Published in final edited form as:
Clin Immunol. 2008 June ; 127(3): 286–297. doi:10.1016/j.clim.2008.02.008.

Umbilical Cord Blood Transplantation: Basic Biology and Clinical Challenges to Immune Reconstitution

Julia Brown[1] and **Vassiliki A. Boussiotis**[1,2]

1*Division for Surgical Services, Cancer Center, Harvard Medical School, Boston, MA 02114*

2*Department of Medicine Division of Hematology and Oncology Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114*

Abstract

Allogeneic stem cell transplantation has continued to evolve as a common procedure for the treatment of hematological malignancies and bone marrow failure. Donor bone marrow and mobilized peripheral stem cells are routinely employed for the reconstitution of immune function in leukemia and lymphoma patients following radiation and/or chemotherapy. Unfortunately, only 30% of patients have an HLA identical sibling donor and the identification of matched unrelated donors, particularly for minorities, can present an exceptional challenge. The transplantation of umbilical cord blood (UCB) represents the most recent strategy to expand the potential donor pool while maintaining an acceptable level of treatment related complications. First utilized in children, UCB transplantation permits a higher degree of HLA disparity while demonstrating a reduction in the incidence and severity of graft versus host disease (GvHD) compared to previous transplantation modalities. Despite the apparent decrease in GvHD, relapse rates remain comparable to transplantation with bone marrow or mobilized peripheral blood suggesting a strong graft versus leukemia/lymphoma (GvL) effect. However, several issues complicate the use of UCB transplantation and its extension to the treatment of adults. Many infections that afflict transplant patients are particularly frequent and more severe in the context of UCB transplantation. UCB T cells are naïve and therefore display less proliferation and IFN-γ production in response to cognate antigen and also appear to demonstrate defects in signal transduction mechanisms. In addition, UCB contain T regulatory cells (Treg) with more potent suppressor function than adult Treg. Furthermore, adult patients often require more total cells and CD34+ progenitors for transplantation than a single UCB unit can provide. Thus, strategies to expand selected subpopulations from UCB and the use of multi-unit transplantation are areas of active research. This review will provide a condensed summary of the clinical history of UCB transplantation and emphasize the advantages and disadvantages of this approach to hematological malignancies in comparison to other methods of hematopoietic stem cell transplantation. Subsequently, it will mainly focus on the current challenges to immune reconstitution presented by UCB transplantation, recent research into their cellular and molecular mechanisms, and experimental approaches to overcome them.

Keywords

Cord Blood transplantation; tolerance; immune reconstitution; post-transplant infections

Correspondence: Vassiliki A. Boussiotis MD, PhD, Massachusetts General Hospital, 55 Fruit Street, GRJ 904, Boston, MA 02114, Tel: 617-643-4037, FAX: 617-726-8623, e-mail: vboussiotis@partners.org.

Introduction

The transplantation of hematopoietic stem cells (HSC) in the context of treatment for high-risk hematologic malignancies has evolved into a standard procedure. Although therapeutic regimens vary depending upon the type of malignancy, HSC are infused into cancer patients following an intense course of chemotherapy and radiation. Traditionally, HSC are harvested from the bone marrow (BM) or peripheral blood of an HLA-matched sibling or unrelated donor. However, only 30% of patients have a potential sibling donor who can meet the stringent requirement of a 6/6 or 5/6 match with the patient's HLA loci (HLA-A, HLA-B, HLA-DRB1) [1]. In the absence of a matched sibling donor, patients must rely on the worldwide network of bone marrow registries to find an HLA-matched donor. Complicating this process is the fact that the majority of registered donors are Caucasian, thereby making the selection process extremely difficult for patients of different or mixed races. The search process for a suitable donor is often lengthy; a recent report cites an average time of 4 months [1]. In the interim, the progression of the patient's malignancy and the toxicity of the chemotherapy required while a matched donor is sought can result in a worsening prognosis.

After HSC transplantation, the additional complications of susceptibility to infection and graft versus host disease (GvHD) provide substantial challenges to immune reconstitution. In particular, acute GvHD frequently develops in the context of allogeneic HSC transplantation. Mature allogeneic T cells that accompany the HSC graft are activated by MHC class I and II antigens expressed by the recipient, resulting in an aggressive T helper type I response that principally targets the skin and gut. Furthermore, chronic GvHD, which is related to acute GvHD but more closely resembles an autoimmune disorder with the development of autoreactive T cells, can result in debilitating and life-threatening disease many months post-transplantation. GvHD and opportunistic infections cause considerable morbidity in transplant patients and treatment protocols for the resolution of each will frequently exacerbate the other (i.e., steroid treatment for GvHD diminishes the immune response to infection).

In an attempt to better address these issues, considerable interest has focused on the use of umbilical cord blood (UCB) as an alternative source of HSC for hematopoietic reconstitution (Table I). Early studies by Knudtzon [2] demonstrated that granulocytic colony-forming cells could be grown in vitro from UCB. Further in vitro studies by Broxmeyer et al. [3] established that UCB contains a sufficient number of hematopoietic stem/progenitor cells to be used for autologous or allogeneic hematopoietic reconstitution. In 1989, Gluckman and colleagues published results from the first successful UCB transplant using cord blood from an HLA-identical sibling in a patient with Fanconii's anemia [4]. Large-scale clinical trials to evaluate UCB transplantation for a variety of hematologic malignancies, bone marrow failure syndromes, and congenital errors of metabolism were soon established.

Transplantation with UCB : clinical studies

Since UCB units typically contain 1–2 logs fewer nucleated cells than a unit of bone marrow [5], pediatric patients were primarily chosen as the first recipients of UCB transplants (Table II). Rocha and colleagues from the Eurocord and International Bone Marrow Transplant Registry [6] compared the incidence of GvHD in children who received either cord blood (113 patients) or bone marrow (2052 patients) from an HLA-identical sibling for the treatment of either malignant or non-malignant disease. Using a multivariate analysis and adjustment for relevant risk factors, the authors found that the risk of acute or chronic GvHD was significantly lower in children who received UCB compared to those who received BM. However, in the first month post-transplantation, UCB recipients demonstrated slower recovery of neutrophil and platelet counts than the BM transplant patients. UCB transplant patients were also more

likely to die of infection. Notably, the two groups had similar rates of death due to relapse [6].

In another study, case reports of related or unrelated UCB transplantation in children from 45 centers revealed the importance of cell dose as a predictor of neutrophil and platelet engraftment [7]. Patients transplanted with unrelated UCB containing less that 37 million nucleated cells/kg required a median of 34 days to reach an absolute neutrophil count of 500 per cubic millimeter and a median of 134 days to obtain a platelet count of 20,000 per cubic millimeter. In patients who received 37 million or more nucleated cells/kg, these numbers were reduced to 25 days and 47 days, respectively. HLA mismatching was also found to be related to the delay in engraftment times. The incidence of GvHD was low in this study, with the degree of HLA mismatch correlated with the incidence of GvHD in patients with related donors. Interestingly, the number of HLA mismatches did not affect the incidence of GvHD in patients given UCB from unrelated donors.

In 1998, Rubinstein and colleagues reported on 562 recipients of UCB transplants from unrelated donors at 98 transplantation centers [8]. Similar to Gluckman et al.[7], this study demonstrated that the number of leukocytes per kilogram in the UCB graft correlated with the time to myeloid engraftment. The kinetics of myeloid engraftment were also related to the degree of HLA mismatch. Conversely, the time to platelet engraftment in this study was not delayed but comparable to that seen in studies of BM transplantation. Event-free survival post-engraftment correlated significantly with the age of the patient and not to the number of leukocytes per kilogram in the graft. Significantly, acute and chronic GvHD in this study was less severe than in transplant patients that received BM from unrelated donors. This observation was seen even in patients that received UCB grafts with up to two HLA mismatched antigens. The authors concluded that UCB is a viable source of HSC for bone marrow reconstitution. Another study during this period commented on the rapid availability of UCB units for transplantation. Wagner et al. [9] found a median time of 3.5 months to receive BM from an unrelated donor, whereas the search for a suitable UCB unit required only 69 days. A more recent study [10] has also demonstrated that the time interval from decision to actual transplantation was 1 month shorter for UCB compared to BM.

