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Overcoming the barriers to umbilical cord blood transplantation

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Abstract

Umbilical cord blood (UCB) transplantation (UCBT) has seen a marked increase in utilization in recent years, especially in the pediatric population; however, graft failure, delayed engraftment and profound delay in immune reconstitution leads to significant morbidity and mortality in adults. The lack of cells available for post-transplant therapies, such as donor lymphocyte infusions, has also been considered a disadvantage. To overcome the cell–dose barrier, the combination of two UCB units is becoming commonplace in adolescent and adult populations, and is currently being studied in pediatrics as well. In some studies, the use of two UCB units appears to have a positive impact on outcomes; however, engraftment is still suboptimal. A possible additional way to improve outcome and extend applicability of UCBT is via ex vivo expansion. Studies to develop optimal expansion conditions are still in the exploratory phase; however, recent studies suggest expanded UCB is safe and can improve outcomes. The ability to transplant across HLA disparities, rapid procurement time and decreased graft-versus-host disease (GvHD) seen with UCBT makes it a promising stem cell source and, while barriers exist, consistent progress is being made to overcome them.

Keywords

adolescent; adult; double-unit cord blood transplant; *ex vivo* expansion; hematopoietic stem cells; umbilical cord blood

Introduction

Umbilical cord blood (UCB) has become an important source of hematopoietic stem cell (HSC) support following myeloablative and non-myeloablative therapies (1–3). UCB is rapidly available and appears to have a lower incidence of graft-versus-host disease (GvHD) despite HLA disparity. This makes it an attractive option for many patients, including

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patients with non-malignant disease where GvHD should be minimized and proceeding to transplant rapidly may be of prime importance. In addition, because of the allowance for greater HLA disparity than bone marrow (BM) or peripheral blood stem cell grafts, UCB has provided a significantly higher chance of finding a donor, especially for minority populations that are currently underrepresented in donor registries.

While the use of UCB as a stem cell source has seen a significant increase in recent years, especially in children and young adults, it is not without drawbacks. One of the major limitations of UCB as an HSC therapy is the low cell dose available for transplantation. It is now well documented that the total nucleated cell (TNC) dose transplanted per kilogram (kg) of body weight of the recipient correlates with outcomes (4–6). As a consequence, UCB transplantation (UCBT) remains significantly more successful in children (5). Also, even in children receiving satisfactory cell doses, there is still often some delay in engraftment of all cell lines compared with traditional stem cell sources (7–9) and in immune reconstitution (10,11), suggesting that, even in the optimal patient population, the low progenitor cell dose given with UCBT could have negative effects on outcomes.

In general there have been two approaches to overcome the obstacle of low TNC cell dose seen with UCBT. One has been to utilize more than one UCB unit in order to achieve a higher number of TNC available for infusion (12–15). Many trials are currently underway assessing the efficacy and outcomes in both adults and children (Table I). The second approach has been to attempt to expand UCB units *ex vivo. Ex vivo* expansion can be performed on either a portion of a UCB unit or the unit in its entirety, with the expanded cells infused either at the time of transplant of ‘unmanipulated’ fraction or given at a separate time. The manipulated UCB could be from either the same unit or, potentially, a different UCB unit. The combination of *ex vivo*-expanded fractions and unmanipulated UCB fractions might prove to be a beneficial strategy (16,17) and clinical trials are currently underway (18–22) (Table II).

Double-unit UCBT

In an effort to overcome the issue of low cell dose with a single cord, case reports of combining cord blood units started appearing in the late 1990s (12,14,21–28). In early studies, up to 12 cord blood units were used per patient but, because of the cost of the units and resource allocation, two units are generally regarded as the standard in multiple cord blood protocols. To date, hundreds of double-unit UCBT (DUCBT) have been performed (29–32). The concept of combining two closely HLA-matched UCB units, while increasing the total cell dose delivered, has raised come concerns, including the possibility of increased rates of GvHD or lack of engraftment as a result of immunoreactivity among the transplanted units. On the other hand, it has been hypothesized that the infusion of multiple units can induce immune tolerance and reduce the risk of rejection and/or GvHD (33).

