The impact of graft-*versus*-host disease prophylaxis in reduced-intensity conditioning allogeneic stem cell transplant in acute myeloid leukemia: a study from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation

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ABSTRACT

The impact of the intensity of graft-*versus*-host-disease immunoprophylaxis on transplantation outcomes in patients undergoing transplantation following reduced-intensity conditioning is unclear. This study addresses this issue in 228 adult patients above 50 years of age with acute myeloid leukemia in first complete remission given peripheral blood stem cells from HLA-identical siblings after fludarabine and 2 days of intravenous busulfan reduced-intensity conditioning. A total of 152 patients received anti-thymocyte globulin, either in combination with cyclosporine A in 86 patients (group 1), or with cyclosporine A and mycophenolate mofetil or short course methotrexate in 66 patients (group 2). The remaining 76 patients did not receive anti-thymocyte globulin but were given cyclosporine A and methotrexate or mycophenolate mofetil (group 3). Incidences of grade II-IV acute graft-*versus*-host-disease were comparable in the three groups (16.5%, 29.5% and 19.5% in groups 1, 2 and 3, respectively, P=0.15). In multivariate analysis, the absence of anti-thymocyte globulin was the only factor associated with a higher risk of chronic graft-*versus*-host-disease (P=0.005), while the use of triple immunosuppression (group 3) was associated with an increased risk of relapse (P=0.003). In comparison to anti-thymocyte globulin and cyclosporine A alone, the other two strategies of graft-*versus*-host-disease prophylaxis were associated with reduced leukemia-free survival and overall survival (P=0.001 for each parameter), independently of the dose of anti-thymocyte globulin. These data suggest that fine tuning of the intensity of this prophylaxis can affect the outcome of transplantation and that anti-thymocyte globulin and cyclosporine A alone should be the preferred combination with the fludarabine-busulfan reduced-intensity conditioning regimen and sibling donors.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) performed with reduced intensity conditioning (RIC) is a therapeutic option in particular for older patients or patients with co-morbidities.[1-4] In this setting, the combination of fludarabine and 2 days of busulfan (Flu-Bu2) is a widely used RIC regimen.[1-3,5] Initially described in HLA identical sibling peripheral blood stem cell transplantation, Flu-Bu2 was combined with anti-T-lymphocyte globulin (ATG) (Fresenius 10 mg/kg/day) and cyclosporine A (CsA) alone for the prophylaxis of graft-*versus*-host-disease (GVHD).[1] However, the best GVHD prophylaxis combination in the Flu-Bu2 RIC regimen has not yet been established. In contrast to allogeneic HSCT with myeloablative conditioning, disease control after RIC relies on the development of an immunological graft-*versus*-leukemia effect (GVL).[1,6,7] Since GVHD and GVL are closely linked[8-10] the possibility of discriminating between them by the use of *in vivo* T-cell depletion remains challenging, particularly in allogeneic HSCT performed after RIC. In this context, ATG can effectively prevent acute and chronic GVHD,[5] but can also increase the risk of infectious complications and of relapse.[3,11] It is now established that the effects of ATG in RIC are dose-dependent,[3] and that intermediate doses of thymoglobulin between 4 and 6 mg/kg seem to prevent GVHD optimally while sparing the GVL effect.[12-14] Apart from ATG, the impact of the post-transplant immunosuppression on transplant outcomes in allogeneic HSCT following RIC has

been poorly explored. While, the combination of CsA and a short course of methotrexate (MTX) after transplantation is considered as the gold standard for GVHD prophylaxis after conventional myeloablative allogeneic HSCT from HLA-identical siblings,[15,16] there is no consensus on the optimal preventive regimen for GVHD prophylaxis after RIC allogeneic HSCT. However, MTX is associated with substantial toxicity, which includes delayed hematopoietic engraftment, worsened mucositis and renal toxicity, and it was thus replaced by mycophenolate mofetil (MMF) specifically in the setting of allogeneic HSCT following RIC.[6,17] Piñana *et al.* compared the transplantation outcome of two non-ATG containing GVHD prophylaxis regimens, CsA + MTX (n=93) and CsA + MMF (n=52), after allogeneic HSCT from HLA-identical siblings following fludarabine-based RIC. Transplantation outcomes were similar in the two groups with the exception of a higher incidence of grade 2-4 mucositis with CsA + MTX.[18]

In this study, we retrospectively investigated the impact of the intensity of the GVHD prophylaxis on the post-transplant outcomes of a homogeneous cohort of 228 adult patients over 50 years old with *de novo* acute myeloid leukemia in first complete remission given haematopoietic stem cells from HLA-identical siblings following fludarabine and 2 days of intravenous busulfan (Flu-ivBu2) RIC. We analyzed the impact of the use of ATG with CsA compared to a combination of ATG and CsA + MTX or MMF *versus* CsA and MTX or MMF with no ATG as post-transplant GVHD prophylaxis.

