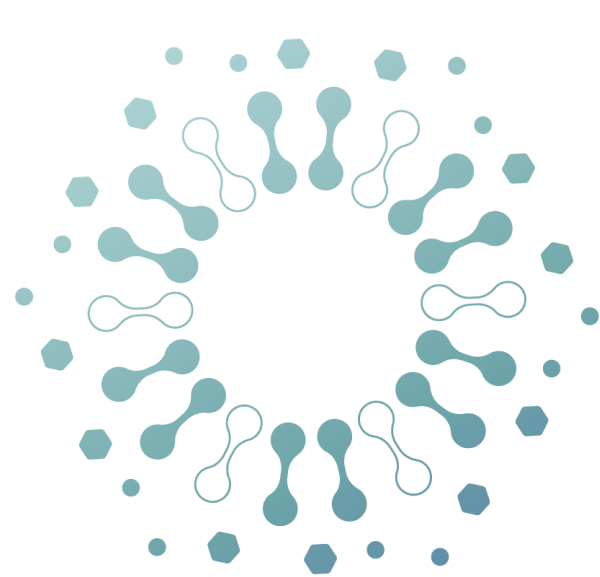




Impact of the ionizable lipid on the efficacy of siRNA-based lipid nanoparticles in breast cancer treatment

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INTRODUCTION

Lipid nanoparticles (LNPs), typically composed of four types of lipids - ionizable lipids and helper lipids, which are essential for endosomal escape, as well as cholesterol and PEGylated lipids for stabilization - are a powerful tool for nucleic acid delivery and hold promise for the treatment of many diseases (figure 1).

