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Allogeneic Hemopoietic Stem Cell Transplants in Patients with Acute Myeloid Leukemia (AML) Prepared with Busulfan and Fludarabine (BUFLU) or Thiotepa, Busulfan, and Fludarabine (TBF): A Retrospective Study

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A B S T R A C T

This is a multicenter retrospective comparison of 2 myeloablative conditioning regimens in 454 patients with acute myeloid leukemia (AML) in remission: busulfan (4 days) and fludarabine (BUFLU) versus thiotepa, busulfan, and fludarabine (TBF). Eligible for this study were patients allografted between January 2008 and December 2018 in 10 transplant centers, with AML in first or second remission: 201 patients received BUFLU, whereas 253 received TBF. The 2 groups (BUFLU and TBF) were comparable for age (P = .13) and adverse AML risk factors (P = .3). The TBF group had more second remissions and more haploidentical grafts. The donor type included HLA-identical siblings, unrelated donors, and family haploidentical donors. The 5-year cumulative incidence of nonrelapse mortality (NRM) was 19% for BUFLU and 22% for TBF (P = .8), and the 5-year cumulative incidence of relapse was 30% and 15%, respectively (P = .0004). The 5-year actuarial survival was 51% for BUFLU and 68% for TBF (P = .002). In a multivariate Cox analysis, after correcting for confounding factors, the use of TBF reduced the risk of relapse compared with BUFLU (P = .03) and the risk of death (P = .03). In a matched pair analysis of 108 BUFLU patients matched with 108 TBF patients, with the exclusion of haploidentical grafts, TBF reduced the risk of relapse (P = .006) and there was a trend for improved survival (P = .07). Superior survival of patients receiving TBF as compared with BUFLU is due to a reduced risk of relapse, with comparable NRM. The survival advantage is independent of donor type and AML risk factors.

INTRODUCTION

The combination of intravenous busulfan and fludarabine (BUFLU) is considered a standard conditioning regimen for patients with acute myeloid leukemia (AML) undergoing an allogeneic transplant (HSCT) in first or second remission [1]. Several retrospective studies have shown relatively low nonrelapse mortality (NRM) and encouraging leukemia control with

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BUFLU [2-6]. Two of these studies [5,6] have compared retrospectively BUFLU with the standard combination of busulfan and cyclophosphamide (BUCY): transplant mortality was reduced in patients receiving BUFLU, which translated in superior overall survival [5,6].

A prospective multicenter randomized study, comparing BUFLU with BUCY in patients with remission AML, has also been conducted [7]. All patients received full-dose busulfan (3.2 mg/kg/day × 4 days), combined with fludarabine 160 mg/m^2 or cyclophosphamide 120 mg/kg. The cumulative incidence of relapse at 5 years was 38% in both groups (P = .7), and NRM was reduced in the BUFLU arm [7]. The editorial that accompanied this publication suggested that BUFLU should be considered the standard conditioning regimen for patients with AML aged 40 to 65 years [1].

The Spanish group headed by Sanz reported some years ago the efficacy of a new conditioning regimen in patients grafted with cord blood units [8]: the combination was a modified BUFLU, with a reduction of the dose of busulfan (3.2 mg/kg/d ×3 days) but with the addition of thiotepa (10 mg/kg): they named this regimen thiotepa, busulfan, and fludarabine (TBF) [8]. In a retrospective Eurocord study, TBF conferred a survival advantage [9]. Since then, the combination has been largely used in Europe as a preparative regimen for patients with hematologic malignancies [10-12]. A retrospective study by the European Group for Blood and Marrow Transplantation (EBMT) has compared TBF or BUFLU as a conditioning regimen for remission AML [13]. Relapse was significantly reduced in patients receiving TBF, but NRM was significantly increased, leading to comparable leukemia-free survival [13]. However, when the authors excluded patients receiving TBF with 4 doses of busulfan, NRM was still greater for TBF patients, but not significantly, whereas relapse remained statistically inferior [13].

Given this background, we sought to test in a real-life setting the role of TBF or BUFLU as a conditioning regimen for patients with AML grafted in first or second remission. We are now reporting the results of this multicenter comparison in 454 consecutive patients with AML.

