Improving allogeneic workflow and cell proliferation using CTS OpTmizer Pro SFM

Introduction

T cell therapy presents immense promise as a treatment for a multitude of diseases. However, a 2019 review on the clinical responses following T cell therapy presents some diseases as being much more challenging than others to successfully treat [1]. Figure 1 shows complete response (CR) rate as a measure of successful treatment. As shown, for some diseases, such as B-cell acute lymphoblastic leukemia (B-ALL) with CR rates over 80%, there has been considerable success, whereas other diseases, with rates at or well below 58%, need significant improvement. Further research in this area suggests that these disparities are often cell based [1].

Disease	Reference	CR/all cases	%CR for all cases
B-ALL (adult)	NCT01044069	44/53	83
B-ALL (pediatric and young adult)	NCT02435849	61/75	81
B-CLL	NCT01865617	4/19	21
DLBCL	NCT02348216	59/101	58
DLBCL	NCT02445248	37/93	40
DLBCL	NCT02631044	33/73	45
MM (anti-BCMA)	NCT02215967	(1/12)(2 VGPR)*	8
MM (anti-BCMA)	NCT02658929	15/33	45

Figure 1. T cell therapy clinical trial responses. Clinical trial response data following T cell therapy for a variety of B cell and plasma cell malignancies. Adapted from Cheng et. al. [1]. * 2 VGPR: two very good partial responses.

Immature T cells have the potential to differentiate into effector cells that can attach to and kill cancer cells. Hence, appropriate levels of T cells often correlate to a healthy immune response. Typically, diseased patients present lower $CD8^+$ cytotoxic T cell numbers and high variability in the size of the central memory T (T_{CM}) cell populations [1] (Figure 2). Differences in T cell characteristics often translate to lower-efficacy treatments. Recent focus has been on the variability and importance of having robust young (T_{CM}) populations for efficacious therapies.

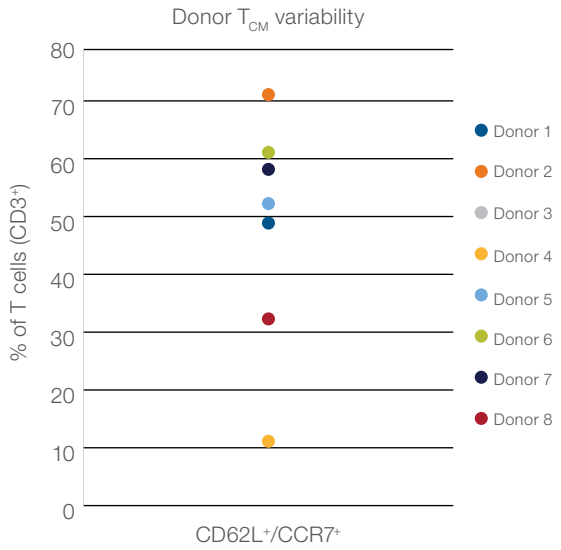

Figure 2. Central memory cell variability across donors. Sampling of T cell populations from 8 donors shows variability in the percentage of cells expressing CD62L and CCR7 phenotypes, which are characteristic of T_{CM} cells. Variability is between 13% and 72%.

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As shown in Figure 3, in the linear T cell differentiation pathway, naive (T_N) cells can differentiate into T_{CM} cells, which are characterized by their high proliferative capacity and enhanced cytokine production. As illustrated on the right side of Figure 3, following prolonged antigen stimulation, the T cell population proliferates and further differentiates and matures into T effector memory (T_{EM}) and T effector (T_{EFF}) cells. Over the years, research results have demonstrated that infusion of the less differentiated cells correlates with higher-efficacy T cell therapies, due to the superior engraftment, persistence, and antitumor immune response conveyed by these younger T cell types [2]. Data indicate that production of a higher T_{CM} count from healthy donors supports and has the potential to improve efficacy.

