The monoculture of cord-blood-derived CD34+ cells by an automated, membrane-based dynamic perfusion system with a novel cytokine cocktail

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SUMMARY

Human leukocyte antigen (HLA)-matched cord blood (CB) transplantation is a procedure for the treatment of certain hematological malignancies, hemoglobinopathies, and autoimmune disorders. However, one of the challenges is to provide a sufficient number of T cell-depleted hematopoietic stem and progenitor cells. Currently, only 4%–5% of the CB units stored in CB banks contain enough CD34+ cells for engrafting 70-kg patients. To support this clinical need, we have developed an automated expansion protocol for CB-derived CD34+ cells in the Quantum system's dynamic perfusion bioreactor using a novel cytokine cocktail comprised of stem cell factor (SCF), thrombopoietin (TPO), fms-like tyrosine kinase 3 ligand (Flt-3L), interleukin-3 (IL-3), IL-6, glial cell line-derived neurotrophic factor (GDNF), StemRegenin 1 (SR-1), and a fibronectin-stromal-cell-derived factor-1 (SDF-1)-coated membrane. In an 8-day expansion of a 2×10^6 positively selected CD34+ cell inoculum from 3 donor lineages, the mean cell harvest and cell viability were 1.02×10^8 cells and 95.5%, respectively, and the mean frequency of the CD45+CD34+ immunophenotype was 54.3%. The mean differentiated cell frequencies were 0.5% for lymphocytes, 15.8% for neutrophils, and 15.4% for platelets. These results demonstrate that the automated monoculture protocol can support the expansion of CD34+ cells with minimal lymphocyte residual.

INTRODUCTION

Human leukocyte antigen (HLA)-8-allele-matched cord blood (CB) transplantation is an allogeneic procedure for the treatment of certain hematological malignancies, hemoglobinopathies, and autoimmune disorders (Horan et al., 2012; Gragert et al., 2014; Barker et al., 2020; Shpall and Rezvani, 2021). CB-derived CD34+ stem cells and progenitor cells are typically selected for hematopoietic reconstitution as a result of their increased capacity for self-renewal and proliferation, longer telomeres, and lower incidence of graft-versus-host disease (GVHD) through a lower frequency of alloreactive T cells along with their ability to achieve rapid engraftment in hematological transplant recipients (Gupta and Wagner, 2020). However, one of the challenges in this setting is to provide a sufficient number of T cell-depleted hematopoietic stem and progenitor cells, which are necessary to support mixed allogeneic hematopoietic stem cell transplantation (HSCT) (Diaz et al., 2021; Singh et al., 2019). Only about 4%–5% of the CB units stored in CB banks contain a sufficient number of CD34+ cells for single-unit grafts ($\geq 1.05 \times 10^7$ CD34+ cells) or for double-unit grafts ($\geq 1.40 \times 10^7$ CD34+ cells) for 70-kg patients (Politikos et al., 2020; Cohen et al., 2020; Barker et al., 2019).

Methods to expand CB-derived CD34+ cells, in either co-culture with mesenchymal stromal cells (MSCs) or with small molecules in combination with various cytokine supplements, frequently rely on inoculums of 4–6×10^6 or more CD34+ cells from CB units (CBUs) (Barker et al., 2019; Corselli et al., 2013; Horwitz et al., 2014). In order to maximize the available inventory of stored CBUs, we have developed a monoculture expansion protocol for low initial seeding of 2×10^6 preselected CB-derived CD34+ cells in the Quantum Cell Expansion System's perfusion-based, 2-chambered, semi-permeable hollow fiber membrane (HFM) bioreactor. All components of a primary cytokine cocktail comprised of recombinant human stem cell factor (SCF), thrombopoietin (TPO), fms-like tyrosine kinase 3 ligand (Flt3L), interleukin-3 (IL-3), and IL-6 were used at one-tenth of the manufacturer's recommended concentration (Lin et al., 2020). This cytokine cocktail was further supplemented with recombinant human glial-cell-line-derived neurotrophic factor (GDNF) to maintain cell viability and combined with the aryl hydrocarbon receptor to upregulate the expression of the anti-apoptotic gene *BCL2* in human CB-CD34+ progenitors and to limit HSC differentiation during CD34+ cell expansion, respectively, when implemented with other HSC cytokines (Boitano et al., 2010; Fonseca-Pereira et al., 2014). The proximity of MSCs and hematopoietic stem and progenitor cells (HSPCs) in the bone marrow sinusoids, coupled with the perivascular support of HSPCs by SCF from CD146+ MSCs, are part of the rationale for their inclusion in hematopoietic co-culture processes (Corselli et al., 2013; Ehninger and Trumpp, 2011; Morrison and Scadden, 2014). However attractive, the co-culture of MSCs and HSPCs adds complexity, time, and potential variability to the stem cell and progenitor expansion process (Hatami et al., 2014). Even so, MSC and HSPC co-culture can serve

to point toward alternative production strategies in CB-derived CD34^{+} cells (Sieber et al., 2018). Automating the hematopoietic cell and progenitor expansion process is central to providing a dependable quantity of selected cells for therapeutic indications. Moreover, the Quantum system (Terumo Blood and Cell Technologies, Lakewood, CO, USA) has a history of supporting the expansion of both adherent MSCs as well as suspension CD3^{+} T cells and regulatory T cells with a perfusion-based HFM bioreactor (Jones et al., 2013; Hanley et al., 2014; Nankervis et al., 2018; Jones et al., 2020; Garcia-Aponte et al., 2021; Cunningham et al., 2022). In our CB-derived CD34^{+} cell expansion method, the intracapillary (IC) HFM lumen of the bioreactor is coated with a mixture of human fibronectin (Fn) and the chemokine stromal cell-derived factor 1 (SDF-1) prior to cell seeding in order to mimic the stimulatory and homing effects of bone-marrow-derived or Wharton's Jelly-derived MSCs (Lapidot and Kollet, 2002). The preselected CB-derived CD34^{+} cells were subsequently propagated under suspension culture conditions, but they were also allowed to adhere to the coated HFM IC surface during this process in order to engage with the Fn-SDF-1-modified surface. Studies by Peled et al. have shown that the immobilized SDF-1 is required to develop integrin-mediated cell adhesion of CD34^{+} cells by VLA-4 integrin to murine endothelial cells (Peled et al., 1999). In this context, Cuchaira et al. have also shown that hydrogel immobilization of SCF and SDF1α, along with the incorporation of the arginine-glycine-aspartic acid (RGD) integrin recognition sequence, onto the cell culture surface recapitulates certain aspects of the bone marrow microenvironment (Mendez-Ferrer et al., 2010; Cuchiara et al., 2013). These underlying studies led us to hypothesize that expanding CB-derived CD34^{+} cells with a modified extracellular matrix protein could be feasible.

