Cell therapy

CTS OpTmizer One SFM supports efficient production of CRISPR-Cas9–transfected CD19⁺ CAR T cells

Abstract

T cell therapies are groundbreaking immunotherapy treatments, but more reliable and scalable clinical-grade media are needed to address major manufacturing limitations. The efficacy of Gibco™ CTS™ OpTmizer™ One SFM (serum-free medium) in improving the production quality of CRISPR-Cas9–transfected chimeric antigen receptor (CAR) T cells was evaluated in this study. We assessed gene editing efficiency and the health and functional attributes of human T cells from a healthy donor, in G-Rex™ plates and a stirred-tank bioreactor (STBR). Our results indicate that CTS OpTmizer One SFM supports T cell expansion, viability, and gene editing efficiency. A 140-fold expansion and higher gene editing efficiency (~80%) were observed in the STBR by day 12. CTS OpTmizer One SFM also maintained higher percentages of memory T cells, enhanced CD8⁺ T cell populations, and increased antitumor activity compared to another serum-free medium. The results of this study demonstrate that CTS OpTmizer One SFM is a robust solution for enhancing the manufacturability of GMP-grade cell therapies.

Introduction

Although T cell therapies collectively represent a phenomenally successful and evolving treatment modality, CAR T cell manufacturing faces daunting challenges throughout each stage of isolation, activation, genetic modification, and expansion. These include manufacturing failures, functional inconsistencies, and compromised cell quality [1]. In addition, stringent regulatory requirements demand cell therapy manufacturing protocols that adhere to Good Manufacturing Practices (GMPs) and minimize the risk of contamination with viruses and pathogens. To realize the full potential of CAR T cell therapy to elicit effective clinical responses in a wider range of patients, it is essential to maximize the quality of ancillary components in the manufacturing process.

Developing a robust T cell therapy manufacturing workflow starts with selecting a refined medium that provides a strong foundation for optimal T cell expansion and GMP-compatible manufacturing of clinical-grade CAR T cells. CTS OpTmizer One SFM (Cat. No. A5757201) is a serum-free and animal origin–free (AOF) formulation that promotes desirable T cell characteristics, including expansion of naive and memory T cell subsets for enhanced proliferation, higher transfection efficiency, and a favorable antitumor response [2]. CTS OpTmizer One SFM is a versatile option that supports GMP manufacturing with closed and automated systems for cell isolation and expansion. As a single-part, GMP-compatible recombinant protein medium, CTS OpTmizer One SFM also reduces variability in performance.

We compared the performance of CTS OpTmizer One SFM and Gibco™ CTS™ OpTmizer Pro SFM (Cat. No. A4966101) for the production of CRISPR-Cas9–transfected human CAR T cells in G-Rex plates and a stirred-tank bioreactor (STBR). T cells from a healthy donor were isolated and activated with Gibco™ CTS™ Dynabeads™ CD3/CD28 magnetic beads (Cat. No. 40203D) on the Gibco™ CTS™ DynaCellect™ Magnetic Separation System (Cat. No. A55867). The T cells were collected after bead removal, and T cell receptor alpha (TCRα) knockout (KO) and CD19 knock-in (KI) were performed using the CRISPR-Cas9 system. The cells were then evaluated for proliferation, viability, and gene editing efficiency. The CD19⁺ CAR T cells were also evaluated for T cell memory phenotype, CD4⁺ and CD8⁺ expression, and cytotoxicity to assess the ability of CTS OpTmizer One SFM to support memory T cell plasticity and T cell–mediated immune responses.

Materials and methods

T cell isolation and activation

On day 0, T cells were isolated from peripheral blood mononuclear cells (PBMCs) from a healthy donor leukopak and activated with CTS Dynabeads CD3/CD28 beads at a 3:1 ratio of beads to target cells, using the CTS DynaCellect Magnetic Separation System. The T cells were collected into CTS OpTmizer One SFM or CTS OpTmizer Pro SFM, seeded in 40 mL G-Rex plates, and incubated for 2 days to facilitate activation. CTS OpTmizer One SFM was supplemented with 4 mM Gibco™ L-glutamine (Cat. No. 25030081) and 100 U/mL Gibco™ PeproTech™ Human IL-2 Recombinant Protein (Cat. No. 200-02-1MG). CTS OpTmizer Pro SFM was supplemented with 2.5% Gibco™ CTS™ Immune Cell Serum Replacement (Cat. No. A2596101), 4.5 mM Gibco™ CTS™ GlutaMAX™-I Supplement (Cat. No. 1286001), 2 mM L-glutamine, and 100 U/mL Gibco™ IL-2 Recombinant Human Protein (Cat. No. CTP0023).

