Preclinical Characterization of a Novel Claudin 18.2 Targeting-ISAC with Robust Potency and Acceptable Safety Profile

ABSTRACT # 1147-D

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INTRODUCTION

Claudin (CLDN) 18.2 is a transmembrane tight junction protein that is expressed in stomach epithelia. CLDN18.2 expression is significantly elevated in gastric and pancreatic adenocarcinomas. Loss of cell polarity in tumors results in CLDN18.2 localization to surfaces that are more readily accessible to biologics and effector cells. This expression pattern makes it an excellent target for immune stimulating antibody conjugate (ISACs), which combine the specificity of a tumor-targeting antibody with potent immune stimulation. The delivery of ISACs to the tumor microenvironment triggers the innate and adaptive immune system to attack CLDN18.2-expressing tumors. T cell priming following phagocytosis of CLDN18.2-expressing tumor cells in the context of immune stimulation results in epitope spreading and the targeting of CLDN18.2-negative tumors cells with durable immunologic memory. These mechanisms differ from other cytotoxic payloads, which rely on the induction of apoptosis or cell death to kill tumor cells. Herein, we describe the development of a Claudin 18.2 ISAC with a TLR7/8 linker-payload.

Claudin 18.2 is an Attractive ISAC Target

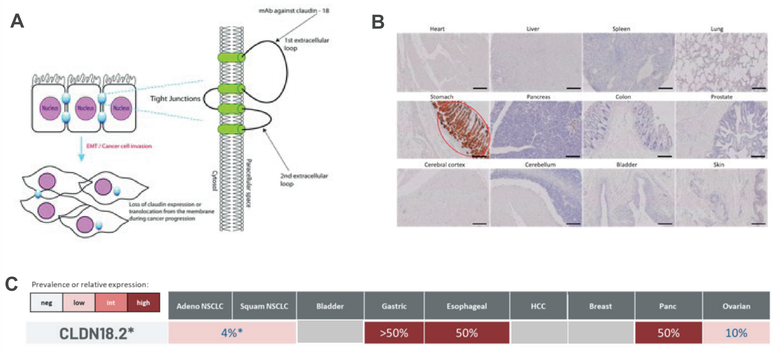
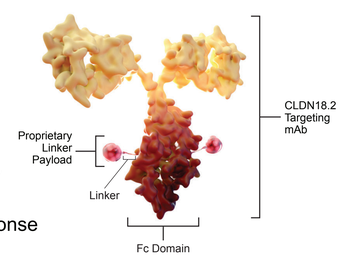


Figure 1. (A) Claudin18.2 (CLDN18.2) is located within tight junction in healthy cells and this controlled localization is lost in cancerous cells.[1] **(B)** CLDN18.2 is solely expressed in the stomach of normal tissue.[2] **C)** High prevalence of CLDN18.2 positive tumors in gastric, esophageal and pancreatic tumors.

Claudin 18.2 Boltbody™ ISAC Program

- **Immune-stimulating antibody conjugates (ISACs):**[3]
  - Antibody against CLDN18.2 directs Boltbody™ ISAC to the tumor
  - Proprietary immune stimulant activates myeloid antigen-presenting cells
  - Myeloid cells kill tumor cells, create a "hot" tumor microenvironment, and initiate an innate & adaptive anti-tumor immune response
- **CLDN18.2 ISAC**
  - Limited normal tissue expression of CLDN18.2 reduces toxicity liabilities
  - Antibody cross reacts with mouse/rat/NHP CLDN18.2 to facilitate tox evaluation
  - Potent activity observed *in vitro* and *in vivo* with CLDN18.2 ISAC
- **Next Generation Linker-Payload**
  - Increased potency with acceptable therapeutic window



