Alexandra Weissman
IDM 2025

Comments to the Editors:

Thank you for the opportunity to review this manuscript. Briefly, this is an original research article detailing the development of an improved liposomal encapsulation of polysaccharides (LEPS) vaccine for pneumococcal pneumonia that builds upon prior work from these authors. The authors highlight some significant problems with the current pneumococcal pneumonia vaccines, namely coverage gaps and off-target effects, and demonstrate a new vaccine approach to ameliorate these issues. The LEPS vaccine detailed in this study has an expanded pneumococcal capsular polysaccharide (CPS) valency and incorporates a higher dose of the surface-displayed virulence-associated protein PncO while eliminating the surface-displayed virulence-associated protein GlpO in order to reduce un-targeted microbiome effects related to GlpO.

The abstract appropriately summarizes the article. The introduction is concise but adequately addresses the burden of disease, highlights problems with existing vaccines, and explains the premise of the current study. Due to the exploratory basic science nature of the study, sampling is limited to mice models. The statistical plan needs to be expanded upon so that proper interpretation of the results is possible. The results are well-written and the corresponding figures are relatively easy to understand. The results pertain to the research question seeking to develop a "best-in-class" pneumococcal vaccine that minimizes off-target antigentic effects and optimizes coverage of vaccine and non-vaccine serotypes.

Main Criticisms/Questions:
It is difficult to discern from the manuscript and figures whether the results are truly significant. More rigorous statistical testing beyond simple 95% confidence intervals, such as a t-test or Mann Whitney depending on normality of the sample, is necessary to fully interpret the results. Specifically, where differences between vaccine types should, or should not be, noted to be statistically different then this needs to be demonstrated.

The authors should state that this is a small, exploratory study and that the data do need to be validated in a larger cohort.

Recommendations:
This is an exploratory basic science evaluation in mice of an expanded LEPS vaccine for pneumococcal pneumonia. The results of this study have a clear and important clinical correlate, they add to the existing literature, and they are worthy of publication. However, interpretation of the results is limited by the lack of rigorous statistical analyses. Incorporation of further statistical analyses to demonstrate any potential statistical differences between vaccine types, displayed for each figure, will improve the research presented. The authors should be clear that these experiments are exploratory, the sample size is small, and further validation in a larger cohort is needed. Following these revisions, the manuscript should be accepted for publication.

Comments to the Authors:

Thank you for the opportunity to review this manuscript. Briefly, this is an original research article detailing the development of an improved liposomal encapsulation of polysaccharides (LEPS) vaccine for pneumococcal pneumonia that builds upon prior work from these authors. The authors highlight some significant problems with the current pneumococcal pneumonia vaccines, namely coverage gaps and off-target effects, and demonstrate a new vaccine approach to ameliorate these issues. The LEPS vaccine detailed in this study has an expanded pneumococcal capsular polysaccharide (CPS)

valency and incorporates a higher dose of the surface-displayed virulence-associated protein PncO while eliminating the surface-displayed virulence-associated protein GlpO in order to reduce un-targeted microbiome effects related to GlpO.

Major Comments:
The results and corresponding figures are clear, but more rigorous statistical testing beyond simple 95% confidence intervals, such as a t-test or Mann Whitney depending on normality of the sample, is necessary to fully interpret the results.

Please add to your discussion/conclusions the limitations of this study: small, exploratory study and that the data do need to be validated in a larger cohort.

Minor Comments:
Figure 4b could be clarified. I assume that the dose-color scheme for 4a applies to 4b, but I should not have to make this assumption.