Three prophylaxis regimens (tacrolimus, mycophenolate mofetil, and cyclophosphamide; tacrolimus, methotrexate, and bortezomib; or tacrolimus, methotrexate, and maraviroc) versus tacrolimus and methotrexate for prevention of graft-versus-host disease with haemopoietic cell transplantation with reduced-intensity conditioning: a randomised phase 2 trial with a non-randomised contemporaneous control group (BMT CTN 1203)

Javier Bolaños-Meade, Ran Reshef*, Raphael Fraser, Mingwei Fei, Sunil Abhyankar, Zaid Al-Kadhimi, Amin M Alousi, Joseph H Antin, Sally Arai, Kate Bickett, Yi-Bin Chen, Lloyd E Damon, Yvonne A Efebera, Nancy L Geller, Sergio A Giralt, Parameswaran Hari, Shernan G Holtan, Mary M Horowitz, David A Jacobsohn, Richard J Jones, Jane L Liesveld, Brent R Logan, Margaret L MacMillan, Marco Mielcarek, Pierre Noel, Joseph Pidala, David L Porter, Iskra Pusic, Ronald Sobecks, Scott R Solomon, Daniel J Weisdorf, Juan Wu, Marcelo C Pasquini†, John Koreth†*

Summary

Background Prevention of graft-versus-host disease (GvHD) without malignant relapse is the overall goal of allogeneic haemopoietic cell transplantation (HCT). We aimed to evaluate regimens using either maraviroc, bortezomib, or post-transplantation cyclophosphamide for GvHD prophylaxis compared with controls receiving the combination of tacrolimus and methotrexate using a novel composite primary endpoint to identify the most promising intervention to be further tested in a phase 3 trial.

Methods In this prospective multicentre phase 2 trial, adult patients aged 18–75 years who received reduced-intensity conditioning HCT were randomly assigned (1:1:1) by random block sizes to tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide (cyclophosphamide 50 mg/kg on days 3 and 4, followed by tacrolimus starting on day 5 and mycophenolate mofetil starting on day 5 at 15 mg/kg three times daily not to exceed 1 g from day 5 to day 35); tacrolimus, methotrexate, and bortezomib (bortezomib 1·3 mg/m² intravenously on days 1, 4, and 7 after HCT); or tacrolimus, methotrexate, and maraviroc (maraviroc 300 mg orally twice daily from day –3 to day 30 after HCT). Methotrexate was administered as a 15 mg/m² intravenous bolus on day 1 and 10 mg/m² intravenous bolus on days 3, 6, and 11 after HCT; tacrolimus was given intravenously at a dose of 0·05 mg/kg twice daily (or oral equivalent) starting on day –3 (except the post-transplantation cyclophosphamide, as indicated), with a target level of 5–15 ng/mL. Tacrolimus was continued at least until day 90 and was tapered off by day 180. Each study group was compared separately to a contemporary non-randomised prospective cohort of patients (control group) who fulfilled the same eligibility criteria as the trial, but who were treated with tacrolimus and methotrexate at centres not participating in the trial. The primary endpoint (GvHD-free, relapse-free survival [GRFS]) was defined as the time from HCT to onset of grade 3–4 acute GvHD, chronic GvHD requiring systemic immunosuppression, disease relapse, or death. The study was analysed by modified intention to treat. The study is closed to accrual and this is the planned analysis. This trial is registered with ClinicalTrials.gov, number NCT02208037.

