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ORIGINAL RESEARCH

Impact of Low-Dose rATG Prior to Matched Sibling Donor Hematopoietic Stem Cell Transplantation for Hematologic Malignancies: Reduced Risk of Chronic Graft-versus-Host Disease and Improved Survival Outcomes

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Purpose: To explore the efficacy of low-dose rabbit antithymocyte globulin (rATG) in matched sibling donor hematopoietic stem cell transplantation (MSD-HSCT) for patients with acute leukemia or myelodysplastic syndrome.

Patients and Methods: We performed a retrospective study of 79 patients with hematologic malignancies who received MSD-HSCT. All patients received standard graft-versus-host disease (GVHD) prophylaxis comprising cyclosporine, mycophenolate mofetil and short-term methotrexate. Among them, 38 were administered 5 mg/kg rATG as part of GVHD prophylaxis. Clinical outcomes including overall survival (OS), GVHD and relapse were analyzed.

Results: No graft failure occurred in the antithymocyte globulin (ATG) or non-ATG group. The cumulative incidences of grade 2–4 and 3–4 acute GVHD at day +100 were 13.3% versus 19.5% (p=0.507) and 5.7% versus 15.2% (p=0.196), respectively. The 2-year cumulative incidences of chronic GVHD (cGVHD) were 35.4% and 60.4% (p=0.039), and those of extensive cGVHD were 12.9% and 40.0% (p=0.015), respectively. In a multivariate analysis, the use of low-dose rATG was an independent protective factor for extensive cGVHD (hazard ratio [HR] 0.256; 95% confidence interval [CI], 0.080 to 0.822, p=0.022). The 2-year OS was 88.1% and 68.4% (p=0.038), respectively, and the use of low-dose rATG was the only protective factor in the multivariate analysis (HR 0.216; 95% CI, 0.059 to 0.792, p=0.021). There was no significant difference between the two groups in terms of the 2-year cumulative incidence of relapse, leukemia-free survival or GVHD-free and relapse-free survival.

Conclusion: Low-dose rATG used in MSD-HSCT as part of the conditioning regimen results in a reduced incidence of cGVHD and improves survival outcomes.

Keywords: low-dose rATG, hematopoietic stem cell transplantation, matched sibling donor, graft-versus-host disease

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Introduction

With the optimization of transplantation regimens and progress achieved in supportive measures, allogeneic hematopoietic stem cell transplantation (allo-HSCT) has provided more opportunities for the therapy of multiple malignant hematological diseases. Graft-versus-host disease (GVHD), which is one of the most common complications after allo-HSCT, is closely related to long-term morbidity, mortality

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and quality of life.[1,2] Despite the emergence of new therapeutic approaches, the response of GVHD to existing treatment is still very limited. Chronic GVHD (cGVHD) can be effectively controlled in only approximately 50% of the patients after initial systemic treatment.[3] Considering the above situation, prophylaxis is the best therapy for GVHD and the key to improving patient prognosis and quality of life.[4]

Extensive exploration and research have been performed in the prophylaxis of GVHD in the past decade, including either pharmacological use of a calcineurin inhibitor in combination with methotrexate (MTX), the mainstream regimen in clinical practice, or T cell depletion. Currently, there are more than 20 GVHD prophylaxis therapies for clinical use worldwide.[5] Despite these advances, the incidence of acute GVHD (aGVHD) is still 30–50%, and that of severe aGVHD (grades 3–4) is approximately 15%[6]; in addition, the incidence of cGVHD is 30–70%.[2]

Reforming traditional prophylaxis has turned out limited improvement in the prevention of GVHD.[7,8] At the end of the 20th century, Resnick et al took the lead in the application of antithymocyte globulin (ATG) or antilymphocyte globulin (ALG) in conditioning regimens.[9] Subsequent studies have reported that the addition of ATG as part of a conditioning regimen reduces the incidence of cGVHD and/or aGVHD and improves the overall survival (OS) without increasing the risk of relapse in unrelated donor hematopoietic stem cell transplantation (URD-HSCT) and haploidentical donor hematopoietic stem cell transplantation (HID-HSCT).[10–12] However, in matched sibling donor hematopoietic stem cell transplantation (MSD-HSCT), which is the best choice of allo-HSCT,[13] even though ATG showed superiority over posttransplantation cyclophosphamide (PT-Cy) in aGVHD and cGVHD prophylaxis without increasing nonrelapse mortality (NRM) in some studies (17.6% versus 26%, 19% versus 47%, and 13% versus 14%),[14,15] its efficacy and safety are yet to be confirmed. Moreover, there is currently no unanimously accepted standard dosage of ATG in the clinic, which may profoundly influence the balance between GVHD and graft-versus-leukemia (GVL) effects.[16] Our transplant center has included rabbit antithymocyte globulin (rATG) in the MSD-HSCT regimen since 2010. Compared to the traditional total dose of 7.5–10 mg/kg in the classic regimen for HID-HSCT,[17,18] we tried a lower dose of 5 mg/kg in total for MSD-HSCT. In this study, we performed a retrospective cohort analysis to evaluate the effect of low-dose rATG on patients who received MSD-HSCT through various outcome parameters, including GVHD occurrence, relapse, and survival.

Materials and Methods

Patients

Consecutive patients (79) aged over 16 years who received their first MSD-HSCT for malignant hematological diseases in the Department of Hematology, Peking University First Hospital between October 2009 and November 2018 were enrolled in this study. No restrictions were applied in terms of remission status or comorbidities. Patients were divided into two groups according to whether they were administered rATG at a cumulative intravenous dose of 5 mg/kg divided over 3 days, starting on day 3 before hematopoietic stem cell transplantation (HSCT). There were 38 patients in the ATG group and 41 patients in the non-ATG group in this study. General data of the patients and donors of the two groups are listed in Table 1. The hematopoietic cell transplant comorbidity index (HCT-CI) was used to evaluate patients' pre-HSCT conditions.[19] The disease risk index (DRI), a combined index considering disease risk, which is determined by the disease and cytogenetics, and stage risk, which is determined by the disease status at transplantation, was used to synthetically evaluate the disease risk.[20]

Acute graft-versus-host disease was defined as GVHD that appeared within 100 days after transplantation. All patients with successful engraftment were included in the aGVHD analysis. Those who survived more than 100 days after transplantation were included in the cGVHD analysis. We ultimately compared all levels of cGVHD and extensive cGVHD between 35 ATG group and 37 non-ATG group patients and the relapse rate between 36 ATG group and 38 non-ATG group patients. The characteristics of the patients are summarized in Table 2.

