related donor. He is alive without graft failure or relapse after the second transplant.

A 17-year-old woman with AML in first relapse received allo-BMT from a matched unrelated donor. Her body weight and BMI were 43.2 kg and 17.3, respectively. Her AUC was 902.7 μmol min/l. Her regimen-related toxicities were grade 4 thrombocytopenia, grade 3 febrile neutropenia and grade 2 nausea, vomiting and stomatitis. She died of disease progression on day 193.

Discussion

It has been reported that a high steady-state concentration of BU causes toxicities including VOD,[5–10] whereas a low steady-state concentration leads to graft rejection[10–15] or relapse/progression of the disease.[11] Targeted dose adjustment of BU to maintain the overall systemic exposure within a proper range may reduce these risks.[4–7,14,15] Although it has been reported that there are ethnic differences in PK for a wide range of drugs,[28] this has not been seriously examined with i.v. BU. Therefore, we conducted this drug bioavailability study in a Japanese population. The data obtained were compared with those published mostly overseas. In this study, all observed treatment-related toxicities were as expected, with a low incidence of severe complications. One patient was clinically diagnosed with VOD. This patient showed body weight gain, liver enlargement and right upper abdominal pain, but had no jaundice. As his body weight returned to the baseline within 2 days, this could have been due to overhydration. One patient who developed graft failure had CML and underwent unrelated BMT following interferon therapy, all of which are well-known risks of graft failure.[10,29] The incidence of relapse and the survival rate in this study were similar to those in previous studies.[11,19]

In studies with an oral preparation of BU, it was unclear whether plasma levels of BU correlate with severe regimen-related toxicities.[4,6–8,11] In the pivotal study for US approval of i.v. BU, plasma levels of BU exceeded 1500 μmol min/l in two of the five patients who developed VOD,[19] whereas in our study there was no case of VOD in three patients who had a level over 1500 μmol min/l. This may suggest an ethnic difference in the PK of BU. On the other hand, a population pharmacokinetic analysis of i.v. BU is rare.[30] Our earlier small-scale study revealed high inter- and intrapatient consistency for i.v. BU pharmacokinetics.[22] However, the value of therapeutic drug monitoring remains crucial. Our study demonstrated no essential difference in PK analysis from earlier published Western data,[19] and this supports the notion that racial factors may not seriously influence the bioactivity of i.v. BU.

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References

1 Litzow MR, Pérez WS, Klein JP, Bolwell BJ, Camitta B, Copelan EA *et al.* Comparison of outcome following allogeneic bone marrow transplantation with cyclophosphamide–total body irradiation versus busulphan–cyclophosphamide conditioning regimens for acute myelogenous leukaemia in first remission. *Br J Haematol* 2002; **119**: 1115–1124.
2 Vassal G, Deroussent A, Hartmann O, Challine D, Benhamou E, Valteau-Couanet D *et al.* Dose-dependent neurotoxicity of high-dose busulfan in children: a clinical and pharmacological study. *Cancer Res* 1990; **50**: 6203–6207.
3 Hassan M, Ljungman P, Bolme P, Ringdn O, Syrckov Z, Bekassy A *et al.* Busulfan bioavailability. *Blood* 1994; **84**: 2144–2150.
4 Schuler U, Schroer S, Kühnle A, Blanz J, Mewes K, Kumbier I *et al.* Busulfan pharmacokinetics in bone marrow transplant patients: is drug monitoring warranted? *Bone Marrow Transplant* 1994; **14**: 759–765.
5 Grochow LB, Jones RJ, Brundrett RB, Braine HG, Chen TL, Saral R *et al.* Pharmacokinetics of busulfan: correlation with veno-occlusive disease in patients undergoing bone marrow transplantation. *Cancer Chemother Pharmacol* 1989; **25**: 55–61.
6 Hassan M, Oberg G, Ehrsson H, Ehrnebo M, Wallin I, Smedmyr B *et al.* Pharmacokinetic and metabolic studies of high-dose busulphan in adults. *Eur J Clin Pharmacol* 1989; **36**: 525–530.
7 Grochow LB. Busulfan disposition: the role of therapeutic monitoring in bone marrow transplantation induction regimens. *Semin Oncol* 1993; **20**(Suppl. 4): 18–25.
8 Dix SP, Wingard JR, Mullins RE, Jerkunica I, Davidson TG, Gilmore CE *et al.* Association of busulfan area under the curve with veno-occlusive disease following BMT. *Bone Marrow Transplant* 1996; **17**: 225–230.
9 Copelan EA, Bechtel TP, Avalos BR, Elder PJ, Ezzone SA, Scholl MD *et al.* Busulfan levels are influenced by prior treatment and are associated with hepatic veno-occlusive disease and early mortality but not with delayed complications following marrow transplantation. *Bone Marrow Transplant* 2001; **27**: 1121–1124.
10 Vassal G, Re M, Gouyette A. Gas chromatographic-mass spectrometric assay for busulfan in biological fluids using a deuterated internal standard. *J Chromatogr* 1988; **428**: 357–361.
11 Slattery JT, Clift RA, Buckner CD, Radich J, Storer B, Bensinger WI *et al.* Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. *Blood* 1997; **89**: 3055–3060.
12 McCune JS, Gooley T, Gibbs JP, Sanders JE, Petersdorf EW, Appelbaum FR *et al.* Busulfan concentration and graft rejection in pediatric patients undergoing hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2002; **30**: 167–173.
13 Bolinger AM, Zangwill AB, Slattery JT, Glidden D, DeSantes K, Heyn L *et al.* An evaluation of engraftment, toxicity and busulfan concentration in children receiving bone marrow transplantation for leukemia or genetic disease. *Bone Marrow Transplant* 2000; **25**: 925–930.
14 Bolinger AM, Zangwill AB, Slattery JT, Risler LJ, Sultan DH, Glidden DV *et al.* Target dose adjustment of busulfan in pediatric patients undergoing bone marrow transplantation. *Bone Marrow Transplant* 2001; **28**: 1013–1018.
15 Deeg HJ, Storer B, Slattery JT, Anasetti C, Doney KC, Hansen JA *et al.* Conditioning with targeted busulfan and cyclophosphamide for hemopoietic stem cell transplantation from related and unrelated donors in patients with myelodysplastic syndrome. *Blood* 2002; **100**: 1201–1207.

16 Kami M, Hamaki T, Maruta Y, Miyakoshi S, Mutou Y. Limitations of oral busulfan in preparative regimen before hematopoietic stem-cell transplantation. *Haematologica* 2002; **87**: ELT10.

17 Andersson BS, Madden T, Tran HT, Hu WW, Blume KG, Chow DS *et al*. Acute safety and pharmacokinetics of intravenous busulfan when used with oral busulfan and cyclophosphamide as pretransplantation conditioning therapy: a phase I study. *Biol Blood Marrow Transplant* 2000; **6**: 548–554.

18 Andersson BS, Gajewski J, Donato M, Giralt S, Gian V, Wingard J *et al*. Allogeneic stem cell transplantation (BMT) for AML and MDS following i.v. busulfan and cyclophosphamide (i.v. BuCy). *Bone Marrow Transplant* 2000; **25** (Suppl 2): S35–S38.

19 Andersson BS, Kashyap A, Gian V, Wingard JR, Fernandez H, Cagnoni PJ *et al*. Conditioning therapy with intravenous busulfan and cyclophosphamide (IV BuCy2) for hematologic malignancies prior to allogeneic stem cell transplantation: a phase II study. *Biol Blood Marrow Transplant* 2002; **8**: 145–154.

20 Shimoni A, Bielorai B, Toren A, Hardan I, Avigdor A, Yeshurun M *et al*. Intravenous busulfan-based conditioning prior to allogeneic hematopoietic stem cell transplantation: myeloablation with reduced toxicity. *Exp Hematol* 2003; **31**: 428–434.

21 Kashyap A, Wingard J, Cagnoni P, Roy J, Tarantolo S, Hu W *et al*. Intravenous versus oral busulfan as part of a busulfan/cyclophosphamide preparative regimen for allogeneic hematopoietic stem cell transplantation: decreased incidence of hepatic venoocclusive disease (HVOD), HVOD-related mortality, and overall 100-day mortality. *Biol Blood Marrow Transplant* 2002; **8**: 493–500.

22 Takama H, Tanaka H, Nakashima D, Ueda R, Takaue Y. Population pharmacokinetics of intravenous busulfan in patients undergoing hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2006; **37**: 345–351.

23 Kaplan EI, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457–481.

24 McDonald GB, Sharma P, Matthews DE, Shulman HM, Thomas ED. Venocclusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. *Hepatology* 1984; **4**: 116–122.

25 Jones RJ, Lee KS, Beschorner WE, Vogel VG, Grochow LB, Braine HG *et al*. Venoocclusive disease of the liver following bone marrow transplantation. *Transplantation* 1987; **44**: 778–783.

26 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al*. 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995; **15**: 825–828.

27 Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE *et al*. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 1980; **69**: 204–217.

28 Xie HG, Kim RB, Wood AJ, Stein CM. Molecular basis of ethnic differences in drug disposition and response. *Annu Rev Pharmacol Toxicol* 2001; **41**: 815–850.

29 Santos GW, Tutschka PJ, Brookmeyer R, Saral R, Beschorner WE, Bias WB *et al*. Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. *N Engl J Med* 1983; **309**: 1347–1353.

30 Nguyen L, Fuller D, Lennon S, Leger F, Puozzo C. I.V. busulfan in pediatrics: a novel dosing to improve safety/efficacy for hematopoietic progenitor cell transplantation recipients. *Bone Marrow Transplant* 2004; **33**: 979–987.

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Unrelated Cord Blood Transplantation for Severe Aplastic Anemia

Ayami Yoshimi,[1] Seiji Kojima,[2] Shuichi Taniguchi,[3] Junichi Hara,[4] Toshimitsu Matsui,[5] Yoshiyuki Takahashi,[2] Hiroshi Azuma,[6] Koji Kato,[7] Tokiko Nagamura-Inoue,[8] Shunro Kai,[9] Shunichi Kato[10]

[1] Department of HSCT Data Management, Nagoya University, School of Medicine, Nagoya, Japan; [2] Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3] Department of Hematology, Toranomon Hospital, Tokyo, Japan; [4] Hematology/Oncology Department of Pediatrics, Osaka General Medical Center, Osaka, Japan; [5] Hematology/Oncology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; [6] Hokkaido Cord Blood Bank, Sapporo, Japan; [7] Tokai Cord Blood Bank, Nagoya, Japan; [8] Tokyo Cord Blood Bank, Tokyo, Japan; [9] Hyogo Cord Blood Bank, Nishinomiya, Japan; and [10] Tokai University Cord Blood Bank, Isehara, Japan; on behalf of the Japan Cord Blood Bank Network (JCBBN)

Correspondence and reprint requests to: Seiji Kojima, MD, Department of Pediatrics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, 466-8550, Japan (e-mail: kojimas@med.nagoya-u.ac.jp).

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ABSTRACT

In the present study we evaluated the feasibility of unrelated cord blood transplantation (UCBT) in patients with severe aplastic anemia (SAA). The outcome of 31 SAA patients (median age 28; range: 0.9-72.3 years old) who received UCBT was analyzed. The cumulative incidences of the neutrophil and platelet recovery after UCBT were 54.8 and 72.2%, respectively (95% confidence interval [CI] = 36.0%-70.3% and 51.3%-85.3%, respectively). The cumulative incidences of grade ≥II acute and chronic graft-versus-host disease (aGVHD, cGVHD) were 17.1% (95% CI = 6.2%-32.8%) and 19.7% (95% CI = 6.2%-38.8%), respectively. Currently, 13 patients are alive, having survived for 33.7 months (median; range: 6-77 months) after UCBT. The probability of overall survival (OS) at 2 years was 41.1% (95% CI = 23.8%-57.7%). A conditioning regimen that included low-dose total body irradiation (TBI) (2-5 Gy), fludarabine, and cyclophosphamide resulted in a favorable OS (80%; 95% CI = 20.4%-96.9%). This result suggests that UCBT using the optimal conditioning regimen can be a salvage treatment for patients without a suitable bone marrow donor and warrants evaluation in further prospective studies.

