Review Article

Unrelated Umbilical Cord Blood Transplantation in Children and Adults

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Abstract

Umbilical cord blood (UCB) has recently been explored as an alternative haematopoietic stem cell (HSC) source for allogeneic immunotherapy in both adults and paediatric patients with haematological malignancies and marrow failure syndromes. The relative ease of procurement, tolerance of 1-2 antigen human-leukocyte antigen (HLA) mismatch and the lower than anticipated risk of severe graft-versus-host disease has made UCB an appealing alternative to marrow-derived HSC. Results from various registries and institutions observed graft cell dose to be the major factor determining engraftment and survival in unrelated UCB transplant recipients. Given that adults are larger than children, there was still limited enthusiasm for the use of UCB in adults. The use of reduced-intensity or nonmyeloablative preparative regimens to allow engraftment of UCB broadens the scope of patients who may benefit from allogeneic immunotherapy, particularly the elderly and medically infirm patients with no matched sibling donor. Further studies on improving graft cell dose such as the use of ex vivo expansion of UCB cells and multiple-unit transplant are currently being pursued, so as to make this potentially curative procedure available to more patients.

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Introduction

Allogeneic haematopoietic stem cell transplantation (HSCT) is an established form of treatment for many high-risk haematological malignancies and marrow failure syndromes. The use of HSCT is still limited by the availability of human leukocyte antigen (HLA) matched donors, even though unrelated donor registries have markedly improved the chances of finding a donor for many patients.[1] Moreover, significant graft-versus-host disease (GVHD) remains a major source of morbidity following HSCT.[2,3] Therefore, clinical investigators have, over the past decade, explored the suitability of umbilical cord blood (UCB) as an alternative source of haematopoietic stem cells (HSC).

History and Rationale for UCB Transplantation

The potential use of umbilical cord as a source of transplantable haematopoietic stem cells was first proposed in the early 1980s in private discussions held by Edward A Boyse, Hal E Broxmeyer, and Judith Bard.[4] The feasibility of this proposal was supported by a number of in vitro studies with human cord blood (CB)[5] and in vivo studies with mouse blood.[6] On the basis of these studies, the first human cord blood transplant was successfully performed on a young patient with Fanconi anaemia in 1988.[7] The success story has spurred interest in further studies of the use of a novel source of stem cells, that has traditionally been discarded. The past 12 years have witnessed an explosion of advances leading to an increased understanding of biological characteristics of UCB, in parallel with its applications in clinical transplantations. UCB banks have been established worldwide for related and unrelated UCB transplantation. It is estimated that more than 70,000 UCB units have been collected, tested and cryopreserved by these banks,[8] and an estimated 2000 patients have undergone UCB transplantation thus far.[9]

The rapid expansion in the use of UCB for transplantation is the culmination of several factors, most of which address the limitations encountered in the use of human leukocyte antigen (HLA) matched related haematopoietic stem cells, and these include: (1) a lack of suitable HLA matched donors; (2) complications of GVHD associated with HLA

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disparities, particularly if bone marrow from HLA-matched unrelated donor is utilised;[2,3] (3) the cumbersome process of identifying, typing, and harvesting an unrelated donor, with the median time interval between the initiation of a search and the donation of marrow being about 4 months.[10]

Unrelated UCB offers many practical advantages as an alternative source of stem cells, including: (1) relative ease of procurement and greater availability compared to unrelated bone marrow graft;[11] (2) absence of risk for mothers and donors; (3) a reduced likelihood of transmitting infections, particularly cytomegalovirus; (4) the ability to store fully tested and HLA-typed cord blood in the frozen state, and prompt availability for immediate use to transplant centres;[12] (5) potentially reduced risk of GVHD;[13] (6) less stringent criteria for HLA matching for donor-recipient selection; and (7) the absence of donor attrition.

Clinical Results of UCB Transplant Using Myeloablative Preparative Regimens

UCB transplantation in both adults and children from both related and unrelated donors after myeloablative preparative regimens has been shown to successfully engraft both paediatric and adult patients with haematological malignancies, marrow failure syndrome and immune deficiencies. The results of several large series have been reported in the literature over the past 7 years.[13-25] The myeloablative preparative regimens employed in these studies were either total body irradiation (TBI)-based or chemotherapy-based, with inclusion of antithymocyte globulin in some of the patients. The data from these UCBT registries, where the majority of the recipients are children, point to a significant delay in the time of neutrophil recovery, with the median time to absolute neutrophil count >500/μL ranging between 22 and 30 days. The overall probability of engraftment was ranged between 80% and 90%. Despite a higher degree of HLA disparity, grade II to IV GVHD in those unrelated UCB recipients is lower than in recipients of unrelated grafts from adult donors. Importantly, the number of nucleated cells in the infused UCB influences the speed of recovery of neutrophils and platelets. In a recently published study, Wagner et al[22] demonstrated the importance of CD34 cell dose in determining the outcome after unrelated UCB transplantation. Patients receiving a CD34 cell dose <1.7 x 10^5 per kg body weight had a slower neutrophil recovery at a median of 34 days (range, 17 to 54 days), smaller likelihood of engraftment and a higher incidence of treatment-related mortality.

