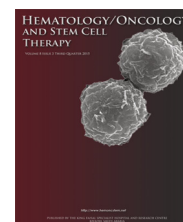


Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/hemonc

ORIGINAL RESEARCH REPORT

Effects of two doses of anti-T lymphocyte globulin-Fresenius given after full-match sibling stem cell transplantation in acute myeloblastic leukemia patients who underwent myeloablative fludarabine/busulfan conditioning



Can Boga^{a,*}, Mahmut Yeral^a, Ciğdem Gereklıoğlu^b, Suheyl Asma^b, Erkan Maytalman^a, Pelin Aytan^a, Ilknur Kozanoğlu^{a,c}, Cagla Sariturk^d, Hakan Ozdogu^a

^a Hematology Department, Adana Adult BMT Center, Faculty of Medicine, Başkent University, Ankara, Turkey

^b Family Medicine Department, Faculty of Medicine, Başkent University, Ankara, Turkey

^c Physiology Department, Faculty of Medicine, Başkent University, Ankara, Turkey

^d Medical Statistics, Adana Hospital, Baskent University, Adana, Turkey

Received 13 March 2017; received in revised form 18 October 2017; accepted 21 January 2018

Available online 20 February 2018

KEYWORDS

Acute myeloblastic leukemia;
Allogeneic stem cell transplantation;
Anti-T lymphocyte globulin;
Antithymocyte globulin;
Graft-versus-host disease

Abstract

Objective/background: Anti-T lymphocyte globulin Fresenius (rATG-F; ATG-Fresenius) and antithymocyte globulin (thymoglobulin), which are included in transplant protocols, are used to reduce the risk of chronic graft-versus-host disease (cGVHD) or suppress allograft rejection. Available clinical studies have been conducted in heterogeneous patient populations and with different administration protocols including stem cell sources. Additionally, the pharmacokinetics of ATG is variable, and the clinically effective dose of rATG-F, in particular, is not exactly known. The aim of the study was to investigate the clinical outcomes of acute myeloid leukemia (AML) patients who underwent hemopoietic peripheral stem cell transplantation from full-matched sibling donors and given two different doses of r-ATG-F.

* Corresponding author at: Department of Hematology, Faculty of Medicine, Baskent University, Yuregir 01250, Adana, Turkey.
E-mail address: drcanboga@hotmail.com (C. Boga).

<https://doi.org/10.1016/j.hemonc.2018.01.004>

1658-3876/© 2018 King Faisal Specialist Hospital & Research Centre. Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Methods: This was a single-center, retrospective chart review conducted between July 2005 and July 2016. Sixty-nine consecutive AML patients who underwent transplant with fludarabine- and busulfan-based conditioning were included in the study. Patients in Group 1 received 15 mg/kg body weight rATG-F to 2013 ($n = 46$), and Group 2 received 30 mg/kg of rATG-F dose beginning in 2013 to reduce to cGVHD ($n = 23$). Cyclosporine and methotrexate were used to treat acute GVHD (aGVHD) prophylaxis. Outcome parameters were compared between the groups.

Results: Although the recommended dose r-ATG-F had led to a decrease in the cumulative incidence of cGVHD (27 [58.7%] vs. 8 [34.8%]; $p = .03$), it also increased the infection rate at 1 year (3 [6.5%] vs. 4 [17.4%]; $p = .02$). The two groups were similar in terms of engraftment time, aGVHD, relapse, nonrelapse mortality, and rATG-F-related toxicity. A Cox regression model revealed that aGVHD III–IV was associated with increased nonrelapse mortality at 1 year (hazard ratio = 18.2; 95% confidence interval, 1.667–199.255; $p = <.02$). No patients developed rATG-F-related severe adverse events (Common Terminology Criteria grade 4 or 5).

Conclusion: Dose difference of rATG-F did not influence survival parameters; however, increasing the dose to 30 mg/kg seems to be effective for reducing cGVHD with an increase in infection rate requiring close monitoring of infections in AML patients who received myeloablative fludarabine/busulfan conditioning.

© 2018 King Faisal Specialist Hospital & Research Centre. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Anti-T lymphocyte globulin (rATG-F; ATG-Fresenius) is a preparation of rabbit polyclonal antibodies against the Jurkat T lymphoblastocytoid cell line [1,2]. Antithymocyte globulin (thymoglobulin) is prepared using more heterogeneous human thymus cells as antigens [1,2]. Both antibody preparations trigger complement-mediated lysis and apoptosis of blood and lymph node cells, and also control the leukocyte/endothelium relationship, increase B-cell apoptosis, regulate dendritic cell functions, and induce T-regulatory cells and natural killer T cells. These effects are associated with antibody-binding to many membrane surface antigens [1–3]. The antibodies of rATG-F are less diverse than those of thymoglobulin; this reflects the manufacturing approach taken. For example, rATG-F does not bind to CD4 or HLA-DR [1–3]. The antibodies reduce the risk of chronic graft-versus-host disease (cGVHD) or suppress allograft rejection when administered in allogeneic hematopoietic stem cell transplantation (HSCT) protocols [4–7]. Most of the available *in vitro* and clinical data are derived from studies performed using Thymoglobulin [1,8–10]. Optimization of dosing is necessary to determine the clinically effective dose of rATG-F. However, it is challenging work because of the highly variable pharmacokinetics of the drugs and likelihood of increased cGVHD risk attributable to reduced dose thymoglobulin [1,2,9]. In the literature, the patient populations were not homogenous: different conditioning regimens including different stem cell sources were used, follow-up was not standardized, and the clinically effective doses of rATG-F were not clear; these issues require attention [4–11]. Therefore, we decided to conduct our study in a homogenous patient group using a standard protocol and follow-up.

The clinical outcomes of acute myeloid leukemia (AML) patients given two different doses of rATG-F and who underwent HSCT using peripheral stem cells from full-matched

sibling donors with myeloablative busulfan/fludarabine-based conditioning regimens were investigated in the present study.

