

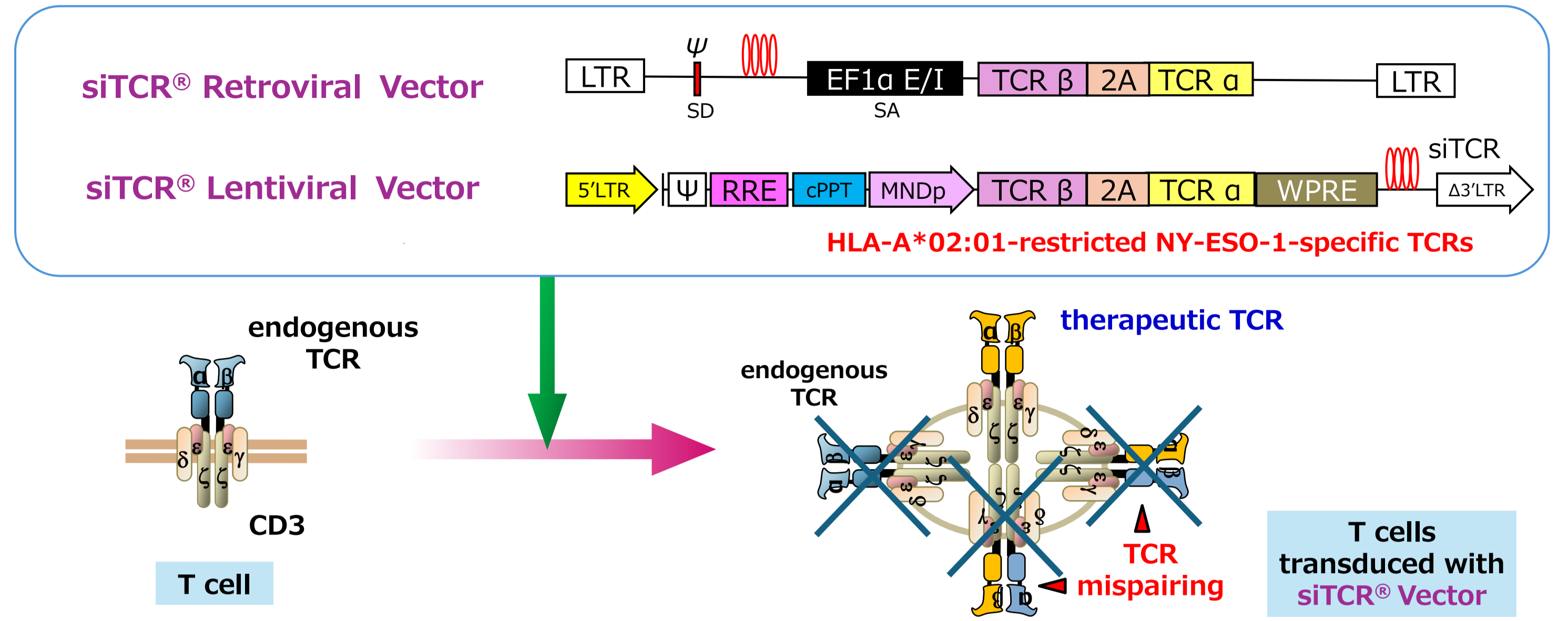
# Development of TCR-T cells targeting solid tumors using novel lentiviral vector and RetroNectin®

