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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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This article is to express our gratitude and honor our esteemed colleague Götz Ulrich Grigoleit who passed away too early and whom we miss.

Running Head: GVHD & GRFS in AML with Treo vs Bu Conditioning

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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 3 MC-FludT.14/L trial compared treosulfan-fludarabine with reduced-intensity busulfan-fludarabine in 352 AML patients (aged 31–70) undergoing alloHCT. The primary endpoint was 24-month event-free survival (EFS); secondary endpoints included overall survival (OS), GVHD incidence, relapse/progression, and non-relapse mortality (NRM). Treosulfan compared to busulfan demonstrated superiority: 24-month EFS was 65% vs. 53% ($p = 0.01$), and OS was 73% vs. 65%. EFS benefits were consistent across AML risk categories and notably higher in patients with hematopoietic cell transplantation comorbidity index >2 (62% vs. 42%, $p = 0.02$). Treosulfan also showed lower NRM 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.

Keywords: treosulfan, busulfan, acute myeloid leukemia, allogeneic, transplantation.

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 (aGVHD) develops in 30% to 50% of patients after alloHCT, while chronic GVHD (cGVHD) is diagnosed in 30% to 70% of patients post-alloHCT.[5] Per 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 aGVHD.[6] cGVHD is a leading cause of morbidity post-alloHCT and is significantly associated with a higher risk of non-relapse mortality (NRM).[7] Ultimately, cGVHD 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, etc.), 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 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 purine analogue fludarabine has been a well-established RIC regimen for patients with AML considered ineligible for myeloablative conditioning 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 regimen (RTC).[21,22] Two phase 2 studies, one phase 3 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 3 study MC-FludT.14/L[21,23] with focus on 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 3 clinical trial. This subgroup analysis is restricted to n = 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.

Study Participants

Adult patients aged >50 years and/or with hematopoietic cell transplantation comorbidity index (HCT-CI) >2 (range 0 to 11) with AML in first or consecutive hematological 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 Index ≥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 Supplementary Section 1.

Randomization and Masking

This phase 3 trial was an open-label study and randomized according to stratification for center, donor type, and risk group for AML following the 2010 European Leukemia Network 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 ciclosporin A (day −1 to day +100), methotrexate (15 mg/m^2 IV day +1, 10 mg/m^2 days +3 and +6) and calcium folinate. In case of MUD, 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) two 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 NRM (probability of dying without relapse or progression), cumulative incidence of aGVHD and cGVHD 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 aGVHD, cGVHD 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 cGVHD, relapse, progression, or death.

Statistical Analysis

The subgroup analysis was performed based on data of the n = 352 patients with AML followed up for at least two years post-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 analysis methods applied in this AML subgroup analysis were prospectively defined in the protocol and consistent with the analysis consisting of both AML and MDS patients. Efficacy analyses adhered to the intent-to-treat principle. p-values of <0.05 are considered statistically significant. All analyses were performed with SAS software (Version 9.4).

RESULTS

Enrolment

A total of n = 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 >CR1 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' HCI-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 compared to the busulfan treatment group (Table 2, Figure 1). EFS was significantly higher in patients allocated to treosulfan (61.9%) as compared to patients receiving busulfan (42.2%) in patients with HCT-CI score >2, p = 0.022 (Table 2).

Kaplan-Meier estimates of OS at 24 months were 72.8% (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 was statistically significantly favoring treosulfan (p = 0.030; 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).

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 [95% 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). Cumulative incidence of primary or secondary graft failure at 24 months was very low (0 patients after treosulfan versus 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 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-months GRFS and CRFS in favor of the treosulfan group was further reflected in the GRFS and CRFS analysis per AML risk groups, where 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 deceased after allogeneic HCT, 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% patients in the treosulfan group and 14.3% patients in the busulfan group). The incidence of aGVHD was similar in both treatment groups (Supplementary Table 6 and Supplementary Figure 2). Subgroup analyses by AML risk groups and HCT-CI did not demonstrate any significant safety differences between the two treatment groups. The incidence of treatment-emergent adverse events in the treosulfan and busulfan treatment groups were comparable (Supplementary Table 4) 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 extensive cGVHD 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 HR 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 of extensive cGVHD. These promising results were achieved in patients in complete remission at time of transplant, which was considered standard of care. For patients with relapsed or refractory AML, a recent EBMT analysis was able to show that higher-dosed treosulfan-fludarabine was associated with better outcome than MAC busulfan-fludarabine conditioning in AML patients with active disease. 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 cGVHD 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 five patients in the busulfan-group), and higher complete donor chimerism rates as

compared to patients receiving busulfan. GVHD prophylaxis used in this study in case of alloHCT from an unrelated donor included ATG. However, several recent publications find 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 MUD and MMUD.[27–29] Therefore, PTCy is increasingly used in alloHCT from MSD, MUDs, MMUDs, and haploidentical donors. The cumulative incidence of extensive cGVHD 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 RIC and MAC conditioning regimens, where incidence of extensive cGVHD was between 21% and 49%. cGVHD rates after PTCy-based prophylaxis in comparative analyses had a tendency of being lower, although not statistically significant.[30–32] In a recently published EBMT analysis for patients with AML, the 2-year incidence of severe cGVHD 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 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 from their disease risk as well as in patients with HCT-CI >2. In general, efficacy endpoints including EFS and OS showed larger differences between treatment groups favoring treosulfan in patients with HCT-CI >2. Additionally, the analysis of relapse/progression, NRM, GRFS, and CRFS favored treosulfan compared to busulfan, too. This allows the interpretation that, while all patients benefitted from the RTC regimen, this benefit is especially pronounced in patients with worse HCT-CI, 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 intensity settings 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 treosulfan may have a similar antileukemic efficacy and lower NRM yielding into 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 HCT centers worldwide.[35,43–49] Whether treosulfan or melphalan would be the

best partner for PTCy in this usually elderly patient population deemed at risk for 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 of trial conduct. First, MRD status prior to alloHCT was not assessed, as MRD monitoring was not standard of care during the recruitment period (2013–2018), and ELN MRD 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 for standard conditioning therapies, with promising data not only in event-free survival and overall survival but also regarding GRFS and CRFS.

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TABLES

Table 1: Baseline Demographics and Disease Characteristics of Patients with AML

AML Subgroup of MC-FludT.14/L Trial	Busulfan (N=168)	Treosulfan (N=184)
Sex		
Male; n (%)	92 (54.8%)	104 (56.5%)
Female; n (%)	76 (45.2%)	80 (43.5%)
Age, years	59.6 (6.3)	59.4 (6.6)
≥50 years; mean (SD)	159 (94.6%)	174 (94.6%)
≥60 years; mean (SD)	94 (56.0%)	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		
Low risk; n (%)	18 (10.7%)	19 (10.3%)
Intermediate risk; n (%)	76 (45.2%)	68 (37.0%)
High risk; n (%)	50 (29.8%)	72 (39.1%)
In case >CR1 allocation to high risk AML for results analysis; n (%)	24 (14.3%)	25 (13.6%)
Donor to patient sex		
Female to Female; n (%)	42 (25.0%)	36 (19.5%)
Female to Male; n (%)	26 (15.5%)	38 (20.7%)
Male to Female; n (%)	34 (20.2%)	44 (23.9%)
Male to Male; n (%)	66 (39.3%)	66 (35.9%)
Donor type		

AML Subgroup of MC-FludT.14/L Trial	Busulfan (N=168)	Treosulfan (N=184)
MRD; n (%)	50 (29.8%)	45 (24.5%)
MUD; n (%)	89 (53.0%)	111 (60.3%)
MMUD (9/10 HLA mismatch); n (%)	29 (17.3%)	28 (15.2%)
Stem cell source		
Peripheral blood; n (%)	163 (97.0%)	177 (96.2%)
Bone marrow; n (%)	5 (3.0%)	7 (3.8%)
CD34; median (Q1, Q3)	6.1 (4.9, 7.9)	6.0 (5.0, 7.6)
Karnofsky performance score		
60; n (%)	0 (0.0%)	3 (1.6%)
70; n (%)	3 (1.8%)	7 (3.8%)
80; n (%)	27 (16.1%)	32 (17.4%)
90; n (%)	75 (44.6%)	67 (36.4%)
100; n (%)	63 (37.5%)	75 (40.8%)
HCT-CI Score		
Patients with HCT-CI score >2; n (%)	101 (60.1%)	105 (57.1%)
Mean (SD)	2.90 (1.94)	2.88 (1.95)

AML, acute myeloid leukemia; BMI, body mass index; HCT-CI, hematopoietic cell transplantation comorbidity index; HCT, hematopoietic stem cell transplantation; MMUD, mismatched unrelated donor; MRD, matched related donor; MUD, matched unrelated donor; Q1, first quartile 1; Q3, third quartile; SD, standard deviation.

