Peer Review File
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Reviewer A

Abstract and Background (Lines 29-34)

Comment 1: Clarity and Scope: The abstract provides a clear overview but lacks specific details about the methodology and key findings. This might limit the reader's immediate understanding of the study's significance.

Suggestion: Include a brief mention of the study design and key results in the abstract for a more comprehensive overview.

Reply 1: Thanks for your suggestion. We revised as advised.

Changes in the text: Page 3, line 39-43 and 47-52.

Methods (Lines 35-137)

Comment 1: Study Population (Lines 98-107): The inclusion criteria and patient characteristics are well-defined. However, the rationale for choosing patients with wild-type epidermal growth factor receptor/anaplastic lymphoma kinase is not explicitly stated.

Suggestion: Clarify the reason for this specific patient selection to strengthen the study's focus.

Reply 1: In clinical practice, immunotherapy is primarily used for patients without driver gene mutations. Patients with driver gene-positive status do not benefit as much from immunotherapy as patients with driver gene-negative status. Thus, patients with driver gene-negative status were included in our study.

Changes in the text: N/A.

Comment 2: PD-L1 mRNA Detection (Lines 121-133): The methodology for mRNA detection is detailed, but there's no discussion about the potential for technical variability or validation of the technique.

Suggestion: Discuss the reliability and validation of the PD-L1 mRNA detection method used.

Reply 2: Thanks. We used the Yunying RNA extraction kit (Yunying Medicine, Shanghai, China) in our study, which has great variability or validation and was used widely in Chian. Thus, we did not discuss it.

Changes in the text: N/A.

Results (Lines 142-183)

Comment 1: Statistical Analysis (Line 135): The use of the Kaplan-Meier method and the definition of PFS and OS are appropriate. However, the choice of median PD-L1 mRNA as a cut-off value might introduce bias.

Suggestion: Justify the choice of median value as a cut-off and consider alternative methods to

define high and low PD-L1 expression groups.

Reply 1: Thanks. Currently, there is no gold standard for predicting the efficacy of immunotherapy, so this study did not employ other methods to calculate the optimal cutoff value. Additionally, considering the limited sample size, binary categorization is performed for comparison as described in many previous studies.

Changes in the text: N/A.

Comment 2: Correlation Analysis (Lines 155-165): The lack of significant correlation between TEP-derived mRNA and tumoral PD-L1 expression is noteworthy but not sufficiently explored.
Suggestion: Discuss potential reasons for the lack of correlation and its implications on the study findings.

Reply 2: Thanks. We added this discussion.

Changes in the text: Page 10, line 236-243.

Discussion (Lines 185-216)

Comment 1: Interpretation of Results (Lines 191-200): The discussion appropriately highlights the importance of TEP-derived PD-L1 mRNA in predicting immunotherapy response. However, the implications of the findings in the broader context of NSCLC treatment are not deeply explored.
Suggestion: Expand the discussion to include how these findings could influence current treatment protocols or future research directions.

Reply 1: Thanks. We added this discussion as advised.

Changes in the text: Page 10, line 223-227.

Comment 2: Limitations (Lines 209-216): The acknowledgment of limitations such as sample size and lack of comparison with other biomarkers is good. However, there is no mention of how these limitations could impact the study's conclusions.
Suggestion: Discuss how these limitations might affect the generalizability or applicability of the findings.

Reply 2: Thanks. We added this discussion as advised.

Changes in the text: Page 10-11, line 248-249 and 251-255.

Comment 3： The paper presents a potentially significant finding in the realm of NSCLC treatment. To enhance the paper's impact and clarity:
Expand the abstract to include key methodological details and findings.
Clarify the rationale behind specific patient selections.
Address the reliability and validation of the PD-L1 mRNA detection technique.
Justify the statistical choices, particularly the median value as a cut-off for PD-L1 mRNA expression.

Explore and discuss the lack of correlation between TEP-derived mRNA and tumoral PD-L1 expression more thoroughly.

Broaden the discussion to contextualize the study's findings within the larger landscape of NSCLC research and treatment.

Elaborate on how the study's limitations may affect its conclusions and future research directions.

Reply 3: Thanks for your advice. We modified these questions mentioned above according to your suggestions.

