

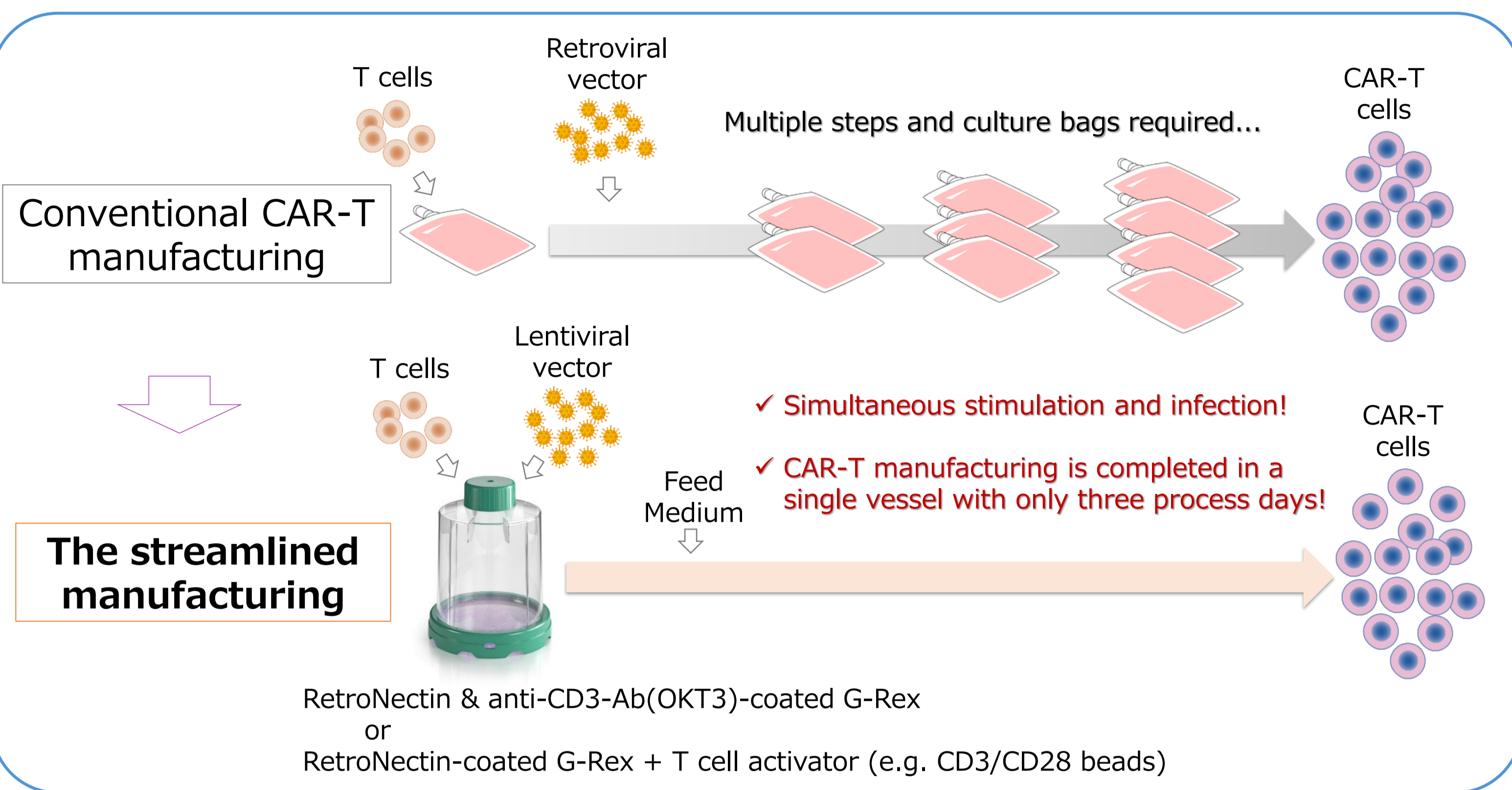
Streamlined CAR-T Cell Manufacturing via Integration of RetroNectin[®], G-Rex[®], and Lentiviral Vectors

