

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

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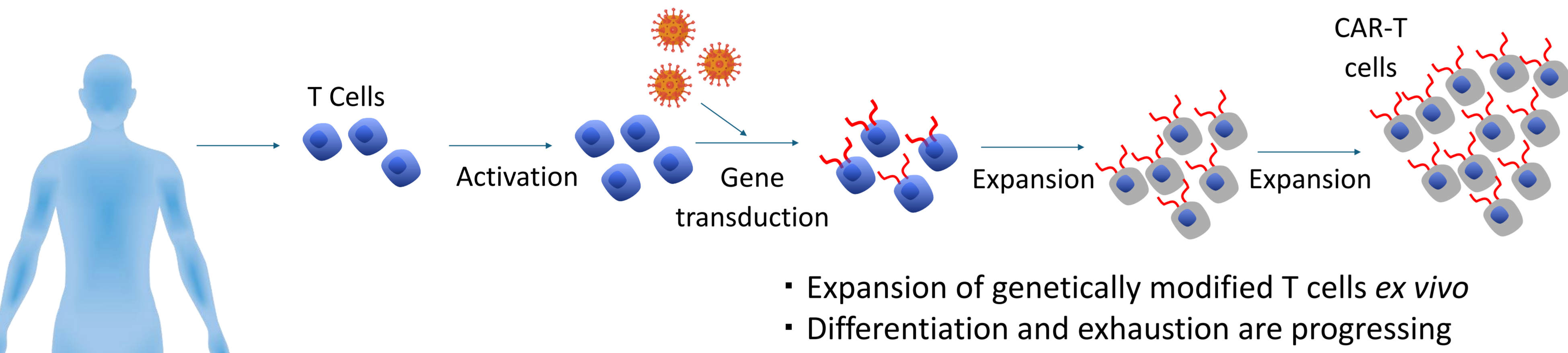


Abstract

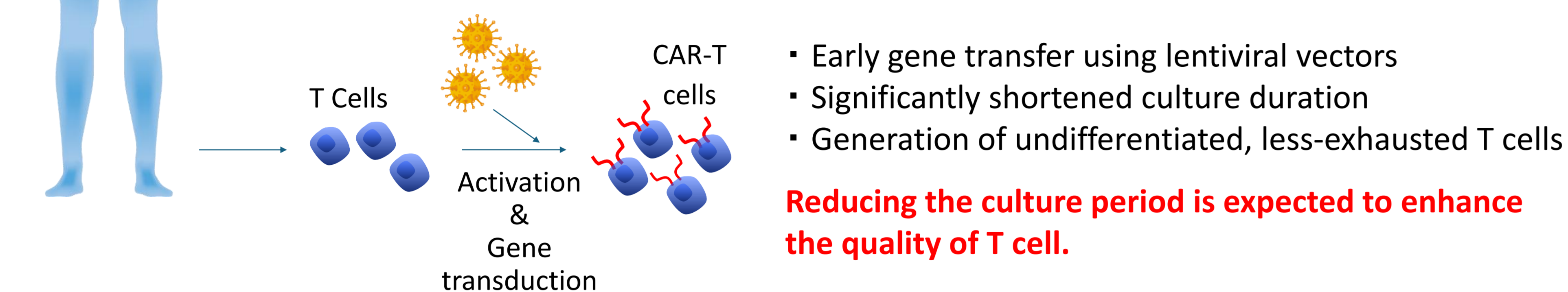
CAR-T cell therapies have shown remarkable efficacy in the treatment of hematologic malignancies. However, there are still many challenges in the cell manufacturing process. Most CAR-T cell manufacturing protocols expand T cells for 9 to 14 days. It is known that extended *ex vivo* culture period results 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

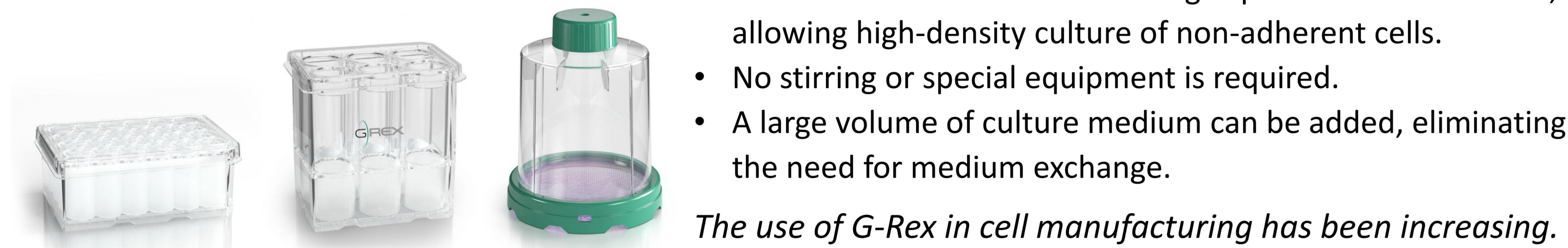
Conventional method : 9 - 14 days culture



