Advancing Allogenic Cell Therapy with Automated iPSC Processing and Engineering: Benefits of Modular Approach.

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Introduction and Methods

Induced pluripotent stem cell (iPSC)-derived natural killer (iNK) cells have emerged as a promising platform for next-generation immunotherapy, offering a homogeneous, scalable and versatile approach for consistent large-scale manufacturing of off-the-shelf allogeneic therapies. This approach involves multiple steps including iPSC culturing and banking followed by gene editing and differentiation to tumor specific iNK cells to enhance cytotoxicity, persistence, and tumor-targeting specificity, while minimizing risks of graft-versus-host disease. We developed closed automated protocols for cell harvest, wash, concentration, gene delivery, and editing milestone to enable iNK-based cell therapy manufacturing.

In our current workflow, we cultured and expanded iPSCs up to a billion cells in a 10-layer cell factory (CF) system for master cell bank preparation. Manual processing of iPSCs in 10-layer CF system can be labor intensive and prone to contamination. Utilizing CTS™ Rotea™ counterflow centrifugation system minimized human intervention at multiple stages, including removal of media, washing of cells, addition of cell detachment media, collecting, concentrating iPSCs and delivering cells to collection bags. Using this protocol, we processed the harvesting of entire iPSC culture in 10-layer CF system in a single batch to create a master bank for cell therapy development. The iPSCs prepared using this method-maintained good viability, expansion rate, genomic stability and pluripotency characteristics. Banked cells were subsequently used for generating anti Mesothelin CAR-iPSC cells using GMP grade reagents, CTS™ StemFlex™ media and CTS™ HiFi Cas9. The Cas9 RNP and anti-mesothelin CAR DNA payloads were delivered using either Neon NxT or clean room compatible CTS™ Xenon electroporation system. Results show successful generation of engineered CAR-iPSC with reproducible KI efficiency of up to 15%. We established the optimal target gene and promoter combination to ensure stable expression of transgene during iPSC to iNK differentiation steps. With methods developed through this work, we successfully generated potent CAR-NK cells that were maintained and expanded in CTS™ NK-Xpander™ media. The workflows described here, using clean room compliant automated cell processing, gene delivery platforms, and GMP-compatible iPSC and NK media systems, enable clinical-scale iNK cell therapy manufacturing.

iPSC derived cell therapy approaches have shown promise in preclinical and early clinical trials for conditions like spinal cord injury, heart disease, and diabetes. Allogeneic cell therapy for cancer treatment is a developing field, and in very recent years, iPSCs have drawn unique interest for the development of clinical manufacturing of CAR-NK and CAR-T cells. iPSC culture, expansion and differentiation involves multiple steps with several manual touch points. Automation can mitigate concerns associated with manual processing however there are limited clean room compatible automated workflow solutions available. Using closed automated systems like Rotea can reduce manual steps, processing time, and human error in iPSC processing. The modular gene delivery platform utilized here seamlessly integrates into the iPSC to iNK manufacturing workflow.

Figure 1. iPSC cell processing and engineering workflow .

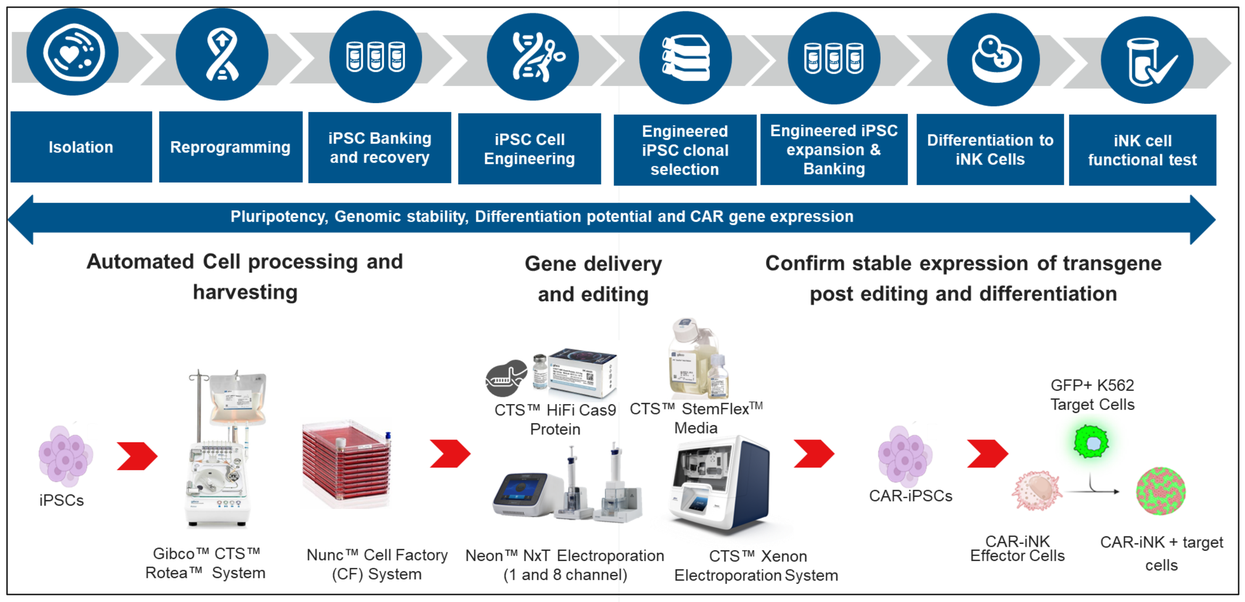
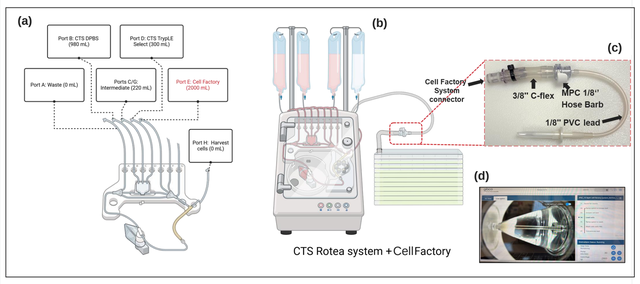


