Review

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Real-world studies of crizotinib in patients with ROS1-positive non-small-cell lung cancer: experience from China

Hua Zhong[1], Jun Lu[1], Mengzhao Wang*[,2] & Baohui Han**[,1]

[1]Department of Pulmonary Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

[2]Department of Respiratory & Critical Care Medicine, Peking Union Medical Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing, China

*Author for correspondence: Tel.: +86 13911235467; mengzhaowang@sina.com

**Author for correspondence: Tel.: +86 13817833343; 18930858216@163.com

The treatment of non-small-cell lung cancer (NSCLC) has progressed from histology-oriented cytotoxic therapy to the era of molecular biology-oriented targeted therapy and immunotherapy. As the first tyrosine kinase inhibitor (TKI) targeting the *ROS1* pathway, crizotinib is widely used as a first-line regimen for *ROS1*-rearranged NSCLC. However, due to the paucity of solid data from randomized, controlled phase III clinical studies, clinicians often require more systematic, real-world data-based guidance for its optimal clinical use. As one of the leading countries of real-world research on crizotinib, China has contributed significantly to data on standardization of the therapeutic use of crizotinib, including its clinical treatment patterns, the timing and duration of treatment and drug resistance monitoring and management.

Plain language summary: As the game-changer for the treatment of advanced *ROS1*-positive non-small-cell lung cancer (NSCLC), crizotinib is currently one of the most recommended first-line treatment options for these patients. However, as crizotinib was initially approved for the treatment of *ALK*-positive NSCLC, data from large-scale randomized, controlled trials of crizotinib in *ROS1*-positive NSCLC patients are limited. Consequently, the availability of comprehensive and large-scale, real-world evidence and clinical practice experience data from China is particularly important for its optimal use. In this review, we summarize the clinical efficacy and safety of crizotinib, drug resistance mechanisms and related mutation patterns and treatment options after drug resistance based on real-world experience in China to help clinicians in this country and elsewhere in the world make clinical decisions.

Shareable abstract: A series of real-world studies in China has contributed significantly to standardization of the clinical use of crizotinib. This article summarizes the practical experience of crizotinib in *ROS1*-positive NSCLC in China, and provides more evidence for clinicians to make optimal clinical decisions.

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In 2022, there were estimated to be about 870,982 new lung cancer cases and 766,898 lung cancer-related deaths in China [1]. As one of the most common driver genes in non-small-cell lung cancer (NSCLC), *ROS1* (*ROS* Proto-Oncogene 1) rearrangement is found in 0.9 to 2.6% of patients with NSCLC. The histological and clinical features associated with *ROS1*-positive NSCLC include female sex, adenocarcinoma histology, younger age, a never or light smoking history and advanced node stages [2].

After decades of development, the treatment of NSCLC has progressed from histology-oriented cytotoxic therapy to the era of molecular biology-oriented targeted therapy and immunotherapy. As the first tyrosine kinase inhibitor (TKI) targeting the *ROS1* pathway, crizotinib is widely used as a first-line regimen for *ROS1*-rearranged NSCLC. However, due to the lack of solid evidence from large-scale randomized, controlled trials, clinicians may still require systematic, real-world data-based guidance for the optimal clinical application of crizotinib, especially regarding the standardization of testing, timing and duration of treatment and drug resistance monitoring and management. As one of the leading countries of real-world research on crizotinib, China has contributed significantly

Table 1. Search strategy summary.

Items	Specification
Databases	PubMed, Embase and major Chinese databases (Wanfang and CNKI)
Search keywords/terms	*ROS* Proto-Oncogene 1 (ROS1) with or without rearrangement; non-small-cell lung cancer (NSCLC); Crizotinib with or without resistance; entrectinib, lorlatinib, repotrectinib and other second- or third-line agents
Timeframe	2014–2024
Inclusion criteria	Real-world studies; randomized controlled trials; observational studies; retrospective studies; prospective studies
Selection criteria	Studies were selected based on their representativeness, statistically significant sample sizes and credibility of evidence.

CNKI: China National Knowledge Infrastructure.

to standardization of the clinical use of crizotinib. This article aims to summarize the practical experience of crizotinib in China and impart this knowledge to clinicians in this country and elsewhere in the world. We focused on studies published between 2014 and 2024, identified through PubMed, Embase and major Chinese databases (Wanfang and CNKI). Articles were selected based on their relevance, statistical robustness and credibility to provide a comprehensive overview. The search strategy is described in Table 1.

Molecular mechanism of action of crizotinib on *ROS1* pathways

Crizotinib is an orally available, multiple-signaling TKI targeting *ROS1*, anaplastic lymphoma kinase (*ALK*) and the mesenchymal-epithelial transition receptor (*MET*). It inhibits ATP-dependent cellular processes by forming a complex with respective protein kinase domains, leading to strong suppression of *ROS1, MET, ALK* and their downstream pathways [3].

