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Transplantation of Two Partially HLA-Matched Umbilical Cord Blood Units To Enhance Engraftment in Adults with Hematologic Malignancy

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Abstract

Limited umbilical cord blood (UCB) cell dose compromises the outcome of adult UCB transplantation. Therefore, to augment graft cell dose, we evaluated the safety of the combined transplantation of two partially HLA-matched UCB units. Twenty-three patients with high-risk hematologic malignancy [median age 24 years (range: 13-53)] received two UCB units [median infused dose 3.5 x 10^7 NC/kg (range 1.1-6.3)] after myeloablative conditioning. All evaluable patients (n = 21) engrafted at a median of 23 days (range 15-41). At day 21, engraftment was derived from both donors in 24% and a single donor in 76%, with one unit predominating in all patients by day 100. While neither nucleated or CD34+ cell dose, nor HLA-A,B,DRB1 match, predicted which unit would predominate, the predominating unit had a significantly higher CD3+ dose ($p < 0.01$). Incidences of grade II-IV and III-IV acute GVHD were 65% (95%CI: 42-88%) and 13% (95%CI: 0-26%), respectively. Disease-free survival was 57% (95%CI: 35-79) at one year, with 72% (95%CI: 49-95) of patients alive if transplanted in remission. Therefore, transplantation of 2 partially HLA-matched UCB units is safe, and may overcome the cell dose barrier that limits the use of UCB in many adults and adolescents.

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Introduction

Unrelated donor umbilical cord blood transplantation (UCBT) has become a standard therapeutic option for pediatric patients with hematological malignancies[1-5]. Compared to unrelated donor bone marrow (BM), UCB has the advantages of rapid availability[6], and low risk of severe acute graft-versus-host disease (GVHD) despite donor-recipient HLA disparity[1-5, 7, 8]. However, UCBT in adults is severely limited by graft cell dose[5, 9]. For example, Laughlin et al found that cryopreserved nucleated cell (NC) dose is a major determinant of neutrophil recovery and that higher CD34+ cell dose is associated with improved survival in adult UCB recipients[9]. Further, Wagner et al have observed that recipients of UCB with infused cell doses of $< 1.8 \times 10^7$ NC/kg or $< 1.7 \times 10^5$ CD34+ cells/kg recipient body weight, have a markedly inferior engraftment and survival[5].

Therefore, we investigated the safety and efficacy of UCBT using UCB units from 2 partially HLA-matched donors as a method of augmenting cell dose in high-risk adults and adolescents with hematological malignancies. While bi-directional immune rejection could potentially lead to graft failure from either donor unit, we hypothesized that double unit UCBT may enhance the availability of UCBT for adults and be associated with improved incidence of engraftment and more rapid neutrophil recovery. The primary end-point of this phase I/II study was safety as measured by donor-derived neutrophil engraftment.

Methods

Patients were eligible for UCBT if they had no 5-6/6 HLA-A,B,DRB1 matched related donor, and no suitable volunteer donor available. UCB was given priority over volunteer donors for patients requiring transplant within 3 months of referral. Patients were eligible for double unit UCBT if no single 4-6/6 HLA-A,B,DRB1 matched UCB unit of adequate cell dose was available. From January 2000 to December 2001, all adult patients without a single unit containing a cryopreserved dose of at least 2.5×10^7 NC/kg were eligible for double unit UCBT in a phase I/II prospective study. In January 2002, as safety with double unit UCBT had been demonstrated, in an effort to increase graft cell dose for all UCBT recipients this cell dose threshold was raised such that all adults without a single unit containing a cryopreserved dose of at least 3.5×10^7 NC/kg were eligible for double unit UCBT. The treatment plan was reviewed and approved by the University of Minnesota Institutional Review Board. Written informed consent was obtained from all patients prior to transplantation.

UCB Unit Selection

UCB units were obtained from the New York Blood Center (n = 13), the St. Louis Cord Blood Bank (n = 19), the American Red Cross and other UCB banks of the National Marrow Donor Program (NMDP) (n = 8) and Netcord (n = 6). Since the goal in graft selection was to maximize the total NC dose of the graft, the largest available UCB unit that was 4-6/6 HLA-A,B,DRB1 matched to the recipient was selected (UCB#1). UCB#2 had to be both 4-6/6 HLA-A,B,DRB1 matched to the recipient *and* to UCB#1. The minimum allowed cryopreserved graft cell dose was 1.5×10^7 NC/kg (UCB#1 $\geq 1.0 \times 10^7$

NC/kg; UCB#2 $\geq$ 0.5 x 10^7 NC/kg). HLA disparity between each unit and the recipient, and between the two units, were not necessarily at the same loci.

