

The Simple CAR-T Cell Manufacture:

P-3236

Simultaneous T cell activation and transduction in RetroNectin®-coated G-Rex

Yasunori Amaishi, Izumi Maki, Seina Inui, Sachiko Okamoto
CDM Center 3, Takara Bio Inc.



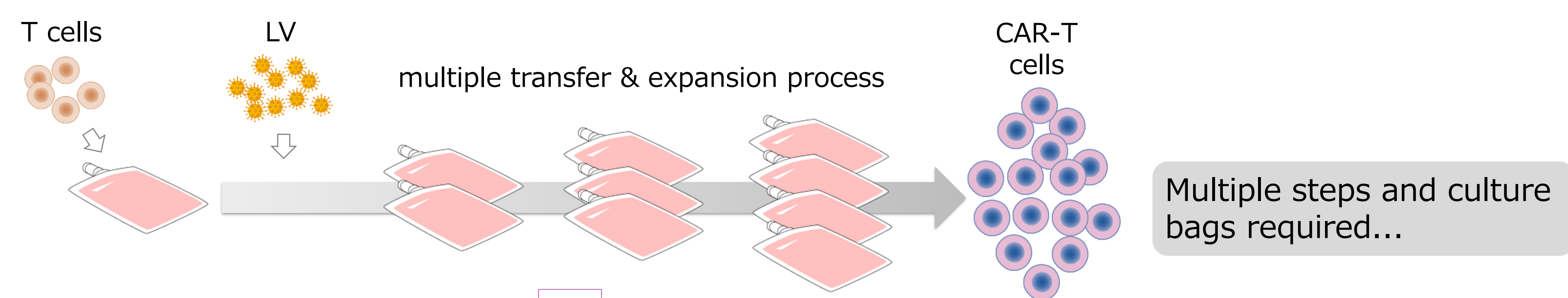
Abstract

CAR-T cell therapy has shown high anti-tumor activity against hematologic malignancies, and several therapeutic agents have been marketed. However, the manufacturing process for CAR-T cells is complex and costly, presenting challenges that require improvements.

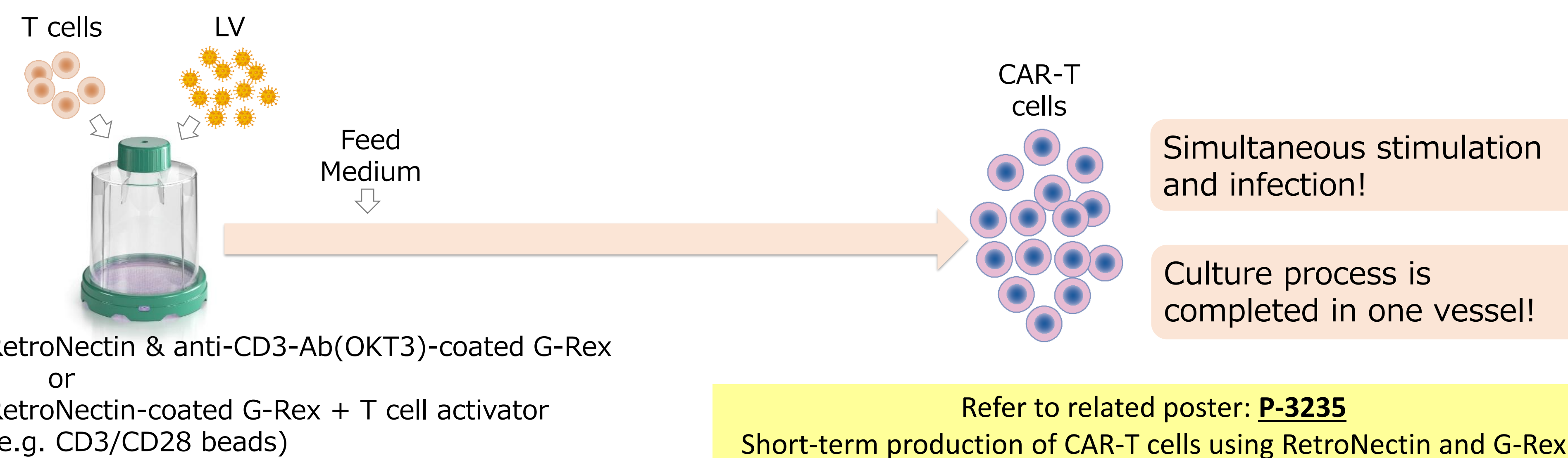
RetroNectin® is a recombinant protein containing functional domains of human fibronectin, which promotes viral gene transfer to cells. Additionally, co-stimulation with RetroNectin and anti-CD3 antibodies can efficiently proliferate undifferentiated T cells.

This study developed a method to produce high-quality CAR-T cells using a simple process by combining RetroNectin and G-Rex bioreactor, which allows high-density culture of cells without the need for agitation or medium exchange with its gas-permeable membrane. By coating the G-Rex with RetroNectin, simultaneous T cell activation and gene transfer were achieved, eliminating the need for medium exchange and reducing the process to three operation days. This method simplifies the manufacturing and provides a promising platform for CAR-T cell manufacturing.

