Background Chimeric Antigen Receptor T-cells (CAR-T) rely on antigen-dependent activation to generate anti-tumor responses in vivo. However, excess activation leads to therapeutic failure through exhaustion and depletion of CAR-T cells. Thus, control of CAR-T cell activation in vivo is desired to promote durable effector function while limiting maturation. We hypothesize that c-Jun N-terminal Kinase (JNK) signaling contributes to the over-activation of CAR-T cells in vivo, and that controlling JNK activity can improve the efficacy and durability of CAR-T cell therapy.

Methods A HER2-targeting CAR designed from trastuzumab was used in the study. CAR-T cells were generated from human peripheral blood mononuclear cells and used to assess the impact of JNK signaling on their functional longevity. JNK activity was suppressed with pharmacologic inhibitor SP600125 or shRNA knockdown of JNK 1 and 2. NFAT-responsive reporter cells were used to quantify NFAT activity in response to T-cell stimulation. The activation and cytokine production of CAR-T cells was assessed by flow cytometry following stimulation with HER2+ cells. Maintenance of cytotoxicity and exhaustion marker expression was assessed by a serial killing assay using SKOV3 ovarian cancer cells as the target. In vivo anti-tumor efficacy was evaluated in a xenograft model with SKOV3 cells, with daily intraperitoneal administration of SP600125.

Results JNK suppression with SP600125 or shRNA weakened the NFAT-dependent reporter responses upon T-cell stimulation. Correspondingly, JNK suppression also decreased antigen-dependent upregulation of activation markers (CD25, CD69, and CD137) by CAR-T cells. Though CAR-T interferon gamma production was reduced by SP600125, CAR-T cells maintained their ability to produce interferon gamma in response to antigen stimulation. JNK suppression had no impact on the short-term cytotoxicity of CAR-T cells. JNK suppression prolonged cytotoxicity and decreased upregulation of exhaustion markers in the in vitro serial challenge model. JNK suppression also showed prolonged anti-tumor activity in the xenograft model.

Conclusions We have identified excess JNK activation as a putative signal preferentially driving T-cell exhaustion over effector function. These results suggest that optimal control of JNK activation in CAR-T cells is a viable strategy for improving the efficacy of CAR-T cell therapy by extending the effective life of the adoptive cells.

Ethics Approval The study was approved by the UAB Institutional Animal Care and Use Committee (IACUC) under IACUC21938.