Hallmarks of Boltbody™ ISAC Platform

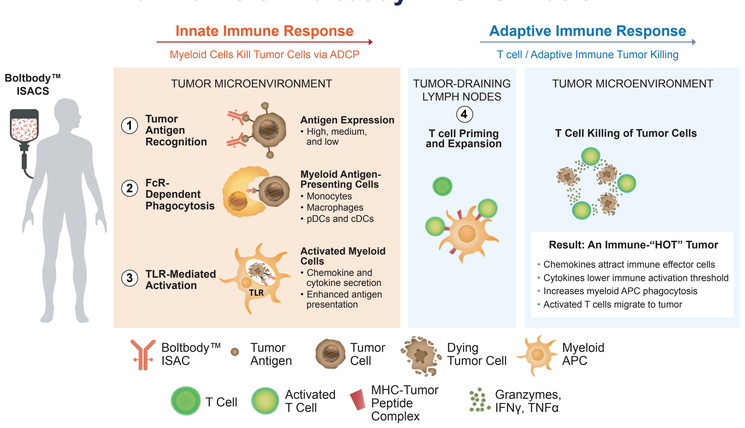


Figure 2. Proposed mechanism of Boltbody™ ISACs

REFERENCES

1. Kumar, *Frontiers in Pharmacology*. 2018.
2. Jiang et al, *JNCI*. 2019.
3. Ackerman SE, et al. *Nature Cancer*. 2021.

RESULTS

P424 Selected as Lead CLDN18.2 Antibody

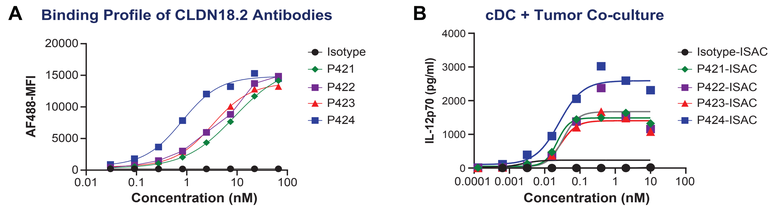


Figure 3. (A) Binding of fluorescently labeled CLDN18.2 antibodies to human PA-TU-899S (human pancreatic cancer cell line) was measured by flow cytometry. **(B)** Conventional dendritic cell (cDC)-enriched primary human cells were co-cultured with PA-TU-8988S tumor cells (10:1 E:T ratio) in the presence of indicated mAbs or ISACs. After 18 hours, supernatants were collected and analyzed for cytokine secretion. Data shown as mean with SEM for n=3 donors. **(C)** Summary table comparing the properties of different CLDN18.2 antibodies tested. P424 was selected as lead antibody based upon superior binding, cDC activity, reduced off target activity and longer half-life in mice.

C

Antibody	FACS Binding	cDC Activity	Off Target Activity	Half-life in mouse (mAb)
P421	2	2	3	2
P422	2	3	2	2
P423	2	2	2	2
P424	1	1	1	1

1- Superior, 2-Comparable, 3-Inferior

Antigen Dependent Immune Activation of CLDN18.2-ISAC

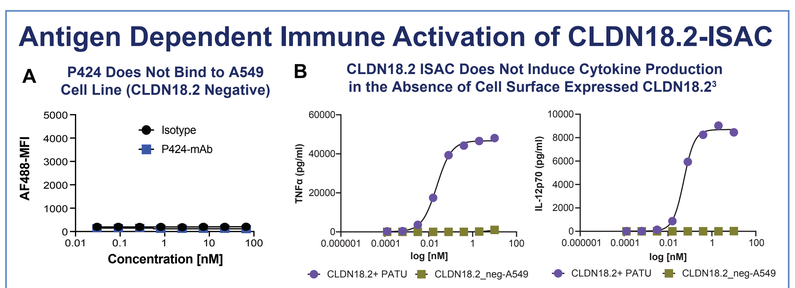


Figure 4. (A) Binding of fluorescently labeled antibodies to human A549 (human lung cancer cell line) was measured by flow cytometry. **(B)** Conventional dendritic cell (cDC)-enriched primary human cells were co-cultured with PA-TU-8988S (human pancreatic cancer) tumor cells or A549-CLDN18.2 negative (lung cancer cell line) at a 10:1 E:T ratio in the presence of indicated mAbs or ISACs. After 18 hours, supernatants were collected and analyzed for cytokine secretion. Data shown as mean with SEM for n=3 donors.

