A Novel RNA Lipid Nanoparticle Platform: Gene-edited CAR T Cells for Off-the-Shelf Cancer Therapy

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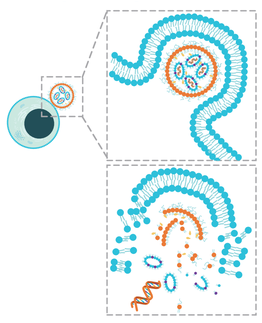
Introduction

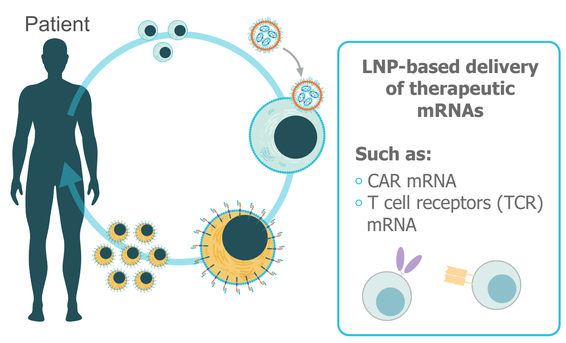
LNPs for gentle and efficient RNA delivery to primary T cells



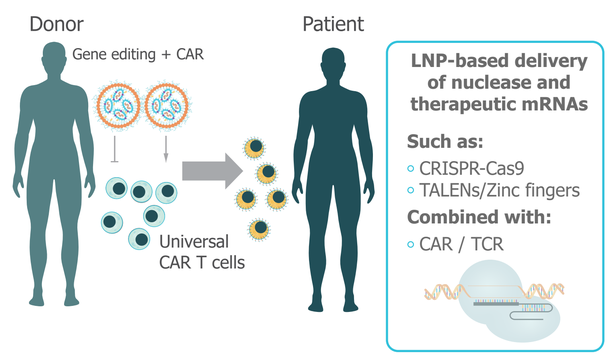
- Engineering T cells with expressions of chimeric antigen receptor (CAR) redirect these cells to target tumours, making this a promising cell-based cancer therapy [1].
- To engineer universal CAR T cells for allogeneic cell therapy, gene editing approaches [2] can be used to remove risks associated with graft-versus-host disease [3].
- Complex gene editing in primary immune can be challenging for some conventional methods [4].
- The LNPs use endogenous uptake pathways to deliver therapeutic RNAs, making this technology gentle to the cells.
- Here we report on the use of a novel lipid nanoparticle (LNP) reagent in a validated protocol to achieve successful complex gene editing in primary T cells with high efficiency while maintaining high cell viability.