Methods

Patients and data collection

This was a retrospective study performed by the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT), and approved by its scientific board. The EBMT registry is a voluntary working group of more than 500 transplant centers, which are required once a year to report all HSCT and their follow-up. The study included patients with *de novo* acute myeloid leukemia above 50 years old in first complete remission who underwent allogeneic HSCT with hematopoietic stem cells from HLA-identical siblings following Flu-ivBu2, between 2004 and 2014. Patients given *ex vivo* T-cell-depleted grafts were excluded. GVHD prophylaxis regimens were dependent on centers' habits and protocols. Acute GVHD was graded using established criteria.[19] Chronic GVHD was classified as limited or extensive according to usual criteria.[20] For the purpose of this study, all necessary data were collected according to the EBMT guidelines, using the EBMT Minimum Essential Data forms. The list of institutions reporting data included in this study is provided in *Online Supplementary Table S1*.

Statistical analysis

The starting time for all endpoints was the date of the transplant. To evaluate the probability of relapse, patients dying from either direct toxicity of the procedure or any other cause not related to leukemia were censored. The non-relapse mortality was defined as death while in complete remission. Patients were censored at the time of relapse or of the last follow-up. Cumulative incidence curves were used for relapse incidence and non-relapse mortality in a competing risk setting, as death and relapse were competing events.[21] For the estimates of the cumulative incidence of chronic GVHD, death was considered as a competing event.

Overall and leukemia-free survival rates (starting from the date of transplant) were calculated using the Kaplan–Meier estimates. Univariate analyses were done using the Gray test for cumulative incidence functions and the log rank test for overall and leukemia-free survival. Associations of patient and graft characteristics with grade II-IV acute GVHD were evaluated using multivariate logistic regression, and with other outcomes (chronic GVHD, relapse, non-relapse mortality, leukemia-free survival, overall survival) by multivariable analyses, using Cox proportional hazards. Factors included in the Cox models included the type of GVHD prophylaxis, patients' age, year of transplantation, time from diagnosis to transplantation, female donor to male recipient *versus* other gender combinations, and poor *versus* intermediate risk cytogenetics. All tests were two-sided. The type I error rate was fixed at 0.05 for determination of factors associated with time to event outcomes. Statistical analyses were performed with SPSS 19 (SPSS Inc, Chicago, IL, USA) and R 2.13.2 (R Development Core Team, Vienna, Austria) software packages.

Results

Patients and graft-versus-host disease prophylaxis

Between January 2004 and January 2012, 228 patients fulfilled the criteria for this study. Among those, three groups were established according to the GVHD prophylaxis regimen used, which was dependent on centers' habits and protocols. The patients' characteristics are summarized in Table 1. Briefly, 86 patients (38%) received ATG and CsA alone (ATG + CsA group), 66 patients (29%) received ATG and a combination of CsA and MMF (n=45) or MTX (n=21) (ATG + CsA + MMF/MTX) and 76 patients (33%) did not receive ATG but were given CsA and MTX (n=60) or MMF (n=16) (No ATG group). The median dose of intravenous busulfan received was 6.4 mg/kg in each group. Information on the brand of ATG was available for 144/152 patients (95%), among whom 92% had received thymoglobulin. Mean doses of thymoglobulin were 5 mg/kg in the ATG + CsA group and 5.5 mg/kg in the ATG + CsA + MMF/MTX group (*P*=0.013), with 4% and 29% of patients receiving doses >6 mg/kg, respectively (Table 1). Apart from GVHD prophylaxis, the three groups displayed minor differences. The median year of allogeneic HSCT was 2010 for the No ATG group and 2009 for the ATG + CsA + MMF/MTX group (*P*=0.008). Time from diagnosis to transplant was 150 *versus* >173 days, respectively (*P*=0.01) and median time from first complete remission to transplantation was 80 *versus* >120 days in the No ATG *versus* other groups, respectively (*P*=0.002). Other patient and donor characteristics did not differ between the three groups (Table 1). Cytogenetic data were available for 73.3% of the patients and missing in 10%, 33% and 39% of the ATG + CsA, ATG + CsA + MMF/MTX and No ATG groups, respectively. Among those with available data, the proportions of patients with poor cytogenetics were similar in the three groups. Fms-like tyrosine kinase 3-internal-tandem duplication status was available for 31% of the patients with similar proportions of mutated cases in the three groups.

