Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-23-597

Reviewer A

This research was well conducted and supports the conclusion that poorer NSCLC outcomes can apply when antibiotics are provided before or during ICI therapy of NSCLC. This is evident both from the univariate and multivariate analyses reviewed. The research was well executed and, to the extent feasible, considered such matters as potential confounding, evidence for heterogeneity between studies, and evidence for selection bias, Limitations include questions of generalizability beyond NSCLC and the extent to which similar results would apply irrespective of antibiotic type, timing, and protocol, and whether results would apply to effects of non-ICI systemic therapies.

Comment 1: Antibiotic use could signal the presence of co-morbidity which may itself have a negative effect on outcomes. How strong is the evidence that this or other confounding explained the poorer outcomes?

Reply 1: We thank the reviewer for their comments. Most of the included studies that considered ECOG performance status or comorbidities still reported a significant association between OS or PFS and antibiotic use in those treated with immunotherapy, after adjusting for other health predictors. However, it is possible that baseline health status differences have not fully been accounted for. We have included reference to a study showing that patients who develop infections may have specific clinical characteristics that predict their infection risk (reference 45). Since each of the studies included adjusted for difference covariates, it is difficult to properly adjust for all relevant covariates in the pooled HR for OS and PFS. Future studies should focus on these confounders.

Changes in text: Lines 276-286.

Comment 2: Are there trade-offs between adverse effects of antibiotics and their benefits that could vary with ECOG status?

Reply 2: We thank the reviewer for their comment. This is an interesting point. Unfortunately, none of the literature we reviewed stratified by ECOG status, only included it in the multivariable models. This could be an interesting point to explore in further research.

Changes in text: None.

I recognize that this review and meta-analysis can only take the evidence available so far. I repeat that I regard the research to have been well executed. I am left wondering about next steps and whether prospects still exist for ethical application of prospective RCTs in relevant clinical cohorts to address remaining questions.

Reviewer B

The authors of this paper aim to perform a more robust analysis of the impact of antibiotics on patients with NSCLC who are receiving immunotherapy. Prior investigation has demonstrated alteration of gut microbiota following antibiotic therapy and has also demonstrated the detrimental association between antibiotic use and immunotherapy across multiple different tumour types, including NSCLC. This evidence is already incorporated into guidance for management of these patients and therefore the novelty of the research is in the study methodology rather than the clinical question.

The criteria used to select articles for inclusion was appropriate given the addressed clinical question. It is especially important the inclusion of controlled studies.

Given the up-to-date nature of the study, the appropriateness of the inclusion criteria, and the correct execution of the article search, it is improbable that any significant or pertinent studies were missed. The process used for the article search is also replicable. Additionally, authors used a funnel plot to demonstrate that there was no publication bias.

Authors appraised the validity of the study by using the MMAT scoring system to ensure the quality of the included the studies. In addition, they used the PRISMA guidelines to report their findings. Finally, the literature screening was independently conducted by two reviewers who consulted with a third author to resolve discrepancy.

The assessment of studies is reproducible. Q and I2 statistics were used to test heterogeneity across studies and both tests reported low heterogeneity. However, we find differences between studies regarding sample size, lines of treatment, antibiotic regimen and adjustment variables.

This meta-analysis shows a statistically significant difference between patients undergoing immunotherapy who take antibiotics compared with those who do not take any during their cancer treatment. Both univariate OS (pooled HR 1.56, 95% CI 1.23-1.99) and PFS (pooled HR 1.22, 95% CI 1.03-1.44) were significantly lower in patients receiving antibiotic treatment in conjunction with ICI when pooled estimates were calculated.

Of note, many of the individual studies included in this meta-analysis did not report statistically significant effects. Among the 19 studies selected, only 8 showed worse OS and PFS, 4 only showed worse OS, 1 showed worse PFS and 6 did not show a statistically significant difference in survival.

There were 12 studies which performed multivariate analysis for survival (12 for OS and 10 for PFS). The pooled HR for OS was 1.67 (95% CI 1.23-2.27) and the pooled HR for PFS was 1.64 (95% CI 1.16-2-32)

The results from this meta-analysis are precise. They include pooled HR for both univariate and multivariate analysis (when feasible) with 95% confidence intervals.

The findings from this study may pose challenges when attempting to integrate them into routine patient care.

Comment 3: Firstly, very different adjustment variables were used between studies. Consequently, it may be difficult to interpret the pooled survival HR.

Reply 3: Thank you for the comment. We agree that this is a significant limitation that is mentioned in the discussion. As immunotherapies become more widely utilized and more data is published, future meta-analyses could attempt to select for studies that adjust for the same covariates.

Changes in text: No changes made, this limitation was already included in the discussion.

Comment 4: Secondly, antibiotics are strongly related to patient's general well-being, as infections in cancer patients are a major cause of morbidity and mortality. Consequently, the observed decrease in survival rates among patients taking antibiotics could be attributed to their poorer health status and comorbidities. While many studies have adjusted their results based on the ECOG performance status, this may not be sufficient to fully account for this confounding factor and it is an important area to further explore.

Reply 4: We agree with the reviewer's comment. Unfortunately, not all of the studies included performance status indicators or comorbidities as covariates, and we acknowledge that even for those that did, it may not fully account for baseline health differences. This is an important avenue to explore in further research.

