Busulfan plus cyclophosphamide versus busulfan plus fludarabine as a preparative regimen for allogeneic haemopoietic stem-cell transplantation in patients with acute myeloid leukaemia: an open-label, multicentre, randomised, phase 3 trial

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Summary

Background The standard busulfan–cyclophosphamide myeloablative conditioning regimen is associated with substantial non-relapse mortality in patients older than 40 years with acute myeloid leukaemia who are undergoing allogeneic stem-cell transplantation. Because the combination of busulfan plus fludarabine has been proposed to reduce non-relapse mortality, we aimed to compare this treatment with busulfan plus cyclophosphamide as a preparative regimen in these patients.

Methods We did an open-label, multicentre, randomised, phase 3 trial for patients with acute myeloid leukaemia at 25 hospital transplant centres in Italy and one in Israel. Eligible patients were aged 40–65 years, had an Eastern Cooperative Oncology Group performance status less than 3, and were in complete remission. Patients were randomly assigned 1:1 to receive intravenous busulfan plus cyclophosphamide or busulfan plus fludarabine. Treatment allocations were not masked to investigators or patients. Randomisation was done centrally via a dedicated web-based system using remote data entry, with patients stratified by donor type and complete remission status. Patients allocated to busulfan plus cyclophosphamide received intravenous busulfan 0·8 mg/kg four times per day during 2 h infusions for four consecutive days (16 doses from days –9 through –6; total dose 12·8 mg/kg) and cyclophosphamide at 60 mg/kg per day for two consecutive days (on days –4 and –3; total dose 120 mg/kg). Patients allocated to busulfan plus fludarabine received the same dose of intravenous busulfan (from days –6 through –3) and fludarabine at 40 mg/m² per day for four consecutive days (from days –6 through –3; total dose 160 mg/m²). The primary endpoint was 1-year non-relapse mortality, which was assessed on an intention-to-treat basis; safety outcomes were assessed in the per-protocol population. This trial has been completed and is registered with ClinicalTrials.gov, number NCT01191957.

Findings Between Jan 3, 2008, and Dec 20, 2012, we enrolled and randomly assigned 252 patients to receive busulfan plus cyclophosphamide (n=125) or busulfan plus fludarabine (n=127). Median follow-up was 27·5 months (IQR 9·8–44·3). 1-year non-relapse mortality was 17·2% (95% CI 11·6–25·4) in the busulfan plus cyclophosphamide group and 7·9% (4·3–14·3) in the busulfan plus fludarabine group (Gray's test p=0·026). The most frequently reported grade 3 or higher adverse events were gastrointestinal events (28 [23%] of 121 patients in the busulfan plus cyclophosphamide group and 26 [21%] of 124 patients in the busulfan plus fludarabine group) and infections (21 [17%] patients in the busulfan plus cyclophosphamide group and 13 [10%] patients in the busulfan plus fludarabine group had at least one such event).

Interpretation In older patients with acute myeloid leukaemia, the myeloablative busulfan plus fludarabine conditioning regimen is associated with lower transplant-related mortality than busulfan plus cyclophosphamide, but retains potent antileukaemic activity. Accordingly, this regimen should be regarded as standard of care during the planning of allogeneic transplants for such patients.

Funding Agenzia Italiana del Farmaco.

Introduction

Allogeneic stem-cell transplantation is the most effective way to control leukaemia relapse for patients with acute myeloid leukaemia. In younger adults (ie, aged 18–40 years) with intermediate-risk or poor-risk acute myeloid leukaemia, allogeneic stem-cell transplant after myeloablative conditioning should be regarded as the treatment of choice for patients in their first complete

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Panel: Research in context

Evidence before this study

We searched PubMed for articles that were published in English before June 1, 2015, and about conditioning regimens for allogeneic haemopoietic cell transplantation in acute myeloid leukemia using the keywords "acute myeloid leukemia (AML)", "allogeneic transplantation", and "conditioning regimen". In younger adults with intermediate or poor-risk acute myeloid leukaemia, allogeneic stem-cell transplant following myeloablative conditioning is the consolidation treatment of choice for patients in remission. Before we started our trial, the HOVON-SAKK meta-analysis of several donor versus no donor studies indicated no survival advantage for patients with acute myeloid leukaemia who had a donor but were aged older than 40 years compared with younger patients. With the intent to reduce the high non-relapse mortality, which represents the major reason for transplant failure, several reduced intensity conditioning regimens have been proposed to minimise transplant toxicity. Although the feasibility of transplants in older patients using these programmes has been confirmed by many phase 2 studies, concerns have been raised about a significantly increased risk of relapse. At the time we designed our study, no abstracts or manuscripts had been reported with results from randomised trials comparing myeloablative with reduced intensity or reduced toxicity conditioning regimens in patients with acute myeloid leukaemia. For this reason we planned a randomised comparison between the standard myeloablative programme based on intravenous busulfan and cyclophosphamide and a reduced toxicity regimen based on the same myeloablative dose of busulfan and fludarabine. The latter regimen has been proposed to be similarly effective to busulfan plus cyclophosphamide, with a remarkably good toxicity profile, low non-relapse mortality, and a low relapse rate in older patients with acute myeloid leukaemia.

Added value of this study

Two randomised trials comparing the same conditioning regimens in younger patients have been reported and provided conflicting results regarding the efficacy and toxicity of busulfan plus fludarabine. Additionally, a randomised trial (prematurely stopped because of slow accrual of patients) to compare reduced intensity total body irradiation plus fludarabine with standard total body irradiation plus cyclophosphamide did not identify any significant difference in terms of non-relapse mortality, relapse incidence, disease-free survival, or overall survival in patients with acute myeloid leukaemia with a median age of 45 years. To our knowledge, our study is the first randomised trial specifically designed to compare these regimens in older patients with acute myeloid leukaemia undergoing transplantation in their first or subsequent haematological remission with a related or unrelated donor. Non-relapse mortality was significantly reduced with the busulfan plus fludarabine regimen compared with the busulfan plus cyclophosphamide regimen in all subgroups independent of patient age and sex, donor type, and acute myeloid leukaemia risk biology (European Leukemia Network score). This is probably because of reduced organ toxicity in the busulfan plus fludarabine group. The reduced non-relapse mortality did not come at the cost of an increased incidence of relapse.

Implications of all the available evidence

In older patients with acute myeloid leukaemia, the reduced toxicity of busulfan plus fludarabine, although still myeloablative, conferred low transplant-related mortality while preserving potent antileukaemic activity. Accordingly, we recommend that it should be considered as a standard of care in the planning of an allogeneic transplant for such patients.

remission.[1] Whether selection of transplant candidates can be improved by prediction of non-relapse mortality remains an issue under active investigation.[2,3] In younger patients (ie, aged 18–40 years) the combination of a myeloablative dose of intravenous busulfan with cyclophosphamide is a standard preparative regimen, which compares favourably with the combination of cyclophosphamide and ablative doses (usually 12 Gy) of total body irradiation.[4–6] However, myeloablative regimens are associated with substantial treatment-related toxicity in patients older than 40 years. In the HOVON-SAKK meta-analysis of several donor versus no donor studies,[7] no advantage was detected for patients older than 40 years compared with younger patients.[7] In the late 1990s, non-relapse mortality in older patients was the major driver for the development of reduced intensity conditioning (RIC) regimens, which aimed to minimise regimen-related toxicity while securing engraftment and providing a platform for the graft-versus-leukaemia effect.[8,9] During the past two decades, the proportion of allogeneic grafts performed after RIC regimens has grown rapidly, resulting in a substantial rise in the median age of patients undergoing transplantation. However, after this initial enthusiasm, it was reported that RIC regimens were associated with a significantly increased risk of relapse.[10] Overall, the benefit of reduced non-relapse mortality that RIC regimens provide is counterbalanced by an increased risk of relapse.[11]

