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Enabling Efficient Ex Vivo Expansion of Long-term Human Hematopoietic Stem and Progenitor Cells For Cell Therapy

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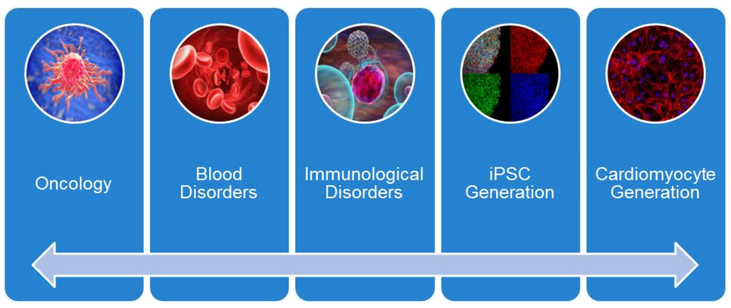
ABSTRACT

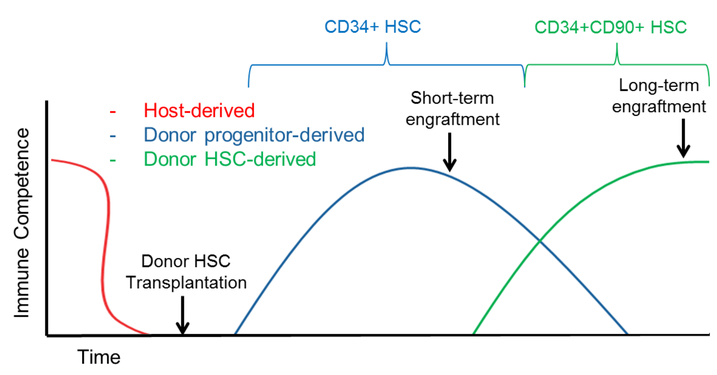
Umbilical-cord blood (UCB) is an important source of hematopoietic stem and progenitor cells (HSPC) for transplantation into patients lacking a suitable HLA-matched donor. However, due to the limited cell dose in each cord blood unit, individuals >60 kg are restricted from the use of UCB-based therapy. Ex vivo expansion of UCB CD34+ cells is one strategy employed to increase the hematopoietic cell dosage. A major limitation of current systems used for the expansion of HSPC is that ex vivo culture leads to expansion and differentiation at the expense of the most primitive pluripotent long-term stem cells. This has limited the clinical application of ex vivo expanded HSPC, since short-term progenitor cells only provide transient protection, ultimately reducing the long-term positive health outcomes, increasing the duration of hospitalizations, and health care costs per patient. Development of a culture system that expands both short-term and long-term HSPC would facilitate immune protection during the early phase of recovery, and provides a suitable solution for transfusion-independent hematopoiesis. Therefore, we sought to develop a HSPC culture medium that enables the expansion of both long-term and short-term HSPC, while maintaining their functional properties. To this end, we conducted several iterative rounds of Design of Experiments (DOE) involving multifactorial analysis, and mathematical modeling methods. Definitive Screening DOEs allowed us to identify optimal combinations and concentrations of essential media components, small molecules, and growth factors. The performance of candidate HSPC expansion media were evaluated after 7 days of culture, with the following attributes assessed: (1) viability of cells; (2) numbers of total nucleated cells; (3) percentages and numbers of $CD34^+$ cells; (4) percentages and numbers of $CD34^+CD90^+CD45RA^-$ cells; (5) expression of aldehyde dehydrogenase by expanded $CD34^+$ cells; and (6) colony-forming unit (CFU) assays. As the transplantation of HSPC in immuno-deficient mice is the gold standard in determining whether the expanded cells are engraftable, we plan to conduct these studies with the lead candidate HSPC expansion medium. Taken together, we seek to highlight our design philosophy in HSPC culture media development. We believe that our efforts are critical for the successful utilization of hematopoietic stem cell transplants in translational cell therapies.

INTRODUCTION

The goal of this research was to develop a hematopoietic stem and progenitor cell (HSPC) expansion media that fulfills the needs for customers interested in therapies that treat cancer, blood disorders, immunological disorders, in addition to generation of iPSC and cardiomyocyte.



