95°C; and 40 cycles consisting of 15 seconds at 95°C and 1 minute at 60°C. Data analysis was performed using ABI Prism sequence detection software (Applied Biosystems). Separate standard curves were generated for the test and reference *GAPDH* genes. Threshold cycle number (Ct) was determined, and the starting gene copy number relative to the reference was determined for each well using a standard curve. Normalized gene expression levels are given as the ratio between the mean value for the target gene and that for the *GAPDH* gene in each sample.

Western blotting

For Western blotting, 5×10^5 MSCs were collected and washed in PBS, and lysed in 100 μL cell lysis buffer for 30 minutes on ice. Cell lysis buffer consisted of 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM ethylenediamine tetraacetic acid, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, and 1 mM Na_3VO_4 containing a 1:50 dilution of protease inhibitor cocktail. Samples were centrifuged at 14,000*g* for 10 minutes at 4°C, and the supernatants were removed, snap-frozen, and stored at −30°C. After determination of the cell protein concentration using the Bio-Rad protein assay (Bio-Rad, Richmond, CA, USA), 20 μg cell protein samples was resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis for 60 minutes at 150 V, followed by electrophoretic transfer to nitrocellulose membranes at 0.5 mA/cm^2 for 120 minutes.

Membranes were then blocked overnight at 4°C in Tris-buffered saline containing 0.1% Tween 20 (pH 7.6, TBST) with 5% bovine serum albumin, and were then incubated with the respective primary antibodies at room temperature for 1 hour. Expression of *GATA-2* was analyzed using a rabbit polyclonal anti – *GATA-2* antibody (1:200; sc-9008, Santa Cruz Biotechnology, Santa Cruz, CA, USA). For control purposes, expression of actin was analyzed using a goat polyclonal anti-actin antibody (1:1,000; sc-1615, Vector Laboratories, Burlingame, CA, USA). After washing in TBST, secondary antibodies (anti-rabbit immunoglobulin G or anti-goat immunoglobulin G, 1:2,000; Vector Laboratories) were used to detect specific primary antibodies. Streptavidin-horseradish peroxidase conjugate (1:1,000; Life Technologies, GIBCO BRL, Gaithersburg, MD, USA) was used for detection of secondary antibodies.

Interferon-γ (IFN -γ) in MSC culture

MSCs (8×10^4/well) derived from normal subjects (n = 5) were suspended in six-well culture plates and cultured at 37°C in a 5% CO_2 incubator. At confluence, IFN-γ was added at a dose of 0, 10, 100, or 1,000 IU/mL. After 24 hours, stimulated MSCs were washed, trypsinized, and collected to extract RNA for detection of *GATA-2* expression by real-time PCR.

Statistical analysis

Statview version 5.0 (Abacus Concepts Inc., Berkeley, CA, USA) was used for all statistical analyses. To compare each group of gene expression, Fisher's protected least significant difference was used, and *t*-test was used to compare *GATA-2* expression after IFN-γ stimulation. *P* values <0.05 were defined as indicating significance.

Results

MSCs are typically identified by a combination of cell surface markers and their potential for multilineage differentiation. As shown in Figure 1, cultured MSCs expressed CD29, CD44, CD90, CD73, CD105, and CD166 surface markers, but did not express hematopoietic markers such as CD45, CD34, and CD14. Expression of surface markers did not differ between AA patients and normal subjects.

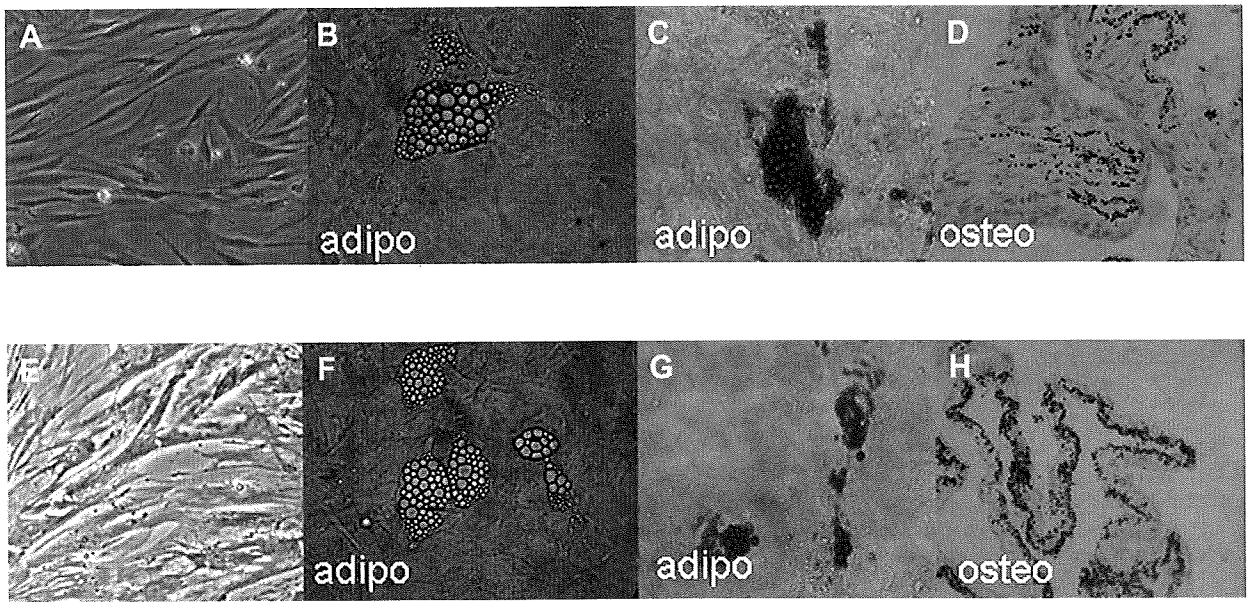

Figure 2. Cultured cells from normal subjects and patients with aplastic anemia were tested for their ability to differentiate. Derived cells from normal subjects (**A** – **D**) and patients (**E** – **H**) were each shown to differentiate into adipogenic (Adipo) and osteogenic (Osteo) lineages. (**A**) and (**E**) show original MSCs; adipogenesis was seen under inverted microscope (**B**,**F**) and Oil Red staining (**C**,**G**). Osteogenesis was indicated by an increase in calcium deposition (**D**,**H**).

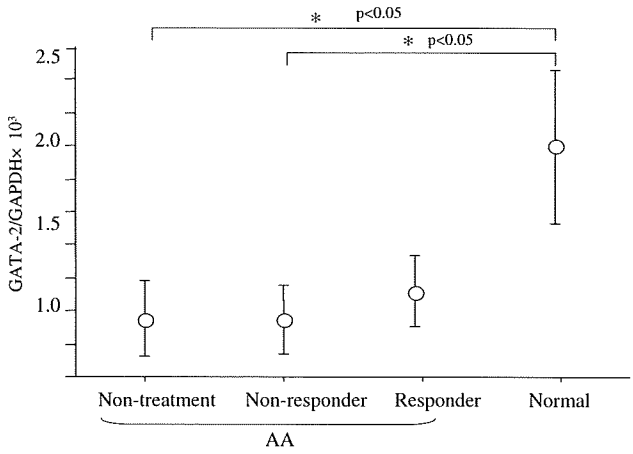

Figure 3. Expression of *GATA-2* in mesenchymal stem cells (MSCs) from aplastic anemia (AA) patients and normal subjects. Expression level was standardized with glyceraldehydes phosphate dehydrogenase (GAPDH) and was shown as a relative ratio. MSCs derived from nontreated patients and immunosuppressive therapy (IST) nonresponders expressed significantly lower amounts of *GATA-2* than normal subjects ($p < 0.05$). On the other hand, IST responder-derived MSCs expressed similar amounts of *GATA-2* when compared with normal subjects.

Cultured MSCs retained their capacity to differentiate into adipogenic and osteogenic lineages (Fig. 2).

GATA-2 expression was measured in third-passage MSCs from AA patients and normal subjects by real-time PCR. *GATA-2* expression levels were found to be significantly different between AA patients and normal subjects (Fig. 3). *GATA-2* levels in MSCs from AA patients before treatment were significantly lower than those in normal subjects ($0.95 \pm 0.23 \times 10^{-3}$ vs $2.00 \pm 0.47 \times 10^{-3}$; $p < 0.05$). Notably, *GATA-2* levels were not significantly different between normal subjects and IST responders ($2.00 \pm 0.47 \times 10^{-3}$ vs $1.12 \pm 0.22 \times 10^{-3}$; $p = 0.07$), while nonresponders had significantly lower *GATA-2* expression levels when compared to normal subjects ($0.95 \pm 0.21 \times 10^{-3}$ vs $2.00 \pm 0.47 \times 10^{-3}$; $p < 0.05$). We also demonstrated that the protein levels of *GATA-2* in MSCs derived from AA patients were lower than those from normal subjects by Western blot analysis (Fig. 4). In nontreated AA patients, both responders and nonresponders to IST, protein levels were lower than those in normal subjects.

