Original Article

Long-Term Outcome After a Treosulfan-Based Conditioning Regimen for Patients With Acute Myeloid Leukemia: a Report From the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation

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BACKGROUND: Allogeneic hematopoietic cell transplantation (HCT) is a curative therapy for patients with acute myeloid leukemia (AML). However, post-HCT relapse and regimen-related toxicity remain significant barriers to long-term survival. In recent years, new conditioning regimens have been explored to improve transplantation outcomes in patients with AML. Treosulfan combines a potent immunosuppressive and antileukemic effect with a low toxicity profile. **METHODS:** To investigate the role of treosulfan-based conditioning, the European Society for Blood and Marrow Transplantation Acute Leukemia Working Party performed a registry analysis of 520 adult patients with AML who received treosulfan-based conditioning and underwent HCT between 2000 and 2012, including 225 patients in first complete remission, 107 in second or later complete remission, and 188 with active/advanced disease 188 (88 with primary refractory disease). The median patient age was 57 years (range, 20-73 years). Donors were human leukocyte antigen-identical siblings (n = 187), unrelated donors (n = 235), or mismatched related donors (n = 98). Conditioning regimens included treosulfan (42 g/m^2 [n = 396], 36 g/m^2 [n = 109], or 30 g/ m^2 [n = 15]) with fludarabine or alkylating agents followed by infusion of hematopoietic stem cells (bone marrow, n = 52; peripheral blood, n = 468). **RESULTS:** At a median follow-up of 61 months, the 5-year overall survival, leukemia-free survival, relapse incidence, and nonrelapse mortality rates were 38%, 33%, 42%, and 25%, respectively. The incidence of grade II-IV acute and chronic graft-versus-host disease was 24% (grade III-V, 11%) and 38%, respectively. Only 11 patients (2%) developed veno-occlusive disease, with two deaths (0.4%) from veno-occlusive disease. **CONCLUSIONS:** Treosulfan-based conditioning regimens provide an acceptable long-term survival with favorable nonrelapse mortality and a very low risk of veno-occlusive disease. Further studies are needed to optimize the treosulfan-based conditioning regimen for patients with AML. ***Cancer*** **2017;123:2671-9**.

KEYWORDS: acute myeloid leukemia, allogeneic stem cell transplantation, conditioning regimen, graft-versus leukemia effect, toxicity, treosulfan.

INTRODUCTION

Busulfan-based and total body irradiation (TBI)-based preparative regimens are widely regarded as standard conditioning therapies for allogeneic hematopoietic cell transplantation (HCT) in patients with acute myeloid leukemia (AML).[1-5] A significant challenge in the management of patients with high-risk AML is the relatively high transplantation-related mortality associated with intensive HCT conditioning regimens. Thus, there is a need for conditioning regimens that reduce the incidence of relapse without significantly increasing the risk of transplantation-related mortality.

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Traditionally, high-intensity conditioning regimens have been standard practice for eradicating AML with HCT. Busulfan is an effective immunosuppressive and antileukemic agent. However, acute and late toxicities are major challenges associated with this potentially curative therapy.[3,4,6-10] The common myeloablative conditioning regimens used in AML include cyclophosphamide plus TBI, cyclophosphamide plus busulfan, or fludarabine plus busulfan. The use of high-dose busulfan and TBI are associated with substantial hepatic toxicity, pulmonary toxicity, neurotoxicity, and cardiotoxicity.[1-4,6,8] Consequently, it is important to identify less toxic conditioning regimens that maintain the antileukemic, immunosuppressive, and myeloablative characteristics of conventional conditioning therapies.

Treosulfan (a water-soluble, bifunctional alkylating agent) has demonstrated efficacy as an antileukemic and immunosuppressive agent.[11] In contrast to busulfan, treosulfan does not require enzymatic activation and thus bypasses hepatic metabolism. Pharmacokinetic studies of both single and multiple intravenous infusions of treosulfan have exhibited low interpatient and intrapatient variability.[11-13] In vitro studies have demonstrated strong proapoptotic effects in human AML cells,[11,14,15] and work from murine xenograft models has revealed potent in vivo activity of treosulfan against acute and chronic leukemia, human B-lymphoblast and T-lymphoblast cell lines, and various solid tumors, myelomas, and lymphomas.[11,14,16]

Dose-limiting toxicities of treosulfan include bone marrow suppression, which occurs at a dose of 10 g/m^2, suggesting that the drug may be effective for HCT conditioning. In clinical trials with autologous HCT, the maximum tolerated cumulative dose of treosulfan could be escalated from 10 to 47 g/m^2 before mucositis, diarrhea, dermatitis, or metabolic acidosis became dose-limiting.[17] In those studies, no episodes of severe hepatotoxicity or central nervous system toxicity were observed. Moreover, treosulfan targets both committed and uncommitted hematopoietic stem cells and thus has profound antileukemic and immunosuppressive properties.[11,16,18] Although data are limited, recent published studies have reported encouraging results with acceptable nonhematologic toxicity.[11,17,19-24] However, to date, no large trials have studied the value of treosulfan-based conditioning in HCT as a potential alternative to the commonly used busulfan-based or TBI-based regimens for patients with AML. In this large, registry-based analysis, we report the safety and efficacy of treosulfan-based conditioning for patients with AML who undergo allogeneic HCT.

