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Busulfan or Treosulfan Conditioning Platform for Allogeneic Stem Cell Transplantation in Patients Aged >60 Y With Acute Myeloid Leukemia/ Myelodysplastic Syndrome: A Subanalysis of the GITMO AlloEld Study

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Background. The conditioning regimens with different alkylators at different doses can influence the outcome of allogeneic stem cell transplantation (SCT), but conclusive data are missing. **Methods.** With the aim to analyze real-life allogeneic SCTs performed in Italy between 2006 and 2017 in elderly patients (aged >60 y) with acute myeloid leukemia or myelodysplastic syndrome, we collected 780 first transplants data. For analysis purposes, patients were grouped according to the type of alkylator included in the conditioning (busulfan [BU]-based; n = 618; 79%; treosulfan [TREO]-based; n=162; 21%). **Results.** No significant differences were observed in nonrelapse mortality, cumulative incidence of relapse, and overall survival, although in the TREO-based group, we observed a greater proportion of elderly patients ($P < 0.001$); more active diseases at the time of SCT ($P < 0.001$); a higher prevalence of patients with either hematopoietic cell transplantation-comorbidity index ≥3 ($P < 0.001$) or a good Karnofsky performance status ($P = 0.025$); increased use of peripheral blood stem cells as graft sources ($P < 0.001$); and greater use of reduced intensity conditioning regimens ($P = 0.013$) and of haploidentical donors ($P < 0.001$). Moreover, the 2-y cumulative incidence of relapse with myeloablative doses of BU was significantly lower than that registered with reduced intensity conditioning (21% versus 31%; $P = 0.0003$). This was not observed in the TREO-based group. **Conclusions.** Despite a higher number of risk factors in the TREO group, no significant differences were observed in nonrelapse mortality, cumulative incidence of relapse, and overall survival according to the type of alkylator, suggesting that TREO has no advantage over BU in terms of efficacy and toxicity in acute myeloid leukemia and myelodysplastic syndrome.

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During the past 2 decades, significant improvements have been recorded in the efficacy and safety of allogeneic stem cell transplantation (allo-SCT) for treating several high-risk hematological malignancies, especially in patients aged >60 y.[1,2] The improved selection of patients and donors using high-resolution HLA typing, the more effective prophylaxis and therapy of graft versus host disease (GVHD), and the improvement of anti-infective supportive treatments have led to a significant amelioration of transplant outcomes and, in particular, of nonrelapse mortality (NRM).[3-8] In contrast, in the same time frame, there has been a progressive decrease in cumulative incidence of relapse (CIR) thanks to advances in conditioning regimens that reduce the toxicity while maintaining or improving the antileukemic activity.[9-13]

All these relevant changes have enabled the transplant age limit to be extended from 45 to 75 y and ensure a higher probability of cure to a larger number of patients, even those aged >60 y, and mainly affected by acute myeloid leukemia (AML) or high-risk myelodysplastic syndromes (MDSs). These latter diseases not only occur predominantly in the elderly but continue to be orphans of potential curative drugs. Thus, the issue of disease eradication remains an unmet clinical need and AML and MDSs remain the most frequent indication of allo-SCT worldwide.[14]

Recently, the Italian Group for Bone Marrow Transplantation, Haematopoietic Stem Cells and Cell Therapy (GITMO) conducted a registry-based retrospective study (AlloEld) reporting the distribution, characteristics, safety, and efficacy of allo-SCTs performed in Italy from 2000 to 2017 in patients aged >60 y.[2] Briefly, this survey showed a significant increase in the number of transplants in patients aged >60 y over time, but also the clinicians' greater propensity to use allo-SCT to cure diseases such as AML and MDSs. In this patient setting, the transplants increased significantly between 2012 and 2017 and reached 27% of the transplants registered in the GITMO database. During this time, the conditioning regimens varied significantly in both intensity and combinations, and they moved from standard myeloablative conditioning (MAC) to reduced-intensity conditioning (RIC) and, finally, from RIC to reduced-toxicity MAC.[9-13] Focusing on the conditioning regimen, total body irradiation was progressively abandoned in favor of regimens based on alkylators such as busulfan (BU), thiotepa, and treosulfan (TREO), which is used even more in RIC and haploidentical settings.[2]

In the MC-FludT.14/L trial, TREO 10 g/m^2/d for 3 d was randomly compared with BU 3.2 mg/kg/d for 2 d, both in combination with fludarabine, in AML and MDS patients, and it was demonstrated to be superior to BU in terms of long-term outcomes.[15] Following these results, TREO, in combination with fludarabine, was approved in Europe as a conditioning regimen for elderly patients with AML/MDS, and important retrospective studies confirmed its efficacy and limited toxicity.[16] Consequently, the use of TREO as an alkylator increased over time, but we have no data concerning its real-life use, at least in Italy.

