207 CLAUDIN18.2-TARGETING CAB-T CELLS HAVE SUPERIOR SAFETY OVER TRADITIONAL CAR-T, WHILE MAINTAINING POTENT *IN VIVO* ANTI-TUMOR EFFICACY

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Background We have developed a proprietary T cell-based anti-CD3 T cell engager delivery platform (named CAB-T), which includes two elements: a chimeric CD3e/signaling component and a CD3-based bispecific T cell engager (TCE). We have previously shown the efficacy *vs.* safety advantages of the CAB-T system over a traditional second-generation CAR-T format by targeting claudin18.2 (CLDN18.2) (poster no. 206, SITC2021). However, a comprehensive comparison between CAB-T *vs.* CAR-T had not been investigated. Here, in multiple independent *in vivo* tumor mouse models, we aimed to assess the safety and efficacy of our CAB-T platform using CLDN18.2-CAB-T cells.

Methods A sequence encoding for an anti-human CLDN18.2 scFv was engineered into CAB-T (CLDN18.2-CAB-T) and CAR-T (CLDN18.2-CAR-T) expression systems and generated via lentiviral transduction of T cells followed by cell expansion. Cytokine release and TCE levels were determined by ELISA and a cytometric bead array, while T cell killing was tested by the detection of LDH release. *In vivo* studies using CLDN18.2+ tumor cells were tested in humanized mouse models (NCG or NOG mice). T cell infiltration into tumors or normal gastric tissue were detected by immunohistochemistry with an anti-FLAG antibody.

Results Both CLDN18.2-targeting CAB-T and CAR-T cells displayed excellent dose-dependent *in vivo* anti-tumor efficacy towards CLDN18.2-positive tumor cells. However, CLDN18.2-CAB-T cells had a superior safety profile over its CAR-T equivalent. More specifically, mid (1 x 10^6 cells) to high (5 x 10^6 cells) single doses of CLDN18.2-CAR-T cells were lethal to mice, whereas all the dose levels of CAB-T cells were safe. CLDN18.2-CAB-T cells released less inflammatory cytokines than CLDN18.2-CAR-T, including IL-6, TNFα and IL-2, in *in vitro* co-culture assays with NUGC4 tumor cell targets. Mice treated with CLDN18.2-CAB-T also showed higher levels of serum GM-CSF and IFNγ compared to those treated with CLDN18.2-CAR-T. Meanwhile, CLDN18.2-CAR-T induced severe immune cell infiltration in normal gastric tissue, whereas CLDN18.2-CAB-T did not. This suggests that CAB-T may provide a wider therapeutic dosing window than traditional CAR-T platforms.

Conclusions We show that CLDN18.2-CAB-T has superior safety traits over CLDN18.2-CAR-T while maintaining high levels of *in vivo* anti-tumor efficacy, suggesting that CAB-T cells may offer better safety for patients in the clinic. CLDN18.2-CAB-T is currently undergoing an investigator-initiated trial to test its safety and efficacy for subjects with CLDN18.2+ solid tumors.

Ethics Approval All mice were maintained under specified pathogen-free conditions, and all studies were approved by the Animal Care and Use Committee of HUST-Suzhou Institute for Brainsmatics.

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