The Simple CAR-T Cell Production: RetroNectin® can Promote Simultaneous T-cell Activation & Transduction in G-Rex Bioreactor

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COI disclosure

Y.A., I.M., S.I. and S.O. are employees of TAKARA BIO INC.
T.Q. and M.H. are employees of TAKARA BIO USA.

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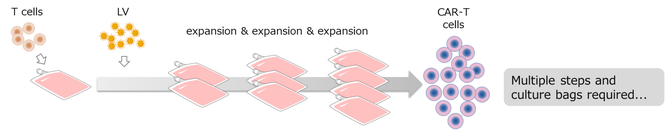
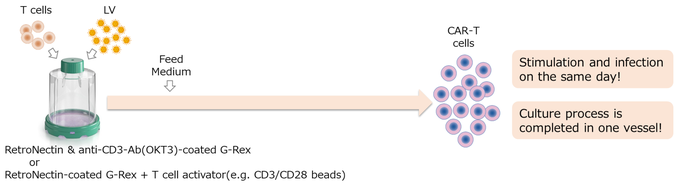
Background

CAR-T cell therapy has demonstrated high efficacy against hematologic malignancies, achieving partial or complete remission in many patients. A variety of approaches are being developed to target various antigens, making it a promising therapy for the various kind of tumors. However, there are still many challenges in the cell manufacturing process, especially when using autologous T cells.
The process is complex and carries the risk of manufacturing failures, leading to high production costs and limiting the number of patients who can receive the therapy.
Therefore, there is a need for a simple and efficient method to produce high-quality CAR-T cells.
In this study, we have established a simple CAR-T manufacturing method by combining RetroNectin and G-Rex.

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

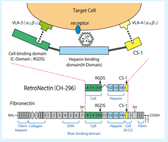
A DMF (18898) was submitted for the liquid format RetroNectin GMP grade on March 15, 2019.

RetroNectin Pro

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

Coating protocol of G-Rex

1. Dilute to the following concentrations using saline or citrate buffer (e.g., ACD-A) .
   - RetroNectin: 25μg/mL
   - anti-CD3 mAb: 5μg/mL
   (optional; in case of RN/OKT3 stimulation)
2. Add the coating solution to G-Rex and coat at 37°C .
   (The coating time ranges from 1 to 48 hours.)
3. After removing the solution and washing with saline, T cell culture can be started immediately.



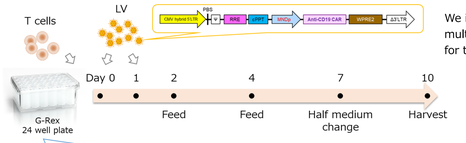
RetroNectin® GMP grade | Anti-CD3 mAb GMP grade

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

Summary

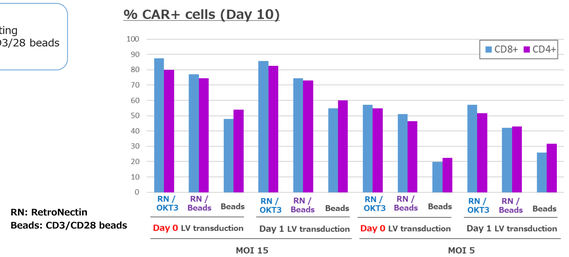
- **By coating the membrane of G-Rex with RetroNectin, we were able to perform simultaneous activation and efficient transduction of T cells with lentiviral vectors.**
- **CAR-T cells cultured in the RetroNectin-coated G-Rex showed higher proliferation rates and a more undifferentiated phenotype compared to uncoated conditions.**
- **This effect was consistent with both stimulation methods, including CD3/CD28 activation beads and RetroNectin/anti-CD3 Ab stimulation method, indicating a beneficial effect regardless of the T cell stimulation method.**

Optimization of Transduction step



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

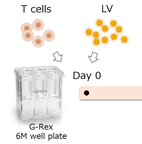
T cell set up conditions
- RetroNectin & OKT3 coating
- RetroNectin coating & CD3/28 beads
- CD3/28 beads alone