KEY WORDS

Unrelated cord blood transplantation • Severe aplastic anemia

INTRODUCTION

Over the last 2 decades, the outcome of patients with severe aplastic anemia (SAA) has dramatically improved regardless of whether patients received immunosuppressive therapy (IST) or bone marrow transplantation (BMT) [1-3]. BMT from an HLA-matched sibling is curative in the majority of younger patients with SAA, and is currently recommended as first-line treatment [4]. IST, with a combination of antithymocyte globulin (ATG) and cyclosporine (CSA), has been an alternative therapy for patients without an HLA-matched sibling. BMT from an unrelated donor (UD) is used as a salvage therapy for patients who fail to respond to IST or who experience a relapse of the disease. However, in general, the results of UD-BMT have been inferior to those achieved with an HLA-matched sibling.

The report the Center for International Blood and Marrow Transplant Research (CIBMTR) on UD-BMT (n = 231), for the period 1988-1998, showed that the overall survival (OS) rates for matched and mismatched UD-BMT in patients with SAA were 39% and 36%, respectively [5]. The Japan Marrow Donor Program (JMDP) reported a favorable outcome with 56% survival rate in 154 patients with SAA who received UD-BMT between 1993 and 2000 [6]. In

the recent 2 reports from the European Group for Blood and Marrow Transplantation (EBMT) and the French Society of Bone Marrow Transplantation and Cellular Therapy (SFGM-TC), the outcomes of UD-BMT for SAA before and after 1998 were compared. The results demonstrated improved OS rates of UD-BMT since year 1998 (32% versus 57% for EBMT and 29% versus 50% for SFGM-TC) [7,8]. The authors speculated that the better HLA matching because of the introduction of high-resolution HLA typing may have contributed to the improved outcomes. In pediatric series, 90% OS rates have been recently reported for UD-BMT patients, which is comparable to that observed for BMT from a matched sibling [9,10].

Treatment approaches for patients who lack a suitable unrelated bone marrow donor remain a great challenge. Cord blood has been used as an alternative source of HSCT, and it has the advantages of rapid availability on demand and a low incidence of graft-versus-host disease (GVHD). There were only a few reports on unrelated cord blood transplantation (UCBT), which included patients with SAA. The results showed poor outcome and high incidence of graft failure [11,12]. However, a few small series and case reports of successful UCBT for SAA have recently been reported [13-17]. Because of the possible reporting bias, the general efficacy of UCBT is still unknown. Therefore, we decided to further examine this procedure by using the database of the Japan Cord Blood Bank Network (JCBBN). We identified 31 patients with acquired SAA who received UCBT and analyzed the outcome.

PATIENTS AND METHODS

Patients

From September 1998 until February 2006, 53 patients with acquired SAA received UCBT through JCBBN. Twenty-two patients who received UCBT as a salvage therapy for the engraftment failure after previous HSCT were excluded, and the remaining 31 patients were included in this study. Patient characteristics and the cord blood units are summarized in Table 1. Patients were eligible for UCBT if they had no HLA-identical related or unrelated bone marrow donor. Patients who could not wait for UD-BMT because of unstable diseases were also considered to be eligible for UCBT. Cord blood units with 0 to 2 HLA locus mismatches by serology in HLA-A, HLA-B, and HLA-DRB1 were searched and then the unit with the largest cell dose was selected. At least 2.0×10^7/kg mononuclear cells (MNCs) were given in all patients.

The age of the patients ranged 0.9 to 72.7 years (median 27.9 years), and there were 8 patients older than 50 years of age. There were 25 patients who had been previously treated with IST, including ATG + CSA (n = 13), ATG only (n = 4), or CSA only (n = 8). In 4 patients, androgen had been given. The remaining 2 patients were given only supportive therapy. All patients or their guardians gave informed consent for transplantation and submission of the data to the JCBBN.

Table 1. *Patient and Donor Characteristics (n = 31)*

Characteristic	
Median patient age, years (range)	27.9 (0.8-72.7)
Sex (male/female)	
Patient (n)	11/20
Donor (n)	14/17
Etiology of aplastic anemia	
Idiopathic/hepatitis associated (n)	30/1
Disease duration before UCBT: median, days (range)	337 (31-5063)
1 year or less/ 1-3 year/3 year or more/unknown (n)	13/4/8/5
Red blood cell transfusions before UCBT	
Less than 20 times/20 or more times/unknown (n)	8/21/2
Platelet transfusions before UCBT	
Less than 20 times/20 or more times/unknown (n)	7/22/2
HLA mismatches (serologic): GVHD direction (n = 31)	
0/1/2 (n)	4/18/9
HLA mismatches (serologic): rejection direction (n = 31)	
0/1/2 (n)	6/17/8
HLA mismatches (DNA typing): GVHD direction (n = 22)	
0/1/2/3/4 (n)	2/6/6/6/2
HLA mismatches (DNA typing): rejection direction (n = 22)	
0/1/2/3/4 (n)	1/5/12/3/1

UCBT indicates unrelated cord blood transplantation; GVHD, graft-versus-host disease.

Recipient-Donor HLA Matching

Data were available for 31 patients with serology-based recipient-donor HLA matching and for 22 patients who underwent high-resolution DNA typing for class I-HLA-A, HLA-B, and DRB1 (Table 1). The HLA disparities for both GVHD and rejection directions are shown in Table 1.

Transplantation Procedure

Characteristics of the transplantation procedures are listed in Table 2. The conditioning regimens varied according to the individual centers used. The 3 most commonly used regimens were: TBI (4-5 Gy) + fludarabine (FLU; 120-175 mg/m^2) + Melphalan (MEL) (80-120/mg/m^2) (n = 12), TBI (2-4 Gy) + FLU (90-250/mg/m^2) and cyclophosphamide (CY; 50-100 mg/kg or 2250/mg/m^2) (n = 5), and TBI (10-12 Gy) + CY (120-200 mg/kg) + ATG (n = 3). Of the 25 patients given irradiation, 24 received TBI

Table 2. *Transplant Procedures (n = 31)*

	No. of Patients
Conditioning Regimen	
TBI (4-5 Gy) + MEL+ FLU	12
TBI (2-4 Gy) + CY + FLU	5
TBI (10-12 Gy) + CY + ATG	3
Others	11
Radiation	
TBI/TAI	25/1
No radiation	7
ATG	
Yes/No	7/24
GVHD prophylaxis	
CSA	6
CSA + others (MTX/steroid/MMF)	10
Tacrolimus	7
Tacrolimus + others (MTX/steroid)	8
MNC cell dose	
$\geq 2.0 \times 10^7$/kg, $< 3.0 \times 10^7$/kg	15
$\geq 3.0 \times 10^7$/kg	16
CFU-GM cell dose	
$< 2.0 \times 10^4$/kg	14
$\geq 2.0 \times 10^4$/kg	15
Unknown	2
CD34 cell dose	
$< 1.0 \times 10^5$/kg	10
$\geq 1.0 \times 10^5$/kg	15
Unknown	6

TBI indicates total body irradiation; TAI, thoracoabdominal irradiation; MEL, melphalan; FLU, fludarabine; CY, cyclophosphamide; ATG, antithymocyte globulin; CSA, cyclosporine; MTX, methotexrate; MMF, mycophenolate mofetil; MNC, mononuclear cell; CFU-GM, colony-forming unit granulocyte-macrophage.

and 1 underwent thoracoabdominal irradiation. A total of 7 patients were administered with ATG, either horse ATG (Lymphoglobulin 30-75 mg/kg in 5 patients) or rabbit ATG (Thymoglobulin 10 mg/kg in 2 patients). GVHD prophylaxis also varied according to the individual centers (Table 2). To facilitate the recovery of neutrophils, all patients received recombinant human granulocyte colony-stimulating factor. The number of mononucleated cells, colony-forming units of granulocyte-macrophage (CFU-GM), and CD34-positive cells of the cord blood units at the time of freezing are shown in Table 2.

Definitions and Statistical Analysis

The status of all patients was evaluated based on the last follow-up report, which was performed using the standardized forms provided by the JCBBN. All results were analyzed as of June 2008.

Date of engraftment was defined as the first of the 3 consecutive days where the neutrophil recovery was $>0.5 \times 10^9$/L. Platelet recovery was defined as the first of the 3 consecutive days where the unsupported platelet count was $>50 \times 10^9$/L. Chimerism was evaluated in 12 patients, with fluorescent in situ hybridization for the Y chromosome performed in 6 sex-mismatched grafts and quantitative polymerase chain reaction analysis for microsatellite DNA markers performed in 6 sex-matched transplantations. Acute and chronic GVHD (aGVHD, cGVHD) were diagnosed and graded according to standard clinical criteria [18,19].

Probability of OS was estimated according to the Kaplan-Meier method. GVHD and engraftment were assessed using the cumulative incidence procedure, and death was the competing event. Univariate comparisons among various groups were made using the log-rank test. The variables evaluated included age of the patient, donor sex, sex mismatch, disease duration before UCBT, the number of pre-UCBT transfusions for red cells and platelets, IST before UCBT, HLA matching by serology and high-resolution DNA typing for both GVHD and rejection directions, the number of mononuclear cells, CFU-GM, CD34-positive cells of the cord blood units at the time of freezing, conditioning regimens, and the administration of ATG and GVHD prophylaxis (single agent versus ≥2 agents, MTX versus no MTX, or CSA versus tacrolimus). All statistical analyses were carried out with version 10 of the STATA software (StataCorp, College Station, TX).

RESULTS

Engraftment

Sustained engraftment was observed in 17 patients. The cumulative incidences of the neutrophil and platelet recovery after UCBT were 54.8 and 72.2%, respectively (95% confidence interval [CI] = 36.0%-70.3% and 51.3%-85.3%, respectively; Figure 1). The median times to achieve a neutrophil count $\geq 0.5 \times 10^9$/l and a platelet count $\geq 50 \times 10^9$/l were 19 days (range: 12-35 days) and 59 days (range: 39-145 days), respectively. Chimerism analysis results were available in 8 patients with sustained neutrophil engraftment. All of these patients showed complete donor chimerism with more than 99% donor cells. No mixed chimerism was observed. There were 7 patients who failed to achieve sustained engraftment among patients who survived more than 28 days after UCBT. Five patients did not achieve a primary engraftment. Although 3 of them underwent a second UCBT, all died of infections, with (n = 1) or without (n = 2) engraftment of the second graft. Autologous recovery was noted in 1 patient, which was proven by the chimerism analysis that demonstrated 100% recipient cells. One patient had achieved engraftment on day 19, but she suffered from late graft failure at day 176 and received second HSCT at day 203. The patient was still alive at the time of the last follow-up.

Results of the univariate analysis for engraftment are shown in Table 3. The GVHD prophylaxis with a single agent (CSA or tacrolimus) exhibited a significantly better engraftment rate than that seen for the other methods (75.0% versus 33.3%, $P = 0.02$).

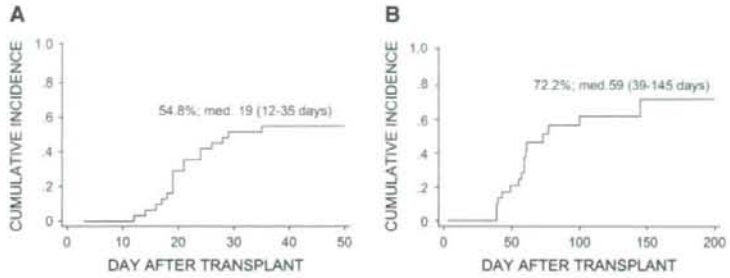

Figure 1. (A) Cumulative incidence of sustained donor neutrophil engraftment ($>0.5 \times 10^9$/L) and (B) platelet engraftment ($>50 \times 10^9$/L) after unrelated cord blood transplantation in patients with aplastic anemia.

