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Impact of Conditioning Regimen on Outcomes for Children with Acute Myeloid Leukemia Undergoing Transplantation in First Complete Remission. An Analysis on Behalf of the Pediatric Disease Working Party of the European Group for Blood and Marrow Transplantation

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A B S T R A C T

Hematopoietic stem cell transplantation (HSCT) represents the cornerstone of treatment in pediatric high-risk and relapsed acute myeloid leukemia (AML). The aim of the present study was to compare outcomes of pediatric patients with AML undergoing HSCT using 3 different conditioning regimens: total body irradiation (TBI) and cyclophosphamide (Cy); busulfan (Bu) and Cy; or Bu, Cy, and melphalan (Mel). In this retrospective study, registry data for patients > 2 and <18 years age undergoing matched allogeneic HSCT for AML in first complete remission (CR1) in 204 European Group for Blood and Marrow Transplantation centers between 2000 and 2010 were analyzed. Data were available for 631 patients; 458 patients received stem cells from a matched

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sibling donor and 173 from a matched unrelated donor. For 440 patients, bone marrow was used as stem cell source, and 191 patients received peripheral blood stem cells. One hundred nine patients received TBICy, 389 received BuCy, and 133 received BuCyMel as their preparatory regimen. Median follow-up was 55 months. Patients receiving BuCyMel showed a lower incidence of relapse at 5 years (14.7% versus 31.5% in BuCy versus 30% in TBICy, $P < .01$) and higher overall survival (OS) (76.6% versus 64% versus 64.5%, $P = .04$) and leukemia-free survival (LFS) (74.5% versus 58% versus 61.9%, $P < .01$), with a comparable nonrelapse mortality (NRM) (10.8% versus 10.5% versus 8.1%, $P = .79$). Acute graft-versus-host disease (GVHD) grades III and IV but not chronic GVHD, was higher in patients receiving BuCyMel. Older age at HSCT had an adverse impact on NRM and the use of peripheral blood as stem cell source was associated with increased chronic GVHD and NRM as well as lower LFS and OS. Among pediatric patients receiving HSCT for AML in CR1, the use of BuCyMel conditioning proved superior to TBICy and BuCy in reducing relapse and improving LFS.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has historically being used to consolidate remission or treat relapse in acute myeloid leukemia (AML) in children and adults [1,2]. The 2 most common conditioning regimens used in this context have been based either on total body irradiation (TBI) or on busulfan (Bu) [3-12].

Thomas and colleagues were the first to describe the use of TBI for AML and reported a reduced toxicity by using a fractionated TBI schedule [13,14]. Santos first described the use of Bu-based regimens associated with cyclophosphamide (Cy) 200 mg/kg [15]. This regimen showed a low relapse rate but increased transplantation-related mortality, which could be corrected reducing the Cy dose to 120 mg/kg as described by Tutschka et al [16]. Blaise and Ringden published 2 randomized trials, which compared TBICy120 versus BuCy120 and showed higher nonrelapse mortality (NRM) and higher relapse incidence in the busulfan arm [17,18]. Nevertheless, further updates on these studies provided longer follow-up and failed to document a worse outcome in Bu-treated patients [19]. De Berranger et al. also concluded there was no advantage of 1 regimen over the other when considering only a pediatric population with AML in first complete remission (CR1) at HSCT [20]. A more recent prospective study from Ishida et al. confirmed no significant outcome differences in children with AML in CR1/CR2 receiving TBICy or intravenous BuCy [21]. In the recent literature, a single retrospective study compared intravenous- (i.v.) administered Bu as opposed to oral Bu, acknowledging the potential role of more predictable drug distribution for i.v. Bu [22]. Copelan et al. documented lower NRM and relapse in patients with AML in CR1 treated with i.v. BuCy as compared to those treated with TBICy and lower incidence of relapse as compared to both TBICy– and oral BuCy-treated patients. Overall, these data have resulted in a reduced use of TBI-based conditioning regimen in the AML pediatric population, as long-term TBI-related toxicity represents a significant burden for surviving children. In the attempt of improving the incidence of relapse in high-risk patients, in 1994 Locatelli et al. proposed the use of Bu associated with Cy 120 mg/kg and melphalan (Mel) 140 mg/m^2 as a conditioning regimen for the treatment of pediatric myelodysplastic syndrome [23]. In that context, 6 out of 8 described patients survived long term and a single child died from potentially Bu-related toxicity (idiopathic pneumonia). Intensification of chemotherapy regimens has often reported significant results in the context of AML [24], and the proposed combination of alkylating agents, though potentially associated with higher risk of acute toxicity, could in theory provide higher stem cell toxicity, thus targeting residual leukemic progenitor cells. BuCyMel has, therefore, been incorporated into national prospective/retrospective studies. The Italian national protocol for AML from Associazione Italiana di Ematologia e Oncologia Pediatrica enrolled 141 patients conditioned with either oral or i.v. Bu who underwent transplantation in CR1 for standard or high-risk cytogenetics. This study reported a remarkably low transplantation-related mortality of 7% and a leukemia-free survival (LFS) of 73%, with a relapse incidence of 17% at 8 years [25].