Materials and methods

Study design

This was a single-center, retrospective chart review conducted between July 2005 and July 2016. A total of 69 consecutive primary or treatment-related AML patients who were older than 18 years, in remission, and who underwent peripheral stem cells transfer from HLA full-matched donors were included. Group 1 was composed of 46 patients who received reduced-dose rATG-F to 2013, and Group 2 had 23 patients who received the recommended rATG-F dose beginning in 2013. The conditioning regimens featured 15 mg/kg of rATG-F (Grafalon; Fresenius, Bad Homburg, Germany) for Group 1 and 30 mg/kg of rATG-F (Grafalon) for Group 2, given over the final 3 days prior to donor cell infusion, in combination with 150 mg/kg of fludarabine and 9.6–12.8 mg/kg of busulfan. Cyclosporine and methotrexate were used to treat acute GVHD (aGVHD) prophylaxis.

Data were obtained from forms created to evaluate transplant patients. The forms met the Joint Accreditation Committee: International Society for Cellular Therapy and the European Group for Blood and Marrow Transplantation (JACIE) Accreditation criteria for the Nucleus electronic data management system (version 9.3.39; Monad Software Co., Ankara, Turkey). All data were checked by a Data Audit Group.

The primary endpoints included probabilities of overall survival (OS) and disease-free survival (DFS), and the cumulative incidences of relapse, nonrelapse mortality (NRM), and cGVHD. The secondary endpoints included the cumulative incidence of aGVHD, infection rate, and proportion of patients for whom immunosuppressive drugs could be dis-

continued by Year 2. For patients aged 18 years and below, and those with another cancer or who received unrelated or HLA-unmatched transplants, a second transplant were excluded.

Local Ethics Committee approval was obtained prior to study commencement (KA16/284).

Stem cell collection

All donors were given 10 $\mu\text{g}/\text{kg}/\text{day}$ filgrastim (Neupogen; Amgen-Roche, Thousand Oaks, CA, USA) in two equal doses for 5 days prior to peripheral stem cell collection, with the aim of mobilizing stem cells from the bone marrow into peripheral blood. An apheresis device using a continuous flow technique (Cobe Spectra V. 7.0; Terumo BCT, Lake-wood, CO, USA) was used to collect stem cells.

Transplantation procedure

All patients received an ablative conditioning regimen (9.6–12.8 mg/kg busulfan via intravenous route, 150 mg/kg fludarabine). In addition, Group 1 cases received 15 mg/kg rabbit ATG (Grafalon, Fresenius, Germany) and Group 2 cases received higher doses of rabbit ATG (30 mg/kg) with the aim of reducing cGVHD rates. For aGVHD prophylaxis, all patients received cyclosporine (3 mg/kg/day intravenously beginning at Day–2; the target concentration was ≥ 200 ng/mL to the time of drug discontinuation) and methotrexate (10 mg/m² intravenously on Day 2; 8 mg/m² intravenously on Days 4 and 8); folinic acid was given 24 h after each dose of methotrexate. Acute GVHD was scored using the standard criteria based on the pattern of severity of abnormalities in the skin, gastrointestinal tract, and liver [12]. The initial dose of methylprednisolone was 2 mg/kg when GVHD developed. cGVHD was diagnosed according to standardized criteria [13]. All patients were followed up in rooms with a laminar airflow. Irradiated, leukocyte-free blood products were used when transfusions were needed. The threshold hemoglobin level was < 8 g/L for administration of an erythrocyte suspension, and the threshold platelet level was $< 20 \times 10^9/\text{L}$ for administration of concentrated platelets. All workflow during chemotherapy, and antimicrobial drug use, were conducted in accordance with standard operating procedures (SOPs) prepared prior to JACIE accreditation (SOP of bone marrow transplantation clinical unit: BMT-CU 032). All patients received acyclovir for herpes prophylaxis, sulfamethoxazole for *Pneumocystis jiroveci* prophylaxis, and empirical cephalosporin–clavulonate and carbapenem until microbiological data were obtained if febrile neutropenia was evident; glycopeptides were given when Gram-positive bacterial infection was suspected. Viral and antifungal prophylaxis was delivered in accordance with SOPs prepared prior to JACIE accreditation (SOP: BMT-CU 002). Preemptive ganciclovir was given in patients exhibiting cytomegalovirus reactivation.

Minimal residual disease analysis

All flow cytometry measurements were performed using the blue (wavelength = 488 nm) and red (wavelength = 633 nm) lasers of an eight-parameter Becton Dickinson FACS CANTO

II device (BD Bioscience, San Jose, CA, USA). Leukemia-related immune typing was performed at the time of diagnosis. Three tubes containing diagnostic markers of AML (CD45APC H7, CD13 APC, CD33 PerCp, CD7 PE, CD19 AlexaFluor, CD56 PE, CD123 PerCp, CD34 PE-Cy7, CD11b Alexa Fluor, HLA DR APC, CD38 FITC, CD15 FITC, CD64 PE, and CD14 Alexa Fluor) were used. Each tube received approximately 10^6 cells, which were evaluated using FACS DIVA Software (BD Bioscience). Minimal residual disease (MRD) was defined as the presence of cell groups exhibiting abnormal antigen expression during maturation of bone marrow cell lines. If possible, the same monoclonal antibodies and fluorochromes were used for MRD follow-up after diagnosis. The MRD bone marrow-positive cutoff was 0.1%. All antibodies were supplied by BD Bioscience.

Response criteria

Normalization of cell counts in the peripheral blood, absence of abnormal blasts in the peripheral blood, and a bone marrow blast count $\leq 5\%$ were the full response criteria. As an institution standard, bone marrow aspiration was performed every 2–4 months until 2 years after transplantation and whenever needed thereafter. Toxicities were determined using the criteria of the National Cancer Institute.

