Enhanced Gene Knock-In Efficiency Using a GMP-Grade Ionizable Lipid FL-0445 in Lipid Nanoparticle Formulations for Ex Vivo T Cell and HSPC Engineering

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Background

- Current gene delivery methods, such as viral vectors and electroporation, face challenges including high cost, safety concerns, and reduced cell viability.
- Lipid nanoparticles (LNPs) offer advantages in ease of manufacturing and preservation of cell viability; however, achieving sufficiently high gene knock-in efficiency remains a major hurdle.
- Here, we developed **LNPs for *ex vivo* gene knock-in**, utilizing the ionizable lipid FL-0445 obtained from FUJIFILM Corporation.
- **FL-0445 has been employed in a Phase III vaccine clinical trial, is available as a GMP-grade material, and thus presents strong translational potential.**

Formulation Optimization

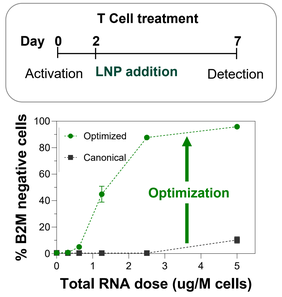
LNP formulation optimization enhanced *ex vivo* gene knock-out efficiency to > 95%.

Canonical LNP formulation

Lipid component screening
Sterol, Phospholipid, PEG-lipid

Lipid ratio optimization
by Design of Experiment (DoE) methodology

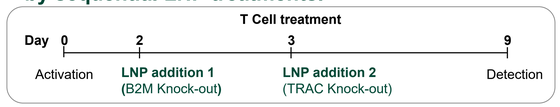
B2M knock-out evaluation
in human primary T cell

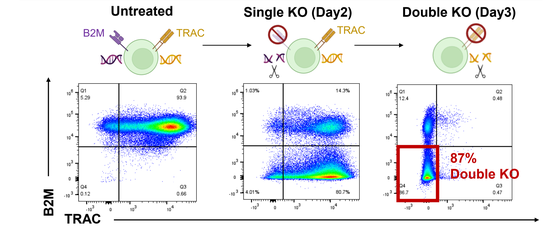


- T cells were isolated from human primary PBMC using bead-based separation and activated on Day 0 with CD3/CD28/CD2 stimulation.
- On Day 2, LNPs encapsulating gene editing nucleic acids were added to the cells in the presence of ApoE.

Multiple Gene Knock-out (T cell)

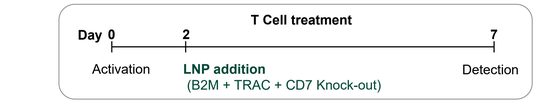
> 85% Double knock-out (B2M+TRAC) was achieved by sequential LNP treatments.

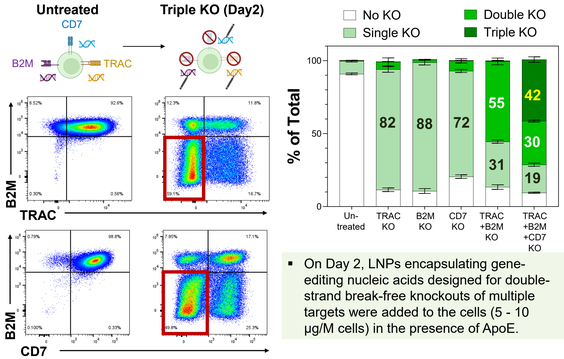




- LNPs encapsulating gene editing nucleic acids were prepared.
- On Day 2, LNPs encapsulating gene editing nucleic acids for B2M knock-out were added to the cells in the presence of ApoE (10 μg/M cells).
- On Day 3, following medium replacement, LNPs encapsulating gene editing nucleic acids for TRAC knock-out were added to the cells (10 μg/M cells), also in the presence of ApoE.

> 40% Simultaneous Triple knock-out (B2M+TRAC+CD7) was achieved with a double-strand break-free enzyme.





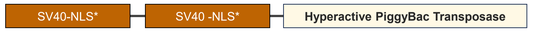
- On Day 2, LNPs encapsulating gene-editing nucleic acids designed for double-strand break-free knockouts of multiple targets were added to the cells (5 - 10 μg/M cells) in the presence of ApoE.