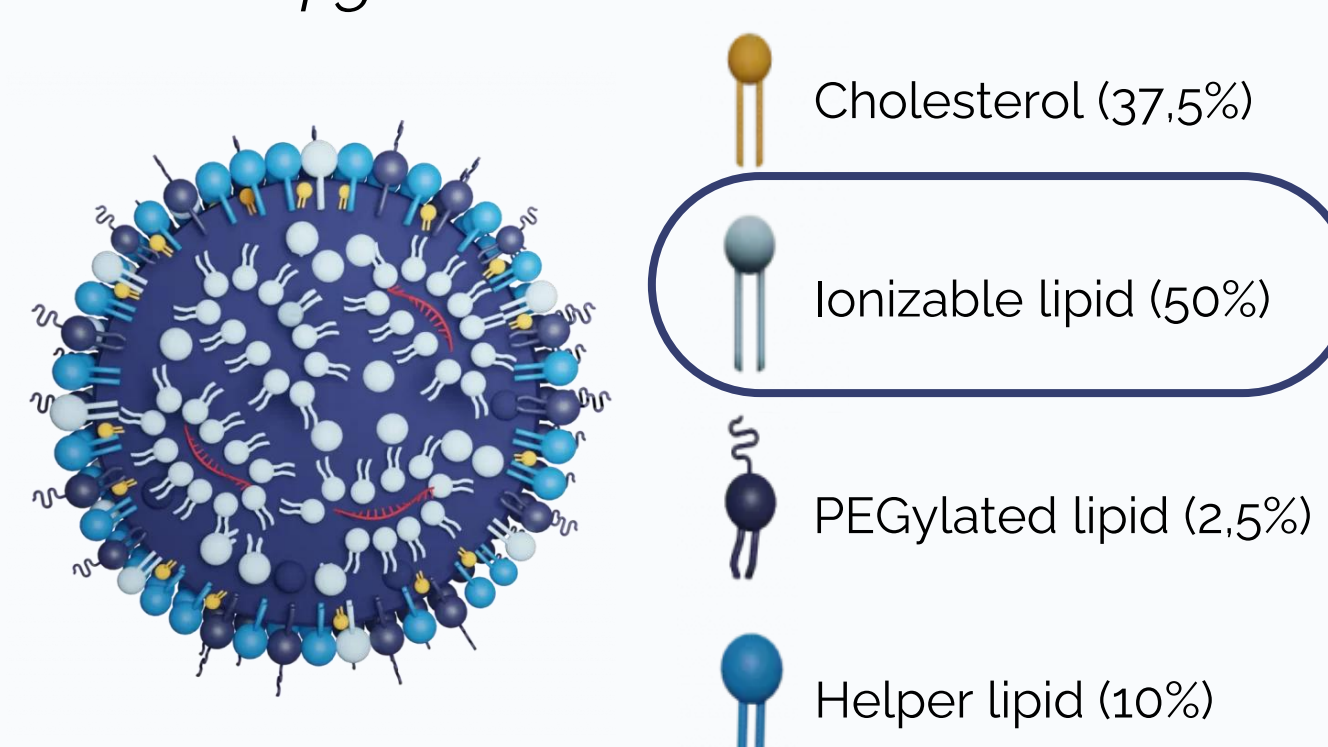


Figure 1 – Composition of the lipid nanoparticles (LNPs)

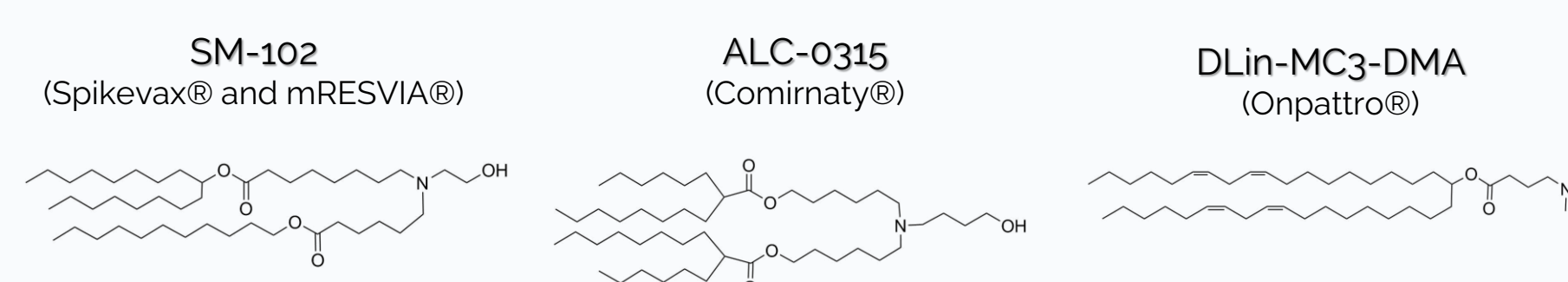
Although currently four formulations are approved and available in Belgium, clinical translation of LNPs for applications beyond vaccination or liver targeting remains limited ; less than 2% of genetic material escapes the endosome to reach the cytoplasm and exert its effect. ¹

The main limitation in gene delivery lies in overcoming two key barriers : cellular uptake and endosomal escape. To address this, optimizing the lipid composition – particularly by modifying ionizable lipids – represents a central strategy explored in this study.

OBJECTIVE

Screening of **different ionizable lipids** (figure 2) to identify optimal LNP formulations for efficient siRNA delivery into **breast cancer cells**.