In this study, we evaluated the distribution, characteristics, safety, and efficacy outcomes of allo-SCTs performed in Italy from 2006 to 2017 using the BU and TREO regimens in patients aged >60 y with AML and MDSs.

MATERIALS AND METHODS

This study is a subanalysis of 780 unselected first transplants performed in patients with AML or MDSs included in the GITMO AlloEld study (ClinicalTrials.gov: NCT04469985): a retrospective, nationwide analysis of allo-SCTs performed in the elderly (patients aged >60 y) between 2000 and 2017.[2]

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For analysis purposes, the transplants performed in the time frame 2000 to 2005 (n = 6) were excluded to restrict the evaluation to patients undergoing more homogeneous transplant procedures. The patients of the present series represent 45% of the 1740 first transplants performed between 2006 and 2017 in the 30 Italian GITMO Centers participating in the study. The data were extracted from the European PROMISE database. The classification of the intensity of each conditioning regimen (RIC or MAC) was given by each Center at the time of data insertion in the PROMISE database. Following approval by local ethical committees, additional queries were submitted to each center to minimize missing data. All the patients included in this study provided informed consent for data registration in the PROMISE database. The study was conducted in compliance with current national and European legislation on clinical trials, in accordance with the Declaration of Helsinki and the principles of good clinical practice.

Statistical Analysis

Dichotomous variables were summarized as numbers and percentages and compared using the chi-square or Fisher exact test; continuous variables were summarized as median and range and compared using the Wilcoxon rank-sum or *T* test. Median follow-up was assessed with the method of reverse Kaplan-Meier method.[17]

Overall survival (OS) was calculated according to the Kaplan-Meier method, from the date of the transplant to the date of death or last follow-up; the log-rank test was used to detect significant differences among subgroups. NRM, CIR, and cumulative incidence of acute GVHD (aGVDH) and chronic GVDH (cGVHD) were calculated on the basis of competing risk models, and the Gray test was used to assess statistical differences among subgroups. Death without the event of interest was considered as a competitive risk.

Cox and Fine-Gray proportional hazard regressions were used for univariate and multivariate analyses of OS and NRM/CIR, respectively.

The following variables were included in the regression models: age at SCT (5-y interval), female donor versus male recipient, complete remission (CR) status at SCT, donor type (versus Matched Related Donor), PBSC, GVHD prophylaxis (versus antithymocyte globulin/anti T-lymphocyte globulin), in vivo T-cell depletion, SCT period (2012–2017), cytomegalovirus patient's serology, type of alkylator (TREO versus BU), MAC regimen, hematopoietic cell transplantation-comorbidity index (HCT-CI) ≥3, and Karnofsky performance status (KPS) 90 to 100. All resulting variables associated with OS and NRM with $P < 0.05$ in univariate analysis underwent multivariate analysis. Taking into account the correlation between GVHD prophylaxis and in vivo T-cell depletion ($P < 0.05$), only T-cell depletion was considered for multivariate analysis.

All *P* values of <0.05 were considered statistically significant. Statistical analysis was performed with EZR software (v.1.54).[18]

RESULTS

Clinical and Transplant characteristics of the study population

For analysis purposes, 2 groups of patients were considered, according to the alkylator included in the conditioning regimen: BU-based group (n = 618; 79%) and TREO-based group (n = 162; 21%). The median follow-up was 4.1 y (range, 0–12.7 y). The distribution of AML and MDSs in the 2 groups was super imposable (73% versus 75% and 27% versus 25%; $P = 0.352$). Concerning transplant time, one-third and two-thirds of the transplants in the TREO-based group were performed in the time frames 2006 to 2011 and 2012 to 2017, respectively, whereas >80% of the transplants in the BU-based group were performed between 2012 and 2017 ($P < 0.001$).

Patients' and transplants' characteristics are reported in Table 1. We observed several significant demographic differences. In particular, in the TREO-based group, we observed a greater proportion of (1) elderly patients (median age 65.04 versus 63.93; $P < 0.001$), (2) active diseases at the time of SCT (33% versus 28%; $P < 0.001$), (3) high-risk patients because of HCT-CI ≥3 (42% versus 28%; $P = 0.014$), (4) patients with a good KPS (100; 33% versus 27%; $P = 0.025$), (5) use of RIC regimens (61% versus 50%; $P = 0.013$), and (6) haploidentical donors (41% versus 27%; $P < 0.001$).

