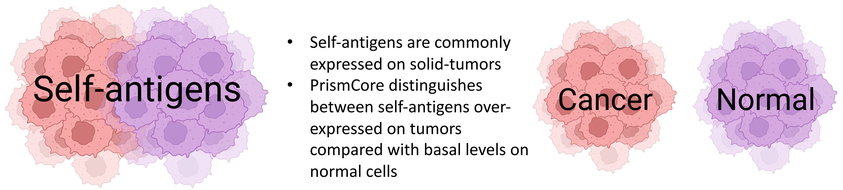
Selective recognition of over-expressed self-antigens in solid tumors using calibrated CAR-T therapy

Farzad Haerizadeh[1], Helena Jiang[2], Laurence Cooper[1]; Bio4t2® [1]San Diego, CA, USA; [2]Shanghai, Peoples Republic of China

Abstract #408212

Background:

- While effectiveness of CAR-T in hematological cancers has been well proven and products commercialized, their utility as an effective treatment of solid malignancies has yet to be established.
- Future development depends on identifying targets for CAR-T that differentiate between normal and malignant cells.
- Bio4t2® uses its PrismCore™ platform to identify targets for CAR-T for treating multiple solid tumor types by calibrating T-cell activity against self-antigens only when present at elevated densities.
- The platform develops calibrated CAR-Ts that exhibit differentiative response to level of self-antigens on tumors and not normal structures using three principles: 1) identification of targets uniformly expressed on solid tumors, 2) over-expression on the surface of invasive cancers compared to healthy cells, and 3) elevated levels linked to tumor aggressiveness.
- Here in we report development and preclinical data of our first novel CAR-T (B4t2-001) recognizing a high value self-antigen BT-001 that is a key regulator of tumor migration, invasion, and metastasis in tumors.
- Clinical trial underway evaluating B4t2-001 (clinicaltrials.gov: NCT05621486).



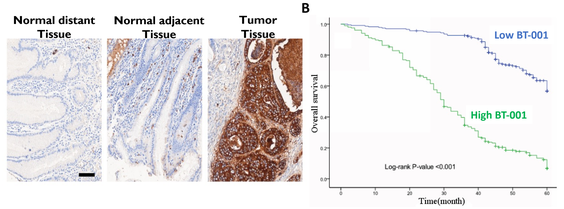
- Self-antigens are commonly expressed on solid-tumors
- PrismCore distinguishes between self-antigens over-expressed on tumors compared with basal levels on normal cells

Table1: *Percentage of cases expressing BT-001 (>*75% of solid tumors)

Indication	Annual Global Cases (Millions) (2022) *		BT-001
	New cases	Deaths	
Lung	2.2	1.7	>85%
Colorectal	1.9	0.93	>55%
Gastric	1.1	0.76	>85%
Pancreatic	0.5	0.46	>90%
Breast	2.2	0.68	>70%

* WHO, International Agency for Research on Cancer

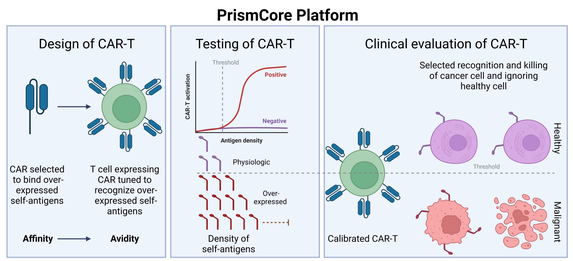
Figure 1. BT-001 antigen target



A) Bt-001 is homogeneously overexpressed in malignant tumors compare to adjacent tissues and normal cells. **B)** BT-001 antigen is critical for patient survival, demonstrated in Gastric cancer patients in this figure.

Methods:

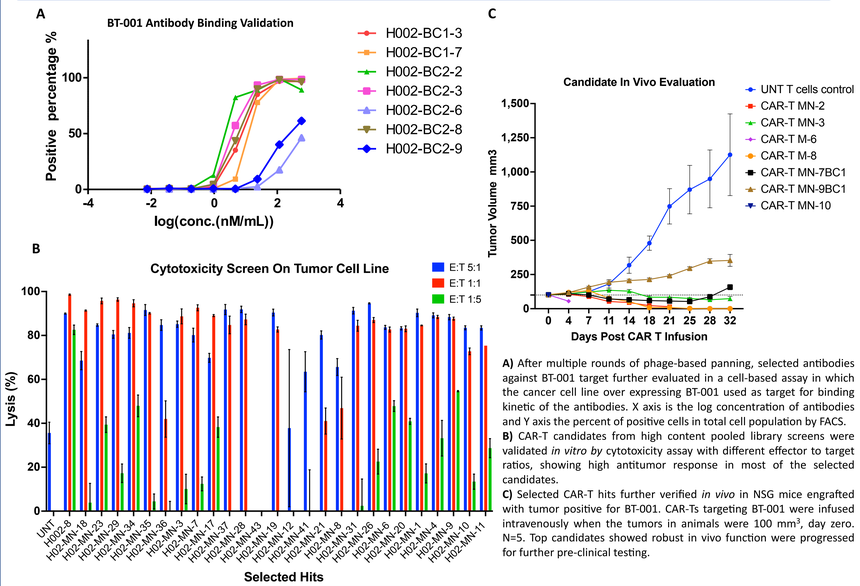
- PrismCore generates CAR-T by combining computational biology with empirical observations to target over-expressed self-antigens. Proto-libraries of CARs are created using synthetic single-domain antibodies and structural/signaling components. Bio-screening is performed using combinatorial libraries generated through computational simulation from synthetic and naturally occurring DNA modules. Lead CAR-T are identified through iterative *in vitro* and *in vivo* testing and evaluated for safety in non-human primates (NHP).



