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REVIEW

Current treatment and novel insights regarding ROS1-targeted therapy in malignant tumors

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Abstract

Background: The proto-oncogene ROS1 encodes an intrinsic type I membrane protein of the tyrosine kinase/insulin receptor family. ROS1 facilitates the progression of various malignancies via self-mutations or rearrangements. Studies on ROS1-directed tyrosine kinase inhibitors have been conducted, and some have been approved by the FDA for clinical use. However, the adverse effects and mechanisms of resistance associated with ROS1 inhibitors remain unknown. In addition, next-generation ROS1 inhibitors, which have the advantage of treating central nervous system metastases and alleviating endogenous drug resistance, are still in the clinical trial stage.

Method: In this study, we searched relevant articles reporting the mechanism and clinical application of ROS1 in recent years; systematically reviewed the biological mechanisms, diagnostic methods, and research progress on ROS1 inhibitors; and provided perspectives for the future of ROS1-targeted therapy.

Results: ROS1 is most expressed in malignant tumours. Only a few ROS1 kinase inhibitors are currently approved for use in NSCLC, the efficacy of other TKIs for NSCLC and other malignancies has not been ascertained. There is no effective standard treatment for adverse events or resistance to ROS1-targeted therapy. Next-generation TKIs appear capable of overcoming resistance and delaying central nervous system metastasis, but with a greater incidence of adverse effects.

Conclusions: Further research on next-generation TKIs regarding the localization of ROS1 and its fusion partners, binding sites for targeted drugs, and coadministration with other drugs is required. The correlation between TKIs and chemotherapy or immunotherapy in clinical practice requires further study.

KEYWORDS

gene fusion, ROS1 gene, tumor-targeted therapy, tyrosine kinase inhibitor

Shizhe Li and He Zhang contributed equally to this work and share first authorship.

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1 | INTRODUCTION

The proto-oncogene ROS1 (c-ROS) was first identified in glioblastoma cells in 1987[1] and is located at position 6q22 on the long arm of chromosome 6.[2] The ROS1 protein encoded by the *ROS1* gene is a type I intrinsic transmembrane tyrosine kinase receptor belonging to the insulin receptor family.[3] The self-mutation or rearrangement of ROS1 can activate tyrosine kinase, which promotes the proliferation of various malignant tumors.[4] ROS1 was subsequently detected in non-small cell lung cancer (NSCLC) in 2007 as a chromosomal rearrangement[5] and contains fusion genes, such as SLC34A2-ROS1,[6] SDC4-ROS1, and CD74-ROS1.[7] The fusion of ROS1 can lead to autophosphorylation, mediating tumor progression through the mitogen-activated protein kinase (MAPK) pathway or RAS phosphorylation.[8,9]

Clinical trials of ROS1-directed tyrosine kinase inhibitors (TKIs) have been conducted, showing considerable results. Currently, several ROS1-targeted drugs, such as crizotinib[10] and ceritinib,[11] have been approved by the FDA for use in a variety of malignancies, especially ROS1-positive NSCLC.[3] Although progress has been made with the use of ROS1 inhibitors in clinical practice, presistant challenges such as drug resistance and adverse reactions (including central nervous system [CNS] metastases) remain unresolved. Remon et al.[12] suggested that next-generation TKIs with high affinity and selectivity could be effective against drug resistance and CNS metastasis in ROS1-targeted therapy. However, these drugs have not been utilized in clinical practice, as they are still undergoing clinical trials. Therefore, elucidation of the biological mechanism of the *ROS1* gene, including the mechanism of *ROS1* gene mutation, clarification of the concept of targeted therapy, and development of next-generation inhibitors are crucial. In this study, ROS1-related articles were retrieved from PubMed/MEDLINE and Web of Science (search keywords: "ROS1" and "malignant tumors") to review the biological mechanisms, diagnostic modalities, and treatment effectiveness of ROS1 inhibitors to provide new insights into their clinical applications, therapeutic strategies, and directions for future development.

2 | BIOLOGICAL MECHANISMS OF THE *ROS1* GENE

2.1 | Nonmalignant biological mechanisms of the *ROS1* gene

ROS1 contains 47 exons, of which exons 1–34 encode the largest extracellular N-terminal domain of the human receptor tyrosine family.[13] Springer et al.[14] found that this domain is composed of three YWTD domains and scattered type III fibronectin (FN3). The YWTD domain has a round and folded "propeller" shape, which can closely interact with surrounding structures, and change the structure of ROS1. In addition, ROS1 and the anaplastic lymphoma kinase (ALK) domains are very similar at the amino acid level, sharing 77% identity at the ATP-binding site.[15] Considering the similarity between ROS1 and ALK, ALK inhibitors are also commonly used in ROS1-mutant tumors.[16] The signaling pathways involving ROS1 are related to the differentiation and proliferation of normal cells. ROS1 can activate and bind to the SH2 domain through the intracellular autophosphorylation of specific tyrosines, acting with specific adaptor proteins to mediate the RAS/RAF/MEK/ERK,[17] PI3K/AKT/mTOR,[18] and JAK/STAT3[19] signaling pathways (Figure 1). ROS1 can activate both PTPN6 (SHP1) and PTPN11 (SHP2). Despite their structural homology, PTPN6 often inhibits cell activity through a negative regulatory pathway, whereas PTPN11 mainly acts as a positive signal transducer.[20,21]

2.2 | Malignant biological mechanisms of the *ROS1* gene

ROS1 can facilitate the progression of various tumors through its overexpression, mutation amplification, or gene fusion. The overexpression of ROS1 was initially discovered in neurological tumors, such as primary gliomas[22] and meningiomas.[23] Cheng et al.[24] found that *ROS1* gene expression in the cytoplasm was positively correlated with the development of oral squamous cell carcinoma (OSCC). It has been suggested that ROS1 can mediate the invasiveness of OSCC by enhancing mitochondrial bioactivity and increasing cellular ATP levels.[25] Boyard et al.[26] found that the rearrangement and overexpression of ROS1 can activate the JAK/STAT pathway in inflammatory hepatocellular adenoma and promote its progression. Bajrami et al. suggested that ROS1 is highly expressed in E-cadherin (CDH1)-deficient breast cancer,