GVHD prophylaxis more frequently included posttransplant cyclophosphamide in the TREO group (32% versus 21%; $P < 0.001$). Of note, BU and TREO were used in combination with another alkylator in 309 of 618 (50%) and in 61 of 162 (38%) of the cases, respectively ($P = 0.005$). Thiotepa was combined with BU and TREO in 300 of 309 (97%) and 26 of 61 (43%) cases, respectively ($P < 0.001$); the other 35 cases of combination in the TREO group included melphalan (35/61; 47%)

NRM, CIR, OS, and GVHD incidence

Analysis of the BU-based versus TREO-based groups shows the cumulative incidence of NRM at 2 y to be 31.4% versus 28.8% ($P = 0.354$; Figure 1A), the CIR at 2 y 25.7% versus 26.8 (0.589; Figure 1B), and the probability of OS at 2 y was 45.6% versus 53.2%, respectively ($P = 0.221$; Figure 1C). The median OS of the BU-based versus TREO-based group was 541 (422–700) versus 847 (595–1465) d ($P = 0.221$). No differences in terms of CIR, NRM, and OS were observed considering patients with BU and TREO alone or in combination with the thiotepa or melphalan as a second alkylator (data not shown). Moreover, the 1 y, 2 y, and 5 y OS of AML versus MDS irrespective of conditioning platform was 57.5% versus 60.6%, 46.1% versus 50.4%, and 36.7% versus 37.6% ($P = 0.512$; data not shown).

Interestingly, within MAC and RIC regimens, the CIR was quite similar for the 2 alkylators, with the lowest CIR observed with myeloablative dose BU (CIR at 2 y 21%) and with TREO at reduced-intensity dose (CIR at 2 y was 28%; Figure 2).

Moreover, focusing on the 2 alkylators used in MAC versus RIC regimens, we observed that the 2-y CIR with BU at myeloablative dose was significantly lower than what was observed with reduced-intensity dose (21% versus 31%; $P = 0.0003$; Figure 3A). A similar significant difference was not observed in the TREO-based group (2 y CIR in MAC versus RIC regimens: 26% versus 28%; $P = 0.773$; Figure 3B).

The cumulative incidence rate of grade ≥II aGVHD at 100 d in BU versus TREO-based groups was 20% and 22%, respectively ($P = 0.365$; Figure 4A). Similarly, no differences were observed in the incidence of extensive cGVHD at 2 y in the BU-based versus TREO-based group (11% versus 14%, respectively; $P = 0.226$; Figure 4B).

Univariate and Multivariate Analyses

We performed univariate and multivariate analyses on OS, CIR, and NRM (Table 2).

TABLE 1.

Patient and transplant characteristics of the 780 allo-SCTs included in the analysis

Factor	Total (n = 780)	BU-based (n = 618; 79%)	TREO-based (n = 162; 21%)	*P*
Median age (range)	64.13 (59.6–77.80)	63.93 (59.6–73.51)	65.04 (60.07–77.80)	<0.001
Disease status at SCT				<0.001
CR	416 (53%)	334 (54%)	82 (50%)	
PR	69 (9%)	65 (11%)	4 (3%)	
NR	226 (29%)	173 (28%)	53 (33%)	
Frontline	61 (8%)	42 (7%)	19 (12%)	
Missing	8 (1%)	4 (1%)	4 (2%)	
Lines of therapy				<0.001
0	12 (2%)	12 (3%)	0 (0%)	
1 or 2	367 (47%)	332 (53%)	35 (22%)	
≥3	59 (7%)	53 (8%)	6 (4%)	
Missing	342 (44%)	221 (36%)	121 (74%)	
HCT-CI				0.014
0	279 (36%)	234 (43%)	45 (32%)	
1 or 2	196 (25%)	159 (29%)	37 (26%)	
≥3	211 (27%)	152 (28%)	59 (42%)	
Missing	94 (12%)	73 (12%)	21 (13%)	
KPS				0.025
100	220 (28%)	166 (27%)	54 (33%)	
90	326 (42%)	265 (43%)	61 (38%)	
80	175 (22%)	143 (23%)	32 (20%)	
<80	47 (6%)	39 (6%)	8 (5%)	
Missing	12 (2%)	5 (1%)	7 (4%)	
SC source				<0.001
PBSC	536 (69%)	397 (64%)	139 (86%)	
BM	229 (29%)	212 (34%)	17 (10%)	
UCB	15 (2%)	9 (1%)	6 (4%)	
Combination with another alkylator	370 (47%)	309 (50%)	61 (38%)	0.005
Thiotepa	326 (88%)	300 (97%)	26 (43%)	<0.001
Melphalan	44 (12%)	9 (3%)	35 (47%)	
Conditioning intensity				0.013
MAC	372 (47%)	309 (50%)	63 (39%)	
RIC	406 (52%)	307 (50%)	99 (61%)	
Missing	2 (<1%)	2 (<1%)	0 (0%)	
GVHD prophylaxis				<0.001
CnI±MMF	206 (27%)	187 (30%)	19 (12%)	
In vivo T-cell dep (ATG/ATLG)	366 (47%)	289 (47%)	77 (48%)	
PT-Cy	182 (23%)	130 (21%)	52 (32%)	
Other	23 (3%)	10 (1%)	13 (8%)	
Missing	3 (1%)	2 (<1%)	1 (<1%)	
Donor				<0.001
Sibling	223 (29%)	196 (32%)	27 (17%)	
MUD	183 (23%)	144 (23%)	39 (24%)	
MMUD	115 (15%)	96 (16%)	19 (12%)	
Haplo	235 (30%)	168 (27%)	67 (41%)	
UCB	15 (2%)	9 (1%)	6 (4%)	
Missing	9 (1%)	5 (<1%)	4 (2%)	

