Transplantation of Unrelated Donor Umbilical Cord Blood in 102 Patients with Malignant and Non-Malignant Diseases: Influence of CD34 Cell Dose and HLA Disparity on Treatment-Related Mortality and Survival

Short title: Unrelated Donor Umbilical Cord Blood Transplant

Scientific Section: Clinical Observation, Intervention, & Therapeutic Trials

John E. Wagner, M.D.¹, Juliet N. Barker, M.B.B.S. (Hons)², Todd E. DeFor, M.S.¹,³, K. Scott Baker, M.D.¹,³, Bruce R. Blazar, M.D.¹, Cindy Eide, M.S.¹, Anne Goldman, Ph.D.³, John Kersey, M.D.¹, William Krivit, M.D.¹, Margaret MacMillan, M.D.¹, Paul J. Orchard, M.D.¹, Charles Peters, M.D.¹, Daniel J. Weisdorf, M.D.², Norma K.C. Ramsay, M.D.¹ and Stella M. Davies, M.B.B.S., Ph.D.¹,²

From the Blood and Marrow Transplant Program of the Departments of Pediatrics¹ and Medicine², and Biostatistical Support Group³ of the University of Minnesota Cancer Center and School of Medicine, Minneapolis, Minnesota, U.S.A.

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Address correspondences to: Dr. John E. Wagner, Mayo Mail Stop 366, University of Minnesota, 420 Delaware Street, S.E., Minneapolis, Minnesota 55455, U.S.A.; phone number (612) 626-2961; fax number (612) 626-4074; email address: wagner002@tc.umn.edu

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ABSTRACT

Rationale. The potential benefits of unrelated donor marrow transplantation are offset by the immunologic complications of graft-versus-host disease (GVHD) and infection. Therefore, cryopreserved umbilical cord blood (UCB) was used as a strategy to reduce the risks of GVHD and treatment-related mortality (TRM) and improve survival.

Objectives. Data on 102 patients (median age 7.4 years) transplanted between 1994 and 2001 for the treatment of malignant (n=65; 68% high-risk) and non-malignant diseases (n=37) were evaluated. Log-rank tests and Cox regression analyses were used to determine the effects of various demographic, graft-related and treatment factors on engraftment, GVHD, TRM, relapse and survival.

Findings. As of October 15, 2001, the median follow-up was 2.7 (0.3-7.2) years. Incidences of neutrophil and platelet engraftment were 0.88 (0.81-0.95) and 0.65 (0.53-0.77), respectively. Notably, incidences of severe acute and chronic GVHD were 0.11 (0.05-0.17) and 0.10 (0.04-0.16), respectively. At 1 year after transplantation, proportions of TRM and survival were 0.30 (0.21-0.39) and 0.58 (0.48-0.68), respectively. In Cox regression analyses, CD34 cell dose was the one factor consistently identified as significantly associated with rate of engraftment, TRM and survival. Despite low incidence of GVHD, leukemia relapse was 0.17 (0.00-0.38) and 0.45 (0.28-0.61) at 2 years for patients with standard and high-risk disease, respectively.

Conclusions. There is a high probability of survival in recipients of HLA 0-2 antigen disparate UCB grafts containing ≥1.7 x 10^5 CD34+ cells/kilogram. Therefore, graft selection should be
based principally on CD34 cell dose when multiple UCB units exist with ≤2 HLA antigen disparity.

Corresponding author’s email address: wagne002@tc.umn.edu

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Introduction

Transplantation of hematopoietic stem cells (HSC) derived from bone marrow and umbilical cord blood (UCB) of sibling and unrelated donors has been used successfully to treat patients with high-risk or recurrent hematological malignancies, bone marrow failure syndromes, selected hereditary immunodeficiency states and metabolic disorders (1-10). Successful use of HSC transplant therapy, however, has been limited by a lack of HLA matched donors and the high-risk of graft-versus-host disease (GVHD) after transplantation. While there are currently 7 million HLA-A, B and DR typed donors registered in marrow donor registries around the world, more than 30% of patients requiring transplant therapy are still unable to find an HLA 0-1 antigen disparate marrow donor (11), with even greater proportions of unsuccessful searches in patients of non-Northern European descent. For those transplanted with unrelated donor marrow, HLA 1 antigen disparity has clearly been shown to increase the risk of treatment-related mortality (TRM) and adversely affect survival attributable in part to high-risks of GVHD and opportunistic infection in every age group (12-16).

To potentially alleviate the shortage of suitable donors and reduce the length of the unrelated marrow donor search process, repositories of banked, HLA typed UCB have been developed since 1993 (17,18). In 1996, Kurtzberg et al. (6) and Wagner et al. (7) reported preliminary clinical results of unrelated donor UCB transplantation suggesting that banked unrelated donor UCB contained sufficient numbers of HSC to achieve engraftment in the majority of cases with lower than anticipated risk of severe acute GVHD in children and smaller adults.

Since these initial reports, more than 40,000 UCB units have been stored and more than 2000 UCB transplants have been performed worldwide (8). However, there have been few reports on the clinical outcomes after UCB transplantation especially from single centers or consortiums.
that utilize uniform treatment plans and methods of assessment (6,7,9,19). Therefore, we report
the results of UCB transplantation at the University of Minnesota with analysis of the influence
of various demographic, treatment and graft characteristics, particularly the effect of HLA
disparity and CD34 cell dose, on rate of hematopoietic recovery and probabilities of engraftment,
GVHD, TRM, relapse and survival.