ROS1 is a transmembrane tyrosine kinase located on 6q22.1, belonging to the insulin receptor family. The macromolecule contains an N-terminal (the extracellular domain), a single-channel hydrophobic transmembrane channel and a C-terminal (the intracellular tyrosine kinase domain). Currently, a total of 14 *ROS1* gene fusion patterns have been found in lung cancer, including CD74, SLC34A2, SDC4, etc [4]. *ROS1* activation promotes cell proliferation and survival through MAPK/ERK, PI3K/AKT, JAK/STAT3 and SHP1/2 pathways. Amino acid sequence analysis shows that *ROS1* has 64% homology with *ALK* in the tyrosine kinase region and the homology of the ATP binding site in the catalytic domain of *ROS1* kinase and *ALK* kinase is as high as 84% [3]. Because crizotinib used as an *ALK* tyrosine kinase inhibitor targets the ATP binding site in the *ALK* kinase catalytic region, crizotinib is presumed to have an anticancer effect in the treatment of NSCLC with fusion mutations of *ROS1*, which has been demonstrated in a series of preclinical and clinical trials.

Although the binding domains are highly homologous, the efficacy spectrum of crizotinib in *ROS1*-positive and *ALK*-positive NSCLC still exhibits some differences. For example, the efficacy of crizotinib is more consistent in patients with different *ROS1* fusion subgroups and the long-term survival data in *ROS1*-positive patients receiving crizotinib appears to be better than that in *ALK*-positive patients. Furthermore, not all *ALK* inhibitors can inhibit both *ROS1* and *ALK* activity.

The possible reasons for the difference in efficacy of crizotinib in *ROS1*-positive and *ALK*-positive NSCLC may include:

- Crizotinib has different binding sites and binding characteristics with *ROS1* and *ALK* kinase domains and has proved to have a stronger binding affinity and inhibitory effect on *ROS1* in molecular (*in vitro*) research studies [5]. Molecular experiments have found that the equilibrium dissociation constant (K_d) of crizotinib binding to the *ROS1* kinase domain is 0.4 nmol/l, which is significantly lower than its K_d value for binding to *ALK* (4.4 nmol/l); this means that the binding affinity of crizotinib to *ROS1* is significantly higher than that of *ALK*. The IC_{50} (concentration required to inhibit a cell line by 50%) of crizotinib (PF-02341066) on *c-MET* and *ALK* has been found to be 11 and 24 nmol/l, respectively, in cell-based assays, while the IC_{50} on *ROS1* (wild-type/without mutation) is 3.9–5.4 nmol/l [6]. The Ki value of crizotinib (concentration required to produce half maximal inhibition) in *ROS1*-positive cell lines is 0.6 nmol/l.
- *ROS*1 is relatively conservative in its evolution. Previous studies have shown that *ROS1* gene rearrangements, rather than *ROS1* mutations, are involved in the pathogenesis of NSCLC because no mutations in the kinase domain of *ROS1* were found in newly diagnosed patients with NSCLC. It is speculated that the gene sequence of the kinase region of *ROS1* is relatively stable, whereas mutations are the primary cause of drug resistance and treatment failure.

Table 2. Summary of data from reported clinical trials of crizotinib.

Study (*ROS1*-positive patients, n)	Phase	Treatment line	ORR	mPFS (months)	mOS (months)	mDOR (months)	OS rate	Ref.
PROFILE 1001 (50)	Ib	13% first line	72%	19.3	51.4	24.7	12 months: 85% 48 months: 51%	[8]
EUCROSS (34)	II	21% first line	70%	20	NR	19	12 months: 83% 24 months: 63%	[9]
OO12-01 (127)	II	18.9% first line	72%	15.9	32	19.7	12 months: 83.1%	[10]
METROS (26)	II	76% first line	65%	22.8	NR	21.4	6 months: 96.2% 12 months: 79.2%	[11]
AcSé (34)	II	3% first line	69%	5.5	17	–	–	[12]

mDOR: Median duration of response; mOS: Median overall survival; mPFS: Median progression-free survival; NR: Not reached; ORR: Objective response rate; OS rate: Overall survival rate.

Progress of research on crizotinib (Table 2)

In 2011, crizotinib was initially approved as the first *ALK*/*ROS1*/*MET* multi-pathway TKI for *ALK*-positive NSCLC. In *ROS1*-rearranged cell lines, crizotinib also showed a potent apoptotic effect (IC_{50} of 60 nmol/l), allowing researchers to explore the potential effect of crizotinib in *ROS1*-positive NSCLC. The PROFILE 1001 phase I clinical trial (n = 50) demonstrated encouraging results. Patients with *ROS1*-rearranged NSCLC who received crizotinib treatment achieved an objective response rate (ORR) of 72%, a 12-month OS rate of 85% and a median PFS of 19.2 months [7]. In an additional >3-year follow-up analysis, the long-term effectiveness and safety of crizotinib were also confirmed. The median PFS and OS of patients reached 19.3 months and 51.4 months, respectively, and the median 48-month OS rate was 51% [8].

