BioResearch

Validation of Cytokines for the Chemically Defined Manufacturing of Human CD34 Positive Hematopoietic Precursor Cells

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Introduction

Human CD34 positive hematopoietic precursor cells (hHPC) have been successfully used over the years to treat disorders of the blood and immune system. Although hHPC can be easily isolated form cord blood, bone marrow and peripheral blood, their in vitro expansion is challenging. For instance, using current methodology hHPC tend to lose their pluripotency during in vitro expansion.

Therefore the identification of an optimal, ideally chemically defined culture medium for the scalable expansion of high-quality hHPC according to GMP standards is an important task during the development of hHPC-based autologous or allogeneic cell therapies. Chemically defined media performance robustness is highly dependent on the quality of recombinant cytokines used for serum replacement.

In order to find appropriate suppliers of high quality recombinant proteins for chemically defined hHPC medium, recombinant human Flt3-ligand, stem cell factor and thrombopoietin from five different commercial suppliers were tested in combination with chemically defined medium containing only human proteins.

Materials and Methods

Expansion cell culture: 20,000 CD34 positive hHPC (Lonza, 2M-101C) from three independent donors (Donor 1-3) were cultured in duplicate for seven days in 200 µl HPGM (Lonza, PT-3926) supplemented with different concentrations of recombinant human Flt3-ligand (rhFlt3), stem cell factor (rhSCF) and thrombopoietin (rhTPO) from five different suppliers (A-E) with one exception. rhFlt3 and rhSCF from supplier E were used in combination with rhTPO from supplier A. Viable cells were counted using a Cedex XS device (Roche).

Myeloid differentiation: After seven days of expansion cell culture, 25,000 cells were transferred into 200 µl myeloid differentiation medium and cultured for another seven days. HPGM supplemented with rhGM-CSF (10 ng/ml), rhG-CSF (10 ng/ml), rhIL-6 (10 ng/ml), rhIL-3 (10 ng/ml) and rhSCF (100 ng/ml) was used as myeloid differentiation medium.

Flow cytometry: Cells were stained according to standard procedures against the indicated marker proteins and analyzed by flow cytometry in the presence of 250 ng/ml propidium iodide and Calibrite APC Beads (BD) using a FACScalibur device (BD) equipped with two lasers and CellQuestPro Software (BD). Antibodies used were the following: mouse-anti-human CD3 (IgG1, APC coupled, BD, 555335), mouse-anti-human CD34 (IgG1, PE coupled, BD, 345802) and mouse-anti-human CD206 (IgG1, APC coupled, BD, 550889).

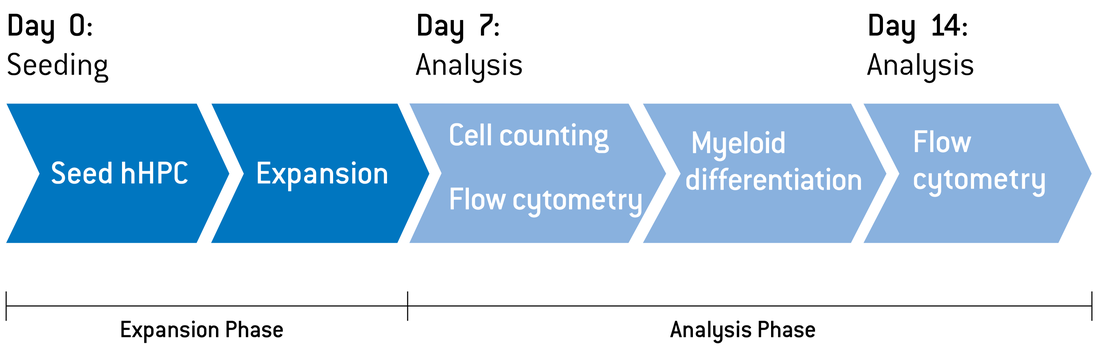

Figure 1. **Experimental set-up.** Details see Materials and Methods.

