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Role of antithymocyte globulin in matched sibling donor peripheral blood stem cell transplantation for hematologic malignancies

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Abstract

Background: High incidence of chronic graft-versus-host disease (GVHD) has been a major drawback of matched sibling donor peripheral blood stem cell transplantation (MSD -PBSCT). This study aimed to investigate the safety and efficacy of antithymocyte globulin (ATG) as a standardized part of GVHD prophylaxis in patients receiving MSD -PBSCT.

Methods: A total of 72 patients with hematological malignancies receiving MSD -PBSCT who displayed similar baseline characteristics were either given rabbit ATG (n=42) or no ATG (n=30), in addition to cyclosporine, methotrexate, and mycophenolate mofetil as a standard GVHD prophylaxis regimen. Either patients or donors aged ≥40 years were included in the study. Thymoglobulin was administered at a daily dose of 1.5 mg/kg on day −5 and 3.5 mg/kg on day −4 prior to transplant (the total dose was 5 mg/kg)

Results: After a median follow-up of 874 days, the 3-year cumulative incidence of chronic GVHD (cGVHD) was 37.3% in the ATG group and 52.1% in the non -ATG group. The 3-year overall and disease-free survival probability were 71.0% and 62.0% (ATG versus non -ATG, $P=.262$) and 66.7% and 58.4% (ATG versus non -ATG, $P=.334$). No difference was found in the 2-year cumulative incidence of nonrelapse mortality and relapse between the ATG and non -ATG groups. This significant reduction in the incidence of cGVHD without increased relapse risk and nonrelapse mortality led to a 3-year GVHD-free, relapse-free survival probability of 66.7% and 40.0% in the ATG and non-ATG groups, respectively.

Conclusions: These data suggested that rabbit antithymocyte globulin in the current protocol for GVHD prophylaxis was well tolerable and efficacious.

The clinical trial was registered on January 1, 2016 (ClinicalTrials.gov Identifier NCT02677181). https://clinicaltrials.gov/ct2/show/NCT02677181.

Abbreviations: GVHD = graft-versus-host disease, MSD-PBSCT = matched sibling donor peripheral blood stem cell transplantation, rATG = rabbit antithymocyte globulin.

Keywords: ATG, graft-versus-host disease, peripheral blood, relapse, stem cell transplantation

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LD and LW authors contributed equally to this study.

This clinical trial was approved by the Chinese People's Liberation Army General Hospital Ethics Committee. All procedures involving human participants were conducted according to the declaration of Helsinki. Written informed consent was signed by all individual participants.

All data published here are under the consent for publication.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

The use of *in vivo* T-cell depletion with rabbit antithymocyte globulin (rATG) is a promising strategy for graft-versus-host disease (GVHD) prophylaxis.[1] However, side effects such as viral reactivation and disease relapse may increase non-relapse mortality (NRM).[2,3] The administration of an appropriate regimen of rATG is critical for effective GVHD prevention. Lower rATG exposure might lead to a higher risk of GVHD while higher rATG exposure and excessive T-cell depletion can result in delayed immune reconstitution with increased risk of infections, disease relapse, and higher non-relapse mortality.[4,5] As the half-life of antithymocyte globulin (ATG) is 5–14 days, the timing of ATG administration such as day –9 or day –5, also affects the outcome.[6,7] High level of rATG before stem cell infusion contributed to low incidence of cGVHD and graft failure.[4,5] Level of rATG after graft infusion may have impact on cGVHD, infection and early immune recovery.[4,5]

The optimal dosage of ATG and its timing of treatment have not been defined, especially in matched sibling donor peripheral blood stem cell transplantation (MSD -PBSCT).[8] Moreover, distinct brands of ATG products differ in effects on the outcomes because of their manufacturing process, including the animal source and the cell line used for immunization.[9] Anti-human thymocyte globulin was produced in rabbits (rATG, Sanofi, Paris, France) against human thymocytes, and anti-T-lymphocyte globulin (ATLG, rabbit, Fresenius, Germany) was produced by immunizing rabbits with a Jurkat cell line.[10] Kroger et al. found that a low dose of ATLG, given at 10 mg/kg on days 3, 2, and 1 before myeloablative allogeneic PBSCT from a human leukocyte antigen (HLA)-identical donor for patients with acute leukemia in remission, decreased the risk of chronic GVHD (cGVHD), but did not improve the overall survival (OS) and relapse-free survival.[11] We have reported that rATG of 10 mg/kg might reduce cGVHD in patients receiving haploidentical PBSCT without in vitro T-cell depletion.[12] The incidence of extensive cGVHD was 17.1%, in patients receiving haploidentical PBSCT in our center.[12] The ATG regimen was given at a total dose of 10 mg/kg (day −5 to −2) before graft infusion. The non-relapse mortality and relapse were acceptable in the haploidentical PBSCT setting. The incidence of acute GVHD (aGVHD) grades II–IV was high in patients receiving stem cells from donors aged ≥40 years.[12] With thymoglobulin doses <6 mg/kg, it was reported that incidence of relapse did not increase.[9] Therefore, we proposed that 5 mg/kg of rATG may be efficacious and tolerable in the MSD setting.

In the prospective study (ClinicalTrials.gov Identifier: NCT02677181), rATG was used for GVHD prophylaxis. The present study aimed to evaluate the impact of 5 mg/kg rATG (the total dose 5 mg/kg and the timing as days −5 and −4 before graft infusion), in patients or donors aged ≥40 years receiving matched sibling donor peripheral blood stem cell transplantation (MSD-PBSCT) on cGVHD, compared with no rATG in patients or donors aged ≥40 years, and also evaluated the tolerability and efficacy of this prophylactic strategy.

2. Methods

This study was approved by the Ethics Committee of the Chinese People's Liberation Army General Hospital and conducted in accordance with the principles of the Declaration of Helsinki. All patients signed informed consent.