Table 2: Event-free Survival and Secondary Outcomes of Patients with AML Stratified by Risk Group and HCT-CI Score

			Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [b]
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

			Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [b]
		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

			Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [b]
	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

[a] Based on Kaplan-Meier estimates.
[b] Adjusted for donor type as factor, and risk group and center as strata using Cox regression model.
AML, acute myeloid leukemia; CI, confidence interval; CRFS, chronic GRFS; EFS; event-free survival; GRFS, GVHD-free and relapse-free survival; GVHD, Graft-versus-host disease; HCT-CI; hematopoietic cell transplantation - specific comorbidity index; N, total number of patients; NRM; non-relapse mortality; OS, overall survival.

FIGURE LEGENDS

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 Cox regression model.

[b] for testing difference of Treosulfan compared to Busulfan.

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 Fine and Gray model.

[b] based on test of Gray.

Figure 3: GVHD-free and relapse-free Survival of Patients with Acute Myeloid Leukemia

Note: GVHD-free 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 Cox regression model.

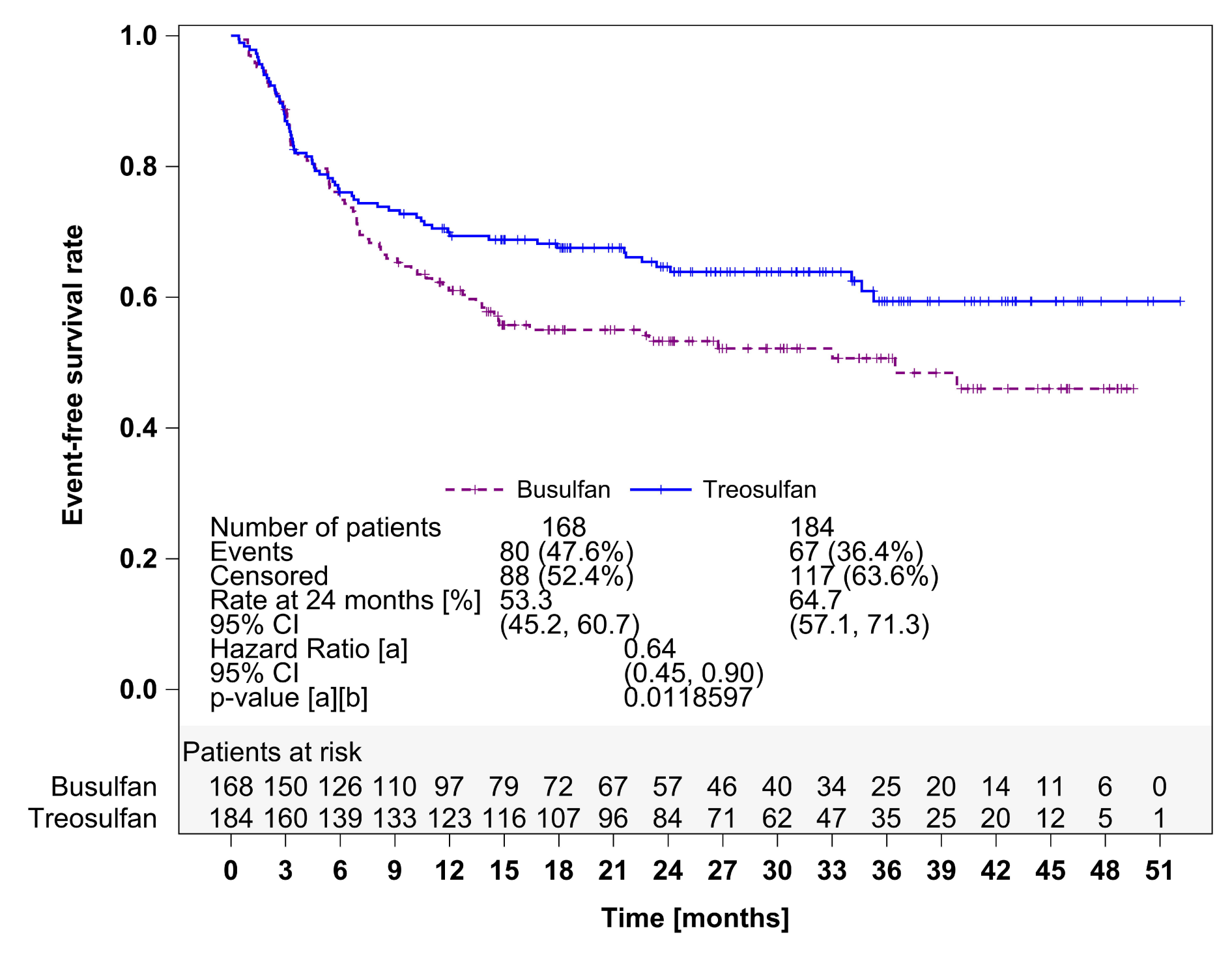
[b] for testing difference of Treosulfan compared to Busulfan.

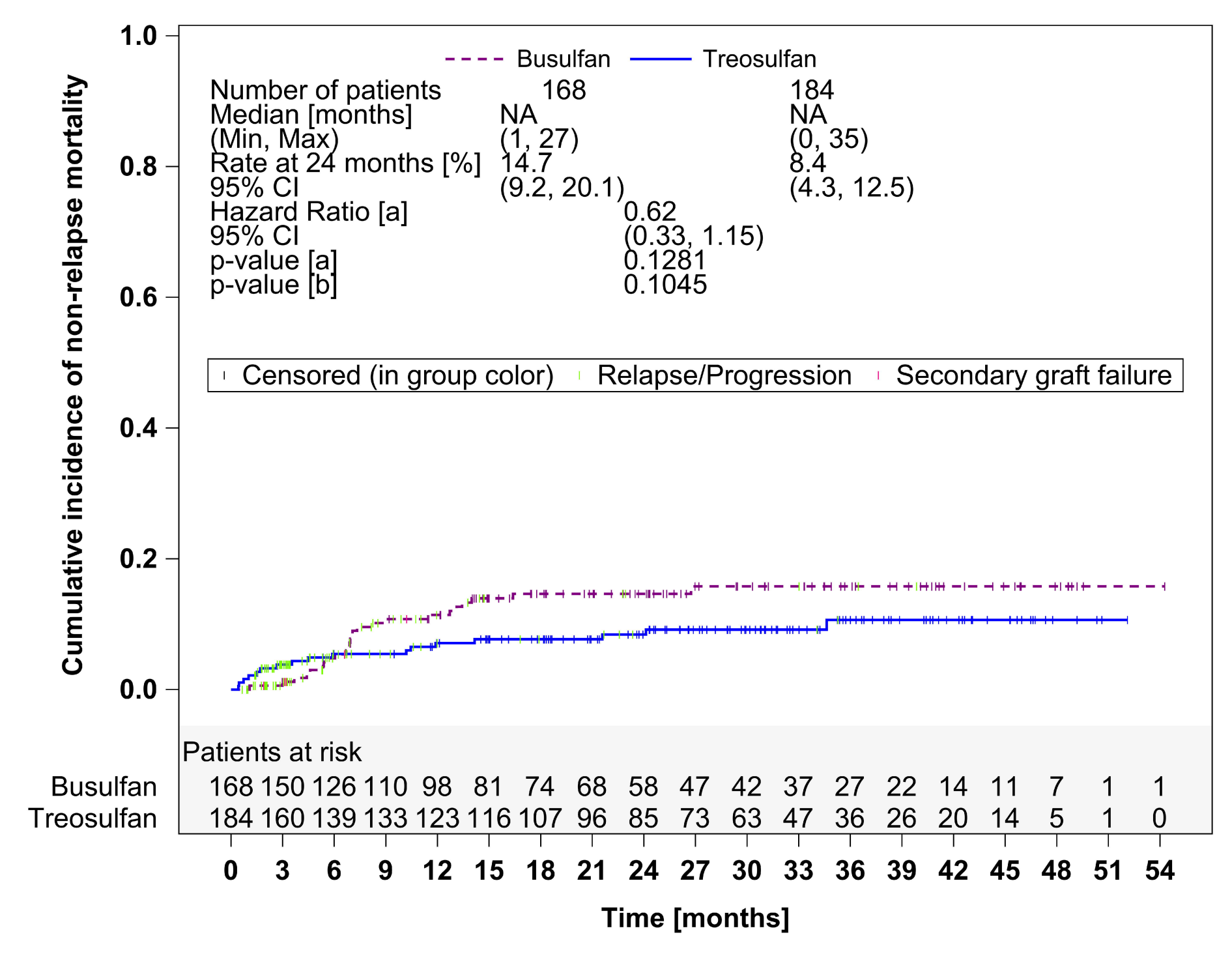
Figure 4: Chronic GVHD-free and relapse-free Survival of Patients with Acute Myeloid Leukemia