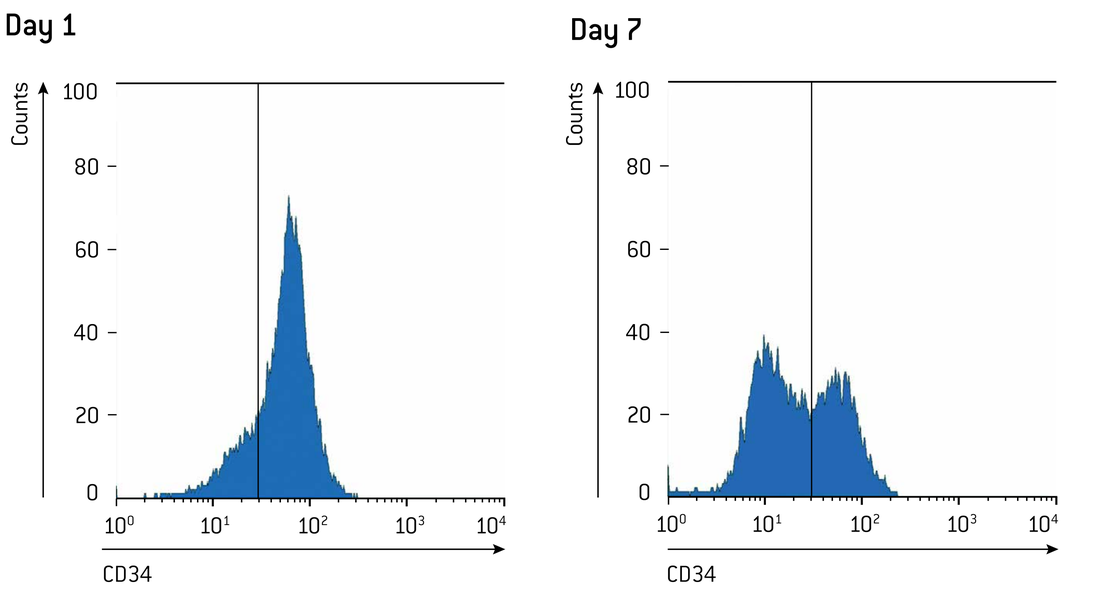

Figure 2. **Flow cytometric analysis of CD34 expression on hematopoietic precursor cells** that were cultured for one day or seven days in HPGM supplemented with the rhFlt3, rhSCF and rhTPO. Cells were stained against CD34 and analyzed using flow cytometry. Even though the total number of CD34 positive hHPC is increasing over time (see Figure 3) a decrease in the percentage of CD34 positive cells as well as a decrease in the mean fluorescence intensity is observed over seven days of culture.

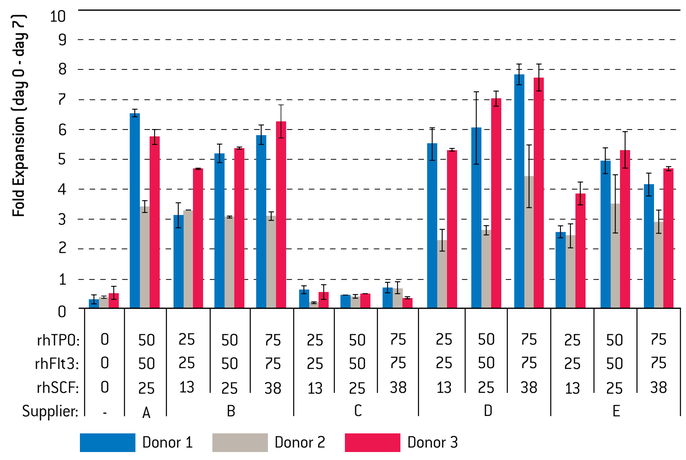

Figure 3. **Proliferation of CD34 positive hematopoietic precursor cells** in HPGM supplemented with the indicated concentrations of rhFlt3, rhSCF and rhTPO (ng/ml) from five different suppliers (A-E). The number of viable cells was counted on day seven and the percentage of CD34 positive cells was determined using flow cytometric analysis. The total number of viable CD34 positive cells was calculated and expressed as fold expansion during seven days of culture. (n = 2) An up to eightfold expansion of hHPC in cytokine supplemented HPGM was observed. Cell proliferation rate was highly donor dependent as well as dependent on the cytokines used. No expansion of CD34 positive hHPC was observed without cytokines or with cytokines from supplier C.

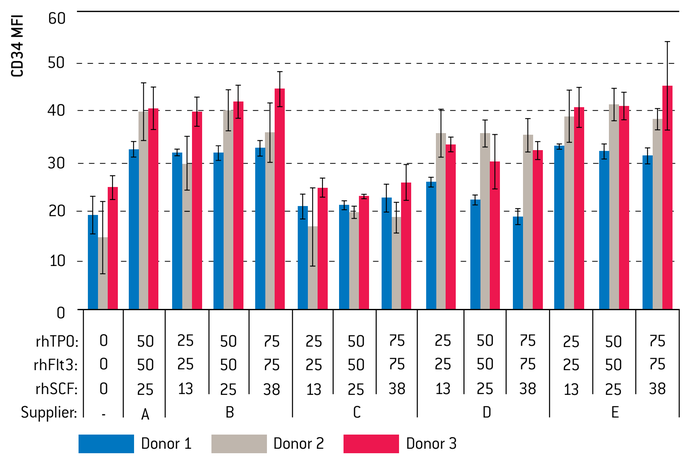

Figure 4. **CD34 expression on hematopoietic precursor cells** that were cultured for seven days in HPGM supplemented with the indicated concentrations of rhFlt3, rhSCF and rhTPO (ng/ml) from five different suppliers (A-E). Cells were stained against CD34 and analyzed using flow cytometry. Expression of CD34 is shown as mean fluorescence intensity (MFI; n=2). Lower MFI were observed in cells that were expanded in HPGM supplemented with no cytokines or cytokines from supplier C. Wu et al., 2001 could show that a decrease in the MFI of CD34 is paralleled by a loss in numbers of colony-forming units.

