Peer Review File

Reviewer A:

Comment 1: This study, it is extremely similar the recently published article in Cancer Immunology and Immunotherapy by Wilson et al which also performed a systematic review and meta-analysis of the effect of antibiotic timing on the effectiveness of immune-checkpoint blockade. The method adopted of splitting the studies into 3 groups by the timing of antibiotic exposure are reproduced from this recent article. Even Figure 1 appears to be a reproduction of the figure generated in this recent publication, albiet with updated data from additional studies published in late 2019. The authors of this paper even reproduce several discussion points made by Wilson et al. Although this paper includes updated data, the overall conclusions of this paper are the same as those by Wilson et al- that the timing of antibiotic exposure can influence immunotherapy effectiveness, and that exposure immediately prior to initiation of immunotherapy has the greatest impact. Therefore, I am not sure that this paper contributes any new insights to the published literature.

Reply: We categorized the antibiotic exposure windows according to the definition in studies, analysed the impact of using antibiotic during the treatment of immunotherapy to the efficacy of ICIs independently, which was completely different from Wilson's. The pooled results showed that there was no significe difference between patients with or without antibiotic in OS, what's more important, antibiotic use has positive impact on PFS. Tinsley et al noted that retrospective studies which failed to show any association between antibiotic therapy and ICI efficacy. Francesco et al found that Eastern Cooperative Oncology Group performance status (PS) 2 was the only factor independently impacting on both PFS and OS. Even though Hopkins et al found the negative impact of antibiotic exposure, but the authors themselves are cautious in their interpretation of results, with a special situation was detected that ECOG performance status was generally low in the cohort. Our results suggested that during the treatment of ICI, if ATB are required, perhaps, it may not cause the negative impact of efficacy of ICI. As we all known, patients with infection may cause bad performance status, the findings about negative impact of antibiotic use which may be confounded by overall health status of patients that necessitates antibiotic use. The impact of antibiotic therapy has been scarcely evaluated through registration studies and further prospective evaluation in this setting is needed. The above discussions were also added in the discussion part of our revised manuscript.

Changes: The change could be seen in the WORD file marked in red (Page10, Line196-207).

Minor:

Comment 2: There are some grammatical inconsistencies in terms of was and were and tenses throughout that could benefit from editorial review

Reply: Thank you for your kindly reminding. we have corrected form of was and were as.

Changes: The change could be seen in the WORD file marked in red (Page5, Line85 and 99; Page7, Line 128 and 144; Page8, Line 147).

Comment 3: There is blurring between the introduction, results and methods sections. For example, the definitions of antibiotic windows adopted in this study are defined in the introduction rather than the methods. The type of meta-analysis performed (random effects), is provided within the results rather than the methods. There is no description of the plan to preform subgroup analysis on antibiotic timing even within group 1 studies.

Reply: Thank you for your useful suggestions. According to your suggestion, we have grouped the definition of antibiotics in the methods. Due to we aim to research the effect of antibiotic exposure in a shortly time (within 2 months before or after ICIs) around initiation of antibiotic, wo did not preform subgroup analysis in Group 1.

Changes: The change could be seen in the WORD file marked in red (Page5, Line100-105).

Specific comments:

Comment 4: Line 72- group 1 was administered with ATBx within a time frame of months before immunotherapy – this is vague, please define how many months. If you then go on to perform further subdivision within group 1 this should also be described in the methods.

Reply: We thank reviewer for pointing out this issue. According to the definition of antibiotic timing of the publications, we defined the Group 1 as antibiotic use within 2 months before or 2 months after ICI (shown in the Figure 1). In such group, we did not do the subgroup analysis.

Changes: The change could be seen in the WORD file marked in red (Page 2, Line 32-33; Page5, Line102-103, Page9, Line184-185).

Comment 5: Line 87- correct grammar " we searched the PROSPERO database without restricted

Reply: We apologize for the grammar errors in our manuscript. we have modified our text as advised.

Changes: The change could be seen in the WORD file marked in red (Page 4-5, Line 84-85).

Comment 6: What is the study registration number? Was this study given ethics approval? This would not normally be required for a review of published studies. Please clarify.

Reply: Thank you for your reminding. We have accessed the application of Prospero, and our registration number is: CRD42020155823. The question about ethics approval is clarified.

