Biol Blood Marrow Transplant 25 (2019) 965–974

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Comparable Outcome with a Faster Engraftment of Optimized Haploidentical Hematopoietic Stem Cell Transplantation Compared with Transplantations from Other Donor Types in Pediatric Acquired Aplastic Anemia

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Article history:
Received 21 August 2018
Accepted 4 January 2019

Keywords:
Children and adolescents
Severe aplastic anemia
Haploidentical stem cell transplantation
Siblings
Unrelated donors

A B S T R A C T

Haploidentical family donors have been used as an alternative source in hematopoietic cell transplantation for patients with severe aplastic anemia. We evaluated and compared the outcomes of transplantation in pediatric acquired severe aplastic anemia based on donor type. Sixty-seven patients who underwent transplantation between 1998 and 2017 were included. Fourteen patients received grafts from matched sibling donors, 21 from suitable unrelated donors, and 32 from haploidentical family donors. Ex vivo $CD3^+$ or $\alpha\beta^+$ T cell–depleted grafts were used for haploidentical transplantation. Sixty-five patients (97.0%) achieved neutrophil engraftment at a median of 11 days. Haploidentical transplantation resulted in significantly faster neutrophil engraftment at a median of 10 days, compared with 14 days in cases of matched sibling donors and 12 days in cases of unrelated donor recipients. Nine patients experienced graft failure, and 5 of 7 who underwent a second transplantation are alive. There was no difference in the incidence of acute or chronic graft-versus-host disease based on donor type. The 5-year overall survival and failure-free survival rates were 93.8% ± 3.0% and 83.3% ± 4.6%, respectively, and there was no significant survival difference based on donor type. The survival outcomes of haploidentical transplantation in patients were comparable with those of matched sibling or unrelated donor transplantation. Optimized haploidentical transplantation using selective T cell depletion and conditioning regimens including low-dose total body irradiation for enhancing engraftment may be a realistic therapeutic option for pediatric patients with severe aplastic anemia.

INTRODUCTION

Severe aplastic anemia (SAA) is a life-threatening disorder for which allogeneic hematopoietic stem cell transplantation (HCT) is a curative treatment. HLA-matched sibling donors (MSDs) are the preferred donors; however, it is not always possible to find an MSD for patients requiring HCT. In the absence of an MSD, the general consensus is frontline immunosuppressive therapy (IST) followed by HCT from a matched unrelated donor (URD) in case of failure or relapse. The limited availability of suitable matched donors has promoted the development of haploidentical HCT, which offers the option of immediate transplantation to any patient in need of an allograft [1].

HCT using a haploidentical family donor (HFD-HCT) was initially complicated by a high incidence of graft-versus-host disease (GVHD), graft rejection, or delayed immune reconstitution, resulting in high treatment-related mortality [2]. Because graft rejection is mediated by host T cells and reduced by donor T cells, which also mediate GVHD, various approaches have been adopted to optimize T cell depletion [3,4].

Ex vivo techniques to manipulate T cells have evolved from positive selection of $CD34^+$ hematopoietic stem cells to deplete $CD3^+$ cells, and the abundant B cells after T cell manipulation can be further depleted to lower the risk of developing post-transplant lymphoproliferative disorder [3]. Ex vivo graft

Financial disclosure: See Acknowledgments on page 973.

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https://doi.org/10.1016/j.bbmt.2019.01.010
1083-8791/

manipulation based on selective depletion of TCR $\alpha\beta^+$ T lymphocytes is a more sophisticated approach that allows transfer of not only high numbers of $CD34^+$ cells and mature donor natural killer (NK) cells to the recipient, but also TCR $\gamma\delta^+$ T cells, which exert protective effects against life-threatening infections [2,5].

We used the $CD3^+$ T cell depletion method to eliminate T cells for haploidentical transplantation (CD3-HFD-HCT), and the early results of our trial on 12 patients with SAA who successfully received CD3-HFD-HCT have been previously reported [6,7]. We are currently using ex vivo TCR $\alpha\beta^+$ T cell depletion in the HFD-HCT setting to enhance immune recovery and mitigate GVHD (TCR$\alpha\beta$-HFD-HCT). Immunosuppressive drugs are not used in TCR$\alpha\beta$-HFD-HCT to alleviate potential toxicity and drug interactions while limiting the final infused $\alpha\beta^+$ T cell dose to $\leq 5 \times 10^4$/kg.

In the present study we evaluated the outcome of HCT in children and adolescents with acquired SAA and compared the outcomes of transplantation based on the donor and methods of graft manipulation in HFD-HCT.

METHODS

Patients

Sixty-seven patients with acquired SAA received HCT at Asan Medical Center between March 1998 and March 2017. Fourteen patients received grafts from MSDs, 21 from suitable URDs, and 32 from HFDs. For URDs HLA-10/10 matched donors were preferred, but a single-allele mismatched donor was also accepted [8].

Grafts for HFD-HCT were CD3 depleted in 16 patients until January 2013 and $\alpha\beta^+$ T cell depleted in 16 patients thereafter. Donor-specific anti-HLA antibodies were tested in haploidentical donor candidates beginning October, 2011, and those with high mean fluorescence intensity of more than 10,000 were not selected as final donors [9].

All protocols were approved by the Institutional Review Board, and the HFD-HCT trials were registered at www.clinicaltrials.gov (NCT01105273, NCT02014506). All patients and donors provided written informed consent.

Conditioning Regimens and GVHD Prophylaxis

Eleven patients for MSD- or URD-HCT received cyclophosphamide (CY, 200 mg/kg) with rabbit antithymocyte globulin (ATG, 3 mg/kg/day, days −4 to −2), and the other 24 patients received a combination of fludarabine (150 mg/m^2), CY (120 mg/kg), and ATG (2.5 mg/kg/day, days −3 to −1) (Table 1). The first 6 patients undergoing HFD-HCT received fludarabine (150 mg/m^2), CY (120 mg/kg), and ATG (2.5 mg/kg/day, days −3 to −1), and the following patients received additional low-dose total body irradiation (LD-TBI, 400 cGy; 200 cGy/day, days −3 to −2) with a modified ATG schedule (2.5 mg/kg/day, days −8 to −6).

Cyclosporine or tacrolimus with mycophenolate mofetil was used for GVHD prophylaxis in HFD-HCT, and cyclosporine with methotrexate was used in other patients. After day 90 the dose of calcineurin inhibitors was gradually reduced by 5% a week and discontinued at 8 months if the patient was not under GVHD treatment. No pharmacologic prophylaxis has been used in TCR$\alpha\beta$-HFD-HCT since November 2015, whereas the $\alpha\beta^+$ T cell dose has been targeted at $\leq 5 \times 10^4$ cells/kg.

