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Demonstrating Key Processes of the T Cell Therapy Workflow in Chemically Defined Media

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BACKGROUND

Although technologies used in the generation of immunotherapies are diverse and rapidly evolving, they share the goal of efficiently generating sufficient cell numbers with high viability and consistent clinical quality. The elimination of undefined media components such as serum-derived proteins reduces variability, facilitating safe and efficacious translation for clinical use.

Automated manufacturing processes and associated closed system solutions are vital for the manufacture of cell therapies, as they minimize risk of contamination, reduce manual cell handling, and result in significantly higher cell numbers and more efficient cell expansion, leading to superior therapeutic outcomes. Chemically defined (CD) media are uniquely amenable to consistent and reproducible scale-up due to the lack of undefined media components, enabling manufacturers to reliably make products of high quality and reduced variability when transitioning from preclinical development to cGMP manufacturing of cell-based therapies.

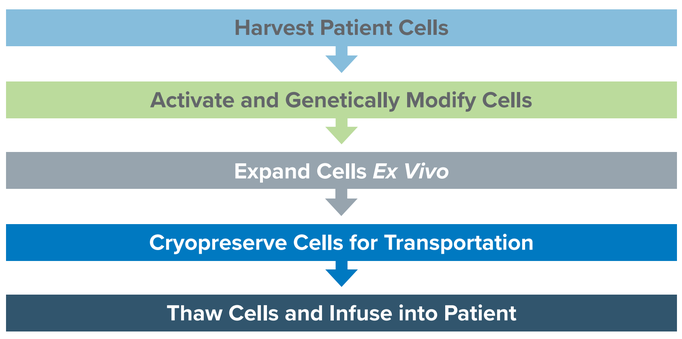

Figure 1. Cell therapy manufacturing process. The workflow for generating clinical-grade cellular therapy products requires a diverse range of effective technologies. Maximizing cell quality and expansion is essential.

MATERIALS AND METHODS

Media and Cytokines: PRIME-XV T Cell CDM (Catalog # 91154), PRIME-XV FreezIS (Catalog # 91139), PRIME-XV FreezIS DMSO-Free (Catalog # 91140), CTGrade GMP rh IL-2_{C126S} (Catalog # 500-01), CTGrade GMP rh IL-7 (Catalog # 500-07), CTGrade GMP rh IL-15 (Catalog # 500-08), and CTGrade GMP rh IL-21 (Catalog # 500-09).

Cell Source: Human peripheral blood mononuclear cells (PBMCs) were activated with αCD3/αCD28 beads. Cultures were initiated without further T cell enrichment.

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 in 24-well G-Rex vessels.

G-Rex Culture System: Cells were expanded in either G-Rex 24-Well Plates with a culture volume and 7 mL and a surface area of 2 cm^2, or in G-Rex 100 M culture vessels with a culture volume of 1 L and a surface area of 100 cm^2. Wilson Wolf-recommended culture protocols were followed.

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

Superantigen Stimulation: On day 13 of expansion, PBMCs were re-stimulated with staphylococcal enterotoxin B (SEB), incubated with a protein transport inhibitor, fixed, and stained intracellularly for cytokines and other functional markers. Control PBMCs were treated identically but stimulated with phosphate buffered saline (PBS).

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

Cryopreservation: Freshly isolated PBMCs were frozen in various freezing media overnight using an isopropanol freezing container, followed by transfer to liquid nitrogen for a minimum of seven days.

SUMMARY

PRIME-XV T Cell CDM supports cell activation, lentiviral transduction, and subsequent scalable expansion of human PBMC-derived T cells in G-Rex Cell Culture Systems and in Quantum hollow fiber bioreactors. PRIME-XV cryopreservation media supports DMSO-free cryopreservation and wash-free thawing. Our data indicate that the expanded cells maintain robust functionality in response to antigenic stimulation, and the omission of a post-thaw wash step streamlines the manufacturing process and simplifies production scaling.

Conclusion: Compatible with multiple culture and activation methods, chemically defined media PRIME-XV FreezIS DMSO-Free and PRIME-XV T Cell CDM work in tandem with CTGrade GMP recombinant human cytokines to provide a powerful toolset for the key processes of T cell therapy manufacturing.