Transplant Procedure

All 79 patients and donors were matched at loci of HLA-A, HLA-B, HLA-C, DRB1, and DQB1 by means of high-resolution polymerase chain reaction and sequence-based typing methods. The conditioning regimens included busulfan (Bu)/cyclophosphamide (Cy), total body irradiation (TBI)/Cy or Bu/fludarabine (Flu) and were administered according to the type of disease. Granulocyte colony-stimulating factor (G-CSF) was used to mobilize stem cells in donors at a dose

Table 1 Clinical Features of Recipients and Donors

Variables	ATG Group (n=38)	Non-ATG Group (n=41)	*P* value
Patient's age, median, years (range)	42 (17–63)	37 (12–59)	0.310
Patient's age, n (%)			0.220
≤40 years	17 (44.7)	24 (58.5)	
>40 years	21 (55.3)	18 (41.5)	
Gender, n (%)			0.587
Male	19 (50)	23 (56.1)	
Female	19 (50)	18 (43.9)	
Time between diagnosis and HSCT, months			0.426
Median (range)	6 (3–53)	6 (2–11)	
Diagnosis, n (%)			0.018
MDS/AML	30 (78.9)	22 (53.7)	
ALL	8 (21.1)	19 (46.3)	
Cytogenetic risk, n (%)			0.760
Low	1 (2.6)	0(0.0)	
Moderate	30 (78.9)	31 (75.6)	
High	7 (18.4)	6 (14.6)	
Missing	0 (0.0)	4 (9.8)	
Disease status at transplantation, n (%)			0.486
CR1	25 (65.8)	32 (78.0)	
CR2	1 (2.6)	2 (4.9)	
PR	5 (13.2)	2 (4.9)	
NR or relapse or untreated MDS	7 (18.4)	5 (12.2)	
Stage risk, n (%)			0.898
Low (any CR/PR/ untreated MDS)	33 (86.8)	36 (87.8)	
High (NR/active relapse)	5 (13.2)	5 (12.2)	
DRI, n (%)			0.831
Moderate	29 (76.3)	29 (70.7)	
High	7 (18.4)	7 (17.1)	
Very High	2 (5.3)	1 (2.4)	
Missing	0 (0.0)	4(9.8)	
With CNSL, n (%)	3 (7.9)	2 (4.9)	0.930
HCT-CI, n (%)			0.842
0	17 (44.7)	21 (51.2)	
1–2	14 (36.8)	13 (31.7)	
≥3	7 (18.4)	7 (17.1)	
Conditioning regimen, n (%)			0.607
TBI/Cy	4 (10.5)	7 (17.1)	
Others(Bu/Flu or Bu/Cy)	34 (89.5)	34 (82.9)	
Donor's age, median, years (range)	39.5 (22–62)	39.0(10–57)	0.168
Donor's age, n(%)			0.941
≤40 years	21 (55.3)	23 (56.1)	
>40 years	17 (44.7)	18 (43.9)	
Donor–recipient ABO match, n (%)			0.485
Match	23 (60.5)	25 (61)	
Minor mismatch	6 (15.8)	9 (22.0)	
Major mismatch	7 (18.4)	4 (9.8)	
Bidirectional mismatch	2 (5.3)	3 (7.3)	
Donor–recipient gender match, n (%)			0.082
Female to male vs others	5 (13.2)	12 (29.3)	
Others	33 (86.8)	29 (70.7)	
Graft			0.494
BM+PB	38 (100.0)	39 (95.1)	
PB	0 (0.0)	2 (4.9)	
MNCs, median, × 10^8/kg (range)	10.75 (7.13–20.26)	10.61 (3.76–20.01)	0.941

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin; BM, bone marrow; Bu, busulfan; CNSL, central nervous system leukemia; CR, complete remission; CR1, first complete remission; CR2, second complete remission; Cy, cyclophosphamide; DRI, disease risk Index; Flu, fludarabine; HCT-CI, hematopoietic cell transplant comorbidity index; HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; MNCs, mononuclear cells; no., number of patients; NR, non-remission; PB, peripheral blood; PR, partial remission; TBI, total body irradiation.

of 10 μg/(kg*d) and injected subcutaneously for 4–5 days. Bone marrow stem cells (BMSCs) were collected on the fourth day, and peripheral blood stem cells (PBSCs) were collected on the fifth day. If the number of stem cells was insufficient on the fifth day, a higher dose of G-CSF was injected, and further collection was performed on the sixth day. Mobilized BMSCs and PBSCs were transplanted on the day of collection. All patients received standard GVHD

Table 2 Engraftment, aGVHD, cGVHD, Relapse, and Other Complications After MSD-HSCT

Variables	ATG Group (n=38)	Non-ATG Group (n=41)	*P* value
Graft failure, n (%)	0	0	
Days to engraftment—median (range)			
Absolute neutrophil count ≥0.5 × 10^9/L	12 (8–24)	12 (10–21)	0.980
Platelet count ≥20 × 10^9/L	16 (9–94)	13 (5–31)	0.060
aGVHD within 100 days after transplantation,n (%)	10 (26.3)	10 (24.4)	0.844
Overall grades of aGVHD, n (%)			0.094
0–1	5 (13.2)	2 (4.9)	
2–4	5 (13.2)	8 (19.5)	
3–4	2 (5.3)	6 (14.6)	
Days of cGVHD onset, median (range)	186 (101–416)	164 (101–514)	0.815
Severity according to revised Seattle criteria, n (%)			0.089
No	23 (60.5%	17 (41.5)	
Limited	8 (21.1)	8 (19.5)	
Extensive	4 (10.5)	12 (29.3)	
Missing	3 (7.9)	4 (9.8)	
Days of relapse onset. median (range)	137 (85–1204)	111(48–1376)	0.451
Relapse	7 (18.4)	13 (31.7)	0.153
Missing	2 (5.3)	3 (7.3)	
Cytomegalovirus reactivation, n (%)	18(47.4)	20 (48.8)	0.906
Epstein–Barr virus reactivation, n (%)	16 (42.1)	18 (43.9)	0.721
Post-transplantation lymphoproliferative disorder, n (%)	0	0	
Hemorrhagic cystitis, n (%)	8 (21.1)	4 (9.8)	0.176