Encouraging results have been reported by a German retrospective study [26] too, where the same conditioning regimen was applied to pediatric patients with AML in ≥CR2. NRM was limited at 12%, 5-year LFS was 56%, and relapse was 33%. In both studies, the authors underscored the possible beneficial effect of intensified chemotherapy as a key factor in obtaining satisfactory long-term outcomes.

Concern regarding possible conditioning-related toxicity emerged from other studies. Strahm et al. reported a significant increase in NRM for children with myelodysplastic syndrome undergoing HSCT without previous chemotherapy. Overall, NRM at 5 years was as high as 21% with an increased risk of developing life-threatening toxicity in patients above the age of 12 (33% NRM) [27]. Similarly, but in other contexts, BuCyMel has been reported as severely toxic ([28], and R. Pieters, personal communication with regard to the Interfant 99 protocol amendment; May 23, 2012), raising possible concerns about the risk/benefit ratio even for patients with high risk of relapse. To the best of our knowledge, there are no data specifically comparing BuCyMel with BuCy or TBICy conditioning regimens for pediatric patients with AML in CR1. The current retrospective registry study aims to investigate the role of BuCyMel in this context and its possible prognostic impact.

PATIENTS AND METHODS

Data Source

We searched the registry of the European Group for Bone and Marrow Transplantation (EBMT) for demographic, laboratory, and clinical data. Data were provided and approved for this study by the institutional review board of the Pediatric Disease Working Party of the EBMT group registry. EBMT is a voluntary working group, including over 500 transplantation units that are requested to report all consecutive HSCT and follow-up visits once each year. Audits are routinely performed to ensure the accuracy of the data. According to EBMT rules, patients gave informed consent for data entry into the EBMT registry database and use for analysis in accordance with the Declaration of Helsinki. The participating centers are listed in the supplementary information, Appendix S1.

Patients

All patients ages >2 to <18 years of age undergoing a first allogeneic HSCT from a matched sibling or unrelated donor for AML in CR1 in 1 of the EBMT centers between 2000 and 2010 were eligible for this study. Patients below

the age of 2 were excluded from the study as they were mostly conditioned without TBI. Patients receiving HSCT from umbilical cord blood were excluded from the study. Patients with AML French-American-British subtype acute promyelocytic leukemia (M3) subtype and juvenile myelomonocytic leukemia were excluded from the study.

CR was defined by the absence of physical signs of leukemia or detectable leukemia cells on blood smears, bone marrow with normal hematopoiesis, and <5% leukemia blasts cells (morphologically identified).

Transplantation Procedure

HLA compatibility with an adult related or unrelated donor was defined as by high/medium-resolution typing for HLA-A, B, -C, -DR, and -DQ loci. HLA compatibility with a sibling donor was defined by low-resolution typing for HLA-A, -B, -C, -DR, and -DQ. Donors were defined as *HLA-identical sibling* if they inherited the same parental haplotypes as their recipients. Donor-recipient pairs were defined as HLA matched if they were ≥9/10 (adult donor) matched at allelic or antigen levels. The indication for transplantation in in CR1 was defined per each center's protocols. Patients received 1 of the following conditioning regimens: (1) Cy 120 mg/kg with TBI with a hyperfractionated regimen (7 to 14.4 Gy, median dose 12); (2) Bu, median dose of 16 mg/kg (range, 8 mg/kg to 23 mg/kg) with 120 or 200 mg/kg Cy; or (3) Bu, median dose of 16 mg/kg (range, 10 mg/kg to 20 mg/kg) with 120 mg/kg Cy and 140 mg/m^2 Mel. Patients receiving Bu with Cy 120 mg/kg or 200 mg/kg were considered part of the same conditioning group, as analysis of the outcome data did not differ between patients receiving the 2 different doses of Cy, as reported in Supplementary Table S1.

Among the participating centers using Bu-based conditioning regimens, for the years considered for the present study, 31% were performing Bu-targeted administration based on the cumulative predicted area under the curve, but specific data for the patients in this study are not available. *Neutrophil engraftment* was defined as granulocyte count $>.5 \times 10^9$/L for 3 consecutive days after HSCT.

Study Endpoints

The primary endpoint of the study was 5-year LFS and overall survival (OS) after HSCT in children with AML in CR1 at HSCT. *LFS* was defined as the time from the date of transplantation to the date of relapse or death from any cause, whichever comes first. *OS* was defined as the time from HSCT to death, regardless of the cause. Secondary endpoints included the incidences of acute and chronic graft-versus-host disease (GVHD), relapse, and NRM. GVHD was diagnosed and graded according to internationally accepted criteria [29,30]. *NRM* was defined as any death occurring in complete remission.