MATERIALS AND METHODS

Study Design and Data Collection

This is a retrospective, multicenter analysis with data provided by the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT) group registry, which is a voluntary working group of more than 500 transplantations centers that are required to report all consecutive stem cell transplantations and follow-ups on an annual basis. Since 1990, registry patients have provided informed consent authorizing the use of their personal information for research purposes. The ALWP of the EBMT group approved this study. The study protocol was approved by the institutional review board at each site and complied with country-specific regulatory requirements. The study was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines.

Eligibility criteria for this analysis included adult patients (age $\geq$18 years) with AML who received a treosulfan-based conditioning regimen followed by a related or unrelated donor (URD) bone marrow transplantation or granulocyte–colony-stimulating factor-mobilized peripheral blood stem cells transplantation between the years 2000 and 2012 (n = 520 patients; n = 19 transplantation centers). Treosulfan was combined with different chemotherapeutic agents, most frequently with fludarabine (94%) or cyclophosphamide (3.5%). The most frequent dose range for treosulfan was from 36 to 42 g/m^2 (Table 1). Standard dosages were used for the other chemotherapeutic agents in the majority of patients (median dosages: cyclophosphamide, 120 mg/kg; interquartile range [IQR], 78-120 mg/kg; fludarabine, 150 mg/m^2; IQR, 150-150 mg/m^2; thiotepa, 10 mg/kg; IQR, 10-10 mg/kg; and melphalan. 140 mg/m^2; IQR, 140-140 mg/m^2).

The variables investigated included recipient and donor characteristics (age, sex, cytomegalovirus serostatus); disease features (including remission status, active/advanced disease); transplantation-related factors, including conditioning regimen, immunosuppression (in vivo T-cell depletion vs none), stem cell source (bone marrow or peripheral blood), graft-versus-host disease (GVHD) prophylaxis; and outcome variables, including acute and chronic GVHD, relapse, nonrelapse mortality (NRM), leukemia-free survival (LFS), overall survival (OS), and causes of death. The choice of conditioning and GVHD prophylaxis depended on transplantation center protocols and strategies for transplantation.

Statistical Analysis

The primary endpoints of this study were LFS and OS. Secondary endpoints included disease relapse incidence

TABLE 1. Patient, Disease, and Transplantation Characteristics

Characteristic	No. of Patients (%)
Patient characteristics, n = 520	
Recipient age at HCT: Median [IQR], y	57 [47-63]
Recipient sex	
Man	266 (51)
Woman	254 (49)
Follow-up: Median [range], mo[a]	61 [2-163]
Interval from diagnosis to HCT: Median [range], d	218 [60-2358]
Year of transplantation: Median [range], y	2009 [2000-2012]
Donor sex	
Man	308 (60)
Woman	210 (40)
Unknown	2
Female donor to male recipient	105 (20)
Missing	2
Disease characteristics	
Disease status at HCT	
CR1	225 (43)
≥CR2	107 (21)
Active/advanced disease, n = 188	188 (36)
Primary refractory	88
First refractory relapse	86
Second or later refractory relapse	14
Secondary AML	136 (26)
Cytogenetics	
Good	34 (7)
Intermediate	234 (45)
Poor	74 (14)
NA/missing	42 (8)
KPS at HCT	
<90%	143 (28)
Missing	8
Transplantation characteristics	
Donor type	
Related	285 (55)
Unrelated	235 (45)
CMV serology	
Patient positive	391 (76)
Missing	4
Donor positive	271 (53)
Missing	8
Stem cell source	
BM	52 (10)
PB	468 (90)
Conditioning regimen	
Treosulfan and fludarabine	484 (94)
In vivo T-cell depletion	279 (52)
ATG	264
Campath	15

Abbreviations: AML, acute myeloid leukemia; ATG, antithymocyte globulin; BM, bone marrow; CMV, cytomegalovirus; CR1, first complete remission; ≥CR2, second or later complete remission; HCT, allogeneic cell transplantation; IQR, interquartile range; KPS, Karnofsky performance status; NA, not applicable; PB, peripheral blood.

[a] Some percentages do not add up to 100% because of rounding.

(RI), NRM, engraftment, incidence and severity of acute and chronic GVHD, and risk of veno-occlusive disease of the liver (VOD).[10] The starting point for time-to-event analysis was "date of transplantation." OS was defined as the time to death from any cause, LFS was defined as survival without relapse or progression, and RI was defined as the time to onset of leukemia recurrence. For OS, LFS, and RI, patients were censored at the time of last follow-up. NRM was the competing risk, and patients who survived in continuous complete remission (CR) were censored at last contact. NRM was defined as death without relapse/progression (relapse was the competing risk). Competing risks considered for GVHD were relapse or death. Standard definitions were used to define remission status.[3,4,25] The groups were compared using the chi-square method for qualitative variables, and the Mann-Whitney test was applied for continuous parameters.

Univariate comparisons were done using the log-rank test for OS and LFS and the Gray test for RI, NRM, and GVHD cumulative incidence. Multivariate analyses were performed using Cox proportional hazards model for all endpoints. All factors known as potentially related to the outcomes were included in the model with stepwise selection. All tests were 2-sided. The Type I error rate was fixed at .05 for determination of factors associated with time-to-event outcomes. Statistical analyses were performed using the software packages SPSS 22.0 (IBM Corp., Armonk, NY) and R 3.2.3 (R Development Core Team, Vienna, Austria).

