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Multi-Arming and Regulator Dial Gene Circuits to Address Key Disease Challenges in HCC

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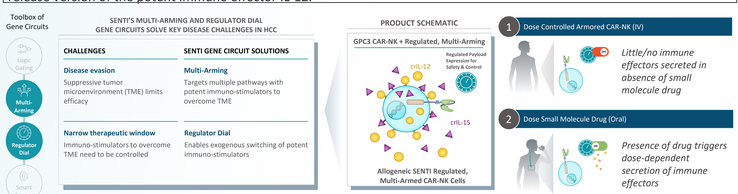
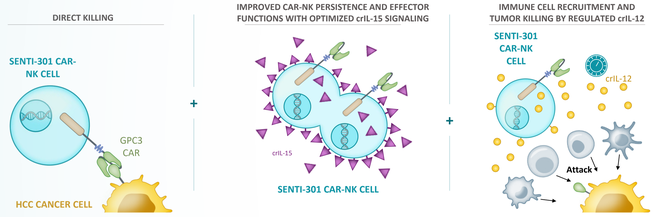
SENTI-301 Aims to Safely Overcome the Immunosuppressive Tumor Microenvironment for Patients with R/R HCC

Background: Hepatocellular carcinoma (HCC) is the 6th most common cancer worldwide and the 2nd leading cause of cancer associated mortality, it represents a major health problem, and its effective treatment is a critical unmet need. Successful cell therapies, including chimeric antigen receptor (CAR)-T and CAR-NK cells, for liquid tumors have yet to translate into solid tumors. A major obstacle for cell therapy in solid tumors is the immunosuppressive tumor microenvironment (TME), which can impair the function of endogenous as well as therapeutic immune cells. To overcome this challenge, Senti Bio is developing SENTI-301, a multi-armed CAR-NK therapy with proprietary **calibrated release (cr) interleukin (IL)-15** (crIL-15) and a Regulator Dial gene circuit to strictly control the expression of the proprietary cleavable release version of the potent immune effector IL-12.

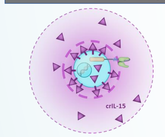


SENTI-301 Multi-Arming Approach is Designed to Attack Cancer in Multiple Complementary Ways



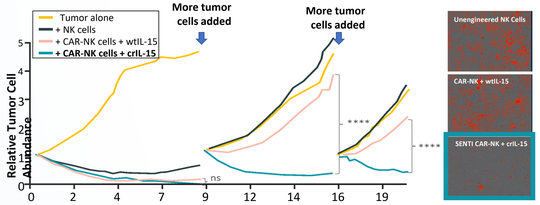
SENTI's crIL-15 Enhances NK Cell Persistence and Tumor Killing

crIL-15 Enables Both Autocrine and Paracrine Signaling



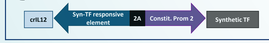
Senti Bio's novel crIL-15 technology enables both autocrine and paracrine signaling, by simultaneously expressing membrane-bound and secreted IL-15. IL-15 is tethered at the membrane via a linker that is recognized and cleaved by endogenous proteases, releasing secreted IL-15.

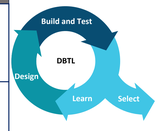
crIL-15 Improves CAR-NK Cell Persistence and Serial Killing



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 secondary and tertiary re-challenges.
Microscopy images from the tertiary re-challenge show Senti Bio's CAR-NK + crIL-15 cells with enhanced killing (red=tumor cells).

SENTI-301 Screening Strategy Using Senti's Synthetic Biology Platform

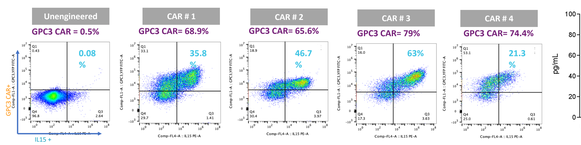
	Parameter Tested	# of constructs Tested
Vector 1 	• Promoter • CAR co-stim • Alternative IL-15 sequences • Vector Backbone • UTR modifications	156
Vector 2 	• Promoter • Zinc-fingers • Alternative crIL-12 • Effector Domain Orientation • Vector Backbone	90



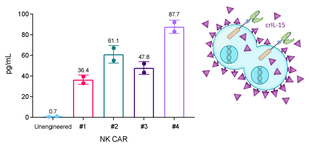
SENTI-301 is an allogeneic product of NK cells engineered with two retroviral vectors to express a GPC3 CAR and a crIL-15 (vector 1), and a Regulator Dial gene circuit containing a synthetic transcription factor, with a genome-orthogonal zinc finger DNA binding domain linked to a transcriptional activation domain via an NS3 protease and an NS3 protease–cleavable linkers, and a Regulator-Dial TF-responsive element to control expression of crIL-12 by grazoprevir (GRZ), an FDA-approved NS3 inhibitor (NS3i), for precise and tunable control of dose, timing and duration of crIL-12 expression.
We screened 156 constructs to optimize expression of GPC3-CAR and crIL-15 (vector 1) and 90 constructs to optimize the Regulator Dial gene circuit (vector 2) to control expression of potent immune effector, crIL-12.

