Cell therapy

CTS OpTmizer One Serum-Free Medium supports enhanced key quality attributes in CD19 CAR T cells more consistently than other commercially available T cell media

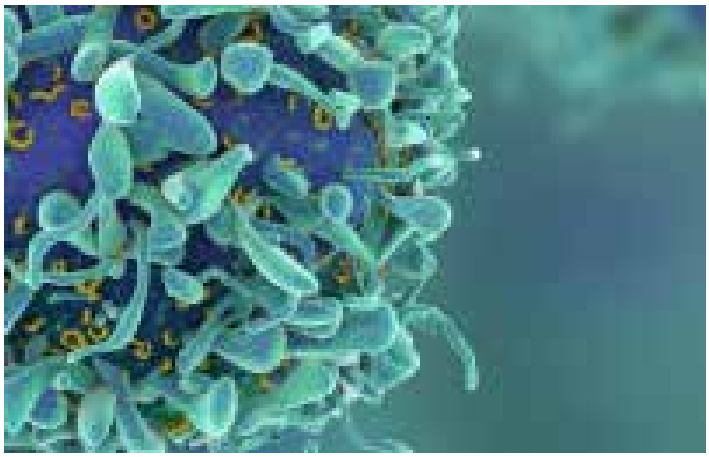
Abstract

CAR T cell therapies require tightly controlled and reproducible manufacturing conditions to ensure clinical safety and efficacy. The cell culture medium is a critical factor that can affect therapeutic outcomes, as it directly influences cell viability, expansion, transduction efficiency, and phenotype. This application note describes the performance of Gibco™ CTS™ OpTmizer™ One Serum-Free Medium (SFM) in a comparison with two other commercially available T cell culture media. The performance of CTS OpTmizer One SFM was better than or comparable to that of the other media with less donor-to-donor variability in several key quality attributes.

Introduction

Selection of an optimal cell culture medium is crucial for establishing a successful CAR T cell manufacturing process. Cell culture media formulations influence key T cell characteristics that can collectively determine the efficacy and persistence of the final cell therapy product [1]. Importantly, medium performance variability can contribute to batch inconsistencies and increase the risk of process failures and regulatory challenges. Serum-containing media have traditionally been used to support T cell growth for cell therapy, but their undefined compositions add contamination risk, regulatory risk, and suboptimal process reproducibility. A serum-free T cell medium is a strategic option that can help ensure consistency and compliance while maintaining optimal T cell performance.

Serum-free and animal origin–free (AOF) media formulations like CTS OpTmizer One SFM offer greater consistency and control to enable fine-tuning of cell therapy end products. This application note presents a comparative analysis of CTS OpTmizer One SFM against a commercially available chemically defined (CD) medium and a commercially available xeno-free medium supplemented with 5% human serum. CAR T cells generated with each medium were assessed for expansion, viability, transduction efficiency, and T cell phenotype. Our results highlight CTS OpTmizer One SFM as a high-performance, serum-free medium for reliable and scalable CAR T cell manufacturing.



Materials and methods

Media

T cell activation and expansion were evaluated using CTS OpTmizer One SFM (Cat. No. A5757201) supplemented with 4 mM Gibco™ L-Glutamine (Cat. No. 25030081) and 10 ng/mL Gibco™ Human IL-2 Recombinant Protein (Cat. No. PHC0021). In each assay, the performance of CTS OpTmizer One SFM was compared to the performance of the CD T cell medium (Alt Medium 1) and the xeno-free T cell medium supplemented with 5% human serum (Alt Medium 2 + Serum). Each medium was supplemented per the manufacturer's recommendation in addition to IL-2.

T cell isolation and activation

T cells were isolated from leukopaks from four healthy donors using the Gibco™ CTS™ DynaCellect™ Magnetic Separation System (Cat. No. A55867), and one-step isolation and activation was performed with Gibco™ CTS™ Detachable Dynabeads CD3/CD28 (Cat. No. A56996). After isolation, T cells from each donor were seeded at a density of 1.0 x 10^6 cells per well in G-Rex™ 24-well plates (Wilson Wolf) with 1 mL of CTS OpTmizer One SFM, Alt Medium 1, or Alt Medium 2 + Serum on day 0. All conditions were tested in duplicate or triplicate. On day 1, the T cells were transduced with CD19 CAR lentivirus at a multiplicity of infection (MOI) of 5 and maintained under standard culture conditions.