RESULTS

Patient and Transplantation Characteristics

Five hundred twenty patients with AML (median age, 57 years; IQR, 47-63 years) who received a treosulfan-based conditioning regimen followed by HCT from a related donor (n = 285) or an URD (n = 235) between 2000 and 2012 were included in this analysis (patient characteristics are summarized in Table 1). Patients received treosulfan at a dose of 42 g/m^2 (n = 396), 36 g/m^2 (n = 109), or 30 g/m^2 (n = 15) over 3 days. Four hundred eighty-four patients (94%) received a treosulfan and fludarabine-based conditioning regimen (Table 1). In total, 225 patients (43%) were in first CR (CR1), 107 (21%) were in second CR or later (≥CR2), and 188 (36%) had active/advanced disease (primary refractory, n = 88) before HCT (Table 1).

Engraftment

Twenty patients (4%) patients died before day 60 (18 of whom died before day 30) without attaining engraftment. The remaining 490 patients (96%) attained engraftment, with a median day to an absolute neutrophil count (ANC) ≥500/μL of 16 days (IQR, 13-20 days). The engraftment rates were 94% at day 30 for neutrophils and 93% at day 180 for platelets. One patient had graft rejection.

TABLE 2. Engraftment and Graft-Versus-Host Disease Data

Variable	No. of Patients (%)[a]		
	All Patients	Treosulfan 36 g/m^2	Treosulfan 42 g/m^2
Engraftment			
Yes	490 (96)	96 (91)	376 (96)
No	20 (4)	9 (9)	14 (4)
Unknown	10	4	6
Time to PMN cells ≥500/μL: Median [IQR], d	16 [13-20]	15 [12-16]	15 [12-18]
Acute GVHD: [95% CI], %			
Grade II-IV	24 [21-28]	18 [1-26]	26 [21-30]
Grade III-IV	11 [8-14]	6 [2-11]	12 [9-15]
Unknown	10	4	6
Chronic GVHD: [95% CI], %	38 [34-43][b]	30% [22-39][b]	41% [36-46][b]
Limited	89	17	71
Extensive	104	15	87
Unknown	3	1	2

Abbreviations: GVHD, graft-versus-host disease; IQR, interquartile range; MAC, myeloablative conditioning; PMN, polymorphonuclear cells; RIC, reduced-intensity conditioning.
[a] Some percentages may not add up to 100% because of rounding.
[b] The 5-year cumulative incidence is indicated.

Acute GVHD

Acute GVHD (grade II-IV) was observed in 123 patients (24%), and grade III and IV acute GVHD was observed in 54 patients (11%). There were no significant differences in the frequency of acute GVHD in the treosulfan dosing groups (grade II-IV: 18% of patients in the 36 g/m^2 group vs 26% of those in the 42 g/m^2 group [$P = .11$]; grade III-IV: 6% of patients in the 36 g/m^2 group vs 12% of those in the 42 g/m^2 group [$P = .06$]) (Table 2).

In multivariate analysis, the factor associated most significantly with increased risk of grade II-IV acute GVHD was active disease (hazard ratio [HR], 1.66; 95% confidence interval [CI], 1.11-2.49; $P = .015$). In contrast, T-cell depletion was associated with a reduced risk of acute GVHD (HR, 1.71; 95% CI, 1.17-2.50; $P = .006$) (Table 3).

Chronic GVHD

The 5-year cumulative incidence of chronic GVHD was 38% (95% CI, 34%-43%) (Table 4). Low-dose treosulfan was associated with a lower incidence of chronic GVHD in univariate analysis (30%; 95% CI, 22%-39%) in the group that received 36 g/m^2 versus 41% (95% CI, 36%-46%) in the group that received 42 g/m^2 ($P = .03$).

In multivariate analysis, the risk factor most significantly associated with chronic GVHD was belonging to the poor cytogenetic risk group (HR, 1.52; 95% CI, 1.01-2.27; $P = .044$). Consistent with our findings in acute GVHD, T-cell depletion reduced the risk of chronic GVHD (HR, 0.70; 95% CI, 0.51-0.97; $P = .03$) (Table 3).

TABLE 3. Multivariate Analysis: Patients Who Received Treosulfan >30 g/m^2

Variable	*P*	HR [95% CI]
Relapse		
Active disease (Ref, CR1)	< .0001	3.34 [2.40-4.65]
Poor cytogenetic group	.04	1.71 [1.19-2.45]
URD (Ref, MSD)	.0001	0.49 [0.34-0.71]
Other relative (Ref, MSD)	.019	0.56 [0.35-0.91]
In vivo T-cell depletion	.023	1.54 [1.06-2.24]
NRM		
Age at SCT (by +10 years)	.006	1.29 [1.07-1.54]
Active disease (Ref, CR1)	.001	2.12 [1.37-3.28]
KPS ≥90%	.010	0.59 [0.40-0.88])
Acute GVHD		
Active disease (Ref, CR1)	.015	1.66 [1.11-2.49][a]
Secondary AML	.049	1.49 [1.00-2.21][a]
In vivo T-cell depletion	.006	1.71 [1.17-2.50][a]
Chronic GVHD		
Poor cytogenetic group	.044	1.52 [1.01-2.27]
In vivo T-cell depletion	.03	0.70 [0.51-0.97]
LFS		
Active disease (Ref, CR1)	< .0001	2.95 [2.27-3.83]
≥CR2 (Ref, CR1)	.003	1.58 [1.16-2.15]
Poor cytogenetic group	.003	1.55 [1.16-2.08]
URD (Ref, MSD)	.037	0.74 (0.56-0.98]
OS		
Active disease (Ref, CR1)	< .0001	2.80 [2.16-3.62]
≥CR2 (Ref, CR1)	.003	1.63 [1.19-2.25]
Poor cytogenetic group	.0004	1.70 [1.27-2.29]