HLA Typing

Prior to 8/03 HLA-A,B typing was performed using the standard two-stage complement-dependent microcytotoxicity assay, and antigens were assigned as defined by the World Health Organization HLA nomenclature committee. From 8/03 HLA-A,B antigen typing methodology was changed to using a reverse polymerase chain reaction (PCR)/ sequence specific oligonucleotide method using a Luminex flow cytometer and bead technology. HLA-DRB1 allele typing was determined using PCR and sequence specific primer technology (One Lambda, Inc).

Treatment Plan

All patients received myeloablative conditioning using cyclophosphamide 120 mg/kg (60 mg/kg/day on at 1000am on days –7 and –6 for 2 doses), and total body irradiation (1320 cGy in 8 fractions) and immunosuppression with cyclosporine-A (CSA) from day –3 for at least 6 months. In addition, the first 2 patients received anti-thymocyte globulin (ATGAM, Pharmacia) 15 mg/kg every 12 hours on days -3 to –1 and methylprednisone (MP) 1 mg/kg every 12 hours days 5-19. As part of an institutional practice change for all adult UCB recipients, all subsequent patients (n = 21) received low dose fludarabine 75 mg/m^2 (25 mg/m^2/day at 900am on days –8 to – 6 for 3 doses) and mycophenolate mofetil 1 gm twice daily from days –3 to +30 as a substitute for the ATG and MP. UCB units were thawed using the method of Rubinstein et al[10], and infused in series after hydration and pre-medication of acetaminophen and diphenhydramine. Granulocyte-colony stimulating factor (5 µg/kg/day) was administered to all patients from day 1 until achieving an absolute neutrophil count (ANC) $\geq$ 2.5 x 10^9/l for 2 consecutive days.

Donor Chimerism Analysis

Donor chimerism was determined serially on BM and/or blood at days 21, 60, 100, 180, 360, and 720 after transplant, with additional time points as needed. Method of analysis was quantitative PCR of informative polymorphic variable number tandem repeat (VNTR) or short tandem repeat (STR) regions in the recipient and donor[11, 12]. Blood was separated into neutrophil and mononuclear fractions provided the total WBC was > 1.0 x 10^9/L. DNA was amplified with fluorescent PCR primers for markers found to distinguish the 2 donor and recipient alleles. Fluorescent PCR products were separated using an Applied Biosystems 373 Sequencer or an Applied Biosystems 3100 Genetic Analyzer and GeneScan software (Applied Biosystems, Foster City, CA) was used to correlate allele peak areas to the percentage of donor or recipient DNA (accuracy +/- 5%). One VNTR/STR marker could distinguish the recipient and donors in 90% of cases. For remaining patients, analysis of 2 different VNTR was required.

Statistical Analysis

The primary end-point was safety as measured by the incidence of donor-derived neutrophil engraftment (by one or both donor units). Secondary end-points included the incidences of platelet engraftment, acute and chronic GVHD, relapse, and transplant-

related mortality, and the probability of survival as well as a description of the contribution of each unit to engraftment over time. Time of neutrophil recovery was designated as the first of 3 consecutive days with an ANC above 0.5 x 10^9/l. Sustained donor engraftment was defined as neutrophil recovery with both myeloid and lymphoid donor hematopoiesis beyond day +42 without secondary graft failure. Complete chimerism was defined as marrow reconstitution of donor origin of $\geq$ 90%. Two patients with refractory AML were censored from the engraftment analysis due to death from infection prior to day 28 after transplant. The first patient died on day 13 without neutrophil recovery. The second patient, who died on day 25, had neutrophil recovery but the assessment of engraftment status was complicated by the fact that granulocyte transfusions were administered prior to death and a day 21 BM biopsy was not performed to assess donor chimerism due to the severity of the patient's illness.

Diagnosis of GVHD was based on standard clinical criteria with histopathological confirmation when possible[13]. Patients were evaluated for acute GVHD daily during hospitalization, at least weekly after discharge to 100 days, and at least every 3 months thereafter. GVHD grading was assigned retrospectively by independent review. The cumulative incidence of engraftment and GVHD was calculated by treating deaths from other causes as competing risks[14]. In order to compare the effect of cell dose and viability of each UCB unit on unit predominance, a matched paired t-test was performed whereas z-score test statistics for normalized data were used to test the association between ABO blood group, sex match and order of unit infusion and unit predominance[15]. The statistical endpoints of survival and disease-free survival were estimated by the Kaplan-Meier method[16]. Event times for survival were measured from transplantation day to date of death or last contact. Median follow-up of survivors is 10 months (3.5 months to 2.5 years). The analysis was performed as of March 15, 2004.

Results

UCB Graft Availability

During the study period 26 patients met eligibility criteria for double UCBT using myeloablative conditioning. Three patients (12%) received a single unit graft due to inability to identify a suitable second unit.