When there was a lower number of transfusions (<20 times) of red cells and platelets prior to the HSCT, there was a trend for a better chance of successful engraftment compared to cases where there were higher number of transfusions (≥20 times), although this was not statistically significant. The number of infused MNCs, CFU-GM, and CD34 had no impact on the engraftment.

Table 3. *Outcome following Unrelated Cord Blood Transplantations for Aplastic Anemia: Univariate Analysis*

Covariates	2-Year-OS (%) (95% CI)	P	Engraftment (%) (95% CI)	P
Recipient age				
<20 year (n = 9)	44.4 (13.6-71.9)	.18	44.4 (13.6-71.9)	.76
20- 40 year (n = 12)	56.3 (24.4-79.1)		66.7 (33.7-86.0)	
>40 year (n = 10)	20.0 (3.0-47.5)		50.0 (18.4-75.3)	
Disease duration before UCBT				
<1 year (n = 13)	35.7 (13.0-59.4)	.34	57.1 (28.4-78.0)	.67
≥1 year (n = 12)	47.6 (18.2-72.4)		58.3 (27.0-80.1)	
RBC transfusions before UCBT				
<20 (n = 8)	62.5 (22.9-86.1)	.26	75.0 (31.5-93.1)	.08
≥20 (n = 21)	31.4 (13.1-51.7)		47.6 (25.7-66.7)	
Platelet transfusions before UCBT				
<20 (n = 7)	57.1 (17.2-83.7)	.28	85.7 (33.4-97.9)	.05
≥20 (n = 22)	35.0 (16.1-54.7)		45.4 (24.4-64.3)	
HLA matching by serologic typing (GVHD direction)				
0-1 mismatched (n = 22)	49.2 (27.3-68.0)	.34	63.6 (40.3-79.9)	.10
2 mismatched (n = 9)	22.2 (3.4-51.3)		33.3 (78.3-62.3)	
HLA matching by serologic typing (Rejection direction)				
0-1 mismatched (n = 23)	43.5 (23.3-62.1)	.64	52.2 (30.5-70.0)	.59
2 mismatched (n = 8)	37.5 (8.7-67.4)		62.5 (22.9-86.1)	
Conditioning regimen				
TBI + CY + FLU (n = 5)	80.0 (20.4-96.9)	.02	75.0 (40.8-91.2)	.17
TBI + MEL + FLU (n = 12)	46.9 (17.6-71.9)		80.0 (20.4-96.9)	
Others (n = 14)	21.4 (5.2-44.8)		28.6 (08.8-52.4)	
ATG				
No (n = 24)	48.9 (27.8-67.0)	.007	66.7 (44.3-81.7)	.19
Yes (n = 7)	14.3 (0.7-46.5)		14.3 (0.7-46.5)	
GVHD prophylaxis				
CSA or tacrolimus only (n = 13)	54.6 (27.4-75.3)	.07	75.0 (46.3-89.8)	.02
CSA or tacrolimus+others (n = 18)	26.7 (8.3-49.6)		33.3 (12.2-56.4)	
MTX				
No (n = 20)	38.5 (17.7-59.1)	.93	60.0 (35.7-77.6)	.24
Yes (n = 11)	45.5 (16.7-70.7)		45.5 (16.7-70.7)	
MNC				
2×10^7/kg-3×10^7/kg (n = 15)	45.0 (19.4-67.8)	.61	60.0 (31.8-79.7)	.70
≥3×10^7/kg (n = 15)	37.5 (15.4-59.8)		50.0 (24.-71.0)	
CD34				
<1×10^5/kg (n = 15)	45.7 (14.3-73.0)	.32	70.0 (32.9-89.2)	.52
≥1×10^5/kg (n = 15)	33.3 (12.2-56.4)		53.3 (26.3-74.4)	

GVHD indicates graft-versus-host disease; TBI, total-body irradiation; CY, cyclophosphamide; Mel, melpharan; Flu, fludarabine; ATG, antithymocyte globulin; CSA, cyclosporine; MTX, methotrexate; MNC, mononuclear cell; CFU-GM, colony-forming unit-granulocyte macrophage; UCBT, unrelated cord blood transplantation.

GVHD and Viral Infections

Acute GVHD (≥grade II) was observed in 5 patients (grade II; n = 4, grade III; n = 1) on days 8 through 56, and was lethal in the 1 patient with grade III aGVHD. Chronic GVHD was observed in 4 patients (extensive: n = 1, limited: n = 3; de novo n = 2, progression from aGVHD n = 2) on days 124 through 213. Figure 2 depicts the cumulative incidence of grade II-IV aGVHD (17.1%; 95% CI = 6.2%-32.8%) and cGVHD (19.7%; 95% CI = 6.2%-38.8%). Viral reactivations were commonly observed in this study. CMV reactivation was noted in 9 patients, and 1 of them developed CMV disease. Epstein-Barr virus (EBV) reactivation was noted in 1 patient, having developed cerebral infarction, which was considered to be related with EBV. Adenovirus induced cystitis occurred in 1 patient.

Survival

Of the 31 total patients, 13 are presently alive, with survival durations of 6 to 77 months (median 33.7 months) after the transplantations. The probability of OS at 2 years was 41.1% (95% CI = 23.8%-57.7%). The results of univariate analysis of the factors influencing survival are shown in Table 3. The conditioning regimen and the administration of ATG were the only factors that were significantly related to the survival. The conditioning regimen, which included low-dose TBI, FLU, and CY, resulted in better outcomes than were seen for the other regimens (Table 3 and Figure 3). The administration of ATG was associated with poor outcome (Table 3 and Figure 3). There were 5 out of 7 patients given ATG that died before engraftment because of infections (n = 3) or hepatic veno-occlusive disease (VOD) (n = 2). In the 2 other patients, 1 demonstrated autologous recovery, whereas the other patient has had sustained engraftment and is currently still alive. There tended to be a better outcome noted for GVHD prophylaxis with a single agent (either CSA or tacrolimus) compared to prophylaxis with 2 or more agents. The outcome for the patients aged 40 years and older was inferior to that seen for the younger patients, although this was not statistically significant.

In the 18 patients who died, the causes of death were graft failure (n = 7), bacterial/fungal infections (n = 3), EBV-related cerebral infarction (n = 1), VOD (n = 3), aGVHD (n = 1), acute respiratory distress syndrome (n = 1), encephalopathy (n = 1), and cardiac toxicity (n = 1).

DISCUSSION

The outcome of 31 patients with SAA who received UCBT was analyzed in this study. This is the first report on a nationwide multicentral study that focused on UCBT for SAA as far as we know. The overall survival rate was 41%, which is comparative to the results of the large registry-based analysis of UD-BMT for SAA by CIBMTR [5], but inferior to the results of some recent reports of UD-BMT [6,20]. The incidence and the severity of aGVHD and cGVHD were considerably lower in this study, which is advantageous for UCBT. The major problem encountered, however, was still the high incidence of engraftment failure after UCBT. In the present study the conditioning regimen with the low-dose TBI, FLU, and CY resulted in better outcome (80% survival rate) compared to other regimens. This regimen and the selection of optimal donor with better HLA match and higher cell dose may improve the outcome of UCBT for SAA.

Previous reports on the conditioning regimen of UCBT for SAA are limited. Mao et al. [13] reported on 9 patients with SAA who were conditioned with ATG and CY (60 mg/kg) prior to undergoing UCBT. A total of 7 out of 9 of these patients survived with hematologic recovery. However, a donor-recipient mixed chimerism was present in all patients. There are a few case reports of UCBT for SAA using more intensified regiments, which resulted in successful engraftment along with complete chimerism [14-16,21].

Radiation-containing regimens are efficient in achieving better engraftments and widely used within the UD-BMT settings for patients with SAA, although these regimens are associated with significant early and late toxicities, including secondary malignancies [22]. Recent study by Deeg et al. [20] to define the optimal TBI dose in combination with CY (200 mg/kg) and

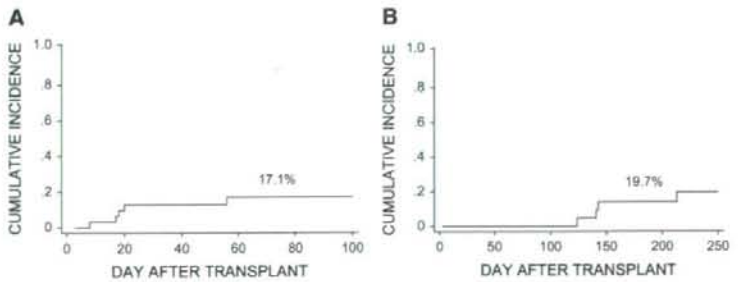

Figure 2. Cumulative incidence of ≥ grade II aGVHD (A) and cGVHD (B) in patients with aplastic anemia who received UCBT.

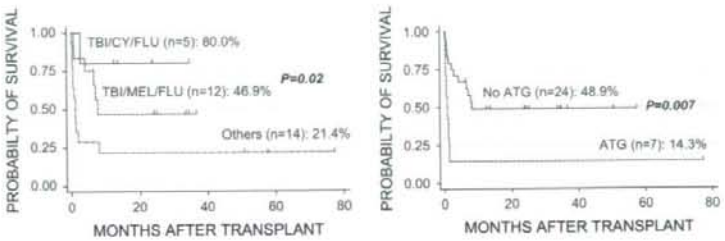

Figure 3. Probability of survival after conditioning regimens in patients with aplastic anemia, who received unrelated cord blood transplantation. TBI: total body irradiation, CY: cyclophosphamide, MEL: melphalan, FLU: fludarabine, ATG: antithymocyte globulin.

ATG for use with UD-BMT in patients with SAA showed that 2 Gy was sufficient to allow engraftment without increasing toxicities. This finding was also supported by a Japanese study on UD-BMT in patients with SAA, which reported that in a small group given a conditioning regimen of low-dose TBI (2-5 Gy), CY (200 mg/kg), and ATG, there was a 90% survival rate [7].

Fludarabine is currently widely used for nonmyeloablative transplants for a variety of diseases including SAA [23-26]. In the recent study on UD-SCT from the Severe Aplastic Anemia Working Party of the EBMT (SAA WP-EBMT), they designed a non-TBI regimen that used FLU (120 mg/m^2), CY (1200 mg/m^2), and ATG [27]. In this study, a total of 38 both pediatric and adults patients with SAA were included (36 BMT and 2 PBSCT patients) and the 2-year survival rate was 73%, with a low incidence of aGVHD and cGVHD. Therefore, this result suggests that a FLU containing regimen might be effective for use with UD-HSCT in SAA. The authors suggested that the conditioning regimen might need to be modified for adults through the addition of a low dose of TBI, as there was a significantly lower engraftment rate seen in the adult patients (82% overall, 68% in adults). Overall, these findings in previous reports and in this study suggest that the conditioning regimen that included the low-dose TBI and FLU resulted in favorable outcomes. In present study, the 7 patients given ATG were poor. Only 1 of them achieved engraftment and is alive. However, the number of patients given ATG was too small to reach any definitive conclusions and the benefit of ATG in UCBT for SAA should be evaluated in a large prospective study.

The GVHD prophylaxis using a single agent (CSA or tacrolimus) exhibited a better engraftment rate and a marginally better survival rate compared to that seen when 2 or more immunosuppressive agents were used. In the latter group, steroid, MTX, or mycophenolate mofetil (MMF) were given in addition to CSA or tacrolimus. Because of the limited number of patients and the highly heterogeneous regimen of the GVHD prophylaxis in this study, it is difficult to define the optimal GVHD prophylaxis based on the current results. However, the low incidence and severity of GVHD that we noted in our study suggests that a single agent, regardless of whether it is tacrolimus or CSA, may be effective enough to prevent GVHD in UCBT for SAA.

