Biol Blood Marrow Transplant 25 (2019) 1567–1575

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Comparable Outcomes of First-Line Hematopoietic Stem Cell Transplantation from Unrelated and Matched Sibling Donors in Adult Patients with Aplastic Anemia: A Retrospective Single-Center Study

Yuping Zhang[1,2,a], Liangliang Wu[1,2,a], Wenjian Mo[1,2], Ming Zhou[1,2], Yumiao Li[1,2], Xiaowei Chen[1,2], Caixia Wang[1,2], Shiyi Pan[1,2], Shilin Xu[1,2], Wei Zhou[1,2], Ruiqing Zhou[1,2], Shunqing Wang[1,2,*]

[1] *Department of Hematology, Guangzhou First People's Hospital, School of Medicine, South China University of Technology, Guangzhou, Guangdong, China*
[2] *Department of Hematology, Guangzhou First People's Hospital, Guangzhou Medical University, Guangzhou, Guangdong, China*

Article history:
Received 26 December 2018
Accepted 19 March 2019

Key Words:
First-line
Unrelated donor
Matched sibling donor
Adult aplastic anemia
Hematopoietic stem cell transplantation

A B S T R A C T

To explore the feasibility of upfront unrelated donor (URD) hematopoietic stem cell transplantation (HSCT) in the treatment of adult aplastic anemia (AA), we conducted a retrospective, single-center study and compared the outcomes of adult patients who underwent first-line URD HSCT or matched sibling donor (MSD) HSCT between August 2012 and June 2018. In all, 23 URD HSCT recipients had an increased cumulative incidence of grade II acute graft-versus-host disease (aGVHD) (21.7% versus 3.4%; P =.007), but similar rates of secondary graft failure (8.7 ± 6.0% versus 6.9 ± 3.4%; P = .764), chronic GVHD (cGVHD) (18.2% versus 8.8%; P = .285), extensive cGVHD (9.1% versus 3.5%; P = .328), 5-year estimated overall survival (87.0% versus 94.2%; P = .501), and 5-year estimated failure-free survival (82.0% versus 89.3%; P = .404) compared with 58 MSD HSCT recipients treated during the same period. After using propensity score matching to reduce the influence of potential confounders, the 2 groups were well balanced in terms of pretransplantation clinical factors. The median survival time was similar, and no significant differences in the aforementioned outcomes were observed between the 2 groups. Our results suggest that URD HSCT may be an effective and feasible option for first-line therapy in adult AA patients who lack an MSD.

INTRODUCTION

Aplastic anemia (AA), characterized by peripheral pancytopenia, bone marrow (BM) hypocellularity, and hematopoietic stem cell niche number impairment, is a potentially fatal disorder for which immunosuppressive therapy (IST) and allogeneic hematopoietic stem cell transplantation (allo-HSCT) are the principal interventions [1–3]. According to the treatment guidelines, upfront HSCT from an HLA-matched sibling donor (MSD) is recommended for patients with severe AA (SAA) age <35 years [4]. If an MSD is not available, horse antithymocyte globulin (ATG)-based IST is indicated, and allo-HSCT from an unrelated donor (URD) is considered the standard treatment after IST failure.

Although a recent study has shown that IST as a first-line therapy achieves an excellent overall survival (OS) rate, this treatment is also associated with a high rate of failure and poor quality of life, as indicated by the far lower event-free survival (EFS) rate, which includes the risk of relapse and weaker reconstitution of hematopoiesis [5]. Moreover, between 10% and 15% of patients who undergo IST for AA will go on to develop clonal evolution [6,7]. Prospective studies have shown that rabbit ATG is inferior to horse ATG in terms of response rate [8,9]; however, horse ATG is not available in China.

With the availability of high-resolution HLA molecular typing, the development of less-toxic conditioning regimens, and improvements in supportive care, the outcomes of matched unrelated donor (MUD) HSCT for SAA have improved significantly [10–14]. The largest recent study, reported by the European Group for Blood and Marrow Transplantation (EBMT), showed that OS for MUD HSCT is not statistically inferior to that of MSD HSCT [14]. In view of this finding, upfront URD HSCT has become an attractive first-line option following the temporary withdrawal of horse ATG. Dufour et al [12] published a report of 29 children from a UK cohort, which included 24 patients who underwent MUD HSCT and 5 patients who underwent mismatched unrelated donor (MMUD) HSCT, and indicated that the outcome of first-line URD HSCT in pediatric patients with SAA was similar to that of MSD HSCT. However, that study investigated the outcome of upfront URD HSCT in children [12], and no similar studies have focused on adults.

Financial disclosure: See Acknowledgments on page 1573.
* Correspondence and reprint requests: Shunqing Wang, MD, PhD, 1 Panfu Road, Guangzhou, Guangdong 510180, People's Republic of China
E-mail address: eywangshq@scut.edu.cn (S. Wang).
[a] Y.Z. and L.W. contributed equally to this work.

https://doi.org/10.1016/j.bbmt.2019.03.020
1083-8791/

Propensity score matching (PSM), which is often applied to balance observed characteristics, is typically used in situations with a limited number of treated subjects and a larger number of comparator subjects [15]. Traditional methods of adjustment (ie, matching, stratification, and covariance adjustment) are often limited, because they can use only a limited number of covariates for adjustment; however, PSM, which provides a scalar summary of the covariate information, does not have this limitation [15].

As such, we retrospectively compared the long-term outcomes of adults with SAA who underwent URD HSCT or MSD HSCT as a first-line treatment, using the raw data and PSM analyses.

METHODS

Patients

Between August 2012 and June 2018, a total of 81 consecutive adults (age >14 years) with AA underwent MSD or MUD/MMUD HSCT as a first-line treatment. Twenty-three patients who lacked an MSD but had an MUD/MMUD and 58 patients with an MSD were enrolled. All patients in this study met the following inclusion criteria: (1) had acquired AA according to the International AA Study Group Criteria [16]; (2) voluntarily underwent allo-HSCT as the first-line treatment; and (3) had not undergone ATG-based IST or had undergone IST with cyclosporine A (CsA) within 6 months before transplantation. The protocol of this retrospective study was approved by the Ethics Committee of Guangzhou First People's Hospital. All treatments were performed after obtaining written informed consent from patients or their caretakers according to the Declaration of Helsinki.

