Peer Review File

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Reviewer A

The synergy of combination TKI plus IO has demonstrated benefit in multiple tumor types e.g., HCC, RCC, and is of particular interest in NSCLC. This research will likely be of broad interest to readers. My concern with the manuscript is this phenotypic retrospective analysis is exploratory in nature but presented in a narrative which suggests much greater significance. Tighter narrative is needed in both background and discussion. As there is no planned prospective subset analyses in this type of observational research all observations of patient cohorts should simply be addressed in tables and noted. There needs to be redress to the limitations of such research with specific attention to unusual features of the patient population as compared to ph 3 RCT beyond those who initially were driver mutation positive e.g., 40% of patients without mets, half of patients in 3L or greater, extent of prior IO and TKI exposure, and Unusually long PFS in late line compared to published RCT. I think there will be interest in the combo of TKI+IO in a/mNSCLC but only if it is presented with much more humility.

Reply: Thanks for your suggestions. We have modified the main text as advised to make the description more objective. We have summarized the subgroup analyses of the observation group in Table S2. In addition, we added appropriate descriptions of deficiencies, such as the retrospective and exploratory nature of the study, the potential immaturity of OS, and the heterogeneity of patients in the observation group (see Page 14, line 447-456).

Reviewer B

The paper titled “Combination of multitarget angiogenesis inhibitor and immune checkpoint inhibitor as late-line treatment for advanced lung adenocarcinoma in driver-negative patients: a retrospective comparative cohort study” is interesting. The combination of the multitargeting tyrosine kinase inhibitor anlotinib and PD-1 blockade demonstrated significant benefits in the second- and subsequent-line treatment of driver-negative patients with advanced LUAD. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) In the introduction of the manuscript, it is necessary to clearly indicate the characteristics and evaluation criteria of immunotherapy and the impact of immunotherapy on tumor micrometastasis.

Reply: We have added the relevant description in the introduction section (see Page 3-4, line 81-96).

2) What are the predictors of efficacy of immunotherapy? What is the application value of PD-1 inhibitors in neoadjuvant treatment of LUAD? It is recommended that relevant information be added to the discussion.

Reply: We have added the relevant description in the discussion section (see Page 13-14, line 424-428).

3) What are the advantages of combination therapy? It is recommended to add relevant comparative analysis.

Reply: We have included the corresponding description in the discussion section (see Page 12-13, line 373-396).

4) With the discovery of new drug targets and the continuous emergence of new combination treatment options, what breakthroughs will there be in the treatment of LUAD in the future? What inspiration can this study provide? It is recommended to add relevant content to the discussion.

Reply: In this study, we found the combination of antiangiogenic drugs and immunotherapy could provide superior efficacy to immune monotherapy, and even patients who underwent previous immunotherapy also achieved good survival after receiving the combination therapy. Other immune combinations therapeutic options are being explored, such as the combination of lag-3 monoclonal antibodies and PD-1/ PD-L1 inhibitors, which suggests that the "chemotherapy-free" approach is feasible. We have added the relevant description in the discussion section (see Page 14, line 442-445).

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Compare the efficacy and safety of programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) inhibitors for advanced non-small cell lung cancer: a Bayesian analysis, Transl Lung Cancer Res, PMID: 32953506". It is recommended to quote this article.

Reply: Thanks for your suggestions. We have added quotes from related articles.

6) What are the highlights and significance of this study? What is the author's next research plan? It is recommended to add relevant content to the discussion.

Reply: We have included the corresponding description in the discussion (see Page 12-13, line 373-396). Subsequent studies will investigate biomarkers to better define populations who will benefit the most from this combination treatment, which was stated in the discussion (see Page 14, line 430-432).

7) Is there a difference in the efficacy of immunotherapy for patients with different PD-1 expression levels? In the treatment plan, is there any difference in the efficacy of different immune checkpoint inhibitors? It is recommended that relevant information be added to the discussion.