Statistical Analysis

Patient data were collected through the EBMT database. Data were checked for consistency and final analysis was performed by means of the statistical software R version 3.2.3.

Descriptive analyses results are reported as medians and ranges for continuous variables and as number and percent for categorical variables. Kaplan-Meier estimators and confidence intervals were used to estimate LFS and OS and the log-rank test (discrete variables) was used for comparison [31]. Patients were censored at last follow-up if no events occurred. The cumulative incidences of relapse and NRM were estimated allowing for competing risks by the Fine-Grey method [32]. The Cox proportional hazard regression models were used to evaluate the impact on relapse, LFS, and OS of the following variables: age at transplantation, conditioning regimen, donor type (sibling versus unrelated), time from diagnosis to transplantation, stem cell source, presence of GVHD, and year of transplantation. Confidence intervals are reported at the 95% and statistical tests were performed at the .05 level (2-sided).

RESULTS

Patient and Transplantation Characteristics

Six hundred thirty-one patients between the age of 2 and 18 were identified via the EBMT registry as HSCT recipients from matched related or unrelated donors for AML in CR1 at HSCT between 2000 and 2010, following 1 of the 3 above-mentioned myeloablative conditioning regimens.

The median follow-up for the patients' cohort is 55 months (range, 2 to 173.8). Patients were reported by 204 centers. Patient and transplantation characteristics according to the conditioning regimen are summarized in Table 1. Patients who received TBI were significantly older and underwent transplantation earlier in time than patients receiving Bu-based conditioning.

Overall, there were no significant differences among the 3 groups of patients in terms of transplantation characteristics. Cytogenetic remission status at time of transplantation was available only for a minority of patients (overall 190 patients; ie, 30% of the cohort). According to the available data, a similar percentage of patients were in a cytogenetic remission at transplantation among the 3 conditioning regimen groups (88.2% versus 89.8% versus 92.9%, $P = 1.00$).

Data about the route of Bu administration were available for 66% of the reported patients. Oral administration was significantly more frequent in patients receiving BuCyMel than in those receiving BuCy conditioning (85.5% versus 44.5%, $P < .001$).

Outcomes

Outcomes per conditioning regimen are shown in Table 2. Engraftment was achieved in 608 patients (98.4%), with no differences across conditioning regimens. NRM in the whole cohort of patients was 10.1% (95% confidence interval [CI], 7.7 to 12.8), with no significant difference according to con-

Table 1
Patient and Transplantation Characteristics according to the Myeloablative Conditioning Regimen

Characteristic	TBICy (n = 109)	BuCy (n = 389)	BuCyMel (n = 133)	Total (n = 631)	*P* Value (95% CI)
Age at HSCT, median (range), yr	14.8 (2.1-17.9)	11.7 (2-18)	9.8 (2.1-17.9)	11.9 (2-18)	.0001
Year of HSCT, median	2005	2007	2007	2007	.0001
Diagnosis to HSCT median, range	4.7 (2-20.5)	4.8 (.8-66.6)	5.3 (1.8-50.1)	4.9 (.8-66.6)	.0345
Patient sex: male	64 (58.7%)	206 (53%)	79 (59.4%)	349 (55.3%)	1.00
Donor sex: male	68 (63.6%)	212 (55.5%)	69 (52.3%)	349 (56.2%)	1.00
Female donor to male recipient	22 (20.6%)	87 (22.8%)	33 (25%)	142 (22.9%)	1.00
Donor type					
MSD	74 (67.9%)	302 (77.6%)	82 (61.7%)	458 (72.6%)	.148
MUD	35 (32.1%)	87 (22.4%)	51 (38.3%)	173 (27.4%)	
In vivo T cell depletion: yes	29 (26.6%)	67 (17.2%)	42 (31.6%)	138 (21.9%)	.127
Stem cell source					.127
BM	82 (75.2%)	250 (64.3%)	108 (81.2%)	440 (69.7%)	
PB	27 (24.8%)	139 (35.7%)	25 (18.8%)	191 (30.3%)	
Busulfan					<.0001
Missing		153	23		
Oral	NA	105 (44.5%)	94 (85.5%)		
Intravenous	NA	131 (55.5%)	16 (14.5%)		

MSD indicates matched sibling donor; MUD, matched unrelated donor; BM, bone marrow; PB, peripheral blood.

