

ORIGINAL ARTICLE

Transplantation of unrelated donor umbilical cord blood utilizing double-unit grafts for five teenagers with transfusion-dependent thalassemia

T-H Jaing¹, C-P Yang¹, I-J Hung¹, S-H Chen¹, C-F Sun² and R Chow³

¹Divisions of Hematology and Oncology, Department of Pediatrics, Chang Gung Children's Hospital, Chang Gung University, Taoyuan, Taiwan; ²Department of Clinical Pathology, Chang Gung Memorial Hospital, Taoyuan, Taiwan and ³StemCyte Taiwan National Cord Blood Center, Linko, Taiwan

To augment graft cell dose, we evaluated the safety of the combined transplantation of two partially HLA-matched umbilical cord blood (UCB) units. Five patients with transfusion-dependent thalassemia, median age 11.1 years (range 10–13.1), received 2 UCB units after myeloablative conditioning. Cord blood units were a 4/6-HLA-match or better with the recipient, and contained a minimum combined pre-freeze CD34 cell dose of 3.0×10^5 /kg. All patients engrafted at a median of 15 days (range 12–19). Four patients with durable trilineage engraftment showed acute grade I–III GVHD; none developed extensive chronic GVHD until the date of last contact. The median times to red blood cell transfusion independence and platelet engraftment were 32 and 49 days after transplant, respectively. With a median follow-up of 18.5 months (range 11–32), four patients transplanted with UCB from two different partially HLA-matched donors were transfusion-independent. Therefore, transfusion of two partially HLA-matched UCB units is safe, and may overcome the cell-dose barrier that limits the use of UCB in long-term recipients of multiple transfusions for thalassemia.
Bone Marrow Transplantation (2007) **40**, 307–311; doi:10.1038/sj.bmt.1705737; published online 18 June 2007
Keywords: cell dose; double-unit cord blood transplantation; enhanced engraftment; teenager; transfusion-dependent thalassemia

Introduction

SCT still remains the only cure currently available for patients with thalassemia.^{1–4} Umbilical cord hematopoietic stem cells are increasingly used as an alternative to bone marrow; advantages include ready availability, no risk to

the donor, low rate of viral contamination and low risk of GVHD. Disadvantages include low stem-cell dose for larger patients and lack of stem cells for boost infusions following the initial procedure. In multiply transfused patients, rejection of donor cells was a major clinical problem due to sensitization of transplant candidates to HLAs via blood transfusions. We hypothesized that two partially matched umbilical cord blood (UCB) units could facilitate engraftment without crossed immunological rejection. We discuss the newer strategies being pursued to improve the safety and efficacy of double-unit UCB transplantation (UCBT).

Patients and methods

Patients

Five patients with transfusion-dependent thalassemia underwent transplantation with two unrelated UCB units at Chang Gung Children's Hospital between August 2004 and August 2006. The thalassemia patients were diagnosed before or around 1 year of age and then received regular monthly transfusion. All but one of our patients received more than 100 transfusions. No patient was splenectomized before transplantation. The diagnosis of each transfusion-dependent thalassemia participant was confirmed by DNA sequencing. Patients included in this study had to be over 10 years of age, and did not have donor relatives with acceptable HLA compatibility. After written informed consent was given by their caregivers, five teenagers were eligible for double-unit UCBT because no single 5-6/6 HLA-A, B, DRB1-matched UCB unit was available that had total nucleated cells of $>3.0 \times 10^7$ /kg and pre-freeze CD34 dose $>2.0 \times 10^5$ cells/kg. We did not assess risk in our patients according to the Pesaro risk classification because we did not routinely perform pretransplantation liver biopsy. The median age of the recipients was 11.1 years (range 10–13.1 years) with weight between 29.5 and 36.5 kg.