2.1. Study design

Consecutive patients with non-Hodgkin lymphoma (NHL) /acute leukemia (AML/ALL)/myelodysplastic syndrome (MDS) who received allogeneic MSD-PBSCT at the study center from August 1, 2015, to December 30, 2018, were eligible.

The sample size was 128, which was estimated with PASS software (NCSS, Kaysville, UT). The sample size was calculated on the basis of the assumption that the 3-year event rate (cGVHD) in the no ATG arm was 50% according to our previous reports.[12] This study proposed to reduce the 3 year incidence of cGVHD from 50% to 23%. Under the hypothesis, the study would have 90% power at a two-sided significance level of 0.05. This prospective, randomized, phase III study was registered as a clinical trial (ClinicalTrials.gov identifier: NCT02677181). In consideration of sample lost to follow-up, death during early treatment, and other unexpected factors, an additional 10% sample size was required; thus, the final sample size was approximately 70 patients in each group. Interim analysis would be performed after the completion of treatment of the first 60 patients in both group. If the *P* value for the difference of the incidence of cGVHD between the two groups by fisher exact test was less than 0.0011, study termination for no ATG arm could be considered. The purpose of early termination for no ATG arm, based on significant reduction of cGVHD in ATG group for cGVHD prophylaxis, was to benefit more patients. If there was a good safety profile and the P value for the difference of incidence of cGVHD between the two groups was more than 0.0011, the study would be continued and the final *P* value cutoff should be .0246. The alpha splitting is based on the Lan-DeMets O'brien-Fleming approximation spending function. If this happens, the study can drop the non -ATG arm and enroll all the rest of patients to the ATG arm. Patients were randomly assigned 1:1 between non -ATG versus ATG group. Baseline parameters were assessed before the start of conditioning regimen, Figure 1.

The inclusion criteria were as follows:

(1) the age of either patients or donors was 40 to 70 years;
(2) patients diagnosed with AML or MDS or NHL; and
(3) indication for MSD-PBSCT.

All patients in this study received myeloablative conditioning regimens. The conditioning regimen consisted of an intravenous administration of busulfan (3.2 mg/kg per day on days −10 to −8; Otsuka Pharmaceutical Company, China), 1,3-bis(2-chlor-

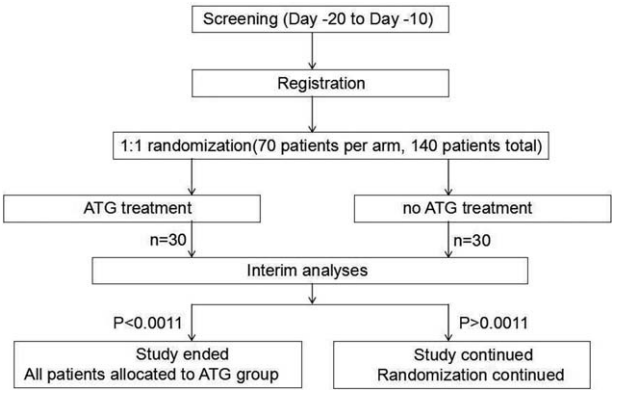

Figure 1. Flow chart illustrating patient selection for analysis. ATG = antithymocyte globulin.

oethyl)-1-nitrosourea (Carmustine; 250 mg/m^2 once on day −5; Jinyao Tianjin Company, China), cytarabine (2 g/m^2 per day on days −7 to −6; Pfizer Company, USA), and cyclophosphamide (60 mg/kg per day on days −4 to −3; Baxter Company) for PBSCT from matched siblings.

2.2. HLA matching and graft collection

Donors and patients were typed by sequence-based typing methods at HLA-A, -B, C, DP, DRB1, and DQB1.[13] For all the patients, the source of donor cells was granulocyte colony-stimulating factor (G-CSF)-primed peripheral stem cells alone. All donors were subcutaneously administered 5 μg/(kg · day) G-CSF (Filgrastim, Kirin, Japan) from day1 to day 5 of mobilization. PBSC was collected on day 5 of mobilization and day 6 if the target numbers for mononuclear cells did not reach above 5×10^8/kg or the number of CD34+ cells did not reach above 2×10^6/kg. The unmanipulated PBSCs alone were infused into the recipient on the day of collection.

2.3. rATG and GVHD prophylaxis

Patients in the ATG group were administered with rATG, at a daily dose of 1.5 mg/kg on day −5 and 3.5 mg/kg on day −4 prior to transplant (the total dose was 5 mg/kg).

All transplant recipients without organ dysfunction received cyclosporine A (CsA), mycophenolate mofetil, and short-term methotrexate for GVHD prophylaxis.[14] From day −10 of transplantation, CsA was given at a dose of 2 mg/kg intravenously, targeting concentration levels of 150 to 250 ng/mL during the first 3 month. After 3 months, the dose of CsA was tapered by 25% for patients without relapse every 2 weeks. Once the patients relapsed after transplantation, CsA was tapered immediately. Tacrolimus was administered in patients showing cyclosporine toxicity or intolerance. Mycophenolate mofetil at a dose of 500 mg was administered twice daily from days −10 to +14. Methotrexate at a dose of 15 mg/m^2 was administered on day +1, and methotrexate at a dose of 10 mg/m^2 was administered on days +3, +6, and +11. All patients received viral prophylaxis with ganciclovir (day -10 ~ day -2 before stem cell infusion) and acyclovir (day +1 ~ day +180 after stem cell infusion).[15] Epstein–Barr virus (EBV) and cytomegalovirus (CMV)CMV DNA in plasma were monitored by real-time quantitive polymerase chain reaction twice weekly until 3 months after transplantation. CMV reactivation was defined as either two consecutive viral loads $>1 \times 10^3$ copies/mL or a single viral loads $>1 \times 10^4$ copies/mL. Pre-emptive therapy for CMV reactivation with ganciclovir lasted for at least 2 weeks until CMV DNA load decreased below 1000 copies/mL. EBV reactivation was defined as viral loads $>1 \times 10^3$ copies/mL. Patients who are only diagnosed as EBV DNAemia were not treated with rituximab. The treatment for EBV posttransplant lymphoproliferative disorders included administration of rituximab, chemotherapy, and EBV -specific cytotoxic lymphocyte infusion.

