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

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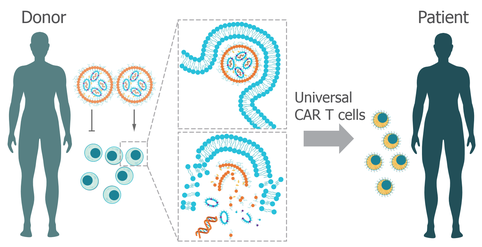
Introduction

- Engineering T cells to express chimeric antigen receptor (CAR) redirects 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 can be used to remove risks associated with graft-versus-host disease [2,3].
- However, conventional delivery methods such as viral vectors and electroporation have performance and manufacturing shortcomings that limit their utility for the multi-step engineering of T cells [4].
- Lipid nanoparticles (LNPs) solve these shortcomings, using endogenous uptake pathways to deliver therapeutic RNAs, making this technology gentle to cells. LNPs are fully synthetic and can be rapidly scaled-up using microfluidics [5,6].
- Here we report on the use of a novel LNP reagent in an optimized protocol to achieve successful multi-step engineering of primary T cells with high efficiency and high cell viability.

LNP-based Off-the-Shelf T Cell Therapy

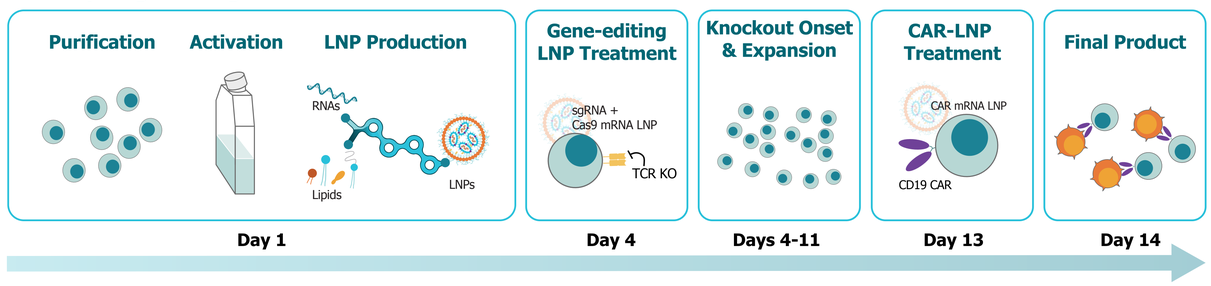


LNP-based delivery of nuclease and therapeutic mRNAs

Gene editing with:	Combined with:
CRISPR-Cas9	CAR / TCR mRNA
TALENs/Zinc fingers	

Material and Methods

Multi-step Engineering for Gene-Edited CAR T Cells



Materials

Lipid nanoparticle reagent, GenVoy-ILM™ T Cell Kit for mRNA, Precision NanoSystems ULC, 1000683 and 1001144 ● CleanCap® Cas9 mRNA (5moU), Trilink, L-7206 ● CleanCap® CD19-CAR mRNA (wt), Trilink, custom order ● 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 (NIS0003) and Ignite (NIN0001), Precision NanoSystems ULC ● BioTek™ Synergy™ H1 plate reader ● Cyto-FLEX V3-B3-R0, Beckman Coulter, C09747

Results

Expression on CD19 CAR with High Efficiency and High Cell Viability

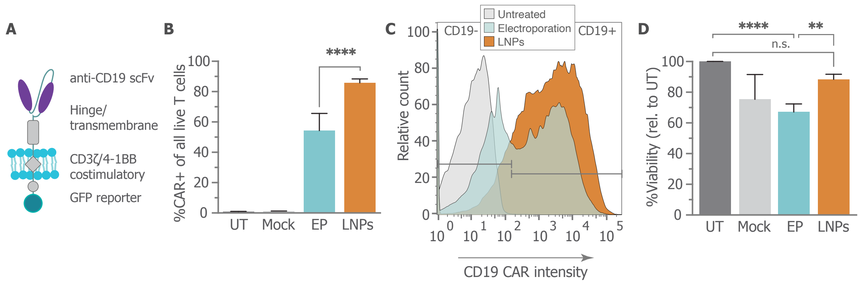


Figure 1. A) Schematic illustration of the custom CAR mRNA construct. **B)** CAR transfection efficacy 24 h post LNP addition in primary T cells. Assessed using flow cytometry with n=2 sample replicates. **C)** Surface expression profile of CAR analyzed by flow cytometry. **D)** Cell viability normalized to untreated (UT) cells. Significance evaluated using one-way ANOVA.