Cord blood selection and characteristics

Units were considered initially if their HLA-A, -B, and -DR phenotypes matched at least four of the patient's submitted six antigen HLA phenotypes at a serologic or intermediate-

Correspondence: Dr T-H Jaing, Divisions of Hematology and Oncology, Department of pediatrics, Chang Gung University and Children's Hospital, 5 Fu-Shin Street, Kwei-Shan, 333, Taoyuan, Taiwan.
E-mail: jaing001@cgmh.org.tw
Received 4 January 2007; revised 1 May 2007; accepted 2 May 2007; published online 18 June 2007

DNA level of resolution. The compatibility of HLA-A, -B, and -DRB1 was further confirmed by high-resolution PCR technique with sequence-specific primers (PCR-SSP) (GVHD mismatches were not included, and only one HLA-DRB1 single-allele mismatch was allowed). HLA disparity between each unit and the recipient and between two units did not necessarily have to be at the same loci. The total graft nucleated cell dose had to exceed $4.0 \times 10^7/\text{kg}$, with one unit containing a minimum cell dose of $2.0 \times 10^7/\text{kg}$. Each unit was required to have a dose of cryopreserved CD34 cells of at least 1.5×10^5 cells/kg, such that the total CD34 cell dose was 3.0×10^5 cells/kg or more. Searches for unrelated cord blood donors were processed through the StemCyte Cord Blood Bank, where 12 000 cord blood units are available locally in Taiwan.

Conditioning regimen and transplantation procedure

Before the UCB transplant procedure, all patients were placed in a high-efficiency particulate air-filtered room in the bone marrow transplantation unit. The preparative regimens consisted of oral busulfan 3.5 mg/kg/day (day -9 to -6), intravenous cyclophosphamide 50 mg/kg/day (day

-5 to -2) and antithymocyte globulin (Pharmacia-Upjohn, Peapack, NJ, USA) 30 mg/kg/day (day -4 to -1). During treatment, patients received phenytoin for prophylaxis against seizures. Mesna (50 mg/kg) was administered intravenously on the days of cyclophosphamide infusion. GVHD prophylaxis comprised cyclosporine (CsA) (2.5 mg/kg intravenously every 8 h) from day -3 with a course of methylprednisolone (1 mg/kg intravenously every 12 h on days 5-19, decreasing 25% thereafter every other day). The level of CsA was adjusted to between 200 and 400 ng/ml, as measured by radioimmunoassay. The CsA dose was tapered beginning at least 60 days after demonstrating engraftment and full donor chimerism by short tandem repeat (STR) analysis.

In this study, all patients received 2 units of cord blood. However, the DNA of only one of the two donors was eventually detectable in patients with durable engraftment. The numbers of infused total nucleated and CD34⁺ cells are shown in Table 1. The UCB units were thawed with gentle agitation in a 37°C waterbath and without further processing before infusing into the patients. Granulocyte colony-stimulating factor (filgrastim, Kirin, Gunma, Japan) 10 µg/kg/day was given intravenously on day 1 after transplantation and on each day thereafter, until

Table 1 Transplant characteristics of patients receiving double-unit CBT for thalassemia

UPN	6	12	16	18	23
Age in years at CBT/gender	11.4/male	10.6/male	10/male	11.1/female	13.1/female
Diagnosis	β/β thalassemia	β/β thalassemia	β/β thalassemia	β/β thalassemia	β thalassemia/Hb E
Weight at CBT, in kg (percentile for age and gender)	29.5 (5-10)	36.5 (50-75)	30 (25-50)	34 (25-50)	35.5 (10)
Height at CBT, in cm (percentile for age and gender)	127 (<5)	129 (<5)	132.5 (25)	142 (25-50)	139 (<5)
Genotype	IVS II-654 and codon 41/42	IVS II-654 and codon 17	Homozygous codon 41/42	IVS II-654 and codon 27/28	Codon 41/42 and Hb E
Pretransplant serum ferritin level (µg/l)	2125	970.8	1802.8	503.6	733.5
<i>HLA alleles</i>					
Patient	A0201, A2402, B1525, B5801, DRB1 0301, 1405	A1101, —, B1301, B4601, DRB1 0901, 1501	A0201, A11, B1301, B4001, DRB1 1101, 1501	A0207, A0201, B4601, —, DRB1 0901, 1405	A0207, A1101, B1301, B4601, DRB1 0803, 1602
Donor A	A0207, A3303, B5801, —, DRB1 0301, 1405	A02, A1102, B1301, B4601, DRB1 0901, 1501	A2402, A1101, B1301, B4001, DRB1 0405, 1501	A0207, A1102, B46, —, DRB1 0901, —	A0207, A1101, B1301, B4601, DRB1 0803, 1602
<i>TNC (× 10⁷/kg)</i>					
Pre-freeze	6.31	3.61	9.88	7.73	5.37
Post-thaw	0.97	2.67	6.83	6.11	3.99
<i>CD34 (× 10⁵/kg)</i>					
Pre-freeze	3.97	3.21	4.83	3.27	1.70
Post-thaw	0.56	1.27	3.61	1.30	2.40
Donor B	A0201, —, B5601, B5801, DRB1 0301, 1401	A1101, —, B40, B4601, DRB1 0901, 1501	A0201, A1101, B5021, B4001, DRB1 1101, —	A0207, A1101, B4601, B4001, DRB1 0901, 1405	A0207, A1101, B1301, B4601, DRB1 0803, 1602
<i>TNC (× 10⁷/kg)</i>					
Pre-freeze	4.73	4.26	4.83	3.43	2.78
Post-thaw	2.28	2.00	3.61	2.19	2.32
<i>CD34 (× 10⁵/kg)</i>					
Pre-freeze	1.71	1.54	1.53	1.71	1.50
Post-thaw	1.75	0.97	1.31	0.47	1.55

