Stemline™ Hematopoietic Stem Cell Expansion Medium, a Serum-Free Medium for the Expansion of CD34+ Hematopoietic Stem Cells and Progenitors

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Abstract

The use of human hematopoietic stem cells (HSC) for repopulation of the hematopoietic system following ablative, high-dose chemotherapy is becoming a more popular option for the treatment of some cancers in the clinical setting. HSC can be obtained from three primary sources: umbilical cord blood (CB), bone marrow or peripheral blood. Due to the reduced incidence and severity of graft versus host disease, CB samples have become the source of choice. However, due to the low number of cells obtained from a given CB sample, there are frequently too few cells for transplantation of adult patients. In order to solve this problem, clinicians need to be able to grow and expand the cells *ex vivo* in the proper serum-free cell culture medium/cytokine combination. Sigma-Aldrich, in collaboration with Stemgenix, has developed Stemline™ Hematopoietic Stem Cell Expansion Medium (marketed as HSC GEM™ by Stemgenix) to address this concern. Stemline™/HSC GEM™ routinely exhibits a greater overall expansion of total nucleated cells from CB than any of the competitor's products. In order to determine the functionality of the expanded cells, flow cytometric analysis and colony-forming assays have also shown Stemline™/HSC GEM™ to be superior for the expansion of both the early and late progenitor populations. NOD/SCID mouse studies are also underway in order to determine the long-term engraftment potential of the expanded cells. Overall, the increased expansion, along with the ability to produce the medium in a state-of-the-art cGMP facility with an available Device Master File (DMF), makes Stemline™/HSC GEM™ the best choice for clinicians seeking a serum-free product for their clinical hematopoietic stem cell applications.

Introduction

Hematopoietic stem cells (HSC) have the ability to repopulate the hematopoietic system by differentiating into all of the necessary erythroid, lymphoid, and myeloid lineages. Due to this rare ability, HSC are used as therapeutic agents in the treatment of malignant and benign diseases of the blood forming and immune systems. There have been many advances in the area of clinical HSC research, but the availability of suitable cells for transplantation still remains a major limiting factor.

HSC can be isolated from three different sources: umbilical cord blood (CB), bone marrow, and mobilized peripheral blood. CB is currently the preferred source because it has been shown to have a lower risk of graft versus host disease (GVHD), presumably due to its immunological naiveté. However, because the volume of CB is limited, each umbilical cord has only enough cells to successfully transplant a small child. In order to transplant an adult, the HSC from CB must be expanded *ex vivo.* The expansion must be performed in a manner to ensure that the HSC not only differentiate along appropriate hematopoietic lineages, but also self-renew, leaving undifferentiated stem cells in the expanded culture. The differentiated cells will allow for short-term engraftment that will reduce the effects of neutropenia and thrombocytopenia in the patient. The undifferentiated cells will allow for long-term engraftment that will establish a new, permanent hematopoietic system for the patient. In order to expand these very specific cell types, an optimized serum-free medium and cytokine cocktail are needed.

To this end, Stemline™ Hematopoietic Stem Cell Expansion Media were developed for the expansion of HSC. They are serum-free media that allow for expansion of both differentiated and undifferentiated HSC. Stemline™ and Stemline™ II are both able to expand HSC from CB, bone marrow, and mobilized peripheral blood. In bench-scale and clinical-scale expansions, both media have shown promising results in expanding a mixed population of cells that remain fully functional. The original medium, Stemline™, expands CD34+ cells better than or equal to other commercially available serum-free HSC media. Stemline™ II is a newer version of the medium that has an increased expansion potential for CD34+ cells.

Materials & Methods

Cell Preparation

For all experiments, cryopreserved, human CD34+ cells were obtained from independent suppliers (Stemgenix; Amherst, New York; AllCells, LLC; Berkeley, California) and were handled in a manner consistent with the manufacturer's instructions with regard to storage and reconstitution. Cells were counted using either a hemocytometer or Guava Personal Cytometer (Guava Technologies; Hayward, California) to determine cell density and viability.

