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Improved Nonrelapse Mortality and Infection Rate with Lower Dose of Antithymocyte Globulin in Patients Undergoing Reduced-Intensity Conditioning Allogeneic Transplantation for Hematologic Malignancies

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We sought to reduce the risk of infectious complications and nonrelapse mortality (NRM) associated with the use of antithymocyte globulin (ATG) without compromising control of acute graft-versus-host disease (aGVHD) in patients undergoing reduced-intensity conditioning (RIC) transplantation. As part of an ongoing quality improvement effort, we lowered the dose of rabbit ATG from 7.5 mg/kg of ATG (R-ATG) (n = 39) to 6.0 mg/kg of ATG (r-ATG) (n = 33) in association with fludarabine (Flu) and busulfan (BU) RIC transplantation and then monitored patients for adverse events, relapse, and survival. Of the 72 mostly high risk (82%) patients studied, 89% received unrelated donor allografts, 25% of which were HLA-mismatched. No differences in posttransplantation full donor-cell chimerism rates were observed between the 2 ATG-dose groups ($P > .05$). When R-ATG versus r-ATG patients were compared, we observed no significant difference in the cumulative incidence of grade II-IV aGVHD (32% versus 27%; $P = .73$) or grade III-IV aGVHD (23% versus 11%; $P = .28$). However, the r-ATG group had significantly less cytomegalovirus (CMV) reactivation (64% versus 30%; $P = .005$) and bacterial infections (56% versus 18%; $P = .001$), a better 1-year cumulative incidence of NRM (18% versus 3%; $P = .03$), and a trend for better 1-year overall survival (OS) (64% versus 84%; P = .07) compared to R-ATG patients. A seemingly modest reduction in the dose of rabbit ATG did not compromise control of aGVHD or achievement of donor chimerism, but led to a significant decrease in the risk of serious infections and NRM in high-risk RIC allograft recipients.

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INTRODUCTION

Graft-versus-host disease (GVHD) remains one of the main factors limiting the wider applicability of allogeneic hematopoietic stem cell transplantation (HSCT) [1-3]. A variety of strategies have been developed to prevent severe GVHD following either myeloablative (MA) or reduced-intensity conditioning (RIC) transplantation, including ex vivo T cell depletion (TCD) [4,5], in vivo TCD with antithymocyte globulin (ATG) [1,6-10], calcineurin inhibitors [11], and monoclonal antibodies (mAbs) [12-16], but no consensus has been reached on the superiority of one modality over another.

In vivo TCD by ATG administration is performed by using 1 of the 3 commercially available ATG products (Thymoglobulin®, ATGAM®, and ATG-Fresenius®) [17]. Despite reducing the risk for GVHD, preliminary data suggest that higher ATG doses seem to be associated with increased risk of disease relapse, infectious complications, and nonrelapse mortality (NRM) [6,10]. However, only few studies have

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tried to evaluate the optimal dose intensity of ATG following MA [17-19], or RIC HSCT [20], and the results of these trials have mostly been inconclusive because of the heterogeneity of the utilized conditioning regimens and ATG administration schedules and the relatively few patients analyzed.

We report here the results of quality improvement efforts designed to compare outcomes associated with 2 moderate doses of rabbit ATG in patients undergoing HSCT following uniform RIC with fludarabine (Flu) and busulfan (BU). Our data suggest that a seemingly "modest" reduction in rabbit ATG dose from 7.5 mg/kg to 6 mg/kg leads to a marked reduction in the rates of infectious complications, and provides a significant NRM benefit without compromising GVHD control, engraftment kinetics, and posttransplantation survival.

PATIENTS AND METHODS

Patient Population

Seventy-two consecutive patients with hematologic malignancies undergoing allogeneic HSCT following uniform RIC with Flu, BU, and rabbit ATG, between January 2006 and December 2008, were included. All patients had adverse risk (precluding the use of MA conditioning), which that was defined by the presence of at least 1 of the following features: (1) age >55 years; (2) Karnofsky performance score (KPS) ≤70; (3) hematopoietic cell transplantation-comorbidity index (HCT-CI) >2 (4) baseline diagnosis of Hodgkin disease (HD), or chronic lymphocytic leukemia (CLL); and (5) prior history of autologous transplantation. This retrospective analysis was approved by the OSU institutional review board and Clinical Scientific Review Committee.