Conversely, pretreatment levels of *PPARγ* in MSCs from AA patients were significantly higher than in those from normal subjects ($2.86 \pm 0.84 \times 10^{-2}$ vs $0.95 \pm 0.23 \times 10^{-2}$; $p < 0.05$). Although expression levels of *PPARγ* decreased in response to IST ($1.71 \pm 0.57 \times 10^{-2}$; $p = 0.38$), they remained high in nonresponders ($3.2 \pm 0.68 \times 10^{-2}$; $p < 0.05$) (Fig. 5).

In order to investigate the mechanism of downregulation in *GATA-2* expression, we cultured MSCs derived from normal subjects in the presence of IFN-γ. After IFN-γ stimulation with IFN-γ, *GATA-2* expression was downregulated based on the concentration of IFN-γ (Fig. 6). *GATA-2* expression was $12.37 \pm 0.66 \times 10^{-3}$ in the absence of IFN-γ, and was significantly lower in the presence of 10 IU/mL IFN-γ ($8.96 \pm 0.33 \times 10^{-3}$; $p < 0.05$), 100 IU/mL IFN-γ ($6.53 \pm 0.44 \times 10^{-3}$; $p < 0.01$) and 1,000 IU/mL IFN-γ ($6.03 \pm 0.52 \times 10^{-3}$; $p < 0.01$) when compared with control levels.

Discussion

MSCs isolated from human BM are capable of differentiating into several cell lineages. When cultured in adipogenic medium, MSCs differentiate into adipocytes and accumulate lipid vesicles in the cytoplasm. Because *GATA-2* is a transcriptional factor expressed in HSCs and various other stem cells, we hypothesized that *GATA-2* is also expressed in MSCs isolated from BM. To date, there have been no reports regarding *GATA-2* expression in human MSCs. *GATA-2* serves as a gatekeeper at the onset of adipocyte differentiation and is expressed in preadipocytes, but is downregulated when cells differentiate into mature adipocytes [14]. Preadipocytes are the main components of stromal cells, which form the

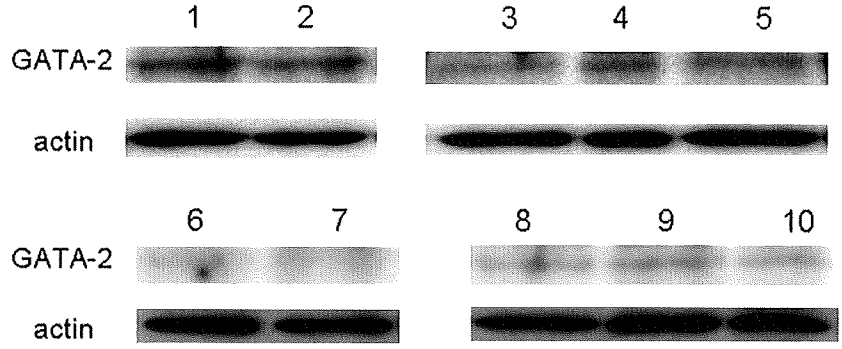

Figure 4. Western blot analysis of *GATA-2* protein in treated or nontreated patients and normal subjects. Whole-cell protein extracts were analyzed using a specific polyclonal anti – *GATA-2* antibody. Upper lanes show *GATA-2* expression; lower lanes show actin expression. Mesenchymal stem cells (MSCs) derived from normal subjects (lanes 1 and 2), nontreated patients (lanes 3, 4, and 5), immunosuppressive therapy (IST) nonresponders (lanes 6 and 7) and IST responders (lanes 8, 9, and 10) were used in Western blotting.

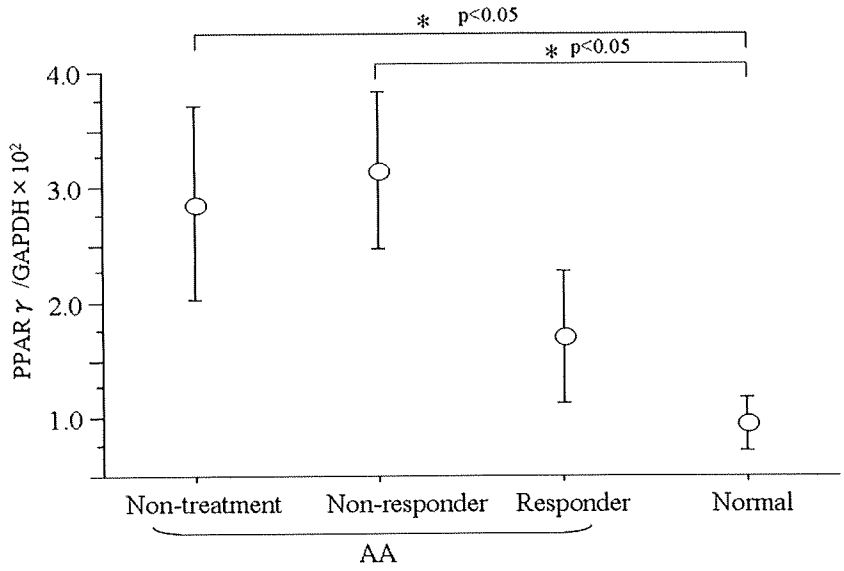

Figure 5. Expression of peroxisome proliferator-activated receptor−γ (*PPARγ*) in mesenchymal stem cells (MSCs) from patients with aplastic anemia (AA) and normal controls. Expression levels were standardized against glyceraldehydes phosphate dehydrogenase (GAPDH) and are shown as relative ratios. *PPARγ* expression of nontreated patients and immunosuppressive therapy (IST) nonresponders was significantly higher when compared with normal subjects ($p < 0.05$). However, in IST responders, *PPARγ* expression did not significantly differ from that in normal subjects.

microenvironment for hematopoiesis in the BM. In this study, we demonstrated that *GATA-2* is expressed in MSCs and that its expression in MSCs is significantly lower in AA patients when compared with normal subjects. *GATA-2* expression in purified CD34-positive cells was also markedly lower in AA patients [7], thus suggesting the presence of a common regulatory mechanism in both HSCs and MSCs.

IFN-γ plays a crucial role in immune-mediated suppression of HSCs in AA. In long-term culture of human BM, in which stromal cells were engineered to constitutively express IFN-γ, the output of long-term culture-initiating cells was markedly diminished, despite low concentrations of IFN-γ in the medium [15]. This implies a pathophysiological role for IFN-γ in the BM microenvironment of AA patients. Okitsu et al. [16] recently discussed the regulation of adipocyte differentiation in BM stromal cells by *GATA-2* using a mouse preadipocyte stromal cell line. The addition of IFN-γ to culture medium significantly suppressed expression of *GATA-2* in the cell line. In addition, they demonstrated that regulatory elements of the *GATA-2* gene in the stromal cell line are the same as those in hematopoietic cells [16]. These findings suggest that a common signal simultaneously suppresses expression of *GATA-2* in both HSCs and MSCs. We also found that when IFN-γ was added to culture medium, *GATA-2* expression of normal MSCs is downregulated.

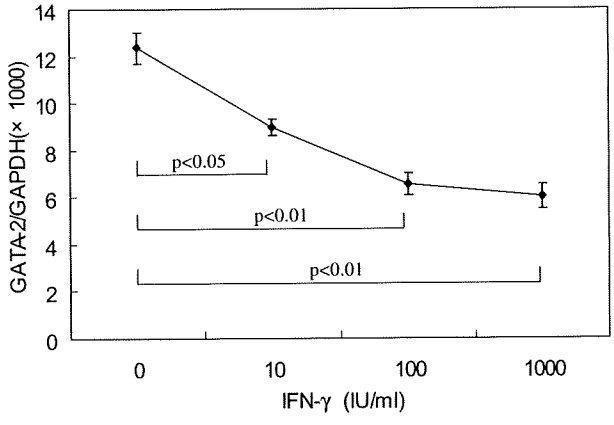

Figure 6. *GATA-2* expression after stimulation with interferon-γ (IFN-γ). Mesenchymal stem cells (MSCs) were stimulated with IFN-γ at various concentrations (0, 10, 100, 1000 IU/mL). In this figure, the relationship between IFN-γ concentration and *GATA-2* expression is shown. When MSCs were stimulated with different concentrations of IFN-γ, *GATA-2* expression was downregulated in a dose-dependent manner.

