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G-CSF-Mobilized Blood and Bone Marrow Grafts as the Source of Stem Cells for HLA-Identical Sibling Transplantation in Patients with Thalassemia Major

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A B S T R A C T

As an inherited anemia, thalassemia major (TM) is currently only curable with allogeneic hematopoietic stem cell transplantation (allo-HSCT). Here we report an allo-HSCT protocol for patients with TM who received a combination of granulocyte colony-stimulating factor-primed bone marrow and peripheral blood stem cells (G-BM & PBSCs) from a matched sibling donor (MSD). The conditioning regimen consisted of i.v. busulfan, cyclophosphamide, fludarabine, and antithymocyte globulin. Chimerism analysis was performed for all patients. Immunosuppressive treatment was terminated if rejection was suspected, and donor lymphocyte infusion was administered once no response was observed. A total of 184 patients with TM were enrolled in the study between July 2007 and July 2018. The cumulative incidence of grade II-IV acute graft-versus-host disease (GVHD) was 13.1%, and that of moderate or severe chronic GVHD was 5.7%. The cumulative incidence of graft rejection was .6%. In the total cohort, the 3-year overall survival, thalassemia-free survival, and GVHD-free, relapse-free survival were 97.8%, 97.3%, and 89.5%, respectively. Collectively, our results indicate that G-BM & PBSCs from an MSD is be a good stem cell source for patients with TM undergoing allo-HSCT.

INTRODUCTION

A major global health problem, thalassemia occurs primarily in developing countries. Most patients with thalassemia major (TM) are born in resource-constrained countries, and many die as teenagers due to inadequate transfusion and iron chelation therapy [1]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only established curative option for TM. A previous study predicted that >90% of patients will TM could survive after allo-HSCT, with a thalassemia-free survival (TFS) of roughly 80% [2].

Traditionally, bone marrow (BM) is the standard stem cell source for transplantation in patients with TM, with cord blood and peripheral blood stem cells (PBSCs) as alternative sources. Although overall survival (OS) and TFS are similar in recipients of PBSC transplantation (PBSCT) and recipients of BM transplantation (BMT), PBSCT is associated with earlier engraftment, a lower rate of graft rejection (GR), greater convenience, and higher incidences of acute and chronic GVHD compared with BMT [3]. In the Beijing protocol for haploidentical hematopoietic stem cell transplantation, the coinfusion of G-CSF-mobilized BM and G-CSF-mobilized PBSCs (G-BM&PBSCs) at a 1:1 ratio with mononuclear cells (MNCs) is a standard procedure [4,5]. The G-BM&PBSC graft design is thought to improve outcomes by favorably balancing engraftment kinetics, graft failure rate, and GVHD, as well as the graft-versus-leukemia effect [5]. However, little information is available on outcomes in G-BM&PBSC graft transplantation in patients with TM.

In the present study, we describe a method of performing allo-HSCT from a matched sibling donor (MSD) that was designed to decrease GR and GVHD. The protocol has 3 elements: a combination of G-BM&PBSC for HSCT, an antithymocyte globulin (ATG)- and fludarabine (Flu)-containing myeloablative conditioning regimen, and close chimerism monitoring and early intervention for preventing GR.

METHODS

Patients

In this prospective, single-center investigation, we evaluated the safety and efficacy of G-BM&PBSC transplantation for patients with TM. A total of 184 patients with TM were enrolled in the study between July 2007 and July 2018. These patients were prepared for transplantation in accordance with treatment protocol GX-07-TM, which was approved by the local Institutional Review Board, and the outcome data were reported to the Chinese Bone Marrow

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Transplant Registry. Written informed consent were obtained from parents of all patients and their donors, in accordance with the Declaration of Helsinki. The inclusion criteria were as follows: (1) a diagnosis of TM with hemoglobin electrophoresis, a genetic diagnosis of β-thalassemia by DNA analysis, and blood transfusion dependence; (2) a cardiac ejection fraction >50%; (3) normal pulmonary function tests and pulmonary examination results; and (4) normal kidney function. The exclusion criteria were (1) aspartate aminotransferase level >4-fold the upper limit of normal range in our institution's laboratory criteria; (2) uncontrolled bacterial, viral, or fungal infection; (3) positive serology for HIV; and (4) cytomegalovirus (CMV) or Epstein-Barr virus (EBV) copy number ≥200 copies/mL in blood by quantitative PCR. Patients positive for hepatitis C or hepatitis B virus were also excluded. Table 1 presents patient characteristics.

