



# Biology of Blood and Marrow Transplantation

journal homepage: [www.bbmt.org](http://www.bbmt.org)



Clinical Research: Pediatric

## Improved Outcome of a Reduced Toxicity-Fludarabine, Cyclophosphamide, plus Antithymocyte Globulin Conditioning Regimen for Unrelated Donor Transplantation in Severe Aplastic Anemia: Comparison of 2 Multicenter Prospective Studies



Hyoung Jin Kang<sup>1,†</sup>, Kyung Taek Hong<sup>1,†</sup>, Ji Won Lee<sup>1</sup>, Hyery Kim<sup>1</sup>, Kyung Duk Park<sup>1</sup>, Hee Young Shin<sup>1</sup>, Soo Hyun Lee<sup>2</sup>, Keon Hee Yoo<sup>2</sup>, Ki Woong Sung<sup>2</sup>, Hong Hoe Koo<sup>2</sup>, Jae Wook Lee<sup>3</sup>, Nak Gyun Chung<sup>3</sup>, Bin Cho<sup>3</sup>, Hack Ki Kim<sup>3</sup>, Kyung Nam Koh<sup>4</sup>, Ho Joon Im<sup>4</sup>, Jong Jin Seo<sup>4</sup>, Hyun Joo Jung<sup>5</sup>, Jun Eun Park<sup>5</sup>, Young Ho Lee<sup>6</sup>, Young Tak Lim<sup>7</sup>, Yeon Jung Lim<sup>8</sup>, Sun Young Kim<sup>8</sup>, Eun Sun Yoo<sup>9</sup>, Kyung Ha Ryu<sup>9</sup>, Jae Hee Lee<sup>10</sup>, Jeong-A Park<sup>11</sup>, Sang Kyu Park<sup>12</sup>, Hyo Seop Ahn<sup>1,\*</sup> on behalf of The Korean Society of Pediatric Hematology-Oncology

<sup>1</sup> Department of Pediatrics, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea

<sup>2</sup> Department of Pediatrics, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Republic of Korea

<sup>3</sup> Department of Pediatrics, The Catholic University of Korea College of Medicine, Seoul, Republic of Korea

<sup>4</sup> Department of Pediatrics, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Republic of Korea

<sup>5</sup> Department of Pediatrics, Ajou University School of Medicine, Suwon, Republic of Korea

<sup>6</sup> Department of Pediatrics, Hanyang University College of Medicine, Seoul, Republic of Korea

<sup>7</sup> Department of Pediatrics, Pusan National University School of Medicine, Pusan, Republic of Korea

<sup>8</sup> Department of Pediatrics, Chungnam National University College of Medicine, Daejeon, Republic of Korea

<sup>9</sup> Department of Pediatrics, Ewha Woman's University School of Medicine, Seoul, Republic of Korea

<sup>10</sup> Department of Pediatrics, Chung-Ang University Hospital, Seoul, Republic of Korea

<sup>11</sup> Department of Pediatrics, Inje University Haeundae Paik Hospital, Pusan, Republic of Korea

<sup>12</sup> Department of Pediatrics, University of Ulsan College of Medicine, Ulsan University Hospital, Ulsan, Republic of Korea

### Article history:

Received 14 February 2016

Accepted 4 April 2016

### Key Words:

Severe aplastic anemia  
Unrelated donor  
Fludarabine  
Cyclophosphamide  
Thymoglobulin  
Antithymocyte globulin (ATG)

