# Abstract:

2799

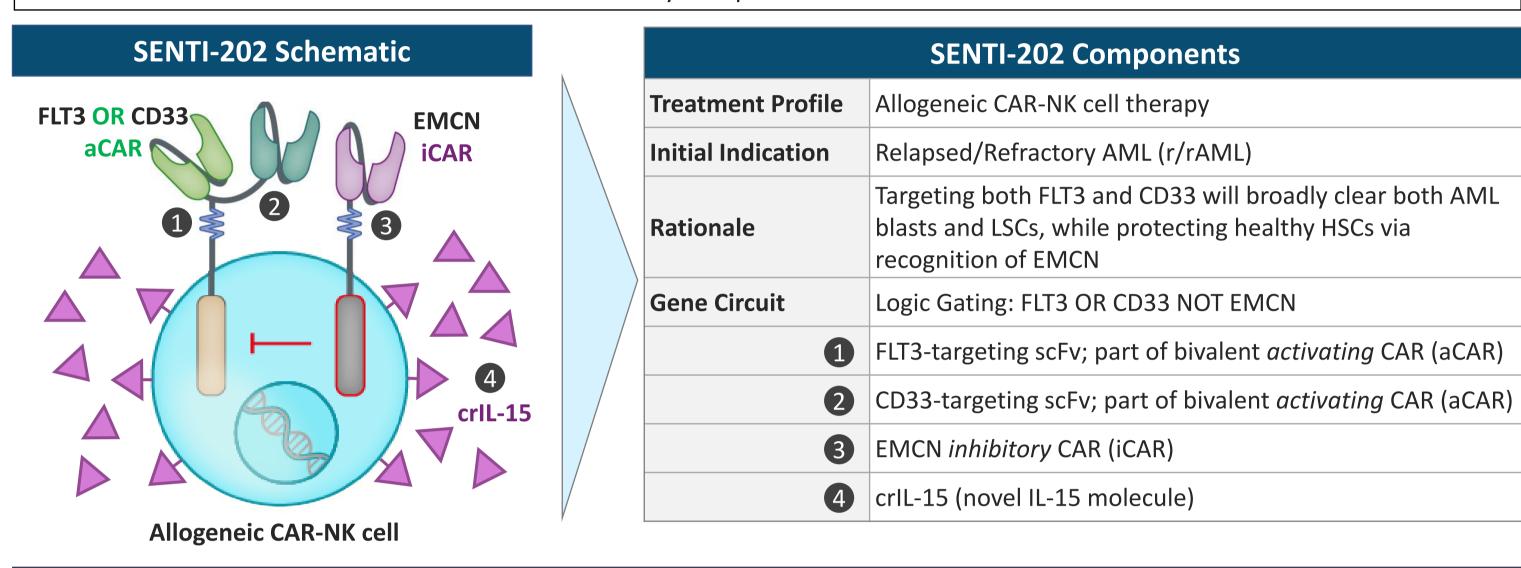
# FLT3 OR CD33 NOT EMCN Logic Gated CAR-NK Cell Therapy (SENTI-202) for Precise Targeting of AML

Brian S. Garrison, PhD¹, Han Deng, PhD¹, Gozde Yucel, PhD¹, Nicholas W. Frankel, PhD¹, Michelle Hung, PhD¹, Mercus Gainer¹, Kathryn Loving, PhD¹, Jenny Chien¹, Tiffany Pan, MS¹, Wesley Gorman¹, Nelia Leemans, MS¹, Alice Lam¹, Poornima Ramkumar, PhD¹, Travis Wood¹, Wilson Wong, PhD², Philip Lee, PhD¹, Tim Lu, MD PhD¹, Gary Lee, PhD¹ <sup>1</sup> Senti Bio, South San Francisco, CA; <sup>2</sup> Department of Biomedical Engineering, Boston University, Boston, MA