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

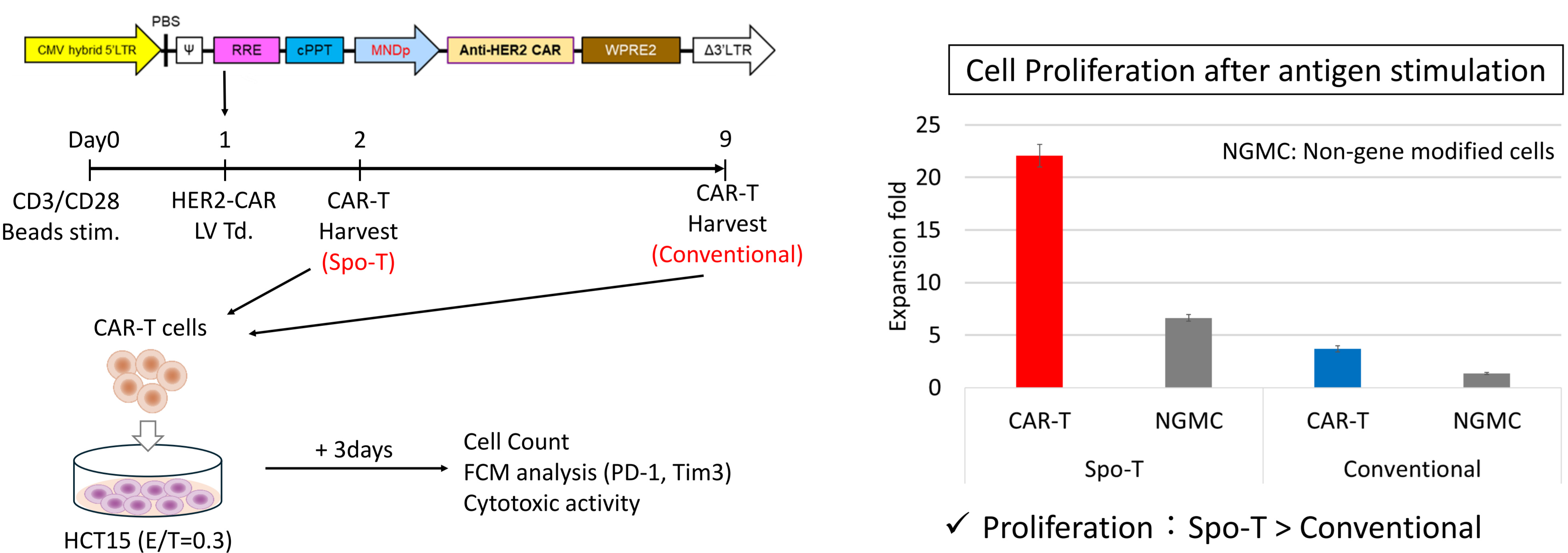


G-Rex[®] bioreactor (Wilson Wolf)