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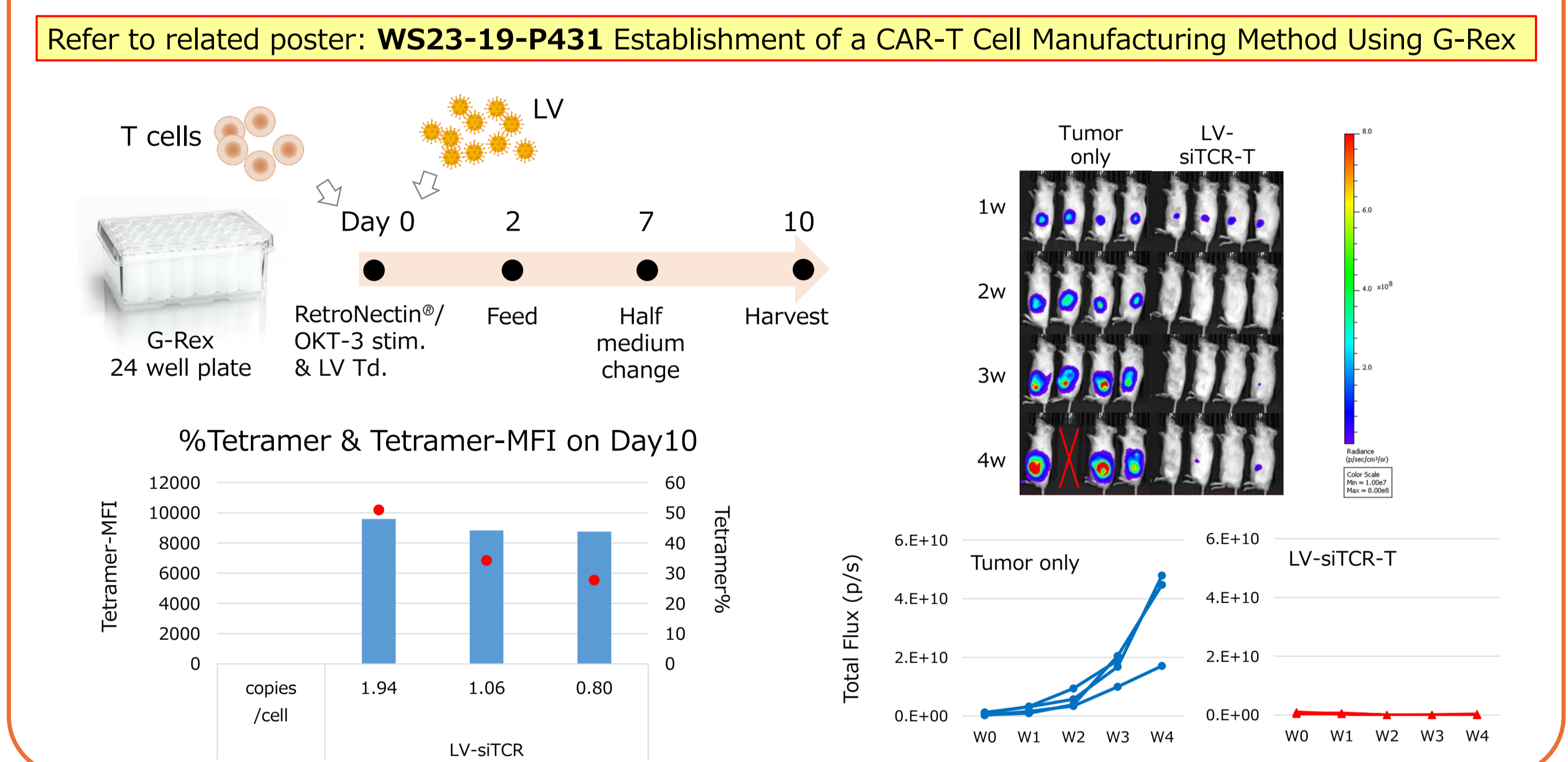