One initial concern was that double-unit UCB infusions could lead to a ‘graft-versus-graft’ effect, preventing engraftment. Early case reports demonstrated that both UCB units contributed to hematopoiesis (14,34); however, it has been shown that typically only one unit contributes to long-term hematopoiesis (15,35). In ablative studies, 76% of patients

showed hematopoiesis from a single cord by day 21 and all patients by day 100, whereas in the reduced intensity setting 57% of patients had a single cord only at day 21, 9% at day 100 and all at 1 year (36). One study suggested that longer mixed chimerism was associated with a higher risk of chronic GvHD (37); however, this has not been examined further in larger studies. Predicting which UCB unit will ultimately provide durable hematopoiesis has been challenging. The initial Barker *et al.* (36) study found a link between a higher CD3 dose and UCB predominance; however, this association disappeared as more patients were accrued. They also reported no link between infused cell dose and unit predominance. In contrast, other studies have suggested that both a higher TNC and $CD34^+$ cell dose are associated with cord predominance (25,38). The question of order of unit infusion has been asked, with contradictory results (39,40).

Better matching at HLA class 1 (HLA-A, -B, -C) has been associated with improvement in time to neutrophil and platelet engraftment (38,39). Engraftment, however, does not seem to have any relation to matching between UCB units. Class I matching had no effect on acute (a)GvHD (39). Analysis on the predominant cord HLA compared with the patient HLA typing found no effect of HLA match on which UCB unit eventually predominated (38).

DUCBT: ablative and reduced intensity trials

Barker *et al.* (41) published the first large study of myeloablative DUCBT, with no graft failures, a 54% disease-free survival (DFS) at 3 years and only 13% grade III or IV aGvHD and 23% chronic (c) GvHD. In DUCBT recipients conditioned with fludarabine, melphalan and anti-thymocyte globulin (ATG), the 100-day treatment-related mortality (TRM) was 14%, with 1-year DFS of 67%. Acute GvHD grade II–IV occurred in 40% of patients (37). While the use of DUCBT has been suggested to reduce risk of relapse (42) and outcomes in some populations have been reported as comparable to related donors (29), others report no benefit of two units over a single unit (43). Further studies are warranted. Certainly many adult patients will not have a single unit that is satisfactory, and in those patients double cord blood transplant is certainly promising.

Successful DCBT following reduced-intensity conditioning has now also been investigated by many for patients who have factors precluding an ablative regimen (35,37,40). In a study of 95 adult patients (44) who received either a double ($n = 78$) or single ($n = 17$) UCBT with Cyclophosphamide/Fludarabine/Total Body irradiation conditioning, TRM for DUCBT recipients was 19% at day 180 and 26% at 3 years. The 3-year survival was 45%; the event-free survival (EFS) was 38%, and was better in patients receiving two units. The incidence of grade III–IV aGvHD was 22% and cGvHD 23%. Graft failure remains a major issue, with higher rates of graft failure occurring in patients with limited pre-transplant chemotherapy (31,35). Particular regimens may alter outcomes, as in a series by Barker *et al.* (13): the 1-year DFS was 24% for the busulfan regimen and 41% for the cyclophosphamide regimen.

