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SPOTLIGHT

INNOVATOR INSIGHT

Improving allogeneic manufacturing workflows

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Allogeneic T-cell therapies have the potential to improve both the efficacy and accessibility of life-changing cellular therapeutics. However, before this new paradigm can be fully established, there remains a need for improved manufacturing workflows to enable consistent production of highly efficacious allogeneic T-cell therapies. One of the core components of these workflows is the media used for the expansion of healthy donor T cells. In particular, a medium that can rapidly facilitate high levels of T-cell proliferation, maintain the desired central memory T (T_{CM}) phenotype, and increase immune responses by enhancing the production of cytokines such as interferon gamma (INFγ) is a much-needed solution. Gibco™ CTS™ OpTmizer™ Pro Serum-free Medium (SFM), a novel medium formulated for allogeneic workflows, has been developed to meet this need. The following article details the potential of CTS OpTmizer Pro SFM to improve both workflow efficiency and overall therapeutic efficacy.

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INTRODUCTION

The development of cellular therapeutics utilizing T cells has been a major revolution in the treatment of a variety of hematological malignancies. This is particularly true for autologous cell therapies – with over 500 clinical trials currently ongoing worldwide, including a number of therapies which have already achieved both US Federal Drug Administration (FDA) and European Commission (EC) approval [1]. However, as these current treatments are patient-specific, time-consuming

and expensive to produce on a dose-by-dose basis, moving to the development of allogeneic T-cell therapies has the potential to make these cutting-edge therapies more accessible. Through the innovation of a novel medium formulated specifically for allogeneic T-cell therapy manufacturing, the shift toward allogeneic therapies can be accelerated and their efficacy and accessibility can be increased.

Increasing therapeutic efficacy with allogeneic cell therapies

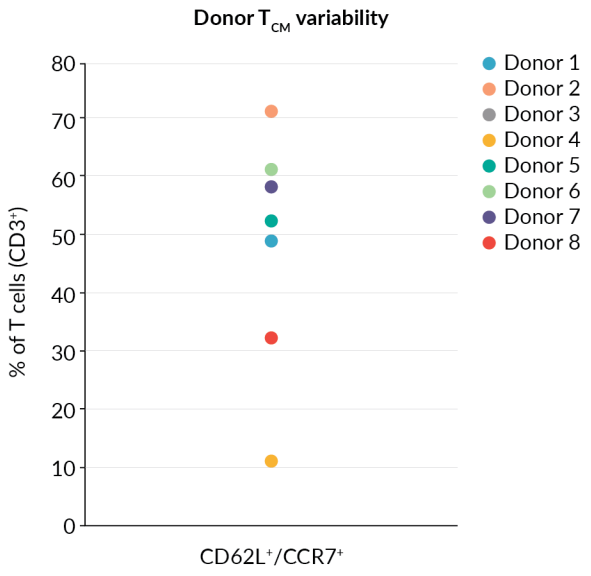
In addition to the well-documented logistical and economic benefits of using allogeneic over autologous T-cell therapies [2], the overall efficacy of the therapeutic intervention is a key factor. This provides an opportunity for allogeneic T-cell therapies, as current autologous treatments often lead to highly variable clinical responses depending on the disease being treated. For example, when looking at clinical trial data, some diseases such as B-cell acute lymphoblastic leukemia have a complete response (CR) – used as a measure of successful treatment – of around 80% whereas others have a CR of below 58% [3].

As a result, a lot of research has been focused on identifying the cause of this variance and it is now widely accepted that this difference is primarily cell based. The results have highlighted the need to use a robust population of young central memory T (T_{CM}) cells, due to the increased engraftment, persistence, and anti-tumor immune response exhibited by these younger T-cell types [4]. This presents an issue for the manufacture of efficacious autologous therapies using cells obtained from diseased patients as they typically present with highly variable T_{CM} populations (Figure 1). This variability usually results from a combination of the patient's disease and any other treatments they may have been exposed to, such as radio- and chemotherapy. To increase efficacy and, crucially, improve clinical outcomes, more suitable donor cells are required.

