Preclinical development of SENTI-202, an off-the-shelf logic gated CAR-NK cell therapy, for the treatment of CD33/FLT3+ hematologic malignancies including AML

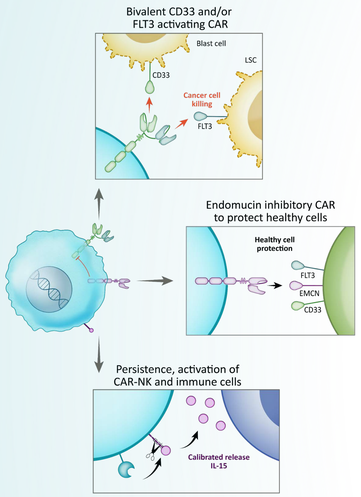
Senti Biosciences, Inc. South San Francisco, CA

Alba Gonzalez, Enping Hong, Gozde Yucel, Elizabeth Leitner, Pearley Chinta, Han Deng, Ian Li, Alice Lam, Abla Bakir, Brandon Lee, Papia Chakraborty, Carmina Blanco, Chen-Ting Lee, Niran Almudhfar, Mengxi Tian, Wenqi Song, Andrew Banicki, Otto Contreras, Martin Gieldin, Brian Garrison, Timothy K. Lu, Kanya Rajangam

AACR Annual Meeting 2023, Orlando, FL
Abstract #3195

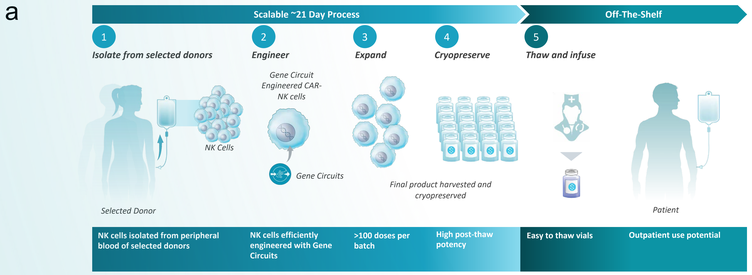
SENTI-202 Logic Gated CAR-NK cells

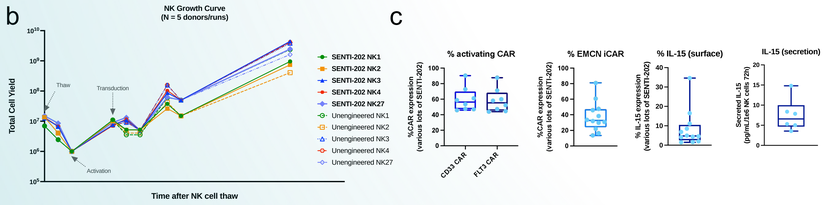
NOT Gated CAR-NK cells for the treatment of CD33/FLT3+ malignancies engineered to reduce on-target/off-tumor toxicities



Patients with CD33 and/or FLT3 expressing malignancies, including myeloid malignancies such as Acute Myeloid Leukemia (AML), Myelodysplastic Syndrome (MDS) or Multiple Myeloma (MM) have very poor prognosis and high clinical unmet need. CD33 and FLT3 are well validated targets for myeloid malignancies, but current therapies targeted against those antigens present considerable limitations. On one hand, the presence of CD33 negative leukemic stem cells (LSCs) can contribute to eventual relapse; on the other hand, expression of CD33 and/or FLT3 by the normal hematopoietic stem cells and progenitor cells (HSCs/HPCs) can lead to bone marrow toxicities, prolonged thrombocytopenia/ neutropenia, and infectious complications.

SENTI-202 is undergoing preclinical development to address these challenges and provide a broader therapeutic window, increasing the anti-tumor activity and safety for the treatment of CD33 and/or FLT3 malignancies. SENTI-202 is a first in class Logic-gated CAR-NK product engineered with an OR and a NOT Logic Gate gene circuit approach to enhance therapeutic efficacy and safety, with additional arming via expression of calibrated release IL-15 (crIL-15). A dual targeting activating CAR (aCAR) that recognizes both CD33 and FLT3 tumor antigens improves the anti-tumor activity, ensuring the targeting of AML blasts and LSCs. Additionally, an inhibitory CAR (iCAR) that recognizes endomucin (EMCN), a protective antigen expressed on the surface of healthy cells prevents the CAR-mediated cytotoxicity of healthy target cells including HSCs/HSPCs, improving the safety potential and reducing on-target/off-tumor toxicities. crIL-15 provides NK cell activation and persistence.

