Aptamer-Targeted Lipid Nanoparticles for Immunotherapeutics Delivery for Glioblastoma

Osama Hassuneh[1], Alexandra R. Paul[1] and Khuloud T. Al-Jamal[1]

[1] JC STEM Lab of Nanomedicine for Advanced Therapy, Department of Pharmacology and Pharmacy, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China.

INTRODUCTION

Glioblastoma (GB) is the most lethal brain tumour, with less than 5% survive after 3 years of diagnosis. Tumour cells migrate deep into healthy brain tissue, rendering conventional therapies ineffective[1]. While immunotherapeutics have emerged as a promising approach, their lack of tissue specificity causes suboptimal responses and off-target toxicities[2].

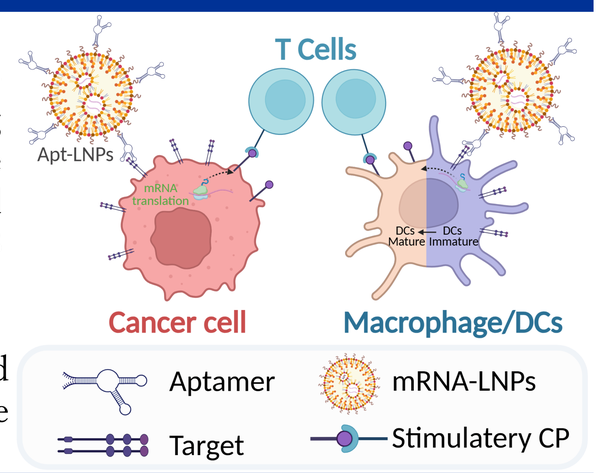
Lipid Nanoparticles (LNPs) are efficient nucleic acid (NA) delivery vehicles. However, achieving precise tumour targeting remains a critical challenge[3]. To address this, we developed an Aptamer-LNP platform that uses synthetic single-stranded oligonucleotides to recognise GB biomarkers and enable receptor-mediated transcytosis across the blood-brain barrier (BBB).

Aim:

To establish an immune and tumour cells-targeted delivery system, maximising immunotherapies' potency while minimising off-target toxicities, and providing a new, efficient modality for GB treatment.

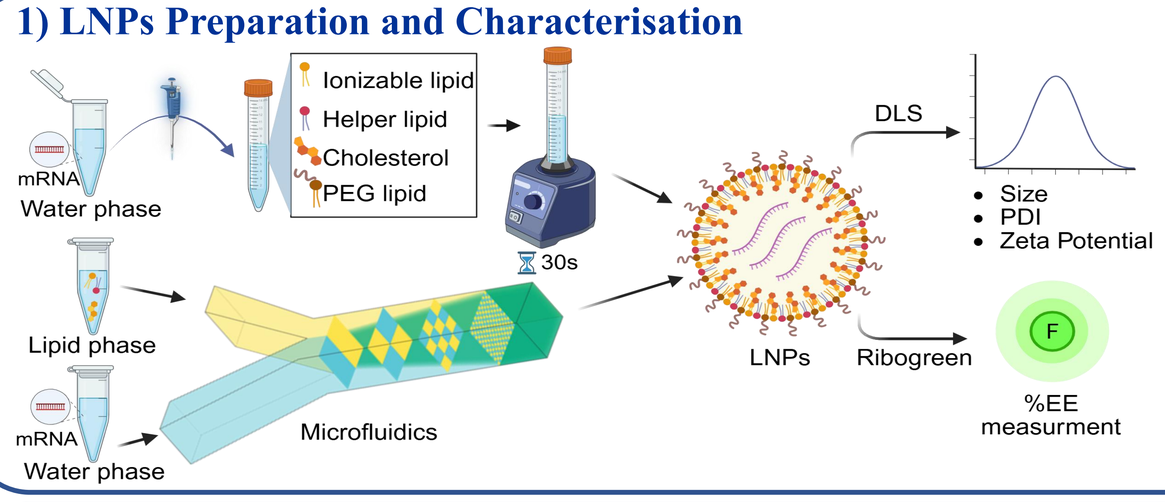
Hypothesis:

