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A Real-World Study: Therapeutic Outcomes of ROS1-Positive Advanced NSCLC

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ABSTRACT

Background: ROS1 gene rearrangement is an important target for NSCLC treatment. There is not yet sufficient real-world data on ROS1 diagnostic methods, treatment selection, and clinical outcomes in the Chinese population.

Methods: A single-center retrospective collection of patients with a diagnosis of ROS1-positive advanced NSCLC from July 2011 to November 2021 was performed to document the method of ROS1 testing, treatment options, efficacy, and resistance to ROS1 inhibitors in these patients.

Results: The method of ROS1 testing and initial treatment selection were significantly correlated with time. ROS1 testing shifted from FISH (67% pre-2019) to NGS (96.3% post-2019; $p < 0.001$). First-line ROS1-TKI use increased from 60.0% to 92.0% ($p = 0.041$). The vast majority of patients (90.0%) chose crizotinib as the initial ROS1 inhibitor, with objective response rates (for patients with target lesions) and median progression-free survival of 82.8% (95% CI: 68.1%–97.9%) and 18.7 months (95% CI: 8.9–28.4 months), respectively. CNS was the most common site of progression for crizotinib (60%, 13/26, including 11 intracranial progressions alone). Compared to patients who received a chemotherapy-based regimen ($n = 8$) as first-line therapy, patients who received ROS1-TKI ($n = 32$) as first-line therapy had significantly longer median PFS (18.3 months vs. 3.7 months, $p < 0.001$). For ROS1 inhibitor-resistant patients, 48.3% of patients underwent rebiopsy throughout the course of their disease, with G2032R being the most common secondary ROS1 mutation (7/8).

Conclusion: With the innovation of diagnostic and therapeutic methods and the expansion of the scope of the health insurance coverage, more and more patients are benefiting from new technologies and targeted drugs. Although crizotinib has brought excellent therapeutic data for ROS1-positive patients, better brain protection strategies should be explored for ROS1-positive patients in the future. In addition, the low rate of rebiopsy in real-world ROS1-positive patients should also be emphasized in clinical practice.

Abbreviations: ALK, anaplastic lymphoma kinase; ALK+, ALK-positive; CI, confidence interval.; CNS, central nervous system; FISH, fluorescence in situ hybridization; HR, hazard ratio; NGS, next-generation sequencing; NSCLC, nonsmall cell lung cancer; OS, overall survival; PFS, progression-free survival; RECIST 1.1, Response Evaluation Criteria in Solid Tumors version 1.1; ROS1, c-ros oncogene 1; ROS1+, ROS1-positive; RT-PCR, reverse transcription polymerase chain reaction.

Hanqi Yuan and Zihua Zou contributed equally to this study.

1 | Introduction

The c-ros oncogene 1 (ROS1) is a reconfigurable monomeric receptor tyrosine kinase; its rearrangement leads to downstream signal transduction dysregulation and abnormalities, which can serve as a carcinogenic driver oncogene mutation. In nonsmall cell lung cancer (NSCLC), the rearrangement of ROS1 is an important therapeutic target; its incidence in NSCLC is 1%–2%, commonly seen in young, nonsmoking lung adenocarcinoma patients [1]. The kinase domain of ROS1 shares a high degree of homology (over 70% similarity) with the kinase domain of anaplastic lymphoma kinase (ALK) [2]; therefore, many ALK inhibitors are also effective against the ROS1 target. As the first ROS1 inhibitor, crizotinib was approved by the US Food and Drug Administration (FDA) for the treatment of advanced ROS1-positive NSCLC due to its excellent therapeutic data demonstrated in the PROFILE 1001 study [3]. Subsequently, OO-1201 [4], EUROS-1 [5], and EUCROSS [6] studies showed the effectiveness of crizotinib in treating ROS1-positive NSCLC in different ethnic groups. Therefore, crizotinib is currently the most successful targeted drug in the ROS1 field. In recent years, with the rapid development of pharmaceutical technology, many new therapeutic drugs targeting ROS1 have emerged, including entrectinib [7], lorlatinib [8], repotrectinib [9], and taletrectinib [10]. However, before 2024, only crizotinib and entrectinib are listed and included in the scope of national medical insurance reimbursement. It is precisely because of the excellent efficacy of ROS1 inhibitors that current guidelines recommend that advanced nonsquamous NSCLC patients (especially lung adenocarcinoma) complete ROS1 gene testing before treatment, and for advanced ROS1-positive NSCLC patients, ROS1 inhibitor treatment should be initiated as early as possible. However, it should be noted that the accessibility of detection methods, detection prices, drug accessibility, and drug prices are several important factors that determine how clinicians diagnose and treat. In the real-world setting, especially in the Chinese population, there is no sufficient data on ROS1 detection methods, initial treatment options, and the CNS efficacy of crizotinib. In addition, there is also limited discussion on the progression pattern, execution of rebiopsy, and possible drug resistance mechanisms of ROS1 inhibitors. Therefore, we exploited single-center data to retrospectively analyze the ROS1 detection methods, treatment options, and clinical outcomes of advanced ROS1-positive NSCLC patients in China, as well as the progression pattern and possible drug resistance mechanisms.

2 | Methods

2.1 | Patient Selection

This was a single-center retrospective study in the real world. The research subjects were advanced NSCLC patients diagnosed with ROS1 fusion gene positive from July 2011 to November 2021. All patients had received treatment with ROS1 inhibitors. All patients completed imaging examinations at baseline and underwent imaging evaluations approximately every 3–4 months during follow-up. There were no specific exclusion criteria for this study.