In comparison to the studies on paediatric patients, the clinical data on the use of UCB transplant in adult patients are relatively limited: 6 are peer-reviewed published manuscripts[16,23-27] and the remaining are either in the abstract form[28-31] or have been integrated into studies which were conducted on predominantly paediatric populations.[8,14,15,22] Several recently published data on using unrelated UCB for transplantation in adult recipients have shown that UCB contained sufficient number of HSC to achieve engraftment with lower than anticipated risk of severe acute GVHD.[16,23-25,28,29] The observed primary graft failure rate is approximately 10% and the median day to neutrophil engraftment (ANC >500/μL) is similar to paediatric patients (range, 25 and 28 days). Transplant-related mortality within the first 100 days of transplant is in the range of 40% to 50%. The number of UCB HSC required to provide durable engraftment in adult recipients is not firmly established but the graft cell dose appears equally important for engraftment and survival.

Comparison of Outcomes of Unrelated UCB Transplantation in Children and Adults

Haematopoietic Recovery and Engraftment

Comparative studies by Eurocord in paediatric patients have suggested that as compared to the allogeneic marrow transplant recipients, UCB transplant recipients have lower engraftment rates and more delayed haematopoietic recovery.[13,32] The median times for neutrophil recovery to ANC recovery and platelet recovery (>20,000/μL) in unrelated UCB recipients were significantly delayed, being 32 days (range, 11 to 56) and 81 days (range, 16 to 159) respectively for unrelated UCBT recipients, as compared to 18 days (range, 10 to 40) and 29 days (range, 8 to 141) for unrelated marrow recipients.[32] The higher risk of graft failure and the delay in haematopoietic recovery may be related to several factors, including the lower nucleated cell and CD34+ cell dose in the UCB graft as compared to the marrow allograft,[33-35] or factors such as immaturity of stem cells, which might need more cell divisions before differentiation to marrow progenitors, or to the lack of subpopulations facilitating engraftment.[36] However, a study performed by the University of Minnesota reported a different outcome in terms of engraftment. In their matched-pair analysis comparing 26 0 to 3 HLA-mismatched unrelated UCB recipients to 26 matched unrelated unmanipulated marrow recipients, neutrophil recovery was significantly delayed in the unrelated UCB recipients, but there was no significant difference in terms of overall engraftment rate at day 45 and platelet recovery.[37]

The important correlation between nucleated cell dose and rate of engraftment in unrelated UCBT patients has been demonstrated by the data from New York Blood Bank and the Eurocord registry.[14,15] A recent study published by the investigators from the University of Minnesota has shown that recipients of UCB graft containing CD34+ cells more than 1.7 x 10^5/kg had a similar incidence of engraftment

to that observed in unrelated marrow allograft recipients.[22]

The concern of limited cell dose giving rise to higher risk of primary graft failure in adult UCB recipients is further confounded by the greater disproportion between nucleated cell dose in UCB grafts and adult body weight, giving rise to a relatively lower number of infused cells per kilogram of the recipient's body weight. However, the available data on adult recipients of unrelated UCB transplantation thus far have shown that UCB contains sufficient number of HSC to achieve engraftment. The observed primary graft failure rate was approximately 10% to 20%, the median day to neutrophil engraftment (ANC >500/μL) ranged between 22 and 32 days, and the probability of engraftment by day 60 ranged between 70% and 100%. These results seem comparable to that observed among the paediatric series, in which median time for neutrophil engraftment ranged between 25 and 32 days, and probability of myeloid engraftment ranged between 80% and 90%.[8,14,15,32,37] Similar to paediatric patients, the neutrophil and platelet recovery in adult UCB recipients are significantly delayed as compared to the marrow allograft recipients.

In 2 of the largest adult series, nucleated cell dose was found to be associated with the rate of neutrophil and platelet recovery.[23,29] In the multi-centre study by Laughlin et al, [23] neutrophil engraftment was faster in adult patients transplanted with a cryopreserved nucleated cell dose above 1.87 x 10^7/kg. In the Eurocord analysis, an infused nucleated cell dose of more than 1.7 x 10^7/kg was associated with more rapid neutrophil recovery.[29]

Ooi et al[38] in a non-matched-pair analysis, performed a clinical comparison of 8 unrelated UCB transplant recipients with 8 unrelated marrow transplant recipients. All patients in both groups were adult patients with acute leukaemia in complete remission, who received the same conditioning regimen, GVHD prophylaxis and supportive treatment. The median time to absolute neutrophil count >0.5 x 10^9/L (33 days of UCBT versus 20.5 days of BMT; P <0.05) and platelet to >50 x10^9/L (48 days of UCBT versus 25.5 days of BMT; P <0.05) were significantly longer in the UCB transplant group.

