Background and Future Considerations for Human Cord Blood Hematopoietic Cell Transplantation, Including Economic Concerns

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ABSTRACT

Cord blood (CB) has been used since 1988 as a source of hematopoietic stem cells (HSCs) and progenitor cells for hematopoietic cell transplantation (HCT) to treat patients with malignant and nonmalignant disorders. CB has both advantages and disadvantages when compared with other tissue sources of HSCs such as bone marrow and mobilized peripheral blood, which are also being used in the setting of HCT. This short review focuses on some historical information, as well as current efforts that are being assessed to enhance the efficacy of CB HCT. Also of importance are the costs of CB, and the feasibility and economics of using such to be identified, and newly confirmed improvements worldwide for the greatest number of patients. In this context, simple methods that would not necessarily entail the need for selected cell-processing facilities to ex vivo expand or improve the CB graft's functional activity may be of interest, with one such possibility being the use of an orally active inhibitor of the enzyme dipeptidylpeptidase 4, alone or in combination with other new and innovative approaches for improving HSC engraftment and in vivo repopulating capability of CB.

Numbers of hematopoietic stem cells are greatly decreased in the blood of a newborn within 24–36h after birth and quickly reach numbers associated with adults.

INTRODUCTION

October 2013 marks the 25th anniversary of the first umbilical cord blood (CB) transplant [1], which was used to treat and save the life of a child with Fanconi anemia [2], an eventually fatal genetic disorder. This transplant was successful and cured the hematological manifestations of Fanconi anemia by replacing the recipient's genetically diseased blood cells with HLA-matched CB from the patient's sister. The recipient of this first CB transplant is alive and well 25 years later. This procedure opened up the door to what has now been over 30,000 CB transplants performed with sibling, related, or unrelated donor cells that have been either fully or partially HLA-matched with the recipients who manifested a wide range of malignant and nonmalignant disorders [3]. Although it was believed by some after the first CB transplant that CB transplantation would have limited application [4], it is now known that all such disorders currently treated with a bone marrow (BM) transplant can be just as successfully treated with a CB transplant [1,3]. The long-term engrafting cells in CB or BM that allow for such life-saving replacement therapy in these settings are the hematopoietic stem cells (HSCs), very rare populations of cells that have the capacity to make

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Advantages and Disadvantages of Using Cord Blood for Hematopoietic Cell Transplantation Compared with Bone Marrow

Advantages	*Disadvantages*
Ease and safety of obtaining cells without harm to baby or mother	Slower time to neutrophil, platelet, and immune cell recovery
Efficient storage as units of HLA-typed cells in cryopreserved form in CB banks	Higher rate of graft failure
Available for immediate use after storage	Limited number of CB cells collected from babies at birth
Elicitation of a lower level of GVHD than BM cells	

more of themselves (self renew) or to differentiate into all types of blood cells [5–8].

Advantages and disadvantages of CB

There are advantages and disadvantages of using CB, compared with BM, for hematopoietic cell transplantation (HCT) [1,3]. The advantages of CB include the ease and safety of obtaining the cells without harm to the baby or mother; their efficient storage as units of HLA-typed cells in cryopreserved form in CB banks that are available for immediate use; and their elicitation of a lower level of graft-versus-host disease (GVHD) than BM cells, which has allowed them to be more forgiving in terms of partial HLA mismatches between donor cells and recipients. However, there are disadvantages of CB compared with that of BM, and these include a slower time to neutrophil, platelet, and immune cell recovery, plus a higher rate of graft failure. Some but perhaps not all of the disadvantages of CB as a source of transplantable HSCs may relate to the limited numbers of CB cells collected from babies at birth. What is collected at birth is all that is available because numbers of HSCs and their immediate progeny, hematopoietic progenitor cells (HPCs), which have limited or no self-renewal capacity but can differentiate down multiple or more restricted blood cell lineages than HSC [5–8], are greatly decreased within 24–36h after birth. These cells quickly reach numbers associated with adults [9], which are extremely limited unless they are mobilized from the marrow to the blood by agents such as granulocyte colony-stimulating factors (G-CSF), AMD3100 (now known as Plerixafor), or the mobilizing agents alone or in combination [10]. This mobilization procedure is used to obtain cells from children and adults for HCT [11–13].