2.4. Immune monitoring

Recovery of immune cells after transplantation was monitered in a subset of patients on days +30, +60, +90, +180, +240 and +360. Multi-parameter flow cytometry was performed.[13] Cell subsets characterized by CD3$^+$, CD4$^+$, CD8$^+$, and CD56/CD16$^+$ were monitored at each time point (Wilcoxon rank sum test). Recovery of immune cells were analyzed with time dependent multivariable Cox proportional hazards regression models.

2.5. Definitions and endpoints

Outcome data reported in this article were updated on December 1, 2019. The time after transplantation was recorded as "+" days, and the time prior to transplantation was recorded as "–" days. The primary endpoint was the incidence of cGVHD. The secondary endpoints were NRM, and relapse, GVHD-free, relapse-free survival (GRFS), incidence of aGVHD, engraftment, disease-free survival (DFS), and OS.Neutrophil engraftment was defined as absolute neutrophil count more than 0.5×10^9/L for 3 consecutive days,[16] and platelet engraftment was defined as platelet count more than 20×109/L for 7 consecutive days without transfusion. Diagnoses of GVHD were defined according to published criteria. According to the National Institutes of Health (NIH) criteria, the grade of cGVHD was defined as "mild," "moderate," or "severe".[15–18] Relapse was defined by morphological evidence of disease in the peripheral blood, marrow, or extramedullary sites. DFS was defined as survival without disease relapse or progression. GRFS was defined as being alive with neither grade III-IV aGVHD nor moderate-to-severe cGVHD nor disease relapse at any time point.

2.6. Statistical analysis

Patient-, disease- and transplant-related characteristics were compared using the Chi-square tests or Fischer exact test for categorical variables, and the Mann–Whitney test for continuous variables. Cumulative incidence was estimated for relapse, NRM, and GVHD (grades II–IV or III–IV aGVHD and cGVHD of any severity or extensive). Competing risks were relapse or death for cGVHD or aGVHD, NRM for relapse, and relapse for NRM. The 95% confidence interval (CI) for the differences were calculated using the log-rank and Wilson score methods. OS, DFS, and GRFS were computed using the Kaplan–Meier method. Univariate and multivariate analyses were performed using the Cox proportional hazards regression analysis. The covariates were diagnosis, donor–recipient sex matching, time from diagnosis to transplantation (<6 months vs ≥6 months), status at the time of transplantation (complete remission vs others), cytogenetic risk, nucleated and CD34+ cells transplanted, blood group and compatibility, disease risk index,[17] and the effect of ATG. All tests were two-sided and the P values < 0.05 were considered as statistically significant. Statistical analysis was conducted using the R statistical software (cmprsk package) and SPSS 20.0.

3. Results

3.1. Patient characteristics

A total of 72 consecutive patients (32 in the non-ATG group and 40 in the ATG group) underwent stem cell transplantation and were included in this analysis (Table 1). No difference was observed in the baseline characteristics between the two groups, including the rate of disease risk according to the disease risk index.[19] The dosage of stem cells was not different between the two groups. The median follow-up was 874 days for the whole study population, 962 days for patients alive at the last follow-up, and 391 days for those who died.

Table 1

Clinical features of stem-cell transplant recipients and donors.

Characteristic	ATG group	No-ATG group	*P* value
No. of patients	42	30	
Patient's age, median, year (range)	47.6 (38–62)	45.6 (36–63)	.184
Donor's age, median, year (range)	47.3 (27–59)	46.3 (35–58)	.352
Sex			
Male	24	18	.808
Female	18	12	
Time between diagnosis and stem-cell transplantation, days			.895
Median (range)	180.9 (83–344)	249.0 (38–989)	
Diagnosis, no. (%)			.319
Acute myeloid leukemia	21	14	
CR	17	12	
NR	4	2	
Acute lymphoid leukemia	12	5	
CR	12	5	
MDS	9	9	
CR	1	1	
Untreated	8	8	
NHL	0	2	
CR	0	2	
High cytogenetic risk, no. (%)			.634
Low	4	5	
Intermediate	21	15	
High	17	10	
Disease risk index, no./total no. (%)			.707
Low	4	3	
Intermediate	26	21	
High	12	6	
Very high	0	0	
Conditioning regimen			
Bu/Cy	38	28	.693
TBI/Cy	2	0	
Bu/Flu	2	2	
Donor's age, median, year (range)	47.3 (27–59)	46.4 (35–58)	.317
Donor–recipient ABO match			.503
Match	25	17	
Major mismatch	7	8	
Minor mismatch	8	5	
Bidirectional mismatch	2	0	
Donor–recipient sex match			.746
Female to male	15	8	
Female to female	10	6	
Male to female	8	7	
Male to male	9	9	
Graft			
MNCs, median, $\times 10^8$/kg (range)	10.2 (6.2–22.5)	10.8 (1.6–8.1)	.454
$CD34^+$, median, $\times 10^6$/kg (range)	3.6 (1.6–8.2)	3.7 (1.9–9.2)	.732

AML = acute myeloid leukemia, ATG = anti-thymocyte globulin, CR = complete remission, MDS = myelodysplastic syndrome, MNC = mononuclear cells, NHL = non-Hodgkin lymphoma, NR = nonremission, SCT = hematopoietic stem-cell transplantation, WBC = white blood cell.

