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RESEARCH ARTICLE

Low-dose thymoglobulin for prevention of chronic graft-versus-host disease in transplantation from an HLA-matched sibling donor

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Abstract

Despite the proven efficacy of anti-T-cell or antithymocyte globulin (ATG) for chronic graft-versus-host disease (GVHD) prevention in transplantation from an unrelated donor, dosing protocols and the effects of ATG on relapse and infection remain controversial. In the setting of transplantation from an HLA-matched sibling (MSD-T), few randomized studies have been conducted. We conducted a prospective, single-center, open-label, randomized study of low-dose thymoglobulin (2.5 mg/kg) for chronic GVHD prevention. A total of 120 patients with acute leukemia were randomly assigned in a 1:1 ratio. After a median follow-up of 27 months, the cumulative incidence of chronic GVHD in the ATG and non-ATG groups was 25.0% and 65.4% ($p < 0.001$), respectively. The ATG group had an increased relapse rate compared with the non-ATG-group (20.0% vs. 9.3%; $p = 0.055$), with risks that differed according to cytogenetic subgroup (high-risk, 29.6% vs. 9.3%, $p = 0.042$; non-high-risk, 12.2% vs. 9.2%, $p = 0.596$). Chronic GVHD-free and relapse-free survival (cGRFS) was higher in the ATG group (46.7% vs. 19.4%; $p = 0.070$), and the difference was significant in a cytogenetic non-high-risk subgroup (45.5% vs. 0%; $p = 0.038$). No differences were observed in other survival outcomes. Improved physical components in quality-of-life scores were observed in the ATG group at 12 months after transplantation. A higher rate of Epstein–Barr virus reactivation was observed in the ATG group (21.8% vs. 5.1%; $p = 0.013$), whereas no between-group differences for other complications. In conclusion, the low-dose thymoglobulin effectively prevented chronic GVHD in MSD-T, resulting in improvement in quality-of-life and cGRFS, whereas the necessity of caution for high-risk acute leukemia.

Byung-Sik Cho and Gi-June Min were equally contributors.

1 | INTRODUCTION

Chronic graft-versus-host disease (GVHD) is a leading cause of late non-relapse mortality (NRM) and poor quality of life (QOL) in long-term survivors after allogeneic hematopoietic stem cell transplantation (allo-HSCT).[1,2] Preventing chronic GVHD without compromising antitumor capacity remains a challenge.[2] Currently, two distinct anti-T-cell or antithymocyte globulin (ATG) products, rabbit (anti-human) anti-T lymphocyte globulin and thymoglobulin are recommended as a part of GVHD prophylaxis.[3] The two formulations, which differ in the cell line used for immunization, reduced the incidence of chronic GVHD after unrelated donor transplantation (URD-T) in randomized controlled trials (RCTs).[4–8] However, the optimal dosing protocols and the effects on relapse, infection, or survival remain controversia.[3] Moreover, in the setting of human leukocyte antigen (HLA)-matched sibling donor transplantation (MSD-T), few RCTs reported the efficacy for prevention of chronic GVHD.[9,10] An RCT by Kroger et al. reported that the use of low-dose Grafalon (30 mg/kg) resulted in a reduced incidence of chronic GVHD without changes in acute GVHD, but with an increased relapse trend.[3,9,11] Given the potential risks of relapse[7,12–14] and infectious complication[15] by in vivo T-cell depletion, the optimal dose of each ATG product warrants further investigation in the setting of MSD-T. We hypothesized that the addition of low-dose thymoglobulin (2.5 mg/kg) to standard GVHD prophylaxis would prevent chronic GVHD without an increase in relapse rates in the setting of MSD-T. Here, we report the results of a randomized study designed to investigate the efficacy of low-dose thymoglobulin to prevent chronic GVHD for patients with acute leukemia who undergo MSD-T.

2 | METHODS

2.1 | Patients and donors

Patients were eligible if they were 19 to 65 years of age, had acute myeloid or lymphoblastic leukemia in complete morphologic remission (CR), and had an ECOG performance score < 2. All donors were matched by high-resolution DNA matching for HLA-A, HLA-B, HLA-C, and HLA-DR alleles. Peripheral blood stem cells were acceptable as a graft source. Cytogenetic risk for each disease was determined by 2017 National Comprehensive Cancer Network guidelines.[16]

2.2 | Transplant procedures

Patients were treated with myeloablative conditioning (MAC) or reduced-intensity conditioning (RIC) regimens by age and/or comorbidities. The MAC regimens consisted of cyclophosphamide 120 mg/kg combined with 1320 cGy of fractionated total body irradiation (TBI) or busulfex (12.8 mg/kg). The RIC regimens consisted of busulfex (6.4 mg/kg) and fludarabine (150 mg/m^2) with 400 cGy of fractionated TBI or busulfex (9.6 mg/kg) and fludarabine (150 mg/m^2). Regimens were determined by the treating physician before random assignment of patients. Immune reconstitution was evaluated by multi-parameter flow cytometry in patients who consented to supply peripheral blood samples at months 1, 3, 6, 9, and 12 after allo-HSCT. This study did not allow to perform prophylactic or pre-emptive donor lymphocyte infusion.