One of the most important factors that determine the success of UCBT is the cell dose in the CB [11,28-30]. In the present study, a minimum of 2×10^7/kg MNCs were infused in all patients. In this condition, the dose of MNCs, CFU-GM, and CD34 had no impact on engraftment and survival. One of the benefits of UCBT is that it can overcome the HLA barrier. Despite the HLA disparity in the majority of the patients, the incidence of GVHD was quite low in this study. There was a tendency for better HLA matching to result in a better outcome, although this was not statistically significant. Selection of the CB units with higher cell dose and better HLA match may be essential to improve the outcome of UCBT for SAA.

In our study there were also 8 patients who were older than 50 years of age, which is generally considered to be over than the cutoff age for transplantation. Because of the poor outcome of UCBT in older patients (OS = 20% in group with age >40 years old), UCBT cannot be recommended for older patients at present, and repeated IST should be considered in these patients [31,32].

In summary, this first multicentral study focused on the UCBT for SAA suggests that UCBT can be an alternative treatment for SAA patients who failed to IST and have no suitable bone marrow donor. The results may be improved by using the optimal conditioning regimen such as low-dose TBI, FLU, and CY and by donor selection of better HLA match and higher cell dose.

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REFERENCES

1. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. *Blood*. 2006; 108:2509-2519.
2. Kojima S, Frickhofen N, Deeg HJ, et al. Aplastic anemia. *Int J Hematol*. 2005;82:408-411.
3. Marsh JC. Management of acquired aplastic anaemia. *Blood Rev*. 2005;19:143-151.
4. Storb R, Blume KG, O'Donnell MR, et al. Cyclophosphamide and antithymocyte globulin to condition patients with aplastic anemia for allogeneic marrow transplantations: the experience in four centers. *Biol Blood Marrow Transplant*. 2001;7:39-44.
5. Passweg JR, Perez WS, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. *Bone Marrow Transplant*. 2006;37:641-649.
6. Kojima S, Matsuyama T, Kato S, et al. Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. *Blood*. 2002; 100:799-803.
7. Viollier R, Socie G, Tichelli A, et al. Recent improvement in outcome of unrelated donor transplantation for aplastic anemia. *Bone Marrow Transplant*. 2008;41:45-50.
8. Maury S, Balere-Appert ML, Chir Z, et al. Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient. *Haematologica*. 2007;92:589-596.
9. Kennedy-Nasser AA, Leung KS, Mahajan A, et al. Comparable outcomes of matched-related and alternative donor stem cell transplantation for pediatric severe aplastic anemia. *Biol Blood Marrow Transplant*. 2006;12:1277-1284.
10. Kojima S, Inaba J, Kondo M, et al. Unrelated donor marrow transplantation for severe acquired aplastic anemia using cyclophosphamide, antithymocyte globulin, and total body irradiation. *Blood*. 1995;85:291-292.
11. 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.
12. Neudorf SM, Blatt J, Corey S, et al. Graft failure after an umbilical cord blood transplant in a patient with severe aplastic anemia. *Blood*. 1995;85:2991-2992.
13. Mao P, Zhu Z, Wang H, et al. Sustained and stable hematopoietic donor-recipient mixed chimerism after unrelated cord blood transplantation for adult patients with severe aplastic anemia. *Eur J Haematol*. 2005;75:430-435.
14. Ohga S, Ichino K, Goto K, et al. Unrelated donor cord blood transplantation for childhood severe aplastic anemia after a modified conditioning. *Pediatr Transplant*. 2006;10:497-500.
15. Lim SH, Zhang Y, Wang Z, et al. Umbilical cord blood transplant in hepatitis C-associated severe aplastic anemia. *Bone Marrow Transplant*. 2004;33:565-567.
16. Kusumi E, Miyakoshi S, Murashige N, et al. Successful reduced-intensity stem cell transplantation (RIST) with mismatched cord blood in a 70-year-old patient with severe aplastic anemia (SAA). *Bone Marrow Transplant*. 2003;32:1111-1112.
17. Lau FY, Wong R, Chui CH, Cheng G. Successful engraftment in two adult patients with severe aplastic anemia using nonmyeloablative conditioning followed by unrelated HLA-mismatched cord blood transplantation. *J Hematother Stem Cell Res*. 2001;10:309-311.
18. 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.
19. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med*. 1980;69:204-217.
20. Deeg HJ, O'Donnell M, Tolar J, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. *Blood*. 2006;108:1485-1491.
21. Shaw PH, Haut PR, Olszewski M, Kletzel M. Hematopoietic stem-cell transplantation using unrelated cord-blood versus matched sibling marrow in pediatric bone marrow failure syndrome: one center's experience. *Pediatr Transplant*. 1999;3: 315-321.
22. Champlin RE, Horowitz MM, van Bekkum DW, et al. Graft failure following bone marrow transplantation for severe aplastic anemia: risk factors and treatment results. *Blood*. 1989;73: 606-613.
23. Giralt S, Logan B, Rizzo D, et al. Reduced-intensity conditioning for unrelated donor progenitor cell transplantation: long-term follow-up of the first 285 reported to the national marrow donor program. *Biol Blood Marrow Transplant*. 2007;13: 844-852.
24. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. *Blood*. 2001;97: 631-637.
25. Kang HJ, Shin HY, Choi HS, Ahn HS. Fludarabine, cyclophosphamide plus thymoglobulin conditioning regimen for unrelated bone marrow transplantation in severe aplastic anemia. *Bone Marrow Transplant*. 2004;34:939-943.
26. George B, Mathews V, Shaji RV, et al. Fludarabine-based conditioning for allogeneic stem cell transplantation for multiply transfused patients with Fanconi's anemia. *Bone Marrow Transplant*. 2005;35:341-343.
27. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. *Bone Marrow Transplant*. 2005;36: 947-950.
28. 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.
29. 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.
30. 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.
31. Tichelli A, Passweg J, Nissen C, et al. Repeated treatment with horse antilymphocyte globulin for severe aplastic anaemia. *Br J Haematol*. 1998;100:393-400.
32. Di Bona E, Rodeghiero F, Bruno B, et al. Rabbit antithymocyte globulin (r-ATG) plus cyclosporine and granulocyte colony stimulating factor is an effective treatment for aplastic anaemia patients unresponsive to a first course of intensive immunosuppressive therapy. Gruppo Italiano Trapianto di Midollo Osseo (GITMO). *Br J Haematol*. 1999;107:330-334.

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ORIGINAL ARTICLE

Unrelated cord blood transplantation in CML: Japan Cord Blood Bank Network analysis

T Nagamura-Inoue[1,2], S Kai[3,4], H Azuma[5,6], M Takanashi[7,8], K Isoyama[9,10], K Kato[11,12], S Takahashi[13], S Taniguchi[14], K Miyamura[15], K Aoki[16], M Hidaka[17], F Nagamura[18], A Tojo[1,2,13], XM Fang[19] and S Kato[20,21], for Japan Cord Blood Bank Network

[1]*Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, University of Tokyo, Tokyo, Japan;* [2]*Tokyo Cord Blood Bank, Tokyo, Japan;* [3]*Department of Transfusion Medicine, Hyogo Medical University, Nishinomiya, Japan;* [4]*Hyogo Cord Blood Bank, Nishinomiya, Japan;* [5]*Hokkaido Red Cross Blood Center, Sapporo, Japan;* [6]*Hokkaido Cord Blood Bank, Sapporo, Japan;* [7]*Japanese Red Cross Tokyo Metropolitan Blood Center, Tokyo, Japan;* [8]*The Metro Tokyo Red Cross Cord Blood Bank, Tokyo, Japan;* [9]*Department of Paediatrics, Showa University Fujigaoka Hospital, Yokohama, Japan;* [10]*Kanagawa Cord Blood Bank, Yokohama, Japan;* [11]*Department of Paediatric Haematology/Oncology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan;* [12]*Tokai Cord Blood Bank, Nagoya, Japan;* [13]*Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, University of Tokyo, Tokyo, Japan;* [14]*Department of Hematology, Toranomon Hospital, Tokyo, Japan;* [15]*Department of Haematology/Oncology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan;* [16]*Department of Hematology, Kitakyusyu City Medical Center Hospital, Kitakyusyu, Japan;* [17]*Department of Hematology and Collagen Disease, National Hospital and Organization Kumamoto Medical Center, Kumamoto, Japan;* [18]*Division of Clinical Trial Safety Management, Research Hospital, The Institute of Medical Science, University of Tokyo, Tokyo, Japan;* [19]*Division of Biostatistics, Kitasato University Graduate School, Tokyo, Japan;* [20]*Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan and* [21]*Tokai University Cord Blood Bank, Isehara, Japan*

We analysed 86 patients with CML who received unrelated cord blood transplantation (UCBT), identified through a registry of the Japan Cord Blood Bank Network. At transplantation, the median patient age was 39 years (range, 1–67 years); 38 patients were in chronic phase (CP), 13 in the accelerated phase (AP) and 35 in blast crisis (BC). Median duration from diagnosis to UCBT was 1.5 years (range, 0.2–14.6 years). A nucleated cell (NC) dose of more than 3.0×10^7 per kg was sufficient to achieve neutrophil (91%) and platelet recovery (86%), whereas the lower dose of NC achieved only 60 and 61%, respectively. The duration and type of pre-transplant treatment did not affect neutrophil or platelet recovery. Results of multivariate analysis indicated that older patients (>50 years) had a higher incidence of transplant-related mortality. Advanced-disease stage and lower doses of NCs were significantly associated with lower leukaemia-free and event-free survival. At 2-year survival for patients in CP, AP and BC was 71, 59 and 32%, respectively ($P = 0.0004$). A pre-transplant European Group for Blood and Marrow Transplantation scoring system was effective in predicting the outcome of UCBT. We conclude that UCBT is a reasonable alternative therapy for patients with CML.

Correspondence: Dr T Nagamura-Inoue, Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail: tokikoni@ims.u-tokyo.ac.jp
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Introduction

Recent clinical research on unrelated cord blood transplantation (UCBT) has encouraged the use of human umbilical cord blood (CB) as a source of haematopoietic SCT (HSCT) in patients with haematopoietic malignancies.[1–5] In Japan, more than 3500 UCBTs have been performed through 11 CB banks in the Japan Cord Blood Bank Network (JCBBN). As a component of quality management and promotion, the JCBBN established a common registry for studying the results of UCBT. However, the clinical application of UCBT for CML has not been established because treatment with interferon (IFN)-α and molecular-targeting reagents, such as imatinib mesylate (imatinib), have induced complete cytogenetic remission and improved long-term survival without the need for allogeneic haematopoietic transplantation.[6–8] Imatinib has now replaced IFN as the first-line therapy for CML and induces a molecular remission (MR) as well as a complete cytogenetic response (CCyR) in the vast majority of patients newly diagnosed with CML who are in the chronic phase (CP) of the disease.[9–10] Imatinib induces complete haematological responses not only in patients in CP but also in those in the accelerated phase (AP) of the disease and in blastic crisis (BC).[11] Although its long-term

efficacy and feasibility were confirmed in the International Randomized Study of Interferon and STI571 (IRIS),[12] sustained administration of IFN is recommended for preventing disease progression and recurrence from minimal residual disease, which cannot be readily eradicated by imatinib alone.[13,14] Some patients who show point mutations in the BCR-ABL oncogene are resistant to imatinib,[15] and might also be resistant to the new derivatives, dasatinib and nilotinib.[16–19]

Patients who are resistant to IFN or imatinib may be candidates for HSCT. Both BMT and PBSC transplantation have provided reasonable results in the patients, even in those who were treated with IFN or imatinib before transplantation.[20,21] Furthermore, minimal residual clones of Ph1 were eradicated by post-HSCT donor lymphocyte infusion.[22,23] Use of UCBT in patients with CML was first reported by Rubinstein *et al.*[3] in the context of haematopoietic malignancies. CML appeared to fall into the group of diseases deemed unfavourable for the application of UCBT because of relatively higher graft failure; however, Sanz *et al.*[24] reported in 2001 that all seven evaluable cases engrafted and four patients with CML-CP remained alive with MR for more than one and a half years.