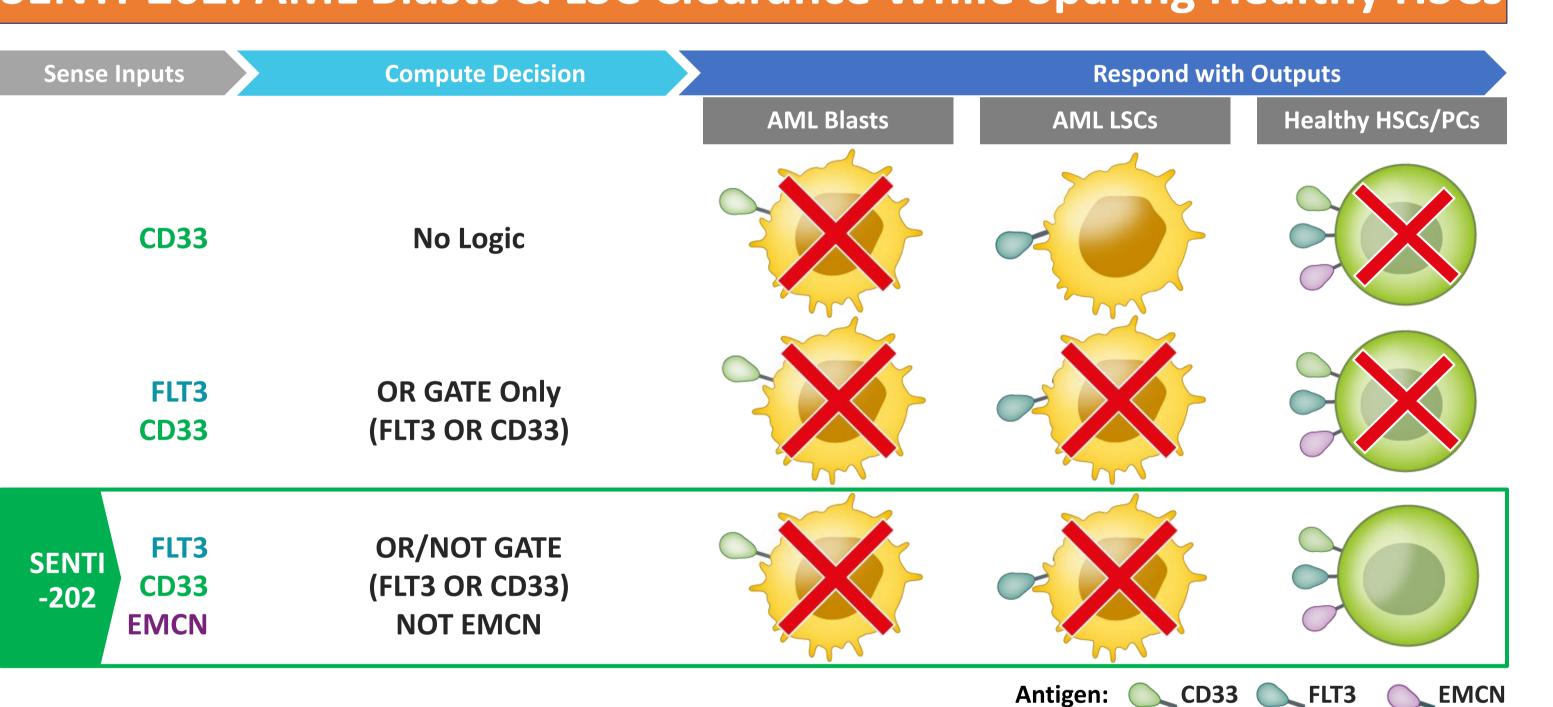
## SENTI-202: Designed to Address Unmet Needs in AML Treatment

While chimeric antigen receptor (CAR) cell therapies have provided extraordinary clinical responses in some hematological malignancies, developing effective CAR cell therapies for acute myeloid leukemia (AML) has been challenging due to: (a) the lack of a single target antigen robustly expressed across both AML leukemic stem cell (LSC) and leukemic blast cell subpopulations, and (b) the lack of truly AML-specific target antigens, since current proposed targets are also expressed on healthy tissues and may result in off-tumor toxicity. Using Logic Gated gene circuits, Senti Bio is engineering its SENTI-202 CAR-NK cell therapy to overcome these long-standing challenges to treating patients with AML

| SENTI-202 is a novel off-the-shelf NK cell product candidate engineered to incorporate both OR and NOT Logic Gated CAR gene circuits, wherein the OR GATE is designed to increase AML LSC/blast tumor clearance (to prevent relapse), and the NOT GATE is designed to protect healthy hematopoietic stem cells (HSCs) from off-tumor toxicity, enabling regeneration of a healthy hematopoietic system and mitigating the need for a bone marrow transplant. SENTI-202 is also engineered to express calibrated release IL-15 (crIL-15), a novel engineered IL-15 molecule that enables autocrine and paracrine signaling that imbues NK cells with enhanced anti-tumor activity and persistence.

