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Reduced Relapse Incidence with FLAMSA–RIC Compared with Busulfan/Fludarabine for Acute Myelogenous Leukemia Patients in First or Second Complete Remission: A Study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation

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A B S T R A C T

Busulfan/fludarabine (BuFlu) is a widely used conditioning regimen for patients with myeloid malignancies. The sequential FLAMSA (fludarabine + Ara-C + amsacrine chemotherapy) protocol followed by either cyclophosphamide and total body irradiation (FLAMSA-TBI) or cyclophosphamide and busulfan (FLAMSA-Bu) has shown remarkable activity in high-risk acute myelogenous leukemia (AML) patients. Here we compare the outcomes of AML patients transplanted in first complete remission (CR1) or second complete remission (CR2) after conditioning with BuFlu or FLAMSA. Eligible patients had their first allogeneic stem cell transplantation for AML in CR1 or CR2 between January 2005 and June 2016. Donors were matched related or unrelated with up to 1 mismatch. Conditioning consisted of either BuFlu or FLAMSA. Propensity score matching was applied and comparisons were performed using weighted Cox regression. BuFlu conditioning was used in 1197 patients, whereas FLAMSA-TBI and FLAMSA-Bu were used in 258 and 141 patients, respectively. Median follow-up of survivors was 24.72 months. In univariate analysis, relapse incidence (RI) was 30.3%, 21.9%, and 23.1% in the BuFlu, FLAMSA-TBI, and FLAMSA-Bu groups, respectively ($P < .01$), and nonrelapse mortality at 2 years was 16.1%, 16.4%, and 26.7%, respectively ($P < .01$). Leukemia-free survival (LFS) at 2 years was 53.6%, 61.6%, and 50.1%, respectively ($P = .03$). Weighted Cox regression revealed that FLAMSA-TBI compared with BuFlu was associated with lower RI (hazard ratio [HR], .64; 95% confidence interval [CI], .42 to .98; $P = .04$) and a trend for better LFS (HR, .72; 95% CI, .49 to

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1.06; $P = .09$). These results suggest that compared with BuFlu, conditioning with FLAMSA-TBI leads to reduced RI at 2 years in AML patients transplanted in CR1 or CR2.

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INTRODUCTION

Relapse is the most important cause of failure in the treatment of acute myelogenous leukemia (AML). The European Leukemia Net recommends allogeneic stem cell transplantation (alloSCT) in AML patients in first complete remission (CR1) after a careful risk-benefit assessment [1]. Here, disease specific and transplantation specific risk factors have to be evaluated before a recommendation for an alloSCT can be given. Generally, alloSCT is recommended if disease relapse risk exceeds 35% to 40% without the procedure. Reduced-intensity conditioning (RIC) before alloSCT was developed to facilitate the use of alloSCT in patients who are not able to tolerate conventional myeloablative conditioning (MAC) due to advanced age or comorbidities [2]. This group of patients (ie, >60 years of age and thus with higher frequency of comorbidities that otherwise would preclude alloSCT due to high treatment-related mortality) are those with the highest incidence of the disease [3]. The prototype RIC regimen consists of a combination of busulfan at reduced dose and fludarabine (BuFlu). Although well tolerated with a nonrelapse mortality (NRM) rate at 4 years of around 21%, higher relapse incidence (RI) compared with myeloablative regimens is a concern [4]. So far, the use of RIC regimens in fit AML patients is not well defined. The FLAMSA (fludarabine + Ara-C + amsacrine chemotherapy)-RIC regimen consists of 4 days of antileukemic chemotherapy, followed after 3 days of rest by a RIC regimen [5]. All patients receive in vivo T cell depletion to reduce the risk of acute graft-versus-host disease (aGVHD) or chronic GVHD (cGVHD). In addition, early tapering of immunosuppression and use of prophylactic/adjuvant donor lymphocyte infusions starting on day 120 after alloSCT are part of the protocol. The aim is to transplant in aplasia after maximum reduction of leukemic blasts and to harness the graft-versus-leukemia effect. FLAMSA-RIC was originally developed for relapsed/refractory disease, where it showed promising efficacy with 2-year leukemia-free survival (LFS) and overall survival (OS) rates of 40% and 42%, respectively [5]. A more recent registry study on 267 patients showed a GVHD-free relapse-free survival (GRFS) rate at 3 years of 17.8% and an LFS of 25.6% [6]. In addition, FLAMSA has also been used with encouraging results in high-risk AML patients in CR1 and in AML patients with complex karyotype transplanted as soon as possible following FLAMSA-RIC irrespective of response [7,8]. More recently, the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT) performed a retrospective registry study on 265 intermediate- or poor-risk AML patients transplanted in CR1 or second complete remission (CR2) after FLAMSA-RIC. Again, promising results were reported with a 2-year LFS of 52.8% and OS of 56.1% [9]. Several modifications of the originally published FLAMSA-RIC protocol have been developed. One is the FLAMSA-Bu variant, in which the 4-Gy total body irradiation (TBI) in the original protocol is replaced by 8 doses of i.v. busulfan (.8 mg/kg) given over 2 days. Other published modifications include the use of treosulfan instead of TBI or clofarabine replacing fludarabine and amsacrine [10-12].

