Author response to reviewer comments v.1

We would like to thank you and the reviewers of the Therapeutic Advances in Respiratory Disease for taking the time to review our article. We made some corrections and clarifications in the manuscript after going over the reviewer's comments. All authors have read and approved the revised manuscript. The changes are summarized below.

Reviewer 1' comments

1. The authors need to justify why this paper is clinically relevant. Both of these drugs were rejected by the US FDA and have not been approved or made available anywhere in the world. Clinicians reading this paper get nothing out of it at the moment because they cannot prescribe these drugs. The authors should consider how any findings contribute to our knowledge and how clinicians might use their findings

R: Thank you for your comment. We agree to your point that the analysis of combined effect of commercially unavailable drugs may accompany limitations. However, we made an approach to these drugs from the following perspectives.

Inhaled antibiotics proved its efficacy in cystic fibrosis (CF)-associated bronchiectasis. On the other hand, several inhaled antibiotics including tobramycin, colistin, or aztreonam lysinate failed to show efficacy in patients with non-cystic fibrosis bronchiectasis (NCFB). Recently, several RCTs of inhaled ciprofloxacin agents firstly demonstrated clinical efficacy in reducing exacerbation frequency in NCFB. However, due to heterogeneous findings between individual studies, these agents could not get FDA approval. The evaluation of combined effect of inhaled ciprofloxacin in NCFB may have limited applicability as this drug is not yet permitted for clinical use among the patients.

Nevertheless, we think that the result of our meta-analysis has its worth in suggesting direction of future treatment modalities for NCFB with chronic respiratory infection. Clinicians and researchers may wonder whether these inhaled ciprofloxacin agents would be effective when they are evaluated together with the manner of meta-analysis. Evidences suggested by this meta-analysis would be an important background for following clinical studies for inhaled antibiotic agents for NCFB, not only limited to ciprofloxacin. In addition, compared to Western population in which CF is the dominant type of bronchiectasis, a major proportion of Asian bronchiectasis patients are NCFB (many of them are sequelae from previous pulmonary tuberculosis or NTM infections) and many of them suffer from chronic pseudomonal infection. Therefore, considering limited treatment options in NCFB patients with chronic respiratory infections, demonstration of positive combined effect of the two different forms of inhaled ciprofloxacin has its clinical value for treatment of this patients group in near future. We presented the limited treatment options for NCFB patients in the introduction section as following:

"However, the study results of inhaled antibiotics including tobramycin, colistin and aztreonam lysinate in patients with NCFB were not as satisfactory as those in patients with CF." (Line 63-64)

We also commented about the positive but controversial findings of inhaled ciprofloxacin RCTs, and need for a systematic review and meta-analysis:

"However, despite several positive outcomes in terms of exacerbation, efficacy of the inhaled ciprofloxacin in these RCTs was controversial. A systematic review with meta-analysis of these two

inhaled ciprofloxacin agents will help better assess of their effects and safety in treatment of patients with NCFB." (Line 70-72)

"Due to these controversial results, it was necessary to perform a meta-analysis including overall studies, for further evaluation of these agents." (Line 231-232)

In addition, we additionally described the following sentences to suggest implication of the present study for the clinicians and researchers in the discussion section:

"Although these drugs are not currently available, the present meta-analysis suggests summary of existing controversies and firstly demonstrated clinical efficacy of inhaled antibiotics in treating NCFB patients. This finding would be a background for further studies about inhaled antibiotics for NCFB." (Line 284-287)

2. The pooling of these two sets of study programmes is hard to justify. Although these both studied ciprofloxacin compounds in bronchiectasis, this is one of the few similarities between them. The liposomal Cipro programme only looked at patients with Pseudomonas while the dry powder programme included patients with multiple pathogens. The characteristics of the patients and the exacerbation rates in the studies were very different. For this reason, it is questionable how meaningful the combined effect estimates are. The authors may disagree, but they need to justify their pooling of these studies in the paper.

