Optimization of a Phenol Red-Free T Cell Expansion Medium to Improve Performance and Workflow Flexibility

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ABSTRACT

Adoptive immunotherapy displays immense potential as an immunotherapeutic strategy for patients with advanced malignancies. Phenol red has been a mainstay in media used for the *ex vivo* expansion of primary T cells. Those in the field prefer to keep phenol red out of the growth media because of potential regulatory concerns and the knowledge that it can interfere with sensors that are crucial to automated production workflows. To better serve customers, Thermo Fisher Scientific formulated a phenol red-free version of CTS™ OpTmizer™ T-Cell Expansion Serum-Free Medium (SFM) for use in T cell therapy manufacturing work flows. The design for CTS OpTmizer T-Cell Expansion SFM, No Phenol Red, is identical to that of the parent product, with the lone difference being the removal of phenol red from the basal medium. Here, we present data confirming that the removal of phenol red from the basal formulation does not affect T cell growth, viability, or phenotype. We also show that the additions of GlutaMAX™ can enhance viability, and the addition of CTS Immune Cell Serum Replacement (ISCR), a chemically defined serum substitute, can improve cell growth during expansion, while still maintaining a desirable phenotype. Finally, we display the results of a comparison between CTS OpTmizer T-Cell Expansion SFM, No Phenol Red and a popular serum-containing culture medium in several vessels, demonstrating that CTS OpTmizer without phenol red is as effective as the serum-containing counterpart.

INTRODUCTION

Removing phenol red alleviates regulatory concerns and adds manufacturing flexibility.

- CTS OpTmizer T Cell Expansion SFM is now available phenol red-free.
- CTS OpTmizer SFM, No Phenol Red is serum-free and xeno-free.
- CTS OpTmizer SFM, No Phenol Red is provided in a bag or bottle and can be customized to fit workflow needs.
- CTS OpTmizer SFM, No Phenol Red has batch-to-batch consistency, security of supply, regulatory support (DMF), and scalability for the clinic and commercialization
- The use of phenol-red containing media can lead to regulatory delays when expanding manufacturing into some regions and countries.
- Phenol red can interfere with the sensors involved in the automated process controls associated with scaled manufacturing.

MATERIALS AND METHODS

T Cell Isolation

Primary human T cells from normal donors were negatively isolated from PBMCs with Dynabeads™ Untouched™ Human T Cells kit (Thermo Fisher Scientific).

Media

CTS OpTmizer T Cell Expansion SFM and CTS OpTmizer SFM, No Phenol Red were supplemented with 2mM L-glutamine and 2.6% of OpTmizer T-Cell Expansion Supplement. 4mM GlutaMAX™ and 2.5% CTS ICSR (Thermo Fisher) were added where indicated. Competitor basal medium was supplemented with 5% human AB serum (hABs) (Gemini Bio-Products) and 2mM L-glutamine (Thermo Fisher).

Seeding

G-Rex® experiments: $5x10^6$ T cells were seeded in 100 ml of the indicated medium. Plate and bag experiments: T cells were seeded $1x10^6$ cells/ml of indicated medium.

Activation

T cells were activated with CTS Dynabeads Human T- Cell Expander CD3/CD28 (Thermo Fisher Scientific) at a ratio of 3 beads per T cell in the presence of 100 IU/ml of rIL-2 for plate and G-Rex® experiments and 300 IU/ml of rIL-2 for bag experiments.

Expansion

T cells were maintained at $5x10^5$ cells/ml in static plates and bag cultures, and counted on days 3, 5, 7, and 10 using a Beckman-Coulter Vi-Cell analyzer and rIL-2 was replenished on these same days. For the HyPerforma Rocker Bioreactor, the cells were activated in static bags and transferred to rocker bags on day 3 following stimulation. DO and pH were maintained at 25% and >7.00, respectively, and the density was maintained at $5x10^5$ cells/ml on days 5 and 7 up to 5 L of medium.

Flow Cytometry

Cellular phenotype was assessed on day 10 by staining T cells with αCD3-Pacific Orange, αCD4-FITC, αCD8-Pacific Blue, αCD62L-APC, and αCCR7-PE (Thermo Fisher Scientific).