Conditioning Regimen and GVHD Prophylaxis

The GX-07-TM protocol used in this study consisted of busulfan (Bu), cyclophosphamide (Cy), Flu, and ATG. The regimen was as follows: (1) Bu 1 mg/kg, given i.v. 4 times daily for 4 days (days −9 to −6); (2) Flu 50 mg/m^2/day, administered i.v. for 3 days (days −12 to −10); (3) Cy 50 mg/kg/day, administered i.v. for 4 days (days −5 to −2); and (4) ATG (Thymoglobulin), 2.5 mg/kg/day, given i.v. for 4 days (days −4 to −1). All patients received 30 mg/kg hydroxyurea orally once daily for 2 months before transplantation. The GVHD prophylaxis regimen included cyclosporine A, methotrexate, and low-dose, short-course mycophenolate mofetil [6].

Stem Cell Collection

Donors were given G-CSF s.c. at a dose of 10 μg/kg/day for 5 consecutive days (days −3 to +1). G-BM was harvested on day 4 after G-CSF, and PBSCs were harvested on day 5 after G-CSF [4,5]. The BM was harvested at a target volume of 10 mL/kg donor weight. The recommended ratio of MNCs from the source in the graft PB to BM was 1 to 2:1. All donors were safe, and no serious adverse events were observed after donation. The median duration of hospitalization in all donors was 6 days (range, 6–8 days). Table 1 presents the infused stem cell dose per graft.

Evaluation of Chimerism and Therapeutic Interventions

Chimerism was first assessed on day +30, followed by an assessment every 1 to 2 months in the first year and then at 3 to 6 months beyond the first year for those with complete chimerism (CC) or, more frequently, with mixed chimerism (MC). In a patient exhibiting a >5% change in MC toward rejection, immunosuppressive treatment (IST) was terminated immediately, and the MC rate was followed. If a patient displayed continuously decreasing donor cells after the termination of IST and a decrease in donor cells to <75%, donor lymphocyte infusion (DLI) was given. The GVHD prophylaxis for DLI was oral cyclosporine A (5 mg/kg).

Table 1
Characteristics of Patients and Donors

Variable	Value
Age, yr, median (range)	5 (2-19)
Male sex, n (%)	128 (69.6)
Age>7 yr, n (%)	60 (32.6)
Liver size >5 cm, n (%)	16 (8.7)
Age >7 yr and liver size >5 cm, n (%)	16 (8.7)
Splenectomy, n (%)	24 (13.0)
Serum ferritin, ng/mL, median (range)	3025 (624-10,667)
Serum ferritin >5000 ng/mL, n (%)	36 (19.6)
Pesaro classification, n (%)	
Class I	24 (13.0)
Class ≥II	160 (87.0)
Alanine aminotransferase, IU/L, median (range)	26 (9-189)
Donor age, yr, median (range)	9 (3-24)
Female donor-male recipient, n (%)	84 (45.7)
ABO incompatibility, n (%)	64 (34.8)
Infused MNCs, × 10^8/kg, median (range)	
BM	7.5 (1.5-15.0)
PB	8.1 (1.5-15.3)
Total	15.5 (5.5-23.5)
Infused CD34$^+$ cells, × 10^6/kg, median (range)	
BM	6.1 (1.1-17.7)
PBSCs	4.3 (1.1-14.2)
Total	11.0 (3.4-27.1)

Definitions

Neutrophil engraftment and platelet engraftment were defined as the first of 3 consecutive days of an absolute neutrophil count >.5 × 10^9/L and an unsupported platelet count of >20 × 10^9/L, respectively. Transplantation-related mortality (TRM) was defined as death related to the transplantation instead of to the recurrence of thalassemia. OS was defined as the interval from the date of transplantation to the date of death or last follow-up. TFS was defined as the time from the date of transplantation to either recurrence of transfusion-dependent thalassemia or death from any cause. GR was defined as undetectable DNA of donor origin on at least 2 occasions no less than 1 week apart. A >95% donor profile by short tandem repeat PCR analysis was considered to indicate CC. The proportion of residual host cells (RHCs) in the recipient whole blood was used to classify the MC levels as follows: level 1, RHCs <10%; level 2, RHCs 10% to 25%; and level 3, RHCs >25% [7,8]. Acute and chronic GVHD were classified by the Glucksberg and National Institutes of Health classification schemes [9,10]. In this study, relevant events, including relapse, death from any cause, grade II-IV acute GVHD, and chronic GVHD requiring systemic treatment, were used for the calculation of GVHD-free and relapse-free survival (GRFS) [11].

