Transplantation of cord blood stem cells for treating hematologic diseases and strategies to improve engraftment

Umbilical cord blood (CB) offers an alternative source of hematopoietic stem cells for the treatment of malignant and nonmalignant hematologic diseases. Umbilical CB has high levels of repopulating hematopoietic stem cells and is depleted of immune cells. It elicits low cell dose, and delayed and poor engraftment to the bone marrow. Umbilical CB is used for pediatric transplants as well as in adults. CB transplantation is associated with a high rate of mortality and morbidity, particularly in adults. To improve the success rate, patients are treated with a double CB dose. However, such a strategy limits the availability of CB transplants for patients and the conditions are still suboptimal. Novel avenues are being devised and considered; among them the expansion and propagation of CB stem cells *in vitro*, and the improvement of homing and engraftment of CB stem cells to the bone marrow. Such strategies would overcome the low cell dose in CB units by improving the delay and engraftment of CB stem cells to the bone marrow. Therefore, it would reduce the risk of early infections, improve the success rate of CB transplantation and make CB transplantation available to more patients. Umbilical CB offers a promising model for cellular therapy and regenerative medicine.

KEYWORDS: cancer ■ glycan ■ homing ■ leukemia ■ selectin ■ stem cell ■ therapy

Philippe Taupin

School of Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland
Tel.: +35 317 005 284
Fax: +35 317 005 412
philippe.taupin@dcu.ie

Umbilical cord blood (CB) offers an alternative source of hematopoietic stem cells (HSCs) to treat patients with malignant and nonmalignant hematologic diseases, such as leukemia, lymphoma, myeloma, Fanconi anemia, myelodysplasia and sickle cell anemia [1]. Umbilical CB contains a low cell dose, and the first transplantation CB was performed in children with Fanconi anemia, by Gluckman and collaborators at the hospital Saint Louis in Paris in 1989 [2]. Since then, CB transplantations have been performed with success in children, but also in adults. However, low cell dose, graft failure, poor engraftment in the bone marrow (BM), and delay in engraftment and immune reconstitution lead to significant morbidity and mortality, particularly in adults.

The rate of morbidity and mortality after CB transplants remains high in adults, with 47% of patients dying within 100 days of the unrelated transplant [3]. To improve the rate of success of CB transplantation, double doses of CB units are being infused into patients. However, this limits the availability of CB for therapy, conditions are still suboptimal and it does not address the main limitations of CB for the treatment of hematologic diseases. CB is considered a standard source of HSCs for pediatric transplants, and the transplantation of HSCs from CB is a viable alternative even in adults, as demonstrated by the number of adult patient transplants exceeding the number in child patients in the last 2–3 years [4–7].

Umbilical CB contains three- to six-times more repopulating hematopoietic progenitor and stem cells than the BM and mobilized peripheral blood (MPB) [8]. It is depleted of immune cells, particularly T lymphocytes, and has decreased natural killer cell activity, reduced alloproliferative, allostimulatory and allocytolytic capacity of mononuclear cells, and a lower frequency of alloreactive cytotoxic T lymphocyte precursors in CB mononuclear cells [9,10]. This allows for greater disparity of HLA antigens when matching donors to recipients than with BM and MPB. Currently, worldwide donor registries include over 14 million donors, including 8 million Americans, and the CB inventory represents only a small fraction of them. The registry of potential BM donors meets the needs of an estimated 60% of Caucasians in the USA, and only 5–15% of minorities. The chance that siblings will be a match is only 25%. Hence, umbilical CB provides an alternative model for unrelated allogeneic transplantation of HSCs and for potentially treating more patients, particularly minorities who are underrepresented in BM registries. Novel strategies are being devised and considered to address the main limitations of umbilical CB for transplantation, and are

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necessary in order to take full advantage of its potential for cellular therapy and regenerative medicine [11]. Among them, four strategies are being primarily reviewed and discussed in the manuscript; the direct delivery of umbilical CB to the BM, the transplantation of a double unit of CB, the fucosylation of selectin glycoprotein ligands *ex vivo*, and the expansion and propagation of CB stem cells *in vitro*.

HSCs & CB stem cells

The BM is the primary source of HSCs in the adult body. HSCs reside in the extravascular space of the endosteum region and in periarterial sites of the BM, primarily, but also that of other organs, from which they give rise to the differentiated lineages of the blood and immune systems [12]. Hematopoietic progenitor and stem cells, identified and characterized as populations highly enriched in $CD34^+$ and $CD34^+$ $CD38^{-/low}$ cells in various species, including rodents and humans, reconstitute the pool of HSCs of the BM after irradiation. Owing to their potential to generate the main phenotypes of the blood and immune systems, HSCs represent a model of choice for treating a broad range of hematopoietic diseases. Populations of $CD34^+$ hematopoietic progenitor and stem cells, capable of repopulating irradiated BM after transplantation, have been identified and characterized in umbilical CB [13,14]. The umbilical CB contains three- to six-times more repopulating hematopoietic progenitor and stem cells than the BM and MPB [8]. Hence, the umbilical CB demonstrates a greater potential to reconstitute the recipient BM than BM and MPB.

