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Reduced Risk of Chronic Graft-Versus-Host Disease (cGVHD) by Rabbit Anti-Thymocyte Globulin (ATG) in Patients Undergoing Matched Sibling Donor Transplantation in Hematological Malignancies

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Background: With the addition of anti-thymocyte globulin (ATG) to GVHD prophylaxis in patients undergoing transplantation of peripheral blood stem cells (PBSCT), the incidence of cGVHD decreases. However, the optimal dose and timing of ATG remain undetermined.

Material/Methods: In this historical controlled trial, data from 85 patients who had hematological malignancies and underwent matched sibling donor (MSD)-PBSCT were used to analyze the effectiveness of rabbit ATG (rATG) for prophylaxis of GVHD. Forty patients received 5 mg/kg rATG used for days -5 to -2, and 45 patients did not receive ATG.

Results: All patients had successful engraftment except for 2 in the non-ATG group, who had platelet engraftment failure. The 2-year cumulative incidence of chronic GVHD (cGVHD) in the ATG group versus non-ATG group was 19.3% (95% CI, 8.4-33.6%) versus 61.4% (95% CI, 45.4-73.9%) ($P<0.001$), and in those with moderate to severe cGVHD it was 11.0% (95% CI, 3.4-23.6%) versus 31.8% (95% CI, 18.8-45.6%) ($P=0.029$), respectively. The 2-year cumulative incidence of non-relapse mortality and relapse (CIR) were 0% versus 15.5% (95% CI, 6.8-27.5%) ($P=0.018$), and 53.3% (95% CI, 35.6-68.1%) versus 26.7% (95% CI, 14.9-40.0%) ($P=0.019$), respectively. No differences were found in other survival outcomes. In the multivariate analysis, ATG was an independent protective factor for moderate to severe cGVHD (HR=0.314, 95% CI, 0.103-0.958, $P=0.042$), and was an independent poor risk factor for CIR (HR=2.337, 95% CI, 1.133-4.822, $P=0.022$).

Conclusions: ATG in our strategy was effective for prophylaxis of cGVHD, whereas the relapse rate was increased in patients with rATG.

Keywords: Graft vs Host Disease • Peripheral Blood Stem Cell Transplantation • Recurrence • Thymoglobulin

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Background

Hematopoietic stem cell transplantation (HSCT) is the only way to cure patients who have malignant hematological diseases. However, graft-versus-host disease (GVHD), as the main complication after HSCT, impairs life quality and longevity [1-3]. Despite the use of calcineurin inhibitor combined with methotrexate (MTX) as GVHD prophylaxis, 30-50% of patients develop acute GVHD (aGVHD) [4], and 30-70% still have chronic GVHD (cGVHD) [1]. Another effective way to reduce GVHD is the depletion of T cells. Studies have shown that in vivo removal of T cells by ATG can reduce GVHD and prolong the survival of patients receiving HSCT from unrelated donors or haploidentical donors (HID-HSCT) without increasing relapse rates [5,6]. With the addition of anti-thymocyte globulin (ATG) to GVHD prophylaxis, the incidence of cGVHD also decreased [7-9].

Infusion of donor graft derived from peripheral blood stem cells increases the incidence of cGVHD [10,11]. Adding ATG to GVHD prophylaxis decreases GVHD in PBSCT patients [12-14]. A similar result was found in a study at our center [15]. Patients undergoing HID-transplantation from peripheral blood stem cells (PBSCT) were given 10 mg/kg rabbit ATG (rATG), divided across 4 days (from -5 to -2). We found a decreased incidence of severe cGVHD in these patients compared with patients undergoing PBSCT from matched sibling donor (MSD) (5.8% vs 21.2%, *P*=0.049) [15]. This result demonstrated the effectiveness of rATG in alleviating severe cGVHD in patients receiving HID-HSCT. Deeg et al showed that ATG at doses of 4.5 to 6.0 mg/kg seemed efficient for GVHD prevention in patients undergoing PBSCT [14]. However, the optimal dose and timing of ATG remain undetermined. In this historical controlled trial, to study whether rATG could reduce cGVHD in MSD-PBSCT setting, we cut down the total dose of rATG (obtained from hyperimmune sera of rabbits immunized with human thymocytes) to 5 mg/kg administered over days -5 to -2 in these patients. This trial was registered at ClinicalTrials.gov. Identifier: NCT05214066. The purpose of this study was to investigate the effectiveness of rATG divided across 4 days in MSD-PBSCT patients.

Material and Methods

Patients

Patients with myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML)/acute lymphocytic leukemia (ALL) who received MSD-PBSCT in the Hematology Department of the General Hospital of the Chinese people's Liberation Army (PLA) from November 20, 2014, to December 31, 2020, met the conditions of this study. Patients' ages were 12 to 65 years. The exclusion criteria were: (1) uncontrolled infections; (2) severe pulmonary, cardiac, hepatic or renal diseases; and (3) AML patients with t (15;17). Patients receiving a total of 5 mg/kg ATG on days -5 to -2 for GVHD prophylaxis were enrolled between January 2019 and December 2020. This trial was registered at ClinicalTrials.gov (Identifier: NCT05214066). The expected sample size was 40. Patients in the historical controlled group were enrolled between November 2014 and December 2018. There were no apparent between-group changes in donor selection, conditioning regimen, and other treatment options. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Chinese PLA General Hospital. All patients signed written informed consent.