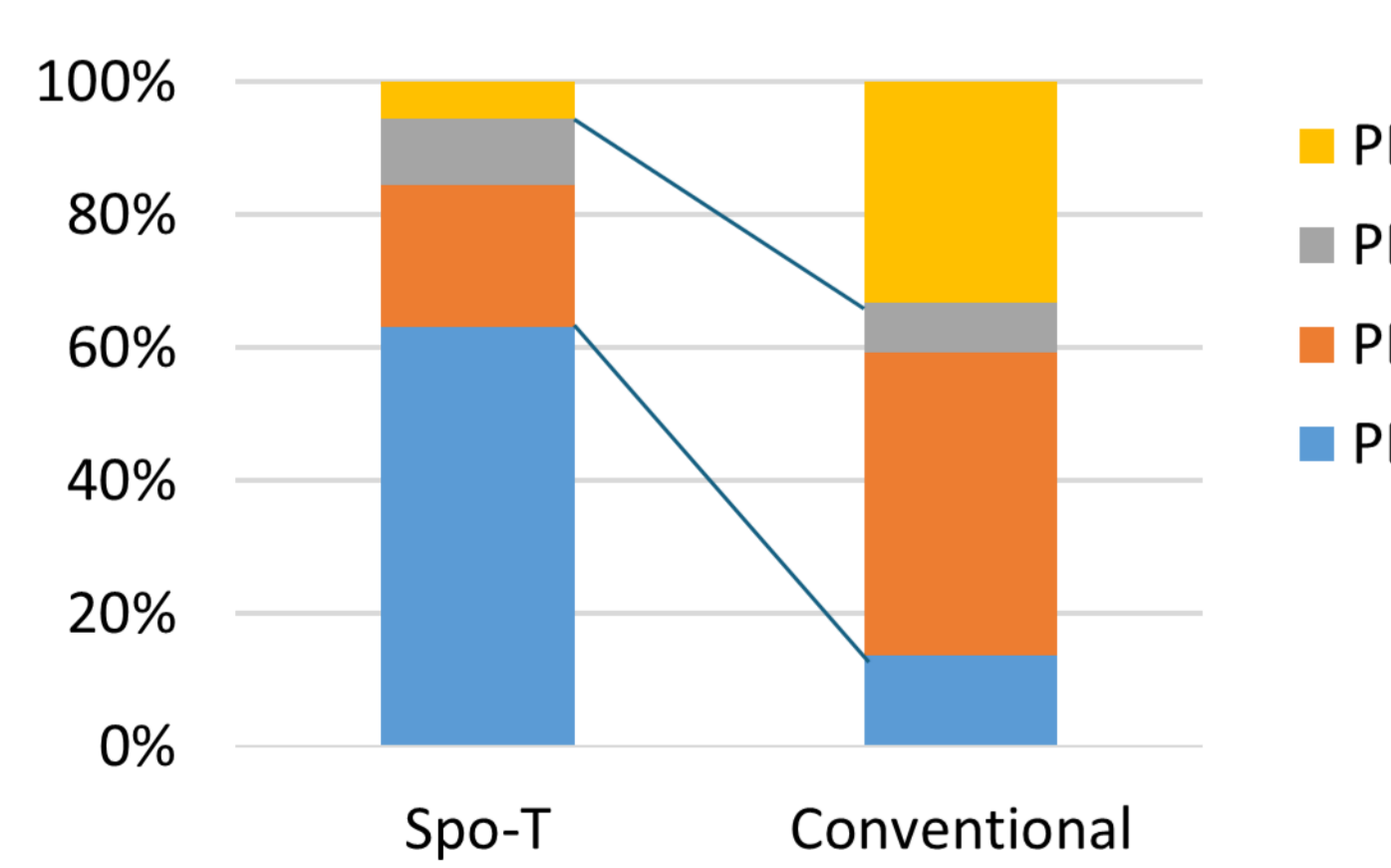


Comparison of Spo-T[®] Method and Conventional Method

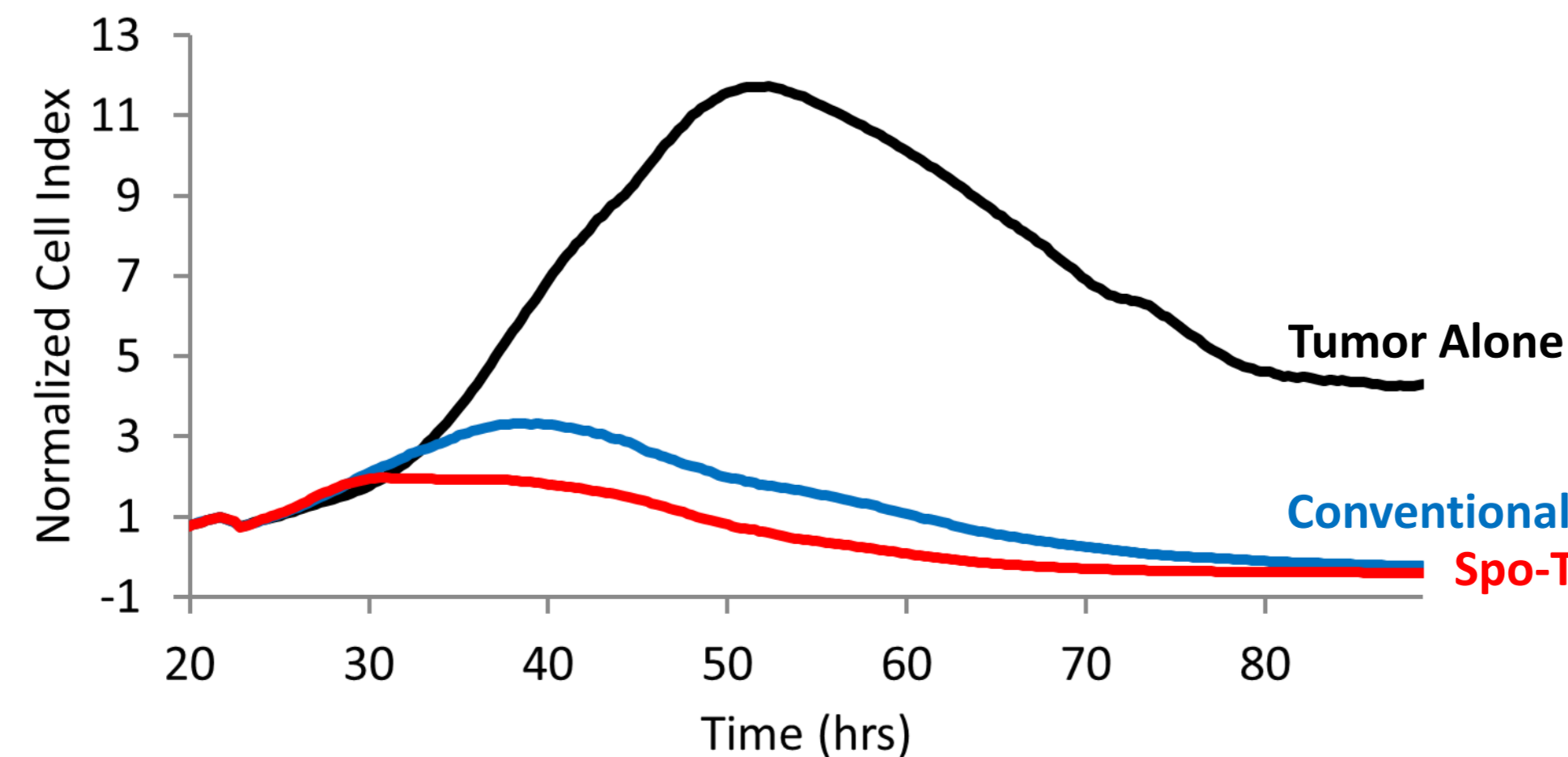
HER2 CAR-T cells were co-cultured with antigen expressing cells (HCT15). Cell proliferation and the exhaustion marker expression after antigen stimulation were measured. The cytotoxicity was measured in real time using xCelligence RTCA label-free system.



Exhaustion marker expression



