# #303 *PiggyBac* transposon-mediated HER2-CAR-T cells exert anti-tumor efficacy against cholangiocarcinoma.

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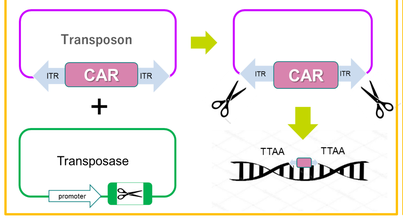
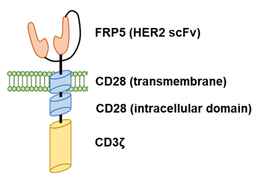
## Introduction

Cholangiocarcinoma (CCA) is an aggressive cancer often diagnosed at a late stage, posing challenges in finding effective treatments for most patients and results in poor prognosis. HER2 overexpression has been reported in cholangiocarcinoma, making it an attractive target for the treatment. We have developed a non-viral HER2-CAR-T cells (BP2301) using *PiggyBac* transposon characterized by CAR-T cells comprising abundance of stem cell memory-like T cells (Tscm). Currently, a phase 1 clinical trial is ongoing to evaluate the safety and efficacy of BP2301 in HER2-expressing sarcoma and gynecologic malignancies. In the present study, we evaluated the anti-tumor efficacy of BP2301 using a CCA xenograft model with patient-derived cell transplantation.

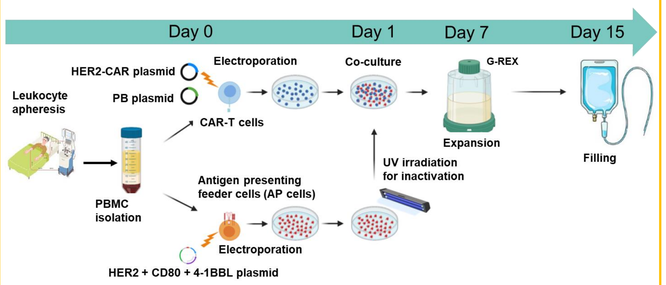
### 1. *piggyBac* Transposon System is adopted for transducing in BP2301.



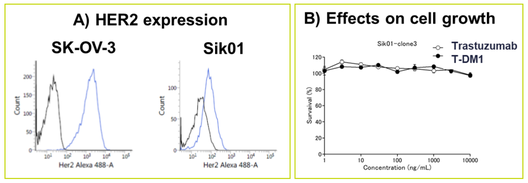

### 2. CAR comprises FRP5, CD28 and CD3ζ.




### 3. BP2301 GMP manufacturing process was optimized.

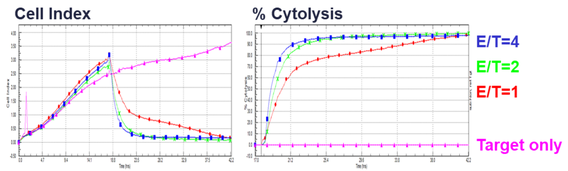



### 4. Although CCA cells (Sik01 cells) are expressing HER2, these cells do not respond T-DM1.



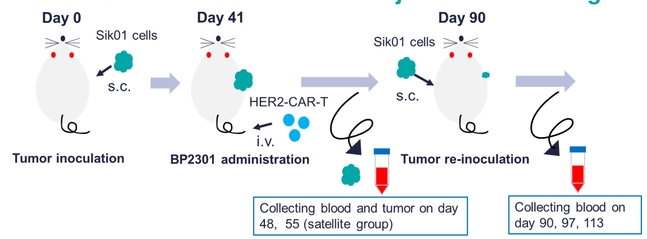

A) HER2 protein on SK-OV-3 or Sik01 cell surface was detected by flowcytometric analysis using anti-HER2 antibody. B) Effects on cell growth were tested in the culture of Sik01 cells adding 1 – 10000 ng/mL T-DM1 or trastuzumab.

