Preclinical development and characterization of allogeneic CAR T cells targeting Claudin18.2-positive tumors

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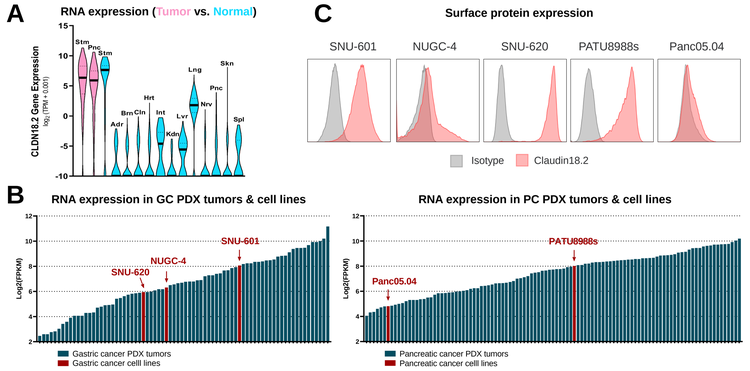
Abstract #283

Background: Claudin18.2 (CLDN18.2) has emerged as a promising therapeutic target, with high expression in many types of epithelial cancers including stomach, esophagus, pancreatic, and ovarian cancers. Multiple CLDN18.2-directed monoclonal antibodies and autologous CAR T cell therapies have entered clinical trials and demonstrated promising results. Here, we describe the preclinical development of a novel allogeneic, off-the-shelf CLDN18.2 CAR T product candidate with the potential to provide clinical benefit with a single treatment and overcome many of the challenges facing autologous CAR T cells.

Methods: A panel of allogeneic fully-human scFv-based CAR T cells targeting both human and murine CLDN18.2 were generated. To enable off-the-shelf use, TALEN® gene editing was used to knock out (KO) the *TRAC* locus and *CD52* KO was also used to permit CD52-directed lymphodepletion regimens. Binding specificity was studied utilizing cell lines and candidates were characterized using a variety of in vitro functional assays and in vivo xenograft mouse models. Rituximab-based off-switches were also evaluated to provide control over CAR T function.

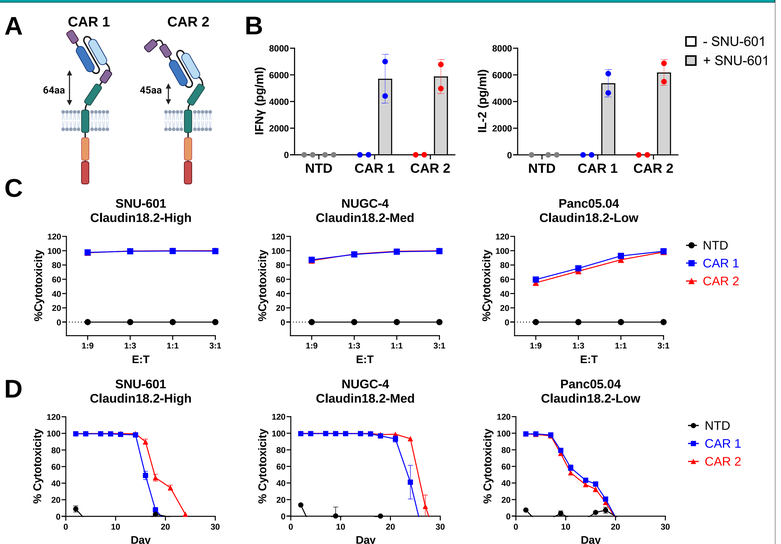
Results: A subset of CARs showed highly specific binding to CLDN18.2 and evaluation of these CARs in multiple rituximab off-switch formats identified candidates with potent activity in both short-term and repeat stimulation in vitro cytotoxicity assays. Lead allogeneic CAR T cell candidates exhibited cytokine release upon target exposure and displayed robust antitumor activity at low cell doses in vivo against both subcutaneous and intraperitoneal gastric cancer models. Safety evaluation was also performed, including body weight measurement. Taken together, these data support the existence of a therapeutic window and the potential to target CLDN18.2 with allogeneic off-the-shelf CAR T cells.

