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Engineering Smarter Medicines

Scalable GMP-Ready Manufacturing Process for Gene Circuit Engineered Allogeneic CAR-NK Cell Therapy for Cancer

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Abstract

Allogeneic CAR natural killer (NK) cell therapy has shown promise in recent years for treating cancer in patients with low risk of graft-vs-host disease or other serious side effects commonly seen in CAR-T therapies[1]. Senti Biosciences uses its synthetic biology platform to program next-generation cell and gene therapies with gene circuits. From a broad toolbox of proprietary gene circuit platform technologies, Senti's internal pipeline is initially focused on Logic Gating and Multi-Arming to increase the applicability of CAR-NK cells against a wide range of liquid and solid tumors. We believe we have established a scalable process for efficient production of allogeneic CAR-NK cell products that includes the ability to perform four necessary steps in the CAR-NK cell manufacturing process.

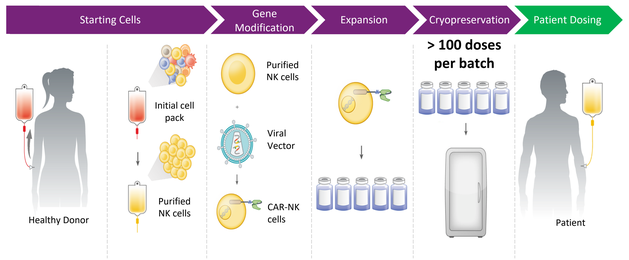
Objectives

- Leverage Senti's cell/gene therapy and manufacturing expertise to develop a platform CAR-NK manufacturing process capable of generating large numbers (hundreds of patient doses) of final product doses per manufacturing batch.
- Apply GMP-friendly methods and materials at every unit operation.

Methods

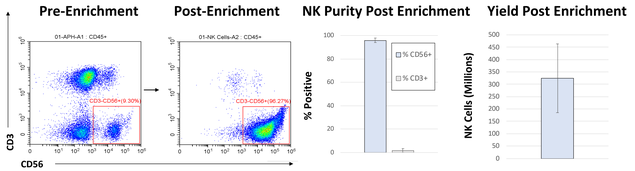
Purified NK cells were isolated via an automated process from healthy donor adult leukapheresis material via CD3 depletion and CD56 selection, and then cryopreserved for later use. Upon thaw, NK cells were activated using freshly irradiated gene modified feeder cells in a 1L G-Rex® closed system culture vessel. 7-10 days later, NK cells were transduced with retroviral vectors coding for the target CAR and gene circuit components. Transduced NK cells were expanded further in G-Rex culture vessels for a total of approximately 21 days. When scaled into multiple 5L G-Rex units, we are targeting > 3,000-fold NK cell expansion for an equivalent of ~$6x10^{10}$ CAR-NK cells. Expanded cells can then be volume reduced, harvested and formulated into cryopreservation medium using an automated cell processing system. Formulated cells are then filled in vials and stored in liquid nitrogen vapor phase.

We Have Developed a Process That's Designed to Enable the Scaling of CAR-NK Manufacturing



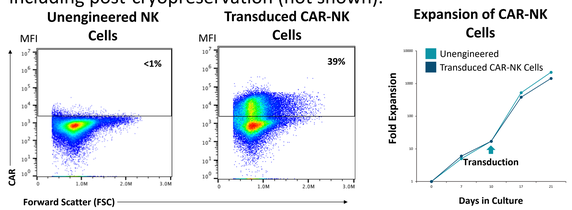
Enrichment of NK Cells from Adult Peripheral Blood using CliniMACS® Prodigy

Multiple enrichments (n=9) from healthy donor leukapheresis products have been performed with >95% NK cell purity and <2% T cells on average. Purified NK cells are cryopreserved and thawed with minimal loss of viability.



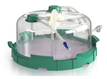
Transduction of CAR-NK Cells

Chimeric antigen receptor (CAR) is efficiently transduced into NK cells using retroviral vector. Transduced cells retain >90% viability and similar growth rate to unmodified NK after 21 days. CAR expression is stable including post-cryopreservation (not shown).

