Addition of sirolimus to standard cyclosporine plus mycophenolate mofetil-based graft-versus-host disease prophylaxis for patients after unrelated non-myeloablative haemopoietic stem cell transplantation: a multicentre, randomised, phase 3 trial

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Summary

Background Acute graft-versus-host-disease (GVHD) after non-myeloablative human leucocyte antigen (HLA)-matched, unrelated donor, allogeneic haemopoietic stem cell transplantation (HSCT) is associated with considerable morbidity and mortality. This trial aimed to evaluate the efficacy of adding sirolimus to the standard cyclosporine and mycophenolate mofetil prophylaxis therapy for preventing acute GVHD in this setting.

Methods This multicentre, randomised, phase 3 trial took place at nine HSCT centres based in the USA, Denmark, and Germany. Eligible patients were diagnosed with advanced haematological malignancies treatable by allogeneic HSCT, had a Karnofsky score greater than or equal to 60, were aged older than 50 years, or if they were aged 50 years or younger, were considered at high risk of regimen-related toxicity associated with a high-dose pre-transplantation conditioning regimen. Patients were randomly allocated by an adaptive randomisation scheme stratified by transplantation centre to receive either the standard GVHD prophylaxis regimen (cyclosporine and mycophenolate mofetil) or the triple-drug combination regimen (cyclosporine, mycophenolate mofetil, and sirolimus). Patients and physicians were not masked to treatment. All patients were prepared for HSCT with fludarabine (30 mg/m² per day) 4, 3, and 2 days before receiving 2 or 3 Gy total body irradiation on the day of HSCT (day 0). In both study groups, 5·0 mg/kg of cyclosporine was administered orally twice daily starting 3 days before HSCT, and (in the absence of GVHD) tapered from day 96 through to day 150. In the standard GVHD prophylaxis group, 15 mg/kg of mycophenolate mofetil was given orally three times daily from day 0 until day 30, then twice daily until day 150, and (in the absence of GVHD) tapered off by day 180. In the triple-drug group, mycophenolate mofetil doses were the same as in the standard group, but the drug was discontinued on day 40. Sirolimus was started 3 days before HSCT, taken orally at 2 mg once daily and adjusted to maintain trough concentrations between 3–12 ng/mL through to day 150, and (in the absence of GVHD) tapered off by day 180. The primary endpoint was the cumulative incidence of grade 2–4 acute GVHD at day 100 post-transplantation. Secondary endpoints were non-relapse mortality, overall survival, progression-free survival, cumulative incidence of grade 3–4 acute GVHD, and cumulative incidence of chronic GVHD. Efficacy and safety analyses were per protocol, including all patients who received conditioning treatment and underwent transplantation. Toxic effects were measured according to the Common Terminology Criteria for Adverse Events (CTCAE). The current study was closed prematurely by recommendation of the Data and Safety Monitoring Board on July 27, 2016, after 168 patients received the allocated intervention, based on the results of a prespecified interim analysis for futility. This study is registered with ClinicalTrials.gov, number NCT01231412.

Findings Participants were recruited between Nov 1, 2010, and July 27, 2016. Of 180 patients enrolled in the study, 167 received the complete study intervention and were included in safety and efficacy analyses: 77 patients in the standard GVHD prophylaxis group and 90 in the triple-drug group. At the time of analysis, median follow-up was 48 months (IQR 31–60). The cumulative incidence of grade 2–4 acute GVHD at day 100 was lower in the triple-drug group compared with the standard GVHD prophylaxis group (26% [95% CI 17–35] in the triple-drug group *vs* 52% [41–63] in the standard group; HR 0·45 [95% CI 0·28–0·73]; p=0·0013). After 1 and 4 years, non-relapse mortality increased to 4% (95% CI 0–9) and 16% (8–24) in the triple-drug group and 16% (8–24) and 32% (21–43) in the standard group (HR 0·48 [0·26–0·90]; p=0·021). Overall survival at 1 year was 86% (95% CI 78–93) in the triple-drug group and 70% in the standard group (60–80) and at 4 years it was 64% in the triple-drug group (54–75) and 46% in the standard group (34–57%; HR 0·62 [0·40–0·97]; p=0·035). Progression-free survival at 1 year was 77% (95% CI 68–85) in the triple-drug group and 64% (53–74) in the standard drug group, and at 4 years it was 59% in the triple-drug group (49–70) and 41% in the standard group (30–53%; HR 0·64 [0·42–0·99]; p=0·045). We observed no difference in the cumulative incidence of grade 3–4 acute GVHD (2% [0–5] in the triple-drug group *vs* 8% [2–14] in the standard group; HR 0·55 [0·16–1·96]; p=0·36) and

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chronic GVHD (49% [39–59] in triple-drug group *vs* 50% [39–61] in the standard group; HR 0·94 [0·62–1·40]; p=0·74). In both groups the most common CTCAE grade 4 or higher toxic effects were pulmonary.

Interpretation **Adding sirolimus to cyclosporine and mycophenolate mofetil resulted in a significantly lower proportion of patients developing acute GVHD compared with patients treated with cyclosporine and mycophenolate mofetil alone. Based on these results, the combination of cyclosporine, mycophenolate mofetil, and sirolimus has become the new standard GVHD prophylaxis regimen for patients treated with non-myeloablative conditioning and HLA-matched unrelated HSCT at the Fred Hutchinson Cancer Research Center.**

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Introduction

In 1997, we developed a minimum-intensity non-myeloablative regimen consisting of fludarabine and low-dose total body irradiation to condition older or medically unfit patients with haematological malignancies before haemopoietic stem cell transplantation (HSCT) from human leucocyte antigen (HLA)-matched related or unrelated donors.[1,2] This regimen is well tolerated, can be used in the outpatient setting, and relies almost entirely on graft-versus-tumour effects for eradicating the under-lying malignancies. Depending on disease and disease burden, and the extent of comorbidities, 5-year overall survival has ranged from 25% to 60%.[3] In our 2013 study[3] of this regimen, overall 5-year non-relapse mortality among the first 1092 patients was 24·5%, which in large part was associated with or preceded by graft-versus-host disease (GVHD).[3] To enable engraftment and control GVHD, the standard postgrafting immunosuppression after non-myeloablative conditioning combines a calcineurin inhibitor and mycophenolate mofetil, which exert their immunosuppressive effects by selectively blocking cytokine transcription and inhibiting lymphocyte proliferation. Our study[3] showed that with this approach, acute GVHD conveyed no significant graft-versus-tumour effect.