Umbilical CB is depleted of immune cells, particularly of T lymphocytes. It also contains a high proportion of 'naive' T cells that express the $CD45RA^+/CD45RO^-$, $CD62L^+$ phenotype. The CB produces increased amounts of IL-10, an anti-inflammatory cytokine, and expresses little cytotoxic activity. The CB, in contrast to BM and MPB, elicits low immunogenicity. This allows for a greater disparity of HLA antigens when matching donors to recipients with CB, compared with BM and MPB, and potentially permits treatment of more patients [15].

Selectins & the homing of HSCs

Hematopoietic stem cells migrate to their niches in the BM, a process referred as homing [12]. The homing contributes to the replenishment and maintenance of the pool of hematopoietic progenitor and stem cells in the BM and other tissues, and to the recruitment and mobilization of HSCs during injuries, particularly during immune responses and stress-induced recruitment of leukocytes. During development, this process is involved in the seeding of fetal HSCs to the BM, which involves the rolling and arresting of hematopoietic progenitor and stem cells through the blood vessels and across the endothelial vasculature, and complex molecular and cellular interactions.

P- & E-selectins & selectin glycoprotein ligands

P- and E-selectins are membrane-bound C-type lectins involved in the homing of HSCs to the BM. They are expressed on endothelial cells and BM vessels in mice and humans (Figure 1) [16–19]. P- and E-selectins bind ligands that are cell-surface proteo-*O*-glycan-conjugated ligands, or selectin glycoprotein ligands (SGLs). SGLs are expressed on various cell types, particularly $CD34^+$ hematopoietic progenitor and stem cells of the BM, in mice and humans (Figure 1). P-SGL-1 is the best characterized P-selectin ligand. It is a mucin expressed on $CD34^+$ cells and on leukocytes [20–23]. P- and E-selectins elicit differential binding affinity to P-SGL-1.

Selectin glycoprotein ligands have a characteristic N-terminal glycan determinant; a N-terminal glycan capped with a sialylated Lewis x (sLex) and a site for fucosylation (NeuAca2–3Galb1–4[Fuca1–3]GlcNAcb1-R). The enzymes α1–3 fucosyltransferase (FT)VI and FTVII are responsible for α1–3 FT activity [24–28]. These enzymes are present on the cell surfaces of various cell types, particularly hematopoietic cells of the BM of mice and humans, where they fucosylate their substrates. SGLs on $CD34^+$ cells of the BM elicit N-terminal glycan determinants with an α2–3-linked sialic acid and an α1–3-linked fucose. The N-terminal glycan determinant of SGLs corresponds to the binding site of hematopoietic cells to P- and E-selectins (Figure 1) [20,23]. The site for fucosylation is most likely to occur at a core 2 *O*-glycan linked to a threonine in the N-terminal binding site for P-selectin on SGLs [21,23]. The residues of the binding site of SGLs to P- and E-selectins include fucose, galactose, sialic acid and sulphated tyrosines [29–31]. P-SGL-1 that are capped with sLex and that are sulphated bind with strong affinity to P- and E-selectins [30]. Hence, post-translational modifications, particularly fucosylation, of SGLs on hematopoietic cells and HSCs underlie their binding to P- and E-selectins.

Homing of hematopoietic progenitor & stem cells of the BM & CB

CD34$^+$ hematopoietic progenitor and stem cells isolated and purified from human BM or MPB, and administered intravenously, in irradiated nonobese diabetic/severe combined immune deficiency (NOD/SCID) mice, home to the BM of those mice. Their rolling activities on microvessels of and homing to the BM are diminished after the injection of blocking monoclonal antibodies to P-selectin or to P-SGL-1. Their homing capabilities are also impaired in irradiated NOD/SCID mice deficient in P- and E-selectins [22]. By contrast, CD34$^+$ hematopoietic progenitor and stem cells isolated and purified from human CB demonstrate poor rolling activities on microvessels of and poor homing capabilities to the BM after transplantation into irradiated NOD/SCID mice [22]. Hence, HSCs of the BM and CB demonstrate different capabilities of homing to the BM. CD34$^+$ hematopoietic progenitor and stem cells of the BM and MPB roll on endothelial cells and BM vessels, and home to the BM, a process mediated by the interaction of SGLs with selectins, in contrast to their CB counterpart. HSCs of the BM bind to P- and E-selectins on endothelial cells and BM vessels for rolling and homing to the BM in mice and humans. P-SGL-1 underlies the tethering of leukocytes to E-selectin in flow studies *in vitro*, and the tethering and rolling of leukocytes to, and on, P-selectin *in vivo* [32]. The interaction of SGLs with selectins also plays an important role in the homing of leukocytes to lymphoid tissues and their recruitment to sites of inflammation [18,20,21,23,33].