Here, we report on the automated monoculture expansion of CB-derived HSCs and progenitor cells beginning with mixed, positively selected CB-derived CD34^{+} cells. These cells were resuspended in serum-free medium and supplemented with a defined hematopoietic cytokine cocktail and expanded under a programmed, but modifiable, perfusion protocol for a period of 8 days in order to minimize T cell differentiation in the Fn-SDF-1-coated HFM bioreactor system (Lang et al., 2011; Schaniel et al., 2021). Quantum-system-expanded CB-derived CD34^{+} cells generated a sufficient quantity of cells to support both single- and double-unit minimal CD34^{+} dose equivalency while conserving the CD34^{+} phenotype with a minimal frequency of lymphocytes. Furthermore, these CB-derived expanded progenitor cells demonstrated their ability to differentiate into mature hematopoietic colony-forming units (CFUs) under methylcellulose assay conditions.

RESULTS AND DISCUSSION

Three master lots of CB-derived, preselected, mixed CD34^{+} cells were expanded in a 2.1 m^2 HFM bioreactor with a 124 mL culture volume and harvested using an automated suspension cell protocol. Cells were introduced into the IC side of the HFM bioreactor through a defined perfusion protocol and maintained within the lumen of the bioreactor with a custom counter-flow fluidics program.

CB-CD34^{+} cell expansion
Based an initial cell seeding of thawed 2.0×10^6 CD34^{+} cells, the mean harvest yield of CD34^{+} cells was 1.02×10^8 cells (range: 4.02×10^7–1.61×10^8 cells) with a mean cell viability by trypan blue exclusion of 95.5% (range: 93.3%–96.8%) as enumerated by Vi-CELL XR cell analyzer over the course of the 8-day expansion period (Figures 1A and 1B). The cell expansion yield of 4.0×10^7–1.6×10^8 cells exceeds the minimum CD34^{+} cell dose of 1.5×10^5 cells/kg for a single-unit graft and a 1.0×10^5 cells/kg for double-unit graft, which translates into minimum doses of 1.1×10^7 CD34^{+} cells and 1.4×10^7 CD34^{+} cells, respectively, for a 70-kg patient (Politikos et al., 2020). In the automated expansion of the three (3) CB-derived CD34^{+} cell lineages, the mean cell population doubling was 5.4 (range: 4.3–6.3), the mean cell population doubling time was 34.9 h, and the mean-fold increase was 51.0-fold (range: 20.1- to 80.5-fold); (Figure 1). The IC medium input perfusion flow rates were adjusted in response to glucose and lactate metabolite levels and ranged from 0.1 to 0.2 mL/min (Figure 1D). To put the results of this cell expansion protocol into perspective, Barker et al. have shown, in an audit of US public CB banks, that a median CBU contains about 4.4×10^6 CD34^{+} cells up to a maximum of about 2×10^7 CD34^{+} cells (Barker et al., 2019). These data suggest that average expansion yields from a single CBU, which are on the order of 2.2×10^8 to 1.0×10^9 CB-derived stem or progenitor cells, are potentially feasible with an automated 8-day monoculture cell expansion protocol by simply increasing the cell inoculum from 2×10^6 cells up to 4.4×10^6–2×10^7 cells with a full CBU CD34^{+} cell fraction. This would have the effect of increasing the cell seeding density from 1.6×10^4 to 3.6×10^4–1.6×10^5 cells/mL in the perfusion bioreactor and entertain the possibility of a shorter expansion time frame, which could reduce the potential for cell differentiation (Ahrens et al., 2011; Liedtke et al., 2021). In comparing the CD34^{+} harvests after 8-day expansion with the precryopreservation technique across various UCB donors, these data also suggest a relationship between expansion yields and precryopreservation cell viability, which ranged from 84% to 98% (Figure 1C). Overcoming a broad range of precryopreservation cell viability is central to the robust expansion of HSC progenitor cells for
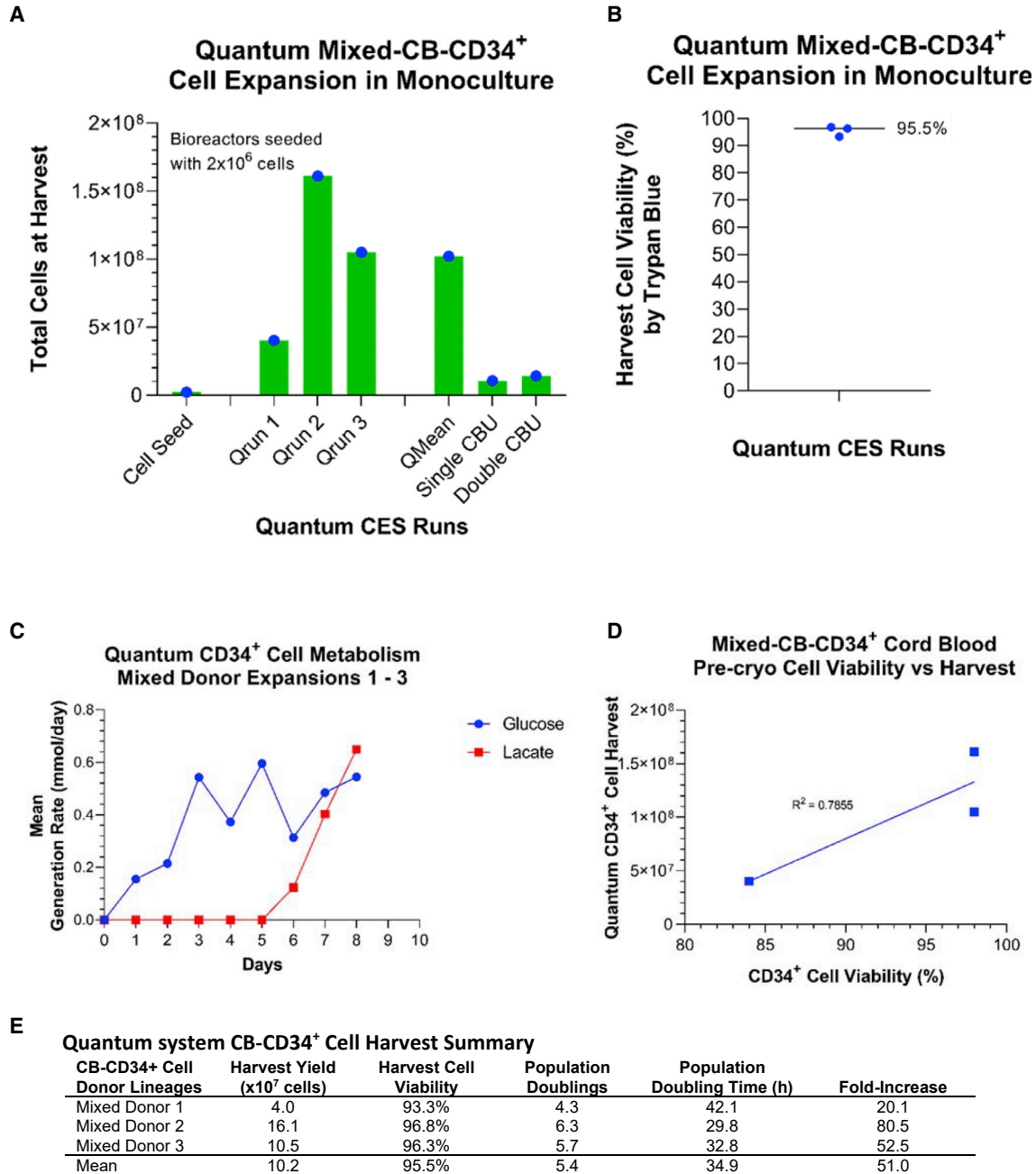
Figure 1. Quantum system CB-derived mixed CD34⁺ cell expansion results

(A) CD34⁺ cell harvest yield from three different donor cell lineages after 8 days of monoculture compared with the minimum single and double CBU CD34⁺ cell dosing guidelines for a 70-kg patient.

