

CONTINUOUS FLOW TRANSFECTION WITH FLOWFECT TECHNOLOGY AS AN EFFICIENT, NON-VIRAL TECHNOLOGY FOR SCALABLE, CRISPR-MEDIATED ENGINEERING OF PRIMARY T-CELLS

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INTRODUCTION

Kytopen Flowfect® technology is a continuous flow cellular engineering technology that combines mechanical, electrical, and chemical forces to drive a multitude of payloads into cells for the development of cell therapies. Incorporating continuous flow, it facilitates the delivery of biomolecules across a wide range of volumes and cell concentrations. Small volume runs on the Flowfect Discover™ 96-well platform enable design, build, test and learn (DBTL) discovery in a cost-effective manner, whereas large scale runs on the Flowfect Tx™ GMP platform support clinically-focused efforts, especially where scalability, speed, and consistency are paramount.

Here we present non-viral RNP-based CAR engineering of primary T cells on both Flowfect™ platforms, firstly, demonstrating the utility of the Flowfect™ Discover platform for rapid optimization of CAR engineered T cells and secondly, the seamless transition to the Flowfect Tx™ GMP platform for manufacturing-scale applications. Furthermore, we demonstrate the specific influence the homology-directed repair (HDR) donor template format has on cell health and uptake. Notably, the reduced backbone of the Aldevron Nanoplasmid™ vector shows significant benefits over the standard miniplasmid.

The combination of these two, the Flowfect® technology and Aldevron's Nanoplasmid™ vector with RNP-CAR KI construct, enables a genome-engineering harmonization effect to achieve best-in-class non-viral CAR T-cell engineering performance in knock-in efficiency, cell health, and expansion at manufacturing scale in a rapid timeframe.