Since rates of acute GVHD were highest among unrelated recipients, we chose the unrelated HSCT setting for attempts to improve acute GVHD prevention by adding sirolimus as a third immunosuppressive agent. The rationale behind adding sirolimus was that its mode of action is different from that of a calcineurin inhibitor and mycophenolate mofetil, namely by blocking cytokine-mediated signal transduction pathways through inhibiting the mammalian target of rapamycin (mTOR). In our 2014 multicentre, randomised phase 2 trial[4] involving 208 unrelated recipients, we compared three different post-transplantation immunosuppressive regimens that were based on a backbone of different calcineurin inhibitor and mycophenolate mofetil schedules, one of which was a triple-drug regimen that included an 80-day course of sirolimus.[4] Results showed a significantly lower incidence of grade 2 acute GVHD, a lower use of systemic steroids, and a reduction in risk of cytomegalovirus (CMV) reactivation compared with the standard double-drug regimen, without an increasing the risk of relapse and without differences in the incidence of chronic GVHD.

To confirm our previous findings, we conducted a multicentre phase 3 trial in which unrelated HSCT recipients were randomly assigned to receive standard

Research in context

Evidence before this study

Graft-versus-host disease prophylaxis after human leucocyte antigen-matched unrelated non-myeloablative haemopoietic stem cell transplantation has been developed through successive clinical trials headed by the Fred Hutchinson Cancer Research Center. Different combinations of a calcineurin inhibitor and mycophenolate mofetil have been tested, yielding a 52–77% cumulative incidence of grade 2–4 acute graft-versus-host disease. The results of a phase 2 randomised trial that we recently published have shown that the addition of sirolimus to a combination of mycophenolate mofetil and a calcineurin inhibitor could reduce the cumulative incidence of grade 2–4 acute graft-versus-host disease.

Added value of this study

This phase 3 trial is the latest in a series of randomised trials designed to further investigate the addition of sirolimus to standard graft-versus-host disease prophylaxis with mycophenolate mofetil and a calcineurin inhibitor. This study is the first phase 3 trial showing that combining sirolimus, cyclosporine, and mycophenolate mofetil for acute graft-versus-host disease prophylaxis significantly improves overall survival and progression-free survival, in addition to reducing the cumulative incidence of acute graft-versus-host disease.

Implications of all the available evidence

This phase 3 trial is the latest in a series of randomised trials designed to further investigate the addition of sirolimus to standard graft-versus-host disease prophylaxis with mycophenolate mofetil and a calcineurin inhibitor. In light of the available evidence, our study sets a new standard for graft-versus-host disease prophylaxis after human leucocyte antigen-matched unrelated nonmyeloablative haemopoietic stem cell transplantation.

GVHD prophylaxis consisting of cyclosporine and mycophenolate mofetil, or a triple-drug combination of cyclosporine, mycophenolate mofetil, and sirolimus. However, given the late development of acute GVHD following discontinuation of sirolimus on day 80 in our phase 2 trial,[4] we attempted to further exploit the immunomodulatory effects of the drug and extended its period of administration to 180 days in the current trial.

Methods

Study design and participants

This multicentre phase 3 trial took place at nine HSCT centres (appendix p 8) based in the USA, Denmark, and Germany. The study was designed in accordance with the CONSORT 2010 Statement[5] and the protocol is available online. The initial study was designed in September, 2010, as a phase 2 trial with three study groups: a standard group with mycophenolate mofetil and cyclosporine, a group with sirolimus added to mycophenolate mofetil and cyclosporine, and a group with cyclosporine and sirolimus. However, an external review board recommended a two-group definitive phase 3 design and the protocol was modified on August 23, 2011, to revise primary and secondary endpoints, sample size and study power, and remove some prespecified stopping rules. Before this change was implemented, six patients had been enrolled in the cyclosporine and sirolimus group. These patients have not been included in the analyses presented.

Eligible patients were diagnosed with advanced haematological malignancies treatable by allogeneic HSCT, had a Karnofsky score greater than or equal to 60, were aged older than 50 years, or if they were aged 50 years or younger, were considered at high risk of regimen-related toxicity associated with a high-dose pre-transplantation conditioning regimen (>40% risk of non-relapse mortality predicted by the haemopoietic cell transplant comorbidity index). Permitted diseases and complete eligibility criteria are described in full in the appendix (pp 2–3).

Donors were unrelated, high-resolution matched for HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 at the allele level or mismatched at no more than a single-allele disparity for either HLA-A, HLA-B, or HLA-C. Patients and donors were not routinely typed for HLA-DP at all centres. Granulocyte colony stimulating factor mobilised blood cells were the sole graft source.

The study was approved by the Institutional Review Boards (IRBs) at the Fred Hutchinson Cancer Research Center (which acted as the coordinating centre) and at each of the collaborating centers. All patients signed IRB-approved consent forms before enrolment.

Randomisation

Patients were allocated to the two study groups by an adaptive randomisation scheme stratified by transplantation centre.[6] Patients were enrolled by study staff at participating sites after confirming eligibility with the coordinating center. Randomisation for all sites was done by the clinical statistics department at the Fred Hutchinson Cancer Research Center. Patients and physicians were not masked to treatment.