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



Abstract

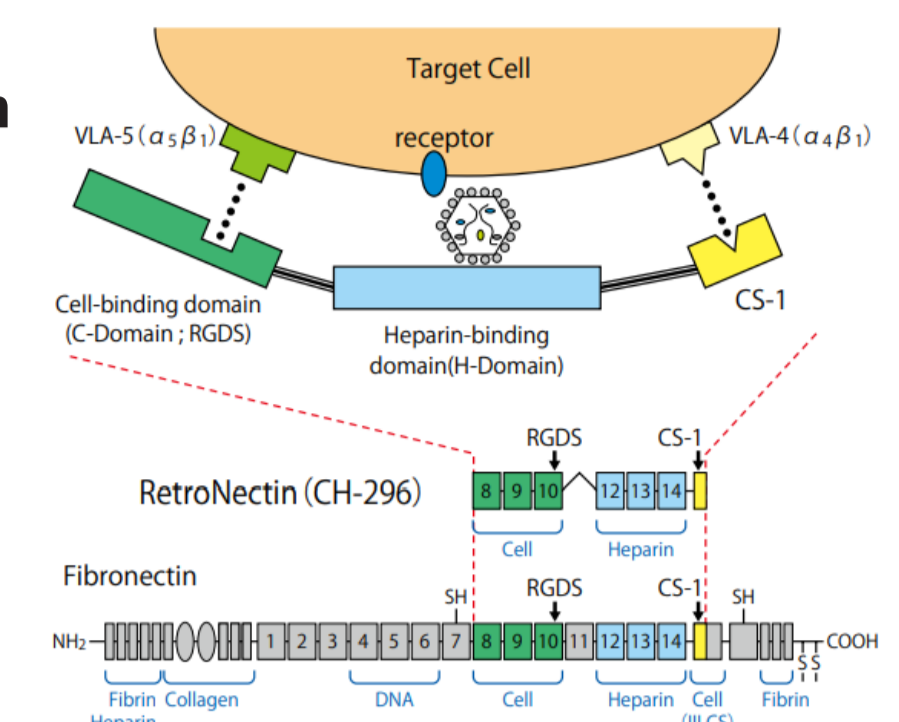
CAR-T therapy is highly effective for hematologic malignancies but faces challenges due to complex, costly autologous cell manufacturing. This study introduces a simplified process using RetroNectin and the G-Rex bioreactor. RetroNectin enhances viral gene transfer, while G-Rex supports high-density culture. Coating G-Rex with RetroNectin enables simultaneous T-cell activation and lentiviral transduction, reducing vector use and labor. An optimized protocol streamline production to three operational days, and the Spo-T method achieves high-quality CAR-T cells in just two operational days. This approach improves efficiency and quality, offering promise for clinical applications.



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 domain.
- 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)

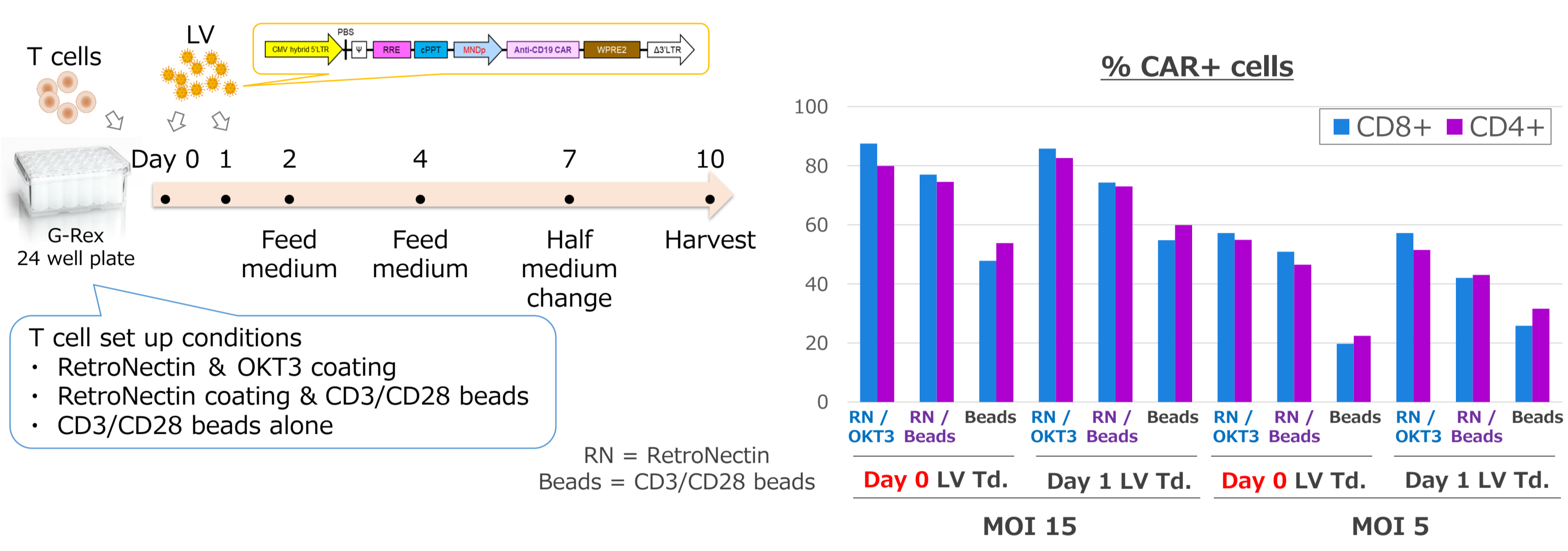


- The bottom surface is made of a gas-permeable membrane, allowing high-density culture of non-adherent cells.
- No stirring or special equipment is required.
- A large volume of culture medium can be added, eliminating the need for medium exchange.