Serum-Free Expansion Medium Preparation

Stemline™ Hematopoietic Stem Cell Expansion Media (Sigma-Aldrich; St. Louis, Missouri)/HSC GEM™ (Stemgenix; Amherst, New York), IMDM (Sigma-Aldrich; St. Louis, Missouri), X-VIVO 15 (BioWhittaker; Walkersville, Maryland),

HPGM (BioWhittaker; Walkersville, Maryland), QBSF-60 (Quality Biological, Gaithersburg, Maryland), StemPro-34 (Life Technologies; Grand Island, New York), and StemSpan H3000 (StemCell Technologies; Vancouver, British Columbia) were purchased fresh, aliquoted and stored according to the manufacturer's recommendations. For each experiment, a 10 ml volume of each expansion medium was warmed to 25 °C. One ml of each medium was pipetted in triplicate in 24-well culture plates (Corning/Costar; Corning, New York) to which SCF, TPO and G-CSF (Sigma-Aldrich; St. Louis, Missouri) were added to a final concentration of 100 ng/ml each. Sterile PBS (Sigma-Aldrich; St. Louis, Missouri) was added to unused wells to maintain humidity. Plates were incubated at 37 °C and 5% CO_2 for 15 minutes prior to the addition of the revived CD34+ cells. Viable recovered CD34+ cells were added to each well at 1.0 x 10^4 cells/ml and allowed to proliferate in a humidified incubator at 37 °C and 5% CO_2 for 10 days. Following the incubation period, the expanded total nucleated cells were counted.

Flow Cytometry

Initially, Fc receptors on cells were blocked to prevent non-specific antibody binding by incubating for 15 minutes with FcR (Miltenyi; Auburn, California). Cells were then stained with CD34-FITC and CD38-PE (Becton Dickinson; San Jose, California) by incubating with antibodies for 30 minutes on ice in the dark. Single color controls were used to set the color compensation and PI was included to determine cell viability. 50,000 events were collected for each sample on a BD FACScan.

Clinical-Scale Expansion

A 2-step, clinical-scale assay (McNiece, et al., *Experimental Hematology* 2000. 28: 1181-1186) using Teflon® culture bags (American Fluoroseal, Inc.; Gaithersburg, Maryland) was set up for a comparison study between Stemline™/HSC GEM™ and Stemline™ II/HSC GEM™ II. For clinical-scale studies, CB CD34+ cells were cultured for 7 days in 100 ml Teflon® culture bags containing 50 ml of each culture medium plus cytokine concentrations as previously described. Cells were harvested from these bags and a 10 ml aliquot was transferred to a second 100 ml Teflon® bag containing 90 ml of each selected medium plus cytokines and cultured for an additional 7-day culture period. At the end of the culture protocol, cells were harvested, counted by hemocytometer, viability tested, and assayed for functional hematopoietic activity *in vivo* and *in vitro*.

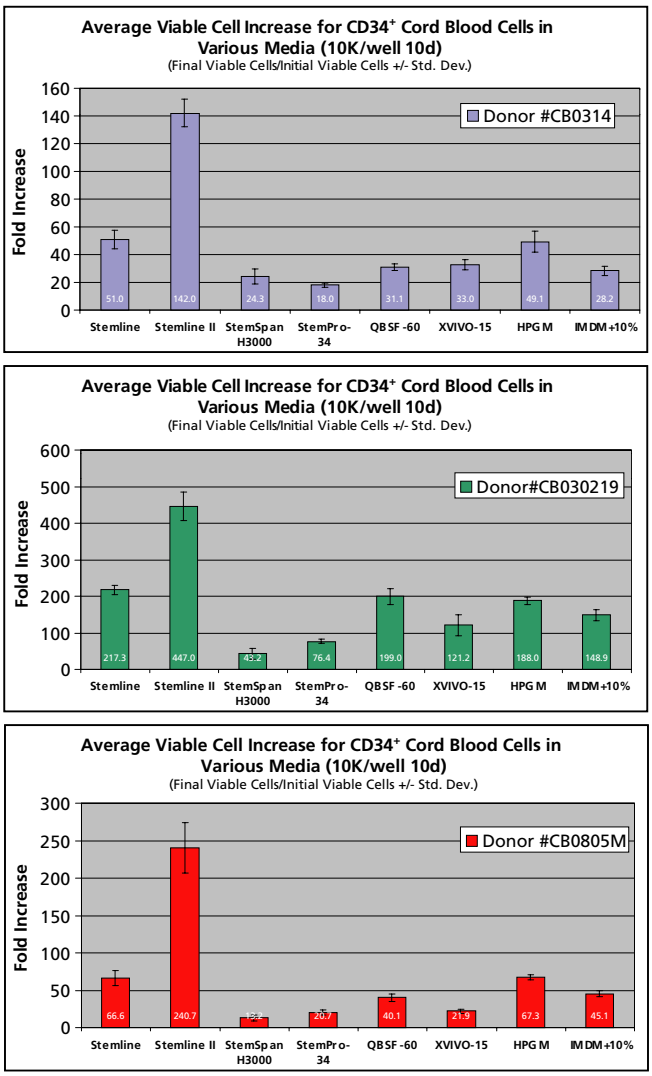
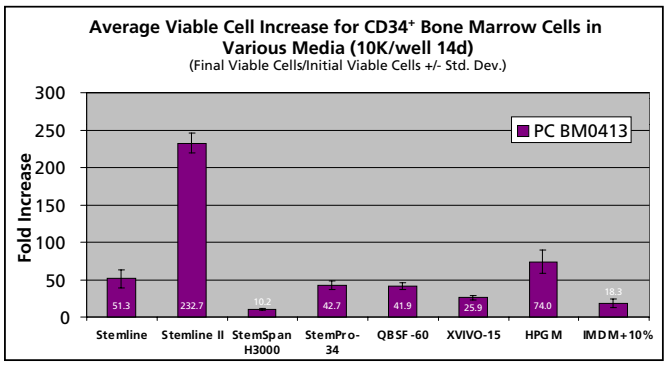


