Author’s response to reviews

Title: Efficacy of mepolizumab for patients with severe asthma and eosinophilic chronic rhinosinusitis

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Author’s response to reviews:

PULM-D-18-00375
Efficacy of mepolizumab for patients with severe asthma and eosinophilic chronic rhinosinusitis

Dear Editor and Reviewers,

Thank you very much for reviewing our manuscript, “Efficacy of mepolizumab for patients with severe asthma and eosinophilic chronic rhinosinusitis (PULM-D-18-00375).” We greatly appreciate the valuable comments of the reviewers. I attach here our revised manuscript as well as our point-by-point responses to the editor and reviewer comments.

We believe that the manuscript has been significantly improved as a result of your suggestions, and we hope that we have adequately addressed all of your comments.
Thank you in advance for your kind consideration of this paper.
RESPONSE TO EDITOR
We wish to express our appreciation to the editor and reviewers for the insightful comments, which have helped us significantly improve the paper.
Comment #1
Can you describe the statistics that demonstrate goodness of fit for the model?
Why did you include blood eosinophils and FeNO as categorical and not continuous variables?
Response #1

We appreciate the editor's comment.
We included these variables as categorical variables for consistency with previous studies. The cut-off values we applied are based on RCTs (Eosinophil, FeNO: Pavord ID et al. Lancet 651-9, 2012. Appendix) or ATS guidelines (FeNO: AJRCCM, 602-15, 2011).
In this study, we selected six variables that were previously identified as predictive factors or that had p<0.10 in the univariate analyses in this study. However, we also constructed multivariate models with fewer variables. We obtained similar results with the four variables as follows; age (≥ 65) and gender as fundamental factors, ECRS and OCS both which showed p<0.10. The present study represents exploratory clinical research to clarify predictive factors in real life. The number of cases in this study is small, and we hope the results will be validated in a larger cohort in the future.

Based on the editor’s comments, we reperformed the multivariate logistic regression analysis with fewer variables; the results are shown in the revised Table 3.

We have replaced the original text describing the results with the following (p 10, lines 3-6):
“Logistic regression analysis was performed to evaluate potential predictive factors (in a multivariate model), including age (≥ 65 years), gender (male), and other variables that achieved a value of P<0.10 in the univariate models. The cut-off value of FeNO (≥ 50) was applied based on RCTs11 and guidelines20.”
In addition, we have deleted the following from the original text: “, blood eosinophil counts (≥150/mm3) and BMI(≥25kg/m2)” (p 13, line 13).

RESPONSE TO REVIEWER 1
We wish to express our appreciation to the reviewer for the insightful comments, which have helped us significantly improve the paper.
Comment #1
FENO. Did you perform 1 or 2 measurements? It might be worthy to cite the ERS technical standard document on exhaled breath analyses as well.
Response #1
We appreciate the reviewer's comment.
The ATS/ERS statement (ref 17) recommends that repeated, reproducible exhalations should be performed to obtain at least two NO plateau values that agree within 10% of each other and that FeNO be calculated as the mean of two values for online measurement. However, if the duration of exhalation is sufficient and the procedure is performed correctly, NIOX-VERO® does not require repeated assessments. In the present study, experienced technicians measured FeNO and remeasured if necessary.

Based on the reviewer’s comments, we have added the following text (p 8, line 13):
“Experienced technicians measured FeNO and remeasured if necessary.”

Comment #2
I understand that this is a retrospective study. However, a post-hoc power analysis on the primary outcome is still warranted.

Response #2
We appreciate the reviewer's comment.
Since the present study is small in size, a power analysis is warranted. Thus, we performed a post hoc power analysis regarding the following primary outcomes in every group, which are shown in Table 2: daily dose of OCS, change of OCS and exacerbation. The power was over 0.80 for all variables except daily dose of OCS in all patients (0.62) and exacerbation in the non-ECRS group (0.31). In addition, the power was over 0.80 for the change from baseline of OCS dose, shown in Table 4. Therefore, the comparisons of primary outcomes between the ECRS and non-ECRS groups were warranted.

Comment #3
Results. The abstract is clear that one patient has been excluded due to pregnancy. This has to be clarified in the text as well in the Assessment of all patients paragraph.

Response #3
We apologized for the confusion arising from our insufficient explanation.
Based on the reviewer’s comments,
1) we have moved the revised statement “We excluded one pregnant woman who had received only one injection of mepolizumab from evaluation” from “Evaluation of the response to mepolizumab” to “Assessment of all patients” section (p 10, lines 10-11) and
2) we have revised the patient numbers and characteristics in the text and in Table 1.

RESPONSE TO REVIEWER 2
We wish to express our appreciation to the reviewer for the insightful comments, which have helped us significantly improve the paper.

