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Development of *piggyBac* transposon-mediated HER2-CAR-T cells for the treatment of solid tumors

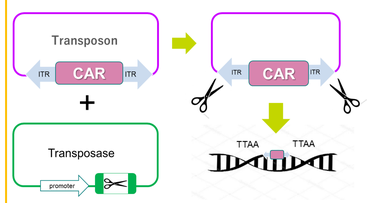
Koichiro Shioya[1*], Tomio Matsumura[1], Yuta Ohira[1], Naomi Komatsuzaki[1], Manaka Shinagawa[2], Miyuki Tanaka[3], Shigeki Yagyu[4], Yozo Nakazawa[3], Lilin Zhang[1**]

1; BrightPath Biotherapeutics Co., Ltd., Tokyo, Japan, 2;Department of Obstetrics and Gynecology, Shinshu University School of Medicine, Matsumoto, Japan, 3;Department of Pediatrics, Shinshu University School of Medicine, Matsumoto, Japan. 4;Center for Advanced Research of Gene and Cell Therapy, Shinshu University, Matsumoto, Japan. *Contact 1: shioya_k@brightpathbio.com, **Contact 2: zhang_l@brightpathbio.com

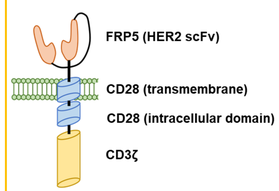
Introduction

Although chimeric antigen receptor (CAR)-T therapies have achieved remarkable success in the treatment of hematologic malignancies, the outcome for patients with solid tumors remains poor. There are several reasons behind this, including exhaustion of CAR-T cell, poor homing and penetration in the tumor, and the lack of persistence in the immunosuppressive tumor microenvironment. To solve these problems, we have developed HER2-CAR-T cells (BP2301) using the *piggyBac* (PB) transposon-based gene transfer system.

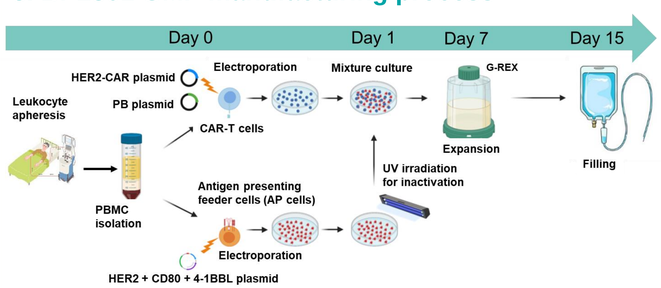
1. *piggyBac* Transposon System



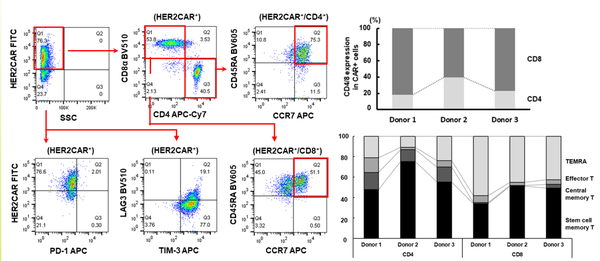
2. CAR structure



3. BP2301 GMP manufacturing process

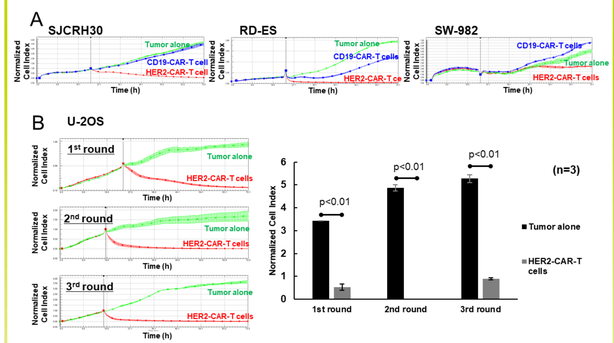


4. BP2301 exhibited memory-like phenotype [1)]



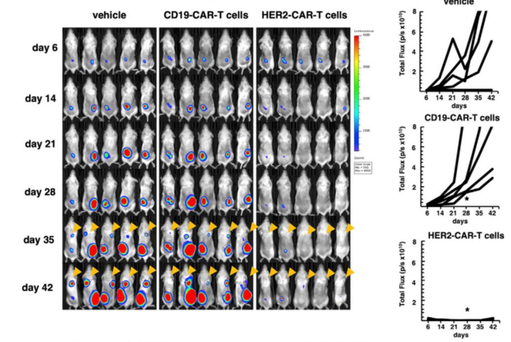
HER2-CAR was detected by recombinant human ErbB2/HER2-Fc chimera protein with a goat anti-human IgG Fc fragment-specific antibody. Antibodies of CD4, CD8, CD45RA, CCR7, PD-1, TIM-3, LAG3 and CD3 were used for the characterization of HER2-CAR+ cells.