Transplantation Protocols and Supportive Care

For URD HSCT, patients received either fludarabine (Flu; 30 mg/m^2/day from day -7 to day -4) plus cyclophosphamide (CY; 40 mg/kg/day from day -7 to day -4) plus rabbit ATG (2.5 mg/kg/day from day -5 to day -2) or busulfan (BU; .8 mg/kg/day from day -7 to day -6) plus CY (40 mg/kg/day from day -5 to day -2) plus rabbit ATG (2.5 mg/kg/day from day -5 to day -2). The conditioning therapy for the MSD HSCT recipients consisted of CY (50 mg/kg/day from day -5 to day -2) plus rabbit ATG (2.5 mg/kg/day from day -5 to day -2), Flu (30 mg/m^2/day from day -7 to day -4) plus CY (40 mg/kg/day from day -7 to day -4) plus rabbit ATG (2.5 mg/kg/day from day -5 to day -2), or BU (.8 mg/kg/day from day -7 to day -6) plus CY (40 mg/kg/day from day -5 to day -2) plus rabbit ATG (2.5 mg/kg/day from day -5 to day -2). The transplantation procedure, including HLA typing, stem cell harvesting, and graft-versus-host disease (GVHD) prophylaxis, has been described in our previous reports [17,18]. In patients who underwent URD HSCT, mobilized peripheral blood stem cells (PBSCs) were the sole stem cell source, whereas patients who underwent MSD HSCT received both BM and PBSCs. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed based on clinical symptoms and/or skin, oral mucosa, liver, or gut biopsies and were graded according to standard clinical criteria [19,20].

All patients were routinely monitored to assess the DNA levels of viruses including cytomegalovirus (CMV) and Epstein-Barr virus (EBV) in the peripheral blood using RT-PCR twice weekly for at least 3 months after transplantation. CMV viremia and EBV viremia were defined as CMV-DNA >500 copies/mL and EBV-DNA >1000 copies/mL, respectively. Diagnoses of CMV disease and EBV disease were defined as described previously [21,22]. For antiviral prophylaxis, acyclovir was administered to all patients. Ganciclovir (Cymevene, 5 mg/kg every 12 hours; Roche Pharma, Basel, Switzerland) or foscarnet (FOS, 60 mg/kg every 8 hours; Jiangsu Chia-Tai Tianqing Pharma, Lianyungang, China) was initiated in patients with CMV viremia as a preemptive therapy and continued until 2 consecutive negative results were obtained. CMV enteritis was treated by combined i.v. and intravitreal injections of ganciclovir. For patients with EBV disease, preemptive therapy with rituximab was given at a dose of 375 mg/m^2 weekly for a total of 4 doses [21].

Definitions and Assessment of Engraftment

Neutrophil and platelet engraftment was defined as the first day of an absolute neutrophil count $\geq .5 \times 10^9$/L for 3 consecutive days and a platelet count $\geq 20 \times 10^9$/L for the first day of 7 consecutive days without platelet transfusion, respectively. Hematopoietic chimerism analysis of BM cells was performed after allo-HSCT using DNA fingerprinting and short tandem repeat PCR. The study's primary endpoint was failure-free survival (FFS) after first-line therapy, defined as survival with response. Death, primary or secondary graft failure (GF), and relapse were considered treatment failure [23]. The secondary endpoint of the study was OS, defined as the time from transplantation to death or the last follow-up. In addition, primary GF was defined as the absence of myeloid engraftment until day +28 post-transplantation. Initial engraftment and the absence of graft function, followed by recurrent pancytopenia with obviously hypocellular BM and without moderate to severe aGVHD, was considered secondary GF [24].

Statistical Analysis

The date of the last follow-up for all surviving patients was September 30, 2018. Differences in categorical variables were evaluated using the chi-square test. Fisher's exact test was used for analysis when at least 1 factor was related to fewer than 5 individuals. The Mann-Whitney U test and Student t test, as appropriate, were used to compare differences in continuous variables between groups. OS and FFS were estimated by the Kaplan-Meier method and compared between groups using the log-rank test. The cumulative incidence (CI) of engraftment and GVHD were estimated in competing-risk models, with early death as the competing event. To adjust for variations in clinical characteristics among recipients in the 2 groups, PSM was applied in this study using 1:1 nearest-neighbor matching. Univariate and multivariate analyses were performed to determine whether these selected factors were predictive of the endpoints. All factors with $P < .1$ in the univariate analysis were evaluated in the multivariate analysis using the Cox regression model with a backward stepwise selection approach. Data were analyzed using SPSS version 13.0 (SPSS, Chicago, IL), Prism 5.0 (GraphPad Software, La Jolla, CA), and R version 3.5.1 (http://www.r-project.org). P values <.05 were considered significant.

RESULTS

Results in Patients Who Underwent Upfront URD HSCT

Patient Characteristics

Twenty-three patients with SAA underwent HSCT from a URD, which included 16 MUDs and 7 MMUDs, as a first-line treatment. Of the 7 patients who underwent MMUD HSCT, 2 received a transplant with a single mismatch at HLA-A, 2 received a transplant with a single mismatch at HLA-C, and 3 received a transplant with a single mismatch at HLA-DQB1. Seven patients (30.4%) were treated between 2012 and 2016 and 16 patients (69.6%) were treated between 2017 and 2018. More patient characteristics are listed in Table 1.

Engraftment

All 23 patients achieved primary engraftment. The median time to neutrophil and platelet engraftment following transplantation was 11 days (range, 8 to 14 days) and 11 days (range, 9 to 30 days), respectively. One patient experienced secondary GF on day +44 after developing CMV pneumonia and died on day +76. Another patient experienced secondary GF on day +135 and achieved engraftment after umbilical cord blood infusion. The CI of secondary GF was 8.7 ± 6.0%.

GVHD

Five of the 23 patients (21.7%) developed grade II aGVHD, and none developed grade III-IV aGVHD. Four of these 5 patients underwent MMUD HSCT (2 with HLA-C mismatch and 2 with HLA-DQB1 mismatch), and 1 patient underwent MUD HSCT. Twenty-two patients who survived longer than 100 days after transplantation were evaluated for the incidence of cGVHD. Four patients (18.2%) developed cGVHD (2 limited and 2 extensive). Two of the 4 patients underwent MMUD HSCT, and the other 2 patients underwent MUD HSCT. The CI of grade II aGVHD at 100 days was 21.7 ± 8.8%. The 5-year CI of cGVHD and extensive cGVHD was 18.2 ± 8.6% and 9.1 ± 26.3%, respectively.

Immune Reconstitution and Complications

CMV and EBV viremia were observed in 19 (82.6%) and 5 (21.7%) patients, respectively. Of the 19 patients with detected CMV viremia, 4 (21.1%) progressed to CMV disease, including 3 who developed CMV enteritis and 1 who developed CMV pneumonia. The 3 patients with CMV enteritis recovered after systemic and i.v. antiviral therapy, and 1 patient died on day +76 due to CMV pneumonia. Three patients developed EBV-associated post-transplantation lymphoproliferative disorder (PTLD) and received therapy with 4 doses of rituximab. The treatment of EBV-PTLD was successful in 2 patients. Except for 1 patient with a maintained PTLD status, no patients died of EBV disease by the end of the follow-up period.