Findings Between Nov 17, 2014, and May 18, 2016, 273 patients from 31 US centres were randomly assigned to the three study arms: 89 to tacrolimus, methotrexate, and bortezomib; 92 to tacrolimus, methotrexate, and maraviroc; 92 to tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide; and six were excluded. Between Aug 1, 2014, and Sept 14, 2016, 224 controls received tacrolimus and methotrexate. Controls were generally well matched except for more frequent comorbidities than the intervention groups and a different distribution of types of conditioning regimens used. Compared with controls, the hazard ratio for GRFS was 0·72 (90% CI 0·54–0·94; p=0·044) for tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide, 0·98 (0·76–1·27; p=0·92) for tacrolimus, methotrexate, and bortezomib, and 1·10 (0·86–1·41; p=0·49) for tacrolimus, methotrexate, and maraviroc. 238 patients experienced grade 3 or 4 toxicities: 12 (13%) had grade 3 and 67 (73%) grade 4 events with tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide; ten (11%) had grade 3 and 68 (76%) had grade 4 events with tacrolimus, methotrexate, and bortezomib; and 18 (20%) had grade 3 and 63 (68%) had grade 4 events with tacrolimus, methotrexate, and maraviroc. The most common toxicities were haematological (77 [84%]

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*Joint first authors

†Joint last authors

Department of Oncology (J Bolaños-Meade MD, Prof R J Jones MD) **and Department of Medicine** (Prof R J Jones), **Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD, USA** (J Bolaños-Meade, Prof R J Jones); **Department of Medicine, Columbia University Medical Center, New York, NY, USA** (R Reshef MD); **Department of Biostatistics** (R Fraser PhD, M Fei MSc, Prof B R Logan PhD) **and Department of Medicine** (Prof P Hari MD, Prof M M Horowitz MD, M C Pasquini MD), **Medical College of Wisconsin, Milwaukee, WI, USA; Department of Internal Medicine, University of Kansas Medical Center, Westwood, KS, USA** (Prof S Abhyankar MD); **Department of Hematology and Oncology, Winship Cancer Institute of Emory University, Atlanta, GA, USA** (Z Al-Kadhimi MD); **Department of Stem Cell Transplant and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX, USA** (Prof A M Alousi MD); **Department of Medicial**

Oncology, Dana-Farber Cancer Institute, Boston, MA, USA (Prof J H Antin MD, J Koreth MD); Department of Medicine, Stanford University, Palo Alto, CA, USA (S Arai MD); The Emmes Corporation, Rockville, MD, USA (K Bickett BSc, J Wu MSc); Department of Medicine, Massachusetts General, Hospital, Boston, MA, USA (Y-B Chen MD); Department of Medicine, University of California, San Francisco, San Francisco, CA, USA (Prof L E Damon MD); Department of Internal Medicine, The Ohio State University, Columbus, OH, USA (Y A Efebera MD); Office of Biostatistics Research, National Institutes of Health, Bethesda, MD, USA (N L Geller PhD); Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA (Prof S A Giralt MD); Department of Medicine (S G Holtan MD, Prof D J Weisdorf MD), Department of Pediatrics (Prof M L MacMillan MD), University of Minnesota, Minneapolis, MN, USA; Department of Pediatrics at George Washington University, Children's National Medical Center, Washington, DC, USA (Prof D A Jacobsohn MD); Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA (Prof J L Liesveld MD); Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA (Prof M Mielcarek MD); Department of Medicine, Mayo Clinic Arizona, Phoenix, AZ, USA (Prof P Noel MD); Blood and Marrow Transplantation and Cellular Immunotherapy, H Lee Moffitt Cancer Center, Tampa, FL, USA (J Pidala MD); Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA (Prof D L Porter MD); Department of Medicine, Washington University School of Medicine, Saint Louis, MO, USA (I Pusic MD); Department of Hematology and Medical Oncology, Cleveland Clinic, Cleveland, OH, USA (R Sobecks MD); and Blood and Marrow Transplant Program at Northside Hospital, Atlanta, GA, USA (S R Solomon MD)

for tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide; 73 [82%] for tacrolimus, methotrexate, and bortezomib; and 78 [85%] for tacrolimus, methotrexate, and maraviroc) and cardiac (43 [47%], 44 [49%], and 43 [47%], respectively).

