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Reviewer A

This is a very important contribution for assessing the treatment outcome and managing ALK-TKI resistance in the real-world setting especially including the strengthens of the study with a large number of evaluated patients and a long follow-up. Well written and organized paper, both regarding the text and tables.

Comment 1: Line 9: "genomic alterations" can be here replaced by "rearrangement" as other genomic alterations beyond rearrangement, like amplification and mutation are rare as de novo alterations and they do not count 4-7%, but rearrangements do.

Reply 1: Thank you for this valuable comment. We have now rewritten this sentence and used "rearrangement" instead of "genomic alterations".

Change in text: We have modified our text as advised (see Page 2, line 32).

Comment 2: line 20: it may be confusing for readers that median follow-up was 54.0 months form diagnosis and 45.0 from initial ALK-TKI. What happened in the gap of 10 months?

Reply 2: Thank you for your insightful comment regarding the median follow-up periods reported in our manuscript. We understand that the gap between the median follow-up from diagnosis (54.0 months) and from initial ALK-TKI therapy (45.0 months) may raise questions.

The 10-month gap observed is due to the fact that some patients included in our study were treated before ALK-TKI was approved as the first-line treatment for advanced ALK-positive NSCLC in Sweden. During this period, these patients initially received chemotherapy before transitioning to ALK-TKI therapy upon its approval and availability. This accounts for the delay between diagnosis and the commencement of ALK-TKI treatment.

Change in text: We have added a clarifying statement in the manuscript to explain this gap (see page 10, lines 321-324).

Comment 3: line 39: "There are more than 19 different ALK fusion partners described...". There are actually more than 90 ALK fusion partners described now. Please reword the sentence and add this reference: Ou SI, Zhu VW, Nagasaka M. Catalog of 5' Fusion Partners in ALK-positive NSCLC Circa 2020. JTO Clin Res Rep. 2020;1(1):100015. Published 2020 Feb 19. doi: 10.1016/j.jtocrr.2020.100015.

Reply 3: Thank you for your valuable feedback.
We acknowledge that more than 90 ALK fusion partners have been described to date.

Change in text: We have reworded the sentence accordingly and included the suggested reference (see page 3, line 64).

Comment 4: Line 64: "...the median overall survival for patients with metastatic ALK-positive NSCLC to over five years". The current median OS is actually longer and counts for seven years. Please rephrase the sentence and you can use here your reference nr 31 and additionally "Pacheco, J.M., Camidge, D.R., 2019. Is long-term survival possible for patients with stage IV ALK+ non-small cell lung cancer? Expert Review of Respiratory Medicine 13, 399–401. Doi.org/10.1080/17476348.2019.1596028".

Reply 4: Thank you for your insightful comment. We appreciate your feedback on the current median overall survival (OS) for patients with metastatic ALK-positive NSCLC. We have updated the manuscript to reflect the most recent data indicating that the median OS now extends to approximately seven years.

Change in text: We have modified our text as advised and added the recommended reference (see page 3, line 88).

Comment 5: line 93-99: please unify the formulations, as it was used both "as first-line treatment" and "treatment-naive" a little confusing what was the difference between both phrases. Is it the same context or do you mean "first-line treatment" and "ALK-TKI treatment naive"?

Reply 5: Thank you for your valuable comment and for pointing out the potential confusion in the terminology used. You are correct that the terms "first-line treatment" and "treatment-naive" need to be clarified to ensure consistency and clear understanding. There is no difference, we mean the same context. We have now revised the manuscript for clarity and we only use the term "first-line treatment".

Change in text: We have revised the manuscript as advised for clarity (see page 4, lines 120-122).

Comment 6: line162: 20% of patients had brain metastases at the diagnosis. It seems a little lower than can be expected in ALK-positive population. How have they been diagnosed? By CT or MRI? Have these patients were symptomatic or asymptomatic?

Reply 6: Thank you for your thoughtful comment. We acknowledge that the 20% incidence of brain metastases at diagnosis in our cohort may seem lower than expected for the ALK-positive population.

In Sweden, routine CT/MRI of the brain at the time of diagnosis for ALK-positive NSCLC patients (diagnosed before 2015) was not performed if the patients were asymptomatic. This practice is reflected in our data, particularly in the cohort of patients who received crizotinib as their initial ALK-TKI, where only 8.1% (6 patients) were reported to have brain metastases at diagnosis. This lower percentage is likely due to the absence of routine brain imaging in asymptomatic patients rather than a true lower incidence of brain metastases. The higher incidence of 31.6% observed in the second-generation ALK-TKI cohort aligns more closely with other reported ALK-positive cohorts, as routine brain imaging at diagnosis was implemented for all ALK-positive NSCLC patients.

