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Sequential Intensified Conditioning Regimen Allogeneic Hematopoietic Stem Cell Transplantation in Adult Patients with Intermediate- or High-Risk Acute Myeloid Leukemia in Complete Remission: A Study from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation

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A B S T R A C T

Post-transplant relapse is the leading cause of treatment failure in acute myeloid leukemia (AML) patients after reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation (allo-HSCT). To improve their outcome, we evaluated the outcome of a sequential intermediate-intensity conditioning regimen combining fludarabine, cytosine arabinoside, amsacrine, cyclophosphamide, and either total body irradiation or busulfan (FLAMSA) in patients with intermediate or high-risk AML in first or second complete remission (CR). A total of 265 patients (median age, 55 years; range, 19 to 76) with AML who underwent allo-HSCT using a FLAMSA regimen were included. At the time of transplant, 216 (81.5%) were in CR1 and 49 (18.5%) in CR2. Cytogenetic was intermediate in 114 (43%) and poor in 42 (15.8%) patients, whereas 109 patients (41.1%) had a secondary AML. With a median follow-up of 46 months (range, 1 to 145), the Kaplan-Meier estimate of overall and leukemia-free survival at 2 years were 56.1% (95% CI, 49.7% to 62.6%) and 52.8% (95% CI, 46.4% to 59.2%), respectively. At 2 years, the cumulative incidences of relapse and nonrelapse mortality were 22.8% (95% CI, 17.6% to 28.4%) and 24.0% (95% CI, 18.8% to 29.5%), respectively. In multivariate analysis, patient age and cytogenetics were the only parameters with a significant impact on overall survival. These data suggest that the FLAMSA sequential intermediate conditioning regimen provides an efficient disease control in intermediate- and high-risk AML patients, including those in CR2 and with secondary AML.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective postremission consolidation treatment, potentially curative, in acute myeloid leukemia (AML) patients [1,2]. Reduced-intensity conditioning (RIC) regimens

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have been developed to control or overcome toxicity and nonrelapse mortality (NRM) associated with allo-HSCT [2]. RIC regimens rely on the graft-versus-leukemia effect mediated by the graft's immune cells [3]. RIC allo-HSCT is now widely used for AML patients with intermediate- or high-risk cytogenetics, particularly in older or heavily pretreated patients and in those with medical comorbidities [2].

Although a significant proportion of patients are cured after RIC allo-HSCT, relapse after transplant is still the leading cause of treatment failure in the RIC setting. In patients transplanted in complete remission (CR), AML cytogenetic status and prior myelodysplastic syndrome or cytotoxic therapy are strong predictors of relapse. Therefore, the effectiveness of different intermediate-intensity conditioning regimens to enhance graft-versus-leukemia while safely minimizing NRM has been evaluated [2,4,5]. One such strategy is the so-called sequential conditioning regimen that combines a short course of intensive chemotherapy followed by a RIC allograft. Thus, the Munich group developed the FLAMSA sequential strategy combining a short course of intensive chemotherapy to improve disease control using fludarabine 30 mg/m^2/day, intermediate-dose cytosine arabinoside 2 g/m^2/day, and amsacrine 100 mg/m^2/day from day −12 to −9, followed, after a 3-day rest, by RIC using 4 Gy total body irradiation (TBI) on day −5, cyclophosphamide 40 to 60 mg/kg/day on days −4 and −3, and antithymocyte globulin (ATG) from days −4 to −2. This strategy has shown encouraging results in relapsed or refractory AML patients [6,7]. In addition, Schmid et al. [8] reported an effective disease control and a low NRM with this strategy in 23 patients with high-risk AML in CR. Thereafter, 4 Gy TBI has been replaced by i.v. busulfan (Bu) 6.4 mg/kg total dose (or equivalent oral dose) to decrease the toxicity associated with TBI in elderly patients or in patients with severe comorbidities [9,10].

Larger studies are needed to evaluate the role of TBI or Bu-based FLAMSA sequential regimen in patients with AML in CR. We report here on 265 patients with AML in first (CR1) or second CR (CR2) in which a FLAMSA sequential allo-HSCT, TBI, and Bu-based FLAMSA are compared. In addition, the contribution of prophylactic donor lymphocyte infusion (DLI) was assessed in the subgroup of patients who were alive and free of disease at 6 months.