Impact of the type of graft-versus-host disease prophylaxis on engraftment and the incidence of acute and chronic graft-versus-host disease

Engraftment data and incidences of GVHD according to GVHD prophylaxis regimens are summarized in Table 2.

All patients engrafted. However, time for engraftment was slightly different between the three groups with a median for absolute neutrophil count >0.5x10^9/L of 16.5 days (range, 1-25), 19 days (range, 4-27), and 17 days (range, 3-26) in the No ATG, ATG + CsA alone and ATG + CsA + MMF/MTX groups, respectively (*P*=0.03). We did not observe any significant impact of the type of GVHD prophylaxis on the 100-day incidence of grade II to IV acute GVHD, which occurred in 19.4%, 16.5% and 29.2% of the patients in the No ATG, ATG + CsA alone and ATG + CsA + MMF/MTX groups, respectively (*P*=0.15) (Table 2). Grade III-IV acute GVHD occurred in four (5.6%), nine (10.5%) and three (4.6%) patients in the No ATG, ATG + CsA alone and ATG + CsA + MMF/MTX groups, respectively (*P*=0.3).

By contrast, the cumulative incidence of chronic GVHD at 1 year after transplantation was significantly higher in the No ATG group than in the ATG + CsA alone and ATG + CsA + MMF/MTX groups (55±7 % *versus* 32±5 % and 20±6 %, respectively, *P*<0.001). Grade of chronic GVHD was extensive in 55% of the cases in both No ATG and ATG + CsA + MMF/MTX groups and in 41% of the cases in the ATG + CsA alone goup (Table 2 and Figure 1).

Non-relapse mortality, relapse incidence, leukemia-free survival and overall survival

The median follow up of the cohort was 19 (1-82) months. There was no difference between the groups in terms of non-relapse mortality with 2-year cumulative incidences of 15±4 %, 15 ± 5% and 23±6% in the ATG + CsA alone, ATG + CsA + MMF/MTX and No ATG groups, respectively, (*P*=0.23) (Table 3 and Figure 2A). The 2-year cumulative incidence of relapse was higher in the ATG + CsA + MMF/MTX group than in either the ATG + CsA alone or the No ATG group (48±7% *versus* 20±5% and 30±6%, respectively, *P*=0.01) (Table 3 and Figure 2B). Two-year leukemia-free survival was significantly better in the ATG + CsA alone group in comparison to the ATG + CsA + MMF/MTX group or the No ATG group (64±6%, 37±7 % and 47±7% , respectively (*P*=0.001) (Table 3 and

Table 1. Patients' characteristics.

Variables	ATG + CsA	ATG + CsA + MMF/ MTX	No ATG CsA + MTX/ MMF	All	*P*-value[a] (3 groups)
Number	86	66	76	228	
Median recipient age, year (range)	58.5 (50-70)	59 (50-70)	60.6 (50-76)	59 (50-76)	0.28
Median year of SCT, year (range)	**2009.5 (05-12)**	**2010 (05-12)**	**2009 (04-12)**	**2010 (04-12)**	**0.008**
Recipient gender male, n. (%)	50 (58.1)	35 (53)	49 (64.5)	134 (58.8)	0.38
Donor gender, male, n. (%)	57 (66,3)	36 (48,6)	35 (53,0)	128 (56,6)	0.06
Female donor, male recipient, n. (%)	17 (19.8)	19 (28.8)	24 (32.4)	60 (26.5)	0.17
Median time from diagnosis to CR1, day (range)	46 (13-176)	53 (17-245)	60 (21-385)	48 (13-385)	0.19
Median time from CR1 to SCT, day (range)	**124 (20-261)**	**120 (45-264)**	**80 (21-290)**	**114 (20-290)**	**0.002**
Median time from diagnosis to SCT, day (range)	**173 (74-443)**	**186 (91-764)**	**150 (28-626)**	**170 (28-764)**	**0.01**
Source of stem cells, PBSC, n. (%)	83 (96.5)	62 (93.9)	70 (92.1)	215 (94.3)	0.48
Cytogenetics , n. (%)					
Intermediate risk[b]	58 (75.3)	32 (72.7)	39 (84.8)	127 (77.2)	
Poor risk[c]	19 (24.7)	12 (27.3)	7 (15.2)	38 (22.8)	0.34
Missing	9 (10.4)	22 (33.3)	30 (39.4)	61 (26.7)	
FLT3-ITD status, n. (%)					
Mutated	13 (32%)	8 (32%)	3 (43%)	24 (33%)	0.85
Missing	46	41	69	156	
Dose of iv busulfan in mg/kg, median (range)	6.4 (6.1-6.9)	6.4 (6.23-6.4)	6.4 (6-6.76)	6.4 (6-6.89)	0.47
In vivo T-cell depletion, n. (%)					*P* (1 *vs.* 2)
None	0	0	76 (100)		
Rabbit ATG:	78 (98%)	55 (86%)	135 (92%)		0.01
Horse ATG	2 (2%)	9 (14%)	11 (8%)		
Brand missing	6	2	8		
If rabbit ATG:					*P* (1 *vs.* 2)
Mean dose (mg/kg) (range)	**5 (2.5-10)**	**5.5 (2.5-11.5)**		**5.2 (2.5-11.5)**	**0.013**
ATG < 6 mg/kg	75 (96%)	39 (71%)		114	
ATG ≥ 6 mg/kg	3 (4%)	16 (29%)		19	
ATG dose unknown	2	0		2	
Post-transplant immunosuppression, n. (%)					
CsA alone	86 (100)	0	0		<0.0001
CsA + MMF	0	45 (68)	16 (21)		
CsA + MTX	0	21 (32)	60 (79)		