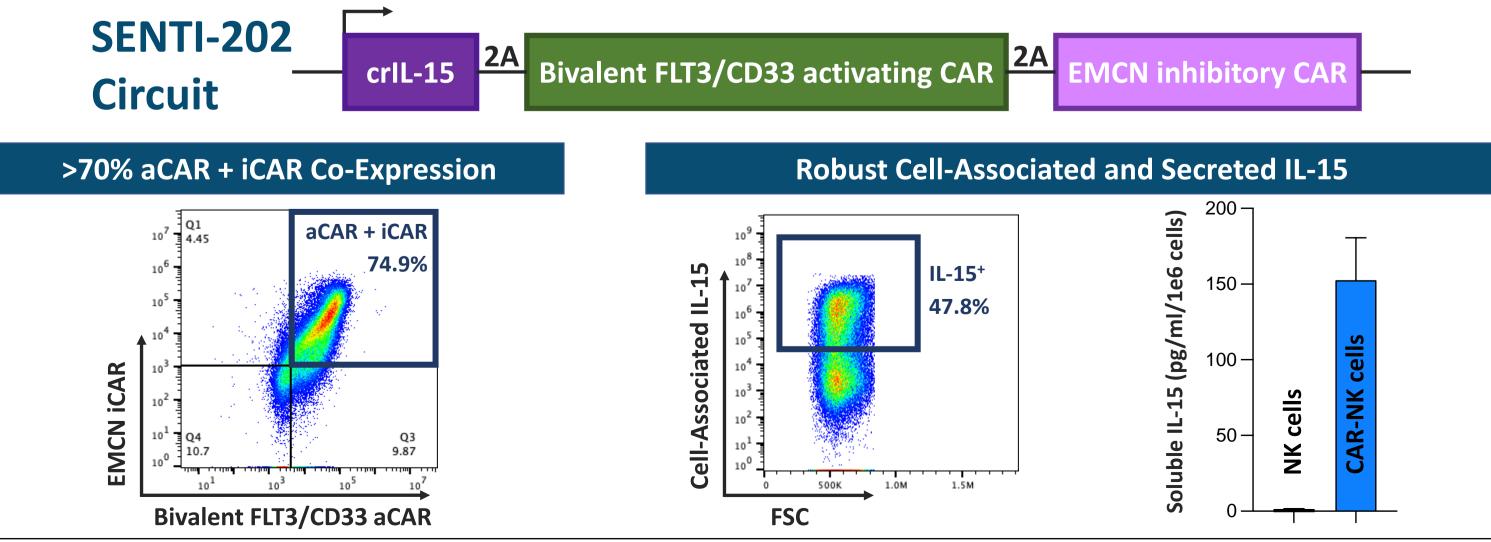


# SENTI-202: AML Blasts & LSC Clearance While Sparing Healthy HSCs



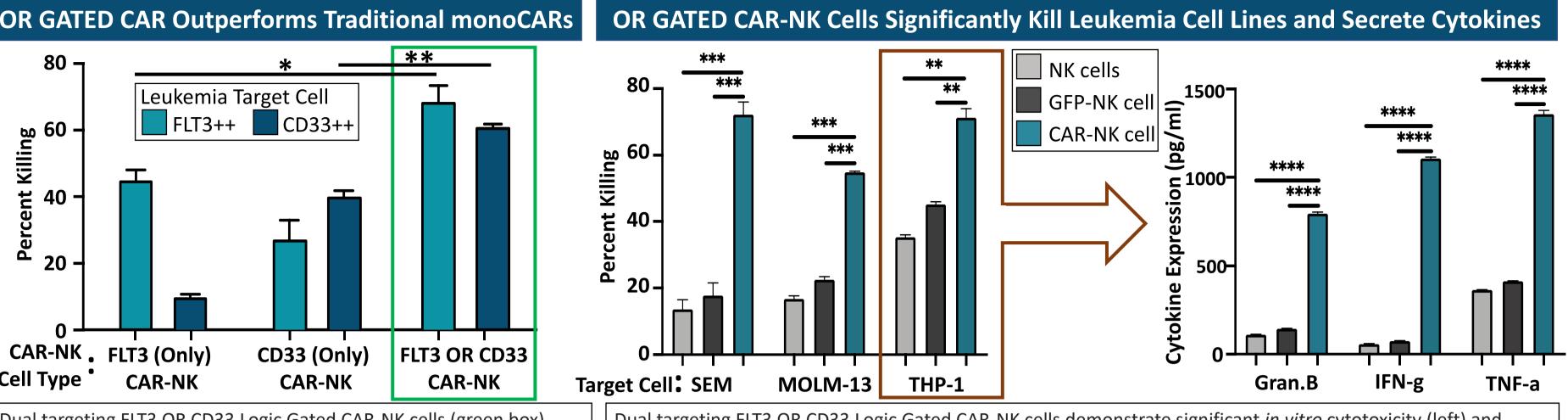
respond capabilities. By concurrently targeting AML tumor-associated antigens FLT3 and CD33 with a bivalent activating CAR (aCAR), SENTI-202 is designed to target both AML blasts and leukemic stem cells (LSCs), leading to potentially greater efficacy and deeper more sustained remissions. By targeting the safety antigen Endomucin (EMCN) with an inhibitory CAR (iCAR), SENTI-202 is designed to protect hematopoietic stem cells (HSCs) and progenitor cells (PCs) from off-tumor toxicity, potentially enabling a curative cell therapy approach in the absence of transplantation.