Quality Improvement Program

As part of our continuous quality improvement program, we audit the rates of serious bacterial, fungal, and viral infections as well as rates of NRM on a quarterly and semiannual basis, respectively. We seek to maintain rates of serious infections (defined as cytomegalovirus (CMV)/Epstein-Barr virus (EBV) reactivation requiring preemptive therapy, bacteremias, or invasive fungal/viral/protozoal infections) below 50% and NRM below 15% at 1 year in recipients of RIC transplantation. Our standard of care (SOC) protocol for recipients of RIC transplantation and unrelated donor or mismatched allografts incorporated a total dose of ATG of 7.5 mg/kg (R-ATG) given over 3 days. When our quality review committee observed that these patients had rates of serious infections above 50% and NRM approaching or >15%, we made a programmatic decision to reduce the total dose of rabbit ATG to 6.0 mg/kg (r-ATG)and continued to monitor clinical events in these patients.

Conditioning Regimen, GVHD Prophylaxis, and Supportive Care

The conditioning regimen consisted of i.v. Flu 30 mg/m^2 on days −7 to −3 (total dose; 150 mg/m^2) and i.v. Bu 0.8 mg/kg/dose × 8 doses, on days −4 to −3 (total dose; 6.4 mg/kg) [21]. The first cohort of 39 consecutive patients received rabbit ATG (Thymoglobulin®, Genzyme; Cambridge, MA) (R-ATG group) at 2.5 mg/kg/day, on days −4 to −2 (total dose; 7.5 mg/kg). The second cohort of 33 patients (r-ATG) were given ATG at 2.0 mg/kg/day on the same schedule (total dose; 6.0 mg/kg).

All patients received standard prophylaxis of GVHD with tacrolimus (0.03 mg/kg/day i.v., commencing on day −2) and minidose methotrexate (MTX; 5 mg/m^2 on days +1, +3, +6, and +11) as previously described [22]. Blood levels of tacrolimus were monitored weekly until day +90 to maintain levels between 5 and 15 ng/mL. From day +90 onward tacrolimus was tapered at the discretion of the treating physician if no GVHD appeared.

All patients were treated in HEPA-filtered rooms, and received fungal (fluconazole, or posaconazole), herpes zoster/herpes simplex (intravenous acyclovir or oral valacyclovir), bacterial, and *Pneumocystis jiroveci* prophylaxis (trimethoprim/sulfamethoxazole [TMP-SMX] or dapsone). Weekly monitoring for CMV and EBV reactivation by quantitative RT-PCR was conducted. Preemptive ganciclovir or valganciclovir were administered to patients with CMV reactivation (defined as ≥4000 copies/mL, reconfirmed within 24 hours from initial detection); preemptive single i.v. dose of rituximab (375 mg/m^2) was administered to all patients with evidence of EBV reactivation (defined as ≥4000 copies/ml, reconfirmed within 24 hours from initial detection). EBV PCR was rechecked 1 week after rituximab administration. For patients with negative EBV PCR at this stage, no further rituximab doses were given, whereas patients with increasing or persistently positive EBV PCR received 3 additional weekly rituximab doses. Urine and/or serum BK-virus PCR was obtained in all suspected cases of hemorrhagic cystitis. The time of neutrophil engraftment was considered the first of 3 successive days with absolute neutrophil count (ANC) ≥0.5 × 10^9/L after posttransplantation nadir; the time of platelet engraftment was considered the first of 3 consecutive days with platelet count ≥20 × 10^9/L, in the absence of platelet transfusion.

GVHD Assessment and Treatment

Patients achieving neutrophil engraftment were evaluable for acute GVHD (aGVHD (acute GVHD)) that was graded using standard criteria [23]. Patients were evaluable for chronic GVHD (cGVHD) if engraftment occurred and the patient survived for 100 days posttransplantation. The diagnoses of cGVHD and extensive cGVHD were made as previously described [24-26]. Corticosteroids comprised the first-line therapy of acute (grade II-IV) and extensive cGVHD. Second-line treatment was at the discretion of treating physicians and included mycophenolate mofetil, extracorporeal photopheresis, and infliximab.

Statistical Analysis

Baseline categoric variables were compared by using Fisher's exact test, whereas continuous variables were compared by Wilcoxon rank-sum test or 2-sample *t*-test as appropriate. Overall survival (OS) and progression free survival (PFS) were estimated using the Kaplan-Meier method. OS was defined as the time from transplant to death, and surviving patients were censored at last follow-up. PFS was defined as the time from transplantation to disease progression/relapse and/or death. OS and PFS data were analyzed by the log-rank test. NRM was defined as death from any cause other than disease progression or relapse. Cumulative incidences of NRM and relapse were calculated with relapse or death as a competing event, respectively [27]. Comparisons between estimates of cumulative incidence were made by using the Gray's test. The cumulative incidence of aGVHD or cGVHD was calculating with relapse or death without relapse or GVHD as competing events [20,27]. Cox proportional hazards models were constructed for a cumulative incidence of aGVHD, cGVHD, or NRM, relapse, and for OS and PFS, using a limited backward selection procedure. Variables considered in the model were those significant at $\alpha = 0.20$ level from the univariable models. Variables remaining in the final models were significant at $\alpha = 0.05$ level. Estimates for hazard ratios (HR) and corresponding 95% confidence intervals (CI) were obtained for each significant prognostic factor. All *P*-values are 2 sided. All analyses were run using Stata 10.1, Stata Corporation, College Station, TX. *P*-values based on Gray's test were estimated using R-project version 2.8.1, The R Foundation for Statistical Computing, 2008.

