SENTI-202 CD33 OR FLT3 NOT EMCN Logic-Gated Gene Circuit Components Selectively Target AML while Protecting Human HSC/HPCs from Off-Tumor Toxicity in a Humanized Mouse Model

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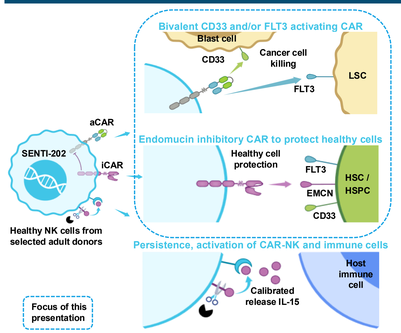
Abstract

There are no approved chimeric antigen receptor (CAR) natural killer (NK) or T cell therapies for acute myeloid leukemia (AML), due in-part to the scarcity of tumor-specific cell surface targets, which creates potential safety challenges with on-target off-tumor toxicity. SENTI-202 is an innovative CAR NK cell therapy that expresses a potent CD33 OR FLT3 NOT EMCN logic-gated gene circuit, in conjunction with IL-15. The bivalent CD33 OR FLT3 activating CAR targets 2 AML antigens, CD33 & FLT3, enabling targeting of AML stem cells & blasts (Gonzalez, 2023; Kaveri, 2024) and venetoclax-resistant AML cells (Muftuoglu, 2023). The NOT EMCN inhibitory CAR (iCAR) protects hematopoietic stem and progenitors (HSC/HPCs) from off-tumor toxicity (even if the healthy cells are FLT3$^+$/CD33$^+$), which is a major technological advancement that could enhance safety and hematopoietic recovery in patients. The iCAR recognizes the healthy cell marker endomucin (EMCN), which is expressed on up to 76% of HSCs (Reckzeh, 2018) but not AML cells. Here, we used CD33 OR FLT3 NOT EMCN CAR NK cells to validate iCAR-driven HSC/HPC protection in a humanized mouse model by comparing CD33 OR FLT3 NOT EMCN NK (88% CAR$^+$) to (1.) Non-engineered NK and (2.) CD33 OR FLT3 NOT HER2 (iCAR control) NK (89% CAR$^+$). First, we confirmed expected in vitro anti-AML activity (observed 50-70% killing; E:T=1:1) and iCAR-mediated model healthy cell preservation activity (observed ~50% preservation; E:T=1:1), using EMCN$^-$ and EMCN$^+$ cell lines, consistent with our prior results showing the EMCN iCAR preserves primary human HSCs from off-tumor toxicity in vitro (Garrison, 2022). Here, using a humanized mouse model we demonstrated iCAR-mediated in vivo protection of human HSC/HPCs from off-tumor toxicity for the first time in the field. Following successful human HSC/HPC engraftment, mice were given 2 CAR NK cell injections on d0 & d7, then HSC/HPC frequency was assessed on d14. While treatment with the iCAR control NK cells led to a significant decrease in HSC/HPC frequency compared to non-engineered NK (18.15% versus 28.6% of hCD45$^+$; p value=0.00015), treatment with CD33 OR FLT3 NOT EMCN NK cells resulted in a significantly increased HSC/HPC frequency versus iCAR control NK cells (34.2% versus 18.15% of hCD45$^+$; p value=0.00000355). This demonstrates that the EMCN iCAR protects human HSC/HPCs from off-tumor toxicity in vivo and suggests that the EMCN iCAR may increase the SENTI-202 therapeutic window. In conclusion, the SENTI-202 CD33 OR FLT3 NOT EMCN logic-gated gene circuit enables exceptional efficacy against AML while protecting HSC/HPCs from off-tumor toxicity, demonstrating the potential of SENTI-202 as a targeted and precise therapy for AML. SENTI-202 is currently in Phase 1 clinical development in patients with relapsed/refractory hematologic malignancies including AML (NCT06325748). Efficacy and clinical results were presented at AACR abstract # CT014 (oral minisymposium # CTMS01) and correlative results at AACR abstract # CT143.