METHODS

Eligibility

This is a retrospective study on patients with AML undergoing an allogeneic HSCT between January 2008 and December 2018, aged 18 years or older, in first or second remission (CR1 or CR2). Secondary AML, treatment-related AML, and AML with trilineage dysplasia were also included. Excluded were patients with AML in CR1 or CR2 with European Leukemia Net favorable genetic abnormalities (14). Nine transplant centers in Italy and 1 center in Israel contributed all their consecutive patients with AML who had undergone an allogeneic transplant, using either 1 of the 2 conditioning regimens under study. We therefore studied 454 consecutive patients with AML grafted in CR1 or CR2 and prepared with either TBF (n = 253) or BUFLU (n = 201).

Patients

Characteristics of patients, donor, disease stage, and transplant are summarized in Table 1. There were no significant differences in patients' age and

Table 1
Clinical Characteristics of Patients

Characteristic	BUFLU	TBF	*P* Value
Number of patients	201	253	
Sex (M/F), n	90/111	130/123	.16
Age, median (range), yr	53 (17-68)	50 (18-72)	.13
Donor age, median (range), yr	30 (16-68)	34 (16-67)	.11
Disease stage, n (%)			
CR1	168 (84)	183 (73)	.007
CR2	33 (16)	69 (27)	
Adverse risk factors, n			
Complex cytogenetics	27	31	
Del7	7	5	
FLT3 ITD	31	26	
Primary induction failure	6	20	
Secondary AML	11	10	
Total patients with adverse factors, n (%)	81 (40)	92 (36)	.3
MRD negative	94 (47)	106 (42)	.3
MRD positive	78 (39)	116 (46)	
MRD unknown	28 (14)	30 (12)	
Interval diagnosis: HSCT	202 (126-868)	192 (111-2542)	
Donor type, n (%)			
HLA-identical sibling	78 (39)	53 (21)	<.001
Family haploidentical	4 (2)	145 (57)	
Matched unrelated	89 (44)	38 (15)	
Mismatched unrelated	30 (15)	17 (7)	
CMV-positive recipient, n (%)	121 (60)	157 (62)	.4
CMV-negative donor, n (%)	48 (24)	66 (26)	
CMV serostatus unknown, n (%)	32 (16)	30 (12)	
Cell source, n (%)			
Bone marrow	35 (17)	195 (77)	<.001
Peripheral blood	166 (83)	58 (23)	
Follow-up: median days (range)	467 (5-3122)	551 (5-3073)	<.001

Primary induction failure: no CR after 1 course of induction chemotherapy. Secondary AML: AML secondary to previous chemo/radiotherapy.
ITD indicates internal tandem duplication; CMV, cytomegalovirus.

sex in the 2 groups or for AML adverse factors: the latter included complex karyotype, secondary AML, deletion of chromosome 7, FLT3 internal tandem duplication, or failure to achieve remission after a first course of induction chemotherapy. The proportion of patients with AML adverse factors was 40% for BUFLU and 36% for TBF ($P = .3$) (Table 1). Minimal residual disease (MRD) was assessed in each center by multicolor flow cytometry: a cutoff of 0.1% was taken as a positive MRD and was comparable in both groups.

Donors

Donors included HLA-identical matched siblings (SIBs), unrelated donors (UDs), and family HLA haploidentical members (HAPLO) (Table 1). A haploidentical related donor (HAPLO) was chosen when a suitable sibling or an unrelated donor was either temporarily or definitively unavailable; second remission patients also were more frequently assigned to a HAPLO graft (27% compared with 16% CR2 patients receiving UD grafts). The proportion of HAPLO donors was higher in the TBF group (Table 1). Cytomegalovirus serostatus is outlined in Table 1.

HLA Typing

SIB donors were HLA genotypically matched with their recipient. UDs were matched at 4 loci (A, B, C, DRB1) in high-resolution HLA typing in 127 patients and mismatched at 1 locus (7/8) in 47 patients (Table 1).

Follow-up

The median follow-up was 467 days (5 to 3122) for BUFLU and 551 days (5 to 3073) for TBF (Table 1).