RESULTS

Patient Characteristics

The baseline characteristics of 72 consecutive patients included in this analysis are shown in Table 1. The 2 ATG groups did not differ significantly for age, histologic diagnosis, donor source, KPS, comorbidity scores, donor/recipient CMV status, and stem-cell dose infused. Approximately 25% of the patients in each group received allografts from HLA-mismatched unrelated donors. More patients in the r-ATG group had high-risk disease at the time of transplantation (*P* = .02), whereas a higher proportion of R-ATG patients received filgrastim (granulocyte-colony stimulating factor [G-CSF]) to promote neutrophil engraftment (*P* = .001). Routine use of G-CSF administration following allografting was discontinued at our center (in October 2006), after the publication of large registry data showing no improvement in allogeneic transplantation outcomes with growth factor administration [28].

Engraftment and Chimerism

Median time to neutrophil engraftment was significantly longer in the r-ATG group compared to the R-ATG group (18 vesus 15 days; *P* = .01), likely because of the omission of G-CSF in the r-ATG group (Table 2). A trend for longer median time to platelet engraftment was seen in R-ATG group compared to the r-ATG group (17.5 versus 15 days; *P* = .06). One patient each in both groups experienced secondary graft failure, which resolved with G-CSF administration. One R-ATG patient had secondary graft rejection, with no such events observed in the r-ATG group. Primary graft failure was not observed. Rates of complete donor-cell chimerism at days +30, +60, +180, and +360 for R-ATG patients were 59%, 63%, 84%, and 91%, respectively, and for r-ATG patients were 43%, 78%, 92%, and 100%, respectively (*P* = NS).

GVHD

All 72 patients were evaluable for aGVHD (Table 3). The median time to onset of aGVHD was 44 days and 38 days for the R-ATG and r-ATG groups, respectively. While accounting for competing events, the day +120-cumulative incidence of grade II-IV aGVHD was 32% for R-ATG group and 27% for r-ATG group (*P* = .73) (Figure 1A). The day +120-cumulative incidence of grade III-IV aGVHD for R-ATG patients and r-ATG patients was 23% and 11%, respectively (*P* = .28) (Figure 1B). On univariate analysis, baseline diagnosis of lymphoma (Hodgkin [HL] and non-Hodgkin [NHL]) was the only variable associated with aGVHD (HR = 3.44; 95% CI = 1-11; *P* = .04).

Sixty-three patients surviving for at least 100 days posttransplantation were evaluable for cGVHD (R-ATG group = 34, r-ATG group = 29) (Table 3). The median time to onset of cGVHD for R-ATG and r-ATG groups was 207 days and 160 days respectively. The day +400-cumulative incidence of cGVHD was 44% (n = 15) for R-ATG group and 61% (n = 13) for r-ATG group (*P* = .09) (Figure 1C). The day +400-cumulative incidence of extensive cGVHD for the patients in R-ATG group

Table 1. Patient Characteristics at the Time of Transplantation

	ATG (7.5 mg/kg) N = 39	ATG (6 mg/kg) N = 33	*P*-Value
Median age; years (range)	56 (24-70)	55 (24-69)	.66
Male (%)	71.8	63.6	.61
Diagnosis			
AML/MDS	18 (46.2%)	9 (27.3%)	.06
NHL/Hodgkin disease	12 (30.8%)	9 (27.3%)	
Chronic lymphocytic leukemia	4 (10.3%)	12 (36.4%)	
Others	5 (12.8%)	3 (9.1%)	
Disease risk‡			
Standard risk	11 (28%)	2 (6%)	.02
High risk	28 (72%)	31 (94%)	
Prior autografting	6 (15%)	1 (3%)	.11
Donors			
Sibling	5 (12.8%)	3 (9.1%)	.71
Unrelated	34 (87.2%)	30 (90.9%)	
Degree of HLA match			
8/8 match*	31 (79%)	29 (87%)	.34
10/10 match†	29 (74%)	25 (75%)	.99
Median KPS; (range)	90 (70-100)	90 (80-100)	.17
Median HCT-CI; (range)	1 (0-4)	2 (0-4)	.11
Cytomegalovirus status			
Patient and/or donor seropositive	27 (69.2%)	22 (66.7%)	.99
Both patient and donor seronegative	12 (30.8%)	11 (33.3%)	
Graft source			
Bone marrow	4 (10.3%)	—	.12
Peripheral blood stem cells	35 (89.7%)	33 (100%)	
Patients receiving G-CSF	29 (74.4%)	11 (33.3%)	*.001*
Median $CD34^+$ cell dose (10^6 cells/kg recipient), (range)	7.02 (0.69-10.2)	7.2 (2.13-10.0)	.93
Median $CD3^+$ cell dose (10^7 cells/kg recipient) (range)	2.33 (0.19-4.24)	2.32 (0.45-4.83)	.64