Statistics

A comparison was made between HLA-matched sibling donor transplants using reduced dose rATG-F (Group 1) and recommended dose rATG-F (Group 2) in the allo-HSCT cohort including AML patients. Baseline categorical variables were compared using Fisher's exact test, whereas continuous variables were compared using Mann–Whitney *U* test or two-sample *t* test as appropriate. OS and DFS 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. DFS was defined as the time from transplantation to disease relapse and/or death. Probabilities of DFS and OS were calculated as described previously [14]. OS and DFS data were analyzed using 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 [15]. Comparisons between estimates of cumulative incidences were made by using Gray's test. The cumulative incidence of aGVHD or cGVHD was calculated with relapse or death without relapse or GVHD as a competing event for both [16]. Cox proportional hazards models were constructed for a cumulative incidence of aGVHD, cGVHD, or rATG-F dose as the factors influencing relapse and mortality. Variables considered in the model were those significant at a .20 level from the univariable models. Variables remaining in the final models were significant at a .05 level. Estimates for hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were obtained for each significant prognostic factor. All *p* values are two sided. Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA), and *p* values based on Gray's test were estimated using NCSS version 11.0.9.

Results

The baseline patient-, disease-, and transplantation-related characteristics are shown in Table 1. There was no significant difference across the two groups in terms of patient sex, age, risk of disease, type of disease, postinduction disease status, comorbidity, and transplantation risk.

When the patients were evaluated with regard to outcome parameters, the median days of neutrophil engraftment was 12.4 ± 1.6 (95% CI, 11.8–12.8) in Group 1 compared with 12.2 ± 0.5 (95% CI, 11.4–13.1) in Group 2 ($p = .84$; Table 2). The median days of platelet engraftment was also similar in both groups (12.1 ± 1.1 vs. 12.3 ± 1.9 ; $p = .89$; Table 2).

Although the mean OS was found to be 47 months and 17 months, the mean DFS was found to be 48 months and 14 months in Group 1 and Group 2, respectively. Univariate analysis revealed the expected 1-year OS rates as 76.1% and 86.9% for patients in Group 1 and Group 2, respectively ($p = .86$; Table 2). The expected DFS was also similar between the groups (76.1% vs. 82.6%; $p = .86$; Table 2). Kaplan–Meier curve construction, followed by application of the log-rank test, showed that neither OS nor DFS was affected by the dose ($p = .06$ for OS and $p = .11$ for DFS; Fig. 1).

Among recipients of Group 1, the cumulative incidence of NRM at 1 year was 4.3% compared with 4.3% in Group 2, and NRM was found not to be influenced by rATG-F dose ($p = .73$; Table 2; Fig. 2A). The cumulative incidence of relapse at 1 year was similar in Group 1 and Group 2 (13% vs. 13%; $p = .71$; Table 2; Fig. 2B).

When the patients were evaluated with regard to aGVHD and cGVHD, it was noted that whereas cumulative incidence of aGVHD II–IV at 100 days was found to be 13.0% and 17.4% ($p = .51$; Table 2), it was 8.7% and 0% for aGVHD III–IV in Group 1 and Group 2, respectively ($p = .20$; Table 2). At 180 days, these values were 17.4% for the two groups for aGVHD II–IV ($p = .63$; Table 2; Fig. 2A) and 8.7% versus 0% for aGVHD III–IV in Group 1 and Group 2, respectively ($p = .20$; Table 2; Fig. 2B).

Gray's test revealed that the cumulative incidence of cGVHD at 1 year was 58.7% and 34.8% in Group 1 and Group 2, respectively ($p = .03$; Table 2; Fig. 3C). At 1 year after transplantation, the cumulative incidence of extensive cGVHD was 23.9% in Group 1, whereas it was 34.8% in Group 2 ($p = .36$; Table 2; Fig. 3D).

Although immunosuppressive treatment could be discontinued at 2 years after transplantation in 31 (75.6%) patients in Group 1 and 17 (89.5%) patients in Group 2, there was no statistically significant difference between the two groups ($p = .30$; Table 2).

Infectious complications at 1 year increased in Group 2 (6.6% vs. 17.4%; $p = .02$; Table 2). Three (6.5%) patients in Group 1 and two (8.6%) patients in Group 2 developed invasive fungal infection, whereas 18 (39.1%) and six (26%) patients developed CMV viremia, and six (13.0%) and three (13.0%) patients developed herpes virus infection in Group 1 and Group 2, respectively. No patients developed rATG-F-related severe adverse events (Common Terminology Criteria grade 4 or 5).

A Cox regression model including 69 AML patients revealed that although aGVHD III–IV was associated with increased NRM at 1 year (HR = 18.2; 95% CI, 1.667–199.255; $p < .02$),

Table 1 Baseline patient-, disease-, and transplantation-related characteristics.

Variable	Group 1 (N = 46)	Group 2 (N = 23)	p
Male, n (%)	21 (45.7)	16 (69.6)	.08
Median age, y	40.3 ± 11.6	44.0 ± 11.1	.20
Risk of AML, n (%)			
High	9 (19.6)	7 (30.4)	.37
Intermediate	37 (80.4)	16 (69.6)	
Type of AML, n (%)			
Primary	39 (84.8)	22 (95.7)	.25
AML with MRC	7 (11.6)	1 (4.3)	
Molecular status, n (%)			
MRD negative	16 (34.8)	11 (47.8)	.94
MRD positive	22 (47.8)	12 (52.1)	
No MRD assessment	8 (17.4)	0	
Comorbidity score (Sorrer), n (%)			
High	10 (21.7)	6 (26.1)	.44
Intermediate	18 (39.1)	10 (43.9)	
Low	13 (28.3)	7 (30.4)	
EBMT risk score ≥ 2, n (%)	34 (73.9)	21 (91.3)	.12

Donor source was HLA-matched sibling in all transplants in both groups.

