GvHD and GRFS in patients with acute myeloid leukemia undergoing allogeneic HCT with treosulfan- *versus* reduced-intensity busulfan-based conditioning: a subgroup analysis of a randomized phase 3 trial

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Abstract

Acute myeloid leukemia (AML) is the most common indication for allogeneic hematopoietic cell transplantation (alloHCT), yet graft-*versus*-host disease (GvHD) remains a major post-transplant complication. Conditioning regimens, particularly

reduced-intensity approaches, are critical in optimizing outcomes. This subgroup analysis of the phase III MC-FludT.14/L trial compared treosulfan-fludarabine with reduced-intensity busulfan-fludarabine in 352 AML patients (aged 31–70 years) undergoing alloHCT. The primary endpoint was 24-month event-free survival; secondary endpoints included overall survival, GvHD incidence, relapse/progression, and non-relapse mortality. Treosulfan compared to busulfan demonstrated superiority: 24-month event-free survival was 65% *vs*. 53% (*P*=0.01), and overall survival was 73% *vs*. 65%. Event-free survival benefits were consistent across AML risk categories and notably higher in patients with Hematopoietic Cell Transplantation Comorbidity Index score >2 (62% *vs*. 42%, *P*=0.02). Treosulfan also showed lower non-relapse mortality and relapse rates. GvHD outcomes favored treosulfan, with a significantly lower incidence of extensive chronic GvHD at 24 months (15.1% *vs*. 28.1%, *P*=0.01). GvHD-free and relapse-free survival was also improved (53% *vs*. 40%, *P*=0.02). The safety profile was more favorable with treosulfan. These findings support treosulfan-fludarabine as a more effective and safer conditioning regimen than busulfan-fludarabine for AML patients undergoing alloHCT, particularly those at higher risk.

Introduction

Acute myeloid leukemia (AML) is the most frequent indication for allogeneic hematopoietic cell transplantation (alloHCT)[1] and is a curative treatment option for these patients.[2] Despite improvements in donor selection strategies, graft-*versus*-host disease (GvHD) prophylactic treatments, supportive therapies, conditioning strategies, and maintenance therapies that contribute to better long-term survival and disease control for AML patients undergoing alloHCT,[3] GvHD continues to be a burden for patients and a significant challenge for healthcare providers.[4]

Acute GvHD develops in 30% to 50% of patients after alloHCT, while chronic GvHD is diagnosed in 30% to 70% of patients after alloHCT.[5] According to a recent Center for International Blood and Marrow Transplant Research (CIBMTR) report, in adults undergoing alloHCT, 8-13% of early deaths within 100 days of transplantation were attributed to acute GvHD.[6]

Chronic GvHD is a leading cause of post-alloHCT morbidity and is significantly associated with a higher risk of non-relapse mortality (NRM).[7] Ultimately, chronic GvHD results in increased direct healthcare resource costs and indirect costs associated with productivity loss.[8]

Along with other factors (e.g., donor and graft cell characteristics, disease status of the recipient, recipient comorbidity status, immunosuppressive prophylaxis), the choice of conditioning regimen has an impact on the incidence and severity of GvHD.[9] Reduced intensity conditioning (RIC) regimens are an option for patients for whom myeloablative conditioning (MAC) would not be tolerable due to e.g., age and/or comorbidities, and who would therefore not be eligible for this potential curative treatment approach. With emerging new GvHD prophylactic approaches, the question of the optimal conditioning intensity is becoming increasingly relevant,[10,11] especially for the growing number of older and/or comorbid patients with myeloid malignancies.[12] A dose-reduced intravenous (IV) busulfan-based regimen combined with the purine analogue fludarabine has been a well-established RIC regimen for patients with AML considered ineligible for MAC treatments.[13-15] Treosulfan is considered an alternative treatment for conditioning of patients with AML or myelodysplastic syndrome (MDS). Various prospective and retrospective studies demonstrated that treosulfan, a water-soluble bifunctional alkylating agent, when combined with fludarabine showed a particularly favorable acute organ toxicity profile and allowed rapid donor cell engraftment with complete and sustained donor hematopoietic chimerism after alloHCT.[16-20] Therefore, the combination of treosulfan with fludarabine is referred to as a myeloablative, but reduced toxicity-conditioning (RTC) regimen.[21,22] Two phase II studies, one phase III study, and a dose-range finding study confirmed that treosulfan in combination with fludarabine is a well-tolerated conditioning regimen for AML and MDS patients.[17,20,21,23]

Here, we present a subgroup analysis of the phase III MC-FludT.14/L study [21,23] with focus on the AML patient population. This is the first time that the MC-FludT.14/L AML-specific comparison of the safety and efficacy outcomes for RTC treosulfan-fludarabine *versus* RIC busulfan-fludarabine conditioning treatment is presented in detail, with an emphasis on GvHD outcomes.

Methods

Study design

MC-FludT.14/L was a multinational, multicenter, randomized (1:1), parallel-group, open-label, prospective phase III clinical trial. This subgroup analysis is restricted to 352 AML patients, following the same objectives defined in the published study.[21] The trial protocol was approved by the responsible ethics committees in the participating countries. All patients provided written informed consent. The trial is registered with EudraCT (2008-002356-18) and on *www.clinicaltrials.gov* (NCT00822393).

Study participants

Adult patients aged >50 years and/or with a Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) score >2 (range, 0-11) with AML in first or consecutive hematologic remission or with MDS were enrolled in the trial between

2013 and 2018. For this analysis, only AML patients were included. Patients had a Karnofsky Performance Status ≥60% (median 90%) and received transplants from matched related- or unrelated donors (≥9/10 HLA class I or II allele identities). Full inclusion criteria are presented in *Online Supplementary Section S1*.