RESULTS

Transduction Using PRIME-XV T Cell CDM

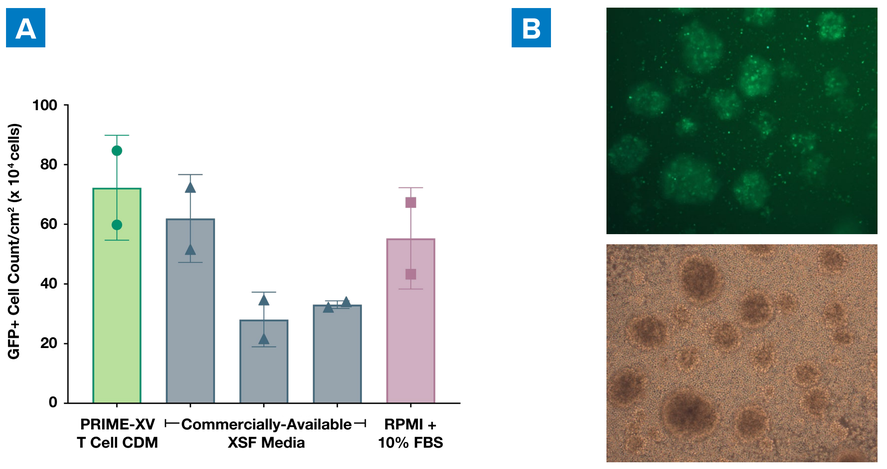

Figure 2. PRIME-XV T Cell CDM supports transduction as well as or better than commercially-available xeno-free, serum-free (XSF) media and FBS-supplemented RPMI. 2.50 x 10^5 PBMCs were transduced in corresponding media in 24-well G-Rex plates **(A)** Total transduced cell count on day 7 post-transduction. **(B)** Fluorescence and bright field microscopy images of the transduced T cell clusters. Results are representative of two healthy donors.

Scale-up of Static Culture Using PRIME-XV T Cell CDM

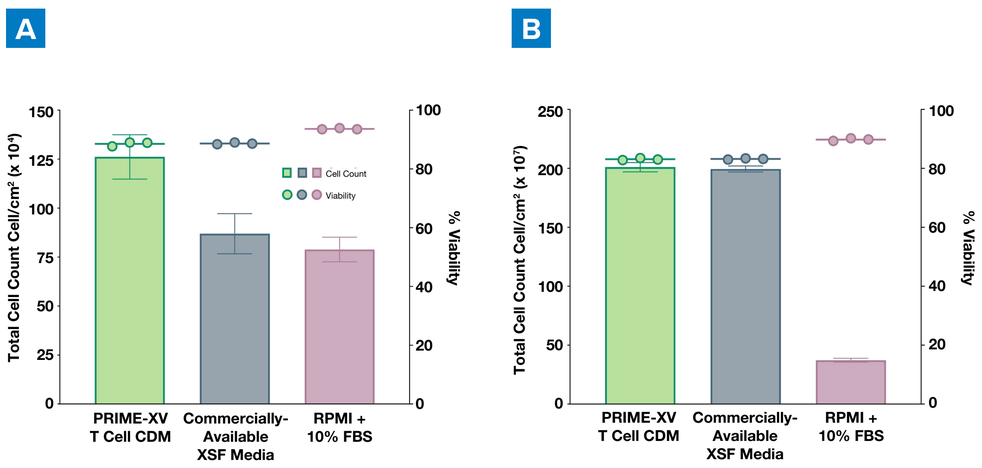

Figure 3. PRIME-XV T Cell CDM supports the scale-up of static culture systems such as G-Rex. Human PBMCs were activated 1:1 with anti-CD3 & anti-CD28 beads and cultured in **(A)** 24-well R series G-Rex plates and **(B)** 1 L G-Rex vessels per Wilson Wolf protocols, and results were obtained on day 11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

