Peer Review File

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Reviewer A

good study
most of the genes are known to be poor prognostic for bladder cancer like TP53,
helps to advance knowledge of bladder cancer in elderly
reply: Thank you for the time and effort you put into our manuscript and are very happy that you recognized our research, thank you again! Reviewer B Authors provide valuable information regarding the prognostic value of a 5-gene signature model associated with aging in bladder cancer samples. Additionally, authors evaluate the association of this risk model with different clinical and molecular parameters related with tumour aggressiveness and drug response. Therefore, the data provided is very interesting for the field, however, some suggestions are provided to improve the manuscript:

Reviewer B

Authors provide valuable information regarding the prognostic value of a 5-gene signature model associated with aging in bladder cancer samples. Additionally, authors evaluate the association of this risk model with different clinical and molecular parameters related with tumour aggressiveness and drug response. Therefore, the data provided is very interesting for the field, however, some suggestions are provided to improve the manuscript:

- The authors' criteria for identifying differentially expressed genes (DEGs) between high- and low-risk groups is 1) an adjusted p-value < 0.05; and 2) an absolute fold-change > 1. However, this fold-change cut-off might not be sufficiently restrictive for identifying true transcriptional alterations. Following analyses should be reconsidered with a more restrictive fold-change (e.g., > 1.5).
reply: Thank you very much for your valuable comments. Since this is the first time for us to explore the difference in the identification of age-related genes between high and low risk groups of bladder cancer, we may still have insufficient experience in the selection of criteria for DEGs. According to your suggestion, we will try to use more stringent criteria in the subsequent analysis.

- Authors should depict the criteria for the selection of the 33 senescence-associated genes.
reply: Thank you very much for your valuable comments. Since we are the first to study the relationship between age-related genes and bladder cancer, our 33 age-related genes are from the previous article study, and we have added the description of 33 age-related genes selected in the "Introduction" section of Line 211-212.

- Abbreviations for bladder cancer (e.g., BC or BLCA) are indistinctly used. Authors should use one through the whole manuscript.
reply: Thank you very much for your valuable comments. According to your suggestion, we have changed all the abbreviation of bladder cancer to BC.

- English writing should be extensively revised (e.g., lines 65-66, lines 501-505). Moreover, gene names (e.g., TP53, RB1, etc.) should be written in italics as indicated by HUGO nomenclature.
reply: Thank you very much for your valuable comments. We have made the modification according to your request. Change in the text: Page 8, Line 379-380, Line 391-392 and Line 397; Page 10, Line 455-458; Page 11, Line 500; Page 12, Line 539; Page 19, Line 653-654; Page 21, Line 717-718; Page 23, Line 804-808;

- First paragraph of the “Results” section might be more suitable to be included in “Materials and methods” section.
reply: Thank you very much for your valuable comments. We have included this paragraph in the "Materials and methods" section according to your suggestion. Change in the text: Page 7, Line 364-369

- Protein-protein interaction and chromosomal location analysis might not add relevant data for this study. Figure 1C and Figure 1E might be repositioned as supplemental figures.
reply: Thank you very much for your valuable comments.Figure 1C further shows the chromosomal locations of age-related genes, aiming to better explain the correlation between age-related gene mutations and bladder cancer. Figure 1E shows that CDKN2A, CDK4, and AGO1 have more interactions with other genes, suggesting their potential significance in the development of BC. We think we can keep these two images here to better demonstrate the interplay between aging genes and bladder cancer, thank you again.

- COX analysis for aging-related genes not linked to prognosis should be included as supplemental data/figure.

reply: Thank you very much for your valuable comments.Because our study was designed to illustrate the relationship between prognostic age-related genes and patients with bladder tumors, we did not perform a COX analysis of prognostic aging-related genes. Please feel free to contact our corresponding authors for data if necessary. Thank you again.

- Graph size should be revised as some gene names and conditions are not properly shown nor easy-to-read (e.g., Figure 2L, Figure 3H, Figure 5A, Figure 5C, Figure 6H, Figure 7F, Figure 7G, Figure 7H).
reply: Thank you very much for your valuable comments.Since the images were compressed in Word and PDF documents, the display was not clear. We have uploaded TIFF vector images of all the original images to the modification system. Thank you again.

