March 23, 2022

Dear Prof. Lian-Sheng Ma,

World Journal of Gastrointestinal Oncology

RE: # NO: 75199, STEAP proteins serve as immunotherapeutic targets in colorectal carcinomas

Dear Prof. Lian-Sheng Ma,

We would like to thank you for providing us an opportunity to revise the manuscript with revised title "**Potential of STEAP4 as a prognostic marker for colorectal cancer**" (ID: 75199). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our research. We do also thank the reviewers for giving us constructive comments and suggestions. We have revised the manuscript according to the reviewer's suggestions. All the changes are highlighted in yellow in the revised manuscript. And the point-to-point responses to the reviewer's comments were followed in the next part of this letter.

I believe the revised manuscript has been largely improved, and will be benefit to the readers of your journal. I hope the new version would be suitable to publish in "***World Journal of Gastrointestinal Oncology***". I look forward to hearing from you.

Best regards,

Jing Liu

Guangdong Provincial Key Laboratory for Diagnosis and Treatment of Breast Cancer

Cancer Hospital of Shantou University Medical College

Shantou 515041, China

The main corrections are in the manuscript and the responds to the reviewers' comments are as follows point-to-point (the replies are marked in blue).

To Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Responses: Thank you again for your positive comments and valuable suggestions to improve the quality of our manuscript. We have revised the manuscript accordingly with tracked changes. And the manuscript has been polished by an English-native speaker with biological background.

Specific Comments to Authors:

(1). The title of research could be more focused on STEAP4, although this is not obligatory. I see that you performed some preliminary research on all four family members but STEAP4 was then analyzed in detail.

Responses: Thanks for your valuable advice. According to the reviewer's suggestion and our findings in this study, the title was changed to "Potential of STEAP4 as a prognostic marker for colorectal cancer" in Page 1, line 1-2. The abstract was also modified accordingly in Page 2, line 7-10.

(2). In the "Core tip" you wrote that "STEAP4 is expected to be a novel therapeutic target for colorectal cancer" while the closing remarks indicate that it is rather a prognostic indicator/biomarker and moreover it was found downregulated in CRC and is presumably tumor suppressor, then how/why to subject it to targeted therapy? This contradicts the sentences like "Immunotherapy serves as an alternative treatment for cancer patients, especially for those whose tumors overexpress antigens recognized by immune cells" (if you would like to subject STEAP4 to this type of therapy then it is not

overexpressed) as well as "STEAPs are present at the intercellular junctions of the prostate secretory epithelium, and are overexpressed in prostate cancer, serving as attractive targets for prostate cancer immunotherapy" (if STEAPs are overexpressed in PCa and serve as attractive targets then STEAP4 cannot be target in CRC since it is downregulated). To sum up, I would remove all sentences where STEAP4 was suggested as therapeutic target in CRC based on data from current study.

Response: Thanks for the reviewer's critical comments and valuable advices. We revised the mentioned ones and changed the demonstration into "STEAP4 being suggested as a therapeutic target for colorectal cancer". Besides, we checked the whole manuscript and revised such demonstration accordingly, in Page 2, line15, Page 3, line 25 and Page 5, line 2.

(3). Could you please provide more details for methodology behind the step of visualizing MSI/MSS subtypes via GENT2? Moreover, I would add link to GENT2 in this location, instead of the section later (alternatively, you can provide link in both sections where GENT2 is mentioned in methodology).

Response: Thanks for your critical comments and professional suggestions. Since the subtypes of colorectal cancer were not differentiated in detail in the previous work, the GEPIA2 database was used to analyze again. As for MSI/MSS, they are different subtypes of colorectal cancer, which can be subdivided into MSI-high, MSI-low and MSS. However, as the biological phenotype of MSI-low is not clear, only MSI-H and MSS are discussed, we modified the manuscript in the following parts. ① In the abstract part, the description of subtypes of CRC was added in Page 3, line 2-4. ② In the Materials and Methods part, we also demonstrated the divided subtypes of CRC based on microsatellite stability and cited related

references accordingly, in Page 5, line 23-29. ③ In the Materials and Methods part, we specifically list the online dataset for MSS/MSI analysis in CRC, in Page 6, line 2. ④ In Results part, we described the subtypes and their relationship with STEAP4, in Page 9, line 8 and line 12-21.