Interpretation **Tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide was the most promising intervention, yielding the best GRFS; this regimen is thus being prospectively compared with tacrolimus and methotrexate in a phase 3 randomised trial.**

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Introduction

Graft-versus-host disease (GvHD) is a frequent cause of morbidity and mortality after allogenic haemopoietic cell transplantation (HCT).[1–3] Over the past few decades, the combination of methotrexate and a calcineurin inhibitor has been the cornerstone of GvHD prevention after HCT.[4] However, despite prophylaxis, over 50% of patients undergoing HCT will have acute or chronic GvHD, or both.[1,5–7] Unfortunately, these outcomes have changed little despite the introduction of agents such as mycophenolate mofetil or sirolimus with methotrexate.[8,9] Moreover, in patients who develop GvHD and do not respond to treatment, survival is poor owing to infectious complications, organ failure, and toxicity of immunosuppressive agents.[10] Therefore, a strategy that minimises not just the incidence of GvHD, but also other adverse events, should translate into better outcomes after HCT.

Novel agents that have shown promising results in the prevention of GvHD include post-transplation bortezomib, pre-transplantation and post-transplanation maraviroc, and post-transplantation cyclophosphamide.

Research in context

Evidence before this study

Since 1986, when the combination of methotrexate and ciclosporine was shown to be superior to ciclosporine alone in preventing graft-versus-host disease (GvHD) in patients undergoing HLA-matched bone marrow transplantation, methotrexate and ciclosporine became the standard of care. Ever since, it has been used continuously after both myeloablative transplantation and reduced-intensity transplantation with bone marrow and with peripheral blood stem cell grafts. Although other strategies have been developed to decrease the frequency of severe GvHD, none has been superior to methotrexate and a calcineurin inhibitor. We searched MEDLINE for articles published in English until Oct 12, 2018, with the terms "cyclophosphamide for graft versus host prophylaxis", "maraviroc for graft versus host prophylaxis", and "bortezomib for graft versus host prophylaxis". We found that, although cyclophosphamide has been used for prevention of GvHD since the 1970s, the drug was only shown to be effective and started to be used in HLA-matched allografts after Luznik and colleagues published their work in 2008 on haploidentical transplantations. Reshef and colleagues reported that maraviroc was effective at blocking lymphocyte chemotaxis and preventing visceral GvHD. Lastly, Koreth and colleagues, and others, showed that bortezomib-based combinations were a promising strategy to prevent GvHD after mismatched transplantations and unrelated donor transplantations. We did not find any study comparing these agents either against each other or against methotrexate and ciclosporine during the time the study was open and recruiting patients.

Added value of this study

In this randomised phase 2 study, treatment with high-dose post-transplantation cyclophosphamide was promising when compared with a non-randomised contemporary control of methotrexate and a calcineurin inhibitor when using a novel composite endpoint that combined severe acute GvHD, chronic GvHD requiring systemic immunosuppression, disease relapse, or death. Patients randomly assigned to receive maraviroc or bortezomib experienced similar outcomes compared with those who received methotrexate and calcineurin inhibitor. Given the nature of the study, no direct comparison between the experimental groups was done for the primary endpoint.

Implications of all the available evidence

To our knowledge, this study is the first randomised clinical trial to show promising outcomes using a novel composite endpoint for patients who received a high-dose post-transplantation cyclophosphamide-based regimen versus the standard combination of methotrexate and calcineurin inhibitor for GvHD prophylaxis. While it has some limitations, based on the design of the clinical trial, results with high-dose post-transplantation cyclophosphamide were sufficiently promising to warrant further study. Therefore, based on these findings, a phase 3 clinical study will be launched to compare high-dose post-transplantation cyclophosphamide with methotrexate and calcineurin inhibitor for GvHD disease prophylaxis (BMT CTN 1703).