All patients received cyclosporine, mycophenolate, and short-term methotrexate for GVHD prophylaxis. Forty patients were treated with the addition of rATG and were assigned to the ATG group. Forty-five patients without rATG were assigned to the non-ATG group. About 92.5% (37/40) of patients in the ATG group and 93.3% (42/45) of patients in the non-ATG group received a busulfan (Bu)/cyclophosphamide (Cy) conditioning regimen. Other patients received total body irradiation (TBI)/Cy, fludarabine (Flu) combined with cytarabine, granulocyte colony-stimulating factor (G-CSF), and Bu (FLAG/Bu), or Bu/Flu conditioning regimens (**Table 1**).

HLA Matching

HLA loci, including HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1, were detected in all patients. All patients and donors matched at these loci.

GVHD Prophylaxis and Therapy

Forty patients received 1 mg/kg rATG on days -5 to -3, and 2 mg/kg on day -2 prior to transplantation. All patients received cyclosporine A (CsA), methotrexate (MTX), and mycophenolate mofetil (MMF) as GVHD prophylaxis. CsA was injected intravenously from day -10 until oral refeeding at a dose of 3 mg/kg, and the target concentration was 150 to 200 ng/mL. After 6 months, it was tapered by 25% every 2 weeks except for patients with relapse. In patients who relapsed within 60 days, CsA was rapidly tapered within 2 weeks. In those who relapsed after 60 days, CsA was immediately discontinued. If patients were intolerant to CsA, they used tacrolimus instead. The usage of MTX was 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11. MMF was administered orally from day -10 to engraftment, at a dose of 250 mg, twice daily [16]. Patients with aGVHD received methylprednisolone (MP). The MP was used at a 2 mg/kg/day dose divided into 2 doses for 7 consecutive days. Then, it was tapered over 8 weeks.

Endpoints and Definitions

The primary endpoint was the incidence of cGVHD. Secondary endpoints were cumulative incidence of aGVHD, NRM, relapse

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Table 1. Clinical characteristics of 85 patients.

Characteristics	ATG group	Non-ATG group	p value
No. of patients	40	45	
Patient's age, y, median (range)	39 (18-60)	37 (12-63)	0.567
Gender, No. (%)			0.411
Male	24 (60.0)	23 (51.1)	
Female	16 (40.0)	22 (48.9)	
Time interval between diagnosis and HSCT, d, median (range)			0.160
<6 m	17 (42.5)	26 (57.8)	
≥6 m	23 (57.5)	19 (42.2)	
Diagnosis, No. (%)			0.013
AML/MDS	23 (57.5)	37 (82.2)	
ALL	17 (42.5)	8 (17.8)	
Disease status at transplantation, No. (%)			0.136
CR			
MRD-	24 (60.0)	16 (35.6)	
MRD+	11 (27.5)	17 (37.8)	
Untreated MDS-AML	3 (7.5)	8 (17.8)	
Refractory/Relapsed	2 (5.0)	4 (8.9)	
Cytogenetic risk, No. (%)			0.116
Favorable	3 (7.5)	6 (13.3)	
Intermediate	21 (52.5)	30 (66.7)	
Poor	16 (40.0)	9 (20.0)	
Disease Risk Index, No. (%)			0.124
Low/intermediate	24 (60.0)	34 (75.6)	
High/very high	16 (40.0)	11 (24.4)	
Conditioning regimen, No. (%)			0.066
Bu/Cy	37 (92.5)	42 (93.3)	
TBI/Cy	3 (7.5)	0 (0.0)	
FB	0 (0.0)	1 (2.2)	
FLAG/Bu	0 (0.0)	2 (4.4)	
Donor's age, y, median (range)	38.5 (12.0-59.0)	36.0 (11.0-58.0)	0.546
Donor-recipient ABO match, No. (%)			0.145
Match	22 (55.0)	31 (68.9)	
Major mismatch	5 (12.5)	8 (17.8)	
Minor mismatch	12 (30.0)	6 (13.3)	
Bidirectional mismatch	1 (2.5)	0 (0.0)	

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Table 1 continued. Clinical characteristics of 85 patients.

Characteristics	ATG group	Non-ATG group	p value
Donor-recipient gender match, No. (%)			0.458
Male to male	8 (20.0)	12 (26.7)	
Female to female	9 (22.5)	14 (31.1)	
Male to female	7 (17.5)	8 (17.8)	
Female to male	16 (40.0)	11 (24.4)	
Graft, median (range)			
MNCs, $\times 10^8$/kg,	11.65 (6.17-25.60)	8.99 (4.75-18.23)	<0.001
$CD34^+$ cells, $\times 10^6$/kg	4.65 (2.25-9.17)	3.21 (1.96-9.26)	0.001

ATG – anti-thymocyte globulin; HSCT – hematopoietic stem cell transplantation; MDS – myelodysplastic syndrome; AML – acute myeloid leukemia; ALL – acute lymphoblastic leukemia; CR – complete remission; MRD – minimal residual disease; MNCs – mononuclear cells; no. – number of patients.

(CIR), GVHD-free and relapse-free survival (GRFS), OS, and disease-free survival (DFS). In this study, neutrophils were successfully engrafted in all patients and they were eligible for analysis of aGVHD. Data from patients who were still alive at 100 days were eligible for analysis of cGVHD. In this study, all patients, except 1 who died within 100 days in the non-ATG group, were eligible for analysis of cGVHD.