The correlation between CD34+ cell dose and engraftment has also been evaluated. In the Duke University series, patients receiving more than 1.37 x 10^5/kg CD34+ cell had more rapid platelet recovery.[25] No correlation between CD34+ cell dose and engraftment was discerned in the multi-centre study by Laughlin et al,[23] although in that study, event-free survival was improved in patients who received more than 1.2 x 10^5 CD34+ cells/kg.

The optimal nucleated cell dose and CD 34+ cell dose in UCB graft remains to be determined. In the context of paediatric patients, the recommended nucleated cell doses include 1.0 x 10^7/kg, 1.5 x 10^7/kg and 2.0 x 10^7/kg.[8,39,40] CD34+ cell dose of 1.7 x 10^5/kg has been established as the threshold dose for patients at the University of Minnesota.[22] Based on the clinical data available so far for adult unrelated UCB transplant,[23,29] it is not unreasonable to suggest that UCB should contain a cryopreserved cell dose of at least 1.8 x 10^7 nucleated cells/kg and 1.2 x 10^5 CD34+ cells/kg.

Graft-Versus-Host Disease

Published data from most of the cord blood registries[14,15,19,20,22,32] have shown that despite the infusion of HLA class I and II disparate grafts, the incidence and severity of acute and chronic GVHD among unrelated UCB recipients have thus far been lower than that previously reported in recipients of matched unrelated donor marrow or partially matched family member marrow allografts.[2,41-44] In these series of UCBT recipients, the majority of whom are children, the overall incidence of grade II-IV acute GVHD and grade III-IV acute GVHD ranged between 30% and 50% and 10% and 20%, respectively.

Barker et al[37] in a matched-pair analysis, have shown similar rates of acute and chronic GVHD in paediatric recipients of 0 to 3 HLA-antigen mismatched unrelated donor UCB grafts as compared with those receiving HLA-matched unrelated donor marrow graft. Another comparative study by Rocha et al[32] has demonstrated lower incidence of acute GVHD (hazard ratio: 0.50) and chronic GVHD (hazard ratio: 0.24) in a cohort of paediatric patients receiving mismatched unrelated UCB graft compared to unrelated, unmanipulated bone marrow recipients. The association between HLA-disparity and the risk of GVHD in unrelated UCB recipients remains unclear, with most studies demonstrating no correlation.[8,14,18,19,22] However, in an updated multivariate analysis of data from the largest series published so far, Rubinstein et al[45] have revealed a significant association between acute GVHD and HLA disparity. The incidences of grade III to IV acute GVHD in patients with no mismatch, 1 antigen HLA mismatch and ≥2 antigen mismatch were 8%, 19% and 28%, respectively (P = 0.006).

To date, no matched-pair comparative study has been performed in the adult patient population to compare the incidence of GVHD between unrelated UCB transplant and unrelated donor marrow transplant. The reported series in adults have shown 40% to 60%, and 20% to 22%, incidences of grade II-IV and grade III-IV acute GVHD respectively, and 26% to 90% incidence of chronic GVHD.[16,23-26,28-31] Given the increased age in these adult patients, and age being recognised as a risk factor for GVHD,[46,47] the incidence of acute and chronic GVHD among these adult patients is considered acceptable as compared to the paediatric unrelated UCBT series, which

Table 1. Comparison of Clinical Outcome of unrelated UCBT in children and adults

Variable	Children	Adults
Haematopoietic recovery and engraftment		
Median days to neutrophil recovery	23-30 days	25-32 days
Median days to platelet recovery (>20,000/μL)	54-90 days	26-129 days
Probability of engraftment (%)	80-100 days (day 60)	70-100 days (at day 60)
Factors reported to impact on engraftment	(i) N.C. dose[14,15] (ii) CD34+ cell dose[22]	(i) N.C. dose[23] (ii) CD34+ cell dose influenced platelet recovery[25]
Optimal cell dose		
N.C. dose (x 10^7/kg)	1.0-2.0‡	1.8†
CD34+ cell dose (x 10^5/kg)	1.7*	1.2†
GVHD		
Acute Grade II- IV (%)	30-50	40-60
Acute Grade III-IV (%)	10-20	20-22
Factors reported to impact on GVHD	HLA disparity[45]	
TRM and survival		
100 day TRM (%)	27-39	40-50
Overall survival (%)	29-58 at 1 year 35-53 at 2 year	19-76 at 3 year
Event-free survival (%)		21-53 at 1 year
Factors reported to impact on survival	(i) Disease status at transplant[16,18] (ii) HLA match[15, 22] (iii) N.C. dose[8,14,15] (iv) CD 34+ cell dose[8,22] (v) Age of recipient[8,15] (vi) Grade III-IV GVHD[22] (vii) CMV status[14]	(i) CD34+ cell dose[23] (ii) Age[24,25] (iii) Status at transplant[29] (iv) N.C. cell dose[29]