Figure 2: Expansion of CD34+ cells from cord blood in Stemline™ and Stemline™ II compared to other commercially available, serum-free, HSC media. Assays were set up in 24-well tissue culture plates with each medium tested in triplicate. 1 ml of the appropriate medium was added to each well, and the plate was inoculated with 10,000 cells/well. The cells were counted on day 10 and the fold increase was determined by $cells_{final}/cells_{inital}$. In cord blood, the Stemline™ media outperform the other serum-free HSC media. While Stemline™ already performed better than or equal to the other HSC media, Stemline™ II exhibited approximately a 3 fold increase in expansion over Stemline™.

Product Name	Product Number	Package Size	Physical State/Storage	Required Glutamine Supplementation	Animal Components	Regulatory Items
Stemline™	S0189	500ml	Liquid/4 °C	4mM	Human Serum Albumin	Manufactured cGMP DMF on file
Stemline™ II	S0192	500ml	Liquid/4 °C	N/A	Human Serum Albumin	Manufactured cGMP DMF in process

Figure 1: Basic characteristics of Stemline™ media.



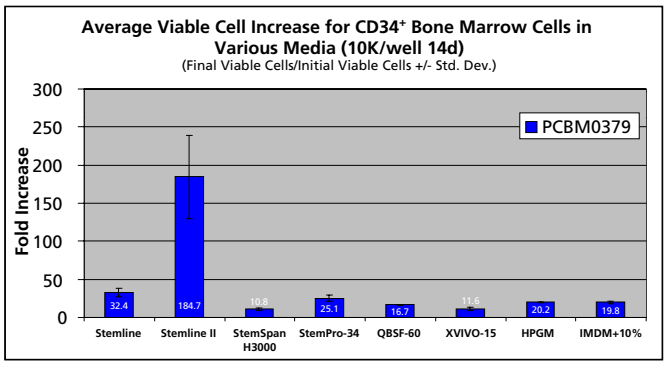


Figure 3: Stemline™ and Stemline™ II bench-scale expansion of CD34+ cells from bone marrow compared to other HSC media. Assays were set up as in Figure 2. The cells were counted on day 14 and the fold increase was determined by ($cells_{final}/cells_{inital}$). For the expansion of bone marrow CD34+ cells, Stemline™ performed as well as or better than the other competitors. However Stemline™ II was vastly superior to the other commercially available serum-free HSC media, giving approximately 5 fold more total nucleated cells.