Conventional CAR-T manufacturing



The Simple manufacturing



Core materials for this study

RetroNectin®

Enhanced gene transduction and T cell expansion

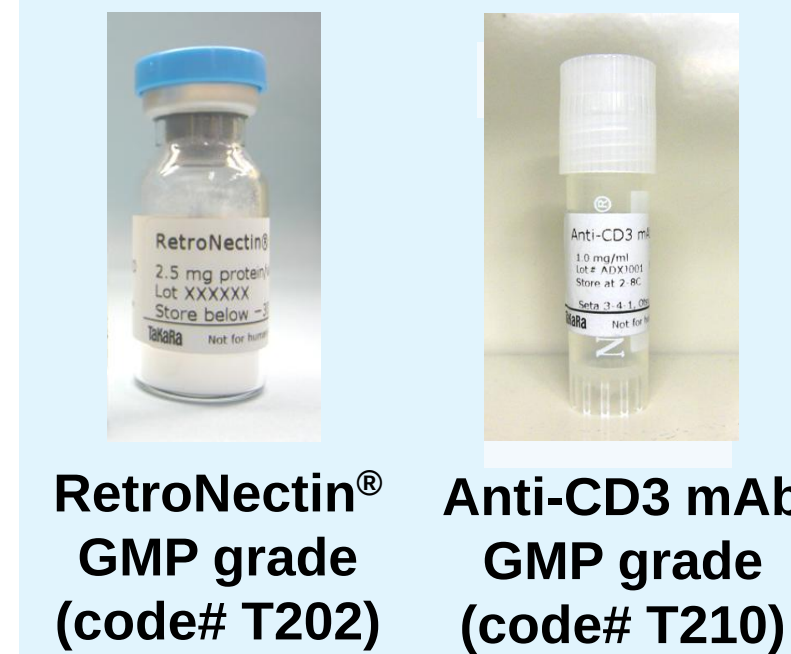
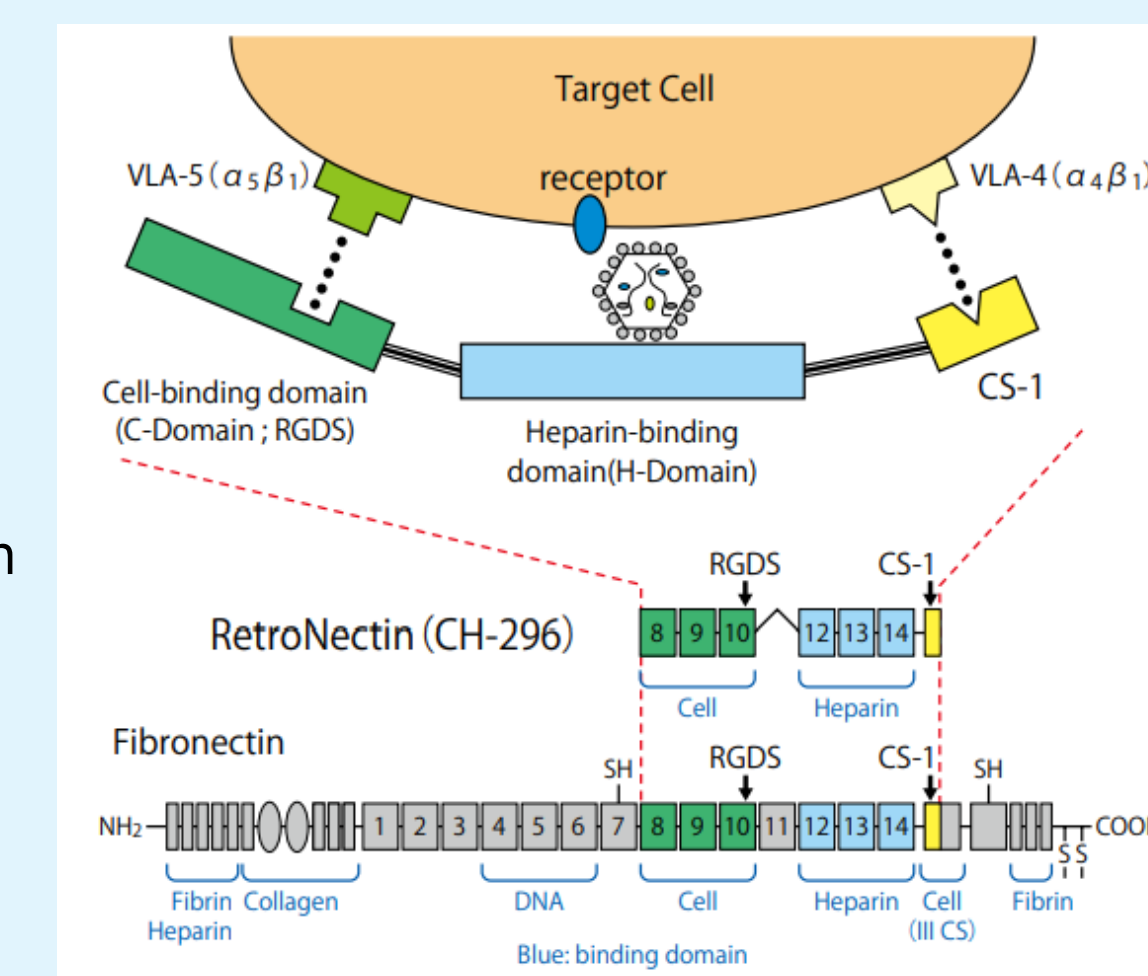
- RetroNectin reagent is a recombinant human fibronectin fragment (rFN-CH-296) that contains three functional domains: the cell-binding domain, the heparin-binding domain, and the CS-1 sequence.
- T cells are conventionally expanded in the presence of interleukin-2 (IL-2) by stimulation with anti-CD3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



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

G-Rex® Bioreactor (Wilson Wolf)



The bottom surface is made of a gas-permeable membrane, allowing high-density culture of non-adherent cells.

