Transfecting αβ T Cells with CRISPR-Cas9 RNP Using a Novel Microfluidic Platform

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Introduction

The introduction of CRISPR-Cas systems has advanced the field of cell and gene therapy by opening possibilities for the targeted engineering of the genome. One application of this technology is to develop chimeric antigen receptor (CAR) T-cell therapy for the detection of cancer antigens. CAR T-cell therapy using αβ T cells is currently limited to autologous applications, as the endogenous T-cell receptors on αβ T cells would induce graft vs. host disease if introduced in unmatched recipients. To expand its applications to allogeneic therapies, that T-cell receptor can be knocked out by targeted editing with CRISPR-Cas9. **We have developed a novel microfluidic platform capable of volume exchange for convective transfection (VECT)**, which can effectively deliver a variety of payloads, such as CRISPR-Cas9 ribonucleoprotein complexes (RNP). We sought to characterize the capability and efficacy of this system for CRISPR-Cas9 knockouts at the T-cell receptor alpha constant (*TRAC*) locus.

Objectives

- Optimize VECT for the delivery of CRISPR-Cas9 RNP in primary human αβ T cells
- Assess the effect of VECT on cell functionality
- Evaluate the editing efficiency of CRISPR-Cas9 knockouts at both the protein and genomic level
- Confirm the physical localization of the CRISPR-Cas9 into the T-cell nucleus

Experimental Design

Human T cells were placed in fresh media with 18 µg/ml of *TRAC* CRISPR-Cas9 RNP and processed with the VECT microfluidic transfection device. For the microscopy images, T cells were transfected in media with 27 µg/ml of fluorescently tagged *TRAC* CRISPR-Cas9 RNP. Cells were cultured for 120 hours after VECT and editing efficiency evaluated with flow cytometry.

Results

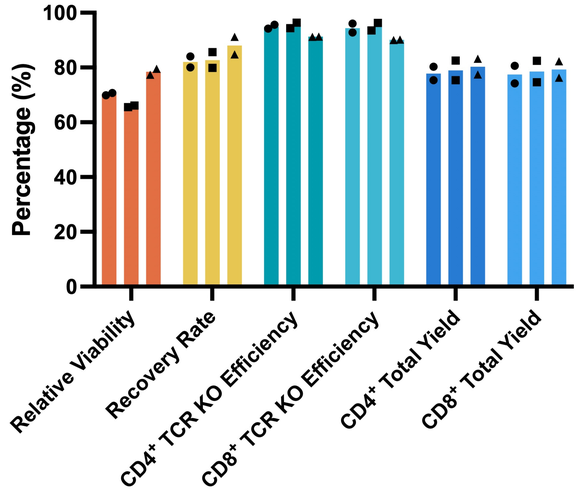

Figure 1: Performance of VECT for RNP-mediated TCR KO in CD4+ and CD8+ cells

The transfection of *TRAC* CRISPR-Cas9 RNP using VECT showed high viability and recovery (up to 80%) and demonstrated high levels of TCR knockout (>80%) in both CD4+ and CD8+ cells across 3 donors.

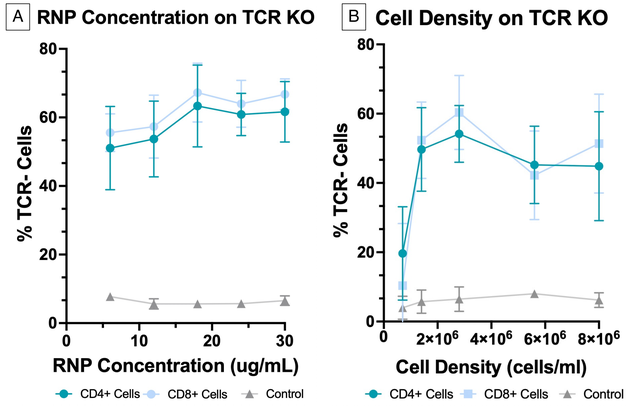

Figure 2: Operational range of cell and payload concentration on TCR knockout

The VECT transfection platform was effective in inducing KOs for a wide range of payload (*Figure 2A*) and cell concentrations (*2B*).

