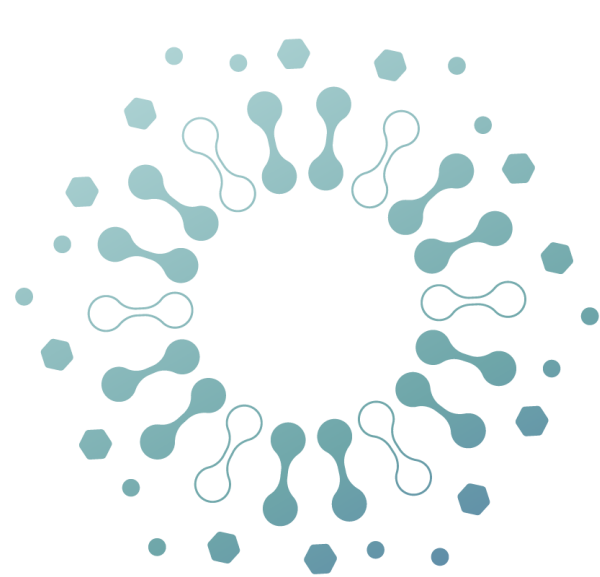




Optimization of siRNA-based lipid nanoparticle formulation to enhance gene delivery and improve efficacy in breast cancer treatment

Alicia Curnel¹, Manon Degey¹, David Torres², Brigitte Evrard¹, Emilien Nicolas², Géraldine Piel¹, Manon Berger¹

¹ **University of Liège, Belgium**, Laboratory of Pharmaceutical Technology and Biopharmacy, Center for Interdisciplinary Research on Medicines (CIRM)

² **CER groupe, Belgium**, Laboratory of Immunology

Introduction

At present, LNPs represent a promising strategy for the delivery of genetic material to treat a wide range of diseases.¹

Four formulations are currently approved and commercially available in Belgium.

However, clinical translation for applications other than vaccination or liver targeting remains challenging. This can, in part, be explained by the limited release of the nanoparticle content into target cells: indeed, less than 2% of the genetic material is able to escape the endosome and reach the cytoplasm.²

The main challenge therefore lies in improving gene delivery by addressing the two major barriers, namely cellular uptake and endosomal escape. To this end, the lipids composition of LNPs can be optimized; in this context, the present study particularly focuses on the modification of the ionizable lipid.

Methods



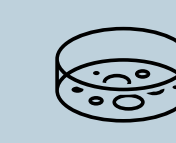
PRODUCTION

The LNPs were produced by a **rapid-mixing** method. They are composed of **ionizable lipid** (8 variants tested (figure 2)), **DSPC**, **cholesterol** and **DSPE-PEG₂₀₀₀** at a molar ratio of 50:10:37.5:2.5 respectively (figure 1), **carrying siRNA**.



CHARACTERIZATION

The LNPs were characterized in terms of size, Pdl and surface charge by **DLS**, encapsulation efficacy (EE) using the **RiboGreen assay**, and the stability in serum by **NTA**.



CELLULAR EFFICACY

The cellular efficacy of LNPs (100nM siRNA) was evaluated in terms of GFP silencing in breast cancer MDA-MB-231 GFP cells by **flow cytometry** and **real-time imaging (Incucyte)**, 72h post-transfection.

Objective



Study of **different ionizable lipids** to identify the most promising LNP formulations for the **efficient delivery of genetic material (siRNA)** into **breast cancer cells**

