Calibrated Release IL15-Expressing Bivalent CD33 and/or FLT3 Logic Gated Gene Circuit CAR-NK Cell Therapy (from SENTI-202 gene circuit) in Venetoclax Resistant Patient Derived Xenograft Acute Myeloid Leukemia Models

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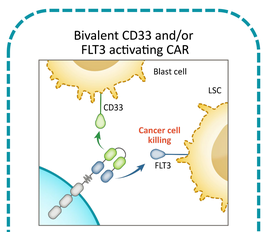
SENTI-202 in Targeting Venetoclax Resistant AML: Efficacy and Strategies

Background: Venetoclax (VEN) as combination therapy has improved response rates and overall survival of patients with acute myeloid leukemia (AML). However, once AML relapses, prognosis is dire with a 5.3 month median survival (Brandwein, 2020). While chimeric antigen receptor (CAR) cell therapies have revolutionized the treatment landscape for B- cell malignancies, development of such therapies for AML has been challenging, in part due to the heterogeneity of the disease. Currently, there is a paucity of individual AML targets that are consistently expressed across AML subpopulations. Furthermore, the expression of these AML targets is not restricted to tumor cell populations, often resulting in off-tumor toxicity against healthy cell populations. SENTI-202 employs OR and NOT Logic Gating along with a calibrated release IL-15 cytokine to overcome these challenges.

SENTI-202 represents an innovative preclinical CAR-NK cell therapy, engineered to exploit a powerful CD33 OR FLT3 NOT EMCN Logic Gate gene circuit, in conjunction with calibrated release IL-15 (crIL15) expression. The CD33 OR FLT3 (OR GATE) activating CAR (aCAR) concurrently targets two AML antigens, thus permitting a broader therapeutic window against potentially VEN-sensisitive (VEN-S) AML LSCs (CD33+/-, FLT3+), blasts (CD33+, FLT3+/-), and more specifically, VEN-resistant (VEN-R) AML with a CD33+ monocytic phenotype (Pei, 2020). The NOT EMCN (NOT GATE) inhibitory CAR (iCAR) is a pivotal safeguard, selectively shielding healthy EMCN+ Hematopoietic Stem Cells and Progenitor Cells (HSCs/PCs) from potential off-tumor toxicity. Previously, SENTI-202 exhibited remarkable efficacy in targeting and eliminating CD33 and/or FLT3 expressing AML cell lines, while concurrently safeguarding healthy HSCs/HSPCs (Garrison et al., ASH, 2022).

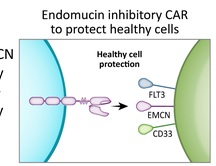
In this presentation, we elucidate the anti-tumor potential of SENTI-202 by using NK cells engineered with only the portion of the SENTI-202 gene circuit responsible for enhancing the capacity of NK cells to recognize and kill tumor cells: (1.) bivalent CD33 and/or FLT3 CAR, and (2.) calibrated release IL15 (crIL15). These cells are referred to as *CD33/FLT3 CAR (crIL15) NK cells* and were assessed in clinically relevant preclinical AML models and VEN-R AML patient-derived xenograft (PDX) models.

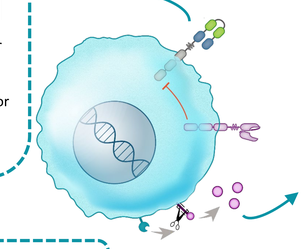
SENTI-202: Mechanism of Action

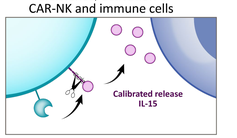


OR GATE: *bivalent* CD33 and/or FLT3 activation → designed to target both AML LSCs and AML blasts to enable the potential for deep and durable responses in acute myeloid leukemia (AML) and other blood cancers

NOT GATE: inhibition by EMCN protective antigen selectively expressed on healthy cells → potential for improved safety and increased therapeutic window







crIL-15 → potential for increased persistence, autocrine and paracrine immune cell activation

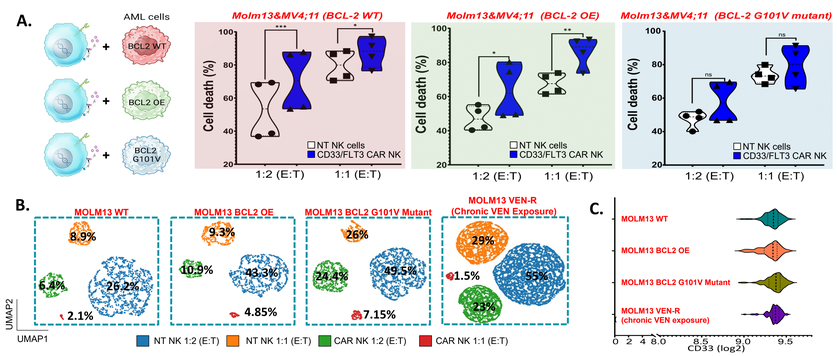
Focus of this presentation:
CD33/FLT3 CAR (crIL15) CAR NK cells

