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

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Late Breaking Abstract
LB028

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

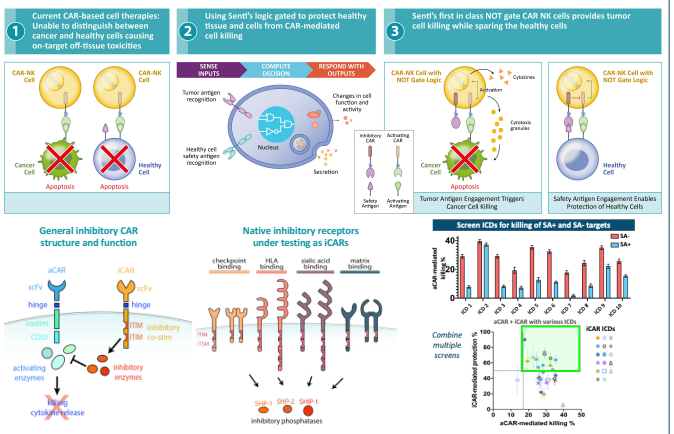

Figure 1. Engineering smarter medicines by using logic-gated CAR-NK cells which can provide selective targeting and avoid on-target/off-tissue toxicities enabling precise targeting of tumor antigens. Senti has developed a first in class NOT gate that can provide selective targeting of tumor cells while 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 cell killing with decreased cytotoxicity 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 (aCAR) is paired with an inhibitory (iCAR) that recognizes a safety antigen in normal cells. Multiple naïve inhibitory domains have been tested for iCARs, to select the ones 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 Wong, Boston Univ. [Cho JW et al Nat Comm 2021].

2. Bioinformatics Antigen Discovery Platform

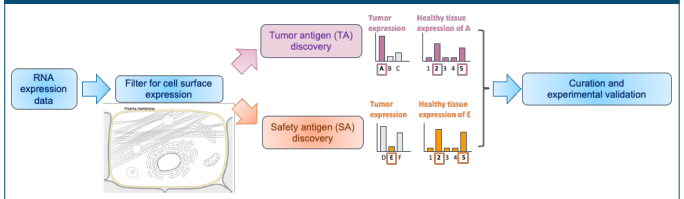

Figure 2. Bioinformatics pipeline for discovery and prioritization of safety antigen for NOT gated CAR NK cells. Senti has developed an internal bioinformatics pipeline to discover and prioritize safety antigens that can work in combination with a tumor target to achieve protection against on-target/off-tissue toxicities. Using transcriptomics data to discover and prioritize 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, subcellular localization, antigen topology (presence of extracellular domain(s)), and antibody availability. Such antigen pairs have can be then validated in primary tissue samples.

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

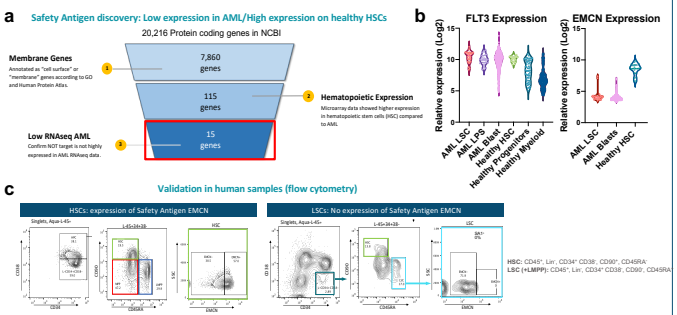

Figure 3. Safety Antigen Discovery Bioinformatics pipeline and target validation for AML. A. Targeting Acute Myeloid Leukemia is particularly challenging due to tumor antigen heterogeneity and expression of AML targets such as Flt3 on the healthy Hematopoietic Stem Cells (HSCs). A. Using Senti's bioinformatics pipeline we discovered Safety Antigens that are uniquely expressed in the membrane of HSCs and not in AML blasts or LSCs. B. Expression of the top Safety Antigen Endomucin (EMCN) in different cell populations including AML tumor cells (LSC, LPS, Blast) and healthy HSCs and progenitors. C. EMCN expression has been validated in human patient samples using flow cytometry using a monoclonal antibody kindly provided by Dr Vestweber, Max Planck Institute.