Results:

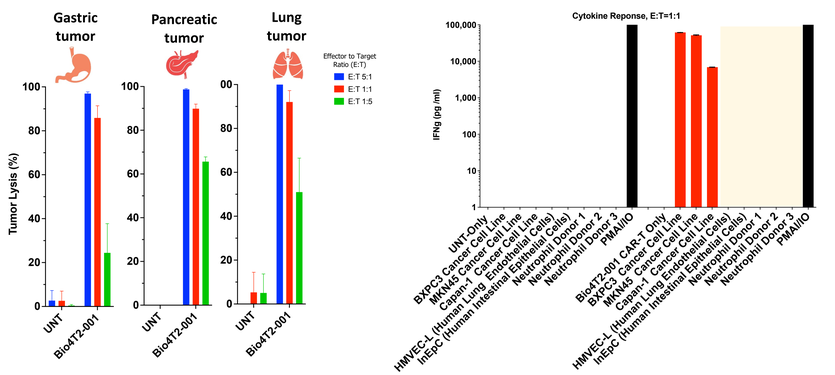
PrismCore platform has identified engineered CAR-T against several self-antigens, with the first CAR-T, termed B4t2-001, advanced to clinical testing against the target BT-001. Libraries (1E10 to 1E11) of camelid antibodies were designed, synthesized, and assessed. Selected antibody variants subsets were combinatorially assembled into CAR-Ts and rigorously screened against gradient of BT-001. Multiple candidates were advanced to further testing in mice to evaluate anti-tumor effect and sustained *in vivo* biological activity. B4t2-001 was evaluated in NHP that express basal levels of BT-001. No toxicity observed despite measurable circulating CAR-T and *in vitro* activity to NHP BT-001 when at high density. The turnaround from target selection to B4t2-001 for clinical evaluation was six months.

Figure 2. screening and selection of calibrated CAR-Ts



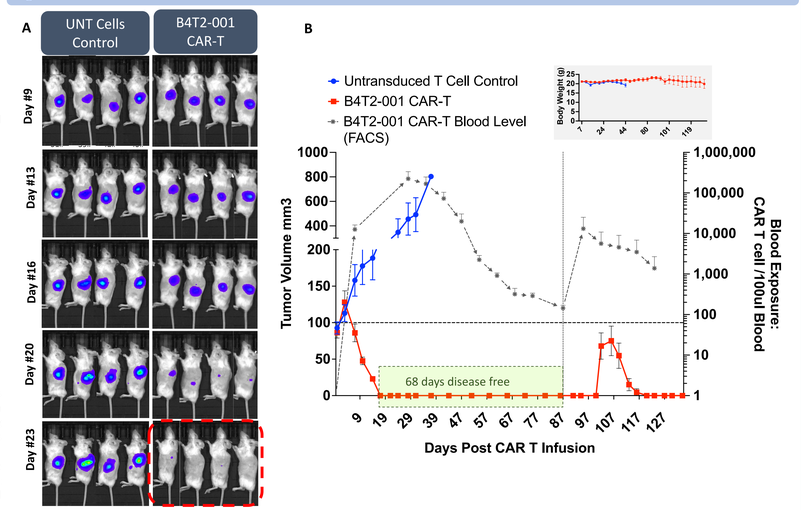
A) After multiple rounds of phage-based panning, selected antibodies against BT-001 target further evaluated in a cell-based assay in which the cancer cell line over expressing BT-001 used as target for binding kinetic of the antibodies. X axis is the log concentration of antibodies and Y axis the percent of positive cells in total cell population by FACS.
B) CAR-T candidates from high content pooled library screens were validated *in vitro by* cytotoxicity assay with different effector to target ratios, showing high antitumor response in most of the selected candidates.
C) Selected CAR-T hits further verified *in vivo* in NSG mice engrafted with tumor positive for BT-001. CAR-Ts targeting BT-001 were infused intravenously when the tumors in animals were 100 mm^3, day zero. N=5. Top candidates showed robust in vivo function were progressed for further pre-clinical testing.

