Treosulfan or busulfan plus fludarabine as conditioning treatment before allogeneic haemopoietic stem cell transplantation for older patients with acute myeloid leukaemia or myelodysplastic syndrome (MC-FludT.14/L): a randomised, non-inferiority, phase 3 trial

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Summary

Background Further improvement of preparative regimens before allogeneic haemopoietic stem cell transplantation (HSCT) is an unmet medical need for the growing number of older or comorbid patients with acute myeloid leukaemia or myelodysplastic syndrome. We aimed to evaluate the efficacy and safety of conditioning with treosulfan plus fludarabine compared with reduced-intensity busulfan plus fludarabine in this population.

Methods We did an open-label, randomised, non-inferiority, phase 3 trial in 31 transplantation centres in France, Germany, Hungary, Italy, and Poland. Eligible patients were 18–70 years, had acute myeloid leukaemia in first or consecutive complete haematological remission (blast counts <5% in bone marrow) or myelodysplastic syndrome (blast counts <20% in bone marrow), Karnofsky index of 60% or higher, and were indicated for allogeneic HSCT but considered at an increased risk for standard myeloablative preparative regimens based on age (≥50 years), an HSCT-specific comorbidity index of more than 2, or both. Patients were randomly assigned (1:1) to receive either intravenous 10 g/m² treosulfan daily applied as a 2-h infusion for 3 days (days –4 to –2) or 0·8 mg/kg busulfan applied as a 2-h infusion at 6-h intervals on days –4 and –3. Both groups received 30 mg/m² intravenous fludarabine daily for 5 days (days –6 to –2). The primary outcome was event-free survival 2 years after HSCT. The non-inferiority margin was a hazard ratio (HR) of 1·3. Efficacy was assessed in all patients who received treatment and completed transplantation, and safety in all patients who received treatment. The study is registered with EudraCT (2008–002356–18) and ClinicalTrials.gov (NCT00822393).

Findings Between June 13, 2013, and May 3, 2016, 476 patients were enrolled (240 in the busulfan group received treatment and transplantation, and in the treosulfan group 221 received treatment and 220 transplanation). At the second preplanned interim analysis (Nov 9, 2016), the primary endpoint was met and trial was stopped. Here we present the final confirmatory analysis (data cutoff May 31, 2017). Median follow-up was 15·4 months (IQR 8·8–23·6) for patients treated with treosulfan and 17·4 months (6·3–23·4) for those treated with busulfan. 2-year event-free survival was 64·0% (95% CI 56·0–70·9) in the treosulfan group and 50·4% (42·8–57·5) in the busulfan group (HR 0·65 [95% CI 0·47–0·90]; p<0·0001 for non-inferiority, p=0·0051 for superiority). The most frequently reported grade 3 or higher adverse events were abnormal blood chemistry results (33 [15%] of 221 patients in the treosulfan group *vs* 35 [15%] of 240 patients in the busulfan group) and gastrointestinal disorders (24 [11%] patients *vs* 39 [16%] patients). Serious adverse events were reported for 18 (8%) patients in the treosulfan group and 17 (7%) patients in the busulfan group. Causes of deaths were generally transplantation-related.

Interpretation Treosulfan was non-inferior to busulfan when used in combination with fludarabine as a conditioning regimen for allogeneic HSCT for older or comorbid patients with acute myeloid leukaemia or myelodysplastic syndrome. The improved outcomes in patients treated with the treosulfan–fludarabine regimen suggest its potential to become a standard preparative regimen in this population.

Funding medac GmbH.

Lancet Haematol 2019

Published Online
October 9, 2019
https://doi.org/10.1016/S2352-3026(19)30157-7

See Online/Comment
https://doi.org/10.1016/S2352-3026(19)30198-X

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Introduction

Allogeneic haemopoietic stem cell transplantation (HSCT) plays a crucial role in the management of adult patients with acute myeloid leukaemia or myelodysplastic syndrome, which become more prevalent with increasing age.[1,2] Together they represent more than 50% of HSCT indications for malignant diseases worldwide.[3] Ultimate success of HSCT is affected by numerous factors, especially disease-specific and patient-specific risks, as well as donor type and transplant source. In addition to the disease stage at HSCT, the primary genetic disease profile is the most important predictor of disease recurrence after HSCT, reflected in the commonly accepted primary disease-risk stratification scores.[4,5] Increasing age, reduced general performance status, and pretransplant comorbidity or organ functional impairment have a strong effect on non-relapse mortality and morbidity. The HSCT-specific comorbidity index (HCT-CI) was primarily developed and validated in patients with acute myeloid leukaemia or myelodysplastic syndrome, allowing approximate stratification of patient subsets with distinct risks of non-relapse mortality.[6]

There are numerous radiochemotherapeutic preparative regimens ranging from minimum effective to maximum tolerable dose intensity. These dose intensities are dichotomised to myeloablative conditioning and reduced-intensity conditioning regimens.[7] In general, it is broadly accepted that myeloablative conditioning is not mandatory for durable donor stem cell engraftment and that reduced-intensity conditioning regimens can substantially ameliorate transplant toxicities, thereby markedly increasing the number of patients aged 50–70 years or comorbid patients eligible for allogeneic HSCT.

A dose-reduced intravenous busulfan-based regimen in combination with the purine analogue fludarabine is currently a well established reduced-intensity conditioning regimen for patients with acute myeloid leukaemia or myelodysplastic syndrome considered ineligible for myeloablative conditioning treatments.[8–11] Two recent clinical trials support that this reduced-intensity conditioning regimen reduces non-relapse mortality compared with myeloablative conditioning regimens in patients with acute myeloid leukaemia or myelodysplastic syndrome.[12,13] However, in one of the trials, this beneficial effect was outweighed by an increased relapse incidence following reduced-intensity conditioning.[13]

Treosulfan, a water soluble, bifunctional alkylating drug, showed strong myelotoxic, immunosuppressive,

Research in context

Evidence before this study

Before we designed this trial, several prospective studies were published comparing different preparative regimens for allogeneic haemopoietic stem cell transplantation (HSCT). We did a systematic literature review in Medline for research articles published from database inception until Dec 31, 2012, in English with the key words "randomised controlled trials", "transplantation", "allogeneic", "transplantation conditioning", and alternative terms. Since the development of reduced-intensity conditioning therapies or even non-myeloablative treatments, the question of the value of these new treatment options has been investigated. Phase 3 trials comparing myeloablative conditioning versus reduced-intensity conditioning regimens provided ambiguous results, depending on patient age and disease status. By 2005, the HSCT-comorbidity index (HCT-CI) was developed, showing that patients with acute myeloid leukaemia or myelodysplastic syndrome with an HCT-CI score higher than 2 were at increased risk for early non-relapse mortality after allogeneic HSCT. Accordingly, this score was proposed to stratify patients for conditioning regimens. Nowadays, reduced-intensity conditioning is preferred compared with myeloablative conditioning, however, despite the rapidly increasing application of the new conditioning regimens, data in older and comorbid patient populations were missing at study start. Phase 1 and 2 trials suggested treosulfan-based conditioning in adult patients with acute myeloid leukaemia or myelodysplastic syndrome had low non-relapse mortality. However, the value of treosulfan-based conditioning in a vulnerable target population was yet to be investigated.