Patient and Graft Characteristics

Twenty-three consecutive patients [median age 24 years (range: 13-53); weight 73 kg (range: 48-120); 57% male; 61% cytomegalovirus antibody positive] were transplanted with two UCB units between January 2000 and October 2003. All patients were considered high-risk for relapse except a single patient with chronic myelogenous leukemia in chronic phase who was refractory to high-dose imatinib and cytarabine. Diagnoses are detailed in Table 1. The median total cryopreserved graft cell dose was 4.8 x 10^7 NC/kg (range 1.6-7.0). Infused graft cell doses are summarized in Table 1. Only 2 patients (9%) received a graft that contained at least one 6/6 HLA-A,B,DRB1 matched unit to the recipient; eleven patients (48%) received a graft with at least one 5/6 HLA matched unit; and ten patients (43%) received a graft with both units being 4/6 HLA matched to the recipient. The units were 6/6 HLA-antigen mismatched to each other in 2 patients, 5/6 in 5, 4/6 in 16.

Double Unit Infusion

No major adverse events resulted from double unit infusion. Fourteen patients had no symptoms, while 3 had nausea and/or emesis, 5 mild to moderate hypertension, 1 dizziness and 1 lip tingling within 2 hours of infusion.

Hematopoietic Recovery and Chimerism

All of the 21 evaluable patients had sustained donor neutrophil engraftment at a median of 23 days (range 15-41) (Figure 1A). All of these 21 patients demonstrated complete donor chimerism with no patient having secondary graft failure. By day 180 after transplant, the cumulative incidence of platelet engraftment to $\geq$ 50 x 10^9/L was 71% (95%CI: 47-95%) with a Kaplan-Meier estimate of 90% (95%CI: 73-100) (Figure 1B).

Serial assessments of the contribution of each unit to hematopoiesis were made beginning at day 21. Of the 21 evaluable patients, hematopoiesis in day 21 BM was accounted for by one donor in 16 (76%) [median donor chimerism 100% (range 73-100)]. While the remaining 5 patients (24%) had engraftment of both donors [median total donor chimerism 91% (range 64-100)], one unit predominated [median 74% (range 42-85) versus 20% (range 15-40) for the non-sustained unit]. Skewed engraftment progressed such that evidence of double unit hematopoiesis was observed in only 2 patients at day 60, and in none of the evaluable patients by day 100 (n = 17, with 2 of the 21 engrafting patients having died from relapse, 1 from pulmonary hemorrhage and 1 from aspergillus infection). Once engraftment was derived from one unit, the other unit never contributed to hematopoiesis at subsequent time points.

Influence of Cell Dose and HLA Disparity on Engraftment

The relative percent viability, infused cell dose and donor-recipient HLA disparity of the UCB units were examined as potential factors influencing which unit would predominate. The median percent viability (as measured by acridine orange and propidium iodide fluorescent dyes) of the predominating unit was 60 (range 51-88) as compared to 61 (range 44-90) in the non-sustained unit (p = 0.39). The median infused cell doses of the predominating unit were 1.8 x 10^7 NC/kg (range 0.7-3.6), 1.5 x 10^5 CD34+/kg (range 0.6-10.4) and 1.9 x 10^4 CFU-GM/kg (range 0.2-4.7). This compared to 1.9 x 10^7 NC/kg (range 0.6-2.9) (p = 0.32), 2.5 x 10^5 CD34+/kg (range 0.5-9.1) (p = 0.93), and 2.0 x 10^4 CFU-GM/kg (0.08-5.7) (p = 0.64) in the non-sustained unit. Notably, in 6 patients the infused CD34+ cell dose of the predominating unit was very low at $\leq$ 1.0 x 10^5/kg (range: 0.6-1.0). In contrast to these non-predictive graft parameters, a higher CD3+ cell dose was associated with the UCB unit that would predominate. The median infused CD3+ cell dose of the predominating unit was 0.6 x 10^7 CD3+/kg (range 0.3-1.1) as compared to 0.4 x 10^7 CD3+/kg (range 0.1-1.3) (p < 0.01) in the non-sustained unit (Figure 2).

Only 8 of the 21 patients with donor engraftment received two UCB units with different degrees of HLA disparity. Of these, the better HLA-matched unit to the recipient predominated in 4 patients, while lesser matched units engrafted over the better matched units in 4 patients. The location of the HLA mismatch (HLA-A and B versus HLA-DRB1 disparity) was not associated with engraftment. Other factors such as ABO

blood group (p = 0.33) and sex match (p = 0.39) failed to demonstrate any relationship with unit predominance. In 6 patients the unit infused first predominated in engraftment whereas in 15 the second unit predominated (p = 0.09).

