# **PiggyBac transposon-mediated HER2-CAR-T cells exert anti-tumor efficacy against cholangiocarcinoma.** #303 Koichiro Shioya<sup>1\*</sup>, Motoya Mie<sup>1</sup>, Yuji Mishima<sup>1</sup>, Tomio Matsumura<sup>1</sup>, Yuta Ohira<sup>1</sup>, Miyuki Tanaka<sup>2</sup>, Shigeki Yagyu<sup>4</sup>, Yozo Nakazawa<sup>3</sup>, Lilin Zhang<sup>1\*\*</sup>

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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 in HER2-expressing sarcoma and



### 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.

# 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.





### 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.

NOG mice were inoculated with 5  $\times$  10<sup>6</sup> Sik01 cells on day 0. HBSS (Vehicle) or  $3.6 \times 10^6$  HER2 CAR T cells (BP2301) were administered intravenously on day 41, respectively (red arrow). BP2301 treated Sik01 tumor bearing mice were

# 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 antihCD3, anti-hCD4 and anti-hCD8a.



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> BP2301 showed potent anti-tumor activity in a CCA xenograft model, and the anti-tumor 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

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