3.2. aGVHD

Among 71 patients who survived (41 in the ATG group vs 30 in the non-ATG group), 4 (6%) developed grade I aGVHD [2 (5%) in the ATG group vs 2 (7%) in the non-ATG group], 17 (24%) developed grade II aGVHD [11 (26%) in the ATG group vs 6 (20%) in the non-ATG group], 2 (3%) developed grade III aGVHD [1 (2%) vs 1 (3%)], and 1 (3%) developed grade IV aGVHD [no (0%) vs 1 (3%)], whereas 47 (65%) did not present any grade of aGVHD [27 (64%) in the ATG group vs 20 (67%) in the non-ATG group] (Table 2). No difference was found between the two groups. Using Gray's test with competing risks, the cumulative incidence of aGVHD grades II–IV was 28.6% (95% CI, 15.8%–42.7%) in the ATG group and 26.7% (95% CI, 12.4%–43.3%) in the non-ATG group (Fig. 2A). The cumulative incidence of grades III–IV aGVHD was 2.4% (95% CI, 0.2%–11.2%) in the ATG group and 6.8% (95% CI, 1.2%–19.8%) in the non-ATG group at day +100 (Fig. 2B). The overall cumulative incidence of grades II–IV and grades III–IV aGVHD was similar between the two groups (Table 3). In the analysis of the variables affecting grades II–IV aGVHD, no risk factors were found to be associated with the occurrence of grades II–IV aGVHD. Four patients with aGVHD grades II–IV died due to disease relapse ($n = 3$, grades II aGVHD) and pneumonia ($n = 1$, grade III aGVHD).

Table 2

Rates of engraftment, infection, aGVHD and cGVHD, and other complications after allogeneic PBSCT from HLA-identical sibling.

Variable	ATG group ($n=42$)	No-ATG group ($n=30$)	P
	Events (range)		
Graft failure, no. (%)	0	0	
Prolonged isolated thrombocytopenia, no. (%)	0	1	
Days to engraftment, median (range)			
Absolute neutrophil count $\geq 0.5 \times 10^9$/L	12 (8–19)	13 (9–32)	.475
Platelet count $\geq 20 \times 10^9$ /L	14 (9–30)	19 (9–38)	.026
Infectious complication, no. (%)			
Cytomegalovirus reactivation, no. (%)	11 (26.2)	6 (20.0)	.587
Epstein–Barr virus reactivation, no. (%)	11 (26.2)	2 (6.7)	.03
Posttransplantation lymphoproliferative disorder, no. (%)	0	0	
Fungal infection, no. (%)	4 (9.5)	13 (43.3)	.001
Overall grades of aGVHD, no. (%)			.762
0	27 (65.9)	20 (66.7)	
I	2 (4.9)	2 (6.7)	
II	11 (26.8)	6 (20.0)	
III	1 (2.4)	1 (3.3)	
IV	0 (0)	1 (3.3)	
II–IV	12 (29.3)	8 (26.7)	.931
III–IV	1 (2.4)	2 (6.7)	.370
Chronic GVHD			
Day of onset			
Median (range)	543 (270–957)	423 (130–1008)	.103
Severity according to revised Seattle criteria, no. (%)			
Limited	4 (9.5)	6 (20.0)	.008
Extensive	4 (9.5)	10 (33.3)	
Severity according to NIH criteria, no. (%)			.330
Mild	3 (7.1)	9 (30.0)	
Moderate	3 (7.1)	3 (10.0)	
Severe	2 (4.8)	4 (13.3)	

ATG = Anti-thymocyte globulin, GVHD = graft-versus-host disease.

Chronic, limited, and extensive grades of GVHD were defined according to the Seattle criteria. Chronic, mild, moderate, and severe grades of GVHD were defined according to the NIH criteria.

3.3. cGVHD

Chronic GVHD was absent in 44 (64.7%) patients [31 (65%) in the ATG group vs 13 (46.4%) in the non-ATG group] (Table 2). The 3-year cumulative incidence of cGVHD was 52.3% (95% CI, 31.0%–69.6%) in the non-ATG group and 37.5% (95% CI, 17.8%–57.2%) in the ATG group (Fig. 2C, $P < .001$). The overall cumulative incidence of extensive cGVHD alone was 19.8% (95% CI, 5.5–40.2) for the ATG group and 38.4% (95% CI, 19.6–57.1) for the non-ATG group after 3 years (Fig. 2D).

According to the NIH criteria, cGVHD severity was mild in 12 patients [17.6%; 3 (7.1%) in the ATG group vs 9 (30.0%) in the non-ATG group], moderate in 6 patients [8.8%; 3 (7.1%) in the ATG group vs 3 (10.0%) in the non-ATG group], and severe in 6 patients [8.8%; 2 (4.8%) in the ATG group vs 4 (13.3%) in the non-ATG group]. In multivariate analysis, the use of ATG was associated with reduced risk of chronic GVHD (hazard ratio, 3.2; 95% CI, 1.3–7.5; $P = .002$) (Table 4). Nineteen patients with cGVHD were alive at the last follow-up.

Of the 41 patients in the ATG group who survived, 3 (7.7%) developed late aGVHD grade II. One patient had *de novo* late aGVHD (skin, day +310), one had persistent (liver) late aGVHD, and one had recurrent late aGVHD (lower gastrointestinal tract, day +103). No patients with late aGVHD died.

Within 3 years after MSD transplantation, 93.5% (38/40) patients stopped immunosuppressive medication in the ATG group, while 72.2% (13/18) discontinued in the non-ATG group.

