Lipid nanoparticle-mediated *in vitro* transfection of microbubble-activated T cells

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Abstract No.
3484

KEY FINDING

Multiple LNP formulations successfully transfect T cells with minimal T cell exhaustion.

ABSTRACT

Immunotherapy of cancer has revolutionized approaches to controlling and curing cancer, with adoptive transfer of chimeric antigen receptor T cells (CAR-T cells) representing a potentially curative treatment for previously unresponsive tumors. CAR-T cell therapy consists of *ex vivo* expansion of autologous T cells, transfection with the chimeric antigen receptor (CAR), and transfer into the patient. The CAR allows those T cells to identify and kill any cells expressing the antigen of interest, typically a surface protein expressed abundantly and primarily by the tumor. While these methods are extremely effective in a subset of patients, there is opportunity for improvement that may broaden the efficacy of this type of therapy. First, the application of selection, activation, and expansion microbubbles has been shown to result in a less "exhausted" phenotype of the T cells at the end of the expansion phase, resulting in better overall T cell responses. In addition, alternative methods of transfection such as lipid nanoparticles (LNPs) may improve both transfection efficiency and viability. LNP lipid components can be tuned to optimally encapsulate different cargo types and transfect different cell types. Therefore, we formulated a variety of different LNPs to encapsulate either mRNA or pDNA encoding a reporter gene and assessed the relative transfection efficiency in microbubble-activated T cells. Additionally, we evaluated the final activation/exhaustion profile of the transfected cells to determine how uptake of different LNPs may affect the killing effectiveness. These results may ultimately suggest process improvements that have the potential to expand the number of CAR-T cell recipients who have improved responses to therapy.

BACKGROUND

CAR-T cell therapy is a groundbreaking form of immunotherapy, with seven US FDA-approved therapies for hematological malignancies and ongoing investigations for solid tumors. This autologous therapy includes harvesting patient's T cells, engineering them to express CAR constructs, and transfusing them back into the patient to target and destroy cancer cells.[1] Lentiviral transduction and electroporation are both used to deliver CAR constructs but have drawbacks. Lipid nanoparticle (LNP)-mediated transfection is being explored to minimize cytotoxicity and insertional mutagenesis. Earlier studies have shown that SM-102- and C14-4-based LNPs were able to successfully transfect immortal T cells and CD3/CD28-activated T cells.[2,3] In this study, we evaluated five LNP formulations to express a reporter gene (eGFP mRNA) in microbubble-CD3/CD28-activated T cells and stimulated with IL-2 and IL-7.

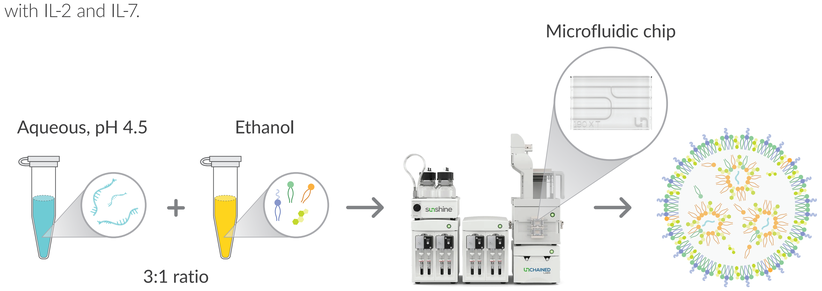

Figure 1 – LNP formulation method using microfluidic device Sunshine from Unchained Labs, where the organic component is mixed with the aqueous component (eGFP mRNA in sodium acetate).

RESULTS

SM-102

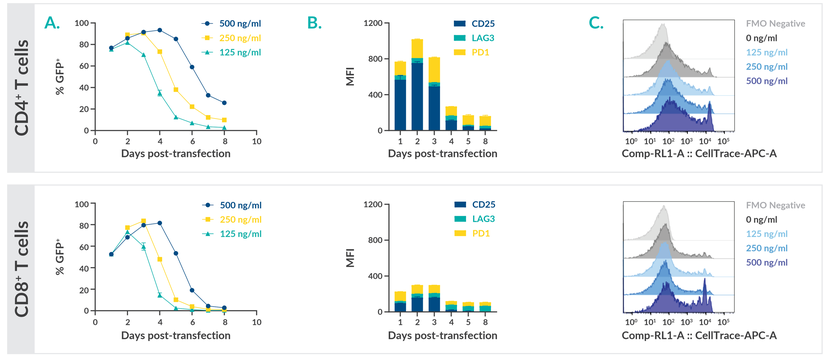

Figure 2 – SM-102 transfects CD4⁺ (top) and CD8⁺ (bottom) T cells after 3 days of activation.
(A) % GFP-expressing cells, **(B)** exhaustion marker expression, and **(C)** proliferation in T cells. While transfection efficiency of SM-102 reached about 80-90%, proliferation was inhibited at the highest concentration tested. Exhaustion markers remained low and stable.