METHODS

Study Design and Data Collection

This retrospective multicenter analysis was performed and approved by the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation registry. The European Group for Blood and Marrow Transplantation is a voluntary working group of more than 500 transplant centers; all centers are required to report annually all stem cell transplantations and follow-up. Use of patients' personal information for research purposes is authorized through the signature of an informed consent by the patients. This study included all adult patients (age > 18 years) with AML in first or second morphologic CR who underwent a bone marrow or granulocyte colony-stimulating factor–mobilized peripheral blood stem cell allo-HSCT from an HLA matched related donor or unrelated donor between 2002 and 2014. In addition, to be eligible patients must have had available cytogenetics data or secondary AML and to have received a so-called sequential conditioning regimen. The latter was defined by the use of a short intensive course of chemotherapy combining fludarabine, intermediate-dose cytosine arabinoside, and amsacrine followed after a 3-day rest by a RIC regimen combining cyclophosphamide and either TBI 4 Gy or i.v. Bu 6.4 mg/kg total dose (or equivalent oral dose of Bu).

Cytogenetics was classified according to the European Leukemia Net [11]. All allogeneic grafts were obtained from HLA-A, -B, -C, -DR, and -DQ matched donors. A single HLA mismatch of 10 was allowed at the antigen or allele level. A list of the participating centers is available online (see supplementary data online).

Statistical Analysis

Endpoints included overall survival (OS), leukemia-free survival (LFS), cumulative incidence of relapse, NRM, and acute and chronic graft-versus-host disease (aGVHD and cGVHD, respectively). All outcomes were measured from the time of allo-HSCT. OS was based on death, regardless of the cause. LFS was defined as survival with no evidence of relapse. NRM was defined as death in CR. Patients alive without relapse were censored at the time of last contact.

OS and LFS rates were calculated by the Kaplan-Meier estimator. Cumulative incidence functions were used to estimate the probabilities of aGVHD and cGVHD, NRM, and relapse to accommodate competing risks. NRM and relapse were the competing risks. For aGVHD and cGVHD, the competing risk was death without the event. For all prognostic analyses, median patient age and median year of transplant were used as a cut-off point.

Univariate analyses were performed using the log-rank test for OS and LFS and Gray's test for cumulative incidences. cGVHD was analyzed as a time-dependent variable. For multivariate regression a Cox proportional hazards model was build. Results were expressed as hazard ratios (HR) with 95% confidence intervals (CI). All tests were 2-sided, and the Type I error rate was fixed at .05. A landmark analysis was conducted 6 months after allo-HSCT on patients alive and free of disease to evaluate the impact of pre-emptive DLIs within the first 6 months on outcome. Patients developing grades II to IV aGVHD or cGVHD before DLI (group DLI) or within the first 6 months (group no DLI) were excluded from the landmark analysis. Statistical analyses were performed with SPSS version 19 (SPSS Inc./IBM, Armonk, NY) and R 3.0.1 (R Development Core Team, Vienna, Austria) software packages.

RESULTS

Patient and Donor Characteristics

A total of 265 patients were included in this study. Patient and donor characteristics are summarized in Table 1. The median age of recipients was 55 years (range, 19 to 76). At transplantation, 216 patients (81.5%) were in CR1 and 49 (18.5%) in CR2. The median time between AML diagnosis and transplantation was 135 days (range, 43 to 225) in patients with AML in CR1 and 627 days (range, 135 to 1701) for those in CR2.

One hundred nine patients (41.1%) had a secondary AML and 156 (58.9%) had a de novo AML, including 114 (43.0%) with intermediate-risk and 42 (15.8%) with high-risk cytogenetics. Of note, no patient had low-risk cytogenetic among de novo AML. Seventy-four donors (27.9%) were matched related and 191 (72.1%) were unrelated . The stem cell source was bone marrow in 14 cases (5.3%) and granulocyte colony-stimulating factor–mobilized peripheral blood stem cells in the remaining 251 (94.7%). All patients except for 7 received in vivo T cell depletion using ATG. The ATG used was Thymoglobulin (Genzyme, Lyon, France) in 102 patients (median total dose, 6 mg/kg; interquartile range, 5 to 7) and ATG Fresenius (Fresenius Biotech GmbH, Munich, Germany) in 129 patients (median total dose, 60 mg/kg; interquartile range, 30 to 60); ATG administered was unknown in 2 patients.

