Lipid Nanoparticles for Gene Editing to Correct Cystic Fibrosis

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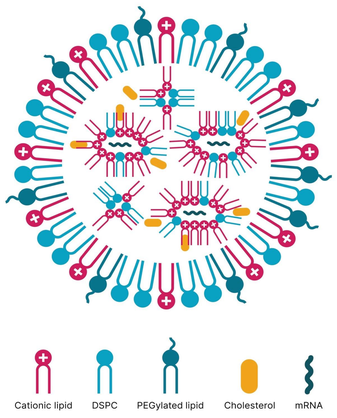
What is an LNP?



EH_1_T48	Molar ratio (%)
MC3	50
DOPE	5
cholesterol	28
DPSC	15
PEG-DSPE	1.5
GM3	0.5

EH_2_T48	Molar ratio (%)
MC3	49.5
DOPE	6
DC-Cholesterol	28
DPSC	15
PEG-DSPE	1.25
GM3	0.25

Image 1:
Each component of a LNP has its own function for determining efficacy. The ionizable lipid, typically cationic, ensures proper nucleic acid encapsulation. The helper lipid ensure stability for fusion. The cholesterol provides mechanical strength during circulation to target. The PEG lipid is key for reducing aggregation.

Introduction

Lipid Nanoparticles (LNPs) present a brand-new avenue for gene-editing capabilities through taking advantage of the diffuse nature of the low-density lipoprotein receptors (LDL-R) across all labile tissues. This development is ushering in a new era in medical treatment. Traditionally, gene editing is achieved through physical or viral delivery methods, but the limitations come in less efficient and quick degradation. LNPs optimize safety and delivery through being composed of natural components which means lower risk in causing unwanted immunogenicity or mutation; additionally, LNPs can be modified substantially to cause a more specific target location. Our group has rationalized to apply the LNP technology to the genetic condition of cystic fibrosis.

Ex Vivo Results

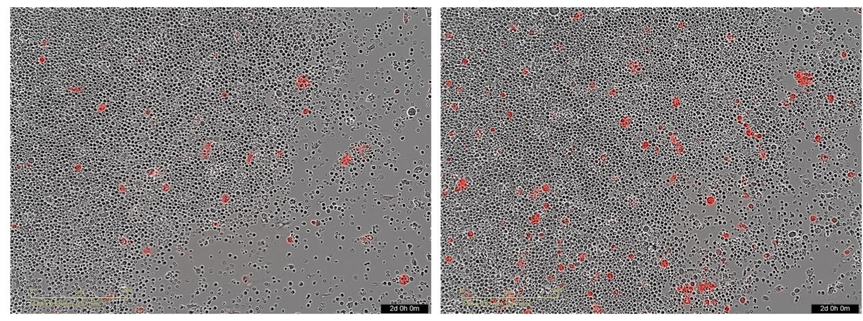
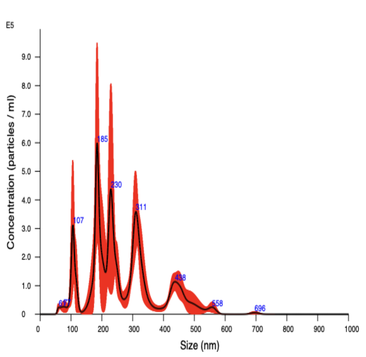


Figure 2:
The EH_1 composition includes GM3 ganglioside at a concentration of 0.5% of total LNP composition yielded 1.02% expression of tdTomato in live bone marrow cells. Lowering the dosage of GM3 to 0.25% (EH_2), the results showed 1.76% of tdTomato expression in the live cells. Ideally, out results aspired to show a 40:1 ratio of nitrogen to phosphate to ensure proper encapsulation.

Results - Measurements



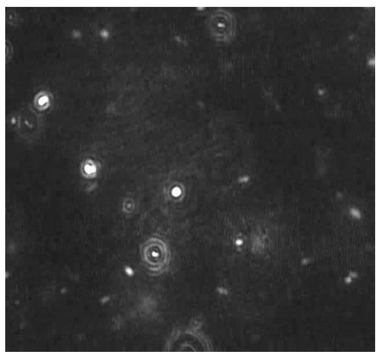


Figure 1a and 1b:
Ideally, LNPs operate best with a diameter of <500 nm, and from the utilization of a NanoSite measurement device. Our current formulations show a mean size of 267.0 nm ± 112.3 nm as a standard deviation. The size is best approximated for widespread delivery as larger sizes demonstrate less efficacy in circulation.