Expansion

On day 3, CTS OpTmizer One SFM, Alt Medium 1, or Alt Medium 2 + Serum and IL-2 were added to a total volume of 8 mL, and the T cells were maintained at 37°C under 5% CO_2. The cells were expanded until day 10 with 60% medium exchanges on days 6 and 8.

Performance metrics

Fold expansion, total cell count, viability, T cell phenotype, and CD19-CAR lentivirus transduction were evaluated. Cell count and viability were measured using a Vi-CELL™ cell counter (Beckman Coulter). Phenotypic analysis (CD4, CD8, $CD27^+/CD62L^+$, $CCR7^+/CD45RA^+$, $CD45RO^+/CD62L^+$) was performed on day 10 using the Invitrogen™ Attune™ NxT Flow Cytometer.

Results

To evaluate the impact of each medium on CAR T cell characteristics, T cells from four different donor leukopaks were isolated, activated, and transduced with anti-CD19 CAR lentivirus in CTS OpTmizer One SFM, a CD medium (Alt Medium 1), or a xeno-free medium supplemented with 5% human serum (Alt Medium 2 + Serum). All three media maintained >80% CAR T cell viability throughout the expansion period. However, CTS OpTmizer One SFM supported enhanced CAR T cell expansion relative to the other media from day 6 through day 10 (Figure 1).

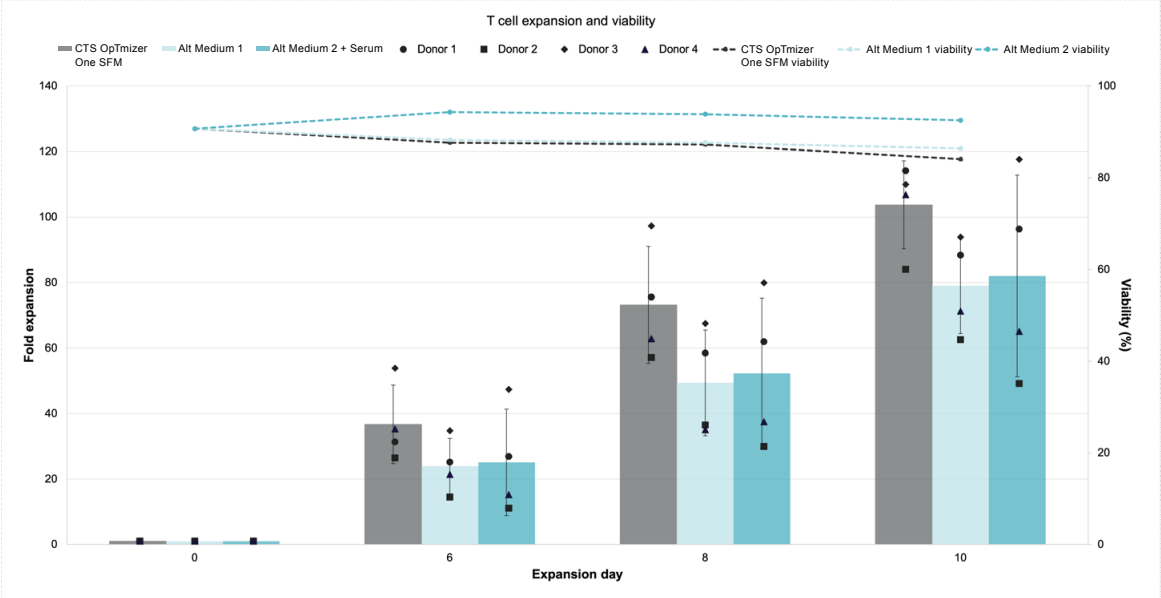


Figure 1. 10-day expansion and viability of T cells in CTS OpTmizer One SFM or an alternative medium. CTS OpTmizer One SFM maintained average cell viabilities of >80% throughout the expansion period, and it consistently supported higher average fold expansion than either of the other media. Each data point represents the mean of technical duplicates or triplicates from the same donor ±SD.