Although DUCBT has shown great promise, particularly for adult patients, this approach continues to be associated with delayed engraftment and a higher rate of engraftment failure compared with marrow and peripheral blood progenitor cell (PBPC) transplantation. Bradstock *et al.* (43) reported neutrophil recovery to 500/μL in a median of 32 days, and de Lima (de Lima *et al.*, American Society of Hematology Annual Meeting, December 2007,

Orlando, FL, USA) in a median of 28 days, for recipients of unmanipulated DUCBT. Majhail *et al.* (29) recently compared the costs of hematopoietic cell transplantation within the first 100 days among recipients of marrow or PBPC transplant versus UCBT (95% of who received two UCB units). Neutrophil recovery was delayed and graft failure was more likely in the UCBT recipients. Within the first 100 days, the absolute costs of myeloablative and non-myeloablative UCBT were significantly higher. The costs were ascribed in large part to delayed engraftment and engraftment failure in the UCBT patients. The authors concluded that strategies to enhance engraftment will decrease the costs of UCBT, underscoring the importance of the *ex vivo* expansion studies described below.

Ex vivo expansion

The goal of *ex vivo* expansion of cord blood is at least two-fold. The primary focus of expansion has been to generate sufficient numbers of HSC to optimize the graft available for transplant. Another important goal is to generate higher numbers of lineage-committed progenitor cells that, although transient, will allow rapid recovery from pancyotpenia, thus decreasing early morbidity and mortality. There is a concern that *ex vivo*-expanded products may possess an inherent reduction in long-term hematopoietic reconstitution potential under certain conditions (45–47). The potential skewing of the UCB product to a more rapidly reconstituting, but short-lived, HSC profile could potentially be exploited to provide a clinical advantage, especially when *ex vivo*-expanded and 'unmanipulated' UCB fractions are combined for transplantation. Clinical data have suggested that UCB that has been subject to *ex vivo* expansion does provide more rapid initial hematopoietic reconstitution, while 'unmanipulated' UCB is the source of long-term sustainable hematopoiesis (17). There are contradictory data regarding whether expansion provides any benefit in terms of outcome (18,19), a discrepancy for which further studies are necessary to elucidate. Currently, there are several different strategies used for *ex vivo* expansion.

Liquid suspension culture

One method of expansion is liquid culture, where UCB cells are cultured with combinations of cytokines, growth factors and other growth-promoting compounds in various flasks, bags or containers. Hematopoietic progenitor cells (primarily $CD133^+$ and $CD34^+$) are isolated from UCB, BM or mobilized peripheral blood (MPB) (48) and then incubated in culture medium. Centers have experimented with various 'cocktails' of growth factors and compounds targeted at stimulating the proliferation of primitive hematopoietic progenitors. Common components used in *ex vivo* HSC expansion protocols include stem cell factor (SCF), interleukin (IL)-3, IL-6 and granulocyte colony-stimulating factor (G-CSF) (48); SCF, thrombopoietin (TPO) and G-CSF (17,49); and Flt-3 ligand (FL), SCF, IL-3, IL-6, IL-11 and G-CSF (50–55). The optimal combination has yet to be defined.

Shpall *et al.* (16) demonstrated the efficacy of *ex vivo* expansion of isolated $CD34^+$ UCB cells in a study where a portion of a UCB unit was thawed and $CD34^+$ cells isolated (Nexell Isolex 300-i device) and cultured *ex vivo* in medium (Amgen) containing SCF, TPO and G-CSF (each at 100 ng/mL) for 10 days. The expansion increased the TNC 56-fold and $CD34^+$ cell count 4-fold; however, there was no significant difference in the time to neutrophil or platelet engraftment. McNiece *et al.* (56) subsequently developed a two-step 14-day cord

expansion protocol, yielding more effective *ex vivo* expansion than the single-step 10-day protocol described above (56), with a >400-fold increase in TNC and >20-fold increase in $CD34^+$ cells (57). This was utilized in a recent 71-patient randomized study comparing DUCBT with transplantation using one unmanipulated UCB unit combined with one unit that was expanded *ex vivo* for 14 days in media containing SCF, G-CSF and TPO (58). The mean fold expansion was 23 for TNC and 2.3 for $CD34^+$ cells. Following reduced intensity conditioning, patients receiving an expanded UCB unit engrafted neutrophils in a median of 7 days (range 4–15 days; $n = 14$) versus 14 days (range 5–32 days; $n = 12$) for those receiving two unmanipulated units ($P = 0.05$). Thirty-four patients (48%) survived for a median of 11.3 (range 2–49) months. Most of the patients on the expanded arm had evidence of expanded UCB chimerism post-transplant; however, by 14 months all patients had a predominance of the unmanipulated cord.

