

UMBILICAL CORD BLOOD TRANSPLANTATION AND BANKING

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■ **Abstract** Umbilical cord blood transplantation (UCBT) is an expanding practice for both pediatric and adult patients. Rapid availability, low risk of infectious disease transmission, lower risk of graft-versus-host disease, and lack of risk for the donor makes UCB an attractive alternative source of hematopoietic stem cells for transplantation. We review the state of the art of pediatric and adult UCBT and important aspects of UCB banking. Current strategies to improve clinical results and expand access to UCBT to a larger number of adult patients are discussed. New approaches to enhance hematopoietic recovery by the use of accessory cells or direct intra-bone marrow injection are also reviewed.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has become the standard of care for a number of high-risk hematological malignancies. However, not all patients who would potentially benefit from HSCT can proceed to transplant because only 50%–60% of patients have an HLA-matched sibling or unrelated donor. Although there are currently seven million adult volunteers registered worldwide, one third of patients still will not find a suitably HLA-matched unrelated donor and thus cannot access this potentially curative therapy. If the patient belongs to an ethnic minority, the probability of not finding an HLA-matched donor is even greater. Moreover, because the search process often takes weeks to months, a significant proportion of patients will die, become higher-risk, or become ineligible for transplantation altogether while they wait for the completion of the donor search. For those patients for whom a donor is identified, transplantation is frequently complicated by acute and chronic graft-versus-host disease (GVHD) and opportunistic infection, which increase transplant morbidity and mortality.

During the past decade, umbilical cord blood (UCB) has been established as an alternative source of hematopoietic stem cells (HSC) for transplantation for both

adult and pediatric patients, and the risks and benefits of UCB have become clear. Compared with adult mobilized peripheral blood stem cells (PBSC) and bone marrow, UCB is enriched with hematopoietic progenitor cells. The collection of UCB after birth carries no risk for the donor. Collected UCB can be cryopreserved and stored in cord blood banks (CBBs) for prolonged periods. As UCB units are HLA-typed, tested for infectious agents, and stored, they are immediately available upon request and can be shipped to any transplant center in the world with relative ease. UCBT is associated with a low incidence of acute GVHD, and partial HLA match between the donor and recipient is tolerable. Although the true impact remains to be determined, there is a negligible risk of infectious disease transmission from UCB itself.

UCB, however, has limitations. First, multiple registry and single-center analyses have shown that the number of progenitor cells is important in engraftment and survival. The lower the cell dose, the poorer the outcome. Because the cell dose in a single unmanipulated UCB unit is fixed, most adults are denied routine access to UCB. A second obstacle is the inability to go back to the donor to collect lymphocytes or additional HSC, in case of disease progression or relapse, or graft failure. Third, there is substantially less experience with UCBT than with bone marrow transplant (BMT), particularly in adult patients.

UMBILICAL CORD BLOOD TRANSPLANTATION IN CHILDREN

Gluckman et al. reported in 1989 the first successful hematopoietic reconstitution in a child with Fanconi anemia using UCB (1). Wagner et al. in 1994 reported the first successful hematopoietic reconstitution in a child with leukemia using UCB and documented the absence of maternal cell contamination. In 1993 and 1994, Kurtzberg and Wagner performed the first transplants using unrelated donor UCB, and patients 2 (acute myelogenous leukemia) and 4 (globoid cell leukodystrophy) are alive and well 13 and 14 years later, respectively (2, 3). Since those pioneering procedures, thousands of children have received UCB grafts for the treatment of numerous malignant and nonmalignant disorders. It is now clear that transplant of HLA-mismatched UCB is acceptable, at least when matched at 4 of 6 HLA A, HLA B, and DRB1 antigens. About 40% of pediatric patients who undergo a related UCBT, and 80% of those with an unrelated donor, receive a mismatched UCB unit (4–8). At pediatric centers, the frequency of transplantation of UCB is rapidly approaching that of bone marrow and has surpassed that of PBSC (personal communication, Center for International Bone Marrow Transplant Research). Supplemental Table 1 summarizes the clinical results with UCBT in the pediatric population. (Follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>.)

From the outset, the clinical studies demonstrated slower but complete hematopoietic reconstitution in the majority of patients (2, 3, 9). Compared with bone

marrow recovery, neutrophil recovery is delayed, with a median time to neutrophil recovery of 22–24 days. Platelet recovery is also slow, with approximately two thirds of patients achieving a platelet count $\geq 50,000/\mu\text{l}$ with transfusion independence at one year post-transplant (4, 5, 7, 8, 10, 11). Although factors associated with the speed of neutrophil recovery have varied between reports, most include nucleated cell dose (7, 11, 12) and some also identify CD34⁺ (5) or CFU-GM dose (13). Wagner et al. demonstrated a positive impact of hematopoietic growth factor administration on speed of neutrophil recovery, but this finding has not been universally observed (4, 5). Hematopoietic recovery is observed in 80%–90% of patients by day 42 (5, 7). Among children, the incidence of graft failure is similar to other HSC sources, but patients with nonmalignant disease may have a higher risk of graft failure (5, 14). It is speculated that patients with nonmalignant disease who do not receive chemotherapy prior to transplantation have a more functional immune system, which may explain this observation. In addition, recent studies suggest that some regimens that do not include total body irradiation (TBI) may be insufficient for consistent engraftment. For example, in very young children with leukemia in whom busulfan, melphalan, and antithymocyte globulin were used, the risk of graft failure was 30% (15).

