ASBMT
American Society for Blood and Marrow Transplantation

The Outcome of Unrelated Hematopoietic Stem Cell Transplants with Total Body Irradiation (800 cGy) and Cyclophosphamide (120 mg/kg) in Adult Patients with Acquired Severe Aplastic Anemia

Jong Wook Lee, Byung Sik Cho, Sung Eun Lee, Ki Seong Eom, Yoo Jin Kim, Hee Je Kim, Seok Lee, Chang Ki Min, Seok Goo Cho, Woo Sung Min, Chong Won Park

To verify the feasibility of 800 cGy of total body irradiation (TBI) with 120 mg/kg of cyclophosphamide (TBI-800/Cy-120) as a conditioning regimen for unrelated stem cell transplantation (u-SCT) in adult patients with severe aplastic anemia, we analyzed 50 consecutive patients who underwent u-SCT, including 26 patients from our previous pilot study. Seventeen patients received transplants from mismatched donors via high-resolution DNA typing (8 of 8). Thirty-eight patients received bone marrow and 12 peripheral blood stem cells (PBSCs). Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and short-course methotrexate. All patients achieved engraftment, and the median days of neutrophil and platelet recovery were 13 days and 20 days, respectively. The 5-year estimated overall survival was 88.0%. The cumulative incidences of acute grade II-IV GVHD (aGVHD) and chronic GVHD (cGVHD) were 46.0% and 50.3%, respectively. Only an HLA-mismatched donor was associated with the occurrence of aGVHD on multivariate analyses, whereas prior aGVHD and the use of PBSCs were associated with the occurrence of cGVHD on univariate analyses. In conclusion, the excellent outcomes of u-SCT with TBI-800/Cy-120 suggest that u-SCT may be applicable to patients with severe aplastic anemia even without prior treatment with immunosuppressive therapy, which will require testing in prospective trials in the future.

Biol Blood Marrow Transplant 17: 101-108 (2011)

KEY WORDS: Aplastic anemia, Unrelated allogeneic stem cell transplantation, TBI-based conditioning regimen

INTRODUCTION

Allogeneic stem cell transplantation (SCT) from an HLA-identical sibling donor (s-SCT) is an established treatment for younger patients with acquired severe aplastic anemia (SAA) [1-5]. For patients with SAA who do not have an HLA-identical sibling donor and cannot acquire an adequate response to immunosuppressive therapy (IST), allogeneic SCT from an unrelated donor (u-SCT) is an alternative therapy, as the outcome in patients with SAA who have failed a single round of antithymocyte globulin (ATG) has been poor [6]. Additionally, u-SCT can be a front-line therapy for very SAA (VSAA), when the patient is able to receive emergent u-SCT without IST. However, data from large retrospective studies suggest that the outcomes of u-SCT remain less favorable compared with s-SCT, because of a higher incidence of transplant-related complications, such as graft rejection, graft-versus-host disease (GVHD), and a variety of organ toxicities. Additionally, the mortality rate in the u-SCT recipients is approximately twice that noted in s-SCT, and long-term survival in these subjects is only approximately 40% to 60% [7-10], even though the outcomes for HLA-matched unrelated donors have recently been reported to rival those of children and young adults receiving s-SCT [9,11].

The optimal conditioning regimen for u-SCT remains uncertain [6]. We have reported early results

From the Division of Hematology, Department of Internal Medicine, Catholic Blood and Marrow Transplantation Center, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Financial disclosure: See Acknowledgments on page 107.

Jong Wook Lee and Byung Sik Cho are cofirst authors.

Correspondence and reprint requests: Jong Wook Lee, MD, PhD, Division of Hematology, Department of Internal Medicine, Catholic Blood and Marrow Transplantation Center, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, Korea (e-mail: jwlee@catholic.ac.kr).

Received February 23, 2010; accepted June 16, 2010

1083-8791/$36.00
doi:10.1016/j.bbmt.2010.06.014

from a pilot prospective study to determine a safe and sufficient dose of total body irradiation (TBI) to be used in combination with 120 mg/kg of cyclophosphamide (Cy) as a conditioning regimen for u-SCT in adult patients with SAA, which demonstrated the superiority of 800 cGy of TBI compared to higher doses of TBI (1000 and 1200 cGy) [12]. However, the limited follow-up period (median, 27 months) and small number (n = 26) of a group of TBI 800 cGy made it impossible to assess the feasibility of the conditioning regimen. Here, to confirm the feasibility of 800 cGy of TBI in combination with 120 mg/kg of Cy (TBI-800/Cy-120), we updated our results regarding the patients included in the original report and extended our experience to a larger number of patients who had received transplants from HLA-matched or HLA-mismatched unrelated donors.

