Biol Blood Marrow Transplant 25 (2019) 1993–2001

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Comparison of Two Doses of Antithymocyte Globulin in Reduced-Intensity Conditioning Allogeneic Hematopoietic Stem Cell Transplantation

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Article history:
Received 15 April 2019
Accepted 17 June 2019

Key Words:
Antithymocyte globulin
Allogeneic transplantation
Reduce-intensity conditioning

A B S T R A C T

The appropriate dose of antithymocyte globulin (ATG) to be used in reduced-intensity conditioning (RIC) allogeneic hematopoietic stem cell transplantation (allo-HSCT) is yet to be determined. We retrospectively analyzed the outcomes of patients who underwent unrelated or mismatch related RIC allo-HSCT for hematologic malignancies and received r-ATG (4.5 mg/kg, 141 patients) versus R-ATG (6 mg/kg, 216 patients). There was a higher incidence of cytomegalovirus ($P < .001$) and Epstein-Barr virus viremia ($P = .03$) in the R-ATG group than in the r-ATG group. The cumulative incidences of acute graft-versus-host disease (aGVHD) grades II to IV at day 180 in the r-ATG and R-ATG groups were 59% and 44% ($P = .006$) and grades III to IV 20% and 12% ($P = .029$), respectively. In multivariable models adjusting for disease diagnosis, the risk of aGVHD grades III to IV did not reach statistical significance ($P = .087$). The respective cumulative incidences of chronic GVHD in the r-ATG and R-ATG groups were 26% and 15% ($P = .10$), respectively. There were no significant differences in relapse rate ($P = .24$), nonrelapse mortality ($P = .96$), progression-free survival ($P = .24$), overall survival ($P = .70$), and GVHD-free relapse-free survival ($P = .24$). In this retrospective analysis, aGVHD incidence was higher in those treated with r-ATG compared with R-ATG, but this did not translate into significant differences of clinical outcome. Given the increasing use of RIC allo-HSCT for treating malignant hematologic conditions, the correct dose and schedule of ATG administration should be defined by prospective randomized controlled trials.

INTRODUCTION

There has been an increasing interest in using reduced-intensity conditioning (RIC) regimens in allogeneic hematopoietic stem cell transplantation (allo-HSCT) in elderly patients and in young patients with poor general condition. This therapeutic approach emphasizes a strong inhibition of the recipient's immune function rather than ablating the bone marrow [1] and relying on the post-transplant graft-versus-tumor effect of the graft for disease control [2]. When compared with myeloablative conditioning, RIC is associated with a decreased incidence of acute graft-versus-host disease (aGVHD), albeit only little impact on the incidence of chronic GVHD (cGVHD), and may be associated with an increased incidence of late aGVHD (ie, aGVHD occurring after day 100), often at the time of conversion from mixed to full donor T cell chimerism [3-8].

Adding antithymocyte globulin (ATG) in 1 of its 3 commercially available preparations (Thymoglobulin [Genzyme, Cambridge, MA], ATGAM [Pharmacia, Piscataway, NJ], and ATG-Fresenius [ATG-F, Fresenius, Germany]) for in vivo T cell depletion has been shown to decrease the incidence of aGVHD and cGVHD, with mixed effects on disease relapse [4,9-14]. These studies have used different preparations and dose intensities of ATG, making it difficult to compare outcomes between them [14]. ATG works through multiple mechanisms, including T cell depletion in the blood and lymphoid tissues by induction of apoptosis or complement-dependent lysis; apoptosis of naive B cells, activated B cells, and plasma cells [15,16]; and induction of regulatory T cells and natural killer cells [17]. These effects could potentially lead to serious infections such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV) and possibly to disease relapse [18-20].

There has been an ongoing effort to define the appropriate dose, schedule, and preparation of ATG that should be added

Financial disclosure: See Acknowledgments on page 2000.

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https://doi.org/10.1016/j.bbmt.2019.06.014
1083-8791/

to RIC regimens to decrease the incidence of GVHD, without significantly increasing the risk of infection and relapse [10]. Preliminary data suggest that higher ATG doses (>7.5 mg/kg) are associated with increased incidence of disease relapse, infections, and nonrelapse mortality (NRM) but that lower doses (≤2.5 mg/kg) do not offer adequate prophylaxis against GVHD [10,12,14]. Therefore, an intermediate level between 2.5 mg/kg and 7.5 mg/kg may provide adequate protection against GVHD without significantly compromising the graft-versus-tumor effect or increasing the risk of infections [9,10,14].

In our previous retrospective analysis (n = 136) of 2 doses of ATG (6 mg/kg and 7.5 mg/kg), we found no significant differences in the cumulative incidences of aGVHD grades II to IV and grades III to IV at 180 days or cGVHD at 24 months. There were no significant differences in relapse rate, NRM, progression-free survival (PFS), and overall survival (OS) [9]. As part of a quality improvement effort to reduce the risk of infectious complications and NRM, the dose of ATG was further reduced at The Ohio State University Comprehensive Cancer Center to 4.5 mg/kg as of December 2013. Now we present our first report on patients who underwent RIC allo-HSCT by comparing patients treated with a low-dose ATG (4.5 mg/kg, r-ATG, n = 141) with patients treated with a high-dose ATG (6 mg/kg, R-ATG, n = 216).

METHODS

Study Design

We retrospectively reviewed 357 consecutive patients who underwent matched unrelated or mismatch related/unrelated RIC allo-HSCT for malignant hematologic diseases at The Ohio State University Comprehensive Cancer Center and received rabbit ATG dose of 6 mg/kg or 4.5 mg/kg as part of their conditioning regimen between October 2007 and September 2016. The study was approved by the institutional review board at The Ohio State University.

