Reviewer A

Comment 1: Abstract: row numbers 43-44; you should specify at which PD-L1 level it applies? 1%, 50%?

Reply 1: We are sorry for our negligence of the clear definition of PD-L1 level used in this study at the beginning of the article. According to the references, the cut-off for PD-L1 positivity or negativity was that PD-L1 stained cells accounted for 1% of tumour cells, or tumour and immune cells, assayed by immunohistochemistry staining methods [1, 2]. Therefore, in order to clarify the grouping criteria clearly, we have made modifications according to the Reviewer's comments.

Changes in the text: We have supplemented and specified a threshold of 1% PD-L1 expression level for grouping criteria in the Abstract-Results of the revised manuscript (see Page 2, line 38-40).

Comment 2: Keywords: you have already NSCLC, PD-L1 clinicopathological characteristics in the title, you should instead use CEA, NSE, WBC TNM etc… You should always avoid using the same words as in the title!

Reply 2: To avoid using the same words as the title, we have modified the Keywords according to the Reviewer's suggestion.

Changes in the text: We have modified our text as advised in the Keywords of the revised manuscript (see Page 3, line 60).

Comment 3: Is there a difference in your outcomes between NSCLC subtypes? ADC vs. SCC?.

Reply 3: Thanks for your thoughtful suggestion. We have conducted subgroup analysis in NSCLC patients, and the results indicated that there was no statistically significant difference in the outcomes between ADC and SCC patients. Therefore, this part of the results was not presented in the article. Additionally, according to another reviewer's comments, considering the relatively small proportion of SCC patients (n=27, 16.5%) and the high prevalence of ADC [4], after careful consideration, we removed SCC and only retained ADC patients for final analysis. Our study has been ongoing until now,

and the cases have been collected until December, 2021. The sample search scope has been extended from the initial September, 2021 to December, 2021 (see Page 8, line 143), with an additional 18 cases added (n＝136) (see Page 8, line 144). In the future, we will conduct a larger sample study to compare the differences in outcomes between the two pathological types.

Changes in the text: After excluding other types of NSCLC, we have re-analyzed and discussed the hematological test results and clinicopathological parameters of the finally included lung adenocarcinoma patients (see Page 8, line 144), and re-analyzed the correlation between these characteristics and PD-L1 expression (see Tables 2-4) in the revised manuscript.

Comment 4: Introduction: row numbers 86-91; you cannot underestimate the importance of NGS in detecting the molecular alterations that show significant correlation with PD-L1 expression and influence the patient´s response to immunotherapy.

Reply 4: It is really true as Reviewer suggested that NGS plays an important role in detecting molecular mutations related to PD-L1 expression and predicting immunotherapy responses, however, due to economic barriers and limited access to this new technology, the popularity of this detection method is relatively low. Therefore, a non-invasive and relatively low-cost method that produces sensitive and accurate predictors are urgently needed to estimate the expression levels of PD-L1. We have already supplemented and elaborated on the importance of NGS in detecting molecular alterations of lung cancer targets in the Introduction part. This reference has been quoted as Ref.19 in the revised manuscript.

Changes in the text : A brief description of the role of NGS in lung cancer has been added in the Introduction (see Page 6, line 98-103) and the paper by Dacic S *et al.* has been cited as Ref. 19 in the revised manuscript.

Comment 5: Introduction: row numbers 115-116. M&M, row number 124; you cannot use "we" in the introduction and M&M. You can only use it in the discussion.

Reply 5: Correction has been made in the revised manuscript.

Changes in the text: We have replaced the "we" in the Introduction and M&M part of the manuscript as required (see Page 7, line 131, 133, 138 and 142; Page 9, line 185).

Comment 6: M&M: subtitle "study design and patients" should be "study design and patient selection" OR "study design and patient collection".

Reply 6: We are very sorry for our incorrect writing, and we have made correction according to the Reviewer's comments (see Page 7, line 139).

Comment 7: Discussion: you may discuss the possibility of combining these haematological assays and liquid biopsy in the future to avoid the invasive methods.

Reply 7: Thank you for your insightful suggestion. As the Reviewer mentioned, liquid biopsy, as a diagnostic tool, is becoming increasingly important in the clinical treatment of lung cancer patients. It is worth noting that liquid biopsy-based detection techniques have been proven to be very helpful in identifying operable tumor markers, especially when tissue biopsy specimens are insufficient or unavailable.