**Patients and methods**

Patients with acute leukemia, other malignancies, bone marrow failure syndromes,
immunodeficiency states or metabolic disorders were eligible for unrelated donor UCB
transplantation if: 1) an HLA-0-1 related or unrelated bone marrow donor was unavailable, and
2) the subject/parent(s) consented to the transplant procedure. Protocols for myeloablative
therapy and the use of banked unrelated donor UCB for transplantation were reviewed and
approved by the institutional review board at the University of Minnesota.

**Patients.** Between July 7, 1994 and September 30, 2001, 152 patients were transplanted with
banked unrelated donor UCB (34 patients 1994-1997; 115 patients 1998-2001). For this
analysis, patients were excluded from analysis if: transplantation occurred after June 30, 2001
(n=18), prior allogeneic HSC transplantation was performed (n=17), a non-myeloablative
cytoreductive regimen was administered (n=10), or the UCB graft was composed of more than
one unit (n=4). One hundred and two patients treated for various malignant and non-malignant
disorders were evaluable. Patient demographic and treatment characteristics are shown in Table
1. Median age of the patients was 7.4 years (range, 0.2-56.9) and median weight was 25.9 kg
(range, 5.0-107.5). Twenty-two were ≥18 years of age. Six patients with malignancy had
relapsed after prior autologous transplant and were included in the analysis.
**HLA typing and unrelated donor selection.** Units were obtained from 6 UCB banks (New York Blood Center Placental Blood Program [n=67]; St. Louis Cord Blood Bank [n=26]; Netcord [n=9, Barcelona, Milano, Dusseldorf and Firenze]). All unrelated donor UCB units were HLA typed by the UCB bank. Prior to transplantation, confirmatory HLA typing of the selected UCB unit and recipient was performed at the transplant center and/or the UCB bank. HLA-A and HLA-B antigens were typed using the standard two-stage complement-dependent microcytotoxicity assay, and antigens were assigned as defined by the World Health Organization (WHO) HLA nomenclature committee (20). HLA-DRB1 type was determined by hybridization of polymerase chain reaction (PCR) amplified DNA with sequence-specific oligonucleotide probes (SSOP) (21) with sequencing if needed. Prior to March 1999, HLA-A, B and DRB1 typing was used to select the closest matched donor unit-recipient pair with preference given to HLA DRB1 matched units; subsequently, priority was given to the HLA 0-2 antigen mismatched unit with the greatest nucleated cell dose.

**Myeloablative regimen and GVHD prophylaxis.** Pretransplant conditioning varied according to the patient's disease, disease status and history of prior radiotherapy. All but 15 patients received cyclophosphamide (CY) and total body irradiation (TBI)-containing regimen; all patients received anti-thymocyte globulin (ATGAM, Pharmacia) prior to unrelated donor UCB transplantation. All patients with malignant disease received CY 120 mg/kg and TBI 1320-1375 cGy.

Prophylaxis for acute GVHD consisted of cyclosporine A and methotrexate (CsA/MTX, n=2) or cyclosporine A and methylprednisolone (CsA/MP, n=100). CsA was initiated on day –3 and continued at least 6 months before initiating a 10% per week taper. MP (1 mg/kg intravenously every 12 hours) was administered on days 5 to 19 with a 25% taper every other day thereafter.
(discontinued by day 26). For the two patients receiving CsA/MTX, MTX was administered intravenously on day 1 (15 mg/m²) and days 3, 6, and 11 (10 mg/m²).

**Transplantation of UCB.** UCB grafts used for transplantation in this study contained a median of 3.1 x 10⁷ nucleated cells (0.7-57.9), 2.8 x 10⁵ CD34 cells (0.4-39.1) and 8.0 x 10⁶ CD3 cells (0.003-80) per kilogram (kg) recipient body weight after thawing. Cryopreserved units of UCB were transported to the transplant center via overnight delivery in a dry shipper previously cooled by liquid nitrogen (temperature <-150°C) before the initiation of the preparative regimen and then maintained in the vapor phase of liquid nitrogen at the transplant center until the day of transplantation. Except for the first 2 patients for whom the unit of UCB was thawed at the bedside and infused intravenously over a 10 to 15 minute period without manipulation, units were thawed in a 37°C water bath with gentle agitation, using the method described by Rubinstein et al. (22). After thawing, an equal volume of dextran/albumin solution was added over 10 minutes, centrifuged at 250 g for 5-10 minutes at 10°C and the supernatant removed. The cell pellet was resuspended in dextran/albumin and immediately infused into the patient over 1 to 2 hours.

**Supportive care.** Patients were hospitalized in single rooms ventilated with high-efficiency particulate air filtration systems. Patients at high-risk for the recurrence of herpes simplex (i.e., IgG titers ≥1:8) received prophylactic intravenous acyclovir. Patients at high-risk for the recurrence of CMV (IgG titer ≥1:8) received prophylactic high dose acyclovir or ganciclovir during cytoreduction followed by high dose acyclovir until neutrophil recovery followed by ganciclovir or acyclovir until day 100. Documented CMV reactivation or infection demonstrated by antigenemia testing after transplantation was treated with therapeutic doses of ganciclovir ± intravenous immunoglobulin (23). Broad spectrum antibiotics were administered for fever
during aplasia and amphotericin B 0.3-1.2 mg/kg/day was added for persistent fever unresponsive to antibiotic therapy. All patients received fluconazole for prophylaxis of yeast infections for 100 days and trimethoprim-sulfamethoxazole for prophylaxis of Pneumocystis carinii after engraftment for 12 months after transplantation and penicillin for prophylaxis of gram positive organisms during treatment of GVHD. Granulocyte-colony stimulating factor (G-CSF, 5 ug/kg/day) from day 0 was administered to all patients since March 1997 (n=80).