AML indicates acute myelogenous leukemia; ATG. antithymocyte globulin; G-CSF, granulocyte colony-stimulating factor; KPS, Karnofsky performance score; HCT-CI, Hematopoietic cell transplantation-comorbidity index; HLA, human leukocyte antigen; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma.

*8/8 match defined by high-resolution allele-level matching at HLA-A, -B, -C and -DRB1.

†10/10 match defined by high-resolution allele-level matching at HLA-A, -B, -C, -DRB1, and -DQB1.

‡Patients with chronic myelogenous leukemia in first chronic phase, acute leukemia in first complete remission, myelodysplastic syndrome with refractory anemia, or refractory anemia with ringed sideroblasts were considered to have low-risk disease. All other patients were placed in the high-risk disease category.

versus those in the r-ATG groups was 40% (n = 12) versus 34% (n = 4), respectively (*P* = .89) (Figure 1D). On univariable analysis, baseline diagnosis of CLL was the only variable associated with cGVHD (HR = 2.82; 95% CI = 1-7.8; *P* = .04).

Infectious Complications

Compared to the R-ATG group, the patients in the r-ATG group had significantly fewer episodes of CMV reactivations (64.1% versus 30.3%; *P* = .005) and bacterial infections (56.4% versus 18.2%; *P* = .001) (Table 4). There was a nonsignificant trend to less BK-virus-associated hemorrhagic cystitis in the r-ATG cohort compared to R-ATG patients (9.1% versus 25.6%; *P* = .12) (Table 4). In the R-ATG group 2 patients developed posttransplant lymphoproliferative disorder (PTLD), and 2 additional patients developed adenoviral infections, whereas no such events were seen in the r-ATG group. Three and 1 invasive fungal infections were reported in the R-ATG and r-ATG patients, respectively.

NRM and Relapse Rate

Median follow-up of surviving patients following HSCT is 15 months. The day 100-cumulative incidence of NRM rate was significantly lower in r-ATG group compared to R-ATG group (0% versus 7.7%; *P* = .03). Similarly, the 1-year cumulative incidence of NRM was significantly lower in r-ATG group compared to R-ATG group (3% versus 18%; *P* = .03)

Table 2. Engraftment Kinetics and Donor-Cell Chimerism Posttransplantation

	ATG (7.5 mg/kg)	ATG (6 mg/kg)	*P*-Value
Neutrophil engraftment (days), median (range)	15 (12-20)	18 (8-32)	.01
Platelet engraftment (days), median (range)	17.5 (10-292)	15 (10-49)	.06
Secondary graft failure	1	1	.99
Secondary graft rejection	1	—	NA
Day +30 chimerism; median			
T cell	97%	91.5%	.15
Myeloid	100%	100%	.78
Day +90 chimerism; median			
T cell	99%	100%	.24
Myeloid	100%	100%	.76
Day +180 chimerism; median			
T cell	100%	100%	.56
Myeloid	100%	100%	.03
Day +360 chimerism; median			
T cell	100%	100%	.84
Myeloid	100%	100%	.11

ATG indicates antithymocyte globulin.

Table 3. Assessment of Graft-versus-Host Disease According to Dose of Antithymocyte Globulin Used

	ATG (7.5 mg/kg) N = 39	ATG (6 mg/kg) N = 33	*P*-Value
Rates of acute GVHD*; %(N)			
Grade I-IV	41% (16)	48.4% (16)	.52
Grade II-IV	30.7% (12)	24.2% (8)	.53
Grade III-IV	20.5% (8)	9% (3)	.17
Cumulative incidence of acute GVHD at day +120† (%)			
Grade II-IV	27%	32%	.73
Grade III-IV	23%	11%	.28
Rates of chronic GVHD;* %(N)			
Overall chronic GVHD	44.1% (15)	44.8% (13)	.95
Limited chronic GVHD	8.8% (3)	31% (9)	.02
Extensive chronic GVHD	35.2% (12)	13.7% (4)	.05
Cumulative incidence of chronic GVHD at day +400† (%)			
Overall chronic GVHD	44%	61%	.09
Limited chronic GVHD	9%	44%	.01
Extensive chronic GVHD	40%	34%	.89

GVHD indicates graft-versus-host disease; ATG, antithymocyte globulin.
*Represents simple rates of GVHD to provide comparison with studies not reporting cumulative incidence of GVHD.
†Represents cumulative incidence of GVHD (at specified time points), while adjusting for competing events (for details please refer to statistical methods).