Randomization and masking

This phase III trial was an open-label study and randomized according to stratification for center, donor type, and risk group for AML following the 2010 European LeukemiaNet criteria.[23,24]

Procedures

Eligible patients were randomized to receive either 10 g/m² body surface area IV treosulfan (days −4, −3, −2) or 3.2 mg/kg IV busulfan (days −4, −3), both combined with 30 mg/m² IV fludarabine (days −6 to −2). Patients received cyclosporine A (day −1 to day +100), methotrexate (15 mg/m² IV day +1, 10 mg/m² days +3 and +6) and calcium folinate. In the case of a matched unrelated donor, patients received Grafalon® (ATG-S-Fresenius) 10 mg/kg IV (days −4, −3, −2) or ATG-thymoglobuline 2.5 mg/kg IV (days −2, −1).

Outcomes

The primary endpoint was event-free survival (EFS) 2 years after alloHCT. Events were defined as relapse, graft failure, or death. Secondary endpoints were overall survival (OS), cumulative incidence of relapse or progression, cumulative incidence of non-relapse mortality (NRM) (probability of dying without relapse or progression), cumulative incidence of acute GvHD and chronic GvHD within 2 years of transplantation, cumulative incidence of engraftment on day +28, incidence of complete donor type chimerism on days +28 and +100, GvHD-free and relapse-free survival (GRFS), chronic GRFS (CRFS), and safety.

GRFS was defined from date of transplant to date of last follow-up without grade III–IV acute GvHD, chronic GvHD requiring systemic treatment, relapse, progression, or death. CRFS was defined from date of transplant to date of last follow-up without either moderate or severe chronic GvHD, relapse, progression, or death.

Statistical analysis

The subgroup analysis was performed based on data of the 352 patients with AML followed up for at least 2 years after transplantation plus additional post-surveillance data. The objectives and methods were prospectively defined for the MC-FludT.14/L clinical trial protocol.[21] All statistical methods applied in this AML subgroup analysis were prospectively defined in the protocol and were consistent with the analysis of both AML and MDS patients. Efficacy analyses adhered to the intent-to-treat principle. *P* values <0.05 are considered statistically significant. All analyses were performed with SAS software (version 9.4).

Results

Enrollment

A total of 352 patients with AML (N=184 in the treosulfan treatment group, N=168 in the busulfan treatment group), who received conditioning treatment and proceeded to alloHCT, were included in this subgroup analysis.

The median age of the 352 patients was 60 years (range, 31-70 years). The majority (94.6%) of the patients were ≥50 years old. A total of 49 patients (13.9%) were in second complete remission or beyond and categorized into the high-risk category. Baseline blast count in the bone marrow at inclusion into the study was <5% for all but one patient. Detailed baseline demographics and disease characteristics are shown in Table 1.

Efficacy

In total 70.7% of patients in the treosulfan treatment group and 63.7% of patients in the busulfan treatment group were alive and censored at the date of last available follow-up. The median follow-up time was 2.5 years. The overall primary and secondary outcome results stratified by low-, intermediate-, and high-risk as well as patients' Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI) score ≤2 or >2 are summarized in Table 2.

Overall, the Kaplan-Meier estimate of EFS at 24 months was significantly higher for the treosulfan treatment group than in the busulfan treatment group (Table 2, Figure 1). EFS was significantly higher in patients allocated to treosulfan (61.9%) than in patients receiving busulfan (42.2%) in patients with a HCT-CI score >2 (*P*=0.022) (Table 2).

Kaplan-Meier estimates of OS at 24 months were 72.8% (95% confidence interval [95% CI]: 65.5-78.8) in the treosulfan treatment group and 64.7% (95% CI: 56.7-71.6) in the busulfan treatment group and statistically significantly favored treosulfan (*P*=0.030; hazard ratio [HR]=0.65; 95% CI: 0.43-0.96). OS was significantly higher in patients in the treosulfan (69.3%) group compared to patients in the busulfan (55.4%) group (*P*=0.029) in patients with an HCT-CI score >2 (Table 2).

The cumulative incidence of NRM at 24 months reached 8.4% (95% CI: 4.3-12.5) in the treosulfan treatment group and 14.7% (95% CI: 9.2-20.1) in the busulfan group (*P*=0.128; HR=0.62; 5% CI: 0.33-1.15) (Figure 2).

Engraftment at 28 days after alloHCT was accomplished in 97.3% in the treosulfan treatment group and 96.4% in the busulfan treatment group (HR=1.08; 95% CI: 0.89-1.31; *P*=0.420). The cumulative incidence of primary or secondary graft failure at 24 months was very low (0 patients after treosulfan *vs*. 5 patients after busulfan [3%]; *data not shown*).

Complete donor chimerism on day +28 and day +100 after alloHCT was assessed as a secondary endpoint in the trial. On day +28, the incidence of complete donor chimerism was 94.5% (95% CI: 90.1-97.3) in the treosulfan group and 87.5% (95% CI: 81.5-92.1) in the busulfan group. On day +100

the incidences were 86.9% (95% CI: 80.9-91.5) and 82.8% (95% CI: 76.1-88.3), respectively.

The Kaplan-Meier estimates of the composite endpoint of GRFS at 24 months were 52.9% (95% CI: 45.2-60.0) for the treosulfan group and 39.6% (95% CI: 31.7-47.4) for the busulfan group (*P*=0.022; HR=0.69; 95% CI: 0.50-0.95) (Figure 3). The Kaplan-Meier estimates of CRFS at 24 months were 53.4% (95% CI: 45.7-60.5) for the treosulfan group and 39.6% (95% CI: 31.7-47.3) for the busulfan group (*P*=0.0164; HR=0.68; 95% CI: 0.49-0.93) (Figure 4).