2.2 | Data Collection

Basic information, clinical and pathological characteristics, ROS1 detection method, treatment selection, efficacy and progression pattern of ROS1 inhibitors, and rebiopsy results were recorded. The selection and imaging evaluation of target lesions were based on the RECIST 1.1 standard. Intracranial efficacy was also evaluated by RECIST 1.1 (namely at most two target lesions ($\geq$1 cm in MRI) could be chosen). The definition of CNS progressive disease was that for patients with a CNS target lesion, the sum of the largest diameter of the target lesion was increased by at least 20% and 5 mm in number, or a new CNS lesion was reported; for patients without a CNS target lesion, at least one nontarget lesion enlarged significantly (at least increased by 5 mm), or a new CNS lesion was found. The type of ROS1 fusion partner was confirmed either at baseline or at the time of disease progression. Survival information was obtained through medical records or telephone follow-up. The data cut-off date was December 12th, 2024. If a patient was lost to follow-up on December 12th, 2024, the data cut-off date for that patient will be the previous follow-up date. Besides, as crizotinib could be used both in advanced ALK+ and ROS1+ NSCLC, patients treated with crizotinib as the initial ALK inhibitor were also collected in this research so as to compare the efficacy of crizotinib and reveal possible distinct clinical outcomes in these two different populations.

2.3 | Definition of the Efficacy-Related Indicators

The efficacy-related indicators included objective response rate (ORR), progression-free survival (PFS), and CNS time to progression (CNS-TTP). ORR includes complete response (CR) and partial response (PR); PFS was calculated from the start date of a treatment regimen to the time of first imaging progression, and CNS-TTP is calculated from the start date of a treatment regimen to the time of first intracranial progression.

2.4 | Statistical Analysis

Statistical analysis was conducted using SPSS 26.0. Baseline characteristics were summarized using means and standard deviations for continuous data and frequencies and percentages for categorical data. The 95% confidence interval (CI) estimation method for ORR adopts the exact method. The comparison between categorical data uses Fisher's exact probability method, while the comparison between continuous data uses the *t*-test. Use the KM method to draw survival curves, and compare the overall survival curves of the two groups using the log-rank method. $p < 0.05$ is considered to have significant statistical differences.

2.5 | Methods of ROS1 Rearrangement Detection

Fluorescence in situ hybridization (FISH) is a cytogenetic technique that uses fluorescent probes to detect specific DNA sequences, including gene rearrangements. For ROS1 fusions, break-apart FISH is commonly employed, where probes flanking the ROS1 gene indicate rearrangement if the signals split

TABLE 1 | Baseline demographics ($N = 40$).

Characteristic	Value
Median age at the time of receiving the first ROS1 inhibitor, *y* (range)	53.2 (25–83)
Male sex, *n* (%)	12 (30%)
Female sex, *n* (%)	28 (70%)
Smoking, *n* (%)	
Nonsmoker	31 (77.5%)
Previous or current smoker	9 (22.5%)
ECOG PS, *n* (%)	
0	6 (15%)
1	24 (60%)
2	10 (25%)
Histologic type, adenocarcinoma, *n* (%)	40 (100%)
Number of previous systemic therapies, *n* (%)	
0	32 (80%)
1 or more	8 (20%)
Initial disease status, *n* (%)	
Distant metastases at first diagnosis	24 (60%)
Recurrence after surgery with distant metastasis	12 (30%)
Recurrence after radical radiotherapy with distant metastasis	4 (10%)
Metastasis, *n* (%)	
Extrathoracic	26 (65%)
Liver	3 (7.5%)
Bone	11 (27.5%)
CNS	8 (20%)
The initial method for detecting ROS1, *n* (%)	
NGS	27 (67.5%)
FISH	10 (25%)
RT-PCR	3 (7.5%)
Fusion pattern for ROS1, *n* (%)	
CD74-ROS1	17 (54.8%)
EZR-ROS1	4 (10%)
GOPC-ROS1	1 (2.5%)
LIRG3-ROS1	1 (2.5%)
RDX-ROS1	1 (2.5%)
SDC4-ROS1	4 (10%)
SLC34A2-ROS1	1 (2.5%)
SNN-ROS1	1 (2.5%)
TPM3-ROS1	1 (2.5%)
Unknown	9 (22.5%)
Initial ROS1 inhibitor, *n* (%)	
Crizotinib	36 (90%)
Brigatinib	1 (2.5%)
Ensartinib	1 (2.5%)
TQB-310	2 (5%)

apart. FISH is considered the historical gold standard for fusion detection due to its reliability in formalin-fixed paraffin-embedded (FFPE) samples. However, it cannot identify the specific fusion partner and may miss small intrachromosomal rearrangements. Reverse transcription polymerase chain reaction (RT-PCR) detects fusion transcripts by converting RNA into complementary DNA (cDNA) followed by PCR amplification using primers specific to known fusion partners. RT-PCR is highly sensitive for detecting common ROS1 fusions (e.g., CD74-ROS1) but requires prior knowledge of the fusion variants and may miss novel or complex rearrangements. It is less commonly used today due to the rise of NGS, but remains useful in resource-limited settings. Next-generation sequencing (NGS), also known as high-throughput sequencing, enables the parallel sequencing of millions of DNA fragments, allowing comprehensive genomic profiling. In ROS1 detection, NGS can identify fusion partners, single-nucleotide variants, and other structural variations with high sensitivity and specificity. Compared to traditional methods, NGS offers the advantage of detecting novel or rare fusion partners without prior knowledge of the breakpoints. However, it requires sophisticated bioinformatics analysis and may have higher costs in some settings.

