Selection of Lead Development Off-the-shelf CAR NK Candidate, SENTI-301A, for GPC3 Program and Expansion of Regulator Dial Gene Circuits to Tackle Key Challenges In Solid Tumors

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Abstract #228

SENTI-301A is an allogeneic, Multi-Armed CAR NK Therapy Engineered to Address Unmet Needs in GPC3 Expressing Solid Tumors

Background:

Senti Bio is developing SENTI-301A, an allogeneic Multi-Armed CAR NK therapy to address unmet needs in the treatment of hepatocellular carcinoma (HCC) and other Glypican-3 (GPC3) expressing tumors.

GPC3 is a membrane-bound protein normally expressed in fetal tissues such as liver and placenta. After birth, GPC3 is not expressed in healthy liver tissue or other human organs but is overexpressed in different tumor types, notably hepatocellular carcinoma (HCC) (*Zheng 2022*).

HCC is the 6th most common cancer worldwide and the 2nd leading cause of cancer-associated mortality (*Llovet 2021*). Cell therapies, including chimeric antigen receptor (CAR) T and CAR NK cells, for liquid tumors have yet to translate into solid tumors such as HCC. A major obstacle for CAR NK cell therapy in solid tumors is the short survival and persistence of NK cells *in vivo*. Effective treatments for solid tumors will likely require novel and multi-functional approaches.

Clinical Unmet Need in GPC3 Expressing Solid Tumors

Unmet Need in Hepatocellular Carcinoma (HCC)

Each year, HCC affects 750,000–1 million people worldwide and is projected to be the third most common cause of cancer death in the US by 2030²

1 Villanueva 2019
2 Shrirakawa 2009

- SENTI-301A is designed to target GPC3 expressing tumors to:
  - address unmet need in HCC: lack of targeted therapies, lack of effective immunotherapies
  - tackle multiple other solid tumor that also express GPC3
- Large overall patient population sizes
- High share of patients with antigen expression

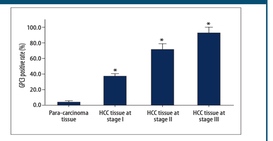
GPC3 Addressable Tumor Types			
Tumor Type	**Annual US Incidence (2014)**	**Est. % GPC3+**	**Clinical Trials for GPC3-Targeted Therapies**
Liver Cancer	42,810	~60-85%	30
Lung SCC	80,087	~45-55%	6
Gastric Cancer	22,220	~11%	3
Esophageal Cancer	18,440	~18%	2

Other common indications for GPC3 targeted therapy not listed in table are NSCLC, ovarian cancer, peritoneal cancer, fallopian tube cancer, and cholangiocarcinoma

Moek 2018

GPC3 Scientific Rationale

- Studies of patient samples have shown that GPC3 tends to be expressed on the cell membrane, making it a viable target for a CAR NK cell therapy product
- GPC3 expression correlates with aggressive and difficult to treat HCC tumors, and increases with advanced stage disease
- GPC3 is co-opted in HCC to promote cancer growth, thus making it more challenging for mutational escape to occur
- GPC3 is a heparan sulfate proteoglycan that acts as a co-receptor for Wnt, contributing to HCC proliferation



GPC3 expression in HCC tissue correlates with negative prognosis. * p< 0.01, compared with para-carcinoma tissue

Sun 2017

SENTI-301A Gene Circuit

To ensure robust NK cell engineering and enable the co-expression of the two distinct genes in SENTI-301A, notably the **GPC3 CAR and crIL-15**, from a single vector, two different approaches were explored:

- The use of bidirectional promoter systems
- Bicistronic messaging

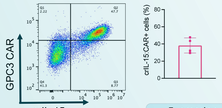
Selection of the lead **Multi-Armed CAR gene circuit** was based on:

1. Measured expression of the two components
2. Functional readouts based on activity of the **crIL-15 and CAR** circuit components

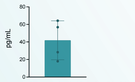
To optimize these readouts, we designed and screened >162 vectors to test the different parameters listed in the table

Parameters tested					
Promoter	**CAR Co-stim**	**IL-15**	**UTR Mods**	**Different Codon Optimization**	**Vector Backbone**
SFFV	CD28	secreted	+/-	CD3ζ	Bidirectional SinVec
SV40	41BB	crIL-15			Bicistronic Sinvec
PGK	OX40	Sushi crIL-15			Bicistronic RetroVec