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

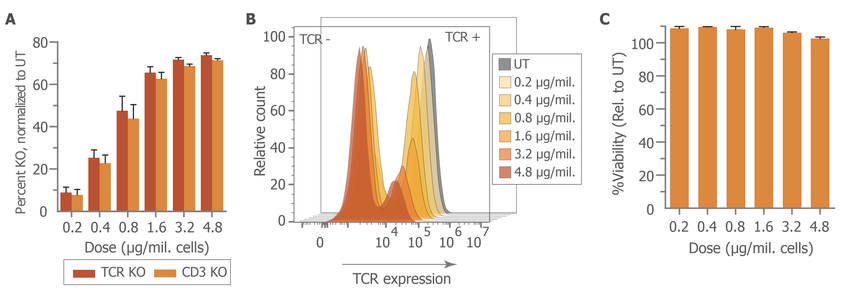


Figure 2. A) Percent TCR (red) or CD3 (orange) knockout of TCR sgRNA treated samples at the indicated 0.2 – 4.8 µg RNA / million cells dose. **B)** Surface expression of TCR as detected by flow cytometry at the indicated RNA dose. **C)** Percent cell viability normalized to untreated (UT) for LNP treated 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

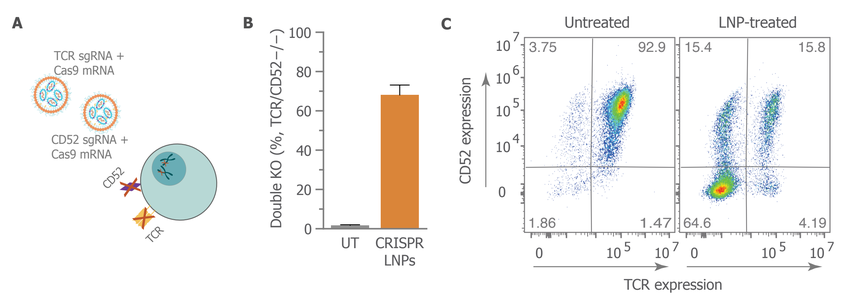


Figure 3. A) Schematic illustration of the experiment. For multiplexing, two LNP samples were prepared, one containing TCR sgRNA and with one CD52 sgRNAs. **B)** Percent TCR and CD52 double negative cells at a 3.2 µg/million cells RNA dose. **C)** Corresponding quadrant analysis of CD52 and TCR levels in the untreated (UT) or LNP treated T cell population.

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

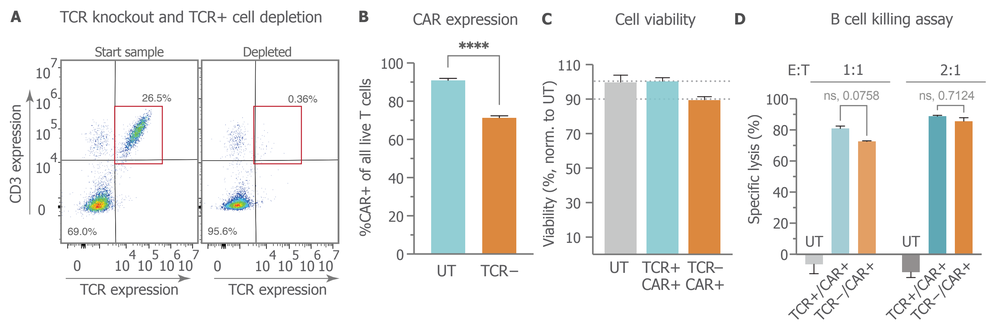


Figure 4. A) TCR KO on Day 7 when treated with TCR sgRNA and Cas9 mRNA at a dose of 3.2 µg RNA/million cells. Starting sample was purified by TCR negative selection prior to downstream applications. **B)** Percent α CD19-CAR expression 24 h after treatment with CAR mRNA LNPs at 3.2 µg RNA/million cells, in the untreated or TCR KO populations. Lower CAR expression is attributed to a lower activation state after TCR knockout. **C)** Percent cell viability normalized to UT after CAR expression or CAR expression and TCR knockout. **D)** Specific lysis of SUP-B15 cells by UT, TCR+/CAR19+, or thhe gene edited TCR−/CAR19+ T cells at the indicated effector (T cell, E) to target (B cell, T) ratios, E:T. Significance evaluated using t-tests (Fig. B) or one-way ANOVA with multiple comparisons (Fig. D).