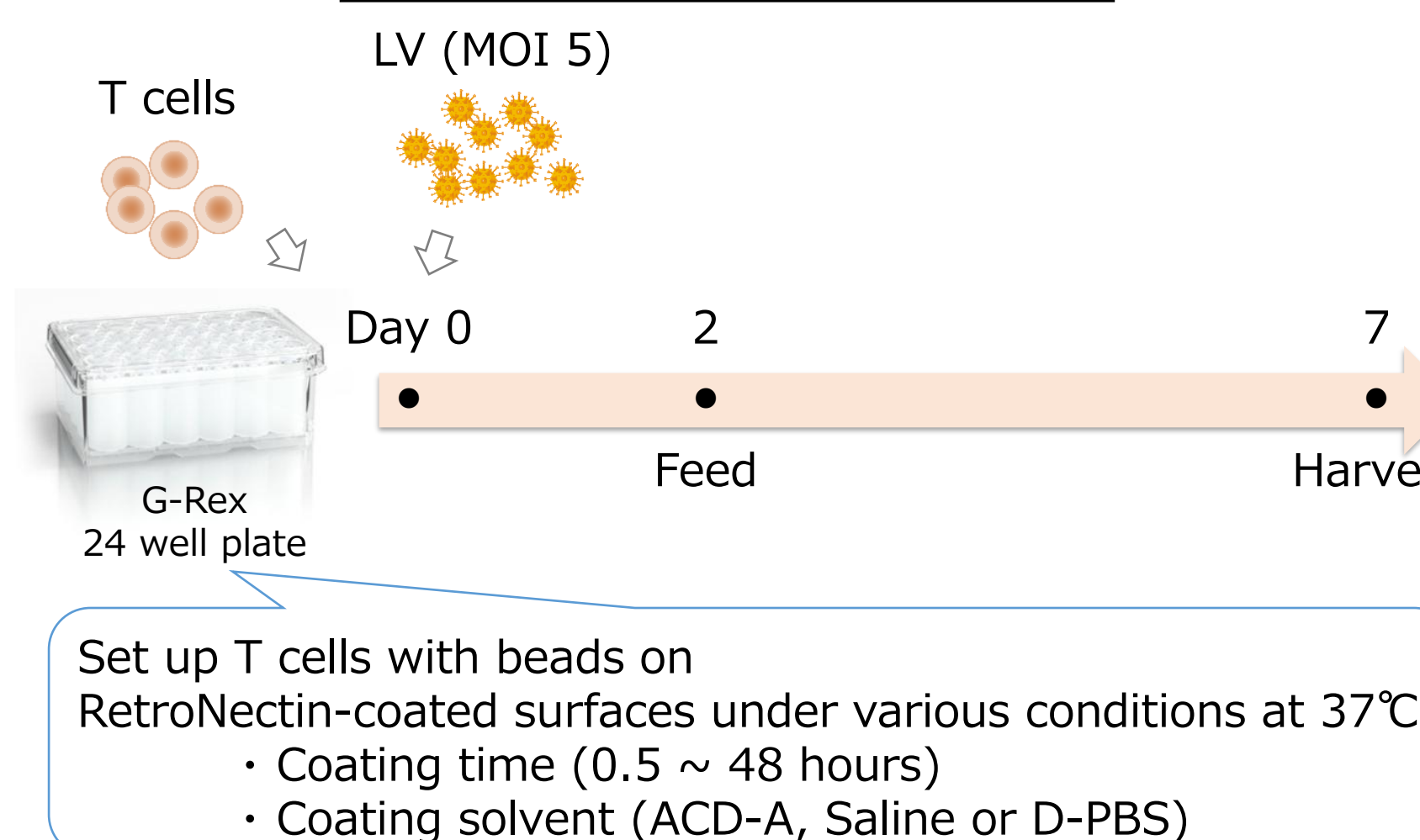
No stirring or special equipment is required.

A large volume of culture medium can be added, eliminating the need for medium exchange.

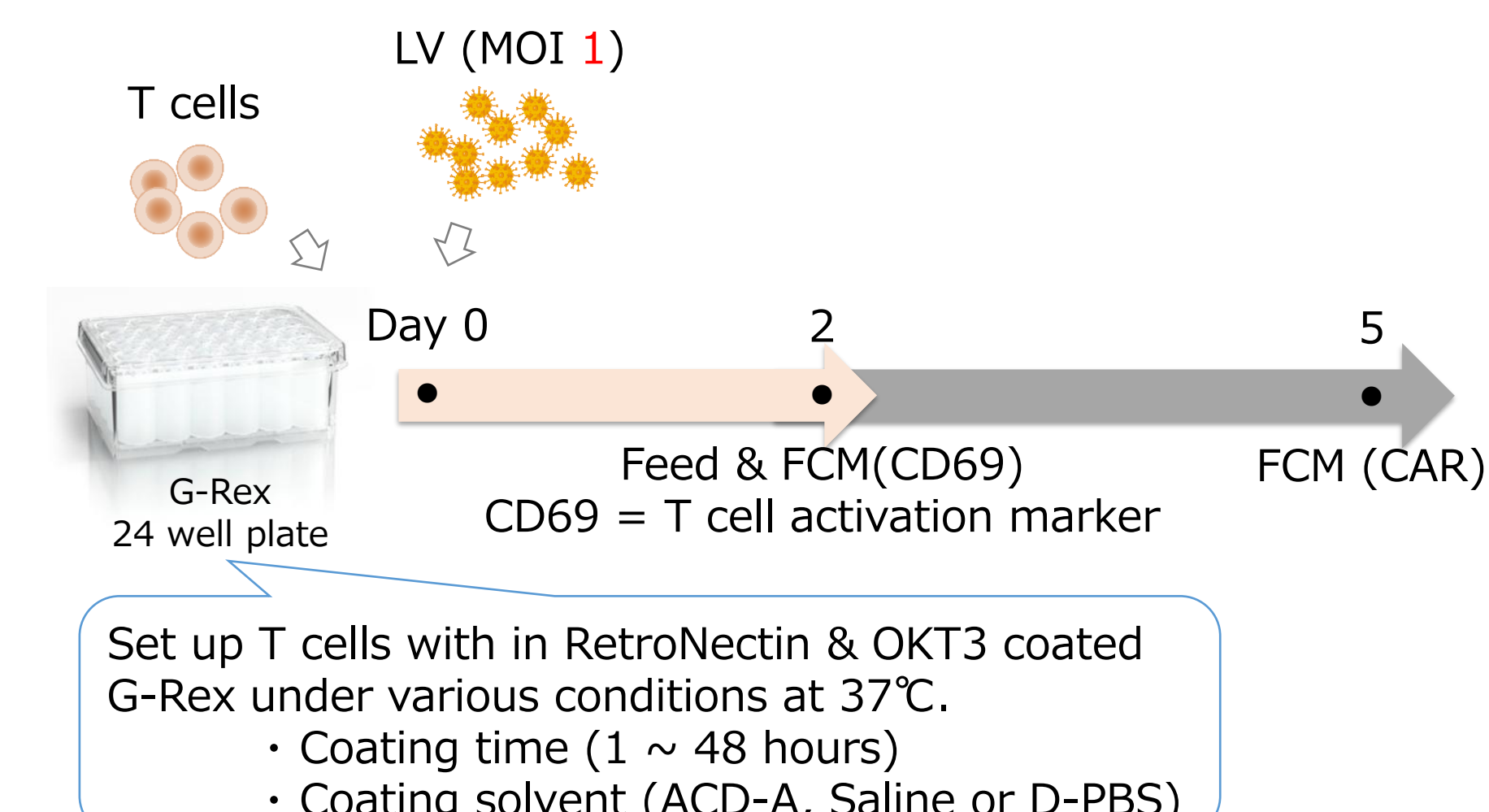
The use of G-Rex in cell manufacturing has been increasing