R: We appreciate your critical comment. Your mention about disparity between two regimens is reasonable and we understand this problem is essential when evaluating the combined effect of the drugs. We are also aware of this dissimilarity and have previously mentioned in the initial version of the manuscript. Nonetheless, we think the present systematic review and meta-analysis has its value because of the following reasons:

First of all, although microbiologic inclusion criteria were different between studies, each study reflects a major subset of NCFB with chronic infection. All studies included culture positive patients: Studies of ILC included patients with P. aeruginosa infection, while studies of DPI also included patients infected with other respiratory pathogens. Nevertheless, P. aeruginosa was the major pathogen which took more than 50% of study patients in the studies of DPI, while heterogeneity of ILC studies also presents (ciprofloxacin sensitive vs non-resistant P. aeruginosa). Considering these points, we think studies of DPI and ILC can be evaluated together, for the context of managing NCFB with chronic infection. Similarly, the pooled analysis of the two drug formulations could be interpreted in the perspective of the evaluation of inhaled ciprofloxacin for NCFB, not of each specific agent. As answered to the comment 1, findings of the present analysis would be a background for further studies about inhaled antibiotics for NCFB. To clearly present these points, we additionally described as following:

We presented different microbiologic inclusion criteria in the result section and supplementary Table 3 in detail:

"Studies of DPI included patients infected with pre-defined respiratory pathogens including P. aeruginosa 13-15, while those of ILC only included patients infected with P. aeruginosa 16, 17. Detailed microbiologic inclusion criteria of individual studies are presented in Supplementary Table 3."(Line 148-151)

We universally applied random effect model in pooled analyses regardless of statistical heterogeneity. This is also the answer to the comment 3, and described in detail in the reply to the comment 3. We also emphasized the heterogeneity of included studies regarding the microbiologic criteria and drug formulation in the discussion section as following:

“Second, there was heterogeneity of included studies in terms of antibiotic formulation and microbiologic inclusion criteria. Concerning methodological heterogeneity, we applied random effect model in pooled analyses despite low statistical heterogeneity. In addition, it should be noted that the positive finding of the pooled analysis might not necessarily mean effectiveness of individual agents.” (Line 287-291)

3. The decision to use a fixed effects model because there was no statistical heterogeneity when the studies were combined is inappropriate. Please refer to the Cochrane handbook for a detailed discussion of why this is inappropriate. The assumptions underlying mixed vs fixed effects models are different. In the fixed effects approach we assume all of the studies are measuring the same underlying effect and differences are due purely to random sampling error. This is clearly not the case for the Cipro studies which were conducted in different patient populations with different definitions of the endpoints. The authors should use random effects.

R: We appreciate your valuable feedback on statistical analysis model. We agree with your comment and applied random effect model throughout the analyses. With the random effect model, the statistical significances of most analyses did not change. Only pooled analysis of any TE-AEs became significant, favoring ciprofloxacin arm (AEs less likely to occur in inhaled ciprofloxacin group). Since serious TE-AE was not different between the two groups, overall findings of the analysis did not change. In the revised manuscript, we replaced the initial values with the new statistical values which were calculated from the analyses using random model. Thank you once again for your constructive comment.

We also revised the method section as following:

“Because studies of two different formulations of inhaled ciprofloxacin agents were included, random-effects models were used regardless of statistical heterogeneity between the studies. (Line 124-126)”

In the result section, we revised the values in the abstract, manuscript, tables, and figures accordingly.

The heterogeneity of primary outcomes increased, but they did not significantly increase. We additionally described this point in the result section:

“Although moderate to substantial heterogeneity was observed, it was not statistically significant by $\chi 2$-test ($P \geq 0.10$). (Line 173-174)”

Also, we additionally commented the heterogeneity of included studies as limitation:

“Second, there was heterogeneity of included studies in terms of antibiotic formulation and microbiologic inclusion criteria. Concerning methodological heterogeneity, we applied random effect model in pooled analyses regardless of statistical heterogeneity. In addition, it should be noted that

the positive finding of the pooled analysis might not necessarily mean effectiveness of individual agents." (Line 287-291)

4. The review was not prospectively registered on the PROSPERO database. Registration of systematic reviews has been mandatory for many years to reduce waste, avoid duplication and promote protocol driven work. There is no real excuse for no registering and the authors should justify this.

R: Thank you for your comment and we also agree that the systematic reviews not being registered on the PROSPERO database is not favorable. We have been a little hasty in performing analysis and submitting the manuscript. We are deeply regretting our hastiness and mentioned your valuable comment about this point in the limitation paragraph of the discussion section:

"Lastly, registration on the PROSPERO database was omitted in the present analysis, which should be necessarily performed for systematic reviews to avoid duplication and promote protocol driven work." (Line 297-299)

5. The authors have missing data for some outcomes which is presumably available e.g QOL-B was done in ORBIT studies but is missing here. Did the authors contact the companies or authors to request missing data?

R: We have sent email requests to the corresponding authors of the studies, asking for the missing data. Unfortunately, we could not receive any response. We additionally commented this point in the result section as following:

"We requested additional data to the authors by e-mail when the original publication did not contain sufficient information, but we could not receive replies." (Line 139-140)

6. I fear figure 5 will be too big to read or digest. Could the authors turn this into a table instead? Or split into more than one figure?