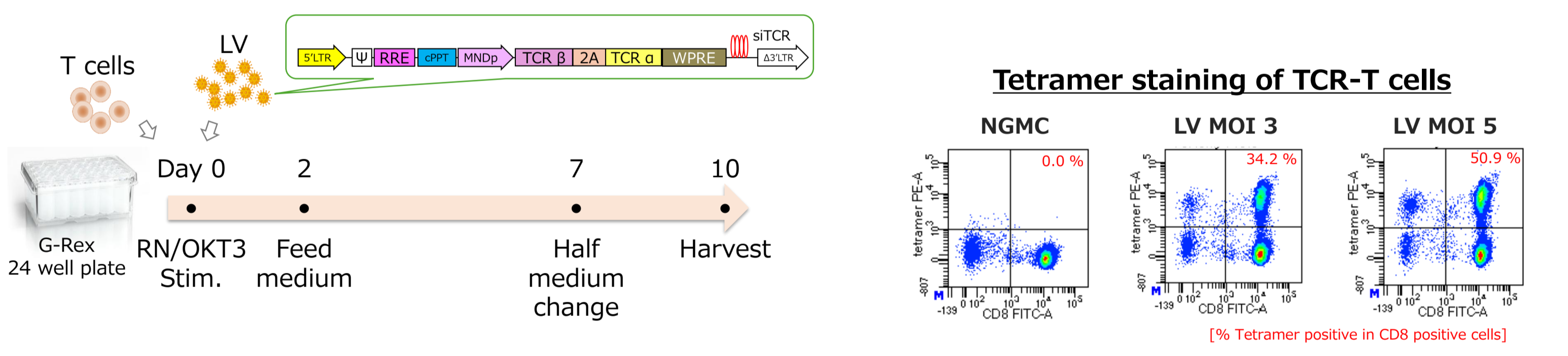
The use of G-Rex in cell manufacturing has been increasing

Optimization of lentiviral transduction

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



- Transduction Efficiency : RN/OCT3 > RN + CD3/CD28 beads > CD3/CD28 beads
- RNs enables to perform high transduction efficiency at low MOI.
- RN coatings provide high Td efficiency comparable to Day 1 infection even on Day 0.

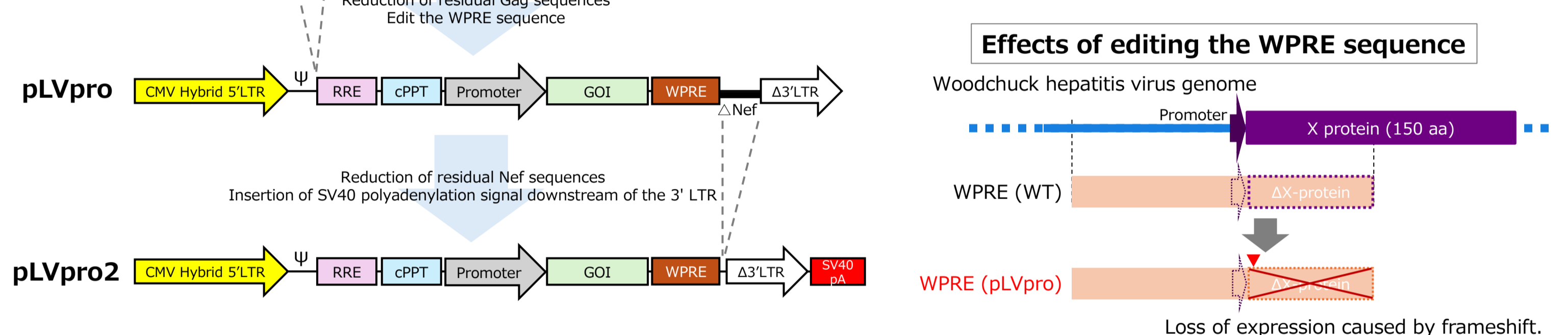


- TCR-T cells can also be easily manufactured using a combination of RetroNectin, G-Rex, and lentivirus

