Freeze, Thaw, and Expand: Scale-Up T Cell Workflow In Chemically Defined Media

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Introduction

The application of T cells as therapeutic products necessitates a reproducible manufacturing process that minimizes cell stress with reduced cell handling, while maximizing scale-up potential with robust cell growth. A major challenge in the field of cell therapy is the difficulty in generating sufficient cell numbers with consistent clinical quality. This can be significantly mitigated by the removal of undefined media components, resulting in a dependable, reproducible manufacturing process and safe translation for clinical use. Maintaining a serum-free environment throughout the manufacturing workflow is critical for the reliable activation, genetic manipulation, expansion, cryopreservation, and recovery of T cells developed for therapeutic use.

FUJIFILM's rational design approach to media development produced chemically defined, animal component-free solutions for T cell manufacture and temperature-controlled storage, with performance equal to or better than commercially available, non-chemically defined media. Compatible with multiple culture and activation methods and a variety of cell sources, PRIME-XV FreezIS DMSO-Free and T cell Chemically Defined Medium (CDM) work in tandem to provide a powerful tool for T cell therapy manufacture.

Methods

Cell source

All data presented was generated using human PBMCs. Cultures were initiated without further T cell enrichment. All cells were expanded in the presence of 200 IU/mL recombinant human IL-2 (Shenandoah Biotechnology Inc).

Transduction

Freshly activated PBMCs were inoculated with PGK-GFP reporter lentivirus at MOI = 5 and incubated in standard tissue culture conditions overnight. The following day, the virus-containing media were replaced with fresh media, and the cells were expanded for up to two weeks.

G-Rex Culture System

R series G-Rex vessels with volumes of 7 and 35 mL, as well as M series vessels with volumes of 100 mL and 1L were used. Wilson Wolf-recommended culture protocols were followed.

Quantum Bioreactor

Cells were expanded in RUO Quantum perfusion bioreactors for nine days following a low-seed T cell culture protocol provided by Terumo BCT.

Flow cytometry

Analysis was performed on a BD FACSymphony A3 using 13- and 18-color panels for surface and function staining, respectively.

Results

FIGURE 1. Cell therapy manufacturing process. The workflow for generating clinical-grade cellular therapy products requires a diverse range of technologies and rigorous reproducibility. Minimizing handling of cells between the manufacturing steps and maximizing cell quality and expansion is essential.

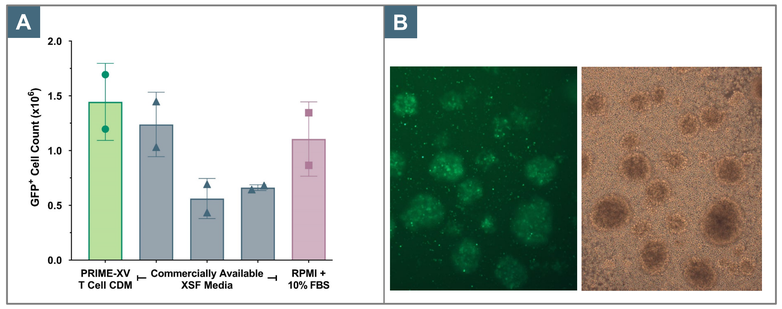

FIGURE 2. PRIME-XV T Cell CDM supports transduction as well as or better than commercially available xeno-free media and FBS-supplemented RPMI. 0.250x10^6 PBMCs were transduced in corresponding media in 24-well G-Rex plates (A) Total transduced cell count on day 7 post-transduction. (B) Fluorescence and bright field microscopy images of the transduced T cell clusters. Data is representative of two healthy donors.

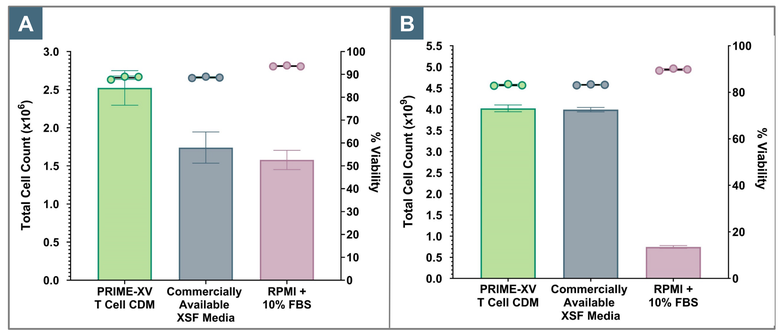

FIGURE 3. PRIME-XV T Cell CDM supports the scale-up of static culture systems such as G-Rex. Human PBMCs were activated 1:1 with anti-CD3 & anti-CD28 beads and cultured in (A) 24-well R series G-Rex plates and (B) 1 L G-Rex vessels per Wilson Wolf protocols, and results were obtained on day 11 of expansion. Results are representative of three healthy donors.