RESULTS

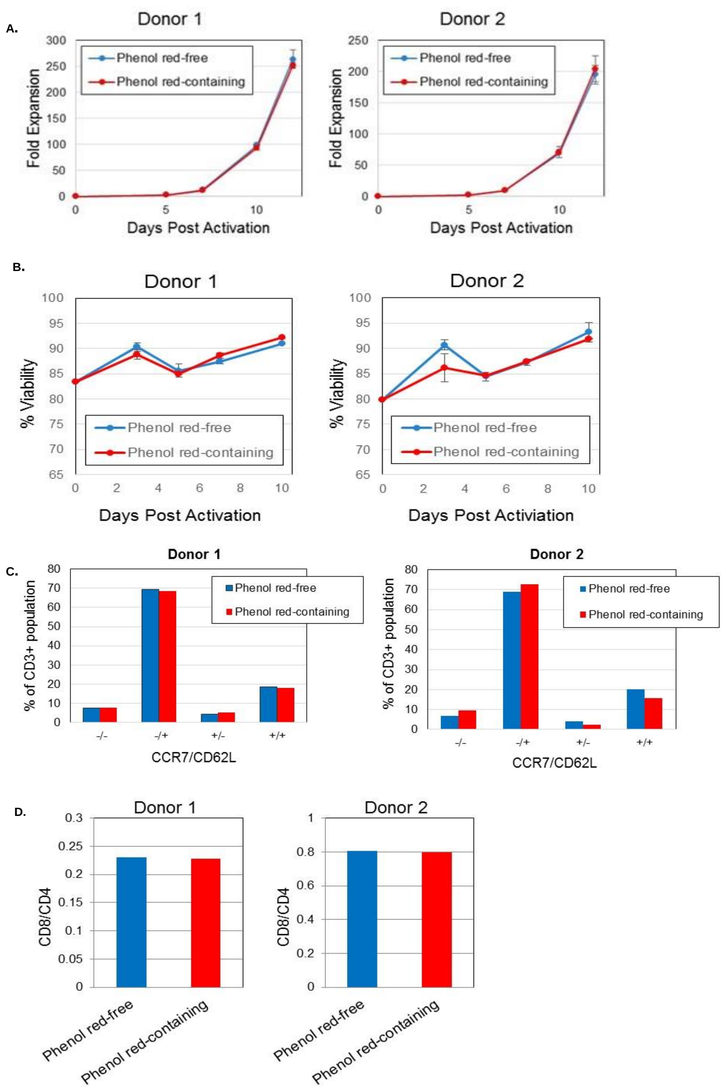

Figure 1. The absence of phenol red did not impact performance. T cells were cultured in complete CTS OpTmizer SFM with or without phenol red for 10 days in static cell culture plates. (A) Cell growth was recorded every 2-3 days and reported in terms of fold expansions, (B) cell viability was monitored throughout expansion of the cells (C) phenotypic characterization was performed on day 10 to determine the CD8:CD4 ratio and (D) the degree of differentiation, as measured by CD62L and CCR7 expression within the CD3+ population. Data represents the mean of three replicates for each independent donors and the error bars depict the standard deviation.