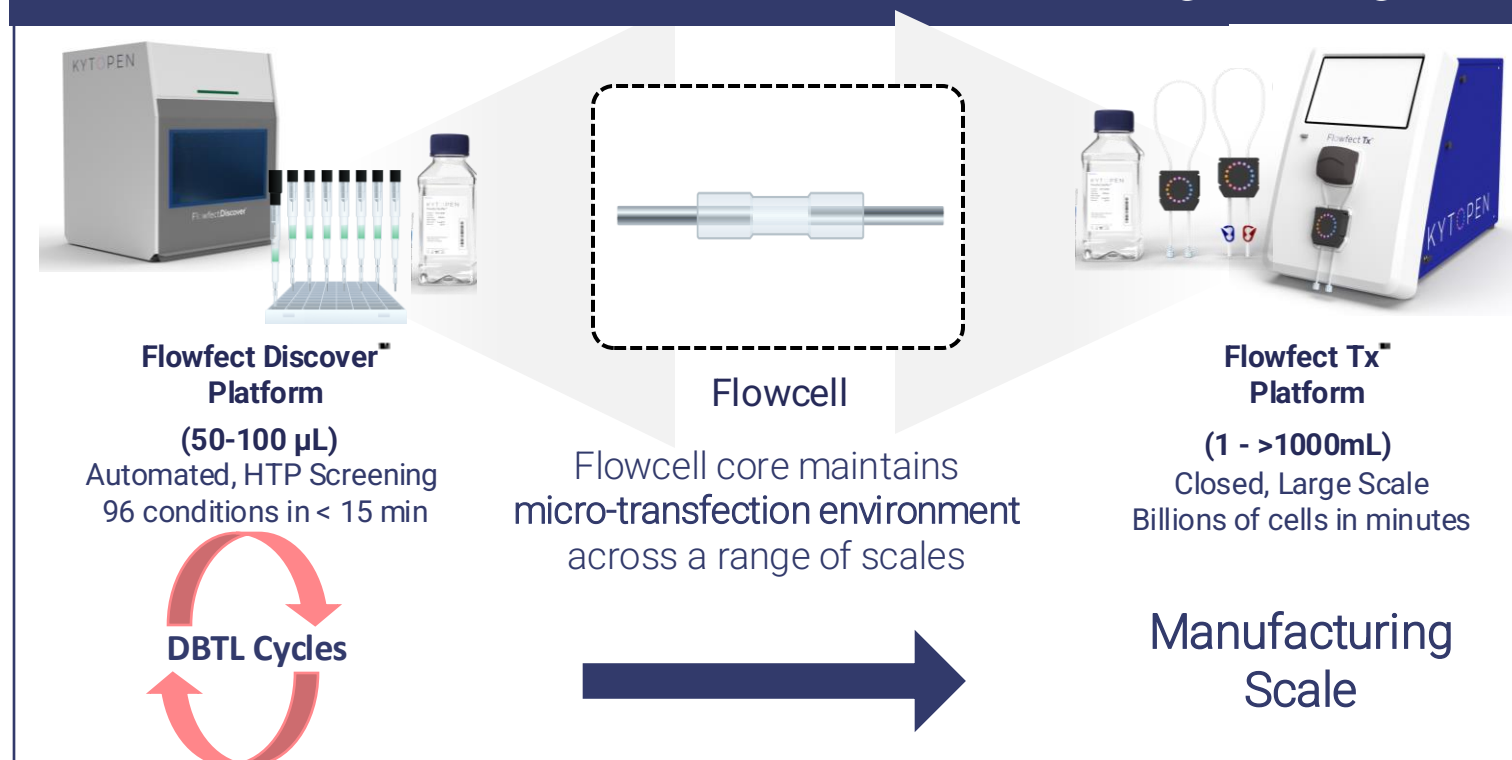
KEY TAKEAWAYS

- Kytopen and Aldevron have partnered to deliver high-quality, non-viral engineered CAR-T cells at therapeutic-scale.
- Harmonization between the Flowfect® technology and Aldevron's Nanoplasmid™ vector consistently produces net yields of CAR-expressing cells 2.5-3X higher than input cells across several donors and replicates.
- Twenty million input cells rapidly generates therapeutically-relevant doses of 45-60 million viable CAR-positive cells within eight days, with scalability to billions of processed cells in minutes on the Flowfect Tx™ GMP continuous flow platform.
- Rapid Design, Build, Test & Learn cycles (DBTL) on the Flowfect Discover™ platform identified CD19 CAR-T cell engineering conditions that could be seamlessly transferred to the large-scale Flowfect Tx™ platform leading to >40% CAR-expressing cells with greater than 70% viability.
- Integrating Aldevron's Nanoplasmid™ vector with the Flowfect® technology boosted CAR-expressing cells to >55% while simultaneously improving T cell health.

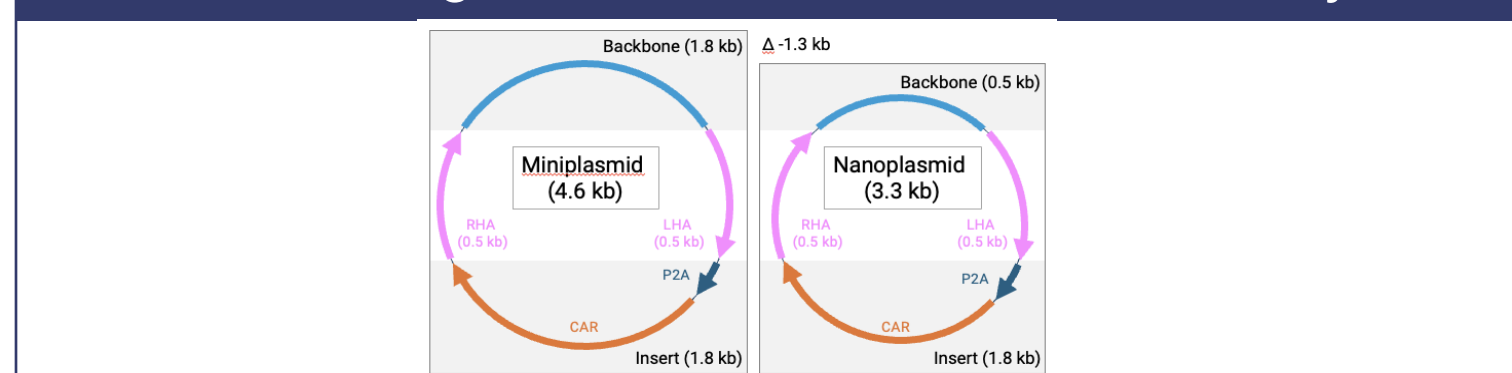
TECHNOLOGY

Flowfect® and Nanoplasmid™ Technology Overview

Flowfect® Technology Drives Rapid DBTL Cycles and Seamless Translation to Scalable T-Cell Engineering



Nanoplasmid™ Enables a Reduced Donor Template Size, Contributing to Increased Transfection Efficiency