Similar to human CD34$^+$ hematopoietic progenitor and stem cells of the BM, human CB CD34$^+$ cells express P-SGL-1 on their surface. As opposed to BM CD34$^+$ cells, most (approximately 75%) of CB CD34$^+$CD38$^{-/low}$ cells lack the sLex and site for fucosylation of the N-glycan determinant of SGLs and do not bind to P- and E-selectins. Hence, SGLs of hematopoietic progenitor and stem cells of the CB express a form of P-SGL-1 that does not bind to P- and E-selectins [22]. It is proposed that the inability of SGLs of CB hematopoietic progenitor and stem cells to bind to P- and E-selectins underlies the diminished rolling and homing activity of the cells on BM vessels and to the BM in NOD/SCID mice [22]. The inability of SGLs of CB hematopoietic progenitor and stem cells to bind to P- and E-selectins originates from the low or absent expression of the N-glycan determinant on hematopoietic progenitor and stem cells of the CB (FIGURE 1) [34,35]. This low or absent expression of the N-glycan determinant – a post-translational event – particularly the fucosylation, may originate from insufficient levels of FTVI to physiologically fucosylate the SGLs on their surface.

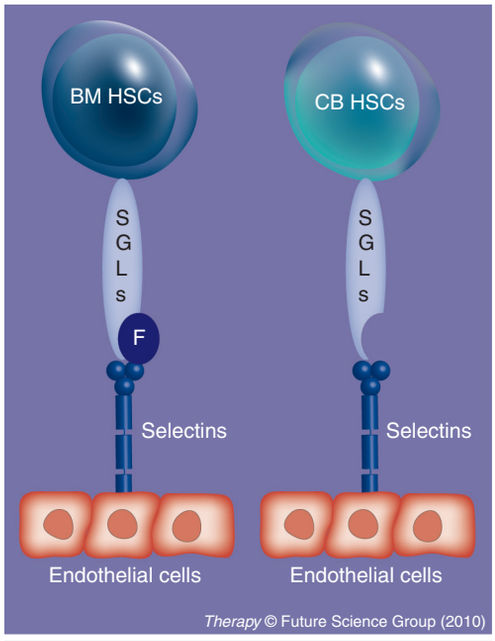

Figure 1. Selectins and selectin glycoprotein ligands on endothelial cells, and on hematopoietic stem cells of the bone marrow and cord blood. P- and E-selectins are membrane-bound C-type lectins involved in the homing of HSCs. They are expressed on endothelial cells and vessels of the BM. SGLs are expressed on CD34$^+$ hematopoietic progenitor and stem cells of the BM and CB. SGLs elicit a characteristic N-terminal glycan determinant; an N-terminal glycan capped with a sialylated Lewis x and a site for fucosylation. SGLs on HSCs of the BM, but not of the CB, have N-terminal glycan determinants with a α1–3-linked fucose (F). The N-terminal glycan determinant of SGLs corresponds to the binding site of HSCs to P- and E-selectins. SGLs, for which the N-terminal glycan determinant is not fucosylated, have a defect in binding to P- and E-selectins. HSCs of the BM and CB elicit different capabilities of homing to the BM. CD34$^+$ hematopoietic progenitor and stem cells of the BM roll on endothelial cells and BM vessels, and home to the BM, a process mediated by the interaction of SGLs selectins, in contrast to their CB counterpart. The defect in fucosylation of SGLs on the surface of CB HSCs underlies their poor capabilities of homing to the BM. Hence, post-translational modifications, particularly fucosylation, of SGLs on HSCs underlie their binding to P- and E-selectins, and their homing capabilities to the BM.
BM: Bone marrow; CB: Cord blood; F: Fucose; HSC: Hematopoietic stem cell; SGL: Selectin glycoprotein ligand.

Overall, the interaction of SGLs with selectins plays an important role in the homing of HSCs and leukocytes. The N-glycan determinant, the sLex and the site for fucosylation on SGLs is critical for the rolling and homing of HSCs on endothelial cells and BM vessels, and to the BM.

Other factors are involved in the process of migration on endothelial cells and homing of HSCs to the BM. These include CD44, chemokines, integrins, lymphocyte function-associated antigen 1, P- and E-selectins, stem cell factor (SCF), stromal-derived factor 1, vascular cell adhesion molecule-1, very late antigen-4/5, and their respective ligands or receptors [17–19,33,36,37].

Transplantation of CB stem cells for the treatment of hematologic diseases

The CB contains three- to six-times more repopulating hematopoietic progenitor and stem cells than the BM and MPB, providing a promising source of transplant for the treatment of hematologic diseases. However, CB transplants are associated with low cell count, graft failure, and delayed engraftment and immune reconstitution, leading to significant morbidity and mortality, particularly in adults. Transplantation-related mortality (TRM) following unrelated CB transplantation is primarily due to infections related to slow engraftment and immune-incompetence.

