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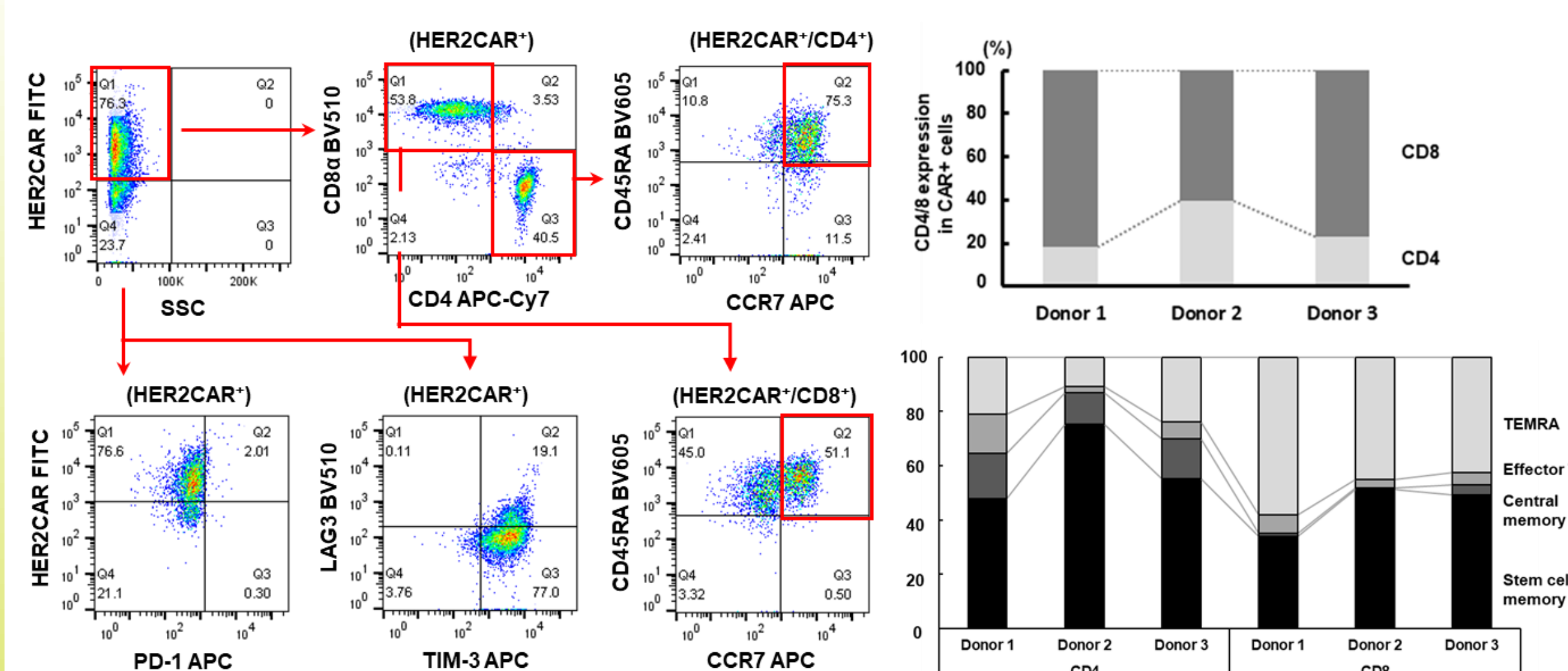
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Introduction