Abbreviations: CI, confidence interval; CR, complete remission; CR1, first complete response; CR2, second complete remission; GVHD, graft-versus-host disease; HCT, allogeneic hematopoietic cell transplantation; HR, hazard ratio; KPS, Karnofsky performance status; LFS, leukemia-free survival; MSD, matched sibling donor; NRM, nonrelapse mortality; OS, overall survival; Ref, reference category; SCT, stem cell transplantation; URD, unrelated donor.
[a] Odds ratio [95% CI].

NRM

The 5-year cumulative incidence of NRM was 25% (95% CI, 21%-29%) (Table 4). In univariate analysis, there was

TABLE 4. Five-Year Outcome After a Treosulfan-Based Conditioning Regimen in Patients With Acute Myeloid Leukemia

Patient Group	Outcome %, [95% CI]					
	Relapse Incidence	Nonrelapse Mortality	Leukemia-Free Survival	Overall Survival	Acute GVHD (Grade III-IV)	Chronic GVHD
All patients	42 [37-46]	25 [21-29]	33 [28-37]	38 [33-42]	11 [8-14]	38 [34-43]
Treosulfan dose, g/m^2						
30	62 [29-83]	22 [2-55]	17 [0-37]	25 [2-48]	7 [0.4-27]	23 [5-50]
36	45 [35-54]	20 [2-54]	35 [26-45]	41 [31-51]	6 [2-11]	30 [22-39]
42	40 [35-45]	27 [3-60]	33 [28-38]	38 [32-43]	12 [9-16]	41 [36-46]
P (36 vs 42 g/m^2)	.25	.17	.83	.68	.06	.03
CR1						
36	39 [24-53]	8.5 [3-19]	53 [38-68]	65 [51-79]	6 [2-16]	39 [25-53]
42	31 [24-38]	20 [9-33]	50 [42-57]	53 [45-61]	10 [6-15]	45 [37-52]
P	.22	.03	.69	.28	.49	.46
Advanced disease						
36	54 [36-69]	33 [18-49]	12 [1.3-23]	14 [1.3-26]	3 [0.2-13]	26 [12-43]
42	55 [46-63]	32 [24-40]	13 [7-19]	18 [12-25]	16 [10-22]	37 [29-45]
P	.90	.77	.60	.97	.04	.18

Abbreviations: CI, confidence interval; CR1, first complete remission; GVHD, graft-versus-host disease.

no significant difference in the incidence of NRM among patients who received 36 g/m^2 versus 42 g/m^2 of treosulfan (20% [95% CI, 2%-54%] vs 27% [95% CI, 3%-60%]; $P = .17$) (Table 4). However, there was a significantly higher incidence of NRM in patients who received the higher dose of treosulfan and underwent transplantation in CR1 (9% [95% CI, 3%-19%] for 36 g/m^2 vs 20% [95% CI, 9%-33%] for 42 g/m^2; $P = .03$) but not in those with active/advanced disease at HCT (33% [95% CI, 18%-49%] vs 32% [95% CI, 24%-40%]; $P = .77$) (Table 4).

In multivariate analysis, the factors associated with an increased risk of NRM were age at HCT (by +10 years: HR, 1.29; 95% CI, 1.07-1.54; $P = .0006$) and disease status at transplantation (for active/advanced disease: HR, 2.12; 95% CI, 1.37-3.28; $P = .001$). Conversely, a Karnofsky performance status $\geq$90% was associated with a decreased risk of NRM (HR, 0.59; 95% CI, 0.40-0.88; $P = .01$) (Table 3).

Relapse

The 5-year cumulative RI was 42% (95% CI, 37%-46%) (Table 4). There was no significant association between RI and treosulfan dose in univariate analysis (45% [95% CI, 35%-54%] in the 36 g/m^2 group vs 40% [95% CI, 35%-45%] in the 42 g/m^2 group; $P = .25$). Analyzing patients in CR1 (39% [95% CI, 24%-53%] vs 31% [95% CI, 24%-38%], respectively; $P = .22$) and those with active/advanced disease did not reveal a significant difference in RI in the 2 treatment groups (54% [95% CI, 36%-69%] vs 55% [95% CI, 46%-63%], respectively; $P = .90$) (Table 4).

In multivariate analysis, the factors associated with an increased risk of relapse included disease status at transplantation (for active/advanced disease: HR, 3.34; 95% CI, 2.40-4.65; $P < .0001$), poor cytogenetics (HR, 1.71; 95% CI, 1.19-2.45; $P = .004$), and T-cell depletion (HR, 1.54; 95% CI, 1.06-2.24; $P = .023$). Patients who underwent HCT from a URD and a mismatched-related donor experienced a significantly lower risk of relapse compared with those who underwent HCT from a matched-related donor (URD: HR, 0.49; 95% CI, 0.34-0.71; $P = .0001$; mismatched-related donor: HR 0.56; 95% CI, 0.35-0.91; $P = .019$) (Table 3).

