DEVELOPMENT OF A CLINICALLY RELEVANT MANUFACTURING PROCESS FOR PRODUCTION OF ANTI-MESO-3 CHIMERIC ANTIGEN RECEPTOR PRIMARY HUMAN NATURAL KILLER CELLS

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Abstract

Purpose: Development of clinically relevant large scale gene editing workflow for production of potent Natural Killer (NK) cells. To achieve this, the payload is required to be delivered to a large number of NK cells. Here we demonstrated use of the CTS™ Xenon™ Electroporation System to deliver CRISPR-Cas9 protein and gRNA targeting the AAVS1 region at a high density and later transduction using AAV6 for delivery of an anti-Meso3 CAR construct.

Methods: NK cells were isolated from PBMCs using the CTS™ Rotea™ Counterflow Centrifugation System and cultured in CTS™ NK-Xpander™ Medium supplemented with IL-2 for 6 days. On day 6, NK cells were electroporated with the CTS Xenon Electroporation System at a density of 50 million cells/mL . The gene edited CAR 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 16 hrs.

Results: Engineered CAR NK cells showed a higher rate of expansion than non-engineered NK cells, and showed anti Meso-3 CAR knock-in efficiency up to 35% over the time of culture (20 days). Furthermore, engineered NK cells (anti-Meso-3 CAR NK) showed a higher cytotoxicity effect in an *in vitro* SKOV3-GFP cell model as compared to non-engineered NK cells.

Introduction

Genetic engineering is a major driving force in advancing immunotherapy and adoptive immunotherapy is a promising approach to treat cancers. The engineering of primary human natural killer (NK) cells in immuno-oncology holds great promise due to the potent antitumor properties of NK cells and their demonstrated safety in an allogeneic setting. NK and T cells are popular cell types used in clinical trials owing to their ability to recognize and destroy malignant cells. NK cells do not rely on a matching human leukocyte antigen to function, protecting allogeneic transfer from graft-versus-host disease. Thus, they have the potential to be safer and more effective than current engineered T cell therapies. A key challenge in the NK cell therapy space is how to harness the ability to expand, modify, and process NK cells in clinically relevant amounts using reagents and instruments that can support regulatory filings. Here we begin to address this pain point.

We have developed CTS NK-Xpander Medium, a feeder-free NK cell culture medium, that expands functional primary human NK cells to clinically relevant levels in two to three weeks. The CTS Rotea system is a flexible/efficient system that can isolate the cells with high viability. The CTS Xenon Electroporation System also supports reliable nonviral cell therapy development and manufacturing by enabling high cell viability and recovery during the ex vivo genetic modification step. By combining the CTS Rotea and CTS Xenon systems together into one workflow, we begin to help to overcome some of the challenges, get closer to a closed and automated workflow, and ultimately support our customers to get therapies to patients faster and safer. Furthermore, this system can be applied to different types of immune/stem cells, which trends show to be increasing in the treatments of various indications.

Materials and Methods

Human PBMCs were isolated from Leukopaks using the CTS Rotea counterflow centrifugation system. Human NK cells were isolated from the PBMCs using a negative isolation kit. Isolated NK cells were cultured with CTS NK-Xpander medium supplemented with human serum and IL-2. On day 6, cells were electroporated with the CTS Xenon system by targeting a CAR construct to the AAVS1 locus with a CRISPR/Cas9 system (CTS™ TrueCut™ Cas9 Protein). After 30 minutes, AAV6 carrying a Meso3 CAR construct was added to the electroporated cells. The post-electroporated (post-EP) cells were cultured for 20 days and analyzed by flow cytometry on the Attune™ flow cytometer.