To address whether patients with CML can be considered applicants for UCBT and to clarify the prognostic factors for UCBT, we analysed 86 registry-based patients with CML who underwent UCBT as the initial HSCT through JCBBN. Our results suggest that UCBT, using an adequate dose of nucleated cells (NCs), may be considered a reasonable therapeutic option for patients with CML, regardless of the duration of treatment.

Patients, materials and methods

Patient selection criteria

Only patients recorded at the JCBBN registry as having undergone UCBT for CML were included in this study. Eighty-six patients who received UCBT as the initial HSCT were analysed. They received UCBT from 1997 to 2006 at CBT centres in Japan. Informed consent was provided according to the Declaration of Helsinki.

Patient characteristics

The characteristics of the 86 patients are listed in Table 1. Median age was 39 years (range, 1–69 years), including 9 children (1–15 years), 58 young adults (16–50 years) and 19 older adults (51 or older). Median weight was 58.3 kg (range, 9–96.1 kg). At UCBT, 38 patients were in CP (7 in first CP, 29 in second CP, 2 in third or subsequent CP), 13 patients were in AP (9 in first AP, 3 in second AP and 1 in third or subsequent AP) and 35 patients were in BC (23 in first BC, 11 in second BC and 1 in third or subsequent BC). A higher percentage of the older adults had advanced stages of the disease compared to the children ($P = 0.03$). In the 19 older adults, only 4 (21%) were in CP, 5 (26%) were in AP and 10 (53%) were in BC; in the 58 young adults, 29 (50%) were in CP, 5 (8.6%) were in AP and 24 (41.4%) were in BC. In contrast, of the nine children, five (56%) were in CP, three (33%) were in AP and one (11%) was in BC at CBT. Patients received transplants at a median time of 1.96 years (range, 0.18–17 years) after they were diagnosed with CML. Sixteen had received chemotherapy

Table 1 Characteristics of patients with CML given an unrelated cord blood transplant

Patients characteristics	n = 86
Gender	
M:F	55:31
Age, years	
Median (range)	39 (1–67)
Children (1–15 years), young adult (16–50 years) and older adult (51–67 years)	9:58:19
Weight, kg	
Median (range)	58.3 (9–96.1)
Duration from diagnosis to CBT, year	
Median (range)	1.96 (0.18–17)
Stage at CBT	
CP (n = 38)	
First	7
Second	29
Third or subsequent	2
AP (n = 13)	
First	9
Second	3
Third or subsequent	1
BC (n = 35)	
First	23
Second	11
Third or subsequent	1
In 38 patients with CP at UCBT	
Haematological response	
Complete	30
Partial	2
No response	2
Unknown	4
Cytogenetic response	
Complete (0%)	16 (7/16 molecular CR)
Partial (<35%)	9
No response (35–100%)	9
Unknown	4
Previous treatment	
Hydrea/BU/Ara-C, other chemotherapy	16
IFN-α-based therapy	22
Imatinib-based therapy	28
IFN-α and imatinib	18
Unknown	2
Pre-transplant risk scoring system of EBMT	
1	1
2	1
3	10
4	18
5	25
6	21
7	10

Abbreviations: AP = accelerated phase; BC = blastic crisis; CBT = cord blood transplantation; CP = chronic phase; EBMT = European Group for Blood and Marrow Transplantation; F = female; Imatinib = imatinib mesylate, M = male.

with BU and hydroxyurea, 22 had received IFN, 28 had received imatinib, 19 had received IFN and imatinib-based treatment and 2 had received an unknown treatment. Of the 38 patients in CP at UCBT, 30 patients showed a complete haematological response; 2, a partial response; 2, no response and 4 had an unknown response. Furthermore, of the 30 patients showing a complete haematological response, 16 had achieved a CCyR, including seven complete MRs; 9 had achieved a partial response; 9 had no response and 4 had an unknown response. Sixteen patients who achieved a CCyR, included only 1 patient in first CP complicated with colon cancer and 15 patients in second or third CP.

Umbilical cord blood characteristics and transplantation procedure

The CB cells were processed and cryopreserved in the cell processing and cryopreservation facilities of 11 public CB banks in Japan. The CB cells were initially processed using hydroxyl ethyl starch (HES)[25] and cryopreserved according to the technical and quality management guidelines of the JCBBN.

The CB characteristics are shown in Table 2. NC dose before cryopreservation was performed for all patients, but the number of GM colony-forming units (GM colonies) and $CD34^+$ cells dose were available only for 78 and 77 patients, respectively. HLA antigen disparities were categorized as either GVHD or rejection direction (Table 2). Low-resolution antigens of HLA-A, -B and -D were identified for all patients by serologic typing. HLA-DRB1 alleles were determined by high-resolution molecular typing using the sequencing-based HLA typing method in 81 of 86 cases. Patients without either an available family donor or an unrelated bone marrow donor were eligible for CBT, when two or less than two antigen mismatched HLA CB units were available.

Conditioning and GVHD prophylaxis regimens varied among the centres. Briefly, the conditioning regimen consisted of myeloablative therapy in 66 cases, reduced intensity in 18 cases and miscellaneous chemotherapy in 2 cases. The myeloablative-conditioning regimen included TBI-containing regimens (TBI $\geqslant$ 8 Gy) or conventional high-dose chemotherapies with CY ($\geqslant 120\,mg\,kg^{-1}$). G-CSF was administered until engraftment in 80 cases, whereas no cytokine was administered in 6 cases. For GVHD prophylaxis, patients received CsA-, tacrolimus- or mycophenolate mofetil-based regimen, except one patient, who received no prophylaxis (Table 2).

Statistical methods

The median duration of follow-up was 24 months (range, 3–67 months). The outcome end points were neutrophil recovery, platelet recovery, GVHD, relapse, transplant-related mortality (TRM), overall survival (OS), leukaemia-free survival (LFS) and event-free survival (EFS). The definitions of the statistical models used were in accordance with the Statistical guidelines for European Group for Blood and Marrow Transplantation (EBMT, http://www.ebmt.org/1WhatisEBMT/whatisebmt2.html). Neutrophil recovery was defined by an ANC of at least 0.5×10^9 per liter for 3 consecutive days, the first being used as the recovery day. Platelet recovery was defined by a non-transfused platelet count of at least 20×10^9 liter for 3 consecutive days. Deaths occurring before day 90 or 180 were considered as competing risks for neutrophil or platelet recovery, respectively. The graft failure rate for neutrophils was calculated for patients living without

Table 2 Transplant characteristics of patients with CML given an unrelated cord blood transplant

Unrelated cord blood characteristics		
No. of evaluable		$n=86$
Nucleated cells per kg recipient	Median (range)	2.5 (1.1–11.6) $\times 10^7$ per kg
$CD34^+$ cells per kg recipient*	Median (range)	0.88 (0.15–7.3) $\times 10^5$ per kg
GM colonies per kg recipient**	Median (range)	18.5 (0.65–102.1) $\times 10^3$ per kg
HLA compatibility with the recipient (%)		
Low-resolution HLA A, B and high-resolution of DRB1 (rejection direction)		
	Identical	10 (12)
	1 Ag mismatch	13 (15)
	2 Ag mismatches	40 (47)
	3 or more Ag mismatches	17 (20)
	Unknown	6 (7)
Low-resolution HLA A, B and high-resolution of DRB1 (GVHD direction)		
	Identical	5 (6)
	1 Ag mismatch	17 (20)
	2 Ag mismatches	42 (49)
	3 or more Ag mismatches	16 (19)
	Unknown	6 (7)
ABO compatibility with the recipient		
	Matched	20 (23)
	Minor incompatibility	32 (37)
	Major incompatibility	34 (40)
Transplantation characteristics		
Conditioning regimen (%)		
	Myeloablative	66 (77)
	Reduced intensity	18 (21)
	Miscellaneous	2 (2)
Post transplantation growth factor (%)		
	G-CSF administration	80 (93)
	No growth factor	6 (7)
GVHD prophylaxis (%)		
	CsA alone	13 (15)
	CsA + sMTX	39 (45)
	CsA + steroids	5 (6)
	CsA + MMF	1 (1)
	Tacrolimus	15 (17)
	Tacrolimus + sMTX	11 (13)
	Tacrolimus + steroids	1 (1)
	No prophylaxis	1 (1)

Abbreviations: Ag = antigen; FK = tacrolimus; MMF = mycophenolate mofetil; sMTX = short-term MTX.
Myeloablative conditioning regimen includes TBI-containing regimens with TBI at 8 Gy or more or conventional high-dose chemotherapies with CY 120 mg kg^{-1} or more.
*$n=77$, **$n=78$.

relapse for more than 90 days. Acute and chronic GVHD were diagnosed and graded at each centre according to the standard criteria.[26] Relapse was defined on the basis of the reappearance of the blast or Ph1 chromosome or BCR-ABL transgene by cytogenetic and/or molecular analysis, including PCR and FISH. TRM was considered a sole cause of non-leukaemic deaths occurring after transplantation; OS was defined as the time between transplantation and death due to any cause; LFS was defined as the time interval from UCBT to a first event, either relapse or death, in patients achievingCR; and EFS was defined as the time interval from UCBT to a first event, either diagnosis of graft failure, relapse or death, in patients achieving CR.

Kaplan–Meier estimates provided approximations of incidence over time for OS, LFS and EFS, whereas Cox models were used to evaluate the combined influence of patient-, CB cell dose-, disease- and transplant-related variables on the outcome. The end points of neutrophil and platelet recovery, acute and chronic GVHD, relapse and TRM were analysed using cumulative incidence curves that estimated incidence according to the Fine and Gray models, in which we first used univariate models that contained each of the variables one at a time. Then all variables with a $P<0.05$ by the likelihood-ratio test were included in a multivariate model. Cause-specific hazard ratios were estimated with 95% confidence intervals (CIs).[27] Statistical analysis was performed with the R Foundation version 2.5.1 (http://www.r-project.org/) and JMP version 6.0.2 software packages (SAS Institute, Cary, NC, USA).

