Peer Review File

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Reviewer A

Comment 1: The authors aim to determine the potential regulatory mechanism of irlncRNAs in PD-1/PD-L1 immunotherapy in TNBC. In a retrospective analysis of 146 biopsies, they identified USP30-AS1 as Inc-RNA associated with immune responses to anti PD-1 therapy. First, the background must focus on the protocol of immunotherapy in TNBC and PDL1 expression.

Reply 1:

Thanks for your kind pointing out and warm advice. To be more clearly and in accordance with your concerns, we have added a more detailed interpretation regarding the background.

Changes in the text:

TNBC is characterized by significant biological heterogeneity. Despite its advances in neoadjuvant and adjuvant therapies, overall TNBC is associated with a higher risk of relapse and disease progression and poorer outcomes [8]. ***(see Page 5, line 11-13)***

The development of immunotherapeutic drugs has revolutionized the field of clinical oncology. (see Page 5, line 16)

However, breast cancer, as a "cold" cancer, is considered poorly immunogenic and immunotherapy is not a priority option [14]. ***(see Page 5, line 23-24)***

There is growing evidence that TNBC exhibits the strongest immunogenicity in breast cancer [15]. Compared to other subtypes, TNBC has been shown to have a higher proportion of tumor infiltrating lymphocytes (TILs) [16], a relatively high tumor mutational burden (TMB) [17] and PD-L1 expression [18]. ***(see Page 5, line 27-30)***

Thus, immunotherapy has emerged as a promising option for the treatment of TNBC and has encouraged the development of additional immunologic agents for the treatment of TNBC patients. ***(see Page 6, line 34-36)***

Comment 2: Second, the methods must describe the patients treatments, if the samples were obtained before or after therapy in adjuvant or neoadjuvant settings, if all patients received the same chemotherapy, stage of TNBC, etc to understand if the analysis is valid.

Reply 2:

We are grateful for the suggestion. To be noticed, our data comes from the public database TCGA. As the largest cancer gene information database, TCGA is comprehensive not only in many cancer types (covering 33 cancer types, more than 30,000 tumor samples, more than 20,000 gene expression information), but also in multiple omics data. It includes gene expression data, miRNA expression data, copy number variation, DNA methylation, SNP, etc. Unfortunately, not all patients have a detailed adjunctive therapy program documented. We queried patient treatment information from another public database called "cBioportal".

Of the 146 TNBC patients, only 1 patient received neoadjuvant therapy and the remaining 145 did not. In addition, 24 patients received adjuvant postoperative pharmaceutical therapy, but the specific chemotherapy regimen was unknown, 1 patient was definitively not receiving

chemotherapy, while the treatment status of the remaining 121 patients was unknown.

Changes in the text:

In addition, the clinical characteristics of all TNBC patients are shown in ***Table 1****. (see Page 7, line 69-70)*

Table 1. Clinical baseline of all 146 TNBC patients from TCGA cohort.

Variables	***TCGA cohort(N=146)***	
Status		
Alive	*125*	*85.61%*
Dead	*21*	*14.38%*
Age		
≥55	*73*	*50%*
<55	*73*	*50%*
AJCC-T		
T1	*37*	*25.34%*
T2	*92*	*63.01%*
T3	*13*	*8.904%*
T4	*4*	*2.739%*
AJCC-N		
N0	*96*	*65.75%*
N1	*31*	*21.23%*
N2	*11*	*7.534%*
N3	*8*	*5.479%*
AJCC-M		
M0	*124*	*84.93%*
M1	*1*	*0.684%*
MX	*21*	*14.38%*
Stage		
Stage I	*26*	*17.80%*
Stage II	*95*	*65.06%*
Stage III	*21*	*14.38%*
Stage IV	*1*	*0.684%*
unknow	*3*	*2.054%*
Postoperative adjuvant chemotherapy		
Yes	*24*	*16.43%*
No	*1*	*0.684%*
NA	*121*	*82.87%*
Neoadjuvant therapy		
Yes	*1*	*0.684%*
No	*145*	*99.31%*

Comment 3: Define high risk and low risk patients.

Reply 3:

We highly agree with your instructive suggestion. we supplemented it and copied below:

Changes in the text:

The TNBC patients in the TCGA cohort were stratified into two categories, namely low-risk and high-risk, based on the risk score calculated using the median value. (see Page 8, line 93-95)

Comment 4: PDL1 expression was found in tumor cells or in granulocytes (infiltrated)?

Reply 4:

Many thanks for your attention. PD-L1 is encoded by the gene CD274, which is located on chromosome 9p24.1, so we determined PDL1 expression based on the level of CD274 in the TCGA-TNBC cohort. In many tumor microenvironments, PDL1 is expressed on both antigen-presenting cells and tumor cells. According to the above definition, we counted the levels of PDL1 in the tumor microenvironment.