However, despite accumulating evidence on activity and tolerability, the exact role of either of the FLAMSA conditioning regimens before alloSCT has not been defined yet because no comparison of FLAMSA-RIC with other commonly used conditioning regimens exists. Therefore, it is unclear, for example, whether using FLAMSA-RIC protocols in patients with AML in CR1 or CR2 is beneficial compared with other, less intensive protocols (eg, the RIC regimen BuFlu). To this end, we compared the outcomes of AML patients transplanted in CR1 or CR2 with either the BuFlu regimen or 1 of 2 FLAMSA-RIC variants (ie, FLAMSA-TBI or FLAMSA-Bu). The hypothesis was that the FLAMSA-RIC protocols, due to their sequential design and higher intensity compared with BuFlu, would lead to reduced RI, which may possibly translate into improved LFS and OS.

METHODS

Study Design

This is a retrospective registry-based analysis on behalf of the ALWP of the EBMT. The EBMT is a nonprofit, scientific society representing >600 transplant centers, mainly in Europe, that are required to report all consecutive stem cell transplantations and follow-ups once a year. Data are entered, managed, and maintained in a central database; each EBMT center is represented in this database. Audits are routinely performed to determine the accuracy of the data. Patients provide informed consent authorizing the use of their personal information for research purposes. Patients were eligible for the study if they had received first alloSCT for AML in CR1 or CR2 after 1 of the following conditioning regimens: (1) BuFlu (ie, fludarabine 5×30 mg/m^2, busulfan $8 \times .8$ mg/kg body weight with or without antithymocite globulin); (2) FLAMSA-TBI (ie, fludarabine 4×30 mg/m^2, amsacrine 4×100 mg/m^2, cytarabine 4×2000 mg/m^2, 400-cGy TBI, cyclophosphamide 2×40 mg/kg body weight [2×60 mg/kg body weight in case of unrelated donors (UDs)], antithymocite globulin 3×10 mg/kg body weight [3×20 mg/kg body weight in case of UDs]); or (3) FLAMSA-Bu, in which TBI is replaced by busulfan $8 \times .8$ mg/kg body weight. Matched related donors or UDs with up to 1 mismatch (9/10) at antigen or allele level were acceptable. For all patients, information on cytogenetic risk at first diagnosis had to be available. Cytogenetic abnormalities were classified according to the Medical Research Council classification system [13,14]. Transplants were done in 134 centers between January 2005 and June 2016. Data were taken from the EBMT registry.

Endpoints and Definitions

The primary endpoint was LFS. Secondary endpoints were OS, refined GRFS, neutrophil engraftment, aGVHD and cGVHD, RI, and NRM. Engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $>.5 \times 10^9$/L. Relapse was defined according to standard hematologic criteria. NRM was defined as death from any cause in the absence of prior disease recurrence. LFS was defined as the time to either relapse or death in remission. OS was defined as the time to death from all causes. aGVHD was graded according to the modified Glucksberg criteria and cGVHD according to the revised Seattle criteria [15,16]. GRFS events have been defined as grade III to IV aGVHD, severe cGVHD, disease relapse, or death from any cause after alloSCT [17]. All time-to-event outcomes were calculated from the date of alloSCT. Patients with no event were censored at last contact.

Statistical Analysis

The 3 groups were compared using the Kruskal-Wallis test for quantitative variables, chi-square test, or the Fisher exact test for categorical variables.

Cumulative incidence was used to estimate the endpoints of NRM, RI, aGVHD, and cGVHD to accommodate for competing risks. To study acute and chronic GVHD, we considered relapse and death to be competing events. Probabilities of OS, LFS, and GRFS were calculated using the Kaplan-Meier method. Univariate analyses were done using the Gray test for cumulative incidence functions and the log-rank test for OS, GRFS, and LFS.

We used propensity score (PS) weighting to control for pretreatment imbalances on observed variables. The following factors were included in the PS model: age at transplant, status at transplantation (CR1/CR2), donor type (matched sibling donor/UD 10/10, UD 9/10), cytogenetics (favorable, intermediate, or adverse), secondary AML, Karnofsky Performance Score at transplant (<90% versus ≥90%), sex matching (female donor to male recipient versus other), CMV donor or recipient, in vivo T cell depletion, and year of transplantation. PS estimation was performed using generalized boosted models [18]. As the research question focused on the effectiveness of