# Single Gene Circuit Expression of all SENTI-202 Components

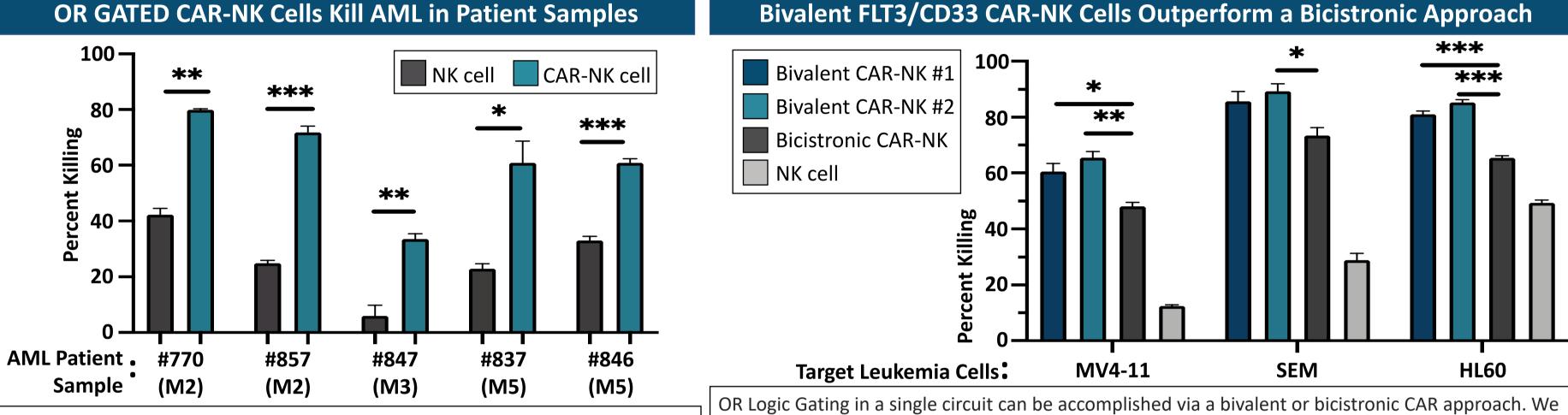


SENTI-202 uses a single gene circuit that encodes for 4 components: (1.) crIL-15, (2.) FLT3 aCAR, (3.) CD33 aCAR, and (4.) EMCN iCAR, which are all expressed in a tricistronic format (see above). FLT3 and CD33 CARs have been combined into a single bivalent FLT3/CD33 aCAR, which is a single aCAR with the ability to bind both FLT3 and CD33. The SENTI-202 circuit enables up to 74.9% of our engineered CAR-NK cells to express both the FLT3/CD33 bivalent aCAR and the EMCN iCAR (left), and express significant levels of IL-15 detected on both the NK cell surface (middle) and in solution (right).

# FLT3 OR CD33 Logic Gated CAR-NK Cells Demonstrate Robust Killing Activity

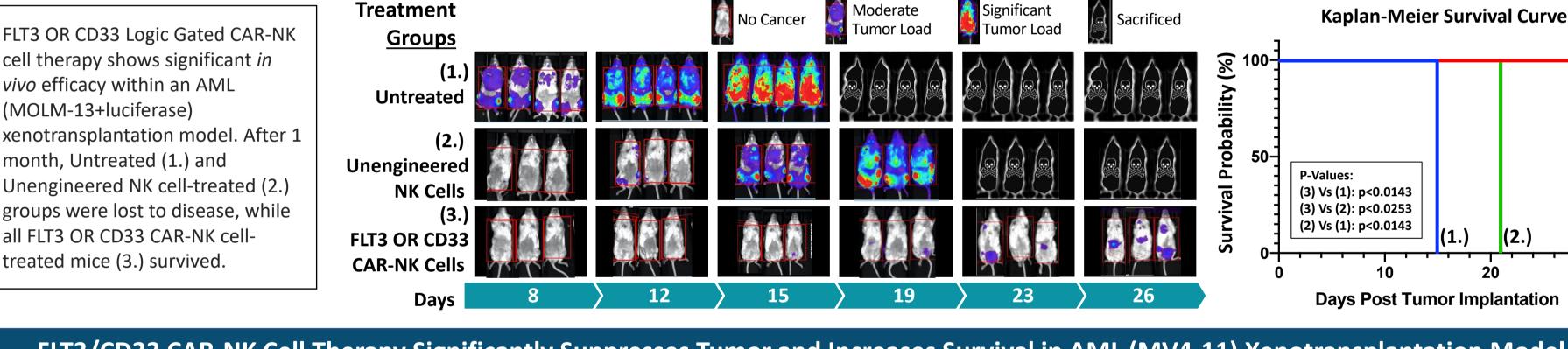


Dual targeting FLT3 OR CD33 Logic Gated CAR-NK cells demonstrate significant in vitro cytotoxicity (left) and Dual targeting FLT3 OR CD33 Logic Gated CAR-NK cells (green box) retion (right; THP-1 data shown) against multiple leukemia cell lines (SEM, MOLM-13, THP-1). show significant killing over FLT3 and CD33 single CAR approaches

