In vitro evaluation of LNPs

Optimization of LNPs by Modulating OZB-Property ionizable lipids for Enhanced mRNA Transfection in Various Cell Lines (Dendritic, macrophage, monocyte, T cells).

After extensive R&D efforts in lipid synthesis, five ionizable lipids were selected for screening the transfection efficiency of LNP-mRNA formulations. In this study, two mRNA reporter genes, enhanced green fluorescent protein (eGFP) and firefly luciferase (Fluc), were used. The selected LNPs were tested on the following cell lines: DC2.4, RAW 264.7, J774, THP-1, Jurkat, MOLT-4. Transfection was performed using LNP-mRNA at doses of 0.1, 0.5, 1, 2, and 5 µg of mRNA, with Luciferase and GFP expression assessed 24 hours post-transfection. The LNP formulated with the ionizable lipid FP105 corresponds to our catalogue product NanOZ. It is important to note that the direct comparison or translation of findings from *in vitro* to *in vivo* conditions is highly complex. This is due to fundamental differences in physiological environments, including factors such as biodistribution, cellular uptake, metabolic stability, and immune system interactions, which cannot be fully replicated *in vitro*.[i]

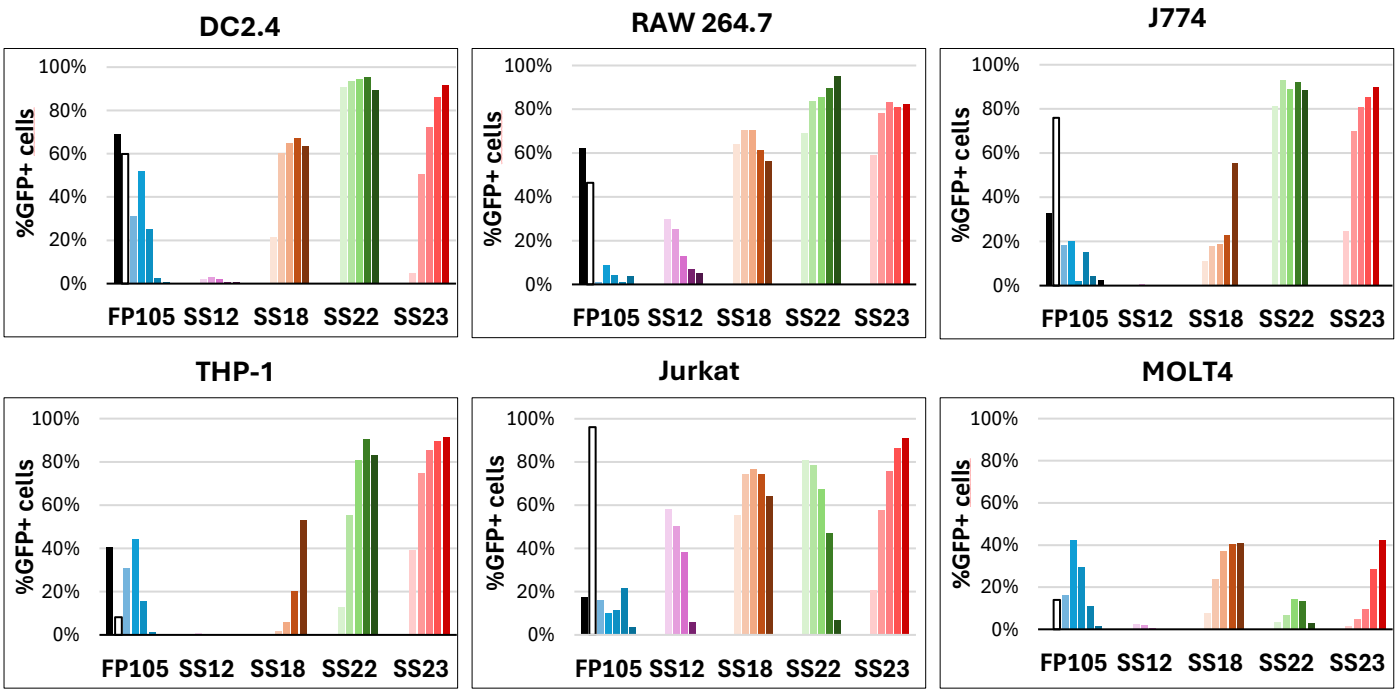

Figure 1: Flow cytometry (FACS) analysis shows the transfection efficiency expressed as the percentage of GFP-positive cells using doses ranging from 0.1, 0.5, 1, 2, and 5 µg of mRNA per well of 24-well plates in various cell lines (DC2.4, RAW 264.7, J774, THP-1, Jurkat, MOLT-4). Transfection efficiency is represented as the percentage of cells expressing GFP. The black bar represents traditional lipoplex (RmesFect), while the white bar represents LNP formulated with the well described and FDA-approved ionizable lipid SM102.