Table 1
Patient Characteristics

		BuFlu (n = 1197)	FLAMSA-TBI (n = 258)	FLAMSA-Bu (n = 141)	Test *P* Value	FLAMSA-TBI versus BuFlu	FLAMSA-Bu versus BuFlu
Follow-up (patients alive)	Median (range)	24.15 (.66-136.59)	40.26 (2.36-121.48)	17.07 (.69-99.61)	$<10^{-3}$	$<10^{-3}$	.089
Patient age, yr	Median (range) (IQR)	58.8 (20.1-76) (52.6-63.4)	47 (18.1-66.8) (40.1-55.6)	59.6 (19.6-74.4) (54-64)	$<10^{-3}$	$<10^{-3}$	.535
Year of transplantation	Median (range)	2012 (2005-2016)	2011 (2005-2016)	2013 (2007-2016)	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$
Status at transplantation	CR1	979 (81.79)	192 (74.42)	113 (80.14)	.025	.007	.633
	CR2	218 (18.21)	66 (25.58)	28 (19.86)			
Cytogenetics	Good risk	89 (7.44)	20 (7.75)	4 (2.84)	.003	.298	.001
	Intermediate	866 (72.35)	175 (67.83)	90 (63.83)			
	Adverse	242 (20.22)	63 (24.42)	47 (33.33)			
Diagnosis	De novo AML	1021 (85.3)	230 (89.15)	106 (75.18)	.001	.106	.002
	Secondary AML	176 (14.7)	28 (10.85)	35 (24.82)			
Donor type	MSD	569 (47.54)	100 (38.76)	39 (27.66)	$<10^{-3}$	.017	$<10^{-3}$
	UD 10/10	497 (41.52)	118 (45.74)	80 (56.74)			
	UD 9/10	131 (10.94)	40 (15.5)	22 (15.6)			
KPS at transplantation	<80	49 (4.42)	10 (4.02)	10 (7.41)	.262	.776	.124
	≥80	1059 (95.58)	239 (95.98)	125 (92.59)			
	Missing	89	9	6			
	<90	301 (27.69)	41 (16.67)	32 (23.7)	.001	$<10^{-3}$	.326
	≥90	786 (72.31)	205 (83.33)	103 (76.3)			
	Missing	110	12	6			
FLT3 status	Negative	360 (63.05)	36 (53.73)	32 (69.57)	.197	.137	.377
	Positive	211 (36.95)	31 (46.27)	14 (30.43)			
	Missing	626	191	95			
NPM1 status	Negative	319 (61.23)	26 (46.43)	36 (78.26)	.005	.032	.022
	Positive	202 (38.77)	30 (53.57)	10 (21.74)			
	Missing	676	202	95			
Previous auto	No previous auto	1162 (97.08)	258 (100)	140 (99.29)	.007	.005	.124
	Previous auto	35 (2.92)	0 (0)	1 (.71)			
Source of SC	BM	59 (4.93)	13 (5.04)	7 (4.96)	.997	.941	.985
	PB	1138 (95.07)	245 (94.96)	134 (95.04)			
Patient sex	Male	633 (52.93)	122 (47.29)	84 (59.57)	.057	.10	.134
	Female	563 (47.07)	136 (52.71)	57 (40.43)			
	Missing	1	0	0			
Donor sex	Donor male	727 (60.89)	172 (66.67)	95 (67.86)	.084	.083	.109
	Donor female	467 (39.11)	86 (33.33)	45 (32.14)			
	missing	3	0	1			
Sex matching	No female to male	958 (80.3)	226 (87.6)	124 (87.94)	.004	.006	.028
	Female to male	235 (19.7)	32 (12.4)	17 (12.06)			
	Missing	4	0	0			
Patient CMV	Negative	411 (34.63)	108 (42.02)	43 (30.5)	.035	.025	.328
	Positive	776 (65.37)	149 (57.98)	98 (69.5)			
	Missing	10	1	0			
Donor CMV	Negative	580 (48.99)	142 (55.69)	64 (45.39)	.084	.052	.419
	Positive	604 (51.01)	113 (44.31)	77 (54.61)			
	Missing	13	3	0			
Donor/recipient CMV	Donor−/recipient−	284 (24.11)	83 (32.68)	33 (23.4)	.051	.042	.292
	Donor+/recipient−	127 (10.78)	25 (9.84)	10 (7.09)			
	Donor−/recipient+	293 (24.87)	58 (22.83)	31 (21.99)			
	Donor+/recipient +	474 (40.24)	88 (34.65)	67 (47.52)			
	Missing	19	4	0			
In vivo TCD	No	146 (12.22)	27 (10.47)	3 (2.13)	.001	.431	$<10^{-3}$

(*continued on next page*)

Table 1 (*Continued*)

		BuFlu (n = 1197)	FLAMSA-TBI (n = 258)	FLAMSA-Bu (n = 141)	Test *P* Value	FLAMSA-TBI versus BuFlu	FLAMSA-Bu versus BuFlu
	Yes	1049 (87.78)	231 (89.53)	138 (97.87)			
	Missing	2	0	0			
aGVHD	Grade I	219 (18.4)	78 (30.71)	26 (18.84)	$<10^{-3}$	$<10^{-3}$	.375
	Grade II	151 (12.69)	44 (17.32)	18 (13.04)			
	Grade III	59 (4.96)	19 (7.48)	9 (6.52)			
	Grade IV	39 (3.28)	5 (1.97)	9 (6.52)			
	Grade unknown	11 (.92)	1 (.39)	2 (1.45)			
	Absence	711 (59.75)	107 (42.13)	74 (53.62)			
	Missing	7	4	3			
	No aGVHD II	930 (78.88)	185 (73.12)	100 (73.53)	.071	$<10^{-3}$	.152
	aGVHD II-IV	249 (21.12)	68 (26.88)	36 (26.47)			
	Missing	18	5	5			
Engraftment	Graft failure	3 (.25)	6 (2.35)	4 (2.88)	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$
	Engrafted	1185 (99.75)	249 (97.65)	135 (97.12)			
	Missing	9	3	2			
DLI	No	1039 (86.95)	202 (78.6)	117 (82.98)	.001	$<10^{-3}$	.421
If DLI	Before relapse	95 (7.95)	43 (16.73)	15 (10.64)			
	After relapse	61 (5.1)	12 (4.67)	9 (6.38)			

Values are n (%) unless otherwise indicated.
MSD indicates matched sibling donor; KPS, Karnofsky Performance Score; FLT3, fms-like tyrosine kinase-3; NPM1, nucleophosmin 1; CMV, cytomegalovirus; SC, stem cell; BM, bone marrow; PB, peripheral blood; TCD, T cell depletion; DLI, donor lymphocyte infusion.