Allo-SCT, allogeneic stem cell transplantation; ATG, antithymocyte globulin; ATLG, anti T-lymphocytes globulin; BM, bone marrow; BU, busulfan; CnI, calcineurin inhibitor; CR, complete remission; GVHD, graft versus host disease; Haplo, haploidentical donor; HCT-CI, hematopoietic cell transplantation comorbidity index; KPS, Karnofsky performance status; MAC, myeloablative conditioning; MMF, mofetil mycophenolate; MMUD, mismatched unrelated donor; MUD, matched unrelated donor; NR, nonresponse; PBSC, peripheral blood stem cell; PR, partial remission; PT-Cy, posttransplant cyclophosphamide; RIC, reduced-intensity conditioning; TBI, total body irradiation; TREO, treosulfan; UCB, umbilical cord blood.

Considering the multivariate analysis on OS, CR status at SCT (hazard ratio [HR] 0.7; 95% confidence interval [CI], 0.6-0.9; $P = 0.013$), and KPS 90 to 100 (HR 0.6; 95% CI, 0.5-0.8; $P < 0.001$) were independently associated with improved outcome, whereas HCT-CI ≥3 (HR 1.8; 95% CI, 1.0-3.0; $P = 0.03$) and haploidentical donor (HR 1.3; 95% CI, 1.0-1.8; $P = 0.031$) were independently associated with impaired outcome.

Moving to the multivariate analysis on the CIR, we observed that factors independently associated with reduced risk of relapse were CR status at SCT (HR 0.7; 95% CI, 0.5-0.9; $P = 0.007$) and the use of MAC regimen (HR 0.7; 95% CI, 0.5-0.9; $P = 0.003$).

Finally, the multivariate analysis of NRM showed that the only independent variable associated with a significantly increased risk of death for causes other than relapse was the

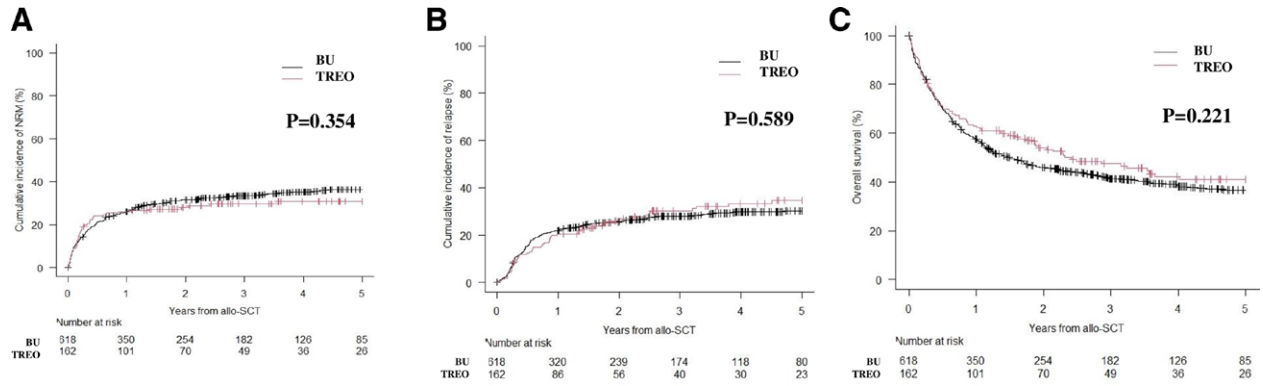

FIGURE 1. Long-term outcomes of the 780 transplants included in this analysis. (A) Cumulative incidence of NRM (BU vs TREO at 2 y 31.4% vs 28.8%); (B) cumulative incidence of relapse (BU vs TREO at 2 y 25.7% vs 26.8%); (C) overall survival (BU vs TREO at 2 y 45.6% vs 53.2%). BU, busulfan; NRM, nonrelapse mortality; TREO, treosulfan.