The definition of neutrophil and platelet engraftment after transplantation was as described in the previous study [16]. Disease risk index was assessed as previously described [17]. Acute GVHD and cGVHD were diagnosed and graded based on the established criteria [18-21]. OS was defined as the time from transplantation to death or last follow-up. DFS was defined as the time from transplantation to relapse, death, or last follow-up. Relapse was defined as reappearance of peripheral blood blasts or >5% blasts detected in bone marrow or extramedullary infiltration. Non-relapse mortality (NRM) was defined as death without disease relapse or progression. GRFS was defined as the absence of grade 3 to 4 aGVHD, moderate to severe cGVHD, relapse, or death.

Statistical Analysis

SPSS 24.0 and R 4.0.3 software was used to do statistical analysis. Continuous variables were exhibited as median (range). The Mann-Whitney U test was used to compare these variables. The chi-square test was used to compare categorical variables. For the expected count of an event <5 or a total number of patients <40, Fisher's exact test was used. The competing risk model was used to calculate the cumulative incidence of GVHD, relapse, and NRM between 2 groups. Gray's test was used to compare the *P* values. Relapse and death were considered competing events for GVHD. When analyzing the cumulative incidence of relapse and NRM by the competing risk model, relapse and NRM were competing events for each other. Kaplan-Meier (K-M) analysis was used to calculate OS and DFS. The log-rank test was used to compare differences between groups. The Cox proportional hazard model was used to perform univariate and multivariate analyses of the cumulative incidence of grades 2-4 aGVHD, cGVHD, relapse, OS, LFS, and GRFS. Parameters with a *P* value <0.2 were used in multivariate analysis. A stepwise backward procedure selection model was used for extracting independent factors in multivariate analysis. A two-sided $P<0.05$ was considered statistically significant.

Results

Clinical Characteristics of Patients

We included 85 patients in this study. Forty patients receiving ATG treatment were assigned to the ATG group, and 45 were in the non-ATG group. The proportion of patients with ALL in ATG group was higher than that in non-ATG group (42.5% vs 17.8%, $P=0.013$, **Table 1**). Salvage HSCT was performed in 2 patients in the ATG group and 4 patients in the non-ATG group who were refractory or relapsed (**Table 1**). The counts of mononuclear cells (MNCs) and CD34-positive cells in the ATG group were higher than those in the non-ATG group (MNCs, $P<0.001$, $CD34^+$ cells, $P=0.001$). The distribution of other baseline characteristics was similar in both groups (**Table 1**).

Engraftment

All patients had successful neutrophil and platelet engraftment, except for 2 in the non-ATG group who had platelet

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Table 2. Engraftment, infection, acute and chronic GVHD, and other complications after MSD-PBSCT.

Outcomes	ATG group	Non-ATG group	p value
Early death, No. (%)	0	0	
Neutrophil engraftment, d, median (range)	12 (8-18)	10 (9-32)	0.060
Platelet engraftment, d, median (range)	12 (7-29)	13 (9-75)	0.084
Cytomegalovirus reactivation at day +180, No. (%)	8 (20.0)	8 (17.8)	0.794
Epstein-Barr virus reactivation at day +180, No. (%)	15 (37.5)	2 (4.4)	<0.001
PTLD, No. (%)	0	0	
Grades of aGVHD, No. (%)			0.784
0	22 (55.0)	28 (62.2)	
1	3 (7.5)	4 (8.9)	
2	13 (32.5)	11 (24.4)	
3	1 (2.5)	2 (4.4)	
4	1 (2.5)	0 (0.0)	
2-4	15 (37.5)	13 (28.9)	
3-4	2 (5.0)	2 (4.4)	
Severity of cGVHD according to revised Seattle criteria, No. (%)*			<0.001
No cGVHD	33 (82.5)	17 (38.6)	
Limited	5 (12.5)	17 (38.6)	
Extensive	2 (5.0)	10 (22.8)	
Severity of cGVHD according to NIH criteria, No. (%)*			0.001
No cGVHD	33 (82.5)	17 (38.6)	
Mild	3 (7.5)	13 (29.5)	
Moderate	3 (7.5)	9 (20.5)	
Severe	1 (2.5)	5 (11.4)	

ATG – anti-thymocyte globulin; PTLD – post-transplantation lympho-proliferative disorder; aGVHD – acute graft-versus-host disease; cGVHD – chronic graft-versus-host disease; no. – number of patients. * 1 patient died within 100 days.

engraftment failure. One of these 2 patients died at day +96 because of aGVHD, and the other failed to have platelet engraftment and died of relapse at day +125. The median time of neutrophil engraftment in the ATG group versus non-ATG group was 12 (range, 8-18) days versus 10 (range, 9-32) days (P=0.060, **Table 2**). The median time of platelet engraftment in the ATG and non-ATG groups were 12 (range, 7-29) days and 13 (range, 9-75) days, respectively (P=0.084, **Table 2**).

Maintenance Therapy After Transplantation

Four patients in the ATG group and 8 in the non-ATG group received prophylactic donor lymphocyte infusion (DLI). None of them developed cGVHD in the ATG group, while 3 out of 4 patients had a relapse. In the non-ATG group, 4 out of 8 patients developed cGVHD, 1 of whom died due to a relapse. The other 4 patients without cGVHD died due to relapse. Three FLT3-ITD$^+$ patients in the ATG group and 5 in the non-ATG group received sorafenib after transplantation. None of them developed cGVHD in the ATG group, but 1 of them eventually relapsed. In the non-ATG group, 3 of them developed cGVHD and 1 of them died of relapse. The remaining 2 patients died of relapse. For Ph+ ALL, 6 patients in the ATG group and 3 in the non-ATG group received tyrosine kinase inhibitor (TKI) after transplantation. None of them developed cGVHD in the ATG group, but 3 of them had a relapse. One out of 3 patients in the non-ATG group developed cGVHD, and none of them relapsed.