The clinical outcome data support the original hypothesis that UCB differs from adult immune cells in ways that make it less likely to cause GVHD. In most reports, the incidence of acute GVHD after UCBT is lower than that expected for BMT (Table 1). Comparative analyses support this claim both for HLA-matched related donor-recipient pairs and for unrelated donor transplants (HLA-matched and -mismatched) (8, 10). Younger recipient age and use of T cell–depleted marrow also decrease the risk of acute GVHD. The influence of HLA mismatch is less consistent, with some studies demonstrating a higher risk of acute GVHD with greater HLA disparity. UCB CD3 cell dose and maternal cell contamination have no demonstrable effect.

In contrast to acute GVHD, where there is general agreement that UCB is associated with lower risk, clinical reports conflict with regard to chronic GVHD. Whereas there appears to be a significantly lower risk of chronic GVHD in recipients of HLA-identical sibling UCB, in the unrelated setting, most comparative studies fail to demonstrate a statistical difference between UCB and bone marrow (7, 8, 10, 11, 16, 19).

Similarly, the treatment-related mortality (TRM) rate after UCBT is not consistent between reports. Comparing UCBT to BMT, Rocha et al. (8) observed a higher TRM within the early period after UCBT. However, most studies have found that cell dose may account for this observation, at least in part. Wagner et al. found that there was a critical threshold (1.7×10^5 CD34/kg) below which patients had a very high TRM (5).

If response to alloantigens is indeed lower after UCBT, it was hypothesized that the relapse rate may be increased. The incidence of relapse after UCBT is similar to that in recipients of unmanipulated marrow (8, 16), suggesting that the graft-versus-leukemia (GVL) effect is intact. Despite the lower T cell dose with

UCB grafts (17), the relapse rate is lower than with T cell–depleted unrelated bone marrow (8). Regardless of stem cell source, relapse risk is lower for patients transplanted earlier in their disease course (4, 11) and for those who have standard-risk acute leukemia (5).

Survival after UCBT is influenced by donor type (i.e., related or unrelated) (7) and treatment indication (i.e., malignant or nonmalignant disease) (4–6, 8, 11) (Table 1). For recipients of sibling donor UCB, survival is in excess of 60% (6), whereas for recipients of unrelated UCB it ranges between 29% and 58% (4, 5, 7, 10, 15). HLA match and higher cell dose (infused CD34⁺ cell dose, CFU-GM or nucleated cells) positively influence survival (5, 7, 12, 13).

Current evidence demonstrates the safety and efficacy UCBT for children. The speed of the donor search, high likelihood of finding a suitably HLA-matched UCB unit (particularly for patients of ethnic and racial minorities) with an adequate cell dose, and lower rate of acute GVHD despite HLA mismatch lead many centers to choose unrelated donor UCB over unrelated adult marrow or peripheral blood. Major endpoints such as TRM, relapse rate, and overall survival are at least comparable, making UCB frequently the preferred alternative HSC source for children who lack a HLA-matched sibling donor.

UMBILICAL CORD BLOOD TRANSPLANTATION IN ADULTS

The encouraging pediatric results prompted the investigation of UCB as an HSC source for adult transplantation (5, 18–28). However, progress has been slow, principally because of the scarcity of units with an adequate cell dose. In 2000–2001, only about one third of adults referred to the University of Minnesota were eligible for UCBT, based on the cell dose requirement of 1×10^7 nucleated cells/kg defined as adequate by most transplant centers in the mid 1990s. In 2004–2005, even with 186,000 units in CBBs, only ~25% of adults meet the current cell dose requirement of 2.5×10^7 nucleated cells/kg. Supplemental Table 2 summarizes the clinical results with UCBT in the adult population. (Follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>.)

As expected based on the early results in children, the rate and incidence of neutrophil and platelet engraftment were lower after UCBT than after PBSCT or BMT, with a median time to neutrophil recovery after UCBT ranging between 22 and 32 days (18–28). In adults, both cryopreserved (19, 21) and infused (21) nucleated cell dose are associated with speed of hematopoietic recovery. Furthermore, the incidence of graft failure in adult recipients of UCB was as high as 35% (18–26). The relatively low nucleated cell dose and greater likelihood of use of a HLA 2-antigen-mismatched graft probably accounts for this slow recovery and high graft failure rate.

The incidence of acute GVHD among adult recipients of UCB is shown in Table 2. Despite a higher degree of HLA mismatch, adult recipients of UCB had

a comparable or lower incidence of acute GVHD compared to adult recipients of HLA-matched, T cell-replete bone marrow (18, 20). Adults who received UCB had a lower incidence of acute GVHD than did those who received HLA-mismatched unrelated marrow (20). Whereas the incidence of chronic GVHD after UCBT has been reported to be as high as 80%, extensive chronic GVHD is as high as 46% (Table 2). Comparative studies with unrelated BMT recipients show conflicting results.