By the end of the expansion period, the average final CAR T cell count was 6.44 x 10^7 in CTS OpTmizer One SFM. This was significantly higher than the average final CAR T cell counts in Alt Medium 1 (3.91 x 10^7) and Alt Medium 2 + Serum (4.61 x 10^7). Less donor-to-donor variability was observed with CTS OpTmizer One SFM on day 10 than either of the other media, particularly Alt Medium 2 + Serum, and CTS OpTmizer One SFM supported the highest average expansion. These results underscore the advantages of CTS OpTmizer One SFM in supporting robust, reliable CAR T cell expansion and sustained viability (Figure 2).

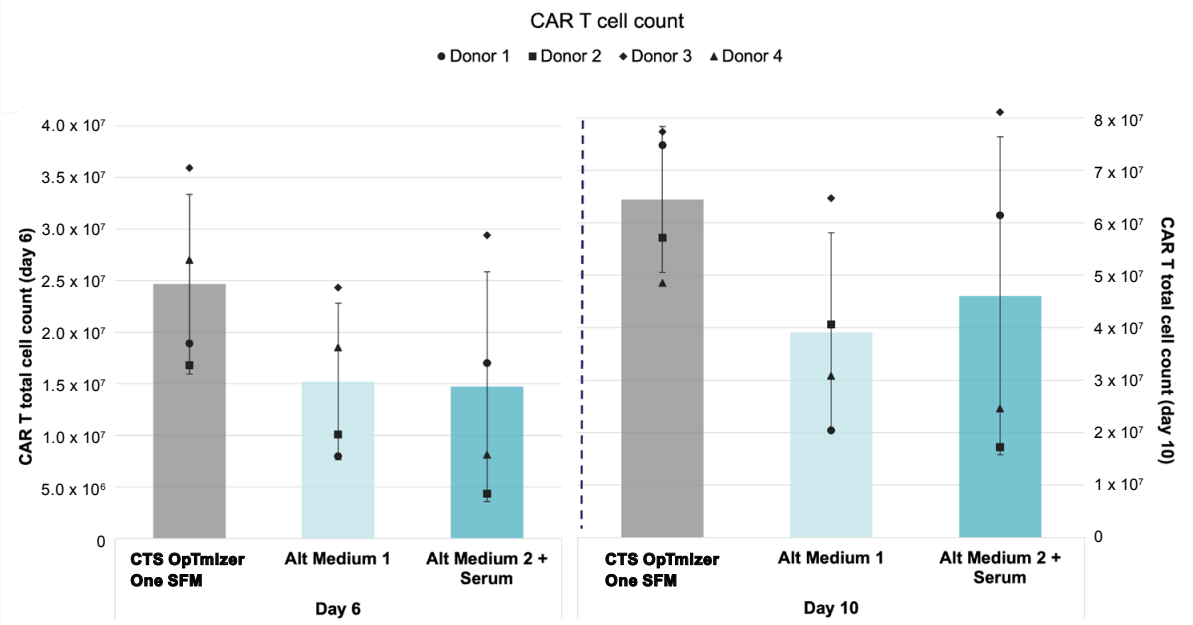


Figure 2. Total CAR T cell growth. CTS OpTmizer One SFM supported higher CAR T cell yields than either Alt Medium 1 or Alt Medium 2 + Serum by day 6 and maintained enhanced growth through day 10. Each data point represents the mean of technical duplicates or triplicates from the same donor ±SD.

Maintaining optimal CAR T cell characteristics while promoting growth can be a major hurdle due to the innate sensitivity of primary T cells to *ex vivo* processes like manipulation and transduction. To assess how well CTS OpTmizer One SFM supported high-quality CAR T cell production compared to the other two media, transduction and phenotype were assessed. Transduction efficiency was high across all three media. CTS OpTmizer One SFM supported a higher percentage of CAR^+ T cells on average (70.14%) than Alt Medium 1 (65.82%) or Alt Medium 2 + Serum (58.41%). Use of CTS OpTmizer One SFM also resulted in less donor-to-donor variability in lentiviral transduction (Figure 3). The expansion performance of CTS OpTmizer One SFM is complemented by the ability to support consistent transduction efficiency, which is advantageous for the lot-to-lot uniformity required in GMP manufacturing.