Figure 2. Closed automated cell harvest and banking.

iPSCs were cultured and expanded to a billion cells in a 10-layer CF system using CTS StemFlex media. The CF system was then integrated with the Rotea system for further processing. Single use kit design for Rotea process (a). The 10-layer CF system was connected to the single-use kit with a custom tubing assembly (b). Customized tubing assembly connects the CF system and the CTS Rotea system (c). iPSCs bed formation in CTS Rotea system cone (d).

Reference for pluripotent stem cell scale out in CF system : https://www.ncbi.nlm.nih.gov/books/NBK571710/



Results

Figure 3. iPSC Pluripotency Characterization.

iPSCs were cultured in 10-layer CF system. After reaching 70% confluency, iPSCs were processed and harvested by using CTS Rotea system. Expression of pluripotency markers (TRA1-60, TRA1-80 and SSEA4) were determined after culturing iPSCs for 2-3 weeks post processing with CTS Rotea system. The upper panel represents iPSCs processed with manual method and the lower panel represents iPSCs processed with CTS Rotea system process. Morphology of the iPSC colonies (a), Viable cells gating (b), expression of TRA1-60 pluripotency marker (c), the expression of TRA1-80 and SSEA4 pluripotency marker (d) and Immunocytochemistry staining for SOX2 and TRA1-60 (e).

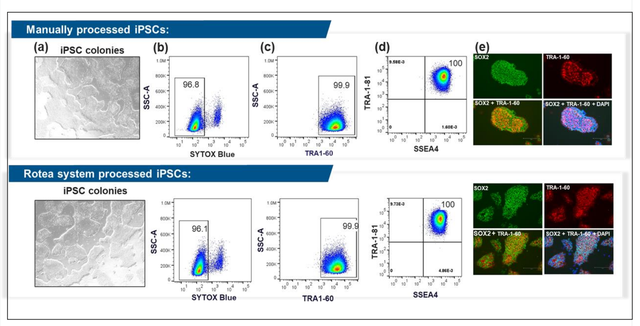


Figure 4. iPSC cell engineering workflow.

The iPSCs were cultured and expanded in CTS StemFlex media. The iPSCs were engineered using Cas9RNP and linear dsDNA for CAR knock-in. Payloads were delivered using Neon NxT or CTS Xenon electroporation system. The gene-edited cells were further cultured for up to 28 days and sorted using the Bigfoot cell sorter. The enriched CAR iPSCs were then differentiated to CAR-iNK cells using CTS™ StemPro™ 34 SFM XF Media. INK Cells were expanded in CTS™ NK-Xpander™ Medium.

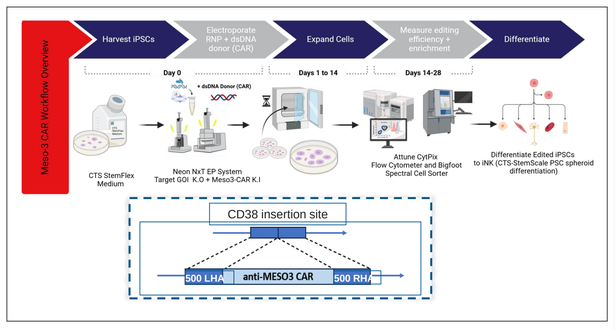


Figure 5. Optimization of electroporation (EP) programs for best EP efficiency.