Conditioning regimens need to be developed that will retain the antileukaemic activity of myeloablative conditioning, while reducing the transplant-related toxicity to the level of reduced RIC regimens: these programmes are tentatively referred to as reduced toxicity regimens.[12] One such regimen is based on the combination of a myeloablative dose of intravenous busulfan (12·8 mg/kg or equivalent) with fludarabine (160 mg/m² or similar), which has been reported to be effective for patients with acute myeloid leukaemia.[13] In a retrospective analysis,[14] the busulfan plus fludarabine regimen was associated with reduced non-relapse

mortality, shortened time to engraftment, and reduced incidence of acute and chronic graft-versus-host disease compared with busulfan plus cyclophosphamide. An increased frequency of relapse with the busulfan plus fludarabine regimen has been noted in some,[15] but not all, studies.[14] However, all of these analyses have been retrospective, and whether the busulfan plus fludarabine regimen represents an improvement for patients with acute myeloid leukaemia is not clear. Two randomised trials investigating these conditioning regimens in younger patients have been reported. In the first,[16] which was done in a patient population that included patients with acute myeloid leukaemia, acute lymphoblastic leukaemia, and other haematological malignancies, the busulfan plus fludarabine regimen did not prove to be a suitable replacement for busulfan plus cyclophosphamide because a higher incidence of relapse was noted in the busulfan plus fludarabine regimen compared with the busulfan plus cyclophosphamide regimen. Conversely, in another trial in young patients with acute myeloid leukaemia, the busulfan plus fludarabine regimen was reported to be associated with less toxicity than the busulfan plus cyclophosphamide regimen, but had similar antileukaemic activity.[17]

In view of this conflicting evidence, we did a randomised trial to compare the standard myeloablative busulfan plus cyclophosphamide regimen with the reduced toxicity busulfan plus fludarabine regimen for older patients with acute myeloid leukaemia undergoing allogeneic stem-cell transplantation.

Methods
Study design and participants
This study was an open-label, multicentre, randomised, phase 3 trial done in 25 hospital transplant centres in Italy and one in Israel (appendix), coordinated by the Gruppo Italiano Trapianto di Midollo Osseo e Terapie Cellulari (GITMO) network. Patients were eligible if they had a diagnosis of acute myeloid leukaemia, were in their first, second, or further complete haematological remission (as established by morphological assessment of the bone marrow), were aged 40–65 years, had an Eastern Cooperative Oncology Group performance status less than 3, and had an HLA-identical matched related or matched unrelated donor as defined by molecular high-resolution typing (four digits) of the HLA gene loci for class I (HLA-A, HLA-B, and HLA-C) and class II (DRB1). If no completely identical donor could be identified according to the minimal degree of matching established by the Italian Bone Marrow Donor Registry, one antigen or allele disparity (class I) or one allele disparity (class II) between the patient and donor was acceptable. Estimated survival of patients at enrolment was more than 3 months. We excluded patients if they were in first complete remission with t(15;17)(q22;q12) or PML/RARα-positive acute promyelocytic leukaemia, or t(8;21)(q22;q22)-positive and inv(16) or t(16;16)(p13;q22)-positive

acute myeloid leukaemia with white blood cell counts less than 20×10^9 cells per L at diagnosis and additional adverse cytogenetic abnormalities. We also excluded previously transplanted patients and patients with uncontrolled infections or severe cardiovascular, renal, hepatic, pulmonary, or psychiatric disorders or any disorder that compromised the ability to give truly informed consent for participation in this study. Patients with another progressive malignant disease or a history of other malignancies within 2 years before study entry were also excluded. After assessment of complete remission, no additional chemotherapy could be given before the start of the conditioning regimen.

We did the study in accordance with the International Conference on Harmonization for Good Clinical Practice and the appropriate regulatory requirements. The study was approved by the ethics committees of the participating centres and all patients and donors provided written informed consent before inclusion. The trial protocol was in accordance with the Declaration of Helsinki and is available online.

Randomisation and masking
Patients were randomly assigned (1:1) with a stratified biased coin algorithm with a variable block size strategy to receive either the busulfan plus cyclophosphamide or the busulfan plus fludarabine conditioning regimen. Randomisation was centralised at the Fondazione Mario Negri Sud (Santa Maria Imbaro, Chieti, Italy) and was done via a dedicated web-based system with remote data entry. Patients were stratified by donor type (matched related donor vs matched unrelated donor) and remission

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For the **full protocol** see http://www.gitmo.it/images/pdf/AMLR2/ProtocolloAMLR2.pdf
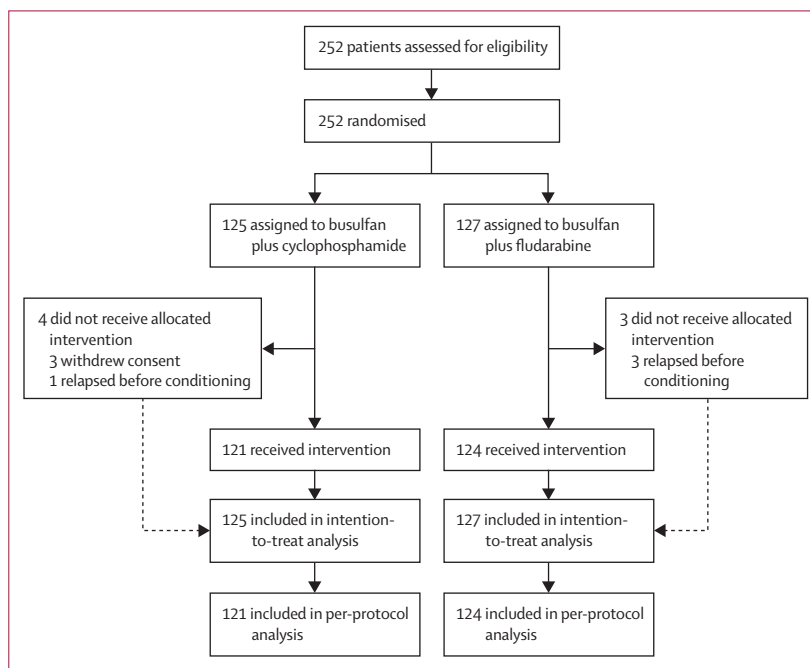
See **Online** for appendix

Figure 1: Trial profile

status (first complete remission *vs* second or further complete remission). Treatment allocations were not masked to the investigators, participants, those assessing outcomes, or those analysing the data.