RIC Regimen

Most patients (n = 311) were given fludarabine intravenously 30 mg/m^2/day over 30 minutes on days −7 to −3 (total dose, 150 mg/m^2) and intravenous busulfan .8 mg/kg per dose over 2 hours every 6 hours for 8 doses on days −4 to −3 (total busulfan dose, 6.4 mg/kg). Thirty-nine patients received conditioning with cyclophosphamide and total body irradiation, 3 with fludarabine and melphalan, and 4 with fludarabine and total body irradiation. In the R-ATG group, patients received 6 mg/kg of rabbit ATG (Thymoglobulin) at a dose of 2 mg/kg/day over 6 hours on days −4 to −2, and in the r-ATG group (Genzyme, Cambridge, MA), patients received 4.5 mg/kg given at 2.25 mg/kg/day on days −3 to −2.

GVHD Prophylaxis

All but 10 patients received standardized GVHD prophylaxis with tacrolimus .03 mg/kg/day intravenously starting on day −2 and methotrexate 5 mg/m^2 on days +1, +3, +6, and +11. Four patients were given tacrolimus with mycophenolate mofetil, 2 patients each were given cyclosporine/mycophenolate mofetil or tacrolimus/sirolimus, and 1 patient each was given cyclosporine/methotrexate and cyclosporine/mycophenolate mofetil. Tacrolimus was switched to an oral form at the time of neutrophil engraftment. Tacrolimus blood levels were monitored initially twice weekly and then once weekly until day +100 to maintain blood levels between 8 and 12 ng/mL. From day 90+ onward the tacrolimus dose was tapered off slowly over 3 months if there was no evidence of GVHD.

Supportive Care

Prophylaxis against fungal infection consisted of fluconazole, voriconazole, or posaconazole initiated on day −1 and continued until day +100 unless continuation of therapy was clinically indicated in accordance with institutional standards. Antiviral prophylaxis consisted of oral valacyclovir for herpes infections and trimethoprim-sulfamethoxazole or dapsone for *Pneumocystis jirovecii*. Both medications were continued until patients were off immunosuppressive therapy and the CD4$^+$ count was ≥200. Routine CMV prophylaxis was not used. Preemptive screening for CMV infection consisted of weekly CMV monitoring by serum quantitative PCR beginning on admission and until day +100 or longer as dictated by the clinical course. Treatment was initiated when the PCR indicated > 4000 copies/mL and outpatient treatment consisted of valganciclovir 900 mg twice daily until CMV was undetectable by PCR.

Monitoring of EBV was done weekly through day +100. If serum EBV PCR levels were greater than 4000 copies/mL on repeated measurement, patients were treated with 1 dose of rituximab at 375 mg/m^2. Any further increase in serum EBV PCR levels were treated with 3 weekly doses of rituximab for a total of 4 doses. Granulocyte colony-stimulating factor was part of standard-of care treatment until September 2007 when it was excluded and only given at the discretion of the treating physician in cases of infections with neutropenia.

GVHD Assessment and Treatment

aGVHD was graded according to the 1994 consensus conference on aGVHD grading [21]. cGVHD was classified into limited versus extensive [22]. Corticosteroids were used as first-line therapy to treat aGVHD (grades II to IV) and extensive cGVHD. Second-line therapy was according to the treating clinician's choice.

Definitions of Outcomes

Cumulative incidence of aGVHD was evaluated within the first 180 days post-transplant (to include aGVHD and delayed aGVHD cases). cGVHD was evaluated within the first 24 months post-transplant.

Time to NRM was defined as the time from transplant to death in the absence of disease relapse. The competing risk for NRM was death due to disease. Time to relapse of malignant disease was defined as the time from transplant to the day of the first diagnostic test that showed relapse, defined as recurrence of the original malignancy, based on standard testing. The competing risk for relapse was death from any cause. PFS was defined as the time from transplant until progression or death, whichever occurred first. GVHD-free, relapse-free survival (GRFS) was defined as the time from transplant until relapse, grades III to IV aGVHD, cGVHD, or death, whichever occurred first. OS was defined as the time from transplant until death. Graft failure was defined as either lack of initial engraftment of donor cells (primary graft failure) or loss of donor cells after initial engraftment (secondary graft failure) [23]. Competing risks for aGVHD and cGVHD were relapse or death. The time to neutrophil engraftment was considered the first of 3 consecutive days with an absolute neutrophil count $\geq .5 \times 10^9$/L. The time to platelet engraftment was considered the first of 7 consecutive days with a platelet count $\geq 20 \times 10^9$/ L without transfusion.

Statistical Analysis

We compared the clinical and demographic variables between the 2 cohorts of patients included in the study (patients who received the smaller dose of ATG [4.5 mg/kg, r-ATG] and the higher dose [6 mg/kg, R-ATG]). The Fisher exact test or chi-square test was used for categorical variables, and the Wilcoxon rank sum test for the continuous variables. PFS, GRFS, and OS estimates were calculated by the Kaplan-Meier method and compared using the log-rank test. Cumulative incidences of aGVHD, cGVHD, relapse, and NRM were analyzed using Gray's test and accounting for competing risks.

As mentioned above, competing risks for aGVHD and cGVHD were relapse or death. The competing risk for relapse was death from any cause, and the competing risk for NRM was death due to disease. Cumulative incidence for infection types was evaluated in the first 180 days of transplant and also analyzed using Gray's test, where the competing risks were grades II to IV aGVHD, cGVHD, relapse, or death before any documented infection. For all analyses patients without an event were censored at the time last evaluated for a particular endpoint.