Experimental Approaches

Effector Cells	Target Cells	Modalities
Non-Transduced (NT) NK cells	VEN-S AML Cells, VEN-R AML Cells	In vitro, In vivo
CD33/FLT3 CAR (crIL15) NK Cells	AML PDX Cells, Primary AML Cells	Single-cell analysis

Bivalent CD33/FLT3 CAR (crIL15) NK Cells Are Highly Effective Against VEN Resistant AML Cells

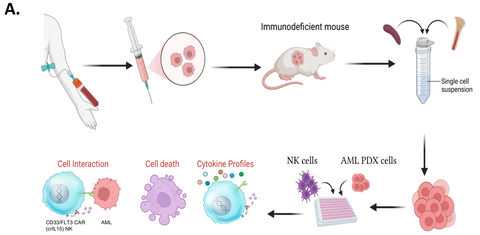
CD33/FLT3 CAR (crIL15) NK Demonstrate Antigen-dependent Cytotoxicity Against VEN-R AML Cells



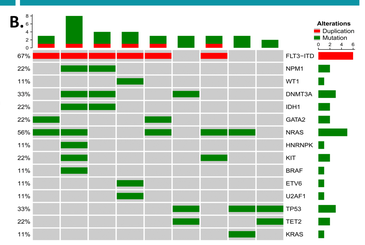
CD33/FLT3 CAR (+crIL15) NK cells demonstrate significant, *in vitro* antigen dependent cytotoxicity against VEN-R AML cells. (A.) Molm13 and MV4-11 AML cell lines, including wild-type (VEN sensitive), BCL2-overexpressing, and G101V-mutated variants, were co-cultured with CD33/FLT3 CAR NK and NT NK cells. (B.) Uniform Manifold Approximation and Projection (UMAP) analysis was conducted to visualize live AML cells after a 24-hour co-culture with either CD33/FLT3 CAR NK or NT NK cells. (C.) VEN-R Molm13 cells continued to express high levels of CD33. p-value: *0.01-0.05, **=0.001-0.01, ***=0.0001-0.001, ****=<0.0001

In Vitro Testing of CD33/FLT3 CAR (crIL15) NK Cell Against PDX AML Cells

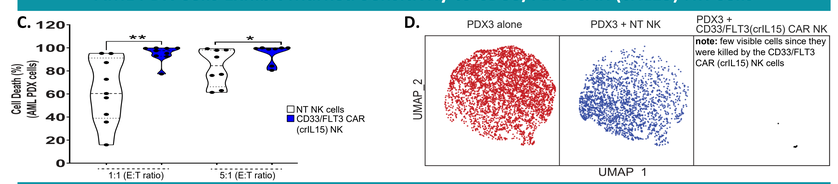
Schema for Evaluating CAR NK Cells Against AML PDXs



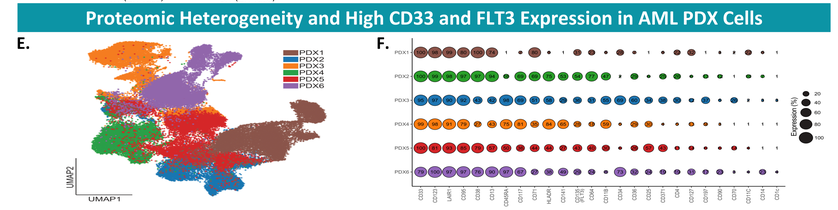
Mutational Profiles of AML PDXs



AML PDX Cells Exhibit Marked Sensitivity to CD33/FLT3 CAR (crIL15) NK Cells



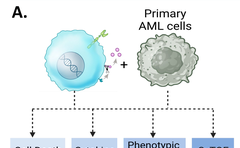
Proteomic Heterogeneity and High CD33 and FLT3 Expression in AML PDX Cells



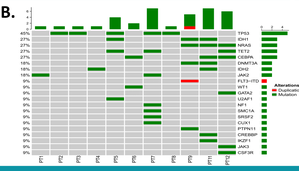
In Vitro Workflow for Assessing CD33/FLT3 CAR (crIL15) NK Efficacy Against AML PDX Cells. (A.) Schematic for evaluating CD33/FLT3 CAR (crIL15) NK efficacy *in vitro* against AML PDX cells(n=9). (B.) Mutation profiles of AML PDX cells. (C.) CD33/FLT3 CAR (crIL15) NK shows significant cytotoxicity against primary AML cells. (D.) UMAP visualization of live PDX cells after co-culture with mock, NT NK, or CD33/FLT3 CAR (crIL15) NK cells. (E.) UMAP visualization demonstrates heterogeneous AML proteomic profiles across 6 PDXs and (F.) Protein expression profiles of surface protein listed on x-axis.
p-value: *0.01-0.05, **=0.001-0.01, ***=0.0001-0.001, ****=<0.0001

