Cell Engineering Solutions for Cell-based Immunotherapy Manufacturing: From Activated to Resting T Cell Engineering

Akshaya Chandrasekaran[1], Ranganatha R Somasagara[1], Arseny Smirnov[1], Gregory Bonello[1], Namritha Ravinder[1]
[1]Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA 92008.

Abstract

Rapid developments in the field of chimeric antigen receptor (CAR) T-based immunotherapies and the need for generating efficient and cost-effective cell therapies have increased the demand for cell and gene therapy manufacturing workflow optimization to help improve patient access while maintaining quality control and performance. Conventional CAR T cell manufacturing workflows involve T cell activation, followed by payload delivery, and *ex vivo* expansion. However, the activation and expansion of CAR T cells could lead to their progressive differentiation, T cell exhaustion, and eventually resistance to CAR T therapies. Accumulating evidence in mice and humans suggests that T cell differentiation negatively correlates with long-term anti-tumor activity, with early memory T cells allowing for greater and persistent anti-tumor effects[1]. More recently, it has been established that a modified cell manufacturing process implementing activation after Cas9 delivery minimizes occurrence and impact of chromosome loss in the manufactured product. Non-activated cells are also desirable as a starting material to help achieve shorter workflow times critical to point-of-care and decentralized therapy manufacturing and reduced costs. Here we demonstrate a flexible, closed, and modular system for the generation of CAR T cell products starting from non-activated/resting and activated T cell populations.

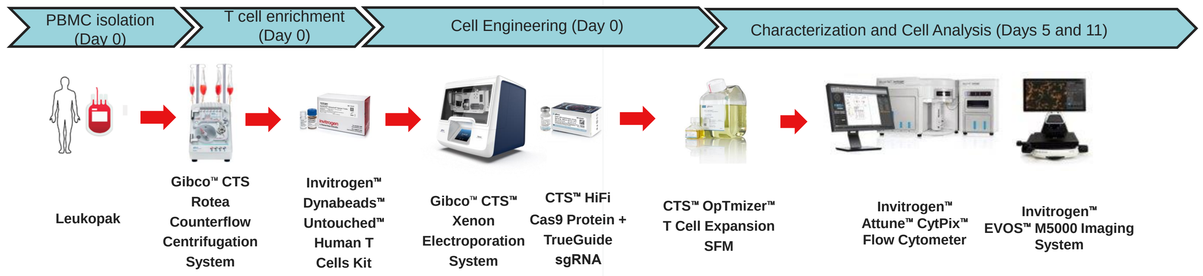
Introduction

Gene-modified cell therapy is a promising cancer treatment strategy. The complexity of cell therapy manufacturing can be associated with the labor-intensive and open protocols that are commonly used today. Thermo Fisher Scientific has developed a workflow solution that allows for physical and digital integration of the end-to-end manufacturing workflow and has the flexibility to scale for a variety of commercial applications. By combining the innovative closed modular components of the workflow, manufacturers can efficiently optimize their workflows in compliance with current regulatory requirements. Prolonged *ex vivo* cultures have been linked to increased T cell exhaustion, underscoring the importance of reducing T cell manufacturing times. Editing non-activated T cells offers the additional benefit of mitigating chromosomal loss and lowering the occurrence of unintended indels compared to editing activated T cells, as indicated by recent studies[2]. While chromosome loss could be an inherent outcome of site-specific gene editing, adjustments to protocols and innovative workflows that facilitate editing of non-activated T cells can significantly minimize both its frequency and impact. Thermo Fisher Scientific has developed a lower conductivity electroporation buffer formulation for the Gibco™ CTS™ Xenon™ Electroporation System that can enable higher energy settings to be applied to cells without adversely affecting viability while facilitating the efficient editing of non-activated T cells, thereby shortening the manufacturing workflow and minimizing occurrence of chromosomal loss and translocations.

