Development of logic-gated CAR-NK cells to reduce target-mediated healthy tissue toxicities

Alba Gonzalez-Junca, Assen Rogueur, Brian Garrison, Nicholas Frankel, Derrick Lee, Marcus Gainer, Alyssa Mullenix, Russell Gordley, Kathryn Loving, Jenny Chien, Gary Lee

Senti Biosciences, Inc. South San Francisco, CA

1. NOT Gate CAR-NK cells can prevent on-target/off-tissue toxicities

- Figure 1. Engineering smarter medicines by using logic-gated CAR-NK cells which can provide selective targeting and avoid on-target/off-tissue toxicities enabling precise targeting of tumor antigens. Senti has developed a first-in-class NOT gate that can provide selective targeting of tumor cells while protecting healthy cells in an antigen-dependent manner. Senti’s proprietary logic-gated circuits allow cells to recognize tumor antigens as well as safety antigens present only in healthy cells. Cells will then compute the information resulting in defined functional outcomes. In this case, the use of a NOT gate allow for antigen-mediated tumor killing with decreased cytokotoxicity and cytokine production in a safety antigen-dependent manner. Here we present the proof of concept of the NOT gate functionality in NK cells, were a tumor-targeting activating CAR (iCAR) is paired with an inhibitory (iCOS) that recognizes a safety antigen in normal cells. Multiple novel inhibitory domains have been tested for (iCOS), to select the one that resulted in robust antigen-mediated inhibition of NK cytotoxicity. NOT-gated logic gene circuits were recently published by Senti’s scientific advisor and collaborator Dr. Weng, Boston Univ. (Zhu NK et al. 2020).

2. Bioinformatics Antigen Discovery Platform

- Figure 2. Bioinformatics pipeline for discovery and prioritisation of safety antigens for NOT-gated CAR-NK cells. Senti has developed an internal bioinformatics pipeline to discover and prioritize safety antigens that can work in combination with a tumor-target to achieve protection against on-target/off-tissue toxicities. Using transcriptomics data to discover and prioritise tumor and healthy tissue antigens we have identified genes differentially expressed in healthy vs tumor tissue and selected leads based on antigens’ co-expression in healthy tissue, subdomain localization, antigen topology (presence of extracellular domains), and antibody availability. Such antigen pairs have been then validated in primary tissue samples.

3. Tumor target and safety antigen pairing on CAR-NK cells for AML

- Figure 3. Safety antigen Discovery. Bioinformatics pipeline and target validation for AML. A. Targeting Acute Myeloid Leukemia is particularly challenging due to tumor antigen heterogeneity and expression of AML targets such as FGFR3 on the healthy hematopoietic stem cells (HSCs). A. Using Senti’s Bioinformatics pipeline we discovered Safety Antigens that are uniquely expressed in the membrane of HSCs and not in AML blasts or LSC. B. Expression of the top Safety Antigens Endomucin (EMCN) in different cell populations, including AML tumor cells (U937, LPS, Blasts) and healthy HSCs and progenitors. C. EMNC expression has been validated in human patient suspensions using flow cytometry using a monoclonal antibody kindly provided by Dr. Vredenburgh, Max Planck Institute.

4. Safety antigen discovery and validation for solid tumors (CEA + CRC)

- Figure 4. NOT gate logic circuit can inhibit NK cell cytotoxicity and cytokine production in a safety antigen-dependent manner. A. NOT-gated NK cells show reduced target killing in a safety antigen-dependent manner. FIIt2 CAR NK cells with a micro-targeted CAR can effectively kill AML cancer cells (IGEN), but the cytokotoxicity is significantly reduced in a Safety Antigen (EMNC) dependent manner. B. Similarly, secretion of cytokines and activation cytokines (Granzyme B and IFN) is also impaired with EMNC iCAR. C. NOT-gated NK cells can also distinguish and spare safety antigen-expressing cells in a mixed co-culture in vitro away using AML target cells. Legend: TαT-NK cells (FITC), iNK-Safety antigen (EMNC).

- Figure 5. VSIG2 discovery and validation as safety antigen to protect healthy epithelial cells from CEA-mediated toxicities. Targeting CEA (CEACAM5) in colorectal cancer (CRC) has demonstrated on-target/off-tissue toxicities in the clinic. Parmentier et al. 2011. NCT00521659 due to CEACAM5 expression on healthy epithelial cells besides tumor cells. A. B. We discovered VSIG2 as Safety Antigen that is uniquely co-expressed in the membrane of healthy epithelial cells. C. D. Using multiplexed IHC we were able to validate co-expression of CEACAM5 and VSIG2 in healthy colon epithelial cells, with no expression of VSIG2 in colorectal tumor samples. Legend: TαT-NK cells (CEACAM5), iNK = Safety Antigen (VSIG2).

Summary

We discovered and validated EMNC as Safety Antigen to pair with FIIt2/CD33 tumor targeting for the treatment of AML, and developed and tested NOT gate inhibitory circuits that work in an antigen-dependent manner in AML models.

We discovered and validated VSIG2 as Safety Antigen to pair with CEA tumor target in treatment of CRC.

Focus and next steps

Proof of concept shows that NOT gated logic circuits can be used in NK cells to prevent target-mediated cytokotoxicity in a Safety Antigen-dependent manner. Opens possibility of targeting multiple tumor antigens that have concerning expression in healthy cells.

Contact: alba.gonzalez@senti.bio

AACR 2021 Late Breaking Abstract L8028