Procedures

All patients were prepared for HSCT with fludarabine (30 mg/m² per day) 4, 3, and 2 days before receiving 2 Gy total body irradiation on the day of HSCT (day 0). Patients who had no previous myelosuppressive chemotherapy, no myelosuppressive chemotherapy within 3–6 months before entering the trial, or a previous allogeneic HSCT with more than 5% CD3 chimerism from the first donor received 3 Gy total body irradiation. Peripheral blood stem cell donor grafts were collected from donors by National Marrow Donor Program standards after 5 days of subcutaneously administered granulocyte colony stimulating factor at a dose of approximately 10 µg/kg. Patients were randomly assigned to standard GVHD prophylaxis with cyclosporine and mycophenolate mofetil, or the triple-drug combination of cyclosporine, mycophenolate mofetil, and sirolimus. In both study groups, 5·0 mg/kg of cyclosporine was administered orally twice daily starting 3 days before HSCT, and (in the absence of GVHD) tapered from day 96 through to day 150. In the standard group, cyclosporine trough concentrations were targeted at 400 ng/mL for the first 28 days and thereafter between 150–350 ng/mL until taper. In the triple-drug group, cyclosporine trough concentrations were targeted at 350 ng/mL for the first 28 days and thereafter at 120–300 ng/mL until taper. In the standard group, 15 mg/kg of mycophenolate mofetil was given orally three times daily from day 0 until day 30, then twice daily until day 150, and (in the absence of GVHD) tapered off by day 180. In the triple-drug group, mycophenolate mofetil doses were the same as in the standard group, but the drug was discontinued on day 40. Sirolimus was started three days before HSCT, taken orally at 2 mg once daily and adjusted to maintain trough concentrations between 3–12 ng/mL through to day 150, and (in the absence of GVHD) tapered off by day 180.

Supportive care, antibiotic prophylaxis, and detection and treatment of CMV reactivation were provided according to local institutional guidelines. Diagnosis, clinical grading, and treatment of acute and chronic GVHD were performed by local investigators according to established criteria.[7,8]

Toxic effects were measured according to the Common Terminology Criteria for Adverse Events (CTCAE; version 4.0). All adverse events that were considered unexpected and related or possibly related to the research (by the principal investigator) and serious or suggesting that research participants were at greater risk of physical or psychological harm, were reported to the IRB within 10 calendar days of investigators becoming aware of the event.

See Online for appendix

For the **protocol** see https://clinicaltrials.gov/ProvidedDocs/12/NCT01231412/Prot_SAP_000.pdf

For more on the **National Marrow Donor Program** see https://bethematch.org

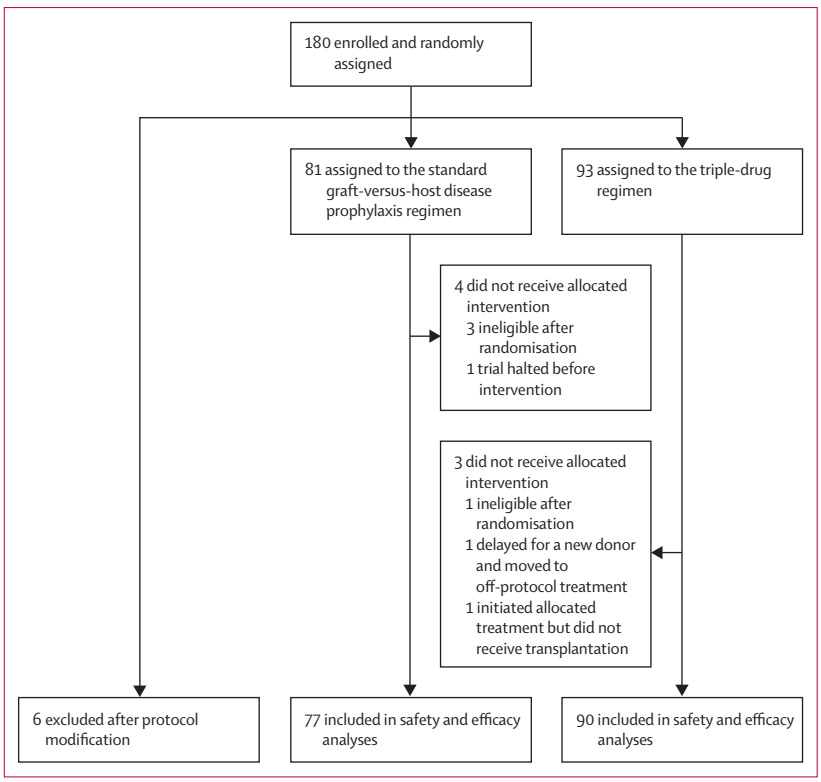

Figure 1: **Trial profile**

Outcomes

The primary objective of the trial was to compare the efficacy of the two prophylactic regimens in reducing the risk of grade 2–4 acute GVHD after HLA-matched unrelated non-myeloablative conditioning HSCT. Efficacy was measured as the cumulative incidence of grade 2–4 acute GVHD at day 100 post-transplant.

Secondary endpoints were non-relapse mortality (time from transplantation to death without progression or relapse of the malignant disease), overall survival (time from transplantation to death from any cause), progression-free survival (time from transplantation to progression of the malignant disease or death from any cause), cumulative incidence of grade 3–4 acute GVHD, and cumulative incidence of chronic GVHD. Exploratory outcomes were chimerism tests, engraftment, acute GVHD organ involvement, incidence of corticosteroid treatment, and infections.

Statistical analysis

The original protocol was approved on Nov 1, 2010. The protocol was modified on August 23, 2011, to change the study design. After the protocol modification the accrual goal was 300 patients, based on 150 patients per group providing 94% power for a difference of 20% (and 74% power for a difference of 15%) in the primary endpoint, at the two-sided 0·05 level of significance. However, the study was closed prematurely by recommendation of the Data and Safety Monitoring Board on July 27, 2016, after 168 patients received the allocated intervention, based on the results of a prespecified interim analysis for futility. The interim futility analysis was designed to stop the trial if the estimated power to show a significant difference between groups for the primary endpoint was less than 33%, given the results at the time and assuming a 15% true difference. Results were analysed on July 1, 2018.

