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ORIGINAL ARTICLE

Clinicopathological characteristics and outcomes of *ROS1*-rearranged patients with lung adenocarcinoma without *EGFR*, *KRAS* mutations and *ALK* rearrangements

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Abstract

Background: c-ros oncogene 1 (*ROS1*) rearrangement presents one of the newest molecular targets in non-small cell lung cancer (NSCLC). *ROS1* rearrangement is predominantly found in adenocarcinoma cases and is exclusive to other oncogenes, such as epidermal growth factor receptor (*EGFR*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*), and anaplastic lymphoma kinase (*ALK*). The aim of this study was to investigate the clinicopathological characteristics and outcomes of *ROS1*-rearranged patients with lung adenocarcinoma without *EGFR* and *KRAS* mutations and *ALK* rearrangements.

Methods: Wild-type *EGFR*/*KRAS*/*ALK* patients with lung adenocarcinoma were selected from Beijing Chest Hospital. Specimens were conducted in tissue microarrays. *ROS1* rearrangement was screened using fluorescence in situ hybridization.

Results: Our study included 127 patients with lung adenocarcinoma without *EGFR* and *KRAS* mutations and *ALK* rearrangements. *ROS1* rearrangement was detected in five (3.9%) of the 127 patients. Compared with *ROS1*-negative patients, the positive rate of *ROS1* in female patients was significantly higher than in male patients (9.8% vs. 0.0%, $P = 0.009$). There were no differences in age, smoking status, stage or histological subtype between *ROS1*-positive and *ROS1*-negative patients. No significant difference in survival was detected between the *ROS1*-positive and *ROS1*-negative patients.

Conclusions: *ROS1* rearrangement is a rare subset of lung adenocarcinoma. In 127 patients with lung adenocarcinoma, 3.9% of *ROS1*-positive patients with wild-type *EGFR*/*KRAS*/*ALK* were found.

Instruction

c-ros oncogene 1 (*ROS1*, located at 6q22) is a receptor tyrosine kinase, which codes for messenger ribonucleic acid (mRNA) and mRNA then translates the protein. The *ROS1* fusion gene as a potential driver in non-small cell lung cancer (NSCLC) was discovered in 2007.[1] ROS1 fusion proteins activate downstream pathways, such as phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR), Janus kinase (JAK)/signal transducer and activator of transcription (STAT), and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK). *ROS1* defines a new molecular subset of NSCLC. The first large sample study conducted by Bergethon *et al.* demonstrated a 1.7% (18 of 1073) frequency of *ROS1* in the general population with NSCLC, predominantly in patients with adenocarcinomas, of younger age, or never-smokers.[2] Other studies have reported

that the prevalence of *ROS1* fusions in NSCLC varies from 0.9 to 3.7%.[3–7] Several gene fusion partners have been discovered, including SLC34A2, CD74, TPM3, SDC4, EZR, and LRIG3.[3] In general, oncogenic driver mutations are mutually exclusive. Several studies have also demonstrated that *ROS1* is mutually exclusive to other oncogenic driver mutations of lung cancer, such as *EGFR*, *KRAS*, *ALK*, and *RET*.[3,7]

In Bergethon *et al.*'s study, a *ROS1*-positive patient with bronchioloalveolar carcinoma treated with crizotinib experienced tumor shrinkage with a near complete response, demonstrating that patients with NSCLC with *ROS1* fusions may benefit from crizotinib treatment.[2] In phase I trial PROFILE 1001, crizotinib demonstrated dramatic anti-tumor activity with a high overall response rate (ORR, 56%) in *ROS1*-positive patients identified using fluorescence in situ hybridization (FISH).[8] Current methods for the detection of *ROS1* fusions are FISH, immunohistochemistry (IHC), and reverse transcriptase polymerase chain reaction (RT-PCR). FISH is currently the most effective diagnostic technology to detect chromosomal rearrangements in tumor tissue. FISH has been used in the diagnosis of *ROS1* rearrangement in lung cancer.[2,3,9]

In our study, we investigated the frequency, clincopathological characteristics, and outcomes of *ROS1*-rearranged patients in wild-type *EGFR*/*KRAS*/*ALK* lung adenocarcinoma.

