259 DISCOVERY OF SYNTHETIC SIGNALING PATHWAY RECEPTORS THAT INCREASE THE POTENCY OF CAR-T CELLS THROUGH OPTIMIZED STAT ACTIVITY

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Background Chimeric antigen receptor (CAR) T cells have limited clinical efficacy in solid tumors due to CAR-T dysfunction induced by chronic antigen stimulation and suppressive signals within the tumor microenvironment. Cytokine-mediated signaling through the Janus-kinase signal transducer and activator of transcription (JAK/STAT) pathway has been shown to regulate T cell differentiation, and increase effector function and persistence. We hypothesized that synthetic biology approaches could be deployed to identify synthetic receptors that improve therapeutic T cell function via regulation of specific JAK/STAT activity.

Methods A library of synthetic receptors designed to engage constitutive JAK/STAT signaling in the absence of external ligands – termed Synthetic Pathway Activators (SPAs) – was developed and screened for the ability to enhance antitumor activity of engineered CAR-T cells. We expressed the SPA library in Integrated Circuit T cells (ICTs) which are engineered T cells that express a logic gate and are generated via non-viral CRISPR-mediated transgene knock-in. We measured cytotoxicity in acute and chronic tumor challenge assays, cytokine production, STAT phosphorylation profiles, and effector/memory phenotyping via flow cytometry. Logic gate constructs expressing lead SPAs were subsequently tested in murine xenograft tumor models to assess antitumor efficacy and pharmacokinetics.

Results Certain Synthetic Pathway Activators (termed class I SPAs) demonstrated increased antitumor efficacy in chronic tumor challenge assays *in vitro*, retained effector function, and maintained markers of stemness upon chronic antigen exposure. This improved antitumor efficacy in vitro translated to improved cell-expansion and potency in xenograft solid tumor models: SPA-expressing cells clear tumors at significantly lower doses than control ICT cells. Importantly, despite their increased proliferative potential, ICTs that express SPAs do not exhibit cytokine-independent outgrowth and contract following tumor clearance in vivo.

Conclusions We have developed a class of SPAs that engage constitutive STAT signaling and significantly enhance the antitumor activity of therapeutic T cells in preclinical assays. SPA-expressing T cells exhibit increased expansion and retention of effector function in the presence of chronic antigen, leading to complete clearance of large xenograft tumors at very low T cell doses. Our lead class I SPA has been incorporated into AB-2100, an Integrated Circuit T cell drug candidate designed to treat clear cell renal carcinoma (ccRCC).

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