### ABSTRACT

Hematopoietic stem cell transplantation (HSCT) is a curative therapy for severe aplastic anemia (SAA); however, the optimal conditioning regimen for HSCT with an unrelated donor has not yet been defined. A previous study using a fludarabine (FLU), cyclophosphamide (Cy), and antithymocyte globulin (ATG) conditioning regimen (study A: 50 mg/kg Cy once daily i.v. on days -9, -8, -7, and -6; 30 mg/m<sup>2</sup> FLU once daily i.v. on days -5, -4, -3, and -2; and 2.5 mg/kg of ATG once daily i.v. on days -3, -2, and -1) demonstrated successful engraftment (100%) but had a high treatment-related mortality rate (32.1%). Therefore, given that Cy is more toxic than FLU, we performed a new phase II prospective study with a reduced-toxicity regimen (study B: 60 mg/kg Cy once daily i.v. on days -8 and -7; 40 mg/m<sup>2</sup> FLU once daily i.v. on days -6, -5, -4, -3, and -2; and 2.5 mg/kg ATG once daily i.v. on 3 days). Fifty-seven patients were enrolled in studies A (n = 28) and B (n = 29), and donor type hematologic recovery was achieved in all patients in both studies. The overall survival (OS) and event-free survival (EFS) rates of patients in study B was markedly improved compared with those in study A (OS: 96.7% versus 67.9%, respectively, *P* = .004; EFS: 93.3% versus 64.3%, respectively, *P* = .008). These data show that a reduced-toxicity conditioning regimen with FLU, Cy, and ATG may be an optimal regimen for SAA patients receiving unrelated donor HSCT.

© 2016 American Society for Blood and Marrow Transplantation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

*Financial disclosure:* See Acknowledgments on page 1459.

<sup>†</sup> These authors contributed equally to this work.

\* Correspondence and reprint requests: Hyo Seop Ahn, Department of Pediatrics, Cancer Research Institute, Seoul National University College of Medicine, 101, Daehak-ro, Jongno-gu, Seoul 03080, Korea.

E-mail address: [hsahn@snu.ac.kr](mailto:hsahn@snu.ac.kr) (H.S. Ahn).

### INTRODUCTION

Bone marrow (BM) transplantation with a matched sibling donor is a curative therapy for severe aplastic anemia (SAA). Studies have demonstrated that the optimal

<http://dx.doi.org/10.1016/j.bbmt.2016.04.003>

1083-8791/© 2016 American Society for Blood and Marrow Transplantation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

conditioning regimen for BM transplantation is the combination of cyclophosphamide (Cy) and antithymocyte globulin (ATG) followed by graft-versus-host disease (GVHD) prophylaxis with methotrexate and cyclosporine [1,2]. However, many patients have no appropriate sibling donor and must choose another treatment, such as immunosuppressive therapy (IST) and/or hematopoietic stem cell transplantation (HSCT) with an alternative donor [3]. Furthermore, because the Cy and ATG regimen is not sufficient for HSCT with an alternative donor [4], other conditioning regimens, such as the inclusion of total body irradiation (TBI) [5,6] or fludarabine (FLU)-based therapies [7], have been used to improve outcomes. In addition, studies have shown promising survival rates using new HLA typing techniques [5] and GVHD prophylaxis [8,9].

We previously reported the results of a phase II, prospective, multicenter clinical trial conducted with a FLU, Cy, and ATG conditioning regimen (study A) [10]. This regimen resulted in good engraftment after unrelated donor (UD) HSCT for patients with SAA; however, the event-free survival (EFS) rate was 67.9%, and all adverse events (except for 1 patient with secondary graft failure) were treatment-related mortalities (TRMs). Consequently, we conducted a newly designed multicenter clinical trial with a reduced-toxicity conditioning regimen (study B) to help improve survival. Here, the aim of the present study was to compare the safety and efficacy of study A and study B and to find the optimal conditioning regimen for SAA patients receiving UD HSCT.

## METHODS

### Patient and Donor Selection

Fifty-seven patients (28 in study A and 29 in study B) with SAA received UD HSCT at multiple centers across Korea. Patients who did not have a prior history of HSCT, who had an Eastern Cooperative Oncology Group performance status of 0 to 2, who were free of significant functional problems in major organs (ejection fraction > 45%, total bilirubin < 2 × upper limit of normal, alanine transaminase < 3 × upper limit of normal, and creatinine < 2 × upper limit of normal), and who did not have any active viral or fungal infection were included in both studies. All patients with congenital aplastic anemia, including Fanconi anemia, were excluded. The clinical characteristics of patients in both studies are summarized in Table 1. The median age, sex, and period between diagnosis and HSCT were not significantly different between participants in study B and study A. However, study B patients were less likely to have a history of IST ( $P = .020$ ).

For donor selection HLA-A, -B, -C, and -DRB1 matching was confirmed by a high-resolution molecular method for all patients and donors. All patients received the designated conditioning regimen after providing informed consent. Both studies were approved by the institutional review board of each center and registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (NCT00737685 and NCT00882323, respectively).