Study

The study was approved by the Institutional Review Board of the Institute of Hematology, Gemelli Hospital (March 18, 2019). All patients and donors provided written informed consent for registration and distribution of anonymous clinical data for research purposes at the national and international level.

Conditioning Regimens

There were 2 conditioning regimens.

BUFLU

Intravenous busulfan 3.2 mg/kg/d (total dose 12.8, mg/kg), combined with fludarabine 40 mg/m^2/d from day −6 through day −3 (total dose 160 mg/m^2), was the conditioning regimen for all 201 patients, independent of age.

TBF

This regimen consisted of thiotepa, busulfan, and fludarabine: thiotepa 5 mg/kg on days −6 and −5 (total 10 mg/kg), plus busulfan 3.2 mg/kg/d (total dose 9.6 mg/kg) combined with fludarabine 50 mg /m^2/d on days −4, −3, and −2 (total dose 150 mg/m^2) (TBF3). For patients older than 60 years or for patients with significant comorbidities, the dose of busulfan in the TBF regimen was reduced to 2 days (TBF2) instead of 3 days: 186 patients received TBF3 and 67 patients received TBF2.

Choice of the Conditioning Regimen

The choice of the conditioning regimen was not predetermined. BUFLU is considered a standard regimen and was used in many centers as a standard of care, especially following the prospective Italian Bone Marrow Transplant Group (GITMO) randomized trial [7]. TBF is a more experimental regimen, initially used almost exclusively in cord blood and HAPLO grafts [9-12]. Given the encouraging results, it has then gradually been transferred also to SIB and UD grafts and is now being used increasingly, at least in Italy. The current comparison was prompted to assess the outcome of BUFLU and TBF in a real-life setting.

Stem Cell Source

On day 0, 230 patients received unmanipulated bone marrow cells, and 224 received granulocyte-colony stimulating factor mobilized peripheral blood progenitor cells (Table 1).

Graft-versus-Host Disease Prophylaxis

SIB transplants received a conventional cyclosporin (CsA) methotrexate regimen. Patients grafted from UDs were given CsA and methotrexate with the addition of rabbit anti-thymocyte globulin (ATG) (Genzyme, Cambridge, MA) 5 to 7.5 mg/kg.

HAPLO grafts received post-transplant cyclophosphamide 50 mg/kg ×2, CsA, and mycophenolate. Therefore, patients grafted from UDs received the same graft-versus-host disease (GVHD) prophylaxis, whether prepared with BUFLU or TBF; the same was true for patients grafted from SIB or HAPLO donors.

Supportive care

Patients were given supportive care according to local standards of care, including monitoring, prophylaxis, and treatment of bacterial, viral, and fungal infections.

Statistical analysis

The NCCS11 package was used for chi-square tables, descriptive statistics, actuarial survival, cumulative incidence reports, and multivariate Cox analysis. Variables included in the multivariate analysis on survival, relapse, and NRM were the following: phase of the disease (CR1 versus CR2), adverse risk factors (no versus yes), conditioning regimen (BUFLU versus TBF), recipient age (≤50 versus >50 years), year of transplant (≤2015 versus >2015), donor type (SIB versus HAPLO, SIB versus UD), stem cell source (bone marrow versus peripheral blood), and ATG used in the conditioning (no versus yes). When calculating the cumulative incidence of NRM, relapse was the competing event and vice versa. The log-rank test was used for differences between survival curves. The Gray test was used for differences between cumulative incidence curves.

Matched pair analysis

To compare more rigorously the 2 conditioning regimens, a matched pair analysis was conducted as follows: HAPLO grafts were excluded because all except 4 had received TBF; 108 TBF patients, grafted from UD or SIB donors, were matched with 108 BUFLU patients, selected among the 197 BUFLU patients who had been grafted from UD and SIB donors. Clinical details of the 2 groups are outlined in Supplementary Table S1. The BUFLU and TBF groups were matched respectively for patient age (48 years for both groups $P = .3$), donor age (37 years versus 32 years, $P = .2$), disease phase (CR1 80% versus 79%, $P = .8$), adverse risk factors (33% versus 31%, $P = .7$), donor type (unrelated 55% versus 54%, $P = .8$), use of ATG (55% versus 54%, $P = .8$), and median follow up (1.1 versus 1.3 years, $P = .6$). There were more peripheral blood grafts in the BUFLU group (72% versus 49%, $P = .01$).