Stem Cell Collection and Graft Manipulation

Sibling donors for bone marrow transplantation were primed with 7.5 μg/kg/day granulocyte colony-stimulating factor as a single evening injection [10-12], and bone marrow was harvested under general anesthesia. Peripheral blood stem cells were collected as described previously [7,13]. Ex vivo T cell depletion was conducted with the CliniMACS system (Miltenyi-BioTec,

Table 1
Patient and Transplant Characteristics

Variables	Total (N = 67)	MSD-HCT (n = 14)	URD-HCT (n = 21)	HFD-HCT (n = 32)	*P*
Study period	1998-2017	1998-2016	2003-2016	2008-2017	
					.593
Median age at transplant, yr (range)	12.2 (.7-21.7)	12.1 (.7-20.1)	8.2 (1.6-19.0)	12.7 (1.4-21.7)	
Sex					.493
Male	46 (68.7)	8 (57.1)	16 (76.2)	22 (68.8)	
Female	21 (31.3)	6 (42.9)	5 (23.8)	10 (31.2)	
Severity of aplastic anemia					.001
Severe	54 (80.6)	13 (92.9)	21 (100)	20 (62.5)	
Very severe	13 (19.4)	1 (7.1)	0 (0)	12 (37.5)	
Previous immunosuppressive therapy					.109
Yes	26 (38.8)	2 (14.3)	8 (38.1)	16 (50.0)	
No	41 (61.2)	12 (85.7)	13 (61.9)	16 (50.0)	
					.523
Median time to HCT after diagnosis, mo (range)	6.1 (1.2-139.0)	4.1 (1.7-109.6)	10.7 (1.6-139.0)	5.2 (1.2-106.8)	
Stem cell source					<.001
Bone marrow	9 (13.4)	6 (42.9)	3 (14.3)	0 (0)	
Peripheral blood	58 (86.6)	8 (57.1)	18 (85.7)	32 (100)	
Donors			NA		NA
Father	5 (7.5)	—		5 (15.6)	
Mother	18 (26.9)	—		18 (56.3)	
Sibling	23 (34.3)	14 (100)		9 (28.1)	
CMV serostatus, recipient−donor					.349
Negative−positive	1 (1.5)	1 (7.1)	—	—	
Positive−negative	3 (4.5)	—	2 (9.5)	1 (3.1)	
Positive−positive	63 (94.0)	13 (92.9)	19 (90.5)	31 (96.9)	
Conditioning regimen					<.001
CY/ATG	11 (16.4)	8 (57.1)	3 (14.3)	0	
Flu/CY/ATG	30 (44.8)	6 (42.9)	18 (85.7)	6 (37.5)	
TBI/Flu/CY/ATG	26 (38.8)	0	0	26 (81.3)	
GVHD prophylaxis					<.001
CSP/MMF	4 (6.0)	0	0	4 (12.5)	
CSP/MTX	35 (52.2)	14 (100)	21 (100)	0	
FK/MMF	19 (28.4)	0	0	19 (59.4)	
None	9 (13.4)	0	0	9 (28.1)	
Graft composition					
$CD34^+$ stem cells, x10^6 cells/kg, median (range)	7.6 (3.0-33.8)	7.8 (4.7-33.8)	8.9 (3.2-25.1)	5.8 (3.0-16.1)	.043
$CD3^+$ T cells, x10^6 cells/kg, median (range)	29.3 (.2-957.0)	203.0 (2.7-923.0)	305.0 (143.0-957.0)	7.7 (.2-90.0)*	<.001

Values are n (%) unless otherwise defined. Flu indicates fludarabine; CSP, cyclosporine; MMF, mycophenolate mofetil; MTX, methotrexate; FK, tacrolimus; NA, not applicable.

* There is a difference in T cell dose according to the T cell−depletion methods; detailed values are described in Table 3.

Bergisch-Gladbach, Germany) per the manufacturer's instructions. One dose of rituximab (375 mg/m^2) was given to patients who received CD3$^+$ or $\alpha\beta^+$ T cell–depleted grafts.

Supportive Care

Patients were given prophylactic antibiotics, fluconazole or micafungin, and acyclovir. For patients undergoing HFD-HCT, cytomegalovirus (CMV) prophylaxis was conducted with ganciclovir (valgancyclovir) ± foscavir as described in our previous study [14]. Granulocyte colony-stimulating factor was given beginning on day 4 or 5 until the absolute neutrophil count reached 3000/μL.

Post-Transplant Assessment

Donor engraftment was determined by using DNA-based short tandem repeat assays from whole blood samples. Viral surveillance included weekly screening for CMV using PCR and/or antigenemia assays and for Epstein-Barr virus using PCR. Preemptive therapy with ganciclovir was initiated when positive results were obtained in the PCR or antigenemia assays. Acute GVHD (aGVHD) was graded per the consensus criteria [15], and chronic GVHD (cGVHD) was defined as limited or extensive [16]. Immune cell recovery was monitored using flow cytometry (Beckman Coulter, Brea, CA) [2].

Definitions and Statistical Analysis

Graft failure (GF) included both primary GF (absolute neutrophil count not exceeding 500/μL within 1 month) and secondary GF (absolute neutrophil count initially exceeding 500/μL, followed by loss of a previously functioning graft) [17]. Failures included GF, transfusion dependency, or death. Overall survival (OS) was measured from the time of HCT to the time of last follow-up or death. Failure-free survival (FFS) was defined as survival without failure. Probabilities of OS and FFS were estimated using the Kaplan-Meier method and compared using the log-rank test. The between-group differences in cumulative incidence (CI) were assessed using the Fine and Gray model [18]. $P < .05$ was considered significant. Statistical analyses were performed using SPSS Statistics 24.0 (IBM Corp., Armonk, NY) and R (3.2.2; http://www.r-project.org/).

RESULTS

Characteristics of Patients and Transplantation

The median age at transplantation was 12.2 years (range, .7 to 21.7) (Table 1). Forty-six patients (68.7%) were male. Of the 67 patients, 13 (19.4%) met the criteria for very SAA, and 26 (38.8%) received IST before HCT. The median time to HCT after diagnosis was 6.1 months in all patients, 4.1 months in MSD-HCT, 10.7 months in URD-HCT, and 5.2 months in HFD-HCT. The median time to HCT was longer in URD-HCT than in other donors, but there was no statistically significant difference ($P = .523$) (Table 1).

In URD-HCT 18 of 21 patients received HCT from HLA-matched donors (HLA-A, -B, -C, -DR, 8/8), whereas 3 patients received HCT from 1- allele mismatched donor (7/8). Most patients (86.6%) received peripheral blood stem cells as the stem cell source. Donors were mothers in 18 patients (56.3%) who underwent HFD-HCT. CMV serostatus of recipients and donors were both positive in 63 patients (94.0%), recipient-only positive in 3 patients (4.5%), and both negative in 1 patient (1.5%). The median doses of infused CD34$^+$ and CD3$^+$ cells were 7.6×10^6/kg (range, 3.0 to 33.8) and 29.3×10^6/kg (range, .2 to 957.0), respectively. The median infused dose of CD34$^+$ cells was the highest in URD-HCT and that of CD3$^+$ T cells was the lowest after ex vivo T cell depletion in HFD-HCT.