ATG: anti-thymocyte globulin; CR1: first complete remission; CsA: cyclosporine A; FLT3-ITD: Fms-like tyrosine kinase 3-internal-tandem duplication; n: number of patients; MMF: mycophenolate mofetil; MTX: methotrexate; PBSC: peripheral blood stem cells; SCT: stem cell transplantation. [a]Calculated with the χ^2 test; [b]Defined as not good risk [including t(8;21); t(15;17) and del(16)], neither poor risk cytogenetics. [c]Defined as 11q23 abnormalities, complex karyotype and abnormalities of chromosomes 5 and 7. Statistically significant results are shown in bold.

Figure 2C). Similarly, the 2-year overall survival was better in the ATG + CsA alone group than in the other two groups (67±5% *versus* 37±7% for the triple immunosuppression group and 50±7% for the No ATG group, *P*=0.001) (Table 3 and Figure 2D). Causes of death included relapse, GVHD, infection in 16 (59%), three (11%) and four (15%) patients of the ATG + CsA alone group, respectively, 20 (69%), three (10%) and four (14%) patients of the ATG + CsA + MMF/MTX group, respectively, and 19 (54%), two (6%) and 13 (37%) patients of the No ATG group, respectively.

Impact of anti-thymocyte globulin dose on transplant outcomes

Among the 152 patients given ATG, the vast majority (n=135) received thymoglobulin, while 11 received horse ATG and in eight the brand of ATG was unknown. Among the p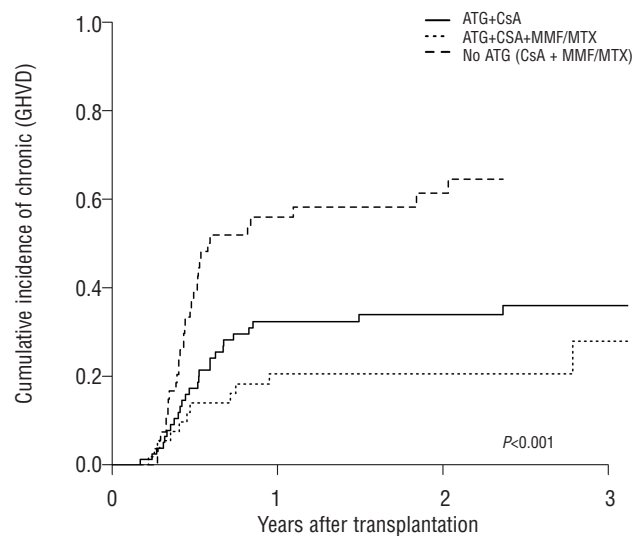atients who received thymoglobulin, patients in the ATG + CsA + MMF/MTX group received higher doses of thymoglobulin than those in the ATG + CsA group (mean dose of 5.5 mg/kg *versus* 5 mg/kg, respectively, *P*=0.013) (Table 1). Since transplant outcomes were worse in the triple immunosuppression group, we sought to determine whether the thymoglobulin dose could have had a role in this phenomenon. In univariate analysis, patients receiving a thymoglobulin dose below 6 mg/kg in the ATG + CsA group (n=75) had similar non-relapse mortality (*P*=0.44), reduced relapse incidence (*P*=0.0009) and improved overall survival (*P*=0.05) and leukemia-free survival (*P*=0.03) in comparison to those receiving similar doses of ATG in the ATG + CsA + MMF/MTX group (n=39) (Table 4). In addition, within the triple immunosuppression group, transplant outcomes were not significantly different in those treated with a thymoglobulin dose above or below 6 mg/kg (*data not shown*).