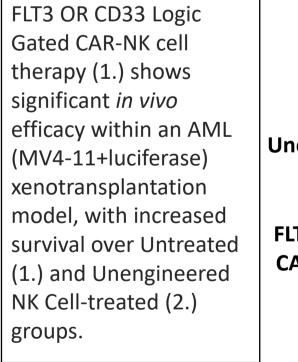


Dual targeting FLT3 OR CD33 Logic Gated CAR-NK cells demonstrate significant in determined that Bivalent FLT3 OR CD33 CAR-NK cells (blue bars) demonstrate significant in vitro vitro cytotoxicity against primary AML cells in fresh patient samples.

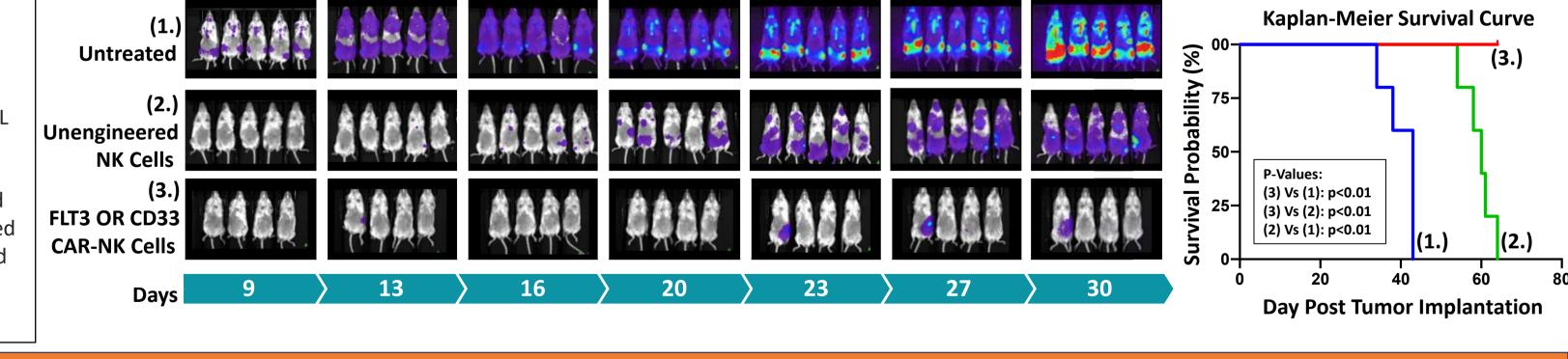
#### cytotoxicity over bicistronic FLT3 OR CD33 CAR-NK cells (dark gray bar) against multiple leukemia lines. FLT3/CD33 CAR-NK Cell Therapy Significantly Suppresses Tumor and Increases Survival in AML (MOLM-13) Xenotransplantation Model



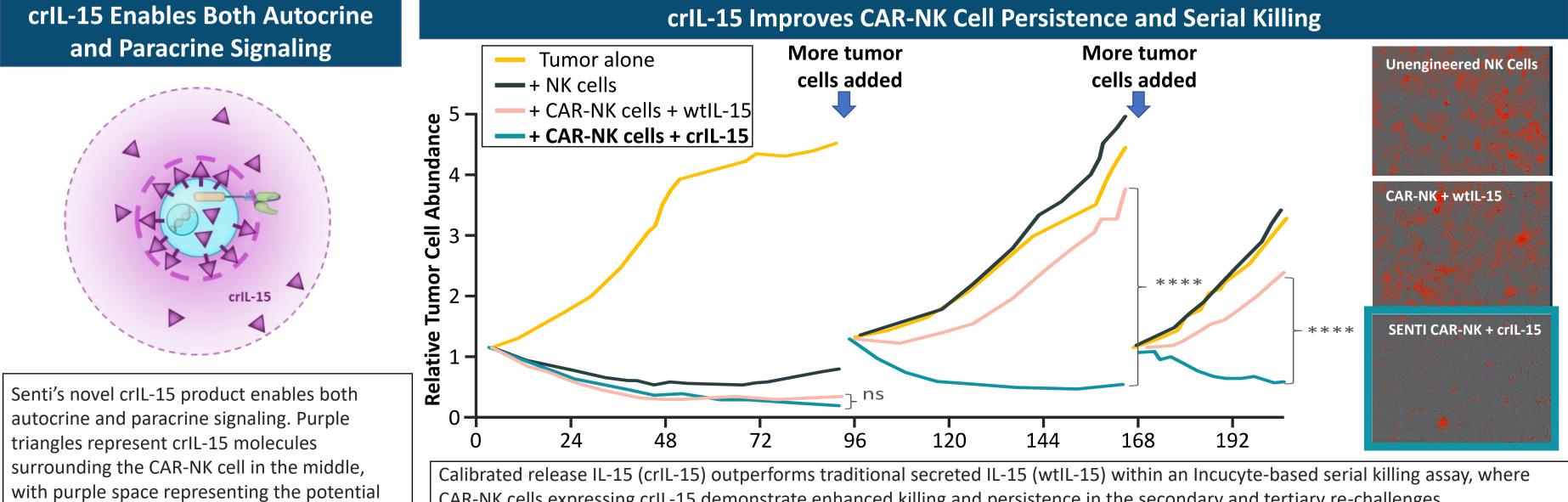
#### FLT3/CD33 CAR-NK Cell Therapy Significantly Suppresses Tumor and Increases Survival in AML (MV4-11) Xenotransplantation Model