LFS

The 5-year LFS rate for the entire cohort was 33% (95% CI, 28%-37%) (Table 4, Fig. 1). There was no significant association between LFS and treosulfan dose in univariate analysis (35% [95% CI, 26%-45%] in the 36 g/m^2 group vs 33% [95% CI, 28%-38%] in the 42 g/m^2 group; $P = .83$). This difference remained nonsignificant when we separately analyzed patients in CR1 (53% [95% CI, 38%-68%] vs 50% [95% CI, 42%-57%]; $P = .69$) and those who had active/advanced disease (12% [95% CI, 1.3%-23%] vs 13% [95% CI, 7%-19%]; $P = .60$).

In multivariate analysis, risk factors for inferior LFS were disease status at transplantation (for active/advanced disease: HR, 2.95; 95% CI, 2.27-3.83; $P < .0001$) and poor cytogenetics (HR, 1.55; 95% CI, 1.16-2.08; $P = .003$). Patients who received URD allografts had

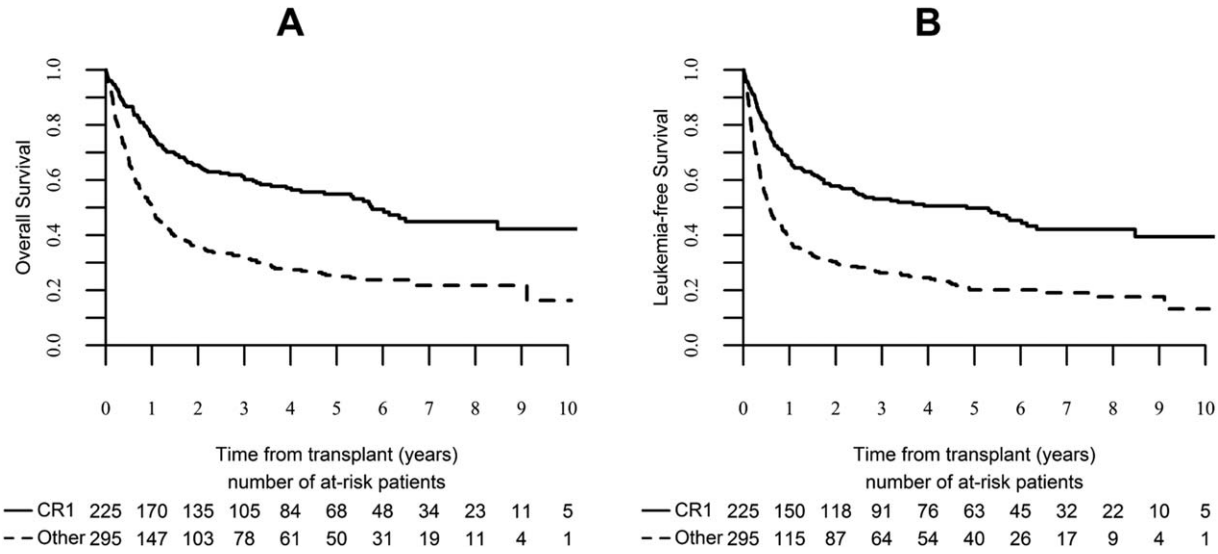

Figure 1. The probabilities of (A) overall survival and (B) leukemia-free survival after a treosulfan-based conditioning regimen for acute myeloid leukemia are illustrated. CR1 indicates first complete remission.

significantly higher LFS (HR, 0.74; 95% CI, 0.56-0.98; $P = .037$) (Table 3).

OS

The 5-year OS rate for the entire cohort was 38% (95% CI, 33%-42%) (Table 4, Fig. 1). There was no significant association between OS and treosulfan dose (41% [95% CI, 31%-51%] in the 36 g/m^2 group vs 38% [95% CI, 32%-43%] in the 42 g/m^2 group; $P = .68$). This difference remained nonsignificant when we separately analyzed patients in CR1 (65% [95% CI, 51%-79%] vs 53% [95% CI, 45%-61%]; $P = .28$) and those with active/advanced disease (14% [95% CI, 1.3%-26%] vs 18% [95% CI, 12%-25%]; $P = .97$).

In multivariate analysis, the factors associated with inferior OS were disease status at transplantation (for active/advanced disease; HR 2.80; 95% CI, 2.16-3.62; $P < .0001$) and poor-risk cytogenetics (HR, 1.70; 95% CI, 1.27-2.29; $P = .0004$) (Table 3).

Causes of Death

In total, 324 patients died after a median follow-up of 61 months (IQR, 34-84 months). Major causes of deaths were progression or recurrence of the original disease (n = 149; 48%) and infection (n = 82; 26%). Causes of deaths are summarized in Table 5.

VOD

VOD occurred in eleven (2.2%) patients with two deaths (0.4%). VOD was classified as mild (n = 4), moderate (n = 2), and severe (n = 3). VOD classification was missing in 2 patients (both remained).