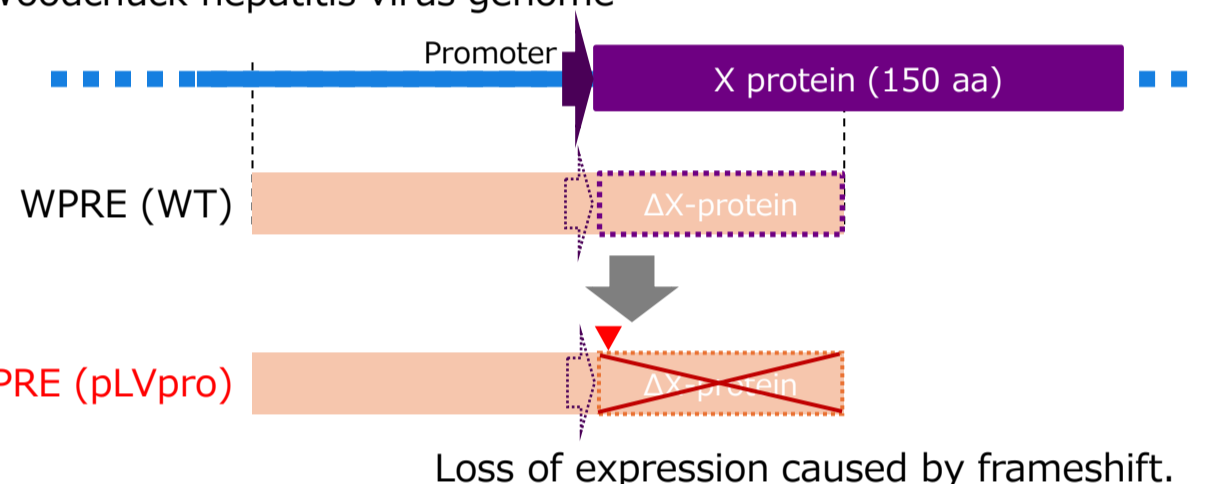
Refer to related poster:
WS23-12-P644
Development of Novel Lentiviral Vectors for TCR-T Cell Therapy Targeting Solid Tumors

LVpro[™] Lentiviral vector production system

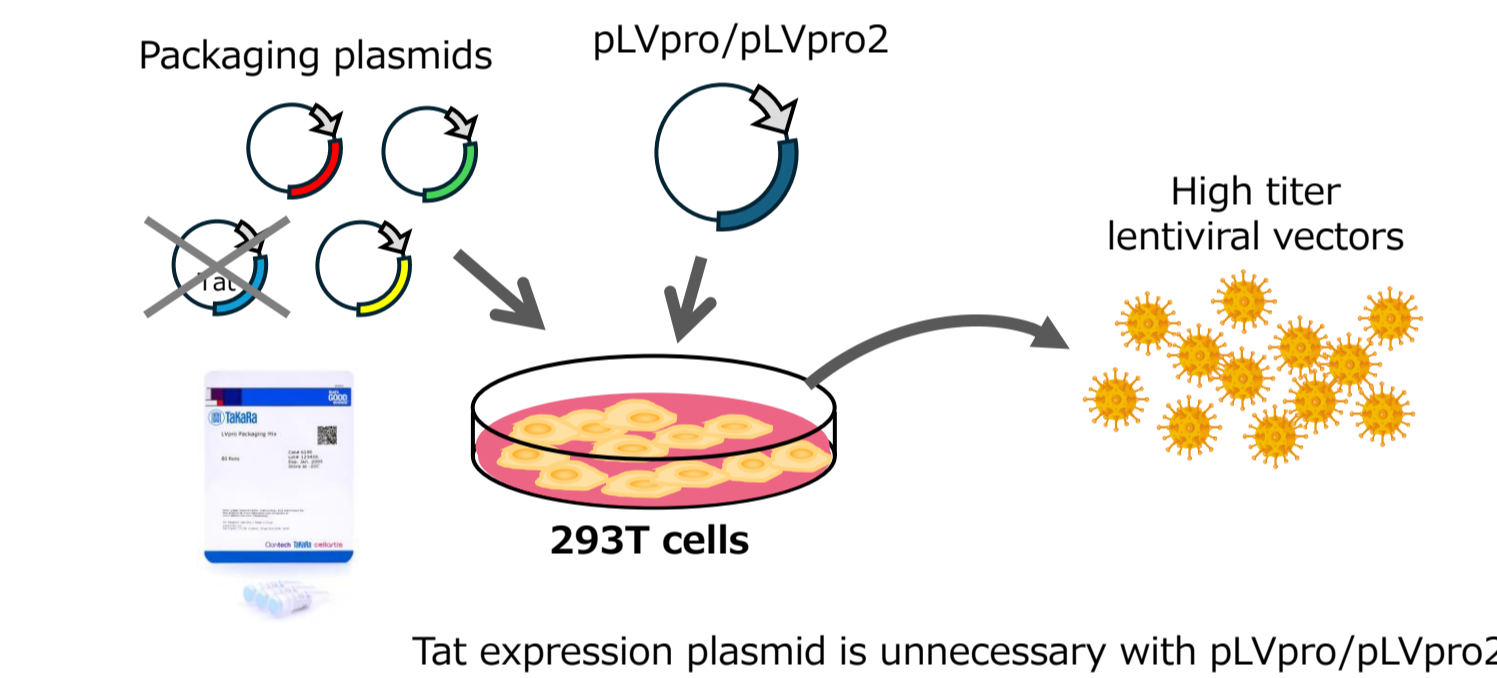
Takara Bio has introduced several improvements to its previously marketed pLV SIN, reducing HIV-derived sequences and developing a high-titer lentiviral vector plasmid. These enhancements are expected to help reduce the cost of viral production.