Statistical Analyses

The median duration of follow-up was 38 months (range, 4 to 137 months). The primary study endpoint was 3-year TFS. Secondary endpoints included the cumulative incidences of GVHD, TRM, GR, OS, and GRFS. Cumulative incidence estimates were used to determine the incidences of GVHD, TRM, and GR. The Kaplan-Meier method was used to evaluate the estimated probabilities of OS, TFS, and GRFS. Results were expressed as a probability or cumulative incidence (%) with 95% confidence interval (95% CI). The effects of the following parameters on OS and TFS were examined: patient characteristics (age, sex, body weight, ferritin level, liver size, splenectomy, ABO compatibility, and risk classification for TM patients), donor characteristics (age, sex, female/male donor-recipient combination), and transplantation-related factors (total number of MNCs, total number of CD34$^+$ cells). Both univariate and multivariate analyses of prognostic factors were carried out using the log-rank test and a stepwise Cox proportional hazards regression model, respectively. All statistical analyses were performed using SPSS 18.0 (SPSS, Chicago, Illinois, USA) except for the cumulative incidence analyses, which were performed using NCSS software (NCSS, Kaysville, Utah, USA).

RESULTS

Engraftment

Table 1 lists the characteristics of patients and donors. All patients received engraftment. The median time to neutrophil and platelet engraftment was 11 days (range, 8 to 22 days) and 14 days (range, 9 to 43 days), respectively.

Therapeutic Interventions and Outcomes of MC

Chimerism analysis was performed for all patients. Seventeen patients (9.2%) exhibited MC, at a median of 5 months (range, 2 to 35 months). IST was terminated in all 17 patients. No patient developed GVHD after discontinuation of IST. Among these 17 patients, 3 progressed to CC, 5 showed persistent mixed chimerism, and 9 displayed continuously decreased donor cells (donor cells <75%). These 9 patients with decreased donor cells received DLI treatment, consisting of 1 infusion each with a median CD3$^+$ cell dose of .7 × 10^8/kg (range, .5 to 1.3 × 10^8/kg) on post-HSCT days ranging from 60 to 457 days. Among the 9 DLI-treated patients, 6 patients developed to grade I-II acute GVHD, which subsequently evolved to CC, 2 patients evolved to CC without GVHD, and 1 patient exhibited GR (Table 2). The median time to GVHD onset was 26 days (range, 21 to 59 days) after DLI. At the last evaluation, 180 patients were alive, and the cumulative rate of GR in all patients was .6% (95% CI, .1% to 3.9%). Five patients with persistent MC (median, 89% donor cells; range, 77% to 93%) were transfusion-independent, with a median hemoglobin value of 124 g/L (range, 102 to 139 g/L).

GVHD

In the total cohort, the cumulative incidence of acute GVHD was 15.3% (95% CI, 10.9% to 21.5%) for all grades, 13.1% (95% CI, 9.0% to 19.1%) for grade II-IV, and 6.0% (95% CI, 3.4% to 10.7%)

Table 2
Interventions and Outcome by MC Level

Variable	Level 1 MC, n (%)	Level 2 MC, n (%)	Level 3 MC, n (%)	CC, n (%)	Rejection, n (%)
Initial level of MC post- HSCT (N = 17)	13 (76.5)	2 (11.8)	2 (11.8)	–	–
Termination of IST (N = 17)	13 (76.5)	2 (11.8)	2 (11.8)	–	–
Level of MC before DLI (N = 9)	–	–	9 (100.0)	–	–
Final chimerism of DLI recipients (N = 9)	–	–	–	8 (88.9)	1 (11.1)
Final chimerism of those with GVHD (N = 6)	–	–	–	6 (100.0)	–
Chimerism status at last follow-up* (N = 17)	4 (23.5)	1 (5.9)	–	11 (64.7)	1(5.9)

* Median follow-up, 49 months (range, 17-127 months).

for grade III-IV (Figure 1). The cumulative incidence of chronic GVHD at 3 years was 9.4% (95% CI, 5.8% to 15.4%) for all-grade and 5.7% (95% CI, 3.0% to 10.9%) for moderate to severe (Figure 2). All the patients with acute GVHD and chronic GVHD responded to IST. No patient died from GVHD.