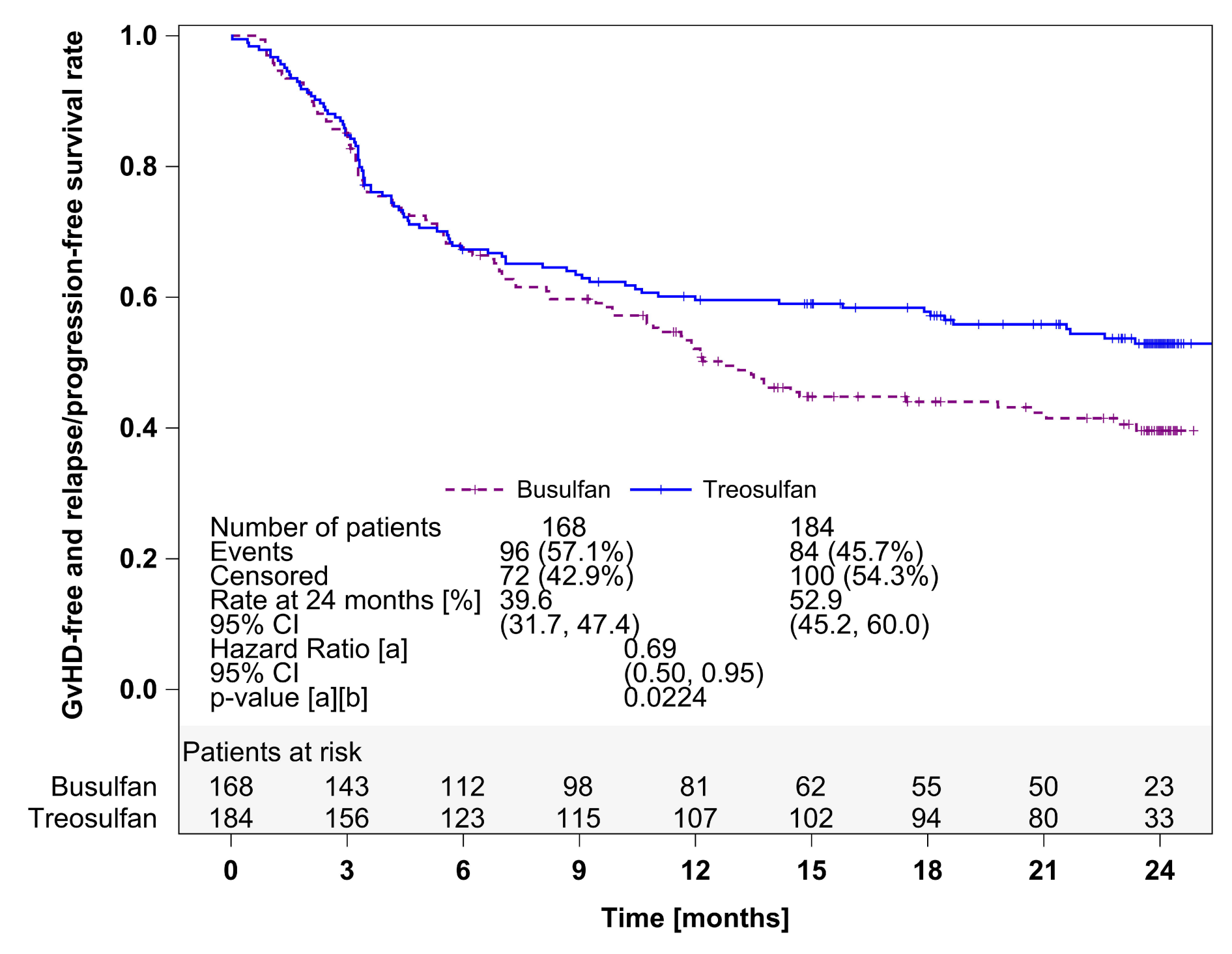
Note: Chronic GVHD-free defined as no extensive chronic GVHD.

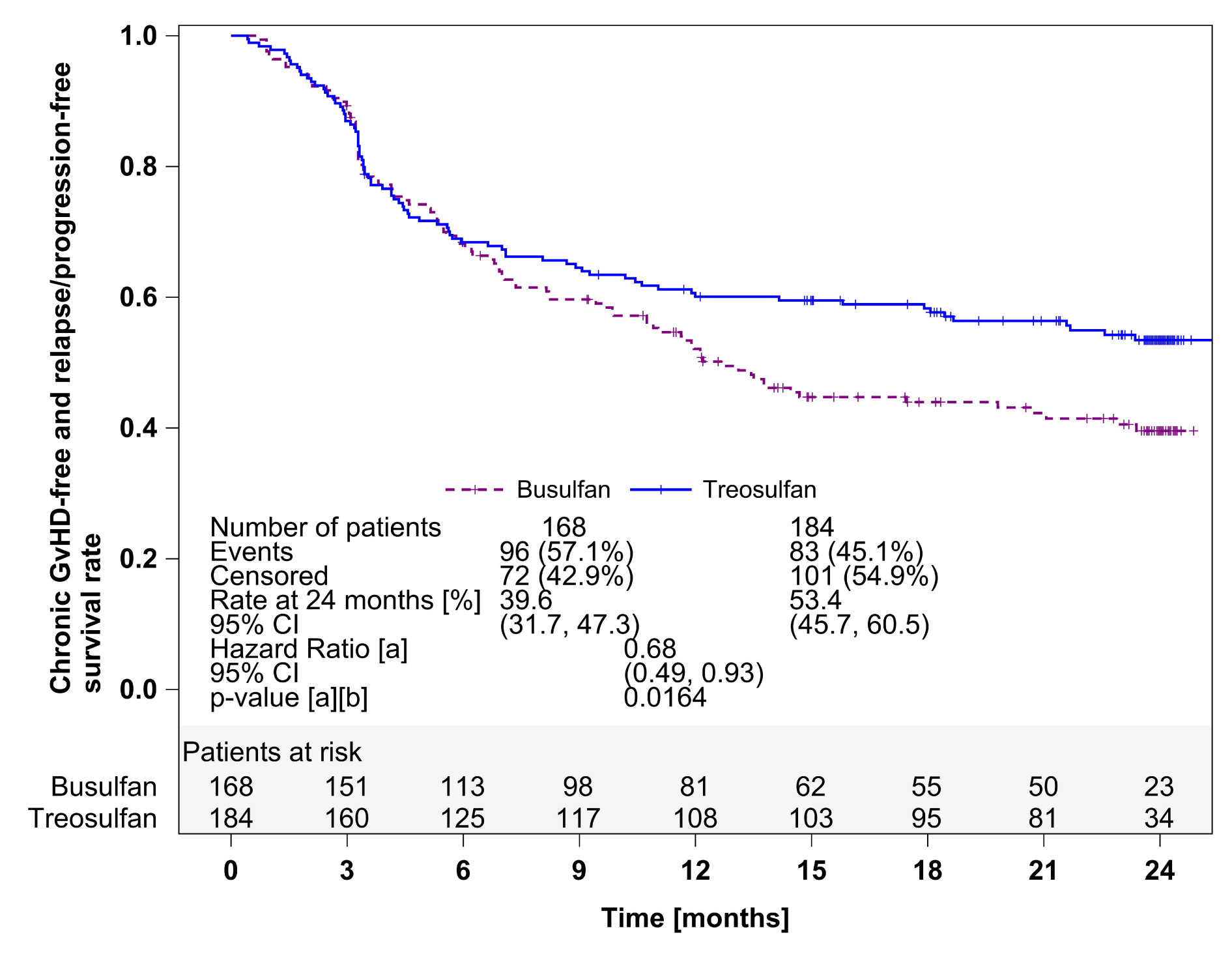
[a] adjusted for donor type as factor, and risk group and center as strata using Cox regression model.

