

NANOSCALE, ANTIGEN-DEPENDENT, IL-12 DELIVERY BY CAR T CELLS PLUS PD-L1 BLOCKADE FOR CANCER TREATMENT

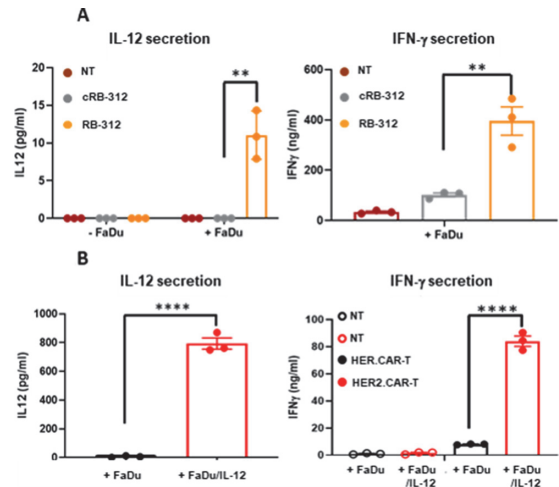
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Background Interleukin(IL)-12 activates T cells pivoting the switch that turns lingering inflammation into acute inflammation and cancer rejection. However, its clinical utilization is limited by severe systemic toxicity. IL-12 is a potent inducer of PD-1 expression in T cells. Here, we present a conditional, antigen-dependent, non-editing CRISPR-activation (CRISPRa) circuit (RB-312) that delivers nanoscale doses of IL-12 for autocrine activation of CAR-T cells. RB-312 was also tested in combination with PD-L1 blocking antibody (atezolizumab).

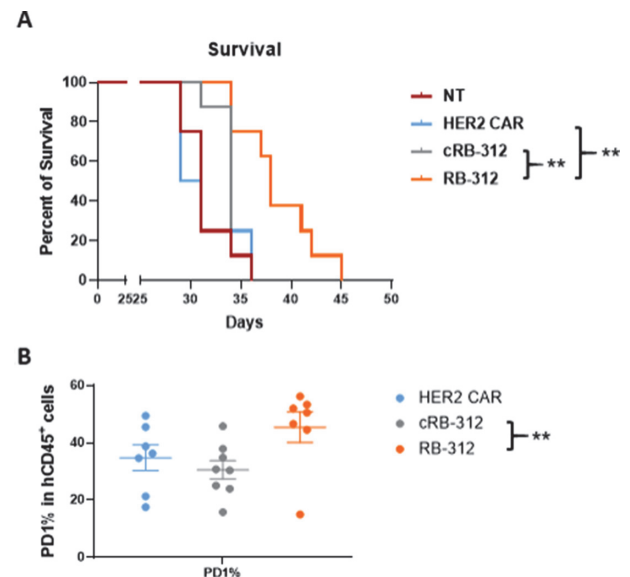
Methods RB-312 is a CAR T cell engineered to express the IL-12 heterodimer via conditional transcription of its two endogenous subunits p35 and p40. The circuit includes two lentiviral constructs with one encoding HER2-specific chimeric antigen receptor and two sgRNAs targeting IL-12A or IL-12B and the other encoding linker for activation of T cells, complexed to dead Cas9 (dCas9)-VP64-p65-Rta transcriptional activator (VPR) (LdCV). Activation of CAR allows the release of dCas9 for nuclear localization and hence conditionally and reversibly induces the secretion of IL-12 p70 heterodimer.

Results RB-312 induced low concentrations of IL-12 upon exposure to HER2+ FaDu cancer cells engineered to overexpress PD-L1 and this resulted in significantly enhanced production of IFN- γ , cytotoxicity and CAR-T proliferation (figure 1A). These effects were comparable to co-culturing conventional HER2 CAR with FaDu cells modified to express high doses of IL-12 (figure 1B). In vivo administration of RB-312 significantly enhanced survival of mice carrying FaDu xenografts compared to mice treated with the respective conventional HER2 CAR or cRB-312 (control lacking the IL-12 sgRNAs, figure 2A). RB-312 induced a strong upregulation of PD-1 in CAR-T cells in vivo (figure 2B). The critical role of the PD-1/PD-L1 interaction was demonstrated in vitro by comparing RB-312 proliferation when exposed to FaDu overexpressing PD-L1 or PD-L1 knock out cells (figure 3A). Indeed, combined treatment of RB-312 and atezolizumab resulted in significant reduction in tumor growth (figure 3B and C) and significantly enhanced survival (figure 3D).

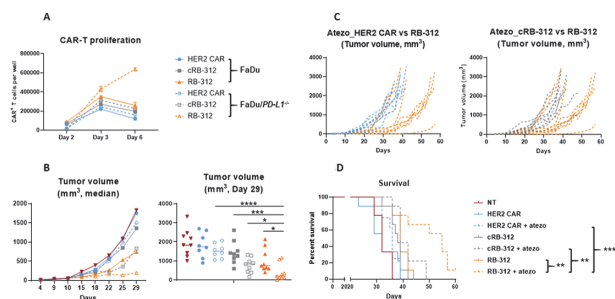
Conclusions We concluded that addition of a Th1 polarizing component such as IL-12 exponentially increases the efficacy of reprogrammed CAR-T cells by combining enhancement of effector functions to cellular fitness. The autocrine effects of nanoscale IL-12 production limit the risk of off-tumor leakage and systemic toxicity. Here, we tested the combination of PD-1/PD-L1 blockade with IL-12-induced CAR-T cell activation demonstrated dramatic synergistic effects. We are currently evaluating the intrinsic combination of IL-12 delivery and PD-L1 resistance for the next generation of RB-312 product eliminating the need for systemic checkpoint blockade.



Abstract 153 Figure 1 Conditional autocrine release of nanoscale-dose p70/IL-12 by RB-312 resulting in enhanced IFN- γ production in vitro after three days of exposure to HER2+ FaDu cells (figure 1A), and the level of IFN- γ production was comparable to co-culturing conventional HER2-specific CAR-T cells with a modified FaDu cell line engineered to constitutively express high doses of IL-12 (FaDu/IL-12, figure 1B)



Abstract 153 Figure 2 Intra-tumoral administration of RB-312 extended survival in mice carrying FaDu xenografts compared to NT (non-transduced T cells), HER2 CAR (conventional HER2 CAR-T cells) and cRB-312 CAR-T cells missing the sgRNAs for the two IL-12 subunits (figure 2A). Analysis of necropsy material demonstrated that PD-1 expression was dramatically increased in RB-312 compared with the respective control cRB-312 (figure 2B)



Abstract 153 Figure 3 RB-312 cellular function in vivo. PD-L1 expression by FaDu cell lines is a critical mechanism of repression of RB-312 function. In vitro CAR-T proliferation of RB-312 upon stimulation with FaDu tumor cells (orange solid lines) or FaDu/PD-L1 knockout tumor cells (orange dashed lines) over 6-day time course (figure 3A). In vivo efficacy of intra-tumoral RB-312 against FaDu tumor cells with (orange solid lines) or without (orange dashed lines) addition of PD-L1 blocking antibody atezolizumab (administered intravenously at 5 mg/kg twice per week), as shown by tumor growth followed till day 29 and scatter plot at day 29 (figure 3B), tumor growth spider plots (figure 3C) and Kaplan-Meier survival curve (figure 3D)

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