Scalable Non-Viral Gene Editing and Feeder-Free Production of CAR-NK Cells

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Abstract

Purpose: The development of clinically relevant, efficient, safe, non-viral gene delivery methods for primary NK cells remains limited. To date, there is very little published data demonstrating high-efficiency (>10%) non-viral gene knock-in for primary NK cells. In this study, we optimized a scalable, non-viral, closed-system gene delivery method for the production of CAR-NK cells.

Methods: NK cells were isolated from PBMC using Rotea counterflow system and culture in NK expander complete media supplemented with IL-2 for 6 days. On day 6, NK cells were electroporated with Neon NxT or Xenon electroporation system at density of 50e06/mL to deliver single strand DNA (ss-DNA), CRISPR-Cas9 gRNA targeting Rab11a/AAVS1 loci. The gene edited NK-cells were expanded and analyzed by flow cytometry for CAR expression. Cells were also further tested for cytotoxicity by co-culturing with SKOV3-GFP for 6 hrs.

Results: The ss-DNA-Rab11a demonstrated up to 25% knock-in efficiency while ant-meso-3 CAR knock-in efficiency up to 15% over the time of culture. The edited NK cells maintained their phenotype and viability. Further, engineered NK cells (anti-meso-3 CAR NK) showed higher cytotoxicity effect towards cancer cells as compared to non-engineered NK cells.

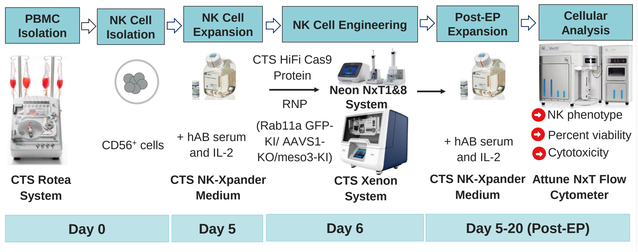
Introduction

Genetic engineering is a major driving force in advancing adoptive immunotherapy, a promising approach for treating cancers. In particular, engineering primary human natural killer (NK) cells holds significant potential owing to their potent antitumor properties and demonstrated safety in an allogeneic applications. NK cells function independently of human leukocyte antigen (HLA) matching, eliminating the risk of graft-versus-host disease in allogeneic settings. As a result, they offer the potential to be safer, more cost-effective, and possibly more efficient than current autologous T-cell therapies. One of the key challenges faced by the cell and gene therapy industry is the ability to efficiently expand, gene edit and process NK cells at clinically relevant scales. Additionally, there is a need for regulatory compliant reagents and automated instruments. Here we have addressed these pain points and developed optimized protocols for non-viral based NK cell engineering and feeder free production of CAR-NK cells.

CTS™ NK-Xpander™ Medium used in this work is capable of producing high yield of hNK cells without the need for feeder cells and it also enables robust expansion of enriched NK cells from qualified donor derived PBMC's. The Gibco™ CTS™ Rotea™ system was used for PBMC isolation and, for wash, concentration and buffer exchange milestones. The Neon™ NxT and Gibco™ CTS™ Xenon™ Electroporation Systems along with CTS™ HiFi™ Cas9 protein and TrueGuide™ synthetic gRNA were used for gene editing milestones. The workflow and protocols developed through this work addresses research use to clinical scale needs for engineered NK cell manufacturing while maintaining high cell viability and/or recovery pre and post *ex vivo* genetic modification steps. Furthermore, combining Rotea and Xenon systems can help overcome some of the challenges faced with manual cell processing and viral based cell engineering protocols.

Materials and Methods

Human PBMCs were isolated from Leukopak using Rotea. Human NK cells were enriched from PBMCs using negative isolation kit. Isolated NK cells were cultured with NK Xpander media supplemented with human serum and IL-2. On day 6, cells were electroporated with Neon NxT/Xenon to deliver CRISPR-Cas9 protein, gRNA and donor DNA. For gene knock in applications either a GFP or CAR construct was used to target Rab11a or AAVS1 locus and CRISPR/Cas9 RNP system was used for all editing experiments. Post-EP cells were cultured for 20 days and analyzed by Attune™ flow cytometry over multiple time intervals.



Results

Figure 1. Isolation of PBMC from leukopak using CTS™ Rotea™ counterflow system

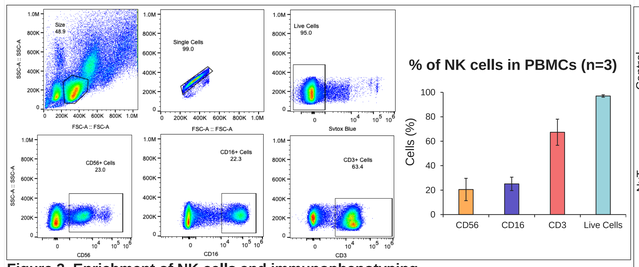


Figure 2. Enrichment of NK cells and immunophenotyping

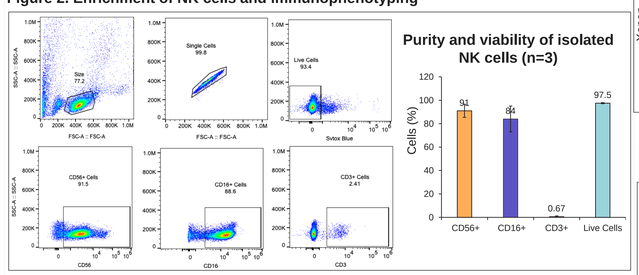


Figure 3. Optimization gene delivery & editing using Neon NxT single & multichannel