The promising results of the PROFILE 1001 study have led to the indications for crizotinib being extended to *ROS1*-rearranged NSCLC in various countries since 2016. Subsequently, a series of retrospective and prospective studies have been conducted to further verify the efficacy and safety of crizotinib as a treatment option for *ROS1*-positive NSCLC. Initial observational data supported the use of crizotinib in the second-line setting for *ROS1*-positive NSCLC, followed by a step-by-step transition to the first-line setting.

In a prospective phase II trial (EUCROSS), 34 *ROS1*-positive patients with NSCLC patients showed an overall ORR of 70%, a median PFS of 20.0 months (95% CI, 10.1_not reached) and a median OS of 'not reached' after a median follow-up of 55.9 month [9]. Brain metastasis (log-rank, p = 0.1805) and TP53 mutation (log-rank, p = 0.015) seemed to be notable risk factors for a poor OS outcome. Among the overall population, 21% of patients received crizotinib as first-line therapy.

In Asia, a prospective study (OO12-01) [10] recruited 127 Chinese patients with *ROS1*-positive, advanced NSCLC and demonstrated an ORR of 72% (including a complete response [CR] rate of 11%), a median OS of 32 months, and a median PFS of 15.9 months, respectively. 18.9% of patients in this trial received crizotinib as first-line treatment. The treatment responses and survival outcomes with crizotinib therapy from eastern and western countries are highly replicable and overlapping, suggesting that crizotinib has consistent efficacy across different ethnicities, allowing real-world research results in different countries to be referenced with each other.

Currently, crizotinib has been approved by the Food and Drug Administration (FDA USA, 2016), European Medicines Agency (EMA, 2016) and the National Medical Products Administration (NMPA, China, 2017) for the treatment of patients with advanced *ROS1*-positive NSCLC. Other approved drugs for this indication include entrectinib (2017 by the FDA and 2022 by the NMPA). Although the clinical evidence was sufficient for crizotinib to be approved for the *ROS1* population in China and for its first-line use, the lack of large phase III randomized, controlled studies had an influence on clinical confidence with crizotinib for the *ROS1*-positive NSCLC population, making supplementation with real-world data in Chinese patients desirable.

Chinese real-world studies of Crizotinib

Clinical features of *ROS1* in China

In 2018, Zhang *et al.* [13] conducted a large-scale retrospective study to explore *ROS1* fusion features in the Chinese population with NSCLC (n = 6066). The results showed an *ROS1* fusion frequency of 2.59% (157/6066) in Chinese patients with NSCLC, which was consistent with former reports. Younger age (positive group: 55.68 ± 11.34 years vs negative group: 61.02 ± 10.44 years; $p < 0.01$), female gender (3.71 % vs men 1.81%; $p < 0.01$), non-smokers (3.33% vs smokers 1.21%; $p < 0.01$), pathology type with adenocarcinoma (2.77% vs squamous carcinoma

Table 3. Summary of data from Chinese real-world studies of crizotinib.

Study (*ROS1*-positive pts, n; %)	Treatment line	Regimen	ORR	DCR	PFS	OS	Ref.
Zhang *et al.* (2309; 2.2%)	Second line	Crizotinib vs chemotherapy	80.0% vs 40.8%† and 25.0%‡	90.0% vs 71.4%† and 47.7%‡	294 days vs 179 days† and 110 days‡	–	[15]
Zeng *et al.* (1466; 1.5%)	73.7% first line	Crizotinib	89%	–	13.6 months	–	[16]
Li *et al.* (2400; 2.0%)	38.9% first line	Crizotinib	83.3%	–	12.6 months	32.7 months	[17]
Liu *et al.* (1842; 1.9%)	50% first line	Crizotinib	71.4% (1st line) 82.4%	94.3% (1st line 100%)	11.0 months	41.0 months	[18]
Zhu *et al.* (2617; 2.56%)	17% first line 83% ≥ second line	Crizotinib	56.52%	78.26%	14.5 months	–	[19]
Xu *et al.* (102; 100%)	100% first line	Crizotinib vs chemotherapy	83.9% vs 56.5% ($p = 0.002$)	96.4% vs 100%	14.9 vs 8.5 months ($p < 0.001$)	NR vs NR	[20]
Shen *et al.* (77; 100%)	100% first line	Crizotinib vs chemotherapy	86.7% vs 44.7% ($p < 0.001$)	96.7% vs 85.1%	18.4 vs 8.6 months ($p < 0.001$)	NR vs 28.4 months	[21]
Zheng *et al.* (56; 100%)	100% first line	Crizotinib	64.7%	94.1%	23.0 months	60.0 months	[22]
Zhang *et al.* (235, 100%)	100% first line	Crizotinib vs chemotherapy	85.7% vs 41.8%	–	18.0 vs 7.0 months ($p < 0.001$)	–	[23]

†Pemetrexed-treated patients.
‡Non-pemetrexed-treated patients.
DCR: Disease control rate; NR: Not reached; ORR: Objective response rate; OS: Overall survival; pt: Patient; PFS: Progression-free survival.