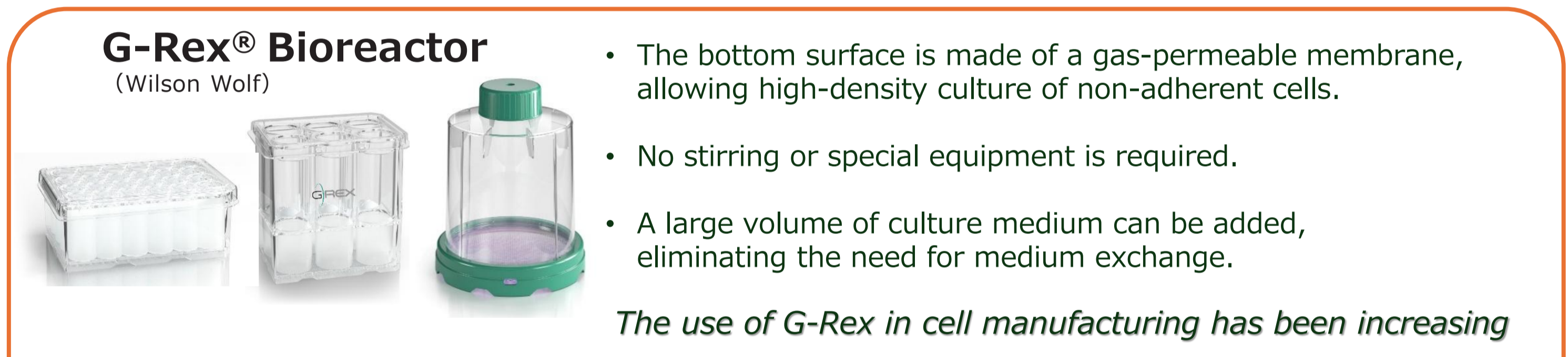
