Background Chimeric antigen receptor (CAR) cell therapy for solid tumors is hampered by the immunosuppressive tumor microenvironment (TME), which can inhibit the function of endogenous and therapeutic immune cells, as well as a paucity of targets. The use of potent immunomodulators to transform the TME, such as interleukin (IL)-12, is limited by the need for regulation to avoid systemic toxicities.

Methods To address these challenges, Senti Bio is developing CAR-NK therapies that include Multi-Arming with calibrated release (cr)IL-15 and a Regulator Dial gene circuit to control the expression of crIL-12. Senti’s proprietary calibrated release technology enables cytokines to be expressed in both membrane-bound and secreted forms, providing multi-factorial activity via autocrine and paracrine stimulation to increase CAR-NK cell function and activation of endogenous immune cells in the TME. In addition, we have designed a Regulator Dial gene circuit that expresses crIL-12 under the control of grazoprevir (GRZ), an FDA-approved small molecule drug.

Results We have tested our Multi-Arming and Regulator Dial gene circuits in combination with a CAR targeting GPC3, a hepatocellular carcinoma (HCC)-relevant target. We observed crIL-15 to promote NK cell persistence and proliferation in an autocrine fashion, while also activating other immune cells in a paracrine manner. In addition, crIL-15 was observed to enhance GPC3 CAR-NK tumor killing in a serial killing assay compared to wild-type secreted IL-15 and showed enhanced antitumor function and increased survival over control groups in vivo.

GPC3 CAR-NK cells containing the Regulator Dial gene circuit expressed low crIL-12 levels in the absence of GRZ (<100 pg/1e6 cells/48h), while induction with GRZ led to a 1000-fold increase of crIL-12 expression under the same conditions. The ON and OFF kinetics of crIL-12 induction were determined in vitro. 4 hours of GRZ-treatment was sufficient to induce >600-fold increase in crIL-12 concentrations. By day 3 post-GRZ removal, crIL-12 reverted to basal levels. Lastly, the role of crIL-12 in reversing immunosuppression was validated in a co-culture assay. Specifically, GRZ-induced crIL12 restored CAR-NK cells that were suppressed in the presence of M2 macrophages.

Conclusions We have engineered off-the-shelf CAR-NK cell therapies that target GPC3 and express crIL-15. To increase the therapeutic window of the potent immune effector IL-12 in the TME, we have designed a Regulator Dial gene circuit to controllably produce crIL-12. These gene circuits have complementary mechanisms of action to enhance CAR-NK antitumor function and potentially overcome the immunosuppressive TME in solid tumors such as HCC.