Biol Blood Marrow Transplant 25 (2019) 1592–1596

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Pediatric

Unrelated Donor Peripheral Blood Stem Cell Transplantation for Patients with β-Thalassemia Major Based on a Novel Conditioning Regimen

Lan Sun[1,†], Na Wang[1,†], Yi Chen[1], Liyuan Tang[1], Chongyun Xing[1,2], Nina Lu[1], Yifen Shi[1], Yongyong Ma[1], Fengyang Lin[1], Kang Yu[1,*], Jianhua Feng[1,3,*]

[1] Department of Hematology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, People's Republic of China
[2] Department of Hematology, the First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, People's Republic of China
[3] Department of Pediatric Hematology-Oncology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, People's Republic of China

Article history:
Received 22 November 2018
Accepted 28 March 2019

Key Words:
Hematopoietic stem cell transplantation
Thalassemia
Unrelated donor PBSCT

A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only available curative treatment for patients with β-thalassemia major (β-TM). However, the problem of finding a suitable sibling donor with well-matched human leukocyte antigens is still a major obstacle to curing these patients. With the progress in high-resolution HLA typing technology and supportive care, outcomes after allogeneic HSCT from an HLA well-matched unrelated donor (UD) now approach those of well-matched sibling donors. However, UD HSCT is hampered by an increased risk of graft-versus-host disease and transplant-related mortality. Here we report the outcome of transplantation in patients with β-TM using a novel WZ-14-TM transplant protocol, based on cyclophosphamide, intravenous busulfan, fludarabine, and antithymocyte globulin, in our center. Forty-eight patients between 2 and 11 years of age with β-TM received HLA well-matched UD peripheral blood stem cell transplantation following the WZ-14-TM protocol. All of the transplanted patients achieved donor engraftment. The incidences of grade II to IV acute and chronic graft-versus-host disease were 8.3% and 8.3%, respectively. The overall survival and thalassemia-free survival rates were both 100%. This encouraging result suggests that the WZ-14-TM protocol is a feasible and safe conditioning regime for patients with β-TM undergoing UD HSCT.

INTRODUCTION

Currently, allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only curative treatment for β-thalassemia major (β-TM) [1]. The widest clinical experience of allo-HSCT in β-TM has been obtained using grafts harvested from a HLA well-matched sibling donor [2,3]. However, only approximately 1 in 3 patients has a matched sibling donor within the family [4]; therefore, alternative transplantation strategies that use allografts from alternative donors are needed. Thanks to the introduction of high-resolution HLA typing, suitable unrelated donors (UDs) with well-matched HLAs can be identified using stringent criteria for compatibility of HLA class I and class II loci, and hematopoietic stem cell transplantation (HSCT) from a suitable UD has been proven to lead to satisfactory results similar to those obtained from well-matched sibling donors in patients with β-TM [5,6].

Considering the specific features of β-TM (such as hyperplastic bone marrow and allosensitization due to multiple blood transfusions), for several years, a myeloablative conditioning regimen of busulfan followed by cyclophosphamide (Bu/Cy) has been considered the gold standard in patients with β-TM undergoing allo-HSCT [7]. On the other hand, given the nonnegligible risk of graft failure (GF) in patients with β-TM who received UD HSCT, the benefit of adding antithymocyte globulin (ATG) and fludarabine (Flu) to the conditioning regimen has been investigated [8,9]. Furthermore, in high-risk patients with β-TM who had a greatly amplified hematopoietic marrow compartment due to a history of poor compliance with adequate blood transfusions and iron chelation therapy, the addition of hydroxyurea (Hu) to a conditioning regimen made an important contribution by reducing erythroid marrow expansion [10]. Since 2001, the conditioning regimen of Bu/Cy ATG + Flu + Hu for HSCT has been performed in patients with β-TM. Nowadays, this conditioning regimen has become dominant in mainland China [11]. The Bu/Cy ATG + Flu + Hu regimen started with a preconditioning phase, during which patients received Hu