Cytotoxicity to tumor cells



Summary

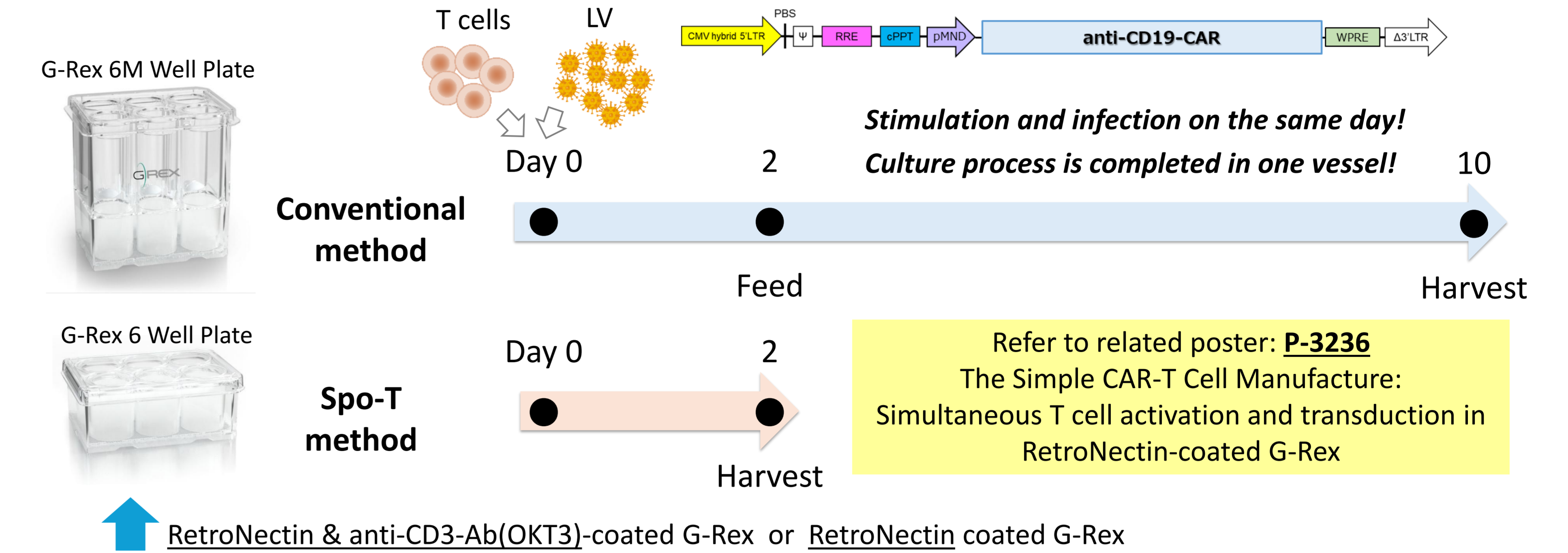
- ✓ We have developed the short-term manufacturing method, Spo-T[®] to produce highly effective CAR-T cells.
 - Extended CAR-T cells survivability and proliferation rates.
 - Strong and sustained antitumor efficacy at low dose.
- ✓ By applying the Spo-T method to the manufacturing process in the RetroNectin[®] 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.