TRM in adult recipients of UCB after a myeloablative regimen has been reported to be as high as 60% (Table 2). Studies comparing recipients of UCB and bone marrow yield conflicting results (18, 20, 23); some studies demonstrate lower TRM, some higher TRM, and some similar TRM. The difficulty in interpreting these retrospective studies comparing UCBT to BMT relates to patient selection. In most studies, adults undergoing UCBT tended to have advanced, high-risk disease and failed to have a HLA-matched unrelated bone marrow donor. Laughlin et al. (20) found that TRM after UCBT was higher than that observed with HLA-matched unrelated-donor BMT, but similar to that observed with HLA-mismatched BMT. Rocha et al. (18), however, reported similar TRM in recipients of HLA-mismatched UCB and HLA-matched unrelated marrow.

As reported for children with malignant disease, the incidence of disease relapse was not increased among adult UCBT recipients (Table 2). However, most series report results for a heterogeneous group of diseases, making disease-specific conclusions impossible. In the two recently published registry-based studies that compared UCBT and unrelated marrow recipients, there was no difference in incidence of relapse for patients with acute leukemia (18, 20). Overall, 25%–30% of the patients were leukemia-free two to three years after UCBT (18, 20).

Overall survival (OS) rate for adults after UCBT ranged between 20% and 35% in those studies (Table 2). Survival is influenced by recipient age, disease status, recipient cytomegalovirus (CMV) serostatus, and UCB graft nucleated cell dose (18–20). It remains to be proven whether survival after UCBT is comparable to that in recipients of HLA-matched unrelated bone marrow. Taken together, the above data suggest that UCBT is a reasonable alternative for adult patients who lack an HLA-matched sibling or unrelated marrow donor. Although there is a growing body of literature on adult UCBT, there is still significantly less experience than in the pediatric setting. Available studies are markedly limited by significant variability in institutional standards regarding patient eligibility, UCB graft selection (cell dose and HLA match requirements), conditioning regimen, and supportive care. Although higher than in the pediatric population, the incidence of both acute and chronic GVHD in the adult population is acceptable. This increased incidence may reflect thymic involution. Larger numbers of patients treated in prospective trials are required before more definitive statements can be made regarding the safety and efficacy of UCBT, but various strategies to overcome the principal limitation of cell dose in the adult population are being tried.

Multiple Umbilical Cord Blood Units

Cell dose is the single most important factor preventing adults and larger adolescents from undergoing UCBT. Barker et al. (29) were the first to report the successful use of two UCB units to overcome the cell-dose limitation. Others (30–32) have subsequently utilized two or more UCB units with variable results. At the University of Minnesota, transplantation of two partially HLA-matched UCB units has been evaluated in the context of a myeloablative (33) and nonmyeloablative (34, 35) preparative therapy (see Nonmyeloablative Umbilical Cord Blood Transplantation, below).

The Minnesota experience in adult and adolescent recipients of “double” UCBT for hematologic malignancies, most often acute leukemia, has been reported (Table 2) (33). The graft consisted of two units that were matched at a minimum of 4 out of 6 HLA A, HLA B, and DRB1 antigens with the recipient and each other (not necessarily at the same HLA loci). In the original cohort, the total graft nucleated cell dose had to exceed $1.5 \times 10^7/\text{kg}$, with the one unit containing a minimum cell dose of $1.0 \times 10^7/\text{kg}$. Median time to neutrophil engraftment was 23 days, and all evaluable patients developed complete chimerism with no secondary graft failure. Notably, double chimerism (i.e., contributions from both UCB units) was detectable in the bone marrow in only $\sim 25\%$ between days 21 and 28. By day 100, hematopoiesis was derived from a single unit in all patients. Although a greater proportion will have “double chimerism” at day 14 in both T cells and myeloid cells, these evaluations have only just been initiated. Importantly, no factor (i.e., total nucleated cell dose, CD34 dose, CD3 dose, HLA match, ABO match, sex match, order of infusion) predicts the long-term engrafting unit; the clinical experience to date suggests that engraftment is random. Further analysis with larger sample sizes is required.

TRM and survival after double UCB transplantation appear superior to those reported in recently published series (18, 20). Kai et al. (30) reported similar results on a smaller number of patients with hematological malignancies who received double UCB grafts, utilizing the same HLA-matching criteria.

The above data suggest that the utilization of two UCB units in combination with fludarabine in the preparative regimen has overcome, at least in part, the cell-dose limitation for UCBT, with improved engraftment and survival. The relative contributions of double UCBT and the benefit of fludarabine to the overall success of the transplant procedure remains to be determined; it is possible that cell dose has a markedly diminished effect in recipients of fludarabine in combination with cyclophosphamide and TBI. Multi-institutional randomized trials have been proposed to address this issue in both children and adults.

Nonmyeloablative Umbilical Cord Blood Transplantation

Nonmyeloablative stem cell transplantation (NST) is based on the understanding that the GVH and GVL effects of allogeneic HSC sources are at least partly responsible for engraftment and long-term disease control, respectively. Storb et al.

(36) developed a canine model of NST that was subsequently transferred to humans (37–39). The use of a reduced-intensity preparative regimen has allowed HSCT of patients who are older, heavily pretreated, and have significant comorbidities that would make them ineligible for a myeloablative preparative regimen.