Risk factors for chronic graft-versus-host disease, non-relapse mortality, relapse incidence, leukemia-free survival and overall survival

In multivariate analysis, the absence of ATG was the only risk factor associated with an increased risk of chronic GVHD [hazard ratio (HR) = 2.48; 95% confidence interval (CI) = 1.32-4.64] (*P*=0.005), while the risks of chronic GVHD in the two ATG groups were similar (HR=1.1; in the ATG + CsA + MMF/MTX group as compared to the ATG + CsA alone group, *P*=0.8) (Table 5). In comparison to ATG + CsA alone, the use of ATG + CsA + MMF/MTX led to a higher risk of relapse (HR=2.97, 95% CI=1.44-6.12, *P*=0.003). The use of ATG + CsA + MMF/MTX or the absence of ATG were associated with worse leukemia-free survival (HR=2.76, *P*=0.001 and HR=2.10, *P*=0.01, respectively) and overall survival (HR=2.96, *P*=0.001 and HR=2.11, *P*=0.01, respectively). The other factors that had a bearing on transplant outcomes were older age (above median) resulting in a higher relapse incidence (HR=2.35, *P*=0.01) and leading to worse leukemia-free survival (HR=1.88, *P*=0.01) and overall survival (HR=1.64, *P*=0.05), and poor cytogenetics associated with higher non-relapse mortality (HR=3.25, *P*=0.004) and, therefore, worse leukemia-free survival (HR=1.71, *P*=0.04) and overall survival (HR=2.03, *P*=0.01) (Table 5).

Figure 1. Cumulative incidence of chronic GVHD according to the GVHD prophylaxis in the three groups of patients (global *P*-value <0.001).

Table 2. Engraftment, acute GVHD and chronic GVHD according to GVHD prophylaxis.

	ATG + CsA	ATG + CsA + MMF/MTX	No ATG (CsA + MTX/MMF)	*P*-value (3 groups)
Number	86	66	76	
Engraftment, n. (%)	86 (100)	66 (100)	76 (100)	
Days for ANC >0.5x10⁹/L, median day (range)	**19 (4-27)**	17 (3-26)	16.5 (1-25)	**0.03**
Day 100 acute GVHD, n. (%)				
Grade 0-I	71 (83,5)	46 (70,8)	58 (80,6)	0.15
Grade II-IV	14 (16,5)	19 (29,2)	14 (19,4)	
1 year cumulative incidence of chronic GVHD (%)	**32±5**	**20±6**	**55±7**	**<0.001**
Grade of chronic GVHD, n. (%)				
Limited	14 (52)	4 (36)	15 (45)	
Extensive	11 (41)	4 (36)	18 (55)	
Missing	2	1	0	

ANC: absolute neutrophil count; ATG: anti-thymocyte globulin; CsA: cyclosporine A; N: number of patients; MMF: mycophenolate mofetil; MTX: methotrexate. Statistically significant results are shown in bold.

Discussion

The long-lasting challenge of allogeneic HSCT in acute myeloid leukemia and other hematologic malignancies is to avoid toxicity and severe GVHD while maintaining the GVL effect. As the median age of patients with acute myeloid leukemia is >65 years and many of them have comorbidities and are, thus, ineligible for allogeneic HCST with myeloablative conditioning, reduced toxicity conditioning and RIC regimens were developed over the last decade. The Flu-ivBu2 RIC is widely used in this setting, essentially because of its low organ toxicity and non-relapse mortality and preserved anti-leukemia effect.[1] The original Flu-ivBu2 RIC protocol used ATG and CsA alone as GVHD prophylaxis. However, the best GVHD prophylaxis remains a subject of debate. The increasing use of matched and mismatched unrelated donors has led to the addition of MMF or MTX to CsA in RIC regimens. Furthermore, the use of *in vivo* T-cell depletion is debatable, as the efficacy of RIC allogeneic HSCT is based on a powerful GVL effect, which is typically mediated by donor T and NK cells, rather than on high doses of radio-chemotherapy. Indeed, in a study from the CIBMTR, performed in patients with myeloid and lymphoid malignancies who underwent RIC allogeneic HSCT from related or unrelated donors, the use of ATG was associated with a higher risk of relapse and lower leukemia-free survival rate.[11] In contrast, a similar recent EBMT study failed to detect either association.[12]