Reply: As presented in the manuscript, in the anlotinib and PD-1 blockade combination therapy group, PD-L1-positive patients achieved longer PFS and OS times than PD-L1-negative or unknown patients (median PFS 8.67 months vs 5.20 months, median OS 21.83 months vs 14.60 months), but the difference did not reach a statistical significance (see Page 8-9, line 250-258). We also have added arguments about this relationship between PD-1 expression levels and the efficacy of combination therapy in the discussion (see Page 13-14, line 424-428).

Reviewer C

1) First, the title needs to indicate efficacy and safety and the control group of nivolumab monotherapy.

Reply: Thanks for your suggestions. The title has been changed to "Comparative efficacy and safety of multitarget angiogenesis inhibitor combined with immune checkpoint inhibitor and nivolumab monotherapy as second-line or beyond for advanced lung adenocarcinoma in driver-negative patients: a retrospective comparative cohort study".

2) Second, the abstract needs some revisions. The background needs to describe the knowledge gaps on the efficacy of antiangiogenic agents and ICIs and explain why the anlotinib + ICIs is potentially effective and safe. The methods need to describe the inclusion of the groups of patients, follow up procedures, and measures of efficacy and safety outcomes. In the results, please briefly summarize the clinical characteristics of the two groups of patients and the baseline comparability of the two groups. The conclusion needs to have comments for the clinical implications of the findings.

Reply: Thanks for your suggestions. We have modified the abstract as suggested.

3) Third, in the introduction of the main text, the authors need to have comments on the limitations and knowledge gaps on the efficacy and safety of antiangiogenic agents and ICIs for lung cancer and explain the clinical needs for assessing the efficacy and safety of anlotinib + ICIs. The authors need to analyze why anlotinib + ICIs is potentially safe and effective.

Reply: Thanks for your suggestions. The efficacy of immune monotherapy is not satisfactory in patients with advanced, treated NSCLC. The combination strategy of anti-angiogenesis inhibitor and immune checkpoint inhibitor counteracts the immunosuppression caused by the upregulation of PD-L1, exerting synergistic antitumor effects. The efficacy and safety of sintilimab combined with anlotinib as first-line therapy for advanced NSCLC has been validated in a phase 1b trial (Chu T, Zhong R, Zhong H, et al. Phase 1b Study of Sintilimab Plus Anlotinib as First-line Therapy in Patients with Advanced NSCLC. J Thorac Oncol. 2021 Apr;16(4):643-652.). We have added relevant comments as advised (see Page 4, line 94-116).

4) Fourth, in the methodology of the main text, please describe the clinical research design, sample size estimation procedures, and how the control group of nivolumab monotherapy was selected. The authors need to describe the assessment of baseline clinical factors and follow up procedures. In statistics, please describe the test of the baseline clinical factors of the two groups and describe the multiple regression method to adjust for confounders to ascertain the independent effect of the combination treatment.