Because BMT and PBSCT have been associated with high incidences of acute and chronic GVHD in addition to the limited availability of HLA-matched unrelated donors, the Minnesota group evaluated the potential benefit of UCBT in the setting of a reduced-intensity preparative regimen. However, it was recognized that, in contrast to sibling or unrelated adult donor HSCT, donor lymphocyte infusions to enhance engraftment or promote GVL would not be possible. In order to meet the cell-dose criteria, approximately three quarters of the patients received two partially HLA-matched UCB units (35). The regimen consisted of cyclophosphamide 50 mg/kg on day –6, fludarabine 40 mg/m² on days –6 to –2, and TBI 200 cGy on day –1 with cyclosporine and mycophenolate mofetil immunoprophylaxis.

To date, the results in the first 59 adult recipients of NST and UCBT have been analyzed (35). Neutrophil recovery was rapid (median of 8 days). The cumulative incidence of sustained donor-derived engraftment was 89%. As observed in recipients of a myeloablative preparative regimen, one unit ultimately predominates with no factor predictive of the long-term engrafting UCB unit. Importantly, the cumulative incidence of acute GVHD may be higher than that previously reported in children or adults after a myeloablative regimen, with 65% of the patients developing grades II–IV acute GVHD and 25% developing grades III–IV acute GVHD. Although the reason is unknown, it is possible that older recipient age or presence of host dendritic cells observed in the setting of NST may be responsible. However, despite the higher incidence of acute GVHD, TRM is low (19%). Thus far, only fitness status at the time of transplant is associated with TRM; age by itself is not a limiting factor. The probability of overall and progression-free survival is 44% and 35% at two years. Insufficient numbers for any one disease group prevent disease-specific evaluations, which are the goal of ongoing multi-institutional trials.

Case reports (40–45) and small series of UCB NST (27, 34, 46, 47) are summarized in Tables 1 and 2.

FUTURE DIRECTIONS

The clinical experience to date indicates that UCBT can result in consistent engraftment and low TRM. UCB is well established in the setting of pediatric HSCT, rapidly becoming a preferred HSC source at many institutions worldwide. In contrast, few centers performing adult HSCT have yet adopted UCBT as routine. Promising phase II clinical data are only now being published, and these studies must be replicated before there is more general use in the adult setting. Moreover, larger numbers of patients need to be treated before we can make statements regarding the use of UCBT for specific diseases. Important obstacles, especially for adult patients, are the inability to find a UCB graft that will provide an adequate

cell dose, and its consequences for engraftment and survival. The successful use of double UCB unit grafts and the potential beneficial effect of fludarabine need further investigation (33).

Other promising strategies, such as the use of UCB combined with T cell-depleted haploidentical related-donor PBSC and coadministration of regulatory T cells to improve the outcome of UCBT in adults, are also being investigated and are summarized below.

T Regulatory (Treg) Cells

A subset of CD4⁺ T cells that coexpress CD25 (IL-2R α chain) has been shown to be important in self-tolerance and prevention of autoimmunity (48–51; see also “CD4⁺CD25⁺ Regulatory T Cells and Their Therapeutic Potential” in this volume). In murine models, the infusion of ex vivo expanded Treg cells can successfully prevent and treat acute GVHD (48, 50, 51), as well as promote high levels of donor chimerism (52). In contrast to peripheral blood-derived Treg cells, UCB-derived Treg cells consistently suppress a third-party mixed lymphocyte reaction (53). Promising preclinical data and development of large-scale manufacture methods will probably lead to phase I–II clinical testing of Tregs in the setting of UCBT in the near future, with the goal of further enhancing engraftment and reducing the risk of GVHD particularly in the older age group.

Ex Vivo Expansion Culture

For the past decade, there has been tremendous interest in the development of ex vivo expansion culture systems in order to enhance engraftment of UCB. Results of several phase I–II clinical trials have been reported (54–59). Most of these studies utilized one UCB unit, first infusing a proportion of the unit that had been unmodified and then infusing the “expanded” portion 10–14 days later. Although no trial has reported significant infusional toxicities, there is also no clear evidence of benefit. The clinical model itself, however, is limiting. Because the expanded product was from the same unit and lacks genetic marking, the contribution of the expanded cells to engraftment cannot be assessed. Further, the infusion of “expanded” cells 10–14 days after the initial infusion minimizes the chance of observing any reduction in the time to neutrophil recovery. For these reasons, trials of ex vivo expansion culture have now been modified, either using units that have been proportioned prior to cryopreservation so that expansion can occur at day –10 to –14, or using the double UCB model with coinfusion of the expanded product with the unexpanded unit. Results of studies using these approaches have not yet been published.