Added value of this study

We report the results of a non-inferiority, phase 3 trial comparing reduced-intensity conditioning with busulfan-fludarabine with a reduced toxicity conditioning regimen, treosulfan plus fludarabine. To our knowledge, our study is the first randomised trial specifically designed to compare these two conditioning regimens in a selected patient population with acute myeloid leukaemia or myelodysplastic syndrome at increased mortality risk for standard myeloablative-conditioning regimens. Treosulfan-based conditioning, although considered myeloablative, showed major improvement of transplantation-related mortality and non-relapse mortality, which is not offset by an increase in relapse incidence. These improvements translated into a clinically meaningful event-free survival and overall survival benefit compared with the reduced-intensity conditioning busulfan and fludarabine treatment.

Implications of all the available evidence

Our treosulfan-based regimen could be used as the preferred standard conditioning therapy for the selected, growing population of older and comorbid patients with acute myeloid leukaemia or myelodysplastic syndrome.

and antileukaemic properties in vitro and in rodents.[14–16] When combined with fludarabine, treosulfan showed a particularly favourable acute organ toxicity profile in patients with acute myeloid leukaemia or myelodysplastic syndrome. Rapid and sustained donor cell engraftment and high proportions of patients with complete donor haemopoietic chimerism were observed. Therefore, the regimen is referred to as a toxicity-reduced but myeloablative conditioning regimen.[17–22]

Our multicentre, open-label, randomised, non-inferiority, phase 3 trial evaluated the efficacy and safety of treosulfan compared with reduced-intensity conditioning busulfan in adult patients with acute myeloid leukaemia or myelodysplastic syndrome at increased mortality risk for standard myeloablative conditioning regimens.

Methods

Study design and participants

This multicentre, open-label, randomised, non-inferiority trial was done in 31 transplantation centres in France, Germany, Hungary, Italy, and Poland (appendix p 1).

We designed the trial based on previous phase 2 trials.[19,21] On Feb 20, 2012 results of a planned interim analysis prompted the independent data monitoring committee to temporarily suspend patient accrual due to concerns about prolonged neutropenia and subsequent serious infectious complications in the treosulfan group (initial dose of 14 g/m² daily on days –6 to –4 of the 6-day regimen). Randomisation of patients was stopped in September, 2012, and the results of this first part of the trial have been shared at the EU Clinical Trials Register. Subsequently, we modified the protocol (Jan 25, 2013) to reduce treosulfan dose (from 14 g/m² to 10 g/m²) and schedule (infusion on days –4 to –2), patient follow-up was extended, and new statistical planning and sample size calculation were implemented. We report in this Article on patients enrolled in the study after this protocol modification.

Patients were eligible if they had acute myeloid leukaemia in first or consecutive complete haematological remission (blast counts <5% in bone marrow) or myelodysplastic syndrome (blast counts <20% in bone marrow) according to WHO 2008 and were indicated for allogeneic HSCT, but considered at increased risk for standard myeloablative conditioning based on age (≥50 years), a HCT-CI score higher than 2, or both.[23] Eligibility also included age between 18 and 70 years, Karnofsky index of 60% or higher, and availability of a human leucocyte antigen (HLA)-identical sibling (matched-related donor) or HLA-identical unrelated donor (matched-unrelated donor) identified by molecular typing of the HLA gene loci A, B, C, DRB1, and DQB1 (one antigen disparity [class I], one allele disparity [class II], or both were accepted).

Exclusion criteria were substantial vital organ function impairment, previous allogeneic HSCT, and active and non-controlled infectious diseases under treatment, including active viral liver infection (see trial protocol in ClinicalTrials.gov). Since this trial was part of the clinical development program to obtain a European marketing authorisation, the European Medicines Agency had decisive influence on its design. The trial protocol is available online.

An independent data monitoring committee supervised trial conduct, safety, and the preplanned interim analyses. Preparation of interim analyses was contracted to an independent biometrical contract research organisation to ensure that medac GmbH had no access to aggregated data until the clinical database was locked; respective firewalls were implemented. The protocol was approved by the responsible ethics committees and competent regulatory authorities in the participating countries. The trial was done in accordance with applicable laws and guidelines, including the Declaration of Helsinki and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, Guideline for Good Clinical Practice (E6). All patients provided written informed consent and identities were kept confidential.

Randomisation and masking

Randomisation was centralised at the sponsor's clinical trial management site. Allocation to treatment groups was based on a detailed registration form signed and submitted by the clinical investigator. A computer-generated and balanced (1:1) randomisation, using a permuted block technique with stratification by donor type (matched-related *vs* matched-unrelated donor), participating centre, and disease risk group was applied. Disease risk group stratification was based on two groups. Risk group 2 comprised patients with genetically unfavourable (adverse) risk acute myeloid leukaemia in first remission, or high or very high-risk myelodysplastic syndrome according to the Revised International Prognostic Scoring System (IPSS-R) for MDS (see trial protocol).[5,24] In addition, patients with acute myeloid leukaemia beyond first complete remission were assigned to risk group 2. All other patients were assigned to risk group 1. Investigators, participating patients, or contracted clinical trial monitors were not masked to individual treatment allocations. However, the sponsor's staff including the biometrical group, investigators, and contracted research organisations were masked to aggregated analyses until the database lock for final confirmatory analysis.

Procedures

The initially registered treosulfan dose of 14 g/m² daily on days –6 to –4 of the 6-day regimen, was changed to 10 g/m² treosulfan daily applied as a 2-h infusion for 3 days (days –4 to –2) after the protocol modification on Jan 25, 2013. The reduced-intensity conditioning reference treatment consisted of 0·8 mg/kg busulfan applied as a 2-h infusion at 6-h intervals on days –4 and –3. Both groups

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See **Online** for appendix

For more on the **results of the first part of the trial** see https://www.clinicaltrialsregister.eu/ctr-search/search?query=eudract_number:2008-002356-18

received 30 mg/m² intravenous fludarabine daily for 5 days (days –6 to –2). The administration of the reference treatment followed the instructions given in the approved Summary of Product Characteristics (Busilvex; Pierre Fabre Médicament, Boulogne, France).

