

P-3235

Short-term production of CAR-T cells using RetroNectin and G-Rex

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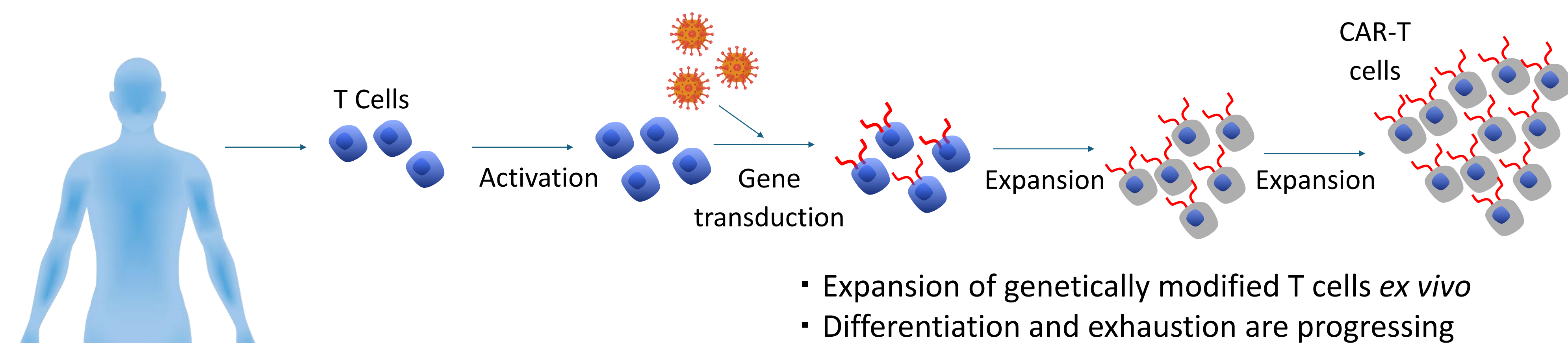


Abstract

CAR-T cell therapies have shown remarkable efficacy in the treatment of hematologic malignancies. However, there are still many challenges in the cell manufacturing process. Most CAR-T cell manufacturing protocols expand T cells for 9 to 14 days. It is known that extended *ex vivo* culture period results in T cell differentiation and exhaustion, which reduces the potency of CAR-T cells after adoptive transfer. Therefore, there is a need to reduce manufacturing time to improve treatment efficacy. Then, we have already developed a short-term production method for CAR-T cell, Spo-T® (Short Period Operation for T-cell production), and reported its higher cytotoxic activity, greater proliferative ability and lower exhaustion compared to the conventional method. In this study, we established a simple and efficient method for CAR-T cell production by combining the Spo-T method with RetroNectin® and G-Rex® (a gas-permeable membrane bioreactor for high-density culture). This approach enables the efficient generation of high-quality CAR-T cells.

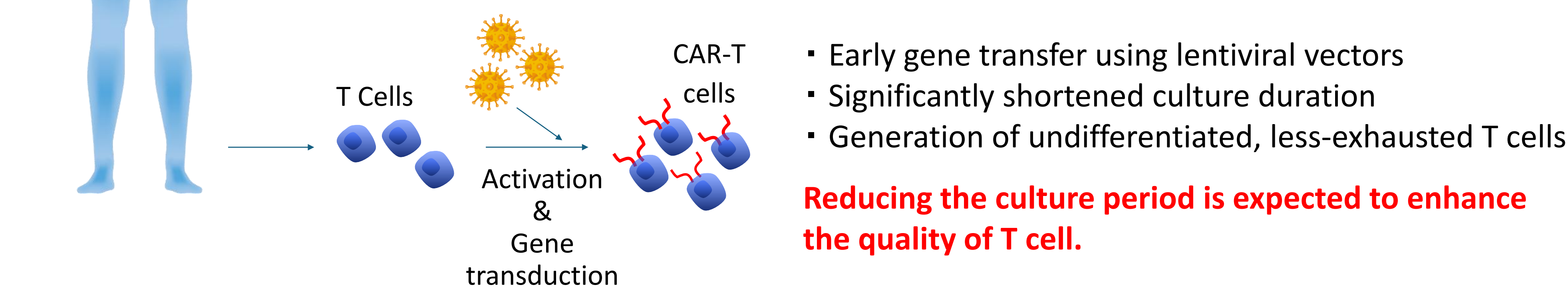
Spo-T® CAR-T cell manufacturing method

Conventional method : 9 - 14 days culture



- Expansion of genetically modified T cells *ex vivo*
- Differentiation and exhaustion are progressing

Spo-T (Short Period Operation for T-cell production) method : 2 days culture



Reducing the culture period is expected to enhance the quality of T cell.

G-Rex® bioreactor (Wilson Wolf)

