Cell therapy

CTS OpTmizer One SFM offers enhanced T cell expansion and high viability while maintaining early memory cell phenotype

Introduction

T cell therapeutics have advanced considerably and shown successful decade-long remissions for some patients with hematological malignancies, such as chronic lymphocytic leukemia (CLL) [1]. However, critical challenges remain that prevent the delivery of long-term treatment success consistently and reliably for many patients. The discovery of factors impacting the success of T cell treatment has revealed some of the effects the media and the cell culture manufacturing process can have on therapeutic success [2,3].

The discovery of these factors indicates that an animal origin–free (AOF) T cell culture medium is preferred to support robust T cell expansion and maintain high T cell viability and early memory phenotype for long-term therapeutic efficacy. A serum-free and AOF medium formulation manufactured to meet high cell therapy quality standards can reduce variability and contaminants, helping to ease the regulatory approval process. Lastly, to facilitate easy use with closed and automated production systems, the medium should be a single-part liquid format and available in bioprocess-ready packaging of various sizes.

Gibco™ CTS™ OpTmizer™ One Serum-Free Medium (SFM) was developed to support the cell therapy industry's need for a novel AOF T cell medium formulation. The basal medium is a single-part formulation manufactured to well-established strict GMP-compliant standards. Additionally, to facilitate use with closed and automated workflows, the medium is available in 1 L, 5 L, and 10 L volumes in bioprocess containers made of Thermo Scientific™ Aegis™ 5-14 film.

Here we outline and discuss the results of experiments conducted to evaluate the performance capabilities of CTS OpTmizer One SFM compared to AOF or xeno-free basal media offerings from other suppliers. Experiments were performed to evaluate the media with cells from healthy donors, as well as cells from diseased donors from two clinical indications, acute myeloid leukemia (AML) and CLL. The performance criteria included activated T cell expansion, cell viability, early memory T cell phenotypes, and CD4:CD8 ratios. Additionally, experiments were executed with healthy donor cells to assess the medium's capability to support transduction of activated T cells with lentivirus (LV) compared to another supplier's medium. Transduction efficiency, transduced early memory phenotype, cell expansion, and viability were evaluated as performance criteria.

Materials and methods

Healthy and diseased donor study

Media: T cell activation and expansion using CTS OpTmizer One SFM (Cat. No. A5757201) were compared with three products from other suppliers: two AOF media formulations (designated CM1 and CM2) and one xeno-free medium (designated CM3). CTS OpTmizer One SFM was supplemented with 4 mM L-glutamine, and the media from other suppliers were supplemented according to each manufacturer's recommendation.

Cells: In the healthy donor experiments, peripheral blood mononuclear cells (PBMCs) from 5 to 11 different donors were seeded in each test medium at 0.5 x 10^6 or 1.0 x 10^6 cells in G-Rex™ 24-well plates (Wilson Wolf). In the diseased donor experiments, PBMCs from 2 donors with AML and 2 donors with CLL were cultured in G-Rex 24-well plates. Seeding densities were adjusted based on the availability of T cells for each donor. Healthy and diseased donor cultures were maintained at 37˚C and 5% CO_2.

Activation: On day 0, cells in healthy and diseased donor experiments were activated using Gibco™ Dynabeads™ Human T-Expander CD3/CD28 (Cat. No. 11141D) at a 3:1 bead:T cell ratio with 100 IU IL-2. The cultures were grown until day 10 with IL-2 replenished on days 3, 5, and 7, and a 50–60% media exchange was performed on days 5 and 7.

Performance criteria: Cell densities and viabilities were measured using a Vi-CELL™ cell counter (Beckman Coulter). T cell phenotype was determined using an Invitrogen™ Attune™ NxT Flow Cytometer. Healthy donor experiments were performed in technical triplicates, diseased CLL donors in duplicates, and AML donors in singlets.

LV transduction study

Media: LV transduction and expansion of activated T cells were evaluated with CTS OpTmizer One SFM and compared with another supplier's AOF medium formulation (CM1). This study was conducted by a third-party independent lab.

Cells: T cells isolated from two healthy donors were seeded at 2.5 x 10^6 in 5 mL of medium per well in G-Rex 6-well plates.

Activation and transduction: On day 0, cells were activated with Dynabeads Human T-Expander CD3/CD28 at a 1:1 ratio with 100 IU of IL-2. Twenty-four hours post-activation, T cells were transduced with LV and expanded until day 14 with medium added on day 4 and 75% media exchanged and IL-2 replenished on days 7, 10, and 12.