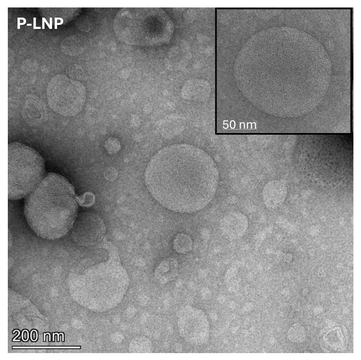
Apt-LNPs can enhance cellular uptake and mRNA transfection in GB and immune cells compared to non-targeted LNPs.

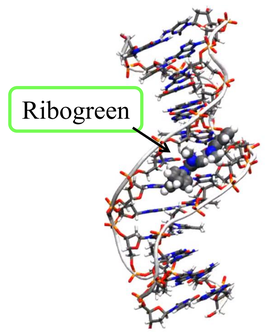


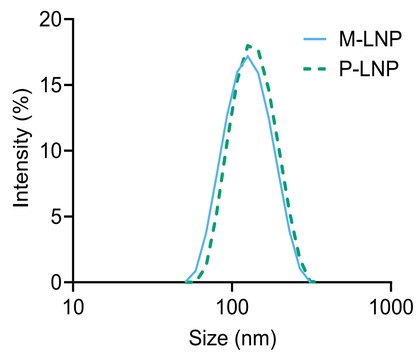
METHODS

1) LNPs Preparation and Characterisation



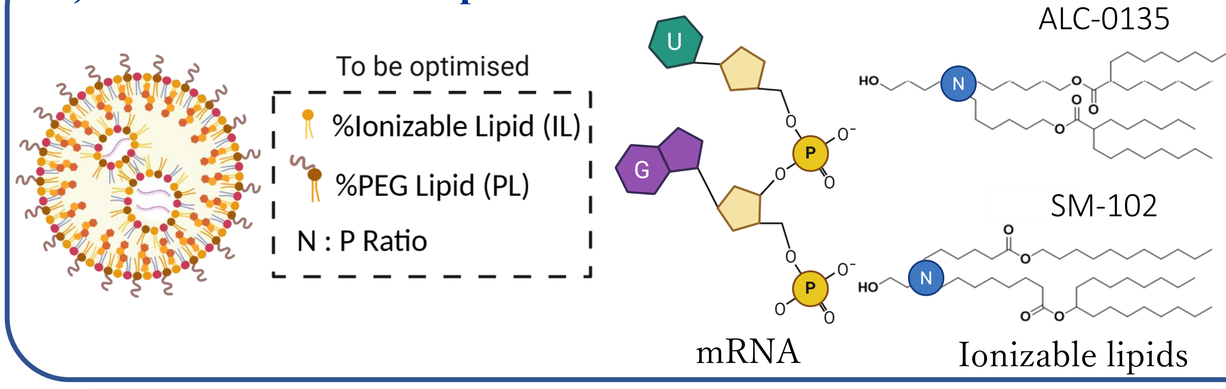


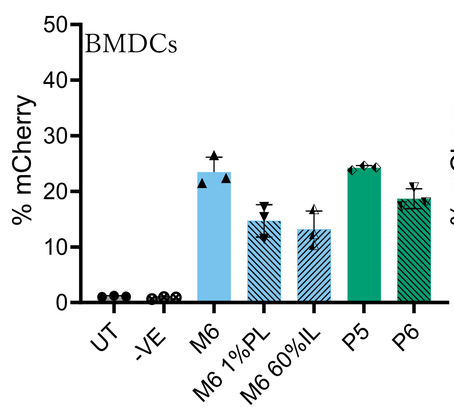


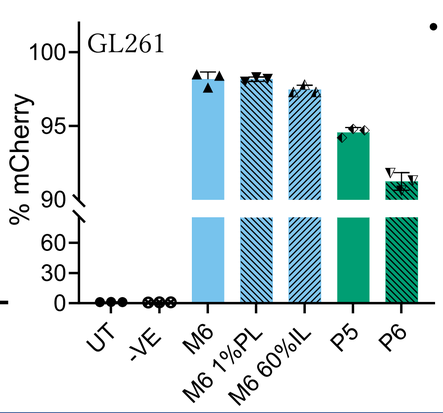


- TEM image of P-LNP, Ribogreen dye interacting with mRNA for quantification (%EE ~ 93%), and size distribution of LNPs.

