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Allogeneic Stem Cell Transplantation for Relapsed/Refractory B Cell Lymphomas: Results of a Multicenter Phase II Prospective Trial including Rituximab in the Reduced-Intensity Conditioning Regimen

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A B S T R A C T

The treatment of patients with refractory/relapsed B cell non-Hodgkin lymphoma (NHL) is evolving because of the availability of novel drugs. Allogeneic stem cell transplantation (alloSCT) can be curative, but its morbidity and mortality remain a matter of concern. We conducted a multicenter prospective phase II trial to evaluate the benefit of including only 1 dose of rituximab in the conditioning regimen before alloSCT. The primary endpoint was progression-free survival. The study enrolled 121 patients with relapsed/refractory B cell lymphomas. The conditioning regimen consisted of thiotepa, cyclophosphamide, fludarabine, and rituximab (500 mg/m^2). Rabbit antithymocyte globulin was administered only in case of unrelated donors. Sixty-seven (55%) and 54 (45%) patients received grafts from related and unrelated donors, respectively. The crude cumulative incidence (CCI) of nonrelapse mortality (NRM) was 21% at 3 years. The CCIs of chronic graft-verus-host disease (GVHD) at 3 years were 54% and 31% in recipients of matched sibling and unrelated grafts, respectively. At a median follow-up of 41 months, the estimated 3-year progression-free and overall survival were 50% and 61%, respectively. Long-term outcome was also evaluated with the composite endpoint of GVHD-free and relapse-free survival (GRFS). This is the first work evaluating the GRFS in a prospective trial of lymphoma patients: the 1-year and 3-year GRFS were 40% and 34%, respectively. AlloSCT can cure a fraction of patients with rather low NRM and an encouraging PFS and GRFS.

INTRODUCTION

Rituximab-based chemoimmunotherapy has improved the survival rate of patients with indolent and aggressive B cell non-Hodgkin lymphoma (NHL) [1,2], but 50% of them fail to respond or relapse and only a fraction could be cured by autologous stem cell transplantation (autoSCT). Despite

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an increase of novel drugs and treatments [3], allogeneic stem cell transplantation (alloSCT) still represents the only chance of cure for patients relapsing after autoSCT or 2 lines of chemo-immunotherapy. In previous studies with reduced-intensity conditioning, we reported a 5-year progression-free survival (PFS) of 57% and 54% in indolent and aggressive lymphomas, respectively [4,5].

Severe acute graft-versus-host disease (GVHD) and extensive chronic GVHD [6,7] are the 2 main complications associated with transplantation and may affect both nonrelapse mortality (NRM) and quality of life. In recent years, a better understanding of GVHD biology prompted the design of novel GVHD prophylaxis regimens, including the use of post-transplantation cyclophosphamide or proteasome inhibitors, but the gold standard is still based on calcineurin inhibitors and methotrexate or mycophenolate mofetil [8-12].

B cells have a role in the pathogenesis of both acute GVHD (for their role as antigen-presenting cells) and chronic GVHD (for the production of autoantibodies from autoreactive B cells) [13]. Rituximab has been introduced during the conditioning regimen in very few trials; however, the results on disease control and prevention of acute or chronic GVHD are still unclear [14-16]. The major experience was derived from the pivotal studies of Khouri [14], which showed rather good disease control with the unexpected finding of a limited incidence of acute and chronic extensive GVHD.

In the present multicenter, prospective, phase II study, we investigated the effect of a single dose of rituximab (500 mg/m^2) in combination with a reduced-intensity conditioning (RIC) regimen on the PFS of refractory/relapsed B cell lymphomas. In addition, overall survival, incidence of acute and chronic GVHD, and GVHD-free, relapse-free survival (GRFS) were evaluated.

MATERIALS AND METHODS

Patient Characteristics

Between December 2007 and December 2015, 121 patients were enrolled in a prospective study (EUDRACT 2007-003657-87) involving 22 Italian hematology divisions. Inclusion criteria were as follows: (1) patients were diagnosed with CD20$^+$ B cell NHL (ie, chronic lymphocytic leukemia [CLL], small lymphocytic lymphoma [SLL], follicular lymphoma [FL], mantle cell lymphoma [MCL], de novo or transformed diffuse large B cell lymphoma [DLBCL]) relapsing or refractory after at least 2 lines of treatment or after failure of autoSCT; (2) MCL and DLBCL were required to have chemosensitive disease; and (3) chemorefractory disease was allowed only for indolent lymphomas. Exclusion criteria were central nervous system localization, positive serologic markers for human immunodeficiency virus, active hepatitis B virus or hepatitis C virus infection, ejection fraction < 45% (or myocardial stroke in the last year), diffusion capacity of the lung for carbon monoxide < 50%, no adequate renal and hepatic functions (clearance of creatinine < 50 mL/minute, serum bilirubin levels >2 the upper normal limit). All the patients received rituximab chemo as salvage before transplantation. The study was approved by the institutional review board of all participating centers.