TABLE 5. Causes of Death

Cause of Death	No. of Deaths (%)
Cardiac toxicity	3
Hemorrhage	5
VOD	2
Infection	82 (26)
IP	3
GVHD	43 (14)
Original disease	149 (48)
Second malignancy	7
Other transplantation-related causes	15
Other	4
Missing information	11
Total deaths	324

Abbreviations: GVHD, graft-versus-host disease; IP, interstitial pneumonitis; VOD, veno-occlusive disease of the liver (sinusoidal obstructive syndrome).

Impact of Treosulfan Dose and Combination With Other Agents on Transplantation Outcome

A higher treosulfan dose (42 vs 36 g/m^2) was associated with increased NRM for patients in CR1 in the univariate analysis. In an adjusted multivariate analysis, there was no impact of treosulfan dose on NRM. In addition, the treosulfan dose had no impact on acute or chronic GVHD, NRM, relapse, LFS, or OS.

Most patients received the combination of treosulfan and fludarabine, so we were unable to analyze the impact of treosulfan in combination with other agents on transplantation outcomes. There was no significant

interaction between treosulfan dose (36 or 42 g/m^2) and disease status at transplantation.

DISCUSSION

TBI-based or busulfan-based ablative conditioning regimens are the most commonly used for patients with AML who undergo HCT, despite significant acute and late toxicities.[1-4,8,9,25-29] In recent years, new conditioning regimens have been explored to improve transplantation outcomes in AML. Treosulfan is a possible alternative to be used in conditioning regimens that have strong immunosuppressive and myelosuppressive effects in preclinical models. Both in vitro studies and in vivo human mouse models of treosulfan have demonstrated significant antileukemic activity, comparable to the activity of other chemotherapeutic agents.[11,16,30] In addition, treosulfan has a lower toxicity profile and the additional advantage of linear pharmacokinetics that do not require the monitoring of drug levels like busulfan.[11,17,19,21,23,24,31]

Hepatotoxicity in our study was low. We observed VOD in only 2.2% of patients (with only 2 deaths related to VOD), similar to earlier reports from studies of treosulfan.[11,32-38] Other myeloablative regimens reportedly were associated with higher VOD rates, especially with a combination of busulfan plus cyclophosphamide or when melphalan was added as a third drug.[10,26,28] In addition, the pulmonary toxicity profile of treosulfan-based regimens was low. Three patients died because of interstitial pneumonitis in our series (Table 5), and 1 patient developed pulmonary dysfunction with VOD (patient survived). Historically, the reported incidence of pulmonary toxicity with TBI-based or busulfan-based regimens was up to 14% with high mortality.[27,39,40] This is noteworthy, because more than one-third of patients in our series had active/advanced disease, which is high risk for organ toxicities.

A recent, retrospective EBMT study[32] evaluated the toxicity profile and outcomes of 71 children with acute lymphoblastic leukemia who underwent HCT after a treosulfan-based conditioning with results that were similar to ours. Those investigators reported a high engraftment rate of 97%, comparable to the data presented here and to earlier studies that used treosulfan with low regimen-related toxicities.[11,32-36,38,41] A very low incidence of graft rejection (only 1 event) and GVHD also was reported in a recent, large series of treosulfan-based, haploidentical HCT.[42] In that study, severe, acute GVHD (grade III or IV) was observed in 14% of patients, comparable to earlier treosulfan studies and our current findings (11%).[11,32,33,35]

The long-term outcomes of this large treosulfan-based series were 5-year OS and LFS rates of 38% and 33%, respectively. Outcomes correlated significantly with disease status at transplantation, as expected, similar to previous studies with TBI-based or busulfan-based conditioning regimens. Patients in CR1 had significantly better OS and LFS compared with patients in $\geq$CR2 (Table 4). A major cause of treatment failure was relapse in 31% of patients at 1 year and in 36% of patients at 2 years. The incidence of relapse was higher in patients beyond CR1 (Table 4), resulting in a 5-year OS rate of 55% for patients who underwent transplantation in CR1, 37% for those who underwent transplantation in $\geq$CR2, and 18% for those with active/advanced disease. These results are comparable to those reported from studies that used busulfan-based or TBI-based ablative conditioning regimens.[3,4]

Our data indicate that treosulfan is an effective conditioning regimen for HCT in patients who have AML in CR at the time of HCT, with a lower toxicity profile compared with that of conventional myeloablative conditioning regimens. This makes treosulfan-based regimens potentially applicable to older patients and/or patients with advanced disease. To our knowledge, this is the largest registry study of treosulfan-based conditioning in an AML cohort with data demonstrating comparable long-term outcomes with a low risk of early organ toxicity and acute GVHD compared with historic data using ablative regimens in AML. These results, which must be confirmed in prospective studies, indicate that treosulfan may represent a viable alternative to TBI-based or busulfan-based conditioning regimens.