Engraftment

Among the 67 patients, 65 (97.0%) achieved initial neutrophil engraftment at a median of 11 days (range, 9 to 30) (Table 2). HFD-HCT showed significantly faster neutrophil engraftment ($P = .022$); the median values of neutrophil engraftment were 10 days in HFD recipients, 12 days in URD, and 14 days in MSD. The CI of neutrophil engraftment at day 14 post-transplant was also significantly higher in HFD-HCT (MSD 57.1% ± 14.0%, URD 71.4% ± 10.3%, and HFD 96.9% ± 3.9%; $P < .001$) (Figure 1). A total of 61 patients with the exception of 6 patients (2 primary GF and 4 secondary GF) achieved platelet engraftment at a median of 18 days post-transplant (range, 13 to 65).

Nine patients experienced GF; 1 URD and 1 HFD recipient experienced primary GF, and 2 MSD and 5 HFD recipients experienced secondary GF (Table 2). The CI of GF was not different in the 3 donor types (MSD 14.3% ± 9.7%, URD 9.8% ± 6.7%, and HFD 18.8% ± 7.0%; $P = .599$). Five HFD recipients who experienced secondary GF received CD3-depleted grafts, and 3

Table 2
Transplantation Outcomes in All Patients

Variables	Total (N = 67)	MSD-HCT (n = 14)	URD-HCT (n = 21)	HFD-HCT (n = 32)	*P*
Median follow-up duration after HCT, yr (range)	5.6 (1.3-19.9)	7.5 (1.7-19.9)	7.2 (1.8-15.2)	5.2 (1.3-8.9)	.006
Engraftment					
Successful engraftment	57 (85.1)	12 (85.7)	19 (90.5)	26 (81.3)	
Primary GF	2 (3.0)	0	1 (4.8)	1 (3.1)	.652
Secondary GF	7 (10.4)	2 (14.3)	0	5 (15.6)	
Transfusion dependency	1 (1.5)	0	1 (4.8)	0	
Neutrophil engraftment	65 (97.0)	14 (100)	20 (95.2)	31 (96.9)	.718
Median days (range)	11 (9-30)	14 (10-17)	12 (11-30)	10 (9-30)	.022
Platelet engraftment	61 (91.0)	14 (100)	20 (95.2)	27 (84.4)	.167
Median days (range)	18 (13-65)	22 (16-64)	18 (15-60)	15.5 (13-60)	.656
aGVHD					
Grade I	4 (6.0)	1 (7.1)	0	3 (9.4)	
Grade II	15 (22.4)	2 (14.3)	7 (33.3)	6 (18.8)	.472
Grade III	5 (7.5)	0	2 (9.5)	3 (9.4)	
Grade IV	0	0	0	0	
cGVHD (assessable)					
Limited	4 (6.0)	0	1 (4.8)	3 (9.4)	.193
Extensive	2 (3.0)	0	2 (9.5)	0	
Other complications					
CMV reactivation	34 (50.7)	1 (7.1)	13 (61.9)	20 (62.5)	.001
CMV disease	6 (9.1)	0	0	6 (18.8)	.030
EBV reactivation	29 (43.9)	1 (7.1)	14 (66.7)	14 (43.8)	.003
PTLD	6 (15.8)	0	3 (14.3)	3 (9.4)	.014
Cystitis	6 (11.3)	0	0	6 (18.8)	.109
Treatment-related mortality	4 (6.0)	1 (7.1)	1 (4.8)	2 (6.2)	.954

Values are n (%) unless otherwise defined. EBV indicates Epstein-Barr virus; PTLD, post-transplant lymphoproliferative disease.

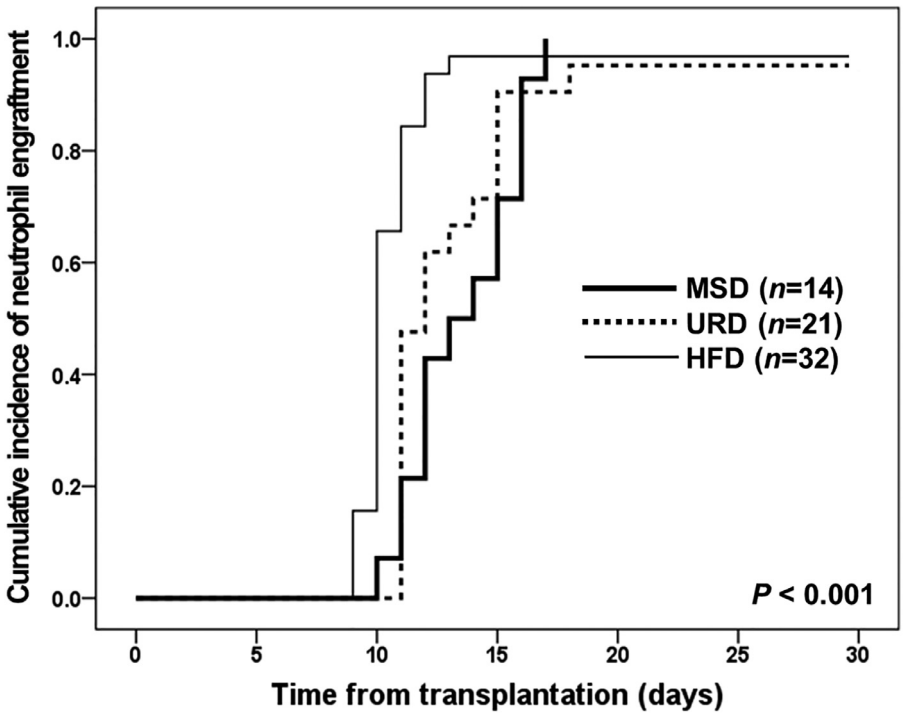

Figure 1. The CI of neutrophil engraftment based on donor type. The CI of neutrophil engraftment at day 14 post-transplant was significantly higher in HFD recipients (MSD, 57.1% ± 14.0%; URD, 71.4% ± 10.3%; and HFD, 96.9% ± 3.9%).

of them underwent LD-TBI–containing conditioning regimens. GF was not correlated with the number of infused $CD34^+$ or $CD3^+$ T cells or infection in multivariate analysis.

aGVHD and cGVHD

Twenty patients developed aGVHD grades II to IV (15 with grade II, 5 with grade III) (Table 2). The CI values of aGVHD grades II to IV and III to IV were 28% ± 5.5% and 7.4% ± 3.2%, respectively. No significant difference was observed between the CIs of aGVHD grades III to IV based on donor type (MSD 0, URD 9.5% ± 6.4%, and HFD 9.4% ± 5.2%; $P = .470$). A combination of methylprednisolone ± calcineurin inhibitors was administered as first-line treatment for aGVHD in all patients and oral beclomethasone dipropionate ± infliximab for persistent gastrointestinal symptoms.

