In Vitro and *In Vivo* Evaluation of Ionizable Lipids in LNP Formulations: Exploring Charge, Composition, and mRNA Delivery Efficiency

Sara Yaser Barham, Sarah Lindsay, Yvonne Perrie
Strathclyde Institute of Pharmacy & Biomedical Sciences , University of Strathclyde, Glasgow, UK

AIM

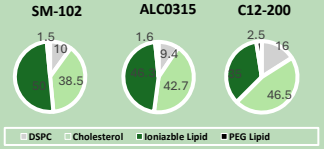
To systematically compare the physicochemical and biological performance of SM-102, ALC-0315, and C12-200 ionizable lipids within standardized LNP formulations, to determine how structural and ionization differences influence mRNA delivery and systemic expression, whilst also exploring the extent of *in vitro–in vivo* correlation to support predictive LNP development strategies.

MATERIAL AND METHOD

1 Manufacturing

Helix Size 0, TFR 10mL/min FRR 3:1

2 Lipid



3 Purification

Spin the column using Tris buffer at pH 7.4, keep it at 4°C, and centrifuge at 2000 GX.

4 *In-vitro* Study

Expression of mRNA GL was visualized using EVOS imaging and quantified with the Hidex reader. For mRNA FLuc fusion, luciferase activity was measured by luminescence quantification using the GloMax system with luciferin substrate.

5 *In-vivo* Study

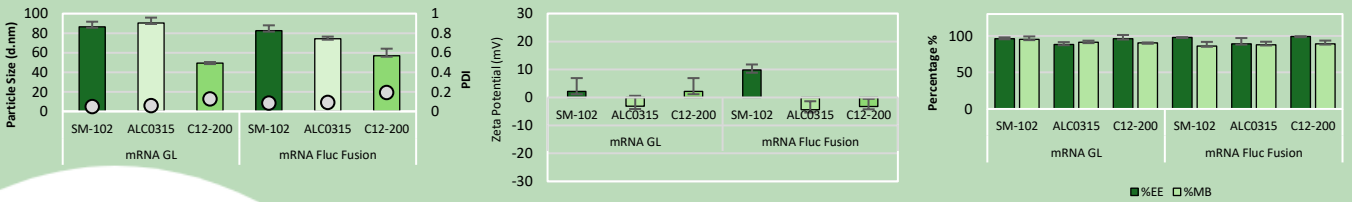
The in vivo expression of mRNA-LNPs was evaluated using BALB/c mice, which received intramuscular injections of 1 µg per dose in one leg

INTRODUCTION

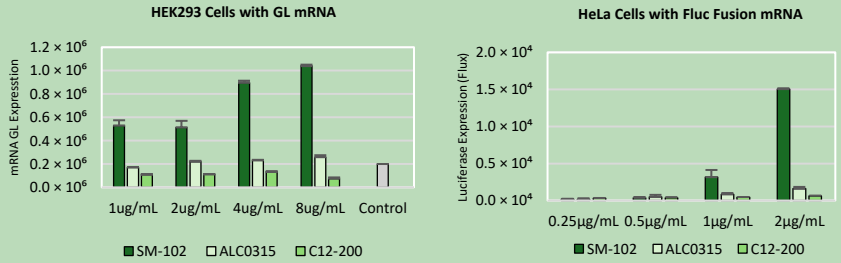
Lipid nanoparticles (LNPs) comprising ionizable lipids, cholesterol, helper phospholipids, and PEGylated lipids represent the current standard for mRNA delivery, providing protection against nuclease degradation while promoting efficient cellular internalization and endosomal escape. Their performance is characterized both *in vitro* — focusing on cellular uptake, transfection efficiency, and cytotoxicity — and *in vivo*, where biodistribution, protein expression profiles, and immunogenic responses are assessed. Systematic evaluation of these parameters is critical to optimize LNP formulations for improved therapeutic efficacy and safety.

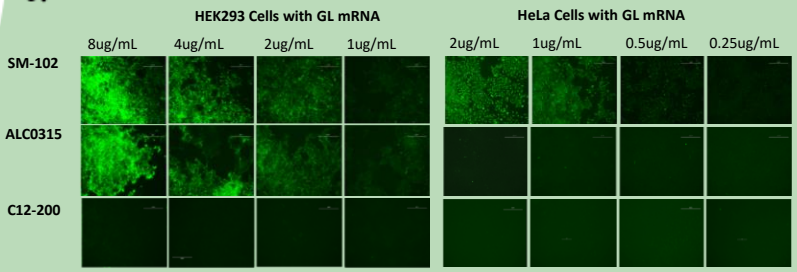
RESULTS

Lipid Composition Effect average Size and EE%

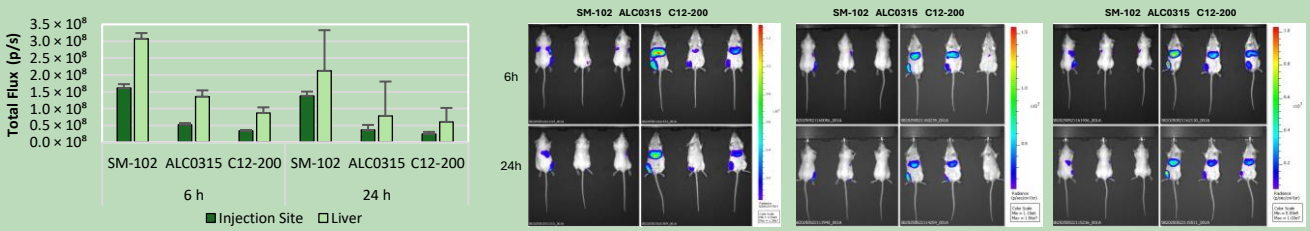


Comparison of mRNA Delivery Efficiency by LNPs in Different Cell Lines





In Vivo Protein Expression Mediated by mRNA LNPs



CONCLUSION

Both in vitro and in vivo studies are essential for evaluating LNPs, even though their results may differ. In vitro testing plays a critical role in confirming compound toxicity before progressing to in vivo experiments and can offer initial insights into how LNPs might behave in a biological system. However, establishing a reliable IVIVC for mRNA-LNPs remains difficult due to the complex nature of cellular uptake, endosomal escape, and immune responses.