Univariable models were fit using either Cox proportional hazards or Fine and Gray models to estimate the association between ATG dose and corresponding outcome. Variables considered for potential confounding effect in association between ATG dose and outcome included patient age and sex, donor sex, disease group, prior autograft, donor relation to patients, degree of HLA match, comorbidity index, graft source, CMV status, and stem cell dose. Confounders were included together with ATG dose in the final multivariable model.

The significance level was set at $\alpha = .05$, and all P values presented are from 2-sided tests. All analyses were conducted using Stata 14 (Stata Corp LLC; 4905 Lakeway Drive, College Station, Texas 77845-4512, USA) (https://www.stata.com) or the cmprsk package in TIBCO (TIBCO Software Inc.; 3307 Hillview Avenue, Palo Alto, CA 94304, USA) Spotfire S version 8.2.0.

RESULTS

Patient Characteristics

Of 357 patients enrolled, 216 received 6 mg/kg ATG (high dose, R-ATG) and 141 received 4.5 mg/kg ATG (low dose, r-ATG). Baseline clinical and demographic characteristics are illustrated in Table 1.

Table 1
Baseline Patient Characteristics at the Time of Transplant

Patient Characteristics	Overall (N = 357)	R-ATG (6 mg/kg) (n = 216)	r-ATG (4.5 mg/kg) (n = 141)	*P*
Median age, yr (range)	57 (19-74)	59 (20-73)	53 (19-74)	.01
Age ≥ 60 yr	145 (41)	99 (46)	46 (33)	.02
Age < 60 yr	212 (59)	117 (54)	95 (67)	
Donor gender				.99
Male	302 (85)	183 (85)	119 (84)	
Female	55 (15)	33 (15)	22 (16)	
Recipient gender				.06
Male	214 (60)	138 (64)	76 (54)	
Female	143 (40)	78 (36)	65 (46)	
Diagnosis				<.001
AML/MDS	192 (54)	117 (54)	75 (53)	
NHL/Hodgkin disease	52 (15)	43 (20)	9 (6)	
CLL	33 (9)	31 (14)	2 (1)	
Other	80 (22)	25 (12)	55 (39)	
Prior transplant				.83
No	334 (94)	201 (93)	133 (94)	
Yes	23 (6)	15 (7)	8 (6)	
Donor				<.001
Unrelated	339 (95)	198 (92)	141 (100)	
Mismatch related	18 (5)	18 (8)	0 (0)	
HLA match				.99
10/10	312 (87)	189 (88)	123 (87)	
<10/10 (9/10, 8/10, 8/8)	45 (13)	27 (13)	18 (13)	
Median comorbidity index (range)	3 (0-12)	3 (0-12)	3 (0-7)	.04
CMV status				.58
Patient and/or donor +ve	214 (60)	132 (61)	82 (58)	
Both patient and donor −ve	143 (40)	84 (39)	59 (42)	
Graft source				.001
Bone marrow	31 (9)	10 (5)	21 (15)	
Peripheral blood stem cells	326 (91)	206 (95)	120 (85)	
Median $CD34^+$ cell dose (range)	7.0 (.96-12.0)	7.2 (1.7-12.0)	6.7 (.96-11.0)	.06

Abbreviations: AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; +ve indicates positive; −ve indicates negative; N indicates number of patients; R-ATG indicates high dose ATG; r-ATG indicates low dose ATG.

There were no significant differences in gender distribution for donors ($P = .99$), recipients ($P = .06$), degree of HLA match ($P = .99$), number of prior autografts ($P = .83$), donor/recipient CMV status ($P = .58$), or CD34 cell dose ($P = .06$) between the 2 groups. However, in the r-ATG group, patients were younger ($P = .01$), had a lower comorbidity index ($P = .04$), and bone marrow was used more often as the graft source ($P = .001$).

Engraftment and Chimerism

Median time to neutrophil engraftment was 16 days (range, 9 to 33) in the r-ATG group versus 16 days (range, 3 to 45) in the R-ATG group ($P = .59$). Time to platelet engraftment was 17 days (range, 10 to 133) in the r-ATG group versus 16 days (range, 8 to 49) in the R-ATG group ($P = .007$). Donor cell chimerism on days +30, +90, +180, and +360 was not significantly different between groups (data not shown). No patients developed secondary graft failure in the r-ATG group versus 2 patients in the R-ATG group.

Graft-versus-Host Disease

All patients were assessed for the presence of grades II to IV aGVHD in the first 180 days and the presence of cGVHD. Cumulative incidence of grades II to IV aGVHD in the r-ATG group was 59% (83 patients) compared with 44% in the R-ATG group (96 patients) ($P = .006$). Cumulative incidences of aGVHD grades III to IV were 20% (28 patients) in the r-ATG group compared with 12% (25 patients) in the R-ATG group ($P = .029$). The median time to onset of aGVHD grades II to IV was 62 days in the r-ATG group but was not reached in the R-ATG group. The limited cGVHD (60 patients [26%] versus 77 patients [15%], $P = .10$) and extensive cGVHD (53 patients [23%] versus 66 patients [13%], $P = .11$) were not significantly different between the 2 groups. Median time to cGVHD onset was not reached in either group (Table 2, Figure 1). In multivariable models adjusting for disease diagnosis, the risk of aGVHD grades II to IV was significantly reduced in the R-ATG versus r-ATG group (hazard ratio [HR], .70; 95% confidence interval [CI], .50 to .96; $P = .029$), whereas the risk of aGVHD grades III to IV was also reduced but did not reach statistical significance (HR, .64; 95% CI, .38-1.07; $P = .087$).