Change in text: We have updated our manuscript to clarify this point and discussed the lower incidence observed in the discussion (see page 10-11, lines 340-348).

Comment 7: line 198-199: "As expected, patients treated with at least three ALK inhibitors had significantly longer median OS compared to those treated with one or two (79.0 months vs 43.0 months)". It can only be "expected" if the patients still had the disease sensitive for ALK-TKI, and not in cases with off-targets resistance of histological transformation. Moreover, why were there only 18.7% of patients in your cohort (yet according to suppl. Table 1 there were 15.6%) treated with several ALK-TKIs? So, for the majority of them it was not possible to receive three or more ALK-TKI. Is this because of poor PS, problem with availability of ALK-TKIs?

Reply 7: Thank you for your comments regarding the interpretation of our findings on ALK inhibitors and the treatment patterns in our cohort.

Regarding your first point, we acknowledge that the expectation of longer median OS with three or more ALK inhibitors assumes ongoing sensitivity to these therapies and excludes cases of acquired off-target resistance or histological transformation. We have clarified this point in our revised manuscript. (see page 7, line 226).

Regarding the treatment patterns observed in our cohort, we acknowledge your observation. The accurate number of patients receiving at least three ALK-TKIs is 25, as indicated in Supplementary Tables 1 and 4, corresponding to 16.7% of the cohort. This discrepancy has been corrected, and Table 1 has been updated accordingly. The lower percentage of patients receiving multiple ALK-TKIs can be attributed to several factors, including patient-specific considerations such as performance status (PS) and the availability of ALK-TKIs during the study period, particularly within the crizotinib group. The ALK-TKI status is depicted in Figure 2 for the overall cohort, as well as for the second-generation ALK-TKI and crizotinib subgroups. Notably, in the second-generation cohort, a majority of patients (66%) were still undergoing treatment at the cut-off period, necessitating treatment with only one or two TKIs due to stable disease.

Change in text: We have changed the manuscript as described above (see page 7, line 226). We have also revised Table 1 as mentioned, as well as Supplementary tables 1 and 4.

Comment 8: line 212-214: "Univariate analysis of PFS showed that patients with baseline brain metastases (BM)or PD-L1 negative tumors had better PFS than those without BM and PD-L1 positive tumors, respectively". How can this finding be clinicallyuseful?

Reply 8: Thank you for your valuable comment. It is clinically relevant to explore the roles of PD-L1 expression and CNS-metastasis for survival in our patient cohort. Firstly, it aligns with previous studies demonstrating an association between high PD-L1 expression and poor prognosis in ALK-rearranged NSCLC patients. For instance, studies have reported that elevated PD-L1 levels are linked with more aggressive disease and reduced overall survival, suggesting that PD-L1 expression can serve as a prognostic biomarker **(Tian X, Li Y, Huang Q, Zeng H, Wei Q, Tian P. High PD-L1 Expression Correlates with an Immunosuppressive Tumour Immune Microenvironment and Worse Prognosis in ALK-Rearranged Non-Small Cell Lung Cancer. Biomolecules. 2023;13(6)).**

Another study showed that ALK fusion variant 3a/b, concomitant mutations, and high PD-L1 expression were associated with poor clinical response to 2nd-generation ALK-TKI **(Li M, Hou X, Chen J, Zhang B, Wang N, Han H, et al. ALK fusion variant 3a/b, concomitant mutations, and high PD-L1 expression were associated with unfavorable clinical response to second-generation ALK TKIs in patients with advanced ALK-rearranged** non-small cell lung cancer (GASTO 1061). Lung Cancer. 2022;165:54-62).

CNS metastasis is often considered a poor prognostic factor in NSCLC patients. The observed better PFS in the univariate analysis is likely attributable to confounding factors, for example, most patients with brain metastases at diagnosis received treatment with a second-generation ALK-TKI, and thus the result did not hold in the multivariable analysis.

It is important to note, however, that while these associations are interesting and could help in stratifying patients more effectively for personalized treatment approaches, our findings were only significant in the univariate analysis and did not hold in the multivariable analysis. Therefore, we cannot conclude that these factors are clinically useful based on our study.

Change in text: We have added a discussion on PD-L1 and CNS involvement regarding PFS in the discussion part of the manuscript (see page 12-13, lines 407-423).

Comment 9: Line 306: "Our survival results" add here OS of 65 months for highlighting the impressive value.

