Published in final edited form as:

Nat Rev Clin Oncol. 2015 March ; 12(3): 163–174. doi:10.1038/nrclinonc.2014.215.

Advances in umbilical cord blood manipulation—from niche to bedside

Troy C. Lund, Anthony E. Boitano, Colleen S. Delaney, Elizabeth J. Shpall, and John E. Wagner

Division of Pediatric Blood and Marrow Transplant, University of Minnesota, MMC 366, 420 Delaware Street SE, Minneapolis, MN 55445, USA (T.C.L., J.E.W.). Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA (A.E.B.). Fred Hutchinson Cancer Research Center, University of Washington, D2-100, 1100 Fairview Avenue N., Seattle, WA 98109, USA (C.S.D.). The University of Texas, MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit Number 423, Houston, TX 77030, USA (E.J.S.)

Troy C. Lund: lundx072@umn.edu

Abstract

The use of umbilical cord blood (UCB) as an alternative haematopoietic cell source in lieu of bone marrow for haematopoietic reconstitution is increasingly becoming a mainstay treatment for both malignant and nonmalignant diseases, as most individuals will have at least one available, suitably HLA-matched unit of blood. The principal limitation of UCB is the low and finite number of haematopoietic stem and progenitor cells (HSPC) relative to the number found in a typical bone marrow or mobilized peripheral blood allograft, which leads to prolonged engraftment times. In an attempt to overcome this obstacle, strategies that are often based on native processes occurring in the bone marrow microenvironment or ‘niche’ have been developed with the goal of accelerating UCB engraftment. In broad terms, the two main approaches have been to expand UCB HSPC *ex vivo* before transplantation, or to modulate HSPC functionality to increase the efficiency of HSPC homing to the bone marrow niche after transplant both of which enhance the biological activities of the engrafted HSPC. Several early phase clinical trials of these approaches have reported promising results.

Introduction

An estimated 30,000 umbilical cord blood (UCB) transplants have been performed worldwide to treat patients with various malignant and nonmalignant diseases.^{1,2} As hoped, the risks of acute and chronic graft-versus-host disease (GVHD) after matched or mismatched UCB transplants are not substantially higher than those observed in patients transplanted with bone marrow, and in many studies overall outcomes are comparable.^{2,3}

Correspondence to: Troy C. Lund, lundx072@umn.edu.

Competing interests: The authors declare no competing interests.

Author contributions: T.C.L. researched data for article. A.B., C.D., E.S. and J.W. reviewed and edited the manuscript before submission. T.C.L. and J.W. substantially contributed to discussion of content. T.C.L. wrote the manuscript.

Units of UCB have a high density of multi-lineage haematopoietic progenitors; however, the total volume of a given UCB transplant is low (generally 60–100 ml), which contributes to delayed haematopoietic recovery.⁴ Unrelated donor or sibling bone marrow as stem-cell sources have a median time to engraftment (most often defined by a neutrophil count 500 cells per μ l of blood) of 16–18 days, mobilized peripheral blood stem cells can engraft in 13–15 days, whereas UCB has a median time to engraftment of greater than 3 weeks (Figure 1).^{5–9} Today, we know that a high dose of total nucleated cells (TNC) and haematopoietic progenitor cells (often measured as granulocyte–macrophage colony-forming units [CFU-GM]), and high numbers of CD34⁺ cells in the UCB graft predict an increased likelihood of successful engraftment, and faster times to neutrophil and platelet recovery.¹⁰ Universally, cell dosages are measured in terms of body weight (in kg) of the recipient. Whereas high UCB-cell doses can be achieved in small children undergoing a UCB transplant, the same cannot be said for adults, who often weigh >70 kg. Thus, new strategies are needed to accelerate and ensure engraftment.^{3,11}

Haematopoietic stem and progenitor cells (HSPC) undergo three main activities after transplant. First, HSPC migrate or ‘home’ to the bone marrow microenvironment or ‘niche’, guided by distinct biological mediators. Second, HSPC undergo expansion and occupy the niche space. Third, HSPC undergo differentiation to reconstitute the haematopoietic system consisting of neutrophils, red blood cells, platelets, lymphocytes, and so on, in a process closely coupled to cell expansion (Figure 2). The two main approaches to increase UCB engraftment have been either to expand UCB *ex vivo* to achieve greater numbers of HSPC before transplantation (that is, increase the cell dose) or to augment homing of the limited number of UCB HSPC to the bone marrow niche. This Review focuses on the approaches to realize these strategies and the results of the various clinical trials of these strategies that have been completed. A summary of ongoing and completed clinical trials involving the approaches covered in this Review is given in Table 1.

Strategies to expand UCB

Cytokine-mediated expansion

The early work of pioneers such as Friedenstein, Dexter and Metcalf (to name a few) focused on increasing understanding of how to grow, maintain and derive the various lineages of HSPC. The conditions for *in vitro* culture of HSPC generally involved (and still involve) isolation of bone marrow cells, followed by incubation in a defined growth medium with the addition of serum (often horse or bovine in origin).¹² The growth medium usually contains a combination of cytokines such as stem cell factor (SCF, also known as Kit ligand), thrombopoietin, IL-3, IL-6 and granulocyte colony-stimulating factor (G-CSF), which have been shown to increase total cell numbers and progenitor cell populations, as measured by CFU assays.^{13–17}

These initial studies led into an early clinical trial by Shpall and colleagues,¹⁸ which evaluated the feasibility of cytokine-mediated UCB expansion in 37 patients undergoing myeloablative UCB transplantation. One portion of UCB that had been previously frozen in two portions (generally the smaller one) was cytokine-expanded for 10 days. Patients received the unmanipulated portion on day 0, with the expanded UCB unit given either

concomitantly (arm 1), or 10 days later (arm 2).¹⁸ The approach of infusing a portion of an unmanipulated UCB unit was used because, at the time, it was unknown if cytokine-expansion would result in graft failure, leaving the patient without a haematopoietic system—a fatal condition.

The data, combined from each arm, showed a TNC expansion of 56-fold and a CD34⁺-cell expansion of fourfold (Table 2).¹⁸ The median time to neutrophil and platelet engraftment was 26 days and 126 days, respectively; no graft failures were observed.¹⁸ Four patients died within 30 days of engraftment due to infection.¹⁸ No statistically significant difference existed between the two arms of the study in terms of engraftment.¹⁸ Acute GVHD was seen in 20 of 30 evaluable patients (66.7%) and chronic GVHD in 14 of 19 evaluable patients (74%).¹⁸ These rates of GVHD are somewhat higher than those typically observed in contemporary studies of UCB transplantation, which might reflect a change in the biology of HSPC after expansion or less-precise HLA typing (compared with current standards), both of which affect GVHD rates. After a median follow-up of 30 months, 12 of 37 patients were alive.¹⁸

Although the patient numbers were small, this early trial showed that UCB could be expanded and safely infused into patients without graft failure. This trial was a pivotal milestone, as it was unclear at the time what the outcomes of expanded UCB would be in the human transplant setting and whether cells would persist long term; however, the overall impact on time to engraftment remained uncertain. First, in one arm, the expanded cells were infused 10 days after infusion of unexpanded cells, before any relevant engraftment had occurred, making it difficult to determine any true clinical effect. Second, measuring the contribution of the expanded population to early and late haematopoietic recovery was impossible because it was derived from the same UCB unit as the unexpanded population. For clinical trials in which a single UCB unit is expanded, it will be difficult to determine the effect on engraftment unless a truly substantial shortening of time to engraftment (that is, a decrease of 7–10 days) is achieved in comparison with historical controls.

Copper-chelation-mediated expansion

Early observations in patients with copper deficiency revealed decreased granulopoiesis and erythropoiesis, and the bone-marrow biopsies of these patients showed a decrease in mature granulocyte numbers, but an increase in the numbers of pro-myelocytes and myelocytes, compared with patients without copper deficiency.^{19,20} These observations led to an early hypothesis that copper deficiency caused a block in myelocyte maturation. If that hypothesis were true, then perhaps a block in maturation could enable populations of more-primitive haematopoietic stem cells to be expanded. Work reported by Peled *et al.* showed that one could lower cellular copper levels using tetra-ethylenepentamine (TEPA), a copper chelator, and extend the lifespan of CD34⁺ cells as well as the number of CFU.²¹ Subsequently, it was shown that UCB CD34⁺ cells exposed to TEPA did indeed have a reduced rate of differentiation and showed increased engraftment in a nonobese diabetic/severe combined immunodeficient (NOD/SCID) murine model, which led to a clinical trial.^{21–23}

As before, UCB units were frozen in two portions, ranging from 50/50 to 20/80 splits, prior to expansion and/or transplantation.²³ The smallest portion was thawed, CD133⁺ cell

selection was performed to enrich HSPC, and these UCB-derived cells were then expanded for 21 days using a cytokine cocktail containing TEPA.²³ A median 161-fold expansion in TNC occurred after expansion.²³ CD34⁺ cell numbers were not measured in the thawed unit before expansion to conserve cells, but when measured in the unmanipulated unit and extrapolated to the pre-expanded portion, the estimated median expansion of CD34⁺ cells was 2.3-fold.²³ After the preparative regimen, patients received the unmanipulated portion of UCB, followed by the expanded cells 24 h later.²³ Engraftment was achieved in nine of 10 patients, with a median time to neutrophil recovery of 30 days.²³ All engrafted patients showed 100% chimerism.²³ Four patients developed grade 2 acute GVHD of the skin, but no grade 3–4 acute GVHD occurred.²³ Four of eight patients surviving for more than 100 days developed chronic GVHD.²³ Six of 10 patients were alive at 180 days (one died of relapse and three from infectious complications).²³

This study showed that TEPA-treated, cytokine-expanded UCB could be safely infused into patients without an increase in nonrelapse mortality (at day 100 in comparison with historical controls). As with cytokine-mediated expansion, whether or not there was any enhancement to engraftment was difficult to determine, and the median time to engraftment of 30 days was within the range for single, unexpanded UCB-unit engraftment; it was suggested that the methotrexate used for GVHD prophylaxis could have promoted some delay in engraftment.²³ A follow-up study with planned enrolment of 100 patients with haematological malignancies undergoing an allogeneic myeloablative transplant is nearing completion. This follow-up study is not randomized for TEPA expansion, but might nevertheless provide additional information about the effectiveness of TEPA-mediated expansion on single UCB-unit engraftment.