R: Thank you for your constructive comment. We agree that the figure contains too much information in one figure. We divided the figure 5 into two parts (figure 5 for (a), (b), (c), and (d); figure 6 for (e) and (f)). After the edit, we hope the figures would be easier to read.

Figure 5. Forest plots presenting pooled analyses of secondary outcomes among NCFB patients treated with inhaled ciprofloxacin versus placebo.

(a) Pulmonary function (FEV1 change). (b) Quality of life (QOL-B RSS). (c) Quality of life (SGRQ). (d) Pathogen eradication.

Figure 6. Forest plots presenting pooled analyses of resistance emergence and adverse effects among NCFB patients treated with inhaled ciprofloxacin versus placebo.

(a) Emergence of resistance. (b) TE-AEs

7. RESPIRE 1 and 2 reported exacerbations and other outcomes with 97.5% and in one case 99.9% confidence intervals because of regulatory requests to adjust for multiple comparisons. The authors have pooled these as if they are 95% confidence intervals. This is statistically problematic. Ideally these should be converted to 95% CI's to map with those in the ORBIT studies. If not, the authors should at least acknowledge that they have done this.

R: We agree that the percentage range of confidence intervals should be coherent among the abovementioned outcomes. Following your comment, we converted the confidence intervals (CIs) of 97.5% and 99.9% into 95% CI's to better analyze the outcome. The statistical significance of the outcomes was maintained after the CI conversion. The changed result is shown both in the figure and the results section of the revised manuscript. Once again, thank you for your constructive feedback.

8. The statement in the discussion about the Kaplain meier weakening over time as evidence of resistance is wrong- you cannot judge anything about resistance from a Kaplain meier which only records the first event. Remove this.

R: We assume that you meant the following sentence in the discussion section "Also, during the one-year study period of the phase III trials, the Kaplan-Meier graphs did not imply gradual weakening of inhaled ciprofloxacin effect." We agree with your comment and removed this sentence in the revised version of the manuscript.

9. There are several statements like this where the authors go beyond the data. I suggest to go through the paper carefully and ensure all statements are evidence based.

R: We agree with the reviewer's comment that the discussion should be more objective based on the findings of the analysis. We reviewed our paper once again and removed the following sentences which might contain assumptions:

"As many of patients with NCFB have post-infectious structural destruction of lung parenchyme, improvement of lung function would not occur after short-term treatment."

"We could assume that resistance will become more evident if treatment duration is longer than one year, eventually hindering the effect of inhaled ciprofloxacin"

"Also, during the one-year study period of the phase III trials, the Kaplan-Meier graphs did not imply gradual weakening of inhaled ciprofloxacin effect."

We appreciate for your precious comments once again.

Reviewer 2' comments

ABSTRACT

1. Since conclusions say longer studies are needed in the future a mention of RCTs duration should be given in the results section.

R: Thank you for the constructive comment. We agree with the reviewer's opinion that the study duration should be present in the result section of the abstract. We described as following:

"Results: Two phase II and four phase III RCTs were included with a total of 1,685 patients. Treatment durations of phase III studies were 48 weeks, while those of phase II studies were shorter." (Line 33-34)

2. Since a long time "NCFB" has been substituted by "bronchiectasis" (see Elborn and Chalmers paper) in order to define a disease by what it is no (non-cf-bronchiectasis). For coherence with current literature I would suggest using the word "bronchiectasis".

R: We agree with the point that "bronchiectasis" is the more general term and can represent "non-CF-bronchiectasis". However, despite the generalizability of the word "bronchiectasis", we think use of the term "NCFB" would be necessary in the present review to emphasize different population of study. As mentioned in the introduction section, efficacy of inhaled antipseudomonal antibiotics was proven by clinical trials with patients with CF-associated bronchiectasis, while the study results in patients with NCFB have not been satisfactory. Inhaled ciprofloxacin agents were the first inhaled antibiotics that showed promising effect in NCFB patient, and the purpose of the present systematic review is to evaluate efficacy of inhaled ciprofloxacin agents in NCFB patients. Therefore, it would be beneficial to use the term "NCFB" to discriminate the study population from the "CF-bronchiectasis". We hope our intention is fully delivered by this explanation.

INTRODUCTION

3. The third sentence of introduction should be mitigated: "exacerbation can produce lung damage progression, qol decrease etc. (not all exacerbations necessarily do it).

R: We agree with the reviewer's opinion. According to the comment, we changed the sentence as following:

"Exacerbation of bronchiectasis can produce damage of lung parenchyme, decreases quality of life, and eventually contribute to increased mortality." (Line 56-58)

4. Please PICOs instead of PICOS

R: According to the reviewer's comment, we changed all "PICOS" to "PICOs".