2.3 | GVHD prophylaxis

All patients received GVHD prophylaxis with cyclosporine (with a target serum trough level of 150 to 300 ng/mL) and methotrexate (10 mg/m^2 on days 1, 3, 6, and 11) regardless of the conditioning intensity in order to suit the primary purpose of the study. In the absence of clinical GVHD, ciclosporine was tapered starting on day 60 or later over a minimum of 24 weeks and then discontinued.

2.4 | Randomization and masking

In this prospective, randomized, single-center, open-label, phase III study, patients were randomly assigned to receive or not receive thymoglobulin (Sanofi/Genzyme, Cambridge, MA) at 1.25 mg/kg/day on days −3 and −2. Patients were randomly assigned in a 1:1 ratio, with stratification by refined disease risk index (DRI)[17] and conditioning intensity. A blocked randomization schedule was generated with SAS (block size; two, four, and six) by independent coordinators in Catholic Medical Center Clinical Research Coordinating Center. The Institutional Review Board of the Catholic Medical Center approved the current study. All analyses were performed according to the Institutional Review Board guidelines and the tenets of the Declaration of Helsinki. This study was registered at the Clinical Research Information Service (KCT0002261).

2.5 | Study end points

The primary end point of this study was the cumulative incidence of chronic GVHD at 2 years as determined by National Institutes of Health (NIH) criteria.[18] The primary end point was analyzed according to the intention-to-treat principle. Secondary end points included engraftment, immune reconstitution, cumulative incidences of acute GVHD,[19] infectious complications, relapse (CIR), and NRM, and disease-free survival (DFS), overall survival (OS), a composite end point of chronic GVHD-free and relapse-free survival (cGRFS), and recipient reported outcomes with the Short Form (36) Health Survey (SF-36).[20] Note, cGRFS is defined as being alive without chronic GVHD and relapse. Neutrophil and platelet engraftment was defined as an absolute neutrophil count of $> 0.5 \times 10^9$/L during the first of three consecutive days or a platelet count of $> 20 \times 10^9$/L without transfusion support during the first of seven consecutive days.

2.6 | Statistical analysis

The sample size was calculated on the basis of expected 2-year rates of chronic GVHD of 30% in the ATG group and 55% in the non-ATG group. We estimated that a sample size of 120 patients (60 patients in each group) would give the study at least 90% power to reject the null hypothesis. Confirmatory testing of the prespecified primary outcome measure, the cumulative incidence of chronic GVHD at 2 years, was performed on the full analysis set at a type I error level of 0.5 (two-sided). All other *p* values are considered descriptively (including those that are not significant). Assuming 5% of lost to follow-up, the planned number of enrolled patients was 126 patients.

Categorical variables were compared using a chi-square test or Fisher exact test and continuous variables were analyzed with a Student *t* test or Wilcoxon rank-sum test. Curves for OS, DFS, and cGRFS were plotted using the Kaplan–Meier method and analyzed with the log-rank test. Multivariate analysis for OS, DFS, and cGRFS included variables with a *p* value < 0.10 (as determined by univariate analysis) that were considered for entry into the model selection procedure based on the Cox proportional hazards model. The cumulative incidence was used to estimate the probability of CIR, NRM, GVHD, and infection, and compared using a Gray test. The CIR and NRM events competed with each other. In other complications, deaths before the occurrence of each complication were treated as competing events. Multivariate analysis for CIR, NRM, GVHD, and

TABLE 1 Clinical characteristics according to ATG use (n = 120)

