Peer Review File

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Reviewer A

Comment 1:

The authors are conducting this study to extract the characteristics of ALK/RET rearrangement lung cancers with GGO, most of which are EGFR mutation-positive lung cancers. In addition, the conclusions obtained are limited due to the very small number of cases.

The identification of genetic profiling of lung cancers that present frosted shadows on images does not affect the choice of treatment for them.

Reply 1:

Thanks for your careful review and constructive suggestions. We are sorry for the small sample size of cases in this study, which mainly due to the relatively low rate of *ALK/RET* rearrangements in early-stage lung cancers, especially in pre-/minimally invasive LUADs. Our previous study which included 98 AIS/MIA revealed that the frequency of *RET* and *ALK* rearrangement in AIS/MIA was only 1% and 0% (Chen H, Carrot-Zhang J, Zhao Y, et al. Genomic and immune profiling of pre-invasive lung adenocarcinoma. Nat Commun. 2019 Nov 29;10(1):5472. PMID: 31784532). Therefore, after collecting a large pre-analysis cohort of LUADs (N=6756, Supplementary Fig. 1), the number of ALK/RET rearrangements cases were still limited. Nevertheless, multi-center clinical trials including larger sample size are still needed for validation of the conclusions obtained in this study, and we have added this main limitation at the end of the Discussion part.

We agree with you that the genetic texting for GGO-featured lung cancers does not affect the choice of treatment. On the contrast, however, the findings in this study suggest that the mixed GGO-featured lung cancers with cystic airspace could possess *ALK/RET* alternations and subsequently rapid progression. And curative surgery should be performed for these patients ASAP.

Changes in the text:

We have added the discussion of the study limitations in the Discussion part, which included "only 12 patients with AIS/MIA with *ALK/RET* rearrangements were included in this study, and these findings must be treated with caution and validated in future multi-center studies with larger sample size". (see Page 12, line 237-240)

Reviewer B

The paper titled "Clinical Characteristics and Progression of Pre-/minimally Invasive Lung Adenocarcinoma Harboring ALK or RET Rearrangements" is interesting. ALK/RET-positive

pre-/minimally invasive lung adenocarcinomas were mostly characterized as mGGOs with cystic airspace developing rapid nodule progression, and no recurrence occurred during long-term follow-up after resection. This provides insights into proper curative surgery timing in the management of patients with gene fusions. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 2:

The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply 2:

Thanks for your constructive suggestions. We have modified the Background section of Abstract as well as Introduction parts to comprehensively describe the clinical problems and needs of this study.

Changes in the text:

We have revised the Abstract and Introduction sections according to the Reviewer's suggestion. (see Page 3, line 41-71; Page 5-6, line 82-101)

Comment 3:

What are the clinical and translational implications of ALK or RET rearrangements in lung adenocarcinoma? It is recommended to add relevant content.

Reply 3:

Thanks for your important question and suggestion. We have added the relevant clinical and translational implications of *ALK/RET* fusions in the Introduction section, including the advance of TKI therapies in these patients as well as the treatment options varied with the progression of patients with *ALK/RET* fusions.

Changes in the text:

We have added the relevant contents in the Introduction section according to the Reviewer's suggestion. (see Page 5-6, line 82-101)

Comment 4:

Some fonts need to be enlarged, as shown in Figure S2.

Reply 4:

Thanks for your kind suggestion. We have enlarged the fonts of Figure S2 as well as Figure 2 to proper size.

Changes in the text:

We have revised the fonts in Figure 2 and Figure S2.

Comment 5:

This study is a retrospective analysis, which is likely to cause some deviations in the results. It needs to be further confirmed by multi-center clinical trials.

Reply 5:

Thanks for your kind suggestion. We have added the discussion of the study limitations in the Discussion part, which included the retrospective nature of this study.

Changes in the text:

We have added the limitation part in the Discussion section. (see Page 12, line 237-240)

Comment 6:

Rearrangements of these 2 genes exist predominantly in lung adenocarcinoma while rarely in non-adenocarcinoma. What is the frequency, clinicopathological characteristics and survival of ALK/RET rearrangements in non-adenocarcinoma NSCLC patients? It is recommended to add relevant content.