Donors and Treatment Plan

Donors age ranged between 18 and 65 years old in the case of identical sibling donors and between 18 and 60 years old in the case of unrelated donors, The availability of HLA-identical or 1-antigen-mismatched (class I) sibling donors or unrelated donors mismatched by 1 antigen or allele at HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1 loci were identified by high- or intermediate-resolution typing.

Patients were registered at time of relapse. At registration, patients were treated with salvage rituximab chemotherapy. The choice of the salvage regimen was left to center preference. All the patients included in this prospective trial received the following drugs intravenously: rituximab (500 mg/m^2, day –6), thiotepa (6 mg/kg every 12 hours for 2 doses, day –5), cyclophosphamide (30 mg/kg, days –4 and –3), and fludarabine (30 mg/m^2, days –4 and –3, administered 4 hours after cyclophosphamide administration). The choice to administer only 1 dose of rituximab was related to the fact that rituximab was already administered with the previous salvage chemo-immunotherapy. In the case of matched related sibling donors, the GVHD prophylaxis consisted of intravenous or oral cyclosporine A, adjusted to maintain blood levels at 200 ng/mL to 300 ng/mL, and a short course of intravenous methotrexate (10 mg/m^2 on day +1, and 8 mg/m^2 on days +3 and +6). Patients with a class I antigen mismatch (sibling donors) or with unrelated donors received intravenous rabbit antithymocyte globulin (ATG, Thymoglobuline, Genzyme-Sanofi; .5 mg/kg on day –4, 3 mg/kg on day –3, 3.5 mg/kg on day –2). On day 0, patients received stem cells > 3×10^8/kg total nucleated stem cells in case of bone marrow and ≥4×10^6/kg CD34$^+$ in case of peripheral blood stem cells. In absence of active acute GVHD, GVHD prophylaxis was administered until day 100 for siblings and day 150 for unrelated donors (details on immunosuppression tapering are given in Supplemental Material). Recommendations for supportive care were previously described [5].

Study Endpoints

The primary endpoint of the study was PFS. The secondary endpoints were NRM, acute GVHD incidence within 100 days of alloSCT, chronic GVHD incidence after 100 days, and overall survival (OS). The study sample size was calculated to estimate the 1-year PFS and corresponding 90% 1-sided confidence interval. In a retrospective series of 115 patients with relapsed/refractory B cell NHL, 1-year PFS was 70%, with relapse of 20% [5]. Assuming no treatment effect on NRM and a 35% relative improvement of relapse rate (from 20% to 13%), at a significance level of 10% (1-sided test) a sample size of 190 assessable patients ensured a 80% probability of detecting a 70% to 77% increase in 1-year PFS. However, because of the expansion of trials incorporating novel agents and an increase of haploidentical donor use, the accrual rate decreased in the last 2 years and the data safety and monitoring committee suggested stopping enrollment; also, a change in PFS was considered very unlikely. Therefore, the primary endpoint of improving PFS was not met. However, we also analyzed our study using the novel composite endpoint of GRFS, which gives more information regarding GVHD.

Response Criteria and Statistical Analysis

The response to therapy was evaluated at 1, 3, and 6 months after alloSCT and every 6 months thereafter and was measured using the International Workshop NHL criteria, as previously described [17]. Acute GVHD was evaluated using the criteria previously described by Glucksberg et al. [18]. Chronic GVHD was diagnosed according to the Seattle criteria [19]. Chronic GVHD was not evaluated by the National Institute of Health criteria because the study was designed before 2007. The incidence of NRM, relapse, and acute and chronic GVHD were estimated in a competing risks setting using cumulative incidence estimates and the curves were compared by means of the Gray test. In the estimation of GVHD, death without GVHD was evaluated as a competing event. NRM and relapse were competing events for each other [20]. We also tested the composite endpoint GRFS [21], introduced in the 2015, which reflects survival free of major complications. GRFS events were defined as grade 3 or 4 acute GVHD or chronic GVHD requiring systemic immunosuppressive treatment at any time, disease relapse, or death from any cause during the first 12 and 36 months after alloSCT. The OS, PFS, and GRFS curves were estimated using the Kaplan-Meier method and the curves were compared by means of the log-rank test. Univariable and multivariable analyses were performed using Fine and Gray (GVHD, NRM, relapse) or Cox regression models (OS, PFS, GRFS) to assess association between baseline characteristics and the endpoints [22] The statistical association level was evaluated by means of Wald tests. In all the analyses, age at transplantation was evaluated as continuous variable using 3-knot restricted cubic spline. In the multivariable analysis for the evaluation of factors influencing acute and chronic GVHD, variables (age, donor type, and donor gender) were chosen based on clinical considerations, and we did not apply any statistical procedures for variable selection. Statistical analyses were performed with SAS (SAS Institute, Cary, NC) and R software (R Development Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/. Last access: July 7th 2016).