The significant advantage in 24-month GRFS and CRFS in favor of the treosulfan group was further reflected in the GRFS and CRFS analysis according to AML risk groups, in which the trend for higher GRFS and CRFS for patients in the treosulfan group compared to the busulfan group was observed in all subgroups (Table 2).

Safety

Overall, 32.7% of patients died after alloHCT, 29.3% of 184 AML patients in the treosulfan group and 36.3% of 168 AML patients in the busulfan treatment group. Relapse and/or progression was the leading cause of death (16.3% patients in the treosulfan group and 19.0% patients in the busulfan group) followed by transplantation-related causes (8.7% of

Table 1. Baseline demographics and disease characteristics of patients with acute myeloid leukemia.

AML subgroup of the MC-FludT.14/L trial	Busulfan, N=168	Treosulfan, N=184
Sex, N (%) Male Female	 92 (54.8) 76 (45.2)	 104 (56.5) 80 (43.5)
Age, years, mean (SD) ≥50 years, N (%) ≥60 years, N (%)	59.6 (6.3) 159 (94.6) 94 (56.0)	59.4 (6.6) 174 (94.6) 94 (51.1)
Weight, kg, mean (SD)	77.6 (17.7)	79.7 (17.1)
BMI, kg/m^2, mean (SD)	26.39 (4.93)	27.15 (5.07)
Body surface area, m^2, mean (SD)	1.896 (0.240)	1.921 (0.231)
Time between diagnosis and HCT, months, median (Q1-Q3)	4.99 (3.65-8.21)	5.26 (3.84-8.62)
Classification of AML, N (%) Low risk Intermediate risk High risk In case of >CR1 allocation to high-risk AML for results analysis	 18 (10.7) 76 (45.2) 50 (29.8) 24 (14.3)	 19 (10.3) 68 (37.0) 72 (39.1) 25 (13.6)
Donor to patient sex, N (%) Female to female Female to male Male to female Male to male	 42 (25.0) 26 (15.5) 34 (20.2) 66 (39.3)	 36 (19.5) 38 (20.7) 44 (23.9) 66 (35.9)
Donor type, N (%) Matched related donor Matched unrelated donor MMUD (9/10 HLA mismatch)	 50 (29.8) 89 (53.0) 29 (17.3)	 45 (24.5) 111 (60.3) 28 (15.2)
Stem cell source, N (%) Peripheral blood Bone marrow	 163 (97.0) 5 (3.0)	 177 (96.2) 7 (3.8)
CD34 cells, x10^6/Kg recipient body weight, median (Q1, Q3)	6.1 (4.9- 7.9)	6.0 (5.0-7.6)
Karnofsky Performance Score, N (%) 60 70 80 90 100	 0 (0.0) 3 (1.8) 27 (16.1) 75 (44.6) 63 (37.5)	 3 (1.6) 7 (3.8) 32 (17.4) 67 (36.4) 75 (40.8)
HCT-CI score Patients with HCT-CI score >2, N (%) Mean (SD)	 101 (60.1) 2.90 (1.94)	 105 (57.1) 2.88 (1.95)

AML: acute myeloid leukemia; SD: standard deviation; BMI: body mass index; HCT: hematopoietic stem cell transplantation; Q1: first quartile; Q3: third quartile; >CR1: second complete remission or beyond; MMUD: mismatched unrelated donor; HCT-CI: Hematopoietic Cell Transplantation-specific Comorbidity Index.

Table 2. Event-free survival and secondary outcomes of patients with acute myeloid leukemia stratified by risk group and Hematopoietic Cell Transplantation-specific Comorbidity Index score.