Already included in approved formulations



Reported in the literature

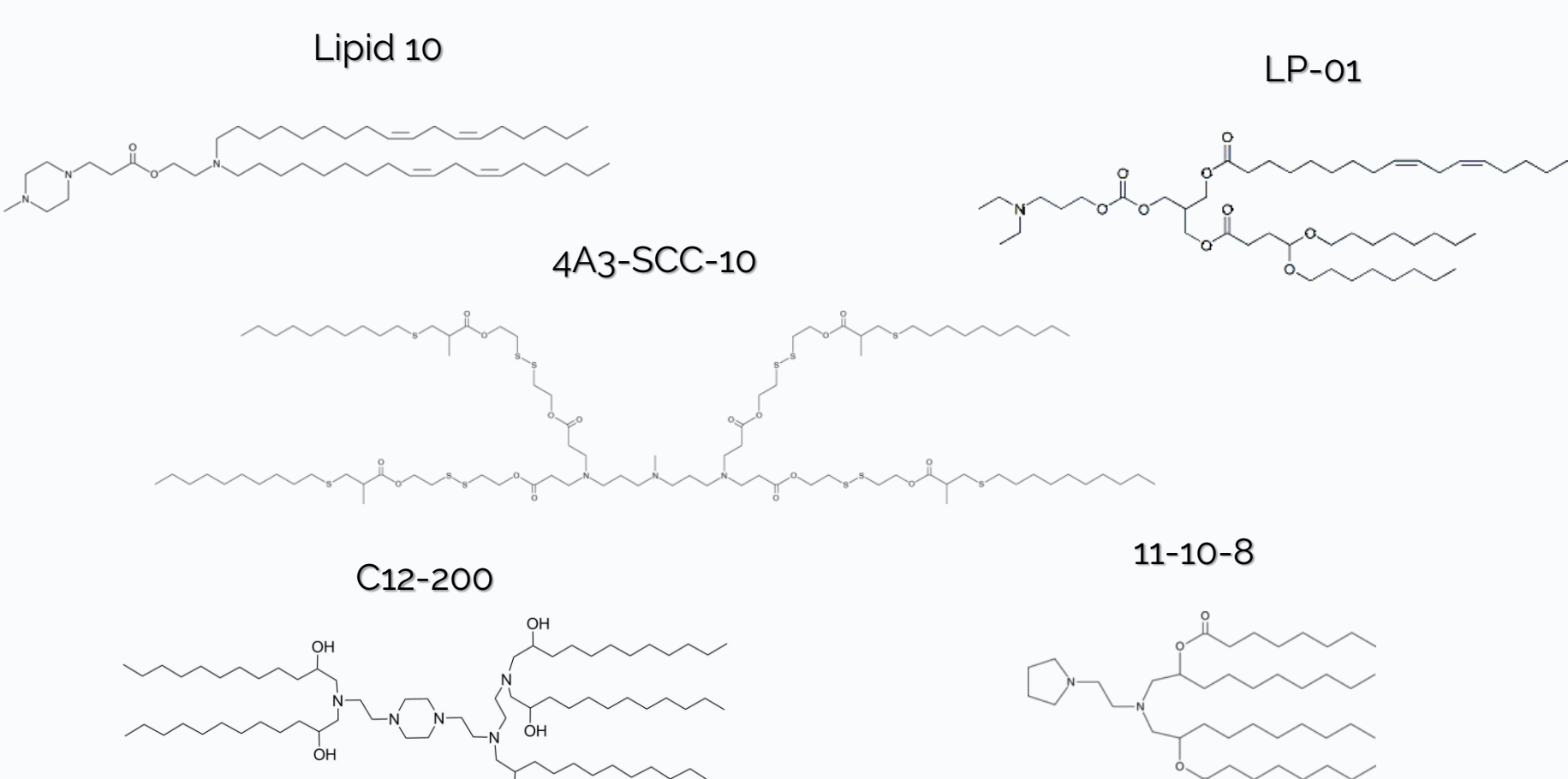


Figure 2 – Structures of the eight ionizable lipids tested for LNP formulation

METHODS

PRODUCTION

LNPs were produced by microfluidics method (TAMARA, Inside Tx®). The lipids were dissolved in ethanol at a molar ratio of 50/10/37.5/2.5 respectively (figure 1) and mixed with siRNA solution in acetate buffer (25mM, pH4) at an N/P ratio of 4.



CHARACTERIZATION

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



CELLULAR EFFICACY

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.



RESULTS AND DISCUSSION

Impact of the ionizable lipid on LNP physicochemical properties

The ionizable lipid did not exhibit any significant impact on the physicochemical properties of the LNPs. Indeed, the results showed no notable differences among the 8 nanoparticles produced. All formulations displayed a size below 150 nm, suitable for intravenous administration, a Pdl of around 0.3, indicating good homogeneity, a near-neutral charge, and a high EE above 70% (figure 3).