FLAMSA-Bu or FLAMSA-TBI if it were to replace BuFlu for patients having the same characteristics of those actually receiving BuFlu, we weighted the FLAMSA-Bu and FLAMSA-TBI groups to match the BuFlu group by estimating the average treatment effect among the treated (ATT), with BuFlu being the treated group. The ATT weights equal 1 for BuFlu, and it equals the ratio of the PS to 1 minus the PS in the 2 FLAMSA groups. We checked the balance between the groups looking to ATT-weighted means. Then, we used ATTs to fit weighted Kaplan-Meier and cumulative incidence. Comparison between groups were performed on cause-specific hazards using Cox proportional hazards model weighted on the PS and factors remaining unbalanced between groups [19] (cytogenetics, Karnofsky Performance Score, and donor type for the comparison between FLAMSA-Bu and BuFlu; patient age for the comparison between FLAMSA-TBI and BuFlu). All tests were 2 sided. The type I error rate was fixed at .05 for determination of factors associated with time to event. Analyses were performed using R statistical software version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). PS analysis was performed using the mnps function of the twang package [20]. Estimation of weighted outcomes at 2 years was done using the rms and npsurv packages, and weighted Cox was done using the survey package.

RESULTS

Patient Characteristics

For the BuFlu group, 1197 patients were eligible, whereas 258 and 141 patients received FLAMSA-TBI and FLAMSA-Bu, respectively. Median follow-up for survivors was 24.7 months. Compared with BuFlu, patients treated with FLAMSA-TBI had a longer follow-up (24.2 versus 40.3 months; $P < .001$). Treatment groups were compared with respect to patient characteristics (Table 1). Some differences were observed. For example, compared with BuFlu, patients were younger and fewer patients were in CR1 in the FLAMSA-TBI group (47 years versus 58.8 years, respectively, $P < .001$; 74.4% versus 81.8%, respectively, $P = .007$). More patients in the FLAMSA-Bu group compared with the BuFlu group had adverse cytogenetics (33.3% versus 20.2%; $P = .001$) or suffered from secondary AML (24.8% versus 14.7%; $P = .002$).

Engraftment and GVHD

Neutrophil engraftment was reached with BuFlu in 99.75% of patients compared with 97.7% in the FLAMSA-TBI group ($P < .001$) and 97.1% in the FLAMSA-Bu group ($P < .001$), respectively. Cumulative incidence of aGVHD grade II to IV and grade III to IV by day 100 after alloSCT were 22.9% (95% confidence interval [CI]. 20.8% to 25%) and 9.1% (95% CI, 7.7% to 10.6%), respectively. Compared with the BuFlu group in which 21.1% of patients experienced aGVHD II to IV, more patients did so in the FLAMSA-TBI group (26.9%; $P < .001$). At 2 years after alloSCT, cumulative incidence of cGVHD was 34% (95% CI, 31.4% to 36.5%). The rates were 34.7% (95% CI, 31.8% to 37.7%), 33.9% (95% CI, 27.6% to 40.3%), and 28% (95% CI, 19.8% to 36.8%) in the BuFlu, FLAMSA-TBI, and FLAMSA-Bu groups, respectively (not significant). Extensive cGVHD at 2 years was diagnosed in 16.5% (95% CI, 14.6 to 18.6) of patients. The rates were 17.3% (95% CI, 15% to 19.8%), 16.2% (95% CI, 11.5% to 21.5%), and 11.1% (95% CI, 6% to 18%) in the BuFlu, FLAMSA-TBI, and FLAMSA-Bu groups, respectively (not significant) (Table 2).

Relapse and NRM

RI at 2 years was 28.3% (95% CI, 25.9% to 30.7%). It was 30.3% (95% CI, 27.5% to 33.1%) in the BuFlu group compared with 21.9% (95% CI, 16.8% to 27.6%) and 23.1% (95% CI, 15.4% to 31.8%) in the FLAMSA-TBI and FLAMSA-Bu groups, respectively ($P = .002$). NRM was 17.1% (95% CI, 15.1% to 19.1%). It was 16.1% (95% CI, 13.9% to 18.5%), 16.4% (95% CI, 12% to 21.4%), and 26.7% (95% CI, 19.1% to 35%) in the BuFlu, FLAMSA-TBI, and FLAMSA-Bu groups, respectively ($P = .007$) (Table 2).

Table 2
Univariate analysis (Kaplan-Meier) outcome at 2 years.

	RI	NRM	LFS	OS	GRFS	aGVHD II-IV	aGVHD III-IV	cGVHD	extensive cGVHD
BuFlu	30.3% [27.5-33.1]	16.1% [13.9-18.5]	53.6% [50.5-56.7]	60% [56.9-63.1]	40.2% [37.1-43.3]	21.6% [19.2-24]	8.5% [7-10.2]	34.7% [31.8-37.7]	17.3% [15-19.8]
FLAMSA-TBI	21.9% [16.8-27.6]	16.4% [12-21.4]	61.6% [55.3-68]	68.3% [62.3-74.4]	46.9% [40.3-53.5]	27% [21.7-32.6]	9.5% [6.3-13.5]	33.9% [27.6-40.3]	16.2% [11.5-21.5]
FLAMSA-Bu	23.1% [15.4-31.8]	26.7% [19.1-35]	50.1% [40.5-59.8]	56.4% [46.8-66.1]	38.1% [28.8-47.4]	26.8% [19.6-34.5]	13.5% [8.3-19.9]	28% [19.8-36.8]	11.1% [6-18]
P Value (global)	.002	.007	.032	.070	.112	.086	.175	.199	.254