**Hematopoietic recovery and engraftment.** Hematologic recovery was defined as time to absolute neutrophil count (ANC) ≥5 x 10^8/L (first of three consecutive laboratory measurements on different days) and platelet count ≥5 x 10^10/L (first of seven days without transfusion support). Donor cell engraftment and remission status in the marrow were assessed on days 21, 60, 100 and at 6 months, 1 year and 2 years after transplantation, with chimerism status determined by quantitative polymerase chair reaction (PCR) analysis of informative polymorphic variable number tandem repeat (VNTR) regions. Complete chimerism was defined as the presence of donor hematopoietic cells only; mixed chimerism was defined as the presence of both donor and host (>10%) hematopoietic cells simultaneously. Autologous recovery was defined as the presence of host hematopoietic cells (>90%).

**Graft-versus-host disease.** Patients were evaluated for acute GVHD daily during initial hospitalization, at least once weekly after initial discharge during the first 100 days, and at routine follow-up evaluations at the transplant centers at 6 months, 1 year and at least yearly thereafter. Diagnosis of acute GVHD was based on clinical criteria with histopathologic confirmation when possible. Overall staging was based on published criteria (24) and assigned by an independent review team retrospectively. Patients with clinical stage >II disease were
treated initially with MP $\geq 48$ mg/m$^2$ intravenously daily for a minimum of 2 weeks prior to 10% taper per week.

**Statistical analysis.** Data regarding transplant patient characteristics, post-transplant complications and outcomes were prospectively collected by the Biostatistical Support Group at the University of Minnesota using standardized collection procedures. Cumulative incidence rates and their 95% confidence intervals (CI) were estimated for engraftment, grade II-IV and grade III-IV acute GVHD, chronic GVHD, TRM, and relapse (for patients with malignant diseases) (25). Kaplan-Meier methods were used to estimate survival (26). Event times for neutrophil engraftment were measured from the date of transplantation to the date of neutrophil recovery with censoring for early death (i.e., died before day 28 without neutrophil recovery, n=5) or evidence of persistent malignant disease (n=3). Patients who had very slow engraftment (i.e., achieved an ANC $\geq 5 \times 10^9$/L after day 42) or failed to have marrow reconstitution of donor origin (<5%) were scored as primary graft failure. Those with severe neutropenia of >1 week duration or autologous recovery after primary engraftment were scored as secondary graft failure. Spearman’s rank correlation coefficient was used to estimate a correlation between nucleated cell dose and CD34 cell dose (27).

Cox proportional hazard models and log rank test statistics were used to evaluate the univariate and multiple effects of risk factors on outcome. Tarone’s test for trend was applied when the alternative hypothesis was a ranked trend rather than differences between unordered categories (28). The outcome variables were: engraftment, GVHD, treatment related mortality, relapse and survival. The following factors were considered potential predictors of outcomes including: recipient age, weight, gender, race, CMV serostatus, diagnosis (malignant versus non-malignant disease) and malignancy risk category; donor-recipient ABO match; donor-recipient HLA match;
presence of class I versus class II disparity; use of G-CSF; conditioning regimen
(TBI+chemotherapy versus chemotherapy alone); graft cell dose (nucleated cells, CD34 cells,
CD3 cells); development of acute GVHD; and, history of prior autologous transplant. All
factors were tested for the proportional hazards assumption with HLA disparity included in every
model (29). Event times were analyzed as of October 15, 2001.

For purposes of these analyses, patients with malignancy were categorized as having disease at
standard-risk or at high-risk for relapse after transplantation. Patients were considered to have
high-risk disease if they had 1) acute leukemia in second remission after short first remission (<3
year for patients with acute lymphocytic leukemia [ALL] (n=14) and <1 year for patients with
acute myelocytic leukemia [AML]) or in third remission and beyond or in relapse (n=22), 2)
chronic myelogenous leukemia (CML) in accelerated phase, 3) juvenile myelomonocytic
leukemia (JMML), or 4) lymphoma in relapse or refractory to therapy; all other patients
(regardless of cytogenetic abnormality) were considered to have standard-risk disease. Effect of
HLA disparity on probabilities of engraftment and acute GVHD took into account graft rejection
and GVHD vectors (i.e., mismatch in the rejection or GVHD direction [15]), respectively. When
both the donor and recipient were heterozygous at the mismatched locus, the disparity was
present for both the graft rejection and GVHD vectors. When the donor was heterozygous and
the recipient was homozygous or displayed a blank at the mismatched locus, HLA was
considered mismatched only in the graft rejection vector and not in the GVHD vector.
Conversely, when the recipient was heterozygous and the donor was homozygous at the
mismatched locus, the disparity was considered only in the GVHD vector and not the graft
rejection vector. Any mismatch, regardless of vector, was considered in the analyses of survival,
treatment-related mortality, and relapse.
Results

