Peer Review File

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Reviewer A

Comment 1: In my view, I believe that the language requires editing because it has major flaws that need to be corrected.

Reply 1: Thanks for your suggestion. Several improvements have been made as advised.

Changes in the text: Relevant modifications can be seen in Page 3, line 10, 13; Page 5, line 92; Page 5, line 110, 111-112; Page 6, line 139, 146; Page 7, line 182, 202; Page 9, line 16-18.

Comment 2: The title of the article is the conclusion and therefore a correction would be appropriate.

Reply 2: Thanks for your suggestion. We agree with your opinion. And the title has been changed to 'In-depth proteomic analysis identifies key gene signatures predicting therapeutic efficacy of anti-PD-1/PD-L1 monotherapy in non-small-cell lung cancer'.

Changes in the text: The change in title can be seen in Page 3, line 1-3.

Comment 3: Also, the genes should be in italics.

Reply 3: Thanks for your suggestion. All the spellings in genes have been checked and corrected.

Changes in the text: We correct our spellings in the text. (see Page 3, line 19-25; Page 8, line 225-226, 236, 246; Page 9, line 289-292, 302-305)

Reviewer B

Comment 1: Please be very specific on cohort composition on patient stage, histological diagnoses, line of therapy, and point of tissue harvest relative to treatment administration, beyond conventional parameters normally included in such tables.

Reply 1: Thanks for your suggestion. We do agree with your opinion, and the detailed information on included patients have been supplemented namely 'Supplementary Table 1'.

Changes in the text: Relevant changes can be seen in file 'Supplementary Table 1'.

Comment 2: Kindly be more transparent with how this cohort without PFS data were treated and the basis by which "possibly longer PFS" was assigned.

Reply 2: Thanks for your suggestion. We agree with your opinion and have explained it in our text that the DCB and NDB reported by GSE13522 cohort were used as surrogate endpoints for PFS due to the lack of PFS data. Patients with DCB in our analytic process were considered with possibly longer PFS, while those with NDB were regarded worse PFS. Thus, the corresponding PFS of the DCB and NDB groups were set to 1 and 0, respectively.

Changes in the text: We added more explanations on the 'Methods' and 'Results' sections to avoid possible misunderstanding. (see Page 5, line 127-128; Page 7, 188-189)

Comment 3: All abbreviations need to be defined and appropriate references provided so the audience can better understand the experimental flow and key findings. (e.g., GSVA, PPI, etc.)

Reply 3: Thanks for your suggestion. We've corrected all the contents related to it.

Changes in the text: We've made relevant modifications as advised. (see Page 6, line 138; Page

5, line 86)

Reviewer C

Comment 1: The last paragraph of the introduction should be in the discussion.
Reply 1: Thanks for your suggestion. We agree with your opinion and we've added more content regarding the last paragraph of the introduction in the first paragraph of the 'Discussion' section.
Changes in the text: Relevant modification can be seen in Page 9, line 262-269.

Comment 2: The authors had to specify when they collected the samples from NSCLC patients. In addition, there are no detail information about the NSCLC patients.
Reply 2: Thanks for your suggestion. We have clarified the information of patients that we included in our analysis in the 'Methods' section: (1) twenty-three patients included in this study were diagnosed as NSCLC without targetable driver gene mutations, and received anti-PD-1 or anti-PD-L1 monotherapy as second-line or above therapy. (2) samples were collected before immunotherapy. In addition, the detailed information for these NSCLC samples have been attached as Supplementary Table 1.
Changes in the text: Relevant modifications can be seen in Supplementary 1.

Comment 3: They also said PD-1 monotherapy (actually, anti-PD-1 monotherapy), but the authors did not consider the differences between anti-PD-1 antibodies. I think they should consider differences between nivolumab and pembrolizumab, for example.
Reply 3: Thanks for your suggestion. We do agree with your opinion that several difference can exist among these anti-PD-1/PD-L1 inhibitors. Nevertheless, due to the limitation of the sample size, statistical power cannot be reached in subgroup analysis. Thus, we consider it as a limitation for our study and add it into our 'Discussion' section.
Changes in the text: Relevant modifications have been made in Page 10, line 313-314.