A single cell-derived colony of undifferentiated human MSCs simultaneously expressed genes characteristic of various committed mesenchymal cell lineages. One explanation for these findings is that MSCs individually entered into distinct differentiation programs, leading to generation of a molecularly heterogeneous population [17]. The adipogenic transcriptional factor *PPARγ* is detectable in human MSCs without external stimuli [11]. We demonstrated the expression of *PPARγ* in MSCs, and its expression was significantly elevated in AA patients when compared to normal subjects. Notably, expression of *GATA-2* increased, while expression of *PPARγ* decreased when patients responded to IST and became transfusion-independent. On the other hand, nonresponders continued to have lower *GATA-2* and higher *PPARγ* expression. This reciprocal expression of *GATA-2* and *PPARγ* is consistent with the

observation that *GATA-2* binds and suppresses the activity of the *PPARγ* promoter.

Taken together with previous reports, these results indicate that the pathological immune response increases levels of IFN-γ in the BM microenvironment, which decreases expression of *GATA-2* in both HSCs and MSCs in AA patients. Decreased *GATA-2* expression in HSCs may compromise hematopoietic function and increased *PPARγ* expression in MSCs may accelerate maturation of preadipocytes, leading to formation of fatty BM.

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Conflict of Interest Disclosure

No financial interest/relationships with financial interest relating to the topic of this article have been declared.

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Tacrolimus/Methotrexate versus Cyclosporine/ Methotrexate as Graft-versus-Host Disease Prophylaxis in Patients with Severe Aplastic Anemia Who Received Bone Marrow Transplantation from Unrelated Donors: Results of Matched Pair Analysis

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Tacrolimus (FK) and cyclosporine (CsA) have been shown to be effective in the prophylaxis of graft-versus-host disease (GVHD). However, no comparative studies have yet been conducted to examine the efficacy of FK/methotrexate (MTX) and CsA/MTX in patients with severe aplastic anemia (SAA) given unrelated donor bone marrow transplantation (U-BMT). We used matched-pair analysis to compare FK/MTX with CsA/MTX in patients with SAA who received U-BMT through the Japan Marrow Donor Program. Forty-seven pairs could be matched exactly for recipient age and conditioning regimens. Forty-five patients achieved engraftment in the FK group and 42 patients in the CsA group. The probability of grade II-IV acute GVHD (aGVHD) was 28.9% in the FK group and 32.6% in the CsA group ($P = .558$). The probability of chronic GVHD (cGVHD) was 13.3% in the FK group and 36.0% in the CsA group ($P = .104$). The 5-year survival rate was 82.8% in the FK group and 49.5% in the CsA group ($P = .012$). The study shows the superiority of FK/MTX over CsA/MTX in overall survival because of the lower incidence of transplantation-related deaths. A prospective randomized study comparing FK/MTX and CsA/MTX is warranted.

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KEY WORDS: Tacrolimus, Cyclosporine, Graft-versus-host disease, Prophylaxis, Aplastic anemia, Unrelated bone marrow transplantation

INTRODUCTION

Bone marrow transplantation (BMT) from a human-leukocyte antigen (HLA)-matched related donor is the treatment of choice for children and young adults with severe aplastic anemia (SAA) [1,2]. However, HLA-matched related donors are available for <30% of patients in developed countries. Immunosuppressive therapy (IST) has been used as an alternative treatment for patients without a HLA-matched related donor [3,4]. For nonresponders to IST, BMT from an unrelated donor (U-BMT) has been indicated [5]. Acute and chronic graft-versus-host disease (aGVHD, cGVHD) contribute to much of the morbidity and mortality associated with U-BMT. Effective prevention of these complications is therefore crucial for the success of U-BMT.

A combination of cyclosporine (CsA) and a short course of methotrexate (MTX) is the standard pharmacologic regimen for the prophylaxis of GVHD after BMT from both HLA-matched siblings and HLA-matched unrelated donors [6,7]. Tacrolimus (FK), a potent macrolide lactone immunosuppressant, inhibits T cell activation by forming a complex with FK binding protein-12, which blocks the serine-threonine phosphatase activity of calcineurin [8]. Although the mechanism of action is similar to that

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of CsA, the potency of FK in vitro is more than 100 times that of CsA [9]. This suggests that FK might also be more effective than CsA as GVHD prophylaxis in high-risk settings. In fact, in randomized studies of GVHD prophylaxis after matched related and unrelated BMT, the incidence of aGVHD was reduced in the treatment group receiving a combination of FK and a short course of MTX (FK/MTX) compared to the control group who received CsA/MTX [10-12]. However, although the number of deaths from GVHD was lower, an increased incidence of relapse was observed in the FK group, resulting in no difference in overall survival (OS) rate between the 2 groups. Nevertheless, because there is no risk of relapse in patients with nonmalignant disease, the results might be different in studies of malignant versus nonmalignant disease. Accordingly, FK/MTX could be associated with a lower incidence of aGVHD/cGVHD and better survival compared to CsA/MTX in patients with acquired SAA who received U-BMT.

METHODS

Patients and Controls

We collected U-BMT data from SAA patients who received FK/MTX for the prophylaxis of GVHD through the Japan Marrow Donor Program (JMDP) database. Forty-seven patients were recruited who underwent BMT between July 1997 and December 2002. For each patient receiving FK/MTX, we selected a control patient who received CsA/MTX for the prophylaxis of GVHD during the same period. Because our previous study identified that recipient age and conditioning regimens were the most important variables associated with treatment failure, we selected control patients matched for these 2 variables [13].

Transplantation data were collected using standardized forms provided by the JMDP. Baseline information and follow-up reports were submitted at 100 days, 6 months, 1 year, and then annually after transplantation. Analysis of patient outcome was performed using data from the last reported follow-up or the date of death.

Recipient-Donor HLA Matching

HLA matching between the recipient and donor was based on HLA serotyping according to the standard technique. In 69 (73%) of the 94 recipient-donor pairs, molecular analyses of HLA-A, -B, and -DRB1 loci were performed using DNA-based methods.

Transplantation Procedures

Various preconditioning regimens were used by individual transplantation centers and classified into 6 categories (Table 1): (1) cyclophosphamide (Cy; 120-200 mg/kg) + total body irradiation (TBI; 2-10 Gy) + limited field irradiation (LFI; 5-8 Gy) + antithymocyte globulin (ATG), (2) Cy + TBI + LFI, (3) Cy + TBI + ATG, (4) Cy + TBI, (5) Cy + LFI + ATG, and (6) Cy + LFI. For the prophylaxis of GVHD, FK was started at a dose of 0.03 mg/kg from day −1 and administered through continuous 24-hour i.v. infusion. Patients were converted from intravenous i.v. to oral intake when it could be tolerated at a ratio of 1:3 in 2 divided doses per day based on the last intravenous dose. Standard doses of CsA were 3 mg/kg by i.v. infusion and 6 mg/kg by oral intake. The MTX doses were 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11 after transplantation in both the FK and CsA groups.

Table 1. Patient/donor Characteristics and BMT Procedure

	Tacrolimus	Cyclosporine	P
Patient number	47	47	
Age (year)			.962
<10	11	11	
11-29	28	27	
>30	8	9	
Sex			.396
Male	31	27	
Female	16	20	
Recipient/donor sex			.71
Male/male	19	19	
Female/female	7	10	
Male/female	12	8	
Female/male	9	10	
HLA matching by DNA typing			.029
A, B, DRB1 match	20	34	
A mismatch	4	3	
B mismatch	7	0	
DRB1 mismatch	7	6	
2 alleles mismatch	3	2	
Unknown*	6	2	
Duration of disease before BMT			.359
1 year or less	6	11	
1-3 year	17	17	
3 year or more	24	19	
RBC transfusions before BMT			1
<20	7	7	
20 or more	38	38	
Unknown	2	2	
Platelet transfusions before BMT			.651
<20	9	7	
20 or more	36	36	
Unknown	2	4	
Conditioning regimens			1
Cy + TBI + LFI + ATG	3	3	
Cy + TBI + LFI	6	6	
Cy + TBI + ATG	18	18	
Cy + TBI	11	11	
Cy + LFI + ATG	3	3	
Cy + LFI	6	6	
Marrow cell dose			.764
<3 x 10^8/kg	14	13	
3 x 10^8/kg or more	30	32	
Unknown	3	2	

BMT indicates bone marrow transplantation; Cy, cyclophosphamide; TBI, total body irradiation; LFI, local field irradiation; ATG, antithymocyte globulin; RBC, red blood cell.
*HLA was serologically matched or 1-antigen mismatched in these donor-recipient pairs.