Table 1
Comparison of Patient Characteristics in the URD and MSD Groups

Characteristic	Before Matching			After Matching		
	URD (N = 23)	MSD (N = 58)	*P*	URD (N = 23)	MSD (N = 23)	*P*
Sex, n (%)			.275			1.000
Male	10 (43.5)	33 (56.9)		10 (43.5)	10 (43.5)	
Female	13 (56.5)	25 (43.1)		13 (56.5)	13 (56.5)	
Age at transplantation, yr, median (range)	26 (14-49)	33 (15-55)	.004	26 (14-49)	30 (17-43)	.071
Age, yr, n (%)			.051			.346
<18	4 (17.4)	2 (3.4)		4 (17.4)	1 (4.3)	
≥18	19 (82.6)	56 (96.6)		19 (82.6)	22 (95.7)	
AA, n (%)			.661			.852
NSAA	3 (13.0)	8 (13.8)		3 (13.0)	4 (17.4)	
SAA	7 (30.4)	24 (41.4)		7 (30.4)	8 (34.8)	
VSAA	13 (56.5)	26 (44.8)		13 (56.5)	11 (47.8)	
ECOG score pretransplantation, median (range)	2 (1-3)	2 (1-3)	.567	2 (1-3)	2 (1-3)	.924
Interval from diagnosis to HSCT, d, median (range)	101 (21-230)	50.5 (23-295)	.006	101 (21-230)	93 (23-295)	.104
Donor-recipient sex match, n (%)			.002			.102
Male-male	9 (39.1)	12 (20.7)		9 (39.1)	5 (21.7)	
Male-female	11 (47.8)	13 (22.4)		11 (47.8)	7 (30.4)	
Female-male	1 (4.4)	21 (36.2)		1 (4.4)	5 (21.7)	
Female-female	2 (8.7)	12 (20.7)		2 (8.7)	6 (26.1)	
ABO match, n (%)			.073			.543
Matched	7 (30.4)	35 (60.3)		7 (30.4)	12 (52.2)	
Minor mismatched	5 (21.7)	9 (15.5)		5 (21.7)	3 (13.0)	
Major mismatched	8 (34.8)	9 (15.5)		8 (34.8)	5 (21.7)	
Different	3 (13.0)	5 (8.6)		3 (13.0)	3 (13.0)	
Period of treatment, n (%)			.028			.753
8/2012-12/2016	7 (30.4)	34 (58.6)		7 (30.4)	8 (34.8)	
1/2017-6/2018	16 (69.6)	24 (41.4)		16 (69.6)	15 (65.2)	
Stem cell source, n (%)			.000			.000
PBSCs	23 (100)	0 (0)		23 (100)	0 (0)	
BM + PBSCs	0 (0)	58 (100)		0 (0)	23 (100)	
Condition regimen, n (%)			.003			.556
CY + ATG	0 (0)	19 (32.8)		0 (0)	1 (4.3)	
FCA	13 (56.5)	21 (36.2)		13 (56.5)	10 (43.5)	
BU/CY + ATG	10 (43.5)	18 (31.0)		10 (43.5)	12 (52.2)	
MNCs, $\times 10^8$/kg, median (range)	10.41 (3.7-18.03)	11.02 (3.95-16.82)	.222	10.41 (3.7-18.03)	10.87 (9.16-15.4)	.448
$CD34^+$ count, $\times 10^6$/kg, median (range)	5.27 (1.36-19.80)	3.87 (1.49-11.0)	.399	5.27 (1.36-19.80)	4.36 (2.48-9.46)	.575
Neutrophil engraftment time, d, median (range)	11 (8-14)	10 (6-18)	.083	11 (8-14)	10 (6-15)	.072
Platelet engraftment time, d, median (range)	11 (9-30)	10.5 (7-36)	.110	11 (9-30)	11 (8-25)	.310
Follow-up in alive patients, d, median (range)	423 (103-2151)	798 (101-2224)	.043	423 (103-2151)	424.5 (103-1950)	.878
CMV viremia, n (%)	19 (82.6)	41 (70.7)	.270	19 (82.6)	14 (60.9)	.189
CMV disease, n (%)	4 (17.4)	2 (3.4)	.051	4 (17.4)	1 (4.3)	.346
EBV viremia, n (%)	5 (21.7)	20 (34.5)	.263	5 (21.7)	7 (30.4)	.738
EBV-PTLD, n (%)	3 (13.0)	2 (3.4)	.136	3 (13.0)	1 (4.3)	.608
OS, % (SE)	87.0 (9.1)	94.2 (3.3)	.501	87.0 (9.1)	95.7 (4.3)	.612
FFS, % (SE)	82.0 (10.2)	89.3 (4.6)	.404	82.0 (10.2)	86.1 (9.8)	.672
CI of aGVHD grade II, % (SE)	21.7 (8.8)	3.4 (2.4)	.007	21.7 (8.8)	8.7 (6.0)	.193
CI of cGVHD, % (SE)	18.2 (8.6)	8.8 (3.8)	.285	18.2 (8.6)	9.1 (6.3)	.434
CI of extensive cGVHD, % (SE)	9.1 (6.3)	3.5 (2.5)	.328	9.1 (6.3)	4.5 (4.5)	.574
CI of secondary GF, % (SE)	8.7 (6.0)	6.9 (3.4)	.764	8.7 (6.0)	4.3 (4.3)	1.000
CI of neutrophil engraftment, n (%)	23 (100)	23 (100)	1.000	23 (100)	23 (100)	1.000
CI of platelet engraftment, n (%)	23 (100)	23 (100)	1.000	23 (100)	23 (100)	1.000

NSAA, indicates nonsevere aplastic anemia; VSAA, very severe aplastic anemia; FCA, fludarabine + cyclophosphamide + antithymocyte globulin; BU, busulfan; MNCs, mononuclear cells.

Survival Outcomes

The cause of treatment failure included secondary GF and death. Two patients died after transplantation, including 1 patient from severe CMV pneumonia on day +76 after experiencing secondary GF and 1 patient from severe varicella virus infection on day +497. With a median follow-up of 423 days (range, 103 to 2151 days), the 5-year estimated OS and FFS rates were 87.0±9.1% and 82.0±10.2%, respectively.

Comparison of Results in Patients Who Underwent First-Line URD HSCT and MSD HSCT

Patient Characteristics

Fifty-eight patients who underwent upfront MSD HSCT were included in this study. Table 1 presents the patient and transplantation characteristics for the 2 groups. The 2 groups were similar in terms of male/female ratio, disease severity, Eastern Cooperative Oncology Group (ECOG) scores before HSCT and the $CD34^+$ cell and mononuclear cell counts. Patients in the URD group were younger than patients in the MSD group (median age, 26 years [range, 14 to 49 years] versus 33 years [range, 15 to 55 years], respectively; $P = .004$). There were 4 patients age <18 years (17.4%; age 14, 15, 16, and 17 years) in the URD group and 2 patients age <18 years (3.4%; age 15 and 17 years) in the MSD group. There were more male donors in the URD group ($P = .002$). As expected, the time to transplantation was significantly longer in the URD group than in the MSD group, with a median interval between diagnosis and transplantation of 101 days (range, 21 to 230 days) and 50.5 days (range, 23 to 295 days), respectively. In addition, among 58 patients who underwent MSD HSCT, 34 (58.6%) were treated between 2012 and 2016 and 24 (41.4%) were treated between 2017 and 2018. Compared with MSD HSCT, more patients underwent URD HSCT in the last 2 years ($P = .028$) (Table 1). The median duration of survival was longer in the MSD group ($P = .043$) (Table 1). The other transplant characteristics that differed significantly between the 2 groups were stem cell source and transplantation conditioning; the use of CY+ATG was more frequent in the MSD group. To reduce the influence of potential confounders, a case-control analysis comparing 23 patients in each group was performed after PSM analysis, which used 3 variables (recipient age at transplant, interval between diagnosis and transplantation and time periods of treatment) to calculate propensity scores. All variables except graft source were balanced between the 2 groups after PSM ($P > .05$) (Table 1).