GVHD and Survival

Incidences of grade II-IV and III-IV acute GVHD were 65% (95%CI: 42-88) and 13% (95%CI: 0-26%), respectively (Figure 3). Two patients had grade III acute GVHD, one involving skin and gut, and one skin and liver. One patient had grade IV acute GVHD affecting the skin only. The 3 patients with grade III-IV acute GVHD were treated with topical corticosteroids and CSA. In addition, 1 received ATG 15 mg/kg every 12 hours for 10 doses and oral prednisone 60 mg/m^2 for 2 weeks with a subsequent taper whereas 2 were enrolled in a blinded randomized study of corticosteroids (methylprednisolone 2 mg/kg days 0-6 with subsequent taper) and daclizumab versus corticosteroids and placebo. All responded to immunosuppression. Five patients have had chronic GVHD (all extensive) for a cumulative incidence of 23% (95%CI: 6-40). Six month transplant-related mortality was 22% (95%CI: 5-39).

With a median follow-up of 10 months (range: 3.5 months-2.5 years), the probability of disease-free survival at 1 year is 57% (95%CI: 35-79). One year disease-free survival for patients transplanted with acute leukemia in remission or with CML (n = 15) versus that for patients with acute leukemia transplanted in relapse or with recurrent MDS (n = 8) is 72% (95%CI: 49-95) versus 25% (95%CI: 0-64%), respectively (p = 0.04) (Figure 4). Causes of death were infection (n = 3), organ failure (n = 2), hemorrhage (n = 1), and relapse (n = 3).

Discussion

As early as 1969 Mathé et al reported the combined transplantation of BM from different donors as a method of increasing graft cell dose[17]. Since the introduction of UCB, and the demonstration of the critical importance of cell dose on UCBT outcome, there has been renewed interest in the augmentation of graft cell dose[18-19]. Zanjani et al have investigated the transplantation of human UCB from multiple donors in a fetal sheep model[18]. Enhanced short-term donor engraftment derived from both donors was demonstrated, although long-term hematopoiesis was derived from a single donor. Further, Chen et al have demonstrated that combining hematopoietic progenitors from two different HLA-mismatched T cell depleted murine BM donors had beneficial effects on myeloid engraftment in murine recipients[20].

Case reports of multi-donor UCBT using up to 12 units have been published[21-24]. The study by Weinreb et al suggested that the unit that was partially HLA-matched would predominate, at least short-term[23]. More recently, De Lima et al demonstrated successful engraftment of both donors in a double unit transplant recipient with advanced acute leukemia[24]. In this study, we have systematically assessed the safety of myeloablative UCBT using two partially matched units with a minimum total cryopreserved graft cell dose of 1.5 x 10^7 NC/kg, and evaluated post-transplant donor chimerism by molecular techniques to document the contribution of each unit to hematopoiesis.

These data demonstrate the feasibility and safety of double unit UCBT. An acceptable double unit graft can be identified for the majority of patients. All evaluable

patients achieved sustained donor engraftment with a low incidence of severe acute GVHD despite HLA-disparity. Further, there was no apparent increase in acute GVHD incidence as compared to single unit UCBT[5, 9]. Interestingly, sustained hematopoiesis was derived from a single donor which predominated as early as 3 weeks after transplant. While the mechanisms that determine the fate of each donor are not known, it is notable that of the factors analyzed only a larger CD3+ cell dose was associated with which unit predominated. Recently, Kim et al have reported on the outcome of double unit UCBT in immunodeficient mice[19]. These investigators found one unit predominated when using unfractionated grafts, whereas depletion of lineage committed cells resulted in sustained double unit engraftment. These data suggest a graft-versus-graft reaction was responsible for single donor predominance. The results of our study would support the hypothesis that donor predominance is immune mediated.

Whether the transplantation of two UCB units improves engraftment as compared with single unit UCBT is not yet known. However, the fact that all evaluable patients engrafted at a median of 23 days in this study is a higher engraftment rate than previously observed by our group[5] and other studies in the literature[9, 25-27]. Prior to double unit UCBT only 30% of adult referrals to the University of Minnesota were eligible for UCBT on the basis of having a single unit with an adequate cell dose. Further, of those patients who proceeded to single unit UCBT, only 72% (95%CI: 51-93) engrafted at a median of 34 days (range 17-54) if the infused cell dose was $< 1.7 \times 10^5$ CD34/kg[5]. In contrast, a double unit graft may be identified for the majority of patients and this approach has been associated with all evaluable patients engrafting despite the low infused CD34+ cell dose (median 1.5×10^5/kg) in the predominating unit. While further study is required to elucidate the biology of double unit UCBT, we speculate that the non-sustained unit may facilitate the engraftment of the predominating unit by immunologic mechanisms.

Double unit UCBT extends access to transplant to many patients who were previously disqualified on the basis of the available cell dose in a single unit. The high engraftment rate and low incidence of severe acute GVHD has resulted in a relatively low transplant-related mortality. Therefore, further investigation of this approach in the context of larger clinical trials is indicated to determine the full impact of double unit UCBT on transplant outcome in adults and larger adolescents.