Table 2
Patient Outcome according to Conditioning Regimen

Outcome	TBICy (n = 109)	BuCy (n = 389)	BuCyMel (n = 133)	Total (n = 631)	*P* Value (95% CI)
Engraftment	100 (95.6-100)	97.9 (95.7-99)	98.4 (93-99.7)	98.4 (96.9-99.1)	.065
aGVHD grade ≥ II	31.1 (22.5-41.1)	29.3 (24.8-34.3)	42.7 (34.2-51.7)	32.5 (28.8-36.4)	.233
aGVHD grade ≥ III	6.8 (3.0-14.0)	9.6 (6.9-13.2)	17.6 (11.7-25.4)	10.8 (8.5-13.6)	.052
cGVHD	27.8 (18.6-37.9)	25.8 (20.7-31.1)	35.2 (26-44.5)	27.5 (23.7-31.7)	1.00
Five-year NRM	8.1 (3.7-14.7)	10.5 (7.4-14.1)	10.8 (6.2-16.8)	10.1 (7.7-12.8)	.79
Five-year relapse	30 (21.4-39)	31.5 (26.5-36.7)	14.7 (9.2-21.5)	27.3 (23.7-31.1)	<.001
Five-year LFS	61.9 (53.1-72.2)	58 (52.8-63.7)	74.5 (67.3-82.4)	62.6 (58.7-66.8)	.005
Five-year OS	64.5 (55.6-74.9)	64 (58.7-69.7)	76.6 (69.6-84.4)	67.2 (63.3-71.3)	.041

Data presented are % (95% CI) unless otherwise indicated.
aGVHD indicates acute graft-versus-host disease; cGVHD, chronic GVHD.

ditioning regimen (Figure 1A). Overall, 51 patients died from nonrelapse-related causes; detailed causes of death are listed in Table 3. Patients above the age of 10 at HSCT had a considerably higher chance of experiencing NRM than younger patients (13.1% versus 5.7%, $P < .01$) as did recipients of peripheral blood stem cells compared with those who received bone marrow (15.4% versus 8%, $P < .01$) and those who underwent transplantation after more than 1 year from diagnosis

