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

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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 myelomas. However, barriers to the clinical translation of CAR-T therapy have been associated with the propensity to lose targeted antigen expression, resulting in tumor escape due to the loss of targeted antigen. The most effective CAR-T cells produce immunomodulatory factors such as interleukin-2 (IL2) and Cluster of Differentiation (CD) ligands (DCLLs) via transduction of human primary T cells via the use of destabilizing 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 approved pharmacologic agents. This conditional stability of DDs can be readily conferred on their potent pharmacological activities. We describe here the implementation of ligand-controlled regulation of IL12 and CD40L expression and activity in human Tumor Antigen Escape and immunosuppressive tumor microenvironment, and tumor escape due to the loss of targeted antigen. We have demonstrated titratable small molecule regulation of both CD40L and IL12 expression and activity in human CAR-T cells in Acute Lymphoblastic Leukemia Model. CD19 CAR-T cells infused into Nalm6 tumor-bearing mice. Mice were dosed orally at the indicated concentrations of the stabilizing ligand, acetazolamide, as a DD selection campaign. Individual domain was fused to GFP and subjected to persistence without exhaustion. Exogenous regulation via safe oral drugs enabled expression of IL12 on T cells in vivo without toxicity or excessive lymphoproliferation. 

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

- A: Activated 106 T cells were transduced with CD19 CAR or CD19 CAR and IL12-042 (IL12-042 Bazedoxifene) in the presence of DOX or in the absence of DOX. DOX was added to the cultured media at 10 μg/ml 12 h before addition of intracellular staining. Intracellular staining was performed using mouse anti-human IL12 peridinin chlorophyll protein (PerCP) and CD19 phycoerythrin (PE). T cells were acquired using a BD LSR II and the data analyzed using FACS DIVA (Becton Dickinson, San Jose) software. 

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

- A: Naïve T cells were transduced with CD40L-042 in the presence or absence of DOX and injected intravenously into NSG mice. Tumor burden was quantified using luciferase readout 3 days after tumor implantation (n=8/group). Tumor burden was quantified by bioluminescence analysis. B: Naïve T cells were transduced with CD40L-042 in the presence of DOX and injected intravenously into NSG mice. Tumor burden was quantified by bioluminescence analysis (n=8/group). Tumor burden was quantified by bioluminescence analysis. C: Naïve T cells were transduced with CD40L-042 in the presence or absence of DOX and injected intravenously into NSG mice. Tumor burden was quantified by bioluminescence analysis (n=8/group). Tumor burden was quantified by bioluminescence analysis.

SUMMARY
- Obsidian’s Destabilizing 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.