Healthy iPSCs are expanded for stable cell growth. The iPSCs are used for transgene editing optimization using the Neon NxT EP system with different EP programs. On day 4 post-electroporation, iPSCs were characterized using the Attune CytPix flow cytometry instrument and data was analyzed and plotted for percent knock-out efficiency (a), percent knock-in efficiency and (b), total gene edited CAR positive cells (c).

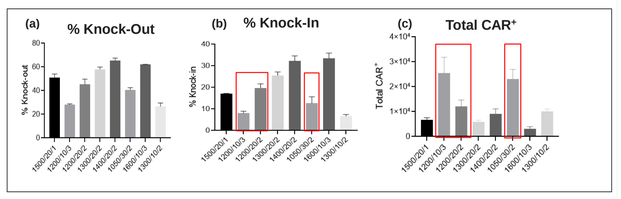


Figure 6. Electroporation scalability from RUO Neon NxT to GMP compatible Xenon EP system and characterization of gene edited iPSCs.

The best-selected EP program from the Neon NxT EP system is scaled up to the CTS Xenon EP system for optimal EP efficiency. The flow cytometry gating strategy for gene-edited iPSCs (a). EP efficiency between Neon NxT and CTS Xenon EP systems for optimal efficiency (b). Characterization of either Neon NxT/ CTS Xenon gene-edited iPSCs includes a Karyostat assay to assess genomic stability (c), Pluritest assay for gene edited iPSCs (d).

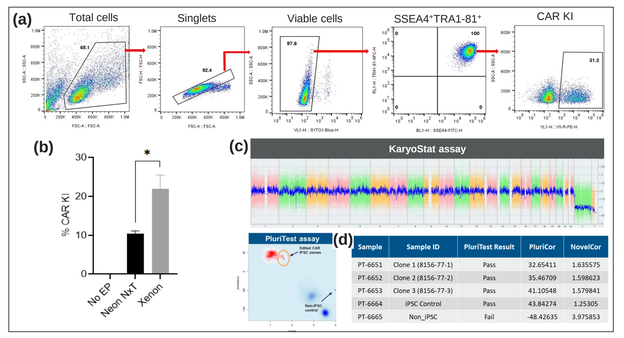
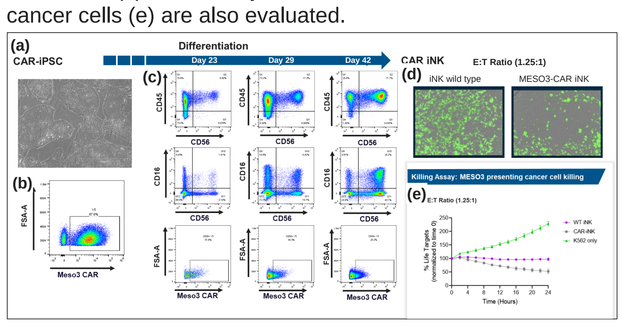


Figure 7. Differentiation of CAR iPSCs to CAR iNK cells. CAR iPSCs are differentiated into CAR iNK cells over 42 days. Before differentiation, CAR iPSCs show compact colonies (a) and express MESO3 CAR (b). During differentiation, CAR iPSCs are evaluated for CD45, CD16, CD56, and MESO3 CAR expression (c). Differentiated iNK cells are assessed for their killing efficiency on cancer cells. GFP-positive cancer cell images with and without CAR iNK incubation (d) and the cytotoxic effect of CAR iNK cells on cancer cells (e) are also evaluated.



Conclusions

iPSC processing using the CTS Rotea System

- Closed system harvesting process using integrated Rotea and CF system reduced the risk of contamination.
- Direct welding of the CTS Rotea system to the Cell Factory System™ enabled.
- Efficient and automated wash and concentration (5-fold) of more than 1 x 10^9 iPSCs.
- Pluripotency and viability was maintained post closed system processing.

iPSC Gene Engineering.

- EP programs, payload concentrations, and cell density were optimized for best EP efficiency using Neon NxT system.
- The Xenon and Neon NxT systems demonstrated scalable performance using the same EP conditions (1200V, 10ms, 3 pulses).
- CAR-iPSC clones-maintained pluripotency and there were no chromosomal aberrations.
- iPSC-derived iNK cells showed stable expression of CAR transgene post-differentiation.
- CAR-iNK cells showed improved target cell killing potential compared to wild-type (WT) NK cells.

Acknowledgements

We would like to thank Omar Farah for his help in preparing the workflow charts.

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