3 | Results

3.1 | Baseline Clinical Characteristics

Forty patients in total were included in our study. All patients included in this study underwent comprehensive molecular profiling. There were no cases with concurrent driver mutations (e.g., EGFR/KRAS/ALK alterations). The baseline characteristics of the patients are shown in Table 1. The median age of the included patients at the time of receiving the first ROS1 inhibitor was 53.2 years (range 25–83 years), with 82.5% (33/40) of patients being under 65 years old. 77.5% (31/40) of patients had an ECOG score of 0–1 when receiving their first ROS1 inhibitor. All patients (40) had distant metastases upon receiving their first ROS1 inhibitor, with bone being the most common site of extrathoracic metastases (11/40). Twenty percent (8/40) of patients had CNS metastases upon receiving their first ROS1 inhibitor (all patients did not receive local treatment for intracranial lesions prior to the first ROS1 inhibitor).

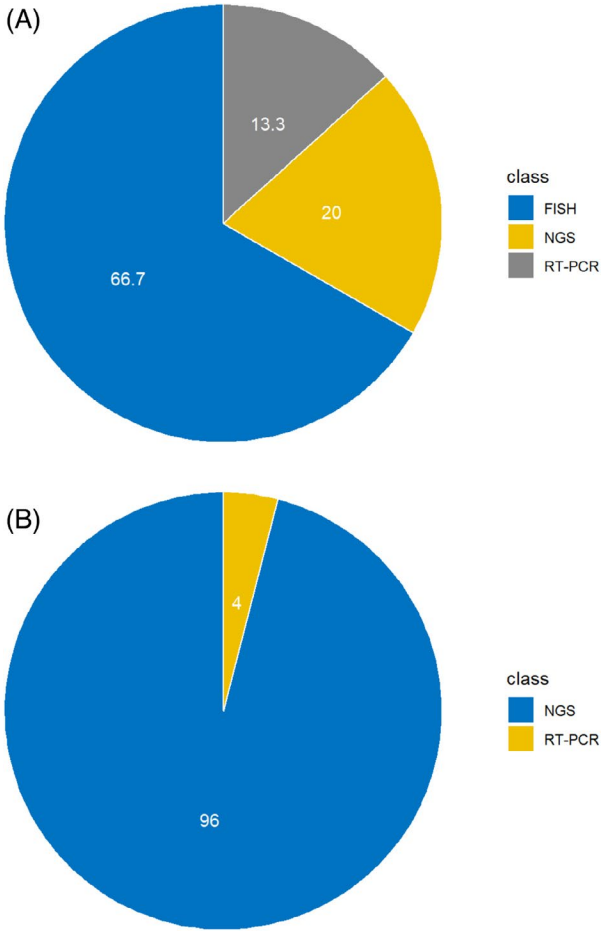

FIGURE 1 | The initial diagnostic method for ROS1. (A) Distribution of methods before 2019. (B) Distribution of methods after 2019.

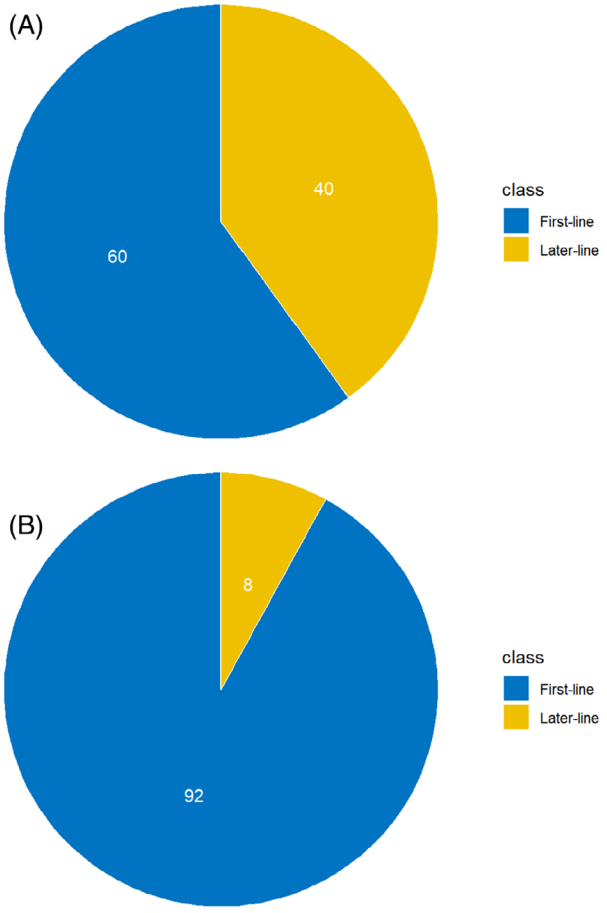

FIGURE 2 | Lines of treatment of ROS1 inhibitor. (A) Distribution of lines of treatment before 2019. (B) Distribution of lines of treatment after 2019.

3.2 | Diagnostic Methods for ROS1 in the Real World Setting

At the initial detection, most (67.4%, 27/40) patients rely on Next generation sequencing (NGS) technology to determine the genetic status of ROS1. Fluorescence in situ hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR) were used in 10 and 3 patients, respectively, for ROS1 detection. The detection method of ROS1 is significantly correlated with time; 15 patients were reported to carry the ROS1 fusion gene before 2019, with FISH being the most commonly used testing method (67%, 10/15) (Figure 1A); 25 patients were diagnosed after 2019, with NGS being the most commonly used detection method (96.3%, 24/25), and only 1 patient (3.7%) using RT-PCR as the detection method ($p < 0.001$) (Figure 1B). The vast majority of patients (37, 92.5%) had their ROS1 gene status identified before initiating systemic therapy, with only 3 patients having their ROS1 gene status identified after multiline therapy. Fusion partners were identified in 31 patients (25 patients identified at baseline and 6 patients identified at progression), with CD74 (17/31) and non-CD74 (14/31) fusion partners accounting for 54.8% and 45.2%, respectively. Among them, non-CD74 fusion partners included EZR (4/14), SDC4 (4/14), TPM3, SNN, LRIG3, GOPC, etc. (Table 1).