Further modifications to this liquid *ex vivo* expansion technique have included, or may include in the future, attempts to optimize further the *ex vivo* culture conditions (52,59–61); the development of serum-free culture systems (51,62,63); the use of tetraethylenepentamine (TEPA), a copper chelator thought to modulate the proliferation and differentiation of primitive hematopoietic progenitors (64); the use of histone deacetylases, thought to promote HSC self-renewal (65); and the use of glycogen synthase kinase (GSK)-3 inhibitors, reported to maintain pluripotency of stem cells (66). A phase I/II trial was conducted by de Lima *et al.* (20) to investigate the potential therapeutic efficacy of TEPA added in liquid UCB expansion. Nine of 10 patients engrafted at a median of 30 days ($n = 9$; range 16–46 days), with 100% donor chimerism despite the low TNC/ kg infused in this study (mean = 1.7×10^7/kg). Nine were alive at day 100, 7 at day 180. No grade III or IV GvHD occurred. Additional studies will be required to investigate the efficacy of TEPA in the expansion of UCB.

In a variation on the liquid culture technique, Delaney *et al.* (21) recently utilized an immobilized engineered form of the Notch ligand delta1 with recombinant cytokines (SCF, FL, IL-6, TPO and IL-3) to stimulate *ex vivo* UCB expansion. Five patients received one unmanipulated UCB unit and a second unit that was CD34-enriched, and cultured for 16 days with the cytokine and ligand combination (67). The CD34 population increased an average of 160-fold, with an average TNC fold increase of 660. The infused TNC/kg $\times 10^7$ average was 2.9 for the unmanipuated cells and 4.6 for the cultured cells, with an infused CD34 cell/kg ($\times 10^5$) of 2.2 (range 1.1–3.4) and 53.4 (range 9.3–133), respectively. All patients engrafted at a median of 14 days (range 7–34), compared with 25 days in the control group. As seen previously, the non-expanded cells were responsible for the durable hematopoiesis. Five of six patients survived in remission for an average of 277 days (range 70–632). These results suggest further that the expanded unit may provide short-term repopulating cells that facilitate and improve the speed of engraftment of the non-cultured unit. This is a promising study, as expansion seems to have favorably affected outcomes. Regardless, the optimal combination of cytokines and growth factors has yet to be defined, and liquid culture is limited to smaller volumes and the static nature of the culture.

Stromal co-culture

The hematopoietic microenvironment is composed of hematopoietic and non-hematopoietic (cellular and extracellular) components (68–70). Complex molecular cues that direct hematopoiesis are provided by the stem cell 'niche' and, at least in part, are responsible for the regulation of differentiation and maturation of HSC (71–78). When cells are expanded *ex vivo*, they lose the support and regulation provided by the microenvironment, and receive only the specific cytokines and growth factors provided in the culture media, thus relying on exogenous direction and potentially driving differentiation at the expense of self-renewal. Third-party (neither donor nor recipient) allogeneic mesenchymal stromal cells (MSC) (78–81) have been show in NOD-SCID mice to promote engraftment of UCB $CD34^+$ cells when co-administered (82,83) and also to possess immunomodulatory activity (84–87). Co-culture of UCB with MSC (even allogeneic) could restore some of the interaction that occurs between the microenvironment of the marrow stroma and the HSC (77,88,89).