Six patients developed cGVHD, 3 URD and 3 HFD recipients (Table 2). The 1-year CI of cGVHD in all patients was 8.8% ± 3.5%. No difference was found in the occurrence ($P = .193$) or CIs of cGVHD based on donor type (MSD 0%, URD 14.3% ± 7.8%, and HFD 9.4% ± 5.3%; $P = .367$). All patients with GVHD were successfully treated, and no GVHD-related deaths were reported.

Second Transplantation

Seven of 9 patients who experienced GF received a second HCT. One URD recipient achieved engraftment after a second URD but died of massive gastrointestinal bleeding caused by mycophenolate mofetil–induced colitis. Five HFD recipients successfully underwent a second HCT from a different HFD with fludarabine (150 mg/m^2) and CY (100 mg/kg) and with additional LD-TBI in 1 patient. Another HFD recipient underwent a second HCT from the same donor, but engraftment was achieved after the third HCT with a different HFD.

Two patients with secondary GF after MSD-HCT did not receive a second HCT; 1 received further IST and a peripheral blood stem cell infusion from the same donor but could not be rescued and died. The other refused further HCT and is currently alive with intermittent transfusions. Currently, 5 of 9 patients with GF are alive with full donor chimerism and no requirement for transfusion.

One URD recipient who initially achieved full donor chimerism after the first HCT became transfusion-dependent and successfully underwent subsequent HFD-HCT 26 months after the initial HCT.

Chimerism

The last chimerism study of 67 patients found that 60 patients showed full donor chimerism and normal peripheral blood counts. Of the 7 patients without full donor chimerism, 5 remained transfusion-free with stable mixed chimerism, ranging from 83% to 94% donor cells, and the other 2 patients showed 100% recipient cells after secondary GF.

Immune Reconstitution

Immune reconstitution of patients with sustained engraftment was evaluated and compared with the normal range in Koreans [19]. The levels of $CD3^+$ T cells were within normal levels 3 months post-transplant in the MSD and URD recipients, whereas the level in the HFD cohort was normal after 6 months (Figure 2A). The average $CD4^+$ T cell counts of MSD, URD, and HFD recipients on day 30 were 93, 183, and 41 cells/μL, respectively, and the levels in the URD cohort were significantly higher than in others ($P < .005$) (Figure 2B). Although the recovery of $CD4^+$ T cells was relatively slow in HFD-HCT, they reached the normal range 9 months post-transplant in all patients (MSD, 482 cells/μL; URD, 456 cells/μL; and HFD, 530 cells/μL). The $CD8^+$ T cell and NK cell recovery was fast and sustained within the normal range after HFD-HCT (Figure 2C,D). B cell recovery was markedly delayed in HFD-HCT compared with the other groups but recovered to normal levels at 12 months (Figure 2E).

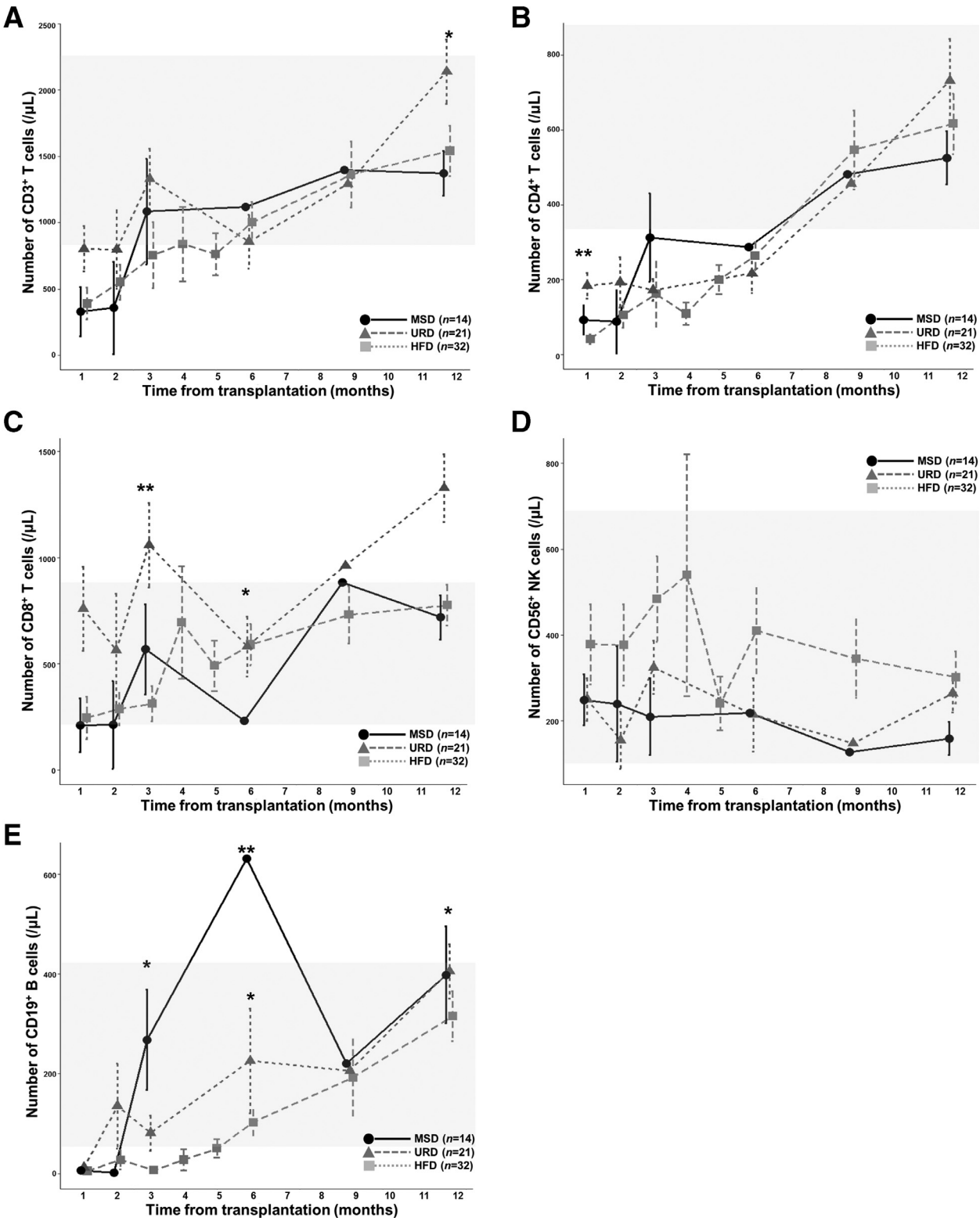

Figure 2. Average counts of immune cells and subsets in patients with SAA after transplantation. Error bars represent the standard deviation of a data set. Normal values from a normal Korean population are shown as gray background. $^{*}P < .05$, $^{**}P < .005$. (A) $CD3^{+}$ T cells, (B) $CD4^{+}$ T cells, (C) $CD8^{+}$ T cells, (D) $CD56^{+}$ NK cells, and (E) $CD19^{+}$ B cells.

