TCR TRANSLATIONAL CANCER RESEARCH
ADVANCES CLINICAL MEDICINE TOWARD THE GOAL OF IMPROVING PATIENTS' QUALITY OF LIFE
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Peer Review File

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Reviewer A

Comment 1: lane 146 space is missing between " beta " and " represents "

Reply 1: We sincerely appreciate your significant comments and apologize for our carelessness. According to your revision suggestions, we have added a space between "β" and "representative"

Changes in the text: Methods, Page 8, line 146

The coefficients of hub genes in the multivariate Cox regression were used to calculate the risk score for each sample. The risk score = β1*X1+β2*X2+…+βn*Xn, where X represents the gene expression values and β represents the regression coefficient of each hub gene included in the ICGs-signature model.

Comment 2: Figure 2 description: not "green" but "blue" dot

Reply 2: In the description of Figure 2, we corrected the green dots to blue.

Changes in the text: Figure Legends, Page 22, line 437

Figure 2 Identification and enrichment analysis of DE-ICGs between TNBC and normal breast tissues in TCGA database. A. Heatmap of DE-ICGs. B. Volcano plot of DE-ICGs; red dot represent up-regulated genes, and the blue dot represent down-regulated genes. C. GO enrichment analysis of DE-ICGs; BP biology process; CC cellular component; MF molecular function. D. KEGG pathway enrichment analysis of DE-ICGs.

Comment 3: lanes 215-16, one sentence?

Reply 3: We made a grammatical correction to "The results showed that in comparison with the other clinical characteristics, the 3-ICGs risk score was a significant indicator for predicting the OS of TNBC patients" to "The results showed that the 3-ICGs risk score was a significant indicator for predicting the OS of TNBC patients compared with other clinical characteristics".

Changes in the text: Results, Page 11, line 216-218

In order to further confirm the weight of 3-ICGs risk score in prognostic prediction. The univariant and multivariant Cox regression analyses were conducted. The results showed that the 3-ICGs risk score was a significant indicator for predicting the OS of TNBC patients compared with other clinical characteristics (Table 3).

Reviewer B

Comment 1: Line 26: investigated -> investigate.

Reply 1: We apologized for our carelessness. We have corrected "investigated" to "investigate".

Changes in the text: Abstract, Page 2, line 26

Triple negative breast cancer (TNBC) is a highly aggressive subtype and only some of patients could benefit from the immunotherapy. The present study aims to investigate the expression pattern and prognostic value of immune checkpoint genes (ICGs) in TNBC and develop a novel ICGs-signature to predict the prognosis and immune status in TNBC.

Comment 2: Line 50: an indicator reflecting immunotherapy response. The subjected included in the study never received ICB. Thus, this statement is too strong. “a potential indicator” would be better.

Reply 2: Thank you for your correction and pointing out the problem. As the reviewer mentioned, we did not have real data on the efficacy of clinical treatment with immune checkpoint inhibitors, so the description of "an indicator reflecting immunotherapy response" is inappropriate. We have revised it to "a potential indicator reflecting immunotherapy response" based on your comments.

Changes in the text: Abstract, Page 3, line 50

A novel ICGs-signature was developed and validated, which may be not only served as a robust prognostic marker, but also a potential indicator reflecting immunotherapy response.

Comment 3: Figure 1 was cited inappropriately. Figure 1 is very important to understand a little complex research story. It should be cited in the Method. As like, Line 95-96 should be moved into the Method.

Reply 3: Thank you very much for your constructive comments. We have moved Figure 1 to Methods section.

Changes in the text: Methods, Page 5, line 99-100

We present the following article in accordance with the TRIPOD reporting checklist (Figure 1). The transcriptome data and clinical characteristics of TNBC samples were obtained from TCGA (https://portal.gdc.cancer.gov/) and METABRIC database (http://molonc.bccrc.ca/aparicio-lab/research/metabric/). The inclusion criteria were: ①Triple-negative breast cancer samples determined by the immunochemistry results of ER, PR and HER2 status; ②Transcriptome data and clinical data were comprehensive and available; ③The overall survival time was longer than 30 days.

After screening, 113 TNBC patients in TCGA database and 286 TNBC patients in METABRIC database were included in our present study. The clinical information of two cohorts was summarized in Table 1. R software (version 4.0.3) was utilized for data collection and processing.

Comment 4: Same in #2, as nobody received ICB, they should not state that "A higher score indicates an increased immunogenicity and a better immunotherapy response." This statement should be removed.

Reply 4: We thank the reviewer for the suggestion. According to your suggestion, we removed the statement "A higher score indicates an increased immunogenicity and a better immunotherapy response" from the article.