References

1. Kurtzberg J, Laughlin M, Graham ML, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med. 1996; 335:157-166.
2. Gluckman E, Rocha V, Boyer-Chammard A, et al. Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. N Engl J Med. 1997; 337:373-381.
3. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N Engl J Med. 1998; 339:1565-1577.
4. Rubinstein P, Stevens CE. Placental blood for bone marrow replacement: the New York Blood Center's program and clinical results. Baillieres Best Pract Res Clin Haematol. 2000; 13:565-584.
5. Wagner JE, Barker JN, DeFor TE, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood 2002; 100:1611-1618.
6. Barker JN, Krepski TP, DeFor TE, Davies SM, Wagner JE, Weisdorf DJ. Searching for Unrelated Donor Hematopoietic Stem Cells: Availability and Speed of Umbilical Cord Blood versus Bone Marrow. Biol Blood Marrow Transplant. 2002; 8:257-260.
7. Rocha V, Cornish J, Sievers EL, et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. Blood. 2001; 97:2962-2971.
8. Barker JN, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. Blood. 2001; 97:2957-2961.
9. Laughlin MJ, Barker J, Bambach B, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. N Engl J Med. 2001; 344:1815-1822.
10. Rubinstein P, Dobrila L, Rosenfield RE, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci U S A. 1995; 92:10119-10122.
11. Scharf SJ, Smith AG, Hansen JA, McFarland C, Erlich HA. Quantitative determination of bone marrow transplant engraftment using fluorescent polymerase chain reaction primers for human identity markers. Blood. 1995; 85:1954-1963.
12. Schichman SA, Suess P, Vertino AM, Gray PS. Comparison of short tandem repeat and variable number tandem repeat genetic markers for quantitative determination of allogeneic bone marrow transplant engraftment. Bone Marrow Transplant. 2002; 29:243-248.
13. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974; 18:295-304.

14. Lin DY. Non-parametric inference for cumulative incidence functions in competing risks studies. Stat Med. 1997; 16:901-910.
15. Snedecor G, Cochran W. Statistical Methods: Iowa State University Press, 1989.
16. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958; 53:457-481.
17. Mathe G, Amiel JL, Schwarzenberg L, et al. Bone marrow transplantation in man. Transplant Proc. 1969; 1:16-24.
18. Zanjani E, Almeida-Porada G, Hangoc G, Broxmeyer HE. Enhanced Short Term Engraftment of Human Cells in Sheep Transplanted with Multiple Cord Bloods: Implications for Transplantation of Adults. Blood. 2000; 96:552a.
19. Kim DW, Chung YJ, Kim TG, Kim YL, Oh IH. Cotransplantation of third-party mesenchymal stromal cells can alleviate single-donor predominance and increase engraftment from double cord transplantation. Blood. 2004; 103:1941-1948.
20. Chen BJ, Cui X, Chao NJ. Addition of a second, different allogeneic graft accelerates white cell and platelet engraftment after T-cell-depleted bone marrow transplantation. Blood. 2002; 99:2235-2240.
21. Ende M, Ende N. Hematopoietic transplantation by means of fetal (cord) blood. Virginia Med J. 1972; 99:276-280.
22. Shen BJ, Hou HS, Zhang HQ, Sui XW. Unrelated, HLA-mismatched multiple human umbilical cord blood transfusion in four cases with advanced solid tumors: Initial studies. Blood Cells. 1994; 20:285-292.
23. Weinreb S, Delgado J, Clavijo O, et al. Transplantation of unrelated cord blood cells. Bone Marrow Transplantation. 1998; 22:193-196.
24. De Lima M, St John LS, Wieder ED, et al. Double-chimaerism after transplantation of two human leucocyte antigen mismatched, unrelated cord blood units. Br J Haematol. 2002; 119:773-776.
25. Cornetta K, Laughlin M, Carter S, et al. Umbilical Cord Blood Transplantation in Adults: Results of a Prospective, Multi-Institutional, NHBLI Sponsored Trial. Blood. 2002; 100:42a.
26. Rocha V, Labopin M, Frassoni F, et al. Results of Unrelated Cord Blood Versus Unrelated Bone Marrow Transplant in Adults with Acute Leukemia. A Matched Pair Analysis. Blood. 2002; 100:42a.
27. Laughlin M, Rubinstein P, Stevens C, et al. Comparison of Unrelated Cord Blood and Unrelated Bone Marrow Transplants for Leukemia in Adults: A Collaborative Study of the International Bone Marrow Transplant Registry and the New York Blood Center. Blood. 2003; 102:244a.

Table 1: Diagnosis and graft characteristics.