Because transplant outcomes are also dependent on recipient age, type of donor, disease risk and status at transplant,[22,23] in order to evaluate the impact of GVHD prophylaxis on transplant outcomes more accurately, in the present study we limited the analysis to a very homo-

Table 3. Transplant outcomes at 2 years after allogeneic hematopoietic stem cell transplantation according to the GVHD prophylaxis.

	N.	LFS	OS	RI	NRM
ATG + CsA	86	64±6	67±5	20±5	15±4
ATG + CsA + MMF/MTX	66	37±7	37±7	48±7	15±5
No ATG (CsA + MMF/MTX)	76	47±7	50±7	30±6	23±6
	P-value	0.001	0.001	0.01	0.23

ATG: anti-thymocyte globulin; CsA: cyclosporine A; LFS: leukemia-free survival; N: number of patients, MMF: mycophenolate mofetil; MTX: methotrexate; NRM: non-relapse mortality; OS: overall survival; RI: relapse incidence.

A — Cumulative incidence of NRM vs Years after transplantation. Legend: ATG+CsA; ATG+CSA+MMF/MTX; No ATG (CsA + MMF/MTX). NS

B — Cumulative incidence of relapse vs Years after transplantation. *P*=0.01

C — Leukemia-free survival vs Years after transplantation. *P*=0.001

D — Overall survival vs Years after transplantation. *P*=0.001
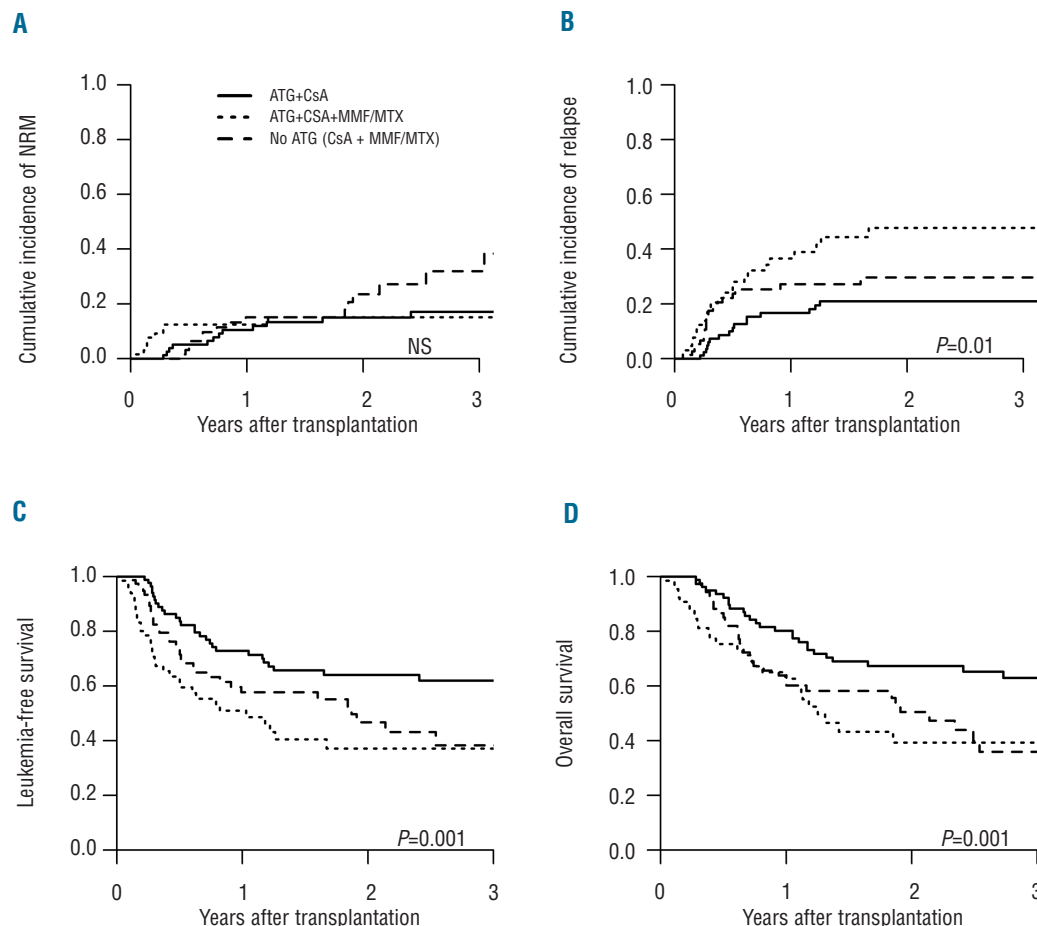
Figure 2. Transplant outcomes according to the GVHD prophylaxis. Cumulative incidence of (A) non-relapse mortality (NRM) (global *P*-value = 0.23), (B) relapse (global *P*-value = 0.01), (C) leukemia-free survival (global *P*-value = 0.001) and (D) overall survival (global *P*-value = 0.001) in the different GVHD prophylaxis groups as indicated.