Intra–Bone Marrow Injection

Animal models show that <20% of HSC intravenously infused ultimately reach the bone marrow space (60). Injection of HSC directly into the bone marrow

microenvironment has been suggested as a potential strategy to improve homing after transplantation (61–65). Cells injected into bone marrow may provide better engraftment by migrating and colonizing other bones after transplantation (62, 64). Thus far, only one small randomized clinical trial has compared intra–bone marrow injection (IBMI) to intravenous infusion in adults undergoing allogeneic BMT. Although IBMI was well tolerated, it had no demonstrable impact on clinical outcome (66). The cell-dose limitation of UCB makes IBMI attractive, as improved homing to the bone marrow would reduce trapping of progenitors in tissues that do not support hematopoiesis.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSC) actively participate in normal hematopoiesis by producing growth factors and cytokines and by providing cell-to-cell interaction (67, 68). In animal models, MSC have been shown to facilitate engraftment of NOD/SCID mice with human UCB (69, 70). Early clinical reports suggest that MSC may be a useful tool to enhance engraftment and to prevent and treat GVHD (71–73). A recent report suggests that the immune modulation of MSC is at least partly mediated through Treg cells (74). One clinical study has explored the coinfusion of parental haploidentical ex vivo expanded MSC with unrelated UCB (75). No serious adverse events were observed. Neutrophil and platelet recoveries tended to be more rapid, but larger numbers of patients in a randomized setting with and without MSC would be required for definitive proof.

Cord Blood Banking

The use of UCB as a source of HSC for transplantation led to the establishment of many cord blood banks (CBBs) worldwide. As UCBT becomes more widely accepted, the number of CBBs and the size of the inventory within some CBBs are likely to increase. Currently, there are at least 15 public CBBs in the United States. The American Association of Blood Banks (AABB) and the Foundation for Accreditation of Cellular Therapy (FACT-NETCORD) have created guidelines pertaining to collection, testing, processing, and banking of UCB for transplantation and provide the accreditation of UCB banks (76, 77). Moreover, the U.S. Food and Drug Administration has issued new Good Tissue Practice regulations, which apply to human cellular and tissue products used for transplantation (78). The overall goal is to provide standards for collection, storage, documentation and labeling, and CBB operations.

At the University of Minnesota, one of the largest single centers using UCB from multiple CBBs, we have recently reviewed the quality of UCB units received from 14 CBBs for clinical UCBT. Of 268 UCB units received, we found quality concerns in 151 (56%) from 12 banks. About half of the units had issues that involved aspects of the medical history and quality control. Labeling and documentation errors were noted in in ~6% of the UCB units. In approximately one third of cases, deviations were felt to pose a potential risk to quality. Among the medical history issues,

a high proportion were related to the potential transmission of infectious and genetic diseases. FACT-NETCORD and AABB guidelines do specify the maternal and family information that should be collected. However, we found variability among CBB staff's interpretations as to which diseases should be considered to pose risks for recipients. In our experience, these quality issues did not improve between 1994 and 2003.

We found UCB units on which tests for transmissible diseases were incomplete, pending, or positive. Despite that, these UCB units were stored and shipped as suitable for transplantation. The transplant center was unaware of these issues prior to shipment in most instances. Although guidelines call for virology screening by CBBs (FACT-NETCORD requires testing for HIV-1 and -2, HTLV/II, HCV, and HBsAg; AABB requires testing for HIV-1 antigen, anti-HBc, and syphilis), this was not always the practice. Bacterial contamination of UCB is also an important concern of quality and safety. Though allowed by both FACT-NETCORD and AABB, units with positive cultures arguably should not be made available for transplant. Other aspects of labor, such as the mother's temperature and use of antibiotics, are important to determine the risk of infection transmission and the unit's suitability for transplantation.

Issues related to shipping, lack of documentation of the processing methods, and improper labeling were also observed. The dry shipper did not always contain a device that monitored temperature throughout the shipment period to document a consistent proper temperature while the UCB unit was in transit (FACT-NETCORD). UCB units partially thawed upon arrival at the transplant center have been reported. Further, in two documented cases, it was proven that units were incorrectly labeled. This risk provides a compelling reason to require testing on attached segments to verify unit identity prior to unit shipment and transplantation.

SUMMARY

The increasing experience with UCBT is changing the standard of care in many institutions. Initial concerns regarding the potency of the GVL effect of UCB have been dismissed by publications from several experienced groups showing relapse rates similar to those associated with other HSC sources. In the pediatric setting, UCBT is now established practice and will soon surpass the number of unrelated adult volunteer donor transplants. Favorable results compared with historical controls have contributed to changes in clinical practice.

In the adult setting, progress has been slower owing to the cell-dose limitation. Engraftment and survival of adult patients who received single UCB unit grafts have been suboptimal. Strategies focused on overcoming this limitation are under intense investigation. Thus far, *ex vivo* expansion of UCB has not yet been shown to influence engraftment, but work continues in this area. Recent registry-based studies suggest that the results of adult UCBT are comparable to those of HLA-matched and -mismatched unrelated-donor BMT. Notably, the utilization of double UCB unit grafts has shown promising results. Engraftment rates and survival seem

to be superior to historical data. However, this strategy still requires validation with larger patient numbers and longer follow-up at multiple institutions. Multicenter trials have been proposed.

The utilization of nonmyeloablative preparative regimens broadens the range of patients who might benefit from UCBT. The fludarabine, cyclophosphamide, TBI regimen proposed at the University of Minnesota has shown encouraging results with high engraftment rates and low TRM for a high-risk group of patients.