**Hematopoietic recovery and engraftment.** Incidence of neutrophil recovery by day 42 was 0.88 (95% confidence interval [CI], 0.81-0.95) with 4 patients engrafting after day 42 (2 of 4 alive at 298 days and 1830 days after transplant). Median time to achieve an ANC ≥5 x 10⁸/L was 23 days (9-54). In univariate analyses, rate of recovery and engraftment of neutrophils were strongly associated with CD34 cell dose (p<0.01) (Figure 1). Notably, with patients grouped according to CD34 cell dose quartiles, rate and likelihood of engraftment were markedly inferior in pediatric and adult patients (with 53% in this quartile >18 years of age) receiving a cell dose <1.7 x 10⁵ CD34 cells/kg (0.72 [CI, 0.51-0.93]) at a median of 34 days (17-54) as compared to other cell doses (p<0.01). A trend toward more rapid and more frequent engraftment was noted in recipients of G-CSF (p=0.09). For those patients receiving G-CSF (n=80), neutrophil engraftment by day 42 was 0.90 (CI, 0.84-0.97) at a median of 21 days (9-54) as compared to 0.80 (0.60-1.00) at a median of 31 days (17-45) in those not receiving G-CSF.

**Figure 1:** Cumulative incidence of neutrophil engraftment after unrelated donor UCB transplantation (n=102): Effect of CD34 cell dose (x 10⁵/kilogram recipient body weight).
While neutrophil recovery was strongly associated with thawed CD34 cell dose, the association with infused nucleated cell dose did not reach statistical significance (p=0.06). However, a correlation between CD34 and nucleated cell doses exists (p<0.01), as shown in Figure 2. With patients grouped according to nucleated cell dose quartiles, rate and likelihood of engraftment was decreased in recipients of <1.8 x 10^7 nucleated cells/kg (0.80 [CI, 0.62-0.97]) occurring at a median of 28 days (15-39) as compared to other cell doses (p=0.07).

![Figure 2: Correlation between infused nucleated cell dose (x 10^7/kilogram recipient body weight) and thawed CD34 cell dose (x 10^5/kilogram recipient body weight).](image)

Incidence of platelet recovery by 6 months was 0.65 (CI, 0.53-0.77). Median time required to achieve a platelet count ≥5 x 10^{10}/L was 86 days (29-276). In univariate analyses, platelet engraftment by 6 months was associated with younger recipient age (p<0.01), higher CD34 cell dose (p<0.01) and lack of grade III-IV acute GVHD (p=0.04) (data not shown).
The proportion of secondary graft failure, as manifested by autologous recovery after primary engraftment, was 0.05 (CI, 0.00-0.10). Autologous recovery was restricted to patients with non-malignant diagnoses, specifically with Hurler syndrome (n=2), Chediak Higashi syndrome (n=1) and metachromatic leukodystrophy (n=1) and occurred 21 to 80 after UCB transplantation. Chimerism was complete in 100% of patients with malignancy who achieved primary engraftment and had no evidence of recurrent disease (i.e., stable mixed chimerism has not been observed).

In Cox regression analysis, both neutrophil (Table 2) and platelet engraftment were associated with CD34 cell dose. Development of severe GVHD was associated with delayed platelet engraftment (data not shown). HLA match and use of G-CSF was not associated with either neutrophil or platelet engraftment.

**Acute and Chronic Graft-versus-Host Disease.** Acute GVHD occurred in 63 patients and scored as grade I (n=24), grade II (n=28), grade III (n=8) and grade IV (n=3) disease (Table 3). By day 100 after transplantation, incidences of grade II-IV and grade III-IV acute GVHD were 0.39 (CI, 0.29-0.49) and 0.11 (CI, 0.05-0.17), respectively. The median time to acute GVHD was 35 days (8-86). Skin and lower gastrointestinal tract were the most likely affected organs with mild disease in the majority of patients (Table 3). Of the 39 patients developing grade II-IV acute GVHD, 24 had a complete response to primary therapy with methylprednisolone. Of those requiring secondary therapy with anti-thymocyte globulin, methyprednisolone 250 mg/m²/day or other investigational agents (n=15), 8 had a complete response with only 2 alive. In Cox regression analysis, acute GVHD was not associated with any predictor, including CD3 cell dose, HLA match or class of HLA disparity (Table 2).
Incidence of chronic GVHD was 0.09 (CI, 0.04-0.14) at 1 year after transplant. Disease was categorized as extensive in 9 patients with involvement of the skin (n=5), liver (n=1), mucous membranes (n=2), lung (n=1) and joints (n=1). All patients with chronic GVHD had extensive disease which was first diagnosed at a median of 5 (2-7) months after transplantation.

**Treatment-Related Mortality.** Incidences of TRM at 1 year and 2 years after unrelated donor UCB transplantation were 0.30 (CI, 0.21-0.39) and 0.35 (CI, 0.25-0.45), respectively. In univariate analyses, TRM was associated with CD34 cell dose with a 1 year TRM of 0.20 (0.10-0.30) for patients receiving $>1.7 \times 10^5$ CD34 cell/kg recipient body weight (Figure 3). In addition, recipient age (p<0.01), nucleated cell dose (p=0.042) and development of grade III-IV acute GVHD (p=0.05) were factors also associated with TRM with favorable outcomes in recipients of younger age, higher cell dose, without history of severe GVHD. TRM was lower in CMV negative recipients (0.26 versus 0.37 at 1 year [p=0.09]) and recipients without a history of prior autologous transplant (0.29 versus 0.50 at 1 year [p=0.06]). TRM was not associated with recipient weight, gender, HLA match (p=0.14), ABO match, diagnosis (malignancy versus non-malignancy), malignancy risk group, preparative therapy (TBI versus no TBI containing regimen), use of G-CSF or recipient race. In Cox regression analyses (Table 2), CD34 cell dose, development of grade III-IV acute GVHD and age were the only factors associated with TRM; there was no association with HLA match.
Figure 3: Cumulative incidence of treatment-related mortality after unrelated donor UCB transplantation (n=102): Effect of CD34 cell dose (x 10^5/kilogram recipient body weight, Panel A) and effect of HLA disparity (Panel B). (mm=mismatch)

