

# Association between preconditioning absolute lymphocyte count and transplant outcomes in patients undergoing matched unrelated donor allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning and anti-thymocyte globulin

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## Abstract

**Background:** Allogeneic stem cell transplantation (alloSCT) offers cure chance for various hematologic malignancies, but graft-versus-host disease (GVHD) remains a major impediment. Anti-thymocyte globulin (ATG) is used for prophylactic T-cell depletion and GVHD prevention, but there are no clear guidelines for the optimal dosing of ATG. It is suspected that for patients with low absolute lymphocyte counts (ALCs), current weight-based dosing of ATG can be excessive, which can result in profound T-cell depletion and poor transplant outcome.

**Methods:** The objective of the study is to evaluate the association of low preconditioning ALC with outcomes in patients undergoing matched unrelated donor (MUD) alloSCT with reduced-intensity conditioning (RIC) and ATG. We conducted a single-center retrospective longitudinal cohort study of acute leukemia and myelodysplastic syndrome patients over 18 years old undergoing alloSCT. In total, 64 patients were included and dichotomized into lower ALC and higher ALC groups with the cutoff of 500/ $\mu\text{l}$  on D-7.

**Results:** Patients with preconditioning ALC <500/ $\mu\text{l}$  were associated with shorter overall survival (OS) and higher infectious mortality. The incidence of acute GVHD and moderate-severe chronic GVHD as well as relapse rates did not differ according to preconditioning ALC. In multivariate analyses, low preconditioning ALC was recognized as an independent adverse prognostic factor for OS.

**Conclusion:** Patients with lower ALC are exposed to excessive dose of ATG, leading to profound T-cell depletion that results in higher infectious mortality and shorter OS. Our results call for the implementation of more creative dosing regimens for patients with low preconditioning ALC.

**Keywords:** absolute lymphocyte count, allogeneic hematopoietic stem cell transplantation, anti-thymocyte globulin, graft-versus-host disease, matched unrelated donors

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## Introduction

Allogeneic stem cell transplantation (alloSCT) offers cure chance for various hematologic malignancies, including acute leukemias and myelodysplastic syndrome (MDS).<sup>1</sup> While the cytotoxicity of conditioning regimens and the graft-versus-leukemia (GVL) effects offer therapeutic advantages, graft-versus-host disease (GVHD) remains a major impediment throughout the alloSCT process.<sup>2</sup>

Currently, various creative combinations of immunosuppressive agents are being used for prophylactic T-cell depletion and consequently GVHD prevention.<sup>3</sup> ATG (anti-thymocyte globulin) is a polyclonal IgG from either rabbit or horse sera that is immunized with human thymocytes or T-cell lines which is widely used for GVHD prophylaxis.<sup>4</sup> It targets donor-derived CD3+ T cells and recipient T cells remaining after cytotoxic conditioning therapy. In many previous trials, the use of ATG has shown to protect against severe chronic GVHD without increasing relapse or nonrelapse mortality (NRM) or diminishing survival outcomes.<sup>5-9</sup>

Unfortunately, there are no clear guidelines for the optimal dosing of ATG. Theoretically, the ideal dose of ATG would deplete T cells and prevent severe GVHD while ensuring adequate immune reconstitution without compromising GVL effects. Insufficient dose of ATG would result in greater risk of GVHD, while excessive dose would lead to impaired engraftment and higher risk of infection and relapse.<sup>10</sup> Recent studies have suggested that absolute lymphocyte count (ALC) is related to the clearance of ATG; thus, low ALC causes higher concentration of ATG that lasts and depletes donor-derived T cells as well as induces regulatory T cells to suppress them.<sup>11,12</sup> In conventional conditioning regimens, however, ATG is administered according to the body weight, which means the delivered dose may be exaggerated in lymphopenic patients. Under the weight-based dosing systems, it is well reported that low ALC at the time of administration of ATG is associated with higher relapse rates, increased infection rates, and worse overall survival (OS).<sup>12-14</sup> A pharmacokinetic-pharmacodynamic study by Admiraal *et al.* analyzed the exposure to ATG according to the ALC and body weight. It reported that only 30% of patients who were

treated with the European Group for Blood and Marrow Transplantation (EBMT) regimen (total 7.5 mg/kg of ATG, from D-3 to D-1) fit in the optimal target attainment of ATG.<sup>12</sup> As a result, efforts have been made to dose ATG according to the ALC on the day of ATG administration.<sup>10,12</sup>

In our previous study that investigated the association between ALC and alloSCT outcomes in matched related donor (MRD) setting, however, we found that preconditioning (D-7) ALC < 500/ $\mu$ l was associated with shorter OS, higher NRM, and higher infection rates compared with ALC  $\geq$  500/ $\mu$ l group, while D-3 ALC was not associated with transplant outcomes.<sup>15</sup> This study investigated the association between preconditioning ALC and alloSCT outcomes in patients undergoing transplant with matched unrelated donor (MUD) and reduced-intensity conditioning (RIC). We aimed to evaluate whether this association is consistently observed in MUD setting and whether D-7 ALC is a better predictor of treatment outcomes compared with D-3 ALC.