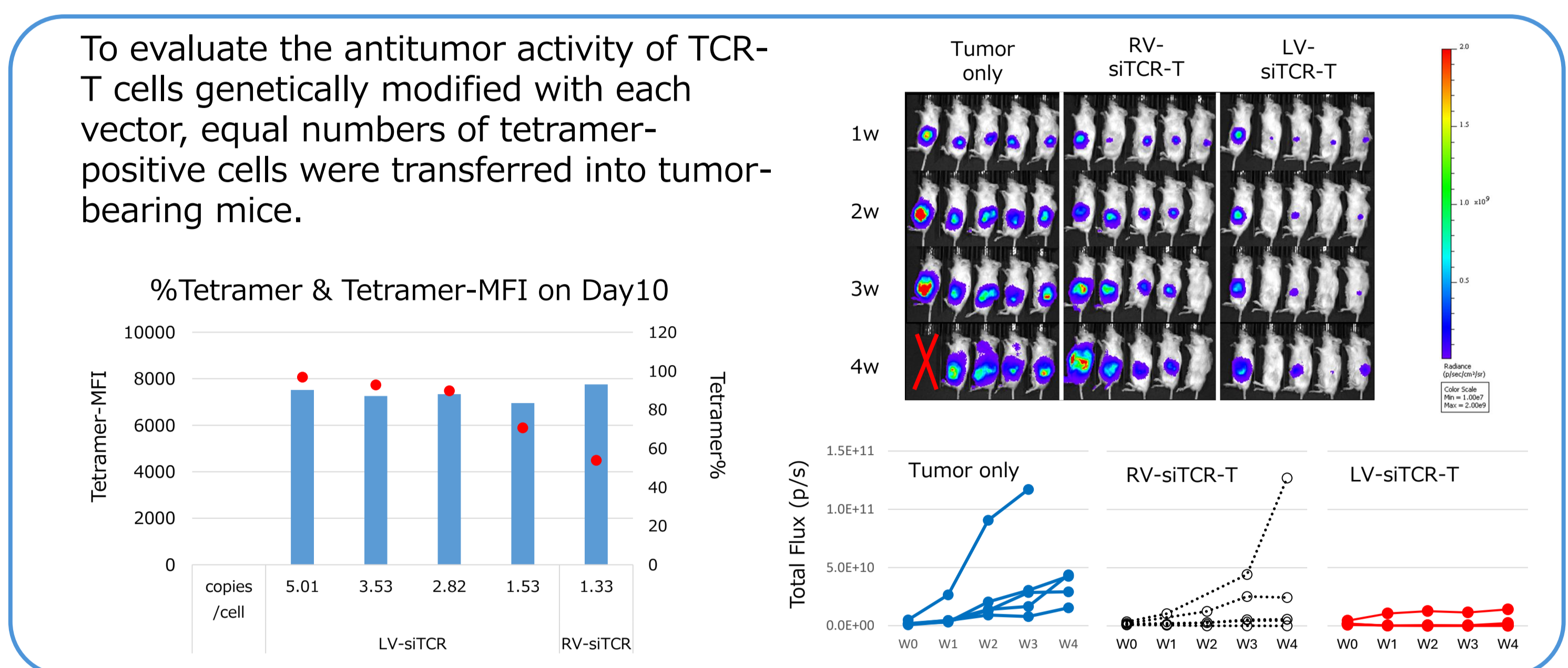
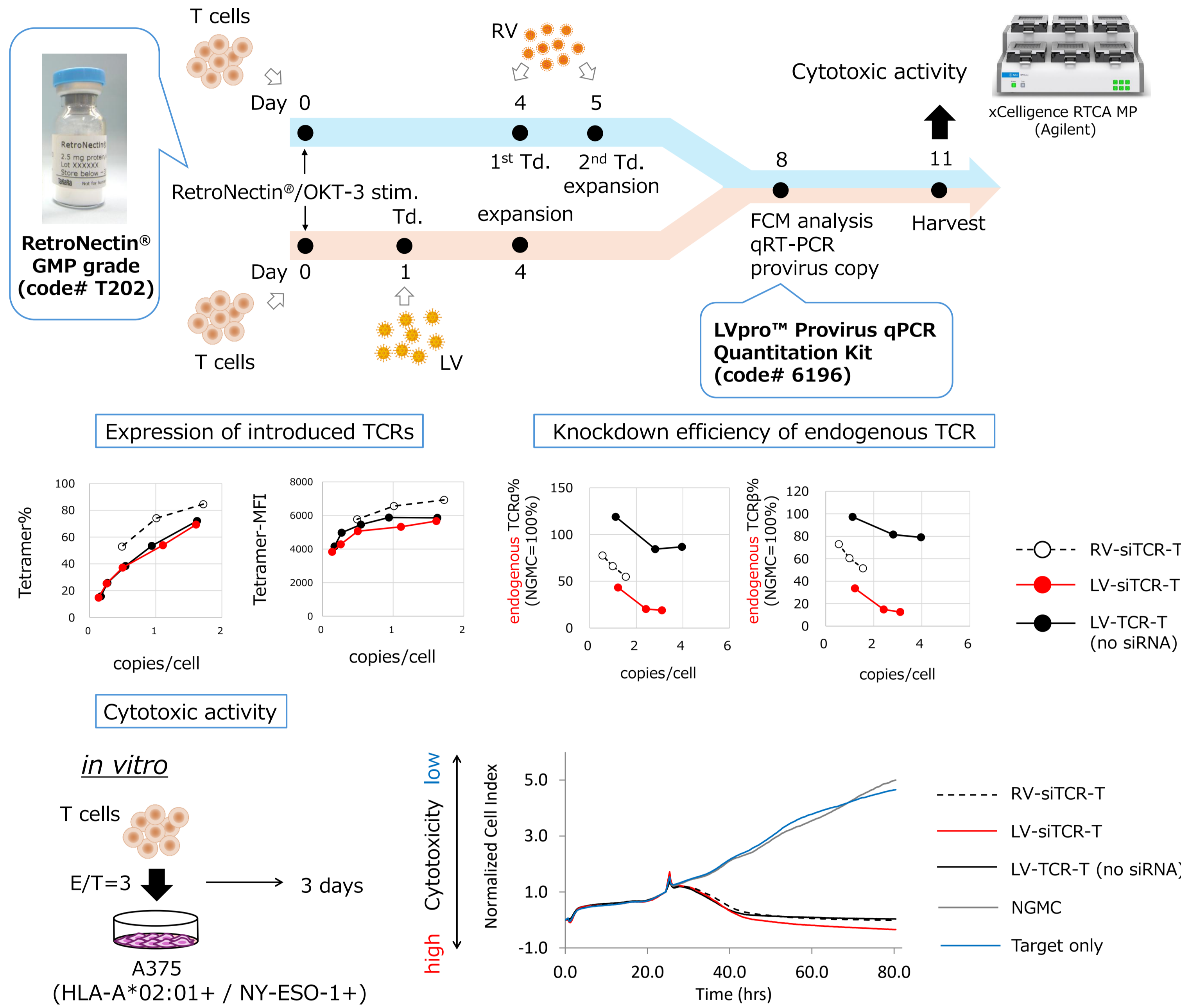
## Abstract