3.3 | Application and Efficacy of ROS1 Inhibitors

All patients were treated with ROS1 inhibitors during the course of their disease, and the choice of first-line treatment also correlated significantly with time. Only 60% (9/15) of patients diagnosed as ROS1-positive before 2019 received ROS1 inhibitors as first-line treatment (Figure 2A), whereas 92% (23/25) of patients after 2019 received ROS1 inhibitors as first-line treatment ($p = 0.041$) (Figure 2B). The vast majority of patients (90%, 36/40) chose crizotinib as their initial ROS1 inhibitor, with other choices including brigatinib ($n = 1$), ensartinib ($n = 1$), and TQB-3101 ($n = 2$) (Table 1).

Thirty-six patients used crizotinib as an initial ROS1 inhibitor, with an ORR for crizotinib = 66.7% (95% CI: 50.5%–82.8%), and in patients with target lesions ($n = 29$), an ORR for crizotinib = 82.8% (95% CI: 68.1%–97.9%). All 8 patients with baseline CNS metastases received crizotinib as initial ROS1 inhibitor. Among the 3 patients with evaluable intracranial lesions, the CNS-ORR was 100%. At the time of data cut-off, median follow-up for the 36 patients was 40.1 months (range 5.7–97.9 months) (calculated from the time of crizotinib initiation), PFS for crizotinib = 18.7 months (95% CI: 8.9–28.4 months) (Figure 3A), and CNS-TTP for patients with CNS baseline metastases treated with crizotinib = 20.7 months (95% CI: 15.8–25.5 months) (median follow-up: 39.0 months, range 24.8–82.8 months) (Figure 3B).

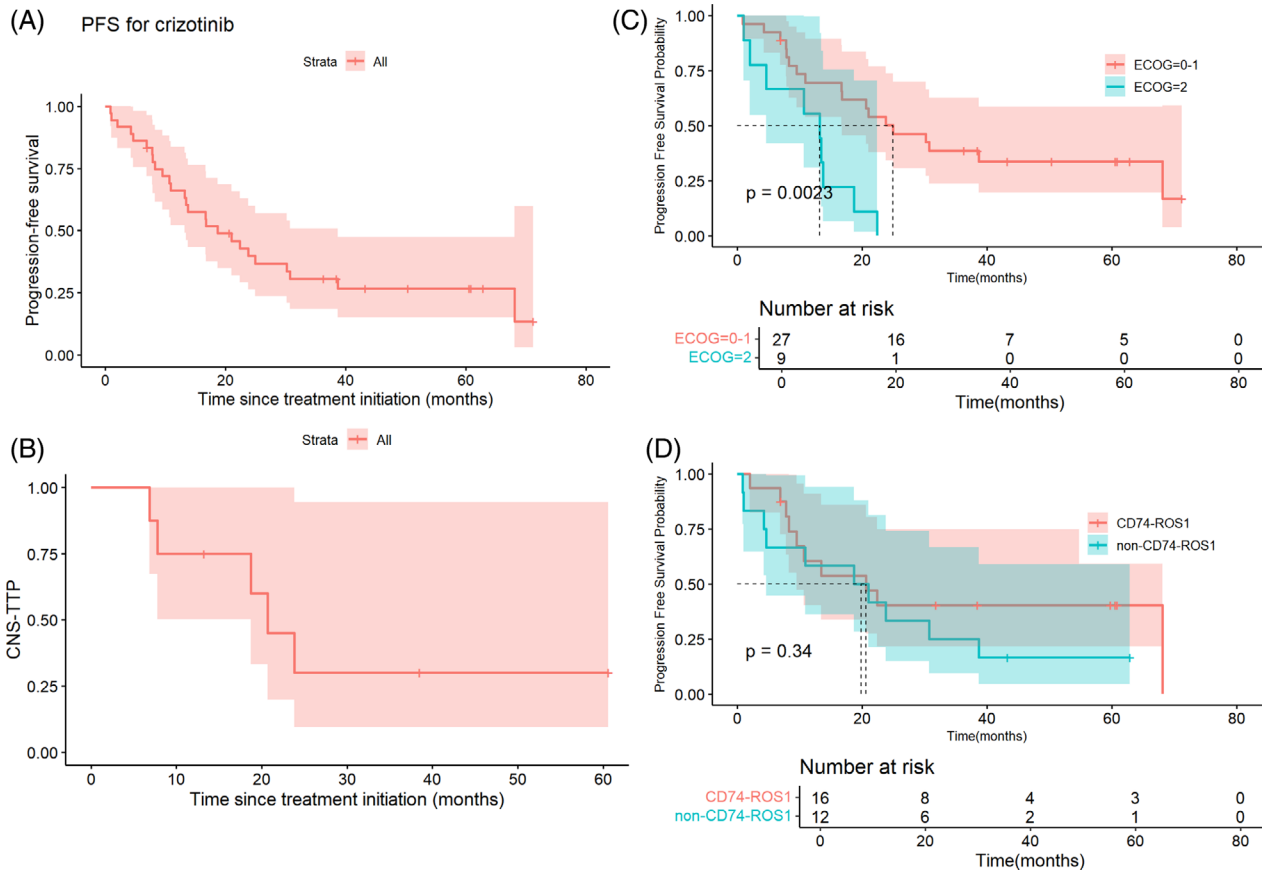

FIGURE 3 | Efficacy of ROS1 inhibitors. (A) PFS for patients receiving crizotinib as the first ROS1 inhibitor. (B) CNS TTP for patients with CNS metastases treated with crizotinib. (C) PFS comparison among patients with different physical status. (D) PFS comparison among patients with different fusion patterns.

