Driving anti-tumor activity in solid tumors with controlled arming of allogeneic CAR-NK

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Senti Biosciences, Inc. South San Francisco, CA

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Abstract #584

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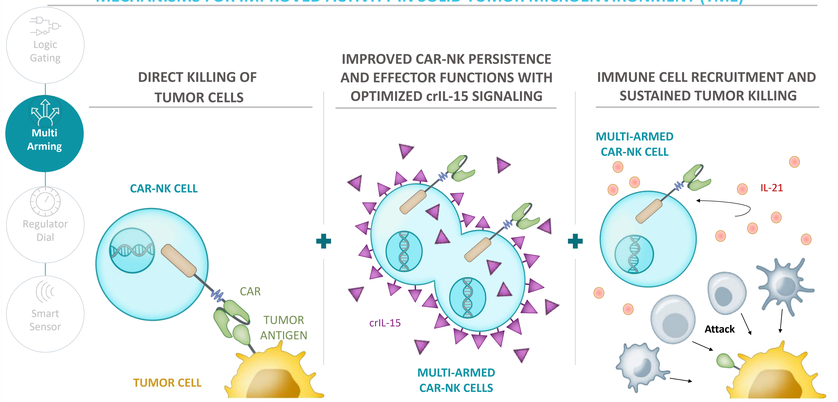
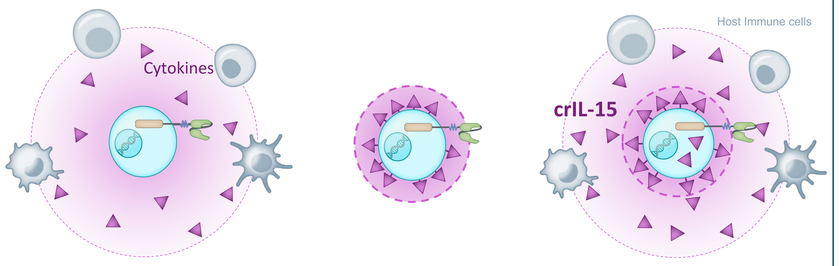
Arming CAR-NK cells for potential treatment of solid tumors

MULTI ARMING OF CAR-NK CELLS DESIGNED TO ATTACK CANCER IN MULTIPLE COMPLEMENTARY MECHANISMS FOR IMPROVED ACTIVITY IN SOLID TUMOR MICROENVIRONMENT (TME)



Despite the recent success and promise of CAR-NK cell therapies for the treatment of hematological malignancies, solid tumors present unique challenges including the presence of a highly suppressive tumor immune microenvironment. Arming CAR-NK cells with multiple strategies is important to increasing the therapeutic success of CAR-NK cells for the treatment of solid tumors. Senti Bio's proprietary gene-circuits may result in more potent products for the treatment of solid tumors.



Secreted soluble cytokines

- **Cytokine is more diffused**
- Greater activation of other endogenous immune cells at the tumor site
- **Weaker** auto-regulation of CAR-NK

Membrane-bound cytokines

- **Cytokine is concentrated on the surface**
- Potent auto-regulation, enhancing CAR-NK cell functions
- Weaker stimulation of other endogenous immune cells at the tumor site

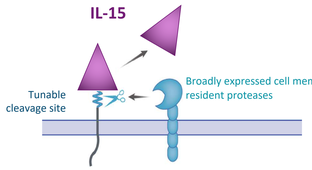
Calibrated release

- Optimized distribution
- Achieve both **potent auto-regulation** and **activation of other endogenous immune cells**
- **Autocrine and paracrine benefits**

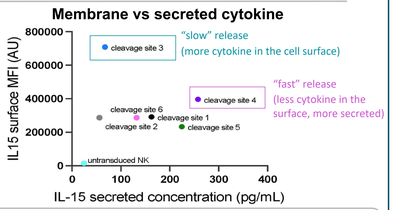
CAR-NK cells armed with Senti's novel Calibrated Release (CR) technology combine the advantage of secreted and membrane-tethered cytokines resulting in optimal stimulation of the CAR-NK cells (autocrine) as well as engagement of the local immune TME (paracrine).

Functional validation

CALIBRATED-RELEASE TECHNOLOGY: crIL-15

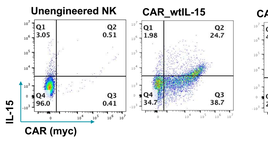


OPTIMIZED CYTOKINE DISTRIBUTION

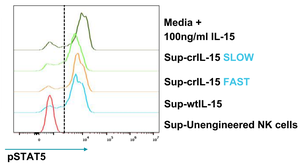


Schematic representation of the concept and design of Calibrated-Release cytokines applied to IL-15. Cytokines are attached to the outside of the cell membrane via a cleavable linker allowing the release upon cleavage by proteases. Different linker sequences result in different kinetics and proportion of cytokine in the surface or soluble.

