Peer Review File

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To reviewer A:

Comment 1: The authors state that no relationship was noted between the % of PD-1 positive CD8 cells and IO benefit, but do not provide details on how this measurement was exactly performed. PD-1 staining of lymphocytes from patients treated with PD-1 inhibitors (as was the majority of patients in this study) is compromised by the fact that the PD-1 molecule on lymphocytes is occupied by the therapeutic PD-1 antibody and not available for the fluorochrome-labeled antibody.

Reply 1: In this analysis, 3 cases to which anti-PD-L1 antibody was administered were included, and there was a tendency that slightly more fluorochrome-labeled anti-PD-1 antibody was bound than the group to which anti-PD-1 antibody was administered. However, no significant difference was detected. Although it was investigated in the literature, it was unclear whether the fluorochrome-labeled anti-PD-1 antibody could bind to the PD-1 molecule to which the therapeutic anti-PD-1 antibody was bound.

Comment 2: One problem with accuracy of flow cytometry measurements on PBMC is the presence of dead cells due to the thawing of samples. What was the PBMC viability in this study? Was there any dye used for live-dead cells discrimination? (e.g. PI, 7AAD etc)?

Reply 2: The PBMC viability of this study was about 80%. Although the nuclei were not dyed, dead cells and live cells could be distinguished by flow cytometer according to the cell size and density. It was confirmed that the discrimination was accurate even by the microscopic findings.

Comment 3: Figure 3B for PFS (left): what was the number of cases in each group? It appears that very few cases were included in the "low" group, which would limit the importance of this finding. PLease add "numbers at risk" data to all survival plots.

Reply 3: We added “number at risk” data to all survival plots.

Comment 4: It would be better (more robust) to test correlations using the Spearman instead of the Pearson's index.

Reply 4: We tried to test correlations Spearman instead of the Pearson's index, but the results were similar to the Pearson's index’s one.

Comment 5: (minor): the quality of Tables: they appear to be Excel screenshots, and Table 3C even has a cell selected in it.

Reply 5: The table was created with the specified Excel software, but I don't think it is screenshots.

To reviewer B:

Major comments:

Comment 1: Please re-write the first paragraph in the Introduction. I cannot fully catch the points. Since we know ICIs block the interaction between PD-1 and PD-L1 for reactivating CD8+ T cells, what kind of specific mechanism for ICIs therapies is urgent to be investigated?

Reply 1: In clinical practice, tumor PD-L1 expression is an excellent biomarker, but it is often found that it does not correlate with clinical efficacy. This analysis was conducted to search for better biomarkers in predicting the effects of ICI.

Changes in the text. Lines 5-7 in Page 6 were added.

Comment 2: In the second paragraph of the Introduction, since PD-L1 expression predicts the efficacy of anti-PD-1 therapy (Reference 6), how to explain that PD-L1 levels are not statistically significant in Table 3B. Meanwhile, what is a complete biomarker?

Reply 2: We believe that PD-L1 expression in tumors is not a complete biomarker. Even in clinical practice, it is often found that the PD-L1 expression of tumors does not match the clinical effect.

Comment 3: In Reference 16, Thomas Duhen et al. have revealed that CD103+CD39+CD8+ T cells appear in tumors but not in peripheral blood (Fig 1e in Reference 16). Why did you still investigate the exhausted tissue-resident memory

phenotype CD8+ T cells as the predictor of ICIs? Did you have positive control for the flow experiment?

Reply 3: From the beginning, we also could not predict whether the CD103 CD39 CD8 T cells would actually correlate. Analysis of the population of cells thought to be involved in the cancer immune response revealed that CD103 CD39 CD8 T cells correlated with the effects of ICI.

Comment 4: Please show the flow results of CR+PR (0.1~1%), SD (0~0.5%), and PD (0%) before and after ICIs therapy in Fig 2A. Meanwhile, the 0.13% in Q6 is actually % in CD8+T, not % in PBMCs.

Reply 4: All the obtained cell proportions were converted to the proportions in PBMC and analyzed.