RESULTS

Patient Characteristics

One hundred twenty-one patients with relapsed/refractory lymphoma were enrolled in this multicenter, prospective, phase II trial (refer to Table 1 for baseline characteristics of patients). The median age was 52 years (range, 24 to 65 years). Diagnoses were de novo or transformed DLBCL (n = 35, of which 2 transformed and 33 de novo), MCL (n = 22), and indolent lymphomas (FL, n = 35; CLL/SLL, n = 29). Sixty-seven out of 121 (55%) underwent transplantation from a related

Table 1
Patient Characteristics (n = 121)

Characteristic	Value
Age at AlloSCT, median (range), yr	52 (23-65)
Gender	
Male	80 (66)
Female	41 (34)
Karnofsky performance status	
≤80	18 (15)
>80	103 (85)
Diagnosis*	
FL	35 (28)
CLL/S CLL	29 (24)
DLBCL	35 (28)
MCL	22 (18)
Bone marrow involvement	
Yes	30 (25)
No	79 (65)
Missing	12 (10)
Extranodal involvement	
Yes	26 (21)
No	78 (64)
Missing	17 (14)
Prior autoSCT	
Yes	74 (61)
No	47 (39)
Disease status at transplantation	
CR	48 (40)
PR	64 (53)
PD/SD	9 (7)
Donor type	
Matched/mismatched related†	67 (55)
Matched unrelated	34 (28)
Mismatched unrelated	20 (17)
Sex mismatched	
Female donor-male recipient	22 (18)
Other combinations	90 (74)
Missing	9 (7)
Stem cell source	
PBSC	101 (83)
BM	20 (17)
Year of allogeneic transplantation	
2007-2011	65 (54)
2012-2015	56 (46)

Data presented are n (%) unless otherwise indicated.
PR indicates partial remission; PD, progressive disease; SD, stable disease; PBSC, peripheral blood stem cells; BM, bone marrow.
* Diagnosis were well balanced: 34 and 30 indolent lymphomas patients received transplants from matched /mismatched related sibling, respectively; 33 and 20 patients with aggressive lymphomas received transplants from matched/mismatched unrelated donors.
† Includes 2 cases mismatched related.

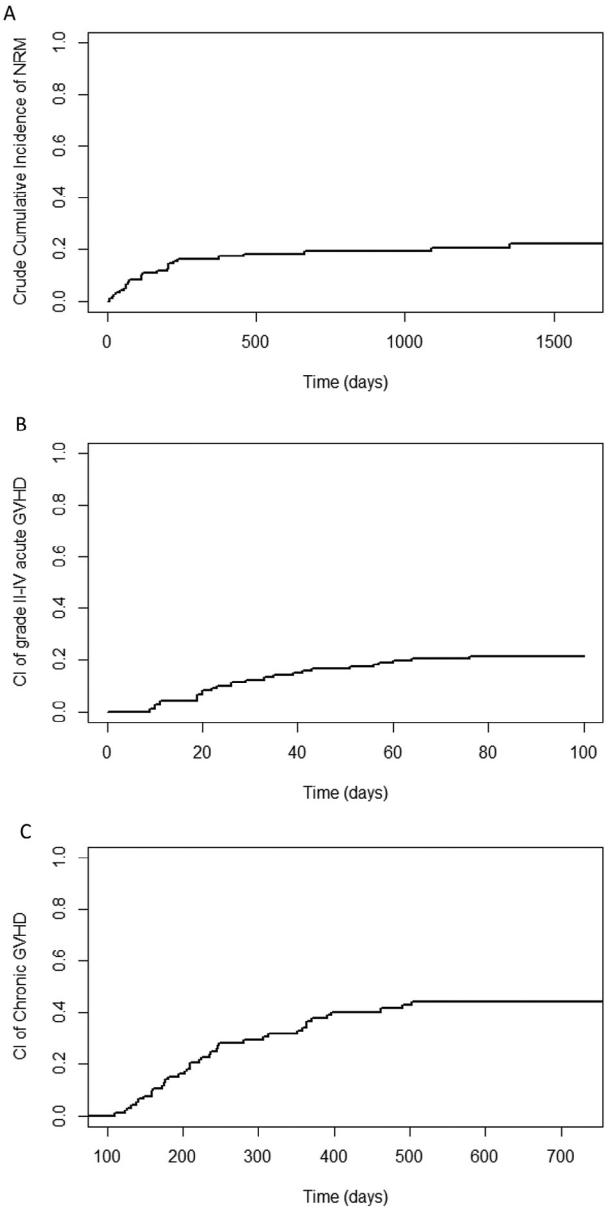

Figure 1. (A) Crude cumulative Incidence (CCI) of NRM. At 3 years, the CCI of NRM was 21% (95% CI, 14% to 29%); (B) CCI of acute GVHD grade II to IV at 100 days: 21% (95% CI,15% to 30%); (C) CCI of limited and extensive chronic GVHD: 27% (95% CI, 195 to 38%) and 17% (95% CI, 11% to 27%), respectively.

