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Design of lipid nanoparticle systems for brain gene therapy

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BACKGROUND

Brain diseases are a significant burden to the Canadian health care system, and can be caused by both heritable and sporadic genetic mutations. The majority of genetic neurodevelopmental and neurodegenerative diseases are caused by either the toxic gain-of-function of a mutant protein, or a loss-of-function mutation.[1,2]

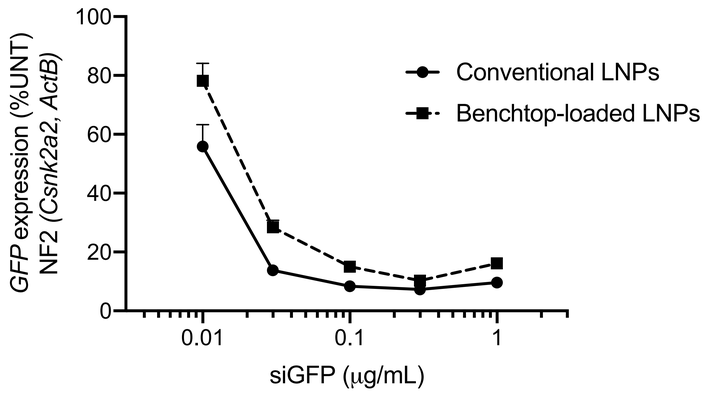
- Brain gene therapy agents must be efficiently delivered to and effective in neurons (the primary cells of interest in the brain)
- Current brain gene therapy approaches are limited by toxicity and immunogenicity
- Neurons are highly amenable to transfection by lipid nanoparticles (LNPs), and LNPs are safe and effective for the treatment of other genetic diseases[3-6]
- *In vivo* LNP administration will occur by direct injection into cerebrospinal fluid or brain tissue, so LNP formulation screening in primary neurons *in vitro* will likely translate accurately to *in vivo* work

PURPOSE

To identify and optimize lipid nanoparticle formulations and doses for the delivery of gene therapy payloads *in vitro* and *in vivo* to treat genetic brain diseases.

"DUMP AND MIX" LNP PREPARATION

Combining preformed vesicles at pH 4 with siRNA by pipette mixing on the benchtop produces LNPs that perform similarly to particles produced using rapid mixing (for details, see abstract #51).



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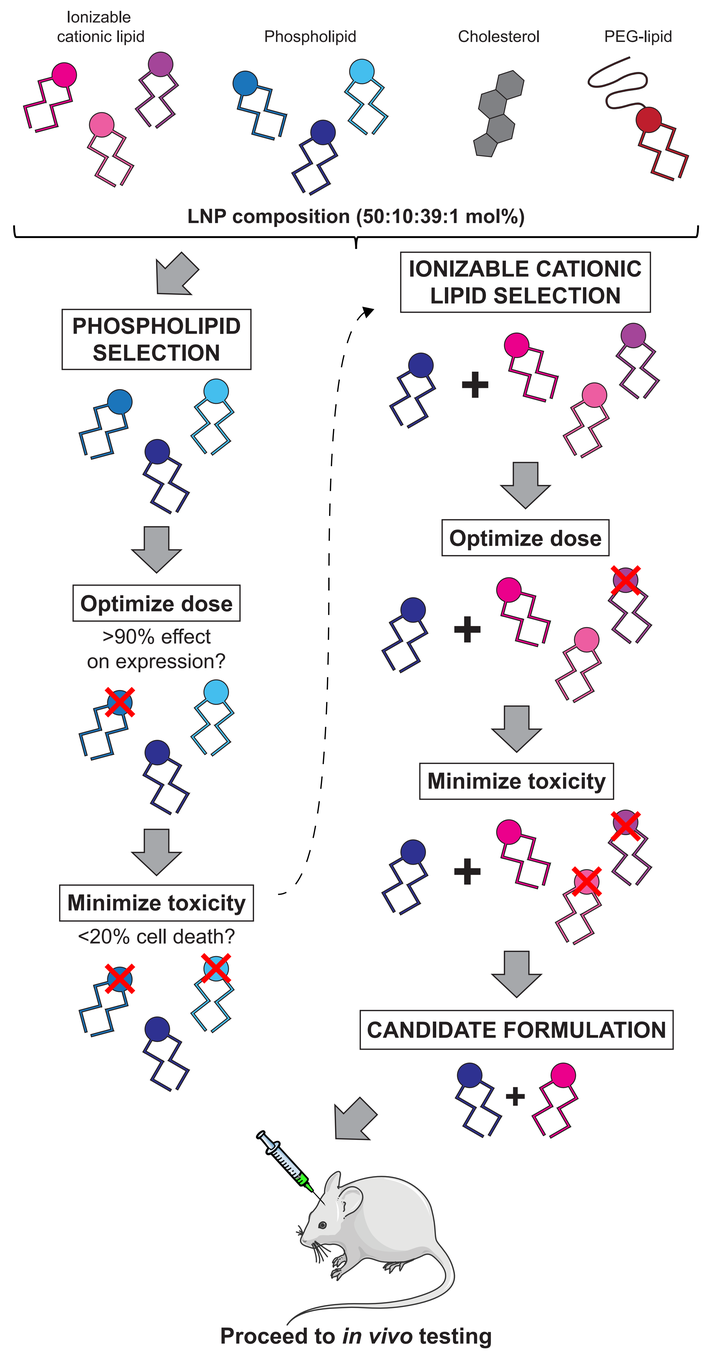
IN VITRO SCREENING STRATEGY