The transformative potential of activating and expanding healthy donors' T cells will have a significant impact on future cell therapy–based treatments for a spectrum of diseases. With this in mind, a next-generation T cell expansion medium, Gibco™ Cell Therapy Systems™ (CTS™) OpTmizer™ Pro Serum Free Medium (SFM), was designed to support robust T cell growth and provide a stronger immune response for allogeneic workflows. Based on our recent study, CTS OpTmizer Pro SFM shows potential to promote strong T cell proliferation, maintain T_{CM} phenotype, and allow for higher-level cell production of interferon gamma (IFNγ) with healthy donor cells utilized in the longer allogeneic workflow applications.

This application note outlines the capabilities of CTS OpTmizer Pro SFM compared with a control medium in both diseased patients' and healthy donors' cells. CTS OpTmizer Pro SFM was tested with cells from six healthy donors in an 18-day expansion workflow, as well as from nine diseased donors in a shorter 10-day workflow to mimic a typical autologous workflow. Diseased and healthy donors' T cells in both workflows were evaluated for proliferation in terms of expansion and the memory cell phenotype markers CD62L, CCR7, and CD27. Following induced T cell activation, healthy donors' cells were additionally evaluated for production of IFNγ.

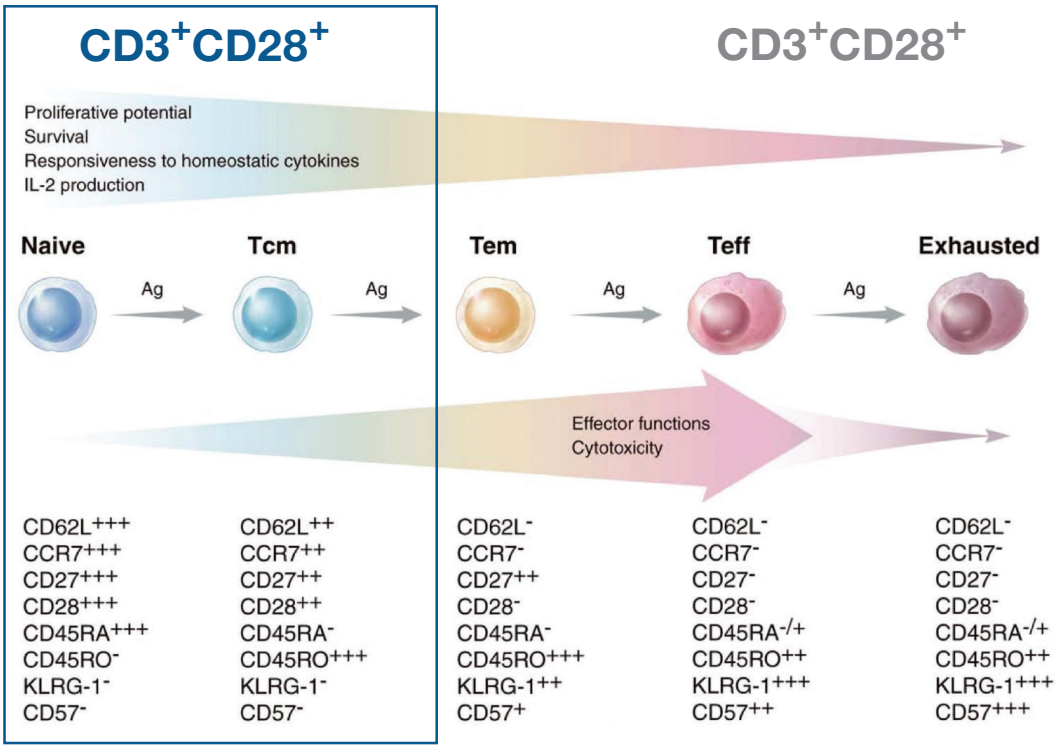

Figure 3. T cell differentiation pathway—younger is better. This diagram illustrates the spectrum of T cell differentiation, with the less differentiated and most therapeutically desirable T_{CM} cells on the left and mature T_{EM} and T_{EFF} cells on the right. Phenotypic changes that follow antigen stimulation are shown to demonstrate T cell differentiation and subset population changes [3].