One hundred fifty-nine patients (60%) were treated with a TBI-based (TBI group) and 106 (40%) with a modified Bu-based FLAMSA regimen (Bu group, 96 i.v. Bu and 10 oral Bu). The comparison between the TBI and Bu groups is shown on Table 1. Compared with the TBI group, patients in the Bu group were significantly older (61 years [range, 25 to 74] versus 52 years [range, 19 to 76]; $P < .0001$), were transplanted more recently (2011 [range, 2005 to 2014] versus 2009 [range, 2002 to 2014]; $P < .0001$), and included more secondary AML (59.4% versus 28.9%; $P < .0001$). The median follow-up among surviving patients was 46 months (range, 1 to 145) and was significantly longer in the TBI group (50 months; range, 1 to 145) compared with that in the Bu group (27 months; range, 3 to 106) ($P = .006$).

Engraftment and GVHD

Engraftment was successful in 153 patients (96.2%) in the TBI and 101 (95.3%) in the Bu group, respectively ($P = .56$).

Table 1
Study Population and Transplant Characteristics

Characteristic	Total (n = 265)	FLAMSA TBI 4 Gy (n = 159)	FLAMSA Bu (n = 106)	*P*
Median patient age, yr (range)	55 (19-76)	52 (19-76)	61 (25-74)	<.0001
Patients < 55 yr	132 (49.6%)	104 (65.4%)	28 (26.4%)	
Patients ≥ 55 yr	133 (50.4%)	55 (34.6%)	78 (73.6%)	
Median year of transplant (range)	2010 (2002-2014)	2009 (2002-2014)	2011 (2005-2014)	<.0001
Patient gender (female)	122 (46.2%)	82 (51.9%)	40 (37.7%)	.02
Donor gender (female)	80 (30.5%)	52 (33.3%)	28 (26.4%)	.23
Female donor to male patient	26 (10.0%)	16 (10.3%)	10 (9.4%)	.81
Karnofsky performance status				
≥90%	179 (70%)	107 (71%)	72 (70%)	.82
80%	65 (26%)	39 (26%)	26 (25%)	
≤70%	10 (4%)	5 (3%)	5 (5%)	
Unknown	11	8	3	
Donor CMV negative to patient CMV positive	51 (19.6%)	34 (21.8%)	17 (16.0%)	.41
Disease status				
CR1	216 (81.5%)	127 (79.9%)	89 (84.0%)	.40
CR2	49 (18.5%)	32 (20.1%)	17 (16.0%)	
Median time from diagnosis to transplant				
CR1 patients, days (range)	135 (43-225)	130 (43-948)	154 (43-827)	.02
CR2 patients, days (range)	627 (135-1701)	631 (135-1508)	547 (166-1701)	.36
Cytogenetic risk				
Intermediate	114 (43.0%)	82 (51.6%)	32 (30.2%)	<.0001
Poor	42 (15.8%)	31 (19.5%)	11 (10.4%)	
Secondary AML	109 (41.1%)	46 (28.9%)	63 (59.4%)	
Donor type				
Matched related donor	74 (27.9%)	50 (31.4%)	24 (22.6%)	.12
Unrelated donor*	191 (72.1%)	109 (68.6%)	82 (77.4%)	
Stem cell source				
BM	14 (5.3%)	10 (6.3%)	4 (3.8%)	.37
PBSC	251 (94.7%)	149 (93.7%)	102 (96.2%)	
In vivo T cell depletion				
No	7 (2.6%)	5 (3.1%)	2 (1.9%)	.53
Yes	258 (97.4%)	154 (96.9%)	104 (98.1%)	
GVHD prophylaxis				
CsA + MMF	216 (81.8%)	129 (81.6%)	87 (82.1%)	.93
Others	48 (18.2%)	29 (18.4%)	19 (17.9%)	

CMV indicates cytomegalovirus; BM, bone marrow; PBSC, peripheral blood stem cells; CsA, cyclosporine A; MMF, mycophenolate mofetil.
* Forty-nine patients received a mismatched unrelated donor: 27 in the TBI group and 22 in the Bu group.