N	23
Diagnosis	
ALL	
CR1	4 (17%)
CR2	1 (4%)
Relapse	3 (13%)
AML*	
CR1	4 (17%)
CR2	3 (13%)
CR3	1 (4%)
MDS	1 (4%)
Relapse	4 (17%)
CML	
CP1	1 (4%)
AP1	1 (4%)
Infused NC x 10^7/kg	
Total	3.5 (1.1-6.3)
Larger Unit	1.9 (0.6-3.6)
Smaller Unit	1.5 (0.5-2.7)
Infused CD34+ x 10^5/kg	
Total	4.9 (1.2-14.5)
Larger Unit	2.9 (0.7-10.4)
Smaller Unit	1.4 (0.5-4.7)
Infused CD3+ x 10^7/kg	
Total	1.0 (0.5-2.2)
Larger Unit	0.6 (0.3-1.3)
Smaller Unit	0.4 (0.1-0.9)

* Patients with AML in 1st CR had disease secondary to prior myelodysplasia. MDS refers to recurrent MDS post-AML induction.

Cell doses are given as median (range).

Abbreviations: ALL = acute lymphoblastic leukemia/ CR = complete remission/ AML = acute myelogenous leukemia/ MDS = myelodysplasia/ CML = chronic myelogenous leukemia/ CP = chronic phase/ AP = accelerated phase/ NC = nucleated cell.

Figure Legends

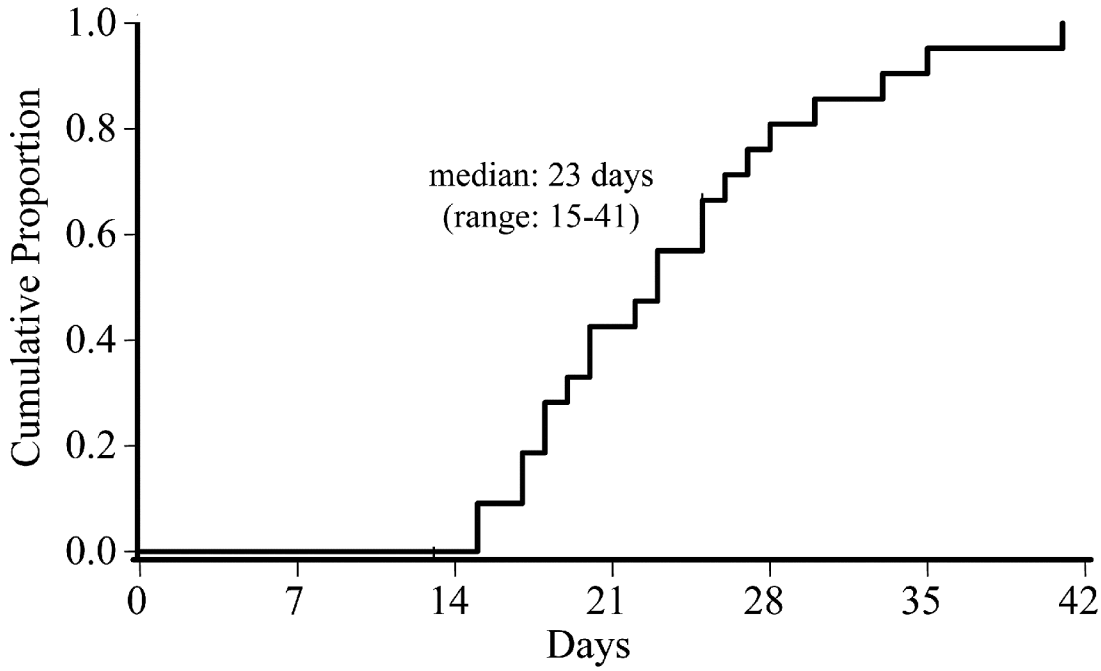
Figure 1A: Cumulative incidence of sustained donor neutrophil engraftment (ANC > 0.5 x 10^9/l) after double unit UCBT. The Kaplan-Meier estimate of neutrophil engraftment was identical to the cumulative incidence.

Figure 1B: Cumulative incidence and Kaplan-Meier estimates of platelet engraftment ($\geq$ 50 x 10^9/L) after double unit UCBT.

Figure 2: Comparison of the infused CD3+ dose of the unit predominating in donor engraftment (y axis) versus the non-sustained unit (x axis). The solid line is a 1:1 reference. The CD3+ cell dose of the predominating unit was significantly greater than that of the non-sustained unit.

Figure 3: Cumulative incidence of grade II-IV and III-IV acute GVHD after double unit UCBT.

Figure 4: Kaplan-Meier probability of disease-free survival after double unit UCBT according to disease status. Patients with acute leukemia in remission or with chronic myelogenous leukemia had a significantly better survival than those patients with acute leukemia transplanted in relapse or secondary AML patients with recurrent myelodysplasia.