5. BP2301 showed persistent cytotoxicity against HER2+ sarcoma in a serial killing assay [1)]



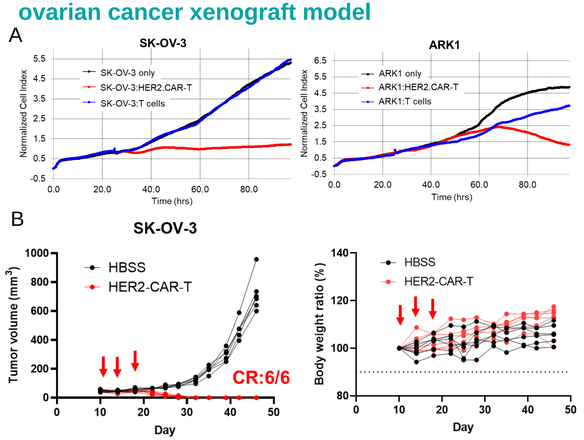
A) *in vitro* cytotoxicity analysis of BP2301 was evaluated using sarcoma cell lines. Tumor cells were seeded on xCelligence E-plates 16 at 0.5-2 × 10^4 cells/well for 18-24h and then BP2301 or CD19-CAR-T cells (negative control) were added at an E:T ratio of 1:1, then real-time impedance was measured for 72 h and presented as the normalized cell index using an xCELLigence RTCA DP system.
B) *in vitro* anti-tumor activity of BP2301 was evaluated in a serial killing assay. BP 2301 was incubated with U-2OS at an E:T ratio of 1:1 for 72 h, and then was collected, incubated with U-2OS again for the next round of killing. Cell impedance was measured for 72h in each round by an xCELLigence RTCA DP system.

6. BP2301 eradicated rhabdomyosarcoma and rejected re-challenged tumor in xenograft model [1)]



Anti-tumor effect of BP2301 was evaluated in SJCRH30-FFluc xenograft model. NSG mice were inoculated with 1 × 10^6 SJCRH30-FFluc cells on day 0. Vehicle, PB-CD19-CAR-T cells or BP2301 (6 × 10^6 CAR+ T cells) were i.v. administered one week after inoculation. Tumor burden was measured as bioluminescence signal intensity (BLI) and presented as total flux (p/s). On day 28, 1 × 10^6 SJCRH30-FFluc cells were re-inoculated onto the mice for tumor re-challenge experiment. Re-challenged tumors are indicated by yellow arrowheads.

7. BP2301 eradicated inoculated tumor in an ovarian cancer xenograft model



A) *in vitro* cytotoxicity analysis of BP2301 was evaluated using gynecological cancer lines. The cancer cells were incubated with BP2301 at an E:T ratio of 1:1 for killing assay based on cell impedance. T cells derived from same donor were used as negative control.
B) Anti-tumor effect of BP2301 was evaluated in SK-OV-3 xenograft model. NSG mice were inoculated with 2 × 10^6 SK-OV-3 cells on day 0. Vehicle or BP2301 (2 × 10^6 CAR+ T cells) were i.v. administered on day 11, 14 and 18, respectively (red arrows).

8. Study design for Phase 1 clinical trial (BP2301-001)

3 + 3 Dose-escalation Design (N=12)

8.3 × 10^5 cells/kg → **2.7 × 10^6 cells/kg**

Lymphodepletion:
3 days regimen (Flu 25 mg/m² + Cy 250 mg/m²)

- **Primary objective :** ・Safety and tolerability
- **Secondary objective :** ・Expansion and persistence of BP2301
  ・Efficacy of BP2301

Key inclusion criteria

- ・Recurrent or advanced Osteosarcoma, Soft tissue sarcoma, Gynecological cancer
- ・5 to 65 years old
- ・KPS ≥ 50%
- ・Adequate organ function
- ・HER2 expression > 1+

Key exclusion criteria

- ・Active infection
- ・Central nervous system diseases
- ・Significant autoimmune diseases
- ・Pregnancy

Conclusion

- A non-viral GMP manufacturing process of BP2301 has been optimized using *piggyBac* transposon system with AP cells.
- BP2301 exhibits stem cell memory phenotype and no significant T-cell exhaustion markers.
- BP2301 showed potent anti-tumor activity *in vitro* and *in vivo*.
- A Phase 1 clinical trial for patients with recurrent or advanced osteosarcoma, soft tissue sarcoma and gynecological cancer is scheduled in the second quarter of 2022.

References

1) Nakamura K., et al. Autologous antigen-presenting cells efficiently expand *piggyBac* transposon CAR-T cells with predominant memory phenotype, Mol Ther Methods Clin Dev. 21 315-324, 2021.

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