Definitions and Statistical Analysis

Engraftment was defined as achievement of a peripheral blood (PB) absolute neutrophil count (ANC) of more than 0.5×10^9/L for 3 consecutive days. In evaluation of engraftment, patients who died before day + 22 without engraftment were not considered evaluable. aGVHD and cGVHD were evaluated according to the standard criteria [14,15]. Patients who died before engraftment were excluded from the analysis of aGVHD. For analysis of cGVHD, only those who survived 100 days after transplantation were included. The probabilities of overall survival and aGVHD and cGVHD were estimated from the time of transplantation according to the Kaplan-Meier product-limit method. The χ^2 test and log-rank statistics were used to assess significance of differences in variables and outcomes between the 2 groups. All probability values were 2 sided, and $P < .05$ was considered significant.

RESULTS

Patient, Donor, and Transplantation Characteristics

Patient, donor, and transplantation characteristics of the study population are summarized in Table 1. There was an imbalance in HLA-A, -B, and -DRB1 allele mismatches, with 21 of the mismatch pairs observed in the FK group and 11 in the CsA group ($P = .029$). Other variables were comparable between the 2 groups.

Engraftment

Engraftment took place in 45 patients (96%) in the FK group and 42 patients (89%) in the CsA group. Three patients, 1 in the FK group and 2 in the CsA group, died before day 21 and were considered not evaluable for engraftment. One of the 46 evaluable patients in the FK group and 3 of the 45 evaluable patients in the CsA group failed to engraft. Another patient in the CsA group experienced late graft failure. The median time to neutrophil recovery was 18 days in the FK group (range: 10-28 days) and 17 days in the CsA group (range: 12-26 days) ($P = .400$).

aGVHD

The probability of grade II-IV aGVHD was 28.9% (range: 15.3%-42.5%) in the FK group and 32.6% (range: 18.4%-46.8%) in the CsA group at 100 days (Figure 1; $P = .558$). aGVHD developed at a median of 30 days (range: 7-76 days) in the FK group and 13 days (range: 5-28 days) in the CsA group after transplantation. The distribution of GVHD grade and organ involvement is presented in Table 2. Despite the imbalance in HLA disparity, the incidence of grade II-IV aGVHD in the FK group was equal to that in the CsA group.

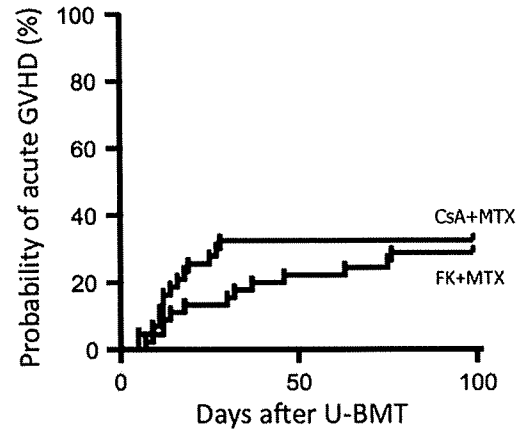

Figure 1. The probability of grade II-IV aGVHD in the FK/MTX group and the CsA/MTX group (28.9% versus 32.6%, $P = .558$).

cGVHD

Thirty-eight patients in the FK group and 35 in the CsA group were evaluable for cGVHD. Five patients in the FK group developed cGVHD at a median period of 4 months (range: 3-5 months) and 10 patients in the CsA group developed cGVHD at a median period of 4 months (range: 2-40 months). Overall, the probability of cGVHD was 13.3% (range: 2.1%-24.5%) in the FK group and 36.0% (range: 15.2%-56.8%) in the CsA group (Figure 2; $P = .104$). Three patients in the FK group and 4 in the CsA group developed an extensive type of cGVHD.

Survival

Of 47 patients in each group, 39 in the FK group survived at 4 to 61 months (median: 26 months), whereas 25 in the CsA group survived at 3 to 61 months (median: 38 months) after transplantation. The OS at 5 years was 82.8% (range: 71.9%-93.6%) in the FK group and 49.5% (range: 32.5%-66.4%) in the CsA group (Figure 3; $P = .012$). Eight patients in

Table 2. Distribution of Grade and Organ Involvement in Acute GVHD

	Tacrolimus (n = 47)	Cyclosporine (n = 47)
Grade		
0	22 (47%)	26 (55%)
I	7 (15%)	3 (6%)
II	8 (17%)	7 (15%)
III	4 (8%)	6 (13%)
IV	2 (5%)	1 (2%)
unevaluable	4 (8%)	4 (8%)
Organ involvement		
skin	5 (38%)	3 (21%)
skin + gut	6 (46%)	7 (50%)
gut + liver	1 (8%)	0 (0%)
skin + gut + liver	1 (8%)	4 (29%)

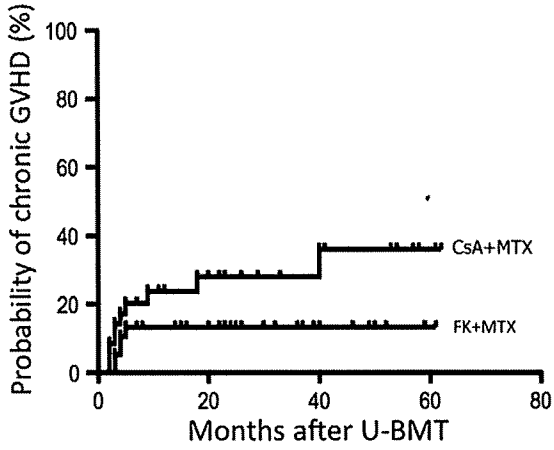

Figure 2. The probability of cGVHD in the FK/MTX group and the CsA/MTX group (13.3% versus 36.0%, $P = .104$).

the FK group and 22 in the CsA group died from transplantation-related toxicities ($P = .002$). Causes of death are summarized in Table 3. Graft failure and bacterial/fungal infection were the major causes of death.

DISCUSSION

Analyses of registration data suggest that the outcome of U-BMT in AA patients has substantially improved over the past 10 years. In analysis of 498 patients registered to the European Group for Blood and Marrow Transplantation (EBMT), 5-year survival increased from 32% ± 8% before to 57% ± 8% after 1998 [16]. Similarly, Maury et al. [17] analyzed the outcome of 89 patients in the French registry and found that 5-year survival increased from 29% ± 7% before and 50% ± 7% after 1998. An optimum conditioning regimen, GVHD prophylaxis and better donor selection may be responsible for these improvements.

In the late 1990s, HLA typing using molecular methods was introduced into clinical use. Matching for 10 alleles by high resolution technology replaced

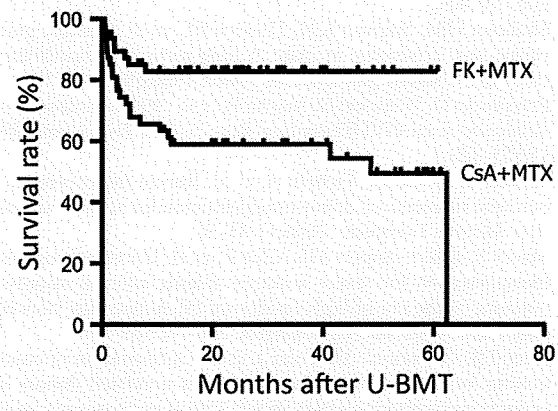

Figure 3. Kaplan-Meier estimates of OS in the FK/MTX group and the CsA/MTX group (82.8% versus 49.5%, $P = .012$).

Table 3. Primary Causes of Death

	Tacrolimus (n = 47)	Cyclosporine (n = 47)
Bacterial/fungal infection	4	4
Graft failure	1	4
Acute GVHD	1	2
Interstitial pneumonitis	0	3
Hemorrhage	1	1
EBLPD	1	1
Heart failure	0	2
Others	0	3
Total	8	22

EBLPD indicates Epstein Barr virus associated lymphoproliferative disorder; GVHD, graft-versus-host disease.

matching for 6 antigens by low resolution technology. A French study revealed that improved survival was associated with high-resolution HLA matching, suggesting that better donor selection might be a major factor in improving prognosis [17]. Recent attempts to improve the outcome in SAA patients include the use of low-dose TBI or a nonirradiation-fludarabine (Flu)-based regimen. In a prospective multicenter study sponsored by the National Marrow Donor Program (NMDP) using low-dose TBI, a low graft rejection rate of 5% and 5-year survival of 55% were achieved in 87 patients [18]. Moreover, a study by the EBMT using Flu, low-dose Cy and ATG showed a lower incidence of aGVHD and cGVHD and 5-year survival of 73% [19]. Although these novel pretransplant conditioning regimens are promising, all analyses failed to show the contribution of new regimens to the improved outcomes because of the small number of patients.

