SMART CAR-T cells resist tumor immunosuppressive microenvironment with enhanced efficacy against solid tumors

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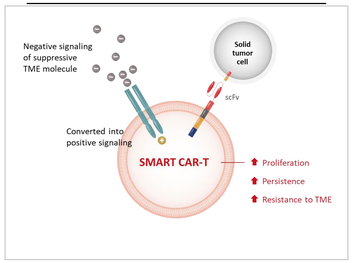
Qi Dong, Wenjie Yin, Manli Yin, Tao Wang, Di Wu, Xinxin Wang†, William Cao, Lianjun Shen*
Gracell Biotechnologies Co., Ltd., Shanghai, China *Corresponding author †Presenting author

Background

- Transforming growth factor-β (TGF-β) is a major mediator of T cell suppression in the tumor microenvironment (TME). It has been shown that co-expression of a dominant-negative TGF-β receptor 2 (dnTGFBR2) in chimeric antigen receptor T (CAR-T) cells increased proliferation of lymphocytes, enhanced cytokine secretion, and maintained long-term efficacy *in vivo*.
- To combat the immunosuppressive TME and further improve the persistence and efficacy of CAR-T cells against solid tumors, we designed CAR constructs with a novel SMART (Suppressive Molecule Activated and Rejuvenated T cells) "switch" module that combines the ectodomain of dnTGFBR2 and an intracellular cytokine receptor signaling domain to convert the suppressive signal mediated by TGFBR2 into pro-T-cell signaling.

Mechanism of action in the TME



Key Features of SMART CAR-T

- More robust cell proliferation and enhanced durability
- Improved resistance to TME (TGF-β)
- Reduced cell exhaustion
- Superior *in vitro* and *in vivo* efficacy even at a lower dose
- Longer immunological memory

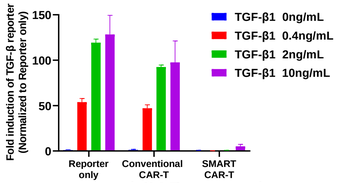
Method

- SMART CAR is a 2nd generation structure that incorporates a CD28 co-stimulatory domain.
- The *in vitro* tumoricidal capacities of SMART CAR-T cells specific to human mesothelin (MSLN) were tested in short-term tumor killing assays and repeated tumor challenge assays in the presence or absence of exogenous of TGF-β1. Cell apoptosis and exhaustion were monitored at various time points.
- Human cell line-derived and patient-derived xenograft (CDX and PDX) models in severe immunodeficient mice were utilized to study the *in vivo* anti-tumor efficacy and preclinical safety profiles of SMART CAR-T cells.

Results

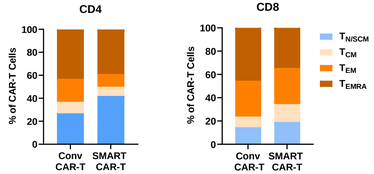
- SMART CAR-T cells and their conventional counterparts displayed comparable efficacy in short-term cytotoxicity assays against multiple tumor cell lines *in vitro*.
- Upon repeated stimulation with target cells, SMART CAR-T cells showed more potent and longer-lasting tumor-specific lysis than the conventional CAR-T. SMART CAR-T cells were more resistant to cell death.
- SMART CAR-T cells exhibited stronger and more durable tumoricidal activities in multiple xenograft mouse models.

Fig. 1 Expression of SMART switch inhibited TGF-β signaling pathway



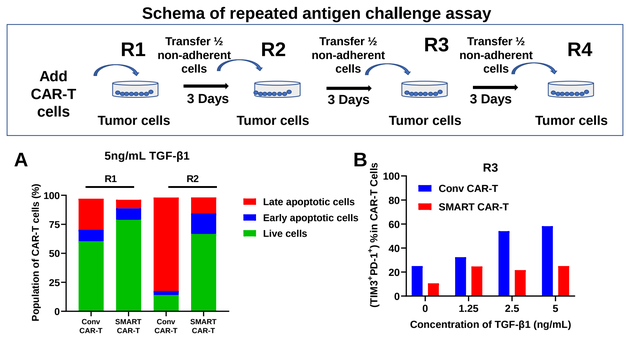
SMART CAR- or conventional CAR-expressing reporter 293T cell lines were treated with indicated levels of human TGF-β1. TGF-β-mediated signaling indicated by luciferase activity was measured after 24h. Similar results were observed for SMART CAR-reporter cells in response to TGF-β2 and TGF-β3.

Fig. 2 SMART CAR-T cells exhibited a younger phenotype



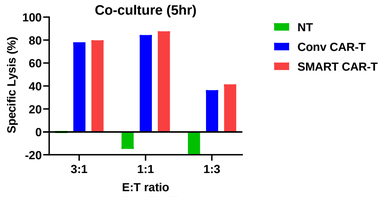
$CD4^+$ and $CD8^+$ SMART or conventional (conv) CAR-T cells were stained for memory surface makers. $T_{N/SCM}$, naïve or stem memory T cells; T_{CM}, central memory T cells; T_{EM}, effector memory T cells; T_{EMRA}, effector memory T cells expressing CD45RA.