3.4. Graft failure and engraftment

No graft failure occurred. One patient in the ATG group died of gastrointestinal hemorrhage before engraftment on day +14. The median time to leukocyte engraftment was 12 days (range, 8–19 days) in the ATG group and 13 days (range, 9–32 days) in the non-ATG group (Table 2). The median time to platelet engraftment in the ATG group was 14 days (range, 9–30 days) in the ATG group and 19 days (range, 9–38 days) in the non-ATG group (Table 2). One patient in the non-ATG group did not achieve platelet engraftment and died of disease relapse after 80 days of transplantation.

3.5. Toxicity, NRM, and relapse

Of the 72 patients, 6 (8.3%) died in remission due to transplant-related reason: 4 (9.5%) of 42 patients in the ATG group and 2 (6.7%) in the non -ATG group. The overall cumulative incidence of non-relapse mortality was 14% (95% CI, 9–21; Fig. 3A). Among these, five patients died of respiratory failure (3 in the ATG group and 1 in the non -ATG group), and 1 patient (ATG group) died of gastrointestinal hemorrhage, and no patients died of GVHD. No difference was observed in the 2-year cumulative incidence of NRM between the 2 groups [ATG 13.0% (95% CI, 0.0%–24.5%) vs non -ATG 8.5% (95% CI, 0.0%–18.3%); $P = .640$] (Fig. 3A).

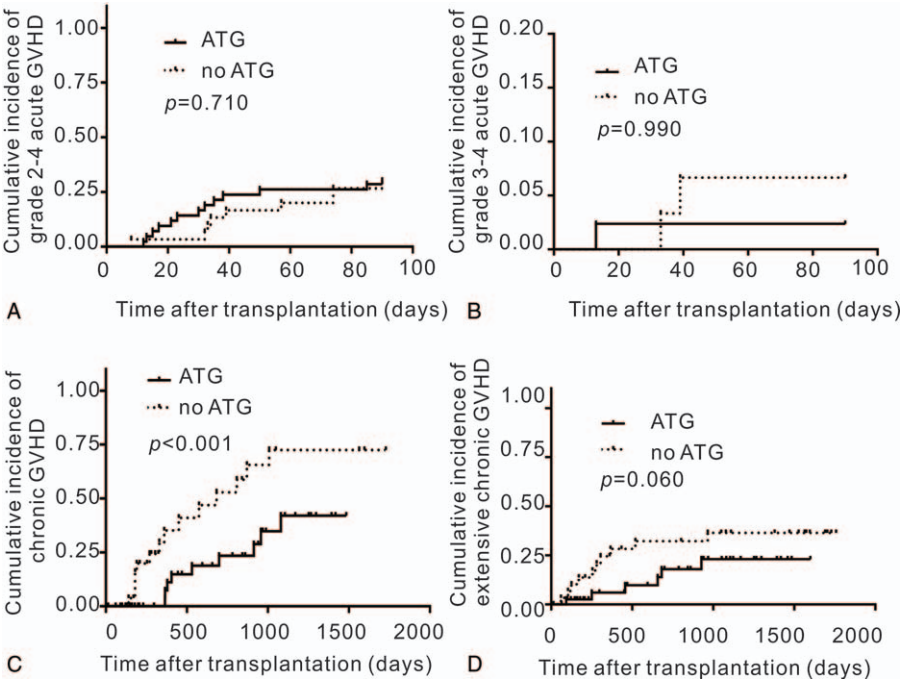

Figure 2. Cumulative incidence of aGVHD and cGVHD after MSD-PBSCT with rATG combined with cyclosporine, mycophenolate, and short-term methotrexate as GVHD prophylaxis compared with the non -ATG group. ATG = antithymocyte globulin, GVHD = graft-versus-host disease.

Table 2 shows the incidence of viral infections (human Epstein–Barr virus, CMV, and disease) and fungal infections in the two groups. No post-transplantation lymphoproliferative disorder was observed (Table 2). Notably, patients receiving the ATG regimen had a higher incidence of viral reactivation compared with the non -ATG group. The difference was significant for the Epstein–Barr virus reactivation [11 (26.2%) of 42 in the ATG group vs 2 (6.7%) of 30 in the non -ATG group; $P = .03$]. No significant difference was founded in the incidence of human CMV reactivation between the two groups [11 (26.2%) of 42 in the ATG group vs 6 (20.0%) of 30 in the non -ATG group; $P = 0.587$]. Clinical Fungal infection [4 (9.5%) of 42 in the ATG group vs 13 (43.3%) of 30 in the non -ATG group; $P < .01$] was significantly higher in the non -ATG group than in the ATG group.

Further, 16 patients undergoing MSD-PBSCT (22.2%) relapsed at 388 (93–966) days post transplantation [6 (14.3%) in the ATG group and 10 (33.3%) in the non -ATG group]. The 3-year cumulative incidence of relapse was not statistically different ($P = .078$) in the two groups [23.1% (95% CI, 4.4%–38.1%) in the ATG group vs 36.5% (95% CI, 15.4%–36.5%) in the non -ATG group], Figure 3B. In multivariate analyses, high-risk disease, according to the disease risk index, was the factor most closely associated with relapse [hazard ratio, 192.0 (95% CI, 3.7–94.9), $P = .009$]. Up to December 1, 2019, 15 relapsed patients died although they received salvage chemotherapy and donor lymphocyte infusion, one relapsed patient (ATG group) was alive in remission. Up to December 1, 2019, no patient had developed a secondary malignancy.

Table 3

Cumulative incidence (95% CI).