Changes in the text: In Discussion of the revised manuscript, we have added the prospect of combining these haematological assays with liquid biopsy to avoid invasive methods in the future (see Page 16-17, line 335-341). At the same time, according to the Reviewer's proposal, we are also considering the combination of the above two as a methodological detection for PD-L1 expression in future research. Several sentences have been added in the Conclusions of the revised manuscript (see Page 17, line 351).

Comment 8: All your results are based on the 1% cutoff of PD-L1 expression. What about 50% cutoff which is important in order to further guide clinical decision-making?

Reply 8: As stated in Comment 1, the cut-off value for PD-L1 positive or negative expression was PD-L1 stained cells accounted for 1% of tumour cells, or tumour and immune cells, assayed by immunohistochemistry staining methods[1-3], and the primary purpose of this study is to screen hematological and clinicopathological markers that can be used to predict PD-L1 positive expression, so we chose the 1% of PD-L1 expression as cut-off value. In the article, we also explained the differences compared to other studies, including the difference in PD-L1 expression threshold as a grouping criterion (see Page 15, line 304-305). As the Reviewer suggested that 50% expression is crucial for the treatment choice of ICIs. Currently, we are planning a subgroup analysis that aims to demonstrate the differences between high and low expression of PD-L1 groups with a cut-off of 50% in positive expression patients, in order to further enrich the research results.

Special thanks to you for your good comments.

References:

1. Ghiringhelli F, Bibeau F, Greillier L, et al. Immunoscore immune checkpoint using spatial quantitative analysis of CD8 and PD-L1 markers is predictive of the efficacy of anti-PD1/PD-L1 immunotherapy in non-small cell lung cancer.

EBioMedicine. 2023;92:104633. doi:10.1016/j.ebiom.2023.104633

2. So WV, Dejardin D, Rossmann E, Charo J. Predictive biomarkers for PD-1/PD-L1 checkpoint inhibitor response in NSCLC: an analysis of clinical trial and real-world data. J Immunother Cancer. 2023;11(2):e006464. doi:10.1136/jitc-2022-006464

3. Rakaee M, Adib E, Ricciuti B, et al. Association of Machine Learning-Based Assessment of Tumor-Infiltrating Lymphocytes on Standard Histologic Images With Outcomes of Immunotherapy in Patients With NSCLC. JAMA Oncol. 2023;9(1):51-60. doi:10.1001/jamaoncol.2022.4933

4. Hu F, Peng J, Niu Y, Mao X, Zhao Y, Jiang L. Clinical predictors of treatment efficacy and a prognostic nomogram in patients with lung adenocarcinoma receiving immune checkpoint inhibitors: a retrospective study. J Thorac Dis. 2022;14(10):4096-4112. doi:10.21037/jtd-22-1270

Reviewer B

Comment 1: Stage of the study patient is unbalanced. Especially Stage1-2 are few, should the authors add these? As advanced lung cancer, re-analyzing only late stage (eg, Stage 3-4).

Reply 1: It is really true as Reviewer suggested that stage of the study patient is unbalanced. Given the relatively small sample size of early lung cancer in this study, we have removed the stage 1-2 lung cancer patients based on your valuable suggestion. Our study has been ongoing until now, and the cases have been collected until December, 2021. The sample search scope has been extended from the initial September, 2021 to December, 2021 (see Page 8, line 143), with an additional 18 cases added (n＝136) (see Page 8, line 144).

Changes in the text: After excluding early cancer patients (stage 1-2) and incorporating new clinical

cases, we have re-written this part according to the Reviewer's suggestion (see Page 1, line 2; Page 2, line 31, 35, 37; Page 3, line 51, 57), and re-analyzed and revised the Results accordingly (see Page 10, line 193, 195-196) in the revised manuscript.

Comment 2: There are multiple histological types of lung cancer in this paper, but the usefulness of tumor markers cannot be stated unless the histological types are unified. It would be better to unify the tissue type (only adenocarcinoma, etc.)

Reply 2: Thanks for raising this critical issue. As Reviewer suggested that only the unified histological type can explain the use and value of tumor markers. In this study, the sample sizes for adenocarcinoma and other pathological types were 127 and 36, respectively. Due to differences in sample size among different pathological types and the high prevalence of adenocarcinoma, we ultimately unified the studied pathological type as adenocarcinoma and conducted the final analysis and research. In addition, we collected an additional 18 advanced adenocarcinoma patients to compensate for the impact of the decrease in patient sample size after excluding other histological types.

Changes in the text: After excluding other types of NSCLC, we have re-analyzed and discussed the hematological test results and clinicopathological parameters of the finally included lung adenocarcinoma patients (see Page 8, line 144), and re-analyzed the correlation between these characteristics and PD-L1 expression (see Tables 2-4) in the revised manuscript.