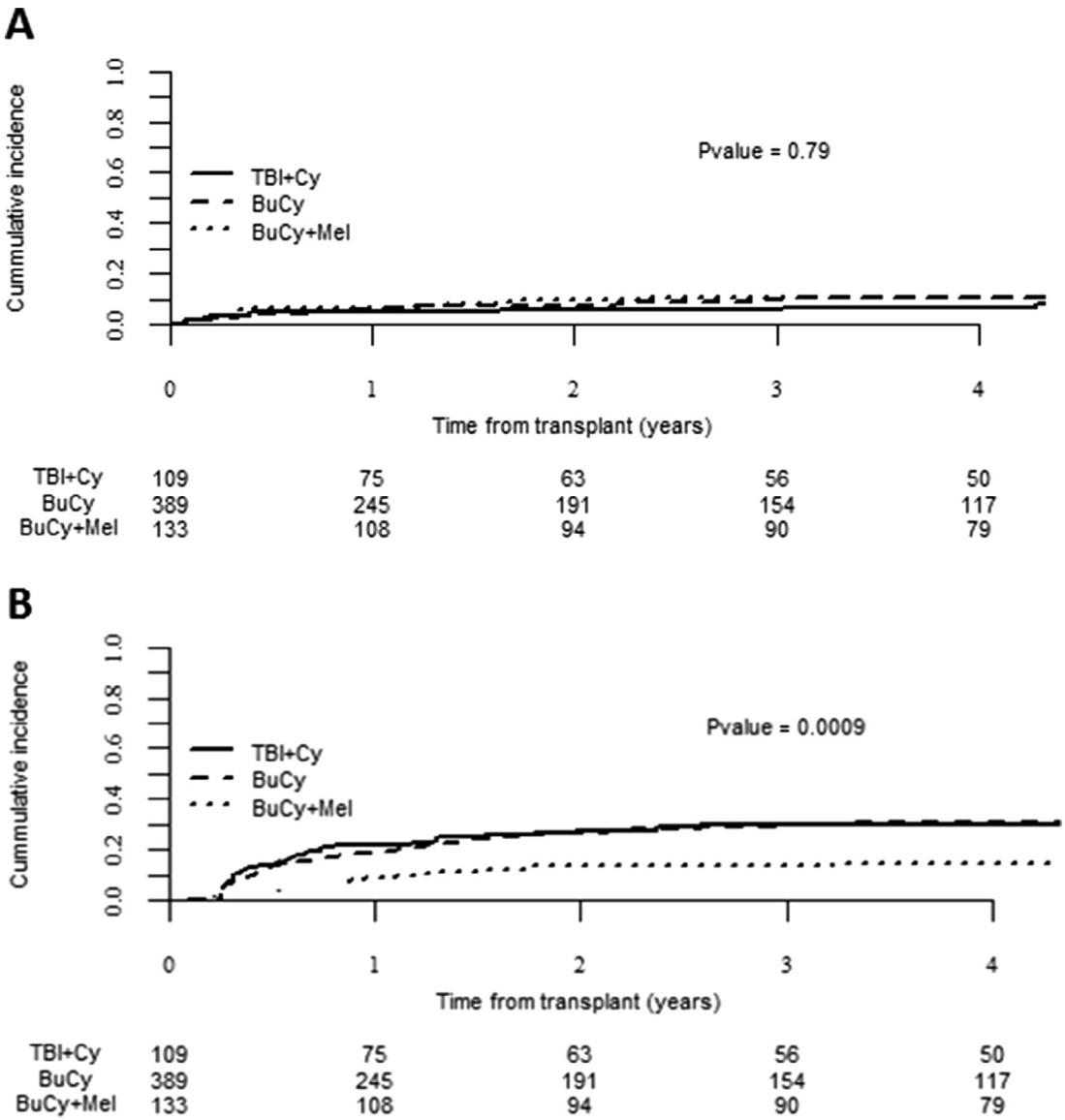

Figure 1. (A) Cumulative incidence of nonrelapse mortality according to conditioning regimen. (B) Cumulative incidence of relapse according to conditioning regimen.

Table 3
Causes of NRM according to Conditioning Regimen

Cause of Death	TBI/Cy (n = 109)	Bu/Cy (n = 389)	Bu/Cy/Mel (n = 133)	Total (n = 631)
Graft failure/rejection	0	1 (.2%)	1 (.7%)	2 (.3%)
Hemorrhage	0	0	1 (.7%)	1 (.2%)
Veno-occlusive disease	0	0	1 (.7%)	1 (.2%)
Infection	6 (5.5%)	6 (1.5%)	6 (4.5%)	18 (2.9%)
Idiopathic pneumonia	0	1 (.2%)	0	1 (.2%)
GVHD related	2 (1.8%)	15 (3.9%)	4 (3.0%)	21 (3.3%)
Cardiac toxicity	0	1 (.2%)	0	1 (.2%)
Other	0	6 (1.5%)	0	6 (1.0%)
Total	8 (7.3%)	30 (7.7%)	13 (9.8%)	51 (8.0%)

Data presented are n (%) unless otherwise indicated.

(15.6% versus 9.6, $P = .01$). One hundred ninety-eight patients (32.5%) developed grade ≥ II acute GVHD, and severe grade III/IV acute GVHD was observed in 66 patients (10.8%). There was a trend toward more frequent severe acute GVHD in patients conditioned with BuCyMel (TBICy, 6.8%; BuCy, 9.6%; BuCyMel, 17.6%; $P = .05$), but the incidence of GVHD-related death in the BuCy-conditioned and BuCyMel-conditioned group of patients was similar among the groups (Table 3). Chronic GVHD occurred in 138 patients (27.5%), with no difference across the conditioning regimen used. Male patients receiving HSCT from a female donor and patients receiving peripheral blood stem cell as a source of graft had a significantly higher chance of developing chronic GVHD (40.8% versus 24.6%, $P < .01$ and 34.9% versus 25.3%, $P = .02$, respectively) (Table 4). OS for the whole population was 67.2% (95% CI, 63.3% to 71.3%). Relapse remained the primary cause of death in our cohort. Overall, relapse incidence was 27.3% (95% CI, 23.7% to 31.1%) (Table 2). Patients conditioned with BuCyMel had a statistically significant lower chance of developing disease relapse than both TBICy- and BuCy-conditioned patients (14.7% versus 30% versus 31.5%, $P < .01$) (Figure 1B), with no other transplantation characteristics prognostic for relapse incidence (Table 4). As a consequence, 5-year LFS was significantly higher in patients conditioned with BuCyMel than in both TBICy- and BuCy-conditioned patients (Figure 2A). In the univariate analysis (Table 4), the only other parameter influencing long-term LFS apart from conditioning regimen was the stem cell source, with bone marrow recipients showing increased survival compared with peripheral blood recipients (65.7 versus 55.3, $P < .01$). BuCyMel conditioning as well as younger age at transplantation, matched sibling donor, and bone marrow as stem cell source were favorable prognostic factors for OS in the univariate analysis as described in Table 4 and Figure 2B.

Table 4
Univariate Analysis for Outcome Variables other than Conditioning Regimen

