ineffective against DIPG and overcome this roadblock by improving the immune synapse (IS), or the interaction between tumor and CAR T cells.

METHODS: In this study, we targeted the tumor associated antigen B7-H3 by generating B7-H3 specific CAR T cells with CD28 signaling domain. We compared *in vitro* and *in vivo* antitumor response of CAR T cells against patient derived DIPGs and non-DIPG brain tumors. We contrasted the IS quality in DIPGs against other brain tumors by confocal microscopy. We further generated IS-enhanced CAR T cells by knocking out RASA2 and compared their performance against control KO in terms of IS-quality, killing kinetics, expansion, cytokine secretion, and *in vivo* tumor control. RESULTS: Our data shows that CAR T cells have potent *in vitro* antitumor activity but fail *in vivo* against DIPGs which correlates with the formation of deficient IS, reflected by small synapses and poor calcium flux and cell polarity. RASA2 deletion in CAR T cells resulted in improved IS quality evidenced by bigger synapses, increased calcium flux, and CAR clustering upon antigen activation. In concordance, RASA2 KO CAR T cells elicited increased expansion, cytokine secretion, and *in vivo* antitumor activity against DIPGs compared to control KO. CONCLUSIONS: This study shows that poor IS formation contributes to CAR T cell failure against DIPGs. Enhancing the IS formation in CAR T cells improves antitumor activity against DIPGs and should be considered for clinical translation.

BACKGROUND: Diffuse intrinsic pontine gliomas (DIPGs) are highly lethal pediatric brain tumors and a leading cause of cancer-related death in children. There is currently no cure for DIPG, highlighting an urgent need for novel therapeutics. Chimeric antigen receptor (CAR)-T-cell therapy offers great promise for DIPG treatment; however, several limitations need to be addressed. The goal of this study is to identify why CAR T cells are