(4). Please add explanation of subfigures in the legends of figures 3 and 5.

Response: Thanks for your valuable advice. The lack of explanation of subfigures does lead to confusion in reading. According to the reviewer's professional suggestions, the explanation of subfigures in the figures 3 and 5 were added respectively, in Page 10 and Page 14.

(5). Put the tables in tabular form, not as pictures.

Response: Thanks for your valuable advice. The image format of the tables does affect reading, the professional proposal of the reviewer on this is very useful. Table 1, 2 and 3 have already been changed in tabular form, in Page 10, 12, and 13 respectively.

(6). Please provide stain/dye type and scale in figure 4.

Response: Thanks for your professional suggestions. Based on the valuable and professional opinion of the reviewer. We have revised the Materials and Methods part to specify the stain/dye type in Page 6, line 24-28. In Figure 4, we also added the scale bar in figure and related figure legend, in Page 11.

(7). Above table 2 I would not write that "STEAP4 expression tended to be lower in CRC" because in the sections you only investigated clinicopathological parameters of CRC patients, not compared to normal tissues. Moreover, "tended to be lower" will not be in line with findings of the previous section

where STEAP4 is clearly decreased in cancerous tissue which was statistically significant.

Response: Thanks for your valuable advice. According to the critical suggestion from the reviewer, we removed such sentence in Page 11, line 12-13.

(8). In table 3, if you put $p<0.05$ below table as a legend, then the p-values below $p<0.01$ should also be denoted as e. g. ** while in the table itself the precise values should be provided. Moreover, please add space between "table" word and the number, this refers to all tables.

Response: Thanks for your valuable advice. According to the reviewer's professional suggestions, we changed the p value as the precise ones and denoted their statistic significance accordingly in Table 3, in Page 12. We also added space between "table" and related number in Page 10, 12 and 13 respecitvely.

(9). What software was used to present Kaplan-Meier curves from Figure 7? I am also wondering whether the re-running the analysis but with DFS instead of OS would reveal some significant observations in terms of survival. Is this possible in your case and your data? Initially, please clarify why OS was used in methodology instead of DFS outcome? Events caused by disease recurrence occur earlier than death from the disease and moreover DFS also include tumors that do not necessarily lead to death, which are not included in OS.

Response: Thanks for your critical comments. We conducted the survival analysis through SPSS 25.0 software, and added this information in Material and Methods part in Page 7, line 22. In addition, the reviewer has provided great and professional suggestions to us. DFS is indeed a significant factor affecting the prognosis of patient. However, the microarray tissue we used did not include the information of distant metastasis of the patients. So we can not

calculate the DFS analysis in this study. According to the suggestions of reviewers, DFS will be a priority research index in our further studies.

(10). Change "[...] assuming a potential tumor suppressor role of STEAP in CRC patients" to "[...] assuming a potential tumor suppressor role of this STEAP member in CRC patients" (it will sound better in my opinion, alternatively write "STEAP4" instead of just "STEAP" in this sentence).

Response: Thanks for your valuable advice. We have changed the demonstration accordingly in Page 17, line 13.

(11). Now, a slightly more complicated suggestion - I think that steps where all STEAPs are considered/visualized should be prior to focusing on STEAP4 only. This would give the deductive reasoning narration.

Response: Thanks for your critical comments. Although its molecular skeleton is similar to other ones, their expression in colorectal cancer is inconsistent. So, based on our preliminary results, we focused on STEAP4 in this study. We also modified the whole manuscript to give solid reasons to investigate STEAP4 prior to others, in Page 2, line 7-10, and Page 17, line 1-8.

(12). Last but not least, the usage of TIMER2.0 and then GEPIA2 for validation (figures 1-2) will most probably lead to the same or similar results as these databases use the same RNA-seq TCGA data, similar to step summarized in figure 6 (TISIDB and TIMER2.0 were compared). I think the slight differences might be due to algorithm that is embedded in these databases to perform analysis. It would be much more preferred to validate the findings from RNA-seq TCGA using e. g. microarrays (see Xena database).