Different from patients with hematologic malignancies, there is no obvious benefit of GVHD for patients with AA. In fact, many studies have indicated adverse effects of aGVHD on the outcome of AA patients, suggesting that the most effective prophylactic regimen for GVHD should be employed for patients with AA. However, trials involved with lessening severe GVHD are limited in patients with AA. In a small number of studies, ex vivo T cell depletion by monoclonal antibodies (mAbs) or in vivo use of alemtuzumab instead of ATG has been attempted with encouraging results [20,21]. Although pharmacologic prevention with CsA/MTX is used as GVHD prophylaxis in the majority of AA patients, the role of alternative pharmacologic agents remains undetermined. Although previous randomized studies comparing CsA/MTX and FK/MTX did not show any survival benefits of FK despite a reduction in the incidence of aGVHD, most patients had malignant disease and only a few with AA were included [10-12].

The aim of the present study was to compare FK and CsA in the prophylaxis of GVHD using matched pair analysis. One drawback was the imbalance of HLA disparity between the 2 groups, with 21 mismatched pairs in the FK group and 11 in the CsA group. Our previous study showed that allelic mismatching of

HLA-A and -B antigens, but not HLA-DRB1 is the most crucial risk factor for survival of AA patients who received transplants from an unrelated donor [13]. More HLA class I mismatched pairs (HLA-A; 4, HLA-B; 7) were included in the FK group than in the CsA group (HLA-A; 3). Despite this disadvantage in terms of HLA disparity, the probability of grade II-IV aGVHD did not differ between the 2 groups. The probability of cGVHD tended to be marginally less in the FK group than in the CsA group ($P = .104$). The duration of CsA or FK after U-BMT may affect the incidences of cGVHD. However, we did not compare the difference of duration in this study because the actual duration of administration of these immunosuppressants was not available in our database.

The duration of follow-up in the FK506 group is less than in the CSP group. Although it may introduce a significant bias in the analysis, the current study showed that 5-year survival was significantly higher in the FK group than in the CsA group. Patients in the FK group showed a significant reduction in treatment-related mortality (TRM), resulting in better OS. To date, results of 3 previous randomized studies comparing FK and CsA have indicated a significantly lower incidence of aGVHD among patients receiving FK, but with no survival benefits having been demonstrated [10-12].

Yanada et al. [22] conducted a retrospective study comparing an FK-based regimen and CsA-based regimen for the prophylaxis of GVHD using registration data of the Japan Society for Hematopoietic Cell Transplantation (JSHCT). In their study, 777 patients who underwent BMT from an unrelated donor were analyzed (FK group: n = 191, CsA group; n = 586). Although the distribution of different diseases was not specified, the majority of the patients appeared to have hematologic malignancies. FK significantly reduced the risk of aGVHD and TRM without any increase in relapse, thus improving OS.

In conclusion, our matched pair analysis showed the superiority of FK/MTX over CsA/MTX in OS. However, our study was retrospective and a further study comparing FK/MTX and CsA/MTX as a prophylaxis of GVHD in AA patients who will receive U-BMT may be warranted.

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Outcome of 125 Children with Chronic Myelogenous Leukemia Who Received Transplants from Unrelated Donors: The Japan Marrow Donor Program

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Because of a small number of patients, only a few studies have addressed the outcome of bone marrow transplantation (BMT) in children with Philadelphia chromosome–positive (Ph+) chronic myelogenous leukemia (CML), who receive graft from a volunteer-unrelated donor (VUD), especially after practical application of imatinib mesylate. The outcomes of BMT from a VUD in 125 children with Ph+ CML were retrospectively reviewed. Patients were identified through the Japan Marrow Donor Program as having undergone BMT between 1993 and 2005 and were aged 1-19 years at the time of transplant (median age, 14 years). The probabilities of 5-year overall survival (OS) and leukemia-free survival (LFS) were 59.3% and 55.5%, respectively. Multivariate analysis identified the following unfavorable survival factors: infused total nucleated cell dose $< 3.14 \times 10^6$ /kg (relative risk [RR] = 2.43; 95% confidence interval [CI] = 1.33-4.44; $P = .004$), advanced phase (RR = 2.43; 95% CI = 1.37-4.31; $P = .004$), and no major cytogenetic response (MCyR) at the time of BMT (RR = 6.55; 95% CI = 1.98-21.6; $P = .002$). Of the 17 patients treated with imatinib, 15 (88%) achieved MCyR at the time of BMT, and this group had an excellent 5-year OS of 81.9%. Disease phase, infused total nucleated cell dose, and cytogenetic response were independent risk factors for survival of unrelated BMT. These findings provide important information for assessing the indications for and improving outcome in unrelated BMT for the treatment of pediatric CML.

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KEY WORDS: Chronic myelogenous leukemia, Children, Unrelated donor, Stem cell transplantation, Bone marrow transplantation, Japan Marrow Donor Program

INTRODUCTION

Philadelphia-positive (Ph+) chronic myelogenous leukemia (CML) is a rare disease in children, accounting for only 3%-5% of all pediatric leukemia, with a incidence of <1 in 100,000 children [1]. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only proved curative treatment for children with Ph+ CML. Reported event-free survival (EFS) in children with Ph+ CML who underwent transplantation in the

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chronic phase with a matched related donor is 60%-75% [2-4]; however, this approach is limited by the availability of HLA-matched family donors. The majority of children who lack an HLA-matched donor receive a transplant from an alternative donor, such as a volunteer-unrelated donor (VUD). EFS is less favorable in this setting, ranging from 30% to 55% [3-5].

Since the introduction of the novel tyrosine kinase inhibitor imatinib mesylate, the treatment for Ph+ CML has been completely revised [6]. Imatinib can induce complete hematologic and cytogenetic remission in the majority of patients, and follow-up data on patients treated only with imatinib indicate that complete cytogenetic and major molecular responses are durable, while drug toxicity is low [7]. The number of transplantations for Ph+ CML has declined rapidly [8]. But, despite significant cytogenetic and molecular responses, there is no evidence that imatinib is curative, and imatinib's long-term side effects remain to be determined. Some patients have successfully stopped imatinib without recurrence, but some who were polymerase chain reaction (PCR)-negative for a period stopped and then experienced recurrence [9,10]. Stopping imatinib may be possible, but effective strategies have yet to be developed.

This is particularly important for pediatric patients, in whom the goal is cure of the disease rather than palliation, and for whom long-term survival is particularly anticipated. The presence of molecular disease and the emergence of resistant clones in patients treated with imatinib suggest the need for caution with regard to abandoning curative therapy by SCT. The need for information on the current status of SCT for Ph+ CML and up-to-date results when considering the treatment of children with Ph+ CML, even in the imatinib era, is evident; however, few studies have specifically analyzed outcomes of SCT in children with Ph+ CML [2-5]. The aim of the present study was to analyze data from 125 children with Ph+ CML who underwent bone marrow transplantation (BMT) from a VUD and identify factors influencing outcome.