* Based on available clinical data[22]

† Based on available clinical data[23,29]

‡ Based on available clinical data[8,39,40]

CMV: cytomegalovirus; GVHD: graft-versus-host disease; HLA: human leukocyte antigen; N.C.: nucleated cell; TRM: transplant-related mortality

has reported 33% to 44%, and 11% to 22%, incidences of grade II-IV and grade III-IV GVHD respectively, and 0% to 25% incidence of chronic GVHD.[14,15,22,32] Also, with many of these patients receiving HLA-mismatched UCB grafts, the incidence of grade III to IV acute GVHD in these unrelated UCB recipients compares favourably with the 35% to 55% incidence reported in recipients of HLA-matched bone marrow from unrelated adult donors who received standard prophylaxis against GVHD.[2,42-44,48] It is noteworthy that the reported incidence of chronic GVHD showed a wide range of 26% to 90%. In comparison, chronic GVHD develops in 55% to 75% of patients receiving HLA-matched bone marrow transplants from unrelated donors.[2,42-44,48] The variability in the reported rate of GVHD may be attributed to the following factors: (1) differences in conditioning regimens and GVHD prophylaxis regimens employed by different centres; (2) differences in supportive care; (3) the transplant centre effect;[49] (4) inequality in the type of patients contributed to the study by each centre; (5) subjective elements and inconsistency involved in the grading of GVHD by different teams. The association between HLA mismatch and GVHD has not been addressed in these adult series except in the report by Laughlin et al, which failed to observe any influence of histocompatibility on severity of acute GVHD.[23]

Toxicity and Transplant-related Mortality

Transplant-related mortality (TRM) is the principal obstacle to successful transplantation outcome in recipients of unrelated donor BMT and is the major reason for evaluating UCB as an alternate source of HSCs.[22] Several series with predominantly paediatric patients receiving unrelated UCB graft have reported 100-day TRM and 1-year TRM in the range between 27% and 39% and between 30% and 44%, respectively.[18,26-32,37] Infection and acute GVHD were the main causes of death within the first day of transplant. Rocha et al[32] have reported in their paediatric studies that the incidence of 100-day TRM in the unrelated marrow recipients was significantly higher as compared with the unrelated UCB recipients. However, no such difference was detected in another series reported by the group from the University of Minnesota.[37] The University of Minnesota series, which consisted predominantly of children, has shown the important association between

TRM with CD34 dose, recipient's age and the development of grade III-IV acute GVHD. However, no correlation could be discerned between HLA and TRM.[22]

A relatively higher incidence of TRM at 100 days has been observed among the adult series, ranging between 43% and 56%. The high non-relapse mortality in these series is partially attributable to the high-risk nature of the patient population. Several prognostic factors have been found to predict higher TRM. The Eurocord data, which showed a higher 180-day TRM in adult unrelated UCB (56%) as compared with the paediatric patients (32%), has found a lower 100-day TRM among patients with disease in chronic phase or remission, number of nucleated cells infused $\geq 2.0 \times 10^7$/kg, and transplant performed after January 1998.[16,40] In the Laughlin series, the first 100 days' TRM was 50%, with nearly half of the deaths caused by infection. Notably, improved EFS was seen among patients receiving UCB graft with CD34+ cell>1.2×10^5/kg.[23] A study from a Spanish centre on 27 adult recipients of unrelated UCB has shown 100% incidence of infectious episodes, 55% incidence of bacteraemia, 58% CMV antigenaemia and 11% incidence of fungal infections. In that study, the reported TRM at day 100 was 37%, with 80% of death related to infections. Remarkably, the study observed more than half of the infections occurring after myeloid recovery.[50]

The increased risk of infection within the first 100 days of transplant may be related to delayed engraftment, GVHD or impaired immune recovery.[15,23] With the data from the University of Minnesota showing the profound influence of CD34 cell dose on the rate of engraftment, TRM and survival, and also the observation that most recipients of UCB with an adequate cell dose do not die of infection,[22] it is believed that prolonged neutropenia is the main cause of an increased risk of infection. However, the Spanish experience,[50] which showed a high incidence of infections after myeloid recovery, suggests the influence of impaired immune recovery and GVHD in causing infections.