The double UCB platform

Another approach to achieve increased numbers of UCB HSPC for transplantation is the use of two donor UCB units. An additional benefit to the use of a double UCB platform that will be reiterated, is that one unit can be culture-expanded or treated (to alter homing, for example), in advance of the scheduled transplant day, while the other unit is left unmanipulated (Figure 3). This strategy also has the unique advantage of traceability, as the manipulated unit is genetically distinguishable from the unmanipulated unit based on HLA genotyping, enabling one to track the individual chimeric contribution from each unit (often through short tandem repeat determination). Furthermore, if the manipulated product were damaged or impaired by the procedure, the second unit would be capable of complete haematopoietic reconstitution alone, increasing clinical trial safety.

The safety of using two UCB units without expansion has been established,²⁴ and the overall outcomes of double UCB transplantation have been shown to be comparable to those of matched-related donor and matched-unrelated donor bone-marrow transplants.^{25–28} Although there was initial concern that two UCB units might react negatively against each other in a ‘graft-versus-graft’ reaction, this reaction has not been observed. Generally, after double UCB infusion, one UCB unit predominates by 100 days following transplant. Many studies have tried to predict which unit will ultimately ‘win out’, and evidence indicates that early CD3⁺ cell engraftment, the presence of anti-HLA antibodies, and a high post-thaw

dose of CD34⁺ cells and TNC can predict which UCB unit will engraft.^{29–32} Another study suggested that the chimerism in CD4⁺-cell, CD8⁺-cell and natural killer (NK)-cell subsets was established by the ‘winning’ UCB unit as early as 11 days post-transplant.³³ Although no single absolute predictor exists, the evidence does suggest that the final engrafting UCB unit establishes itself early in the transplant process.

The idea that one or more cellular subsets can play an important part in predicting UCB engraftment should guide future studies of UCB expansion and homing, as UCB manipulation will undoubtedly change the make-up of the cellular subpopulations therein. Double UCB transplantation is a reasonable alternative to single UCB transplantation if cell dose is limiting or if adequately matched bone-marrow donors cannot be found.

Notch-mediated expansion

The Notch family of receptors (Notch 1–4) and their ligands (Jagged-1 and Jagged-2, and Delta-like (DLL) 1, DLL3 and DLL4) have diverse and key roles in a wide range of biological processes—from organ development to cancer metastases.^{34,35} Early work initially suggested a role for Notch in haematopoiesis, with the detection of human Notch 1 on CD34⁺ human haematopoietic precursor cells.³⁶ Subsequently, several studies determined that the ligands of Notch 1, Jagged-1 and DLL1 could promote expansion of haematopoietic progenitor cells, and that the use of an immobilized form of DLL1 fused to the Fc portion of human IgG₁ (DLL1^{ext}-IgG) was key to ideal activation.^{37,38} By using this immobilized form of DLL1 and a defined cytokine cocktail to expand murine HSPC, it was found that one could achieve a 4–5-fold log increase in cell numbers, with the cells also retaining the capacity for short-term myeloid and lymphoid engraftment when tested in competitive repopulation assays.^{38–40} Further work using human haematopoietic progenitor cells from UCB (that is, CD34⁺CD38[−] cells) that were exposed to immobilized DLL1 in the presence of human cytokines—SCF, Flt3 ligand, IL-6, thrombopoietin and IL-3—resulted in no increase in total cell number, but a 10-fold increase in the number of CD34⁺ cells and an enhanced repopulating ability in an immunodeficient mouse model.⁴¹

The double UCB platform was used in a study of patients undergoing a myeloablative transplant who received one unmanipulated UCB unit along with a second UCB unit previously expanded in the presence of DLL1^{ext}-IgG.⁴² There was a 562-fold increase in the number of TNC and a 164-fold increase in the number of CD34⁺ cells in the expanded UCB.⁴² The median time to engraftment was markedly shortened to 16 days, compared with the historical control of 26 days at the same institution, using the same conditioning and post-transplant immunosuppressive regimens.⁴² Importantly, there were no infusional toxicities, and engraftment occurred in nine of the 10 patients.⁴² Grade 2 acute GVHD was observed in all but one of the evaluable patients, with one patient developing grade 3 acute GVHD.⁴²

Contribution to early myeloid recovery at 7 days post-transplant was derived almost entirely from the expanded UCB unit, although it did not persist beyond 14 to 21 days post-transplant.⁴² The investigators hypothesized that the reduced time to neutrophil recovery suggested a potential facilitating effect exerted by the *ex vivo* expanded cells on the nonmanipulated unit.⁴² Longer-term *in vivo* persistence of progeny from the expanded cell

graft was observed in two of the nine evaluable patients.⁴² In one patient, analysis at day 240 post-transplant revealed that a portion (10–15%) of the donor cells were derived from the expanded graft;⁴² however, by 1 year post-transplant 100% of haematopoietic cells were derived from the unmanipulated graft.⁴² In the second patient, the contribution of the expanded cell population ranged from 25% to 66% of total donor engraftment.⁴² Unfortunately, this patient died from a septic event 6 months after the transplant.⁴² The lack of contribution to long-term engraftment in this study suggests a deficiency of true haematopoietic-stem-cell expansion, and that early myeloid recovery was the result of short-term repopulating progenitor cells.

MSC-mediated expansion

Several niches exist in the bone-marrow microenvironment, including endothelial, osteoblastic, perivascular and others, and they all have various roles in the maintenance of HSPC. Strategies using cytokines and/or Notch-ligand engagement serve to mimic biological growth and maintenance mechanisms that occur in the native bone-marrow environment governed by the niche cells. The best *ex vivo* niche model is that of the mesenchymal stromal cells (MSC), which are derived from the nonhaematopoietic portion of the bone marrow after culture expansion under the proper conditions. Evidence is mounting that MSC arise from perivascular cells in the marrow, and therefore many other vascularized tissues are able to give rise to MSC, as demonstrated in several studies.^{43–45} MSC secrete the majority of the cytokines and ligands that support HSPC growth and expansion, including CSF, GM-CSF, IL-6 and DLL1, which gives credibility to the idea that cytokine-mediated expansion strategies to expand UCB have a basis in the natural interaction between niche-cell-secreted factors and HSPC.^{46,47}

UCB mononuclear cells expand in greater quantities in the presence of MSC than with growth factors alone: the expansion reported in early preclinical studies resulted in a 6–20-fold increase in TNC; an 8–37-fold increase in CD34⁺ cells; and a 3.5–200-fold increase in CFU potential.^{48,49} Finally, MSC from ‘third-party’ donors expanded UCB as well as MSC from donors related to the recipient, enabling an ‘off-the-shelf’ MSC product to be created.^{48,49}

In the most-recently reported clinical trial of MSC-mediated expansion,⁵⁰ MSC were expanded from off-the-shelf mesenchymal precursor cells. The advantage of using off-the-shelf, third-party MSC was that the cells were readily available and grew to numbers required for UCB co-culture within 4 days.⁵⁰ The early results of this clinical trial were reported in a study involving 31 patients undergoing myeloablative haematopoietic-stem-cell transplantation (HSCT). Seven patients received UCB expanded on MSC derived from family members and 24 patients received UCB expanded on third-party MSC; all patients also received a second unmanipulated UCB unit.⁵⁰ MSC-expanded UCB showed a 12.2-fold increase in TNC, a 30.1-fold increase in CD34⁺ cells and a 17.5-fold increase in CFU formation.⁵⁰ The median time to engraftment was 15 days, and 23 of 24 patients who received UCB expanded on third-party MSC achieved engraftment (one patient died of early fungal sepsis before engraftment);⁵⁰ median time to platelet recovery was 42 days.⁵⁰ Early chimerism between days 21–30 showed that 54% of the patients had chimerism from the

unmanipulated unit alone, whereas 46% had chimerism from both UCB units.⁵⁰ At 6 months, only 13% of the patients had chimerism from the expanded unit, and at 1 year, all patients had chimerism prevailing from the unmanipulated unit.⁵⁰ The rate of acute grade 2–4 GVHD was 42% and the incidence of acute grade 3–4 GVHD was 13%.⁵⁰ These data show that MSC-expanded UCB can engraft, albeit temporarily, and do so in an earlier time frame than historical UCB engraftment. Similar to the clinical trial of Notch-mediated expansion,⁴² the unmanipulated UCB product was responsible for long-term engraftment,⁵⁰ which suggests that there is a common process at work in expansion strategies.