RESULTS

Engraftment and GVHD

The median time to a neutrophil count of 0.5 × 10^9/L was 15 days (range, 10 to 42) for BUFLU and 17 days (range, 10 to 64) for TBF ($P = .001$); graft failure was reported in 1 patient (TBF) as a cause of death.

The risk of grade II to IV acute GVHD was 22% (BUFLU) versus 19% (TBF) ($P = .5$); the risk of moderate to severe chronic GVHD was 22% versus 16%, respectively ($P = .2$).

Chimerism

Donor chimerism on day +30 after transplant was available in 326 patients (129 and 197 for BUFLU and TBF, respectively): it was 98% (average) (range, 46% to 100%) for BUFLU and 97% for TBF (range, 6% to 100%) ($P = .4$).

NRM

The 5-year cumulative incidence (CI) of NRM was 19% (95% confidence interval, 14% to 26%) for BUFLU and 22% (95% confidence interval, 16% to 30%) for TBF (Gray test = 0.8) (Supplementary Fig. 1). In univariate analysis, patients over the age of 50 years had higher NRM (hazard ratio [HR], 1.53; $P = .06$), and there was a borderline effect also in multivariate analysis (HR, 1.49; $P = .08$) (Table 2).

Relapse

The 5-year CI of relapse was 30% (95% confidence interval, 23% to 38%) for BUFLU and 15% (95% confidence interval, 10% to 21%) for TBF (Gray's test $P = .0004$) (Fig. 1A). At 8 years, the CI of relapse was respectively 30% versus 17%. In univariate analysis, factors predicting relapse were the conditioning regimen (HR, 0.44; $P = .0002$) and adverse AML risk factors (HR, 1.64; $P = .02$) (Table 2). In multivariate analysis, the use of TBF (HR, 0.53; $P = .03$) and AML risk factors (HR, 1.51; $P = .05$) remained independent predictive variables (Table 2).

When selecting only TBF patients, the CI of relapse at 5 years was 14% for HAPLO and 16% for other donor types (sibling and unrelated) ($P = .8$).

Survival

The 5-year actuarial survival of the entire group of 454 patients was 59% (95% confidence interval, 53% to 64%); it was

Table 2
Univariate and Multivariate Analysis

			Univariate		Multivariate	
Characteristic	Baseline Value	Compared Value	HR	*P*	HR	*P*
NON RELAPSE MORTALITY						
Phase	CR1	CR2	1.01	.9	1.16	.5
Adverse risk	No	Yes	0.91	.7	0.91	.6
Regimen	BUFLU	TBF	0.89	.6	0.98	.9
Recipient age	≤50	>50	1.53	.06	1.49	.08
Year of treatment	≤2015	>2015	1.01	.9	0.97	.5
Donor	SIB	HAPLO	1.17	.5	1.68	.2
		UD	1.25	.4	1.22	.4
Cell source	BM	PB	1.29	.2	1.25	.6
ATG	No	Yes	1.10	.5	1.01	.9
RELAPSE						
Phase	CR1	CR2	0.9	.8	9.3	.08
Adverse risk	No	Yes	1.64	.02	1.51	.05
Regimen	BUFLU	TBF	0.44	.0002	0.53	.03
Recipient age	≤50	>50	1.23	.3	1.14	.5
Year of treatment	≤2015	>2015	0.7	.2	0.95	.8
Donor	SIB	HAPLO	0.58	.07	0.86	.7
		UD	1.15	.5	1.51	.5
Cell source	BM	PB	1.19	.3	0.92	.7
ATG	No	Yes	1.50	.05	1.15	.5
SURVIVAL						
Phase	CR1	CR2	0.9	.9	1.15	.4
Adverse risk	No	Yes	1.19	.2	1.10	.3
Regimen	BUFLU	TBF	0.56	.0008	0.62	.03
Recipient age	≤50	>50	1.38	.05	1.39	.04
Year of treatment	≤2015	>2015	0.89	.5	1.01	.9
Donor	SIB	HAPLO	0.84	.3	1.27	.4
		UD	1.17	.4	1.05	.8
Cell source	BM	PB	1.46	.09	1.20	.4
ATG	No	Yes	1.31	.09	1.01	.7

Abbreviations as in Table 1.