Acute and chronic graft-versus-host disease (GvHD) were diagnosed according to Glucksberg's criteria and the modified Seattle criteria, respectively. Prophylaxis for GvHD was standardised in both groups and based on ciclosporin from day –1 (5 mg/kg daily, concentration adapted) and short course methotrexate (15 mg/m² on day +1, and 10 mg/m² on days +3 and +6). All matched-unrelated donors recipients received anti-T-lymphocyte immune globulin (either ATG Fresenius or Grafalon Neovii at a dose of 10 mg/kg on days –4, –3, and –2; or Thymoglobulin [Sanofi Genzyme] at a dose of 2·5 mg/kg on days –2 and –1), as previously published.[19]

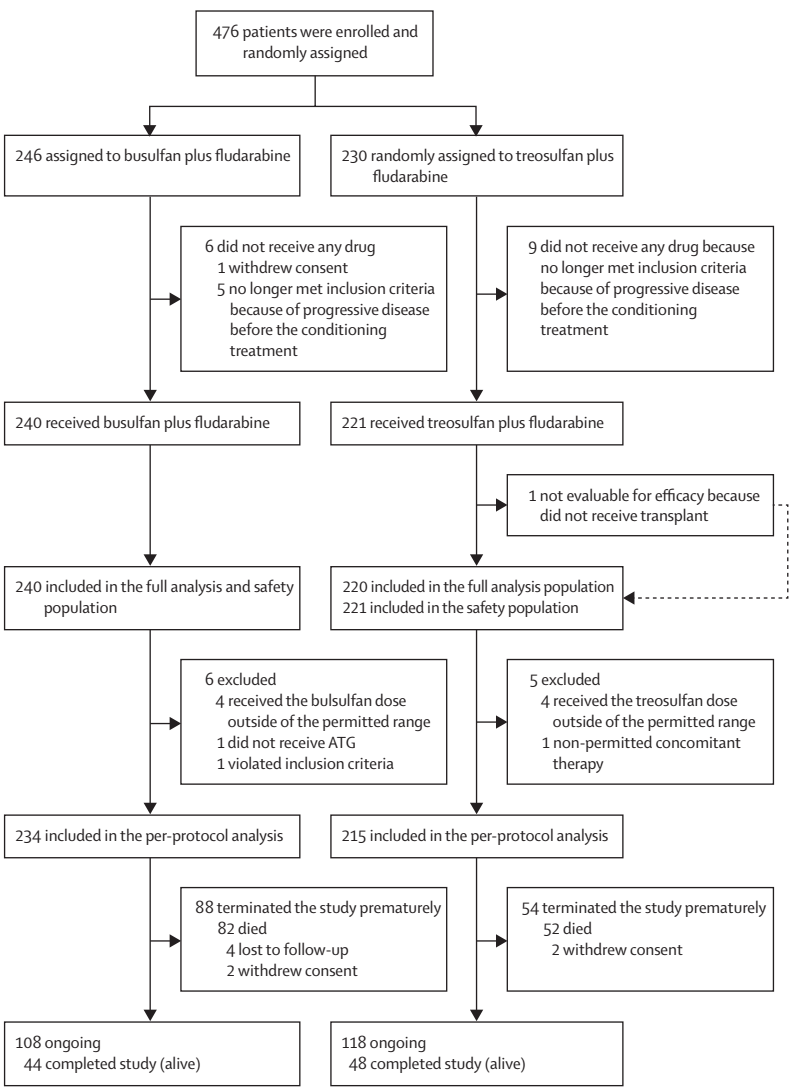

Figure 1: **Trial profile**

Efficacy and safety assessments were documented on days 28 and 100 and months 6, 9, 12, 15, 18, 21, and 24 after HSCT. Acute adverse events were continuously assessed and graded with Common Terminology Criteria for Adverse Events (CTCAE; version 4.03) between day –6 (after start of conditioning treatment) and day +28. The incidence of grade 3–4 mucositis was comparatively evaluated between day –6 and day +28. Patients who had a graft failure or relapse were followed for survival.

For event-free survival, reappearance of acute myeloid leukaemia blasts in peripheral blood or bone marrow, reappearance of cytogenetic abnormalities, or clinically relevant increase of molecular markers (only if a cytogenetic marker was not detectable) were used for event documentation.

Outcomes

The primary endpoint was event-free survival 2 years after HSCT. Event-free survival was defined as the time from allogeneic HSCT to relapse or progression of disease (for acute myeloid leukaemia was based on the usual morphological, cytogenetic, or molecular criteria as outlined in the trial protocol), graft failure (durable decline of neutrophil counts to $0 \cdot 5 \times 10^9$ cells per L or less in the peripheral blood, confirmed by bone marrow aplasia), or death (whichever occurred first).

Secondary endpoints were overall survival, cumulative incidence of relapse or progression, cumulative incidence of non-relapse mortality (probability of dying without relapse or progression), and cumulative incidence of acute and chronic GvHD within 2 years of transplantation; incidence of grade 3–4 mucositis and other grade 3–4 adverse events between day –6 and day +28 after transplantation; cumulative incidence of engraftment on day +28; and incidence of complete donor-type chimerism on days +28 and +100 after transplantation.

In addition, the cumulative incidence of transplantation-related mortality (probability of dying from a specific transplantation-related cause predefined in the protocol according to European Society for Blood and Marrow Transplantation definition) was analysed post-hoc in patients with chronic GvHD.

Statistical analysis

We initially assumed a 12-month event-free survival of 68·5% with busulfan-based conditioning (based on the results of the first confirmatory interim analysis of part 1 on Feb 1, 2012) and an accrual of ten patients per month within the first six months, 15 patients per month thereafter until 24 months after re-start of the trial, and 25 patients per month thereafter. With these assumptions, a sample size of 930 patients provided 80% power to exclude any relevant increased risks of events with treosulfan compared with busulfan (non-inferiority margin of 1·3 for hazard ratio) with a one-sided overall significance level of 0·025.

Three interim analyses were planned after 45, 137, and 239 events, or latest after 220, 460, 700 evaluable patients were enrolled. Results of the second preplanned interim analysis (Nov 9, 2016) covering 460 evaluable patients randomly assigned to the current protocol (modified on Jan 25, 2013), prompted the data monitoring board to recommend stopping further patient recruitment, since the primary trial objective had been accomplished. Accordingly, patient enrolment was closed on Dec 7, 2016. The reported analysis of 476 patients constitutes the final confirmatory analysis of the trial. A close out plan was implemented during which the data of the interim analysis were further clarified and the database was locked on May 31, 2017.

The safety population included all patients who received at least one dose of the study treatment. The full-analysis population evaluated for efficacy outcomes included all randomly assigned patients who received transplantation and had at least one efficacy parameter documented after baseline. The per-protocol population comprised all patients of the full-analysis population without major protocol violations.

For the primary endpoint, an O'Brien–Fleming stopping boundary for efficacy was calculated with a Lan–DeMets α spending function based on number of events observed. If significant non-inferiority was shown for event-free survival, the superiority of treosulfan could be tested at the same significance level.

Kaplan-Meier plots were calculated for event-free survival, overall survival, and transplantation-related mortality, and evaluated by a stratified Cox regression model with donor type as factor and risk group and centre as strata. Cumulative incidences of relapse or progression and non-relapse mortality were calculated using a Fine and Gray model with donor type as factor and risk group as stratum. Death and graft failure were competing risks for cumulative incidence of relapse or progression, whereas relapse or progression and graft failure were competing risks for non-relapse mortality. Conditional cumulative incidences were calculated for analysing reconstitution of neutrophils, leucocytes, and platelets. Duration of neutropenia was compared between the two drugs with the Wilcoxon-Mann-Whitney test.

P values (two-sided unless otherwise stated) for secondary endpoints were explorative, based on a significance level of 0·05.

SAS software (version 9.4) was used for all statistical analyses.

The study is registered with EudraCT (2008–002356–18) and ClinicalTrials.gov (NCT00822393).