sibling (1 mismatched siblings in only 2 cases) and 54 (45%) received a graft from an unrelated donor (34 matched, 20 mismatched). Twelve patients had mismatches in class I and the rest had mismatches in class II. Interestingly, only 3 patients out of 20 (15%) had antigenic mismatches at locus C. It is important to note that most of the patients had a chemosensitive disease (complete remission [CR], n = 48 [39%]; partial remission, n = 61 [51%]) at transplantation.

Cumulative Incidence of NRM and GVHD

At a median follow-up of 41 months (range, 6 to 95 months), 24 patients died (20%) of treatment-related causes: acute GVHD (n = 5), chronic GVHD (n = 3), infections (n = 11), thrombotic microangiopathy (n = 1), cardiovascular complications (n = 3), and liver failure (n = 1). The estimated 1-year and 3-year crude cumulative incidence rates (CCI) of NRM were 16% (95% confidence interval [CI], 11% to 25%) and 21% (95% CI, 14% to 30%), respectively (Figure 1). NRM, by univariate analysis (Table S1), was not affected by donor type (CCI at 1 year: 14% versus 19% in matched sibling donors and unrelated donors, respectively [$P = .325$]) nor by lymphoma subtype (CCI at 1 year: 16% versus 17% in indolent and aggressive lymphomas, respectively [$P = .80$]). When we performed a multivariate analysis, NRM did not appear to be influenced either by age at transplantation, histotype, or by a previously failed autoSCT (Table 2). However, a higher hazard ratio (HR) was estimated for sex mismatch (female donor to male recipient versus other combinations: HR, 2.5; 95% CI, .91 to 6.97; $P = .076$), although results remained nonsignificant. Twenty-six patients (22%) were diagnosed with acute GVHD grade II to IV (n = 16 grade II, n = 9 grade III, and n = 1 grade IV) with an estimated CCI of 22% (95% CI, 15% to 30%). Two patients experienced late-onset acute GVHD beyond day 100 and were excluded from the CCI evaluation. The CCI of

Table 2
Multivariable Analysis on NRM and Relapse

Factor	NRM		Relapse	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age at AlloSCT, yr				
46 versus 57*	1.45 (.73-2.86)	.55	—	—
Histotype subgroup				
Aggressive versus indolent	1.07 (.44-2.60)	.891	3.03 (1.14-8.08)	**.026**
Bone marrow involvement				
Yes versus no	—	—	3.18 (1.16-8.66)	**.024**
Extranodal involvement				
Yes versus no	—	—	.95 (.28-3.18)	.930
Prior AutoSCT				
Yes versus no	1.82 (.66-5.06)	.250	1.11 (.35-3.47)	.860
Donor type				
Unrelated versus related	1.75 (.75-4.08)	.197	.80 (.31-2.05)	.640
Sex mismatched				
Female donor-male recipient versus Other combinations	2.52 (.91-6.97)	**.076**	.12 (.01-1.15)	**.067**

Bold typeface indicates statistical significance.
* Age was evaluated as continuous variable. The 2 values are, respectively, the third and first quartiles of the variable distribution.

acute GVHD was not significantly different in patients receiving grafts from sibling and from unrelated donors (21% versus 22%, respectively).

Ninety-four patients (77%) were evaluable for chronic GVHD, whereas the others 27 were not included because of early death (n = 18), short follow-up (n = 7), or an overlapping syndrome of acute and chronic GVHD (n = 2). The CCI of chronic GVHD at 3 years was 44% (95% CI, 34% to 56%) with a median time to onset of 353 days. Curves related to acute and chronic GVHD are reported on Figure 1. According to the modified Seattle criteria, the CCI of extensive GVHD was 17% (95% CI, 11% to 27%) whereas the CCI of limited form was 27% (95% CI, 19% to 39%) (Figure S1). In the univariable Fine and Gray model, the only factor that was protective against the occurrence of GVHD was transplantation from unrelated donors when rituximab and ATG were combined together (HR, .50; 95% CI, .25 to .99; $P = .05$). The CCIs of chronic GVHD at 3 years were 54% (95% CI, 42 to 1%) and 31% (95% CI, 19% to 49%) for patients allografted from matched sibling and unrelated donors, respectively ($P = .04$). The analysis of association between the occurrence of acute and chronic GVHD with different endpoints was performed by including each GVHD type as time-dependent covariate in univariable Cox model. The occurrence of acute GVHD significantly increased the risk of NRM (HR, 3.33; 95% CI, 1.44 to 7.71; $P = .005$). NRM and acute and chronic GVHD were not affected by donor type (matched sibling, matched and mismatched unrelated) (curves reported in Supplemental Material). There were no cases of Epstein-Barr virus (EBV) lymphoproliferative disorders.

