SENTI-202, a Selective, Off-the-Shelf, Preclinical CAR-NK Cell Therapy with CD33 and/or FLT3 Activating CAR, Healthy Cell Protection from Endomucin (EMCN) Inhibitory CAR and Calibrated Release IL-15 for Hematologic Malignancies Including AML

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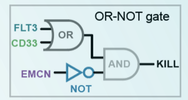
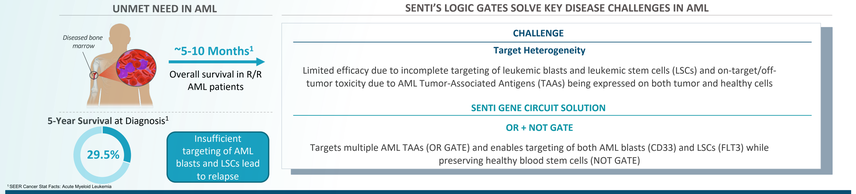


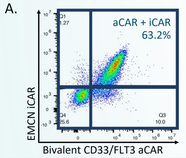
SENTI-202 is an Allogeneic Logic-Gated Preclinical CAR-NK Cell Therapy Engineered to Address Unmet Needs in Hematopoietic Malignancies, Including AML

Background: Patients with CD33+ and/or FLT3+ malignancies, which encompass most myeloid malignancies such as acute myeloid leukemia (AML), have a grim prognosis and a high unmet need. There are no approved cell therapies for patients with AML due to paucity of targets and an immunosuppressive bone marrow milieu. SENTI-202 is a preclinical CAR-NK cell product engineered with a CD33 and/or FLT3 NOT EMCN logic-gated gene circuit and a proprietary calibrated release IL-15 (crIL-15) technology to overcome these challenges. The bivalent CD33/FLT3 activating CAR (aCAR), EMCN inhibitory CAR (iCAR) and crIL-15 are all engineered within a single gene circuit onto a natural killer (NK) cell (healthy adult peripheral blood-derived) to create an off-the-shelf chimeric antigen receptor (CAR) NK cell therapy.
While previous presentations (Garrison et al., ASH, 2021) focused on the performance of individual SENTI-202 components, this presentation focuses on functional data from the complete logic-gated gene circuit and the crIL-15 together in an NK cell (i.e., the final CAR-NK cell with all 3 genetic elements for clinical development).

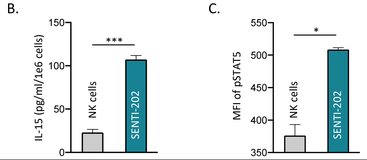


SENTI-202: All Genetic Components Delivered as a Single Transduction

>60% aCAR + iCAR Co-Expression

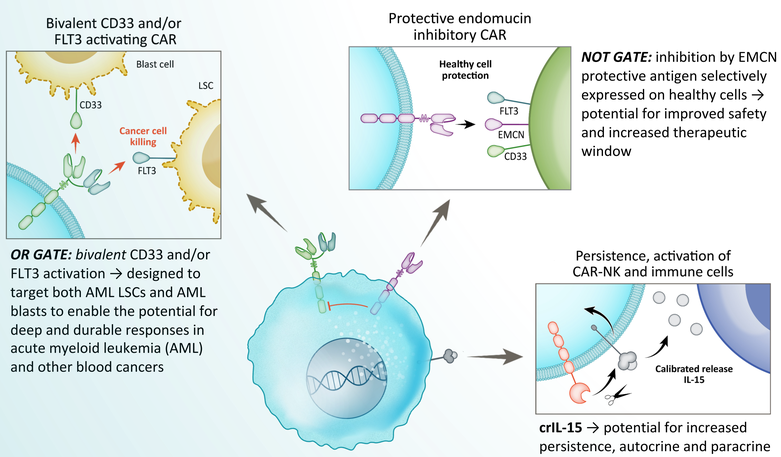


IL-15 Expression and NK Cell Activation



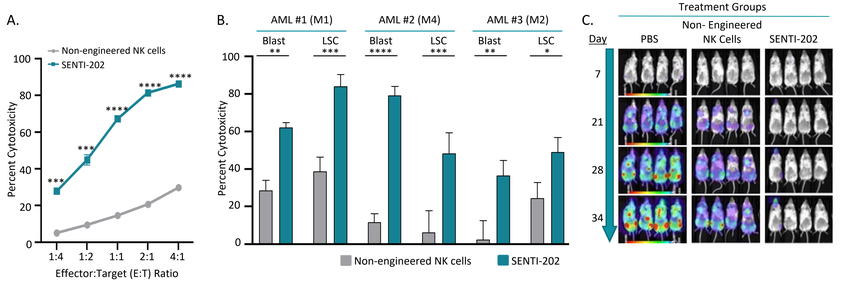
The SENTI-202 gene circuit encodes for 3 cargos: (1.) crIL-15, (2.) bivalent CD33/FLT3 aCAR, and (3.) EMCN iCAR, that are expressed in a tricistronic format (see above) that enables (A.) up to 63.2% co-expression of the bivalent CD33/FLT aCAR and EMCN iCAR, (B.) IL-15 production of up to 107pg/ml/1e6, and (C.) up-regulation of the NK cell IL-15 receptor signaling cascade via pSTAT5. note: 2A=2A self-cleavage peptide. p-value: *=0.01-0.05, ***=0.0001-0.001