51% for BUFLU (95% confidence interval, 43% to 59%) and 68% for TBF (95% confidence interval, 60% to 76%), *P* = .002 (Fig. 1B). The actuarial survival at 8 years was respectively 48% versus 62%.

In univariate analysis, factors predicting survival were the conditioning regimen, with an HR of 0.56 for TBF versus BUFLU (*P* = .0008). In multivariate analysis, after correcting for AML risk factors, year of transplant, disease phase, stem cell source, the use of ATG, and donor type, TBF remained a significant positive predictor of survival, with an HR of 0.62 (*P* = .03), whereas the patient age over 50 years remained a significant negative predictor (HR, 1.39; *P* = .04) (Table 2). When selecting patients receiving only TBF, we found no differences in survival comparing HAPLO

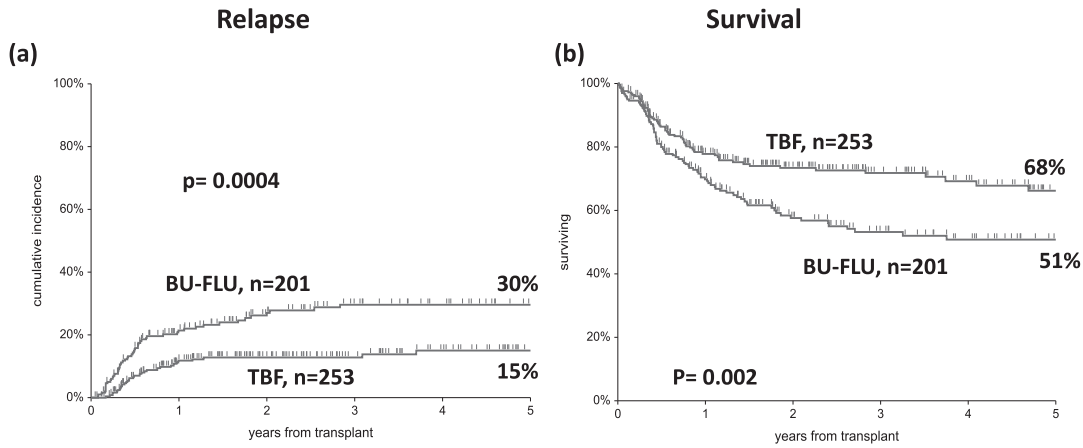

Figure 1. Cumulative incidence of relapse (a) and survival (b) for patients, with AML receiving TBF or BUFLU.

grafts versus transplant from others donors (67% versus 64%, P = .2) (Supplementary Fig. S2). The survival advantage of TBF over BUFLU was seen in patients under the age of 50 years (70% versus 51%, P = .02) and in patients aged 50 years or above (67% versus 50%, P = .07) (Supplementary Fig. S3).

TBF3 compared with TBF2

We then compared patients receiving 3 days of busulfan (TBF3) (n = 186) with patients receiving 2 days of busulfan (TBF2) (n = 67): the median age was 46 years (18 to 61) for TBF3 and 61 years (18 to 70) for TBF2 ($P < .00001$), the proportion of HAPLO grafts was respectively 76% versus 50% (P = .001), and the proportion of patients with AML CR2 was 26% versus 28% (P = .8) and with AML adverse factors 38% versus 27% (P = .08). The median follow-up was comparable (467 versus 547 days) (P = .8). The cumulative incidence of relapse at 5 years was 15% (95% confidence interval, 10% to 22%) for TBF3 compared with 14% (95% confidence interval, 6% to 29%) for the older TBF2 patients (P = .4). The cumulative incidence of NRM at 5 years was respectively 21% (95% confidence interval, 14% to 31%) versus 25% (95% confidence interval, 15% to 40% (P = .4). The actuarial 5-year survival was 65% versus 68% (P = .8) (Supplementary Fig. S4).