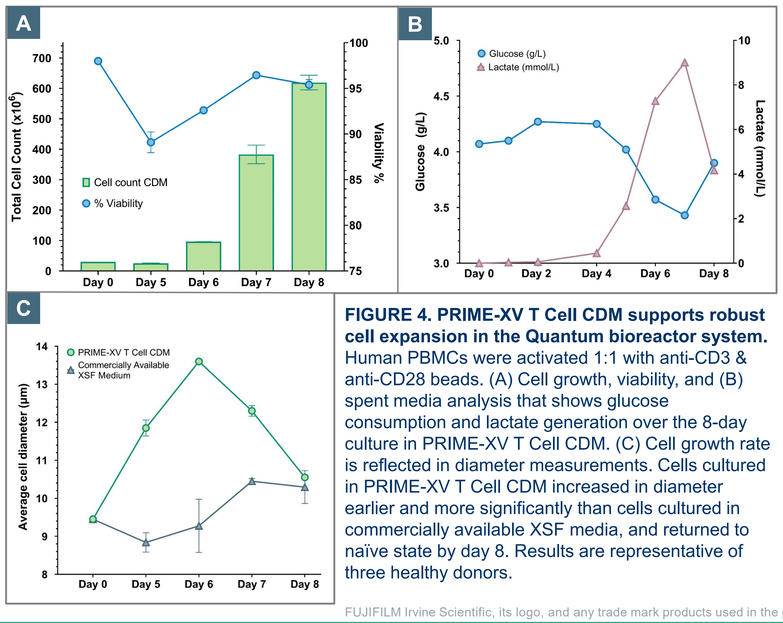

FIGURE 4. PRIME-XV T Cell CDM supports robust cell expansion in the Quantum bioreactor system. Human PBMCs were activated 1:1 with anti-CD3 & anti-CD28 beads. (A) Cell growth, viability, and (B) spent media analysis that shows glucose consumption and lactate generation over the 8-day culture in PRIME-XV T Cell CDM. (C) Cell growth rate is reflected in diameter measurements. Cells cultured in PRIME-XV T Cell CDM increased in diameter earlier and more significantly than cells cultured in commercially available XSF media, and returned to naïve state by day 8. Results are representative of three healthy donors.

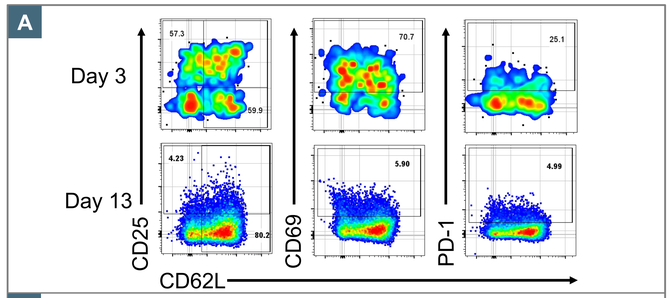

FIGURE 5. Activation and exhaustion markers illustrate healthy cell growth kinetics over two weeks of culture in 24-well G-Rex plate. (A) Representative flow cytometry dot plots of the three markers on days 3 and 13. (B) The early activation marker CD69 peaks on approximately the same day as the exhaustion/cell death marker PD-1, with late-stage activation marker CD25 peaking at the end of the first week of culture. All three drop to naïve cell levels by day 13. Results are representative of three healthy donors.