MATERIALS AND METHODS

Patients

Between April 2001 and February 2009, 50 consecutive adult patients with SAA including the 26 patients in our previous pilot study [12] underwent u-SCT at the Catholic Blood and Marrow Transplantation Center in Korea after the patients had certified their fully informed consent. Patients with SAA or VSAA who did not have an HLA-identical sibling donor and could not acquire an adequate response to IST were enrolled in this study. Most patients (90%) were idiopathic SAA, with an exception of 5 patients (paroxysmal nocturnal hemoglobinuria, n = 2; hepatitis B virus, n = 2; non-A,-B,-C hepatitis-associated, n = 1). As of June 2009, the median follow-up duration for the surviving patients was 50 months (range: 6-104 months). The patients and transplant characteristics are summarized in Table 1. The median age of patients was 28 years (range: 15-53 years) and the interval from the diagnosis to transplantation was prolonged (median: 48 months, range: 2-323 months). The majority of patients received multiple transfusions (median: 64 units, range: 4-346) prior to u-SCT. Compared to patients in the pilot cohort (n = 26), more patients received peripheral blood stem cells (PBSCs), and the age of donors was higher in the additional cohort (n = 24), whereas other characteristics were similar (Table 1).

Transplant Characteristics

All patients received a conditioning regimen of TBI-800/Cy-120, which consisted of the administration of Cy at a dose of 60 mg/kg/day for 2 days (120 mg/kg in total) followed by TBI at a dose of 2 × 200 cGy fraction per day for 2 days. The DNA-based typing for HLA-A, -B, -C, and -DRB1 was used for all donor-recipients. Thirty-three patients (66%) were transplanted from fully matched unrelated donors, and the remaining 17 patients (34%) from mismatched unrelated donors. The stem cell source was bone marrow (BM) in 38 patients (76%) and granulocyte-colony stimulating factor (G-CSF) mobilized PBSC in 12 patients (24%). The transplanted median numbers of total nucleated cells, mononuclear cells, $CD34^+$ cells, and $CD3^+$ cells were 2.52×10^8/kg (range: 0.67-15.26), 0.96×10^8/kg (range: 0.40-10.19), 4.28×10^6/kg (range: 0.82-14.36), and 51.76×10^6/kg (range: 20.45-776.62), respectively. GVHD prophylaxis was attempted by tacrolimus and short-course methotrexate. Tacrolimus was administered to patients until day 180, or later, according to the presence of GVHD.

Supportive Care

All patient transplants were performed in laminar airflow and high-efficiency particulate air-filtered rooms until engraftment. Intravenous (i.v.) access was achieved with a double-lumen tunneled central venous catheter. G-CSF was administered subcutaneously to all patients at a dose of 5 μg/kg per day from day 7 after the transplant until neutrophil recovery. A low dose of heparin (Heparin sodium, Hanlim, Seoul, Korea; continuous i.v., 100 U/kg/day) or lipo-prostaglandin E1 (Eglandin; alprostadil, Welfide, Osaka, Japan; continuous i.v., 1 μg/kg/day) was administered with ursodiol (200 mg thrice a day) for the prevention of veno-occlusive disease (VOD). Transfused blood products were irradiated and leukocyte depleted. For alloimmunized patients prior to transplant (52%), we conducted prophylactic single-donor platelet transfusion from family members of each patient after infusion of stem cells until engraftment of platelets. Antimicrobial prophylaxis consisted of ciprofloxacin (250 mg twice a day) and intraconazole (100-200 mg daily) started at the beginning of the conditioning treatment. Cytomegalovirus (CMV) prophylaxis consisted of high-dose i.v. acyclovir (10 mg/kg thrice a day) until engraftment for all patients. After transplantation and until hospital discharge, patients were monitored for CMV infection or reactivation twice a week with a real-time polymerase chain reaction (PCR)-based assay for CMV DNA using the LightCycler® 2.0 instrument (Roche Diagnostics, Mannheim, Germany) and a CMV antigenemia assay performed using a protocol described previously [13]. Patients were then monitored weekly to biweekly until the cessation of the immunosuppressive drugs. The patients with CMV viremia were treated with ganciclovir (5 mg/kg twice a day) or foscarnet (60 mg/kg thrice a day or 90 mg/kg twice a day). Every patient received *Pneumocystis jirovecci* prophylaxis with sulfamethoxazole/trimethoprim (1 single-strength tablet daily) after engraftment until discontinuation of the immunosuppressant therapy. The other general transplantation procedures