Optimization of coating condition

RetroNectin coating

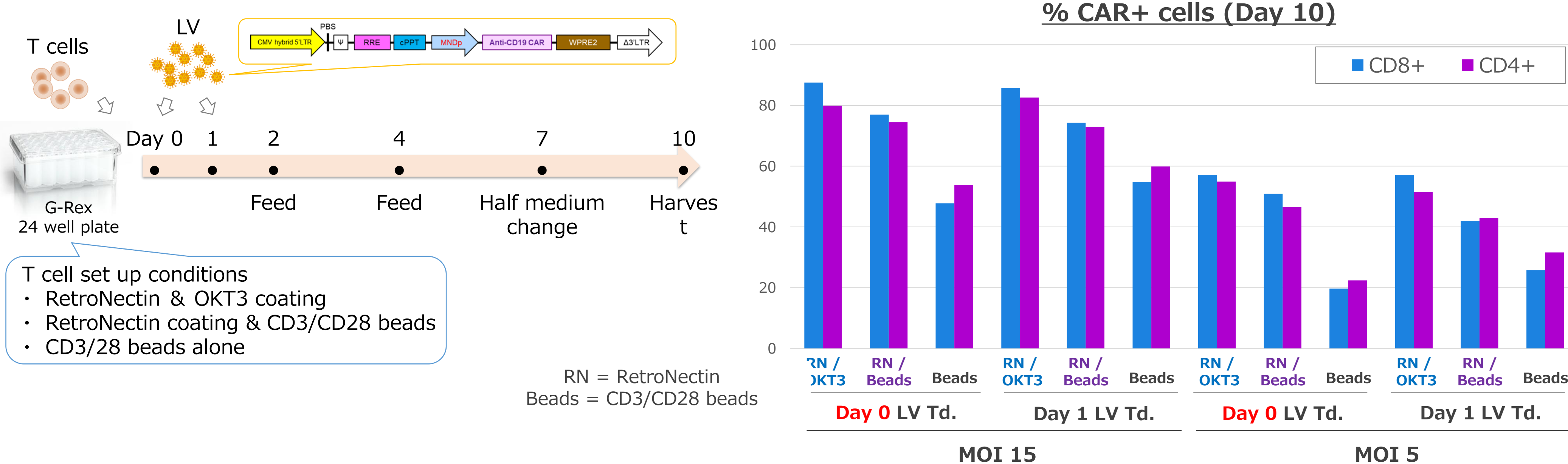


RetroNectin & OKT3 coating



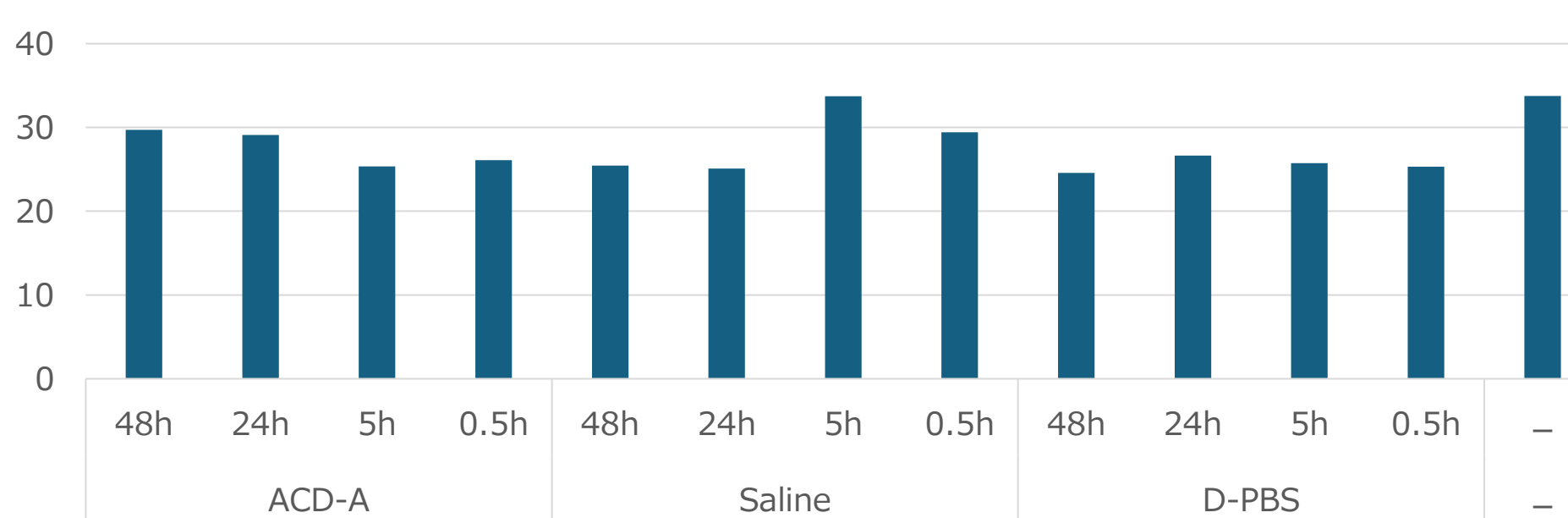
Optimization of lentiviral transduction

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

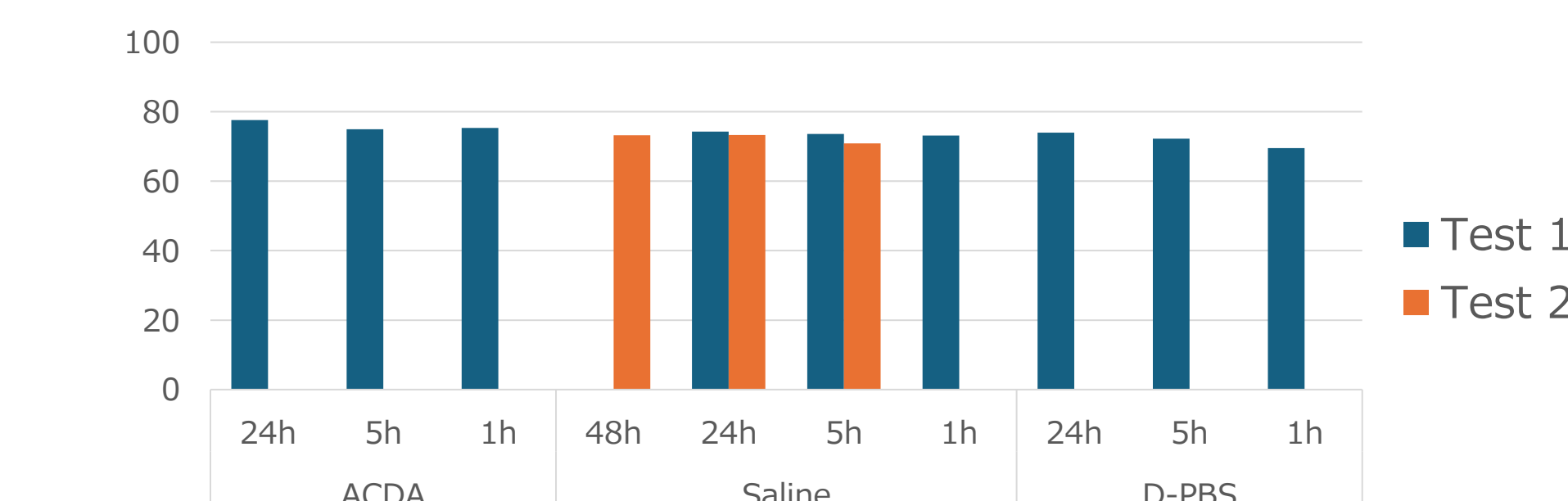