The median time to neutrophil recovery was significantly longer in the TBI group: 17 days (range, 10 to 74) compared with 14 days (range, 8 to 112) in the Bu group ($P < .0001$). The day-30 cumulative incidence of absolute neutrophil count $> .5 \times 10^9$/L was 93.9% (95% CI, 90.1% to 96.2%).

The day 100 cumulative incidence of grades II to IV aGVHD was 28.5% (95% CI, 23.1% to 34.1%), being 30.3% (95% CI, 23,2% to 37.7%) in the TBI group and 25.7% (95% CI, 17.6% to 34.6%) in the Bu group ($P = .45$). At 2 years, the cumulative incidence of cGVHD was 31.8% (95% CI, 25.9% to 37.9%), being 33.5% (95% CI, 25.7% to 41.3%) in the TBI group versus 29.1% (95% CI, 20% to 38.8%) in the Bu group ($P = .79$).

Outcome

Univariate and multivariate analyses of transplantation-related events are summarized in Tables 2 and 3, respectively. At 2 years, the cumulative incidence of NRM was 24.0% (95% CI, 18.8% to 29.5%) (Figure 1A), being 19.4% (95% CI, 13.5% to 26.2%) in the TBI group and 31.1% (95% CI, 24.0% to 38.4%) in the Bu group ($P = .02$). In multivariate analysis, there was no significant difference in NRM between the TBI and the Bu groups (HR, 1.11; 95% CI, .62 to 2.01; $P = .72$). NRM was related mainly to infection (n = 31) and GVHD (n = 19), others causes being hemorrhage (n = 5), sinusoidal obstruction syndrome (n = 2), cardiac toxicity (n = 2), secondary malignancy (n = 1), others (n = 9), and unknown (n = 9). At 2 years, the cumulative incidence of relapse was 22.8% (95% CI, 17.6% to 28.4%) (Figure 1B), with 21.2% (95% CI, 50.7% to 66.8%) in the TBI and 25.7% (95% CI, 32.8% to 53.7%) in the Bu group ($P = .77$). In multivariate analysis, there was no significant difference in relapse incidence between the 2 groups (HR, 1.27; 95% CI, .68 to 2.37; $P = .4$). The only parameter with a significant impact on relapse incidence in multivariate analysis was cytogenetic status: Relapse was significantly increased in patients with poor as compared with intermediate cytogenetics (HR, 1.96; 95% CI, 1.03 to 3.72; $P = .04$).

The Kaplan-Meier estimate of OS at 2 years was 56.1% (95% CI, 49.7% to 62.6%) (Figure 1C), being 62.0% (95% CI, 54.0% to 70.0%) in the TBI and 46.7% (95%CI, 36.1% to 57.3%) in the Bu group ($P = .14$). In multivariate analysis, there was no significant difference in OS between the TBI group and the Bu group (HR, 1.09; 95% CI, .70 to 1.69; $P = .70$). The only parameters with a significant impact on OS in multivariate analysis were cytogenetic status and patient age. OS was significantly lower in patients with poor as compared with intermediate cytogenetics (HR, 1.26; 95% CI, 1.06 to 2.92; $P = .03$) and in older patients (HR, 1.21; 95% CI, 1.01 to 1.45; $P = .04$). The Kaplan-Meier estimate of LFS at 2 years was 52.8% (95% CI, 46.4% to 59.2%) (Figure 1D): 58.8% (95% CI, 50.7% to 66.8%) in the TBI group and 43.2% (95% CI, 32.8% to 53.7%) in the Bu group ($P = .14$). In multivariate analysis, there was no significant difference in LFS between the TBI group and the Bu group (HR, 1.16; 95% CI, .76 to 1.78; $P = .48$). No parameter had a significant impact on LFS in multivariate analysis.