Infectious Complications

There was a significantly lower number of patients in the r-ATG group (13 patients, 9%) who developed CMV reactivation compared with the R-ATG group (57 patients, 26%; $P < .001$) and a lower cumulative incidence of EBV reactivation (14 patients [9%] versus 39 patients [18%], respectively; $P = .03$). No differences were seen between the r-ATG and R-ATG groups in BK virus–associated hemorrhagic cystitis (12 patients [9%] versus 10 patients [4%], respectively; $P = .13$) and *Clostridium difficile*infections (19 patients [13%] versus 17 patients [8%], respectively; $P = .08$). However, higher bacterial

Table 2
Pattern of GVHD

Cumulative Incidence	Overall (N = 357)	R-ATG (6 mg/kg) (n = 216)	r-ATG (4.5 mg/kg) (n = 141)	*P*
aGVHD				
Grades II-IV				.006
No. of events	179	96	83	
Cumulative incidence at day 180	.50 (.45-.55)	.44 (.38-.51)	.59 (.50-.66)	
Grades III-IV				.029
No. of events	53	25	28	
Cumulative incidence at day 180	.15 (.11-.19)	.12 (.07-.16)	.20 (.14-.27)	
cGVHD				
Limited and extensive				.10
No. of events	137	77	60	
Cumulative incidence at 6 mo	.19 (.15-.24)	.15 (.11-.20)	.26 (.19 -.33)	
Cumulative incidence at 12 mo	.35 (.30-.39)	.32 (.26-.38)	.38 (.30-.46)	
Cumulative incidence at 24 mo	.39 (.34-.44)	.36 (.29-.42)	.43 (.35-.51)	
Extensive				.11
No. of events	119	66	53	
Cumulative incidence at 6 mo	.17 (.13-.21)	.13 (.09-.18)	.23 (.16-.30)	
Cumulative incidence at 12 mo	.30 (.25-.35)	.27 (.22-.33)	.34 (.26-.42)	
Cumulative incidence at 24 mo	.34 (.29-.38)	.31 (.25-.35)	.38 (.30-.46)	

Values in parentheses are 95% CI. N indicates number of patients; R-ATG indicates high dose ATG; r-ATG indicates low dose ATG.