0.93%; p = 0.017) and later advanced node stages (1.31%, 1.40%, 2.07% and 3.23% for N0, N1, N2 and N3, respectively; p = 0.027) were correlated with higher *ROS1* fusion frequency. However, the treatment regimens and survival data were not provided in this report.

The study of Zhuang *et al.* [14] was the first research to focus on clinical features and treatment options in NSCLC patients with concomitant mutations of *EGFR, ALK, ROS1, KRAS* or *BRAF* in China. A total of 3774 NSCLC patients were screened in the study and the incidence of *ROS1* rearrangement was 2.1%. 9.1% (8/88) of patients harboring an *ROS1* rearrangement had co-alterations, which occurred more frequently in male patients (p = 0.02). Among patients with *EGFR, ALK* or *ROS1* alterations, 21 patients received TKIs as first-line treatment, with an ORR of 61.9% and a median PFS of 11.8 months. However, only two of them were *ROS1* positive patients harboring coalterations. Therefore, the sample size was too small to compare the efficacy of crizotinib in patients with single *ROS1* rearrangements or combined co-alterations.

Crizotinib in the second-line setting

The first large-scale, retrospective study was conducted in 2016 to comprehensively investigate the efficacy of crizotinib and chemotherapy (including pemetrexed-based and non-pemetrexed-based regimens) in Chinese patients with *ROS1* fusion-positive NSCLC [15]. A total of 2309 patients were screened for *ROS1* alteration and 51 (2.2%) patients were confirmed to harbor an *ROS1* rearrangement. In the *ROS1* fusion-positive group, all crizotinib-treated patients were in the second-line setting or above. The treatment response and survival outcomes of patients in the crizotinib group were improved more significantly than in patients in the pemetrexed-treated and non-pemetrexed-treated groups: the ORR values in the 3 groups were 80.0, 40.8 and 25.0%; disease control rate (DCR) values were 90.0, 71.4 and 47.7%; and the median PFS values were 294 days, 179 days and 110 days, respectively. The results suggested that in the Chinese *ROS1*-rearranged NSCLC population, crizotinib had robust efficacy as a second-line option. Notably, the treatment response was consistent among different *ROS1* fusion partners (in that no distinct correlation was observed).

Crizotinib in the first-line or multi-line (including first-line) settings [Table 3]

In a study conducted by Zeng *et al.* (n = 1466 patients with NSCLC) reported a *ROS1* rearrangement frequency of 1.5% (22 of the 1466 patients) [16]. Among the 19 *ROS1* positive NSCLC patients that received crizotinib treatment, the ORR with crizotinib was 89% and the median PFS was 13.6 months. The median PFS was longer in patients with a single *ROS1* rearrangement than in those harboring concomitant alterations (15.5 vs 8.5 months,

p = 0.0213). It was noted that 73.7% patients in this study received crizotinib as first-line treatment. Therefore, this was the first real-world study demonstrating the efficacy of crizotinib in the first-line setting of Chinese *ROS1*-positive patients with NSCLC.

Li *et al.* compared the efficacy of crizotinib among different types of *ROS1* fusion partners in Chinese patients with *ROS1*-rearranged NSCLC for the first time [17]. A total of 49 patients with *ROS1* rearrangement-positive NSCLC received crizotinib treatment, providing an ORR of 83.3%, a median OS of 32.70 months and a median PFS of 12.63 months. According to different fusion partners, both the median PFS and OS of patients in the non-CD74-*ROS1* group were significantly longer than those in the CD74-*ROS1* group (PFS: 17.63 vs 12.63 months, p = 0.048; OS: 44.50 vs 24.33 months, p = 0.036). In multivariate analysis, only the presence of brain metastases at baseline before treatment was significantly associated with the median OS (p = 0.01). Due to limitations such as a small sample size, imbalanced baseline characteristics of enrolled patients, the retrospective nature of the study and a relatively short follow-up period, the comparative conclusion between the different fusion groups needs to be further explored.

Two additional retrospective studies in 2019 patients further demonstrated that crizotinib is highly effective and well tolerated in real-world clinical settings in the Chinese NSCLC population. In the study conducted by Liu *et al.* [18] (n = 35 *ROS1*-positive patients with NSCLC), 50% of patients received crizotinib as the first-line therapy option. The ORR with crizotinib treatment was 71.4%, the DCR was 94.3% and the estimated median PFS and median OS reached 11.0 months (95% CI, 7.8–14.2) and 41.0 months (95% CI, 22.5–59.5), respectively. The study conducted by Zhu *et al.* [19] in 2617 patients with NSCLC and reported a *ROS1* fusion incidence of 2.56% (67/2617). The ORR, DCR and median PFS of patients receiving crizotinib were 56.52%, 78.26% and 14.5 months, respectively; OS was not reported. The most common adverse events were mild and manageable and included gastrointestinal discomfort (such as diarrhea and vomiting), elevated transaminases and vision disorders.