Characterization of *in vivo* Models for Testing of CLDN18.2-ISAC

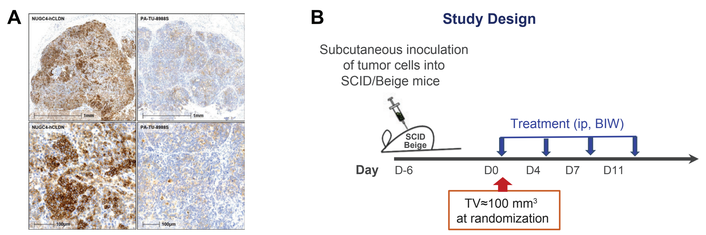
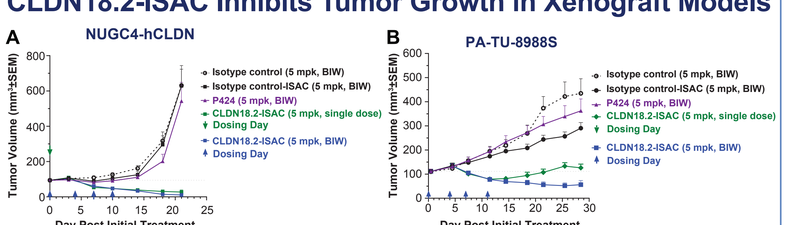


Figure 5. (A) The NUGC4-hCLDN, cell line was engineered to express human CLDN18.2. PA-TU-8988S cells endogenously express human CLDN18.2. To determine the level of CLDN18.2 expression in these models, SCID/beige mice were inoculated with NUGC4-hCLDN or PA-TU-8988S cells. Tumors were harvested and CLDN18.2 expression was evaluated by IHC. Brown staining represent positive CLDN18.2 expression. Expression of CLDN18.2 is lower in PA-TU-8988S compared to NUG4-hCLDN. Not all PA-TU-8988S tumor cells stain positive for CLDN18.2. **(B)** CLDN18.2-ISAC was evaluated in both these models using a similar dosing strategy. Tumors were randomized once the average reaches 100mm^3. Mice received four total doses over two weeks.

CLDN18.2-ISAC Inhibits Tumor Growth in Xenograft Models



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Treatment	NUGC4-hCLDN		PA-TU-8988S	
	%TGI	Mice with no detectable tumor	%TGI	Mice with no detectable tumor
CLDN18.2-ISAC Single dose	96%	3 out of 8	71%	None
CLDN18.2-ISAC BIW	98%	5 out of 8	85%	None

Figure 6. (A & B) SCID/beige mice with NUGC4-hCLDN **(A)** or PA-TU-8988S **(B)** tumors (N=8) were treated as indicated once tumors reached an average 100m^3 as indicated. Significant tumor regression observed in both models. No significant body weight loss was observed in any treatment group. **(C)** Summary of TGI and number of animals without a detectable tumor.

CLDN18.2-ISAC Inhibits Tumor Growth in Syngeneic Model

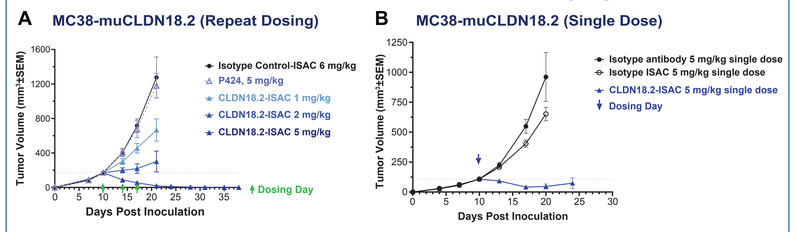

Figure 7. (A) MC38 cells were engineered to express mouse CLDN18.2. C57BL/6 mice with MC38-muCLDN18.2 derived tumors (N=8) were treated as indicated. In the 5 mg/kg group, all eight mice treated had no detectable tumors for > 4 weeks. **(B)** A single dose of the CLDN18.2-ISAC (5 mg/kg) led to no detectable tumor in 4 out 8 mice in the same MC38 tumor model.