Comment 4: To me, from Figure 1B and Figure 1C and Figure 2A and Figure 2B, there are big differences between their NSCLC cohort and the GSE cohort. Please explain why the authors used two different cohorts for this study.
Reply 4: Thanks for your suggestion. We do acknowledge the heterogeneity between the two cohorts. Nevertheless, we also think it is still necessary to conduct the research following this way, and the reasons are listed as follows. (1) we aimed to screen the predictive markers on efficacy of ICI monotherapy through multi-omics data, including the transcriptomic data of the GSE135222 cohort and the proteomic data of the SHFK cohort. Thus, the difference regarding the gene modules between the two cohorts in Figure 1 and Figure 2 could be largely attributed to the discrepancy of two omics, rather than the population difference. (2) By comparing and overlapping the gene modules from both cohorts with multi-omics data, we could further screen the most representative genes that were closely to the treatment efficacy to avoid the potential bias from one cohort.
Changes in the text: We've explained the reasons to the reviewer in the 'Reply' section. No additional modification in the original text is necessary.

Comment 5: Please explain more in detail the below the sentence. I don't understand.
"Since the PFS of patients in the GSE135222 120 cohort was not available, the number 1 was used to represent patients with DCB, 121 indicating a possibly longer PFS." GSE cohorts include the efficacy of PD-L1?
Reply 5: Thanks for your suggestion. We agree with your opinion and have explained it in our text that the DCB and NDB reported by GSE13522 cohort were used as surrogate endpoints for PFS due to the lack of PFS data. Patients with DCB in our analytic process were considered with possibly longer PFS, while those with NDB were regarded worse PFS. Thus, the corresponding PFS of the DCB and NDB groups were set to 1 and 0, respectively.
Changes in the text: We added more explanations on the 'Methods' and 'Results' sections to avoid possible misunderstanding. (see Page 5, line 127-128; Page 7, 188-189)

Comment 6: Why did the authors choose only "43 genes? Please explain how the authors defined "immune-related genes from the literature".
Reply 6: Thanks for your suggestion. We've explained the screening process in the 'Methods' section. The WGCNA network was constructed and the differentially expressed genes were analyzed in the SHFK and GSE135222 cohorts, respectively. And then these 43 gene modules were obtained from the WCGNA network, indicting a close relationship with PFS. In addition, the reference regarding the seven immune-related genes has been attached.
Changes in the text: Relevant modifications have been made in Page 6, line 137.

Comment 7: "Previous studies reported that high expression 248 levels of PD-L1 and TMB were related to better response to ICI for NSCLC populations." – need references
Reply 7: Thanks for your suggestion. Relevant citations have been attached.
Changes in the text: Relevant references have been added in Page 8, line 259.

Comment 8: Figure 3
The authors showed the differences between LUAD and LUSQ. But the authors did not explain the original cohorts they used which kind of NSCLC patients. Does this analysis have any meaning?
Reply 8: Thanks for your suggestion. We do agree with your opinion, and detailed information on included patients have been supplemented as Supplementary Table 3, which included patients' pathological types and makes the comparison between LUAD and LUSQ meaningful.
Changes in the text: Relevant modifications can be seen in Supplementary 3.

Comment 9: What kind of gene mutations/alterations are included? Gene nutation is only one mutation?
Reply 9: Thanks for your suggestion. Please kindly refer to the reference from 'Methods' section about muTarget method (doi: 10.1002/ijc.33283). Here is the explanation of the original article: each mutation was classified into three functional groups, including "all mutations," "protein coding mutations" and "disruptive mutations," based on the sequence ontology generated by snpEff for the canonical transcripts. The all mutations group included any alteration in the gene; protein coding mutations group included those that hit a protein coding

sequence, including missense, synonymous, frameshift, translational start and stop codon-associated mutations; and the disruptive mutations group included only those actually disrupting the protein structure, namely, translational start and stop codon-associated mutations and frameshift mutations.

Changes in the text: Relevant explanations have been made to the reviewer, and no additional information needs to be attached to the original text.