	Busulfan plus cyclophosphamide (n=125)	Busulfan plus fludarabine (n=127)
Sex		
Male	69 (55%)	70 (55%)
Female	56 (45%)	57 (45%)
Age (years)	51 (46–55)	51 (47–57)
40–50	66 (53%)	62 (49%)
51–65	59 (47%)	65 (51%)
Type of acute myeloid leukaemia		
De novo	102 (82%)	103 (81%)
Secondary	22 (18%)	24 (19%)
Unknown*	1 (<1%)	0 (0%)
Disease status at allogeneic stem-cell transplantation		
First complete remission	105 (84%)	108 (85%)
Second or further complete remission	19 (15%)	18 (14%)
Unknown*	1 (<1%)	1 (<1%)
ELN risk		
Good	14 (11%)	13 (10%)
Intermediate 1	61 (49%)	62 (49%)
Intermediate 2	23 (18%)	17 (13%)
Adverse	23 (18%)	32 (25%)
Unknown*	4 (3%)	3 (2%)
HCT-CI		
0	69 (55%)	72 (57%)
1–2	41 (33%)	27 (21%)
≥3†	14 (11%)	28 (22%)
Unknown*	1 (<1%)	0 (0%)
Donor type		
Related	56 (45%)	58 (46%)
Unrelated	69 (55%)	69 (54%)
Graft source		
Bone marrow	41 (33%)	36 (28%)
Peripheral blood	80 (64%)	88 (69%)
Unknown*	4 (3%)	3 (2%)
Donor–recipient sex pair		
Female to male	19 (15%)	23 (18%)
Other	105 (84%)	104 (82%)
Unknown*	1 (<1%)	0 (0%)
Recipient/donor CMV serology		
Negative/negative	8 (6%)	12 (9%)
Negative/positive	10 (8%)	6 (5%)
Positive/negative	31 (25%)	37 (29%)
Positive/positive	75 (60%)	69 (54%)
Unknown	1 (<1%)	3 (2%)

Data are n (%) or median (IQR). ELN=European Leukemia Network. HCT-CI=Hematopoietic Cell Transplantation-Specific Comorbidity Index. *Patients not undergoing allogeneic stem-cell transplantation.

Table 1: Baseline demographic and transplant characteristics

Procedures

The standard treatment group received a myeloablative combination of intravenous busulfan (Laboratoires Pierre Fabre, Boulogne, France) 0·8 mg/kg four times per day during 2 h infusions for four consecutive days (16 doses from day –9 through day –6; total dose of 12·8 mg/kg) with cyclophosphamide 60 mg/kg per day for two consecutive days (on days –4 and –3; total dose of 120 mg/kg). The experimental group received the same myeloablative dose of intravenous busulfan (from day –6 through day –3) combined with fludarabine 40 mg/m² per day for four consecutive days (from day –6 through day –3) for a total dose of 160 mg/m². All patients received fixed doses of busulfan and no pharmacokinetic monitoring was done. No dose reductions or interruptions were allowed. On day 0, patients received either bone marrow cells or granulocyte-colony stimulating factor (G-CSF)-mobilised peripheral blood progenitor cells in their transplantation.

Prophylaxis for graft-versus-host disease was based on conventional ciclosporin A 1·5 mg/kg twice per day by short intravenous infusion starting on day –1 before transplant (to reach target trough level concentration 200 ng/mL or higher) and methotrexate 15 mg/m² given intravenously on day 1, and subsequently at 10 mg/m² intravenously on days 3, 6, and 11. Patients in both treatment groups who received stem cells from unrelated donors were treated with anti-thymocyte immunoglobulin (Genzyme, Cambridge, MA, USA) 0·5 mg/kg intravenously on day –3 and 2·0 mg/kg intravenously on day –2 and, if the donor was identical, 2·5 mg/kg on day –1. In cases with one antigen or allele disparity (class I) or one allele disparity (class II) between donor and recipient, the total dose of anti-thymocyte immunoglobulin could be increased up to 7·5 mg/kg.

After transplantation, patients were followed up until the end of the study. The main outcome data were collected and the main assessments of adverse events were done on days 30, 60, 100, and 180, then at 1 year and 2 years after transplant and once per year thereafter. At the same timepoints, the ratio of donor-derived cells to recipient-derived bone marrow cells, peripheral blood cells, and T lymphocytes (chimerism) was evaluated by molecular analysis of short tandem repeats on DNA isolated from bone marrow and peripheral blood mononuclear cells. The analysis of the chimeric status of peripheral blood T lymphocytes was done after positive selection of CD3-positive cells sorted by the AutoMacs device (Miltenyi Biotec, Bergisch Gladbach, Germany). The achievement of a full haemopoietic donor chimerism (defined as more than 95% of cells being of donor origin) was evaluated on bone marrow cells, peripheral blood cells and T lymphocytes at 30, 60, 100, 180 days and 1 year after transplant. Acute graft-versus-host disease with organ involvement and symptoms was assessed on a weekly basis for the first 3 months after transplant and graded according to the

Glucksberg scale. Chronic graft-versus-host disease was assessed at each follow-up visit and classified as limited or extensive.

Outcomes

The primary endpoint was the cumulative incidence of non-relapse mortality, assessed at 1 year after transplantation. Non-relapse mortality was defined as death from any cause not subsequent to relapse. Secondary endpoints for efficacy and safety were cumulative incidence of relapse, leukaemia-free survival, and overall survival at 1 and 2 years after transplantation, neutrophil and platelet engraftment, haemopoietic chimerism, incidence of rejection and graft failures, incidence of acute and chronic graft-versus-host disease, and cumulative incidence of regimen-related toxic effects, according to Bearman's criteria,[18] and are described further in the appendix. Deaths after relapse were categorised as caused by the disease irrespective of the proximate cause. Haematological relapse was defined via cytological assessment of the bone marrow. Neutrophil engraftment was defined as the number of days after transplantation taken to achieve an absolute neutrophil count of at least 0.5×10^9 cells per L and platelet engraftment was defined as the number of days to maintain an untransfused platelet count of at least 20.0×10^9 cells per L. Graft failure was defined as the absence of donor cells in the bone marrow by day 30 after transplant. Graft rejection was defined as the absence of donor cells in the bone marrow by day 60 after transplant following an initial haemopoietic chimerism. Adverse events were recorded in accordance with the Medical Dictionary for Regulatory Activities (MedDRA) version 10.0 code and graded with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Statistical analysis

We calculated the necessary sample size by assuming that 1-year non-relapse mortality in the busulfan plus cyclophosphamide group would be 25% (range 16–50). To show a reduction in non-relapse mortality of 50% to 12.5% (range 0–30) in the busulfan plus fludarabine group, 240 patients needed to be enrolled and randomly assigned (120 patients in each group) for a power of 80% (type II error 0.2). We used a two-sided alpha-level probability of 0.05 (type I error).