### Conditioning Regimen

The study A conditioning regimen was composed of Cy (50 mg/kg once daily i.v. on days -9, -8, -7, and -6), FLU (30 mg/m<sup>2</sup> once daily i.v. on days -5, -4, -3, and -2), and ATG (Thymoglobulin; SangStat, Lyon, France and Genzyme, Cambridge, MA; 2.5 mg/kg once daily i.v. on days -3, -2, and -1). The regimen used in study B was composed of a reduced dose of Cy (60 mg/kg once daily i.v. on days -8 and -7), an increased dose of FLU (40 mg/m<sup>2</sup> once daily i.v. on days -6, -5, -4, -3, and -2), and the same dose of ATG (2.5 mg/kg once daily i.v. on 3 days). To prevent hemorrhagic cystitis, patients received mesna and adequate hydration.

### GVHD Prophylaxis and Supportive Care

Each institution used their own GVHD prophylaxis protocol. In study A a cyclosporine-based protocol was used more often, whereas a tacrolimus (FK)-based protocol was used more often in study B ( $P = .001$ ). Each protocol included methotrexate regardless of post-transplant, low-dose ATG (1.25 mg/kg once daily i.v. on days 7, 9, and 11) [11,12]. Epstein-Barr viral load monitoring was regularly conducted in study B, although it could not be done uniformly according to the institutions in study A. Supportive care was performed according to the protocols of each institution.

**Table 1**  
Patient Characteristics and Transplantation Data

	Study A (n = 28)	Study B (n = 29)	P
Median age, yr (range)	13.5 (1.2-29.8)	12.9 (6.4-19.8)	.52
Sex			.672
Male	17 (60.7)	16 (55.2)	
Female	11 (39.3)	13 (44.8)	
Interval from diagnosis to transplantation			.889
<1 yr	13 (46.4)	14 (48.3)	
≥1 yr	15 (53.6)	15 (51.7)	
History of previous IST			.02
Yes	14 (50.0)	6 (20.7)	
No	14 (50.0)	23 (79.3)	
Stem cell source			.004
BM	15 (53.6)	5 (17.2)	
PB	13 (46.4)	24 (82.8)	
Median infused cell dose, (range)			
TNC, ×10 <sup>8</sup> /kg	6.8 (1.3-26.5)	11.6 (1.3-218.4)	.137
CD34, ×10 <sup>6</sup> /kg	5.2 (1.2-23.9)	6.4 (1.3-27.5)	.601
HLA disparity, 8/8 (HLA-A, -B, -C, -DR)			.215
0/8	19 (67.9)	15 (51.7)	
≥1/8	9 (32.1)	14 (48.3)	
GVHD prophylaxis			.001
CsA based	20 (71.4)	8 (27.6)	
FK based	8 (28.6)	21 (72.4)	

TNC indicates total nucleated cell; CsA, cyclosporine.

Values are number of cases with percents in parentheses, unless otherwise indicated.

### Assessment of Engraftment and Toxicity

Neutrophil engraftment was defined as the first day of 3 consecutive days with an absolute neutrophil count (ANC) > .5 × 10<sup>9</sup>/L. Platelet recovery was defined as the first day with a platelet count > 20 × 10<sup>9</sup>/L without platelet transfusions. Hematopoietic chimerism was evaluated using a serial analysis of short tandem repeats of BM aspirates at 1, 3, 6, and 12 months post-HSCT. Secondary graft failure was defined as engraftment followed by severe neutropenia (ANC < .5 × 10<sup>9</sup>/L) or the absence of donor cells in the BM or peripheral blood (PB), as demonstrated by a chimerism assay, without subsequent improvement occurring either spontaneously or after treatment with a growth factor. BM or PB stem cell reinfusion, which was administered any time after day 0 if indicated by inadequate hematopoietic function, was taken as a definitive indication of graft failure regardless of ANC values and BM cellularity. In study B the regimen-related toxicity up to 42 days after transplantation was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. In study A the regimen-related toxicity, which was previously graded according to the National Cancer Institute Common Toxicity Criteria for BM transplant recipients (version 2.0), was converted to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