CAR-T cell therapy is highly effective in blood cancers but limited in solid tumors due to the immunosuppressive tumor microenvironment (TME). TCR-T cells can target intracellular antigens like MART-1 and MAGE-A4, showing benefit in targeting solid tumors. However, introducing tumor-specific TCRs risks off-target effects from mispairing with endogenous TCRs. To address this, we developed siTCR technology to knock down endogenous TCRs. HLA-A\*02:01-restricted NY-ESO-1-specific TCRs using this approach showed efficacy in synovial sarcoma, but retroviral systems are less suited for rapid, automated manufacturing. Here, we developed siTCR<sup>®</sup> lentiviral vector enabling efficient endogenous TCR knockdown and strong antitumor activity. However, solid tumors present additional challenges beyond antigen targeting, primarily due to the immunosuppressive TME. Factors such as TGF- $\beta$ , regulatory cells, and inhibitory cytokines can dampen T cell function and limit therapeutic efficacy. To overcome one of these key barriers, we incorporated a TGF- $\beta$  capture receptor, which conferred resistance to TGF- $\beta$ -mediated suppression without impairing T cell function. This platform enhances TCR-T safety and efficacy for solid tumors by minimizing off-target effects and improving resistance to immunosuppressive signals.



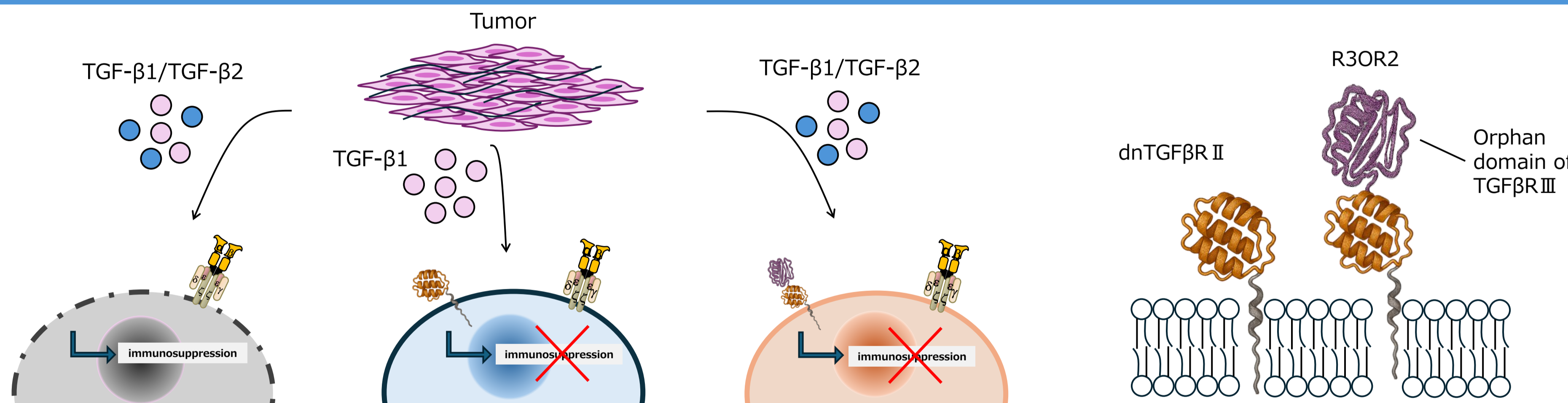
## Production of TCR-T cells transduced with siTCR<sup>®</sup> vector and assessment of cytotoxic function