Performance criteria: LV transduction efficiency, transduced early memory phenotype cell expansion, and viability were evaluated on days 7, 10, and 14.

Results

Healthy donor study

On days 5, 7, and 10, evaluation of cells from 5 to 11 healthy donors showed CTS OpTmizer One SFM supported comparable or higher total cell counts relative to those supported with two AOF media (CM1 and CM2) and one xeno-free medium (CM3) from other suppliers (Figure 1). CTS OpTmizer One SFM supported statistically significant higher total cell counts than CM1 and CM3 on days 7 and 10 (Student's *t*-test, $p < 0.05$). Additionally, with CTS OpTmizer One SFM, day 7 and day 10 cell counts showed greater consistency across the different donors. Throughout the study, comparably high average cell viabilities of 76% to 88% were shown between the media tested (data not shown). The results indicate that through day 10, $CD3^+$ cells with CTS OpTmizer One SFM maintained comparable or higher average expression of $CCR7^+$ $CD62L^+$, $CD27^+$ $CD62L^+$, and $CD45RO^+$ $CD62L^+$ early memory cell phenotype markers (Figure 2). Lastly, the average CD4:CD8 T cell ratios were comparably maintained through day 10 (data not shown).

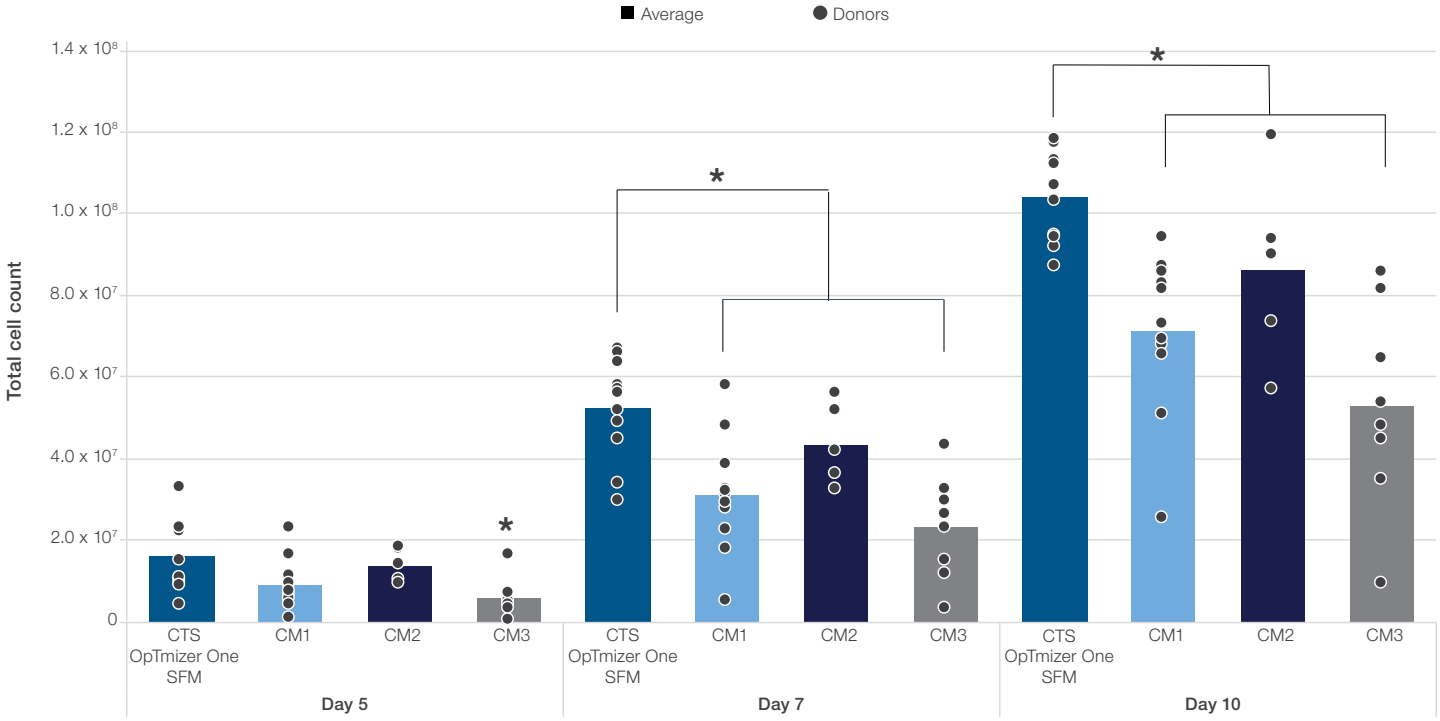

Figure 1. Healthy donor cell expansion. On days 5, 7, and 10, CTS OpTmizer One SFM supported comparable or statistically higher average total cell counts (as indicated) to those produced with CM1, CM2, and CM3 media from other suppliers. On days 7 and 10, generally more consistent results were shown across the donors with CTS OpTmizer One SFM. (CTS OpTmizer One SFM: n = 11 donors, CM1: n = 11 donors, CM2: n = 5 donors, and CM3: n = 8 donors; * $p < 0.05$.)