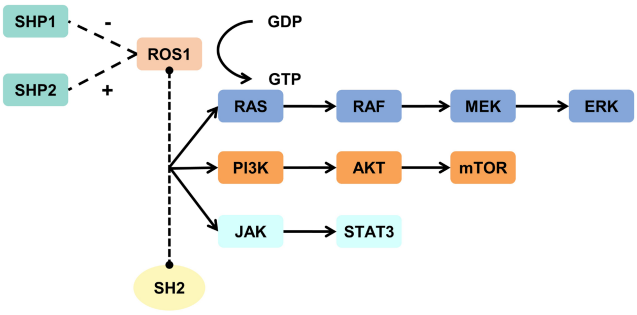

FIGURE 1 Nonmalignant biological signaling pathways involving the *ROS1* gene.

and its inhibitors (such as GSK1363089 and crizotinib) can destroy tumor cells via a synergistic lethal pathway.[27] A clinical trial (NCT04551495) investigating this is currently underway.[28]

Hou et al.[29] studied the cBioPortal for Cancer Genomics and next-generation sequencing (NGS) test results of 177 patients with lung adenocarcinoma and found the frequent occurrence of *ROS1* gene rearrangements in younger patients (<40 years old; $p = 0.035$); however, the significance of these mutations was unclear. Consistently, Wang et al.[30,31] also suggested that *ROS1* gene amplification or rearrangement mutations occurred more frequently in malignant tumors, such as gastrointestinal stromal tumors, gallbladder cancer, and soft tissue sarcoma. However, the significance and impact of these mutations require further investigation.

Compared to the overexpression and amplification of the *ROS1* gene, researchers believe that *ROS1* gene fusion is the primary driving force for tumorigenesis and disease progression.[4,13,32,33] Several *ROS1* fusion genes have been identified in NSCLC, Spitz neoplasms, and glioblastomas (Figure 2). In NSCLC, CD74 is the most common fusion partner of the *ROS1* gene, accounting for approximately 38% of cases, followed by EZR (13%), SDC4 (13%), and SLC34A2 (10%).[7] Gerami et al.[34] found that the PWWP2A-ROS1 fusion had the highest frequency among 16 cases of Spitz neoplasm. In addition, GOPC-ROS1 is the most common fusion in glioblastoma (75%)[35]; Richardson et al.[36] suggested that this fusion may be caused by a microdeletion in chromosome 6q22.1. In conclusion, the high-frequency ROS1 fusion partners differ among cancer types and are related to the intrinsic levels of fusion gene activation and fusion sites. For example, unlike in glioblastomas, *ROS1* gene fusion in NSCLC happens via interchromosomal translocations.[37,38] Differences in fusion partners lead to changes in subcellular localization, which affect downstream signaling pathways. For example, compared to CD74-ROS1, which acts on the endoplasmic reticulum, SDC4-ROS1 and SLC34A2-ROS1 have a stronger ability to activate the MAPK pathway.[39] Furthermore, there exists a mutually exclusive relationship between ROS1 fusions and other oncogenic mutations, a trend observed in NSCLC,[40] gliomas,[41] and Spitz neoplasms.[42] ROS1 is rarely co-mutated with the *ALK*, *EGFR*, or *KRAS* genes.[40] Despite the similarity and high homology of the ROS1 and ALK domains, ROS1/ALK double fusions rarely coexist.[43]

3 | DIAGNOSIS OF *ROS1* GENE MUTATIONS

ROS1 gene mutations can occur in a variety of tumors, including NSCLC (1%–2%),[44] gliomas (6%–7%),[45] and cholangiocarcinomas (1.1%),[46] among which ROS1 fusion NSCLC is the most common. A meta-analysis of 9898 patients with NSCLC indicated that ROS1 fusion occurred in younger female patients with no smoking history and was more prevalent in patients with advanced disease (stage III–IV) than those with early disease ($p < 0.001$).[47] Therefore, the prompt and effective diagnosis of ROS1 mutations is crucial. Several techniques have been reported for detecting ROS1 mutations that can be used for clinical screening or definitive diagnosis, as discussed in the succeeding sections.

3.1 | Immunohistochemical staining

Immunohistochemical staining (IHC) is a useful screening tool for ROS1 fusions. ROS1 IHC can be performed using cellular specimens, paraffin-embedded tissue

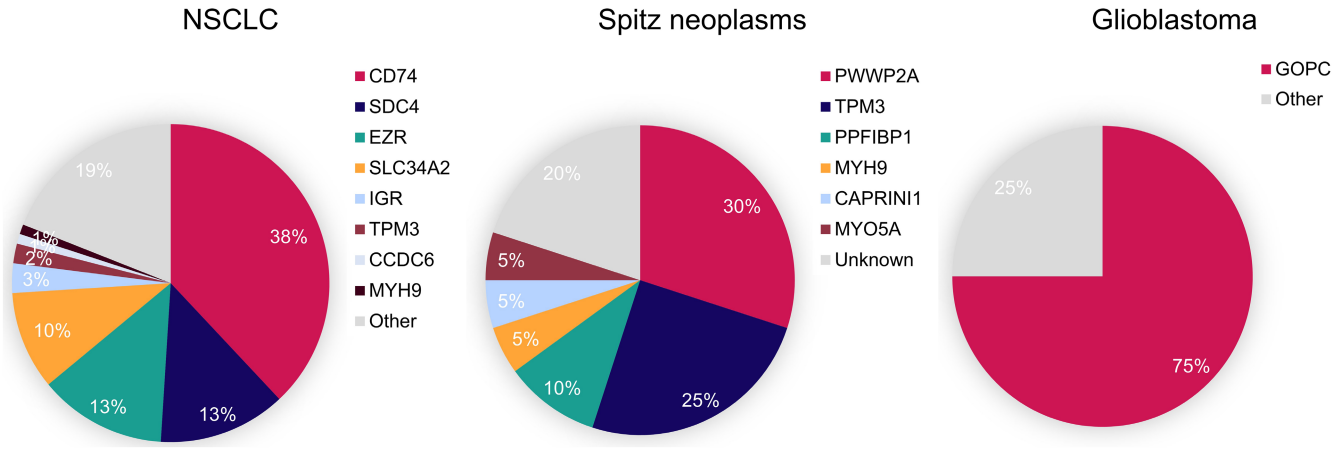

FIGURE 2 Pie chart of ROS1 fusion partner frequencies in non-small cell lung cancer (NSCLC), Spitz neoplasms, and glioblastoma (percentages shown).