Transplantation-Related Events and Causes of Death

Veno-occlusive disease was diagnosed in 16 patients (8.7%), but no deaths were attributed to veno-occlusive disease. Hemorrhagic cystitis was observed in 50 patients (27.2%). CSA-related neurotoxicity was found in 11 (6.0%) patients. CSA was immediately withdrawn when neurotoxicity was diagnosed, and single-agent tacrolimus was replaced after a 7-day washout. Four (2.2%) patients had Bu-related neurotoxicity, all of who were treated successfully with phenytoin. Bacteremia was documented in 19 patients (10.3%). CMV reactivation was observed in 41 patients (22.3%) post-transplantation, with no fatalities. EBV reactivation was observed in 6 patients (3.3%) post-transplantation. Two patients (1.1%) developed post-transplantation lymphoproliferative disorder (PTLD) and were successfully treated with rituximab. No probable or definitive invasive fungal infections were detected. Four patients died of transplantation-related complications (all within 4 months after HSCT), including 2 patients due to hemorrhage and 2 due to infection. The cumulative rate of TRM in all patients at 3 years was 2.2% (95% CI, .8% to 5.7%) (Figure 2).

Survival

A total of 180 patients were alive at the last follow-up, 179 of whom exhibited TFS. In the total cohort, the 3-year Kaplan-Meier estimates for OS, TFS, and GRFS were 97.8% (95% CI, 95.7% to 99.9%), 97.3% (95% CI, 93.6% to 98.9%), and 89.5% (95% CI, 85.1% to 94.0%), respectively (Figure 3). None of the above-mentioned factors had any statistically significant influence on OS or TFS. At the last evaluation, all high-risk patients (age >7 years with hepatomegaly >5 cm; n = 16) were alive and thalassemia-free.

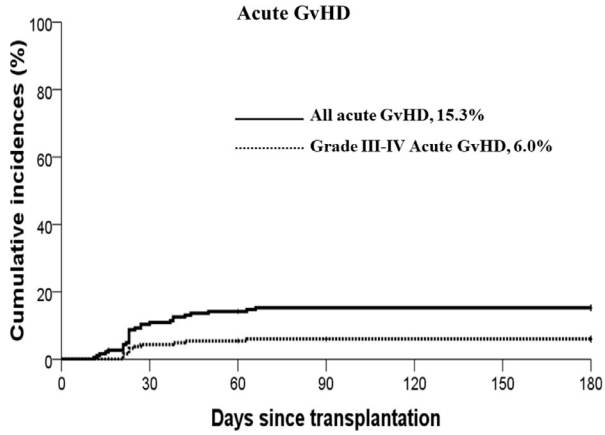

Figure 1. Cumulative incidence of acute GVHD.

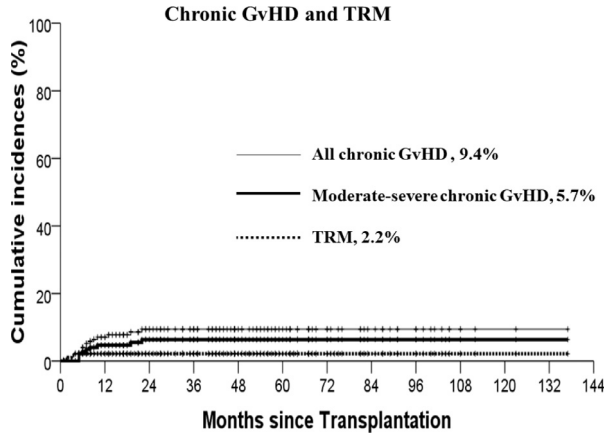

Figure 2. Cumulative incidences of chronic GVHD and TRM.

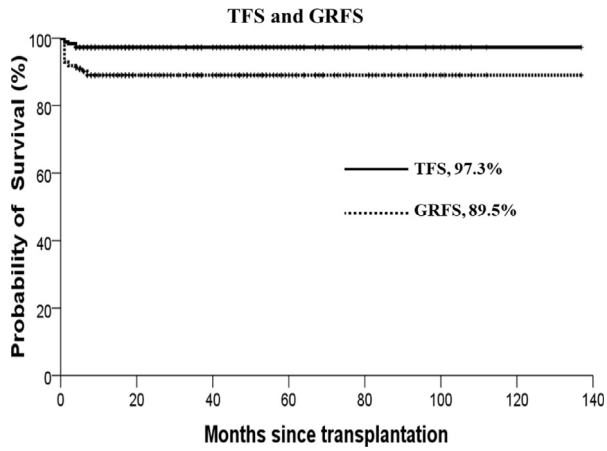

Figure 3. Kaplan-Meier curve of TFS and GRFS.