Characteristics	No ATG (n = 60)	ATG (n = 60)	*p* value
Age, recipient at allo-HSCT, median (range)	44.5 (18–64)	47.5 (20–64)	0.362
Gender, recipient, male (%)	23 (39.0%)	36 (61.0%)	0.018
From female donor to male recipient (%)	17 (28.3%)	16 (26.7%)	0.853
Age, donor at allo-HSCT, median (range)	45.0 (19–66)	47.5 (23–61)	0.783
Refined DRI, high risk	8 (13.3%)	9 (15.0%)	0.793
Cytogenetic risk[a], high risk	25 (41.7%)	27 (45.0%)	0.713
No. of courses of chemotherapy before allo-HSCT	3 (2–6)	3 (2–4)	0.552
Time from diagnosis to allo-HSCT, months	6.8 ± 2.0	6.7 ± 0.9	0.745
Disease type			1.000
AML	33 (55.0%)	33 (55.0%)	
ALL	27 (45.0%)	27 (45.0%)	
ABO-compatibilities			0.760
Match	39 (65.0%)	35 (58.3%)	
Major mismatch	8 (13.3%)	12 (20.0%)	
Major & minor mismatch	6 (10.0%)	7 (11.7%)	
Minor mismatch	7 (11.7%)	6 (10.0%)	
Conditioning regimen			0.613
CY + TBI 1320 cGy	25 (41.7%)	23 (38.3%)	
CY + BU	17 (28.3%)	15 (25.0%)	
FLU + BU	15 (25.0%)	15 (25.0%)	
FLU + BU + TBI 400 cGy	3 (5.0%)	7 (11.7%)	
Conditioning intensity			0.562
MAC	42 (70.0%)	38 (63.3%)	
RIC[b]	18 (30.0%)	22 (36.7%)	
Disease status before HSCT			0.619
CR1	57 (95.0%)	59 (98.3%)	
CR2	3 (5.0%)	1 (1.7%)	
Peripheral blood as stem cell sources	60 (100%)	60 (100%)	1.000
CD34 (10^6/kg) at allo-HSCT (mean ± SE)	6.15 ± 0.37	6.28 ± 0.50	0.837

Note: The proportion of each cell was identical, and the *p* value of chi-square analysis was 1.000.

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, anti-T-cell or antithymocyte globulin; BU, busulfex; CR1, first complete remission; CR2, second complete remission after relapse; CY, cyclophosphamide; DRI, disease risk index; FLU, fludarabine; HSCT, hematopoietic stem cell transplantation; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; TBI, total body irradiation.

[a]Cytogenetic risk was defined according to National Comprehensive Cancer Network consensus guidelines.

[b]RIC consisted of FLU + BU and FLU + BU + TBI 400 cGy.

infection included variables with a p value < 0.1, (as determined by univariate analysis) that were considered for entry into the model selection procedure based on a proportional hazards model for sub-distribution of the competing risk factors. Statistical significance was determined as a p value < 0.05 (two-tailed). All statistics were conducted using SPSS, version 13.0 (SPSS, Inc., Chicago, IL) and

TABLE 2 Treatment outcomes according to ATG use (n = 120)

Characteristics	No ATG (n = 60)	ATG (n = 60)	*p* value
Days of engraftment, median (range)			
Absolute neutrophil count	12 (7–25)	12 (7–23)	0.902
Platelet count	12 (5–35)	14 (9–30)	0.414
Graft failure, no. (%)	0	0	
Chronic GVHD[a]			
Overall grade mild to severe	65.4% (50.8–76.7)	25.0% (14.8–36.5)	<0.001
Overall grade moderate to severe	45.3% (31.6–58.0)	15.0% (7.3–25.2)	0.001
Overall grade severe	11.5% (4.5–22.1)	1.7% (0.1–7.9)	0.057
Day of onset, median (range)	138 (50–706)	125 (45–229)	0.060
Organ involvement			
Skin	10 (26.3%)	0	0.027
Oral mucosa	8 (21.1%)	3 (20.0%)	0.932
Eyes	5 (13.2%)	2 (13.3%)	0.986
Liver	10 (26.3%)	3 (20.0%)	0.630
Gastrointestinal tract	2 (5.3%)	0	0.365
Lung	5 (13.2%)	3 (20.0%)	0.531
Genital	0	1 (6.7%)	0.108
Joints and fascia	4 (10.5%)	0	0.191
Acute GVHD			
Overall grade I–IV	28.3% (17.5–40.1)	36.7% (24.6–48.8)	0.320
Overall grade II–IV	23.3% (13.5–34.7)	31.7% (20.3–43.6)	0.289
Overall grade III–IV	10.0% (4.0–19.2)	15.0% (7.3–25.2)	0.401
Infectious complications			
EBV viral reactivation (≥1000 IU/mL)	5.1% (1.3–12.9)	21.8% (11.8–33.7)	0.013
CMV DNAemia	49.2% (35.6–61.5)	57.1% (43.3–68.8)	0.198
CMV disease	11.8% (5.1–21.4)	5.0% (1.3–12.7)	0.190
Bacterial sepsis	8 (13.3%)	10 (16.7%)	0.609
Fungal infection	16 (26.7%)	11 (18.3%)	0.274
Possible IPA	8 (13.3%)	5 (8.3%)	
Probable IPA	7 (11.7%)	6 (10.0%)	
IA sinus	1 (1.7%)	0	
Pulmonary infection	15 (25.0%)	15 (25.0%)	1.000
Herpes zoster	12 (20.0%)	14 (23.3%)	0.658
HBV reactivation	2 (3.3%)	5 (8.3%)	0.243
Other complications			
Thrombotic microangiopathy	1.7% (0.1–7.9)	3.3% (0.6–10.3)	0.561
Sinusoidal obstruction syndrome	1.7% (0.1–7.9)	0.0% (0.0–0.0)	0.317
Hemorrhagic cystitis, Grade ≥ 2	22.2% (12.5–33.7)	17.0% (8.6–27.7)	0.506
Post-transplantation lymphoproliferative disorder	0	0	

Note: The proportion of each cell was identical, and the p value of chi-square analysis was 1.000.

