Preclinical data supporting the Phase 1 trial design of SENTI-202, a next generation allogeneic logic-gated selective CAR-NK cell therapy, engineered to overcome key limitations of first generation cell therapies in AML

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Abstract

First generation natural killer (NK) and chimeric antigen receptor (CAR) NK cell products are well-tolerated and have clinical activity (~20-60% CR) in patients with AML. CRs generally increase with the inclusion of CARs or cytokines, increased cell doses, and when Flu/Ara-C lymphodepletion (LD) is used for conditioning. Key clinical limitations have been low PK of the infused NK cells, short durability of the observed responses potentially due to immune evasion of leukemic stem cells (LSCs), and manufacturing challenges precluding higher or multiple doses.

SENTI-202 is a next generation allogeneic logic-gated selective CAR NK cell therapy specifically designed to address these limitations and to augment endogenous NK anti-AML activity. SENTI-202 expresses a bivalent activating CAR (aCAR) that targets CD33 and FLT3 on bulk AML blasts and LSCs. As FLT3 is also expressed on healthy hematopoietic stem cells (HSCs), SENTI-202 is further engineered to express an inhibitory CAR that recognizes endomucin expressed on HSCs, thereby conferring protection from aCAR-mediated cytotoxicity even in the presence of FLT3/CD33. SENTI-202 expresses a calibrated-release IL15 that provides both auto- and paracrine cytokine support, mediating expansion, activation, and persistence of SENTI-202 and host immune cells.

SENTI-202 demonstrated robust and specific killing of primary AML blasts, LSCs, and AML cell lines in vitro. SENTI-202 protected HSCs from CAR-mediated cytotoxicity while preserving their function. In vivo, SENTI-202 revealed robust efficacy that increased with higher E:T ratio and with 3 weekly vs 1 dose. Preclinically, 3 doses of SENTI-202 that were > 60-fold the planned starting clinical trial dose were well tolerated in acute and chronic toxicology studies with no CAR NK-related body weight, laboratory, or histopathology findings. SENTI-202 non-clinical PK revealed greater than dose proportional exposure, which was ~2-fold greater compared to non-engineered NK cells. Pretreatment of CD33/FLT3 negative AML cell lines with Ara-C resulted in upregulation of CD33 and FLT3 expression, sensitizing cells to robust SENTI-202-mediated killing, providing additional rationale for the use of Ara-C-based LD. In the presence of exogenous IL2, persistence, cytotoxicity, and serial killing activity of SENTI-202 were increased, supporting the use of low dose IL2 to further augment SENTI-202 clinical activity.

Taken together, these results support the Phase 1 trial design of SENTI-202-101 in patients with R/R CD33 and/or FLT3 positive malignancies including AML, which uses Flu/Ara-C as LD followed by 3 weekly doses of SENTI-202 and includes the option of enrolling patients into cohorts that additionally receive low dose IL2 following SENTI-202 administration.

SENTI-202 demonstrates cytotoxicity against AML/MDS and protection of healthy stem and progenitor cells

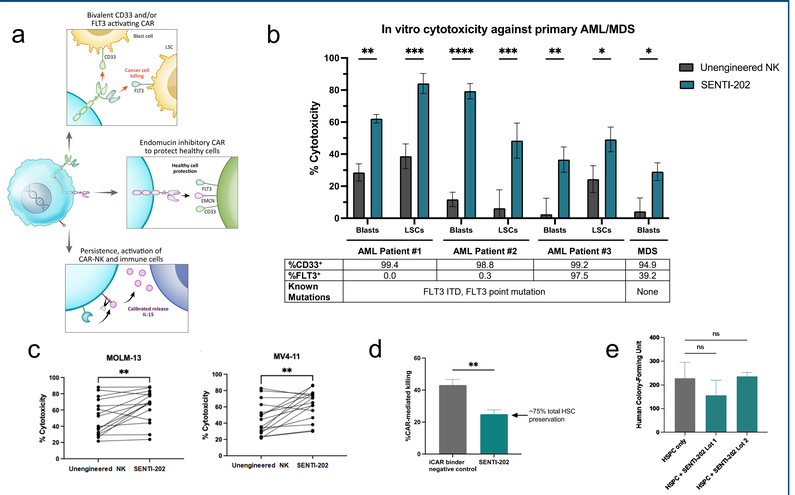
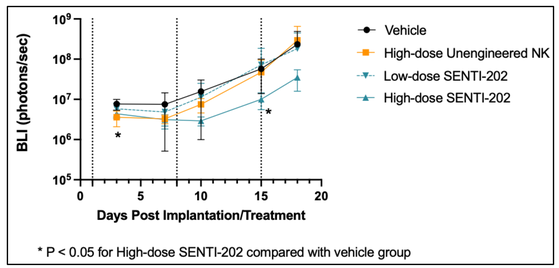
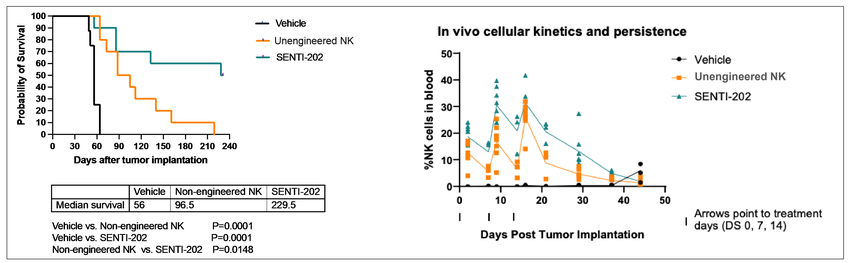