Relapse

Thirty patients relapsed (25%) and 18 of them died of disease (n = 11 aggressive DLBCL, n = 7 CLL). The CCIs of relapse were 19% (95% CI, 13% to 28%) at 12 months and 27% (95% CI, 19% to 37%) at 3 years. In particular, the CCI of relapse at 3 years was 17% and 34% in indolent and aggressive histotypes, respectively ($P = .011$). By multivariate analysis, a positive bone marrow infiltration at the time of transplantation (adjusted HR, 3.18; 95% CI, 1.16 to 8.66; $P = .024$) and aggressive histotypes (adjusted HR; 3.03; 95% CI, 1.14 to 8.08; $P = .026$) were associated with higher relapse risk.

PFS, OS, and GRFS

Seventy-nine (65%) patients are currently alive, with a median follow-up for surviving patients of 41 months (range, 6 to 95 months). The estimated 3-year PFS and OS were 50% and 61%, respectively. (Figure 2). At 3 years, the PFS and OS were as follows in the different subtypes: 70% (95% CI, 52% to 82%) and 76% (95% CI, 59% to 86%) in FL, 60% (95% CI, 40% to 75%) and 66% (95% CI, 45% to 80%) in CLL/SLL, 40% (95% CI, 23% to 57%) and 52% (95% CI, 32% to 68%) in DLBCL, and

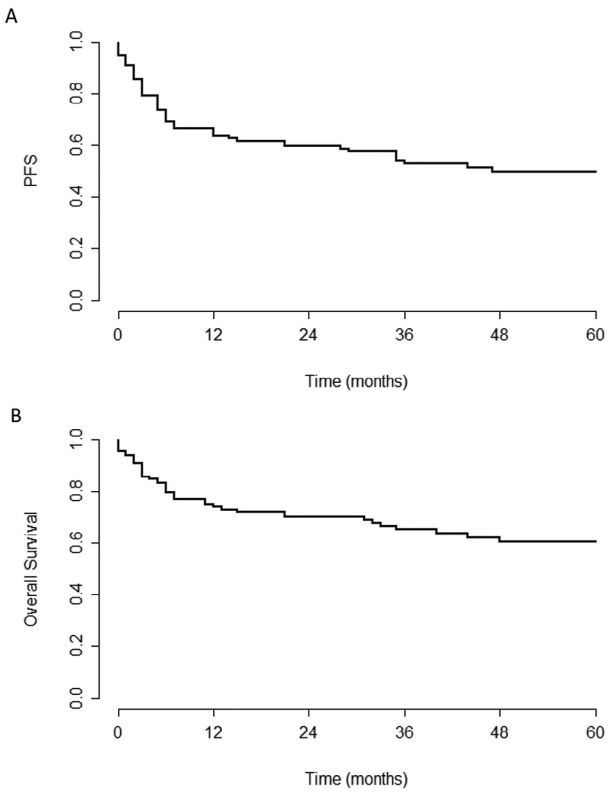

Figure 2. Kaplan-Meier estimates of progression-free survival and overall survival. (A) PFS at 5 years was 49% (95% CI, 40% to 61%). (B) OS at 5 years was 61% (95% CI, 51% to 71%).