Xu *et al.* [20] conducted a study comparing crizotinib with platinum-based chemotherapy as first-line treatment for advanced NSCLC with different *ROS1* fusion partners in 2019 patients (n = 102); 54.9% received crizotinib and 45.1% received chemotherapy as first-line treatment. The results showed that the ORR and median PFS of patients in the crizotinib group were significantly improved compared with those in the chemotherapy group (ORR (83.9 vs 56.5%, respectively, p = 0.002); median PFS 14.9 versus 8.5 months, respectively, p < 0.001. The OS in both groups was not reached. The study was the first large-scale, head-to-head one to demonstrate the efficacy superiority of crizotinib over platinum-based chemotherapy in a real-world, first-line setting in China. As regards different fusion variants, patients carrying CD74 fusion variants achieved a significantly longer median PFS with crizotinib treatment (20.1 vs non-CD74 variants 12.0 months, p = 0.046). This result seemed to be inconsistent with other studies, because the incidence of brain metastases at baseline in the CD74 variant group was lower than that in the non-CD74 variant group (11.8 vs 27.7%), which is contrary to the baseline features reported in other studies. Therefore, this study's conclusion might have been related to a baseline imbalance and this still needs to be explored by larger-scale studies.

Another first-line study conducted by Shen *et al.* [21] also demonstrated an advantage of crizotinib over platinum-pemetrexed chemotherapy (n = 77). Compared with the platinum-pemetrexed chemotherapy group, patients in the crizotinib group had a significantly better ORR (86.7 vs 44.7%, p < 0.001), significantly longer median PFS (18.4 vs 8.6 months, p < 0.001), and numerically longer OS (not reached vs 28.4 months, p = 0.176). There was no statistically significant difference in OS between the two groups, which might be related to the high crossover rate after first-line treatment failure (disease progression) in this study. A total of 37 patients had treatment crossover after failure of first-line treatment. Among them, 30 patients receiving first-line chemotherapy were switched to second-line crizotinib, which might improve the expected survival prognosis of these patients (versus without crossover).

Prognostic & predictive factors of crizotinib treatment

Zheng *et al.* [22] investigated the prognostic factors of first-line crizotinib treatment in 56 Chinese patients with *ROS1*-rearranged NSCLC. The median PFS and OS after first-line crizotinib treatment was 23.0 and 60.0 months, respectively. In univariate analysis, female sex (median PFS: 12.0 vs 24.0 months, p = 0.015) and the presence of >2 baseline metastatic organs (median PFS: 4.0 vs 24.0 months, p < 0.001; median OS: 6.0 vs 60.0 months, p < 0.001) was associated with significantly poor survival outcomes. In multivariate analysis, only the involvement of >2 baseline metastatic organs was an independent risk factor for a shorter PFS (p = 0.008). The study also

explored the mutation profile of crizotinib resistance, showing that a G2032R mutation in the *ROS1* kinase domain was the most common resistance-associated mutation (4/8 patients, 50%).

In 2021, a larger multicenter retrospective study (n = 235) was conducted to further explore the clinical and molecular factors influencing the efficacy of first-line crizotinib therapy in patients with *ROS1*-rearranged NSCLC [23]. The ORR was 85.7% (144/168) in the first-line crizotinib group and 41.8% (28/67) in the chemotherapy group. First-line treatment with crizotinib versus chemotherapy significantly improved the median PFS of Chinese patients with *ROS1*-positive NSCLC (18.0 months vs 7.0 months, $p < 0.001$). Consistent with a series of previous studies, CD74-*ROS1* fusions (17.0 months vs non-CD74 *ROS1* fusions 21.0 months, $p = 0.008$), and the presence of baseline brain metastasis (16.0 vs 22.0 months, $p = 0.03$) were associated with a significantly shorter PFS. The study contributed to the concomitant mutation spectrum of the Chinese *ROS*1 population, showing that TP53 was the most common concomitant mutation with *ROS1* rearrangement (the incidence was 13.1% in the CD74-*ROS1* fusions group and 18.4% in the non-CD74-*ROS1* fusions group). And the presence of TP53 concomitant mutation was associated with a significantly shorter median PFS (6.5 months vs wild-type TP53 group 21.0 months, $p < 0.001$). In addition to TP53, concomitant driver mutations (11.0 months vs 24.0 months, $p = 0.0167$) or concomitant tumor suppressor genes (i.e., TP53, RB1, or PTEN: 9.5 months vs 24.0 months, $p < 0.001$) were also associated with significantly worse PFS outcomes, suggesting that bypass signaling might weaken the efficacy of crizotinib or lead to a risk of resistance. Thus, baseline brain metastatic status and different molecular features could contribute to distinct clinical outcomes from first-line crizotinib therapy.