Table 1. Patients' Characteristics

Characteristics	All Patients	Pilot Cohort	Additional Cohort	*P* Value
Number	50	26	24	
Median age of patient (range), year	28 (15-53)	26 (17-50)	29 (15-53)	.496
≤28 years/>28 years (%)	27 (54)/23 (46)	16 (62)/10 (38)	11 (46)/13 (54)	.266
Median age of donor (range), year	26 (20-40)	23 (20-40)	28 (21-39)	.005
≤26 years/>26 years (%)	28 (56)/22 (44)	18 (70)/8 (31)	10 (42)/14 (58)	.050
Sex of patient (male/female), number (%)	28 (56)/22 (44)	15 (58)/11 (42)	13 (54)/11 (46)	.802
Sex of donor (male/female), number (%)	38 (76)/12 (24)	21 (81)/5 (19)	17 (71)/7 (29)	.411
Donor/patient sex combination, number (%)				.259
Female to female/female to male	6 (12)/6 (12)	1 (4)/4 (15)	5 (21)/2 (8)	
Male to male/male to female	22 (44)/16 (32)	11 (42)/10 (39)	11 (46)/6 (25)	
ABO type of donor/patient, number (%)				.500
Match	22 (44)	9 (35)	13 (54)	
Minor mismatch	9 (18)	5 (19)	4 (17)	
Major mismatch	9 (18)	5 (19)	4 (17)	
Major and minor mismatch	10 (20)	7 (27)	3 (13)	
Severity of disease				
Severe/very severe (%)	41 (82)/9 (18)	24 (92)/2 (8)	17 (71)/7 (29)	.069
Stem cell source				
Bone marrow/peripheral blood (%)	38 (76)/12 (24)	24 (92)/2 (8)	14 (58)/10 (42)	.007
HLA allelic matching/8 loci, HLA-A, -B, -C, -DRB1 (%)				.090
Match	33 (66)	20 (77)	13 (54)	
Mismatch	17 (34)	6 (23)	11 (46)	
1 allele mismatch	9 (53)	4 (67)	5 (45)	
2 allele mismatch	2 (12)	0 (0)	2 (18)	
1 antigen mismatch	3 (18)	2 (33)	1 (9)	
1 allele and 1 antigen mismatch	3 (18)	0 (0)	3 (27)	
Prior treatment, number (%)				.260
Antithymocyte globulin + cyclosporine	46 (92)	25 (96)	21 (88)	
Cyclosporine ± androgen	4 (8)	1 (4)	3 (12)	
Prior transfusions (PRC + platelets), median (range), unit	64 (4-346)	54 (8-252)	89 (4-346)	.164
Number of PRC units transfused, median (range)	25 (0-212)	23 (3-107)	30 (0-212)	.265
Number of platelet transfusions, median (range)	32 (20-282)	21 (0-172)	39 (2-282)	.260
Allo-immunized (anti-platelet antibody), number (%)	26 (52)	15 (58)	11 (46)	.402
Time from diagnosis to transplantation, months, median (range)	48 (2-323)	51 (2-192)	45 (4-323)	.808
Less than 3 years, number (%)	22 (44)	11 (42)	11 (46)	
More than 3 years, number (%)	28 (56)	15 (58)	13 (54)	

were conducted as described in previous reports [14,15].

Definitions

Neutrophil engraftment was defined to have occurred on the first of 3 consecutive days during which the absolute neutrophil count (ANC) was $>0.5 \times 10^9$/L. Platelet engraftment was defined to have occurred on the first of 7 consecutive days with a platelet count of $>20 \times 10^9$/L, without transfusion support. Primary graft failure was defined as failure to achieve a neutrophil count of $>0.5 \times 10^9$/L for 3 consecutive days at any time after transplantation. Secondary graft failure was defined as the development of an ANC of $<0.5 \times 10^9$/L after initial engraftment had already been achieved. Regimen-related toxicity (RTT) was graded by a Bearman score [16]. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and graded using previously published criteria [17,18].

Statistical Analysis

Survival, graft failure, and GVHD were the primary outcomes of interest in this study. We calculated overall survival (OS) from the date of transplantation until the date of death or last follow-up, and the survival curves were plotted using the Kaplan-Meier method and compared by the log-rank test. aGVHD and cGVHD were considered as time-dependent covariates, and their incidences were calculated and plotted using cumulative incidence estimates and compared by the Gray test [19], in which death without aGVHD within 100 days or death without cGVHD were considered competing risks, respectively. The assumption of proportional hazards over time was tested for all explanatory covariates by using a time-dependent covariate. For multivariate analysis, variables with a *P*-value <.1, as determined by univariate analysis, were considered for entry into the model selection procedure on the basis of the Cox proportional hazards model for OS or the semiparametric model called "proportional hazard model for the subdistribution of competing risks" for cumulative incidences of aGVHD and cGVHD [19]. The association between the categoric variables was assessed using either chi-square or Fisher's exact tests. The Mann-Whitney test was utilized to compare the continuous variables. Statistical analyses were conducted using SPSS 13.0

software (SPSS, Chicago, IL, USA) with the exception of the cumulative incidence analyses, which were conducted with R (freely distributed on the web, http://www.r-project.org/).