(Figure 2A). On univariate analysis, no other clinical variable was significantly associated with NRM risk. Causes of NRM are listed in Table 5.

At last follow-up 20 patients relapsed (R-ATG = 12; r-ATG = 8). The cumulative incidence of relapse for R-ATG and r-ATG patients was not significantly different ($P = .85$); the 1- and 2-year rates for the 2 groups were 28% versus 25% and 25% versus 31%, respectively (Figure 2B). On univariate analysis baseline diagnosis of CLL (HR = 0.09; 95% CI = 0.01-0.68; $P = .02$) was the only factor associated with the relapse risk.

OS and PFS

At last follow-up 49 patients were alive (R-ATG group = 21, r-ATG group = 28). Patients in the r-ATG group showed a trend for better 1-year OS compared to patients in the R-ATG group (84% versus 64%; log-rank $P = .07$) (Figure 3A; online only); the 2-year expected OS rates were 84% and 55%, respectively. Univariate analysis identified rabbit ATG dose (HR = 2.40; *P*-value = 0.08), baseline diagnosis of CLL (HR = 0.11; *p*-value = 0.03), and KPS ≥90 (HR = 0.50; *P*-value = 0.09) as variables of interest for multivariable analysis. However, none of these variables demonstrated independent prognostic significance on multivariate Cox regression analysis ($P > .05$). The expected 1-year PFS rates were 53% and 71% for patients in R-ATG group and those in r-ATG group, respectively (log-rank $P = .12$) (Figure 3B; online only). The corresponding estimates of 2-year PFS rates were 42% and 71%, respectively. In a multivariable analysis, baseline diagnosis of CLL (HR = 0.10; 95% CI = 0.02-0.45; $P = .003$) and KPS ≥90 (HR = 0.91; 95% CI = 0.85-0.97; $P = .008$) were the only factors independently associated with a better PFS.

DISCUSSION

As part of our continuous quality improvement program, we sought to reduce the risk of infectious complications and NRM observed in recipients of mostly unrelated RIC allografts by means of a planned dose reduction of rabbit ATG associated with our SOC protocol. We then retrospectively analyzed the impact of this modest ATG dose reduction on clinical outcomes and have made several interesting observations. First, our data support that a relatively small ATG dose reduction to 6 mg/kg had no deleterious effect on engraftment kinetics or donor chimerism. Second, the lower ATG dose did not appear to increase the risk of either aGVHD or cGVHD. Third, the dose reduction was associated with significantly less viral and bacterial infections. These results ultimately translated into a reduction in NRM and similar OS and PFS for patients that received the lower dose of ATG compared with those that received the higher dose. These findings we feel validate the importance of continuous quality monitoring associated with blood and marrow transplant programs.

Over the last 2 decades, various investigators have reported encouraging transplantation outcomes with the inclusion of ATG in transplant conditioning regimens [6,9,29-31], whereas others have reported comparable transplantation results without the use of ATG [32,33]. These divergent data have created considerable controversy about the role (if any) and indications of ATG use with allogeneic HSCT. The lack of randomized data addressing the role of ATG with transplant conditioning has been the key factor fueling this controversy for decades. Recently, a phase III trial, reported in abstract form only [34], has shown significant reduction in the rates of aGVHD and cGVHD for patients randomized to receiving ATG with transplant conditioning. This key study supports the use of ATG, at least for patients receiving unrelated donor allografts following MA conditioning.

ATG prevents development of GVHD, not only through in vivo donor effector TCD, but also via pleiotropic effects on the immune system including depletion and modulation of antigen presenting cells, modulation of cell surface molecules that mediate leukocyte/endothelium interactions and induction of regulatory T cells [35-40]. In addition to dose intensity, the efficacy of ATG in preventing GVHD is intricately dependent on the type of ATG preparation used and on the timing of ATG administration before HSCT [1,17,41]. Unfortunately, in the majority of the studies assessing ATG dose intensity, different ATG doses were administered