specimens, and cell blocks for lung cancer detection.[48] The most commonly used antibody for detecting ROS1 is the D4D6 rabbit monoclonal antibody (Cell Signaling Technology, Danvers, MA, USA), which has a sensitivity and specificity of 89% and 98%, respectively.[49] A multicenter evaluation by Conde et al.[50] found the SP384 antibody (Ventana, Tuscon, Arizona, USA) to be more sensitive (93%) in detecting ROS1 gene rearrangements than the D4D6 antibody. However, Hofman's experimental results suggested that D4D6 is more accurate for ROS1 rearrangements.[51] Therefore, the use of these antibodies in IHC remains controversial. IHC results are mainly reported in the literature as 1+ (weak cytoplasmic staining), 2+ (moderate staining), or 3+ (strong staining) with positive microscopic manifestations of granular cytoplasmic staining.[52] IHC is an inexpensive and highly accurate screening method; however, it lacks clear scoring criteria and can be expressed at different levels in different tumor cells. In addition, normal tissues, such as type II alveolar and osteoclast-type giant cells, can also weakly express ROS1, which interferes with diagnosis.[53]

3.2 | Reverse transcription-polymerase chain reaction

Reverse transcription-polymerase chain reaction (RT-PCR) detects ROS1 rearrangements by identifying fusion mRNA and distinguishing fusion partners.[54] RT-PCR can be used to extract RNA from formalin-fixed paraffin-embedded tissue samples and cellular specimens,[55] requires fewer tissue samples, and can be easily performed within a shorter detection period. However, RT-PCR can only detect known fusion partners; it cannot identify unknown species of fusion genes.[56] Owing to the large number of unknown ROS1 fusion partners,[57] use of RT-PCR has certain limitations in clinical applications.

3.3 | Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) is currently the only technique that can detect almost all ROS1 mutations.[58] It involves using two probes labeled with different fluorescent colors (usually red, green, or orange) to target the 5′ and 3′ ends of ROS1.[59] ROS1 fusion manifests in FISH as a loss of the probe at the 5′ end or a break in the signal, separating the two colors.[60] An advantage of FISH is its ability to detect unknown fusion partners of ROS1. Furthermore, the cycle time, specificity, and sensitivity of FISH are clinically satisfactory.[59] However, FISH is expensive, and the requirements for tissue specimens are stringent, such as tissue sections from infants under 6 months of age.[56] Furthermore, FISH must be detected in more than 50 cell nuclei for diagnostic significance.[61] In addition, some fusion partners of ROS1 (GOPC-ROS1) cannot be detected by FISH owing to the limitations in the probe design[62]; self-designed probes can overcome this limitation.[63,64] However, FISH remains the gold standard for detecting *ROS1* gene mutations.

3.4 | Next-generation sequencing

The range of NGS technologies, from analysis of mutant gene regions to whole-genome sequencing, and their ability to detect multiple variant forms in parallel[65] save time and tissue samples compared to single-target detection. NGS can detect single nucleotide mutations, gene insertions/deletions, and genomic rearrangements, and both DNA and RNA can be used as samples.[66] NGS is currently used to detect *ROS1* gene rearrangements, particularly for ROS1 fusion genes with negative FISH results, such as GOPC-ROS1.[67] Additionally, NGS can be performed using cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA), which are valid tools for early diagnosis.[68] However, limitations exist in employing NGS technology for large-scale clinical applications. First, NGS is expensive when analyzing only a few genes.[69] Second, DNA-only detection is less accurate and does not cover intronic breakpoints with large amounts of repetitive nucleotides, which whole-genome RNA sequencing can overcome.[70] Benayed et al.[71] used an RNA-based genome sequencer to test samples from 2522 patients with lung adenocarcinoma and found an improvement in the detection rate of ROS1 rearrangements compared to that of DNA samples. Finally, no standardized NGS process exists for the different types and stages of tumor progression, so additional clinical information is needed to define rare variants.

In summary, each of these four detection methods has advantages and disadvantages (Table 1), and there is no definitive conclusion regarding which assay should be used to detect ROS1 mutations. Owing to the tendency of ROS1 to be mutually exclusive with genes such as *ALK*, this study suggests that IHC can be used to screen tumor specimens negative for *ALK* expression before conducting FISH on specimens with positive IHC results. By contrast, NGS may be more appropriate for cases requiring early diagnosis and guidance on drug use.

4 | ROS1 KINASE INHIBITORS

All ROS1 inhibitors are multi-kinase inhibitors that can inhibit ALK, MET, and other kinases (such as EGFR, JAK2, and TrkA) in addition to ROS.[72] Early-stage ROS1

TABLE 1 Advantages and disadvantages of ROS1 mutation detection technology.

Diagnostic techniques	Advantages	Limitations	Quotes
IHC	High sensitivity and specificity Wide range of selected materials Low cost	Absence of definitive diagnostic criteria Some normal tissues are also positively expressed, interfering with diagnosis	[48–53]
RT-PCR	Low tissue requirements. Short testing cycles and simplicity in operation.	Unable to detect unknown types of fusion partners	[54–57]
FISH	Known or unknown fusion partners can be detected Short testing period High sensitivity and specificity	High cost Stringent requirements for tissue specimens Undetectable partial fusion genes	[56,58–64]
NGS	Possibility of parallel testing, saving time and tissue samples Partial detection of FISH-negative fusion types Detectable ctDNA for early diagnosis	High cost Requires combined RNA sequencing to complement the range of detection No standardized process	[65–71]

Abbreviations: ctDNA, circulating tumor DNA; FISH, fluorescence in situ hybridisation; IHC, immunohistochemical staining; NGS, next-generation sequencing; RT-PCR, reverse transcription polymerase chain reaction.

TKIs, such as crizotinib, ceritinib, and entrectinib, can be used in patients with ROS1-positive NSCLC who have not received TKI treatment (TKI-naïve).[10,73] Considering resistance mutations are inevitable during treatment with early-stage TKIs, next-generation TKIs such as cabozantinib and taletrectinib have been tested in clinical trials. However, most of these are not approved for ROS1-positive tumors.[74–76]

ROS1 kinase inhibitors primarily affect the kinase domain of ROS1.[77] Conventional kinases can be classified into DFG-in (active, type I) and DFG-out (inactive, type II) kinases, depending on the domain conformation. DFG refers to the activation loop that regulates kinase activity, consisting of aspartic acid (D), phenylalanine (F), and glycine (G).[78] When the domain is in the DFG-in state, aspartate rotates inward to expose the ATP-binding site. Most TKIs (crizotinib, ceritinib, entrectinib, taletrectinib, and repotrectinib) compete for the ATP-binding site, preventing further phosphorylation of the kinase by ATP, thus inhibiting kinase activity.[79] In the DFG-out state, the aspartic acid in the domain is rotated outward and exposes a hydrophobic site that can be occupied by a few TKIs (such as cabozantinib and foretinib) to inhibit tumor activity.[79] As the DFG-out conformation does not need to participate in the catalytic reaction, resulting in less restricted structure and providing greater selectivity in the design of inhibitors. However, the development of type II kinase inhibitors remains challenging because most kinases lack the DFG-out domain conformation.