Organ toxicity associated with the intensive treatment administered to patients before CBT is another leading cause of non-relapse mortality in adult UCB recipients. In the Laughlin series, 35% of the deaths were related to preparative regimens.[23] Because of toxicities from the intensive conditioning regimens to non-marrow organs such as the gut, liver, lung, and heart, UCB transplants using myeloablative preparative regimens have been restricted to patients younger than 50 to 55 years of age, with none of the series reported thus far including patients older than 60 years of age. Such age restrictions are problematic in that many haematologic malignancies typically present after the age of 50,[51] making many patients ineligible for UCB transplants despite having suitably matched unrelated UCB grafts with adequate cell doses. These limitations have given an impetus for exploring the use of nonmyeloablative regimens for UCB transplant, as will be discussed later in this paper.

Finally, given the heterogeneity of the patient population, conditioning regimen and GVHD prophylaxis regimen employed, as well as the supportive care rendered at the different centres, it is difficult to have a reliable evaluation of the possible impact of different pre-transplant variables on TRM. However, among all the different prognostic variables that have been evaluated, cell dose of the UCB graft appears to be the only one that can be manipulated.[22] Future efforts in lowering TRM should therefore focus not only on improving transplant methodology and supportive care, but also on improving UCB cell dose.

Disease Relapse

As with unrelated donor BMT, relapse is another common cause of death after UCB transplantation. Concerns raised about the possibility of an increased risk of leukaemia recurrence in CBT recipients derive from the following considerations: (1) there is a close association of GVL with GVHD in allograft recipients, such that patients developing either acute or chronic GVHD experience a much lower risk of relapse;[52,53] (2) the incidence and severity of both acute and chronic GVHD appeared to be less after transplantation of cord blood progenitors than after marrow transplantation[14,15,19,20,22,32] and (3) immaturity and diminished cytotoxicity of infused cord blood lymphocytes[54,55] could further impair the immune-mediated antileukaemia effect. However, 3 previous reports comparing UCBT and unmanipulated BMT from HLA-identical siblings[13] and from unrelated donors[32,37] among children with leukaemia have shown a similar risk of relapse. The 2-year incidence of relapse in children receiving unrelated UCB transplant ranges between 37% and 40%, with disease status at transplantation being the predominant risk factor.[18,22,32]

In comparison with the paediatric series, the data on adult patient populations are scanty and inconclusive, owing to smaller numbers of patients, shorter duration of follow-up and differences in patient selection. The reported incidence of relapse as the cause of death has ranged widely between 6%[23,28] and 35%.[30] The variability in the relapse rate is likely due to heterogeneity in patient selection.

Survival and Outcome

Two comparative studies in paediatric patients have shown no difference in survival between patients receiving UD-UCBT and UD-BMT.[32,37] Most of the studies involving mainly children with UD-UCBT have reported 1- and 2-year overall survival rates in the range between 29% and

58%, and between 35% and 53%, respectively.[8,14,22,32,37] The prognostic factors which have been found to influence survival include: (i) disease status at transplantation;[20,22] (ii) HLA match;[15,22] (iii) infused nucleated cell dose per kilogram of recipient's weight;[8,14,15] (iii) CD34 cell dose per kilogram of recipient's weight;[8,22] (iv) age of recipient;[8,15] (v) grade III-IV GVHD[22] and (vi) CMV status of recipients.[14]

In contrast to the children series, owing to the heterogeneity of the patient population, limitation of small patient numbers and short duration of follow-up, it is difficult to make a reliable evaluation of the possible impact of various pre-transplantation variables on the survival of the adult UCB recipients. The available series thus far has reported a survival outcome with a wide range, from 19% to 76% 3-year overall survival, and from 21% to 53% 1-year event-free survival. In the Laughlin series, the presence of higher CD 34+ cell dose in the UCB graft was associated with improved event-free survival.[23] In both the Duke University series[25] and the Spanish series,[24] age at transplantation had significant impact on survival. The Eurocord data has shown that good risk status at transplantation and infused nucleated dose of >1 x 10^7/kg are favourable factors for survival.[29] The superior survival of a small group Japanese patients with high-risk myelodysplastic syndrome and de novo AML has provided further evidence that adequate cell dose has critical impact on survival.[26,27] Notably, none of these studies have demonstrated any association between HLA disparity and survival. However, in a recent review update with 861 unrelated UCBT recipients from the Placental Blood program of the New York Blood Center, which includes 181 (21%) patients with age ≥18 years and 170 patients (20%) weighing ≥60 kg, Rubinstein et al[45] have demonstrated in a multivariate analysis that HLA match is an independent predictor of event-free survival in the subset of patients with ALL, AML or CML.