Infections

Viral reactivation was less frequent in MSD-HCT, and CMV disease was significantly more frequent in HFD-HCT (Table 2). CMV reactivation occurred in 34 patients (50.7%). Among these, 6 HFD recipients developed CMV disease: 4 retinitis, 1 pneumonitis, and 1 colitis. After starting CMV prophylaxis in HFD-HCT the incidence of CMV reactivation and disease decreased from 100% to 57.1% and 50% to 14.3%, respectively.

Epstein-Barr virus reactivation occurred in 29 patients (43.9%), mostly in URD and HFD recipients. Six patients developed post-transplant lymphoproliferative disorder and were successfully treated with rituximab ± chemotherapy. BK virus-hemorrhagic cystitis occurred in 6 HFD recipients, and 5 recovered completely after supportive care or cidofovir, except 1 patient who developed BK nephropathy. No serious bacterial or fungal infections occurred during the study period.

Survival and Transplant-Related Mortality

As of May 2018 the estimated 5-year OS and FFS rates were 93.8% ± 3.0% and 83.3% ± 4.6% in all patients. No difference in OS was observed based on donor type (MSD, 92.9% ± 6.9%; URD, 95.2% ± 4.6%; and HFD, 93.4% ± 4.5%; $P = .957$) (Figure 3A). FFS was the lowest in HFD-HCT; however, the difference was not significant based on donor type (MSD, 85.7% ± 9.4%; URD, 90.2% ± 6.6%; and HFD, 78.1% ± 7.3%; $P = .432$) (Figure 3B).

Four patients died of transplant-related causes; thus, the CI of treatment-related mortality at 2 years was 6.1% ± 2.9%. One MSD recipient died 18 months after HCT due to secondary GF-related pancytopenia and complications. One URD recipient experienced primary GF and died of massive gastrointestinal bleeding caused by mycophenolate mofetil–induced colitis. One CD3-HFD-HCT recipient with secondary GF died of pure RBC aplasia followed by a $CD34^+$ stem cell booster given on day 265. One patient who received TCR$\alpha\beta$-HFD-HCT died of CMV pneumonitis on day 157.

Outcomes of HFD-HCT

Among 32 patients who underwent HFD-HCT, grafts were $CD3^+$ depleted in 12 patients, $CD3/CD19^+$ depleted in 4, and $\alpha\beta^+$ T cell depleted in 16 patients (Table 3). The median infused dose of $CD3^+$ T cells was 6.8×10^5/kg (range, 1.6 to 79.5) in 16 CD3-HFD-HCT and that of $\alpha\beta^+$ T cells was 7.0×10^4/kg (range, 1.0 to 66) in 16 TCR$\alpha\beta$-HFD-HCT. Six patients who received CD3-HFD-HCT experienced GF (1 primary and 5 secondary), whereas none in the TCR$\alpha\beta$-HFD-HCT group experienced GF over a median follow-up of 2.4 years. The CIs of aGVHD grades III to IV were comparable between the 2 groups (CD3-HFD-HCT, 12.5% ± 8.5%, and TCR$\alpha\beta$-HFD-HCT, 6.3% ± 6.3%; $P = .570$) (Figure 4A). cGVHD occurred in 3 patients; 2 received $CD3^+$-depleted grafts and 1 $\alpha\beta^+$-depleted grafts. There was no definite risk factor including higher $CD3^+$ or $\alpha\beta^+$ T cells in these patients. The CIs of cGVHD were not different between CD3-HFD-HCT (12.5% ± 8.7%) and TCR$\alpha\beta$-HFD-HCT (6.3% ± 6.3%) (Figure 4B). No patient developed aGVHD grades III to IV or cGVHD after TCR$\alpha\beta$-HFD-HCT without immunosuppressive drugs.

The 5-year OS rates were similar between the 2 groups (CD3-HFD-HCT, 93.8% ± 8.7%, and TCR$\alpha\beta$-HFD-HCT, 93.8% ± 6.1%) (Figure 4C). The median follow-up duration in TCR$\alpha\beta$-HFD-HCT (immunosuppression) was shorter than that in others, but there was no treatment-related mortality at the time of analysis. The 5-year FFS of TCR$\alpha\beta$-HFD-HCT (93.8% ± 6.1%) was significantly higher than that of CD3-HFD-HCT (62.5% ± 12.1%, $P = .032$) (Figure 4D). All TCR$\alpha\beta$-HFD-HCT recipients achieved full donor chimerism on day 14 and showed more stable donor chimerism than the CD3-HFD-HCT cohort (Supplementary Figure S1). In terms of conditioning regimens, patients given an LD-TBI–containing regimen showed higher FFS with a lower incidence of GF (Supplementary Figure S2). The initial trends in the T cell subsets and B cell recovery were similar in both cohorts, but immune cell counts started to increase markedly approximately 6 months after TCR$\alpha\beta$-HFD-HCT (Supplementary Figure S3).

DISCUSSION

Our data demonstrate that ex vivo T cell–depleted HFD-HCT had comparable outcomes in children and adolescents with acquired SAA with MRD- or URD-HCT. Although few reports have compared outcomes of HCT from HFD and MSD in SAA, this is the first report comparing HCT outcomes of consecutive pediatric and adolescents patients with SAA based on different donors including URD [20-22].

In the treatment of SAA, IST with the combination of cyclosporine and horse ATG is still considered the first-line choice for those who lack an HLA-matched family donor. IST with horse ATG has shown better hematologic response and higher survival rates [23-25]. However, rabbit ATG is the only preparation available in Asia since 2007, because horse ATG is no longer supplied. Given the high relapse rate and additional clonal evolution after IST and the comparable outcomes in upfront URD-HCT compared with historical control subjects who had undergone IST [26], we believe it is reasonable to perform transplantation from suitable URDs as first-line treatment in patients lacking an MSD, especially in situations where horse ATG is not available [27].