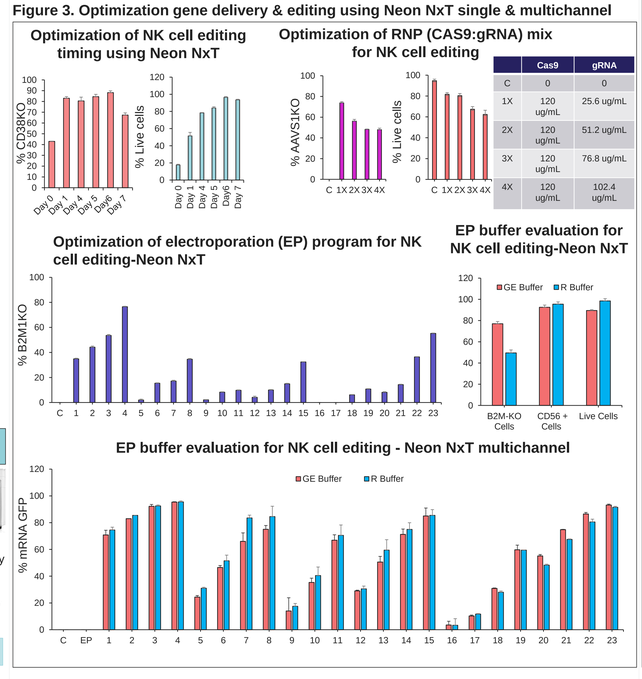


Figure 4. Non-viral transgene knock-in into NK cells (Rab11a GFP)

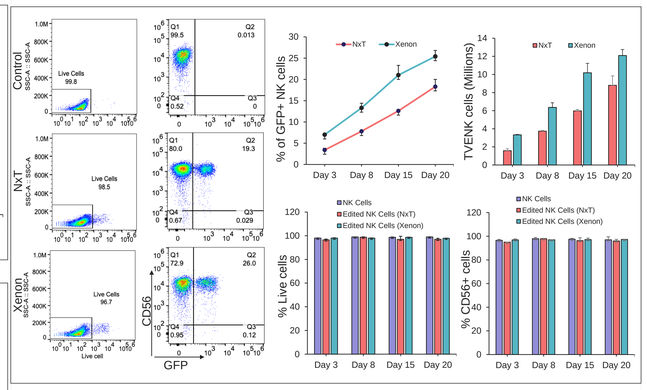
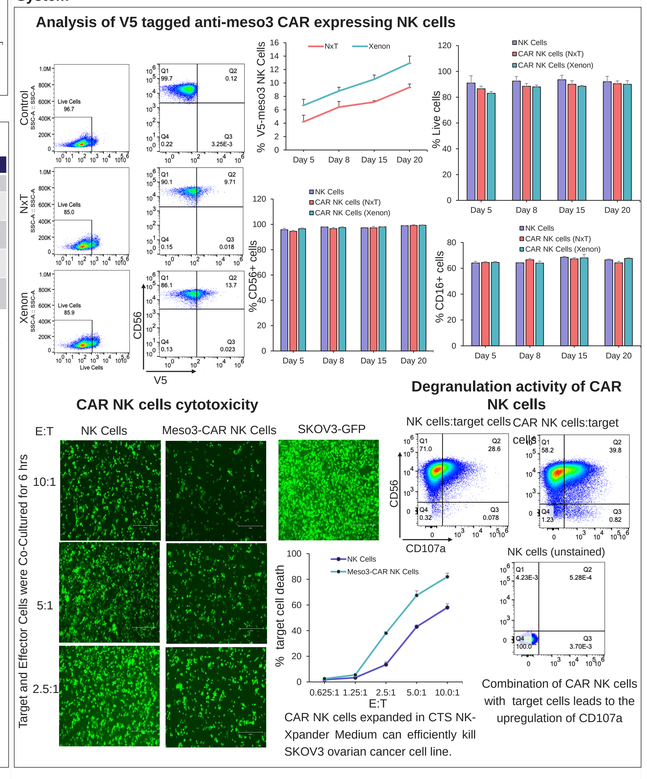


Figure 5. Generation of V5 tagged anti-mesothelin CAR NK cells- Scaling from research use plateform (Neon NxT) to GMP compatible CTS Xenon Electroporation System



Conclusions

- PBMCs were successfully isolated using the CTS Rotea system.
- The Neon NxT 8 channel system delivered GFP mRNA into NK cells with over 90% expression.
- The CTS™ Xenon and Neon™ NxT Electroporation Systems along with CTS™ HiFi Cas9 showed around 15-25% KI efficiency with fully non viral protocol using either ss-DNA Rab11a GFP or ss-DNA meso3 payloads.
- The edited NK cells/CAR NK cells expanded well in CTS NK-Xpander Medium and maintained relevant NK cells phenotype, viability and functionality.
- CAR NK cells were able to kill around 80% of target cells (SKOV3) within 6 hours of co-incubation.
- Data demonstrated here used of modular approaches incorporating Rotea and Xenon instruments to manufacture clinically relevant CAR-NK cells.

Acknowledgements

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