Applications

Autologous Cell Therapy

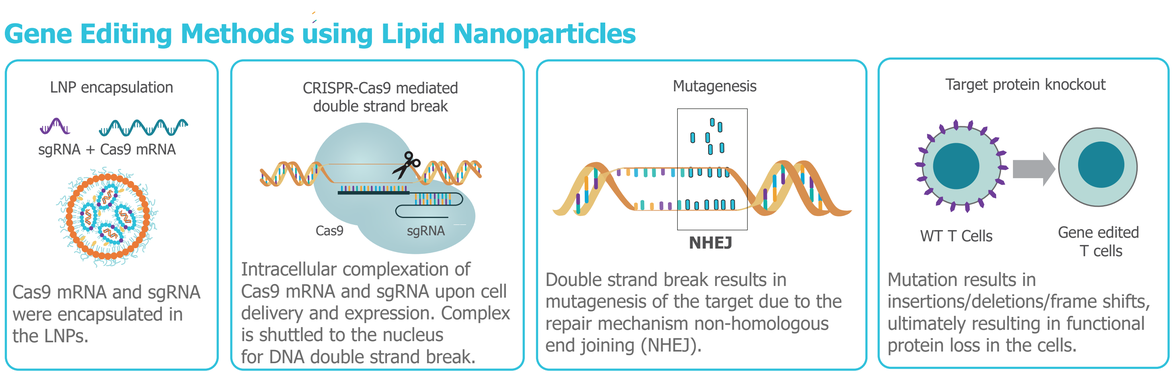


Gene-edited Allogeneic Cell Therapy

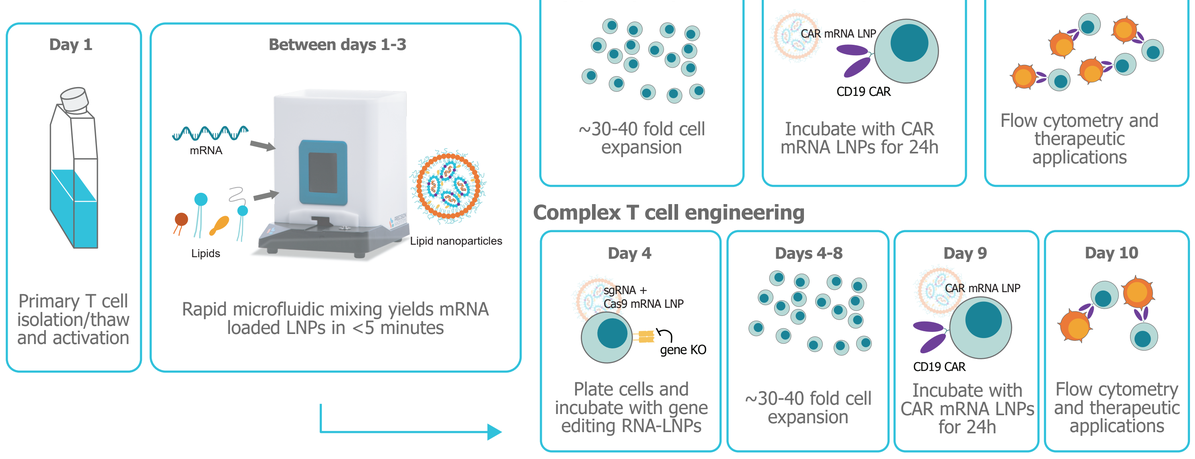


Material and Methods

Gene Editing Methods using Lipid Nanoparticles



Standard Workflow with Minimal Cell Manipulation for Single- or Multi-step T Cell Engineering



Materials

Lipid nanoparticle reagent: GenVoy-ILM T Cell Kit for mRNA, Precision NanoSystems Inc., 1000683 • CleanCap® Cas9 mRNA (5moU), Trilink, L-7206 • CleanCap® CD19-CAR mRNA (wt), Trilink, custom order • CleanCap® GFP mRNA (wt), Trilink L-7601 • sgRNAs Alt-R® CRISPR-Cas9 sgRNA, Integrated DNA Technologies, custom targets • Quant-iT™ RiboGreen® RNA Assay Kit,Thermo Fisher Scientific, R11490 • Cryopreserved T cells, STEMCELL Technologies Inc., 70024 • ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator, STEMCELL Technologies Inc., 10970 • ImmunoCult™-XF T Cell Expansion Medium, STEMCELL Technologies Inc., 10981

Equipment

NanoAssemblr Spark Precision NanoSystems Inc., NIS0003 • BioTek™ Synergy™ H1 plate reader • CytoFLEX V3-B3-R0, Beckman Coulter, C09747

Results

Efficient Delivery of GFP mRNA with High Cell Viability

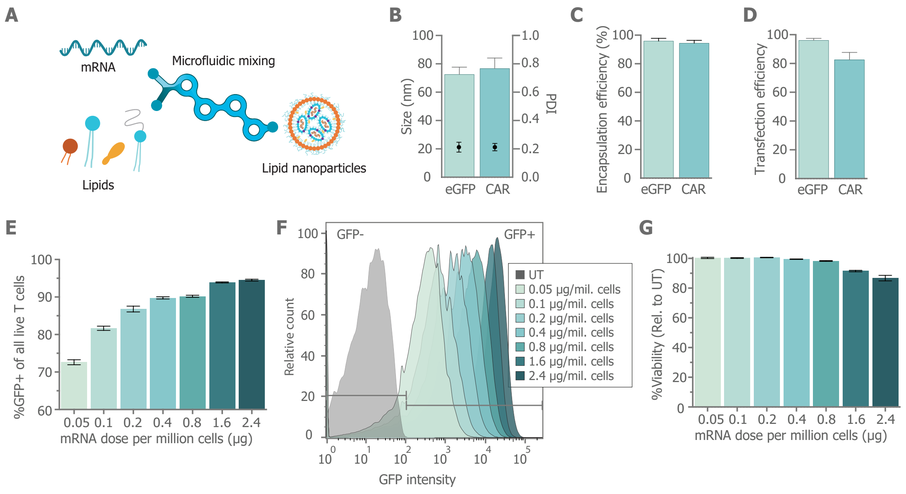


Figure 1. A) Schematic illustration of LNP formation using microfluidic mixing. **B)** Size (Z-average) and PDI, **C)** encapsulation efficiency, and **D)** transfection efficiency in primary T cells of LNPs loaded with either GFP (n=27) or CAR (n=13) mRNA, in n=2 primary cell donors. **E)** Percent GFP expression **F)** Representative GFP flow histogram and **G)** cell viability relative to untreated cells (UT) at indicated treatment dose of 0.025 to 1.2 µg/million cells. For all: 10 µg of RNA was incapsulated per sample, as instructed by the manufacturer.