Both type I and type II ROS1 kinase inhibitors have inhibitory effects on ROS1 fusion-positive tumors,[80] including crizotinib in CD74-ROS1 and SDC4-ROS1 fusion NSCLC,[81] ceritinib in TFG-ROS1 fusion inflammatory myofibroblastoma,[82] and repotrectinib in G2032R-ROS1 and D2033N-ROS1 fusion tumors.[83] Furthermore, studies by Davare and Facchinetti have shown slight differences in the inhibitory effects of TKIs on different ROS1 fusion phenotypes.[84,85] However, a statistical review of clinical trial data by Drilon et al.[13] revealed that differences in the level of inhibition did not affect treatment efficacy.

5 | TREATMENT OF ROS1-MUTANT TUMORS

5.1 | NSCLC

NSCLC accounts for approximately 80% of all lung cancers, and ROS1 fusion-positive NSCLC accounts for 1%–2% of NSCLC cases.[86] Currently, ROS1 kinase inhibitors are the first-line treatment for advanced ROS1 fusion-positive NSCLC[87] (Table 2 and Figure 3).

In the phase I PROFILE 1001 clinical trial, 50 patients with advanced NSCLC treated with standard doses of crizotinib had an objective remission rate (ORR) of 72%, a median duration of remission (DOR) of 17.6 months, and a mean progression-free survival (PFS) of 19.2 months.[88] Based on the significant antitumor activity observed in this trial, crizotinib was the first drug approved by the FDA for advanced ROS1 rearrangement-positive NSCLC.[88] Recent reports from this trial showed an increased DOR of 24.7 months and PFS of 19.3 months after crizotinib treatment, with similar adverse effects, including visual impairment, edema, vomiting, and diarrhea.[89] A phase II single-arm trial (NCT01945021) of 127 patients with ROS1 rearrangement-positive NSCLC in East Asia by Wu et al.[57] reported an ORR of 71.7% and a mean PFS of 15.9 months for crizotinib. Another result from the EUROS1 cohort demonstrated an ORR of 80% and a mean PFS of 9.1 months in patients treated with

TABLE 2 Summary of clinical trials for TKIs in ROS1 rearrangement-positive NSCLC.

Drug	Clinical trial identifier/ Study	Study design	ORR	Median DOR (months)	Median PFS (months)
Crizotinib	NCT00585195	Interventional, parallel-cohort, Open-label Phase I trial	72%	24.7	19.3
	NCT01945021	Interventional Single-group assignment Open-label Phase II Single-arm trial	71.7%	19.7	15.9
	EUROS1	Single-group assignment Open-label	80%	–	9.1
	NCT02183870/EUCROSS	Interventional, Single-group assignment Open-label Multicentre Phase II trial	70%	–	20.0
Ceritinib	NCT01964157	Interventional Open-label Multicentre Phase II trial	62%	21.0	9.3
Entrectinib	ALKA-372-001, STARTRK-1, STARTRK-2	Interventional Open-label Multicentre Phase I/II trial	67.1% 79.2% (CNS)	15.7 12.9 (CNS)	15.7 12.0 (CNS)
Lorlatinib	NCT01970865	Interventional Non-randomized Open-label Multicentre Phase I/II Single-arm trial	41%	25.3	21.0 (TKI-naïve) 8.5 (crizotinib-treated)
Repotrectinib	NCT03093116	Interventional Single-group assignment Open-label Multicentre Phase I/II trial	79% (TKI-naïve) 38% (TKI-treated)	34.1 (TKI-naïve) 14.8 (TKI-treated)	35.7 (TKI-naïve) 9.0 (TKI-treated)
Taletrectinib	NCT02279433, NCT02675491	Interventional Non-randomized Single-group assignment Open-label Multicentre Phase I/IB trial	66.7% (TKI-naïve) 33.3% (TKI-refractory)	23.5 (TKI-naïve) 14.0 (TKI-refractory)	29.1 (TKI-naïve) 14.2 (TKI-refractory)
TQ-B3139	NCT03099330	Interventional Single-group assignment Phase I trial Open-label	66.7%	–	20.2 (partial response) 27.0 (complete response)
Cabozantinib	Sun et al.	–	25%	–	7.4
Brigatinib	Dudnik et al.	–	29% (crizotinib-resistant)	–	21.6 (TKI-naïve) 2.5 (crizotinib-resistant)

crizotinib.[90] In the follow-up EUCROSS cohort phase II study (NCT02183870), the ORR was 70% in 30 patients, and PFS improved to 20.0 months.[91] This suggests that crizotinib is highly effective in NSCLC patients of different ethnicities. However, most patients with advanced NSCLC treated with crizotinib eventually experience

