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Workflow Optimization of Rotea™-to-Xenon™: A Closed Modular and Semi-Automated System for Cell Therapy

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Abstract

Purpose: To provide a closed, GMP-compliant, and non-viral system to overcome the manufacturing challenges and get therapies to patients faster.

Methods: The integration of Thermo Fisher Scientific products such as the CTS™ Rotea, DynaMag™ Magnet, Xenon Electroporation, and OpTmizer™ cell culture media. Human, primary T-cells were activated for 2 of 3 days and then, electroporated with Xenon to generate nonviral CAR T cells. The newly created CAR T-cells were expanded and analyzed 3 days post-EP.

Results: On Day5 (3 days post-EP), the cells were analyzed by Attune™ Flow Cytometer and cell counter. % Knockout (KO) of TCR$\alpha\beta$ and % CAR expression Knock-in (KI) were measured to be >90% and 20-50%, respectively. Viability was measured to be >85%.

Introduction

In recent years, there has been substantial progress in the development and application of cell and gene therapy. However, most clinical studies maintain the use of viral vector systems. Non-viral delivery systems are an attractive alternative due to their reduced cytotoxicity, immunogenicity, and mutagenesis as compared to viral vectors. Electroporation, one of the non-viral approaches, is particularly suitable due to its simplicity of use, ease of large-scale production and lack of specific immune response. Autologous T cell therapy process has many touch points and a very labor-intensive workflow with a lot of open processes and complexity. Due to those challenges, manufacturing practices make gene therapies difficult to scale and meet the therapeutic demand of treatments with large patient populations. Thermo Fisher Scientific has been working to integrate and optimize the whole process into one workflow to better serve the cell therapy industry. The Gibco™ CTS Rotea system is a flexible/efficient system that can isolate cells with high viability and recovery. The newly launched Gibco CTS Xenon Electroporation System offers reliable cell therapy development and manufacturing with high cell viability and efficiency during the ex vivo genetic modification step. By combining Rotea and Xenon systems together into one workflow, this closed, modular, and semi-automated system will help to overcome some of the challenges and ultimately get therapies to patients faster. 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

Sample Preparation – PBMC isolation by Rotea, Activation with CD3/CD28 Dynabeads, and debeading with DynaMag Magnet.

Test Method(s) – Rotea Wash/Conc, Xenon Electroporation, Immunophenotyping, %KO/%KI efficiencies, Viability, and Viable Cell counts for cell growth and expansion.

Data Analysis – Attune Flow Cytometer, cell counter, Prism and FlowJo.

Results

Genome Editing of human primary T-cells using Rotea-Xenon Workflow System.

Human PBMC were isolated from Leukopak by Rotea. PBMC were activated with anti-CD3/CD28 dynabead for 2 or 3 days. Activated cells were debeaded and washed/concentrated by Rotea. Those cells were electroporated with Xenon by targeting a CAR construct to the TRAC locus with CRISPR/Cas9 system. 3 days post-EP, the cells were analyzed by Attune flow cytometry and cell counter.

Figure 1. Comparison of CD4/CD8 ratio and CD69/CD25 activation markers between D2 protocol and D3 protocol.

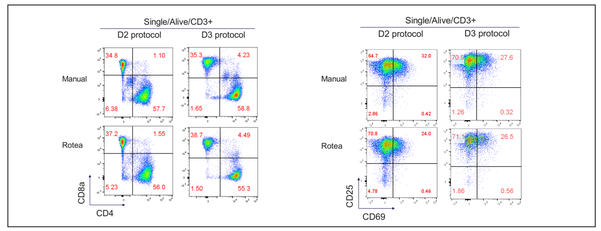


Figure 2. Rotea wash/concentration and non-viral gene editing by Xenon Electroporation. a) 3 days post-EP, cell viabilities were measured by Vi-Cell. b) % Knockout (KO) of TCR$\alpha\beta$ and % GFP expression via Knock-in were calculated by Flow cytometry analysis. (0 ul: No EP, 100 uL: Neon Electroporation, and 1 mL: Xenon Electroporation)