Reply 6:

Thanks for your great suggestions and constructive suggestions. We have reviewed the literature and added the relevant information in the Introduction section. *ALK* fusions and *RET* rearrangements were both rare in non-adenocarcinoma NSCLC, and none of this two gene alterations were reported to be prognostic factors. Clinically, the characteristics of non-adenocarcinoma NSCLC patients with ALK/RET rearrangements were similar with those with lung adenocarcinomas.

Changes in the text:

We have added relevant content in the Introduction section. (see Page 5-6, line 83-87)

Comment 7:

The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Complex genetic alterations contribute to rapid disease progression in an ALK rearrangement lung adenocarcinoma patient: a case report, Transl Cancer Res, PMID: 35116617”. It is recommended to quote this article.

Reply 7:

Thanks for your suggestions and recommendation. We have revised and expanded the Introduction part according to your and other reviewers’ suggestions, and the relevant paper (PMID: 35116617) has been cited in the Introduction section.

Changes in the text:

We have revised the Introduction section and cited new relevant papers. (see Page 6, line 99-101)

Comment 8:

The number of patient samples in this study is too small, and a large sample study should be added for verification.

Reply 8:

Thanks for your constructive suggestions. We are sorry for the small sample size of cases in this study, which mainly due to the relatively low rate of *ALK/RET* rearrangements in early-stage lung cancers, especially in pre-/minimally invasive LUADs. Nevertheless, multi-center clinical trials including larger sample size are still needed for validation of the conclusions obtained in this study, and we have added this main limitation at the end of the Discussion part.

Changes in the text:

We have added the discussion of the study limitations in the Discussion part, which included "only 12 patients with AIS/MIA with ALK/RET rearrangements were included in this study, and these findings must be treated with caution and validated in future multi-center studies with larger sample size". (see Page 12, line 237-240)

Reviewer C

Comment 9:

First of all, my major concern is the very small sample of ALK or RET positive patients, which is not able to answer the research question of their clinical characteristics and progression. Because of this, the authors may consider to write this study as a case series of 12 cases.

Reply 9:

Thanks for your careful review and constructive suggestions. We are sorry for the small sample size of cases in this study, which mainly due to the relatively low rate of *ALK/RET* rearrangements in early-stage lung cancers, especially in pre-/minimally invasive LUADs. Our previous study which included 98 AIS/MIA revealed that the frequency of *RET* and *ALK* rearrangement in AIS/MIA was only 1% and 0% (Chen H, Carrot-Zhang J, Zhao Y, et al. Genomic and immune profiling of pre-invasive lung adenocarcinoma. Nat Commun. 2019 Nov 29;10(1):5472. PMID: 31784532). Therefore, after collecting a large pre-analysis cohort of LUADs (N=6756, Supplementary Fig. 1), the number of *ALK/RET* rearrangements cases were still limited.

However, the clinical discrepancy between AIS/MIA with *ALK/RET* rearrangements and *EGFR* mutations was really significant and we still hope to publish it in the form of original article. Nevertheless, multi-center clinical trials including larger sample size are still needed for validation of the conclusions obtained in this study, and we have added this main limitation at the end of the Discussion part.

Changes in the text:

We have added the discussion of the study limitations in the Discussion part, which included "only 12 patients with AIS/MIA with *ALK/RET* rearrangements were included in this study,

and these findings must be treated with caution and validated in future multi-center studies with larger sample size". (see Page 12, line 237-240)

Comment 10:

Second, the title did not indicate the clinical research design of this study, i.e., a retrospective cohort study.

Reply 10:

Thanks for your kind suggestion. We have modified the study title as you suggested.

Changes in the text:

We have revised the study title.

Comment 11:

Third, the abstract needs some revisions. The background did not indicate the knowledge gap. The methods need to describe the inclusion of subjects, the assessment of their clinical characteristics, the diagnosis method of ALK or RET positive, follow up procedures, and measurement of survival outcome. The results part needs to focus on the findings from the 12 ALK or RET positive patients. The current conclusion needs to be tone down since the sample is very small.

