ARMORED CAR T CELLS SECRETING 4-1BB LIGAND CROSSLINKED TO PD-1 CHECKPOINT INHIBITOR FOR ENHANCED SOLID TUMOR EFFICACY

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Background Chimeric antigen receptor (CAR) T cell therapy has transformed the treatment of hematological malignancies but has yet to achieve similar success in solid tumors due to a lack of persistence and function in the tumor microenvironment. We previously reported the augmentation CAR T cell therapy in an engineered solid tumor model through the secretion of anti-PD-1 scFv, as shown by enhanced CAR T cell antitumor efficacy, expansion, and vitality.¹ We have since matured the platform to create a superior cellular product – CAR T cells secreting single-chain trimeric 4-1BB ligand cross-linked to anti-PD-1 scFv (α PD1-41BBL). 4-1BB signaling promotes cytotoxic T lymphocytes proliferation and survival but targeting 4-1BB with agonist antibodies in the clinic has been hindered by low antitumor activity and high toxicity. CAR T cells using 4-1BB endodomain for costimulatory signals have demonstrated milder anti-tumor response and longer persistence compared to CAR T cells costimulated by CD28 endodomain. We have, for the first time, engineered CAR T cells to secrete a fusion protein containing the soluble trimeric 4-1BB ligand.

Methods We hypothesized that crosslinking the current anti-PD-1 scFv with 4-1BB ligand would provide additional benefits to CAR T cells and is potentially of translational value in the management of tumors resistant to PD-1 blockade due to lack of T cell function. Therefore, we engineered CAR T cells to secrete a novel immunomodulatory fusion protein consisting of anti-PD-1 scFv crosslinked to a single-chain format of trimeric 4-1BB ligand, in which three extracellular domain units of 41BBL are connected with polypeptide linkers. The CAR T cells were then characterized in vitro and subcutaneous tumor models.

Results In vitro and in vivo, CAR19. α PD1-41BBL T cells exhibited reduced inhibitory receptor upregulation, enhanced persistence and proliferation, and a less differentiated memory status compared to CAR T cells without additional 4-1BB:4-1BBL costimulation. Accordingly, CAR19. α PD1-41BBL T cell-treated mice displayed significantly improved tumor growth control and overall survival. Spurred on by our preclinical success targeting CD19 as a model antigen, we produced mesothelin-targeting CAR T cells and confirmed the enhanced solid tumor efficacy and persistence of α PD1-41BBL secreting CAR T cells.

Conclusions Given the significantly better therapeutic efficacy of α PD1-41BBL expressing T cells over α PD1 expressing T cells, we believe that it is of high translational value to adopt secretion of α PD1-41BBL fusion protein to improve CAR T cell solid tumor efficacy, especially given the large number of patients that are PD1/PD-L1 therapy resistant.

REFERENCES

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