RESULTS

Engraftment

All patients had sustained engraftment until the last follow-up. The median time for neutrophils to reach a level of 0.5 $\times$ 10^9/L for 3 consecutive days was 13 days (range: 8-30). The median time for the platelet count to increase above 20 $\times$ 10^9/L, without platelet transfusion, was 20 days (range: 9-200). There was no difference in both neutrophil (P = .545) and platelet (P = .263) engraftment between pilot and additional cohort. One patient showed a delayed platelet recovery until 200 days posttransplant, but his platelet count rose gradually to the normal level thereafter. Only 1 patient showed secondary graft failure at the 15th month after transplantation. The secondary graft failure may have been the result of CMV infection and gancyclovir treatment, rather than a matter of graft rejection. The patient suffered from CMV antigenemia and received gancyclovir prior to the secondary graft failure. The BM chimerism of the patient was revealed as the donor type of 100% at the time of graft failure by PCR analysis of the short tandem repeat sequences. The patient received a booster of BM stem cells from the same donor on the 17th month after the transplant, and the patient recovered from the cytopenia and remained alive with a normal blood count.

RRT

Table 2 shows RRT. With a conditioning regimen of TBI-800/Cy-120, the most frequent RRT equal to or greater than grade II is mucosal toxicity (n = 23) followed by renal toxicity (n = 2) and hepatic toxicity (n = 2). No cardiac and pulmonary toxicity equal to or greater than grade II was noted. None of the 50 patients experienced grade III or IV RRT, which is consistent with our previous report [12].

GVHD

Among 50 patients, 26 developed aGVHD between 11 days and 63 days after transplant, including 3 patients with grade I, 17 with grade II, 4 with grade III, and 2 with grade IV. The cumulative incidence of aGVHD equal to or greater than grade II was 46.0% ± 7.1% (Figure 1A). The results of analyses for factors predicting the cumulative incidence of aGVHD (equal to or greater than grade II) are provided in Table 3. On multivariate analysis, only a transplant from HLA-mismatched donor was identified as a significant factor associated with the occurrence of aGVHD (equal to or greater than grade II) (Figure 1B).

Twenty-three of the 45 evaluable patients experienced cGVHD: 9 patients developed the limited type of cGVHD, and 14 the extensive type. The cumulative incidence of cGVHD was 50.3% ± 7.5% (Figure 2A). On univariate analyses, prior aGVHD (P = .030) and the use of PBSC (P = .024) were associated with the occurrence of cGVHD, but were not shown to be significant on multivariate analyses (Figure 2B and C). Despite more patients receiving PBSCs in the additional cohort, there was no significant difference in the cumulative incidence of cGVHD compared to the pilot cohort (P = .133).

Table 2. Regimen-Related Toxicity

*Grading by Bearman et al.	Mucosal Toxicity	Renal Toxicity	Lung Toxicity	Hepatic Toxicity	Cardiac Toxicity
1	25	3	0	4	0
2	23	2	0	2	0
3	0	0	0	0	0
4	0	0	0	0	0

*Grading by Bearman et al. [16].

Survival

The Kaplan-Meier estimate of OS was 88.0% ± 4.6% (Figure 3A) with a median follow-up of 50 months (range: 6-104) for the surviving patients. At the time of analysis, 6 of the 50 patients had died. The causes of death were aGVHD with sepsis (n = 3), cGVHD with sepsis (n = 1), systemic fungal infection with a brain abscess (n = 1), and thrombotic microangiopathy with a pulmonary hemorrhage (n = 1). There was no difference in OS between the pilot cohort and the additional cohort (Figure 3B), and no other factors were associated with OS. None of the surviving patients developed secondary malignancies after transplantation during a median follow-up period of 50 months (range: 6-104).

