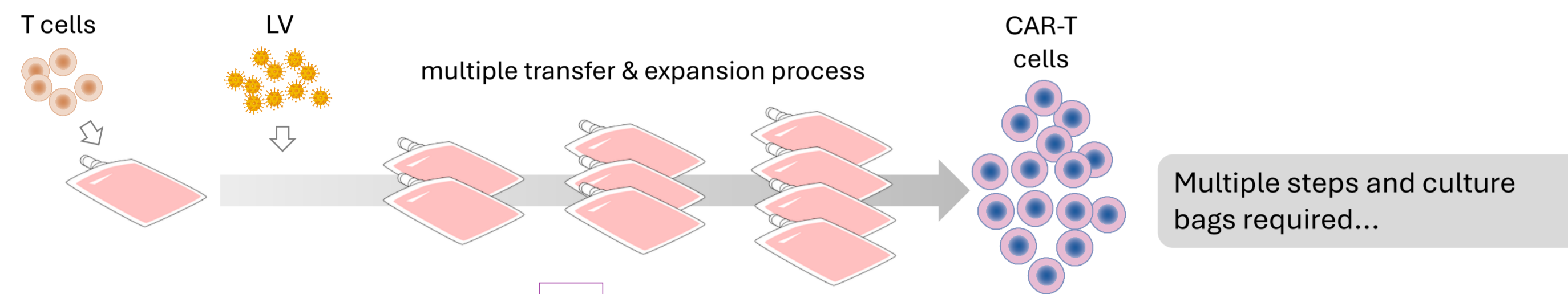


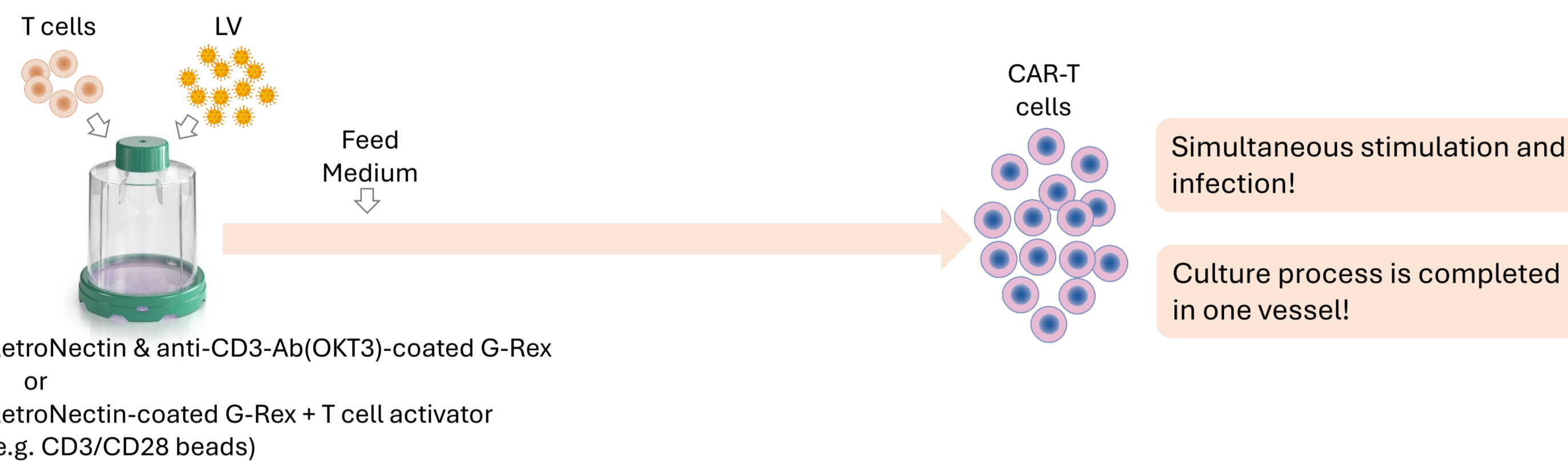
Background

The manufacturing process for CAR-T and TCR-T cell therapies is complex, costly, and resource-intensive, involving multiple steps that increase variability and hinder scalability. To tackle these challenges, a T-cell manufacturing platform combining the viral transduction enhancer RetroNectin® (RN) with the gas-permeable, closed-system bioreactor G-Rex® was developed. This platform simplifies T-cell activation and gene delivery in a single vessel, reducing handling to just three touches—setup, medium fill, and harvest—while promoting robust cell expansion and favorable cell phenotypes. Additionally, introducing an anti-CD28 antibody into the RN/G-Rex system helped correct a CD8-skewed cell population observed with RN/OKT3 stimulation alone. This adjustment improved the CD4/CD8 balance without compromising gene transduction efficiency or functional potency. The enhanced protocol maintains the simplified manufacturing workflow and shows promise for improving in vivo persistence, highlighting a practical and clinically relevant approach to next-generation T-cell production.