The results of univariate analysis suggested that only performance status (ECOG PS) was associated with the efficacy of crizotinib, that is, patients with better performance status had a significantly better outcome of crizotinib (PFS ECOG 0-1: 35.9 months vs. ECOG ≥ 2: 11.1 months, $p = 0.02$, HR = 0.27, 95% CI: 0.11–0.66) (Figure 3C), whereas history of smoking, brain metastasis status, and number of lines of crizotinib were not associated with PFS (Table 1).

In numerical terms, patients who received crizotinib with CD74-ROS1 fusion had longer PFS than those with non-CD74-ROS1 fusion (CD74 fusion: 22.43 months vs. non-CD74 fusion: 18.70 months, $p = 0.340$, Figure 3D). Patients who received crizotinib with CD74-ROS1 fusion had a numerically superior ORR (only for patients with target lesions) than those with non-CD74-ROS1 fusion (92.9% vs. 70.0%, $p = 0.272$). However, neither of them had statistical significance. (Patients with CD74 fusion and patients with non-CD74 fusion were similar in baseline characteristics, Table 2).

3.4 | Comparison Between Chemotherapy and ROS1 Inhibitor as First-Line Therapy

Among these 40 patients, 8 (20%) did not receive ROS1-TKI as first-line therapy. Among the cohort, 3 patients initiated systemic therapy without confirmed ROS1 status, while 5 additional patients did not receive ROS1-TKI as first-line therapy due to drug accessibility issues. These 8 patients received chemotherapy or chemotherapy combined with anti-angiogenic therapy as their first-line therapy. As compared to these 8 patients, patients who received ROS1-TKI ($n = 32$) as first-line therapy had significantly longer median PFS (18.3 months ($n = 32$, 95% CI: 12.9–23.7 months) vs. 3.7 months ($n = 8$, 95% CI: 0.4–7.1 months), $p < 0.001$, Figure 4, HR = 0.215, 95% CI: 0.09–0.51).

3.5 | Comparison of Efficacy of Crizotinib in ROS1+ and ALK+ NSCLC

Crizotinib showed more favorable efficacy in metastatic ROS1+ NSCLC than in metastatic ALK+ NSCLC. For patients who received crizotinib as initial ROS1 or ALK inhibitors (ROS1: $n = 36$, ALK: $n = 36$), ALK+ NSCLC demonstrated shorter PFS compared with ROS1+ NSCLC (10.4 vs. 18.7 m, $p < 0.001$, Figure 5A, HR = 2.90, 95% CI: 1.70–5.03); CNS-TTP was also found to be significantly shorter in ALK+ NSCLC (for patients with baseline CNS lesions: 6.9 vs. 20.7 m, $p = 0.011$, Figure 5B, HR = 4.35, 95% CI: 1.29–14.63). (There was no significant difference in baseline disease burden between metastatic ROS1+ and ALK+ NSCLC, Table 3).

TABLE 2 | Comparison between baseline characteristics of patients with CD74 fusion and non-CD74 fusion.

	CD74-ROS1	Non-CD74-ROS1
Median age at the time of receiving the first ROS1 inhibitor, *y* (range)	50 (25–74)	56 (38–77)
Male sex	31.3% (5/16)	33.3% (4/12)
Female sex	68.7% (11/16)	66.7% (8/12)
Smoking		
Nonsmoker	75.0% (12/16)	75.0% (9/12)
Previous or current smoker	25.0% (4/16)	25.0% (3/12)
ECOG PS		
0	25.0% (4/16)	8.3% (1/12)
1	50.0% (8/16)	66.7% (8/12)
2	25.0% (4/16)	25.0% (3/12)
Histologic type, adenocarcinoma	100.0% (16/16)	100.0% (16/16)
Number of previous systemic therapies		
0	81.3% (13/16)	83.3% (10/12)
1 or more	18.7% (3/16)	16.7% (2/12)
Initial disease status		
Distant metastases at first diagnosis	62.5% (10/16)	58.3% (7/12)
Recurrence after surgery with distant metastasis	25.0% (4/16)	33.3% (4/12)
Recurrence after radical radiotherapy with distant metastasis	12.5% (2/16)	8.3% (1/12)
Extrathoracic metastases	81.3% (13/16)	50.0% (6/12)
CNS metastases	31.3% (5/16)	16.7% (2/12)
Multiple CNS metastases	40.0% (2/5)	100.0% (2/2)
Measurable CNS lesions	40.0% (2/5)	50.0 (1/2)
Liver metastases	18.8% (3/16)	0.0% (0/12)
Bone metastases	37.5% (6/16)	8.3% (1/12)
≥ 3 distant organs involved	43.8% (7/16)	16.7% (2/12)

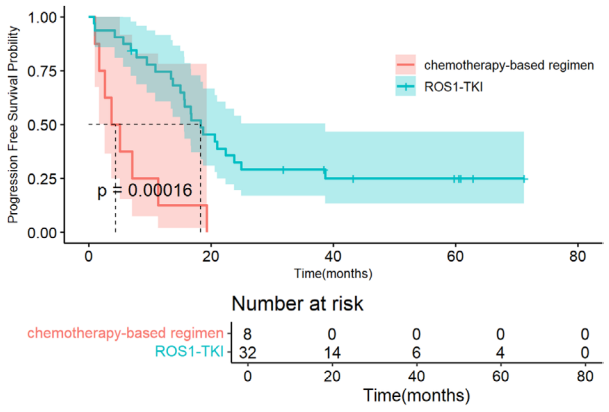

FIGURE 4 | Comparison of PFS of patients who received ROS1 inhibitor as first-line therapy and those who received chemotherapy-based regimen as first-line therapy.

