Process Development and Scale-Up for Gene Circuit Engineered CAR-NK Cell Manufacturing

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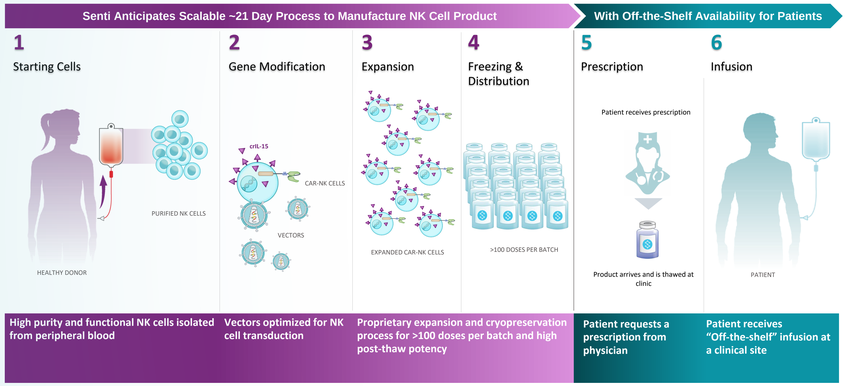
Introduction

Allogeneic CAR natural killer (CAR-NK) cell therapy has shown promise in recent years for treating cancer in patients without the potential of inducing graft versus host disease[1]. Senti Bio is using gene circuits to introduce logic-gating and small molecule regulation of payloads into next-generation allogeneic CAR-NK cell therapies to broaden efficacy in liquid and solid tumors. Here, we describe a scalable v1.0 GMP-ready flask-based manufacturing process for generating clinical scale production of CAR-NK cells to support our SENTI-202 (acute myeloid leukemia) and SENTI-301 (hepatocellular carcinoma) programs.

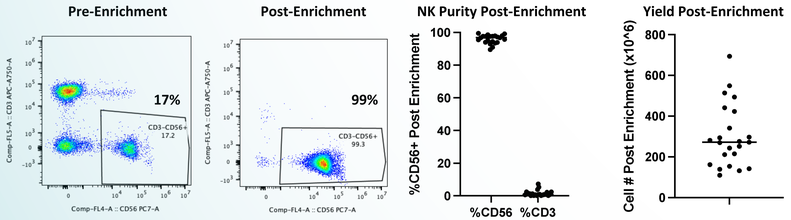
Process Designed to Enable the Scaling of CAR-NK Manufacturing



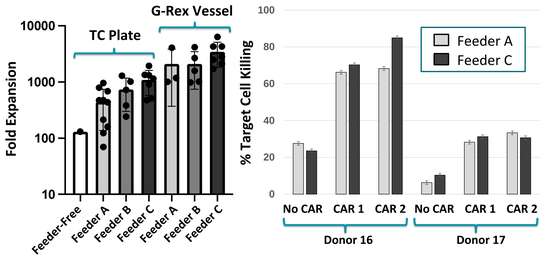
We aimed to develop a v1.0 closed process starting with 25*10^6 enriched NK cells, targeting >40% CAR+ transduction, and obtaining >5,600-fold expansion over 21 days to achieve a batch size of >1*10^11 NK cells.

NK Cell Enrichment From Adult Peripheral Blood Using CliniMACS® Prodigy

To ensure safety, T cells must be depleted from the product to reduce risk of GvHD. Removing T cells at the beginning of the process is the most favorable. Multiple enrichments from healthy donor leukapheresis collections (n=22) have been performed via an automated program on the CliniMACS Prodigy resulting in an average of ~300*10^6 enriched NK cells post isolation at >95% NK cell purity and <2% T cells on average. Purified NK cells are cryopreserved and thawed upon initiation of a production run with minimal loss of viability.



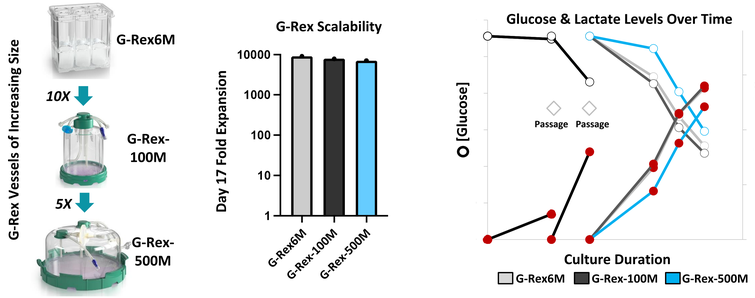
Multiple NK Feeder Systems Have Been Evaluated



Multiple NK feeder systems were evaluated with the initial focus on expanding un-transduced NK cells. After CAR transduction, feeder systems had little-to-moderate impact on killing. NK donor differences have shown to be a key variable in both basal and CAR-mediated cell killing. Donor screening experiments are currently underway. Feeder "C" has been re-engineered and selected for generation of a GMP master cell bank.