Rapid Flowfect Discover™ 96-well Platform for Optimized Translation to the Flowfect Tx™ GMP Platform

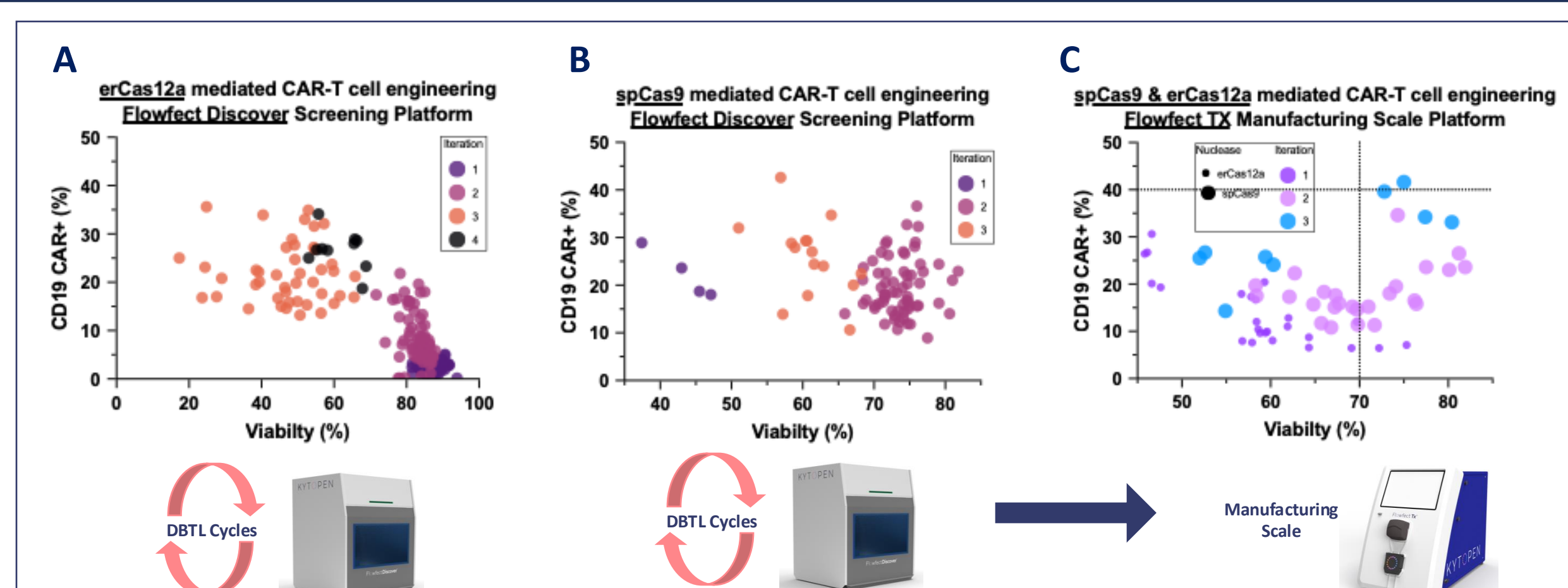


Figure 1. Primary activated T cells were co-transfected with TRAC targeting RNP and promoterless CD19 CAR miniplasmid as the KI donor. Efficiency and viability measurements were taken 8 days post-transfection. A) erCas12a-mediated CD19 CAR KI efficiency compared to viability for samples in the initial experiment (Iteration 1, purple) and in two optimization experiments (Iteration 2 and 3 magenta, orange, respectively). B) spCas9-mediated CD19 CAR KI efficiency compared to viability for samples in the initial experiment (Iteration 1, purple) and in three optimization experiments (Iteration 2 and 3, and 4 magenta, orange and black, respectively). C) Flowfect Tx™ manufacturing-scale performance (1mL) for both erCas12a (small dot) & spCas9 (large dot)-mediated CD19 CAR KI efficiency compared to viability for samples in the initial experiment (Iteration 1, purple) and two additional follow-on iterations (lavender and blue, respectively).

