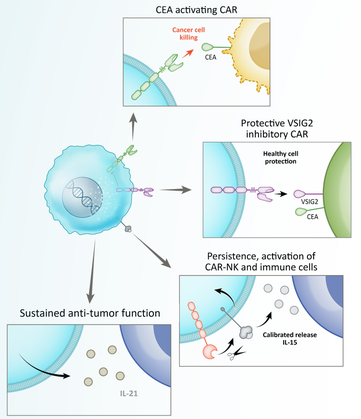
SENTI-401, an Allogeneic Logic-Gated and Multi-Armed CAR-NK Cell Therapy for the Treatment of CEA-Expressing Solid Tumors with Enhanced Selectivity and Activity

SITC 2022, Boston MA
Abstract #221

Senti Biosciences, Inc. South San Francisco, CA

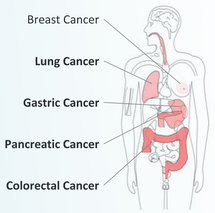
Nicholas Frankel, Maelig Morvan, Assen Roguev, Michelle Hung, Russell Gordley, Miguel Palermo, Tyler Santomasso, Pearley Chinta, Aldo Sotelo, Marcus Gainer, Derrick Lee, Tony Hua, Cheng-Ting Lee, Andrew Banicki, Mengxi Tian, Niran Almudhfar, Otto Contreras, Tim Lu, Alba Gonzalez

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



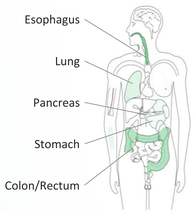
Current antibody-based and CAR-based cell therapies are unable to distinguish between cancer and healthy cells that express the same antigen, resulting in **on-target, off-tumor toxicities** when healthy tissues express the targeted antigen. Senti is developing a **Logic-Gated NOT Gate CAR-NK product for the treatment of CEA-expressing solid tumors**. CEA (CEACAM5) is expressed by 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 [Parkhurst et al 2011]. Using a bioinformatics antigen-pairing pipeline, we 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-gated CAR-NK cells lower the risks 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 persistence and activity while stimulating endogenous immune cells, we **Multi-Armed** CEA CAR-NK cells with calibrated release (cr) IL-15, which achieves both autocrine and paracrine IL-15 signaling, and IL-21. This strategy significantly improved NK anti-tumor activity, resulting in durable NK-mediated anti-tumor activity in vitro (serial killing) and in vivo.

Tumor Types With CEACAM5 Overexpression[1]



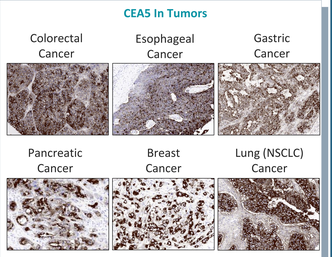
Highest overexpression in GI tumors, particularly CRC, also NSCLC

Healthy Tissues With CEACAM5 Overexpression[1]



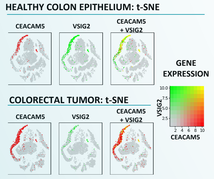
Potential for greatest toxicity risks without protective NOT gate antigen involve lung alveolar cells and GI tract epithelial cells

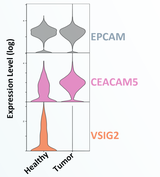
CEA Protein Expression in Solid Tumors



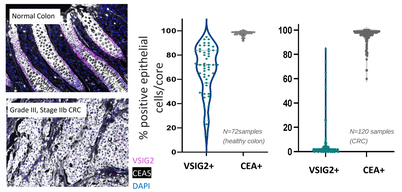
1 Median expression of tumor and normal samples in body map (Log2 (TPM+1) scale). Source: TCGA, Gtex; IHC: Internal Data