2) LNPs Formulation Optimisation

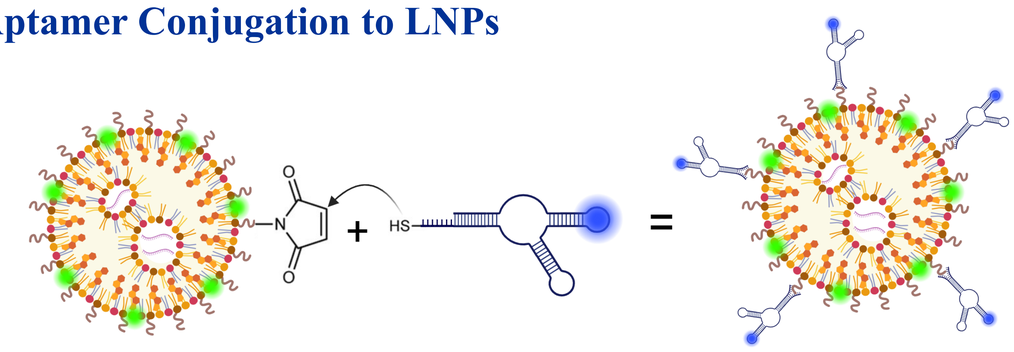


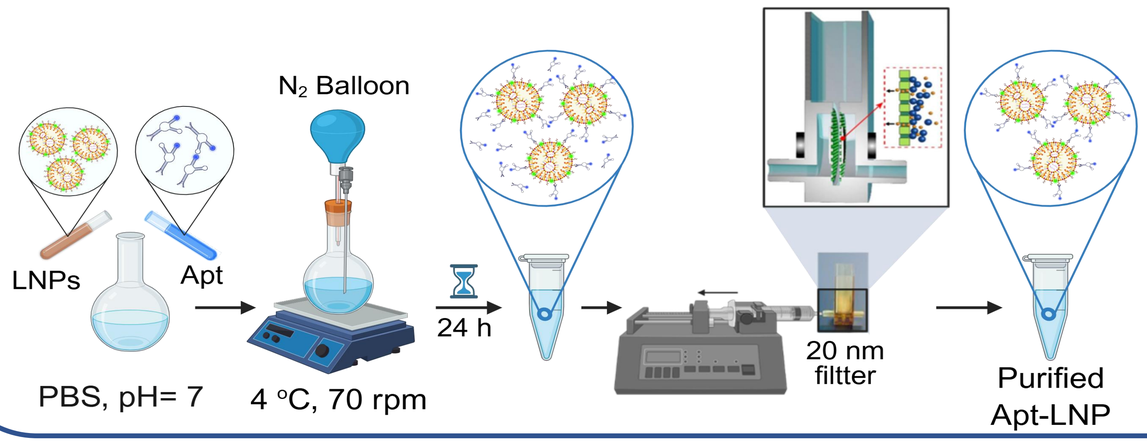


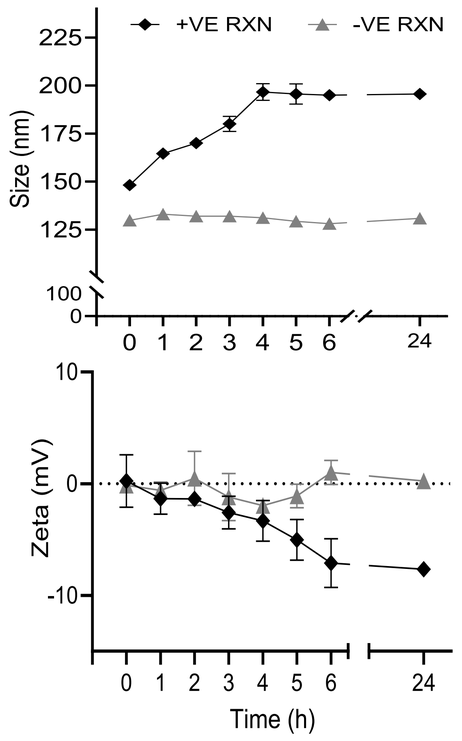


- %mCherry expression of optimised M-LNP and P-LNP in BMDCs and GL261 cells after 1ug/ml of mCherry mRNA treatment for 24h and 48h, respectively. M-LNPs performed better in both cells.