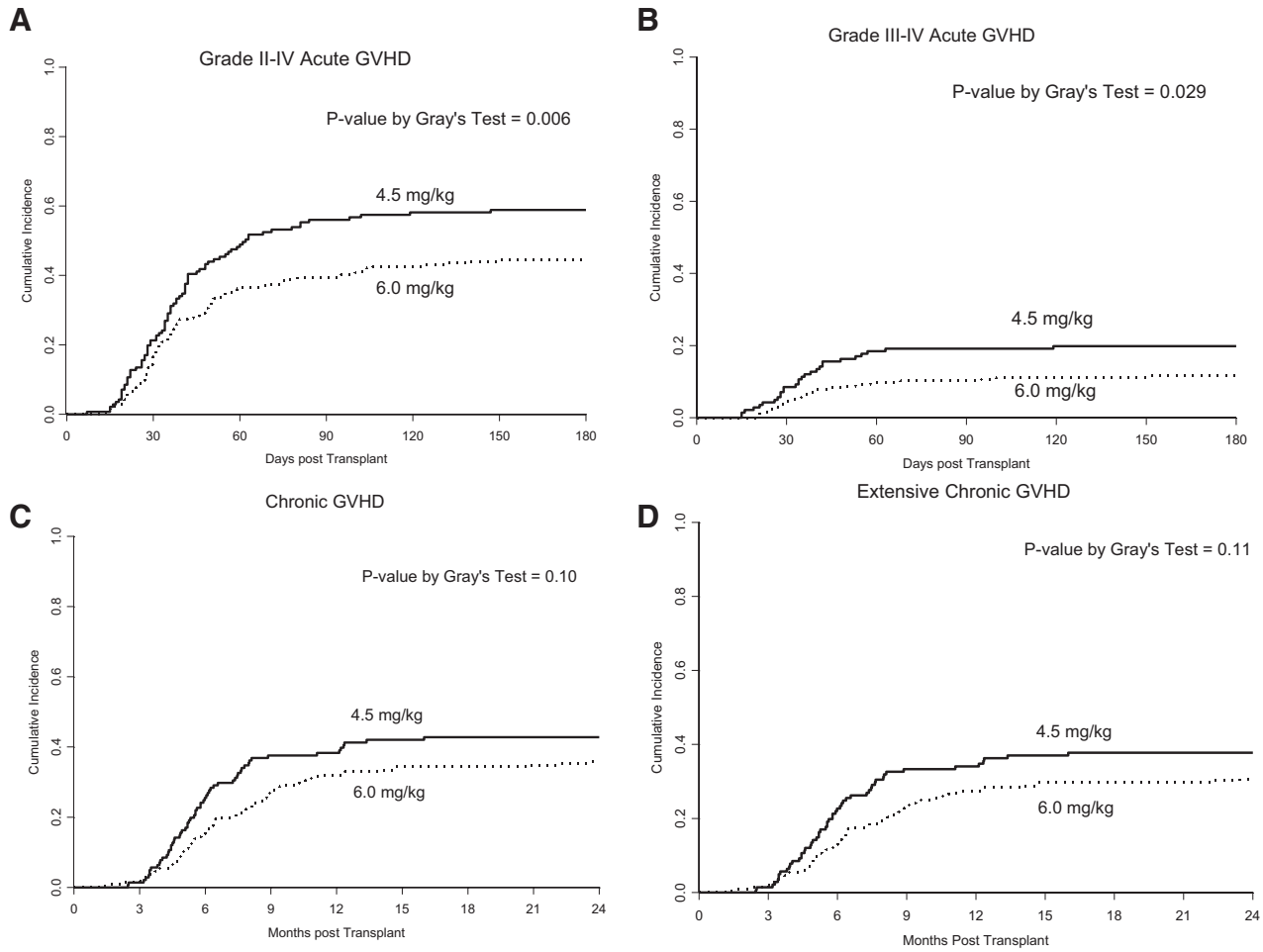

Figure 1. Cumulative incidence of aGVHD and cGVHD according to the dose of ATG administered. (A) Cumulative incidence of grades II to IV aGVHD. (B) Cumulative incidence of grades III to IV aGVHD. (C) Cumulative incidence of cGVHD. (D) Cumulative incidence of extensive cGVHD. Solid line indicates patients who were on r-ATG (4.5 mg/kg), and dotted line indicates patients who were on R-ATG (6.0 mg/kg).

Table 3
Infectious Complications after Transplant

Characteristic	Overall (N = 357)	R-ATG (6 mg/kg) (n = 216)	r-ATG (4.5 mg/kg) (n = 141)	*P*
CMV reactivation				<.001
No. of events	70	57	13	
Cumulative incidence at day 180)	.20 (.16-.24)	.26 (.21-.32)	.09 (.05-.15)	
EBV reactivation				.03
No. of events	53	39	14	
Cumulative incidence at day 180	.15 (.11-.18)	.18 (.13-.23)	.09 (.05-.15)	
BK virus–associated hemorrhagic cystitis				.13
No. of events	22	10	12	
Cumulative incidence at day 180	.06 (.04-.09)	.04 (.02-.07)	.09 (.05-.14)	
Bacterial infections				.01
No. of events	82	42	40	
Cumulative incidence at day 180	.23 (.18-.27)	.18 (.13-.24)	.29 (.22-.37)	
C. difficile infections				.08
No. of events	36	17	19	
Cumulative incidence at day 180	.10 (.07-.13)	.08 (.04-.11)	.13 (.08-.19)	

Values in parentheses are 95% CI. N indicates number of patients; R-ATG indicates high dose ATG; r-ATG indicates low dose ATG.

infections were seen in the r-ATG group (40 patients [29%] versus 42 patients [18%], respectively; $P = .01$) (Table 3, Figure 2). Five patients developed adenovirus infection, 1 in the r-ATG group and 4 in the R-ATG group.

Survival and Relapse

With a median follow-up of 28 months (range, 7 to 51) in the r-ATG group, 63 patients (45%) have died, 39 (62%) from relapsed disease, 13 (21%) from GVHD, and 1 from veno-occlusive disease. With a median follow-up of 66 months (range, 8 to 111) in the R-ATG group, 128 patients (59%) have died, 75 from relapsed disease, 23 from GVHD, and 2 from secondary cancer. There were no significant differences between the r-ATG group and R-ATG group with respect to relapse, PFS, NRM, GRFS, and OS. At 6 months the respective cumulative incidences were relapse (39 patients [24%] versus 85 patients [24%], $P = .24$), PFS (69% versus 66%, $P = .24$), NRM (7% versus 9%, $P = .96$), GRFS (35% versus 48%, $P = .24$), and OS (80% versus 80%, $P = .70$); at 2 years the cumulative incidences were relapse (39 patients [34%] versus 85 patients [28%], $P = .24$), PFS (48% versus 54%, $P = .24$), NRM (17% versus 17%, $P = .96$), GRFS (21% versus 20%, $P = .24$), and OS (55% versus 57%, $P = .70$) (Table 4, Figure 3).

DISCUSSION

This study comes as a continuation of our previous reports comparing different doses of Thymoglobulin as part of an effort to define the optimal dose and preparation of ATG in RIC allo-HSCT. In our current retrospective analysis of 357 patients given ATG (Thymoglobulin), we found an increase in the incidence of grades II to IV aGVHD in the r-ATG group compared with the R-ATG group (59% versus 44%, $P = .006$) and an increase in grades III to IV aGVHD (20% versus 12%, $P = .029$); however, this did not translate into significantly worse clinical outcome. cGVHD at 24 months was diagnosed with similar frequency in the r-ATG group versus R-ATG group (36% versus 43%, $P = .10$) in patients undergoing HSCT. In fact, relapse, NRM, PFS, OS, and GRFS did not differ between the 2 groups. Although more patients in the R-ATG group received a transplant from mismatched donors, had peripheral blood stem cells as the donor source (versus bone marrow), and were ≥60 years old, none of these variables was found to have an association between ATG and outcome. Hence, they were not included in the multivariable analysis. There was a higher incidence of CMV and EBV viremia in the R-ATG group compared with the r-ATG group. In a previous report by Salem et al. [9], when a Thymoglobulin dose of 7.5 mg was compared with a 6-mg/kg dose, there were no statistically significant differences in grades II to IV aGVHD, cGVHD, EBV or CMV infections, relapse rate, NRM, PFS, and OS but was a higher incidence of BK virus–associated hemorrhagic cystitis.