geneous population of patients above 50 years of age, transplanted for acute myeloid leukemia in first complete remission with an HLA-identical sibling after Flu-ivBu2 RIC. In this particular setting, the comparison of ATG and CsA GVHD prophylaxis to prophylaxis not containing ATG showed that the use of ATG was not linked to a higher risk of relapse in patients with equivalent cytogenetic risks. Most of the patients in this study had received thymoglobulin at a dose <6 mg/kg, and these results are consistent with the previously reported preserved GVL effect in patients treated with relatively reduced doses of ATG,[12-14] in contrast with doses >10 mg/kg.[3]

As previously described,[11,12] our study confirms a protective effect of ATG against chronic GVHD. No ATG was actually the only factor associated with an increased risk of chronic GVHD in a multivariate analysis. We did not observe an equivalent protective effect of ATG against acute GVHD. Moreover, the use of ATG is not an all or none phenomenon. The ATG dose also matters. Indeed, two recent studies reported that GVHD prophylaxis with a reduced dose of ATG of 2.5 mg/kg *versus* 5 mg/kg in Flu-ivBu2 RIC allogeneic HSCT with peripheral blood stem cells from matched related or unrelated donors was associated with an increased risk of acute and chronic GVHD.[13,14] Collectively, these data confirm that the optimal dose of thymoglobulin in the Flu-ivBu2 RIC regimen is about 5 mg/kg.

The type and dose of post-transplant immunosuppression in the context of non-ATG-containing, fludarabine-based RIC from HLA-identical sibling donors was recently evaluated by Piñana *et al.*[18] The authors found similar post-transplant outcomes with either a combination of CsA and MTX or of CsA and MMF. In our study, we also observed similar outcomes in the group of patients who received MTX or MMF and CsA without ATG (*data not shown*), but this group had a higher risk of chronic GVHD leading to worse survivals. In the context of ATG-containing regimens, the addition of MMF or MTX to CsA did not reduce the risk of acute GVHD, but significantly increased the risk of relapse, possibly as a consequence of the relatively reduced risk of chronic GVHD, leading to worse leukemia-free and overall survival. Chronic GVHD was previously reported as a very important process medi-

ating the GVL effect, and with a strong correlation with reduced relapse rates, mainly in the RIC setting, compensating for the lower chemotherapy dose and, thus, the lower anti-leukemic effect.[24] Interestingly, despite an increased risk of chronic GVHD due to the absence of ATG, the combination of CsA and MTX or MMF was also associated with a trend towards a higher risk of relapse and lower leukemia-free and overall survival rates, in comparison with the group treated with ATG and CsA. This emphasizes that early post-transplant immunosuppression after RIC in patients with acute myeloid leukemia in first complete remission has an important impact on the equilibrium between GVL and GVHD and, therefore, on final post-transplant outcomes. The use of intermediate doses of ATG (thymoglobulin at 5 mg/kg on average) + CsA alone provided the best outcomes with a 2-year leukemia-free survival rate of 64±6% and 2-year overall survival of 67±5%.

Besides indicating the role of immunosuppression, the results of our study pointed out a negative impact of older age (>60 years old) and poor cytogenetics on leukemia-free survival and overall survival. Older age was associated with a higher risk of relapse, even in this cohort of patients allografted in first complete remission. This is in accordance with previous studies demonstrating poorer results of anti-leukemic therapy in elderly patients with acute myeloid leukemia due to more aggressive biology of the disease, selection for more primitive clones, higher prevalence of multidrug resistance and lower treatment tolerability.[25,26] Although the frequency of poor cytogenetics

Table 4. Transplant outcomes at 2 years after allogeneic hematopoietic stem cell transplantation in the two ATG groups treated with ATG <6 mg/kg.