Comment 5: Please show the flow results of CR+PR, SD, and PD before and after ICIs therapy in Fig 2B. In addition, CD103+CD39+CD8+ T cells also express high PD-1 (Fig 2e in Reference 16), how to explain that there are no PD1+CD8+ T cells increased in Fig 2B?

Reply 5: Since CD103 + CD39 + CD8 + T cells have a small population, it is probable that PD1 + CD8 + T cells as a whole did not increase.

Comment 6: The flow data is shown at 2.74 for PD+CD8+T (left in Fig 2B), but the average is about 11 % in PBMCs (right in Fig 2B). How to connect the data.

Reply 6: The figure on the left in Fig. 2B shows only a typical example when setting

conditions, and the figure on the right in Fig. 2B is the actual result of case analysis.

Comment 7: If pretreatment CD103+CD39+CD8+ T cells result in CR and PR, why patients with CR and PR did not present better PFS or cancer specific-survival probability (Fig 4A and 4B)?

Reply 7: As the reviewers pointed out, pretreatment CD103 + CD39 + CD8 + T cells correlated with CR and PR, but did not lead to prolongation of PFS and cancer-specific survival. In general, cases with CR and PR often show these prolongations. The reason for this was not well understood from this analysis. It may be necessary to increase the number of cases and analyze.

Comment 8: Please also show relationship between the change of CD103+CD39+CD8+T and tumor CD8+ cells; the pretreatment of CD103+CD39+CD8+T and tumor CD8+ cells (Fig 4C). In addition, how to make sure CD103+ cells in tumor tissue are CD8+T cells, not dendritic cells?

Reply 8: In this analysis, a pathologist morphologically differentiated lymphocytes from DCs and counted only CD103-positive lymphocytes as shown in lines 15-18 Page8.

Comment 9: Please separate and compare CR+PR with SD+PD for the parameters in Table 2.

Reply 9: We separated and compared CR+PR with SD+PD for the parameters in Table 2.

Comment 10: In the first paragraph of the Discussion, CD103 is upregulated by TGFbeta (reference 25). However, as we know, TGFbeta suppresses CD8+T cells. What is the mechanism for the up-regulation of CD103 in CD8+ T cells by ICIs?

Reply 10: Though we don't know the relationship with TGFbeta, it was reported that tumor derived IL-33 was crucial for antitumor efficacy of ICIs. Mechanistically, IL-33 increased the accumulation and effector function of tumor resident CD103+ CD8+ T cells and CD103 expression on CD8+ T cells was required for the antitumor efficacy of IL-33 (Cancer Immunol Res. 2020; 8(11): 1381-1392).

Comment 11: Does any evidence support that CD103+CD8+ T cells are activated and proliferated by ICIs? What is the activation mechanism?

Reply 11: It was reported that tumor derived IL-33 was crucial for antitumor efficacy of ICIs. Mechanistically, IL-33 increased the accumulation and effector function of tumor resident CD103+ CD8+ T cells and CD103 expression on CD8+ T cells was required for the antitumor efficacy of IL-33 (Cancer Immunol Res. 2020; 8(11): 1381-1392).

Comment 12: What is the correlation between the Brinkman index and the CD103+CD39+CD8+ T cells?

Reply 12: The relationship between the Brinkman index and CD103 + CD39 + CD8 + T cells has not been clarified. Further analysis is needed.

To reviewer C:

Major comments:

Comment 1: The authors failed to provide the description on the methodology of variable selections for multivariable analysis in the method section.

Reply 1: Multivariate analysis was performed using a general a COX proportional hazards model using the EZR analysis software described in Materials and Methods. The analysis method is shown according to the general paper description method.

Comment 2: The patients background (table 2): The authors should provide data for stage (and metastatic status), TPS and line of ICI treatment as key characteristics of their cohort.

Reply 2: We added data for stage (and metastatic status), TPS. The line of ICI treatment was shown before the correction.

Comment 3: The authors failed to fully explain the limitations of this study.