DISCUSSION

The optimal type and intensity of conditioning regimens of u-SCT for patients with SAA that can both overcome the risk of graft rejection and minimize RRT remain uncertain because of a few prospective trials in which unified regimens were utilized [6]. From the experience of another group demonstrating the limits of u-SCT following 200 mg/kg of Cy and 90 mg/kg of ATG (Cy-200/ATG-90) because of graft failure [20], we thought that, to overcome the graft failure, the use of a more intensive conditioning regimen could guarantee sustained hematopoietic reconstitution. Thus, we conducted a prospective pilot study to determine the optimal dose of TBI with a fixed reduced dose of 120 mg/kg of Cy because of our experience with the congestive heart failure induced by a higher dose (200 mg/kg) of Cy with ATG (1.25 mg/kg for 3 days) and procarbazine (6.25 mg/kg/day for 6 days) in s-SCT [21]. The results demonstrated

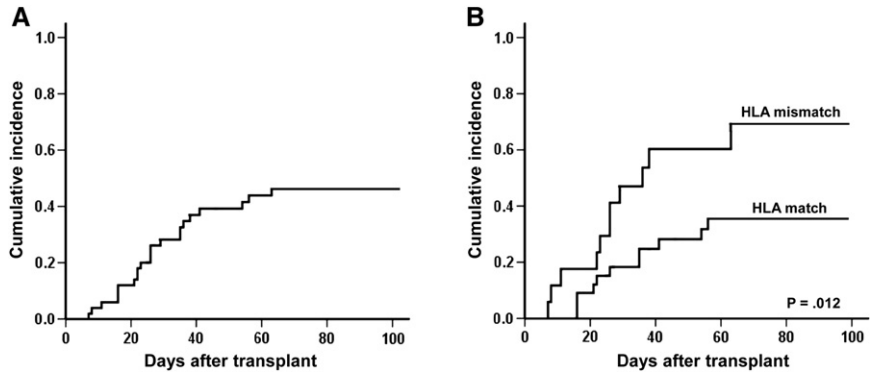

Figure 1. Cumulative incidence of aGVHD. (A) The cumulative incidence of aGVHD of grade II-IV was 46% ± 7.1%. (B) Patients with HLA mismatch had higher cumulative incidences of aGVHD with grade II-IV (70.6% ± 11.1% versus 33.3% ± 8.2%, $P = .012$).

that the group with TBI-800/Cy-120 had a lower incidence of RRT equal to or greater than grade II and better survival than the group treated with 1000 and 1200 cGy of TBI (RRT; 35%, 67%, 80%, survival at 3 years; 92%, 44%, 40%) [12]. The present study, in which the results of the patients included in the original report (the pilot cohort) were updated and our experience was extended to a larger number of patients, revealed excellent OS (88.0%), which is comparable with the results of s-SCT (OS, 64%-89%) [1-5]. No graft rejection was observed in this study. Only 1 patient had secondary graft failure because of CMV infection and gancyclovir treatment, and the patient continues to survive with a normal blood count after receiving a booster with BM stem cells from the same donor. Compared to the pilot cohort, the additional cohort included more donors with older age and patients receiving PBSCs, but there was no significant difference in clinical outcomes.

Outcomes of u-SCT for patients with SAA [7-10] were inferior to those achieved with s-SCT [1-5], which could be explained by the following unfavorable characteristics of u-SCT recipients: longer disease duration, higher iron deposits in tissues, and a greater probability of alloimmunity because of multiple transfusions prior to transplant, and a higher intensity of pretransplant conditioning required to overcome graft rejection [7-10]. Our patients also had those unfavorable characteristics, including prolonged disease duration and multiple transfusions. Nevertheless, this study demonstrates that the TBI-800/Cy-120 regimen effectively improves survival, and also achieves sustained engraftment without inducing unacceptable RRT. In particular, the population of this single center study was confined to adult patients (median 28 years, range: 15-53 years), unlike previous multicenter studies that include children [7-10,22]. Considering that the age of patients at transplant has been identified as an important factor for the outcomes of u-SCT in SAA patients [7,8,22], the survival outcome of this study is relatively encouraging. This encouraging survival result can also be explained, in part, by better donor selection as the result of high-resolution HLA matching. Our previous pilot study showed different survival rates according to the HLA matching method (serologic typing versus DNA-based typing) [12]. Additionally, recent large retrospective studies have demonstrated that the superior outcomes of u-SCT reported in recent years could be attributed to better donor selection because of the routine use of high-resolution DNA typing as well as superior supportive care [9,10].

One of the benefits of the TBI-800/Cy-120 regimen is the relative tolerability of this regimen in SAA patients without unacceptable RRT. Another previous

Table 3. Factors Predicting the Cumulative Incidence of aGVHD (Equal to or Greater Than Grade II)

	Univariate Analysis		Multivariate Analysis	
	Cumulative Incidence ± Standard Error	*P* Value	Hazard Ratio (95% CI)	*P* Value
Cohort		.073		.372
Pilot	34.6% ± 9.3%		1	
Additional	58.3% ± 10.1%		1.5 (0.6-3.6)	
Donor age		.080		.261
Age ≤26 years	35.7% ± 9.1%		1	
Age >26 years	59.1% ± 10.5%		1.7 (0.7-4.0)	
Severity of disease		.018		.163
Severe	39.0% ± 7.6%		1	
Very severe	77.8% ± 13.9%		2.0 (0.8-5.5)	
HLA match		.012		
match	33.3% ± 8.2%		1	.026
mismatch	70.6% ± 11.1%		2.6 (1.1-5.9)	

CI indicates confidence interval; GVHD, graft-versus-host disease.