Relapse

Hematological relapse was detected between 21 and 672 days (median 196) after transplantation in 21 of 65 patients treated for malignant disease. The cumulative incidence of relapse was 0.37 (0.24-0.50) at 2 years. For patients with ALL, the incidence of relapse at 2 years was 0.10 (0.00-0.29) for patients with standard-risk disease (n=14) and 0.43 (0.17-0.61) for patients with high-risk disease (n=14). For patients with high-risk AML (n=22), the incidence of relapse was 0.47 (0.25-0.69) at 2 years as compared to 1 of 4 patients relapsing with standard-risk disease (Figure 4). For the entire group, relapse was not associated with cell dose, HLA match or history of prior acute or chronic GVHD. In Cox regression analysis, relapse was only associated with recipient age (p=0.01) and malignancy risk group (p=0.05).
Survival. With a median follow-up of 2.7 years, the cumulative proportion surviving at 1 year and 2 years after unrelated donor UCB transplantation was 0.58 (0.49-0.70) and 0.47 (0.36-0.57), respectively, with a survival of 0.70 (0.49-0.90) at 1 year in patients with UCB grafts containing $>1.7 \times 10^5$ CD34/kg. Survival in transplant recipients with non-malignant and malignant disease was 0.60 (0.44-0.77) and 0.38 (0.25-0.51) at 2 years, respectively. Of 28 patients with ALL, survival was 0.55 (0.23-0.87) for standard-risk and 0.32 (0.06-0.59) for high-risk patients. Similarly, of 26 patients with AML, survival was 0.33 (0.12-0.54) for high-risk patients and 2 of 4 alive with standard-risk disease. In univariate analyses, survival was associated with recipient age ($p<0.01$), disease ($p=0.04$), CD34 cell dose ($p<0.01$) (Figure 5), recipient CMV serostatus ($p=0.05$) and history of severe acute GVHD ($p<0.01$) with favorable outcomes in recipients of younger age, non-malignant disease, higher cell doses, without history of severe GVHD.

Notably potential risk factors such as HLA match ($p=0.08$), recipient CMV serostatus ($p=0.09$),
malignancy risk group (p=0.10) and history of prior autologous transplant (p=0.07) did not reach statistical significance in univariate analysis. Further, survival was not associated with recipient weight, gender, ABO match, class of HLA disparity, malignancy risk group, preparative therapy (TBI versus no TBI containing regimen), use of G-CSF, or recipient race. In Cox regression analyses, however, HLA match in addition to CD34 cell dose, development of grade III-IV acute GVHD and history of prior autologous transplant were associated with survival (Table 2).

Figure 5: Cumulative proportion surviving after unrelated donor UCB transplantation (n=102): Effect of CD34 cell dose (x 10^5/kilogram recipient body weight, Panel A) and effect of HLA disparity (Panel B). (mm=mismatch)

In this series, 54 patients died. Death was most frequently associated with relapse of malignant disease (n=26) and opportunistic infection (n=15). Other primary causes of death were GVHD (n=7), graft failure (n=3), and interstitial pneumonitis (n=2) and hepatic veno-occlusive disease (n=1). EBV-lymphoproliferative syndrome was diagnosed in 2 patients (30) but was not a primary cause of death in any patient.
Discussion

It has been established that a single UCB unit contains sufficient numbers of HSC for durable engraftment in most patients (2-5,31-33). Importantly, the results presented indicate that the proportion achieving neutrophil engraftment by day 42 after unrelated donor UCB transplantation is similar to that observed after unrelated donor BMT (34) with neutrophil engraftment occurring in 88% and 90% and platelet engraftment occurring in 50% and 55%, respectively. It is noteworthy that UCB units engraft successfully considering the low numbers of CD34 cells infused. In contrast to the typical bone marrow allograft containing a median 3 x 10⁶ CD34 cells/kg, recipients of UCB receive >1-log fewer CD34 cell dose (median 2.7 x 10⁵ CD34 cells/kg). In fact, the incidence of engraftment is similar to that observed after unrelated donor BMT until the CD34 cell dose approaches 1.7 x 10⁵/kg, below which the rate of recovery and incidence of engraftment are unsatisfactory. These data, therefore, support the contention that a threshold dose exists, defining which UCB units are acceptable for each transplant candidate. As a result of these findings, a dose of 1.7 x 10⁵ CD34/kg has now been established as the threshold dose for patients at the University of Minnesota. Arguably, differences in preparative and supportive care therapies as well as methods of CD34 analysis may prevent the establishment of a universally applicable threshold value. Importantly, Thompson et al. (9) and Laughlin et al. (10) did not observe an association between CD34 cell dose and engraftment and survival, possibly due to differences in treatment and methods of CD34 analysis or smaller patient numbers. Nonetheless, these data suggest a need for routine evaluation of each UCB unit using a standardized method of CD34 analysis and quantification by all cord blood banks so that a threshold limit can be defined.
An advantage of UCB is its apparent reduced alloreactive response as compared to BM (35-37). The data would suggest that UCB, despite HLA mismatching, is associated with low GVHD risk. Davies et al. (13) previously reported a probability of grade III-IV acute GVHD of 32 and 49% in recipients of HLA 0 and 1 antigen disparate BM, respectively, at the University of Minnesota, while data presented here demonstrated an incidence of 11% in recipients of HLA 0-2 antigen disparate UCB using the same grading criteria. In addition, the infrequent development of severe acute GVHD and extensive chronic GVHD after unrelated donor UCB transplantation as compared to unrelated BMT is striking (38). While it may be related to treatment factors such as the use of pretransplant ATGAM, the explanation for such a low incidence of GVHD in recipients of unrelated donor UCB is unclear. While the cytotoxic T cell precursor frequency has been found to be similar in UCB and adult peripheral blood (39), the median CD3 cell dose of 8 x 10^6/kg in UCB units make it similar to a marrow graft after modest T cell depletion. However, such a level of T cell depletion nor the choice of immunoprophylaxis fully explains the low incidence of GVHD observed by us and others (40-42). More likely functional differences such as a defective cytotoxic response reported with UCB lymphocytes (43), altered cytokine profile (31,44) or other differences may explain the decreased incidence of severe GVHD. Notably, time to onset of acute GVHD appears to be delayed (median day 33) as compared to that observed after BMT. Reasons for delayed onset of GVHD may be 1) slower pace of peripheral blood cell recovery and 2) prophylactic use of MP on days 5-19 after transplantation. Nonetheless, distribution of involved organs is similar to that after BMT albeit milder in severity.