Major comment #1
One of the major concerns is the duration of therapy: it is not clear to me what time points are compared and how long the follow-up of the patients was.
Response #1
We apologized for the confusion arising from our insufficient explanation.
In the original manuscript, we had shown the number of mepolizumab injections and the duration of observation in Table 1 and addressed these data in the text. Because the data of one pregnant woman had been included in Table 1, giving rise to the confusion, we have excluded these data from Table 1. Because this study is a retrospective study, the examination time was not constant among patients except at the time point just before the start of treatment.
In previous RCTs, a significant reduction of peripheral eosinophil count was confirmed after four weeks, and the effectiveness of treatment on pulmonary function and OCS dose were confirmed after four to eight weeks. However, some studies found no significant differences from placebo.
Because we evaluated most cases after eight weeks in the present study, we considered it appropriate to evaluate and confirm response to treatment.

According to the reviewer’s comment, we have added the following sentences to “Assessment of all patients”:
“The median number of mepolizumab injection was nine (range 2-17), and there was no significant difference between the ECRS and non-ECRS groups. The median duration of observation was 11 months (range 4-17), and there was a significant difference in observation duration between the two groups” (p 10, lines 14-18).

Based on the reviewer’s comments, we have revised the patient characteristics in Table 1 and have added the following text to the footnote in Table 2:
"Blood eosinophil counts at last follow-up were examined at three months (median, range 1-6). Serum IgE tests at last follow-up were examined at six months (median, range 2-6). FeNO tests at last follow-up were examined at nine months (median, range 1-12). Asthma Control Test (ACT) score at last follow-up was examined at six months (median, range 2-12). Pulmonary function tests at last follow-up were conducted at four months (median, range 2-10)."

Major comment #2
Were the two groups comparable? The group with ECRS seemed to have more eos and a lower FEV1/FVC than the group without ECRS. So did they have more severe asthma? Also this group contained 4 patients with EGPA. Could this have influenced the results?

Response #2
We appreciate the reviewer's comment.
It was previously reported that the asthma patients with ECRS had severe symptoms and low pulmonary function. In the present study, four patients with EGPA were in the ECRS group; however, only one of them required OCS maintenance therapy. Not all cases involved low pulmonary function, and there was no significant difference in ACT or exacerbation among levels of asthma severity. Therefore, we considered the two groups comparable.

Major comment #3
Although it is a retrospective study, new privacy guidelines require informed consent to publish patients results. In particular since the number of patients is really low and individual patients could be identified in the study.
Response #3
We appreciate the reviewer's comment.
The ethical committee of our institution stated that patient consent was not necessary for this retrospective study. (We have added this information to Ethics approval and consent to participate.) This determination was based on the ethical guidelines for medical and health research involving human subjects in Japan. Some of the data were specific to a few cases, but we took care to ensure that patient anonymity was preserved.

Minor concerns:
Comment #1
Why was ACOS mentioned in the methods section? I see no need for this.

Response #1
We appreciate the reviewer's comment. Based on the reviewer’s comment, we deleted the text regarding ACO from the methods section.

Comment #2
Line 2 page 16 (discussion) needs to be rephrased.

Response #2
We apologized for the error. We have replaced “were not responded” with “did not respond.”

Comment #3
It is not clear to me why the authors spend quite some space in the discussion on the role of FeNO in anti-IL5 treated patients. This could be shorter.

Response #3
We appreciate the reviewer's comment. It has been reported previously that anti IL-5 therapy does not decrease FeNO level, and we thought it relevant to attempt to explain this phenomenon. We have deleted several sentences and replaced them with the following: “Eosinophil-derived major basic protein (MBP) injures epithelial cells, which activates the innate immune system. Furthermore, group 2 innate lymphoid cells (ILC2s) and eosinophils release large amounts of IL-4, 5 and 1328,29. Hence, it is possible that anti-IL-5 therapy decreases peripheral blood and tissue eosinophils and indirectly inhibits the IL-4/IL-13 pathway, reducing serum IgE and FeNO levels.” (p 16, lines 2-7)

Comment #4
I see no information about ICS/LABA use in these patients. This should perhaps be added to baseline characteristics.

Response #4
We appreciate the reviewer's comment. All patients received ICS/LABA, and the ICS dose was 963µg (222) (400-1600, fluticasone propionate
or equivalent). Thirteen patients (48%) received LAMA, 23 (85%) received LTRA and 17 (63%) received xanthine derivative.

Based on the reviewer’s comment, we have added information regarding baseline therapy to Table 1 and to "Assessment of all patients” in the results section as follows:

“Initial treatment at baseline is shown in Table 1. All patients received ICS/LABA, and 25 patients (93%) received high-dose ICS (≥ 800μg, fluticasone propionate or equivalent). There was no significant difference regarding initial treatment between the ECRS and non-ECRS groups.” (p 10, lines 18-p 11, lines 3)