Materials and methods

T cell isolation—Human primary T cells were negatively isolated from peripheral blood mononuclear cells (PBMCs) with the Invitrogen™ Dynabeads™ Untouched™ Human T Cells Kit. Alternatively, for process development and clinical use, please see the application note titled "One-step isolation and activation of naive and early memory T cells with CTS™ Dynabeads™ CD3/CD28" (Pub. No. COL23050).

- **Medium**—CTS OpTmizer Pro SFM contains two parts: 1 L of CTS OpTmizer Pro SFM Basal Medium and 26 mL of CTS OpTmizer Expansion Supplement. The complete culture medium was created by supplementing with Gibco™ L-Glutamine to a final concentration of 2 mM, per the user manual. No benefit has been found by supplementing CTS OpTmizer Pro SFM with Gibco™ CTS™ Immune Cell Serum Replacement (ICSR, Thermo Fisher Scientific), so we do not recommend that it be added to this culture medium.
- **Activation and stimulation**—T cells were seeded in culture dishes at 1 x 10^6 cells/mL in the indicated medium and activated with Gibco™ Dynabeads™ Human T-Expander CD3/CD28 at a ratio of 3 beads per T cell in the presence of 100 IU/mL of rIL-2.
- **Routine maintenance**—T cells were counted every 2–3 days using a Vi-CELL™ Cell Viability Analyzer (Beckman Coulter). Viable cell density was maintained at 0.25 x 10^6 cells/mL, and rIL-2 was added to the culture to a concentration of 100 IU/mL.
- **Flow cytometry**—On the days indicated, cellular phenotype was assessed using the Invitrogen™ Attune™ NxT Flow Cytometer, by staining T cells with Invitrogen™ CD3 Pacific Orange™, CD4 FITC, CD8 Pacific Blue™, CD62L APC, and CCR7 PE antibodies.
- **Assessment of IFNγ production**—A subset of the T cells expanded in CTS OpTmizer Pro SFM were reseeded at 0.5 x 10^6 cells/mL in the indicated medium and restimulated with Dynabeads Human T-Expander CD3/CD28 at a ratio of 1 bead per T cell in the presence of 100 IU/mL of rIL-2. At day 3 following the restimulation, the spent medium was analyzed for IFNγ production on the Luminex® MAGPIX® system (Thermo Fisher Scientific) using the Invitrogen™ Cytokine Human Magnetic 35-Plex Panel for the Luminex platform (Thermo Fisher Scientific) according to the user manual.

Results

CTS OpTmizer Pro SFM supports excellent proliferation with cells from healthy donors

When T cells from six healthy donors were grown with CTS OpTmizer Pro SFM in an 18-day expansion workflow normalized to the control medium, the average growth of the cells was approximately 20% higher for all donors by day 10, with an increase of over 100% by day 17 (Figure 4), with no negative effect on viability (data not shown).

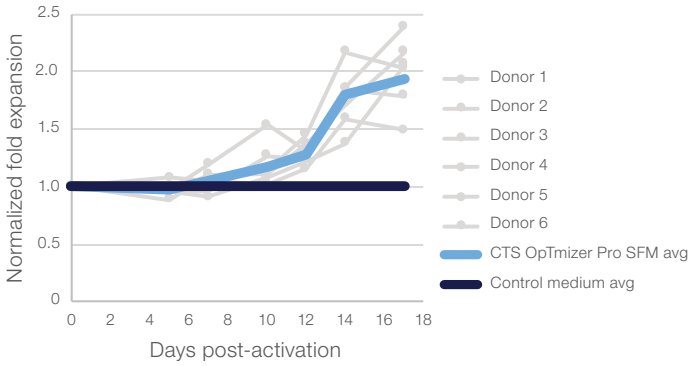

Figure 4. Normalized proliferation in CTS OpTmizer Pro SFM in an 18-day workflow with cells from six healthy donors. Healthy donors' cells tested in an 18-day allogeneic type workflow in CTS OpTmizer Pro SFM demonstrated ~20% higher cell proliferation by day 10, with a ~100% increase by day 17, when compared to a control medium. The average normalized change in cell growth in CTS OpTmizer Pro SFM is represented by the light blue line, with the baseline standard shown in dark blue and individual donors represented by the light gray lines.