We used χ^2 tests or Fisher's exact test to assess categorical variables. Non-relapse mortality and the incidence of relapse incidence were considered to be competing events and we assessed group differences using the Fine and Gray's non-parametric test.[19] We calculated leukaemia-free survival and overall survival using the Kaplan-Meier method and we made comparisons by the log-rank test using censored data. We assessed the effect of treatment on non-relapse mortality and cumulative incidence of relapse at 1 year in prespecified subgroups by fitting a Cox model and calculating cause-specific HRs and 95% CIs. We assessed the effect of treatment on non-relapse mortality and cumulative incidence of relapse at 1 year in prespecified subgroups by fitting a Cox model and calculating HRs and 95% CIs. We did multivariable analyses at the two-tailed 5% significance level and calculated adjusted hazard ratios (HRs) and 95% CIs by fitting Cox models. Non-relapse mortality, cumulative incidence of relapse, leukaemia-free survival, and overall survival were analysed on an intention-to-treat basis. The subgroup
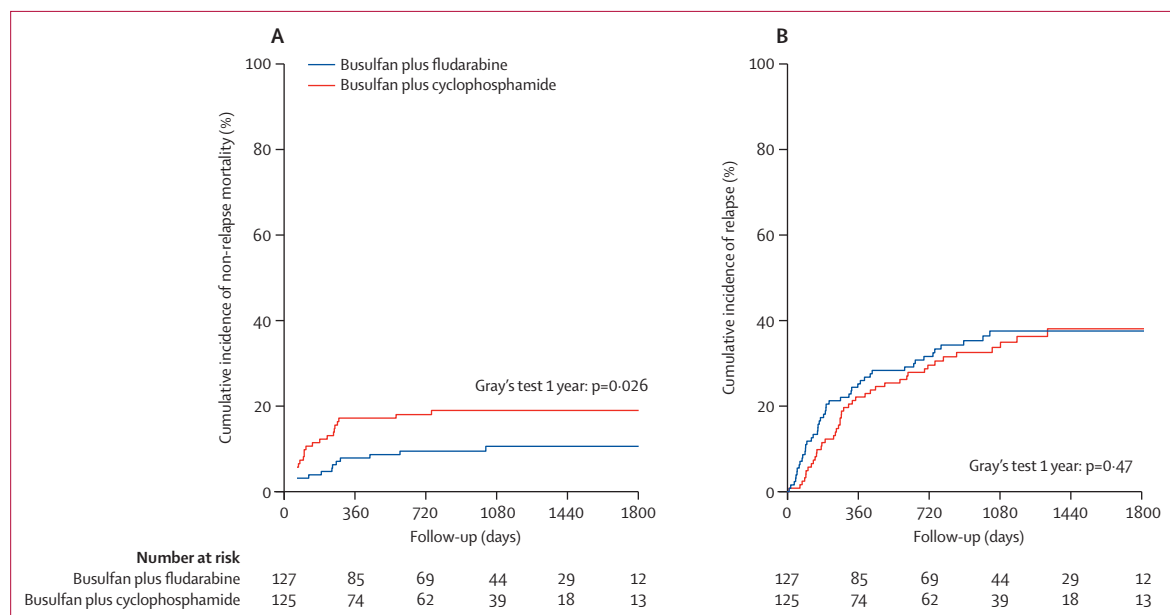

Figure 2: Cumulative incidence of non-relapse mortality (A) and cumulative incidence of relapse (B)

analysis, multivariable analysis, and safety assessments were done in the per-protocol population. All reported p values are two-sided.

One interim analysis was done after half of the patients (n=120) had been accrued, mainly to assess severe adverse effects, which could affect the continuation of the trial, but also to provide a first efficacy profile with an assessment of the adequacy of the sample size

calculation. The numbers of treatment failures and serious adverse events were closely monitored by the Data Safety Monitoring Board for unexpected trends. All analyses were done with SAS version 9.3 and R version 3.1.2 software.

This trial is registered with ClinicalTrials.gov, number NCT01191957.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The Steering Committee delegated the Fondazione Mario Negri Sud as the core data management and statistical facility, which did the centralised data collection, Good Clinical Practice quality monitoring, and analysis of data. The corresponding author (AR) and AM, CB, EO, and RMM had full access to all the data in the study and AR had final responsibility for the decision to submit for publication.

Results

From Jan 3, 2008, to Dec 20, 2012, we enrolled 252 patients and randomly assigned them to receive busulfan plus cyclophosphamide (n=125) or busulfan plus fludarabine (n=127). Four patients had a leukaemia relapse before conditioning (three died subsequently and one received a transplant outside the protocol) and three patients withdrew consent (figure 1). The allocated treatment was delivered to 121 patients in the standard busulfan plus cyclophosphamide group and 124 in the experimental busulfan plus fludarabine group; patients in this per-protocol population were treated with no deviations from the scheduled time and dose administration defined by the study protocol. The main

	Busulfan plus cyclophosphamide (n=121)	Busulfan plus fludarabine (n=124)	p value
Total mortality	50 (41%)	51 (41%)	0·98
Relapse death	27 (22%)	38 (31%)	0·14
Non-relapse death	23 (19%)	13 (10%)	0·060
Graft-versus-host disease	5 (4%)	3 (2%)	0·50
Infection	8 (7%)	7 (6%)	0·75
Viral	0 (0%)	2 (2%)	0·50
Bacterial	5 (4%)	2 (2%)	0·28
Fungal	3 (2%)	1 (<1%)	0·37
Unknown cause	0 (0%)	2 (2%)	0·50
Organ failure	9 (7%)	1 (<1%)	0·0095
Heart	2 (2%)	1 (<1%)	0·62
Lung	4 (3%)	0 (0%)	0·058
Gastrointestinal	1 (<1%)	0 (0%)	0·49
Multiple	2 (2%)	0 (0%)	0·24
Haemorrhage	1 (<1%)	1 (<1%)	1·00
Other	0 (0%)	1 (<1%)	1·00

Data are n (%) for per-protocol population. Comparisons between groups were done with χ^2 tests or Fisher's exact test as appropriate.