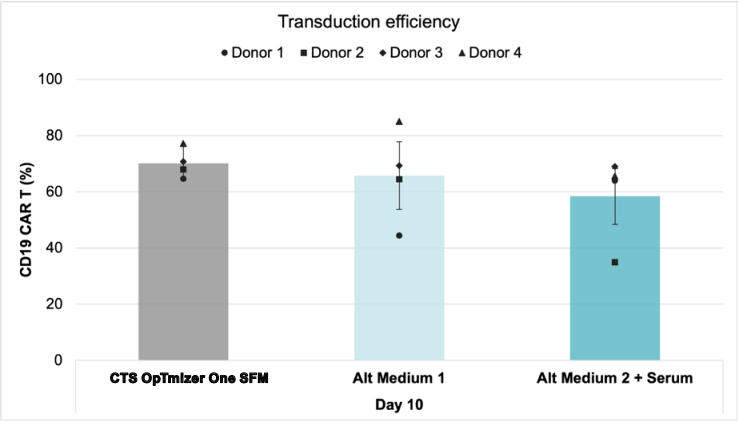


Figure 3. Lentiviral transduction efficiency in CAR T cells cultured in CTS OpTmizer One SFM or an alternative medium. Transduction efficiency was comparable in CAR T cells cultured in CTS OpTmizer One SFM or Alt Medium 1 and higher than it was in CAR T cells cultured in Alt Medium 2 + Serum. Less donor-to-donor variability was observed in cells cultured in CTS OpTmizer One SFM, indicating more consistent transduction. Each data point represents the mean of technical duplicates or triplicates from the same donor ±SD.

Phenotypic analysis on day 10 showed that the frequencies of early memory T cell subsets ($CD27^+/CD62L^+$ and $CCR7^+/CD45RA^+$) in cells cultured in CTS OpTmizer One SFM were comparable to or higher than the frequencies observed in cells cultured in the other media, which contributed to a less variable T cell profile (Figure 4). A key determinant of CAR T cell therapeutic efficacy is retention of a favorable phenotype that enhances *in vivo* persistence and proliferative capacity. Specifically, higher levels of expression of early/stem cell memory markers like CD45RA, CCR7, CD27, and CD62L have been linked to greater persistence and functional longevity in CAR T cells [2,3]. The CD4/CD8 ratio remained consistent across all media, indicating there was no shift in T cell population balance (Figure 5).