Historical perspective

Some personal historical information for CB HCT has been provided elsewhere [1,3,14–22], but in brief, the laboratory of one of the authors, through basic research efforts, established that the limited numbers of CB cells collected at the birth of a baby could serve as a source of transplantable HSCs and HPCs [14]. It was this laboratory-based study—which analyzed numbers of HPCs in CB collections, their cryopreservation, and transportation—that led to the first proof-of-principle CB bank in the Broxmeyer laboratory from which came the HLA-matched CB collections used for the first five, and two subsequent, sibling CB transplants performed [2,3,17,23,24]. The scientific and clinical studies were both published in 1989 [2,14], but it was the scientific article [14], the information for which was available many years before the first transplant was performed, that led us to envision and initiate the collaborations for the first CB HCT. We waited until we knew that the first CB transplant was successful before submitting both articles for publication. In fact, this was a fortuitous circumstance, as the scientific article that came out in print before the clinical study elicited an editorial that said, in no uncertain terms, that our suggestions that CB could serve as a source of transplantable HSCs and HPCs was "off-base" and extremely dangerous, as it was known that CB contained maternal cells and would thus likely elicit fatal maternal cell-induced GVHD [25]. However, before initiating the first CB HCT, we had believed that CB transplantation would elicit less GVHD than that of BM because of the relative immature nature of immune cells in CB versus BM. When the editorial disparaging our scientific article came out, we already knew that the first CB HCT had worked well and, consistent with our beliefs, there was little or no noticeable GVHD [2]. Many subsequent clinical studies have clearly shown that CB elicits less GVHD than BM in related and unrelated HCT (reviewed in [3]). There are maternal cells in CB, but such cells were low in frequency and did not appear to be a counteracting force for the use of CB for HCT.

Economic concerns for the future of CB HCT

There have been numerous clinical studies in children and adults that have highlighted the clinical usefulness of CB for HCT [1,3]. Improvements in CB collections, and in conditioning regimens, have helped to make CB HCT a more efficacious treatment for malignant and nonmalignant disorders. Moreover, as noted below, both scientific laboratory-based and clinical investigations are getting us closer to further improvements to enhance the use of CB for HCT. However, with all the positive aspects in this improvement, there are concerns about the future of CB HCT. This is not a scientific or clinical concern but rather one of an economic nature in that the field has the real potential to price itself out of competition as a source of HSCs for HCT.

