Clinical and molecular factors that impact the efficacy of first-line crizotinib in ROS1-rearranged non-small-cell lung cancer: a large multicenter retrospective study

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Abstract

Background: ROS1-rearranged lung cancers benefit from first-line crizotinib therapy; however, clinical and molecular factors that could affect crizotinib efficacy in ROS1-rearranged lung cancers are not yet well-elucidated. Our retrospective study aimed to compare the efficacy of chemotherapy and crizotinib in the first-line treatment of ROS1-rearranged advanced lung cancer and evaluate various clinical and molecular factors that might impact crizotinib efficacy in real-world practice.

Methods: Treatment responses, survival outcomes, and patterns of disease progression were analyzed for 235 patients with locally advanced to advanced disease who received first-line chemotherapy (n = 67) or crizotinib (n = 168).
Results: The overall response rate was 85.7% (144/168) for first-line crizotinib and 41.8% (28/67) for chemotherapy. Patients treated with first-line crizotinib (n = 168) had significantly longer median progression-free survival (PFS) than chemotherapy (n = 67) (18.0 months vs. 7.0 months, p < 0.001). Patients harboring single CD74-ROS1 (n = 90) had significantly shorter median PFS with crizotinib than those harboring non-CD74 ROS1 fusions (n = 69) (17.0 months vs. 21.0 months; p = 0.008). Patients with baseline brain metastasis (n = 45) had a significantly shorter PFS on first-line crizotinib than those without brain metastasis (n = 123) (16.0 months vs. 22.0 months; p = 0.03). At progression, intracranial-only progression (n = 40), with or without baseline CNS metastasis, was associated with longer median PFS than those with extracranial-only progression (n = 64) (19.0 months vs. 13.0 months, p < 0.001). TP53 mutations were the most common concomitant mutations, detected in 13.1% (7/54) of patients with CD74-ROS1 fusions, and 18.8% (6/32) with non-CD74 ROS1 fusions. Patients with concomitant TP53 mutations (n=13) had significantly shorter PFS than those who had wild-type TP53 (n = 81) (6.5 months vs. 21.0 months; p < 0.001). PFS was significantly shorter for the patients who harbored concomitant driver mutations (n = 9) (11.0 months vs 24.0 months; p = 0.0167) or concomitant tumor suppressor genes (i.e., TP53, RB1, or PTEN) (n = 25) (9.5 months vs 24.0 months; p < 0.001) as compared to patients without concomitant mutations (n = 58).

Conclusion: Our results demonstrate that baseline brain metastatic status and various molecular factors could contribute to distinct clinical outcomes from first-line crizotinib therapy of patients with ROS1-rearranged lung cancer.

Clinical trials registration: CORE, NCT03646994

Keywords: ROS1 gene rearrangements, Crizotinib, Concomitant mutations, Progression associated efficacy

Background
Genomic rearrangements involving ROS proto-oncogene-1 (ROS1) are actionable targets in the treatment of non-small-cell lung cancer (NSCLC) [1]. The overall prevalence of ROS1 fusions is reported to be 2% in NSCLC and up to 3.3% in lung adenocarcinoma [1–4]. ROS1, located at the long arm of chromosome 6q22, encodes one of the receptor tyrosine kinases of the insulin receptor family; however, its exact activating ligand remains unidentified and hence is considered an orphan receptor [4, 5]. Since the amino acid sequence of the kinase domains of ROS1 and ALK are highly homologous, selective inhibitors of ALK, including crizotinib, have shown anti-tumor activity in vitro and have been explored clinically in the treatment of patients with ROS1-rearranged tumors [5–13]. Crizotinib has been approved for use in ROS1-rearranged NSCLC based on the PROFILE 1001 study, which observed an objective response rate (ORR) of 72%, median progression-free survival (PFS) of 19.2 months, and median overall survival of 51.4 months among the 53 patients with ROS1-rearranged NSCLC included in the expansion cohort [8, 14]. Meanwhile, the largest phase II study conducted to date on crizotinib response of ROS1-positive NSCLC demonstrated an ORR of 71.7% with a median PFS of 15.9 months among 127 East Asian patients [13]. Given the rarity of ROS1 fusions, the effect of ROS1 fusion variants and other clinical and molecular factors on the efficacy of crizotinib is not well-elucidated. So far, the real-world studies describing the efficacy of crizotinib in Asian patients with ROS1-rearranged NSCLC mostly involved smaller cohorts.

This retrospective cohort study aimed to explore the efficacy of crizotinib as a first-line treatment for advanced NSCLC with various ROS1 rearrangements. We also investigated clinical and molecular factors that could impact the clinical outcomes of patients with locally advanced to advanced ROS1-rearranged NSCLC from first-line crizotinib therapy.