In further studies involving UCB transplant in children, Barker et al. [11] and Rocha et al. [12] continued to see a delay in neutrophil engraftment but encouraging results regarding acute and chronic GvHD. In a retrospective multicenter study, Rocha et al. [12] also observed a delay in platelet engraftment and re-emphasized the finding of other groups [7,8] that a nucleated cell dose above 0.37×10^8/kg is required to increase the probability of engraftment. This study also demonstrated, similar to the results of Rubinstein et al. [8], no correlation between the outcome of UCB transplantation and the number of HLA mismatches between the UCB unit and recipient. In a single institute matched pair analysis comparing the outcomes of patients receiving HLA-mismatched UCB versus HLA-matched BM, Barker et al. [11] found that the probabilities of engraftment, GvHD and survival were actually comparable between the two groups. These studies suggested that UCB T cells demonstrate a lower level of alloreactivity than T cells in BM transplants. Although beneficial in terms of the reduced incidence of GvHD, this continued finding presented the concern that UCB transplants would mediate a poor graft versus tumor or graft versus leukemia (GvL) effect. However, Rocha et al. [12,13] reported that after 100 days post-transplant, the risk of relapse was comparable in patients receiving UCB versus BM transplant. This finding has been confirmed by several other groups [11,14, 15].

Following these favorable comparisons with BM transplantation in pediatric patients, many clinical studies began to focus on the adoption of UCB transplantation for the treatment of adult malignancies (Table III). A major concern regarding adult patients is the restricted

number of HSC in the UCB graft. In a study involving both children and adults, Wagner et al. [16] followed 102 unrelated donor UCB transplant patients to assess the effect of $CD34^+$ cell dose and HLA mismatch on treatment-related mortality (TRM) and survival. They concluded that the number of $CD34^+$ cells infused per kilogram was significantly related to the rate of engraftment, TRM, and overall survival. In this study, 29% of patients that received 1.7 to 2.7×10^5 $CD34^+$/kg experienced TRM while 68% of those that received less than 1.7×10^5 $CD34^+$/kg had TRM. Furthermore, for each degree of HLA disparity, the authors suggested that a critical number of $CD34^+$ cells/kg must be infused; below this threshold, the probability of survival is significantly decreased. This finding was found to be particularly applicable to recipients of UCB units with 2 HLA mismatches. As a consequence of these results, the authors concluded that UCB graft selection should be based on the $CD34^+$ cell dose when several UCB units are available with an HLA mismatch of 2 or less. In another multi-center study of adult transplant patients, UCB grafts with a relatively high number of nucleated cells were associated with a faster rate of neutrophil recovery [15]. Additionally, infusion of UCB units containing relatively high numbers of $CD34^+$ cells was correlated with improved event-free survival.

Additional studies comparing UCB versus BM transplantation in adults found that patients transplanted with mismatched UCB had similar rates of TRM, treatment failure, overall mortality, and relapse rates compared to patients who received mismatched BM [13,14]. Furthermore, Laughlin et al. [14] reported that the rate of acute GvHD in mismatched UCB transplant patients was comparable to the rate found in HLA-matched BM transplant patients. In a registry-based retrospective study of 682 patients, Rocha et al. [13] found both the rate of acute and chronic GvHD in mismatched UCB patients to be lower than the rates in matched BM recipients. However, the relationship between the relatively small cell dose found in UCB units and the delay in engraftment times and subsequent immune reconstitution for neutrophils and platelets continued to be problematic [13,15,17,18]. Long et al. [17] noted that infection was the primary cause of death in their study of 57 adults with high-risk disease who were transplanted with unrelated UCB. A large multicenter prospective study of UCB transplantation in adults found that in 34 patients 90% experienced infections; two thirds of those studied reported at least 3 infections during the first 6 months post-transplant [19]. These authors suggested that the delay in immune reconstitution in UCB transplant patients versus BMT recipients could play a major role in their increased susceptibility to infection.

Given the critical importance of $CD34^+$ cell dose and total nucleated cell dose to engraftment and immune reconstitution, new strategies have evolved to increase the total cell number provided in UCB transplants. Ex vivo expanded UCB has demonstrated potential for improved neutrophil engraftment despite the infusion of a less than optimal nucleated cell dose [20]. McNiece et al. [20] have developed a two-step ex vivo culture procedure that results in the further expansion of $CD34^+$ cells with a resulting enrichment for mature neutrophils comparable to cultures of ex vivo expanded peripheral blood progenitor cells. Furthermore, human $CD34^+$ UCB cells expanded ex vivo without a murine stromal cell feeder layer have “long-term” engrafting potential as demonstrated by their ability to reconstitute primary and secondary nonobese diabetic-severe combined immunodeficiency (NOD-SCID) mice or preimmune fetal sheep [21]. Such “stroma free” systems are more adaptable for clinical use by avoiding complications from murine feeder cells. Ex vivo expansion of UCB cells to facilitate engraftment and immune reconstitution continues to be an area of active investigation [22].

Another strategy for increasing the total nucleated cell dose of UCB transplants involves the infusion of a single recipient with multiple cord blood units. Studies in NOD-SCID mice have demonstrated that transplantation with two unrelated UCB units can increase engraftment compared to single unit transplants [23]. Barker et al. [18] reported the use of double UCB transplantation in the context of a clinical trial designed to evaluate the efficacy of reduced-

intensity conditioning with UCB transplantation. This initial report was followed by a study devoted exclusively to evaluating the safety of double UCB transplantation for hematological malignancy in adults [24]. Following myeloablative conditioning, 23 patients with high-risk hematologic malignancy were infused with 2 partially HLA-matched UCB units containing a median cell dose of 3.5×10^7 nucleated cells/kg. All of the 21 patients available for analysis demonstrated donor neutrophil engraftment at a median of 23 days post-transplant. Despite the infusion of 2 HLA disparate grafts, these patients did not demonstrate an increase in the incidence of GvHD observed with single unit transplants. Disease-free survival in this high-risk group was 57% at 1 year. Most notably, 24% of patients displayed engraftment from both UCB units and 76% of patients from 1 unit at day 21 while all patients demonstrated reconstitution with only 1 unit by day 100. The predominating unit was found to contain a significantly higher dose of $CD3^+$ cells.

Additional clinical studies of double UCB transplantation have demonstrated the safety and efficacy of this approach in the treatment of hematological malignancies. In a study of 21 patients treated with a nonmyeloablative approach, Ballen et al. [25] found that TRM at day 100 post-transplant was only 14% and the occurrence of severe acute GvHD remained low. As in the study by Barker et al. [24], all of the patients engrafted with 1 UCB unit by 3 months posttransplantation. Relapse rates among these patients were low suggesting that the GvL effect was present despite the low rates of GvHD. Interestingly, patients who displayed mixed chimerism with both cord blood units at 6 weeks post-transplant presented with a higher incidence of chronic GvHD, a finding that may reflect an ongoing graft-versus-graft interaction. A recent report by Brunstein et al. [26] further substantiates the low rates of severe GvHD, reduced TRM, and low relapse rates seen with the double UCB transplant approach. In this study of 110 patients, neutrophil engraftment was notably rapid and occurred at a median of 12 days in 92% of patients. Although many parameters were examined including total $CD34^+$ and $CD3^+$ numbers, the authors found no predictive characteristic for determining which UCB unit would eventually predominate in these patients. Ting et al. [27] has reported that in a group of 10 UCB double transplant patients, the predominant cord blood unit contained a higher number of NK cells compared to the rejected unit.