Most available data about the use of ATG in RIC come from retrospective studies, with only few prospective trials reported. In a randomized controlled phase III trial including 203 patients, an advantage was found in adding rabbit ATG (Thymoglobulin) 4.5 mg/kg to both myeloablative conditioning and nonmyeloablative conditioning regimens because it reduced the need to use immunosuppressants at 1 year (the primary endpoint of the study) (16% versus 37%, $P = .0006$). Incidence of aGVHD at 100 days was 50% with ATG versus 65% without it ($P = .012$). There was a manageable increased incidence of EBV reactivation with ATG (22 patients [33%] versus 2 patients [3%]) [13]. The prospective study by Finke et al. [11] and updated by Socie et al. [8] of 202 patients in which the authors looked at the role of ATG-Fresenius as part of myeloablative conditioning regimens in unrelated allo-HSCT showed that adding ATG-F to a GVHD prophylaxis regimen of cyclosporine and methotrexate led to a drop in the incidence of extensive cGVHD at 3 years from 45% to 12.2% ($P < .0001$) The 3-year relapse rate was similar between patients receiving ATG-F and control subjects (32.6% versus 28.2%), but there was a trend toward reduction in the NRM in the ATG-F group (19.4% versus 33.5%), resulting in a 3-year OS of 55.2% versus 43.3% (HR, .84; $P = .39$) in the ATG-F group versus control group. Soiffer et al. [24] reviewed 1676 adult patients undergoing RIC HSCT for hematologic malignancies. Outcomes after in vivo T cell depletion (584 patients with either rabbit or horse ATG [Thymoglobulin] in different dosing schedules and 213 patients with alemtuzumab) were compared with T cell–replete (879 patients) transplant. The study showed similar rates of aGVHD grades II to IV between the ATG and T cell–replete groups (38% versus 40%) but a lower rate of aGVHD grades II to IV with alemtuzumab (19%; $P < .0001$). The 3-year rate of cGVHD was lowest in the alemtuzumab group, followed by the ATG group, and highest in the T cell–replete group (24% versus 40% versus 52%, respectively; $P < .0001$). However, relapse was

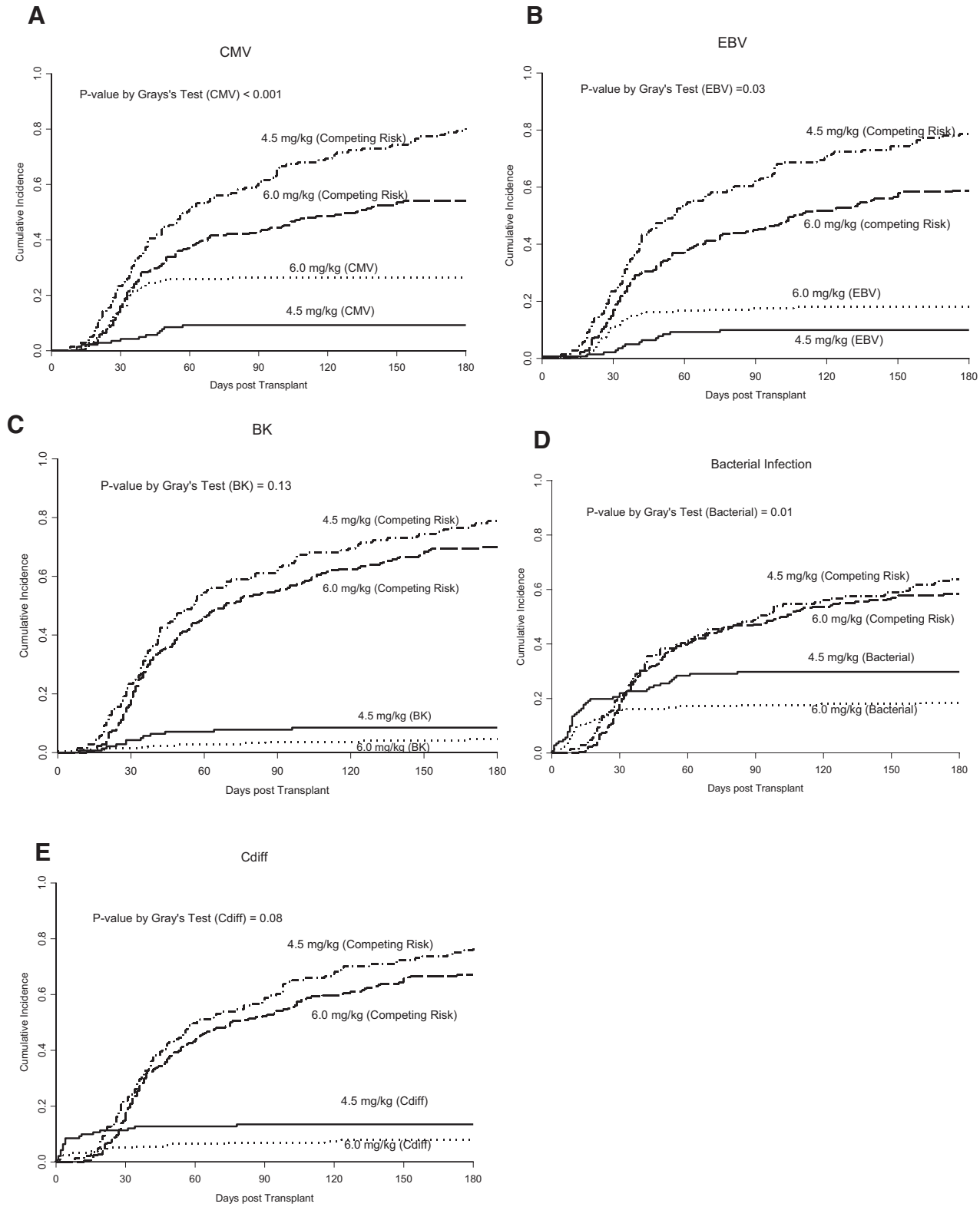

Figure 2. Infectious complications after transplant according to ATG dosing. (A) Cumulative incidence of CMV reactivation. (B) Cumulative incidence of EBV reactivation. (C) Cumulative incidence of BK virus–induced infection. (D) Cumulative incidence of bacterial infection. (E) Cumulative incidence of *C. difficile* infections. Solid line indicates patients who were on r-ATG (4.5 mg/kg), and dotted line indicates patients who were on R-ATG (6.0 mg/kg). Incidence of competing risks between the 2 groups are as indicated by dashed lines.

more frequent with alemtuzumab and ATG compared with T cell–replete regimens (49%, 51%, and 38%, respectively; $P < .001$) with corresponding probabilities of OS (50%, 38%, and 46%, respectively; $P = .008$). The authors suggested adopting a cautious approach to routine use of in vivo T cell depletion with RIC regimens [24].