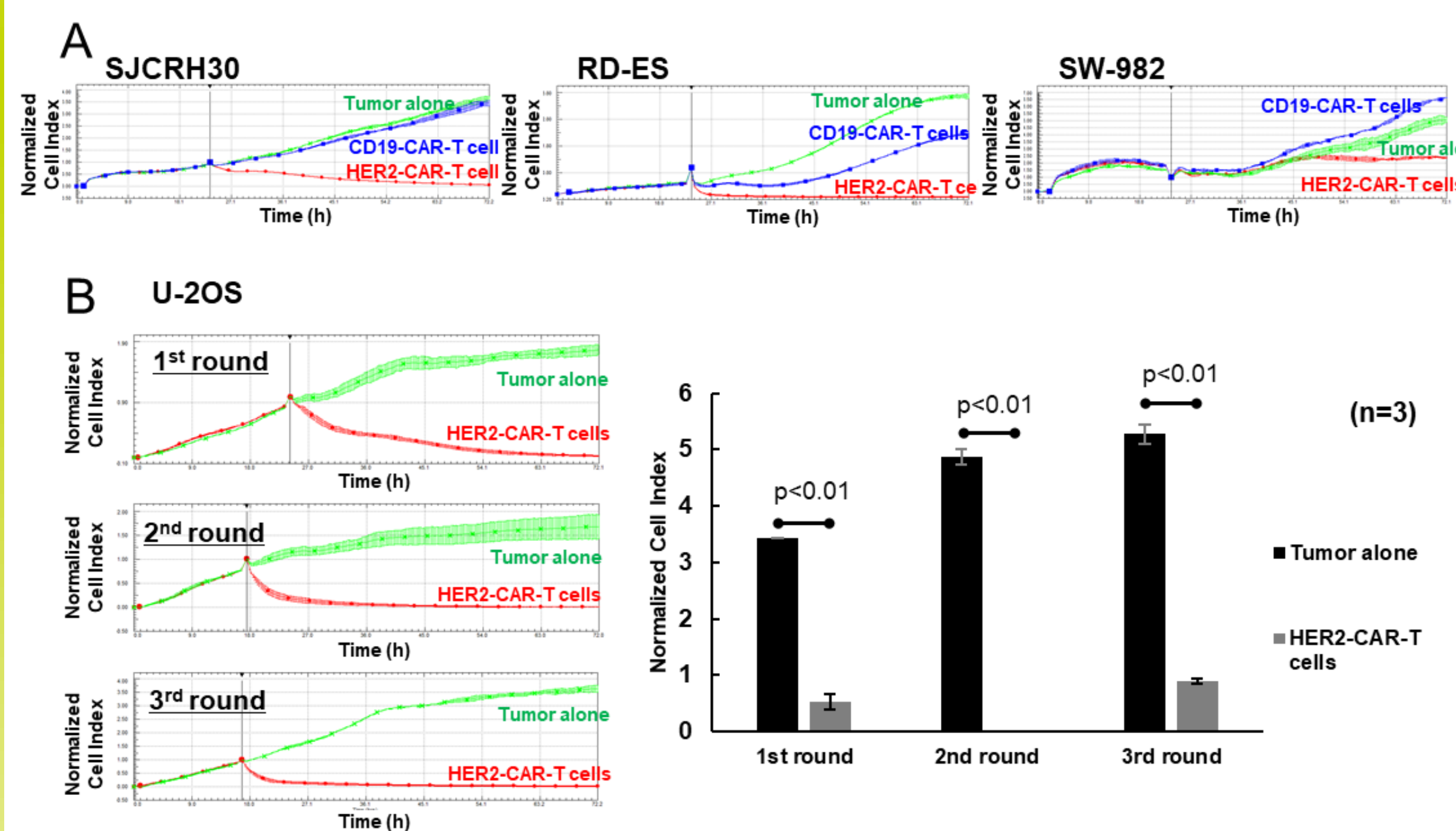
Although chimeric antigen receptor (CAR)-T therapies have achieved remarkable success in the treatment of hematologic malignancies, the outcome for patients with solid tumors remains poor. There are several reasons behind this, including exhaustion of CAR-T cell, poor homing and penetration in the tumor, 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.

4. BP2301 exhibited memory-like phenotype¹⁾



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.

