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Original Article

Clinical characteristics and risk factors for acute graft-versus-host disease in related HLA-haploidentical without *ex vivo* T cell depletion peripheral blood HSCT

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Abstract: Aims: We investigated the clinical features and risk factors for acute graft-versus-host disease (GVHD) in related HLA-haploidentical peripheral blood stem cell transplantation (RH-PBSCT). Methods: The clinical characteristics of 147 patients who received RH-PBSCT and hematologic malignancies were retrospectively analyzed. The RH-PBSCT approach applied myeloablative conditioning and infused hematopoietic stem cells from related HLA-haploidentical donors without ex vivo T cell depletion. Results: The 3-year overall survival rate was 69.4 ± 5.161%, the relapse incidence was 22.4 ± 5.154%, and the non-relapse mortality was 15.0 ± 4.067%. The median time for acute GVHD onset was 44.7 (range: 6-95) days. The cumulative incidences for grade I-IV, grade II-IV, grade II-IV, and acute GVHD were 58.5 ± 4.53%, 34.7 ± 4.69%, and 12.9 ± 3.80% respectively. One-organ involvement GVHD (mostly the skin) was revealed in 57 patients (66.3%). The patient clinical outcomes of grade I acute GVHD were the best, followed by without acute GVHD, grade II acute GVHD, and grade III-IV acute GVHD, with the 3-year overall survival rates being 80.9%, 66.9%, 63.6%, and 46.3% ($P < 0.05$) respectively. Having a child donor was statistically close to being a risk factor (HR = 2.48, 95% CI 0.88-1.96, P = 0.08), but a multivariate analysis showed no significant difference (P = 0.116). Conclusion: The RH-PBSCT approach to treating hematological malignancies leads to moderate acute GVHD incidence and low severe acute GVHD (grade III-IV) incidence. The clinical outcome in mild acute GVHD is better than in severe acute GVHD. No independent risk factor of acute GVHD was identified in our analysis.

Keywords: Acute graft-versus-host disease, peripheral blood stem cell transplantation, HLA haploidentical, risk factors

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is increasingly used for the treatment of hematological malignancies and other conditions [1, 2]. Acute graft-versus-host disease (GVHD) is a significant barrier to the more widespread use of allo-HSCT [3]. Previous studies suggest that about 50% of patients develop grade II-IV acute GVHD and 20% develop severe acute GVHD in allo-HSCT [4]. A high-dose of peripheral blood stem cells (PBSCs) is infused, which significantly increase the incidence of GVHD [5]. However, GVHD is only a secondary cause of transplant failure in allo-HSCT, with the primary causes being engraftment failures and infectious complications.

HLA-haploidentical HSCT, meaning finding a donor among siblings, children or parents that have half matched HLA, provides a new option for patients without a matched donor. Although HLA-haploidentical family donors are readily available and highly-motivated, the clinical outcomes of the application also show the disadvantages of the high rate of engraftment failure, severe GVHD, and a lack of efficacy [6]. In recent years, new strategies such as graft engineering (CD3 selection or *ex vivo* T cell depletion (TCD)) and high doses of cyclophosphamide (Cy) have been used to improve HLA haploidentical transplantation [7-10].

From 2002, we have developed the related HLA-haploidentical peripheral blood stem cell

transplantation (RH-PBSCT) approach in treating hematological malignancies [11]. This is a combined method of r-ATG *in vivo* TCD, a high dose of peripheral blood hematopoietic stem cells mobilized by granulocyte colony stimulation factor (G-CSF), and a strengthened GVHD prophylaxis [12]. The clinical outcomes of the patients who underwent this approach to transplantation and the risk factors of acute GVHD were retrospectively analyzed.