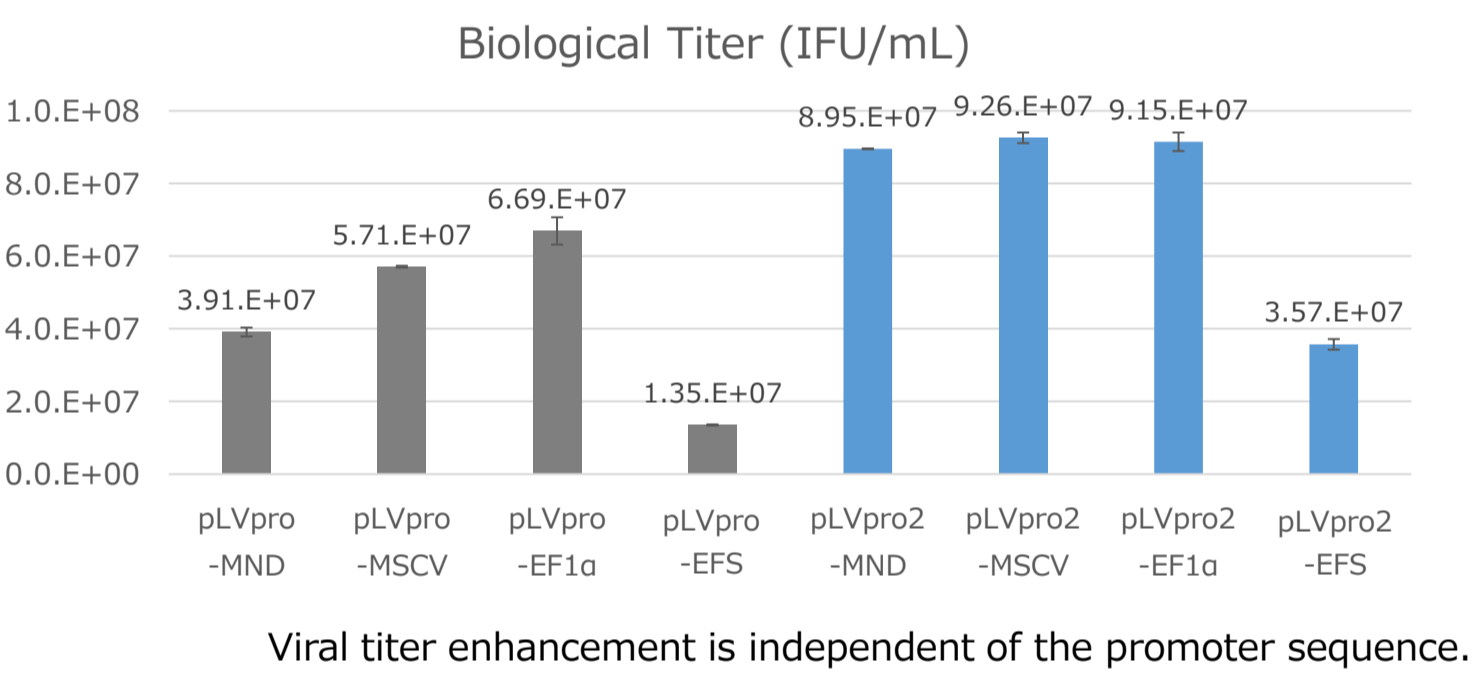
Effects of editing the WPRE sequence



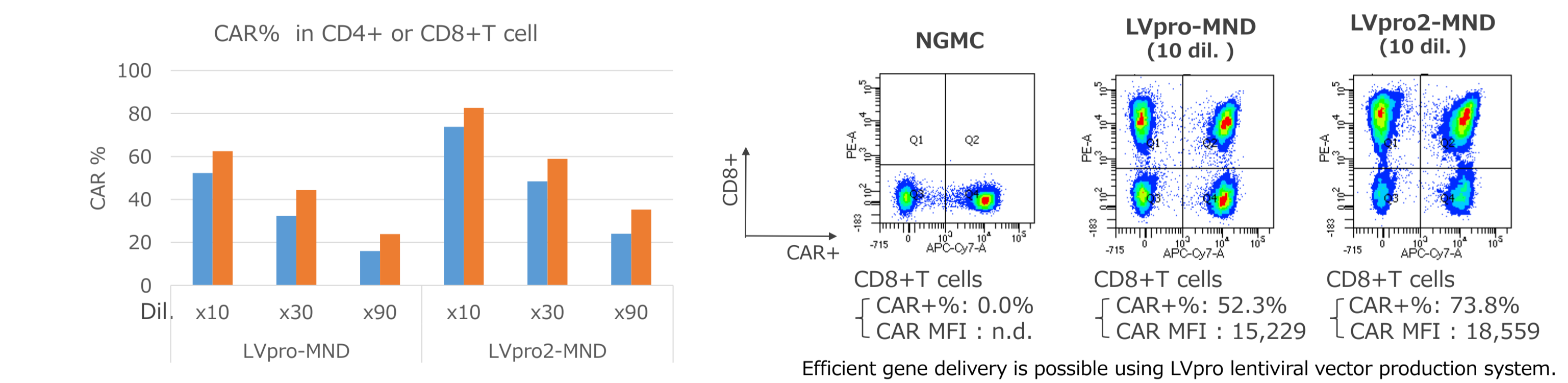
Production of lentivirus using a hybrid LTR-type plasmid