Reply 9: Thank you for your suggestion. We agree that highlighting the specific OS value would emphasize the survival results. We have revised the sentence to include the median OS value.

Change in text: We have added the OS value as suggested (see page 11, line 351).

Comment 10: In 38,7% of the patients in the whole cohort ALK-fusion variant was not identified and in 12% there were other variants. So, more than 50% of the patients were underdiagnosed which may have an impact on response and the type of resistance. This fact should be added to better inform the readers and discuss a little more what was the reason for that? Challenges with implementing RNA-NGS?
Furthermore, there is no information about how many patients were diagnosed with ALK-IHC only, PCR only, NGS only, and how many patients were diagnosed using more methods? Have you detected any co-alterations at the diagnosis, like mutations in TP53 or other?

Reply 10: Thank you for your insightful comments. The cohort included only ALK+ patients diagnosed either with IHC, PCR, and/or NGS. It is not that the fusion variant was not identified, it was not reported in the pathological report since the clinical relevance is unclear with conflicting data in the literature. Some patients in our study were only diagnosed with ALK-IHC and/or FISH and thus the fusion variant was not always tested for (missing data) with PCR/NGS. In some, the fusion variants were detected in the NGS but not specified which type in the pathological report. While we recognize the importance of detailing the diagnostic methods used (ALK-IHC, PCR, NGS, or multiple methods), this information was not included in the original study due to limitations in data collection. Including this information retrospectively would require re-evaluation of patient records and reclassification of diagnostic methods, which was beyond the scope of our current study. Similarly, data on co-alterations such as *TP53* mutations were not systematically collected or analyzed in our cohort. This was a limitation of the retrospective nature of our study. Future studies could be designed to include these important genetic details to provide a more comprehensive understanding of the patient cohort.

Change in text: We have commented on this in the discussion part to better inform the readers and the reason for the missing data on *ALK* fusion status (see page 13, lines 425-429).

Comment 11: Please add the information why in only limited number of patients (n=19) the rebiopsy was performed? Are there any logistic challenges with rebiopsies? Which diagnostic methods (IHC, FISH, NGS) were used in rebiopsies? Have you observed also histological transformations to SCLC, squamous cell carcinoma or EMT?
In pt. Nr 4 with three acquired resistance ALK-mutations; G1202R + F1174L + T1151M, why Brigatinib was used and not Lorlatinib which may more likely be effective?
Some reflections in the discussion will be needed about how rebiopsies helped (or not) to inform the next treatment choice.

Reply 11: Thank you for this important comment. It was actually in 18 patients, there was a typo in the figure 3A which is changed now. NGS was performed in all patients. Rebiopsies can be challenging if the locations of the metastases are not accessible for biopsy and/or could carry risks, including patients with comorbidities. Patients with progression in the CNS is also challenging when it comes to rebiopsies. We did not observe any histological transformations in this limited number of patients. In patient ID 4, lorlatinib was not available by the time the patient progressed on alectinib. However, in the rest of the patients lorlatinib was used specifically when a G120R mutation was detected, which is a known on-target resistance mutation that lorlatinib can overcome.

Change in text: We have commented on this in the discussion part as advised (see page 13, lines 447-451).

Comment 12: Regarding poor prognostic factors.
You have found that crizotinib treatment was one of the poor prognostic factors. Can you explain a little more in the discussion this point. Can you make it clear whether it is treatment with Crizotinib as 1. line or after chemotherapy or both. Furthermore, can it be linked to the devastating progression pattern illustrated in figure 2C with 40% progression in the brain and 36% in multiple sides?

Reply 12: Thank you for your valuable feedback and for highlighting this. We have addressed your concerns in the revised discussion section. In our study, the variable for crizotinib treatment as initial ALK-TKI included patients who received it both as a first-line therapy and after progression on chemotherapy. We did not separate these two groups in our analysis. However, our univariate analysis observed that receiving prior chemotherapy before transitioning to crizotinib did not significantly affect overall survival. This suggests that the poor prognostic impact of crizotinib is consistent regardless of its sequence relative to chemotherapy.

The progression patterns depicted in Figure 2C show a significant rate of brain metastases (40%) and multiple site progressions (36%). These patterns highlight critical limitations of crizotinib, particularly its poor penetration of the blood-brain barrier, resulting in untreated CNS involvement. The multi-site progression reflects the aggressive nature of the disease and the development of resistance mechanisms post-crizotinib treatment.