Results

Neutrophil and platelet recovery

The cumulative incidence of neutrophil recovery on day 90 was 68% (95% CI, 58–78%; Figure 1a). During the first 90 days after transplantation, the competing risk for neutrophil recovery was death ($n=16$). The graft-failure rate for neutrophil recovery was 17% (12 of 70 living patients). For those patients who recovered, the median time to achieve an ANC equal to or greater than 0.5×10^9 per liter was 24 days (range, 6–78 days). The cumulative incidence of neutrophil recovery on day 90 was 91% (95% CI, 77–100%) for the patients who received more than 3.0×10^7 per kg NCs before cryopreservation, and 60% (95% CI, 48–72%) for those who received a lower dose of NCs ($P=0.00005$; Figure 1b). With respect to the dose of GM colonies before cryopreservation, the cumulative incidence of neutrophil recovery was 83% (95% CI, 70–100%) for patients who received more than 25×10^3 per kg of GM colonies and 62.0% (95% CI, 49–75%) for those who received a lower dose ($n=79$, $P=0.025$; Figure 1c). The association of neutrophil recovery with the dose of $CD34^+$ cells before cryopreservation, the rejection-directed disparities of HLA antigens in low and high resolution, disease status at UCBT and type of conditioning regimen (that is, myeloablative or not) was not statistically significant. Note that no statistical significance was found in the association of neutrophil recovery with type of pretreatment (IFN vs imatinib), or with the duration from diagnosis to UCBT (1 year or less vs

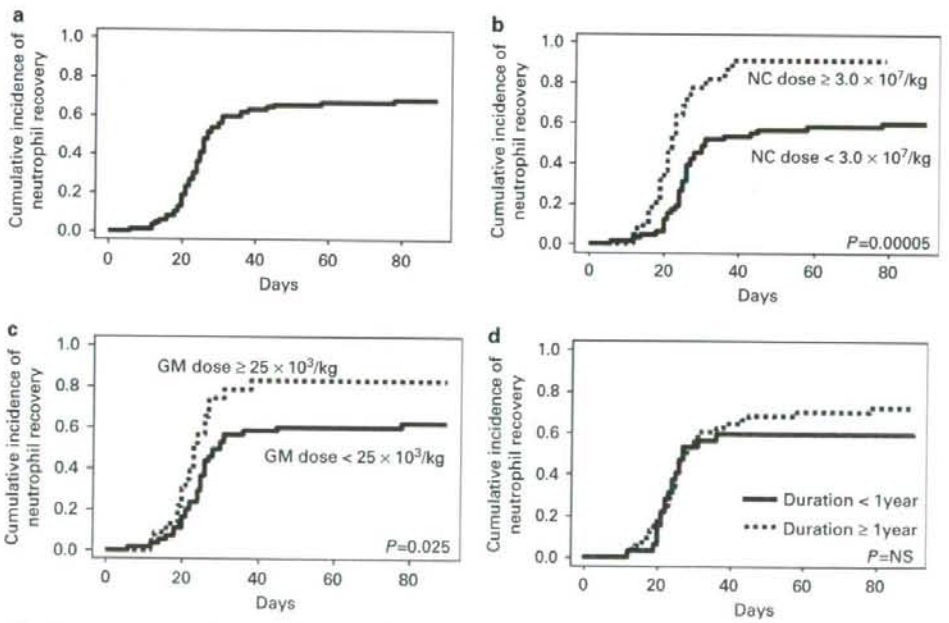

Figure 1 The cumulative incidence of neutrophil recovery. (**a**) Cumulative incidence of neutrophil recovery (**b**) by the dose of nucleated cells (NCs) and (**c**) by the dose of GM colonies before cryopreservation. (**d**) Cumulative incidence of neutrophil recovery by the duration from diagnosis to unrelated cord blood transplantation (UCBT).

longer), as shown in Figure 1d. In a multivariate analysis, only dose of NCs before cryopreservation at greater than 3.0×10^7 per kg was associated with improved neutrophil recovery (Table 3).

On day 180, cumulative incidence of platelet recovery, as indicated by more than 2×10^{10} per liter of platelets in blood, was 69% (95% CI, 59–79%; Figure 2a). During the first 180 days after transplantation, the competing risk for platelet recovery was death ($n = 16$). For those patients who recovered, the median time to achieve platelet recovery was 42 days (range, 26–148 days). In the univariate analysis, the factors statistically associated with platelet recovery were the dose of NC and GM colonies before cryopreservation (Figures 2b and c). On day 180, the incidence of platelet recovery was 86% (95% CI, 76–100%) for the patients who received more than 3.0×10^7 per kg NCs compared to 61% (95% CI, 49–73%) for those who received a lower dose ($P = 0.00002$). Additionally, the incidence of platelet recovery was 83% (95% CI, 66–100%) for patients who received more than 25×10^3 per kg of GM colonies, whereas it was 63% (95% CI, 50–76%) in those who received fewer ($P = 0.0062$). No statistical significance was found in the association of platelet recovery with dose of $CD34^+$ cells, HLA disparities, type of pretreatment or duration from diagnosis to UCBT. Multivariate analysis demonstrated that NC dose was the only factor significantly influenced by platelet recovery (Table 3).

Acute and chronic GVHD

Acute GVHD was observed in 38 of the 58 living patients with neutrophil engraftment (13 had grade I; 18, grade II; 5, grade III; 2, grade IV), whereas 20 had no acute GVHD. The cumulative incidence of acute GVHD at grade II or higher before day 100 was 47% (95% CI, 36–58%). We did not find a statistical association of HLA (AB-DRB1) disparities in a GVHD direction with the incidence of acute GVHD, although a trend towards a lower incidence of severe, acute GVHD was observed in less mismatched transplants. Chronic GVHD was found in 15 of the 57 patients who were alive and obtained engraftment (13 with limited disease; 2 with extensive disease).

TRM and cause of death

The respective 100-day and 1-year cumulative incidences of TRM were 20% (95% CI, 11–29%) and 25% (95% CI, 16–34%), respectively. When we separated the three age groups including children, young adults and older adults, the incidence of TRM in children was comparable to that in the young adults. The cumulative incidence of TRM at 1 year was 11% (95% CI, 0–33%) in children, 20% (95% CI, 9–30%) in young adults and 49% (95% CI, 25–73%) in older adults ($P = 0.026$). No significant difference was observed in TRM incidence by regimen (myeloablative (23%) vs reduced-intensity regimen (26%), $P = 0.5$). Even though the older-adults group received a greater percentage

Table 3 Multivariate analyses of risk factors for the main outcomes after unrelated cord blood transplant for CML

Factors		*Hazard ratio*	*(95% CI)*	P-*value*
Neutrophil recovery				
NC dose ($\times 10^7$ per kg)	<3.0	1		
	>3.0	2.27	1.69–2.84	0.0055
Platelet recovery				
NC dose ($\times 10^7$ per kg)	<3.0	1		
	>3.0	2.4	1.83–2.98	0.0029
TRM				
Age (years)	<50	1		
	>50	4.15	2.89–5.21	0.017
GM colony dose ($\times 10^3$ per kg)	<25	1		
	>25	0.06	0–1.98	0.0052
Disease stage at UCBT	CP to AP	1		
	BC	2.28	1.18–3.38	0.085
LFS				
NC dose ($\times 10^7$ per kg)	<3.0	1		
	>3.0	0.32	0.14–0.78	0.012
Disease stage at UCBT	CP/AP	1		
	BC	1.97	1.09–3.56	0.024
EFS				
NC dose ($\times 10^7$ per kg)	<3.0	1		
	>3.0	0.29	0.12–0.68	0.0045
Disease stage at UCBT	CP/AP	1		
	BC	1.81	1.05–3.12	0.032
Overall survival				
Disease stage at UCBT	CP/AP	1		
	BC	3.23	1.66–6.29	0.00058

Abbreviations: AP = accelerated phase; BC = blastic crisis; CP = chronic phase; CI = confidential interval; EFS = event-free survival; LFS = leukaemia-free survival; NCs = nucleated cells; TRM = transplantation-related mortality; UCBT = unrelated cord blood transplantation.

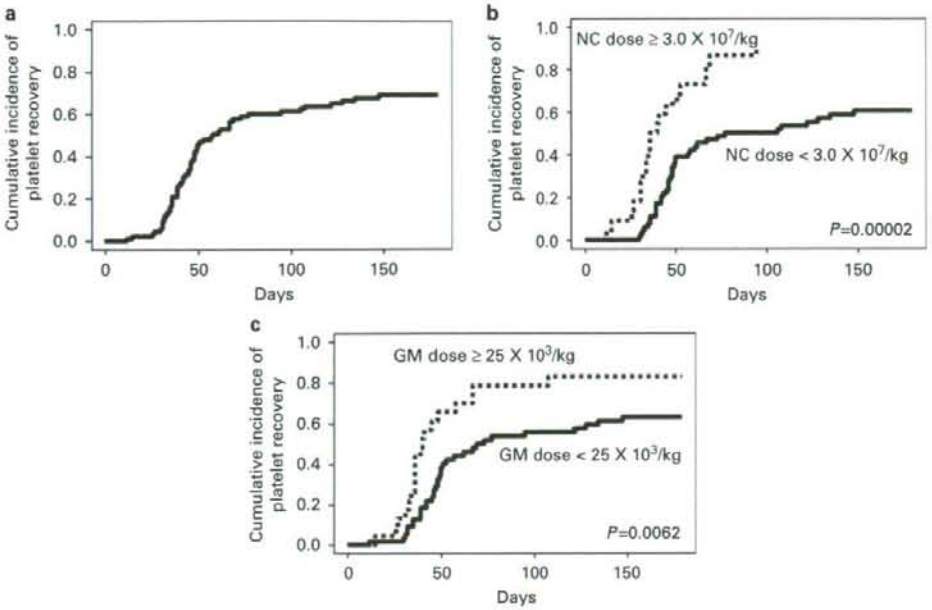

Figure 2 The cumulative incidence of platelet recovery. (**a**) Cumulative incidence of platelet recovery (**b**) by the dose of nucleated cells (NCs) and (**c**) by the GM colonies before the cryopreservation.

of the reduced-intensity regimen (12 patients with reduced-intensity and 7 patients with the myeloablative regimen), our data showed that old age (51 years or older) had a significant influence on the TRM. The cumulative incidence of TRM in older adults at 1 year was 49 ± 24, and 18 ± 10% in those younger than 50 years of age ($P = 0.0086$; Figure 3a). The cumulative incidence of TRM by disease stage at UCBT was 34 ± 16% in BC, 16 ± 21% in AP and 8 ± 9% in CP ($P = 0.019$; Figure 3b). Results of the multivariate analyses showed that older age and the dose of GM colonies were factors associated with a significantly increased risk of TRM. The disease stage at UCBT was only marginally associated with an increased risk of TRM (Table 3).

A total of 38 patients died and their causes of death are shown in Table 4. Five patients died of GVHD-related complications, two of veno-occlusive disease, two of bleeding, nine of infectious complications (bacterial, five; bacteria plus fungus, one; fungus, one; tuberculosis, one; viral encephalitis, one), one of tacrolimus-related encephalopathy and seven of graft failure-related complications. In total, ten patients died of relapse or induction failure, and one died of TRM after a second UCBT to treat the relapse. One patient died of the original malignancy, colon cancer.

Relapse incidence

In total, 29 patients had cytogenetic, molecular and/or haematological relapse after UCBT, as defined in Patients, materials and methods. The 2-year cumulative relapse incidence was 37% (95% CI, 26–48%). The only factor significantly associated with increased relapse incidence was NC-dose before cryopreservation of less than 3.0×10^7 per kg (44 ± 13 vs 9 ± 12%; $P = 0.01$; Figure 3c). Patients with an advanced stage (AP and BC) of disease at transplantation showed a trend towards earlier relapse compared to those in CP at transplantation; however, no significant difference was observed 1 year after UCBT (Figure 3d).

In 29 patients who relapsed after CBT, 21 relapsed haematologically, whereas 8 patients had a molecular/cytogenetic relapse. In total 3 of the 21 patients with haematological relapse received the second HSCT; others received chemotherapy and/or STI or IFN. Five patients achieved haematological remission while still alive, but 15 died of relapse or of the second HSCT-related mortality. Among eight patients with molecular/cytogenetic relapse (one in first CP, six in second CP and one in second AP at the time of CBT), three patients received STI, two chemotherapy and one STI and chemotherapy, when minimal residual disease was found. Only two patients developed into haematological relapse, whereas the others continued in remission.