A study from the St Louis Cord Blood Bank, reported in abstract form, compared the outcome of 23 adults [with median age of 39 years (range, 17 to 66) and median weight 66 kg (range, 41 to 131)] with 83 children [with median age 7 (range, 1 to 16) and median weight 25 kg (range, 6 to 78)].[56] The adult patients received a significantly lower cell dose with median total nucleated cell of 2.7 x 10^7/kg (range, 1.1 to 5.3) and median CD34 cell dose of 1.4 x 10^5/kg (range, 0.2 to 4.4) as compared with the children, who received median total nucleated cell of 5.8 x 10^7/kg (range, 1.3 to 24.8) and median CD34 cell dose of 3.3 x 10^5/kg (range, 0.5 to 20.8). The time to neutrophil and platelet recovery were similar between the 2 groups. The estimated 1-year survival rates of the adults and the children were comparable (64% for adults; 60% for children). Taken together, these results suggest that UCB should be considered as an alternative stem cell source for adults, especially when an unrelated marrow donor is not available in time.

Nonmyeloablative Umbilical Cord Blood Transplantation

While cord blood transplantation from both related and unrelated donor has demonstrated encouraging results in paediatric patients with haematologic malignancies or marrow failure syndromes, there is still limited applicability in larger adults. The use of cord blood for adult patients is still in development. The lower number of haematopoietic stem cells in CB compared with bone marrow, together with preliminary data showing the importance of cell dose for the outcome of UCB transplant have been a cause for caution regarding its use in adult patients.[14,15] Secondly, the majority of UCB transplants involve the use of myeloablative preparative regimens that are associated with considerable morbidity and mortality. Despite having suitable UCB donors, many older patients and patients with co-morbidities will be precluded from receiving UCB transplants because of the unacceptable toxicities from the standard conditioning regimens.

Recently, older recipients of allogeneic HSCT have been treated successfully following a variety of less intense nonmyeloablative (NM) conditioning regimens.[57-60] These encouraging observations were a result of selective lymphoablation using lymphotoxic agents, large progenitor cell doses and drugs to prevent host-versus-graft as well as GVHD. On the basis of these encouraging observations, it has been hypothesised that a reduced intensity preparative regimen would allow engraftment of the UCB stem cells. The clinical outcome of 2 patients with malignant lymphoma using this novel approach was first reported by investigators in Duke University.[61] In their study, 2 patients with relapsed lymphoma who had no matched siblings, partially matched family members, or matched unrelated donors successfully underwent NM conditioning therapy followed by infusion of 4/6 matched, unrelated donor UCB cells at the nucleated cell dose of 2.9 and 6.5 x 10^7/kg, respectively. The conditioning regimens consisted of fludarabine 30 mg/m^2 and cyclophosphamide 500 mg/m^2 daily for 4 days with antithymocyte globulin 30 mg/kg per day for 3 days. Cyclosporine and prednisolone were given for acute GVHD prophylaxis. Both patients had 100% donor engraftment by the third month of transplant and remained in remission 6 to 12 month following transplantation. The favourable outcome demonstrates the feasibility of the mismatched unrelated UCB cells, even with the NM preparative regimens.

Clinical Results of Nonmyeloablative UCB Transplantation

The Duke University Medical Center Experience[62]

Between November 2000 and September 2002, 10 patients with high-risk malignancies underwent NM transplant using UCB at Duke University Medical Center. The median age of these patients was 51 years (range, 19 to 62 years), their median weight was 65.7 kg (range, 49.1 to 99 kg) and the median number of nucleated cells per kilogram infused was 2.07 x 10^7/kg (range, 1.07 to 5.53 x 10^7/kg). All patients received fludarabine 30 mg/m^2 and cyclophosphamide 500 mg/m^2 daily for 4 days (days –5 to –2) with antithymocyte globulin (ATG) 30 mg/kg per day for 3 days (days –3 to –1). Acute GVHD prophylaxis consisted of cyclosporine A (CYA) and methylprednisolone for all patients except 2 who were given cyclosporine and mycophenolate mofetil (MMF). The median time to neutrophil recovery with ANC 500/μL or greater for all the 10 patients was 8 days (range, 0 to 32 days), and the median time to platelet recovery with platelet count exceeding 20,000/μL was 3 days (range, 0 to 61 days). Six (60%) of the 10 patients demonstrated donor chimerism between 4 weeks and 6 months, with subsequent conversion to full donor chimerism achieved in 3 patients. Five patients are alive with 3 remaining free of disease at between 70 and 705 days after transplantation. The estimated overall survival and event-free survival at 2 years for this high-risk group were 36% (95% CI, 16% to 55%) and 27% (95% CI, 12% to 42%), respectively. Importantly, no treatment-related mortality was observed within the first 100 days of transplant.