Donor Template Directly Impacts Viability and Knock-in Performance

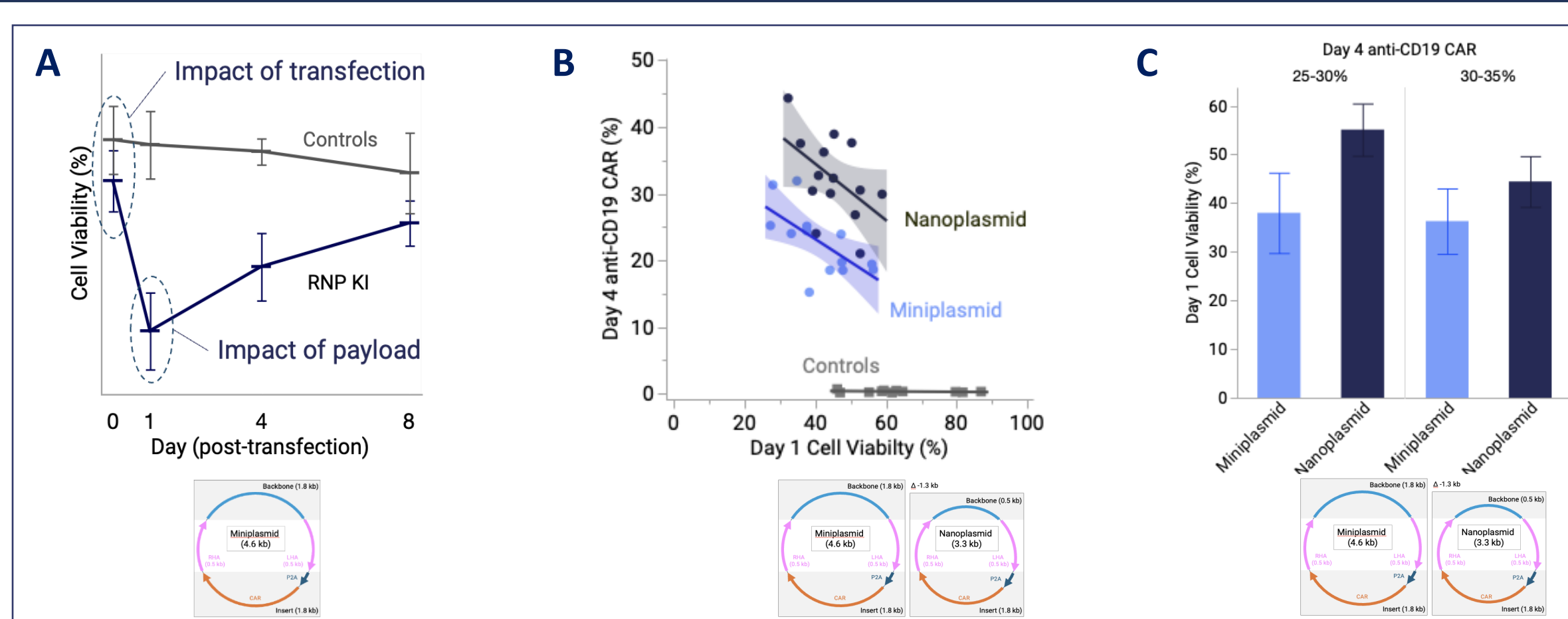
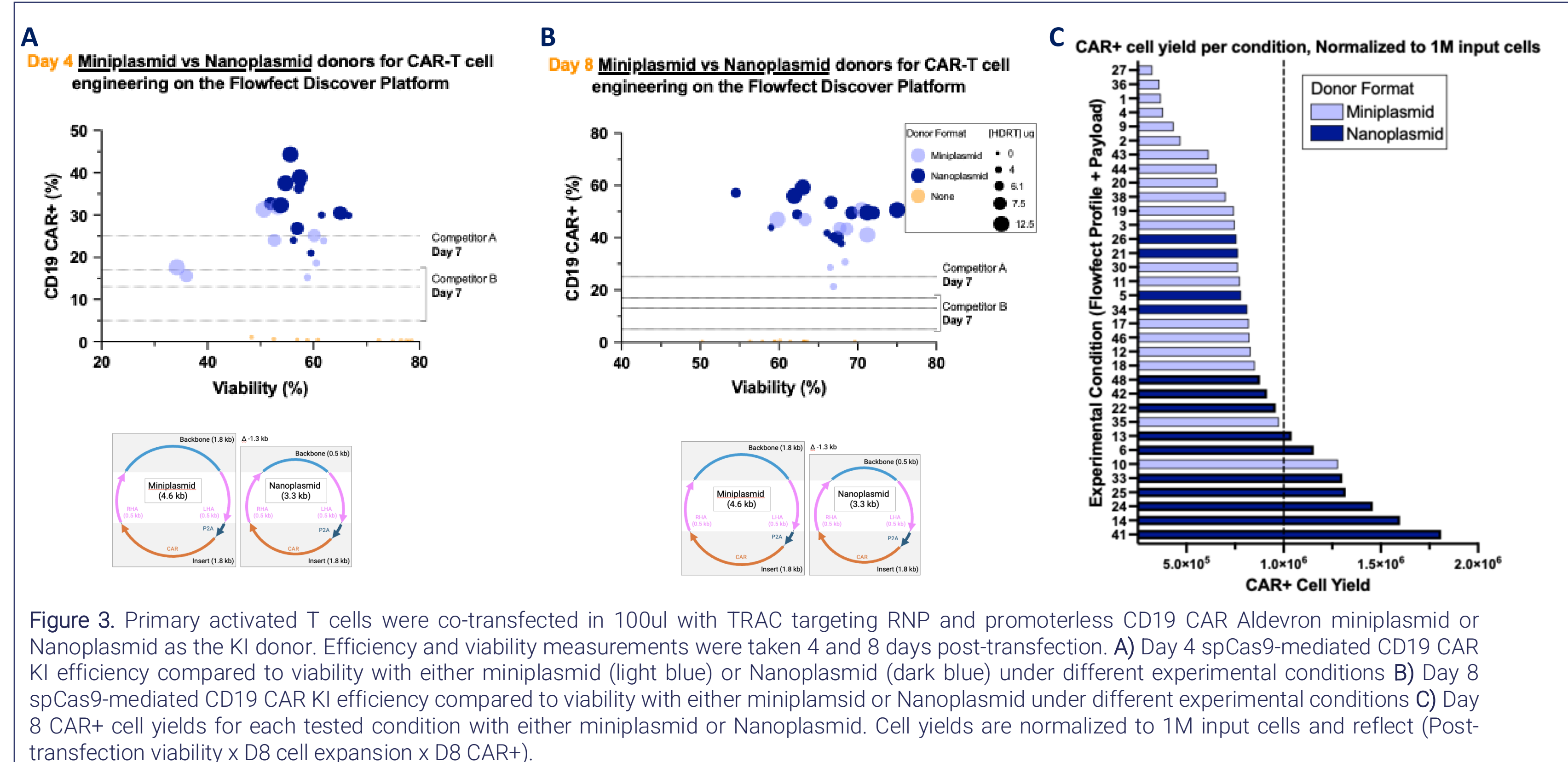