Total nucleated cell count & age of patients

Cord blood has a low total nucleated cell count and a low number of $CD34^+$ cells. The rate of success of unrelated CB transplantation is positively correlated with the nucleated cell dose and the number of $CD34^+$ cells of the CB transplanted per kilogram of body weight of the recipient, and with the younger age of the patient [38–40]. Higher total nucleated cell count, number of $CD34^+$ cells in the graft and younger age of the patient correlate positively with improved engraftment, survival of the graft and outcome for the patient [3,41]. A 20% TRM after 100 days was reported for children with acute myeloid leukemia following unrelated CB transplantation, with a collected nucleated cell dose higher than 5.2×10^7/kg [42]. The rate of success of unrelated CB transplantation for treating hematologic diseases is reported to be higher in children than in adult patients [40]. The transplantation of CB in children still has delays in engraftment and in immune reconstitution, despite receiving sufficient nucleated cell doses [43–47]. Hence, even in children, the transplantation of CB is still suboptimal. Owing to the low nucleated cell dose, unrelated CB transplantation is considered a standard procedure for the treatment of hematologic diseases in children, particularly for children with very poor prognosis acute myeloid leukemia who lack a HLA-identical sibling [1,4,42,48,49].

In adults, the rate of mortality of patients with hematologic diseases who are treated with unrelated, matched, CB transplants is as high as 47% and the rate of disease-free survival is 26% in the first 100 days after myeloablative transplantations [3]. After 3 years, the rate of leukemia-free survival is 23% for adult patients treated with unrelated, matched, CB transplants, it is 33% for patients treated with unrelated matched BM transplants and 19% for unrelated single-antigen mismatched BM transplants [50,51]. In a nonmyeloablative regimen, a 26% unrelated, matched, CB TRM was reported at 3 years [52]. While most studies reveal that, for unrelated transplantation, BM has a higher rate of success in adult patients than CB, others report a TRM rate of 9% for adults with hematologic malignancies following unrelated CB transplantation [53]. Hence, nonmyeloablative conditioning favors CB transplantation as a strategy for treating patients with hematologic diseases, particularly for older individuals, and unrelated CB transplantation could be as effective as unrelated BM or MPB transplantation for treating adult patients.

Early infections & delayed engraftment

As chemotherapy and radiation treatments to kill cancer cells suppress patients' immune systems, the patients are at risk of early infections. After chemotherapy and radiation treatment, impaired recipient thymopoiesis and lack of transferred memory cells contribute to delayed T-cell recovery. This results in an increased risk of opportunistic infections. This is a major risk for patients undergoing HSC transplant. Unrelated CB transplant is associated with delayed engraftment, and, therefore, with delayed reconstitution of the patients' immune system and with higher risks of early infections than BM and MPB transplants [38,54,55]. Hence, early infection is a major risk for patients undergoing CB transplantation and is one of the reasons for the lower success rate of CB transplantation. Patients treated with CB transplants for hematologic diseases face months of recovery and treatments to ensure a successful transplantation therapy.

The delayed engraftment of CB HSCs in patients after transplant reveals that CB cells have poor homing and engraftment capabilities to the BM after intravenous infusion, compared with BM and MBP transplants. In addition, the fact that T lymphocytes of the CB show decreased expression of two enzymes that contribute to eradicating viral infections – granzyme and perforin – is a contributing factor to the risk of early infections, particularly viral infections, in patients treated with a CB transplant [56,57]. Both of these factors contribute to the higher risk of early infections in patients treated with CB transplants.

Graft-versus-host disease

Graft-versus-host disease (GVHD) is the immunological reaction and associated damage that occurs when transplanting immunologically competent cells into a host whose immune system is compromised. The degree of severity of GVHD is related to the HLA disparity between the donor and the recipient, with the severity enhanced with increased HLA disparity. It ranges from mild to fatal [58]. Mild forms of GVHD can also be beneficial to the patients, particularly for cancer patients (e.g., leukemia patients). In mild forms of GVHD, the graft-derived lymphocytes attack the cancer cells, increasing the chance of successful therapy for the patients, a phenomenon referred as graft-versus-tumor effect (e.g., graft-versus-leukemia effect). The transplantation of allogeneic CB has a low risk of severe GVHD, particularly lower risks of grade II, III or IV acute GVHD than after unrelated BM transplantation [38,51,54,55]. The reasons for the low risk of severe GVHD for CB transplants are twofold; first the CB is depleted of immune cells and, second, it produces increased amounts of IL-10, an anti-inflammatory cytokine, and little cytotoxic activity [15]. The increased production of the anti-inflammatory cytokine IL-10 and low cytotoxic activity may downregulate GVHD after CB transplant [59–61].

On the one hand, because allogeneic CB transplantation allows for greater HLA disparity and has a lower risk of severe GVHD, it is appropriate for a broader range of patients than BM or MPB transplantations. On the other hand, as allogeneic CB transplantation has a low risk of GVHD, it is more suited for the treatment of patients with nonmalignant hematologic diseases, for whom GVHD risk must be minimized and for whom there is no need for a graft-versus-tumor effect, than for patients with malignant diseases, such as leukemia [62–64]. In addition, patients with nonmalignant hematologic diseases have a lower risk of relapse and are less likely to be in need of post-transplant therapies. Therefore, allogeneic CB transplantation is particularly suited to a broad range of patients with nonmalignant hematologic diseases, particularly children. Similarly, the autologous transplantation of BM is more suited for the treatment of patients with nonmalignant hematologic diseases [65].