3.6 | Patterns of Progression, Rebiopsy Status, and Mechanisms of Drug Resistance

Twenty-nine patients experienced disease progression following the treatment of initial ROS1 inhibitors (Table 4); 12 had intracranial progression alone, 15 had extracranial progression alone, and 2 had intracranial + extracranial progression. Among them, 26 patients received crizotinib as the initial ROS1 inhibitor, and more than half of the patients experienced intracranial progression after crizotinib treatment (11 of whom had intracranial progression alone and 2 of whom had intracranial + extracranial progression); for patients with baseline CNS metastases (6 patients), 83.3% (5/6) of the patients experienced intracranial progression with crizotinib treatment; for patients without CNS metastases before treatment (20/29), 40% (8/20) developed intracranial progression upon crizotinib; similar proportions of CD74-ROS1 and non-CD74-ROS1 fusions treated with crizotinib developed intracranial progression (31.3% vs. 33.3%, $p = 0.656$, Table 1).

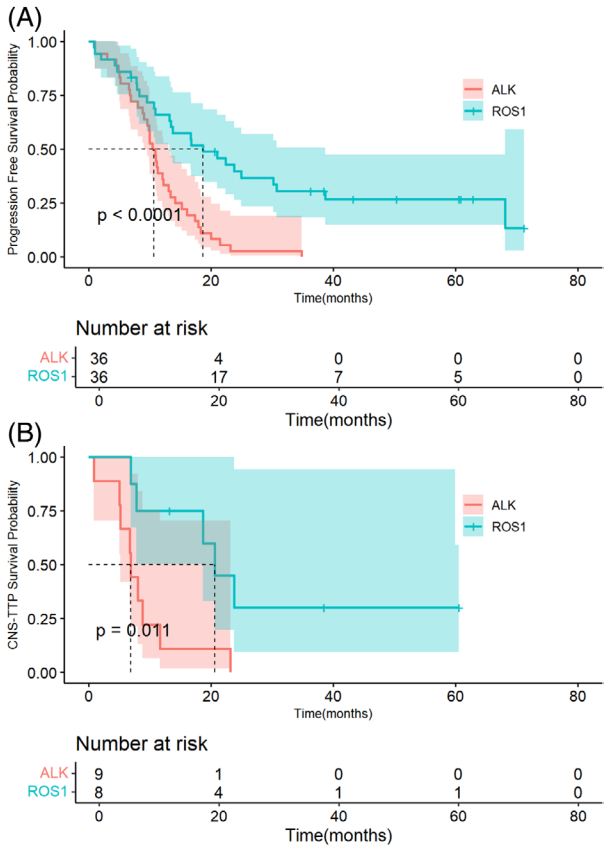

FIGURE 5 | Comparison of efficacy of crizotinib in ROS1+ and ALK+ NSCLC. (A) PFS comparison between ALK+ NSCLC patients and ROS1+ NSCLC patients treated with crizotinib. (B) CNS-TTP comparison between ALK+ NSCLC patients and ROS1+ NSCLC patients with baseline CNS lesions treated with crizotinib.

TABLE 3 | Comparison between baseline disease burden of metastatic ROS-1+ NSCLC and metastatic ALK+ NSCLC.

	ROS1	ALK	*p*
Extrathoracic metastases	63.8% (23/36)	80.5% (29/36)	0.114
CNS metastases	22.2% (8/36)	25.0% (9/36)	0.781
Multiple CNS metastases	62.5% (5/8)	44.4% (4/9)	0.457
Measurable CNS lesions	37.5% (3/8)	44.4% (4/9)	0.772
Liver metastases	8.3% (3/36)	22.2% (8/36)	0.101
Bone metastases	27.7% (10/36)	44.4% (16/36)	0.141
≥ 3 distant organs involved	25.0% (9/36)	36.1% (13/36)	0.306

Among the 29 patients who experienced disease progression, 48.3% of them underwent rebiopsy throughout the course of the disease (Table 2), including 8 cases after the first ROS1 inhibitor resistance and 6 cases following the progression of the second or third ROS1 inhibitor. For patients who did not undergo rebiopsy, 8 of them were due to the inconvenience of obtaining intracranial specimens or the low sensitivity of the blood test, but still, 46.7% (7/15) did not undergo rebiopsy in the presence of extracranial progression.

Among the 14 patients who underwent rebiopsy, ROS1 kinase domain secondary mutation occurred in 8 cases (57.1%) (Figure 6), with G2032R being the most common resistant mutation (7 patients), and K1790T mutation appearing in 1 patient. The proportion of patients with CD74-ROS1 fusion that developed secondary ROS1 mutation was numerically higher than that of patients carrying non-CD74 fusions (71.4% vs. 40%, $p = 0.558$). Other possible mechanisms of resistance included pathological transformation (1 patient had a pathotype of mucoepidermoid adenocarcinoma prior to treatment, while the pathotype of rebiopsy after resistance was mesenchymal origin tumor) and bypass activation (a HER-2 mutation was found on rebiopsy in 1 patient, who had primary resistance to a ROS1 inhibitor, and since the baseline patient had no NGS examination to clarify HER-2 status, it was hypothesized that this patient had HER-2 and ROS1 comutations at baseline leading to primary resistance).

3.7 | Follow-Up Treatment and Overall Survival

Among all 40 patients, a total of 17 patients were subsequently treated with other ROS1 inhibitors, and 18 patients received chemotherapy throughout the course of their disease. At the time of data cutoff, the overall population had a median follow-up (calculated from the time of late diagnosis) of 46.6 months (range 7.7–128.5 months), with a total of 13 deaths and a median OS = 89.0 months (95% CI: 34.7–153.2 months) (Figure 7).