Changes in the text: "Expand the abstract to include key methodological details and findings": **Page 3, line 39-43 and 47-52.**

- "Clarify the rationale behind specific patient selections": **In clinical practice, immunotherapy is primarily used for patients without driver gene mutations. Patients with driver gene-positive status do not benefit as much from immunotherapy as patients with driver gene-negative status. Thus, patients with driver gene-negative status were included in our study.**
- "Address the reliability and validation of the PD-L1 mRNA detection technique": **Thanks. We used the Yunying RNA extraction kit (Yunying Medicine, Shanghai, China) in our study, which has great variability or validation and was used widely in Chian. Thus, we did not discuss it.**
- "Justify the statistical choices, particularly the median value as a cut-off for PD-L1 mRNA expression": **Thanks. Currently, there is no gold standard for predicting the efficacy of immunotherapy, so this study did not employ other methods to calculate the optimal cutoff value. Additionally, considering the limited sample size, binary categorization is performed for comparison as described in many previous studies.**
- "Explore and discuss the lack of correlation between TEP-derived mRNA and tumoral PD-L1 expression more thoroughly": **Page 10, line 236-243.**
- "Broaden the discussion to contextualize the study's findings within the larger landscape of NSCLC research and treatment": **Page 10, line 223-227.**

"Elaborate on how the study's limitations may affect its conclusions and future research directions" **: Page 10-11, line 248-249 and 251-255**

Reviewer B

This paper from Hu et al investigates the value of tumor-educated platelets-derived PD-L1 mRNA as a biomarker for response to immunotherapy in non-small cell lung cancer patients. The paper presents interesting data but needs to undergo major revisions before it is ready for publication.

Major comments:

Comment 1： The Result sections, both in the abstract and in the main paragraph, needs clarity

regarding if the numbers are referring to the whole patient cohort (n=72), or to the subcohort who have only been receiving immunotherapy (n=63). The authors should clarify which cohort is being used to avoid misunderstandings.

Reply 1： Thanks. We revised these questions in the manuscript.

Changes in the text: Page 3 and 8, line 52, 57 and 177.

Comment 2： There is a discrepancy in the definition of responders/patients who have clinical benefit. In the Methods section (page 6, line 137-138) a clinical benefit was defined as having either CR/PR or SD for at least 6 months. At page 6 line 149-150 it is mentioned that 21 patients achieve PR, and 7 patients have a SD of at least 6 months – which would make 28 responders. However, later the authors mention a total of 23 responders, while after dividing into low and high PD-L1 cohort the authors find 24 responders (19 + 5 patients). If this is due to different subcohorts being used or changing definitions of the term responder, is unclear. The authors should clear this up in the text as well as in Figure 3.

Reply 2： Sorry for the confusion. The clinical benefit was defined as having either CR/PR or SD for at least 6 months. Responding to immunotherapy was defined PR or PFS > 6months. Thus, there are different numbers as your question mentioned. We had added the comprehensive information for the “24 responders”. We also deleted the sentence about the 23 responders in order to right description.

Changes in the text: Page 8, line 161-162.

Comment 3： In the Discussion (page 8 line 201-204), the authors mention that TEP PD-L1 mRNA has independent value in predicting immunotherapy outcomes. To support this conclusion a multivariable Cox regression analysis should be conducted both to adjust for baseline variables, line of treatment and combination therapy, as well as to establish TEP PD-L1 mRNA as a biomarker independent of tissue PD-L1 TPS. This could be further investigated by testing both TEP PD-L1 mRNA expression as a continuous and a binary variable.

Reply 3：Thanks for your great advice. We analyzed the relationship between prognosis and clinical-pathological characteristics, such as age, gender, pathology, TPS (PD-L1 expression), with or without chemotherapy, lines of therapy, and expression level of TEP-derived PD-L1 mRNA. Univariate analysis showed that female, lines of therapy are associated with worse prognosis, while high expression level of TEP derived mRNA relates to longer survival (Table S1a and 1b). By using Cox’s hazard regression model, we found that the pathologic type of NSCLC, expression of PD-L1 protein and high expression level of TEP-derived mRNA constitute the independent risk factors.

Changes in the text: Page 7 and 9, line 145-147, 198 -210.

Minor comments:

Comment 1： In the abstract the authors should specify the timing of the blood samples as well as the methods being used to quantify the TEP-derived PD-L1 mRNA

Reply 1：Thanks. We revised it.

Changes in the text: Page 3, line 39-43.

Comment 2： In the Introduction section at page 5, line 91-92, the authors write "However, a few reports have indicated that the mRNA in TEPs can predict the clinical response of advanced NSCLC patients after immunotherapy". The authors should supply this statement with the relevant references.

Reply 2：Thank you. The words should be "few reports", not "a few reports". We corrected it.

Changes in the text: Page 5, line 96.

Comment 3： In the Methods section the authors write "About 5 mL of peripheral blood samples were collected before immunotherapy from all patients." Instead of using the unspecific word "about", could the authors specify the range of mLs of collected blood?