Abbreviations: IVS = intervening sequence; TNC = total nucleated cells.
'—' Indicates the allele is homozygous.

the neutrophil count remained above $1.0 \times 10^9/l$ for 3 consecutive days.

Supportive care and post transplantation follow-up

Blood components were given whenever indicated to maintain hemoglobin and platelet values $>80 \text{ g/l}$ and $20 \times 10^9/l$, respectively. For streptococcal prophylaxis, intravenous cefazolin was given until the neutrophil counts exceeded $0.5 \times 10^9/l$, and oral itraconazole (antifungal prophylaxis) 3 mg/kg daily was prescribed for the month preceding transplantation. Intravenous or oral acyclovir and oral co-trimoxazole were given to prevent CMV reactivation and *Pneumocystis jiroveci* infection. Both acyclovir and co-trimoxazole were continued as prophylaxis until day 180 after transplantation and further continued until T-cell function is restored. Parenteral nutrition was provided for the duration of anorexia. Intravenous immunoglobulin (500 mg/kg) was given at days -6, +7, +21, +35, +56, +77, and +98 following UCBT. The standard CMV pp65 antigenemia assay was performed in parallel. The positive results were quantified by counting the number of pp65-expressing cells per 50 000 leukocytes on the slide.

Myeloid engraftment was defined as 3 consecutive days of an absolute neutrophil count of $\geq 0.5 \times 10^9/l$. The last day of RBC transfusion was recorded as a day of RBC transfusion independence. Platelet engraftment was defined as 7 consecutive days of a platelet count of $\geq 20 \times 10^9/l$ maintained without transfusion. Current methods for measuring hematopoietic chimerism are based on STR polymorphisms that distinguish recipient from donor. Serial STR polymerase chain reaction confirmed the conversion from mixed chimerism to a predominantly donor profile on the day the myeloid engraftment occurred, on days +42, +100, +180, +270 and +360, 1.5 years after transplantation, and yearly, thereafter. An additional test was done if clinically indicated. In patients achieving myeloid engraftment, desferrioxamine was administered at a dose of 40 mg/kg/day as a 24-h intravenous infusion to accelerate the clearance of body iron deposits.

Results

Engraftment and chimerism status

Double cord blood units were used to minimize the risk of developing graft failure in these multiply transfused patients with weights over 30 kg. High-resolution molecular typing demonstrated that four recipient/donor pairs had an HLA 2-4 loci mismatch, and only one patient received an HLA-matched graft (6/6 match). All patients demonstrated primary engraftment with one having late graft failure and autologous recovery. Myeloid engraftment occurred at a median of 15 days (range 12-19 days) after transplantation. Interestingly, the patient with a 'perfect' match and the lowest cell dose had initial mixed donor engraftment but demonstrated rapid autologous recovery by day 42. In patients with successful transplantation, the median time to RBC transfusion independence was 32 days (range 10-120 days). The median number of days to achieve a platelet

count of $>20 \times 10^9/l$ was 49 days (range 25-117 days; Table 2).

Although the precise biologic mechanisms for single-donor predominance in double UCBT are not understood to date, all except patient 5 showed predominance of one cord by day +19 at the time of first STR DNA analysis. Chimerism analysis showed 100% single donor chimerism by day +42 in four of the patients evaluable for long-term follow-up of 18.5 months (range 11-32). In one recipient (patient 5) of 6/6 HLA-matched units, chimerism status on day 16 was donor A 53.4% and donor B 47.1%, which was completely lost on day 42.