LFS, OS, and GRFS

At 2 years, LFS was 54.6% (95% CI, 52% to 57.3%). It was 53.6% (95% CI, 50.5% to 56.7%), 61.6% (95% CI, 55.3% to 68%), and 50.1% (95% CI, 40.5% to 59.8%) in the BuFlu, FLAMSA-TBI, and FLAMSA-Bu groups, respectively (P = .03). OS at 2 years was 61.1% (95% CI, 58.5% to 63.8%). It was 60% (95% CI, 56.9% to 63.1%), 68.3% (95% CI, 62.3% to 74.4%), and 56.4% (95% CI, 46.8% to 66.1%) for the BuFlu, FLAMSA-TBI, and FLAMSA-Bu groups, respectively (not significant). GRFS at 2 years was 41.1% (95% CI, 38.5% to 43.8%). It was 40.2% (95% CI, 37.1% to 43.3%), 46.9% (95% CI, 40.3% to 53.5%), and 38.1% (95% CI, 28.8% to 47.4%) for the BuFlu, FLAMSA-TBI, and FLAMSA-Bu groups, respectively (not significant) (Table 2). There were 475 (39.7%), 95 (36.8%), and 55 (39%) deaths observed in the BuFlu, FLAMSA-TBI, and FLAMSA-Bu groups, respectively (Table 3). On the one hand, infection was the leading cause of death in the FLAMSA-Bu group, accounting for 24 cases (43.6% of deaths), whereas 77 (16.2% of deaths) and 21 (22.1% of deaths) patients died from infection in the BuFlu and FLAMSA-TBI groups, respectively. On the other hand, there were fewer deaths due to disease relapse in the FLAMSA-Bu (n = 13, 23.6% of deaths) group compared with the BuFlu (n = 237, 49.9% of deaths) and FLAMSA-TBI (n = 44, 46.3% of deaths) groups, respectively.

Multivariate Analysis

Weighted probabilities for RI, NRM, LFS, OS, GRFS, aGVHD II to IV, and cGVHD at 2 years were calculated for BuFlu, FLAMSA-TBI, and FLAMSA-Bu groups (Table 4, Figures 1, 2). ATT are given in Supplementary Table S1.

Compared with BuFlu conditioning, FLAMSA-TBI resulted in reduced RI at 2 years (hazard ratio [HR], .64; 95% CI, .42 to .98; P = .04) (Table 4, Figure 1). NRM was comparable (HR, .88; 95% CI, .44 to 1.74; P = .72). In addition, a trend for better LFS with the FLAMSA-TBI conditioning compared with BuFlu was observed (HR, .72; 95% CI, .49 to 1.06; P = .09). However, this did not translate into improved OS (HR, .81; 95% CI, .54 to 1.22; P = .32). Conditioning with FLAMSA-Bu compared with BuFlu had no significant impact on outcome (Table 4, Figure 2). RI (HR, .98; 95% CI,63 to 1.53; P = .93), NRM (HR, 1.45; 95% CI, .88 to 2.41; P = .14), LFS (HR, 1.15; 95% CI, .82 to 1.61; P = .39), and OS (HR, 1.226; 95% CI, .85 to 1.77; P = .27) were all comparable.

DISCUSSION

In the present study, we retrospectively compared alloSCT outcomes after BuFlu, FLAMSA-TBI, or FLAMSA-Bu conditioning in AML patients in CR1 or CR2. FLAMSA-TBI led to a reduction in RI at 2 years and to a trend for improved LFS when compared with BuFlu. However, this did not translate into

Table 3
Causes of Death

	BuFlu (n = 475)	FLAMSA-TBI (n = 95)	FLAMSA-Bu (n = 55)
Cardiac Toxicity	1 (.21)	1 (1.05)	0 (0)
Hemorrhage	3 (.63)	3 (3.16)	2 (3.64)
Failure/Rejection	3 (.63)	0 (0)	0 (0)
VOD	1 (.21)	0 (0)	1 (1.82)
Infection	77 (16.21)	21 (22.11)	24 (43.64)
IP	6 (1.26)	1 (1.05)	1 (1.82)
GVHD	103 (21.68)	12 (12.63)	7 (12.73)
Original Disease	237 (49.89)	44 (46.32)	13 (23.64)
Second Malignancy	13 (2.74)	1 (1.05)	1 (1.82)
Other Transplant Related	31 (6.53)	12 (12.63)	6 (10.91)

Values are n (%).
VOD indicates veno-occlusive-disease; IP, interstitial pneumonia.

Table 4
Weighted probabilities at two years.