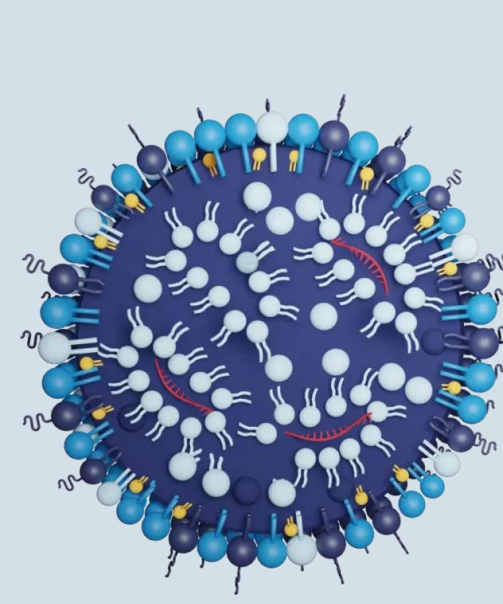


Figure 1 – Composition of the lipid nanoparticles (LNPs)

Already included in approved formulations

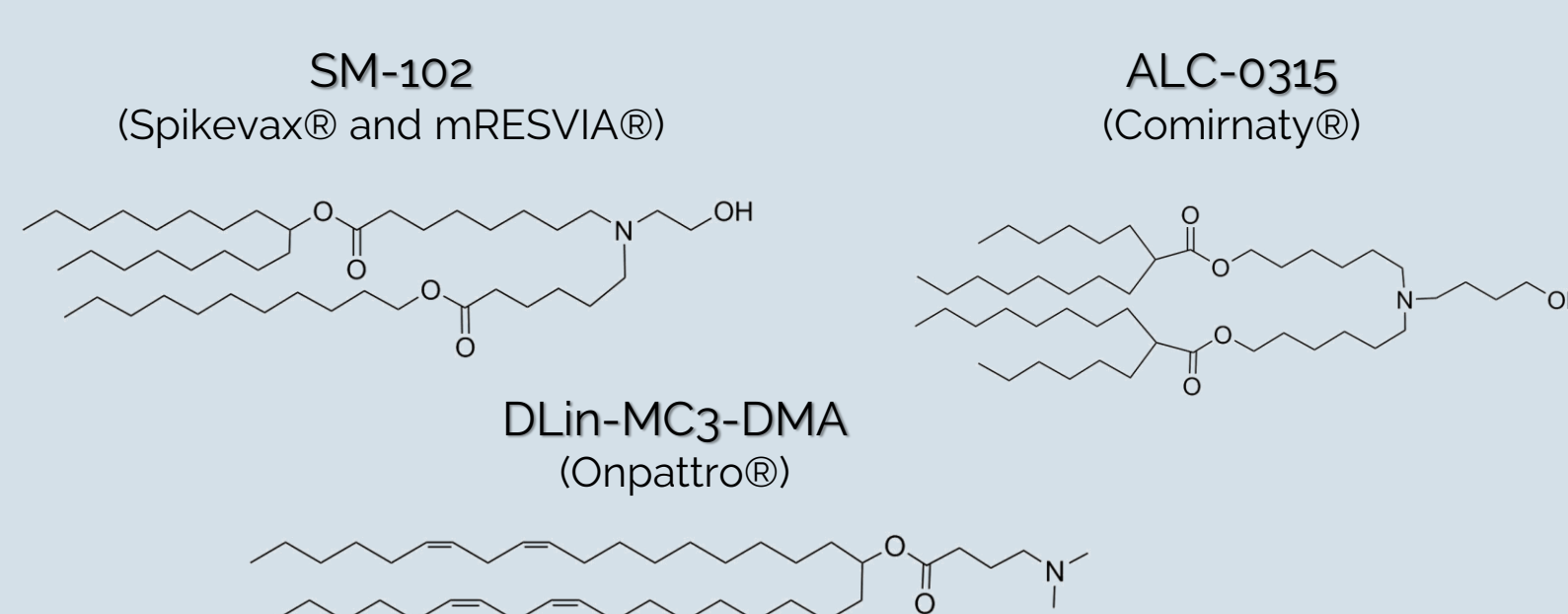
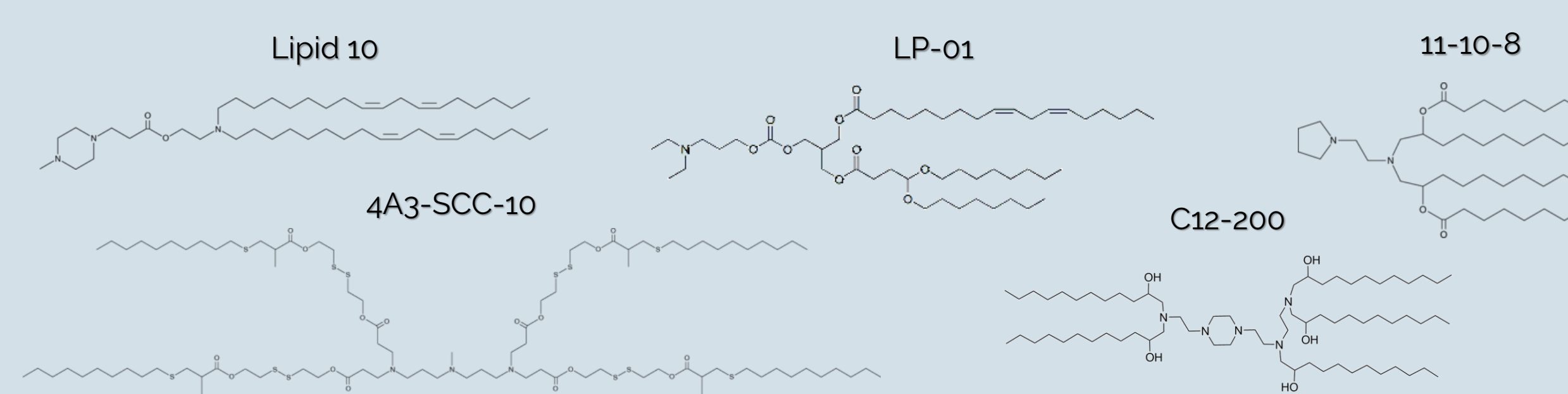


