Author Response 1

We sincerely thank the Reviewers for helpful suggestions and comments, which we have incorporated in the revised manuscript with yellow highlighted text. The following is our Point-By-Point response with bulleted text:

Reviewer: 1

Comments to the Author
This study investigated the possibility to make simvastatin an inhalable drug for use against pulmonary hypertension and possibly other pulmonary conditions. It is an interesting study, but I have some concerns:

Major:
1. It can't be stated in the paper that it shows that "inhaled Sim is safe and effectively and efficiently treats PH". This statement is much too strong. You have problems with cell toxicity, and you can't state that you have found efficient treatment just because you see a difference in response to Ach.
• As suggested by the Reviewer, the revisions have been made.

2. I couldn't understand from the text for how long the rats were treated. Was it just one dose or repeated doses? It says 10 mg/kg/day. Would it be possible to show in this model how far out in the lung the particles reached? This would strengthen the paper significantly.
• As suggested by the Reviewer, the revision has been made.
• Depth of lung penetration of the inhaled particles in vivo was beyond the scope of this focused and comprehensive study. That would be a different study design for a in vivo separate study.
• The in vitro aerodynamic dispersion performance testing using the NGI clearly shows by quantification that these particles have the aerodynamic size needed to reach the deep lung respiratory region.

3. When was that BALF taken (how long after inhalation)? How many repeats of the ELISA for each rat? What is shown in the graph, one value for each rat? Please indicate in the figure whether the differences were significant.
• As suggested by the Reviewer, the revision has been made.

4. The histology needs to be described in much more detail. How many sections were analyzed for each rat lung. How was inflammation etc evaluated? Blinded observer? How long after inhalation was the tissue taken out? Enough time to see possible damage or inflammation? It is not clear to me what you would like to show in the images in Fig 12 b. Why not show high magnification images of Sim inhaled lung vs control?
• As suggested by the Reviewer, the revision has been made.
• Yes, there was a blinded observer.

5. Not enough data is shown from the lamb model. Absolute pressure and PVR values should be shown and not only relative change (for both non-treated and treated controls and non-treated and treated lambs with PH). Would also be good to show this as scatterplots for the reader to see values for individual animals. I guess you also have a lot of other parameters, like for example systemic blood pressure and saturation. Why not show this?
• As suggested by the Reviewer, the revision has been made.

6. Could you please comment on the drug concentration you expect to get in the lung? How close is it to the levels when you see toxicity in cell culture? How close is it to what would been seen with systemic administration?
• A mass of 3-4 mg of simvastatin was delivered as inhalation aerosols in our in vivo rat study which is in the concentration region where in vitro cell viability was maintained.
• Systemic administration was not studied and was beyond the scope of this study. A different study design would be needed for systemic administration as a separate study.

Minor comments:
1. Figure 1: Not so well organized. Difficult to see the what the magnification is (the text is so small) and the scale bars are too small to see clearly. Would be better to show fewer images and to clearly mark what you would like to point the readers attention to.
• As suggested by the Reviewer, the revision has been made for reader clarity.

2. Figure 4: Why show temperatures so close to each other for a (133 and 139) and different values for b (94 and 129)? Are the images comparable when the temperatures are so different? You mention that Raw Sim showed some thermal events before the main thermal event. Can you indicate this in the images? Is it possible to see?
• HSM is for observation not for quantification of phase transition temperatures which is done by DSC, since DSC is adiabatic while HSM is not adiabatic. HSM is used to visualize birefringency and melting, as stated in the text, which is a characteristic of crystallinity. It is not to determine phase transition temperatures.

3. Figure 7. Would be better to have the same scale on the y-axis for a and b.
• The same scale makes it much smaller and unclear to observe the profile and individual trends. Hence, the original scale is maintained which maximizes clarity for the reader.

Reviewer: 2

Comments to the Author
The authors have used healthy rats to test the safety of their aerosol formulation, and the lamb model to test the efficacy of the treatment. What about the usage of some well-known chronic models of pulmonary hypertension, such as for example monocrotaline model in rats or chronic exposure to hypoxia? I would like to see the efficacy of this aerosol formulation in one of those in vivo models. If the authors are not able to perform the suggested experiments they should clearly discuss this point and indicate this as a limitation of the study and future direction of the research.

• As suggested by the Reviewer, the revision has been made by adding some text at the end of the Discussion section.
• We have done the MCT-rat model of PH in other studies, but we have not done MCT-rat PH rat model in this study since we had the large animal model of PH in the validated lamb model. We are not able to perform the MCT rat model at this time, as it would need to be a separate study with a different study design outside the scope of this focused study.