Analyses were done per protocol; all randomly assigned patients who received conditioning and HSCT were included in the efficacy and safety analyses.

Overall survival and progression-free survival were estimated with Kaplan-Meier analyses. Cumulative incidence methods were used to estimate rates of endpoints subject to competing risks.[9] Death was treated as a competing risk for all endpoints; relapse was treated as a competing risk for non-relapse mortality, acute GVHD, and withdrawal of immunosuppression. GHVD was censored for donor lymphocyte infusion. Cox regression was used for all time-to-event endpoints, with competing risks analysis based on event-specific hazard ratios (HRs). All cited p values for time-to-event endpoints refer to HR analyses over the entire period of follow-up. Comparisons of GVHD organ stage and toxic effects were done with a χ^2 test. Comparisons of donor chimerism and peripheral blood counts were done with Wilcoxon rank sum test. All p values are two-sided and without adjustment for multiple comparisons.

All statistical analyses were done with SAS (version 8; Cary, NC, USA). The study is registered with ClinicalTrials.gov, number NCT01231412.

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 corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. BMS, BK, BES, DGM, and RS had access to the raw data.

Results

Participants were recruited between Nov 1, 2010, and July 27, 2016. 180 patients were enrolled and randomly assigned to study treatment. Six patients were excluded after the protocol modification, and four patients in the standard GVHD prophylaxis group and two in the triple-drug group did not receive their allocated treatment. One additional patient in the triple-drug group initiated their allocated treatment, but was excluded from the safety and efficacy analyses after the transplantation was cancelled during conditioning because of complications (bacteraemia and absence of caregiver). 77 patients in the standard GVHD prophylaxis group and 90 in the triple-drug group were included in safety and efficacy analyses (figure 1). Pre-transplantation demographics were evenly distributed among groups, except for

previous transplantations; 23 (30%) patients in the standard group had one previous HSCT compared with 13 (14%) in the triple-drug group (table 1). Before entering the trial, 30 (18%) of 168 patients receiving their allocated treatment had one previous autologous high-dose HSCT. Eight (62%) of the 13 patients with multiple myeloma had one previous autologous high-dose HSCT, one (8%) patient had two, and one (8%) had three. Median follow-up at the time of analysis was 48 months (IQR 31–60).

Except for two rejections in the standard group, all patients had sustained engraftment. One of the two patients had a previous allogeneic HSCT for chronic lymphocytic leukaemia which had been unsuccessful, and rejected the second graft at day 28. The other patient had acute myeloid leukaemia with a second complete remission and rejected the graft at day 158. Near-complete median granulocyte chimerism (95% [IQR 94–100] in triple-drug group *vs* 98% [95–100] in standard group; p=0·12; appendix p 5) and natural killer cell donor chimerism (97% [94–100] in triple-drug group *vs* 95% [88–100] in standard group; p=0·20; data not shown) was achieved in both study groups by day 28. Median donor T-cell chimerism was lower in the triple-drug group on day 28 (79% [59–89] in triple-drug group *vs* 84% [75–95] in standard group; p=0·030; appendix p 5). Among patients surviving to day 100, median donor T-cell chimerism at the last available measurement was lower in the triple-drug group (89% [72–94] in triple-drug group *vs* 93% [76–99] in standard group; p=0·026). 20 (24%) of 84 patients in the triple-drug group and 28 (41%) of 68 patients in the standard group had more than 95% donor T-cell chimerism (p=0·022). Absolute neutrophil counts and platelet count nadirs were similar in both study groups (appendix p 9), while the median number of days with absolute neutrophil count below 500 cells per µL was higher in the triple-drug group (13 days [IQR 8–15] in triple-drug group *vs* 10 days [5–14] in standard group; p=0·033).

Four (5%) of 77 patients in the standard group received donor leucocyte infusion for low donor chimerism, without developing subsequent GVHD. Three (3%) of 90 patients in the triple-drug group received donor leucocyte infusion, two for relapsed acute myeloid leukaemia and non-Hodgkin lymphoma and one for low donor chimerism. The patient in the triple-drug group with low donor chimerism developed acute GVHD after the second donor leucocyte infusion. No patients were on immunosuppressive treatment at the time of donor leucocyte infusion.

The cumulative incidence of grade 2–4 acute GVHD at day 100 was 26% (95% CI 17–35) in the triple-drug group compared with 52% (41–63) in the standard group (HR 0·45 [95% CI 0·28–0·73]; p=0·0013; figure 2). Three patients developed acute GVHD after day 100. Corresponding values for grade 3–4 acute GVHD cumulative incidence were 2% (0–5) in the triple-drug