	RI	NRM	LFS	OS	GRFS	aGVHD II-IV	cGVHD
BuFlu	30.3% [27.5-33.1]	16.1% [13.9-18.5]	53.6% [50.5-56.7]	60% [56.9-63.1]	40.2% [37.1-43.3]	21.6% [19.2-24]	34.7% [31.8-37.7]
FLAMSA-TBI	20% (11.1-28.1)	15.7% (5.4-24.9)	64.3% (58.1-71.1)	69.4% (65.5-73.5)	44.9% (40.7-49.5)	29.0% (16.2-39.8)	38.5% (24.6-49.8)
FLAMSA-Bu	29.3% (16.-40.5)	24.2% (14.-33.2)	46.5% (38.1-56.7)	49.4% (44.8-54.6)	33.5% (29.4-38.2)	26.0% (14.7-35.8)	29.4% (15.9-40.7)
HR (FLAMSA-TBI vs BuFlu)*	0.64 (0.42-0.98)	0.88 (0.44-1.74)	0.72 (0.49-1.06)	0.81 (0.54-1.22)	0.90 (0.65-1.25)	1.47 (0.89-2.42)	1.03 (0.67-1.60)
P (FLAMSA-TBI vs BuFlu)*	0.04	0.72	0.09	0.32	0.52	0.13	0.88
HR (FLAMSA-Bu vs BuFlu)**	0.98 (0.63-1.53)	1.45 (0.88-2.41)	1.15 (0.82-1.61)	1.23 (0.85-1,77)	1.12 (0.84-1.51)	1.13 (0.69-1.83)	0.74 (0.49-1.11)
P (FLAMSA-Bu vs BuFlu)**	0.93	0.14	0.39	0.27	0.43	0.62	0.15

* Adjusted for patient age.
** Adjusted for cytogenetics, Karnofsky performance score and donor type.

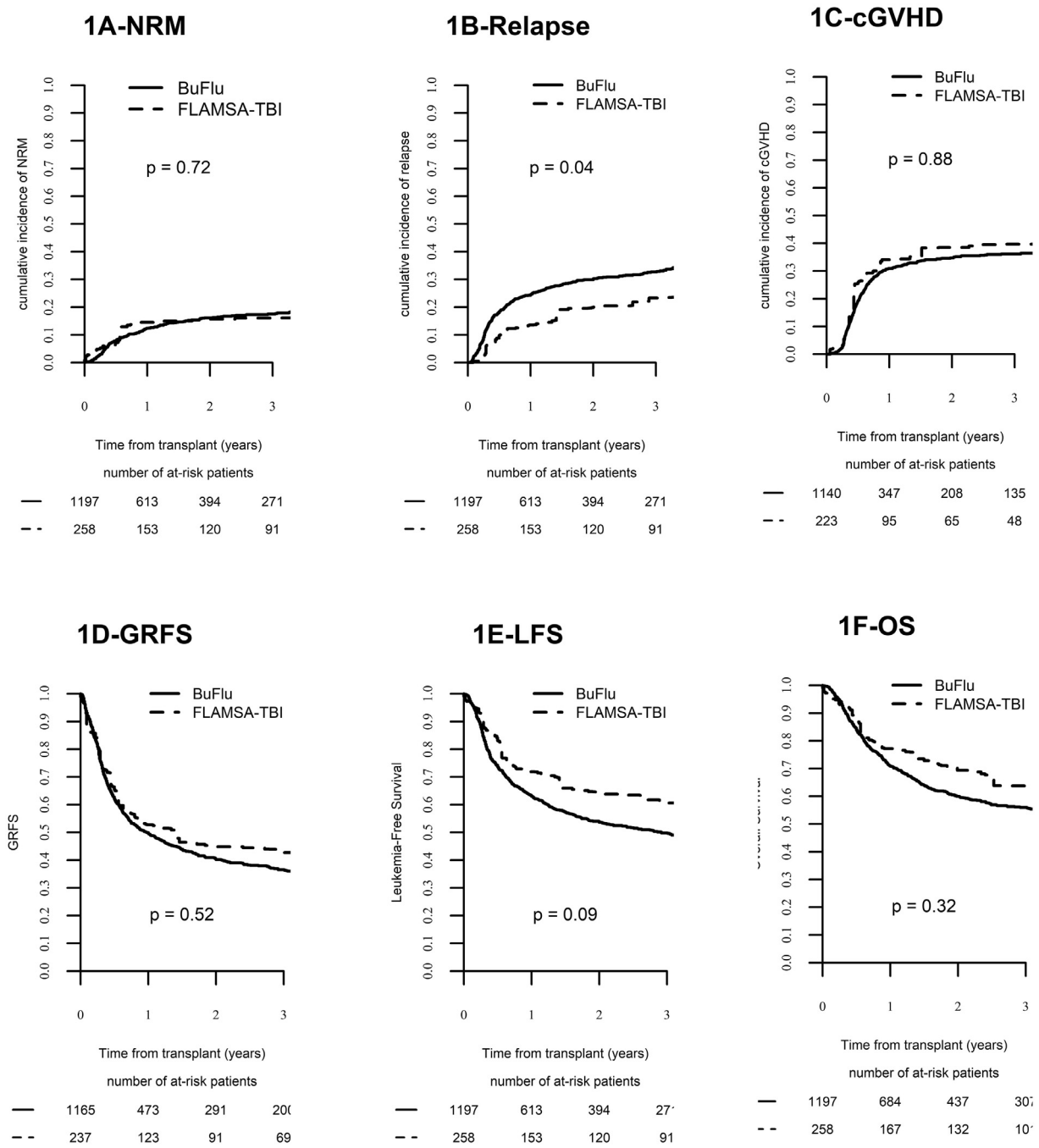

Figure 1. Comparisons of cumulative incidence (CI) of NRM (A), RI (B), and cGVHD (C), and comparisons of probability of GRFS (D), LFS (E), and OS (F) for conditioning with BuFlu (solid line) versus FLAMSA-TBI (dashed line).