Conventional CAR-T manufacturing



The Simple manufacturing



RetroNectin®

Enhanced gene transduction and T cell expansion

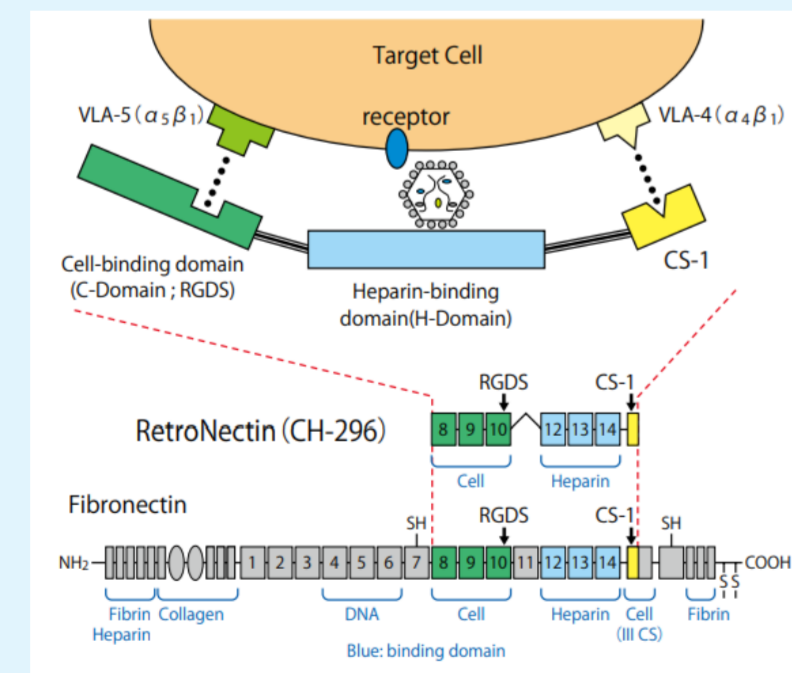
RetroNectin reagent is a recombinant human fibronectin fragment (rFN-CH-296) that contains three functional domains: the cell-binding domain, the heparin-binding domain, and the CS-1 sequence. T cells are conventionally expanded in the presence of interleukin-2 (IL-2) by stimulation with anti-CD3 antibody. The addition of RetroNectin in this stimulation step dramatically increases the efficiency of T cell expansion.

RetroNectin GMP grade registered in DMF

A DMF (18898) was submitted for the liquid format RetroNectin GMP grade on March 15, 2019.

RetroNectin Pro (code# T101)

RetroNectin Pro is a cost-effective new product released in 2024, which uses the same protocol and performs the same as RetroNectin reagent.



RetroNectin® GMP grade (code# T202)



Anti-CD3 mAb GMP grade (code# T210)

High quality is assured as a recombinant protein required for the manufacture of regenerative medical products.