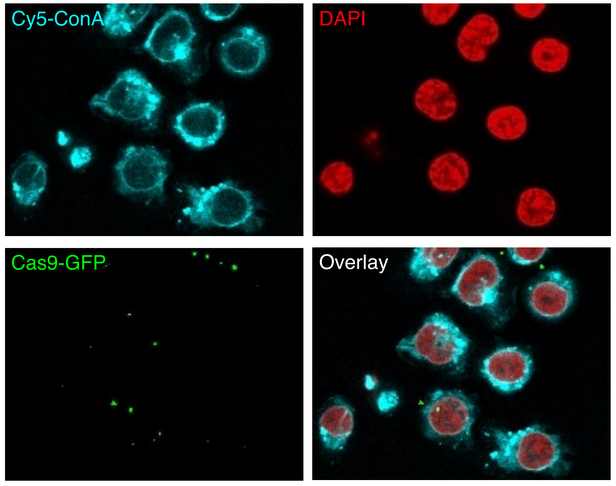

Figure 3: Confocal microscopy imaging of VECT-mediated Cas9-GFP transfection

The delivery of fluorescently tagged *TRAC* Cas9-GFP RNP into the cell was visualized through confocal microscopy and the successful localization of the Cas9-GFP RNP into the nucleus was confirmed. The cell membrane was stained with Cy5-Concanavalin A and the nucleus stained with DAPI.

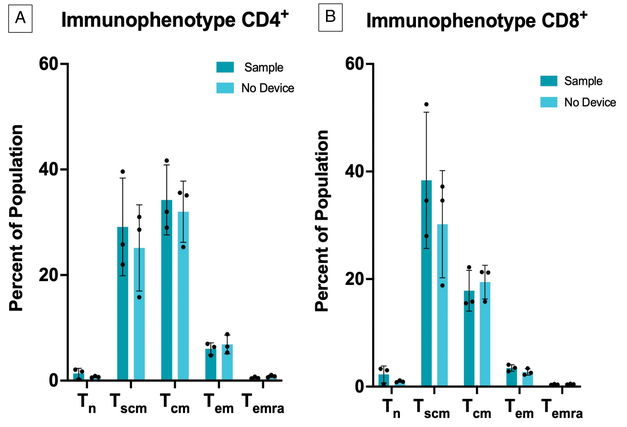

Figure 4: Comparison of cellular immunophenotypes before and after VECT

VECT showed no significant impact on the memory immunophenotypes of CD4+ (*Figure 4A*) and CD8+ cells *(4B)*.

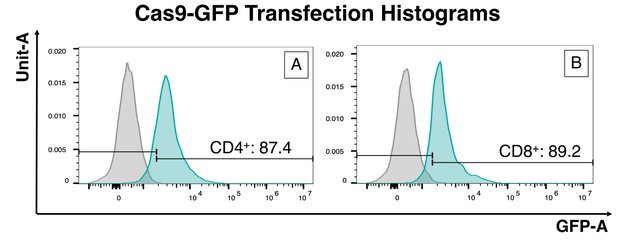

Figure 5: Histogram of Cas9-GFP delivery 24 hours after VECT

Flow cytometry showed high transfection efficiency (>80%, teal) of Cas9-GFP in both CD4+ (*Figure 5A*) and CD8+ cells *(5B*), when gated against negative control (gray).

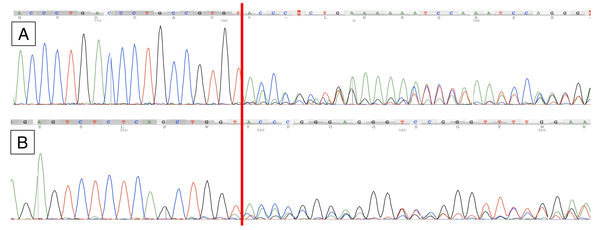

Figure 6: Sanger sequencing chromatograms of edited DNA at the TRAC *locus*

Sanger sequencing of the *TRAC* locus of edited cells showed loss of a clear sequencing signal immediately after the cut site (indicated by the red line) for the forward sequence (*Figure 6A*) and the reverse (*6B*), confirming the introduction of indels via NHEJ.

Conclusions

The VECT microfluidic platform has shown to effectively transfect CRISPR-Cas9 RNPs and induce knockouts at the *TRAC* locus in αβ T cells over a wide operational range and while preserving cell functionality. The genetic edits were assessed at the protein level through flow cytometry and confirmed at the DNA level through sequencing. The deliveries of the *TRAC* CRISPR-Cas9 RNP into the cytoplasm and nucleus were also visualized.

Future Plans

We are continuing to expand the applications for this transfection platform by establishing gene knock-in capabilities through VECT.