CAR-TS ARMORED WITH SMALL MOLECULE-REGULATED IL12 OR CD40L CASSETTES FOR ENHANCED ACTIVITY AGAINST SOLID TUMORS

ABSTRACT
Adaptive cell therapy with chimeric antigen receptor (CAR) modified T cells has demonstrated remarkable clinical efficacy in the treatment of certain B cell malignancies, and more recently in multiple myeloma. However, CAR-T therapy has been less successful in treating solid tumors due to multiple obstacles, including the lack of robust CAR-T cell expansion, the immunosuppressive tumor microenvironment, and tumor escape due to the loss of target antigen. Engineering CAR-T cells to produce immunomodulatory factors such as IL12 and Cluster of Differentiation 40 Ligand (CD40L) has been shown to enhance functional activity by driving T cell expansion, conferring resistance to immunosuppression, improving antigen presentation, and inducing antigen spread. However, clinical utility of both IL12 and activators of the CD40L signaling pathway have been limited by toxicity associated with their potent pharmacological activities. We describe here the implementation of ligand-controlled regulation of IL12 and CD40L in vivo and in vivo in engineered primary human T cells via the use of destabilization domains (DD) technology. DDs are small protein domains that are misfolded and inherently unstable in the cell, but which can be reversibly stabilized by the binding of specific pharmacological agents. This condition stability of DDs can be reversibly controlled to any protein of interest by fusing it to the DD, thus providing fine-tuned, reversible regulation of protein function and activity. We show the transcription of human T cells with either IL12-DD or CD40L-DD fusion construct yields levels in the basal state and a rapid, dose-dependent induction of IL12 or CD40L, protein in the presence of ligand. Moreover, time-dependent, dose-dependent induction of either factor from CAR-T cells can be achieved in mice by oral dosing. Providing precise tuning of the timing and level of expression of these immunomodulatory factors in CAR-T cells may effectively enhance safety and therapeutic efficacy, in particular against solid tumor malignancies.

Figure 1: The Dramatic Success of CAR-Ts has Reinvigorated the Field of Cell Therapy but Substantial Challenges Remain

Figure 2: Small Molecule Regulated Protein Expression Using Destabilizing Domains

Figure 3: CD40L Expression Enhances Anti-Tumor Efficacy of CD19 CAR-T Cells in Acute Lymphoblastic Leukemia Model

Figure 4: Regulation of CD40L Levels and Function in vitro and in vivo

Figure 5: Regulation of CD40L with a Clinically Translatable DD

Figure 6: IL12 Improves Expansion and Efficacy of CD19 CAR in vivo

Figure 7: Drug-dependent Regulation of IL12 in CAR-T Cells in vitro and in vivo with a Clinically Translatable DD

Figure 8: Expression and Regulation of a Membrane-bound Form of IL12 on T Cells

SUMMARY
- Obsidian’s Destabilization Domain (DD) technology provides titratable and reversible regulation of protein levels via exogenous small molecule dosing.
- Multiple human DD families have been created, each of which can be regulated by distinct FDA approved drugs.
- We have demonstrated titratable small-molecule regulation of both CD40L and IL12 expression and activity in human T cells in vitro and in vivo.
- With precise control over levels of immunomodulatory factors produced, we are building next generation CAR-T cell products for enhanced efficacy against solid tumors and more favorable safety profiles.

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