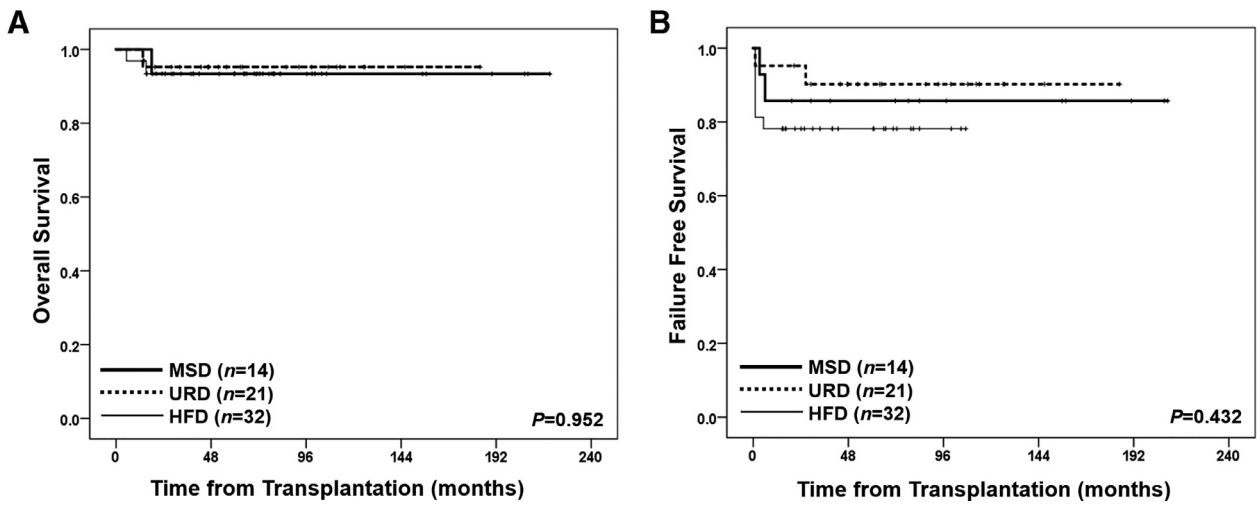

Figure 3. Survival rates of patients based on donor type. (A) OS rate and (B) FFS rate were not different between the MSD, URD, and HFD recipients.

Table 3
Characteristics and Outcomes of HCT

Variables	CD3-HFD-HCT (n = 16)	TCR$\alpha\beta$-HFD-HCT			*P*
		Subtotal (n = 16)	IS(+) (n = 7)	IS(−) (n = 9)	
Median follow-up after HCT, yr (range)	6.5 (5.4-8.9)	2.4 (1.3-5.1)	3.3 (2.8-5.1)	1.4 (1.3-2.5)	<.001
Median age at transplant, yr (range)	14.4 (3.8-21.7)	10.3 (1.4-16.6)	11.6 (2.4-16.6)	7.7 (1.4-16.1)	.012
Severity of aplastic anemia					
Severe	16 (100)	4 (25.0)	2 (28.6)	2 (22.2)	<.001
Very severe	0 (0)	12 (75.0)	5 (71.4)	7 (77.8)	
Previous immunosuppressive therapy					.104
Yes	11 (68.7)	5 (31.3)	2 (28.6)	3 (33.3)	
No	5 (31.3)	11 (68.7)	5 (71.4)	6 (66.7)	
CMV serostatus, recipient−donor					.158
Negative−positive	–	–	–	–	
Positive−negative	–	1 (6.2)	1 (14.3)	–	
Positive−positive	16 (100)	15 (93.8)	6 (85.7)	9 (100)	
Methods of T cell depletion					NA
$CD3^+$ depletion	12	–	–	–	
$CD3^+/CD19^+$ depletion	4	–	–	–	
$\alpha\beta^+$ T cell depletion	–	16	7	9	
Conditioning regimen					.025
Flu/CY/ATG	6 (37.5)	–	–	–	
TBI/Flu/CY/ATG	10 (62.5)	16 (100)	7 (100)	9 (100)	
GVHD prophylaxis					<.001
CSP/MMF	4 (25.0)	–	–	–	
FK/MMF	12 (75.0)	7 (43.7)	7 (100)	–	
None	–	9 (56.3)	–	9 (100)	
Graft composition					
$CD34^+$ stem cells, $x10^6$ cells/kg, median (range)	5.63 (3.00-9.40)	7.72 (3.03-16.14)	8.6 (4.0-15.2)	6.1 (3.0-16.1)	.070
$CD3^+$ T cells, $x10^5$ cells/kg, median (range)	6.8 (1.6-79.5)	264.3 (62-900)	248 (76-766)	281 (62-900)	<.001
$CD3^+$ $\alpha\beta^+$ T cells, $x10^4$ cells/kg, median (range)	NA	7.0 (1.0-66)	22.0 (13-66)	4.0 (1.0-8.0)	NA
$CD3^+$ $\gamma\delta^+$ T cells, $x10^6$ cells/kg, median (range)	NA	21.41 (4.09-87.40)	23.0 (5.2-75.0)	19.8 (4.1-87.4)	NA
$CD19^+$ B cells, $x10^6$ cells/kg, median (range)	26.70 (.00-76.40)	53.60 (19.22-118.95)	63.6 (19.2-116.1)	45.3 (21.6-119.0)	.039
$CD56^+$ NK cells, $x10^6$ cells/kg, median (range)	27.25 (5.29-73.34)	40.01 (17.45-165.60)	38.1 (18.8-124.9)	41.9 (17.5-165.6)	.090
Engraftment					.012
Successful engraftment	10 (62.5)	16 (100)	7 (100)	9 (100)	
Primary GF	1 (6.2)	–	–	–	
Secondary GF	5 (31.3)	–	–	–	
Transfusion dependency	0	–	–	–	
Neutrophil engraftment	15 (93.8)	16 (100)	7 (100)	9 (100)	.597
Median days (range)	10 (9-30)	10 (9-12)	10 (9-10)	10 (9-12)	.424
Platelet engraftment	11 (68.8)	16 (100)	7 (100)	9 (100)	.052
Median days (range)	19 (14-60)	15 (13-20)	15 (14-20)	15 (13-19)	.021
aGVHD					.476
Grade I	1 (6.3)	2 (12.5)	1 (14.3)	1 (11.1)	
Grade II	2 (12.5)	4 (25.0)	2 (28.6)	2 (22.2)	
Grade III	2 (12.5)	1 (6.3)	1 (14.3)	0	
Grade IV	0	0	0	0	
cGVHD (assessable)					.124
Limited	2 (12.5)	1 (6.3)	1 (14.3)	0	
Extensive	0	0	0	0	
Other complications					
CMV reactivation	9 (56.3)	11 (68.8)	4 (57.1)	7 (77.8)	.536
CMV disease	3 (18.8)	3 (18.8)	1 (14.3)	2 (22.2)	.922
EBV reactivation	9 (56.3)	5 (31.3)	1 (14.3)	4 (44.4)	.175
PTLD	2 (12.5)	1 (6.3)	0	1 (11.1)	.625
Cystitis	5 (31.3)	1 (6.3)	0	1 (11.1)	.165
Treatment-related mortality	1 (6.3)	1 (6.3)	1 (1.4)	0	.992

Values are n (%) unless otherwise defined. IS indicates immunosuppressant.