Response: Thanks for your valuable advice and professional suggestion. According to the valuable suggestion, we applied USCS Xena

database to analyze the STEAPs expression differences in CRC and related normal tissues. After the analysis, we found that the results of the expression of STEAPs in CRC and adjacent tissues are consistent with the original Figure 1, supporting our hypothesis. The corresponding UCSC Xena diagram has been rendered in new Figure 2, in Page 9. We also revised the demonstration of Material and Methods in corresponding section, in Page 5, line 20-21, Page 8, line 20 and Page 9, line 5-8.

To Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Responses: Thank you again for your positive comments and valuable suggestions to improve the quality of our manuscript. We have revised the manuscript accordingly with tracked changes. And the manuscript has been polished by an English-native speaker with biological background.

Specific Comments to Authors:

1- There are so many tumor markers used in the diagnosis, prognosis, and predictive factors in colorectal cancer, so what is the novelty of your work?

Response: Thanks for your critical comments. With the increasing incidence and mortality of colorectal cancer patients, immunotherapy can serve as an effective alternative treatment for cancer patients (PMID: 34952144). Although existing immune biomarkers play a role in treatment, further research and exploration are needed for clinical guidance. The STEAP family is closely related to oxidative stress and metal ion accumulation, affecting the occurrence and

development of tumors, especially STEAP1 (PMID: 34778263). However, during the study, it was found that STEAP4, a new member of the STEAP family, was very different from STEAP1-3. STEAP4 promotes androgen-positive prostate cancer and inhibits AR- in prostate cancer. However, the expression of STEAP4 in colorectal cancer differs from that of STEAP1-3, suggesting a different mechanism. Therefore, this study will explore the role of STEAP4 at mRNA and clinical levels. Interestingly, we found dual anti-STEAP1 antibody targeting T cells for cancer immunotherapy (PMID: 34497115). Combined with our study, it is suggested that STEAP4 can be developed as a new therapeutic strategy. According to the reviewer's critical comments, we revised the Discussion part accordingly, in Page 17, line 1-8.

2-As regards images, please add scale bar, annotations, type of stain or dye, type of software program that generated these figures.

Response: Thanks for your professional comments. We added scale bar in Figure 4 and revised the figure legend accordingly in Page 11. To specify the information for IHC experiment, we also revised the Materials and Methods part in Page 6, line line 24-28. For Figure 7, the software used was demonstrated in Page 7, line 22.

To Re-reviewer:

Scientific Quality: Grade A (Good)

Language Quality: Grade A (Minor language polishing)

Conclusion: Accept (High priority)

Responses: Thank you again for your positive comments and valuable suggestions to improve the quality of our manuscript. We have revised the manuscript accordingly with tracked changes. And the

manuscript has been polished by an English-native speaker with biological background.

Specific Comments to Authors:

Dear Authors, thank you for your revisions. Good work! I recommend the manuscript to be published in the World Journal of Gastrointestinal Oncology.

Response: Thanks for your professional comments.

To Editorial Office's Comments:

(1) To Science editor:

1) Some figures have too low resolution, it should be edited;

Response: Thanks for your critical comments. According to the editor's suggestion, we changed the figures with low resolution and provided Image file in editable version.

2) all abbreviations in figure captions should be spelled out;

Response: Thanks for your suggestion. We modified all the abbreviations in the figures.

3) tables should not be figures.

Response: Thanks for your critical comments. We changed all the tables and provided them in table version.

I think that after correcting the manuscript in accordance with these recommendations and the recommendations of the reviewers, this manuscript may be accepted for publication in the World Journal of Gastroenterology or in the ***World Journal of Gastrointestinal Oncology***.

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade C (Good)

Responses: Thank you again for your positive comments and valuable suggestions to improve the quality of our manuscript. We have revised the manuscript accordingly with tracked changes. And the

manuscript has been polished by an English-native speaker with biological background.

(2) To Company editor-in-chief:

I recommend the manuscript to be published in the World Journal of Gastrointestinal Oncology.

Responses: Thank you again for your positive comments and valuable suggestions to improve the quality of our manuscript. We have revised the manuscript accordingly with tracked changes. And the manuscript has been polished by an English-native speaker with biological background.