- ✓ Transduction Efficiency : RN/OKT3 > RN + CD3/CD28 beads > CD3/CD28 beads
- ✓ RNs enables to perform higher transduction efficiency at low MOI.
- ✓ RN coatings provide high Td efficiency comparable to Day 1 infection even on Day 0.

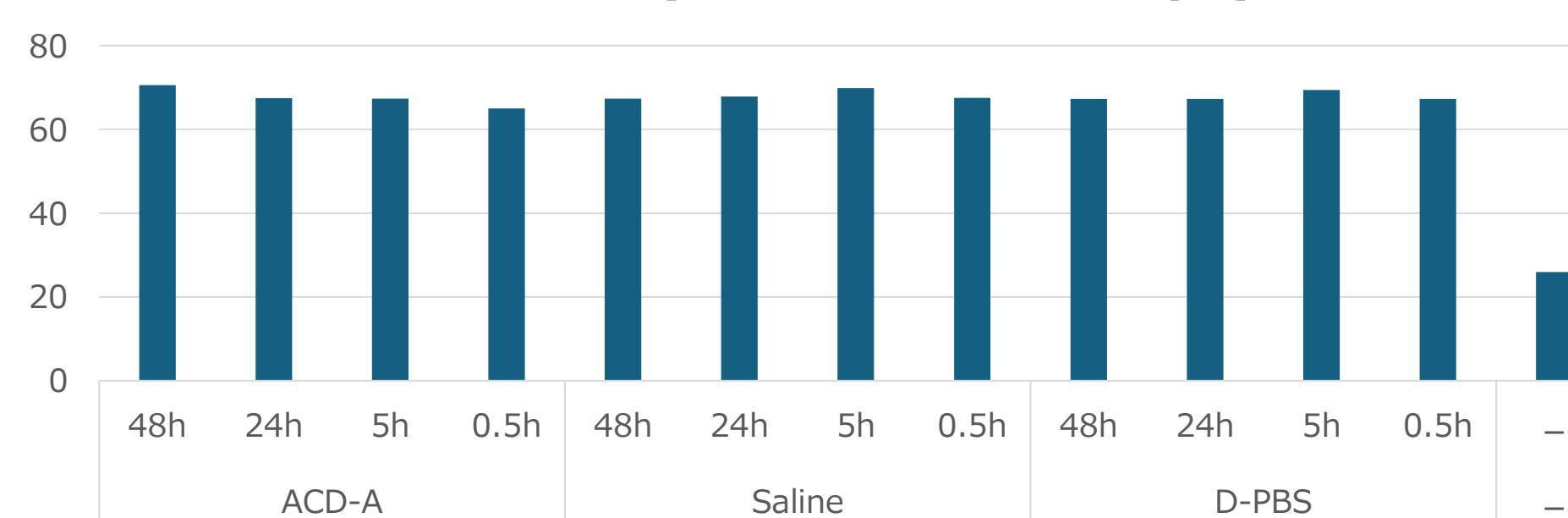
Growth Fold (7days)



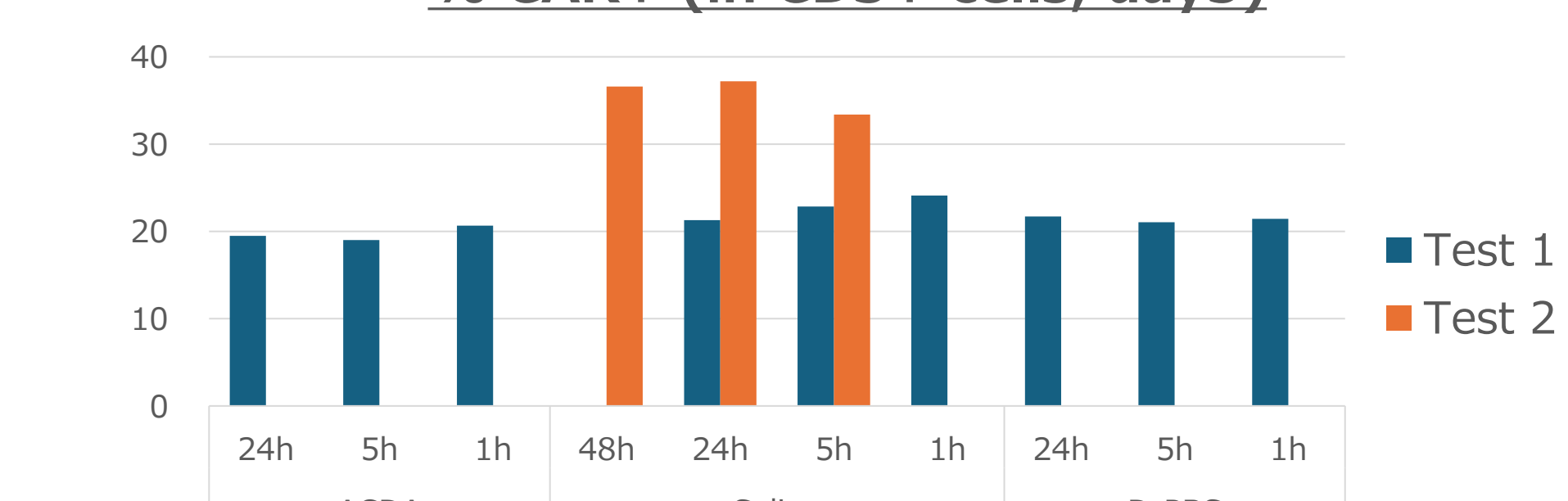
% CD69+ (in CD3+ cells, day2)