AML = acute myeloid leukemia; EBMT = European Group for Blood and Marrow Transplantation; HLA = human leukocyte antigen; MRC = myelodysplasia-related change; MRD = minimal residual disease.

Table 2 Transplant outcome.

	Group 1 (N = 46)	Group 2 (N = 23)	p
Median days of N eng, d ± SD	12.4 ± 1.6 (95% CI, 11.8–12.8)	12.2 ± 0.5 (95% CI, 11.4–13.1)	.85
Median days of P eng, d ± SD	12.1 ± 1.1 (95% CI, 11.4–12.8)	12.3 ± 1.9 (95% CI, 9.2–15.3)	.89
OS, at 1 year, n (%) ^a	35 (82.2)	20 (78.7)	.85
DFS, at 1 year, n (%) ^a	35 (75.8)	19 (82.6)	.11
NRM, at 1 year ^b	6.5% (95% CI, 2.4–10.8)	4.3% (95% CI, N/A)	.73
Relapse at 1 year ^b	17.7% (95% CI, 11.3–24.1)	13.1% (95% CI, 1.8–24.4)	.71
aGVHD at 100 days ^b			
Grade II–IV	15.2% (95% CI, 9.2–21.2)	13% (95% CI, 1.7–24.3)	.63
Grade III–IV	6.7% (95% CI, 2.5–10.9)	0	.20
aGVHD at 180 days ^b			
Grade II–IV	17.4% (95% CI, 11.0–23.8)	17.4% (95% CI, 4.7–30.1)	.64
Grade III–IV	8.7% (95% CI, 3.0–13.4)	0	.20
cGVHD at 1 year ^b			
As a whole	7.3% (95% CI, 2.9–11.7)	11.1% (95% CI, 0.5–21.7)	.03
Extensive	3.8% (95% CI, 0.6–7.1)	16.7% (95% CI, 4.2–29.2)	.36
Cessation of IS at 2 years, n (%) ^c	31 (75.6)	17 (89.5)	.30
Infection at 1 year, n (%) ^c	3 (6.5)	4 (17.4)	.02

aGVHD = acute graft-versus-host disease; cGVHD = chronic graft-versus-host disease; CI = confidence interval; d = days; DFS = disease-free survival; IS = immunosuppressives; N/A = nonavailable with NCCSS system; N eng = neutrophil engraftment; NRM = nonrelapse mortality; OS = overall survival; P eng = platelet engraftment; SD = standard deviation.

^a Kaplan–Meier product limit estimate.

^b Gray's test cumulative incidence estimate.

^c Chi-square test.

it was not influenced by aGVHD II–IV (HR = 2.0; 95% CI, 0.260–16.801; $p = .49$), cGVHD (HR = 1.2; 95% CI, 0.446–3.423; $p = .68$), or different rATG-F doses (HR = 1.1; 95% CI, 0.292–4.355; $p = <.86$; Table 3). By contrast, patients showed no significant difference in the relapse risk following aGVHD II–IV (HR = 1.1; 95% CI, 0.225–5.727; $p = .88$), aGVHD III–IV (HR = 1.6; 95% CI, 0.137–20.195; $p = .69$), cGVHD (HR = 1.9; 95% CI, 0.560–6.488; $p = .30$), or different rATG-F doses (HR = 1.2; 95% CI, 0.314–4.756; $p = .77$; Table 4).

Discussion

Strong evidence indicates that antithymocyte globulin is quite effective for prevention of cGVHD in unrelated allogeneic transplants [4,5,7,11], and the relapse rate is not increased in the setting of myeloablative conditioning [1,8]. Immunosuppressive treatment may be discontinued early in many patients, except in those who were not in complete remission prior to transplantation and who received the reduced-intensity conditioning regimen. Furthermore, infections did not increase in number, and quality of life improved, unless given in high doses [8].

Many studies that were retrospective in nature and used the reduced-intensity conditioning regimen have explored the utility of thymoglobulin in patients receiving transplants from HLA-matched sibling donors [17–21]. One study compared the outcomes of patients who received transplants from related and unrelated donors treated with the fludarabine/busulfan conditioning regimen [22]. The incidence of cGVHD did not vary by donor type; however, the relapse

rate was lower and DFS was longer when the donor was unrelated [22]. A recently published study showed that the use of Thymoglobulin with fludarabine/busulfan myeloablative regimen followed by HLA-identical sibling donor allogeneic HSCT for AML improves overall transplant outcomes owing to the reduced incidence of cGVHD without increased relapse risk, supporting our hypothesis [23].

The retrospective studies of Cornillon et al. [24] and Salem et al. [25] explored the utility of low-dose Thymoglobulin. The first study compared the outcomes of patients given two low doses (2.5 and 5 mg/kg); the latter dose better protected against NRM. Furthermore, Salem et al. [25] and Hamadani et al. [26] used fludarabine/busulfan conditioning and compared two doses (6 mg/kg and 7.5 mg/kg) [25,26]. No significant between-group difference was evident in terms of cGVHD, and the latter reported lower cytomegalovirus and bacterial infection rate. Meanwhile, Socie et al. [11] found that relapse risk did not increase with thymoglobulin doses <6 mg/kg, and CIBMTR data showed controversial results [19]. Thymoglobulin doses of ≥10 mg/kg was found to lead to a poorer OS in a French study [20]. Kanate et al. [27] have found that NRM and survival were not influenced by ATG use. By contrast, cGVHD rates were better among the patients who received Thymoglobulin in their study, which investigated lymphoma patients who underwent haplo transplant using cord blood. The authors considered that prospective studies were required to determine appropriate Thymoglobulin doses.

