

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Differential impact of anti-thymocyte globulin dosing by disease risk index in alternative donor peripheral blood stem cell transplantation in patients with acute leukemia or myelodysplastic syndrome after reduced intensity conditioning

TO THE EDITOR: Chronic graft-versus-host disease (GVHD), one of the major hurdles in the way of successful hematopoietic cell transplantation (HCT), has increased in incidence with the widespread use of peripheral blood (PB) grafts and alternative donors, along with an increased number of older transplant recipients [1]. Although anti-thymocyte globulin (ATG) plays a protective role against GVHD across various transplant settings, including alternative donor PB HCT with reduced intensity conditioning (RIC), its optimal dosing in a specific transplant platform remains largely unknown [2, 3].

We hypothesized that the impact of different ATG doses can depend on the disease risk index (DRI). The present study aimed to explore this hypothesis by comparing transplant outcomes between total ATG doses of 6 mg/kg and 9 mg/kg in a homogenous population stratified by DRI. These patients received PB grafts from alternative donors after a specified RIC regimen for acute leukemia or myelodysplastic syndrome (MDS).

We retrospectively identified 130 eligible patients who had undergone their first HCT between February 2008 and March 2017 at Seoul National University Hospital (SNUH) and Seoul National University Bundang Hospital (SNUBH). The donors included 10/10 human leukocyte antigen (HLA) allele-matched unrelated donors (MUDs), 7/10 or 8-9/10 partially matched unrelated donors (PUDs), and 3-4/6 or 3-7/8 or 6/10 haploidentical familial donors (HIDs), while the graft source consisted of PB stem cells only. Conditioning included the administration of intravenous busulfan at a dose of 3.2 mg/kg on day D-7 and D-6, fludarabine at 30 mg/m² from D-7 to D-2, and rabbit ATG (Thymoglobulin) at 2.0 or 3.0 mg/kg from D-3 to D-1. Cyclosporine A or tacrolimus were additionally used with or without methotrexate. The study protocol was reviewed and approved by the Institutional Review Boards of SNUH and SNUBH.

Baseline characteristics of included patients are summarized in [Supplementary Table 1](#). The median follow-up period for the total population was 35.00 months [95% confidence interval (CI), 30.34-39.66]. In the total population, the GVHD-free, relapse-free survival (GRFS), disease-free survival (DFS), and overall survival (OS) tended to be longer when using the 6 mg/kg dose than when 9 mg/kg was used, but without statistical significance ([Fig. 1A-C](#)). In 99 patients with low/intermediate DRI, those in the 6 mg/kg

group had significantly higher DFS and OS than those in the 9 mg/kg group, while their GRFS was similar. The estimates of 2-year GRFS, DFS, and OS rates were 31% (95% CI, 23-40) vs. 25% (95% CI, 19-30; $P=0.133$; Fig. 1D), 65% (95% CI, 57-74) vs. 43% (95% CI, 37-49; $P=0.017$; Fig. 1E), and 73% (95% CI, 65-81) vs. 53% (95% CI, 46-59; $P=0.018$; Fig. 1F), for the 6 mg/kg group vs. 9 mg/kg group, respectively. In contrast, for 31 patients with a high or

very high DRI the GRFS, DFS, and OS did not differ significantly between the different ATG doses. The estimates of 2-year GRFS, DFS, and OS rates were 20% (95% CI, 7-33) vs. 29% (95% CI, 19-39; $P=0.999$; Fig. 1G), 20% (95% CI, 7-33) vs. 38% (95% CI, 28-49; $P=0.386$; Fig. 1H), and 30% (95% CI, 16-45) vs. 38% (95% CI, 28-49; $P=0.855$; Fig. 1I), for the 6 mg/kg group vs. 9 mg/kg group, respectively. Grade III-IV acute GVHD at day 100 and chronic GVHD