	Standard regimen group (n=77)	Triple-drug regimen group (n=91)
Age (years)	61 (53–67)	63 (58–68)
Sex		
Female	27 (35%)	28 (31%)
Male	50 (65%)	63 (69%)
Donor age (years)	26 (22–34)	25 (22–35)
Single-allele HLA mismatch	4 (5%)	4 (4%)
Sex of patient/donor		
Male/female	23 (30%)	12 (13%)
Other combinations	54 (70%)	79 (87%)
Previous cytomegalovirus infection of patient/donor		
Negative/negative	16 (21%)	37 (41%)
Negative/positive	8 (10%)	8 (9%)
Positive/negative	27 (35%)	28 (31%)
Positive/positive	26 (34%)	17 (19%)
Previous high-dose haemopoietic cell transplantation		
Autologous	19 (25%)	13 (14%)
Allogeneic	4 (5%)	0
Time from first transplantation (days)	297 (93–1188)	94 (75–552)
Number of previous regimens	3 (2–5)	3 (2–4)
Disease histology		
Acute myeloid leukaemia	25 (32%)	41 (45%)
Myelodysplastic syndrome	14 (18%)	15 (16%)
Chronic myeloid leukaemia	1 (1%)	1 (1%)
Acute lymphoblastic leukaemia	6 (8%)	9 (10%)
Non-Hodgkin lymphoma	12 (16%)	15 (16%)
Chronic lymphocytic leukaemia	9 (12%)	5 (5%)
Hodgkin lymphoma	2 (3%)	0
Multiple myeloma	8 (10%)	5 (4%)
Relapse risk (Kahl)[10]		
Low	20 (26%)	30 (33%)
Standard	47 (61%)	46 (51%)
High	10 (13%)	15 (16%)
Haemopoietic cell transplantation comorbidity index		
0	12 (16%)	13 (14%)
1, 2	24 (32%)	31 (34%)
3+	40 (53%)	47 (52%)
Donor recipient ABO match		
Match	41 (53%)	44 (48%)
Major mismatch	15 (19%)	28 (31%)
Minor mismatch	19 (25%)	18 (20%)
Incomplete data	2 (3%)	1 (1%)
Peripheral blood stem cell dose		
CD34+ cells × 10^6 per kg	8·0 (5·6–10·4)	8·0 (6·0–10·7)
CD3+ cells × 10^8 per kg	3·0 (2·3–4·2)	2·8 (2·2–3·6)
Karnofsky performance status		
100	15 (19%)	21 (23%)
90	34 (44%)	38 (42%)
≤80	28 (36%)	32 (35%)

Data are median (IQR) or n (%).

Table 1: **Baseline characteristics of study population assigned to treatment**

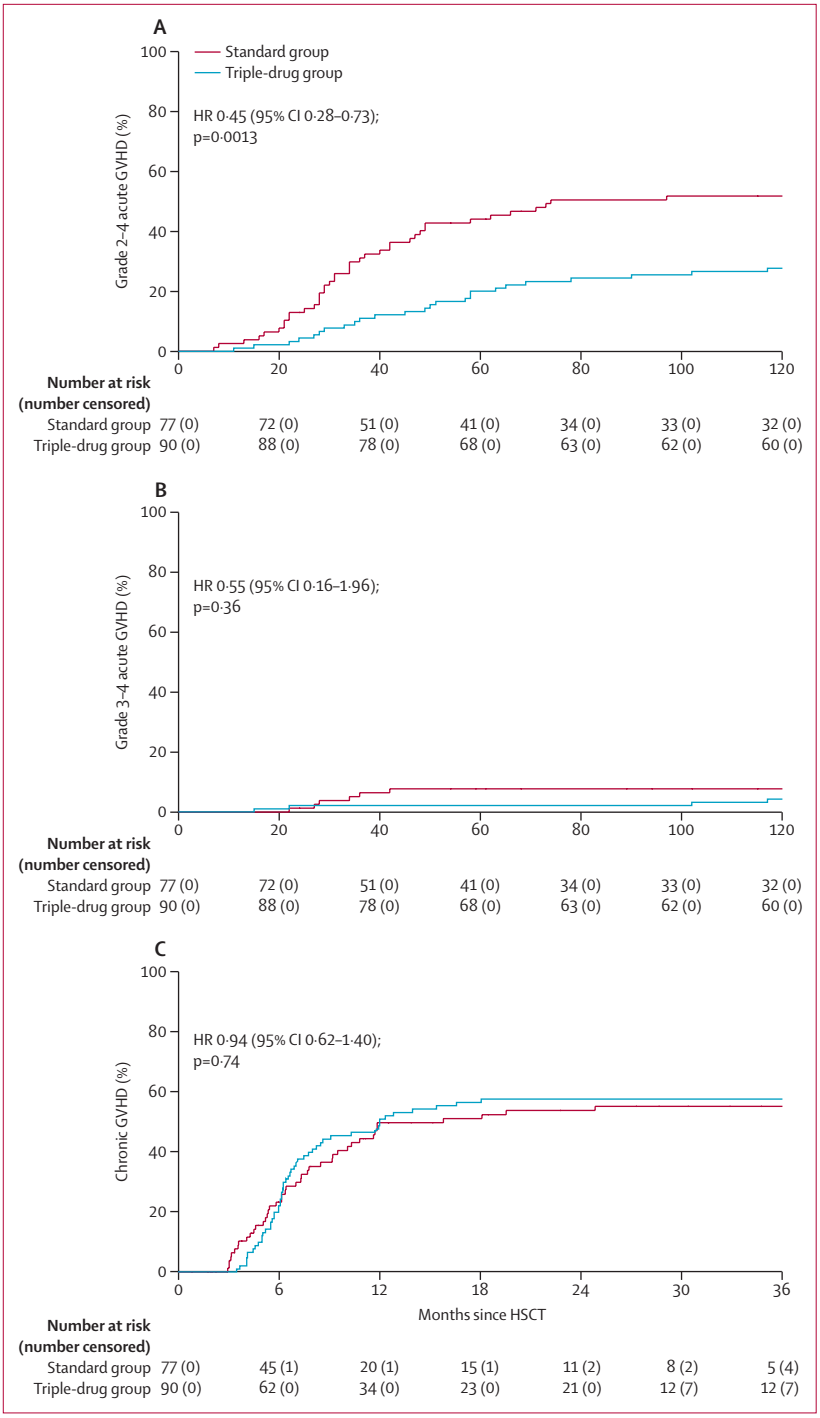

Figure 2: **GVHD**
Cumulative incidence of (A) grade 2–4 acute GVHD, (B) grade 3–4 acute GVHD, and (C) chronic GVHD by treatment group. GVHD=graft-versus-host disease. HSCT=haemopoietic stem cell transplantation.

group compared with 8% (2–14) in the standard group (HR 0·55 [0·16–1·96]; p=0·36; figure 2). Consistent with the difference in acute GVHD, the cumulative incidence of systemic corticosteroid treatment of up to 1 year was 26% (20–38) in the triple-drug group compared with 64% (53–74) in the standard group (HR 0·33 [0·20–0·52]; p<0·0001).