Nicotinamide-mediated expansion

Nicotinamide is a form of vitamin B₃ and the precursor to oxidized nicotinamide adenine dinucleotide (NAD). Several enzymes are dependent on NAD for function, namely the sirtuin family. A prototypical sirtuin family member, SIRT1, has a myriad of biological effects with activities involving cell division, DNA repair and cell-shape control in many tissue types including endothelial, cardiac and skeletal muscle.^{51–54} Importantly, haematopoietic cells from *Sirt1*^{−/−} mice show a decrease in the requirement for growth factors in *in vitro* culture and an increase in growth capacity.⁵³ SIRT1, although dependent on NAD, is inhibited by its precursor, nicotinamide. Peled *et al.* took advantage of the fact that this vitamin can inhibit SIRT1 and used nicotinamide in co-culture with SCF, thrombopoietin, IL-6 and Flt3 ligand to expand CD34⁺CD38[−] cells from UCB over a period of 21 days and increased engraftment in mouse models of HSCT.⁵⁵ Interestingly, NAD regulation can occur through metabolic pathways within MSC.⁵⁶ Furthermore, MSC function can also be affected by NAD levels,^{56,57} once again demonstrating that the nicotinamide-mediated expansion strategy might mimic a native process within the bone-marrow microenvironment.

On the basis of these preclinical studies, a clinical product was developed, NiCord® (Gamida-Cell, Israel), for use in phase I and phase II clinical trials. A pilot safety and efficacy study has been completed in 11 adults with haematological malignancy undergoing myeloablative double UCB transplant, showing a 486-fold expansion in TNC and 72-fold expansion in numbers of CD34⁺ cells after treatment of UCB with NiCord®.⁵⁸ The median time to engraftment was 13 days, with one patient developing graft failure.⁵⁸ Six of the patients showed long-term myeloid engraftment with the NiCord® expanded UCB only, and one patient had mixed chimerism at 3 years after transplantation.⁵⁸ An important difference in the NiCord® trial from other trials is that AC133⁺ cells, which are known to have stem-cell characteristics similar to CD34⁺ cells were selected for expansion from one UCB unit and the AC133[−] cells were cryopreserved until transplant day, when they were co-infused with the expanded UCB.⁵⁸ As in other trials, an unmanipulated UCB was also infused (Figure 3).⁵⁸ This trial is the first to show that an expanded product can have long-term engraftment in a majority of the patients treated. The biological effect of giving the nonselected AC133[−] cells is unknown, although one could theorize that there might be cell populations (T cells and other accessory cells) that facilitate engraftment of the manipulated UCB (or even the unmanipulated UCB, in theory). Whether this approach facilitated the long-term engraftment with the NiCord® product is hard to know based on data from only

11 patients, but its significance in this regard will become more apparent when assessed with larger patient numbers.

SR1-mediated expansion

A study involving an image-based screen has identified a purine derivative, SR1, that promotes the *ex vivo* expansion of HSPC via engagement of the aryl hydrocarbon receptor.^{59,60} The high-throughput screen used CD34⁺ cells derived from primary human mobilized peripheral blood that were cultured in a serum-free medium supplemented with thrombopoietin, SCF, Flt3 ligand and IL-6.⁵⁹ These conditions alone generate robust proliferation, but are also usually accompanied by haematopoietic differentiation and loss of engraftment activity. The state of increased differentiation was visualized by the lack of maintenance of surface markers expressed on HSPC (CD34⁺ and CD133⁺).⁵⁹ Conversely, SR1-treated cultures were found to maintain their undifferentiated phenotype.⁵⁹ In addition, validating experiments revealed that SR1 induced a 670-fold increase in the total number of UCB-derived CD34⁺ cells after 21 days of culture compared with input cell numbers.⁵⁹ Biochemical labelling studies suggested that SR1 promoted symmetric cell divisions without the loss of HSPC function; cells cultured with SR1 for 21 days led to a 17-fold increase in the number of NOD/SCID repopulating cells compared with starting cells or control cultures, and the expanded population retained the ability to sustain multi-lineage reconstitution, as well as engraft in secondary recipients.⁵⁹

The double UCB platform is also being used for the first in-human study using SR1-mediated UCB expansion of positively selected CD34⁺ cells in patients with haematological malignancies undergoing a myeloablative preparative transplant. The strategy has been modified to infuse the CD34⁺ population previously collected and cryopreserved at the time the CD34⁺ cells were positively selected for expansion culture (Figure 3). This approach is based on the preclinical observation in NOD/SCID/gamma mice that expanded UCB cells had an improved long-term competitive advantage if the CD34⁺ cells were re-introduced. The trial is still accruing at the time of this Review.

Strategies to increase UCB HSPC homing

Before discussing homing-based strategies to increase the efficacy of UCB transplantation, we should note that attempts have been made to inject UCB HSPC directly into the bone-marrow space, which would obviate the need for cell homing to this niche. However, cells would still need to migrate from the injection site to other marrow spaces for complete haematopoietic reconstitution. Experiments in mice have shown that there might be an early (within 48 h) advantage to intra-marrow injection of cells, but no clear long-term engraftment advantage.^{61,62} Clinical trials have shown good engraftment rates, but the median time to engraftment remains >20 days.^{63–65} Two studies have suggested that rates of GVHD are reduced with intra-marrow injection.^{64,65} The study authors hypothesize that immediate contact of UCB cells with osteoblasts and MSC alter their biology, although this hypothesis is yet to be proven in any model system.

SDF-1–CXCR4 axis manipulation

Stromal cell-derived factor-1 (SDF-1) is a chemokine originally discovered to be secreted in large amounts by *in vitro* cultured bone marrow stromal cells. Although not termed MSC at the time, these cells were isolated and cultured in a method similar to that used in MSC isolation.⁶⁶ The receptor for SDF-1 is CXC chemokine receptor 4 (CXCR4), which is expressed on a variety of cell types including MSC, endothelial and various subtypes of haematopoietic cells including most HSPC populations. Through CXCR4 engagement, SDF-1 is a potent *in vitro* and *in vivo* chemoattractant for cells including CD34⁺ haematopoietic progenitor cells.^{66–70} The SDF-1–CXCR4 axis has been well established as an important mediator of HSPC homing to the bone marrow after transplantation, but is also important in the retention and maintenance of HSPC within the bone-marrow niche.

A key negative regulator of SDF-1-mediated migration is the cell-surface protein dipeptidyl peptidase 4 (DPP4), which is able to remove the *N*-terminal dipeptide from SDF-1, thereby decreasing its activity and reducing the ability of SDF-1 to recruit CXCR4-expressing cells. The effect of DPP4 inhibition was shown to increase the homing of human lin[–] or CD34⁺ cells to the bone-marrow compartment twofold to threefold after transplantation into NOD/SCID/B2m^{null} mice.⁷¹ Moreover, DPP4 has been shown to regulate the activities of several haematopoietic growth factors including CSF and G-CSF. Therefore, inhibition of DPP4 positively affects not only cellular homing, but also the cellular growth signals governing haematopoiesis in a broader capacity.⁷²

Sitagliptin is an oral DPP4 inhibitor that is used to treat diabetes mellitus and might also hold the potential to augment HSPC homing via its capacity to inhibit DPP4.^{73,74} In a clinical trial, patients undergoing HSCT received 600 mg oral sitagliptin once daily from the day before transplantation to 2 days following transplantation.⁷⁵ Importantly, after chemotherapeutic conditioning, patients received UCB that had been processed for red-cell depletion (RCD; *n* = 17) or plasma depletion (*n* = 7). Plasma-depleted units had 10-fold less CFU activity than the RCD units, and given that CFU activity is associated with engraftment,¹⁰ these groups were analysed separately.⁷⁵ In the RCD group (16 evaluable patients), the median time to neutrophil engraftment was 21 days and the median time to platelet engraftment was 77 days.⁷⁵ As one might expect based on the lower CFU activity, the median time to neutrophil engraftment in the plasma depletion group was 36 days, despite sitagliptin treatment.⁷⁵ There were no unexpected toxic effects from sitagliptin or marked increases in GVHD rates.⁷⁵ Sitagliptin was able to suppress DPP4 activity by ~70–80%, although the effect was transient and return to baseline activity levels occurred within 16 h of administration.⁷⁵ A key finding was that, in the RCD group, DPP4 was substantially less active in the nine patients with neutrophil engraftment in ≤21 days than in the eight patients with engraftment in >21 days.⁷⁵ Although time to neutrophil recovery in this study was not markedly shortened, the findings do suggest that modulation of the SDF-1–CXCR4 pathway via DPP4 suppression is feasible and could potentially be a new modality to improve overall UCB engraftment. Future studies are needed to address the timing and dosage of sitagliptin to optimize DPP4 suppression.