Reply 3：Thanks. We deleted "about".

Changes in the text: Page 6, line 116.

Comment 4： In line with the former comment, the authors write in the Abstract that "This article presented the first data on TEP-derived PD-L1 mRNA in advanced NSCLC patients following immunotherapy […]", however in the Methods section the authors mention that the blood was collected prior to immunotherapy treatment and not following.

Reply 4：Thanks. We revised the description to "In this study, we prospectively detected pre-treatment PD-L1 mRNA and analyzed its prediction value for immunotherapy in advanced NSCLC patients.**" in the former comment.**

Changes in the text: Page 2, line 26.

Comment 5： At page 7 line 157-158, the authors mention that mRNA expression was detected in 72 patients with the number 94.7% being added in a paragraph. However, the whole population of this study is 72 patients, and thus, it should be 100% of the patients who have mRNA expression detected.

Reply 5：Thanks. You are right. We revised it.

Changes in the text: Page 8, line 167.

Comment 6： The authors mention at page 7 line 160-161, the percentage of patients with a PD-L1 TPS of <1%, 1-49% and >50%. However, in Figure 1 the authors use continuous values for the PD-L1 TPS. Please report the median as well as range for these values, both for the

tissue PD-L1 TPS as well as for the TEP-derived mRNA. It would also be relevant to mention the median value that are used to divide the patients into the high and low PD-L1 groups.

Reply 6：Thanks. We added these data in the manuscript.

Changes in the text: Page 8, line 167, 171.

Comment 7： If patients with a SD of at least 6 months is characterized as responders, this information should be included in the comparison in Table 2.

Reply 7：Thanks. We added it in Table 2. We divided SD to two groups (SD≥6 months and SD<6 months) in this table.

Changes in the text: Page 17, line 373.

Comment 8： Figure 1 and Figure 3 needs better clarification of which values is being shown at the y-axis. The reasoning for a cut-off at TPS=5 in Figure 1C is also needed.

Reply 8：Thanks. We added the description of y-axis and x-axis in the legends of Figure 1 and Figure 3. We also reconstruct the Figure 1C. The cut-off was TPS=1 and TPS=50.

Changes in the text: Page18 and 21, line 376-377, 394.

Comment 9： Figure 2 is very confusing. The authors should restructure the figure, so it is clear which plots for PFS and OS analyzed the same patient cohort. Furthermore, the number of patients in each group should be included as well as a number at risk table. It would increase the clarity further if the authors would add headers to the figures.

Reply 9：Thanks. We restructure the Figure 2 according to your advice. We also revised the last two figures showing the difference between high PD-L1 and low PD-L1 in the patients with PD-L1 negative TPS.

Changes in the text: Page 20, line 383-388.

Comment 10： At page 8 line 196-197 the authors write “Previous studies have reported that TEP-derived mRNA could identify NSCLC patients with 96% accuracy” with a reference to papers 29-31. However, none of these papers aim to identify NSCLC patients. Paper 29 shows an association between microRNAs and lung cancer cell invasion, while paper 30 and 31 is focused on prostate cancer patients and gastric cancer patients, respectively. These should be changed to the right references.

Reply 10：Thanks. We revised it.

Changes in the text: Page 9, line 214-216.

Reviewer C

In the realm of non-small cell lung cancer (NSCLC) immunotherapy research, the article titled "PD-L1 mRNA derived from tumor-educated platelets as a potential immunotherapy biomarker in non-small cell lung cancer" offers an intriguing exploration into the use of tumor-educated platelets (TEPs) as a source of programmed death ligand-1 (PD-L1) messenger RNA (mRNA). This study aims to shed light on the role of TEP-derived PD-L1 mRNA in predicting clinical outcomes for advanced NSCLC patients undergoing immunotherapy, providing valuable insights into potential biomarkers for treatment response.

While the research presents a significant contribution to the understanding of unconventional biomarkers, it is essential to critically evaluate the study's methodology, results, and implications. This critique will address both major and minor observations within the article, seeking to enhance our comprehension of the findings and identify areas for further refinement in future research endeavors.

Here are the major remarks :

Comment 1： This study includes a diverse group of patients, encompassing those treated with both single and combination immunotherapy, as well as individuals in both the first and second lines of treatment. These variations introduce numerous potential confounding biases.

I strongly recommend that the analyses be adjusted for these criteria using a minimum of multivariate analysis, and ideally incorporating a propensity score approach.

The tumor proportion score (TPS) of PD-L1 by tumor cells should be included in Tables 1 and 2.

