

## ORIGINAL ARTICLE

# Early engraftment kinetics of two units cord blood transplantation

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**Cord blood transplantation (CBT) is a promising alternative means of allogeneic stem cell transplantation. However, limited cell doses may compromise outcome. To enhance engraftment, CBT has been conducted using two units with promising results. However, little is known about the mechanism of engraftment. Here, we analyzed the early engraftment kinetics of eight patients given two unit umbilical CBT. Early engraftment kinetics revealed dominancy of one of two units from the day of engraftment (absolute neutrophil count  $>0.5 \times 10^9/l$ ). The median value of percentage of the predominant unit by chimerism analysis at the time of engraftment was 88% (60–100%). Two units CBT was found to be a safe, effective and promising alternative treatment option with good engraftment potential. Dominancy occurred early after CBT and is probably influenced by multiple factors.** *Bone Marrow Transplantation* (2006) **38**, 197–201. doi:10.1038/sj.bmt.1705423; published online 19 June 2006  
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### Introduction

Since the first successful transplantation of umbilical cord blood (UCB) was used to treat a patient with Fanconi anemia in 1988,<sup>1</sup> cord blood transplantation (CBT) has become an alternative to bone marrow transplantation in the treatment of a variety of diseases. Cord blood cells have many theoretical advantages as grafts for stem cell transplantation (SCT) because of their immaturity. As compared with those of adults, UCB stem cells produce larger *in vitro* hematopoietic colonies, and can be expanded in long-term culture *in vitro*. The properties of UCB cells should theoretically compensate for the relatively low numbers of cells contained in a single UCB unit, and through rapid expansion reconstitute myeloablated patients

with fewer nucleated cells (by 1–2 logs) more so than the cells of bone marrow. However, low cell numbers compromise outcome if infused cell doses fall below critical limits.<sup>2</sup>

To enhance engraftment, *ex vivo* expansion of UCB cells has been attempted in several studies with limited success.<sup>3</sup> However, recently, the transplantation of two partially human lymphocyte antigen (HLA)-matched UCB units has been attempted with promising engraftment result,<sup>4</sup> but this method is at the early development stage and little is known about the mechanism or kinetics of engraftment. Here, we analyzed early chimerism after transplants of two UCB units to clarify the early engraftment kinetics of this complex form of transplantation with multiple donors.

### Patients and methods

#### *Patient and cord blood unit selection*

Eight patients diagnosed as having acute leukemia (five AML, two ALL) or severe aplastic anemia were given transplants of two UCB units consecutively at Seoul National University Children's Hospital. Patients were eligible for two units CBT when we were unable to secure a single 4-6/6 HLA-A, -B or DRB1 matched UCB unit with a nucleated cell dose of at least  $3.5 \times 10^7/kg$ . Written informed consent was obtained from all patients before transplantation.

Cord blood units were selected based on serologic typing for HLA-A and -B, low-resolution molecular typing for HLA-DRB1 loci and total nucleated cell dose. HLA-A, -B, -C, -DRB1 and -DQB1 loci were confirmed using a high-resolution molecular method (polymerase chain reaction (PCR) single-strand conformation polymorphism).

#### *Treatment*

Patients received various conditioning regimens and graft-versus-host disease (GVHD) prophylaxis according to disease status (Table 1). Patients 1 and 6 received total body irradiation (12 Gy), cytosine arabinoside (12 g/m<sup>2</sup>) and cyclophosphamide (120 mg/kg). Patients 2, 3 and 4 received total body (or lymphoid) irradiation (4 Gy), fludarabine (180 mg/m<sup>2</sup>) and intravenous (i.v.) busulfan (6.4 mg/kg). Patients 5 and 7 received fludarabine (180 mg/m<sup>2</sup>) and i.v. busulfan (12.8 mg/kg). Patient 8 received fludarabine (240 mg/m<sup>2</sup>), i.v. busulfan (12.8 mg/kg) and

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**Table 1** Patient characteristics and transplantation data

