Performance equivalency of CTS OpTmizer T Cell Expansion SFM with and without phenol red

Introduction

Thermo Fisher Scientific offers a formulation of Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free Medium (SFM) without phenol red for customers to use in their T cell therapy manufacturing workflows. This formulation:

- Was developed specifically for T cell expansion, has a proven track record of nearly a decade, is xeno-free, and does not require the addition of serum
- Allows usage of optical sensors in automated workflow solutions
- Is available to support cell therapy manufacturing in regions that encourage use of phenol red–free cell culture media

The design of CTS OpTmizer T Cell Expansion SFM, no phenol red (Cat. No. A3705001 and A3705003), is identical to that of the original CTS OpTmizer T Cell Expansion SFM (Cat. No. A1048501 and A1048503) and includes both a basal medium and expansion supplement. The only formulation modification is the removal of phenol red from the basal medium. No changes were made to the user manual's instructions to add 2 mM L-glutamine and the expansion supplement. Even so, it was necessary to confirm that the exclusion of phenol red in CTS OpTmizer T Cell Expansion SFM does not change its ability to support T cell expansion. To this end, extensive testing was conducted to compare the growth, viability, function, and phenotypes of human T cells cultured with CTS OpTmizer T Cell Expansion SFM with and without phenol red. No significant variations in the cellular products, based on the exclusion of phenol red, were observed.

Materials and methods

T cell activation and expansion

T cells were activated using Gibco™ Dynabeads™ Human T-Expander CD3/CD28 (Cat. No. 11141D) at a ratio of three beads for every cell and cultured in CTS OpTmizer T Cell Expansion SFM formulated with or without phenol red for 10 days in a Thermo Scientific™ Heracell™ 150i incubator set to 5% CO_2 and 37°C. All versions of CTS OpTmizer T Cell Expansion SFM contained the T cell expansion supplement and 2 mM L-glutamine as specified in the user guide. Cells were initially seeded into static culture dishes at 1 x 10^6 cells/mL and maintained at 5 x 10^5 cells/mL every 2–3 days thereafter. Additionally, 100 IU/mL of recombinant IL-2 was added fresh on day 0 and every 2–3 days thereafter for the rest of the expansion phase. Growth and viability were measured using a Vi-CELL™ Cell Viability Analyzer (Beckman Coulter) every 2–3 days, and phenotypes were assessed on day 10 using flow cytometric analysis.

Phenotype analysis by flow cytometry

A total of 2 x 10^6 cells per treatment group were pelleted and stained with the following Invitrogen™ antibodies: Pacific Orange™ CD3, FITC CD4, Pacific Blue™ CD8, eBioscience™ APC CD62L, and eBioscience™ PE CCR7 (Thermo Fisher Scientific). Following staining, the cells were washed with PBS, pelleted by centrifugation (350 x *g* for 5 min), and fixed in 2% paraformaldehyde. Surface expression of the proteins was then assessed on a Gallios™ Flow Cytometer using Kaluza™ Analysis Software (Beckman Coulter).

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Results

Cell growth and viability were nearly identical in the groups grown with and without phenol red (Figure 1A, B). Likewise, the phenotypic outcome of the expansion was similarly unaffected by the absence of phenol red. The extent of differentiation and the ratio of CD8 to CD4 T cells are comparable between the culture conditions (Figure 1C, D). Therefore, phenol red is not essential to the performance of CTS OpTmizer T Cell Expansion SFM.

Conclusion

We conducted extensive testing on CTS OpTmizer T Cell Expansion SFM with no phenol red against the original formulation with phenol red to investigate its ability to expand T cells. This report presents evidence that phenol red is not essential to the performance of CTS OpTmizer T Cell Expansion SFM.