% CAR+ (in CD3+ cells, day7)



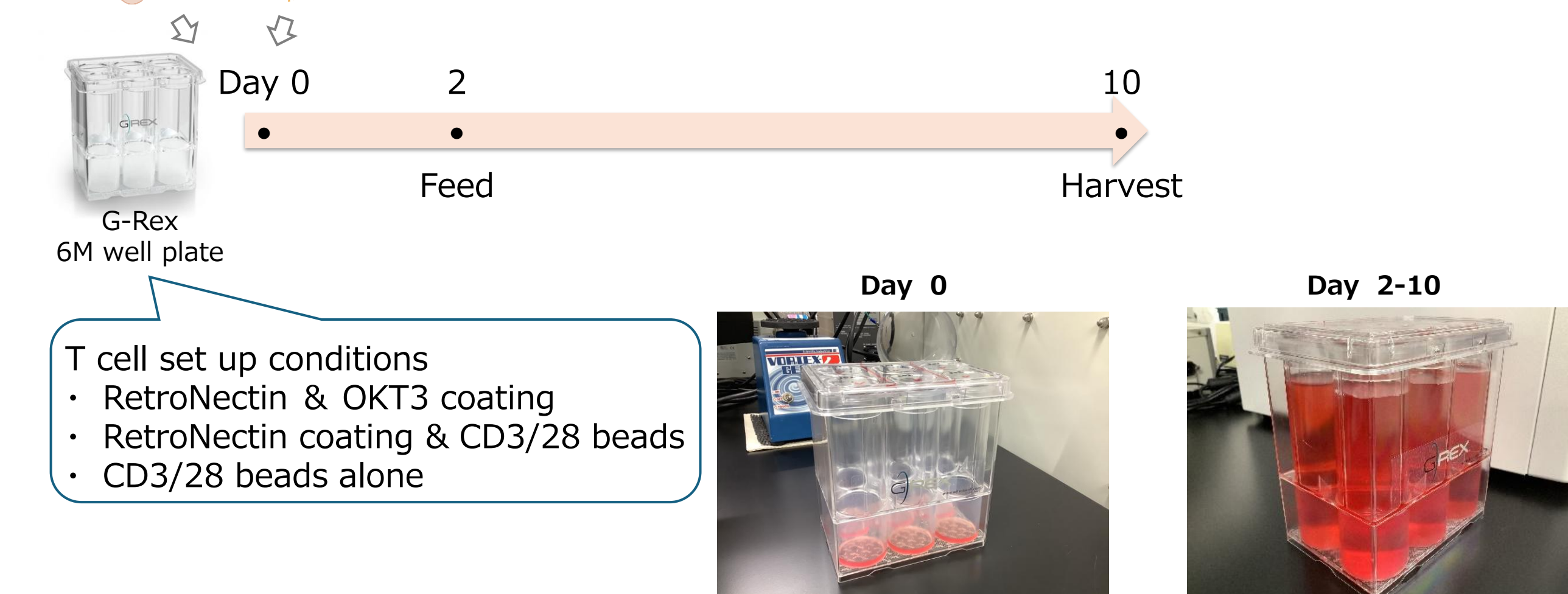
% CAR+ (in CD3+ cells, day5)



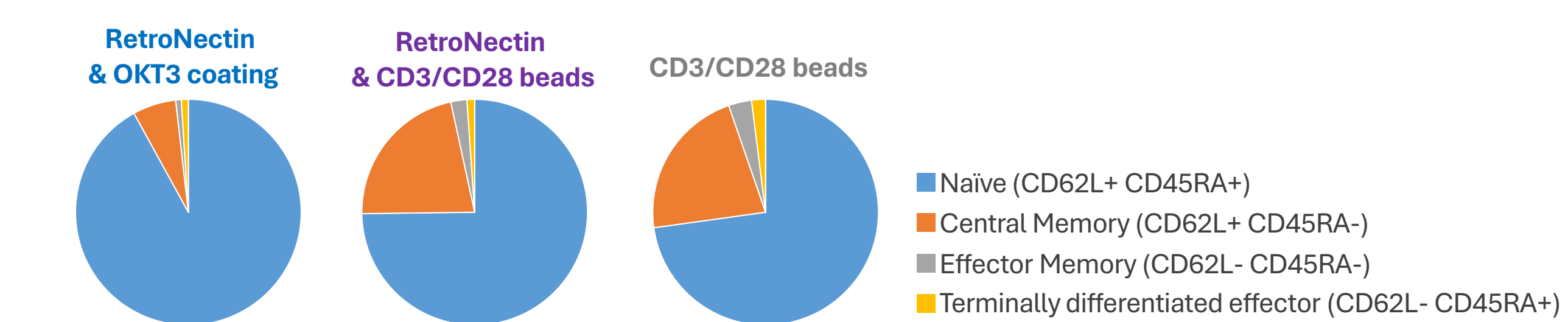
- ✓ RN exhibits stable coating efficiency across various solvents and time conditions, enhancing gene transfection efficiency by approximately threefold
- ✓ RN/OKT3 coating also demonstrates robust coating performance, supporting stable activation and gene transduction.