Patients
Included patients
We retrospectively screened 21,747 consecutive treatment-naïve patients who were diagnosed with lung cancer from August 1, 2018, to March 31, 2020, and submitted samples for molecular detection of ROS1 rearrangements, including next-generation sequencing (NGS), fluorescence in situ hybridization (FISH), and amplification refractory mutation system (ARMS) at various hospitals in Hunan, Hubei, Guangdong, and Zhejiang Provinces in China. All the patients who were analyzed for clinical outcomes from first-line crizotinib met the following criteria: (1) pathologically-confirmed NSCLC, (2) locally advanced/unresectable disease to advanced/metastatic disease; (3) ROS1 rearrangements identified by NGS, and (4) treatment with crizotinib in the first-line setting. Written informed consent was obtained from all the patients for the use of their data for research purposes. All procedures in our study were performed following the ethical standards of the institutional and national research committees, and the Declaration of Helsinki as revised in 2013. Approval was obtained from the Hunan Cancer Hospital Institutional Review Board Committee (approval number: 2017YYQ-
SSB-026). This study was also registered as a clinical trial (CORE, NCT03646994).

**NGS**

Patient samples were submitted for NGS-based analysis to Burning Rock Biotech, a College of American Pathologists-accredited, Clinical Laboratory Improvement Amendments -certified clinical laboratory. Briefly, DNA isolated from the tissue biopsy, blood, or pleural effusion samples obtained from all the patients were processed accordingly for NGS using commercially available panels targeting various cancer-related genes and sequenced on a Nextseq 500 (Illumina, CA, USA) with paired-end reads with a target sequencing depth of 1000X for tissue samples and 10,000X for liquid biopsy samples using optimized protocols [15]. All the gene capture panels used in our study, including 8, 56, 108, 168, and 295 cancer-related genes, interrogated whole exons and critical introns for the 8 classic NSCLC oncoplastic drivers, which includes EGFR, ALK, BRAF, ERBB2, KRAS, MET, RET, and ROS1. To understand the impact of concomitant mutations, including TP53, other tumor suppressor genes, and oncogenic driver genes, on crizotinib outcomes, we selected the patient samples that were assayed using 168-gene or 295-gene panels (n = 94). We only analyzed the 168 genes common between the 2 panels. Table S1 lists the genes included in the 168 gene panel. The sequencing analyses were performed using optimized bioinformatics pipelines for somatic variant calling that involved accurate identification of base substitutions, small insertions-deletions, copy number variations, and genomic rearrangements as described previously [15].

Non-reciprocal/reciprocal translocations are defined by the simultaneous detection of at least one ROS1 fusion that contains the tyrosine kinase domain and another ROS1 fusion involving the 5'-region of ROS1 fused with other gene partners aside from the gene partner fused to the ROS1 tyrosine kinase domain, as previously described with ALK fusions [16].

**Evaluation of treatment efficacy**

Crizotinib was orally administered at a dose of 250 mg twice daily. Chemotherapy with either pemetrexed-cisplatin regimen or docetaxel-cisplatin regimen was administered intravenously. The dose for pemetrexed was 500 mg per square meter of body-surface area (m²), plus carboplatin at a dose of target area under the curve of 5 to 6 mg per milliliter per minute. The dose for docetaxel was 75 mg/m² and 75 mg/m² for cisplatin. The treatment regimens were administered until progressive disease (PD) or unacceptable toxicity. Patients who experienced toxicity were managed by dose reduction or discontinuation as decided by their physicians. The best responses were assessed by the investigators according to the Response Evaluation Criteria in Solid Tumors version 1.1. For patients treated with crizotinib, as local law for drug purchase, all patients were required to undergo radiologic imaging evaluation including chest computed tomography (CT) scanning and brain magnetic resonance imaging (MRI) every 2 cycles. For patients treated with chemotherapy, as they were all treated in hospital, all patients were required to undergo chest radiologic imaging evaluation including chest CT and brain MRI every 2 cycles. The overall response rate (ORR) was calculated as the proportion of patients with complete response (CR) or partial response (PR). The disease control rate (DCR) was calculated as the proportion of patients with CR, PR, and stable disease (SD). Progression-free survival (PFS) was measured from the start of crizotinib administration until the date of PD or death from any causes. Each image was independently evaluated by two radiologists. The data cutoff date was December 31, 2020. The median follow-up duration was 28 (range: 2–59) months.

**Statistical analysis**

Continuous variables were summarized as means and standard deviations or medians with range and compared using unpaired t test or Wilcoxon signed-rank test. Categorical variables were summarized as frequencies with percentages and compared using Chi-squared or Fisher’s exact test, as appropriate. Kaplan-Meier analysis was used to estimate the survival functions and log-rank test to determine the difference in survival outcomes between groups. The Cox proportional hazards model was used for multivariate survival analysis. Variables with a p value < 0.2 in the univariate analysis were included in the multivariate analysis. Schoenfeld residuals were used to check the proportional hazards assumption. All tests were two-sided, and p value < 0.05 was considered statistically significant. All statistical analyses were performed with R (version 3.3.3, the R Foundation for Statistical Computing, Vienna, Austria) and RStudio (version 1.1.383).

**Results**

**Patient characteristics and distribution of ROS1 fusions identified through NGS**

Figure 1 summarizes our study design. Among the 21,747 consecutive treatment-naïve patients diagnosed with various stages of lung cancer who submitted samples for ROS1 detection, 447 were positive for ROS1 rearrangements, revealing an overall prevalence rate of 2.1%. A majority of the ROS1 rearrangements were identified using NGS (59.1%; n = 264), while 23.5% (n = 105) were identified by FISH, and 17.4% (n=78) were identified by ARMS. Of the 264 patients who were identified...
as ROS1 positive using NGS analysis, a majority (95.5%; \(n = 252\)) submitted tissue biopsy samples. The remaining 2.7% (\(n = 7\)) of the patients submitted plasma samples, and 1.8% (\(n = 5\)) submitted pleural effusion samples. Unfortunately, no patient submitted samples for all three molecular assays or multiple sample types to enable further concordance analyses among the detection methods or sample types.