Summary

UCB transplantation has been rigorously examined in the setting of many clinical trials and continues to develop as a viable alternative to the use of bone marrow or mobilized peripheral stem cells for the treatment of hematological malignancies and bone marrow failure. The use of UCB as a stem cell source has revealed certain advantages for transplantation recipients. Relative to other donor sources, UCB units can be obtained quickly and mediate less severe GvHD without compromising GvL effects. However, the total cell numbers in UCB units are at least one log lower than bone marrow or peripheral stem cell sources. New approaches, such as the ex vivo expansion of UCB stem cells and the use of double unit transplantation, have been employed to avoid a prolonged delay in immune reconstitution.

UCB basic biology and implications for the development of GvHD post-UCB transplantation

One of the early clinical observations in the outcome of UCB transplantation has been the fact that UCB permits a greater degree of HLA mismatching without an unacceptably high incidence of GvHD. HLA disparity between the donor and recipient is an important determinant of acute and subsequent chronic GVHD. Notably, a higher incidence of acute and chronic GVHD has been observed in patients transplanted with HLA-matched unrelated grafts when compared with histocompatible sibling grafts, possibly attributable to reactivity of donor T cells with recipient minor histocompatibility antigens. Minor histocompatibility antigen

disparity is expectedly greater between unrelated individuals. Despite the use of HLA class I and II disparate grafts, the incidence and severity of acute GVHD observed in pediatric and adult recipients of UCB grafts is lower when compared to recipients of unrelated adult donor grafts [6,9,11,13,16,28].

Multiple factors intrinsic to the UCB graft may contribute to the reduced GVHD observed after UCB transplantation. These may include reduced graft lymphocyte numbers, altered recognition of recipient self antigens by UCB donor T cells interacting with recipient's antigen-presenting cells (APC) and limited response of these naive donor T cells activated by recipient alloantigen. Subsequently, these changes will result in impaired cytokine production, limited cellular activation and lack of clonal expansion of alloreactive T cells [29–31].

UCB T lymphocytes are typically CD45RA+ and express low levels of activation markers. Several in vitro studies point to the inherent lack of full expression of immunomodulatory cytokines by alloreactive T cells contained in UCB grafts. In primary mixed lymphocyte culture UCB T cells demonstrate proliferative responses to allogeneic stimulation, but less cytotoxic effector function, less proliferation and greater activation-induced cell death (AICD). Further mechanisms potentially underlying UCB immune tolerance includes altered toll-like receptors and adhesion molecule expression on donor graft antigen-presenting cells [31]. Early recovery of NK cells able to activate the granzyme/perforin lytic pathway and Fas/Fas ligand (FasL) activity has also been proposed as contributing to the low incidence of acute GVHD observed after UCB transplantation [32]. Other studies have suggested that UCB graft T cells display reduced expression of nuclear factor of activated T cells-1 (NFAT1), which may be one important molecular mechanism underlying their reduced capacity for cytokine production [33]. The reduced GVHD after UCB transplant reported in clinical studies may be related to these in vitro observations that T cells in the UCB graft respond less fully than mature alloreactive lymphocytes.

The long-term clinical implications of the reduced GvHD in UCB transplantation are currently not well understood. From the present clinical experience it is clear that, despite the decreased incidence and severity of GvHD associated with UCB grafts, GvL immunologic effects are maintained [34]. Clinical reports of allogeneic UCB recipients have not identified increased relapse rates [7–9,14,15]. Interestingly, studies have reported that successful immune reconstitution after UCB transplantation, as determined by development of detectable CMV, VZV and HSV immunity, is associated with reduced relapse rate [35]. These results indicate that despite reduced incidence of GvHD, lymphocytes in the UCB graft are capable of providing significant immunity against leukemia and viral antigens.

UCB basic biology and implications for hematopoietic reconstitution post-UCB transplantation

Graft characteristics known to positively correlate with rapid donor engraftment in recipients of conventional allografts include cell dose, CD34 content, and HLA matching. The rate of donor hematopoietic reconstitution is lower and kinetics of engraftment and myeloid recovery are delayed using UCB compared to bone marrow grafts [14]. This event is thought to be secondary to the low stem cell dose in the UCB graft compared with adult donor grafts [6, 36]. UCB graft characteristics shown to have a predictive value for time to myeloid engraftment include total nucleated graft cell content, CD34 content and colony-forming units (CFU) [15, 37]. UCB HLA compatibility is also predictive of engraftment [15]. Minor histocompatibility disparity in unrelated allogeneic transplantation may contribute to graft rejection and to graft-versus-leukemia effects [38–41]. HLA class I mismatching including HLA-C and NK epitope mismatching are associated with higher rates of graft rejection and severe acute GVHD after unrelated donor transplantation [42,43]. Allele matching for adult unrelated blood and marrow

grafting has improved rates of engraftment and GVHD. Effects of the number and type of graft HLA disparities on UCB donor engraftment have not been fully studied. Perhaps such evaluation for UCB selection will improve engraftment time.

Various groups have attempted to compare HSC in UCB to those of peripheral blood grafts (Table IV). These studies have revealed a less mature phenotype of UCB CD34+ progenitors compared to adult marrow and peripheral blood grafts [12,44]. Interestingly, these studies have shown that the frequency of early stem cells is similar in adult bone marrow grafts, mobilized peripheral blood HSC, and UCB, but the proliferative potential of UCB early stem cells is significantly higher. Cobblestone area-forming cell (CAFC) assays have also shown that UCB CD34+ cells contain the highest frequency of CAFC (3.6- to 10-fold higher than BM CD34+ cells and peripheral blood stem cells, respectively). In addition, the engraftment capacity in vivo, as determined by nonobese diabetic/severe combined immunodeficiency (NOD/SCID) repopulation assay, is also significantly greater than BM CD34+ cells [45,46]. From the above studies it becomes apparent that UCB may have lower CD34+ cell numbers, which have a higher proliferation potential than adult CD34+ cells. Thus, other characteristics of UCB progenitor cells may be responsible for the delayed engraftment of UCB stem cells. Such characteristics may include adhesion molecules, homing properties and maturational stage of UCB progenitor cells [22,47–49]. These factors may have a major contribution in the kinetics and efficiency of engraftment and may explain why CD34 quantification in UCB has not been consistently predictive of time to hematopoietic reconstitution.

Several approaches have been employed to improve rates and kinetics of hematopoietic engraftment in adult UCB recipients. A successful strategy involves infusion of more than one UCB graft. As mentioned in the previous sections, initially, the Minnesota group reported their experience with adults infused with two UCB units in whom 92% demonstrated sustained donor engraftment [18] . Interestingly, although patients developed a transient state of mixed chimerism, eventually one donor dominated over time. Neither cell dose nor HLA disparity were predictive for selection of engraftment. GvHD incidence paralleled that of historic controls and 1-year survival was 33%. These observations were encouraging and further studies using two UCB units in adult recipients followed immediately thereafter. The Dana-Farber/MGH Partners Cancer care group reported their preliminary experience using two UCB units in adult patients following a nonmyeloablative conditioning regimen [25]. This study used a reduced-intensity conditioning regimen of fludarabine, melphalan, and antithymocyte globulin followed by 2 partially matched UCB units. The UCB units were a 4/6 HLA match or better with each other and with the patient and achieved a minimum precryopreservation cell dose of 3.7×10^7 nucleated cells/kg. The median time to an absolute neutrophil count $>0.5 \times 10^9$/L was 20 days, and the median time to an unsupported platelet count $>20 \times 10^9$/L was 41 days. Two patients experienced primary graft failure and underwent a second UCB transplant. One patient had a late graft failure. Acute graft-versus-host disease (GVHD) grade II–IV occurred in 40% of patients. The 100-day TRM was 14%, and the 1-year disease-free survival was 67%. Mixed chimerism was associated with a higher risk of chronic GVHD.