Despite the large number of studies investigating thymoglobulin, few studies have evaluated low-dose rATG-F. The pharmacodynamics of thymoglobulin varies consider-

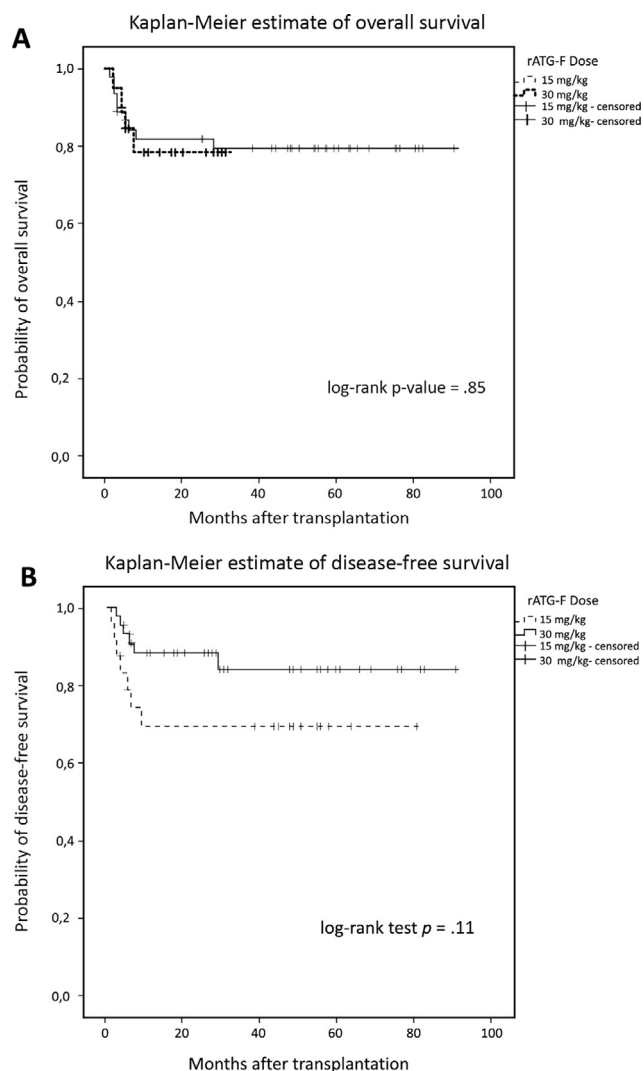


Fig. 1 Overall survival and disease-free survival according to rATG-F dosage group. (A) Kaplan–Meier estimates of overall survival following allogeneic transplantation. (B) Kaplan–Meier estimates of disease-free survival following allogeneic transplantation. Solid curves represent patients receiving rATG-F at 15 mg/kg (Group 1), whereas dashed curves represent patients receiving the 30 mg/kg rATG-F dose (Group 2). rATG-F = anti-T lymphocyte globulin Fresenius.

ably. rATG-F is prepared from more homogenous cells than is thymoglobulin. Placental cells must absorb higher concentrations of immunoglobulin G (IgG) antibodies (compared to other antibodies) prior to cell lysis. The European Group for Blood and Marrow Transplantation considers that 30 mg/kg of rATG-F is equivalent to 7.5 mg/kg of thymoglobulin when an unrelated transplant is contemplated [1,28].

The prospective study of Kröger et al. [29] is the most recent work exploring the use of rATG-F in HLA-matched sibling donors. The study found that the GVHD ratio was lower, and GVHD-free and relapse-free survival were longer, in an rATG-F versus non-rATG-F group. However, all acute leukemia cases were included in the cited study, and the conditioning regimens included cyclophosphamide,

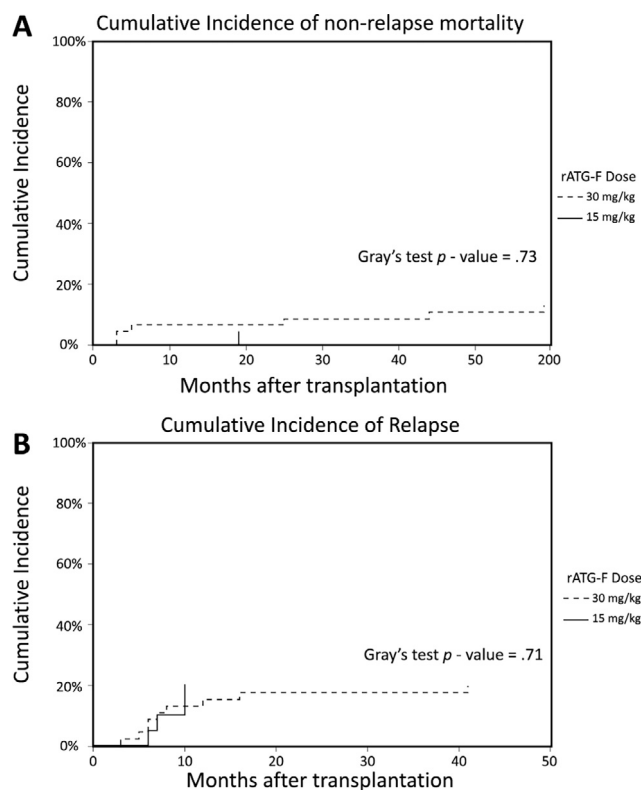


Fig. 2 (A) Cumulative incidence of nonrelapse mortality according to the rATG-F dosage group. (B) Cumulative incidence of disease relapse according to the ATG dosage group. Solid curves represent patients receiving rATG-F at 15 mg/kg (Group 1), whereas the dashed curves represent patients receiving the 30 mg/kg rATG-F dose (Group 2). rATG-F = anti-T lymphocyte globulin Fresenius.

total body irradiation, or combination of busulfan and etoposide. In their study, Ayuk et al. [30] compared 30 mg/kg versus 60 mg/kg rATG-F in patients who underwent unrelated transplant. Although they found that acute and chronic GVHD rates were similar, they noted that transplant-related mortality and DFS were favorable in the low-dose group. We earlier reported the outcomes of allogeneic transplant patients who received peripheral stem cells from HLA-matched siblings, and who were given rATG-F at 7.5–10 mg/kg as part of a busulfan/fludarabine conditioning regimen [31]. Our results were found successful at Years 1 and 3. However, it must be emphasized that the study was retrospective in nature and we did not include a control group.