### Statistical Analyses

Categorical variables and continuous variables were compared using a chi-square test and Student's *t*-test, respectively. Acute and chronic GVHD were computed using the cumulative incidence (CI) function. For both, the competing risks were graft failure and TRM. Overall survival (OS) and EFS were analyzed using the Kaplan-Meier method. The difference in CI curves was examined using a Gray's test, whereas the difference in survival rates was determined using the log-rank test. A Cox proportional hazard regression model was used for the multivariate analysis of prognostic factors affecting survival.  $P < .05$  was considered to be statistically significant. Statistical analyses were performed using R version 3.2.2 ([www.r-project.org](http://www.r-project.org)) and SPSS 20.0 (IBM-SPSS, Armonk, NY).

## RESULTS

### Transplantation and Engraftment Data

HLA disparity as well as the median infused dose of nucleated cells and the median infused dose of CD34<sup>+</sup> cells were not significantly different between study A and study B. However, PB was used more often as a stem cell source in study B than in study A (Table 1). The median number of days

with an ANC  $> .5 \times 10^9/L$  in studies A and B were 15 days (range, 10 to 32) and 12 days (range, 10 to 23), respectively. Platelet recovery without transfusion required a median of 22 days (range, 11 to 179; except for 1 patient with TRM) in study A and of 16 days (range, 8 to 127 days; except in 1 patient with TRM) in study B. All patients achieved donor-type chimerism  $> 90\%$  on day 28 post-HSCT; nevertheless, secondary graft failure occurred in 2 patients (study A: 1 patient, 37 months after HSCT; study B: 1 patient, 3 months after HSCT). One underwent a successful second HSCT and the other achieved donor-type hematologic recovery again after steroid pulse therapy.

### Treatment-Related Mortality

In study A 9 patients died of a TRM, resulting in a CI of 32.1%. TRM was related to post-transplantation lymphoproliferative disease (PTLD;  $n = 3$ ), thrombotic microangiopathy ( $n = 2$ ), chronic GVHD-associated complications ( $n = 2$ ), pneumonia ( $n = 1$ ), and myocardial infarction ( $n = 1$ ). However, only 1 patient died of TRM (caused by pneumonia) in study B (CI, 3.5%).

### Graft-versus-Host Disease

Between study A and B there were no differences in the CI of grades II to IV acute GVHD (46.4% versus 41.3%, respectively,  $P = .771$ ) and grades III to IV acute GVHD (14.8% versus 3.5%, respectively,  $P = .294$ ). Although the CI of chronic GVHD also did not differ significantly between the 2 groups (35.7% versus 37.9%, respectively,  $P = .910$ ), extensive chronic GVHD occurred in 4 patients in study A and 5 patients in study B (14.3% versus 17.2%, respectively,  $P = .754$ ).

### Toxicity and Infection

In study A grades 3 to 4 regimen-related toxicity up to 42 days after HSCT occurred in 18 patients (64.3%). In study B 24 patients (82.8%) showed grades 3 to 4 toxicity, but most of them had febrile neutropenia ( $n = 23$ , 79%) that was treated without mortality. Bladder toxicity, particularly hemorrhagic cystitis, was markedly decreased in patients in study B compared with those in study A (3.4% versus 35.7%, respectively,  $P = .003$ ).

Cytomegalovirus infection occurred in 21 patients in study A and 19 patients in study B (CI: 75.0% versus 65.5%, respectively,  $P = .434$ ), and cytomegalovirus disease occurred in 2 patients in study A and 1 patient in study B (CI: 7.1% versus 3.4%, respectively,  $P = .611$ ). PTLD developed in 5 patients in study A and 2 patients in study B (CI: 17.9% versus 6.9%, respectively,  $P = .253$ ). Three of the 5 patients in study A with PTLD died due to PTLD, whereas in study B 1 patient improved after including rituximab and 1 improved after stopping FK. More information regarding infection and toxicity rates is shown in Table 2.

### Survival Data and Prognostic Factors

To determine the prognostic factors for survival, the total samples in studies A and B were analyzed together. In the univariate analysis, the EFS rate of study B was 93.1%, which is markedly higher when compared with that of study A (64.3%,  $P = .008$ ). Moreover, the OS rate in study B was also higher than that of study A (96.6% versus 67.9%, respectively,  $P = .004$ ; Figure 1). In addition, a history of IST affected the OS rate significantly ( $P = .048$ ).