PATIENTS AND METHODS

Patients

A retrospective analysis was conducted on behalf of the Japan Marrow Donor Program (JMDP) and the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) CML Committee. Data were collected from 125 children (age at transplantation < 20 years) whose donors were identified through the JMDP and who underwent allogeneic BMT from a VUD for Ph+ CML between 1993 and 2005. Table 1 summarizes the patient, donor, and transplant characteristics. Patient characteristics in the first chronic phase (CP1) and in the advanced phase are described separately. All patients or their guardians gave written informed consent for transplantation and submission of data to the JMDP for further research. This study

Table 1. Patient, Donor, and Transplant Characteristics

	CP1 (n = 88)	Advanced Phase (n = 37)	Total (n = 125)
Year of transplantation			
1993-1998	45	22	67
1999-2005	43	15	58
Stage of CML at BMT			
CP1	88	0	88
CP2	0	12	12
CP3	0	1	1
Advance phase	0	11	11
Blast crisis	0	13	13
Cytogenetic response at BMT			
With MCyR	29	4	33
Without MCyR	39	25	64
Unknown	20	8	28
Pretransplantation therapy with IFN-α			
No	22	8	30
Yes	66	29	95
Pretransplantation therapy with imatinib			
No	72	36	108
Yes	16	1	17
Recipient sex, M/F	56/32	25/12	81/44
Donor–recipient sex			
Female donor to male recipient	20	10	30
Other	68	27	95
Median age at BMT, years (range)	13 (1-19)	17 (2 -19)	14 (1-19)
Median time from diagnosis to transplantation, months (range)	14 (2-111)	19 (5-103)	14 (2-111)
Patient CMV antibody			
Negative	25	14	39
Positive	54	21	75
Unknown	9	2	11
ABO mismatch			
Match	41	15	56
Major mismatch	29	11	40
Minor mismatch	17	9	26
Unknown	1	2	3
Recipient–donor HLA DNA typing			
Match (10/10)	33	8	41
1 alleles mismatch	9	5	14
2 alleles mismatch	19	9	28
3 alleles mismatch	8	3	11
4 alleles mismatch	2	2	4
6 alleles mismatch	0	1	1
Unknown	17	9	26
Conditioning regimen			
TBI regimen	66	30	96
Non-TBI regimen	22	7	29
GVHD prophylaxis			
CsA + MTX	59	22	81
Tacrolimus + MTX	28	15	43
MTX alone	1	0	1
Administration of ATG			
No	76	34	110
Yes	12	3	15
Median infused total nucleated cell dose, $\times 10^6$ /kg (range)	315 (27-880)	298.5 (29-750)	314 (27-880)

ATG indicates antithymocyte globulin; BMT, bone marrow transplantation; CML, chronic myelogenous leukemia; CP, chronic phase; CMV, cytomegalovirus; CsA, cyclosporine; IFN, interferon; GVHD, graft-versus-host disease; MCyR, major cytogenetic response; MTX, methotrexate; TBI, total body irradiation.

was approved by the Data Management Committee of the JMDP and by the Ethical Committee of Nagoya University Graduate School of Medicine.

The 125 children in the study included 81 boys (65%) and 44 girls (35%). The median age at the time of BMT was 14 years (range, 1-19 years). Disease phase at the time of transplantation was defined according to International Bone Marrow Transplant Registry (IBMTR) criteria [11]. Eighty-eight patients (70%) underwent transplantation in CP1. Of the 37 children who underwent transplantation in an advanced phase of CML, 12 were in CP2, 1 was in CP3, 11 were in the accelerated phase (AP), and 13 were in blast crisis (BC). Cytogenetic response data at the time of BMT were available for 97 patients (78%), of whom 68 were in CP1 and 29 were in an advanced phase. Major cytogenetic response (MCyR; $\leq$ 35% Ph+ cells) was achieved in 33 patients (29 patients in CP1 and 4 patients in CP2). Ninety-five recipients (76%) were given interferon (IFN)-α, and 17 (14%) were given imatinib before transplantation. The patients treated with imatinib proceeded to BMT regardless of their response, according to each institutes' therapeutic strategy. The median interval from diagnosis to transplantation was 14 months (range, 2-111 months). Fifty-seven patients (46%) underwent transplantation within 12 months, and 68 (54%) did so after 12 months. Imatinib began to be used in Japan in 1999, and its use was approved by the Japanese Health and Welfare Ministry in 2002. In our cohort, 17 patients (16 in CP1, 1 in AP) received imatinib before transplantation.

Transplantation Procedures and Recipient–Donor HLA Matching

All 125 recipients received a BM graft from a VUD identified through the JMDP. Various preconditioning regimens were used by individual centers. Of the 125 recipients, 96 (77%) received a preparative regimen with total body irradiation (TBI). Fifteen recipients (12%) received antithymocyte globulin (ATG). Cyclosporine A (CsA)–based GVHD prophylaxis was used in 81 patients (65%); tacrolimus-based prophylaxis, in 43 (34%). One patient received only methotrexate (MTX) as GVHD prophylaxis. HLA-matching data based on high-resolution DNA typing for HLA-A, -B, -C, -DRB1, and -DQB1 antigens were available in 99 patients (79%). Of these 99 patients, 41 (41%) were fully matched at 10/10 alleles, 14 (14%) were mismatched at 1 HLA allele, 28 (28%) were mismatched at 2 HLA alleles, and 16 (16%) were mismatched at more than 3 HLA alleles.

Definitions, Data Collection, and Statistical Analysis

The outcomes were analyzed on the basis of engraftment, grade II-IV acute and chronic GVHD (aGVHD, cGVHD), treatment-related mortality (TRM), relapse, overall survival (OS), and leukemia-free survival (LFS). The date of engraftment was defined as the first of 3 consecutive days with a neutrophil count exceeding 0.5×10^9 /L. aGVHD and cGVHD were classified according to published criteria [12]. Only patients surviving for >100 days after transplantation were considered eligible for evaluation of cGVHD. Relapse of CML was defined by hematologic or cytogenetic evidence of disease. (Data on molecular evidence of relapse were not available.) Transplantation data were collected using standardized forms provided by the JMDP. After transplantation, patient baseline information and follow-up reports were submitted at 100 days, 6 months, 1 year, and annually thereafter.

Comparisons between groups were performed using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. Survival and time to events were calculated from the date of transplantation. OS and LFS were estimated by the Kaplan-Meier method and compared using the log-rank test. Cumulative incidence curves were created for TRM. The Cox proportional hazard model was used to obtain the estimates and the 95% confidence interval (CI) of the relative risk (RR) for predictive factors and to evaluate predictive factors for TRM, LFS, and OS in a multivariate analysis. The following variables were evaluated: patient age at the time of BMT ($\geq$ 15/< 15 years), patient sex, sex mismatch, year of transplantation (1993-1998/1999-2005), period from diagnosis to transplantation ($\geq$ 12 months/< 12 months), infused total nucleated cell dose ($\geq 314 \times 10^6$/kg/< 314×10^6/kg), TBI-containing regimen (yes/no), use of ATG (yes/no), GVHD prophylaxis (CsA + MTX $\pm$ steroids/FK $\pm$ MTX), full HLA matching (yes/no), disease phase at the time of BMT (CP1/advanced phase), MCyR at the time of BMT (yes/no), ABO mismatch (match/mismatch), recipient cytomegalovirus (CMV) antibody (negative/positive), history of interferon therapy (yes/no), and history of imatinib therapy (yes/no). Variables with more than 2 categories were dichotomized for the final multivariate model. The cutoff points of the variables were chosen to make optimal use of the information, with the proviso that smaller groups contained at least 20% of the patients. The cutoff points of continuous variables were chosen from the 25th, 50th, and 75th percentiles; consequently, the median of continuous variables was dichotomized as follows: age ($\geq$15/< 15 years), year of transplantation (1993-1998/1999-2005), and infused total nucleated cell dose ($\geq 314 \times 10^6$/kg/< 314×10^6/kg). SPSS version 15.0 (SPSS Inc, Chicago, IL) was used for all statistical calculations except estimation of the cumulative incidence, which was performed using Stata version 10.0 (StataCorp, College Station, TX).

Table 2. Patient Clinical Outcomes

	CP1 (n = 88)	Advanced Phase (n = 37)	Total (n = 125)	P Value
Engraftment				.336
Yes/No	85 / 3	34 / 3	119 / 6	
Acute GVHD				.186
None	21	11	32	
Grade I	34	9	43	
Grade II	18	5	23	
Grade III	11	7	18	
Grade IV	4	5	9	
Chronic GVHD				.393
None	49	25	74	
Limited	15	6	21	
Extensive	24	6	30	
5-year TRM (95% CI)	28.3% (23.4-33.2)	56.5% (48.0-65.0)	36.5% (32.5-40.5)	.002
5-year relapse rate (95% CI)	11.8% (8.1-15.5)	29.0% (18.7-39.3)	15.4% (11.7-19.1)	.098
5-year LFS (95% CI)	65.2% (60.0-70.4)	32.4% (24.7-40.1)	55.5% (51.0-60.0)	.001
5-year OS (95% CI)	70.7% (65.7-75.7)	32.4% (24.7-40.1)	59.3% (54.8-63.8)	<.001

GVHD indicates graft-versus-host disease; LFS, leukemia-free survival; OS, overall survival; TRM, treatment-related mortality.

RESULTS

Engraftment

A total of 119 recipients (95%) were successfully engrafted. Neutrophil engraftment occurred at a median of 18 days after BMT (range, 11-37 days). Six patients (5%) experienced primary graft failure (Table 2), all of whom died.

aGVHD and cGVHD

Grade II-IV aGVHD occurred in 50 patients (40.7%; 95% CI = 36.3%-45.1%), and grade III-IV aGVHD occurred in 27 patients (22.6%; 95% CI = 16.1%-31.2%). Fifty-one patients (50.1%; 95% CI = 45.0%-55.2%) developed cGVHD (extensive type, n = 30; limited type, n = 21).