TRM is the principal obstacle to successful transplant outcome in recipients of unrelated donor BMT and is the major reason for evaluating UCB as an alternate source of HSC. Of the 3 risk factors associated with risk of TRM in Cox regression analysis (i.e., recipient age, development of severe acute GVHD, and CD34 cell dose), CD34 cell dose is the only pre transplant variable.
that can be manipulated. Half the patients in this study received a CD34 cell dose >2.7 x 10^5/kg and had a TRM of only 15% at 1 year. This compares favorably with a TRM of 24-51% reported for pediatric recipients of unrelated BMT (45-47). However, patients receiving UCB grafts that contained 1.7–2.7 x 10^5 and <1.7 x 10^5 CD34/kg had higher rates of TRM (29% and 68%, respectively). While an interaction between low cell dose and age >18 years may exist, too few patients in the adult age range prevent further analyses. These results underscore the argument that UCB units containing <1.7 x 10^5 CD34/kg should be considered inadequate for routine use.

Survival in this study is somewhat higher than that reported by registries (3,4,36). While direct comparisons with Registry data are difficult because differences are multifactorial, reflecting differences in eligibility criteria, treatment and supportive care plans and definitions of endpoints. Further investigations are underway as part of a national study sponsored by the National Heart, Lung and Blood Institute (48). Regardless, pediatric patients in this study had a high survival rate (71% and 63% for patients aged 0-1 and 2-17 years, respectively) with poorer results in adults (30%) at 1 year. As might be expected on the basis of results with unrelated donor BMT, HLA mismatch, lower cell doses, history of severe GVHD and history of prior autologous transplantation were all factors associated with poorer survival in univariate analysis. With HLA disparity and CD34 cell dose both being independent risk factors for survival, the major question is how to weigh the relative risks of HLA disparity and CD34 cell dose. The answer will not only aid the clinician in the instruction of prospective patients on the relative importance of UCB CD34 cell dose and HLA disparity but also in the development of a UCB graft selection algorithm. As previously suggested by Rubinstein et al (49), our results indicate that higher CD34 cell dose partially overcomes the negative impact of HLA for each level of HLA disparity. For example, in recipients of HLA 2 antigen disparate UCB grafts, patients transplanted with >1.7 x 10^5 CD34 cells/kg had a higher survival (0.61 [CI, 0.43-0.79], n=30)
than those with a lower cell dose (0.11 [CI, 0.00-0.32], n=9). For each degree of HLA disparity, data presented here indicate that there is a critical infused cell dose below which survival is significantly impaired, particularly in recipients of UCB units with HLA 2 antigen disparity. Resolution of this issue will require larger patient numbers.

As with unrelated donor BMT, it is important to note that the most common causes of death after UCB transplantation were infection and relapse. Due to the profound influence of CD34 cell dose on rate of neutrophil engraftment, TRM and survival, risk of infection is likely related due to the prolonged length of neutropenia in recipients of lower cell doses rather than an impairment of neutrophil function as has been suggested (31). While defects in neutrophil function could play a role, most recipients of UCB with an adequate cell dose do not die of infection. Relapse, however, was the major limiting factor. While decreased GVHD raised the concern that the graft-versus-leukemia (GVL) might be decreased, relapse after unrelated donor UCB transplantation is similar to that previously reported for unrelated BMT patients (46,47). While only a randomized trial with larger numbers of patients will answer the question of relative risk of relapse after UCB transplantation as compared to BMT, these results are similar to those reported by Locatelli et al (50) who similarly observed a 40% incidence of relapse in recipients with acute leukemia with disease status being the only risk factor.