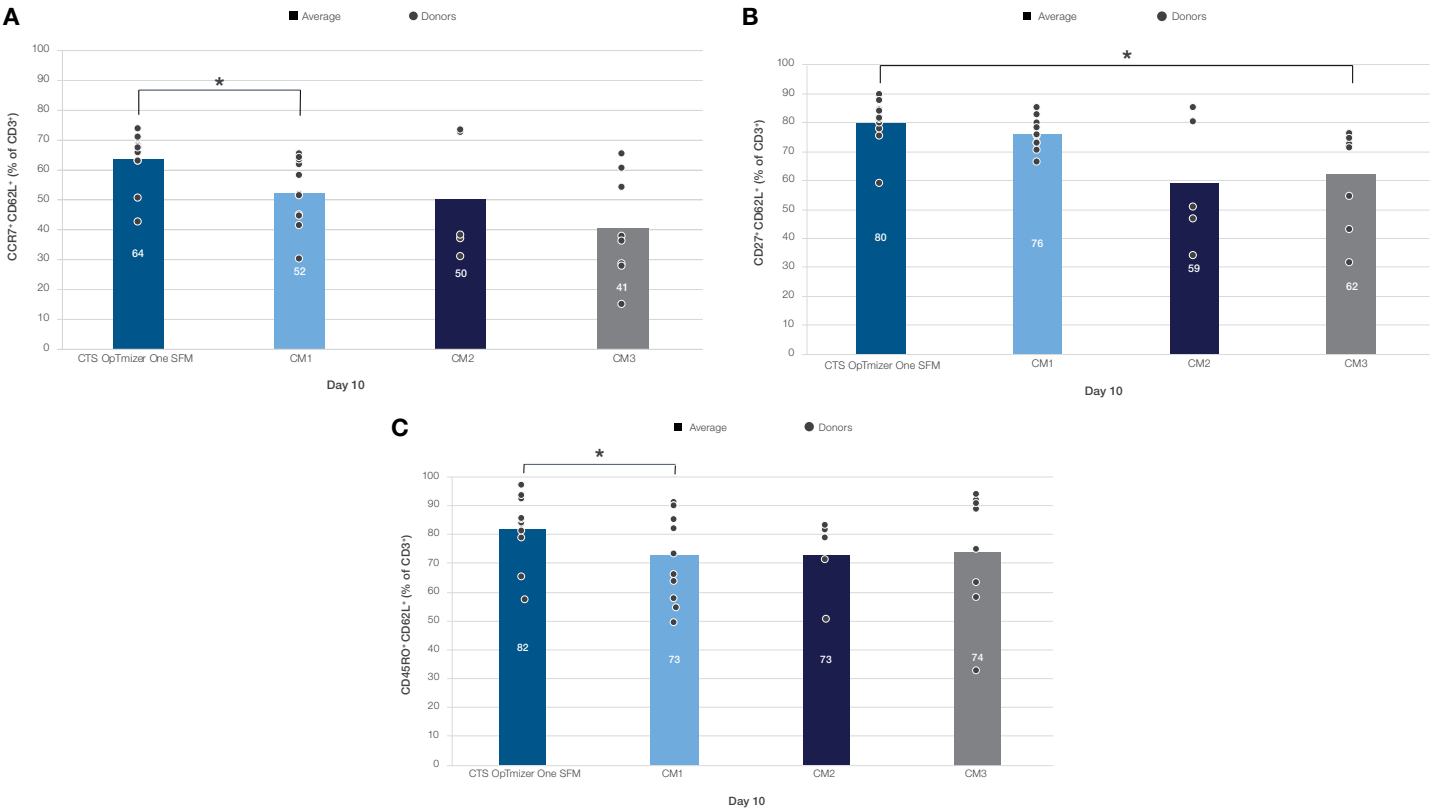

Figure 2. Healthy donor T cell phenotype. By day 10, with CTS OpTmizer One SFM, $CD3^+$ cells demonstrated a comparable, or statistically higher (as indicated) average expression of early memory cell phenotype markers, **(A)** $CCR7^+$ $CD62L^+$, **(B)** $CD27^+$ $CD62L^+$, and **(C)** $CD45RO^+$ $CD62L^+$, relative to cells with the CM1, CM2, and CM3 media from other suppliers. (CTS OpTmizer One SFM: n = 11 donors, CM1: n = 11 donors, CM2: n = 5 donors, and CM3: n = 8 donors; * $p < 0.05$.)

