Electroporation and Lipid Nanoparticles as Delivery Methods for Targeted CRISPR/Cas9 Gene Editing in Primary Human T-Cells

Aaron Hoover and Ming Jin, Sharlene Amador, Chesney Michels, Jeff Cram, Mercedes Segura, Mike Paglia

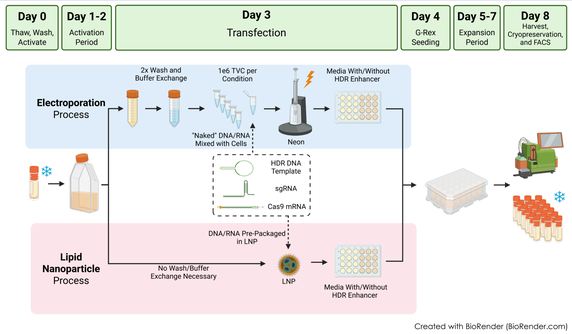
ElevateBio Basecamp®, 200 Smith Street, Waltham MA 02451, US.

Abstract

Chimeric Antigen Receptor (CAR)-T cell therapy has emerged as a revolutionary approach in autoimmune disease and cancer treatment, showing remarkable success in managing B-cell malignancies. However, the reliance on viral vectors for gene editing in CAR-T cell manufacturing presents challenges, including high costs, stringent regulations, and safety concerns such as insertional mutagenesis, all of which pose risks to manufacturing success or patient health. Alternative editing approaches, such as transposon systems and mRNA-based transduction, have attempted to address these risks but are often hindered by issues like random integration and low or transient CAR expression. Recently, the CRISPR-Cas9 editing system has shown promise in producing gene-specific CAR-T cells by employing non-viral transfection methods such as electroporation (EP) and lipid nanoparticles (LNP) to introduce nucleic acids to T-cells, offering a targeted and more precise system for gene knockout and insertion compared to viral vectors.

In this study we examined and compared the effects of non-viral transfection methods for gene knockout/insertion in primary human T-cells and demonstrated the feasibility of an LNP-based transfection process wherein CRISPR/Cas9 nucleic acids and transgene HDR template DNA are formulated together in a single LNP-encapsulated payload.

Materials and Methods



Created with BioRender (BioRender.com)

Cryopreserved T-cells from healthy donors were thawed, washed, and activated before being seeded into flasks. On Day 3 the activated T-cells from each donor were split into two experimental arms for transfection, (1) gene modification payload delivery via electroporation and (2) delivery via pre-formulated lipid nanoparticles, to genetically edit 1e6 total viable cells (TVC) per condition. Electroporation cargo were formulated with CRIPSR/Cas9 mRNA and TRAC sgRNA in combinations with GFP and CD19-CAR HDR templates, respectively. Lipid nanoparticles were formulated at the ratio 1:1:2 or 1:1:4 (Cas9 mRNA: TRAC sgRNA: GFP/CD19-CAR HDR template) for their cargo delivery. Following transfection, edited T-cells were seeded into G-Rex 24-well plates, with and without an HDR enhancer, and allowed to recover overnight. The following day, cells were sampled for cell count and viability (CCV) and transferred to a new G-Rex 24-well plate for expansion. At Harvest, the cells were sampled for their cell count and viability, washed, concentrated, and analyzed via FACS to evaluate T-cell phenotype and transfection editing efficiency (i.e., knockout of TRAC locus and expression of GFP or CD19-CAR).

Note: GFP and CD19-CAR HDR templates are proprietary, circular, single-stranded (ss) DNA templates ("MegaBulb DNA") generated by TouchLight (www.Touchlight.com).

TRAC Knockout Efficiency and Cell Yield

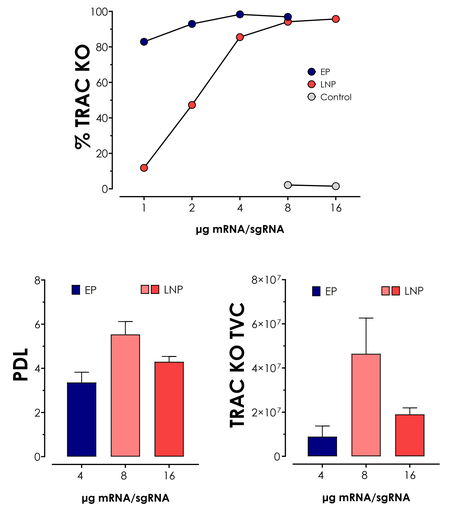


Figure 1. Head-to-head comparison of EP- and LNP-delivered gene editing components targeting knockout of the TRAC locus showed that EP (blue) achieved higher TRAC knockout percentages at lower mRNA/sgRNA concentrations compared to LNP delivery (red), which required 2-4x more RNA cargo to reach comparable editing levels. LNP delivery, however, led to higher population doubling levels (left) across conditions, leading to significantly more total viable TRAC knockout cells (right) at harvest.