Patients	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
Age (years)/sex	13/M	8/F	17/M	6/M	15/M	15/F	15/M	14/M
Body weight (kg)	61.3	34.4	45.0	23.0	60.0	62.9	51.0	57.0
Diagnosis	AML	SAA	AML	AML	AML	ALL Ph+	AML	ALL
Previous SCT	Auto-SCT	CBT	BMT	Auto-SCT	No	No	No	No
Interval from previous SCT (months)	42	3	12	26	—	—	—	—
Pre-CBT status	2nd CR	Engraft failure	3rd CR	2nd CR	1st CR	1st CR	1st CR	1st CR
Conditioning	TBIAcCPM	FluBuTLI	FluBuTBI	FluBuTBI	FluBu	TBIAcCPM	FluBu	FluBuVP
ATG/ALG	Yes	No	No	No	Yes	No	Yes	Yes
GVHD prophylaxis <sup>a</sup>	C, S, M	C, S	C, M	C, M	C, F	C, F	C, F	C, F
Nucleated cells ( $\times 10^7$ /kg)	2.75	4.48	5.39	6.51	3.03	4.23	5.17	4.87
CD34 <sup>+</sup> cells ( $\times 10^5$ /kg)	0.67	1.72	2.32	1.79	0.53	1.56	3.14	1.94
ANC $> 0.5 \times 10^9$ /l	20	18	32	14	19	27	13	18
Platelet $> 20 \times 10^9$ /l	No	112	93	24	33	50	29	30
Follow-up (months)	6	16	11	14	6	13	8	5
Outcomes	Death (CMV)	Live	Death (relapse)	Live	Death (relapse)	Live	Live	Live

Abbreviations: Ac = cytosine arabinoside; Auto = autologous; BMT = bone marrow transplantation; Bu = busulfan; CBT = cord blood transplantation; CPM = cyclophosphamide; Flu = fludarabine; SCT = stem cell transplantation; TBI = total body irradiation; TLI = total lymphoid irradiation; VP = etoposide.

<sup>a</sup>GVHD prophylaxis: C = cyclosporine; F = mycophenolate; M = methotrexate; S = corticosteroid.

etoposide (60 mg/kg). Patients received thymoglobulin (7.5 mg/kg) or lymphoglobulin (30 mg/kg) (both from SangStat, Lyon, France) with conditioning regimens, but these were omitted when irradiation was delivered to the patient after a severe cytomegalovirus (CMV) infection occurred in patient 1, who received thymoglobulin with irradiation. Patients received cyclosporine-based GVHD prophylaxis with methotrexate, prednisolone or mycophenolate.

Supportive care was performed according to the guidelines for SCT of our center.<sup>5</sup> Patients received ciprofloxacin, itraconazole, isoniazid and acyclovir as a prophylaxis for infection. Intravenous immunoglobulin (0.5 g/kg/dose) was infused weekly until day 100, and then monthly until day 180. Daily sulfamethoxazole/trimethoprim medication was discontinued 3 days before CBT and then restarted after white blood cell recovery. When fever exceeded 38°C, broad-spectrum antibiotics were administered. Amphotericin B was given when fever persisted for more than 5 days despite an appropriate antibacterial treatment. CMV treatment with gancyclovir was started when CMV antigenemia was detected on routine weekly examination. Transfusions were given to maintain the hemoglobin level above 8 g/dl and the platelet count above  $20 \times 10^9$ /l. All blood products were irradiated with 20 Gy to avoid the risk of acute GVHD induction. Granulocyte colony-stimulating factor ( $300 \mu\text{g}/\text{m}^2$ ) was administered from 1 day after CBT or from the last methotrexate infusion and then discontinued when the absolute neutrophil count (ANC) increased to more than  $1.0 \times 10^9$ /l for 3 consecutive days. Patients received low-molecular-weight heparin (nadroparine; fraxiparine, Sanofi-synthelabo, Paris, France) and/or lipo-PGE1 (eglandin; alprostadiol, Welfide, Osaka, Japan) as a prophylaxis for veno-occlusive disease.

