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

[1] *Department of Pediatrics, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea*
[2] *Department of Pediatrics, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Republic of Korea*
[3] *Department of Pediatrics, The Catholic University of Korea College of Medicine, Seoul, Republic of Korea*
[4] *Department of Pediatrics, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Republic of Korea*
[5] *Department of Pediatrics, Ajou University School of Medicine, Suwon, Republic of Korea*
[6] *Department of Pediatrics, Hanyang University College of Medicine, Seoul, Republic of Korea*
[7] *Department of Pediatrics, Pusan National University School of Medicine, Pusan, Republic of Korea*
[8] *Department of Pediatrics, Chungnam National University College of Medicine, Daejeon, Republic of Korea*
[9] *Department of Pediatrics, Ewha Woman's University School of Medicine, Seoul, Republic of Korea*
[10] *Department of Pediatrics, Chung-Ang University Hospital, Seoul, Republic of Korea*
[11] *Department of Pediatrics, Inje University Haeundae Paik Hospital, Pusan, Republic of Korea*
[12] *Department of Pediatrics, University of Ulsan College of Medicine, Ulsan University Hospital, Ulsan, Republic of Korea*

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A B S T R A C T

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^2 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^2 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.

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

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† 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 (H.S. Ahn).

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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 GHVD 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 $\times$ upper limit of normal, alanine transaminase $<$ 3 $\times$ upper limit of normal, and creatinine $<$ 2 $\times$ 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 (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^2 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^2 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, $\times 10^8$/kg	6.8 (1.3-26.5)	11.6 (1.3-218.4)	.137
CD34, $\times 10^6$/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) $> .5 \times 10^9$/L. Platelet recovery was defined as the first day with a platelet count $> 20 \times 10^9$/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 $< .5 \times 10^9$/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) 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$^+$ 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 × 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 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).

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.

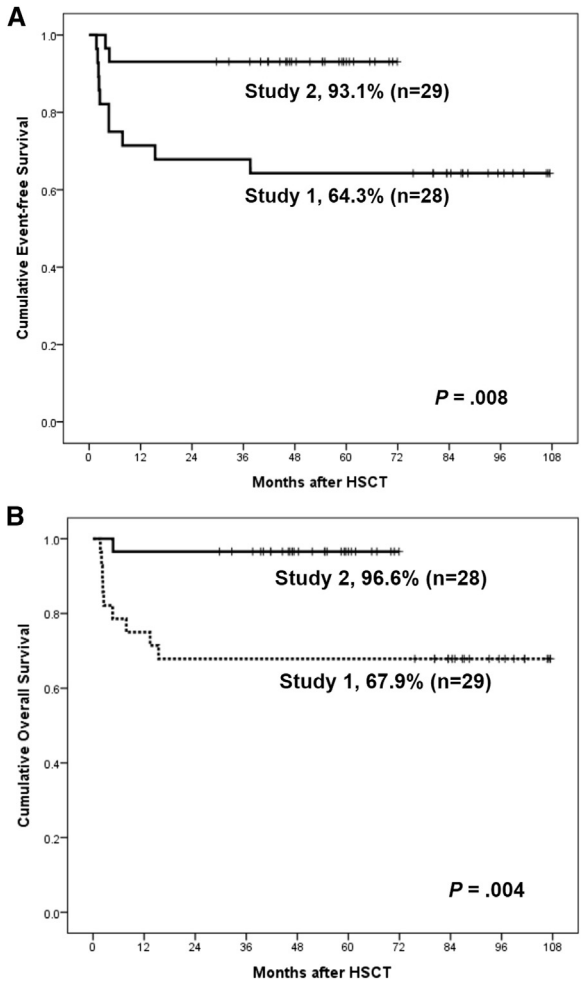

Figure 1. EFS and OS curves according to the study group. (A) EFS by study group. (B) OS by study group. Study B (the reduced-toxicity regimen) showed markedly improved EFS and OS when compared with study A.

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

	EFS				OS			
	No. (%)	P^*	$P^\dagger$	Exp[B] (95% CI)	No. (%)	P^*	$P^\dagger$	Exp[B] (95% CI)
History of previous IST		.149	.776	.776 (.359-3.953)		.048	.370	1.845 (.483-7.043)
Yes (n = 20)	14 (70.0)				14 (70.0)			
No (n = 37)	31 (83.8)				33 (89.2)			
Interval from diagnosis to transplantation		.102	.13	3.126 (.715-13.659)		.238	.496	1.727 (.483-7.043)
<1 yr (n = 27)	24 (88.9)				24 (88.9)			
≥1 yr (n = 30)	21 (70.0)				23 (76.7)			
Stem cell source		.201	.614	1.407 (.373-5.310)		.065	.85	.865 (.192-3.896)
BM (n = 20)	14 (70.0)				14 (70.0)			
PB (n = 37)	31 (83.8)				33 (89.2)			
HLA disparity, 8/8 (HLA-A, -B, -C, -DR)		.660				.540		
0/8 (n = 34)	26 (76.5)				27 (79.4)			
≥1/8 (n = 23)	19 (82.6)				20 (87.0)			
GVHD prophylaxis		.472				.415		
CsA based (n = 28)	21 (75.0)				22 (78.6)			
FK based (n = 29)	24 (82.8)				25 (86.2)			
Age at HSCT		.5				.482		
≤13 yr (n = 29)	24 (82.8)				25 (86.2)			
>13 yr (n = 28)	21 (75.0)				22 (78.6)			
Study group		.008	.027	.15 (.028-.808)		.004	.057	.116 (.013-1.063)
Study A (n = 28)	18 (64.3)				19 (67.9)			
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^2). 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^2, 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^2 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^2) 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 telomeropathies. In future studies, distinguishing the telomeropathies 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.

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