Complement-mediated homing

The serum complement system is considered an arm of the innate immune system. Ratajczak and co-workers⁷⁶ showed that the complement cascade is activated during growth-factor-induced HSPC mobilization and that complement cleavage fragments play a part in the mobilization. Reca *et al.*⁷⁷ reported that UCB CD34⁺ cells express the C3a receptor (C3aR), and that C3a could enhance CD34⁺-cell migration towards SDF-1 *in vitro*. In addition, UCB CD34⁺ cells pretreated with the C3aR antagonist SB290157 showed impaired homing after transplantation into NOD/SCID mice.⁷⁸ In fact, use of C3a to prime HSPC before transplantation might mimic a process that exists natively in the bone-marrow microenvironment to regulate steady state HSPC trafficking, as bone marrow stromal cells have been shown to produce C3a and other complement factors.⁷⁹

A phase I clinical trial investigated the effect of priming UCB cells with C3a before transplanting them into nonmyeloablated patients with high-risk haematological malignancies.⁸⁰ The trial used a double UCB platform, in which one UCB unit was primed with C3a (this unit was the smaller unit transplanted in the first 22 patients) and the other unit was left unmanipulated.⁸⁰ No serious infusional toxicities occurred and median time to engraftment was 6 days, which was not significantly different from the time to engraftment of historical nonmyeloablative UCB transplant control patients.⁸⁰ Survival and mortality were not negatively affected by C3a priming.⁸⁰ Unfortunately, engraftment was skewed toward the C3a-primed UCB in only nine of 27 evaluable patients. In most patients, engraftment by the unmanipulated unit was predominant, and a predictor for which UCB unit engrafted was driven by CD3⁺-cell dose, with a mean of 0.5×10^7 cells per kg compared with 0.8×10^7 cells per kg in the primed and unmanipulated units, respectively.⁸⁰ Unmanipulated UCB units also had a higher TNC dose, with a mean of 0.15×10^8 cells per kg compared with 0.25×10^8 cells per kg in the primed and unmanipulated units, respectively, although there was no difference in CD34⁺-cell dose between the primed and unmanipulated units.⁸⁰

This clinical trial was the first to evaluate a strategy in which one UCB unit is primed by a compound to facilitate homing *a priori*, and although not robust in effect, it does provide data to help us better design the follow-up trials. For example, much of the C3a preclinical data was performed in the myeloablative setting, whereas the clinical trial was carried out using nonmyeloablated recipients,⁸⁰ in whom C3a-primed cells might act differently. In addition, other variables in the priming process might need to be optimized in future trials, such as C3a concentration, incubation temperature (priming was carried out at room temperature) and duration of priming, all of which could have important roles in modulating homing.

Prostaglandin-mediated homing

North *et al.*⁸¹ carried out a high-throughput screen of known biologically active agents in zebrafish and determined that a long-acting form of prostaglandin E2 (16,16-dimethyl-PGE₂, or dmPGE₂) increased the native haematopoietic-stem-cell population. Follow-up studies in mice demonstrated that haematopoietic-cell engraftment was increased in transplantation assays if donor cells were exposed to PGE₂ before adoptive transplant.⁸¹

Further studies showed that human UCB CD34⁺ cells expressed PGE₂ receptors and that dmPGE₂ enhanced the engraftment of UCB cells when transplanted into NOD/SCID mice, with increases in homing and HSPC expansion have contributing roles.⁸¹ Moreover, MSC were found to produce PGE₂ as an effector to inhibit T-cell activity, which accounted, in part, for some of the immunosuppressive qualities that MSC are known to retain.^{82–84} It is not known whether PGE₂ is used directly by bone marrow stromal cells or MSC to maintain haematopoiesis, but *in vitro* data suggest that MSC enhance erythroid-cell production via PGE₂.⁸⁵

In the initial pilot study of prostaglandin-mediated homing, nine patients undergoing a reduced intensity double UCB transplant received a PGE₂-primed UCB unit along with an unmanipulated UCB unit (separated by 4 h).⁸⁶ There was no difference in time to engraftment between recipients of PGE₂-primed UCB and historical controls, nor was there a considerable contribution to chimerism by the primed unit.⁸⁶ Instead of abandoning this approach, the authors discovered that substantial optimization of temperature, time and media formulation was required for most robust PGE₂ pathway activation.⁸⁶ A second cohort of patients undergoing a nonmyeloablative preparative regimen received a PGE₂-primed UCB unit, processed using optimized conditions, along with an unmanipulated UCB unit (Table 2).⁸⁶ Median time to neutrophil engraftment was 17.5 days for recipients of PGE₂-primed UCB versus 21 days for historical controls.⁸⁶ Interestingly, 10 of the 12 patients engrafted 100% with the PGE₂-treated unit, and mild GVHD occurred in only four of the 12 patients.⁸⁶

This study represents the first in which modulation of homing might have altered engraftment, although the engraftment advantage was only marginally significant ($P = 0.045$). The fact that the engrafted cells were derived from PGE₂-primed UCB in the majority of patients is intriguing, but as the authors point out, other potential explanations exist.⁸⁶ That the larger of the UCB units (based on pre-freeze analysis) in each case received PGE₂ priming, and that both TNC and CD34⁺-cell dose are predictors of engraftment in the double UCB transplant setting,^{10,32} were foremost among these explanations.⁸⁶ However, the studies determining TNC and CD34⁺-cell dose as predictors have often used post-thaw analyses,^{10,32} and in the PGE₂ study there was no significant difference in TNC and CD34⁺-cell dose post-thaw between PGE₂-primed units and unmanipulated units.⁸⁶ Future studies will involve larger cohorts of patients and possibly test PGE₂-primed UCB in the myeloablative setting to learn if this initial positive data is maintained.

Predicting engraftment

The results of the UCB expansion clinical trials involving MSC or DLL1 have a common thread: although there was a decrease in the time to neutrophil engraftment as a result of the expanded UCB, long-term chimerism was obtained from the unexpanded UCB. A simple explanation for this result could be a lack of true HSPC expansion, and early myeloid recovery as a result of short-term repopulation of myeloid progenitor cells. Another possibility is that cell cycle also played a part, as HSPC that are quiescent (in G₀) engraft most efficiently and provide long-term immune reconstitution in both murine and human transplant models.^{87,88} Short-term expansion of UCB intentionally drives the cell cycle to

achieve high levels of proliferation, which might lead to engraftment only with transient potential. By contrast, the unmanipulated unit, which contains more cells in a quiescent phase than the expanded UCB, engrafts at a slower rate and provides long-term immune reconstitution.

Previous studies have shown that CD3 chimerism can predict the overall engrafting UCB unit in double UCB transplants.²⁹ Another possible explanation for preferential engraftment of the unmanipulated UCB unit is that the expanded unit is immune-compromised, as it contains few, if any, T cells. Expansion with DLL1 involved isolation of CD34⁺ cells beforehand, and the negative cells (including T cells) were discarded, with the resultant expanded UCB units containing a median of only 0.2% CD3⁺ T cells. The MSC-expanded UCB had almost no CD3⁺ T cells compared with the number in the unmanipulated UCB, which increases the possibility of immune rejection by the T-cell-replete unmanipulated unit. T cells and other cells might also provide unknown 'accessory' functions during engraftment. We know from trials in which only CD34⁺-selected bone marrow cells are transplanted that graft failure and delayed immune reconstitution are considerable problems that can be somewhat ameliorated by giving an 'add-back' T-cell dose just after the CD34⁺-cell transplant.^{89–91}

The add-back strategy will be employed in planned trials of nicotinamide and SR1 expansion, in which the AC133[−] and CD34[−] cells, respectively, are to be cryopreserved and infused into the recipient after the expanded and unexpanded UCB units are given. The biological effect of this strategy remains to be seen, but if appreciable long-term chimerism resulting from the expanded UCB unit is observed, it might suggest an important role for the unexpanded or unselected cells.

Looking ahead

In contrast to the findings of the original expansion strategies explored in the 1990s and early 2000s, it is now clear that primitive haematopoietic progenitors can be manipulated and the engraftment process altered (Figure 4). UCB HSPC can be expanded using one of several unique approaches, although all rely on cytokine supplementation. Even more intriguing is that simple priming of UCB before transplantation might change engraftment dynamics, with the aim that HSPC homing is increased. Thus far, all of these methodologies seem to be safe, without any demonstrable adverse effects.