The U.S. health system is currently in crisis. In the very near future, it is anticipated that there will be a significant increase in the proportion of patients supported by Medicare, and with declining reimbursements by other third-party payers, the practice of such high-cost procedures as HCT is likely to be significantly affected. The acquisition cost from a public CB bank of a single CB unit to a transplantation center is approximately $40,000, and recent new regulations licensing requirements by the FDA for CB banks are likely to further drive the expense up as the increased cost of banking may be passed onto users. Also, beyond the cost of acquisition of a CB unit, the daily cost of a CB transplant procedure is considered higher than that of adult-source stem cell donor transplantation [26], mostly related to more frequent posttransplant complications and longer inpatient hospitalization. As the CB field progresses technologically, therefore, the scientific community needs to be cognizant of these economic considerations. Recently, the use of double CB units to deliver a larger dose of transplanted CB cells has dramatically increased, with apparently no clear benefit on time to engraftment, posttransplant complications, or length of stay [27]. For many transplant programs that have a fixed case rate of reimbursement, the acquisition cost of two CB units for transplantation is already prohibitive. Therefore, while scientifically sound and exciting, currently investigated approaches focusing on accelerating engraftment by co-infusing T cell–depleted haploidentical peripheral blood cells with a CB unit [28–30], or a variety of CB expansion procedures in which one CB unit is expanded and co-infused with an unmanipulated unit [31–34], are in danger of not being economically viable because of the significant added complexity and cost of cellular manipulation, reducing their overall impact on the field. These economic concerns are not meant to cast a negative outlook on these exciting and relatively new treatments with manipulated or ex vivo-cultured cells, and the scientific and clinical investigations are still ongoing to enhance CB HSC and HPC functions and CB HCT, but to make those involved in the field of CB HCT aware that eventually costs will need to be contained. This may entail going back to the use of single CB units for CB HCT, if the circumstance will allow this without compromising treatment outcome. A focus on simple approaches that enhance graft function and engraftment of single CB units, including those that pharmacologically enhance homing [35–42], may be more economically viable and have greater exportability to centers with less resources and so ultimately have greater impact on the field of CB transplantation. The paradigm we have previously mentioned at talks and in print is that simple may trump complicated, especially if it is efficacious and less costly, and that the simpler the procedure the better. Toward this "simpler the better" concept, the next section describes attempts by a number of groups to enhance the functional activities of CB HSC for ex vivo expansion, and in vivo homing and engraftment, as well as our attempts to find a simple and less costly means for some of these approaches.

Means to enhance CB engraftment

Because of limiting numbers of cells obtained in each collection of CB, the use of two CB units per recipient was instituted in order to increase the numbers of CB cells infused [3,43–47]. This procedure increased the numbers of CB transplants performed in adult patients and is used worldwide, although it is not certain that two CB units are better than one. What is known is that the time to neutrophil, platelet, and immune cell reconstitution is not significantly faster with two CB infusions than that obtained when one CB unit is used, and that in the great majority of cases only one CB unit wins out after a month or two, with the other unit gone or present at low or nondetectable frequency. There have been no definitive studies yet that can clearly identify the "winning" CB unit in a double-CB HCT, although it is believed, but not yet definitively proven, that this may be an immune-mediated phenomenon and that T cells or $T8^+$ cells are involved. What is clear is that the use of two CB units for HCT result in more GVHD, at least that of chronic GVHD, than does one CB unit, and thus in a way negates some of the benefits of single-CB unit HCT, which has been associated with a relatively lower amount of GVHD elicited than that of BM or double-CB HCT.

In attempts to make CB HCT a more efficient procedure, a number of different maneuvers have been attempted. This includes, but is not necessarily limited to, (i) collection of more CB via perfusion of placenta [48], (ii) intramarrow infusion of CB to deal with the low homing efficiency of CB HSC during intravenous administration of the cells [27,49,50], or (iii) combining a haplo identical family member's cells or that of an HLA-matched unrelated donor with a single CB unit [28–30]. The first effort has not been widely attempted and would likely present a major logistical problem for the hospitals where the CB is collected. The second procedure is a bit more invasive, and although there is some evidence that it may be beneficial, more data are necessary in order to confirm a benefit. The third procedure is clearly more complicated and more information is required to determine if there is a beneficial effect.

In addition to the above three efforts, others have investigated means to either ex vivo expand CB HSC so that more cells are available, or have evaluated means to enhance the homing efficiency of the HSC so that more HSCs get to the BM microenvironment where the cells can be nurtured and grow. It may be that combinations of such efforts will be more efficacious than that of any one approach.