Transplantation-related events

The grading of acute GVHD was grade I in one patient, grade II in one, and grade III in two. Patients with grade III acute GVHD were treated with methylprednisolone at 48 mg/m^2 per day and both showed good response. Limited chronic GVHD involving skin subsequently developed in four patients who were treated with topical steroids. Asymptomatic CMV reactivation was detected in three patients (patient 1, 3 and 5) on post transplantation day 62, 63 and 56, respectively. The first two patients were successfully treated preemptively with intravenous ganciclovir. Patient 5 developed late-onset hemorrhagic cystitis 24 days post transplant associated with BK viraemia concomitant with CMV reactivation. BK virus-associated hemorrhagic cystitis and simultaneous CMV reactivation were treated simply by stopping immunosuppressive agents after complete autologous recovery had been identified.

Two of these patients (3 and 4) succumbed to their bleeding complications, and alloimmunization contributed significantly to the demise in patient 4. Eight months following UCBT she presented with severe hemolytic anemia and thrombocytopenia (Evans' syndrome). Treatment included high-dose steroids, intravenous immunoglobulins, CsA, and anti-CD20 monoclonal antibody (rituximab), but produced poor response. The patient died of diffuse alveolar hemorrhage 11 months post transplant.

Patient 3 had experienced a complicated post transplantation course that included an episode of massive pericardial effusion. Pericardial drainage of fluid was carried out. Results showed hemorrhagic pericardial effusion. Methicillin-resistant *Staphylococcus aureus* pericardial abscess developed eventually, which necessitated surgical intervention and multiple antibiotic treatment courses in varying combinations including linezolid. Although CsA therapy had to be stopped before the 6-month withdrawal, he was transfusion-independent with full donor chimerism 16 months post transplant.

According to the criteria outlined previously, continuous chelation therapy was performed with desferrioxamine in four patients during the early post transplantation period.

Discussion

UCB has the advantages of rapid availability, and low risk of severe GVHD despite donor-recipient HLA disparity.⁵ Previous mortality risk outweighs the benefit from un-

Table 2 Characteristics of engraftment, transplant-related events, GVHD grading, outcome, and chimerism

UPN	6	12	16	18	23
<i>Days until</i>					
ANC $>0.5 \times 10^9/l$	12	19	19	14	15
RBC transfusion independence	22	42	120	10	NA
Platelet $>20 \times 10^9/l$	55	43	117	25	NA
Transplantation-related complications	CMV reactivation	Pneumonitis	CMV reactivation ICH Pericardial abscess	Evans' syndrome	CMV reactivation BK virus-associated hemorrhagic cystitis
<i>Acute GVHD</i>					
Maximum clinical grade	III	II	III	I	NA
Site(s) involved	Skin	Skin	Skin, gastrointestinal tract	Skin	NA
Chronic GVHD	Limited	Limited	Limited	Limited	NA
Outcome	Transfusion independence	Transfusion independence	Transfusion independence	Died of pulmonary hemorrhage	Autologous recovery by day 52
Months after transplant	32	21	16	11	8
<i>Chimerism status</i>					
Day of myeloid engraftment	100% donor A	6.2% donor A	83.2% donor A	90.2% donor A	53.4% donor A
Day +42 post transplant	100% donor A	93.8% donor B 100% donor B	8.6% donor B 100% donor A	8.4% donor B 100% donor A	47.1% donor B 100% recipient
Day +100 post transplant	100% donor A	100% donor B	100% donor A	100% donor A	NA
Latest follow-up	100% donor A	100% donor B	100% donor A	100% donor A	NA
Lansky play-performance scale	100	100	90	NA	100

Abbreviations: ANC = absolute neutrophil count; ICH = intracranial hemorrhage; NA = not applicable; RBC = red blood cells.

related transplantation and cell dose is a critical factor for UCBT,⁶ although it is difficult to compare the units from different cord blood banks. Laughlin *et al.*⁷ found that dose of cryopreserved nucleated cells is a major determinant of neutrophil recovery and that higher CD34⁺ cell dose is associated with improved survival in adult UCB recipients. The optimal way to deal with delayed engraftment of UCB and its complications has not yet been found.⁸