Priming UCB for better cell homing might have a role in future application, but remains to be proven robust enough as an independent strategy. Currently, expansion of UCB gives the best results in terms of robust early engraftment. However, there are limitations to expansion-based strategies. First, a 10–14 day lead time is required for the expansion, which might not be acceptable for patients with high-risk malignancies and a tenuous remission status. Second, thus far, expanded cells seem to provide only short-term engraftment. That being said, one of the main causes of death after transplant is infection, and the expanded UCB unit as a transient population of myeloid cells could provide clinical benefit. Studies in mouse models have shown that these myeloid cells can be functional and protect recipients against a viral or fungal infection.^{92–94} Therefore, these strategies might be adequate to

provide a clinical benefit, even without a long-term contribution of the expanded UCB unit to eventual engraftment. At this time, it is too early to know the 'functionality' of the manipulated UCB unit in transplant recipients, as the focus has been on time to neutrophil recovery and not on function.

The limitation of homing-based strategies is that only a minimal (if any) decrease in time to engraftment has been seen thus far. Of interest is the combination of DPP4 inhibition and PGE₂ priming. Broxmeyer and Pelus⁹⁵ found that mice treated with sitagliptin and transplanted with PGE₂-primed marrow had markedly better engraftment than those given either treatment alone. This finding suggests that a combination of strategies could be used, leading one to speculate that even priming an expanded product could increase long-term engraftment.

The next and more important questions to be answered are whether UCB manipulation can improve overall outcomes, in terms of survival from malignancy and/or reduction in mortality from infectious causes. To date, the published clinical trials have enrolled patient numbers too low to adequately address these points; therefore, larger trials are required. UCB selection algorithms take into account both HLA match and cell dose, often with a sliding scale supporting higher cell doses when using lesser matched units.⁹⁶ Although HLA type cannot be altered *ex vivo*, it is likely that within the next 5 years the field of UCB manipulation will mature to a point at which a single UCB can be expanded to give every recipient an optimal cell dose upon infusion. Data supporting a single UCB strategy comes from a clinical trial that compared single versus double UCB transplants in 224 children with malignancy.⁹⁷ When matched for age, HLA match and disease, the outcomes were similar for relapse, disease-free survival, infections, grade 2–4 acute GVHD and neutrophil recovery.⁹⁷ Data for adult patients is not available at present, but will be important to obtain, as the physical mass of adults is greater, resulting in relatively reduced cell doses for transplantation.

Most of the clinical trials thus far have used myeloablative conditioning (except for the C3a trial). The development of reduced intensity or nonmyeloablative regimens in the past 15 years has enabled more patients to undergo transplant, while being spared from excess toxicity. One drawback of reduced intensity transplantation is an increased rate of graft failure.⁹⁸ Therefore, another ideal scenario is one in which a patient gets a single manipulated UCB product that has high 'engraftment potential' to enable a reduction in the chemotherapeutic conditioning regimen and to overcome any barriers to engraftment.

Finally, the costs associated with UCB manipulation could be substantial. The cost of an individual UCB unit varies by the specific cell bank and according to the testing that has been undertaken. Best estimates range from US\$34,000 to US\$59,000 for a single UCB unit, which could be doubled when performing a double UCB transplant.^{99,100} What is unknown is the cost of the products used currently to manipulate UCB. All the trials of UCB transplantation are being performed with pharmaceutical sponsorship under an investigational new drug application, and the market or retail price for any product has not been set. Certainly, products will have to compete against each other as well as against unmanipulated UCB. After proving their clinical effectiveness, cost-effectiveness will have

to be demonstrated for each product, weighing the cost of the product against the total cost of hospital care for the patient. Ideally, one would want to see a reduction in in-patient hospital days, a decrease in infection rates and antibiotic use, less time spent in the intensive-care unit (a substantial cost), and so on. Secondary advantageous outcomes that should be evaluated are that more-rapid engraftment might decrease the time a patient spends in the hospital bed, improve strength and reduce hospital stay—all of which would contribute to a reduction in the morbidity associated with transplantation and result in better patient outcomes.

Conclusions

For the first time, the engraftment kinetics of UCB can be altered either through cell expansion or modulation of cell homing. The current standard of performing clinical trials to alter engraftment should utilize the double UCB transplant model—both for safety and to assess effectiveness. The ability to alter expansion or increase homing will be of greatest benefit to adult-sized patients weighing 60 kg or greater. By improving UCB performance one might be able to choose UCB units that provide a lower frozen cell dose, but is better HLA-matched. The true test of efficacy in the ability to decrease transplant related morbidity and mortality will be tested in the next generation of clinical trials.

Acknowledgments

The authors thank T. Glass for her conception and rendition of the bone-marrow microenvironment in Figure 2.

References

1. Kita K, Lee JO, Finnerty CC, Herndon DN. Cord blood-derived hematopoietic stem/progenitor cells: current challenges in engraftment, infection, and *ex vivo* expansion. *Stem cells int.* 2011; 2011:276193. [PubMed: 21603139]
2. Laughlin MJ, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med.* 2004; 351:2265–2275. [PubMed: 15564543]
3. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood.* 2013; 122:491–498. [PubMed: 23673863]
4. Broxmeyer HE, et al. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Natl Acad Sci USA.* 1989; 86:3828–3832. [PubMed: 2566997]
5. Calvet L, et al. Hematologic, immunologic reconstitution, and outcome of 342 autologous peripheral blood stem cell transplantations after cryopreservation in a –80 degrees C mechanical freezer and preserved less than 6 months. *Transfusion.* 2013; 53:570–578. [PubMed: 22804351]
6. Davies SM, et al. Engraftment and survival after unrelated-donor bone marrow transplantation: a report from the national marrow donor program. *Blood.* 2000; 96:4096–4102. [PubMed: 11110679]
7. Eapen M, et al. Higher mortality after allogeneic peripheral-blood transplantation compared with bone marrow in children and adolescents: the Histocompatibility and Alternate Stem Cell Source Working Committee of the International Bone Marrow Transplant Registry. *J Clin Oncol.* 2004; 22:4872–4880. [PubMed: 15520055]
8. Rubinstein P, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med.* 1998; 339:1565–1577. [PubMed: 9828244]
9. Anasetti C, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. *N Engl J Med.* 2012; 367:1487–1496. [PubMed: 23075175]

10. Avery S, et al. Influence of infused cell dose and HLA match on engraftment after double-unit cord blood allografts. *Blood*. 2011; 117:3277–3285. [PubMed: 21149633]
11. Laughlin MJ, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med*. 2001; 344:1815–1822. [PubMed: 11407342]
12. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells *in vitro*. *J Cell Physiol*. 1977; 91:335–344. [PubMed: 301143]
13. Broxmeyer HE, et al. Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation in adults. *Proc Natl Acad Sci USA*. 1992; 89:4109–4113. [PubMed: 1373894]
14. Gabutti V, et al. Expansion of cord blood progenitors and use for hemopoietic reconstitution. *Stem cells*. 1993; 11(Suppl. 2):105–112. [PubMed: 7691315]
15. Mayani H, Dragowska W, Lansdorp PM. Cytokine-induced selective expansion and maturation of erythroid versus myeloid progenitors from purified cord blood precursor cells. *Blood*. 1993; 81:3252–3258. [PubMed: 7685200]
16. Migliaccio AR, Migliaccio G, Durand B, Mancini GC, Adamson JW. The generation of colony-forming cells (CFC) and the expansion of hematopoiesis in cultures of human cord blood cells is dependent on the presence of stem cell factor (SCF). *Cytotechnology*. 1993; 11:107–113. [PubMed: 7686025]
17. Brugger W, Scheding S, Ziegler B, Buhning HJ, Kanz L. Ex vivo manipulation of hematopoietic stem and progenitor cells. *Semin Hematol*. 2000; 37:42–49. [PubMed: 10718158]
18. Shpall EJ, et al. Transplantation of ex vivo expanded cord blood. *Biol Blood Marrow Transplant*. 2002; 8:368–376. [PubMed: 12171483]
19. Zidar BL, Shadduck RK, Zeigler Z, Winkelstein A. Observations on the anemia and neutropenia of human copper deficiency. *Am J Hematol*. 1977; 3:177–185. [PubMed: 304669]
20. Percival SS. Neutropenia caused by copper deficiency: possible mechanisms of action. *Nutr Rev*. 1995; 53:59–66. [PubMed: 7770185]
21. Peled T, et al. Cellular copper content modulates differentiation and self-renewal in cultures of cord blood-derived CD34⁺ cells. *Br J Haematol*. 2002; 116:655–661. [PubMed: 11849228]
22. Peled T, et al. Linear polyamine copper chelator tetraethylenepentamine augments long-term *ex vivo* expansion of cord blood-derived CD34⁺ cells and increases their engraftment potential in NOD/SCID mice. *Exp Hematol*. 2004; 32:547–555. [PubMed: 15183895]
23. de Lima M, et al. Transplantation of *ex vivo* expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial. *Bone Marrow Transplant*. 2008; 41:771–778. [PubMed: 18209724]
24. Barker JN, Weisdorf DJ, Wagner JE. Creation of a double chimera after the transplantation of umbilical-cord blood from two partially matched unrelated donors. *N Engl J Med*. 2001; 344:1870–1871. [PubMed: 11407361]
25. Brunstein CG, et al. Allogeneic hematopoietic cell transplantation for hematologic malignancy: relative risks and benefits of double umbilical cord blood. *Blood*. 2010; 116:4693–4699. [PubMed: 20686119]
26. Scaradavou A, et al. Double unit grafts successfully extend the application of umbilical cord blood transplantation in adults with acute leukemia. *Blood*. 2013; 121:752–758. [PubMed: 23223509]
27. Wagner JE. Should double cord blood transplants be the preferred choice when a sibling donor is unavailable? *Best Pract Res Clin Haematol*. 2009; 22:551–555. [PubMed: 19959107]
28. Verneris MR, et al. Relapse risk after umbilical cord blood transplantation: enhanced graft-versus-leukemia effect in recipients of 2 units. *Blood*. 2009; 114:4293–4299. [PubMed: 19706886]
29. Ramirez P, et al. Factors predicting single-unit predominance after double umbilical cord blood transplantation. *Bone Marrow Transplant*. 2012; 47:799–803. [PubMed: 21946383]
30. Cutler C, et al. Donor-specific anti-HLA antibodies predict outcome in double umbilical cord blood transplantation. *Blood*. 2011; 118:6691–6697. [PubMed: 21940825]
31. Barker JN, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood*. 2005; 105:1343–1347. [PubMed: 15466923]