Abbreviations: aGVHD, acute graft-versus-host disease; ATG, antithymocyte globulin; cGVHD, chronic graft-versus-host disease; MSD-HSCT, matched sibling donor hematopoietic stem cell transplantation; no., number of patients.

prophylaxis consisting of cyclosporine A (CsA), mycophenolate mofetil (MMF) and short-term MTX. CsA was administered intravenously from day −6 until oral refeeding, at a target concentration of 150 to 250 ng/mL. MMF 500 mg was administered orally twice daily from days −11 to +30. MTX 15 mg/m^2 was administered on day +1, and 10 mg/m^2 was administered on days +3, +5, and +11. Intestinal sterilization and antiviral prophylaxis were routinely administered.

Data Collection

The clinical profiles of patients were obtained through retrospective reviews of hospital files and telephone interviews. The date of neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count ≥0.5×10^9/L. The date of platelet engraftment was defined as the first day of 7 consecutive days with an absolute platelet count ≥20×10^9/L without the aid of transfusion. Diagnosis and grading of aGVHD and cGVHD were performed according to established criteria. aGVHD was classified as grade 0–4.[21,22] cGVHD was classified as "limited" or "extensive" according to the Seattle criteria.[23,24] OS was defined as the time from transplantation to death from any cause or last follow-up. Leukemia-free survival (LFS) was defined as survival with no evidence of relapse or disease progression. Relapse was defined as the reappearance of peripheral blood blast or >5% bone marrow blasts or blasts infiltration in extramedullary sites. GVHD-free and relapse-free survival (GRFS) was defined as the absence of grade 3–4 aGVHD, systemic therapy-requiring cGVHD, relapse, or death throughout the follow-up period. NRM was defined as death from any cause other than relapse.

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 (IBM Corporation, USA). Categorical variables were compared using the chi-squared test, and continuous variables were

compared using Mann–Whitney *U* tests. Time-dependent rates including the cumulative incidence of aGVHD, cGVHD, relapse, OS, LFS and GRFS were calculated using the Kaplan–Meier method and compared using the Log rank test. Multivariate analyses were performed with Cox proportional hazard models. Factors with P values <0.2 in the univariate analyses or acknowledged as meaningful in the clinic were included in the multivariate analysis. $P < 0.05$ was considered significant.

Results

Patient Characteristics

The clinical characteristics of 38 patients in the ATG group and 41 patients in the non-ATG group are listed in Table 1. Compared with the non-ATG group, the ATG group had fewer patients with acute lymphoid leukemia (ALL) (21.1% versus 46.3%, p = 0.018). Moreover, the two groups were well balanced in terms of age, gender, HCT-CI, cytogenetic risk, stage risk, DRI, donor–recipient gender match and infused mononuclear cell count.

Table 3 Cumulative Incidence of aGVHD, cGVHD, Relapse, OS, LFS and GRFS of Patients in Two Groups

Variables	ATG Group (n=38)	Non-ATG Group (n=41)	*P* value
aGVHD within 100 days after transplantation			
Grade 2–4	13.3%	19.5%	0.507
Grade 3–4	5.7%	15.2%	0.196
cGVHD			
2 years overall	35.4%	60.4%	0.039
2 years extensive	12.9%	40.0%	0.015
CIR			
1 year	17.1%	23.9%	0.414
2 years	17.1%	26.7%	0.302
OS			
1 year	91.5%	73.7%	0.044
2 years	88.1%	68.4%	0.038
LFS			
1 year	80.6%	71.1%	0.329
2 years	80.6%	68.4%	0.241
GRFS			
1 year	52.6%	35.9%	0.104
2 years	49.5%	30.8%	0.082

Abbreviations: aGVHD, acute graft-versus-host disease; ATG, antithymocyte globulin; cGVHD, chronic graft-versus-host disease; CIR, cumulative incidence of relapse; GRFS, GVHD-free and relapse-free survival; LFS, leukemia-free survival; OS, overall survival.

Engraftment

All patients demonstrated successful engraftment. Chimerism was monitored by short tandem repeat analysis for donors and recipients of the same gender, and fluorescent in situ hybridization was used for donors and recipients of the opposite gender. All patients reached full donor chimerism. The median times to neutrophil engraftment in the ATG group and non-ATG group were 12 (8–24) and 12 (10–21) days (p=0.980), respectively (Table 2). The median times to platelet engraftment in the ATG group and non-ATG group were 16 (9–94) and 13 (5–31) days (p=0.060), respectively (Table 2).

Acute and Chronic GVHD

We did not observe a significant difference in terms of the cumulative incidence of grade 2–4 aGVHD between the ATG group and the non-ATG group (13.3% versus 19.5%, p=0.507) (Table 3 and Figure 1A). There were also no statistically significant differences in the cumulative incidence of grade 3–4 aGVHD, although there was a trend towards a decreased incidence of severe aGVHD in the ATG group (5.7% versus 15.2%, p=0.196) (Table 3 and Figure 1B). In the univariate analysis, donor–recipient gender disparity (female to male) was associated with the occurrence of aGVHD (p=0.001) (Table 4). This factor was still a significant predictor of grade 3–4 aGVHD in the multivariate analysis (hazard ratio [HR], 6.658; 95% confidence interval [CI], 1.470 to 30.161, p=0.014) (Table 5).