SENTI-202: Mechanism of Action



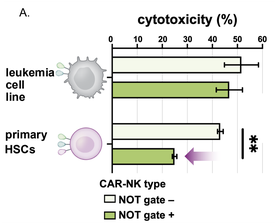
SENTI-202 Gene Circuit Design

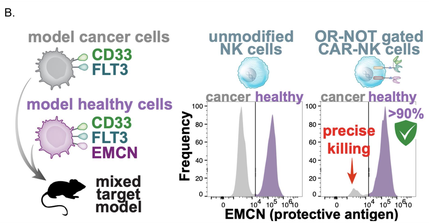
- **OR Logic Gate *KILLS*** leukemia blasts and LSCs via CD33/FLT3 CAR
  - CD33 and/or FLT3 expressed in ~95% of AML patients with CD33 being predominantly expressed on bulk blasts and FLT3 on LSCs
- **NOT Logic Gate *PROTECTS*** healthy HSC/HSPCs from 'off-tumor, on-target' effects
  - Protects HSC/HSPCs via Endomucin (EMCN) inhibitory CAR even when they express CD33 and/or FLT3
  - EMCN is found predominantly on healthy HSC/HSPC cell surface, and rarely on AML
- **Calibrated release IL-15 *ENHANCES*** SENTI-202 and host immune cell activation and persistence

LSC: Leukemic stem cell; HSC: hematopoietic stem cell; HSPC: hematopoietic stem and progenitor cell

SENTI-202 is a First-in-Class Off-the-Shelf Logic-Gated Selective CD33 OR FLT3 NOT EMCN CAR NK Cell Therapy for Blood Cancers

Previous Demonstration of EMCN NOT Gate Activity





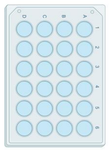
The SENTI-202 EMCN NOT Gate preserves healthy cells from off-tumor toxicity. (A.) EMCN NOT Gate protects primary human HSCs from in vitro off-tumor toxicity. (B.) EMCN NOT Gate enables selective killing of on-target tumor cells within in vivo xenograft, preserving EMCN$^+$ model healthy cells. (Frankle et al., Cell Reports, 2024; ASH 2022) p-value: **≤ 0.01

EMCN NOT Gate (iCAR) Preserves Human HSPCs in Humanized Mouse Model

R&D Scale CAR NK Cell Manufacturing

CD33/FLT3 aCAR + EMCN iCAR (titering)			
Virus (µL)	CD33%	EMCN%	MOI
500	63.5	42.1	33.55
166	58	36.9	11.14
55	47.8	28.1	3.69
18	35.9	19.5	1.21

CD33/FLT3 aCAR + Control iCAR (titering)		
Virus (µL)	CD33%	MOI
500	67.6	35.55
166	62.8	11.80
55	53.6	3.91
18	36.7	1.28

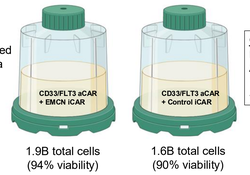


Initial Transduction & Expansion

- 1.3x10^6 cells / well
- 1 plate/construct
- (need total 50e6 for 1L G-Rex)
- 500 µL virus / well
- 2hr Spinoculation
- NK Macs: 1% NK Frozen Supplement + 500 UI/mL IL2

Aspirate off 1.75 mL and feed 2 mL with complete media

4 days to get at least 50e6 cells for 1L G-Rex



1.9B total cells (94% viability)

1.6B total cells (90% viability)

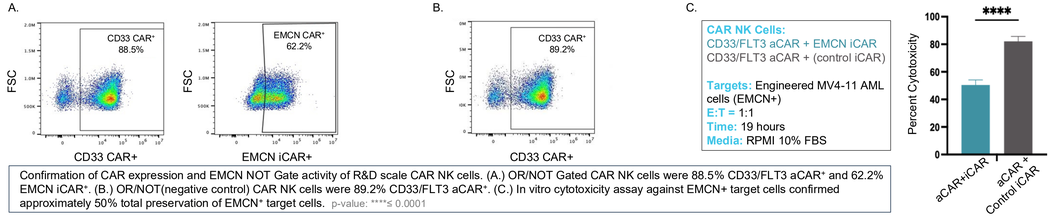
G-Rex Media
NK Macs: 5% Human AB serum and 1% NK Frozen Supplement +IL2 +IL15

OR/NOT Logic Gate Gene Circuits are Expressed and Functional in R&D Scale CAR NK Cells