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AUTHOR CONTRIBUTIONS

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REFERENCES

1. Copelan EA, Hamilton BK, Avalos B, et al. Better leukemia-free and overall survival in AML in first remission following cyclophosphamide in combination with busulfan compared with TBI. *Blood.* 2013;122:3863-3870.
2. Bredeson C, LeRademacher J, Kato K, et al. Prospective cohort study comparing intravenous busulfan to total body irradiation in hematopoietic cell transplantation. *Blood.* 2013;122:3871-3878.
3. Nagler A, Rocha V, Labopin M, et al. Allogeneic hematopoietic stem-cell transplantation for acute myeloid leukemia in remission: comparison of intravenous busulfan plus cyclophosphamide (Cy) versus total-body irradiation plus Cy as conditioning regimen—a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *J Clin Oncol.* 2013; 31:3549-3556.
4. Nagler A, Savani BN, Labopin M, et al. Outcomes after use of 2 standard ablative regimens in patients with refractory acute myeloid leukaemia: a retrospective, multicentre, registry analysis. *Lancet Haematol.* 2015;2:e384-e392.
5. Gupta V, Tallman MS, Weisdorf DJ. Allogeneic hematopoietic cell transplantation for adults with acute myeloid leukemia: myths, controversies, and unknowns. *Blood.* 2011;117:2307-2318.
6. Majhail NS, Brazauskas R, Rizzo JD, et al. Secondary solid cancers after allogeneic hematopoietic cell transplantation using busulfan-cyclophosphamide conditioning. *Blood.* 2011;117:316-322.
7. Rizzo JD, Curtis RE, Socie G, et al. Solid cancers after allogeneic hematopoietic cell transplantation. *Blood.* 2009;113:1175-1183.
8. Al Mumma N, Kahn JM, Jin Z, et al. Survival impact of early post-transplant toxicities in pediatric and adolescent patients undergoing allogeneic hematopoietic cell transplantation for malignant and non-malignant diseases: recognizing risks and optimizing outcomes. *Biol Blood Marrow Transplant.* 2016;22:1525-1530.
9. Sengsayadeth S, Savani BN, Blaise D, Malard F, Nagler A, Mohty M. Reduced intensity conditioning allogeneic hematopoietic cell transplantation for adult acute myeloid leukemia in complete remission—a review from the Acute Leukemia Working Party of the EBMT. *Haematologica.* 2015;100:859-869.
10. Dalle JH, Giralt SA. Hepatic veno-occlusive disease after hematopoietic stem cell transplantation: risk factors and stratification, prophylaxis, and treatment. *Biol Blood Marrow Transplant.* 2016;22:400-409.
11. Danylesko I, Shimoni A, Nagler A. Treosulfan-based conditioning before hematopoietic SCT: more than a BU look-alike. *Bone Marrow Transplant.* 2012;47:5-14.
12. Galaup A, Paci A. Pharmacology of dimethanesulfonate alkylating agents: busulfan and treosulfan. *Expert Opin Drug Metab Toxicol.* 2013;9:333-347.
13. ten Brink MH, Zwaveling J, Swen JJ, Bredius RG, Lankester AC, Guchelaar HJ. Personalized busulfan and treosulfan conditioning for pediatric stem cell transplantation: the role of pharmacogenetics and pharmacokinetics. *Drug Discov Today.* 2014;19:1572-1586.
14. Meinhardt G, Dayyani F, Jahrsdorfer B, Baumgart J, Emmerich B, Schmidmaier R. Treosulfan is an effective inducer of cell death in myeloma cell lines and primary myeloma cells from patients. *Br J Haematol.* 2003;122:892-899.
15. Schmidmaier R, Oellerich M, Baumgart J, Emmerich B, Meinhardt G. Treosulfan-induced apoptosis in acute myeloid leukemia cells is accompanied by translocation of protein kinase C delta and enhanced by bryostatin-1. *Exp Hematol.* 2004;32:76-86.
16. Fichtner I, Becker M, Baumgart J. Antileukaemic activity of treosulfan in xenografted human acute lymphoblastic leukaemias (ALL). *Eur J Cancer.* 2003;39:801-807.
17. Scheulen ME, Hilger RA, Oberhoff C, et al. Clinical phase I dose escalation and pharmacokinetic study of high-dose chemotherapy with treosulfan and autologous peripheral blood stem cell transplantation in patients with advanced malignancies. *Clin Cancer Res.* 2000;6:4209-4216.
18. van Pel M, van Breugel DW, Vos W, Ploemacher RE, Boog CJ. Towards a myeloablative regimen with clinical potential: I. Treosulfan conditioning and bone marrow transplantation allow induction of donor-specific tolerance for skin grafts across full MHC barriers. *Bone Marrow Transplant.* 2003;32:15-22.
19. Glowka FK, Karazniewicz-Lada M, Grund G, Wrobel T, Wachowiak J. Pharmacokinetics of high-dose intravenous treosulfan in children undergoing treosulfan-based preparative regimen for allogeneic haematopoietic SCT. *Bone Marrow Transplant.* 2008;42(suppl 2):S67-S70.
20. Wachowiak J, Sykora KW, Cornish J, et al. Treosulfan-based preparative regimens for allo-HSCT in childhood hematological malignancies: a retrospective study on behalf of the EBMT Pediatric Diseases Working Party. *Bone Marrow Transplant.* 2011;46:1510-1518.
21. Casper J, Holowiecki J, Trenschel R, et al. Allogeneic hematopoietic SCT in patients with AML following treosulfan/fludarabine conditioning. *Bone Marrow Transplant.* 2012;47:1171-1177.
22. Casper J, Knauf W, Kiefer T, et al. Treosulfan and fludarabine: a new toxicity-reduced conditioning regimen for allogeneic hematopoietic stem cell transplantation. *Blood.* 2004;103:725-731.
23. Shimoni A, Hardan I, Shem-Tov N, Rand A, Yerushalmi R, Nagler A. Fludarabine and treosulfan: a novel modified myeloablative regimen for allogeneic hematopoietic stem-cell transplantation with effective antileukemia activity in patients with acute myeloid leukemia and myelodysplastic syndromes. *Leuk Lymphoma.* 2007;48:2352-2359.
24. Kroger N, Shimoni A, Zabelina T, et al. Reduced-toxicity conditioning with treosulfan, fludarabine and ATG as preparative regimen for allogeneic stem cell transplantation (alloSCT) in elderly patients with secondary acute myeloid leukemia (sAML) or myelodysplastic syndrome (MDS). *Bone Marrow Transplant.* 2006;37:339-344.
25. Savani BN, Labopin M, Kroger N, et al. Expanding transplant options to patients over 50 years. Improved outcome after reduced intensity conditioning mismatched-unrelated donor transplantation for patients with acute myeloid leukemia: a report from the Acute Leukemia Working Party of the EBMT. *Haematologica.* 2016;101: 773-780.
26. Carreras E, Diaz-Beya M, Rosinol L, Martinez C, Fernandez-Aviles F, Rovira M. The incidence of veno-occlusive disease following allogeneic hematopoietic stem cell transplantation has diminished and the outcome improved over the last decade. *Biol Blood Marrow Transplant.* 2011;17:1713-1720.