Outcome	Busulfan, N=168	Treosulfan, N=184	Hazard ratio treosulfan/busulfan (95% CI), *P**
EFS at 24 months, % (95% CI)[a]			
Total	53.3 (45.2-60.7)	64.7 (57.1-71.3)	0.64 (0.45-0.90), 0.012
Patient risk group			
Low	59.6 (33.1-78.4)	88.4 (60.8-97.0)	0.14 (0.02-1.33), 0.087
Intermediate	62.9 (50.5-72.9)	75.6 (63.1-84.3)	0.64 (0.33-1.23), 0.181
High	42.0 (30.4-53.2)	52.5 (41.9-62.0)	0.74 (0.47-1.16), 0.194
Patient HCT-CI score			
≤2	69.6 (56.8-79.2)	68.3 (56.4-77.6)	1.22 (0.58-2.59), 0.595
>2	42.2 (32.0-52.0)	61.9 (51.7-70.6)	0.59 (0.37-0.93), 0.022
OS at 24 months, % (95% CI)[a]			
Total	64.7 (56.7, 71.6)	72.8 (65.5-78.8)	0.65 (0.43-0.96)-0.0303
Patient risk group			
Low	76.6 (48.8-90.5)	88.4 (60.8-97.0)	0.17 (0.02-1.70), 0.1326
Intermediate	71.0 (59.0-80.1)	87.2 (75.9-93.4)	0.54 (0.23-1.25), 0.1514
High	55.5 (43.2-66.2)	59.6 (48.9-68.8)	0.79 (0.49-1.27), 0.3250
Patient HCT-CI score			
≤2	78.4 (66.1-86.6)	77.5 (66.3-85.4)	1.27 (0.56-2.92), 0.5663
>2	55.4 (44.8-64.8)	69.3 (59.2-77.4)	0.56 (0.34-0.94), 0.0289
Relapse/progression at 24 months, % (95% CI)[a]			
Total	29.0 (21.9, 36.0)	26.9 (20.3-33.5)	0.82 (0.56-1.22)-0.3296
Patient risk group			
Low	34.4 (11.9-56.9)	11.6 (0.0-26.8)	0.25 (0.05-1.16), 0.0762
Intermediate	18.1 (9.1-27.2)	19.6 (10.0-29.2)	0.96 (0.46-1.98), 0.9071
High	38.7 (27.4-50.0)	34.9 (25.2-44.6)	0.90 (0.55-1.49), 0.6943
Patient HCT-CI score			
≤2	21.2 (11.3-31.0)	27.8 (17.6-38.0)	1.21 (0.63-2.31), 0.5718
>2	34.3 (24.6-44.0)	26.3 (17.7-34.8)	0.69 (0.42-1.13), 0.1414
NRM at 24 months, % (95% CI)[a]			
Total	14.7 (9.2-20.1)	8.4 (4.3-12.5)	0.62 (0.33-1.15), 0.1281
Patient risk group			
Low	6.0 (0.0-17.4)	0.0 (0.0-0.0)	NA (NA-NA), NA
Intermediate	16.1 (7.8-24.5)	4.8 (0.0-10.2)	0.37 (0.12-1.17), 0.0899
High	15.3 (6.9-23.6)	12.5 (5.9-19.2)	0.87 (0.39-1.95), 0.7292
Patient HCT-CI score			
≤2	7.8 (1.2-14.3)	3.8 (0.0-8.1)	0.74 (0.23-2.41), 0.6190
>2	19.3 (11.5-27.1)	11.8 (5.5-18.1)	0.59 (0.28-1.21), 0.1499
GRFS at 24 months, % (95% CI)[a]			
Total	39.6 (31.7, 47.4)	52.9 (45.2-60.0)	0.69 (0.50-0.95)-0.0224
Patient risk group			
Low	40.9 (17.8-62.9)	67.5 (41.4-84.0)	0.30 (0.07-1.27), 0.1028
Intermediate	46.7 (34.5-58.1)	63.4 (50.3-73.9)	0.65 (0.36-1.16), 0.1445
High	32.1 (21.2-43.4)	42.7 (32.4-52.5)	0.76 (0.50-1.17), 0.2096
Patient HCT-CI score			
≤2	51.9 (38.9-63.4)	57.4 (45.2-67.8)	0.98 (0.51-1.89), 0.9502
>2	31.2 (21.7-41.1)	49.6 (39.5-58.8)	0.76 (0.50-1.15), 0.1878
CRFS at 24 months, % (95% CI)[a]			
Total	39.6 (31.7-47.3)	53.4 (45.7-60.5)	0.68 (0.49-0.93), 0.0164
Patient risk group			
Low	40.9 (17.8-62.9)	67.5 (41.4-84.0)	0.30 (0.07-1.27), 0.1028
Intermediate	46.7 (34.5-58.0)	63.4 (50.3-73.9)	0.65 (0.37-1.17), 0.1534
High	32.0 (21.2-43.3)	43.7 (33.4-53.6)	0.73 (0.48-1.12), 0.1492
Patient HCT-CI score			
≤2	51.9 (38.9-63.4)	57.4 (45.2-67.8)	0.98 (0.51-1.89), 0.9502
>2	31.2 (21.7-41.1)	50.5 (40.4-59.8)	0.74 (0.48-1.12), 0.1532

*Adjusted for donor type as factor, and risk group and center as strata using a Cox regression model.[a]Based on Kaplan-Meier estimates. EFS: event-free survival; 95% CI: 95% confidence interval; EFS: event-free survival; HCT-CI: Hematopoietic Cell Transplantation-specific Comorbidity Index; OS: overall survival; NA: not available; NRM: non-relapse mortality; GRFS: graft-*versus*-host disease and relapse-free survival; CRFS: chronic GRFS.

patients in the treosulfan group and 14.3% of patients in the busulfan group). The incidence of acute GvHD was similar in both treatment groups (*Online Supplementary Table S6, Online Supplementary Figure S2*). Subgroup analyses by AML risk groups and HCT-CI score did not demonstrate any significant safety differences between the two treatment groups. The incidences of treatment-emergent adverse events in the treosulfan and busulfan treatment groups were comparable (*Online Supplementary Table S4*) with a tendency for a lower frequency of treatment-emergent adverse events in the treosulfan group as compared to the busulfan group (62.5% and 67.9%, respectively).

A total of 59.9% of patients in the treosulfan treatment group and 54.3% of patients in the busulfan treatment group experienced chronic GvHD. The cumulative incidence at 24 months was 61.1% (95% CI: 53.2-69.0) for the treosulfan treatment group and 54.9% (95% CI: 46.4-63.3) in the busulfan treatment group. The cumulative incidence of

