Comparing lipid nanoparticle mediated delivery of Cas9 mRNA and protein for cystic fibrosis CRISPR gene editing

Ryann A. Joseph[1], Rebecca M. Haley[1], Adele S. Riccardi[1], Marshall S. Padilla[1], Michael J. Mitchell[1]

[1]Department of Bioengineering, University of Pennsylvania

Introduction

- Lipid nanoparticle (LNP) mediated delivery of nucleic acids is a potent technology used for applications including gene editing, protein treatments, vaccines, and more
- LNP-encapsulated CRISPR-Cas9 protein can perform successful gene editing along with a guide RNA (sgRNA) and corrective editing with donor DNA (ssDNA)

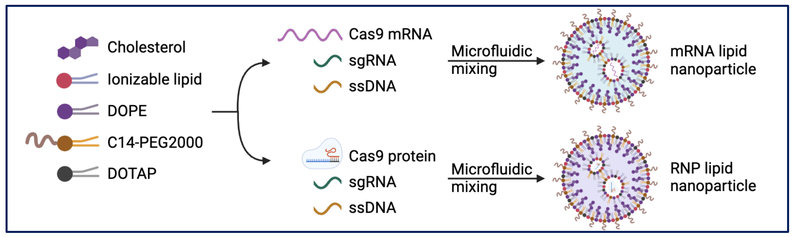

Figure 1. Cas9 protein or mRNA are encapsulated with corresponding nucleic acids alongside lipid excipients and the ionizable lipid of interest

- Cystic fibrosis, an autosomal recessive genetic disorder, causes malfunction in the Chloride ion flux in the airway surface liquid interface. The main organ affected by this disorder is the lungs.
- CF is a valuable therapeutic target for LNP gene editing due to the ability for LNPs to traffic to specific organs intravenously.

Methods

Figure 2. Cas9 protein or mRNA are encapsulated with corresponding nucleic acids alongside lipid excipients and the ionizable lipid of interest

- LNPs were formulated with either Cas9 protein or mRNA (Fig 1) with previously elucidated lung-tropic excipients and varying ionizable lipid
- Editing efficiencies were evaluated with flow cytometry and sanger sequencing of genomic DNA (Fig 2), and organ tropisms were analyzed with IVIS imaging.
- All statistical analyses were performed and graphs were created with GraphPad Prism 10. Organ images were analyzed with Living Image, figures created with BioRender.

Results and Discussion

In vitro screen of EGFP → BFP knock-in

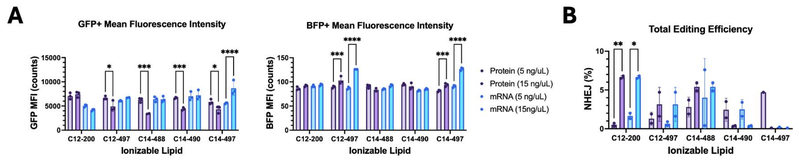

Figure 3. **A** GFP+ and BFP+ mean fluorescence intensities (MFIs) from flow cytometry **B** Total editing efficiency of all formulations and doses.