RN: RetroNectin
Beads: CD3/CD28 beads

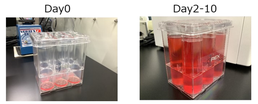
- ✓ **Transduction Efficiency : RN/OKT3 > RN + CD3/CD28 beads > CD3/CD28 beads**
- ✓ **Using RNs enables to perform higher transduction efficiency at low MOI.**
- ✓ **RN coatings provide high Td efficiency comparable to Day 1 infection even on Day 0.**

Comparative evaluation of CAR-T with different stimulation methods



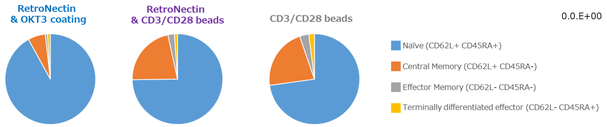
Aiming for a simpler culture system, CAR-T production was attempted by feeding the culture medium to the maximum volume on Day 2.

T cell set up conditions
- RetroNectin & OKT3 coating
- RetroNectin coating & CD3/28 beads
- CD3/28 beads alone

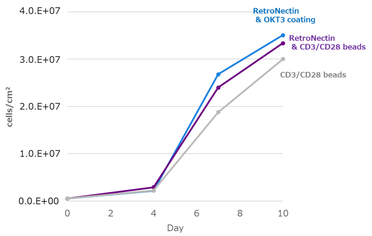


G-Rex bioreactors were kindly provided by ScaleReady.

Immunophenotype



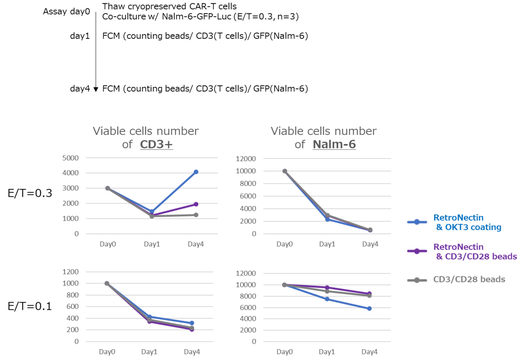
T cell expansion



- ✓ **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**
- ✓ **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.**

Comparison of antigen reactivity

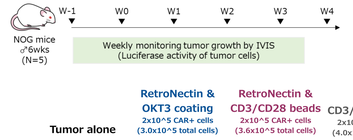
FCM-based Proliferation and Killing Assay

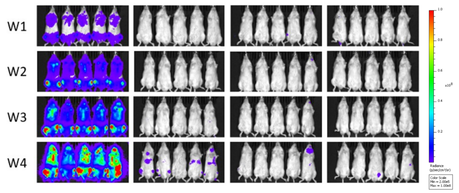


- ✓ **Proliferation : RN/OKT3 > RN + CD3/CD28 beads > CD3/CD28 beads**
- ✓ **Cytotoxicity : RN/OKT3 > RN + CD3/CD28 beads > CD3/CD28 beads**

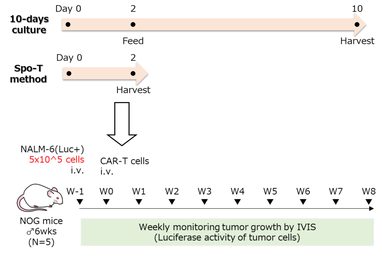
In vivo anti-tumor effect of CAR-T cells produced using RetroNectin & G-Rex

in vivo antitumor test

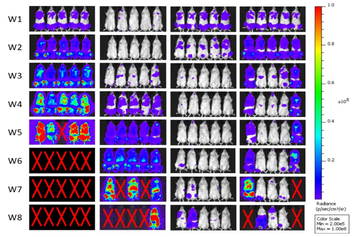




Comparison of anti-tumor effect by production period



In vivo comparison of CAR-T cells produced by Spo-T® method (Short Period Operation for T-cell production) and 10-days culture method in G-Rex.



- ✓ **T cells produced by all stimulation methods showed the same level of anti-tumor effect.**
- ✓ **CAR-T cells produced by the Spo-T method maintained a high anti-tumor effect for a long period of time even with 1/10 the number of cells administered compared to CAR-T cells produced by the 10-days culture method, and demonstrated the significant life-extending effect.**