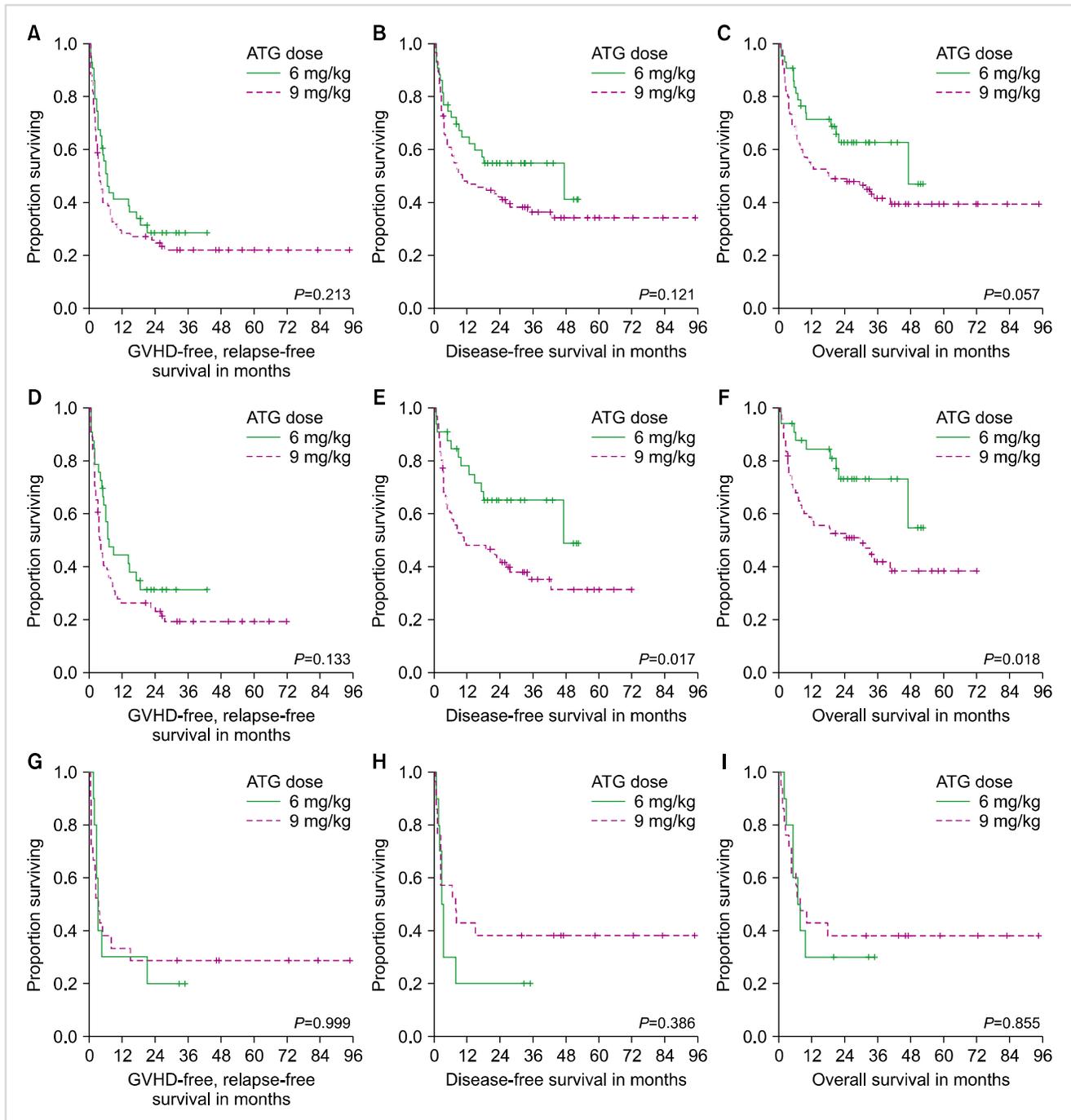


Fig. 1. Graft-versus-host disease (GVHD)-free, relapse-free survival (GRFS), disease-free survival (DFS), and overall survival (OS) by total dose of anti-thymocyte globulin (ATG) in the overall population (A-C), in subgroups with low/intermediate disease risk indices (DRI; D-F), and in subgroups with high/very high DRI (G-I).

Table 1. Cox regression analyses for overall survival.

