



# Off-the-Shelf CAR-NK Cells Engineered to Express Calibrated Release IL-15 Exhibit Enhanced Persistence and Anti-Tumor Activity

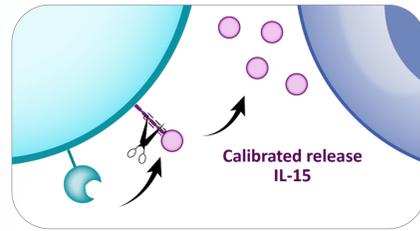
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AACR Annual Meeting  
2023, Orlando, FL  
Abstract Presentation #2902

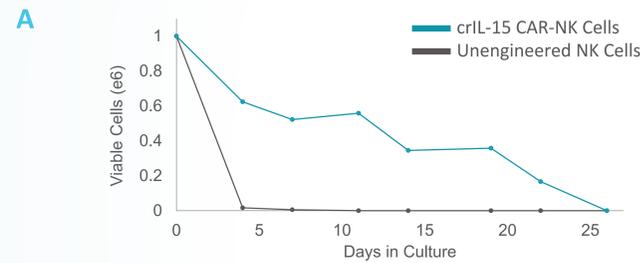
## Arming CAR-NK Cells with Calibrated Release IL15 (crIL-15)

crIL-15: calibrated release of IL15 by local proteases -> enhanced killing activity

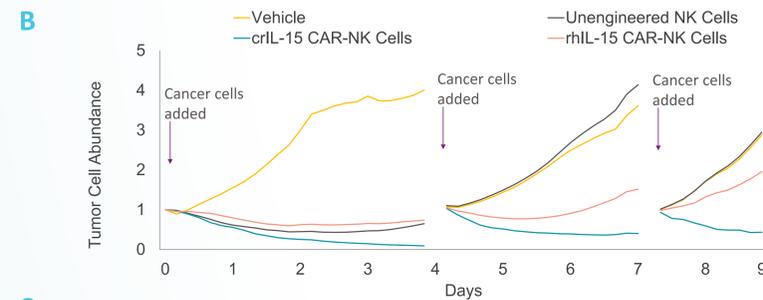


## crIL-15 Increases Persistence and Activation of Both CAR-NK and Other Immune Cells

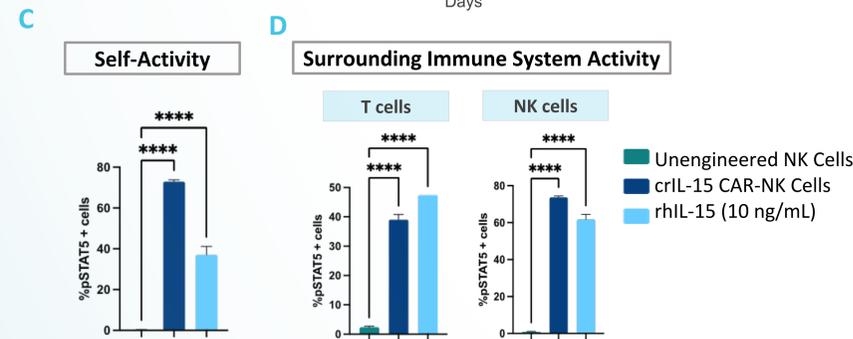
crIL-15 increases persistence of CAR-NK cells