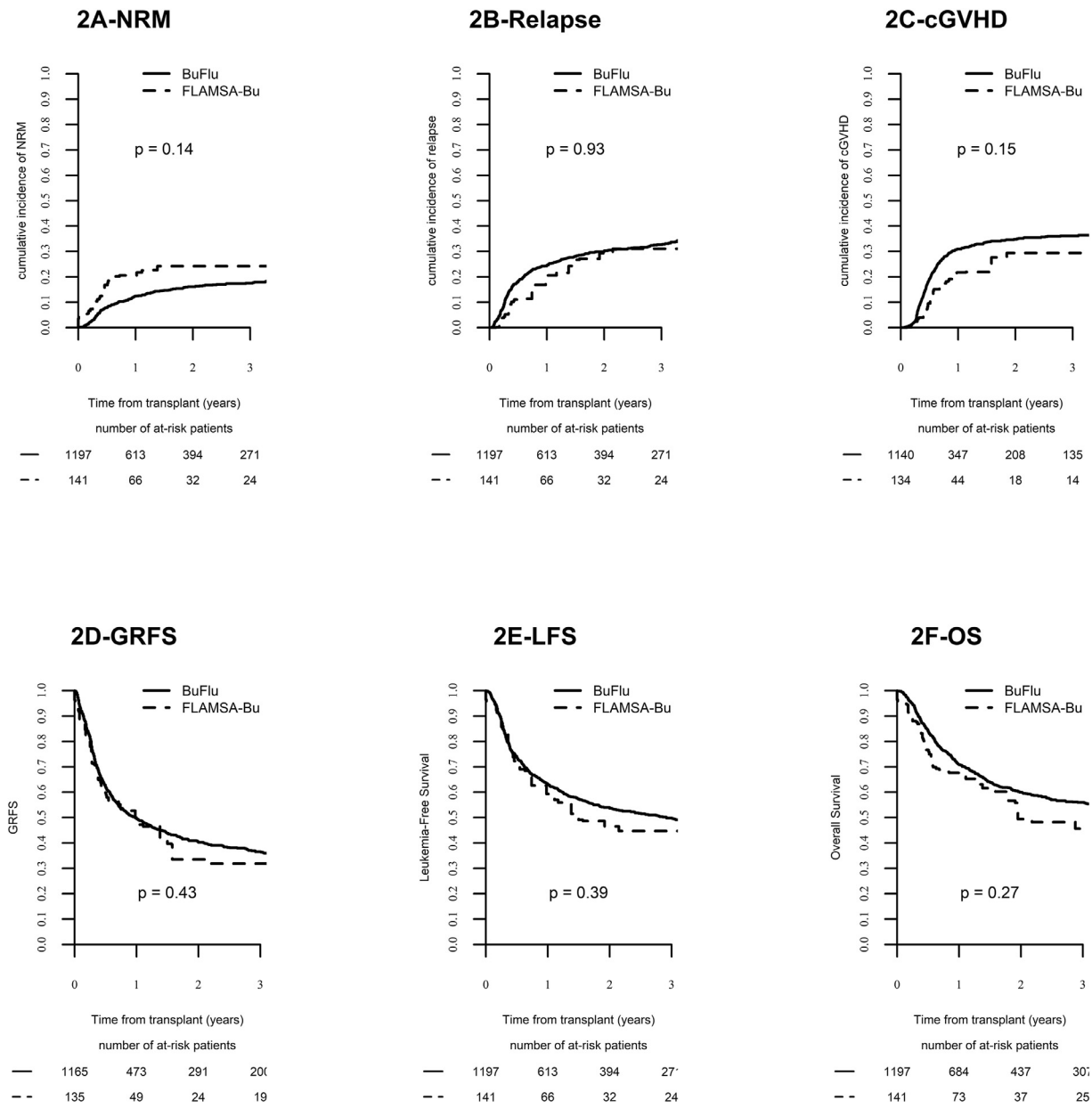

Figure 2. Comparisons of CI of NRM (A), RI (B), and cGVHD (C), and comparisons of probability of GRFS (D), LFS (E), and OS (F) for conditioning with BuFlu (solid line) versus FLAMSA-Bu (dashed line).

improved OS. In univariate analysis FLAMSA-Bu compared with BuFlu showed an increased NRM (16.1% versus 26.7%; $P < .007$). However, this did not remain significant in the Cox proportional hazards model adjusted on the PS. Furthermore, no significant difference could be detected in any other of the endpoints. Infection was more often the cause of death in the FLAMSA-Bu group (43.6% of deaths). The FLAMSA protocols, due to their sequential design, lead to longer time in neutropenia than other regimens do. This may in part explain the higher rate of death secondary to infection in the FLAMSA patient groups. The FLAMSA-Bu cohort comprised patients who were older than those receiving the FLAMSA-TBI conditioning and thus may be more vulnerable during neutropenia. Because relapse and death from other causes are competing risks, this may explain that a reduction in RI by FLAMSA-Bu was not detected in our study.