Claudin18.2 is widely expressed in human gastric and pancreatic cancers, as well as normal stomach



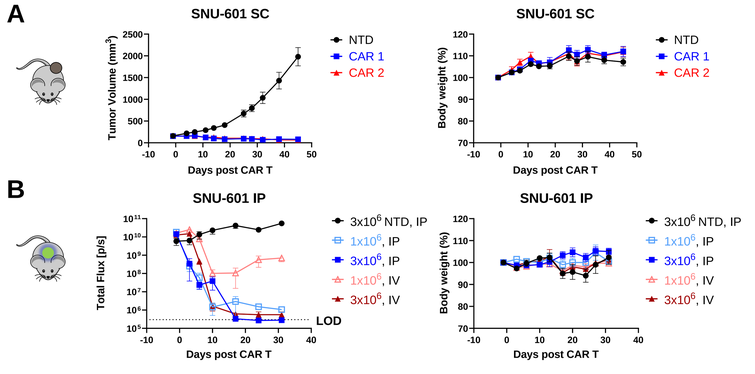
(A) RNAseq data from TCGA and GTEx shows that Claudin18.2 is highly expressed in gastric and pancreatic cancers as well as normal stomach tissue. **(B)** Claudin18.2 RNA analysis suggests that the gastric cancer (GC) and pancreatic cancer (PC) cell lines used covers a range of Claudin18.2 expression of most PDX tumors. **(C)** Claudin18.2 protein expression is detected on gastric and pancreatic tumor cell lines by flow cytometry.

Claudin18.2 CAR T cells are active against Claudin18.2-positive cell lines in vitro



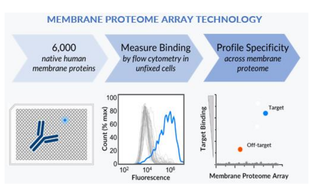
(A) Different Rituximab off-switch (purple) formats were chosen for each CAR based on transduction efficiency and cytotoxicity. **(B)** Claudin18.2 CAR T cells secrete IFNγ and IL-2 when co-cultured with Claudin18.2-positive cell lines. CAR T cells and tumors were co-cultured for 24 hours at the E:T ratio of 1:1. **(C)** Short-term cytotoxicity assay (72-hour co-culture) demonstrates that Claudin18.2 CAR T cells can eliminate cells expressing different levels of Claudin18.2 even at low E:T ratios. **(D)** Long-term killing assays confirm the persistency of killing over an extend period by transferring CAR T cells to freshly plated target cells every 2 to 3 days. Results represent mean ± SEM, n=3 technical replicates. NTD, non-transduced T cells. Experiments were performed three times with CAR T cells from three different donors.

Claudin18.2 CAR T cells show anti-tumor efficacy in subcutaneous and intraperitoneal in vivo models



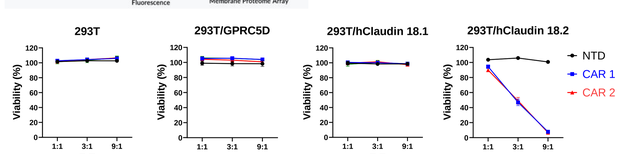
(A) Claudin18.2 CAR T cells induced complete regression of SNU-601 subcutaneous tumors. 1x10^6 CAR$^+$ cells were given to animals by intravenous tail vein injection. None of the animals showed significant loss of body weight. Results represent mean ± SEM. **(B)** Local-regional delivery of Claudin18.2 CAR T cells is superior to systemic delivery in a SNU-601 intraperitoneal xenograft model. Mice were treated with CAR1 via different injection routes (IP: intraperitoneal or IV: intravenous) at two dose levels (1x10^6 or 3x10^6 CAR$^+$). Tumor regression was measured by bioluminescence photometry. None of the animals showed significant loss of body weight. Results represent mean ± SEM. **(C)** Representative bioluminescence images of same mice as in **(B)** on Days -1, 6, 10, and 31 post CAR T treatment.