CD33/FLT3 CAR (crIL15) NK Induced Significant Cell Death in Primary AML Cells

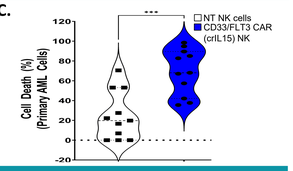
Approach



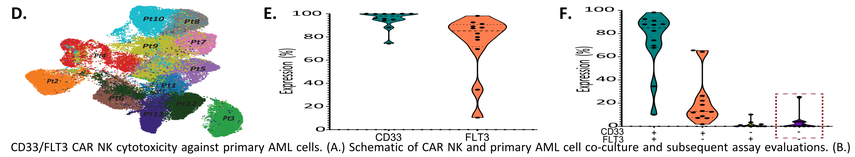
Mutation Profiles of Primary AML Samples



Significant CD33/FLT3 CAR NK Activity Against 1° AML Blasts

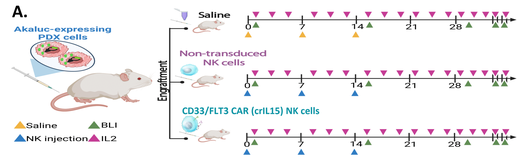


Proteomic Heterogeneity and High CD33 and FLT3 Expression in Primary AML Cells

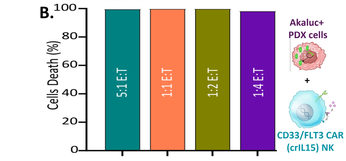


CD33/FLT3 CAR NK cytotoxicity against primary AML cells. (A.) Schematic of CAR NK and primary AML cell co-culture and subsequent assay evaluations. (B.) Mutation profiles of primary AML samples. (C.) CAR NK Elicits Pronounced Cytotoxic Effects on Primary AML Cells (n=12). (D.) UMAP visualization of the AML proteomic profile across 12 AML patients. (E.) Expression of CD33 and FLT3 on AML blast. Each dot represents an individual patient. (F.) Co-expression Analysis of CD33 and FLT3 across patient samples. Each dot represents an individual patient

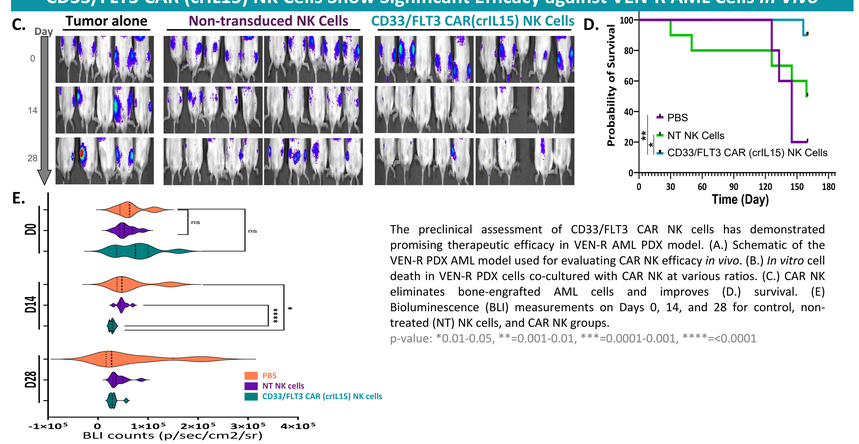
VEN-R PDX Model Schema



CD33/FLT3 CAR(crIL15) NK Cytotoxicity against VEN-R AML PDX1



CD33/FLT3 CAR (crIL15) NK Cells Show Significant Efficacy against VEN-R AML Cells *In Vivo*



The preclinical assessment of CD33/FLT3 CAR NK cells has demonstrated promising therapeutic efficacy in VEN-R AML PDX model. (A.) Schematic of the VEN-R PDX AML model used for evaluating CAR NK efficacy *in vivo*. (B.) *In vitro* cell death in VEN-R PDX cells co-cultured with CAR NK at various ratios. (C.) CAR NK eliminates bone-engrafted AML cells and improves (D.) survival. (E) Bioluminescence (BLI) measurements on Days 0, 14, and 28 for control, non-treated (NT) NK cells, and CAR NK groups.
p-value: *0.01-0.05, **=0.001-0.01, ***=0.0001-0.001, ****=<0.0001

Summary

The CD33/FLT3 CAR and crIL15 components of the SENTI-202 gene circuit were tested against clinically relevant preclinical AML models and VEN-R AML patient-derived xenograft (PDX) models. VEN-R AML has extremely high unmet need with no approved therapies.

- **CD33/FLT3 CAR (crIL15) NK cell therapy demonstrated significant therapeutic efficacy against VEN-R AML cells *in vitro* and *in vivo*.**
- **Additionally, CD33/FLT3 CAR (crIL15) NK cells additionally demonstrated significant:**
  - *in vitro* cytotoxicity against AML cells with genetically and chemically induced VEN resistance
  - preclinical activity against primary AML cells from different genetic makeups
  - *in vitro* cytotoxicity against AML PDX cells
  - *in vivo* activity in VEN-R AML PDX model, resulting in reduction in tumor burden and significantly improved survival

SENTI-202 represents a promising approach for helping relapsed and/or refractory AML patients and is being developed for future clinical applications.