Among the 235 patients with stage IIIB-IV disease, a majority was female (63%; \(n = 147\)), with a median age of 52 years (range: 25–79 years). Except for 1 patient having squamous cell carcinoma, all had adenocarcinoma (99.0%; \(n = 234\)). Of these 235 patients, 168 received crizotinib and 67 received platinum-based doublet chemotherapy regimen as first-line therapy. Baseline clinical characteristics including local treatment strategies were statistically similar among the subgroups (Table 1).

We further explored the various ROS1 fusion variants and gene partners among the 235 patients with NGS data who received first-line therapy. A majority of the patients (93.6%, 220/235) were detected with only a single ROS1 fusion partner, with CD74-ROS1 as the most common (59.1%, 130/220), followed by SDC4-ROS1 (13.2%, 29/220), and EZR-ROS1 (11.4%, 25/220) (Table S2, Fig. 2A). Five previously unreported ROS1 fusion
|                      | All ROS1 fusion lung cancer patients (n = 447, n, %) | NGS identified ROS1 fusion lung cancer patients (n = 264, n, %) | Locally-advanced/advanced ROS1 fusion lung cancer patients (n = 235, n, %) | Crizotinib, n = 168 | Chemotherapy, n = 67 | Non-reciprocal/reciprocal ROS1 translocation (n = 9) | p | Non-reciprocal/reciprocal ROS1 translocation (n = 6) | p |
|----------------------|-----------------------------------------------|----------------------------------------------------------|-----------------------------------------------|---------------------|---------------------|-----------------------------------------------|---|-----------------------------------------------|---|
| **Age (Median range)** | 52 (25–79)                                    | 52 (25–79)                                               | 52 (27–79)                                    | 52 (27–79)          | 52 (29–79)          | 52 (27–79)                                    | 0.868 | 54 (37–74)                                    | 0.793 |
| **Sex**              |                                               |                                                          |                                               |                     |                     |                                               | 0.443 | 0.487                                         | 0.662 |
| Female               | 293 (66)                                      | 166 (63)                                                 | 147 (63)                                      | 63 (38)             | 61 (38)             | 38 (42)                                       | 2.33 | 2 (2)                                         | 0.331 |
| Male                 | 154 (34)                                      | 98 (37)                                                  | 88 (37)                                       | 105 (62)            | 98 (62)             | 52 (58)                                       | 46 (67) | 7 (98)                                         | 0.837 |
| Clinical stage       |                                               |                                                          |                                               |                     |                     |                                               | 0.474 | 0.535                                         | 0.395 |
| III                  | 30 (7)                                        | 23 (9)                                                   | 14 (6)                                        | 10 (6)              | 9 (6)               | 4 (4)                                         | 5 (7) | 1 (1)                                         | 1 (16.7) |
| IIia                 | 0 (0)                                         | 0 (0)                                                    | 0 (0)                                         | 0 (0)               | 0 (0)               | 0 (0)                                         | 0 (0) | 0 (0)                                         | 0 (0) |
| IIib                 | 9 (2)                                         | 5 (2)                                                    | 2 (1)                                         | 2 (1)               | 1 (1)               | 1 (1)                                         | 0 (0) | 0 (0)                                         | 0 (0) |
| IIic                 | 21 (5)                                        | 18 (7)                                                   | 12 (5)                                        | 8 (5)               | 8 (5)               | 3 (3)                                         | 5 (7) | 1 (1)                                         | 1 (16.7) |
| IV                   | 417 (93)                                      | 241 (91)                                                 | 221 (94)                                      | 158 (84)            | 150 (84)            | 86 (96)                                       | 64 (93) | 8 (99)                                         | 0.833 |
| Smoking status       |                                               |                                                          |                                               |                     |                     |                                               | 0.284 | 0.487                                         | 0.33 |
| Smoker or former smoker | 89 (20)                                    | 62 (23)                                                  | 54 (23)                                       | 32 (19)             | 29 (18)             | 19 (21)                                       | 10 (14) | 2 (2)                                         | 0.933 |
| Nonsmoker            | 358 (80)                                      | 202 (77)                                                 | 181 (77)                                      | 136 (81)            | 130 (82)            | 71 (79)                                       | 59 (86) | 7 (98)                                         | 0.893 |
| ECOG PS score        |                                               |                                                          |                                               |                     |                     |                                               | 0.943 | 0.938                                         | 0.697 |
| 0–1                  | 430 (96)                                      | 253 (96)                                                 | 226 (96)                                      | 162 (86)            | 153 (86)            | 86 (96)                                       | 67 (97) | 9 (100)                                        | 6 (100) |
| ≥ 2                  | 17 (4)                                        | 11 (4)                                                   | 9 (4)                                         | 6 (4)               | 6 (4)               | 4 (4)                                         | 2 (3) | 0 (0)                                         | 0 (0) |
| Tumor histology      |                                               |                                                          |                                               |                     |                     |                                               | 0.998 | 0.974                                         | 0.666 |
| Adenocarcinoma       | 445 (99)                                      | 262 (99)                                                 | 234 (99)                                      | 166 (99)            | 157 (99)            | 89 (99)                                       | 68 (99) | 9 (100)                                        | 6 (100) |
| Squamous cell carcinoma | 2 (1)                                        | 2 (1)                                                   | 1 (1)                                         | 2 (1)               | 1 (1)               | 1 (1)                                         | 0 (0) | 0 (0)                                         | 0 (0) |
| Presence of brain metastasis at |                     |                                                          |                                               |                     |                     |                                               | 0.83  | 0.735                                         | 0.928 |

