Developing LNPs for Pediatric Acute Myeloid Leukemia through Two Biological Mechanisms

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Introduction

Pediatric acute myeloid leukemia (AML) is the second most prevalent cancer in children with a 70% survival rate and a 25-35% chance of relapse.[1] Standard-of-care for pediatric AML includes chemotherapy and bone marrow transplantations.[1] There is a need for new therapeutics that are effective and safer than current options. In this project, we are developing a nanoparticle-based therapy for gene regulation in pediatric AML. Towards this goal, we are developing lipid nanoparticles (LNPs) to deliver therapeutic nucleic acids to AML cells to halt disease progression. LNPs are a powerful delivery method due to their ability to encapsulate and deliver nucleic acids with high safety profiles and translational potential. Here, we developed LNPs to deliver Wilms Tumor 1 (WT1) and β-Catenin siRNA to AML cells. WT1 is a transcription factor that is often mutated in pediatric AML and causes increased cell proliferation and survival.[2] β-Catenin is often overexpressed in AML and drives cancer progression.[3] We screened an established ionizable lipid, and our novel ionizable lipids, complexed into LNPs to identify formulations with high delivery. Using our top formulation, we have successfully delivered WT1 and β-Catenin siRNA, leading to their downregulation. Ongoing work is evaluating the ability of this LNP platform to halt AML proliferation and survival. Ultimately, this platform will provide a safer and more effective approach to pediatric AML treatment.

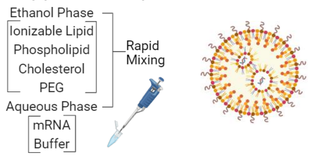

Figure 1. Schematic showing LNP formulation technique.

LNP Design Influences Physicochemical Characteristics

A.

Lipid Component	Library
Ionizable Lipid (%)	20-60
Phospholipid (%)	10-30
PEG (%)	2-5
Cholesterol (%)	20-68

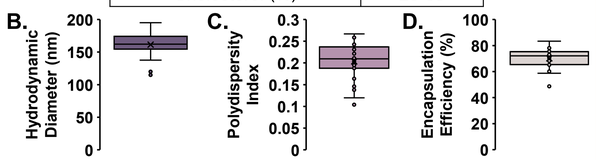

Figure 2. (A) Ranges of each lipid component in the LNP library, **(B)** hydrodynamic diameter, **(C)** polydispersity index, and **(D)** siRNA encapsulation efficiency of LNPs.

LNP-Mediated Delivery of Cy5-Labelled siRNA to Kasumi-1 AML Cells

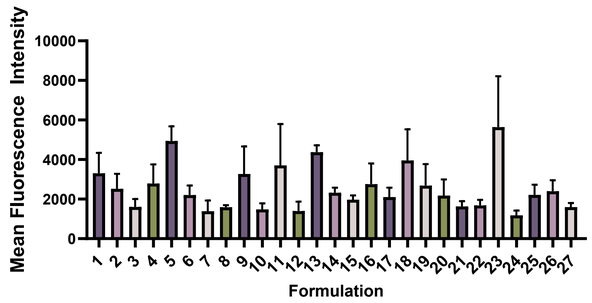

Figure 3. Mean fluorescence intensities following LNP-mediated delivery of Cy5-labelled siRNA. LNPs were formulated with C12-200 as the ionizable lipid, and cells were analyzed by flow cytometry 4 hours after LNP treatment (200 ng).

New Ionizable Lipids in LNPs Facilitate siRNA Delivery to AML Cells

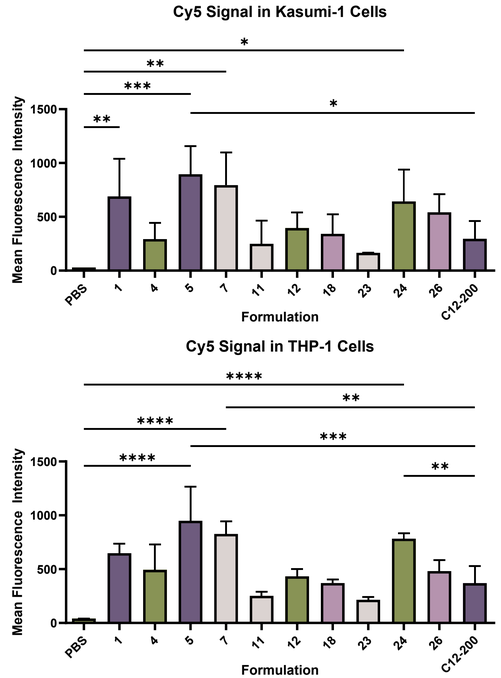

Figure 4. Mean fluorescence intensities following LNP-mediated delivery of Cy5-labelled siRNA. LNPs were formulated with our lab's proprietary ionizable lipids and cells were analyzed by flow cytometry 4 hours after LNP treatment (10 ng). *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001.