A second interesting approach to enhance hematopoietic recovery in adult UCB recipients has been proposed by Fernandez et al. [50], who attempted simultaneous transplantation of a low number of highly purified peripheral blood CD34+ cells from an HLA haplo-identical donor along with UCB. All patients had prompt myeloid recovery and the median time to an absolute neutrophil count $>0.5 \times 10^9$/L was 10 days. One interesting observation was that after a transient initial phase of mixed chimerism and engraftment of haplo-identical HSC there was a subsequent progressive replacement by UCB. However, a major concern with this strategy is the potential development of severe acute GvHD that could be triggered by the haplo-identical CD34+ cells in this setting.

A third strategy was proposed in an attempt to shorten the time interval to attain donor-derived hematopoietic recovery after UCB transplantation, and allow transplantation of adult recipients. This approach involves expansion of UCB in cytokines in vitro prior to infusion [51–53]. Early clinical trials based on infusion of a proportion of the UCB graft expanded ex vivo in cytokine-based conditions have failed to demonstrate more rapid hematopoietic recovery in UCB recipients, suggesting that cytokine based expansion may result in differentiation of early self-renewable stem cells. Reports from the Duke University program have shown that infusion of ex-vivo expanded UCB did not alter the time to myeloid, erythroid or platelet engraftment [53]. Shpall et al. reported their experience at the University of Colorado with infusion of ex vivo expanded cells along with the UCB [51]. Although this approach allowed engraftment in all patients, the median time to reach an absolute neutrophil count 0.5 $\times 10^9$/L was 28 days and did not differ from that observed in adult patients infused with non-expanded UCB [15]. These observations suggest that not simply the number of CD34+ cells but other components or properties of the UCB govern engraftment.

UCB basic biology and implications for immune reconstitution post-UCB transplantation

Following allogeneic transplantation, all patients experience a period of profound immunodeficiency. Immune reconstitution of T- and B-cell compartments following allogeneic transplantation may require as long as 12–24 months. The slow process of immune reconstitution together with post-engraftment immunosuppression creates an immunologic environment whereby the host is susceptible to opportunistic infections [54–56] as well as virally induced malignancies [57,58]. Recipients of unrelated donor or HLA non-identical transplants appear to have a higher rate of infectious complications than recipients of matched sibling allogeneic grafts [59–61]. GvHD and its treatment further delays immune recovery after allogeneic transplantation and may account for the higher rate of delayed immune reconstitution in recipients of unrelated versus related donor grafts.

Several reports outlining the kinetics of immune recovery after UCB transplantation in adults and children point to slow T-cell recovery [60],[62–66] compared with published reports after conventional graft allogeneic transplantation [66–71]. Recent studies have evaluated the immune function of adult UCB patients during the first two years post-transplant. Immune recovery in these early series of adult UCB transplant recipients was marked by profound lymphopenia and immunodeficiency during the first 6–12 months after transplant. However, when immune recovery was attained, generally 9–12 months after transplant, recovery of both T- and B-cell function was noted. Enumeration of T, B and NK cells revealed absolute lymphocyte counts measured in the early post-transplant time period were very low but absolute numbers of circulating NK cells did not differ from that of adult controls, a finding consistent with previous observations in BMT recipients [72,73]. B cells (both proportions and absolute numbers) increased beginning 6 months post-UCB transplant and persisted at 1 year follow-up. The mechanism and significance of this B-cell ‘rebound’ phenomenon observed post-transplant in adult UCB recipients is unclear. Work from the Duke University program reported that T-cell recovery was comparable in adults and children following UCB transplant [64]. This event was associated with delayed emergence of thymic recent emigrants cells (TREC) in adults (18 months) vs. children (12 months) as well as lower levels than expected for age. That study also reported skewing of T-cell repertoires in these patients persisting until 2–3 years after transplant.

The immediate consequence of the impaired rate and quality of immune recovery after UCB transplantation is directly associated with higher infection rates in adult UCB recipients. The Case Western Reserve University transplant group evaluated patients with hematologic disorders treated with myeloablative regimen and transplantation with either UCB or HLA-

matched unrelated (MUD) grafts, for life-threatening infections, hematologic reconstitution, GvHD, relapse and event-free survival [60]. The median duration of neutropenia after transplantation was longer (29 vs. 14 days) in the UCB group. Overall infection rates were higher in UCB recipients, particularly at early time points (prior to day 50) after transplantation. Event-free survival at 3-year follow-up was 0.25 in UCB and 0.35 in MUD recipients.

Recent reports indicate that the rate of infections during the early post-transplantation period is higher in adult patients transplanted with HLA mismatched unrelated UCB, while overall rates of infections at later time points are similar to that observed in unrelated adult donor transplant recipients [60]. Mechanisms responsible for this event may be related to the prolonged duration of neutropenia and lymphopenia after infusion of smaller numbers of total graft nucleated cells and CD34+ cells. Saavedra et al. reported a high incidence of bacteremia (55%) in 27 adults at early time points after UCB transplant. Ten patients (37%) died prior to day 100. Infection was a direct cause of death in 4 patients [74]. Investigators from Japan reported cytomegalovirus (CMV) infection following UCB in 28 adults compared with sibling matched (R-BMT) and URD BM recipients. CMV antigenemia was observed in 19 (79%) of UCB patients at median 42 days [75]. A higher proportion of UCB patients treated with preemptive gancyclovir therapy required a second course of treatment compared with R-BMT and URD BM patients, suggesting that CMV-specific immunity after UCB may be delayed.

In addition to the delayed engraftment and cellular reconstitution, increased infection rates in UCB recipients may be related with other cell populations that are present in the UCB. Extensive studies have shown that UCB is a superior source for isolation of T regulatory cells (Treg) that have a more potent suppressor function that Treg isolated from adult peripheral blood [76]. Fresh $CD4^+CD25^+$ cells isolated from UCB expressed high levels of Foxp3 mRNA and protein that was further upregulated after in vitro culture. Cell lines were readily generated from $CD4^+CD25^+$ (Treg) isolated from UCB, by repetitive stimulation with anti-CD3/CD28 coated beads and IL-2 culture. Moreover, UCB-Treg cell lines displayed impaired activation of Ras, MEK1/2 and Erk1/2, whereas activation of Akt was retained and activation of Rap1 was enhanced [77]. Treg also displayed increased expression of $p27^{kip1}$ and had high susceptibility to apoptosis. This event was reversed by IL-2, which induced activation of Erk1/2, upregulation of Bcl-x_L and phosphorylation of Bad at Ser^{112}, a site specifically phosphorylated by Erk. These UCB-derived Treg cell lines had potent suppressive function when added in HLA-mismatched allo-MLRs, in which $CD4^+$ responder cells were stimulated with allogeneic DC that generate a very robust proliferation. Interestingly, the inhibitory activity of UCB-derived Treg cell lines was persistently superior to the inhibitory activity of PBMC-derived Treg in all responder: stimulator ratios tested. UCB Treg cell lines also markedly reduced cytokine and chemokine accumulation in MLRs. These observations may have potentially significant clinical implications because these highly suppressive UCB Treg may be able to inhibit responses of effector cells in UCB recipients.

Summary

The unique biology of the HSC and lymphocytes found in UCB grafts provides the explanation for the variations in clinical outcomes seen in HSC versus UCB transplantation. The lower incidence and severity of GvHD seen in UCB recipients is a direct consequence of the reduced proliferation, cytokine production, and cytotoxicity to alloantigens displayed by UCB lymphocytes. The prolonged delay to neutrophil and platelet reconstitution seen in UCB patients can be attributed to relatively small numbers of total nucleated cells and CD34+ stem cells found in UCB grafts. However, the substantial proliferative and engraftment potential of UCB CD34+ cells can offset these disadvantages to a degree that allows UCB to be a viable source of HSC for transplantation. In terms of immune function, UCB recipients remain highly vulnerable to infection. Further basic research and new methodologies are necessary to address

the prolonged times to engraftment and the possible influence of T regulatory cells on mechanisms of immune suppression.