Materials and methods

Patients

Patients who had been tested for *EGFR*, *KRAS*, and *ALK* status at the Beijing Chest Hospital, China, between 2005 and 2013, were selected. Patients without *EGFR* and *KRAS* mutations and *ALK* rearrangements were enrolled in the study. *EGFR* and *KRAS* status were tested using DNA sequencing, while *ALK* rearrangements were tested using FISH. Nonsmokers were those who had smoked <100 cigarettes in their lifetime. Tumor node metastasis (TNM) stage was assessed using the 7th edition of the American Joint Committee for Cancer (AJCC) staging system.[10] The histological subtype of lung adenocarcinoma was classified using criteria from the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS).[11] Responses were evaluated using standard Response Evaluation Criteria in Solid Tumors (RECIST).[12] Evaluation of response included complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). ORR included CR and PR. Progression-free survival (PFS) was calculated from the first day of treatment to the date of disease progression. Overall survival (OS) was calculated from the date of diagnosis to the date of death as a result of any cause. This study was given formal approval by the institutional review board of the Peking Union Medical College Hospital and the Beijing Chest Hospital.

Methods

Formalin-fixed paraffin-embedded (FFPE) specimens were conducted in tissue microarrays (TMA) containing 2-mm-diameter three cores for each patient. Several TMAs used in this study were from a research published in the Journal of Cancer Research and Clinical Oncology.[13] Eighty-eight surgical samples and 52 biopsy tissues from metastatic lymph nodes were used in TMAs. FISH was performed on 4-μm-thick slides of FFPE TMA with break apart FISH probes for ROS1 (Vysis LSI ROS1 [Tel] SpectrumOrange and LSI ROS1 [Cen] SpectrumGreen Probe kit, Abbott Molecular, Chicago, IL, USA) according to the manufacturer's instructions on ThermoBrite Elite (Leica, Richmond, CA, USA). At least 100 tumor cells were scored. A specimen was defined as a *ROS1*-positive tumor if >15% of tumor cells showed a split signal. Two pathologists assessed the results of FISH under an Olympus fluorescence microscopy (Tokyo, Japan) equipped with orange/green/4′, 6-diamid -ino-2-phenylindole filters. Images were captured using the VideoTesT Image analysis system (Saint Petersburg, Russian Federation).

Statistical analysis

The Fisher's exact test was used for analysis on the association of *ROS1* rearrangement with clinicopatholgoical characteristics. Continuous data was analyzed by Wilcoxon rank sum test. The Kaplan–Meier method was used to estimate PFS and OS, and the difference between groups was compared using the log-rank test. SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) was used for all data analysis. All *P*-values were two-tailed and $P < 0.05$ was considered statistically significant.

Results

Patients

A total of 140 patients with lung adenocarcinoma with wild-type *EGFR*/*KRAS*/*ALK* status were enrolled and *ROS1* testing was performed using FISH. The results for 13 patients could not be included because of FISH testing failure or FFPE quality; 127 patients' data were available for evaluation. Of the 127 patients, the median age was 61 years (range: 26–82); 76 patients (59.8%) were men; 67 patients (52.8%) were nonsmokers; 65 patients (51.2%) were in advanced disease; and 75 (59.1%) patients had an acinar subtype. The characteristics of the 127 patients are shown in Table 1.

Table 1 Basic characteristics of 127 patients with lung adenocarcinoma

Characteristic	*N* (%)
Age, years	
Median	61
Range	26–82
Gender	
Male	76 (59.8)
Female	51 (40.2)
Smoking status	
Non-smokers	67 (52.8)
Smokers	60 (47.2)
Stage	
IA	15 (10.2)
IB	5 (3.9)
IIA	5 (3.9)
IIB	4 (3.1)
IIIA	33 (24.4)
IIIB	20 (15.7)
IV	45 (36.2)
Histologic subtype	
Lepidic predominant	1 (0.8)
Acinar predominant	75 (59.1)
Papillary predominant	21 (16.5)
Micropapillary predominant	8 (6.3)
Solid predominant	16 (12.6)
Invasive mucinous adenocarcinoma	4 (3.1)
Colloid variant	2 (1.6)