T cell transfection and expansion

The beads were manually removed from culture on day 2. The isolated T cells were collected, and TCRα KO and CD19 KI were performed using CRISPR-Cas9 technology and the Gibco™ CTS™ Xenon™ Electroporation System (Cat. No. A50301). After CAR transfection, some of the T cells were inoculated into a 300 mL STBR to a density of 3.6×10^5 cells/mL. Other transfected T cells were inoculated into 40 mL G-Rex plates to a density of 4.0×10^5 cells/mL. T cells were expanded in CTS OpTmizer One SFM in the STBR, while T cells in the G-Rex plates were expanded in either CTS OpTmizer One SFM or CTS OpTmizer Pro SFM.

On day 5, fresh medium was added up to the full working volumes in the STBR and G-Rex plates. On days 7 and 9, half the volume was removed from the STBR and replaced with fresh medium. Medium exchange was also performed in the G-Rex plates. The cells were expanded for 12 days to reflect clinically relevant workflows in T cell therapy manufacturing. Fold expansion, cell viability, and total cell counts were measured on days 5, 7, 9, and 12 using a Vi-CELL™ cell viability analyzer (Beckman Coulter). Transfection efficiency, early memory phenotype, and expansion of $CD4^+$ and $CD8^+$ T cells were evaluated using the Invitrogen™ Attune™ NxT Flow Cytometer.

Cytotoxicity

On day 12, cells from all three $CD19^+$ transfected conditions and the control T cells transduced with empty vector and grown in the STBR were harvested and evaluated for cytotoxic activity. The cells were co-cultured overnight in the presence of $CD19^+$ targets at effector:target cell ratios ranging from 0.312:1 to 10:1, and cytotoxicity was evaluated using a luminescence-based assay.

Results

CTS OpTmizer One SFM supports efficient expansion of CD19⁺ CAR-transfected T cells in an STBR

Enriched CD3⁺ T cells were obtained after positive selection with CTS Dynabeads CD3/CD28 beads. The T cells were isolated at >97.7% purity, which increased to >99% after 12 days of expansion (data not shown). The impact of using an AOF medium on the manufacturability, expansion, and viability of CAR T cells was assessed on days 2, 5, 7, 9, and 12 following electroporation. Cell viability remained near or above 90% through day 12 in both the STBR and G-Rex plates (Figure 1). CAR T cells cultured in the STBR with CTS OpTmizer One SFM had the highest yield by day 12, with 140-fold expansion. CTS OpTmizer One SFM also supported the highest total cell count of 5.6 x 10^9 in the STBR (Table 1). The KI and KO efficiencies in the STBR and G-Rex plates were comparable on day 5. By day 12, it was clear that CTS OpTmizer One SFM in the STBR supported more efficient CRISPR-Cas9–mediated CD19⁺ CAR transfection. With CTS OpTmizer One SFM the percentage of CD19⁺ CAR T cells exceeded 80% in the STBR, compared to ~57% in the G-Rex plates (Figure 2).

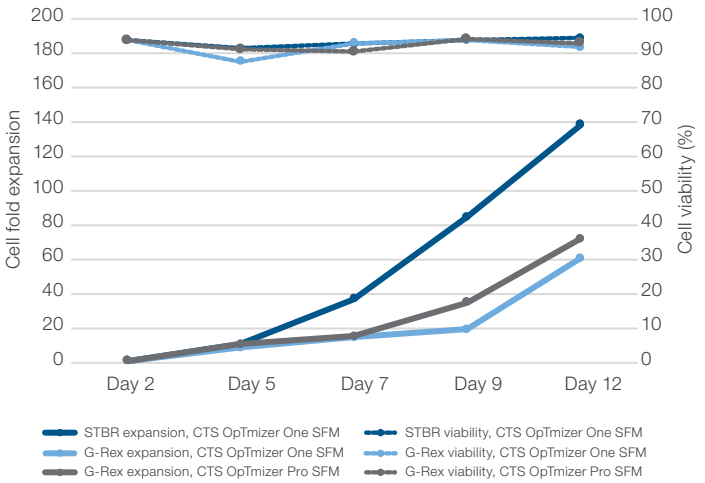

Figure 1. Fold expansion and viability of T cells cultured in a STBR and G-Rex plates. T cells were cultured for 12 days with CTS OpTmizer One SFM in a STBR and G-Rex plates, or with CTS OpTmizer Pro SFM in G-Rex plates only. T cells cultured with CTS OpTmizer One SFM in the STBR underwent a 140-fold expansion. The fold expansion of T cells in the G-Rex plates was similar in both media. Cell viability was consistently high across culture conditions, with percentages ranging from 92% to 95% on day 12.