Financial disclosure: See Acknowledgments on page 1595.
* Correspondence and reprint requests: Jianhua Feng or Kang Yu, Department of Hematology, the First Affiliated Hospital of Wenzhou Medical University, Ouhai Zone, Wenzhou 325000, Zhejiang, People's Republic of China.
E-mail addresses: yukang62@126.com (K. Yu), wzfjh@126.com (J. Feng).
† These authors contributed equally to this work.

https://doi.org/10.1016/j.bbmt.2019.03.028
1083-8791/

(30 mg/kg/d) and azathioprine (3 mg/kg/d) for 3 to 4 weeks before Bu conditioning, as well as Flu (30 mg/m^2/d) from day −16 through day −12. This was followed by intravenous Bu (total 11.2 to 12.8 mg/kg, divided into 16 fractions from days −9 to −6) combined with Cy (total 120 to 200 mg/kg, divided into 4 fractions between days −5 and −2). For graft-versus-host disease (GVHD) prophylaxis, patients received ATG (horse ATG, total 90 to 100 mg/kg, 3 or 4 fractions, started at day −4, or rabbit ATG, total 7.5 to 11.5 mg/kg), cyclosporine A (CsA) starting at 2.5 to 3.0 mg/kg intravenously daily on day −1 with a mean (SD) plasma concentration of 200 (50) ng/mL or in combination with short-term methotrexate (15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +5, and +11), or mycophenolate mofetil (15 mg/kg bid starting on day +1). With the Bu/Cy ATG + Flu + Hu preparative regimen, the mean (SD) long-term overall survival (OS) and thalassemia-free survival (TFS) after HSCT were 92.9% (6.9%) and 85.7% (9.4%), respectively. Although this conditioning regimen was effective in the treatment of patients with β-TM, it was associated with a 14.3% GF rate [11]. The toxicity of conditioning regimens is also a difficulty we faced during HSCT for β-TM. For example, the dose-limiting toxic effect of Cy is heart toxicity, while Bu is known to potentiate Cy-induced toxicity. Furthermore, Loushin et al. [12] reported a case of severe cardiopulmonary reaction to ATG infusion requiring intubation and cardiopulmonary resuscitation.

However, none of these difficulties stop the advancement of allo-HSCT for β-TM. In the present study, we report our experience in allo-HSCT from well-matched UDs, using the WZ-14-TM HSCT protocol based on intravenous Bu, Cy, Flu and ATG, in a cohort of 48 patients with β-TM.

METHODS

Patients and Donors

This study included 48 patients with β-TM who received UD peripheral blood stem cell transplantation (PBSCT) between August 2014 and June 2018 at the Department of Hematology, the First Affiliated Hospital of Wenzhou Medical University. Before transplantation, all patients had received regular packed RBC transfusions every 2 to 5 weeks. The hemoglobin threshold for transfusion was 90 g/L. The dose of RBCs transfused was 10 to 15 mL/kg body weight of packed RBCs. In addition, all patients had been compliant with regular iron chelation therapy before allograft. Iron chelation was started when ferritin exceeded 1000 μg/L, and the iron chelator consisted of either oral deferasirox (in most patients) or subcutaneous infusion of deferoxamine. We could not group the patients according to the Pesaro risk classification because we did not routinely perform liver biopsies in our pretransplant evaluation. However, based on the patient's age, liver size, and ferritin level, we categorized the pediatric patients with β-TM into low-risk, medium-risk, and high-risk groups (Table 1) [6]. Donor-recipient histocompatibility was determined by high-resolution typing for HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 alleles. Only donors fully matched or with no more than 2 antigenic mismatches were selected. HLA antibody screening was not routinely performed in the patients with β-TM who had a well-matched UD for allo-HSCT. This study received the approval of the institutional review board from Wenzhou Medical University, and informed consent was obtained from the parents of the patients.

Conditioning Regimen

The WZ-14-TM conditioning regimen consisted of Cy (day −10 to day −9), intravenous Bu (day −8 to day −5), Flu (day −6 to day −2), and rabbit ATG (Fresenius, Graefelfing, Germany; day −4 to day −1). The therapeutic drug monitoring of Bu and Cy was not performed in the present study; the doses were adjusted based on the patient's risk stratification and age (Table 2).