The proportion of patients in the triple-drug group was lower for acute GVHD of skin (18% *vs* 55%; p<0·0001), gut (28% *vs* 38%; p=0·17), and liver (1% *vs* 2%; p=0·65). Acute GVHD organ stages are summarised in the appendix (p 10). Cumulative incidences of chronic GVHD at 1 year were similar in both study groups (49% [95% CI 39–59] in triple-drug group *vs* 50% [39–61] in standard group; HR 0·94 [0·62–1·40]; p=0·74; figure 2), as were the sites of chronic GVHD (data not shown). Patients affected by chronic GVHD in the triple-drug group had a lower non-relapse mortality after 1 year compared with those in the standard group (4% [95% CI 0–9] in triple-drug group *vs* 15% [4–26] in standard group; HR 0·45 [0·19–1·10]; p=0·080; appendix p 6), however this difference was not significant. Chronic GVHD was preceded by acute GVHD in 27 (64%) of 42 patients in the standard group and in 15 (28%) of 53 patients in the triple-drug group. After 3 years, the cumulative incidence of patients off immunosuppressive therapy was 17% (95% CI 9–25) in the triple-drug group compared with 15% (7–23) in the standard group (HR 1·05 [0·52–2·12]; p=0·90).

Non-relapse mortality was 3% (95% CI 0–7) at day 100 in both study groups. After 1 and 4 years, non-relapse mortality increased to 4% (0–9) and 16% (8–24) in the triple-drug group and 16% (8–24) and 32% (21–43) in the standard group (HR 0·48 [0·26–0·90]; p=0·021; figure 3). Overall, 41 (25%) of 167 patients had non-relapse mortality (16 [18%] in triple-drug group and 25 [32%] in standard group). Non-relapse mortality was most commonly related to GVHD (eight patients had GVHD with infection, 13 had GVHD without infection). In the triple-drug group, four (4%) of 90 patients had non-relapse mortality unrelated to GVHD or infection (one was related to cardiomyopathy, one to lung embolism, one to pulmonary complications, and one to multi-organ failure), compared with six (8%) of 77 patients in the standard group (one was related to cardiomyopathy, one to renal failure, one to multi-organ failure, one to brain hemorrhage, one to secondary malignancy, and one to an unknown cause). Two [2%] patients in the triple-drug group and six [8%] in the standard group died of infection.

No differences in cumulative incidence of relapse or progression were observed between study groups at 1 year after HSCT (19% [95% CI 11–27] in triple-drug group *vs* 21% [12–30] in standard group) or at 4 years after HSCT (25% [16–34] in triple-drug group *vs* 27% [17–37] in standard group; HR 0·85 [0·47–1·56]; p=0·61; figure 3). Progression-free survival at 1 year was 77% (95% CI 68–85) in the triple-drug group and 64% (53–74) in the standard drug group, and at 4 years it was 59% in the triple-drug group (49–70) and 41% in the standard group (30–53%; HR 0·64 [0·42–0·99]; p=0·045; figure 3). Overall survival at 1 year was 86% (95% CI 78–93) in the triple-drug group