OR/NOT Gated CAR NK Cells: CD33/FLT3 aCAR + EMCN iCAR NK Cells

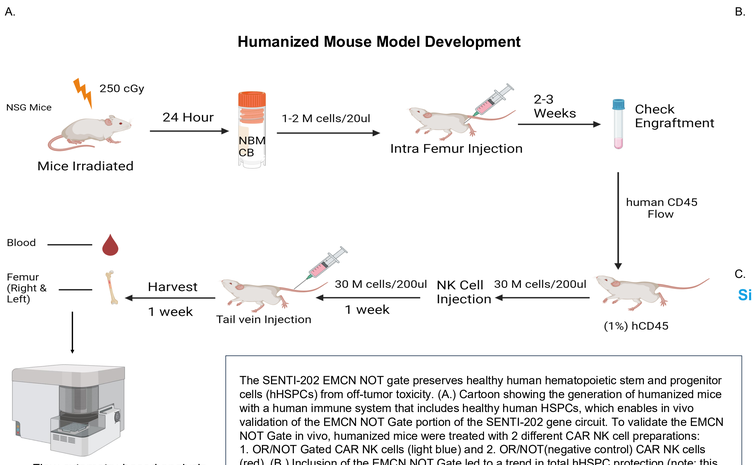
OR/NOT(control) CAR NK Cells: CD33/FLT3 aCAR + iCAR(neg. control) NK Cells

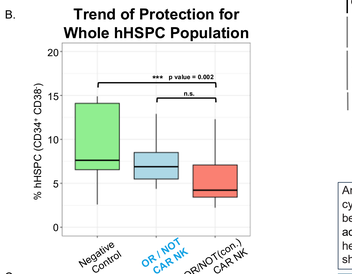
Confirmation of In Vitro Protection of EMCN$^+$ Target Cells

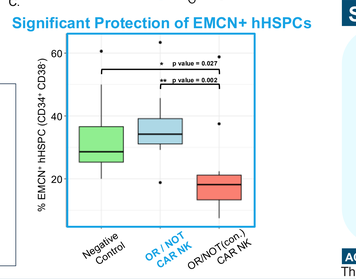


Confirmation of CAR expression and EMCN NOT Gate activity of R&D scale CAR NK cells. (A.) OR/NOT Gated CAR NK cells were 88.5% CD33/FLT3 aCAR$^+$ and 62.2% EMCN iCAR$^+$. (B.) OR/NOT(negative control) CAR NK cells were 89.2% CD33/FLT3 aCAR$^+$. (C.) In vitro cytotoxicity assay against EMCN+ target cells confirmed approximately 50% total preservation of EMCN$^+$ target cells. p-value: ****≤ 0.0001