Figure 2. Primary activated T cells were co-transfected in 100ul with TRAC targeting RNP and promoterless CD19 CAR Aldevron miniplasmid or Nanoplasmid™ as the KI donor. Efficiency and viability measurements were taken at the indicated days post transfection. A) Diagram illustrating parameters affecting overall desired cell therapy outcomes in delivery of payloads to cells (non-exhaustive), as well as data from CD19 CAR KI experiments demonstrating the impact payload composition can exhibit on primary T cell health, across time. B) Day 4 spCas9-mediated CD19 CAR KI efficiency compared to day 1 viability with either miniplasmid (blue) or Nanoplasmid (red) under different experimental conditions. C) Miniplasmid versus Nanoplasmid day 1 viabilities grouped within two specific ranges (25-30% and 30-35%) of day 4 CAR positivity.

RESULTS

Enhanced synergy between Kytopen's Flowfect Discover™ platform and Aldevron Nanoplasmid™ donor templates for superior engineering of CAR-T cells

Seamless Transition from the 96-well Flowfect Discover™ Platform to the Flowfect Tx™ GMP Platform with the Nanoplasmid™ Vector Delivers 2-3x Engineered Cell Output with Flowfect Tx platform and Nanoplasmid™



CONCLUSIONS

With its continuous flow gene-editing technology, Kytopen is uniquely poised to offer best-in-class systems and expertise for non-viral delivery of a wide array of payloads for the development of cell therapies.

Here, we demonstrated specific capabilities for RNP-directed CAR-T cell engineering. The effectivity of the Flowfect™ platforms for non-viral engineering of CAR-T cells was further enhanced by partnering with Aldevron, a leading global manufacturer of DNA, RNA, and proteins used in cell and gene therapies. By leveraging Aldevron's Nanoplasmid™ technology and integrating it into Kytopen's plug-and-play Flowfect Tx™ GMP platform, we highlight the value of harnessing a third dimension of the transfection process, namely, the mechanical component, continuous flow, in addition to the critical parameters of payload design and quality.

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