CONTEXT-DEPENDENT REVERSIBLE MODULATION OF cJUN EXPRESSION BY CAR T CELLS FOR CANCER TREATMENT

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Background Overexpression of canonical AP-1 factor cJUN was shown to prevent CAR T cell exhaustion and improve anti-tumor potency in vivo (1). However, its clinical utilization is limited by potential for transformation and oncogenic risk. Here, we present a conditional, antigen-dependent, non-editing CRISPR-activation (CRISPRa) circuit (RB-339) that delivers context-dependent upregulation of endogenous cJUN increasing CAR-T cell resistance to exhaustion.

Methods RB-339 is a CAR T cell engineered to conditionally turn on the transcription of the cJUN endogenous gene. The circuit includes a lentiviral construct encoding an anti-HER2 (4D5) single chain variable fragment, with CD28 and CD3 ζ co-stimulatory domains linked to a tobacco etch virus (TEV) protease and a single guide RNA (sgRNA) targeting the promoter region of cJUN. A second construct encodes linker for activation of T cells, complexed to nuclease-deactivated/dead Cas9 (dCas9)-VP64-p65-Rta transcriptional activator (VPR) via a TEV-cleavable linker (LdCV). Activation of CAR allows the release of dCas9 for nuclear localization and conditionally and reversibly induces the expression of cJUN. RB-339 was compared in vitro to control (cRB-339, lacking the cJUN sgRNA) and CAR-T cells engineered to constitutively express cJun.

Results RB-339 induced cJUN upregulation upon stimulation with HER2-coated beads and this resulted in significantly elevated expression over a 6-day time course compared to the control cRB-339 (figure 1A-B). When HER2-coated beads were removed at day 3, cJUN expression returned to baseline parallel to cRB-339. The conditional upregulation of cJUN in RB-339 contrasted with the constitutive overexpression in the transgene carrying cells that was irrespective of antigen stimulation (figure 1C). Upon exposure to HER2+ FaDu cancer cells, RB-339 peaked at day 2 and declined afterwards when FaDu cells were killed at day 3; cJUN increased again at day 4 upon restimulation with FaDu cells at day 3 (figure 2). Such a dynamic induction of cJUN resulted in significantly enhanced CAR-T cells proliferation in RB-339 compared to the respective conventional CAR-T cells or cRB-339 (figure 3).

Abstract 154 Figure 1 Conditional upregulation of cJUN by RB-339 in vitro. RB-339 and its control cRB-339 were stimulated by HER2-coated or BSA-coated beads for either six days or three days followed

by removal of beads at day 3 (figure 1A-B). Intracellular expression of cJUN was detected at indicated time points. Intracellular cJUN expression in overexpressed cJUN-CAR (figure 1C)

Abstract 154 Figure 2 Kinetics of cJUN upregulation in RB-339 upon exposure to HER2+ FaDu tumor cells. RB-339 and its control cRB-339 were stimulated by FaDu tumor cells for six days with or without restimulation at day 3

Abstract 154 Figure 3 Conditional upregulation of cJUN resulted in enhanced CAR-T proliferation in RB-339 in vitro after 6-day co-culture with FaDu tumor cells, compared to conventional HER2 CAR or cRB-339

Conclusions We conclude that CAR-T engineered to conditionally express the canonical AP-1 factor cJUN increases expansion potential similarly to CAR-T cells engineered to constitutively express the cJun transgene. However, the context-dependent upregulation of cJUN limits the risk of oncogenic transformation. We are currently combining inducible and reversible cJUN and IL-12 upregulation for the generation of the next RB-339 product.

REFERENCE

1. Lynn RC, Weber EW, et al. c-Jun overexpression in CAR T cells induces exhaustion resistance. *Nature* 2019; **576**(7786):293–300.

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