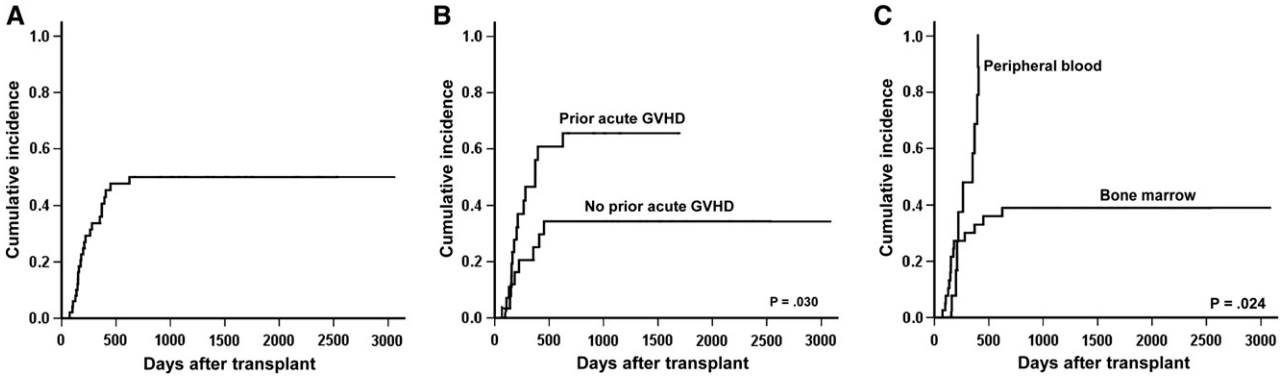

Figure 2. Cumulative incidence of cGVHD. (A) The cumulative incidence of cGVHD was 50.3% ± 7.5%. (B, C) Patients with prior aGVHD (65.1% ± 1.0% versus 35.7 ± 10.2%, $P = .030$) or those who received peripheral blood stem cells (100% versus 38.1% ± 8.0%, $P = .024$) had a higher cumulative incidence of cGVHD.

prospective trial of Deeg and colleagues [22] to determine optimal conditioning regimens for u-SCT of SAA patients showed that a treatment consisting of 200 cGy of TBI in combination with Cy-200/ATG-90 (TBI-200/Cy-200/ATG-90) resulted in better outcomes than higher doses of TBI. They demonstrated that the application of low doses of TBI might allow for sustained engraftment without inducing unacceptable RRT. However, the incidence of RRT equal to or greater than grade III was 21%, which is higher than that reported in this study (0%), despite the fact that lower doses of TBI (200 cGy, 400 cGy, and 600 cGy) were employed than in this trial. This suggests that higher doses of chemotherapy (Cy 200 mg/kg) might affect RRT, especially compared to additional radiation. Deeg et al. [22] have also suggested that the pulmonary toxicity that occurred in their trial, which was a major toxicity in their conditioning regimen, may have been induced by high-dose Cy (200 mg/kg) even in combination with low-dose TBI (200 cGy), whereas no pulmonary toxicity occurred in our study. On the basis of this indirect comparison, we suggest that reduced dose of Cy (120 mg/kg) coupled to higher doses of irradiation (800 cGy) may prove more acceptable for u-SCT of SAA than lower doses of irradiation (200-600 cGy) coupled to a high-dose of Cy (200 mg/kg) and ATG (90 mg/kg). However, the potential late effects of high-dose irradiation, including the development of new malignancies [23,24], remain a matter of concern. In this study, none of the surviving patients developed secondary malignancies over a median follow-up period of 50 months (range: 6-104). However, particularly in young patients, the development of malignancies related to irradiation should be monitored through longer follow-up.