We evaluated a novel siTCR<sup>®</sup> lentiviral vector encoding an HLA-A\*02:01-restricted NY-ESO-1-specific TCR in comparison with retroviral vector.



- ✓ TCR expression : siTCR<sup>®</sup>-LV vector < siTCR<sup>®</sup>-RV vector
  - ✓ Knockdown efficiency : siTCR<sup>®</sup>-LV vector > siTCR<sup>®</sup>-RV vector
  - ✓ Cytotoxic activity (in vitro/in vivo) : siTCR<sup>®</sup>-LV vector  $\geq$  siTCR<sup>®</sup>-RV vector
- siTCR<sup>®</sup>-LV vector showed high transduction efficiency in T cells, successfully suppressed endogenous TCR, and exhibited cytotoxicity equivalent to siTCR<sup>®</sup>-RV vector. Furthermore, TCR-T cells transduced with siTCR<sup>®</sup>-LV vector can be easily manufactured using G-Rex.

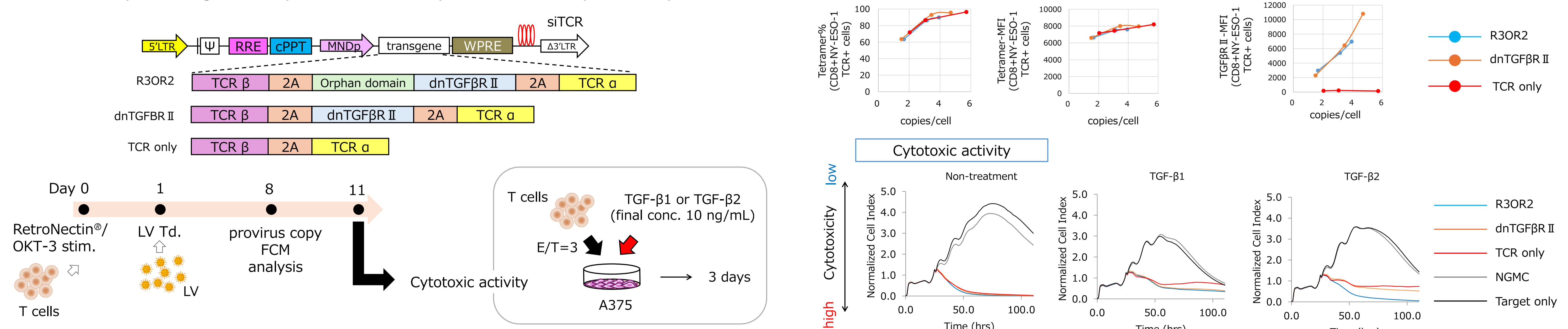
## Concept and improvement of dominant negative TGF $\beta$ II



TGF- $\beta$  is a key immunosuppressive cytokine in the tumor microenvironment, promoting immune evasion and cancer progression. Conventional strategies employ a dominant-negative TGF $\beta$  II (dnTGF $\beta$  II) lacking the signaling domain to block TGF- $\beta$  signaling; however, its low affinity for TGF- $\beta$ 2 limit efficiency. To address this, we engineered a chimeric receptor combining dnTGF $\beta$  II with the high-affinity orphan domain of TGF $\beta$  III, enhancing inhibition both TGF- $\beta$ 1 and TGF- $\beta$ 2 pathways.

## Functional assessment of a novel TGF- $\beta$ resistance receptor in TCR-T cells against TGF- $\beta$ 1 and TGF- $\beta$ 2

We evaluated the cytotoxic activity of NY-ESO-1 TCR-T cells co-expressing the optimized TGF- $\beta$  resistance receptor, alongside comparisons of TCR expression and receptor co-expression.



- ✓ siTCR<sup>®</sup>-LV vector co-expressing TGF- $\beta$  resistance receptor exhibited TCR expression levels comparable to TCR only vector.
- ✓ The newly modified siTCR<sup>®</sup>-LV vector co-expressing R3OR2 receptor exhibited
  - TGF $\beta$  II expression comparable to that of dnTGF $\beta$  II co-expression vector.
  - cytotoxic activity while maintaining TGF- $\beta$ 1/TGF- $\beta$ 2 resistance equivalent to or greater than that of dnTGF $\beta$  II co-expression vector.

### COI Disclosure Information

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

### Summary

- ◆ siTCR<sup>®</sup>-LV vector efficiently transduced T cells, efficiently knocked down endogenous TCR, and exhibited cytotoxic activity equivalent to that of siTCR<sup>®</sup>-RV vector.
- ◆ Using G-Rex enables simultaneous stimulation and infection, allowing us to produce siTCR<sup>®</sup>-LV vector-transduced TCR-T cells within a single culture vessel through just three processes.
- ◆ The newly developed TGF- $\beta$  resistance receptor minimized the immunosuppressive effects not only of TGF- $\beta$ 1 but also of TGF- $\beta$ 2.