Vector 1: Expression and *In Vitro* Functional Analysis of Top Constructs

GPC3 CAR and membrane-bound crIL-15 expression

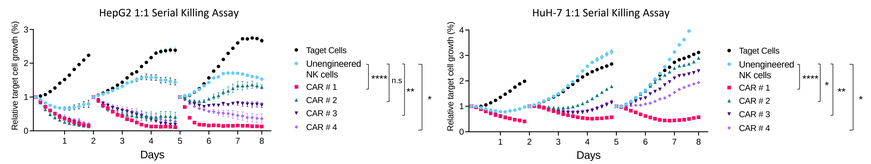


Secreted crIL-15



Senti Bio's four top V1 constructs demonstrated high GPC3 CAR expression (>50%), membrane associated crIL-15 (>20%), secreted crIL-15

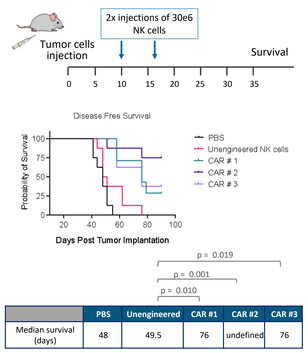
GPC3 CAR-crIL-15 Serial functional assay



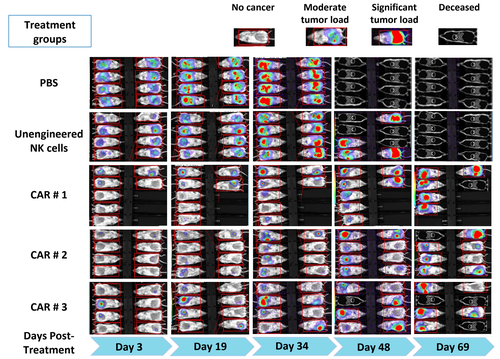
NK cells transduced with GPC3 CAR-crIL-15 NK cells demonstrate improved serial killing compared to unengineered NK cells.

GPC3 CAR- crIL-15 *In Vivo* Antitumor Function

HCC Intraperitoneal (IP) Xenograft Model

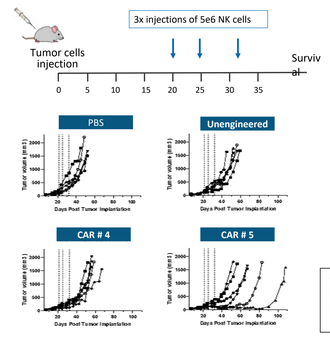


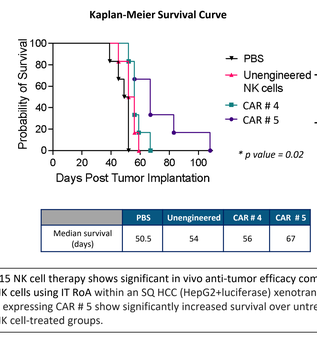
	PBS	Unengineered	CAR #1	CAR #2	CAR #3
Median survival (days)	48	49.5	76	undefined	76



GPC3 CAR- crIL-15 NK cell therapy shows statically significant *in vivo* anti-tumor efficacy compared to unengineered NK cells in an IP HCC (HepG2+luciferase) xenotransplantation model. All 3 groups treated with GPC3-crIL-15 engineered NK cells show significant increased survival over untreated (PBS) and unengineered NK cell-treated groups.

HCC subcutaneous xenograft model





	PBS	Unengineered	CAR # 4	CAR # 5
Median survival (days)	50.5	54	56	67

GPC3 CAR- crIL-15 NK cell therapy shows significant in vivo anti-tumor efficacy compared to unengineered NK cells using IT RoA within an SQ HCC (HepG2+luciferase) xenotransplantation model. NK cells expressing CAR # 5 show significantly increased survival over untreated (PBS) and unengineered NK cell-treated groups.