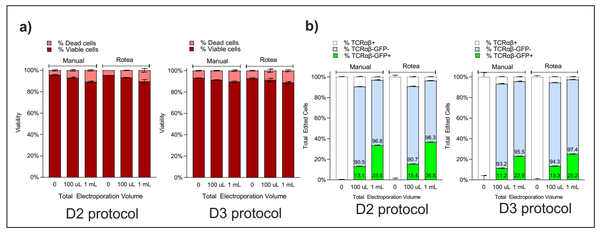
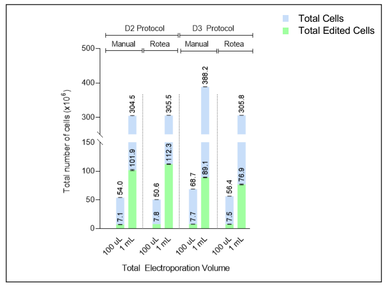


Figure 3. Experimental Condition and Calculation of Total Edited Cells.

Sample ID		Run
Cell density per mL in EP buffer (GE buffer)		50M
Payload	Cas9V2 (5 mg/mL)	120 ug
	gRNA (3.2 ug/uL)	30 ug
	GFP (2ug/uL)	80 ug

CTS Xenon Genome Editing Buffer

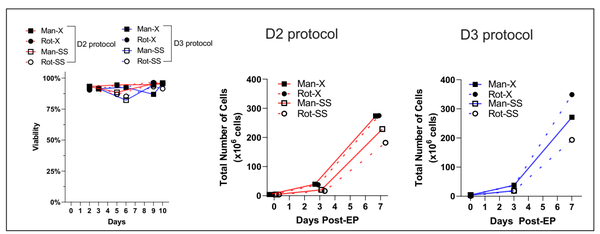


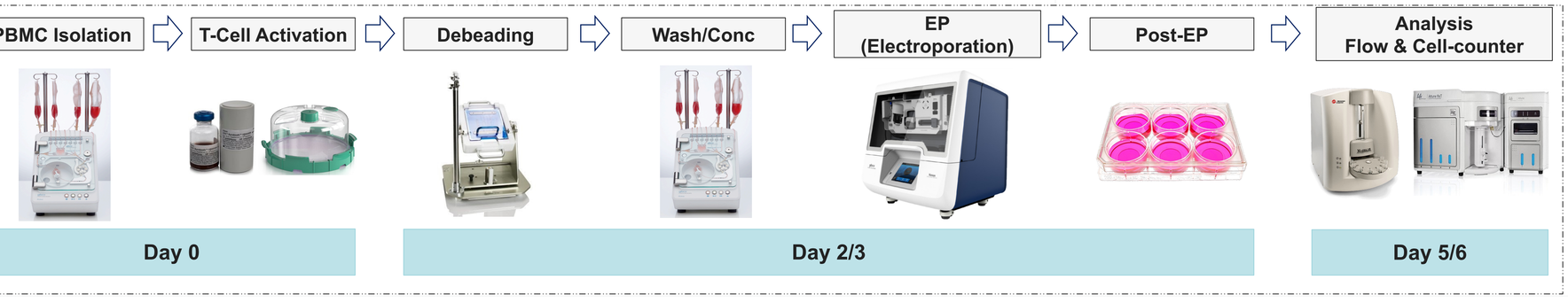
** Total edited cells = % total edited cells X total number of cells

CTS Xenon SingleShot Chamber

CTS TrueCut™ Cas9 protein

Figure 4. Cell health and growth: Viability and Expansion up to 7 days post-EP





Conclusions

A Rotea-Xenon workflow successfully supports CAR-T manufacturing process.

- KI efficiencies were higher with 2 days of activation than with 3 days of activation, although KO efficiencies were similar.
- Cell viability and Immunophenotyping data show no significant difference between 2 days of activation and 3 days of activation.
- Newly generated CAR-T cells were expanded up to 7 days post-EP with no adverse effect.

References

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3. https://www.thermofisher.com/us/en/home/clinical/cell-gene-therapy/cell-therapy/cell-therapy-manufacturing-solutions/xenon-electroporation-system/resources.html.

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