	ATG group ($n = 42$)		No-ATG group ($n = 30$)			
Variable	**Events**	**Incidence or probability* (95% CI)**	**Events**	**Incidence or probability* (95% CI)**	**Hazard ratio (95% CI)**	***P***
Grades II–IV aGVHD	12	28.6% (95% CI, 15.8%–42.7%)	8	26.7% (95% CI, 12.4%–43.3%)	0.82 (95% CI, 0.28–2.39)	.710
Grades III–IV aGVHD	1	2.4% (95% CI, 0.2%–11.2%)	2	6.8% (95% CI, 1.2%–19.8%)	0.00 (95% CI, 0.00–29.9)	.990
Chronic GVHD	9	37.5% (95% CI, 17.8%–57.2%)	15	52.3% (95% CI, 31.0%–69.6%)	0.31 (95% CI, 0.13–0.72)	<.001
Extensive chronic GVHD	4	19.8% (95% CI, 5.5–40.2)	10	38.4% (95% CI, 19.6–57.1)	0.27 (95% CI, 0.06–1.08)	.060
Overall survival	9	71.0% (95% CI, 56.3%–89.4%)	12	62.0% (95% CI, 46.5%–82.6%)	0.60 (95% CI, 0.30–1.40)	.262
Disease-free survival	10	66.7% (95% CI, 51.4%–86.7%)	12	58.4% (95% CI, 42.8%–79.7%)	0.70 (95% CI, 0.30–1.50)	.334
Non relapse mortality	4	13.0% (95% CI, 0.0%–24.5%)	2	8.5% (95% CI, 0.0%–18.3%)	1.50(95% CI, 0.30–8.10)	.640
Relapse	6	23.1% (95% CI, 4.4%–38.1%)	10	36.5% (95% CI, 15.4%–36.5%)	0.03 (95% CI, 0.01–1.44)	.078
Extensive cGVHD-free, relapse-free survival	10	66.7% (95% CI, 51.4%–86.7%)	17	40.0% (95% CI, 25.3%–63.4%)	0.05 (95% CI, 0.00–0.26)	<.001

ATG = anti-thymocyte globulin, GVHD = graft-versus-host disease.

Chronic, limited, and extensive grades of GVHD were defined according to the Seattle criteria.

Chronic, mild, moderate, and severe grades of GVHD were defined according to the NIH criteria.

* *P* means *P* value. 95% CI (cumulative incidence).

Table 4

Univariate analysis of transplant outcomes for the risk factors in all patients.

	Grades II-IV aGVHD % (95% CI)	P	Chronic GVHD % (95% CI)	P	Extensive chronic GVHD % (95% CI)	P	NRM % (95% CI)	P	OS HR(95% CI)	P	DFS % (95% CI)	P	GRFS % (95% CI)	P
Disease Risk Index														
Low	1.0		1.0		1.0		1.0		1.0		1.0		1.0	
Intermediate	1.1 (0.3–4.8)	.893	0.5 (0.2–1.9)	.344	1.1 (0.2–9.7)	.998	0.4 (0.0–4.1)	.457	0.8 (0.2–3.5)	.755	0.9 (0.2–3.9)	.857	1.2 (0.3–5.1)	.819
High	0.8 (0.1–4.3)	.784	0.6 (0.1–2.6)	.477	1.2 (0.1–8.7)	.967	0.9 (0.1–10.0)	.837	1.5 (0.3–7.4)	.591	1.6 (0.3–7.9)	.536	2.0 (0.4–9.3)	.393
High cytogenetic risk														
Low	1.0		1.0		1.0		1.0		1.0		1.0		1.0	
Intermediate	0.8 (0.2–3.1)	.790	0.4 (0.1–1.2)	.107	1.2 (0.1–9.5)	.894	0.4 (0.0–4.8)	.492	0.8 (0.2–2.7)	.672	0.8 (0.2–2.9)	.742	0.9 (0.3–3.3)	.914
High	0.9 (0.2–3.5)	.784	0.2 (0.1–0.8)	.022	1.4 (0.2–12.1)	.736	0.9 (0.1–8.2)	.890	0.5 (0.1–2.0)	.341	0.6 (0.2–2.5)	.530	1.1 (0.3–3.9)	.926
Remission status														
Complete remission	1.0		1.0		1.0	1.0			1.0					
Advanced stage of disease	1.6 (0.6–3.8)	.317	1.5 (0.6–3.6)	.335	1.0 (0.3–3.1)	.963	13.1 (1.5–112.5)	.019	2.9 (1.2–6.8)	.016	2.7 (1.2–6.2)	.022	1.7 (0.8–3.8)	.156
Group														
No ATG	1.0		1.0		1.0		1.0		1.0					
ATG	0.8 (95% CI, 0.3–2.4)	.710	0.3 (95% CI, 0.1–0.7)	<.001	0.3 (95% CI, 0.1–1.1)	.060	1.5 (0.3–8.1)	.640	0.6 (0.3–1.4)	.262	0.7 (0.3–1.5)	.334	0.1 (0.0–0.3)	<.001
Time from diagnosis to transp														
< 6 mo	1.0		1.0		1.0		1.0		1.0					
>6 mo	1.4 (0.1–1.2)	.097	1.1 (0.4–2.7)	.831	1.0 (0.3–3.3)	.974	1.1 (0.2–6.0)	.913	0.6 (0.3–1.4)	.489	1.3 (0.5–3.1)	.553	0.9 (0.4–2.0)	.737

*P means P value. (cumulative Incidence 95% CI).

3.6. Survival

On the day of analysis, 51 (70.8%) patients were alive in CR (complete remission). The 3-year OS probability was 63.4% (95% CI, 51.6%–78.1%) for the whole study population: 71.0% (95% CI, 56.3%–89.4%) for the ATG group and 62.0% (95% CI, 46.5%–82.6%) for the non-ATG group ($P=0.262$, Fig. 3C). The 3-year DFS probability was 63.0% (95% CI, 51.5–76.9) for the whole study population: 66.7% (95% CI, 51.4%–86.7%) for the ATG group and 58.4% (95% CI, 42.8%–79.7%) for the non-ATG group ($P=0.334$, Fig. 3D). The 3-year GRFS probability was 54.1% (95% CI, 42.4%–69.1%) for the whole study population: 66.7% (95% CI, 51.4%–86.7%) for the ATG group and 40.0% (95% CI, 25.3%–63.4%) for the non-ATG group ($P<.001$, Fig. 3E). In univariate and multivariate analyses, the non-ATG group was a risk factor for GRFS (Tables 4 and 5).