Diseased donor study

Cells from two diseased donors with AML showed CTS OpTmizer One SFM supported a more consistent and stronger expansion of 284-fold by day 10 relative to the CM1 and CM3 media (Figure 3). The media also supported comparably high 85% viability and more consistent maintenance of early memory cell phenotype than the other suppliers' media. Similarly, by day 10, cells from two CLL diseased donors demonstrated a higher average expansion of 80-fold with CTS OpTmizer One SFM compared to slightly more than 30-fold for the CM1 medium (Figure 3). Comparably high 84% to 88% viability was shown between the test media with more consistent and comparable or higher early memory cell phenotype maintained with CTS OpTmizer One SFM (data not shown).

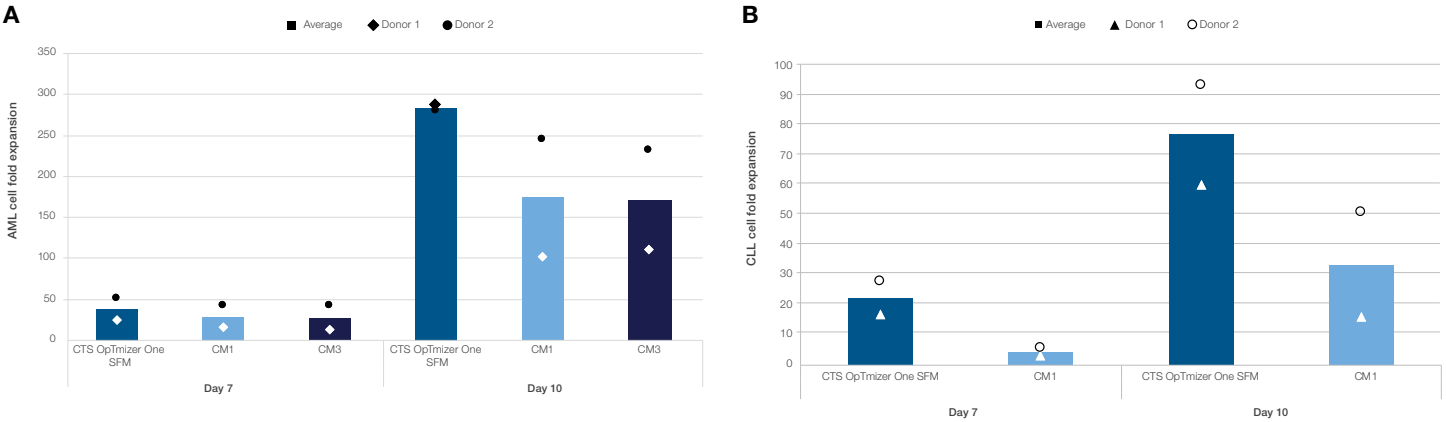

Figure 3. Diseased donor cell expansion. (A) By day 10, AML donor cells achieved more consistent and robust 284-fold expansion with CTS OpTmizer One SFM compared to those with the CM1 and CM3 media from other suppliers. **(B)** CLL donor cells by day 10 showed a higher near 80-fold average expansion with CTS OpTmizer One SFM compared to those with the CM1 medium. (n = 2 for each AML and CLL clinical indication.)

LV transduction study

From day 7 to day 14, assessments of LV-transduced cells from two healthy donors showed that the cells with CTS OpTmizer One SFM achieved and maintained consistent and comparable or higher transduction efficiencies to those with the CM1 medium (Figure 4). Cell transduction efficiency was based on positive expression of green fluorescent protein (GFP^+). Both media supported comparably high GFP^+ cell count expansion and maintenance of early memory phenotype $CCR7^+$ and $CD27^+$ marker expression to day 14 (Figure 5). Cell viabilities remained comparably strong near 95% until day 14 (data not shown).