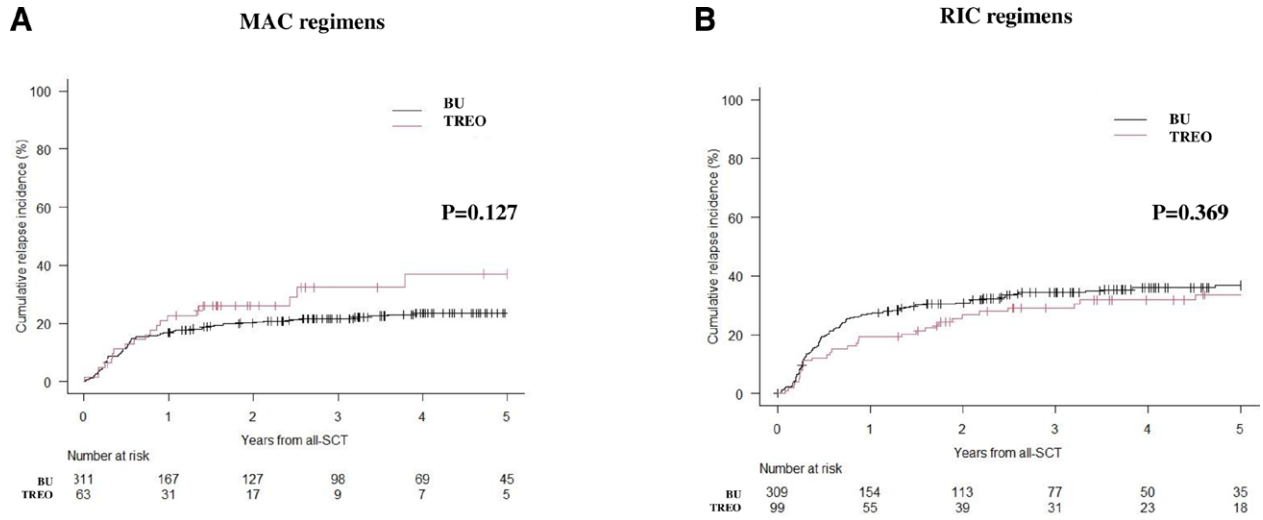

FIGURE 2. Cumulative incidence of relapse according to alkylator and conditioning intensity. (A) MAC regimens (BU vs TREO at 2 y 21% vs 26%; $P=0.127$). (B) RIC regimens (BU vs TREO at 2 y 31% vs 27%; $P=0.37$). BU, busulfan; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; TREO, treosulfan.