Engraftment and GVHD

No between-group differences were found in the CI of neutrophil engraftment (100% versus 100%; P=1.000), platelet engraftment (100% versus 100%; $P = 1.000$), or secondary GF (8.7 ± 6.0% versus 6.9 ± 3.4%; P =.764). The CI of grade II aGVHD was significantly higher in the URD group (21.7% versus 3.4%; $P = .007$) (Table 1). However, no patient in either group developed grade III-IV aGVHD. After PSM, the CI of aGVHD was higher in the URD group compared with the MSD group, but the difference was not statistically significant (21.7% versus 8.7%; $P = .193$) (Table 1). In addition, the CIs of cGVHD and extensive cGVHD were higher in the URD group both before and after PSM, but the differences were not statistically significant (before PSM: 18.2% versus 8.8% [$P = .285$] and 9.1% versus 3.5% [$P = .328$], respectively; PSM: 18.2% versus 9.1% [$P = .434$] and 9.1% versus 4.5% [$P = .574$], respectively). After PSM, there was no significant between-group difference in the CI of secondary GF (8.7% versus 4.3%; $P = 1.000$).

Immune Reconstitution and Complications

Figure 1 shows the immune reconstitution outcomes. Within 1 year after transplantation, the $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^-CD19^+$, and $CD16^+CD56^+$ cell counts and the IgA, IgG, and IgM concentrations were comparable in the 2 groups. In the MSD group, 41 patients (70.7%) experienced CMV viremia, and 2 of these 41 patients (4.9%) developed CMV disease. There were no between-group differences in the incidence of CMV viremia (82.6% versus 70.7%; $P = .270$) or CMV-related disease (17.4% versus 3.4%; $P = .051$). In addition, a higher (albeit not significantly so) rate of CMV disease was observed in the URD patients with CMV viremia compared with the MSD recipients with CMV viremia (21.1% versus 4.9%; $P = .074$). The incidences of EBV viremia and EBV-PTLD were 21.7% and 13.0%, respectively, in the URD group and 34.5% and 3.4%, respectively, in the MSD group, with no significant differences between the 2 groups (Table 1). After PSM, there were no significant between-group differences in the incidence of CMV or EBV viremia or the level of immune reconstitution (Table 1 and Figure 1).

Survival Outcomes

The 5-year OS rate was 87.0 ± 9.1% after URD HSCT and 94.2 ± 3.3% after MSD HSCT ($P = .501$) (Table 1 and Figure 2). The 5-year FFS rate did not differ significantly between the first-line URD HSCT group and the MSD HSCT group (82.0 ± 10.2% versus 89.3 ± 4.6%; $P = .404$) (Table 1 and Figure 2). After PSM, the 5-year OS and FFS rates were 95.7% and 86.1%, respectively, in the MSD group. Moreover, no significant between-group difference was found in the 5-year OS (87.0 ± 9.1% versus 95.7 ± 4.3%; $P = .612$) or FFS (82.0 ± 10.2% versus 86.1 ± 9.8%; $P = .672$) (Table 1 and Figure 2). The univariate and multivariate analyses demonstrated a significant difference in OS and FFS among patients with an ECOG score of 3 pretransplantation (Tables 2 and 3).

DISCUSSION

In this retrospective study, we compared the outcomes of consecutive adults with AA undergoing first-line URD or MSD HSCT. The survival outcomes in the URD HSCT group were not inferior to those in the MSD HSCT group. In addition, poor performance (ie, ECOG score 3) pretransplantation was confirmed as a predictive factor in both the URD and MSD groups. Considering the important role of baseline patient characteristics in the outcomes, we matched the patients in the groups according to their baseline characteristics using PSM, which resulted in the similar characteristics except stem cell source in the 2 groups.

As reported previously [25,26], the risk of GF is increased in transplantation with URDs compared with HLA-identical sibling donors. With advances in conditioning regimens, the incidence of GF in URD HSCT for SAA has been reduced to <10% [12,14,27]. In this study, no patient experienced primary GF, and there was no difference in median myeloid or platelet engraftment times between the URD and MSD groups. Furthermore, the secondary GF rate of 8.7% in the URD group was comparable to that in the MSD group. Several factors may contribute to this encouraging engraftment outcome. First, conditioning regimens consisting of BU or Flu, which have been shown to have the capacity to reduce graft rejection [28,29], were adopted in our study. Second, compared with Yagasaki et al [27], who used GVHD prophylaxis consisting of CsA and short-term methotrexate, we also used mycophenolate mofetil, which has been shown to be effective in promoting engraftment and in GVHD prophylaxis [30–32]. Other possible reasons for the low incidence may be our treatment of all recipients with ATG before graft infusion, which depletes the T cells of patients in vivo [33], and our use of high-resolution genomic HLA typing.

Because SAA is a nonmalignant disease, avoiding GVHD is a major challenge in HSCT, particularly in URD transplantation. In our study, all patients with AA received an ATG-based conditioning regimen, and GVHD prophylaxis consisted of CsA, mycophenolate mofetil, and low-dose methotrexate. Compared with