DICUSSSION

5. The first negative sentence is not appropriate at the beginning of a discussion. Moreover, it is referred to a secondary outcome and I would suggest start highlighting primary outcomes and main results or message of the paper.

R: We appreciate for the precious comment. We agree with the reviewer's opinion. According to the comment 5 and 11, we added the following sentence to the beginning of the discussion section, and replaced the previous initial sentence on antibiotic resistance at the end before limitations.

"The present meta-analysis evaluated clinical efficacy of inhaled ciprofloxacin agents in NCFB patients, individual studies of which were controversial." (Line 213-214)

We tried to explain the background of the review in the first paragraph of the discussion section, and then emphasize the main finding of the analysis in the second paragraph. We believe that this flow may also highlight the main massage of the paper.

6. Ref 23 is probably not appropriate, I would better use a specific paper ref to describe which antibiotics have not reported positive results.

R: According to the reviewer's comment, we cited the references 10-12 instead of the reference 23. In addition, the sentence has been changed to following to comment specific agents:

"While other antibiotics such as tobramycin, colistin, and aztreonam for inhalation had not been proven efficacious in NCFB patients 10-12, inhaled ciprofloxacin agents showed promising results in recent RCTs 10, 12, 14, 15." (Line 217-218)

7. I would mention the statistical differences in primary outcomes between respire 1 and 2.

R: According to the reviewer's comment, we additionally mentioned the statistical differences in primary outcomes between respire 1 and 2 as following:

"Statistical analysis for the two RESPIRE studies were almost same, except that α corrections were differently applied (a significance level of 0.025 for each treatment arm in RESPIRE 1, and 0.049 for the 14-day on/off arm and 0.001 for the 28-day on/off arm in RESPIRE 2) 15." (Line 223-226)

8. When discussing impact of cipro on QoL I would suggest to slightly modify the text as follows: "did not lead improvements in QoL according to used outcome measures (questionnaires)". In particular I would suggest that it is not clear whether the used questionnaires are the best option to capture measure of change related to inhaled cipro.

R: We appreciate for the precious comment. According to the reviewer's opinion, we revised the sentence as following:

"However, while treatment of inhaled ciprofloxacin showed significant improvement in exacerbations, it did not lead to improvements in quality of life according to used outcome measures (questionnaires)." (Line 245-246).

Also, we mention the limitation of questionnaires in the limitation paragraph as following:

"Also, among the secondary outcomes, it is not clear whether the questionnaires objectively reflected the effects of inhaled ciprofloxacin on quality of life." (Line 292-293)

9. When discussing eradication, I would suggest differences in terms of definition of eradication used by the different trials in the analysis. Also, it is important to state that a consensus definition of eradication is not available in Bronchiectasis.

R: We agree with the reviewer's comment. Since the studies of DPI and ILC differ in terms of pre-defined pathogens, we additionally presented target pathogens in the result section:

"Studies of DPI included patients infected with pre-defined respiratory pathogens including P. aeruginosa 13-15, while those of ILC only included patients infected with P. aeruginosa 16, 17. Detailed microbiologic inclusion criteria of individual studies are presented in Supplementary Table 3."(Line 148-151)

Also, according to the reviewer's opinion, we further added the following sentence to the discussion section:

"In addition, it should also be taken into consideration that definitions of eradication vary among the included studies, in terms of evaluation timing and targeted pathogens." (Line 262-264)

10. In general, some discussion should be focused in heterogeneity of results…

R: We agree with the reviewer's comment. Although most analysis did not show statistically significant heterogeneity, there are heterogeneity of included studies in terms of antibiotic formulation and microbiologic inclusion criteria. Concerning this point, we applied random effect model in pooled analyses regardless of statistical heterogeneity in the revised manuscript. We commented this point in the discussion section as following:

"Second, there was heterogeneity of included studies in terms of antibiotic formulation and microbiologic inclusion criteria. Concerning methodological heterogeneity, we applied random effect model in pooled analyses regardless of statistical heterogeneity. In addition, it should be noted that the positive finding of the pooled analysis might not necessarily mean effectiveness of individual agents." (Line 286-290)

11. The initial sentence on antibiotic resistance should be at the end before limitations in my opinion.

R: We agree with the reviewer's opinion and changed accordingly.

We hope the revised manuscript will better meet the requirements of your journal for publication. We would like to thank the editor and the reviewers of the Therapeutic Advances in Respiratory Disease once again for their constructive review of our paper.