392 ENGINEERING T CELLS TO CATALYZE ENDOGENOUS ANTICANCER IMMUNITY

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Background Pancreatic ductal adenocarcinoma (PDAC) is a severe disease, with a 5-year survival rate of only 12%. Disease-targeted immune cells are increasingly employed in adoptive cell therapy (ACT) of various cancers and infectious diseases, and represent an attractive option for PDAC therapy. T cells can replicate within a patient to robustly generate tumor-specific cells that actively migrate throughout the body to eradicate disease. However, the PDAC tumor microenvironment (TME) provides several challenges for T and other immune cells, including hypoxia, limited metabolic substrates, and upregulated inhibitory ligand expression. Costimulatory signals, particularly those in the tumor necrosis factor receptor family (TNFR), can initiate distinct gene expression programs in immune cells to counter inhibitory obstacles and enhance antitumor functions. TNFR signals enhance the antitumor functions of multiple types of immune cells, including conversion of pro- to anti-cancer macrophages, maturation of dendritic cells (DC), and exhaustion resistance in T cells. We have developed novel membrane-bound fusion proteins, Dual Costimulatory Receptors (DCRs), that combine a CD40L ligand ectodomain (to elicit an endogenous anticancer response) with a costimulatory endodomain (to enhance T cell robustness and function). We hypothesized that this approach would address two critical objectives: to enable T cells to attack cancer more powerfully and durably AND to safely deliver activation signals specifically to other immune cells in the TME and lymphoid organs, where tumor-targeted T cells naturally localize.

Methods We evaluated CD40L DCRs with novel *in vitro* assays that we developed to reliably predict *in vivo*antitumor therapeutic efficacy. DCR-T cells were evaluated for metabolic reprogramming and challenged with tumor in hypoxic and nutrient-limited conditions. T cells were also assessed for exhaustion resistance with multiple stimulation and serial killing assays. DCR-T cells were co-cultured with immature DC to determine cell-extrinsic functionality.

Results CD40L DCR-T cells showed significantly increased function in assays measuring T cell proliferation, cytotoxicity, cytokine production, and exhaustion resistance. Co-culture of human DCR-T cells with immature DCs resulted in DC maturation, including upregulation of costimulatory ligand and MHC molecules. *In vivo* studies with KPC-derived cell lines demonstrated enhanced therapeutic efficacy and altered DC programming with CD40L DCR-T cell therapy.

Conclusions Here we report a first-in-class fusion protein strategy that enhances both engineered T and endogenous immune cell antitumor function, supporting clinical translation.

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