3.7. Outcomes of the randomized first 60 patients by interim analysis

Using Gray's test with competing risks, the cumulative incidence of aGVHD grades II–IV was 20.0% (95% CI, 7.9%–35.9%) in the ATG group and 26.7% (95% CI, 12.4%–43.3%) in the non -ATG group ($P=.480$). The cumulative incidence of grades III–IV was 0 in the ATG group and 6.8% (95% CI, 1.2%–19.8%) in the non-ATG group at day +100 ($P=0.150$). Chronic GVHD developed in 2 (6.7%) patients in the ATG group and 14 (46.7%) patients in the non -ATG group ($P<.0011$). The cumulative incidence of cGVHD was 44.5% (95% CI, 24.8%–66.0%) in the non-ATG group and 5.7% (95% CI, 0.3%–33.9%) in the ATG group after 2 years ($P<.0011$). The 2-year OS probability was 69.3% (95% CI, 52.1%–92.1%) for the ATG group and 62.0% (95% CI, 46.5%–82.6%) for the non -ATG group ($P=.382$). The 2-year DFS probability was 69.5% (95% CI, 52.4%–92.1%) for the ATG group and 62.3% (95% CI, 46.9%–82.7%) for the non -ATG group ($P=.526$). The 2-year GRFS probability was 54.1% (95% CI, 42.4%–69.1%) for the whole study population: 69.5% (95% CI, 52.4%–92.1%) for the ATG group and 52.0% (95% CI, 36.6%–73.9%) for the non-ATG group ($P=.073$).

3.8. Immune reconstitution

14 patients in the ATG group and 13 patients in the no-ATG group were studied for immune reconstitution at all time points. On day +100, median CD3 +, CD4 +, CD8 +, and CD56/CD16 + counts were 914 (642–1465), 189 (63–488), 686 (483–1355), and 138 (75–250), respectively in the ATG group. On day +100 in the no-ATG group, median CD3 +, CD4 +, CD8 +, and CD56/CD16 + counts were 1600 (768–2137), 210 (201–274), 878 (290–1490), and 315 (111–546), respectively. There were no differences between the ATG and no-ATG group. There was no association between CD3+, CD4+, CD8+ or CD56+ cell counts at all time points and relapse, the occurrence of GVHD and CMV/EBV reactivation.

4. Discussion

The administration of a 5-mg/kg total dose of rATG (days −5 to −4) as GVHD prophylaxis in this study demonstrated the followings:

(1) lower incidence of cGVHD in patients with rATG,
(2) improved GRFS rates in patients with rATG,
(3) no effect of rATG on the incidence or the severity of aGVHD, CMV reactivation, relapse, and NRM and,
(4) higher incidence of EBV reactivation, but no PTLD, in patients with rATG.

High cGVHD rates have been recognized as one of the major drawbacks of MSD-PBSCT.[20,21] rATG has been used for *in vivo* T-cell depletion with the goal of improving transplant outcomes.[22,23] In a meta-analysis of 6 randomized trials with 568 transplantation patients, the incidence of severe aGVHD (grades III–IV) and aGVHD (grades II–IV) were significantly reduced