Table 2
Transplant-Related Events: Univariate Analysis

	NRM	RI	OS	LFS
Conditioning				
TBI group	19.4% (13.5-26.2)	21.2% (15-28.2)	62% (54-70)	58.8% (50.7-66.8)
Bu group	31.1% (24-38.4)	25.7% (17-35.2)	46.7% (36.1-57.3)	43.2% (32.8-53.7)
P	**.02**	.77	.14	.14
Patient age				
<55 yr	17.8% (11.5-25.3)	23.9% (16.4-32.1)	62.2% (53.2-71.2)	57.9% (48.8-67.1)
≥ 55 yr	29.7% (21.8-38)	21.8% (14.9-29.5)	50.6% (41.6-59.7)	48.1% (39.2-57)
P	**.008**	.90	**.005**	**.02**
Status at transplant				
CR1	23.2% (17.6-29.4)	23% (17.3-29.3)	57.5% (50.4-64.6)	53.2% (46.1-60.4)
CR2	27.1% (21.1-33.5)	22% (11.2-35.2)	50.6% (36.1-65.2)	50.8% (36.3-65.3)
P	.68	.70	.98	.99
Patient gender				
Male	28.2% (20.8-36.1)	23.8% (16.8-31.4)	49.4% (40.7-58.1)	47.6% (38.9-56.2)
Female	18% (12-25.1)	21.7% (14.4-30.1)	65.4% (56.2-74.5)	59.8% (50.3-69.3)
P	.052	.54	**.049**	.10
Donor gender				
Male	22.9% (16.9-29.5)	23.7% (17.5-30.5)	55.1% (47.4-62.8)	53.4% (45.7-61.1)
Female	27.5% (21-34.3)	21.6% (12.7-32)	56.6% (44.7-68.4)	49.4% (37.5-61.4)
P	.22	.58	.39	.48
Female to male				
Yes	23.6% (18.1-29.5)	23% (17.5-29)	56% (49.1-62.8)	53.2% (46.3-60)
No	27.7% (21.9-33.7)	24% (9.4-42.2)	54.5% (34.6-74.4)	46.2% (26.3-66.1)
P	.32	.95	.28	.34
Donor				
MRD	13.8% (7-22.8)	30.6% (20.3-41.5)	62.5% (51-73.9)	54.4% (42.7-66.1)
UD	28.4% (18.5-39.1)	19.5% (13.8-26)	53.5% (45.8-61.2)	52.1% (44.4-59.8)
P	**.04**	**.047**	.54	.94
Year				
<2010	13.9% (8.3-20.9)	23.5% (16.2-31.6)	66.3% (57.6-75)	61.9% (53-70.9)
≥2010	33.3% (25-41.8)	23.3% (16-31.6)	46.3% (37.1-55.5)	43.4% (34.2-52.5)
P	**.0008**	.61	**.003**	**.02**
Cytogenetics				
Intermediate	20.6% (12.6-30)	21.7% (14.5-29.8)	61.2% (52-70.4)	56.9% (47.5-66.2)
Poor	19.6% (11.8-28.9)	35.2% (20.6-50.2)	47.3% (31.6-62.9)	45.2% (29.6-60.7)
Secondary AML	30.3% (20.9-40.3)	19.1% (11.4-28.3)	53.7% (42.9-64.5)	50.6% (39.8-61.4)
P	.10	.08	.46	.69

Bold denotes statistical significance. Values in parenthesis represent 95% CI.
RI indicates relapse incidence; MRD, matched related donor; UD, unrelated donor.

Prophylactic DLI

Ninety-six patients alive and disease free at 6 months and without a history of grades II to IV aGVHD or cGVHD before DLI were eligible for the landmark analysis. Of these, 21 received preemptive DLIs within the first 6 months, based on physician decision, whereas 75 did not. The outcome was significantly improved in the former group of patients. The 2-year rates of LFS were 95% (95% CI, 86% to 100%) in the DLI group versus 76% (95% CI, 67% to 86%) in the no DLI group ($P = .03$), and the 2-year rates of OS were 100% (95% CI, 100% to 100%) in the DLI group versus 81% (95% CI, 72% to 91%) in the no DLI group ($P = .10$). The cumulative incidence of NRM and relapse were 0% and 5% (95% CI, 0% to 20%), respectively, in the DLI group versus 4% (95% CI, 1% to 11%) and 19% (95% CI, 11% to 29%), respectively, in the no DLI group ($P = .18$ and .11 respectively). The 2-year cumulative incidence of cGVHD was significantly higher in the DLI group, 26% (95% CI, 9% to 46%) versus 15% (95% CI, 8% to 24%) in the no DLI group ($P = .48$).