Resistance analysis of crizotinib treatment

Zhang *et al.* [24] conducted a study focusing on the treatment failure patterns and resistance mechanisms of crizotinib treatment in Chinese patients with *ROS1*-positive NSCLC (n = 49). At baseline, the most common fusion partners were CD74-*ROS1* (57.1%), followed by SDC4-*ROS1* (20.4%), EZR-*ROS1* (12.2%), SLC34A2-*ROS1* (4.1%), TPM3-*ROS1* (4.1%) and CCDC6-*ROS1* (2%). After progression on first-line crizotinib therapy, 61.2% (30/49) of patients developed secondary *ROS1* point mutations, of which *ROS1* G2032R was the most common (28.5%; 14/49). Other common mutations included G2032K (8.3%; 4/49), G2026M (6.1%; 3/49), L2086F (6.1%; 3/49), S1986Y (4.1%; 2/49), S1986F (2%; 1/49), L1174F (2%; 1/49) and L2155S (2%; 1/49). The most common progression types and sites were extracranial-only (67.3%), intracranial-only (22.4%) and both intracranial and extracranial progression (10.2%). Patients with extracranial-only progression had a significantly higher frequency of point mutations compared with patients with intracranial-only progression (72.7% vs 15.2%, respectively; $p = 0.001$).

Resistance mechanism, management & salvage treatment of crizotinib

The mechanism of resistance of *ROS1* with crizotinib is mainly due to secondary mutation or bypass activation. Secondary mutations are usually mutations in the kinase region of *ROS1*, accounting for 50–60%, which is higher than the 20–25% mutation rate in the kinase region of *ALK* [25]. The most common drug resistance mutation in *ROS1* is G2032R, which is similar to G1202 in *ALK*. G2032R is the first drug-resistance mutation discovered and it leads to drug-binding damage independently of drug dose but does not alter the onchogenic kinase activity. In an analysis of 17 samples resistant to crizotinib treatment, the incidence of G2032R mutation was 41%, emphasizing that G2032R plays an important role in acquired resistance of *ROS1* [26]. Another common resistance-related mutation is D2033N, which is similar to *ALK* D1203N and affects the electrostatic effect of the D2033 residue for binding ability to crizotinib. Other drug resistance-related mutations include S1986Y/F and L2026M [25].

Activation of bypass signaling is another important mechanism of crizotinib resistance and was identified in around 45% of crizotinib-resistant *ROS1*-rearranged NSCLC malignancies. Common bypass activation related to crizotinib resistance included the *EGFR* pathway and the *KIT* pathway [3]. Just as independent upregulation of *EGFR* activity was not controlled by crizotinib, *KIT* pathway activation could lead to crizotinib resistance through promoting uncontrolled auto-phosphorylation and cell proliferation *in vitro*. Therefore, the addition of *EGFR* inhibitors such as erlotinib and gefitinib, or *KIT* inhibitors such as ponatinib might be beneficial in this subgroup of crizotinib-resistant patients. Last, upregulation of MAPK signaling plus the amplification of TP53 and HER2 have been reported in crizotinib-resistant cells, which might play a key role in bypass mechanisms.

Entrectinib is an emerging TKI with high blood–brain barrier (BBB) penetration that has demonstrated robust *ALK* and *ROS1* inhibition, as well as effective TRKA, TRKB and TRKC signaling suppression. However, entrectinib has a similar resistance profile to crizotinib, showing limited efficacy against G2032R, D2033N and L2026M *ROS1*

mutants. Furthermore, a meta-analysis in 2022 showed that crizotinib and entrectinib have comparable efficacy in *ROS1*-positive NSCLC and an adjusted simulated treatment comparison found non-significant trends favoring crizotinib over entrectinib: ORR risk ratio = 1.04 (95% CI 0.85–1.28); mean difference in the median duration of response = 16.11 months (95% CI -1.57–33.69); mean difference in the median PFS = 3.99 months (95% CI -6.27–14.25); 12-month OS risk ratio = 1.01 (95% CI 0.90–1.12). Thus, entrectinib may not be an optimal choice after the occurrence of crizotinib resistance [27].

If disease progression is observed while receiving crizotinib treatment, it is suggested that a second-line choice is lorlatinib. This drug is approved for *ALK*/*ROS1*-positive NSCLC that has progressed on other *ALK* inhibitors, and appears to overcome acquired resistance to crizotinib in *ROS1*-positive NSCLC. A phase I/II trial in *ROS-1* positive NSCLC patients (n = 69, 48 of whom had previously received *ROS1* TKI therapy) showed that whether crizotinib was used or not, lorlatinib could bring treatment benefit [28]. A phase II study in China (NCT05297890) is also ongoing, which may complement evidence from the Chinese population.