Materials and methods

Patients

In all, 147 patients who had hematologic malignancies and received RH-PBSCT at the First Affiliated Hospital of Xinjiang Medical University from Jan 2004 to Dec 2014 were included. The median age was 27 years (range 3-50 years). The clinical characteristics are shown in **Table 1**. Among the cohort, 65 patients were diagnosed with acute myelocytic leukemia (AML: CR1 42 cases, CR2 16 cases, CR3 7 cases); 54 patients with acute lymphoblastic leukemia (ALL: CR1 39 cases, CR2 11 cases, CR3 4 cases); 23 patients with chronic myelogenous leukemia (CML: CP 19 cases, AP 4 cases); and 5 patients with myelodysplastic syndrome (MDS, RAEB-1 2 cases, RAEB-2 3 cases). The inclusion criteria were: (1) age under 50; (2) patient received a graft from related HLA-haploidentical/mismatched donor as he/she was in need of immediate transplantation but had no HLA-matched donor, or umbilical cord blood (UCB); (3) no active infection, no obvious organ dysfunction before transplantation. A prior written and informed consent was obtained from each patient. The study protocol was approved by the ethics committee of the First Affiliated Hospital of Xinjiang Medical University.

Conditioning regimen, PBSCs mobilized and collections

Two myeloablative conditioning regimens were used. 143 patients in RH-PBSCT received the modified condition regimen combine r-ATG, which consisted of Ara-c 2-4 g m^{-2} day^{-1} (days -9, -8); busulfan (Bu) 4 mg kg^{-1} day^{-1} orally or 3.2 mg kg^{-1} day^{-1} (days -7 to -5); cyclophosphamide (Cy) 1.8 mg m^{-2} day^{-1} (days -3, -2); r-ATG (thymoglobuline; Sangstat, Lyon, France) 2.5 mg kg^{-1} day^{-1} (days -4 to -1). Nine patients with only 1 HLA mismatch loci were given r-ATG 2.5 mg kg^{-1} day^{-1} (days -4 to -3). Before 2004, four patients received total-body irradiation (TBI) + Cy preparative regimen, which consisted of TBI 8.5 Gy day^{-1} (day -1), Cy 1.8 mg kg^{-1} day^{-1} (days -5, -4).

The only source of stem cells for all patients were G-CSF mobilized PBSCs. PBSC was mobilized for 4 days by an intraperitoneal injection of 7-10 μg/kg G-CSF. The PBSC product was prepared by applying a blood cell separator (COBE Spectra Blood) on day 0 and was infused into to the recipient patient at the time of transplantation (within 1 hour). If the donor weight was lower than that of the recipient in excess of 10 kg, the expected number of PBSC was reached by increasing the circulation amount or by collecting for four consecutive days.

GVHD prophylaxis

All patients with HLA-mismatched PBSCT were given an intensive and delayed GVHD prophylaxis regimen as follows: the classic regimen (CSA/Tac + MTX), patients with HLA 2 or 3 loci mismatched combined with mycophenolate mofetil (MMF 1000 mg twice a day, orally on day -2 to +100; Novartis, Switzerland), anti-CD25 monoclonal antibody (Basiliximab, 20 mg a day intravenously on day 01 and +2), and a short course of glucocorticoid (dexamethasone 5 mg per day +1 to +30). Within 30 days post-transplant, CsA or Tac was administered intravenously, with 2.5 mg/kg i.v. CsA (drug concentration of 300-400 ng/ml) for 4 hours twice daily or for 24 hours once daily and 0.02 mg/kg i.v. Tac (drug concentration of 10-15 ng/ml) for 24 hours once daily. Oral CsA or Tac was administered from day 30 to 180 post-transplant, with 4-5 mg/kg oral CsA (drug concentration of 150-250 ng/ml) daily, and 0.1 mg/kg oral Tac (drug concentration of 5-10 ng/ml) daily. Patients with HLA 1 locus mismatched combined half a dose of MMF (500 mg once daily, +1 to +30) and a short course of glucocorticoid.

Infection prophylaxis and supportive care

All patients received prophylactic antibiotics to prevent bacterial and fungal infections if their neutrophile (ANC) count was less than 0.5 × 10^9/L. Third-generation cephalosporin/carbapenem was given to all patients until hematopoietic reconstitution. Fluconazole/Micafungin was given to all patients from day 9 to day 30. Sulfamethoxazole was administered for prophylaxis against *Pneumocystis jiroveci* infection.

