Peer Review File

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Response to Reviewer A

General comment. The paper titled "The treatment of anlotinib combined with PD-1 inhibitors in advanced non-small cell lung cancer" is interesting. Anlotinib combined with PD-1 inhibitors has good efficacy and a well-tolerated safety profile in the treatment of advanced NSCLC patients. However, there are several minor issues that if addressed would significantly improve the manuscript.

Response: Thank you for your positive feedback. We are submitting a revised manuscript to address these concerns. Detailed point-by-point responses to these concerns are provided hereinunder.

Specific Comments

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

Reply 1: We appreciate the reviewer's careful review of our manuscript. We have added the reference to the revised manuscript.

Changes in the text: Page 7, line 212-216

"…Neoadjuvant nivolumab plus chemotherapy resulted in significantly longer disease-free survival than chemotherapy alone (20)"

Comment 2: 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 2: We have added the reference to the revised manuscript.

Changes in the text: Page 3, line 72-77

"…In addition to the traditional gold standards overall survival(OS) and objective response rate (ORR), the unique evaluation standards for ICIs such as treatment-free time survival (5) and durable responses (6), which are distinctively clinical benefits of ICIs."

Comment 3: 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 NSCLC in the future? What inspiration can this study provide? It is recommended to add relevant content to the discussion.

Reply 3: We have modified the revised manuscript as advised.

Changes in the text: Page 8, line 259-263

"…the efficacy and safety assessment of anlotinib combined with PD-1 inhibitors should be explored in prospective clinical trials with larger sample sizes."

Comment 4: What are the advantages of combination therapy? It is recommended to add relevant comparative analysis.
Reply 4: We have added the reference to the revised manuscript.
Changes in the text: Page 7-8, line 218-232
"...a mOS of 27.0 months, an ORR of 40.0%, and a DCR of 82.5% (24)."

Comment 5: 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 5: We have modified the revised manuscript as advised.
Changes in the text: Page 8, line 258-262
"Anlotinib could stimulate the infiltration of the innate immune cells…the efficacy and safety assessment of anlotinib combined with PD-1 inhibitors should be explored in prospective clinical trials with larger sample sizes."

Comment 6: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Efficacy, prognosis and safety analysis of anti-PD-1/PD-L1 inhibitor rechallenge in advanced lung cancer patients: a cohort study, Transl Lung Cancer Res, PMID: 35832441". It is recommended to quote this article
Reply 6: We have added the reference to the revised manuscript.
Changes in the text: Page 3, line 77-78
"ICIs rechallenge might be an effective therapy for patients who discontinue treatment due to immune-related adverse events (AEs) (7). "

Comment 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 7: We have modified the revised manuscript as advised.
Changes in the text: Page 8, line 245-246
"There was no significant difference in clinical efficacy between patients with different PD-1 expression levels and different PD-1 inhibitors."

Response to Reviewer B

Comment 1: First, the title needs to indicate efficacy and safety and the clinical research design of this study, i.e., a retrospective cohort study
Reply 1: We agree with the reviewer's comments. As recommended, we have highlighted this in the revised manuscript
Changes in the text: Page 1, line 3-4
"Efficacy and safety analysis of anlotinib combined with PD-1 inhibitors in advanced non-small cell lung cancer： a retrospective cohort study"

Comment 2: Second, my major concern for this study is no a control group receiving PD-1 inhibitors only, so the current conclusion is not convincing because of no reference group undergone standard treatment.

Reply 2: We agree with the reviewers that this is indeed a limitation of this study, and we add an explanatory in the revised manuscript

Changes in the text: Page 7-8 line 255-259

"this is an observational study without setting a matched control group for PD-1 monotherapy or PD-1 inhibitors combined with chemotherapy, our results in line with some of previous studies and can provide a degree of real-world understanding of anlotinib combined with PD-1 inhibitors in advanced NSCLC patients."

Comment 3: Third, the abstract needs some revisions. The background did not explain why anlotinib + PD-1 inhibitors is potentially effective and safe for advanced NSCLC. The methods did not describe the inclusion of subjects, follow up procedures, and measurements of safety outcomes. The results need to first briefly summarize the clinical characteristics of the study sample. The conclusion needs to be tone down since this is not a RCT and no comparison reference for efficacy and safety

Reply 3: Thank you for pointing this out, we have modified our revised manuscript as advised.

Changes in the text: Page 2 line 37-58

"…Anlotinib combined with PD-1 inhibitors has potentially good efficacy and a tolerated safety profile in the treatment of advanced NSCLC patients."