Table 2: Causes of death
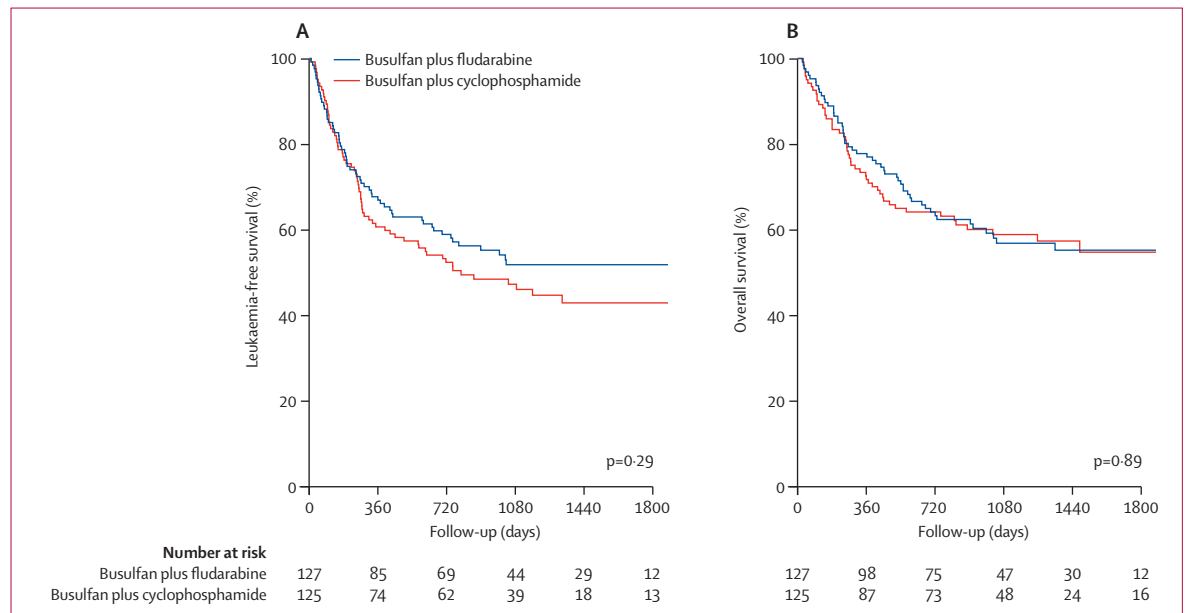
Figure 3: Kaplan-Meier curves of leukaemia-free survival (A) and overall survival (B)
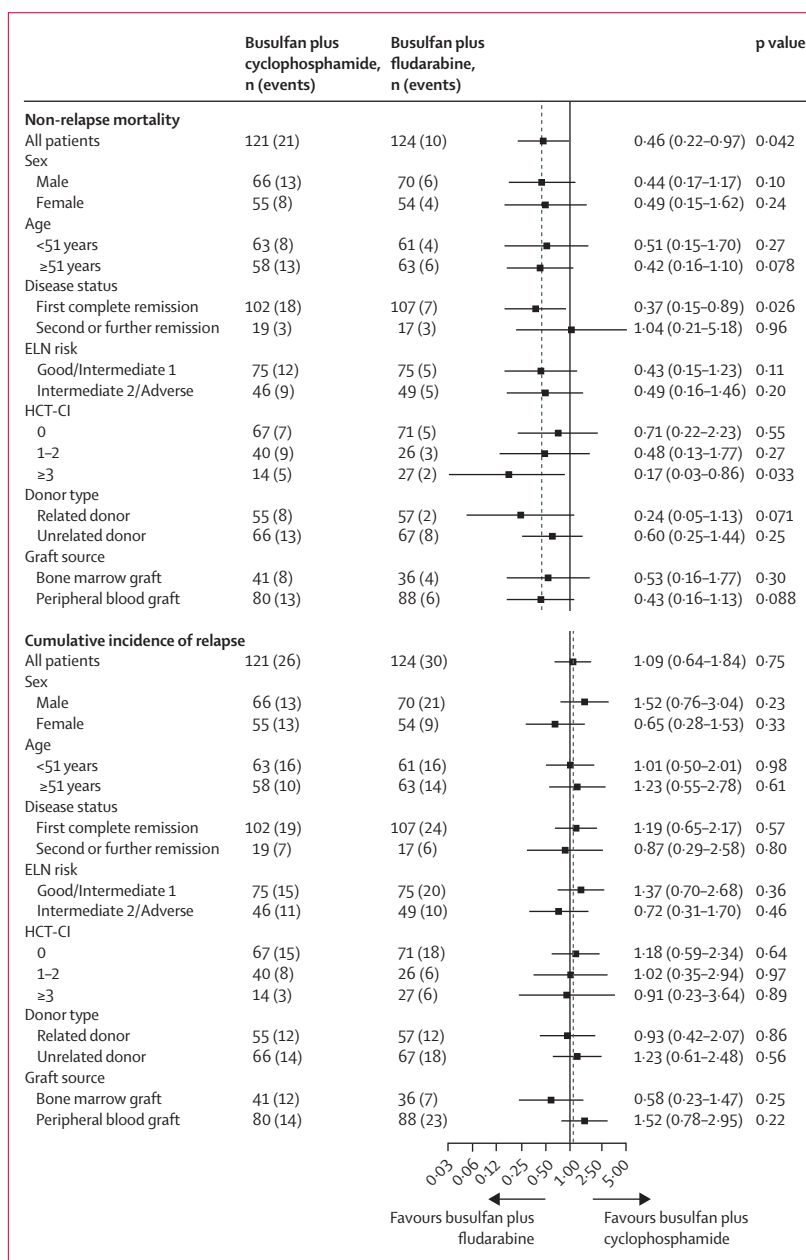
clinical features were balanced between the groups (table 1). Fewer patients with a Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) score[20] of 1–2, and more patients with a score of 3 or more, were allocated to the busulfan plus fludarabine group than to the busulfan plus cyclophosphamide group.

Median follow-up was 27·5 months (IQR 9·8–44·3). At 1 year, overall non-relapse mortality in the busulfan plus cyclophosphamide group was 17·2% (95% CI 11·6–25·4) compared with 7·9% (4·3–14·3) in the busulfan plus fludarabine group (Gray's test p=0·026; figure 2). At 1 year, non-relapse death occurred in 21 patients in the busulfan plus cyclophosphamide group and 10 patients in the busulfan plus fludarabine group. At 2 years, non-relapse mortality in the busulfan plus cyclophosphamide group was 18·0% (12·3–26·4) versus 9·5% (5·5–16·3) in the busulfan plus fludarabine group, and at 5 years this was 19·0% (13·1–27·5) in the busulfan plus cyclophosphamide group versus 10·6% (6·3–17·8) in the busulfan plus fludarabine group (Gray's test p=0·047 at 2 years and p=0·050 at 5 years; figure 2). At 1 year, the cumulative incidence of relapse was 22·1% (95% CI 15·8–30·9) in the busulfan plus cyclophosphamide group and 25·2% (18·6–34·1) in the busulfan plus fludarabine group (Gray's test p=0·47; figure 2). In both groups, the cumulative incidence of relapse remained similar at 2 years (29·6% [22·4–39·0] in the busulfan plus cyclophosphamide group vs 31·6% [24·4–40·9] in the busulfan plus fludarabine group; Gray's test p=0·59) and 5 years (38·1% [29·7–48·8] in the busulfan plus cyclophosphamide group vs 37·6% [29·7–47·4] in the busulfan plus fludarabine group; Gray's test p=0·70; figure 2). All causes of death are reported in table 2.

Leukaemia-free survival for busulfan plus cyclophosphamide versus busulfan plus fludarabine was 60·7% (95% CI 52·6–70) versus 66·9% (59·2–75·6) at 1 year (p=0·38), 52·4% (44·3–62·1) versus 58·9% (50·9–68·2) at 2 years (p=0·36), and 42·9% (34·4–53·6) versus 51·8% (43·6–61·7) at 5 years (p=0·29; figure 3). Relapses occurred in 43 patients in the busulfan plus cyclophosphamide group and in 46 patients in the busulfan plus fludarabine group. The proportion of patients who survived after relapse was significantly higher in the busulfan plus cyclophosphamide group (16 [35%]) than in the busulfan plus fludarabine group (six [13%]; p=0·0083). Overall survival was similar between the busulfan plus cyclophosphamide group and the busulfan plus fludarabine group at 1 year (71·7% [64·1–80·2] vs 77·0% [70·0–84·7]; p=0·36), 2 years (64·2% [56·1–73·4] vs 62·4% [54·5–71·5]; p=0·99)) and 5 years (54·8% [45·5–66·0] vs 55·2% [46·7–65·4]; p=0·89; figure 3). In our Kaplan Meier analysis, HR was 0·83 (0·58–1·17) for leukaemia-free survival and 0·97 (0·66–1·43) for overall survival.