Table 1. Patient characteristics

	Patients (n = 147)
Patients median age, years (range)	27 (3-50)
< 40, no. (%)	122 (83.0)
≥ 40, no. (%)	25 (17.0)
Male/Female, no. (%)	91/56 (61.9/38.1)
Underlying disease, no. (%)	
AML	65 (44.3)
ALL	54 (36.7)
CML	23 (15.6)
MDS	5 (3.4)
Disease status, no. (%)	
High risk	80 (54.4)
Standard risk	67 (45.6)
Disease status at the time of transplantation, no. (%)	
CR1/CR2	113 (76.9)
CML (CP)	19 (12.9)
Advanced	15 (10.2)
Donor median age, years (range)	35.6 (16-56)
< 40, no. (%)	87 (59.2)
≥ 40, no. (%)	60 (40.8)
HLA mismatched, no. (%)	
3 loci	93 (63.3)
2 loci	39 (26.5)
1 locus	15 (10.2)
Donor-patient sex match, no. (%)	
Male to Male	55 (37.5)
Female to Female	28 (19.0)
Male to Female	36 (24.5)
Female to Male	28 (19.0)
Donor-patient relationship, no. (%)	
Sibling	77 (52.4)
Mother to child	29 (19.7)
Father to child	29 (19.7)
Child to parent	9 (6.2)
Cousin	3 (2.0)
Donor-patient ABO match, no. (%)	
Matched	94 (63.9)
Minor mismatched	30 (20.4)
Major mismatched	23 (15.7)
Conditioning regimen, no. (%)	
Ara-c 2.0 + Bu/Cy + r-ATG	33 (22.5)
Ara-c 4.0 + Bu/Cy + r-ATG	110 (74.8)
TBI + Cy + r-ATG	4 (2.7)

Note: AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndromes; CR, complete remission; CP, chronic stage; Ara-c, arabinoside; Bu, busulfan; Cy, cyclophosphamide; r-ATG, rabbit antihuman thymocyte immunoglobulin. TBI, total body irradiation.

Acyclovir/ganciclovir was given from day 5 until day 180 to prevent viral infections. All blood products were irradiated (2500 cGy) before infusion. G-CSF (300 ug d^{-1}) was given from day +5 to ANC ≥ 0.5 × 10^9/L, The patients of HLA-mismatched used human immunoglobulin (0.4 g kg^{-1} days +1, +8, +24, +31) after transplantation.

Definitions

The first of three consecutive days with a neutrophil level ≥ 0.5 × 10^9/L and a platelet level ≥ 20 × 10^9/L with evidence of donor hematopoiesis was defined as engraftment. Patients who did not achieve ANC ≥ 0.5 × 10^9/L after transplantation were considered to have primary graft failure. Patients with initial engraftment in whom a severely hypocellular marrow and ANC < 0.5 × 10^9/L recurred for more than three days were considered to have secondary or late graft failure. Acute GVHD refers to the clinical complications that occurred within 100 days post-transplant, with skin, liver and gastrointestinal tract involvement, manifesting such as rash, liver dysfunction and diarrhea. It affects one or multiple organs simultaneously or successively. The severity of acute GVHD was determined by the degrees (or stages) of involvement of each main target organ, as per the modified Seattle Glucksberg criteria [13]. Acute GVHD and chronic GVHD were diagnosed and graded based on a previously published standard.

Follow-up and outcome measures

After transplantation, HLA-matching and chimerisms were tested monthly for 12 months to

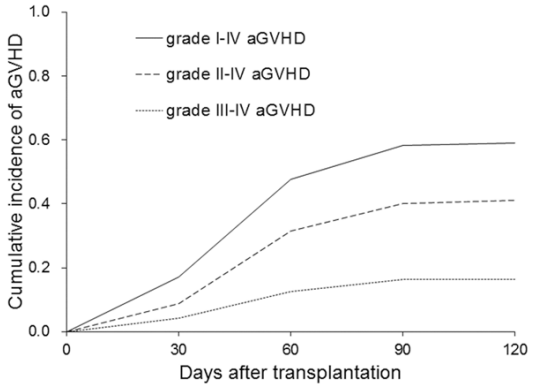

Figure 1. Cumulative incidence of grade I-IV aGVHD, grade II-IV aGVHD, and grade III-IV aGVHD. aGVHD: acute graft-versus-host disease.

monitor engraftment. ABO blood type or sex chromosomes were monitored for patients who had a donor with a different blood type or a different gender. Patients were followed if there were genetics or molecular biology abnormalities. The above indexes were followed every three months from 12 to 24 months post-transplant.

