# EXPANSION OF HUMAN HEMATOPOIETIC STEM-PROGENITOR CELLS IN XENO-FREE SERUM-FREE STEMPRO™ HSC EXPANSION MEDIUM (PROTOTYPE) Thermo

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# ABSTRACT

Development of ex vivo culture systems to expand harvested human hematopoietic stem-progenitor cells (HSPCs) remains a critical translational research quest that would enhance clinical hematopoietic stem cell (HSCs) transplantation and gene therapies. To address the problem that HSCs generally die or differentiate rapidly in current ex vivo culture media, we developed a xeno-free, serum-free medium -- StemPro™ HSC Expansion Medium (Prototype) -- by extensive iterative modifications of medium constituents. Culture of primary human CD34+ cells immunopurified from healthy cord blood, mobilized peripheral blood and bone marrow in StemPro™ HSC Expansion Medium (Prototype) supplemented with FLT3L, KITL (also known as SCF), TPO, IL3, and IL6 (FKT36), resulted in massively increased numbers of immunophenotype-defined HSPCs, as compared to either uncultured day 0 cells or cells cultured in industry-standard culture media containing FKT36. For example, culture of primary human CD34+ cells from mobilized peripheral blood (mPB) for 7 days in FKT36-containing StemPro™ HSC Expansion Medium (Prototype) resulted in ~100-fold increased numbers of CD34+CD45+Lin- cells and ~2000-fold increased numbers of CD34+Lin-CD90+CD45RA- cells (an early HSPC immunophenotype), as compared to uncultured day 0 cells. The ex vivo-cultured CD34+ cells contained high frequencies of aldehyde dehydrogenase-containing cells and formed erythroid and non-erythroid hematopoietic colonies in vitro. In an ongoing in vivo hematopoietic chimera experiment, ex vivo-cultured mPB CD34+ HSPCs harbored robust in vivo-engrafting capacity at the 8-week post-transplant shortterm HSC time point evaluated to date. Thus, it appears that StemPro<sup>™</sup> HSC Expansion Medium (Prototype) supports HSPC expansion that includes selfrenewal of short-term and long-term HSC.

## RESULTS

Figure 1: Phenotypic Characterization of Expanded HSC Cultured in StemPro HSC Expansion Medium (Prototype).



**Figure 1:** Purified human CD34<sup>+</sup> mPB samples cultured for 7 days in StemPro HSC Expansion Medium (Prototype) were assessed by flow cytometry for expression of CD45, Lineage Negative (CD3, CD14, CD16, CD19, CD20, CD56), CD34, CD90, and CD45RA. Doublets and dead cells were excluded from analysis, and gates identifying CD34<sup>+</sup> cells and CD90<sup>+</sup>CD45RA<sup>-</sup> cells were demarcated based on fluorescent minus one (FMO) controls.

# Figure 2: StemPro HSC Expansion Medium (Prototype) provides a ~100-fold increase in CD34<sup>+</sup> cells and ~2000-fold increase in CD34<sup>+</sup>CD90<sup>+</sup>CD45RA<sup>-</sup> cells using mPB from donors tested.

#### Figure 4: StemPro HSC Expansion Medium (Prototype) Enables Reprogramming of CD34<sup>+</sup> cells into iPSC with CTS CytoTune 2.1





Figure 4: StemPro HSC Enables Reprogramming of CD34+ cells into iPSC with CTS CytoTune 2.1.

### 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<sup>+</sup> cells, at the expense of the most primitive multipotent long-term stem cells CD34<sup>+</sup>CD90<sup>+</sup>CD45RA<sup>-</sup> cells that provide life-long immunity.



# **MATERIALS AND METHODS**

Process Workflow for Expansion of CD34<sup>+</sup> cells with StemPro HSC Expansion Medium (Prototype)





**Figure 2:** Expansion of CD34<sup>+</sup> mPB samples in StemPro HSC Expansion Medium (Prototype) has been demonstrated to show significantly higher levels in expansion of (A) CD34<sup>+</sup> cells and (B) TNC, with cells demonstrating (C) > 80% viability. Expanded cells were (D) > 60% CD34<sup>+</sup>, with significantly higher levels of (E) percent CD34<sup>+</sup>CD90<sup>+</sup>CD45RA<sup>-</sup> and (F) fold-increase in CD34<sup>+</sup>CD90<sup>+</sup>CD45RA<sup>-</sup> long term HSC. Percentages of CD34<sup>+</sup> and CD34<sup>+</sup>CD90<sup>+</sup>CD45RA<sup>-</sup> shown are from the total live TNC population. Error bars denote standard deviation (A – C) and standard error (D – E).

Figure 3: CD34<sup>+</sup> cells Expanded in StemPro HSC Expansion Medium (Prototype) Engraft & Exhibit Multi-lineage Chimerism at 6 months post-transplant



#### Figure 5: StemPro HSC Expansion Medium (Prototype) Enables CRISPR-Cas9 Gene Editing of CD34<sup>+</sup> cells with Neon electroporation



Figure 5: StemPro HSC Enables CRISPR-Cas9 Gene Editing of CD34+ cells with Neon Electroporation.