Acute and Chronic GVHD

There were no significant differences observed in the cumulative incidence of grades 2-4 aGVHD (P=0.231, **Figure 1A**) and

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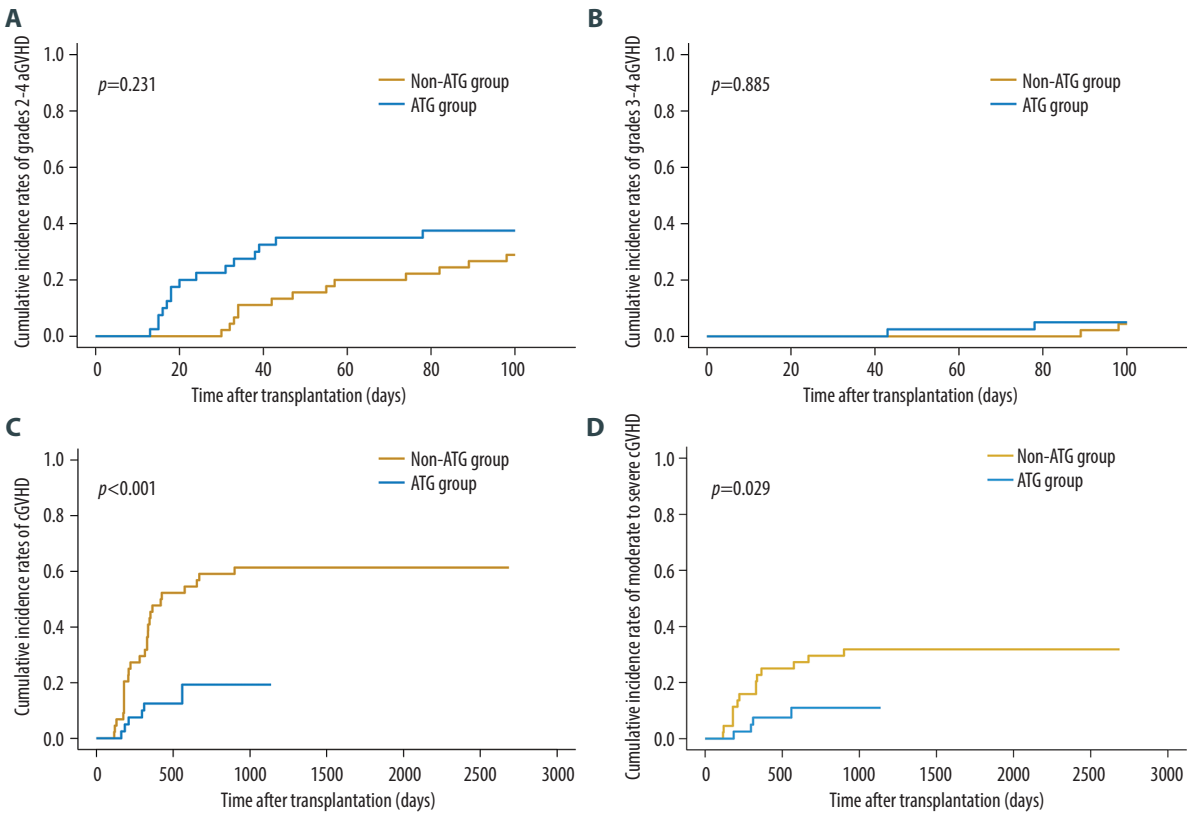

Figure 1. (**A-D**) Comparison of cumulative incidence of aGVHD and cGVHD between ATG group and non-ATG group (R version 4.0.3). aGVHD – acute graft-versus-host disease; cGVHD – chronic graft-versus-host disease.

grades 3-4 aGVHD (*P*=0.885, **Figure 1B**) between the ATG group and non-ATG group. The median time to onset was 26.5 days (range, 13.0-78.0 days) in the ATG group and 47 days (range, 16-98 days) in the non-ATG group. The 100-day cumulative incidence of grades 2-4 aGVHD in the ATG group versus non-ATG group was 37.5% (95% CI, 22.9-52.1%) and 28.9% (95% CI, 16.6-42.4%), respectively. The 100-day cumulative incidence of grades 3-4 aGVHD and grades 3-4 aGVHD was 5% (95% CI, 0.9%-14.8%) and 4% (95% CI, 0.8-13.3%), respectively. One patient died of severe aGVHD on day +96 in the non-ATG group. In the univariate and multivariate analysis, there were no risk factors associated with grades 2-4 aGVHD (**Tables 3, 4**).