Role of the funding source

The sponsor was involved in the study design, data collection, analysis, and interpretation. The corresponding author had full access to all the data in the study

	Busulfan plus fludarabine group (n=240)	Treosulfan plus fludarabine group (n=220)
All patients		
Sex		
Male	149/240 (62%)	130/220 (59%)
Female	91/240 (38%)	90/220 (41%)
Age, years		
Median	61·0 (56·5–64·0)	60·0 (55·0–65·0)
≥50	229/240 (95%)	205/220 (93%)
Comorbidity		
HCT-CI score	3·0 (1·0–4·0)	3·0 (1·0–4·0)
HCT-CI score >2	140/240 (58%)	131/220 (60%)
Donor type		
Matched related donor	59/240 (25%)	52/220 (24%)
Matched unrelated donor	181/240 (75%)	168/220 (76%)
Graft source		
Peripheral blood	235/240 (98%)	214/220 (97%)
Bone marrow	5/240 (2%)	6/220 (3%)
Diagnosis		
Acute myeloid leukaemia	138/240 (58%)	155/220 (71%)
Myelodysplastic syndrome	102/240 (43%)	65/220 (30%)
Patients with acute myeloid leukaemia (n=293)		
Time between diagnosis and HSCT, months	5·14 (3·52–8·25)	5·32 (3·88–9·36)
Complete remission		
First complete remission	117/138 (85%)	133/155 (86%)
Consecutive remission	21/138 (15%)	22/155 (14%)
Risk group for stratification		
Risk group I	74/138 (54%)	70/155 (45%)
Risk group II	64/138 (46%)	85/155 (55%)
Risk group*		
Low risk	13/138 (9%)	15/155 (10%)
Intermediate risk	61/138 (44%)	55/155 (36%)
High risk	43/138 (31%)	63/155 (41%)
Not applicable (if > complete remission 1)	21/138 (15%)	22/155 (14%)
Patients with myelodysplastic syndrome (n=167)		
Time between diagnosis and HSCT, months	7·59 (4·88–14·09)	7·62 (4·47–16·99)
Cause		
De novo	80/102 (78%)	51/65 (78%)
Therapy related	22/102 (22%)	22/65 (22%)
Treated		
No	42/102 (41%)	34/65 (52%)
Yes	60/102 (59%)	31/65 (48%)
Risk group for stratification		
Risk group I	47/102 (46%)	29/65 (45%)
Risk group II	55/102 (54%)	36/65 (55%)
Risk group based on IPSS-R		
Very low risk	1/102 (1%)	5/65 (8%)
Low risk	16/102 (16%)	13/65 (20%)
Intermediate risk	30/102 (29%)	11/65 (17%)
High risk	24/102 (24%)	16/65 (25%)
Very high risk	31/102 (30%)	20/65 (31%)

Data are n (%), n/N (%), or median (IQR). HCT-CI=haemapoietic cell transplantation-comorbidity index. HSCT=haemapoietic stem cell transplantation. IPSS-R=Revised International Prognostic Scoring System. *Based on the European Leukemia Network.

Table 1: **Baseline characteristics (full-analysis population)**

and had final responsibility for the decision to submit for publication.

Results

Between June 13, 2013, and May 3, 2016, 476 patients indicated for allogeneic HSCT were randomly assigned to treosulfan plus fludarabine (n=230) or busulfan plus fludarabine (n=246). 461 patients received treatment and were analysed for safety (treosulfan 221, busulfan 240) and 460 patients were transplanted and included in the efficacy analyses (treosulfan 220, busulfan 240; figure 1). Baseline characteristics are presented in table 1. 347 (75%) of 460 patients were aged between 50 and 65 years, and only 48 (14%) of 347 patients had no documented comorbidities. Distribution of those patients was balanced (busulfan 28 [15%] of 186 patients, treosulfan 20 [12%] of 161 patients) and overall survival outcome in these 48 patients was comparable between the treatment

	Busulfan plus fludarabine group (n=240)	Treosulfan plus fludarabine group (n=220)	HR (95% CI)	p value
Follow-up,* months	17·4 (6·3–23·4)	15·4 (8·8–23·6)	..	..
Event-free survival				
Patients with event	100 (42%)	68 (31%)	..	..
Death†	41 (17%)	23 (10%)	..	..
Relapse or progression†	51 (21%)	45 (20%)	..	..
Primary graft failure†	1 (<1%)	0	..	..
Secondary graft failure†	7 (3%)	0	..	..
24-month event-free survival (95% CI)	50·4% (42·8–57·5)	64·0% (56·0–70·9)	0·65 (0·47–0·90)	<0·0001‡ for non-inferiority; 0·0051‡ for superiority
Overall survival				
Patients with event	82 (34%)	52 (24%)	..	..
24-month overall survival (95% CI)	56·4% (48·4–63·6)	71·3% (63·6–77·6)	0·61 (0·42–0·88)	0·0082‡
Relapse or progression				
Patients with event	51 (21%)	45 (20%)	..	..
Cumulative relapse or progression incidence at 24 months (95% CI)	23·3% (17·6–29·0)	24·6% (17·8–31·3)	0·87 (0·59–1·30)	0·50§
Transplantation-related mortality				
Patients with event¶	45 (19%)	23 (10%)	..	..
GvHD	18 (8%)	10 (5%)	..	..
Haemorrhage	1 (<1%)	1 (<1%)	..	..
Renal failure	0	5 (2%)	..	..
Cardiac toxicity	4 (2%)	1 (<1%)	..	..
Interstitial pneumonitis	0	1 (<1%)	..	..
Central nervous system toxicity	1 (<1%)	0	..	..
Veno-occlusive disease or hepatic sinusoidal obstruction syndrome	1 (<1%)	0	..	..
Infection	30 (13%)	19 (9%)	..	..
Multiple organ failure	5 (2%)	5 (2%)	..	..
Other transplantation-related cause	1 (<1%)	0	..	..
Patients with event later than 6 months after transplantation¶	26 (11%)	5 (2%)	..	..
GvHD	7 (3%)	3 (1%)	..	..
Renal failure	0	1 (<1%)	..	..
Cardiac toxicity	4 (2%)	1 (<1%)	..	..
Central nervous system toxicity	1 (<1%)	0	..	..
Infection	17 (7%)	3 (1%)	..	..
Multiple organ failure	2 (1%)	1 (<1%)	..	..
24-month transplantation-related mortality (95% CI)	28·2% (21·4–36·5)	12·1% (8·1–17·7)	0·54 (0·32–0·91)	0·020‡
Non-relapse mortality				
Patients with event	41 (17%)	23 (10%)	..	..
24-month cumulative non-relapse mortality incidence (95% CI)	22·6% (16·2–28·9)	11·4% (7·0–15·9)	0·60 (0·36–1·01)	0·053§

(Table 2 continues on next page)