Delivery of CD19 CAR mRNA with High Efficiency and High Cell Viability

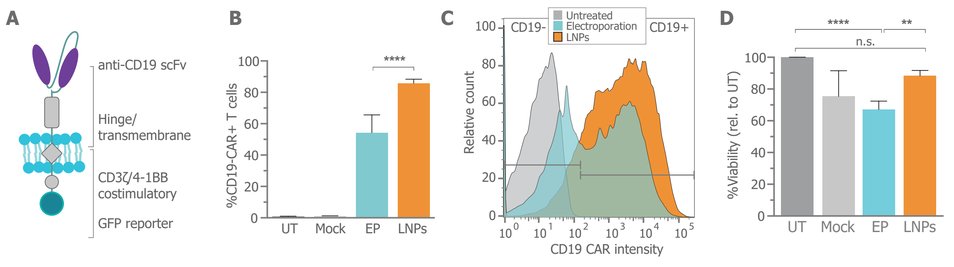


Figure 2. A) Schematic illustration of the custom CAR construct. **B)** CAR transfection efficacy in primary T cells assessed using flow cytometry. N=2 sample replicates. **C)** Surface expression of CD19 CAR as detected by flow cytometry. **D)** Cell viability normalized to untreated (UT) cells. Significance evaluated using one-way ANOVA with multiple comparisons.

Efficient Knock Out TCR through Co-Delivery of Cas9 mRNA and sgRNA

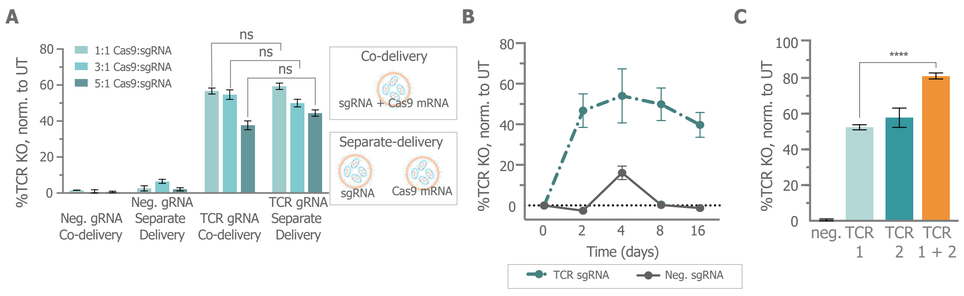


Figure 3. A) Cas9 mRNA and sgRNA weight ratios were varied during microfluidic encapsulation as indicated. A total of 10 µg RNAs were encapsulated per sample. Primary T cells were treated with LNPs at an encapsulated mRNA dose of 3.2 µg/million cells. **B)** Kinetics of TCR KO onset was performed with detection over the course of 16 days. **C)** TCR KO was performed with two different sgRNAs, along with the combination of guides 1 and 2 at half the mol%. Significance evaluated using t-tests among selected groups.

Knock Out of TCR and CD3 in a Dose-dependent Manner

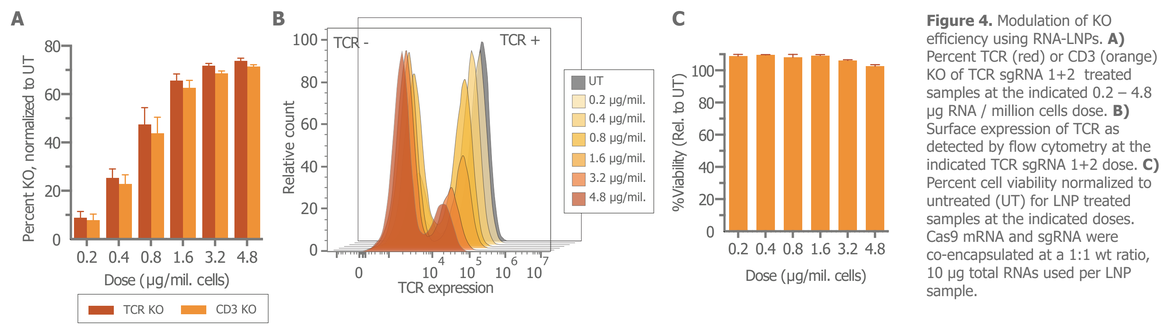


Figure 4. Modulation of KO efficiency using RNA-LNPs. **A)** Percent TCR (red) or CD3 (orange) KO of TCR sgRNA 1+2 treated samples at the indicated 0.2 – 4.8 µg RNA / million cells dose. **B)** Surface expression of TCR as detected by flow cytometry at the indicated TCR sgRNA 1+2 dose. **C)** Percent cell viability normalized to untreated (UT) for LNP treated samples at the indicated doses. Cas9 mRNA and sgRNA were co-encapsulated at a 1:1 wt ratio, 10 µg total RNAs used per LNP sample.