Expression of CAR and crIL-15 at the membrane

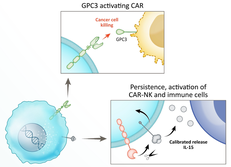


Secreted crIL-15*



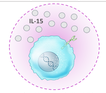
*secreted crIL-15/1e6 cell in 24 hours

Expression variability is observed due to donor-to-donor variability



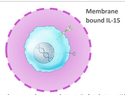
SENTI-301A Multi-Arming Approach is Designed to Attack Cancer in Multiple Complementary Ways

1. Cytokine Secretion Only (Paracrine)



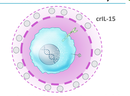
Secreted IL-15 (sIL-15) can activate neighboring immune cells present in the local tumor microenvironment (TME)

2. Membrane-bound Only (Autocrine)

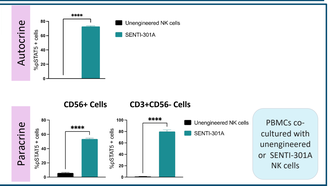


Membrane-bound IL-15 (mbIL-15) on the cell surface leads to autocrine activity and increases NK cell function activity/function

3. Calibrated Release (cr) (Paracrine AND Autocrine) Combines 1 & 2



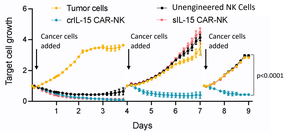
- Secreted IL-15 activates neighboring immune cells present in the local TME
- Membrane-bound IL-15 on the cell surface increases NK cell function activity/function



PBMCs co-cultured with unengineered or SENTI-301A NK cells

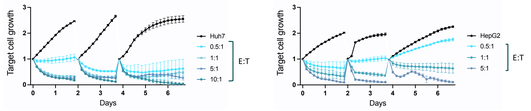
SENTI's crIL-15 Enhances NK Cell Persistence and Tumor Killing

crIL-15 autocrine and paracrine signaling improves CAR-NK cell persistence and serial killing



Calibrated release IL-15 (crIL-15) outperforms secreted wild-type IL-15 (sIL-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 with additional cancer cells.
crIL-15 is used in all Senti's pipeline products (*Garrison 2021 ASH, Garrison 2022 ASGCT, Frankel 2022 SITC*)

SENTI-301A antitumor function against GPC3 expressing cell lines in vitro

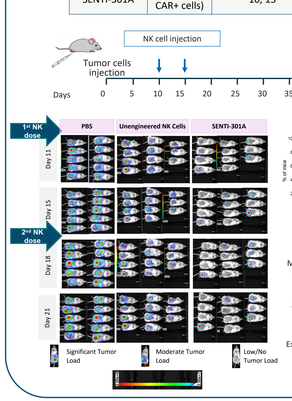


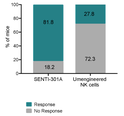
SENTI-301A *In Vivo* Antitumor Function

GPC3 Expressing Intraperitoneal (IP) Xenograft Models

HepG2 (6e6 cells injected)

Group Name	NK Cells Per Dose	Dose (Days Post-tumor Inoculation)	#Mice
PBS	-	10, 15	10
Unengineered	40e6	10, 15	11
SENTI-301A	20e6 (# of CAR+ cells)	10, 15	11





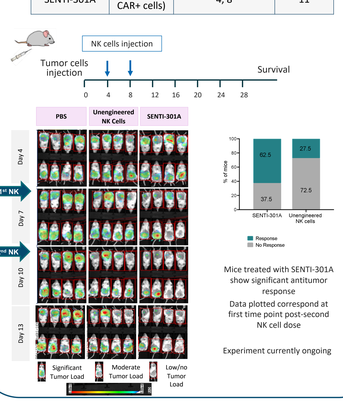
Mice treated with SENTI-301A show significant antitumor response

Data plotted correspond at first time point post-second NK cell dose

Experiment currently ongoing

Huh7 (2e6 cells injected)

Group Name	NK Cells Per Dose	Dose (Days Post-tumor Inoculation)	#Mice
PBS	-	4, 8	8
Unengineered	40e6	4, 8	8
SENTI-301A	20e6 (# of CAR+ cells)	4, 8	11



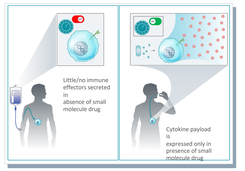
Mice treated with SENTI-301A show significant antitumor response

Data plotted correspond at first time point post-second NK cell dose

Experiment currently ongoing

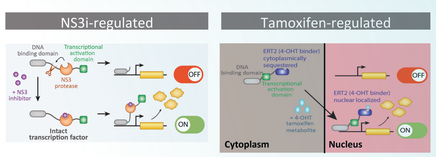
SENTI-301A cells show significant *in vivo* anti-tumor activity in IP xenotransplantation models expressing GPC3. Mice treated with SENTI-301A show significant tumor reduction over untreated (PBS) and unengineered NK cell-treated groups.