Consistent with results in the intention-to-treat population, in the per-protocol population, non-relapse mortality was improved for all subgroups of patients given busulfan plus fludarabine, compared with patients given busulfan plus cyclophosphamide, while subgroups of patients treated with either conditioning regimen had a similar cumulative incidence of relapse (figure 4). The prognostic effect of the conditioning regimen and all baseline characteristics on non-relapse mortality, cumulative incidence of relapse, leukaemia-free survival, and overall survival was assessed in the per-protocol population at 1 year after transplantation (table 3). By

	Busulfan plus cyclophosphamide, n (events)	Busulfan plus fludarabine, n (events)			p value
Non-relapse mortality					
All patients	121 (21)	124 (10)		0·46 (0·22–0·97)	0·042
Sex					
Male	66 (13)	70 (6)		0·44 (0·17–1·17)	0·10
Female	55 (8)	54 (4)		0·49 (0·15–1·62)	0·24
Age					
<51 years	63 (8)	61 (4)		0·51 (0·15–1·70)	0·27
≥51 years	58 (13)	63 (6)		0·42 (0·16–1·10)	0·078
Disease status					
First complete remission	102 (18)	107 (7)		0·37 (0·15–0·89)	0·026
Second or further remission	19 (3)	17 (3)		1·04 (0·21–5·18)	0·96
ELN risk					
Good/Intermediate 1	75 (12)	75 (5)		0·43 (0·15–1·23)	0·11
Intermediate 2/Adverse	46 (9)	49 (5)		0·49 (0·16–1·46)	0·20
HCT-CI					
0	67 (7)	71 (5)		0·71 (0·22–2·23)	0·55
1–2	40 (9)	26 (3)		0·48 (0·13–1·77)	0·27
≥3	14 (5)	27 (2)		0·17 (0·03–0·86)	0·033
Donor type					
Related donor	55 (8)	57 (2)		0·24 (0·05–1·13)	0·071
Unrelated donor	66 (13)	67 (8)		0·60 (0·25–1·44)	0·25
Graft source					
Bone marrow graft	41 (8)	36 (4)		0·53 (0·16–1·77)	0·30
Peripheral blood graft	80 (13)	88 (6)		0·43 (0·16–1·13)	0·088
Cumulative incidence of relapse					
All patients	121 (26)	124 (30)		1·09 (0·64–1·84)	0·75
Sex					
Male	66 (13)	70 (21)		1·52 (0·76–3·04)	0·23
Female	55 (13)	54 (9)		0·65 (0·28–1·53)	0·33
Age					
<51 years	63 (16)	61 (16)		1·01 (0·50–2·01)	0·98
≥51 years	58 (10)	63 (14)		1·23 (0·55–2·78)	0·61
Disease status					
First complete remission	102 (19)	107 (24)		1·19 (0·65–2·17)	0·57
Second or further remission	19 (7)	17 (6)		0·87 (0·29–2·58)	0·80
ELN risk					
Good/Intermediate 1	75 (15)	75 (20)		1·37 (0·70–2·68)	0·36
Intermediate 2/Adverse	46 (11)	49 (10)		0·72 (0·31–1·70)	0·46
HCT-CI					
0	67 (15)	71 (18)		1·18 (0·59–2·34)	0·64
1–2	40 (8)	26 (6)		1·02 (0·35–2·94)	0·97
≥3	14 (3)	27 (6)		0·91 (0·23–3·64)	0·89
Donor type					
Related donor	55 (12)	57 (12)		0·93 (0·42–2·07)	0·86
Unrelated donor	66 (14)	67 (18)		1·23 (0·61–2·48)	0·56
Graft source					
Bone marrow graft	41 (12)	36 (7)		0·58 (0·23–1·47)	0·25
Peripheral blood graft	80 (14)	88 (23)		1·52 (0·78–2·95)	0·22

0·03 0·06 0·12 0·25 0·50 1·00 2·50 5·00

← Favours busulfan plus fludarabine   Favours busulfan plus cyclophosphamide →

Figure 4: **Non-relapse mortality and cumulative incidence of relapse at 1 year after transplantation**
HRs and 95% CIs are calculated from Cox regression models. The dotted line represents the point estimated for non-relapse mortality and cumulative incidence of relapse for the per-protocol population. HR=hazard ratio. ELN=European Leukaemia Network. HCT-CI=Hematopoietic Cell Transplantation-Specific Comorbidity Index.

univariate analysis (data not shown) and multivariate analysis, disease status at transplant was the only factor that significantly affected 1-year cumulative incidence of relapse, overall survival, and leukaemia-free survival, whereas the conditioning regimen was the only factor that significantly affected non-relapse mortality at 1 year (table 3).

Median time to neutrophil engraftment was 16 days (IQR 14–18) in the busulfan plus cyclophosphamide group and 17 days (15–20) in the busulfan plus fludarabine group (Gray's test p=0·55). Median time to platelet engraftment was 20 days (15–25) for patients given busulfan plus cyclophosphamide and 17 days (14–21) for patients given busulfan plus fludarabine (Gray's test p=0·002; appendix). Two graft failures and two graft rejections occurred in patients receiving busulfan plus cyclophosphamide, whereas one graft rejection occurred in the busulfan plus fludarabine

group. No difference in haemopoietic chimerism existed between unfractionated mononuclear cells obtained from bone marrow and peripheral blood in either treatment group. However, when we compared the haemopoietic chimerism of purified peripheral blood T lymphocytes at early timepoints (days 30 and 60), full donor chimerism was significantly higher in the busulfan plus cyclophosphamide group (30/36 [83%] at 30 days; 29/36 [81%] at 60 days) than in the busulfan plus fludarabine group (16/38 [42%] at 30 days, p=0·00026; 23/40 [58%] at 60 days, p=0·031). A progressive increase in T-lymphocyte donor chimerism gradually developed at later timepoints in the busulfan plus fludarabine group, at days 100, 180, and 365 after transplantation (appendix). Despite the early delay in the achievement of full donor T lymphocyte chimerism in the busulfan plus fludarabine group, the number of donor lymphocyte infusions was not different between study groups (18 in the busulfan

	1-year non-relapse mortality			1-year cumulative incidence of relapse			1-year overall survival			1-year leukaemia-free survival		
	Events (%)	HR (95% CI)	p value	Events (%)	HR (95% CI)	p value	Events (%)	HR (95% CI)	p value	Events (%)	HR (95% CI)	p value
Regimen												
Busulfan plus cyclophosphamide (n=121)	21 (17%)	1·0	..	26 (21%)	1·0	..	35 (29%)	1·0	..	47 (39%)	1·0	..
Busulfan plus fludarabine (n=124)	10 (8%)	0·42 (0·19–0·91)	0·027	30 (24%)	1·09 (0·64–1·86)	0·75	27 (22%)	0·69 (0·41–1·15)	0·15	40 (32%)	0·79 (0·51–1·21)	0·28
Age (years)												
<51 (n=124)	12 (10%)	1·0	..	32 (26%)	1·0	..	28 (23%)	1·0	..	44 (36%)	1·0	..
≥51 (n=121)	19 (16%)	1·57 (0·76–3·26)	0·22	24 (20%)	0·81 (0·48–1·38)	0·44	34 (28%)	1·30 (0·79–2·16)	0·30	43 (36%)	1·03 (0·67–1·57)	0·91
Disease status at allogeneic stem-cell transplantation												
First complete remission (n=209)	25 (12%)	1·0	..	43 (21%)	1·0	..	47 (23%)	1·0	..	68 (33%)	1·0	..
Second or further complete remission (n=36)	6 (17%)	1·46 (0·58–3·69)	0·42	13 (36%)	2·08 (1·07–4·02)	0·030	15 (42%)	1·91 (1·03–3·53)	0·039	19 (53%)	1·81 (1·06–3·09)	0·031
ELN risk												
Good/intermediate 1 (n=150)	17 (11%)	1·0	..	35 (23%)	1·0	..	39 (26%)	1·0	..	52 (35%)	1·0	..
Intermediate 2/adverse (n=95)	14 (15%)	1·32 (0·64–2·71)	0·46	21 (22%)	0·98 (0·56–1·70)	0·94	23 (24%)	0·99 (0·58–1·68)	0·96	35 (37%)	1·10 (0·71–1·70)	0·68
HCT-CI												
0 (n=138)	12 (9%)	1·0	..	33 (24%)	1·0	..	30 (22%)	1·0	..	45 (33%)	1·0	..
1–2 (n=66)	12 (18%)	1·76 (0·78–3·97)	0·17	14 (21%)	0·82 (0·43–1·56)	0·54	18 (27%)	1·13 (0·63–2·05)	0·68	26 (39%)	1·10 (0·67–1·79)	0·72
≥3 (n=41)	7 (17%)	2·25 (0·86–5·90)	0·10	9 (22%)	0·96 (0·45–2·03)	0·91	14 (34%)	1·72 (0·90–3·30)	0·10	16 (39%)	1·29 (0·72–2·31)	0·40
Donor type												
Related (n=112)	10 (10%)	1·0	..	24 (21%)	1·0	..	22 (20%)	1·0	..	34 (30%)	1·0	..
Unrelated (n=133)	21 (16%)	1·53 (0·71–3·34)	0·28	32 (24%)	1·09 (0·62–1·90)	0·76	40 (30%)	1·37 (0·80–2·35)	0·26	53 (40%)	1·21 (0·77–1·90)	0·40