Workflow to optimize LNP formulation and dosage for efficient delivery of gene therapy cargoes to primary cortical neurons *in vitro*.



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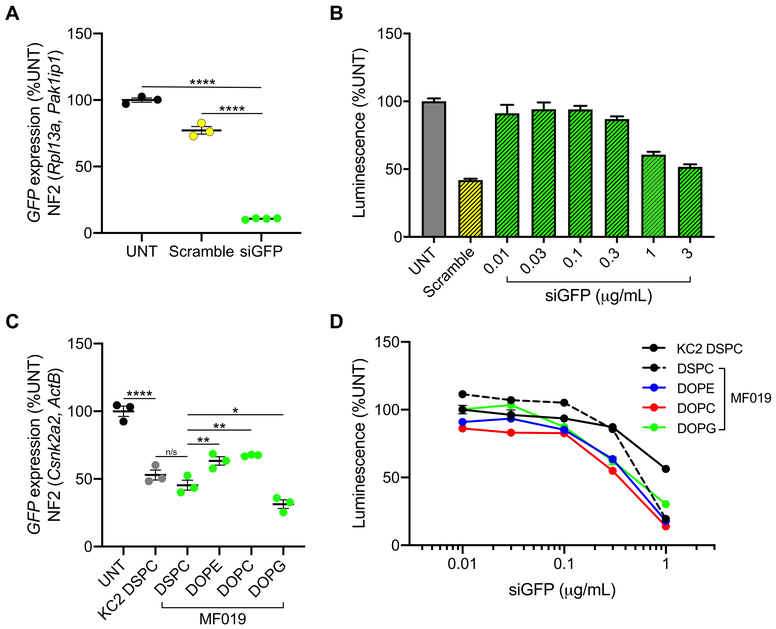
RESULTS

siRNA dose and phospholipid type affect GFP reporter knockdown efficiency and primary neuronal cell viability *in vitro*.



(A) LNP-mediated GFP knockdown (measured by RT-qPCR) is efficient at 3 μg/mL siGFP. (B) siRNA dose impacts primary neuronal viability *in vitro*. (C) In LNPs prepared using the ionizable lipids DLin-KC2-DMA (KC2) or MF019 (proprietary lipid), phospholipid species impacts GFP knockdown efficiency at 0.01 μg/mL siGFP (measured by RT-qPCR). (D) Higher doses result in decreased primary neuronal viability *in vitro,* regardless of phospholipid species.

FUTURE DIRECTIONS

- Assess the impact of ionizable lipid on GFP reporter knockdown efficiency and primary neuronal viability *in vitro.*
- Optimize LNP formulations for the delivery of mRNA to primary cortical neurons *in vitro.*
- Evaluate the performance of optimized neuronal LNP formulations in other primary brain cell types *in vitro*.
- Evaluate the performance of *in vitro*-optimized LNPs *in vivo*.

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