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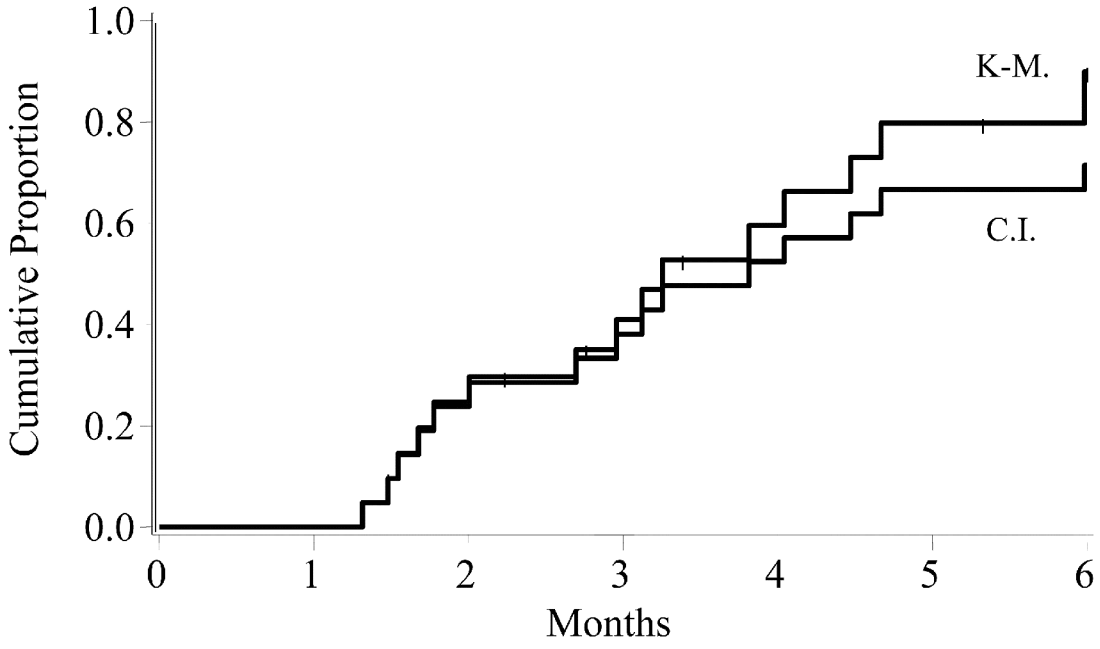

Figure 1B: Cumulative incidence and Kaplan-Meier estimates of platelet engraftment ($\geq$ 50 x 10^9/L) after double unit UCBT.

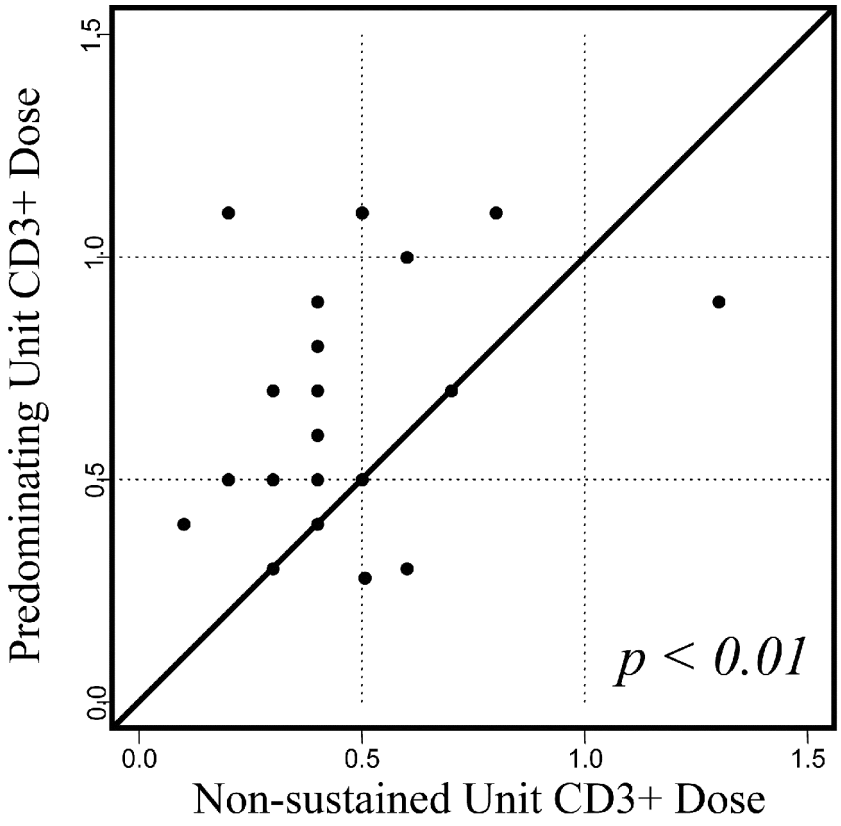

Figure 2: Comparison of the infused CD3+ dose of the unit predominating in donor engraftment (y axis) versus the non-sustained unit (x axis). The solid line is a 1:1 reference. The CD3+ cell dose of the predominating unit was significantly greater than that of the non-sustained unit.