c-ros oncogene 1 rearrangement

Of the 127 patients, five (3.9%) were *ROS1*-positive and 122 (96.1%) were *ROS1*-negative. The median age of the *ROS1*-positive patients was 53 years (range: 41–62) and the median age of the *ROS1*-negative patients was 62 years (range: 26–82). Although the median age of the *ROS1*-postitive patients was younger, there was no significant difference ($P = 0.114$). All five of the *ROS1*-positive patients were women. The frequency of *ROS1* rearrangement in the female patients was significantly higher than in the male (5/51, 9.8%; 0/76, 0.0%, $P = 0.009$). The five female patients were non-smokers, but there was no difference in smoking status between the two groups (5/67, 7.5%; 0/60, 0.0%, $P = 0.059$). Although the five female *ROS1*-positive patients were in advanced disease (one was stage IIIB and four were stage IV), no difference in *ROS1* rearrangement was found between patients with early stage (I-IIIA) and advanced stage (IIIB-IV) (0/62, 0.0%, 5/65, 7.7%, $P = 0.058$). The histological subtype of the five female *ROS1*-positive patients was acinar predominant, in which one tumor contained signet cell features. There was no difference in the frequency of *ROS1* rearrangement in the acinar subtype compared with the non-acinar subtype (5/75, 6.7%; 0/52, 0.0%, $P = 0.078$). The association of clinicopathological characteristics of *ROS1* rearrangement is shown in Table 2. Figure 1 shows the images of *ROS1* rearrangement using FISH.

Table 2 Association of *ROS1* rearrangement with clinicopathological characteristics

	ROS1-positive		*ROS1*-negative		
Variable	*N* = 5	%	*N* = 122	%	*P*
Age, years					
Median	53		62		0.114
Range	41–62		26–82		
Gender					
Male	0	0.0	76	62.3	0.009
Female	5	100.0	46	37.7	
Smoking status					
Non-smokers	5	100.0	62	50.8	0.059
Smokers	0	0.0	60	49.2	
Stage					
I-IIIA	0	0.0	62	50.8	0.058
IIIB-IV	5	100.0	60	49.2	
Histologic subtype					
Acinar	5	100.0	70	57.4	0.078
Non-acinar	0	0.0	52	42.6	

ROS1, c-ros oncogene 1.

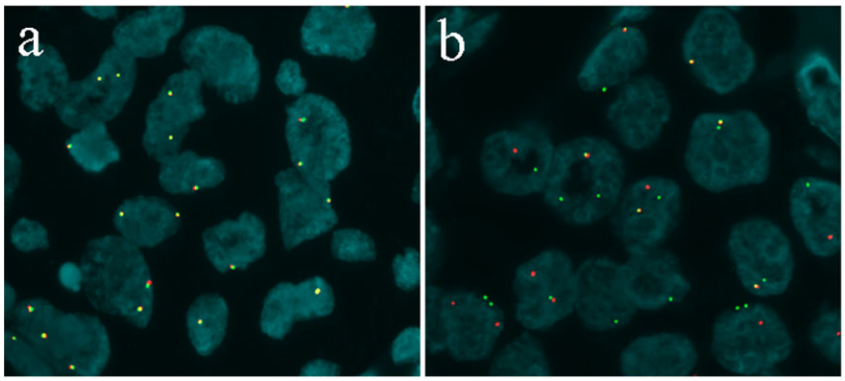

Figure 1 Images of c-ros oncogene 1 (*ROS1*) rearrangement using fluorescence in situ hybridization (FISH) (1000×). (**a**) A *ROS1*-negative tumor with intact signals; (**b**), a *ROS1*-positive tumor with split signals.

Table 3 Response and survival of patients according to genotypes

	n	*ROS1* positive	*ROS1* negative	*P*
No. of patients evaluated in first line chemotherapy	56	3	53	
CR		0 (0.0)	0 (0.0)	
PR		1 (33.3)	11 (20.8)	
SD		2 (66.7)	25 (47.2)	
PD		0 (0.0)	17 (32.1)	
ORR		1 (33.3)	11 (20.8)	0.586
PFS, month (95% CI)		7.8 (2.039–13.561)	3.5 (2.686–4.314)	0.200
No. of patients evaluated in any-line TKIs therapy	27	2	25	
CR		0 (0.0)	0 (0.0)	
PR		0 (0.0)	2 (8.0)	
SD		0 (0.0)	10 (40.0)	
PD		2 (100.0)	13 (52.0)	
ORR		0 (0.0)	2 (8.0)	0.573
PFS, month (95% CI)		0.9	2.5 (1.031–3.969)	0.040
Overall survival, month (95% CI)		12.1 (3.297–20.903)	8.0 (4.720–11.280)	0.687

CI, confidence interval; CR, complete response; ORR, overall response rate; PD, progressive disease; PFS, progression-free survival; PR, partial response; ROS1, c-ros oncogene 1; SD, stable disease; TKIs, tyrosine kinase inhibitors.