Table 1
Risk Classification of Patients with β-TM According to the NF Classification

Group	Ferritin, μg/L	Hepatomegaly, cm	Age, y
Low risk	<3000	<2.5 under the costal margin	<4
Medium risk	Neither low- nor high-risk group		
High risk	> 5000	>4	>8

Data from Li et al. [6].
NF, nanfang hospital.

GVHD Prophylaxis

With respect to GVHD prophylaxis, patients received CsA starting at 1.5 to 3.0 mg/kg intravenously daily on day −7 with a mean (SD) targeted plasma concentration of 200 (50) ng/mL, in combination with short-term methotrexate (15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11) and mycophenolate mofetil (CellCept, Shanghai, China; 15 mg/kg bid starting on day −7).

Supportive Care and Infection Prophylaxis

Recombinant human granulocyte colony-stimulating factor (5 μg/kg/d) was administered from day +1 until the absolute neutrophil count was $>0.5 \times 10^9$/L for 3 consecutive days. For infectious disease prophylaxis, the patients received broad-spectrum antibacterial until the neutrophil level was $>1.0 \times 10^9$/L, as well as acyclovir and antifungal agents for 6 months. Trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii*prophylaxis was required for 1 year. Cytomegalovirus (CMV) surveillance of blood using quantitative RT-PCR was mandated weekly for 6 months.

Definitions and Endpoints

Neutrophil engraftment was defined to occur on the first of 3 consecutive days in which the absolute neutrophil count was $>0.5 \times 10^9$/L. Platelet engraftment was defined to occur during the first of 7 consecutive days in which the platelet count was $>20 \times 10^9$/L without transfusion. Hemoglobin engraftment was defined to occur on the first of 3 consecutive days in which the hemoglobin level was >90 g/L. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and graded using established criteria [13,14]. Chimerism of donor/recipient DNA in peripheral blood was analyzed using short tandem repeat PCR on days +14, +30, +60, +90, +120, +150, +180, and +365 after transplantation and yearly thereafter. Mixed chimerism (MC) was defined as the presence of >5% residual host cell at any time point post-transplantation, whereas rejection was defined as >90% residual host cell in peripheral blood with relapse of β-TM or RBC transfusion dependence [15-17]. OS was measured from the day of transplantation until death from any cause. TFS was measured from the day of transplantation until either thalassemia recurrence with transfusion dependence or death from any cause.

Statistical Methods

All patients were monitored until October 31, 2018. The OS and TFS were estimated by the Kaplan-Meier method. Statistical tests were performed with STATA Version 12.0 software (StataCorp LP, College Station, Texas).

RESULTS

Patient Characteristics and Engraftment

The characteristics of the 48 recipients and donors are shown in Table 3. The median age of the patients at transplantation was 4 years (range, 2 to 11). The median total CD34$^+$ cell dose and mononuclear cell dose in the infused product were 14.80×10^6/kg (range, 3.00 to 50.10) and 14.35×10^8/kg (range, 1.89 to 53.80), respectively. The median time to achieve neutrophil engraftment was 13 days (range, 10 to 23). The median platelet and hemoglobin recovery times were 12 days (range, 8 to 31) and 11 days (range, 3 to 47), respectively.

aGVHD and cGVHD

The incidences of aGVHD and cGVHD are shown in Table 4. Sixteen (33.3%) of 48 patients evaluable after day 30 developed aGVHD. Three patients (6.2%) experienced grade II to III aGVHD. One patient (2.1%) had grade IV gut aGVHD but showed resolution of GVHD after the administration of high-dose steroids and basiliximab (Simulect, Novartis Pharma, Basel, Switzerland). The most affected organ was skin, with mild to moderate disease severity in most patients. Four (8.3%) of 48 patients evaluable after day 100 developed cGVHD, and the severity of cGVHD was as follows3 mild and 1 severe. Of the 3 patients with cGVHD of mild severity, 1 had skin GVHD alone, 1 showed skin and oral GVHD, and another had skin and hepatic GVHD. All 3 patients showed rapid resolution of cGVHD with oral methylprednisolone administration. Obliterative bronchiolitis was diagnosed by clinical symptoms and high-resolution computed tomography in 1 patient (2.1%). The patient showed resolution of obliterative bronchiolitis after treatment with corticosteroids, azithromycin, and supportive care.