Changes: The change could be seen in the WORD file marked in red (Page 5, Line 85-89).

Comment 7: At least one of the referenced studies does not contain the data required to be included in this meta-analysis. The abstract referenced by Lalani et al did not include the HR for PFS or OS. Please explain where the data was obtained and reference accordingly.

Reply: Thank for your carefully reviewing. When we check this issue, we found that Lalani et al had published the full text, so we updated the included data of Lalani (DOI: 10.1016/j.euo.2019.09.001). Please see the specific data in our revised Table 1.

Changes: The change could be seen in the Table 1, Figure 3A and 3B.

Comment 8: In line 171 you state that "Neither study had stratified the ATB exposure time well" in reference to the studies by Wilson et al and Huang. You then go on to state "For the first time, we have divided the included cohorts into three groups in accordance with the different definitions of the ATB exposure window to avoid the overlapping definitions of ATB time in different studies as much as possible. This is misleading. Wilson et al also divided the exposure window into 3 groups, with quite similar definitions to current study. The argument that your windows are not overlapping is also incorrect. As illustrated Figure 1, your definition of antibiotic exposures in group 1 very much overlaps with your definitions in group 2 and 3. Please revise this section.

Reply: We thank reviewer for pointing out this issue. We apologized for the inappropriate statement here. What we wanted to express here is that we investigated the effects of ATB exposure on the PFS and OS during ICI for the first time, and a completely different situation was founded that antibiotic use was positive to the PFS of patient with ICI. The above discussions were added in the discussion part of our revised manuscript.

Changes: The change could be seen in the WORD file marked in red (Page 9, Line 177-184).

Comment 9: The statement : "Wilson et al. have found that exposure to ATBs during the first 60 days of ICB and at any time during ICB does not affect the PFS or the OS of patients" is also misleading. This study found that antibiotics within 42 days prior to ICI affected PFS and OS, that antibiotics 60 days before and up to 42 days after also affected PFS and OS. The authors are perhaps trying to convey that when a very broad definition of antibiotic exposure is adopted (antibiotic exposure anytime within the window 60 days before anytime after initiation of immunotherapy), the effect of antibiotics appears was lost. If this case, please reframe and reword.

Reply: Thank you for providing these important insights. We have revised our manuscripts as your advice.

Changes: The change could be seen in the WORD file marked in red (Page 9, Line 174-177).

Comment 10: Line 201: clarify language, I think you mean to say mice transplanted with the microbiota of non-responders had inferior response to ICI, rather than the non-responder mice had inferior response to ICI.

Reply: We apologize for the misstatement, and our manuscript has been rephrased about this formulation.

Changes: The change could be seen in the WORD file marked in red (Page 11, Line 219-220).

Comment 11: Figure 1- some of the study identifiers have years while others do not. Consider editing for consistency, and making DO into Do

Reply: Thank you for censoring carefully! The figure 1 has been re-editing, as we updated the literature retrieval.

Changes: Please see the results in Fig. 1.

Comment 12: The authors have omitted the following paper: Hemadri A LH, Lin Y, Rose A, Sander C, Najjar Y, Zarour HM, Kirkwood JM, Davar D: Association of

medication and antibiotic use with response and survival in advanced melanoma receiving PD-1 inhibtors. In: American Society of Clinical Oncology. vol. 37: Journal of Clinical Oncology; 2019.

Reply: Thank you for your comments. In fact, this article was included in our screening researches, but it was excluded because of lacking HR for PFS or OS.

The authors try to assess if ATBs may affect the efficacy of ICIs. However, the study, even if the argument is of great interest, has several metholodical limitations.

Comment 1: I strongly reccomend to use PRISMA to help authors to improve the reporting of their systematic reviews and meta-analyses.

Moreover, I have some concerns about the groups analyzed: Group 3 seems somehow a subgroup of Group 1. Maybe, it would be more interesting a comparison between group 2 and Group 3 (with ATBs start at ICI initiation, after 1 month and after 3 months).

The duration of ATB therapy may have a great influence: has a treatment last 5 days the same effect of a treatment last 1 months? Are the authors sure that is correct to use retrospective studies for their systematic review since the principal endpoint of their study is the time of ATB administration?