Despite significant progress in the past decade, there remains a need for new strategies to promote engraftment and facilitate lympho-hematopoietic recovery. Coinfusion of Treg cells and IBMI are likely to reach clinical trials soon.

Expansion and greater reliability of the CBBs are essential for the success of UCBT strategies. Expanding the UCB pool and adopting FACT-NETCORD and AABB proposed quality standards should provide clinicians high-quality information and UCB units for a safer UCBT procedure.

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LITERATURE CITED

1. Gluckman E, Broxmeyer HA, Auerbach AD, et al. 1989. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N. Engl. J. Med.* 21: 1174–78
2. Kurtzberg J, Laughlin M, Graham ML, et al. 1996. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. *N. Engl. J. Med.* 335:157–66
3. Wagner JE, Rosenthal J, Sweetman R, et al. 1996. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. *Blood* 88:795–802
4. Michel G, Rocha V, Chevret S, et al. 2003. Unrelated cord blood transplantation for childhood acute myeloid leukemia: a Eurocord Group analysis. *Blood* 102:4290–97
5. Wagner JE, Barker JN, DeFor TE, et al. 2002. 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* 100:1611–18
6. Rocha V, Wagner JE Jr, Sobocinski KA, et al. 2000. 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.* 342:1846–54
7. Gluckman E, Rocha V, Boyer-Chamard A, et al. 1997. 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.* 337:373–81
8. Rocha V, Cornish J, Sievers EL, et al. 2001. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood* 97:2962–71
9. Wagner JE, Kernan NA, Steinbuch M, et al. 1995. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. *Lancet* 346:214–19

10. Barker JN, Davies SM, DeFor T, et al. 2001. 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* 97:2957-61
11. Locatelli F, Rocha V, Chastang C, et al. 1999. Factors associated with outcome after cord blood transplantation in children with acute leukemia. Eurocord-Cord Blood Transplant Group. *Blood* 93:3662-71
12. Rubinstein P, Carrier C, Scaradavou A, et al. 1998. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N. Engl. J. Med.* 339:1565-77
13. Migliaccio AR, Adamson JW, Stevens CE, et al. 2000. Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity. *Blood* 96:2717-22
14. Locatelli F, Rocha V, Reed W, et al. 2003. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. *Blood* 101:2137-43
15. Wall D, Carter C, Kernan NA, et al. 2005. Busulfan/melphalan/ATG followed by unrelated donor cord blood transplantation for treatment of infant leukemia and leukemia in young children: the COBLT experience. *Biol. Blood Marrow Transplant.* 11:637-46
16. Jacobsohn DA, Hewlett B, Ranalli M, et al. 2004. Outcomes of unrelated cord blood transplants and allogeneic-related hematopoietic stem cell transplants in children with high-risk acute lymphocytic leukemia. *Bone Marrow Transplant.* 34:901-7
17. Chao NJ, Emerson SG, Weinberg KI. 2004. Stem cell transplantation (cord blood transplants). *Hematol. (Am. Soc. Hematol. Educ. Program)*:354-71
18. Rocha V, Labopin M, Sanz G, et al. 2004. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N. Engl. J. Med.* 351:2276-85
19. Rocha V, Arcese W, Sanz G, et al. 2000. Prognostic factors of outcome after unrelated cord blood transplant (UCBT) in adults with hematologic malignancies. *Blood* 96:587a (Abstr.)
20. Laughlin MJ, Eapen M, Rubinstein P, et al. 2004. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N. Engl. J. Med.* 351:2265-75
21. Laughlin MJ, Barker J, Bambach B, et al. 2001. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N. Engl. J. Med.* 344:1815-22
22. Sanz GF, Saavedra S, Planelles D, et al. 2001. Standardized, unrelated donor cord blood transplantation in adults with hematologic malignancies. *Blood* 98:2332-38
23. Takahashi S, Iseki T, Ooi J, et al. 2004. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematological malignancies. *Blood* 104:3813-20
24. Cornetta K, Laughlin M, Carter S, et al. 2005. Umbilical cord blood transplantation in adults: results of the prospective Cord Blood Transplantation (COBLT). *Biol. Blood Marrow Transplant.* 11:149-60
25. Ooi J, Iseki T, Takahashi S, et al. 2004. Unrelated cord blood transplantation for adult patients with de novo acute myeloid leukemia. *Blood* 103:489-91
26. Long GD, Laughlin M, Madan B, et al. 2003. Unrelated umbilical cord blood transplantation in adult patients. *Biol. Blood Marrow Transplant.* 9:772-80
27. Koh LP, Chao NJ. 2004. Umbilical cord blood transplantation in adults using myeloablative and nonmyeloablative preparative regimens. *Biol. Blood Marrow Transplant.* 10:1-22
28. Goldberg SL, Chedid S, Jennis AA. 2000. Unrelated cord blood transplantation in