4 | Discussion

ROS1 is an important therapeutic target in NSCLC; current guidelines recommend that all patients with advanced nonsquamous NSCLC (especially lung adenocarcinoma) should have their ROS1 genes tested before systemic therapy. Our study suggested that the majority of patients (more than 90%) underwent the detection of ROS1 before systemic treatment; only three patients were identified with the ROS1 fusion gene after multiple courses of treatment, and the anti-tumor treatments in these three patients were mainly concentrated before 2018, which also suggested that in recent years, with the increasing attention paid by clinicians to rare targets, the diagnostic and treatment specifications recommended by the guideline had been well implemented in clinical practice. In addition, we also found that the detection methods of ROS1 showed a significant correlation with time; before 2019, FISH was the most dominant detection method, while after 2019, NGS became the most dominant detection method. There is no doubt that, whether for point mutations or rearrangement fusions, NGS has a clear superiority over ARMS, RT-PCR, FISH, and other detection methods because it can detect hundreds of genetic abnormalities at the same time; it greatly reduces sample loss and shortens turn-around testing time, which indicates that the rapid development and upgrading of testing technology has greatly improved the efficiency of clinical work. In addition, the choice of first-line treatment also showed a significant correlation with time, with only half of the

TABLE 4 | Efficacy and progression in patients receiving ROS1 inhibitors.

Treatment	*n*	Best response	ORR DCR	Median PFS (95% CI)	Median OS (95% CI)	PD event	Patterns of progression	Rebiopsy	Timing of rebiopsy
Crizotinib	36	PR: 24 Pts (66.7%) SD: 10 Pts (27.8%) PD: 2 Pts (5.5%)	ORR: 66.7% DCR: 94.4%	18.7 months (8.9–28.4)	89.0 months (33.0–153.5)	26 (72.2%)	Intracranial: 11 Pts (42.3%) Extracranial: 13 Pts (50.0%) Intracranial + extracranial: 2 Pts (7.7%)	11 (42.3%)	After the first ROS1 inhibitor resistance: 6 Pts (54.5%) After the second ROS1 inhibitor resistance: 3 Pts (27.3%) After the third ROS1 inhibitor resistance: 2 Pts (18.2%)
Brigatinib	1	PR: 1 Pts (100.0%)	ORR: 100.0% DCR: 100.0%	18.3 months	83.7 months	1 (100.0%)	Extracranial: 1 Pts (100.0%)	1 (100.0%)	After the second ROS1 inhibitor resistance: 1 Pts (100.0%)
Ensartinib	1	PR: 1 Pts (100.0%)	ORR: 100.0% DCR: 100.0%	15.7 months	67.8 months	1 (100.0%)	Extracranial: 1 Pts (100.0%)	1 (100.0%)	After the first ROS1 inhibitor resistance: 1 Pts (100.0%)
TQB-3101	2	PR: 1 Pts (50.0%) SD: 1 Pts (50.0%)	ORR: 100.0% DCR: 50.0%	15.0 months (15.0–15.6)	46.9 months (15.0–46.9)	1 (50.0%)	Intracranial: 1 Pts (100.0%)	1 (100.0%)	After the first ROS1 inhibitor resistance: 1 Pts (100.0%)
Total	40	PR: 27 Pts (67.5%) SD: 11 Pts (27.5%) PD: 2 Pts (5.0%)	ORR: 67.5% DCR: 95.0%	16.8 months (13.0–20.5)	89.0 months (34.7–153.2)	29 (72.5%)	Intracranial: 12 Pts (41.4%) Extracranial: 15 Pts (51.7%) Intracranial + extracranial: 2 Pts (6.9%)	14 (48.3%)	After the first ROS1 inhibitor resistance: 8 Pts (57.1%) After the second ROS1 inhibitor resistance: 4 Pts (28.6%) After the third ROS1 inhibitor resistance: 2 Pts (14.3%)

Abbreviations: CI, confidence interval; CR, complete response; DCR, disease control rate; mo, months; ORR, overall response rate; PD, progressive disease; PFS, progression-free survival; PR, partial response; Pts, patients.

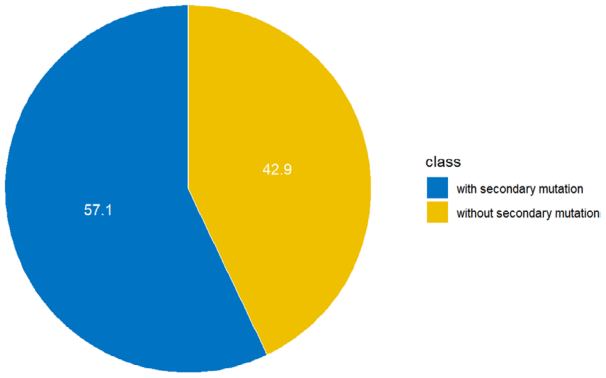

FIGURE 6 | Comparison of ROS1 kinase domain secondary mutation results in rebiopsy.

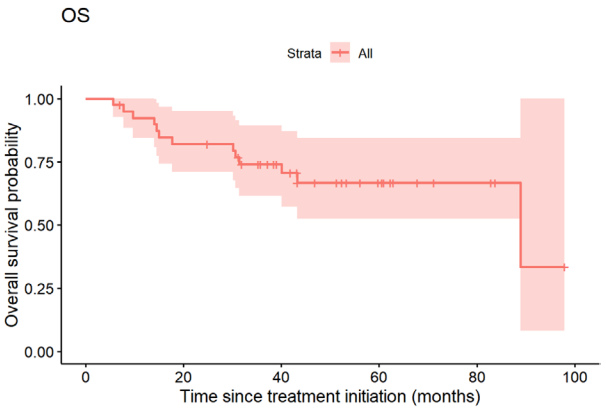

FIGURE 7 | OS in patients receiving ROS1 inhibitors.

patients choosing ROS1 inhibitors as first-line treatment before 2019. While after 2019, the proportion of patients selecting ROS1 inhibitors (including the choice of marketed drugs (crizotinib had been the most common application) and participation in clinical trials) as first-line treatment had been more than 85%, which was obviously due to the increasing drug accessibility, the expansion of indications for health insurance reimbursement, and the widespread conduct of clinical trials.