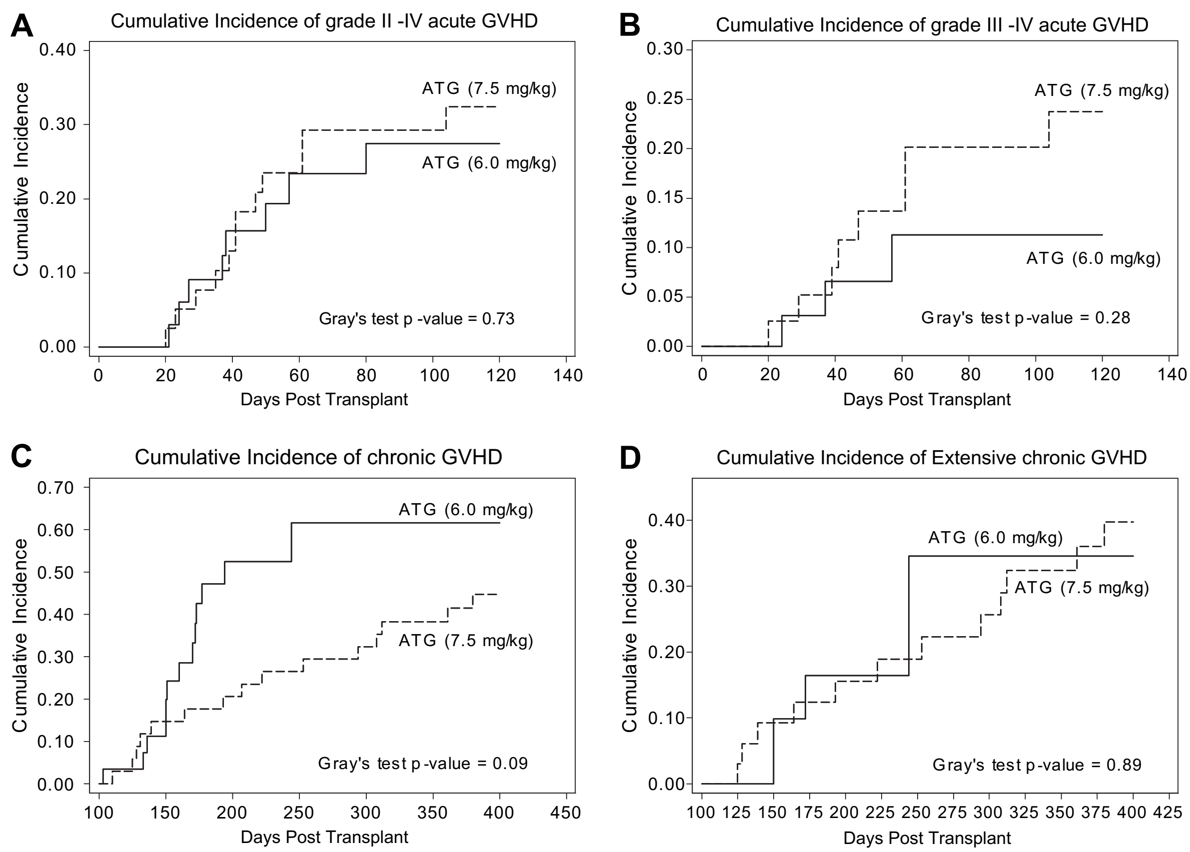

Figure 1. Cumulative incidence of GVHD according to the ATG dosage group. (A) cumulative incidence of grade II-IV aGVHD. (B) Cumulative incidence of grade III-IV aGVHD. (C) Cumulative incidence of cGVHD. (D) Cumulative incidence of extensive cGVHD. Solid curves represent patients receiving ATG at 6.0 mg/kg, whereas the dashed curves represent patients getting the 7.5 mg/kg ATG dose.

on different schedules, making interpretation of the efficacy results difficult [6,17,20]. Thus, in our study, when we elected to reduce the 7.5 mg/kg total dose of rabbit ATG in patients undergoing RIC HSCT, we decided to keep the ATG preparation and administration schedule uniform, to facilitate comparison of the 2 ATG dose levels. This allowed us to investigate the impact of an apparently lower ATG dose on GVHD control rates, graft function, infection, and other transplantation outcomes.

Table 4. Infectious Complications Postallogeneic Transplantation

	ATG (7.5 mg/kg) N (%)	ATG (6 mg/kg) N (%)	*P*-Value
CMV reactivation	25 (64.1)	10 (30.3)	*.005*
EBV reactivation	10 (25.6)	7 (21.2)	.78
Adenovirus infections	2 (5.1)	0	.49
BK-virus associated hemorrhagic cystitis	10 (25.6)	3 (9.1)	.12
Bacterial infections	22 (56.4)	6 (18.2)	*.001*
Gram-positive bacteria	*14*	*3*	
Gram-negative bacteria	*8*	*3*	
Invasive fungal infections	3 (7.7)	1 (3)	.62
Aspergillus fumigatus	*1*	*1*	
Histoplasma Capsulatum	*1*	—	
Candida albicans	*1*	—	

CMV indicates cytomegalovirus; EBV, Epstein-Barr virus; ATG, antithymocyte globulin.