Table 1 Baseline clinicopathological characteristics of patients harboring ROS1 fusions

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|                | All ROS1 fusion lung cancer patients (n = 447, n, %) | NGS identified ROS1 fusion lung cancer patients (n = 264, n, %) | Locally-advanced/advanced ROS1 fusion lung cancer patients (n = 235, n, %) | Crizotinib, n = 168 | Non-reciprocal/reciprocal ROS1 translocation (n = 9) | Chemotherapy, n = 67 | Non-reciprocal/reciprocal ROS1 translocation (n = 6) | p     |
|---------|---------------------------------------------------|---------------------------------------------------------------|-------------------------------------------------------------------------|---------------------|-------------------------------------------------|---------------------|-------------------------------------------------|------|
| baseline | Present                                           |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 129 (29)                                          | 69 (26)                                                       | 58 (25)                                                   | 45 (27)             | 42 (26)                                         | 23 (26)             | 19 (28)                                         | 3 (33) |
|         | 318 (71)                                          | 195 (74)                                                      | 177 (75)                                                  | 123 (73)            | 117 (74)                                        | 67 (74)             | 53 (72)                                         | 6 (67) |
|         | Method used for evaluating brain metastasis       |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | CT                                                |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 169 (38)                                          | 98 (37)                                                       | 86 (37)                                                   | 64 (38)             | 62 (39)                                         | 42 (47)             | 20 (29)                                         | 2 (22) |
|         | MRI                                               |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 278 (62)                                          | 166 (63)                                                      | 149 (63)                                                  | 104 (62)            | 97 (61)                                         | 48 (53)             | 49 (71)                                         | 7 (78) |
|         | Local therapy received for management of brain metastasis |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | None                                              |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 89 (69)                                           | 50 (72)                                                       | 44 (76)                                                   | 31 (69)             | 29 (69)                                         | 15 (65)             | 14 (74)                                         | 2 (67) |
|         | WBRT                                              |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 22 (17)                                           | 10 (14)                                                       | 8 (14)                                                    | 8 (18)              | 7 (17)                                          | 5 (22)              | 2 (11)                                          | 1 (33) |
|         | SBRT                                              |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 18 (14)                                           | 9 (14)                                                        | 6 (10)                                                    | 6 (13)              | 6 (14)                                          | 3 (13)              | 3 (15)                                          | 0 (0)  |
|         | Disease status                                    |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | Non-progression                                   |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 232 (52)                                          | 146 (55)                                                      | 124 (53)                                                  | 57 (34)             | 51 (32)                                         | 29 (32)             | 22 (32)                                         | 6 (67) |
|         | Progression                                       |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 215 (48)                                          | 118 (45)                                                      | 111 (47)                                                  | 111 (66)            | 108 (68)                                        | 61 (68)             | 47 (68)                                         | 3 (33) |
|         | Site of progression                               |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | Brain                                             |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 93 (43)                                           | 53 (45)                                                       | 48 (43)                                                   | 48 (43)             | 47 (44)                                         | 25 (41)             | 22 (47)                                         | 1 (33) |
|         | Non-brain                                         |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 122 (57)                                          | 65 (55)                                                       | 63 (57)                                                   | 63 (57)             | 61 (56)                                         | 36 (59)             | 25 (53)                                         | 2 (67) |
|         | Method used for evaluating brain metastasis       |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | CT                                                |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 169 (38)                                          | 98 (37)                                                       | 86 (37)                                                   | 64 (38)             | 62 (39)                                         | 42 (47)             | 20 (29)                                         | 2 (22) |
|         | MRI                                               |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 278 (62)                                          | 166 (63)                                                      | 149 (63)                                                  | 104 (62)            | 97 (61)                                         | 48 (53)             | 49 (71)                                         | 7 (78) |
|         | Local therapy received for management of brain metastasis |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | None                                              |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 89 (69)                                           | 50 (72)                                                       | 44 (76)                                                   | 31 (69)             | 29 (69)                                         | 15 (65)             | 14 (74)                                         | 2 (67) |
|         | WBRT                                              |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 22 (17)                                           | 10 (14)                                                       | 8 (14)                                                    | 8 (18)              | 7 (17)                                          | 5 (22)              | 2 (11)                                          | 1 (33) |
|         | SBRT                                              |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 18 (14)                                           | 9 (14)                                                        | 6 (10)                                                    | 6 (13)              | 6 (14)                                          | 3 (13)              | 3 (15)                                          | 0 (0)  |
|         | Disease status                                    |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | Non-progression                                   |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 232 (52)                                          | 146 (55)                                                      | 124 (53)                                                  | 57 (34)             | 51 (32)                                         | 29 (32)             | 22 (32)                                         | 6 (67) |
|         | Progression                                       |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 215 (48)                                          | 118 (45)                                                      | 111 (47)                                                  | 111 (66)            | 108 (68)                                        | 61 (68)             | 47 (68)                                         | 3 (33) |
|         | Site of progression                               |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | Brain                                             |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 93 (43)                                           | 53 (45)                                                       | 48 (43)                                                   | 48 (43)             | 47 (44)                                         | 25 (41)             | 22 (47)                                         | 1 (33) |
|         | Non-brain                                         |                                                               |                                                          |                     |                                                 |                     |                                                 |      |
|         | 122 (57)                                          | 65 (55)                                                       | 63 (57)                                                   | 63 (57)             | 61 (56)                                         | 36 (59)             | 25 (53)                                         | 2 (67) |

