Double Umbilical Cord Blood Transplantation after Novel Myeloablative Conditioning Using a Regimen of Fludarabine, Busulfan, and Total Lymphoid Irradiation

Sameem Abedin1,*, Edward Peres2, John E. Levine1, Sung Choi1, Gregory Yanik1, Daniel R. Couriel1

1Blood and Marrow Transplant Program, University of Michigan Health System, Ann Arbor, Michigan
2Henry Ford Health System, Detroit, Michigan

Article history:
Received 16 May 2014
Accepted 9 July 2014

Key Words:
Umbilical cord blood
Myeloablative Conditioning
Busulfan

ABSTRACT
We conducted a pilot study evaluating double umbilical cord blood transplantation (dCBT) after myeloablative conditioning with fludarabine and busulfan 3.2 mg/kg i.v. × 4, followed by total lymphoid irradiation at 400 cGy (FluBu4/TLI) for any indicated hematological disorder for patients without a suitable donor. Twenty patients with predominantly high-risk disease underwent dCBT according to protocol. The regimen was well tolerated, with mucositis as the primary observed toxicity (n = 19). The cumulative incidence of neutrophil engraftment was 89% (95% confidence interval [CI], 64% to 97%), with a median time to recovery of 16 days (range, 12 to 31 days). All evaluable patients with neutrophil engraftment achieved complete donor chimerism by day 40. The cumulative incidence of grades III and IV acute graft–versus-host disease (GVHD) at day 100 was 10% (95% CI, 2% to 27%), and the cumulative incidence of chronic GVHD was 35% (95% CI, 16% to 55%) by the end of the study. At 1 year, the cumulative incidence of treatment-related mortality (TRM) was 35% (95% CI, 16% to 55%). The leading cause of nonrelapse mortality was acute GVHD (n = 4), followed by graft failure (n = 2) and chronic GVHD (n = 1). TRM was significantly associated with a pretransplantation hematopoietic cell transplantation–specific comorbidity index score ≥ 3 (P = .005). At 1 year, disease relapse occurred in 6 patients and overall survival was 40% (95% CI, 19% to 60%). We conclude that FluBu4/TLI is an adequate preparative regimen before dCBT, providing high engraftment rates and relatively early neutrophil recovery. The best survival outcomes were seen in patients without significant comorbidities before transplantation, and outcomes are comparable to previously published dCBT studies.

INTRODUCTION
Umbilical cord blood (UCB) has become an increasingly utilized alternative cell source for hematopoietic stem cell transplantation. Less stringent criteria for HLA matching allows for greater access to suitable units, and a suitable UCB unit can be made available in days [1,2]. In adults and larger children, single-unit UCB transplantation has been linked to slower neutrophil recovery and higher likelihood of graft failure because of low cell dose [3-6]. To overcome single-UCB cell dose limitations, a double-umbilical cord blood transplantation (dCBT) strategy was introduced and successfully validated in several adult studies [7-9].

At this time, the optimal myeloablative conditioning regimen before dCBT in regards to engraftment, treatment-related mortality (TRM), or prevention of relapse remains unknown. Most successful trials to date have utilized total body irradiation (TBI)–based myeloablative conditioning regimens in combination with cyclophosphamide and/or fludarabine [10-12]. Although these regimens may exert a potent antitumor effect and sufficient immunosuppression to facilitate mismatched unrelated cord blood engraftment, the toxicity with this approach may be contributing to the overall increased TRM seen with dCBT. Fludarabine and busulfan 3.2 mg/kg i.v. × 4 (FluBu4) is a widely used myeloablative regimen for bone marrow and peripheral hematopoietic stem cell transplantation, with favorable tolerability and lower TRM, compared with other myeloablative regimens [13-15].

Experience with FluBu4 in the setting of UCB transplantation is limited. In a previous study (Duke, MD Anderson Cancer Center) where FluBu4 was utilized, engraftment was low [15,16]. To augment immunosuppression without significantly adding toxicity, we conducted a pilot study to assess the safety and efficacy of performing dCBT using FluBu4 followed by low-dose total lymphoid irradiation (TLI) at 400 cGy (FluBu4/TLI) as our preparative regimen.

PATIENTS AND METHODS
Study Design
This was a pilot study conducted at the University of Michigan Health System with institutional review board approval. The primary objective was to estimate the 1-year survival rate for patients receiving dCBT using a conditioning regimen of FluBu4/TLI. Our initial target sample size was 30 subjects, set for feasibility. Stopping rules in this study were graft failure >10% at 35 days and all-cause mortality >50% at 100 days. These conditions were not met during our study. Between October 2008 and February 2012, a total of 21 consecutive patients were eligible and enrolled. All patients signed informed consent according to institutional guidelines. The median follow-up for survivors was 2.65 years (range, 1.63 to 4.36 years).