Table 2
Conditioning Regimens of WZ-14-TM

	Cy, mg/kg/d	i.v. Bu, mg/kg/d	Flu, mg/m^2/d	ATG, mg/kg/d
Conditioning	Day −10 to −9	Day −8 to −5	Day −6 to −2	Day −4 to −1
Regimen for low-risk group	60		40	5
1-2 yr		4.4		
>2 yr		4.0		
Regimen for medium-risk group	55		40	5
<6 yr		3.6		
>6 yr		3.2		
Regimen for high-risk group	50		40	5
<8 yr		3.0		
>8 yr		2.8		

Transplantation-Related Complications

None of our patients developed veno-occlusive disease of the liver. Four patients (8.3%) developed hemorrhagic cystitis, and 4 patients (8.3%) had CsA-related neurotoxicity with seizures. All clinical signs improved after symptomatic treatment. CMV DNAemia occurred in 19 (39.6%) of the 48 patients. Sepsis was documented in 2 patients (4.2%). No patients died of CMV infection or sepsis in this study. Epstein-Barr virus DNA levels were monitored weekly for the first 6 months of transplantation in 42 patients. Epstein-Barr virus DNA replication was detected in 12 patients (28.6%) and treated with preemptive therapy with ganciclovir and intravenous immunoglobulin. No patient developed post-transplant lymphoproliferative disease. Autoimmune hemolytic anemia was diagnosed in 2 patients (4.2%) and was confirmed by a positive direct Coombs test. One patient showed resolution of autoimmune hemolytic anemia after the initial therapy with corticosteroids, CsA, and intravenous immunoglobulin, and the other patient had a complete resolution following the subsequent therapy with anti-CD20 monoclonal antibody (rituximab) and bortezomib (Velcade, Millenium Pharmaceuticals, Cambridge, MA). Immune thrombocytopenia was diagnosed in 3 patients (6.3%). All patients had complete resolution of immune thrombocytopenia after initial treatment with corticosteroids and intravenous immunoglobulin, with the exception of 1 patient who was subsequently treated with recombinant human thrombopoietin. Nephrotic syndrome was confirmed by laboratory testing in 1 patient (2.1%). The patient was treated with corticosteroids and had a complete resolution. The detailed results are listed in Table 4.

Table 3
Characteristics of Patients and Transplants

Characteristic	Total (n = 48)
Patient age, median (range), yr	4 (2-11)
Donor age, median (range), yr	31 (20-47)
Male patient sex, n (%)	23 (47.9)
Female patient sex, n (%)	25 (52.1)
Serum ferritin, median (range), μg/L	1826 (409-4960)
Risk group, n (%)	
Low risk	13 (27.1)
Medium risk	30 (62.5)
High risk	5 (10.4)
β-Globin gene mutation, n (%)	
Homozygous	18 (37.5)
Compound heterozygous	30 (62.5)
Donor/patient ABO match, n (%)	
Match	15 (31.3)
Mismatch	33 (68.7)
Donor/patient sex match, n (%)	
Match	26 (54.2)
Mismatch	22 (45.8)
HLA matching status, n (%)	
10/10 of the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci are matched	28 (58.3)
9/10 matched	16 (33.3)
8/10 matched	4 (8.3)
Infused cells, median (range)	
CD34$^+$ cells, $\times 10^6$/kg	14.80 (3.00-50.10)
Mononuclear cells, $\times 10^8$/kg	14.35 (1.89-53.80)

Chimerism and Follow-up

The median duration of the follow-up treatment was 14 months (range, 4-52). No patient was lost to follow-up. Among the 48 patients included in this study, 6 (12.5%) were observed to have MC at some time point in the post-transplantation period with a median follow-up of 14 months (range, 4-52). At the time of the first chimerism evaluation (day +14 post-transplantation), all patients had achieved complete chimerism (CC). Subsequently, 3 patients developed MC between 1 and 6 months, 2 patients developed MC between 6 months and 1 year, and 1 of the remaining 43 patients with CC developed MC at 13 months

Table 4
Engraftment and Complications of the Patients.