Abbreviations: ATG, anti-T-cell or antithymocyte globulin; CI, cumulative incidence; CMV, cytomegalovirus; EBV, Epstein Barr Virus; GVHD, graft versus host disease; IA, invasive aspergillosis; IPA, invasive pulmonary aspergillosis.

[a]According to the National Institutes of Health (NIH) criteria for organ involvement, which rates the extent and severity of chronic GVHD for each organ or site and takes organ function into account, the maximum involvement of the organs listed was ≥2 on a scale of 0 to 3, with 0 indicating no disease, 1 mild disease, 2 moderate disease, and 3 severe disease.

R-software (version 3.4.1, R Foundation for Statistical Computing, 2017).

3 | RESULTS

3.1 | Patient characteristics

From April 2017 and January 2019, a total of 121 patients were enrolled, of whom 120 were randomly assigned to the ATG and non-ATG groups (Figure S1) and one patient was excluded due to relapse before randomization. Four patients (three in the ATG group and one in the non-ATG group) withdrew consent after transplant because of study sampling. All four patients were censored at the time of withdrawal. Demographic characteristics of the enrolled patients are listed in Table 1. There were no significant differences between the two groups other than sex; the ATG group comprised more males (61% vs. 39%, $p = 0.018$). The median follow-up period for survivors was 27 months.

3.2 | Engraftment and immune reconstitution

The median time for neutrophil and platelet engraftment was not significantly different between the ATG and non-ATG groups (Table 2). After myeloid recovery, all patients achieved sustained, full donor chimerism (mean 98.8%, range 90%–100%), by day 30 after allo-HSCT with no evident difference between groups ($p = 0.247$). No recipient experienced secondary engraftment failure.

Post-transplantation immune reconstitution in peripheral blood was analyzed in 46 patients of the ATG group and 50 of the non-ATG group who survived for at least 3 months without relapse between 1 and 12 months after transplant (Table S1). The ATG group had significantly fewer CD4 T cells after transplant compared with the non-ATG group, whereas no significant differences were observed in other subsets.