Patient Selection
Patients from 0 to 65 years of age, with a diagnosis of an incurable malignant or nonmalignant hematological disorder, with no suitable matched related or unrelated donor were eligible for the study. Patients with acute myelogenous leukemia (AML) or acute lymphoblastic leukemia could not have >10% blasts in their pretransplantation bone marrow. In patients with AML or myelodysplastic syndrome (MDS), there could not be any evidence of fibrosis of the marrow. Before transplantation, patients with non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia had to be...
in at least partial remission and patients with multiple myeloma (MM) had to be in at least a very good partial response.

Other routine transplantation eligibility criteria included performance status using Karnofsky or Lanksy criteria of 70% or higher, no significant medical comorbidities/conditions, negative serologies for hepatitis B, hepatitis C, and human immunodeficiency virus, and no uncontrolled infection.

Finally, patients were required to have 2 partially HLA-matched UCB units, with a minimum 4/6 HLA match between UCB units and patient, and 3/6 match between UCB units. One unit must have been able to deliver a precryopreserved total nucleated cell dose of at least 2.5 x 10^9 per kilogram and the second unit, at least 2 x 10^9 per kilogram.

**Treatment Plan**

All patients received fludarabine 40 mg/m^2 i.v. over 30 minutes daily for 4 days and each dose was immediately followed by busulfan 3.2 mg/kg i.v. over 4 hours for 4 days (days -5 to -2). Busulfan kinetics were performed on all patients to achieve a steady-state concentration of 600 to 900 ng/mL. TLI at 400 cGy was performed in 1 session on day -1 or day 0. No antithymocyte globulin (ATG) was given. All patients then underwent dCBT on day 0 and the UCB units were administered sequentially.

**Supportive Care**

All supportive care measures followed institutional clinical practice guidelines. Levetiracetam 1000 mg twice daily was given 12 hours before and the UCB units were administered sequentially.

Granulocyte colony-stimulating factor was given beginning on day and for 48 hours after i.v. busulfan administration for seizure prophylaxis. Guidelines. Levetiracetam 1000 mg twice daily was given 12 hours before and the UCB units were administered sequentially.

**TLI at 400 cGy was performed in 1 session on day -1 or day 0. No antithymocyte globulin (ATG) was given. All patients then underwent dCBT on day 0 and the UCB units were administered sequentially.**

**HLA Typing and Chimerism Analysis**

HLA typing was done with combined HLA class I (A, B, and C) intermediate-resolution, and class II (DR, DQ) high-resolution tests. Bone marrow or blood was collected after transplantation for donor chimerism assay studies on days >30, >60, >100, >180, and >365. Chimerism was determined by comparative analysis of donor and recipient microsatellite markers, utilizing multiplex polymerase chain reaction and differential fluorescence analysis. The threshold for detection of residual recipient cells was 5% of all nucleated cells.

**Clinical Variables**

At time of data analysis, each patient’s pretransplantation comorbidities were reviewed and scored using the hematopoietic cell transplant-specific comorbidity index (HCT-CI) [17]. Additionally, each patient’s pretransplantation disease characteristics were reviewed, and patients were assigned a disease risk score of low, intermediate, or high, using a previously validated disease risk index scale [18].

Engraftment was defined by the first of 3 consecutive days with an ANC >500/µL. Failure to achieve an ANC >500/µL within 35 days of stem cell infusion was defined as primary engraftment failure. Platelet engraftment was defined by the first of 7 consecutive days with a platelet count >20,000/µL without transfusion support. One patient with biopsy-confirmed leukemic relapse on day 15 was excluded from engraftment analysis.

History and physical examinations were performed on days >30, >60, >100, >180, and >365 and as clinically indicated to assess for GVHD. Acute and chronic GVHD were diagnosed and scored using previously established criteria [19,20].

Overall survival (OS) was calculated from the day of transplantation, and TRM was defined as death from any cause other than relapse. Event-free survival (EFS) was calculated from day of transplantation until relapse or death.