Concluding remarks and future directions

Lower incidence of acute GVHD in UCB transplant recipients would be expected to be associated with higher rates of malignancy relapse, similarly to the highest rates of infectious complications. However, relapse rates after UCB transplant remain low. The mechanisms underlying the strong graft versus-leukemia (GVL) effects mediated by UCB have not been delineated. Clinical reports of allogeneic UCB recipients have not identified increased relapse rates, despite the fact that the majority of patients have advanced disease at the time of transplant. The unique immunologic features of UCB that allow HSC transplantation in the absence of full HLA compatibility, resulting in low incidence of GvHD but efficient GvL, make UCB an attractive approach for patients requiring allogeneic stem cell grafts. Further understanding of the mechanisms responsible for delayed engraftment and immune reconstitution will improve the outcome of UCB transplantation and will possibly alter our choice priority between peripheral blood mobilized stem cells and UCB as a HSC source in adult patients.

Acknowledgements

This work has been supported by NIH grants: CA104596 and AI 43552

References

1. Barker JN, Wagner JE. Umbilical-cord blood transplantation for the treatment of cancer. Nat Rev Cancer 2003;3:526–532. [PubMed: 12835672]
2. Knudtzon S. In vitro growth of granulocytic colonies from circulating cells in human cord blood. Blood 1974;43:357–361. [PubMed: 4811820]
3. Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Arny M, Thomas L, Boyse EA. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci U S A 1989;86:3828–3832. [PubMed: 2566997]
4. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Socie G, Lehn P, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med 1989;321:1174–1178. [PubMed: 2571931]
5. Barker JN, Wagner JE. Umbilical cord blood transplantation: current practice and future innovations. Crit Rev Oncol Hematol 2003;48:35–43. [PubMed: 14585482]
6. Rocha V, Wagner JE Jr, Sobocinski KA, Klein JP, Zhang MJ, Horowitz MM, Gluckman E. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources. N Engl J Med 2000;342:1846–1854. [PubMed: 10861319]
7. Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R, Ortega J, Souillet G, Ferreira E, Laporte JP, Fernandez M, Chastang C. Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. N Engl J Med 1997;337:373–381. [PubMed: 9241126]
8. Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, Migliaccio AR, Berkowitz RL, Cabbad M, Dobrila NL, Taylor PE, Rosenfield RE, Stevens CE. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N Engl J Med 1998;339:1565–1577. [PubMed: 9828244]
9. Wagner JE, Rosenthal J, Sweetman R, Shu XO, Davies SM, Ramsay NK, McGlave PB, Sender L, Cairo MS. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood

from unrelated donors: analysis of engraftment and acute graft-versus-host disease. Blood 1996;88:795–802. [PubMed: 8704232]

10. Dalle JH, Duval M, Moghrabi A, Wagner E, Vachon MF, Barrette S, Bernstein M, Champagne J, David M, Demers J, Rousseau P, Winikoff R, Champagne MA. Results of an unrelated transplant search strategy using partially HLA-mismatched cord blood as an immediate alternative to HLA-matched bone marrow. Bone Marrow Transplant 2004;33:605–611. [PubMed: 14743192]
11. Barker JN, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. Blood 2001;97:2957–2961. [PubMed: 11342417]
12. Rocha V, Cornish J, Sievers EL, Filipovich A, Locatelli F, Peters C, Remberger M, Michel G, Arcese W, Dallorso S, Tiedemann K, Busca A, Chan KW, Kato S, Ortega J, Vowels M, Zander A, Souillet G, Oakill A, Woolfrey A, Pay AL, Green A, Garnier F, Ionescu I, Wernet P, Sirchia G, Rubinstein P, Chevret S, Gluckman E. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. Blood 2001;97:2962–2971. [PubMed: 11342418]
13. Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A, Jacobsen N, Ruutu T, de Lima M, Finke J, Frassoni F, Gluckman E. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med 2004;351:2276–2285. [PubMed: 15564544]
14. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, Stevens C, Barker JN, Gale RP, Lazarus HM, Marks DI, van Rood JJ, Scaradavou A, Horowitz MM. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med 2004;351:2265–2275. [PubMed: 15564543]
15. Laughlin MJ, Barker J, Bambach B, Koc ON, Rizzieri DA, Wagner JE, Gerson SL, Lazarus HM, Cairo M, Stevens CE, Rubinstein P, Kurtzberg J. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. N Engl J Med 2001;344:1815–1822. [PubMed: 11407342]
16. Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, Goldman A, Kersey J, Krivit W, MacMillan ML, Orchard PJ, Peters C, Weisdorf DJ, Ramsay NK, Davies SM. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood 2002;100:1611–1618. [PubMed: 12176879]
17. Long GD, Laughlin M, Madan B, Kurtzberg J, Gasparetto C, Morris A, Rizzieri D, Smith C, Vredenburgh J, Halperin EC, Broadwater G, Niedzwiecki D, Chao NJ. Unrelated umbilical cord blood transplantation in adult patients. Biol Blood Marrow Transplant 2003;9:772–780. [PubMed: 14677117]
18. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, Miller JS, Wagner JE. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. Blood 2003;102:1915–1919. [PubMed: 12738676]
19. Cornetta K, Laughlin M, Carter S, Wall D, Weinthal J, Delaney C, Wagner J, Sweetman R, McCarthy P, Chao N. Umbilical cord blood transplantation in adults: results of the prospective Cord Blood Transplantation (COBLT). Biol Blood Marrow Transplant 2005;11:149–160. [PubMed: 15682076]
20. McNiece I, Kubegov D, Kerzic P, Shpall EJ, Gross S. Increased expansion and differentiation of cord blood products using a two-step expansion culture. Exp Hematol 2000;28:1181–1186. [PubMed: 11027837]
21. Lewis ID, Almeida-Porada G, Du J, Lemischka IR, Moore KA, Zanjani ED, Verfaillie CM. Umbilical cord blood cells capable of engrafting in primary, secondary, and tertiary xenogeneic hosts are preserved after ex vivo culture in a noncontact system. Blood 2001;97:3441–3449. [PubMed: 11369635]
22. Peled T, Landau E, Mandel J, Glukhman E, Goudsmid NR, Nagler A, Fibach E. Linear polyamine copper chelator tetraethylenepentamine augments long-term ex vivo expansion of cord blood-derived CD34+ cells and increases their engraftment potential in NOD/SCID mice. Exp Hematol 2004;32:547–555. [PubMed: 15183895]
23. Nauta AJ, Kruisselbrink AB, Lurvink E, Mulder A, Claas FH, Noort WA, Willemze R, Fibbe WE. Enhanced engraftment of umbilical cord blood-derived stem cells in NOD/SCID mice by

cotransplantation of a second unrelated cord blood unit. Exp Hematol 2005;33:1249–1256. [PubMed: 16219548]

24. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, McGlave PB, Miller JS, Verfaillie CM, Wagner JE. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood 2005;105:1343–1347. [PubMed: 15466923]
25. Ballen KK, Spitzer TR, Yeap BY, McAfee S, Dey BR, Attar E, Haspel R, Kao G, Liney D, Alyea E, Lee S, Cutler C, Ho V, Soiffer R, Antin JH. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. Biol Blood Marrow Transplant 2007;13:82–89. [PubMed: 17222756]
26. Brunstein CG, Barker JN, Weisdorf DJ, DeFor TE, Miller JS, Blazar BR, McGlave PB, Wagner JE. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. Blood 2007;110:3064–3070. [PubMed: 17569820]
27. Ting DT, Ballen K, Spitzer TR, Haspel RL, Dorn M, Preffer F, Boussiotis V, McAfee S, Attar E, Antin JH, Soiffer RJ, Cutler C, Ho V, Dey BR. Effects of Cord Blood Cell Subset Populations in the Development of the Dominant Cord Blood Unit in Non-Myeloablative Sequential Double Cord Blood Transplantation (DCBT). Blood 2006;108 (supp)abstract
28. Michel G, Rocha V, Chevret S, Arcese W, Chan KW, Filipovich A, Takahashi TA, Vowels M, Ortega J, Bordigoni P, Shaw PJ, Yaniv I, Machado A, Pimentel P, Fagioli F, Verdeguer A, Jouet JP, Diez B, Ferreira E, Pasquini R, Rosenthal J, Sievers E, Messina C, Iori AP, Garnier F, Ionescu I, Locatelli F, Gluckman E. Unrelated cord blood transplantation for childhood acute myeloid leukemia: a Eurocord Group analysis. Blood 2003;102:4290–4297. [PubMed: 12920027]
29. Kadereit S, Deeds LS, Haynesworth SE, Koc ON, Kozik MM, Szekely E, Daum-Woods K, Goetchius GW, Fu P, Welniak LA, Murphy WJ, Laughlin MJ. Expansion of LTC-ICs and maintenance of p21 and BCL-2 expression in cord blood CD34(+)/CD38(−) early progenitors cultured over human MSCs as a feeder layer. Stem Cells 2002;20:573–582. [PubMed: 12456965]
30. Chalmers IM, Janossy G, Contreras M, Navarrete C. Intracellular cytokine profile of cord and adult blood lymphocytes. Blood 1998;92:11–18. [PubMed: 9639493]
31. Liu E, Law HK, Lau YL. Tolerance associated with cord blood transplantation may depend on the state of host dendritic cells. Br J Haematol 2004;126:517–526. [PubMed: 15287945]
32. Brahmi Z, Hommel-Berrey G, Smith F, Thomson B. NK cells recover early and mediate cytotoxicity via perforin/granzyme and Fas/FasL pathways in umbilical cord blood recipients. Hum Immunol 2001;62:782–790. [PubMed: 11476901]
33. Kadereit S, Mohammad SF, Miller RE, Woods KD, Listrom CD, McKinnon K, Alali A, Bos LS, Iacobucci ML, Sramkoski MR, Jacobberger JW, Laughlin MJ. Reduced NFAT1 protein expression in human umbilical cord blood T lymphocytes. Blood 1999;94:3101–3107. [PubMed: 10556195]
34. Howrey RP, Martin PL, Driscoll T, Szabolcs P, Kelly T, Shpall EJ, Bearman SI, Slat-Vasquez V, Rubinstein P, Stevens CE, Kurtzberg J. Graft-versus-leukemia-induced complete remission following unrelated umbilical cord blood transplantation for acute leukemia. Bone Marrow Transplant 2000;26:1251–1254. [PubMed: 11149743]
35. Cohen G, Carter SL, Weinberg KI, Masinsin B, Guinan E, Kurtzberg J, Wagner JE, Kernan NA, Parkman R. Antigen-specific T-lymphocyte function after cord blood transplantation. Biol Blood Marrow Transplant 2006;12:1335–1342. [PubMed: 17162216]
36. Paulin T. Importance of bone marrow cell dose in bone marrow transplantation. Clin Transplant 1992;6:48–54. [PubMed: 10150090]
37. Wagner JE, Kurtzberg J. Cord blood stem cells. Curr Opin Hematol 1997;4:413–418. [PubMed: 9358998]
38. Faber LM, van Luxemburg-Heijs SA, Veenhof WF, Willemze R, Falkenburg JH. Generation of CD4 + cytotoxic T-lymphocyte clones from a patient with severe graft-versus-host disease after allogeneic bone marrow transplantation: implications for graft-versus-leukemia reactivity. Blood 1995;86:2821–2828. [PubMed: 7670118]
39. Faber LM, van Luxemburg-Heijs SA, Willemze R, Falkenburg JH. Generation of leukemia-reactive cytotoxic T lymphocyte clones from the HLA-identical bone marrow donor of a patient with leukemia. J Exp Med 1992;176:1283–1289. [PubMed: 1402674]
40. Falkenburg JH, Faber LM, van den Elshout M, van Luxemburg-Heijs SA, Hooftman-den Otter A, Smit WM, Voogt PJ, Willemze R. Generation of donor-derived antileukemic cytotoxic T-lymphocyte

responses for treatment of relapsed leukemia after allogeneic HLA-identical bone marrow transplantation. J Immunother Emphasis Tumor Immunol 1993;14:305–309. [PubMed: 8280712]

41. Mommaas B, Stegehuis-Kamp JA, van Halteren AG, Kester M, Enczmann J, Wernet P, Kogler G, Mutis T, Brand A, Goulmy E. Cord blood comprises antigen-experienced T cells specific for maternal minor histocompatibility antigen HA-1. Blood 2005;105:1823–1827. [PubMed: 15498856]
42. Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K, Urbani E, Negrin RS, Martelli MF, Velardi A. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood 1999;94:333–339. [PubMed: 10381530]
43. Flomenberg N, Baxter-Lowe LA, Confer D, Fernandez-Vina M, Filipovich A, Horowitz M, Hurley C, Kollman C, Anasetti C, Noreen H, Begovich A, Hildebrand W, Petersdorf E, Schmeckpeper B, Setterholm M, Trachtenberg E, Williams T, Yunis E, Weisdorf D. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. Blood 2004;104:1923–1930. [PubMed: 15191952]
44. Gomi S, Hasegawa S, Dan K, Wakabayashi I. A comparative analysis of the transplant potential of umbilical cord blood versus mobilized peripheral blood stem cells. Nippon Ika Daigaku Zasshi 1997;64:307–313. [PubMed: 9283200]
45. Ng YY, van Kessel B, Lokhorst HM, Baert MR, van den Burg CM, Bloem AC, Staal FJ. Gene-expression profiling of CD34+ cells from various hematopoietic stem-cell sources reveals functional differences in stem-cell activity. J Leukoc Biol 2004;75:314–323. [PubMed: 14634063]
46. Theunissen K, Verfaillie CM. A multifactorial analysis of umbilical cord blood, adult bone marrow and mobilized peripheral blood progenitors using the improved ML-IC assay. Exp Hematol 2005;33:165–172. [PubMed: 15676210]
47. Roy V, Verfaillie CM. Expression and function of cell adhesion molecules on fetal liver, cord blood and bone marrow hematopoietic progenitors: implications for anatomical localization and developmental stage specific regulation of hematopoiesis. Exp Hematol 1999;27:302–312. [PubMed: 10029170]
48. Peled A, Kollet O, Ponomaryov T, Petit I, Franitza S, Grabovsky V, Slav MM, Nagler A, Lider O, Alon R, Zipori D, Lapidot T. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. Blood 2000;95:3289–3296. [PubMed: 10828007]
49. Zheng Y, Watanabe N, Nagamura-Inoue T, Igura K, Nagayama H, Tojo A, Tanosaki R, Takaue Y, Okamoto S, Takahashi TA. Ex vivo manipulation of umbilical cord blood-derived hematopoietic stem/progenitor cells with recombinant human stem cell factor can up-regulate levels of homing-essential molecules to increase their transmigratory potential. Exp Hematol 2003;31:1237–1246. [PubMed: 14662330]
50. Fernandez MN, Regidor C, Cabrera R, Garcia-Marco JA, Fores R, Sanjuan I, Gayoso J, Gil S, Ruiz E, Little AM, McWhinnie A, Madrigal A. Unrelated umbilical cord blood transplants in adults: Early recovery of neutrophils by supportive co-transplantation of a low number of highly purified peripheral blood CD34+ cells from an HLA-haploidentical donor. Exp Hematol 2003;31:535–544. [PubMed: 12829030]
51. Shpall EJ, Quinones R, Giller R, Zeng C, Baron AE, Jones RB, Bearman SI, Nieto Y, Freed B, Madinger N, Hogan CJ, Slat-Vasquez V, Russell P, Blunk B, Schissel D, Hild E, Malcolm J, Ward W, McNiece IK. Transplantation of ex vivo expanded cord blood. Biol Blood Marrow Transplant 2002;8:368–376. [PubMed: 12171483]
52. Kollet O, Peled A, Byk T, Ben-Hur H, Greiner D, Shultz L, Lapidot T. beta2 microglobulin-deficient (B2m(null)) NOD/SCID mice are excellent recipients for studying human stem cell function. Blood 2000;95:3102–3105. [PubMed: 10807775]
53. Jaroscak J, Goltry K, Smith A, Waters-Pick B, Martin PL, Driscoll TA, Howrey R, Chao N, Douville J, Burhop S, Fu P, Kurtzberg J. Augmentation of umbilical cord blood (UCB) transplantation with ex vivo-expanded UCB cells: results of a phase 1 trial using the AastromReplicell System. Blood 2003;101:5061–5067. [PubMed: 12595310]
54. Baddley JW, Salzman D, Pappas PG. Fungal brain abscess in transplant recipients: epidemiologic, microbiologic, and clinical features. Clin Transplant 2002;16:419–424. [PubMed: 12437621]