Regarding cGVHD, 12 patients developed cGVHD in the ATG group, including 4 patients with extensive cGVHD. Twenty patients developed cGVHD in the non-ATG group, including 12 patients with extensive cGVHD (Table 2). The median times to onset were 186 (101–416) days and 164 (101–514) days (p=0.815) in the ATG group and non-ATG group, respectively (Table 2). The cumulative incidence of overall cGVHD at 2 years after transplantation was significantly higher in the non-ATG group than in the ATG group (60.4% versus 35.4%, p=0.039) (Table 3 and Figure 1C), and the cumulative incidence of 2-year extensive cGVHD was also lower in the ATG group (12.9% versus 40.0%, p=0.015) (Table 3 and Figure 1D). In the univariate analysis, diagnosis of ALL, absence of ATG, female donor–male recipient and aGVHD were associated with the occurrence of cGVHD (Table 4). In

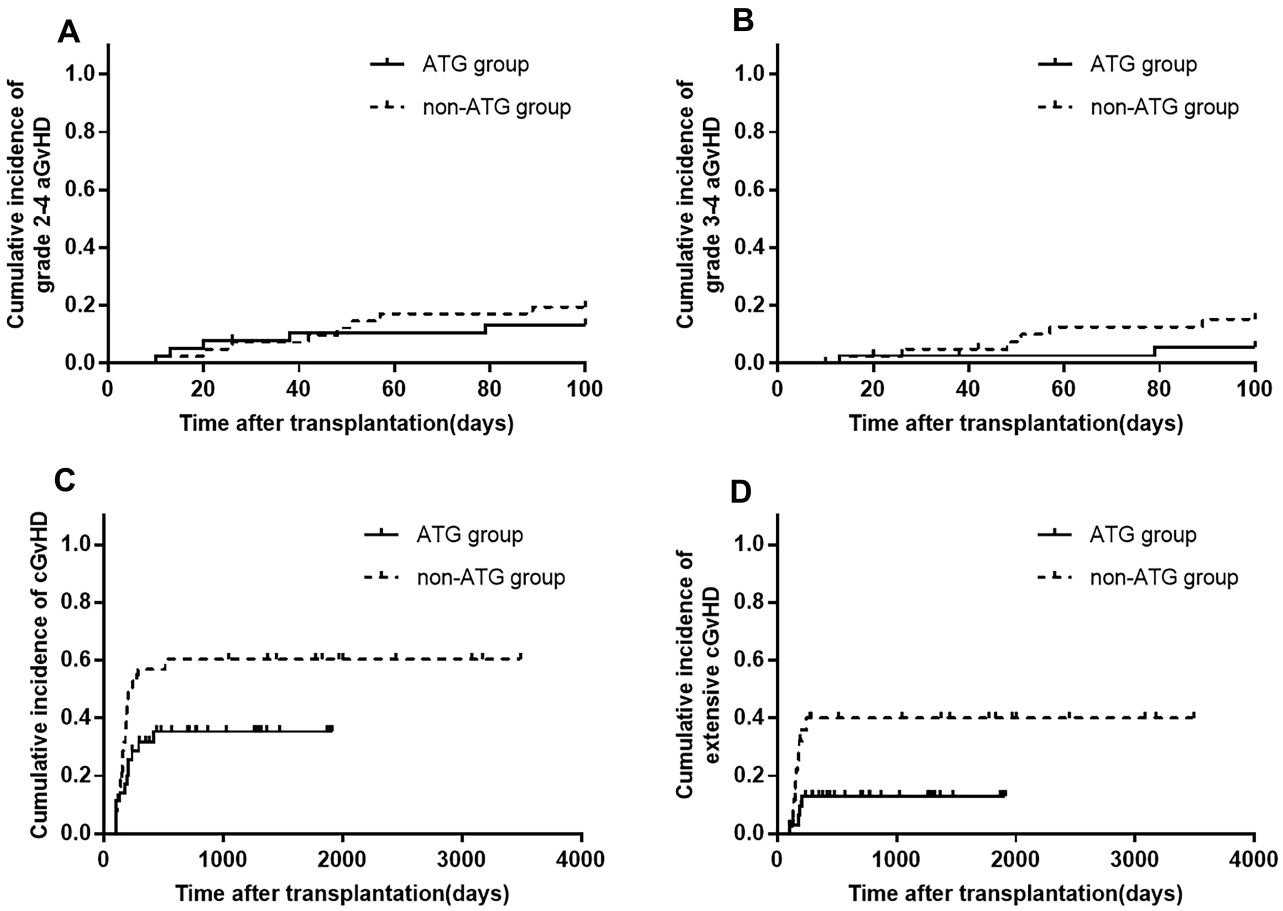

Figure 1 Cumulative incidence of aGVHD and cGVHD after MSD-HSCT with or without low-dose rATG in conditioning regimens. (**A**) The cumulative incidence of grade 2–4 aGVHD was similar between ATG group and non-ATG group (13.3% versus 19.5%, p=0.507). (**B**) The cumulative incidence of grade 3–4 aGVHD demonstrated no statistical significance although there was a trend toward decreasing the incidence of severe aGVHD in ATG group (5.7% versus 15.2%, p=0.196). (**C**) The ATG group had a significantly lower incidence of overall cGVHD than non-ATG group (35.4% versus 60.4%, p=0.039). (**D**) The ATG group had a significantly lower incidence of extensive cGVHD than non-ATG group (12.9% versus 40.0%, p=0.015).

Abbreviations: aGVHD, acute graft-versus-host disease; ATG, antithymocyte globulin; cGVHD, chronic graft-versus-host disease; rATG, rabbit antithymocyte globulin.

the multivariate analysis, use of ATG was the only factor associated with a decreased risk of extensive cGVHD (HR, 0.256; 95% CI, 0.080 to 0.822, p=0.022) (Table 5).

Survival

The median follow-up durations after transplantation among survivors were 774 (61–1912) days and 1420 (53–3544) days in the ATG group and non-ATG group, respectively. At the time of analysis, the 2-year OS was significantly better in the ATG group than in the non-ATG group (88.1% versus 68.4%, p=0.038) (Table 3 and Figure 2A). In the univariate analysis, the addition of ATG was associated with OS benefit (Table 6). In the multivariate analysis, the use of ATG was also a beneficial prognostic factor for OS (HR, 0.216; 95% CI, 0.059 to 0.792, p=0.021) (Table 7). The 2-year LFS of patients in the ATG group and non-ATG group was similar (80.6% versus 68.4%, p=0.241) (Table 3 and Figure 2B). The 2-year GRFS showed a beneficial trend in the ATG group (49.5% versus 30.8%, p=0.082) (Table 3 and Figure 2C).