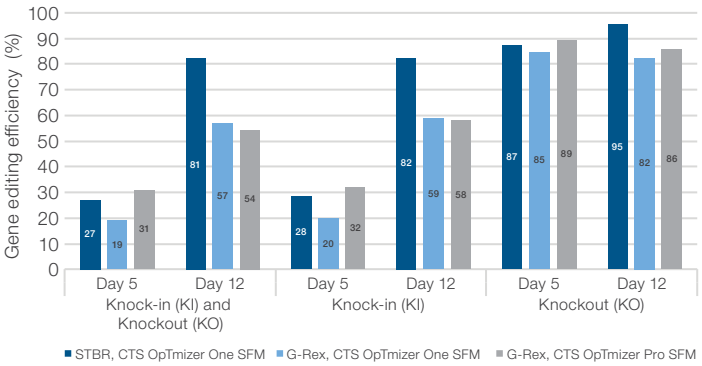

Figure 2. Efficiency of CRISPR-Cas9–mediated gene editing in human T cells. CD19 knock-in (KI) and TCRα knockout (KO) efficiency were evaluated on days 5 and 12. The highest editing efficiency was observed with CAR T cells cultured in CTS OpTmizer One SFM in the STBR.

Table 1. Total T cell counts in culture after 12 days of expansion. CTS OpTmizer One SFM supported the highest cell count in the STBR, with the average cell count reaching 5.55 x 10^9 cells/mL by day 12.

		T cell count (cells/mL)				
Bioreactor	**Medium**	**Day 2**	**Day 5**	**Day 7**	**Day 9**	**Day 12**
STBR	CTS OpTmizer One SFM	4.00 x 10^7	4.26 x 10^8	1.49 x 10^9	3.39 x 10^9	5.55 x 10^9
G-Rex plate	CTS OpTmizer One SFM	4.00 x 10^6	3.45 x 10^7	5.90 x 10^7	7.70 x 10^7	2.44 x 10^8
G-Rex plate	CTS OpTmizer Pro SFM	4.00 x 10^6	4.22 x 10^7	6.11 x 10^7	1.40 x 10^8	2.89 x 10^8

CTS OpTmizer One SFM maintains the efficacy and key quality attributes of CD19⁺ CAR-transfected T cells cultured in a STBR

Given the clinical importance of maximizing the persistence and efficacy of CAR T cells during *ex vivo* manufacturing, we assessed the impact of CTS OpTmizer One SFM on expression of T stem cell memory (TSCM; CCR7⁺, CD62L⁺, CD45⁺), T central memory (TCM; CCR7⁺, CD62L⁺, CD45⁻), and CD4⁺/CD8⁺ T cell populations on days 5 and 12 [3]. CTS OpTmizer One SFM maintained a higher percentage of T cells with stem cell memory and central memory phenotypes than did CTS OpTmizer Pro SFM (Figure 3). On day 12, higher percentages of CD8⁺ CAR T cells were observed in cultures grown with CTS OpTmizer One SFM in the STBR and G-Rex plates than in cultures grown in CTS OpTmizer Pro SFM (Figure 4).

To determine whether an *ex vivo* manufacturing process performed with CTS OpTmizer One SFM would support CAR T cell function and antitumor activity, cytotoxicity was evaluated against CD19⁺ targets. CTS OpTmizer One SFM supported expansion of CAR T cells in the STBR and G-Rex plates, and cells cultured with CTS OpTmizer One SFM had observably higher cytotoxicity than cells cultured with CTS OpTmizer Pro SFM (Figure 5). This enhancement may have been due to the higher percentage of CD8⁺ T cells expanded from cultures grown with CTS OpTmizer One SFM. These data demonstrate that CTS OpTmizer One SFM supports the growth, phenotype, and functionality of CAR T cell end products across tested manufacturing platforms.

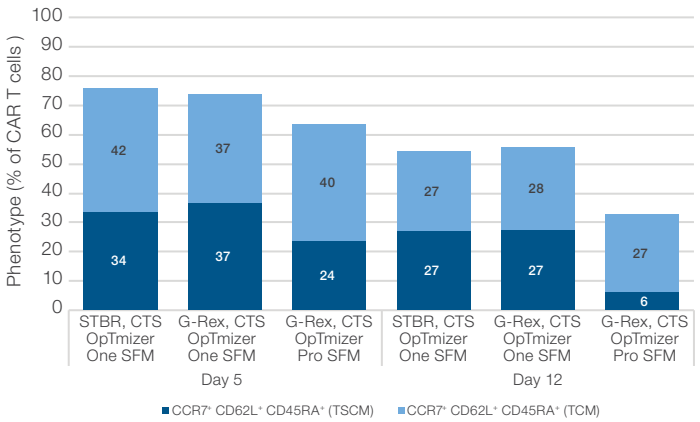

Figure 3. Effect of OpTmizer One SFM on the stem cell memory and central memory phenotypes of CD19⁺ CAR T cells. Characterization of CD19⁺ CAR T cells generated in CTS OpTmizer One SFM and CTS OpTmizer Pro SFM in a STBR and G-Rex plates showed that naive and early memory T cell subsets were maintained by cultures on days 5 and 12 of expansion in CTS OpTmizer One SFM.