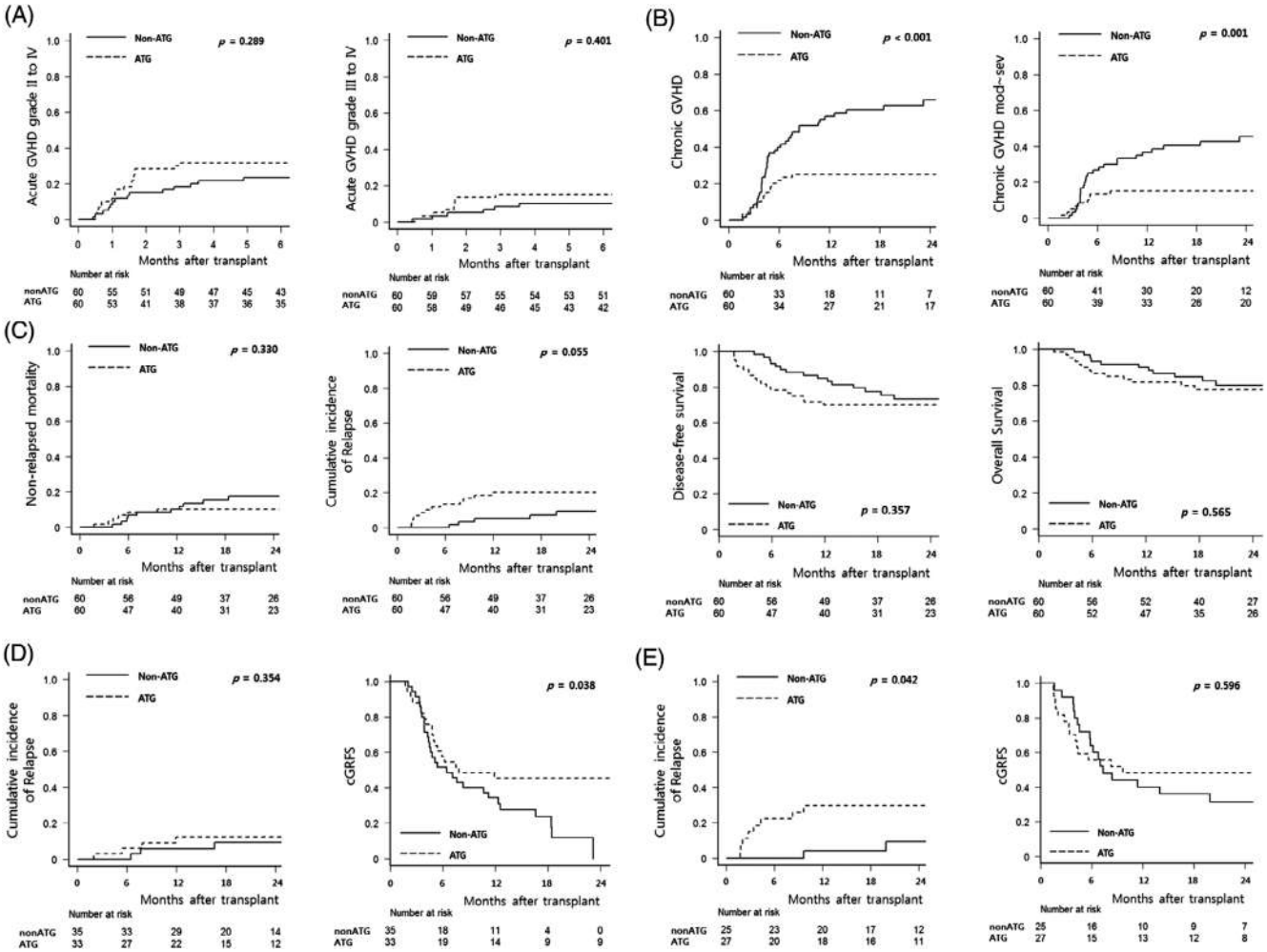

FIGURE 1 Outcomes according to ATG use. (A) Grade II–IV acute GVHD and grade III to IV acute GVHD, (B) chronic GVHD and moderate–severe chronic GVHD, (C) survival outcomes, including non-relapse mortality, relapse, disease-free survival, and overall survival, (D,E) relapse and cGRFS according to ATG use in (D) non-high-risk and (E) high-risk cytogenetic subgroup. ATG, anti-T-cell or antithymocyte globulin; cGRFS, chronic graft-versus-host disease-free and relapse-free survival; GVHD graft-versus-host disease

3.3 | Acute and chronic GVHD

No significant differences in the cumulative incidence of acute GVHD ($p = 0.320$; Table 2) were observed between the ATG and non-ATG groups. The cumulative incidence of grades II to IV acute GVHD for the ATG and non-ATG groups was 31.7% (95% confidence interval [CI], 20.3% to 43.6%) and 23.3% (95% CI, 13.5% to 34.7%), respectively ($p = 0.289$; Figure 1A). No significant differences in grades III and IV acute GVHD were observed between the two groups (15.0% for ATG group versus 10.0% for non-ATG group; $p = 0.401$; Figure 1A). The cumulative incidence of chronic GVHD at 2 years in the ATG and non-ATG groups was 25.0% (95% CI, 14.8% to 36.5%) and 65.4% (95% CI, 50.8% to 76.7%), respectively ($p < 0.001$; Figure 1B). The difference remained significant when adjusted by refined DRI and conditioning intensity (Table S2). The 2-year cumulative incidence of moderate–severe chronic GVHD by NIH consensus criteria was 15% (95% CI, 7.3% to 25.2%) for the ATG group and 45.3% (95% CI, 31.6% to 58.0%) for the non-ATG group ($p = 0.001$; Figure 1B). Twenty (33%) of 60 patients in the ATG group were free from immunosuppressive treatment (IST) at 12 months compared with seven (12%) of 60 in the non-ATG group ($p = 0.004$). Median time to stop all IST without resumption was 5.3 months (range, 2.3–11.8 months) in the ATG group and 9.6 months (range, 4.3–17.8 months) in the non-ATG group. With respect to organ involvement of chronic GVHD, skin involvement was significantly less frequent in the ATG group than non-ATG group (0% vs. 26.3%; $p = 0.027$), but no differences were observed in other organs (Table 2).

3.4 | Toxicity and viral reactivation

The grade > 2 infusion reactions in the ATG group were 33%, and one patient in the ATG group did not complete 2 days of infusion because of an infusion reaction on the second day. The grade > 2 infusion reactions in the ATG group were 42%. The rate of Epstein–Barr virus (EBV) reactivation was significantly higher in the ATG group (21.8%; 95% CI, 11.8% to 33.7%) compared with the non-ATG group (5.1%; 95% CI, 1.3% to 12.9%; $p = 0.013$), whereas no difference was seen in cytomegalovirus reactivation (Table 2). No post-transplantation lymphoproliferative disorder was observed during follow-up. Other infectious complications were not significantly different between the two groups. No difference was found in the incidence of sinusoidal obstruction syndrome, hemorrhagic cystitis, or transplant-associated thrombotic microangiopathy.

