Female	Never	IIb	Adenocarcinoma	*SDC4-ROS1* (SD2;R32)	Alive
5	80	Male	Smoker	IIb	Adenocarcinoma	*SLC34A2-ROS1* (S4;R32)	Dead
6	67	Male	Smoker	IV	Adenocarcinoma	*SLC34A2-ROS1* (S4;R32)	Dead
7	50	Female	Never	IIb	Adenocarcinoma	*SLC34A2-ROS1* (S13del;R32) *SLC34A2-ROS1* (S13del;R34)	Dead
8	64	Male	Smoker	Ib	Adenosquamous	*SLC34A2-ROS1* (S4;R32)	Dead

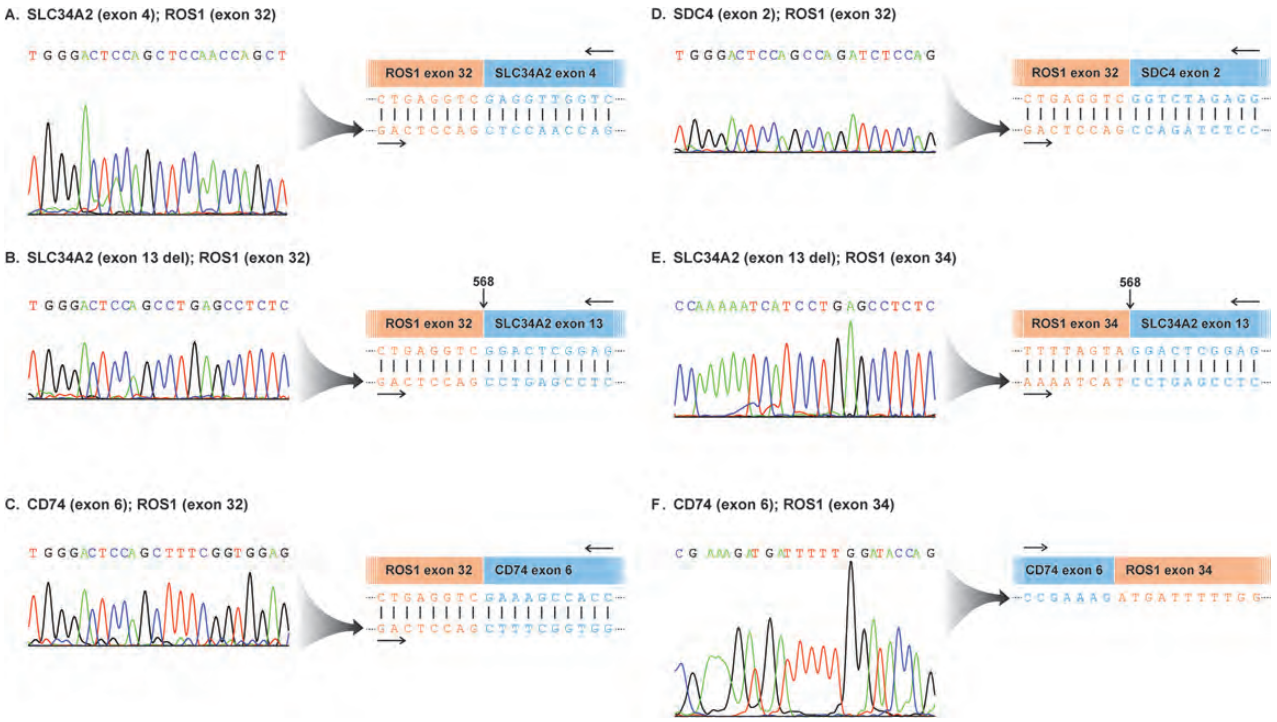

Figure 1. The variants of *ROS1* fusions identified by a multiplex RT–PCR were validated by direct sequencing. (A). S4;R32 in three cases, (B) S13del;R32 and (E) S13del;R34 in one case, (C) C6;R32 in one case, (D) SD2;R32 in one case, and (F) C6;R34 in two cases.

that no specific clinicopathological feature was significantly associated with the *ROS1* fusion status. *ROS1* fusion-negative patients had a significantly better overall survival than those harboring *ROS1* fusions, with a *P* value of 0.04.