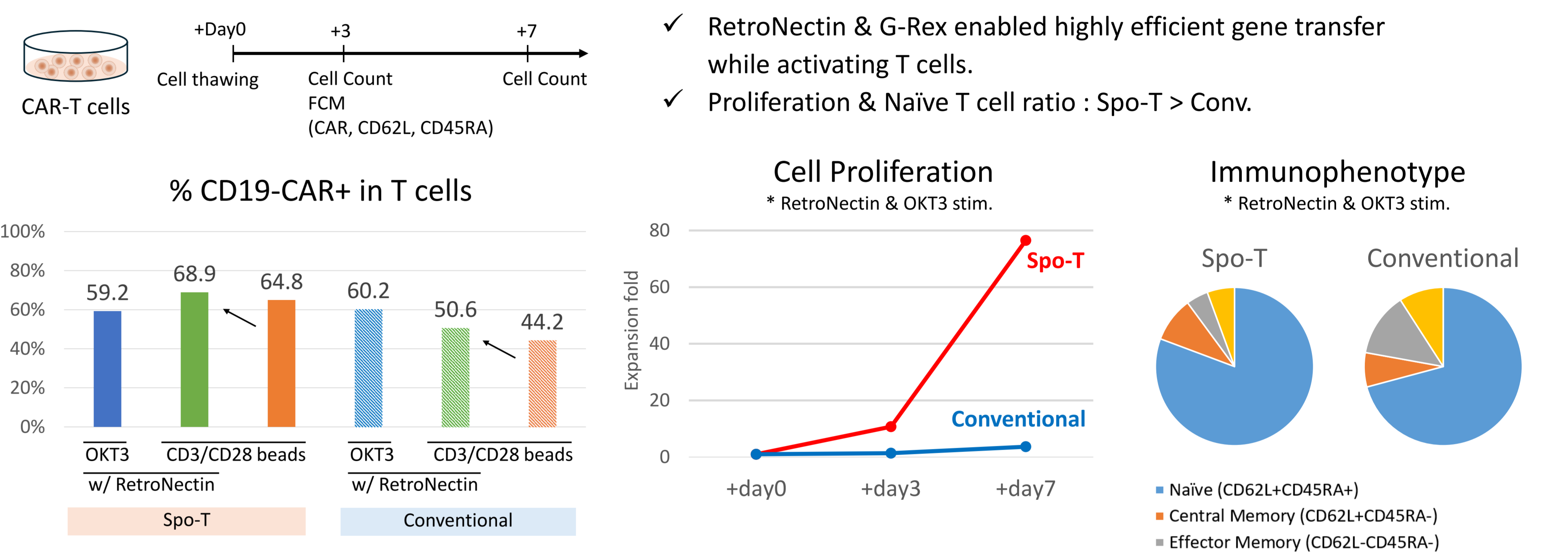
Adaptation of the Spo-T[®] method to RetroNectin[®] & G-Rex[®] Bioreactor

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.