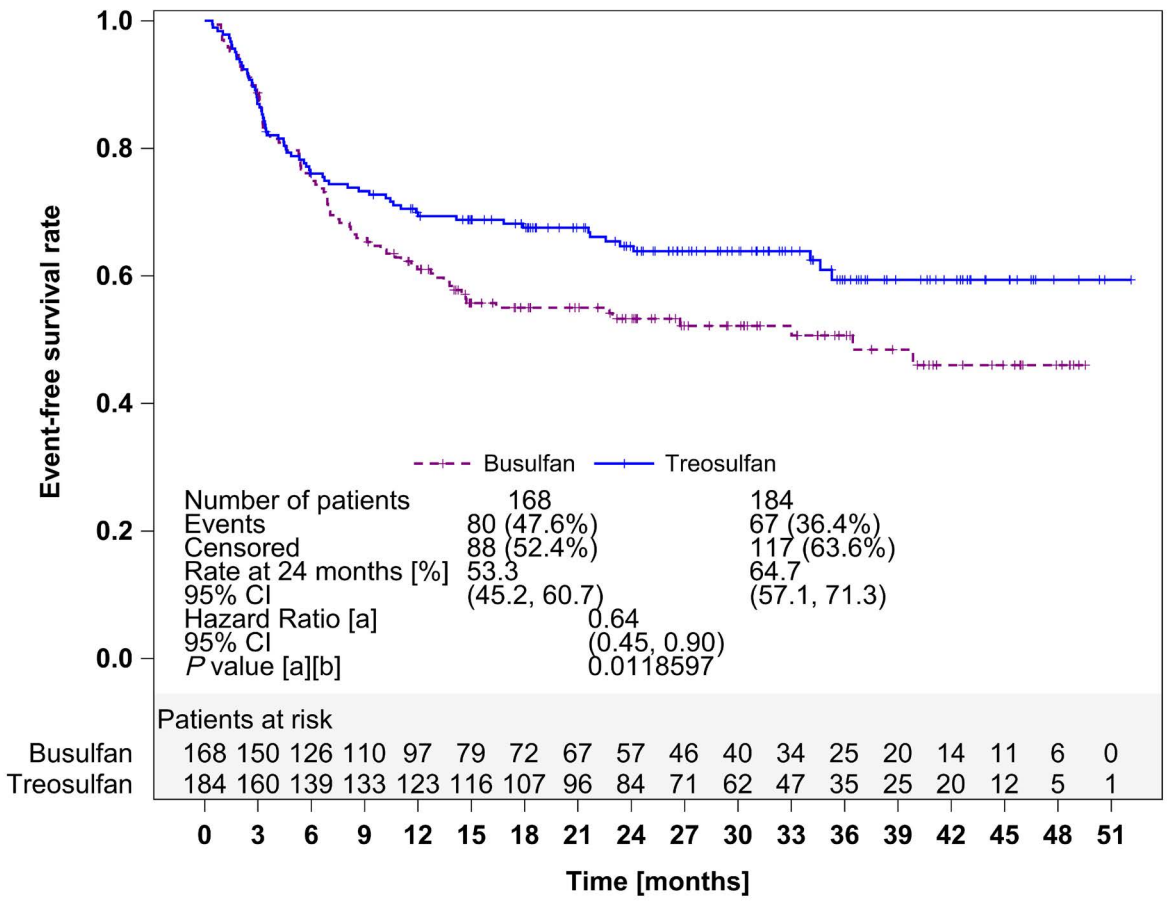


Figure 1. Kaplan-Meier estimate of event-free survival of patients with acute myeloid leukemia. [a] Adjusted for donor type as factor, and risk group and center as strata using a Cox regression model. [b] For testing difference of treosulfan compared to busulfan. 95% CI: 95% confidence interval.