Devillier et al. [25] reported that rabbit ATG (Thymoglobulin) at 5 mg/kg compared with 2.5 mg/kg in RIC allo-HSCT led

Table 4
Survival and Relapse Post-Transplant

Outcome	Overall (N = 357)	R-ATG (6 mg/kg) (n = 216)	r-ATG (4.5 mg/kg) (n = 141)	*P*
Median follow-up, mo (range)	47 (7-111)	66 (8-111)	28 (7-51)	
NRM				.96
No. of events	77	53	24	
Cumulative incidence at 6 mo	.08 (.06-.12)	.09 (.05-.13)	.07 (.04-.12)	
Cumulative incidence at 12 mo	.13 (.10-.17)	.14 (.10-.19)	.11 (.07-.17)	
Cumulative incidence at 24 mo	.17 (.13-.21)	.17 (.12-.22)	.17 (.11-.24)	
Relapse				.24
No. of events	124	85	39	
Cumulative incidence at 6 mo	.24 (.20-.29)	.24 (.19-.30)	.24 (.17-.31)	
Cumulative incidence at 12 mo	.30 (.25-.35)	.32 (.25-.38)	.27 (.20-.34)	
Cumulative incidence at 24 mo	.32 (.27-.37)	.34 (.28-.41)	.28 (.21-.35)	
PFS				.24
No. of events	204	137	67	
Median, mo	25 (15-40)	22 (10-36)	38 (15-NR)	
PFS at 6 mo	.67 (.62-.72)	.66 (.59-.72)	.69 (.60-.76)	
PFS at 12 mo	.57 (.52-.62)	.54 (.47-.60)	.62 (.53-.69)	
PFS at 24 mo	.51 (.45-.56)	.48 (.42-.55)	.54 (.46-.62)	
GRFS				.24
No. of events	294	182	112	
Median, mo	4.9 (4.4-5.6)	5.3 (4.7-6.4)	4.3 (3.4-5.3)	
GRFS at 6 mo	.43 (.38-.48)	.48 (.41-.54)	.35 (.28-.43)	
GRFS at 12 mo	.25 (.21-.30)	.26 (.21-.32)	.24 (.17-.31)	
GRFS at 24 mo	.21 (.17-.25)	.21 (.16-.27)	.20 (.14-.27)	
OS				.70
No. of events	191	128	63	
Median, mo	43 (25-64)	42 (20-64)	38 (23-NR)	
OS at 6 mo	.80 (.75-.84)	.80 (.74-.85)	.80 (.73-.86)	
OS at 12 mo	.66 (.61-.70)	.63 (.57-.69)	.69 (.61-.76)	
OS at 24 mo	.26 (.51-.61)	.55 (.48-.61)	.57 (.49-.65)	
Cause of death, n (%)	(n = 191)	(n = 128)	(n = 63)	.007
Disease	114 (60)	75 (59)	39 (62)	
Infection	18 (9)	14 (11)	4 (6)	
GVHD	36 (19)	23 (18)	13 (21)	
Second cancer	2 (1)	2 (2)	0 (0)	
Cardiac	1 (1)	1 (1)	0 (0)	
Respiratory	7 (4)	1 (1)	6 (10)	
VOD	2 (1)	1 (1)	1 (2)	
Other	11 (6)	11 (9)	0 (0)	

Values in parentheses are 95% CI unless otherwise defined. VOD indicates veno-occlusive disease; NR, not reached; N indicates number of patients; R-ATG indicates high dose ATG; r-ATG indicates low dose ATG.

to lower incidence of grades II to IV aGVHD at 100 days (8.8% versus 30.2%, $P = .038$) and less extensive cGVHD (12% versus 60.4%, $P < .001$) with no differences in the relapse rate at 24 months (28.5% versus 18.9%, $P = .64$) or NRM at 1 year (19.6% versus 17%, $P = .56$). Bashir et al. [26] in a phase II comparative trial found no differences between rabbit ATG (Thymoglobulin) 4.5 mg/kg and ATG 7.5 mg/kg in aGVHD, cGVHD, RFS, treatment-related mortality, and OS. Further, in a study by Binkert et al. [27] lower dose ATG was shown to reduce treatment-related mortality without increasing disease relapse, reduce incidence of cGVHD, and increase OS (75% versus 50%) compared with the higher ATG dose. Although the incidence of grades II to IV aGVHD reported in our study (59% in r-ATG and 44% in R-ATG) was higher than those published by some using the same dose of rabbit ATG (17.6% by Baron et al. [20], 50% by Walker et al. [13], and 38% by Soiffer et al. [24]), most aGVHD cases in our study were grade II, with only 20% and 12% grades III to IV aGVHD cases occurring in the r-ATG and R-ATG groups, respectively. In our study there was a significantly decreased risk in the incidence of CMV and EBV reactivation at 180 days in the r-ATG group compared with the R-ATG group (9% versus 26%, $P < .001$, and 9% versus 18%, $P = .03$, respectively). No significant differences were found between the 2 groups in the incidence of BK virus–associated hemorrhagic cystitis ($P = .13$) and *C. difficile* infection ($P = .08$).

