POTENT T CELL COSTIMULATION MEDIATED BY A NOVEL COSTIMULATORY ANTIGEN RECEPTOR (COSTAR) WITH DUAL CD28/CD40 SIGNALING DOMAINS TO IMPROVE ADOPTIVE CELL THERAPIES

Martina Sykora, Leyuan Bao, Cynthia Chauvin-Fleurence, Milena Kalaitidou, Michelle Le Brocq, Robert Hawkins, Gray Kueberuwa, John Bridgeman*, Rubén Alvarez-Rodríguez. Instil Bio, Inc., Dallas, TX, USA

Background Costimulatory signals are a critical component to mount an effective anti-tumor response. Prolonged TCR stimulation in the absence of costimulatory signals can lead to T cell anergy and dysfunction. The tumor microenvironment evades immune surveillance by creating a suppressive environment characterized by high expression of coinhibitory receptors and lack of antigen presenting cells providing costimulatory signals. Third-generation CAR-T therapies containing two costimulatory domains have improved performance in animal models over second-generation CAR-T designs containing a single costimulatory domain, indicating the additive nature of some of these signaling pathways. Here we describe a synthetic CoStAR containing dual CD28 and CD40 domains designed to enhance the activity of T cells in the context of adoptive cell therapy.

Methods Healthy donor T cells were transduced with CoStAR receptors targeting the tumor-associated antigen CEA. Anti-CEA CoStAR T cells were then challenged with CEA+ tumor cells expressing a membrane anchored anti-CD3 antibody to provide signal 1 through TCR/CD3 complex cross linking. CoStAR signaling domains consisted of CD28 alone or a fusion of CD28 and CD40. Activity was measured by quantifying expression of activation markers, cytokine secretion, proliferation, and analysis of gene expression profiles.

Results Anti-CEA CoStAR-expressing T cells containing both CD28 and CD40 domains displayed increased cell activation, proliferation (>400-fold improvement over a 42-day serial coculture), and cytokine expression (eg, IL-2, ~14-fold; TNF α , ~2-fold) when compared to T cells expressing either CD28-only CoStAR or no CoStAR. Immunosuppressive cytokines (eg, IL-10 and IL-4) did not increase beyond levels observed with CD28-only CoStAR.

Conclusions The combination of CD28 and CD40 in the synthetic costimulatory antigen receptor CoStAR gives rise to superior T cell activity when compared to receptors consisting of a CD28 domain alone, including improvement in secretion of pro-inflammatory cytokines and long-term proliferative capacity. The novel design of the CoStAR molecule, including CD28 and CD40 signaling motifs, may further improve the performance of T-cell-based therapies, including tumor-infiltrating lymphocytes (TIL). Similar observations with an analogous anti-FOLR1 CoStAR have been observed, indicating broad applicability of the CoStAR platform across target molecules and tumor indications. Instil plans to initiate its first-in-human clinical trial with ITIL-306, an investigational anti-FOLR1 CoStAR TIL product in 1H 2022.

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