Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-23-10

Reviewer A

Comment 1: lines 66-67: ROS1 rearrangements have been associated with a lower incidence of extrathoracic metastases, including CNS metastases at initial diagnosis and during treatment, compared to ALK rearrangements: there is actually a controversy in the literature about this, other studies have reported similar frequencies of brain metastases between ALK and ROS1 (PMID 29981925, which is also included in the manuscript as ref. 13)

Reply: Thank you for raising this point. As you point out, there is some debate about the pattern of progression, including CNS metastasis.

Thus, we have added the following sentences:

"In contrast, similar frequencies of CNS metastases have been reported in patients with *ALK* and *ROS1* rearrangements." (see Page 5, lines 68-69).

Comment 2: Figure 1B / line 111: for ALK NSCLC, it has been shown that EML4-ALKI v3 is associated with more metastases at initial diagnosis than variants 1+2 (e.g. PMID 29363116, 30023099). Do you have any information about the ALK fusion variants of the analyzed patients? How was the ALK status determined?

Reply: Thank you for raising this point. Unfortunately, no information on the ALK variant was available in this study, as ALK rearrangement status in most of the patients was identified by FISH and IHC. Additionally, we had already described this point as a study limitation; "although the prognostic impact of distinct fusion genes on clinical outcomes has been reported, details of the fusion types were unavailable. (see Page 11, lines 189–190).

Reviewer B

Comment 1: A full English review is required. There are many grammatical mistakes, repetitive words and terms.

Reply: The manuscript has been re-reviewed by a native English speaker.

Comment 2: Line 33 Crizotinib is the standard treatment for ROS1- and ALK-rearranged non-small cell lung cancer (NSCLC) patients. ; it´s not truth for ALK. Since new generation TKI, such alectinib and brigatinib have been developed, incorpotating in the guidilines.

Reply: Thank you for raising this point. As pointed out, second generation ALK-TKIs, alectinib and brigatinib, are the standard of care for ALK-rearranged NSCLC patients, but not crizotinib. Thus, we have changed the following sentence to accurately state this point: "Crizotinib has been approved for *ROS1*- and *ALK*-rearranged non-small cell lung cancer (NSCLC) patients." (see Page 3, lines 30–31).

Comment 3: Line 43 extrathoracic metastases > Here, extrathoracic includes the CNS mets?? Or CNS were not considered in this group? I recommend to be clearer, like: extratoracic and extra-CNS metastases. Please, add this information in line 108.

Reply: As you pointed out, "extrathoracic" includes CNS metastases. We have added "including CNS metastases" to the relevant sentence in the manuscript. (see Page 7, line 121).

Comment 4: Line 64 The progression-free survival (PFS) of crizotinib in 64 advanced ROS1-positive NSCLC patients was longer than that in ALK-positive NSCLC patients, based on the results of several clinical trial > there is no study design for this comparison. This, we should not say that. I recommend the authors to cite the PFS in trial with both drugs, and do not mention this comparison.

Reply: Thank you for raising this point. We totally agree with the reviewer's comment. We have modified the text as follows:

"crizotinib produced median progression-free survival (PFS) duration of 15.8 to 22.8 month in patients with advanced *ROS1*-positive NSCLC, while the median PFS of 7.7 to 10.9 months in patients with advanced *ALK*-positive NSCLC, based on the results of several clinical trials" (see Page 5, lines 60–63).

Comment 5: I suggest the authors to explore more deeply the choose for crizotinib in ALK rearranged, instead of the new generation drugs, including historical aspects and the time of study beginning.

Reply: Thank you for your great suggestion. The second generation *ALK*-TKIs, alectinib and brigatinib, have been the standard of care for *ALK*-rearranged NSCLC patients, although crizotinib was first approved for patients with advanced non-small cell lung cancer (NSCLC) with *ALK* rearrangements. In *ROS1*-rearranged NSCLC patients, crizotinib and entrectinib are still the standard of care, and the development of next-generation *ROS1* TKIs is underway to overcome the resistance of these TKIs.

In this study, to evaluate the distinct clinical features in *ROS1*- and *ALK*-positive NSCLC patients, we investigated the baseline metastatic spread, crizotinib efficacy, and progression patterns during crizotinib treatment in *ROS1*- and *ALK*-positive NSCLC patients, although the second-generation inhibitors are the standard of care in *ALK*-positive NSCLC.

(see Page 5, line 71-78).

"In *ALK*-positive NSCLC patients, next-generation *ALK* inhibitors have been designed to more strongly inhibit the ALK native kinase, be active on crizotinib-induced resistance mutations, and have better blood-brain barrier (BBB) penetration to control or prevent CNS involvement. The second-generation inhibitors, alectinib and brigatinib, have become the standard first-line treatment. In contrast, crizotinib and entrectinib are still the standard of care in *ROS1* positive NSCLC patients, but due to the resistance of these TKIs, the development of next-generation ROS1 TKIs is underway.