The primary outcome measures were the engraftment rate and the cumulative incidence of acute GVHD. The secondary outcome measures included the relapse incidence, the 3-year cumulative overall survival (OS), and the cumulative non-relapse mortality (NRM). From day 1 to day 100 post-transplant, the cumulative incidence of acute GVHD, target organs, and onset time of acute GVHD were determined. The OS and relapse incidence were followed from day 0 to the date of death or the end of the follow-up period.

Statistical analysis

SPSS19.0 software was used for the statistical analysis. Survival was analyzed using Kaplan-Meier and the differences in survival were evaluated by a Log-rank test. A chi-square test or a fisher exact test was used to analyze the effect of the ages of donor and recipient, primary disease diagnosis, number of HLA mismatched loci, relationships of the donor and recipient, and infusion doses of MNC and CD34. The risk factors of acute GVHD were assessed by univariate analysis, and $P < 0.1$ was assumed to be statistically significant; and then COX regression was used for multivariate analysis, and $P < 0.05$ was assumed to be statistically significant.

Results

Engraftment

The engraftment rate was 97.9% in the 144 patients, with a median neutrophil recovery time of 13 days (8-25 days) and a median platelet recovery time of 17 days (10-31 days). The mean infusion doses of MNC and CD34 were 15.17 (9.25-24.6) × 10^8/kg and 8.16 (3.24-15.52) × 10^6/kg respectively. Three patients of HLA-mismatched PBSCT suffered primary graft failure, and one patient survived for 32 months until the preparation of this report and the other two died on days 50 and 72 post transplantation.

Incidence of acute GVHD

The incidence of acute GVHD (grade I-IV) was 58.5 ± 4.53% (86/147), of which 35 were grade I. The cumulative incidence of grade II-IV and grade III-IV acute GVHD were 34.7 ± 4.69% (51/147) and 12.9 ± 3.80% (19/147) respectively (**Figure 1**). As shown in **Table 2**, the median time for acute GVHD onset was 44.7 (range 6-95) days, with the majority being within 60 days. As for organ involvement, one-organ involvement GVHD was revealed in 57 patients (of them, 47 were affected in the skin), two-organ involvement (most commonly, the skin and the liver) was revealed in 24 patients, and three-organ involvement was revealed in 5 patients (all were severe acute GVHD). About 53.5% (46/86) of patients who had grade I-IV acute GVHD developed chronic GVHD and 52.9% (27/51) of patients with grade II-IV acute GVHD developed chronic GVHD. The infection incidence was 61.6% (53/86) in patients with acute GVHD.

No difference was found in the cumulative incidence of grade II-IV acute GVHD among the AML, ALL, CML, and MDS groups (31.8% vs 28.6% vs 21.7% vs 25.0%, $P = 0.431$) (**Figure 2A**). However, the cumulative incidence of acute GVHD (grade III-IV) was significantly higher in patients with ALL (7.6% vs 24.1% vs 4.3% vs 0, $P = 0.048$, **Figure 2B**). No significant differences were observed in the occurrence rate of grade II-IV and grade III-IV acute GVHD between patients with different HLA mismatched loci (**Figure 2C** and **2D**). Similarly, the occurrence rate of grade II-IV and grade III-IV acute GVHD showed no significant difference among