Baseline characteristics that are not included in this table were analysed only in univariate analysis. HR=hazard ratio. ELN=European Leukemia Network. HCT-CI=Hematopoietic Cell Transplantation-Specific Comorbidity Index.

Table 3: Prognostic factors for outcomes at 1 year after transplantation

	Busulfan plus cyclophosphamide (n=121)				Busulfan plus fludarabine (n=124)			
	Grade 1–2	Grade 3	Grade 4	Grade 5	Grade 1–2	Grade 3	Grade 4	Grade 5
Infection	58 (48%)	13 (11%)	4 (3%)	8 (7%)	43 (35%)	6 (5%)	0	7 (6%)
Gastrointestinal	27 (22%)	27 (22%)	0	1 (<1%)	19 (15%)	26 (21%)	0	0
Metabolic/laboratory	26 (21%)	8 (7%)	1 (<1%)	0	18 (15%)	2 (2%)	0	0
Pulmonary/upper respiratory	16 (13%)	8 (7%)	1 (<1%)	4 (3%)	7 (6%)	2 (2%)	1 (<1%)	0
Cardiac arrhythmia/cardiac general	10 (8%)	1 (<1%)	0	3 (2%)	8 (6%)	3 (2%)	0	0
Neurology	10 (8%)	3 (2%)	1 (<1%)	0	5 (4%)	1 (<1%)	0	0
Constitutional symptoms*	10 (8%)	1 (<1%)	0	0	3 (2%)	2 (2%)	0	0
Allergy/immunology	9 (7%)	1 (<1%)	0	0	2 (2%)	0	0	0
Blood/bone marrow	7 (6%)	13 (11%)	3 (2%)	0	7 (6%)	8 (6%)	1 (<1%)	1 (<1%)
Dermatology/skin	7 (6%)	1 (<1%)	0	0	5 (4%)	0	0	0
Renal/genitourinary	7 (6%)	3 (2%)	1 (<1%)	0	3 (2%)	0	0	0
Musculoskeletal/soft tissue	6 (5%)	3 (2%)	0	0	4 (3%)	0	0	0
Haemorrhage/bleeding	6 (5%)	2 (2%)	0	1 (<1%)	4 (3%)	0	0	1 (<1%)
Endocrine	5 (4%)	1 (<1%)	0	0	2 (2%)	1 (<1%)	0	0
Hepatobiliary/pancreas	2 (2%)	1 (<1%)	0	0	4 (3%)	1 (<1%)	0	0
Ocular/visual	2 (2%)	1 (<1%)	0	0	3 (2%)	1 (<1%)	0	0
Pain	2 (2%)	3 (2%)	0	0	1 (<1%)	0	0	0
Vascular	2 (2%)	2 (2%)	0	0	1 (<1%)	0	0	0
Coagulation	1 (<1%)	0	2 (2%)	0	0	0	0	0
Secondary malignancy	0	0	0	0	5 (4%)	3 (2%)	0	0
Multi-organ failure	0	0	0	2 (2%)	0	0	0	1 (<1%)

Data are number of patients (%). *Includes general symptoms of fever, fatigue, and insomnia. Only grade 1–2 adverse events that occurred in more than 10% of patients in any group are reported, whereas all grade 3, 4, and 5 adverse events that occurred after treatment allocation are reported.

Table 4: Adverse events

plus cyclophosphamide group *vs* 14 in the busulfan plus fludarabine group, χ² p=0·41).

At least one grade 3 or worse adverse event was reported for 62 (51%) of 121 patients in the busulfan plus cyclophosphamide group and 46 (37%) of 124 patients in the busulfan plus fludarabine group (table 4). The most frequently reported grade 3 or higher adverse events were gastrointestinal events (28 [23%] patients in the busulfan plus cyclophosphamide group *vs* 26 [21%] patients in the busulfan plus fludarabine group) and infections (21 [17%] patients in the busulfan plus cyclophosphamide group and 13 [10%] patients in the busulfan plus fludarabine group had at least one such event). Adverse events with an outcome of death were reported for 19 (16%) patients in the busulfan plus cyclophosphamide group and 10 (8%) in the busulfan plus fludarabine group. Eight secondary malignancies were diagnosed in six patients, all of whom received busulfan plus fludarabine (table 4). The most common conditioning regimen-related toxic effects assessed shortly (within 28 days) after transplantation are shown in table 5. Four patients (two in each treatment group) had a diagnosis of venous occlusive disease. The 100-day cumulative incidence of grade II–IV acute graft-versus-host disease was 28·1% (95% CI 21·1–37·4) in the busulfan plus cyclophosphamide group versus 19·4% (13·5–27·8) in

	Busulfan plus cyclophosphamide (n=121)			Busulfan plus fludarabine (n=124)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
Mucosa	8 (7%)	23 (19%)	1 (<1%)	11 (9%)	23 (19%)	1 (<1%)
Liver	9 (7%)	5 (4%)	0	4 (3%)	1 (<1%)	1 (<1%)
Gut	7 (6%)	4 (3%)	2 (2%)	3 (2%)	1 (<1%)	0
Bladder	0	3 (2%)	2 (2%)	0	0	0
Heart	1 (<1%)	2 (2%)	1 (<1%)	1 (<1%)	0	0
Kidney	2 (2%)	0	0	2 (2%)	0	0
Central nervous system	0	1 (<1%)	1 (<1%)	0	2 (2%)	0
Lung	1 (<1%)	0	0	0	1 (<1%)	0

Data are number of patients (%). Toxic effects were judged with Bearman's criteria.[18]

Table 5: Regimen-related toxic effects by organ system within 28 days after transplantation in the per-protocol population

the busulfan plus fludarabine group (Gray's test p=0·12), whereas grade III–IV acute graft-versus-host disease was significantly more common in the busulfan plus cyclophosphamide group (12 [10%] patients) than in the busulfan plus fludarabine group (three [2%] patients; Gray's test p=0·014; appendix). At 1 year, the cumulative incidence of chronic graft-versus-host disease was 27·5% (20·6–36·9) in the busulfan plus cyclophosphamide group and 25·8% (19·1–34·8) in the busulfan plus fludarabine group.