Materials and Methods

Our formulations for lipid nanoparticles came from utilization of multiple gangliosides, natural lipid components, that can be varied with the other components of an ionizable lipid, cholesterol, amphipathic phospholipid, and a polyethylene-glycol component. We have characterized 4 different compositions that target 2 different tissues: hemopoietic stem cells. To test the application, we have bred tdTomato mice, in which the red fluorescent protein expression requires Cre recombinase (Cre) to cut out the stop codon before the gene. For ex vivo experiments, we will extract the bone marrow cells from the mouse tibia and femur. LNPs packed with the Cre mRNA will be added to the cells and cultured for 24 and 48 hours. Then fluorescent cell percentage will be determined via flow cytometry. For in vivo experiments, the formulated LNPs packed with the Cre mRNA will be injected into the tdTomato mice via I.V. administration. Tissues and cells with fluorescence will be examined. Furthermore, our group decided on the utilization of gangliosides (GT1b and GM3) to be included in the composition due to their natural applicability to native tissues which present a new horizon for reduction of an immune system response.

In Vivo Results

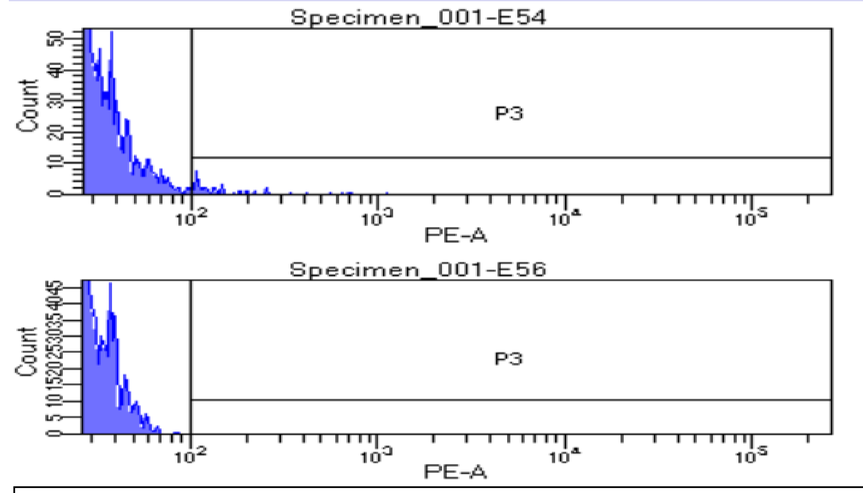


Figure 3a and 3b:
For the diagrams shown above detail a control (E56;3b) and applied to mouse stem cells (E54;3a), the results show for our control no level of statistically significant levels of expression. For applied cells (E54), there were 51 events of activity from distinguished Population 3 and a Parent cell percentage of 0.5. These results were collected via application of the listed compositions and measured via flow cytometry.

Conclusion and Future Directions

Ideally, LNPs present a new horizon concerning how we deliver gene-editing therapies. Confirmation of the devised compositions will confirm the experiments to move to the in vivo phase which can present more details about the intracellular effects as a therapy. In a more longitudinal aspect, we hope to develop a prime editing mechanism for application to correct *G542X* for CF, a premature termination mutation to which there is no effective modulator therapy. From the preliminary confirmations of our group's LNP compositions, we decided to move ahead with in-vivo experimentation on neonatal and adult mice with the inclusion of GT1b ganglioside with the retained composition The biggest horizon for our group is to intermingling of the developed *G542X* genome and the ganglioside usage for more widespread application, especially for epithelial tissues, for treatment of systemic CF disorders.

Acknowledgement and Citation for Image 1: Malvern Panalytical. (2025). *Lipid nanoparticle formulation*. Lipid nanoparticle formulation | Malvern Panalytical. https://www.malvernpanalytical.com/en/industries/biologics/lipid-nanoparticles