Current antibody-based and CAR-based cell therapies are unable to distinguish between cancer and healthy cells that express the same antigen, resulting in off-target, off-tumor toxicities when healthy tissues express the targeted antigen. Senti is developing a Logic-Gated NOT CEA CAR-NK product for the treatment of CEA-expressing solid tumors. CEA (CEACAM5) is expressed on multiple solid tumors including CRC, NSCLC, gastric cancer and breast cancer. Past therapies targeting CEA resulted in dose-limiting on-target toxicities in the clinic [Pattabiraman et al 2013]. Using a bistable antigen-activating platform, we have identified and validated VSIG2 as a Protective Antigen (PA) expressed on the surface of CEA+ healthy epithelial cells. By adding an inhibitory CAR (iCAR) that recognizes VSIG2, NOT-activated CAR-NK cells lower the risk of on-target off-tumor toxicities by reducing NK-cell cytotoxicity and cytokine production in a Protective Antigen-dependent manner. To improve anti-tumor killing activity by enhancing NK proliferation and activity while stimulating endogenous immune cells, we Multi-Armed CEA CAR-NK cells with Senti’s proprietary crIL-15 and paracrine IL-21 signaling, and anti-IL-15 blocking. This strategy significantly improved anti-tumor activity, resulting in durable, NK-mediated anti-tumor activity in vitro (serial killing) and in vivo.

**Senti-401: Logic-Gated CAR-NK Cells Incorporating NOT GATE and Multi-Arming Gene Circuits for the Treatment of CEA-Solid Tumors**

**NOT GATE Gene Circuit Optimization: Design-Build-Test-And-Learn Approach to Optimize Tumor-Cell Killing and NOT GATE Protection**

Optimization of CEA activating CARs (iCARS). Various CEA-iCARS were tested in CEA-NK cells. Constructs were selected based on expression and function in NK cells, using CEA-CRC target cell lines in an image-based serial killing assay (hours). Tumor-associated antigen specificity was determined using a mixed target cell assay with WT DMEM solid CRC and CEA-OV (SIP) target cells.

**Multi-Arming With crIL-15 and IL-21 Results in Improved NK Cell Function, Serial Killing and Durable In Vivo Anti-Tumor Function**

Arming CEA CARs with the combination of Senti’s proprietary crIL-15 and IL-21 results in improved anti-tumor activity of NK cells. In vitro, CEA CAR-NK cells expressing crIL-15 and IL-21 had sustained serial killing even in the presence of immunosuppressive cytokine TGFβ (20ng/ml). In vivo, CEA CAR-NK cells secreting crIL-15 and IL-21 had durable tumor control for 100+ days.

**SENTI-401, an Allogeneic Logic-Gated and Multi-Armed CAR-NK Cell Therapy for the Treatment of CEA-Expressing Solid Tumors with Enhanced Selectivity and Activity**

**SENTI-401** is an off-the-shelf CAR-NK product that incorporates a CEA-targeting iCAR along with NOT Logic-Gate and Multi-Arming gene circuits, intended to treat CEA solid tumors. The NOT GATE gene circuit reduces the risk of off-target, off-tumor toxicities by incorporating an iCAR that prevents cytokotisity of target cells in a VSIG2-dependent manner, potentially enabling a safer therapeutic window that could allow for improved anti-tumor function and lesser off-tumor toxicities. The combination of crIL-15 and IL-21 results in durable anti-tumor function of the CAR-NK cells.

As next steps, Senti is continuing to optimize the expression and activity of the overall gene circuit in order to define its final lead development candidate for clinical evaluation.

Contact: alba.gonzalez@sentibio.com