Expansion in Bioreactors Using PRIME-XV T Cell CDM

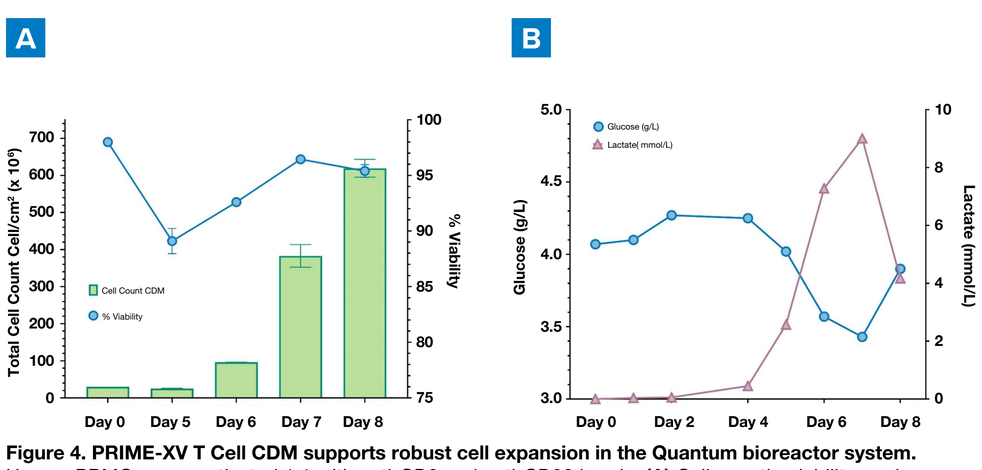

Figure 4. PRIME-XV T Cell CDM supports robust cell expansion in the Quantum bioreactor system. Human PBMCs were activated 1:1 with anti-CD3 and anti-CD28 beads. **(A)** Cell growth, viability, and **(B)** spent media analysis that shows glucose consumption and lactate generation over the 8-day culture in PRIME-XV T Cell CDM. Results are representative of three healthy donors.

IL-2-Independent Expansion with CTGrade GMP rh Interleukins

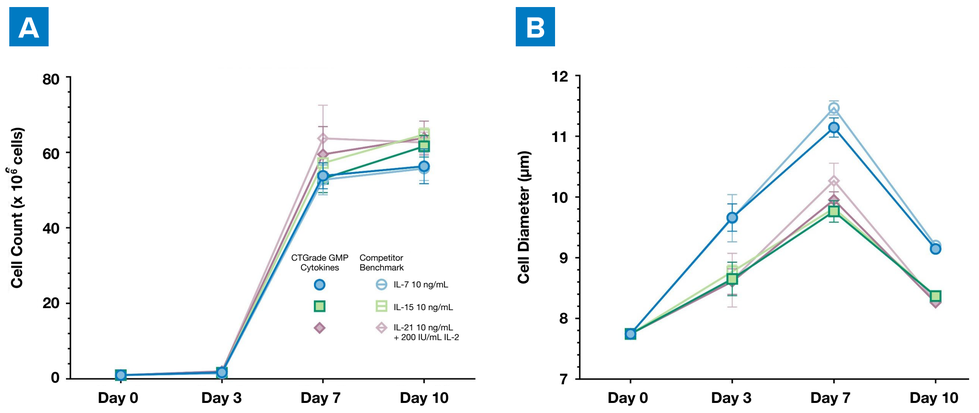

Figure 5. Fresh human PBMCs show robust IL-2-independent expansion and healthy activation kinetics in cytokine-supplemented PRIME-XV T Cell CDM. (A) By day 10, all CTGrade GMP rh IL-containing conditions had comparable levels of expansion. **(B)** The increase in cell diameter post-activation is more pronounced in conditions containing IL-7, but otherwise follows expected kinetics. Results are representative of three donors, three lots and two different concentrations of CTGrade GMP rh IL-7, rh IL-15, and rh IL-21.