The RIC regimen BuFlu has a favorable tolerability, and in a randomized study even at myeloablative dosage, NRM at 1 year was shown to be only 7.9% [21]. The question whether more intense BuFlu is more effective has been addressed by several studies. Chen et al. [22], in a monocentric retrospective study, compared 2 different busulfan dosages (3.2 mg/kg body weight versus 6.4 mg/kg body weight) in combination with fludarabine (120 mg/kg body weight) in AML and myelodysplastic syndrome (MDS) patients. No significant differences in outcomes were found. In a subset of patients with high clinical disease risk and nonadverse (favorable or intermediate) cytogenetics, the regimen with 6.4 mg/kg body weight of busulfan was associated with a trend toward improved PFS. Furthermore, the ALWP of the EBMT retrospectively compared 2 variants of BuFlu (FB2 and FB4), which differ in dose intensity, in AML patients in CR1 and CR2. NRM at 2 years was 16% and

21%, respectively. In AML patients in CR1 <50 years of age, an increase in RI was detected with FB2 compared with FB4. In those patients ≥50 years of age, LFS and OS were significantly higher with FB2, although RI and NRM both were comparable for FB2 and FB4. In the study investigating AML patients in CR2, the more intense regimen FB4 resulted in better LFS and OS. Interestingly, this was not explained by reduced RI for FB4 [23,24].

Retrospective studies comparing RIC with MAC in AML patients mostly showed improved NRM with RIC but higher RI and comparable LFS and OS [3]. Scott et al. [25] recently published the results of a prospective randomized trial comparing RIC (busulfan with fludarabine or busulfan with melphalan) with MAC (busulfan/cyclophosphamide, busulfan/fludarabine, or cyclophosphamide/TBI) in AML or MDS patients. Treatment-related mortality was significantly lower for the RIC group, with 4.4% in the RIC group versus 15.8% in the MAC group. In AML patients, cumulative incidence of relapse was 15.9% (95% CI, 9.7% to 23.5%) with MAC and 51% (95% CI, 41.2% to 60%) with RIC. In addition, 18-month RFS and OS were significantly better with MAC compared with RIC. In contrast to our study, the marked difference in RI obtained with RIC versus MAC did translate into an improved OS. The smaller difference in RI between FLAMSA-TBI, which is of intermediate intensity, and BuFlu in our study compared with the more marked difference in the study comparing RIC versus MAC may explain that this difference did not translate into an OS advantage in our study. The previously mentioned studies suggest that in AML patients, dose intensity of the conditioning regimen is important. Nonmyeloablative BuFlu shows a favorable NRM, but more intense protocols lead to lower RI. However, MAC is associated with higher NRM in most studies, and LFS and OS are comparable after RIC and MAC [4]. In the previously mentioned randomized trial, TRM even in the MAC group was remarkably low. FLAMSA is a sequential conditioning protocol of intermediate intensity. A recent retrospective study by the ALWP of the EBMT in 265 intermediate- or high-risk AML patients in either CR1 or CR2 transplanted after FLAMSA conditioning showed 2-year LFS and OS of 52.8% and 56.1%, respectively [8]. In this study, NRM was reported to be 24%. Thus, despite its increased intensity compared with other RIC regimens in this high-risk population, FLAMSA appears to have a favorable tolerability. Nevertheless, despite its favorable toxicity and antileukemic activity, FLAMSA-RIC did not result in improved LFS or OS compared with BuFlu in our study.

Blaise et al. [26] compared BuFlu with the NMA regimen developed in Seattle, combining fludarabine (4×30 mg/m^2) with TBI (2Gy) in various diseases. NRM at 1 year was 17% and 11% with BuFlu and Flu-TBI, respectively. The cumulative incidence of progression or relapse at 1 year was 14% after BuFlu compared with 37% after Flu-TBI ($P < .01$). However, this difference did not translate into different LFS or OS. The ALWP of EBMT retrospectively compared BuFlu with fludarabine/melphalan conditioning in AML patients [27]. RI was significantly higher for BuFlu patients compared with fludarabine/melphalan patients. However, in agreement with our study, this did not translate into different LFS or OS. Shimoni et al. [28] reported a comparison of 2 BuFlu regimens of different intensity with 2 fludarabine/treosulfan regimens. No differences in RI, NRM, LFS, and OS were seen in patients in CR.

More recently, a prospective study comparing RIC BuFlu with myeloablative fludarabine/treosulfan in AML patients in CR or MDS patients was presented [29]. Patient age was ≥50 years, or patients had a hematopoietic cell transplantation comorbidity index >2. In this study, event-free survival and OS rates at 24 months were significantly better in the fludarabine/treosulfan group (64% and 72.5%, respectively) compared with the BuFlu group (50.4% and 56.4%, respectively). Interestingly, RI was similar in both groups. The favorable results for the fludarabine/treosulfan combination may be explained by reduced treatment-related mortality in this group compared with the BuFlu group of 11.3% versus 28.2%, respectively.

Our study has some limitations due to its retrospective nature, such as some degree of heterogeneity in the GVHD prophylaxis, the theoretical possibility of patient selection and center effect, having only the Karnofsky Performance Score and not the Sorror comorbidity score (hematopoietic cell transplantation comorbidity index) available, and some missing data, mainly on molecular markers and minimal residual disease.

However, until results are available from a well-designed randomized clinical trial, the results of our study are of clinical importance, suggesting at least noninferiority of FLAMSA-TBI compared with BuFlu in terms of RI, NRM, LFS, and OS in AML patients transplanted in CR1 or CR2. Currently, it is unclear whether the benefit of FLAMSA-TBI might in part depend on comorbidity and may be more prominent in certain subsets of patients (eg, those with minimal residual disease positivity at time of transplantation). Therefore, prospective trials comparing the FLAMSA platform with other conditioning regimens are warranted, in which in addition to other established risk factors, comorbidity score and minimal residual disease status at time of transplantation have to be assessed.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.bbmt.2018.07.007.

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