For stromal co-culture, mononuclear cells (MNC) are isolated by density separation and co-cultured with established MSC monolayers in medium containing fetal bovine serum (FBS) and a growth factor cocktail (e.g. SCF, TPO and G-CSF) (89). The non-adherent cells are removed from the co-culture after 7 days and subjected to a secondary expansion on an additional MSC monolayer. The original adherent layer that is then composed of MSC and HSC is re-fed with fresh medium containing growth factors. The culture is then continued for an additional 7 days (total 14 days) (89). A 10–20-fold increase in TNC and a 16–37-fold increase in $CD34^+$ cells has been reported using co-culture expansion. HSC (defined as $CD34^+$ and $CD133^+$) are detected in the non-adherent and adherent fractions of the co-culture. Co-administration of third-party MSC with the UCB-derived HSC may aid engraftment (82,83) and provide immunomodulatory benefits (86,87,90), therefore it may prove clinically beneficial to re-infuse both non-adherent and adherent cells from the expansion process.

In a current clinical trial using UCB expanded on MSC, a median fold expansion of 12 was seen in both the TNC and $CD34^+$ subsets. After myeloablative therapy, the median time to neutrophil engraftment was 14.5 days (range 12–23) and platelet engraftment 30 days (range 25–51). Two of six patients developed grade II aGvHD. Five of the six patients were alive and in complete remission at a median follow-up of 1 year, with accrual continuing (22). As with the development of liquid *ex vivo* expansion, optimization of culture conditions for this approach will continue, including the growth factor cocktail utilized, the length of co-culture and the development of potentially more effective stromal cell lines to support the HSC expansion (91).

Continuous perfusion culture systems

Automated, continuous perfusion culture systems, or 'bioreactors', are also being investigated for the *ex vivo* expansion of HSC, rather than the use of 'static' culture (culture flasks or bags) (18,19,92–99). These systems were designed to allow larger volumes as well as to provide improved nutrient delivery and gas exchange. Therefore, a continuous perfusion of culture medium that removes mature cells could protect the cultured cells from

toxic byproducts (95). Expansion trials using bioreactors are ongoing and have shown variable results (17,96).

Cell delivery and homing

One of the hypothesized reasons for lower rates of engraftment of cord blood is that homing to the BM may not be as effective as for other stem cell sources. Two recent trials were designed to overcome the need to home to BM by giving the UCBT directly into the BM space rather than infusing intravenously (97,98). The initial study suggested improved rates of engraftment, specifically of platelets (98). The second study, which gave two units and randomized the units to intrabone or intravenous infusion, failed to show benefit of the intrabone infusion (97). In murine models, investigators are attempting to enhance stem cell homing to the marrow space by using tumor necrosis factor (TNF)-alpha (99), co-infusion of MSC (100) and short-term culture of UCB cells (101) to alter the homing signals on the UCB cells and marrow stroma. This certainly would be an interesting area for further investigation in clinical studies.

Summary

Recently, trials have shown improved outcomes for UCBT. In pediatric patients, cord blood may even emerge as the preferred stem cell source. In adult patients more obstacles still exist; however, progress continues to be made. Combining cord blood units has allowed higher cell doses to be achieved, reduced graft failure rates and improved outcomes. Current clinical trials have demonstrated that the use of expanded UCB can be safe and recent results suggest the potential for improved outcomes; however, the optimal expansion conditions have yet to be identified. *Ex vivo* expansion technology could have further-reaching clinical applications. With cell sorting and manipulation of culturing techniques, it is possible to expand particular subsets of UCB-derived cells, such as T cells (102) and natural killer (NK) cells (103). The *ex vivo*-expanded cells could then be available as a platform for adoptive immunotherapy to target either tumor or infectious pathogens. In addition, *ex vivo* expansion could allow gene transfer technologies to be available in the UCB setting. With the rapidly evolving field of cord blood transplantation, important improvements in the safety, efficacy and application of UCBT may be observed in the near future.

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Table I

Summary of clinical trials of double (or multiple)-unit UCBT.

