THE DEVELOPMENT OF 'CHIMERIC CD3E FUSION PROTEIN' AND 'ANTI-CD3-BASED BISPECIFIC T CELL ACTIVATING ELEMENT' ENGINEERED T (CAB-T) CELLS FOR THE TREATMENT OF SOLID MALIGNANCIES

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Background Cancer immunotherapy has achieved unprecedented success in the complete remission of hematological tumors. However, serious or even fatal clinical side-effects have been associated with CAR-T therapies to solid tumors, which mainly include cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), macrophage activation syndrome, etc. Furthermore, CAR-T therapies have not yet demonstrated significant clinical efficacy for the treatment of solid tumors. Here, we present a novel T cell therapeutic platform: a Chimeric CD3e fusion protein and anti-CD3-based bispecific T cell activating element (BiTA) engineered T (CAB-T) cells, which target tumor antigens via the secretion of BiTAs that act independently of MHC interactions. Upon BiTA secretion, CAB-T cells can simultaneously achieve anti-tumor cytotoxic effects from the CAB-T cells and simultaneously activate bystander T cells.

Methods CAB-T cells were generated by co-expressing a chimeric CD3e fusion protein and an anti-CD3-based bispecific T cell activating element. The chimeric CD3e contains the extracellular domain of CD3e, a CD8 transmembrane domain, 4-1BB costimulatory domain, CD3z T cell activation domain and a FLAG tag, while the BiTA element includes a tumor antigen targeting domain fused with an anti-CD3 scFv domain and a 6x His-tag. CAR-T cells were generated as a control. Cytokine release activity, T cell activation and exhaustion markers, T cell killing activity and T cell differentiation stages were analysed. We also tested their tumor growth inhibition activity, peripheral and tumor tissue distribution, and their safety-profiles in humanized mouse models.

Results CAB-T cells have similar or better in vitro killing activity compared with their CAR-T counterparts, with lower levels of cytokine release (IL-2 and IFN γ). CAB-T cells also showed lower levels of exhaustion markers (PD-1, LAG-3 and TIM-3), and higher ratios of naive/Tscm and Tcm T cell populations, after co-culture with their target tumor cells (48h). In in vivo studies, CAIX CAB-T and HER2 CAB-T showed superior anti-tumor efficacy and tumor tissue infiltration activity over their corresponding CAR-T cells. For CLDN18.2 CAB-T cells, similar in vivo anti-tumor efficacy was observed compared to CAR-T after T cell infusion, but blood glucose reduction and animal mortality was observed in the mice administered with CAR-T cells.

Conclusions The advantages of CAB-T in in vitro and in vivo studies may result from TCR signal activation of both the engineered CAB-T cells and the non-engineered bystander T cells via cross-bridging by the secreted BiTA molecules, thus offering superior anti-tumor efficacy with a potential better safety-profile compared to conventional CAR-T platforms.

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