Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-1169

Reviewer A

introduction:

I recommend to delete sentence 71-72-73.

What others found in their research should be elaborated on. What did others found in peripheral blood; prognostic factors? Sentence 222 to 239 can be used for this. This feels more introduction then discussion.

REPLY: This paper discussed the clinical significance of lymphocyte subsets in lung cancer, so we first introduce the current situation of lung cancer at the beginning of the article, which we think is appropriate. The original 222 to 239 sentences are more appropriate as an introduction, so follow your suggestion, we wrote this part in the introduction and deleted it in the discussion section.

Results:

The author writes in sentence 176/177 about number of lymphocyte subsets. In the table percentages are shown. Probably the author means percentages? It is unclear what percentage of what. %CD4+ is the percentage of CD4 cells of CD3 cells? for instance?

Sadly the authors only show CD3, CD4 and CD8. Recent research show more in-depth analyses of lymphocyte subsets. It is unclear what kind of CD4 or CD8 cells especially in- or decrease.

Is there an explanation for the high response rate to immunotherapy? Are there differences between SD and PR? Or is the difference between PR and PD more pronounced?

REPLY: The number of lymphocyte subsets described in sentence 176/177 refers to the proportion of this type of cell in the total number of peripheral blood lymphocytes, not the absolute number of this type of cell. Specifically, the percentages of CD3+T, CD4+T and CD8+T cells in the paper represent the proportion of such cells in the total number of peripheral blood lymphocytes.

This study was a retrospective study, and our present studies did not differentiate lymphocytes in more detail. We are glad to adopt your suggestion and make a more detailed classification of lymphocytes in the follow-up study in order to obtain more accurate results. Unfortunately, this study did not make a more detailed distinction between CD4+T or CD8+T cell subtypes.

All patients included in this study were PD-L1 positive, and more than half of the patients were PD-L1 positive > 50%, so they may have a high response rate to immunotherapy. The difference between the lymphocyte subsets of SD and PR patients was not obvious before medication, and the change trend was roughly the same after medication. Therefore, this study included SD and PR patients in the same group to compare with PD patients.

Changes in the text: see Page 10, line 304-307.

sentence 188 seem to miss the last bit.

REPLY: We have amended the sentence 188“8 patients (26.7%) achieved PD.”

Reviewer B

The present study, which focused changing in T lymphocyte subsets for non-small cell lung cancer patients before and after receiving atezolizumab but I have some comments.

1. Were was the line of use for atezolizumab? What was the ratio of stage IVA and IVB? Were there any difference between the PR/SD group and the PD group in the line of use for atezolizumab and the stage?

REPLY 1: The criteria for the use of atezolizumab in this study were: EFGR gene, ALK, ROS1 rearrangement negative or unknown, PD-1 expression (PD-1≥ 1%), PS (physical activity status) score 0-2 stage IVA non-small cell lung cancer. Details in line 142-144. All patients in this study were stage IVA. There was no difference between the PR/SD group and the PD group for atezolizumab monotherapy use criteria and stage.

Changes in the text: see Page 5, line 146-149.

2. What was the content of the prior treatment for atezolizumab?

REPLY 2: Patients had not received any other antitumor therapy before atezolizumab, details in line 141-142.

3. The changing of T lymphocyte subsets is easier to understand using a boxplot.

REPLY 3: Follow your advice, we converted tables to boxplots for representing the changing of T lymphocyte subsets, details in figure 2, 3, 4.

4. How did changing T lymphocyte subsets correlate with PD-L1 expression?

REPLY 4: The patients included in this study were all PD-L1 positive and grouped with PD-L1≥ or ＜ 50%. Statistically, it was found that there was no significant correlation between changes in T lymphocyte subsets and PD-L1 expression levels. Therefore, we do not describe the relationship between the two in the text.

Changes in the text: None.

5. As for statistical analysis, since the changing T lymphocyte subsets is a continuous variable, the Mann-Whitney U test may be more appropriate.

REPLY 5: The statistical method has been modified and the values recalculated, details in statistical analysis and result sections.

6. Regarding the discussion part, I think there have been previous reports on immunotherapy

and the changing of T lymphocytes, but there is no detailed description of the new findings obtained in this study. It should describe how it is useful in clinical practice.

REPLY 6: In response to this issue, we have added some content to the discussion, details in line 305-318.

Reviewer C

I have two primary concerns regarding this study.

1) Firstly, the sample size is very small, specifically with only 8 PD cases, making the main findings unreliable. Consequently, the authors should reconsider their current conclusion.
   REPLY 1): This study is a retrospective study with phased results. The inclusion criteria, exclusion criteria, follow-up process and data processing of this study were strictly standardized. Although the sample size is small, we still believe that the conclusion is reliable. We tone down the current conclusion as your advice.
   Changes in the text: see Page 11, line 339-343.