Table 2. Characteristics of acute GVHD

	aGVHD grade		
	I~IV N (%)	II~IV N (%)	III~IV N (%)
Days from transplant to acute GVHD	44.7 d (6-95 d)		
Days 1-30	26 (30.2)	13 (25.5)	7 (36.8)
Days 31-60	44 (51.2)	28 (54.9)	9 (47.4)
Days 61-100	16 (18.6)	10 (19.6)	3 (15.8)
Involvement organs			
Only skin	47 (54.7)	21 (41.2)	4 (21.1)
Only gut	5 (5.8)	4 (7.8)	3 (15.7)
Only liver	5 (5.8)	4 (7.8)	1 (5.3)
Skin and Liver	12 (14.0)	11 (21.7)	4 (21.1)
Skin and Gut	7 (8.1)	7 (13.7)	3 (15.7)
Liver and Gut	5 (5.8)	-	-
Skin and Liver and Gut	5 (5.8)	4 (7.8)	4 (21.1)
Chronic GVHD			
Yes	46 (53.5)	27 (52.9)	11 (57.9)
No	40 (46.5)	24 (47.1)	8 (42.1)
Infection			
Yes	53 (61.6)	37 (72.5)	16 (84.2)
No	33 (38.4)	14 (27.5)	3 (15.8)

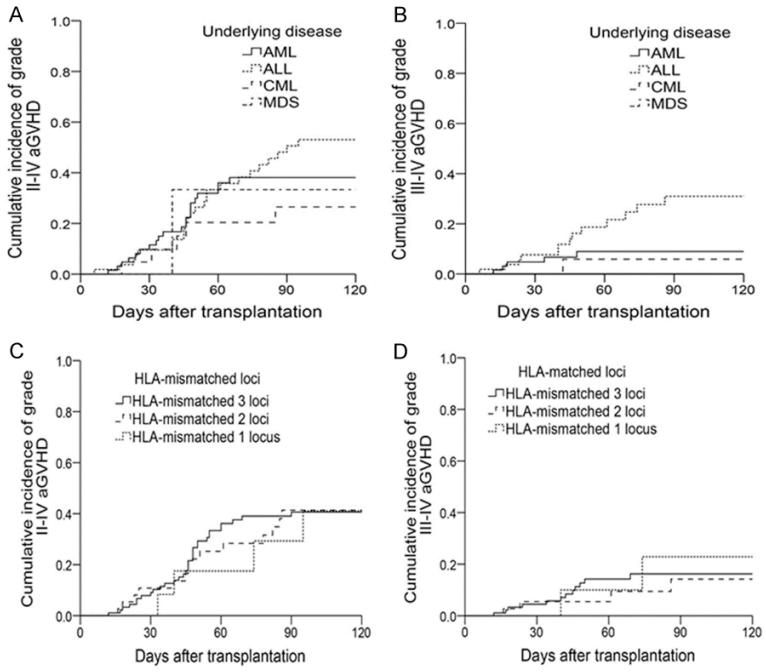

Figure 2. Comparison of the cumulative incidence of aGVHD. Comparison of the cumulative incidence of II-IV aGVHD (A) and III-IV aGVHD (B) in underlying disease. Comparison of the cumulative incidence of II-IV aGVHD (C) and III-IV aGVHD (D) in HLA mismatched patients. aGVHD: acute graft-versus-host disease.

patients with different donor-patient relationships, ABO-match statuses, MNC infusion doses and CD34 infusion doses (**Figure 3A-D**). These results suggest that the occurrence rate of grade II-IV acute GVHD was not high in our study, and particularly, the occurrence rate of grade III-IV acute GVHD was low.

OS, relapse, and NRM

The 3-year OS rate was 69.4 ± 4.0%. The 3-year relapse rate was 25.6 ± 4.1%, and the NRM incidence was 16.0 ± 3.2%. The 3 year OS was significantly lower, but the NRM was significantly higher in patients with grade II-IV acute GVHD compared with patients without acute GVHD and grade I acute GVHD (53.3 ± 7.9% vs 72.0 ± 5.0 %, χ^2 = 5.092, P = 0.024; 26.1 ± 6.8 % vs 10.9 ± 3.3%, χ^2 = 4.506, P = 0.034). However, no significant differences were observed in the relapse rate between patients with grade II-IV acute GVHD and the other patients (35.7 ± 8.6% vs 25.0 ± 5.0%, χ^2 = 1.259, P = 0.262).