Current systems used for the ex vivo expansion of HSPC result in the expansion and differentiation of $CD34^+$ cells, at the expense of the most primitive pluripotent long-term stem cells $CD34^+CD90^+CD45RA^-$ cells that provide life-long immunity.



Adapted from Bhattacharya et al.

MATERIALS AND METHODS

Figure 1. Process Workflow for Expansion of $CD34^+$ cells

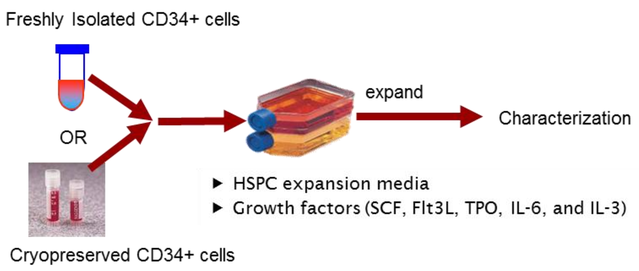


Figure 1: $CD34^+$ cells were expanded with HSPC expansion media and characterized to determine the TNC, % Viability, phenotype and function.

Figure 2. Experimental Workflow for Development of HSPC Expansion Medium

A. Input-Output Control Diagram for HSPC Expansion Medium Development

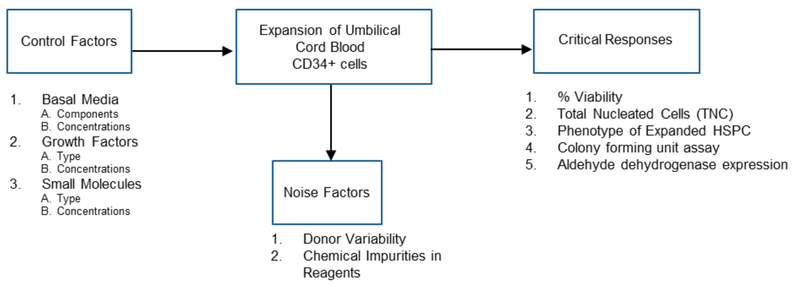


Figure 2: Process Map that outlines input variables that may influence outputs. Illustrates potential Noise Factors that increase variability during medium development.

RESULTS

Figure 3. Analysis of Existing Solutions in Expansion of Umbilical Cord Blood $CD34^+$ cells.

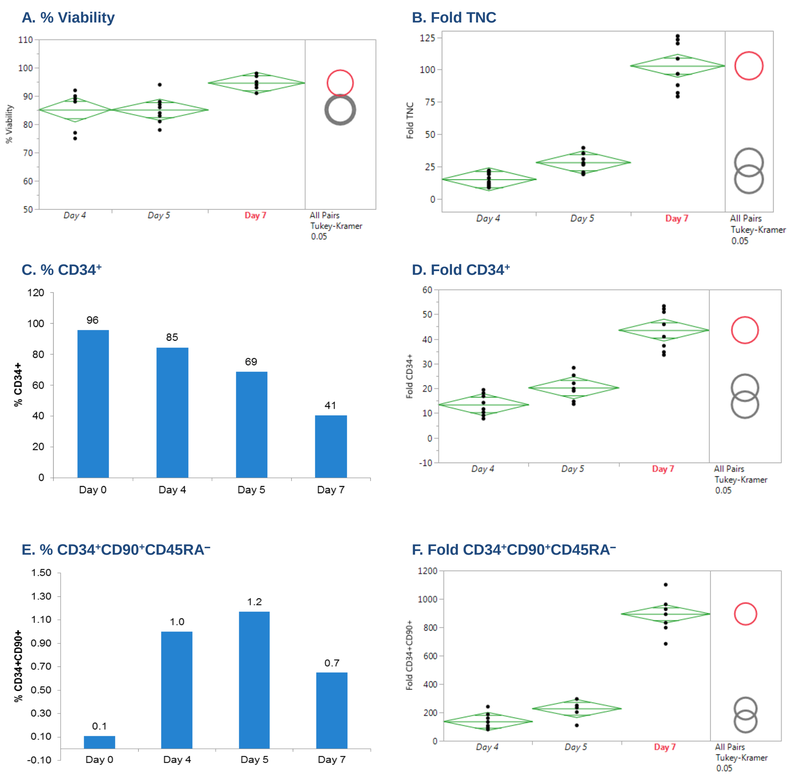


Figure 3: Representative results of current solutions demonstrated that the highest increase in HSPC was observable at 7 days post expansion. Viability of cells is highest at Day 7 (A). While the number of TNC increases with time (B), the % of $CD34^+$ decreases due to differentiation of HSPC (C). However, the overall number of $CD34^+$ was highest at Day 7 post-expansion (D). Although the highest frequency of $CD34^+CD90^+CD45RA^-$ cells was observed at Day 5 (E), the highest fold increase in the number of $CD34^+CD90^+CD45RA^-$ cells was observed at Day 7 (F).

