

# Claudin 18.2 - 4-1BB bispecific antibody induced potent tumor inhibition through tumor-specific 4-1BB activation.

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## Introduction

Claudin 18.2 (CLDN18.2) is a gastric-specific membrane protein. In the healthy tissue, CLDN18.2 expression is restricted to the gastric mucosa as a component of tight junction. However, it is ectopically expressed at significant levels in a variety of primary lesion and metastases of epithelial tumor entities, including gastric, pancreatic, esophageal, and lung adenocarcinoma cells. Taken together, CLDN18.2 is believed to be an ideal tumor antigen for immunotherapy. Here, we reported the development of a bispecific antibody CLDN18.2-4-1BB (TJ-CD4B). TJ-CD4B showed stronger binding capability to CLDN18.2 when compared with benchmark CLDN18.2 monoclonal antibody (IMAB362). Functional evaluation of TJ-CD4B indicated the activation of 4-1BB signaling was solely dependent on CLDN18.2 expression on the cell. Furthermore, TJ-CD4B showed superior 4-1BB activity than benchmark 4-1BB monoclonal antibody (Urelumab) in the presence of cells expressing a wide range of CLDN18.2 level including CLDN18.2-low cells. Using a humanized 4-1BB mouse model, TJ-CD4B showed strong tumor growth inhibition (TGI) of CLDN18.2 expressing tumor cells, with an increase of tumor infiltrating lymphocytes. Our data suggested TJ-CD4B activated 4-1BB in a CLDN18.2-dependent manner, thus addressing the safety concern associated with 4-1BB-based therapies. TJ-CD4B is a promising IO therapeutic option for gastric and other CLDN18.2-positive tumors.

## RESULTS

### Design of anti-CLDN18.2-4-1BB BsAb

#### Anti-CLDN18.2 mAb

- Highly potent anti-CLDN18.2 mAb
- Benefit patients with wide range of CLDN18.2 expression.