Largescale cell production and *in vivo* anti-tumor test

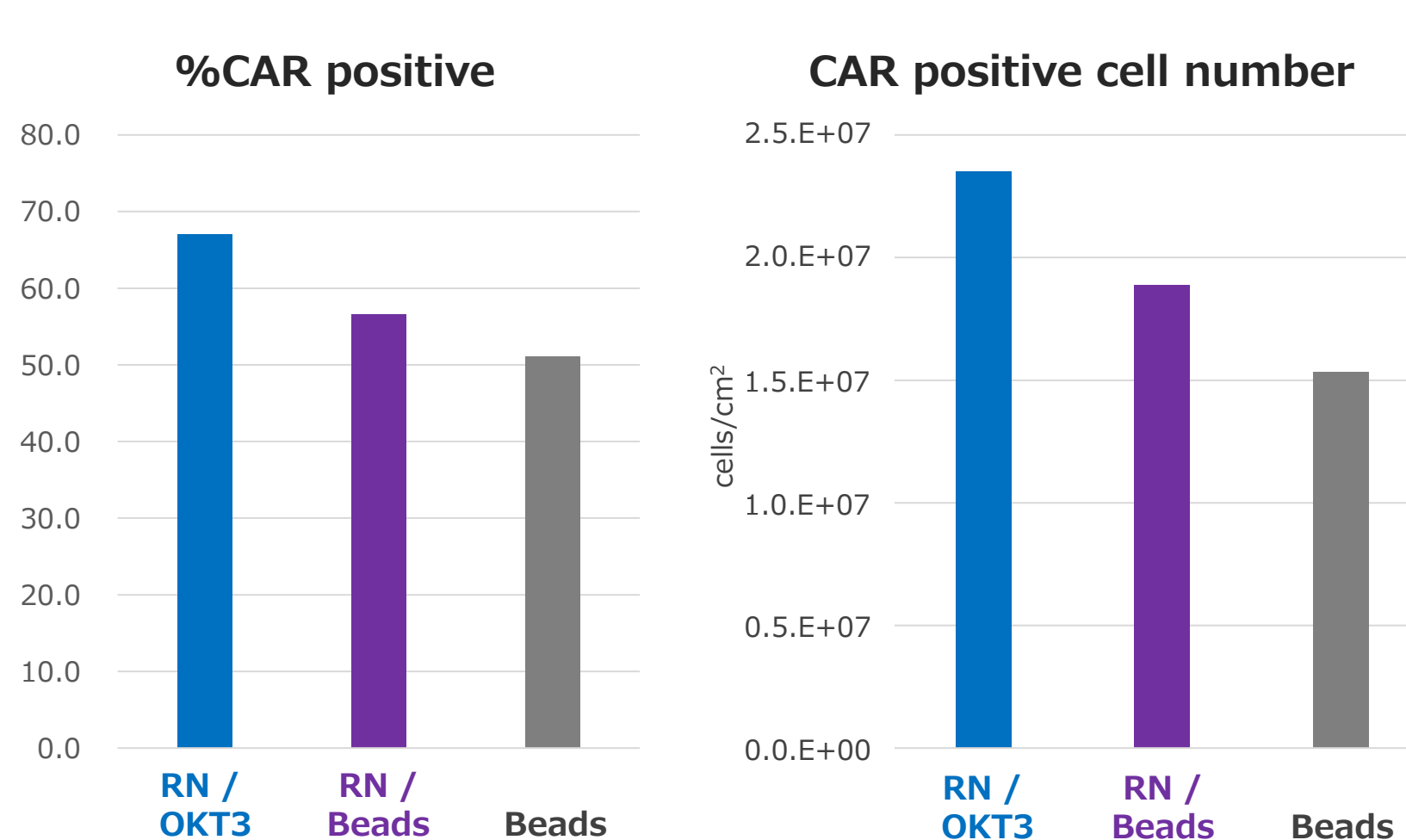
To further simplify the manufacturing process, the culture medium was added to its maximum volume on day 2.