In this study, to evaluate the distinct clinical features in *ROS1*- and *ALK*-positive NSCLC patients, we investigated the baseline metastatic spread, crizotinib efficacy, and progression patterns. "

Comment 6: Some references are no correct, such as #10. Need be reviewed.

Reply: Thank you for raising this point. We have re-checked all citations. As you indicated, there were several errors in the references cited, and we have corrected the citations correctly.

Comment 7: Since crizotinib has not been the first line recommendation for ALK rearrangements, why the inclusion period was so long?

Reply 7: Thank you for the comments. As I already responded above (Comment 5), to evaluate the distinct clinical features in *ROS1*- and *ALK*- positive NSCLC patients, we focused on the *ROS1*- and *ALK*- positive NSCLC patients treated with crizotinib. Most *ALK* patients initiated

crizotinib from 2011 to 2017, because the second-generation inhibitors are the standard of care in ALK positive NSCLC. In contrast, all ROS1-positive NSCLC patients intiated crizotinib from 2015 to 2019, because crizotinib is still the standard of care in *ROS1*-positive NSCLC patients. Therefore, the inclusion period was long due to the difference in standard of care for *ROS1*- and *ALK*- positive NSCLC patients (see Figure 2).

Comment 8: Line 91 The OS for patients with undocumented dates of death were calculated from the date of their last follow-up visit. > this concept is equivocal. Patients who died, but the date was not identified, should be censored from the data of the last visit. We should not considered the last visit as the death date.

Reply: Thank you for raising this point. As you pointed out, the description was wrong. We have changed the sentence to "Patients with undocumented dates of death were censored from the data of the last visit." (see Page 6, lines 97-98)

Comment 9: The authors should mention the inclusion and exclusion criteria.

Reply: Thank you for raising this point. "We retrospectively reviewed and included all advanced *ROS1*- and *ALK*-positive NSCLC patients who had been treated with crizotinib at the National Cancer Center Hospital from January 2011 to March 2021. There were no exclusion criteria. " (see Page 6, lines 85-87)

Comment 10: The authors should clarify which criteria for rate of disease control was used.

Reply: Thank you for raising this point.

We have added the following text: "Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The objective response rate (ORR) was defined as the proportion of patients who had a complete response (CR) or partial response (PR). The disease control rate (DCR) was defined as the proportion of patients who achieved CR, PR, or stable disease." (see Pages 6–7, lines 98-102)

Comment 11: Line 107 Most patients had been treated with crizotinib as an initial tyrosine kinase inhibitor (TKI) > these data is not shown in the table. For me, the first line with crizotinib was mandatory, was it not?

Reply: Thank you for your question. In this study, the first line with crizotinib was not mandatory. We added the information on the treatment line of therapy in Table 1.

We have added the following sentences to the Results section: “In *ROS1*-positive NSCLC, crizotinib was administered to 10 patients (38%) as a first-line treatment, 6 patients (23%) as a second-line treatment, and 10 patients (38%) as a third-line or later treatment. In *ALK*-positive NSCLC, crizotinib was administered to 23 patients (55%) as a first-line treatment, 11 patients (26%) as a second-line treatment, and 8 patients (19%) as a third-line or later treatment. “(see Page 7, lines 114–118)

Comment 12: Line 116 The percentage of brain metastases was also lower in ROS1- positive patients, although the difference was not statistically significant > I recommend to remove the word "also".

Reply: Thank you for your great suggestion. We have removed “also.”

Comment 13: Line 143 > the authors need also discuss about diffences in trial design, power of the trial, differences of Phase 2 and 3 trials. I recommend to state that its not permitted direct comparisons between trials

Reply: We totally agreed with your suggestion. The direct comparison between clinical trials is challenging due to the varying trial designs. Thus, we have added the following text: “Although direct comparison between clinical trials is challenging due to the varying trial designs”, and revised to “the differences in the clinical outcomes with crizotinib treatment between patients with *ROS1*- and *ALK*-positive NSCLC could be related to the difference in inhibitory effects of this drug.” (see Page 9, lines 156–158)

Comment 14: Line 159 > the authors should discuss about tumor biology and carcinogenesis that could be associated to brain metastasis in patients with this tumor profile.

Reply: Thank you for your suggestion. We have added the following sentences: “the biology of brain metastasis is poorly understood, metastases to the CNS, unlike to other distal organ sites, involve the breach of the BBB. BBB effectively prevents the free exchange of substances between the blood and the interstitial fluid of the brain. The CNS is considered a sanctuary site for metastatic cancer cells because many therapeutic agents cannot cross the BBB. A better

understanding of molecular biology of lung cancer metastasis to the brain and efficient drug delivery across the BBB will be essential to find the appropriate treatment strategy for prevention of brain metastasis." (see Page 10, lines 181–187)