Overall, unrelated CB transplantation is promising for the treatment of hematologic diseases, particularly for children and adults with acute leukemia who lack a HLA-matched BM donor. Despite CB having a higher potential to reconstitute the recipients' BM, there are still limitations in the use of CB as therapy for the treatment of hematologic diseases, beside the low nucleated cell dose in the CB. The main limitations of CB for transplantation are the delayed engraftment and immune reconstitution, leading to significant morbidity and mortality, particularly in adults. CB transplantation is more suited for the treatment of nonmalignant hematologic diseases for a broad range of patients, particularly children. Strategies aimed at improving the nucleated cell dose, homing and engraftment of CB must be devised and developed to improve the rate of success of CB transplantation, and to reduce the risk of early infections and the mortality and morbidity rate after CB transplantation, particularly in adults.

Strategies to improve the outcome of CB transplantation for the treatment of hematologic diseases

Several strategies are being devised and proposed to improve the outcome of CB transplantation for the treatment of hematologic diseases. Among them are the direct delivery of CB stem cells to the BM, the increase of doses or units of CB transplanted, the improvement of the engraftment and homing of CB stem cells to the BM, and the expansion and propagation of CB stem cells *in vitro* to generate cell lines for transplantation.

Direct delivery of CB stem cells to the BM

To overcome the poor engraftment and homing capabilities of CB stem cells to the BM, a strategy has been proposed to directly transplant umbilical CB into the BM cavities. Studies report mixed results and benefits for the patients [66,67]. Further investigations are required to prove the benefits of a strategy that is more invasive for the patients than the intravenous infusion of CB stem cells.

Transplantation of double CB units

To overcome the low nucleated cell dose present in CB, transplantation of either pooled or sequential multiple units of umbilical CB has been proposed [54,68–70]. The use of multiple CB units for allogeneic transplantation requires the matching of the various donors to the recipient HLA antigens, as well as the various donors to each other, a difficult and less probable task as the number of units increases. The transplanted units must be closely HLA matched to the recipients to reduce the risk of GVHD. They must be closely HLA matched to each other to reduce the risk of immunoreactivity among them or graft-versus-graft effect, which would prevent engraftment of the units. Therefore, the use of two CB units is considered as the standard for multiple CB unit transplantation. Double CB units achieve a nucleated cell dose of 0.23×10^8 nucleated cells/kg [54]. They are generally composed of a matched and a partially matched CB unit to the recipient HLA antigens.

Double CB unit transplantation has been applied successfully to improve the outcome of CB transplantation in children and adults in need of HSC transplant. Successful therapies with double CB unit transplantation have been reported with no graft failures and 54% disease-free survival at the 3-year mark in myeloablative transplantations [71]. Double CB unit transplantation improves the outcome of the transplantation in humans [72–74]. It improves myeloid and platelet engraftment rates, but not the time to engraftment and does not trigger immunologic rejection in patients [68]. Cases of chronic GVHD have been reported [75]. Conflicting data have been reported on the contribution of each CB unit to the transplant. Some studies report that only one unit contributes to long-term hematopoiesis, while others report that both CB units contribute to hematopoiesis, with the unit comprising a higher nucleated cell and $CD34^+$ cell dose being associated with cord predominance in the transplant [72,73,75–77]. The failure of one unit to engraft results from the immune rejection mediated by effector $CD8^+$ T cells that develop after CB transplantation [73]. Despite only one unit engrafting in most patients, double CB unit transplantation improves the outcome of the transplantation by increasing the probability of one/the most viable CB unit engrafting [74].

Double CB unit transplantation increases the nucleated cell count of the transplant, and improves engraftment of the cells and immune reconstitution in patients. It is associated with an improved rate of success of CB transplantation [78–80]. Nonetheless, the engraftment is still suboptimal. Double CB unit transplantation is still associated with delayed engraftment and a higher rate of engraftment failure than BM and MPB transplantations. Several strategies have been devised and used successfully to circumvent problems associated with engraftment in double CB transplantation. Among them are the use of a reduced-intensity conditioning regimen of fludarabine, melphalan and antithymocyte globulin, which leads to a 14% TRM after 100 days [81], and the use of specific antibodies, such as rituximab [82]. Reduced-intensity or nonmyeloablative regimens use lower doses of pretransplant chemotherapy drugs and/or radiation than the traditional high-dose, myeloablative regimens, reducing the toxicity of these treatments. Rituximab is a monoclonal antibody against the protein CD20, a phosphoprotein expressed on the surface of B cells. It is used in transplants involving incompatible blood groups. Hence, double CB unit transplantation represents a promising strategy for the treatment of hematologic diseases in children and adults. It is particularly suited for patients for whom a perfectly single matched unit is not available, and may reduce the risk of relapse in patients treated for hematologic malignancies owing to higher graft-versus-tumor effect, than with single CB unit transplantation.