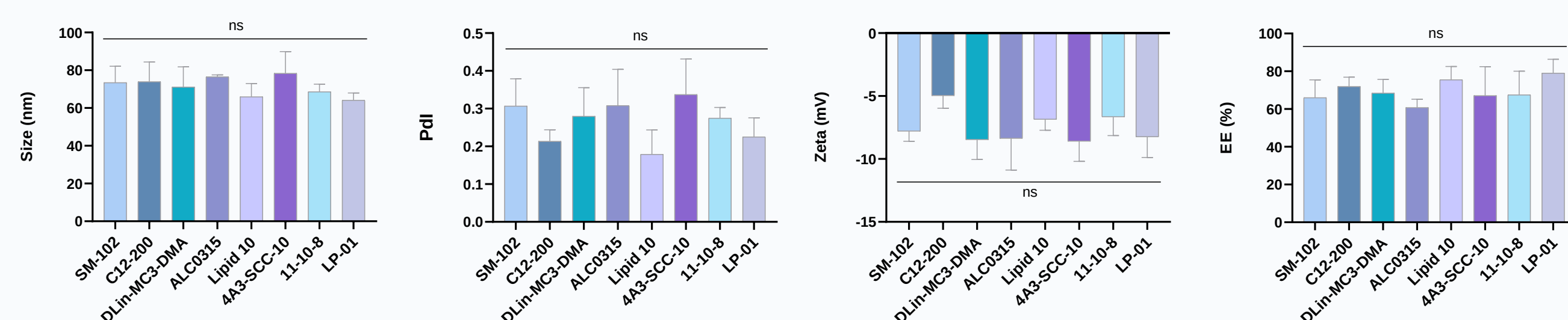


Figure 3 – Impact of the ionizable lipid on LNP physicochemical properties including size, Pdl, zeta potential and encapsulation efficacy.

LNP stability in serum medium

Although nanoparticle stability in serum – linked to protein corona formation – primarily depends on the type of PEGylated lipid used (long-chain PEGylated lipids such as DSPE-PEG₂₀₀₀ reducing its formation), the 8 formulations were evaluated using NTA. The results consistently demonstrated that all LNPs maintained serum stability, regardless of the ionizable lipid employed, a key parameter to consider in the context of IV administration for tumor accumulation (figure 4).

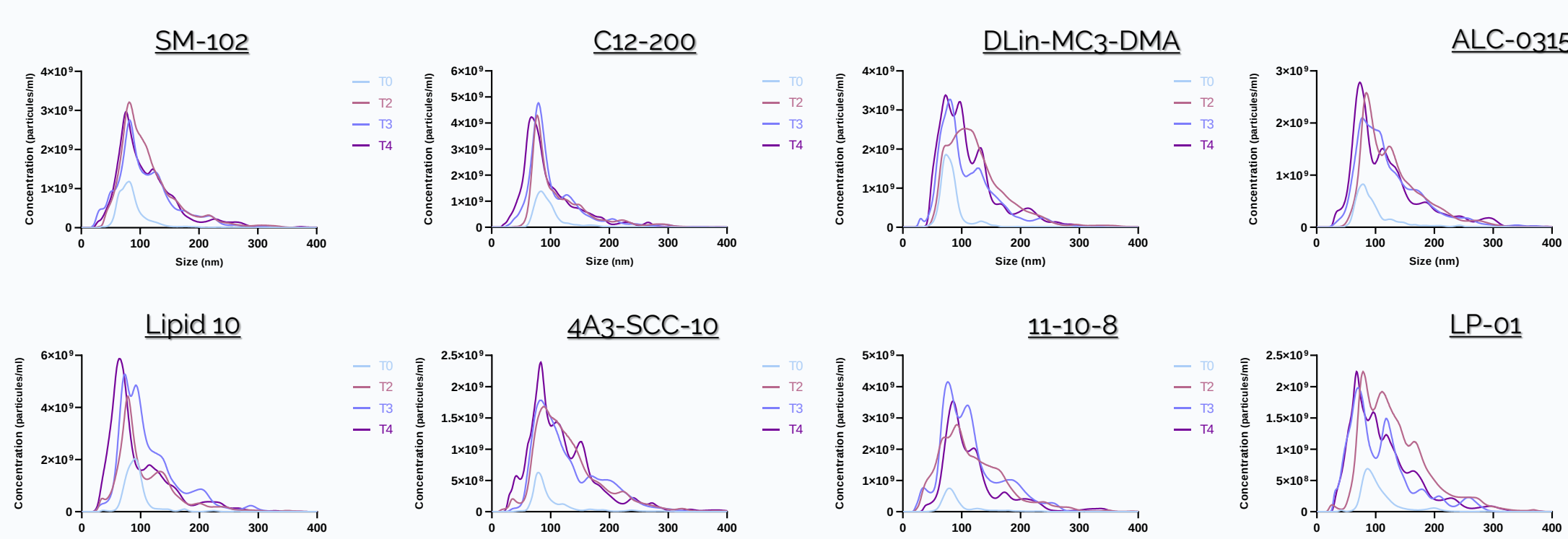


Figure 4 – Impact of the ionizable lipid on LNP stability in serum medium, evaluated using an established protocol at LTPB, employing NTA.