Cotransplantation of two partially HLA-matched unrelated UCB is an attractive option, especially for older children who are typically not eligible for single UCBT because of limitations of cell dose. If no single 5-6/6 HLA-A, -B, -DRB1 matched UCB is available that had a TNC of $>3.0 \times 10^7$ cells/kg, the patient is considered for double UCBT. Besides being more readily available, an acceptable double unit graft can be identified for the majority of patients. HLA disparity between each unit and the recipient and between two units does not necessarily have to be at the same loci.⁹ It is difficult to extrapolate the results of studies correlating post-thaw cell dose and transplant outcome to establish criteria for product selection using data obtained on products before cryopreservation.¹⁰ The engraftment failure occurred in patient 5 who used HLA-matched grafts, but the pre-freeze CD34⁺ cell doses were the smallest among these five patients. Wagner *et al.*⁵ observed a threshold infused CD34 cell dose of $\geq 1.7 \times 10^5/kg$ was required for durable hematopoietic engraftment, below which the rate of recovery and incidence of

engraftment are unsatisfactory. Together, these data suggest the graft selection should be principally based on HLA match for those units with an acceptable cell dose.

Although the use of myeloablative conditioning might be linked to infertility, its benefits should be weighed against its potential risks, and diligent long-term follow-up should be carried out especially in thalassemic patients with iron overload, in whom the risk for infertility is already increased. After transplantation of cord blood, engraftment usually seems slower than after transplantation of marrow or peripheral blood. A consensus is emerging that UCB grafts of higher cell doses should be selected wherever possible to optimize engraftment.¹¹ Although CD34 quantification in UCBT has not been consistently predictive of time to donor hematopoietic engraftment,¹² CD34 cell dose may play a critical role in engraftment. Thus, there are concerns about reaching a sufficient cell dose for engraftment.

UCBT has problems related to risk of graft failure or relatively late engraftment in patients with thalassemia.^{13,14} To our knowledge, this study is the first to suggest that the utilization of double-unit UCBT has shown promising results in teenagers with transfusion-dependent thalassemia, although the clinical effectiveness of HSCT over traditional transfusion and chelation therapy has been reported previously.¹⁵ Notably, tolerability and safety were also acceptable in the therapeutically challenging clinical setting of our study. Phlebotomy was not routinely

performed in our study, as it may stimulate the hyperplastic bone marrow after HSCT, and the residual recipient cells may also be stimulated.¹⁶

Patients with transfusion-dependent thalassemia are at high risk of graft failure, either because of major prior alloimmunization or an insufficient amount of UCB stem cells.¹⁷ Our conditioning regimens were generally well tolerated and the median day of neutrophil engraftment was 15 days (12–19 days) after transplantation. Four patients had sustained engraftment despite one, whose case was subsequently complicated by fatal Evans' syndrome. Although agreement on the minimum acceptable and the optimal UCB graft cell doses are not yet unanimous,^{14,18} our result suggested that higher cell doses may partially overcome the negative impact of HLA disparity. Ideally, since no standardized treatment concept exists, these data will be used to design larger comparative studies.

The present study provides evidence suggesting double-unit UCBT is an acceptable therapeutic approach in thalassemic patients with multiple-transfusion aged over 10 years. With strategies that maximize cell dose – using non-red cell reduced plasma depleted UCB, no post-thaw wash, and double cord transplantation – promising results may be achieved with UCBT in selected patients with thalassemia.

Acknowledgements

We are indebted to the patients and their caretakers for participating in the externally audited results. We would like to thank Professor Delon Wu for his continued support of this program. This work was supported in part by grants from Chang Gung Memorial Hospital (CMRP G33020) and National Science Council (95-2314-B-182A-106).

Material contained in this paper has been presented at the following meetings:

1. Jaing TH. Unrelated cord blood transplantation for hemoglobinopathies. 4th Annual International Umbilical Cord Blood Transplantation Symposium; Los Angeles, CA, USA; May 19–20, 2006.
2. Jaing TH, Tan P, Chan LL, Rosenthal J, Wang B, Gjertson D, Petz L, Chow R. Unrelated Cord Blood Transplantation for Hemoglobinopathy. 11th Congress of the Asia Pacific Bone Marrow Transplantation; Nagoya, Japan; October 27–29, 2006.
3. Jaing TH, Tan P, Rosenthal J, Wang B, Gjertson D, Petz L, Chow R. Unrelated Cord Blood Transplantation (CBT) for Thalassemia. 48th American Society of Hematology Annual Meeting, Orlando, FL, USA; December 9–12, 2006.