### 5. BP2301 has potent killing activity against Sik01 cells.

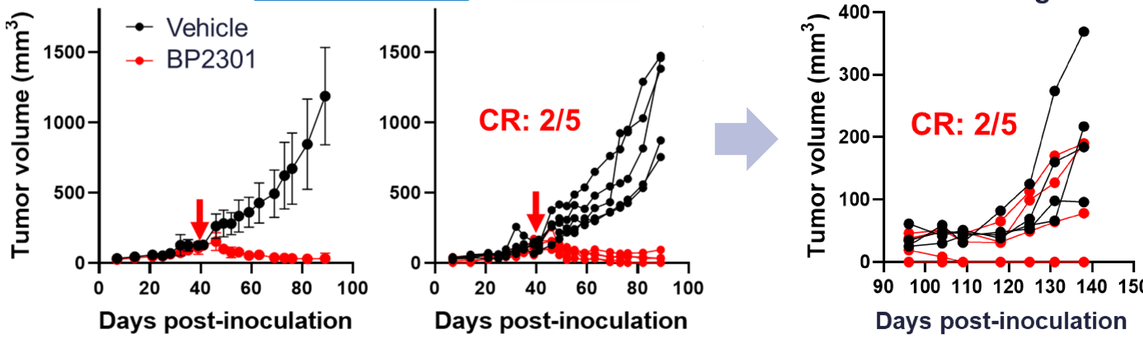



*In vitro* killing activity of BP2301 was evaluated using Sik01 cells. The cancer cells were co-cultured with BP2301 at an E:T ratio of 1:1 - 4:1 for killing assay based on the cell impedance.

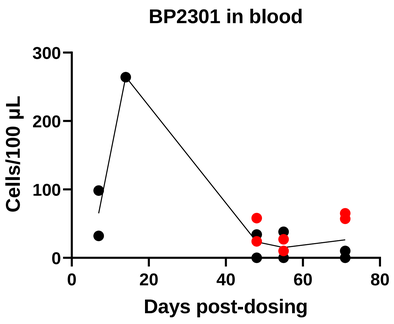
### 6. BP2301 eradicated tumor and rejected re-challenge tumor in Sik01 xenograft model.




NOG mice were inoculated with 5 × $10^6$ Sik01 cells on day 0. HBSS (Vehicle) or 3.6× $10^6$ HER2 CAR T cells (BP2301) were administered intravenously on day 41, respectively (red arrow). BP2301 treated Sik01 tumor bearing mice were re-inoculated with 5 × $10^6$ Sik01 cells on day 90.



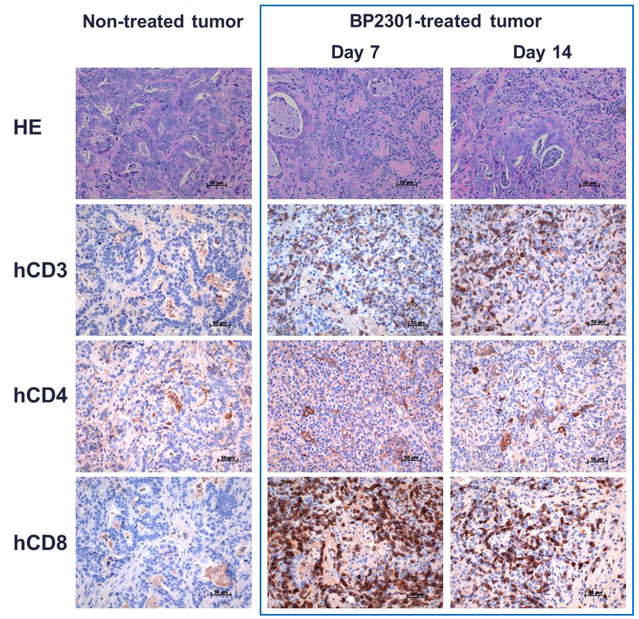

### 7. BP2301 was detected in the peripheral blood of CR mice for up to 71 days after dosing.




| Days post-dosing | BP2301 in blood (cells/100 µL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Satellite group 1 | | Satellite group 2 | | Anti-tumor group | | | | |
| | No.1 | No.2 | No.1 | No.2 | No.1 | No.2 | No.3 | No.4 | No.5 |
| 7 | 98.4 | 32.0 | | | | | | | |
| 14 | | | 264.2 | 265.2 | | | | | |
| 48 | | | | | 0 | 34.1 | 0 | 24.1 (CR) | 58.2 (CR) |
| 55 | | | | | 0 | 38.0 | 0 | 27.0 (CR) | 10.2 (CR) |
| 71 | | | | | 0 | 10.0 | 0 | 57.0 (CR) | 65.2 (CR) |

The blood from the BP2301 treated mice were collected and analyzed for pharmacokinetics on day 7, 14, 48, 55 and 71 after dosing. BP2301 was detected by flowcytometric analysis. Red dots indicate samples from CR mice.

### 8. The tumor was collapsed and infiltrated by a larger amount of CD8 positive cells.




The tumors from the BP2301 treated mice were excised on day 7 and 14 after dosing. The specimens were immunostained with anti-hCD3, anti-hCD4 and anti-hCD8a.

## Conclusion

- BP2301 showed potent anti-tumor activity in a CCA xenograft model, and the anti-tumor activity is correlated with long-term persistence of BP2301 after adoptive transfer.
- BP2301 exhibits remarkable homing and expansion abilities in the tumor site.
- Our results provide support for the future development of BP2301 as a potential treatment option for CCA patients with HER2 expression.

## Acknowledgement

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