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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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.

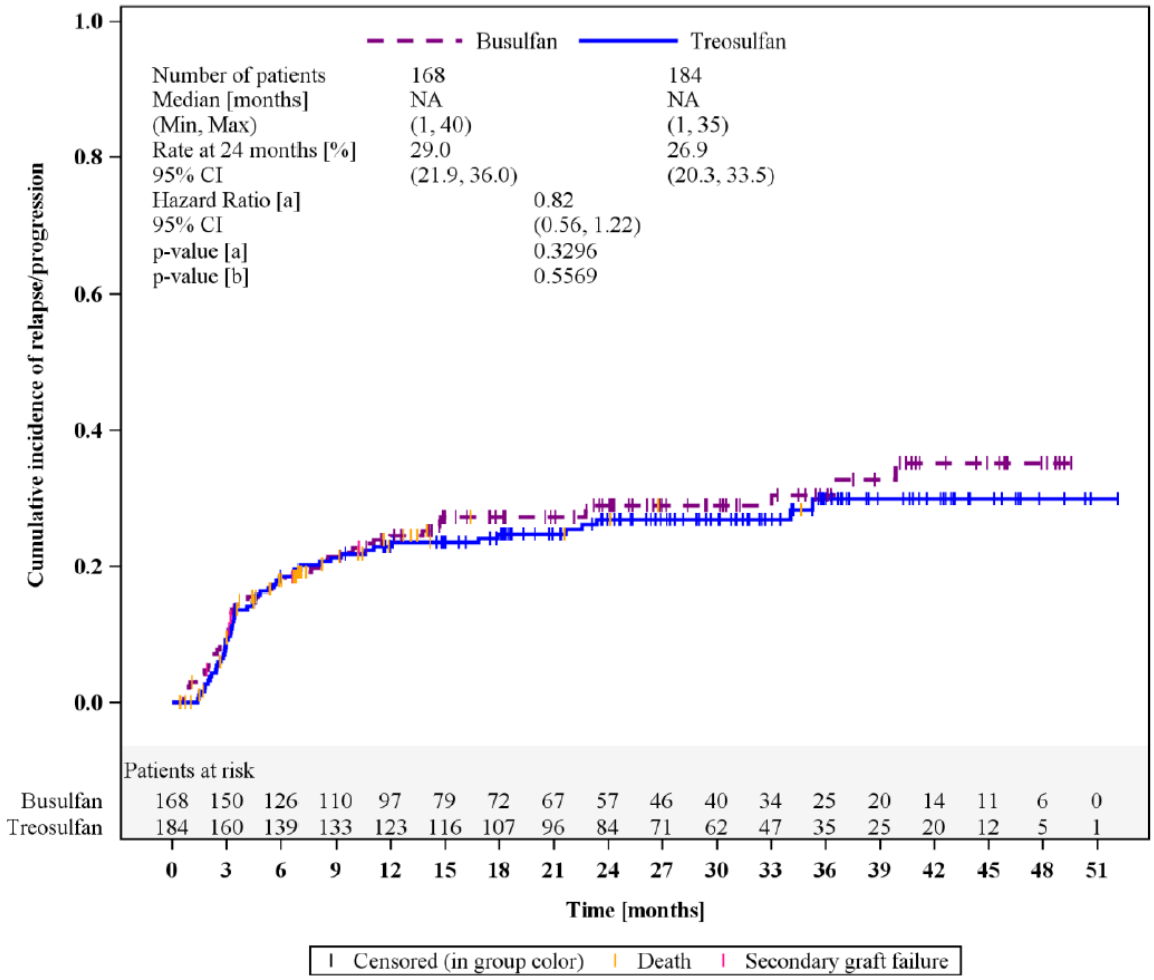
Supplementary Table 3: GRFS and CRFS in Patients with AML

	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)