We found that reduced-dose rATG-F yielded sufficient outcomes in allogeneic stem cell transplant patients receiving cells from HLA-matched sibling donors. Recommended dose rATG-F was found to reduce cumulative incidence of cGVHD while leading to a mild increase in infection rates without influencing transplant outcomes. A similar increase in infection rate was observed with increasing dose of Thymoglobulin. In the study of Ayuk et al. [30] reporting that NRM is associated with infections, fatal infections were detected in contrast to the findings of our study, which revealed that NRM seems to be associated with aGVHD III–IV.

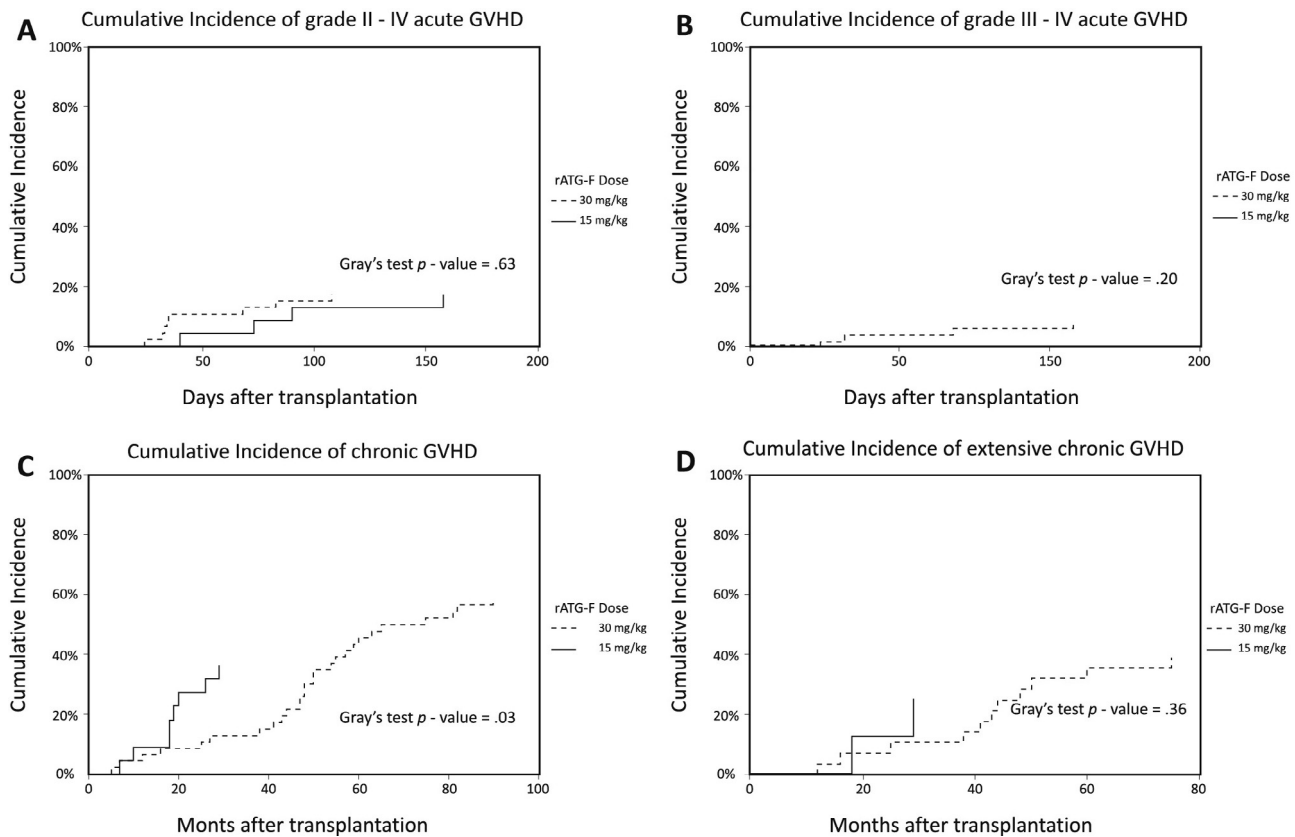


Fig. 3 Cumulative incidence of GVHD according to the rATG-F dosage group. (A) cumulative incidence of grade II–IV aGVHD. (B) Cumulative incidence of grade III–IV aGVHD. (C) Cumulative incidence of cGVHD as a whole. (D) Cumulative incidence of extensive cGVHD. Solid curves represent patients receiving rATG-F at 15 mg/kg (Group 1), whereas dashed curves represent patients receiving the 30 mg/kg rATG-F dose (Group 2). aGVHD = acute graft-versus-host disease; cGVHD = chronic graft-versus-host disease; rATG-F = anti-T lymphocyte globulin Fresenius.

Table 3 Cox regression model for GVHD and rATG-F dose and the factors influencing NRM.

Analysis of maximum likelihood estimates								
Parameter	<i>B</i>	Standard error	Wald	<i>df</i>	<i>p</i>	Hazard ratio	95% CI hazard ratio	
							Lower	Upper
aGVHD II–IV	.738	1.063	.482	1	.49	2.092	.260	16.801
aGVHD III–IV	2.903	1.220	5.659	1	.02	18.226	1.667	199.255
cGVHD	.212	.520	.166	1	.68	1.236	.446	3.423
rATG-F dose	.121	.689	.031	1	.86	1.128	.292	4.355

aGVHD = acute graft-versus-host disease; cGVHD = chronic graft-versus-host disease; *B* = beta (standardized regression coefficients); CI = confidence interval; *df* = degree of freedom; rATG-F = rabbit anti-T lymphocyte globulin Fresenius.