In the ATG group, 17.5% (7/40) of patients had cGVHD, 4 of whom had preceding aGVHD. The median time to develop cGVHD was 297 days (range, 162-559 days). One patient with cGVHD died of relapse. In the non-ATG group, 60% (27/45) of patients had cGVHD. Among them, 11 patients had preceding aGVHD. The median time to develop cGVHD was 316 days (range, 116-900 days). Six out of 27 patients with cGVHD died because of pneumonia, and another 4 died of relapse. The two-year cumulative incidence of cGVHD in the ATG group was lower than that in the non-ATG group (19.3% [95% CI, 8.4-33.6%] versus 61.4% [95% CI, 45.4-73.9%], *P*<0.001, **Figure 1C**). The two-year cumulative incidence of moderate to severe cGVHD was also lower in the ATG group than that in the non-ATG group (11.0% [95% CI, 3.4-23.6%] versus 31.8% [95% CI, 18.8-45.6%], *P*=0.029, **Figure 1D**). In the univariate analysis, absence of ATG was a poor risk factor for overall cGVHD and moderate to severe cGVHD (**Tables 3, 5, Figure 1C, 1D**). In the multivariate analysis, ATG was an independent protective factor for occurrence of overall cGVHD and moderate to severe cGVHD (ATG vs non-ATG, overall cGVHD, HR=0.251, 95% CI, 0.108-0.582, *P*=0.001; moderate to severe cGVHD, HR=0.314, 95% CI, 0.103-0.958, *P*=0.042. **Tables 3-5**).

Virus Infection, Relapse and NRM

In the ATG group, 8 patients experienced cytomegalovirus (CMV) reactivation. Fifteen patients experienced Epstein-Barr virus (EBV) reactivation. In the non-ATG group, 8 and 2 patients experienced CMV and EBV reactivation, respectively (**Table 2**). The percentage of patients with EBV reactivation was 37.5% in the ATG group and 4.4% in the non-ATG group (*P*<0.001, **Table 2**). No patients had post-transplantation lymphoproliferative disorder (PTLD) in either group.

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Table 3. Univariate analysis of risk factors for grades 2-4 aGVHD and cGVHD in all patients.

	Grades 2-4 aGVHD			Chronic GVHD		
	HR	95% CI	*p*	HR	95% CI	*p*
ATG vs non-ATG	1.573	0.748-3.307	0.232	0.222	0.093-0.530	0.001
Recipient age (≥ median vs < median)	0.962	0.457-2.021	0.918	0.930	0.427-2.027	0.856
Donor age (≥ median vs < median)	1.140	0.542-2.396	0.730	1.040	0.531-2.039	0.908
Donor-recipient gender match						
Female to male vs others	1.091	0.493-2.412	0.830	0.719	0.335-1.542	0.397
Donor-recipient ABO match						
Mismatch vs match	1.858	0.885-3.902	0.101	1.153	0.576-2.308	0.688
Diagnosis (ALL vs others)	1.243	0.562-2.748	0.591	0.396	0.153-1.023	0.056
Time interval between diagnosis and HSCT						
≥6 m vs <6 m	1.806	0.845-3.859	0.127	0.679	0.344-1.339	0.264
Cytogenetic risk						
High vs favorable/intermediate	1.854	0.868-3.964	0.111	0.325	0.126-0.843	0.021
Disease status at HSCT						
Untreated/refractory/relapsed vs CR	0.595	0.206-1.715	0.336	1.531	0.692-3.386	0.293
Disease risk index						
High/very high vs low/intermediate	1.556	0.728-3.322	0.254	0.313	0.121-0.810	0.017
MNC (≥ median vs < median)	1.315	0.622-2.781	0.473	0.535	0.268-1.068	0.076
CD34 (≥ median vs < median)	2.026	0.935-4.391	0.073	0.474	0.234-0.958	0.038
aGVHD (Grades 2-4 vs grades 0-1)				0.828	0.403-1.704	0.608

ATG – anti-thymocyte globulin; aGVHD – acute graft-versus-host disease; GVHD – graft-versus-host disease; HSCT – hematopoietic stem cell transplantation; ALL – acute lymphoblastic leukemia; CR – complete remission; MNCs – mononuclear cells.

Twenty patients relapsed after transplantation in the ATG group. By the end of the follow-up, 11 patients had died of relapse. The two-year CIR was 53.3% (95% CI, 35.6-68.1%). In the non-ATG group, 12 patients relapsed and died. The two-year CIR was 26.7% (95% CI, 14.9-40.0%). In the univariate analysis, use of ATG was an inferior factor associated with CIR (**Table 5, Figure 2A**). In the multivariate analysis, ATG treatment was an independent poor risk factor for CIR (HR=2.337, 95% CI, 1.133-4.822, *P*=0.022, **Table 4**).

In the ATG group, no patients died because of NRM. In the non-ATG group, 7 patients died because of NRM: 1 of them died due to aGVHD and the other 6 died because of pneumonia and eventual respiratory failure. The two-year NRM was 15.5% (95% CI, 6.8%-27.5%). The rate of NRM was lower in the ATG group than that in the non-ATG group (*P*=0.018, **Figure 2B**).

Survival

In the ATG group, 50% (20/40) of patients survived with CR status. About 22.5% (9/40) of patients relapsed and were still alive at the end of follow-up. The median follow-up was 811 days (range, 458-1137 days). In the non-ATG group, 57.8% (26/45) of patients survived with CR status. The median follow-up was 1906.5 days (range, 1290.0-2686.0 days). The two-year OS was 71.9% (95% CI, 54.9-83.4%) in the ATG group and 62.2% (95% CI, 46.5-74.6%) in the non-ATG group (*P*=0.315, **Figure 2C**). The two-year DFS was 46.7% (95% CI, 29.7-62.1%) versus 62.2% (95% CI, 46.5%-74.6%) in the ATG group versus non-ATG group (*P*=0.284, **Figure 2D**). The two-year GRFS was 76.5% (95% CI, 67.1-83.6%) versus 66.9% (95% CI, 55.7-76.0%) in the ATG group versus non-ATG group (*P*=0.306, **Figure 2E, Supplementary Table 1**). In the univariate and multivariate analysis, no risk factors were found to be associated with OS and DFS (**Supplementary Table 2, Table 4**). In the

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Table 4. Multivariate analysis of risk factors for transplant outcomes in all patients.