EMCN NOT Gate (iCAR) Preserves Human HSPCs Against Off-Tumor Toxicity In Vivo

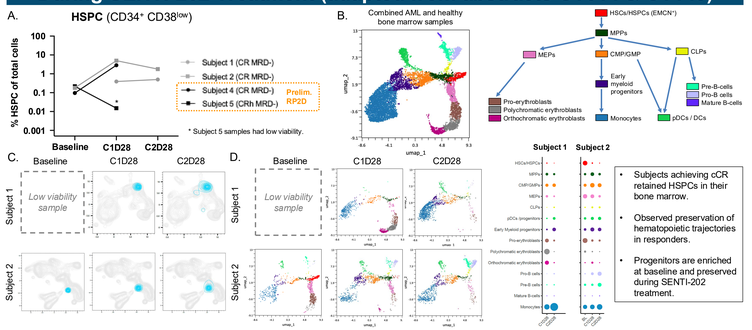






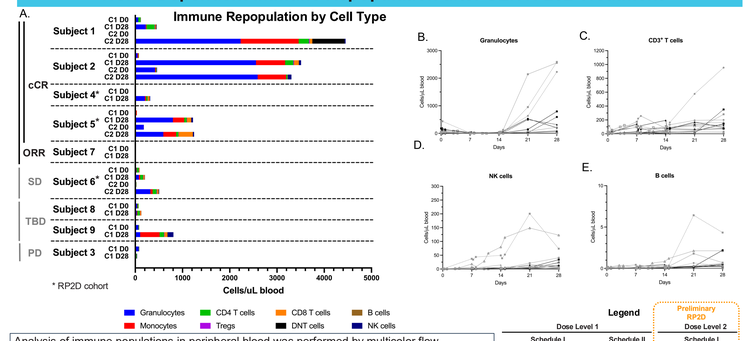
The SENTI-202 EMCN NOT gate preserves healthy human hematopoietic stem and progenitor cells (hHSPCs) from off-tumor toxicity. (A.) Cartoon showing the generation of humanized mice with a human immune system that includes healthy human HSPCs, which enables in vivo validation of the EMCN NOT Gate portion of the SENTI-202 gene circuit. To validate the EMCN NOT Gate in vivo, humanized mice were treated with 2 different CAR NK cell preparations: 1. OR/NOT Gated CAR NK cells (light blue) and 2. OR/NOT(negative control) CAR NK cells (red). (B.) Inclusion of the EMCN NOT Gate led to a trend in total hHSPC protection (note: this cell population contains both EMCN$^+$ and EMCN$^-$ HSPCs). (C.) Focusing analysis on the EMCN$^+$ hHSPC sub-population, EMCN$^+$ HSPCs were significantly protected from off-tumor toxicity, demonstrating that the EMCN NOT Gate works as designed to protect hHSPCs within an in vivo setting. p-value: *≤ 0.05; **≤ 0.01; ***≤ 0.001

Maintenance of HSPCs in AML Patient Responders During SENTI-202 Treatment (adapted from abstracts CT014 & CT143)

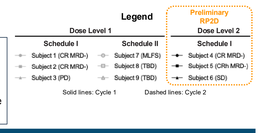


Analysis of HSPCs and hematopoietic differentiation in the bone marrow of trial subjects. (A.) HSPCs were identified as CD34$^+$CD38^{-low} hematopoietic cells, and the proportion of HSPCs in responder bone marrow was either increased or maintained during SENTI-202 treatment. (B.) CyTOF analysis identified cell populations in the classical hematopoietic differentiation hierarchy in healthy and diseased samples. Representative analysis was performed on responder subjects 1 and 2, showing that (C.) EMCN$^+$ hematopoietic populations (blue contours) were retained after SENTI-202 treatment, and (D.) UMAP analysis of subjects 1 and 2 showed the preservation of hematopoietic trajectories in responders, with progenitor cell types enriched at baseline and more differentiated populations appearing after treatment.

HSPC-Dependent Immune Repopulation after SENTI-202 Treatment



Analysis of immune populations in peripheral blood was performed by multicolor flow cytometry. (A.) Stacked bar plots showing the concentration of cells measured in blood at the beginning (pre-dose 1) and end (Day 28 / EOT) of each treatment cycle. Subjects that achieved cCR showed robust immune recovery by the end of each cycle, indicating normal hematopoiesis. Recoveries of (B.) granulocytes, (C.) T cells, (D.) NK cells, and (E.) B cells are shown over each treatment cycle are shown for all subjects. note: EOT = end of treatment.



SUMMARY

- SENTI-202 is a First-in-Class Off-the-Shelf Logic-Gated Selective CD33 OR FLT3 NOT EMCN CAR NK Cell Therapy for Blood Cancers.
- *CD33/FLT3 OR Logic Gate Gate KILLS* leukemia blasts and LSCs via bivalent CD33/FLT3 activating CAR (aCAR).
- *EMCN NOT Logic Gate PROTECTS* healthy HSC/HSPCs from 'off-tumor, on-target' effects in vitro and enables selective killing in vivo of on-target tumor cells by protecting EMCN$^+$ model healthy cells from 'off-tumor, on-target' effects.
- Shown here, larger R&D scale CAR NK cell preparations maintain aCAR and iCAR expression, as well as function.
- The EMCN NOT Gate protects human HSPCs from 'off-tumor, on-target' effects within an in vivo humanized mouse model.
- In the on-going SENTI-202 clinical trial for AML, HSPCs were either increased or maintained after SENTI-202 treatment.
- EMCN$^+$ HSPCs were detectable throughout treatment and differentiated into normal hematopoietic lineages during response in patients' bone marrows.
- Multiple immune populations increased in peripheral blood after SENTI-202 treatment in patients.

ACKNOWLEDGEMENTS

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