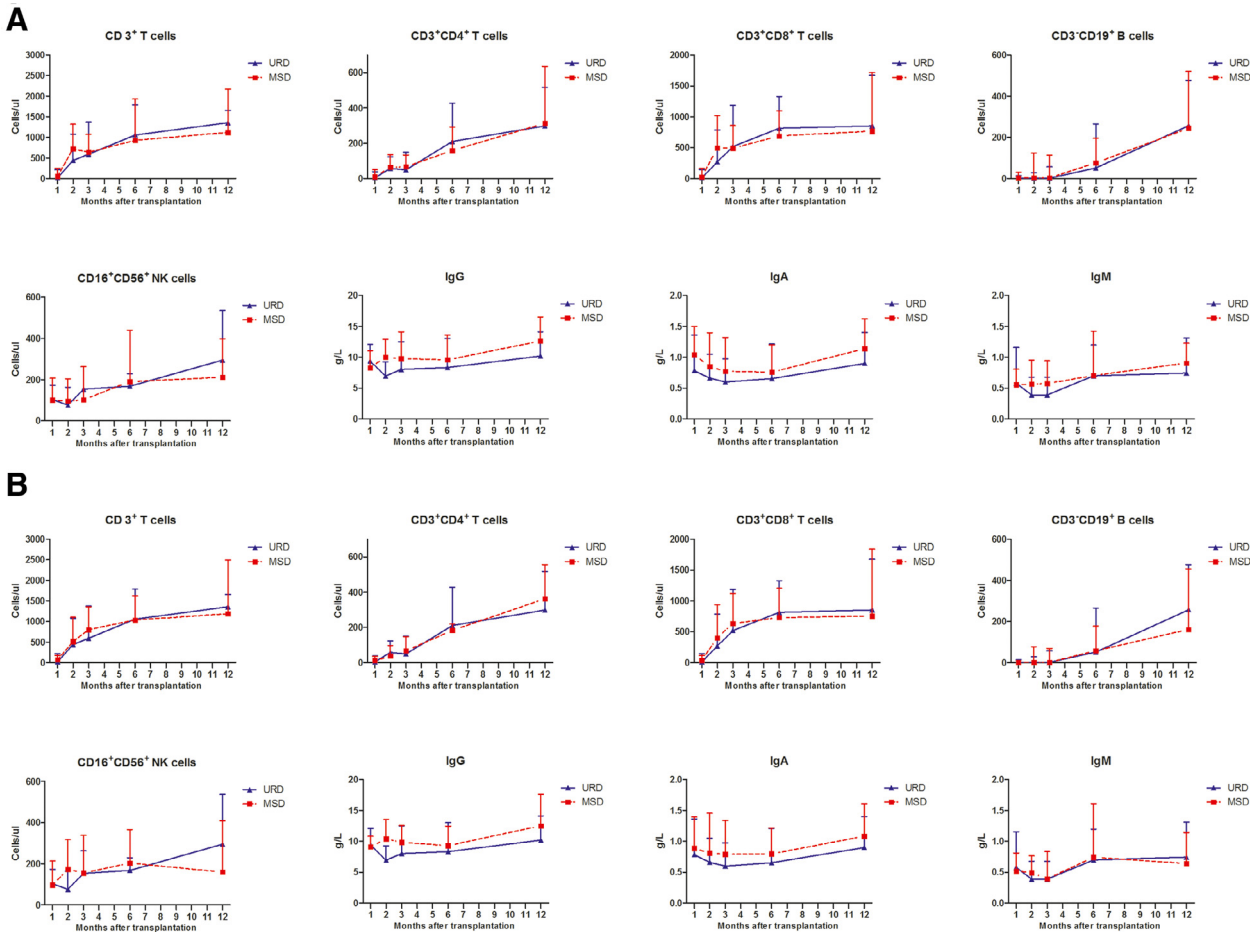

Figure 1. Immune reconstitution before (A) and after (B) propensity score matching. Within 1 year after transplantation, the $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^-CD19^+$, and $CD16^+CD56^+$ cell counts and the IgA, IgG, and IgM concentrations were comparable in the URD and MSD groups.

a patient population treated with URD HSCT with total body irradiation and CY as the preparative regimen (grade II-IV aGVHD, 46%; cGVHD, 50%) [34], our URD patients had a 21.7% CI of aGVHD (no patients developed grade III-IV aGVHD) and a, 18.2% CI of cGVHD, and the use of ATG contributed to this low incidence [35,36]. A small number of pediatric AA patients underwent first-line URD transplantation, and the rate of GVHD was low with the alemtuzumab-based regimen (Flu + Cy + alemtuzumab) [12]. Given the excellent outcomes of HSCT using alemtuzumab [12,37], alemtuzumab may be an optimal alternative to ATG for AA patients who undergo upfront URD HSCT. In a comprehensive report on more than 1000 transplantations, the CIs of aGVHD and cGVHD were significantly higher after MUD HSCT than after MSD HSCT [14]. In our study, the CI of aGVHD was higher in the URD group compared with the MSD group before and after PSM; however, the difference was not statistically significant after PSM. In addition, the CI of cGVHD was higher (but not significantly so) in the URD group compared with the MSD group. Single-locus HLA-C [38] or HLA-DQB1 [39] mismatch was associated with a significantly higher incidence of aGVHD. It is important to realize that 4 of 5 patients with aGVHD in the URD group had received a transplant with 1 mismatched allele (2 with an HLA-C mismatch and 2 with an HLA-DQB1 mismatch), which may have contributed to the higher incidence of aGVHD [38–40]. Moreover, patients who receive G-CSF-primed BM have a lower incidence of aGVHD and cGVHD compared with those who receive PBSCs [41,42]. In our institution, only G-CSF-primed PBSCs were the source of stem cells in the URD group because the Chinese Marrow Donor Program and Tzu Chi's Bone Marrow Bank provide only G-CSF-primed PBSCs. However, patients in the MSD group received a mixture of G-CSF-primed PBSCs and G-CSF-primed BM as grafts, which is likely associated with the lower incidence of GVHD in that group [11,42].

In the present study, the incidence of CMV and EBV infection was comparable in the 2 groups before and after PSM. However, the incidence of CMV viremia was as high as 82.6% in the URD group and 70.7% in the MSD group. Complications of infections, particularly those of viral infections, are known to be closely associated with the ATG dose in conditioning regimens [43–45]. In one study, compared with the 6.0 mg/kg ATG dose, the 7.5 mg/kg ATG dose was associated with a higher CMV reactivation rate (30.3% versus 64.1%, respectively; $P = .005$) [45]. In the present study, in both the URD and MSD groups, all patients received 10.0 mg/kg ATG, which may have contributed to the high incidence of CMV viremia. Moreover, the PCR cutoff for positive CMV viremia was 500 copies/mL, lower than the cutoff in a previous study (1000 copies/mL) [46], and this low cutoff may also be an important contributing factor in the high rate of CMV viremia in our institution [47].

