238 POTENTIATING CAR T CELL THERAPY THROUGH SYNTHETIC IL-9R SIGNALING

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Background Chimeric antigen receptor (CAR) T cell therapy has shown limited success in treating solid tumors. One approach for improving antitumor efficacy in solid tumors is to equip CAR T cells with synthetic cytokine receptors to enhance cell-autonomous function. Our lab recently published that an orthologous IL-2/IL-9 receptor could augment antitumor immunity.[1] Here we have engineered mouse and human CAR T cells targeting the tumor antigen mesothelin to express an authentic IL-9 receptor (IL-9R), providing them with an IL-9 signal (CAR-IL9R T cells).

Methods For *in vitro* studies, T cells were isolated from mouse splenocytes or human donors, activated, transduced, expanded and harvested for *in vitro* assays. For *in vivo* studies, we established a syngeneic model in which C57BL/6 mice were implanted with PDA7940b, a pancreatic ductal adenocarcinoma cell line derived from KPC mice. Animals were then treated with intravenous injection of CAR-IL9R T cells and intratumoral injection of an adenovirus encoding murine IL-9 (Ad-mIL9). Control groups included mice treated with CAR T cells, CAR-IL9R T cells, or Ad-mIL9. At the time of peak CAR T cell expansion, tumors were collected and analyzed by flow cytometry and immunohistochemistry. To determine the antitumor efficacy of human IL-9 signaling CAR T cells, we established a xenograft flank tumor model by implanting AsPC-1 tumor cells in NSG mice and treated them with CAR-IL9R T cells combined with an adenovirus encoding human IL-9 (Ad-hIL9).

Results CAR-IL9R T cells displayed a unique STAT signaling profile, a shift towards a stem cell-like memory T cell phenotype, and improved effector function *in vitro* compared with control CAR T cells. In our syngeneic model, tumor progression was significantly reduced, overall survival was improved, and higher numbers of CAR T cells were detected in tumors harvested from mice treated with CAR-IL9R + Ad-mIL9 compared with control groups. No significant changes in the tumor microenvironment were observed across the treatment groups, suggesting that the improved antitumor efficacy of IL-9 signaling CAR T cells is largely due to T cell-intrinsic mechanisms. Supporting this hypothesis, treatment with CAR-IL9R T cells + Ad-hIL9 significantly improved antitumor efficacy in immunodeficient NSG mice. Ongoing studies seek to understand the mechanisms underlying the gain-of-function of IL-9 signaling CAR T cells.

Conclusions CAR T cells provided with an IL-9 signal display a unique T cell phenotype combining the beneficial functional characteristics of stem cell-like memory and effector T cells, leading to enhanced *in vivo* antitumor activity supported by cell-intrinsic mechanisms.

REFERENCE

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