Peer Review File

Reviewers' Comments

Comment 1: First, English language of this paper was problematic and there are many unclear sentences. Please have this paper edited by native English-speaking professionals after revisions.
Reply 1: Thanks for your advice, we have asked a naive English speaker to polish our manuscript.
Changes in the text: Whole manuscript

Comment 2: First of all, the authors needs revise their title to make it clear and accurate.
Reply 2: Thanks for your advice, we have changed our manuscript title into "A single-sample mRNA molecular classification of bladder cancer predicting prognosis and response of immunotherapy".
Changes in the text: The title section

Comment 3: Second, in the background part of abstract, the authors should have comments on the clinical significance of the current research topic and the knowledge gap to be filled in this study.
Reply 3: Thanks for your advice, we have modified our text as advised and enriched the content in the background section, also we re-wrote the introduction section to make this topic more valuable.
Changes in the text: The background section in the abstract (Page 2 Line 15-20)

Comment 4: The methods part is not informative. Please briefly indicate the research design of this study, how the samples were obtained, how the SNP was tested, and how the clinical prognosis outcomes were assessed.
Reply 4: Thanks for your advice, we have we have modified our text and added corresponding descriptions of these aspects. Research design and number of samples were added into the method part. However, single nucleotide polymorphism actually reflected changes of single base pair, leading to mutation of specific gene. Thus the analysis of SNP was also based on the RNA sequencing detection. On the other hand, the clinical outcomes recorded in the database were collected according to the protocols from NCI's Biospecimen Research Database (https://brd.nci.nih.gov/brd/). Considering the complexity these two parts, we chose added the relevant description into the main text.
Changes in the text: The methods section in the abstract (Page 2 Line 21 - Page 3 Line 7); The methods section in the main text (Page 6 Line 6-8 and Line 17-19).

Comment 5: The results part needs to use detailed figures and their corresponding P values to describe the main findings.
Reply 5: Thanks for your advice, we have modified our text as advised and added main results in this section
Changes in the text: The result section in the abstract (Page 3 Line 8 - Page 4 Line 1)

Comment 6: The conclusion should not only have comments on the clinical significance, please consider the summary of the findings and suggestions on possible clinical implications.

Reply 6: Thanks for your advice. Except for present summary, we also emphasize the importance of molecular stratification and its application prospect.

Changes in the text: The result section in the abstract (Page 3 Line 8 - Page 4 Line 1)

Comment 7: Third, in the part of introduction, the authors should have a brief overview of MIBC to indicate why the molecular stratification of MIBC deserved to be studied. It is also necessary to have a review on prognostic predictors of MIBC and have comments on knowledge gaps in molecular stratification of MIBC. The authors need to provide insights on the clinical significance of the research topic and detailed comments on the "guide the use of immunotherapy".

Reply 7: Thanks for your comprehensive suggestions. To make our topic more valuable and more intelligible, we have modified our text and enriched the content in the introduction section.

Changes in the text: The introduction section in the text (Page 4 Line 10 - Page 5 Line 19)

Comment 8: Fourth, in the methodology part, please indicate the research design of this study, such as a bioinformatic analysis? Please also consider to exclude the confounding effects of clinical factors such as TMN stage and gender.

Reply 8: Thanks for your suggestions. We have added corresponding descriptions in the method section, revealing our study design. As for the confounding effects, we attempted to create a novel molecular stratification independent of clinical characteristics. As a result, in our analysis, we first conducted the molecular stratification based on immune related genes. And then we analyzed the correlation between molecular stratification and clinical factors such as TMN stage, gender, smoking status. From this point of view, clinical factors could be separated according to the molecular stratification. Indeed, the potential effects of clinical factors should not be ignored, so we added this point in our limitation.

Changes in the text: The discussion section in the text (Page 21 Line 9-12)

Comment 9: Finally, on significant limitation should be discussed, that is no empirical validation evidence for the prognostic role of the novel clustering criterion for patient population in the real-world practice.

Reply 9: Thanks for your suggestions. We have added this point in our limitation.

Changes in the text: The discussion section in the text (Page 21 Line 14-18)