References

- 1 La Nasa G, Caocci G, Argioli F, Giardini C, Locatelli F, Vacca A *et al*. Unrelated donor stem cell transplantation in adult patients with thalassemia. *Bone Marrow Transplant* 2005; **36**: 971–975.
- 2 Bosi A, Bartolozzi B, Guidi S. Allogeneic stem cell transplantation. *Transplant Proc* 2005; **37**: 2667–2669.
- 3 Fleischhauer K, Locatelli F, Zecca M, Orofino MG, Giardini C, De Stefano P *et al*. Graft rejection after unrelated donor

hematopoietic stem cell transplantation for thalassemia is associated with nonpermissive HLA-DPB1 disparity in host-versus-graft direction. *Blood* 2006; **107**: 2984–2992.

- 4 Hongeng S, Pakakasama S, Chaisiripoomkere W, Chuan-sumrit A, Sirachainan N, Ungkanont A *et al*. Outcome of transplantation with unrelated donor bone marrow in children with severe thalassemia. *Bone Marrow Transplant* 2004; **33**: 377–379.
- 5 Wagner JE, Barker JN, DeFor TE, Barker KS, Blazar BR, Eide C *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 Benito AI, Diaz MA, Gonzalez-Vicent M, Sevilla J, Madero L. Hematopoietic stem cell transplantation using umbilical cord blood progenitors: review of current clinical results. *Bone Marrow Transplant* 2004; **33**: 675–690.
- 7 Laughlin MJ, Barker J, Bambach B, Koc ON, Rizzieri DA, Wagner JE *et al*. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med* 2001; **344**: 1815–1822.
- 8 Goldstein G, Toren A, Nagler A. Human umbilical cord blood biology, transplantation and plasticity. *Curr Med Chem* 2006; **13**: 1249–1259.
- 9 Majhail NS, Brunstein CG, Wagner JE. Double umbilical cord blood transplantation. *Curr Opin Immunol* 2006; **18**: 571–575.
- 10 Moroff G, Eichler H, Brand A, Kekomaki R, Kurtz J, Letowska M *et al*. Multiple-laboratory comparison of in vitro assays utilized to characterize hematopoietic cells in cord blood. *Transfusion* 2006; **46**: 498–500.
- 11 Grewal SS, Barker JN, Davies SM, Wagner JE. Unrelated donor hematopoietic cell transplantation: marrow or umbilical cord blood? *Blood* 2003; **101**: 4233–4244.
- 12 Tse W, Laughlin MJ. Umbilical cord blood transplantation: a new alternative option. *Hematology (Am Soc Hematol Educ Program)* 2005, 377–383.
- 13 Locatelli F, Rocha V, Reed W, Bernaudin F, Ertem M, Grafakos S *et al*. Related umbilical cord blood transplantation in thalassemia and sickle cell anemia. *Blood* 2003; **101**: 2137–2143.
- 14 Goussetis E, Peristeri J, Kitra V, Kattamis A, Petropoulos D, Papassotiriou I *et al*. Combined Cord-Blood and Bone Marrow Transplantation for Beta-Thalassemia. *Pediatr Hematol Oncol* 2000; **17**: 307–314.
- 15 Ho WL, Lin KH, Wang JD, Hwang JS, Chung CW, Lin DT *et al*. Financial burden of national health insurance for treating patients with transfusion-dependent thalassemia in Taiwan. *Bone Marrow Transplant* 2006; **37**: 569–574.
- 16 Li CK, Lai DH, Shing MM, Chik KW, Lee V, Yuen PM. Early iron reduction programme for thalassaemia patients after bone marrow transplantation. *Bone Marrow Transplant* 2000; **25**: 653–656.
- 17 Bornstein R, Flores AI, Montalbán MA, del Rey MJ, de la Serna J, Gilsanz F. A modified cord blood collection method achieves sufficient cell levels for transplantation in most adult patients. *Stem Cells* 2005; **23**: 324–334.
- 18 Migliaccio AR, Adamson JW, Stevens CE, Dobrila NL, Carrier CM, Rubinstein P. Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity. *Blood* 2000; **96**: 2717–2722.