We screened for known *ROS1* fusions, except *FIG-ROS1*, *KDELR2-ROS1* and *CCDC6-ROS1*, in NSCLC using a multiplex RT–PCR, and identified *SLC34A2-ROS1* fusion in four cases, *CD74-ROS1* fusion in three cases and *SDC4-ROS1* fusion in one case. The former two appeared to occur in NSCLC with higher frequency when compared with other fusion patterns. A total of nine known fusion partners for *ROS1* were identified in NSCLC at present. Among them, *SLC34A2* and *CD74* were the first to be found and the most investigated partners as far. Then, Takeuchi et al. identified additional four fusion partners for *ROS1* in NSCLC: *TPM3*, *SDC4*, *EZR* and *LRIG3* [12]. Lately, *FIG-ROS1*, *KDELR2-ROS1* and *CCDC6-ROS1* fusions were originally described in NSCLC by Rimkunas et al., Govindan et al. and Seo et al., respectively [20–22]. Moreover, Davies et al. observed a more potent inhibition of crizotinib in Ba/F3 cells transduced with *SDC4-ROS1* (SD2;R32) compared with HCC78 cells (a NSCLC cell line harboring *SLC34A2-ROS1*), with the IC50 values of 31 and 775 nmol/l, respectively. The difference in sensitivity to crizotinib between *SDC4-ROS1* expressing Ba/F3 cells and HCC78 cells may be partially due to fusion partner identity. Besides that, the scholars also believed that reduced sensitivity to *ROS1* inhibitor may be partially attributed to *EGFR* bypass activation [13]. It is very important to be aware of the discrepancy in frequency and sensitivity to *ALK/ROS1* inhibitors between different *ROS1* fusion patterns and is very essential to determine the definitive patterns of *ROS1* fusions in clinical practice. The finding from this study is consistent

with most previous studies [6, 13, 20], although we cannot draw a hasty conclusion for the frequency and identity of each fusion pattern on the basis of such small number of *ROS1* fusion-positive cases.

ROS1 fusions were shown to occur in eight NSCLC patients in our study, with the prevalence of 2.0% which was slightly higher than some previous reports. Bergethon et al. identified *ROS1* fusion in 1.7% (18 out of 1073) of the NSCLC patients using FISH. Of 14 *ROS1* fusion-positive cases with sufficient tissue, 5 were found to harbor *CD74-ROS1*, 1 to harbor *SLC34A2-ROS1*, and 8 were still unknown by RT–PCR with a panel of PCR primers including *CD74-ROS1* (C6;R32/34) and *SLC34A2-ROS1* (S4;R32/34) [6]. Then, Takeuchi et al. identified 13 *ROS1* fusion-positive cases from 1476 NSCLC patients using FISH, with the prevalence of 0.9% (including *CD74-ROS1* in three cases, *SLC34A2-ROS1* in one, *SDC4-ROS1* in three, *TPM3-ROS1* in two, *EZR-ROS1* in two, *LRIG3-ROS1* in one and an unknown pattern in one) [12]. Similarly, 1.2% (5 out of 428) of evaluable NSCLC samples were found using FISH in a recent study by Davies et al. [13]. Recently, *ROS1* fusions were detected in 1.6% (9 out of 556) of Chinese patients with NSCLC using IHC by Rimkunas et al. [20]. There seems to be no significant difference in *ROS1* fusion expression between different ethnicities. Accordingly, we consider that the slightly high frequency of *ROS1* fusions in our study is at least partially attributed to different screening methods. Moreover, the definitive fusion patterns of *ROS1* cannot be determined using FISH. In our study, all *ROS1* fusion-positive samples identified by a RT–PCR were validated by direct sequencing. Therefore, we believe that the RT–PCR alone was capable of detecting samples with *ROS1* fusions and could be routinely applied in clinical practice.

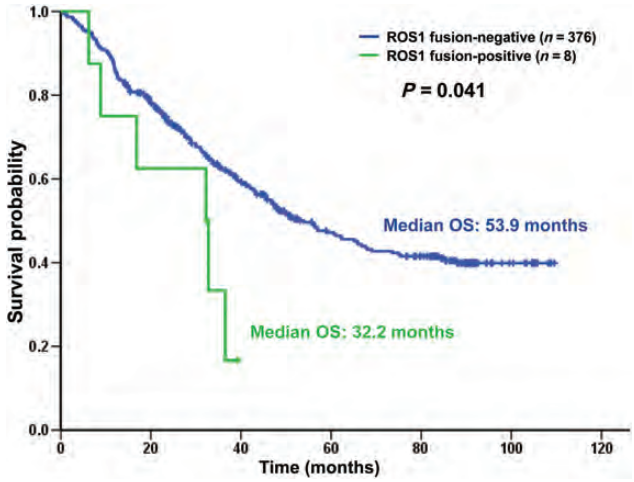

Figure 2. The comparison of overall suvival between *ROS1*-negative and *ROS1*-positive patients using the Kaplan–Meier method.