[b] for testing difference of Treosulfan compared to Busulfan.


1.0
0.8
0.6
0.4
0.2
0.0
Event-free survival rate
Busulfan
Treosulfan
Number of patients 168 184
Events 80 (47.6%) 67 (36.4%)
Censored 88 (52.4%) 117 (63.6%)
Rate at 24 months [%] 53.3 64.7
95% CI (45.2, 60.7) (57.1, 71.3)
Hazard Ratio [a] 0.64
95% CI (0.45, 0.90)
p-value [a][b] 0.0118597
Patients at risk
Busulfan 168 150 126 110 97 79 72 67 57 46 40 34 25 20 14 11 6 0
Treosulfan 184 160 139 133 123 116 107 96 84 71 62 47 35 25 20 12 5 1
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51
Time [months]


Busulfan
Treosulfan
Number of patients 168 184
Median [months] NA NA
(Min, Max) (1, 27) (0, 35)
Rate at 24 months [%] 14.7 8.4
95% CI (9.2, 20.1) (4.3, 12.5)
Hazard Ratio [a] 0.62
95% CI (0.33, 1.15)
p-value [a] 0.1281
p-value [b] 0.1045
Censored (in group color)
Relapse/Progression
Secondary graft failure
Cumulative incidence of non-relapse mortality
1.0
0.8
0.6
0.4
0.2
0.0
Patients at risk
Busulfan 168 150 126 110 98 81 74 68 58 47 42 37 27 22 14 11 7 1 1
Treosulfan 184 160 139 133 123 116 107 96 85 73 63 47 36 26 20 14 5 1 0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54
Time [months]


GvHD-free and relapse/progression-free survival rate
1.0
0.8
0.6
0.4
0.2
0.0
Busulfan
Treosulfan
Number of patients 168 184
Events 96 (57.1%) 84 (45.7%)
Censored 72 (42.9%) 100 (54.3%)
Rate at 24 months [%] 39.6 52.9
95% CI (31.7, 47.4) (45.2, 60.0)
Hazard Ratio [a] 0.69
95% CI (0.50, 0.95)
p-value [a][b] 0.0224
Patients at risk
Busulfan 168 143 112 98 81 62 55 50 23
Treosulfan 184 156 123 115 107 102 94 80 33
0 3 6 9 12 15 18 21 24
Time [months]


Chronic GvHD-free and relapse/progression-free survival rate
1.0
0.8
0.6
0.4
0.2
0.0
Busulfan
Treosulfan
Number of patients 168 184
Events 96 (57.1%) 83 (45.1%)
Censored 72 (42.9%) 101 (54.9%)
Rate at 24 months [%] 39.6 53.4
95% CI (31.7, 47.3) (45.7, 60.5)
Hazard Ratio [a] 0.68
95% CI (0.49, 0.93)
p-value [a][b] 0.0164
Patients at risk
Busulfan 168 151 113 98 81 62 55 50 23
Treosulfan 184 160 125 117 108 103 95 81 34
0 3 6 9 12 15 18 21 24
Time [months]

MC-FLUDT.14/L AML SUBGROUP ANALYSIS SUPPLEMENTAL DATA

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1 INCLUSION CRITERIA FOR MC-FLUDT.14/L CLINICAL TRIAL

Patients had to meet all of the following criteria:

1. Patients with acute myeloid leukaemia (AML) according to the World Health Organisation (WHO) 2008, (AML in complete remission [CR] at transplant, i.e., blast counts <5% in bone marrow) [1] or myelodysplastic syndrome (MDS) according to WHO 2008, (MDS with blast counts < 20% in bone marrow during disease history) [1] indicated for allogeneic haematopoietic progenitor cell transplantation but considered to be at increased risk for standard conditioning therapies according to the following criteria:

   - patients aged ≥50 years at transplant

   and/or

   - patients with an HCT-CI score >2 (according to Sorror et al. 2005) [2]

2. Availability of a human leukocyte antigen (HLA)-identical sibling donor (MRD) or HLA-identical unrelated donor (MUD). Donor selection was based on molecular high-resolution typing (4 digits) of class II alleles of the DRB1 and DQB1 gene loci and molecular (at least) low-resolution typing (2 digits) of class I alleles (i.e., antigens) of the HLA- A, B, and C gene loci.

   In case no class I and class II completely identical donor (10 out of 10 gene loci) could be identified, one antigen disparity (class I) and/or one allele disparity (class II) between patient and donor were acceptable. Conversely, the disparity of two antigens (irrespective of the involved gene loci) could not be accepted. These definitions for the required degree of histocompatibility applied to the selection of related as well as unrelated donors.

3. Adult patients of both genders, 18 – 70 years of age.
4. Karnofsky Performance Score ≥60%.

5. Written informed consent.
6. Men capable of reproduction and women of childbearing potential must have been willing to consent to using a highly effective method of birth control such as condoms, implants, injectables, combined oral contraceptives, intrauterine devices, sexual abstinence, or vasectomized partner while on treatment and for at least 6 months thereafter.

2 STATISTICAL ANALYSIS METHODS

The subgroup analysis was performed based on data of the n = 352 patients with AML followed up for at least 2 years post-transplantation and included additional post-surveillance data with respect to survival per methods prospectively defined for the MC-FludT.14/L clinical trial protocol [3]. Descriptive statistics were applied to summarize all efficacy and safety endpoints. Fisher's exact test and Cochran-Mantel-Haenszel tests were used for binary endpoints, such as donor type chimerism. All time to event endpoints were measured from the day of allogeneic hematopoietic cell transplantation (alloHCT) (except chronic graft-versus-host disease [cGVHD] from day +100) to the time of event or competing event. The probability of event over time for event-free survival (EFS) and overall survival (OS) was estimated by Kaplan-Meier estimators. The probability of event over time with competing risks for non-relapse mortality (NRM) and incidence of acute GVHD (aGVHD) and cGVHD was estimated by cumulative incidence functions. Cox proportional hazards models for EFS, OS, GVHD-free and relapse-free survival (GRFS), chronic GRFS (CRFS), and Fine and Gray models for NRM, and incidence of aGVHD and cGVHD were applied to adjust statistical analysis for covariates in multivariate analyses with donor type (MRD/MUD) as factor, and risk group (same strata used in the randomization) and center as strata. All statistical analysis methods applied in this AML subgroup analysis were prospectively defined in the protocol and consistent with the analysis consisting of both AML and MDS patients. p-values of <0.05 are considered statistically significant. All analyses were performed with SAS software (Version 9.4).

3 TREATMENT SCHEDULE

Treatment schedule for **test** arm (i.v. treosulfan):

Day	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**+1**	**+3**	**+6**
Treosulfan i.v. (study medication) (10 g/m² within 120 min)			X	X	X					
Fludarabine i.v. (30 mg/m² within 30 min)	X	X	X	X	X					
Applies to Germany, Hungary, Italy, Poland: ATG-S-Fresenius / Grafalon® i.v. (10 mg/kg in case of MUD only)			X	X	X					
Applies to France only: *ATG-Thymoglobuline i.v.* *(2.5 mg/kg in case of MUD only)*					*X*	*X*				
Allogeneic stem-cell transplantation							X			
Ciclosporin-A i.v. (3 mg/kg/day start, 5 mg/kg/day p.o. ...)*						X	X	X	X	X
Methotrexate i.v. (mg/m²/day)								15	10	10
Ca-Folinate i.v. (mg/m²; 6 hours after MTX)								15	10	10

* Ciclosporin-A dose levels adapted to the standards of the participating center; treatment starts i.v.

Abbreviations: ATG = anti-thymocyte globulin, Ca = calcium; i.v. = intravenous; MRD = matched related donor; MTX = Methotrexate; MUD = match unrelated donor; PO = per os, oral(ly).