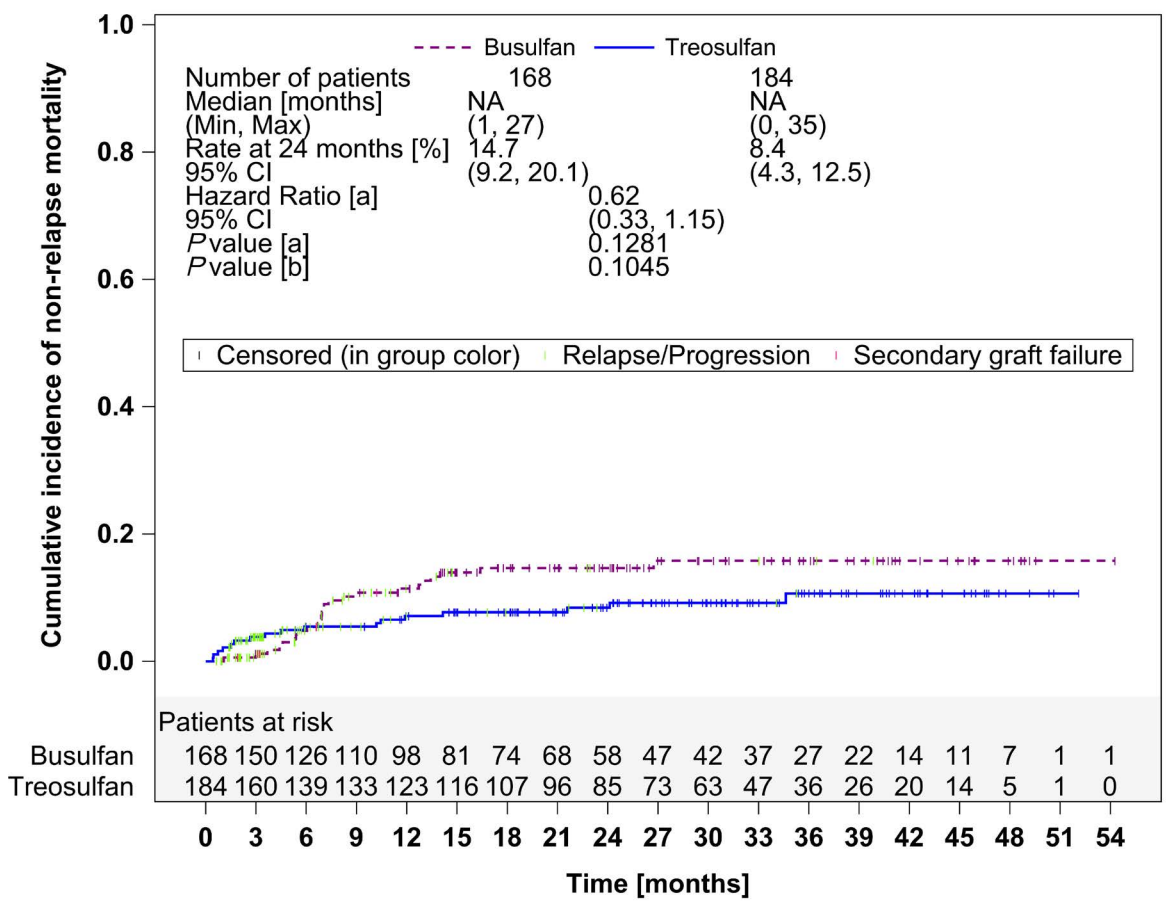


Figure 2. Cumulative incidence of non-relapse mortality of patients with acute myeloid leukemia. [a] Adjusted for donor type as factor and risk group as stratum using a Fine and Gray model. [b] Based on the Gray test. NA: not available; Min: minimum; Max: maximum; 95% CI: 95% confidence interval.

extensive chronic GvHD at 24 months was 15.1% (95% CI: 9.4-20.9) in the treosulfan group and 28.1% (95% CI: 20.3-35.9) in the busulfan group, with a hazard ratio of 0.53 (95% CI: 0.31-0.88), which significantly favors the treosulfan group.

Discussion

This AML subgroup analysis of the MC-FludT.14/L trial demonstrated the substantial curative potential of alloHCT with treosulfan-based conditioning in elderly and/or comorbid AML patients. Improved survival outcome with RTC treosulfan compared to RIC busulfan plus fludarabine conditioning was consistently evident in all AML risk group categories and was mainly related to reduction of NRM and extensive chronic GvHD. These promising results were achieved in patients in complete remission at the time transplant, which was considered standard of care. For patients with

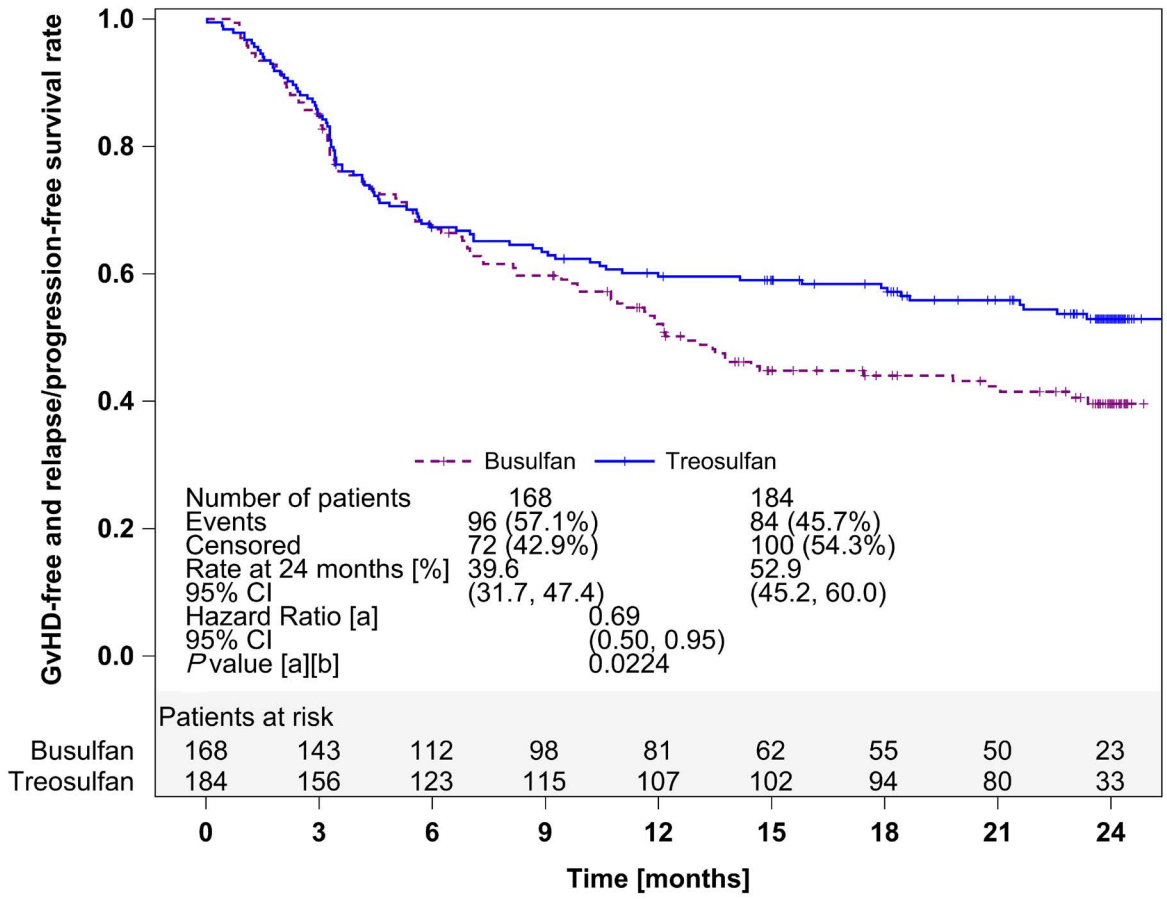


Figure 3. Graft-*versus*-host disease-free and relapse-free survival of patients with acute myeloid leukemia. Note: graft-*versus*-host disease (GvHD)-free is defined as no acute GvHD of at least grade III and no extensive chronic GvHD. [a] Adjusted for donor type as factor, and risk group and center as strata using a Cox regression model. [b] For testing difference of treosulfan compared to busulfan. 95% CI: 95% confidence interval.