Similar to a previous study [48], our present study indicates a higher incidence of CMV disease in the URD group compared with the MSD group, but the difference was only borderline statistically significant ($P = .051$). The lack of statistical significance may be related to our small sample size. Yagasaki et al [27]

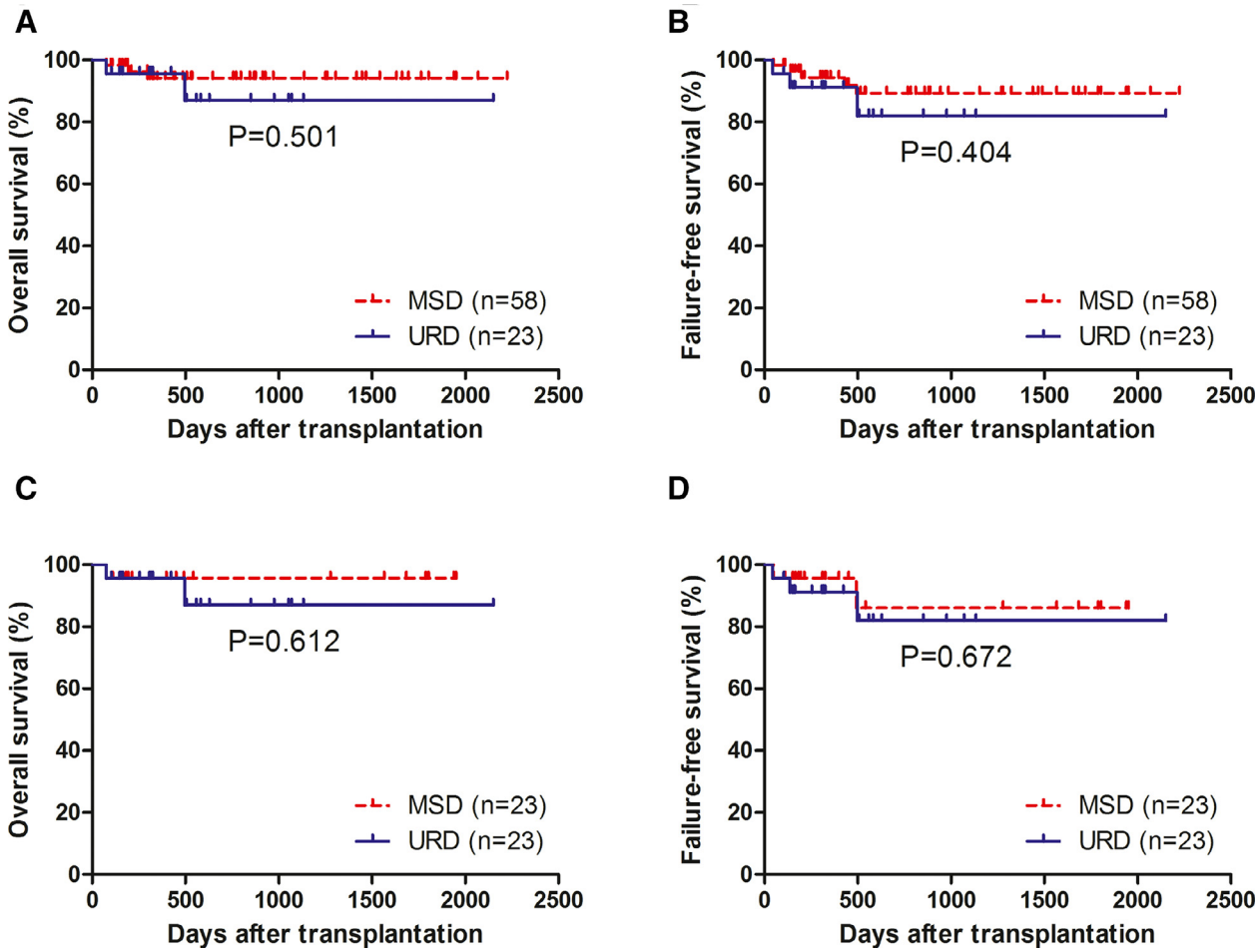

Figure 2. Survival outcomes of the 2 cohorts after transplantation. (A) OS of the 2 cohorts before PSM. (B) FFS of the 2 cohorts before PSM. (C) OS of the 2 cohorts after PSM. (D) FFS of the 2 cohorts after PSM.

reported that the rate of EBV reactivation was as high as 55% after URD HSCT in AA patients. In their study, 24 of 31 patients underwent URD HSCT after failing ATG-based IST, which may be related to the higher risk of EBV reactivation [49]. Similar to the rate of infection-related complications, at 1 year after transplantation, the $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^-CD19^+$, and $CD16^+CD56^+$ cell counts and IgA, IgG, and IgM concentrations were comparable in the 2 groups.

In a small-sample study of pediatric patients (n = 8) diagnosed with SAA, Mackarel et al [13] found superior outcomes with salvage MUD transplantations, and these outcomes were similar to those of MSD HSCT [13]. Moreover, a large EBMT study also found that the OS for MUD HSCT is approaching that of MSD HSCT [14]. The patients in both of those studies underwent URD HSCT as a second-line therapy, however [13,14]. In our present retrospective study, we found comparable survival outcomes, including OS and FFS rates, were comparable in the first-line URD HSCT and MSD HSCT groups before and after PSM. These results are consistent with those of a previous study reporting similar outcomes for upfront URD HSCT and MSD HSCT in pediatric SAA patients [12]. In addition, univariate and multivariate analyses identified poor performance (ECOG score 3) as an adverse factor for survival in patients who underwent HSCT, which has also been reported in several previous studies [17,50,51]. Patient age is a predictor of survival in URD HSCT [5,14,52,53]. Younger age is associated with better outcomes in children and adolescents undergoing URD HSCT after failed IST [5,53]. In addition, several reports have identified the interval from diagnosis to transplantation as another very strong predictor [14,52]. According to our data, the interval from diagnosis to transplantation had borderline significance ($P = .051$) as a predictor of FFS in URD transplantation; however, these 2 factors were not found to be predictive of OS or FFS in a recent study. Future studies involving a larger number of upfront URD HSCTs are needed to further examine this issue.

Our work has several limitations, including the small number of patients, retrospective design, inclusion of adolescents (4 of 23 patients; 17.4%), and lack of comparison with first-line IST. Furthermore, when URD HSCT should be performed in patients for whom an MSD is not available remains to be determined. Finally, PSM also harbors certain limitations, including an inability to account for potential unmeasured confounding factors and inability to mimic the design of a randomized trial [54]. A prospective randomized controlled trial comparing adults undergoing upfront URD HSCT with those undergoing first-line MSD HSCT or IST is needed.

In conclusion, our data indicate that in adults with AA, the outcomes of first-line URD HSCT were not inferior to those of MSD HSCT. This retrospective study shows that upfront URD HSCT is an effective and feasible choice for patients age 14 to 55 years with AA who lack an MSD and supports a potential change in the AA treatment algorithm published in 2016 [4].