Protective Antigen Discovery Platform Results



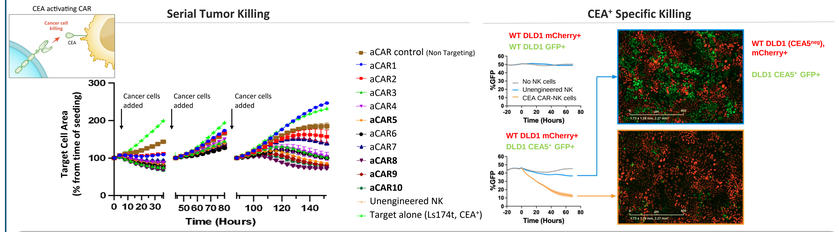


Protective Antigen Tissue Validation Using Multiplexed IHC

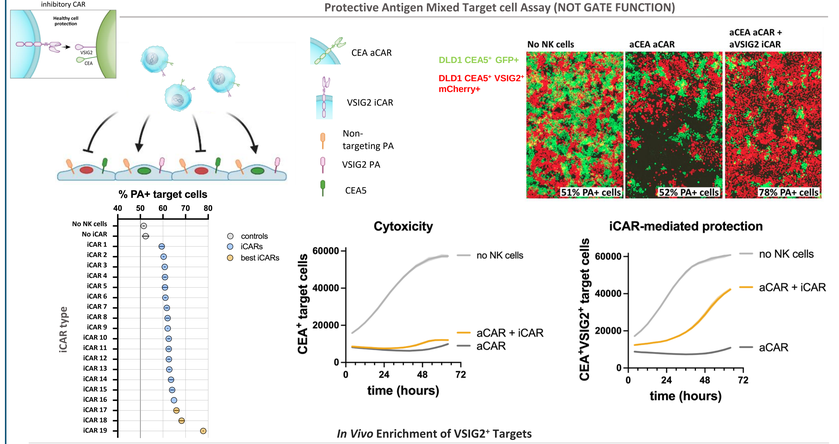


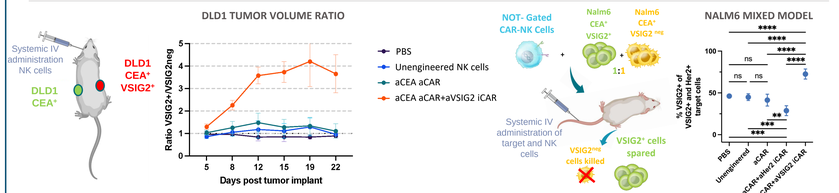
CEA (CEACAM5) expression in tumor and healthy tissues (TCGA and Gtex gene-expression data sets). IHC validation of CEA expression in various solid tumors. Bioinformatics pipeline prioritizes Protective Antigens that are expressed in the membrane of healthy epithelial cells that also express the tumor-associated antigen. Using this approach, we identified and validated VSIG2 as a protein uniquely expressed on the membrane of CEA⁺ healthy epithelial cells and not expressed on the membrane of tumor cells.

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



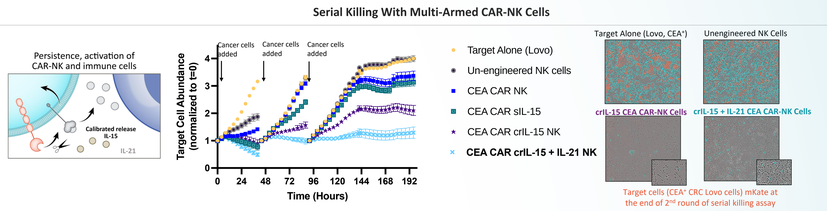
Optimization of CEA activating CARs (aCARs). Various CEA aCARs were tested in CAR-NK cells. Constructs were selected based on expression and function in NK cells, using CEA⁺ CRC target cell lines in an image-based serial killing assay (Incucyte). Tumor-associated antigen specificity was determined using a mixed target cell assay with WT (CEA5^{neg} mCherry) and CEA5⁺ DLD1 (GFP) target cells.

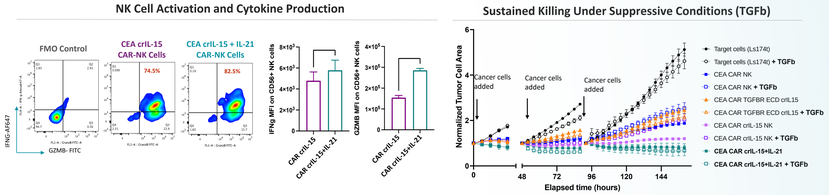


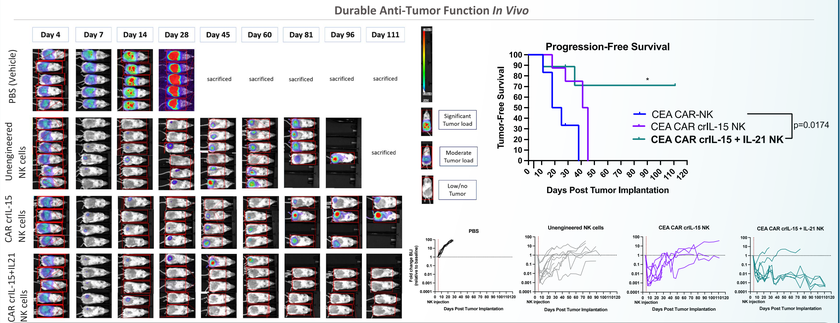


Optimization of VSIG2 inhibitory CARs (iCARs). Target cells expressing CEA and VSIG2 (Protective Antigen, PA) or an off-target PA were mixed at 1:1 ratio and co-cultured with CEA CAR-NK cells expressing different VSIG2-targeting iCARs. Best iCARs were ranked based on selective enrichment of VSIG2⁺ target cells (red). CEA NOT VSIG2 NK cells were able to selectively kill CEA⁺ targets (green) while sparing CEA⁺/VSIG2⁺ targets (red).
CEA NOT VSIG2 CAR-NK cells were used in vivo to treat mice with dual flank DLD1 tumors, or a mixture of Nalm6 target cells, where one target expresses CEA only and the other expresses CEA along with the PA VSIG2. VSIG2⁺ tumors grew faster than VSIG2^{neg} tumors, indicating protection of VSIG2⁺ targets when mice were treated with the NOT-gated NK 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 the immune-suppressive cytokine TGFb (20pg/mL). In vivo, CEA CAR-NK cells secreting crIL-15 and IL-21 had durable tumor control for 100+ days.

SENTI-401, a Multi-Armed Logic-Gated CAR-NK Cell Therapy Product for the Potential Treatment of CEA⁺ Solid Tumors

- **SENTI-401** is an off-the shelf CAR-NK product that incorporates a CEA-targeting aCAR 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 on-target, off-tumor toxicities by incorporating an iCAR that prevents cytotoxicity of target cells in a VSIG2⁺ dependent manner, potentially enabling a wider 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