Reply 1： Thanks. We analyzed the relationship between prognosis and clinical-pathological characteristics, such as age, gender, pathology, TPS (PD-L1 expression), with or without chemotherapy, lines of therapy, and expression level of TEP-derived PD-L1 mRNA. Univariate analysis showed that female, lines of therapy are associated with worse prognosis, while high expression level of TEP derived mRNA relates to longer survival (Table S1a and 1b). By using Cox's hazard regression model, we found that the pathologic type of NSCLC, expression of PD-L1 protein and high expression level of TEP-derived mRNA constitute the independent risk factors. Besides, we added the tumor proportion score (TPS) of PD-L1 by tumor cells in Tables 1 and 2.

Changes in the text: Page 7 and 9, line 145-147, 198 -210.

Here are some minor comments:

Comment 1： The figure 2 is not clear enough, and the figures should be grouped in a more logical manner.

Reply 1： Thanks. We revised figure 2 according to your and other reviewers' suggestions.

Changes in the text: Page 20, line 383-388.

Comment 2： "Figure 3: It is essential to clearly distinguish between partial response and progression without progression. I recommend creating a figure with each dissociated.
Reply 2：Thanks. We were confused about this question. We think it is enough to analyze the data using clinical benefits and response.
Changes in the text: N/A.

Comment 3： Subject to modifications in accordance with these recommendations and based on the results, this paper could then be considered for publication.
Reply 3：Thanks for your suggestion.
Changes in the text: N/A.

Reviewer D

The authors investigate the value of blood platelet derived PDL1 mRNA for immunotherapy response prediction. Using a 72-patients cohort, the identified no correlation with the tissue expression levels. However, when the cohort was splitted into a high and low platelet PDL1 subset, the responses were significantly different between both groups. The work is of interest, because minimally invasive biomarkers for immunotherapy response prediction are of relevance. Though, some important questions remain

Comment 1： - The authors employ a two-step differential centrifugation protocol to isolate platelets but as stated in the methods section also exosomal RNA. How pure are the preparations? Have the authors investigated leucocyte contamination, and can they rule-out contribution of exosomal RNA to the platelet RNAs? Have the authors for example investigated the presence of PDL1 in blood platelets using FISH/confocal microscopy?
Reply 1：Thank you for your attention and valuable suggestions. We acknowledge that there may be omissions in the description of our methods that could cause confusion for readers. Indeed, we have thoroughly checked and ensured that the platelet samples post-purification are nearly free of leukocyte contamination. Each platelet-rich plasma sample underwent blood smearing and was subjected to Giemsa staining to assess its quality. Only samples with minimal impurities, such as leukocytes, were allowed to proceed to the subsequent steps of RNA extraction. Besides, microscopic images of 9 randomly selected samples are reviewed and presented for your reference (Fig. Ref_1).