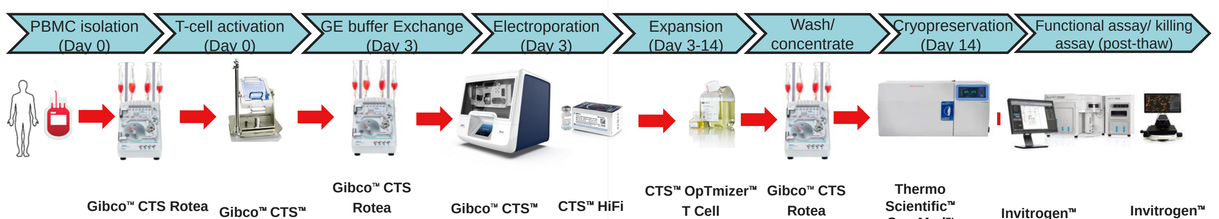
Materials and methods

We use an automated counterflow centrifugation method where PBMCs are isolated from fresh leukopaks using the Gibco™ CTS™ Rotea™ Counterflow Centrifugation System and then downstream T cell enrichment is performed using the Invitrogen™ Dynabeads™ Untouched™ Human T Cells Kit. Electroporation (EP) reactions for payload delivery are set up and optimized at small scale using Invitrogen Neon™ NxT™ Electroporation System and then scaled up using CTS™ Xenon™ Electroporation System. Transfection efficiency and cell viability are measured by flow cytometry and resting T cell phenotype is monitored pre- and post-EP to monitor maintenance of stemness in comparison to the starting population. To enable optimal delivery in resting T cells, electroporations are performed using CTS™ Xenon™ Lower Conductivity (LC) Buffer. For the activated T cell protocol, PBMCs are isolated from frozen leukopaks using the Rotea™ system and activated using CD3/CD28 CTS™ Dynabeads™ for 72 hours. The activated T cells are then resuspended in CTS™ Xenon™ Genome Editing (GE) Buffer and electroporated to deliver the Cas9 RNP complex and donor DNA expressing anti-CD19 CAR. The Cas9 RNP complex was prepared using GMP grade CTS™ HiFi Cas9 protein and TrueGuide™ sgRNA. Post 14-day expansion, cells are washed and concentrated using Rotea™ and analyzed for transgene expression and cryopreserved for downstream functional studies in a CryoMed™ Controlled-Rate Freezer. Targeted Amplicon-seq validation assay (TAV-seq) confirmed no detectable off-targets in CAR T cells and functional cancer cell cytotoxic assays against CD19 presenting-Nalm-6 cells showed effective killing by anti CD19 CAR-T cells.

Non-activated T cell gene editing workflow



Activated T cell gene editing workflow



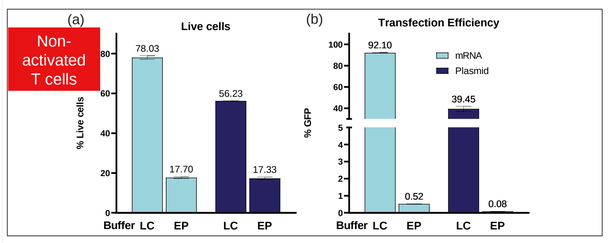


Figure 1. LC buffer outperforms R buffer in Resting T Cells. CTS Lower Conductivity (LC) Electroporation Buffer outperforms CTS Electroporation Buffer (EP) for both mRNA and DNA based payloads in resting T cells as observed from a) cell viabilities 24 hours (mRNA) or 3 days (plasmid) post-electroporation, and b) % GFP mRNA or plasmid transfection efficiency using CTS Electroporation Buffer or CTS Lower Conductivity Electroporation Buffer on Xenon electroporation system.

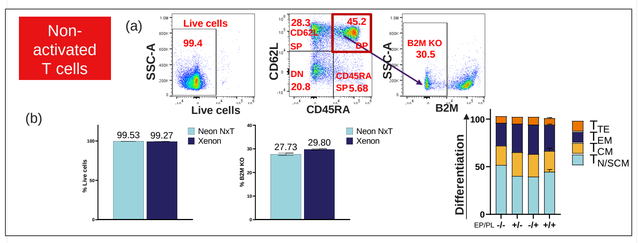


Figure 2. LC buffer demonstrates successful Cas9 RNP delivery in resting T cells. a) The CTS Xenon Lower Conductivity Electroporation Buffer demonstrates successful delivery of CTS™ HiFi Cas9 Protein and TrueGuide B2M Synthetic gRNA using higher energy settings on the Xenon electroporation system as indicated by a) representative flow plots for b) maintenance of cell viability 5 days post-EP, scalable B2M KO, and maintenance of stemness markers post-EP.

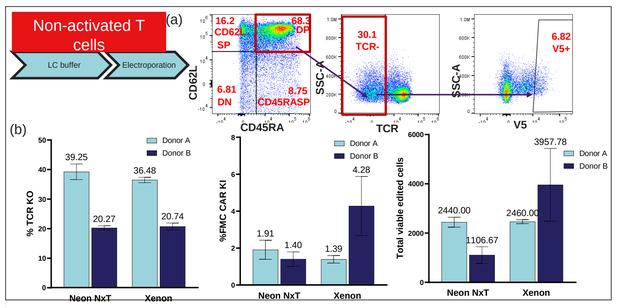


Figure 3. LC buffer enables activation-agnostic gene editing of non-activated T cells. Non-activated T cells were resuspended in T buffer or LC buffer and electroporated with CTS HiFi Cas9, TrueCut gRNA and CD19 CAR dsDNA using Neon NxT electroporation system or large-scale CTS Xenon electroporation system. (a) The expression of cell surface markers (CD45RA, CD62L, TCR CD19 CAR) were determined at Days 5 and 11 using flow cytometry (b). % TCR KO efficiency, % CD19 CAR KI efficiency, and total viable edited cells 11 days post-EP were measured.