This study demonstrates that LNPs formulated with our proprietary ionizable lipids, especially SS22 and SS23, achieve excellent *in vitro* transfection efficiency across multiple cell lines, including DC2.4, RAW 264.7, J774, THP-1, and Jurkat. The transfection efficiency, measured as the percentage of GFP-positive cells, consistently exceeds 80% in all tested cell lines (Figure 1). Additionally, the mean

fluorescence intensity, which reflects the level of gene expression, exceeds 1×10^7 relative units for SS22 in RAW 264.7 and THP-1 cells, demonstrating robust mRNA delivery and expression. Likewise, SS23 achieves luminescence intensity surpassing 1×10^7 relative luminescence units in J774 and THP-1 cells, further highlighting its efficiency in transfection (Figure 2).

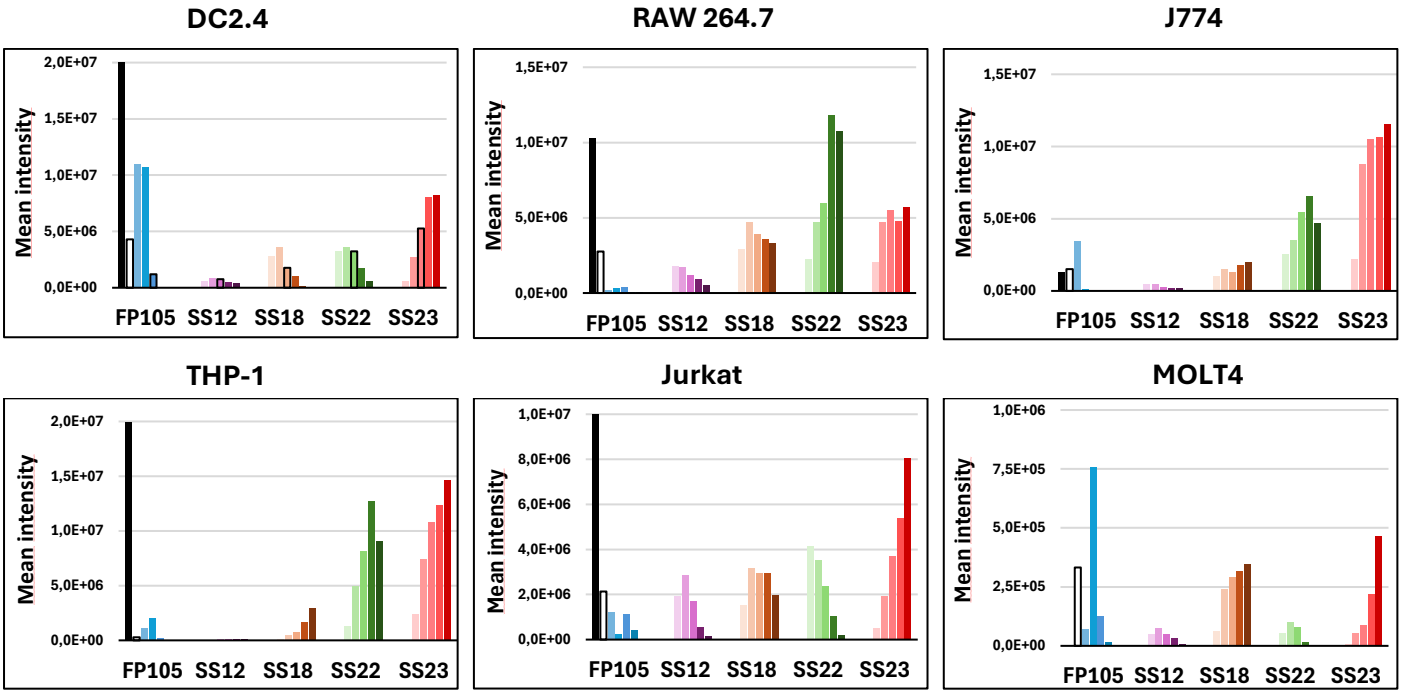

Figure 2: Flow cytometry (FACS) analysis shows the transfection efficiency expressed as the luminescence intensity of FLuc using doses ranging from 0.1, 0.5, 1, 2, and 5 µg of mRNA per well of 24-well plates in various cell lines (DC2.4, RAW 264.7, J774, THP-1, Jurkat, MOLT-4). The black bar represents traditional lipoplex (RmesFect), while the white bar represents LNP formulated with the well described and FDA-approved ionizable lipid SM102.

