

List of Required Correction to Thesis: Laura Bartlett

1. Include more detailed description of lung structural changes across parenchymal and airway compartments in COPD (Chapter 1)

As extracellular matrix remodelling and the role of epithelial cells in COPD are covered by the introduction, I have added a section between these two to cover changes to lung structure (Section 1.1.3.3). In particular, I have focused this section on changes to the structure of lung parenchyma and vascular compartments which are known to contribute to inefficient gas exchange and inflammatory microenvironments within the lungs in COPD patients. I believe that this new section provides rationale and justification for the histological analysis performed in chapter 5.

2. Include section discussing the interplay of various cell types in the pathophysiology of COPD (Chapter 1)

I have added a section discussing the interplay of immune cell types in COPD pathophysiology (Section 1.1.4.9). I have focused this on the interplay between neutrophils and macrophages, T cells, macrophages and neutrophils, and DCs and T cells, given that the interplay between T cell subsets is discussed in a previous section.

3. Expand the section on the 3Rs to give accurate descriptions of each R, relating back to current models of COPD (Chapter 1)

This section has been expanded to provide further examples of each R in the context of COPD research, in particular focusing on examples of where each R may be used in a model system and highlighting areas of improvement for each R in COPD research.

4. Clearly define in the aims which R the proposed new model aims to address (Chapter 1)

The discussion of the aims of the PhD have been changed to reflect the fact that the project is designed to focus upon refinement of COPD model systems, and all references to other Rs have been removed.

5. Amend table 2.1.1 to include which cell type the marker is detecting (Chapter 2)

This table has now been updated to provide full details of which cell type each marker is for.

6. Include a detailed section on data and statistical analysis including approaches taken to minimise bias (ARRIVE guidelines) (Chapter 2)

Small sections on data analysis and statistics were included at the end of each section of the methods depending on techniques (e.g. flow cytometry, RPPA, RT-qPCR and histology). These sections have now been moved to a more detailed section dedicated entirely to the

discussion of data and statistical analysis (Section 2.2.6). A section has also been added at the beginning of this section to discuss approaches taken to minimise bias according to ARRIVE guidelines for randomisation and blinding, as well as a section on power calculations for determining group sizes using the pilot study data.

7. Reword figure legends in chapter 3 to better clarify what a negative control is and remove reference to positive controls where a positive control has not been included (Chapter 3)

Negative controls have been clarified in figure legends (dH₂O, no template control (NTC) and no reverse transcriptase (NRT)) and all references to positive controls have been removed. These are now referred to as “samples”. I have also noticed that there is a typo where primers for NRF2 are mistakenly referred to as NOX2, which is discussed in a previous section of the same chapter. I have therefore corrected this to ensure clarity of which primers are being discussed.

8. Describe which PCR primers demonstrated non-specific binding and make clear that these primers will not be used in future experiments due to their non-specificity (Chapter 3)

I have added a short concluding section to the second to last paragraph of the discussion of chapter 3 to state that primers for TLR4, IRF3, MMP9, MMP28, Akt1, mTOR, Serpina1a, CYLD, NFKBIA, CRP and NRF2 were not used for future experiments due to nonspecific binding due to the presence of consistent peaks at the wrong temperature. I have justified the use of other primers as they either displayed no nonspecific binding or minimal instances of nonspecific binding which could be justified as human or experimental error.

9. Figure 3.3.12: add details in the legend of what this stain is (Chapter 3)

The figure legend has been updated to include the details of the staining method used.

10. Include error bars or individual data points to all longitudinal data as well as statistical analysis (Chapter 4)

Error bars have been added to all longitudinal data. Statistical analysis was not performed on longitudinal data due to a large number of variables (time, sex, age, CSE dose) in addition to low and variable cell numbers between individuals and weeks, and therefore is not included in figures. As the clustering analysis allowed us to isolate data based on these variables and standardise event counts, statistical analysis was performed and I have updated these figures to reflect this.

11. Include event count number or range in all flow cytometry figure legends (Chapter 4)

All figure legends now include the event count range for longitudinal data, and exact count number for clustering analysis.

12. In the introduction section of Chapter 4 there is an exceptionally long paragraph discussing individual immune cells. Cut this down into individual paragraphs on each immune cell type to improve readability (Chapter 4)

This paragraph has now been separated into smaller paragraphs per cell type to improve readability.

13. Remove all RT-qPCR data from primers that showed non-specific binding in the validation chapter as the results are meaningless when the melt curves show more than one peak (Chapter 5)

All irrelevant RT-qPCR data has been removed from this chapter, and chapter 6 has also been updated to reflect this.

14. Add a paragraph to the discussion section focused on biological vs statistical significance following our discussions in the viva (Chapter 5)

I have added a section to the discussion to discuss biological and statistical significance and highlighted that while statistical significance is observed in reported data, it would be unwise to conclude biological significance given the small sample size and the fact that MLI analysis was conducted on collapsed alveoli. I have also edited the wording of the concluding paragraph in the discussion to suggest that further work is required before the model can be concluded as successful in encapsulating COPD in mice. I have also reworded sections of chapter 6 to reflect this, so that it is evident that further work is required in order to refine and validate the model.

15. Add a section to the discussion highlighting the limitations of performing mean linear intercept measurements on collapsed alveoli and that more accurate measurements are taken from lungs inflated/insufflated (Chapter 5)

I have added a section to the discussion highlighting the limitations of performing MLI on collapsed alveoli and suggested that repeats are necessary on inflated/insufflated lungs to determine a more accurate estimation of MLI and degree of airway remodelling.

16. Add either error bars or individual data points to all data (Chapter 5)

Error bars and individual data points have been added to all data.

17. Perform picosirius red staining on lung tissue sections to assess fibrosis/remodelling due to the inability of H+E staining to detect these features and amend the results and discussion sections in chapter 5 accordingly (Chapter 5)

Picrosirius red staining has been performed and analysed, and chapter 5 has been updated accordingly. Materials and methods for this technique have also been added to chapter 2, and chapter 6 has also been edited to include this data and remove all reference to airway remodelling based on H&E staining.

18. Include a table in the future directions section of Chapter 6 showing the results of a power calculation using your data to determine group sizes in future experiments (Chapter 6)

I have added a table as part of the future directions to provide evidence of suggested group sizes for all future experiments. Given the wide variety and quantity of data, this was performed using three hallmark characteristics of COPD: MLI analysis, neutrophil counts and total peripheral immune cell counts following 12 weeks of sham/CSE exposure. RT-qPCR data was not used for the power calculations due to concerns raised regarding the specificity and validity of primers as discussed during the viva. A power calculation was not performed using longitudinal data due to the complexities of performing statistical analysis on repeated measures. The section regarding power calculations in the materials and methods chapter (Section 2.2.6.7) summarises the methods and approaches used to perform the analysis.