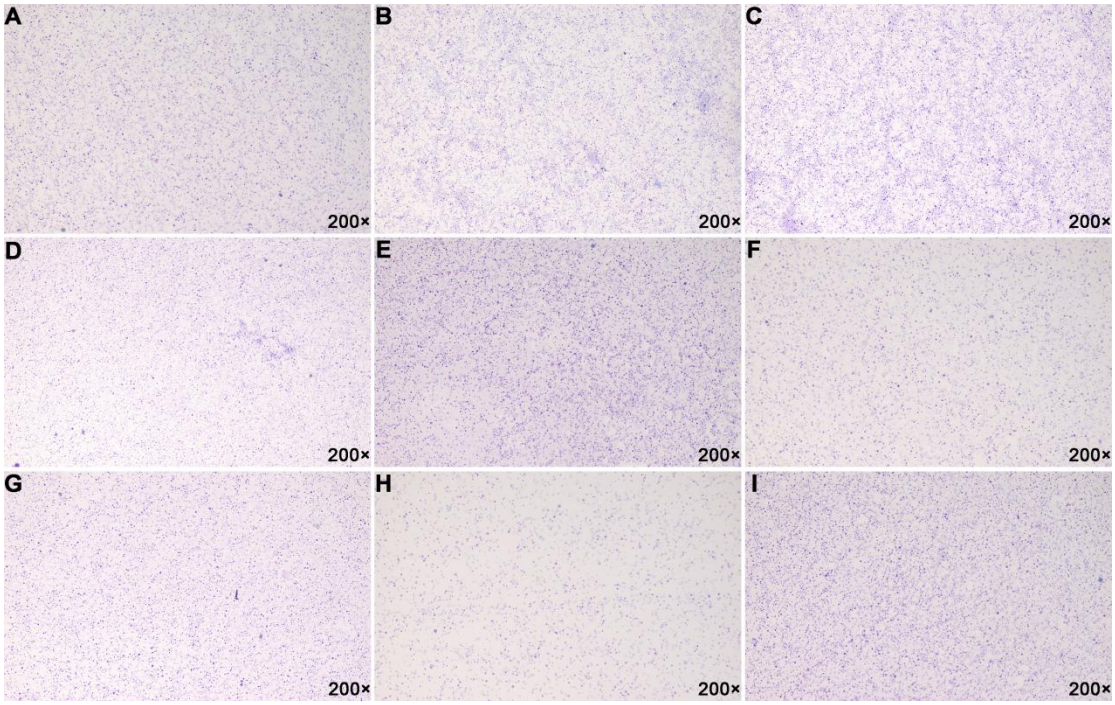


Fig. Ref_1 Giemsa staining results of 9 samples

However, we have not rigorously excluded extracellular vesicle (EV) RNA from platelets. This decision is rooted in the challenge of accurately distinguishing EVs released by platelets from those released by other cells under current technological conditions. Platelets are a major source of EV vesicle release1,2, and precise differentiation between EVs from platelets and those from other cells is deemed difficult. Eliminating EVs from the samples would not only escalate the economic and time costs of detection but also compromise the potential clinical translational application value. Moreover, studies have confirmed that platelet-secreted EVs play a crucial role in oncology3.4, potential bias may caused by the removal of platelet-derived· EVs. Therefore, we believe that, for our study, the meticulous exclusion of platelet EVs is not a necessary step.

In accordance with your suggestion, the following statement has been added in the revised manuscript:"10 μL of each obtained sample was used for the subjecting Swiss Giemsa staining. Samples with high purity, devoid of impurities such as leukocytes in the field of view, proceeded to the next steps of the operation."

Reference:

1. Boilard E, Bellio M. Platelet extracellular vesicles and the secretory interactome join forces in health and disease. Immunol Rev. 2022;312(1):38-51. doi:10.1111/imr.13119

2. Karimi N, Dalirfardouei R, Dias T, Lötvall J, Lässer C. Tetraspanins distinguish separate extracellular vesicle subpopulations in human serum and plasma - Contributions of platelet extracellular vesicles in plasma samples. J Extracell Vesicles. 2022;11(5):e12213. doi:10.1002/jev2.12213

3. Dovizio M, Bruno A, Contursi A, Grande R, Patrignani P. Platelets and extracellular vesicles in cancer: diagnostic and therapeutic implications. Cancer Metastasis Reviews. 2018;37(2-3):455-467. doi:10.1007/s10555-018-9730-4

4. Lazar S, Goldfinger LE. Platelets and extracellular vesicles and their cross talk with cancer. Blood. 2021;137(23):3192-3200. doi:10.1182/blood.2019004119

Changes in the text: Page 6, 10 and 11, line 121-123,253-254.

Comment 2：- How stable and consistent is GAPDH expression, used as a reference, in platelets? As far as I am aware, b-actin is a better, more stable, reference marker.

Reply 2： Thank you very much for your suggestion. GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) and β-actin (also known as ACTB) are commonly used as reference genes in gene expression studies. Although β-actin is shown to be more suitable as a reference gene in some studies, GAPDH can also be used as a reference gene in platelets. According to the research by Tatjana et al, both GAPDH and β-actin can be used as internal reference genes to study the expression of platelet serotonin (5HT)1. Additionally, another study found that GAPDH in platelets exhibits more stable expression compared to β-actin as an internal reference gene2. Furthermore, a Comparative Study also showed the feasibility of using GAPDH as an internal reference gene in platelets3. Therefore, based on the existing conditions in our laboratory, we have chosen GAPDH as the internal reference gene.

Reference:

1. Bordukalo-Niksic T, Cicin-Sain L, Jernej B. Expression of brain and platelet serotonin transporters in sublines of rats with constitutionally altered serotonin homeostasis. Neurosci Lett. 2004;369(1):44-49.

2. Wen X, Yang G, Dong Y, et al. Selection and Validation of Reference Genes for Pan-Cancer in Platelets Based on RNA-Sequence Data. Frontiers In Genetics. 2022;13:913886. doi:10.3389/fgene.2022.913886

3. Rox JM, Bugert P, Müller J, et al. Gene expression analysis in platelets from a single donor: evaluation of a PCR-based amplification technique. Clinical Chemistry. 2004;50(12):2271-2278.

Changes in the text: N/A.

Comment 3： - How much time was there between tissue collection and platelet collection, and may this have influence the negative correlation in Fig 1? Is it possible that the reference is not 'up to date'?

