Peer Review File

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Reviewer A

This is an interesting review on an important area. However, the style in which the review is written, with long convoluted sentence structures makes it difficult to follow. Further, the figure is overly complicated and doesn't contribute to the readers understanding of the review. The paragraphs addressing current trials investigating the therapeutic potential of microbiome modulation are better written and more interesting.

If the structure of the writing could be addressed this could be an interesting review. While an important area to be reviewed, the writing style of this review was difficult to understand, and it made the main messages difficult to take home. The figure and table were poorly formatted and difficult to interpret. There were redundant paragraphs and repetitive information so the review didn't flow particularly well.

Reply: Thank you for your insightful comment. We reorganized the chapters by numbering and by changing the titles. We also shortened the long sentences that were difficult to understand. The table has been arranged to make it easier to view. In addition, the English was reviewed and proofread by a native English speaker. Please find the editing certificate attached to the supplementary material.

Reviewer B

The manuscript is a very timely summary of the biological basis of microbiome on cancer development, effect on the immune system and tumour microenvironment and effect on the benefit and toxicity to anti-PD(L)1 therapeutics and other systemic therapy.

Overall, the scientific content is detailed and up to date. In the Discussion Section, the authors have provided a good summary and some of the current limitations of the identification of the taxonomy of the bacteria in the gut and other organs. But inclusion of future research directions in microbiome will be valuable.

The quality of writing needs substantial editing to improve the readability of the manuscript. The manuscript is a good attempt to summarize the current status of microbiome in cancer immunotherapy and cancer biology. The manuscript will benefit from extensive English editing, and it will be impossible to list them point by point.

With such efforts in summarizing the current status of microbiome, the discussion on further research directions is very anemic.

Reply: Thank you for your insightful comment. We have supplemented the discussion section with a reference to the new clinical trials presented in this review and clarified the last phrase and result. In addition, the English was reviewed and proofread by an English native speaker again. Additionally, we have added some lines on further research directions.

Changes in the text: Page 17, lines 26-30

In this review, we have described a number of new clinical trials that intervene in the gut

microbiome. It is hoped that some of these trials may lead to new therapies that improve the efficacy of ICIs or influence the attenuation of resistance to these drugs.

Page 19, lines 26-28

To moderate the gut microbiota to improve the efficacy of immunotherapy, an interventional trial is ongoing. → To improve the efficacy of immunotherapy, it is important to conduct intervention trials that modulate the gut microbiota.

Page 20, lines 4-5

Future studies of the oncobiome will have the potential to enhance threatment. It is important to study the oncobiome to enhance its efficacy in pharmacotherapy and adjuvant treatment.

Reviewer C

It deals with a very important and timely topic of the microbiome and its influence on the development, course and treatment of cancer. It is a very interesting topic, but article seems to be a bit unfocused. Oncobiome somehow doesn't resonate in this article.

Below are some comments on the manuscript.

1. *Page 3, line 26-27: 'Immune checkpoint inhibitors are key drugs for both SCLC and NSCLC patients.'*
   Are ICIs for SCLC really the key? The authors probably mean that for many years the only therapeutic strategy was chemotherapy, without the possibility of targeted treatment, and now there are more and more reports of ICIs being effective in SCLC. But from the sentence cited here, this does not follow. For NSCLC, TKIs are also crucial when targeted murine is identified. For NSCLC, TKIs are also crucial when targeted mutations are identified. This sentence should be reworded, expanded.

Reply 1: Thank you for your insightful suggestion. We have rewritten the sentence as follows.

Changes in the text: Page 3, lines 23-26.

For SCLC, chemotherapy has long been the only treatment strategy, and for NSCLC, targeted therapies such as MTAs with gene mutated patients have been used. Therefore, the advent of ICIs has had a profound impact on lung cancer treatment.

2. *Page 4, line 17: 'Microbiota and cancer development' There are some statements that are very general, e.g.: 'The first effect is mutagenesis of the colonic epithelium, consequent to the production of bacterial toxins and other molecules that either damage DNA directly, disrupt the systems that maintain genomic integrity, or stress cells in other ways that indirectly impair the fidelity of DNA replication and repair.' What kind of toxins? What is the origin of these toxins, from what species or strains? '...other ways that indirectly impair the fidelity of DNA replication and repair.' What other ways?*

Reply 2: Thank you for your comment. We have added the sentences below. Changes in the text: Page 6, lines 20-24.

For example, *E. coli* carries the *pks* locus, which demonstrably mutagenizes the human genome and is implicated in conveying hallmark-enabling mutations. Secretion of butyrate acid, a metabolite of *Porphyromonas* sp. has been shown to contribute to tumorigenesis by inducing senescence of fibroblasts and epithelial cells.

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3. *Page 4, line 20-21: 'The gut microbiota and the intestine are in perfect balance via genes,*

proteins, and metabolites.' It is not quite clear what the authors mean: protein genes or metabolites, bacterial or human, or both? The reader can guess, but that's not the point of the article, to guess. And is there really a perfect balance between the microbiota and the gut?

Reply 3: Thank you for your comment. We have rewritten the sentence as follows.
Changes in the text: Page 4, Lines 23-24
The gut microbiota and the intestine are in an exquisite balance via genes, proteins, and metabolites of both bacteria and humans.

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4. *Page 5, line 6-8: 'In addition to the aforementioned cancer types, the gut microbiota is thought to have an effect on other cancers through interactions with the tumor microenvironment (TME)' What 'other' cancers? Again, not very precise.*

Reply 4: We have added the specific cancer types to the sentence.
Changes in the text: Page 6, line 25
In addition to the aforementioned cancer types, the gut microbiota is thought to have an effect on other cancers, such as hematologic tumors or lung cancer, through interactions with the tumor microenvironment (TME).

