Peer Review File

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Reviewer A

Comment 1: First, the title needs to indicate the prognostic role of TIM-3 and the clinical research design, i.e., a retrospective cohort study.
Reply 1: We have modified our text as advised (see Page 1, line 2-3)
Changes in the text: "Increased TimIM-3 expression in tumor-associated macrophages predicts a poorer prognosis in non-small cell lung cancer:a retrospective cohort study"

Comment 2: Second, the abstract needs further revisions. The background did not indicate the clinical significance of this research focus. The methods did not describe the inclusion of subjects, the assessment of baseline clinical factors, follow up procedures, and measurements of prognosis outcomes. The results need to first briefly summarize the clinical characteristics of the study sample, and quantify the findings by reporting outcome values, effect size measures such as HR, and accurate P values. The conclusion is overstated since prognostic biomarkers are still far from therapeutic target. Please have comments on the clinical implications of the findings.
Reply 2: We have modified our text as advised (see Page 2-3, line 40-68)
Changes in the text: "…, Our results demonstrated that high TIM-3 expression in TAMs was an independent predictor of worse prognosis in patients."

Comment 3: Third, the introduction of the main text needs to review known prognostic biomarkers of NSCLC, analyze the limitations and knowledge gaps of prior studies, indicate the potential strengths of TIM, and further explain the clinical significance of the research focus on TIM-3.
Reply 3: We have modified our text as advised (see Page 3, line 77-94)
Changes in the text: "…, we need prognostic biomarkers of NSCLC that can take into account cost, operability and practicality."

Comment 4: Fourth,the methodology of the main text needs to indicate the clinical research design, sample size estimation, and follow up details. In statistics, the purpose of multiple Cox regression analysis should focus on the test of the independent TIM-3-OS association, not to identify prognostic factors. Please ensure $P<0.05$ is two-sided.
Reply 4: We have modified our text as advised (see Page 5, line 147-158)
Changes in the text: "…, Diaminobenzidine(DAB) was used for color development, and the sections were stained with hematoxylin."

Comment 5: Finally, please consider to cite the below papers: 1. Domen A, Deben C, De Pauw I, Hermans C, Lambrechts H, Verswyvel J, Siozopoulou V, Pauwels P, Demaria M, van de Wiel M, Janssens A, Hendriks JMH, Van Schil P, Vermorken JB, Vandamme T, Prenen H, Peeters M, Lardon F, Wouters A. Prognostic implications of cellular senescence in resected non-small cell lung cancer. Transl Lung Cancer Res 2022;11(8):1526-1539. doi: 10.21037/tlcr-22-192. 2. Lu D, Ma Z, Huang D, Zhang J, Li J, Zhi P, Zhang L, Feng Y, Ge X, Zhai J, Jiang M, Zhou X, Simone CB 2nd, Neal JW, Patel SR, Yan X, Hu Y, Wang J. Clinicopathological characteristics and prognostic significance of HDAC11 protein expression in non-small cell lung cancer: a retrospective study. Transl Lung Cancer Res 2022;11(6):1119-1131. doi: 10.21037/tlcr-22-403. 3. Li Z, Fan L, Wu Y, Niu Y, Zhang X, Wang B, Yao Y, Chen C, Qi N, Wang DD, Lin PP, Tang D, Gao W. Analysis of the prognostic role and biological characteristics of circulating tumor cell-associated white blood cell clusters in non-small cell lung cancer. J Thorac Dis 2022;14(5):1544-1555. doi: 10.21037/jtd-22-423.

Reply 5: We have modified our text as advised (see Page 3, line 77-94)

Changes in the text: "…, we need prognostic biomarkers of NSCLC that can take into account cost, operability and practicality."

Reviewer B

Comment 1: The authors are requested to provide some relevant in vivo data in support of their observations.

Reply 1: We added some content (see Page8-9, line 264-272)

Changes in the text: "…, This study can also prove the feasibility of our research from the side."

Comment 2: The authors are also requested to find out the intricate molecular mechanism(s) associated with their observations.