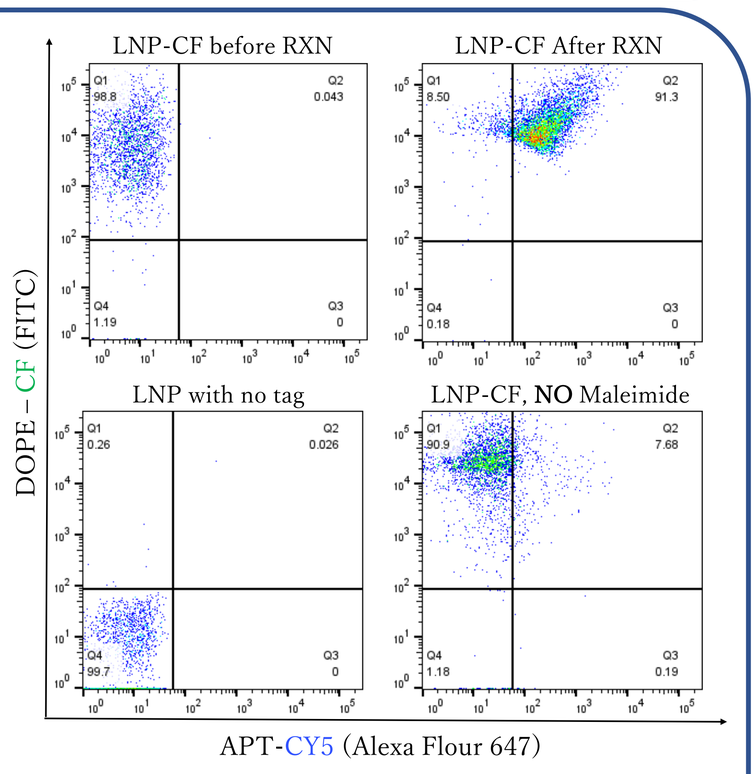
3) Aptamer Conjugation to LNPs





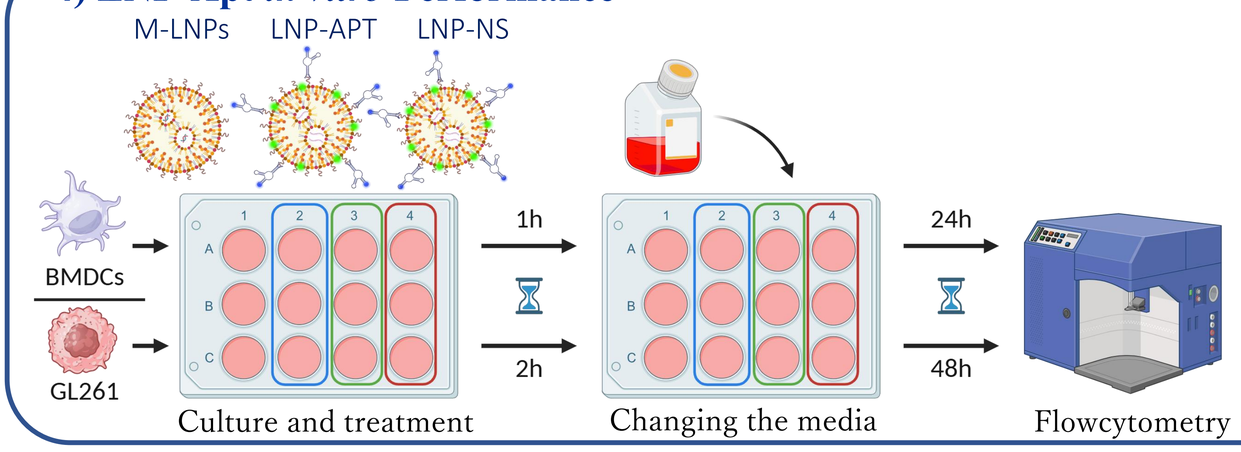


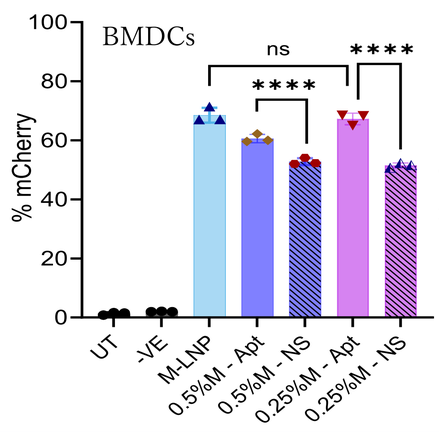
- Size and surface charge don't change for LNPs without maleimide (-VE RXN).

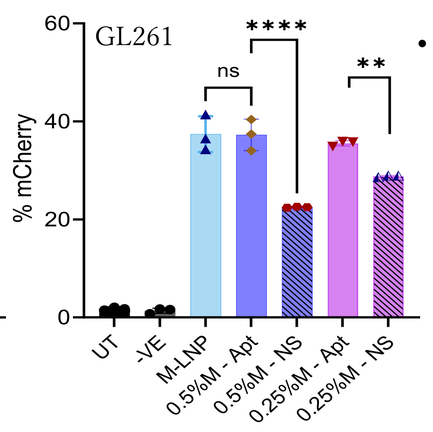


- The CY5 signal is exclusive to LNPs with maleimide, proving selective thiol-maleimide click chemistry.

4) LNP-Apt *in vitro* Performance







- Transfection efficiency of LNP-Apt vs. non-specific aptamer LNPs (NS) after only 1h and 2h of treatment, followed by 24h and 48h incubation for BMDCs and GL261, respectively.

CONCLUSION & FUTURE WORK

- ✓ This study established a reliable framework for developing high-purity, aptamer-functionalised LNPs (Apt-LNP) for glioblastoma and immune cells targeting.
- ✓ Functional validation showed that Apt-LNPs significantly accelerated uptake in BMDCs and GB cells, leading to a significant increase in transfection efficiency.
- ✓ Future work includes validating Apt-LNPs targeting efficiency in a GB mouse model via Intravenous (IV) and intracranial (ICV) administration routes.

References:
1. Singh, S., et al., Glioblastoma at the crossroads: current understanding and future therapeutic horizons. Signal Transduction and Targeted Therapy, 2025. 10(1): p. 213.
2. Kichloo, A., et al., Systemic adverse effects and toxicities associated with immunotherapy: A review. World J Clin Oncol, 2021. 12(3): p. 150-163
3. Barbier, A.J., et al., The clinical progress of mRNA vaccines and immunotherapies. Nature Biotechnology, 2022. 40(6): p. 840-854.

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LKS Faculty of Medicine
Department of Pharmacology & Pharmacy
香港大學藥理及藥劑學系

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