Impact of the ionizable lipid on LNP cellular efficacy in breast cancer

The cellular efficacy results obtained by flow cytometry revealed differences in GFP expression silencing. Specifically, formulations containing the ionizable lipids SM-102, C12-200 and ALC-0315 proved to be the most effective, achieving GFP knockdown levels of 82.8%, 85.9% and 81.5%, respectively. Among the other LNPs, only the formulation with 11-10-8 showed a notable GFP silencing of 63.4%, while in the remaining 4 formulations, GFP suppression did not exceed 30% (figure 5).

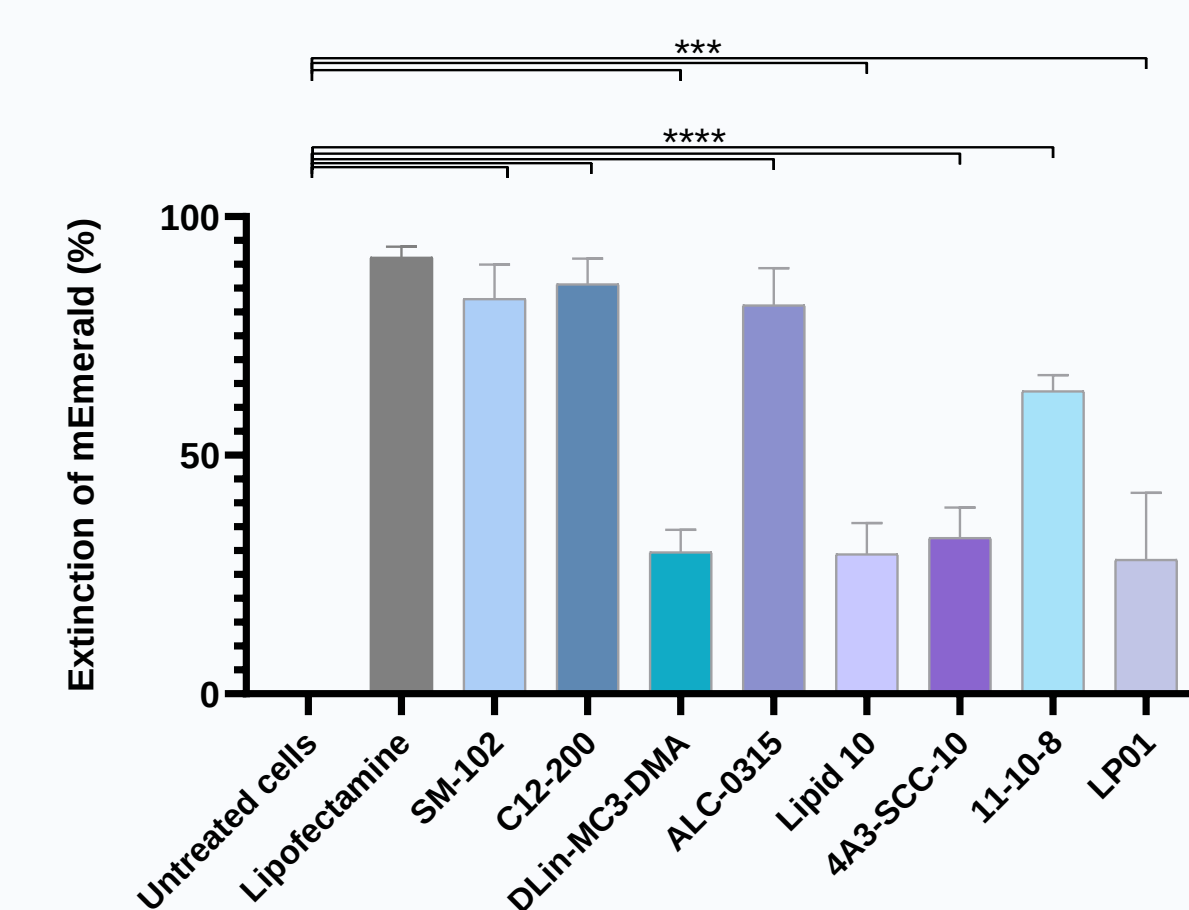


Figure 5 – Impact of the ionizable lipid on LNP cellular efficacy in breast cancer cell lines assessed by flow cytometry 72 hours post-transfection and expressed as normalized MFI.

These results can also be correlated with the images obtained by real-time imaging (Incucyte), since it is possible to identify certain LNP formulations that appear to efficiently silence the GFP fluorescence compared to untreated cells (figure 6).