Table 2
Univariate Analysis of Factors Associated with Survival Outcomes in Upfront HSCT (URD and MSD)

Variable	Number	Probability of Survival			
		5-yr OS, % (SE)	*P*	5-yr FFS, % (SE)	*P*
Total	81	92.4 (3.3)		87.4 (4.3)	
Donor type			.501		0.404
URD	23	87.0 (9.1)		82.0 (10.2)	
MSD	58	94.2 (3.3)		89.3 (4.6)	
Sex			.795		.794
Male	41	90.7 (5.2)		87.4 (6.1)	
Female	24	94.7 (3.6)		87.5 (5.9)	
Age, yr			.623		.492
<30	40	93.4 (4.6)		83.2 (7.1)	
≥30	41	91.3 (4.9)		91.8 (4.6)	
Interval from diagnosis to HSCT, d			.695		.051
<90	55	94.2 (3.3)		94.2 (3.3)	
≥90	26	88.2 (8.0)		72.3 (11.0)	
ECOG score			.001		.026
0-2	69	96.2 (2.7)		90.2 (4.3)	
3	12	69.8 (14.9)		72.2 (13.8)	
MNCs, $\times 10^8$/kg			.636		.735
<12	56	93.0 (4.0)		85.9 (5.5)	
≥12	25	91.2 (6.0)		91.2 (6.0)	
$CD34^+$ cells, $\times 10^6$/kg			.089		.055
<5	47	97.1 (2.8)		94.2 (4.1)	
≥5	34	86.1 (6.7)		78.41 (8.1)	
AA			.580		.851
SAA and NSAA	42	93.7 (4.4)		89.0 (5.2)	
VSAA	39	91.2 (4.9)		86.5 (6.4)	
Fludarabine-based regimen			.126		.382
Yes	34	87.5 (5.9)		84.4 (6.4)	
No	47	97.1 (2.9)		88.6 (6.5)	
aGVHD			.800		.798
Grade I-II	15	88.9 (10.5)		93.3 (6.4)	
None	66	92.9 (3.5)		86.8 (4.7)	
Donor age, yr			.887		.377
<30	30	93.3 (4.6)		84.6 (7.3)	
≥30	51	91.8 (4.6)		89.3 (5.2)	
Sex mismatch			.502		.820
Yes	46	95.7 (3.0)		89.7 (5.1)	
No	35	89.0 (6.1)		85.4 (6.8)	

Table 3
Multivariate Analysis of Factors Associated with Survival Outcomes in the URD And MSD Groups

Outcome	Hazard Ratio (95% Confidence Interval)	*P*
OS		
ECOG score 3	11.092 (1.796-68.502)	.010
FFS		
ECOG score 3	11.019 (1.769-68.630)	.010

ACKNOWLEDGMENTS

The authors thank American Journal Experts (www.journalexperts.com) for providing editorial assistance to the authors during the preparation of this manuscript.

Financial disclosure: This work was supported by research funding from the General Guidance Project of the Guangzhou Health and Family Planning Commission (20181A011002), the Nature Science Foundation of Guangdong Province (2018A0303130179), the Special Fund for Clinical Research of the Chinese Media Association (16010050621), the Fundamental Research Fund for the Central Universities (D2175060), and the National Natural Science Foundation of China (81600147).

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

Authorship statement: Y.Z. and L.W. performed the research, analyzed and interpreted the data, and wrote the manuscript. S.W. designed the study and supervised the analysis. Y.Z. and L.W. contributed equally to this study. All other authors participated in the collection of patient data.

REFERENCES

1. Young NS. Aplastic anemia. *N Engl J Med*. 2018;379:1643–1656.
2. Wu L, Mo W, Zhang Y, et al. Impairment of hematopoietic stem cell niches in patients with aplastic anemia. *Int J Hematol*. 2015;102:645–653.
3. Medinger M, Drexler B, Lengerke C, Passweg J. Pathogenesis of acquired aplastic anemia and the role of the bone marrow microenvironment. *Front Oncol*. 2018;8:587.
4. Killick SB, Bown N, Cavenagh J, et al. Guidelines for the diagnosis and management of adult aplastic anaemia. *Br J Haematol*. 2016;172:187–207.
5. Dufour C, Pillon M, Passweg J, et al. Outcome of aplastic anemia in adolescence: a survey of the Severe Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation. *Haematologica*. 2014;99:1574–1581.