#### Assessment of engraftment and chimerism analysis

Myeloid engraftment was defined as the first of 3 consecutive days with an ANC of  $0.5 \times 10^9$ /l, and platelet

recovery was defined to have occurred when the platelet count reached  $20 \times 10^9$ /l without transfusion. Hematopoietic chimerism of peripheral blood was evaluated by serial analysis of short tandem repeats from day 7, and when DNA could be obtained from blood samples. These analyses were performed at 3–4 days interval until ANC reached  $1.0 \times 10^9$ /l, and then weekly for 3 months after CBT.

The analytic method used was based on the quantitative amplification of informative polymorphic short tandem repeat (STR) regions in the recipient and donor using AmpFISTR profiler PCR amplification kit (Applied Biosystems, Foster City, CA, USA) as per the manufacturer's instructions. Briefly, DNA from two donors and recipient were each amplified with fluorescence-conjugated PCR primers for representative alleles. Subsequently, fluorescent PCR products were separated on ABI PRISMR 310 Genetic Analyzer (Applied Biosystems), and GeneScan software (Applied Biosystems) was used to correlate allele peak areas to the percentage of donor or recipient DNA.

## Results

### Patient characteristics

Patients' (M:6; F:2) median age and body weight were 14 years (6–17 years) and 54.5 kg (23.0–62.9 kg), respectively. Patients 1–4 received CBT as a salvage treatment after the failure of previous transplants. Patient characteristics and transplantation results are summarized in Table 1.

### Engraftment and outcome

The median number of infused total nucleated cells, which was obtained by summing numbers in paired units before freezing was  $4.68 \times 10^7$ /kg (2.75–6.51  $\times 10^7$ /kg), and the median number of days required to reach an ANC of more than  $0.5 \times 10^9$  or  $1.0 \times 10^9$ /l were 19 days (14–32 days) and 22 days (16–34 days), respectively. Spontaneous platelet

recovery to more than  $20 \times 10^9$  or  $50 \times 10^9/l$  required a median 33 days (24–112 days) and 43 days (24–123 days), respectively, excluding one patient (who died before recovery). CMV antigenemia occurred in six patients. Two patients progressed to CMV disease and one of them died owing to severe systemic CMV disease. Grade II acute GVHD occurred in four patients but resolved after treatment. Two patients relapsed 5 months after CBT.