(B) Harvest cell viability by trypan blue dye exclusion.

(C) Correlation of precryopreservation cell viability with CB-derived CD34⁺ cell harvest yield. Pearson's correlation coefficient of $R^2 = 0.8863$.

(D) Mean of CD34⁺ normalized glucose consumption rate (mmol/day) and lactate generation rate (mmol/day).

(E) Quantum system CB-CD34⁺ cell harvest summary table.

These results are from 3 independent cell expansion experiments, which are based on 3 separate pooled CB-derived CD34⁺ cell lineages. Each donor cell harvest and cell viability by trypan blue value is the mean of 50 replicate measurements per expansion sample as acquired by a Beckman Coulter Vi-CELL XR Cell Analyzer.

engraftment (Lee et al., 2008). Monitoring the glycolytic metabolism shows that the glucose consumption rate ranged from 0 to a high of 0.596 mmol/day on day 5 and that the lactate generate rate ranged from 0 to a high of 0.650 mmol/day on day 8 (Figure 1D). The difference in peak days for these two metabolites can be attributed to media flow rate adjustments, differential expression of enzymes controlling glycolytic flux, and the demand for biosynthetic metabolites during cell expansion (Tanner et al., 2018).

CB-CD34+ cell immunophenotyping

Over the three (3) CB-derived donor cell lineages, flow cytometry revealed the mean frequency of the CD45+CD34+ immunophenotype to be 54.3% (range: 51.9%–57.9%) and the mean frequency of the more primitive CD133+CD38− immunophenotype to be 31.8% (range: 25.9%–39.0%) at harvest on day 8 of automated culture (Figure 2A) (Radtke et al., 2015). These results compare favorably with other CD34+ cell 7-day expansion protocols using StemRegenin 1 (SR-1) (CD34+ cells 10%–25%) media as shown by Tao et al. and 21-day CD34+CD38− expansion protocols using nicotinamide (CD34+ cells 0.2–4.4%) media as shown by Peled et al. in UCB-derived cell culture (Tao et al. 2017; Peled et al. 2012). Upon further analysis, the mean frequency of the differentiated cell lineages was 0.5% for lymphocytes (CD3+, CD19+, CD56+), 27.7% for neutrophils (CD34+CD15+), and 26.5% for platelets (CD34+CD41a+). The fact that biomarkers for both neutrophils and platelets are present in an expanded CB-derived CD34+ cell population can be attributed, in part, to the cytokine composition of the expansion media, which contains the interleukins IL-3 and IL-6. Although used to support CD34+ cell expansion, both cytokines have also been implicated in the development of myeloid cell lineages (Koller et al., 1993). In our study, the CD34+CD15+ immunophenotype defines immature neutrophils, which is consistent with the identification strategy used by E. Dick et al. in their study of *ex-vivo*-expanded bone-marrow-derived neutrophils (Dick et al., 2008). This generates an overall CD34+C15+ immature neutrophil frequency of 15.8% in our expanded progenitor cell population (Figure 2B). In addition, our identification of CD34-derived platelets is analogous to previous work by R. Feng et al., which has shown that CD34+CD41a+ platelets are dual biomarkers that predict platelet recovery after autologous blood stem cell transplantation (Feng et al., 1998). With this definition, our results generate an overall CD34+CD41a+ platelet immunophenotype frequency of 15.4% in the expanded CB-derived progenitor cell population (Figure 2B).

In vitro CB-CD34+ clonal differentiation

The MethoCult CD34+ cell differentiation assay of harvested cells generated hematopoietic progenitor lineages of granulocyte-erythroid-macrophage-megakaryocyte (GEMM), granulocyte-macrophage (GM), and burst-forming-unit erythroid (BFU-E) CFUs. After 14 days of methylcellulose-based cell culture, the CB-derived CD34+ cell differentiated CFUs averaged 56% for the GM, 23% for GEMM, and 21% for BFU-E progenitor lineages of the total CFUs across the three (3) expanded CB-derived CD34+ cell lines (Figure 3A and 3B). These CFU results are comparable to prior studies with methylcellulose H4034 cytokine differentiation of electroporated, genetically unmodified CB-derived CD34+ cells by Chicaybam et al., where the majority of the lineages were GM-CFU (60%) clones followed by BFU-E (36%) and GEMM-CFU (10%) clones, and by Pavel-Dinu et al., where the majority of both the genetically modified and unmodified clones were also GM-CFU (60%) followed by BFU-E (18–20%) and GEMM-CFU (5%) clones (Chicaybam et al., 2016; Pavel-Dinu et al., 2019). The differences in the relative distribution of the CFU clones among these studies can be attributed to variations in the donor CBU cell sources, stem cell selection methods, and the cytokine cocktail formulations used in the expansion of the CB-derived CD34+ cells prior to differentiation (Rheaume et al., 2020). In as much as we expanded positively selected CD34+ mixed donor cell populations in a perfusion system as a proof of concept and myeloid differentiation of CD34+ cells being dependent on GM-CSF and G-CSF, we only detected granulocyte, macrophage (myeloid), or GEMM/GM colonies in the differentiation of CB-derived CD34+ cells where the stimulatory medium contained recombinant human (rh)-GM-CSF and rh-G-CSF (Stec et al., 2012; D'Aveni et al., 2015). These monocyte growth factors were not present in the media used to expand CB-derived CD34+ cells. However, the clonal results do suggest that the monocyte lineage should be present under differentiation conditions since we have observed GEMM colonies in the presence of the hematopoietic agents G-CSF and GM-CSF.