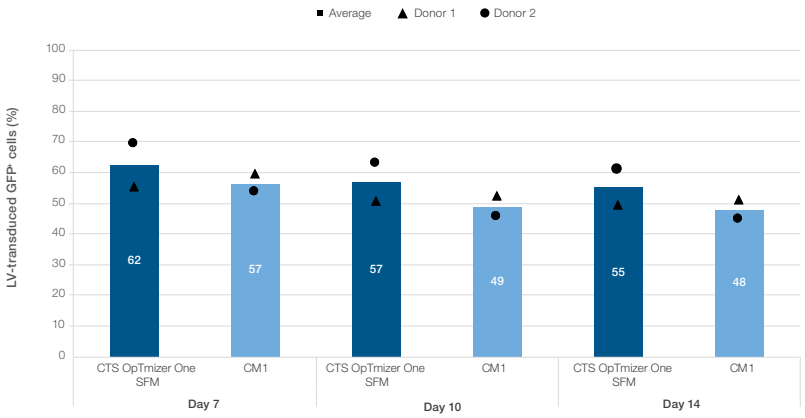

Figure 4. LV transduction efficiency. From day 7 to day 14, GFP^+ cells maintained consistent and comparable or higher LV transduction efficiencies with CTS OpTmizer One SFM relative to CM1 medium. (n = 2 for each medium.)

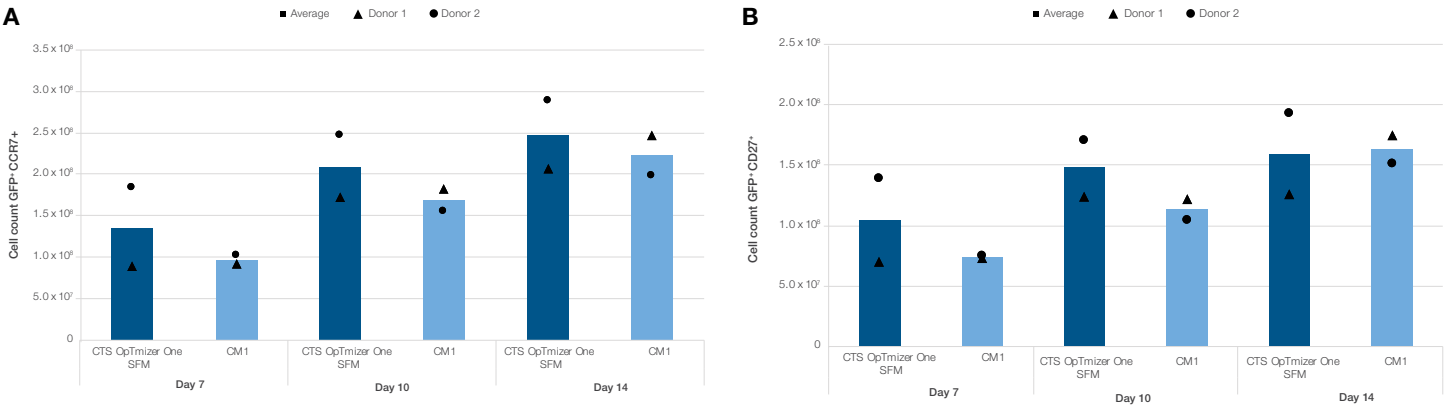

Figure 5. Cell expression and maintenance of early memory phenotype from LV-transduced donor cells. On days 7 to 14, CTS OpTmizer One SFM and CM1 medium from another supplier supported comparable robust GFP^+ T cell counts and maintained expression of early memory phenotype markers, **(A)** $CCR7^+$ and **(B)** $CD27^+$. (n = 2 for each medium.)

Conclusions

Compared to media from other suppliers, CTS OpTmizer One SFM supported more consistent enhanced T cell expansion, while maintaining high cell viability, early memory cell phenotype, and CD4:CD8 ratios with healthy and diseased AML and CLL donor cells. Moreover, the LV transduction experiment results with healthy donor T cells demonstrate that CTS OpTmizer One SFM supports comparable or higher transduction efficiencies relative to an AOF medium from another supplier. In addition, the CTS OpTmizer One SFM is a single-part and animal component–free formulation that helps to increase consistency.

Overall, these results demonstrate that CTS OpTmizer One SFM shows strong capability and the potential to be the AOF medium that helps manufacturers meet the challenge to deliver efficacious long-term treatment success reliably and consistently to more patients.

References

1. Melenhorst JJ, Chen GM, Wang M et al. (2022) Decade-long leukaemia remissions with persistence of $CD4^+$ CAR T cells. *Nature* 602(7897):503–509. **doi.org/10.1038/s41586-021-04390-6**
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