In vivo biodistribution screen

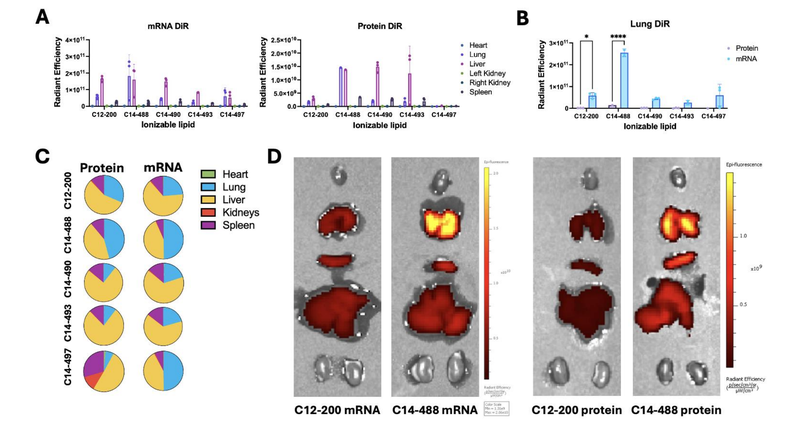

Figure 4. **A** Radiance efficiency of DiR in mRNA and protein formulations in all organs (heart, lung, liver, kidneys, spleen) **B** Radiance efficiency of DiR in lungs between protein and mRNA formulations **C** Representative charts for organ tropism proportions **D** Images of organ fluorescence for mRNA and protein top and base particles.

In vivo screen of functional editing in Ai9 mice

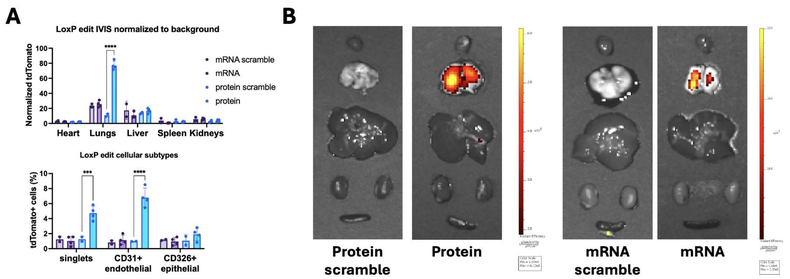

Figure 5. **A** Normalized Radiance efficiency of TdTomato+ in organs and % TdTomato+ cells in cell subtypes **B** Images of organ fluorescence for mRNA and protein scrambled control and top performer at same radiance efficiency scale.

In vitro corrective editing for f508del mutation

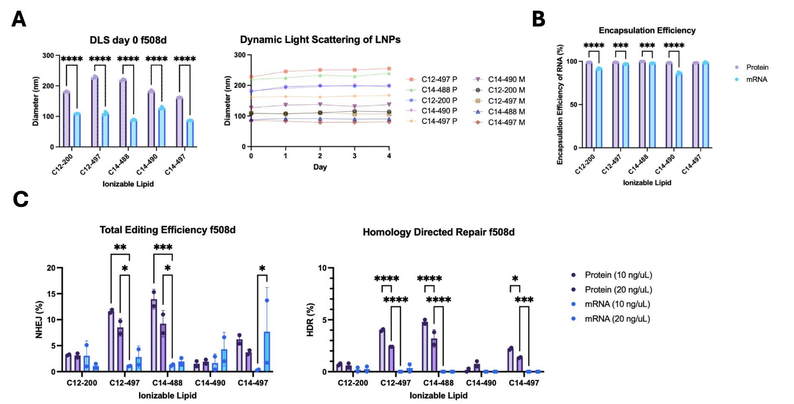

Figure 6. **A** Dynamic light scattering data for all protein and mRNA formulations **B** Encapsulation efficiency of total RNA **C** Total editing efficiency in all formulations and homology directed corrective repair in 16HBE-f508d cells.

Conclusions

- LNPs formulated with either Cas9 protein or mRNA were able to exhibit overall editing and corrective editing in reporter cell lines (H1299) and therapeutically-relevant lines (16HBEge), and protein formulations outperformed mRNA formulations.
- Formulations exhibited high lung-tropism when administered intravenously, with top performer C14-488 outperforming industry standard C12-200. C14-488 particles showed high editing capability *in vivo*, and protein formulations outperformed mRNA formulations.
- Further investigation can be done to optimize formulations for higher editing efficiencies. Additional next steps include performing CFTR-specific functionality assays.

Acknowledgements

This work was funded by the AWE Program, Center for Undergraduate Research and Fellowships under the Jumpstart for Juniors program at the University of Pennsylvania and the Cystic Fibrosis Foundation through the Mitchell Lab.