Conclusion

Various strategies for cell processing and expansion of CB-derived CD34+ have been proposed to reduce the incidence of GVHD and to increase the cell dose for engraftment in HSCT (Diaz et al., 2021; Lang et al., 2014). More recent protocols involve both the selection of CD34+ cells and the retention of CD34− fraction, which may contain immune modulatory cell types for co- or sequential administration (Cohen et al., 2020). In our study, we chose to expand 2×10^6 preselected CB-derived CD34+ cells, or the equivalent of one-third to one-half of a CBU CD34+ cell fraction, under novel cytokine conditions, which include Fn-immobilized SDF-1 and cell culture medium containing the requisite soluble SCF, TPO, Flt-3L, IL-3, and IL-6 cocktail at 10% of the manufacturer's recommendations with the addition of SR-1 and GDNF in an automated perfusion HFM bioreactor
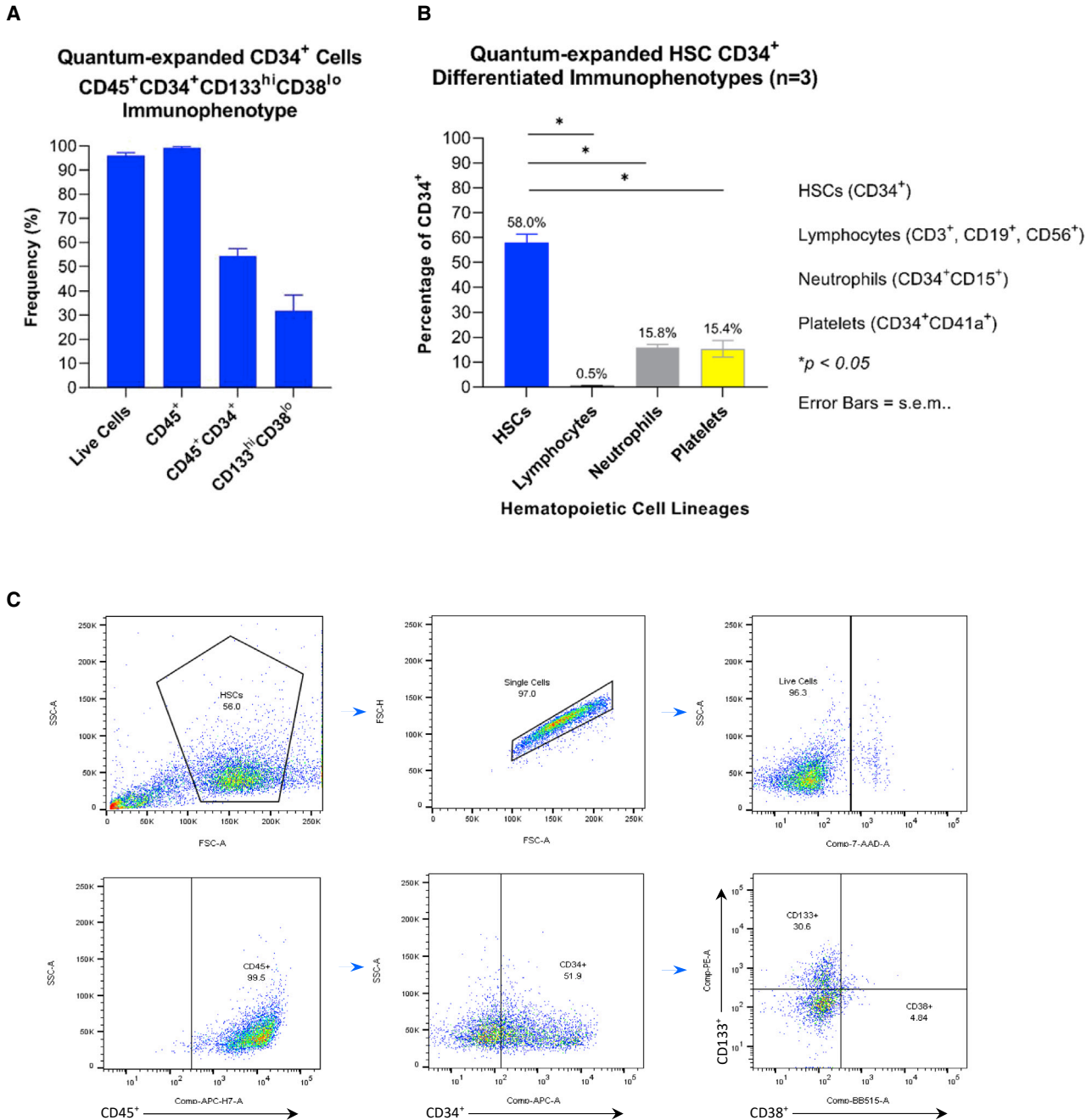
Figure 2. Immunophenotype frequency of Quantum-expanded CB-derived CD34⁺ cells at harvest

(A) Mean frequency of HSC immunophenotype displayed in blue bar chart with error bars based on SEM.

(B) The immunophenotype frequency of differentiated hematopoietic cell lineages is presented using representative biomarkers: HSCs (CD34⁺), lymphocytes (CD3⁺, CD19⁺, CD56⁺), neutrophils (CD34⁺CD15⁺), platelets (CD34⁺CD41a⁺), error bars based on SEM, p < 0.05.

(C) The FMO gating strategy (FSC-A versus SSC-A → singlets FSC-H versus FSC-A → live cells SSC-A versus & 7-AAD-A → SSC-A versus CD45-APC-H7 → SSC-A versus CD34-APC → CD133-PE versus CD38-BB515) (C) was verified with Streck CD-Chex-CD34 Level 3 peripheral blood reference standard.

The immunophenotype results are based on 3 independent cell expansion experiments with 10,000 events acquired per sample.

A Quantum CD34⁺ Differentiated Cells MethoCult CFUs

GEMM
CFU-granulocyte, erythroid, macrophage, megakaryocyte

GM
CFU-granulocyte, macrophage

BFU-E
Burst-forming unit-erythroid

E
CFU-erythroid

SCT H4034 Optimum Media Formulation
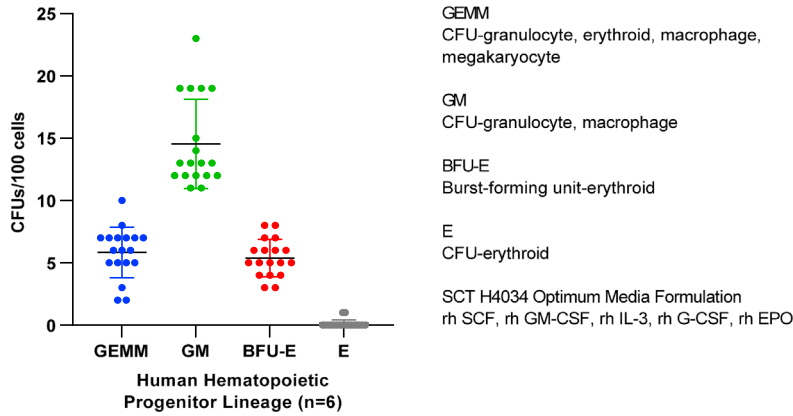rh SCF, rh GM-CSF, rh IL-3, rh G-CSF, rh EPO

Figure 3. Methocult assay of CB-derived human CD34⁺ cells

(A) Differentiated colony-forming units (CFUs) from Quantum-expanded CD34⁺ cells 14 days post-harvest. The mean CFU values for each progenitor colony type are based on 6 replicates across each pooled donor cell line over 3 independent cell expansion experiments. Error bars based on standard deviation.