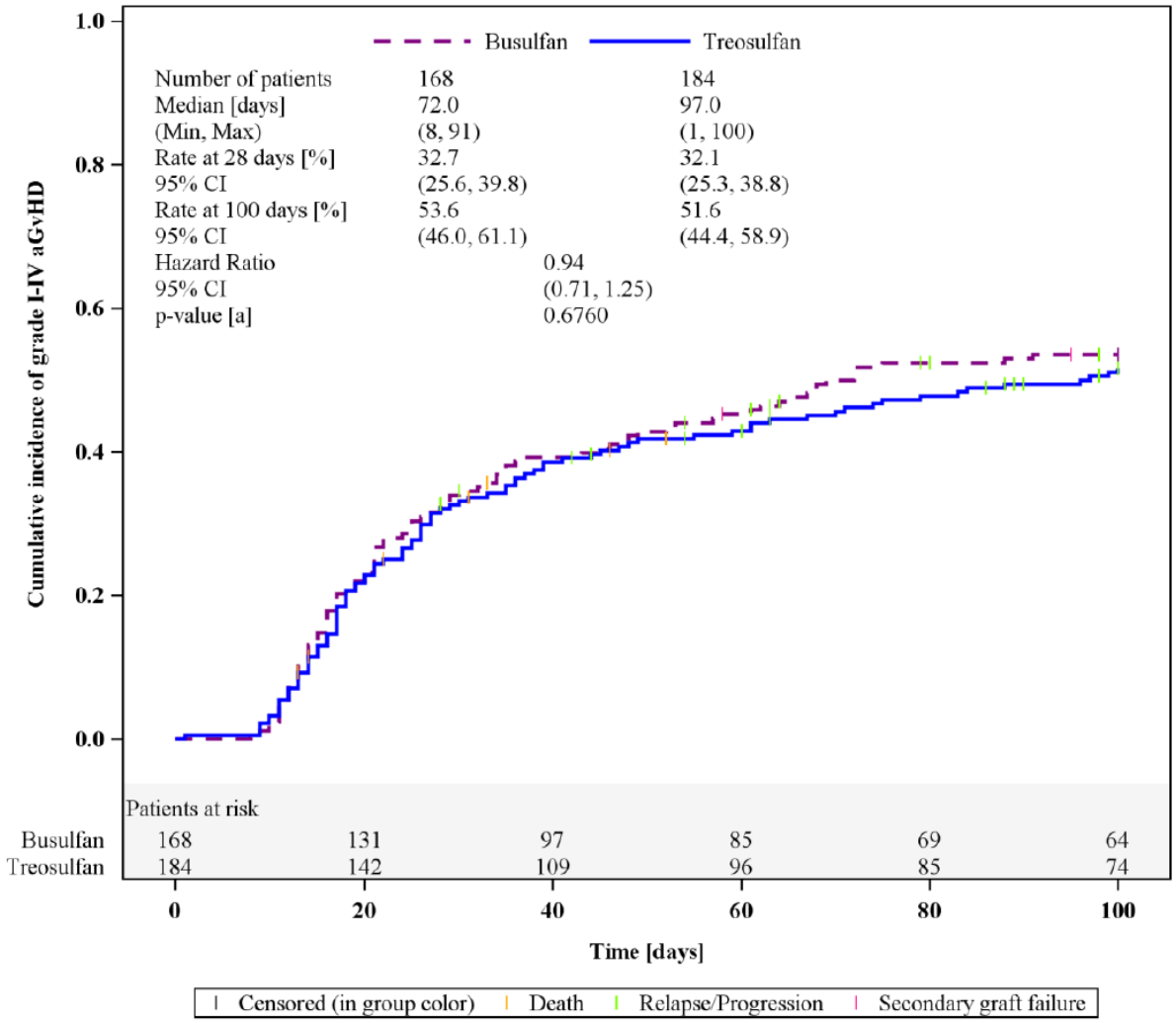
CTCAE System Organ Class CTCAE Preferred Term	Busulfan (N=168)	Treosulfan (N=184)	Total (N-352)
Patients with any event	114 (67.9%)	115 (62.5%)	229 (65.1%)
Gastrointestinal disorders			
Any event	85 (50.6%)	86 (46.7%)	171 (48.6%)
Mucositis oral	63 (37.5%)	55 (29.9%)	118 (33.5%)
Nausea	48 (28.6%)	39 (21.2%)	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.

6 REFERENCES

1. Swerdlow SH. WHO classification of tumours of haematopoietic and lymphoid tissues / edited by Steven H. Swerdlow [and others]. 4th ed. Lyon, France: International Agency for Research on Cancer; 2008.

2. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, Storer B. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106:2912–9. doi:10.1182/blood-2005-05-2004.

3. Beelen DW, Stelljes M, Reményi P, Wagner-Drouet E-M, Dreger P, Bethge W, et al. Treosulfan compared with reduced-intensity busulfan improves allogeneic hematopoietic cell transplantation outcomes of older acute myeloid leukemia and myelodysplastic syndrome patients: Final analysis of a prospective randomized trial. Am J Hematol. 2022;97:1023–34. doi:10.1002/ajh.26620.