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partners were identified from our cohort, including MYH9-ROS1 (n = 2), AQP4-ROS1 (n = 1), CTNN2-ROS1 (n = 1), PHACTR3-ROS1 (n = 1), and PTM-ROS1 (n = 1). Six percent (6.4%, 15/235) of the patients with ROS1-rearranged NSCLCs were detected with more than 1 genomic rearrangement involving ROS1, of which at least 1 of the rearrangements retained the kinase domain of ROS1, while the other ROS1 fusion did not retain the kinase domain, which was referred to as non-reciprocal/reciprocal translocation. Among them, a majority had CD74-ROS1 (47.1%; n = 8) as the ROS1 fusion which retained the kinase domain. Interestingly, 1 patient was detected with 3 fusions (CD74-ROS1, SDC4-ROS1, and SLC34A2-ROS1), which all retained the kinase domain. Table S3 summarizes the detailed breakpoint information for the non-reciprocal/reciprocal ROS1 translocations detected from our cohort.

Comparison between chemotherapy and crizotinib as first-line therapy
Clinical outcomes were analyzed for the 235 patients with advanced or locally advanced ROS1-rearranged NSCLCs who received first-line therapy of either chemotherapy (n = 67; pemetrexed and carboplatin regimen (97%; n = 65), docetaxel and cisplatin regimen (n = 2)) or crizotinib (n = 168). As compared to patients who received chemotherapy (n = 67), patients who received crizotinib (n = 168) had significantly better ORR (85.7% (n = 144) vs 44% (n = 28), p = 0.003; Fig. 2B) and significantly longer median PFS (18.0 months vs. 7.0 months, p < 0.001, Fig. 2C).

Impact of ROS1 fusion types on crizotinib efficacy
Next, we explored the clinical impact of ROS1 fusion variants among the 168 patients with NGS data who received first-line crizotinib. Baseline clinicopathologic
characteristics were similar for the patients regardless of ROS1 fusion (Table 1).

PFS was significantly shorter for patients with single CD74-ROS1 fusion (n = 90) than those with single non-CD74 ROS1 fusions (n = 69) (17.0 months vs 21.0 months; p = 0.008; Fig. 2D). PFS was comparable among patients with single CD74-ROS1 fusions (n = 90) and those with single SDC4-ROS1 (n = 23; 16.0 months [95% confidence intervals (CI): 14.2–17.7 months]; p = 0.175), or single EZR-ROS1 (n=21; 19.0 months [95% CI: 12.7–25.2 months]; p = 0.07), but was significantly longer for patients with uncommon non-CD74 ROS1 fusions (n = 25; 22.0 months [95% CI: 14.5–29.0 months]; p = 0.02) (Figure S1A). The ORR (88.9% vs 82.6%; p = 0.92) and DCR (96.7% vs 97.1%; p = 0.96) was similar for patients with CD74-ROS1 and non-CD74 ROS1 fusions (Table S4). With first-line crizotinib therapy, the ORR was 91.3% for patients with SDC4-ROS1 (n = 23), 85.7% for EZR-ROS1 (n = 21), 91.7% for TPM3-ROS1 (n = 12), and 88% for other uncommon non-CD74 ROS1, including SLC34A2-ROS1 (n = 3), LRIG3-ROS1 (n = 3), MYH9-ROS1 (n = 1), Ccdc6-ROS1 (n = 1), AQP4-ROS1 (n = 1), CTNND2-ROS1 (n = 1), PHACTR3-ROS1 (n = 1), GOPC-ROS1 (n = 1), and PTM-ROS1 (n = 1), which were grouped together due to small numbers. As compared to patients with single ROS1 fusion (both CD74-ROS1 and non-CD74 ROS1) (n = 159), patients with non-reciprocal/reciprocal ROS1 translocations (n = 9) had comparable ORR (77.8% vs 86.2%; p = 0.951; Table S3), DCR (100.0% vs 96.9%; p = 1.0; Table S4), and PFS (not reached vs 18.0 months; p = 0.116; Figure S1B) with crizotinib. Baseline clinicopathologic characteristics were similar for patients with single ROS1 fusion and non-reciprocal/reciprocal ROS1 translocations (Table S5). Table S6 lists the treatment outcomes on first-line crizotinib therapy of the 159 patients with single ROS1 fusion grouped according to ROS1 fusion partners. Table S7 and S8 list the detailed clinicopathologic features and clinical outcomes of the 13 patients with uncommon non-CD74 ROS1 fusions and the 15 patients with non-reciprocal/reciprocal ROS1 translocations, respectively.