The grade of acute GVHD showed an impact on 3-year OS with a statistical difference (grade I: 80.9 ± 5.2%; non acute GVHD, 66.9 ± 8.3%; grade II: 63.6 ± 8.9%; grade III-IV: 46.3 ± 13.8%; χ^2 = 9.587, P = 0.022) (**Figure 4A**). The grade of acute GVHD showed no significant impact on relapse incidence (grade I acute GVHD: 22.5 ± 6.1%; non acute GVHD: 28.4 ± 8.1%, grade II-IV acute GVHD: 25.5 ± 8.6%; and grade III-IV acute GVHD: 46.4 ± 17.9%; χ^2 = 3.046, P = 0.385) (**Figure 4B**). These results indicate that the clinical outcomes of patients with grade I acute GVHD are the best, better than the outcomes of grade III-

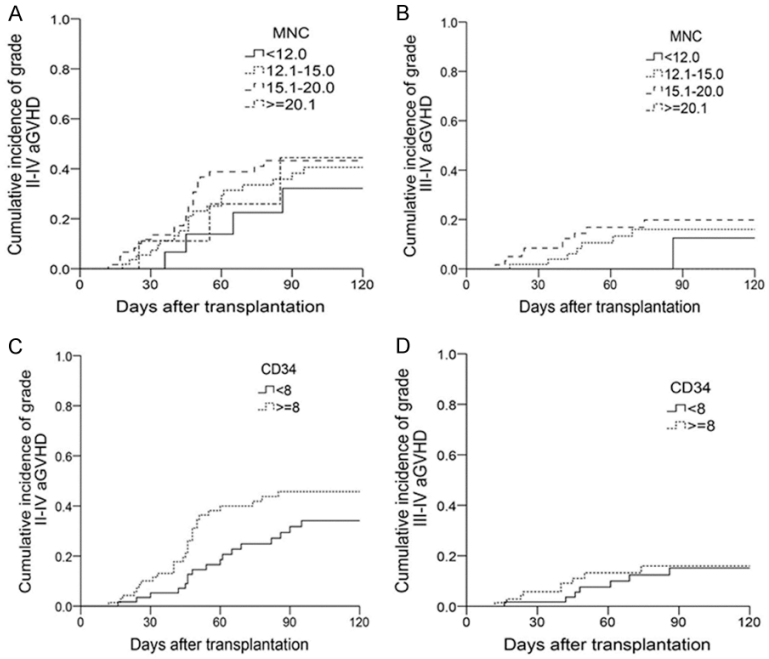

Figure 3. Comparison of the cumulative incidence of aGVHD. Comparison of the cumulative incidence of II-IV aGVHD (A) and III-IV aGVHD (B) in an MNC infusion dose. Comparison of the cumulative incidence of II-IV a GVHD (C) and III-IV aGVHD (D) in a CD34 infusion dose. aGVHD: acute graft-versus-host disease.

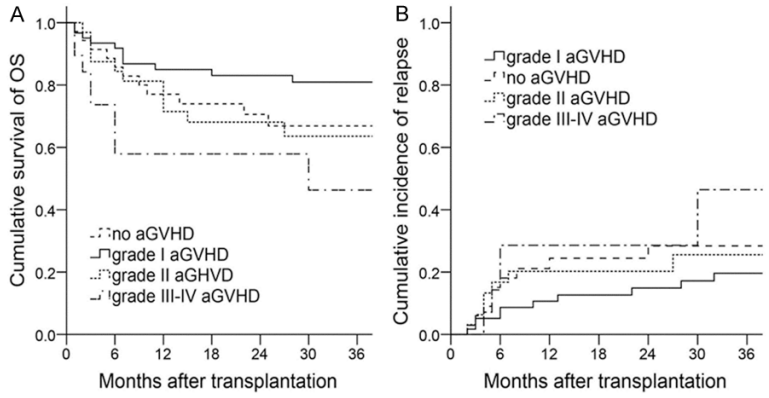

Figure 4. Comparison of OS and relapse. Comparison of cumulative survival of 3-year OS in patients with aGVHD (A). Comparison of the cumulative incidence of 3-year relapse in patients with aGVHD (B). aGVHD: acute graft-versus-host disease.