## Methods

### *Patients and study design*

This is a single-center retrospective, longitudinal cohort study of acute leukemias and MDS patients over 18 years old undergoing alloSCT with RIC from an MUD between January 2011 and December 2018. One locus mismatch (9/10) was allowed and hematopoietic stem cell allografts were harvested *via* peripheral blood in all cases. There was no restriction on the disease status. Patients undergoing second alloSCT or receiving preemptive donor lymphocyte infusions were excluded. Those with previous history of solid organ transplantation were also excluded. At the end, a total of 64 patients were identified and their medical records were reviewed for demographics, baseline disease characteristics, factors related to alloSCT, response to alloSCT, adverse events, and survival outcomes. This study was conducted according to Declaration of Helsinki and was approved by the institutional review board of Seoul National University Hospital (IRB no. H-1906-001-103). The reporting of this study conforms to the STROBE statement.<sup>16</sup>

### Procedures

All patients underwent identical RIC with busulfan, fludarabine, and ATG: busulfan 3.2 mg/kg intravenous (IV) for D-7 and D-6; fludarabine 30 mg/m<sup>2</sup> from D-7 to D-2; and rabbit ATG (Thymoglobulin; Sanofi-Aventis, Korea) 2.5 mg/kg/day from D-3 to D-1 for a total of 7.5 mg/kg. GVHD prophylaxis consisted of cyclosporine A at a starting dose of 3 mg/kg at 48 h before stem cell infusion, with adjustments to achieve the target serum trough level of 250–400 ng/ml. The choice of calcineurin inhibitors (tacrolimus or cyclosporine) was left to the attending physician's preference. The serum cytomegalovirus (CMV) antigen level was monitored weekly and intravenous immunoglobulin was administered for 6 months after alloSCT. Micafungin was administered for fungal infection prophylaxis. The pre-conditioning ALC was measured at D-7 (i.e. conditioning initiation). The serum level of ATG and busulfan could not be measured.

### Definitions

The European Group for Blood and Marrow Transplantation (EBMT) risk score<sup>17</sup> was used to assess HSCT risks. The Glucksberg standard criteria<sup>18</sup> were used to grade acute GVHD. Chronic GVHD was classified as mild, moderate, or severe according to the 2014 National Institutes of Health consensus criteria.<sup>19</sup> NRM was defined as death without progression of underlying disease. Relapse was defined by the morphological evidence of disease in the peripheral blood, bone marrow, or extramedullary sites. The relapse-free survival (RFS) was defined as the time from stem cell infusion to relapse or death from any cause. The OS was defined as the time from stem cell infusion to death of any cause. Neutrophil recovery was defined as absolute neutrophil count (ANC) > 500/μl for three consecutive measurements. Platelet recovery was defined as seven consecutive measurements of >20,000/μl without transfusion. Time to neutrophil engraftment and time to platelet recovery were defined as the time from stem cell infusion to neutrophil engraftment or platelet recovery.

### Statistical analysis

Differences between groups were assessed using a Student's *t* test or one-way analysis of variance for continuous variables and Pearson's chi-square test for categorical variables, as indicated. The RFS and OS curves were estimated using the

Kaplan–Meier method. If patients survived without death or progression, the survival was censored at the latest date of follow-up when no death or progression was confirmed. The *p* values of <0.05 were considered statistically significant. These data were analyzed using the Statistical Package for the Social Sciences software (IBM® SPSS® Statistics, version 22.0) and the statistical software R (www.r-project.org).

Cumulative incidence curves were used in competing-risk setting to calculate the probability of acute and chronic GVHD and NRM. For GVHD, death without an event was considered as the competing event. For NRM, relapse was considered as the competing event. Associations between potential prognostic factors and survival outcomes were evaluated using the Cox's proportional hazard regression models. With stepwise backward procedure, predictors achieving a *p* value below 0.05 were considered then sequentially removed if the *p* value in the multiple model was above 0.05. For this part of the analyses, SAS Enterprise Guide 6.1 Version and the statistical software R (www.r-project.org) were used.

## Results

### Patient characteristics

The baseline characteristics are shown in Table 1. For analyses, patients were divided into two groups: those with ALC < 500/μl *versus* those with ALC ≥ 500/μl at D-7. The cutoff of 500/μl was set as described in our previous study.<sup>15</sup> There were no differences between the two groups with regard to body weight, age, underlying diseases, disease status at alloSCT, and infused CD34 cell dose. Overall, cyclosporine was more often used, but there were no significant differences between the two groups regarding the choice of calcineurin inhibitor (CIN). Methotrexate was used in 35.9% of the patients. There was no female to male transplantation in ALC < 500/μl group *versus* six (12.2%) cases in ALC ≥ 500/μl group, but the overall modified EBMT (the European Group for Blood and Marrow Transplantation) score<sup>17</sup> was comparable between the two groups.