	Busulfan plus fludarabine group (n=240)	Treosulfan plus fludarabine group (n=220)	HR (95% CI)	p value
(Continued from previous page)				
Engraftment of neutrophils (>0·5 x 10^9 cells per L)				
Patients with event	236 (98%)	217 (99%)	..	..
28-day conditional cumulative incidence of neutrophil engraftment (95% CI)	96·2% (94·1–98·3)	96·8% (93·5–100·0)	1·09 (0·92–1·28)	0·34§
Engraftment of leucocytes (>1·0 x 10^9 cells per L)				
Patients with event	237 (99%)	217 (99%)	..	..
28-day conditional cumulative incidence of leukocyte engraftment (95% CI)	96·7% (94·3–99·0)	99·5% (96·8–100·0)	1·14 (0·97–1·34)	0·12§
Engraftment of platelets (>20 x 10^9 cells per L)				
Patients with event	232 (97%)	215 (98%)	..	..
28-day conditional cumulative incidence of platelet engraftment (95% CI)	97·9% (96·2–99·6)	96·8% (94·2–99·3)	0·86 (0·73–1·02)	0·077§
Incidence of complete chimerism (95% CI)‖				
Day +28 visit	82·0% (76·5–86·7)	93·5% (89·3–96·4)	..	0·0080**
Day +100 visit	78·2% (72·1–83·5)	86·4% (81·0–90·8)	..	0·021**
Acute GvHD (grade 2-4)				
Patients with event	141 (59%)	114 (52%)	..	..
Cumulative incidence at 100 days (95% CI)	58·8% (52·5–65·0)	52·1% (45·5–58·7)	0·83 (0·65–1·06)	0·13††
Acute GvHD (grade 3-4)				
Patients with event	23 (10%)	14 (6%)	..	..
Cumulative incidence at 100 days (95% CI)	9·6% (5·9–13·3)	6·4% (3·2–9·6)	0·66 (0·34–1·27)	0·21††
Chronic GvHD††				
Patients with event	103/190 (54%)	91/179 (51%)	..	..
Cumulative incidence at 24 months (95% CI)	60·7% (53·1–68·4)	60·1% (49·8–70·3)	0·91 (0·69–1·20)	0·52††
Extensive chronic GvHD‡‡				
Patients with event	42/190 (22%)	28/179 (16%)	..	..
24-month cumulative incidence (95% CI)	26·1% (19·2–33·1)	18·4% (12·0–24·8)	0·68 (0·42–1·09)	0·11††

Data are n (%) or median (IQR) unless otherwise specified. HR=hazard ratio. GvHD=graft-versus-host disease. HSCT=haemopoietic stem cell transplantation. *Based on reverse Kaplan-Meier estimates for overall survival. †Only if this event occurred first. ‡Adjusted for donor type (factor), and risk group and centre (strata) using Cox regression model. §Adjusted for donor type (factor) and risk group (stratum) using Fine and Gray model. ¶Multiple transplantation-related causes per patient when applicable. ‖Odds ratio 3·21 (95% CI 1·69–6·09) at +28 days; 1·89 (1·11–3·19) at +100 days. **Stratified Cochran–Mantel–Haenszel test adjusted for donor type and risk group as strata. ††Patients are at risk if they have survived 100 days after end of HSCT without relapse and graft failure. ‡‡Test of Gray.

Table 2: **Study outcomes (full-analysis population)**

groups (24-month overall survival 69·0% [95% CI 37·8–86·8] for busulfan *vs* 87·5% [58·6–96·7] for treosulfan). A higher proportion of patients with myelodysplastic syndrome was observed in the busulfan group (table 1). However, this imbalance was outweighed by stratified randomisation: treatment groups were balanced regarding disease risk categories, determined according to disease status and cytogenetic or molecular risk (table 1; appendix p 3).

Median follow-up was 15·4 months (IQR 8·8–23·6) for the treosulfan group and 17·4 months (6·3–23·4) for the busulfan group (table 2). At 2 years after HSCT, event-free survival in the full analysis population was 64·0% (95% CI 56·0–70·9) for treosulfan versus 50·4% (42·8–57·5; one-sided p<0·0001 for non-inferiority) for busulfan (HR 0·65 [95% CI 0·47–0·90]; figure 2, table 2). These results were comparable to those in the per-protocol population (HR 0·67 [0·48–0·93]; p<0·0001; appendix p 4). Although a considerable treatment effect in favour of treosulfan was observed, the rigid prespecified significance level for superiority was formally not met within this interim analysis (one-sided p=0·0051 for superiority). The event-free survival benefit of treosulfan was consistently shown throughout the predefined exploratory subgroup analyses, including patients aged 50 years or older, with HCT-CI score higher than 2, patients with unfavourable risk (risk group 2), patients with matched unrelated donor grafts, and analyses by disease type (figure 3; appendix pp 6, 7).

The 2-year overall survival, transplantation-related mortality, and non-relapse mortality were all improved in the treosulfan group compared with the busulfan group (figure 2; table 2). A significantly improved transplantation-related mortality was further observed in the treosulfan group for patients with chronic GvHD (6·1% [2·6–14·2]) compared with the busulfan group (32·3% [22·8–44·5], HR 0·24 (0·09–0·63); p=0·0041; appendix p 9). The most

frequent causes of late death (>6 months after transplantation) were infection and GvHD (table 2). The overall benefit of treosulfan was consistently shown throughout the exploratory subgroup analyses, including patients aged 50 years or older, patients with unfavourable disease risk, patients with matched unrelated donor grafts, and patients with either acute myeloid leukaemia or myelodysplastic syndrome (appendix p 8).

There was no difference between treatment groups regarding disease recurrence or progression after HSCT (p=0·50; figure 2, table 2). The prespecified disease risk category had a comparable effect on both trial groups. For risk group 1, the 2-year incidence of relapse or progression was 13·1% (95% CI 6·6–19·6) in the busulfan group and 9·8% (3·2–16·5) in the treosulfan group (p=0·37). For risk group 2, the 2-year relapse or progression incidence was 33·6% (24·6–42·6) in the busulfan group and 37·5% (26·4–48·6) in the treosulfan group (p=0·80).

At day 28 after HSCT, 96·8% (95% CI 93·5–100·0) of treosulfan-treated patients and 96·2% (94·1–98·3) of busulfan-treated patients achieved neutrophil engraftment (p=0·34). However, the median duration of neutropenia of $0·5 \times 10^9$ cells per L or less was longer in the treosulfan group (14·0 days *vs* 12·5 days, p=0·00020). One patient in the busulfan group developed primary graft failure and seven patients developed secondary graft failure, whereas no patients in the treosulfan group developed graft failure (table 2). Platelet recovery to more than 20×10^9 cells per L on day 28 after HSCT was achieved in 97% and 98% of patients, respectively (p=0·077). Incidence of complete donor haemopoietic chimerism on day 28 after HSCT was in favour of treosulfan treatment (p=0·0080; table 2). This difference was confirmed at day 100 after HSCT (p=0·021; table 2).