Changes in the text: Methods, Page 8, line 162-163

To further explore the correlation between the risk score and immune status of TNBC patients, a single sample gene set enrichment analysis (ssGSEA) was conducted by R software. Immunophenoscore (IPS) IPS, which is a machine learning-based algorithm, was used for the quantitative evaluation of tumor immunogenicity. It was calculated based on the Z-score of representative cell type gene expression including: immunomodulators, effector cells, immunosuppressive cells and MHC molecules. The IPS (ranges 0–10) is calculated based on the gene expression in the above representative cell types. The IPS of patients were downloaded from The Cancer Immunome Atlas (TCIA). The IPS in the high-risk group and low-risk group was

analyzed. The expression level of four immune checkpoint genes (PD1, CTLA-4, PD-L1, PD-L2) was also compared between two groups.

Comment 5: Line 163-164: It seems weird. They calculated IPS scores using 3 core genes with ecoefficiency values for OS. Are there any results with TCIA database? This statement should be removed.

Reply 5: We appreciate your comments and apologize that we were not able to explain Immunophenoscore (IPS) clearly. IPS refers to four main parts (effector cells, immunosuppressive cells, MHC molecules, and immunomodulators) determining the immunogenicity, and is calculated without bias using machine learning methods. The IPS (ranges 0–10) is calculated based on the gene expression in representative cell types. We downloaded the IPS of patients from The Cancer Immunome Atlas (PMID: 28052254). Other authors have also employed IPS in their researches (PMID: 34368124, PMID: 33834038). According to your comments, we have made specific explanations for the parts that were not clearly stated.

Changes in the text: Methods, Page 8, line 162-164

To further explore the correlation between the risk score and immune status of TNBC patients, a single sample gene set enrichment analysis (ssGSEA) was conducted by R software. Immunophenoscore (IPS) IPS, which is a machine learning-based algorithm, was used for the quantitative evaluation of tumor immunogenicity. It was calculated based on the Z-score of representative cell type gene expression including: immunomodulators, effector cells, immunosuppressive cells and MHC molecules. The IPS (ranges 0–10) is calculated based on the gene expression in representative cell types. The IPS of patients were downloaded from The Cancer Immunome Atlas (TCIA). The IPS in the high-risk group and low-risk group was analyzed. The expression level of four immune checkpoint genes (PD1, CTLA-4, PD-L1, PD-L2) was also compared between two groups.

Comment 6: Figure 5A-B were cited prior to Figure 4 in the Results. It should be corrected and numbered accosting the appearance sequence.

Reply 6: Thank you so much for your valuable suggestions. We have corrected the order of the image citations and numbered them in order of appearance sequence.

Changes in the text: Results, Page 10, line 202-213; Page 22, line 449-456

After risk scores of each sample in training set were calculated, 113 patients in TCGA database were divided into low-risk group (n=56) and high-risk group (n=57) by the median score (median score = -0.91, Figure 4A-B). Kaplan-Meier survival curve showed that patients in the high-risk group had worse OS than those in the low-risk group ($p < 0.001$, Figure 4C). The predictive value of the 3-ICGs signature was assessed by ROC curve. The AUC of the ROC curve for predicting 1-, 2- and 3-year OS were 0.925, 0.822 and 0.835, respectively (Figure 4D). The risk scores of patients in METABRIC cohort were also calculated and the patients were then divided into low-risk group (n=143) and high-risk group (n=143) (Figure 4E-F). In the validation cohort, patients in the high-risk group exhibited a worse prognosis than those in the high-risk group (Figure 4G). ROC curves also indicated a fairly good predictive value of 3-ICGs signature in the validation cohort (Figure 4H). Moreover, higher pathological stages and lower expression level of these hub genes were found in the high-risk group (Figure 5).

Comment 7: Line 243-245: This study just evaluated pembrolizumab monotherapy in metastatic TNBC. Thus, pCR cannot be assessed.

Reply 7: Thank you for your kind reminder. Indeed, this study only evaluated the effectiveness of pembrolizumab monotherapy in metastatic TNBC and does not fully prove our point. Therefore, we have revised the cited references (PMID: 31095287, PMID: 32053137.).

Changes in the text: Discussion, Page 12, line 245

In recent years, immunotherapy for malignancies developed rapidly. Immunotherapy is expected to improve the outcomes of TNBC patients. However, TNBC patients could hardly achieved complete pathological remission from immune checkpoint inhibitor therapy(16,17).

Comment 8: Still, 3 gene signature should be validated in the trial and evaluated for its reproducibility in clinical setting. These statements are too early. It should be removed or modified.

Reply 8: We sincerely appreciate your significant comments. As the reviewer mentioned, all clinical and transcriptomic data collected in our study were based on publicly available datasets, and the accuracy of the model remains to be further

investigated in vitro or in vivo studies. Therefore, it is indeed too early to apply the gene signature to clinical use and we have removed that part of the statement).

Changes in the text: Results, Page 14, line 297

Since the 3-ICGs signature contains only three genes, it is cost-effective and easy-to-use in clinical practice. The score of 3-ICGs signature could guide the immunotherapy, surveillance strategy and clinical decision making. However, there were some limitations in our study. All the clinical and transcriptome data collected in our study were based on publicly available datasets, the accuracy of the model should by further verified in clinical practice. A further in vitro or in vivo experimental study is necessary to be conducted to demonstrate the result of the present study findings.