55. Baddley JW, Stroud TP, Salzman D, Pappas PG. Invasive mold infections in allogeneic bone marrow transplant recipients. Clin Infect Dis 2001;32:1319–1324. [PubMed: 11303267]
56. Collin BA, Leather HL, Wingard JR, Ramphal R. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. Clin Infect Dis 2001;33:947–953. [PubMed: 11528564]
57. Haque T, Taylor C, Wilkie GM, Murad P, Amlot PL, Beath S, McKiernan PJ, Crawford DH. Complete regression of posttransplant lymphoproliferative disease using partially HLA-matched Epstein Barr virus-specific cytotoxic T cells. Transplantation 2001;72:1399–1402. [PubMed: 11685111]
58. Crawford DH, Mulholland N, Iliescu V, Hawkins R, Powles R. Epstein-Barr virus infection and immunity in bone marrow transplant recipients. Transplantation 1986;42:50–54. [PubMed: 3014694]
59. van Kraaij MG, Verdonck LF, Rozenberg-Arska M, Dekker AW. Early infections in adults undergoing matched related and matched unrelated/mismatched donor stem cell transplantation: a comparison of incidence. Bone Marrow Transplant 2002;30:303–309. [PubMed: 12209352]
60. Hamza NS, Lisgaris M, Yadavalli G, Nadeau L, Fox R, Fu P, Lazarus HM, Koc ON, Salata RA, Laughlin MJ. Kinetics of myeloid and lymphocyte recovery and infectious complications after unrelated umbilical cord blood versus HLA-matched unrelated donor allogeneic transplantation in adults. Br J Haematol 2004;124:488–498. [PubMed: 14984500]
61. Yoo JH, Lee DG, Choi SM, Choi JH, Park YH, Kim YJ, Kim HJ, Lee S, Kim DW, Lee JW, Min WS, Shin WS, Kim CC. Infectious complications and outcomes after allogeneic hematopoietic stem cell transplantation in Korea. Bone Marrow Transplant 2004;34:497–504. [PubMed: 15286689]
62. Locatelli F, Maccario R, Comoli P, Bertolini F, Giorgiani G, Montagna D, Bonetti F, De Stefano P, Rondini G, Sirchia G, Severi F. Hematopoietic and immune recovery after transplantation of cord blood progenitor cells in children. Bone Marrow Transplant 1996;18:1095–1101. [PubMed: 8971378]
63. Thomson BG, Robertson KA, Gowan D, Heilman D, Broxmeyer HE, Emanuel D, Kotylo P, Brahmi Z, Smith FO. Analysis of engraftment, graft-versus-host disease, and immune recovery following unrelated donor cord blood transplantation. Blood 2000;96:2703–2711. [PubMed: 11023501]
64. Klein AK, Patel DD, Gooding ME, Sempowski GD, Chen BJ, Liu C, Kurtzberg J, Haynes BF, Chao NJ. T-Cell recovery in adults and children following umbilical cord blood transplantation. Biol Blood Marrow Transplant 2001;7:454–466. [PubMed: 11569891]
65. Inoue H, Yasuda Y, Hattori K, Shimizu T, Matsumoto M, Yabe M, Yabe H, Tsuchida F, Tanaka Y, Hosoi G, Sako M, Kato S. The kinetics of immune reconstitution after cord blood transplantation and selected CD34+ stem cell transplantation in children: comparison with bone marrow transplantation. Int J Hematol 2003;77:399–407. [PubMed: 12774932]
66. Giraud P, Thuret I, Reviron D, Chambost H, Brunet C, Novakovitch G, Farnarier C, Michel G. Immune reconstitution and outcome after unrelated cord blood transplantation: a single paediatric institution experience. Bone Marrow Transplant 2000;25:53–57. [PubMed: 10654015]
67. Roux E, Helg C, Dumont-Girard F, Chapuis B, Jeannet M, Roosnek E. Analysis of T-cell repopulation after allogeneic bone marrow transplantation: significant differences between recipients of T-cell depleted and unmanipulated grafts. Blood 1996;87:3984–3992. [PubMed: 8611731]
68. Kook H, Goldman F, Giller R, Goeken N, Peters C, Comito M, Rumelhart S, Holida M, Lee N, Trigg M. Reconstruction of the immune system after unrelated or partially matched T-cell-depleted bone marrow transplantation in children: functional analyses of lymphocytes and correlation with immunophenotypic recovery following transplantation. Clin Diagn Lab Immunol 1997;4:96–103. [PubMed: 9008290]
69. Foot AB, Potter MN, Donaldson C, Cornish JM, Wallington TB, Oakhill A, Pamphilon DH. Immune reconstitution after BMT in children. Bone Marrow Transplant 1993;11:7–13. [PubMed: 8431713]
70. Pavletic ZS, Joshi SS, Pirruccello SJ, Tarantolo SR, Kollath J, Reed EC, Bierman PJ, Vose JM, Warkentin PI, Gross TG, Nasrati K, Armitage JO, Kessinger A, Bishop MR. Lymphocyte reconstitution after allogeneic blood stem cell transplantation for hematologic malignancies. Bone Marrow Transplant 1998;21:33–41. [PubMed: 9486492]
71. Gratama JW, Fibbe WE, Visser JW, Kluin-Nelemans HC, Ginsel LA, Bolhuis RL. CD3+, 4+ and/or 8+ T cells and CD3+, 4-, 8- T cells repopulate at different rates after allogeneic bone marrow transplantation. Bone Marrow Transplant 1989;4:291–296. [PubMed: 2543471]

72. Ault KA, Antin JH, Ginsburg D, Orkin SH, Rappeport JM, Keohan ML, Martin P, Smith BR. Phenotype of recovering lymphoid cell populations after marrow transplantation. J Exp Med 1985;161:1483–1502. [PubMed: 3159819]

73. Hercend T, Takvorian T, Nowill A, Tantravahi R, Moingeon P, Anderson KC, Murray C, Bohuon C, Ythier A, Ritz J. Characterization of natural killer cells with antileukemia activity following allogeneic bone marrow transplantation. Blood 1986;67:722–728. [PubMed: 3081066]

74. Saavedra S, Jarque I, Sanz GF, Moscardo F, Jimenez C, Martin G, Plume G, Regadera A, Martinez J, De La Rubia J, Acosta B, Peman J, Perez-Belles C, Gobernado M, Sanz MA. Infectious complications in patients undergoing unrelated donor bone marrow transplantation: experience from a single institution. Clin Microbiol Infect 2002;8:725–733. [PubMed: 12445010]

75. Tomonari A, Iseki T, Ooi J, Takahashi S, Shindo M, Ishii K, Nagamura F, Uchimaru K, Tani K, Tojo A, Asano S. Cytomegalovirus infection following unrelated cord blood transplantation for adult patients: a single institute experience in Japan. Br J Haematol 2003;121:304–311. [PubMed: 12694253]

76. Godfrey WR, Spoden DJ, Ge YG, Baker SR, Liu B, Levine BL, June CH, Blazar BR, Porter SB. Cord blood CD4(+)CD25(+)-derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. Blood 2005;105:750–758. [PubMed: 15374887]

