Background: Chimeric antigen receptor T (CAR-T) cells have performed poorly in patients with advanced solid cancers. A critical hurdle for CAR-T cell efficacy is the immunosuppressive tumor microenvironment (TME). CAR-T cell function is profoundly inhibited by transforming growth factor-beta (TGF-β) enriched in the TME. Current strategies to address this issue focus on the abrogation of TGF-β signaling. However, these strategies can have toxic side effects due to an imbalance in T cell homeostasis induced by complete blockade of TGF-β signaling.

Methods: We engineered a novel chimeric switch receptor comprising a TGF-β receptor domain and a T cell costimulatory domain to initiate T cell costimulation upon TGF-β binding. To maintain T cell homeostasis balance, we optimized the intracellular sequence of the switch receptor to reduce its effect on endogenous TGF-β signaling and decrease the potential side effects. The switch receptor was co-expressed in LeY-specific CAR-T cells (switch CAR-T). The in vitro function of switch CAR-T cells were investigated in the presence of TGF-β. We also performed RNAseq to explore genes involved in the switch receptor activation. Finally, we demonstrated the anti-tumor efficacy of switch CAR-T cells in vivo.

Results: In the presence of TGF-β, switch CAR-T cells showed significantly enhanced cytotoxicity and higher levels of TNF secretion compared with conventional CAR-T cells (figure 1). In the presence of both CAR stimulation and TGF-β, but not TGF-β alone, switch CAR-T cells also had significantly higher proliferation and increased mitochondrial biogenesis, indicating an antigen-specific response. Furthermore, both switch and conventional CAR-T cells had equivalent levels of SMAD2 phosphorylation in response to TGF-β, indicating that switch CAR-T cells retained endogenous TGF-β signaling (figure 2). RNAseq analysis showed that switch CAR-T cells have a unique gene expression profile in response to CAR stimulation and TGF-β. Finally, tumor-bearing mice treated with switch CAR-T cells showed significantly better tumor control compared with conventional CAR-T cells (figure 3). This finding was associated with decreased TGF-β and increased IFN-γ levels within the tumor.

Conclusions: The novel switch receptor activated CAR-T cells in response to immunosuppressive TGF-β leading to improved CAR-T cell function. This effect also required CAR-T cell activation through CAR-stimulation, suggesting a tumor-specific response. Furthermore, these switch CAR-T cells provided improved in vitro anti-tumor control. By fine-tuning the intracellular sequence of the switch receptor, we also successfully retained the endogenous TGF-β signaling. Switch CAR-T cells can preserve this important homeostatic mechanism and will be safe to use in the clinic.