Reply 3: We added the limitation of this study. The follow-up period was also limited to the median observation period 220 days.

Changes in the text. Lines 16-17 in Page 18 were added.

Comment 4: Table 3A: The authors failed to provide the explanation on the cutoff values for variables in the univariate and multivariable analysis. Also, the cutoffs for

some variables (age, brinkman, line, TPS) are not clinically reasonable. These points seem severely impaired the comprehensibility of data.

Reply 4: As described in Materials and Methods, the cut off value was calculated from the ROC curve using EZR software. We think that this method is a common analytical method and is widely accepted. It is thought that this is a valid method for research analysis targeting the cohort of this study.

Minor comments:

Comment 1: Page7, line 9-23: These sentences seem redundant not fully explaining the background on the background of this study's topic. Rather, the authors should show what is known/unknown from the findings of preceding studies. The comprehensive explanation including other types of cancer might be warranted.

Reply 1: I'm very sorry, but I didn't know which part was being pointed to.

Comment 2: Page 8, line 8, "The pathological stage (p-stage) was determined~": The authors should not know pathological stage for all the cases. For advanced cases, we would only know clinical stage (c-stage). Please clarify this point.

Reply 2: As you pointed out, c-stage is important for this analysis, so we corrected the text.

Changes in the text. Line 5 in Page 8 were added.

Comment 3: Material and methods: The authors should describe the methodology of

adverse event evaluation in the method section, if they would present the data on irAE in the results section.

Reply 3: The cases enrolled in this prospective study were evaluated by the attending physician based on Common terminology criteria for adverse events (CTCAE) version 6 and recorded in the medical record. Information from medical records was collected and analyzed.

Comment 4: Page8, line 11, 12, “PBMCs of the patients were collected and cryopreserved before ICI therapy, and at 1 month and 2 months after the start of ICI therapy”: The authors are encouraged to show the allowance of period of blood sampling, not just as “before” or “1 month”. Also, I recommend define the timepoints after ICI start in the unit of weeks (e.g., 1 month -> 4 (3) weeks). This point can affect the reproducibility of data.

Reply 4: We have changed the time point after the start of ICI to be defined on a weekly basis.

Comment 5: Material and methods: The authors should provide the clear definition on PFS and “cancer specific survival”.

Reply 5: Progression-free survival (PFS) is the period during which the cancer does not progress and remains stable during treatment (after treatment). Cancer-specific survival is defined as the proportion of patients who survive a certain period of time (usually 5 to 10 years) after being diagnosed with cancer, with cancer death as the endpoint. Since

the above commonly used terms were used, they were not described in the text.

Comment 6: Page 10, line 6-12: Please show the catalog numbers for the kits the authors used.

Reply 6: We added the catalog numbers for the kits in the text.

Comment 7: Page 10, “TILs were evaluated according to the method of a previous report”: Please add brief explanation of TILs analysis.

Reply 7: We added brief explanation of TILs analysis.

1. Calculate the ratio of the area where immune cells (lymphocytes, plasma cells) exist to the whole including not only hot spots but also stromal tissues, and use it as the median value.

2. Exclude lymphoid follicles (tertiary lymphatic structure) with granulocytes and germinal centers.

3. Exclude lymphocyte colonies away from the infiltrate of the cancer, even without germinal centers. Exclude necrosis and artifacts.

4. Exclude lymphocyte infiltration away from the stroma close to the tumor even at the tumor border.

5. Exclude lymphocyte infiltration in normal lung tissue, even in close proximity to the tumor.

6. Exclude lymphocytes that have infiltrated into the tumor tissue.

Changes in the text. Lines 13-21 in Page 10 were added.

Comment 8: Page 14, irAE: The authors should present the grade of irAE.

Reply 8: Data on the grade of irAE was also performed in this analysis, but since there was no particular difference, it was not presented.

Comment 9: Figure 3A, Are the ROC curves drawn using time dependent ROC program. The methodology on ROC analysis seems not clearly explained.

Reply 9: ROC analysis was calculated using EZR software as explained in the text. Since the analysis is based on the ROC curve, which is widely used, it is presented according to the general description.