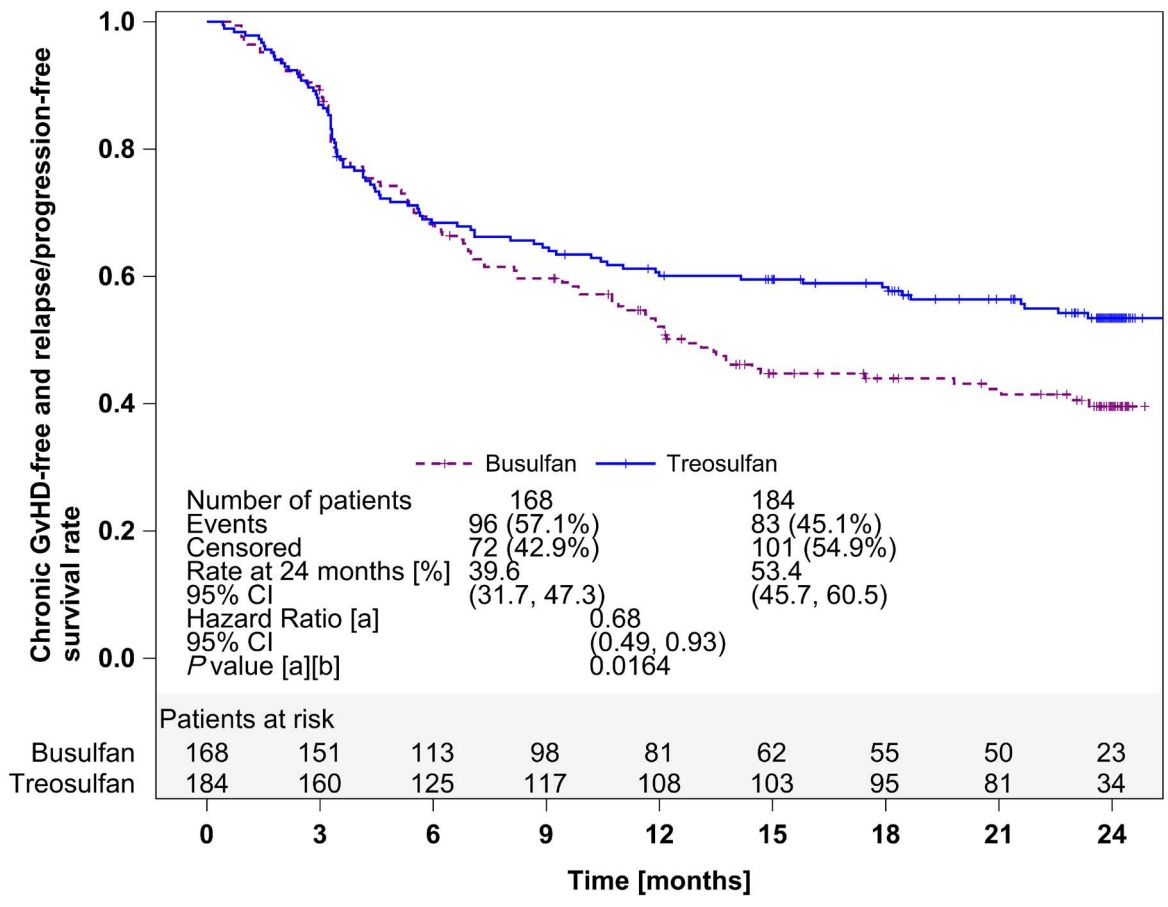


Figure 4. Chronic graft-*versus*-host disease-free and relapse-free survival of patients with acute myeloid leukemia. Note: chronic graft-*versus*-host disease (GvHD)-free is defined as no extensive chronic GvHD. [a] Adjusted for donor type as factor, and risk group and center as strata using a Cox regression model. [b] For testing difference of treosulfan compared to busulfan. 95% CI: 95% confidence interval.

relapsed or refractory AML, a recent European Blood and Marrow Transplant Group (EBMT) analysis was able to show that higher-dose treosulfan-fludarabine was associated with better outcomes than myeloablative busulfan-fludarabine conditioning in AML patients with active disease. The conditioning regimen was the only independent predictor of leukemia-free survival, OS, and GRFS.[25] Thus, using treosulfan-based conditioning in patients even with active AML might be a promising alternative which would have to be assessed in a prospective trial.