6. Scheinberg P, Nunez O, Wu C, Young NS. Treatment of severe aplastic anaemia with combined immunosuppression: anti-thymocyte globulin, ciclosporin and mycophenolate mofetil. *Br J Haematol*. 2006;133:606–611.
7. Saracco P, Quarello P, Iori AP, et al. Cyclosporin A response and dependence in children with acquired aplastic anaemia: a multicentre retrospective study with long-term observation follow-up. *Br J Haematol*. 2008;140:197–205.
8. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N Engl J Med*. 2011;365:430–438.
9. Marsh JC, Bacigalupo A, Schrezenmeier H, et al. Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia from the EBMT Severe Aplastic Anaemia Working Party. *Blood*. 2012;119:5391–5396.
10. Clesham K, Dowse R, Samarasinghe S. Upfront matched unrelated donor transplantation in aplastic anemia. *Hematol Oncol Clin North Am*. 2018;32:619–628.
11. 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.
12. Dufour C, Veys P, Carraro E, et al. Similar outcome of upfront-unrelated and matched sibling stem cell transplantation in idiopathic paediatric aplastic anaemia. A study on behalf of the UK Paediatric BMT Working Party, Paediatric Diseases Working Party and Severe Aplastic Anaemia Working Party of EBMT. *Br J Haematol*. 2015;171:585–594.
13. Mackarel J, Iatan M, Kumar L, Storey L, O'Marcaigh A, Smith O. In support of upfront stem cell transplantation as first-line therapy for paediatric patients with idiopathic severe aplastic anaemia who lack a sibling donor. *Br J Haematol*. 2017;177:806–808.
14. Bacigalupo A, Socié 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.
15. D'Agostino Jr. RB. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. *Stat Med*. 1998;17:2265–2281.
16. Marsh JC, Ball SE, Cavenagh J, et al. Guidelines for the diagnosis and management of aplastic anaemia. *Br J Haematol*. 2009;147:43–70.
17. Xu S, Wu L, Zhang Y, et al. Allogeneic hematopoietic stem cell transplantation for the treatment of severe aplastic anemia patients with infection: a single-center retrospective study. *Biol Blood Marrow Transplant*. 2018;24:2532–2539.
18. Wu L, Mo W, Zhang Y, et al. Vascular and perivascular niches, but not the osteoblastic niche, are numerically restored following allogeneic hematopoietic stem cell transplantation in patients with aplastic anemia. *Int J Hematol*. 2017;106:71–81.
19. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825–828.
20. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2005;11:945–956.
21. van der Velden WJ,, Mori T, Stevens WB, et al. Reduced PTLD-related mortality in patients experiencing EBV infection following allo-SCT after the introduction of a protocol incorporating pre-emptive rituximab. *Bone Marrow Transplant*. 2013;48:1465–1471.
22. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis*. 2002;34:1094–1097.
23. Xu LP, Wang SQ, Wu DP, et al. Haplo-identical transplantation for acquired severe aplastic anaemia in a multicentre prospective study. *Br J Haematol*. 2016;175:265–274.
24. Champlin RE, Horowitz MM, van Bekkum DW, et al. Graft failure following bone marrow transplantation for severe aplastic anemia: risk factors and treatment results. *Blood*. 1989;73:606–613.
25. Davies SM, Ramsay NK, Haake RJ, et al. Comparison of engraftment in recipients of matched sibling of unrelated donor marrow allografts. *Bone Marrow Transplant*. 1994;13:51–57.
26. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. *Bone Marrow Transplant*. 2005;36:947–950.
27. Yagasaki H, Takahashi Y, Hama A, et al. Comparison of matched-sibling donor BMT and unrelated donor BMT in children and adolescent with acquired severe aplastic anemia. *Bone Marrow Transplant*. 2010;45:1508–1513.
28. Dulley FL, Vigorito AC, Aranha FJP, et al. Addition of low-dose busulfan to cyclophosphamide in aplastic anemia patients prior to allogeneic bone marrow transplantation to reduce rejection. *Bone Marrow Transplant*. 2003;33:9–13.
29. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. *Blood*. 2014;124:344–353.
30. Ostronoff F, Ostronoff M, Souto-Maior AP, et al. Prospective trial of mycophenolate mofetil-cyclosporine A prophylaxis for acute GVHD after G-CSF stimulated allogeneic bone marrow transplantation with HLA-identical sibling donors in patients with severe aplastic anemia and hematological malignancies. *Clin Transplant*. 2009;23:33–38.
31. Neumann F, Graef T, Tapprich C, et al. Cyclosporine A and mycophenolate mofetil vs cyclosporine A and methotrexate for graft-versus-host disease prophylaxis after stem cell transplantation from HLA-identical siblings. *Bone Marrow Transplant*. 2005;35:1089–1093.
32. Storb R, Yu C, Wagner JL, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood*. 1997;89:3048–3054.
33. Remberger M, Watz E, Ringdén O, Mattsson J, Shanwell A, Wikman A. Major ABO blood group mismatch increases the risk for graft failure after unrelated donor hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:675–682.
34. Lee JW, Cho BS, Lee SE, et al. The outcome of unrelated hematopoietic stem cell transplants with total body irradiation (800 cGy) and cyclophosphamide (120 mg/kg) in adult patients with acquired severe aplastic anemia. *Biol Blood Marrow Transplant*. 2011;17:101–108.
35. Park SS, Kwak DH, Jeon YW, et al. Beneficial role of low-dose antithymocyte globulin in unrelated stem cell transplantation for adult patients with acquired severe aplastic anemia: reduction of graft-versus-host disease and improvement of graft-versus-host disease-free, failure-free survival rate. *Biol Blood Marrow Transplant*. 2017;23:1498–1508.
36. Finke J, Bethge WA, Schmoor C, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol*. 2009;10:855–864.
37. Marsh JC, Gupta V, Lim Z, et al. Alemtuzumab with fludarabine and cyclophosphamide reduces chronic graft-versus-host disease after allogeneic stem cell transplantation for acquired aplastic anemia. *Blood*. 2011;118:2351–2357.
38. Kanda Y, Kanda J, Atsuta Y, et al. Impact of a single human leucocyte antigen (HLA) allele mismatch on the outcome of unrelated bone marrow transplantation over two time periods. A retrospective analysis of 3003 patients from the HLA Working Group of the Japan Society for Blood and Marrow Transplantation. *Br J Haematol*. 2013;161:566–577.
39. Petersdorf EW, Longton GM, Anasetti C, et al. Definition of HLA-DQ as a transplantation antigen. *Proc Natl Acad Sci U S A*. 1996;93:15358–15363.
40. Morishima Y, Kashiwase K, Matsuo K, et al. Biological significance of HLA locus matching in unrelated donor bone marrow transplantation. *Blood*. 2015;125:1189–1197.
41. Serody JS, Sparks SD, Lin Y, et al. Comparison of granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood progenitor cells and G-CSF-stimulated bone marrow as a source of stem cells in HLA-matched sibling transplantation. *Biol Blood Marrow Transplant*. 2000;6:434–440.
42. Alousi A, Wang T, Hemmer MT, et al. Peripheral blood versus bone marrow from unrelated donors: bone marrow allografts have improved long-term overall and graft-versus-host disease-free, relapse-free survival. *Biol Blood Marrow Transplant*. 2019;25:270–278.
43. Kennedy VE, Chen H, Savani BN, et al. Optimizing antithymocyte globulin dosing for unrelated donor allogeneic hematopoietic cell transplantation based on recipient absolute lymphocyte count. *Biol Blood Marrow Transplant*. 2018;24:150–155.
44. Bacigalupo A, Lamparelli T, Bruzzi P, et al. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood*. 2001;98:2942–2947.
45. Hamadani M, Blum W, Phillips G, et al. Improved nonrelapse mortality and infection rate with lower dose of antithymocyte globulin in patients undergoing reduced-intensity conditioning allogeneic transplantation for hematologic malignancies. *Biol Blood Marrow Transplant*. 2009;15:1422–1430.
46. George B, Pn N, Devasia AJ, et al. Post-transplant cyclophosphamide as sole graft-versus-host disease prophylaxis is feasible in patients undergoing peripheral blood stem cell transplantation for severe aplastic anemia using matched sibling donors. *Biol Blood Marrow Transplant*. 2018;24:494–500.
47. Peres RM, Costa CR, Andrade PD, et al. Surveillance of active human cytomegalovirus infection in hematopoietic stem cell transplantation (HLA sibling identical donor): search for optimal cutoff value by real-time PCR. *BMC Infect Dis*. 2010;10:147.
48. Takenaka K, Gondo H, Tanimoto K. The Fukuoka Bone Marrow Transplantation Group. Increased incidence of cytomegalovirus (CMV) infection and CMV-associated disease after allogeneic bone marrow transplantation from unrelated donors. *Bone Marrow Transplant*. 1997;19:241–248.
49. Buyck HCE, Ball S, Junagade P, Marsh J, Chakrabarti S. Prior immunosuppressive therapy with antithymocyte globulin increases the risk of EBV-related lymphoproliferative disorder following allo-SCT for acquired aplastic anaemia. *Bone Marrow Transplant*. 2009;43:813–816.

50. Xu LP, Jin S, Wang SQ, et al. Upfront haploidentical transplant for acquired severe aplastic anemia: registry-based comparison with matched related transplant. *J Hematol Oncol*. 2017;10:25.
51. Passweg JR, Pérez WS, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. *Bone Marrow Transplant*. 2006;37:641–649.
52. Devillier R, Dalle JH, Kulasekararaj A, et al. Unrelated alternative donor transplantation for severe acquired aplastic anemia: a study from the French Society of Bone Marrow Transplantation and Cell Therapies and the EBMT Severe Aplastic Anemia Working Party. *Haematologica*. 2016;101:884–890.
53. Dufour C, Pillon M, Sociè G, et al. Outcome of aplastic anaemia in children. A study by the Severe Aplastic Anaemia and Paediatric Disease Working Parties of the European Group for Blood And Bone Marrow Transplant. *Br J Haematol*. 2015;169:565–573.
54. Johnson SR, Tomlinson GA, Hawker GA, Granton JT, Feldman BM. Propensity score methods for bias reduction in observational studies of treatment effect. *Rheum Dis Clin North Am*. 2018;44:203–213.