Reply: Thank you for providing these important insights. Wo are sorry for making confuse here. According to your advice, we categorized the definition of antibiotic exposure window into new three groups, drop the publications which defined the timing as several time before and/or during ICIs, as shown in the methods. Kapoor et al found that compared with median OS for patients who received > 10 days of antibiotic, the median OS was prolonged in those who received ≤10 days of antibiotics. It seems that duration of ATB may play an important role. However, existing retrospective studies are rarely included the relationship about the duration of ATB and efficacy of ICIs. It deserves more research in the future. As shown in our studies, the question about the impact of antibiotic exposure window in efficacy of ICIs was controversial, we hope that our research could provide inspiration to the clinician.

Changes: The change could be seen in the WORD file marked in red (Page 2, Line 32-33; Page5, Line102-103, Page9, Line184-185) and Figure 1.

Huang et al present a meta-analysis of studies for the effect of antibiotics on response to immune checkpoint inhibitors for a variety of cancers. This is an important question with strong clinical relevance; however, the authors fall short of providing a meaningful contribution to the field. There are two main problems: a fast-moving field and a confusing result that leaves the reader with more questions that aren't addressed.

Comment 1: First, there have been many papers on this topic, including several other meta-analyses, and the torrent of papers has not slowed since the authors stopped assimilating papers. Clearly the authors had to freeze their analysis somewhere, but the last paper included seems to be in 2019, and 10 months is a long time in this field. An update might be warranted in this case, because the primary contribution of this paper remains confusing.

The primary contribution of this manuscript is in the stratification of the timing of ATB exposure into 3 groups: all times, pre ICBs, and post ICBs. The timing is a critical aspect of this problem that has been addressed in other studies but not in other meta-analyses. However, the authors chose overlapping groups, which eliminates the value of stratification. Even more confusingly, only the "all times" group showed a significant effect of ATB – neither the "pre ICBs" or "post-ICB" showed a significant effect. How is that possible? Either before should be important or after – this result suggests that neither before or after is important, but only both before AND after? This paradox should be discussed, or, more appropriately, defining the groups such that this paradox isn't possible.

Reply: Thank you for your useful suggestions. We conducted a research in the Pubmed and the Embase databases again, and 9 new data of studies were available, and several studies were updated their data. Since the relevant research started in 2017, we researched the articles of the last five years form the electronic database. We did a redistribution of the timing of ATB, avoiding the overlapping as possible as we can do. The primary objective of this meta-analysis is to explore the effect of antibiotic exposure window on efficacy of ICIs. Our pooled results showed that antibiotic use in shortly time (within before or after 2 months) around the initiation of immunotherapy was remarkably related to the efficacy of ICIs. During the long-term treatment of ICIs, the effect of antibiotic exposure seems to be eliminated. The above discussions were also added in the discussion part of our revised manuscript.

Changes: The corresponding results are showed in Table1, Fig.1, Fig. 2, the change could be seen in the WORD file marked in red (Page 2-3, Line 32-49; Page5, Line102-103, Page9, Line184-185, Page10, Line 198-207).

Major:

Comment 2: The weighting system isn't well defined. For example, how sample size is accounted for isn't well described, and the few prospective studies appear to be weighted similarly to the retrospective analyses.

Reply: Thank you for your comments. In this meta-analysis, we use HR to evaluate the effect of the time window of ATB exposure on the survival of patients with immunotherapy, which corrected sample size already. The sample size may not be considered in the pool analysis. The weights are shown in the forest figure.

Minor:

Comment 3: 156: Is that p-value correct? The CI is outside 0? The text implies that the p-value cited is a typo. Either that p-value is wrong or the text has to change – line 157 sets the PFS in contrast to OS, but by the p-values neither has a significant effect.

Reply: Thank for your carefully reviewing. We apologize that we show the p-value of I^2 in this inappropriate way, which caused a misunderstanding here. Please see the revised manuscript.

Changes: The change could be seen in the WORD file marked in red (Page 2, Line 35-44; Page7, Line145-146, Page8, Line153-157 and 162-164).

Comment 4: 171: "Neither study has stratified the ATB exposure time well" This sentence is vague. Did they stratify or not? If they did, why wasn't it done well?

Reply: Thank you for your comments. In fact, Huang et al didn't do the subgroup research about the effect the antibiotic timing to the efficacy of ICI. The above discussions were revised in the discussion part of our manuscript.
Changes: The change could be seen in the WORD file marked in red (Page 9, Line 177-185).