CTS OpTmizer Pro SFM recommended for allogeneic workflows

T cells from nine diseased donors were grown in a 10-day expansion workflow representative of a typical autologous workflow, using a control medium and CTS OpTmizer Pro SFM. The average cell proliferation for all donors in CTS OpTmizer Pro SFM was normalized to that in the control medium. While a modest growth benefit was shown using CTS OpTmizer Pro SFM compared to the control, from day 5 through day 10 (Figure 5), the data show individual donors were highly inconsistent and unpredictable. These results suggest that CTS OpTmizer Pro SFM may not be the optimal medium to improve cell expansion for diseased cells in autologous workflows. Additional studies are needed to fully understand the factors that influence whether a donor's cells will benefit.

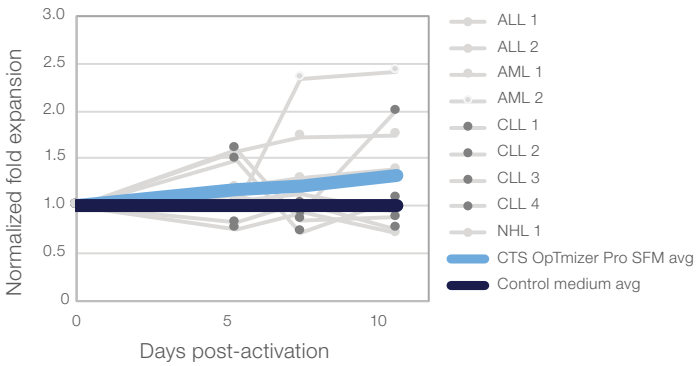

Figure 5. Normalized proliferation in CTS OpTmizer Pro SFM in a 10-day workflow with cells from nine diseased donors. Diseased donors' cells tested in a 10-day autologous type workflow demonstrated, on average, slightly higher growth in CTS OpTmizer Pro SFM; however, the growth of cells from individual patients was highly inconsistent and unpredictable. The average change in expansion with CTS OpTmizer Pro SFM is shown in light blue, normalized to expansion with the control medium (dark blue). The individual patients' expansion is represented by the light gray lines.

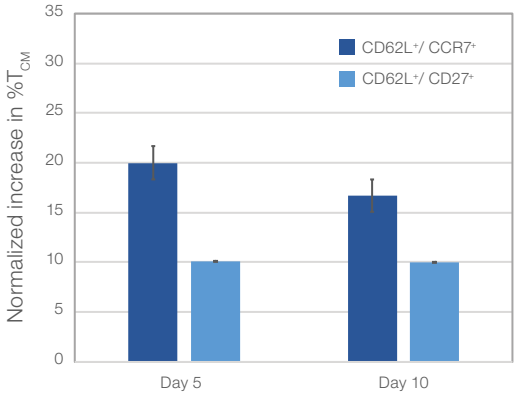

Figure 6. CTS OpTmizer Pro SFM maintains T_{CM} phenotype with healthy donor cells. Six healthy donor cells tested in an 18-day allogeneic workflow utilizing CTS OpTmizer Pro SFM demonstrated a 10–20% increase in the size of the central memory subset when evaluated on days 5 and 10, following normalization with a control medium. T_{CM} cells express CD62, CCR7, and CD27 as indicated in the legend.

CTS OpTmizer Pro SFM maintains T_{CM} phenotype and enables robust IFNγ production with healthy donor cells

The T cell phenotype was evaluated in cells from the same six healthy donors discussed previously, by assessing the expression of central memory markers—CD62L, CCR7, and CD27. The donor cell populations grown in CTS OpTmizer Pro SFM displayed a 10–20% normalized increase in the size of the central memory subset on days 5 and 10 (Figure 6). Additionally, as shown in Figure 7, the healthy donor cells grown in CTS OpTmizer Pro SFM demonstrated a robust average of a 187% normalized increase in IFNγ production, compared to the same cells grown with the control medium. This increase will likely act as a catalyst to boost overall immune response and provide more efficacious patient therapies by enhancing stimulation of macrophages, neutrophils, and natural killer (NK) cells.