Numerous studies have shown the efficacy of ATG for GVHD prophylaxis [4,6-10,19,31,42]. However, higher ATG doses (thymoglobulin dose-equivalents ≥7.5-15 mg/kg) have been associated with increased risk of relapse, infectious complications, and NRM [8,31,43]. Despite these data, only a handful of studies have attempted to define the optimal dose intensity of ATG. Bacigalupo et al. [6] reported outcomes of patients receiving either 15 mg/kg or 7.5 mg/kg of ATG (along with MA conditioning) compared to patients not receiving thymoglobulin. Although no direct dose comparisons were planned in that study, rates of aGVHD appeared worse (69%) with the lower 7.5 mg/kg ATG dose, compared to the higher 15 mg/kg dose (37%). However, the 15 mg/kg ATG was associated with significantly more lethal infectious complications.

Meijer et al. [18] compared progressively lower doses of ATG (8 mg/kg, 6 mg/kg, and 4 mg/kg) in patients undergoing NA HSCT, and reported significantly higher rates of aGVHD and trends toward higher rates of cGVHD with the lower ATG doses. A limitation of this study, however, was the relatively small number of patients receiving the lower 4 mg/kg (n = 9) and 6 mg/kg (n = 13) ATG dose. In contrast, Ayuk et al. [17] reported no difference in

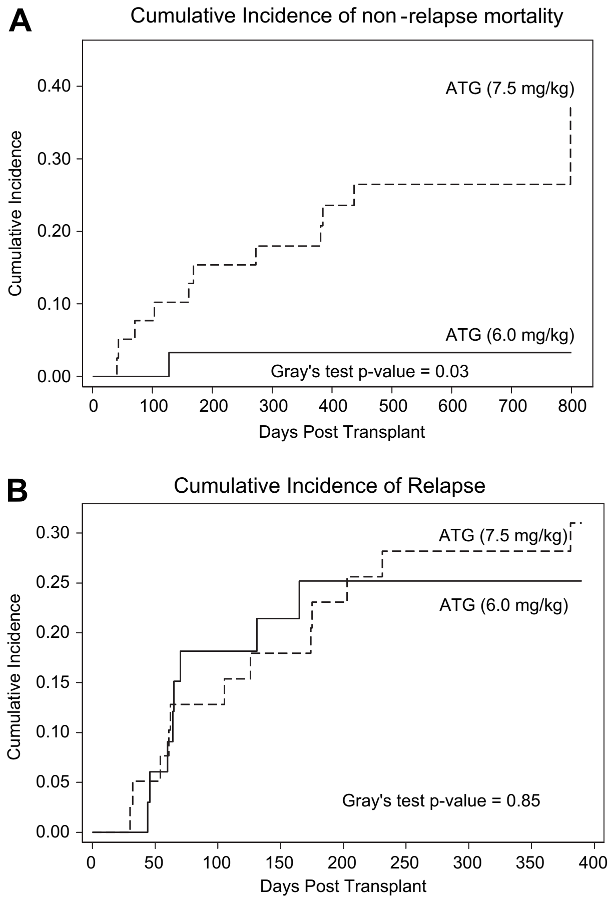

Figure 2. (A) Cumulative incidence of NRM according to the ATG dosage group. (B) Cumulative incidence of disease relapse according to the ATG dosage group. Solid curves represent patients receiving ATG at 6.0 mg/kg, whereas the dashed curves represent patients getting the 7.5 mg/kg ATG dose.

aGVHD and cGVHD rates in patients receiving 2 different doses of ATG-Fresenius (30 mg/kg versus 60 mg/kg) with MA conditioning. In the only series comparing ATG dose intensity in patients undergoing (matched sibling) RIC-HSCT, Mohty et al. [20] observed a significant increase in rates of aGVHD and cGVHD when ATG dose was reduced from ≥7.5 mg/kg to 2.5 mg/kg. Our analysis, in contrast,

Table 5. Causes of Nonrelapse mortality According to Dose of Antithymocyte Globulin Administered

Cause of death	ATG (7.5 mg/kg) N = 11	ATG (6 mg/kg) N = 1
GVHD with sepsis	1	1
GVHD without sepsis	1	—
Sepsis	1	—
Posttransplant lymphoproliferative disorder	2	—
Second malignancy	2	—
Adenoviral pneumonia	1	—
Cerebral toxoplasmosis	1	—
JC-viral progressive multifocal leukoencephalopathy	1	—
Cardiac toxicity	1	—

ATG indicates antithymocyte globulin; GVHD, graft-versus-host disease.

shows that a less aggressive ATG dose reduction may not compromise aGVHD control. This is a potentially important observation given that most of our patients received allografts from unrelated donors, had high-risk disease at baseline, and approximately a quarter of the patients received transplants from HLA-mismatched unrelated donors.