When compared to SM102-LNP, SS22 and SS23 LNPs exhibit comparable transfection efficiency in DC2.4 and RAW 264.7 cells. Notably, in J774 and THP-1 macrophage/monocyte-derived cell lines, SS22 and SS23 LNPs outperform the standard SM102-LNP, demonstrating superior transfection efficiency. These findings highlight the potential of SS22 and SS23 as highly effective ionizable lipids for LNP-based mRNA delivery, particularly in immune cell applications.

Furthermore, the LNPs were evaluated using the PANC-1 cancer cell line (Figure 3). Once again, LNPs formulated with the ionizable lipids SS22 and SS23 demonstrated the highest efficiency for in vitro transfection, achieving over 90% cell transfection at 0.1 µg of mRNA (GFP).

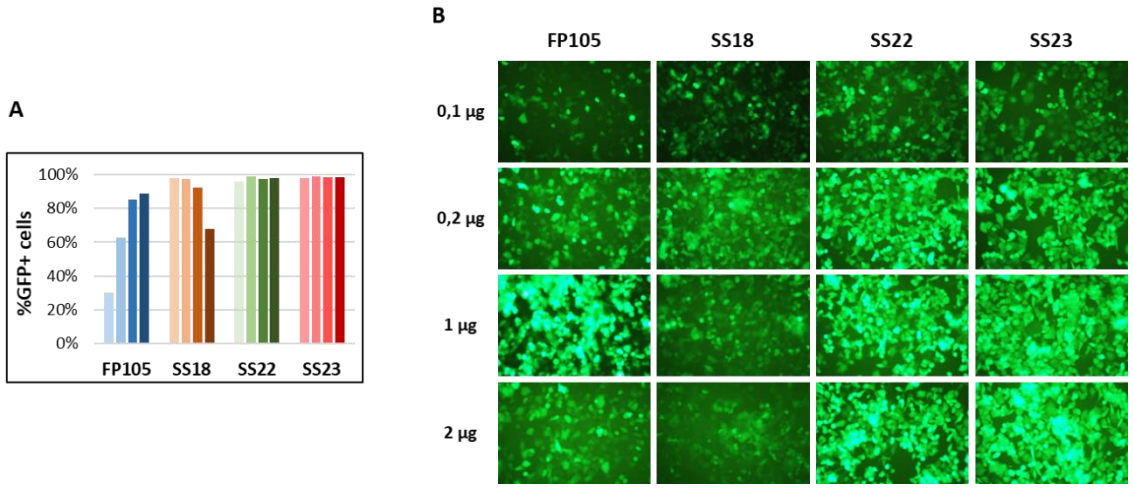

Figure 3: Transfection efficiency of LNP-mRNA (GFP) in PANC-1 cell line. **A** FACS analysis shows the transfection efficiency expressed as the percentage of GFP-positive cells using doses ranging from 0.1, 0.2, 1, and 2 µg of mRNA per well of 24-well plates. **B** Fluorescent microscopy images of PANC-1 transfected cells, 24h post transfection.

These findings underscore the versatility of our ionizable lipids for LNPs in facilitating mRNA transfection across diverse cell types, supporting their application in vaccine development, immunotherapy, oncology, and targeted gene therapies.

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[i] Lindsay, S.; Hussain, M.; Binici, B.; Perrie, Y. Exploring the Challenges of Lipid Nanoparticle Development: The In Vitro–In Vivo Correlation Gap. *Vaccines* **2025**, *13*, 339.