Bortezomib resulted in significant protection from acute GvHD in murine models with no adverse effects on long-term donor reconstitution.[11] The drug was effective in single-centre studies of mismatched unrelated donor reduced-intensity HCT.[12,13] C-C chemokine receptor type 5 (CCR5) is a chemokine receptor that is important in GvHD pathogenesis in murine models.[14,15] Maraviroc, a CCR5 antagonist, inhibits lymphocyte chemotaxis without impairing T-cell function, and showed promising results in a study of reduced-intensity HCT, primarily through reduction of severe acute GvHD in the liver and gut.[16,17] Finally, post-transplantation cyclophosphamide allows transplantation between matched and mismatched donor–recipient pairs with low incidences of chronic GvHD, presumably via killing of activated effector T cells and upregulation of regulatory T cells.[18–21]

The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) analysed several single-centre GvHD prevention approaches and selected three promising interventions to be analysed prospectively in a multicentre setting, focusing on recipients of reduced-intensity conditioning regimens.[22] The aim of the present study was to evaluate new approaches for GvHD prophylaxis compared with contemporary controls using a novel composite primary endpoint to identify the most promising intervention to be further tested in a phase 3 clinical trial.

Methods

Study design and participants

This was a BMT CTN randomised phase 2, open-label, multicentre trial comparing each of tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide; tacrolimus, methotrexate, and bortezomib; and tacrolimus, methotrexate, and maraviroc with a non-randomised prospective contemporary tacrolimus and methotrexate control (control group). HCT centres not participating in the clinical trial were recruited to participate in the control arm using data collected by the Center for International Blood and Marrow Transplant Research (CIBMTR). The CIBMTR is a research collaboration between the National Marrow Donor Program/Be The Match and the Medical College of Wisconsin. The CIBMTR represents an international network of transplant centres that submit transplant-related outcomes data. It has been collecting HCT outcomes data for over 40 years and has an extensive prospectively collected longitudinal database of detailed information related to patients, transplants, and diseases.[23] The CIBMTR data were collected in compliance with Health Insurance Portability and Accountability Act regulations and with all applicable federal regulations pertaining to the protection of human research participants, as determined by a continuous review by the National Marrow Donor Program Institutional Review Board and the Medical College of Wisconsin. Centres participating in the control cohort provided supplemental information specific for this trial, including dates of grades 3 and 4 acute GvHD and use of corticosteroids, in addition to standard CIBMTR data collection forms.

Eligible patients in all groups were 18–75-year-old candidates for reduced-intensity conditioning with a related 6/6 match for HLA-A and HLA-B at intermediate resolution, and HLA-DRB1 at high resolution; or unrelated donors who were HLA matched 7/8 or 8/8 HLA-A, HLA-B, HLA-C, and HLA-DRB1 at high resolution using DNA-based typing. Eligible diseases were acute leukaemia, chronic myelogenous leukaemia, and myelodysplasia with no circulating blasts and with less than 5% blasts in the bone marrow; chronic lymphocytic leukaemia or small lymphocytic lymphoma; and follicular, marginal zone, diffuse large-B cell, Hodgkin, or mantle cell lymphoma with chemosensitive disease at the time of transplantation.

Exclusion criteria were previous allogeneic transplantation; left ventricular ejection fraction less than 45%; hepatitis B or C; HIV; transformed lymphoma; other cancer diagnosis, planned therapy after transplantation, including tyrosine kinase inhibitors; uncontrolled bacterial or fungal infections; poor Karnofsky performance status (<70%); creatinine clearance less than 50 mL/min; diffusing capacity of the lungs for carbon monoxide less than 40% or forced expiratory volume in 1 s less than 50%; and inability to withhold agents that interact with hepatic cytochrome P450 enzymes or glutathione S-transferases involved in bortezomib or busulfan metabolism, or both, from day –5 to day 7.

The control cohort had the same eligibility criteria as patients enrolled in the clinical trial, which was applied in two stages to reach the final population. In the first stage, eligibility criteria included centres that agreed to participate in the control cohort and patient age, diseases, conditioning regimens, and tacrolimus-based GvHD prophylaxis. All consecutive patients who received a stem cell transplant from Aug 1, 2014, to Sept 14, 2016, at participating centres were included in the pool of potential controls. The second phase assessed all reported data for consistencies in the data and applied additional eligibility criteria not included in the earlier entry forms (eg, excluding transformed lymphoma and presence of any viral infections before transplantation).

The study was approved by the institutional review board at each institution and all patients provided written informed consent before being admitted into the clinical study.