Variables	HR	95% CI	*p*
Grades 2-4 aGVHD			
Donor-recipient ABO match			
Mismatch vs match	1.978	0.933-4.192	0.075
Time interval between diagnosis and HSCT			
≥6 m vs <6 m	2.032	0.943-4.380	0.070
CD34 (≥ median vs < median)	1.976	0.911-4.289	0.085
Chronic GVHD			
Diagnosis (ALL vs others)	0.791	0.288-2.173	0.650
ATG vs non-ATG	0.251	0.108-0.582	0.001
Disease risk index			
High/very high vs low/intermediate	0.414	0.158-1.083	0.072
Moderate to severe cGVHD			
ATG vs non-ATG	0.314	0.103-0.958	0.042
Recipient age (≥ median vs < median)	0.428	0.161-1.143	0.090
Extensive cGVHD			
ATG vs non-ATG	0.269	0.073-0.995	0.049
CIR			
Diagnosis (ALL vs others)	1.536	0.728-3.241	0.260
ATG vs non-ATG	2.337	1.133-4.822	0.022
Time interval between diagnosis and HSCT			
≥6 m vs < 6 m	0.520	0.253-1.071	0.076
GRFS			
Disease risk index			
High/very high vs low/intermediate	1.654	0.979-2.792	0.060
CD34 (≥ median vs < median)	1.785	1.067-2.984	0.027
OS			
Time interval between diagnosis and HSCT			
≥6 m vs <6 m	0.559	0.266-1.176	0.125
DFS			
Time interval between diagnosis and HSCT			
≥6 m vs <6 m	0.593	0.311-1.133	0.114

ATG – anti-thymocyte globulin; aGVHD – acute graft-versus-host disease; cGVHD – chronic graft-versus-host disease; HSCT – hematopoietic stem cell transplantation; ALL – acute lymphoblastic leukemia; CR – complete remission; MNCs – mononuclear cells.

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Table 5. Univariate analysis of risk factors for moderate to severe cGVHD and CIR in all patients.

	Moderate to severe cGVHD			CIR		
	HR	95% CI	*p*	HR	95% CI	*p*
ATG vs non-ATG	0.301	0.099-0.918	0.035	2.158	1.051-4.430	0.036
Recipient age (≥ median vs < median)	0.407	0.152-1.084	0.072	1.405	0.687-2.875	0.352
Donor age (≥ median vs < median)	0.591	0.229-1.526	0.278	1.633	0. 798-3.343	0.180
Donor-recipient gender match						
Female to male vs others	1.438	0.556-3.720	0.454	1.125	0.542-2.334	0.752
Donor-recipient ABO match						
Mismatch vs match	0.907	0.340-2.420	0.846	1.405	0.699-2.827	0.340
Diagnosis (ALL vs others)	0.765	0.250-2.343	0.639	1.701	0.830-3.489	0.147
Time interval between diagnosis and HSCT						
≥6 m vs <6 m	0.737	0.291-1.872	0.521	0.577	0.282-1.181	0.132
Cytogenetic risk						
High vs favorable/intermediate	0.434	0.125-1.499	0.187	1.147	0.543-2.424	0.719
Disease status at HSCT						
Untreated/refractory/relapsed vs CR	1.300	0.428-3.953	0.644	1.170	0.506-2.706	0.714
Disease risk index						
High/very high vs low/intermediate	0.418	0.121-1.443	0.168	1.610	0.794-3.264	0.187
MNC (≥ median vs < median)	0.789	0.311-2.001	0.618	1.159	0.579-2.322	0.677
CD34 (≥ median vs < median)	0.829	0.326-2.107	0.694	1.548	0.763-3.141	0.226
aGVHD (Grades 2-4 vs grades 0-1)	0.700	0.249-1.968	0.499	2.158	1.051-4.430	0.036

ATG – anti-thymocyte globulin; aGVHD – acute graft-versus-host disease; cGVHD – chronic graft-versus-host disease; CIR – cumulative incidence of relapse; HSCT – hematopoietic stem cell transplantation; ALL – acute lymphoblastic leukemia; CR – complete remission; MNCs – mononuclear cells.

multivariate analysis, high count of $CD34^+$ cells were related to GRFS (HR=1.785, 95% CI, 1.067-2.984, *P*=0.027, **Table 4**).

Discussion

GVHD is the leading cause affecting long-term survival after HSCT [22,23]. The current regimens for GVHD prophylaxis are CsA and MTX. However, the incidence of grades 2-4 aGVHD remains 19-40% in patients with MSD-HSCT, and that of cGVHD is 40-60% [16,24,25]. A mainstream approach to the removal of T cells in vivo and to reducing GVHD is post-transplant cyclophosphamide (PTCy) [26,27]. Another effective strategy for T cell depletion in vivo is ATG [28]. ATG effectively targets alloreactive T cells from the graft, leading to T cell depletion and decreased incidence of GVHD [29]. PTCy appears to be more effective in GVHD prophylaxis compared with ATG [26,27]. However, there is no difference in OS between the 2 regimens [26,27]. These may be due to the relatively higher incidences of graft failure and relapse [30,31]. Due to the absence of prospective randomized trials directly comparing the efficacy of these 2 approaches, it remains unclear which regimen is better. In our study, we used a strategy for GVHD prophylaxis with 5 mg/kg rATG divided over 4 days in 40 patients. We found that the 2-year cumulative incidence of cGVHD in ATG group was 23.2%, lower than that of the non-ATG group.