The cumulative incidences of grade II-IV aGVHD and cGVHD in this trial were 46.0% and 50.3%, respectively, a similar range as reported in other recent trials of u-SCT (aGVHD; 37%-75%, cGVHD; 22%-57%) [8-10,22], whereas the incidences were higher than those reported with s-SCT (aGVHD; 11%-30%, cGVHD; 12-35%) [1-5]. Among the 6 patients that died, 4 patients died of aGVHD (n = 3) and cGVHD (n = 1), which illustrated the need for novel approaches to GVHD prevention and therapy. We demonstrated that HLA-mismatch was a significant independent factor predictive of the occurrence of aGVHD, and prior aGVHD and the use of PBSCs were possible risk factors that predicted the occurrence of cGVHD. The increasing use of PBSCs in u-SCT is a world-wide trend because of donors' priority to choose stem cell source, and our data also include more patients receiving PBSCs in the recent cohort. First, it will be needed to educate potential donors pertaining to harmfulness of PBSCs to patients and persuade them to donate BM. Additionally, strategies for the prevention of GVHD should be incorporated into our conditioning regimen for patients who will receive transplantation from HLA-mismatched donors or PB as a source of stem cells. Several trials in patients with SAA using the anti-CD52 antibody alemtuzumab [25], rabbit ATG [26], or in vitro T cell depletion with TIPB9 or OKT3 [27] reported reduction in the incidence of aGVHD to as low as 11%, albeit generally at the expense of increased graft failure rates. Recently, we reported the successful prevention of aGVHD using low-dose ATG (thymoglobulin; Genzyme, Cambridge, MA; 1.25 mg/kg/day for 2 consecutive days, day −3 and day −2) after mismatched u-SCT for acute myelogenous leukemia. The results showed that patients who received low-dose ATG developed less aGVHD than those who did not receive it (8% versus 29%, $P = .038$), and this translated into improved OS at 2 years (68% versus 38%, $P = .043$) and reduced nonrelapse mortality at 2 years (16% versus 44%, $P = .013$) [28]. Based on the results of our previous study, we will begin prospective trials to evaluate the feasibility of low-dose ATG as a method to prevent aGVHD in the setting of u-SCT for SAA patients, who receive stem cells from HLA-mismatched donors or PBSCs.

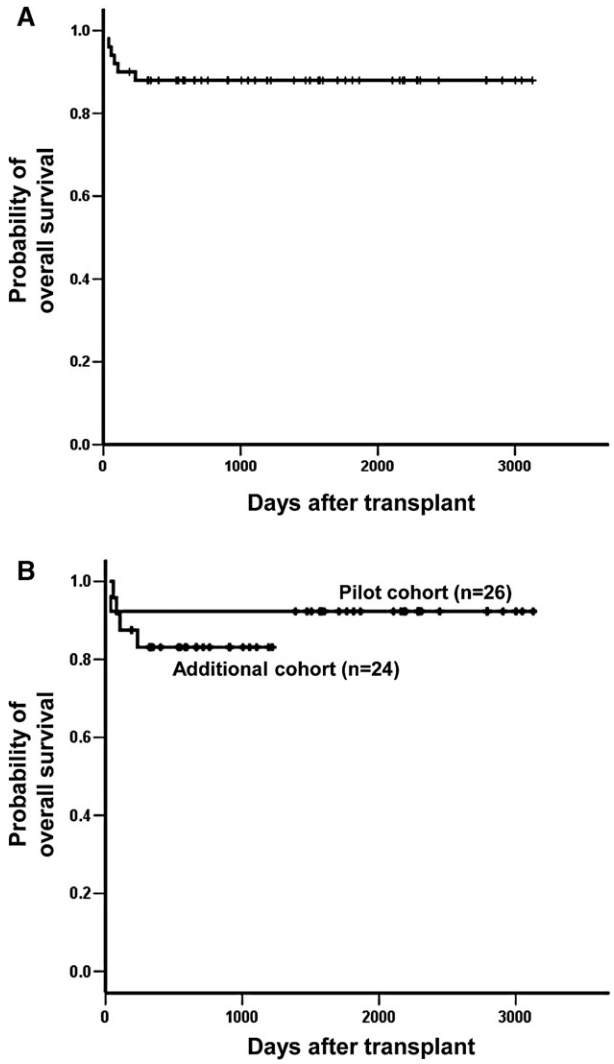

Figure 3. OS. (A) With a median follow-up duration of 50 months (range: 6-104), the 5-year estimates of OS was 88.0% ± 4.6%. (B) There was no significant difference in OS ($P = .365$) between pilot cohort (92.3% ± 5.2%) and additional cohort (83.1% ± 7.7%).

In conclusion, a TBI-800/Cy-120 conditioning regimen resulted in excellent outcomes of unrelated transplants in adult SAA patients who had not responded to IST, despite unfavorable risk factors such as multiple transfusions and long disease duration. These results were comparable to those of s-SCT. Additional strategies for the prevention of severe GVHD, for example, the addition of ATG, particularly for HLA-mismatched transplants and, in the case of PB as a stem cell source, will be helpful to improve outcomes in the future. Whether upfront u-SCT should be carried out in patients with SAA is currently a matter of debate. However, considering the encouraging survival rates of u-SCT in this study, it might be argued that u-SCT should be performed even without prior IST, to improve transplant outcomes, if early identification of a matched unrelated donor would be guaranteed. This should be investigated in prospective trials in the future.