Randomisation and masking

Patients were approached for this study after the decision to proceed with transplantation was made and a suitable HLA-matched donor identified. Transplantation physicians evaluated patient eligibility for randomisation into this study. Eligibility criteria were verified and

Correspondence to:
Dr Javier Bolaños-Meade, Department of Oncology, Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD 21231, USA
fbolano2@jhmi.edu

ineligible patients were off study and no further follow-up was obtained. Transplant centre personnel registered the patient in EMMES AdvantageEDC (Electronic Data Capture, an internet-based data entry system; EMMES, Rockville, MD, USA) which upon completion of registration informed the study centre of the results of randomisation.

All patients were randomly assigned within 7 days before the initiation of conditioning therapy. Patients were randomly assigned (1:1:1) to tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide; tacrolimus, methotrexate, and bortezomib; or tacrolimus, methotrexate, and maraviroc, using permuted blocks with random block sizes, stratified by donor type (HLA-matched sibling *vs* matched unrelated *vs* mismatched unrelated) and disease risk (high *vs* low). Disease risk was modelled based on the risk for GvHD-free, relapse-free survival (GRFS); patients with chronic lymphocytic leukaemia and myelodysplastic syndrome were considered high risk and all the others were standard risk according to these criteria. No masking took place.

Procedures

Conditioning regimens allowed in this trial included fludarabine (120–180 mg/m²) and busulfan (≤8 mg/kg orally or 6·4 mg/kg intravenously); fludarabine (90–120 mg/m²) and cyclophosphamide (120 mg/kg or 2250 mg/m²); fludarabine (120–180 mg/m²) and melphalan (≤150 mg/m²); fludarabine (90 mg/m²) and total body irradiation 200 cGy; and fludarabine (150 mg/m²), cyclophosphamide (29 mg/kg), and total body irradiation (200 cGy). Donor cells were mobilised with filgrastim and collected by leukapheresis to a target stem cell dose of between 2×10^6 and 10×10^6 CD34+ cells per kg based on actual recipient bodyweight. Cells were administered on day 0 per institutional standards.

Patients in the clinical trial were randomly assigned to one of three GvHD prophylaxis regimens: post-transplantation cyclophosphamide (50 mg/kg on days 3 and 4) followed by tacrolimus and mycophenolate mofetil (15 mg/kg three times daily not to exceed 1 g three times daily) starting on day 5 (mycophenolate mofetil was stopped on day 35); tacrolimus, methotrexate, and bortezomib (1·3 mg/m² intravenously on days 1, 4, and 7 after HCT); or tacrolimus, methotrexate, and maraviroc (300 mg orally twice daily from day –3 to day 30 after HCT). Patients receiving methotrexate were administered a 15 mg/m² intravenous bolus on day 1 and a 10 mg/m² intravenous bolus on days 3, 6, and 11 after HCT. Tacrolimus was given intravenously at a dose of 0·05 mg/kg twice daily (or oral equivalent) starting on day –3, with a target level of 5–15 ng/mL. Tacrolimus was recommended to continue at least until day 90 and to be completely tapered off by day 180.

Drugs for prophylaxis against *Pneumocystis jirovecii*, fungal, and herpetic infections, and use of growth factors, intravenous immunoglobulin, blood products, and other supportive care were per institutional standards. Dose adjustments of study drugs were allowed. Tacrolimus dose reductions were made if toxicity was present or whole blood concentrations were above the recommended range (15 ng/mL) in the absence of toxicity. Patients with severe intolerance of tacrolimus could be given ciclosporine (trough concentration 200–400 ng/mL) or sirolimus (trough concentration 3–8 ng/mL). Methotrexate dose reductions owing to worsening creatinine clearance after initiation of conditioning regimen, high serum concentrations, or development of oral mucositis were done according to institutional practices. Maraviroc dose was reduced by 50% if grade 3 or higher liver toxicity not attributable to other causes such as infection, GvHD, toxicity from other drugs, or sinusoid obstructive syndrome or veno-occlusive disease was present, or if severe mucositis, nausea, or other complications that preclude administration of an oral medication occurred. For coadministration of cytochrome P450 3 (CYP3) inhibitors with or without a potent CYP3A inducer (ketoconazole, itraconazole, or clarithromycin) the dose of maraviroc was 150 mg twice daily. For potent CYP3A inducers without a potent CYP3A inhibitor (rifampicin, carbamazepine, phenobarbital, and phenytoin) the dose of maraviroc was 600 mg twice daily. Bortezomib dose reductions to 1 mg/m² occurred in the presence of grade 1 or 2 neuropathy. For grade 2 with pain or grade 3, the drug was held until resolution. For grade 4, it was discontinued. For post-transplantation cyclophosphamide, no dose adjustments were made, but no immunosuppressants were allowed until at least 24 h after completion of the drug.