### Outcomes of alloSCT

Neutrophil engraftment was seen in 93.3% of the ALC < 500/μl group and 98.0% of the ALC ≥ 500/μl group (*p* = 0.368) (Table 2). There

**Table 1.** Baseline characteristics.

Variables	All patients (N = 64)	ALC < 500/ $\mu$ l (n = 15)	ALC $\geq$ 500/ $\mu$ l (n = 49)	p
Body weight, kg, median (range)	62.7 [46.4–93.6]	61.8 [47.9–84.0]	63.1 [46.4–93.6]	0.945
Age, years, median (range)	53 [20–68]	48 [20–68]	53 [23–67]	0.287
Age $\geq$ 60 years, n (%)	16 (25.0)	4 (26.7)	12 (24.5)	0.865
Sex, male, n (%)	31 (48.4)	9 (60.0)	22 (44.9)	0.306
Diagnosis, n (%)				
Acute myeloid leukemia	36 (56.3)	12 (80.0)	24 (49.0)	0.086
Myelodysplastic syndrome	16 (25.0)	1 (6.7)	15 (30.6)	
Acute lymphoblastic leukemia	12 (18.8)	2 (13.3)	10 (20.4)	
1MMUD (9/10), n (%)	20 (31.3)	4 (26.7)	16 (31.3)	0.662
Disease status, n (%)				
CR1	23 (35.9)	2 (13.3)	21 (42.9)	0.096
CR >1	26 (40.6)	9 (60.0)	17 (34.7)	
Non-CR	15 (23.4)	4 (26.7)	11 (22.4)	
Female to male match, n (%)	6 (9.4)	0	6 (12.2)	0.155
Modified EBMT score, n (%)				
1–3	28 (43.8)	6 (40.0)	22 (44.9)	0.738
4–6	36 (56.3)	9 (60.0)	27 (55.1)	
Calcineurin inhibitor, n (%)				
Cyclosporine	37 (57.8)	11 (73.3)	26 (53.1)	0.164
Tacrolimus	27 (42.2)	4 (26.7)	23 (46.9)	
Methotrexate, n (%)	23 (35.9)	5 (33.3)	18 (36.7)	0.810
ALC at D-7, cells/ $\mu$ l, median (range)	1018.1 [96.8–15,534.9]	351.0 [96.8–477.6]	1139.6 [512.0–15,534.9]	0.021
ALC at D-3, cells/ $\mu$ l, median (range)	67.0 [4.0–1054.0]	49.0 [4.0–221.0]	141.1 [11.0–1054.0]	0.020
Infused CD34 cells, $\times 10^6$ /kg, median (range)	4.94 [1.00–12.68]	5.06 [1.00–9.19]	4.92 [1.00–12.68]	0.607
ALC, absolute lymphocyte count; CR, complete remission; EBMT, The European Group for Blood and Marrow Transplantation; MMUD, mismatch unrelated donor.				

were no differences between the two groups regarding the immune reconstitution: time to neutrophil engraftment and time to platelet recovery. There were no differences between the two groups with regard to relapse rates (Figure 1(a)). But lower ALC was significantly associated with higher mortality rate (66.7% in ALC < 500/ $\mu$ l group

versus 34.7% in ALC  $\geq$  500/ $\mu$ l group,  $p = 0.026$ ; Table 2). Also, patients of ALC < 500/ $\mu$ l presented with significantly shorter OS (median 201 days in ALC < 500/ $\mu$ l group versus not reached in ALC  $\geq$  500/ $\mu$ l group,  $p = 0.019$ ) (Figure 1(b)). NRM was higher in the ALC < 500/ $\mu$ l group but statistically insignificant ( $p = 0.215$ ) (Figure 1(c)).

**Table 2.** Transplantation outcomes.

Variables	ALC < 500/ $\mu$ l (n = 15)	ALC $\geq$ 500/ $\mu$ l (n = 49)	p
Neutrophil engraftment, n (%)	14 (93.3)	48 (98.0)	0.368
Time to neutrophil engraftment, days (median, range)	12 [5–28]	13 [3–26]	0.723
Platelet engraftment, n (%)	15 (100)	47 (95.9)	0.427
Time to platelet recovery, days (median, range)	15 [7–32]	15 [3–33]	0.760
Acute GVHD, any, n (%)	6 (40.0)	22 (44.9)	0.738
Grade II–IV acute GVHD	3 (20.0)	16 (32.7)	0.348
Grade III–IV acute GVHD	3 (20.0)	8 (16.3)	0.741
Chronic GVHD, any, n (%)	1 (6.7)	22 (44.9)	0.007
Moderate-severe chronic GVHD	1 (6.7)	7 (14.3)	0.435
CMV reactivation, n (%)	6 (40.0)	29 (59.2)	0.192
CMV disease	4 (26.7)	8 (16.3)	0.369
Mortality, n (%)	10 (66.7)	17 (34.7)	0.026
Cause of death, n (%)			0.195
Disease progression	2 (20.0)	3 (17.6)	1.000
Infection	7 (70.0)	7 (41.2)	0.236
GVHD related	0 (0)	6 (35.3)	0.057
Others	1 (10.0)	1 (5.9)	1.000

ALC, absolute lymphocyte count; CMV, cytomegalovirus; GVHD, graft-versus-host disease.

In univariate analyses (Table 3), the following factors were recognized as adverse prognostic factors for OS: older age ( $p = 0.015$ ), 1MMUD ( $p = 0.028$ ), non-CR ( $p = 0.020$ ), higher modified EBMT score ( $p = 0.010$ ), and preconditioning ALC < 500/ $\mu$ l ( $p = 0.023$ ). In multivariate analyses, 1MMUD ( $p = 0.022$ ) and preconditioning ALC < 500/ $\mu$ l ( $p = 0.016$ ) were recognized as adverse prognostic factors for OS.