Knock Out of Both CD52 and TCR in a Multiplex Gene Editing Approach

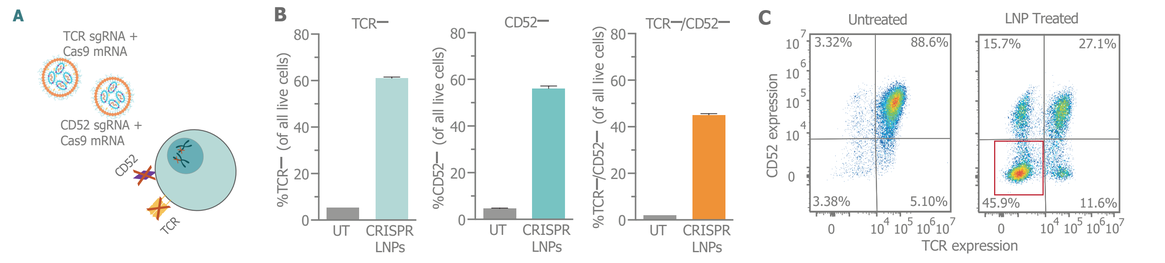


Figure 5. A) Schematic illustration of the experiment. For multiplexing, two LNP samples were prepared, one containing TCR sgRNAs 1+2, and with one CD52 sgRNAs 1+2. LNPs were encapsulated at a 1:1 Cas9 mRNA : sgRNA ratio wt ratio, as before. **B)** Percent TCR negative, CD52 negative or double negative cells at 3.2 µg/million cells RNA dose. **C)** Quadrant analysis of CD52 and TCR levels in untreated or LNP treated T cell population at 3.2 µg/mil treatment dose.

Generated Gene-edited CAR T Cells are Highly Functional for Cancer Therapy

Figure 6. A) TCR KO on Day 7 when treated with TCR sgRNA 1+2 and Cas9 mRNA at a dose of 3.2 µg/million cells. Starting sample was purified by TCR negative selection prior to downstream application use. **B)** Percent CD19 CAR expression 24h after treatment with CAR mRNA LNPs at 3.2 µg RNA/million cells, in the untreated or TCR KO populations. Lower CAR expression is attributed to a lower activation state after TCR knockout. **C)** Specific lysis of SUP-B15 cells by UT (grey), TCR+/CAR19+, or gene edited TCR−/CAR19+ T cells at the indicated effector (T cell) to target (B cell) ratios, E:T. Significance evaluated using t-tests (Figs A and B) or one-way ANOVA with multiple comparisons (Fig C).

Conclusions

- We present utility of a novel LNP reagent, GenVoy-ILM T Cell Kit for mRNA, for gene delivery and multiplex gene editing approaches in both autologous and allogeneic cell therapy research and development.
- Specifically, we demonstrated a 81.5 ± 4.3% CD19 CAR expression and 74.7 ± 5.1% TCR gene knock-out using CRISPR-Cas9, while maintaining >90% cell viability.
- RNA LNPs can be produced rapidly using PNI's scalable microfluidic technology from discovery to clinical translation, and can be easily integrated into any existing cell culture workflow with a validated protocol.

References

[1] Feins, S., Kong, W., Williams, E. F., Milone, M. C., & Fraietta, J. A. (2019). An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer. American journal of hematology, 94(S1), S3-S9. [2] Rafiq, S., Hackett, C. S., & Brentjens, R. J. (2020). Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nature reviews Clinical oncology, 17(3), 147-167. [3] Zhao, J., Lin, Q., Song, Y., & Liu, D. (2018). Universal CARs, universal T cells, and universal CAR T cells. Journal of hematology & oncology, 11(1), 1-9. [4] Atsavapranee, E. S., Billingsley, M. M., & Mitchell, M. J. (2021). Delivery technologies for T cell gene editing: Applications in cancer immunotherapy. EBioMedicine, 67, 103354.

Acknowledgments

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