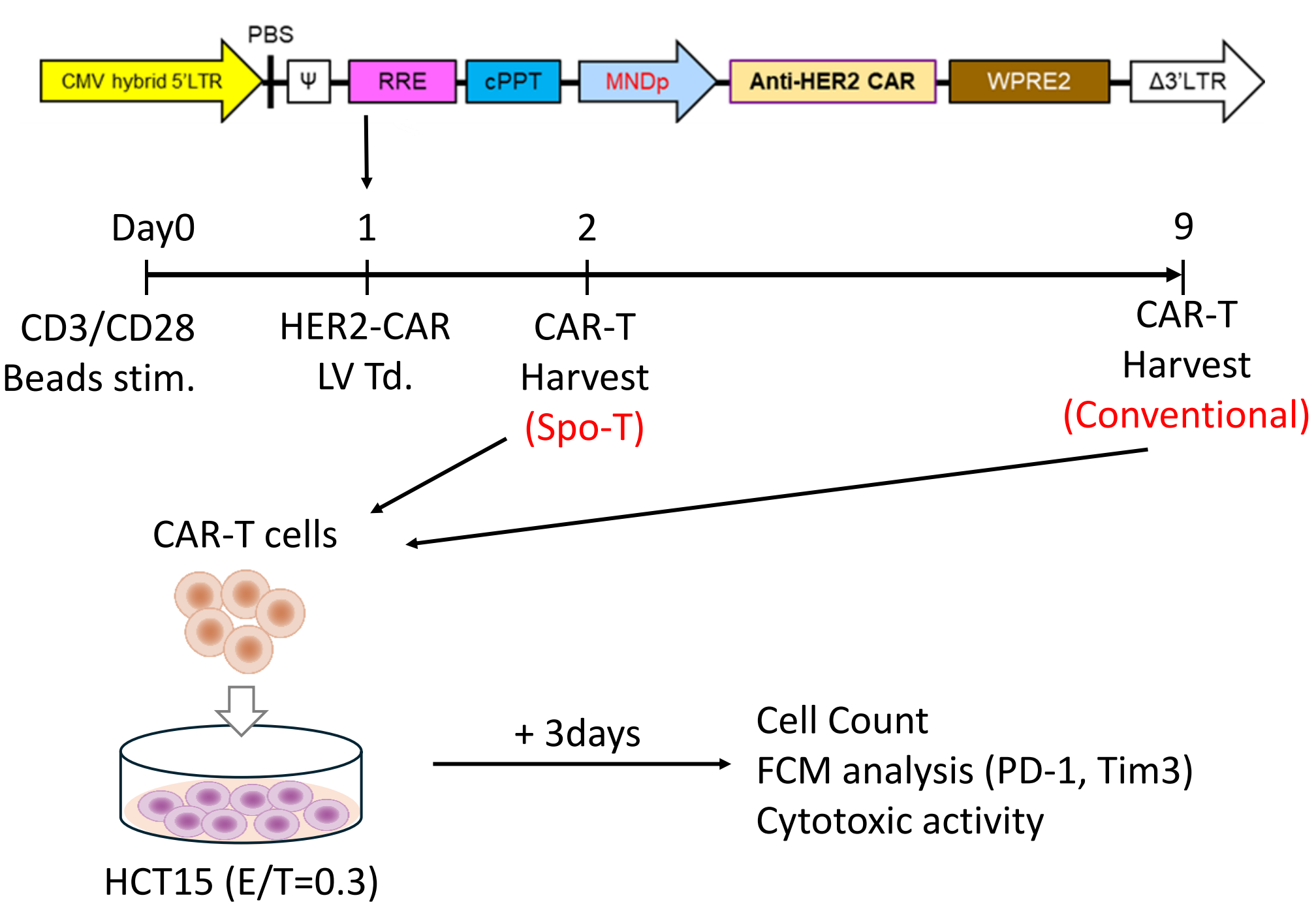


- The bottom surface is made of a gas-permeable membrane, allowing high-density culture of non-adherent cells.
- No stirring or special equipment is required.
- A large volume of culture medium can be added, eliminating the need for medium exchange.

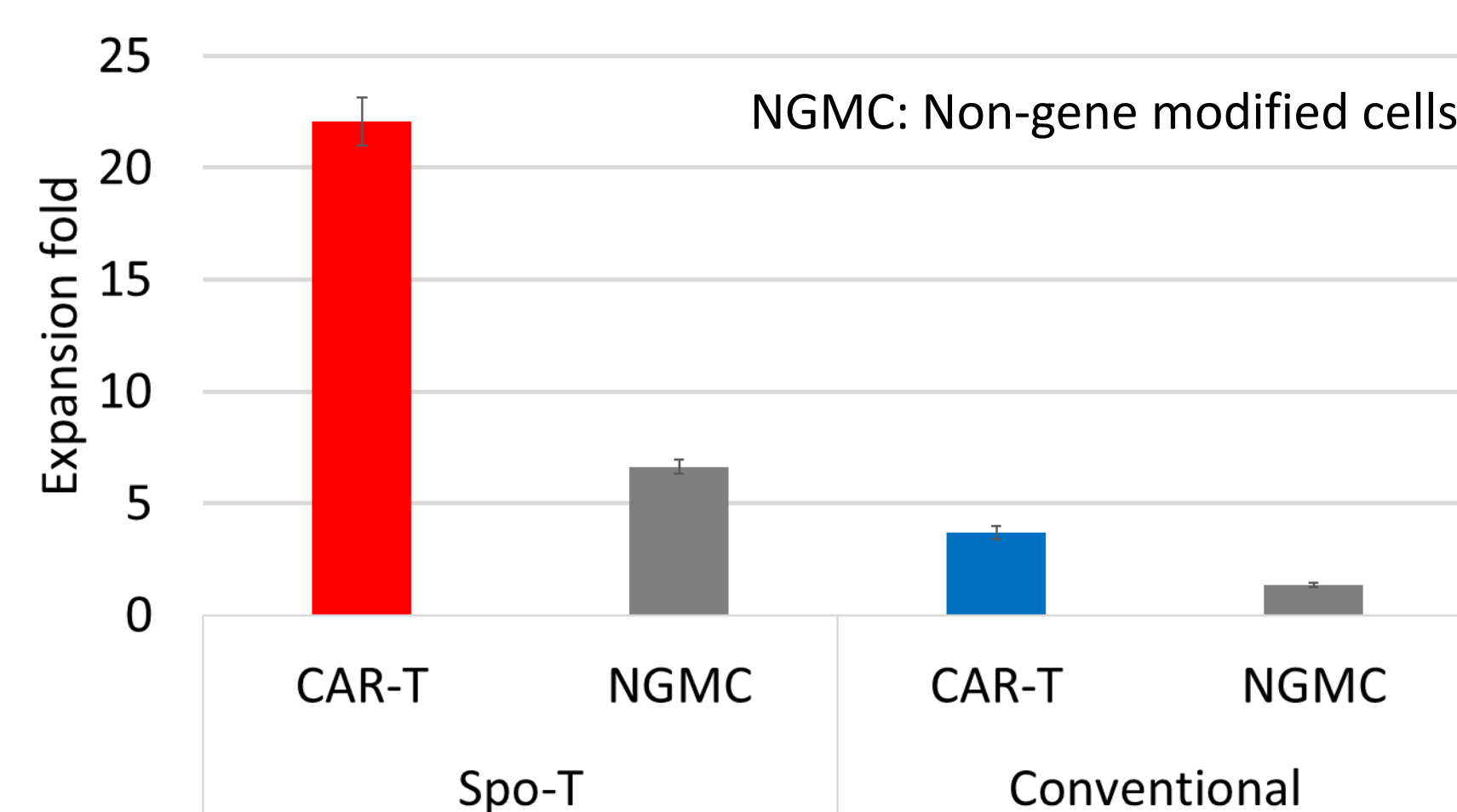
The use of G-Rex in cell manufacturing has been increasing.

Comparison of Spo-T® Method and Conventional Method

HER2 CAR-T cells were co-cultured with antigen expressing cells (HCT15). Cell proliferation and the exhaustion marker expression after antigen stimulation were measured. The cytotoxicity was measured in real time using xCelligence RTCA label-free system.