Ex vivo expansion of HSCs for CB HCT

Efforts to expand HSCs have been ongoing for decades, but while successes were

apparent for mouse BM cells in this endeavor, successful efforts for similar outcomes with human cells have been more limited. As our knowledge of the microenvironment and growth factors and cytokines that nurture HSC function has increased [5,6], so too have our efforts of using this knowledge for ex vivo expansion of human HSC. There have now been a few reports published that have assessed such ex vivo expansion efforts in a clinical setting to overcome the low cell numbers present in single-CB HCT. One such maneuver has been to use the notch ligand, Delta1, during the ex vivo culture of CB with selected cytokines [31]. This culture condition resulted in increased short-term repopulating HSC and a time to neutrophil engraftment more rapid than historical control numbers using nonmanipulated CB. While encouraging, the ex vivo-cultured cells were part of a double-CB HCT in which one unit was ex vivo cultured and the other unit was unmanipulated. After a month or so, all donor cells were from the nonmanipulated CB unit. Thus, at present, the use of such ex vivo notch ligand cultured cells with only an increase in short-term repopulating HSCs would not likely be useful as a single source of cells for HCT as it would be associated with the inherent risk of late-term graft failure. Additional efforts to ex vivo expand CB for HCT have utilized coculture of CB with mesenchymal cells during the ex vivo culture period [32]. Such efforts have also shown some effectiveness in shortening the time to neutrophil engraftment compared with historical controls using nonmanipulated CB, but again these studies were performed in context of a double-CB HCT in which one unit had been ex vivo expanded with selected cytokines along with coculture with mesenchymal cells, and the other CB unit was not manipulated. Thus, it is not yet clear if such ex vivo-cultured cells could by themselves provide both short- and long-term repopulating HSC, and thus save the life of a recipient in need of an HCT.

Several other efforts have been published dealing with ex vivo manipulation of human CB cells. This includes use of nicotinimide [33], aryl hydrocarbon receptor antagonists [34], or the mTOR inhibitor rapamycin [35], each in combination with selected cytokines during the culture phase of the ex vivo expansion culture system. It remains to be seen if both short- and long-term engrafting HSCs are expanded, and if so, how effectively these efforts can be translated to a clinical CB HCT setting, and if these manipulated units can be used effectively in the setting of a single manipulated CB unit, without the need for a second nonmanipulated CB unit in order to maintain long-term engraftment in recipients.

Technological advances will likely result in a manipulated CB unit with both short- and long-term HSC repopulating capacity that can be used by itself, without need for a double-CB HCT, and it may be that those studies mentioned above, or others yet to be done or reported, will satisfy the criteria for rapid and long-term engraftment with a single-CB unit HCT. However, regardless of whether or not this is accomplished, these ex vivo culture maneuvers will have to be done in selected cell processing units, and these efforts will be time-consuming and will add extra expenses to an already-expensive health-care procedure. Thus, while such efforts to learn more about enhancing CB HSC function and such use for clinical efficacy are underway, it is also important to look for simpler maneuvers that may incur less expense, and that will be able to be used in an expanded clinical setting that may not require a sophisticated cell processing center but rather could be done in any reputable transplant unit worldwide.

Enhancing homing and HCT engrafting capabilities of CB/HSCs

Treatment of donor CB populations containing HSCs, before infusion of these cells into animal models, has shown preclinical efficacy in an animal model of HCT. Such approaches have assessed fucosylation [36], pretreatment of cells with a modified prostaglandin (PG) E2 [37–39], or pretreatment of cells with an inhibitor of the enzyme dipeptidylpeptidase (DPP) 4 [40,41,51]. Fucosylation of CB cells has enhanced the homing and engrafting capability of these treated cells in sublethally irradiated NS2 mice [36]. Pretreatment of mouse BM or human CB cells for minutes with a modified PGE2 molecule has, respectively, enhanced the engraftment of these cells into lethally irradiated congenic mice and sublethally irradiated immune-deficient mice [37,38], with efforts in nonhuman primates [39] and humans [52] showing safety, with a modest decrease to the time to engraftment seen in a human study in context of a double-CB transplant including one manipulated and one unmanipulated CB unit [52].

Use of DPP4 inhibition to enhance homing/engraftment of HSCs and CB HCT

Our own laboratory has focused on the use of DPP4 inhibitors to enhance preclinical efforts at HCT [40,41,51]. DPP4 is a member of the prolyl oligopeptidase family [53]. It is a cell surface serine protease that has the capacity to cleave with selectivity, proline, alanine, and perhaps with less efficiency serine and other specific amino acids that are in an N-terminal penultimate position of the start site of a protein.