Outcomes

Fifty-six patients received palliative chemotherapy, including three *ROS1*-positive patients and 53 *ROS1*-negative patients. Of the three *ROS1*-positive patients who received chemotherapy, one achieved PR and two achieved SD. Of the 53 *ROS1*-negative patients who received chemotherapy, 11 (20.8%) achieved PR, 25 (47.2%) SD, and 17 (32.1%) PD. There was no difference in the ORR between the *ROS1*-positive and negative patients (1/3, 33.3%; 11/53, 20.8%, $P = 0.586$). The median PFS of the three *ROS1*-positive patients was 7.8 months, compared with 3.5 months for the *ROS1*-negative patients ($P = 0.200$). The PFS of the two *ROS1*-positive patients who received a pemetrexed regimen in the second line was 2.0 and 4.5 months.

Of the 127 patients, 27 patients received epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) treatment, including two *ROS1*-positive patients (one patient received Gefitinib treatment in the first line and another patient received Erlotinib in the third line) and 25 *ROS1*-negative patients in all lines. One *ROS1*-positive patient who received gefitinib in the first line achieved PD, and PFS was 0.9 months. Another *ROS1*-positive patient who received erlotinib in the third line achieved PD, and PFS was 1.2 months. Of the twenty-five *ROS1*-negative patients who received TKIs, two achieved (8.0%) PR, 10 (40.0%) SD, and 13 (52.0%) PD. The ORR was 8.0% and the PFS for these patients was 2.5 months. There was no difference in the ORR (0/2, 0.0%; 2/25, 8.0%, $P = 0.573$) between the *ROS1*-positive and *ROS1*-negative patients. The *ROS1*-positive patients had significantly poorer PFS than the *ROS1*-negative patients (0.9 months vs. 2.5 months, $P = 0.040$) (Table 3). Figure 2 shows computed tomography scans of the chest at pretreatment and after treatment of the *ROS1*-positive patient who received gefitinib in the first line. The fifth *ROS1*-positive patient did not receive anti-tumor therapy.

Survival analysis was performed because all of the *ROS1*-positive patients were in advanced disease stage (IIIB or IV). The last follow-up was performed on 31 December 2013. Of the 65 patients with advanced disease, 63 (96.9%) patients had died and two (3.1%) had been lost to follow-up. The median OS of the 65 advanced stage patients was 8.0 months (95% confidence interval [CI] 5.313–10.687). The median OS of the five *ROS1*-positive patients was 12.1 months (range: 1.8–22.1 months). The median OS of the 60 *ROS1*-negative patients was 8.0 months (range: 0.6–37.4 months). There was no significant difference in the OS between the *ROS1*-positive and *ROS1*-negative patients (12.1 months, 95% CI 3.297–20.903; 8.0 months, 95% CI 4.720–11.280, $P = 0.687$) (Fig 3).

Discussion

In this study, *ROS1* rearrangement was detected in 127 patients with lung adenocarcinoma with *EGFR*/*KRAS*/*ALK* wild type using FISH. The *ROS1* positive rate was 3.9% (5 of 127). The frequency of *ROS1* rearrangement in women was significantly higher than in men ($P = 0.009$).

In previous studies, the frequency of *ROS1* rearrangement among an unselected NSCLC population was reported at 0.6–3% and 1.2–4.5% among patients with adenocarcinoma.[2,3,5,7,9,14–18] The data of these studies is shown in Table 4. The varying results maybe a result of the enrolled population and testing methods of different studies. In a selected population, Kim *et al*. reported that the frequency of the *ROS1* fusion gene in *EGFR*/*KRAS*/*ALK*-negative and

