Peer Review File
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Reviewer A

The paper titled “Efficacy and regulatory strategies of gut microbiota in immunotherapy: a narrative review” is interesting. This article reviewed the effects and mechanisms of gut microbes on tumor immunotherapy to further explore the medical value of gut microbes in tumor immunotherapy. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What is the main pattern of gut microbiota regulating anti-tumor immunity? How do metabolites affect local and systemic anti-tumor immune responses? Suggest adding relevant content.

Response: Thank you for this very pertinent comment, in that our review really does not summarize and generalize about gut microbiota regulating anti-tumor immunity and does not provide an exhaustive description of how do metabolites affect local and systemic anti-tumor immune responses. A concise summary of the aspects of gut microbiota regulating anti-tumor immunity and an exhaustive description of how do metabolites affect local and systemic anti-tumor immune responses would greatly enhance the manuscript's readability of the manuscript. Hence the addition on pages 7-8, lines 208-263. Again, thank you for this extremely helpful comment on our manuscript.

2) How to improve the age utilization rate of precision medicine in microbial communities? Suggest adding relevant content.

Response: After careful discussion among our team, we agree that this comment of yours has greatly enhanced the integrity of our manuscript, and we thank you sincerely for it. We have made content additions on pages 19-20, lines 674-698.

3) What is the intricate crosstalk among the gut microbiome, cancer immune response, and immunotherapy? Suggest adding relevant content.

Response: Thank you for your tangential when suggestion, in our manuscript we really did not have a description of the intricate crosstalk between the gut microbiome, cancer immune response, and immunotherapy, and we think that this section is well worth adding.
Hence the addition on pages 10-11, lines 336-358 of the article. Once again, we are grateful to you for this comment that helped us greatly with the manuscript.

4) This study is based on the analysis and summary of the literatures. It is suggested to add clinical experimental research, which may be more meaningful.

Response: Our team believes that it is necessary and urgent to conduct prospective clinical trial

studies on gut flora and cancer immunotherapy. Due to the relatively long clinical trial period, it is more difficult to supplement prospective clinical trial studies at this time, but we have already passed the relevant ethical review. This comment will largely improve our manuscript, and we thank you again for your suggestion.

5) What is the function and mechanism of gut microbiota in other directions? It is recommended to add analysis and comparison.

Response: Thanks to your suggestion, we have added lines 468-493 on page 14 of the article and discussed and compared the function and mechanism of gut microbiota in other ways.

6) There are many databases. Why did the author only select PubMed databases in this study for searching? Please explain the reason.

Response: Thank you for your meticulous review, we made a mistake in describing this detail when writing the manuscript and did not just use the PubMed database. When searching for published studies, PubMed was used as the primary database for primary searches, and databases such as American Medical Association, Elsevier ScienceDirect, and others were used for secondary reference searches. After all, many studies are not searchable in the PubMed database, which is a biomedical abstract-type database. We apologize for this error, which caused a misunderstanding of your review, and we have corrected it in Table 1.

7) How does the gut microbiota affect the efficacy of cancer immunotherapy? What are the main manifestations? Suggest adding relevant content.

Response: Thank you for this comment, the generalization that the gut microbiota affects the efficacy of cancer immunotherapy was indeed missing in our review. The addition of lines 402-465 on pages 12-14 makes our manuscript more logically coherent. Your review comments are essential to improve the academic quality of our article.

Reviewer B

1. The author's name does not match the references. Please revise.

1)

Mager et al. studied the efficacy of ICIs in the treatment of MC38 tumor models and found that Bifidobacterium pseudocolonica and Lactobacillus johnsonii significantly improved the ICI efficacy against PD-L1 and anti-CTLA-4 (46).

46. Sivan A, Corrales L, Hubert N, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science (New York, NY) 2015;350:1084-9.

Response: We apologize for the errors in our article that have caused you review your manuscript, but we have changed the corresponding errors on Page 9, Line 305.

2)

A meta-analysis by Lise et al. found that the use of antibiotics before or during ICI treatment reduced the OS of NSCLC patients by more than six months (89).

89. Lurienne L, Cervesi J, Duhalde L, et al. NSCLC Immunotherapy Efficacy and Antibiotic Use: A Systematic Review and Meta-Analysis. Journal of Thoracic Oncology : Official Publication of the International Association For the Study of Lung Cancer 2020;15:1147-59.

Response: We apologize for the errors in our article that have caused you review your manuscript, but we have changed the corresponding errors on Page 17, Line 593.

3)

Additionally, in a later study, Vétizou, who was aware that tumors in antibiotic-treated or sterile (GF) mice do not respond significantly to the CTLA-4 blockade, showed that when colonized by two Bacteroidesspecies and one Burkholderia (Proteus) species, the anti-cancer response of CTLA-4Ab was restored in mice transplanted with colon and melanoma tumors (43).

43. Chen F, Zang Z, Chen Z, et al. Nanophotosensitizer-engineered Salmonella bacteria with hypoxia targeting and photothermal-assisted mutual bioaccumulation for solid tumor therapy. Biomaterials 2019;214:119226.

Response: We apologize for the errors in our article that have caused you review your manuscript, but we have changed the corresponding errors on Page 10, Line 312.

2. The authors mentioned "studies...", while only one reference was cited. Change "Studies" to "A study" or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

However, studies have shown that the overall response rates are less than 30% for most tumor types (45).

Response: Thank you for your valuable suggestion, we have re-examined the corresponding section and made changes accordingly in accordance with your suggestion. See Page 6, Lines 202-203 for details.