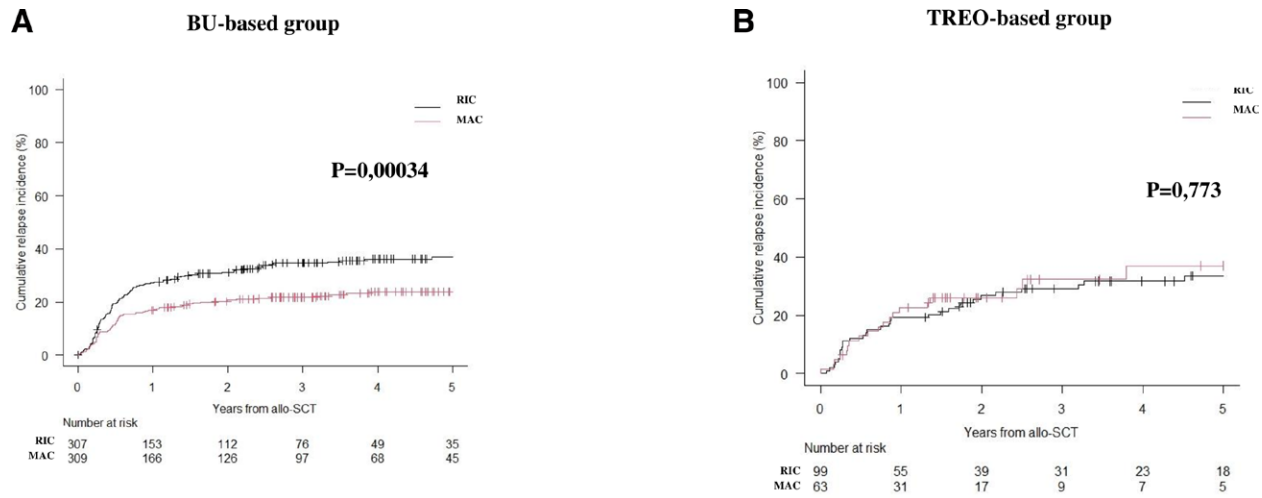

FIGURE 3. Cumulative incidence of relapse according to alkylator and conditioning intensity. (A) BU-based (MAC vs RIC at 2 y 21% vs 31%; $P=0.0003$). (B) TREO-based) (MAC vs RIC at 2 y 26% vs 28%; $P=0.773$). BU, busulfan; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; TREO, treosulfan.

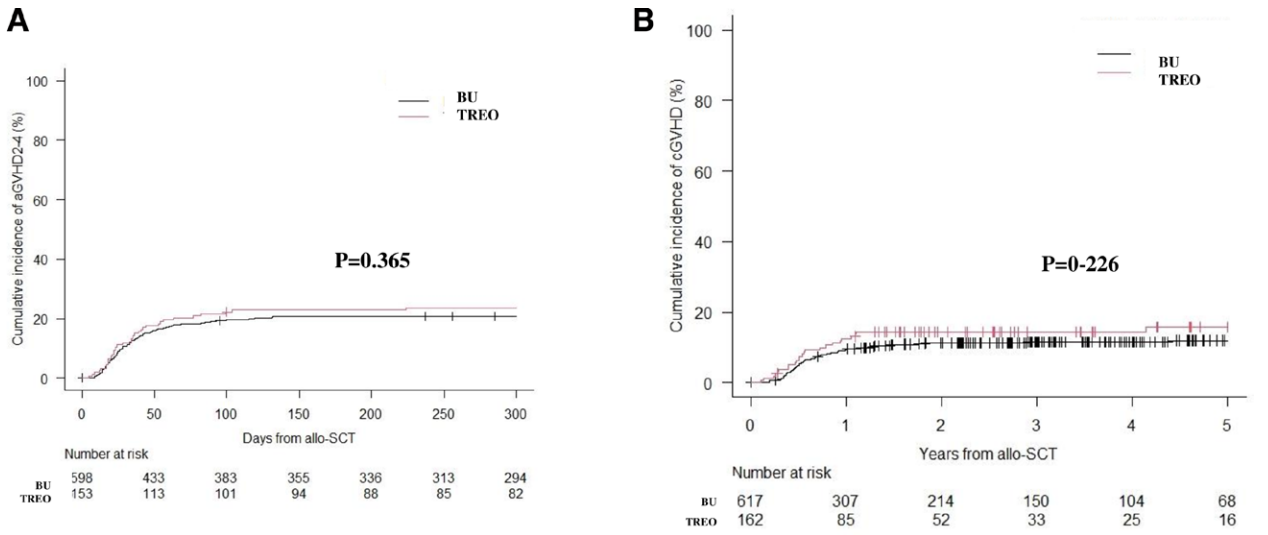

FIGURE 4. Cumulative incidence of aGVHD grade ≥2: (A) BU vs TREO at 100 d 20% vs 22%; cumulative incidence of extensive cGVHD. (B) BU vs TREO at 2 y 11% vs 14%. BU, busulfan; aGVDH, acute graft versus host disease; cGVDH, chronic graft versus host disease; TREO, treosulfan.

TABLE 2.