Changes in text: Lines 276-286.

Comment 5: Thirdly, there could be discrepancies in interpreting OS and PFS in this group of patients. Four studies in the univariate analysis indicated worse OS but no differences in PFS. This could suggest that the use of antibiotics doesn't affect the efficacy of immunotherapy, but is associated with poorer outcomes due to its strong correlation with comorbidity and mortality.

Reply 5: We thank the reviewer for their comment. It is possible that this is what the data is suggesting. If we had data on cancer specific mortality, we could possibly test this hypothesis, but as of now, there is a gap in the literature addressing this.

Changes in text: this future direction is included in lines 286-88.

Comment 6: Unfortunately, there is a lack of information on the outcome of the infection following the antibiotic treatment. This data would be extremely useful in evaluating the effectiveness of antibiotics and distinguishing between patients who recovered from the infection and those who had to discontinue their treatment due to the severity of their infection.

Reply 6: We agree with the reviewer that this would be valuable information. Unfortunately, none of the studies included information on whether patients had stopped antibiotic treatment due to worsening health conditions or simply due to completion of treatment. We believe this is an important idea to explore in further research, but it is a bit out of the scope of our meta-analysis.

Changes in text: this future direction is included in lines 286-88.

The main conclusion of the present study is that antibiotic use should be taken into consideration when using ICIs to treat NSCLC. It is important to acknowledge that antibiotics have the potential to modify the gut microbiota, which could subsequently influence the clinical outcomes in NSCLC patients receiving ICIs. Nevertheless, infections also contribute to the mortality rate among oncology patients. Consequently, it would be imprudent to discontinue antibiotic treatment in patients with severe infections solely based on these findings.

This manuscript is well-written and the research was well-executed relating to the specific question under investigation. However, the added value to clinical community from these findings is limited, as the conclusion mirrors those of previously published research on this topic.

Reviewer C

In this meta-analysis, Authors investigated the impact of antibiotic treatment on overall survival and progression free survival of patients affected by Non-Small-Cell-Lung-Cancer (NSCLC) treated with immune-checkpoint-inhibitors (ICIs).

Overall, the meta-analysis was conducted on nineteen studies deriving from "real-world" case series demonstrating that the concurrent use of antibiotics and ICIs in the context of patients affected by NSCLC may be associated with reduced overall survival and progression free survival.

The analysis has been properly conducted and data are well presented.

Overall, I suggest only minor revisions before publication:

Comment 7: I think that 108-133 of introduction may be shortened. In fact, detailed discussion of results deriving from previous studies should be discussed in discussion section.

Reply 7:

Changes in text: we have moved the details on previous meta-analyses from the introduction to the discussion, thus shortening the introduction as suggested by the reviewer.

Comment 8: Despite previous studies suggested that the cause of reduced OS and PFS derives from microbiota alteration, at least other two possible explanations need to be investigated: i) patients who receive antibiotics probably suffered for infections. This could explain reduced survival; ii) antibiotic use for infection may be associated with ICIs discontinuation or delay in administration; this could be associated with reduced effectiveness of therapy. These points should be discussed.

Reply 8: We thank the reviewer for their comments. We agree that those who experience infections may have differing underlying health conditions than those who do not, and furthermore that infections may lead to delay in ICI treatment. We have now addressed this in the limitations section.

Changes in text: Lines 276-288.

Comment 9: Some recent works highlighted the importance of early identification of infections in patients with NCLCs and importance of multidisciplinary management of these complications, including appropriate antimicrobial stewardship (DOI: 10.1093/ofid/ofab187) (https://doi.org/10.1111/sji.13303). Given important results of this meta-analysis these two works should be cited and discussed.

Reply 9: We thank the reviewer for sharing these studies with us. We have included relevant information in the discussion.

Changes in text: Lines 280-286.

Reviewer D

'Studies on a variety of cancers have suggested a reduced overall survival (OS) and progression-free survival (PFS) in patients treated with ICIs that have also been treated with antibiotics.'

The article has an element of scientific discussion, which is very good.

Comment 10: It would be good if the authors provided whether during or before immunotherapy antibiotics were administrated and for what time, e.g. one month or 2 weeks.

Reply 10: We believe this is covered in column 6 of table 1. The inclusion criteria based on antibiotic regimens differed in each study, though all were within 3 months prior to or after starting immunotherapy.

Changes in text: No changes made, since the information is already in table 1.

Comment 11: The writing of PD-1, and PD-L1 needs to be standardized.

Reply 11: .

Changes in text: we went through the text and the tablecarefully to make sure the writing is standardized.

Comment 12: In Table 1, the line of treatment column: "alone or combined" is not fully understood.

Reply 12: Thank you for the comment. We have edited Table 1 to reflect that the Zhao et al paper focused on all lines of treatment.

Changes in text: Table 1, row 8, column 5.

Comment 13: The reader could be disappointed because the authors did not describe how the microbiota changes under the influence of antibiotics, and its composition most likely affects the shortening of OS or PFS.

Reply 13: We thank the reviewer for this comment. This meta-analysis cannot answer this question. Microbiota changes following antibiotic use depend on the type of antibiotic administered. Since the studies included in this meta-analysis did not select for any specific antibiotic/antibiotic class, we believe this is an excellent point for further observational studies. We added this as a future direction in the discussion.

Changes in text: line 305.