Figure 2 – Structures of the eight ionizable lipids tested for LNP formulation

Reported in the literature



Results and discussion

❖ Impact of the ionizable lipid on LNP physicochemical properties

The ionizable lipid **does not affect the physicochemical properties of LNPs**; indeed, the results showed no significant differences among the 8 nanoparticles produced. All formulations have a **size below 150 nm** (suitable for IV injection), a **Pdl around 0.3** (indicating formulation homogeneity), a **near-neutral charge**, and a high **encapsulation efficacy (EE) over 70%**, expressing the amount of siRNA encapsulated in the LNP relative to the total amount of siRNA in the sample (figure 3).

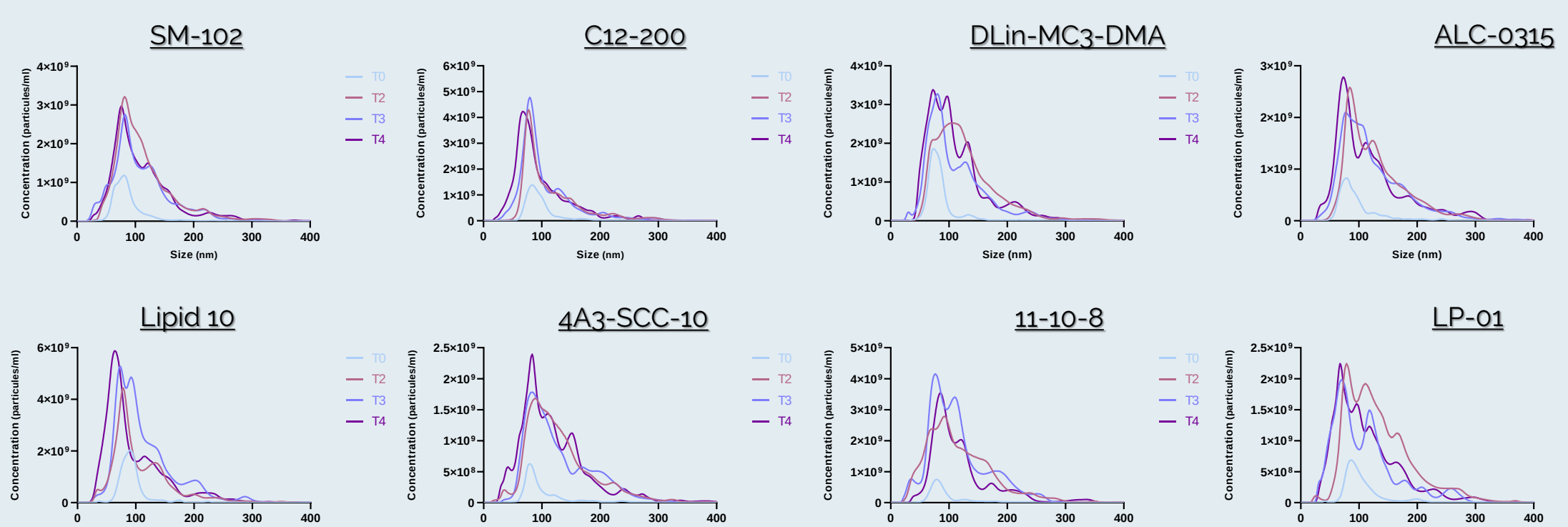


Figure 4 – Impact of the ionizable lipid on LNP stability in serum medium, evaluated using an established protocol at LTPB, employing NTA.

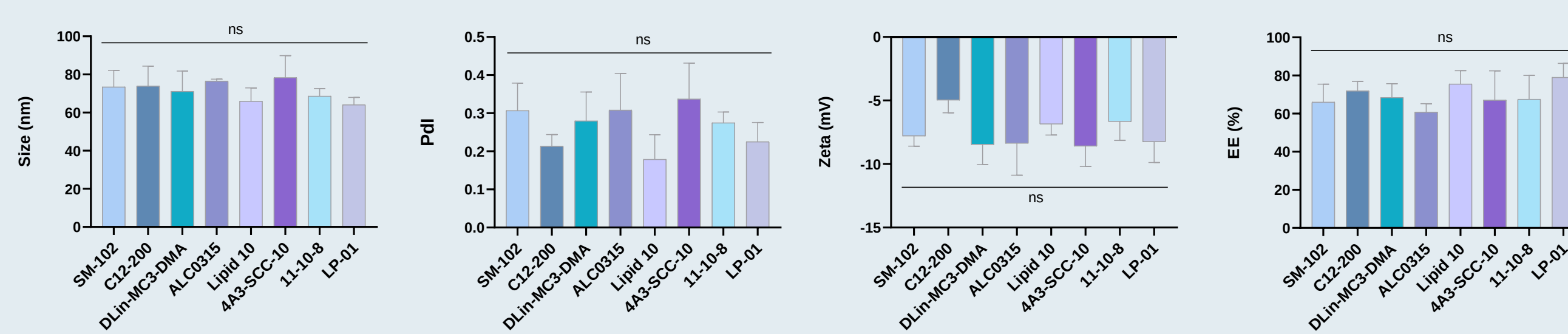


Figure 3 – Impact of the ionizable lipid on LNP physicochemical properties including size, Pdl, zeta potential and encapsulation efficacy.

❖ LNP stability in serum medium

Although nanoparticle stability in serum, associated with the formation of the protein corona, largely depends on the PEGylated lipid used — with long-chain PEGylated lipids like DSPE-PEG₂₀₀₀ limiting its formation — 8 LNP formulations were tested, employing NTA³. The results consistently showed that **all LNPs exhibit stability in serum, regardless of the ionizable lipid used** (figure 4). This serum stability is an important factor to consider in the context of IV administration for tumor accumulation.