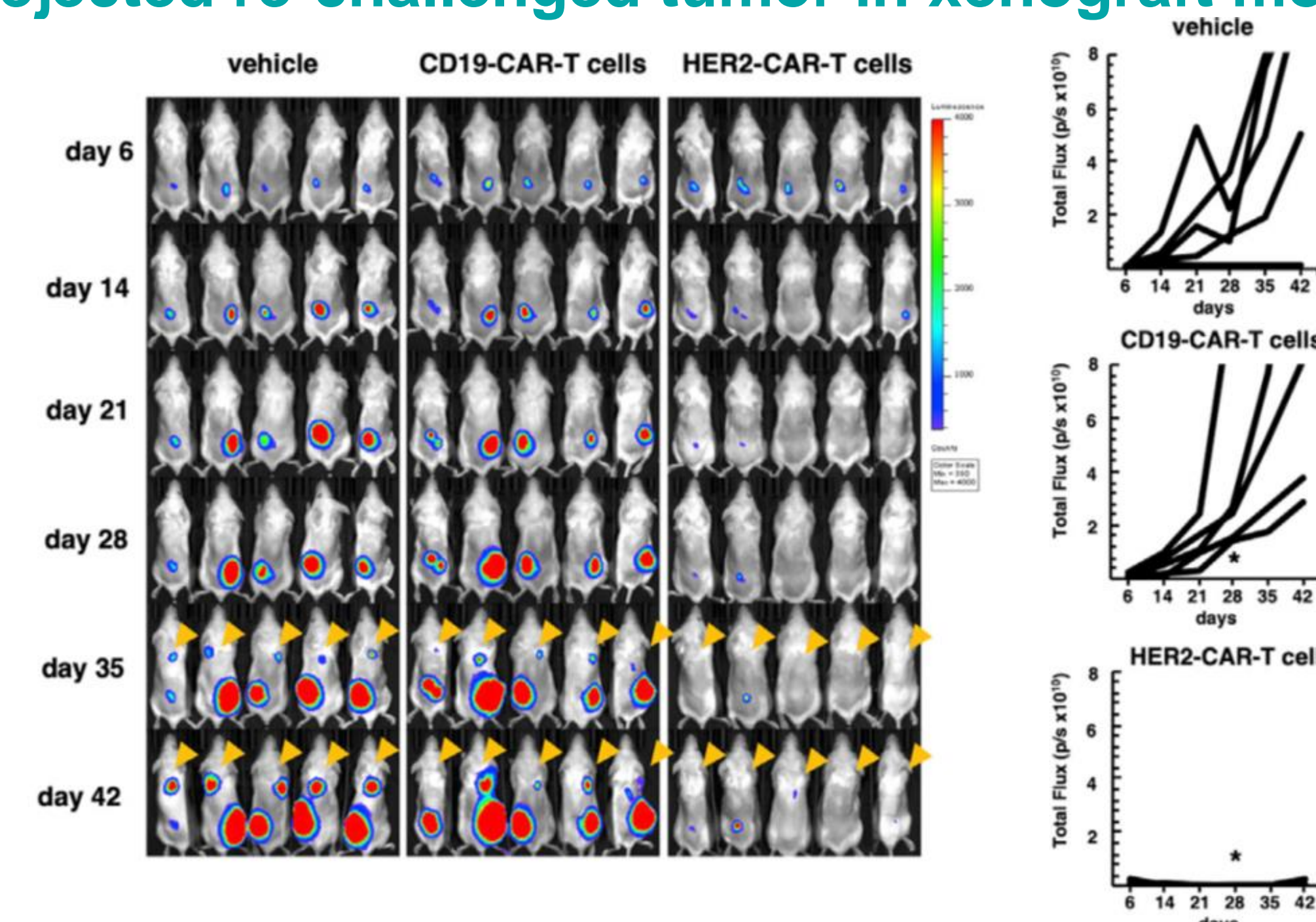
5. BP2301 showed persistent cytotoxicity against HER2+ sarcoma in a serial killing assay¹⁾



A) *in vitro* cytotoxicity analysis of BP2301 was evaluated using sarcoma cell lines. Tumor cells were seeded on xCelligence E-plates 16 at $0.5-2 \times 10^4$ 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.