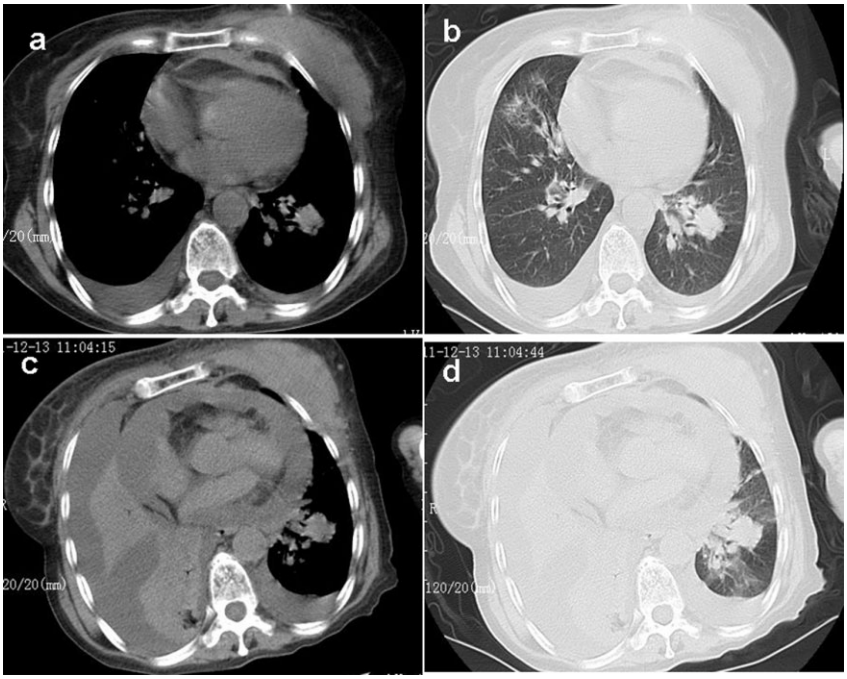

Figure 2 Computed tomography scans of the chest at pretreatment and after treatment in a c-ros oncogene 1 (*ROS1*)-positive patient who received gefitinib in first line therapy. (**a,b**) Pretreatment of gefitinib, (**c,d**) progression of disease after about one month.

never-smoking patients with lung adenocarcinoma from Korea was 5.7% (6 of 105).[6] Kim *et al.* reported 8.3% (5 of 60) of *ROS1* fusion in *EGFR*/*KRAS*/*ALK*-negative and non-smoking patients with lung adenocarcinoma.[19] Mescam-Mancini *et al.* screened the *ROS1* rearrangement in 121 triple *EGFR*/*KRAS*/*ALK* wild-type patients with lung adenocarcinoma and diagnosed 7.4% *ROS1* positive cases.[20] Our result was slightly lower than these studies, which may be related to the population studied and the sample size; for example, Kim *et al.* and Mescam-Mancini *et al.* enrolled never-smoking patients with the triple wild type.[19,20]

Bergethon *et al.* identified that patients with *ROS1*-rearranged tumors were predominantly patients with adenocarcinomas, of younger age, or never-smokers. This study reported 18 *ROS1*-positive tumors, of which seven tumors were acinar predominant subtype, five were papillary predominant, five were solid, and one was bronchioloalveolar carcinoma.[2] Cai *et al.* found that *ROS1* fusions had no specific clinicopathological feature.[14] Warth *et al.* reported that *ROS1* expression was found predominantly in women, at early tumor stages, in adenocarcinoma, and a distinct histomorphological growth pattern strongly facilitated case enrichment (lepidic, acinar, solid).[15] Go *et al.* also found that *ROS1* rearrangement occurred predominantly in women.[16] Yoshida *et al.* reported that *ROS1* was associated with non-smoking female patients, one-third of *ROS1*-positive NSCLC patients had a mucinous cribriform pattern, and one-third had a solid signet-ring structure.[7] In the present study, the frequency of *ROS1* rearrangement was significantly higher in women than in men, which was consistent with previous studies.[7,15,16] The histological subtype was predominantly acinar without any significant difference, which was also similar to previous studies.[2] There were no differences in smoking status or histological subtype in this study, possibly a result of the small sample size or population studied, which therefore warrants further study.