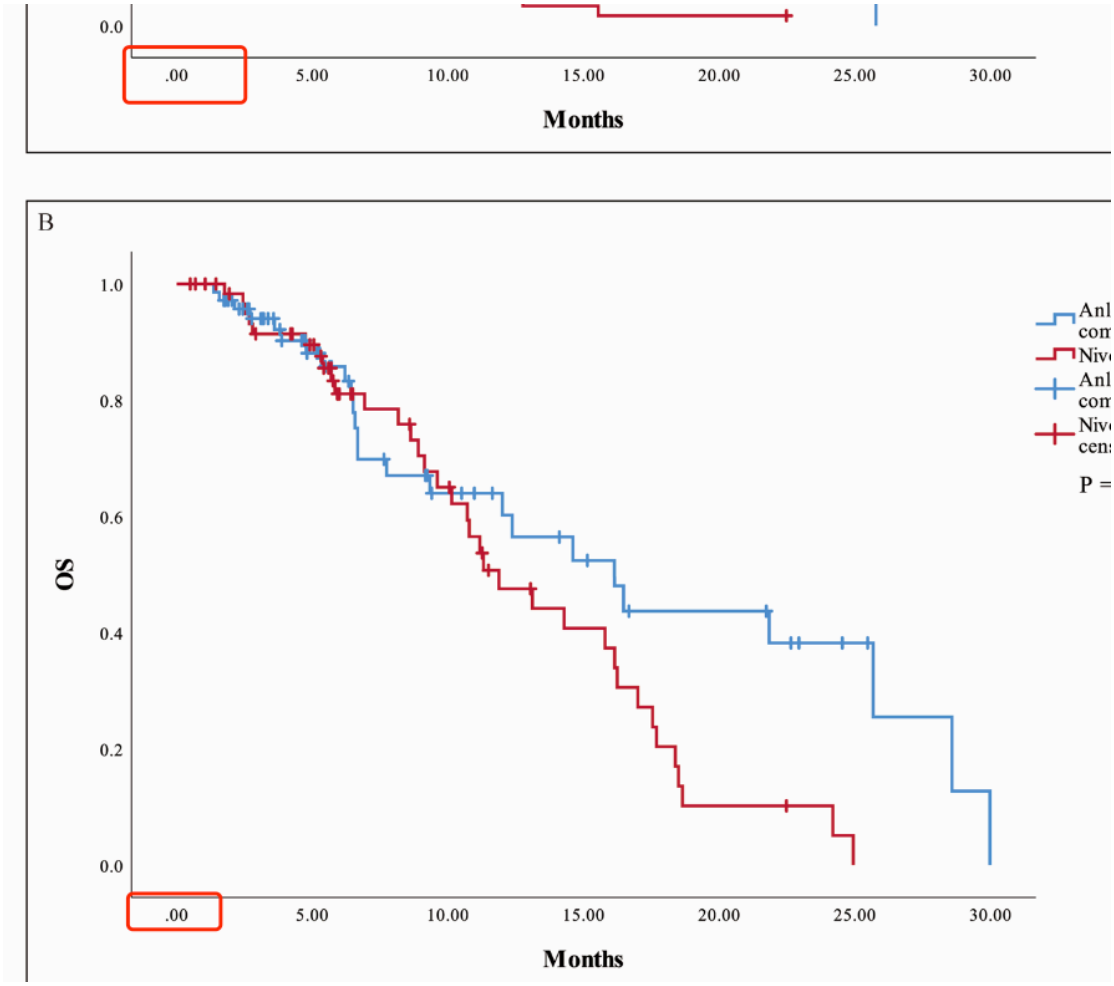
Reply: In this study, patients with advanced driver-negative LUAD who had received anlotinib in combination with ICI from October 2018 to July 2021 at Shanghai Chest Hospital as second- and subsequent-line treatment were included as the observation group and patients with nivolumab monotherapy as second-line treatment were included as the control group in the same time. The process of patient screening is shown in Figure 1. The number of cases in the area during the study period determined the sample size. The patients were followed up with chest CT every 2 to 3 months, with additional abdominal ultrasound and cranial MRI, and bone ECT being conducted as necessary until endpoints. The baseline characteristics of the observation and control groups and their comparison are shown in Table 1. Due to the

retrospective nature of this study, this combination warrants further clinical verification.