Fucosylation of selectin glycoprotein ligands *ex vivo*

To overcome the poor engraftment capabilities of CB stem cells to the BM, it has been proposed to fucosylate the SGLs expressed on CB cells *ex vivo*, prior to transplantation. CB $CD34^+$ hematopoietic progenitor and stem cells express forms of SGLs that lack the proper N-glycan determinant, the sLex and the site for fucosylation. The lack of proper N-glycan determinant of SGLs on the surface of HSCs results in the inability of the SGLs to bind to P- and E-selectins, and poor rolling and homing capabilities of CB HSCs on BM vessels, and to the BM [22]. The fucosylation of the N-glycan determinant of SGLs has been reported to be critical for the homing and rolling activities of HSCs *in vivo*. Rolling and homing on blood and BM vessels, and to the BM are key steps for therapies involving the transplantation of HSCs [12]. It is proposed that improved homing capabilities of CB HSCs would improve their engraftment to the BM. *Ex vivo* fucosylation, by FTVI, of SGLs on the surface of CB HSCs would restore

the binding properties of SGLs, and the homing and rolling potential of CB HSCs to the BM. Therefore, *ex vivo* fucosylation of SGLs expressed on CB cells would improve their engraftment to the BM. As a consequence, it would reduce the delayed engraftment and reduce the risk of early infections associated with CB transplants, thereby improving the rate of success of CB transplantation.

The treatment of human CB CD34$^+$ hematopoietic progenitor and stem cells *ex vivo* by FTVI in the presence of GDP fucose increases the yield of fucosylation of sLex determinants on the surface of the cells, particularly on P-SGL-1 (Figure 2). It results in improved binding of the cells to fluid-phase P- and E-selectins, and improves cell rolling on P- and E-selectins under flow *in vitro* [83]. It enhances the homing of the cells to their engraftment in the BM, through more effective rolling interactions of CD34$^+$ cells with P- and E-selectins in BM vessels, in irradiated NOD/SCID mice after infusion [83]. Hence, the fucosylation of CB CD34$^+$ hematopoietic progenitor and stem cells *ex vivo*, by FTVI, improves the homing of CB HSCS to and their engraftment in the BM [83].

A blocking antibody to the P- and E-selectin-binding region of P-SGL-1 does not inhibit the binding of CB CD34$^+$ cells to fluid-phase P-selectin after fucosylation *ex vivo* [83]. This reveals that surface fucosylation creates additional binding sites for P- and E-selectin on other regions of P-SGL-1, or on other glycoproteins or glycolipids. It further reveals that CB HSCs may not necessarily roll on P- and E-selectins, after their surface fucosylation; they may roll on other glycoproteins or glycolipids. In addition, surface fucosylation of CB cells does not adversely affect their ability to repopulate the BM after homing.

Hence, the surface fucosylation of CB stem cells has important consequences for therapeutic applications involving CB transplants for treating hematologic diseases. The force fucosylation of the SGLs, and other glycoprotein or glycolipid sites expressed on the surface of human CB cells, is proposed to improve the homing of CB HSCs to the BM and their engraftment to the BM. It is a simple and efficient procedure performed *ex vivo* prior to transplantation. It involves a 30 min incubation, at 37°C, of the CB cells with FTVI. Short-term biochemical treatment with exogenous FTVI and GDP fucose only transiently increases fucosylated glycans on CB cells, which decline as glycoproteins and glycolipids turn over and as cells divide. Owing to the fact that the increased fucosylation is transient, it is less likely to affect the long-term functions of HSCs and accessory cells after they enter the BM of conditioned recipients [83]. It would improve homing of CB HSCs to the BM and their engraftment in the BM. However, forced