Parameters	N	Univariable hazard ratio (95% CI)	P	Multivariable hazard ratio (95% CI)	P
Patient age		1.00 (0.98–1.02)	0.979		
Patient sex					
Female	62	1		1	
Male	68	1.92 (1.16–3.16)	0.011	1.85 (1.11–3.09)	0.019
Female-to-male combination					
No	109	1			
Yes	21	1.54 (0.84–2.83)	0.162		
Diagnosis					
AML	78	1			
ALL	19	0.89 (0.43–1.83)	0.747		
MDS	33	1.17 (0.67–2.05)	0.589		
HCT-CI scores		1.33 (1.06–1.67)	0.012	1.36 (1.06–1.76)	0.018
Disease risk index					
Low/intermediate	99	1		1	
High/very high	31	1.65 (0.98–2.80)	0.062	4.14 (1.52–11.26)	0.005
Timing of transplant					
2013–2018	120	1			
2008–2012	10	1.11 (0.48–2.57)	0.814		
Donor type					
MUD	55	1		1	
HID	31	1.22 (0.69–2.16)	0.492	1.29 (0.71–2.33)	0.399
PUD	44	1.30 (0.68–2.48)	0.430	0.87 (0.43–1.75)	0.687
CD34 ⁺ cells ($\times 10^6$)/kg		0.91 (0.82–1.01)	0.084		
ATG total dose					
6 mg/kg	43	1		1	
9 mg/kg	87	1.72 (0.98–3.02)	0.060	2.56 (1.19–5.51)	0.016
ATG total dose \times disease risk index ^{a)}				0.26 (0.08–0.85)	0.026
Calcineurin inhibitor use					
Cyclosporin A	68	1			
Tacrolimus	52	1.00 (0.60–1.68)	0.998		
Methotrexate use					
Yes	79	1			
No	51	0.93 (0.57–1.53)	0.782		

^{a)}Interaction between ATG total dose and disease risk index.

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin; CD34⁺, cluster of differentiation 34-positive; CI, confidence interval; HCT-CI, hematopoietic cell transplantation comorbidity index; HID, haploidentical familial donors; MDS, myelodysplastic syndrome; MUD, matched unrelated donors; OS, overall survival; PUD, partially-matched unrelated donors.

requiring systemic therapy after 2 years of HCT were more frequently noted in the ATG 6 mg/kg group than in the 9 mg/kg group, with a cumulative incidence of 23% vs. 16% ($P=0.023$) and 41% vs. 21% ($P=0.025$), respectively. The cumulative incidence estimates of relapse and non-relapse mortality at a 2-year time point were numerically lower when an ATG dose of 6 mg/kg was used than when 9 mg/kg was used, with the cumulative incidence being 17% vs. 24% ($P=0.517$) and 29% vs. 34% ($P=0.311$), respectively, albeit without statistical significance (Supplementary Table 2).

Subsequent multivariable analyses showed that patient sex (male), a higher HCT-comorbidity index (HCT-CI), the 9 mg/kg ATG dose, and high or very high DRI were independently associated with a worse survival, with hazard

ratios (HRs) of 1.85 (95% CI, 1.11–3.09; $P=0.019$), 1.36 (95% CI, 1.06–1.76; $P=0.018$), 4.14 (95% CI, 1.52–11.26; $P=0.005$), and 2.56 (95% CI, 1.19–5.51; $P=0.016$), respectively, while donor type did not harbor this association. Additionally, the interaction between ATG dose and DRI was a significant predictor of OS, with a HR of 0.26 (95% CI, 0.08–0.85; $P=0.026$; Table 1).

Our findings are consistent with those of previous studies. Remberger *et al.* reported a lower incidence of GVHD and a higher incidence of relapse with an ATG dose of 8 mg/kg compared to 6 mg/kg in RIC HCT from a PB or bone marrow (BM) graft of MUDs, in accordance with our study (Supplementary Table 2) [4]. Chang *et al.* [5] conducted a prospective randomized trial comparing ATG doses of 6 mg/kg and 10 mg/kg in HCT from both PB and BM grafts

from HIDs. These researchers found that although a higher ATG dose was associated with better GVHD prevention, it increased the risk of infectious complications, which was similarly observed in the results of the current analysis (Supplementary Table 2); however, Chang *et al.* [5] only included standard-risk disease and used myeloablative conditioning. A recent Korean study also suggested that ATG doses ranging from 2.5 to 7.5 mg/kg were associated with better survival in comparison to doses ranging from 9 to 12 mg/kg in recipients of mismatched HCT for acute leukemia or MDS [6].