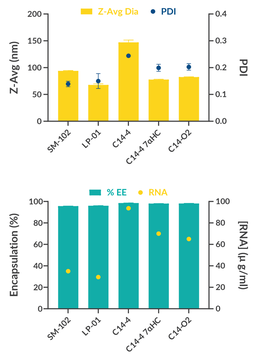

Table 1 – Molar ratios of lipid components in organic phase.

	SM-102	LP-01	C14-4	C14-4 7αHC	C14-O2
Ionizable Lipid	50.0	45.0	35.0	35.0	35.0
Cholesterol	38.5	44.0	46.5	23.3	46.5
7α-Hydroxy cholesterol (7αHC)	-	-	-	23.3	-
DSPC	10.0	9.0	-	-	-
DOPE	-	-	16.0	16.0	16.0
DMG-PEG(2000)	1.5	2.0	2.5	2.5	2.5

Figure 3 – Biophysical characterization of GFP-encoding LNPs.
LNPs based on the noted lipids were formulated with eGFP mRNA. Percent molar ratios of lipid components are shown in Table 1. **(A)** Diameter and polydispersity index (PDI) were measured by dynamic light scattering. **(B)** Encapsulation efficiency (% EE) and RNA concentration were measured using a fluorescent assay. All LNPs are within the desirable size and monodispersity range, with C14-4 being the largest. Encapsulation of the payload is more than 98%, and the concentration of encapsulated eGFP-mRNA in each formulation was used to normalize each experiment.

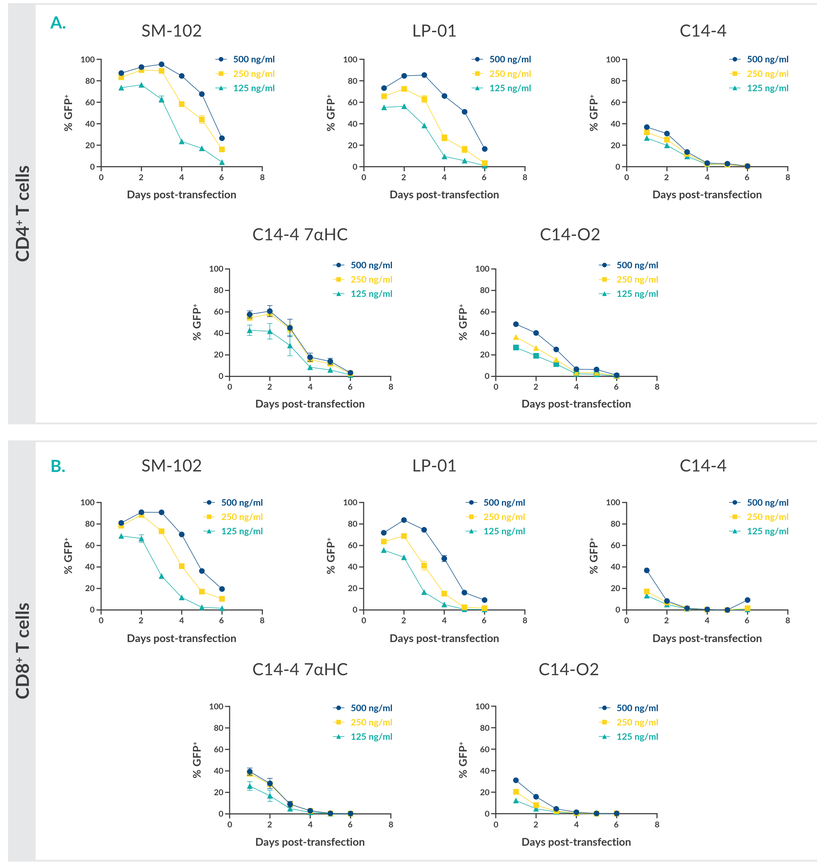

FIGURE 4 – Differential transfection efficiency with different LNPs and T cells.
% GFP expression and exhaustion markers were analyzed by flow cytometry on CD4⁺ **(A)** and CD8⁺ **(B)** T cells gated on Live/CD3⁺ cells. SM-102 and LP-01 resulted in high GFP expression. C14-4 7αHC seems promising and can be further evaluated and optimized. Exhaustion markers remained low and stable as seen in SM-102 (data not shown).

CONCLUSIONS

- SM-102-based transfection of T cells did not affect exhaustion marker expression, but higher concentrations negatively impacted proliferation.
- A variety of formulations successfully transfected T cells with GFP mRNA.
- Substituting 50% of cholesterol with 7α-hydroxy cholesterol (7αHC) improves the transfection efficiency of C14-4 LNPs.
- We have demonstrated a complete workflow from formulating LNPs, transfecting activated T cells, and assessing expression of reporter genes, T cell markers, and proliferation by flow cytometry.

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References

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3. Patel, S.K., Billingsley, M.M., Frazee, C., *et al.* Hydroxycholesterol substitution in ionizable lipid nanoparticles for mRNA delivery to T cells. *J. Control. Release* **347**, 521-532 (2022).