For a long time in the past, the medical community generally believed that only PDL1 expressed on the tumor cells played a role in suppressing activated T cells. However, it has now been shown that some of the PDL1-positive patients do not respond to immune checkpoint inhibitors, whereas some of the PDL1-negative patients do respond to immune checkpoint inhibitors, which may be related to the fact that the analysis of the statistics of PDL1 expression did not include the expression of PDL1 on non-tumor cells [1, 2].

Importantly, however, specific information on this component was not detailed in the TCGA, so in subsequent studies, scientists will need to update the data so that we can make further distinctions.

1. Tang H, Liang Y, Anders RA, Taube JM, Qiu X, Mulgaonkar A, Liu X, Harrington SM, Guo J, Xin Y et al: PD-L1 on host cells is essential for PD-L1 blockade-mediated tumor regression. The Journal of clinical investigation 2018, 128(2):580-588.

2. Yarchoan M, Hopkins A, Jaffee EM: Tumor Mutational Burden and Response Rate to PD-1 Inhibition. The New England journal of medicine 2017, 377(25):2500-2501.

Comment 5: PD1 expression was obtained from which cells? My concern is the lack of information to reach a valid conclusion.

Reply 5:

Thank you for the kind remind. PD1 is expressed on infiltrating immune cells in the tumor microenvironment. As Arlene and Kristen mentioned, “Furthermore, PD1 is expressed by subsets of tolerant T cells, regulatory T(Treg) cells, T follicular helper (Tfh) cells, T follicular regulatory (Tfr) cells and memory T cells and several other cell types” [3].

3. Sharpe AH, Pauken KE: The diverse functions of the PD1 inhibitory pathway. Nature reviews Immunology 2018, 18(3):153-167.

Comment 6: Please provide the Helsinki approval by the local committee.

Reply 6:

Thanks for your kindly pointing out and warm advice. TCGA belongs to public database. The patients involved in the database have obtained ethical approval. Users can download

relevant data for free for research and publish relevant articles. Our study is based on open source data, so there are no ethical issues and other conflicts of interest.

Reviewer B

1. The citation of ***Ref 18*** is missing in the main text, please check and revise.
   Reply:
   Thanks for your kind pointing out. The citation of *Ref 18* can be found on line 63 of the manuscript.

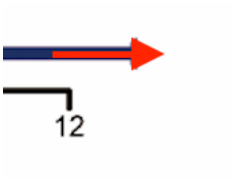
   62 programmed cell death protein 1/programmed cell death ligand 1 (PD-1/PD-L1) pathway could induce

   63 immune responses and improve clinical outcomes in TNBC patients (2, 18, 19). Thus, immunotherapy

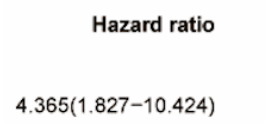
2. Please check if any references should be cited in the following sentence since you mentioned "studies":
   - Previous studies have revealed that TNBC is critically related to the expression of PD-L1 in the tumor microenvironment (TME).

   Reply:
   Thanks for your kind pointing out and warm advice. We revised it.

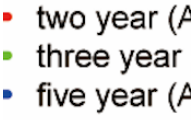
3. Figures and tables
   (1) Figure 1B: please indicate what the colorful dots stand for.
   (2) It is suggested to use arrow to those data that larger than the number in the current x-axis in Figure 1C, 2E, 3B-3C,



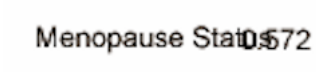
   (3) Please add "(95% CI)" after Hazard ratio in Figure 1C, 2E, 3B-3C, .



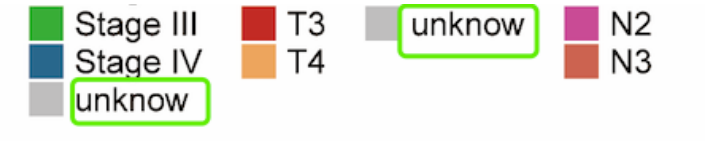
   (4) Please revise "year" to "years" in Figure 2D.



   (5) There is an overlapping in Figure 3B, please check and revise.



   (6) Figure 3D: it should be "unknown"



(7) Please indicate the meaning of "*" in Figure 3D, and please add a unit to Age in Figure 3D-3E.

(8) Please indicate the meaning of "**" "***" in Figure 4B.

(9) Please add a unit to Age in Table 1.

Reply:

Thanks for your kindly pointing out and warm advice. We revised the Figures and Tables point by point, and we will enclose the revised Figures.

In Figure 3D, the "*" has no special meaning and we have removed it.