Stromal cell–derived factor (SDF)-1/CXCL12, a member of the chemokine family of molecules, is a potent chemoattractant, with chemotactic activity, for a number of cell types, including HSCs and HPCs, which express the SDF-1/CXCL12 receptor CXCR4 [54]. SDF-1/CXCL12 has also been implicated in the homing in vivo to the BM microenvironment of infused HSCs and HPCs [55]. This chemokine has a DPP4 truncation site, and we have shown that DPP4 truncates SDF-1/CXCL12 and that truncated SDF-1/

CXCL12 is not chemotactic, but it does block chemotaxis of the full-length, nontruncated form of SDF-1/CXCL12 [56]. Moreover, inhibition of DPP4 on HSC/HPC with a small peptide (diprotin A [ILE-PRO-ILE] or the dipeptide VAL-PYR) greatly enhances the chemotaxis of these cells to full-length SDF-1/CXCL12 [56]. With this information, we subsequently demonstrated that inhibition of DPP4 by pulse-exposure of donor mouse BM cells to diprotin A or VAL-PYR, or functional deletion of CD26 in CD26 knock-out (−/−) donor mouse BM cells, greatly increased the homing and engrafting capability of limiting numbers of long-term marrow repopulating and self-renewing HSCs [40]. These studies were confirmed by us in both competitive and noncompetitive HSC assays in lethally irradiated congenic mouse recipients, and were also confirmed by others [57–59]. Such studies were, in addition, confirmed by us [51] and others [42] for engraftment of human CB $CD34^+$ cells, or human G-CSF-mobilized peripheral blood $CD34^+$ cells [60] in sublethally irradiated NOD/SCID mice. We have taken this effort into the clinic and have had encouraging, but not yet definitive, results regarding enhancing time to neutrophil engraftment by in vivo inhibition of DPP4 to enhance engraftment of single-CB unit HCT in adults with high-risk hematological malignancies [61]. In this trial, the patients were treated in vivo one time per day for a few days with an FDA-approved orally administered and active DPP4 inhibitor, sitagliptin, just before and after administration of the single CB unit. Sitagliptin is being used to treat type 2 diabetes, and there was evidence from our studies and those of others that in vivo inhibition of DPP4 to recipient mice [41,60,62] could enhance engraftment of nonmanipulated donor cells. It was clear from our published report [61] that we had not optimized the use of sitagliptin to most efficiently reduce in vivo DPP4 levels, and it is possible that a more optimized schedule of sitagliptin administration may improve time to blood-cell recovery after CB HCT. Such efforts are currently ongoing and are part of an National Institutes of Health (NIH)–funded multicenter clinical trial.