IV acute GVHD, and even better than patients without acute GVHD, but patients with grade III-IV have the worst clinical outcomes.

Risk factors of acute GVHD

A univariate analysis showed that age, gender, the relationship between the donor and recipient, disease status, and HLA mismatched loci were not risk factors. Having a child donor was statistically close to being a risk factor (HR = 2.48, 95% CI 0.88-1.96, P = 0.08), but multivariate analysis showed no significant difference (P = 0.116). Neither MNC infusion dose, CD34 infusion dose, neutrophil recovery time, nor infection was a risk factor for grade II-IV acute GVHD (**Table 3**). These results suggest that children donors are close to being a risk factor for acute GVHD.

Discussion

Acute GVHD is a major complication of allo-HSCT. Factors that influence the development of acute GVHD include unrelated donors, peripheral blood stem cells, and transplantations from HLA-haploidentical/mismatched donors [14-16]. To face these challenges, we developed a strategy of RH-PBSCT, which features a high dose of PBSCs without ex vivo TCD and an enhanced in vivo TCD with r-ATG to reduce GVHD. As shown here, this approach demonstrates feasibility by a high engraftment rate (97.9%), a moderate grade II-IV acute GVHD cumulative incidence (34.7%), and a low mortality rate after transplantation. As in other studies [17], this study also finds that the skin and liver are the main organs involved in acute GVHD.

We assume the low incidence of severe acute GVHD may be due to the following: First, a high-dose of CD34 positive cells can not only produce an “anti-tumor” effect, but it also induces apoptosis, promotes engraftment, and reduces GVHD through the alloreactive T lymphocytes [18, 19]. Our approach of r-ATG in the conditioning regimen continuously removes T lymphocytes in vivo to prevent GVHD without increas-

Table 3. Multivariate analysis of acute GVHD

	Grade II~IV aGVHD		
	HR	95% CI	P
Patients age			
< 40	1.00	-	
≥ 40	2.27	0.65-1.87	0.19
Patients sex			
Male	1.00	-	
Female	1.52	0.72-3.23	0.26
Donor age			
< 40	1.00	-	
≥ 40	0.89	0.35-2.26	0.80
Underlying disease			
AML	1.00	-	
ALL	1.07	0.13-2.67	0.94
CML	1.28	0.15-1.40	0.81
MDS	0.52	0.05-1.72	0.56
Disease status			
Standard risk	1.00	-	
High risk	1.74	0.77-3.90	0.87
HLA mismatched			
3 loci	1.00	-	
2 loci	0.84	0.32-2.20	0.72
1 locus	1.25	0.51-3.04	0.62
Donor-patient sex match			
Male to male	1.00	-	
Male to female	1.07	0.36-1.72	0.55
Female to female	1.29	0.48-2.22	0.97
Female to male	0.52	0.62-2.26	0.61
Donor-patient relationship			
Sibling	1.00	-	-
Mother to child	2.32	1.15-1.65	0.32
Father to child	1.29	0.54-3.06	0.55
Child to parent	2.48	0.88-1.96	0.08
Cousin	2.52	0.61-3.45	0.23
ABO match			
Matched	1.00	-	-
Minor matched	0.58	0.28-1.19	0.13
Major matched	1.01	0.38-2.67	0.99
MNC, 10^8/kg			
< 12	1.00	-	-
12.1-15	1.10	0.11-2.02	0.94
15.1-20	0.28	0.05-1.53	0.11
≥ 20.1	1.26	0.51-3.14	0.62
CD34, 10^6/kg			
< 8	1.00	-	-
≥ 8	1.44	0.72-2.87	0.29
NE 0.5 × 10^9/L time*			
< day 13	1.00	-	-
≥ day 13	1.30	0.76-2.48	0.28
PLT 20 × 10^9/L time*			
< day 17	1.00	-	-
≥ day 17	0.96	0.54-1.70	0.89
Infection			
No	1.00	-	-
Yes	1.32	0.47-3.64	0.60