effective area of crlL-15.

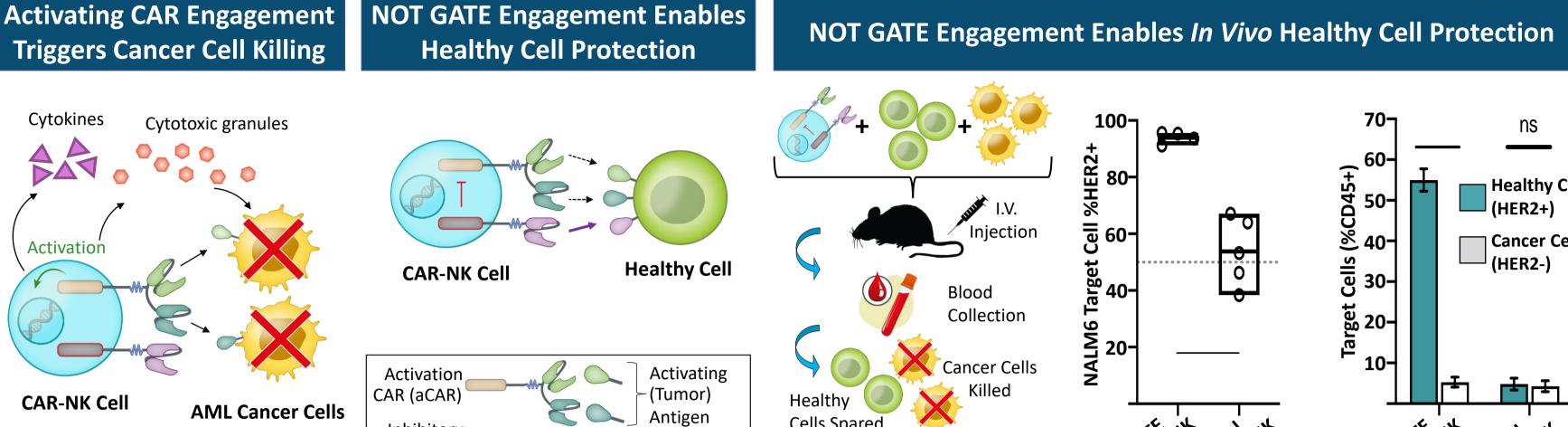


# Senti's Novel crlL-15 Enhances NK Cell Persistence and Tumor Killing



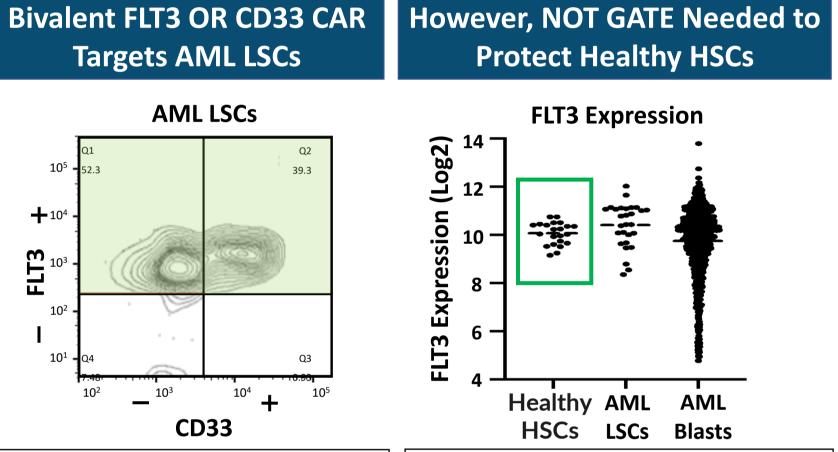
Calibrated release IL-15 (crIL-15) outperforms traditional secreted IL-15 (wtIL-15) within an Incucyte-based serial killing assay, where CAR-NK cells expressing crIL-15 demonstrate enhanced killing and persistence in the secondary and tertiary re-challenges. Microscopy images from the tertiary re-challenge show Senti's CAR-NK + crIL-15 cells with enhanced killing (red=tumor cells)