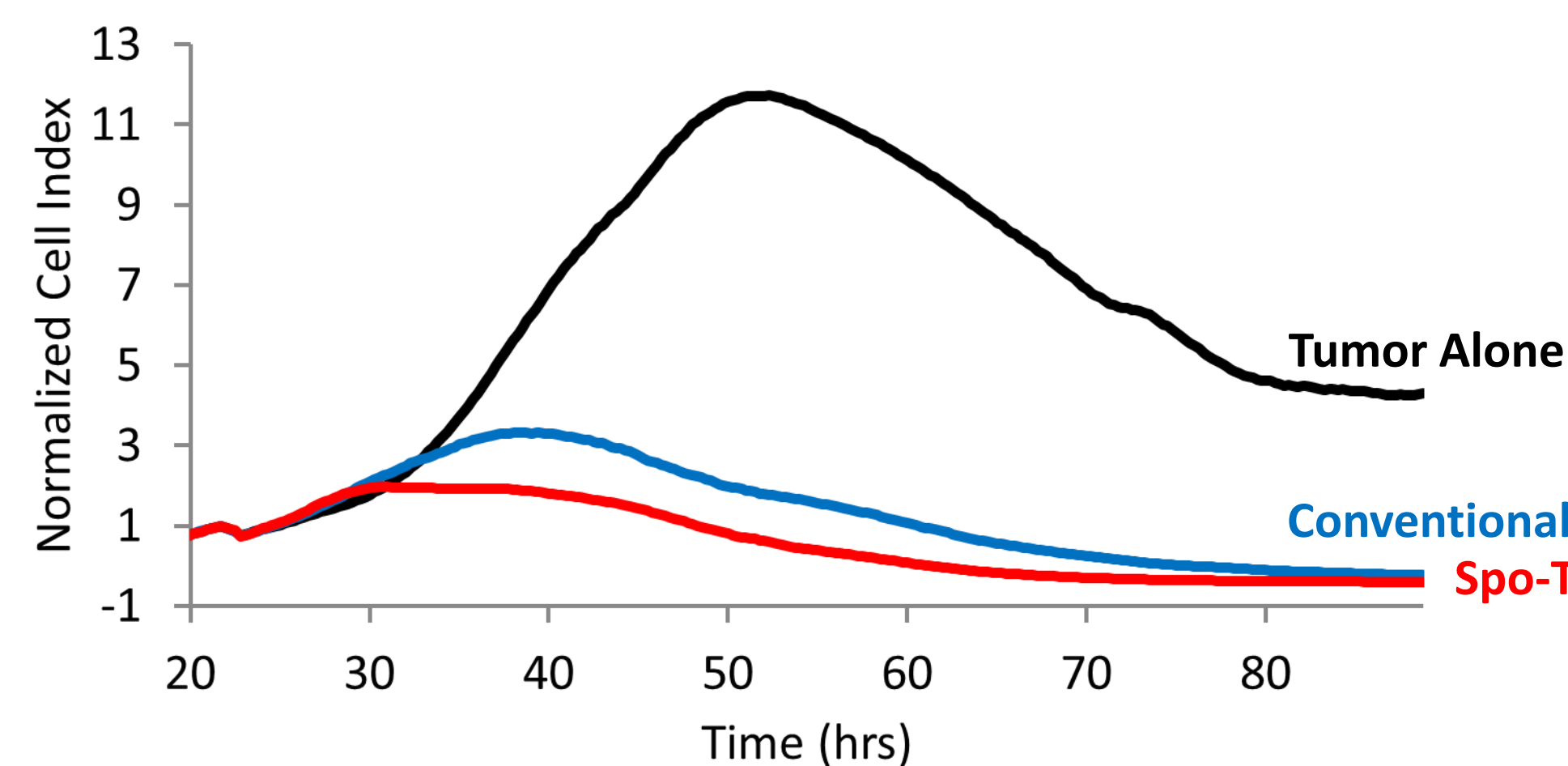


Cell Proliferation after antigen stimulation



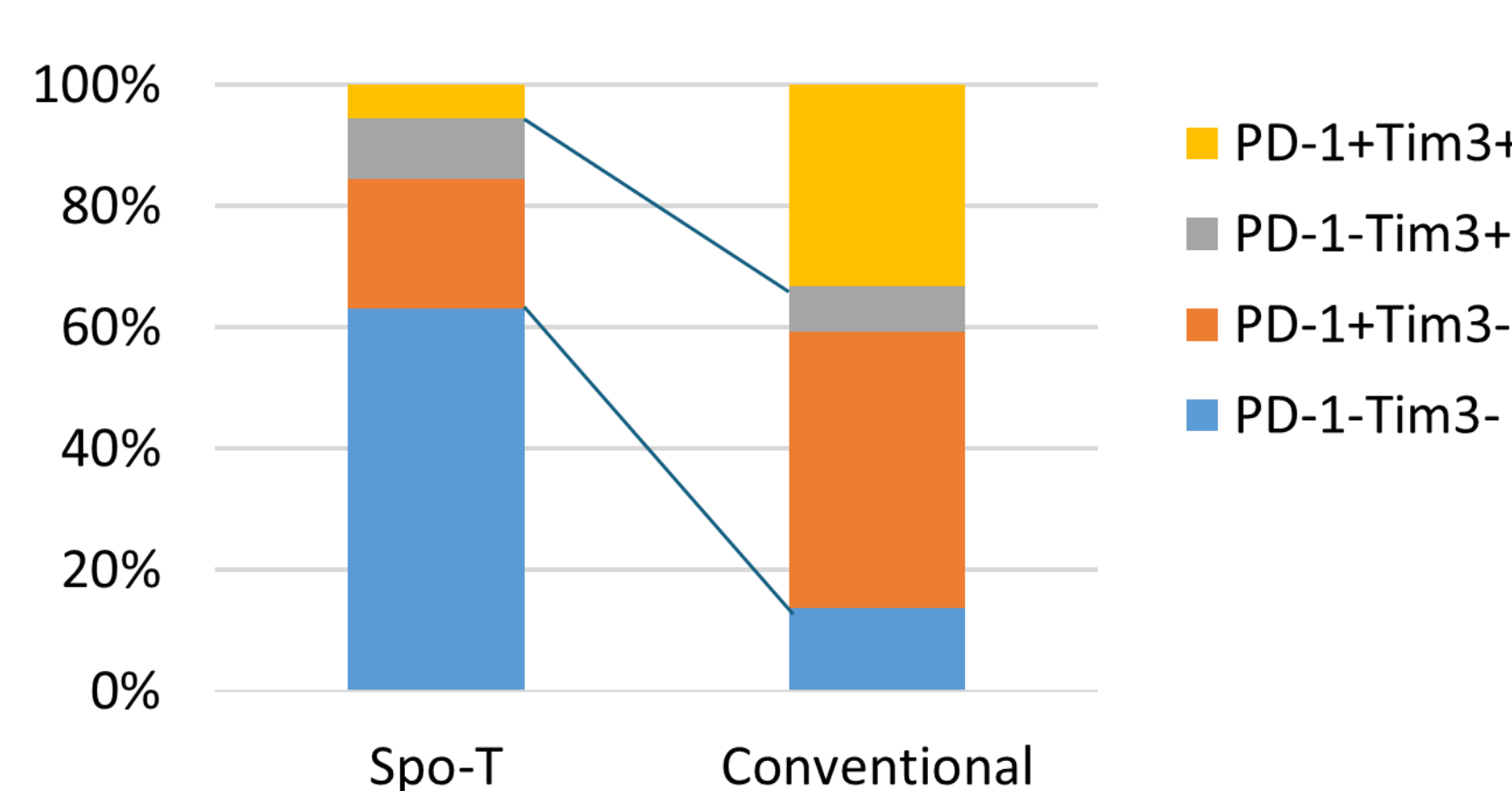
✓ Proliferation : Spo-T > Conventional

Cytotoxicity to tumor cells



✓ Cytotoxicity : Spo-T > Conventional

Exhaustion marker expression



✓ Exhaustion : Spo-T < Conventional

Summary

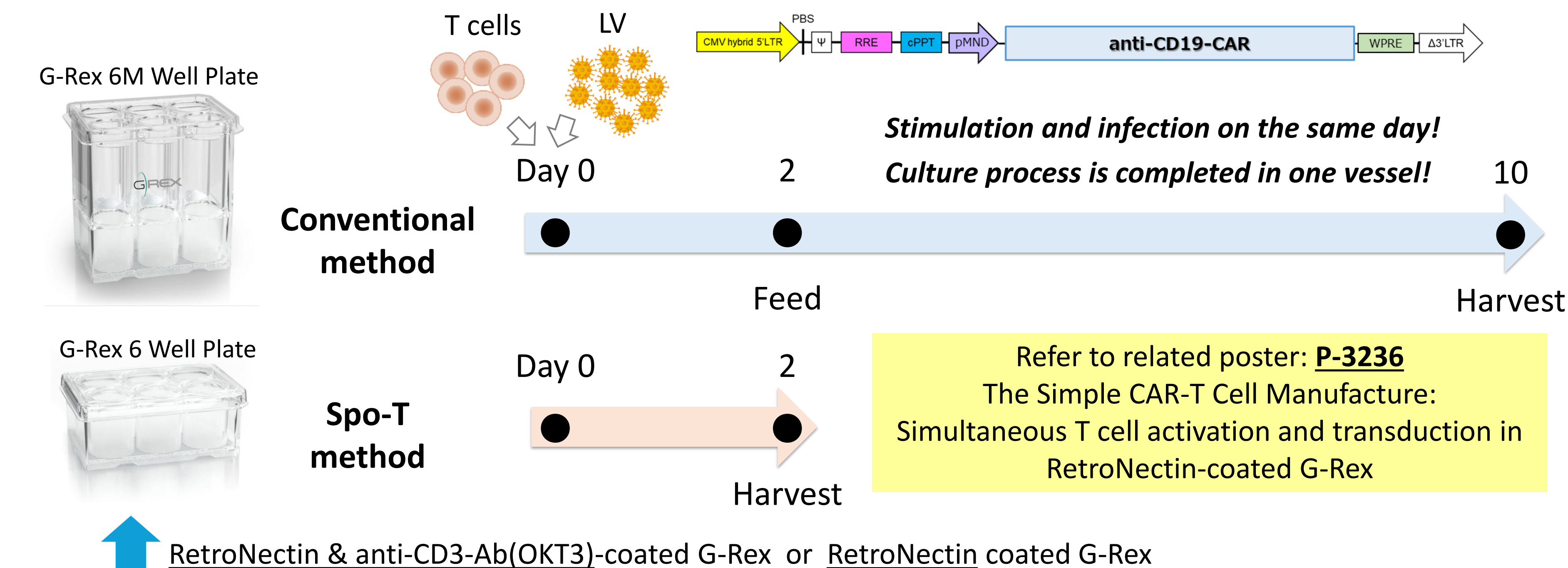
- ✓ We have developed the short-term manufacturing method, **Spo-T®** to produce highly effective CAR-T cells.
 - Extended CAR-T cells survivability and proliferation rates.
 - Strong and sustained antitumor efficacy at low dose.