Reply 2: We added some content (see Page 9-10, line 295-304)

Changes in the text: "…, This brings inspiration for our next research(29)."

Reviewer C

Minor comments:

Comment 1: Please correct type in title "prognosisin"; Abstract, line 39: the word "NSCLC" is not needed

Reply 1: We have modified our text as advised

Comment 2: In Table 1 authors show stage but only split it to two groups: Stage Ia and Ib-IV. This is not appropriate. Use multiple stages or the early-stage vs advanced

stage (I-IIIa vs IIIb-IV) distinction. When this is done, please evaluate whether there is any different regarding TIM3 expression in early vs late stage NSCLC.
Reply 2: We have modified our text as advised (see Table1)

Comment 3: Authors state in the limitations section ,Third, there is still a lack of direct evidence as to whether TIM-3 is involved in the development of lung cancer by modulating TAM functions" humbleness is respected, but this is not really a limitation, because proving causality was not within the scope of the demonstrated study. However it can be included in another part of the discussion to and expanded with some speculation or potential citations where other researchers tried to find evidence to this (other cancers maybe?)
Reply 3: We have modified our text as advised. We have deleted the limitations section.

Comment 4: In Figure 2 and 3 legends, Authors use the word "correlations" incorrectly describing Kaplan Meier curves. KM analysis shows comparison of survival probability, not correlation.
Reply 4: We have modified our text as advised (see Figure2-3)
Changes in the text: "The comparison of survival probability between TIM-3-positive TAMs and CD68-positive or CD163-positive TAMs."

Comment 5: Please specify median (or mean) survival in survival analyses apart from p-values and HR
Reply 5: We have modified our text as advised

Major comments:
Comment 1: The Discussion of the manuscript lacks an essential comparison, due to the fact, that most of these correlations between TAM markers (CD68, CD163) and TIM3 in the TME have been already elucidated in small-cell lung cancer (SCLC), the closest cancer entity to NSCLC:
1) The widespread stromal expression of TIM3 in highly infiltrated and TAM-rich „hot SCLC tumors" (https://pubmed.ncbi.nlm.nih.gov/32506804/; https://pubmed.ncbi.nlm.nih.gov/34200100/
2) and the negative prognostic role of CD68+ TAM density in SCLC https://pubmed.ncbi.nlm.nih.gov/35978199/
The authors should include discussion and comparison with SCLC that -anyway- nicely coalesces and underpins the Authors' current results, that similar immunological mechanisms act in the two lung cancer types.
Reply 1: We have modified our text as advised (see Page 9, line 273-282)

Changes in the text: "…, This will also become the focus of our next research."

Comment 2: Despite Authors state in the limitations section that they did not performdouble fluorescent stainingto unequivocally prove colocalization of TIM3 and TAM markers, they should include some close-up images of cells with macrophage-like morphology in the stroma stained with TIM3. These images can be inserted as insets in Figure 1.

Reply 2: We have modified our text as advised (see Figure)

Reviewer D

1. Please check all abbreviations in the main text, such as below. All abbreviated terms should be full when they first appear.

178 Conway China) was used for secondary antibody culture. DAB was used for color
179 development, and the sections were stained with hematoxylin.Tumor stroma was
180 defined as the area where tumor stromal cells accounted for more than 70% of the
181 total cells. All slides were reviewed by 2 experienced pathologists who were blinded
182 to the clinical records, and the evaluation of IHC was based on consensus. The

Response: we have modified our text as advised (see Page 5, line 138-143)

2. The below reference should be 18. Please revise.

147 diagnoses were made according to the World Health Organization classifications. The
148 tumor-node-metastasis (TNM) classification of patients was determined according to
149 the 8th edition of the Union for International Cancer Control (15). We measured OS

Response: we have modified our text as advised (see Page 4, line 115)

3. Please add citation of references for the two previous reports.

97 in distinct types of cancer.In NSCLC, only two previous reports demonstrated a
98 negative prognostic value of individual immunohistochemical senescence markers,
99 such as lipofuscin accumulation and high $p21^{WAF1/Cip1}$ and high Ki67 , however, both
100 in rather histological heterogenous patient populations(5).Therefore, we need