CLDN18.2-ISAC Induced T-cell Memory and Epitope Spreading

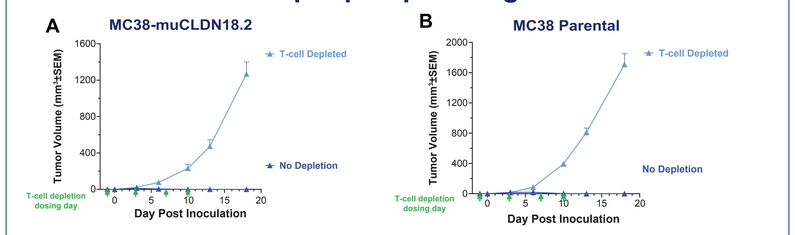

Figure 8. Tumor free mice (26 days from final treatment with CLDN18.2-ISAC) were rechallenged with **(A)** MC38-muCLDN18.2 cells and **(B)** parental MC38 cells. Each mouse was subcutaneously inoculated with 1 million MC38-muCLDN18.2 cells on right flank and 1 million MC38 parental cells on left flank (N=8). To investigate the role of T cells, half of the mice were administered with depleting anti-CD4 and anti-CD8 antibodies. The depleting antibodies were given one day prior to inoculation of tumor cells. The rechallenged mice did not have detectable tumor growth for either cell lines, suggestive of epitope spreading. Depletion of T cells led to tumor growth, demonstrating a critical role of T cells in rejection and involvement of an adaptive immune response.

CLDN18.2-ISAC Was Tolerated in Mice Following Two Doses at 60 mg/kg

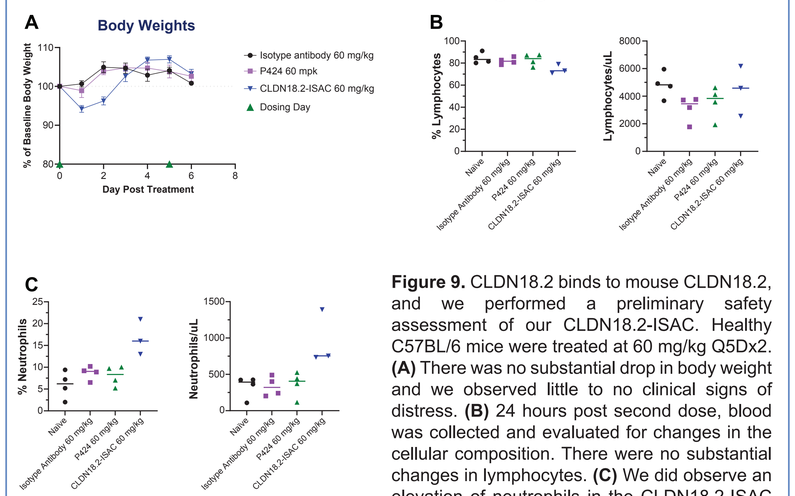

Figure 9. CLDN18.2 binds to mouse CLDN18.2, and we performed a preliminary safety assessment of our CLDN18.2-ISAC. Healthy C57BL/6 mice were treated at 60 mg/kg Q5Dx2. **(A)** There was no substantial drop in body weight and we observed little to no clinical signs of distress. **(B)** 24 hours post second dose, blood was collected and evaluated for changes in the cellular composition. There were no substantial changes in lymphocytes. **(C)** We did observe an elevation of neutrophils in the CLDN18.2-ISAC treated group.

CONCLUSIONS

We believe that this is the first reported CLDN18.2-ISAC that demonstrates potent anti-tumor activity, induction of immunologic memory with epitope spreading, and an acceptable safety profile in preclinical studies. A CLDN18.2-ISAC may offer benefits beyond other CLDN18.2-targeting therapeutics in development.