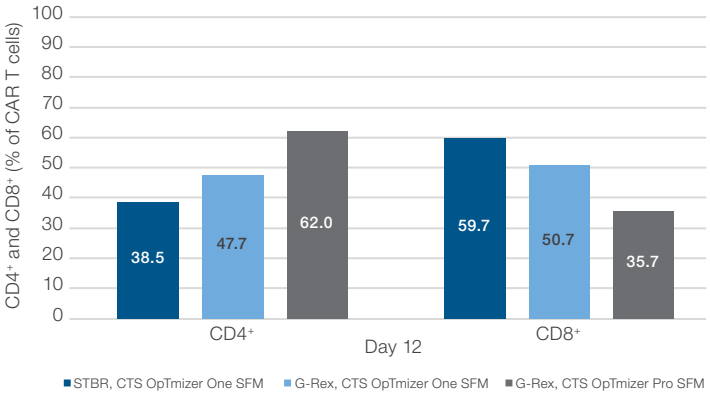

Figure 4. Effect of CTS OpTmizer One SFM on CD4⁺ and CD8⁺ CD19⁺ CAR T cell expansion. By day 12, CTS OpTmizer One SFM was supporting similar expansion of CD4⁺ and CD8⁺ CAR T cells in the STBR and G-Rex plates. The percentage of CD8⁺ CAR T cells reached 59.7% in culture with CTS OpTmizer One SFM, while it reached only 35.7% in CTS OpTmizer Pro SFM.

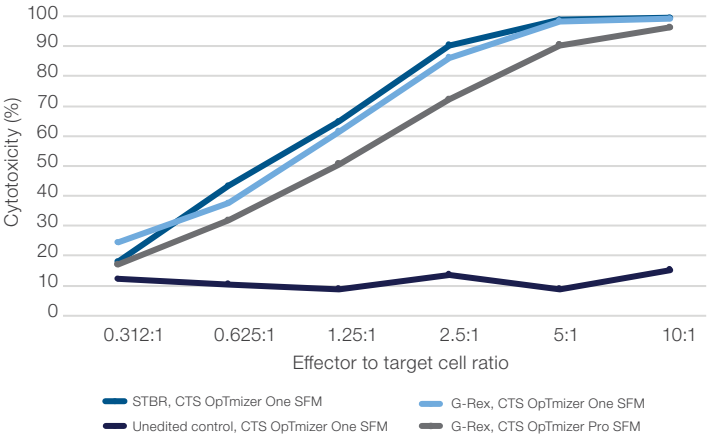

Figure 5. Cytotoxicity of CD19 transfected CAR T cells cultured in CTS OpTmizer One SFM. The cytotoxic activity of CD19 CAR T cells was assessed against CD19⁺ target cells. CAR T cells cultured in CTS OpTmizer One SFM demonstrated a greater capacity to kill CD19⁺ target cells than CAR T cells cultured in CTS OpTmizer Pro SFM.

Conclusion

CTS OpTmizer One SFM can support a simplified and reliable manufacturing process by enabling consistent or improved healthy donor T cell viability, expansion, and transfection efficiency in stirred-tank and plate bioreactor formats. CTS OpTmizer One SFM promotes the outgrowth of naive and central memory T cell phenotypes and $CD8^+$ lymphocyte expansion, thereby enhancing effector function as demonstrated by increased antitumor activity. CTS OpTmizer One SFM is a GMP-compliant medium that can help enable a seamless transition from process development to commercial-scale production. It is suitable for closed, automated systems and is available in large-volume formats. High-cost risks associated with contamination can be further mitigated by using CTS OpTmizer One SFM with closed, automated platforms like the CTS DynaCellect system. These results highlight CTS OpTmizer One SFM as an efficient GMP-grade AOF medium that can produce CAR T cell products with quality that matches or exceeds the quality of CAR T cells manufactured with a GMP-grade non-AOF formulation.

Further insights

The effectiveness of CTS OpTmizer One SFM in increasing cell viability and expansion in an electroporation workflow may be improved by adding a serum substitute such as 2.5% CTS Immune Cell Serum Replacement.

References

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