Healthy donors have been shown to display more consistent, and often higher T_{CM} counts. Therefore, allogeneic workflows could play a key role in improving efficacy through the activation and expansion of cells with more desirable characteristics.

FIGURE 1

Central memory cell variability across donors.



Pre-expansion sampling of T-cell populations in 8 donors for the percentage of cells expressing CD62L and CCR7 phenotypes – characteristic of T_{CM} cells. Variability is between 13% and 72%.

The power of cell expansion media

Much like autologous therapies, manufacturing allogeneic therapies with maximal efficacy cannot be achieved without the use of an optimal T-cell expansion medium. Due to its role in modulating the growth and differentiation of the cells, media can have a profound effect on the overall therapeutic efficacy of the manufactured dose.

One of the biggest challenges when manufacturing T-cell therapies is overcoming the tendency for the naïve T cells to be overstimulated and pushed too far through the differentiation pathway during expansion. If this happens, by the time the cells have been developed into the final therapeutic product, a large proportion of them will be exhausted, reducing the overall therapeutic efficacy. To avoid this situation, the medium used needs

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to be able to drive cells toward early memory phenotypes – such as central memory (T_{CM}) and stem cell memory T cells (T_{SCM}) – and maintain them at this stage to avoid further differentiation and exhaustion.

An additional way that cell expansion media can reduce overstimulation and thus improve efficacy is to eliminate the disparity between workflow lengths for autologous and allogeneic therapies. Using current media, allogeneic workflows require an additional 3 to 4 days compared to autologous therapies to generate enough cells for a therapeutic dose. As each additional day means further differentiation, there is a vital need for a medium that can reduce workflow length by facilitating the production of a greater number of T cells with early memory phenotypes in a shorter period of time.

Gibco CTS OpTmizer Pro SFM

Gibco CTS OpTmizer Pro Serum-Free Media (SFM) is a first-of-its-kind media solution specifically formulated to facilitate the expansion of human T lymphocyte cultures within allogeneic T-cell therapy manufacturing workflows.

By targeting healthy donor cell metabolism, the medium has the potential to enable strong proliferation, as well as robust maintenance of the desired T_{CM} phenotype, ultimately resulting in the production of more efficacious therapeutic products. Crucially, it has the capacity to achieve these results in a shorter time compared to existing T-cell expansion media.

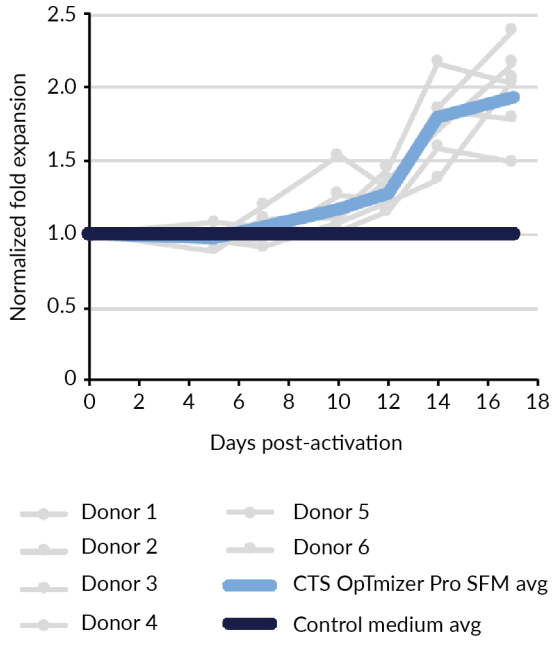
Additionally, the medium has been formulated to enhance overall workflow productivity. Most notably, it eliminates the need for serum, which can improve consistency, lower costs, reduce supply and contamination risks, and ease regulatory concerns. It is also highly versatile – supporting T-cell activation using either Dynabead™ magnetic beads, soluble antibodies, nanomatrices, or stimulatory antibody-presenting cell protocols.

CTS OpTmizer Pro SFM supports T-cell activation using Dynabeads magnetic beads, soluble antibodies, and stimulatory antibody-presenting cell protocol and comes in enhanced design and packaging making it compatible with closed systems.