Interestingly, in the present study, the survival benefit of the 6 mg/kg ATG dose over the 9 mg/kg dose was apparent only in patients with low or intermediate DRI, but not in those with high or very high DRI. Indeed, RIC HCTs for hematologic malignancies are expected to exert its main therapeutic effects through graft-versus-leukemia (GVL) effects, and a close association between chronic GVHD and GVL has been noted previously [7, 8]. Because patients with high or very high DRI are at a higher risk of relapse than those with low or intermediate DRI, an ATG dose of 6 mg/kg may have been excessive for them.

Our study has several limitations. First, the retrospective nature of this study may render this analysis hypothesis-generating at most. Second, this study did not address the interaction between ATG dose and donor types. Although donor type was not significantly associated with survival in the multivariable analysis, subtle differences may have not been detected due to the limited number of patients included in the study.

In conclusion, the present study suggests that a total ATG dose of 6 mg/kg is more suitable than that of 9 mg/kg in RIC PB HCT from alternative donors in patients with acute leukemia or MDS and low or intermediate DRI, while those with high or very high DRI may require a more cautious strategy on GVHD prophylaxis.

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Supplementary Methods

Definition

Acute graft-versus-host disease (GVHD) was diagnosed and graded clinically according to the Glucksberg's criteria [1], and chronic GVHD according to the National Institutes of Health Consensus criteria [2]. Relapse was defined by any evidence of the disease after the hematopoietic cell transplantation (HCT), and non-relapse mortality (NRM) was defined as death from any cause except for relapse. The HCT comorbidity index (HCT-CI) was applied according to a previous study by Sorror *et al.* [3]. The disease risk index (DRI) consisted of the disease and stage risk, each of which was derived from a diagnosis with the cytogenetics data and remission status at the time of the HCT, respectively, and has been shown to successfully risk stratify heterogeneous allogeneic transplant recipients [4]. For the

current study, the DRI was collapsed into a 2-group system of low/intermediate and high/very high risk, as proposed by the original study [4].

Statistical endpoints included GVHD-free, relapse-free survival (GRFS), disease-free survival (DFS), and overall survival (OS). GRFS is a composite endpoint encompassing ongoing morbidity from GVHD in addition to relapse and death [5, 6]. GRFS events were defined as grade III-IV acute GVHD, chronic GVHD requiring systemic immunosuppressive treatment, relapse, or death. DFS was calculated as the time from the HCT to relapse or death, and OS was defined as the time from the HCT to death.

Statistical methods

Patient and treatment characteristics were compared with Chi-square test for categorical variables and t-test for continuous variables. Survival curves were plotted using the

Supplementary Table 1. Patients and treatment characteristics.

Variables	Overall (N=130)	By ATG doses		P ^{a)}
		6 mg/kg (N=43)	9 mg/kg (N=87)	
Patient age, median (range)	52 (16–68)	55 (18–67)	52 (16–68)	0.219
Patient sex, N (%)				0.264
Male	68 (52.3%)	19 (44.2%)	49 (56.3%)	
Female	62 (47.7%)	24 (55.8%)	38 (43.7%)	
HCT-CI total scores, median (range)	0 (0–5)	0 (0–4)	0 (0–5)	0.411
Disease, N (%)				0.073
AML	78 (60.0%)	28 (65.1%)	50 (57.5%)	
MDS	19 (14.6%)	2 (4.7%)	17 (19.5%)	
ALL	33 (25.4%)	13 (30.2%)	20 (23.0%)	
Disease risk index				1.000
Low/intermediate	99 (76.2%)	33 (76.7%)	66 (75.9%)	
High/very high	31 (23.8%)	10 (23.3%)	21 (24.1%)	
Time from diagnosis to HCT in mo, median (range)	5.92 (2.07–197.43)	5.46 (2.07–31.50)	6.07 (2.57–197.43)	0.450
Female donor-to-male patient, N (%)	21 (16.2%)	5 (11.6%)	16 (18.4%)	0.464
Donor type				0.008
HID	55 (42.3%)	25 (58.1%)	30 (34.5%)	
PUD	31 (23.8%)	4 (9.3%)	27 (31.0%)	
MUD	44 (33.8%)	14 (32.6%)	30 (34.5%)	
ABO incompatibility				0.592
Matched	48 (36.9%)	16 (37.2%)	32 (36.8%)	
Major mismatch	36 (27.7%)	9 (20.9%)	27 (31.0%)	
Minor mismatch	27 (20.8%)	10 (23.3%)	17 (19.5%)	
Bidirectional	18 (13.8%)	8 (18.6%)	10 (11.5%)	
Infused CD34 ⁺ cells in ×10 ⁶ /kg, median (range)	5.14 (0.97–12.68)	5.59 (1.80–12.68)	4.97 (0.97–12.09)	0.093
CNI use				< 0.001
Cyclosporin A	68 (52.3%)	12 (27.9%)	56 (64.4%)	
Tacrolimus	52 (40.0%)	30 (69.8%)	22 (25.3%)	
Switch	10 (7.7%)	1 (2.3%)	9 (10.3%)	
Methotrexate use				0.005
Yes	79 (60.8%)	34 (79.1%)	45 (51.7%)	
No	51 (39.2%)	9 (20.9%)	42 (48.3%)	