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5. *Page 6, line 29-31, '…local microbiota…' what kind of microorganisms? Moreover, page 7, line 1-2: '…signature of lower airway dysbiosis was most prevalent …' What does the signature of dysbiosis mean? It should be either write dysbiosis or list the most important bacteria. Later, the authors point out Veillonella parvula, but does the signature only cover one type of bacteria?*

Reply 5: We have added the names of specific bacteria and the definition of dysbiosis.
Changes in the text: Page 8, line 27.
They found that the local microbiota, such as *Herbaspirillum* and *Sphingomonadaceae,* which are associated with tumor growth, could promote inflammation and cancer progression via lung-resident γδ T cells.
Page 9, Lines 2-3
In addition, Tsay et al. found that the signature of lower airway dysbiosis, an imbalance between the types of organisms present in a person's natural microflora, was most prevalent in the group of lung cancer patients with stage IIIB-IV tumor node metastasis and was associated with poor prognosis, as shown by decreased survival among subjects with early-stage disease.

6. *'de novo' should be written in italics.*
Reply 6: We have rewritten the words in italics.
Changes in the text: Page 7, Lines 26-27, *de novo*

7. *Page 8, line 25: '…distant effects with microbial metabolites.' lack of citation.*

Reply 7: We have added the citation.
Changes in the text: Page 10, Line 28.

8. *Page 10, line 13: Maybe to make it uniform, the headline 'Response of the gut microbiota to cancer chemotherapy' convert to: 'The role of the gut microbiota in chemotherapy'?*

Reply 8: We have changed the headline as you suggested.
Changes in the text: Page 10, Line 15
The role of the gut microbiota in chemotherapy.

9. *Page 11, line 3: '…found that the gut microbiota promotes the development of…' the whole gut microbiota affects it? A very vague statement.*

Reply 9: We have added the word "diversity" to the sentence.

Changes in the text: Page 13, Line 7

Shen et al. found that the diversity of gut microbiota promotes the development of chemotherapy-induced mechanical hyperalgesia (57).

10. *Page 11, line 6: 'Restoration of the microbiota…' How it was restored?*

Reply 10: We have rewritten the sentence because it was confusing.

Changes in the text: Page 13, Lines 10-11

Germ-free mice did not suppress mechanical hyperalgesia.

11. *Page 11, line 9-10: 'Several studies of the association between gut microbiota and immune related adverse events are underway.' - Like many other studies. Is this sentence necessary?*

Reply 11: We have deleted the sentence.

Changes in the text: Page 11, Lines 9-10 deleted.

12. *Page 11, line 17-19: 'It is said that bacterial taxa within the Ruminococcaceae family, including Firmicutes, have been associated with immunotherapy-induced colitis (56).' It is not entirely clear what the authors intended to express - Firmicutes is a phylum of bacteria which includes genus Ruminococcaceae.*

Reply 12: We have deleted "including Firmicutes".

Changes in the text: Page 13, Line 23

Bacterial taxa within the *Ruminococcaceae* family have been reported to be associated with immunotherapy-induced colitis (59).

13. *Page 11, line 22-24: 'Therapeutic strategies modulating the microbiome are currently being evaluated to boost ICI responses or circumvent primary resistance to ICI, although without patient stratification based on the degree of dysbiosis.' This sentence needs to be reworded. It is not quite clear what the authors want to express in it.*

Reply 13: We have rewritten the sentence because it was confusing.

Changes in the text: Page 13, Lines 27-31

Therapeutic strategies that modulate the microbiome are currently being evaluated to enhance the ICI response or to avoid primary resistance to ICIs (60).

14. *Page 11, line 21: 'Targeting the microbiota' Maybe replace that headline with something like: 'Modulating the composition of the microbiome'?*

Reply 14: We have changed the headline as you suggested.

Changes in the text: Page 11, Line 20

4) Modulating the composition of the microbiome

15. *Page 15, lines 17-19: 'In this review, microbiomes are introduced as ecosystems involving existing bacteria or fungi, and they have a profound impact on human health and diseases, including cancer phenotypes.' Yes, the authors described bacteria, but not fungi. Next: 'However, he also considers…' Who is this 'he'?*

Reply 15: We have changed the term "In this review" to "In addition" because it was a continuation of the Hallmarks of Cancer review.

Changes in the text: Page 17, Lines 22-25

In addition, microbiomes are introduced as ecosystems involving existing bacteria or fungi, and they have a profound impact on human health and diseases, including cancer phenotypes (72) (5). However, Hanahan also considers~

16. *Page 16, lines 12-17: 'Butyrate disrupts the intestinal barrier (72) and leads to dysbiosis, and bacteria invade to affect cellular energy, metabolism, histone modifications, cell cycle progression, and tumor-promoting inflammation associated with immunosuppressive adaptive immune responses via the multifarious routes of the bacterial immunomodulatory factors that activate damage sensors on epithelial or resident immune cells.'*
Quite a bold statement considering that butyric acid, which belongs to SCFAs, is considered beneficial in sealing the gut and promoting gut microbial diversity. The article cited in item 73 is from 1976. Some more recent literature can be found.

Reply 16: We have deleted the bold sentences and summarized them in the preamble.

Changes in the text: Page 18, Line 16

In addition, metabolites such as butyrate from the microbiome induce complex physiologic effects in nascent epithelial and fibroblastic cells (75) (29).