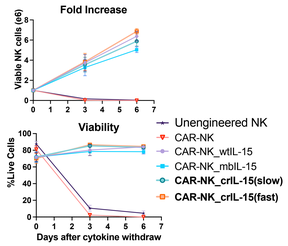
a



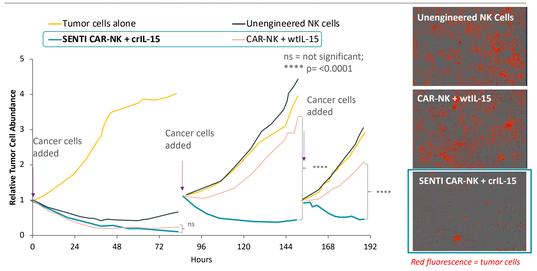
b Soluble form of calibrated release IL-15 generates pSTAT5 signaling in resting PBMCs (T cells)



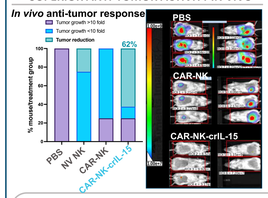
c CRIL-15 ENABLES EXPANSION AND SURVIVAL OF CAR-NK CELLS



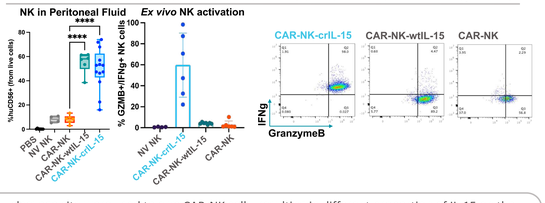
d CRIL-15 IMPROVES NK PERSISTENCE AND SERIAL KILLING



e CRIL-15-ARMED CAR-NK CELLS HAVE SUPERIOR ANTI-TUMOR ACTIVITY *IN VIVO*



f CRIL-15-ARMED CAR-NK CELLS HAVE INCREASED PERSISTENCE AND ACTIVITY *IN VIVO/EX VIVO*

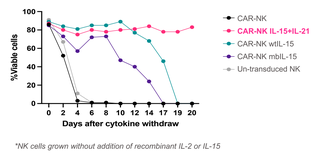


a. Different versions of crIL-15 with a fast or slow cleavage site were used to arm CAR-NK cells, resulting in different proportion of IL-15 on the surface of the NK cells. b. Cleaved forms of crIL-15 have demonstrated function and can induce pSTAT5 in resting PBMCs. c-d. All forms of crIL-15 favor expansion and survival of CAR-NK cells in the absence of cytokines in the media and result in superior killing of tumor targets in a serial killing assays (three rounds of killing). e-f. CAR-NK cells armed with crIL-15 have superior anti-tumor activity and persistence *in vivo* (up to day 18 post treatment) and demonstrate increased activation and IFNg production upon stimulation with target cells *ex vivo (ex vivo* assay on day 6 post treat.)

Combinatorial Arming of CAR-NK cells

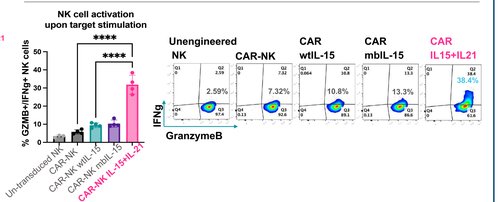
CAR-NK CELLS ARMED WITH IL-15 + IL-21 HAVE IMPROVED SURVIVAL AND FUNCTION

a *IN VITRO* SURVIVAL IN THE ABSENCE OF CYTOKINES

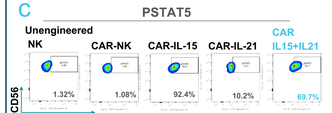


*NK cells grown without addition of recombinant IL-2 or IL-15

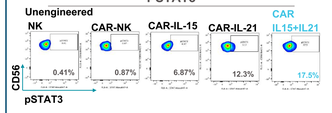
b NK CELL ACTIVATION *Co-culture with targets at 1:1 ratio*



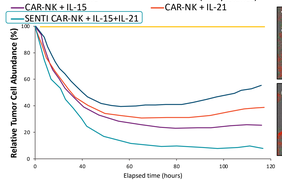
c PSTAT5



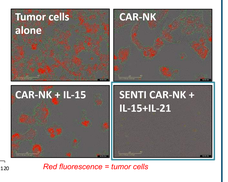
PSTAT3



d *IN VITRO* TUMOR CELL KILLING



TUMOR CELL KILLING *(day 5 post co-culture, 2:1 ratio)*



The combination of IL-15 + IL-21 resulted in synergistic effects in the arming of CAR-NK cells. a. CAR-NK cells armed with the combination of IL-15 + IL-21 had much more prolonged survival than CAR-NK cells armed with IL-15, in the absence of recombinant cytokines, up to 20 days in culture. b. NK cells armed with IL-15 + IL-21 also had superior activity compared to CAR-NK cells armed with IL-15, after co-culture with target cells, with increased proportion of NK cells with IFNg and GZMB production. c. CAR-NK cells armed with IL-15 + IL-21 activate both pSTAT5 and pSTAT3 signaling pathways. d. The combination of IL-15 + IL-21 also increased the killing capacity of CAR-NK cells resulting in almost complete ablation of tumor target cells (*in vitro*).

Summary and next steps

Calibrated Release Cytokine Technology

Senti Bio has developed a novel technology to regulate the proportion of surface vs soluble cytokines to arm allogeneic CAR-NK cells. In the case of IL-15, we have shown that optimal cytokine distribution can result in superior activity (killing and persistence) of CAR-NK cells as well as paracrine activation of other immune cells.

Multi-Armed CAR NK cells for the Potential Treatment of Solid Tumors

Senti Bio is currently advancing two programs for the potential treatment of solid tumors that incorporate Multi-Armed CAR-NK cells: **SENTI-301** is intended for the treatment of Hepatocellular Carcinoma and **SENTI-401** for the treatment of Colorectal Cancer. Both programs apply the Calibrated Release Technology and multi-arming to improve therapeutic potential in solid tumors.

Broad applicability of Multi-Arming and tunable cytokine release technology
to potentiate the function of allogeneic CAR-NK cells and maximize autocrine and paracrine benefits of cytokines with the aim of increasing the therapeutic window of CAR-NK cells for the treatment of solid tumors, increasing **NK cell activity and persistence** as well as **recruiting** and **activating the local immune TME.**

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