crIL-15 increases CAR-NK serial killing ability

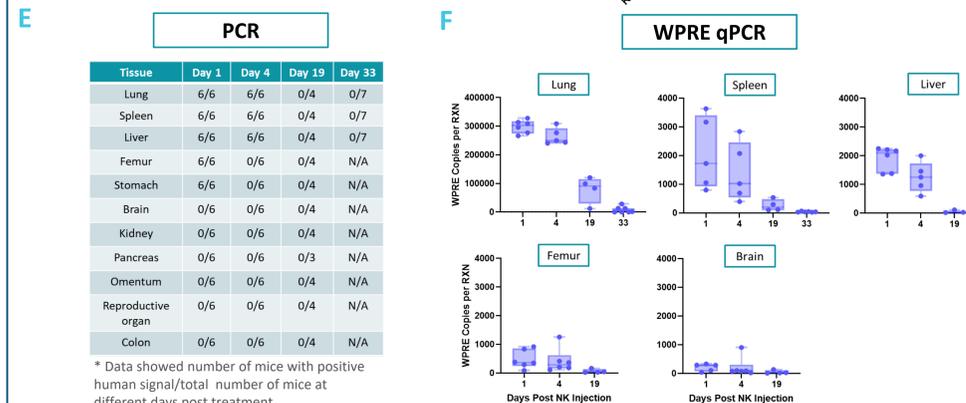
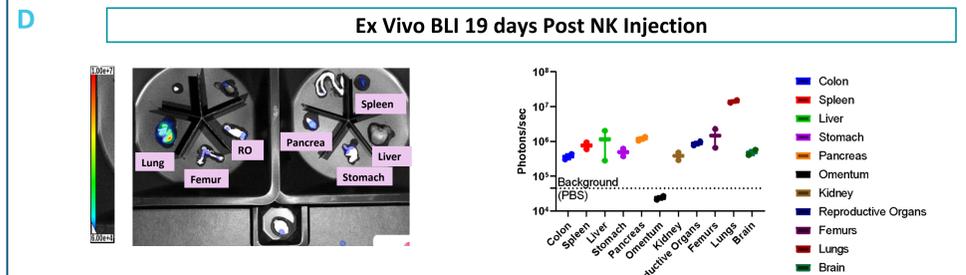
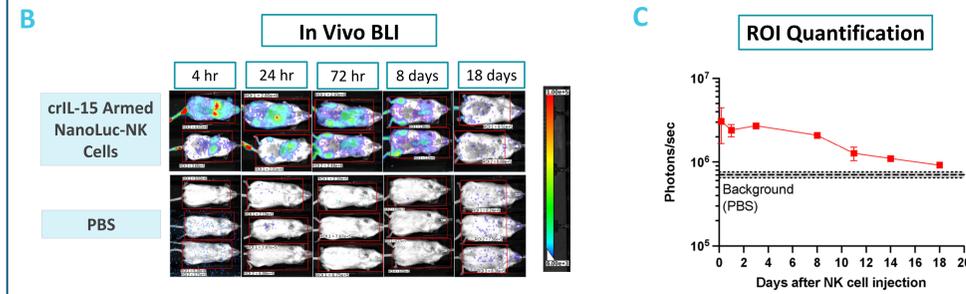
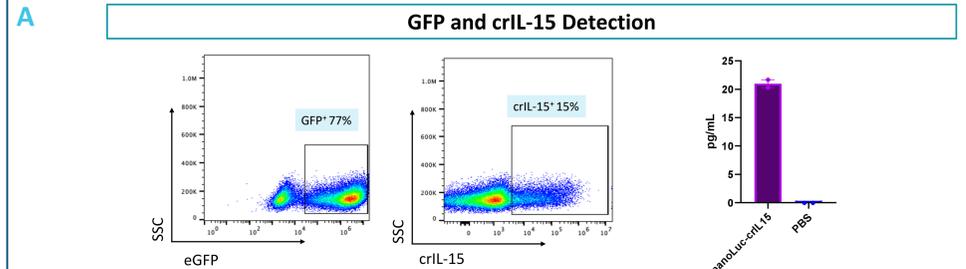