Impact of various molecular factors on crizotinib efficacy in ROS1-rearranged NSCLCs
Numerous reports have implicated the presence of other concomitant mutations in various genes including TP53 in the poor response of ALK-rearranged NSCLC to crizotinib therapy [17, 18]; however, very limited reports have explored the clinical impact of other concomitant mutations on crizotinib therapy of ROS1-rearranged NSCLC. We then analyzed the genomic profile of 94 patients with ROS1 fusions whose samples were sequenced using a panel with at least 168 genes and evaluable for survival outcomes on first-line crizotinib to investigate the correlation between the presence of certain concomitant mutations at baseline and crizotinib efficacy. For this analysis, concurrent mutations in only the 168 genes common across the gene panels used for NGS were analyzed. Among the 54 evaluable patients with CD74-ROS1 fusions, 21 patients (38.9%) were detected with other concomitant mutations, with TP53 (13.1%, 7/54) as the most common co-occurring mutation. Other co-occurring mutations in classic NSCLC oncogenic drivers, including EGFR L858R (n=1), MET amplification (n = 1), and KRAS G12D (n = 4), were detected from tissue samples of 6 patients. Meanwhile, among the 32 patients with non-CD74 ROS1 fusions, 14 patients (43.7%) had other concomitant mutations, with TP53 (18.8%, 6/32) as the most common. Concurrent mutations in classic NSCLC oncogenic drivers, including MET amplification (n = 3), were detected from tissue samples of 3 patients with non-CD74 ROS1 fusion. The distribution of concurrent mutations detected among patients with CD74-ROS1 fusions (n = 54) was not statistically different from those with non-CD74 ROS1 fusions (n = 32) (38.9% vs. 43.7%; p = 0.774; Table S9).

In general, as compared to patients with no co-occurring mutations (n = 58), PFS was significantly shorter for those who harbored concomitant driver mutations (n = 9) (11.0 months vs 24.0 months; p = 0.0167; Fig. 3A) or concomitant tumor suppressor genes (i.e., TP53, RB1, or PTEN) (n = 25) (9.5 months vs 24.0 months; p < 0.001; Fig. 3A). Patients with concomitant TP53 mutations (n = 13) had significantly shorter PFS than those who had wild-type TP53 (n = 81) (6.5 months vs. 21.0 months; p < 0.001; Fig. 3B). Multivariate analysis consistently demonstrate the impact of harboring concomitant TP53 mutations (p = 0.028; hazard ratio: 0.41, 95% CI: 0.19–0.91) and harboring any concomitant mutations (p = 0.048; HR = 0.537, 95% CI: 0.290–0.995; Table S10) on PFS. These data suggest that the presence of concomitant mutations contributes to poor survival outcomes with first-line crizotinib therapy. Table S11 summarizes the clinical information of the 9 patients with concomitant driver mutations.

Impact of baseline brain metastasis on crizotinib and chemotherapy efficacy
Since a substantial number of patients with advanced-stage NSCLC present with brain metastasis at initial presentation and that crizotinib is known to have limited penetrability to the blood-brain barrier, we assessed the clinical impact of baseline brain metastasis on crizotinib and chemotherapy efficacy. Among the 235 patients with advanced or locally advanced ROS1-rearranged NSCLCs, brain metastasis at presentation was detected from 58 patients (24.6%), which were detected using either MRI or CT scanning. Of them, 10 patients received whole-
brain radiotherapy (WBRT) and 9 patients received stereotactic body radiotherapy (SBRT) for the management of their brain metastasis (Table 1). As compared to patients without brain metastasis at baseline, patients with baseline brain metastasis had a trend of shorter median PFS on first-line chemotherapy (4.0 months (n = 16) vs 7.0 months (n = 51); p = 0.09; Fig. 4A) and significantly shorter PFS on first-line crizotinib (16.0 months (n = 45) vs. 22.0 months (n = 123); p = 0.03; Fig. 4B).

However, patients with brain metastasis at baseline had significantly longer median PFS with crizotinib therapy than with chemotherapy (16.0 months (n = 45) vs. 4.0 months (n = 16); p < 0.0001). The ORR (77.8% vs. 87.8%; p = 0.642) and DCR (95.6% vs. 97.5%; p = 0.933) with crizotinib therapy were comparable between patients with or without brain metastasis at baseline (Table S 4). No difference in the presence of baseline brain metastasis was found among patients with CD74-ROS1 and non-CD74 ROS1 fusions (25.6% (23/90) vs. 27.5% (19/69); p = 0.830; Table 1). These findings indicate that despite the presence of brain metastasis at presentation, patients with ROS1-rearranged NSCLC still benefit more from crizotinib than chemotherapy.