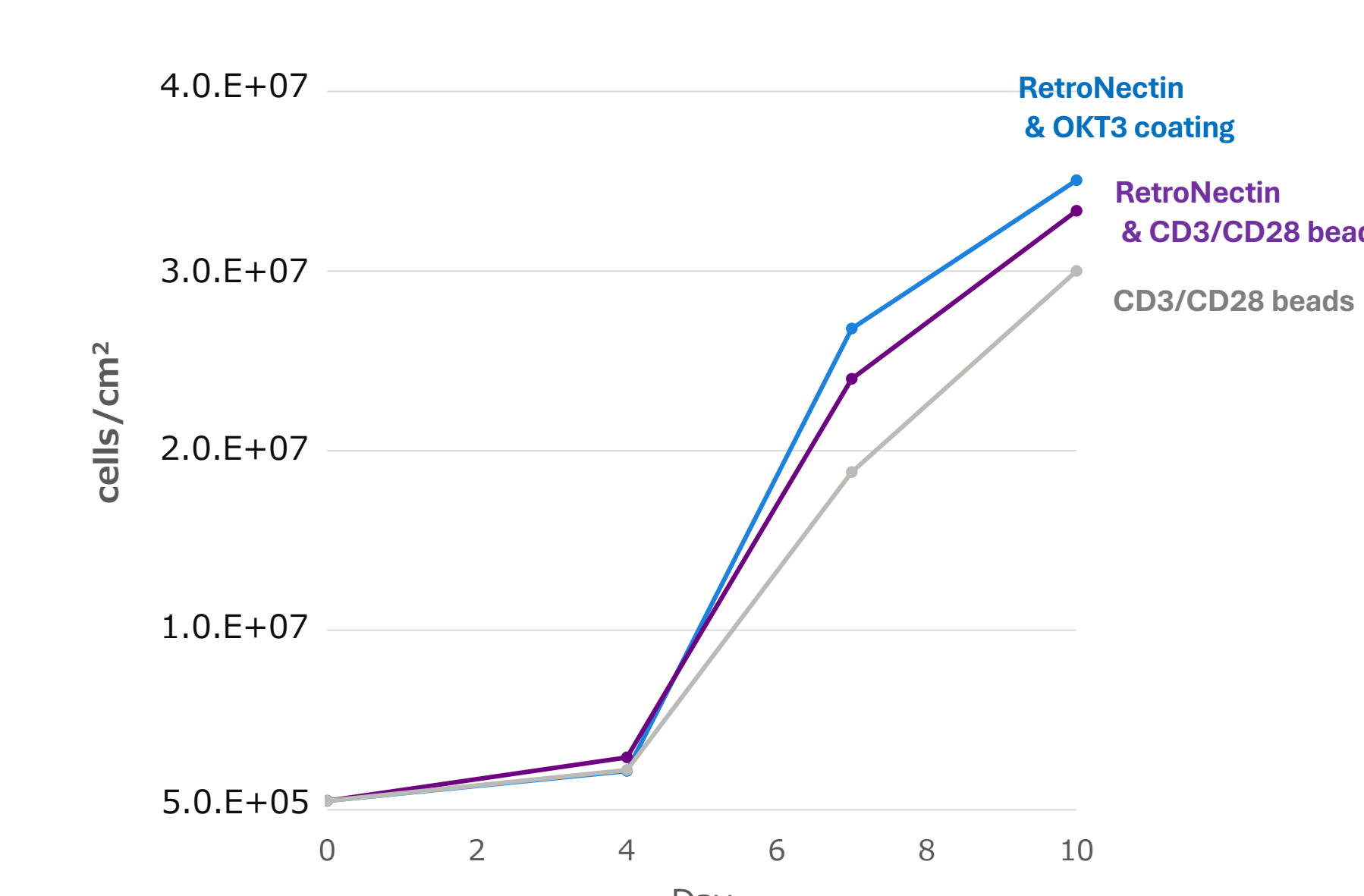
Immunophenotype



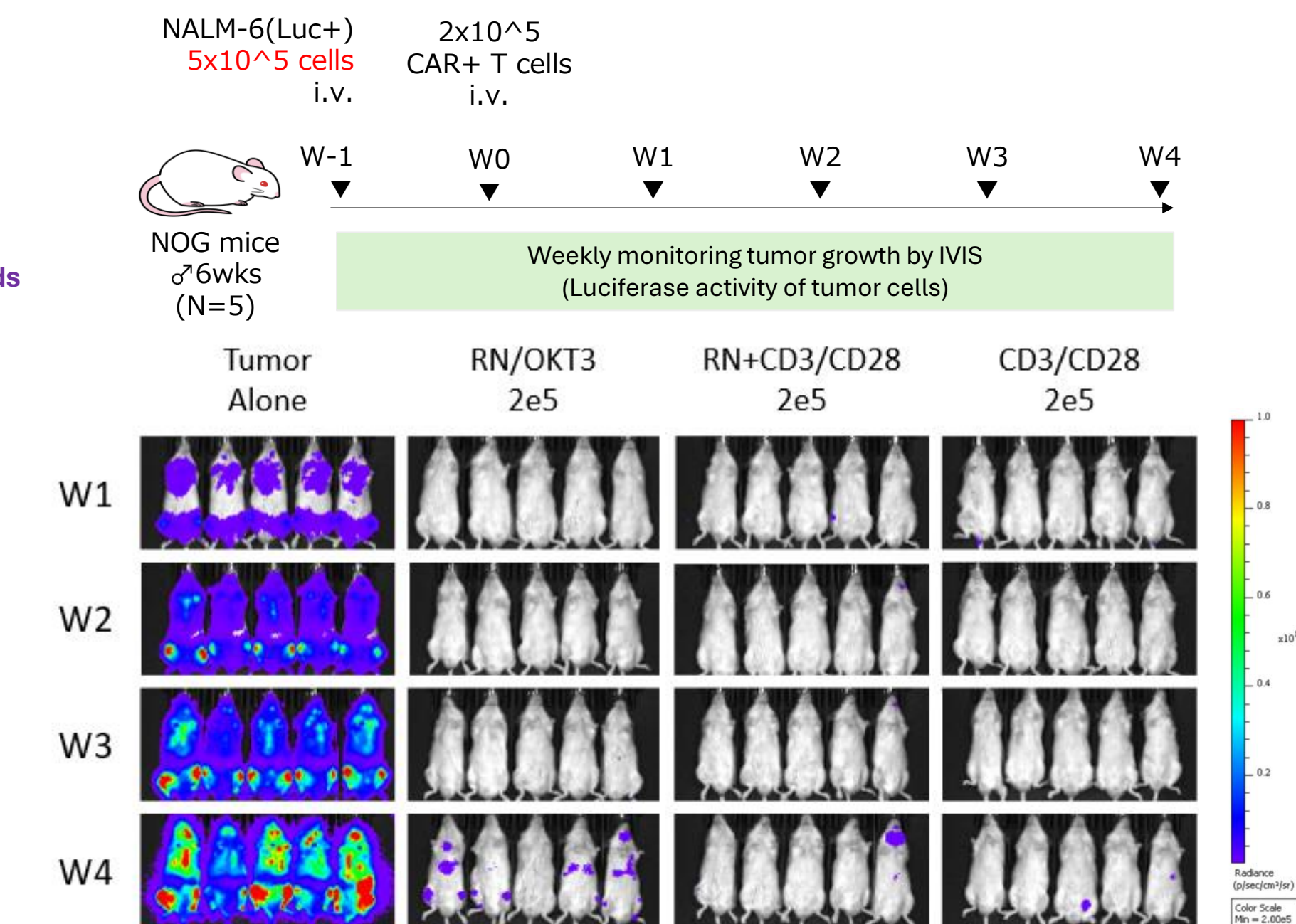
Transduction efficiency



T cell expansion



in vivo antitumor activity



- ✓ Proliferation and Transduction Efficiency: RN/OKT3 > RN + CD3/CD28 beads > CD3/CD28 beads
- ✓ T cell stemness (Ratio of CD62L positive Naïve & Central memory): RN/OKT3 > RN + CD3/CD28 beads ≥ CD3/CD28 beads
- ✓ T cells produced by all stimulation methods showed the same level of anti-tumor effect.
- ✓ 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.

COI Disclosure Information

Lead Presenter: Yasunori Amaishi
Principal Researcher: Sachiko Okamoto

We have no financial relationships to disclose.
Y.A, I.M, S.I, S.O are employees of Takara Bio Inc.

Summary

- ◆ 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.
- ◆ CAR-T cells cultured in the RetroNectin/OKT3-coated G-Rex showed higher proliferation rates and a more undifferentiated phenotype compared to uncoated conditions.
- ◆ We have established a method for manufacturing CAR-T cells in a single vessel over three process days, regardless of the cell stimulation method.