G-Rex® Bioreactor

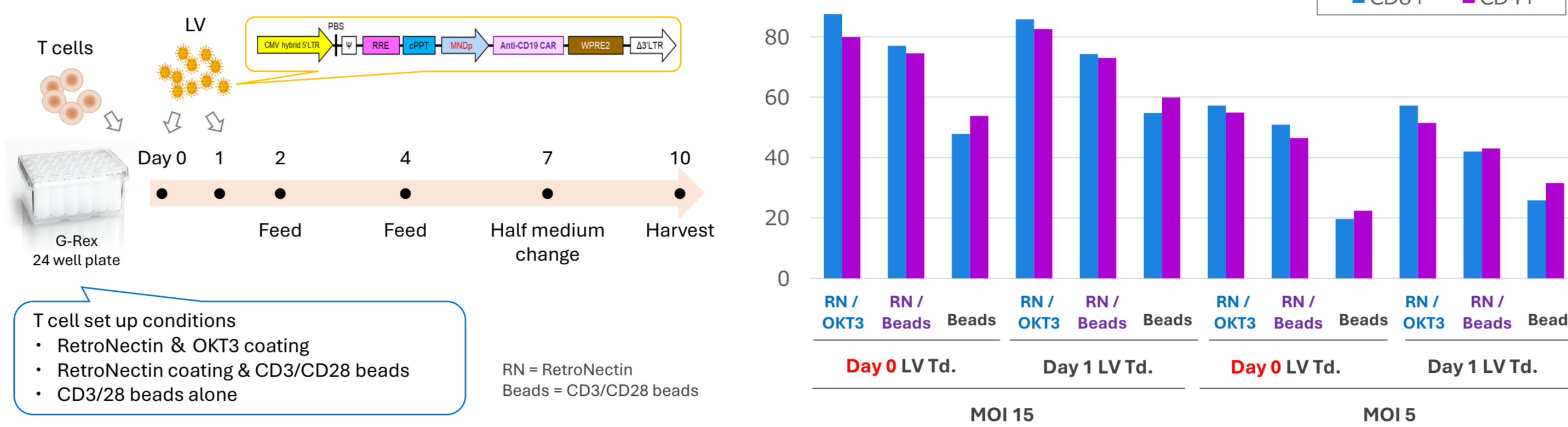


- The bottom surface is made of a gas-permeable membrane, allowing high-density culture of non-adherent cells.
- No stirring or special equipment is required.
- A large volume of culture medium can be added, eliminating the need for medium exchange.

The use of G-Rex in cell manufacturing has been increasing

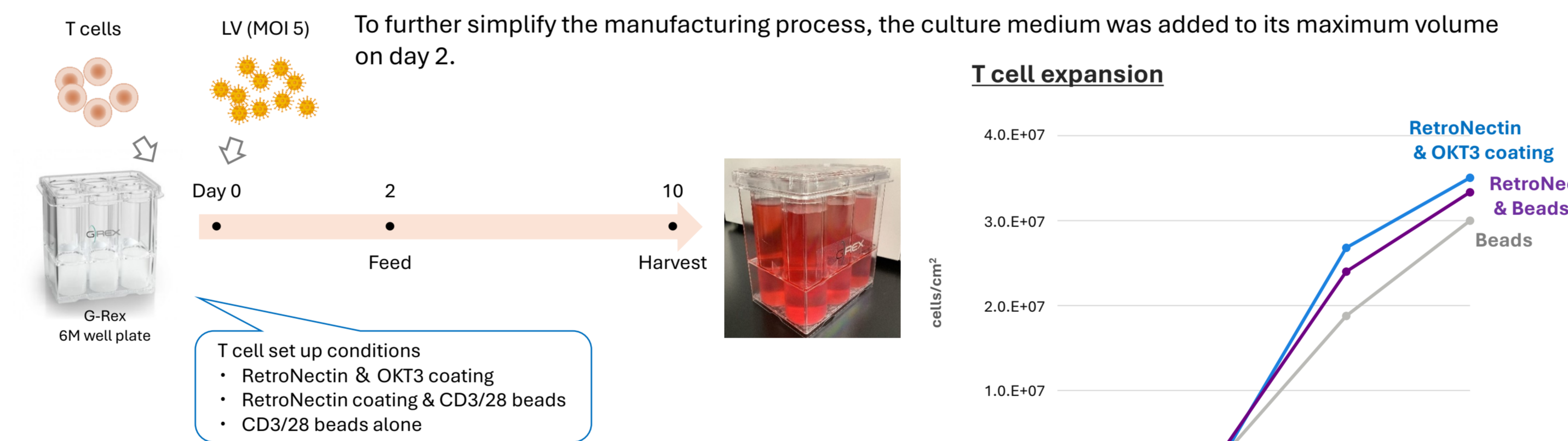
CAR-T cell manufacturing process using G-Rex system

We investigated the optimal infection timing and multiplicity of infection (MOI) of lentiviral vectors to produce CAR-T cells using G-Rex.