Characteristic	Total (n = 48)
Engraftment, median day (range)	
ANC $>0.5 \times 10^9$/L	13 (10-23)
PLT $>20 \times 10^9$/L	12 (8-31)
Hb >90 g/L	11 (3-47)
GVHD, n (%)	
Acute, grades II-IV	4 (8.3)
Chronic	4 (8.3)
Procedure-related complications, n (%)	
Hemorrhagic cystitis	4 (8.3)
Seizure	4 (8.3)
CMV DNAemia	19 (39.6)
EBV DNAemia*	12 (28.6)
Sepsis	2 (4.2)
Autoimmune hemolytic anemia	2 (4.2)
Immune thrombocytopenia	3 (6.3)
Nephrotic syndrome	1 (2.1)

ANC indicates absolute neutrophil count; PLT, platelet; Hb, hemoglobin; EBV, Epstein-Barr virus.
* EBV DNA levels were monitored in 42 patients.

post-transplantation. None of the 6 patients who developed MC post-transplantation rejected the graft or evolved to have >95% donor chimerism, but all remained in persistent MC throughout follow-up. After a median follow-up of 14 months, all patients had survived and had achieved RBC transfusion independence. The rates of OS and TFS were both 100% (Figure 1).

DISCUSSION

In August 2014, we began using WZ-14-TM in hopes of lowering the GF rate and transplant-related mortality, thus improving TFS in patients with β-TM undergoing UD transplantation. We modified the classic conditioning regimen based on Bu/Cy through the addition of Flu and ATG to decrease the risk of GF. In an attempt to reduce transplantation-related toxicity, the cumulative dose of Cy in the standard Bu/Cy conditioning regimen was reduced from 200 mg/kg to 120 to 100 mg/kg. Similar to the study by Li et al. [6], Cy was first administrated during the process of conditioning to reduce conditioning-related heart toxicity by avoiding the concurrent administration of ATG and Cy. Furthermore, considering that all the 48 patients with β-TM had received regular RBC transfusion and iron chelation therapy before transplantation, Hu was not given before conditioning. Compared with the Bu/Cy ATG + Flu + Hu conditioning regimen [11], our present results showed that the WZ-14-TM protocol effectively decreased transplant-related mortality and improved OS and TFS in patients with β-TM undergoing UD transplantation.

Long-term blood transfusion can result in an immune response against allo-HLA, and thus patients with β-TM undergoing HSCT typically display a relatively high incidence of graft rejection. In our data, no graft rejection was observed. The following factors are most likely to contribute to our results: (1) the intensity of hematopoietic (Bu and Cy) and immune suppression (Flu and ATG) in the WZ-14-TM regime. (2) It was anticipated that the HSCT with mobilized peripheral blood might engraft quicker because of the high number of hematopoietic stem cells. Mathews et al. [18] demonstrated that PBSCT was associated with faster engraftment and a lower incidence of MC on day +28 post-transplantation than bone marrow. (3) It is possible that a more rapid engraftment of graft with granulocyte colony-stimulating factor administered post-HSCT might reduce the risk of primary GF. (4) Before the year 2000, most patients with β-TM accepted RBC transfusions with no leuco-depletion, which induced elevated pretransplant panel reactive antibody levels and thus increased the risk of graft rejection [19]. Meanwhile, inadequate and irregular blood transfusions led to the expansion of the erythroid compartment, which is an unfavorable risk factor for engraftment [6,10]. To reduce the rejection rate as well to improve engraftment, we adopted the WZ-14-TM protocol for allo-HSCT in patients with β-TM aged 14 years or younger. However, our result is limited and needs to be validated in future studies with larger sample sizes.