As opposed to a previous study by Bergethon et al., there is no statistically significant difference in age, smoking history and pathological stage in this study. Moreover, *ROS1* fusion-negative patients had a significantly better survival than *ROS1* fusion-positive patients (53.9 versus 32.2 months, $P = 0.041$) on survival analysis. A multivariate analysis identified three independent predictors for better prognosis: age <65 years, low pathological stage and *ROS1*-negative status. Takeuchi et al. demonstrated that negative kinase-fusion status was one of the independent predictors of poor prognosis on a multivariate analysis of 1116 patients with LADC containing 71 fusion-positive adenocarcinomas. However, only 13 of 71 harbored *ROS1* fusions and all patients assessed had adenocarcinoma histology [12]. Besides that, a study conducted by Lee et al. found that 22% of NSCLC were positive for *ROS1* expression and *ROS1* expression was an independent factor for worse prognosis in patients with stage I adenocarcinoma (HR: 1.942, 95% CI: 1.164–3.239, $P = 0.011$) on a multivariate analysis. Moreover, compared with primary NSCLC, *ROS1* expression occurred significantly more frequent in recurrent lesions (38% versus 19%, $P < 0.001$) [25]. Although we consider that *ROS1* fusions may be associated with poor survival in NSCLC patients, the low frequency of *ROS1* fusion-positive patients may have a potential impact on the statistical evaluation of this study. Therefore, the association between *ROS1* fusions and poor prognosis still needs a further study with a large sample size to confirm.

Of interest, we also identified *ROS1* fusions in 2.7% (1 out of 37) of patient with adenosquamous carcinoma. The frequency of *ROS1* fusions in patients with adenosquamous carcinoma here may not reflect the prevalence in general population of NSCLC patients based on a small number of *ROS1* fusion-positive samples. Besides that, no case from 119 patients with squamous cell carcinoma histology was found to harbor *ROS1* fusions in our study. A recent study by the Cancer Genome Atlas Research Network profiled 178 lung squamous cell carcinomas and identified a large number and variety of DNA

Table 4. Univariate and multivariate analyses of prognostic factors in patients with non-small-cell lung cancer (NSCLC)

Variables	Univariate		Multivariate	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age < 65 (versus ≥65)	1.439 (1.088–1.903)	0.011	1.489 (1.124–1.972)	0.004
Female (versus male)	1.397 (1.047–1.862)	0.023	1.301 (0.975–1.737)	0.058
Stage I/II (versus III/IV)	2.845 (2.146–3.773)	<0.001	2.947 (2.218–3.917)	<0.001
ROS1 negative (versus positive)	2.292 (1.012–5.192)	0.047	2.569 (1.259–5.241)	0.01
Never/light smokers (versus smokers)	1.292 (0.978–1.708)	0.072	–	–[a]
Non-LADC (versus LADC)	0.857 (0.649–1.131)	0.274	–	–

[a]Smoking status was not calculated as a prognostic factor for multivariate analysis because of correlation with gender.
LADC, lung adenocarcinoma.

alterations, including a mean of 165 genomic rearrangements per tumor. However, no recurrent rearrangement predicted to generate fusion proteins was detected [26]. In fact, gene fusions, including *EML4-ALK* and *RET* fusions (data not shown), have been identified in lung squamous cell carcinoma. Additionally, two *ROS1* fusion-positive patients with squamous cell carcinoma were identified in 428 assessable NSCLC patients by Davies et al. [13]. We also have demonstrated that *ROS1* fusions do not occur exclusively in LADC. However, for the cases with adenosquamous carcinoma histology, we cannot determine whether the occurrence of *ROS1* fusions is attributed to the presence of the adenocarcinoma component or squamous component. No *ROS1* fusion-positive sample with squamous cell carcinoma identified may be due to the relatively small number of samples with squamous cell carcinoma histology.

In conclusion, the prevalence of *ROS1* fusions in NSCLC is low, even in Chinese patients. The *ROS1*-negative status is one of the three independent factors that are indicators of better prognosis. Additionally, with further research on fusion gene, the fusion patterns of *ROS1* become increasingly complex. However, do all patterns of *ROS1* fusions identified in NSCLC serve as an oncogenic driver and have a similar sensitivity to *ALK*/*ROS1* inhibitors? There is no definitive answer so far. Given the demonstrated good clinical efficacy of crizotinib in the treatment of NSCLC harboring *ROS1* fusions, our study provides new insights into the definition of the NSCLC population most likely to benefit from crizotinib in the absence of a standard diagnostic approach for NSCLC harboring *ROS1* fusions at present. An expanded study on *ROS1* fusions in Chinese NSCLC patients is ongoing.

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disclosure

The authors have declared no conflicts of interest.

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