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 cell 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 lymphodepleting regimens. Binding specificity was studied using 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 cytokassay 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.

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

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

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

**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.