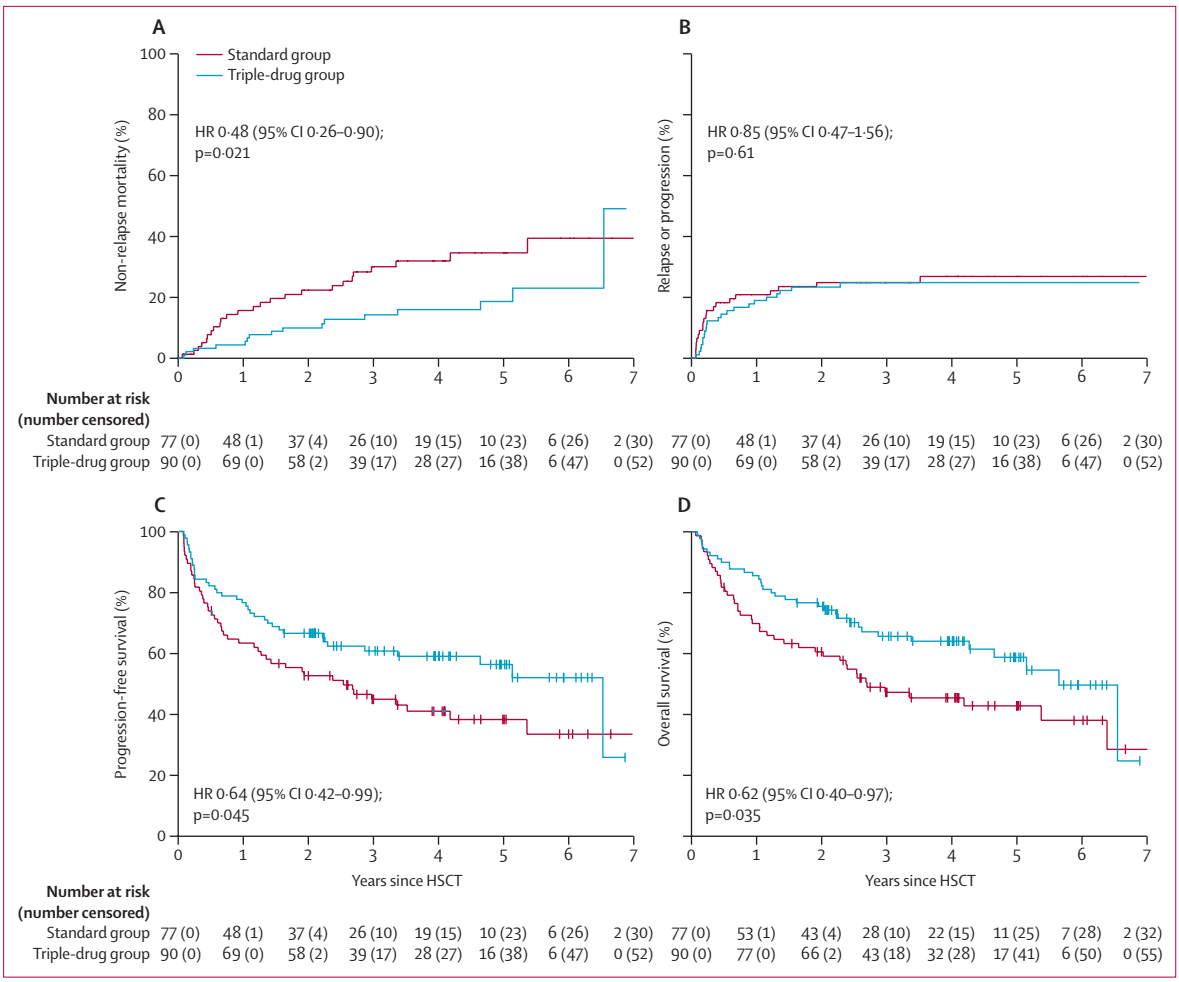

Figure 3: **Survival and progression outcomes**
(A) Non-relapse mortality, (B) relapse or progression, (C) progression-free survival, and (D) overall survival by treatment group. HSCT=haemopoietic stem cell transplantation.

and 70% (60–80) in the standard group and at 4 years it was 64% (54–75) in the triple-drug group and 46% (34–57%) in the standard group; HR 0·62 [0·40–0·97]; p=0·035; figure 3).

No differences were observed in the cumulative incidence of non-haemopoietic toxic effects by day 100 post-transplantation (25% [95% CI 16–34] in triple-drug group *vs* 34% [24–45] in standard group; HR 0·84 [0·49–1·44]; p=0·52; table 2). In both groups the most common CTCAE grade 4 or higher toxic effects were pulmonary (table 2). Four patients in the triple-drug group had hypertriglyceridaemia (CTCAE grade ≥3) and one patient had microangiopathy (CTCAE grade 3). No patient had veno-occlusive disease of the liver. Cumulative incidences of bacterial infections (45% [95% CI 35–56] in triple-drug group *vs* 37% [26–48] in standard group; HR 1·05 [0·69–1·62]; p=0·81), fungal infections (12% [6–19] in triple-drug group *vs* 18% [9–26] in standard group; HR 0·77 [0·38–1·58]; p=0·48), and viral infections (29% [19–38] in triple-drug group *vs* 41% [29–52] in standard group; HR 0·64 [0·41–1·01]; p=0·056) at 1 year were similar in both study groups. Among patients who were CMV-positive or had CMV-positive donors (53 [59%] of 99 patients in triple-drug group and 61 [79%] of 77 patients in standard group; table 1), the cumulative incidence of CMV reactivation or CMV infection up to 1 year after transplantation was lower in the triple-drug group (38% [95% CI 25–51] in triple-drug group *vs* 69% [57–81] in standard group; HR 0·35 [0·21–0·60]; p=0·0001; appendix p 7).

We noted four baseline characteristics (table 1) that by chance were imbalanced by more than 10 percentage points between groups and could plausibly be related to reported outcomes: female donor to male patient sex mismatch, patient/donor CMV seropositivity, previous HSCT, and Kahl[10] risk group. Adjustment for these factors did not materially alter HR results compared with unadjusted results (appendix p 11) and did not alter any conclusions derived from the unadjusted results. No significant departures from proportional hazards were

	Standard GVHD prophylaxis group (n=77)		Triple-drug group (n=90)	
	Grade 3	Grade 4	Grade 3	Grade 4
Renal and urinary disorder	6 (8%)	0	9 (10%)	0
Hepatic	9 (12%)	1 (1%)	3 (3%)	1 (1%)
Gastrointestinal	3 (4%)	0	1 (1%)	1 (1%)
Cardiac	6 (8%)	0	1 (1%)	1 (1%)
Pulmonary	5 (6%)	3 (4%)*	5 (5%)	5 (6%)
Coagulation	2 (3%)	0	1 (1%)	0
Blood and lymphatic system	3 (4%)	0	0	0
Neurology	2 (3%)	0	1 (1%)	0
Dermatology	1 (1%)	0	0	0

Grade 1–2 events were not recorded in this trial. All grade 3 and 4 adverse events are shown. *One patient had a grade 5 fatal adverse event due to pulmonary complications unrelated to GVHD. GVHD=graft-versus-host disease.

Table 2: **Adverse events**

noted for any endpoint, based on a test of interaction of treatment with (log) time.

Discussion

The current trial shows that addition of sirolimus to cyclosporine and mycophenolate mofetil reduces the cumulative incidence of acute GVHD after unrelated HLA-matched non-myeloablative conditioning HSCT. The phase 3 trial design was based on results of a preceding phase 2 trial,[4] and sought to definitively test the hypothesis that a triple-drug combination of mycophenolate mofetil, cyclosporine, and sirolimus would control acute GVHD significantly better than the standard combination of mycophenolate mofetil and cyclosporine. If the primary aim of the study was met, we expected improvements in the secondary endpoints of non-relapse mortality, overall survival, and progression-free survival.