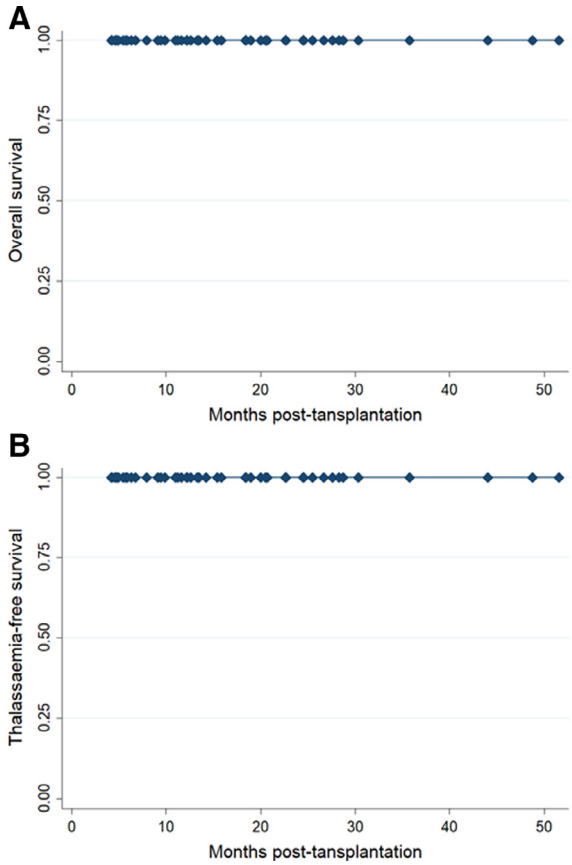

Figure 1. (A) Overall survival and (B) thalassemia-free survival of patients with β-thalassemia major treated by HLA well-matched unrelated donor peripheral blood stem cell transplantation based on the WZ-14-TM conditioning regimen.

Furthermore, we observed a lower incidence of GVHD (grade II to IV aGVHD, 8.3%; cGVHD, 8.3%) than in previous studies, in which 37% to 42% of the patients with β-TM undergoing HLA well-matched UD HSCT developed grade II to IV aGVHD, and 14% to 27% developed cGVHD [5,20-22]. The low incidence of GVHD in our study may be related to the combination of ATG, CsA, mycophenolate mofetil, and methotrexate for GVHD prophylaxis [23-25], as well as that the patients included in our study were young with a median age of 4.0 years. The incidence of CMV DNAemia in our cohort was high. The reason might be related to the use of strong immunosuppressive agents, including Flu and ATG, in the conditioning regimen. These patients with CMV DNAemia were treated with antiviral therapy, and no patient died of a CMV infection.

In summary, our study demonstrates that treatment of β-TM patients with HLA well-matched UD PBSCT based on the WZ-14-TM protocol is feasible and safe. UD PBSCT in patients with β-TM yielded an excellent engraftment rate, OS, and TFS when the WZ-14-TM protocol was used. Considering the limitations of our single-center study, the results of UD PBSCT using the WZ-14-TM protocol should be reassessed in the future in multicenter studies.

ACKNOWLEDGMENTS

Financial disclosure: This work was supported by the Public Welfare Science and Technology Project of Wenzhou (Nos. Y20160099, Y20150034), the Natural Science Foundation of Zhejiang Province (No. LQ14H080002), and the National Natural Science Foundation of China (No. 81502793).

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

REFERENCES

1. Gaziev J, Lucarelli G. Hematopoietic stem cell transplantation for thalassemia. *Curr Stem Cell Res Ther*. 2011;6:162–169.
2. Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. *Haematologica*. 2014;99:811–820.
3. Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. *N Engl J Med*. 1990;322:417–421.
4. Delfini C, Donati M, Marchionni D, et al. HLA compatibility for patients with thalassemia: implications for bone marrow transplantation. *Int J Cell Cloning*. 1986;4:274–278.