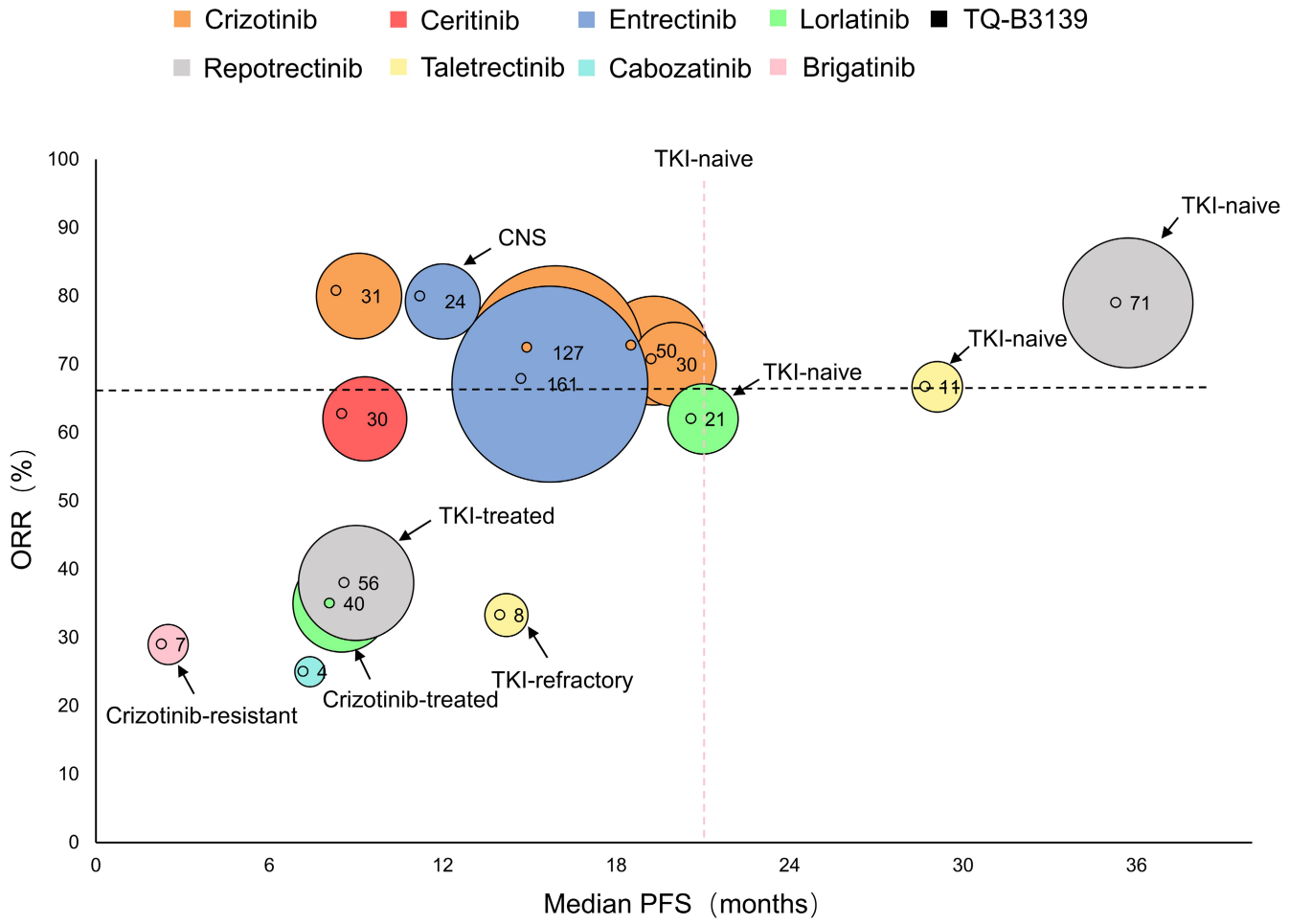

FIGURE 3 Activity of tyrosine kinase inhibitors (TKIs) in clinical trials involving ROS1 rearrangement-positive non-small cell lung cancer (NSCLC), comparing the objective remission rates (ORRs) and progression-free survival (PFS). Unclear ORRs or PFS are indicated with dashed lines.

disease progression owing to ROS1 resistance mutations or CNS metastases.

Ceritinib is currently approved for patients with ALK rearrangement-positive NSCLC resistant to crizotinib[92,93]; however, it is also effective in patients with ROS1 rearrangement-positive NSCLC. A phase II trial (NCT01964157) comprising 30 patients with advanced ROS1 rearrangement-positive NSCLC not treated with crizotinib indicated that after a standard dose of ceritinib treatment (750 mg once daily), patients exhibited an ORR of 62%, a DOR of 21 months, and a mean PFS of 9.3 months; the common adverse events reported were gastrointestinal symptoms (78% diarrhea, 59% nausea, and 56% anorexia).[94] As a selective ROS1 inhibitor, ceritinib is 20 times more effective than crizotinib.[95] However, despite promising trial data, ceritinib may not work against crizotinib-resistant mutations, including G2032R, D2033N, and L2086F.[80,96] In addition, ceritinib has a higher incidence of adverse effects than crizotinib.[94,97,98]

Entrectinib is a multi-kinase inhibitor that inhibits ROS1, ALK, and TRK.[99] According to an analysis of three phase I and II clinical trials (ALKA-372-001, STARTRK-1, and STARTRK-2), entrectinib had an ORR of 67.1% and a DOR and PFS of 15.7 months. For those who developed CNS metastases, the ORR was 79.2%, DOR was 12.9 months, and PFS was 12.0 months, with primary adverse effects including weight gain and neutropenia.[100,101] Unlike crizotinib, entrectinib has a weaker interaction with P-glycoprotein (P-gp) and can thus cross the blood–brain barrier, yielding ideal blood concentrations in the CNS and efficacy in patients with NSCLC who develop intracranial metastases.[102,103] Based on the positive clinical trial results and the specificity of the drug itself, entrectinib has become the second targeted agent approved by the FDA for treating patients with advanced ROS1 rearrangement-positive NSCLC.[104] However, similar to ceritinib, entrectinib appears to be ineffective in crizotinib-resistant cases, particularly in the absence of activity in G2032R- and L2026M-mutant tumor cells.[73,105]

Other next-generation TKIs also play an indispensable role in the treatment of ROS1 rearrangement-positive NSCLC, including lorlatinib, repotrectinib, taletrectinib,

