

Peer Review File

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Reviewer A

Comment 1: The authors have shown that RAB4B is positively correlated with patient outcome in some cancers but negatively correlated with patient outcome in some other cancers. However, all the results are derived from TCGA. It is beneficial to see if these results can be reproduced on more other cohorts.

Reply 1: Thank you for your valuable feedback on our manuscript. In our revised manuscript, we another patient prognosis data queue through online Kaplan-Meier Plotter (<https://kmplot.com/>), emphasizing the importance of validating our results in other patient queues, which will undoubtedly enhance the overall quality and reliability of our study.

Changes in the text: We have modified our text as advised (see Page 4, line 100-101 and Page 7, line 184-189).

Comment 2: The authors indicated that RAB4B might also serve as a biomarker for ICB therapy. However, this was only done by comparison with the TIDE score, which is indirect and not very convincing. There are many datasets with both RNAseq data and ICB response information. The authors may want to directly test the predictive role of RAB4B on these datasets.

Reply 2: We agree that relying solely on the comparison with the TIDE score may not provide the most convincing evidence for the predictive role of RAB4B in ICB therapy. In our revised manuscript, we have incorporated your suggestion and performed additional analysis using available online datasets with ICB response information, providing stronger support for our hypothesis.

Changes in the text: We have modified our text as advised (see Page 9, line 248-256).

Comment 3: Only univariate Cox analysis of the association between RAB4B expression and survival metrics was performed. It is beneficial to also show the results from multi-variate Cox analysis to exclude influence of potential confounding factors such as tumor histology, tumor stage, age, and gender.

Reply 3: We appreciate your suggestion regarding the inclusion of multivariate Cox analysis to account for potential confounding factors such as tumor histology, tumor stage, age, and gender. In our revised manuscript, we have conducted the multivariate Cox analysis based on your suggestions and presented the results, and we believe will enable us to better elucidate the independent prognostic significance of RAB4B expression.

Changes in the text: We have made corresponding supplements and modifications in the main text(see Page 4, line 103-105 and Page 7-8, line 208-217).

Comment 4: Multiple testing correlation should be used when presenting p values

throughout the manuscript.

Reply 4: We acknowledge the importance of multiple testing correction in controlling Type I errors. However, due to the nature of our current study, which primarily focuses on exploratory analyses across various cancer types to identify potential associations between RAB4B expression and prognosis, clinical characteristics, and immune responses, we may not be able to implement this correction for the following reasons. These analyses are intended to generate hypotheses and provide support for future research, and at this stage, strict multiple testing correction may not be necessary. Additionally, some of our results were obtained through online analysis tools, making it challenging to apply rigorous multiple testing correction. Nevertheless, we appreciate your suggestion, and in our revised manuscript, we will explicitly state the limitations of our study regarding multiple testing.

Changes in the text: We have already explained the limitations of our research in terms of multiple tests in the discussion section (see Page 13, line 389-391).

Comment 5: Line 105: "data was downloaded" should be "data were downloaded".

Reply 5: Thank you for your comment. We have modified our text as advised (see Page 4, line 110)

Reviewer B

Comment 1. In the sentences below, you refer to "studies" but have only one citation. Please check and revise.

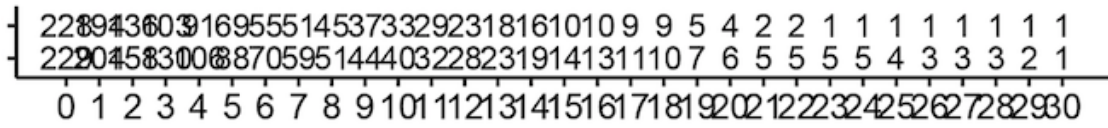
422 30). On the other hand, **studies** have shown that MSI, independent of tumor
423 histology and completely dependent on tumor gene composition, is a pan-cancer
424 biomarker for predicting immunotherapy response (31). A previous report found
104 cholangiocarcinogenesis (14). Immunological **studies** have shed light on the
105 molecular link between RAB4B transcriptional activation and major
106 histocompatibility complex class II genes, further demonstrating the essential
107 functions of RAB4B in mediating endocytic recycling, thereby enhancing the antigen
108 presentation capacity of antigen-presenting cells (9). Nevertheless, it is still

Reply 1: Thank you for bringing this to our attention. We apologize for the oversight in the previous version of the manuscript. We have made changes in the manuscript.

Comment 2. Check whether "." should be * in Figure 2A.

Reply 2: We apologize for any confusion caused by the initial image with the ".". The updated image should accurately reflect the intended content. We want to clarify that the "." were present in the original image that was downloaded from the website. However, upon further investigation and multiple confirmations, we have made the necessary updates and replaced the image with a new version, which no longer contains the ".".

Comment 3. Numbers are overlapping in Figure 3. For example,



Reply 3: Thank you for bringing up the issue regarding Figure 3 in the manuscript. We appreciate your keen observation and attention to detail. Upon careful examination, we have identified the problem of number overlapping in Figure 3. We apologize for this oversight, as it may hinder the readability and interpretation of the data. To address this issue, we will make the necessary adjustments to the figure, such as resizing or repositioning the numbers, as well as ensuring appropriate spacing between them. These modifications will ensure that the data points and values are clearly visible and distinguishable. We appreciate your valuable feedback, which has helped us identify and rectify this problem. Our objective is to present the data accurately and comprehensively, and your input is instrumental in achieving this goal.

Comment 4. The number does not match Figure 4D.