3.5 | NRM and relapse

The 2-year cumulative incidence of NRM was 10.0% (95% CI, 4.0% to 19.2%) in the ATG group and 17.5% (95% CI, 8.9% to 28.6%) in the non-ATG group ($p = 0.330$; Figure 1C). Older age was the only significant factor associated with increased NRM ($p = 0.035$; Table S3) and remained significant in multivariate analysis (Table S5). The 2-year CIR was higher in the ATG group (20.0%; 95% CI, 11.0% to 31.0%) than in the non-ATG group (9.3%; 95% CI, 3.3% to 19.0%; $p = 0.055$; Table S3), with risks that differed according to cytogenetic subgroup (non-high-risk, 12.2% vs. 9.2%, $p = 0.596$, Figure 1D; high-risk, 29.6%

TABLE 3 Quality of life scores by ATG use

SF-36 scale	No ATG (n = 52) at 6 months	ATG (n = 45) at 6 months	*p* value	No ATG (n = 47) at 12 months	ATG (n = 40) at 12 months	*p* value
Physical component summary						
Physical Function (PF)	23.48 ± 5.0	22.78 ± 5.1	0.572	22.86 ± 5.6	25.44 ± 5.6	0.061
Role Physical (RP)	5.90 ± 1.7	5.61 ± 1.7	0.478	5.78 ± 1.8	6.84 ± 1.3	0.007
Body Pain (BP)	4.29 ± 2.5	4.08 ± 2.0	0.711	4.59 ± 2.4	3.63 ± 2.0	0.074
General Health (GH)	17.47 ± 3.0	16.56 ± 2.4	0.170	16.76 ± 2.3	15.72 ± 2.1	0.052
Mental component summary						
Vitality (VT)	16.84 ± 2.5	16.31 ± 2.6	0.391	16.30 ± 2.6	15.94 ± 3.1	0.606
Social Function (SF)	5.97 ± 0.7	5.97 ± 1.3	0.986	5.51 ± 1.0	5.94 ± 0.6	0.034
Role Emotion (RE)	4.94 ± 1.3	4.94 ± 1.3	0.978	5.11 ± 1.2	5.41 ± 1.0	0.298
Mental Health (MH)	21.97 ± 3.1	21.14 ± 2.6	0.238	21.00 ± 2.8	21.03 ± 2.9	0.680

Note: The questionnaire was developed from the Medical Outcomes Study Short Form-36 (SF-36) Quality of Life Scale and the Social Support Scale (Korean Version). The SF-36 scale includes eight domains, which are physical functioning (PF), role-physical (RP), body pain (BP), general health (GH), vitality (VT), social functioning (SF), role-emotional (RE), and mental health (MH), and there're 10 items in the Social Support Scale. Values are expressed as means ± SD.

vs. 9.3%, $p = 0.042$, Figure 1E; Table S4). Refined DRI was the only significant factor associated with increased CIR ($p = 0.038$; Table S3) and remained significant in multivariate analysis (Table S5).

3.6 | Survival outcomes

The 2-year probability of DFS was 70.0% (95% CI, 56.6% to 79.9%) in the ATG group and 73.2% (95% CI, 59.3% to 83.0%) in the non-ATG group ($p = 0.357$; Figure 1C). The 2-year probability of OS was 77.5% (95% CI, 64.2% to 86.3%) in the ATG group and 80.0% (95% CI, 66.6% to 88.5%) in the non-ATG group ($p = 0.565$; Figure 1C). Older age was the only significant factor associated with inferior OS ($p = 0.024$; Table S3) and remained significant in multivariate analysis (Table S5). The probability of 2-year cGRFS was 46.7% (95% CI, 33.7% to 58.6%) in the ATG group and 19.4% (95% CI, 9.8% to 31.5%) in the non-ATG group ($p = 0.070$). Improvement in cGRFS was significant in the non-high-risk cytogenetic subgroup (45.5% vs. 0%; $p = 0.038$; Figure 1D), whereas no difference in high-risk cytogenetic subgroup (48.1% vs. 31.5%; $p = 0.596$; Figure 1E; Table S4).