Together, these results provide clear justification for the further development of UCB banks worldwide. Not only should banks focus on the collection of larger units with greater numbers of CD34+ cells but also UCB units from ethnic and racial minorities. Greater HLA disparity between donor and recipient adversely affects survival which has particular relevance for patients of ethnic minority descent. Targeting collection centers with large minority populations may help reduce the degree of HLA disparity for minority patients and serve as an important
adjunct to marrow donor registries worldwide. In addition, these results suggest that there
should be routine quantitation of CD34 cells preferably by UCB banks using a standardized
procedure between banks so that rapid decisions can be made as to the optimal unit for a specific
patient. While small units should not be routinely prescribed, such units might be made available
for phase I clinical trials of ex vivo expansion or multi unit UCB transplantation or when an
alternative hematopoietic stem cell source is not available.

In summary, we have demonstrated the importance of graft CD34 cell dose in determining
outcome after unrelated donor UCB transplantation. Even in recipients of HLA 2 antigen
disparate grafts, data would suggest that higher CD34 cell dose significantly impacts survival.
Therefore, these data suggest that the choice of UCB graft should be based primarily on CD34
cell dose and secondarily on degree HLA disparity. The tolerability of HLA-2 antigen disparate
grafts will likely increase the availability of HSC transplantation, particularly for patients with
infrequent HLA haplotypes. The importance of cell dose on transplant outcomes provide the
most compelling argument for focusing on the collection of larger UCB grafts and for
investigating ex vivo HSC expansion and transplantation of multiple UCB units in future clinical
trials.

ACKNOWLEDGEMENTS
We gratefully thank the many faculty, nurses, housestaff and clinical post-doctoral fellows of the
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families treated at the Fairview-University Medical Center, Minneapolis, Minnesota. We also
acknowledge Dr. Kathryn Chaloner, co-director of the Biostatistical Support Group for her
careful review of the manuscript.
Legends

Figure 1: Cumulative incidence of neutrophil engraftment after unrelated donor UCB transplantation (n=102): Effect of CD34 cell dose (x 10^5/kilogram recipient body weight).

Figure 2: Correlation between infused nucleated cell dose (x 10^7/kilogram recipient body weight) and thawed CD34 cell dose (x 10^5/kilogram recipient body weight).

Figure 3: Cumulative incidence of treatment-related mortality after unrelated donor UCB transplantation (n=102): Effect of CD34 cell dose (x 10^5/kilogram recipient body weight, Panel A) and effect of HLA disparity (Panel B). (mm=mismatch)

Figure 4: Cumulative incidence of relapse in recipients with ALL and AML stratified by disease risk. (HR=high risk; SR=standard risk)

Figure 5: Cumulative proportion surviving after unrelated donor UCB transplantation (n=102): Effect of CD34 cell dose (x 10^5/kilogram recipient body weight, Panel A) and effect of HLA disparity (Panel B). (mm=mismatch)
References


21. Petersdorf EW, Smith AG, Mickelson EM, Martin PJ, Hansen JA.  Ten HLA-DR4 alleles defined by sequence polymorphism within the DRB1 first domain.  Immunogenetics 1991; 33: 267-.


Table 1  Patient demographic and treatment characteristics

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient’s age (years)</td>
<td>7.4 (0.2 – 56.9)</td>
</tr>
<tr>
<td>Median (range)</td>
<td></td>
</tr>
<tr>
<td>Recipient’s weight (kg)</td>
<td>25.9 (5.0 – 107.5)</td>
</tr>
<tr>
<td>Median (range)</td>
<td></td>
</tr>
<tr>
<td>Recipient’s sex</td>
<td>60 (59%) /42 (41%)</td>
</tr>
<tr>
<td>Male/Female</td>
<td></td>
</tr>
<tr>
<td>Recipient’s race</td>
<td>80 (78%) /22 (22%)</td>
</tr>
<tr>
<td>Caucasian/Non-Caucasian</td>
<td></td>
</tr>
<tr>
<td>Recipient’s CMV serostatus</td>
<td>61 (60%) /41 (40%)</td>
</tr>
<tr>
<td>Negative/Positive</td>
<td></td>
</tr>
<tr>
<td>Recipient’s Diagnosis</td>
<td>28 (27%)</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>Malignant Disease</strong></td>
<td>8 CR1, 10 CR2, 6 &gt;CR3, 4 early relapse</td>
</tr>
<tr>
<td><strong>ALL</strong></td>
<td>14 (14%)</td>
</tr>
<tr>
<td><strong>AML</strong></td>
<td>5/6 antigens</td>
</tr>
<tr>
<td><strong>CML</strong></td>
<td>1 (1%)</td>
</tr>
<tr>
<td><strong>JMML</strong></td>
<td>3 (3%)</td>
</tr>
<tr>
<td><strong>NHL</strong></td>
<td>1 (1%)</td>
</tr>
<tr>
<td><strong>Hodgkins</strong></td>
<td>2 (2%)</td>
</tr>
<tr>
<td><strong>Non-Malignant Disease</strong></td>
<td>4 (4%)</td>
</tr>
<tr>
<td><strong>Severe Aplastic Anemia</strong></td>
<td>1 (1%)</td>
</tr>
<tr>
<td><strong>Fanconi Anemia</strong></td>
<td>3 (3%)</td>
</tr>
<tr>
<td><strong>Blackfan Diamond</strong></td>
<td>1 (1%)</td>
</tr>
<tr>
<td><strong>Osteopetrosis</strong></td>
<td>5 (5%)</td>
</tr>
<tr>
<td><strong>MDS (RA)</strong></td>
<td>21 (21%)</td>
</tr>
<tr>
<td><strong>Immune Deficiency</strong></td>
<td>6 (6%)</td>
</tr>
<tr>
<td><strong>Metabolic Disorders</strong></td>
<td>2 (2%)</td>
</tr>
</tbody>
</table>