Central nervous system (CNS)-related progression is associated with better prognosis

We further investigated the pattern of disease progression and the prognostic role of CNS progression among the patients whose disease progressed from either chemotherapy or crizotinib therapy. Although the frequency of CNS progression was similar between the two treatment groups (crizotinib 28.6% (48/168) vs. chemotherapy 28.4% (19/67)), the median time of the appearance of CNS progression was significantly slower on crizotinib therapy than on chemotherapy (30.0 months vs 22.0 months; p < 0.001; Fig. 4C). Of the 168 patients who received crizotinib, disease progression was observed in 112 patients and the treatment of 56 patients was still ongoing as of the data cut-off. Of the relapsed patients, 35.7% (n = 40) patients had intracranial-only progression, 7.1% (n = 8) patients were simultaneously detected with intracranial and extracranial progression, while 57.1% (n = 64) patients had extracranial-only progression, including the enlargement of the primary lung lesion, and development of other extracranial metastatic lesions. Patients with both intracranial and extracranial progression (n = 8) were omitted from further analysis but their clinical details were summarized in Table S 12. Patients who had intracranial-only progression (n = 40) achieved significantly longer PFS than those with extracranial-only progression (n = 64) (23.0 months vs 13.0 months, p < 0.001; Fig. 4D). The baseline clinical characteristics were similar between the patients with intracranial only and extracranial only progression on crizotinib therapy (Table S 13).

We further compared the survival outcomes of the patients based on the pattern of disease progression and the presence of baseline brain metastasis. Of the 40 crizotinib-treated patients who experienced intracranial-only progression, 16 patients had baseline CNS metastasis. Meanwhile, of the 64 crizotinib-treated patients with extracranial-only progression, 16 patients had baseline CNS metastasis (Table S13). The PFS were statistically different among the patients with or without baseline CNS metastasis who progressed with intracranial and extracranial metastasis (p = 0.007; Fig. 4E). Patients with baseline CNS metastasis who experienced intracranial-only progression with crizotinib therapy (n = 16) had significantly longer median PFS than those who experienced extracranial-only progression (n = 18) (17.0 months vs 8.0 months; p = 0.04; hazard ratio: 0.59, 95%CI: 0.23–0.95; Fig. 4E). Consistently, patients...
without baseline CNS metastasis who experienced intracranial-only progression with crizotinib therapy (n = 24) had longer median PFS than those who experienced extracranial-only progression (n = 46) (26.5 months vs 13.5 months; p < 0.001; hazard ratio: 0.51, 95%CI: 0.31–0.83; Fig. 4E). We found no difference in the pattern of progression with first-line crizotinib therapy among patients with CD74-ROS1 and non-CD74 ROS1 fusions (intracranial progression 41.0% vs. 46.8%; extracranial progression 59.0% vs. 53.2%; p = 0.679;
Table 1). And there was also no difference for the local treatment (Table 1). Taken together, these data indicate that patients with CNS progression can still benefit from crizotinib therapy.

Discussion

The current guidelines of the National Comprehensive Cancer Network recommend the use of ROS1 targeted inhibitors such as crizotinib and entrectinib as the preferred first-line treatment for ROS1 rearrangement-positive NSCLC [19]. However, in real-world clinical practice, the use of targeted inhibitors can be restricted by the patient’s socio-economic status for molecular testing services and access to various treatment modalities. Hence, we aimed to investigate the efficacy of crizotinib and chemotherapy in patients with ROS1-rearranged NSCLC to gain a better understanding of the advantages of crizotinib therapy for this subset of patients in the real-world setting. In our retrospective study, we screened the molecular data of a large cohort of lung cancer patients from various hospitals in 4 provinces of China and revealed a prevalence of 2.1% for ROS1 rearrangements among the Chinese patients with lung cancer. The prevalence we have derived from our cohort was consistent with the reported prevalence of 2.2% in another smaller cohort of Chinese patients with lung cancer [20, 21]. To the best of our knowledge, our study included the largest sample obtained from multiple centers, which reflects the actual prevalence of ROS1 rearrangements in our population and could help identify effective therapeutic management of ROS1-rearranged NSCLCs.

Consistent with previous observations [5], chromosomal rearrangements involving ROS1 in our cohort were mostly identified from younger patients with a median of 52 years old, females (63–66%), never smokers (75–76%), and with adenocarcinoma histology (97–98%). Our study has provided real-world clinical evidence on the efficacy of crizotinib in the first-line setting as reported in numerous clinical trials and other studies [8, 13, 14, 21–23]. The clinical outcomes we have observed from the 168 crizotinib-treated patients were consistent with another study that reported the better ORR, DCR, and PFS with first-line crizotinib as compared to chemotherapy for ROS1-rearranged NSCLC (ORR 80.0% vs 40.8%; DCR 90.0% vs 71.4%; PFS 9.8 months vs 6.0 months) [21, 24]. Moreover, our results that demonstrated better survival outcomes with first-line crizotinib for patients with non-CD74 ROS1 fusions than those who harbor CD74-ROS1 fusions (21.0 months vs 17.0 months) were consistent with a prior report that demonstrated a PFS of 17.6 months and 12.6 months, respectively [12]. Meanwhile, another study reported an opposite trend of longer PFS with crizotinib for patients with CD74-ROS1 than non-CD74-ROS1 (20.1 months vs. 12.0 months) [24], while no difference in PFS with chemotherapy was observed between CD74-ROSI and non-CD74-ROSI (8.6 months vs. 4.3 months) [24]. Since some of the ROS1 fusion partners are not as common as CD74, survival outcome data for patients with these fusion partners remains limited. Our results demonstrated a median PFS of 16.0 months for patients with SDC4-ROSI, 19.0 months each for patients with EZR-ROSI and TPM3-ROSI, and 22.0 months for patients with other uncommon non-CD74 ROS1 fusion partners. Our study is the first to provide data on the treatment and survival outcomes on first-line crizotinib for patients harboring various novel/uncommon non-CD74 ROS1 fusion partners as detailed in Table S7.