(B) Representative images of CFU-granulocyte, erythroid, macrophage, megakaryocyte, CFU-granulocyte and macrophage, and BFU-erythroid lineages. Scale bar: 200 μm.

**B   Colonies Derived from Human Hematopoietic Progenitors**
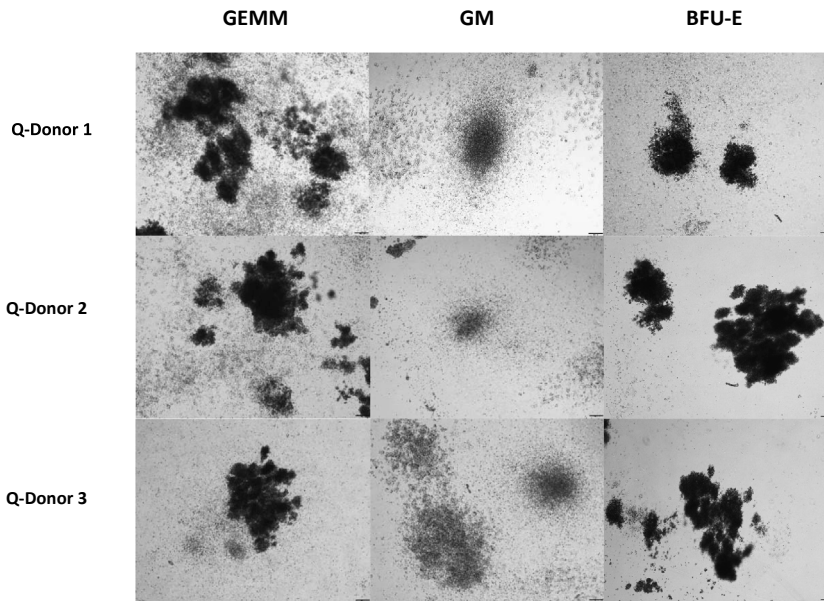
system (Figures 4A and 4B). The perfusion of the media across the HFM permits the concentration of both medium and large molecular weight molecules during the automated 8-day expansion of CD34⁺ cells. The purpose of this cytokine composition and perfusion protocol is to recreate the simulatory microenvironment of the bone marrow sinusoids with the goal of generating conditions, which are conducive to the expansion of both CD45⁺CD34⁺ hematopoietic stem cells and CD133⁺CD38⁻ progenitor cells. *In vitro* functionality of the expanded CD34⁺ cells was confirmed by clonal differentiation in GM-CFU, GEMM-CFU, and BFU-E assays.

These results, taken as a whole, demonstrate that the Quantum system perfusion monoculture protocol can support the cGMP expansion of preselected CB-derived CD34⁺ cells for both single and double CBU dose equivalency of an average of 1.02×10^8 cells with minimal lymphocyte residual and harvest cell viability on the order of 95% while employing a reduced seeding density for cell expansion. In addition, this automated CD34⁺ cell protocol provides a manufacturing strategy to broaden the available CBUs in CB banks, which can be considered for HSCT development.
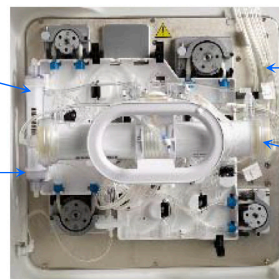
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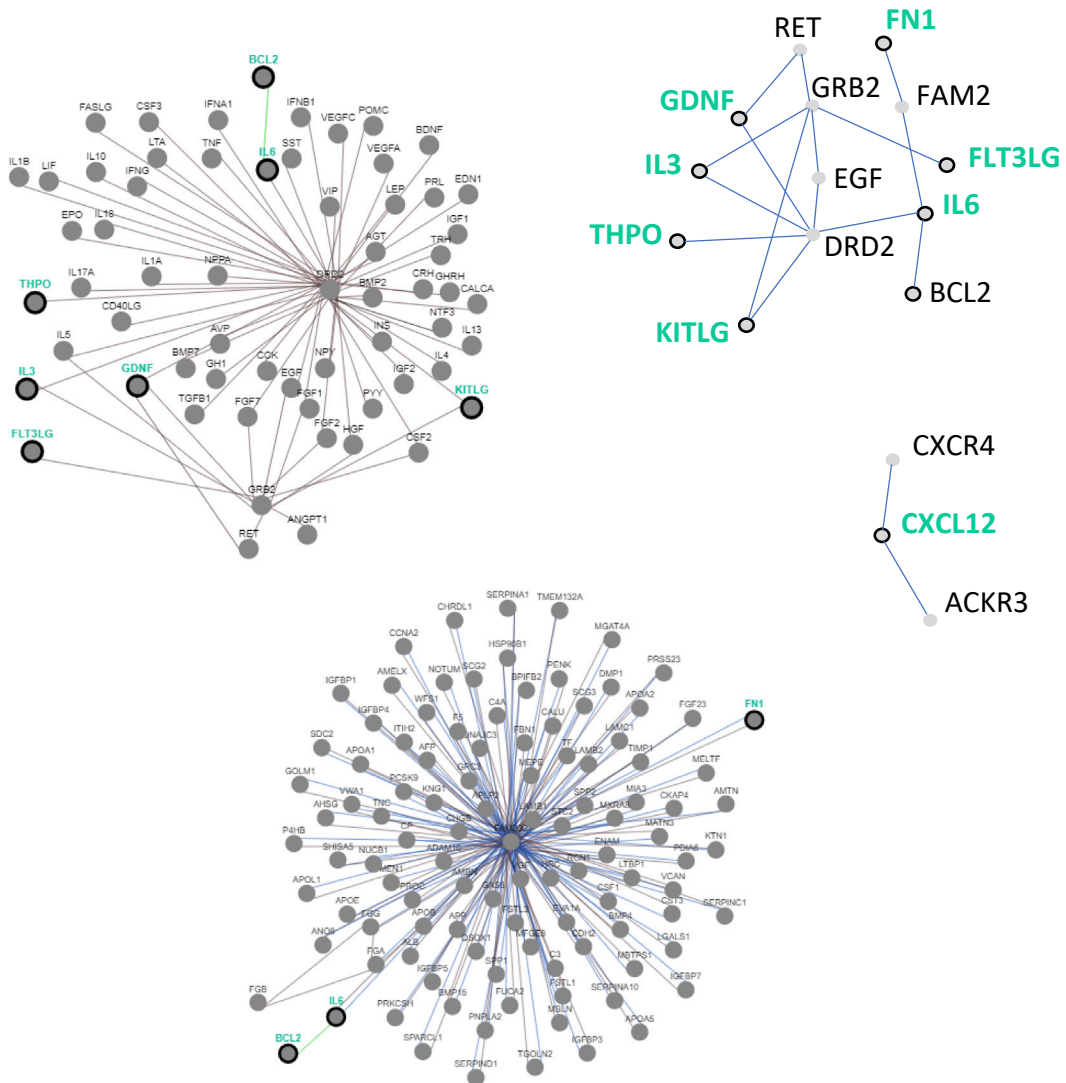
B