However, in the multivariate analysis, which included all factors with a  $P < .250$  in the univariate analysis, only the study group was identified as a significant prognostic factor

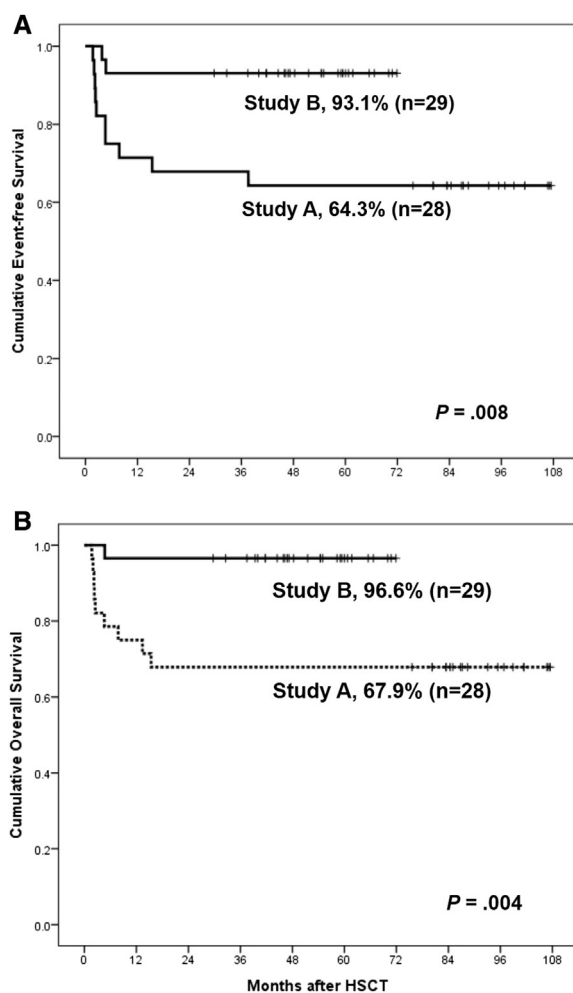
**Table 2**  
Infection and Organ Toxicity Rates for Patients in Study A and Study B

	Study A (n = 28)	Study B (n = 29)	P
Veno-occlusive disease	0 (.0)	1 (3.4)	1.000
CMV infection			
Antigenemia	21 (75.0)	19 (65.5)	.434
Disease	2 (7.1)	1 (3.4)	.611
PTLD	5 (17.9)	2 (6.9)	.253
Organ toxicities, grades 3-4			
Bladder	10 (35.7)	1 (3.4)	.003
Cardiac	1 (3.6)	0 (.0)	.491
CNS	3 (10.7)	0 (.0)	.112
GI	7 (25.0)	6 (20.7)	.698
Hepatic	5 (17.9)	9 (31.0)	.358
Febrile neutropenia	13 (46.4)	23 (79.3)	.010
Pulmonary	4 (14.3)	1 (3.4)	.194
Renal	3 (10.7)	0 (.0)	.112
Skin	1 (3.6)	3 (10.3)	.611

CMV indicates cytomegalovirus; CNS, central nervous system; GI, gastrointestinal.

Values are number of cases with percents in parentheses.

for EFS (exp [B] .150,  $P = .027$ ). In the present study, other factors such as the period between diagnosis and HSCT, stem cell source, HLA disparity, GVHD prophylaxis, and age at HSCT were not significantly associated with OS or EFS (Table 3).



**Figure 1.** EFS and OS curves according to the study group. (A) EFS by study group. (B) OS by study group. Study B (the reduced-toxicity regimen) showed markedly improved EFS and OS when compared with study A.