Comparable frequencies of acute adverse events (all grades) were observed in both treatment groups (table 3). None of the patients required a dose reduction or discontinuation due to drug-related toxicity. Mucositis of grade 3 or higher was comparable between the groups (busulfan 18 [7·5%] of 240 patients, treosulfan 10 [4·5%]

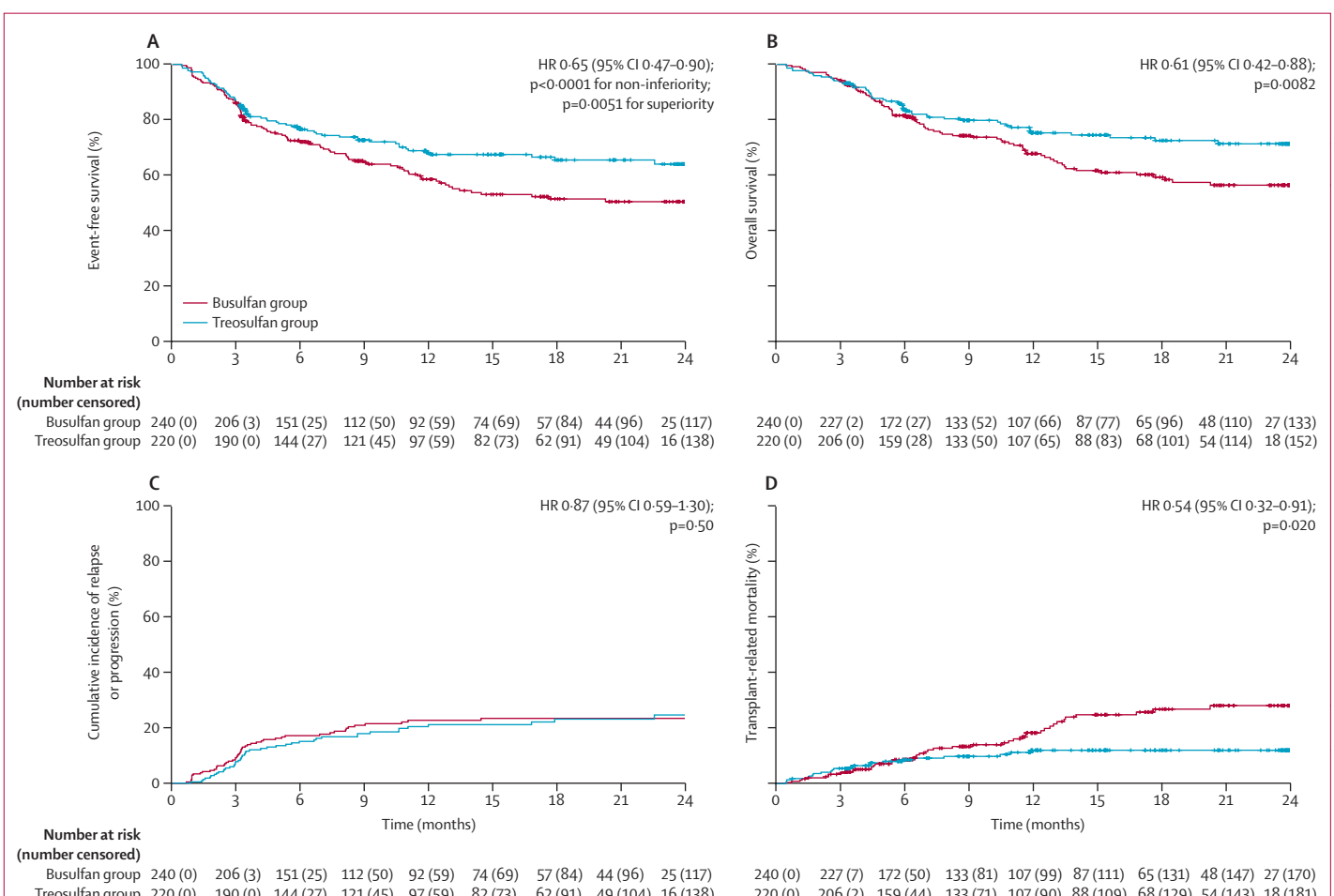

Figure 2: **Event-free survival (A), overall survival (B), cumulative incidence of relapse or progression (C), and cumulative incidence of transplantation-related mortality (D) in the full-analysis population**
HR=hazard ratio.

of 221 patients). No significant differences between the treatment groups were observed for adverse events of grade 3 or higher. The proportion of patients with serious adverse events was low in both groups (18 [8%] patients in the treosulfan group and 17 [7%] patients in the busulfan group, appendix p 5). Drug-related serious adverse events were reported in six (3%) of 221 patients of the treosulfan group and eight (3%) of 240 patients in the busulfan group. Most commonly reported drug-related serious adverse events were infections (four [2%] in the treosulfan group and four [1·7%] in the busulfan group) and hepatobiliary disorders (none in the treosulfan group and three [1%] in the busulfan group).

52 (24%) patients in the treosulfan group died: 26 (12%) of relapse, 23 (10%) of transplantation-related causes, and two (1%) of other causes (suicide and sepsis). The cause of death was unknown for one (<1%) patient. Transplantation-related causes of death were infection in eight (4%) patients; GvHD in four (2%); GvHD in combination with either infection (two [1%]) or with infection and multiple organ failure (one [<1%]), infection and renal failure (one [<1%]), infection, interstitial pneumonitis, and renal failure (one [<1%]), or infection, renal failure, and multiple organ failure (one [<1%] patient). In addition, three (1%) patients in the treosulfan group died of infection with multiple organ failure, one (<1%) of haemorrhage with renal and multiple organ failure, and one (<1%) of infection with cardiac toxicity and renal failure. In the busulfan group, 82 (34%) died: 36 (15%) of relapse, 45 (19%) of transplantation-related causes, and one (<1%) of a secondary malignancy. Transplantation-related causes of death were infection in 17 (7%) patients, GvHD in eight (3%), GvHD in combination with either infection (seven [3%]), multiple organ failure (two [1%]), or graft failure and infection (one [<1%]). In addition, four (2%) patients in the busulfan group died of cardiac toxicity, three (1%) of infection with multiple organ failure, one (<1%) of infection with CNS toxicity, and one (<1%) each of haemorrhage and infection with hepatic veno-occlusive disease.

The cumulative incidence of acute and chronic GvHD was comparable between the treatment groups; and so was extensive chronic GvHD at 2 years (18·4% [95% CI 12·0–24·8] *vs* 26·1% [19·2–33·1]; p=0·11; table 2).