Note: AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndromes; MNC, mononuclear cell. NE, neutrophil. PLT, platelet. *The median time of NE 0.5 × 10^9/L was 13 days. The median time of PLT 20 × 10^9/L was 17 days.

ing the relapse risk [20]. Second, anti-CD25 monoclonal antibodies and the combination of multi-drugs of MMF and Glu adding to the conventional prophylaxis intensified the GVHD prophylaxis. Anti-CD25 monoclonal antibodies can inhibit alloreactivity and have a high affinity to lymphocytes, and they inhibit interleukin-2 mediated cytotoxic T lymphocyte's activation and proliferation before lymphocyte recovery [21]. Thirdly, G-CSF mobilizing the donor stem cells before infusion into the recipient can facilitate Th0 to Th2 conversion, down regulate IL-2 and IFN-γ secretion, thereby ameliorating the severity of GVHD [22]. In addition, we believe that the early diagnosis of acute GVHD and active interventions also contribute to a decrease in severe GVHD incidence.

Aversa et al. is one of the early research groups that applied the ATG myeloablative conditioning regimen in *ex vivo* TCD haploidentical peripheral blood HSCT. The engraftment rate increased, and the incidence of GVHD decreased significantly in their approach, but the relapse incidence and infectious complications were also significantly increased [23]. A Peking University group reports that the cumulative incidences of grade II-IV and grade III-IV acute GVHD were about 40% and 20%, and the OS was 70% [24, 25]. Bartolomeo et al. reported that using the intensified myeloablative regimen of G-CSF mobilized related HLA halpoidentical bone marrow transplant without *ex vivo* TCD, which showed good treatment efficacy in 80 patients [26]. However, reports on myeloablative RH-PBSCT without *ex vivo* TCD with a large number of patients are limited. The use of ATG and intensified GVHD prophylaxis often results in an increased relapse rate, an increased infection rate, and NRM [27]. In this study, patients with

grade II-IV acute GVHD had significantly lower 3-year OS but a significantly higher NRM. However, there was no difference in relapse incidence between patients with grad II-IV acute GVHD and other patients. The grade of acute GVHD showed an impact on 3-year OS but no impact on relapse incidence.

Age, donor-recipient gender, CMV serostatus, ABO compatibility, disease, disease status, transplant source, donor type, HLA matching between donor and recipient, conditioning regimen intensity and GVHD prophylaxis have been shown to be risk factors for developing aGVHD [28, 29]. Previous studies have suggested that the ages of the donor and recipient, female donors, HLA locus incompatibility, and stem cell sources may increase acute GVHD incidence [30-33]. However, our univariate analysis showed that the above factors were not risk factors of grade II-IV acute GVHD; but a pediatric donor was close to being statistically significant (HR = 2.48, 95% CI 0.88-1.96, P = 0.08), but not significant in the multivariate analysis (P = 0.116). Studies have found that the incidence of GVHD in CML patients is higher than it is in other diseases, and they found that the mechanism may be related to increased serum tumor necrosis factor [34, 35]. Our results showed that primary disease was not a risk factor for acute GVHD, while higher grade III-IV acute GVHD incidence was revealed in ALL patients. This is possibly a result of low concentrations of CsA/Tac administered in ALL patients aiming to avoid a relapse. A high dose of CD34 cells (8.0×10^6/kg) is suggested to be a risk factor for II-IV acute GVHD [33, 36, 37]. Our univariate analysis showed that neither MNC nor CD34 was a risk factor.

In conclusion, our approach of RH-PBSCT without ex vivo TCD is safe and effective to treat patients with severe hematological malignancies. The severe acute GVHD incidence (manifested mainly in the skin), the disease-free survival rate, and the OS rate are acceptable and comparable to other approaches. No independent risk factor is identified for acute GVHD in our approach.

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Disclosure of conflict of interest

None.

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