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

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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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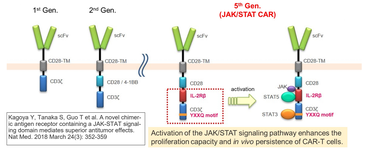
Background

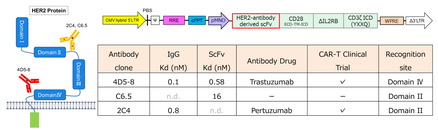
Human epidermal growth factor receptor 2 (HER2)-specific antibody or antibody-drug conjugate therapy is widely used for the treatment of HER2-positive tumors, and the development of CAR-T therapy using targeted antibody technology is expected. One of the attractive solution is CAR-T therapy. In fact, 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.



Kagoya Y, Tanaka S, Guo T et al. A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. Nat Med. 2018 March 24(3): 352-359



Antibody clone	IgG Kd (nM)	ScFv Kd (nM)	Antibody Drug	CAR-T Clinical Trial	Recognition site
4D5-8	0.1	0.58	Trastuzumab	✓	Domain Ⅳ
C6.5	n.d.	16	–	–	Domain Ⅱ
2C4	0.8	n.d.	Pertuzumab	✓	Domain Ⅱ

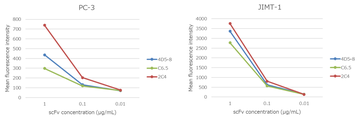
Summary

- **HER2-JAK/STAT-CAR-T cells derived from the 4D5-8 and 2C4 antibodies demonstrated high anti-tumor 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 anti-tumor activity in both in vitro and in vivo settings compared to those produced using conventional methods.**

Reactivity of anti-HER2 scFv fusion protein against tumor cell lines

Flow cytometry (FCM) analysis of HER2 expression by staining with scFv fusion proteins in each tumor cell line.

PC3: HER2 weakly expressing cell line.
JIMT-1: HER2 positive trastuzumab resistance cell line.



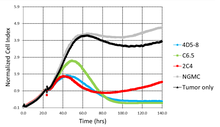
✓ **The reactivity is correlated with the affinity of each scFv.**

HER2 - JAK/STAT CAR scFv Comparative Tests : *in vitro*

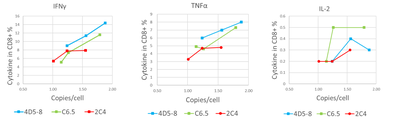
PC3

Killing assay using xCELLigence (Effector/Target = 1)

Lenti-viral Vector HER2-JAK/STAT-CAR	LV proviral copy number	% CAR+
4D5-8	2.55	90.2%
C6.5	2.02	84.5%
2C4	2.72	94.2%



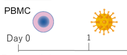
Intracellular Cytokine Staining: co-cultured for 5hrs



✓ **Cytotoxicity: 4D5-8, 2C4 > C6.5**
✓ **Cytokine: 4D5-8 > 2C4, C6.5**

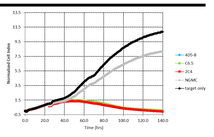
The affinity of scFv correlated with the anti- tumor activity and cytokine secretion.

JIMT-1

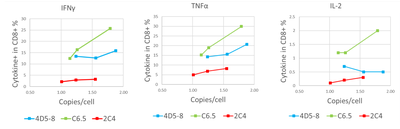


Killing assay using xCELLigence (Effector/Target = 0.3)

Lenti-viral Vector HER2-JAK/STAT-CAR	LV proviral copy number	% CAR+
4D5-8	1.62	65.2%
C6.5	1.65	47.5%
2C4	1.66	66.4%



Intracellular Cytokine Staining: co-cultured for 5hrs



✓ **Cytotoxicity : 4D5-8 = 2C4 = C6.5**
✓ **Cytokine: C6.5 > 4D5-8 > 2C4**

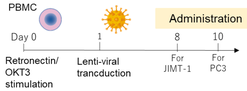
The cytotoxicities were too strong to observe any differences. An inverse correlation was observed between cytokine secretion and scFv affinity.

HER2 - JAK/STAT CAR scFv Comparative Tests : *in vivo*

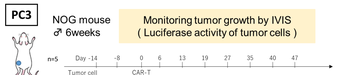
***In vivo* antitumor test**

Anti-tumor activity was also compared *in vivo* test.
Tumor cells were subcutaneously inoculated into the left dorsal region.
HER2-JAK/STAT-CAR-T cells with different scFv were intravenously administered approximately one week after tumor cell inoculation.
The tumor growth was monitored by IVIS weekly.

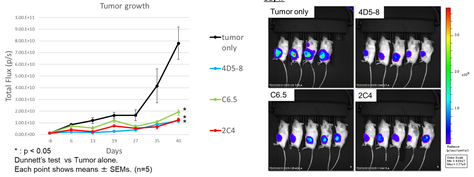
HER2-JAK/STAT-CAR –T cells manufacturing schedule



PC3



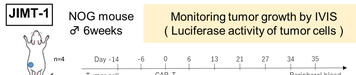
Lenti-viral Vector HER2-JAK/STAT-CAR	LV proviral copy number	% CAR+
4D5-8	2.26	66.3%
C6.5	2.26	58.1%
2C4	2.25	77.7%



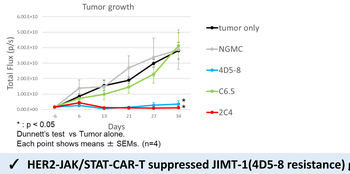
*: p < 0.05
Dunnett's test vs Tumor alone.
Each point shows means ± SEMs. (n=5)