Event-free, leukaemia-free and overall survival

Estimated 2-year EFS, LFS and OS were 34 ± 6, 38 ± 6 and 53 ± 6%, respectively (Figure 4a). Results of univariate and multivariate analyses showed that the disease stage at UCBT significantly influenced the LFS, EFS and OS (Table 3). Two-year LFS was 52% (95% CI, 37–73%) for patients in CP at the time of transplantation, 38% (95%

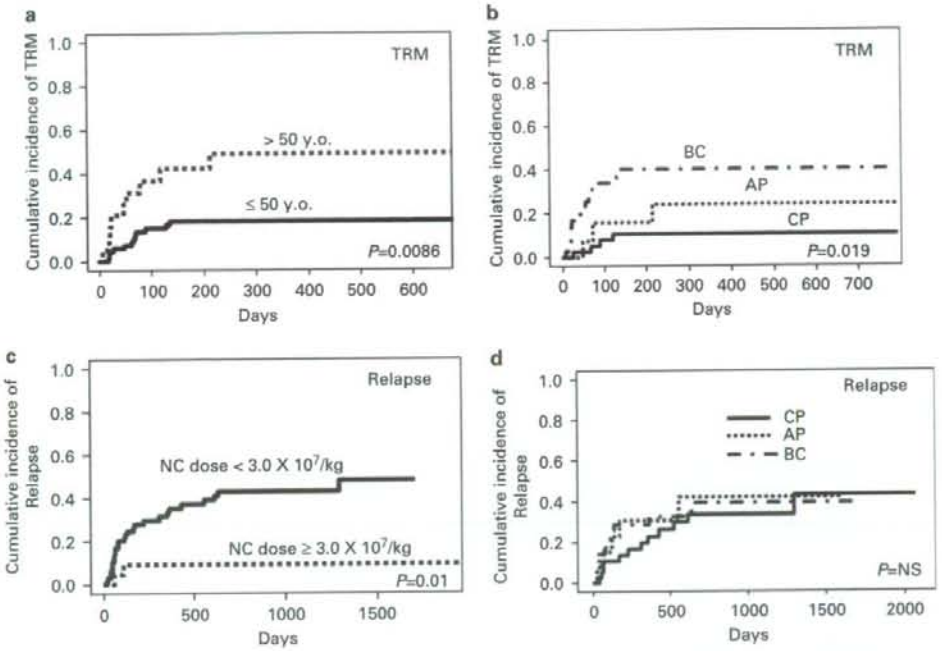

Figure 3 The cumulative incidence of transplant-related mortality (TRM) and relapse. Cumulative incidences of TRM are shown by age (**a**) and by the stage at unrelated cord blood transplantation (UCBT) (**b**). Cumulative incidences of relapse are shown by the dose of nucleated cells (NCs) before cryopreservation (**c**) and by disease stage at UCBT (**d**).

Table 4 Cause of death

Causes	n = *38*
Transplantation-related mortality	26
GVHD	5
VOD	2
Bleeding	2
Infection (5 bacterial, 1 bacteria plus fungus, 1 fungus, 1 tuberculosis, 1 viral encephalitis)	9
Tacrolimus-induced encephalopathy	1
Graft failure-related complications	7
Relapse or induction failure	10
Others	2
Post-second UCBT for relapse, TRM	1
Other malignancy	1

Abbreviations: TRM = transplantation-related mortality; UCBT = unrelated cord blood transplantation; VOD = veno-occlusive disease.

CI, 17–84%) for those in AP and 22% (95% CI, 10–48%) for those in BC ($P = 0.0004$; Figure 4b). The probability of 2-year OS for patients in CP, AP and BC was 71% (95% CI, 56–90%), 59% (95% CI, 37–94%) and 32% (95% CI, 20–55%), respectively ($P = 0.0004$; Figure 4e). The probability of survival remained higher for the patients in CP to AP at UCBT compared to LFS because the molecular or cytogenetic relapse in CP or AP might not be fatal. Note that NC-dose before cryopreservation also significantly affected the LFS and EFS (Table 3). Two-year EFS was 20% (95% CI, 12–35%) for the patients who received less than 3.0×10^7 per kg NCs before cryopreservation, whereas it was 68% (95% CI, 48–96%) for those who received more than 3.0×10^7 per kg NCs ($P = 0.0005$; Figure 4c). A marginally significant influence on 2-year EFS was observed if a patient was in the youngest age group (74; 95% CI, 48–100%) compared to those of young adults (33; 95% CI, 22–49%) and older adults (15; 95% CI, 3–72%; $P = 0.049$; Figure 4d). Hence, no significant influence by age was found in multivariate analysis for LFS, EFS and overall survival.

Pre-transplant risk scoring system of EBMT and TRM, LFS and OS

The pre-transplant risk scoring system was established by the EBMT to evaluate patients with CML who are candidates for HSCT.[28,29] We adjusted the data of the patients enrolled in the JCBBN to conform to the EBMT scoring system. The scores of all patients were raised by one point because of unrelated donors. When the EBMT scoring system was applied, 1 had a score of 1, 10 had scores of 3, 18 had scores of 4, 25 had scores of 5, 21 had scores of 6 and 10 had scores of 7 (Table 1). The outcome of TRM and survival had a tendency to be correlated with the pre-transplant scores. We compared the patients with scores of 0–4 ($n = 30$) vs the patients with scores of 5–7 ($n = 56$). The patients with a score of 5–7 had a significantly higher incidence of TRM and an unfavourable survival rate. The 1-year cumulative incidences of TRM were 7%

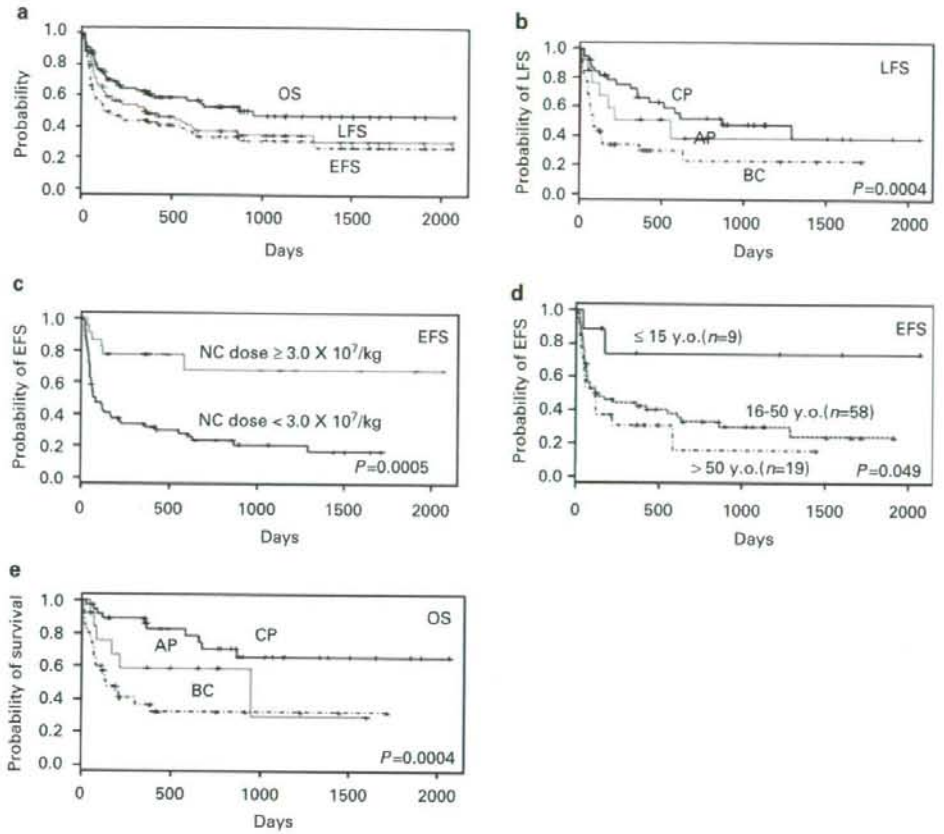

Figure 4 Kaplan–Meier estimate of leukaemia-free survival (LFS), event-free survival (EFS) and overall survival (OS). Overall LFS, EFS and OS are shown in (**a**). (**b**) LFS is shown by disease stage (chronic phase, CP; accelerated phase, AP and blast crisis, BC) at unrelated cord blood transplantation (UCBT). (**c**) EFS is shown by the dose of nucleated cells (NCs), NC dose of 3×10^7 per kg or more vs a lower dose. (**d**) Probability of EFS by age showed a marginally significant difference between children and adults. (**e**) OS is indicated by disease stage at UCBT.

(95% CI, 0–16%) for the patients with a score of 0–4 and 34% (95% CI, 21–47%) for those with a score of 5–7 ($P = 0.005$; Figure 5a). The 2-year survival rate was 66% (95% CI, 48–89%) for patients with a score of 0–4 and 47% (95% CI, 35–63%) for those with a score of 5–7 (Figure 5b).

Discussion

Reports of UCBT in patients with CML are limited. Sanz *et al.*[24] reported on nine cases from a single institute, but the remaining cases were reported only as part of a heterogeneous series of patients with different diseases[3] or as anecdotal case studies. To our knowledge, this retrospective registry-based analysis is the first that was specifically designed to describe the results of UCBT in patients with CML.

As expected, administering higher doses of NC and GM colonies before cryopreservation produced significantly quicker and more effective myeloid and platelet engraftment; however, the dose of GM colonies was not statistically significant because of missing data. Patients (14 adults and 8 children) who received more than 3.0×10^7 per kg NCs achieved the most successful myeloid and platelet engraftments. On the basis of these data, adult patients and their physicians are encouraged to obtain units containing a higher NC count. In contrast to previous studies,[30,31] $CD34^+$ cells did not have a significant influence on the incidence of myeloid engraftment. As with the dose of GM colonies, $CD34^+$ cell data were missing for several patients and different assay systems for $CD34^+$ analysis were used in each bank. Engraftment was influenced neither by duration from diagnosis to UCBT nor by prior therapy for CML. The patient with the longest duration from diagnosis to UCBT (17.6 years) achieved successful engraftment. Although the disease stage of CML at UCBT tended to influence the engraftment rate, no significant difference was detected (data not shown; $P = 0.08$). These results are encouraging for patients

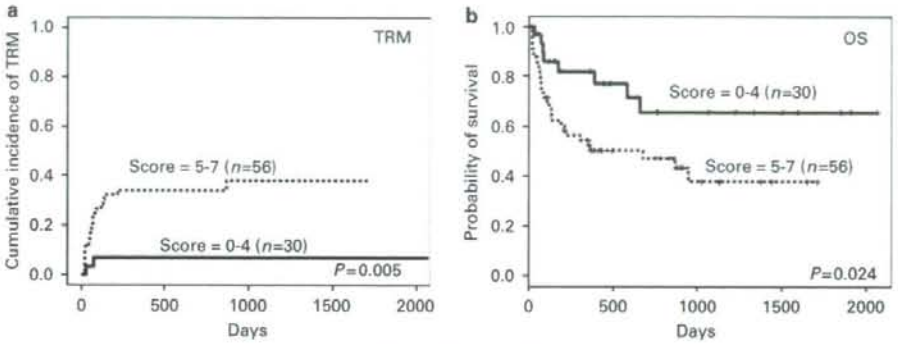

Figure 5 Transplant-related mortality (TRM) and overall survival (OS) according to the pre-transplant risk score of the European Group for Blood and Marrow Transplantation (EBMT). (**a**) TRM by pre-transplant risk score of the EBMT (0–4 vs 5–7). (**b**) Survival by pre-transplant risk score of the EBMT (0–4 vs 5–7).

with CML who are treated with imatinib or IFN before UCBT.