SENTI-202: Mechanism of Action



SENTI-202 Possesses Significant Preclinical Anti-Tumor Activity

SENTI-202 Demonstrates *In Vitro* and *In Vivo* Anti-Tumor Activity

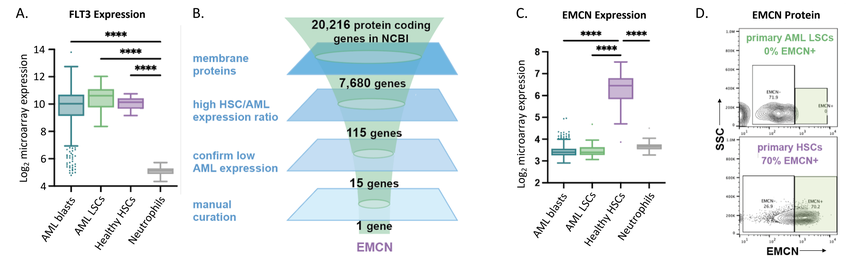


SENTI-202 demonstrates significant *in vitro* CAR-dependent anti-tumor cytotoxicity against (A.) SEM leukemia cells which express CD33 and FLT3, and (B.) AML LSCs and AML blasts from primary patient samples. (C.) SENTI-202 cell therapy shows significant *in vivo* activity within an AML (MV4-11) xenotransplantation model. p-value: *=0.01-0.05, **=0.001-0.01, ***=0.0001-0.001, ****=<0.0001

Potential Need for NOT GATE for Increased Healthy HSC Protection

HSCs Express FLT3

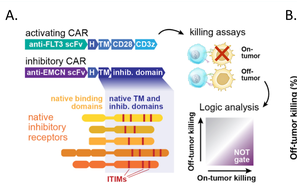
Identification of the Healthy HSC Marker EMCN



SENTI-202 includes a NOT GATE (iCAR) to protect healthy HSCs from potential off-tumor toxicity. (A.) The tumor-associated target antigen FLT3 is expressed on both AML LSCs and healthy HSCs, which necessitates the use of an iCAR to help protect healthy HSCs from potential off-tumor toxicity. (B.) We identified EMCN as a potential HSC-specific iCAR target using a multi-step bioinformatics pipeline, and validated EMCN expression as a healthy HSC surface marker that is not expressed on AML cells using (C.) transcriptomics and (D.) flow cytometry. p-value: ****=<0.0001

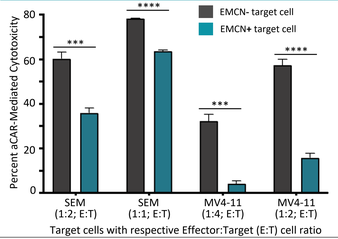
SENTI-202 Inhibitory CAR Design and Function

LIR1 Identified as Optimal iCAR ICD for SENTI-202



Identification of optimal iCAR intracellular domain (ICD) for SENTI-202. (A.) We developed a systematic approach to identify the optimal SENTI-202 iCAR ICD using a screen of native inhibitory proteins in the context of an anti-EMCN iCAR, which were co-expressed and assayed for function in concert with FLT3 aCARs. Constructs were evaluated based on HIGH on-tumor (FLT3+EMCN-) killing and LOW off-tumor (FLT3+EMCN+) killing. Killing was CAR-mediated (basal NK cytotoxicity subtracted). (B.) Comparative analyses between NOT gates with different iCAR ICDs identified LIR1 as the optimal ICD for SENTI-202.