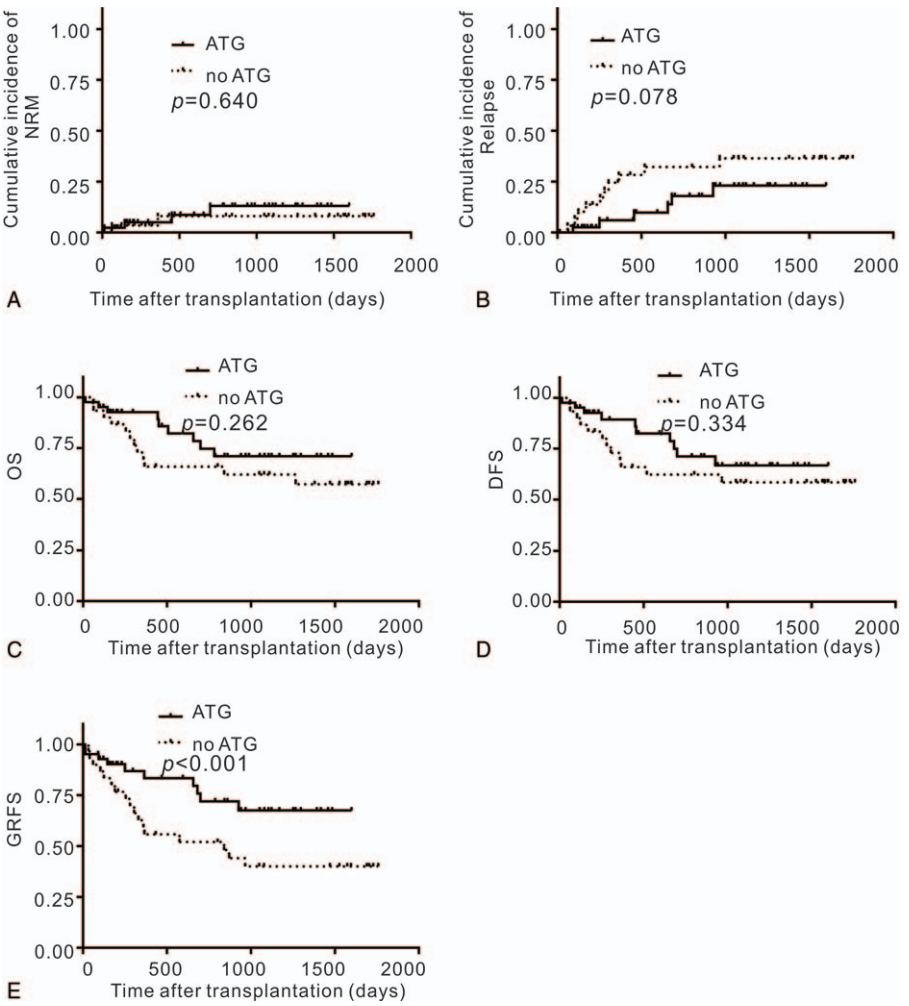

Figure 3. Cumulative incidence of NRM, relapse, OS, DFS, and GRFS after MSD-PBSCT with rATG combined with cyclosporine, mycophenolate, and short-term methotrexate for GVHD prophylaxis compared with the non -ATG group. ATG = antithymocyte globulin, DFS = disease-free survival, GRFS = severe GVHD-free, relapse-free survival, NRM = non-relapse mortality, OS = overall survival.

(from 18% to 10% for severe aGVHD and from 36% to 19% for aGVHD) with the addition of high doses of ATG.[24] However, this benefit did not result in improved survival.[25] One randomized study using 10 mg/kg of rabbit ATLG in sibling myeloablative transplantation showed decreased cGVHD incidence. The incidence of cGVHD and extensive cGVHD were significantly reduced (from 68.7% to 32.2% for cGVHD and from 33.3% to 6.0% for extensive cGVHD) with the addition of ATLG. Despite relatively low cGVHD incidences in these patients, OS and progression-free survival did not improve in both study groups. Therefore, the optimal ATG regimen that achieves a balance between immunosuppressive activity and minimum toxicity remains to be elucidated.[26] In this study, the cumulative incidence of cGVHD was 37.3% in the ATG group and 52.1% in the non-ATG group after 3 years. The overall cumulative incidence of extensive cGVHD alone was 19.5% for the ATG group and 38.4% for the non -ATG group after 3 years. The significantly lower incidence of cGVHD in the ATG group resulted in a significantly higher rate of survival free of cGVHD and relapse among patients who received ATG compared with those who did not receive ATG (66.7% vs 40.0%), as the incidence of relapse was not statistically different. In the present study, the rates of GRFS were significantly higher in the ATG group than in the non -ATG group. Thus, 5-mg/kg of rATG on days −5 to −4 may lead to the reduction of the incidence of severe or extensive cGVHD and improve survival.

rATG targets alloreactive T cells at the expense of potentially increasing the risk of infections after transplantation and delayed immune reconstitution.[6] rATG administration persists for long periods *in vivo* and causes relapse and infection.[27,28] Experimental studies have showed that rATG induced apoptosis toward different hematologic malignancies, antibody-dependent cell-mediated cytotoxicity, and complement-dependent cytotoxicity.[29] Socie et al. found that relapse was not related with Thymoglobulin doses less than 6 mg/kg,[22,30,31] which was consistent with the finding of the present study. This study confirmed that the 3-year cumulative incidence of relapse was not statistically different in the two groups. No difference was observed in the cumulative incidence of NRM between the ATG and non -ATG groups (13.0% vs 8.5%). rATG is considered a risk factor for EBV reactivation and posttransplant lymphoproliferative disorder. Notably, patients receiving the ATG regimen had a

Table 5

Multivariate analysis of OS, GRFS, DFS, or GVHD for the risk factors of transplant outcomes in all patients.

		Univariate			Multivariate	
GRFS	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Group						
No ATG	1.0			1.0		
ATG	0.4	0.2–0.9	<.001	0.1	0.0–0.5	.002
OS						
Group						
No ATG	1.0			1.0		
ATG	0.6	0.3–1.4	.262	0.2	0.1–0.9	.032
DFS						
Group						
No ATG	1.0			1.0		
ATG	0.7	0.3–1.5	.334	0.2	0.1–0.9	.031
Chronic GVHD						
Group						
No ATG						
ATG	0.3	0.1–0.7	<.001	0.1	0.0–0.4	.002
Extensive chronic GVHD						
Group						
No ATG	1.0			1.0		
ATG	0.2	0.1–0.6	.060	0.0	0.0–0.6	.028

95% CI = cumulative incidence 95% CI, ATG = anti-thymocyte globulin, DFS = disease-free survival, GRFS = severe GVHD-free, relapse-free survival, GVHD = graft-versus-host disease, OS = overall survival.
Chronic, limited, and extensive grades of GVHD were defined according to the Seattle criteria.
Chronic, mild, moderate, and severe grades of GVHD were defined according the NIH criteria.
*P means P value.

greater incidence of Epstein–Barr virus reactivations compared with those in the non -ATG group, but not CMV reactivation. No posttransplantation lymphoproliferative disorder was observed in this study. Fungal infection (9.5% vs 43.3%) was significantly higher in the non -ATG group than in the ATG group. The development of cGVHD (7 cGVHD in 13 patients with fungal infection) and high dosage of corticosteroids were the major reason for the high incidence of fungal infection in the non -ATG group.

5. Conclusions

In summary, this study showed that the incorporation of rATG (at 5-mg/kg of the total dose administered from days −5 to −4) in adult patients undergoing MSD-PBSCT resulted in a significantly lower rate of cGVHD, and improved GRFS, compared with those in the non -ATG group. The rates of relapse were similar in the two groups. Whether 5 mg/kg of rATG for MSD-PBSCT patients is the optimal dose or not need more evidence. Novel approaches of optimizing ATG dosage based on pharmacokinetic analysis, should be explored further.[32–34]

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Author contributions

All authors read and approved the final manuscript.
Data Collection: WH, SW
Data Interpretation: FL, XG
Funds Collection: DL
LD recruited patients, collected data, did the data analysis and interpretation of the data and drafted the manuscript.
Literature Search: LW, YL
Manuscript Preparation: LD, LY
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