- adults: a single institution experience. *Blood* 96:208a (Abstr.)
29. Barker JN, Weisdorf DJ, Wagner JE. 2001. Creation of a double chimera after the transplantation of umbilical-cord blood from two partially matched unrelated donors. *N. Engl. J. Med.* 344:1870–71
30. Kai S, Misawa M, Iseki T, et al. 2004. Double-unit cord blood transplantation in Japan. *Blood* 104:5166a (Abstr.)
31. De Lima M, St John LS, Wiedner ED, et al. 2002. Double-chimerism after transplantation of two human leucocyte antigen mismatched, unrelated cord blood units. *Br. J. Haematol.* 119:773–76
32. Fanning L, Hamza N, Tary-Lehman M, et al. 2003. High rate of graft failure after infusion of multiple (3–5) umbilical cord blood (UCB) units in adults with hematologic disorders: role of HLA disparity and UCB graft T cell-cross immune reactivation. *Blood* 104:195a (Abstr.)
33. Barker JN, Weisdorf DJ, DeFor TE, et al. 2005. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood* 105:1343–47
34. Barker JN, Weisdorf DJ, DeFor TE, et al. 2003. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. *Blood* 102:1915–19
35. Barker J, Weisdorf DJ, DeFor TE, et al. 2004. Non-myeloablative umbilical cord blood transplantation (UCBT): low transplant-related mortality in 59 high-risk adults. *Blood* 104:825a (Abstr.)
36. Storb R, Yu C, Wagner JL, et al. 1997. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood* 89:3048–54
37. Slavin S, Nagler A, Naparstek E, et al. 1998. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 91:756–63
38. McSweeney PA, Bearman SI, Jones RB, et al. 2001. Nonmyeloablative hematopoietic cell transplants using cord blood. *Blood* 98:666a (Abstr.)
39. Giralt S, Thall PF, Khouri I, et al. 2001. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. *Blood* 97:631–37
40. Ohwada C, Nakaseko C, Ozawa S, et al. 2004. Second cord blood transplantation (CBT) with reduced-intensity conditioning for graft failure after the first CBT for AML. *Bone Marrow Transplant.* 34:999–1000
41. Rizzieri DA, Long GD, Vredenburgh JJ, et al. 2001. Successful allogeneic engraftment of mismatched unrelated cord blood following a nonmyeloablative preparative regimen. *Blood* 98:3486–88
42. Nobili B, Rossi G, De Stefano P, et al. 2002. Successful umbilical cord blood transplantation in a child with dyskeratosis congenita after a fludarabine-based reduced-intensity conditioning regimen. *Br. J. Haematol.* 119:573–74
43. Nakazawa Y, Sakashita K, Kinoshita M, et al. 2004. Successful unrelated cord blood transplantation using a reduced-intensity conditioning regimen in a 6-month-old infant with congenital neutropenia complicated by severe pneumonia. *Int. J. Hematol.* 80:287–90
44. Yamada T, Tomonari A, Takahashi S, et al. 2004. Unrelated cord blood transplantation with a reduced-intensity conditioning regimen following autologous transplantation for multiple myeloma. *Int. J. Hematol.* 80:377–80
45. Ando T, Yujiri T, Tominaga T, et al. 2005. Autografting followed by a reduced-intensity conditioning unrelated donor cord blood transplantation for a patient with

- refractory multiple myeloma: successful engraftment with minimal toxicity. *Eur. J. Haematol.* 74:175–79
46. Miyakoshi S, Yuji K, Kami M, et al. 2004. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. *Clin. Cancer Res.* 10:3586–92
 47. Del Toro G, Satwani P, Harrison L, et al. 2004. A pilot study of reduced intensity conditioning and allogeneic stem cell transplantation from unrelated cord blood and matched family donors in children and adolescent recipients. *Bone Marrow Transplant.* 33:613–22
 48. Taylor PA, Lees CJ, Blazar BR. 2002. The infusion of ex vivo activated and expanded CD4⁺CD25⁺ immune regulatory cells inhibits graft-versus-host disease lethality. *Blood* 99:3493–99
 49. Taylor PA, Noelle RJ, Blazar BR. 2001. CD4⁺CD25⁺ immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. *J. Exp. Med.* 193:1311–18
 50. Cohen JL, Trenado A, Vasey D, et al. 2002. CD4⁺CD25⁺ immunoregulatory T cells: new therapeutics for graft-versus-host disease. *J. Exp. Med.* 196:401–6
 51. Hoffmann P, Ermann J, Edinger M, et al. 2002. Donor-type CD4⁺CD25⁺ regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. *J. Exp. Med.* 196:389–99
 52. Taylor PA, Panoskaltis-Mortari A, Swedin JM, et al. 2004. L-Selectin^{hi} but not the L-selectin^{lo}CD4⁺25⁺ T-regulatory cells are potent inhibitors of GVHD and BM graft rejection. *Blood* 104:3804–12
 53. Godfrey WR, Spoden DJ, Ge YG, et al. 2005. Cord blood CD4⁺CD25⁺-derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. *Blood* 105:750–58
 54. Shpall EJ, Quinones R, Giller R, et al. 2002. Transplantation of ex vivo expanded cord blood. *Biol. Blood Marrow Transplant.* 8:368–76
 55. McNiece IK, Almeida-Porada G, Shpall EJ, et al. 2002. Ex vivo expanded cord blood cells provide rapid engraftment in fetal sheep but lack long-term engrafting potential. *Exp. Hematol.* 30:612–16
 56. Pecora AL, Stiff P, Jennis A, et al. 2000. Prompt and durable engraftment in two older adult patients with high risk chronic myelogenous leukemia (CML) using ex vivo expanded and unmanipulated unrelated umbilical cord blood. *Bone Marrow Transplant.* 25:797–99
 57. Kogler G, Nurnberger W, Fischer J, et al. 1999. Simultaneous cord blood transplantation of ex vivo expanded together with non-expanded cells for high risk leukemia. *Bone Marrow Transplant.* 24:397–403
 58. Jaroscak J, Goltry K, Smith A, et al. 2003. Augmentation of umbilical cord blood (UCB) transplantation with ex vivo-expanded UCB cells: results of a phase I trial using the Astrum Replicell System. *Blood* 101:5061–67
 59. Fernandez MN, Granena A, Millan I, et al. 2000. Evaluation of engraftment of ex vivo expanded cord blood cells in humans. *Bone Marrow Transplant.* 25(Suppl. 2):S61–67
 60. Cui J, Wahl RL, Shen T, et al. 1999. Bone marrow cell trafficking following intravenous administration. *Br. J. Haematol.* 107:895–902
 61. Zhong JF, Zhan Y, Anderson WF, et al. 2002. Murine hematopoietic stem cell distribution and proliferation in ablated and nonablated bone marrow transplantation. *Blood* 100:3521–26
 62. Wang J, Kimura T, Asada R, et al. 2003. SCID-repopulating cell activity of human cord blood-derived CD34⁺ cells assured by intra-bone marrow injection. *Blood* 101:2924–31
 63. Kushida T, Inaba M, Hisha H, et al. 2001. Intra-bone marrow injection of allogeneic bone marrow cells: a powerful new strategy for treatment of intractable autoimmune