**Table 3**  
Univariate and Multivariate Analyses of EFS and OS among the Total Sample

	EFS				OS			
	No. (%)	<i>P</i> <sup>*</sup>	<i>P</i> <sup>†</sup>	Exp[B] (95% CI)	No. (%)	<i>P</i> <sup>*</sup>	<i>P</i> <sup>†</sup>	Exp[B] (95% CI)
History of previous IST								
Yes (n = 20)	14 (70.0)	.149	.776	.776 (.359–3.953)	14 (70.0)	.048	.370	1.845 (.483–7.043)
No (n = 37)	31 (83.8)				33 (89.2)			
Interval from diagnosis to transplantation								
<1 yr (n = 27)	24 (88.9)	.102	.13	3.126 (.715–13.659)	24 (88.9)	.238	.496	1.727 (.483–7.043)
≥1 yr (n = 30)	21 (70.0)				23 (76.7)			
Stem cell source								
BM (n = 20)	14 (70.0)	.201	.614	1.407 (.373–5.310)	14 (70.0)	.065	.85	.865 (.192–3.896)
PB (n = 37)	31 (83.8)				33 (89.2)			
HLA disparity, 8/8 (HLA-A, -B, -C, -DR)								
0/8 (n = 34)	26 (76.5)	.660			27 (79.4)	.540		
≥1/8 (n = 23)	19 (82.6)				20 (87.0)			
GVHD prophylaxis								
CsA based (n = 28)	21 (75.0)	.472			22 (78.6)	.415		
FK based (n = 29)	24 (82.8)				25 (86.2)			
Age at HSCT								
≤13 yr (n = 29)	24 (82.8)	.5			25 (86.2)	.482		
>13 yr (n = 28)	21 (75.0)				22 (78.6)			
Study group								
Study A (n = 28)	18 (64.3)	.008	.027	.15 (.028–.808)	19 (67.9)	.004	.057	.116 (.013–1.063)
Study B (n = 29)	27 (93.1)				28 (96.6)			

CI indicates confidence interval.

\* Univariate analysis.

† Multivariate analysis.

## DISCUSSION

This study aimed to investigate the safety and efficacy of a reduced-toxicity conditioning regimen for SAA patients receiving UD HSCT. Although the results of study A demonstrated good engraftment, the TRM rate was relatively high [10]. Therefore, the dosage of the medications used in the conditioning regimen was changed and re-evaluated in a second prospective clinical trial. The second regimen (study B) demonstrated a definitive benefit for EFS and OS without decreasing the engraftment potency.

Recently, FLU-based conditioning regimens for SAA patients receiving UD HSCT have shown promising results [7,13–17], and a 95% failure-free survival rate has been reported with a FLU, Cy, and alemtuzumab conditioning regimen [16]. However, the exact dose of each conditioning medication has not yet been fully defined. For example, in a retrospective study from the European Group for Blood and Marrow Transplantation [7], graft failure/rejection was reported in 17% of the total sample, which may be explained by a lower than conventional dose of Cy (1200 mg/m<sup>2</sup>). On the other hand, Tolar et al. [18] proposed that a Cy dose combined with FLU should range between 50 and 100 mg/kg to prevent excessive organ toxicity. Fortunately, recent studies showed that Cy 50 mg/kg is the most desirable dose in combination with TBI 2 Gy, FLU 120 mg/m<sup>2</sup>, and ATG for UD HSCT for SAA [19,20].

In study B we reduced the dose of Cy (120 mg/kg divided into 2 doses) and increased the dose of FLU (200 mg/m<sup>2</sup> divided into 5 doses). Reducing the dose of Cy resulted in a dramatic effect on EFS. Moreover, hemorrhagic cystitis decreased, representing an overall reduction in conditioning regimen toxicity. On the other hand, the dose of FLU in study B was relatively higher than in other studies [7,16]. Because our conditioning regimen was a non-TBI-containing regimen, we selected the relatively higher cumulative FLU dose in study B. However, it did not cause TRM or severe organ toxicity and showed engraftment outcomes similar to study A. We also

have previously reported that high cumulative FLU doses (200 to 240 mg/m<sup>2</sup>) have acceptable toxicity and predictable pharmacokinetics in children [21]. Although 1 patient in study B showed secondary graft failure, donor-type chimerism was achieved again after steroid pulse therapy.

A conditioning regimen in addition to the use of radiation has also been shown to improve outcomes and enhance engraftment [5,6]. Nevertheless, many concerns about radiation, including secondary malignancy [22] and endocrine problems [23], make it difficult to use, especially in children. However, a European Group for Blood and Marrow Transplantation study has documented that a FLU-based regimen without TBI could be sufficient for children because of the relative better outcome of children when compared with that of adults [7]. Improved outcomes resulting from a FLU-based conditioning regimen without TBI would protect many patients from radiation toxicity.