**Statistical Analyses**

OS and EFS were estimated using standard Kaplan-Meier methods, and asymptotic 95% confidence intervals (CI) were generated based on Greenwood’s variance formula. Secondary outcomes include neutrophil engraftment, platelet engraftment, acute GVHD (aGVHD), and chronic GVHD (cGVHD), estimated using cumulative incidence estimates, with death and relapse as competing risks. Additionally, an estimate of TRM was made based on cumulative incidence estimates, with relapse as a competing risk. Comparisons of time-to-event curves between subgroups were performed using the log-rank test. A Cox proportional hazard model was used to assess

---

**RESULTS**

**Patient Characteristics**

A total of 21 patients were enrolled between October 2008 and February 2012; 20 underwent UCB transplantation according to protocol, and 1 did not because of disease progression. Patient characteristics are summarized in Table 1. The majority were male (55%, n = 11); median age was 49 (range, 13 to 64 years) and median weight was 85 kg (range, 40 to 135 kg) at time of transplantation.

AML was the predominant diagnosis (45%, n = 9), most with high-risk cytogenetics (n = 6), followed by NHL (n = 5). The remaining diagnoses included MDS (n = 3), acute lymphoblastic leukemia in early relapse (n = 1), MM (n = 1), and acquired aplastic anemia (n = 1). Overall disease risk was low in 2 patients (10%), intermediate in 8 patients (40%), and high in 10 patients (50%).

The median number of total nucleated cells (TNC) infused was 5.32 x 10^9/kg (range, 4 to 15.6) of actual recipient body weight. The median number of TNCs in the larger UCB unit was 3.94 x 10^9/kg (range, 2.1 to 4.4). HLA matching between recipient and donor and between donor units are described in Table 1.

**Engraftment and Chimerism**

The cumulative incidence of neutrophil engraftment was 89% (95% CI, 64% to 97%), with a median time to recovery of 16 days (range, 12 to 31 days). The cumulative incidence of platelet engraftment was 73% (95% CI, 48% to 88%), with a
median time to recovery of 43 days (range, 26 to 86 days). Figure 1 illustrates these results.

Two patients (10%) failed to engraft by day 35 and experienced primary graft failure. Before transplantation, 1 had a diagnosis of aplastic anemia; the second patient had MDS. The first patient demonstrated absence of chimerism on day 28 bone marrow biopsy, and the second patient had a day 30 bone marrow biopsy with donor chimerism and findings suggestive of hemophagocytic lymphohistiocytosis. These 2 patients died within 80 days of transplantation.

Patients were evaluated for donor chimerism if they achieved neutrophil engraftment and were in remission at time of evaluation. All evaluable patients achieved complete donor chimerism on bone marrow chimerism studies performed on day 30, and this persisted at day 100. Single donor dominance was also seen in all but 1 patient on bone marrow chimerism studies at day 30 and day 100. Peripheral blood chimerism studies on days 30, 60, 100, 180, and 365 revealed complete donor chimerism in all patients, and single donor dominance in all but 1 case. In the majority of patients (n = 12, 71%), the dominant cord blood graft was that with the higher TNC. However, cell dose was not statistically associated with engraftment.

GVHD

The cumulative incidences of grade II to IV and grade III and IV aGVHD at day 100 was 40% (95% CI, 19% to 60%) and 10% (95% CI, 2% to 27%), respectively (Figure 2). Sites affected by aGVHD included the skin, gastrointestinal tract, and liver. By the end of the study, 6 patients developed cGVHD and the cumulative incidence of cGVHD was 35% (95% CI, 16% to 65%). Onset was de novo in 4 patients, quiescent in 2 patients, and progressive in 1 patient. Using National Institutes of Health criteria, 4 patients had mild cGVHD, 2 patients had moderate cGVHD, and 1 patient had severe cGVHD [20]. Sites affected by cGVHD included the skin, mouth, eyes, lungs, and kidney.

Infection

Within the first 100 days, 27 documented infections occurred in 16 patients. Bacterial infections included coagulase-negative Staphylococcus bacteremia (n = 2), vancomycin-resistant Enterococcus bacteremia (n = 1), Escherichia coli bacteremia (n = 1), Achromobacter baumannii bacteremia (n = 1), and Pseudomonas species pneumonia (n = 1). Nine patients developed human herpes virus–6 viremia, 5 developed BK virus cystitis, 1 had human metapneumovirus pneumonia, and 3 developed cytomegalovirus (CMV) viremia. Three patients receiving high-dose corticosteroids for treatment for aGVHD died of infection as a secondary cause of death.