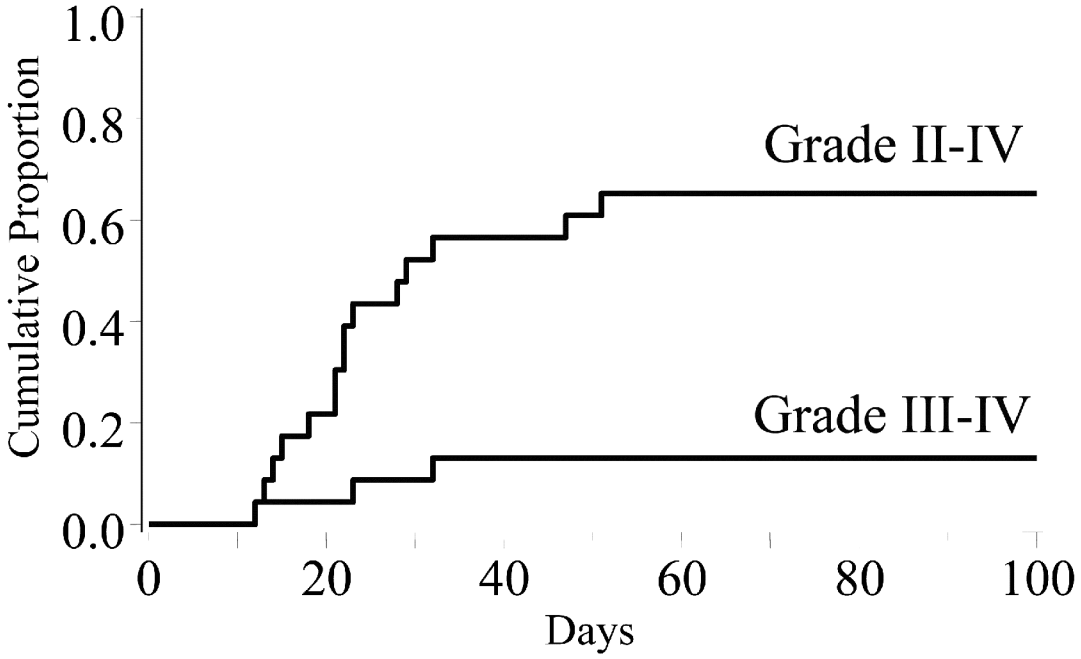

Figure 3: Cumulative incidence of grade II-IV and III-IV acute GVHD after double unit UCBT.

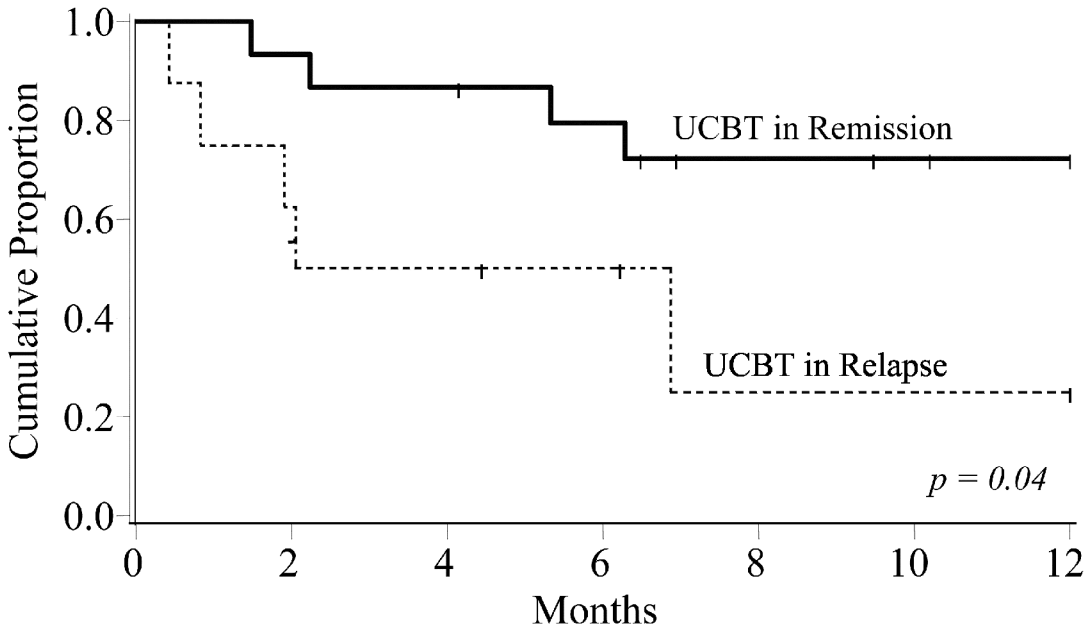

Figure 4: Kaplan-Meier probability of disease-free survival after double unit UCBT according to disease status. Patients with acute leukemia in remission or with chronic myelogenous leukemia had a significantly better survival than those patients with acute leukemia transplanted in relapse or secondary AML patients with recurrent myelodysplasia.