Quantum Disposable Set

Gas Transfer Module (GTM)

Gas Inlet

Inlet and Outlet Lines

Hollow-fiber Bioreactor

EXPERIMENTAL PROCEDURES

CB-derived CD34$^+$ cell selection and expansion

Human, mixed, CB-derived (CB) CD34$^+$ cells were purchased from STEMCELL Technologies (Vancouver, BC, Canada). The vendor had previously isolated CD34$^+$ cells from the CB of healthy donors under the auspices of an Institutional Review Board (IRB) approval or other regulatory authority-approved consent protocols. The cells were isolated using a Ficoll preparation of mononuclear cells and subsequent StemSep positive immunomagnetic selection, which involves the depletion of undesirable MNCs, red blood cells, lymphocytes, and platelets. Approximately 2×10^6 CB-derived CD34$^+$ cells (2 mL) were thawed at 37°C in a water bath and washed in 23 mL and resuspended in 50 mL of serum-free GMP SCGM medium (CellGenix GmbH, Freiburg, Germany) supplemented with StemSpan CD34 Supplement 10X (STEMCELL Technologies), which contains rh Flt3l, SCF, TPO, IL-3, and IL-6 at a concentration of 1% by volume, GDNF at 10 ng/mL (R&D Systems, Minneapolis, MN, USA), SR-1 at 0.75 μM (STEMCELL Technologies), and PSN antibiotic mixture 100X at 1% by volume (Thermo Fisher Scientific, Waltham, MA, USA) in complete medium. Base medium was formulated with serum-free GMP SCGM supplemented with 0.75 μM SR-1 and PSN antibiotic mixture 100X at 1% by volume as previously described.

Prior to seeding the CD34$^+$ cell inoculum, the Quantum system HFM bioreactor (Terumo Blood and Cell Technologies) was coated overnight with a mixture of 5 mg human plasma-derived Fn or 0.23–0.24 μg/cm^2 (Corning Life Sciences, Corning, NY, USA) and recombinant human stromal-cell-derived factor 1 (SDF-1) at 0.075 ng/cm^2 (R&D Systems, Minneapolis, MN, USA) in 100 mL PBS without (w/o) Ca^{2+}-Mg^{2+} (Lonza Group, Walkersville, MD, USA) at a temperature of 37°C and mixed gas (5%CO$_2$, 20%O$_2$, balance N$_2$). CB-derived CD34$^+$ cells were seeded in suspension into the coated HFM bioreactor in 50 mL complete medium after IC (complete) medium and EC (base) medium exchange/conditioning, expanded in monoculture, and harvested on day 8 of cell culture using the Quantum system automated tasks as outlined in Tables S1–S3. Default tasks were used for the Quantum system priming, IC media/EC media exchange, and media conditioning tasks. In-process glucose and lactate levels were monitored by i-STAT Analyzer G and CG4+ cartridges (Abbott Point-of-Care, Princeton, NJ, USA). During cell expansion, the Quantum system IC and EC inlet flow rates were adjusted in response to the glucose consumption and lactate generation rates and the nature of the automated task.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.stemcr.2022.10.006.

REFERENCES

Ahrens, I., Domeij, H., Topcic, D., Haviv, I., Merivirta, R.M., Agrotis, A., Leitner, E., Jowett, J.B., Bode, C., Lappas, M., and Peter, K. (2011). Successful in vitro expansion and differentiation of cord blood derived CD34+ cells into early endothelial progenitor cells reveals highly differential gene expression. PLoS One 6, e23210. https://doi.org/10.1371/journal.pone.0023210.

Barker, J.N., Kempenich, J., Kurtzberg, J., Brunstein, C.G., Delaney, C., Milano, F., Politikos, I., Shpall, E.J., Scaradavou, A., and Dehn, J. (2019). CD34(+) cell content of 126 341 cord blood units in the US inventory: implications for transplantation and banking. Blood Adv. 3, 1267–1271. https://doi.org/10.1182/bloodadvances.2018029157.

Barker, J.N., Mazis, C.M., Devlin, S.M., Davis, E., Maloy, M.A., Naputo, K., Nhaissi, M., Wells, D., Scaradavou, A., and Politikos, I. (2020). Evaluation of cord blood total nucleated and CD34(+) cell content, cell dose, and 8-allele HLA match by patient Ancestry. Biol. Blood Marrow Transplant. 26, 734–744. https://doi.org/10.1016/j.bbmt.2019.11.017.

Boitano, A.E., Wang, J., Romeo, R., Bouchez, L.C., Parker, A.E., Sutton, S.E., Walker, J.R., Flaveny, C.A., Perdew, G.H., Denison, M.S., et al. (2010). Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science 329, 1345–1348. https://doi.org/10.1126/science.1191536.

Chicaybam, L., Barcelos, C., Peixoto, B., Carneiro, M., Limia, C.G., Redondo, P., Lira, C., Paraguassú-Braga, F., Vasconcelos, Z.F.M.D., Barros, L., and Bonamino, M.H. (2016). An efficient electroporation

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Figure 4. Cytokine interaction bioinformatics and Quantum system

(A) Proposed cytokine gene product relationships derived from www.pathwaycommons/pcviz/ network visualizer detailing the interaction of stem cell factor (KITLG), thrombopoietin (THPO), fms-related tyrosine kinase 3 ligand (FLT3LG), interleukin-3 (IL-3), IL-6, glial cell derived neurotrophic factor (GDNF), stem-cell-derived factor 1 (CXCL12), and fibronectin (FN1) in relation the anti-apoptotic gene Bcl-2 are summarized.

(B) Quantum Cell Expansion System with hollow fiber membrane bioreactor disposable set.

protocol for the genetic modification of mammalian cells. Front. Bioeng. Biotechnol. *4*, 99. https://doi.org/10.3389/fbioe.2016.00099.

Cohen, S., Roy, J., Lachance, S., Delisle, J.S., Marinier, A., Busque, L., Roy, D.C., Barabé, F., Ahmad, I., Bambace, N., et al. (2020). Hematopoietic stem cell transplantation using single UM171-expanded cord blood: a single-arm, phase 1-2 safety and feasibility study. Lancet Haematol. *7*, e134–e145. https://doi.org/10.1016/S2352-3026(19)30202-9.

Corselli, M., Chin, C.J., Parekh, C., Sahaghian, A., Wang, W., Ge, S., Evseenko, D., Wang, X., Montelatici, E., Lazzari, L., et al. (2013). Perivascular support of human hematopoietic stem/progenitor cells. Blood *121*, 2891–2901. https://doi.org/10.1182/blood-2012-08-451864.

Cuchiara, M.L., Horter, K.L., Banda, O.A., and West, J.L. (2013). Covalent immobilization of stem cell factor and stromal derived factor 1alpha for in vitro culture of hematopoietic progenitor cells. Acta Biomater. *9*, 9258–9269. https://doi.org/10.1016/j.actbio.2013.08.012.