Claudin18.2 CAR T cells are specific to Claudin18.2 and show no off-target risks



Membrane Proteome Array Results

Clone	On-target hits	Off-target hits	Cytotoxicity results
CAR 1	Claudin 18.2	GPRC5D	De-risked
CAR 2	Claudin 18.2	-	-



(A) Membrane proteome array is used to assess the binding profile of Claudin18.2 CARs and identify potential off-target hits for CAR 1. **(B)** Short-term killing assay demonstrates an acceptable safety profile for Claudin18.2 CARs. 293T cells expressing human GPRC5D, Claudin18.1, Claudin18.2 were co-cultured with Claudin18.2 CARs for 72 hours. Results represent mean ± SEM, n=3 technical replicates. Experiments were performed three times with CAR T cells from two different donors.

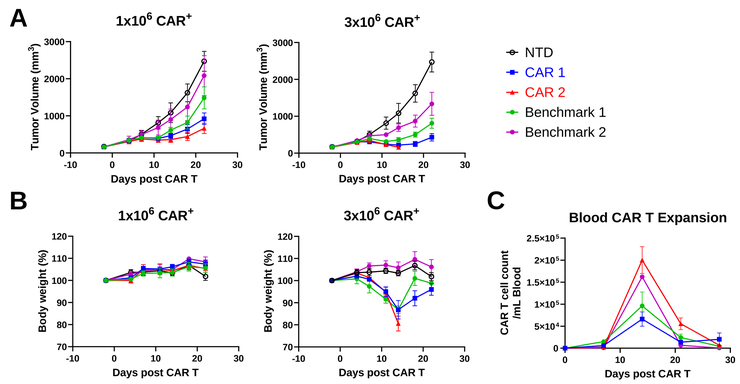
Histopathology analysis identify expected on-target toxicity with Claudin18.2 CAR T cells at high doses

(A) Schematic of in vivo safety evaluation using NUGC4 subcutaneous model. Each Claudin18.2 CAR T treatment group was harvested at different time point due to rapid body weight loss. **(B)** Histopathology analysis showed infiltration and tissue damage in the stomach, which matches the normal tissue expression of Claudin18.2.

B

		Treatment				
Tissue	**Findings**	NTD, 1x10^7, (n=3)	**CAR 1** 1x10^7, (n=3)	**CAR 2** 1x10^7, (n=3)	**Benchmark 1** 1x10^7, (n=3)	**Benchmark 2** 1x10^7, (n=3)
Stomach (Claudin18.2+ /Claudin18.1-)	Infiltrate, mononuclear /mixed cell	0	Moderate (n=2)	Moderate (n=2) Marked (n=1)	Moderate (n=3)	Moderate (n=3)
	Ulcer/Erosion	0	Mild (n=1)	Moderate (n=1)	0	Marked (n=3)
	Hyperplasia	0	Mild (n=2)	Minimal (n=1)	Mild (n=1)	Mild (n=1) Moderate (n=2)
Lung (Claudin18.2- /Claudin18.1+)	Infiltrate, mononuclear /mixed cell	0	Moderate (n=2) Marked (n=1)	Moderate (n=1) Marked (n=2)	Mild (n=1) Moderate (n=2)	Mild (n=3)

Superior activity of Claudin18.2 CAR T cells over benchmarks support the existence of a therapeutic window



(A, B) Claudin18.2 CAR T cells induced a dose-dependent anti-tumor responses and body weight loss in a NUGC4 subcutaneous xenograft model. 1x10^6 or 3x10^6 CAR$^+$ cells were given to animals by intravenous tail vein injection. **(A)** Differences in efficacy remain consistent at both dose levels. **(B)** The level of toxicity, measured by loss of body weight, is positively correlated with anti-tumor efficacy. None of the animals showed body weight loss at the 1x10^6 dose. **(C)** Claudin18.2 CAR T cells exhibited expansion in the blood. Blood was collected from groups treated with 3x10^6 CAR$^+$ cells. Results represent mean ± SEM.

Conclusions

- Claudin18.2 RNA is expressed in gastric and pancreatic tumors and normal tissue expression is limited to the stomach.
- The efficacy and safety data suggest that allogeneic Claudin18.2 CARs may be an effective and clinically valuable treatment for patients with Claudin18.2-positive cancers.