Peer Review File

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Reviewer A

This interesting article focuses on the use of immune checkpoint inhibitors in the treatment of bladder tumours, however the authors wanted to highlight not only the encouraging results they have provided in the treatment of bladder cancer, but above all how unfortunately not all patients respond well to such treatment.
Therefore sought to assess the prediction of response to treatment and how this may be strongly correlated with PD-L1 expression.
For this reason, they analysed patients with bladder cancer who were treated with atezolizumab and divided them into 2 groups, one responding to treatment and one group that did not respond to treatment.
Analysis of these data showed how the expression of 2 particular CXCL9-CXCL10 mRNAs can help select patients suitable for treatment with immune checkpoint inhibitors.
Although this article offers some very interesting insights there are some shortcomings.
For example, there is no real assessment of what an effective screening method might be to select patients who are candidates for such treatment, even more so, there is no mention of how treatment with immune checkpoint inhibitors may impact prognosis and survival in these patients.
Reply: Thanks for your suggestion. The main purpose of this study is to select molecular markers that can effectively predict the response to treatment with Atezolizumab in patients with bladder urothelial carcinoma via bioinformatics based on datasets. Unfortunately, due to clinical information limitations, we cannot provide relevant validation results at present, and we only analyzed the overall survival of patients in two datasets using CXCL9/10. We aim to address this gap in future research with our own clinical data.

1. Regarding the topics covered, it would be interesting to take a cue from this article 10.3390/ijms23031133, as it could give new insights to the article and enrich it with potential.
2. Another interesting article that could expand on the importance of this study is 10.3390/cancers14102545
Reply：Through the study of the two researches, we realized the important significance of molecular markers in the study of immune checkpoint inhibitors in bladder cancer adjuvant and neoadjuvant treatment. In our future research, we will also focus on the study of CXCL9/10 in immune checkpoint inhibitor adjuvant or neoadjuvant therapy for bladder cancer. This was mentioned at the end of the Discussion section, and we appropriately cited the two aforementioned articles. Once again, we thank the reviewer for your suggestions.

Reviewer B

This manuscript is interesting and offers some new data and novel findings in the field of er biomarkers of response to therapy with ICI (immune checkpoint inhibitors) and their possible clinical application. However, it needs a number of changes before being accepted for publication. The changes needed are detailed in the text below.

TITLE: The most important finding of this paper, which is also its main strength, is that the novel panel of miRNAs (CXCL-9/10) proposed by the Authors can be used as biomarkers to accurately predict patients responsive to Atezolizumab and could acquire prognostic significance. This is the real novel concept of this paper.
I think therefore that this fact should be clearly stated in the title of the paper, which is at the moment a bit generic and not very attention-catching. A proposed change for the title, which would make it a real hit, is:
Comment 1: A NOVEL GENES-BASED SIGNATURE WITH PROGNOSTIC VALUE AND PREDICTIVE ABILITY TO SELECT PATIENTS RESPONSIVE TO ATEZOLIZUMAB TREATMENT IN BLADDER CANCER: AN ANALYSIS ON DATA FROM REAL-WORLD STUDIES
Reply 1: We have modified our title as advised.
Changes in the text: see page 1, line 2-3.

ABSTRACT

Comment 2: Line 36: …and the prognostic impact of bladder cancer patients treated with Atezolizumab: re-phrase, unclear
Reply 2: We have modified our text as advised.
Changes in the text: see page 2, line 34-36.

Comment 3: Line 47-48: re-phrase, unclear
Reply 3: We have modified our text as advised.
Changes in the text: see page 2, line 46-48.

Comment 4: KEYWORDS: Add immune checkpoint inhibitors (ICIs) to the Keywords.
Reply 4: We have modified our text as advised.
Changes in the text: see page 2, line 50.

HIGHLIGHT BOX
Comment 5: Use the expression “patients responsive”, rather than “patients adapted”
Reply 5: We have modified our text as advised.
Changes in the text: see page 3, line 53 in the highlight box.