Figure 3. Pre-clinical Evaluation of B4t2-001 CAR-T



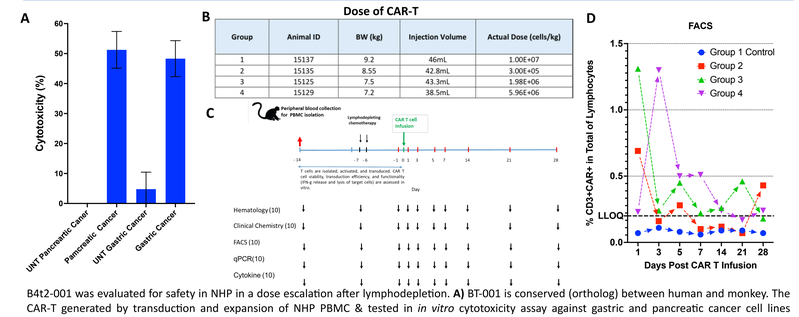
A) *In vitro* antitumor activity of B4T2-001 against multiple solid tumor targets, including gastric, pancreatic and NSCLC cancer models expressing the BT-001 target antigen. Very efficient anti-tumor activity is observed in all effectors to target ratios tested. The assay was done using target cells that are tagged with luciferase.
B) B4T2-001 CAR-T exhibits no response to normal human primary cells in vitro by interferon gamma response test. Left panel, Untransduced control t cells (UNT), right panel, B4t2-001 CAR-T cells. Effector to target ratio 1:1, 24 hrs incubation. PMA/ Ionomycin as positive control, black column.

Figure 4. In vivo evaluation of B4t2-001 CAR-T



A) Pancreatic cancer orthotopic model showing B4t2-001 antitumor response nearly 10 days post CAR-T infusion. In this study, BxPC3 pancreatic tumor cell line tagged with luciferase reporter were injected/seeded inside pancreas organ of each animal (NSG mice) in each study arm. After 9 days when the tumors established in the organ, the CAR-T infused IV (intravenous), from day 16 post tumor engraftment that is only 10 days post CAR T infusion, a robust antitumor activity was observed and the tumors in all animals in the CAR T arms were eradicated. Control arm left and CAR T arm in right panel.
B) In a tumor re-challenge study conducted on a gastric cancer CDX model, the long-term persistence and effective protection of B4t2-001 CAR-T were demonstrated. After achieving complete response in the CAR-T treated arm, animals from that arm were re-engrafted with a fresh set of tumor cells nearly 2 months later. However, B4T2-001 CAR-T cells were not re-administered upon tumor re-engraftment. Ten days after re-engraftment, the tumors in all animals started to regrow, but were then eliminated by CAR-Ts. Before tumor re-engraftment, the CAR T blood level was undetectable, but it spiked upon re-engraftment of new tumor cells, which correlates well with the anti-tumor response for the secondary tumor. These findings highlight the superior persistence and protective capacity of B4T2-001 CAR-T in vivo, resulting in the eradication of tumors. The blue line denotes the untreated arm, the red line represents the CAR T infused arm, and the gray line shows the blood level of the CAR-T analyzed by FACS. NSG mice, N=5, Error bars=SEM.

Figure 5. Safety evaluation in non-human primates (NHP)



B4t2-001 was evaluated for safety in NHP in a dose escalation after lymphodepletion. **A)** BT-001 is conserved (ortholog) between human and monkey. The CAR-T generated by transduction and expansion of NHP PBMC & tested in *in vitro* cytotoxicity assay against gastric and pancreatic cancer cell lines expressing BT-001 at 5:1 effector to target ratio. Compared to UNT, NHP CAR-T showed robust tumor killing response. **B)** Grouping and dosing (The dose is according to CAR positive T cells of B4T2-001 CAR T in NHP study using Naïve monkeys. **C)** Schematic diagram of dosing and blood collection of NHP safety study. **D)** CAR-T engraftment after infusion was evaluated by FACS, CAR-T was transiently detected above LLOQ of the assay in all monkeys infused with B4t2-001 NHP-CAR-T. The best engraftment was observed in group 4 or highest dose level. No clinical adverse events, including hematological toxicity, were observed in animals tested.

Conclusion:

We report on PrismCore platform for development of calibrated CAR-T. To date, three high-value targets and associated CAR-Ts have been developed. B4t2-001 in pre-clinical testing demonstrated target density-dependent response, potency, durability of effect and safety in preclinical models and is undergoing phase I clinical evaluation (NCT05621486).

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