Scalable Microfluidic Technology Allows for Discovery to Pre-clinical Translation

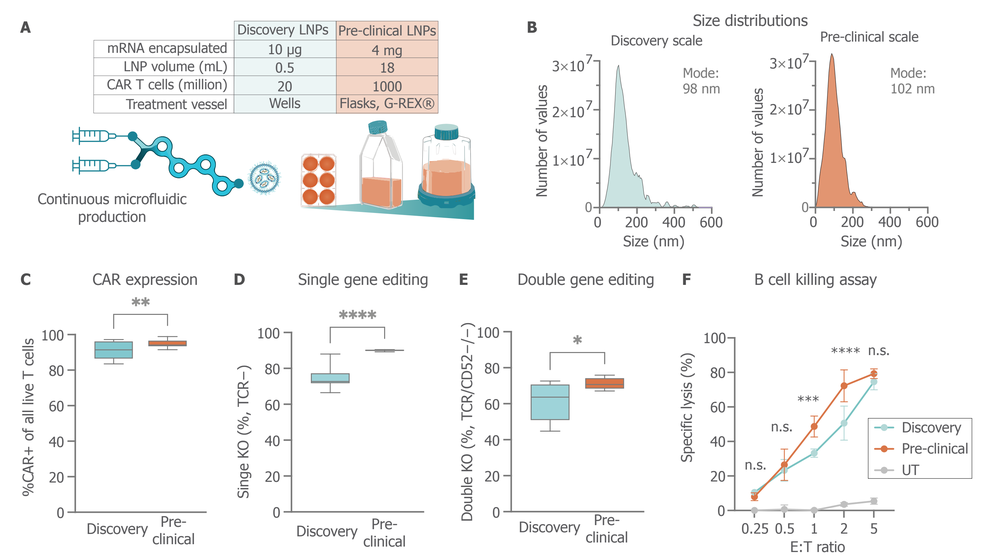


Figure 5. A) Seamless scale-up allows for the production of discovery scale LNPs at 10 µg RNA, to pre-clinical scale at 4 mg RNA. **B)** Nanoparticle Tracking Analysis (NTA) shows agreeable size distribution between the two scales. **C)** α CD19-CAR transfection efficiency 24 h post mRNA LNP addition for both scales. **D)** Single target (T cell receptor, TCR) knockout, and **E)** double target (TCR and CD52) knockout through sgRNA and Cas9 mRNA delivery. **F)** Functional killing of CD19+ B cells (SUP-B15) in 16 h co-coculture experiment for both scales. For all: a dose of 3.2 µg RNA/million cells was applied to human primary T cells. Average gene expression and gene knockout shows at least n=8 LNPs and n=2 donors. Functional killing shows n=2 LNPs and n=2 donors. Error bars represent standard deviation with significance evaluated using t-tests among selected groups.

Conclusions

- The presented LNP reagent, GenVoy-ILM T Cell Kit for mRNA, is versatile for gene expression, gene editing, or the combination of both in a multi-step approach.
- LNPs show exceptional performance with 81.5 ± 4.3% CD19 CAR expression and 74.7 ± 5.1% TCR gene knockout in primary T cells. LNPs are gentle and maintain >90% cell viability.
- LNPs are easily scalable using PNI's microfluidic technology, where both the discovery and preclinical LNPs show excellent performance.

References

[1] Feins, S., et al., An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer. Am J Hematol, **2019**. 94(S1): p. S3-S9. **[2]** Rafiq, S., C.S. Hackett, and R.J. Brentjens, Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat Rev Clin Oncol, **2020**. 17(3): p. 147-167. **[3]** Zhao, J., et al., Universal CARs, universal T cells, and universal CAR T cells. J Hematol Oncol, 2018. 11(1): p. 132. **[4]** Atsavapranee, E.S., M.M. Billingsley, and M.J. Mitchell, Delivery technologies for T cell gene editing: Applications in cancer immunotherapy. EBioMedicine, **2021**. 67: p. 103354. **[5]** Absalon, J., K. Koury, and W.C. Gruber, Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. Reply. N Engl J Med, **2021**. 384(16): p. 1578. **[6]** Baden, L.R., et al., Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med, **2021**. 384(5): p. 403-416.

Acknowledgments

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