Case reports suggest that cabozantinib may be effective in *ROS1*-positive patients who have developed resistance to crizotinib. Ceritinib is also showing effectiveness, but may not be able to overcome acquired resistance spectrum to crizotinib. For example, a phase II trial (n = 28) in evaluable advanced *ROS1*-rearranged patients with NSCLC found that ceritinib treatment achieved an ORR of 62%, a duration of response (DOR) of 21 months, and a DCR of 81%. The overall median PFS was 9.3 months, the median PFS in crizotinib-naive patients was 19.3 months, and the median OS was 24 months [29].

Although alectinib and brigatinib are effective in *ALK*-positive NSCLC, they have little clear *ROS1* inhibitory activity. In addition, early clinical trials of drug candidates such as cabozantinib, ceritinib, repotrectinib and taletrectinib (DS-6051b) for *ROS1* NSCLC are ongoing [6].

The TRIDENT-1 study (n = 127) showed that repotrectinib achieved promising efficacy for *ROS1* fusion-positive patients with NSCLC, regardless of treatment lines [30]. For *ROS1* TKI-naive patients (n = 71), the ORR was 79%, the median PFS was 35.7 months and the median DOR was 34.1 months. For patients who had received one *ROS1* TKI, the ORR was 38%, the median PFS was 9.0 months, the median OS was 25.1 months and the median DOR was 14.8 months. 59% of patients harboring *ROS1* G2032R responded to repotrectinib. Therefore, the US FDA has recently approved repotrectinib for the treatment of locally advanced or metastatic *ROS1*-positive NSCLC.

Another phase II study (TRUST-I: n = 173) which was conducted in China, showed that taletrectinib also achieved encouraging efficacy in TKI-naive patients or crizotinib-pretreated patients [31]. For TKI-naive patients, the ORR was 91%; neither median PFS nor DOR was reached until a maximum follow-up of 23.5 month. For crizotinib-pretreated patients, the ORR was 52%, median PFS was 7.6 months and median DOR was 10.6 months. 67% (8/12) of patients harboring the G2032R mutation had a response. Preclinical studies had shown that taletrectinib has excellent blood–brain barrier penetration and high intracranial activity and this phase II study further confirmed that its notable intracranial ORR in both TKI-naive and crizotinib-treated patients (88% and 73%, respectively). Therefore, for crizotinib-treated patients especially those harboring a *ROS1* G2032R mutation, repotrectinib and taletrectinib may be alternative treatment options that can be considered.

For *ROS1*-positive NSCLC patients who choose not to have any targeted therapy options for subsequent-line treatment, potential treatment options include: chemotherapy, immunotherapy, chemotherapy combined with immunotherapy and multidrug combinations containing anti-angiogenic agents.

Preclinical studies have demonstrated that PD-L1 expression was significantly up-regulated in experimental cells after forced expression of *ROS1* fusion, and was eliminated when HCC78 (crizotinib-sensitive) cell lines and xenograft mouse models were treated with crizotinib [32]. Also, in HCC78CR (crizotinib-resistant) cells, PD-L1 expression was not affected by crizotinib and was persistently highly expressed after treatment. The correlation between *ROS1*-fusion and PD-L1 overexpression suggests that PD-L1/PD-1 inhibitors could be a second-line choice for the crizotinib-resistant NSCLC with *ROS1* rearrangement. On the other hand, mouse xenograft models with Ba/F3 *ROS1* fusion showed more $CD3^+$ $PD\text{-}1^+$ T cells in both blood and tissues, and more sensitivity than the cells with Ba/F3 *ROS1*-G2032R resistant mutations after anti-PD-L1 therapy [33]. This mouse model suggested that the use of anti-PD-1/PD-L1 therapy in *ROS1*-G2032R resistant mutations might be less efficient, and further research is needed to explore more clinical situations where immunotherapy is applicable.

Platinum-based therapy as a second-line treatment with or without bevacizumab for patients who have received prior crizotinib has also been recommended in several guidelines. However, the response rate is not encouraging. An *in vitro* study found that the IC_{50} of cisplatin in a crizotinib-resistant cell line (HCC78CR) was also significantly

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Table 4. IC_{50} values of crizotinib versus newer TKIs targeting different ROS1 mutations.

ROS1 mutations	IC_{50} values (nmol/l)								
	Crizotinib	Entrectinib	Lorlatinib	Repotrectinib	Carbozantinib	Ceritinib	Brigatinib	Taletectinib	Alectinib
Parental	840.5§	1801.0§	>3000§	1218.0§	>3000§	1117.0§	>3000§	>3000§	1207.0§
Non-mutants	5.4†	2.7†	0.7†	2.0†	2.8†	16.4†	9.4†	2.6†	995.4§
G2032R	609.6§	463.3§	196.6‡	23.1†	17.5†	346.4§	472.7§	53.3‡	1091.0§
L2000V	37.1†	25.9†	2.5†	10.1†	7.6†	124.9‡	78.9‡	29.8†	985.0§
L2086F	536.8§	440.0§	>3000§	587.9§	3.6†	226.9§	159.3‡	1265.0§	672.5§
S1986F/L2000V	159.4‡	36.1†	2.4†	7.2†	5.1†	86.9‡	62.5‡	20.3†	1080.0§
S1986F/L2086F	469.7§	344.2§	>3000§	241.2§	1.3†	154.8‡	48.5†	662.6§	919.9§
G2032R/L2086F	498.6§	335.4§	>3000§	248.9§	5.0†	573.9§	450.9§	744.2§	1254.0§
S1986F/G2032R	594.4§	718.5§	990.6§	65.1‡	70.1‡	614.7§	717.0§	105.4‡	1137.0§
S1986F G2032R/L2086F	562.8§	1111.0§	2131.0§	1178.0§	9.4†	1116.0§	1341.0§	2432.0§	1150.0§