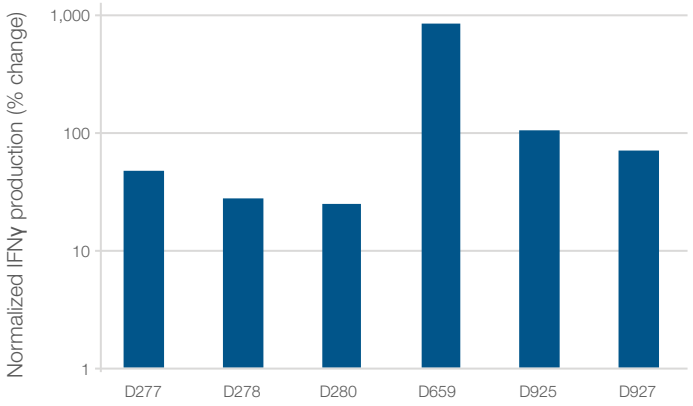

Figure 7. CTS OpTmizer Pro SFM enables robust IFNγ production with healthy donor cells. Six healthy donor cells grown in CTS OpTmizer Pro SFM demonstrated an average increase of 187% in IFNγ production at day 16 when normalized to the same cells grown with the control medium.

Conclusion

Although T cell therapy shows great promise in treating a diverse range of diseases, including oncological and autoimmune, a significant number of indications still need improvement. Research has demonstrated that frequent disparities in patients' treatment response is cell based and is specifically related to the reduced T_{CM} population. As a result, there has been a greater emphasis on utilizing T cells from healthy donors in allogeneic workflows. Healthy donor cells provide the necessary robust, young, and less differentiated T cell populations that can help provide more efficacious therapies. To support the need for larger central memory populations, Thermo Fisher Scientific developed CTS OpTmizer Pro SFM.

The data show that CTS OpTmizer Pro SFM may not be ideal for diseased cells used in an autologous workflow. When T cells from six healthy and nine diseased donors were expanded in CTS OpTmizer Pro SFM, the medium was shown to support expansion, maintain memory cell phenotype, and stimulate cytokine production following the immune response in the healthy donors' cells. The results of testing the diseased patients' cells with CTS OpTmizer Pro SFM demonstrated a slight increase in average cell

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growth; however, the results obtained on the basis of an individual patient were inconsistent and unpredictable. Until we understand more about the unique factors this population's cells present, this medium may not be optimal for autologous workflows. Results clearly demonstrate that CTS OpTmizer Pro SFM is most effective at supporting the expansion of healthy donor cells in allogeneic workflows.

Following normalization with the control medium, approximate average increases of 20% and 100% in cell proliferation were observed by day 10 and 17, respectively. These results were associated with the maintenance and increase of T_{CM} population subsets, indicated by the expression of CD62L, CCR7, and CD27. A normalized 10–20% increase in the size of the central memory subset was observed when using CTS OpTmizer Pro SFM, evaluated on day 5 and 10. Additionally, normalized to the control medium, CTS OpTmizer Pro SFM supports an average increase of 187% in IFNγ production, suggesting stimulation of both the innate and adaptive immune responses to combat diseases. Using an allogeneic workflow, CTS OpTmizer Pro SFM is well poised to significantly improve T cell expansion for healthy donor cells. Ultimately, this finding will potentially help decrease the overall workflow time and make T cell therapy more efficacious and an available off-the-shelf option for treatment.

References

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2. Klebanoff CA, Gattinoni L, Restifo NP (2006) CD8+ T-cell memory in tumor immunology and immunotherapy. *Immunol Rev* 211:214–224.

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