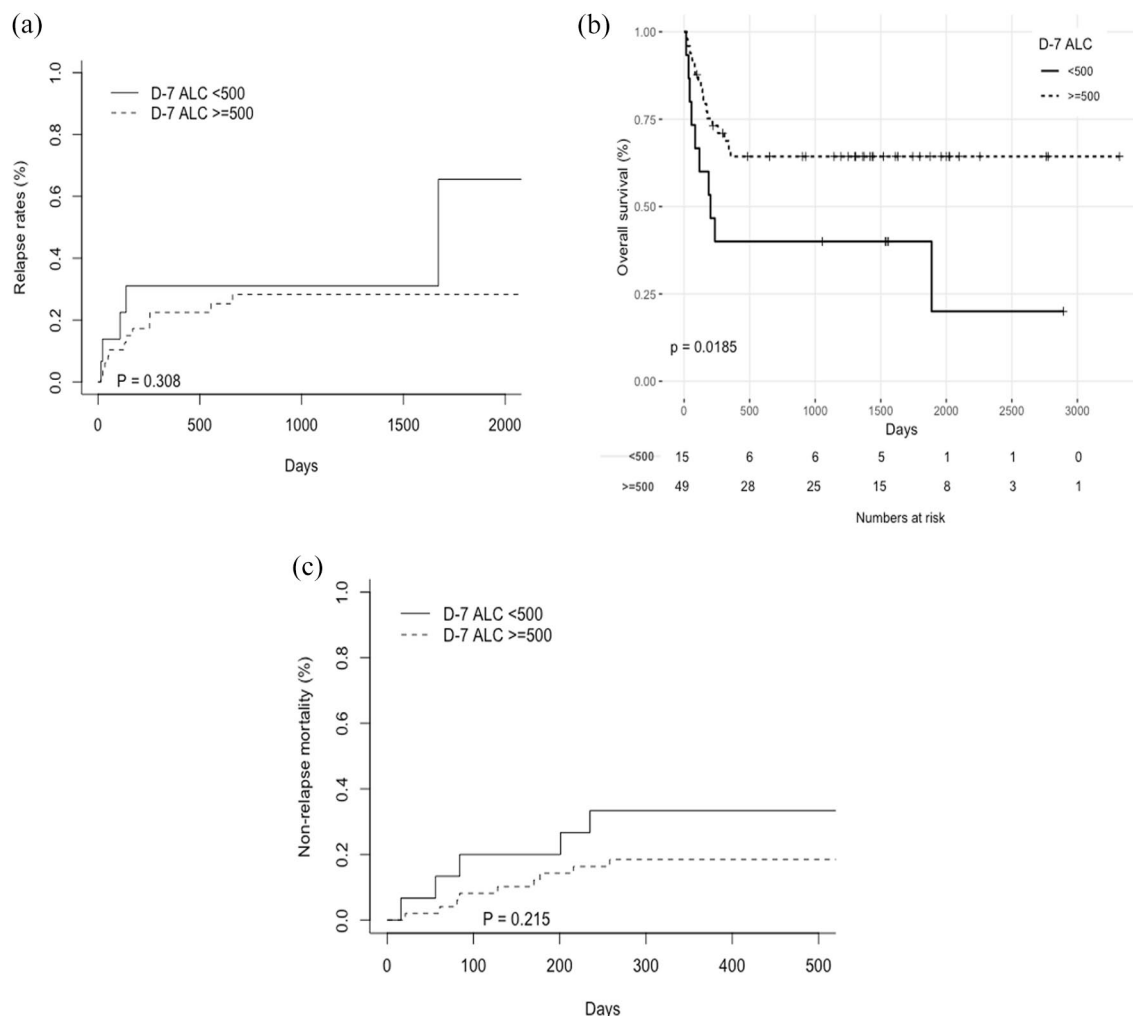
#### GVHD and other complications

There were no differences in acute GVHD occurrence according to preconditioning ALC (Table 2). The cumulative incidence of grade II–IV acute GVHD ( $p = 0.348$ ) and grades III–IV acute GVHD ( $p = 0.70$ ) were also similar between the two groups (Figure 2(a) and (b)). The use of

cyclosporine *versus* tacrolimus did not affect the incidence of acute GVHD ( $p = 0.355$ ; data not shown).

On the contrary, more chronic GVHD occurrence was noted in ALC  $\geq$  500/ $\mu$ l group ( $p = 0.003$ ). The cumulative incidence of chronic GVHD of any grade was higher in ALC  $\geq$  500/ $\mu$ l group (Figure 2(c)), but there was no difference in moderate-severe chronic GVHD between the two groups (Figure 2(d)). The use of cyclosporine *versus* tacrolimus did not affect the incidence of acute GVHD ( $p = 0.711$ ; data not shown).

In ALC < 500/ $\mu$ l group, the major cause of death was infection (70.0%) (Table 2). GVHD was one of the leading causes of death in ALC  $\geq$  500/ $\mu$ l group (35.3%), while no patient died of GVHD



**Figure 1.** Transplantation outcomes according to preconditioning absolute lymphocyte counts (ALCs) at D-7. (a) Relapse rates. (b) Overall survival. (c) Nonrelapse mortality.

in  $A_{LC} < 500/\mu\text{l}$  group. One patient in  $A_{LC} < 500/\mu\text{l}$  group expired due to gastrointestinal bleeding, while one patient in  $A_{LC} \geq 500/\mu\text{l}$  group expired due to intracranial hemorrhage.