Outcomes

The primary endpoint was the composite endpoint of GRFS, defined as the time to onset of any of the following events from time of HCT: grade 3–4 acute GvHD, chronic GvHD requiring systemic immunosuppressive treatment, disease relapse or progression, death from any cause, or loss to follow-up or end of 1 year, whichever came first.[22] Systemic immunosuppression was defined as continuation of drugs in patients with chronic GvHD or addition of new drugs to treat this complication, which included non-topical immunosuppression medications, extracorporeal photopheresis, or prednisone doses of 10 mg and higher or equivalent.

Secondary endpoints were cumulative incidence of grades 2–4 and 3–4 acute GvHD, cumulative incidence of chronic GvHD, 1-year immunosuppression-free survival, neutrophil and platelet recovery, donor cell engraftment, disease relapse or progression, transplant-related mortality (TRM), toxicities, infections, disease-free survival (DFS), GvHD-free survival, overall survival, and causes of death.

To accommodate the unique design of comparing the arms within a randomised phase 2 trial to a contemporary

control cohort, we compared each arm with the control for all endpoints with the exception of donor cell engraftment, toxicities, infections, and causes of death, which were compared across the three randomised arms.

Acute GvHD was graded according to the Keystone consensus,[24] and chronic GvHD was diagnosed and evaluated per the National Institutes of Health consensus conference.[25] Immunosuppression were defined as use of any systemic immunosuppression. Corticosteroids were included as systemic immunosuppression when patients were receiving doses higher than 10 mg of prednisone daily or equivalent. Immunosuppression-free survival was defined as being patients who were off systemic immunosuppression at 1 year (plus or minus 15 days) after transplantation. Neutrophil engraftment recovery was defined as the first of three consecutive measurements with an absolute neutrophil count of 500 cells per µL or greater. Platelet engraftment recovery was defined as the first day of a sustained platelet count of more than 20 000 cells per µL, with no platelet transfusion in the preceding 7 days. Donor cell engraftment was assessed by chimerism studies in the blood or bone marrow and was defined based on the proportion of donor chimerism as full (>95%), mixed (5–95%), or graft failure (<5%). Disease relapse was defined by either morphological or cytogenetic evidence of acute leukaemia or myelodysplastic syndrome, or radiological or clinical evidence of lymphoma confirmed histologically (appendix pp 2–3). Disease progression applied to lymphoproliferative diseases not in remission at the time of transplantation and was defined as an increase in the size of disease at previous sites of disease or at new sites (appendix p 3). Toxicities were defined as the development of grade 3 or higher events according to the Common Terminology Criteria for Adverse events (version 4). The number of events, grade, and organism group (bacteria, virus, or fungus) were provided for infections. DFS comprised disease relapse or progression and death as events. GvHD-free survival was defined as grades 3–4 acute GvHD, chronic GvHD requiring immunosuppression, and death.