Causes of death

We recorded 148 deaths, 82 in the BUFLU and 66 in the TBF group. Leukemia relapse was the most frequent cause of death in 70 of 454 patients (15%): it was recorded in 46 BUFLU (23%) and 24 TBF patients (9%) (P = .00005). Transplant-related causes of death were as follows in the BUFLU and TBF groups, respectively: GVHD (16 and 11 patients), infections (5 and 18 patients), and other transplant-related causes (15 and 13 patients); one of these was reported as sinusoidal obstruction syndrome in the BUFLU arm.

Matched pair analysis

The CI of relapse at 5 years, in the matched pair analysis, with the exclusion of HAPLO grafts, was 33% (95% confidence interval, 24% to 44%) for 108 BUFLU patients and 17% (95% confidence interval, 9% to 29%) for 108 TBF patients (Gray test P = .006) (Fig. 2A); the 5-year actuarial survival was 52% for BUFLU (95% confidence interval, 40% to 63%) and 64% for TBF (95% confidence interval, 51% to 78%) (P = .07) (Fig. 2B); the NRM was respectively 15% (95% confidence interval, 10% to 25%) and 22% (95% confidence interval, 13% to 36%) (P = .56) (Gray test P = .5).

In a multivariate Cox analysis, using the same variables as with the entire population, the use of TBF had a protective effect on mortality (HR, 0.56; P = .02) and on relapse (HR, 0.31; P = .0008). There was no effect on NRM (HR, 1.2; P = .56).

DISCUSSION

Relapse is a major problem in patients with AML undergoing an allogeneic HSCT [15] and has remained unchanged, unlike NRM, which has been significantly reduced [16]. Intensification of the preparative regimen is one way to attempt a better control of leukemia: unfortunately, reduction of leukemia relapse may come with increased NRM. In a prospective randomized study comparing total body irradiation 12 Gy versus 15.75 Gy in patients with AML [17], relapse was reduced from 40% with 12 Gy to 15% with 15.75 Gy, but NRM was increased from 18% (12 Gy) to 38% (15.75 Gy). The net result was identical 10-year survival for both 12-Gy and 15.75-Gy patients [17]. Thus, intensification of the conditioning regimen usually increases the risk of NRM.

In the present retrospective study, we report a significant reduction of post-transplant leukemia relapse in patients with AML in first or second remission receiving TBF as compared with BUFLU, with no detrimental effect on NRM: this resulted in improved 5-year survival. The TBF regimen is not really an intensification as compared with the conventional BUFLU, but rather a modification: instead of 4 doses of busulfan (in the BUFLU), the TBF regimen has 3 doses of intravenous busulfan but combines busulfan with thiotepa, 2 very strong myeloablative agents, both capable of allowing engraftment in a mismatched animal model [18,19]. The combination of the 2 alkylating agents appears to be very effective: this is true also for the older patients receiving only 2 days of busulfan and thiotepa. Indeed, we could not find a significant difference in the risk of relapse or survival when comparing patients receiving 3 days or 2 days of busulfan, despite the fact that TBF2 patients were 15 years older as compared with TBF3 patients: this resulted in comparable survival for TBF3 and TBF2, both superior to BUFLU. This suggests that 2 days of busulfan combined with 2 days of thiotepa delivers enough myeloablation in patients with AML and is tolerated also in patients up to the