- ✓ By applying the Spo-T method to the manufacturing process in the RetroNectin® coated G-Rex® bioreactor, we successfully simplified the workflow and achieved the production of high-quality CAR-T cells.

COI Disclosure Information

Lead Presenter: Seina Inui, Principal Researcher: Sachiko Okamoto
We have no financial relationships to disclose. S.I, Y.A, I.M, S.O are employees of Takara Bio Inc.

Adaptation of the Spo-T® method to RetroNectin® & G-Rex® Bioreactor

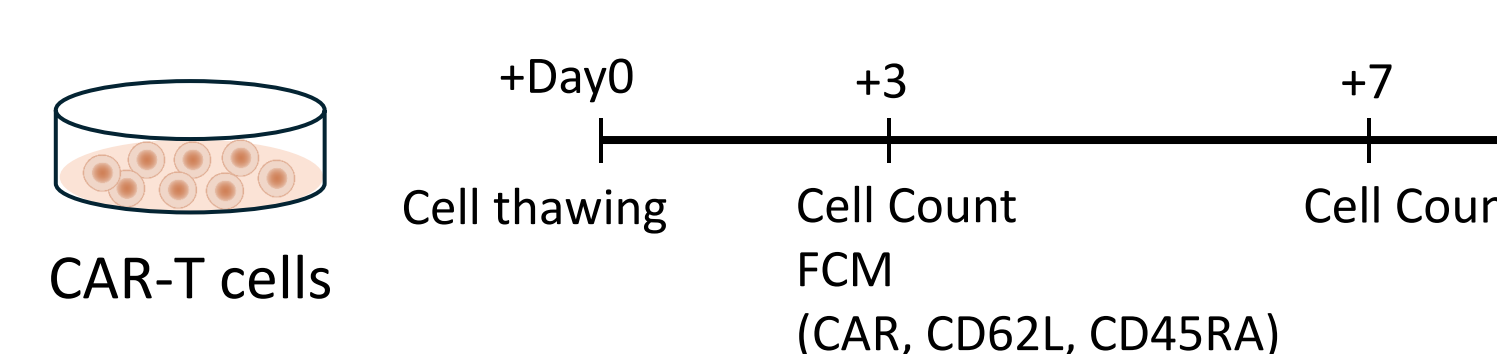
CD19 CAR-T cells were manufactured by G-Rex Bioreactor. For CAR-T cell generation, T cells were stimulated using CD3/CD28 beads or RetroNectin & anti-CD3-Ab(OKT3), and simultaneously transduced with anti-CD19 CAR lentiviral vector at MOI of 5.