Variables	Five-Year Relapse	Five-Year NRM	Five-Year LFS	Five-Year OS	Five-Year cGVHD
Patient sex					
Male	26.6 (21.7-31.6)	10 (6.9-13.7)	63.5 (58.2-69.2)	67.7 (62.4-73.3)	34.6 (28.7-40.7)
Female	28.3 (22.8-33.9)	10.1 (6.8-14.2)	61.6 (55.8-67.9)	66.5 (60.8-72.7)	20.5 (15.4-26.1)
P Value	.48	.85	.47	.56	**.002**
Donor sex					
Male	28.6 (23.7-31.6)	9 (6.1-12.4)	62.4 (57.2-68.1)	67.4 (62.3-73)	25.6 (20.4-31.1)
Female	25.9 (20.5-31.6)	11.8 (8-16.4)	62.3 (56.4-68.8)	66.5 (60.5-72.9)	31.5 (23.2-38.1)
P Value	.37	.73	.48	.89	.15
Female donor to male recipient					
Yes	25.2 (18-32.9)	11.4 (6.4-17.9)	63.5 (55.5-72.5)	67.3 (59.4-76.2)	40.8 (30.9-50.4)
No	28.1 (23.9-32.5)	9.9 (7.3-12.9)	62 (57.5-66.8)	66.9 (62.4-71.7)	24.6 (20.2-29.2)
P Value	.51	.99	.53	.73	**.002**
Donor type					
MSD	26.6 (22.3-31)	9.4 (6.8-12.6)	64 (59.4-68.9)	69.6 (65.1-74.3)	27.2 (22.6-32.1)
MUD	29.3 (22.3-36.6)	11.6 (7.2-17.1)	59.1 (51.9-67.4)	60.6 (53-69.2)	30.1 (22.3-38.3)
P Value	.33	.18	.05	**.02**	.29
Age at HSCT, yr					
≤10	26.5 (20.9-32.3)	5.7 (3.3-9.1)	67.8 (62-74.1)	73.5 (67.9-79.5)	25.6 (19.5-32.1)
10	27.9 (23.2-32.9)	13.1 (9.7-17.1)	59 (53.8-64.6)	62.8 (57.5-68.4)	29.7 (24.4-35.1)
P Value	.67	**.009**	.06	**.02**	.47
Stem cell source					
BM	26.4 (22.1-30.8)	8 (5.6-10.9)	65.7 (61.1-70.6)	71.8 (67.4-76.5)	25.3 (20.7-30.1)
PB	29.3 (22.5-36.4)	15.4 (10.3-21.6)	55.3 (48-63.6)	55.7 (48.1-64.4)	34.9 (27-42.9)
P Value	.27	**.007**	**.006**	**< .0001**	**.02**
Time from dx to HSCT					
>1 Year	28 (24.2-32)	9.6 (7.3-12.4)	62.3 (58.3-66.7)	67 (63-71.3)	28.2 (24-32.5)
<1 Year	18.4 (8.5-31.3)	15.6 (6.8-27.8)	66 (53.3-81.7)	68.8 (55.8-84.8)	26.7 (12-43.4)
P Value	.24	**.02**	.58	.59	.94
Year of HSCT					
≤ 2007	25.9 (21.3-30.7)	9.2 (6.4-12.6)	65 (60-70.3)	69.9 (65.1-75)	28.6 (23.3-34)
> 2007	29.8 (23.8-36.1)	11.6 (7.8-16.4)	58.5 (52.2-65.6)	62.5 (55.6-70.2)	26.5 (20.7-32.8)
P Value	.40	.48	.27	.13	.74

Bold typeface indicates statistical significance.