Cunningham, A.W., Jones, M., Frank, N., Sethi, D., and Miller, M.M. (2022). Stem-like memory T cells are generated during hollow fiber perfusion-based expansion and enriched after cyropreservation in an automated modular cell therapy manufacturing process. Cytotherapy *24*, 1148–1157. https://doi.org/10.1016/j.jcyt.2022.07.009.

D'Aveni, M., Rossignol, J., Coman, T., Sivakumaran, S., Henderson, S., Manzo, T., Santos e Sousa, P., Bruneau, J., Fouquet, G., Zavala, F., et al. (2015). G-CSF mobilizes CD34+ regulatory monocytes that inhibit graft-versus-host disease. Sci. Transl. Med. *7*, 281ra42. https://doi.org/10.1126/scitranslmed.3010435.

Diaz, M.A., Gasior, M., Molina, B., Pérez-Martínez, A., and González-Vicent, M. (2021). Ex-vivo" T-cell depletion in allogeneic hematopoietic stem cell transplantation: new clinical approaches for old challenges. Eur. J. Haematol. *107*, 38–47. https://doi.org/10.1111/ejh.13636.

Dick, E.P., Prince, L.R., and Sabroe, I. (2008). Ex vivo-expanded bone marrow CD34+ derived neutrophils have limited bactericidal ability. Stem Cell. *26*, 2552–2563. https://doi.org/10.1634/stemcells.2008-0328.

Ehninger, A., and Trumpp, A. (2011). The bone marrow stem cell niche grows up: mesenchymal stem cells and macrophages move in. J. Exp. Med. *208*, 421–428. https://doi.org/10.1084/jem.20110132.

Feng, R., Shimazaki, C., Inaba, T., Takahashi, R., Hirai, H., Kikuta, T., Sumikuma, T., Yamagata, N., Ashihara, E., Fujita, N., and Nakagawa, M. (1998). CD34+/CD41a+ cells best predict platelet recovery after autologous peripheral blood stem cell transplantation. Bone Marrow Transplant. *21*, 1217–1222. https://doi.org/10.1038/sj.bmt.1701273.

Fonseca-Pereira, D., Arroz-Madeira, S., Rodrigues-Campos, M., Barbosa, I.A.M., Domingues, R.G., Bento, T., Almeida, A.R.M., Ribeiro, H., Potocnik, A.J., Enomoto, H., and Veiga-Fernandes, H. (2014). The neurotrophic factor receptor RET drives haematopoietic stem cell survival and function. Nature *514*, 98–101. https://doi.org/10.1038/nature13498.

Garcia-Aponte, O.F., Herwig, C., and Kozma, B. (2021). Lymphocyte expansion in bioreactors: upgrading adoptive cell therapy. J. Biol. Eng. *15*, 13. https://doi.org/10.1186/s13036-021-00264-7.

Gragert, L., Eapen, M., Williams, E., Freeman, J., Spellman, S., Baitty, R., Hartzman, R., Rizzo, J.D., Horowitz, M., Confer, D., and Maiers, M. (2014). HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N. Engl. J. Med. *371*, 339–348. https://doi.org/10.1056/NEJMsa1311707.

Gupta, A.O., and Wagner, J.E. (2020). Umbilical cord blood transplants: current status and evolving therapies. Front. Pediatr. *8*, 570282. https://doi.org/10.3389/fped.2020.570282.

Hanley, P.J., Mei, Z., Durett, A.G., Cabreira-Hansen, M.d.G., Cabreira-Harrison, M.d.G., Klis, M., Li, W., Zhao, Y., Yang, B., Parsha, K., et al. (2014). Efficient manufacturing of therapeutic mesenchymal stromal cells with the use of the Quantum Cell Expansion System. Cytotherapy *16*, 1048–1058. https://doi.org/10.1016/j.jcyt.2014.01.417.

Hatami, J., Andrade, P.Z., Bacalhau, D., Cirurgião, F., Ferreira, F.C., Cabral, J.M.S., and da Silva, C.L. (2014). Proliferation extent of CD34(+) cells as a key parameter to maximize megakaryocytic differentiation of umbilical cord blood-derived hematopoietic stem/progenitor cells in a two-stage culture protocol. Biotechnol. Rep. *4*, 50–55. https://doi.org/10.1016/j.btre.2014.07.002.

Horan, J., Wang, T., Haagenson, M., Spellman, S.R., Dehn, J., Eapen, M., Frangoul, H., Gupta, V., Hale, G.A., Hurley, C.K., et al. (2012). Evaluation of HLA matching in unrelated hematopoietic stem cell transplantation for nonmalignant disorders. Blood *120*, 2918–2924. https://doi.org/10.1182/blood-2012-03-417758.

Horwitz, M.E., Chao, N.J., Rizzieri, D.A., Long, G.D., Sullivan, K.M., Gasparetto, C., Chute, J.P., Morris, A., McDonald, C., Waters-Pick, B., et al. (2014). Umbilical cord blood expansion with nicotinamide provides long-term multilineage engraftment. J. Clin. Invest. *124*, 3121–3128. https://doi.org/10.1172/JCI74556.

Jones, M., Varella-Garcia, M., Skokan, M., Bryce, S., Schowinsky, J., Peters, R., Vang, B., Brecheisen, M., Startz, T., Frank, N., and Nankervis, B. (2013). Genetic stability of bone marrow-derived human mesenchymal stromal cells in the Quantum System. Cytotherapy *15*, 1323–1339. https://doi.org/10.1016/j.jcyt.2013.05.024.

Jones, M., Nankervis, B., Roballo, K.S., Pham, H., Bushman, J., and Coeshott, C. (2020). A comparison of automated perfusion- and manual diffusion-based human regulatory T cell expansion and functionality using a soluble activator complex. Cell Transplant. *29*. 963689720923578. https://doi.org/10.1177/0963689720923578.

Koller, M.R., Bender, J.G., Miller, W.M., and Papoutsakis, E.T. (1993). Expansion of primitive human hematopoietic progenitors in a perfusion bioreactor system with IL-3, IL-6, and stem cell factor. Biotechnology *11*, 358–363. https://doi.org/10.1038/nbt0393-358.

Lang, J., Weiss, N., Freed, B.M., Torres, R.M., and Pelanda, R. (2011). Generation of hematopoietic humanized mice in the newborn BALB/c-Rag2null Il2rgammanull mouse model: a multivariable optimization approach. Clin. Immunol. *140*, 102–116. https://doi.org/10.1016/j.clim.2011.04.002.

Lang, P., Teltschik, H.M., Feuchtinger, T., Müller, I., Pfeiffer, M., Schumm, M., Ebinger, M., Schwarze, C.P., Gruhn, B., Schrauder,

A., et al. (2014). Transplantation of CD3/CD19 depleted allografts from haploidentical family donors in paediatric leukaemia. Br. J. Haematol. *165*, 688–698. https://doi.org/10.1111/bjh.12810.