G-Rex® System Scalability Demonstrated - 100mL to 5L Vessels

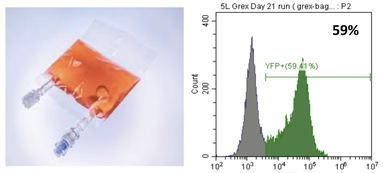
Optimized iterations of different irradiated gene-modified feeder lines and varying culture vessels (tissue culture plates and G-Rex® vessels) have led to continual improvements in fold expansion. G-Rex6M (100mL culture) acts as a natural scale-down model before verifying conditions at 5L scale. Target levels of glucose consumption and lactate production act as triggers for passaging to the next culture vessel(s). 5L G-Rex expansions achieved as high as >16,000-fold and/or >2.1*10^10 NK cells per vessel during early process development runs.



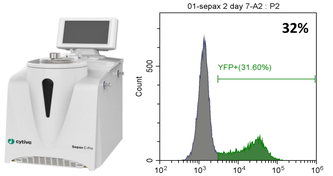
Off-the-Shelf Technologies Enable Robust Transduction

All unit operations have been designed to be GMP compatible. Proof of concept for the transduction unit operation has been demonstrated using two different closed system methods: retronectin-coated gas permeable bags, and via the Sepax® using Spinoculation software. Depending on Multiplicity of Infection (MOI) and viral vector titer, either method can be utilized in manufacturing.

Retronectin-Coated Gas Permeable Bags



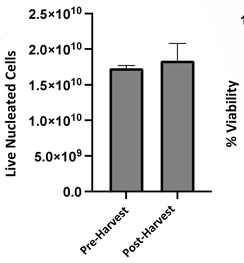
Sepax-Enabled Transduction

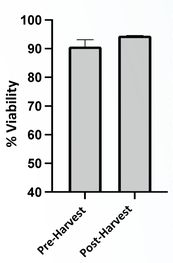


Scaled Up Expansion & Harvest Process is Reproducible and Robust

Volume Reduction + Harvest

Cryopreservation via CRF

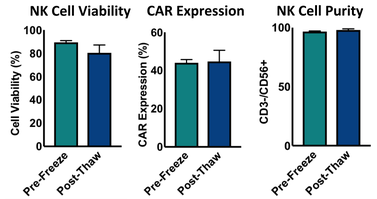


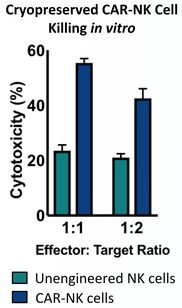


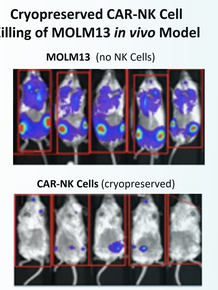
After optimization of the NK cell splitting schedule, multiple G-Rex-500M NK suspension cultures were volume reduced and harvested using closed system methodology. These devices have achieved harvest recoveries of >95% while maintaining high viability (>90%). Cells are then vialed and cryopreserved in a controlled rate freezer.

Cryopreserved Cells Retain Functionality

CAR-NK cells – fresh and post-thaw – maintain similar cell viability, CAR expression and NK cell purity (CD3-/CD56+). Cryopreserved/thawed CAR-NK cells added to cancer target cells retain high potency *in vitro* and *in vivo*.







Summary

Several process parameters have been optimized to produce a reproducible, scalable, GMP-friendly allogeneic CAR-NK manufacturing process applicable towards different tumor associated antigens. Donor screening programs are being developed to ensure the best starting material is used to generate product for optimal therapeutic efficacy.

Rigorous development around feeder cell optimization has resulted in the ability to reproducibly activate and expand NK cells.

We continue to optimize different parameters of the allogeneic CAR-NK manufacturing process.

We have designed this manufacturing process to be used in support of multiple allogeneic CAR-NK products across Senti's proprietary product 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