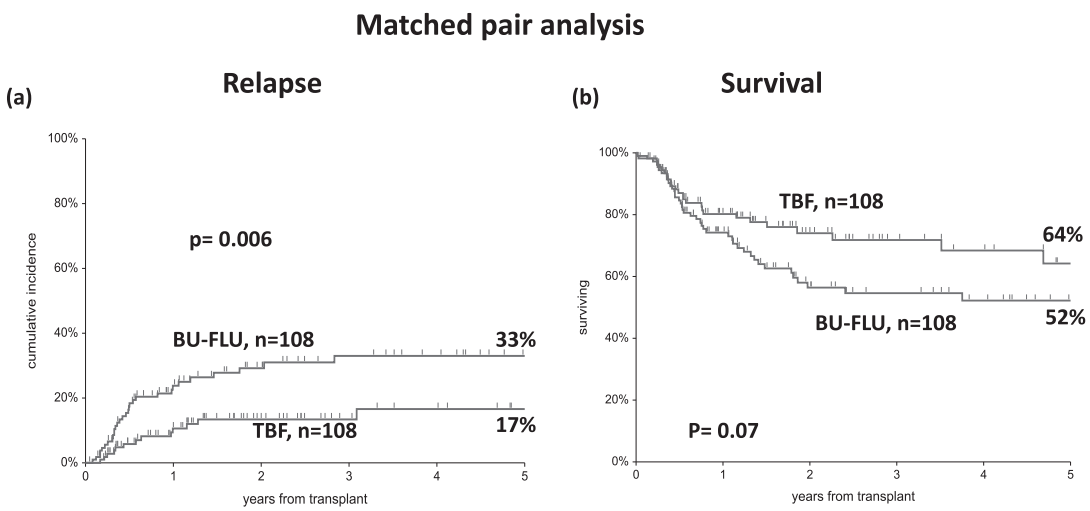

Figure 2. Cumulative incidence of relapse (a) and survival (b) for patients with the matched pair analysis.

age of 70. The TBF is currently used in Europe in a variety of transplant settings [8-13], and we have reported encouraging results in HAPLO transplants [20].

We are particularly impressed with the low incidence of leukemia relapse with TBF (15%) compared with BUFLU (30%), and this study, although retrospective, is a large multicenter, multinational real-life setting. The difference of relapse is not due to a poor performance of the BUFLU arm: indeed, in the randomized trial of BUFLU versus BUCY for patients with AML in first or second remission [7], the cumulative incidence of relapse at 5 years in the BUFLU arm was 38%, whereas in this study, it was 30%. We therefore confirm the relapse risk of BUFLU recorded in the prospective study, and we report a significantly inferior relapse risk with the TBF regimen, as also confirmed by the EBMT study [13]. The increased risk of NRM in the EBMT study for TBF patients, compared with BUFLU patients [13], was possibly due to the inclusion of patients receiving 4 doses of busulfan in addition to thiotepa and fludarabine.

One important question concerns confounding factors: the proportion of patients with adverse AML risk factors, such as complex karyotype, secondary AML, deletion of chromosome 7, FLT3 internal tandem duplication, or failure to achieve remission after a first course of induction chemotherapy, was comparable in the BUFLU and TBF arms. In addition, the proportion of patients with positive MRD, as assessed by multicolor flow cytometry, was comparable in the 2 groups. There were more HAPLO transplants in the TBF arm, and this could be a bias, although the difference in relapse was still there when excluding HAPLO grafts (30% for BUFLU versus 17% for TBF). However, to better study the impact of the conditioning regimen, we ran a matched pair analysis, with the exclusion of HAPLO grafts, on 108 BUFLU versus 108 TBF patients: again, relapse was significantly reduced in the TBF group (P = .006), and there was a trend for improved survival, confirmed in multivariate analysis.

Conversely, when selecting only patients receiving TBF, there was no difference in relapse between HAPLO and other donor types.

Survival at 5 years was 68% for TBF, compared with 51% for BUFLU: again, the difference is not due to poor performance of the BUFLU arm, because in the randomized BUFLU versus BUCY study, the 5-year survival in the BUFLU arm was quite comparable (55%), and that study excluded hypoplastic AML with additional cytogenetic abnormalities and secondary AML (7). The survival difference was still there when excluding HAPLO grafts in the matched pair analysis (52% versus 64%) (P = .07), which reached statistical significance in the multivariate analysis. Finally, when selecting only TBF patients, the 5-year survival was absolutely identical for patients receiving a graft from a HAPLO or another donor. This was confirmed in a multivariate Cox analysis on survival: after correcting for confounding factors, the difference in relapse and survival appears not due to a difference in donor type or a difference in GVHD prophylaxis.

In conclusion, the conditioning regimen TBF appears to reduce the risk of relapse in patients with AML in first or second remission undergoing an allogeneic HSCT as compared with a conventional BUFLU. A prospective randomized trial comparing TBF and BUFLU in patients with AML is currently being activated.

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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.2019.12.725.

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