Reply 11:

Thanks for your constructive suggestions and detailed explanation. We have revised the abstract section according to your suggestions. The knowledge gap concerning the clinical and translational application of *ALK/RET* rearrangements were added in the background. Data collection, sequencing methods and analysis methods were described in the methods part. The description order of the results was revised to focus on the the 12 *ALK/RET* positive patients. And we addressed the limitation of the small sample size of this study in the conclusion part to avoid unnecessary misunderstanding.

Changes in the text:

We have revised the Abstract of this study. (see Page 3, line 40-71)

Comment 12:

Fourth, the introduction of the main text is inadequate for the review of what has been known on the characteristics and outcomes of ALK or RET positive patients and analyze the limitations of prior studies.

Reply 12:

Thanks for your constructive suggestion. We have added the information of the clinical and translational applications of *ALK/RET* alternations in patients with lung adenocarcinoma as well as non-adenocarcinoma NSCLC, including the prevalence and clinical characteristic

discrepancy, the prognostic value and TKI treatment efficacy, and the knowledge gap concerning *ALK/RET*-positive pre-/minimally invasive lung adenocarcinomas.

Changes in the text:

We have modified the introduction of the main text for better reviewing the knowledge gap of this topic. (see Page 5-6, line 83-101)

Comment 13:

Fifth, the methodology of the main text needs to accurately describe the clinical research design, sample size estimation, measurement of clinical characteristics, and follow up procedures. The authors need to explain whether it is appropriate to merge ALK+ and RET+ patients as a group without considering their clinical heterogeneity. In statistics, the authors need to explain why authors compared ALK/RET+ with EGFR+ because the comparison is not the focus. Because of the small sample, most analysis should be descriptive.

Reply 13:

Thanks for your careful review and detailed suggestion. We have modified the Methods section to accurately describe the information you mentioned which was missing in the paper. Considering the relative low frequency and similar clinical characteristics (both of them were correlated with never-smokers, younger age and advanced-stage patients), we combined patients with *ALK* fusions and *RET* fusions as a group, and compared it with *EGFR*-positive patients, the latter of which stands for the most common targetable driver mutation even in early-stage lung adenocarcinoma (Chen H, Carrot-Zhang J, Zhao Y, et al. Genomic and immune profiling of pre-invasive lung adenocarcinoma. Nat Commun. 2019 Nov 29;10(1):5472. PMID: 31784532).

Changes in the text:

We have added the detailed information in the Methods section according to the reviewer's suggestions. (see Page 7-9, line 118-165)

Comment 14:

Finally, please consider to review and cite some related papers:

1. Feng J, Li Y, Wei B, Guo L, Li W, Xia Q, Zhao C, Zheng J, Zhao J, Sun R, Guo Y, Brcic L, Hakozaki T, Ying J, Ma J. Clinicopathologic characteristics and diagnostic methods of RET rearrangement in Chinese non-small cell lung cancer patients. Transl Lung Cancer Res 2022;11(4):617-631. doi: 10.21037/tlcr-22-202. 2. Galletti A, Russano M, Citarella F, Di Fazio G, Santo V, Brunetti L, Vincenzi B, Tonini G, Santini D. RET-mutated non-small cell lung cancer treated with pralsetinib: a case series. Precis Cancer Med 2022;5:20. 3. Zia V, Lengyel CG, Tajima CC, de Mello RA. Advancements of ALK inhibition of non-small cell lung cancer: a literature review. Transl Lung Cancer Res 2023;12(7):1563-1574. doi: 10.21037/tlcr-22-619. 4. Chen MF, Chaft JE. Early-stage anaplastic lymphoma kinase (ALK)-positive lung cancer: a narrative review. Transl Lung Cancer Res 2023;12(2):337-345. doi: 10.21037/tlcr-22-631.

Reply 14:

Thanks for your suggestion. We have reviewed the above and other relevant papers and cited them in our study.

Changes in the text:

We have cited several new papers in the Introduction section. (see Page 5-6, line 83-101)