32. Haspel RL, et al. Preinfusion variables predict the predominant unit in the setting of reduced-intensity double cord blood transplantation. *Bone Marrow Transplant.* 2008; 41:523–529. [PubMed: 18037942]
33. Somers JA, et al. Double umbilical cord blood transplantation: a study of early engraftment kinetics in leukocyte subsets using HLA-specific monoclonal antibodies. *Biol Blood Marrow Transplant.* 2013; 19:266–273. [PubMed: 23041604]
34. Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. *Development.* 2011; 138:3593–3612. [PubMed: 21828089]
35. Sethi N, Kang Y. Notch signalling in cancer progression and bone metastasis. *Br J Cancer.* 2011; 105:1805–1810. [PubMed: 22075946]
36. Milner LA, Kopan R, Martin DI, Bernstein ID. A human homologue of the *Drosophila* developmental gene, Notch, is expressed in CD34⁺ hematopoietic precursors. *Blood.* 1994; 83:2057–2062. [PubMed: 7512837]
37. Varnum-Finney B, et al. Pluripotent, cytokine-dependent, hematopoietic stem cells are immortalized by constitutive Notch1 signaling. *Nat Med.* 2000; 6:1278–1281. [PubMed: 11062542]
38. Varnum-Finney B, et al. Immobilization of Notch ligand, Delta-1, is required for induction of notch signaling. *J Cell Sci.* 2000; 113:4313–4318. [PubMed: 11069775]
39. Dallas MH, Varnum-Finney B, Martin PJ, Bernstein ID. Enhanced T-cell reconstitution by hematopoietic progenitors expanded *ex vivo* using the Notch ligand Delta1. *Blood.* 2007; 109:3579–3587. [PubMed: 17213287]
40. Varnum-Finney B, Brashem-Stein C, Bernstein ID. Combined effects of Notch signaling and cytokines induce a multiple log increase in precursors with lymphoid and myeloid reconstituting ability. *Blood.* 2003; 101:1784–1789. [PubMed: 12411302]
41. Delaney C, Varnum-Finney B, Aoyama K, Brashem-Stein C, Bernstein ID. Dose-dependent effects of the Notch ligand Delta1 on *ex vivo* differentiation and *in vivo* marrow repopulating ability of cord blood cells. *Blood.* 2005; 106:2693–2699. [PubMed: 15976178]
42. Delaney C, et al. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat Med.* 2010; 16:232–236. [PubMed: 20081862]
43. Musina RA, Bekchanova ES, Sukhikh GT. Comparison of mesenchymal stem cells obtained from different human tissues. *Bull Exp Biol Med.* 2005; 139:504–509. [PubMed: 16027890]
44. Crisan M, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell.* 2008; 3:301–313. [PubMed: 18786417]
45. Corselli M, Chen CW, Crisan M, Lazzari L, Peault B. Perivascular ancestors of adult multipotent stem cells. *Arterioscler Thromb Vasc Biol.* 2010; 30:1104–1109. [PubMed: 20453168]
46. Weber JM, Calvi LM. Notch signaling and the bone marrow hematopoietic stem cell niche. *Bone.* 2010; 46:281–285. [PubMed: 19679213]
47. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem.* 2006; 98:1076–1084. [PubMed: 16619257]
48. Robinson SN, et al. Superior *ex vivo* cord blood expansion following co-culture with bone marrow-derived mesenchymal stem cells. *Bone Marrow Transplant.* 2006; 37:359–366. [PubMed: 16400333]
49. McNiece I, Harrington J, Turney J, Kellner J, Shpall EJ. *Ex vivo* expansion of cord blood mononuclear cells on mesenchymal stem cells. *Cytotherapy.* 2004; 6:311–317. [PubMed: 16146883]
50. de Lima M, et al. Cord-blood engraftment with *ex vivo* mesenchymal-cell coculture. *N Engl J Med.* 2012; 367:2305–2315. [PubMed: 23234514]
51. Tang BL. Sirt1's systemic protective roles and its promise as a target in antiaging medicine. *Transl Res.* 2011; 157:276–284. [PubMed: 21497775]
52. Borradaile NM, Pickering JG. NAD⁺, sirtuins, and cardiovascular disease. *Curr Pharm Des.* 2009; 15:110–117. [PubMed: 19149606]
53. Narala SR, et al. SIRT1 acts as a nutrient-sensitive growth suppressor and its loss is associated with increased AMPK and telomerase activity. *Mol Biol Cell.* 2008; 19:1210–1219. [PubMed: 18184747]

54. Ou X, et al. SIRT1 deficiency compromises mouse embryonic stem cell hematopoietic differentiation, and embryonic and adult hematopoiesis in the mouse. *Blood*. 2011; 117:440–450. [PubMed: 20966168]
55. Peled T, et al. Nicotinamide, a SIRT1 inhibitor, inhibits differentiation and facilitates expansion of hematopoietic progenitor cells with enhanced bone marrow homing and engraftment. *Exp Hematol*. 2012; 40:342–355 e1. [PubMed: 22198152]
56. Fruscione F, et al. Regulation of human mesenchymal stem cell functions by an autocrine loop involving NAD⁺ release and P2Y₁₁-mediated signaling. *Stem Cells Dev*. 2011; 20:1183–98. [PubMed: 20964598]
57. Li Y, He J, He X, Li Y, Lindgren U. Nampt expression increases during osteogenic differentiation of multi- and omnipotent progenitors. *Biochem Biophys Res Commun*. 2013; 434:117–123. [PubMed: 23537654]
58. Horwitz ME, et al. Umbilical cord blood expansion with nicotinamide provides long-term multilineage engraftment. *J Clin Invest*. 2014; 124:3121–3128. [PubMed: 24911148]
59. Boitano AE, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science*. 2010; 329:1345–1348. [PubMed: 20688981]
60. Dvorak Z, Vrzal R, Starha P, Klanicova A, Travnicek Z. Effects of dinuclear copper(II) complexes with 6-(benzylamino)purine derivatives on AhR and PXR dependent expression of cytochromes P450 CYP1A2 and CYP3A4 genes in primary cultures of human hepatocytes. *Toxicol In Vitro*. 2010; 24:425–429. [PubMed: 19854261]
61. van Os R, et al. Engraftment of syngeneic bone marrow is not more efficient after intrafemoral transplantation than after traditional intravenous administration. *Exp Hematol*. 2010; 38:1115–1123. [PubMed: 20643182]
62. Castello S, et al. Intra-bone marrow injection of bone marrow and cord blood cells: an alternative way of transplantation associated with a higher seeding efficiency. *Exp Hematol*. 2004; 32:782–787. [PubMed: 15308330]
63. Brunstein CG, et al. Intra-BM injection to enhance engraftment after myeloablative umbilical cord blood transplantation with two partially HLA-matched units. *Bone Marrow Transplant*. 2009; 43:935–940. [PubMed: 19139736]
64. Frassoni F, et al. The intra-bone marrow injection of cord blood cells extends the possibility of transplantation to the majority of patients with malignant hematopoietic diseases. *Best practice & research Clin Haematol*. 2010; 23:237–244.
65. Rocha V, et al. Unrelated cord blood transplantation: outcomes after single-unit intrabone injection compared with double-unit intravenous injection in patients with hematological malignancies. *Transplantation*. 2013; 95:1284–1291. [PubMed: 23507699]
66. Tashiro K, et al. Signal sequence trap: a cloning strategy for secreted proteins and type I membrane proteins. *Science*. 1993; 261:600–603. [PubMed: 8342023]
67. Kim CH, Broxmeyer HE. *In vitro* behavior of hematopoietic progenitor cells under the influence of chemoattractants: stromal cell-derived factor-1, steel factor, and the bone marrow environment. *Blood*. 1998; 91:100–110. [PubMed: 9414273]
68. Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34⁺ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34⁺ progenitors to peripheral blood. *J Exp Med*. 1997; 185:111–120. [PubMed: 8996247]
69. Bleul CC, Fuhlbrigge RC, Casasnovas JM, Aiuti A, Springer TA. A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). *J Exp Med*. 1996; 184:1101–1109. [PubMed: 9064327]
70. Peled A, et al. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science*. 1999; 283:845–848. [PubMed: 9933168]
71. Christopherson KW 2nd, Paganessi LA, Napier S, Porecha NK. CD26 inhibition on CD34⁺ or lineage[−] human umbilical cord blood donor hematopoietic stem cells/hematopoietic progenitor cells improves long-term engraftment into NOD/SCID/Beta2null immunodeficient mice. *Stem Cells Dev*. 2007; 16:355–360. [PubMed: 17610365]