#### IgG1(N297A)

- No ADCC or CDC
- Minimize FcγR mediated 4-1BB clustering

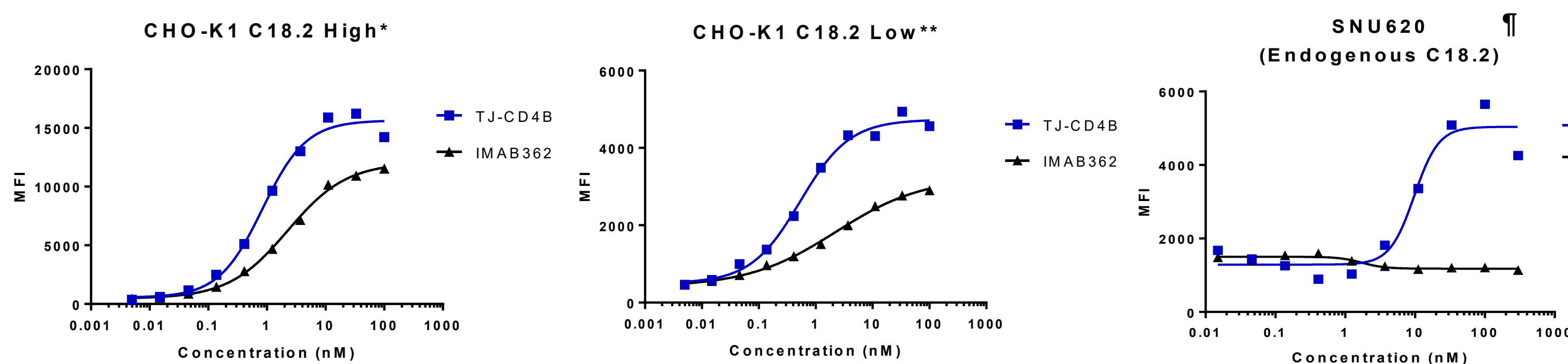
#### Unique 4-1BB binding epitope

- CLDN18.2 dependent 4-1BB activation
- Potent 4-1BB agonism

#### Anti-4-1BB scFv

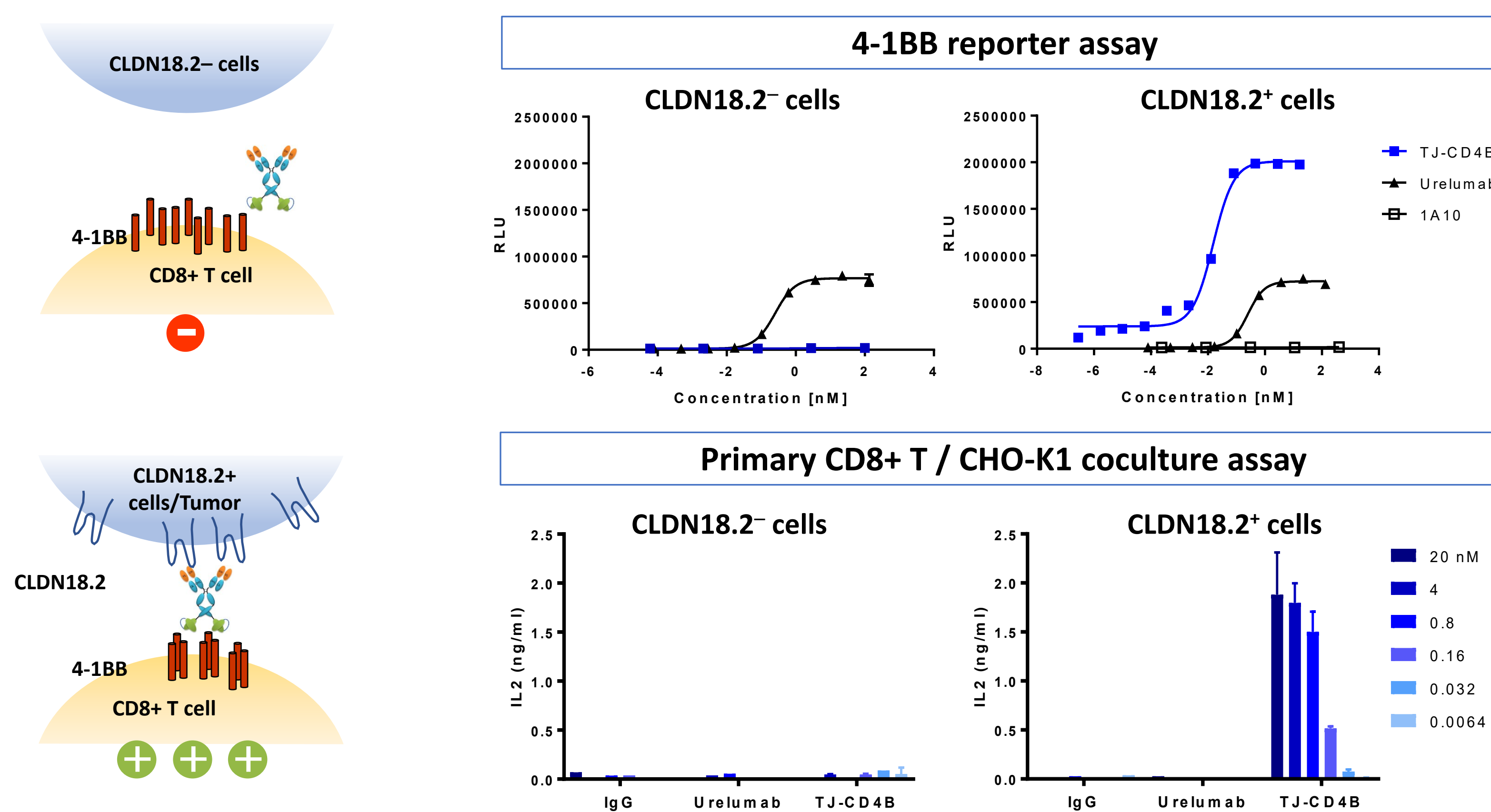
**Generation of anti-CLDN18.2-4-1BB bispecific antibody.** Lead CLDN18.2 mAbs were identified for binding to human CLDN18.2. Binders with high affinity were fused with anti-4-1BB antibody to generate different formats of anti-CLDN18.2-4-1BB bispecific antibodies. Final candidates were selected based on *in vitro* and *in vivo* functional evaluation and drug developability.