By addressing these points, we aim to provide our hypothesis of why crizotinib is associated with poor prognosis and how it relates to the observed progression patterns. We hope this revised explanation meets your expectations and clarifies the points raised.

Change in text: We have revised the manuscript accordingly to enhance clarity and context for the readership (see page 12, lines 390-396).

Comment 13: Line 386: “PD-L1 expression”. Why is lacking data of PD-L1 expression is considered as a limitation? Has PD-L1 status any relevance in ALK-positive NSCLC?

Reply 13: Thank you for your comment. As previously addressed in response to comment 8, the consideration of PD-L1 expression data as a limitation stems from emerging evidence in the field. Some studies have reported that elevated PD-L1 levels are associated with more aggressive disease and reduced overall survival in ALK-positive NSCLC patients, suggesting that PD-L1 expression may be a prognostic biomarker. The lack of comprehensive PD-L1 expression data in our cohort limits our ability to fully explore these associations and provide insights into the prognostic significance of PD-L1 in this specific patient population. This is why we considered the absence of such data as a limitation in our study. We hope this clarifies the rationale behind our assessment.

Change in text: We have commented on this in the discussion part (see page 12-13, lines 407-420).

Comment 14: Regarding Supplementary Figure 3. Histogram showing subsequent therapy after progression on initial ALK-TKI. 67% of patients received 2nd gen ALK-TKI after crizotinib, 42% received Lorlatinib after 2nd gen ALK-TKI and 24% received Lorlatinib after crizotinib + 2nd gen ALK-TKI. Can you comment on this? Is this decreasing percentage of patients due to poor PS, adverse events, or challenges with the availability of the drug?

Reply 14: Thank you for your insightful comment regarding Supplementary Figure 3. The observed decreasing percentage of patients receiving lorlatinib after progression on initial ALK-TKI can be primarily attributed to the fact that many patients in the crizotinib group did not live long enough to receive lorlatinib after progressing on both crizotinib and second-generation ALK-TKIs. Additionally, lorlatinib was not available at the time of progression for some patients. This trend is reflected in Supplementary Table 4, which shows that lorlatinib was used in later lines of therapy in the crizotinib group compared to those who started with a second-generation ALK-TKI as initial treatment.

Change in text: We have now commented on this in the discussion part (see page 14, lines 459-463).

Reviewer B

This manuscript reports clinical response and outcomes from 150 ALK fusion positive NSCLC patients who received first-line crizotinib or second line treatments. Resistance mechanisms were profiles using biopsy/NGS. Manuscript is succinct and important contribution from >10 years of treatment information. Real world data, such as presented in this manuscript are necessary for translational studies. My primary suggestions pertain to enhancing the quality of the figures prior to publication to improve the impact of the findings.

Comment 1: Minor editing for text format and grammar is recommended.

Reply 1: Thank you for your feedback. We have carefully reviewed the manuscript and made the necessary corrections to improve the text format and grammar throughout the document.

Change in text: The grammar corrected is highlighted in yellow in the text.

Comment 2: Spell out all abbreviations when they first occur in manuscript (e.g., COPD)

Reply 2: Thank you for your feedback. We have reviewed the manuscript and ensured that all abbreviations are spelled out when they first occur and used only the abbreviations if repeated in the text.

Change in text: We have changed the text as advised (see page 4, line 113; page 5, line 142; page 6, lines 189-190).

Comment 3: I would revise the figures for improved visual clarity - use sans serif font, increase font size on the Figure Legend, X- and Y-axis titles, and the comments on mOS

Reply 3: Thank you for your suggestion regarding the figures. We have revised figure 1 to improve visual clarity by using a sans serif font, increasing the font size of the Figure Legend and X- and Y-axis titles, and enhancing the comments on median overall survival.

Change in text: We have revised Figure 1 as suggested.

Comment 4: Harmonize graph fonts. In Figure 2, the text in pie charts looks like Calibri, but in Figure 3, text in pie charts looks like Times New Roman.

Reply 4: Thank you for your feedback on harmonizing the graph fonts. We have reviewed the figures and noticed the discrepancy you pointed out. We have now updated Figure 3 to ensure that all text in the pie charts uses the same font.

Change in text: We have updated the text in the pie chart in Figure 3 to ensure it is harmonized as advised.

Comment 5: Font sizes need to be bigger in the swimmer's plot.

Reply 5: Thank you for your feedback regarding the font sizes in the swimmer's plot. We have increased the font sizes to enhance readability and improve the overall clarity of the figure. Additionally, we have uploaded the file as a separate **.png** figure (figure 3B).

Change in text: See above.