Comment 6: In this study, we report that CXCL9/10mRNA can be used as molecular markers to predict response to Atezolizumab treatment, with a potential prognostic significance in bladder cancer patients.
Reply 6: We have modified our text as advised.
Changes in the text: see page 3, line 53 in the highlight box.

INTRODUCTION

Comment 7: We do know that a number of patients are nonresponsive to immunotherapy. The importance of finding biomarkers of response to treatment (or of resistance to treatment) in this respect would be crucial. A recent study showing the possibility of utilizing upfront biomarkers of resistance to ICI therapy in bladder cancer should be added to the references as a relevant publication offering a practical possible solution, in the next future, to this problem (Mancini M. et al: Cancers, 2021, doi.org/10.3390/cancers13236016). This paper should be also cited in the Discussion section of the manuscript.

In Urological tumors it has been shown that miRNA can be useful biomarkers, but the vast amount of available research data is not yet usable in clinical practice. However, we clearly know at present that different cancers can have very different clinical behavior in different patients. Utilization of biomarkers can be a very promising strategy. Epigenetic-based biomarkers, such as abherrant DNA methylations, deregulated expression of chromatin structure proteins and miRNAs or nt-RNAs or IncRNAs could have a high impact on clinical practice in Oncology. Despite this, transfer from laboratory to clinic practice is still slow. This is why clinically significant translational research, like your study, is the future of oncological research.

Line 60-74: Re-phrase, being more descriptive. Is it only the high cost that make the selection of patients upfront important? No, it is only the high toxic effects of the therapy and the oncological disadvantage of selecting the wrong oncological treatment in unresponsive patients. Despite these therapies can improve the prognosis of the disease, a number of patients show a low or no response to treatments, partly because of high tumor heterogeneity. With the development of more advanced methodologies and technologies, molecular subtyping of bladder cancer cases has provided some clues for selecting upfront, before starting treatment, which patients will respond to the appropriate therapies.

Encouraging data from clinical studies indicated that immunotherapeutic strategies could become a major treatment strategy in bladder cancer. In this respect, the possibility of identifying early on, before starting treatment, which patients will respond to immunotherapy, is a major clinical need. A recent paper showed the possibility of patient-specific immune profiling of bladder cancer cases in order to select which patients will respond to immunotherapy, but no specific biomarkers are currently used yet in clinical practice

Reply 7: We have modified our text as advised.

Changes in the text: see page 3-4 and page 6, line 56-64, 70-80, and line 153-154.

DISCUSSION

Comment 8: Use “patients responsive/who will respond” rather than “adapted”

Reply 8: We have modified our text as advised.

Changes in the text: see page 6, line 150.

Comment 9: Line 150-157: re-phrase, unclear

Reply 9: We have modified our text as advised.

Changes in the text: see page 6-7, line 158-166.

Comment 10: Line 173-175: re-phrase, unclear. Needs English language revision.
Reply 10: We have modified our text as advised.
Changes in the text: see page 6-7, line 187-192.

CONCLUSION
Comment 11: We propose a novel genetic signature, which also seems to be able to reliably stratify the risk profile of bladder cancer patients, with prognostic significance. Moreover, our signature appears to be able to indicate which patients will show a better response to immunotherapy. These results could significantly improve the possibility of designing patient-oriented treatment strategies in the next future. Based on real-world data sets, CXCL9 and CXCL10 mRNA were found to be upregulated in bladder cancer patients who showed treatment response to Atezolizumab. Moreover, CXCL9 and CXCL10 mRNA expression was positively correlated with both PD-L1 and TMB. CXCL9 and CXCL10 mRNA seem to have a stronger ability than PD-L1 mRNA to predict responsiveness to Atezolizumab treatment. Patients with high CXCL9 and CXCL10 mRNA also show better overall survival in the Atezolizumab treated cohort. CXCL9 and CXCL10 mRNA can be used as molecular markers not only to predict treatment response, but also to acquire a prognostic value in bladder cancer patients treated with Atezolizumab
Reply 11: We have modified our text as advised.
Changes in the text: see page 8, line 206-216.