The capacity of CTS OpTimizer Pro SFM to improve allogeneic manufacturing workflows and enable the production of efficacious therapeutic products is demonstrated in the following experiments.

FIGURE 2

Normalized healthy donor T-cell proliferation in CTS OpTmizer Pro SFM.



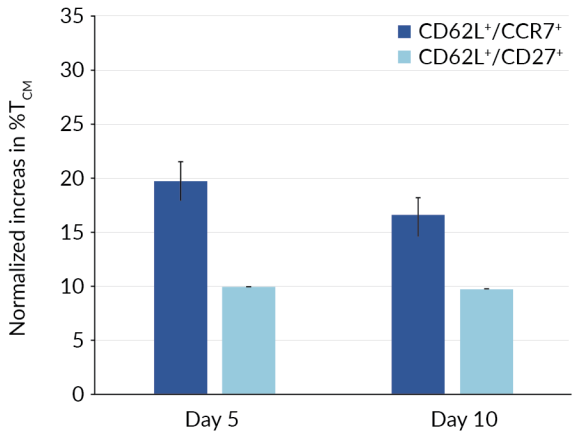
Healthy donors T cells expanded in an 18-day allogeneic-type workflow using CTS OpTmizer Pro SFM demonstrated ~20% higher cell proliferation by day 10, with a ~100% increase by day 17, when compared to a control medium. The average normalized change in cell growth in CTS OpTmizer Pro SFM is represented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

PROLIFERATION OF HEALTHY DONOR T CELLS

To assess the suitability of CTS OpTmizer Pro for use in allogeneic T-cell therapy manufacturing workflows, its ability to promote healthy donor T-cell proliferation was measured and compared to an industry standard, serum-free T-cell expansion medium.

FIGURE 3

Healthy donor T-cell phenotype maintenance in CTS OpTmizer Pro SFM.



Six healthy donor cells expanded in an 18-day allogeneic workflow using 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.

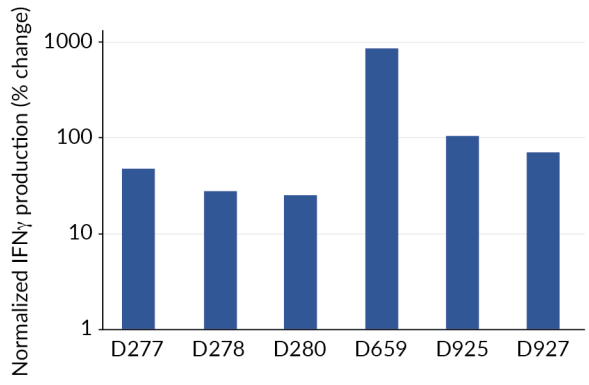
Human primary T cells were first negatively isolated from peripheral blood mononuclear cells (PBMCs) with the Invitrogen™ Dynabeads™ Untouched™ Human T Cells Kit. These cells were then seeded in culture dishes at 1 x 10^6 cells/mL in the indicated medium and activated with Gibco™ Dynabeads™ Human T-Expander CD3/CD28 at a ratio of 3 beads per T cell in the presence of 100 IU/mL of recombinant interleukin-2 (rIL-2). In all experiments using CTS OpTmizer Pro SFM, the complete culture medium was created by supplementing with Gibco L-Glutamine to a final concentration of 2 mM, per the user manual.

Throughout the experiments, T cells were counted every 2–3 days using a Vi-CELL™ Cell Viability Analyzer (Beckman Coulter). Viable cell density was also maintained at 0.25 x 10^6 cells/mL, and rIL-2 was added to the culture to a concentration of 100 IU/mL. In this first experiment, T cells from 6 healthy donors were grown as described above with CTS OpTmizer Pro SFM in an 18-day expansion workflow.

Normalized to the control medium, the average growth of the cells was increased by approximately 20% by day 10 for all donors on average, which rose further to over 100% by day 17 (Figure 2). The normalized results for each individual donor are represented by the gray lines. Moreover, all conditions displayed robust expansion, and no negative effect on viability was observed.

FIGURE 4

Healthy donor cell IFNγ production in CTS OpTmizer Pro SFM.