The Data and Safety Monitoring Board halted the trial early after 168 of the planned 300 patients received the allocated intervention, because there was a significant survival advantage among patients in the triple-drug group. 106 (63%) of the 168 patients had been enrolled at the Fred Hutchinson Cancer Research Center. The root cause for the improvement in overall survival was likely the significant 26% reduction in acute GVHD (the primary study endpoint), given the well-established high mortality associated with it. Importantly, the reduced incidence of acute GVHD was not offset by an increase in relapse or progression. This finding was consistent with previous data[3] showing no significant association between acute GVHD and graft-versus-tumour effects in this HSCT setting. The 2% incidence of grade 3–4 acute GVHD was gratifyingly low in the triple-drug group but not significantly different from the 8% incidence observed in the standard group which, in turn, was similar to our previous data.[4] The premature closure of the trial precluded more definitive assessment of differences in grade 3–4 acute GVHD between the two study groups; the generally high incidence of grade 2–4 acute GVHD historically among patients at the Fred Hutchinson Cancer Research Center is likely due to the aggressive use of endoscopy in an attempt at diagnosing and treating gut GVHD early.[11] Of note, the 2% incidence of grade 3–4 acute GVHD we observed in patients taking the triple-drug regimen is lower than the 13% incidence seen in the previously reported triple-drug cohort.[4] We believe this is due to the extension of sirolimus administration from the previous 80 days to the current 180 days. Although the incidence of chronic GVHD was similar in the two study groups and similar to previous observations among unrelated recipients,[4,12] patients receiving the triple-drug regimen who were affected by chronic GVHD had lower non-relapse mortality, suggesting less severe chronic GVHD manifestation compared with patients in the standard group.

Overall, the addition of sirolimus to a calcineurin inhibitor and mycophenolate mofetil was safe and well tolerated. Veno-occlusive disease has nearly never been observed after non-myeloablative conditioning with fludarabine plus 2–3 Gy total body irradiation. Adverse events described for sirolimus after high-dose conditioning such as hypertriglyceridemia and transplantation-associated microangiopathy were rare. In this study we found no evidence that sirolimus increases the risk of developing transplant-associated thrombotic microangiopathy; the generally low incidence we observed was likely due to attending physicians' attention to the risk factors (such as calcineurin inhibitor and sirolimus concentrations) in patients entered on the protocol. Although the cumulative incidences of bacterial and fungal infections were similar in both study groups, CMV reactivation was significantly lower in the triple-drug group. This could have been caused by the specific antiviral activity of sirolimus or by lower steroid use due to the reduced incidence of acute GVHD, or a combination of these factors.[13]

This study has several limitations. Although a significant difference was observed for the primary endpoint, the trial's power was limited by its premature closure, which precluded definitive assessment of differences in grade 3–4 acute GVHD. Additionally, an imbalance in some pre-transplantation baseline characteristics was observed. However, adjustment for these factors did not materially alter HR results compared with unadjusted results.

Sirolimus has been investigated in allogeneic HSCT for almost two decades, but it has yet to move beyond clinical trials for a wider use as standard GVHD prophylaxis. Sirolimus added to tacrolimus or to a combination of low-dose methotrexate or anti-thymocyte globulin has been the focus of several prospective single-arm trials.[14–24] The results are difficult to compare because of heterogeneity

in study populations and treatment regimens; observed rates of grade 2–4 acute GVHD range from 16 to 77% and observed cumulative incidences of chronic GVHD range from 32 to 77%.[14–24] Only three randomised trials have been published, all involving myeloablative preparative regimens before HSCT, in which sirolimus was added to tacrolimus in place of or in addition to methotrexate. One of these trials[25] showed significant reductions in grade 2–4 acute GVHD and in moderate to severe chronic GVHD without a concurrent reduction in non-relapse mortality. The second trial[26] showed a reduction in grade 2–4 acute GVHD without a concurrent reduction in non-relapse mortality. The third trial[27] reported no differences in acute or chronic GVHD. None of the three trials showed improvements in overall survival.

In a study among patients receiving reduced-intensity preparative regimens, Armand and colleagues[28] randomly assigned 139 patients to receive either a triple combination of calcineurin inhibitor, methotrexate, and sirolimus, or double combinations of calcineurin inhibitor with mycophenolate mofetil or calcineurin inhibitor with methotrexate. The triple combination resulted in a significant reduction in cumulative incidence of grade 2–4 acute GVHD (9% in triple-combination group *vs* 25% in double-combination group), without showing differences in chronic GVHD or in overall survival.[28]

More recently, GVHD prophylaxis with sirolimus has been combined with post-transplantation cyclophosphamide in patients with hematologic malignancies given myeloablative conditioning before HLA-mismatched or HLA-matched unrelated HSCT. Results were promising, with 23–25% cumulative incidences of grade 2–4 acute GVHD, 13–16% chronic GVHD, and 6–14% non-relapse mortality at days 100 to 180 post-transplant, and with 35–36% relapse at 1 to 2 years after transplantation.[29,30]

To our knowledge, our study is the first phase 3 trial showing that combining sirolimus, cyclosporine, and mycophenolate mofetil for acute GVHD prophylaxis significantly improves overall survival and progression-free survival. This survival improvements seem to be driven by the reduced cumulative incidence of grade 2–4 acute GVHD and non-relapse mortality without increasing the risk of relapse. These results could set a new standard for GVHD prevention after unrelated HSCT with non-myeloablative conditioning regimens. Further studies are needed to establish whether these promising results of GVHD prophylaxis with calcineurin inhibitor, mycophenolate mofetil, and sirolimus translate into other donor-recipient settings or into other more intense conditioning regimens.

Contributors

BMS conceived, designed, and supervised the study, reviewed enrollment of patients, provided patients, analysed and interpreted data, drafted and revised the manuscript, and provided funding. BK drafted the protocol, drafted and revised the manuscript, and analysed and interpreted data. BS designed the study, did statistical calculations, and revised the manuscript. GO, MMa, AL, JAG, SLP, TC, WAB, MP, AW, and MMi provided patients and revised the manuscript. PJM and MEDF provided patients, reviewed GVHD scoring, and revised the manuscript. FRA provided patients, provided funding, and revised the manuscript. DGM designed the study, reviewed enrollment of patients, provided patients, and revised the manuscript. RS designed study, provided funding, provided patients, and revised the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

The study is a therapeutic clinical trial. There is no present intention to seek an IRB waiver of consent to share individual patient-level data.

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