

Utilization of Lipid Nanoparticles to Show Proof-Of-Concept of Gene-Editing of Lung Cells for Future Studies Regarding Cystic Fibrosis

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Acknowledgements:

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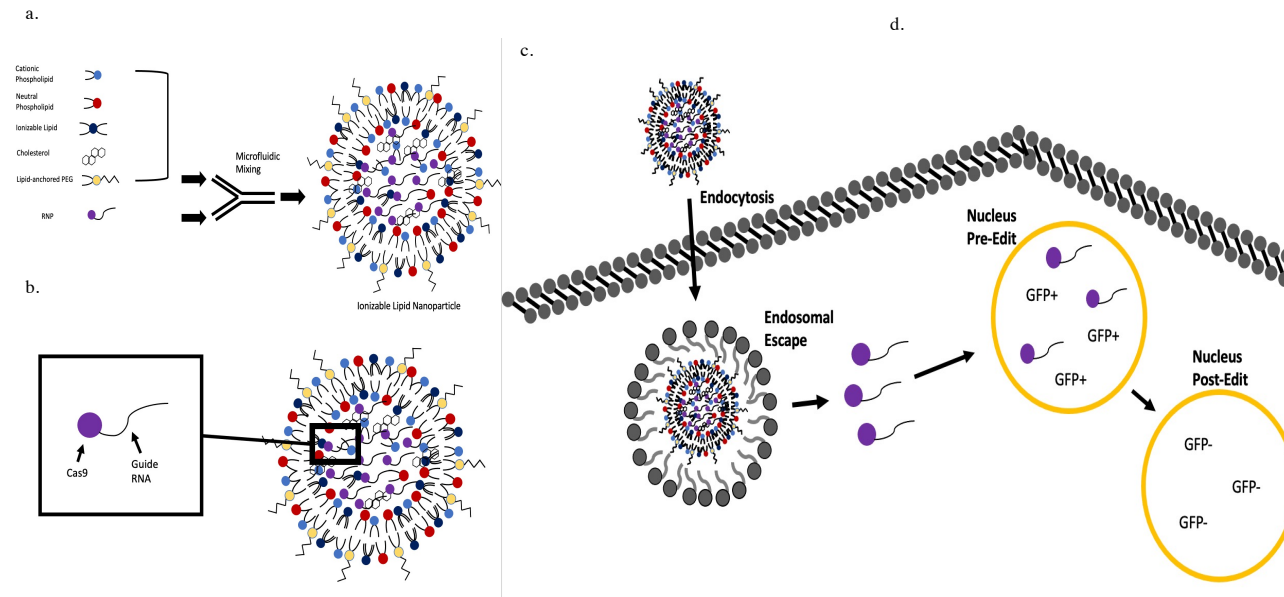
Background

- Cystic fibrosis (CF) is a genetic disorder; alters functionality of cells that produce mucus, sweat and digestive juices
- CF decreases life expectancy by ~30 years
- CRISPR Cas9 gene editing technology; complex of Cas9 and guide RNA
 - Guide RNA encodes for DNA needing to be edited out
 - Cas9 cuts out DNA strand
- Lipid nanoparticle technology
 - Encapsulating cargo
 - Transfecting cell
- Toxicity of gene editing

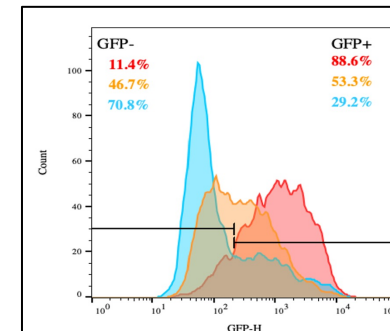
Goals

- Show proof-of-concept that lipid nanoparticles can transfect and edit in lung cells
- Optimize lipid nanoparticle formulation to maximize efficacy & minimize toxicity

Methods & Results



	DLS - Zavg	DLS - PDI	Zeta Potential		Nano Drop
Average	98.26 nm	.147	22.4	Concentration (ng/μl)	72.16
St. Dev.	0.81	0.002	1.082	Ratio	2.05



Mean : GRN-B-HLin	SampleID	\$WELLID
634	RNP LNPs	B04
576	CRISPRmax	B08
1903	control	A01

Discussion

- A basic, non-optimized LNP formulation was used to show proof-of-concept
- GFP expressing human non-small cell lung carcinoma cell line was used
- Ability to transfect and edit human non-small cell lung carcinoma cells shows proof-of-concept that the LNPs can edit lung cells – where CF gene is present
- 98.26 nm is large compared to LNPs encapsulating RNA due to the larger size of the Cas9-guideRNA cargo
- Positive Zeta potential, according to SORT, allows LNPs to navigate to the lungs
- Commercial agent CRISPRmax had a higher editing success rate than the LNP formulation
- CRISPRmax shown to be toxic to cells

Future Work

- Optimize LNP formulation
- Work with different lung cell lines to ensure functionality