Normalized to the same cells grown using a control medium, cells from 6 healthy donors grown in CTS OpTmizer Pro SFM demonstrated an average increase of 187% in IFNγ production at day 16.

HEALTHY DONOR T_{CM} PHENOTYPE MAINTENANCE

The T-cell phenotype was also assessed in cells from the same 6 healthy donors by evaluating the expression of central memory markers – CD62L, CCR7, and CD27. This was conducted using the Invitrogen™ Attune™ NxT Flow Cytometer, by staining T cells with Invitrogen™ CD3 Pacific Orange™ dye, CD4 FITC dye, CD8 Pacific Blue™ dye, CD62L APC, and CCR7 PE antibodies.

Normalized to a control medium, healthy donor cells grown in CTS OpTmizer Pro SFM displayed a 10–20% increase in the size of the central memory subset when evaluated on days 5 and 10 (Figure 3). All donors showed normalized increases in the size of the early memory population at days 5 and 10 when expanded in CTS OpTmizer Pro (individual data not shown).

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HEALTHY DONOR T-CELL IFNγ PRODUCTION

Finally, to determine the capacity of CTS OpTmizer Pro SFM to produce T cells capable of stimulating a robust immune response, production of interferon gamma (IFNγ) by the cells was evaluated. IFNγ production is a particularly useful parameter to measure as the cytokine can stimulate both the innate and adaptive immune response and has the potential to boost overall therapeutic efficacy by further stimulating macrophages, neutrophils, and natural killer cells and enhancing host cytokine release [5].

To measure this parameter, 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 Invitrogen™ Luminex™ MAGPIX™ system using the Invitrogen™ Cytokine Human Magnetic 35-Plex Panel for the Invitrogen™ Luminex platform (Thermo Fisher Scientific) according to the user manual.

When normalized to the same cells grown in the control medium, healthy donor T cells grown in CTS OpTmizer Pro SFM showed a 187% increase in IFNγ production by day 16 (Figure 4). It is important to note that this observation was not associated with a significant shift in the CD8/CD4 ratio, which was not significantly altered by growth in CTS OpTmizer Pro.

CONCLUSION

These results demonstrate the capacity of CTS OpTmizer Pro SFM to facilitate high levels of T-cell proliferation, robust maintenance of T_{CM} phenotypes, and improved INFγ production using healthy donor T cells – all of which are hallmarks of efficacious T-cell therapies. Furthermore, they indicate that CTS OpTmizer Pro SFM is capable of producing a greater number of T cells with early memory phenotypes in a shorter period of time compared to existing media.

Combined, these results suggest that CTS OpTmizer Pro SFM could have a two-fold effect on the feasibility of allogeneic T-cell therapies. First, it could improve the efficacy of allogeneic treatments by facilitating the production of a large population of desirable T-cell phenotypes and potentially reducing overall workflow time. Second, through this improvement, it could open the possibility of these therapies becoming more prevalent and eventually becoming life-changing, off-the-shelf treatment options for hematological malignancies.

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BIOGRAPHIES

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Dr Zynda has been with Thermo Fisher Scientific for almost 5 years. He serves as a Senior Scientist in R&D for the department of Cell Culture and Cellular Medicine and has been focused Cell therapy process development and product development. He first began studying T-cell biology in 2005 at Roswell Park Cancer Institute, where he received a PhD in Molecular and Cellular Biophysics and Biochemistry. During his academic years, he elucidated mechanisms by which tumor cells to evade the immune system and went on to apply this knowledge in drug development and cell therapy manufacturing.

Dr. Aditi Singh
Global Product Manager, Thermo Fisher Scientific

Aditi Singh has been with Thermo Fisher scientific for over 6 years. She is currently working as Global Product Manager managing products utilized in T Cell Therapy manufacturing workflow utilized in research, process development and clinical settings. Her goal through this role is to improve patient care by making personalized medicine (/cell therapy) more accessible to cancer patients. Aditi has a Ph.D degree in Cell and Molecular Biology from University of Heidelberg, Germany where she worked on infectious disease and diagnosis.

AUTHORSHIP & CONFLICT OF INTEREST

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