Figure 1. (a) SENTI-202 is an off-the-shelf, logic gated gene circuit enabled CAR NK cell therapy with three engineered components: a bivalent activating CAR against validated AML specific targets CD33 and/or FLT3 (OR gate), an inhibitory CAR recognizing endomucin (EMCN) (NOT gate), and calibrated release IL15 (crIL15). (b) SENTI-202 demonstrated robust killing of primary AML blasts, LSCs, and MDS cells derived from patient samples. (c) Multiple lots of SENTI-202 also showed killing of the AML cell lines MOLM-13 and MV4-11. (d) SENTI-202 preserved the viability of 75% of HSCs after co-culture, and (e) further preserved the colony-forming function of HSPCs. Various statistical tests were used in this figure. ns = not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

SENTI-202 demonstrates significant in vivo anti-tumor activity in mouse models of AML





	Vehicle	Non-engineered NK	SENTI-202
Median survival	56	96.5	229.5

Vehicle vs. Non-engineered NK P=0.0001
Vehicle vs. SENTI-202 P=0.0001
Non-engineered NK vs. SENTI-202 P=0.0148

Figure 2. (a) A higher E:T ratio is important for in vivo SENTI-202 anti-tumor activity. High-dose (15 x 10^6 CAR+ cells), but not low-dose (7.5 x 10^6 CAR+ cells), SENTI-202 showed significantly higher anti-tumor activity than vehicle control and unengineered NK cells in the MV4-11 AML mouse model. (b) Three weekly doses, but not a single dose (separate but similar study, not shown), of SENTI-202 significantly improved survival compared to vehicle and unengineered NK cells in the MV4-11 AML mouse model. In general, higher levels (~2-fold) of SENTI-202 than unengineered NK cells were detected in mouse peripheral blood (right panel). Here, SENTI-202 was dosed IV at a dose level that was >60-fold higher than the Phase 1 clinical starting dose of 1 x 10^9 CAR+ cells. No adverse clinical symptoms were observed when mice were dosed with SENTI-202 at the planned clinical dosing regimen (3 weekly IV doses). Treatment with SENTI-202 was well-tolerated, with no significant differences in treatment-associated body weight changes.

IL-2 further enhances SENTI-202 function and persistence

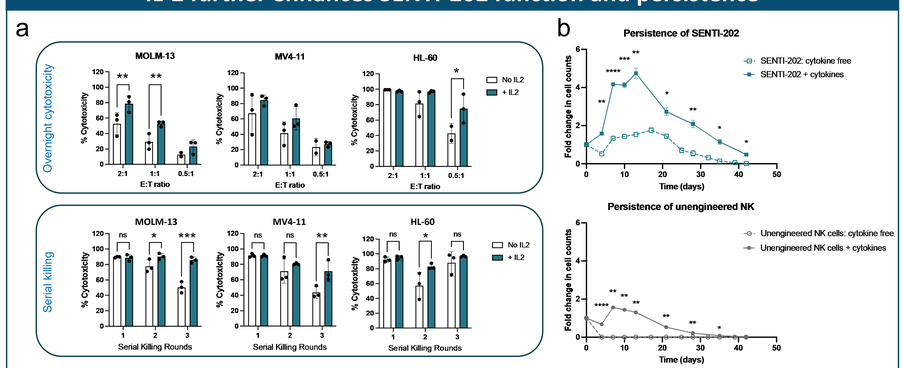


Figure 3. The addition of exogenous IL-2 enhanced the cytotoxicity and persistence of SENTI-202 against multiple AML cell lines. (a) The addition of IL-2 in an overnight cytotoxicity assay significantly enhanced the killing of MOLM-13 and HL-60 cell lines (top panel), and its addition during a serial rechallenge assay significantly augmented the killing of MOLM-13, MV4-11, and HL-60 cell lines, particularly during the later rounds of serial killing (bottom panel). (b) The addition of the cytokines IL-2 and IL-15 (which signal through the same receptor complex) enhanced the in vitro persistence of both SENTI-202 and unengineered NK cells, substantially increasing the peak proliferation of SENTI-202. Statistical analysis performed with multiple unpaired t-tests. ns = not significant; * p < 0.05; ** p < 0.01; *** p < 0.0001.