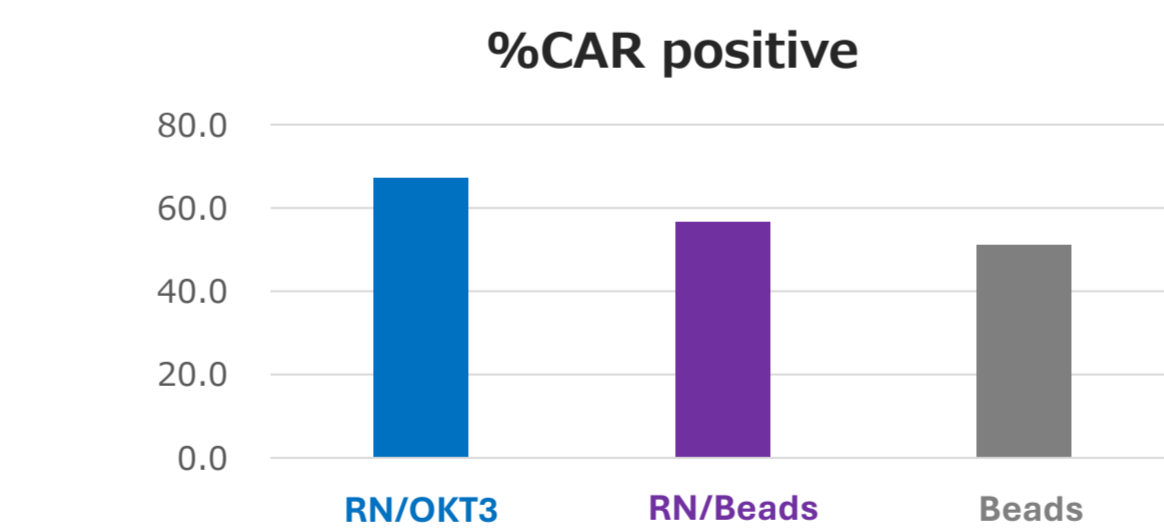


- ✓ Transduction Efficiency: RN/OKT3 > RN + CD3/CD28 beads > CD3/CD28 beads
- ✓ RNs enables to perform higher transduction efficiency at low MOI.
- ✓ RN coatings provide high Td efficiency comparable to Day 1 infection even on Day 0.

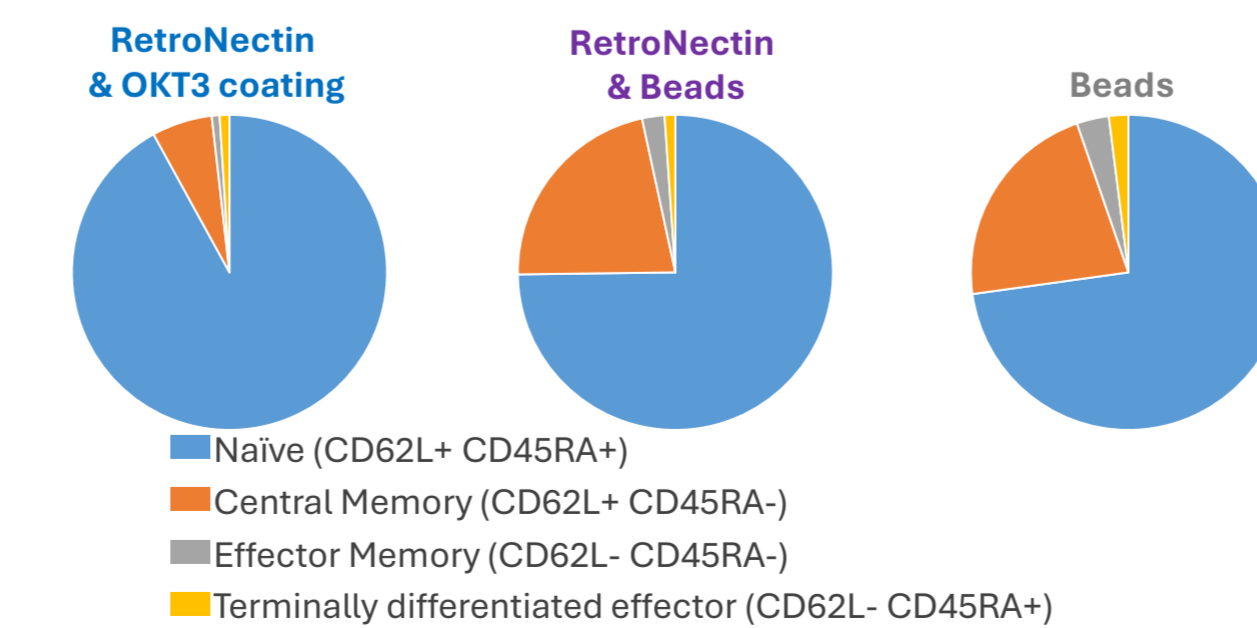
Largescale cell production and in vivo anti-tumor test