In the present study, no difference in the efficacy of chemotherapy was observed between the *ROS1*-positive and *ROS1*-negative patients. A small case study reported that NSCLC patients harboring *ROS1* rearrangements might show a significantly prolonged PFS from pemetrexed-based therapy.[21] In our study, the two *ROS1*-positive patients who received second line pemetrexed therapy had PFS of two and 4.5 months, which were not shorter than the routine data of second line chemotherapy. The exact efficacy of pemetrexed on *ROS1*-positve patients requires a large sample size study. In Bergethon *et al.*'s study, a *ROS1*-positive patient was treated with first-line erlotinib without response. Another study showed that EGFR-TKI treatment in patients with *ROS1* resulted in a significantly reduced PFS.[6] In accordance with previous studies, we observed that two of the five *ROS1*-positive patients did not receive any benefit from TKI treatment, with PFS rates of 0.9 and 1.2 months, which was significantly shorter than the 2.5 months of PFS in *ROS1*-

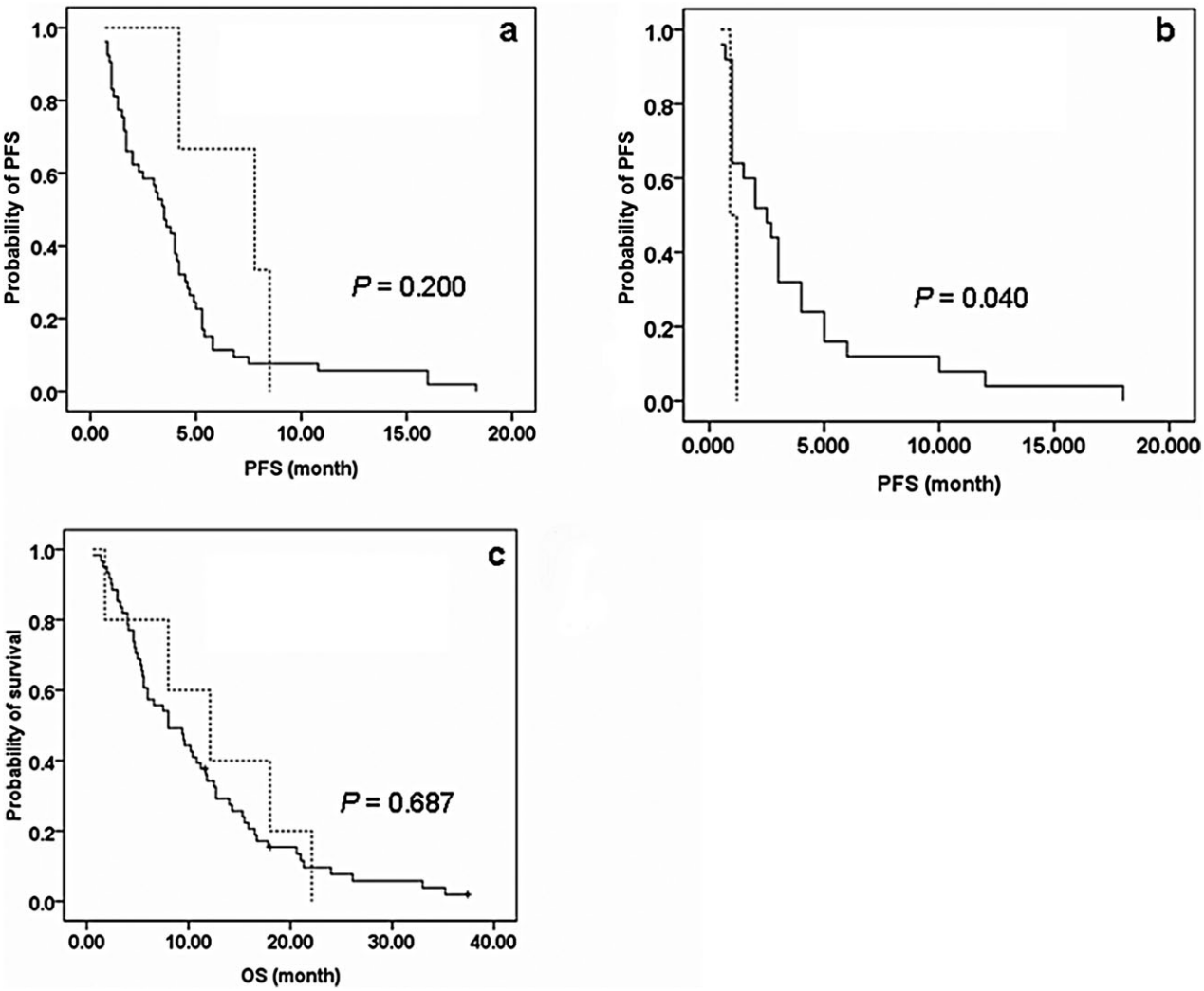

Figure 3 Kaplan-Meier curve of progression-free survival (PFS) of patients who received palliative chemotherapy and epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs); overall survival (OS) of advanced patients according to c-ros oncogene 1 (*ROS1*) status. (**a**) PFS of patients who received palliative chemotherapy in the first line. (**b**) PFS of patients who received EGFR-TKIs in all lines. (**c**) OS of advanced patients. ---, *ROS1* positive; ——, *ROS1* negative.