Transplantation Outcomes

At day 100 and at 1 year, the cumulative incidence of TRM was 25% (95% CI, 9% to 45%) and 35% (95% CI, 16% to 55%), respectively. TRM was not associated with CMV status (P = .07) or disease risk (P = .69). Univariate analysis revealed that TRM was significantly associated with a pre-transplantation HCT-CI score ≥ 3 (P = .005). Five patients had an HCT-CI score ≥ 3. The 1-year TRM in this subgroup was estimated at 89% (95% CI, 49% to 99%). In comparison, 15 patients had an HCT-CI score <3, and 1-year TRM in this subgroup was estimated at 23% (95% CI, 8% to 56%). Univariate analysis also revealed that TRM was significantly associated with age ≥50 years (P = .04). On multivariate analysis, including CMV status, HCT-CI score, and age ≥50 as variables, the strongest predictor of TRM was an HCT-CI score ≥ 3 (P = .05). These patients were 5 times as likely to die from nonrelapse causes (hazard ratio, 5.6; 95% CI, 1 to 32). On multivariate analysis, age was not a significant predictor of TRM.

With a median follow-up of 2.65 years among survivors, disease relapse was observed in 6 patients (30%). By disease state, relapse occurred in 4 patients with AML demonstrating high-risk cytogenetic features before transplantation, 1 patient with NHL who underwent transplantation in complete remission (CR) after failed autologous transplantation, and 1 patient with MM who underwent transplantation in CR after failed autologous transplantation. Relapse occurred in 0 of 2 patients with low-risk disease, 2 of 8 patients with intermediate-risk disease, and 4 of 10 patients with high-risk disease before transplantation; however, the difference in relapse rate between these groups was not statistically significant (P = .65).

Estimated OS at 1 year was 40% (95% CI, 19% to 60%), and 7 (35%) patients are in continuous CR with a median follow-up of 2.35 years after transplantation (range, 1.32 to 4.36 years) (Figure 1). The EFS at the end of this study was 35% (95% CI, 16% to 55%). EFS was not significantly associated with disease risk, age ≥ 50, or HCT-CI score ≥ 3. The main cause of death outside relapse was GVHD. Four patients died during
DISCUSSION

DCBTextends access to transplantation for adolescents and adults without an available related or unrelated donor. The EFS and OS after dCBT in adults are comparable to related and unrelated donor transplantations for a variety of malignant and nonmalignant hematological conditions [7-10,21]. Previ-
ously reported successful myeloablative conditioning regi-
mens for dCBT include cyclophosphamide (120 mg/kg), TBI (13.2 Gy) with fludarabine (75 mg/m²), and TBI (13.5 Gy) with fludarabine (160 mg/m²) [10-12]. Given the high engraftment rates and low TRM seen with myeloablative FluBu4 in the setting of related or unrelated donor transplantation, we assessed whether performing dCBT using FluBu4 followed by TLI at 400 cGy would achieve similar results. In our study, we report favorable engraftment and toxicities using FluBu4/TLI. Moreover, despite treating a high-risk population in this study, a sizable number of patients enjoy a prolonged EFS and OS.

The cumulative incidence of neutrophil engraftment in our study was 89%, consistent with previously reported values of 80% to 91% [10-12]. Further, compared with the low engraftment rates reported utilizing FluBu4 for dCBT (Duke, MD Anderson Cancer Center), the addition of TLI has resulted in excellent engraftment [15,16]. Finally, we observed relatively rapid engraftment at a median of 16 days. Delayed engraftment has been associated with decreased survival in dCBT. In a study comparing dCBT with related and unrelated peripheral blood stem cell transplantation, Bruinstein et al. noted patients who received a dCBT had an increase in nonrelapse mortality compared with those with other donor sources, and the strongest factor implicated was delayed neutrophil recovery, especially if recovery was delayed beyond 26 days after transplantation [8]. In our study, only 3 of 17 had neutrophil recovery later than 26 days and the latest neutrophil recovery occurred was 31 days.

We decided to utilize TLI in this study, and we omitted ATG because of the adverse effects of ATG, including delayed immune reconstitution. Our study was not designed to quantify immune reconstitution; however, we did not experience any deaths primarily due to infection and we experienced a low relapse rate. Of note, our total infused cell dose (median, 5.32 × 10⁹/kg; range, 4 to 9.6 × 10⁹/kg) was higher than in previous studies and may have contributed to better engraftment rates, as well. Although we did experience high engraftment, we also had 2 cases of graft failure. Two cases are too few to analyze; however, 1 graft failure occurred in a patient with aplastic anemia, which is known to be less likely to engraft [22].