In terms of efficacy, we focused on analyzing the therapeutic efficacy of crizotinib as an initial ROS1 inhibitor, and our findings (radiographic response and progression-free survival) were similar to those of previous research [3–6, 11, 12], which further confirmed the superior efficacy of crizotinib in the treatment of ROS1-positive NSCLC. In addition, in the univariate analysis, we only found that performance status was associated with the PFS of crizotinib; that is, patients with better performance status experienced significantly better outcomes under crizotinib, which suggested that more effective treatment regimens should be explored in the future for patients with worse ECOG PS (≥ 2 points).

Moreover, we also analyzed the progression pattern of crizotinib and found that CNS was the most common site of failure in crizotinib treatment (all patients with baseline CNS metastases showed intracranial progression during crizotinib treatment, and nearly half of the patients without baseline CNS metastasis were found to have new brain lesions following the treatment of crizotinib), which was similar to the outcome of patients with ALK-positive NSCLC [13]. But the CNS progression-free survival of crizotinib in ROS1-positive NSCLC patients could reach beyond 18 months, which was significantly longer than that of ALK-positive NSCLC, whether in our study or comparing with the historical data [14], and previous research conducted by Gainor [15] showed that the cumulative risk of intracranial progression of ROS1-positive NSCLC patients treated with crizotinib was significantly lower than that of ALK-positive NSCLC patients. Therefore, we can infer that the aggressiveness of ROS1-positive NSCLC for CNS progression is weaker than that of ALK-positive NSCLC under the treatment of crizotinib. But due to the relatively poor ability of crizotinib to cross the blood–brain barrier, the percentage of ROS1-positive patients experiencing CNS progression is considerably high, so we should pay attention to CNS protection in these patients. Fortunately, next-generation ROS1 inhibitors like repotrectinib [16] or taletrectinib [10] presented favorable intracranial efficacy, whether in the first-line setting or after the resistance to crizotinib. Therefore, next-generation ROS1-TKI should be the preferred treatment option in advanced ROS1 + NSCLC, especially for those with CNS metastases.

In terms of rebiopsy status and mechanism of resistance, our study suggested that in the real-world setting, clinicians paid insufficient attention to rebiopsy. Nearly 50% of patients did not undergo rebiopsy to identify the potential mechanism of resistance after the occurrence of extracranial progression during the treatment of the first ROS1 inhibitor. According to our study, a high percentage of patients occurred ROS1 kinase domain secondary mutation during ROS1 inhibitor treatment (around 60%, similar to the results of previous studies [15]). G2032R was the most common site. It is noteworthy that because of the resistance of G2032R to several ROS1inhibitors, including lorlatinib, entrectinib, and brigatinib [17], the current sequential treatment strategy has not achieved much success in ROS1-positive NSCLC. However, for ALK-positive patients, several clinical studies suggested that lorlatinib, brigatinib, and ensartinib are effective against the G1202R mutation [18–20]. Thankfully, several drugs targeting the G2032R mutation have been successful in preclinical trials (typically represented by repotrectinib [16] and taletrectinib [10]), and it is hoped that medications targeting the G2032R mutation will benefit more patients in the future.

We also explored the clinical outcomes of different ROS1 fusion types receiving ROS1 inhibitors. Our results suggested that patients with CD74 fusion and non-CD74 fusion had similar PFS of crizotinib, which is different from the results of previous studies (retrospective analyses from Hunan Cancer Hospital [12] and Shanghai Chest Hospital [21] suggested that patients with CD74 fusion had worse PFS of crizotinib). The difference might be related to the fact that we included fewer patients in this study. Additionally, our study suggested that similar proportions of patients with CD74 fusion and non-CD74 fusion receiving crizotinib experienced intracranial progression, which was similar to the findings of previous studies, suggesting that more appropriate CNS protection strategies need to be explored in patients with different ROS1 fusion partners. Finally, we also found that patients with CD74-ROS1 fusion receiving ROS1 inhibitors had a greater chance (71.4% vs. 40.0%, $p = 0.558$, numerically higher but not statistically significant) of developing ROS1 kinase

domain secondary mutation, suggesting that it is essential to explore different sequential therapeutic strategies for different ROS1 fusion types in the future.

We have some limitations in our study. First, it was a single-center retrospective study with a small sample size, and there was selection and retrospective bias; our findings have inherent limitations in generalizability to other populations. Second, due to the small sample size, we did not explore the efficacy of sequential therapy following the resistance of crizotinib in real-world settings thoroughly. We hope to further expand the data in the future to improve the shortcomings in this area.

5 | Conclusion

With the innovation of diagnostic and therapeutic technologies and the expansion of the scope of the health insurance catalog, more and more patients are benefiting from new technologies and targeted drugs. Although crizotinib has brought excellent clinical data for ROS1-positive NSCLC patients, better CNS protection strategies for ROS1-positive patients should be explored in the future. Also, the low rate of rebiopsy in ROS1-positive NSCLC patients in the real-world setting should be emphasized in the future.

Author Contributions

Zihua Zou and Puyuan Xing were responsible for study conception and design. Hanqi Yuan, Zihua Zou, Xuezhi Hao, Yan Li, Junling Li, and Jianming Ying collected the data. Hanqi Yuan and Zihua Zou assembled the data. Hanqi Yuan and Zihua Zou analyzed the data. Hanqi Yuan and Zihua Zou drafted the report. Hanqi Yuan drew illustrations. All authors critically reviewed drafts of the manuscript and read and approved the final manuscript.

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Ethics Statement

This study was approved by Ethics Committee of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College (19/096-1880).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated and analyzed during this study are available from the corresponding authors on reasonable request.

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