A previous study used 10 mg/kg ATG (ATG-Fresenius) as GVHD prophylaxis before HLA-identical sibling transplantation in acute leukemia patients [32]. The 2-year incidence of cGVHD was lower in the ATG group compared with non-ATG group. No differences were found in the rates of aGVHD, relapse, 2-year relapse-free survival, and OS between groups [32]. Another study revealed that 4.5 mg/kg ATG in MSD-HSCT

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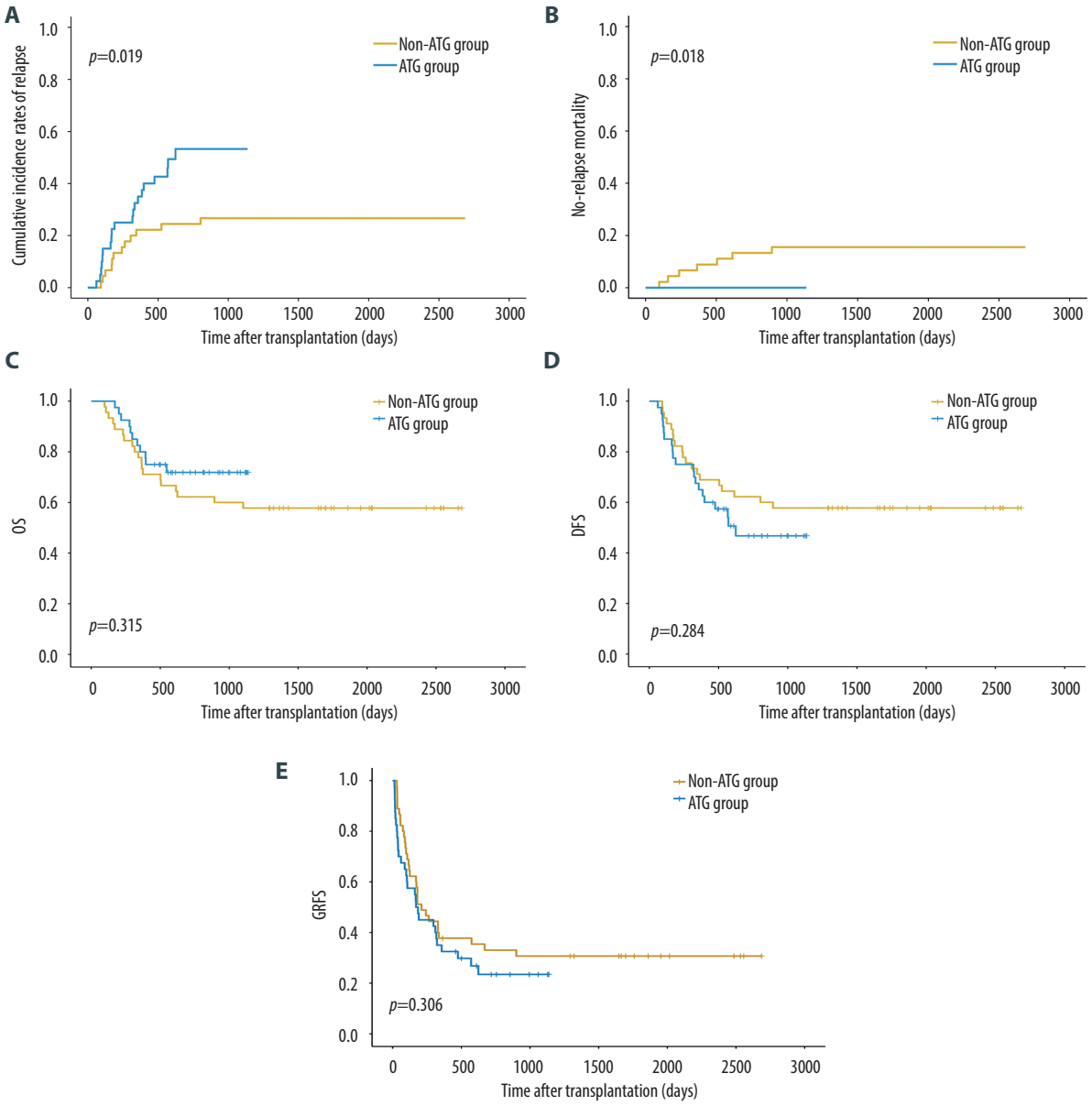

Figure 2. (**A-E**) Comparison of cumulative incidence of relapse, non-relapse mortality, OS, DFS, and GRFS (R version 4.0.3). OS – overall survival; DFS – disease-free survival; GRFS – GVHD-free relapse-free survival; GVHD – graft-versus-host disease.

patients could reduce the risk of overall cGVHD, extensive cGVHD, and grades 2-4 aGVHD but not increase relapse and not improve NRM, OS, or LFS [33]. Other studies investigated 5 mg/kg rATG divided over 3 days [25] or 2 days [16] for GVHD prophylaxis in patients receiving MSD-HSCT, and found a lower incidence of overall cGVHD and extensive cGVHD and improved OS in the ATG group. Our results showing the benefits of rATG in reducing the risk of cGVHD were similar to previous studies. Our study showed that rATG reduced the risk of total cGVHD and moderate to severe cGVHD, but not grades 2-4 aGVHD.