- Results from Figure 7I-L are not properly depicted on Figure legend. Furthermore, Y axis title turns out graphs to be difficult-to-follow. Y axis and figure legends should be corrected to be auto explicative. In this regard, it might be relevant to evaluate whether this risk model might predict response to immunotherapy (e.g., based on PD-1, PD-L1 or CTLA-4) in bladder cancer patients or any type of immune hot tumour (e.g., melanoma).
reply: Thank you very much for your valuable comments. We have modified the legend according to your requirements and explained in detail the content shown on the Y axis of Figure 7I-L. Thank you again. Change in the text: Page 21, Line 699-701

Reviewer C

This manuscript is interesting and offers some new data and novel findings in the field of prediction of prognosis and response to therapy with ICI (immune checkpoint inhibitors) and its possible clinical application. However, it needs a number of changes before being accepted for publication. Better conceptualisation and clarification of unclear sentences are highly necessary. 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 aging-associated genes (5-gene signature) proposed by the Authors, can be used for prognostic prediction and to accurately predict response to immunotherapy in bladder cancer. 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. A proposed change for the title, is:
RELIABLE PROGNOSTIC DEFINITION AND IMMUTHERAPY RESPONSE PREDICTION IN BLADDER CANCER, BASED ON A NOVEL AGING-ASSOCIATED 5-GENE SIGNATURE MODEL

reply: Thank you very much for your valuable comments. We have revised the title according to your suggestion, so that the novelty of the article can be expressed more clearly. Change in the text: Page 1, Line 1-2;

ABSTRACT

GENERAL PRINCIPLE: DO NOT USE ABBREVIATIONS IN THE ABSTRACT
Reply: Thank you very much for your valuable comments. According to your suggestion, we have made corrections. Change in the text: Page 2, Line 60-74

Start the Abstract with: Bladder Cancer is a urological tumor which can be associated with a poor prognosis.
Reply: Thank you very much for your valuable comments. According to your suggestion, we have added this sentence to the Abstract. Change in the text: Page 2, Line 60-61

Line 39: …in this study…
Reply: Thank you very much for your valuable comments. According to your suggestion, we have made corrections.

Line 39: BLCA? Do not use abbreviations in the Abstract. Later in the Introduction you can choose to use either BC or BLCA to mean bladder cancer, but not two different abbreviations for the same disease.
Reply: Thank you very much for your valuable comments. According to your suggestion, we have made corrections. We have made corrections and We have made corrections and unified the abbreviations into one.

Line 42: immunotherapy response
Reply: Thank you very much for your valuable comments. We have made changes.

Line 44: variations were revealed in…
Reply: Thank you very much for your valuable comments. We have made changes.

Line 47: Erase “To conclude” and start with “Our findings indicate a strong correlation between genes related to aging and the prognosis of bladder cancer. This prediction model can offer…
Reply: Thank you very much for your valuable comments. According to your suggestion, we have made corrections. Change in the text: Page 2, Line 71-74

Line 50-51: re-phrase, unclear
Reply: Thank you very much for your valuable comments. According to your suggestion, we have revised the wording of the sentence. Change in the text: Page 2, Line 71-74

KEYWORDS: Add immune checkpoint inhibitors (ICIs) to the Keywords.
Reply: Thank you very much for your valuable comments. We have added the keyword. Change in the text: Page 2, Line 76-77;

INTRODUCTION

Line 58-59: The main aetiological factor is tobacco smoking, at present.
Reply: Thank you very much for your valuable comments. We have made changes.

Line 66-67: What does it mean? Re-phrase, unclear
Reply: Thank you very much for your valuable comments. We have made changes. Change in the text: Page 3, Line 141-142;

Line 67-68: to address its high recurrence and progression rate. In the case of MIBC, the potentially metastatic spread of the tumor and the high mortality rate are a key clinical problem. Since the rise of immunotherapy, the prognosis of BC patients has improved dramatically, in selected responsive cases. But, in general, only a few patients benefit from immunotherapy, and immune resistance and lack of response to immunotherapy are relevant clinical issue. 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.
Reply: Thank you very much for your valuable comments. We have added this reference to the "Discussion " section.