77. Li L, Godfrey WR, Porter SB, Ge Y, June CH, Blazar BR, Boussiotis VA. CD4+CD25+ regulatory T-cell lines from human cord blood have functional and molecular properties of T-cell anergy. Blood 2005;106:3068–3073. [PubMed: 16020508]

78. Cairo MS, Wagner JE. Placental and/or umbilical cord blood: an alternative source of hematopoietic stem cells for transplantation. Blood 1997;90:4665–4678. [PubMed: 9389681]

Table I

Umbilical cord blood vs. bone marrow or mobilized stem cell transplantation (HSC)

	UCB	HSC
Advantages:		
Availibility of donor grafts	Large Supply	Limited supply
Availibility of graft	Rapid	Prolonged
Optimal degree of match	4/6 or Higher	9/10 or 10/10
Risk of GvHD	Lower risk	Higher risk
Risk of viral transmission	Very low risk	Higher risk
Risk to donor	No risk	Higher risk
Disadvantages		
Number of nucleated cells	Limited numbers	Higher numbers (1–2 logs)
Risk of infection	Higher than HSC	High
Speed of neutrophil/platelet recovery	Prolonged	Faster than UCB
Possibility of donor lymphocyte infusion	Not possible	Possible
Risk of genetic disease transmission	Higher than HSC	Very low

Table II

Umbilical cord blood transplantation in pediatric patients - selected clinical trials

	Gluckman et al. [7]	Rocha et al. [6]	Rubinstein et al. [8]	Wagner et al. [9]	Dalle et al. [10]	Rocha et al. [12]
Dates of clinical trial (yrs)	1988–1996	1990–1997	1992–1998	1994–1995	1996–2002	1994–1998
Patients enrolled	143	113	562	18	36**	99
Age in yrs (median)# (range)	0.2–45*	5 (<1–15)	< 2 - ≥18	2.7 (0.1–21.3)	7.5 (0.1–19.5)	6 (2.5–10)
Patients with hematologic malignancies	95	61	378	13	30	99
Patients without hematologic malignancies	48	52	184	5	6	0
Myeloablative (M) or nonmyeloablative (N)	M	M	M	M	M	M
Degree of HLA match (%)						
matched (6/6)	69	100 (HLA-ID sibs)	7	7	6	8
1 or 2/6	62		86	10	94	84
3/6 or more	12		7	1		8
Median cell dose (range):						
Nucleated cells/kg ($\times 10^8$)	0.37 (0.07–3.0)	0.47 (1.0–3.6)	range: 0.07 - >1.0	0.41 (0.14–4.0)	0.25 (na)	0.38 (0.24–3.6)
CD34+ cells/kg ($\times 10^5$)	2 (0–45)	na	na	na	na	na
Days to neutrophil recovery (median) (range)	30 (8–56)	26 (na)	28 (10–120)	24 (16–53)	28 (16–49)	32 (11–56)
Days to platelet recovery (median) (range)	56 (9–180)	44 (na)	90 (16–250)	54 (39–130)	43 (18–59)	81 (16–159)
No. given hematopoietic growth factors	104	45	na	7	23	54
Incidence of acute GvHD:	In 143 patients:	In 107 patients	In 399 patients:	In 13 patients	In 36 patients	In 99 patients
Grade I–II (%)	related=13; unrelated=12	29	78	61	3	35
Grade III–IV (%)	related=5; unrelated=20	2	22	11	11	21
Patients with cGvHD/surviving patients	related 8/56; unrelated 0/23	5/93	39/158	na	3/36	5/43
Incidence of relapse (%)	related=14; unrelated=8	na	26	11	17	38
Treatment related mortality (TRM) (%)	related=14; unrelated=45	31	39	28	27 at day 100	39 at day 100
Overall survival (2–3yrs) (%)	63 (related donor) 73 (unrelated; 6/6 match)	46 (malignant) 86 (nonmalignant)	61% at 100 days	65% at 6 months	59	35

na = not available

* 84% of patients ≤ 15 yrs.

when indicated

** data given is for UCB transplants only

Table III

Umbilical cord blood transplantation in adult patients - selected clinical trials

	Rocha et al. [13]	Laughlin et al. [14]	Wagner et al. [16]	Barker et al. [24]	Ballen et al. [25}	Brunstein et al. [26]
Dates of clinical trial (yrs)	1998–2002	1996–2001	1994–2001	2000–2003	2003–2005	2001–2005
Patients enrolled	98	150	102	23	21	110
Age in yrs (median)[#](range)	24.5 (15–55)	na (16–60)	7.4 (0.2–56.9)	24 (13–53)	49 (24–63)	51 (17–69)
Patients with hematologic malignancies	98	150	65	23	19	106
Patients without hematologic malignancies	0	0	37	0	2	4
Myeloablative (M) or nonmyeloablative (N)	M	M	M	M	N	N
Degree of HLA match (%)				**Double UCB trial:	**Double UCB trial:	**Double UCB trial:
matched (6/6)	6	0	14	each cord 4-6/6 matched	each cord 4-6/6	each cord 4-6/6 matched
1 or 2/6	90	100	84	to each other and to the	matched to each other	to each other and to the
3/6 or more	4	0	2	recipient	and to the recipient	recipient
Median cell dose (range):						
Nucleated cells/kg ($\times 10^8$)	0.23 (0.09-0.6)	0.22 (0.10–0.65)	0.31 (0.07–5.79)	0.35 (0.11–0.63)	0.4 (2.9–5.1)	0.37 (0.15–0.68)
CD34+ cells/kg ($\times 10^5$)	1.1 (0.8–8.8)	na	2.8 (0.4–39.1)	4.9 (1.2–14.5)	1.9 (0.6–9.7)	4.9 (0.7–16.6)
Days to neutrophil recovery (median) (range)	26 (14–80)	27 (25–29)	23 (9–54)	23 (15–41)	20 (15–34)	12 (0–32)
Days to platelet recovery (median) (range)	na	60 (54–71)	86 (29–276)	na	41 (21–55)	49 (0–134)
No. given hematopoietic growth factors	na	na	80	23	na	110
Incidence of acute GvHD:	In 98 patients:	In 150 patients:	In 63 patients:	In 23 patients:	In 19 patients:	In 110 patients
Grade I–II (%)	13	Grades II, III, IV: 41	51	52	53	37
Grade III–IV (%)	13		17	13	5	22
Patients with cGvHD/surviving patients	18/61	35/69	9/59	5/23	5/16	23/110
Incidence of relapse (%)	23	17	37	13	10	31
Treatment related mortality (TRM) (%)	44 (2 yrs)	63	30 (1 yr)	22 (6 months)	14 (100 days)	19 (180 days)
Overall survival (2–3yrs) (%)	63	19	58 (1 yr)	57 (1 yr)	71 (1 yr)	45 (3 yrs)

na = not available

Table IV

Biology of umbilical cord blood cells versus bone marrow or mobilized peripheral stem cells (HSC)

	UCB		HSC
T cell response to alloantigens/mitogens	Modest response		Vigorous response
Proportion of CD4+ CD45RA+ cells	Majority of T cells		Dual population of CD45RA+/CD45RO+
Proportion of hematopoietic progenitor cells	Greater than HSC		Reduced
CD34+ proliferation rate in response to cytokines in vitro	Greater than HSC		Reduced
Production of hematopoietic factors* from activated mononuclear cells	Significantly less than HSC		Higher amounts
Production of IL-2, IFN-γ, TNF-α from activated T cells	Significantly less than HSC		Higher amounts
Allogeneic cytotoxic activity of activated T cells	Significantly less than HSC		Higher amounts
Expression of NFAT1	Reduced		Higher
Natural killer cell activity	——	Comparable	——

*G-CSF, GM-CSF, IL-3, M-CSF, TGF-β1, MIF-1α [78]