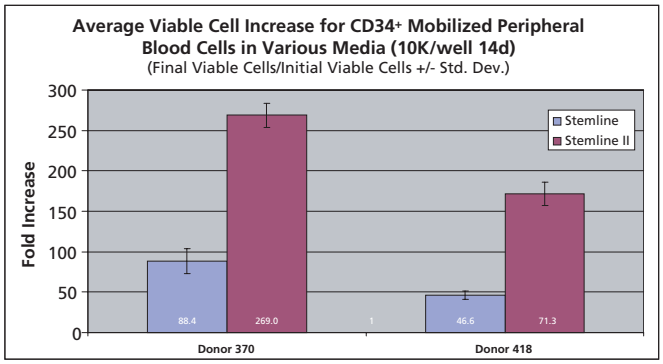


Figure 4: Bench-scale expansion of CD34+ cells from mobilized peripheral blood in Stemline™ and Stemline™ II. Assays were set up as in Figure 2. The cells were counted on day 14 and the fold increase was determined by ($cells_{final}/cells_{inital}$). In mobilized peripheral blood, both Stemline™ products consistently exhibit high levels of expansion of total nucleated cells.

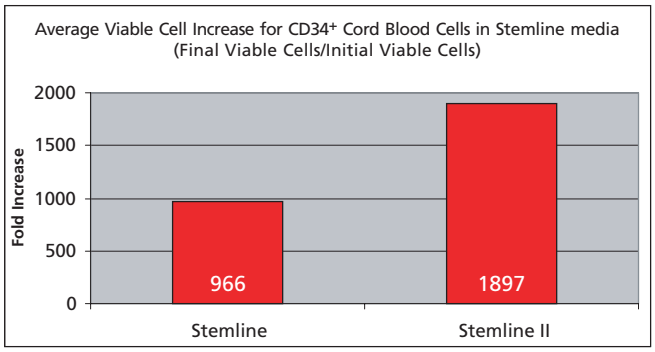


Figure 5: Comparison of Stemline™ II to Stemline™ in a clinical-scale expansion of CD34+ cells from cord blood. A two step clinical-scale expansion was performed to compare cell growth in Stemline™ and Stemline™ II. Briefly, the cells were seeded into 100 ml bags and incubated for 7 days. On day 7, a portion of the expanded cells was inoculated into a fresh 100 ml bag for an additional 7 days. Both media demonstrate increased potential for expanding CD34+ cells from cord blood, supporting excellent growth and high viability (>80%).

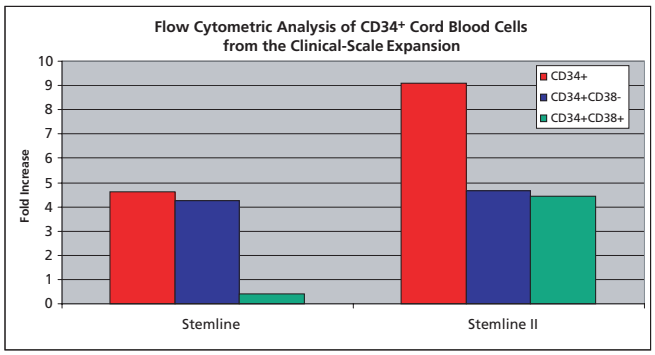


Figure 6: Flowcytometric analysis on CD34+ cord blood cells from clinical-scale expansion. After the two step expansions were complete, a sample of cells from the Stemline™ and Stemline™ II cultures was analyzed by flow cytometry for expression of CD34 and CD38. The majority of the CD34+ cells expanded in Stemline™ remained undifferentiated, early progenitors ($CD34^+38^-$), while cells expanded in Stemline™ II contained both early (34^+38^-) and late progenitor (34^+38^+) phenotypes. Both media expand high levels of early progenitors, which is important for long term engraftment. Stemline™ II also expands high levels of the late progenitors required for early engraftment and amelioration of the post-transplant nadir in mature myeloid cells.

Conclusions

Bench-Scale Expansions

- Stemline™ and Stemline™ II are capable of expanding CD34+ cells from umbilical cord blood, adult bone marrow, and mobilized peripheral blood in bench-scale expansions. The Stemline™ media expand CD34+ cells from all three sources better than the serum-free commercially available competitors.

Clinical-Scale Expansions

- Both Stemline™ media were able to expand CD34+ cells from cord blood in a clinical-scale expansion.
- Flow cytometric analysis of the clinical-scale expansions reveals that Stemline™ and Stemline™ II expand comparable numbers of early progenitor cells ($CD34^+/CD38^-$).
- Stemline™ II also has the additional benefit of a higher capacity for the expansion of the $CD34^+/CD38^+$ late progenitors required for short-term engraftment.

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