DISCUSSION

Intermediate-intensity conditioning regimens have been developed to decrease disease recurrence while minimizing NRM after a RIC regimen. This retrospective study is the largest so far to evaluate the so-called FLAMSA sequential intermediate-intensity conditioning regimen in AML patients in CR.

The risk of relapse was notably high in our patients: 57% had an unfavorable karyotype or a secondary AML, and 18.5% were in CR2. Notably, the cumulative incidences of relapse and LFS were 22.8% and 52.8%, respectively. These results compare favorably with previous studies evaluating RIC regimens for AML, with relapse incidences up to 41% and LFS below 50% [12-17] but with the results of myeloablative conditioning regimens showing relapse incidences ranging from 24% to 29% [12,16]. Our results are also comparable with those of other intermediate conditioning regimens, combining fludarabine, ATG, and 3 days of Bu [4], associated with a cumulative incidence of relapse of 29.1% and an LFS of 57% [5]. Overall, this regimen is associated with a good disease control in intermediate- and high-risk AML compared with that achieved in previous studies.

In patients with a median age of 55 years and aged up to 76 years, we reported a 2-year cumulative incidence of NRM of 24%. This may seem high for a RIC regimen. Russel et al. [16] reported a reduced NRM of 6% after RIC compared with 22% after myeloablative conditioning in nonfavorable AML in CR1; however, the RIC regimen was nonmyeloablative with low-dose TBI in most patients, leading to a cumulative incidence of relapse of 36% after RIC transplant, compared with only 22.8% in our study. Scott et al. [18] recently reported the preliminary results of the BMT CTN 0901 randomized protocol comparing myeloablative conditioning versus RIC in AML and myelodysplastic syndrome in CR. NRM was significantly lower after RIC allo-SCT (4.4% versus 15.8% after myeloablative conditioning), whereas corresponding relapse rates were 48.3% versus 13.5%. NRM rates are lower in this

Table 3
Transplant-Related Events: Multivariate Analysis

Outcome	HR (95% CI)	*P*
NRM		
Bu versus TBI-based conditioning	1.11 (.62-2.01)	.72
Age at transplant (per 10 years)	1.18 (.93-1.50)	.18
Status at transplant (CR2 vs. CR1)	1.31 (.69-2.50)	.41
Unrelated donor vs. MRD	1.77 (.92-3.39)	.09
Cytogenetic		
Poor vs. intermediate	1.07 (.47-2.41)	.87
Secondary AML vs. intermediate	1.32 (.73-2.39)	.35
Patient gender (female vs. male)	.68 (.39-1.19)	.18
Female donor to male patient vs. others	1.45 (.65-3.26)	.37
Year of transplant	1.11 (.99-1.24)	.07
Relapse incidence		
Bu vs. TBI based conditioning	1.27 (.68-2.37)	.44
Age at transplant (per 10 years)	1.23 (.96-1.58)	.11
Status at transplant (CR2 vs. CR1)	1.24 (.62-2.48)	.54
Unrelated donor vs. MRD	.62 (.36-1.07)	.08
Cytogenetic		
Poor vs. intermediate	**1.96 (1.03-3.72)**	**.04**
Secondary AML vs. intermediate	.89 (.48-1.68)	.73
Patient gender (female vs. male)	.82 (.48-1.41)	.48
Female donor to male patient	.81 (.34-1.95)	.64
Year of transplant	.98 (.87-1.09)	.69
OS		
Bu vs. TBI based conditioning	1.09 (.70-1.69)	.70
Age at transplant (per 10 years)	**1.21 (1.01-1.45)**	**.04**
Status at transplant (CR2 vs. CR1)	1.35 (.83-2.19)	.23
Unrelated donor vs. MRD	1.11 (.73-1.69)	.63
Cytogenetic		
Poor vs. intermediate	**1.76 (1.06-2.92)**	**.03**
Secondary AML vs. intermediate	1.17 (.75-1.84)	.49
Patient gender (female vs. male)	.71 (.48-1.07)	.10
Female donor to male patient vs. others	1.15 (.63-2.10)	.65
Year of transplant	1.08 (.99-1.18)	.07
LFS		
Bu vs. TBI based conditioning	1.16 (.76-1.78)	.48
Age at transplant (per 10 years)	1.19 (1.00-1.41)	.05
Status at transplant (CR2 vs. CR1)	1.23 (.77-1.98)	.39
Unrelated donor vs. MRD	.98 (.66-1.47)	.94
Cytogenetic		
Poor vs. intermediate	1.50 (.92-2.47)	.11
Secondary AML vs. intermediate	1.09 (.71-1.68)	.68
Patient gender (female vs. male)	.76 (.52-1.11)	.16
Female donor to male patient vs. others	1.10 (.61-1.99)	.76
Year of transplant	1.04 (.97-1.13)	.28