Studies have found that the combination of specific bacterial strains in the gut microbiome is significantly associated with a patient's response to treatment(64).

Response: Thank you for your valuable suggestion, we have re-examined the corresponding section and made changes accordingly in accordance with your suggestion. See Page 11, Line 538 for details.

Studies have shown that the incidence of tumors in mouse models after treatment with Clostridium butyricum and 1,2-dihydrohydrochloric acid was reduced due to a decrease in the number of Th2 and Th17 cells, which in turn inhibited CD4+ and CD8+ T lymphocytes, blocking the cell cycle, reducing the secretion of inflammatory factors, and promoting the apoptosis of tumor cells (93).

Response: Thank you for your valuable suggestion, we have re-examined the corresponding section and made changes accordingly in accordance with your suggestion. See Page 18, Lines 617-618 for details.

Studies have shown that cyclooxygenase-2 promotes tumor angiogenesis, while probiotics inhibit carcinogenesis by reducing the expression of cyclooxygenase-2 (94).

Response: Thank you for your valuable suggestion, we have re-examined the corresponding section and made changes accordingly in accordance with your suggestion. See Page 18, Lines 622-623 for details.

3. Please check if citations are needed in this sentence, as you mentioned "studies".

Fortunately, some studies have shown that differences in gut microbes in cancer patients are related to the effectiveness of immunotherapy, which suggests a direction for further research.

Response: Due to the negligence of our review, we have added the citation to the appropriate place in a timely manner, and we have made additional corrections on Line 204 on Page 6 in response to this error. Give you detailed review comments.

Fortunately the studies using gut flora in improving gastrointestinal disorders and modulating immune function are relatively well defined, so it is feasible to draw on them to extrapolate the role of gut flora for tumor immunotherapy.

Response: Due to the negligence of our review, we have added the citation to the appropriate place in a timely manner, and we have made additional corrections on Line 479 on Page 14 in response to this error. Give you detailed review comments.

4. Ref. (43) was not cited properly. Table 3 includes references (44-51). Please revise.

199 direction for further research. **Table 3** provides list of microorganisms that have been
200 (43)shown to play a role in immunotherapy.Gut microbes influence the sensitivity of
201 tumors to various therapies, especially immunotherapy. Records of immunotherapy

Response: In accordance with your suggestion, we have rechecked the section and found that it had been misimported, and we have made changes to it. Line207.

5. We cannot open 'figdraw.com'. Please revise.

maturity and number of IL-1β, thereby promoting neutrophil recruitment. Figure 1 created by figdraw.com.

risk of creating drug-resistant superbugs. Figure 2 created by figdraw.com

Response: Thanks for the reminder, we've added links to our websites in the appropriate places to make it easier for readers to click and use. Specifically, see Figure 1 Legend and Figure 2 Legend.

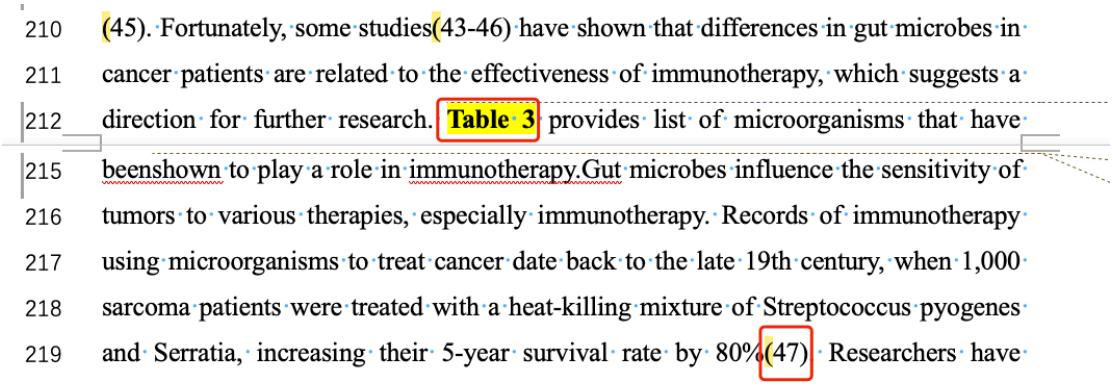
6. Please check the coherence (or grammar errors) of this sentence.

In a study(73)exploring the relationship between gut microbes and gastric cancer treatment, researchers patients with higher relative abundance of lactobacilli had higher microbiome diversity and significantly better responses to anti-PD-1/PD-L1 immunotherapy.

Response: Thank you very much for finding errors in our manuscripts. We re-read the paragraph you mentioned and found that the paragraph was indeed ambiguous. To this end, we have made modifications. The specific changes are made on Page 13, Lines 451-454

7. Some references in the text are out of order. The references should be cited in order of their appearance in the text. Table 3 includes References 48-55. Ref. 47

was not cited properly. Please revise.



Response: Thank you for your careful review, the citation errors in the paper are due to our failure to update the citation order. We should complete re-updating the citation order of the paper according to your suggestion. We apologize for any inconvenience caused to your review. See lines 216 on page 7 for details.

8. The citation of Ref. 55 in the main text was missing. Please indicate where you would like to cite Ref. 55 in the main text, which should be cited between Ref. 54 and Ref. 56.

Response: Thank you for reviewing the manuscript in the midst of your hundred names, and we apologize for the inconvenience caused to your review process by the lack of obvious notation of the location of reference 55 in the text. We have changed the position of reference 55 in the text, which is on Line 216 on Page 7 of the manuscript.