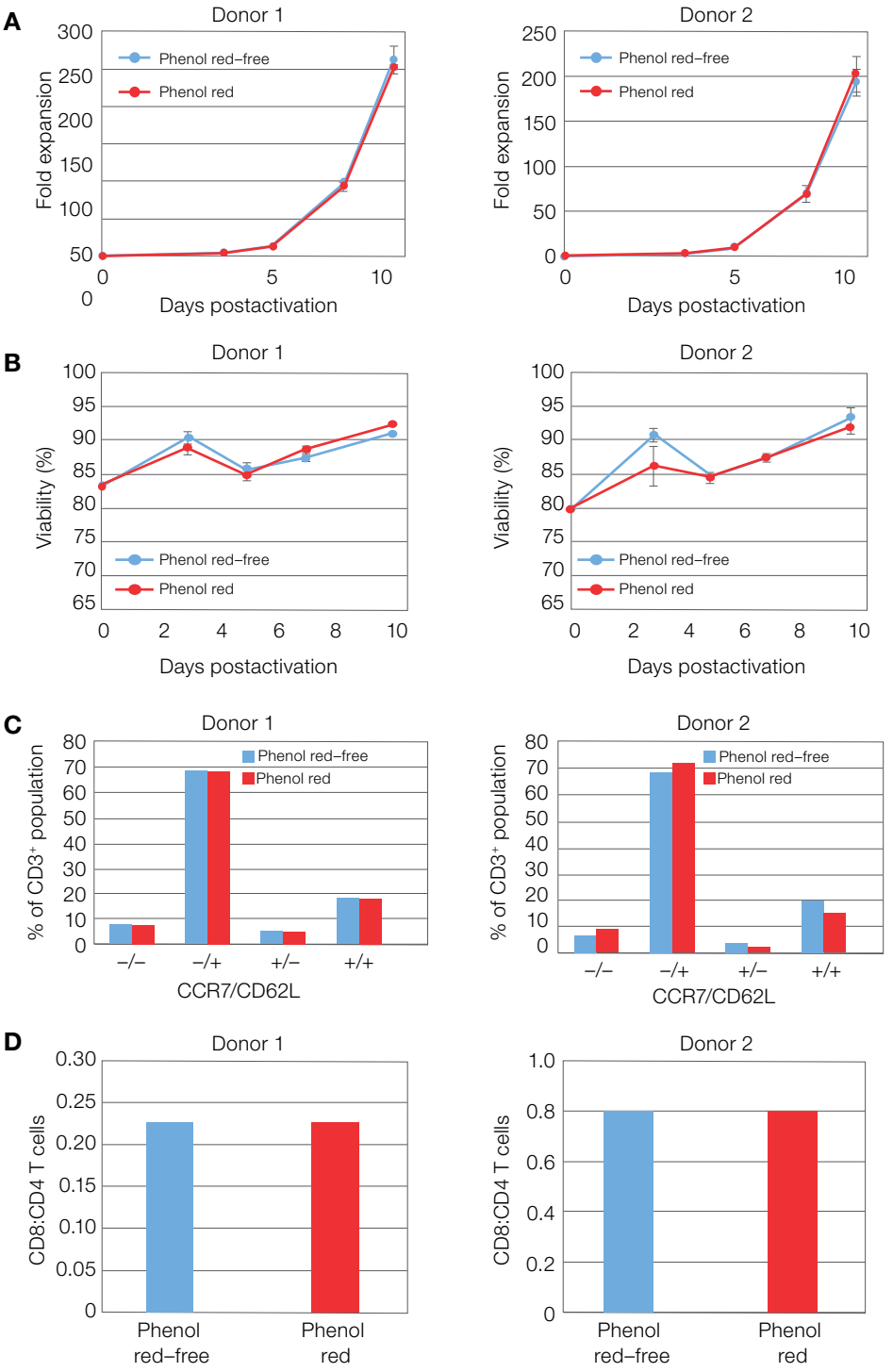

Figure 1. The presence or absence of phenol red in CTS OpTmizer T Cell Expansion SFM is inconsequential for T cell expansion. Human T cells from two independent donors were activated and expanded in CTS OpTmizer T Cell Expansion SFM with or without phenol red for 12 days. Endpoints assessed were **(A)** cell growth, **(B)** cell viability, **(C)** differentiation status as monitored by CCR7 and CD62L expression, and **(D)** CD8:CD4 T cell ratios. All experiments were done in triplicate, and the error bars in panels A and B indicate the standard deviation between the replicates.

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