Response: we have modified our text as advised (see Page 3, line 75-79)

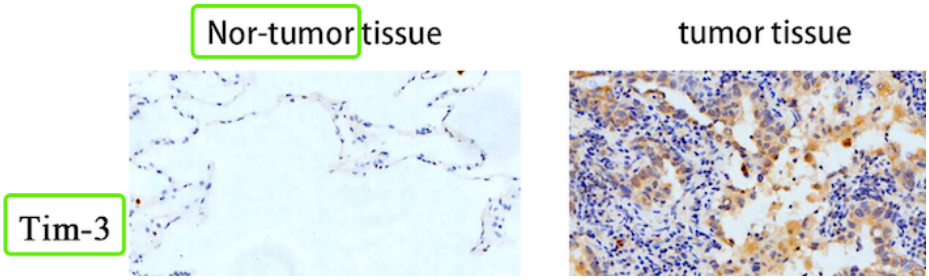
4. Table 1:
The data below in your main text is inconsistent with your Table 1.

As shown in Table 1, high TIM-3 expression in TAMs was more frequently identified in patients with higher CEA levels (>5 ng/mL; TIM-3, 64.29% *vs.* 35.71%, P=0.048), lymph node metastasis (positive; TIM-3, 70.16% *vs.* 29.84%, P=0.010), high CD68 expression (TIM-3, 62.60% *vs.* 37.40%, P=0.018), and high CD163 expression (TIM-3, 67.79% *vs.* 32.21%, P<0.001). No statistically significant differences were found in

CD68 expression, n (%)			0.018
Low	52 (44.83)	64 (55.17)	
High	40 (30.30)	92 (69.70)	

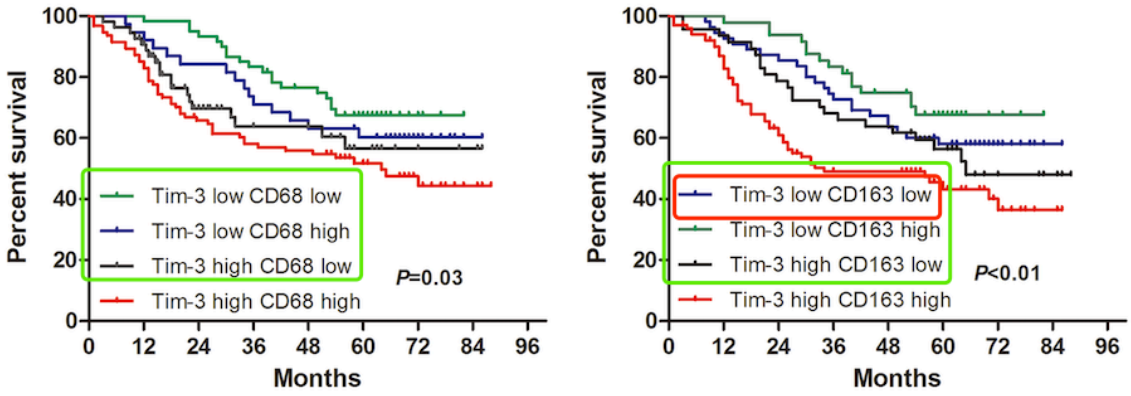
Response: we have modified our text as advised (see Page 6, line 186)

5. Figure 1:
Please revise the below two words to "Non-tumor" and "TIM-3".



Response: The picture has been modified as required and the new picture has been uploaded.

6. Figure 2:
1) Please revise all "Tim-3" to "TIM-3".
2) Figure 2B: The colorful lines don't match with the labels. For example, in the right image below, the green line should be "TIM-3 low CD163 low", not the blue line.



Response: The picture has been modified as required and the new picture has been uploaded.

7. Figure 3:

Figure 3 has the same issues with Figure 2. Please revise.

Response: The picture has been modified as required and the new picture has been uploaded.