Fig. 3 SMART CAR-T cells were insensitive to TGF-β-mediated apoptosis and exhaustion



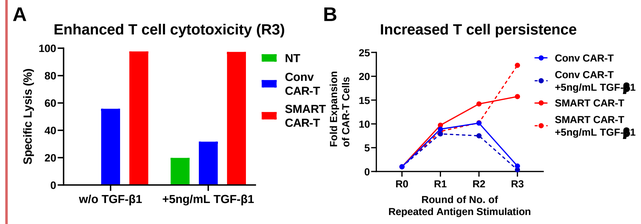
SMART or conventional (conv) CAR-T cells were repeatedly challenged with antigen-expressing target cells in TGF-β1-replete cultures. CAR-T cells were stained for (**A**) apoptotic markers (Annexin V and 7-AAD) in R1 and R2 or (**B**) exhaustion markers (TIM3 and PD-1) in R3.

Fig. 4 SMART CAR-T demonstrated uncompromised short-term cytotoxic capacities *in vitro*



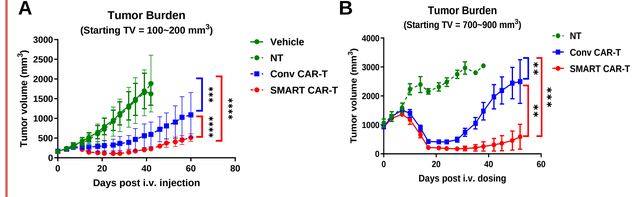
SMART or conventional (conv) CAR-T cells, or non-transduced T-cells (NT) were co-cultured with target antigen-expressing cells at the indicated ratios. Specific lysis of target cells was calculated 5hr later.

Fig. 5 SMART CAR-T cells showed longer-lasting tumor-specific lysis in multi-round killing assays



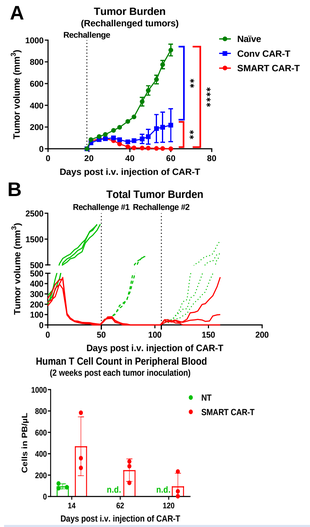
SMART or conventional (conv) CAR-T cells, or non-transduced T-cells (NT) were cultured as shown in **Fig. 3**, but at an E:T ratio of 1:3. (**A**) Specific lysis of tumor cells were quantified with luciferase-based imaging. (**B**) CAR-T cell expansion was determined by flow cytometry.

Fig. 6 SMART CAR-T cells were more efficacious than conventional CAR-T cells in CDX mouse models



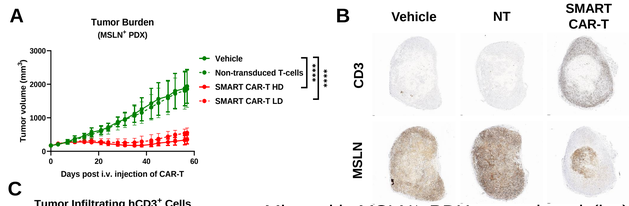
Immunodeficient mice with (**A**) regular or (**B**) large volumes of established MSLN-expressing tumors were dosed (i.v.) with SMART or conventional (conv) MSLN-targeting CAR-T cells on day 0. Animal body weights remained in the normal range throughout the experiments (data not shown). Mean±SD; n=5 per group in (**A**) or n=4~7 per group in (**B**). One-way ANOVA. **, p<0.01; ***, p<0.005; ****, p<0.001.

Fig. 7 SMART CAR-T cells were more durable in response to tumor rechallenges in CDX mouse models



Mice with target-expressing CDX previously dosed (i.v.) with SMART or conv CAR-T cells were rechallenged (s.c.) with the same tumor cell lines. (**A**) Mice were rechallenged with $MSLN^+$ tumor cells on day 19. Volumes of the rechallenged tumors are shown. (**B**) Mice were rechallenged with $CLDN18.2^+$ tumor cells on day 50 and day 106. Total tumor volumes of individual mice (top) and peripheral human T cell counts (bottom) 2 weeks post tumor inoculations are shown. Green solid lines, mice injected with NT cells; green dashed lines, naïve mice (batch #1); green dotted lines, naïve mice (batch #2). Mean±SD and n=4~5 per group in (**A**), or n=3 per group in (**B**). One-way ANOVA. **, p<0.01; ****, p<0.001.

Fig. 8 SMART CAR-T cells efficiently suppressed tumor growth in a PDX mouse model



Mice with $MSLN^+$ PDX were dosed (i.v.) with a high or low dose of SMART CAR-T cells. Tumor volumes (**A**), representative IHC results of extracted tumors on day 15 (**B**), and tumor infiltrating human $CD3^+$ cell counts (**C**) are shown. Mean±SD; n=4~6 per group in (**A**) or n=2 per group in (**B-C**). One-way ANOVA. ****, p<0.001.

Conclusions

SMART CAR-T cells comprising of dnTGFBR2 and a cytokine receptor signaling domain resisted the immunosuppressive TME and maintained long-term proliferation and cytotoxicity both *in vitro and in vivo*. The enhanced preclinical efficacy and safety profile of SMART CAR-T cells against solid tumors warrants further investigation in clinical trials.