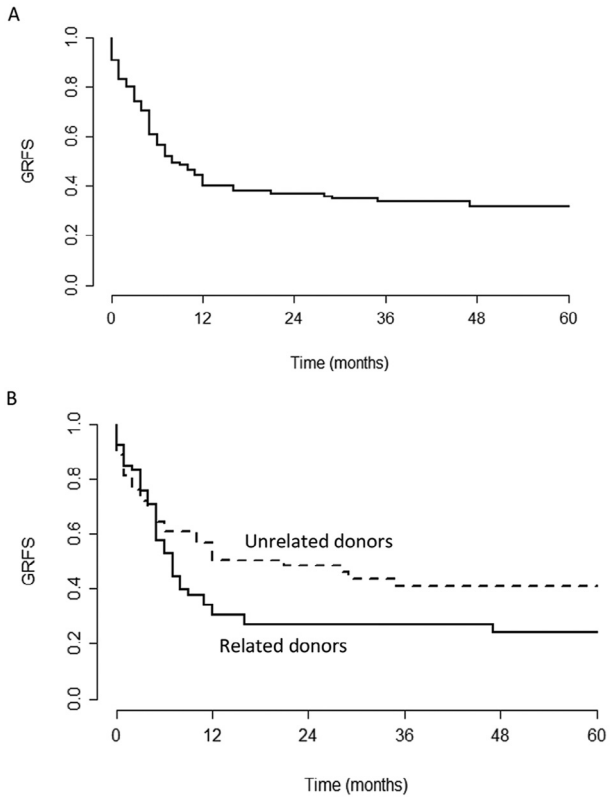

Figure 3. Adjusted Kaplan-Meier estimates of GVHD-free and relapse-free survival (GRFS) at 3 years after allogeneic stem cell transplantation. (A) GRFS for all the patients: 34% (95% CI, 26% to 44%); (B) GRFS according to donor type: 27% and 41% in patients allografted from matched sibling donors and unrelated donors, respectively ($P = .09$).

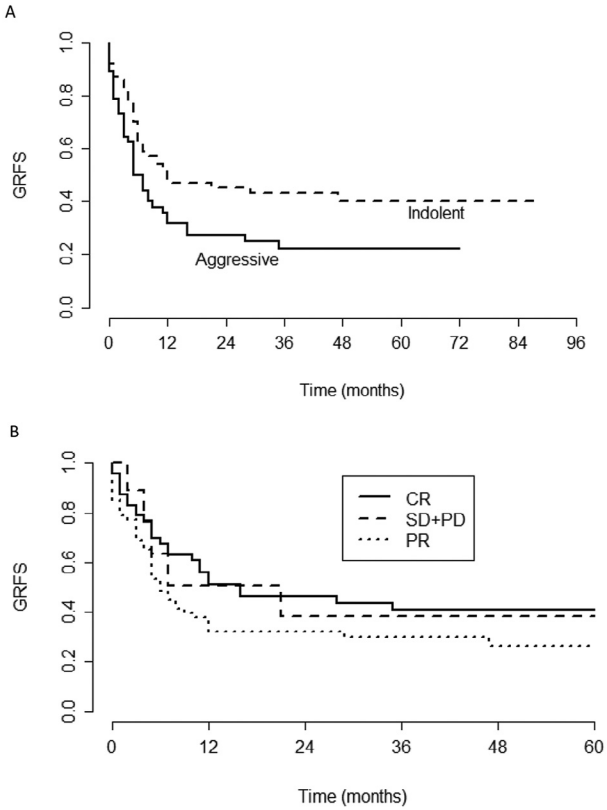

Figure 4. Adjusted Kaplan-Meier estimates of graft-versus-host disease-free and relapse-free survival (GRFS) at 3 years after allogeneic stem cell transplantation. (A) GRFS upon histotype: 43% indolent (95% CI, 32% to 58%) versus 22% aggressive lymphomas (95% CI, 13% to 38%), ($P = .02$); (B) GRFS upon pretransplantation disease status: 41% (95% CI, 28% to 59%) and 30% (95% CI, 20% to 44%) for patients in complete and partial remission, respectively ($P = .185$).

52% (95% CI, 28% to 71%) and 66% (95% CI, 39% to 83%) in MCL. In the 2 cohorts (indolent and aggressive), we evaluated the impact of different prognostic factors on PFS and OS: (1) time from diagnosis to alloSCT, (2) previous autoSCT; and (3) disease status at time of allograft (CR versus others). Neither of these factors influenced significantly PFS or OS. In the indolent subtype, patients in CR had a better PFS although the difference was not statistically significant (3 year PFS: 73%; 95% CI, 50% to 86% versus 52%; 95% CI, 35% to 67%; $P = .099$) but the status of disease did not influence OS. In the aggressive subtype, there was again a better survival for patients who were allografted while in CR (3 year PFS: 75%; 95% CI, 49% to 88% versus 43%; 95% CI, 23% to 61%; $P = .091$) but the difference was not statistically significant.

Univariate analysis for all the patients is shown in the Supplementary Methods (Table S2). The multivariate analysis for the PFS and OS showed that patients affected by an aggressive histotype had a worst outcome (PFS: HR, 3.30; $P = .003$; OS: HR, 3.73;$P = .007$). Bone marrow infiltration at time of transplantation increased the risk of disease relapse and death (PFS: HR, 4.78; $P < .001$; OS: HR, 6.00; $P < .001$). The occurrence of acute GVHD was associated with shorter OS (HR, 2.31; 95% CI, 1.20 to 4.44; $P = .012$).