Line 73-74: Erase, unnecessary

Reply: Thank you very much for your valuable comments. We have deleted the corresponding sentence.

Line 74: Cellular senescence can be induced by different stimuli, both in vivo and in vitro.

Reply: Thank you very much for your valuable comments. According to your suggestion, we have made corrections.

Line 79: Senescence is associated to the development of several degenerative pathologies…

Reply: Thank you very much for your valuable comments. According to your suggestion, we have made changes.

Line 81: Bases on the available data, it is hypothesized that a close relationship between senescence and carcinogenesis may exist.

Reply: Thank you very much for your valuable comments. According to your suggestion, we have made changes.

Line 84: At the same time, immune cells are attracted, with the purpose of killing senescent cells

Reply: Thank you very much for your valuable comments. According to your suggestion, we have made changes to the sentence.

Line 87: tumors

Reply: Thank you very much for your valuable comments. We have made corrections.

Line 89: enhancing tumor invasion and distant metastasis

Reply: Thank you very much for your valuable comments. We have made corrections.

Line 97: will help us find new therapeutic strategies and select the most effective treatments upfront.

Reply: Thank you very much for your valuable comments. According to your suggestion, we have made changes to the sentence.

Line 93: …early in life, but, over time, it may disrupt normal tissue structure and function, and drive…

Reply: According to your suggestion, we have made changes to the sentence.

Line 95-96: re-phrase, unclear
Reply: Thank you very much for your valuable comments. We have made changes to this sentence.

Line 98: Erase, pleonastic
Reply: Thank you very much for your valuable comments. We have made changes.

Line 99: In this study, we integrated 33 senescence-associated genes that has been characterized in previous studies, and developed a 5-gene model. The data derive from the TCGA database.
Reply: Thank you very much for your valuable comments. According to your suggestion, we have made changes to the sentence.

Line 104-105: Erase, pleonastic
Reply: Thank you very much for your valuable comments. According to your suggestion, we have deleted the sentence. RESULTS

RESULTS

Line 205-209: Move to Materials and Methods, these are not results.
Reply: Thank you very much for your valuable comments. We have included this paragraph in the "Materials and methods" section according to your suggestion.
Change in the text: Page 7, Line 364-369

Line 232: This implies
Reply: Thank you very much for your valuable comments. We have made corrections.

Line 238-240: Re-phrase, unclear
Reply: Thank you very much for your valuable comments. We have made changes.

Line 375: Non-negligible: what does it mean? Use a more specific adjective here (important, key, significant). This is a very important statement in your paper.
Reply: Thank you very much for your valuable comments. According to your suggestion, we have made changes to this adjective.

Line 450-451: Erase. Conclude the paragraph with: In conclusion, individuals in the high-risk group showed a better response to immunotherapy.
Reply: Thank you very much for your valuable comments. According to your suggestion, we have made changes.

Line 494-496: Re-phrase, unclear
Reply: We have made changes.

Line 556-558: erase, pleonastic
Reply: According to your advice, we have deleted the corresponding sentence.

Line 562-565: Therefore, immunotherapy (you could also use ICI treatment) provides new treatment alternatives for BC patients, but only a small proportion of patients respond. Assessment upfront of immunotherapy resistance and lack of response are currently highly relevant fields of investigational research and biomarkers identification (cit. Mancini M., et al, Cancers 2021)
Reply: Thank you very much for your valuable comments. We have read the article you suggested and quoted it accordingly. Change in the text: Page 24, Line 821

Line 573: Erase “clinic”. State the Conclusions more clearly, mentioning the real novel findings of this study, and their possible clinical application in the real world. Expand on the possible use of the novel gene-signature identified in this study, clarifying what this adds to the current clinical practice. Also, the future directions of the research started by the Authors should be stated at the end of the Conclusions.
Reply: Thank you very much for your valuable comments. According to your suggestion, we have made changes. Change in the text: Page 24, Line 827-832