27. Sengsayadeth SM, Srivastava S, Jagasia M, Savani BN. Time to explore preventive and novel therapies for bronchiolitis obliterans syndrome after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2012;18:1479-1487.
28. Richardson PG, Ho VT, Cutler C, Glotzbecker B, Antin JH, Soiffer R. Hepatic veno-occlusive disease after hematopoietic stem cell transplantation: novel insights to pathogenesis, current status of treatment, and future directions. *Biol Blood Marrow Transplant.* 2013;19: S88-S90.
29. Baron F, Ruggeri A, Beohou E, et al. RIC versus MAC UCBT in adults with AML: a report from Eurocord, the ALWP and the CTIWP of the EBMT. *Oncotarget.* 2016;7:43027-43038.
30. Munkelt D, Koehl U, Kloess S, et al. Cytotoxic effects of treosulfan and busulfan against leukemic cells of pediatric patients. *Cancer Chemother Pharmacol.* 2008;62:821-830.
31. Palmer J, McCune JS, Perales MA, et al. Personalizing busulfan-based conditioning: considerations from the American Society for Blood and Marrow Transplantation Practice Guidelines Committee. *Biol Blood Marrow Transplant.* 2016;22:1915-1925.
32. Boztug H, Zecca M, Sykora KW, et al. Treosulfan-based conditioning regimens for allogeneic HSCT in children with acute lymphoblastic leukaemia. *Ann Hematol.* 2015;94:297-306.
33. Gyurkocza B, Gutman J, Nemecek ER, et al. Treosulfan, fludarabine, and 2-Gy total body irradiation followed by allogeneic hematopoietic cell transplantation in patients with myelodysplastic syndrome and acute myeloid leukemia. *Biol Blood Marrow Transplant.* 2014;20:549-555.
34. Boztug H, Sykora KW, Slatter M, et al. European Society for Blood and Marrow Transplantation analysis of treosulfan conditioning before hematopoietic stem cell transplantation in children and adolescents with hematological malignancies. *Pediatr Blood Cancer.* 2016; 63:139-148.
35. Kroger N, Bornhauser M, Stelljes M, et al. Allogeneic stem cell transplantation after conditioning with treosulfan, etoposide and cyclophosphamide for patients with ALL: a phase II study on behalf of the German Cooperative Transplant Study Group and ALL Study Group (GMALL). *Bone Marrow Transplant.* 2015;50:1503-1507.
36. Schmitt M, Trenschel R, Sayer HG, et al. Conditioning with treosulfan and fludarabine for patients with refractory or relapsed non-Hodgkin lymphoma. *Mol Clin Oncol.* 2014;2:773-782.
37. Burroughs LM, Nemecek ER, Torgerson TR, et al. Treosulfan-based conditioning and hematopoietic cell transplantation for nonmalignant diseases: a prospective multicenter trial. *Biol Blood Marrow Transplant.* 2014;20:1996-2003.
38. Slatter MA, Boztug H, Potschger U, et al. Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases. *Bone Marrow Transplant.* 2015;50:1536-1541.
39. Savani BN, Montero A, Wu C, et al. Prediction and prevention of transplant-related mortality from pulmonary causes after total body irradiation and allogeneic stem cell transplantation. *Biol Blood Marrow Transplant.* 2005;11:223-230.
40. Williams KM, Chien JW, Gladwin MT, Pavletic SZ. Bronchiolitis obliterans after allogeneic hematopoietic stem cell transplantation. *JAMA.* 2009;302:306-314.
41. Shimoni A, Shem-Tov N, Volchek Y, Danylesko I, Yerushalmi R, Nagler A. Allo-SCT for AML and MDS with treosulfan compared with BU-based regimens: reduced toxicity vs reduced intensity. *Bone Marrow Transplant.* 2012;47:1274-1282.
42. Cieri N, Greco R, Crucitti L, et al. Post-transplantation cyclophosphamide and sirolimus after haploidentical hematopoietic stem cell transplantation using a treosulfan-based myeloablative conditioning and peripheral blood stem cells. *Biol Blood Marrow Transplant.* 2015;21:1506-1514.