†IC_{50} ≤50 nmol/l.
‡IC_{50} 50 nmol/l to <200 nmol/l.
§IC_{50} ≥200 nmol/l.
Data taken from Lin *et al.* [6].

higher than in the parental line HCC78 (25.86 vs 10.08 μmol/l), suggesting that the use of chemotherapy in crizotinib-resistant patients may still be controversial.

Table 4 shows IC_{50} values of *ROS1* TKIs targeting different mutations. The lower the IC_{50} value, the more potent the drug is against that mutation (adapted from reference [6]).

Prospect & conclusion

Because crizotinib was initially approved for the treatment of *ALK*-positive NSCLC, and its indication was then expanded to *ROS1*-positive NSCLC, there has been a lack of solid phase III randomized, controlled clinical trial data on *ROS1*-positive NSCLC, making comprehensive and large-scale, real-world evidence and practice experience in China particularly important.

The experience from real-world studies in China that can be summarized includes the clinical efficacy and safety of crizotinib; risk factors for drug resistance; drug resistance mechanism and related mutation patterns; treatment options after drug resistance, etc. In conclusion, crizotinib has changed the treatment landscape of advanced *ROS1*-positive NSCLC and it is the preferred first-line option for these patients. The benefit of crizotinib over standard chemotherapy has also been strongly demonstrated by a series of real-world studies in China, although a large proportion of patients have experienced disease progression and the development of resistance to crizotinib. Newer generation TKIs or other agents to overcome *ROS1* resistance are therefore required. Also required is higher BBB penetration of novel agents, a deeper understanding of molecular mechanisms in drug resistance and disease progression processes, the exploration of more effective combination regimens and the exploration for more accurate predictive biomarkers for optimal benefit populations. Among the newer generation of TKIs, entrectinib has unique activity due to its high CNS penetration, but its resistance spectrum is similar to that of crizotinib and thus it may not be the best salvage treatment option for crizotinib resistance. Other emerging drugs such as repotrectinib and taletrectinib can overcome the resistance caused by the common G2032R mutation in crizotinib-treated patients. In this regard, the high CNS penetration of taletrectinib is notable, but these data need further confirmation from large-scale clinical studies. In future, a more comprehensive understanding of *ROS1* molecular rearrangement is essential for the design of more precise and effective combination therapies and individualized long-term management strategies.

Future perspective

A large proportion of patients have reported disease progression and resistance to crizotinib. A new generation of agents to overcome *ROS1* resistance is therefore required. Also required is higher penetration of the CNS, more effective combination regimens and exploration of predictive biomarkers for optimal benefit populations. In future, a more comprehensive understanding of *ROS1* molecular rearrangement is essential for the design of more precise and effective combination therapies and individualized long-term management strategies.

Summary points

- The treatment of non-small cell lung cancer (NSCLC) has progressed from histology-oriented cytotoxic therapy to the era of molecular biology-oriented targeted therapy and immunotherapy.
- As one of the most common driver genes in NSCLC, *ROS1* rearrangement is found in 0.9%–2.6% of patients with NSCLC.
- Crizotinib is an orally available, multiple-signaling tyrosine kinase inhibitor (TKI) targeting *ROS1*, as well as anaplastic lymphoma kinase (*ALK*) and the mesenchymal-epithelial transition receptor (*MET*).
- Although crizotinib has been widely used for *ROS1*-rearranged NSCLC, data from large-scale randomized, controlled trials of crizotinib are limited.
- Consequently, real-world studies become important for the optimal clinical use of crizotinib.
- Chinese real-world studies of crizotinib have reported its efficacy and safety in *ROS1*-positive NSCLC.
- In addition, the mechanisms of resistance to crizotinib, and treatment options for resistance have also been explored, as a large proportion of patients have experienced disease progression and the development of resistance.

Author contributions

H Zhong: conceptualization, literature searching for relevant studies, analysis of the data, writing-original draft; J Lu: literature searching, data curation and writing original draft; M Wang and B Han: conceptualization, funding acquisition, resources, supervi-

sion, writing – review and editing. All authors participated in the critical review of the manuscript, and all have read and approved the final version submitted for publication.

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