TQ-B3139, cabozantinib, and brigatinib. The ORR of lorlatinib in a single-arm phase I-II trials (NCT01970865) was 62% for TKI-naïve cases and 35% for crizotinib-treated cases, PFS was 21.0 months for TKI-naïve cases compared to 8.5 months for crizotinib-treated cases; the common adverse effects include hypertriglyceridemia (19%) and hypercholesterolemia (14%).[106] A phase I–II trial of repotrectinib (NCT03093116) showed an ORR of 79%, PFS of 35.7 months, and DOR of 34.1 months in TKI-naïve cases. Repotrectinib was also shown to be effective in patients who had previously received ROS1 TKI and had never received chemotherapy, with ORR of 38%, PFS of 9.0 months, and DOR of 14.8 months. The main adverse reactions were dizziness (58%), dysgeusia (50%), and paresthesia (30%).[107] Phase I studies of taletrectinib in the United States (NCT02279433) and Japan (NCT02675491) showed an ORR of 66.7% and a mean PFS of 29.1 months in patients with TKI-primary ROS1-mutated NSCLC compared with an ORR of 33.3% and a PFS of 14.2 months in crizotinib-resistant cases; its adverse effects included abnormal liver function (72.7%) and gastrointestinal symptoms (50%).[108] The clinical activity of TQ-B3139 as a novel TKI was validated. A phase I trial involving four patients with ROS1 fusion NSCLC (NCT03099330) showed that TQ-B3139 had an ORR of 66.7%, along with a PFS of 20.2 months (partial response) or 27.0 months (complete response).[109] The results of trials with cabozantinib (ORR, 25%; PFS: 4.9–13.8 months)[110] and brigatinib (ORR, 29%)[111] have also demonstrated potent antitumor activity. Next-generation TKIs also performed well in crizotinib-resistant NSCLC, such as repotrectinib for the G2032R/D2033N fusion mutation,[83] lorlatinib and taletrectinib for the G2032R fusion mutation,[112,113] and cabozantinib and brigatinib for the CD74 fusion mutation.[114,115] Despite these promising results, only repotrectinib has been approved by the FDA as a first-line agent for ROS1 rearrangement-positive NSCLC.[103] Further trials are necessary to demonstrate the efficacy and safety of next-generation TKIs for clinical use.

The role of immunotherapy in ROS1-positive NSCLC is not well-defined. Choudhury et al. found that ROS1 regulates the expression of programmed cell death 1 ligand 1 (PD-L1) by activating the MEK-ERK and ROS1-SHP2 pathways. However, most ROS1-positive NSCLC cells do not express PD-L1 and have a low mutation load.[116] Immunotherapy combined with chemotherapy elicited a higher ORR in patients with ROS1-positive NSCLC compared with immunotherapy alone.[117,118] In addition, a phase II clinical trial of Atezolizumab (NCT04042558) showed that Atezolizumab with or without bevacizumab and the "platinum-Pemetrexed" chemotherapy regimen are effective in NSCLC patients with metastatic EGFR mutation or ALK/ROS1 rearrangement after TKI failure.[119] This suggests that immunotherapy combined with chemotherapy is a potential treatment strategy for patients with ROS1-positive NSCLC, especially those for whom TKI therapy has failed.

5.2 | Non-NSCLC tumors

ROS1 kinase inhibitors are also effective in non-NSCLC tumors; however, owing to the low incidence of ROS1 rearrangements, no relevant TKIs are currently approved for treating non-NSCLC tumors. This has also been mentioned in the literature as a case report (Table 3). For example, crizotinib has shown good or partial remission in YWHAE1-ROS1 fusion inflammatory myofibroblastoma (IMT),[120] GOPC-ROS1 fusion Spitz naevi, high-grade serous ovarian cancer,[67,121] RDX-ROS1 fusion intrahepatic cholangiocarcinoma,[122] and TJP1-ROS1 fusion malignant peripheral nerve sheath tumors(MPNST).[123] Cases on the use of entrectinib for ER+/HER2-breast cancer, ARCN1-ROS1 and ZCCHC8-ROS1 fusion pediatric glioma, GOPC-ROS1 fusion limbal melanoma, and SLC4A4-ROS1 fusion metastatic pancreatic cancer have also been reported.[28,124–127] Among the next-generation TKIs, lorlatinib has been suggested to be potentially effective in ROS1 p.L1950F point-mutated pancreatic cancer and TFG-ROS1 fusion IMT of the chest wall,[128,129] with no relevant case reports for other TKIs.

6 | CHALLENGES OF ROS1-TARGETED THERAPY

6.1 | Adverse effects related to ROS1 inhibitors

Adverse events (AEs) are a major challenge in ROS1-targeted therapy. The use of most ROS1 kinase inhibitors in clinical practice has been limited because of the AEs associated with their mechanism of action (Table 4), such as edema, visual disorders, gastrointestinal symptoms, and, in severe cases, liver function abnormalities, neutropenia, and even life-threatening effects.[130,131] Some AEs are related to the unique systems in which TKIs act. For example, entrectinib inhibits TRK and acts on the nervous system, resulting in dizziness, weight gain, and cognitive impairment during targeted therapy.[132] Moreover, crizotinib can cause peripheral edema in patients because of MET kinase inhibition.[133,134] However, other adverse reactions still lack clear mechanisms to explain their occurrences, such as abnormal liver function,[135] manifestations of ocular toxicity associated with crizotinib treatment,[136] hyperlipidemia, and peripheral neuropathy associated with lorlatinib treatment.[131] There is currently no standardized

TABLE 3 Overview of ROS1 kinase inhibitor therapy in non-NSCLC tumors.

Drug	Study	Tumor histology	Fusion/Mutation	Response
Crizotinib	Comandini et al.	IMT	YWHAE1-ROS1	PR
	Robertson et al.	Spitz nevi	GOPC-ROS1	PR
	Dong et al.	High-grade serous ovarian cancer	GOPC-ROS1	PR
	Jakubowski et al.	Intrahepatic cholangiocarcinoma	RDX-ROS1	PR
	Li et al.	MPNST	TJP1-ROS1	SD
Entrectinib	Agostinetto et al.	ER+/HER2-breast cancer (invasive lobular carcinoma)	E-cadherin defect	PR
	Mayr et al.	Pediatric high-grade glioma	ARCN1-ROS1	PR
	Papusha et al.	Infant hemispheric glioma	ZCCHC8-ROS1	PR
	Couts et al.	Acral lentiginous melanoma	GOPC-ROS1	PR
	Pishvaian et al.	Metastatic pancreatic cancer	SLC4A4-ROS1	SD
Lorlatinib	Velthaus et al.	Pancreatic cancer	ROS1 p.L1950F mutation	SD
	Carcamo et al.	IMT of the chest wall	TFG-ROS1	PR

Abbreviations: IMT, inflammatory myofibroblastoma; MPNST, malignant peripheral nerve sheath tumor; PR, partial response; SD, stable disease.

clinical treatment process to mitigate the damage caused by AEs. Bauer et al.[131] suggested that adjusting drug doses or symptomatic supportive treatment could manage mild to moderate AEs (grade <3). Although next-generation TKIs are considered to have potential value in reducing the incidence of AEs owing to their high affinity,[13] recent studies have demonstrated that next-generation TKIs do not have an absolute advantage in treating AEs.[137,138]