### NOT GATE Significantly Reduces CAR-NK Cell On-Target/Off-Tumor Toxicity

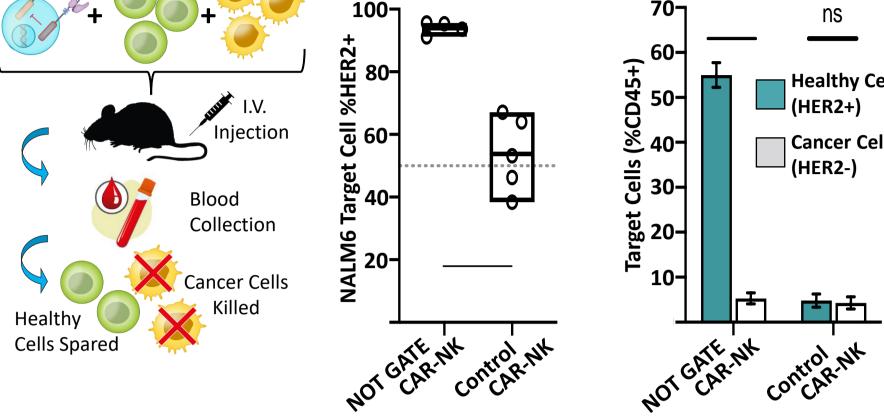


(Left) OR/NOT GATED CAR-NK cells recognize and kill cancer cells that express aCAR targeted tumor-associated antigens (e.g. FLT3, CD33; green) and do not express iCAR-targeted safety antigens (e.g. EMCN; purple).

(Right) OR/NOT GATED CAR-NK cells do not kill healthy cells expressing the iCARtargeted safety antigen; iCAR engagement results in inhibition of NK cell activation.

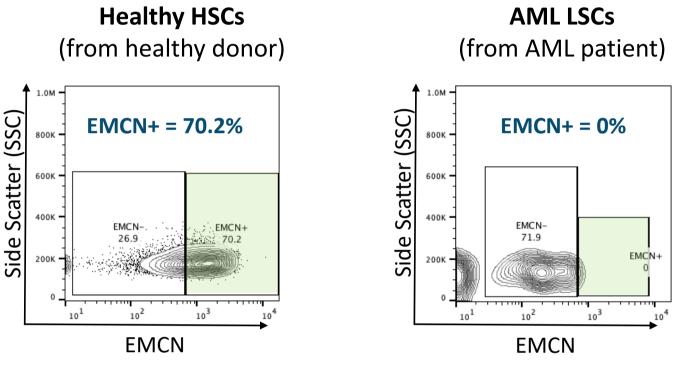


Targeting FLT3 enables more While a FLT3 CAR enables targeting of comprehensive targeting of AML LSCs, AML LSCs, this may lead to off-tumor toxicity against healthy HSCs, which also a cell population thought to express FLT3. ntribute to relapse in AML patients



Using HER2 as a model iCAR target antigen, we demonstrate in vivo NOT GATE protection of HER2+ target cells against CAR-NK cell toxicity. NOT GATED CAR-NK cells and NALM6 target cells (50% HER2+ and 50% HER2-) are injected (left), and at day 21 peripheral HER2+ target cells are protected from killing. Analysis of HER2+/HER2- target cell frequencies as part of total CD45+ shows a similar result (right)

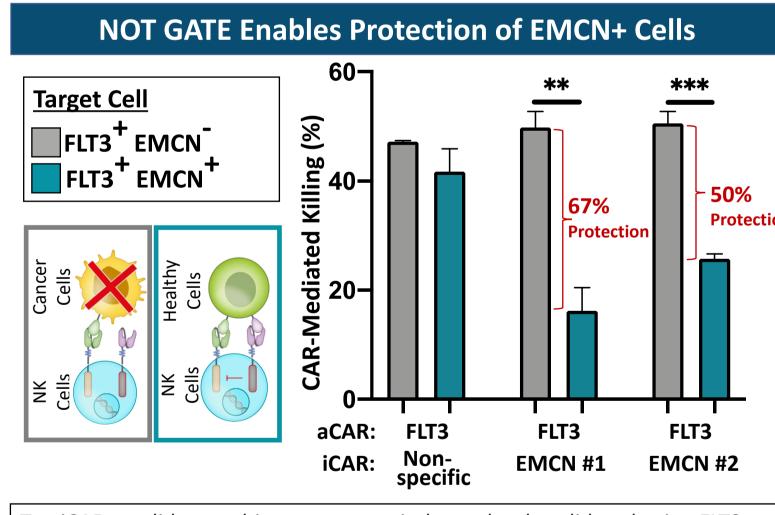
#### **EMCN Differentiates Healthy HSCs from AML LSCs AML LSCs Healthy HSCs**