Titer improvement of pLVpro2 compared to pLVpro

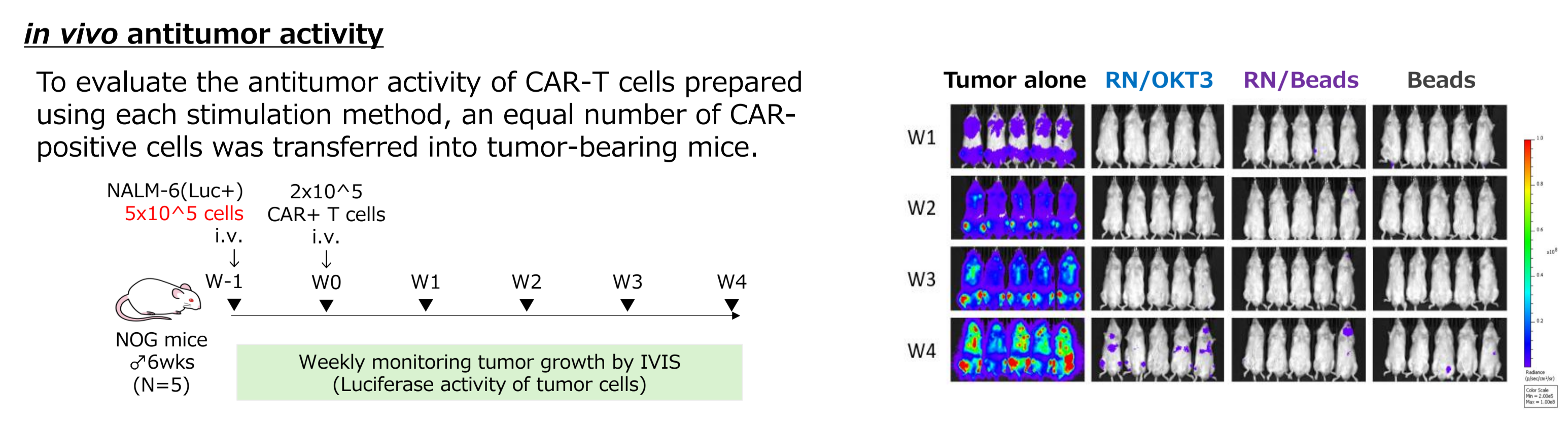
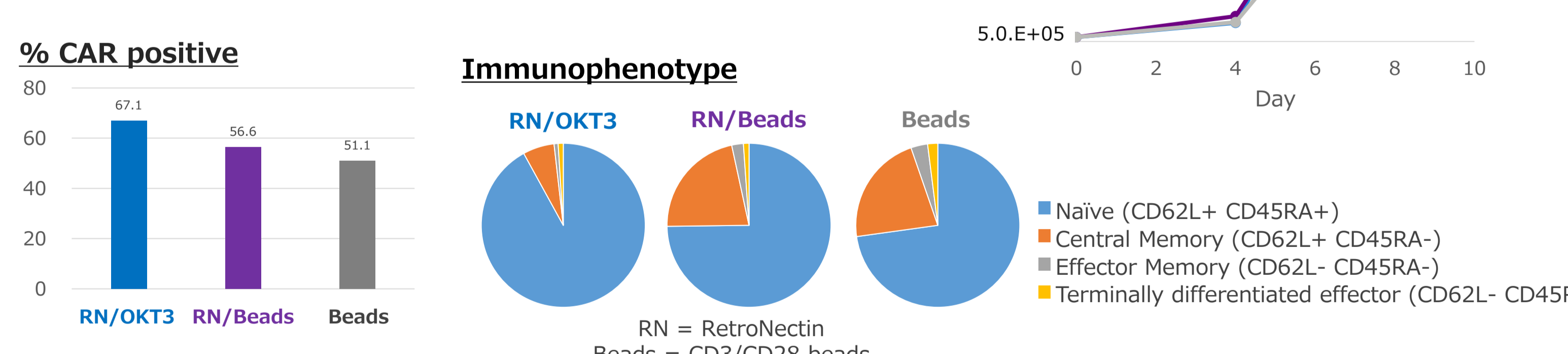
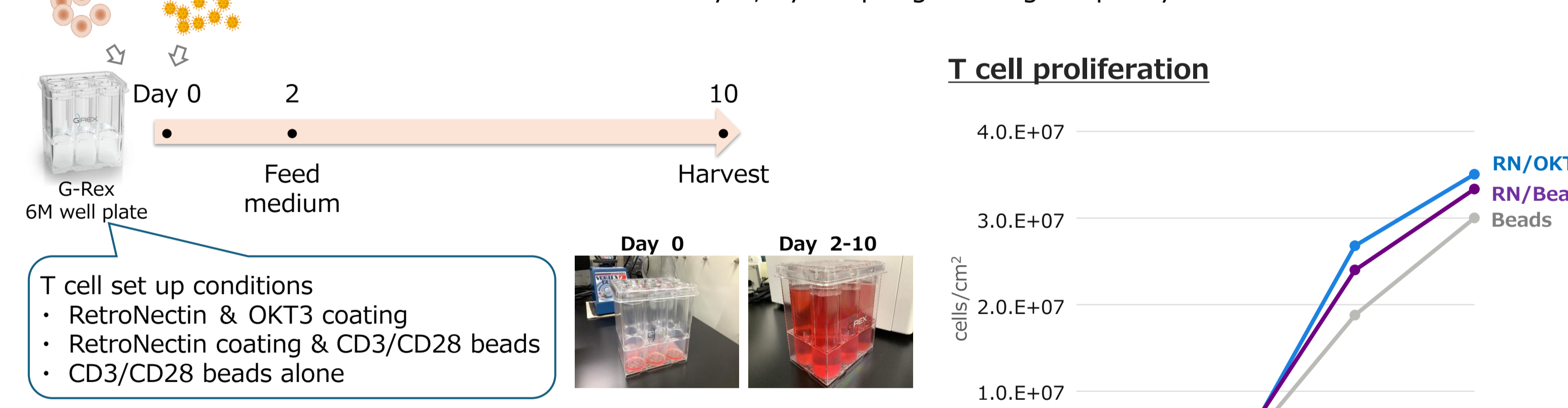