Statistical analysis

The trial was designed to randomly assign 270 patients across the three GvHD prophylaxis groups (n=90 each), and also collect comparable data on the concurrent non-randomised CIBMTR control cohort of about 270 patients. The planned sample size of 540 patients had at least 80% power to identify a treatment as promising (hazard ratio [HR] relative to concurrent non-randomised control group significant at one-sided 5% significance level) when its GRFS at 1 year was 15% better than controls, based on a simulation study that also accounted for a futility stopping rule. The incidences of acute and chronic GvHD, GvHD requiring immunosuppression, relapse or progression, TRM, and haematological recovery were calculated for each group using the cumulative incidence estimator, along with 90% CIs. GRFS, immunosuppression-free survival, DFS, GvHD-free survival, and overall survival were estimated using the Kaplan-Meier method along with 90% CIs.

Because the control group was non-randomised, multivariate analyses were done using Cox proportional hazards regression models for GRFS, acute and chronic GvHD, chronic GvHD requiring immunosuppression, relapse, treatment-related mortality, DFS, and GvHD-free and overall mortality (1–overall survival) after adjusting for age, disease, and donor HLA matching. Additional variables, including sex, ethnic origin, conditioning regimen, Karnofsky performance score, HCT comorbidity index, disease status, donor and recipient cytomegalovirus status, time to transplantation, donor and recipient sex match, and donor and recipient ABO match were also considered in each model using a stepwise model building strategy with a significance level of 10%. 90% CIs for each HR compared with the control group were calculated. Adjusted survival curves were estimated for each treatment group as well as controls. Comparisons with the control group were analysed by modified intention to treat, in which randomly assigned patients were analysed according to their randomised group, but only patients who received a transplant were included in the analysis. This restriction was because the control group was a non-randomised cohort enrolled at the time of transplantation. Planned interim analyses for futility were done after the first 30 evaluable patients in each group based on an anticipated 6-month GRFS in the control group of 45–50%. Additional safety monitoring was done for TRM at 100 days after HCT. All analyses were done in SAS/STAT (version 9.4).

See Online for appendix

This study is registered with ClinicalTrials.gov, number NCT02208037.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. RF and MF had full access to all the data in the study and JB-M had final responsibility for the decision to submit for publication.

Results

From Nov 17, 2014, to May 18, 2016, 279 patients from 31 US centres, and from Aug 1, 2014, to Sept 14, 2016, 403 controls from 32 US centres were enrolled (figure 1; appendix pp 9,10). Of the 279 patients, 89 were assigned to tacrolimus, methotrexate, and bortezomib; 92 to tacrolimus, methotrexate, and maraviroc; 92 to tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide; and six were excluded. The final control population used to compare with each group in the randomised trial was 224 control patients. Median age was 64 years (IQR 59–68) for all groups and controls. Patient characteristics were balanced in participants enrolled in the randomised trial. Comparing each