Figure 4. Expansion of $CD34^+$ Umbilical Cord Blood with Our HSPC Expansion Medium at Day 7 post-expansion

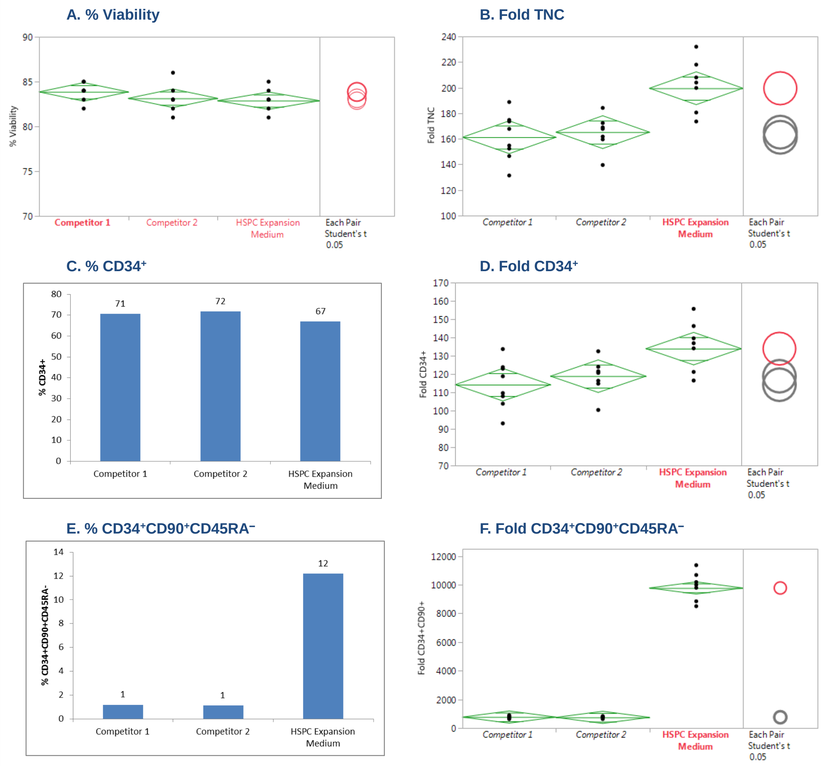


Figure 4: Representative performance of HSPC Expansion Medium when cultured with $CD34^+$ UCB lot of cells. 7 day expansion with our HSPC Expansion Medium resulted in high % viability, with the highest fold TNC, highest fold $CD34^+$, and more importantly significantly high fold expansion of long-term $CD34^+CD90^+CD45RA^-$ compared to available solutions.

Figure 5. Aldehyde dehydrogenase (ALDH) Assay to assess whether expanded cells are bona-fide stem cells

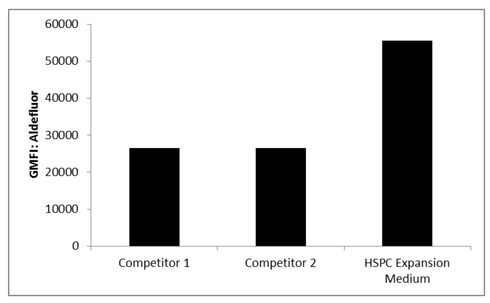


Figure 5: Our HSPC Expansion Medium had the highest ALDH expression as demonstrated by high ALDEFLUOR™ staining. This shows that HSPC expanded with our media system contain highest frequency of bona-fide stem cells.

CONCLUSIONS

- We successfully developed an HSPC Expansion Medium that expands both short term $CD34^+$ cells, and primitive long-term engraftable hematopoietic stem cells ($CD34^+CD90^+CD45RA^-$).

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TRADEMARKS/LICENSING

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