MSD-HCT in children with SAA shows an estimated 10-year OS of 90% [28,29]. The results of URD-HCT have been improved to over 90% [30-32]. However, the management of patients lacking a suitable matched donor has not been fully established. The options for alternative donors include cord blood or HFD. Although several studies have reported the feasibility of cord blood transplantation in acquired SAA, high GF rates and delayed immune recovery have been reported to be major problems [33,34].

Recent advances in T cell manipulations, optimal conditioning, and better supportive care have significantly improved the outcomes of HFD-HCT [7,35-37]. Xu et al. [38] reported that T cell–replete HFD-HCT using ATG in 52 children with SAA showed 3-year OS and FFS rates of 84.5% and 82.7%, respectively. In another study using reduced-intensity conditioning and post-transplant CY, the 1-year OS was 67.1% in 16 patients with SAA [36]. In our study the 5-year OS and FFS rates of the HFD-HCT cohort were 93.4% and 78.1%, respectively, and OS was higher than that in reports of HFD-HCT using in vivo T cell depletion [36,38]. In addition, FFS of the TCR$\alpha\beta$-HFD-HCT group was markedly improved to 93.8% in our study.

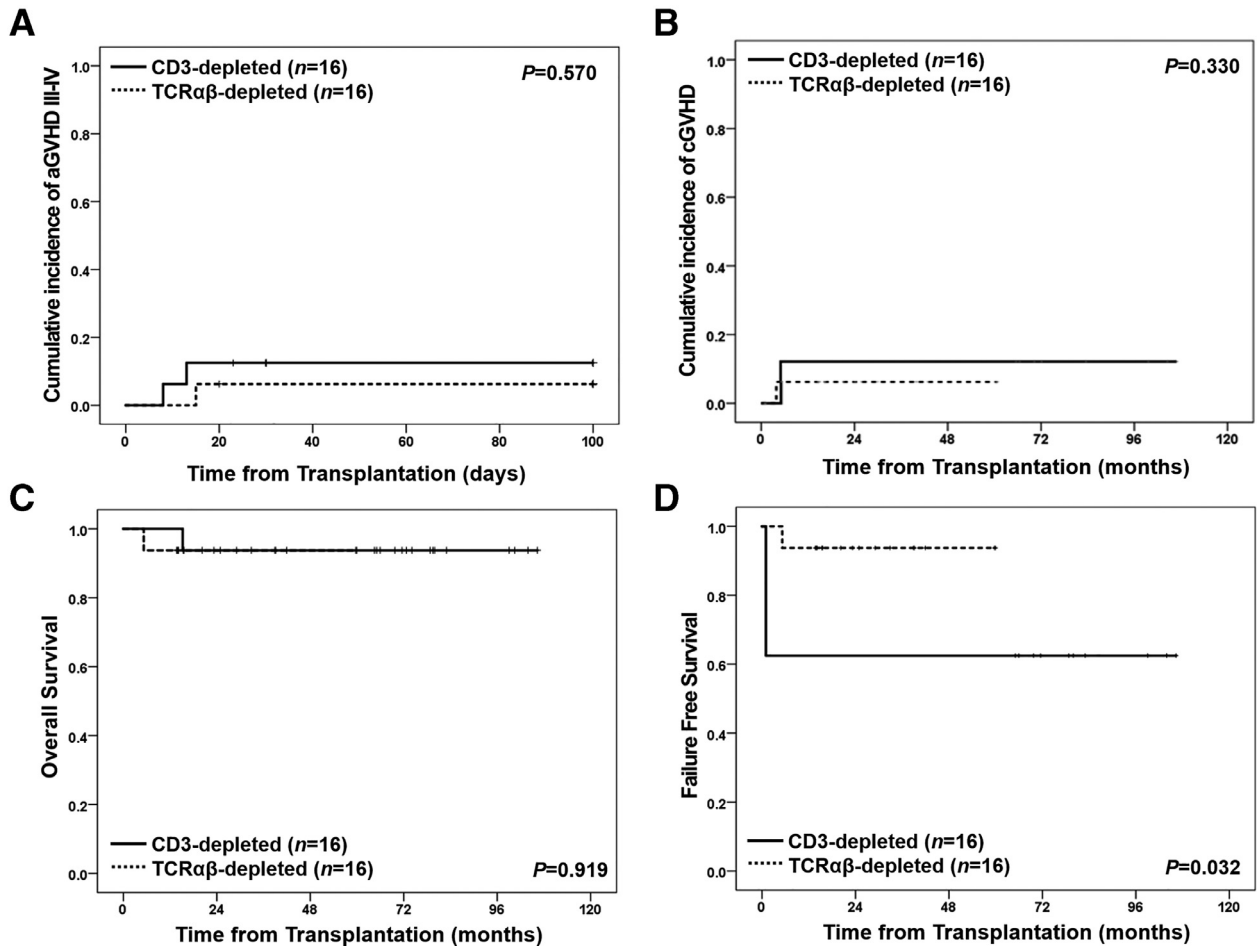

Figure 4. Outcomes of HCT using the T cell–depletion method. (A) CI of aGVHD grades III to IV. (B) CI of cGVHD. (C) OS rate. (D) FFS rate.

One of the major considerations of HCT in patients with SAA is engraftment. The number of $CD34^+$ cells has been suggested as the most reliable predictor of rapid engraftment [39]. In addition, graft source, intensity of the conditioning, age, and higher $CD3^+$ T cell number are correlated with rapid engraftment [39]. In our study we could not find any cut-off values of $CD3^+$ or $\alpha\beta^+$ T cells that influenced engraftment, and the infused $CD34^+$ cell counts were lower in the HFD-HCT cohort. Therefore, the main factor for faster neutrophil engraftment in HFD-HCT might be related to a large infusion of other committed progenitors including $CD8^+$ T cells and NK cells [39,40].

GF occurs more frequently in SAA than in malignancies. Studies regarding HFD-HCT have reported GF rates of 0 to 25%, and these rates were comparable with those of MSD-HCT. Lang et al. [5] reported a 12% graft rejection rate in a series of 41 pediatric patients with malignant disease who underwent TCR$\alpha\beta$-HFD-HCT. Locatelli's group reported a 17.4% GF in 23 pediatric patients with nonmalignant disease who underwent TCR$\alpha\beta$-HFD-HCT [3]. The GF rate of T cell–replete HFD-HCT was 3% in 89 patients including children and adults with SAA [20]. In our study GF rates of MSDs, URDs, and HFDs were 14.3%, 9.6%, and 18.7%, respectively, and there was no difference between donor types. These encouraging engraftment results might be due to various factors, but 1 reason is optimized conditioning including LD-TBI.

In our study 3 of 6 patients who received chemotherapy-only conditioning for HFD-HCT showed GF. A reduced-toxicity regimen is usually insufficient to ensure engraftment in nonmalignant diseases [41]. TBI-based conditioning for HFD-HCT ensures engraftment, particularly in patients with reduced-intensity conditioning [42,43]. Although no standardized TBI dose exists for conditioning in SAA, the Seattle group recommends 200 cGy TBI and Korean researchers recommend 800 cGy based on a study of Korean adults with SAA [44,45]. Given that the GF rate was somewhat high in our initial trial [7], we introduced TBI at a dose of 400 cGy as a conditioning regimen, and GF only occurred in 3 of 26 patients (11.5%) after incorporating LD-TBI.