Relapse

Seventeen patients (11 recipients in CP1 and 6 in an advanced phase) experienced a relapse. The 5-year cumulative incidence of relapse was 19.7% (95% CI = 15.1%-24.3%). The median time for occurrence of relapse for the entire study cohort was 7 months (range, 1-97 months).

Survival

LFS

The 5-year LFS rate was 55.5% (95% CI = 51.0%-60.0%) for the entire cohort (Figure1). The LFS rate was significantly higher in children undergoing BMT in CP1 (65.2%; 95% CI = 60.0%-70.4%) than those undergoing BMT in an advanced phase (32.4%; 95% CI = 28.7%-36.1%; $P = .001$) (Table 2).

On univariate analysis, the following factors were significantly associated with LFS: age at the time of BMT ($P = .047$), infused total nucleated cell dose ($P = .002$), disease phase ($P = .002$), and cytogenetic response at the time of BMT ($P = .001$). Multivariate analysis also identified infused total nucleated cell dose (RR = 2.320; 95% CI = 1.326-4.061; $P = .003$), disease phase (RR = 2.051; 95% CI = 1.187-3.545; $P = .010$), and cytogenetic response at the time of BMT (RR = 2.890; 95% CI = 1.264-610); $P = .012$) as independent risk factors for LFS.

OS

The 5-year OS rate was 59.3% (95% CI = 54.8%-63.8%) for the entire cohort (Figure 1). The OS rate was significantly higher in the children undergoing BMT in CP1 (70.7%; 95% CI = 65.7%-75.7%) than in those undergoing BMT in an advanced phase (32.4%; 95% CI = 24.7%-40.1%; $P < .001$) (Table 2).

On univariate analysis, the following risk factors were significantly associated with OS: age at the time of BMT ($P = .037$), interval between diagnosis and

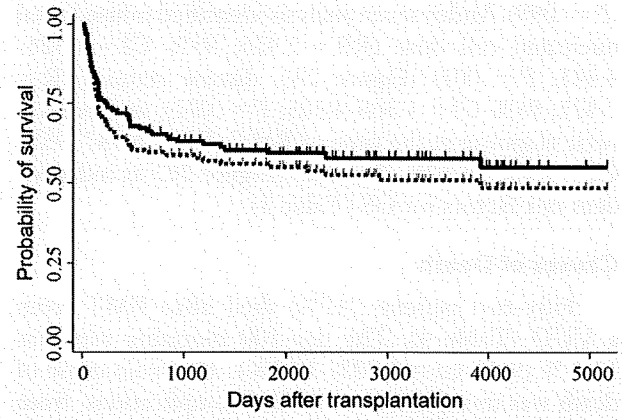

Figure 1. OS and LFS in children with Ph+ CML. In Kaplan-Meier curves graph, solid line shows the probabilities of OS (5-year OS = 59.3%; 95% CI = 54.8%-63.8%) and the dotted line shows that of LFS (5-year LFS = 55.5%; 95% CI = 51.0%-60.0%).

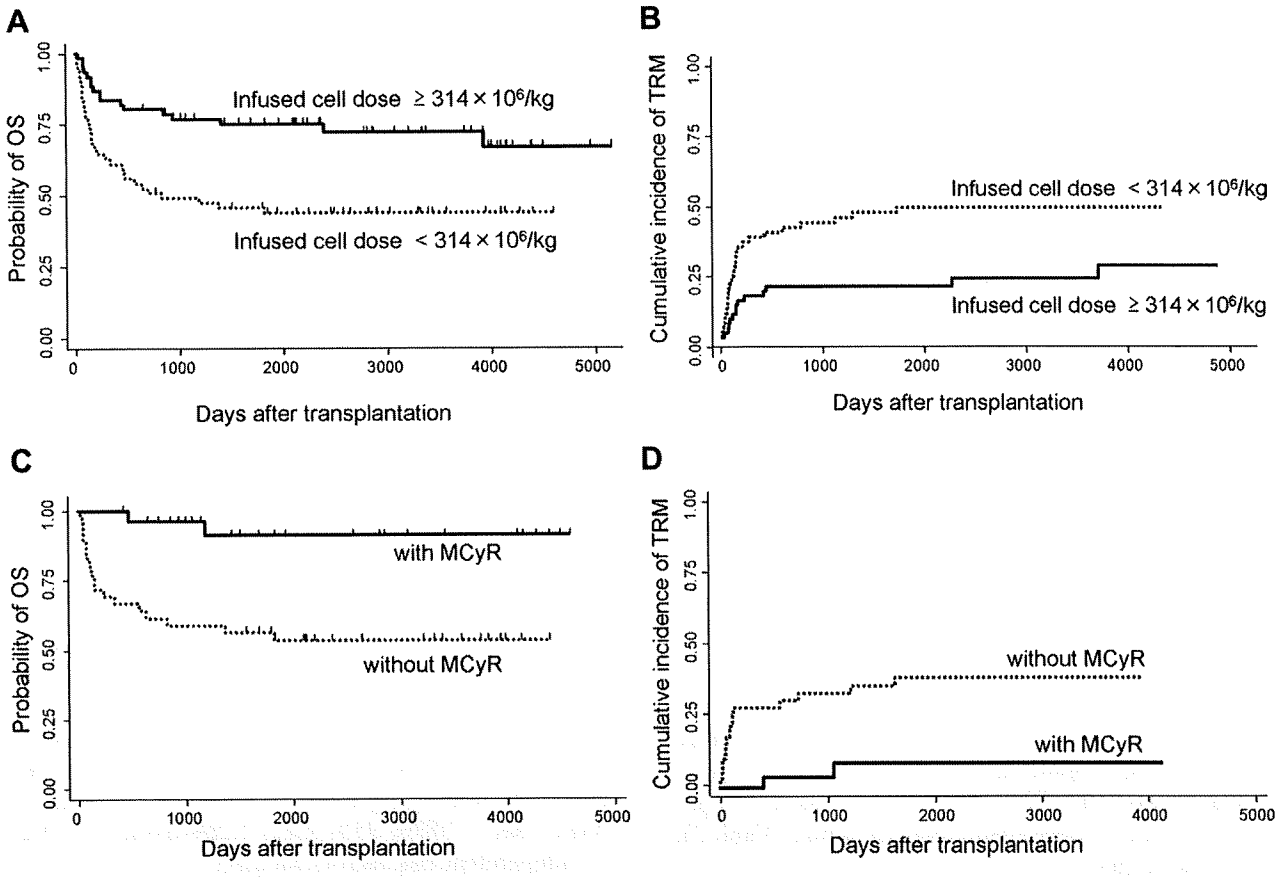

Figure 2. A and B, Relationship among infused total nucleated cell dose, OS (A), and TRM (B) in children with Ph+ CML. In the entire cohort, OS was significantly higher for children who received a higher infused total nucleated cell dose than those who received a lower dose ($\geq 314 \times 10^6$/kg vs $< 314 \times 10^6$/kg; $P = .001$). TRM was significantly higher for children who received a lower cell dose than for those who received a higher cell dose ($\geq 314 \times 10^6$/kg vs $< 314 \times 10^6$/kg; $P = .003$). Solid lines show the probabilities of OS and TRM for children who received a higher infused total nucleated cell dose and the dotted lines show the probabilities for those who received a lower infused total nucleated cell dose. C and D, OS (C) and TRM (D) of Ph+ CML children in CP1 with or without an MCyR. OS was significantly higher for children who achieved MCyR at the time of BMT (n = 29) than for those who did not (n = 39) (OS; $P < .001$) (C). TRM was also significantly higher for children who did not achieve MCyR ($P = .005$) (D). The solid lines show the probabilities of OS and TRM for children with MCyR at the time of BMT, and the dotted lines show the probabilities for those without.

BMT ($P = .042$), infused total nucleated cell dose ($P = .002$), disease status ($P < .001$), and cytogenetic response at the time of BMT ($P = .002$). A history of imatinib therapy before BMT marginally affected OS ($P = .099$). Multivariate analysis identified infused total nucleated cell dose (RR = 2.426; 95% CI = 1.326-4.441; $P = .001$) (Figure 2A), disease status (RR = 2.427; 95% CI = 1.368-4.305; $P = .002$), and cytogenetic response at the time of BMT (RR = 6.547; 95% CI = 1.982-21.629; $P = .002$) (Figure 2C) as independent risk factors for OS (Table 3).