Gene Knock-in (T cell, HSPC)

To enhance gene knock-in efficiency, donor DNA with a shorter backbone and mRNA containing double nuclear localization signals (NLS) were used.

- CD19 ScFv-CD8-4-1BB-CD3ζ Nanoplasmid DNA (npDNA, 3,548 bp), purchased from Aldevron via their custom Nanoplasmid vector synthesis service, was used as donor DNA.
- Gene insertion was mediated by Hyperactive PiggyBac Transposase mRNA, which includes two NLS sequences added to the N-terminus of the transposase. The mRNA was purchased from TriLink through their custom mRNA synthesis services.
- npDNA (10 μg/M cells) and Transposase mRNA (2.5 μg/M cells) were separately encapsulated in LNPs and added to the cells simultaneously in the presence of ApoE.
- Experiments utilized T cells isolated from human primary PBMCs or bone marrow-derived human primary CD34⁺ cells.

Schematic diagram of the Transposase mRNA construct

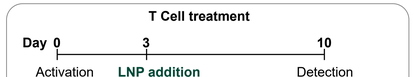


NLS of SV 40 large T antigen: **KKKRKV***

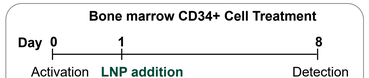
*Lu, J., et.al. (2021). Types of nuclear localization signals and mechanisms of protein import into the nucleus. *Cell Communication and Signaling*, *19*(1).

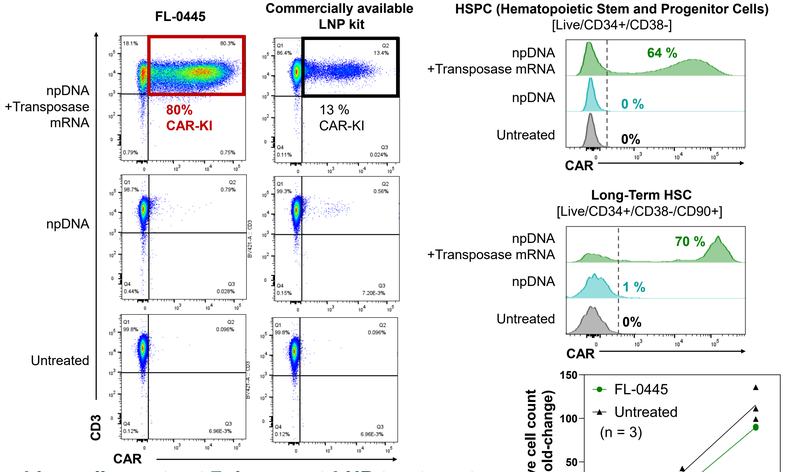
> 70% CD19-CAR knock-in with Transposase was demonstrated in both human primary T cells and Long-Term HSCs.

Human Primary T cell



Human Primary HSPC

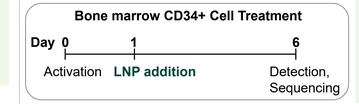




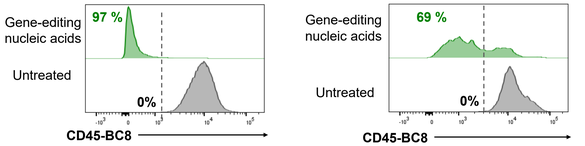
Live cell counts at 7 days post-LNP treatment showed no significant difference compared to the untreated group (data from T cell treatment).

Epitope Editing (HSPC)

> 95% knock-out of the target epitope was demonstrated in HSPCs with a double-strand break-free enzyme.



- LNPs encapsulating gene-editing nucleic acids designed for epitope editing at the BC8 epitope of CD45 were prepared and added to the cells (10 μg/M cells) in the presence of ApoE.
- Sanger sequencing was performed without separating LT-HSCs from the HSPC population.



**Wellhausen, N., et.al. (2023). Epitope base editing CD45 in hematopoietic cells enables universal blood cancer immune therapy, *Science Translational Medicine*, 15(714)

Summary

- FL-0445 LNP with an optimized formulation demonstrates broad applicability for *ex vivo* gene editing, achieving high editing efficiency.

	T cell	HSPC	NK cell***
Knock-in	✓	✓	✓
Knock-out	✓	✓	✓

***Data not presented

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