Reviewer's report

Title: The effect of the novel phosphodiesterase-4 inhibitor MEM 1414 on the allergen induced responses in mild asthma

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Reviewer: Gail Gauvreau

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Comments for the Authors:
Data showing effects of PDE4 in the allergen challenge model have already been described in at least 4 other publications which show a similar magnitude of protection on the LAR, so this observation is not novel. This study adds some mechanistic information showing PDE4 administration attenuates LPS-stimulated whole blood assays for TNFα at 8 hours and LTB4 at 24 hours post-challenge.

Major Compulsory

1. The abstract indicates measurements of blood or FeNO are made at 4h post-challenge, however this is not mentioned in the methods or results in the body of the manuscript.
2. Abstract - why was a cut-off of p<0.08 chosen for LTB4 at 24 hours? Suggest inserting actual p-value if it is not < or = 0.05.
3. Study Design 1st paragraph - Washout period. Please confirm if minimum washout was 2 weeks (2nd sentence) or 3 weeks (same paragraph, 2nd to last sentence).
4. Figure 1 indicates subjects were clinically stable at the start of each treatment period. How was it determined whether all subjects had washed out from allergen (ie: had returned to baseline airway hyperreactivity and inflammation) after the screening before the start of the 1st treatment period, and before the start of the 2nd treatment period? Figure 1 shows a methacholine at visit A1 and metacholine (please correct spelling) at Visit B1, which is not mentioned in the text. If stability was assessed by methacholine and/or FEV1, what value was considered to be “baseline” to be eligible to continue into the treatment period?
5. Considering controversy about what FeNO is actually measuring, why didn’t the authors directly measure airway inflammatory cells?
6. Methods. The authors state that the potency of MEM 1414 was quantified as the EC50. Why is potency of drug measured in ex vivo expts if drug is given in vivo? The methods do not indicate what experiments were performed to measure MEM 1414 EC50. Was the EC50 for MEM 1414 calculated from LPS-induced TNF-alpha measured from serum PK samples, or were separate in vitro studies conducted to measure the EC50 for MEM 1414 for these outcomes? The only EC50 reported in the results appears to be for LPS concentrations. Please clarify the MEM 1414 EC50 measurement/results.
7. Methods. LPS challenges - The authors indicate that “Cytokine data (TNF#, IL-6, LTB4) were collected in each treatment period at the following times: post-dose/pre-allergen challenge and 3 and 8 hours post allergen challenge on day 6 and at 24 hours post-dose/post-challenge on day 7.” Please reword this to clarify that blood was collected at these time points for ex vivo measurements of LPS-stimulated cytokines. The current wording is somewhat obscure.

8. The authors state that measures of AHR were underpowered. What about the other discrepancies, as others have found significant inhibition of EAR, airway inflammation. Please discuss these differences. The current study shows no separation at all of EAR and FeNO between the two treatment arms.

9. Discussion, first sentence. The authors state that MEM 1414 is comparable in magnitude of effect to other oral and inhaled anti-inflammatory agents. This sentence is not true. Please clarify that MEM 1414 is comparable in magnitude of effect on LAR as compared to other PDE4 inhibitors, but not effective in reducing other outcomes as compared to other PDE4 inhibitors. MEM 1414 is certainly not comparable to other anti-inflammatory agents such as ICS (see refs 21 and 22) and anti-leukotrienes. This sentence should be adjusted accordingly.

10. Discussion para 5. “Studies using inhaled corticosteroids have shown both attenuation [18, 22, 23] and no attenuation [20] of methacholine reactivity post allergen challenge.” Reference 20 is not the best reference since ICS was administered after challenge.

11. Discussion 6th para, 2nd sentence. This statement is quite controversial as FeNO does not always reflect asthma control by ICS in all phenotypes of asthma.

12. Figure 5 – other studies have reported increases in FeNO during the LAR. Can the authors comment on why this increase was not observed in the current study? Did allergen induce a significant increase from baseline in the placebo arm at the 24h time point? It is unclear whether allergen challenge caused any change in FeNO. If no increase was observed, one would be unable to assess drug inhibition of a “non-response”.

13. Figure 5 - If FeNO was log-normalized for analysis, it would be best to plot on a log y-axis.

14. Figure 6 – is it necessary to show all concentrations of LPS or is there a way of plotting which illustrates the EC50, since the EC50 values are not reported in the results.

Minor Essential Revisions

1. Discussion 3rd para, first sentence should be divided into 2 sentences.

2. Discussion 6th para, 1st sentence “Reducing FeNO levels by specific iNOS inhibition does inhibit the EAR or LAR”. Should this say “does not inhibit”?

3. Table 1 could be summarized in 2 sentences in the results.

4. Table 2 is not needed to show this negative result. The difference and p-value could be reported in the result section.

5. Figure3 – asterisks are missing from the figure.
6. Figure 4 not needed. Could simply report as not significant in the text.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.