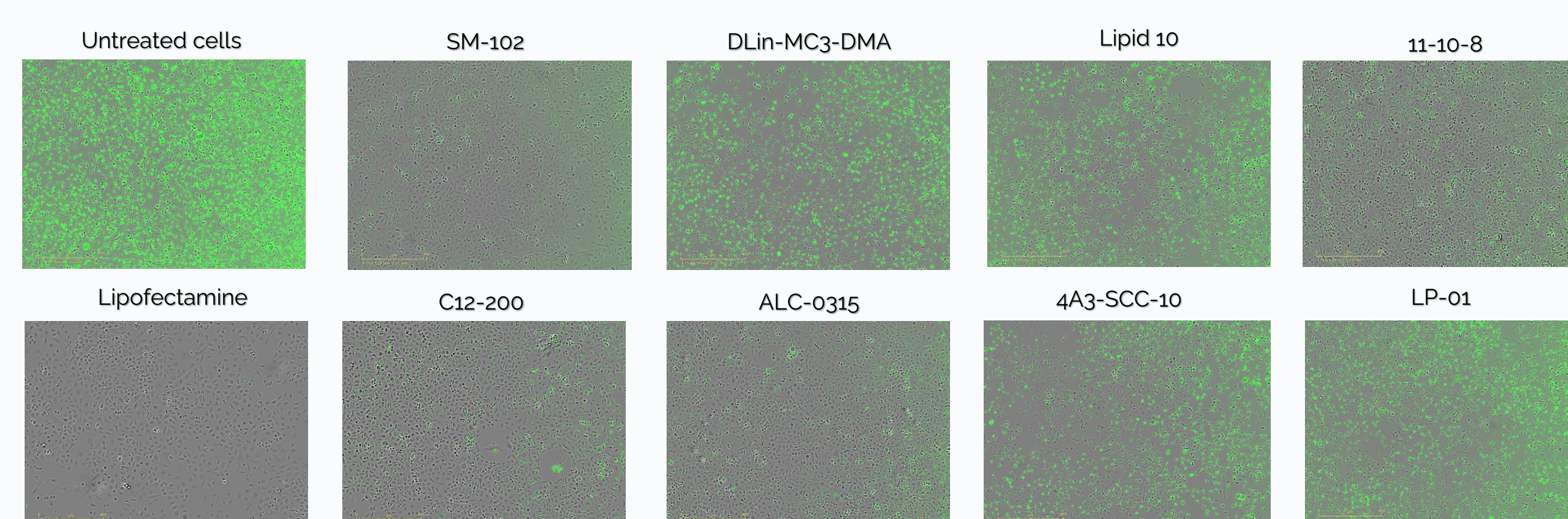


Figure 6 – Impact of the ionizable lipid on LNP cellular efficacy in breast cancer cell lines assessed by Incucyte 72 hours post-transfection

CONCLUSION & PERSPECTIVES

Although no differences in physicochemical properties have been observed between the 8 LNP formulations, the cellular efficacy tests have identified certain ionizable lipids as more promising, which can be explained by their optimal pKa and specific structural features.²

Subsequently, comprehensive efficacy analyses will be conducted on all formulations, as well as in vitro safety assessments on the most promising ones. In vivo studies of biodistribution and immunogenicity will ultimately be performed in collaboration with the CER.

REFERENCES

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