#### Infection-related mortality

Infection-related mortality rate was 46.7% in  $A_{LC} < 500/\mu\text{l}$  group and 14.3% in  $A_{LC} \geq 500/\mu\text{l}$  group. More detailed information on the infection-related mortality is provided in Table 4. In total, 14 patients died of infection, seven patients from each group. The onset of infection from the day of transplantation showed no difference between the two groups (median 77 days in  $A_{LC} < 500/\mu\text{l}$  group versus 106 days in  $A_{LC} \geq 500/\mu\text{l}$  group,  $p = 0.805$ ). In  $A_{LC} < 500/\mu\text{l}$

group, the causative pathogens included CMV, candida, and mucor; in  $A_{LC} \geq 500/\mu\text{l}$  group, however, most pathogens were bacteria (Table 5). The site of infection was mostly pneumonia with occasional cases of central line-associated bloodstream infection, enterocolitis, brain abscess, cholecystitis, and rhinocerebral infection, with no significant difference between the two groups.

Few patients were being treated with steroid at the onset of infection: three patients in  $A_{LC} < 500/\mu\text{l}$  group and two patients in  $A_{LC} \geq 500/\mu\text{l}$  group. The median dose of steroid administered at the time of infection was both 0 with no significant difference ( $p = 0.535$ ). The mean dose of steroid was calculated by dividing the cumulative steroid doses administered from



**Table 3.** Univariate and multivariate analyses for overall survival.

	Univariate		Multivariate	
	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>
Age, $\geq 60$ versus $< 60$ years	2.616 (1.208–5.664)	0.015	2.601 (0.915–4.644)	0.081
Sex, male versus female	0.516 (0.236–1.128)	0.097		
Diagnosis, acute leukemias versus MDS	0.814 (0.328–2.018)	0.657		
HLA, MUD versus 1MMUD	0.303 (0.104–0.876)	0.028	0.288 (0.099–0.836)	0.022
Disease status, non-CR versus CR	2.543 (1.160–5.574)	0.020	1.752 (0.735–4.176)	0.206
Infused CD34 cell count, $< 5.0$ versus $\geq 5.0$	0.846 (0.395–1.810)	0.666		
Modified EBMT score, 4–6 versus 1–3	3.373 (1.342–8.481)	0.010	2.167 (0.755–6.220)	0.151
Preconditioning ALC, $\geq 500/\mu\text{l}$ versus $< 500/\mu\text{l}$	0.403 (0.184–0.882)	0.023	0.380 (0.173–0.533)	0.016
CNI, tacrolimus versus cyclosporine	1.554 (0.696–3.471)	0.282		

ALC, absolute lymphocyte count; CI, confidence interval; CNI, calcineurin inhibitors; CR, complete remission; EBMT, The European Group for Blood and Marrow Transplantation; HR, hazard ratio; MDS, myelodysplastic syndrome; MMUD, mismatch unrelated donor; MUD, matched unrelated donor.

D100 to death by the duration from D100 to death. It showed no differences between the two groups (18.7 mg in ALC  $< 500/\mu\text{l}$  group versus 12.5 mg in ALC  $\geq 500/\mu\text{l}$  group,  $p = 0.857$ ).

The cumulative incidence of cGVHD was higher in the ALC  $\geq 500/\mu\text{l}$  group, which entails the possibility of more abundant usage of systemic steroids that can confound the relationship with infectious mortality. Among the patients who died of infection, however, the incidence of grade II–IV aGVHD and moderate-severe chronic cGVHD showed no statistical significance (grade II–IV aGVHD, 42.9% versus 14.3%,  $p = 0.559$ ; moderate-severe chronic cGVHD, 14.3% versus 0%,  $p = 1.000$ ; Table 5).