72. Broxmeyer HE, et al. Dipeptidylpeptidase 4 negatively regulates colony-stimulating factor activity and stress hematopoiesis. *Nat Med.* 2012; 18:1786–1796. [PubMed: 23160239]
73. Christopherson KW 2nd, Hangoc G, Broxmeyer HE. Cell surface peptidase CD26/dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34⁺ progenitor cells. *J Immunol.* 2002; 169:7000–7008. [PubMed: 12471135]
74. Christopherson KW 2nd, Hangoc G, Mantel CR, Broxmeyer HE. Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science.* 2004; 305:1000–1003. [PubMed: 15310902]
75. Farag SS, et al. *In vivo* DPP-4 inhibition to enhance engraftment of single-unit cord blood transplants in adults with hematological malignancies. *Stem Cells Dev.* 2013; 22:1007–1015. [PubMed: 23270493]
76. Ratajczak MZ, Kim C, Ratajczak J, Janowska-Wieczorek A. Innate immunity as orchestrator of bone marrow homing for hematopoietic stem/progenitor cells. *Adv Exp Med Biol.* 2013; 735:219–232. [PubMed: 23402030]
77. Reca R, et al. Functional receptor for C3a anaphylatoxin is expressed by normal hematopoietic stem/progenitor cells, and C3a enhances their homing-related responses to SDF-1. *Blood.* 2003; 101:3784–3793. [PubMed: 12511407]
78. Wysoczynski M, et al. Defective engraftment of C3aR^{-/-} hematopoietic stem progenitor cells shows a novel role of the C3a-C3aR axis in bone marrow homing. *Leukemia.* 2009; 23:1455–1461. [PubMed: 19357704]
79. Ratajczak J, et al. Mobilization studies in mice deficient in either C3 or C3a receptor (C3aR) reveal a novel role for complement in retention of hematopoietic stem/progenitor cells in bone marrow. *Blood.* 2004; 103:2071–2078. [PubMed: 14604969]
80. Brunstein CG, et al. Complement fragment 3a priming of umbilical cord blood progenitors: safety profile. *Biol Blood Marrow Transplant.* 2013; 19:1474–1479. [PubMed: 23892047]
81. North TE, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature.* 2007; 447:1007–1011. [PubMed: 17581586]
82. Yagi H, et al. Mesenchymal stem cells: mechanisms of immunomodulation and homing. *Cell Transplant.* 2010; 19:667–679. [PubMed: 20525442]
83. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood.* 2005; 105:1815–1822. [PubMed: 15494428]
84. English K, et al. Cell contact, prostaglandin E₂ and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4⁺CD25^{High} forkhead box P3⁺ regulatory T cells. *Clin Exp Immunol.* 2009; 156:149–160. [PubMed: 19210524]
85. DeGowin RL, Gibson DP. Prostaglandin-mediated enhancement of erythroid colonies by marrow stromal cells (MSC). *Exp Hematol.* 1981; 9:274–280. [PubMed: 7227477]
86. Cutler C, et al. Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation. *Blood.* 2013; 122:3074–3081. [PubMed: 23996087]
87. Jetmore A, et al. Homing efficiency, cell cycle kinetics, and survival of quiescent and cycling human CD34⁺ cells transplanted into conditioned NOD/SCID recipients. *Blood.* 2002; 99:1585–1593. [PubMed: 11861272]
88. Orschell-Traycoff CM, et al. Homing and engraftment potential of Sca-1⁺lin⁻ cells fractionated on the basis of adhesion molecule expression and position in cell cycle. *Blood.* 2000; 96:1380–1387. [PubMed: 10942381]
89. Geyer MB, et al. T cell depletion utilizing CD34⁺ stem cell selection and CD3⁺ addback from unrelated adult donors in paediatric allogeneic stem cell transplantation recipients. *Br J Haematol.* 2012; 157:205–219. [PubMed: 22313507]
90. Lang P, et al. Transplantation of highly purified peripheral-blood CD34⁺ progenitor cells from related and unrelated donors in children with nonmalignant diseases. *Bone Marrow Transplant.* 2004; 33:25–32. [PubMed: 14704654]
91. Peters C, et al. Transplantation of highly purified peripheral blood CD34⁺ cells from HLA-mismatched parental donors in 14 children: evaluation of early monitoring of engraftment. *Leukemia.* 1999; 13:2070–2078. [PubMed: 10602431]

92. Arber C, et al. Protection against lethal *Aspergillus fumigatus* infection in mice by allogeneic myeloid progenitors is not major histocompatibility complex restricted. *J Infect Dis.* 2005; 192:1666–1671. [PubMed: 16206084]
93. Arber C, et al. Common lymphoid progenitors rapidly engraft and protect against lethal murine cytomegalovirus infection after hematopoietic stem cell transplantation. *Blood.* 2003; 102:421–428. [PubMed: 12663447]
94. BitMansour A, et al. Myeloid progenitors protect against invasive aspergillosis and *Pseudomonas aeruginosa* infection following hematopoietic stem cell transplantation. *Blood.* 2002; 100:4660–4667. [PubMed: 12393415]
95. Broxmeyer HE, Pelus LM. Inhibition of DPP4/CD26 and dmPGE₂ treatment enhances engraftment of mouse bone marrow hematopoietic stem cells. *Blood Cells Mol Dis.* 2014; 53:34–38. [PubMed: 24602918]
96. Barker JN, Byam C, Scaradavou A. How I treat: the selection and acquisition of unrelated cord blood grafts. *Blood.* 2011; 117:2332–2339. [PubMed: 21149636]
97. Wagner, JE., et al. No survival advantage after double umbilical cord blood (UCB) compared to single UCB transplant in children with hematological malignancy: results of the blood and marrow transplant clinical trials network (BMT CTN 0501) randomized trial. Presented at the 54th American Society of Hematology Annual Meeting and Exposition; 2012. abstract 359
98. Wahid SF. Indications and outcomes of reduced-toxicity hematopoietic stem cell transplantation in adult patients with hematological malignancies. *Int J Hematol.* 2013; 97:581–598. [PubMed: 23585244]
99. Bart T. Cost effectiveness of cord blood versus bone marrow and peripheral blood stem cells. *Clinicoecon Outcomes Res.* 2010; 2:141–147. [PubMed: 21935324]
100. Majhail NS, et al. Costs of pediatric allogeneic hematopoietic-cell transplantation. *Pediatr Blood Cancer.* 2010; 54:138–143. [PubMed: 19693941]

Biographies

Dr. Troy C. Lund gained his MD in 2002 from the University of Minnesota, MN, USA. Dr. Lund is an Assistant Professor in the Division of Pediatric Blood and Marrow Transplant at the University of Minnesota. He holds a PhD in Medical Sciences from the University of South Florida, Tampa, FL, USA. He has a clinical focus on the strategies to overcome graft failure after transplant and a laboratory focus on the mechanisms governing hematopoietic cell homing/engraftment.

Anthony Boitano did his undergraduate work at Washington State University and obtained his Ph.D. from the University of Michigan. He was a post-doctoral fellow in the Schultz lab at The Scripps Research Institute. Anthony's currently an investigator in the Regenerative Medicine Department at the Genomics Institute of the Novartis Research Foundation where he is interested in identifying molecular tools that regulate hematopoietic stem cell self-renewal and lineage fate. With these tools, he hopes to manipulate hematopoietic stem cells to generate therapeutic doses of manufactured cells and blood products for clinical use.

Dr. John E. Wagner gained his MD in 1981 from Jefferson Medical College, Philadelphia, PA, USA. Currently, Dr. Wagner is Director of the Blood and Marrow Transplant Program in the Department of Pediatrics at the University of Minnesota. He performed the first umbilical cord blood transplant for leukemia worldwide in 1990, and since then his work has focused on the development of novel cellular approaches to enhance the efficacy of cord blood, including ex vivo expansion culture. He has published more than 250 papers in the transplant field.

Key points

Umbilical cord blood (UCB) transplantation is limited by cell doses, especially for adult recipients

UCB can be expanded in vitro greater than 500-fold.

UCB can be 'primed' just before infusion to influence cellular homing

Cell expansion or modulation of cell homing can alter engraftment kinetics in recipients

These strategies can increase the rapidity of engraftment and the next generation of clinical trials will determine the clinical efficacy of such approaches

Review criteria

All articles identified were English-language, full-text papers. The references chosen were based on the outcomes of pivotal clinical trials in which UCB was manipulated to enhance engraftment, either via expansion methodology or by priming to enhance homing. We also searched the reference lists of identified articles for further papers that reported the preclinical studies leading up to the clinical trials. Finally, basic science contributions to determining the mechanisms of each strategy were also reviewed in detail.