Comment 10: Please show the number at risk for all K-M curves.

Reply 10: We added the number at risk for all K-M curves.

Comment 11: Table 2: What doses “1.5Th “line mean? Dose SD mean SD with confirmation or not?

Reply 11: The durvalmab used as a consolidation therapy after chemoradiation therapy is treated as the 1.5th line. SD shows the statistical standard deviation.

To reviewer D:

In their manuscript autors focused on analyzed the association between the immune-

related molecular expression in peripheral blood mononuclear cells (PBMCs) and lung cancer tissues, and the effects of ICI monotherapy.

The work is very interesting and provides valuable information, but I have some reservations about the size of the group.

I think it would be justified to expand the research group before the publication of the work, it could have a significant impact on the results. Also consideration of other checkpoints could be of importance including PD-L2, CTLA-4, CD80/86.

Besides, I have no comments. The manuscript is well, concisely and coherently organized and presented. The style, language and grammar are accurated and appropriated. The manuscript cited appropriately the latest, important and authoritative references in the introduction and discussion sections.

The author prepared the manuscript according to the appropriate research methods and reporting and the manuscript meeted the requirements of ethics.

The Editor should be considered for publication of such a small research group.

Reply: We would like to express our sincere gratitude for the encouraging comments.

Post-revision Comments:

To reviewer A:

Comment 1: The analysis of PD-1 in blood cells of patients under PD1 inhibitors is not valid, the text sjould be modified to based conclusions only on the 3 patients treated with PD-L1 inhibitors, and this acknowledged as a limitation.

Reply 1: As a reviewer mentioned, only 3 cases with anti-PD-L1 antibody administration were included in this analysis, and the results of anti-PD-L1 antibody administration cases were not well reflected. Therefore, we added this acknowledgement as a limitation.

Changes in the text. Lines 16-18 in Page 18 were added.

Comment 2: -> the death rate of 80% is another limitation, if rare populations with a frequency much below 20% are analyzed

Reply 2: As a reviewer mentioned, the death rate of 80% is another limitation, if rare populations with a frequency much below 20% are analyzed. Therefore, we added this acknowledgement as a limitation.

Changes in the text. Lines 18-19 in Page 18 were added.

Comment 3: Please show the Spearman results, at least to the reviewers, or as supplements or replace Pearson with Spearman if the results are the same

Reply 3: We added the Spearman results. In the Spearman results, although there is a significant difference, it was decideded that the correlation between the relationship of pretreatment CD103+ CD39+ CD8+ cells and CD103+ cells in tumor tissue is not strong because ϱ is a low value.

Changes in the supplemental files. Pearson and Spearman results were attached.

To reviewer B:

Comment 1: should have lead to replacement of Figure 2B left with a figure from actual patients

Reply 1: We replaced Figure 2B left.

Changes in the Figure. Figure 2B left was replaced.

Comment 2: comment 7 (should have lead to rephrasing of limitations)

Reply 2: As the reviewer B pointed out, pretreatment CD103 + CD39 + CD8 + T cells correlated with CR and PR, but did not lead to prolongation of PFS and cancer-specific survival. In general, cases with CR and PR often show these prolongations in lung cancer patients treated with ICIs. The reason for this was not well understood from this analysis. It may be necessary to increase the number of cases and analyze. Therefore, we added this acknowledgement as a limitation.

Changes in the text. Lines 19-24 in Page 18 were added.

Comment 3: Figure 3A includes 1 single “low” patient -> this is not sufficient for conclusions, more cases would be needed or this analysis removed and many more.

Reply 3: Case accumulation has been completed for this analysis, and it is difficult to add further cases. Analysis of the correlation between the change in CD103+ CD39+ CD8+ cells in PBMCs and PFS may have a limitation because there was only one case with fewer than the cut-off for the change in CD103+ CD39+ CD8+ cells in PBMCs.

Changes in the text. Line 24 in Page 18, Lines 1-3 in Page 19 were added.