Results from Other Transplant Centres

A similar approach has also been taken by investigators in the University of Colorado Health Sciences Center. McSweeney et al[63] reported 4 patients, age range between 25 and 78 years, with advanced haematological malignancies with receiving 5 to 6/6 HLA matched UCB after being conditioned with fludarabine 30 mg/m^2 x 3 and TBI 200 cGy. Cyclosporin and MMF were used as post-grafting immunosuppression. The ranges of nucleated cell dose and CD34+ cells were 0.75 to 1.3 x 10^7/kg and 1.0 to 4.0 x 10^4/kg, respectively. Two of the 3 evaluable patients had stable engraftments. Mild biopsy-proven skin GVHD developed in 1 patient but resolved spontaneously.

Cairo et al[64] demonstrated the feasibility of reduced intensity allogeneic transplantation using 1-2 antigen mismatched UCB with median nucleated cell dose of 5 x 10^7/kg (range, 0.22 to 9.5) and median CD34+ cell dose of 1.95 x 10^5/kg (range, 0.11 to 3.7), on 6 children and adolescent patients with both malignant and non-malignant diseases. All patients were below 21 years old and they were conditioned with fludarabine-based regimen. Engraftments occurred in all except 1 patient, and survival of >50% was attained.

Transplantations using RIC regimen have also been evaluated by investigators from the University of Minnesota on a cohort of high-risk patients with haematological malignancies.[65] In their study, unrelated UCB grafts with a median nucleated cell dose of 3.7 x 10^7 per kg (range, 1.6 to 6.0 x 10^7 /kg) were infused into 43 patients [median age of 49.5 years (range, 22 to 65 years)], after receiving 2 types of nonmyeloablative conditioning regimens: fludarabine 200 mg/m^2, TBI 200 cGy and busulphan 8 mg/kg (Flu/Bu/TBI) for the initial 21 subjects; fludarabine 200 mg/m^2, TBI 200 cGy and cyclophosphamide 50 mg/kg (Flu/Cy/TBI) for the subsequent 22 subjects. All patients received GVHD prophylaxis with cyclosporin A and MMF. The median time to neutrophil recovery of more than 0.5 x 10^9/L was 26 days (range, 12 to 30 days) for the Flu/Bu/TBI recipients, but only 9.5 days (range, 5 to 28 days) for the Flu/Cy/TBI recipients. The cumulative incidence of engraftment for Flu/Bu/TBI and Flu/Cy/TBI recipients were 76% and 94%, respectively. Despite the use of 1-2 HLA-antigen mismatched graft in 93% of the recipients, the cumulative incidence of grade II to IV GVHD and grade III to IV GVHD for the entire cohort of patients were 44% and 9%, respectively. The disease-free survival of these high-risk subjects was also favourable: 24% at 1 year for Flu/Bu/TBI recipients; and 41% at 1 year for Flu/Cy/TBI recipients.

In the experience reported by Ballen et al,[66] 6 patients with solid tumours and 1 patient with lymphoma, who do not have family matched donors, received unrelated UCB transplant after conditioning with 100 cGy TBI. No GVHD prophylaxis was given to any of these patients. The median number of CD34+ cells/kg and CD3+ cells/kg were 3.1 x 10^4/kg (range, 1.1 to 10.7 x 10^4/kg) and 1.7 x 10^6/kg (range, 0.5 to 3.7 x 10^6/kg). However, none of these patients achieved a tumour response or showed evidence of donor chimerism.

Novel Strategies in UCB Transplantation

Ex Vivo Expansion and Transplantation of Multiple Units of UCB

One of the major limitations of using UCB as the source of stem cell for transplantation is the low cell dose, which not only adversely affects both the rate of haematopoietic recovery and probability of survival,[14,15,67] but also results in higher risk of graft failure as compared with bone marrow transplantation. To circumvent the limitations of UCB transplants, studies have been done to look into the possibility of expanding ex vivo the UCB progenitors to improve engraftment. This area of investigation seems particularly interesting, since in vitro studies have shown

that expansion can be increased in UCB compared with BM cells. The use of cytokine cocktails including stem cell factor (SCF), granulocyte colony stimulating factor (G-CSF), and megakaryocyte growth and differentiation factor (MGDF) is effective in preclinical studies.[68] In a report from the University of Colorado, the infusion of UCB which was expanded ex vivo in conjunction with the unexpanded fraction in adults (weighing between 54 and 116 kg) and paediatric patients with high-risk malignancies following myeloablative therapy, resulted in a low incidence of engraftment failure and equivalent times to engraftment of neutrophils and platelet as reported for smaller paediatric patients.[69] Notably, the protocols employed in this study consisted of both an expanded fraction and an unexpanded fraction. The reason for including the unexpanded fraction is the concern that ex vivo expansion may exhaust long-term engrafting cells. The same group of investigators have also addressed this issue of short- and long-term engrafting potential of ex vivo expanded CB by doing an experiment using a fetal sheep xenogeneic transplant model. In that study, it has been demonstrated that although ex vivo expanded cells may be able to provide rapid short-term engraftment, the long-term potential of expanded cells may be compromised. It is for this reason that transplantation of the unexpanded CB products is included in the studies to ensure durable long-term donor engraftment.[70] The overall benefit of this strategy has not been fully determined and deserves further investigation.