### Superior CLDN18.2 binding potency than benchmark antibody

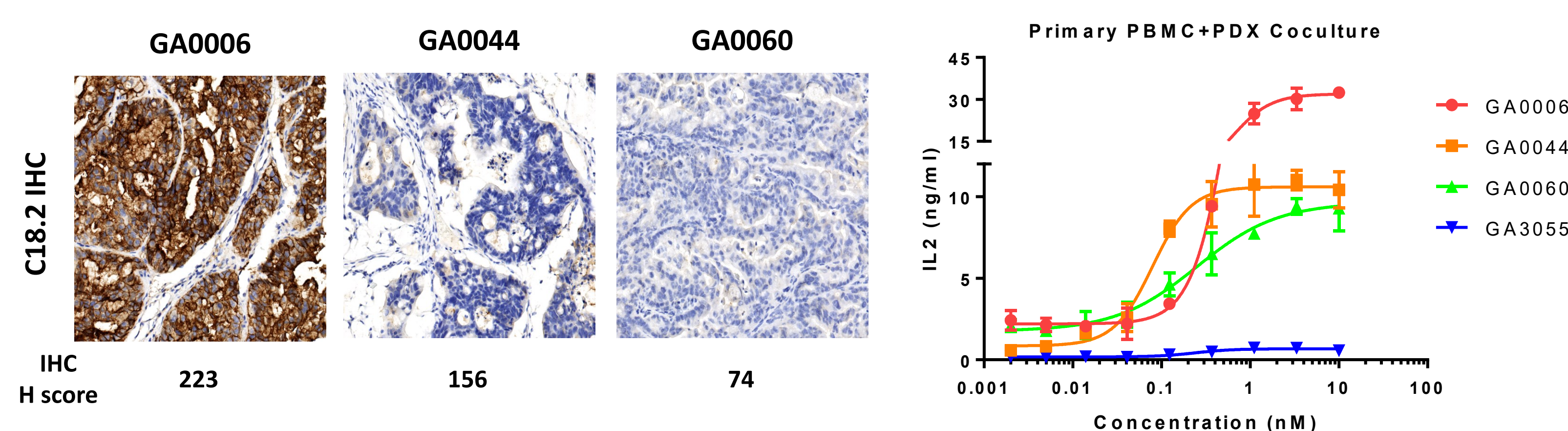


**Cell-based binding of CLDN18.2 by CLDN18.2-4-1BB bispecific antibodies.** Antibodies were 3-fold serially diluted and incubated with  $1 \times 10^5$  CHO-K1-C18.2 and SNU620 for 30 minutes at 4 °C in FACS buffer. Then, APC conjugated-anti-human IgG antibody was added at 4°C for another 30 minutes. After washing, MFI of APC was evaluated by FACS. \* FACS-sorted CLDN18.2 higher expression clone; \*\* FACS-sorted CLDN18.2 lower expression clone; † SNU620 is a gastric carcinoma cell line with very low CLDN18.2 expression on the cell surface. IMAB362 is the benchmark CLDN18.2 monospecific antibody.