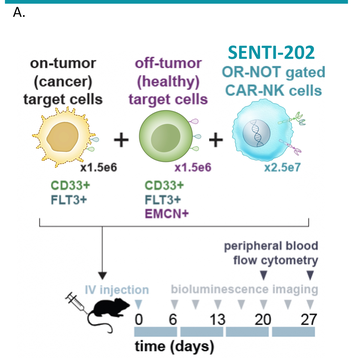
EMCN iCAR Protects EMCN+ Cells



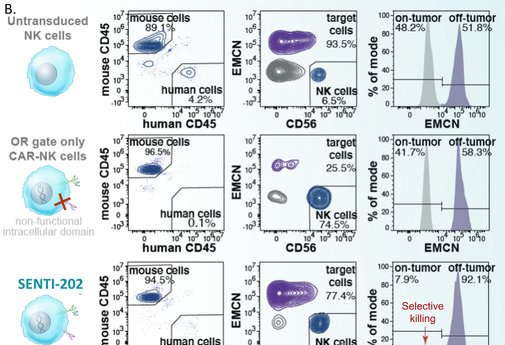
SENTI-202 possesses robust killing activity against leukemia cell lines (SEM, MV4-11), which lack EMCN expression. However, once these cells are engineered to express EMCN, the SENTI-202 EMCN iCAR then provides significant protection from aCAR-mediated cytotoxicity. p-value: ***=<0.0001-0.001, ****=<0.0001

In Vivo: SENTI-202 iCAR Protects Cells Expressing the Healthy Cell Marker EMCN

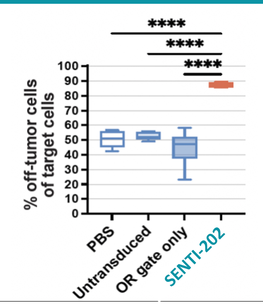
In Vivo Assessment of NOT logic gating



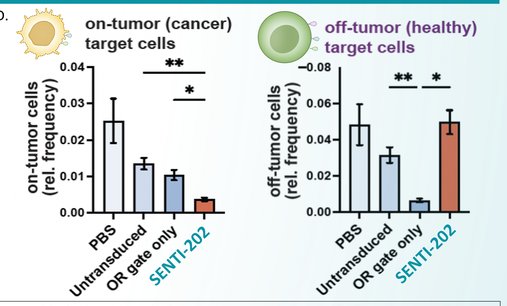
OR/NOT Logic Gate Enables Precise On-Tumor Cell Killing



NOT Gate Protects Off-Tumor Cells

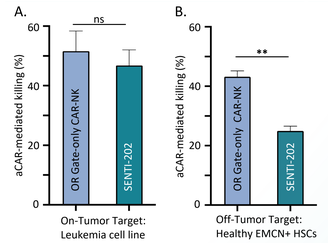


Precise On-Tumor Killing with Off-Tumor Protection



The EMCN NOT gate enables SENTI-202 to avoid on-target off-tumor toxicity *in vivo*. (A.) The *in vivo* mixed target study design. "On-Tumor" target cells (EMCN- FLT3+ CD33+ cancer model) and "Off-Tumor" target cells (EMCN+ FLT3+ CD33+ healthy model) were mixed with NK cells and injected into mice. (B.) Peripheral blood flow cytometry analysis from representative mice showing selective killing of On-Tumor cells by SENTI-202. (C.) The SENTI-202 NOT gate enables specific protection and enrichment of Off-Tumor target cells (EMCN+ healthy model), compared to the OR gate-only control. (D.) Abundance of target cells of each type relative to all CD45+ blood cells showing that On-Target Tumor cells are reduced by NK cells (especially in OR gate only and SENTI-202 groups), but notably, "Off-Tumor" healthy target cells are only spared by SENTI-202 since they possess the EMCN iCAR (NOT gate).
p-value: *=0.01-0.05, **=0.001-0.01, ****=<0.0001

EMCN iCAR Protects Healthy Human HSCs from aCAR-Mediated Killing



(A.) SENTI-202 CAR-NK cells possess similar cytotoxicity potential as OR gate-only control CAR-NK cells against leukemia target cells. However, (B.) only the SENTI-202 cells (with the EMCN iCAR) were able to provide significant protection to the healthy EMCN+ HSCs.
p-value: **=0.001-0.01

Summary

- **SENTI-202, a preclinical off-the-shelf CAR-NK cell therapy, is engineered with a CD33 OR FLT3 NOT EMCN logic-gated gene circuit and crIL-15 for the treatment of patients with hematologic malignancies, including AML**
- **SENTI-202 has demonstrated:**
  - Significant *in vitro* cytotoxicity against leukemia cell lines, and AML LSCs and blast cells from primary patient tumor samples
  - Significant *in vivo* activity within an AML (MV4-11) xenotransplantation model
  - Significant *in vitro* protection of EMCN+ CD33+ FLT3+ model healthy cells, which would otherwise be killed in the absence of a NOT gate
  - Significant increase *in vivo* of killing precision by demonstrating robust on-tumor killing of EMCN- CD33+ FLT3+ cells with concurrent protection and expansion of EMCN+ CD33+ FLT3+ healthy model cells
  - Significant protection of primary healthy human EMCN+ HSCs from off-tumor toxicity

Clinical evaluation of SENTI-202 is planned (IND expected in 2023) to evaluate the safety and efficacy in patients with hematologic malignancies with high unmet need, including AML.

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