TP53 is one of the most frequent concomitant mutations in NSCLC, and has been associated with poor prognosis of not only patients with EGFR-mutant NSCLC treated with EGFR-TKI but also patients with ALK-rearranged NSCLC treated with crizotinib or chemotherapy [17, 18, 25, 26]. TP53 is a tumor suppressor gene and plays a critical role in regulating cell proliferation, and its loss-of-function promotes uncontrolled cell proliferation, tumor growth, and drug resistance [27–29]. Moreover, the detection of various concomitant mutations represents the molecular heterogeneity of the tumor, which could contribute to variable inhibitor response [30]. Our findings demonstrate poorer survival outcomes of patients with ROS1-rearranged NSCLC who harbored concomitant mutations in TP53, other tumor suppressor genes such as PTEN and RB1, and other oncogenic drivers such as EGFR, MET amplification, and KRAS. Our findings were consistent with the subgroup analysis of EUROCROSS study on the shorter PFS of patients with ROS1-rearranged lung cancers with concomitant TP53 mutations, treated with crizotinib than their wild-type counterpart [31].

Our study is limited by its retrospective nature; hence, some data are not available for analysis. Since the cohort with survival outcomes was based on the patients who submitted samples for NGS, inherent sampling bias might exist including the economic capability of the patients to submit samples for NGS and the decision on receiving chemotherapy or targeted therapy, which could limit our conclusion. Our study only analyzed the DNA-based NGS analysis of ROS1 fusion variants and does not include in vitro functional assays or RNA-based analysis of patient samples to confirm the transcription of novel ROS1 fusions or reciprocal/non-reciprocal ROS1 translocations that we have identified from our cohort. Further in vitro experiments are necessary to provide functional evidence for these ROS1 fusions.

Conclusions

Our study provided real-world clinical evidence of the distinct efficacy of crizotinib among NSCLCs with
various \textit{ROS1} fusion partners including some novel non-
\textit{CD74} \textit{ROS1} fusions. Our findings demonstrate that as
compared to chemotherapy, crizotinib is a better first-
line therapy for patients with \textit{ROS1}-rearranged NSCLC,
with or without brain metastasis at presentation. More-
over, concurrent mutations may contribute to the poor
clinical outcomes with crizotinib; hence, a better un-
derstanding of the patient’s mutation landscape is necessary
for optimal treatment planning. Our study contributes
to the understanding that baseline clinical and molecular
factors could impact the survival outcomes of patients
with locally advanced to advanced \textit{ROS1}-rearranged
NSCLC treated with first-line crizotinib.

Abbreviations
ARMS: Amplification refractory mutation system; CI: Confidence intervals;
CNS: Central nervous system; CR: Complete response; CT: Computed
tomography; DCR: Disease control rate; EGFR: Epidermal growth factor
receptor; FISH: Fluorescence in situ hybridization; HR: Hazard ratio;
MRI: Magnetic resonance imaging; NGS: Next-generation sequencing; NSCLC:
Non-small-cell lung cancer; ORR: objective response rate; PFS: Progression-
free survival; PD: Progressive disease; PR: Partial response; ROS1: ROS proto-
oncogene-1; SD: Stable disease; TNK: Tyrosine kinase inhibitor; WBRT: Whole-
brain radiotherapy

Supplementary Information
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Additional file 1: Figure S1. Kaplan Meier curves comparing the
progression-free survival (expressed in months) of patients with (A) single
\textit{CD74-ROS1} and various single non-\textit{CD74} \textit{ROS1} fusions; and (B) single
\textit{ROS1} fusions and non-reciprocal/reciprocal \textit{ROS1} translocations. The risk
table below summarizes the number of patients included per time point

Additional file 2: Table S1. List of genes included in the 168-gene panel
(Lung Plasma, Burning Rock Biotech). Table S2. Distribution of \textit{ROS1}
fusions among the 220 patients with single \textit{ROS1} fusion and 15 patients
with non-reciprocal/reciprocal \textit{ROS1} translocations. Table S3. Detailed
breakpoint information for the non-reciprocal/reciprocal \textit{ROS1} transloca-
tions detected in the cohort. Table S4. Response to crizotinib of \textit{ROS1}-
rearranged lung cancers. Table S5. Clinicopathologic characteristics of the
patients with single \textit{ROS1} fusions and non-reciprocal/reciprocal \textit{ROS1}
translocations. Table S6. Treatment outcomes on first-line crizotinib ther-

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Availability of data and materials
The datasets used and/or analyzed during the current study are available
from the corresponding author upon reasonable request.

Declarations
Ethics approval and consent to participate
Approval of the study protocol was obtained from the Hunan Cancer
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Consent for publication
Not applicable

Competing interests
Analyz Lizaso declared that she is employed by Burning Rock Biotech. Sai-
Hong Ignatius Ou has stock ownership and was on the scientific advisory
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ogy, and has received speaker honorarium from Merck, Roche/Genentech,
Astra Zeneca, Takeda/ARRAD and Pfizer; has received advisory fees from
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