EX VIVO Expansion of CD34+ Cells in Stemline™ II Hematopoietic Stem Cell Expansion Medium Generates a Large Population of Functional Early and Late Progenitor Cells

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Abstract: Hematopoietic stem cell replacement therapy is an area of research lacking an optimal culture system that allows for the *ex vivo* expansion of $CD34^+$ cells for transplant. The necessity for expansion is due to the lack of sufficient material from umbilical cord blood (UCB), the preferred source of $CD34^+$ cells. The final composition of the expanded cells is also very important to a successful transplant. The material must contain both early and late progenitor cells to ensure the long-term engraftment that is required in patients with genetic disorders and those that have gone through high dose chemotherapy.

Stemline™ II Hematopoietic Stem Cell Expansion Medium was developed to facilitate the development of this replacement therapy. Culturing $CD34^+$ cells in Stemline™ II consistently yields higher levels of expansion than seen with other commercially available serum-free media. Analysis of the expanded material for lineage indicating markers by flow cytometry demonstrates that the material contains the required early and late progenitors. Furthermore, NOD/SCID studies confirm that the expanded cells are capable of long-term engraftment and are therefore functional. Together, this data supports the conclusion that culturing $CD34^+$ cells in Stemline™ II Hematopoietic Stem Cell Expansion Medium is a key step in developing the optimal culture system.

Key words: Hematopoietic, Stem Cell, Expansion, Serum Free, Medium

R. Smith (ed.), Cell Technology for Cell Products, 339–341.

1. 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, HSCs 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. 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 HSCs. They are serum-free media that allow for expansion of both differentiated and undifferentiated HSCs. 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.

2. RESULTS

In bench-scale experiments, Stemline™ media expand $CD34^+$ cells from all three sources better than other commercially available serum-free media. The expanded material contains the proper cell types, both early progenitors and late progenitors. Figure 1 has two representative graphs demonstrating the superior expansion characteristics of the Stemline™ media when using $CD34^+$ cells from cord blood. Similar results were seen in mobilized peripheral blood and bone marrow (data not shown).

The expansion capabilities of the Stemline™ media were also tested on a clinical scale. The expansion of $CD34^+$ cells cultured in both media was performed in clinical bags and the final material analyzed by flow cytometry. The assays demonstrated that both media are able to support the expansion of these cells, but Stemline™ II was able to achieve a 377-fold increase in cell number compared to a 238-fold increase achieved by Stemline™. The majority of the cells expanded in Stemline™ remained undifferentiated ($CD34^+$, $CD38^-$), while cells expanded in Stemline™ II contained both early and later progenitors ($CD34^+$, $CD38^+$) (data not shown).

A sample of cells from the Stemline™ and Stemline™ II clinical cultures were prepared for transplantation into NOD/SCID mice. A high percentage of the mice survived transplantation with cells expanded from both media (higher with Stemline™ II), all of which contained a small number of $CD45^+$ human cells as proof of engraftment (an even smaller number of which were also $CD34^+$). A summary of NOD/SCID results is in Table 1.

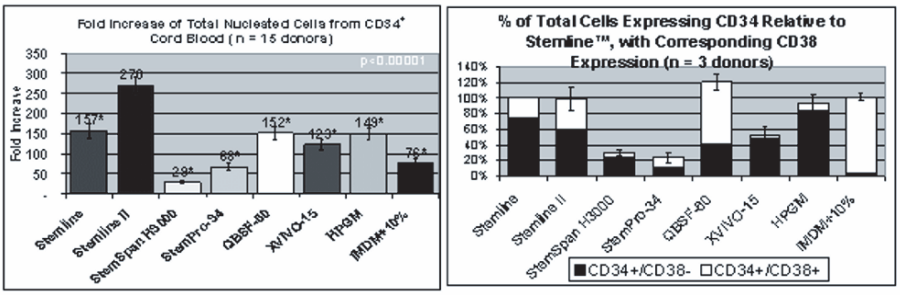

Figure 1. Expansion and characterization of $CD34^+$ cells from cord blood in Stemline™ and Stemline™ II compared to other commercially available, serum-free, HSC media.

Table 1. Summary data from the transplantation of expanded cells in the NOD/SCID mouse model.

	Stemline™			*Stemline™ II*		
Injected Cells	*600,000*	*1,800,000*	*5,400,000*	*600,000*	*1,800,000*	*5,400,000*
Survival Rate	*5/10 50%*	*3/10 30%*	*3/7 43%*	*7/10 70%*	*6/10 60%*	*6/7 86%*
Average % $CD45^+$	*0.064 ± 0.061*	*0.017 ± 0.006*	*0.143 ± 0.081*	*0.036 ± 0.013*	*0.018 ± 0.019*	*0.108 ± 0.162*
Average % $CD45^+$/ $CD34^+$	*0.000 ± 0.000*	*0.003 ± 0.006*	*0.007 ± 0.006*	*0.011 ± 0.009*	*0.002 ± 0.004*	*0.010 ± 0.000*

3. DISCUSSION

The excellent performance of the Stemline™ media for the expansion and maintenance of functionality of $CD34^+$ cells makes both media superior to all other commercially available, serum-free, HSC media. Both media expanded enough functional, early progenitors to achieve long-term engraftment in NOD/SCID mice. The higher survival rate in Stemline™ II may be explained by the higher levels of the late progenitors required for early engraftment and amelioration of the post-transplant nadir in mature myeloid cells. Additionally, Stemline™ media have Device Master Files (DMF) and are formulated in a state-of-the-art cGMP facility making them well suited for clinical applications.