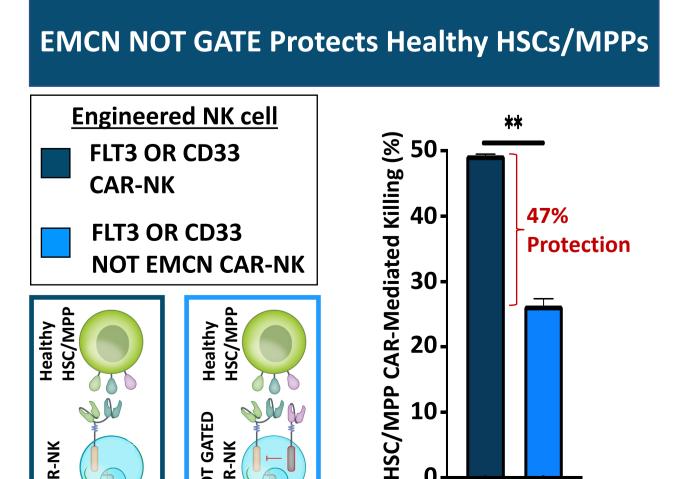
To design and employ a NOT GATE (iCAR) for protection of healthy HSCs from off-tumor toxicity, we first used a proprietary NOT GATE paired-antigen discovery platform to identify Endomucin (EMCN) as a potential NOT GATE safety antigen robustly expressed on healthy HSCs (left) but not expressed on AML LSCs (right).

# Senti has Identified Multiple iCAR Architectures for CAR-NK Cells

Using an in vitro NOT GATE cytotoxicity screen, we identified at least 9 different NOT GATE architectures that are functional within the context of CAR-NK cells. All NOT GATED CAR-NK cells consisted of a FLT3 aCAR and an EMCN iCAR with a unique architecture (FLT3 NOT EMCN CAR-NK cells), which were then mixed with FLT3+ SEM target cells (+/- EMCN-expressing versions), and in the presence of a functional iCAR there is reduced killing of the +EMCN target cells (or increased protection of the EMCN+ target cells).



Top iCAR candidate architectures were independently validated using FLT3 NOT EMCN CAR-NK cells against FLT3+ target cells (+/- EMCN-expressing populations). We observed a significant decrease in killing of EMCNexpressing target cells, indicating the NOT GATE enabled up to 67% protection of EMCN+ cells. Importantly, we believe 10-20% protection of healthy HSCs may be clinically relevant and allow full reconstitution of a healthy hematopoietic system in patients.



FLT3 OR CD33 NOT EMCN CAR-NK cells demonstrated 47% protection of healthy HSCs/MPPs compared to equivalent CAR-NK cells without the EMCN NOT GATE within an in vitro cytotoxicity assay. CAR-NK groups show similar killing of MV4-11 AML cells (not shown). Note: MPP = Multipotent Progenitor

#### Summary SENTI-202 is an allogeneic Logic Gated FLT3 OR CD33 NOT EMCN CAR-NK cell therapy product candidate designed to treat r/rAML patients in need

#### SENTI-202 uses a single gene circuit expressed from a tricistronic design encoding:

- 1. crlL-15: enhances CAR-NK cell activity and persistence
- 2. Bivalent FLT3/CD33 activating CAR (aCAR): enables targeting of AML LSCs and blast cells 3. EMCN inhibitory CAR (iCAR): enables protection of healthy HSCs from off-tumor toxicity

#### FLT3 OR CD33 Logic Gated CAR-NK cells demonstrate:

- Enhanced cytotoxicity over more traditional FLT3 or CD33 single CAR approaches across multiple cell lines Significant in vitro cytotoxicity against AML patient tumor samples and cell lines
- Significant in vivo efficacy within AML (MOLM-13 and MV4-11) xenotransplantation models
- Bivalent FLT3/CD33 CARs possess significant killing over a bicistronic FLT3/CD33 CAR approach crlL-15 provides CAR-NK cells with enhanced killing activity and persistence

#### **NOT GATE Logic Gated CAR-NK cells demonstrate:**

- Significant in vivo protection of safety antigen-expressing target cells (model antigen = HER2)
- Multiple iCAR architectures are functionally active in FLT3 NOT EMCN CAR-NK cells Significant in vitro protection of healthy HSC/MPP cells from aCAR-mediated toxicity