Senti's Regulator Dial Gene Circuits Can Be Regulated by Different FDA-Approved Drugs to Enable Broad Applications to Multiple Solid Tumors



Multiple Drug Switches:

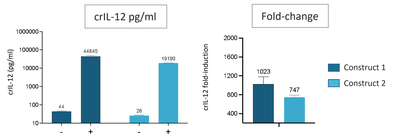
- HCV protease inhibitors
- IMiDs
- Tamoxifen



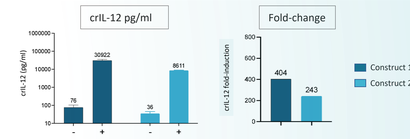
Regulator Dials are engineered with high sensitivity to enable real-time control of protein expression *in vivo* with clinically feasible concentrations of FDA-approved drugs, enabling the potential for improved efficacy and control over highly potent cell therapies.

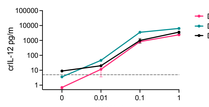
Regulated crIL-12

Engineered NK cells + GRZ (24 hours)

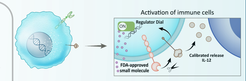


Engineered NK cells + GRZ (72 hours)

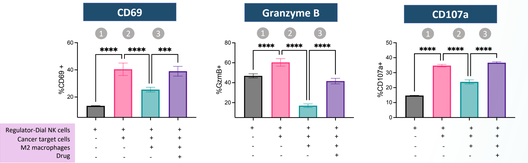


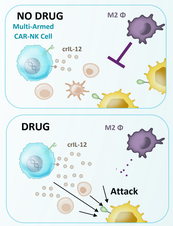


GRZ-responsive Regulator Dials demonstrate low crIL-12 basal levels in the absence of GRZ (<100 pg/1e6 cells in 24h), while induction with 10nM of GRZ lead to an increased IL-12 expression by ~390-fold (30,000 pg/1e6 cells p<0.0001) in 24h. crIL-12 expression is regulated by GRZ in a dose-response manner and show low donor-to-donor variability



Induced crIL-12 Restores the Therapeutic Activity of Engineered Immune Cells





Drug-induced crIL-12 efficiently restores activity of engineered NK cells in co-culture assay with immunosuppressive M2 MΦ

1. Engineered NK cells are activated by co-culture with cancer cells
2. In the presence of M2 MΦ, the activity of NK cells is significantly inhibited
3. Drug-induced crIL-12 significantly restored the activity of engineered NK cells

Summary

SENTI-301A is an off-the-shelf Multi-Armed CAR NK cell-based therapy optimized to efficiently and robustly target GPC3 expressing solid tumors

- **SENTI-301A gene circuit encodes for**
  1. **GPC3 CAR**: to enable targeting of HCC cells
  2. **crIL-15**: to enhance CAR NK cell activity and persistence through autocrine activity, and to enhance TME stimulation through paracrine activity
- Calibrated release IL-15 (crIL-15) increased persistence and activation of both CAR NK and immune cells in tumor milieu
- SENTI-301A lead candidate was selected through Senti's Design-Build-Test-Learn development engine and demonstrated:
  1. Robust expression of GPC3 CAR at the cell membrane and membrane-bound and secreted crIL-15
  2. **Effective in vitro serial killing of GPC3+ cell line in serial killing assays**
  3. Effective **in vivo antitumor responses in HepG2 (81.8%) and Huh7 (62.5%) NSG mouse models**
- **SENTI-301A is planned for clinical development with IND in 2023**

Senti's Regulator Dial gene circuit portfolio has been expanded to include regulation by multiple FDA-approved drug classes, thus potentially expanding its applications across multiple solid tumors:

1. Control the release of potent immunostimulatory payloads with FDA-approved small molecules
2. Increase the therapeutic window of systemic IL-12 to enable the potential for effective and safe modulation of the immunosuppressive tumor microenvironment
3. Regulator Dial gene circuit has potential to be used in combination with multiple CAR NK products