	N	LFS	OS	RI	NRM
ATG + CsA	75	63±6	67±5	22±5	15±4
ATG + CsA + MMF/MTX	39	41±9	46±10	51±10	8±4
	P-value	0.03	0.05	0.0009	0.44

ATG: anti-thymocyte globulin; CsA: cyclosporine A; LFS: leukemia-free survival; N: number of patients; MMF: mycophenolate mofetil; MTX: methotrexate; NRM: non-relapse mortality; OS: overall survival; RI: relapse incidence

Table 5. Multivariate analyses of chronic transplant outcomes.

	Chronic GVHD		NRM		Relapse		LFS		OS	
	HR (95% CI)	*P*[a]	HR (95% CI)	*P*[a]	HR (95% CI)	*P*[a]	HR (95% CI)	*P*[a]	HR (95% CI)	*P*[a]
ATG + CsA	1		1		1		1		1	
ATG + CsA + MMF/MTX	1.11 (0.50-2.46)	0.80	2.51 (0.87-7.21)	0.09	**2.97 (1.44-6.12)**	**0.003**	**2.76 (1.53-4.98)**	**0.001**	**2.96 (1.57-5.60)**	**0.001**
No ATG (CsA+MMF/MTX)	**2.48 (1.32-4.64)**	**0.005**	2.34 (0.97-5.60)	0.06	2.01 (0.96-4.20)	0.06	**2.10 (1.2-3.68)**	**0.01**	**2.11 (1.18-3.75)**	**0.01**
Age >median	0.95 (0.55-1.65)	0.87	1.25 (0.58-2.70)	0.57	**2.35 (1.26-4.37)**	**0.01**	**1.88 (1.16-3.04)**	**0.01**	**1.64 (1.00-2.69)**	**0.05**
Year of SCT >median	1.06 (0.50-2.25)	0.88	0.81 (0.26-2.52)	0.72	1.44 (0.70-2.93)	0.32	1.24 (0.68-2.24)	0.48	1.12 (0.59-2.13)	0.72
Poor *versus* interm. cytogenetics	1.43 (0.74-2.76)	0.29	**3.25 (1.44-6.91)**	**0.004**	1.18 (0.60-2.35)	0.63	**1.71 (1.03-2.82)**	**0.04**	**2.03 (1.21-3.41)**	**0.01**
Female donor/male recipient	1.31 (0.74-2.33)	0.35	1.12 (0.50-2.50)	0.79	0.77 (0.40-1.47)	0.42	0.85 (0.52-1.41)	0.53	1.08 (0.65-1.79)	0.77
Time from diagnosis to SCT > median	0.96 (0.57-1.63)	0.89	0.86 (0.41-1.83)	0.70	1.35 (0.75-2.42)	0.31	1.12 (0.71-1.78)	0.62	0.91 (0.57-1.45)	0.68

ATG: anti-thymocyte globulin; CsA: cyclosporine A; CI: confidence interval; HR: hazard ratio; interm. cytogenetics: intermediate cytogenetics; LFS: leukemia-free survival; MMF: mycophenolate mofetil; MTX: methotrexate; NRM: non-relapse survival; OS: overall survival; P: P-value; RI: relapse incidence; SCT: stem cell transplantation. [a]Statistically significant factors are in bold.

increases with age, this factor was not associated with a higher risk of relapse in this series of patients but was associated with increased non-relapse mortality. An absence of impact of poor cytogenetics on relapse incidence should be considered with caution because of the limited numbers of patients with documented poor cytogenetics.

We recognize that this study has several limitations. First, the study was retrospective and the reason for the choice of GVHD prophylaxis was not known but mainly dependent on the habits and protocols of each center. Second, the study included relatively small numbers of patients per group and cytogenetic data were imperfectly covered over the groups. However, the study was performed on a very homogeneous cohort of patients with acute myeloid leukemia older than 50 years, in first complete remission, receiving peripheral blood stem cells from HLA-identical siblings. Despite its limitations and the need for prospective randomized studies, the results of this study suggest that, in this particular setting, the best GVHD prophylaxis is the combination of an intermediate dose of ATG and CsA. Omission of ATG from the prophylactic regimen is associated with an increased risk of chronic GVHD without limiting the risk of relapse, while addition of MMF or MTX increased the risk of relapse.

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