*Early engraftment kinetics*

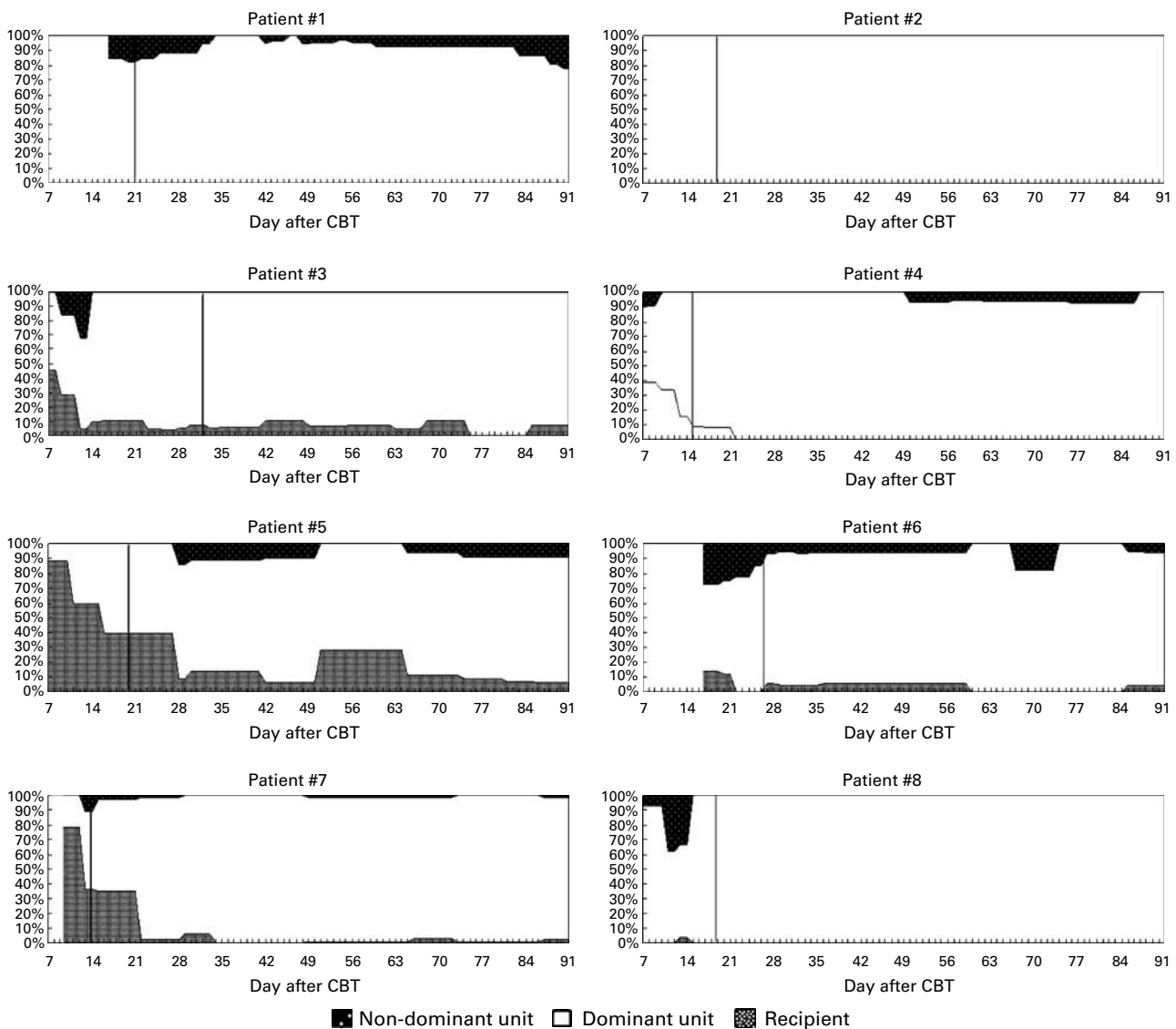
Early engraftment kinetics revealed a dominance of one of two administered units in each patient from the day of engraftment ( $>0.5 \times 10^9/l$ ). The median values of the percentage of the dominant unit, non-dominant unit and recipient by chimerism analysis on the days of engraftment were 89.5% (60–100%), 0% (0–16%) and 5% (0–40%), respectively. All patients achieved complete

donors chimerism ( $>90\%$ ) at day 28. The median values of the percentage of the dominant unit, non-dominant unit and recipient at day 28 were 94.5% (80–100), 0% (0–12%) and 2.5% (0–8%), respectively (Figure 1).

*Characteristics of dominant and non-dominant units*

The numbers of total nucleated cells, CD34<sup>+</sup> cells and CD3<sup>+</sup> cells infused, and the donor-recipient HLA disparities of the UCB units were viewed as factors that potentially influence dominancy. Units with a higher number of total nucleated cells and CD34<sup>+</sup> cells predominated in two and four of eight patients, respectively, and units with higher numbers of CD3<sup>+</sup> cells predominated in three of seven patients (Table 2).

In terms of the degree of HLA mismatch in pairs of serotype of HLA-A, -B, and low-resolution genotype of HLA-DRB1, the better HLA-matched unit predominated



**Figure 1** Engraftment kinetics analyzed by the serial chimerism of recipient, dominant unit and non-dominant unit from peripheral blood after two units CBT. The vertical line represents the day of engraftment (ANC  $>0.5 \times 10^9/l$ ).