One major advantage in the use of a DPP4 inhibitor, either sitagliptin or a next-generation and more effective DPP4 inhibitor, is the simplicity of the treatment: the recipients take a pill (e.g., sitagliptin) perhaps two times a day for a few days in the midst of the conditioning regimen used to pretreat patients for an HCT. This does not entail manipulation of the CB unit to be infused, or additional invasive procedures beyond that usually used in an HCT. When the clinical trial with sitagliptin was first envisioned in its planning stages, and after it had already been initiated and a number of patients were treated as noted above, our belief was that inhibition of DPP4 would mainly enhance the homing process of the infused cells, most likely through, but not necessarily limited to, enhancing the effectiveness of SDF-1/CXCL12 as a homing molecule by preventing its truncation by CD26/DPP4-expressing donor HSC/HPC. However, since this clinical trial initiation, we have found that DPP4 can truncate and inactivate a number of hematopoietically active cytokines such as the colony-stimulating factors (CSFs: granulocyte [G], macrophage [M]-CSF, G-CSF, interleukin [IL]-3, and erythropoietin [EPO]) [41] among a number of other cytokines and growth factors for hematopoietic cell regulation that have putative and perhaps active DPP4 truncation sites, such as thrombopoietin, IL-1, IL-2, IL-5, IL-6, IL-8, IL-10, and IL-13; subsets of IL-17, IL-22, IL-23, IL-27, IL-28, IL-29, and inhibin/activin; a number of isoforms of vascular endothelial growth factor; and many other proteins [63,64]. At least for GM-CSF, G-CSF, IL-3, and EPO, DPP4 truncation produces a less active CSF, but one that can block and dampen the activity of the full-length form of their respective protein, an effect at least for GM-CSF that entails the truncated GM-CSF binding with higher affinity to the GM-CSF receptor than full-length GM-CSF and this truncated GM-CSF blocking receptor binding of full-length GM-CSF in competitive fashion [41]. The truncated GM-CSF results in greatly reduced intracellular signaling involving reduced phosphorylation of JAK2 and STAT5, and the truncated GM-CSF blocks full JAK2 and STAT5 phosphorylation by full-length GM-CSF [41]. Inhibiting DPP4 enhances the CSF activities of GM-CSF and EPO in vivo and in vitro, and enhances the CSF in vitro activity of G-CSF and IL-3 [41]. In total, it is likely that endogenous inhibition of DPP4 will enhance the nurturing as well as homing capacities of HSCs to and within the BM microenvironment. Preclinical data have shown that $CD26^{-/-}$, or DPP4 inhibition by oral administration of sitagliptin to mice, greatly enhances the speed of recovery and amplitude of hematopoiesis in mice subjected to the stress of lower (400 cGy) or higher (650 cGy) nonlethal doses of radiation, and nonlethal doses of drugs such as 5-flurouracil or cyclophosphamide [41]. It is based on these findings that we are optimistic that correct timing and dosing of a DPP4 inhibitor has the real potential to significantly and greatly enhance time to engraftment of single-CB unit HCT in adults. It is also possible that it can have this effect in children, although DPP4 inhibitors have not yet been approved for treatment in children, so safety studies will need to be done in this context. Thus far, sitagliptin has been used for CB HCT in nonremission, end-stage patients. It is likely that improvements in context of time to engraftment of single-CB unit HCT in patients given sitagliptin may be substantially enhanced if the recipients are in remission, and engraftment may also be enhanced as newer efforts are undertaken to improve the conditioning regimens used to prepare patients for an HCT.

It may also be that combinations of treatments would enhance CB HCT. One could consider donor CB cells pulse-treated for a short time (minutes to an hour) with either a DPP4 inhibitor or PGE2 before infusion of the cells into recipients pretreated with a DPP4 inhibitor. Such preclinical efforts are currently ongoing.

Concluding thoughts

The field of CB HCT has come a long way since our initial scientific laboratory [14] and clinical studies [2,3]. However, efforts are still ongoing to improve the use and efficacy of CB for HCT. It is important

Keywords
(in order of appearance)

cord blood (CB)
bone marrow (BM)
hematopoietic stem cells (HSCs)
hematopoietic cell transplantation (HCT)
human leukocyte antigen (HLA)
hematopoietic progenitor cells (HPCs)
graft-versus-host disease (GVHD)
granulocyte colony-stimulating factors (G-CSF)
prostaglandin E2 (PGE2)
dipeptidylpeptidase (DPP)
stromal cell-derived factor (SDF)
colony-stimulating factor (CSF)
granulocyte (G)
macrophage (M)
interleukin (IL)
erythropoietin (EPO)
thrombopoietin (TPO)
vascular endothelial growth factor (VEGF)

with all efforts to improve this life-saving treatment, to not only consider the science involved but also consider the costs of the procedures. It would certainly be counterproductive to demonstrate an improved setting for CB HCT and then find that the costs to use this improvement are so prohibitive that sources of HSCs and HPCs other than those found in CB are used instead.

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