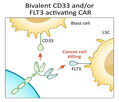




(a) SENTI-202 is an off-the-shelf cell therapy that uses healthy donor derived NK cells from peripheral blood mononuclear cells (PBMCs) that are expanded, engineered, and characterized in our optimized GMP-suitable process. All SENTI-202 components are stably expressed within a single expression g-retroviral vector. (b) Expansion and (c) expression of all components of the gene circuit is shown in multiple lots of SENTI-202.

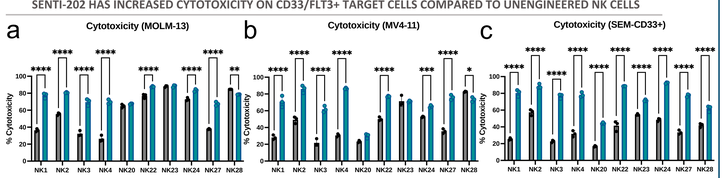
Preclinical Pharmacology of SENTI-202

In vitro cytotoxicity and cytokine production of SENTI-202



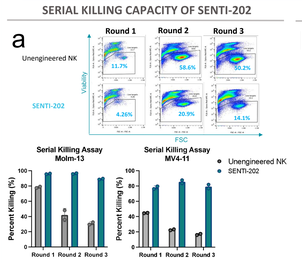
- Unengineered NK
- SENTI-202

SENTI-202 HAS INCREASED CYTOTOXICITY ON CD33/FLT3+ TARGET CELLS COMPARED TO UNENGINEERED NK CELLS

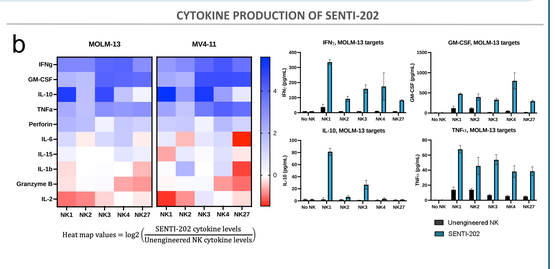


Various lots of SENTI-202 manufactured using different NK donors were used for in vitro cytotoxicity assays. SENTI-202 demonstrated increased cytotoxicity against the AML target cell lines (a) Molm-13, (b) MV4-11, and (c) the SEM cell line engineered to express CD33 (SEM-CD33+) compared to donor-matched unengineered NK cells.

SERIAL KILLING CAPACITY OF SENTI-202

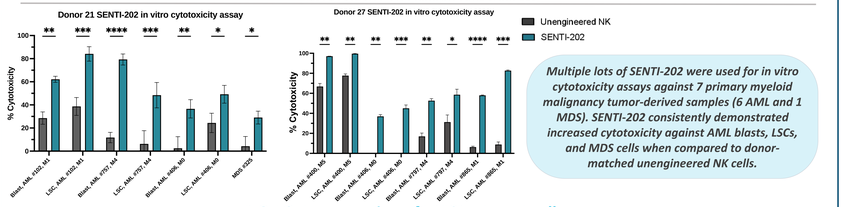


CYTOKINE PRODUCTION OF SENTI-202



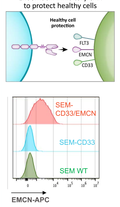
(a) SENTI-202 demonstrated increased serial killing capacity, retaining the ability to kill AML target cells over multiple rounds of rechallenge. (b) SENTI-202 also demonstrated increased cytokine and cytotoxic molecule production during cytotoxicity assays compared to unengineered NK cells.

CYTOTOXICITY ON PRIMARY AML SAMPLES

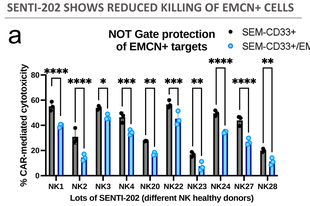


Multiple lots of SENTI-202 were used for in vitro cytotoxicity assays against 7 primary myeloid malignancy tumor-derived samples (6 AML and 1 MDS). SENTI-202 consistently demonstrated increased cytotoxicity against AML blasts, LSCs, and MDS cells when compared to donor-matched unengineered NK cells.