- diseases in MRL/lpr mice. *Blood* 97:3292–99
64. Mazurier F, Doedens M, Gan OI, et al. 2003. Rapid myeloerythroid repopulation after intrafemoral transplantation of NOD-SCID mice reveals a new class of human stem cells. *Nat. Med.* 9:959–63
65. Yahata T, Ando K, Sato T, et al. 2003. A highly sensitive strategy for SCID-repopulating cell assay by direct injection of primitive human hematopoietic cells into NOD/SCID mice bone marrow. *Blood* 101:2905–13
66. Hagglund H, Ringden O, Agren B, et al. 1998. Intraosseous compared to intravenous infusion of allogeneic bone marrow. *Bone Marrow Transplant.* 21:331–35
67. Deans RJ, Moseley AB. 2000. Mesenchymal stem cells: biology and potential clinical uses. *Exp. Hematol.* 28:875–84
68. Noort WA, Kruisselbrink AB, in't Anker PS, et al. 2002. Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34⁺(+) cells in NOD/SCID mice. *Exp. Hematol.* 30:870–78
69. in't Anker PS, Noort WA, Kruisselbrink AB, et al. 2003. Nonexpanded primary lung and bone marrow-derived mesenchymal cells promote the engraftment of umbilical cord blood-derived CD34⁺ cells in NOD/SCID mice. *Exp. Hematol.* 31:881–89
70. Kim DW, Chung YJ, Kim TG, et al. 2004. Cotransplantation of third-party mesenchymal stromal cells can alleviate single-donor predominance and increase engraftment from double cord transplantation. *Blood* 103:1941–48
71. Koc ON, Gerson SL, Cooper BW, et al. 2000. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. *J. Clin. Oncol.* 18:307–16
72. Le Blanc K, Rasmusson I, Sundberg B, et al. 2004. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 363:1439–41
73. Frassoni F, LeBopen M, Bacigalupo A. 2002. Expanded mesenchymal stem cells, co-infused with HLA-identical hematopoietic stem cell transplant, reduce acute and chronic graft versus host disease: a matched-pair analysis. *Bone Marrow Transplant.* 29:75
74. Maccario R, Podesta M, Moretta A, et al. 2005. Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4⁺ T-cell subsets expressing a regulatory/suppressive phenotype. *Haematologica* 90:516–25
75. MacMillan ML, Ramsay NKC, Atkinson K, et al. 2002. Ex-vivo culture-expanded parental haploidentical mesenchymal stem cells to promote engraftment in recipients of unrelated donor umbilical cord blood: results of a phase I–II clinical trial. *Blood* 100:836a (Abstr.)
76. 2001. *Net Cord—Foundation for the Accreditation of Cellular Therapy (FACT-NETCORD), formerly Foundation for the Accreditation of Hematopoietic Cell Therapy (FAHCT), International Standards for UCB Collection, Processing, Testing, Banking, Selection and Release.* 2nd ed.
77. 2001. *Standards for UCB Services, American Association of Blood Banks,* Bethesda, MD
78. Food and Drug Administration. 2001. Current Good Tissue Practice for manufacturers of human cellular and tissue-based products; inspection and enforcement. *Fed. Regist.* 66:1508–59
79. Hamza NS, Fanning N, Tary-Lehmann M, et al. 2003. High rate of graft failure after infusion of multiple (3–5) umbilical cord blood (UCB) units in adults with hematologic disorders: role of HLA disparity and UCB graft T cell cross immune reactivation. *Blood* 102:680a (Abstr.)

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