# Figure 6: StemPro HSC Expansion Medium (Prototype) Enables ~2500 fold Expansion of Megakaryocyte & Erythroid Progenitors





**Cell Culture**. Three human single donor purified CD34<sup>+</sup> mobilized peripheral blood (mPB) samples were cultured in StemPro HSC Expansion Medium (Prototype) (StemPro<sup>™</sup> HSC Basal and 50X Supplement) and three commercial media, all supplemented with SCF, Flt3L, TPO, IL-3, and IL-6 (SFT36) cytokines. Cells were cultured for 7 days, following which total nucleated cells (TNC) and % viability were determined using a Countess II, and phenotype assessed using the Attune NxT flow cytometer.

**iPS Reprogramming of CD34+ cells with CTS CytoTune 2.1.** Single donor, cord-blood derived, CD34+ cells obtained were thawed and cultured in either OpTmizer™ containing SCF, IL-3, and GM-CSF; or StemPro™ HSC containing SCF, Flt3L, TPO, IL-3, and IL-6. During each day of culture, half the media was removed and replaced with fresh media containing cytokines. Cells were transduced with CTS™ CytoTune™ 2.1-iPS Sendai Reprogramming Kit at an MOI of 5-5-3 (KOS-LMyc-Klf4) in the presence of polybrene. Three days later, cells were plated onto rhVTN-N coated plates containing no cytokines. Seven days after transduction, half the media was removed and replaced with Essential 8™ Medium. The following day, and every day thereafter, cells were fed with Essential 8™ Medium. Sixteen days after transduction, reprogramming efficiency was determined by staining cells for Alkaline Phosphatase using the Vector Red Alkaline Phosphatase Substrate Kit. The number of AP positive colonies was counted, and reprogramming efficiency was determined relative to the number of cells plated on Day 3 after transduction.

**CRISPR-Cas9 Gene Editing of CD34+ cells with Neon Electroporation**. The donor DNA was prepared by PCR which contained a promoterless puro\_2A\_EmGFP cassette flanked by 35nt homology arm on each side. The gRNA targeting the n-terminus of ACTB locus (GCGGCGATATCATCATCCATGG) was synthesized. Single donor, cord-blood derived, CD34+ cells obtained from AllCells were thawed and cultured in StemPro HSC expansion media containing SCF, FLT3, TPO, IL3 and IL6 growth factors. For Neon electroporation, cells were washed then re-suspended in Resuspension Buffer R. To form the RNP (ribonucleoprotein) complexes, 1.2 µg TrueCut<sup>TM</sup> Cas9 Protein v2 and 300 ng gRNA was added to Resuspension Buffer R. Donor DNA and the cell suspension was added to the RNP complexes and then used for electroporation. The efficiency of inframe insertion of a promoterless puro\_2A\_EmGFP fragment into the n-terminus of ACTB locus was measured by Attune<sup>TM</sup> NxT Acoustic Focusing Cytometer at 72 hours post transfection. The percentages of EmGFP-positive cells, CD34-positive cells, and CD90-positive cells were determined. For the colony formation assay, the unstained cells and sorted cells were plated onto MethoCult<sup>TM</sup> medium at a cell density of 500 cells/ml medium in a 6-well plate. Upon incubation at 37° C, in 5% CO<sub>2</sub>, with  $\geq$  95% humidity for approximately 14 days, the number of white and red blood cell colonies were counted under the microscope.



**Figure 3:** LT-HSC-derived NRG engraftment capacity, assessed at 6 months post-transplant, was ~10-fold less on a per transplanted cell basis in cultured vs non-cultured cell populations; but there were 100-fold more total cultured cells. Therefore, the engraftment capacity of the entire 7d cultured cell population was 10-fold greater than that of the d0 population.

**Figure 6:** StemPro HSC Expansion Medium (Prototype) enables Megakaryocyte & Erythroid Progenitor expansion over a 7 day culture period.

# CONCLUSIONS

- We successfully developed xeno-free, serum-free StemPro™ HSC Expansion Medium (Prototype) that:
  - Expands short-term CD45<sup>+</sup>Lin<sup>-</sup>CD34<sup>+</sup> cells and long-term primitive longterm hematopoietic stem cells (CD45<sup>+</sup>Lin<sup>-</sup>CD34<sup>+</sup>CD90<sup>+</sup>CD45RA<sup>-</sup>).
  - Maintains long-term engraftment capacity, with cells exhibiting multi-lineage chimerism at 6 months post-transplant, with 10-fold greater engraftment capacity with StemPro HSC cultured cells versus non-cultured cells.
  - Enables iPSC reprogramming of expanded CD34<sup>+</sup> cells with CTS CytoTune 2.1
  - Enables CRISPR-Cas9 Gene Editing of CD34 + cells with Neon electroporation.
  - Enables expansion of Megakaryocyte & Erythroid Progenitors.

#### REFERENCES

- 1. Bhattacharya D, Ehrlich RI, Weissman IL. *European Journal of Immunology*, 2008 Aug; 38(8): 2060–2067.
- . Doulatov S, Notta F, Laurenti E, Dick JE. Hematopoiesis: a human perspective. *Cell Stem Cell*, 2012 Feb 3;10(2):120-36.
- Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science, 2011 Jul 8;333(6039):218-21.

#### ACKNOWLEDGEMENTS

We would like to thank Tracee Crossett, Anne Chen, Melissa Cross, Nicole McCarthy, and Konstantin Slavashevich for their assistance and helpful discussions.

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