crIL-15 activates NK and resting immune cells



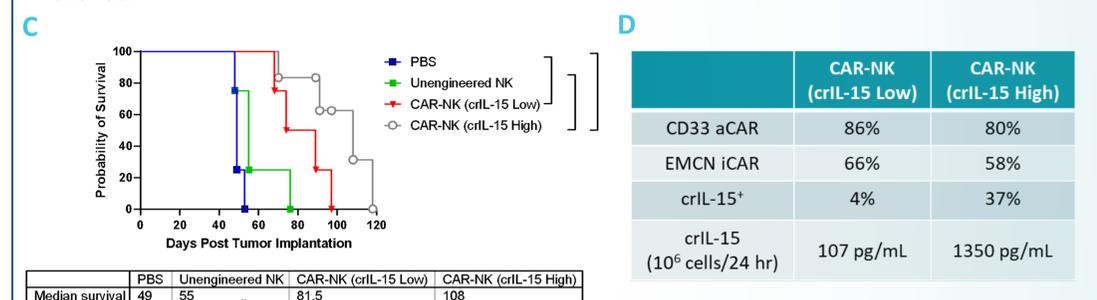
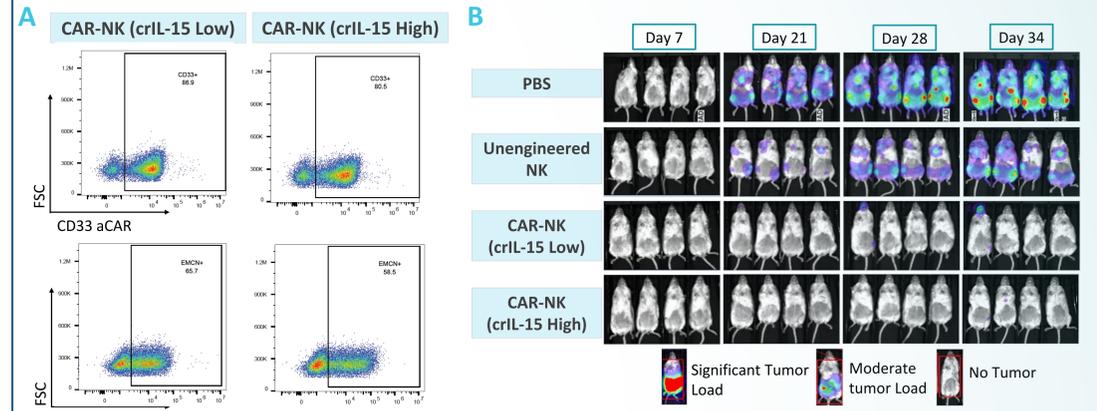
A. crIL-15 CAR-NK cells exhibited increased survival and expansion without exogenous cytokine support compared to unengineered NK cells. B. crIL-15 CAR-NK cells outperformed recombinant human IL-15 (rhIL-15) CAR-NK cells using an incu-cyte-based serial killing assay. crIL-15 CAR-NK cells demonstrated enhanced target cell killing and persistence in the secondary and tertiary re-challenges with additional cancer cells. C-D. crIL-15 pathway activation of NK and resting immune cells by phospho-STAT5 (pSTAT5) detection.

## Biodistribution and Persistence of crIL15 NK Cells In Vivo



A. NK cells were engineered to express GFP, NanoLuc and crIL-15. Expression was detected by flow cytometry and Luminex. B-F. crIL-15 armed NanoLuc NK cells (20 x 10<sup>6</sup>) were intravenously injected into NSG mice. NK cells biodistribution and persistence was examined by bioluminescence imaging (B-D), human/mouse duplex PCR (E) and WPRE qPCR (F). crIL-15 NK cells were detected in the lung, femur, spleen, liver and stomach 1 day post injection and lasted up to 20 days in the lung.

## Anti-Tumor Activity of crIL15 CAR-NK Cells In Vivo



A. NK cells were engineered to express a FLT3 and/or CD33 activating CAR, an EMCN inhibitory CAR and crIL-15. B-C. MV4-11-Fluc AML tumor cells were co-injected intravenously with CAR-NK cells expressing low or high crIL-15 or dose matched unengineered NK cells into NSG-Tg (Hu-IL15) mice. Tumor growth was monitored by bioluminescence imaging. CAR-NK cells significantly reduced MV4-11 tumor burden and prolonged mouse survival compared to unengineered NK cells. D. The improved anti-tumor functions correlated with IL-15 levels produced by the CAR-NK cells.

## Summary

Senti has designed the calibrated release technology to simultaneously stimulate surrounding immune cells and promote CAR-NK cell expansion, persistence, and tumor killing.

- Effects of crIL-15 on CAR-NK cell functions in vitro:
  - crIL-15 increased in vitro persistence and target tumor cell killing ability of CAR-NK cells.
  - crIL-15 activated both NK and other immune cells.
- Effects of crIL-15 on CAR-NK cell functions in vivo:
  - crIL-15 CAR-NK cells significantly reduced MV4-11 tumor burden and prolonged mouse survival compared to unengineered NK cells.
  - The improved anti-tumor function correlated with IL-15 levels produced by the CAR-NK cells.

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