Treatment schedule for **reference** arm (i.v. busulfan):

Day	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**+1**	**+3**	**+6**
Phenytoin p.o. (mg) (3 x per day)*		200	100	100	100					
Busulfan i.v. (study medication) (4 x 0.8 mg/kg/d within 120 min)			X	X						
Fludarabine i.v. (30 mg/m² within 30 min)	X	X	X	X	X					
Applies to Germany, Hungary, Italy, Poland: ATG-S-Fresenius / Grafalon® i.v. (10 mg/kg in case of MUD only)			X	X	X					
Applies to France only: *ATG-Thymoglobuline i.v.* *(2.5 mg/kg in case of MUD only)*					*X*	*X*				
Allogeneic stem-cell transplantation							X			
Ciclosporin-A i.v. (3 mg/kg/day start, 5 mg/kg/day p.o. ...)**						X	X	X	X	X
Methotrexate i.v. (mg/m²/day)								15	10	10
Ca-Folinate i.v. (mg/m²; 6 hours after MTX)								15	10	10

* Phenytoin can be replaced by adequate benzodiazepine treatment in accordance with SmPC Busilvex®

** Ciclosporin-A dose levels adapted to the standards of the participating center; treatment starts i.v.

Abbreviations: ATG = anti-thymocyte globulin, Ca = calcium; i.v. = intravenous; MRD = matched related donor; MTX = Methotrexate; MUD = match unrelated donor; PO = per os, oral(ly); SmPC = summary of product characteristics.

4 EFFICACY

Supplementary Table 1: Event-free Survival and Secondary Outcomes of Patients with AML

	Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [b]
EFS			
Patients without event, n (%)	88 (52.4%)	117 (63.6%)	
EFS at 24 months, % (95% CI) [a]	53.3 (45.2, 60.7)	64.7 (57.1, 71.3)	0.64 (0.45, 0.90), 0.012
Patients at low risk			
Patients without event, n (%)	10 (55.6%)	17 (89.5%)	
EFS at 24 months, % (95% CI) [a]	59.6 (33.1, 78.4)	88.4 (60.8, 97.0)	0.14 (0.02, 1.33), 0.087
Patients at intermediate risk			
Patients without event, n (%)	47 (61.8%)	50 (73.5%)	
EFS at 24 months, % (95% CI) [a]	62.9 (50.5, 72.9)	75.6 (63.1, 84.3)	0.64 (0.33, 1.23), 0.181
Patients at high risk			
Patients without event, n (%)	31 (41.9%)	50 (51.5%)	
EFS at 24 months, % (95% CI) [a]	42.0 (30.4, 53.2)	52.5 (41.9, 62.0)	0.74 (0.47, 1.16), 0.194
OS			
Patients without event, n (%)	107 (63.7%)	130 (70.7%)	
OS at 24 months, % (95% CI)	64.7 (56.7, 71.6)	72.8 (65.5, 78.8)	0.65 (0.43, 0.96), 0.0303
Patients at low risk			
Patients without event, n (%)	13 (72.2%)	17 (89.5%)	
OS at 24 months, % (95% CI) [a]	76.6 (48.8, 90.5)	88.4 (60.8, 97.0)	0.17 (0.02, 1.70), 0.1326
Patients at intermediate risk			
Patients without event, n (%)	54 (71.1%)	58 (85.3%)	
OS at 24 months, % (95% CI) [a]	71.0 (59.0, 80.1)	87.2 (75.9, 93.4)	0.54 (0.23, 1.25), 0.1514
Patients at high risk			
Patients without event, n (%)	40 (54.1%)	55 (56.7%)	
OS at 24 months, % (95% CI) [a]	55.5 (43.2, 66.2)	59.6 (48.9, 68.8)	0.79 (0.49, 1.27), 0.3250
Relapse/progression			
Patients without event or with competing event, n (%)	118 (70.2%)	134 (72.8%)	
Relapse/progression at 24 months, % (95% CI) [a]	29.0 (21.9, 36.0)	26.9 (20.3, 33.5)	0.82 (0.56, 1.22), 0.3296
Patients at low risk			
Patients without event or with competing event, n (%)	11 (61.1%)	17 (89.5%)	
Relapse/progression at 24 months, % (95% CI) [a]	34.4 (11.9, 56.9)	11.6 (0.0, 26.8)	0.25 (0.05, 1.16), 0.0762
Patients at intermediate risk			

	Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [b]
Patients without event or with competing event, n (%)	61 (80.3%)	54 (79.4%)	
Relapse/progression at 24 months, % (95% CI) [a]	18.1 (9.1, 27.2)	19.6 (10.0, 29.2)	0.96 (0.46, 1.98), 0.9071
Patients at high risk			
Patients without event or with competing event, n (%)	46 (62.2%)	63 (64.9%)	
Relapse/progression at 24 months, % (95% CI) [a]	38.7 (27.4, 50.0)	34.9 (25.2, 44.6)	0.90 (0.55, 1.49), 0.6943
NRM			
Patients with event, n (%)	25 (14.9%)	17 (9.2%)	
NRM at 24 months, % (95% CI) [a]	14.7 (9.2, 20.1)	8.4 (4.3, 12.5)	0.62 (0.33, 1.15), 0.1281
Patients at low risk			
Patients with event, n (%)	1 (5.6%)	0 (0.0%)	
NRM at 24 months, % (95% CI) [a]	6.0 (0.0, 17.4)	0.0 (0.0, 0.0)	NA (NA, NA), NA
Patients at intermediate risk			
Patients with event, n (%)	12 (15.8%)	4 (5.9%)	
NRM at 24 months, % (95% CI) [a]	16.1 (7.8, 24.5)	4.8 (0.0, 10.2)	0.37 (0.12, 1.17), 0.0899
Patients at high risk			
Patients with event, n (%)	12 (16.2%)	13 (13.4%)	
NRM at 24 months, % (95% CI) [a]	15.3 (6.9, 23.6)	12.5 (5.9, 19.2)	0.87 (0.39, 1.95), 0.7292

[a] Based on Kaplan-Meier estimates.
[b] Adjusted for donor type as factor, and risk group and center as strata using Cox regression model.
AML, acute myeloid leukemia; CI, confidence interval; N, total number of patients; NA, not applicable; NRM, non-relapse mortality; OS, overall survival.

Supplementary Table 2: Event-free Survival and Secondary Outcomes of Patients with AML Stratified by HCT-CI Score ≤2 and >2

	Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [b]
EFS			
Patients with HCT-CI score ≤ 2			
Patients without event, n (%)	44 (65.7%)	51 (64.6%)	
EFS at 24 months, % (95% CI) [a]	69.6 (56.8, 79.2)	68.3 (56.4, 77.6)	1.22 (0.58, 2.59), 0.595
Patients with HCT-CI score > 2			
Patients without event, n (%)	44 (43.6%)	66 (62.9%)	
EFS at 24 months, % (95% CI) [a]	42.2 (32.0, 52.0)	61.9 (51.7, 70.6)	0.59 (0.37, 0.93), 0.022
OS			
Patients with HCT-CI score ≤ 2			
Patients without event, n (%)	51 (76.1%)	57 (72.2%)	
Overall survival at 24 months, % (95% CI) [a]	78.4 (66.1, 86.6)	77.5 (66.3, 85.4)	1.27 (0.56, 2.92), 0.5663
Patients with HCT-CI score > 2			
Patients without event, n (%)	56 (55.4%)	73 (69.5%)	
Overall survival at 24 months, % (95% CI) [a]	55.4 (44.8, 64.8)	69.3 (59.2, 77.4)	0.56 (0.34, 0.94), 0.0289
Relapse/progression			
Patients with HCT-CI score ≤ 2			
Patients without event or with competing event, n (%)	51 (76.1%)	56 (70.9%)	
Relapse/progression at 24 months, % (95% CI)	21.2 (11.3, 31.0)	27.8 (17.6, 38.0)	1.21 (0.63, 2.31), 0.5718
Patients with HCT-CI score > 2			
Patients without event or with competing event, n (%)	67 (66.3%)	78 (74.3%)	
Relapse/progression at 24 months, % (95% CI)	34.3 (24.6, 44.0)	26.3 (17.7, 34.8)	0.69 (0.42, 1.13), 0.1414
NRM			
Patients with HCT-CI score ≤ 2			
Patients with event, n (%)	6 (9.0%)	5 (6.3%)	
NRM at 24 months, % (95% CI)	7.8 (1.2, 14.3)	3.8 (0.0, 8.1)	0.74 (0.23, 2.41), 0.6190
Patients with HCT-CI score > 2			
Patients with event, n (%)	19 (18.8%)	12 (11.4%)	
NRM at 24 months, % (95% CI)	19.3 (11.5, 27.1)	11.8 (5.5, 18.1)	0.59 (0.28, 1.21), 0.1499
GRFS			
Patients with HCT-CI score ≤ 2			
Patients with event	31 (46.3%)	32 (40.5%)	
Death [c]	2 (3.0%)	2 (2.5%)	
Relapse/Progression [c]	12 (17.9%)	20 (25.3%)	

	Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [b]
Acute GVHD ≥ Grade III [c]	2 (3.0%)	1 (1.3%)	
Patients without event	36 (53.7%)	47 (59.5%)	
GRFS at 24 months [%] (95% CI) [a]	51.9 (38.9, 63.4)	57.4 (45.2, 67.8)	0.98 (0.51, 1.89), 0.9502
Patients with HCT-CI score > 2			
Patients with event	65 (64.4%)	52 (49.5%)	
Death [c]	11 (10.9%)	9 (8.6%)	
Relapse/Progression [c]	31 (30.7%)	26 (24.8%)	
Acute GVHD ≥ Grade III [c]	7 (6.9%)	4 (3.8%)	
Patients without event	36 (35.6%)	53 (50.5%)	
GRFS at 24 months [%] (95% CI) [a]	31.2 (21.7, 41.1)	49.6 (39.5, 58.8)	0.76 (0.50, 1.15), 0.1878
CRFS			
Patients with HCT-CI score ≤ 2			
Patients with event	31 (46.3%)	32 (40.5%)	
Death [c]	2 (3.0%)	3 (3.8%)	
Relapse/Progression [c]	12 (17.9%)	20 (25.3%)	
Extensive chronic GVHD [c]	17 (25.4%)	9 (11.4%)	
Patients without event	36 (53.7%)	47 (59.5%)	
CRFS at 24 months [%] (95% CI) [a]	51.9 (38.9, 63.4)	57.4 (45.2, 67.8)	0.98 (0.51, 1.89), 0.9502
Patients with HCT-CI score > 2			
Patients with event	65 (64.4%)	51 (48.6%)	
Death [c]	13 (12.9%)	11 (10.5%)	
Relapse/Progression [c]	32 (31.7%)	26 (24.8%)	
Extensive chronic GVHD [c]	20 (19.8%)	14 (13.3%)	
Patients without event	36 (35.6%)	54 (51.4%)	
CRFS at 24 months [%] (95% CI) [a]	31.2 (21.7, 41.1)	50.5 (40.4, 59.8)	0.74 (0.48, 1.12), 0.1532

a Based on Kaplan-Meier estimates.
b Adjusted for donor type as factor, and risk group and center as strata using Cox regression model.
c only if this event occurred first.
AML, acute myeloid leukemia; CI, confidence interval; CRFS, chronic GRFS; EFS; event-free survival; GRFS, GVHD-free and relapse-free survival; GVHD, Graft-versus-host disease; HCT-CI; hematopoietic cell transplantation - specific comorbidity index; N, total number of patients; NRM; non-relapse mortality; OS, overall survival.

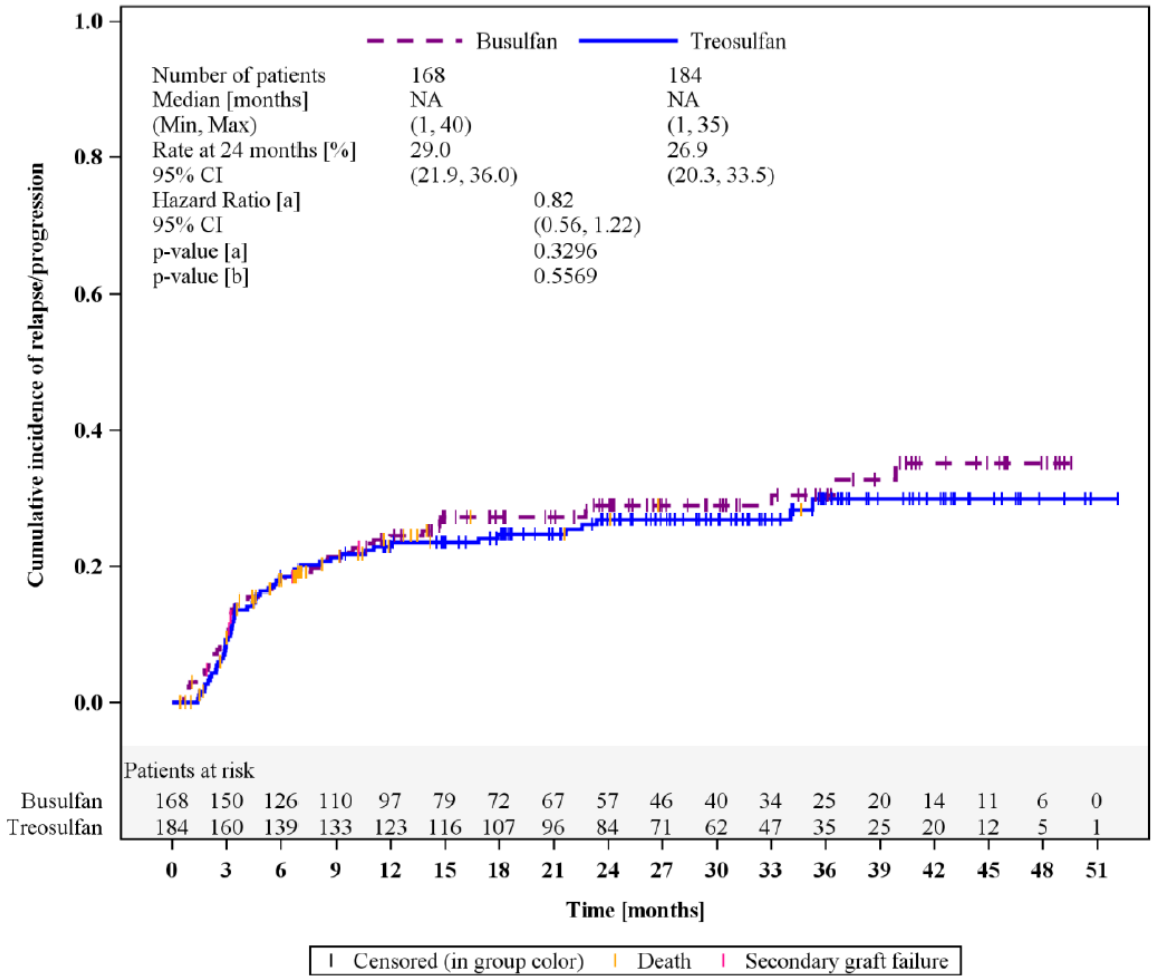
Supplementary Table 3: GRFS and CRFS in Patients with AML

	Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [c]
GRFS			
Patients with event	96 (57.1%)	84 (45.7%)	
Death [a]	13 (7.7%)	11 (6.0%)	
Relapse/Progression [a]	43 (25.6%)	46 (25.0%)	
Acute GVHD ≥ Grade III [a]	9 (5.4%)	5 (2.7%)	
Extensive chronic GVHD [a]	31 (18.5%)	22 (12.0%)	
Patients without event	72 (42.9%)	100 (54.3%)	
GRFS at 24 months [%] (95% CI) [b]	39.6 (31.7, 47.4)	52.9 (45.2, 60.0)	0.69 (0.50, 0.95), 0.0224
Patient at low risk			
Patients with event	10 (55.6%)	6 (31.6%)	
Death [a]	1 (5.6%)	0 (0.0%)	
Relapse/Progression [a]	5 (27.8%)	1 (5.3%)	
Acute GVHD ≥ Grade III [a]	0 (0.0%)	0 (0.0%)	
Extensive chronic GVHD [a]	4 (22.2%)	5 (26.3%)	
Patients without event	8 (44.4%)	13 (68.4%)	
GRFS at 24 months [%] (95% CI) [b]	40.9 (17.8, 62.9)	67.5 (41.4, 84.0)	0.30 (0.07, 1.27), 0.1028
Patient at intermediate risk			
Patients with event	38 (50.0%)	24 (35.3%)	
Death [a]	8 (10.5%)	2 (2.9%)	
Relapse/Progression [a]	13 (17.1%)	13 (19.1%)	
Acute GVHD ≥ Grade III [a]	3 (3.9%)	1 (1.5%)	
Extensive chronic GVHD [a]	14 (18.4%)	8 (11.8%)	
Patients without event	38 (50.0%)	44 (64.7%)	
GRFS at 24 months [%] (95% CI) [b]	46.7 (34.5, 58.1)	63.4 (50.3, 73.9)	0.65 (0.36, 1.16), 0.1445
Patient at high risk			
Patients with event	48 (64.9%)	54 (55.7%)	
Death [a]	4 (5.4%)	9 (9.3%)	
Relapse/Progression [a]	25 (33.8%)	32 (33.0%)	
Acute GVHD ≥ Grade III [a]	6 (8.1%)	4 (4.1%)	
Extensive chronic GVHD [a]	13 (17.6%)	9 (9.3%)	
Patients without event	26 (35.1%)	43 (44.3%)	
GRFS at 24 months [%] (95% CI) [b]	32.1 (21.2, 43.4)	42.7 (32.4, 52.5)	0.76 (0.50, 1.17), 0.2096
CRFS			
Patients with event	96 (57.1%)	83 (45.1%)	
Death [a]	15 (8.9%)	14 (7.6%)	
Relapse/Progression [a]	44 (26.2%)	46 (25.0%)	

	Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [c]
Extensive chronic GVHD [a]	37 (22.0%)	23 (12.5%)	
Patients without event	72 (42.9%)	101 (54.9%)	
CRFS at 24 months [%] (95% CI) [b]	39.6 (31.7, 47.3)	53.4 (45.7, 60.5)	0.68 (0.49, 0.93), 0.0164
Patient at low risk			
Patients with event	10 (55.6%)	6 (31.6%)	
Death [a]	1 (5.6%)	0 (0.0%)	
Relapse/Progression [a]	5 (27.8%)	1 (5.3%)	
Extensive chronic GVHD [a]	4 (22.2%)	5 (26.3%)	
Patients without event	8 (44.4%)	13 (68.4%)	
CRFS at 24 months [%] (95% CI) [b]	40.9 (17.8, 62.9)	67.5 (41.4, 84.0)	0.30 (0.07, 1.27), 0.1028
Patient at intermediate risk			
Patients with event	38 (50.0%)	24 (35.3%)	
Death [a]	8 (10.5%)	2 (2.9%)	
Relapse/Progression [a]	13 (17.1%)	13 (19.1%)	
Extensive chronic GVHD [a]	17 (22.4%)	9 (13.2%)	
Patients without event	38 (50.0%)	44 (64.7%)	
CRFS at 24 months [%] (95% CI) [b]	46.7 (34.5, 58.0)	63.4 (50.3, 73.9)	0.65 (0.37, 1.17), 0.1534
Patient at high risk			
Patients with event	48 (64.9%)	53 (54.6%)	
Death	6 (8.1%)	12 (12.4%)	
Relapse/Progression	26 (35.1%)	32 (33.0%)	
Extensive chronic GVHD [a]	16 (21.6%)	9 (9.3%)	
Patients without event	26 (35.1%)	44 (45.4%)	
CRFS at 24 months [%] (95% CI) [a]	32.0 (21.2, 43.3)	43.7 (33.4, 53.6)	0.73 (0.48, 1.12), 0.1492

a Only if this event occurred first.
b Based on Kaplan-Meier estimates.
c Adjusted for donor type as factor, and risk group and center as strata using Cox regression model.
AML, acute myeloid leukemia; CI, confidence interval; CRFS, chronic GRFS; GRFS, GVHD-free and relapse-free survival; GVHD, Graft-versus-host disease; N, total number of patients.

Supplementary Figure 1: Cumulative Incidence of Relapse/progression of Patients with AML



[a] adjusted for donor type as factor and risk group as stratum using Fine and Gray model
[b] based on test of Gray

5 SAFETY

Supplementary Table 4: Treatment-emergent Adverse Events (Full Analysis Set of 352 AML Patients)

	Busulfan (N=168)	**Treosulfan (N=184)**
Any adverse event n (%)		
Patients with any adverse event	161 (95.8%)	168 (91.3%)
Patients with AEs of at least CTCAE Grade 3	80 (47.6%)	95 (51.6%)
Drug-related adverse events n (%)		
Patients with any drug-related adverse event	114 (67.9%)	115 (62.5%)
Patients with drug-related AEs of at least CTCAE Grade 3	43 (25.6%)	44 (23.9%)
Serious adverse events n (%)		
Patients with any serious adverse event	8 (4.8%)	10 (5.4%)
- Results in death	1 (0.6%)	4 (2.2%)
- Life-threatening	5 (3.0%)	4 (2.2%)
- Hospitalization or prolongation of hospitalization	2 (1.2%)	5 (2.7%)
- Disability/incapacity	0 (0.0%)	0 (0.0%)
- Congenital anomaly or birth defect	0 (0.0%)	0 (0.0%)
Drug-related serious adverse events n (%)		
Patients with any drug-related serious adverse event	3 (1.8%)	3 (1.6%)
Maximum CTCAE grade of adverse events n (%)		
Patients with AEs of a maximum CTCAE Grade 1	30 (17.9%)	28 (15.2%)
Patients with AEs of a maximum CTCAE Grade 2	51 (30.4%)	45 (24.5%)
Patients with AEs of a maximum CTCAE Grade 3	73 (43.5%)	84 (45.7%)
Patients with AEs of a maximum CTCAE Grade 4	6 (3.6%)	7 (3.8%)
Patients with AEs of a maximum CTCAE Grade 5	1 (0.6%)	4 (2.2%)

AE, adverse event; AML, acute myeloid leukaemia; CTCAE, Common Terminology Criteria for Adverse Events.

Supplementary Table 5: Patients with Treatment-related Treatment-emergent Adverse Events by CTCAE SOC and PT Occurring in at least 5% of Patients in Either Treatment Group (Full Analysis Set of 352 AML Patients)