As expected, we found that an advanced disease stage at UCBT was significantly associated with lower LFS, EFS and OS. Moreover, although the disease stage of CML at UCBT did not have a significant influence on the cumulative incidence of relapse, it seemed to influence TRM (Figure 3b). However, in the multivariate analysis, the only significant factors that influenced higher TRM were age of 50 years or older and dose of GM colonies at less than 25×10^3 per kg (Table 3). The collinearity between older age and advanced stage of the disease may have had a large influence on this part of the analysis. In comparison to previous studies on unrelated BMT,[32–34] OS in UCBT seemed comparable to that in unrelated BMT. Two-year survival rates varied approximately 44–77% for patients in CP with unrelated BMT, whereas a 71% survival rate was observed for those in CP with UCBT. The finding that young age (1–15 years) indicated high EFS (74% at 2 years) with only marginal significance by univariate, but not by multivariate, analysis may have occurred because children included a relatively low percentage of the advanced disease cohort at CBT and a relatively high dose of NCs compared to the adults. Note that an NC dose higher than 3.0×10^7 per kg before cryopreservation improved the outcome of myeloid and platelet engraftment, the relapse rate and LFS. Dini *et al.*[35] reported that a NC dose higher than 3×10^8 per kg was associated with LFS in unrelated BMT, although the NC dose in UCBT was one log less than in UBMT; however, the dose of GM colonies did not influence the relapse and DFS. This discrepancy in the influence of the dose of GM colonies on engraftment and relapse suggests that non-myeloid stem cells in NC, such as T cells, are important in the immune system after HSCT.[36]

The most likely causes of death in UCBT for patients with CML were related to transplantation rather than relapse. The high incidence of rejection-related complications, such as infections and bleeding, remains to be resolved; however, unexpectedly, the incidence of GVHD as the cause of death was relatively higher in UCBT. CML is the only haematopoietic malignancy shown to respond effectively to immunotherapy with donor lymphocyte infusion after allogeneic HSCT.[22,23] Some unintended factors by physicians might promote the development of GVHD, although we could not assess this concern in detail in this retrospective registry-based analysis. To clarify this important issue, carefully designed prospective trials are needed.

We also demonstrated that the pre-transplant risk scoring system established by the EBMT for CML[28] can be used to predict overall risk for UCBT. This scoring system is based on previously reported pre-transplant risk factors including histocompatibility, disease stage at the time of transplantation, age and sex of the donor and recipient and time from diagnosis to transplantation. According to this scoring system, we classified the patients using a 7-level risk score. Although a greater number of patients are needed to properly evaluate this scoring system, the group that scored 0–4 showed significantly better LFS, OS and TRM compared to the group that scored 5–7, but the TRM of the higher scoring group was much lower (40%) compared to a previous report (75%).[28] The lower TRM we observed may explain why advanced GVHD was limited and why imatinib, not intensive chemotherapy, was initiated prior to CBT.[37] This scoring system might provide a rational basis for counselling before UCBT as well as other HSCT; however, it does not address donor-side haematopoietic unit data, which is absolutely essential in the selection of CB.

The finding that the duration from diagnosis to UCBT did not affect the main outcome encouraged us to use targeted therapy, such as imatinib, even for patients with advanced-stage CML before transplantation. Furthermore, the patients with cytogenetic/molecular relapse after CBT could be started on imatinib therapy as we describe in this study. Recent evidence suggests that kinase inhibitors, such as imatinib, suppress T-cell functions and immunoglobulin production by B cells.[38,39] At present, how long patients will need to take imatinib is unclear, but the combination of imatinib and HSCT clearly results in improved survival in patients with CML.

We conclude that UCBT is an encouraging alternative treatment for patients with CML who do not have an identical sibling and/or those who cannot be expected to obtain a favourable cytogenetic response with imatinib treatment.

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References

1 Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A *et al.* Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N Engl J Med* 1989; **321**: 1174–1178.

2 Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D *et al.* Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Natl Acad Sci USA* 1989; **86**: 3828–3832.

3 Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, Migliaccio AR *et al.* Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med* 1998; **339**: 1565–1577.

4 Gluckman E, Rocha V, Arcese W, Michel G, Sanz G, Chan KW *et al.* Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice. *Exp Hematol* 2004; **32**: 397–407.

5 Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A *et al.* Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 2004; **351**: 2276–2285.

6 Talpaz M, Kantarjian H, Kurzrock R, Trujillo JM, Gutterman JU. Interferon-alpha produces sustained cytogenetic responses in chronic myelogenous leukemia. Philadelphia chromosome-positive patients. *Ann Intern Med* 1991; **114**: 532–538.

7 Lee MS, Kantarjian H, Talpaz M, Freireich EJ, Deisseroth A, Trujillo JM *et al.* Detection of minimal residual disease by polymerase chain reaction in Philadelphia chromosome-positive chronic myelogenous leukemia following interferon therapy. *Blood* 1992; **79**: 1920–1923.

8 Kantarjian HM, Cortes JE, O'Brien S, Giles F, Garcia-Manero G, Faderl S *et al.* Imatinib mesylate therapy in newly diagnosed patients with Philadelphia chromosome-positive chronic myelogenous leukemia: high incidence of early complete and major cytogenetic responses. *Blood* 2003; **101**: 97–100.

9 Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. *Blood* 2005; **105**: 2640–2653.

10 Cortes J, Talpaz M, O'Brien S, Jones D, Luthra R, Shan J *et al.* Molecular responses in patients with chronic myelogenous leukemia in chronic phase treated with imatinib mesylate. *Clin Cancer Res* 2005; **11**: 3425–3432.

11 Kantarjian H, Talpaz M, O'Brien S, Giles F, Faderl S, Verstovsek S *et al.* Survival benefit with imatinib mesylate therapy in patients with accelerated-phase chronic myelogenous leukemia—comparison with historic experience. *Cancer* 2005; **103**: 2099–2108.

12 Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N *et al.* Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med* 2006; **355**: 2408–2417.

13 Cortes J, O'Brien S, Kantarjian H. Discontinuation of imatinib therapy after achieving a molecular response. *Blood* 2004; **104**: 2204–2205.

14 Merante S, Orlandi E, Bernasconi P, Calatroni S, Boni M, Lazzarino M. Outcome of four patients with chronic myeloid leukemia after imatinib mesylate discontinuation. *Haematologica* 2005; **90**: 979–981.

15 Bartholomeusz GA, Talpaz M, Kapuria V, Kong LY, Wang S, Estrov Z *et al.* Activation of a novel Bcr/Abl destruction pathway by WP1130 induces apoptosis of chronic myelogenous leukemia cells. *Blood* 2007; **109**: 3470–3478.

16 Ray A, Cowan-Jacob SW, Manley PW, Mestan J, Griffin JD. Identification of Bcr/Abl point mutations conferring resistance to the Abl kinase inhibitor AMN107 (Nilotinib) by a random mutagenesis study. *Blood* 2007; **109**: 5011–5015.

17 Hochhaus A, Erben P, Ernst T, Mueller MC. Resistance to targeted therapy in chronic myelogenous leukemia. *Semin Hematol* 2007; **44** (1 Suppl 1): 15–24.

18 Kimura S, Ashihara E, Maekawa T. New tyrosine kinase inhibitors in the treatment of chronic myeloid leukemia. *Curr Pharm Biotechnol* 2006; **7**: 371–379.

19 Weisberg E, Catley L, Wright RD, Moreno D, Banerji L, Ray A *et al.* Beneficial effects of combining nilotinib and imatinib in preclinical models of BCR-ABL+ leukemias. *Blood* 2007; **109**: 2112–2120.

20 Weisser M, Schleuning M, Haferlach C, Schwerdtfeger R, Kolb HJ. Allogeneic stem-cell transplantation provides excellent results in advanced stage chronic myeloid leukemia with major cytogenetic response to pre-transplant imatinib therapy. *Leuk Lymphoma* 2007; **48**: 295–301.

21 Koh LP, Hwang WY, Chuah CT, Linn YC, Goh YT, Tan CH *et al.* Imatinib mesylate (STI-571) given concurrently with nonmyeloablative stem cell transplantation did not compromise engraftment and resulted in cytogenetic remission in a patient with chronic myeloid leukemia in blast crisis. *Bone Marrow Transplant* 2003; **31**: 305–308.

22 Simula MP, Marktel S, Fozza C, Kaeda J, Szydlo RM, Nadal E *et al.* Response to donor lymphocyte infusions for chronic myeloid leukemia is dose-dependent: the importance of escalating the cell dose to maximize therapeutic efficacy. *Leukemia* 2007; **21**: 943–948.

23 de Weger RA, Tilanus MG, Scheidel KC, van den Tweel JG, Verdonck LF. Monitoring of residual disease and guided donor leucocyte infusion after allogeneic bone marrow transplantation by chimaerism analysis with short tandem repeats. *Br J Haematol* 2000; **110**: 647–653.

24 Sanz GF, Saavedra S, Jimenez C, Senent L, Cervera J, Planelles D *et al.* Unrelated donor cord blood transplantation in adults with chronic myelogenous leukemia: results in nine patients from a single institution. *Bone Marrow Transplant* 2001; **27**: 693–701.

25 Rubinstein P, Dobrila L, Rosenfield RE, Adamson JW, Migliaccio G, Migliaccio AR *et al.* Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci USA* 1995; **92**: 10119–10122.

26 Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA *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.

27 Michel G, Rocha V, Chevret S, Arcese W, Chan KW, Filipovich A *et al.* Unrelated cord blood transplantation for childhood acute myeloid leukemia: a Eurocord Group analysis. *Blood* 2003; **102**: 4290–4297.

28 Gratwohl A, Hermans J, Goldman JM, Arcese W, Carreras E, Devergie A *et al*. Risk assessment for patients with chronic myeloid leukaemia before allogeneic blood or marrow transplantation. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Lancet* 1998; **352**: 1087–1092.

29 Gratwohl A, Brand R, Apperley J, Crawley C, Ruutu T, Corradini P *et al*. Allogeneic hematopoietic stem cell transplantation for chronic myeloid leukemia in Europe 2006: transplant activity, long-term data and current results. An analysis by the chronic leukemia working party of the European Group for Blood and Marrow Transplantation (EBMT). *Haematologica* 2006; **91**: 513–521.

30 Nagamura-Inoue T, Shioya M, Sugo M, Cui Y, Takahashi A, Tomita S *et al*. Wash-out of DMSO does not improve the speed of engraftment of cord blood transplantation: follow-up of 46 adult patients with units shipped from a single cord blood bank. *Transfusion* 2003; **43**: 1285–1295.

31 Wagner JE, Barker JN, DeFor TE, Baker 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.

32 Reiter E, Greinix HT, Keil F, Brugger S, Rabitsch W, Schulenburg A *et al*. Long-term follow-up of patients after related- and unrelated-donor bone marrow transplantation for chronic myelogenous leukemia. *Ann Hematol* 1999; **78**: 507–513.

33 Barker JN, Davies SM, DeFor TE, Burns LJ, McGlave PB, Miller JS *et al*. Determinants of survival after human leucocyte antigen-matched unrelated donor bone marrow transplantation in adults. *Br J Haematol* 2002; **118**: 101–107.

34 Morishima Y, Yabe T, Matsuo K, Kashiwase K, Inoko H, Saji H *et al*. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. *Biol Blood Marrow Transplant* 2007; **13**: 315–328.

35 Dini G, Lamparelli T, Rondelli R, Lanino E, Barbanti M, Costa C *et al*. Unrelated donor marrow transplantation for chronic myelogenous leukaemia. *Br J Haematol* 1998; **102**: 544–552.

36 Molldrem JJ, Lee PP, Wang C, Felio K, Kantarjian HM, Champlin RE *et al*. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. *Nat Med* 2000; **6**: 1018–1023.

37 Deininger M, Schleuning M, Greinix H, Sayer HG, Fischer T, Martinez J *et al*. The effect of prior exposure to imatinib on transplant-related mortality. *Haematologica* 2006; **91**: 452–459.

38 Seggewiss R, Lore K, Greiner E, Magnusson MK, Price DA, Douek DC *et al*. Imatinib inhibits T-cell receptor-mediated T-cell proliferation and activation in a dose-dependent manner. *Blood* 2005; **105**: 2473–2479.

39 Appel S, Balabanov S, Brummendorf TH, Brossart P. Effects of imatinib on normal hematopoiesis and immune activation. *Stem Cells* 2005; **23**: 1082–1088.