An advantage of low-dose rATG-F may lie in its effect on natural cell counts. The T-suppressor effect persists for >1 year if rATG-F is used in addition to lympholytic medications. However, killer cell counts were reported to match the prior levels if the dose was reduced [32,33]. Positive correlations were evident between the natural killer cell count, and DFS and OS, in transplant patients for whom low-dose rATG-F had been added to the busulfan/fludarabine conditioning regimen ($p = <.01$ and $p = .02$, respectively). Natural killer cell numbers $>1.5 \times 10^7/\text{kg}$ correlated with a good prognosis [34].

We sought to investigate the effectiveness of different doses of rATG-F. We studied homogenous patients undergoing an identical busulfan/fludarabine conditioning regimen and obtained peripheral stem cells using standardized operating procedures; these represent the strengths of our study. The absence of a control group to which rATG-F was not given may be a limitation of our study.

Dose difference of rATG-F did not influence survival parameters; however, recommended dose seems to be effective for reducing cGVHD with a slight increase in infection rate in AML patients who received myeloablative flu-

Table 4 Cox Regression Model for GVHD and rATG-F dose as the factors influencing relapse.

Analysis of maximum likelihood estimates								
Parameter	B	Standard error	Wald	df	p	Hazard ratio	95% CI hazard ratio	
							Lower	Upper
aGVHD II–IV	.126	0.826	0.023	1	.88	1.134	0.225	5.727
aGVHD III–IV	.508	1.274	0.159	1	.69	1.662	0.137	20.195
cGVHD	.645	0.625	1.064	1	.30	1.906	0.560	6.488
rATG-F dose	.201	0.693	0.084	1	.77	1.222	0.314	4.756

aGVHD = acute graft-versus-host disease; cGVHD = chronic graft-versus-host disease; B = beta (standardized regression coefficients); CI = confidence interval; df = degree of freedom; rATG-F = rabbit anti-T lymphocyte globulin Fresenius.

darabine/busulfan conditioning. We may conclude that recommended dose rATG-F may be used effectively with careful monitoring of infections.

In conclusion, reduced dose rATG-F seems sufficient to reduce the incidence of cGVHD, and does not affect the cumulative incidence of aGVHD without changing the outcome in allogeneic transplant AML patients receiving cells from HLA-matched donors subjected to fludarabine/busulfan conditioning regimes. Increasing the dose of r-ATG-F to 30 mg/kg seems to be more effective for reducing cGVHD with a slight increase in infection rate. Our results support the notion that individualized rATG-F use and dose adjustment could improve clinical outcomes. An increase in the rate of infection suggests that close monitoring of infection may be necessary. Prospective controlled randomized studies are required to draw any conclusive findings.

Conflicts of interest

All authors declare no conflicts of interest.

References

- [1] Mohty M. Mechanism of action of antithymocyte globulin: T-cell depletion and beyond. *Leukemia* 2007;21:1387–94.
- [2] Tsuge I, Kojima S. Comparison of antibody specificities of four anti-thymocyte/anti-lymphocyte globulin products. *Curr Ther Res* 1995;56:671–7.
- [3] Leitner J, Grabmeier-Pfistershammer K, Majdic O, Ziabinger G, Steinberger P. Interaction of antithymocyte globulins with dendritic cell antigens. *Am J Transpl* 2011;11:138–45.
- [4] Walker I, Panzarella T, Couban S, Couture F, Devins G, Elemetry E, et al. Pretreatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with haematological malignancies undergoing haematopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. *Lancet Oncol* 2016;17:164–73.
- [5] Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol* 2009;10:855–64.
- [6] Bacigalupo A, Lamparelli T, Barisione G, Bruzzi P, Guidi S, Alessandrino PE, et al. Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. *Biol Blood Marrow Transpl* 2006;12:560–5.
- [7] Huang W, Zhao X, Tian Y, Cao T, Li Y, Liu Z, et al. Outcomes of peripheral blood stem cell transplantation patients from HLA-mismatched unrelated donor with antithymocyte globulin (ATG)-Thymoglobulin versus ATG-Fresenius: a single-center study. *Med Oncol* 2015;23:465.
- [8] Storek J, Mohty M, Boelens JJ. Rabbit anti-T cell globulin in allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transpl* 2015;21:959–70.
- [9] Preville X, Nicolas L, Flacher M, Revillard J. A quantitative flow cytometry assay for the preclinical testing and pharmacological monitoring of rabbit antilymphocyte globulin (rATG). *J Immunol Methods* 2000;245:45–54.
- [10] Bunn D, Lea C, Bevan D, Higgins RM, Hendry BM. The pharmacokinetics of anti-thymocyte globulin (ATG) following intravenous infusion in man. *Clin Nephrol* 1996;45:29–32.
- [11] Socie G, Schmoor C, Bethge WA, Ottinger HD, Stelljes M, Zander AR, et al. Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. *Blood* 2011;117:6375–82.
- [12] Rowlings PA, Przepioroka D, Klein JP, Gale RP, Passweg JR, Henslee-Downey PJ, et al. IBMTR severity index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. *Br J Haematol* 1997;97:855–64.
- [13] Lee SJ, Klein JP, Barrett AJ, Ringden O, Antin JH, Cahn JY, et al. Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. *Blood* 2002;100:406–14.
- [14] Zhang X, Loberiza FR, Klein JP, Zhang MJ. A SAS macro for estimation of direct adjusted survival curves based on a stratified Cox regression model. *Comput Methods Programs Biomed* 2007;88:95–101.
- [15] Lacobelli S. Suggestions on the use of statistical methodologies in studies of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transpl* 2013;48:S1–S37.
- [16] Mohty M, Bay JO, Faucher C, Choufi B, Bilger K, Tournilhac O, et al. Graft-versus-host disease following allogeneic transplantation from HLA-identical sibling with antithymocyte globulin-based reduced-intensity preparative regimen. *Blood* 2003;102:470–6.
- [17] Devillier R, Crocchiolo R, Castagna L, Fürst S, El Cheikh C, Faucher J, et al. The increase from 2.5 to 5 mg/kg of rabbit anti-thymocyte-globulin dose in reduced intensity conditioning reduces acute and chronic GVHD for patients with myeloid malignancies undergoing allo-SCT. *Bone Marrow Transpl* 2012;47:639–45.
- [18] Baron F, Lapobin M, Blaise D, Lopez Coral L, Vigouroux S, Craddock C, et al. Impact of in vivo T-cell depletion on outcome of AML patients in first CR given peripheral blood stem cells and reduced-intensity conditioning allo-SCT from a HLA-identical sibling donor: a report from the Acute Leukemia