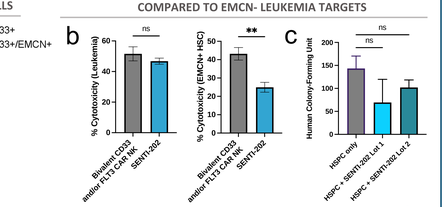
NOT Gate protection of EMCN+ target cells



SENTI-202 SHOWS REDUCED KILLING OF EMCN+ CELLS



SENTI-202 SHOWS PROTECTION OF EMCN+ HSCs COMPARED TO EMCN- LEUKEMIA TARGETS

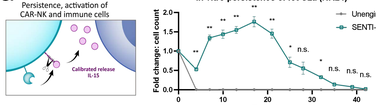


Various lots of SENTI-202 were used for in vitro cytotoxicity assays against target cells with or without expression of the protective antigen EMCN. (a) SENTI-202 showed reduced cytotoxicity when co-cultured with EMCN+ target cells, and (b) demonstrated protection of EMCN+ HSCs, (c) while preserving the myeloid colony forming activity of the protected HSCs.

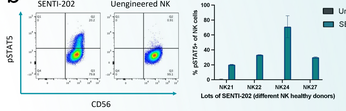
Pharmacokinetics and Pharmacodynamics of SENTI-202

In vitro persistence and pSTAT5 signaling

SENTI-202 CAR-NK CELLS HAVE INCREASED PERSISTENCE IN VITRO



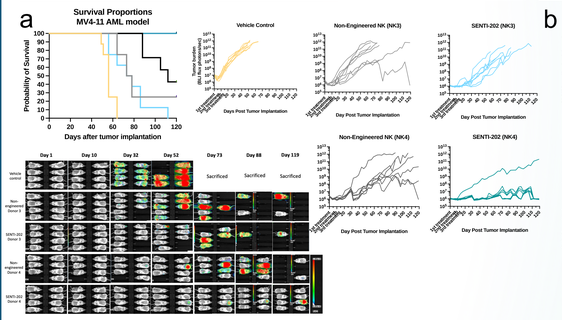
SENTI-202 CAR-NK CELLS HAVE INCREASED PSTAT5



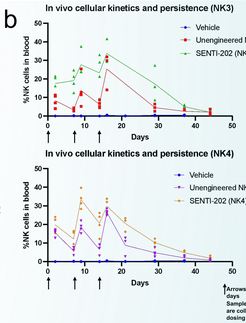
SENTI-202 is engineered with crIL-15, which releases wild type IL-15 by the action of endogenous proteases to provide autocrine and paracrine support to SENTI-202 and other immune cells. Consistent with this feature, (a) SENTI-202 has increased persistence compared to unengineered NK cells when cultured in cytokine-free conditions, and (b) multiple lots of SENTI-202 demonstrated endogenous phospho-STAT5 signaling by flow cytometry.

SENTI-202 in vivo anti-tumor function and pharmacokinetics

SENTI-202 REDUCES TUMOR AML TUMOR GROWTH AND PROLONGS MICE SURVIVAL



SENTI-202 IN VIVO KINETICS AND PERSISTENCE



In vivo treatment of SENTI-202 (a) decreases MV4-11 tumor burden and prolongs survival, with donor-to-donor differences in in vivo performance. (b) SENTI-202 cellular PK is evaluated in vivo via flow cytometry, showing persistence over multiple doses and eventual clearance.

Summary and next steps

SENTI-202 is a First In Class OR/NOT Logic Gated Off-The-Shelf CAR-NK Cell Therapy

SENTI's novel Logic-gated gene circuits have enhanced tumor targeting. The CD33/FLT3 OR Gate activating CAR successfully enables the targeting of primary AML blasts, LSCs, and MDS cells, while the NOT Gate decreases the killing of HSCs while preserving their function by an inhibitory CAR that detects a protective antigen, EMCN, found in healthy HSCs.

Preclinical development and in-house manufacturing capabilities

SENTI's state of the art manufacturing facility and optimized GMP-compatible process enables large scale production of SENTI-202 product from various healthy NK donors. Selected NK donors have been evaluated pre-clinically with demonstrated activity and tolerability. IND-enabling studies are underway and on track for IND-submission in 2023.

SENTI-202 clinical evaluation

Phase 1 dose-escalation evaluation of SENTI-202 safety and efficacy is being planned in patients with CD33 and/or FLT3 expressing malignancies.

Contact: kanya.rajangam@sentibio.com