Bold denotes statistical significance.

study, including in the myeloablative conditioning group, consisting of a majority of reduced toxicity regimens combining fludarabine and Bu. However, this study includes only selected patients up to 65 years, whereas in our cohort patients up to age 76 years were treated. Therefore, our NRM is comparable with the NRM reported in some of the largest studies on RIC regimen for AML [12,13]. Regarding conventional myeloablative conditioning regimen, similar rate of NRM have been reported in prospective studies: 22% in the study by Russel et al. [16] and 25.7% in the study by Lee et al [19]. However, in these prospective studies, patients were selected and younger with a median age of 42 and 41 years old, respectively, compared with a median age of 55 years in our study. Therefore, our NRM rate compared favorably with traditional myeloablative regimens. Previous reports evaluating a FLAMSA sequential regimen reported a 2-year NRM rate of 22% both in relapse/refractory AML [7] and in AML in CR [8]. Of note, we reported only 2 deaths related to cardiac toxicity, a known side effect of amsacrine [20], and there was no increase in the mortality related to sinusoidal obstruction syndrome, with only 2 deaths reported. Taken together, the low cumulative incidence of relapse and the NRM led to an OS rate of 56.1% at 2 years, which compared favorably with result of the previously cited studies evaluating both RIC (OS ranged from 36% to 55%) [12-16] and myeloablative regimens (OS ranged from 57% to 53%) [12,16].

The patients' outcome was similar using either the TBI or the Bu-based FLAMSA regimen. Although NRM was higher in patients receiving Bu in univariate analysis, after adjustment for patient age, among others, there was no difference in NRM between the 2 conditioning regimens in multivariate analysis. Given TBI is a the major risk factor of long-term complications [21] and the favorable safety profile associated with the use of i.v. Bu [22], i.v. Bu appears as an effective alternative to TBI in the FLAMSA sequential approach.

Attention must be paid to elderly patients when using a FLAMSA sequential regimen. Although McClune et al. [23] reported no impact of patient age on the outcome after RIC regimen allo-SCT, older age at transplant was associated with a significantly lower OS in our multivariate analysis. This difference seems to be related to an increased NRM in elderly patients; however, given the retrospective nature of this analysis, we were not able to identify the exact nature of those deaths. Ultimately, careful screening of comorbidities must be performed in elderly patients before using intermediate-intensity conditioning such as the FLAMSA sequential regimen.

In multivariate analysis, poor-risk cytogenetic status was associated with a significant increase in relapse and a decrease in OS. Therefore, despite the increased cytotoxicity, the FLAMSA sequential regimen does not overcome the bad prognosis of poor-risk cytogenetic. Development of a new strategy to decrease relapse risk in those patients remains indispensable. In our study, some patients received prophylactic DLIs based on physician decision, and although we recognize that given the retrospective nature of our study the exact reason guiding the decision to give prophylactic DLI is unknown, use of prophylactic DLI seems to be a valuable option to decrease relapse risk. Indeed, in a landmark analysis of patients alive and disease-free at 6 months, LFS was significantly improved in patients who received prophylactic DLIs compared with those who did not. Furthermore, other strategies, such as early administration of the hypomethylating agent azacytidine or of FLT3-specific tyrosine kinase inhibitor, seem promising [24,25].

Given its retrospective nature, our study does have several obvious biases. Molecular marker and minimal residual disease before transplant were not available for all patients, precluding the evaluation of their prognostic value. Because it was not a prospective study, the choice of the allocation to the FLAMSA regimen was based on physician preference, and we cannot exclude a bias in patient selection. However, the homogeneity of the data in terms of disease and transplant characteristics strengthen our study and make the conclusion more robust.