267 $P = 0.034$), PAAD (HR = 0.590, $P = 0.006$), PCPG (HR = 0.137, $P = 0.031$), and UVM

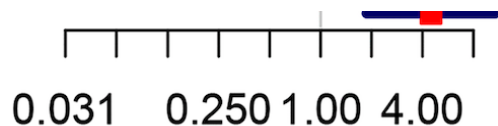
Reply 4: Thank you for pointing out the error in the manuscript. After further investigation, we have confirmed that there is an incorrect description in the manuscript, while the figure itself is accurate. We sincerely apologize for any confusion caused.

We will promptly make the necessary corrections to the manuscript to ensure consistency with the data presented in the figure. We will carefully review the relevant text in the manuscript to ensure accuracy and consistency, and rectify any incorrect statements accordingly.

We greatly appreciate your correction and your thorough review. Your valuable feedback is crucial for improving our research.

Thank you again for your correction and for your patience in reviewing our work.

Comment 5. Scales and numbers do not correspond. Please revise Figure 4A-4C.

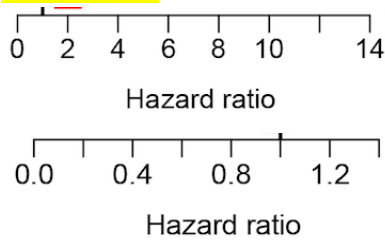


Reply 5: Thank you very much for your review and suggestions. Regarding this issue, we would like to provide the following explanation:

The x-axis values for Figures 4A-4C are as follows: (0.01562, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16). The x-axis of the forest plot is not evenly spaced; instead, it is multiplied by 2 in order to better visualize the data changes. In certain cases, the data may exhibit exponential growth or decay. If we were to use evenly spaced intervals on the x-axis, it would result in larger data points

clustering together while smaller data points would be compressed, making it difficult to observe details and trends. By multiplying the x-axis values by 2, we gradually expand the intervals between them, allowing for a better representation of the data changes. This approach ensures a more even distribution of data points on the plot and enables a more intuitive observation of growth or decay trends.

Comment 6. Numbers are missing in Figure 4D.

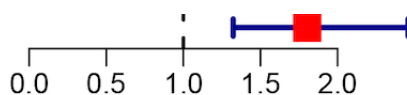


Reply 6: Thank you for bringing up the issue regarding missing numbers in Figure 4D of the manuscript. We appreciate your careful review and attention to detail.

Upon close examination, we have identified an oversight in the labeling of numbers in Figure 4D. We sincerely apologize for this mistake and any confusion it may have caused.

To rectify this issue, we will revise Figure 4D to include the missing numbers and ensure that all data points are appropriately labeled. We will also review the entire manuscript to ensure consistency and accuracy in the presentation of figures and their corresponding labels.

Comment 7. Lines are out of range in Figure 4. For example,





Reply 7: Thank you very much for your suggestions. In Figure 4, some lines exceeded the range. To address this issue, we reanalyzed the data and resolved the problem. However, during the analysis, a few individual results changed. After confirmation, these modifications were made in the manuscript.

Comment 8. Add the age unit in Figure 5B.

Reply 8: Thank you for your feedback regarding Figure 5B in the manuscript. We appreciate your attention to detail and suggestion for improvement. Upon careful consideration, we agree that adding the age unit to Figure 5B would enhance the comprehensibility of the graph. We apologize for the oversight in not including this important information initially. In response to your suggestion, we will revise Figure 5B to include the appropriate age unit. This addition will provide readers with a clear understanding of the temporal scale represented in the figure.

Comment 9. Check if these group categories are correct in Figure 5B.

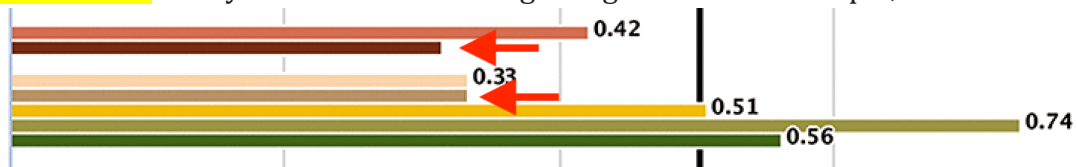
age  ≥ 65  < 65

Reply 9: Thank you for your time and effort in reviewing our manuscript. We performed data analysis after redefining the groups, and the figure has been updated to reflect the correct version.

Comment 10. Indicate what *, **, and *** in Figure 6A and 6B represent.



Reply 10: We apologize for any confusion caused by not explicitly stating the meaning of these symbols in the figure legends. To address this, we will revise the figure legends in Figure 6A and 6B to include a clear explanation of the symbols and their corresponding levels of significance.

Comment 11. Many numbers are missing in Figure 6C. For example,



Reply 11: We greatly appreciate your thorough review and your assistance in ensuring the completeness and accuracy of the information presented in the picture. Your attention to detail is invaluable in maintaining the integrity of our research. Upon careful consideration and in response to your feedback, we have taken the necessary steps to address the issue. We have meticulously added the missing numbers one by one, ensuring their accuracy by referring to the original data.

Comment 12. A space is missing after the comma, and words are out of the border in Figure 6C.

 **Prat2017_PD1_NSCLC-HNSC-Melanoma_Nanostring**
Pos=2, Neg=12 

Reply 12: Thank you for your careful review of Figure 6C and for pointing out the formatting issues. We appreciate your attention to detail and your feedback. We apologize for words extending beyond the border in Figure 6C. These formatting errors were unintentional and have resulted from an oversight during the preparation of the figure. To address these issues, we will resize the text to fit within the borders of the figure. The modification will enhance the clarity and presentation of the information in Figure 6C. However, this figure is downloaded from TIDE, after ", " can not add space, thank you for your understanding.

Comment 13. Provide an overview caption for Figure 8 and note that each subfigure should have its own caption as well.

Reply 13: Thank you very much for your valuable suggestions, and we have revised the manuscript according to your comments. Hope to get your approval.