**Treatment**

| TBI/no TBI | 87 (85%) / 15 (15%) |
| G-CSF/no G-CSF | 80 (78%) / 22 (22%) |

ALL=acute lymphocytic leukemia; AML=acute myelocytic leukemia; CML=chronic myelogenous leukemia (CP=chronic phase; AP=accelerated phase); JMML=juvenile myelomonocytic leukemia; NHL=non-Hodgkin lymphoma; SAA=severe aplastic anemia; FA=Fanconi anemia; BD=Blackfan Diamond syndrome; MDS (RA)=myelodysplastic syndrome (refractory anemia); TBI=total body irradiation; no TBI=any conditioning regimen without TBI; G-CSF=prophylactic granulocyte colony stimulating factor. * Maximal degree of HLA disparity (rejection/GVHD vector).
Table 2   Cox regression analyses of factors potentially associated with neutrophil engraftment, treatment-related mortality and survival after unrelated donor UCB transplantation.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>RELATIVE RISK (95% (CI)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neutrophil Recovery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34 dose (x 10^5/kg)</td>
<td>Model (X^2=25.6, p&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>&lt;1.7</td>
<td>1.0</td>
<td>0.14</td>
</tr>
<tr>
<td>1.7 – 2.7</td>
<td>1.7 (0.8 – 3.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2.8 – 5.4</td>
<td>2.6 (1.3 – 5.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>&gt;5.4</td>
<td>4.7 (2.2 – 9.8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Growth factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Yes</td>
<td>3.4 (0.8 – 2.1)</td>
<td></td>
</tr>
<tr>
<td>HLA match</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/6 and 5/6</td>
<td>1.0</td>
<td>NS</td>
</tr>
<tr>
<td>4/6</td>
<td>0.9 (0.6 – 1.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Acute GVHD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3 dose (x 10^6/kg)</td>
<td>Model (X^2=0.9, p=NS)</td>
<td></td>
</tr>
<tr>
<td>&lt;8</td>
<td>1.0</td>
<td>NS</td>
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<tr>
<td>≥8</td>
<td>0.7 (0.2 – 1.9)</td>
<td>NS</td>
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<tr>
<td>Age (by decade)</td>
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</tr>
<tr>
<td>1.0 (0.98 – 1.03)</td>
<td>NS</td>
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<tr>
<td>HLA match</td>
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<tr>
<td>6/6 and 5/6</td>
<td>1.0</td>
<td>NS</td>
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<tr>
<td>4/6</td>
<td>1.2 (0.5 – 2.7)</td>
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<tr>
<td><strong>Treatment-related mortality</strong></td>
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</tr>
<tr>
<td>CD34 dose (x 10^5/kg)</td>
<td>Model (X^2=29.2, p&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>≤1.7</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>&gt;1.7</td>
<td>0.2 (0.1 – 0.5)</td>
<td>0.03</td>
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<tr>
<td>Age (by decade)</td>
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<tr>
<td>1.3 (1.0 – 1.6)</td>
<td>NS</td>
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<tr>
<td>Grade III-IV acute GVHD</td>
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</tr>
<tr>
<td>No</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Yes</td>
<td>4.4 (1.7 – 11.4)</td>
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<tr>
<td>HLA match</td>
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<tr>
<td>6/6 and 5/6</td>
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<td>4/6</td>
<td>1.6 (0.6 – 3.9)</td>
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<td><strong>Survival</strong></td>
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<td>CD34 dose (x 10^5/kg)</td>
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<tr>
<td>≤1.7</td>
<td>1.0</td>
<td>&lt;0.01</td>
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<tr>
<td>&gt;1.7</td>
<td>0.3 (0.1 – 0.5)</td>
<td>&lt;0.01</td>
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<tr>
<td>Grade III-IV acute GVHD</td>
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<tr>
<td>No</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Yes</td>
<td>3.5 (1.5 – 7.9)</td>
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<tr>
<td>6/6 and 5/6</td>
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<td></td>
</tr>
<tr>
<td>4/6</td>
<td>2.4 (1.2 – 4.7)</td>
<td>0.01</td>
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</table>
Table 3   Clinical grade of acute GVHD by organ system

<table>
<thead>
<tr>
<th>SEVERITY</th>
<th>SKIN</th>
<th>UPPER GI</th>
<th>LOWER GI</th>
<th>LIVER</th>
<th>OVERALL</th>
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<tr>
<td>No GVHD</td>
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<td></td>
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<tr>
<td>Grade 1</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Grade 2</td>
<td>23</td>
<td>7</td>
<td>3</td>
<td>0</td>
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</tr>
<tr>
<td>Grade 3</td>
<td>7</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>8</td>
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<tr>
<td>Grade 4</td>
<td>3</td>
<td>1</td>
<td>3</td>
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<td>3</td>
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<tr>
<td>Total</td>
<td>57</td>
<td>13</td>
<td>14</td>
<td>5</td>
<td>102</td>
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Transplantation of unrelated donor umbilical cord blood in 102 patients
with malignant and non-malignant diseases: influence of CD34 cell dose
and HLA disparity on treatment-related mortality and survival

John E Wagner, Juliet N Barker, Todd E DeFor, K S Baker, Bruce R Blazar, Cindy Eide, Anne Goldman,
John Kersey, William Krivit, Margaret MacMillan, Paul J Orchard, Charles Peters, Daniel J Weisdorf,
Norma K Ramsay and Stella M Davies