For the entire cohort of patients, the 1-year and 3-year GRFS were 40% (95% CI, 32% to 50%) and 34% (95% CI, 26% to 44%), respectively (Figure 3A). The GRFS at 3 years was significantly better in patients with indolent as opposed to aggressive lymphomas (43%; 95% CI, 32% to 58% versus 22%; 95% CI, 13% to 38%; HR 1.69; $P = .02$), mainly because of a lower relapse risk (Figure 4). These figures remained unchanged at 5 years (data not shown). The 3-year GRFS was 41% and 30% in patients in CR and partial remission at the time of transplantation, respectively ($P = .075$). There was a trend for a better outcome in CR patients. The 3-year GRFS was 27% and 41% in patients allografted from matched sibling and unrelated donors, respectively ($P = .096$) (Figure 3B). Distribution of individual components of GRFS is given in Supplementary Methods (Figure S5 and S6). Main clinical characteristics were not different between patients receiving sibling or matched unrelated donor transplants; therefore, the difference in GRFS was caused by lower chronic GVHD requiring systemic therapy in patients allografted from unrelated donors. In multivariable model, the same factors affecting PFS and OS significantly influenced GRFS (histotype: HR, 2.02; $P = .026$; bone marrow infiltration: HR: 2.70; $P < .004$) (Table 3).

DISCUSSION

In this large, multicenter, prospective, phase II trial, we explored the effect of the inclusion of rituximab in a RIC regimen for B cell lymphomas. Our findings showed that: (1) 3-year NRM was low (21%); (2) PFS was not improved compared with our previous data without rituximab; (3) extensive chronic GVHD was low despite the high proportion of patients (45%) allografted from unrelated donors; (4) because

Table 3
Multivariable Analysis on PFS, OS, GRFS

Factor	PFS		OS		GRFS	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Age at AlloSCT						
46 versus 57*	1.63 (.93-2.85)	.234	1.70 (.91-3.18)	.22	1.62 (1.02-2.55)	.113
Histotype subgroup						
Aggressive versus Indolent	3.30 (1.49-7.30)	**.003**	3.73 (1.42-9.78)	**.007**	2.02 (1.09-3.77)	**.026**
Prior autoSCT						
Yes versus No	1.34 (.60-3.02)	.476	1.20 (.48-3.00)	.696	1.55 (.78-3.05)	.208
Bone marrow involvement						
Yes versus no	4.79 (2.12-10.82)	**<.001**	6.00 (2.10-14.15)	**<.001**	2.70 (1.37-5.32)	**.004**
Extranodal involvement						
Yes versus no	.87 (.40-1.90)	.728	1.07 (.45-2.53)	.884	1.54 (.82-2.89)	.177
Donor type						
Unrelated versus related	1.28 (.59-2.76)	.529	1.35 (.56-3.3.30)	.506	.79 (.41-1.51)	.472
Sex mismatched						
Female donor-male recipient versus other combinations	1.21 (.48-3.04)	.690	1.50 (.53-4.26)	.449	1.41 (.68-2.91)	.352

Bold typeface indicates statistical significance.
* Age was evaluated as continuous variable. The 2 values are, respectively, the third and first quartiles of the variable distribution.

of rituximab inclusion, there were no EBV-related post-transplantation lymphoproliferative disorders (PTLD); and (5) a lower incidence of chronic GVHD did not translate in a higher relapse rate.

In a previous trial, we showed that alloSCT is an effective option for relapsed/refractory lymphomas. We conducted a clinical trial in 170 patients with different B and T lymphoma subtypes allografted only from matched sibling donors with a RIC regimen and we obtained an encouraging NRM (14%) with PFS of 56%, but the trial was complicated by a CCI of 14% for severe acute GVHD (overall 35%) and 25% of chronic extensive form (overall 49%) [5]. Most of reports show that approximately 50% of patients can be free of progression after alloSCT. To improve PFS, we designed the present trial including rituximab in the conditioning regimen; however, we show that while there was no benefit in terms of disease control, there was an advantage in terms of chronic GVHD occurrence. In general, when chronic GVHD decreases, there is an increased relapse rate; interestingly, this was not the case with the addition of rituximab.

Despite several studies conducted over the last decade that showed a significant decrease in NRM after alloSCT with RIC regimens [5], further progress is needed to improve GVHD prophylaxis, as GVHD is a major determinant of morbidity and mortality. To improve the above reported results (using also unrelated donors), we performed the present trial, which included a single dose of rituximab (500 mg/m^2) administered during the conditioning. The rationale for the single pretransplantation rituximab administration relies on the assumption that circulating CD20$^+$ B cells of the recipient had already been depleted by rituximab-supplemented salvage chemo-immunotherapy, whereas pretransplantation rituximab will probably work largely by depleting the donor's alloreactive B cells (and thus also preventing EBV reactivation) in the first 3 months after transplantation. In the current study, the incidence of acute GVHD (21% and 22% in transplantations from related and unrelated donors, respectively) was lower than in our previous study without rituximab (35%), which included only patients allografted from HLA-matched siblings and who received the same conditioning regimen and GVHD prophylaxis [5]. The 3-year CCI of chronic GVHD was 31% versus 54% in patients allografted from unrelated and related donors, respectively. This finding is clinically relevant and could be related to the high-dose ATG used or to a potential synergistic effect of rituximab and ATG. This result substantially contributes in generating a 41% GRFS in patients allografted from unrelated donors. The dose of ATG in our trial was not associated with unacceptable risk of infection and derived from a previous Italian trial [23] that evaluated 2 different GVHD prophylaxis strategies (ATG at 7.5 mg/kg versus alemtuzumab) in patients who received a graft from unrelated donors (8/8 or 7/8 [a single allele mismatch was allowed]). For all patients, the cumulative incidence of acute and chronic GVHD were 44% and 25%, respectively.