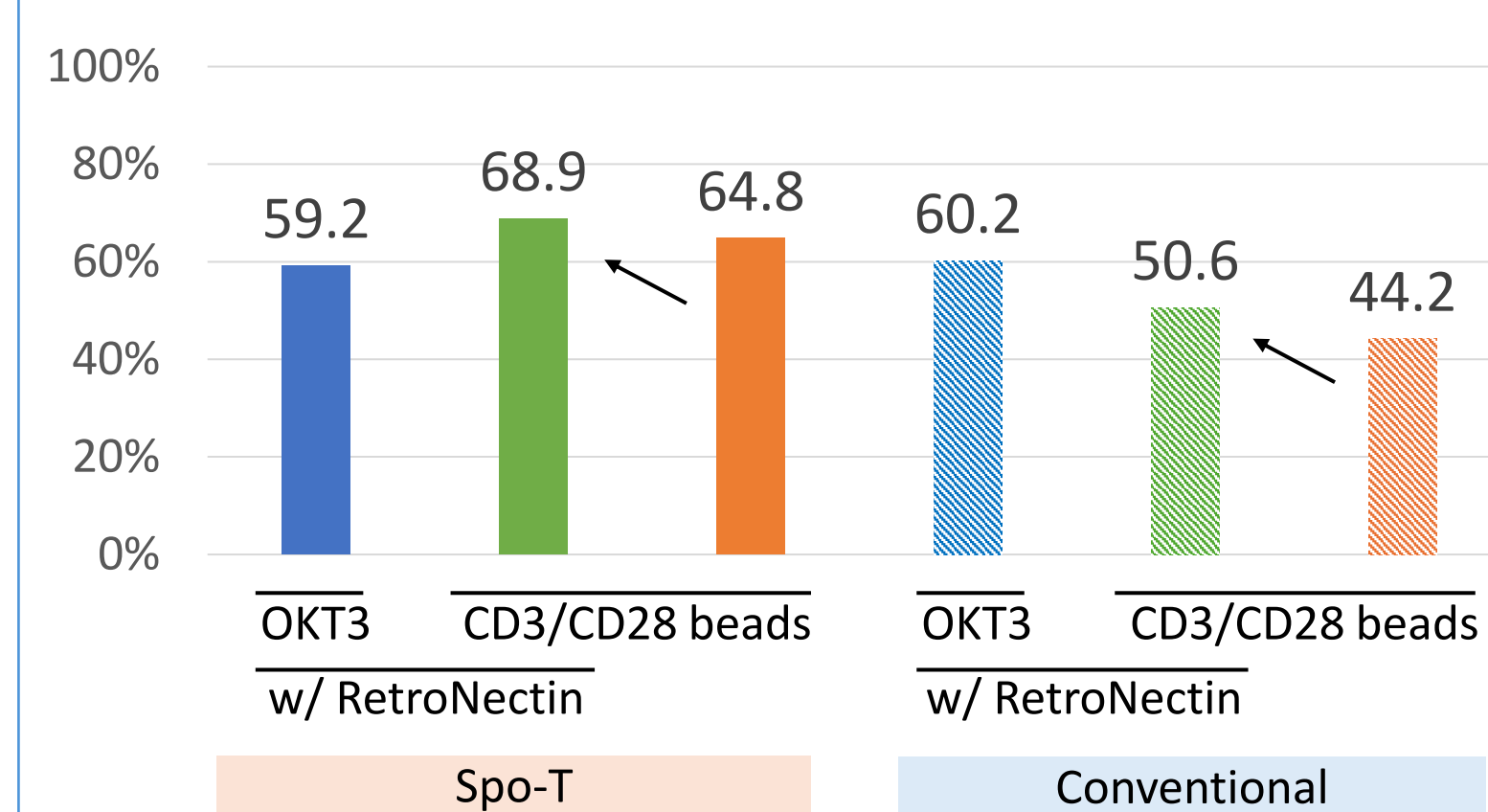
Refer to related poster: **P-3236**
The Simple CAR-T Cell Manufacture:
Simultaneous T cell activation and transduction in RetroNectin-coated G-Rex

In vitro effector function after thawing

After thawing, CAR-T cells were cultured for an additional 3 to 7 days.