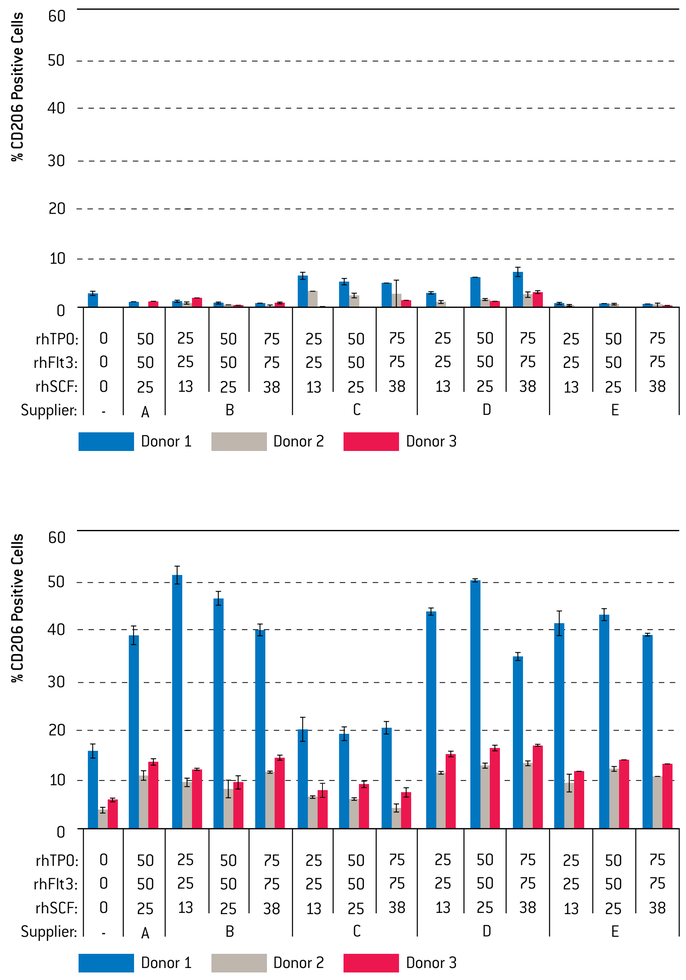

Figure 5. **Expression of CD206 on un-induced hematopoietic precursor cells** that were expanded for seven days in HPGM supplemented with the indicated concentrations of rhFlt3, rhSCF and rhTPO (ng/ml) from five different suppliers (A-E). Cells were stained against CD206 and analyzed using flow cytometry. The percentage of CD206 positive cells is shown (n=2). Slight up-regulation of the myeloid marker CD206 is observed in cells cultured with supplier C or D cytokines. Low expression of the lymphoid marker CD3 was observed during expansion culture (data not shown).

Figure 6. **Myeloid differentiation of hematopoietic precursor cells** that were expanded for seven days in HPGM supplemented with the indicated concentrations of rhFlt3, rhSCF and rhTPO (ng/ml) from five different suppliers (A-E) and subsequently induced toward myeloid defferentiation. Cells were transferred into myeloid differentiation medium and monitored on day 7 by staining against CD206 and analyzed using flow cytometry (n=2). The percentage of CD206 positive cells is shown. The pre-expanded cells were able to undergo myeloid differentiation, even though donor-to-donor variability was observed. Less myeloid differentiation was observed for cells pre-cultured without cytokines or cytokines from supplier C.

Summary

- Using chemically defined media an up to eightfold expansion of CD34 positive hHPC in chemically defined HPGM supplemented with rhFlt3, rhSCF and rhTPO was achieved. At least a substantial subpopulation of cells remains able to undergo myeloid differentiation after seven days of expansion.
- Some variability between different hHPC lots was observed likely due to donor-to-donor variation. Therefore cells from different donors have to be used during a media optimization process in order to develop a largely donor-independent process.
- The cytokines tested supported the proliferation of hHPC except cytokines from supplier C. Only small differences in the performance of the cytokines from suppliers A, B, D and E were detected with respect to cell proliferation and maintenance of differentiative ability after seven days of culture.
- For the selection of appropriate cytokine suppliers further factors will be taken into account, like the price per cytokine quantity required and the lot-to-lot variability of different cytokine batches from a single supplier.
- This report shows that qualifying suppliers of critical raw materials at the beginning of any cell manufacturing process development is essential for material quality and consistency of results.

References

MH Wu, SL Smith, GH Danet, AM Lin, SF Williams, DN Liebowitz and ME Dolan (2001) *Optimization of Culture Conditions to Enhance Transfection of Human CD34⁺ Cells by Electroporation*. Bone Marrow Transplantation 27, 1201–1209.

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