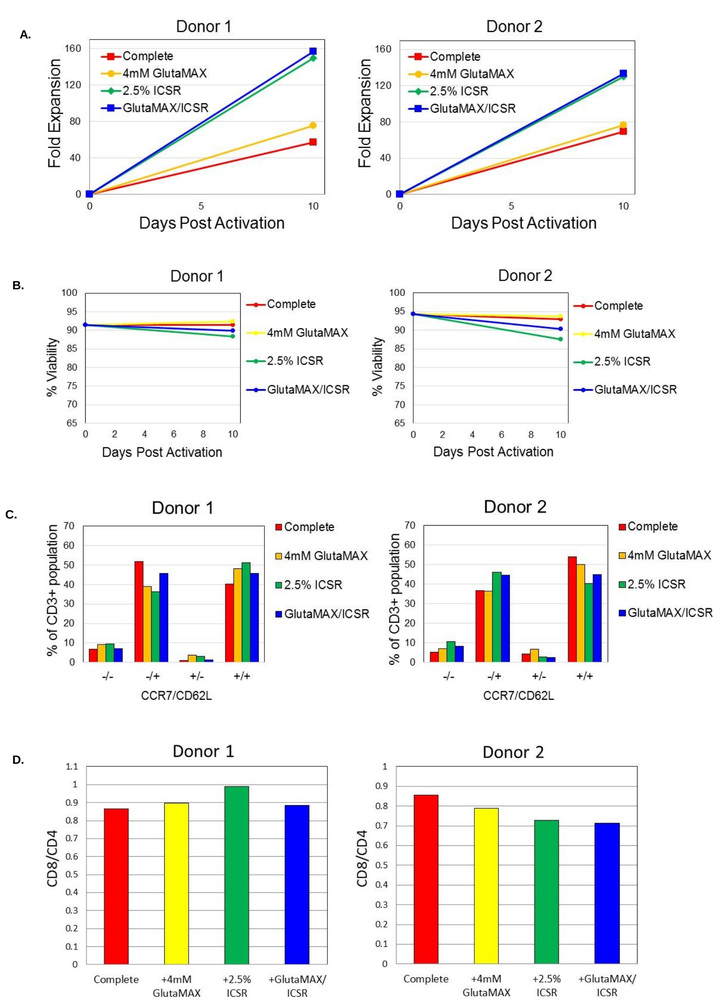

Figure 2. Addition of CTS ICSR and GlutaMAX increases the performance of CTS OpTmizer SFM, No Phenol Red. T cells were cultured in differentially supplemented CTS OpTmizer SFM, No Phenol Red for 10 days in G-Rex vessels. The control group was the complete medium, formulated as instructed in the insert. Variations on this control included the addition of 4mM GlutaMAX, the addition of 2.5% CTS ICSR, and the addition of both 4mM GlutaMAX and 2.5% ICSR. (A) Cell growth was measured over time and reported as fold expansions, (B) cell viability was monitored along with expansion of the cells (C) phenotypic characterization was performed on day 10 to determine the CD8-to-CD4 ratio and (D) the degree of differentiation, as measured by CD62L and CCR7 expression within the CD3+ population. Data shown for two independent T cell donors.

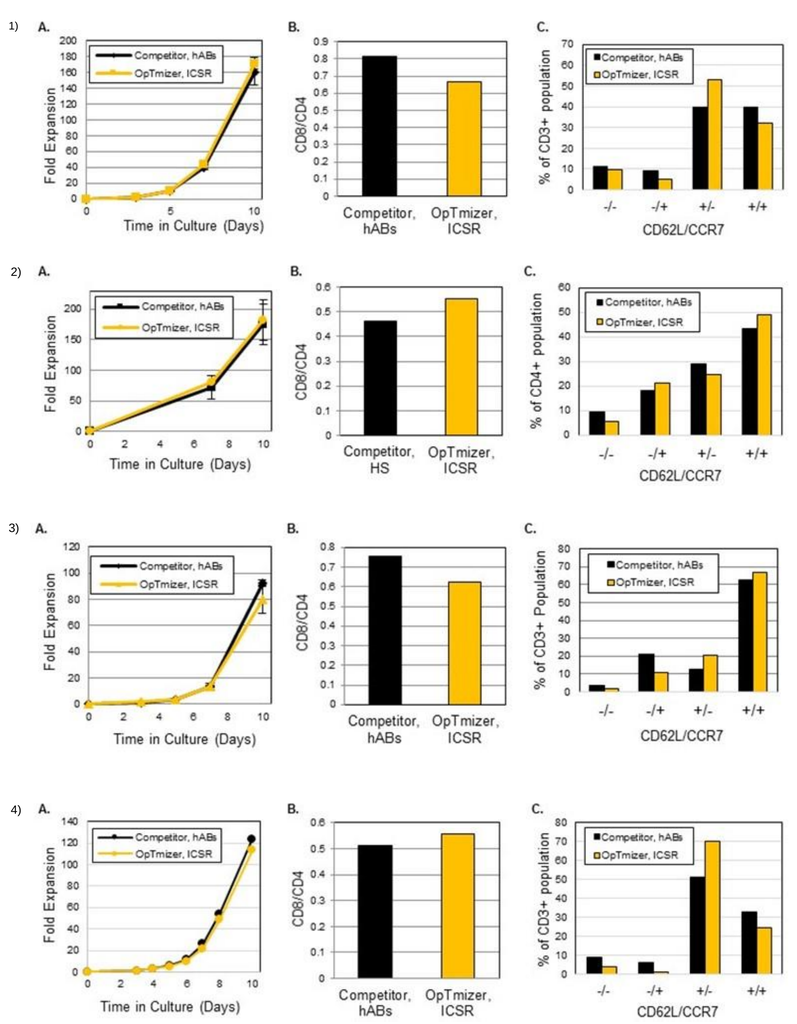

Figure 3. Increased cell performance was seen in several different culture vessels. T cells were cultured in CTS OpTmizer SFM, No Phenol Red with GlutaMAX and 2.5% CTS ICSR for 10 days in (1) static cell culture plates, (2) G-Rex® vessels, (3) static culture bags, and (4) the HyPerforma Rocker Bioreactor. For all vessels, (A) cell growth was measured over time and reported as fold expansions. (B) phenotypic characterization was performed on day 10 to determine the CD8:CD4 ratio and (C) the degree of differentiation, as measured by CD62L and CCR7 expression within the CD3+ population. These test employed competitor media containing 5% hABs as a benchmark. Data represent the mean of three experimental replicates and error bars are the standard deviations of each data set.

CONCLUSIONS

- The removal of phenol red from CTS OpTmizer SFM has no effect on its performance.
- The addition of GlutaMAX™ can enhance viability, and the addition of CTS ISCR can improve cell growth during expansion, while preserving the desirable central memory phenotype.
- CTS OpTmizer SFM, No Phenol Red supplemented with GlutaMAX and CTS ICSR can support T cell expansion in static culture plates, G-Rex® vessels, static culture bags, and rocking bioreactors.
- CTS OpTmizer SFM, No Phenol Red supplemented with GlutaMAX and CTS ICSR can expand cells as well as serum-containing medium.

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