Relapse and NRM

A total of 7 patients in the ATG group and 13 in the non-ATG group relapsed after transplantation. The median relapse time (137 versus 111 days after transplantation, p=0.451) was similar (Table 2). The 1-year and 2-year cumulative incidence of relapse (CIR) in the ATG and non-ATG groups were not significantly different (17.1% versus 23.9%, p=0.414, and 17.1% versus 26.7%, p=0.302) (Table 3 and Figure 2D). In the univariate and multivariate analyses, no risk factors were identified for the occurrence of relapse or NRM (Tables 6 and 7). NRM occurred in 1 of the 38 patients in the

Table 4 Univariate Analyses for the Risk Factors of Cumulative Incidence of GVHD in All Patients

Variables	aGVHD Grade 2–4		aGVHD Grade 3–4		cGVHD All		cGVHD Extensive	
	%	*P*	%	*P*	%	*P*	%	*P*
Patient's age		0.374		0.953		0.669		0.418
≤40 years	19.7		10.4		48.6		30.5	
>40 years	13.2		10.8		46.9		21.4	
Diagnosis		0.760		0.846		0.067		0.039
ALL	18.5		11.7		64.7		39.1	
Others	15.5		10.3		38.5		18.8	
Cytogenetic risk		0.928		0.460		0.596		0.752
Moderate	16.6		9.0		52.2		28.5	
High	15.4		15.4		37.7		23.8	
Stage risk		0.811		0.312		0.065		0.362
Low	16.1		9.2		52.5		28.6	
High	20.0		20.0		11.1		11.1	
DRI		0.756		0.275		0.425		0.527
Moderate	15.7		7.5		54.5		29.7	
High	14.3		14.3		28.4		12.5	
Very high	33.3		33.3		33.3		33.3	
HCT-CI		0.244		0.753		0.556		0.234
0	10.7		10.7		51.0		35.6	
1–2	18.5		7.9		44.8		21.6	
≥3	28.6		16.1		49.2		11.1	
Conditioning regimen		0.461		0.835		0.241		0.129
TBI/Cy	9.1		9.1		72.2		44.4	
Others	17.8		11.0		44.4		23.1	
ATG		0.507		0.196		0.039		0.015
Yes	13.3		5.7		35.4		12.9	
No	19.5		15.2		60.4		40.0	
Donor's age		0.964		0.341		0.784		0.751
≤40 years	16.1		7.3		44.8		27.3	
>40 years	17.1		14.6		52.1		24.8	
Gender match		0.001		0.001		0.021		0.113
Female to male	41.2		32.7		70.2		39.8	
Others	9.8		5.1		42.0		22.5	
aGVHD								
All grade					68.4	0.008	60.0	0.032
Grade 2–4					66.7	0.015	47.6	0.072
Grade 3–4					85.7	0.000	71.4	0.003

Abbreviations: aGVHD, acute graft-versus-host disease; ALL, acute lymphoblastic leukemia; ATG, antithymocyte globulin; cGVHD, chronic graft-versus-host disease; Cy, cyclophosphamide; DRI, disease risk Index; HCT-CI, hematopoietic cell transplant comorbidity index; TBI, total body irradiation.

ATG group and 3 of the 41 patients in the non-ATG group (Table 8). Among these patients, one patient died of fungal infection in the ATG group, while one patient died of cGVHD and two died of infection in the non-ATG group.

Other Complications

There were no differences between the ATG group and non-ATG group regarding cytomegalovirus (CMV) reactivation (47.4% versus 48.8%, p=0.906), Epstein–Barr virus (EBV) reactivation (42.1% versus 43.9%, p=0.721) or

Table 5 Multivariate Analyses for the Risk Factors of Grade 3–4 aGVHD, All and Extensive cGVHD in All Patients

aGVHD Grade 3–4	**HR**	**95% CI**	***P***
ATG Yes	0.561	0.106–2.971	0.496
Gender match Female to male	6.658	1.470–30.161	0.014
Patient's age >40 years	1.397	0.314–6.218	0.661
Conditioning regimen TBI/Cy	1.085	0.115–10.188	0.943
cGVHD all	**HR**	**95% CI**	***P***
Diagnosis ALL	1.357	0.585–3.149	0.477
Stage risk High	0.268	0.036–2.001	0.199
Conditioning regimen TBI/Cy	1.574	0.537–4.611	0.409
ATG Yes	0.490	0.235–1.019	0.056
Gender match Female to male	1.778	0.792–3.990	0.163
aGVHD All grade	2.176	1.028–4.609	0.042
cGVHD extensive	**HR**	**95% CI**	***P***
Diagnosis ALL	2.081	0.608–7.128	0.243
Stage risk High	0.600	0.072–5.033	0.638
Conditioning regimen TBI/Cy	1.901	0.476–7.595	0.363
ATG Yes	0.256	0.080–0.822	0.022
Gender match Female to male	1.787	0.533–5.997	0.347
aGVHD All grade	2.772	0.953–8.064	0.061

Abbreviations: aGVHD, acute graft-versus-host disease; ALL, acute lymphoblastic leukemia; ATG, antithymocyte globulin; cGVHD, chronic graft-versus-host disease; CI, confidence interval; Cy, cyclophosphamide; HR, hazard ratio; TBI, total body irradiation.

hemorrhagic cystitis (21.1% versus 9.8%, p=0.176) (Table 2). No posttransplantation lymphoproliferative disorders occurred in either group.