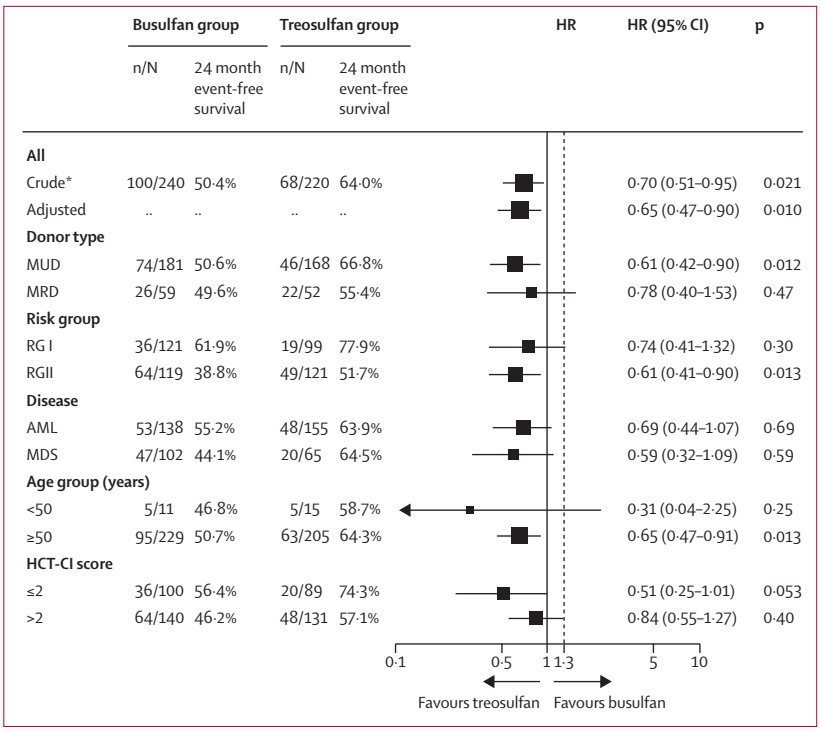

Figure 3: **Event-free survival stratified by prognostic factors (full-analysis population)**
MUD=matched unrelated donor. MRD=matched related donor. RG I=risk group 1. RG II=risk group 2. AML=acute myeloid leukaemia. MDS=myelodysplastic syndrome. HR=hazard ratio. HCT-CI=haemopoietic stem cell transplantation-specific comorbidity index. *Not adjusted; others parameters are adjusted for donor type as factor, and risk group and study centre as strata, using Cox model.

Discussion

We compared a new treosulfan-based preparative regimen with a widely accepted reduced-intensity conditioning busulfan-based regimen, preceding allogeneic HSCT in older or comorbid patients with acute myeloid leukaemia or myelodysplastic syndrome at increased mortality risk after standard myeloablative regimens. In this Article, we report the final confirmatory analysis of the trial in 476 patients randomly assigned after the treosulfan dose and schedule were modified (protocol version Jan 25, 2013). We observed that event-free survival was more than 10% in favour of treosulfan, justifying early termination of patient enrolment after confirmatory analysis at the second planned interim analysis. This difference was predominantly attributable to the substantial reduction of transplantation-related mortality (almost 2 times lower for treosulfan compared with busulfan), which is in accordance with previous phase 2 trials.[18,19,21] Substantially reduced transplantation-related mortality and non-relapse mortality following treosulfan-treatment translated into a favourable overall survival, with a 2-year overall survival estimate of more than 70%. The overall survival benefit of treosulfan was consistently shown throughout all major exploratory subgroup analyses, including patients aged 50 years or older, patients with unfavourable disease risk, and patients with matched-unrelated donor grafts. Overall survival for our busulfan reference group (56·4% [95% CI 48·4–63·6] at 2 years) met expectations for this reduced-intensity conditioning regimen in patients with acute myeloid leukaemia or myelodysplastic syndrome strictly selected for increased mortality risk after standard myeloablative conditioning regimens.[6,8–11,25,26]

We assume that the high proportion of older patients (median patient age in the range of 60 years), of comorbid patients (HCT-CI>2), and of patients with unfavourable disease risk features in both study groups might have contributed to the inferior survival observed in the reference group compared with previous studies. This

	Busulfan plus fludarabine group (n=240)				Treosulfan plus fludarabine group (n=221)			
	Grade 1–2	Grade 3	Grade 4	Grade 5	Grade 1–2	Grade 3	Grade 4	Grade 5
Patients with any event	98 (41%)	116 (48%)	12 (5%)	3 (1%)	88 (40%)	98 (44%)	14 (6%)	6 (3%)
Gastrointestinal disorders	140 (58%)	34 (14%)	5 (2%)	0	126 (57%)	20 (9%)	4 (2%)	0
General disorders and administration site conditions	116 (48%)	12 (5%)	0	0	116 (52%)	4 (2%)	0	0
Musculoskeletal and connective tissue disorders	60 (25%)	7 (3%)	0	0	72 (33%)	10 (5%)	0	0
Nervous system disorders	65 (27%)	8 (3%)	0	0	55 (25%)	5 (2%)	0	0
Skin and subcutaneous tissue disorders	64 (27%)	4 (2%)	0	0	61 (28%)	4 (2%)	0	0
Abnormal blood chemistry results	31 (13%)	33 (14%)	2 (1%)	0	28 (13%)	31 (14%)	2 (1%)	0
Vascular disorders	40 (17%)	25 (10%)	2 (1%)	0	32 (14%)	21 (10%)	1 (<1%)	0
Infections and infestations	36 (15%)	13 (5%)	7 (3%)	2 (1%)	26 (12%)	21 (10%)	5 (2%)	6 (3%)
Respiratory, thoracic, and mediastinal disorders	46 (19%)	6 (3%)	2 (1%)	1 (<1%)	36 (16%)	3 (1%)	1 (<1%)	2 (1%)
Metabolism and nutrition disorders	31 (13%)	12 (5%)	1 (<1%)	0	29 (13%)	16 (7%)	0	0
Blood and lymphatic system disorders	0	29 (12%)	0	0	0	31 (14%)	2 (1%)	0
Cardiac disorders	13 (5%)	6 (3%)	0	1 (<1%)	26 (12%)	6 (3%)	1 (<1%)	0
Renal and urinary disorders	22 (9%)	1 (<1%)	0	0	17 (8%)	0	3 (1%)	0
Psychiatric disorders	21 (9%)	1 (<1%)	1 (<1%)	0	15 (7%)	2 (1%)	0	0
Immune system disorders	18 (8%)	1 (<1%)	0	0	13 (6%)	2 (1%)	0	0
Eye disorders	25 (10%)	0	0	0	8 (4%)	0	0	0
Ear and labyrinth disorders	18 (8%)	1 (<1%)	0	0	12 (5%)	0	0	0
Injury, poisoning, and procedural complications	4 (2%)	2 (1%)	0	0	3 (1%)	1 (<1%)	0	0
Hepatobiliary disorders	3 (1%)	1 (<1%)	1 (<1%)	0	2 (1%)	1 (<1%)	0	0
Reproductive system and breast disorders	4 (2%)	1 (<1%)	0	0	3 (1%)	0	0	0
Surgical and medical procedures	0	0	0	0	0	1 (<1%)	0	0

Data are n (%). Only grade 1–2 adverse events that occurred in 10% of patients or more in any group are reported, whereas all grade 3, 4, and 5 adverse events are reported.

Table 3: **Adverse events in the safety population**

assumption is supported by exploratory analysis of these adverse prognostic factors on event-free survival (figure 3) and overall survival (data not shown), which were each in favour of the treosulfan regimen compared with the busulfan regimen. In particular, increasing patient age is generally confirmed as one of the most decisive adverse outcome factors for allogeneic HSCT, irrespective of the intensity of busulfan-based regimens.[27] In contrast, survival data of prospective randomised trials published in 2017, with identical or similar busulfan regimens, were generated exclusively in myeloablative conditioning eligible and younger patients with acute myeloid leukaemia and myelodysplastic syndrome (median age of patients treated with reduced-intensity conditioning of 51 years and 55 years, respectively).[12,13] Accordingly, slightly higher overall survival estimates can be expected for those trials.