Expansion with CTGrade GMP rh IL-2_{C126S}

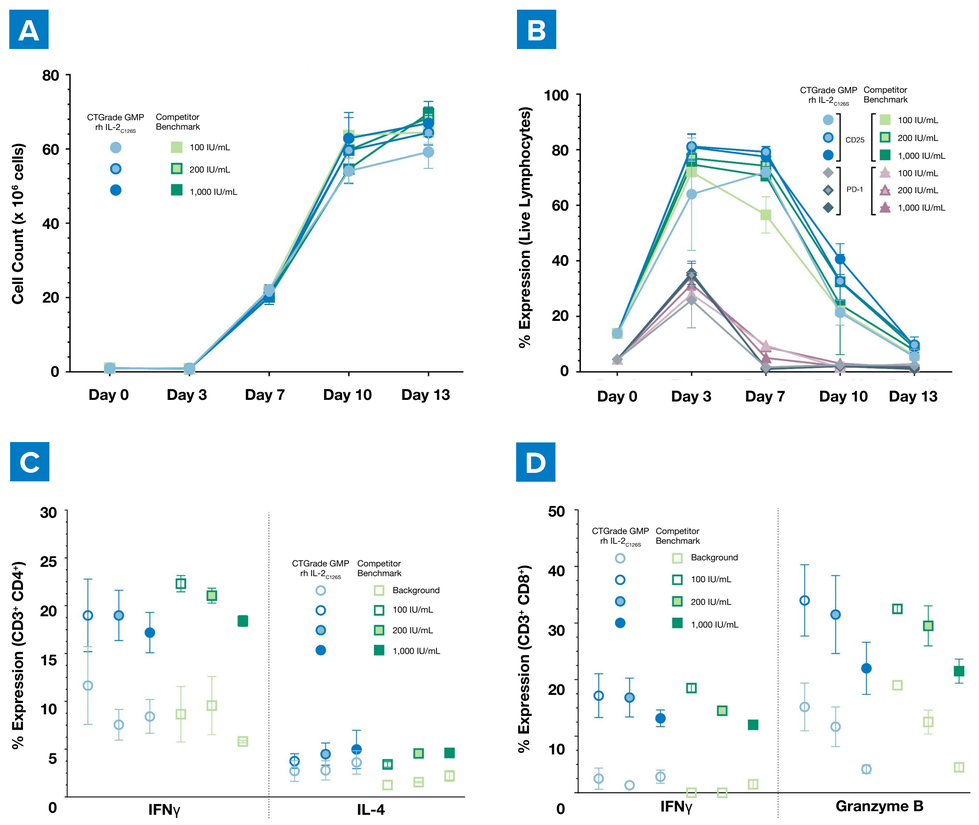

Figure 6. Fresh human PBMCs show robust expansion, healthy activation kinetics, and retain full function in CTGrade GMP rh IL-2_{C126S} supplemented PRIME-XV T Cell CDM. (A) By day 13, all conditions showed similar growth patterns. **(B)** The day three increase in PD-1 is accompanied by a drop in viability (data not shown), which fully recovers by day 7 post-expansion with an accompanied decrease of PD-1 to baseline values. The activation marker CD25 remains high through the first week of expansion, with a mild decrease in levels at the lowest IL-2 concentration, returning to baseline by day 13. **(C)** $CD4^+$ cells provide a strong Th1 and mild Th2 response following superantigen stimulation. **(D)** $CD8^+$ cells secrete inflammatory cytokines and cytolytic molecules following superantigen stimulation. Strength of $CD4^+$ and $CD8^+$ response is independent of IL-2 concentration. Results are representative of three donors and three lots of CTGrade GMP rh IL-2_{C126S} run in duplicates per condition.

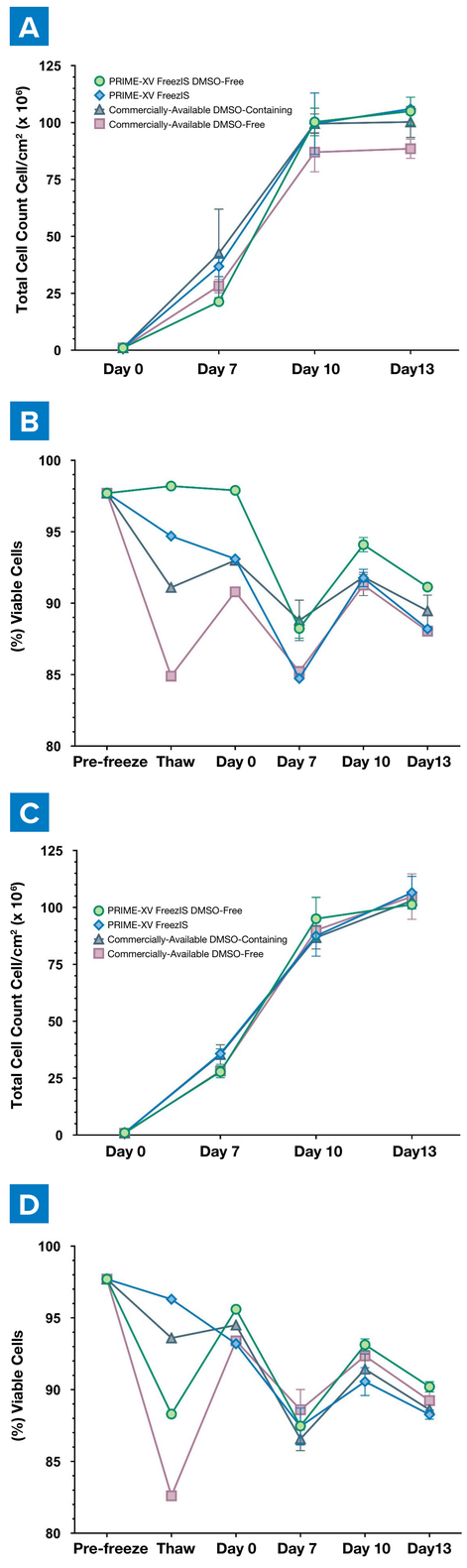

Figure 7. Human PBMCs frozen in PRIME-XV FreezIS DMSO-Free do not require a centrifugation step post-thaw. (A-B) Cell thawed directly into PRIME-XV T Cell CDM and cultured for 13 days yielded 100-fold expansion, and cells thawed from PRIME-XV FreezIS DMSO-Free specifically maintained a higher viability throughout the culture. **(C-D)** Cells that were centrifuged to wash out the cryoprotectant post-thaw displayed a similar growth curve, but DMSO-Free viability at thaw and post-rest was significantly lower. Results are representative of three healthy donors.