Another avenue of research is the possibility of using several cord blood units in order to increase the stem cell yield. In a sheep xenograft model of human haematopoiesis, a combination of human UCB units enhanced the short-term, but not long-term, repopulating capacity of human UCB cells.[71] Barker et al[72] first reported the successful transplantation of 2 partially HLA-matched units of UCB into a 53-year-old, 83-kg woman with accelerated phase of chronic myelogenous leukaemia and no bone marrow donor. Double chimera with both units contributing to haematopoiesis was attained based on an RFLP analysis performed 60 days after transplantation. The same group of investigators recently updated the clinical outcome of 23 high-risk adult patients [median age 47 years (range, 18 to 60)] with haematological malignancies.[73] Using both myeloablative and nonmyeloablative conditioning regimens, they demonstrated a high incidence of engraftment (94%) without an increase in severe GVHD (cumulative incidence of grade II-IV and III-IV acute GVHD were 47% and 10%, respectively). The data support the principle that the transplantation of 2 immunologically distinct UCB units is not associated with crossed immunological rejection. These observations provide the most compelling argument for focusing future investigations on evaluating the efficacy of ex vivo expansion of 2 or more units of UCB in larger clinical trials, and also to explore the potential advantages of the transplantation of multiple units of UCB following nonmyeloablative preparative regimens.[73]

Embryo Selection to Create a UCB Donor

A novel approach in paediatric UCB transplant for families with children afflicted with genetic disorders such as Fanconi anaemia is embryo selection using preimplantation genetic diagnosis (PGD). In this procedure, after in vitro conception, non-affected embryos are selected by PGD and implanted for the purpose of obtaining a related UCB donor. Grewal and colleagues[74] from the University of Minnesota recently reported on the first successful matched sibling donor transplantation in which the sibling donor was created by this technique. The transplant resulted in the cure of a 3-year-old child with Fanconi anaemia with marrow failure, and no matched sibling donor. While PGD may be associated with ethical concerns, it could potentially be applied to a variety of other disorders such as haemoglobinopathies, immune deficiencies, inborn error of metabolism, and even selected malignancies.

Conclusions

UCB is a viable alternative to bone marrow and peripheral blood as a source of stem cells capable of haematopoietic reconstitution for both paediatric and adult patients, when unrelated marrow donor is not available. The advantages of UCB include the relative ease of procurement, tolerance of 1-2 antigen HLA mismatch and lower than anticipated risk of severe GVHD, even when HLA-disparate grafts are infused. UCB represents a highly convenient HSC source that may significantly extend the HSC donor pool, allowing the potentially curative allogeneic immunotherapy to be offered to a greater proportion of patients who do not have a matched sibling or unrelated donor. The results thus far suggest that unrelated UCB transplants can result in long-term disease-free survival in many of these patients. However, transplant-related mortality remains the main obstacle for successful UCB transplantation, particularly in adult patients receiving myeloablative preparative regimens. Clinical experience in both paediatric and adult patients has also documented the paramount importance of graft cell dose in determining engraftment and survival. It is hoped that the advantage of a lower GVHD without any apparent increase in relapse in UCB transplant will offset any adverse impact of reduced cell dose on survival. With the profound influence of UCB cell dose (both nucleated cell dose and CD34+ cell dose) on engraftment and survival, future efforts to improve the outcome of adult patients need to focus on augmenting UCB cell dose. UCB transplantation

following non-ablative preparative regimen is an exciting new approach which provides an option for patients who are otherwise excluded from conventional haematopoietic stem cell transplantation, including the elderly or medically infirm patients with no matched sibling donor**.** Preliminary results have shown that such an approach can be associated with timely engraftment with full donor chimerism. Comparison between the myeloablative approach and the non-ablative approach will be needed before this therapy can be considered for younger patients eligible for myeloablative transplant. At the moment, the use of non-ablative UCB transplant cannot be encouraged outside of clinical trials or selected patients. The future challenge will be to develop strategies to optimise the chance of early and durable engraftment, as well as to minimise the risk of GVHD and transplant-related death.

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