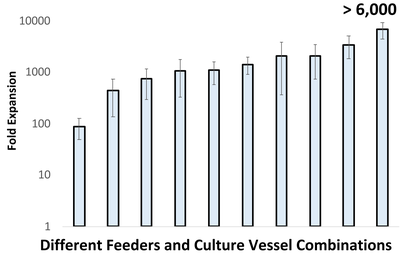


Screening of Different NK Culture Conditions Can Lead to Substantially Increased Fold Expansion

Optimized combinations of irradiated gene modified feeder lines and culture vessels lead to increased expansion.

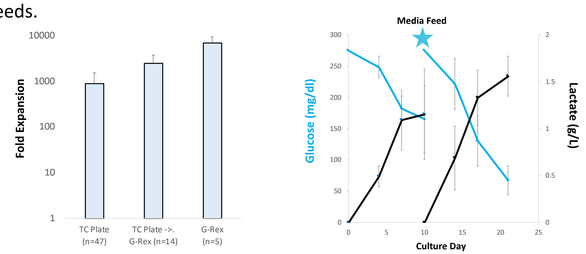


G-Rex (5L, Closed System)



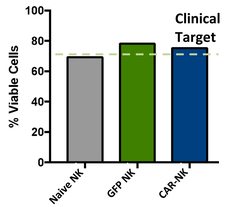
G-Rex® (Wilson Wolf) Enables Robust Expansion of NK cells

G-Rex® scales directly from 100mL to 5L. Gas permeable bottom and large liquid volume minimized media exchange. Tracking glucose & lactate concentration indicates ~10 days of robust expansion between feeds.

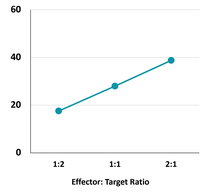


Cryopreserved Cells Retain Function

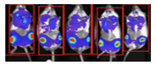
Cryopreserved CAR-NK Retain High Post-Thaw Viability



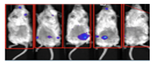
Cryopreserved CAR-NK Cell Killing of MOLM13 *in vivo*



Untreated Control (no NK Cells)



(cryopreserved)



Images taken 10 days after single CAR-NK dose

Cryopreserved CAR-NK directly added to cancer target cells retain high potency in vitro and in vivo, and similar to freshly prepared NK cells.

Process designed to generate >100 doses per batch

Starting Cells NK cells isolated/cryopreserved from donor blood

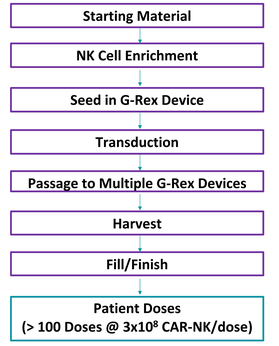
Retroviral Vector from stable producer cell line

Transduction with stable expression

Expansion >3000-fold via G-Rex

Cryopreserve in vials and stored in LN2 vapor phase

Continued Optimization: We are continuing to improve methods of expansion including investigating bioreactors for further scale-up (i.e for commercial scale).



Conclusions

Several expansion and transduction optimization studies have been performed to achieve process qualification readiness.

Transduction efficiency was optimized by comparing healthy donors, transduction enhancers and culture vessels, and measured by flow cytometry and vector copy analysis. Functional assessment was performed via *in vitro* and *in vivo* studies.

CAR-NK cells were evaluated for growth characteristics using different culture vessels, several irradiated feeder cell lines and various media formulations.

We believe the result is a robust allogeneic CAR-NK manufacturing-ready production process suitable for translation to GMP clinical manufacturing.

We have designed this manufacturing process to be used to support multiple allogeneic CAR-NK products across Senti's internal allogeneic CAR-NK cell pipeline.

References

1. Liu E, Marin D, Banerjee P, et.al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. N Engl J Med. 2020;382(6);545-553. doi: 10.1056/NEJMoa1910607