✓ **HER2-JAK/STAT-CAR-T suppressed PC3(HER2 weakly positive) growth.**
✓ **The anti-tumor activity correlated with the affinity of scFv. (4D5-8, 2C4 > C6.5)**

JIMT-1

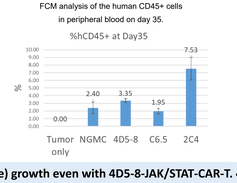


Lenti-viral Vector HER2-JAK/STAT-CAR	LV proviral copy number	% CAR+
4D5-8	2.55	74.8%
C6.5	2.02	76.9%
2C4	2.72	71.2%



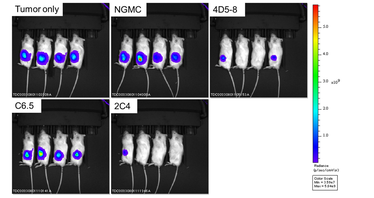
*: p < 0.05
Dunnett's test vs Tumor alone.
Each point shows means ± SEMs. (n=4)

FCM analysis of the human CD45+ cells in peripheral blood on day 35.



Day34

Tumor regression was confirmed in two individuals from the 4D5-8 treatment group and three individuals from the 2C4 treatment group.



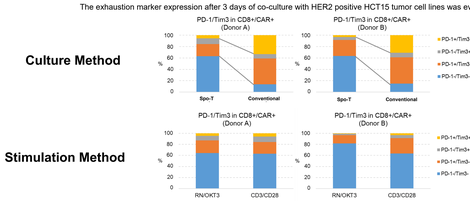
✓ **HER2-JAK/STAT-CAR-T suppressed JIMT-1(4D5-8 resistance) growth even with 4D5-8-JAK/STAT-CAR-T. 4D5-8 and 2C4 could completely eradicate the tumor.**
✓ **The anti-tumor activity correlated with the affinity of scFv . (2C4 > 4D5-8 >> C6.5)**
✓ **The proportion of CAR-T cells in the blood also correlated with the affinity of the scFv. (2C4 > 4D5-8 > C6.5)**

Improved CAR-T cell production process (Spo-T™ Method)

Another challenge in CAR-T cell therapy is the cost of T cell manufacturing and the differentiation and exhaustion of T cells during the manufacturing process. Anti-HER2 (2C4)-JAK/STAT-CAR-T cells prepared using the Spo-T™(Short period operation for T-cell production) method showed higher cytotoxic activity and lower exhaustion than those using conventional methods.
Furthermore, these cells demonstrated long-term survival and inhibited tumor engraftment in an in vivo experiment using a mouse model simulating tumor recurrence.

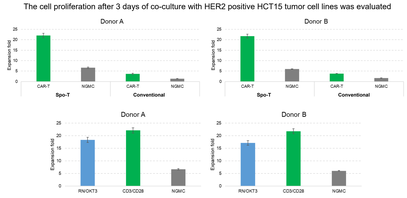
Exhaustion marker expression

The exhaustion marker expression after 3 days of co-culture with HER2 positive HCT15 tumor cell lines was evaluated



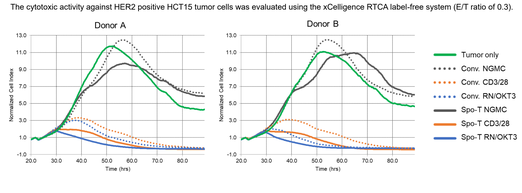
Growth after antigen stimulation

The cell proliferation after 3 days of co-culture with HER2 positive HCT15 tumor cell lines was evaluated



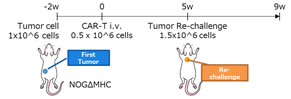
Cytotoxicity

The cytotoxic activity against HER2 positive HCT15 tumor cells was evaluated using the xCelligence RTCA label-free system (E/T ratio of 0.3).



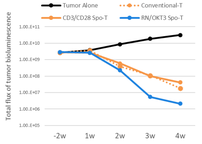
***In vivo* anti-tumor test**

JIMT-1



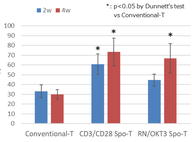
Anti-tumor effect against First Tumor

After subcutaneous transplantation of Luciferase-expressing JIMT-1 cells, tumor growth was observed by measuring luminescence upon administration of Luciferin.



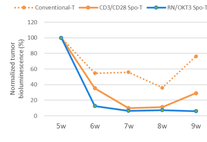
Peripheral blood lymphocyte

At the second and fourth week after CAR-T administration, peripheral blood lymphocytes were stained with antibodies, and the number of human lymphocytes (CD45 positive) was measured using a flow cytometer.



Inhibition of Re-challenged Tumor growth

After 5 weeks of CAR-T administration, JIMT-1 cells were re-implanted subcutaneously, and the growth and progression of the tumors were observed the next day, with the initial signal set as 100%



✓ **CAR-T cells manufactured using the Spo-T™ method exhibit extremely high anti-tumor activity, similar to CAR-T cells produced using conventional manufacturing methods.**
✓ **These cells are capable of proliferating and surviving in the body for extended periods, suggesting that they may maintain long-term anti-tumor activity more effectively than cells produced using conventional methods.**
✓ **CAR-T cells stimulated using RetroNectin™ & OKT3 in Spo-T™ method showed higher antitumor activity than CD3/CD28 stimulation.**