Lapidot, T., and Kollet, O. (2002). The essential roles of the chemokine SDF-1 and its receptor CXCR4 in human stem cell homing and repopulation of transplanted immune-deficient NOD/SCID and NOD/SCID/B2m(null) mice. Leukemia *16*, 1992–2003. https://doi.org/10.1038/sj.leu.2402684.

Lee, S., Kim, S., Kim, H., Baek, E.J., Jin, H., Kim, J., and Kim, H.O. (2008). Post-thaw viable CD34(+) cell count is a valuable predictor of haematopoietic stem cell engraftment in autologous peripheral blood stem cell transplantation. Vox Sang. *94*, 146–152. https://doi.org/10.1111/j.1423-0410.2007.01009.x.

Liedtke, S., Korschgen, L., Korn, J., Duppers, A., and Kogler, G. (2021). GMP-grade CD34(+) selection from HLA-homozygous licensed cord blood units and short-term expansion under European ATMP regulations. Vox Sang. *116*, 123–135. https://doi.org/10.1111/vox.12978.

Lin, H., Damen, J.E., Walasek, M.A., Szilvassy, S.J., Turhan, A.G., Louis, S.A., Eaves, A.C., and Wognum, A.W. (2020). Feeder-free and serum-free in vitro assay for measuring the effect of drugs on acute and chronic myeloid leukemia stem/progenitor cells. Exp. Hematol. *90*, 52–64.e11. https://doi.org/10.1016/j.exphem.2020.08.004.

Méndez-Ferrer, S., Michurina, T.V., Ferraro, F., Mazloom, A.R., Macarthur, B.D., Lira, S.A., Scadden, D.T., Ma'ayan, A., Enikolopov, G.N., and Frenette, P.S. (2010). Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature *466*, 829–834. https://doi.org/10.1038/nature09262.

Morrison, S.J., and Scadden, D.T. (2014). The bone marrow niche for haematopoietic stem cells. Nature *505*, 327–334. https://doi.org/10.1038/nature12984.

Nankervis, B., Jones, M., Vang, B., Brent Rice, R., Jr., Coeshott, C., and Beltzer, J. (2018). Optimizing T cell expansion in a hollow-fiber bioreactor. Curr. Stem Cell Rep. *4*, 46–51. https://doi.org/10.1007/s40778-018-0116-x.

Pavel-Dinu, M., Wiebking, V., Dejene, B.T., Srifa, W., Mantri, S., Nicolas, C.E., Lee, C., Bao, G., Kildebeck, E.J., Punjya, N., et al. (2019). Gene correction for SCID-X1 in long-term hematopoietic stem cells. Nat. Commun. *10*, 1634. https://doi.org/10.1038/s41467-019-09614-y.

Peled, A., Grabovsky, V., Habler, L., Sandbank, J., Arenzana-Seisdedos, F., Petit, I., Ben-Hur, H., Lapidot, T., and Alon, R. (1999). The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. J. Clin. Invest. *104*, 1199–1211. https://doi.org/10.1172/JCI7615.

Peled, T., Shoham, H., Aschengrau, D., Yackoubov, D., Frei, G., Rosenheimer G, N., Lerrer, B., Cohen, H.Y., Nagler, A., Fibach, E., and Peled, A. (2012). Nicotinamide, a SIRT1 inhibitor, inhibits differentiation and facilitates expansion of hematopoietic progenitor cells with enhanced bone marrow homing and engraftment. Exp.

Hematol. *40*, 342–355.e1. https://doi.org/10.1016/j.exphem.2011.12.005.

Politikos, I., Davis, E., Nhaissi, M., Wagner, J.E., Brunstein, C.G., Cohen, S., Shpall, E.J., Milano, F., Scaradavou, A., Barker, J.N., et al. (2020). Guidelines for cord blood unit selection. Biol. Blood Marrow Transplant. *26*, 2190–2196. https://doi.org/10.1016/j.bbmt.2020.07.030.

Radtke, S., Görgens, A., Kordelas, L., Schmidt, M., Kimmig, K.R., Köninger, A., Horn, P.A., and Giebel, B. (2015). CD133 allows elaborated discrimination and quantification of haematopoietic progenitor subsets in human haematopoietic stem cell transplants. Br. J. Haematol. *169*, 868–878. https://doi.org/10.1111/bjh.13362.

Rhéaume, M.È., Rouleau, P., Tremblay, T., Paré, I., and Loubaki, L. (2020). Short-Term exposure of umbilical cord blood CD34+ cells to human platelet lysate and cytokines enhances engraftment. Transfusion *60*, 2348–2358. https://doi.org/10.1111/trf.15991.

Schaniel, C., Papa, L., Meseck, M.L., Kintali, M., Djedaini, M., Zangui, M., Iancu-Rubin, C., and Hoffman, R. (2021). Evaluation of a clinical-grade, cryopreserved, ex vivo-expanded stem cell product from cryopreserved primary umbilical cord blood demonstrates multilineage hematopoietic engraftment in mouse xenografts. Cytotherapy *23*, 841–851. https://doi.org/10.1016/j.jcyt.2021.04.001.

Sieber, S., Wirth, L., Cavak, N., Koenigsmark, M., Marx, U., Lauster, R., and Rosowski, M. (2018). Bone marrow-on-a-chip: long-term culture of human haematopoietic stem cells in a three-dimensional microfluidic environment. J. Tissue Eng. Regen. Med. *12*, 479–489. https://doi.org/10.1002/term.2507.

Shpall, E.J., and Rezvani, K. (2021). Cord blood expansion has arrived. Blood *138*, 1381–1382. https://doi.org/10.1182/blood.2021012725.

Singh, J., Chen, E.L.Y., Xing, Y., Stefanski, H.E., Blazar, B.R., and Zúñiga-Pflücker, J.C. (2019). Generation and function of progenitor T cells from StemRegenin-1-expanded CD34+ human hematopoietic progenitor cells. Blood Adv. *3*, 2934–2948. https://doi.org/10.1182/bloodadvances.2018026575.

Stec, M., Baran, J., Szatanek, R., Mytar, B., Baj-Krzyworzeka, M., Gozdzik, J., Siedlar, M., and Zembala, M. (2012). Interactions of monocyte subpopulations generated from cord blood CD34(+) hematopoietic progenitors with tumor cells: assessment of antitumor potential. Exp. Hematol. *40*, 914–921. https://doi.org/10.1016/j.exphem.2012.07.008.

Tanner, L.B., Goglia, A.G., Wei, M.H., Sehgal, T., Parsons, L.R., Park, J.O., White, E., Toettcher, J.E., and Rabinowitz, J.D. (2018). Four key steps Control glycolytic flux in mammalian cells. Cell Syst. *7*, 49–62.e8. https://doi.org/10.1016/j.cels.2018.06.003.

Tao, L., Togarrati, P.P., Choi, K.D., and Suknuntha, K. (2017). StemRegenin 1 selectively promotes expansion of multipotent hematopoietic progenitors derived from human embryonic stem cells. J. Stem Cells Regen. Med. *13*, 75–79.