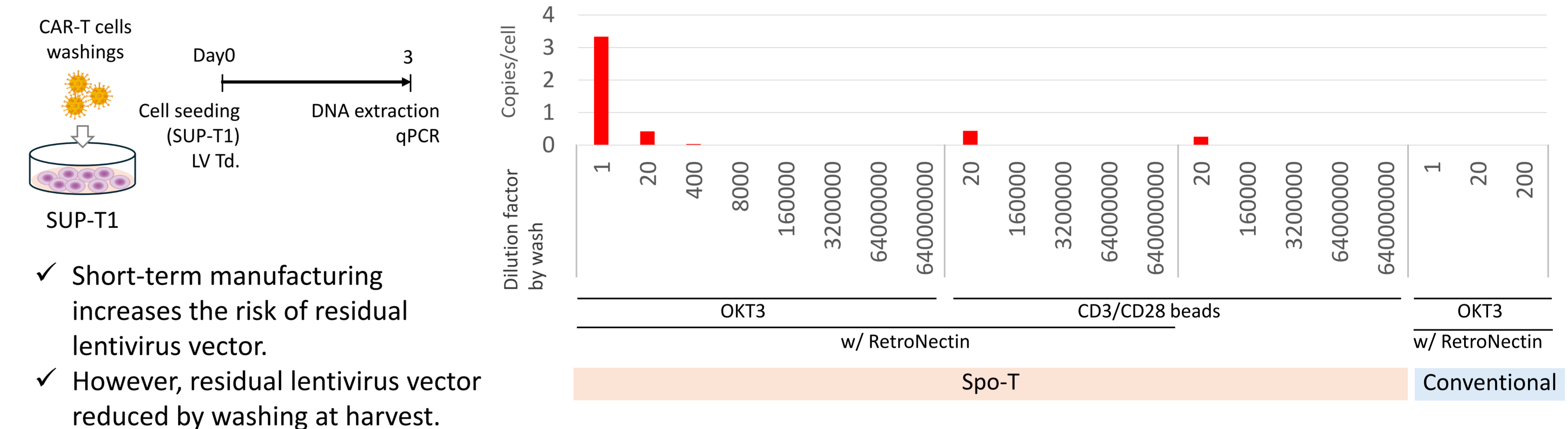
In vitro effector function after thawing

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

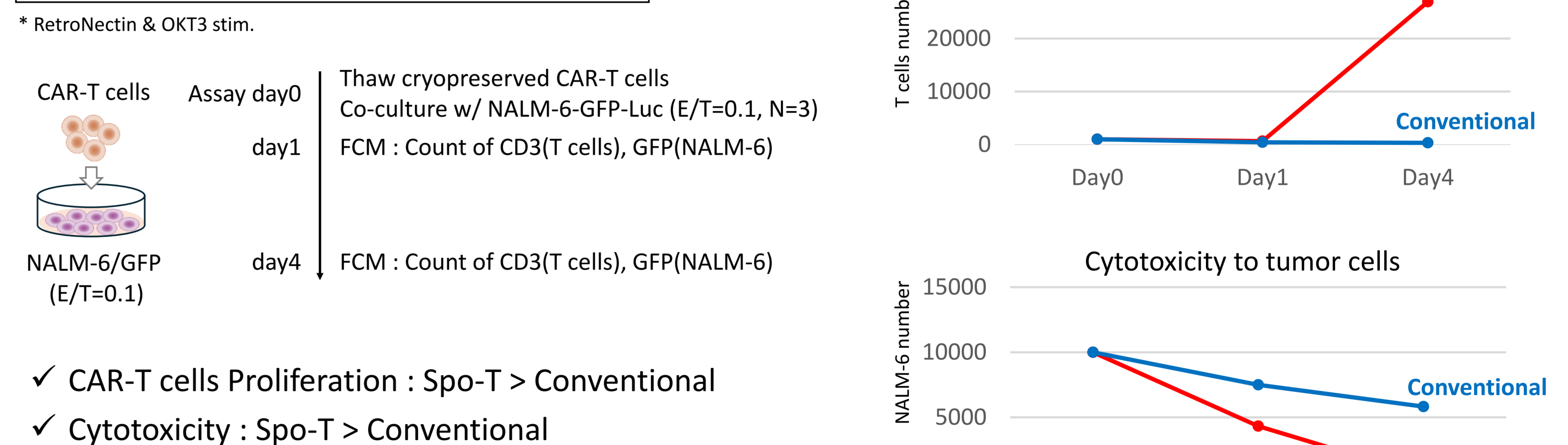


Residual lentiviral vector

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.



in vitro Proliferation and Cytotoxicity Assay



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).