At the end of our study, with a median observation time of 2.35 years after transplantation, the estimated OS was 35%. The majority of deaths (n = 6, 30%) were secondary to relapse, reflecting the large proportion of patients with high-risk disease. Our overall TRM of 35% was not different from previously published experience using standard myeloablative conditioning [10-12]. On the other hand, our TRM among patients with an HCT-CI score of 3 or more was high at 89%. Healthier patients, regardless of age, appeared to fare better with a TRM of 23%. The rates of both aGVHD and cGVHD were comparable with other dCBT studies and lower than expected in mismatched unrelated transplantation from other sources [8]. Only 10% of patients developed grade III and IV aGVHD, and 30% developed cGVHD by the end of this study.

In summary, our study suggests FluBu4/TLI is an adequate preparative regimen before dCBT, providing high engraftment rates and relatively early neutrophil recovery. Compared with FluBu4 alone, the addition of TLI at 400 cGy may have contributed to improved engraftment. Although larger studies in a randomized control setting are required to accurately compare different myeloablative regimens, our outcomes appear in line with previous successful dCBT trials.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

REFERENCES

Outcomes of Thalassemia Patients Undergoing Hematopoietic Stem Cell Transplantation by Using a Standard Myeloablative versus a Novel Reduced-Toxicity Conditioning Regimen According to a New Risk Stratification

Usanarat Anurathapan1, Samart Pakakasama1, Pimsiri Mejkaruskul1, Nongnuch Sirachainan1, Duantida Songdej1, Ampaiwan Chuanumrit1, Pimlak Charoenkwan2, Arnee Jetsrisuparb3, Kleebabai Sanpak4, Bunchoo Ponttanakul4, Piya Rujkijyanont5, Arunotai Meekaewkunchorn6, Rosarin Sruamsiri7, Artit Ungkanont8, Surapol Issaragrisil1, Borje S. Andersson15, Suradej Hongeng1,16

1 Department of Pediatrics, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
2 Department of Pediatrics, Chiangmai University Hospital, Chiangmai, Thailand
3 Department of Pediatrics, Khonkaen University, Khonkaen, Thailand
4 Department of Pediatrics, Siriraj Hospital, Mahidol University, Bangkok, Thailand
5 Department of Pediatrics, Phramongkutklao Hospital, Bangkok, Thailand
6 Queen Sirikit National Institute of Child Health, Bangkok, Thailand
7 Center of Pharmaceutical Outcomes Research, Department of Pharmacy Practice, Naresuan University, Phitsanulok, Thailand
8 Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
9 Department of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand
10 Department of Stem Cell Transplantation and Cellular Therapy, University of Texas MD Anderson Cancer Center, Houston, Texas

ABSTRACT

Improving outcomes among class 3 thalassemia patients receiving allogeneic hematopoietic stem cell transplantations (HSCT) remains a challenge. Before HSCT, patients who were ≥ 7 years old and had a liver size ≥ 5 cm constitute what the Center for International Blood and Marrow Transplant Research defined as a very high-risk subset of a conventional high-risk class 3 group (here referred to as class 3 HR). We performed HSCT in 98 patients with related and unrelated donor stem cells. Seventy-six of the patients with age < 10 years received the more conventional myeloablative conditioning (MAC) regimen (cyclophosphamide, busulfan, ± fludarabine); the remaining 22 patients with age ≥ 10 years and hepatomegaly (class 3 HR), and in several instances additional comorbidity problems, underwent HSCT with a novel reduced-toxicity conditioning (RTC) regimen (fludarabine and busulfan). We then compared the outcomes between these 2 groups (MAC versus RTC). Event-free survival (86% versus 90%) and overall survival (95% versus 90%) were not significantly different between the respective groups; however, there was a higher incidence of serious treatment-related complications in the MAC group, and although we experienced 6 graft failures in the MAC group (8%), there were none in the RTC group. Based on these results, we suggest that (1) class 3 HR thalassemia patients can safely receive HSCT with our novel RTC regimen and achieve the same excellent outcome as low-standard-risk thalassemia patients who received the standard MAC regimen, and further, (2) that this novel RTC approach should be tested in the low-standard-risk patient population.

© 2014 American Society for Blood and Marrow Transplantation.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment for thalassemia patients [1]. A myeloablative conditioning regimen (MAC) of busulfan (Bu) followed by 4 days of cyclophosphamide (Cy) (BuCy4) has, for several years, been considered the standard of care for HSCT in severe thalassemias [2]. The outcome of HSCT is dependent on patients’ pretransplantation risk...