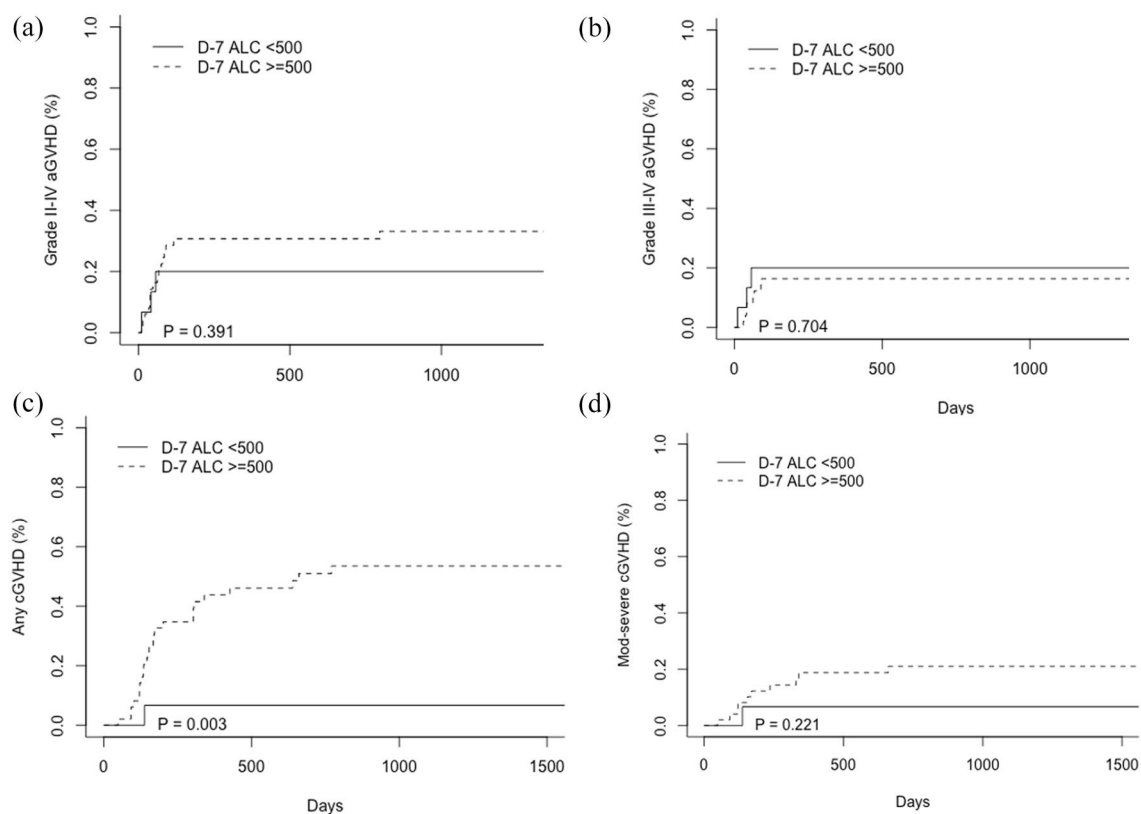
### D-3 ALC

Previous papers have investigated the association between transplant outcomes and ALC on the day of ATG administration (i.e. D-3). We performed a subanalysis using our data with D-3 ALC as in Figure 3. The median of D-3 ALC was  $63/\mu\text{l}$  (range = 4–1054/ $\mu\text{l}$ ). With the cutoff of  $100/\mu\text{l}$ , there was no difference in OS between the two groups ( $p = 0.901$ ). Relapse rates, NRM, and the incidence of acute and chronic GVHD did not differ significantly either. Similar trends were

also observed with the cutoff of  $50/\mu\text{l}$  or  $63/\mu\text{l}$  which is not presented in this manuscript.

### Discussion

The importance of our study lies in that we showed the role of preconditioning ALC in RIC-alloSCT setting according to donor types. In our previous study,<sup>15</sup> we reported that in MRD setting, patients with lower preconditioning ALC of  $< 500/\mu\text{l}$  were associated with lower incidence of both acute and chronic GVHD but suffered from higher rates of infection and shorter OS. Furthermore, preconditioning ALC was recognized as a prognostic factor for OS. In this study, we showed that in MUD setting, patients with lower preconditioning ALC of  $< 500/\mu\text{l}$  were also associated with higher infection rates and shorter OS, but there were no differences in the incidence of acute GVHD or moderate-severe chronic GVHD. Chronic GVHD of any grade was more prevalent in the ALC  $\geq 500/\mu\text{l}$  group, which is probably because alloSCT from MUD is already more prone to GVHD compared with that from MRD. Despite the lack of difference in GVHD occurrence, lower preconditioning ALC was recognized as an independent risk factor for shorter OS, again reinforcing the detrimental effect of profound T-cell depletion by ATG.



**Figure 2.** GVHD outcomes according to preconditioning absolute lymphocyte counts (ALC) at D-7. (a) Cumulative incidence of grade II–IV acute graft-versus-host disease (GVHD). (b) Cumulative incidence of grade III–IV acute GVHD. (c) Cumulative incidence of any chronic GVHD. (d) Cumulative incidence of moderate-severe chronic GVHD.

**Table 4.** Characteristics of infection-related mortality and the use of systemic steroids.

	ALC <500/ $\mu$ l (n = 7)	ALC $\geq$ 500/ $\mu$ l (n = 7)	p
Onset, median (range) in days	77 (12–198)	106 (35–181)	0.805
Steroid dose at the onset of infection, median (range) in prednisolone (mg)	0 (0–80)	0 (0–20)	0.535
Mean steroid dose, median (range) in mg <sup>a</sup>	18.7 (17.9–31.8)	12.5 (3.1–60.0)	0.857
Grade II–IV aGVHD, n (%)	3 (42.9%)	1 (14.3%)	0.559
Moderate-severe cGVHD, n (%)	1 (14.3%)	0 (0.0%)	0.100

ALC, absolute lymphocyte count; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.  
<sup>a</sup>Mean steroid dose = (cumulative steroid doses administered from D100 to death) / (duration from D100 to death).