Univariate and multivariate analyses on OS, CIR, and NRM

	Univariate analysis		Multivariate analysis	
CIR	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age (5 y interval)	1.1 (0.1- 1.3)	0.107	–	–
Donor (F to M)	1.3 (1.0-1.6)	0.029	1.1 (0.9-1.5)	0.283
CR at SCT	0.6 (0.5-0.7)	<0.001	0.7 (0.6-0.9)	0.013
Donor (vs MRD)				
MUD	0.9 (0.7-1.1)	0.352	0.9 (0.7-1.2)	0.615
MMUD	1.0 (0.8-1.4)	0.604	1.2 (0.9-1.7)	0.281
Haplo	1.4 (1.1-1.8)	0.004	1.3 (1.0-1.8)	0.031
UCB	2.1 (1.2-3.9)	0.010	1.9 (0.9-4.0)	0.077
Source PBSC	0.9 (0.7-1.0)	0.202	–	–
GVHD prophylaxis (vs ATG/ATLG-based)				
PT-Cy	1.0 (0.8-1.3)	0.718	–	–
Other	1.0 (0.8-1.3)	0.669	–	–
In vivo T-cell depletion	1.0 (0.8-1.2)	0.781	–	–
SCT time (2012–2017)	0.9 (0.7-1.1)	0.283	–	–
CMV patient's positivity	1.1 (0.8-1.5)	0.412	–	–
TREO-based	0.9 (0.7-1.0)	0.222	–	–
MAC	0.9 (0.8-1.1)	0.440	–	–
HCT-CI ≥3	1.4 (1.2-1.7)	0.0007	1.8 (1.0-3.0)	0.03
KPS 90 or 100	0.6 (0.5-0.7)	<0.0001	0.7 (0.5-0.9)	0.02
CIR	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age (5 y interval)	0.9 (0.7-1.2)	0.500	–	–
Donor (F to M)	1.1 (0.8-1.5)	0.530	–	–
CR at SCT	0.7 (0.5-0.9)	0.003	0.7 (0.5-0.9)	0.007
Donor (vs MRD)				
MUD	0.6(0.4-0.9)	0.011	0.7 (0.5-1.2)	0.190
MMUD	0.7 (0.7-0.5)	0.050	0.8 (0.5-1.4)	0.540
Haplo	0.7 (0.7-0.5)	0.017	0.8 (0.5-1.1)	0.200
UCB	0.9 (0.4-2.1)	0.820	0.9 (0.4-2.4)	0.890
Source PBSC	1.1 (0.8-1.5)	0.390	–	–
GVHD prophylaxis (vs ATG/ATLG-based)				
PT-Cy	0.9 (0.7-1.3)	0.670	–	–
Other	1.4 (1.0-1.9)	0012	–	–
In vivo T-cell depletion	0.7 (0.5-0.9)	0.004	0.8 (0.6-1.2)	0.390

(Continued)

TABLE 2. (Continued)

Univariate and multivariate analyses on OS, CIR, and NRM

	Univariate analysis		Multivariate analysis	
CIR	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
SCT time (2012–2017)	0.7 (0.5-0.9)	0.012	0.8 (0.6-1.1)	0.110
CMV patient's positivity	1.3 (1.0-1.8)	0.079	–	–
TREO-based	1.1 (0.8-1.5)	0.610	–	–
MAC	0.6 (0.5-0.8)	0.001	0.7 (0.5-0.9)	0.003
HCT-CI ≥3	1.3 (0.9-1.7)	0.120	–	–
KPS 90–100	0.7 (0.5-0.9)	0.015	0.9 (0.6-1.2)	0.320
NRM	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age (5 y interval)	1.2 (1.0-1.5)	0.041	1.1 (0.9-1.4)	0.270
Donor (F to M)	1.3 (1.0-1.6)	0.096	–	–
CR at SCT	0.7 (0.6-1.0)	0.015	0.8 (0.6-1.1)	0.180
Donor (vs MRD)				
MUD	1.2 (0.8-1-7)	0.380	1.1 (0.7-1.7)	0.600
MMUD	1.5 (1.0-2.2)	0.046	1.5 (0.9-2.3)	0.090
Haplo	1.9 (1.4-2.6)	<0.001	1.6 (1.0-2.3)	0.020
UCB	2.3 (1.0-5.2)	0.045	2.0 (0.-9-4.8)	0.080
Source PBSC	0.8 (0.6-1.0)	0.110	–	–
GVHD prophylaxis (vs ATG/ATLG-based)				
PT-Cy	1.0 (0.8-1.4)	0.660	–	–
Other	0.8 (0.6-1.0)	0.073	–	–
In vivo T-cell depletion	1.3 (1.0-1.8)	0.033	1.1 (0.8-1.6)	0.410
SCT time (2012–2017)	1.0 (0.7-1.4)	0.850	–	–
CMV patient's positivity	1.1 (0.8-1.7)	0.480	–	–
TREO-based	0.9 (0.6-1.2)	0.400	–	–
MAC	1.2 (0.9-1.5)	0.180	–	–
HCT-CI ≥3	1.3 (1.0-1.7)	0.05	–	–
KPS 90–100	0.7 (0.5-0.9)	0.007	–	–

ATG, antithymocyte globulin; ATLG, anti T-lymphocytes globulin; CI, confidence interval; CIR, cumulative incidence of relapse; CMV, cytomegalovirus; CR, complete remission; GVHD, graft versus host disease; Haplo, haploidentical donor; HCT-CI, hematopoietic cell transplantation comorbidity index; HR, hazard ratio; KPS, Karnofsky performance status; MAC, myeloablative conditioning; MRD, Matched Related Donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; NRM, nonrelapse mortality; OS, overall survival; PBSC, peripheral blood stem cells; PT-Cy, posttransplant cyclophosphamide; SCT, stem cell transplantation; TREO, treosulfan; UCB, umbilical cord blood.

use of a haploidentical donor (HR 1.6; 95% CI, 1.0-2.3; $P = 0.020$), whereas a KPS 90 to 100 significantly reduced the NRM (HR 0.7; 95% CI, 0.53-0.93; $P = 0.015$).