**Table 2** Characteristics of dominant and non-dominant cord blood units

Patients	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
<i>Nucleated cells (<math>\times 10^7/\text{kg}</math>)</i>								
Dominant unit	1.33	2.27	2.59	3.56	1.23	1.79	2.24	2.37
Non-dominant unit	1.42	2.21	2.80	2.95	1.80	2.44	2.93	2.50
<i>CD34<sup>+</sup> cells (<math>\times 10^5/\text{kg}</math>)</i>								
Dominant unit	0.49	0.57	1.38	1.40	0.25	0.46	1.27	1.29
Non-dominant unit	0.18	1.15	0.94	0.39	0.28	1.10	1.87	0.65
<i>CD3<sup>+</sup> cells (<math>\times 10^7/\text{kg}</math>)</i>								
Dominant unit	0.50	0.30	0.50	0.98	0.13	0.41	0.19	0.18
Non-dominant unit	N/A	0.36	0.22	0.51	0.21	0.28	0.31	0.39
<i>Serotype (HLA-A, -B) and low-resolution genotype (HLA-DR) mismatch</i>								
Dominant unit	1/6	0/6	1/6	1/6	0/6	1/6	0/6	1/6
Non-dominant unit	2/6	1/6	1/6	2/6	1/6	1/6	1/6	1/6
<i>High-resolution genotype (HLA-A, -B, -C, -DR, and -DQ) mismatch</i>								
Dominant unit	N/A	5/10	3/10	6/10	0/10	4/10	0/10	1/10
Non-dominant unit	N/A	5/10	3/10	3/10	1/10	5/10	3/10	4/10

Abbreviations: HLA = human lymphocyte antigen; N/A = not available.

in five of eight patients. In another two with the same HLA mismatch of six serotype and low-resolution genotype, units better matching high-resolution genotypes HLA-A, -B, -C, -DRB1 and -DQB1 predominated (Table 2).

## Discussion

Two units CBT is a promising method with a better engraftment potential,<sup>4</sup> and was recently proposed to have a better graft-versus-leukemia effect, and thus to offer a better chance of survival than single unit CBT.<sup>6</sup> However, the mechanisms underlying this advantage and the kinetics of this engraftment are not understood.

Our preliminary results showed the dominance of one unit in all transplants, which concurs with a previous report by the Minnesota group,<sup>4</sup> although mixed chimerism occurred in the first case at about 3 months after CBT during CMV treatment with gancyclovir. It has also been reported that mixed chimerism may be maintained for as long as 100 days after two units CBT.<sup>7</sup> The most dramatic result of the present study was that dominance was found to occur early after transplantation. However, the exact time for determining dominance has not been determined, and it may even occur just after the infusion of the two units.

It is not known why one unit dominates over the other. Barker *et al.*<sup>4</sup> reported that a larger CD3<sup>+</sup> cells dose is associated with dominance, but with some exceptions, and it is possible that the unit with the larger number of CD3<sup>+</sup> cells might defeat the weaker unit. However, in present study, less than half of the cases analyzed showed dominance of the unit with more CD3<sup>+</sup> cells. Nevertheless, the present study suggests that the degree of HLA mismatch is also important.

Multiple factors associated with outcomes of CBT could influence the determination of dominance, such as those factors that affect the recipient environment (underlying

disease, previous treatment, conditioning regimen, use of antithymocyte globulin/antilymphocyte globulin, and the method of GVHD prophylaxis), the characteristics of each UCB unit and HLA disparity.<sup>8</sup>

For this reason, the selection of the two units requires consideration of the above factors. However, optimal selection guidelines that take into account influencing factors are unavailable. The selection of one strong unit (with high numbers of total nucleated and/or CD3<sup>+</sup> cells) and one weak unit to activate the strong one, and thus create dominance of one unit, might result in a better outcome than the currently favored combination of two strong units, which presents the possibility of persistent mixed chimerism.

In conclusion, two units CBT produced promising results, and the determination of dominance was found to be an early event influenced by multiple factors.

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