❖ Impact of the ionizable lipid on LNP cellular efficacy

The flow cytometry results revealed differences in GFP silencing. Formulations containing the ionizable lipids **SM-102**, **C12-200** and **ALC-0315** were found to be more effective, with **GFP silencing exceeding 80%**. Next, the formulation with the lipid **11-10-8**, which showed **over 60% GFP silencing**, while the other LNPs displayed less than 30%. These results can also be correlated with the images obtained by Incucyte, since it is possible to identify certain LNP formulations that appear to efficiently silence the GFP fluorescence compared to untreated cells (figure 5).

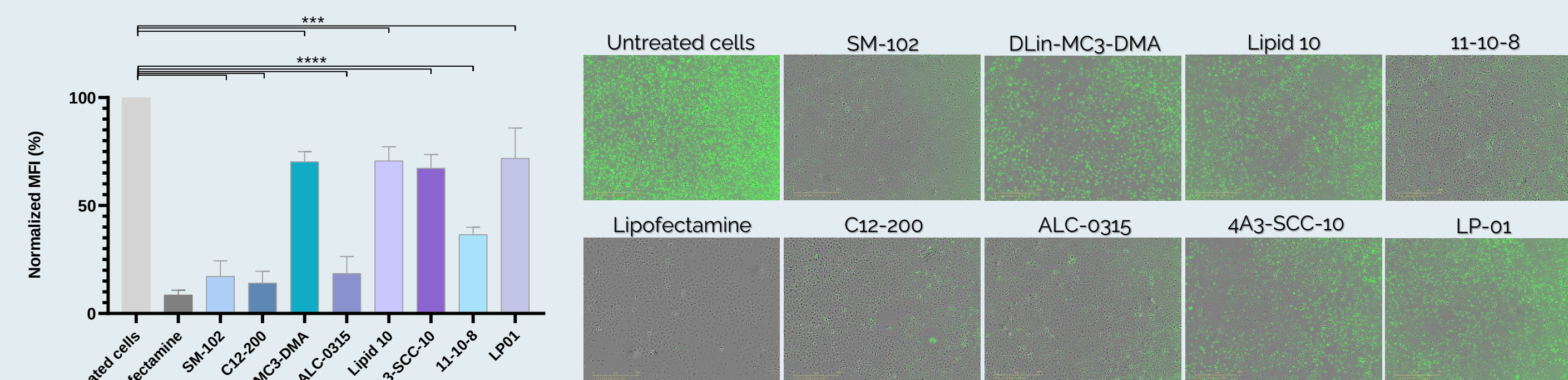


Figure 5 – Impact of the ionizable lipid on LNP cellular efficacy in breast cancer cell line, assessed by real-time imaging (Incucyte) and flow cytometry

Conclusion

Although no significant difference has been observed in terms of physicochemical properties between the formulations, **some LNP formulated with particular ionizable lipid showed better gene extinction efficacy**.

Future studies will focus on cellular uptake mechanisms and endosomal escape to explain the differences observed *in vitro*, along with *in vitro* evaluations of cellular toxicity and hemocompatibility.

References

- (1) El Moukhtari S.H., et al. Lipid Nanoparticles for siRNA Delivery in Cancer Treatment. *J. Control. Release Off. J. Control. Release Soc.* **2023**, 361, 130–146. <https://doi.org/10.1016/j.jconrel.2023.07.054>.
- (2) Gilleron J., et al. Image-Based Analysis of Lipid Nanoparticle-Mediated siRNA Delivery, Intracellular Trafficking and Endosomal Escape. *Nat. Biotechnol.* **2013**, 31 (7), 638–646. <https://doi.org/10.1038/nbt.2612>.
- (3) Berger M., et al. Effect of PEG Anchor and Serum on Lipid Nanoparticles: Development of a Nanoparticles Tracking Method. *Pharmaceutics* **2023**, 15 (2), 597. <https://doi.org/10.3390/pharmaceutics15020597>.