Reference	*n*	Intensity of conditioning regimen	Median age in years (range)	No. of UCB units	Total combined TNC ($\times 10^7$/kg)	Total combined CD34 ($\times 10^5$/kg)	Days to ANC >500	Days to platelets >20 000	Follow-up (range)	aGvHD (grade II–IV)
38	10	Reduced intensity conditioning (RIC)	55 (28–67)	5–7	6.3 (3.8–10)	5.7 (1.1–11.9)	18	61	34 days	30%
14	1	RIC	19	2	4.36	0.96	27	72	148 days	None
12	1	Ablative	43	12	318		31		43 days	None
26	1	Ablative	15	2	2.66	0.8	35	59	479 days	None
25	1	Ablative	44	2	2.33	5.3	29	37	364 days	None
37	38	RIC	49 (24–63)	2	2.15 (1.48–3.38)	1.13 (1.48–8.60)	20	43	487 days	–
21	5	Ablative	11(10–13)	2	6.31	3	15	49	18.5 months (11–32 months)	80%
22	4	RIC	15 (5–38)	2	4.6 (2.9–6.5)	3.15 (1.5–5.7)	23 (15–31)		12 months (1.5–25 months)	75%
24	1	RIC	30	2	3.3		25	42	22 months	Yes
104	26	RIC	41	2	3.02 (1.2–7.9)	0.91 (0.14–5.15)	17 (3–54)		18 months, 1-year PFS 57%, OS 65%	24%
21	20	Ablative	11 (10–13)	2	5.68 (2.18–13.11)	3.8 (0.49–21.7)	18 (11–48)	46	15 month	75%
23	6	Ablative	22 (14–32)	2	1.67 (0.8–2.67)	0.54 (0.24–0.75)	30 (21–50)	61 (28–72)	21 days– >52 months	66%
34	23	Ablative	24 (13–53)	2	3.5 (1.1–6.3)	4.9 (1.2–14.5)	23 (15–41)		10 months, 1-year DFS 57%	65%
36	53	RIC	49 (19–67)	2	4.6 (2.9–6.8)	2.4 (0.5–11.5)	21 (18–28)	42 (41–56)		–
35	21	RIC	49	2	4.0 (2.9–5.1)	1.9 (0.6–9.7)	20	41	1-year DFS 67%	Yes
42	85	RIC		2	3.7 (1.5–6.8)	4.9 (0.7–16.6)	12		3-year OS 45%	22%

Table II

Summary of clinical trials utilizing UCB that has been expanded *ex vivo*.

Type of expansion	Reference	Subjects	Cytokines	Days in culture	Fold-expansion		Days to absolute neutrophil count (ANC) >500	Days to platelets >20 000	Survival (median length) and aGvHD
					TNC	CD34+			
Liquid suspension	16	*n* = 37 adults and children	SCF, TPO, G-CSF	10	56	4	28	106	32% survival (minimum 17 months), 67% grade II–IV, 40% grade III–IV
	57	*n* = 35 adults and children	SCF, TPO, G-CSF	14	23	2.3	14	34	48% survival (11 months), 43% grade II–IV, 7% grade III–IV
	20	*n* = 10 adults and children	SCF, FL, IL-6, TPO, TEPA	21	219	6	30	48	30% survival (25 months), 44% grade II, no grade III–IV
	21	*n* = 5 adults& children	Notch ligand delta1, SCF, FL, IL-6, TPO, IL-3	16	660	160	14		83% survival (277 days)
Stromal co-culture	22	*n* = 6 adults and children	SCF, TPO, G-CSF	14	12	12	14.5	30	83% survival (12 months), 33% grade II, no grade III–IV
Continuous perfusion system	18	*n* = 27 children and a few adults	PIXY321, FL, EPO	12	2.4	0.5	22	71	39% survival (41 months)n 36% grade II–IV, 22% grade III–IV
	17	*n* = 2 adults	PIXY321, FL, EPO	12	2.2	1.6, second did not expand	28	56	100% survival (13 months), no