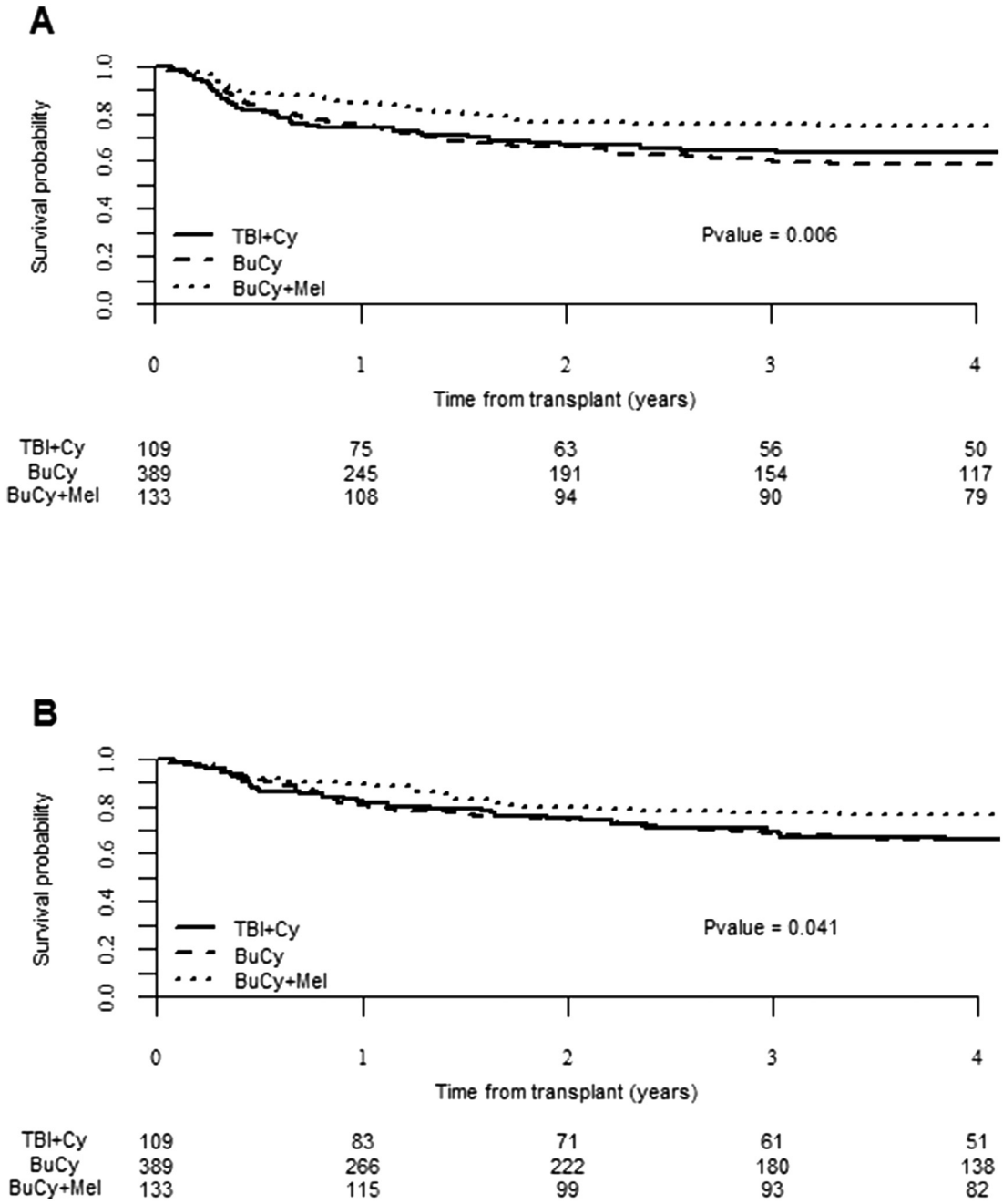

Figure 2. (A) Leukemia-free survival estimation according to conditioning regimen. (B) Overall survival estimation according to conditioning regimen.

Multivariate Analysis

Results from the multivariate analysis are summarized in Table 5. In our cohort of patients, BuCyMel as a conditioning regimen was demonstrated to be the only significant factor in reducing the relapse risk with an odds ratio (OR) of .44 (95% CI, .25 to .8; $P < .01$). Older age at HSCT, longer gap between diagnosis and HSCT, peripheral blood as stem cell source, and severe acute GVHD statistically increased the risk for NRM, with OR of 1.1 (95% CI, 1.04 to 1.19), 1.7 (95% CI, 1.15 to 2.41), 2.1 (95% CI, 1.13 to 3.8), and 4.2 (95% CI, 2.01 to 8.68), respectively. Overall, BuCyMel as a conditioning regimen had a favorable prognostic impact on LFS with an OR of .57 (95% CI, .35 to .94) when compared with TBICy and BuCy. Receiving HSCT from peripheral blood stem cells had a negative impact on both LFS and OS in our cohort of patients, with OR of 1.40 (95% CI, 1.03 to 1.9) and 1.83 (95% CI, 1.32 to 2.54), respectively. Another negative prognostic factor on LFS and OS proved to be receiving HSCT from a matched unrelated as opposed to a matched sibling donor, with OR of 1.46 (95% CI, 1.05 to 2.03) and 1.56 (95% CI, 1.09 to 2.22), respectively.

Discussion

The optimal conditioning regimen for children with AML is still debated.

Although older studies pointed at a superiority of TBI-based regimens over Bu-based regimens [17,18], more recent

Table 5
Multivariate Analysis according to Cox Regression Model

Risk Factor	OR (95% CI) and *P* value			
	Relapse	NRM	LFS	OS
BuCy versus TBICy	.96 (.63-1.46) .84	1.63 (.69-3.83) .27	1.07 (.73-1.56) .72	1.04 (.69-1.58) .84
BuCyMel versus other conditioning	**.44 (.25-0.8)** **< .01**	1.22 (.45-3.32) .70	**.57 (.35-0.94)** **.03**	.61 (.36-1.03) .07
Age at HSCT >10 yr	1.00 (.97-1.04) .82	**1.11 (1.04-1.19)** **< .01**	1.03 (1-1.06) .08	1.03 (1-1.06) .09
MUD versus MSD	1.44 (.96-2.13) .07	1.61 (.85-3.06) .15	**1.46 (1.05-2.03)** **.03**	**1.56 (1.09-2.22)** **.02**
Time from dx to HSCT (>1 yr)	.81 (.5-1.3) .38	**1.66 (1.15-2.41)** **< .01**	1.1 (.81-1.49) .55	1.14 (.84-1.55) .39
aGVHD	.44 (.18-1.09) .08	**4.17 (2.01-8.68)** **< .01**	1.11 (.65-1.89) .70	1.52 (.88-2.61) .13
Stem cell source PB versus BM	1.24 (.86-1.78) .25	**2.07 (1.13-3.8)** **.02**	**1.40 (1.03-1.9)** **.03**	**1.83 (1.32-2.54)** **< .01**
Year of HSCT before 2007	1 (.95-1.06) .97	.99 (.9-1.1) .91	1 (.95-1.05) .99	1.01 (.96-1.06) .72