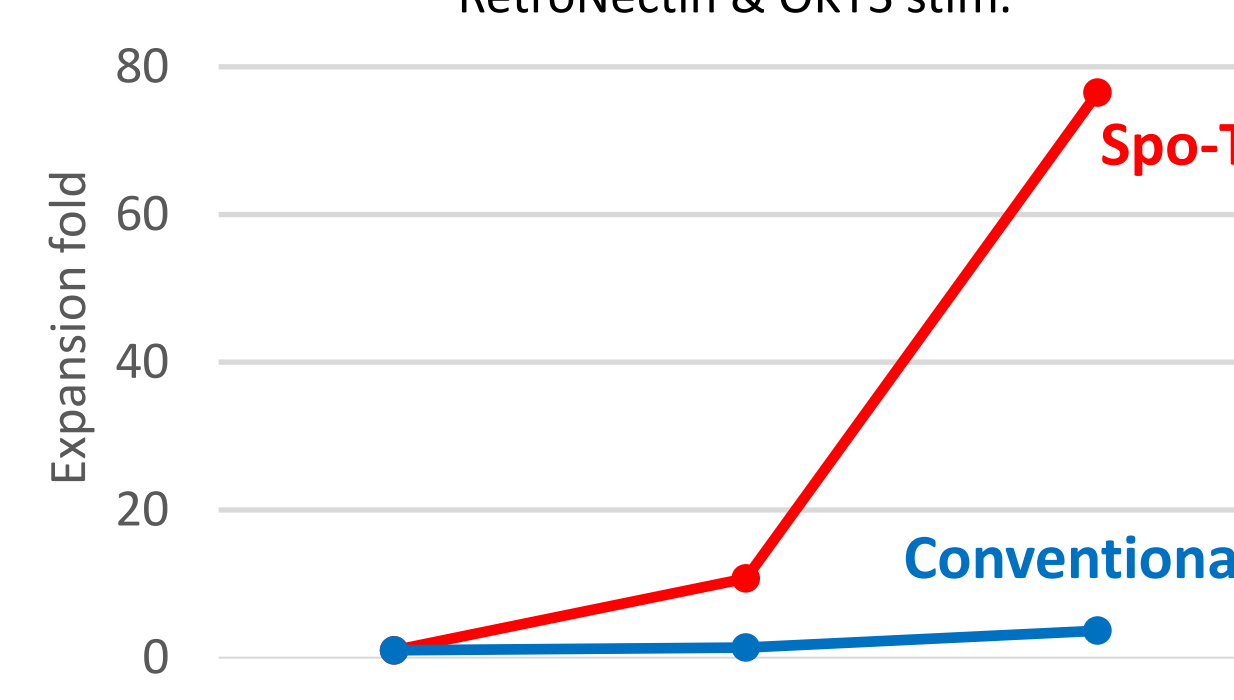


% CD19-CAR+ in T cells



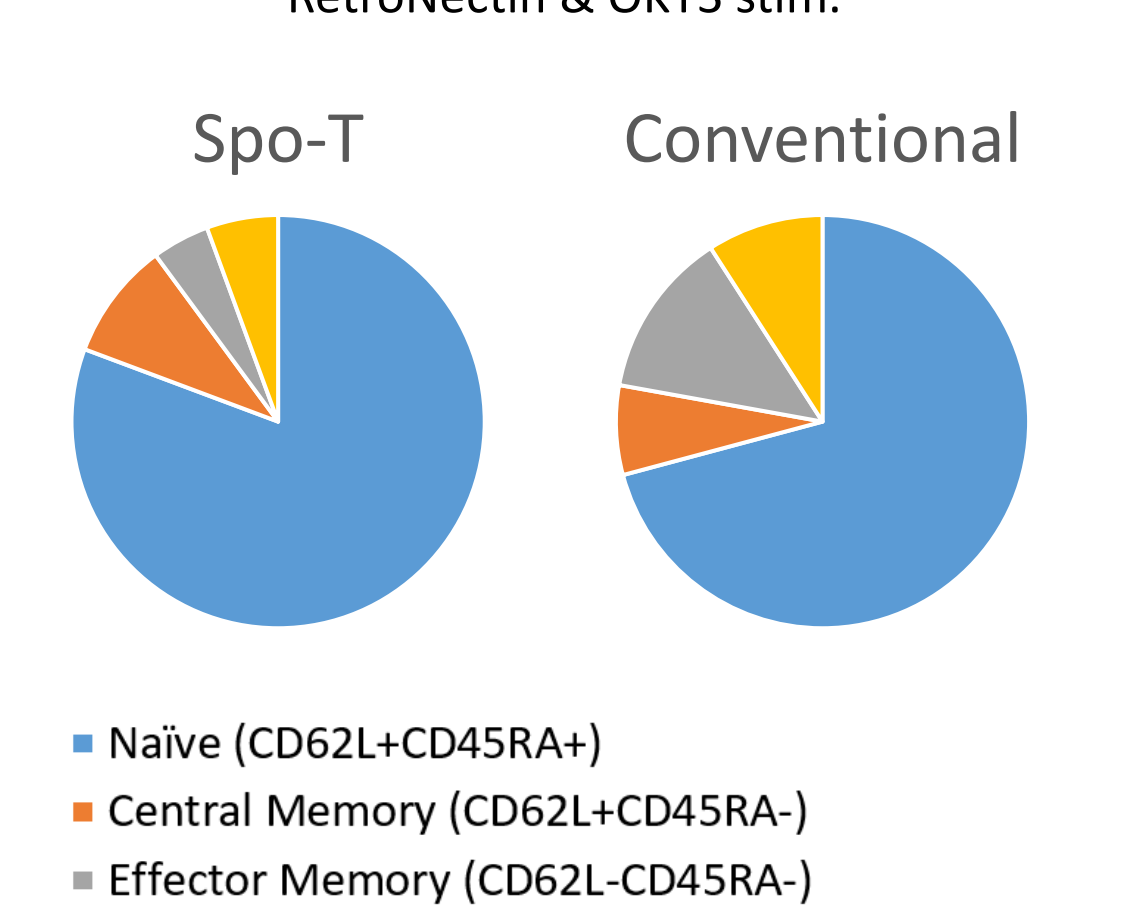
Cell Proliferation

* RetroNectin & OKT3 stim.



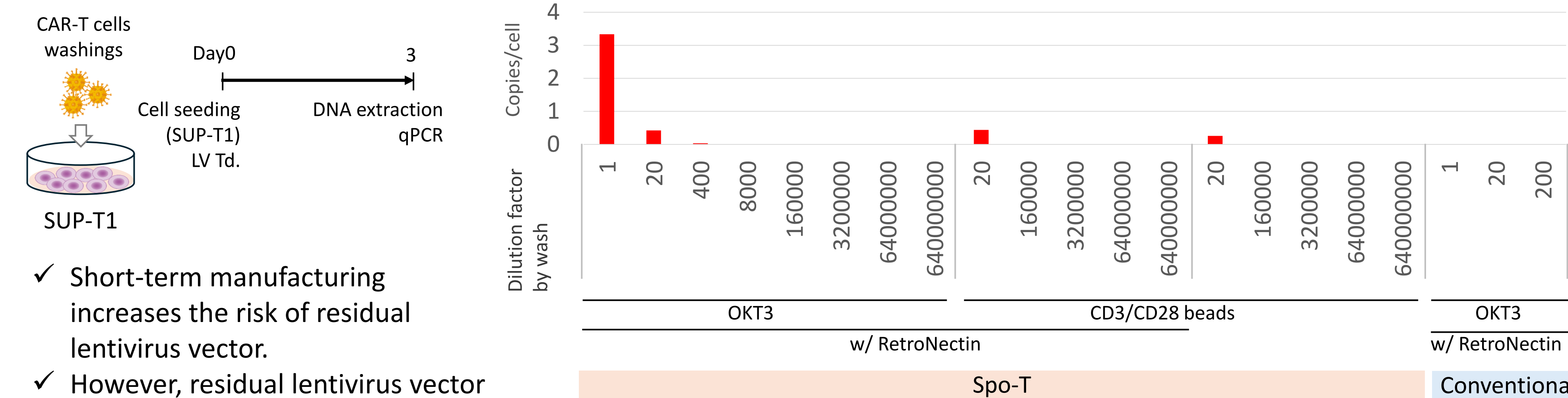
Immunophenotype

* RetroNectin & OKT3 stim.