6.2 | Resistance to ROS1 inhibitors

The prolonged use of ROS1 inhibitors often leads to the development of drug resistance in tumor cells during disease progression. The mechanisms leading to the development of drug resistance in tumors mainly include mutations in the kinase domain and activation of collateral signaling.[139,140] Point mutations in the structural domain of the ROS1 kinase can transform the drug target and reduce its inhibitory effect, leading to resistance to targeted therapies.[141] G2032R was the first identified and most common drug resistance mutation in ROS1.[142] The mutation results in the substitution of glycine with an arginine that still enables ATP binding but conflicts with the piperidine ring structure of crizotinib, causing resistance to ROS1 kinase inhibition, mediating epithelial-mesenchymal transition, and upregulating Twist 1, contributing to tumor progression.[141,143,144] Another mutation, D2033N, causes drug resistance by affecting the solvent front region of the ATP-binding site.[145] In addition, other drug resistance mutations, such as L2155S, affect protein function to enhance drug resistance,[143] and S1986F/Y (parallel mutation) and L2026M, promote kinase activity by blocking the critical binding site.[85,146] This mechanism of action accounts for 50%–60% of ROS1-resistant tumors.

The activation of bypass signaling pathways (e.g., the EGFR pathway) has been shown to confer drug resistance by reducing tumor dependence on ROS1 activity and increasing its dependence on the self.[147,148] Including previously reported mutations in the KIT and MAPK pathways, autophosphorylation or alterations in the MEK/ERK pathway can render tumor cells resistant to crizotinib[8,149] (Figure 4).

In addition, resistance to ROS1 inhibitors can lead to the phenotypic transformation of tumor cells.[150] Lin et al. reported a case of a patient with NSCLC whose pathological classification transformed into adenocarcinoma after ROS1 inhibitor resistance; this phenotypic change may be related to retinoblastoma-1 (RB1) and TP53 inactivation.[151]

The current mainstream response in response to kinase inhibitor resistance is to replace next-generation TKIs or combine them with other kinase inhibitors. Yun et al.[76] found that repotrectinib has a targeted therapeutic effect against the G2032R mutation with a similar response observed with foretinib.[84] Lorlatinib has therapeutic effects against other mutations, such as L2026M and S1986F/Y.[152] In particular, combinations of kinase inhibitors appeared to be more effective against resistance due to the activation of bypass signaling pathways. Dziadziuszko et al.[149] discovered that combining crizotinib with ponatinib could overcome resistance caused by mutations in the KIT pathway in vitro. Vaishnavi et al.[140] detected the expression of the EGFR pathway in SLC34A2-ROS1 fusion HCC78 cells, and the coadministration of crizotinib with the EGFR inhibitor gefitinib reduced drug resistance in HCC78 cells.[153] Aside from point mutations in the structural domain of ROS1 and the activation of bypass signaling pathways, some unknown drug resistance

TABLE 4 Summary of AEs associated with clinical trials of ROS1 inhibitors.

Drug	Study	Overall AEs in ≥20% of patients	Treatment-related AEs (≥ grade 3)
Crizotinib	PROFILE 1001	Vision disorder (87%) Nausea (51%) Oedema (47%) Diarrhea (45%) Vomiting (38%) Elevated transaminases (36%) Constipation (34%) Bradycardia (21%) Fatigue (21%)	Hypophosphatemia (15%) Neutropenia (9%) Vomiting (4%) Elevated transaminases (4%)
	NCT01945021	Elevated transaminases (55.1%) Vision disorder (48%) Nausea (40.9%) Diarrhea (38.6%) Vomiting (32.3%) Constipation (29.9%) Neutropenia (29.1%) Leukopenia (22.8%) Oedema (22.8%)	Neutropenia (10.2%) Elevated transaminases (5.5%) Leukopenia (2.4%) Nausea (1.6%)
	EUCROSS	Vision disorder (65%) Diarrhea (56%) Oedema (50%) Bradycardia (47%) Nausea (41%) Increased ALT (35%) Vomiting (32%) Leukopenia/neutropenia (32%) Increased AST (26%) Increased blood creatinine (21%)	Leukopenia/neutropenia (9%) Nausea (3%) Vomiting (3%) ALT increased (3%) Dysgeusia (3%) Pulmonary embolism (3%)
Ceritinib	NCT01964157	Diarrhea (78%) Nausea (62%) Anorexia (59%) Vomiting (53%) Cough (47%) Pain (41%) Fatigue (38%) Dyspnoea (25%)	Fatigue (16%) Pneumonia (12%) Dry mouth (3%) Pleural effusion (3%)
Entrectinib	NCT02097810, NCT02568267	Dysgeusia (43.4%) Dizziness (34.8%) Constipation (31.4%) Fatigue (30%) Weight gain (28.6%) Diarrhea (26.7%)	Weight gain (8.1%) Increased ALT (3.3%) Diarrhea (2.9%) Increased AST (2.4%) Decreased neutrophil count (2.4%) Neutropenia (1.9%) Rash (1.4%)
Lorlatinib	NCT01970865	Hypercholesterolaemia (72%) Hypertriglyceridemia (39%) Peripheral oedema (39%) Peripheral neuropathy (39%) Cognitive effects (22%)	Hypercholesterolaemia (13%) Hypertriglyceridemia (6%) Weight gain (6%) Increased lipase (4%) Cognitive effects (2%) Increased AST (2%)
Repotrectinib	NCT03093116	Dizziness (58%) Dysgeusia (50%) Paresthesia (30%) Constipation (26%) Anemia (26%) Ataxia (20%)	Anemia (4%) Increased blood creatine kinase leve (4%) Dizziness (3%) Weight increase (2%)

TABLE 4 (Continued)

Drug	Study	Overall AEs in ≥20% of patients	Treatment-related AEs (≥ grade 3)
Taletrectinib	NCT02279433, NCT02675491	Increased ALT (72.7%) Increased AST (72.7%) Nausea (59.1%) Diarrhea (54.5%) Vomiting (36.4%) Increased blood creatinine (31.8%)	Increased ALT (22.7%) Increased AST (13.6%) Diarrhea (4.5%)