Bold typeface indicates statistical significance.
Dx indicates diagnosis.

studies failed to identify significant differences between the 2 strategies, possibly because intravenous Bu and targeted Bu exposure were introduced [20,21]. Overall, the equivalence between TBICy and BuCy preparative regimens became of paramount importance in pediatrics, as BuCy allowed avoiding TBI exposure in children, which invariably leads to severe long-term sequelae. Nevertheless, the available studies have so far failed to identify a conditioning regimen capable of affecting the post-HSCT relapse risk, which still represents the most frequent and less treatable complication for AML patients. To our knowledge, none of the above-mentioned comparative studies included a significant number of patients exposed to BuCyMel as preparative regimen.

BuCyMel has shown remarkable results in pediatric prospective and retrospective studies. Nonetheless, concerns have been raised about its possible severe toxicity profile [25-28]. Admittedly, Bu had been administered with inconsistent therapeutic drug monitoring (TDM) in some of the studies, reporting increased toxicity. This might have had a strong impact on Bu-related comorbidities, as significant data are available, suggesting that toxicity correlates to cumulative Bu exposure [33].

To understand if BuCyMel compared favorably with other conditioning regimens in children with AML in CR1, we retrospectively analyzed registry data from a large cohort of patients.

The current study suffers from intrinsic limitations due to his retrospective nature. Moreover, we had limited available information about the cytogenetic risk/status of the who underwent transplantation patients as well as no details about Bu TDM.

However, BuCyMel was associated with a significant reduction of relapse incidence compared with both TBICy and BuCy. Moreover, the use of BuCyMel did not increase the rate of NRM. This allowed for LFS and OS of 74.5% and 76.6%, respectively, which are in keeping with other cohorts of children who underwent transplantation for AML in CR1 with BuCyMel. The better outcomes in BuCyMel-conditioned patients might simply rely on the intensification of the chemotherapy or might be related to some increased graft-versus-leukemia effect, as patients conditioned with BuCyMel tended towards a higher incidence of severe GVHD, and previous papers have clearly shown an association between Mel administration and increased gut GVHD [34]. Our data also confirmed previously reported observations. In line with recent data from Ishida et al., we demonstrated that TBICy and BuCy provide similar outcomes in pediatric patients with AML in CR1 [21]. As documented by Locatelli et al. and by Beier et al., we report that older pediatric patients experience a significantly higher risk of NRM [25,26]. Interestingly, though, in our study no Bu-specific toxicity was found to be the cause for increasing deaths in these patients, as infections and GVHD were the prominent causes of toxic deaths. In this regard, it would be useful to compare other conditioning strategies to BuCyMel. Bartelnik et al. have, for instance, showed in smaller numbers that no significant outcome difference was documented between pediatric patients with myeloid malignancies treated with BuCyMel and those treated with Bu with fludarabin, but less toxicity was documented in the latter group of patients [35]. More recently, data about the use of treosulfan-based conditioning are becoming available for pediatric patients affected with malignancies, and they seem promising in terms of reduced toxicity, though efficacy has not been extensively examined yet in patients in first CR at transplantation [36]. Other groups (United Kingdom and France) are currently investigating in a randomized prospective study the use of Bu/fludarabine (reduced intensity) versus BuCy in pediatric AML in CR1 (NCT 02724163). As data become available, it will be interesting to compare these alternative approaches to BuCyMel, with the specific aim of finding the right balance between toxicity and efficacy.

In our data, we were also able to confirm that peripheral blood as stem cell source in non T cell–depleted grafts significantly increases the risk of GVHD and NRM, and should, therefore, be avoided whenever possible, as it undermines the long-term survival of treated patients [37-39].

According to our multivariate analysis, recipients of sibling donor grafts had a superior survival compared with matched unrelated recipients, which is unusual considering recently published data. To explain this result, we should underscore that the definition of matched unrelated donor in our study was quite broad and included some 9/10 HLA–matched donors, thereby possibly accounting for higher toxicity risk.

Overall, we were able to demonstrate that increasing the intensity of conditioning regimen for children with AML in CR1 might be a key factor in preventing relapse. We showed that this could be achieved with BuCyMel administration,

witnessing some toxicity but without significantly increasing the risk of NRM in the context of adequate supportive care, particularly in younger patients. These data support the use of BuCyMel with appropriate TDM as a preparative regimen for AML patients in CR1 at higher risk of relapse (ie, those with high-risk cytogenetics).

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APPENDIX. SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2016.11.022.

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