Residual lentiviral vector

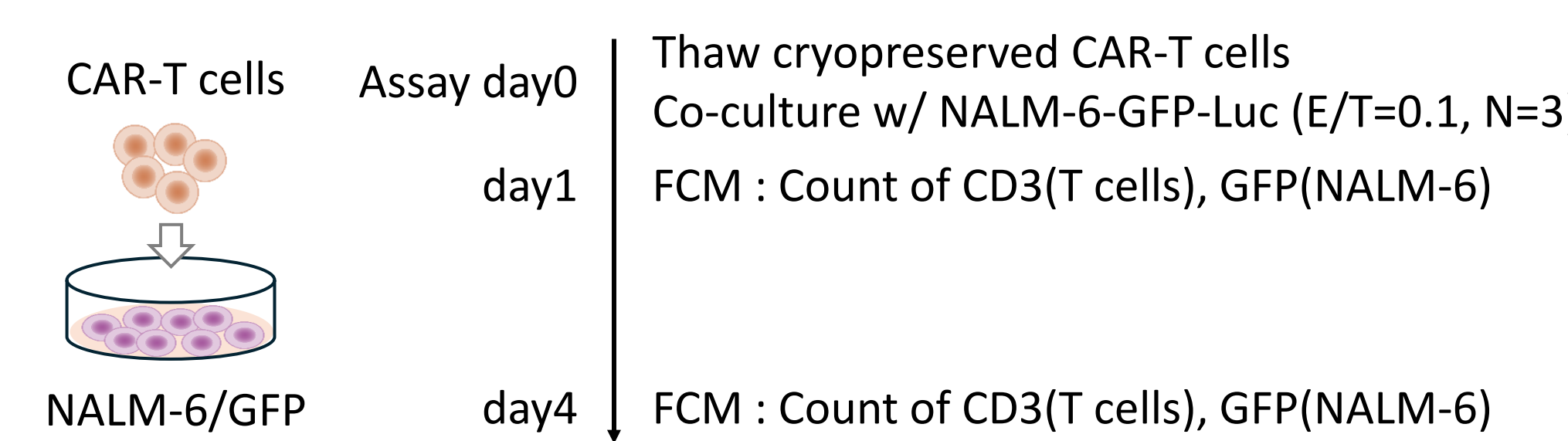
At the harvest step of CAR-T cells (day 2 or 10) washings were collected, and infectivity in the indicator cell line (SUP-T1) was evaluated by qPCR.



- ✓ Short-term manufacturing increases the risk of residual lentivirus vector.
- ✓ However, residual lentivirus vector reduced by washing at harvest.

in vitro Proliferation and Cytotoxicity Assay

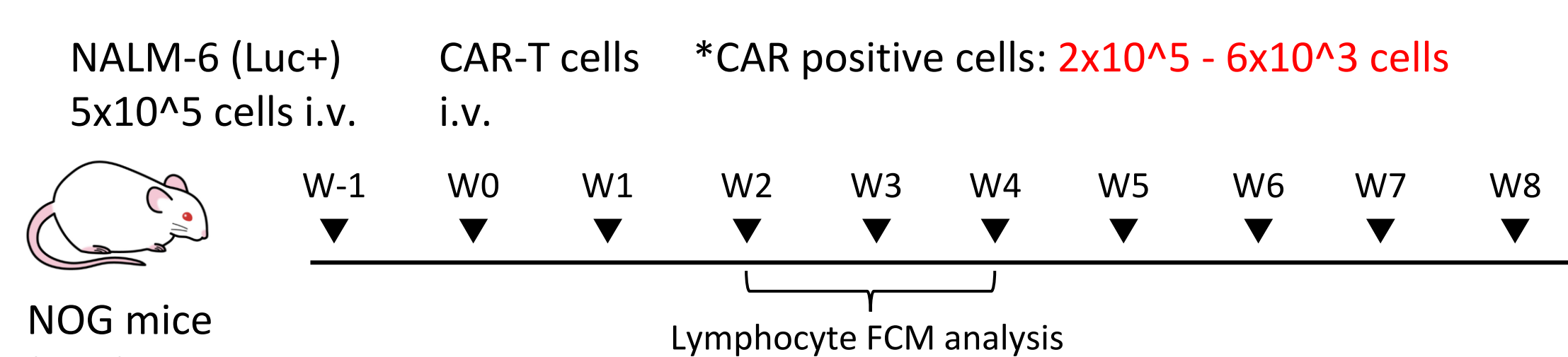
* RetroNectin & OKT3 stim.