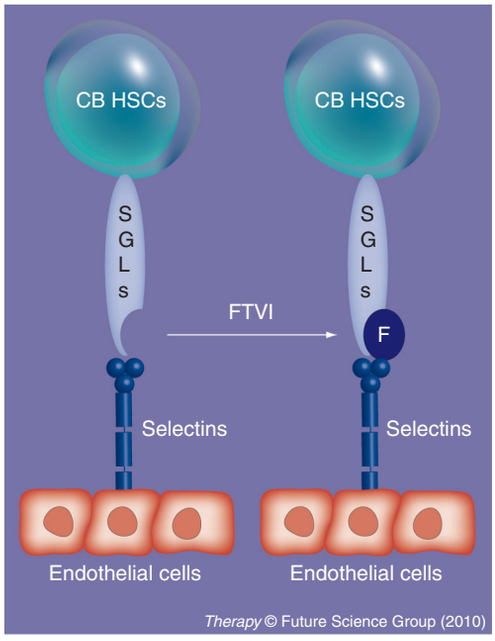

Figure 2. Fucosylation of selectin glycoprotein ligands on the surface of hematopoietic stem cells of the cord blood *ex vivo*. HSCs of the CB elicit a defect in binding to P- and E-selectins and poor homing capabilities to the bone marrow. The defect in fucosylation of SGLs on the surface of HSCs of the CB underlies their inability to bind to P- and E-selectins, and their poor capabilities of homing to the bone marrow. The enzyme α1–3 FTVI is responsible for α1–3 fucosyltransferase activity. The defect in fucosylation of SGLs on the surface of CB HSCs may originate from insufficient levels of FTVI to physiologically fucosylate the SGLs on their surface. The treatment of human CB CD34$^+$ hematopoietic progenitor and stem cells *ex vivo* by FTVI in the presence of guanosine diphosphate fucose increases the yield of fucosylation of the N-glycan determinant of SGLs on the surface of the HSCs (F). *Ex vivo* fucosylation of SGLs by FTVI on the surface of CB HSCs would restore the binding properties of SGLs, and the homing and rolling potential of CB HSCs to the bone marrow. Surface fucosylation of CB stem cells would improve their engraftment to the bone marrow. As a consequence, it would reduce the delayed engraftment and reduce the risk of early infections associated with CB transplants, thereby improving the success rate of CB transplantation.
CB: Cord blood; F: Fucose;
FTVI: Fucosyltransferase VI; HSC: Hematopoietic stem cell; SGL: Selectin glycoprotein ligand.

fucosylation of CB HSCs might improve rolling on selectins, but does not increase entry into the BM if the cells also lack integrins, chemokine receptors or other molecules essential for homing [84]. Many factors, including SCF, fetal liver tyrosine kinase-3 ligand, erythropoietin, granulocyte colony-stimulating factor (G-CSF) and IL-11, are involved in the osteoblastic niche, and in the interaction between the niche and the stem cells [85]. The modulation of these factors may affect the homing and engraftment of CB stem cells to the BM, and particularly the rolling on selectins and entry into the BM. In addition, *ex vivo* manipulations on the graft are hampered by difficulties, such as stem cell loss and dendritic cell activation, and are extremely expensive if performed under good manufacturing practice-grade conditions. Hence, forced fucosylation of CB HSCs represents an alternative strategy for improving engraftment of CB stem cells to the BM. However, it remains to be further confirmed and validated, particularly in humans, before being brought to therapy.

Expansion & propagation of CB stem cells *in vitro*

Another strategy to overcome the low nucleated cell dose present in CB is to expand and propagate CB stem cells *in vitro*, in order to generate populations of CB stem and progenitor cells for transplantation. Stem cells are self-renewing multipotent cells that generate a large number of differentiated progenies through a transient amplifying population [86]. Three strategies have been proposed and are being considered for the expansion and propagation of CB stem cells *in vitro*.

The first strategy is the expansion and propagation of progenitor and stem cells from CB *in vitro*, or liquid culture. Progenitor and stem cells are isolated from umbilical CB and cultured *in vitro*, in the presence of growth factors and cytokines [5,87,88]. Several protocols and cocktails of growth factors/cytokines have been reported for propagating CB-derived progenitor and stem cells *in vitro*. Among the latter are [71,87]:

- SCF, IL-3, IL-6 and G-CSF
- SCF, thrombopoietin and G-CSF
- Fetal liver tyrosine kinase-3 ligand, SCF, IL-3, IL-6, IL-11 and G-CSF

δ-1, a membrane-bound ligand of the Notch receptor, induces a 100-fold increase in the number of human $CD34^+$ $CD38^-$ CB cells and promotes their lymphoid differentiation *in vitro* [89]. The cytokine pleiotrophin also promotes the expansion of repopulating populations of human CB, $CD34^+$ $CD38^-$ Lin^- cells, *in vitro* and *in vivo*. As well as its use *in vitro* to promote the expansion and propagation of human CB stem cells, pleiotrophin may be used to promote hematopoiesis *in vivo* [90]. Generally, CB-derived progenitor and stem cells expand and propagate more rapidly and generate a larger number of progenies *in vitro* than their BM counterpart. The second strategy involves the co-culture of progenitor and stem cells from the CB with mesenchymal stromal cells *in vitro*, or stromal co-culture. The microenvironment or niche controls the developmental potential of stem cells [91]. Mesenchymal cells from the marrow stroma elicit immuno-modulatory activity on and promote the engraftment of CB $CD34^+$ cells when co-administered in NOD/SCID mice [71,92,93]. CB progenitor and stem cells co-cultured with mesenchymal stromal cells, in presence of fetal bovine serum and a growth factor cocktail (such as SCF, thrombopoietin and G-CSF), promote the growth and expansion of CB-derived progenitor and stem cells by 10–20-fold, and of $CD34^+$ cells by 16–37-fold [71,94]. The third strategy involves the culture of progenitor and stem cells from the CB *in vitro* in bioreactors, or continuous perfusion culture systems.