Discussion

GVHD is a key factor that affects long-term survival and quality of life after transplantation.[25,26] Despite the emergence of new therapeutic and supportive approaches to GVHD in the past decade, treatment outcomes have been only moderately improved. Initial systemic treatment is effective in approximately 50% of cGVHD patients, and the failure-free survival rate after second-line treatment is less than 60% at 6 months.[3] Therefore, GVHD prophylaxis is the key to improving the prognosis of HSCT patients.[4]

Pharmacological GVHD prophylaxis in the form of the calcineurin inhibitor CsA in combination with MTX is currently the main regimen used in clinical practice; however, it imparts significant toxicities, including nephrotoxicity, neurotoxicity, hypertension, seizures, hypertrichosis, mucositis, etc.[5,27] Replacing CsA with tacrolimus does not significantly reduce the occurrence of GVHD, suggesting that optimizing conventional immunosuppressive regimens has limited benefit for enhancing the prevention of GVHD.[7,8] Another strategy employed as GVHD prophylaxis is T cell depletion in vitro, which may result in a higher incidence of graft failure and relapse, delayed immune reconstitution, and a higher incidence of CMV or EBV reactivation at the same time.[28] Some studies have shown that the use of PT-Cy can reduce the occurrence of aGVHD and cGVHD,[29,30] but its disadvantage lies in the uncertainty of drug dosage and duration of administration. Excessive dosage will increase the risk of delayed immune reconstruction, infection or relapse.[31]

Given these studies and efforts, the incidence of aGVHD remains at 30–50%, and that of severe aGVHD (grades 3–4) is approximately 15%; in addition, the incidence of cGVHD is 30–70% after allo-HSCT.[2,6] Even for MSD-HSCT, which avoids the influence of HLA incompatibility (an important factor leading to the occurrence of GVHD and the reason why MSD-HSCT is generally believed the best choice for allo-HSCT, the incidences of aGVHD and cGVHD are 45–70% and 30–70%, respectively.[32]

At the end of the 20th century, Resnick et al took the lead in the application of the nonmyeloablative stem cell transplantation conditioning regimen based on ATG or ALG.[9] Although there are different impacts of ATG across different donor types, notable results of existing clinical studies have confirmed the benefits of the addition of ATG as part of a conditioning regimen in many types of HSCT. Walker et al conducted a randomized open-label

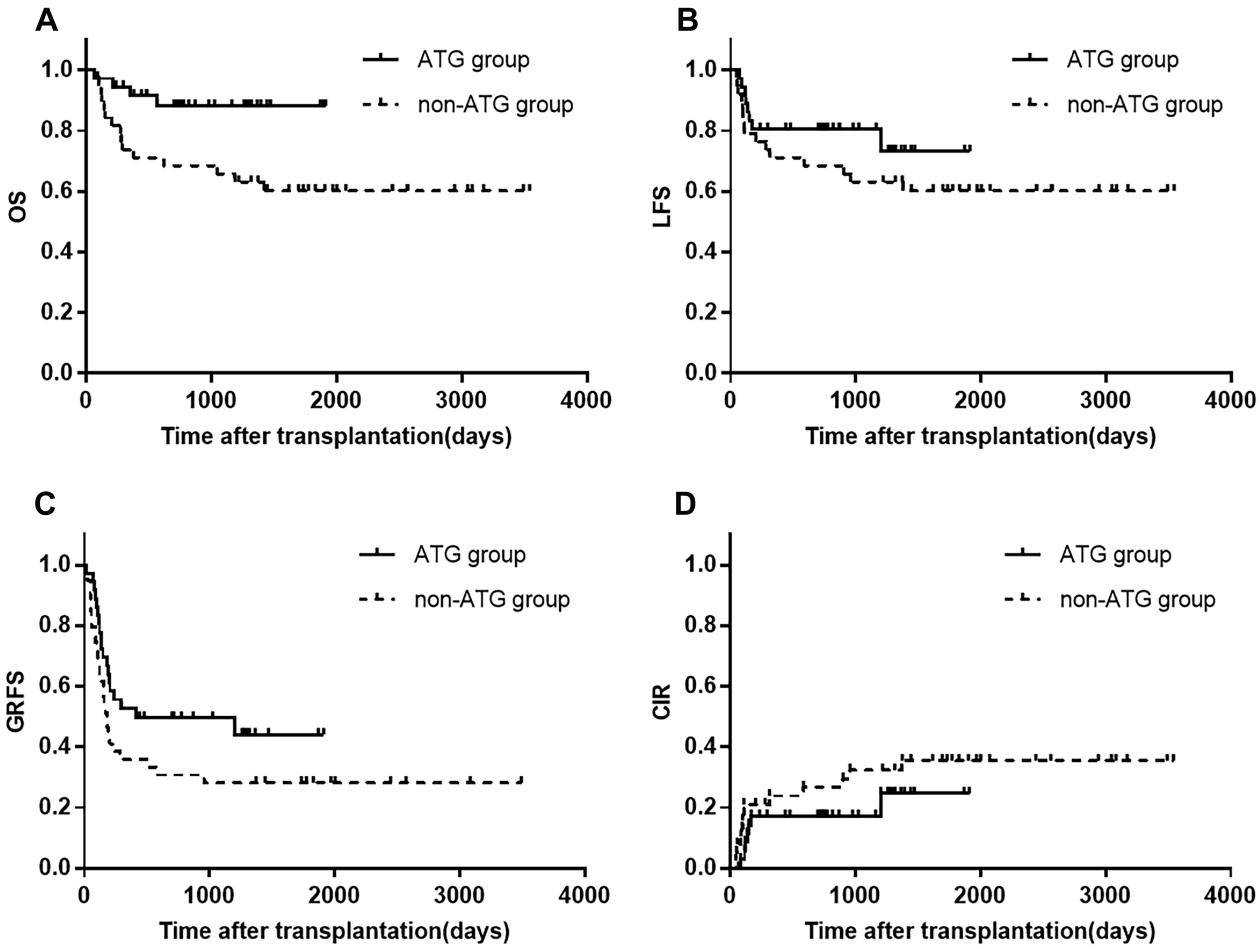

Figure 2 Cumulative incidence of OS, LFS, GRFS and CIR after MSD-HSCT with or without low-dose rATG in conditioning regimens. (**A**) The 2-year OS was significantly improved in ATG group than in non-ATG group (88.1% versus 68.4%, p=0.038). (**B**) The 2-year LFS of ATG group and non-ATG group were similar (80.6% versus 68.4%, p=0.241). (**C**) The 2-year GRFS showed a beneficial trend in the ATG group (49.5% versus 30.8%, p=0.082). (**D**) There was no significant difference between the two groups in 2-year CIR (17.1% versus 26.7%, p=0.302).