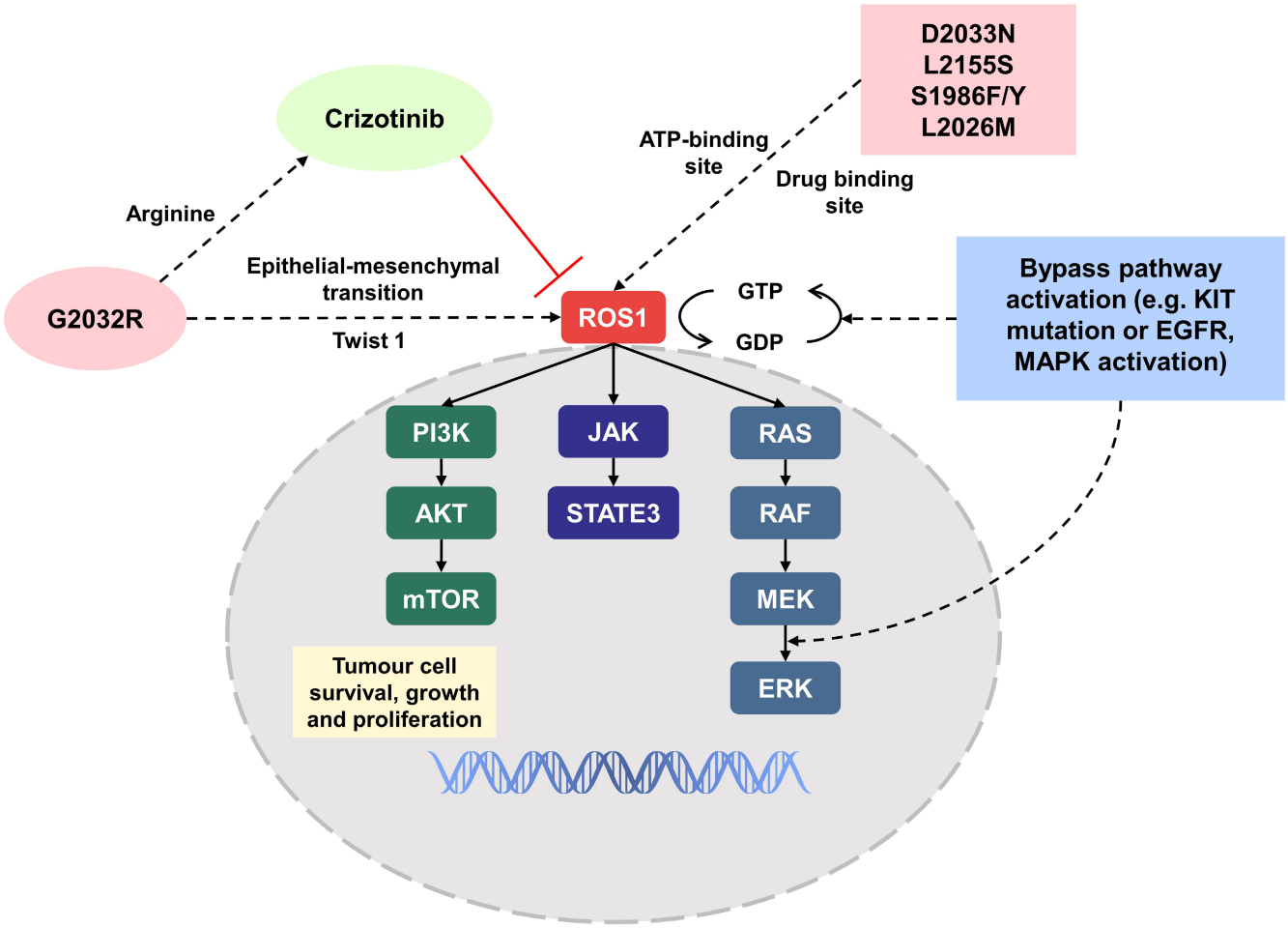

FIGURE 4 Molecular mechanisms of resistance to ROS1 tyrosine kinase inhibitors (TKIs), depicting the two main drug resistance patterns.

mechanisms exist, such as lorlatinib resistance in patients with ROS1 fusion-positive NSCLC.[80] Therefore, when developing next-generation TKIs, future studies should focus on epigenetics, RNA, and proteins to elucidate the mechanisms of ROS1 resistance.

6.3 | Central nervous system metastases

CNS metastases are common in ROS1-mutant malignancies. CNS metastases occur in approximately 36% of patients with stage IV ROS1-positive NSCLC.[154] Li et al.[155] found a higher incidence of CNS metastasis in patients with NSCLC having CD74-ROS1 fusion compared to non-CD74-ROS1 fusion cases ($p = 0.020$), suggesting that the type of fusion partner is associated with CNS metastasis. Jun et al.[156] discovered that CD74-ROS1 confers a higher metastatic potential to tumor cells by activating the phosphorylation of the plasma membrane protein E-Syt1. Due to its poor ability to cross the blood–brain barrier, crizotinib cannot be used as the first drug of choice for CNS metastasis.[157] However, next-generation TKIs have exhibited great potential in cases of CNS metastases, including ceritinib, which showed an ORR of 25% in patients with CNS metastases in

a phase II study,[94] and lorlatinib, which showed an ORR of 50%.[106] Among these, entrectinib is the most effective, with an ORR of 55% and a mean PFS of 13.6 months.[100] In contrast to TKI replacement, CNS metastases are sensitive to radiotherapy. Hence, they can be an option for controlling the progression of CNS metastases when patients cannot tolerate targeted therapy or have few lesions.[73]

7 | PROSPECTS AND CONCLUSIONS

As a proto-oncogene, ROS1 is mostly expressed in malignant tumors such as NSCLC. Chromosomal rearrangements resulting in ROS1 fusions drive tumor progression, and an appropriate diagnosis should be made on a clinical basis. Only a few ROS1 kinase inhibitors are currently approved for use in NSCLC; however, the efficacy of other TKIs for NSCLC and other malignancies has not been ascertained, as they are still being tested in clinical trials. Next-generation TKIs appear capable of overcoming resistance and delaying CNS metastasis owing to their high affinity; however, they are associated with a greater incidence of adverse effects. Further research on next-generation TKIs regarding the localization of ROS1 and its fusion partners, binding sites for targeted drugs (type I vs. type II), and coadministration with other drugs is required. Moreover, the correlation between TKIs and chemotherapy or immunotherapy in clinical practice requires further study.

AUTHOR CONTRIBUTIONS

Shizhe Li: Writing – original draft (equal). **He Zhang:** Methodology (equal); software (equal). **Ting Chen:** Data curation (equal); supervision (equal). **Xiaowen Zhang:** Writing – review and editing (equal). **Guanning Shang:** Writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

The materials that support the conclusion of this review have been included within the article.

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