In this study we performed first-line UD HSCT for patients without previous IST (37 of 57 patients, 64.9%) Although IST has been considered as a first-line treatment for patients without a matched sibling donor [3], recent studies have shown that the outcomes of HSCT with a UD are comparable with those with a matched sibling donor [24,25]. Because of the poor failure-free survival of IST [16], early UD HSCT after IST failure would be a good option to improve patient outcomes.

As a stem cell source for SAA patients with HSCT, PB has been shown to be a negative predictor of survival [25,26]. However, in the present study PB as a stem cell source did not negatively affect survival. Furthermore, study B, which showed better outcomes, had more PB donors than study A. Although this finding is limited because of the small sample size, FK-based GVHD prophylaxis [9] and the homogeneity of immunogenetics in the Asia-Pacific population [24] might alleviate the negative effects of PB. Many donors prefer PB to BM, and our data suggest that UD HSCT with PB could be acceptable in cases when only PB is available.



However, the present study had a limitation in that the characteristics of the 2 study groups were different regarding previous IST history, stem cell source, GVHD prophylaxis regimen, and timing of the HSCT. The higher incidence of IST history in study A might be a negative prognostic factor. Moreover, recent advances in supportive care, such as early detection of Epstein-Barr virus infection, could affect the outcome of study B. Although the multivariate analysis demonstrated that the conditioning regimen was the only prognostic factor of survival, a small sample size made it difficult to compare the 2 different conditioning regimens accurately. Moreover although it is well known that some telomere diseases, such as dyskeratosis congenita, exist in SAA patients, this study could not evaluate the telomero-pathies. In future studies, distinguishing the telomero-pathies from other SAA patients will be needed for more delicate assessment [27].

In this study we report the results of 2 phase II, prospective, multicenter clinical trials examining a conditioning regimen for SAA in children receiving UD HSCT. The comparison between study A and study B demonstrated that a reduced-toxicity FLU, Cy, and ATG conditioning regimen may be an optimal regimen for reducing TRM and enhancing engraftment.

#### ACKNOWLEDGMENTS

**Financial disclosure:** This work was sponsored by the Korean Society of Pediatric Hematology-Oncology and supported by a grant (11172MFDS288) from Ministry of Food and Drug safety in 2011 and by a grant (03-2015-0040) from the Seoul National University Hospital Research Fund.