5. La Nasa G, Argiolu F, Giardini C, et al. Unrelated bone marrow transplantation for beta-thalassemia patients: the experience of the Italian Bone Marrow Transplant Group. *Ann N Y Acad Sci*. 2005;1054:186–195.
6. Li C, Wu X, Feng X, et al. A novel conditioning regimen improves outcomes in beta-thalassemia major patients using unrelated donor peripheral blood stem cell transplantation. *Blood*. 2012;120:3875–3881.
7. Lucarelli G, Polchi P, Galimberti M, et al. Marrow transplantation for thalassaemia following busulphan and cyclophosphamide. *Lancet*. 1985;1:1355–1357.
8. Lawson SE, Roberts IA, Amrolia P, Dokal I, Szydlo R, Darbyshire PJ. Bone marrow transplantation for beta-thalassaemia major: the UK experience in two paediatric centres. *Br J Haematol*. 2003;120:289–295.
9. Anurathapan U, Pakakasama S, Mekjaruskul P, et al. Outcomes of thalassemia patients undergoing hematopoietic stem cell transplantation by using a standard myeloablative versus a novel reduced-toxicity conditioning regimen according to a new risk stratification. *Biol Blood Marrow Transplant*. 2014;20:2066–2071.
10. Sodani P, Gaziev D, Polchi P, et al. New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. *Blood*. 2004;104:1201–1203.
11. Li XY, Sun X, Chen J, et al. Hematopoietic stem cell transplantation for children with β-thalassemia major: multicenter experience in China. *World J Pediatr*. 2018;14:92–99.
12. Loushin MK, Hasinoff IK, Belani KG. A delayed cardiopulmonary reaction to an intravenous immunosuppressant thymoglobulin after pancreas transplant. *Anesth Analg*. 2001;93:1260–1261.
13. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995;15:825–828.
14. Martin PJ, Lee SJ, Przepiorka D, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: VI. The 2014 Clinical Trial Design Working Group Report. *Biol Blood Marrow Transplant*. 2015;21:1343–1359.
15. Andreani M, Testi M, Battarra M, et al. Relationship between mixed chimerism and rejection after bone marrow transplantation in thalassaemia. *Blood Transfus*. 2008;6:143–149.
16. Andreani M, Testi M, Gaziev J, et al. Quantitatively different red cell/nucleated cell chimerism in patients with long-term, persistent hematopoietic mixed chimerism after bone marrow transplantation for thalassemia major or sickle cell disease. *Haematologica*. 2011;96:128–133.
17. Hsieh MM, Wu CJ, Tisdale JF. In mixed hematopoietic chimerism, the donor red cells win. *Haematologica*. 2011;96:13–15.
18. Mathews V, George B, Viswabandya A, et al. Improved clinical outcomes of high risk β thalassemia major patients undergoing a HLA matched related allogeneic stem cell transplant with a treosulfan based conditioning regimen and peripheral blood stem cell grafts. 2013;8:e61637.
19. Xu LH, Fang JP, Huang WG, et al. Marrow graft rejection by repeated transfusions of allogeneic donor spleen cells. *Bone Marrow Transplant*. 2007;40:691–698.
20. La Nasa G, Giardini C, Argiolu F, et al. Unrelated donor bone marrow transplantation for thalassemia: the effect of extended haplotypes. *Blood*. 2002;99:4350–4356.
21. La Nasa G, Caocci G, Argiolu F, et al. Unrelated donor stem cell transplantation in adult patients with thalassemia. *Bone Marrow Transplant*. 2005;36:971–975.
22. Hongeng S, Pakakasama S, Chuansumrit A, et al. Outcomes of transplantation with related- and unrelated-donor stem cells in children with severe thalassemia. *Biol Blood Marrow Transplant*. 2006;12:683–687.
23. Bacigalupo A. Antilymphocyte/thymocyte globulin for graft versus host disease prophylaxis: efficacy and side effects. *Bone Marrow Transplant*. 2005;35:225–231.
24. Iravani M, Mousavi A, Gholibeikian S, et al. Cyclosporin A and mini short-term methotrexate vs cyclosporin A as graft-versus-host disease prophylaxis in patients with beta thalassemia major undergoing allogeneic blood and marrow transplantation. *Bone Marrow Transplant*. 2005;35:1095–1099.
25. Pidala J, Tomblyn M, Nishihori T, et al. ATG prevents severe acute graft-versus-host disease in mismatched unrelated donor hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2011;17:1237–1244.