2) Secondly, I am concerned about the clinical implications of using changes in T lymphocyte subsets as potential predictors. These changes coincided with the remission or progression of lung cancer, but predictors should ideally be observed before treatment outcomes occur.
   REPLY 2): This paper preliminarily proved that the changes of lymphocyte subsets may be related to the sensitivity of immunotherapy for lung cancer patients, but the precise molecular mechanism related to immunotherapy was still unclear. The analysis of the changes of lymphocyte subsets after immunotherapy may provide ideas for exploring the molecular mechanism of immunotherapy.
   Changes in the text: None.

3) Third, the title did not indicate the clinical research design of this study such as a prospective cohort study.
   REPLY 3): This study is a retrospective study, which has been changed in the title.
4) Fourth, the abstract needs some revisions. The background did not explain why changes in T lymphocyte subsets can predict the treatment outcomes and what the knowledge gap is. The methods need to describe the follow up procedures and measurement of treatment outcomes. The results need to briefly summarize the clinical characteristics of the study sample and quantify the findings by using statistics such as levels of T lymphocyte subsets and accurate P values. Please tone down the current conclusion as I argued above.
   REPLY 4): It has been modified in the text according to your request.
5) Fifth, in the introduction of the main text, the authors did not explain the clinical needs for using T lymphocyte subsets to predict the treatment outcomes, did not review what has been

known on the predictors of treatment outcomes, explain why T lymphocyte subsets alone could accurately predict outcomes, and what the potential clinical significance of this research focus is.

REPLY 5): This paper preliminarily proved that the changes of lymphocyte subsets may be related to the sensitivity of immunotherapy for lung cancer patients, but the precise molecular mechanism related to immunotherapy was still unclear. The analysis of the changes of lymphocyte subsets after immunotherapy may provide ideas for exploring the molecular mechanism of immunotherapy. In this study, we found that the proportion of CD4+T, CD8+T cells and the ration of CD4+T /CD8+T lymphocytes changed with the treatment cycle. Therefore, it is necessary to monitor lymphocyte subsets with the cycle during immunotherapy. By analyzing the periodic changes of lymphocyte subsets, it may indicate the patient's response to immunotherapy in the course of follow-up treatment, and provide a possible auxiliary indicator for judging the prognosis. However, assessing the association between peripheral blood lymphocyte subsets and PFS in immunotherapy patients with NSCLC will require the recruitment of additional NSCLC patients for monitoring in future studies.

Changes in the text: see Page 11, line 318-331.

6) Sixth, in the methodology of the main text, the authors need to clearly indicate the clinical research design of this study and procedures for the sample size estimation, as well as the follow up procedures. In statistics, please ensure $P<0.05$ is two-sided and describe the calculation of the 95%CIs of AUC and sensitivity and specificity. Please also describe the covariates adjusted in the multiple Cox regression analysis.

REPLY 6): This study is a retrospective study, the results presented in the paper are phased, and our follow-up period is based on the chemotherapy cycle. In statistics, all P values are two-sided, and the calculation method of 95%CIs of AUC is Wilson/Brown.

Changes in the text: see Page 6-7, line 182-190. And figure2, 3, 4, 5.

7) Seventh, please review and cite several related papers: 1. Zou Y, Ren X, Zhang H, Wang Y, Wang H, Bai R, Zhang Z, Sun G, Xu L. Efficacy and safety of durvalumab + chemotherapy vs. atezolizumab + chemotherapy in the treatment of small-cell lung cancer: a retrospective comparative cohort study. J Thorac Dis 2023;15(6):3339-3349. doi: 10.21037/jtd-23-588. 2. Xu L, Luo Y, Tian J, Fang Z, Zhu W, Zhang B, Wu J, Li Y. A validated nomogram integrating baseline peripheral T-lymphocyte subsets and NK cells for predicting survival in stage I–IIIA non-small cell lung cancer after resection. Ann Transl Med 2022;10(5):250. doi: 10.21037/atm-21-6347. 3. Xu X, Wang D, Chen W, Li N, Suwinski R, Rossi A, Rosell R, Zhong J, Fan Y. A nomogram model based on peripheral blood lymphocyte subsets to assess the prognosis of non-small cell lung cancer patients treated with immune checkpoint inhibitors. Transl Lung Cancer Res 2021;10(12):4511-4525. doi: 10.21037/tlcr-21-899.

REPLY 7): References have been cited in appropriate places in the article.