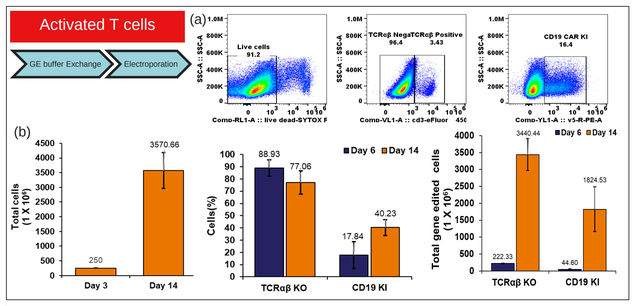


Figure 4. GE buffer enables gene editing of activated T cells. Activated T cells were resuspended in GE buffer using CTS Rotea™ System and electroporated with CTS TrueCut Cas9, TrueCut gRNA and CD19 CAR dsDNA using closed large scale CTS Xenon Electroporation System. The expression of cell surface markers (CD3, CD19 CAR) were determined at Day 6 and Day 14 using flow cytometer (a). Fold expansion, editing efficiency, total edited cells were calculated on Day 6 and Day 14 (b).

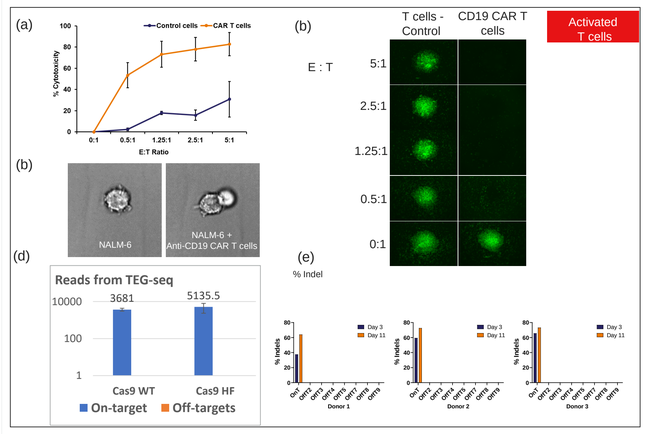


Figure 5. CAR T cells generated from activated T cells are functional and cytotoxic. Anti-CD19 CAR T cells and NALM-6 cancer cells were co-cultured at different effector to target cell ratios for 18 hrs. (a) Cytotoxicity was evaluated using Invitrogen™ Attune™ CytPix™ Flow Cytometer. (b) The cell images were collected by EVOS M5000 Imaging System and (c) single cell-cell interaction was captured by Attune Cytpix cytometer. No off-targets were detected through a genome-wide off-target screen in the T cells edited using TrueGuide TRAC sgRNA and CTS™ Cas9. (d) Both wild-type and high-fidelity versions of Cas9 were tested via target-enriched GUIDE-seq (TEG-seq) for on- and off-target reads. (e) Targeted Amplico-seq validation (TAV-seq) confirmed no detectable off-target for predicted top off-target sequences 3- and 11-days post electroporation.

Conclusions

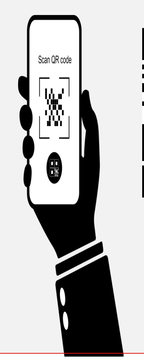
In summary, we established a CAR T cell engineering workflow using automated instrumentation for cell processing and gene delivery thereby minimizing human intervention in the cell therapy manufacturing process. Non-activated or activated T cells can be used as starting material in our end-to-end optimized T cell engineering workflow that has the flexibility to be scaled up for a variety of clinical applications. The transfection reagents and gene editing payloads, in combination with the closed automated workflows described here, can help reduce labor-intensive open system hurdles and help improve the engineering of hard-to-transfect primary cells used for the development of efficient adoptive immunotherapies.

References

1. Meyran D, Zhu JJ, et al. T_{STEM}-like CAR-T cells exhibit improved persistence and tumor control compared with conventional CAR-T cells in preclinical models. Sci Transl Med. 2023 Apr 5;15(690):eabk1900.doi: 10.1126/scitranslmed.abk1900
2. Tsuchida CA, Brandes N, et al. Mitigation of chromosome loss in clinical CRISPR-Cas9-engineered T cells. Cell. 2023 Oct 12;186(21):4567-4582.e20. doi: 10.1016/j.cell.2023.08.041.

Trademarks/licensing

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.



Learn more at thermofisher.com/celltherapy