Transduction efficiency



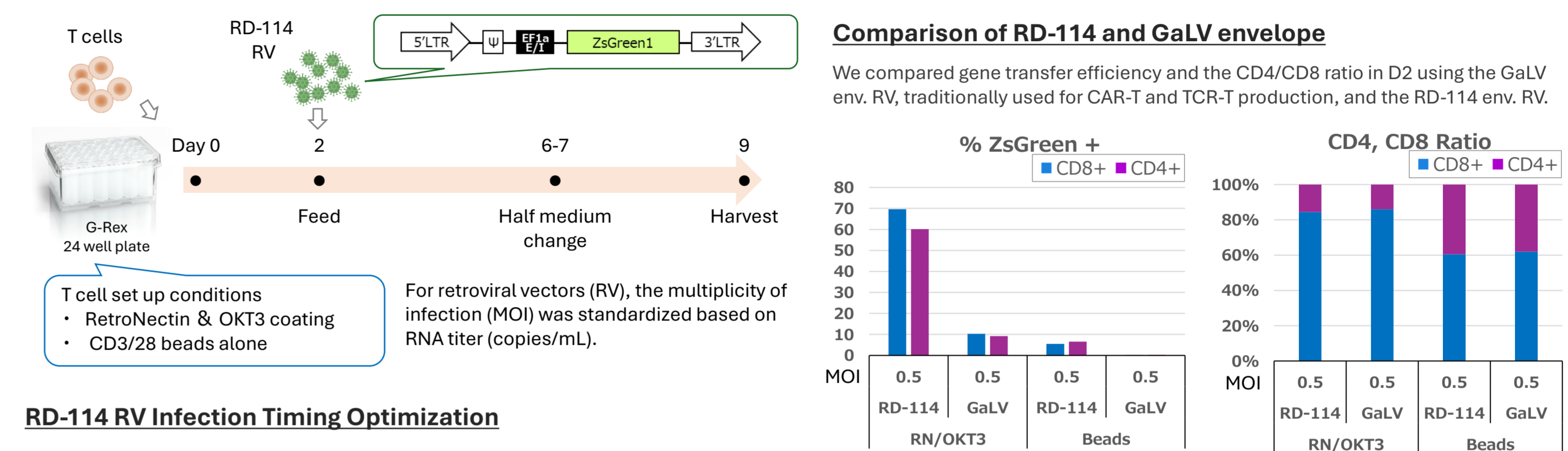
Immunophenotype



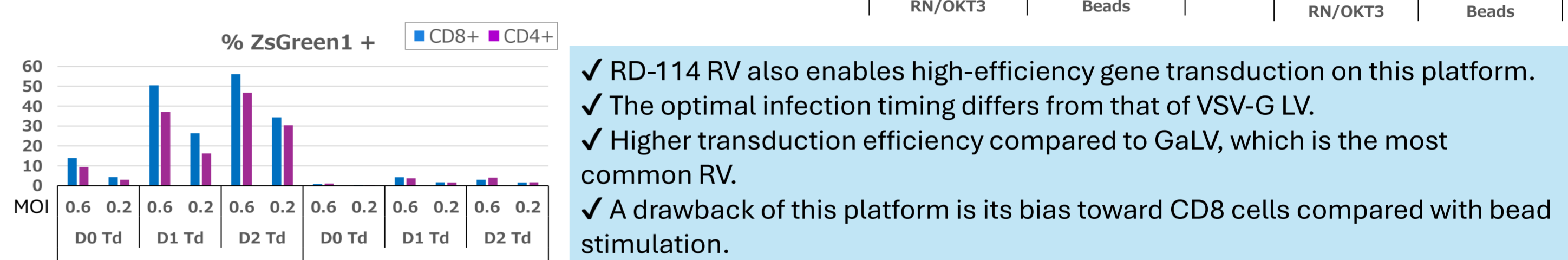
- ✓ By pre-coating with RetroNectin and OKT3 (anti-CD3 Ab), high-quality and high-efficiency CAR-T cells can be prepared without using other T cell activators.