**Conflict of Interest Statement:** There are no conflicts of interest to report.

**Authorship Statement:** H.J.K. and K.T.H. contributed equally to this work.

#### REFERENCES

- Storb R, Leisenring W, Anasetti C, et al. Long-term follow-up of allogeneic marrow transplants in patients with aplastic anemia conditioned by cyclophosphamide combined with antithymocyte globulin. *Blood*. 1997;89:3890-3891.
- Aljurf M, Al-Zahrani H, Van Lint MT, Passweg JR. Standard treatment of acquired SAA in adult patients 18–40 years old with an HLA-identical sibling donor. *Bone Marrow Transplant*. 2012;48:178-179.
- Marsh JCW, Ball SE, Cavenagh J, et al. Guidelines for the diagnosis and management of aplastic anaemia. *Br J Haematol*. 2009;147:43-70.
- 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.
- Perez-Albuern ED, Eapen M, Klein J, et al. Outcome of unrelated donor stem cell transplantation for children with severe aplastic anemia. *Br J Haematol*. 2008;141:216-223.
- Deeg HJ. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. *Blood*. 2006;108:1485-1491.
- Bacigalupo A, Socie G, Lanino E, et al. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA working party. *Haematologica*. 2010;95:976-982.
- Hardinger KL, Rhee S, Buchanan P, et al. A prospective, randomized, double-blinded comparison of thymoglobulin versus atgam for induction immunosuppressive therapy: 10-year results. *Transplantation*. 2008;86:947-952.
- Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood*. 2000;96:2062-2068.
- Kang HJ, Shin HY, Park JE, et al. Successful engraftment with fludarabine, cyclophosphamide, and thymoglobulin conditioning regimen in unrelated transplantation for severe aplastic anemia: a phase II prospective multicenter study. *Biol Blood Marrow Transplant*. 2010;16:1582-1588.
- Bacigalupo A, Lamparelli T, Milone G, et al. Pre-emptive treatment of acute GVHD: a randomized multicenter trial of rabbit anti-thymocyte globulin, given on day+7 after alternative donor transplants. *Bone Marrow Transplant*. 2010;45:385-391.
- Bacigalupo A, Oneto R, Lamparelli T, et al. Pre-emptive therapy of acute graft-versus-host disease: a pilot study with antithymocyte globulin (ATG). *Bone Marrow Transplant*. 2001;28:1093-1096.
- Ashizawa M, Akahoshi Y, Nakano H, et al. A combination of fludarabine, half-dose cyclophosphamide, and anti-thymocyte globulin is an effective conditioning regimen before allogeneic stem cell transplantation for aplastic anemia. *Int J Hematol*. 2014;99:311-317.
- Anderlini P, Acholonu SA, Okoroji G-J, et al. Fludarabine, cyclophosphamide, and antithymocyte globulin for matched related and unrelated allogeneic stem cell transplant in severe aplastic anemia. *Leuk Lymph*. 2015;52:137-141.
- Kudo K, Muramatsu H, Yoshida N, et al. Second allogeneic hematopoietic stem cell transplantation in children with severe aplastic anemia. *Bone Marrow Transplant*. 2015;50:1312-1315.
- Samarasinghe S, Steward C, Hiwarkar P, et al. Excellent outcome of matched unrelated donor transplantation in paediatric aplastic anaemia following failure with immunosuppressive therapy: a United Kingdom multicentre retrospective experience. *Br J Haematol*. 2012;157:339-346.
- Chaudhry M, Ali N. Reduced-intensity conditioning hematopoietic stem cell transplantation: looking forward to an international consensus. *Blood Res*. 2015;50:69.
- Tolar J, Deeg HJ, Arai S, et al. Fludarabine-based conditioning for marrow transplantation from unrelated donors in severe aplastic anemia: early results of a cyclophosphamide dose deescalation study show life-threatening adverse events at predefined cyclophosphamide dose levels. *Biol Blood Marrow Transplant*. 2012;18:1007-1011.
- Anderlini P, Wu J, Gersten I, et al. Cyclophosphamide conditioning in patients with severe aplastic anaemia given unrelated marrow transplantation: a phase 1–2 dose de-escalation study. *Lancet Haematol*. 2015;2:e367-e375.
- Kulasekararaj AG, Marsh JC. Sweet spot for cyclophosphamide: a balancing act. *Lancet Haematol*. 2015;2:e350-e351.
- Ju HY, Lee JW, Hong CR, et al. Pharmacokinetics of fludarabine in pediatric hematopoietic stem cell transplantation. *Blood*. (ASH Annual Meeting Abstracts) 2014;124:2466.
- Pierga JY, Socie G, Gluckman E, et al. Secondary solid malignant tumors occurring after bone marrow transplantation for severe aplastic anemia given thoraco-abdominal irradiation. *Radiother Oncol*. 1994;30:55-58.
- Eapen M, Ramsay NK, Mertens AC, et al. Late outcomes after bone marrow transplant for aplastic anaemia. *Br J Haematol*. 2000;111:754-760.
- Chen J, Lee V, Luo CJ, et al. Allogeneic stem cell transplantation for children with acquired severe aplastic anaemia: a retrospective study by the Viva-Asia Blood and Marrow Transplantation Group. *Br J Haematol*. 2013;162:383-391.
- Bacigalupo A, Socie G, Hamladji RM, et al. Current outcome of HLA identical sibling versus unrelated donor transplants in severe aplastic anemia: an EBMT analysis. *Haematologica*. 2015;100:696-702.
- Eapen M, Le Rademacher J, Antin JH, et al. Effect of stem cell source on outcomes after unrelated donor transplantation in severe aplastic anemia. *Blood*. 2011;118:2618-2621.
- Townsend DM, Dumitriu B, Young NS. Bone marrow failure and the telomero-pathies. *Blood*. 2014;124:2775-2783.