The impact of rituximab on B cell depletion has been previously evaluated in different trials with conflicting results. First, the dose, timing, and duration of antibody administration was variable. Second, these studies are not easily comparable to each other not only for the type of patients enrolled, but also for differences in the conditioning regimens as well as in the type and dose of the drugs used for GVHD prophylaxis (eg, ATG) [14,15]. Khouri et al. [14] were the first to explore the administration of high-dose rituximab (before and after transplantation) and reported results with a mature follow-up. They showed an impressive low incidence (10%) of grade III and IV acute GVHD and of extensive chronic GVHD (36%), which appeared to be not only related to patient selection (mainly matched sibling donors) but also to antibody administration. In that study, rituximab-naive patients were included. In a more recent paper [24], the same authors explored a new reduced-conditioning regimen (bendamustine, fludarabine, and rituximab), but they also introduced also a low-dose ATG for recipients of unrelated grafts. The 2-year cumulative incidence of chronic GVHD was 26% in patients who received a graft from unrelated donors, which is comparable to our results. Recently, Laport et al. [25] performed a prospective trial using the fludarabine, cyclophosphamide and rituximab regimen in a population of 65 patients who received grafts from matched sibling and unrelated donors. The authors did not use ATG and the cumulative incidence of chronic GVHD for unrelated recipients was 66%. Despite that, the study was interesting because they found associations between higher rituximab serum concentration with better outcome and lower serum concentration with severe acute GVHD. Cutler et al. [15] explored different rituximab infusions after day 100, demonstrating a 30% incidence of chronic GVHD requiring systemic corticosteroids (lower than 48% observed in the historical control) in patients who received transplants from related and unrelated donors.

In contrast to the above studies, Glass et al. [16] evaluated the effect of post-transplantation rituximab in a randomized trial and did not observe any significant effect on either acute or chronic GVHD. However, the major limitation of this prospective trial was the use of ATG in a minority of patients who received grafts from unrelated donors and the discontinuation of rituximab in a substantial number of patients (61%) allocated to the rituximab group.

Recent randomized trials, performed mainly in myeloid malignancies, strongly supported the use of ATG as GVHD prophylaxis in patients receiving alloSCT not only from unrelated donors [26] but also from HLA-identical siblings [27]. The benefit was mainly related to a reduction of the severe forms of chronic GVHD.

EBV reactivation is common (33%) after allogeneic transplantation from unrelated donors when the GVHD prophylaxis includes ATG, as recently reported also in the paper of Walker et al. [26]. High levels of EBV DNAemia increase the risk of PTLD. The use of prophylactic rituximab (200 mg on day +5) has been explored in a retrospective study [28] in recipients of transplants from alternative donor (the GVHD prophylaxis included rabbit ATG at dose of 6 mg/kg to 10 mg/kg) and was associated with a lower rate of EBV DNAemia and the absence of cases of PTLD, as compared with the control group. In our study, the administration of rituximab efficiently prevented EBV reactivation and PTLD.

Our trial expands the knowledge about the role of transplantation in relapsed/refractory aggressive lymphomas. In fact, we demonstrated that at 3 years, 54% of patients failing an autoSCT and 43% of patients not in CR are alive. These data compare favorably with the results reported by Glass et al. [16] in patients with refractory disease at time of alloSCT (estimated 3-year OS of 38%) and with those described by T. Fenske et al. [29] in retrospective study enrolling patients progressing after autoSCT (estimated 3-year OS of 37%).

In recent years, a number of novel agents for lymphomas became available. Major advances occurred in relapsed/refractory CLL, MCL, and FL using the Bruton tyrosine kinase inhibitors (ibrutinib); the inhibitor of BCL-2, venetoclax; and phosphatidylinositol-3-kinase delta inhibitor, idelalisib [30-33]. All of these studies have intrinsic limitations mainly because of the very short follow-up. Further, long-term efficacy and safety data are lacking. The question of which strategy (RIC alloSCT or new drugs) is better for the treatment of transplantation-eligible patients is now open and appropriately designed prospective clinical trials will be required to challenge the 34% of patients who are alive and free of any complication after alloSCT.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2017.03.031.

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