Reviewer D

1. Figure 2 and 3

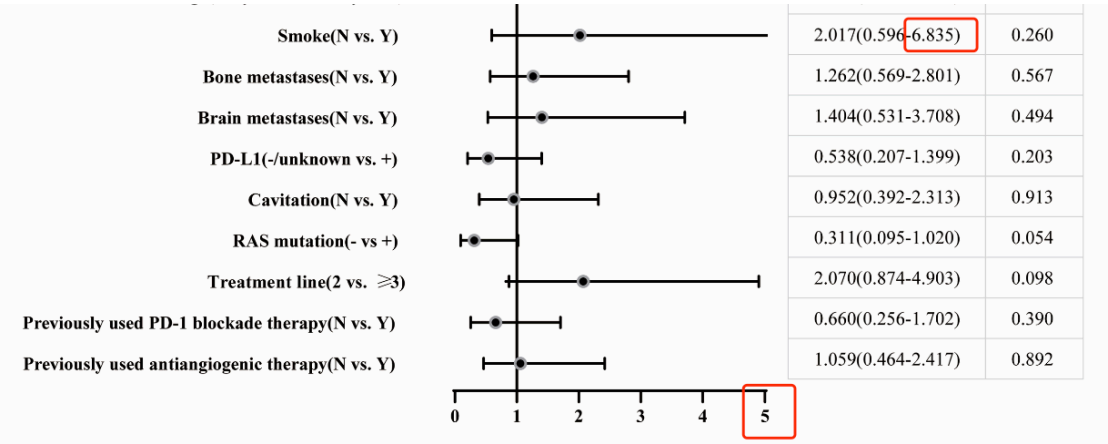
Please revise the .00 in x-axis to 0.00.

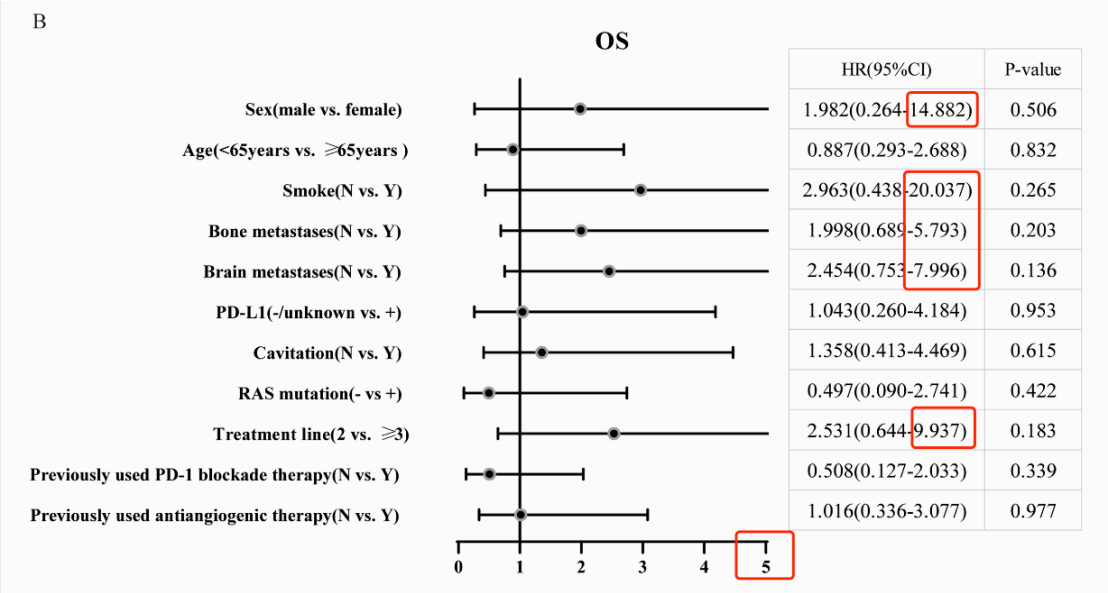


Reply: The revised figures are attached and have replaced the original figures in the main manuscript.

2. Figure 4

To standardize the results, the part that exceeds the horizontal coordinates should be indicated by arrows.





Reply: The revised figure is attached and have replaced the original one in the main manuscript.

3. References/Citations

a) There are 2 reference lists in the file, please keep the correct one and delete another one.

b) Please double-check if more studies should be cited as you mentioned “studies”. OR use “study” rather than “studies”.

474 accordance with the conclusions reported in previous studies: the combination of
475 antiangiogenic agents and ICIs did not lead to an increase in side reactions. [43].

Reply: We have modified the reference section as advised. Reference 43 is a review and we added another review to support the viewpoint mentioned in the text.