Causes of Death

Fifty-two patients (42%) died after BMT from a VUD (Table 4). The day-100 mortality rate was 15.2 % (95% CI = 12.0%-18.4%). The main cause of death was transplantation-related complications, from which 46 patients (37%) died between day 8 and 10 years (median, 4 months) after transplantation. These included 18 transplantation-related deaths occurring before day 100 after transplantation. Death was associated with treatment-resistant GVHD in 14 patients (9 with aGVHD and 5 with cGVHD). Infection was the cause of death in 12 patients. Six patients died from recurrent CML between 3 and 28 months (median, 13 months) after transplantation.

Univariate analysis revealed that infused cell dose ($P = .013$), disease phase ($P = .006$), and cytogenetic response at the time of BMT ($P = .001$) were significant risk factors for TRM. The interval between diagnosis to BMT ($P = .083$) and HLA mismatch ($P = .087$) were marginally associated with TRM. In the multivariate model, infused cell dose (RR = 2.347; 95% CI = 1.195-4.610; $P = .013$) (Figure 2B) and cytogenetic response at the time of BMT (RR = 9.055; 95% CI = 2.151-38.127; $P = .003$) (Figure 2D) were independent risk factors for TRM (Table 3).

Effects of HLA Compatibility

The influence of HLA compatibility between recipient and donor on aGVHD, TRM, and OS was assessed by univariate analysis. aGVHD (grade II-IV)

Table 3. Risk Factors for TRM and OS on Multivariate Analysis

Covariates	RR (95% CI)	P value
TRM		
Infused cell dose		
$\geq 314 \times 10^6$/kg	(1)	
$< 314 \times 10^6$/kg	2.347 (1.195-4.610)	.013
Cytogenetic response at BMT		
With MCyR	(1)	
Without MCyR	9.055 (2.151-38.127)	.003
OS		
Infused total nucleated cell dose		
$\geq 314 \times 10^6$/kg	(1)	
$< 314 \times 10^6$/kg	2.426 (1.326-4.441)	.004
Disease phase at BMT		
CP1	(1)	
Advanced phase	2.427 (1.368-4.305)	.002
Cytogenetic response at BMT		
With MCyR	(1)	
Without MCyR	6.547 (1.982-21.629)	.002

BMT indicates bone marrow transplantation; MCyR, major cytogenetic response; OS, overall survival; TRM, treatment-related mortality.

was less frequent in patients with fully matched donors than in those with mismatched donors (RR = 2.044; 95% CI = 1.055-3.961; P = .034). TRM (RR = 1.902; 95% CI = 0.894-4.045; P = .095) and OS (RR = 1.572; 95% CI = 0.817-3.027; P = .176) tended to be worse in mismatched transplantation, but the difference was not statistically significant. In the analysis of each single allele mismatch, only the HLA-A allele mismatch significantly affected OS (RR = 2.837; 95% CI = 1.347-5.977; P = .006). HLA-C mismatch marginally affected OS (RR = 1.639; 95% CI = 0.945-2.843; P = .078), whereas HLA-B, -DRB1, and -DQB1 mismatch were not significant. On multivariate analysis, HLA compatibility was not identified as an independent risk factor for acute GVHD, TRM, or OS.

Table 4. Causes of Death

	CP1 (n = 88)	Advanced Phase (n = 37)	Total (n = 125)
TRM	26	20	46
Infections			
Bacterial	4	1	5
Fungal	1	0	1
Viral	3	1	4
Pneumocystis jirovecii	1	0	1
Unknown	0	1	1
Rejection	0	1	1
Acute GVHD	5	4	9
Chronic GVHD	4	1	5
Idiopathic interstitial pneumonitis	6	4	10
Cardiac failure	0	1	1
Respiratory failure	0	1	1
Renal failure	1	1	2
Hemorrhage	0	2	2
Secondary malignancy	1	0	1
Unknown	0	2	2
Relapse	1	5	6

CP indicates chronic phase; GVHD, graft-versus-host disease; TRM, treatment-related mortality.

Effect of Cytogenetic Response at Transplantation

Cytogenetic response data were available in 68 of 88 patients (77%) who underwent transplantation in CP1. Sixteen patients received imatinib, 35 received IFN-α, and 3 received neither imatinib nor IFN-α. MCyR at the time of BMT was achieved in 15 of the 16 patients (94%) treated with imatinib and in 14 of the 35 patients (40%) treated with IFN-α.

Patients with MCyR at the time of BMT (n = 29) had significantly better OS and LFS than those without MCyR (n = 39): 5-year OS = 91.4%, 95% CI = 85.4%-97.4% versus 53.4% and 45.3%-61.5% (P = .001); 5-year LFS = 81.0 %, 95% CI = 73.2%-88.8% versus 50.9% and 42.8%-59.0% (P = .02) (Figure 2C). Although no significant difference in relapse rate was seen between the 2 patient groups (P = .91), TRM was significantly lower in those who achieved MCyR at the time of BMT (n = 29) than in those who did not (n = 39): 5-year TRM = 9.6 %, 95% CI = 3.0%-16.2% vs 41.0% and 32.7%-49.3% (P = .005) (Figure 2D).

Effect of Pre-BMT Imatinib Therapy

In this cohort, 17 patients received imatinib before transplantation, and 15 of them (88.2%) achieved MCyR in CP1 before transplantation. This percentage was significantly higher than that in the patients who did not receive imatinib (88.2% vs 22.2%; $P < .01$). A history of imatinib therapy had a positive effect on survival (5-year OS = 81.9 %, 95% CI = 72.4%-91.4% vs 56.4% and 51.6%-61.2%; P = .086), but this effect was not statistically significant.

DISCUSSION

Because of the small number of patients, to date only a few studies have addressed the outcome of children with Ph+ CML undergoing BMT with a VUD [3-5]. The number of patients in the present study is comparable to that of the largest previous study, which included 132 children with CML undergoing BMT from a VUD [4]. Furthermore, unlike that previous study, our data set contains detailed information on infused total nucleated cell dose, high-resolution HLA compatibility, and cytogenetic response at the time of BMT. Until now, these variables have not been evaluated in a pediatric CML population.

In clinical settings [13-15], as well as in animal models [16,17], larger cell dose is recognized as an important predictor of a favorable outcome for allogeneic BMT. When an adult patient with CML receives a transplant from a VUD, a lower infused total nucleated cell dose is associated with an increased incidence of TRM [18]. Our findings also demonstrate an association between lower infused total nucleated cell dose

and lower OS and LFS and a higher incidence of TRM. These correlations are independent of recipients' age. Moreover, all 6 patients who experienced graft failure were in the lower infused total nucleated cell dose group. Based on our findings, we recommend BM harvest teams attempt to collect a higher number of nucleated cells for infusion in CML patients undergoing BMT from a VUD.

Cytogenetic response to previous treatment with IFN-α [19] and imatinib [20] has been reported to be predictive for survival after allogeneic SCT in Ph+ CML. In the multivariate model of our entire cohort, MCyR at the time of BMT was an independent predictive factor for transplantation outcome. Furthermore, subgroup analysis of the patients in CP1 confirmed that the lower TRM rate in patients with MCyR at the time of BMT contributed to a better survival rate (Figure 2C), suggesting that MCyR is important for better transplantation outcome in CP1 CML as well. Recently, the Center for International Blood and Bone Marrow Transplant Research reported a significantly lower TRM and a better OS in imatinib-treated patients undergoing allogeneic SCT [21]. In our cohort, the imatinib-treated patients tended to have a higher OS ($P = .086$), but the difference was not statistically significant; however, our imatinib-treated group was small (17 of 125 patients), which may have reduced the statistical power.

We have now multiple treatment modalities for pediatric CML, including allogeneic SCT, imatinib, and, more recently, second-generation tyrosine kinase inhibitors. Although only few small studies have analyzed the data on pediatric imatinib monotherapy [22,23], those studies have reported comparable results to adult large clinical trials [24-26]. Growth disturbance as a side effect of imatinib in a pediatric CML patient was reported recently [27]; this effect could be a serious drawback to long-term imatinib therapy in the future. Of course, allogeneic SCT also has potential long-term sequelae, including growth retardation. We are currently planning a study comparing the long-term outcomes and complications of therapy with tyrosine kinase inhibitors and allogeneic SCT in the imatinib era.

In summary, disease phase, infused total nucleated cell dose, and cytogenetic response at the time of BMT were found to be independent risk factors for OS, LFS, and TRM in BMT from a VUD for the treatment of pediatric CML. These results provide important information for evaluating indications and improving outcome in children with CML undergoing unrelated BMT.

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