- Working Party of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transpl* 2014;49:389–96.
- [19] Soiffer RJ, Lerademacher J, Ho V, Kan F, Artz A, Champlin R, et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. *Blood* 2011;117:6963–70.
- [20] Michallet M, Le QH, Mohty M, Prebet T, Nicolini F, Boiron JM, et al. Predictive factors for outcomes after reduced intensity conditioning hematopoietic stem cell transplantation for hematological malignancies: a 10-year retrospective analysis from the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire. *Exp Hematol* 2008;36:535–44.
- [21] Atta EH, de Oliveira DC, Bouzas LF, Nucci M, Abdelhay E. Less graft-versus-host disease after rabbit antithymocyte globulin conditioning in unrelated bone marrow transplantation for leukemia and myelodysplasia: comparison with matched related bone marrow transplantation. *PLoS ONE* 2014;9:107155.
- [22] Ruggeri A, Battipaglia G, Labopin M, Ehninger G, Beelen D, Tischer J, et al. Unrelated donor versus matched sibling donor in adults with acute myeloid leukemia in first relapse: an ALWP-EBMT study. *J Hematol Oncol* 2016;9:89.
- [23] Rubio MT, D'Aveni-Piney M, Labopin M, Hamladji HM, Sanz MA, Blaise D, et al. Impact of in vivo T cell depletion in HLA identical allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission conditioned with a fludarabine iv-busulfan myeloablative regimen: a report from the EBMT Acute Leukemia Working Party. *J Hematol Oncol* 2017;10:31.
- [24] Cornillon J, Balsat M, Cabrespine A, Tavernier-Tardy E, Hermet E, Mulliez A, et al. Impact of ATG dose on the outcome of patients undergoing reduced intensity conditioning followed by allogeneic hematopoietic stem cell transplantation for hematological malignancies. *Acta Haematol* 2016;136:193–200.
- [25] Salem G, Ruppert AS, Elder P, Hofmeister CC, Benson DM, Penza S, et al. Lower dose of antithymocyte globulin does not increase graft-versus-host disease in patients undergoing reduced-intensity conditioning allogeneic hematopoietic stem cell transplant. *Leuk Lymphoma* 2015;56:1058–65.
- [26] Hamadani M, Blum W, Phillips G, Elder P, Andritsos L, Hofmeister C, et al. Improved nonrelapse mortality and infection rate with lower dose of antithymocyte globulin in patients undergoing reduced-intensity conditioning allogeneic transplantation for hematologic malignancies. *Biol Blood Marrow Transpl* 2009;15:1422–30.
- [27] Kanate AS, Mussetti A, Kharfan-Dabaja MA, Ahn KW, DiGilio A, Beitinjaneh A, et al. Reduced-intensity transplantation for lymphomas using haploidentical related donors vs HLA-matched unrelated donors. *Blood* 2016;7:938–47.
- [28] Giebel S, Labopin M, Socié G, Socié G, Labopin M, Esteve J, et al. Anti-thymocyte globulin as graft-versus-host disease prevention in the setting of allogeneic peripheral blood stem cell transplantation: a review from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Haematologica* 2017;102:139–49.
- [29] Kröger N, Solano C, Wolschke C, Bandini G, Patriarca F, Pini M, et al. Antilymphocyte globulin for prevention of chronic graft-versus-host disease. *N Engl J Med* 2016;374:43–53.
- [30] Ayuk F, Diyachenko G, Zabelina T, Wolschke C, Fehse B, Bacher U, et al. Comparison of two doses of antithymocyte globulin in patients undergoing matched unrelated donor allogeneic stem cell transplantation. *Biol Blood Marrow Transpl* 2008;14:913–9.
- [31] Kasar M, Asma S, Kozanoglu I, Maytalman E, Boga C, Ozdogu H, et al. Effectiveness of fludarabine- and busulfan-based conditioning regimens in patients with acute myeloblastic leukemia: 8-year experience in a single center. *Transpl Proc* 2015;47:1217–21.
- [32] Eiermann TH, Freitag S, Cortes-Dericks L, Sahn H, Zander AR. Jurkat cell-reactive anti-thymocyte globulin assessed ex vivo by flow cytometry persists three weeks in circulation. *J Hematother Stem Cell Res* 2001;10:385–90.
- [33] Kho MM, Bouvy AP, Cadogan M, Kraaijeveld R, Baan CC, Weimar W. The effect of low and ultra-low dosages Thymoglobulin on peripheral T, B and NK cells in kidney transplant recipients. *Transpl Immunol* 2012;26:186–90.
- [34] Yeral M, Kasar M, Boga C, Kozanoglu I, Ozdogu H, Sariturk C. Clinical relevance of apheretic graft composition in patients with acute myeloblastic leukemia who received a busulfan–fludarabine–antithymocyte globulin conditioning regimen for allogeneic transplant. *Exp Clin Transpl* 2015;13:453–60.