The *in vitro* expansion and propagation of CB stem cells may benefit the transplantation of CB for the treatment of hematologic and immune diseases. This is achieved by generating and expanding CB stem cells in an unlimited fashion, thereby overcoming the low nucleated cell count present in CB – the main limiting factor in CB transplantation. It may also benefit the use of CB stem cells for regenerative medicine and gene therapy, for the treatment of a broad range of diseases and injuries [95,96]. With this aim in mind, it is important to note the ability of CB stem cells to generate and differentiate into other phenotypes, including the neuronal lineages, to produce neurotropic factors/cytokines, and to modulate immune and inflammatory reactions that may contribute to extending their potential for regenerative medicine, particularly for the treatment of neurological diseases and disorders [97]. One of the main advantages of expanding CB stem cells *in vitro* is the availability of the generated stem cells for the recipients for post-transplant therapies. In one schema, it is proposed to transplant an unmanipulated CB unit to the patient and

expand the same unit for later use [98–102]. The main limitations regarding the expansion and propagation of CB stem cells for therapeutic use are: the risk of altering the developmental and therapeutic potential of the cells, particularly stem cell loss and dendritic cell activation, the fact that optimal culture conditions remain to be established, and the high cost associated with generating cell lines under good manufacturing practice-grade conditions [103–105].

Other strategies are also being considered to improve the engraftment of CB stem cells to the BM, such as *ex vivo* graft engineering to improve T-cell recovery, pharmacologic interventions to preserve thymopoiesis, the reduction of the toxicity of the conditioning regimens and the transplantation of two populations of stem cells, as well as other methods of improving homing [106–108]. Delaney and collaborators recently reported the development of a Notch-mediated expansion of human $CD34^+$ CB progenitor cells *ex vivo* [109]. Cells were cultured for 17–21 days on immobilized engineered Notch ligand in the presence of cytokines. The Notch-mediated expansion of CB progenitor cells resulted in over a 100-fold increase in the absolute number of stem/progenitor cells *ex vivo* and in the rapid engraftment of stem cells to the BM of humans in the clinical trial [109]. These strategies are at different stages of therapeutic application, from basic research to advanced phases of clinical studies. Hence, several strategies are being devised and considered, and are promising to improve the engraftment of CB stem cells to the BM. In all, CB is considered a standard source of HSCs for pediatric transplants, but its use is not limited to children. More than 80% of CB transplants are performed in adults with malignancies, and results in nonmalignant disorders have been improved by the use of reduced-intensity conditioning and double CB transplant.

Conclusion

Umbilical CB is enriched in repopulating HSCs, is depleted of immune cells and is easily accessible. It provides a model choice for treating hematologic and BM diseases. CB transplant is considered a standard procedure for the treatment of hematologic diseases in children. It is more suited for the treatment of nonmalignant hematologic diseases for a broad range of patients, including adults. There are, however, limitations to the use of umbilical CB for transplantation: low cell dose, delayed engraftment and early infections, particularly. Current strategies to improve the rate of success of CB transplantation, particularly in adults, involve the infusion of double CB doses. However, this limits the availability of CB units for therapy and the engraftment is still suboptimal. Future strategies to improve the rate of success of CB transplantation, particularly in adult patients, involve the forced fucosylation of CB stem cells *ex vivo*, the expansion and propagation of CB stem cells *in vitro*, reduced-intensity conditioning, and the use of Notch-mediated expanded CB progenitor cells *ex vivo*. Forced fucosylation of CB stem cells *ex vivo* and Notch-mediated expanded CB progenitor cells *ex vivo* are being considered to improve the homing and engraftment capabilities of CB stem cells to the BM, whereas the expansion and propagation of CB stem cells *in vitro* are aimed at providing cell lines for cellular therapy and regenerative medicine. These strategies will give the opportunity to treat more patients. Strategies aiming at improving the homing and engraftment capabilities of the CB stem cells to the BM may also be applied to other types of stem cells, such as embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells, neural stem cells and very small embryonic-like stem cells, to improve their engraftment and therapeutic potential, particularly when administered intravenously [110–112]. Future investigations will aim at validating the novel technologies that will not only take advantage of the full potential of umbilical CB for therapy, but will also enhance it.

Future perspective

In 5–10 years, CB stem cell therapy will be the model of choice for the treatment of malignant and nonmalignant hematologic diseases. Improvement in engraftment and immune reconstitution after CB transplantation will lead to more successful treatments, not only in children, but also in adults. CB stem cell therapy will also be a model of choice for regenerative medicine.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject natter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or parents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Umbilical cord blood (CB) offers an alternative source of hematopoietic stem cells for the treatment of malignant and nonmalignant hematologic diseases.
- Umbilical CB is used for pediatric and adult transplants, but is associated with high rates of mortality and morbidity, particularly in adults.
- Various strategies are being developed and considered to improve CB transplantation, among them are double CB transplantation and improving the homing and engraftment capabilities of CB stem cells.
- Administration of double doses of CB improves the rate of success of transplantation, but the conditions are still suboptimal.
- *Ex vivo* fucosylation of CB stem cells prior to transplantation provides a novel strategy for improving homing and engraftment to the bone marrow, but it remains to be confirmed and validated. Another strategy for improving homing and engraftment consists of using Notch-mediated expanded CB progenitor cells *ex vivo*.
- The expansion and propagation of CB stem cells *in vitro* provides an alternative strategy for treating hematologic diseases, as well as for regenerative medicine, but conditions for expansion and growth of the cells remain to be established.

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