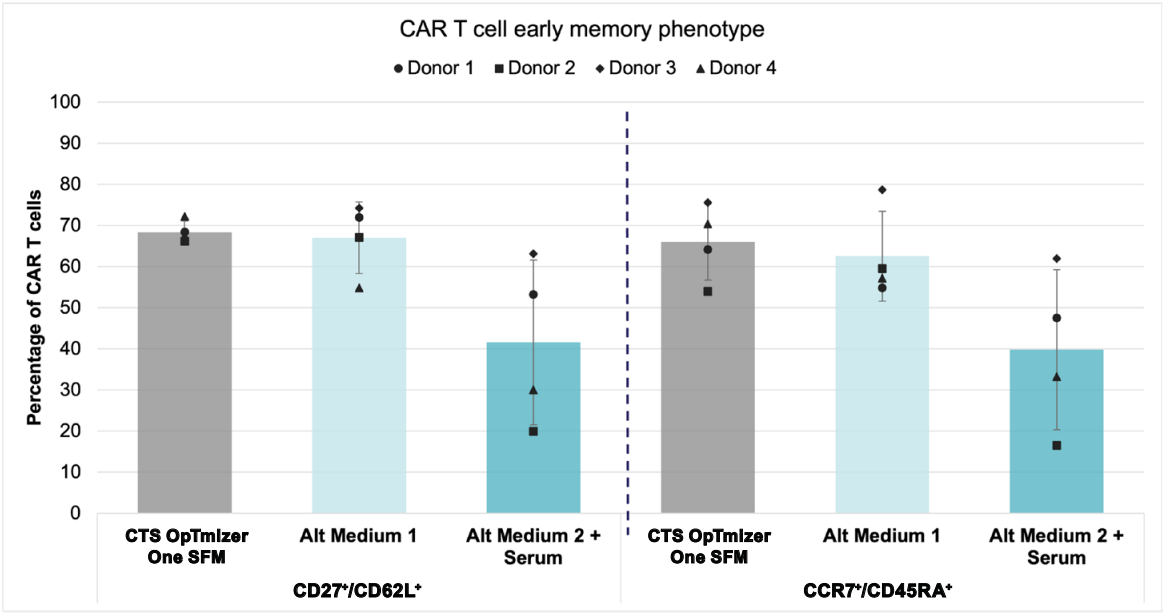


Figure 4. CTS OpTmizer One SFM maintains an early memory phenotype in expanded CAR T cells. CTS OpTmizer One SFM supported comparable or higher frequencies of $CD27^+/CD62L^+$ and $CCR7^+/CD45RA^+$ CAR T cells relative to Alt Medium 1 and Alt Medium 2 + Serum. Each data point represents the mean of technical duplicates or triplicates from the same donor ±SD.

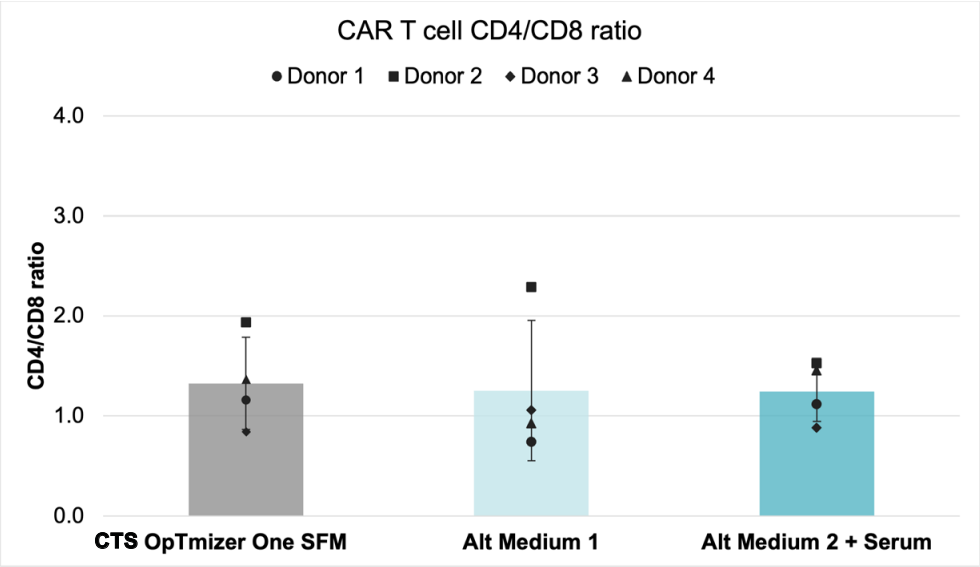


Figure 5. CTS OpTmizer One SFM maintains balanced CD4/CD8 ratios in CAR T cell cultures. The CD4/CD8 ratios in expanded CAR T cells were comparable across media, indicating there was no skewing of the T cell subset composition. Each data point represents the mean of technical duplicates or triplicates from the same donor ±SD.

Conclusion

The data obtained in this study support the use of CTS OpTmizer One SFM as a high-performance, serum-free medium for CAR T cell manufacturing. Key benefits include:

- CTS OpTmizer One SFM consistently supports enhanced expansion and high counts of CAR T cells across donors while maintaining >80% viability.
- CTS OpTmizer One SFM supports high lentiviral transduction efficiency in T cells with minimal donor-to-donor variability.
- In addition to maintaining robust CAR T cell expansion, CTS OpTmizer One SFM preserves comparable or higher frequencies of cells with the early memory phenotype and maintains a balanced CD4/CD8 ratio.

CTS OpTmizer One SFM is a reliable, animal origin–free medium for CAR T cell manufacturing that can help manufacturers streamline processes for regulatory review. The findings of this study make a strong case for using CTS OpTmizer One SFM in T cell manufacturing workflows that require consistent performance for CAR T cell therapy.

References

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