野見

Enhancing the Efficacy of Anti-HER2-JAK/STAT-CAR-T Cells Through Shortening the Cell Production Period

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Background

Human epidermal growth factor receptor 2 (HER2)-specific antibody or antibody-drug conjugate therapy is widely used for the treatment of HER2-positive tumors, and the development of CAR-T therapy using targeted antibody technology is expected. One of the attractive solution is CAR-T therapy. In fact, while CAR-T therapy has demonstrated high efficacy in targeting hematological tumors, its clinical effectiveness in solid tumors remains inadequate due to cell exhaustion and depletion of naïve/memory subsets. JAK/STAT CAR-T cells, a next-generation CAR-T cells, show lower expression of exhaustion markers compared to conventional CAR-T cells, exhibits antigen-specific high cell proliferation, and maintain long-term cytotoxic activity against continuous antigen stimulation.

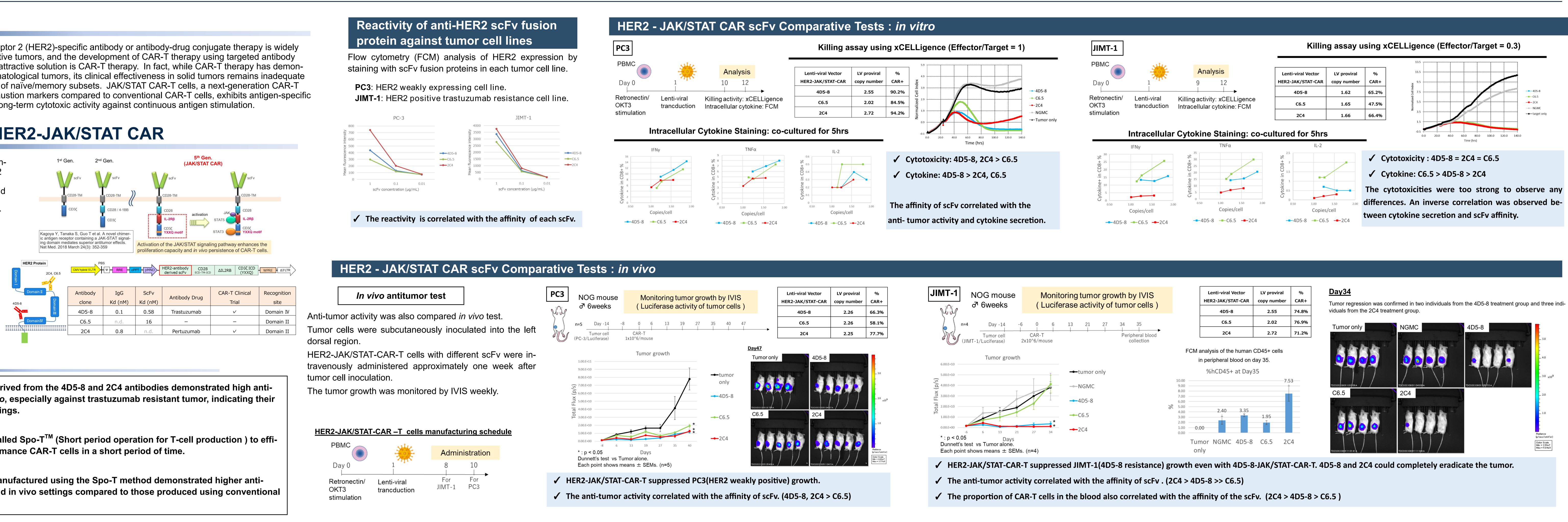
Development of HER2-JAK/STAT CAR

In this study, we utilized three humanized antibodies that recognize HER2 to construct anti-HER2-JAK/STAT-CAR expression lentiviral vectors and Fc fusion proteins.

The 4D5-8 and 2C4 clones were derived from antibodies already approved for clinical use, namely, Trastuzumab and Pertuzumab, respectively

The C6.5 clone is widely used in research targeting HER2. Each clone exhibits different epitope recognition and affinity

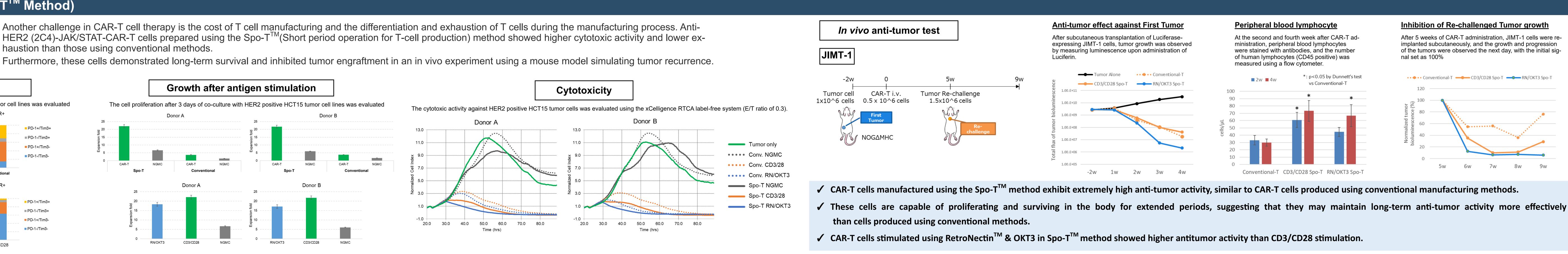
toward the HER2 molecule.



Summary

- HER2-JAK/STAT-CAR-T cells derived from the 4D5-8 and 2C4 antibodies demonstrated high antitumor activity *in vitro* and *in vivo*, especially against trastuzumab resistant tumor, indicating their potential efficacy in clinical settings.
- We have developed a method called Spo-T[™] (Short period operation for T-cell production) to efficiently manufacture high-performance CAR-T cells in a short period of time.
- HER2-JAK/STAT-CAR-T cells manufactured using the Spo-T method demonstrated higher antitumor activity in both in vitro and in vivo settings compared to those produced using conventional methods.

Improved CAR-T cell production process (Spo-T[™] Method) D9~D14 Harvest _____ haustion than those using conventional methods. Isolation Exhaustion marker expression ession after 3 days of co-culture with HER2 positive HCT15 tumor cell lines was evaluated The exhaustion marker expre PD-1/Tim3 in CD8+/CAR+ PD-1/Tim3 in CD8+/CAR+ (Donor A) (Donor B) ■ PD-1-/Tim3+ Culture Method PD-1+/Tim3-PD-1-/Tim3-Spo-T Conventional Spo-T Conventional PD-1/Tim3 in CD8+/CAR+ PD-1/Tim3 in CD8+/CAR+ (Donor A) (Donor B) PD-1+/Tim3+ ■ PD-1-/Tim3+ **Stimulation Metho** PD-1+/Tim3-PD-1-/Tim3-RN/OKT3 CD3/CD28 CD3/CD28



COI disclosure

Y.A., R.N., K.U., S.I., Y.K. and S.O. are employees of TAKARA BIO INC

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