Reply 3：Thanks for your great question. The time interval was within one year. The question "may this have influenced the negative correlation in Fig 1?" should be studied

in the further research. We also discussed your idea in our manuscript. Besides, we added some great references which is "up to date".
Changes in the text: Page 10 and 14, line 241-242, 362-364.

Comment 4： - Is there a difference in outcome/results between the use of PD1 inhibitors versus PDL1 inhibitors?
Reply 4：Thanks. Some previous studies reported that there was not significant difference between the use of PD1 inhibitors versus PDL1 inhibitors. Besides, this difference is not the topic of our manuscript.
Changes in the text: N/A.

Comment 5： - What tumor stage were these patients in? Because traces of earlier stage tumors are more difficult to detect as compared to advanced stage tumors. Were earlier stage tumors overrepresented in the PDL1 low group?
Reply 5：Thanks. All patients in the study were advanced NSCLC patients, which was shown in the Methods part.
Changes in the text: N/A.

Comment 6： - What is the biological mechanism explaining this observation? Can the author speculate on this in the revised manuscript? How is this data interpretated in light of this recent study (PMID 37270827)?
Reply 6：Thanks. We added the related discussion according to your suggestion.
Changes in the text: Page 10, line 228-235.

Comment 7： - An independent validation cohort should be included, confirming their finding of a cutoff value predicting response to PD(L)1 inhibitors.
Reply 7：Thanks for your suggestion. The number of samples of our study was limited. We will include the independent validation cohort in the next study as you advised.
Changes in the text: N/A.

Reviewer E

The authors present a novel method using tumor-educated platelet (TEP)–derived PD-L1 messenger RNA as a surrogate biomarker to tissue-based assay in patients with advanced non-small cell lung cancer (NSCLC). The liquid biopsy methods in cancer diagnostics are promising and carry numerous benefits. The knowledge of TEP-based methods and their clinical utility in NSCLC is still scarce. Thus, the topic is relevant and important.
The study shows the potential usefulness of the assay in predicting response to immune

checkpoint inhibitors. Namely, patients with high PD-L1 mRNA levels had higher response rates and longer survival than their low mRNA level counterparts.

The study is interesting but has several limitations. The most important of these, in addition to the small patient size, is the high heterogeneity of the studied population, which included patients administered:

- six PD-1 and PD-L1 inhibitors
- with or without chemotherapy
- in the first- and second-line settings.

Hence, the results should be interpreted cautiously, particularly regarding subset analyses, and conclusions should be nuanced.

Specific comments

Comment 1： Key findings: In the statement "This research presents the data of tumor-educated platelet (TEP)–derived programmed death ligand 1 (PD-L1) messenger RNA (mRNA) in advanced non-small cell lung cancer (NSCLC) patients following immunotherapy and discusses the potential advantage of using it as a surrogate biomarker for predicting the progression-free survival (PFS) and overall survival (OS) of NSCLC patients following immunotherapy" – what do you mean by "following immunotherapy" in both cases?

Reply 1：The word means patients treated by ICIs, including nivolumab, pembrolizumab, atezolizumab, durvalumab, tremelimumab, and camrelizumab.

Changes in the text: N/A.

Comment 2： Key findings: The conclusion: "we found (that) the expression of TEP PD-L1 mRNA was much higher in the treatment-naïve patients than those who progressed after first-line chemotherapy, which supports the early use of immune checkpoint inhibitors (ICIs) in the treatment of advanced NSCLC" is illogical and should be removed.

Reply 2：Thanks. We deleted it.

Changes in the text: N/A.

Comment 3： Line 59-60" The statement: "Every year, more than one million people are diagnosed with or die from lung cancer" is awkward, as it implies the total number of diagnosed cases and deaths.

Reply 3：Thanks. We revised this sentence.

Changes in the text: Page 5, line 66.

Comment 4： Lines 73-75: "Tumor programmed death ligand-1 (PD-L1) expression, the tumor mutational burden (TMB), deficient mismatch repair, tumor infiltrating lymphocytes, and related gene expression signatures have been identified as potential predictors….". Actually, only tumoral PD-L1 expression is an accepted assay in clinical use; others are still investigational.

Reply 4：Thanks. The sentence means that these are potential predictors, not confirmed as the clinical predictors.
Changes in the text: N/A.

Comment 5: Line 137: "OS was defined as the time from ICI initiation until the date of death." This is incorrect – the Kaplan-Mayer curve also includes censored values (not all patients have died).
Reply 5: Thanks. We corrected the sentence to "OS was defined as the time from ICI initiation until the date of death or last follow-up date".
Changes in the text: Page 7, line 142.