DISCUSSION

Initial reports of patients with TM undergoing MSD-HSCT have shown that outcomes are significantly hampered by an increased GR rate and GVHD. A TFS rate of approximately 83% was reported in a European Society for Blood and Marrow Transplant survey comprising 1061 cases of MSD-HSCT performed over the last decade [12]. Previous studies have demonstrated a TFS after BMT for TM ranging from 64% to 92% [13-15]. MC after MSD-HSCT occurs in 23% to 35% of TM patients [7,8,16]. Post-MSD-BMT, GR occurs in 6% to 23% of TM patients [7,8,16], while the risk of developing grade II-IV and grade III-IV acute GVHD is 28% to 57% and 9% to 22%, respectively [16-18]. The probability of developing chronic GVHD after MSD-BMT is 6% to 44% [16-18]. In the present study, all 184 patients achieved complete donor engraftment. The

cumulative incidence of the grade II-IV and grade III-IV acute GVHD in all patients was 13.1% and 6.0%, respectively. The cumulative incidence of the moderate-severe chronic GVHD at 3 years in all patients was 5.7%. Although the number of MNCs in the grafts was $>15 \times 10^8$ cells/kg in all patients (Table 1), the incidence of GVHD was lower than the expected values. The MC rate (9.2%) after transplantation and the graft failure rate (.6%) remained low. The probabilities of 3-year OS (97.8%), TFS (97.3%), and GRFS (89.5%) were also satisfactory.

Several factors might contribute to such a low incidence of GVHD and GR. First is the use of G-BM&PBSCs as the graft source in the present study. Ghavamzadeh et al [3] reported that patients receiving PBSCT have a shorter median time for neutrophil and platelet engraftments compared with the patients receiving BMT (3 days and 11 days shorter, respectively), and the GR rate is lower (5.7% versus 18.4%), showing higher incidence of acute GVHD (33.3% versus 50.8%) and chronic GVHD (26.1% versus 51.4%). Zhao et al [5] reported that coinfusion of G-BM&PBSC graft resulted in similar engraftment kinetics and lower rates of grade III-IV acute GVHD compared with PBSC grafts. Second, intensified immunosuppression was used to prevent both GVHD and GR. In the present study, the classic preparative regimen based on Bu and Cy was modified through the addition of ATG and Flu, which have been shown to be highly effective in immunosuppression. ATG can induce depletion of host T lymphocytes and infuse donor T lymphocytes in vivo, leading to reduced incidence of GR and GVHD [15,19]. The addition of Flu to the conditioning regimen has been shown to provide additional immunosuppression for engraftment without increasing toxicity in patients with Fanconi's anemia undergoing HSCT [20]. Third, we evaluated the course of MC in TM patients and intervened early in TM patients showing decreased donor cells by discontinuing IST or DLI. Importantly, our study showed that early intervention effectively prevented GR, and only 1 patient exhibited GR.

The grafts used in the present study were G-BM&PBSC grafts, which were intended to be used to combine the advantages of both elements. PBSCs have more $CD34^+$ cells and T cells than steady-state BM, and they have been shown to accelerate engraftment and decrease GR [3,21]. Morton et al [22] reported that G-BM transplantation leads to reduced severity of acute GVHD and less subsequent chronic GVHD, and engraftment is comparable to that of PBSCT. Unfortunately, the present study was not designed to compare the outcomes of coinfusion of G-BM&PBSCs grafts with PB grafts or BM grafts. A related randomized trial is needed to clarify the rationale for this regimen.

Conditioning regimens containing ATG have been indicated to increase infection risk [23]. In the present study, the incidences of CMV reactivation, EBV reactivation, HC, and bacteremia were 22.3%, 3.3%, 27.2%, and 10.3%, respectively. Two patients (1.1%) developed to PTLD and were successfully treated with rituximab. Although the TRM in the present study was low (2.2%), appropriate monitoring of viral infections should be considered.

The pretransplantation disease stage of patients is an independent factor determining prognosis. Using the Pesaro classification, TFS for class I, II, and III patients was 85% to 94%, 80%, and 65% to 70%, respectively [24]. For the high-risk patients age at least 7 years with hepatomegaly of at least 5 cm, the 5-year TFS is only 23.93% [25]. In the present study, all high-risk patients (age >7 years with hepatomegaly >5 cm; $n = 16$) were alive and thalassemia-free. Patient age, liver size, splenectomy, serum ferritin, and Pesaro classification were not risk factors for OS and TFS. Our results suggest that our protocol is a reasonable approach not only for low-risk patients, but also for high-risk patients with an MSD. However, further studies are needed to investigate the details with more high-risk TM patients.

Collectively, in the present study, we effectively and safely prevented GR and GVHD using an approach combining coinfusion of G-BM&PBSC, ATG, and Flu- intensified conditioning regimens, close chimerism monitoring, and early intervention, and such an approach ensured a high TFS rate in patients undergoing allo-HSCT for TM with an MSD. This treatment strategy could benefit patients with TM.

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