RD-114 Pseudotyped Retroviral Vectors Fit Well within This Platform

We also considered whether retroviral vectors fit this method, starting with optimizing the timing of infection.

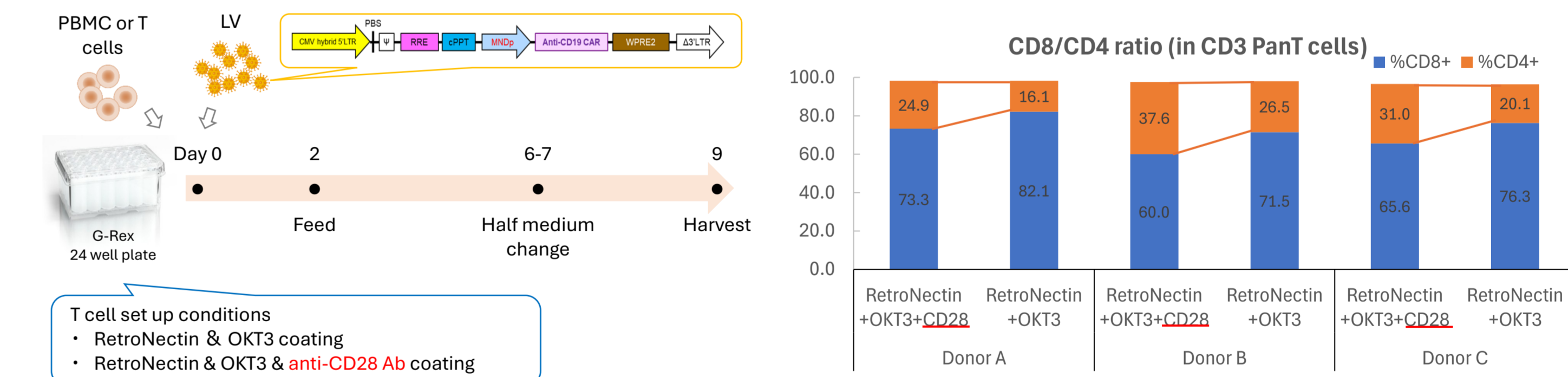


RD-114 RV Infection Timing Optimization



Optimization of coating condition

To optimize T-cell stimulation using the G-Rex system, we investigated the addition of an anti-CD28 antibody to the immobilization conditions. Furthermore, we compared the differences between PBMCs and separated T cells as starting materials.



Using a design of experiments (DOE) approach, we determined that an OKT3:anti-CD28 antibody ratio of 1:2 is optimal for immobilization.

	(μg/cm ²)		
	RN	OKT3	aCD28 Ab
RetroNectin+OKT3 +anti-CD28 Ab	5.0	1.0	2.0
RetroNectin+OKT3	5.0	1.0	-

- ✓ Pre-coating with an anti-CD28 antibody in addition to RetroNectin and OKT3 improved CD4 ratio and cell growth.
- ✓ Coating with CD28 antibody showed stable growth with or without T cell isolation of the starting material.

Summary

- ◆ We have established a method for manufacturing CAR-T cells in a single vessel over three process days, regardless of the cell stimulation method.
- ◆ By coating the membrane of G-Rex with RetroNectin, we were able to perform simultaneous activation and efficient transduction of T cells with lentiviral vectors.
- ◆ RD-114 envelopes can also be used to apply retrovirus vectors to this platform.
- ◆ We developed a new manufacturing method by adding anti-CD28 antibody to G-Rex pre-coated with RetroNectin and anti-CD3 antibodies, improving the CD4/CD8 imbalance.