The incidence of chronic GVHD was significantly higher in ALC  $\geq$  500/ $\mu$ l group, which accounted for 35.3% of mortality, as the second leading cause of death following infection. In contrast, in

ALC < 500/ $\mu$ l group, no patient died for GVHD. It could be inferred that the exaggerated effect of ATG in low ALC group resulted in less GVHD and less GVHD-related mortality. Considering



**Table 5.** Detailed information on infection-related mortality cases.

ALC group	Pathogen	Onset (day)	Site	Steroid dose (mg)	Mean steroid dose (mg) <sup>a</sup>
ALC < 500/ $\mu$ l	VRE, <i>Candida tropicalis</i>	160	CLABSI	80	31.8
	Mucor	139	Rhinocerebral infection	80	18.7
	CMV versus fungal	35	Pneumonia	15	
	<i>Escherichia coli</i> , <i>Enterococcus faecium</i>	42	Bacteremia	0	
	PCP versus CMV versus fungal	181	Pneumonia	0	17.9
	CMV	106	Enterocolitis	0	
	CMV versus <i>E. faecium</i>	46	Pneumonia	0	
ALC $\geq$ 500/ $\mu$ l	CMV	77	Pneumonia	0	60.0
	PCP, <i>Corynebacterium</i>	198	Pneumonia	0	12.5
	CMV versus fungal	142	Pneumonia	0	3.1
	<i>Aspergillus</i>	68	Pneumonia	10	
	<i>Klebsiella pneumoniae</i>	194	Brain abscess	0	20.1
	<i>E. faecium</i>	44	Cholecystitis	20	
	VRE, IRAB	12	Pneumonia	0	

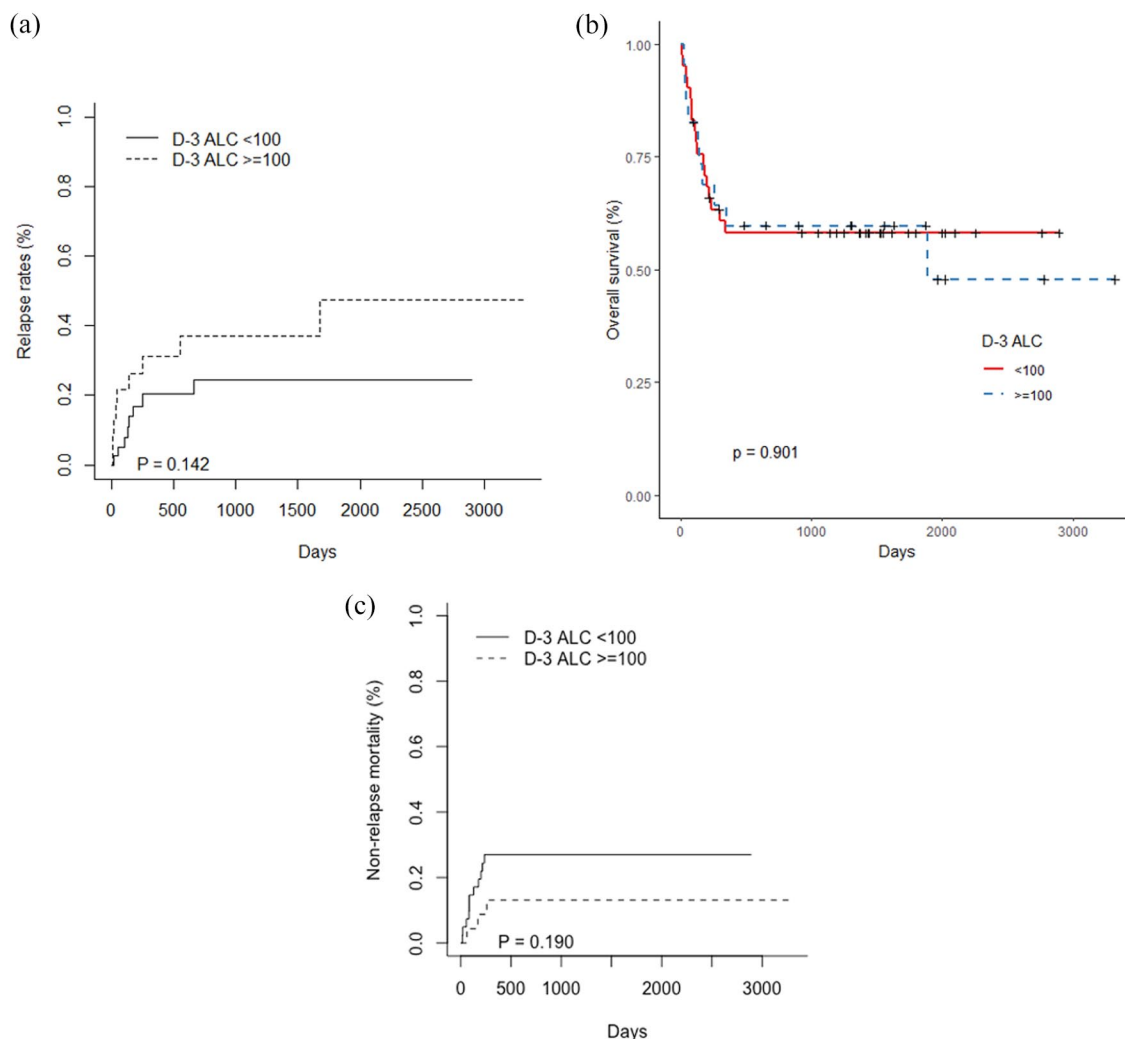
ALC, absolute lymphocyte count; CLABSI, central line-associated bloodstream infection; CMV, *cytomegalovirus*; IRAB, imipenem-resistant *Acinetobacter baumannii*; PCP, *Pneumocystis jirovecii* pneumonia; VRE, vancomycin-resistant *Enterococci*.  
<sup>a</sup>Mean steroid dose = (cumulative steroid doses administered from D100 to death) / (duration from D100 to death).

the compromise of OS, however, the reduction of GVHD did not compensate for the increased risk of lethal infections.