Transduction of primary T cells using CD19-CAR-expressing LVpro vector



Scale up of cell production and *in vivo* anti-tumor test

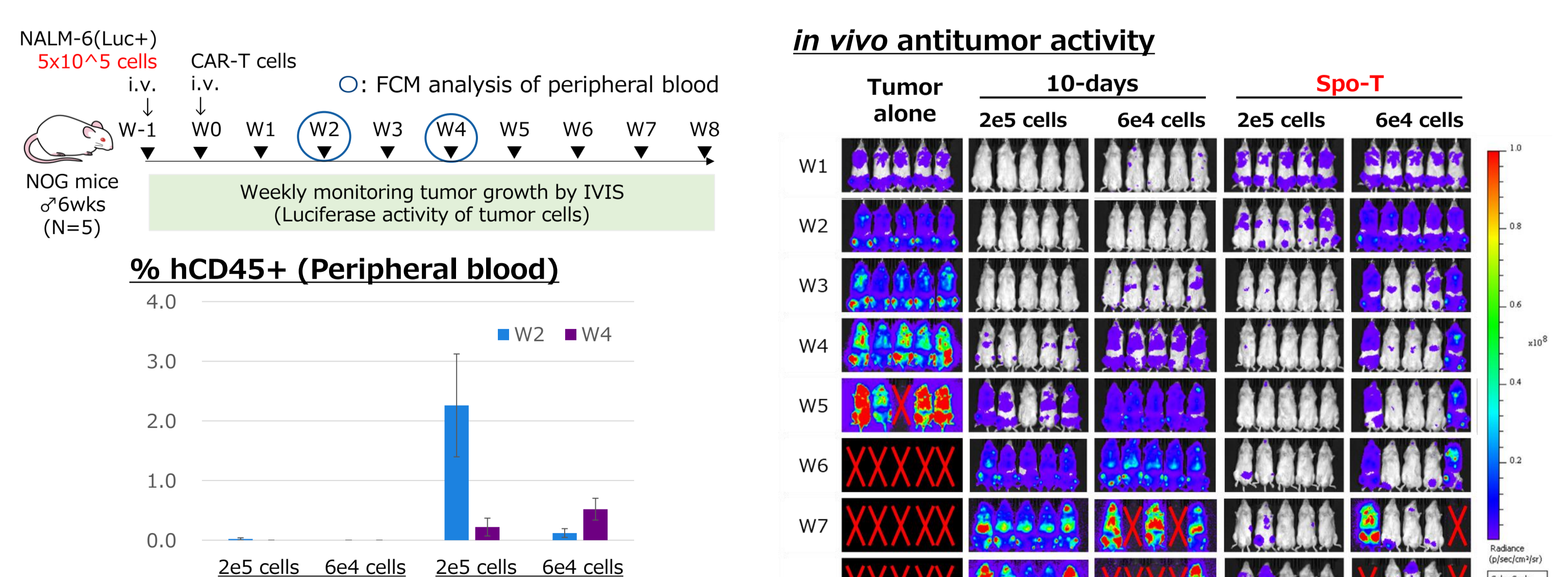
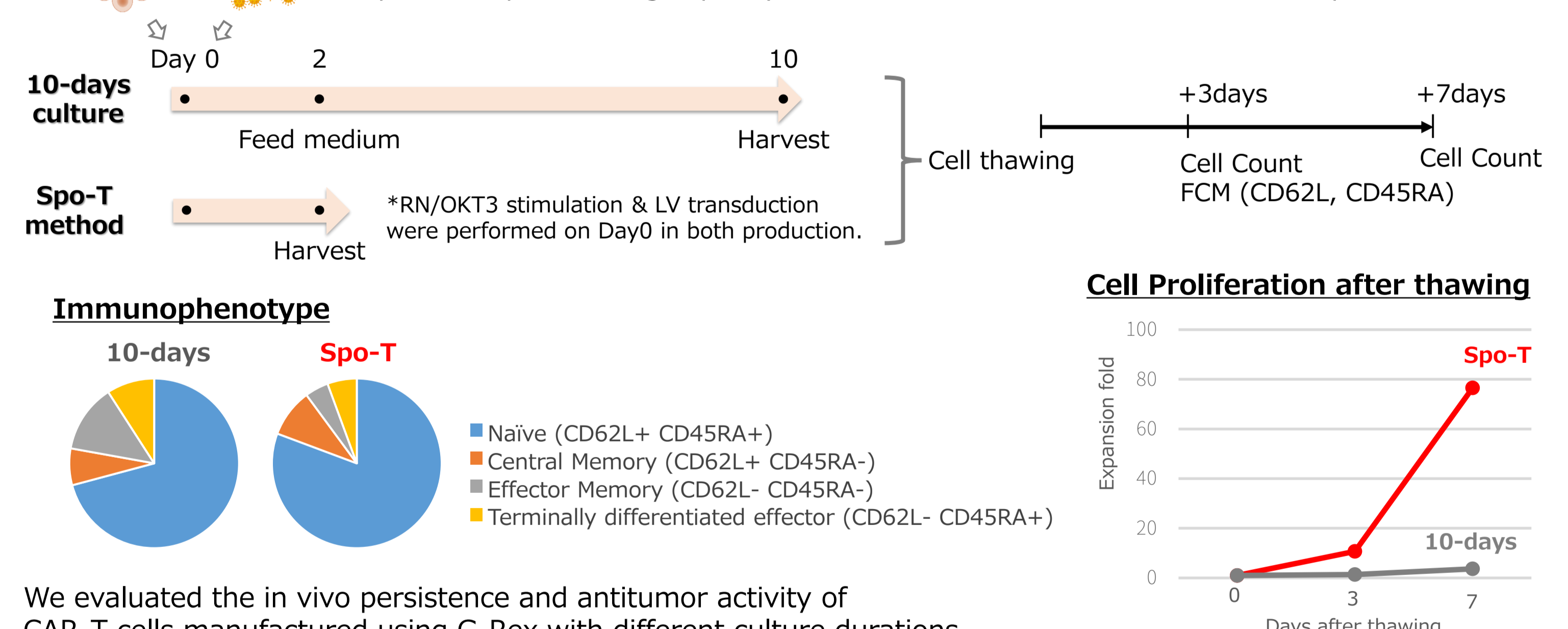
To further simplify the manufacturing process, the culture medium was added to its maximum volume on day 2, by adopting the large-capacity G-Rex M series.



- Proliferation & Td. Efficiency: RN/OCT3 > RN + CD3/CD28 beads > CD3/CD28 beads
- T cell stemness : RN/OCT3 > RN + CD3/CD28 beads ≥ CD3/CD28 beads
- T cells produced by all stimulation methods showed the strong anti-tumor effect.

Shortening of the culture period (Spo-T[®] method)

We are working not only to simplify the process but also to shorten the manufacturing period to produce high-quality CAR-T cells, a method we have named Spo-T method.



- G-Rex Spo-T CAR-T cells showed higher proliferation and stemness.
- Spo-T CAR-T cells showed long-term survivability and anti-tumor effect in tumor bearing model.

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/OCT3-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 with only three process days, regardless of the cell stimulation method.

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.