3.7 | Impact of absolute lymphocyte count (ALC) at the time of ATG administration

Given the association of ALC on the first day of ATG administration and post-transplantation globulin pharmacokinetics and transplantation outcomes,[21,22] we conducted an unplanned exploratory analysis that investigated the interaction of ALC on day −3 (at the time of ATG initiation) with the effect of ATG. We dichotomized the ALC level on day −3 (> 0.1 vs. ≤ 0.1×10^9/L) by the median value (0.104×10^9/L; range, 0–3.089×10^9/L) and evaluated outcomes by ATG use in each ALC group. The ATG use reduced chronic GVHD more efficiently in patients with day −3 ALC ≤ 0.1×10^9/L (66.7% vs. 9.7%; $p < 0.001$) than patients with day −3 ALC > 0.1×10^9/L (63.2% vs. 41.4%; $p = 0.261$; Figure S2A). Note, cGRFS was significantly improved by ATG use in patients with day −3 ALC ≤ 0.1×10^9/L (67.7% vs. 18.0%; $p = 0.002$), whereas no differences in patients with day −3 ALC > 0.1×10^9/L (24.1% vs. 21.0%; $p = 0.410$; Figure S2B). We found that TBI conditioning had significant effects on ALC on day −3 with 66.7% of patients who received TBI having an ALC ≤ 0.1×10^9/L compared with 31.8% of patients who received non-TBI regimens ($p < 0.001$; Table S6). Multivariate analysis for the effects of ATG on chronic GVHD and cGRFS adjusted by TBI conditioning in each ALC group confirmed the benefit of ATG in patients with day −3 ALC ≤ 0.1×10^9/L, whereas no benefit in patients with day −3 ALC > 0.1×10^9/L (Table S7). No significant differences of acute GVHD, CIR, NRM, DFS, and OS by ATG use were observed in each ALC group (Figure S3).

3.8 | Quality of life

We obtained responses to an SF-36 questionnaire from recipients surviving without relapse at 6 and 12 months after transplantation (Table 3). Forty (67%) of 60 patients in the ATG group and 47 (78%) of 60 patients in the non-ATG group completed an SF-36 questionnaire at 12 months after transplantation. The reason of the failure to retrieve the QOL form was not different between the two groups (Table S8). No significant between-group differences were evident at 6 months after allo-HSCT. However, at 12 months after allo-HSCT, the ATG group reported better scores for the physical components of the survey, including physical role and function, body pain, and general health, while there were no significant differences in the mental component summary, including vitality, emotional role, and mental health.

4 | DISCUSSION

We performed a prospective, single-center, open-label, randomized study of thymoglobulin (2.5 mg/kg) for prevention of chronic GVHD in MSD-T. The low-dose thymoglobulin significantly reduced the incidence of chronic GVHD and improved QOL without significant impacts on acute GVHD. The benefit was more prominent in patients with acute leukemia without high-risk cytogenetic features, in whom cGRFS was significantly improved by ATG use. In addition, those effects varied according to ALC on the first day of ATG administration. The low-dose ATG reduced chronic GVHD more efficiently and improved cGRFS in patients with low ALC at the time of ATG administration.

The two ATG products currently recommended for GVHD prophylaxis[3] are polyclonal immunoglobulin G antibodies, raised in rabbits by immunization with human thymocytes (thymoglobulin) or with a Jurkat T-cell line (rabbit (anti-human) anti-T lymphocyte globulin).[23] Unlike the proven efficacy of each ATG for chronic GVHD prophylaxis in several RCTs of URD-T,[4–8] only two RCTs were available for MSD-T.[9,10] An RCT by Kroger et al.[9] used a dose of 30 mg/kg of Grafalon, which was lower than the current recommendations for URD-T (60 mg/kg),[3] and in another RCT by Chang et al.,[10] 4.5 mg/kg of thymoglobulin was used, which is the lowest standard dose currently recommended for URD-T (4.5–6.0 mg/kg).[3] Given that each RCT was powered to evaluate different primary end points (chronic GVHD for Kroger et al.[9] and grade II to IV acute GVHD for Chang et al.[10]), both studies demonstrated a reduced incidence of chronic GVHD by each type of ATG, suggesting efficacy of each type of ATG in MSD-T. Our RCT, the first evaluation of low-dose thymoglobulin powered to evaluate the efficacy for chronic GVHD prevention, showed that 2.5 mg/kg thymoglobulin was sufficient to prevent chronic GVHD (25% vs. 65%) in MSD-T as effectively as 4.5 mg/kg thymoglobulin dose reported by Chang et al. (28% vs. 52%).[10] The efficacy was supported by sustained lower numbers of CD4 T cells in the ATG group. Moreover, the current RCT demonstrated improvement in QOL, mainly in the physical domains, following the use of low-dose thymoglobulin, which is consistent with recent long-term data for low-dose Grafalon by Kroger et al.[11] These data provide strong evidence for a benefit of low-dose ATG for chronic GVHD prevention.

