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%. Several gene fusion partners have been discovered, including SLC34A2, CD74, TPM3, SDC4, EZR, and LRIG3. 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.

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. 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). 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.

In our study, we investigated the frequency, clinicopathological 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. Non-smokers 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. The histological subtype of lung adenocarcinoma was classified using criteria from the International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society/European Respiratory Society (ATS/ERS). Responses were evaluated using standard Response Evaluation Criteria in Solid Tumors (RECIST). 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. 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 microscope (Tokyo, Japan) equipped with orange/green/4’, 6-diamidino-2-phenylindole filters. Images were captured using the VideoTestT 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 clinicopathological 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 non-smokers; 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)        |
| II A                           | 5 (3.9)        |
| II B                           | 4 (3.1)        |
| III A                          | 33 (24.4)      |
| III B                          | 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**-positive 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.

![Figure 1](image_url)

**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 2 Association of **ROS1** rearrangement with clinicopathological characteristics

| Variable                  | **ROS1**-positive | **ROS1**-negative | $P$  |
|---------------------------|-------------------|-------------------|------|
| Age, years                | N = 5             | N = 122           |      |
| Median                    | 53                | 62                | 0.114|
| Range                     | 41–62             | 26–82             |      |
| Gender                    |                   |                   |      |
| Male                      | 0                 | 76                | 0.009|
| Female                    | 5                 | 46                |      |
| Smoking status            |                   |                   |      |
| Non-smokers               | 5                 | 62                | 0.059|
| Smokers                   | 0                 | 40                |      |
| Stage                     |                   |                   |      |
| I-IIIA                    | 0                 | 62                | 0.508|
| IIIB-IV                   | 5                 | 60                |      |
| Histologic subtype        |                   |                   |      |
| Acinar                    | 5                 | 70                | 0.078|
| Non-acinar                | 0                 | 52                |      |

**ROS1**, c-ros oncogene 1.
ROS1 in lung adenocarcinoma

| 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 | 0.586 |
| CR  | 0 (0.0) | 0 (0.0) | 0.573 |
| PR  | 1 (33.3) | 11 (20.8) | 0.040 |
| SD  | 2 (66.7) | 25 (47.2) | 0.200 |
| PD  | 0 (0.0) | 17 (32.1) | 0.040 |
| ORR | 1 (33.3) | 11 (20.8) | 0.040 |
| 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 | 0.586 |
| CR  | 0 (0.0) | 0 (0.0) | 0.573 |
| PR  | 0 (0.0) | 2 (8.0) | 0.573 |
| SD  | 0 (0.0) | 10 (40.0) | 0.573 |
| PD  | 2 (100.0) | 13 (52.0) | 0.573 |
| ORR | 0 (0.0) | 2 (8.0) | 0.573 |
| PFS, month (95% CI) | 0.9 | 2.5 (1.031–3.969) | 0.573 |
| 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 to be 0.6–3% and 1.2–4.5% among patients with adenocarcinoma. 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
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-positive 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 ROSI-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 ROSI-

Figure 2 Computed tomography scans of the chest at pretreatment and after treatment in a c-ros oncogene 1 (ROSI)-positive patient who received gefitinib in first line therapy. (a,b) Pretreatment of gefitinib, (c,d) progression of disease after about one month.
negative patients treated with TKIs \((P = 0.040)\). These results demonstrate that \(\text{ROS1}\)-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 \(\text{ROS1}\)-positive and \(\text{ROS1}\)-negative patients.\(^2\) Yoshida et al. also reported that the OS rate of \(\text{ROS1}\)-positive patients was similar to \(\text{ROS1}\) fusion-negative cancer patients.\(^7\) There was also no significant survival difference between the \(\text{ROS1}\) fusion-positive and \(\text{ROS1}\) fusion-negative cohorts in a surgical group study.\(^18\) In our study, there was no significant difference in the survival between the \(\text{ROS1}\)-positive and \(\text{ROS1}\)-negative patients among the 65 advanced patients analyzed. Takeuchi et al. reported that negative fusion status (\(\text{ALK}, \text{ROS1}, \text{and RET}\)) was an indicator of poor prognosis.\(^3\) However, Kim et al. reported that the disease-free survival time of \(\text{ALK}\) or \(\text{ROS1}\)-positive patients was significantly poorer than fusion-negative patients.\(^19\) Cai et al. demonstrated that \(\text{ROS1}\) fusion-negative patients might have a better survival than \(\text{ROS1}\) fusion-positive patients.\(^14\) The variation in results of survival outcomes may be a result of the small sample size of \(\text{ROS1}\)-positive patients. Although we found that \(\text{ROS1}\) rearrangement was not related to survival in patients with lung adenocarcinoma, its role in predicting survival is undetermined because of the low number of \(\text{ROS1}\)-positive cases. The prognostic value of \(\text{ROS1}\) in patients with lung adenocarcinoma requires further investigation with a larger number of cases with \(\text{ROS1}\) rearrangement.
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.

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

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

### 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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