DISCUSSION

In this study, we report the real-life data on the use of BU and TREO in the conditioning platforms of allo-SCT performed in elderly patients in Italy between 2006 and 2017.

The outcome in terms of NRM, CIR, and OS was comparable in the 2 groups (Figure 1), although in the TREO-based group we observed a greater proportion of elderly and frail patients with nonresponsive diseases at the time of SCT and a higher use of RIC regimens and of haploidentical donors. Moreover, the CIR was comparable across the 2 alkylators both in the myeloablative and reduced-intensity setting (Figure 2), but we observed that the 2-y CIR with myeloablative dose was significantly lower than what was observed with reduced intensity for BU-based conditioning only (21% versus 31%; $P = 0.0003$; Figure 3A).

These observations are very interesting because they suggest that the 2 alkylators were considered different a priori and were used in a sort of "customized" way. In particular, TREO was preferred for older and frail patients, even before the published results of the MC-FludT.14/L trial, showing the superiority of TREO 10 g/m^2/d for 3 d versus BU 3.2 mg/kg/d for 2 d, both in combination with fludarabine used in the same setting of AML and MDS patients.[15]

This may not be surprising because BU and TREO are and have been considered as 2 very different alkylators. The pharmacological profile of TREO is very peculiar: it is a prodrug that is rapidly activated at pH >5 and, differently from other alkylators such as BU, does not require enzymatic activation or hepatic metabolism.[19] Because of its characteristics, TREO shows a very low inter- and intrapatient variability in adult patients, and, moreover, drug level monitoring or dose adjustments are not strictly required.[20] Furthermore, TREO has intense cytotoxicity mediated by caspases against AML leukemic cells[21]; the incidence of severe mucositis, gastrointestinal toxicity, and hepatic veno-occlusive disease is reduced compared to that observed with the other alkylators agents; and the low proinflammatory cytokine release facilitates stem cell engraftment and reduces the risk of GVHD.[22]

The main limitation of our study is its retrospective nature, with many important information missing and selection biases (eg, the higher proportion of BU-based regimens between 2012 and 2017). Moreover, we cannot conclude that TREO-based conditioning regimens are more effective and less toxic than BU-based, and these were not the aims of this analysis. We can expect that the lower gastrointestinal toxicity of TREO may reduce bacterial translocation and, thus, infection

complications, but we do not have proof of this hypothesis. However, at present, literature data confirm that TREO at a dose of 10 g/m²/d for 3 d in combination with fludarabine is the gold-standard RIC regimen for older AML and MDS patients,[15] particularly if frail, whereas there is no evidence that TREO at a possibly higher dose (14 g/m²/d for 3 d) in combination with fludarabine is more effective than BU (3.2 mg/kg/d for 4 d) in MAC regimen, in this subset of patients.

Concerning this issue, Shimoni et al,[16] in a retrospective European Society for Blood and Marrow Transplantation study, reported that the combination of fludarabine and TREO 14 g/m²/d for 3 d or TREO 12 g/m²/d for 3 d resulted in equal levels of OS and leukemia-free survival, with respect to fludarabine combined with BU 3.2 mg/kg/d for 4 d or BU 3.2 mg/kg/d for 2 d, although patients in the BU and TREO group were different in number, age, and disease status at transplant.

Furthermore, a recent meta-analysis comparing fludarabine and TREO versus fludarabine and BU in patients with AML and MDS showed that the combination of fludarabine and TREO was associated with improved OS and reduced aGVHD.[23] The majority of the studies included in this metanalysis are retrospective and include transplants performed at least 10 to 15 y ago, when the transplant procedures were very different from those currently performed, and were not planned to address the issue of safety and efficacy of TREO in specific patients' settings.

In conclusion, our real-life data suggest that TREO-based regimens were empirically preferred in patients who were frail, with high-risk disease, and undergoing transplant from alternative donors (matched unrelated donor or haploidentical). The transplant outcomes in this setting of patients with poor prognosis were not inferior to those of patients with more favorable characteristics who were transplanted with BU-based regimens. Moreover, the BU-based MAC regimens were mainly deserved to more fit patients and induced a lower relapse incidence. The question of the efficacy and toxicity of TREO-based MAC regimens (eg, fludarabine and TREO 14g/m2/d for 3 d) is open and still unanswered.

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