Limitations of the study are its retrospective nature and the differences in some patient characteristics between the 2 groups. Nonetheless, the findings suggest that for patients undergoing matched unrelated or mismatch related/unrelated RIC HSCT, an ATG dose of 4.5 mg/kg does not compromise survival outcomes. Validation by a large randomized controlled trial is needed.

In summary, our study showed that patients receiving the smaller dose of rabbit ATG (4.5 mg/kg) had a higher incidence of aGVHD grades II to IV and III to IV. However, in multivariable models adjusting for disease diagnosis, the risk of aGVHD

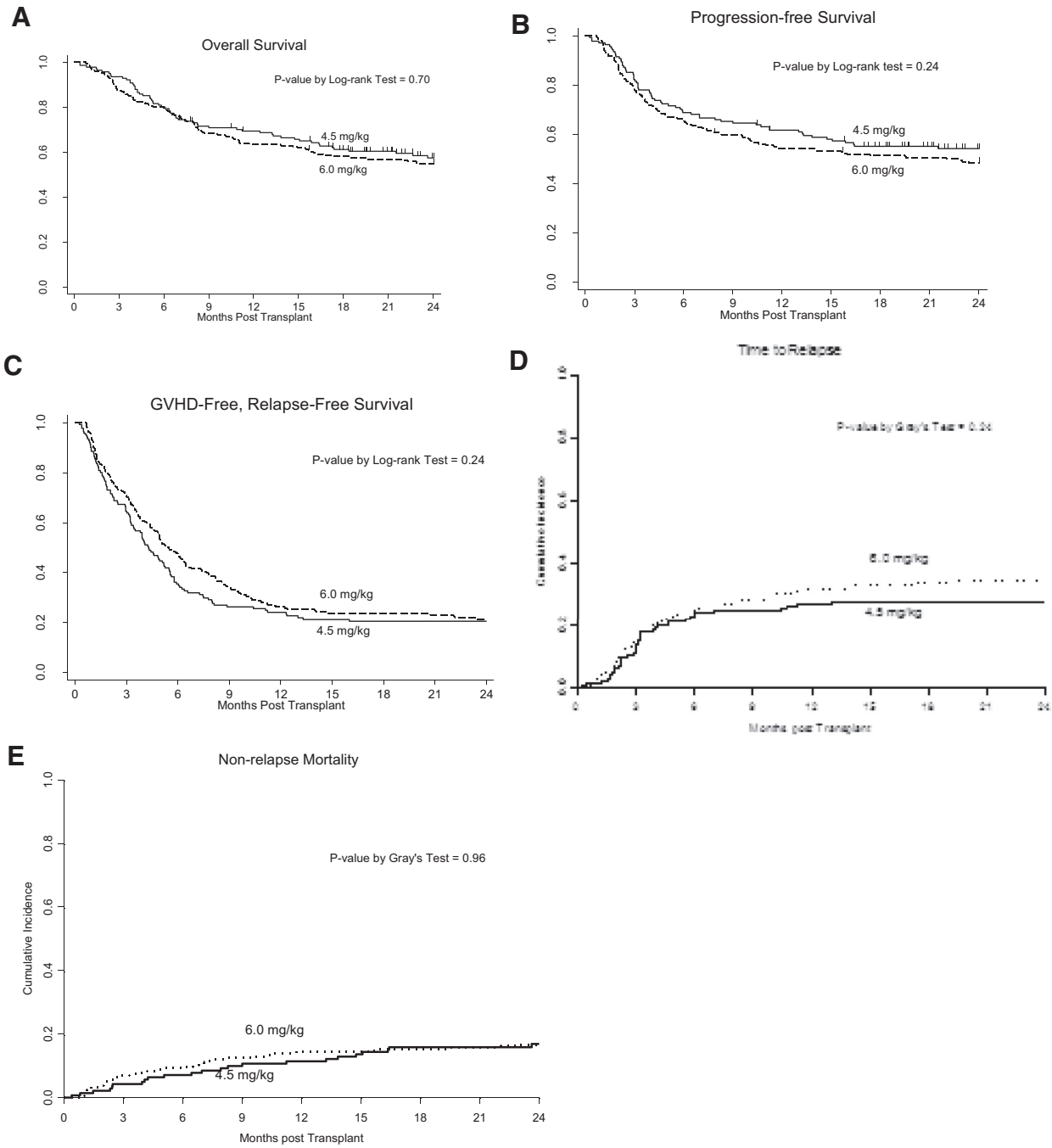

Figure 3. Outcomes from transplantation according to ATG dosing. (A) Kaplan-Meier estimate of OS. (B) Kaplan-Meier estimate of PFS. (C) Kaplan-Meier estimate of GRFS. (D) Cumulative incidence of relapse. (E) Cumulative incidence of NRM. Solid line indicates patients who were on r-ATG (4.5 mg/kg), and dotted line indicates patients who were on R-ATG (6.0 mg/kg).

grades II to IV was reduced in the R-ATG versus r-ATG group (HR, .70; 95% CI, .50 to .96; $P = .029$), whereas aGVHD grades III to IV did not reach statistical significance (HR, .64; 95% CI, .38 to 1.07; $P = .087$). In addition, there was no statistical significance in the risk of cGVHD. With the exception of bacterial infection, which was higher in the r-ATG group, infectious complications were lower with r-ATG. The reason for this in unclear as one would have expected the opposite. Overall, there was no significant differences in relapse, NRM, GRFS, OS, and PFS between the 2 groups.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: H.I and N.S are co–first authors.

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