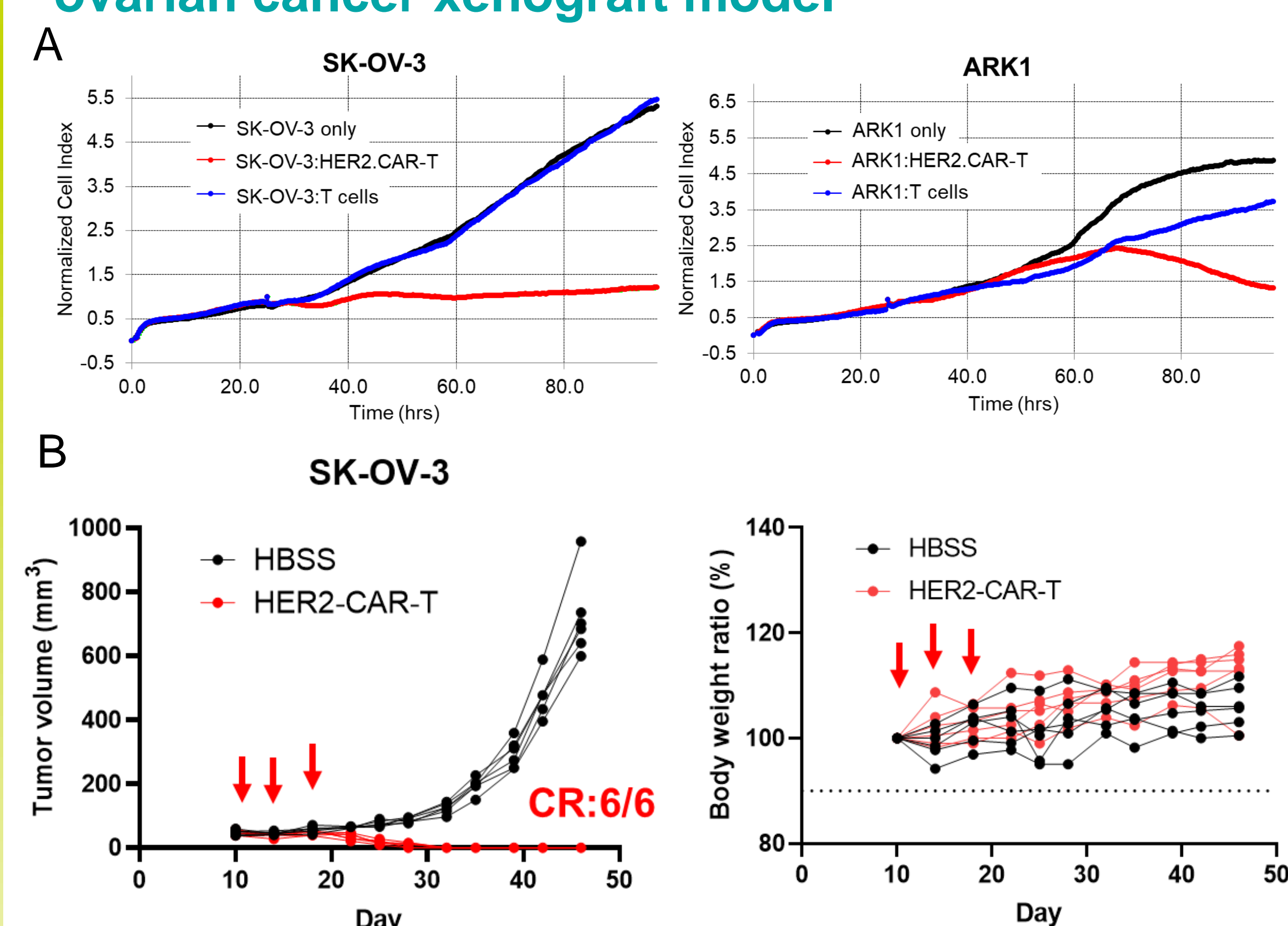
B) *in vitro* anti-tumor activity of BP2301 was evaluated in a serial killing assay. BP 2301 was incubated with U-2OS at an E:T ratio of 1:1 for 72 h, and then was collected, incubated with U-2OS again for the next round of killing. Cell impedance was measured for 72h in each round by an xCELLigence RTCA DP system.

6. BP2301 eradicated rhabdomyosarcoma and rejected re-challenged tumor in xenograft model¹⁾



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 \rightarrow 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 \geq 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

- 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.