Intriguingly, the efficacy of low-dose thymoglobulin for chronic GVHD prevention may be related to an interaction between ALC and

ATG. Previous studies in the setting of URD-T reported the importance of ALC on the first day of ATG administration by affecting the remaining ATG level at transplantation.[7,21,22,24] We found that low-dose thymoglobulin reduced chronic GVHD more efficiently in patients with low ALC at the time of ATG administration. Moreover, the low-dose thymoglobulin significantly improved cGRFS only in patients with low ALC, whereas no differences in patients with high ALC. These data provide evidence for varying ATG effects according to ALC on the first day of ATG administration in MSD-T, and suggest the necessity of higher doses than 2.5 mg/kg thymoglobulin in patients with high ALC to further prevent GVHD and improve cGRFS, which should be confirmed in RCTs.

With respect to acute GVHD prevention, neither current RCT nor Kroger et al.[9] showed significant effects of 2.5 mg/kg thymoglobulin and 30 mg/kg rabbit (anti-human) anti-T lymphocyte globulin in MSD-T, respectively. Both used lower doses than is current recommended for URD-T.[3] However, an RCT by Chang et al. reported that a higher dose of thymoglobulin (4.5 mg/kg) effectively prevented grade II to IV acute GVHD in MSD-T.[10] These findings suggest that a lower dose of ATG may be insufficient to prevent acute GVHD in MSD-T, which was supported by the reported efficacy of higher doses of ATG, 4.5 mg/kg of thymoglobulin[15] and 60 mg/kg of rabbit (anti-human) anti-T lymphocyte globulin,[5,7] for acute GVHD prevention in URD-T. Doses of between 2.5 and 4.5 mg/kg thymoglobulin and between 30 and 60 mg/kg rabbit (anti-human) anti-T lymphocyte globulin may reduce the incidence of acute GVHD in MSD-T, but this needs to be subjected to RCTs with different dosing regimens.

The major concern associated with T-cell depletion is the potential harm of disease relapse, and chronic GVHD has been correlated with decreased incidence of relapse.[25] The preventive effect of ATG against chronic GVHD did not adversely affect relapse rates in previous RCTs,[5,7,9,10,15] but was associated with a trend of higher relapse rates in some studies,[7,9] given that no previous RCTs were powered to detect difference in relapse by ATG use. An increased risk of relapse by ATG use was reported in one registry trial,[14] but no differences were reported in another registry trial.[26] A recent meta-analysis found an increased risk of relapse following ATG use.[13] In the context of these conflicting results, the current RCT revealed that the addition of low-dose thymoglobulin in MSD-T resulted in increased post-transplant relapse. In exploratory sub-group analysis, the relapse risk was significantly higher in patients with high-risk cytogenetic features, whereas no significant difference was observed in those without such features. This contradicts an RCT by Chang et al. reporting that a higher dose of thymoglobulin did not increase post-transplant relapse.[10] The discrepancy between the two RCTs may be caused by different characteristics of the two cohorts, including patients' age, disease type, stem cell sources, and transplantation procedures. In this context, an RCT by Kroger et al., which involved a cohort similar to ours, showed a trend of increased relapse rate in patients who received low-dose rabbit (anti-human) anti-T lymphocyte globulin .[9,11] Moreover, discrepancies in the 2-years CIR in each group of RCTs of MSD-T, including ours, suggest a need for RCTs powered to detect differences in relapse rates by ATG use. Taken together, the benefit of ATG in MSD-T needs to be cautiously determined in patients with acute leukemia who have high-risk cytogenetic features. With respect to NRM, we did not observe a significant between-group difference in NRM. The benefit of ATG in GVHD prevention did not translate into lower NRM and better OS in previous RCTs.[9,10] Collectively, cGRFS tended to improve in the ATG group, with a significant improvement in patients without high-risk cytogenetic features, supporting the findings of other two RCTs in MSD-T that demonstrated significant improvement by ATG use.[9,10]

With respect to infectious complications, most previous RCTs did not show significant differences by ATG use.[5,7,9,10] However, a higher incidence rate of EBV reactivation was reported in an RCT of thymoglobulin (4.5 mg/kg) in URD-T.[15] The current study with low-dose thymoglobulin also showed that EBV reactivation was more common in the ATG group, with no differences in other infectious complications. Despite no increase in post-transplantation lymphoproliferative disorder in both studies, EBV monitoring and consideration of pre-emptive or prophylactic treatment with rituximab need to be considered.

In conclusion, this study shows that low-dose thymoglobulin effectively prevented chronic GVHD in MSD-T, resulting in improvement in QOL and cGRFS. This finding should be applied with caution to patients with acute leukemia who have high-risk cytogenetic features due to their increased risk of relapse. Further investigation powered to detect differences in relapse as well as GVHD by ATG use is needed for the personalized optimal dosing of thymoglobulin without compromising the graft-versus-leukemia effect.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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