### CLDN18.2-dependent 4-1BB agonism



### Primary PBMC / PDX coculture assay

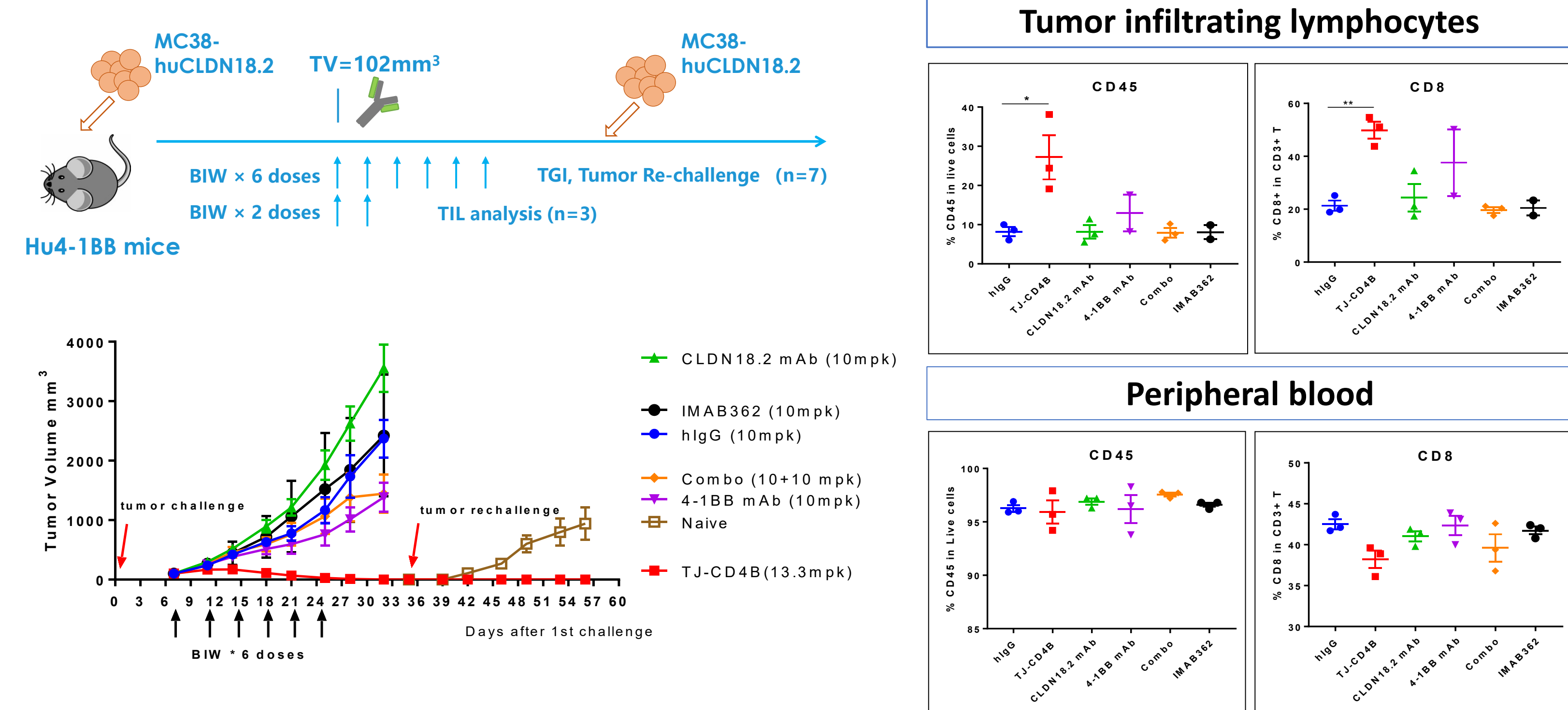


**4-1BB reporter assay.** GloResponse™ NFκB-luc2/4-1BB Jurkat cell at a density of  $2.5 \times 10^4$  cells per well were mixed with  $2.5 \times 10^4$  target cells in a white 96-well plate. Antibodies were serially diluted and added to a white 96-well assay plate. After 6 hours' incubation at 37 °C, luminescence was obtained by adding the substrate of luciferase and measured by a microplate reader. Urelumab is the benchmark 4-1BB mono-specific antibody. 1A10 is a 4-1BB human IgG, the sequence of which was used to generate TJ-CD4B.

**Primary CD8+T assay.** Human CD8+ T cells ( $1 \times 10^5$ ) stimulated with 0.5 μg/ml human anti-CD3 antibody were co-cultured with CHO-K1-CLDN18.2 or CHO-K1. Serially diluted antibodies were added and incubated with co-culture. After 48 hours, levels of IL2 in the culture medium were measured using IL-2 LANCE Ultra TR-FRET Detection Kit (PerkinElmer).

**Primary PBMC assay.** Gastric adenocarcinoma(GA) PDX-derived cells were coculture with human PBMC ( $1 \times 10^5$ ) from the same donor. Coculture was stimulated with 0.5 μg/ml human anti-CD3 antibody and incubated with serially diluted antibodies. After 48 hours, levels of IL2 in the culture medium were measured using IL-2 LANCE Ultra TR-FRET Detection Kit (PerkinElmer).

### Significant tumor growth inhibition and increased TIL



**In vivo efficacy study.** MC38-hCLDN18.2 cells were subcutaneously implanted into hu4-1BB mice. When the tumor grew to an average of 102 mm<sup>3</sup>, mice (n=7/group) were intraperitoneally treated with human IgG, CLDN18.2 mAb and 4-1BB mAb alone or in combination, IMAB362 and BsAb at equal molar amount twice weekly for 6 doses. For the re-challenge study, a second dose of MC38-hCLDN18.2 cells were injected in the contralateral flank after the initial tumor challenge. Tumor growth was monitored by volumetric measurement. *Ex vivo* analysis of FACS analysis of intratumoral and peripheral CD45+ cells and CD8+ T cells were performed in a subgroup (n=3/group). \* n<0.05; \*\*n <0.01

## CONCLUSIONS

- TJ-CD4B is a bispecific antibody that can recognize cells expressing wide range of CLDN18.2.
- TJ-CD4B activated 4-1BB signaling to enhance T cell activation in a CLDN18.2-dependent manner.
- TJ-CD4B showed significant inhibition of tumor growth (7/7 tumor free) in a syngeneic mouse model. Furthermore, TJ-CD4B can induce long-term protective immunological memory.
- *Ex vivo* analysis suggested TJ-CD4B increased CD8+ T cell number in tumor microenvironment with no impact on peripheral lymphocytes.