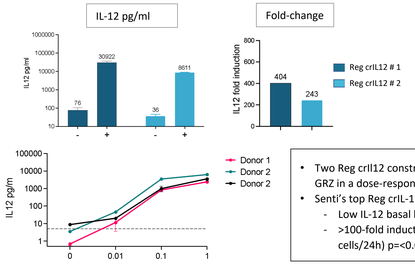
Senti Bio's Regulator Dial Enables Physician Real-Time Control of Protein Expression *In Vivo* to Improve Efficacy and Reduce Side Effects

1. Regulator DNA binding domain is linked to the transcriptional activation domain by a protease cleavable linker. When the protease is active, the DNA binding domain is not linked to the transcriptional activation domain, and is unable to activate transcription = **expression is OFF**

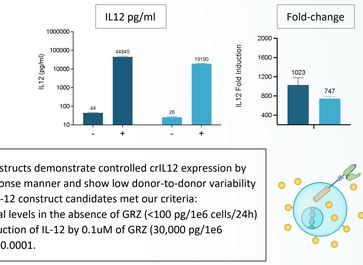
2. NS3i small molecule suppresses protease activity, resulting in an intact transcription factor. Regulator Dial transcription factor can activate transcription = **expression is ON**

Regulated crIL-12

Reg crIL-12 (V2) NK cell + GRZ (24 hours)



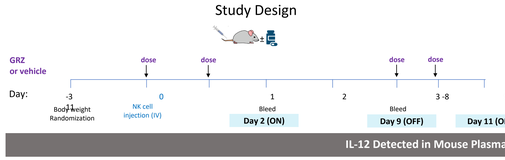
Reg crIL-12 (V2) NK cells + GRZ (72 hours)



- Two Reg crIl12 constructs demonstrate controlled crIL12 expression by GRZ in a dose-response manner and show low donor-to-donor variability
- Senti's top Reg crIL-12 construct candidates met our criteria:
  - Low IL-12 basal levels in the absence of GRZ (<100 pg/1e6 cells/24h)
  - >100-fold induction of IL-12 by 0.1uM of GRZ (30,000 pg/1e6 cells/24h) p=<0.0001.

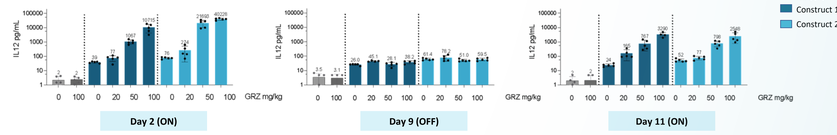
Regulator Dial Enables ON/OFF/ON Regulation of IL-12 *In Vivo*

Study Design



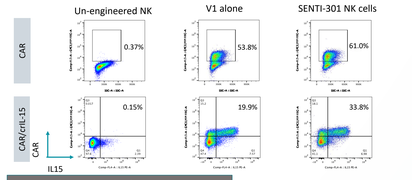
- IL-12 OFF → ON: On day 2, mice treated with GRZ show significant IL-12 induction in a GRZ dose dependent manner
- IL-12 ON → OFF: There was no significant difference between the vehicle and GRZ groups at day 9
- IL-12 OFF → ON: Significant induction of IL-12 via GRZ compared to vehicle control

IL-12 Detected in Mouse Plasma

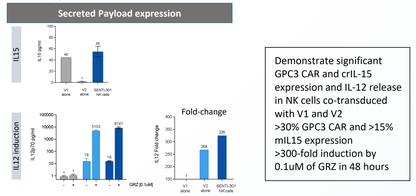


SENTI-301 Gene Circuits Expression in NK Cells

Membrane-Bound Protein expression



Secreted Payload expression



Demonstrate significant GPC3 CAR and crIL-15 expression and IL-12 release in NK cells co-transduced with V1 and V2
>30% GPC3 CAR and >15% mIL15 expression
>300-fold induction by 0.1uM of GRZ in 48 hours

Summary

- SENTI-301 is an allogeneic multi-armed CAR-NK cell therapy with a Regulator Dial gene circuit designed to tackle existing challenges of current immunotherapies for the solid tumor indication, HCC.
- SENTI-301 provides a potentially improved, safe and more efficacious treatment option for patients with HCC.

- **SENTI-301 is an allogeneic NK cell product including:**
  1. **GPC3 CAR**: to enable targeting of HCC cells.
  2. **crIL-15**: to enhance CAR-NK cell activity and persistence.
  3. **crIL-12**: to remodel the TME via the recruitment and activation of immune cells, as well as the inhibition or reprogramming of immunosuppressive cells.
  4. **Regulator Dial**: to strictly regulate the expression of the potent immune effector IL-12 under the control of an FDA-approved small molecule.

- SENTI-301 gene circuit constructs were screened through Senti Bio's Design-Build-Test-Learn engine:
  1. **crIL-15 demonstrated enhanced killing activity and persistence of GPC3 CAR-NK cells**
  2. **The Regulator Dial crIL-12 demonstrated significant secretion of IL-12 in GRZ-dependent manner, while showing no/low secretion of IL-12 in the absence of this drug. This optimized gene circuit provides tunable ON/OFF/ON expression kinetics.**

- NK cells co-transduced with the GPC3 CAR-crIL-15 and Regulator Dial crIL-12 vectors successfully express all the SENTI-301 components