Figure 1.

Median times to neutrophil engraftment of mobilized PBSC, unrelated donor marrow and single UCB transplants after a myeloablative preparative regimen (transplant is on day 0). Engraftment is most often defined as an absolute neutrophil count >500 cells per μl for three consecutive days. The range is indicated by the orange box, with a line at the median. Abbreviations: PBSC, peripheral blood stem cells; UCB, umbilical cord blood.

Figure 2.

Activities of HSPC required for successful umbilical cord blood engraftment. HSPC home toward the bone marrow (1), expand within the bone-marrow microenvironment (2) and differentiate into mature cell lineages (3). Listed below each activity are the mediators that have been used in strategies to modulate UCB engraftment. Note that MSC are present in the marrow 'niche' and perhaps also take a *de novo* form as perivascular cells.

Abbreviations: DLL1, Delta-like ligand; G-CSF; granulocyte colony-stimulating factor; GM-CSF; granulocyte–macrophage colony-stimulating factor; HSPC, haematopoietic stem and progenitor cells; MSC, mesenchymal stromal cells; PGE₂, prostaglandin E2; SCF, stem cell factor; SDF-1, stromal cell-derived factor-1; TEPA, tetra-ethylenepentamine; TPO, thrombopoietin.

Figure 3.

General schema of a double UCB transplant platform. Before transplant, one UCB unit is thawed and manipulated by either priming with an agent to affect homing on the day of transplant (1–2 h) or expanded for 7–21 days before transplantation. In the two most-recent trials of UCB expansion, one UCB unit will be thawed, followed by positive selection of the HSPC of interest (AC133⁺ or CD34⁺ cells) and subsequently expanded with nicotinamide or SR1, respectively, for 21 days. The unselected AC133[–] or CD34[–] cell fraction is re-cryopreserved. After 5 days of preparative chemotherapy (typically), the unmanipulated UCB unit is infused, followed by the expanded UCB unit and then the thawed ‘unselected’ cells. Abbreviations: HSPC, haematopoietic stem and progenitor cells; UCB, umbilical cord blood.

Figure 4.

UCB manipulation offers improved time to neutrophil engraftment. Shown are the median times to neutrophil recovery from published clinical trials testing UCB manipulation involving expansion mediated by MSC, TEPA, Notch and cytokines, and priming of UCB by C3a, PGE₂ and sitagliptin. Note that the trials of factors in red text were performed in patients receiving a reduced intensity conditioning regimen, whereas those in black text were performed in myeloablated patients. Abbreviations: MSC, mesenchymal stromal cells; PBSC, peripheral blood stem cells; PGE₂, prostaglandin E2; TEPA, tetra-ethylenepentamine; UCB, umbilical cord blood.

Table 1
Ongoing or completed clinical trials of UCB manipulation listed in the ClinicalTrials.gov database*

Manipulation	Strategy	ClinicalTrials.gov identifier	Status per ClinicalTrials.gov
<i>Expansion</i>			
MSC	Double UCB expanded with myeloablative preparative regimen; MSC can be from a related family member or third party	NCT00498316	Currently recruiting participants
TEPA	Single UCB expanded with myeloablative preparative regimen	NCT00469729	This study is ongoing, but not recruiting participants
TEPA	Single UCB expanded with a reduced intensity preparative regimen.	NCT01484470	This study is currently recruiting participants
Nicotinamide	Double UCB platform with AC133 ⁺ cells selected and expanded; AC133 ⁻ cells are also infused	NCT01221857	This study is ongoing, but not recruiting participants
Nicotinamide	Double UCB platform with AC133 ⁺ cells selected and expanded; AC133 ⁻ cells are also infused	NCT01590628	This study is currently recruiting participants
Nicotinamide	Single UCB platform with AC133 ⁺ cells selected and expanded; AC133 ⁻ cells are infused	NCT01816230	This study is currently recruiting participants
SR1	Double UCB platform with CD34 ⁺ cells selected and expanded; CD34 ⁻ cells are also infused	NCT01474681	This study is currently recruiting participants
<i>Homing</i>			
C3a	Double UCB platform with a reduced intensity preparative regimen	NCT00963872	Study terminated (Lack of efficacy after interim analysis)
Sitagliptin	Single UCB with myeloablative preparative regimen	NCT00862719	This study is ongoing, but not recruiting participants.
Sitagliptin	Single UCB with myeloablative preparative regimen (multicentre trial)	NCT01720264	This study is currently recruiting participants.
PGE ₂	Double UCB platform with a reduced intensity preparative regimen	NCT00890500	Completed
PGE ₂	Single UCB platform with a reduced intensity preparative regimen	NCT01527838	Completed

* Patients in all trials had (or have) haematological malignancies, apart from trial NCT01590628, which is including patients with severe sickle-cell disease. Abbreviations: MSC, mesenchymal stromal cells; PGE₂, prostaglandin E₂; TEPA, tetra-ethylenepentamine; UCB, umbilical cord blood.

Table 2

Summary of UCB Manipulation Trial Data

Expansion strategy	Expansion	Cell dose [‡]			Day of engraftment [§]	Acute GVHD events
		Unmanipulated	Manipulated	Total		
Cytokine-based (n = 37) ¹⁸	TNC: 56-fold CD34 ⁺ cells: 4-fold	TNC: 1.2 × 10 ⁷ CD34 ⁺ cells: 7.4 × 10 ⁴	not given	TNC: 1.0 × 10 ⁷ CD34 ⁺ cells: 10.4 × 10 ⁴	Neutrophils: 28 Platelets: 108	Grade II–IV in 20 of 30 evaluable patients
TEPA (n = 10) ²³	TNC: 161-fold CD34 ⁺ cells: 2.3-fold	TNC: not given CD34 ⁺ cells: 0.4 × 10 ⁵	TNC: 0.6 × 10 ⁶ CD34 ⁺ cells: 1.2 × 10 ⁵	not given	Neutrophils: 30 Platelets: 48	Grade II in 4 of 9 patients
Notch-based with Delta ^{ext} -IgG (n = 10) ⁴²	TNC: 562-fold CD34 ⁺ cells: 164-fold	TNC: 3.3 × 10 ⁷ CD34 ⁺ cells: 2.4 × 10 ⁵	TNC: 4.6 × 10 ⁷ CD34 ⁺ cells: 6 × 10 ⁶	not given	Neutrophils: 16 Platelets: not given	Grade II in all 10 patients
MSC-based (n = 7 family MSC donor; n = 24 3 rd party MSC donors) ³⁰	TNC: 12.2-fold CD34 ⁺ cells: 30.1-fold	TNC: 2.3 × 10 ⁷ CD34 ⁺ cells: 0.4 × 10 ⁵	TNC: 5.8 × 10 ⁷ CD34 ⁺ cells: 1.0 × 10 ⁶	TNC: 8.3 × 10 ⁷ CD34 ⁺ cells: 1.2 × 10 ⁶	Neutrophils: 15 Platelets: 42	Grade II–IV in 13 patients
Nicord® (n = 11) ⁵⁸	TNC: 486-fold CD34 ⁺ cells: 72-fold	TNC: 2.6 × 10 ⁷ CD34 ⁺ cells: 0.6 × 10 ⁵	TNC: 2.5 × 10 ⁷ 3.5 × 10 ⁶	not given	Neutrophils: 13 Platelets: 33	Grade II in 5 patients
C3a priming (n = 29) ⁸⁰	NA	TNC: 2.5 × 10 ⁷ CD34 ⁺ cells: 3.4 × 10 ⁵	TNC: 1.5 × 10 ⁷ CD34 ⁺ cells: 3.2 × 10 ⁵	TNC: 4.0 × 10 ⁷ CD34 ⁺ cells: 7.6 × 10 ⁵	Neutrophils: 7 Platelets: not given	not given
DPP4 inhibition (n = 17) ⁵⁵	NA	NA	TNC: 2.4 × 10 ⁷ CD34 ⁺ cells: 1.0 × 10 ⁵	NA	Neutrophils: 21 Platelets: not given	Grade II in 1 patient
dmPGE2 priming (n = 12) ⁸⁶	NA	TNC: 1.7 × 10 ⁷ CD34 ⁺ cells: 0.6 × 10 ⁵	TNC: 1.8 × 10 ⁷ CD34 ⁺ cells: 0.7 × 10 ⁵	not given	Neutrophils: 17.5 Platelets: 43	Grade I–II in 4 patients

* Values shown are the reported medians.

[‡] Cell doses are cells/kg recipient weight.[§] Typically defined as an absolute neutrophil count >500 cells per µl for three consecutive days and a platelet count > 20,000 per µl for three consecutive days. Abbreviations: dmPGE₂, 16,16-dimethyl-prostaglandin E₂; DPP4, dipeptidyl peptidase 4; GVHD, graft-versus-host disease; MSC, mesenchymal stromal cells; NA, not applicable; TEPA, tetra-ethylene-pentamine; TNC, total nucleated cells.