Abbreviations: ATG, antithymocyte globulin; CIR, cumulative incidence of relapse; GRFS, GVHD-free and relapse-free survival; LFS, leukemia-free survival; no., number of patients; OS, overall survival; rATG, rabbit antithymocyte globulin.

multicenter study of 203 patients with hematological malignancy given matched or one-locus-mismatched URD-HSCT and found that compared with the standard GVHD prophylaxis of CsA or tacrolimus plus MTX or MMF, addition of ATG at a dose of 4.5 mg/kg brought a lower 2-year cumulative incidence of cGVHD (26.3% versus 41.3%, p=0.032), an improved 2-year OS (70.6% versus 53.3%, p=0.022) and an improved 1-year GRFS (45.4% versus 24.7%, p=0.0034), and no significant difference was observed in relapse or NRM between groups.[10] In a retrospective analysis of 268 patients undergoing HID-HSCT by El-Cheikh et al, the results showed that addition of 2.5/5 mg/kg ATG led to lower incidence of grade II–IV aGVHD (12% versus 22%, p=0.029), a higher 1-year OS (79% versus 69%, p=0.029) and a higher 1-year NRM (8% versus 23%, p=0.005) than PT-Cy only, with no significant difference in cGVHD, relapse, LFS, or GRFS between the groups.[12] For matched sibling donor peripheral blood stem cell transplantation (MSD-PBSCT), a prospective multicenter study of 168 patients by Kröger et al pointed out that the addition of 10 mg/kg ATG 3 days before MSD-PBSCT could reduce the incidence of cGVHD.[33] The 2-year cumulative incidences of cGVHD was 32.2% versus 68.7% (p<0.001), and the 2-year cumulative incidence of extensive cGVHD was 7.6% versus 52.4% (p<0.001). There was no statistically significant difference in the incidence of grade 2–4 aGVHD. Recently, a prospective multicenter open-label randomized controlled clinical trial by Chang et al showed that addition of 4.5 mg/kg ATG in MSD-HSCT, sources of graft including peripheral blood and bone marrow and peripheral blood + bone marrow, could reduce not only

Table 6 Univariate Analyses for the Risk Factors of OS, LFS, GRFS and CIR in All Patients

Variables	OS		LFS		GRFS		CIR	
	%	*P*	%	*P*	%	*P*	%	*P*
Patient's age		0.290		0.611		0.795		0.773
≤40 years	73.7		67.4		34.4		20.7	
>40 years	68.0		63.9		37.4		31.1	
Diagnosis		0.683		0.537		0.236		0.547
ALL	71.1		68.2		21.8		28.5	
Others	71.3		64.8		42.0		31.9	
Cytogenetic risk		0.381		0.639		0.506		0.226
Moderate	70.7		65.9		34.1		29.3	
High	90.0		80.0		54.5		40.0	
Stage risk		0.196		0.363		0.764		0.226
Low	73.0		66.9		34.0		29.3	
High	54.9		60.0		50.0		40.0	
DRI		0.674		0.525		0.718		0.444
Moderate	71.1		65.8		32.3		30.0	
High	71.6		63.6		50.0		36.4	
Very high	100		100		66.7		0.0	
HCT-CI		0.844		0.741		0.880		0.461
0	64.3		62.8		38.0		32.3	
1–2	75.0		63.1		29.5		36.9	
≥3	78.6		78.6		35.7		15.4	
Conditioning regimen		0.749		0.504		0.643		0.658
TBI/Cy	78.8		78.8		25.0		21.2	
Others	69.8		64.0		37.1		32.3	
ATG		0.021		0.241		0.094		0.300
Yes	88.1		73.2		44.0		24.7	
No	60.2		60.2		28.2		35.6	
Donor's age		0.357		0.636		0.502		0.855
≤40 years	70.2		65.8		39.0		32.5	
>40 years	69.7		65.3		31.9		29.0	
Gender match		0.256		0.105		0.034		0.321
Female to male	59.1		46.4		11.7		45.2	
Others	74.1		71.5		41.4		27.2	
aGVHD								
All grade	85.0	0.267	61.3	0.695			30.0	0.859
Grade 2–4	76.9	0.888	51.3	0.241			35.9	0.713
Grade 3–4	62.5	0.343	50.0	0.260			25.0	0.964
cGVHD								
Overall	75.6	0.439	73.8	0.232			20.9	0.084
Extensive	68.6	0.819	70.0	0.678			19.2	0.204

Abbreviations: CIR, cumulative incidence of relapse; GRFS, GVHD-free and relapse-free survival; LFS, leukemia-free survival; no., number of patients; OS, overall survival.

Table 7 Multivariate Analyses for the Risk Factors of OS, LFS, GRFS and CIR in All Patients

OS	HR	95% CI	P
Diagnosis ALL	0.544	0.168–1.767	0.311
Stage risk High	1.638	0.457–5.871	0.449
ATG Yes	0.216	0.059–0.792	0.021
aGVHD Grade 3–4	1.151	0.249–5.309	0.857
cGVHD Extensive	1.059	0.308–3.647	0.927
LFS	**HR**	**95% CI**	***P***
Diagnosis ALL	0.783	0.294–2.089	0.626
Gender match Female to male	3.158	1.086–9.186	0.035
ATG Yes	0.517	0.191–1.398	0.193
aGVHD Grade 3–4	0.882	0.213–3.653	0.862
cGVHD Overall	0.383	0.137–1.066	0.066
GRFS	**HR**	**95% CI**	***P***
Diagnosis ALL	1.330	0.666–2.658	0.419
Gender match Female to male	1.931	1.015–3.676	0.045
Conditioning regimen TBI/Cy	1.006	0.392–2.586	0.990
ATG Yes	0.658	0.362–1.195	0.169
CIR	**HR**	**95% CI**	***p***
Diagnosis ALL	0.757	0.234–2.450	0.642
Stage risk High	0.595	0.074–4.809	0.626
Cytogenetic risk High	1.087	0.223–5.297	0.918
ATG Yes	0.544	0.171–1.728	0.302
cGVHD Overall	0.428	0.138–1.330	0.142

Abbreviations: aGVHD, acute graft-versus-host disease; ALL, acute lymphoblastic leukemia; ATG, antithymocyte globulin; cGVHD, chronic graft-versus-host disease; CI, confidence interval; CIR, cumulative incidence of relapse; Cy, cyclophosphamide; GRFS, GVHD-free and relapse-free survival; HR, hazard ratio; LFS, leukemia-free survival; OS, overall survival; TBI, total body irradiation.