However, our study showed higher rates of relapse and EBV infection in the ATG group than those in the non-ATG group. The use of rATG before HSCT leads to depletion of T cells [28], immunosuppression, infection, and relapse [34]. Previous studies found that the relapse rate increased in patients with high-dose ATG [35] but not in patients receiving low-dose ATG [32,33]. Nevertheless, a recent prospective, single-center, randomized study showed that the relapse rate was increased in patients with a 2.5 mg/kg ATG to prevent GVHD [36], especially in cytogenetic high-risk patients. In our study, we found that the percentage of cytogenetic high-risk patients was higher in the ATG group than in the

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non-ATG group, which might lead to a higher relapse rate in the ATG group. Another reason might be the lower prophylactic DLI in the ATG group. Although we found that in the ATG group the risk of EBV infection was increased, no patients developed PTLD. These might be due to adequate and timely antiviral therapy.

In the ATG group, we also found a lower rate of NRM. These might be due to the lower incidence of cGVHD in the ATG group. We discovered that cGVHD occurred in 6 out of 7 patients who died of NRM in the non-ATG group. These patients eventually died of pneumonia infection and respiratory failure after long-term immunosuppressive regimen treatment. These might be the reason for the higher NRM in patients without ATG treatment.

This study also has some limitations. First, it was a singer-center study and patient selection may have been biased. Second, it was a non-randomized, non-concurrent control study, the distribution of baseline characteristics of patients between the 2 groups may have been uneven, and many confounding factors may have affected the results. In addition, the sample size is not very large, so the data acquired in our study may be insufficient to prove the reliability of the conclusions. Multicenter, large-sample, prospective, randomized, controlled studies are still needed to verify these results.

Conclusions

In summary, our study shows that 5 mg/kg rATG divided over 4 days can reduce the risk of cGVHD, whereas the relapse rate was higher in patients with rATG. The strategy of rATG in our study need to be cautiously used in clinical practice. More studies are needed to investigate the optimal dose and timing of rATG in transplant patients.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

Supplementary Tables

Supplementary Table 1. Univariate analysis of risk factors for GRFS in all patients.

Variables	HR	95% CI	*p*
ATG vs non-ATG	1.299	0.785-2.149	0.309
Recipient age (≥ median vs < median)	1.054	0.636-1.747	0.839
Donor age (≥ median vs < median)	1.155	0.698-1.912	0.576
Donor-recipient gender match			
Female to male vs others	1.190	0.702-2.020	0.518
Donor-recipient ABO match			
Mismatch vs match	1.336	0.798-2.236	0.271
Diagnosis (ALL vs others)	1.333	0.779-2.281	0.294
Time interval between diagnosis and HSCT			
≥6 m vs <6 m	0.945	0.571-1.563	0.826
Cytogenetic risk			
High vs favorable/intermediate	1.615	0.950-2.745	0.076
Disease status at HSCT			
Untreated/refractory/relapsed vs CR	0.803	0.427-1.512	0.498
Disease risk index			
High/very high vs low/intermediate	1.730	1.027-2.913	0.039
MNC (≥ median vs < median)	1.407	0.848-2.333	0.186
CD34 (≥ median vs < median)	1.846	1.107-3.079	0.019

ATG – anti-thymocyte globulin; HSCT – hematopoietic stem cell transplantation; ALL – acute lymphoblastic leukemia; CR – complete remission; MNCs – mononuclear cells; GRFS – GVHD-free and relapse-free survival; GVHD – graft-versus-host disease.

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Supplementary Table 2. Univariate analysis of risk factors for OS and DFS in all patients.

	OS			DFS		
	HR	95% CI	*p*	HR	95% CI	*p*
ATG vs non-ATG	0.683	0.323-1.444	0.318	1.411	0.749-2.657	0.287
Recipient age (≥ median vs < median)	1.324	0.638-2.751	0.451	1.214	0.641-2.297	0.552
Donor age (≥ median vs < median)	0.992	0.485-2.031	0.983	1.147	0.611-2.155	0.669
Donor-recipient gender match						
Female to male vs others	0.909	0.416-1.985	0.810	0.965	0.489-1.905	0.918
Donor-recipient ABO match						
Mismatch vs match	1.196	0.575-2.488	0.631	1.415	0.751-2.667	0.283
Diagnosis (ALL vs others)	0.979	0.434-2.205	0.959	1.463	0.749-2.856	0.265
Time interval between diagnosis and HSCT						
≥6 m vs <6 m	0.559	0.266-1.176	0.125	0.593	0.311-1.133	0.114
Cytogenetic risk						
High vs favorable/intermediate	0.593	0.243-1.452	0.253	1.135	0.575-2.242	0.715
Disease status at HSCT						
Untreated/refractory/relapsed vs CR	1.351	0.579-3.151	0.486	1.262	0.599-2.661	0.540
Disease risk index						
High/very high vs low/intermediate	0.821	0.366-1.846	0.634	1.341	0.696-2.583	0.381
MNC (≥ median vs <median)	0.875	0.427-1.794	0.716	0.881	0.469-1.653	0.693
CD34 (≥ median vs <median)	1.018	0.496-2.092	0.960	1.402	0.742-2.648	0.298

ATG – anti-thymocyte globulin; HSCT – hematopoietic stem cell transplantation; ALL – acute lymphoblastic leukemia; CR – complete remission; MNCs – mononuclear cells; OS – overall survival; DFS – disease-free survival.

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