Although LD-TBI enhanced engraftment, GF can be affected by many factors such as disease, prior therapy, conditioning regimen, cell dose, and T cell–depletion technique [13,46]. Multivariate analysis was performed with factors that might influence the engraftment, but no single factor including LD-TBI was found to be important in the present study. Analysis of a larger number of patients is needed, with monitoring of long-term complications related to TBI.

The second concern for HFD-HCT is the risk of fatal GVHD. Because SAA is a nonmalignant disease, another goal in these patients is avoidance of GVHD after successful engraftment. Xu et al. [38] reported the outcomes of T cell–replete HFD-HCT in 52 pediatric SAA patients. The CIs of aGVHD grades II to IV and III to IV and cGVHD were 39.2%, 13.7%, and 34.2%, respectively. In the present study none developed grade IV disease. Notably, the incidence rates of aGVHD or cGVHD in HFD-HCT were comparable with those of MSD or URD recipients. Additionally, no GVHD-related death was reported at the time of analysis.

GVHD prevention using pharmacologic agents has effectively prevented fatal GVHD, but there are serious side effects and drug interactions that require serial blood level monitoring for several months post-transplant. The $\alpha\beta^+$ T cell–depletion strategy could offer a realistic preventive strategy to avoid such hurdles. In particular, a depletion strategy using an anti-TCR$\alpha\beta^+$ monoclonal antibody resulted in an approximately 4-log reduction in $\alpha\beta^+$ T cells during most of the depletion procedures [47,48]. To date, the optimal T cell dose for HFD-HCT that is sufficient to prevent GVHD while ensuring successful engraftment has not been determined. A German study suggested that $<5 \times 10^4$ T cells/kg is safe and effective for preventing GVHD after HLA nonidentical transplantation without prophylaxis [49]. However, in that study patients who received fewer than 5×10^4 CD3$^+$ T cells/kg experienced GF, whereas those who received $>8 \times 10^4$ CD3$^+$ T cells/kg developed aGVHD without GF.

We have made continuous modifications since early 2011 to determine the optimal dose in the HFD-HCT setting [1]. Our previous study with CD3-depleted HCT showed a rather high incidence of GF in the early period of the study. Therefore, we modified the target dose of T cells in different ranges to improve the outcomes. Another practical issue was that the reduction of T cells is less effective with the CD3-depletion method and frequently leads to residual T cells in grafts exceeding the threshold of 5×10^4/kg. We gradually reduced the target dose of T cells from 1 to 6×10^6/kg to 6 to 8×10^5/kg to decrease the risk of severe GVHD and ensure stable engraftment [13,50]. Target grafts were adjusted to contain $\leq 5 \times 10^4$ $\alpha\beta^+$ T cells/kg for the final infused graft, and post-transplant immunosuppressants were eliminated in TCR$\alpha\beta$-HFD-HCT. In the present study patients who underwent HCT with this adjusted $\alpha\beta^+$ T cell dose did not develop grades III to IV aGVHD or cGVHD even without post-transplant immunosuppressants. Although the follow-up duration was short and the number of patients limited, early clinical outcomes were similar between the TCR$\alpha\beta$-HFD-HCT (immunosuppression postitive) cohort and the TCR$\alpha\beta$-HFD-HCT (immunosuppression negative) cohort, regardless of post-transplant immunosuppression (Table 3) [51].

Rapid immune reconstitution is critical for reducing post-transplant morbidity and mortality. Pei et al. [52] reported immune reconstitution data of 46 children and 35 adults with SAA who underwent in vivo T cell–depleted HFD-HCT and found that CD8$^+$ T cells were within the normal range after 1 month and CD3$^+$ T cell counts after 3 months post-transplant. In our study the recovery of CD3$^+$ T cells was also fast, but the recovery was delayed to 6 months after HCT. Post-transplant CD4$^+$ T cell count is an important marker for evaluating the restoration of immune competence post-transplant [53]. In our study all patients showed a very rapid CD4$^+$ T cell recovery with average counts of 482 cells/μL (MSD), 456 cells/μL (URD), and 546 cells/μL (HFD) at 9 months post-transplant. B cell recovery was slower in HFD-HCT, and recovery began 6 months after transplantation. This was because the B cell–depletion effect derived from prophylactic rituximab administered in HFD-HCT lasted for a specific period. In the present study the TCR$\alpha\beta$-HFD-HCT cohort showed better FFS with faster immune reconstitution than the CD3-HFD-HCT cohort. Depleting $\alpha\beta^+$ T cells produces grafts containing many $\gamma\delta^+$ lymphocytes and other effector cells including NK cells [47]. These graft properties might contribute to more stable engraftment and faster immune reconstitution than in the CD3-HFD-HCT cohort.

In our study 7 patients underwent a second transplantation after GF. When we plan a second transplantation in a patient lacking a matched donor, a different family donor other than the first is usually selected for the second transplantation. Although there are few reports on the role of donor changes in non-malignancy, the presence of donor-specific anti-HLA antibodies at the time of stem cell infusion increases the risk of GF in HFD-HCT [9]. Therefore, it is reasonable to change the donor for the second HFD-HCT after GF.

The limitations of the present study are that the number of patients was relatively small and the follow-up duration and basic characteristics of each group were not identical. In particular, patients who underwent TCR$\alpha\beta$-HFD-HCT without post-transplant GVHD prophylaxis needed a longer follow-up for assessing late outcomes.

In conclusion, our data show that optimizing HDF-HCT using ex vivo T cell depletion with LD-TBI conditioning yields excellent outcomes in children and adolescents with acquired SAA, and the survival rates and transplantation-related toxicities were comparable with those of HCT with MSDs or URDs. Also notable is that HFD-HCT conferred faster engraftment without delayed immune reconstitution. Considering the advantage of readily available sources of HFD in almost all patients in need of an allograft, our study suggests that HFD-HCT using ex vivo $\alpha\beta^+$ T cell depletion with an optimized conditioning regimen including LD-TBI is a realistic therapeutic option for SAA.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship Statement: H.J.I. was the principal investigator, conceived and designed the study, analyzed the data, and wrote the article. H.K. analyzed the data and wrote the article. H.J.I., J.J.S., K.N.K., and H.K. treated the patients and collected the clinical data. S.H.K. and J.W.Y. collected the clinical data. E. S.C. was responsible for collecting the mononuclear cells and acquiring the related data. Y.-U.C., S.J., and C.-J.P. performed the flow cytometric analysis. J.J.S. and K.N.K. critically reviewed and revised the article.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2019.01.010.

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