Discussion

In this study, the combination of busulfan plus fludarabine, compared with busulfan plus cyclophosphamide, was associated with significantly lower non-relapse mortality for older (median age 51 years) patients with acute myeloid leukaemia who underwent allogeneic haemopoietic stem-cell transplant from an HLA-matched related or unrelated donor. Non-relapse mortality in patients receiving the busulfan plus fludarabine regimen was lower in all subgroups analysed compared with that for patients in the busulfan plus cyclophosphamide group, especially for patients in their first complete remission and in those with an HCT-CI of 3 or greater, and was independent of patient age and sex, donor type, graft type, and acute myeloid leukaemia risk biology. Chemotherapy-related organ toxic effects were less common in patients treated with the busulfan plus fludarabine regimen than those treated with the busulfan plus cyclophosphamide conditioning; the primary endpoint of the study was probably met because of the reduced organ toxicity of the busulfan plus fludarabine regimen. Fludarabine has been postulated to be associated with reduced toxic effects in several organs, including the heart, lungs, and liver, while the myeloablative dose of busulfan was predicted to preserve the antileukaemic effect of the regimen. Most importantly, the reduced non-relapse mortality did not come at the cost of a significantly increased incidence of relapse, which was similar between the busulfan plus fludarabine and busulfan plus cyclophosphamide groups, both overall and in subgroup analysis.

Our results concur with those reported in other clinical trials. In a phase 2 study,[21] patients aged 50–70 years were given transplants for myeloid malignancies after a busulfan plus fludarabine myeloablative conditioning regimen. For patients in complete remission at the time of the transplant, 1-year transplant-related mortality was 19%, with a 2-year event-free survival of 71% and an overall survival of 68%. Additionally, in a randomised clinical trial[17] done in younger (median age of 30 years) patients with acute myeloid leukaemia, non-relapse mortality at 5 years was lower in patients treated with busulfan plus fludarabine (10%) compared with those given busulfan plus cyclophosphamide (19%). As in our study, the overall incidence of relapse was superimposable between the study groups and, although not significant, the patients who received fludarabine had improved disease-free survival and overall survival compared with those who received cyclophosphamide.

The lower non-relapse mortality at 1 year in favour of the busulfan plus fludarabine group was maintained up to 5 years. Therefore, the reduction of the antileukaemic power of a conditioning regimen should be carefully assessed for patients with acute myeloid leukaemia up to the age of 65 years. Notably, the lower transplant-related mortality is not a consequence of poor performance in the control group. In fact, the busulfan plus cyclophosphamide regimen was better tolerated than initially postulated when the trial was designed and non-relapse mortality in this group was similar to that reported in large international non-randomised studies[4–6] that were done in patients of similar or younger median age with myeloid malignancies. The overall good performance of both treatment groups is emphasised by three further findings. First, most patients had intermediate-risk or high-risk acute myeloid leukaemia and only six patients with a favourable cytogenetic profile had received transplants in their first remission. In this respect, our results are similar to those reported in a comparable set of older patients with acute myeloid leukaemia who were enrolled into studies in the HOVON-SAKK analysis.[22] Second, patients receiving transplants from related or unrelated donors were equally represented in both treatment groups and no difference existed in the treatment outcome according to stem cell source or donor type. Our results also support the notion that transplantations from matched related donors and matched unrelated donors produce similar outcomes for patients with acute myeloid leukaemia.[23] All transplants from unrelated donors in our study included an intermediate dose of antithymocyte immunoglobulin as part of the conditioning regimen and this did not translate into any significant increase of relapse or transplant-related complications. Third, the incidence of venous occlusive disease was very low in both treatment arms: the reduction of this life-threatening complication is probably related to the use of an intravenous formulation of busulfan.[24] Importantly, after busulfan plus fludarabine was given to patients, the speed and robustness of haematological engraftment was similar to, if not better than, that observed in the control group, despite the fact that full T-cell engraftment was delayed. This finding did not translate into an increase in early or late graft failures and we speculate that the significantly reduced incidence of grade III–IV acute graft-versus-host disease reported in the busulfan plus fludarabine group might be at least partly related to the kinetics of T-cell reconstitution in this group.

Several studies[25–27] have reported that reduction of the myeloablative component of chemotherapy-based conditioning regimens is associated with a significant increase in relapse incidence, although in a recent randomised trial,[28] no difference in relapse was detected in a comparison of total body irradiation-based conditioning regimens (8 Gy vs 12 Gy) for patients with acute myeloid leukaemia. A phase 3 study (ClinicalTrials.gov, number NCT01339910) by the Bone Marrow Transplant Clinical Trials network to compare myeloablative conditioning with reduced intensity conditioning in patients with acute myeloid leukaemia and myelodysplastic syndrome, aged 18–65 years, was closed prematurely because of an excess of relapse in the reduced intensity programme.

Our results contrast with those published by Lee and colleagues[16] who compared a busulfan plus fludarabine

conditioning regimen with busulfan plus cyclo-phosphamide in 126 younger adults (median age 41 years) eligible for myeloablative conditioning. Although severe (grade 3 or higher) infection and gastrointestinal adverse events were significantly more common in the busulfan plus cyclophosphamide group, the frequency of hepatic adverse events was similar between the two groups. Moreover, overall non-relapse mortality was similar between the two groups and the busulfan plus cyclophosphamide group had improved 2-year overall survival and event-free survival compared with the busulfan plus fludarabine group. Beyond the substantial difference in the median age of the patients in this study and ours, we must emphasise the fact that Lee and colleagues' study had a smaller number of patients and seemed to lack a formal sample size calculation. Furthermore, only 70 of 126 patients in that study had a diagnosis of acute myeloid leukaemia.

Our study has several limitations that should be taken into consideration. Most patients were in their first complete remission and it is possible that before being randomly assigned to a myeloablative conditioning regimen they had been selected for enrolment on the basis of a favourable HCT-CI score and good performance status.[29] Accordingly, the generalisability of our results should be critically assessed when decisions are made about the conditioning regimen for older patients with acute myeloid leukaemia, especially when severe comorbidities or poor performance status are present. Additionally, we recognise that for patients who relapse after transplantation, the outcome was better in the busulfan plus cyclophosphamide group than in the busulfan plus fludarabine group. The reduced success of the rescue treatment for disease relapse after busulfan plus fludarabine treatment might be a matter of chance, but alternative explanations include the possible selection of more aggressive leukaemic cell clones that have survived a purine analogue-containing chemo-therapy and the increased incidence of infectious complications. Finally, although non-relapse mortality was lower in the busulfan plus fludarabine group than in the busulfan plus cyclophosphamide group, overall survival was not different between the groups, which emphasises the fact that leukaemia relapse after transplantation remains an unmet clinical need for patients with acute myeloid leukaemia. For this reason, innovative post-engraftment treatments with cells or drugs should now be regarded as an integral part of the allogeneic stem-cell transplantation platform, and clinical trials that have been appropriately designed to address the efficacy and toxic effects of such new approaches are needed.[30]

Our results support myeloablative busulfan plus fludarabine as a highly effective conditioning regimen for patients aged 40–65 years with acute myeloid leukaemia, conferring lower transplant-related mortality than with standard treatment. These results might be useful in the planning of allogeneic transplants for older patients with acute myeloid leukaemia.

Contributors
AR designed the clinical trial. AR, AG, AM, ABa, and AN wrote the manuscript. AM, RMM, and EO were in charge of the collection and management of data. RMM and CB did the statistical analysis. AG, MCM, ABu, BB, IC, SS, RR, MM, GMi, PCh, DP, SG, FP, AMR, GS, MP, ET, WA, GMa, AMC, AN, DR, PCo, EPA, GFT, RS, NM, EO, ABa, and ABo enrolled and managed patients enrolled into this trial. All authors reviewed and approved the final draft.

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