Comment 6: Line 100: "wild type epidermal growth factor receptor/anaplastic lymphoma kinase" – consider "wild type epidermal growth factor/anaplastic lymphoma kinase receptor".
Reply 6: Thank you. We revised it.
Changes in the text: Page 6, line 107.

Comment 7: Line 158-159. PD-L1 expression was examined in the archived tissue of only 58% of patients. PD-L1 status is a routine diagnostic method in patient selection for PD-1 and PD-L1 immune checkpoint inhibitors. Thus, these data should be available for all patients.
Reply 7: Thanks. Some patients in the study was treated by immunotherapy as the second-line therapy, when it was not necessary to examine the PD-L1 expression. Thus, we only collected the PD-L1 expression of 58% of patients.
Changes in the text: N/A.

Comment 8: Line 155-161: The authors should explain in Discussion the lack of correlation between TEP-derived PD-L1 mRNA and PD-L1 TPS and, if possible, compare the predictive value of both assays.
Reply 8: Thanks. We added the discussion about the finding. We did not compare the predictive value of both assays because we had compared the correlation about these.
Changes in the text: Page 10, line 236-243.

Comment 9： Line 156-157. Forty-three patients underwent dynamic monitoring of PD-L1 mRNA derived from TEPs after immunotherapy, but the data for this monitoring are not presented.
Reply 9：Due to the research topic focusing on "the predictive value of PD-L1 mRNA derived from TEPs before immunotherapy", we did not analyze the data related to the dynamic monitoring. Thus, we deleted this sentence. Thanks for your advice.
Changes in the text: Page 8, line 165-166.

Comment 10： Line 169-170: provide percentage values of response to treatment

Reply 10：Thanks. We added the percentage values.

Changes in the text: Page 8, line 180-182.

Comment 11： There is no data on median follow-up.

Reply 11：Thanks. We added it.

Changes in the text: Page 7, line 155.

Comment 12： The percentage and time values should be rounded to decimals.

Reply 12：Thanks. We revised as advised.

Changes in the text: N/A.

Comment 13： Table 1 should include data on first-line chemotherapy in patients who were administered immunotherapy in the second line.

Reply 13：Because we did not analyzed drugs for patients who were administered immunotherapy or a combination of immunotherapy and chemotherapy in the second line, we could not include the data.

Changes in the text: N/A.

Comment 14： Table 1: the list of checkpoint inhibitors does not include tremelimumab mentioned in Methods (line 103).

Reply 14：Thanks. We corrected "Durvalumab" to "Tremelimumab".

Changes in the text: Page 16, Table 1.

Comment 15： Table 2: "Patient background" replace by "Patient characteristics". Add the third column (Total). What do P-values refer to?

Reply 15：Thanks. We revised the word and added the column. The P-value means the statistical difference of chi square test.

Changes in the text: Page 17, Table2.

Comment 16： Figure 1. y-axis: what do you mean by values? PD-L1 TEP? Make it clear.

Reply 16：Thanks. We added the information in the legend of Figure 1.

Changes in the text: Page 18, line 377.

Reviewer F

1. Figure 1

Please unify 'PDL1' to 'PD-L1'.

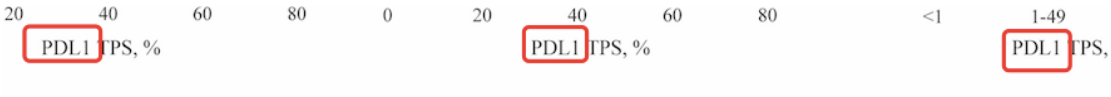
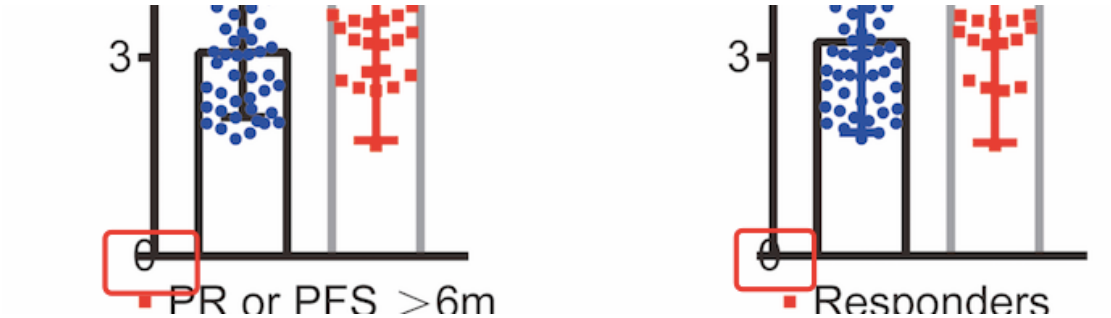


Figure 1. The correlation of PD-L1 TPS (X-axis) and TEP-derived mRNA value (Y-axis). (A) PD-L1 TPS and TEP-derived mRNA in all patients. (B) PD-L1 TPS (≥ 1%) and corresponding TEP-derived

A: Thanks. We revised it.