ACKNOWLEDGMENTS

Financial disclosure: The authors declare no conflict of interest.

REFERENCES

1. Locatelli F, Bruno B, Zecca M, et al. Cyclosporin A and short-term methotrexate versus cyclosporin A as graft versus host disease prophylaxis in patients with severe aplastic anemia given allogeneic bone marrow transplantation from an HLA-identical sibling: results of a GITMO/EBMT randomized trial. *Blood*. 2000;96:1690-1697.
2. Kim HJ, Park CY, Park YH, et al. Successful allogeneic hematopoietic stem cell transplantation using triple agent immunosuppression in severe aplastic anemia patients. *Bone Marrow Transplant*. 2003;31:79-86.
3. Ades L, Mary JY, Robin M, et al. Long-term outcome after bone marrow transplantation for severe aplastic anemia. *Blood*. 2004; 103:2490-2497.
4. Kahl C, Leisenring W, Deeg HJ, et al. Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anaemia: a long-term follow-up. *Br J Haematol*. 2005;130:747-751.
5. Champlin RE, Perez WS, Passweg JR, et al. Bone marrow transplantation for severe aplastic anemia: a randomized controlled study of conditioning regimens. *Blood*. 2007;109:4582-4585.
6. Young NS, Scheinberg P, Calado RT. Aplastic anemia. *Curr Opin Hematol*. 2008;15:162-168.
7. 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.
8. 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.
9. 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.
10. 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.
11. 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.
12. Kim SY, Lee JW, Lim J, et al. Unrelated donor bone marrow transplants for severe aplastic anemia with conditioning using total body irradiation and cyclophosphamide. *Biol Blood Marrow Transplant*. 2007;13:863-870.
13. Choi SM, Lee DG, Choi JH, et al. Risk-adapted preemptive therapy for cytomegalovirus disease after allogeneic stem cell transplantation: a single-center experience in Korea. *Int J Hematol*. 2005;81:69-74.
14. Lee S, Kim YJ, Min CK, et al. The effect of first-line imatinib interim therapy on the outcome of allogeneic stem cell transplantation in adults with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood*. 2005;105:3449-3457.
15. Min CK, Kim SY, Lee MJ, et al. Vascular endothelial growth factor (VEGF) is associated with reduced severity of acute graft-versus-host disease and nonrelapse mortality after allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2006;38: 149-156.

16. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. *J Clin Oncol*. 1988;6:1562-1568.
17. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
18. 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.
19. Gray R. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16:1141-1154.
20. Deeg HJ, Anasetti C, Petersdorf E, et al. Cyclophosphamide plus ATG conditioning is insufficient for sustained hematopoietic reconstitution in patients with severe aplastic anemia transplanted with marrow from HLA-A, B, DRB matched unrelated donors. *Blood*. 1994;83:3417-3418.
21. Min CK, Kim DW, Lee JW, Han CW, Min WS, Kim CC. Supplemental peripheral blood stem cells to decrease marrow rejection in adult patients with severe aplastic anemia. *Am J Hematol*. 2002;69:15-22.
22. 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.
23. Socie G, Henry-Amar M, Bacigalupo A, et al. Malignant tumors occurring after treatment of aplastic anemia. European Bone Marrow Transplantation-Severe Aplastic Anaemia Working Party. *N Engl J Med*. 1993;329:1152-1157.
24. Deeg HJ, Socie G, Schoch G, et al. Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. *Blood*. 1996;87:386-392.
25. Gupta V, Ball SE, Yi QL, et al. Favorable effect on acute and chronic graft-versus-host disease with cyclophosphamide and in vivo anti-CD52 monoclonal antibodies for marrow transplantation from HLA-identical sibling donors for acquired aplastic anemia. *Biol Blood Marrow Transplant*. 2004;10:867-876.
26. 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.
27. Bunin N, Aplenc R, Iannone R, et al. Unrelated donor bone marrow transplantation for children with severe aplastic anemia: minimal GVHD and durable engraftment with partial T cell depletion. *Bone Marrow Transplant*. 2005;35:369-373.
28. Kim HJ, Min WS, Cho BS, et al. Successful prevention of acute graft-versus-host disease using low-dose antithymocyte globulin after mismatched, unrelated, hematopoietic stem cell transplantation for acute myelogenous leukemia. *Biol Blood Marrow Transplant*. 2009;15:704-717.