The incidence of extensive chronic GvHD and of the composite endpoint CRFS in the present analysis significantly favored the treosulfan-fludarabine regimen, which was also seen in one retrospective registry analysis.[26] This suggests a lower extramedullary toxicity profile of treosulfan which is in part reflected by a better immunosuppressive potency in the marrow when considering the higher engraftment rates, lower rates of graft failure (no patient in the treosulfan-group as compared to 5 patients in the busulfan-group), and higher complete donor chimerism rates as compared to those in patients receiving busulfan. The GvHD prophylaxis used in this study in the case of alloHCT from an unrelated donor included ATG. However, several recent publications report use of post-transplant cyclophosphamide (PTCy) as prophylactic treatment to be a promising alternative with regard to OS and GRFS not only from haploidentical donors but also from matched unrelated and mismatched unrelated donors.[27-29] Therefore, PTCy is increasingly used in alloHCT from matched sibling, matched unrelated, mismatched unrelated, and haploidentical donors. The cumulative incidence of extensive chronic GvHD at 2 years after treosulfan-based conditioning combined with ATG-based prophylaxis of 15.1% in the present analysis compares favorably to published data with other reduced intensity and myeloablative conditioning regimens, with which the incidence of extensive chronic GvHD was between 21% and 49%. Chronic GvHD rates after PTCy-based prophylaxis in comparative analyses had a tendency of being lower, although the difference was not statistically significant.[30-32] In a recently published EBMT analysis of patients with AML, the 2-year incidence of severe chronic GvHD after treosulfan-based conditioning before HCT from haploidentical donors with PTCy was 12%, showing that PTCy is a promising approach which might further improve transplantation outcomes and consequently have a positive impact on health-related quality of life in patients and reduction of costs due to necessary healthcare resource utilization.[8,33]

The outcome after the treosulfan-fludarabine regimen was also significantly better compared to busulfan-fludarabine with regard to the composite endpoint of GRFS. GRFS at 2 years after treosulfan-based conditioning was at least comparable if not better than reported rates after other conditioning regimens combined with ATG-containing GvHD prophylaxis: 52.9% in this study compared to 21%-49.3% reported in the literature.[30-32,34] Saraceni *et al*. reported a 2-year GRFS after haploidentical HCT with PTCy and treosulfan-based conditioning of 53% which is well in line with the present analysis.[35] Treosulfan-based conditioning in the present analysis resulted in favorable GRFS in all AML patients independently of their disease risk as well as in patients with an HCT-CI score >2. In general, efficacy endpoints including EFS and OS showed larger differences between treatment groups favoring treosulfan in patients with an HCT-CI score >2. The analysis of relapse/progression, NRM, GRFS, and CRFS also favored treosulfan compared to busulfan. This allows the interpretation that, while all patients benefited from the RTC regimen, this benefit is especially pronounced in patients with a worse HCT-CI score, underlining its standing as a reduced toxicity regimen.

Another important RIC regimen, frequently reported about and advised by international recommendations based on retrospective non-randomized data, is melphalan in combination with fludarabine.[10,11,36-40] In a recently published matched-pair analysis of the EBMT, AML/MDS patients treated with different conditioning intensities based on the recently proposed intensity weighted Transplant Conditioning Intensity risk scheme were compared.[41] Patients receiving conditioning with fludarabine and melphalan (intermediate intensity), or with busulfan and cyclophosphamide (high intensity), or with fludarabine and treosulfan (low intensity) as part of the prospective MC-FludT.14/L study were compared. The analysis demonstrated superiority of the treosulfan-containing RTC over busulfan- as well as melphalan-based conditioning regarding both OS and NRM.[42] These data suggest that treosulfan may have a similar antileukemic efficacy and lower NRM resulting in higher OS than melphalan or busulfan when used as part of a conditioning regimen.

Nowadays, the combination of either treosulfan or melphalan for RTC or RIC, respectively, in AML/MDS patients for alloHCT followed by PTCy is established in many transplant centers worldwide.[35,43-49] Whether treosulfan or melphalan would be the better partner for PTCy in this usually elderly patient population deemed at risk of a higher transplantation-associated toxicity is not clear yet and will be assessed in the upcoming randomized ETAL5/RELEVANT trial (EUCT number: 2023-507879-21-00).

This study has several limitations that reflect the standard clinical practices at the time the trial was conducted. First, minimal residual disease status prior to alloHCT was not assessed, as minimal residual disease monitoring was not standard of care during the recruitment period (2013–2018), and European LeukemiaNet minimal residual disease guidelines were only published in 2018.[50] Secondly, no specific data on T-cell chimerism was collected. Furthermore, no data were captured on the use of post-transplant maintenance therapy, which was not routinely performed in clinical practice at the time. These factors may have influenced relapse dynamics and long-term outcomes.

The optimal conditioning regimen for patients with AML is

yet to be determined, considering the increasing numbers of HCT for elderly AML patients all over the world. Nonetheless, our analysis confirms the clinically relevant benefit of the treosulfan-fludarabine regimen as a well-tolerated and effective preparative regimen for alloHCT compared to a busulfan-based regimen in elderly and/or comorbid AML patients considered to be at increased risk with standard conditioning therapies, with promising data not only in EFS and OS but also regarding GRFS and CRFS.

Disclosures

FS has been a speaker for Link/Clinigen, Medscape, medac, Servier and JAZZ; has provided consultancy and/or participated in advisory boards for medac, Pierre Fabre and Astellas and has received travel support from medac, Servier and Johnson & Johnson. DWB has been a speaker for, provided consultancy for, participated in advisory boards for and received travel support from medac GmbH. JM, BS, IK and XL are employers of medac GmbH. GSt, MS, MM, PR, TL, FC, E-MW-D, CJ, CS, FP, GSo, IH, AR, KS-E, DR, GW, BG, HL-W, MB, JC, EH, FB, API, DK and WB do not have any conflicts of interest to disclose.

Contributions

FS, MS, DWB and WB designed the study, contributed patients and were primarily responsible for writing the manuscript; they had access to full trial data. XL provided all statistical support and contributed to the data analysis and the manuscript. IK contributed to the study design and the manuscript. All authors had access to full trial data, contributed patients, helped with data cleaning and edited the manuscript.

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Data-sharing statement

The data underlying this study are not separately available from the corresponding author, due to being only a subgroup analysis of another published work.

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