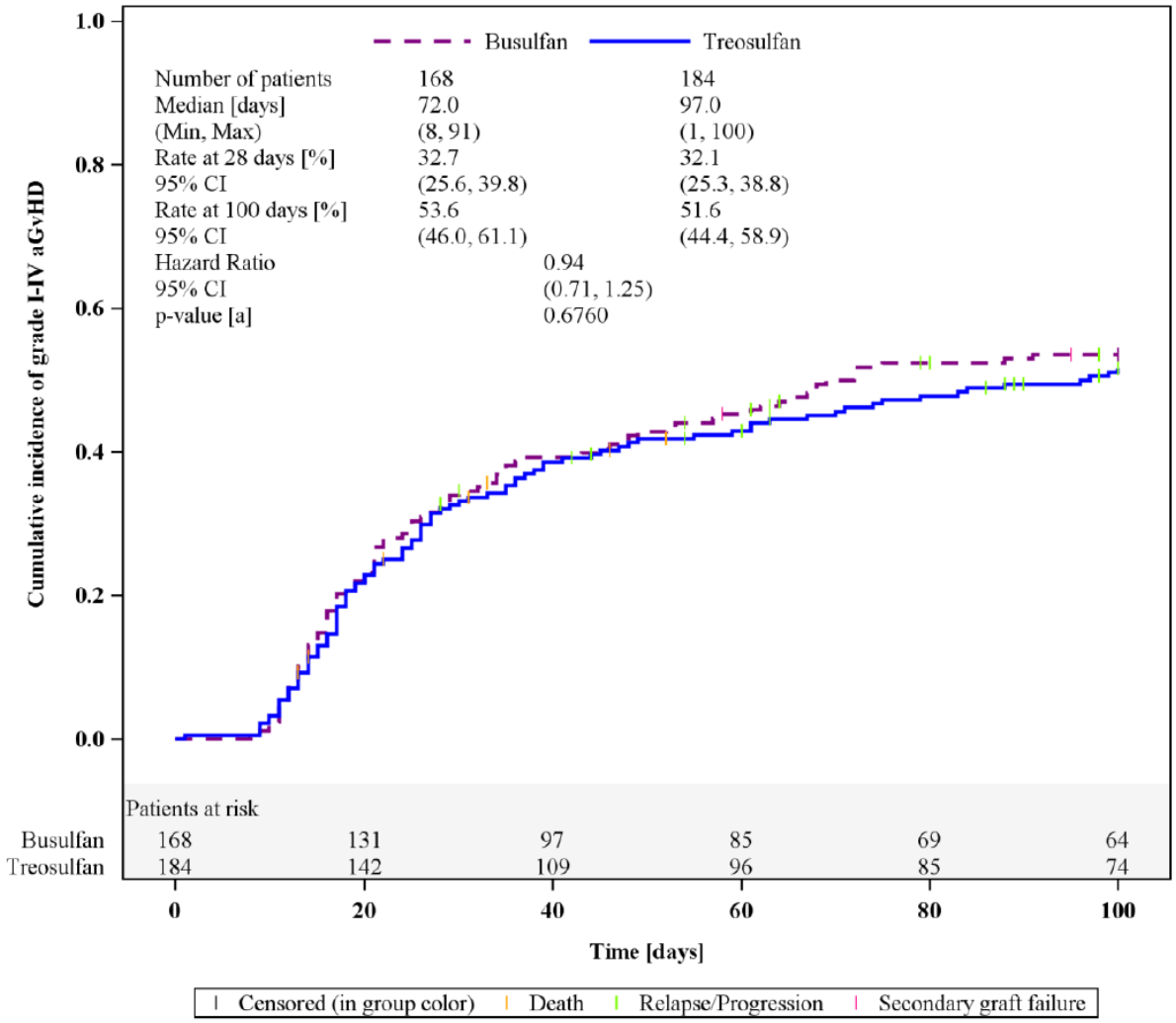
CTCAE System Organ Class CTCAE Preferred Term	Busulfan (N=168)	Treosulfan (N=184)	Total (N-352)
Patients with any event	114 (67.9%)	115 (62.5%)	229 (65.1%)
Gastrointestinal disorders			
Any event	85 (50.6%)	86 (46.7%)	171 (48.6%)
Mucositis oral	63 (37.5%)	55 (29.9%)	118 (33.5%)
Nausea	48 (28.6%)	39 (21.2%)	87 (24.7%)
Vomiting	20 (11.9%)	25 (13.6%)	45 (12.8%)
Diarrhea	14 (8.3%)	10 (5.4%)	24 (6.8%)
Abdominal pain	9 (5.4%)	6 (3.3%)	15 (4.3%)
Investigations			
Any event	21 (12.5%)	37 (20.1%)	58 (16.5%)
GGT increased	16 (9.5%)	12 (6.5%)	28 (8.0%)
Alanine aminotransferase increased	8 (4.8%)	17 (9.2%)	25 (7.1%)
Aspartate aminotransferase increased	6 (3.6%)	14 (7.6%)	20 (5.7%)
Blood bilirubin increased	4 (2.4%)	11 (6.0%)	15 (4.3%)
General disorders and administration site conditions			
Any event	30 (17.9%)	22 (12.0%)	52 (14.8%)
Fatigue	14 (8.3%)	11 (6.0%)	25 (7.1%)
Fever	15 (8.9%)	7 (3.8%)	22 (6.3%)
Skin and subcutaneous tissue disorders			
Any event	18 (10.7%)	21 (11.4%)	39 (11.1%)
Nervous system disorders			
Any event	22 (13.1%)	14 (7.6%)	36 (10.2%)
Headache	12 (7.1%)	11 (6.0%)	23 (6.5%)
Metabolism and nutrition disorders			
Any event	10 (6.0%)	12 (6.5%)	22 (6.3%)
Anorexia	6 (3.6%)	11 (6.0%)	17 (4.8%)
Respiratory, thoracic and mediastinal disorders			
Any event	8 (4.8%)	14 (7.6%)	22 (6.3%)
Infections and infestations			
Any event	9 (5.4%)	12 (6.5%)	21 (6.0%)
Vascular disorders			
Any event	8 (4.8%)	11 (6.0%)	19 (5.4%)
Blood and lymphatic system disorders			
Any event	9 (5.4%)	7 (3.8%)	16 (4.5%)

CTCAE System Organ Class CTCAE Preferred Term	Busulfan (N=168)	Treosulfan (N=184)	Total (N-352)
Febrile neutropenia	9 (5.4%)	7 (3.8%)	16 (4.5%)
CTCAE, Common Terminology Criteria for Adverse Events; SOC, system organ class; PT, preferred term.			

Supplementary Table 6: Acute Graft-versus-host-disease and Chronic Graft-versus-host-disease

	Busulfan (N=168)	Treosulfan (N=184)	Hazard ratio (treosulfan/busulfan) (95% CI), p-value [a]
Acute GVHD			
Acute GVHD Grade I-IV			
Patients with event, n (%)	90 (53.6%)	95 (51.6%)	
Cumulative incidence at 100 days, % (95% CI)	53.6 (46.0, 61.1)	51.6 (44.4, 58.9)	0.94 (0.71, 1.25), 0.6760
Acute GVHD Grade II-IV			
Patients with event, n (%)	28 (16.7%)	29 (15.8%)	
Cumulative incidence at 100 days, % (95% CI)	16.7 (11.0, 22.3)	15.8 (10.5, 21.0)	0.95 (0.57, 1.60), 0.8547
Acute GVHD Grade III–IV			
Patients with event, n (%)	9 (5.4%)	5 (2.7%)	
Cumulative incidence at 100 days, % (95% CI)	5.4 (2.0, 8.8)	2.7 (0.4, 5.1)	0.50 (0.17, 1.50), 0.2111
Chronic GVHD			
Patients with event, n (%)	76 (54.3%)	94 (59.9%)	
Cumulative incidence at 24 months, % (95% CI)	54.9 (46.4, 63.3)	61.1 (53.2, 69.0)	1.16 (0.86, 1.56), 0.3447
Extensive chronic GVHD			
Patients with event, n (%)	37 (26.4%)	23 (14.6%)	
Cumulative incidence at 24 months, % (95% CI)	28.1 (20.3, 35.9)	15.1 (9.4, 20.9)	0.53 (0.31, 0.88), 0.0128
[a] Based on test of Gray. CI, confidence interval; GVHD, Graft versus-host disease; N, total number of patients.			

Supplementary Figure 2: Cumulative Incidence of Grade I-IV Acute GVHD of Patients with AML



[a] based on test of Gray

Supplementary Table 7: Summary of Detailed Causes of Deaths incl. Post-surveillance (Full Analysis Set of 352 AML Patients)

	Busulfan (N=168)	Treosulfan (N=184)	Total (N=352)
Survival status at trial termination n (%)			
Alive [a]	107 (63.7%)	130 (70.7%)	237 (67.3%)
Dead	61 (36.3%)	54 (29.3%)	115 (32.7%)
If dead, cause of death n (%)			
Relapse/progression	32 (19.0%)	30 (16.3%)	62 (17.6%)
Transplantation related [b]	24 (14.3%)	16 (8.7%)	40 (11.4%)
GvHD	11 (6.5%)	5 (2.7%)	16 (4.5%)
Haemorrhage	0 (0.0%)	2 (1.1%)	2 (0.6%)
Renal failure	0 (0.0%)	2 (1.1%)	2 (0.6%)
Cardiac toxicity	2 (1.2%)	0 (0.0%)	2 (0.6%)
Interstitial pneumonitis	0 (0.0%)	1 (0.5%)	1 (0.3%)
Central nervous system toxicity	1 (0.6%)	0 (0.0%)	1 (0.3%)
Infection	17 (10.1%)	12 (6.5%)	29 (8.2%)
Bacterial	7 (4.2%)	8 (4.3%)	15 (4.3%)
Viral	4 (2.4%)	6 (3.3%)	10 (2.8%)
Fungal	6 (3.6%)	1 (0.5%)	7 (2.0%)
Parasitic	1 (0.6%)	0 (0.0%)	1 (0.3%)
Unknown	2 (1.2%)	0 (0.0%)	2 (0.6%)
Multiple organ failure	1 (0.6%)	3 (1.6%)	4 (1.1%)
Unknown	5 (3.0%)	6 (3.3%)	11 (3.1%)
Other	0 (0.0%)	2 (1.1%)	2 (0.6%)

[a] The status ‘alive’ is displayed for all patients who did not terminate the trial due to death.

[b] Multiple transplantation related causes per patient possible

AML, acute myeloid leukaemia; GVHD, Graft versus-host disease.

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