The correlation between low ALC and higher mortality has been reported in various studies.<sup>13,14</sup> Our study is in line with these findings. Under the current weight-based dosing regimen, patients with lower ALC are exposed to excessive amount of ATG, which can lead to profound T-cell depletion and increased risk of fatal infections. A study reported that high ATG level at day 7 of transplantation was associated with lower T-cell counts that persisted for a year.<sup>20</sup> This prolonged T-cell depletion is thought to be the leading cause of more infections and higher infectious mortality, as patients with lower ALC are not likely to be exposed to immunosuppressants as the incidence of moderate-severe chronic GVHD was low in this group. It calls for the implementation of novel dosing regimens based on lymphocyte counts,

which has been proposed by various investigators.<sup>10,12</sup> It is also worth noting that although ATG use was associated with increased NRM, death due to acute GVHD was improved, indicating that more vigorous infection control measurement can lead to better transplantation outcomes. As noted in Table 5, the cause of infection differs per time post-transplant; thus, close monitoring with timely intervention with adequate antimicrobial treatment is important.

Previous studies have shown the association between the ALC on the day of ATG administration (i.e. D-3 ALC) and transplant outcomes. In the current study, however, low D-3 ALC was not associated with shorter OS. In addition, D-7 ALC showed no correlation with D-3 ALC. In our study population, D-3 ALC was much lower than previous studies. The median D-3 ALC of this study was 63/ $\mu$ l (range = 4–1054/ $\mu$ l). Previous studies that reported the correlation between D-3



**Figure 3.** Transplantation outcomes according to absolute lymphocyte counts (ALC) at D-3. (a) Relapse rates. (b) Overall survival. (c) Nonrelapse mortality. (d) GVHD.

ALC and transplant outcomes in MUD settings, however, had median D-3 ALC of 1150/ $\mu\text{l}$  (range = 0–5814/ $\mu\text{l}$ )<sup>13</sup> and 200/ $\mu\text{l}$  (range = 100–6000/ $\mu\text{l}$ ).<sup>14</sup> The heterogeneity of conditioning regimens does not fully explain this discrepancy, as previous study regimens included TBI (total body irradiation) which is reported to further reduce D-3 ALC, while the current study included BuFlu (RIC) regimen only.<sup>21</sup> The age, disease status, and EBMT score were all comparable with other studies, as well as transplant outcomes, including OS, relapse rate, and GVHD incidence. It could be explained by differing ethnicity, as current study only involved Asians and there has been insufficient data regarding the Asian population. It is widely accepted that ethnic differences

affect the susceptibility to and tolerability of chemotherapeutic agents, including busulfan.<sup>22</sup> We could also hypothesize that the administration of conditioning regimens such as busulfan and fludarabine can significantly alter the lymphocyte count in Asian population, so preconditioning ALC can better represent the true immune characteristics of the patients. It, however, remains no more than a hypothesis at this point and further studies regarding both D-7 ALC and D-3 ALC are warranted with patients with diverse ethnic backgrounds.

Also, several studies have reported that lower dose of ATG could successfully prevent GVHD and improve the quality of life in Asians. The

incidence of GVHD is also lower in Asians compared with that of Caucasian populations.<sup>23–25</sup> Therefore, lower dose of ATG might be appropriate to Asian patients, especially for those with extremely low ALC, although prospective studies or comparative studies are warranted.

The strength of this study is, as a single-center study, an identical conditioning regimen was applied to all study participants. Previous studies possessed limitations owing to heterogeneity of conditioning regimen and its intensity. In particular, the use of corticosteroids and total body irradiation can significantly alter the number of circulating lymphocytes.<sup>26</sup> By ensuring less heterogeneity of the study population, we could maximize its statistical power despite the limited number of patients enrolled. Unfortunately, however, this study is not without faults. First, instead of our true target, CD3+ T cells, we measured ALCs which also included B cells. Second, we could not analyze the effect of donor-driven T-cell counts. Third, we did not obtain the serum level of ATG. Several pharmacokinetic–pharmacodynamic studies have proven the association of ATG level and ALC, as well as the relationship of ATG level and transplant outcomes. Finally, limited number of patients in each group calls for further large-scale study.

In conclusion, with alloSCT in MUD setting, lower preconditioning ALC of <500/μl is associated with shorter OS and higher infectious mortality with no significant differences in the incidence of acute GVHD or moderate-severe chronic GVHD. Low preconditioning ALC is recognized as an independent adverse prognostic factor for OS. It calls for the implementation of novel dosing regimens which administers lower dose of ATG for patients with low preconditioning ALC.

#### Author contributions

JMB and JH conceptualized the study. JMB set methodology. JS and JMB performed formal analysis and investigation. All authors performed data curation. JMB and JH supervised the study. SJ, JMB and JH wrote original manuscript. All authors reviewed, edited and revised the manuscript.

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