Results

Figure 1. Isolation of PBMCs from Leukopak using the CTS Rotea system. PBMCs were stained to verify CD56, CD16 and CD3 cell surface expression, and percentage marker cell surface expression was determined by flow cytometry

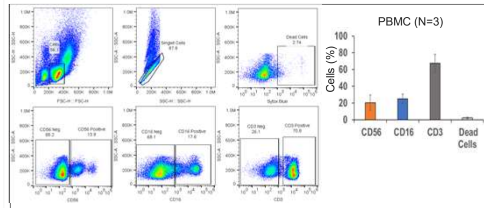
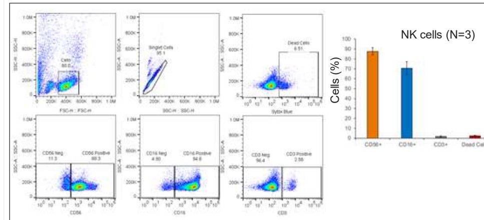


Figure 2. Enrichment of NK cells and immunophenotyping. NK cells were stained to verify CD56, CD16 and CD3 cell surface expression, and percentage marker cell surface expression was determined by flow cytometry



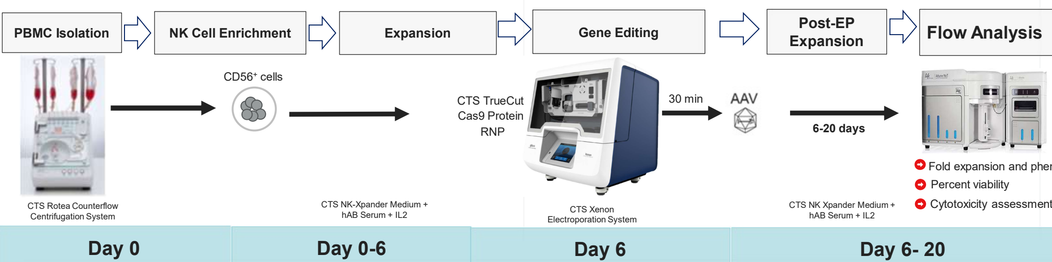


Figure 3. NK cell genome editing using the CTS Xenon electroporation system. a) Anti-Meso3 CAR-NK (% V5 positive) expression was determined by flow cytometry analysis (100 uL: Invitrogen™ Neon™ Transfection System and 1 mL: CTS Xenon electroporation system with the CTS™ Xenon™ SingleShot cartridge, 50M/ml of NK cells), b) expression of anti-Meso3 CAR NK over 20 days, c) cell expansion over 20 days, d) Edited NK cell immunophenotype over 20 days, e) viability of cells over 20 days post-electroporation

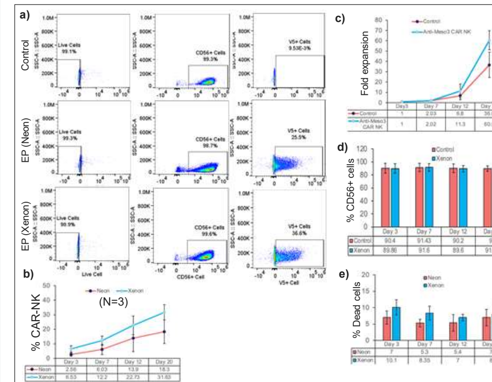
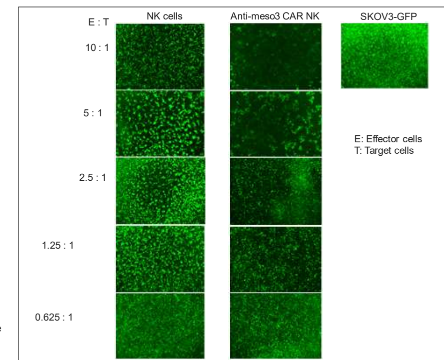


Figure 4. Anti-meso3-CAR NK dependent cytotoxicity assay



Conclusions

- We successfully developed a feeder-free NK cell expansion medium, CTS NK-Xpander Medium, that expands human primary enriched NK cells to clinically relevant levels
- The CTS Xenon Electroporation System was used to edit clinically relevant volumes of NK cells and generate up to 36% anti-Meso-3 knock-in efficiency
- Control (non-electroporated) and electroporated NK cells demonstrated no significant difference in cell viability and immunophenotyping data
- The expanded CAR-NK cells maintained their phenotype and functionality and were able to kill SKOV3 cancer cells within 6 hours of co-incubation
- Data demonstrated feasibility of using a modular approach with the CTS Rotea and CTS Xenon systems, representing a step toward support of cell therapy developers who seek to manufacture clinically relevant CAR NK cells using closed, automated systems.

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