LNPs Enable Delivery of WT1 siRNA to Pediatric AML Cells

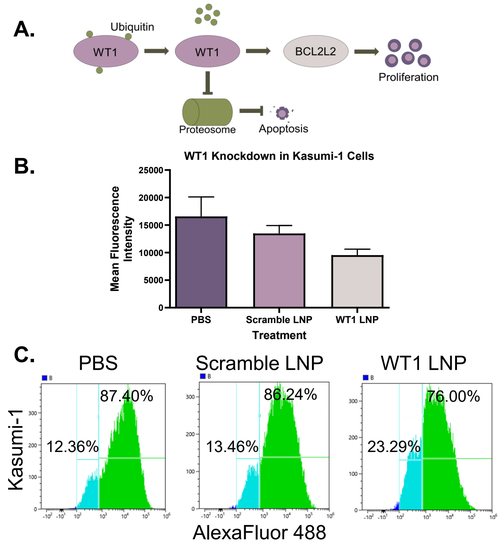

Figure 5. (A) WT1 protein signaling pathway and **(B, C)** flow cytometry histograms and quantification showing WT1 expression in Kasumi-1 AML cells following treatment with PBS or LNPs for 96 hours.

LNPs with Encapsulated β-Catenin siRNA Inhibit Protein Expression

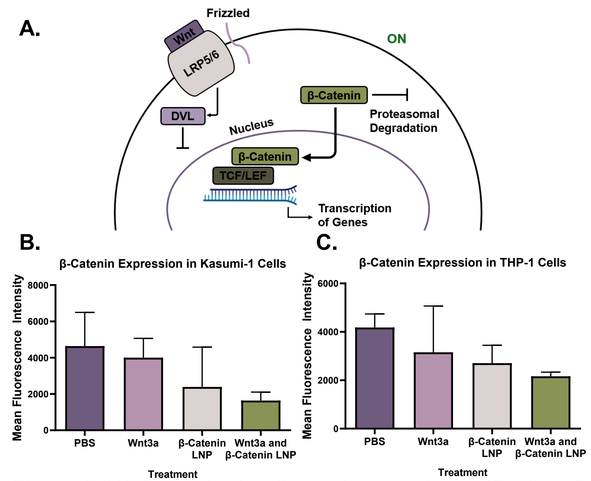

Figure 6. (A) β-catenin signaling pathway and quantification of β-catenin expression by flow cytometry in **(B)** Kasumi-1 and **(C)** THP-1 cells. Cells were pre-treated with PBS or Wnt3a ligands for 24 hours followed by PBS or LNPs for 24 hours.

LNPs with Encapsulated β-Catenin siRNA Inhibit Protein Expression

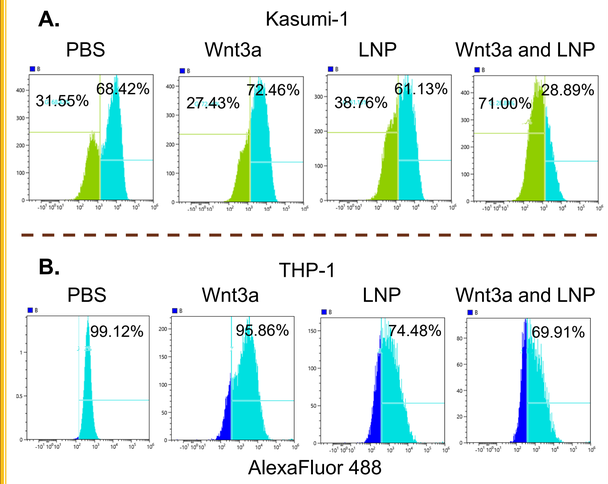

Figure 7. Flow cytometry histograms showing β-catenin expression in **(A)** Kasumi-1 and **(B)** THP-1 cells. Cells were pre-treated with PBS or Wnt3a ligands for 24 hours followed by PBS or LNPs for 24 hours.

Conclusions and Future Directions

We are developing an LNP platform for siRNA delivery to AML cells. First, we evaluated how LNP formulation parameters and ionizable lipid structure influence delivery to AML cells. We used our top LNP platform to deliver therapeutic WT1 or β-Catenin siRNA to inhibit expression of these oncogenic proteins. In ongoing work, we are evaluating the impact of delivery on cellular survival and proliferation. This offers a multifaceted approach to pediatric AML treatment to improve patient outcomes.

References

[1] Johnson. *Leukemia,* vol. 36,4 (2022).[2] Glienke. *Leukemia,* vol. 21,10 (2007). [3] Wagstaff. *Biosci Rep,* vol. 42(4) (2022).

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