Significantly fewer r-ATG patients in our analysis received G-CSF postallografting, which may explain acceptable aGVHD control rates in this group. It must be pointed out, however, that although some studies suggest an increased risk of aGVHD with post-HSCT G-CSF administration [44], others have found no such association [28,45]. Moreover, in our analyses G-CSF was not independently associated with an increased risk of aGVHD ($P = .63$). We also found no negative correlation between attainment of full donor chimerism and lower ATG dose. The median time to neutrophil engraftment was longer in the r-ATG patients, which is likely because of less frequent use of G-CSF in this group.

In our study, the higher 7.5 mg/kg ATG dose was associated with frequent CMV reactivations, bacterial infections, and lethal infectious complications, leading

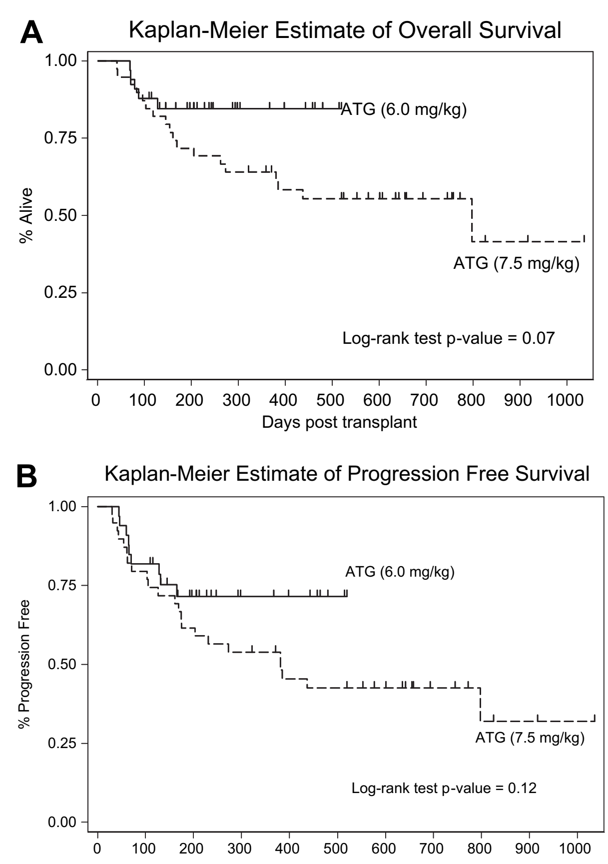

Figure 3. OS and PFS according to ATG dosage group. (A) Kaplan-Meier estimates of OS following allogeneic transplantation, (B) Kaplan-Meier estimates of PFS following allogeneic transplantation. Solid curves represent patients receiving ATG at 6.0 mg/kg, whereas the dashed curves represent patients getting the 7.5 mg/kg ATG dose.

to significantly higher NRM rates. However, unlike previous studies, where the benefit of fewer infectious complications attained with lower ATG doses (2.5-4 mg/kg) was offset by unacceptable rates of GVHD [18,20,43], we demonstrated here that "conservative" ATG dose deescalation to 6 mg/kg can significantly reduce the rates of infectious events and NRM while maintaining acceptable GVHD control rates. The 1-year NRM rate of 3% in the r-ATG cohort is encouraging, especially when considering the high-risk characteristics of patients included at baseline.

In summary, our findings suggest that for patients undergoing RIC HSCT, a total dose of ATG as low as 6 mg/kg dose appears to be associated with significantly fewer infectious complications and lower rates of NRM, without compromising GVHD control or survival when compared to higher administered doses. Our data highlight the fact that the relative ATG dose intensity has significant impact on transplantation outcomes, underscoring the need to systematically determine and employ the ATG dose with "best" therapeutic index, not only in clinical practice, but also in future clinical trials. We must acknowledge that these findings were made in the context of a quality improvement effort rather than a prospective hypothesis driven research protocol. It also involved a heterogeneous group of high-risk patients. Nevertheless, the present study is one of the larger analyses that have attempted to address the question of defining the best therapeutic index for ATG in patients undergoing HSCT. Based on our analysis, we conclude that validation of these results would be warranted in a larger, prospective trial evaluating the impact of varying doses of ATG.

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