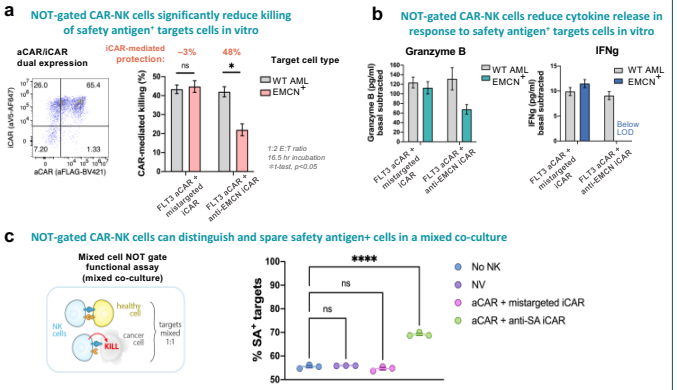

Figure 4. NOT gate logic circuit can inhibit NK cell cytotoxicity and cytokine production in a safety antigen dependent manner. A. NOT-gated NK cells show reduced target cell killing in a safety-antigen dependent manner. FLT3CAR NK cells with a miss-targeted iCAR can effectively kill AML cancer cells (SEM), but the cytotoxicity is significantly reduced in a Safety Antigen (EMCN) dependent manner. B. Similarly, secretion of cytotoxic and activation cytokines (GranzymeB and IFNg) is also impaired with EMCN iCAR. C. NOT-gated NK cells can also distinguish and spare safety-antigen expressing cells in a mixed co-culture in vitro assay using AML target cells. Legend: TA= Tumor antigen (Flt3), SA= Safety antigen (EMCN).

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

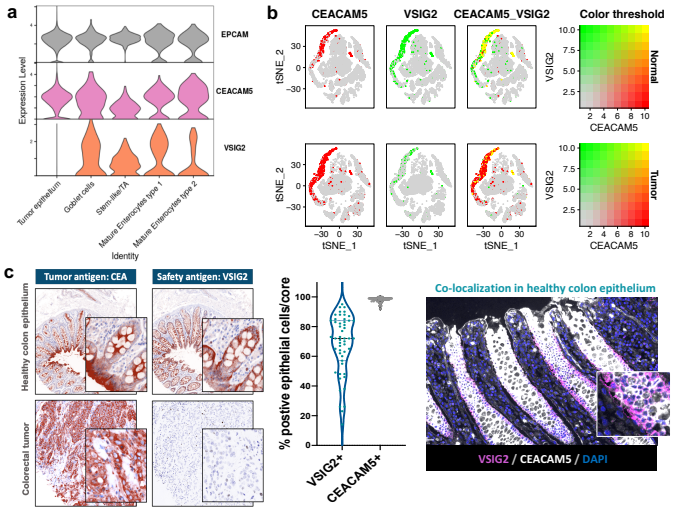

Figure 5. VSIG2 discovery and validation as safety antigen to protect healthy epithelial cells from CEA-mediated toxicities. Targeting CEA (CEACAM5) in Colorectal Cancer (CRC) has demonstrated on-target/off-tissue toxicities in the clinic [Parkhurst et al 2011, NCT00923806] due to CEACAM5 expression on healthy epithelial cells besides tumor cells. A-B. We discovered VSIG2 as a 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: TA= Tumor Antigen (CEACAM5) / SA = Safety Antigen (VSIG2)

Summary

NOT gated CAR NK cells for the treatment of AML

We discovered and validated EMCN as Safety Antigen to pair with Flt3/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.

NOT gated CAR NK cells for the treatment of metastatic CRC

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 cytotoxicity in a Safety Antigen dependent manner.

Opens possibility of targeting multiple tumor antigens that have concerning expression in healthy cells.

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