GFP and CD19-CAR Knock-in Efficiency

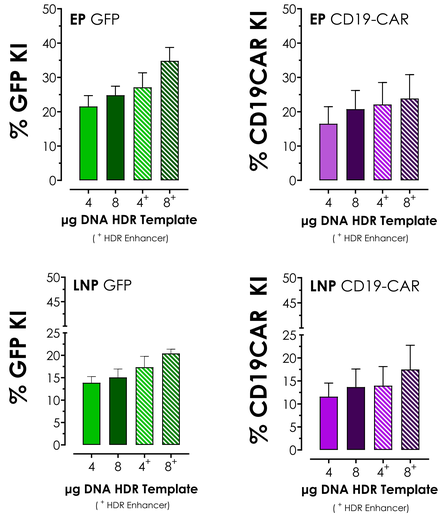


Figure 2. Electroporated cell groups (top) demonstrated a consistent increase in both GFP and CD19-CAR expression with increasing HDR template payload, an effect further enhanced by the addition of HDR enhancer. In contrast, LNP-mediated delivery (bottom) of corresponding amounts of HDR template resulted in lower knock-in efficiencies. HDR enhancer addition appeared to moderately improve the efficiency of LNP-mediated HDR template delivery, but the overall efficiency remained significantly lower compared to EP.

Cell Viability, Population Doubling Level, & Transgene-Positive Cell Yield

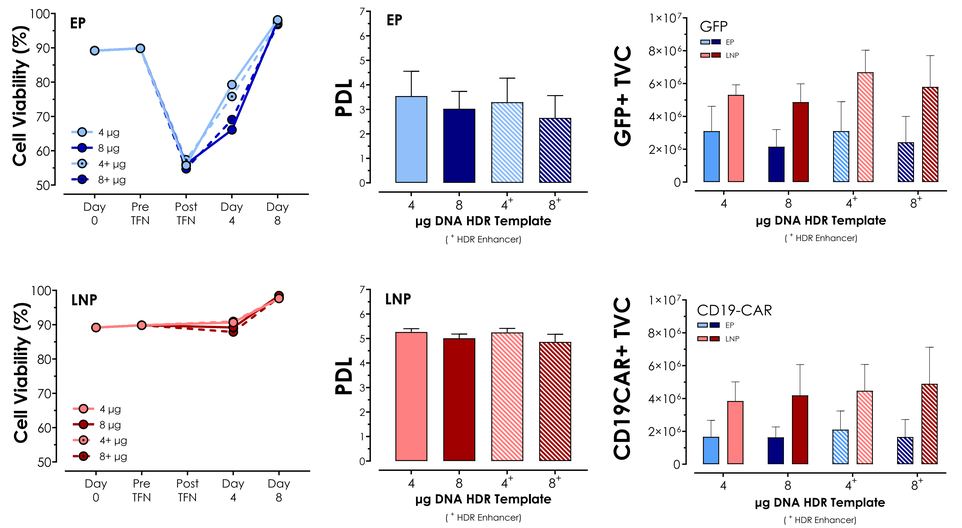


Figure 3. Cell viability kinetics (left) demonstrated that EP groups experienced a significant post-transfection (TFN) viability drop with DNA template addition, while LNP groups maintained a consistently high cell viability. This cellular stress impacted population doubling levels (center), which declined with increasing template concentration in EP but remained stable across LNP conditions. The critical manufacturing implications are shown in the right panels, where total GFP+ and CD19-CAR+ cell yields are compared. Despite EP showing higher percentage transgene expression, the superior expansion of LNP-treated cells resulted in comparable or higher total edited cell yields. The addition of HDR enhancer (hatched bars) improved outcomes for both delivery methods but provided greater relative benefit to the EP approach.

T-Cell Memory Phenotype Analysis

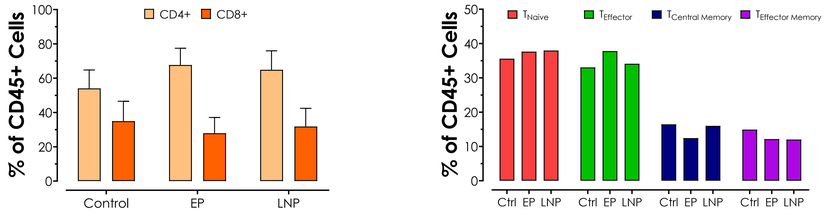


Figure 4. The distribution of CD4+/CD8+ T-cell subsets (left panel) remained relatively consistent across groups. Similarly, memory phenotype distributions (right panel) showed comparable proportions of naive, central memory, effector memory, and terminal effector cells regardless of delivery method.

Conclusion

This study demonstrated the feasibility of using non-viral transfection methods for ex-vivo gene knockout and insertion in primary human T-cells. Both electroporation and LNP-mediated delivery methods effectively facilitated the knockout of the TRAC locus and insertion of HDR templates at the targeted site, albeit with varying efficiencies and cell health outcomes. EP allowed for higher gene knockout/knock-in efficiencies compared to LNP at similar payload concentrations; however, LNP-delivered transfection preserved the viability and expansion capacity of the cells, generating up to 4-fold higher total viable edited cells. The addition of HDR enhancer improved gene editing outcomes across both delivery platforms, enabling higher template integration efficiency without compromising cell viability. Additionally, neither gene editing approach altered the phenotypic characteristics of the engineered T-cells, preserving critical CD4/CD8 ratios and memory/effector phenotypes.

These findings highlight the potential of non-viral delivery systems as alternatives to traditional viral vector approaches for next-generation cell therapies. By understanding and optimizing the balance between editing efficiency and cell preservation, both EP and LNP platforms represent promising technologies for the development of safer and more cost-effective CAR-T cell therapies with reduced manufacturing complexity.

Learn More

EMAIL
bcbd@elevate.bio