The incidence of disease recurrence within 2 years after allogeneic HSCT was comparable between the treatment groups in our trial. Cumulative incidence of relapse or progression was equally affected by disease risk category in both groups. These results are consistent with most reports on disease risk adjusted cumulative incidence of relapse or progression of the reduced-intensity conditioning busulfan regimen in patients with acute myeloid leukaemia or myelodysplastic syndrome and justify the conclusion that the antileukaemic efficacy of the treosulfan regimen is equivalent to the reduced-intensity conditioning busulfan regimen.[8,12] Transplantation-related mortality reached a plateau within the second post-transplantation year for treosulfan, whereas it further increased for busulfan. Late transplantation-related mortality is strongly associated with the manifestations of (extensive) chronic GvHD and associated inherent infectious complications.[28] In our study, chronic GvHD was associated with a significantly higher transplantation-related mortality due to infections in busulfan-treated patients. This unexpected observation might point to a prolonged dysfunction of anti-infectious immunity in patients developing chronic GvHD in the busulfan group and might explain why patients administered busulfan

remained more susceptible to late fatal infectious complications. In comparison to busulfan, improved immune reconstitution has previously been described for treosulfan in rodents and is further suggested by clinical results obtained in treosulfan conditioned children with primary immunodeficiency. Since monitoring of immune reconstitution has not been implemented in this comparative trial protocol, the favourable effect of the treosulfan regimen on late transplantation-related mortality deserves further investigation.[29,30]

This study has several limitations. It is current consensus that measurable residual disease is an independent prognostic indicator for the post-transplant relapse risk in acute myeloid leukaemia. Validated and standardised quantitative molecular residual disease evaluation was only established for a minority of patients with acute myeloid leukaemia and was generally not applicable in patients with myelodysplastic syndrome at the time of this trial's design. Therefore, we decided not to implement measurable residual disease at study entry and, thus, not to use it for stratified randomisation. Because of the vulnerable patient population and the different treatment schedules applied, blinding of the trial was considered unfeasible. Open-label trial designs bear the risk of bias. To reduce potential bias, we chose a robust primary endpoint, which was considered being independent from the subjective view of the patient or the investigator. In addition, investigators and trial personnel were masked for aggregated data analyses until database lock. Nevertheless, a potential risk of bias cannot be completely ruled out. When setting up this trial, we implemented patient age, transplant type, and HCT-CI to assess patients' comorbidities, as well as myelodysplastic syndrome and acute myeloid leukaemia specific disease risk scoring (based on cytogenetic and molecular markers for acute myeloid leukaemia and IPSS-R for myelodysplastic syndrome) to adjust for transplantation-related risks. Other disease-specific risk scores as the disease risk index have been developed meanwhile but were not implemented in our study.

As discussed, non-relapse mortality in the busulfan group of our study appears somewhat higher than reported by others.[12,13] However, indirect data comparison is of limited validity and the reason to do sufficiently dimensioned, prospective, comparative studies like ours. Finally, the eligibility criteria of our trial restrict the results and conclusions to patients with acute myeloid leukaemia and myelodysplastic syndrome at increased mortality risk for myeloablative conditioning.

In conclusion, the improvement of the new treosulfan regimen was consistently detectable for event-free survival, overall survival, transplantation-related mortality, and non-relapse mortality, which suggests that this regimen has the potential to become a standard preparative regimen before allogeneic HSCT in patients with acute myeloid leukaemia and myelodysplasic syndrome at increased mortality risk for myeloablative conditioning.

Contributors

DWB, FC, JC, JB, and UP conceived and designed the study. AK and JB provided global project management. DWB, UP, MT, and CH analysed the data. DWB, FC, PD, MS, JC, FS, CH, MT, JB, and UP interpreted the data. DWB acted as coordinating principal investigator and corresponding author. MM (Poland), PR (Hungary), FC (Italy), and MM (France) acted as national coordinating investigators. DWB, RT, MS, CG, TM, PR, E-MW-D, BH, PD, TL, WB, RV, FC, JP, FS, JS, CJ, CG-T, MM, HL-W, KS-E, SD, GUG, SM, CS, UH, FP, MM, AR, MCM, DN, G-NF, IH, NRW, DR, GS, RPL, EH, DW, BG, JC, GW, HM, NB, AH, MB, GS, MV, SG, API, JF, FB, MDM, and MM actively admitted study patients and contributed clinical data from their site. DWB, PD, JB, UP, HAM, MT, and CH were responsible for the preparation and writing of the manuscript. All authors reviewed and approved the final version of the manuscript.

Declaration of interests

DWB received honoraria for consultation, a grant for study patient documentation, speaker fees, and travel support from medac GmbH, during the conduct of the study. UP, CH, MT, AK, HAM, and JB are employees of medac GmbH. JB has licensed an employee's invention (PCT/EP00/10871) to medac GmbH. WB received personal fees from medac GmbH, Neovii, Miltenyi Biotech, and Jazz Pharma, outside the submitted work. AR received personal fees from Amgen, Pfizer, and Novartis, outside the submitted work. EH received personal fees from Novartis, MaatPharma, and Apceth, outside the submitted work. PD reports grants from medac GmbH, outside the submitted work. TM received support for advisory board membership from AbbVie, Bristol-Myers Squibb, Janssen-Cilag, Novartis, Pfizer, Takeda, Novartis, Merck, Sharp & Dohme, and Pfizer, outside the submitted work. FS received travel support from medac GmbH and Neovii; personal fees from Janssen and Jazz Pharma; and grants from Astellas, outside the submitted work. UH received non-financial support from medac GmbH, outside the submitted work. FP reports honoraria from Celgene, Janssen, and Jazz; and travel grants from medac GmbH and Neovii, outside the submitted work. DN received grants from Novartis and non-financial support from Amgen, outside the submitted work. IH reports grants and non-financial support from medac GmbH and Novartis. DW obtained grants and personal fees from medac GmbH, and personal fees from Neovii, outside the submitted work. BG reports fees for study documentation from medac GmbH; personal fees and non-financial support from Jazz and Roche; non-financial support from Celgene; and grants, personal fees, and non-financial support from Riemser, outside the submitted work. JC received grants and personal fees from medac GmbH, outside the submitted work. JF reports grants from Novartis and medac GmbH; and grants, personal fees, and other support from Neovii and Riemser, outside the submitted work. SM received non-financial support from European Society for Blood and Marrow Transplantation/European Hematology Association, International Academy for Clinical Hematology, Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie, and International Society Cell & Gene Therapy; and personal fees and non-financial support from Celgene, Miltenyi, Kiadis, Bellicum, and Jazz Pharma. RT, MS, CG, PR, BH, TL, WV, FC, JP, JS, CJ, CG-T, MMi, HL-W, KSE, SD, GUG, CS, MMe, MCM, GNF, NRW, DR, GS, RPL, GW, NB, MB, GS, MV, SG, API, FB, MDM, MMa, HM, and UP declare no competing interests.

Data sharing

Individual participant data will not be shared.

Acknowledgments

We thank the patients, their families, the nurses, and investigators' teams who participated in the MC-FludT.14/L trial. We thank the members of the independent Data Monitoring Committee for their invaluable work and guidance throughout the duration of the trial. We also thank the committed support of Sandra Mahn and Anke Witt for clinical data management and statistical programming (medac GmbH).

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