^{a)}P-value by χ^2 test for categorical variables and t-test for continuous variables.

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin; CNI, calcineurin inhibitor; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation comorbidity index; HID, haploidentical familial donors; MDS, myelodysplastic syndrome; MUD, matched unrelated donors; PUD, partially-matched unrelated donors.

Supplementary Table 2. Cumulative incidence of individual failure events and crude incidence of infectious complications.

	ATG 6 mg/kg (N=43)	ATG 9 mg/kg (N=87)	P
100-day acute graft-versus-host disease, grade III-IV	23.3%	16.1%	0.023 ^{a)}
2-year chronic graft-versus-host disease requiring systemic treatment	41.2%	21.0%	0.025 ^{a)}
2-year non-relapse mortality	16.6%	24.3%	0.517 ^{a)}
2-year relapse	28.8%	33.7%	0.311 ^{a)}
Fungal infection	2.3%	25.3%	0.003 ^{b)}
CMV infection	0	3.4%	0.541 ^{b)}

^{a)}P-value by Gray test. ^{b)}P-value by χ^2 test.

Abbreviations: ATG, antithymocyte globulin; CMV, cytomegalovirus.

Kaplan-Meier method, and compared with the log-rank test. Cumulative incidence was estimated for individual failure events and compared using the Gray test, with death without GVHD being a competing risk for GVHD, relapse for NRM, and NRM for relapse. Cox proportional hazards models were applied to identify risk factors significantly related to survival, in which variables with statistical significance from the univariate analyses were subsequently used to construct multivariate analyses, along with the donor types. All *P*-values were 2-sided and values of <0.05 were considered statistically significant. The analyses were done with two statistical software packages: SPSS version 23.0 (IBM Corp, Armonk, NY, USA) and R 3.5.1.

Supplementary Results

Baseline characteristics

The characteristics of the included 130 patients are shown in [Supplementary Table 1](#). Overall, the median recipient age was 52 years (range, 16-68 yr) and 52.3% of the patients were males. Median HCT-CI scores were 0 both in the overall and in each of the two groups. The most common diagnosis was acute myeloid leukemia (AML, 60.0%), followed by acute lymphoblastic leukemia (ALL, 25.4%), and myelodysplastic syndrome (MDS, 14.6%). The DRI was low/intermediate in 76.2% and high/very high in 23.8% of the patients. Patients received a HCT after a median of 5.92 months (range, 2.07-197.43 mo) from their diagnosis of hematologic malignancy, and about 16.2% of the cases consisted of the combination of a male recipient and a female donor. The median dose of the infused CD34⁺ cells was 5.14×10^6 /kg (range, $0.97-12.68 \times 10^6$ /kg). Although these characteristics were generally comparable among the groups with different anti-thymocyte globulin (ATG) doses, haploidentical familial donors (HIDs) were more common in the 6 mg/kg group than in the 9 mg/kg groups, with 58.1%

vs. 34.5%, respectively ($P=0.008$). In addition, tacrolimus or methotrexate was more frequently used in the 6 mg/kg group than in the 9 mg/kg groups, with 64.4% vs. 27.9% for tacrolimus ($P<0.001$) and 79.1% vs. 51.7% ($P=0.005$) for methotrexate, respectively.

Incidence of infectious complications

Both fungal infection and cytomegalovirus infection were less frequently observed with a total ATG dose of 6 mg/kg compared to 9 mg/kg, with a crude incidence of 2% vs. 25% ($P=0.003$) and 0 vs. 3% ($P=0.541$), respectively ([Supplementary Table 2](#)).

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