2. Figure 3

The number '0' is overlapped. Please revise.



A: Thanks. We revised it.

3. The textual contents in '**Table S1a and 1b**' file is duplicated with the main text. Please confirm if those contents are necessary in 'Table S1a and 1b' file. Otherwise, please delete them.

144 expression or low PD-L1 expression. Using Cox regression analysis, we also analyzed the relationship
145 between prognosis and clinical-pathological characteristics, such as age, gender, pathology, TPS (PD-L1
146 expression), with or without chemotherapy, lines of therapy, and expression level of TEP-derived PD-
147 L1 mRNA. A two-sided P value <0.05 level was considered statistically significant.

191 derived mRNA expression than the other patients (median: 3.0 vs. 3.5 months, P=0.012).

192 Univariate analysis showed that female, lines of therapy are associated with worse prognosis, while high
193 expression level of TEP derived mRNA relates to longer survival (Table S1a and 1b). By using Cox's
194 hazard regression model, we found that the pathologic type of NSCLC, expression of PD-L1 protein and
195 high expression level of TEP-derived mRNA constitute the independent risk factors.

We analyzed the relationship between prognosis and clinical-pathological characteristics, such as age, gender, pathology, TPS (PD-L1 expression), with or without chemotherapy, lines of therapy, and expression level of TEP-derived PD-L1 mRNA. Univariate analysis showed that female, lines of therapy are associated with worse prognosis, while high expression level of TEP derived mRNA relates to longer survival (**Table S1a and 1b**). By using Cox's hazard regression model, we found that the pathologic type of NSCLC, expression of PD-L1 protein and high expression level of TEP-derived mRNA constitute the independent risk factors.

A: Thanks. We deleted it in the 'Table S1a and 1b' file.

4. Please revise 'Table S1a and 1b' to 'Table S1 and Table S2'.

Univariate analysis showed that female, lines of therapy are associated with worse prognosis, while high expression level of TEP derived mRNA relates to longer survival (Table S1a and 1b). By using Cox's

Table S1a

Univariate overall survival analysis for the patients by Cox' s proportional hazards model

Characteristics	P value	HR (95% CI)
Gender	0.0046	3.5 (1.5-8.2)
Age	0.056	0.95 (0.9-1)
Pathology	0.32	1.6 (0.63-4.2)
TPS	0.94	1 (0.98-1)
Chemotherapy	1	1.8e-08 (0-Inf)
Lines of therapy	0.041	8.3 (1.1-62)
Expression level	0.0089	0.23 (0.076-0.69)

Table S1b

Multivariate Cox analysis for the patients

A: Thanks. We revised it.

5. Please spell out the following abbreviations in the Table S1 and S2: TPS, HR, CI.

A: Thanks. We added it.

6. Table 1 and Table 2

Please indicate the unit of age.

Age (median)			61 (42–75)	
Age median (range)	61 (42-75)	61 (48-75)	60 (42-72)	0.131

A: Thanks. We added it.

7. Table 2

It seems that the P value was in wrong box. Please check all P value and revise.

Table 2 Patient characteristics and the effect of ICI treatment for the high and low PD-L1 mRNA group.

Characteristics	Total (n=72)	High PD-L1 group (n=36)	Low PD-L1 group (n=36)	P value (χ^2 test)
Gender				
Male	54	29	25	
Female	18	7	11	0.414
Age median (range)	61 (42-75)	61 (48-75)	60 (42-72)	0.131
Pathology				
Adeno	49	23	26	
Squamous	23	13	10	0.613
PD-L1 TPS (%)				

A: Thanks. We revised it.

8. The author's name does not match the citation. Please check and revise.

Chiara et al. reported that PD-L1 on platelets represent an easy-to-use clinical approach to predict ICI responsiveness (29).

29. Colarusso C, Falanga A, Terlizzi M, et al. High levels of PD-L1 on platelets of NSCLC patients contributes to the pharmacological activity of Atezolizumab. Biomed Pharmacother. 2023; 168:115709.30. Mok TSK, Wu YL, Kudaba I, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet 2019;393:1819-30.

A: Thanks. We revised it. It is "Chiara Colarusso".