Overall, our results suggest that the sequential FLAMSA conditioning regimen using either TBI or Bu may be a valid approach for intermediate- or high-risk AML patients transplanted in CR. This regimen is associated with a low incidence of relapse, although disease progression is still expected in patients with high-risk cytogenetic. However, elderly patients have an increased risk of NRM, and particular attention should be paid to comorbidities, supportive care, and dose of TBI or Bu used in these patients. Our results pave the way for future studies that should compare such sequential approaches with standard approaches and include minimal residual monitoring to decipher the exact impact of the chemotherapy included in our sequential regimen before the RIC

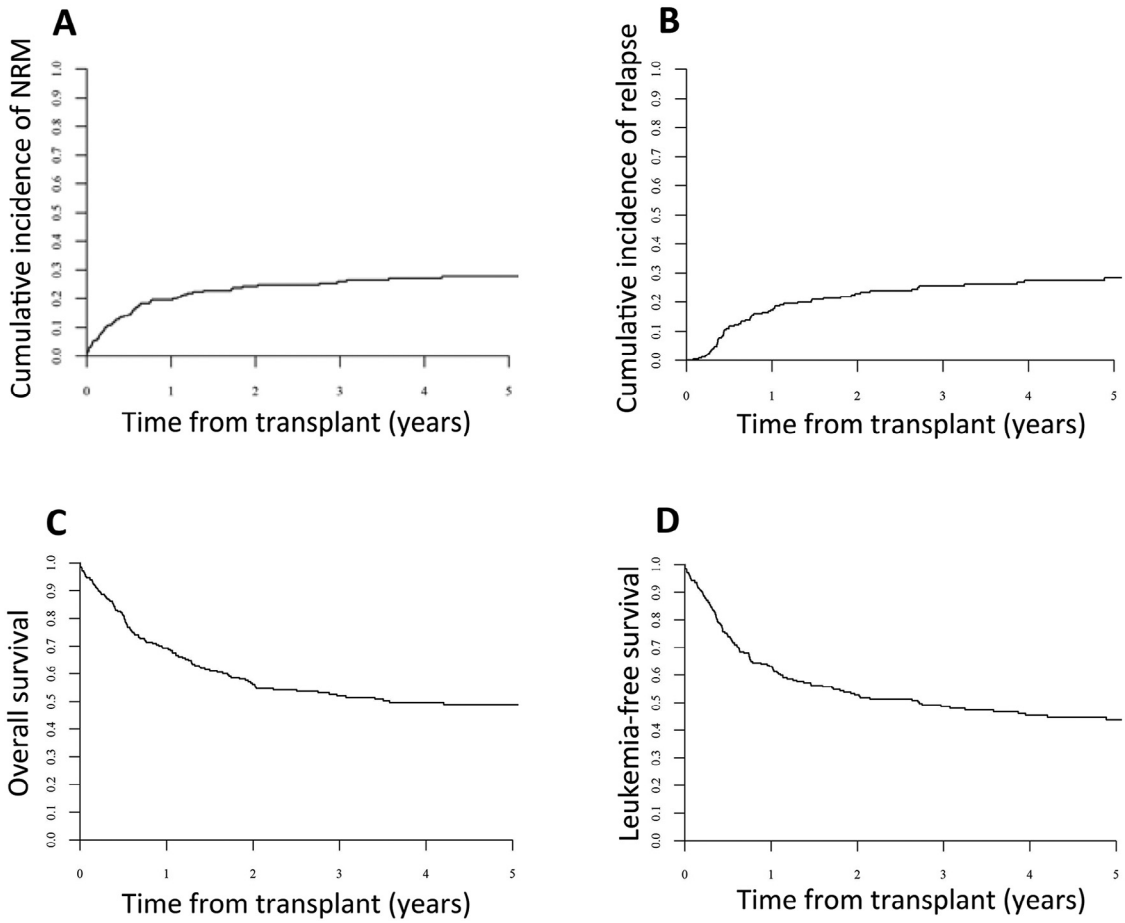

Figure 1. Outcome after allo-HSCT. (A) Cumulative incidence of NRM. (B) Cumulative incidence of relapse. (C) OS. (D) LFS.

allo-SCT. Overall, our study provides a framework for further refinement of intermediate-intensity conditioning designed to improve disease control without increasing toxicity in AML in CR.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2016.11.002.

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