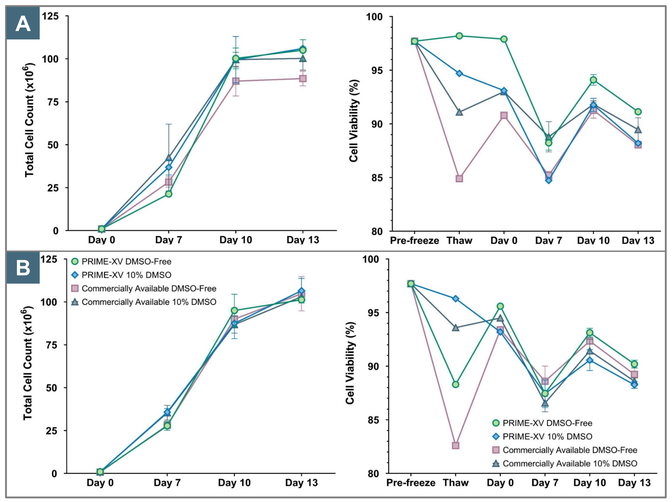

FIGURE 6. Human PBMCs frozen in PRIME-XV FreezIS DMSO-Free do not require a centrifugation step post-thaw. (A) Cell thawed directly into PRIME-XV T Cell CDM and cultured for 13 days yielded 100-fold expansion, and cells thawed from PRIME-XV FreezIS DMSO-Free specifically maintained a higher viability throughout the culture. (B) Cells that were centrifuged to wash out the cryoprotectant post-thaw displayed a similar growth curve, but DMSO-free viability in early days of culture was significantly lower. Results are representative of three healthy donors.

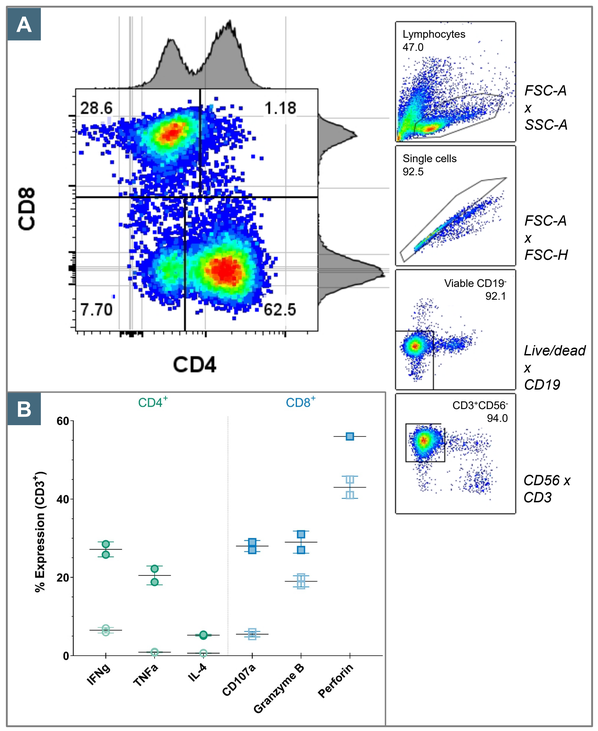

FIGURE 7. Cells expanded in PRIME-XV T Cell CDM maintain Th1, Th2, and cytotoxic functionality. Staphylococcus enterotoxin B (SEB) stimulation on day 14 post-expansion demonstrates the cells' ability to produce cytokines essential for a variety of T cell functions. (A) Representative gating strategy for identifying CD4$^+$ and CD8$^+$ subsets. (B) CD4$^+$ expression of Th1 (IFNg, TNFa) and Th2 (IL-4) and CD8$^+$ expression of cytotoxic markers show significant increase in SEB-stimulated cells (filled shapes) compared to background expression levels of unstimulated cells (open shapes).

Conclusion

FUJIFILM Irvine Scientific's PRIME-XV T-cell Chemically Defined Medium supports T-cell activation and expansion to the same degree or better than commercially available xeno-free media. Though magnitude of expansion and relative performance of benchmark media differed between cell donors, these results were representative of the trend seen across all runs. We demonstrate that T cells frozen in PRIME-XV FreezIS DMSO-Free cryopreservation medium may be directly thawed into PRIME-XV T Cell CDM, avoiding the mechanical stress cells undergo during post-thaw centrifugation. The omission of a post-thaw wash step also has the benefit of streamlining the manufacturing process and simplifying production upscaling. PRIME-XV T Cell CDM supports cell activation, lentiviral transduction, and subsequent expansion in G-Rex Cell Culture Systems and Quantum hollow fiber bioreactors.

Chemically defined media perform consistently, scale reliably, and are more likely to translate safely to clinical use. Our chemically defined cryopreservation and cell culture media provide solutions for *ex vivo* workflow in the field of cell therapy, paving the future for autologous and allogeneic therapy alike.