Ara-C treatment upregulates CD33 and FLT3 expression in KG-1a AML cells, sensitizing them to SENTI-202-mediated cytotoxicity

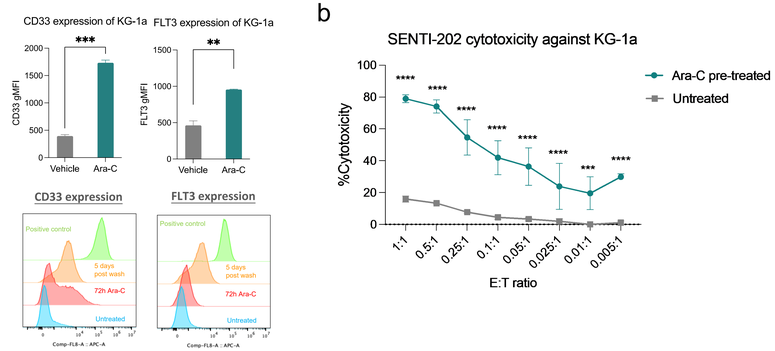


Figure 4. (a) Treatment of the CD33/FLT3 non-expressing cell line KG-1a with the AML standard of care chemotherapy drug Ara-C significantly increased the expression of both CD33 and FLT3 (top panel). This increase was durable even after Ara-C was washed away from target cells (bottom panel). (b) Ara-C pre-treatment significantly sensitized KG-1a cells to SENTI-202-mediated cytotoxicity.

SENTI-202-101 Phase 1 clinical trial design, with starting dose of 1 x 10^9 CAR+ NK cells weekly for 3 weeks per 28-day cycle following disease specific Flu/Ara-C lymphodepletion (ClinicalTrials.gov ID NCT06325748)

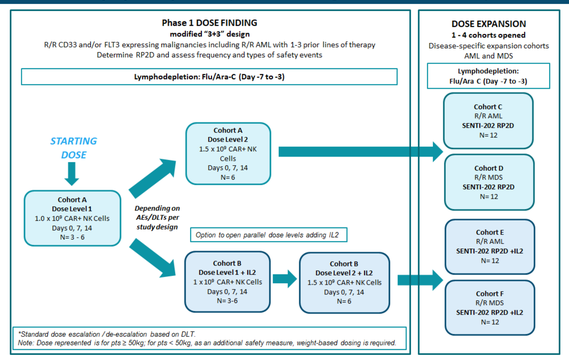


Figure 5. "3+3" Phase 1 clinical trial design of SENTI-202-101, enrolling patients with R/R CD33 and/or FLT3 malignancies with a focus on AML

Summary and next steps

- SENTI-202 is an AML target specific CAR NK that demonstrated robust and specific killing of primary AML blasts, LSCs, and AML cell lines in vitro.
- SENTI-202 protected HSCs from CAR-mediated cytotoxicity while preserving their function.
- SENTI-202 showed robust in vivo anti-tumor activity that increased with higher E:T ratio and with 3 weekly doses vs 1 dose.
- 3 doses of SENTI-202 that were > 60-fold the planned starting clinical trial dose were well-tolerated.
- Pretreatment of CD33/FLT3 negative AML cell lines with Ara-C resulted in upregulation of CD33 and FLT3 expression, sensitizing cells to SENTI-202-mediated killing, providing additional rationale for the use of Ara-C-based LD.
- IL-2 enhanced the cytotoxicity, serial killing, and persistence of SENTI-202, supporting the use of low dose IL2 to further augment SENTI-202 clinical activity.
- These results support the Phase 1 trial design of SENTI-202-101 in patients with R/R CD33 and/or FLT3 positive hematologic malignancies including AML, which uses Flu/Ara-C as LD followed by 3 weekly doses of SENTI-202 and includes the option of enrolling patients into cohorts that additionally receive low dose IL-2 following SENTI-202 administration.
- The SENTI-202-101 trial **(ClinicalTrials.gov ID NCT06325748)** is commencing enrollment in Q2 2024.

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