Table 8 Causes of Death

	ATG Group	Non-ATG Group
Death for all reason	4	15
Relapse	3	12
Non-relapse	1	3

Abbreviation: ATG, antithymocyte globulin.

the risk of overall cGVHD (27.9% versus 52.5%, p<0.001) and extensive cGVHD (8.5% versus 23.2%, p=0.029) but also the risk of grade 2–4 aGVHD (13.7% versus 27.0%, p=0.007).[34] However, in regard to survival and efficacy, Kröger et al and Chang et al observed no significant between-group differences in relapse, NRM, LFS or OS, while GRFS in the study of Chang et al was much better in the ATG group than that in the non-ATG group (38.7% versus 24.5%, p=0.003). In cord blood transplantation (CBT), the use of ATG may need further confirmation and exploration. Several studies of adult patients receiving CBT have indicated that ATG increases NRM and decreases OS and GRFS, with unclear protective effects on aGVHD and cGVHD.[35,36] Above all, we can see that the effect of ATG in reducing the risk of cGVHD is most obvious in MSD-HSCT (reduced by more than 45%), especially in extensive cGVHD (reduced by more than 60%).

Our center has used low-dose rATG as part of conditioning regimens for MSD-HSCT since 2010. The cumulative incidence of cGVHD, especially the extensive cGVHD, was effectively controlled to some extent, and the OS was significantly better than that in the non-ATG group. Our results are consistent with the conclusions from most other centers that the addition of ATG during the

pretreatment of MSD-HSCT does not significantly reduce the risk of aGVHD but provides a benefit in reducing the incidence of cGVHD. The risk of relapse did not increase, and the OS was effectively improved, as were LFS and GRFS, although no statistical significance was found in the univariate or multivariate analyses (Tables 6 and 7 and Figure 2A–D). We have noticed that the benefit of ATG in preventing aGVHD after MSD-HSCT was once considered controversial,[15,33] but it has been confirmed recently in the study of Chang et al.[34] Considering that the transplant procedures, including conditioning regimen and GVHD prophylaxis, were almost the same in our two studies, we analyzed whether the difference in the aGVHD result might be partly due to patient age (40–60 years old), which is thought to be associated with an increased risk of aGVHD.[32]

Although many studies have confirmed the protective effects of ATG, the issue is that an insufficient dose of ATG cannot exert an effective immunosuppressive function, while high-dose ATG will produce an inhibitory effect on host immune function, resulting in delayed immune reconstruction, increasing the risk of infection and relapse, and ultimately negatively affecting survival.[16] Devillier et al conducted a study of 87 patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) who received MSD-PBSCT to compare the efficacy of two doses of rATG and found that when the rATG dose was raised from 2.5 mg to 5 mg/kg, GVHD was significantly reduced without increasing the relapse rate.[37] A retrospective analysis by Crocchiolo et al also showed that, compared with the dose of 2.5 mg/kg, ATG at a dose of 5 mg/kg was significantly correlated with reduced incidence and severity of GVHD without impairing disease control.[38] Collectively, these data confirm that the optimal dose of ATG is approximately 5 mg/kg, and our results also support the rationality of rATG at this dose.

Conclusion

In summary, our data suggest that rATG is efficacious and safe at a total dose of 5 mg/kg administered over 3 days (day −3 to −1) in MSD-HSCT for both GVHD prophylaxis and survival improvement without increasing the risk of other complications or decreasing the GVL effect. However, as this was a small-sample retrospective study, there are obvious limitations of our research that should be considered when interpreting our results. The optimal timing and dosing of rATG and a rational strategy for drug concentration monitoring still warrant further investigation.

Abbreviations

aGVHD, acute graft-versus-host disease; ALG, antilymphocyte globulin; ALL, acute lymphoblastic leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplantation; AML, acute myeloid leukemia; ATG, antithymocyte globulin; BM, bone marrow; BMSCs, bone marrow stem cells; Bu, busulfan; CBT, cord blood transplantation; cGVHD, chronic graft-versus-host disease; CI, confidence interval; CIR, cumulative incidence of relapse; CMV, cytomegalovirus; CNSL, central nervous system leukemia; CR, complete remission; CR1, first complete remission; CR2, second complete remission; CsA, cyclosporine A; Cy, cyclophosphamide; DRI, disease risk Index; EBV, Epstein–Barr virus; Flu, fludarabine; G-CSF, Granulocyte colony-stimulating factor; GRFS, GVHD-free and relapse-free survival; GVHD, graft-versus-host disease; GVL, graft versus leukemia; HCT-CI, hematopoietic cell transplant comorbidity index; HSCT, hematopoietic stem cell transplantation; HID-HSCT, haploidentical donor hematopoietic stem cell transplantation; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; LFS, leukemia-free survival; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MNCs, mononuclear cells; MSD-HSCT, matched sibling donor hematopoietic stem cell transplantation; MSD-PBSCT, matched sibling donor peripheral blood stem cell transplantation; MTX, methotrexate; NR, non-remission; NRM, non-relapse mortality; OS, overall survival; PB, peripheral blood; PBSCs, peripheral blood stem cells; PR, partial remission; PT-Cy, posttransplantation cyclophosphamide; rATG, rabbit antithymocyte globulin; TBI, total body irradiation; URD-HSCT, unrelated donor hematopoietic stem cell transplantation.

Ethics and Consent

In accordance with the Declaration of Helsinki and the approval of Ethics Committee of Peking University First Hospital for research and treatment, a signed informed consent was obtained from all adult patients and from the guardians of minor patients for participation in this study. All data used in this manuscript were anonymized.

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Disclosure

The authors report no conflicts of interest in this work.

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