negative patients treated with TKIs ($P = 0.040$). These results demonstrate that *ROS1*-positive patients do not receive any benefit from EGFR-TKIs.

In an analysis of survival, Bergethon *et al.* reported that there was no difference in OS of *ROS1*-positive and *ROS1*-negative patients.[2] Yoshida *et al.* also reported that the OS rate of *ROS1*-positive patients was similar to *ROS1* fusion-negative cancer patients.[7] There was also no significant survival difference between the *ROS1* fusion-positive and *ROS1* fusion-negative cohorts in a surgical group study.[18] In our study, there was no significant difference in the survival between the *ROS1*-positive and *ROS1*-negative patients among the 65 advanced patients analyzed. Takeuchi *et al.* reported that negative fusion status (*ALK*, *ROS1*, and *RET*) was an indicator of poor prognosis.[3] However, Kim *et al.* reported that the disease-free survival time of *ALK* or *ROS1*-positive patients was significantly poorer than fusion-negative patients.[19] Cai *et al.* demonstrated that *ROS1* fusion-negative patients might have a better survival than *ROS1* fusion-positive patients.[14] The variation in results of survival outcomes may be a result of the small sample size of *ROS1*-positive patients. Although we found that *ROS1* rearrangement was not related to survival in patients with lung adenocarcinoma, its role in predicting survival is undetermined because of the low number of *ROS1*-positive cases. The prognostic value of *ROS1* in patients with lung adenocarcinoma requires further investigation with a larger number of cases with *ROS1* rearrangement.

Table 4 The frequency of *ROS1* rearrangement in previous studies

Author	*N*	Histology	Population	Method	Frequency of *ROS1* (%)
Bergethon *et al.*[2]	1073	NSCLC	Unselected	FISH	1.7
	694	Adenocarcinoma	Unselected	FISH	2.6
Cai *et al.*[14]	392	NSCLC	Unselected	RT-PCR	2
	231	Adenocarcinoma	Unselected	RT-PCR	3
Takeuchi *et al.*[3]	1476	NSCLC	Unselected	FISH	0.9
	1116	Adenocarcinoma	Unselected	FISH	1.2
Davis *et al.*[9]	428	NSCLC	Unselected	FISH	1.2
Warth *et al.*[15]	1478	NSCLC	Unselected	FISH	0.6
Yoshida *et al.*[7]	799	NSCLC	Unselected	RT-PCR	1.9
	569	Adenocarcinoma	Unselected	RT-PCR	2.5
Go *et al.*[16]	451	NSCLC	Unselected	FISH	1.8
	236	Adenocarcinoma	Unselected	FISH	3.4
Rimkunas[5]	556	NSCLC	Unselected	IHC	1.6
	246	Adenocarcinoma	Unselected	IHC	3.3
Cha *et al.*[17]	111	Adenocarcinoma	Unselected	FISH	4.5
Chen *et al.*[18]	492	Adenocarcinoma	Unselected	RT-PCR	2.4

FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer; ROS1, c-ros oncogene 1; RT-PCR, reverse transcriptase polymerase chain reaction.

Conclusion

In conclusion, *ROS1*-rearrangement presents a relatively rare subset of lung cancer. A 3.9% *ROS1*-positive rate was found in *EGFR*/*KRAS*/*ALK* wild-type patients with lung adenocarcinoma. A clearer understanding of the clinicopathological characteristics and outcomes of *ROS1*-positive patients may be achieved using a large sample size of *ROS1*-positive patients. Because of the promising response of crizotinib in *ROS1*-positive patients, detection of *ROS1*-rearrangement status is recommended in patients with wild-type *EGFR*/*KRAS*/*ALK*.

Disclosure

No authors report any conflict of interest.

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