Comment 4: Fourth, in the introduction of the main text, the authors did not explain why anlotinib + PD-1 inhibitors is potentially safe. Please also have a brief review on the efficacy and safety of antiangiogenic agents + PD-1 inhibitors and analyze the knowledge gaps on anlotinib + PD-1 inhibitors. The authors emphasized that this is a real-world study so please explain the strengths and clinical needs for the real-world data. My second major concern for this study is the problematic research design, a real-world study, which is often characterized by a large-scale sample, but 42 patients are a small sample

Reply 4: We agree with the reviewer's comments. As recommended, we have added the reference and an explanatory in the revised manuscript

Changes in the text: Page 3-4 line 83-96; Page 8 line 253-255

"…Anlotinib could stimulate the infiltration of the innate immune cells (14). The therapy of Anlotinib combined with programmed cell death 1 (PD-1) inhibitors is efficacy, durability, and safety (15).…the relatively small sample size may affect the results, descriptive data for efficacy and safety profile are needed to be confirmed in future large-scale studies."

Comment 5: Fifth, in the methodology of the main text, please describe the sample size estimation and details of follow up procedures. It is problematic to exclude cases without complete medical records. The authors need to consider this limitation in their

discussion. In statistics, please consider multiple Cox regression analysis to identify factors associated with the prognosis outcomes.

Reply 5: We have modified our revised manuscript as advised.

Changes in the text: Page 4-5 line 110-112，119-129

"Patients without complete medical records or follow-up information affected the evaluation of efficacy and safety were excluded from the study…PFS was defined as the time from which the oral administration of anlotinib was started to PD or to the last follow-up"

Response to Reviewer C

General comment. This study discussed the efficacy and safety of anlotinib combined with PD-1 for patients with advanced lung cancer. This is a well-written paper containing interesting results which merit publication. For the benefit of the reader, however, a number of points need clarifying and certain statements require further justification as listed below.

Response. We appreciate the reviewer's encouragement and helpful comment. We are submitting a revised manuscript to address these concerns. Detailed point-by-point responses to these concerns are provided hereunder.

Specific Comments

Comment 1: The number of the patients was relatively small, especially in the subgroup analysis according to the PD-L1 expression levels, which might make the results unreliable

Reply 1: We agree with the reviewers that this is indeed a limitation of this study, and we add an explanatory in the revised manuscript.

Changes in the text: Page 8-9 line 253-261

"…our study enriches the clinical evidence for the efficacy and safety of anlotinib combined with PD-1 inhibitors in patients with advanced NSCLC"

Comment 2: Involving the patients' datasets, please describe the patients' previous treatment strategies in detail.

Reply 2: We agree with the reviewer's comments. As recommended, we have modified our revised manuscript as advised.

Changes in the text: in the text: Page 5 line 152-153

Comment 3: Due to the limitation of small sample size, adding a matched control group using PD-1 inhibitors combined with chemotherapy or PD-1 monotherapy might be preferable and persuasive by comparing the efficacy and safety

Reply 3: We agree with the reviewers that this is indeed a limitation of this study, and we add an explanatory in the revised manuscript.

Changes in the text: Page 8-9 line 253-261

"…our study enriches the clinical evidence for the efficacy and safety of anlotinib combined with PD-1 inhibitors in patients with advanced NSCLC"

Comment 4: The limitations of the study were not well illustrated in discussion part, which should be added

Reply 4: Thank you for pointing this out, which we did not fully acknowledge in our original manuscript. We have modified our revised manuscript as advised.

Changes in the text: Page 8-9 line 253-263

"...the efficacy and safety assessment of anlotinib combined with PD-1 inhibitors should be explored in prospective clinical trials with larger sample sizes"

Comment 5: The results required thorough elaboration. For example, in the discussion part, the author stated that 'the PFS times of the patients treated with anlotinib combined with PD-1 inhibitors as a first-, second-, and third-line and above therapy were 17.753, 11.244, and 4.57 months, respectively, while the DCRs were 100%, 83.3%, and 64.3%, respectively. Thus, this treatment appears to have a better effect as an early line treatment than posterior lines'. This conclusion was not reliable for that the log-rank test didn't show significant differences in PFS between different treatment lines subgroups. Also, there exists similar real-world study published in ESMO 2022, in which the DCR in the second line reached 92.3%, compared to 91.3% in the first line, which was a little different to your research findings, which should be explained and summarized carefully

Reply 5: Thank you for pointing this out, and we have added PFS information for patients in first/second lines vs. third line and above therapy and revised the description in the manuscript

Changes in the text: Page 8, line 238-242

"Further sub-combined analysis of mPFS for first/second line therapy and third line and above therapy were 17.753 and 4.570 months (P=0.055, HR 0.416, 95% CI:0.166-1.047). Numerical values show a difference and P-values are close to statistically significant. Thus, this treatment appears to have a better effect as an early line treatment and especially used before the third line."

Comment 6: The plots illustrating the survival curves are preferable to be added

Reply 6: We have modified our revised manuscript as advised

Changes in the text: Page 15, Figure1

"Figure 1 -revised Comparison of survival curves among patients with First/Second line and Third-line and above in the overall population."