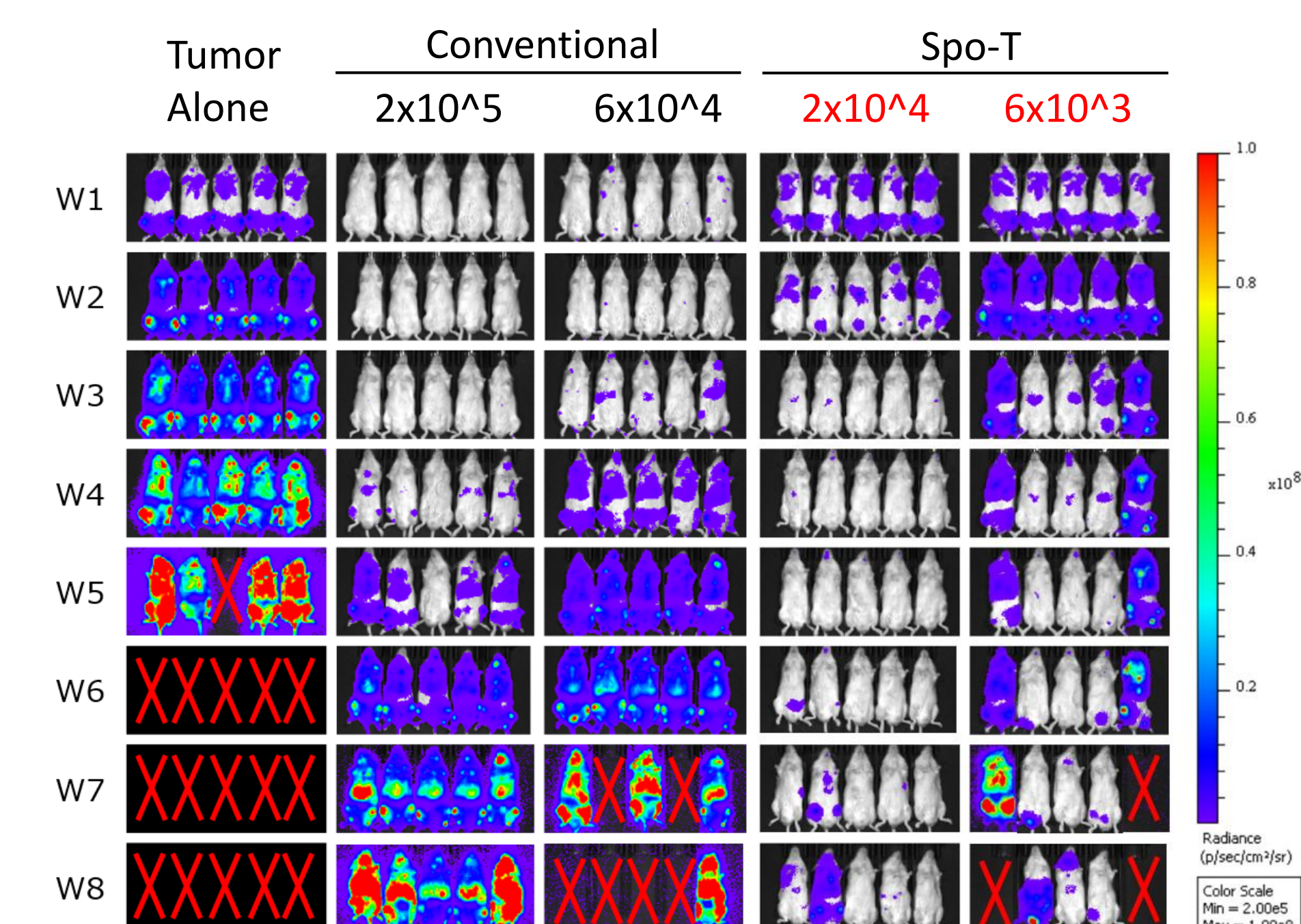
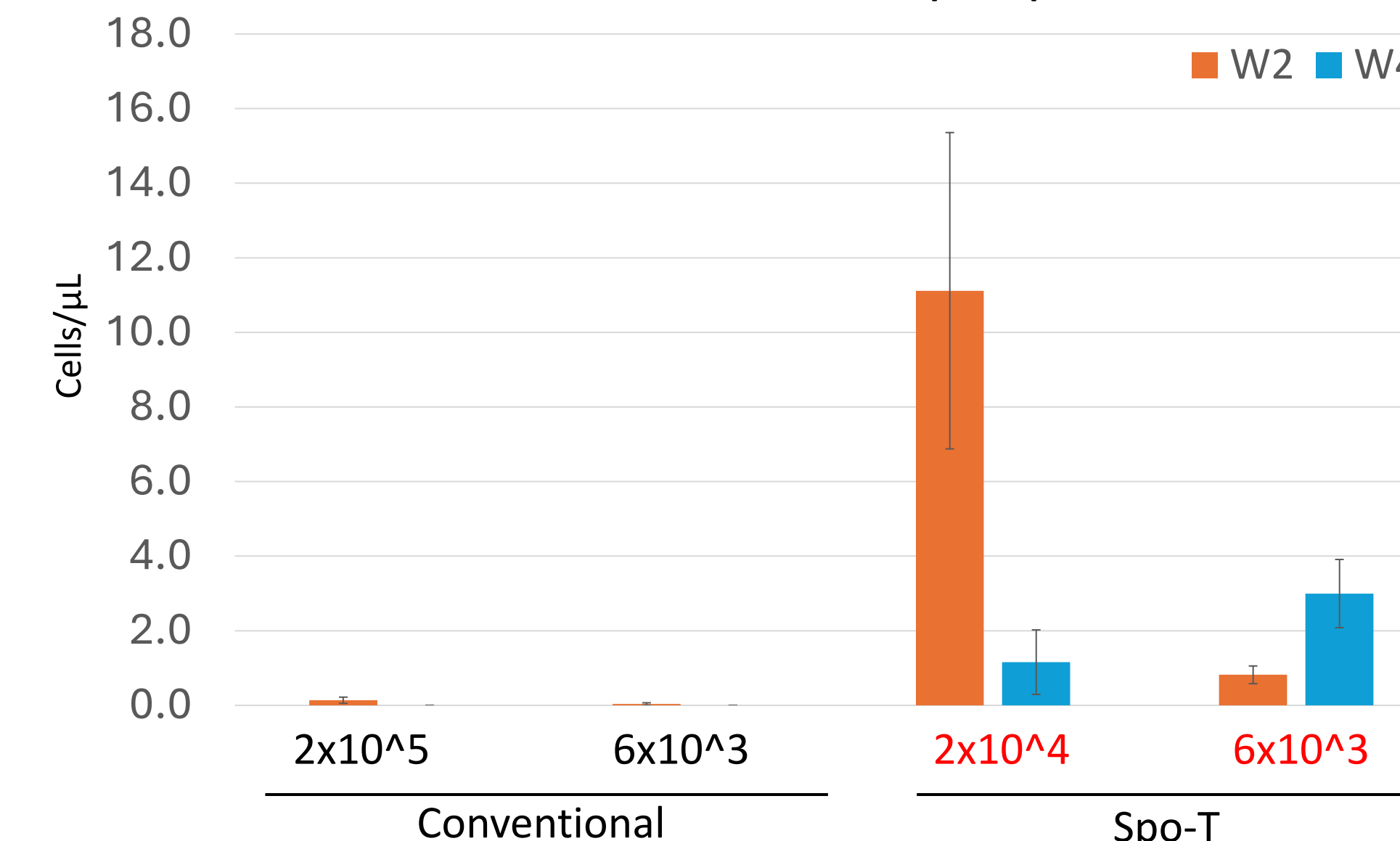
- ✓ CAR-T cells Proliferation : Spo-T > Conventional
- ✓ Cytotoxicity : Spo-T > Conventional

in vivo Comparison of anti-tumor effect

Tumor bearing NOG mice were treated with Spo-T CAR-T cells or conventional CAR-T cells (RetroNectin & OKT3 stimulation) at respective doses. Tumor growth was monitored weekly using in vivo imaging system (IVIS).



hCD45+ cells number in peripheral blood



- ✓ Spo-T CAR-T exhibited higher survivability in peripheral blood than conventional CAR-T.
- ✓ Spo-T CAR-T showed high and long-term antitumor effect at 1/10 doses compared to conventional CAR-T, and significantly extended mouse survival rate.