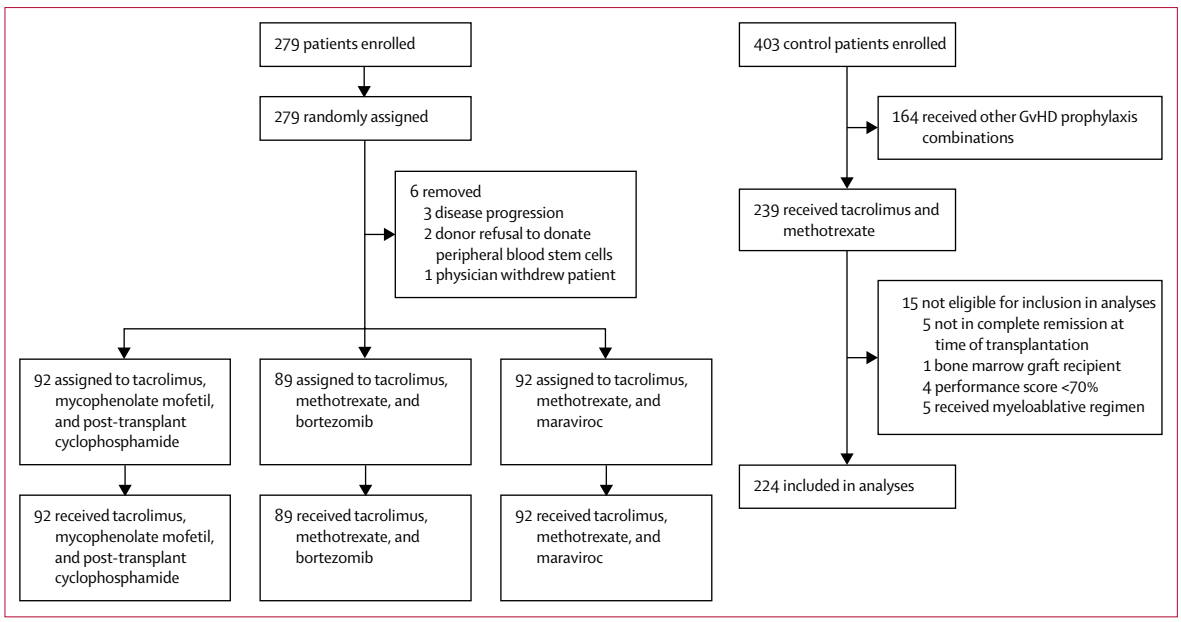

Figure 1: **Trial profile**
GvHD=graft-versus-host disease.

treatment group with controls, a higher proportion of patients in the control group had a HCT comorbidity index of at least 3, and controls had a different distribution of types of reduced intensity conditioning regimens and minor differences in the distribution of diseases (table 1).

Adjusted 1-year Kaplan-Meier estimates for GRFS were 34% (90% CI 28–40) for the control group compared with 43% (34–54) for tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide (HR 0·72, 95% CI 0·54–0·94; p=0·044); 35% (27–47) for tacrolimus, methotrexate, and bortezomib (0·98, 0·76–1·27; p=0·92); and 28% (20–38) for tacrolimus, methotrexate, and maraviroc (1·10, 0·86–1·41; p=0·49; table 2 and figure 2A). Additional covariates in the GRFS model included age, disease, donor type, and conditioning regimen (appendix p 5).

Neutrophil recovery at day 28 and platelet recovery at day 60 did not differ in any group compared with controls (appendix p 6). At day 100, 62 (75%) of 83 patients in the tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide group, 63 (73%) of 86 in the tacrolimus, methotrexate, and bortezomib group, and 56 (65%) of 86 in the tacrolimus, methotrexate, and maraviroc group were full donor chimeras (p=0·36; appendix p 7). Graft failure by day 100 occurred in three patients (4%) of 92 in the tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide group, five (6%) of 89 in the tacrolimus, methotrexate, and bortezomib group, and three (4%) of 92 in the tacrolimus, methotrexate, and maraviroc group (p=0·65; appendix p 7).

The cumulative incidence of acute GvHD grades 2–4 at day 180 was 30% (90% CI 25–36) for the control group compared with 27% (20–35) for tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide, 26% (19–34) for tacrolimus, methotrexate, and bortezomib, and 32% (24–40) for tacrolimus, methotrexate, and maraviroc (table 2). Corresponding cumulative incidences of acute GvHD grades 3–4 were 13% (90% CI 9–16) compared with 2% (0–5), 8% (4–13), and 9% (4–14; figure 2B; table 2).

The cumulative incidence of chronic GvHD at 1 year was 38% (90% CI 33–43) for the control group compared with 28% (20–36) for tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide, 39% (30–48) for tacrolimus, methotrexate, and bortezomib, and 43% (35–52) for tacrolimus, methotrexate, and maraviroc (table 2). Corresponding cumulative incidences of chronic GvHD requiring immunosuppression were 37% (90% CI 31–42) for the control group compared with 22% (15–30), 29% (22–38) and 33% (25–41; figure 2C; table 2). Multivariate analysis of GvHD models are shown in table 2.

1-year cumulative incidences of disease relapse or progression and TRM, and 1-year estimates of DFS and overall survival were similar across groups (table 2; appendix pp 12, 13). For patients alive and relapse free at 1 year, there were 75 (56%) of 135 patients in the control group, compared with 40 (71%) of 56 in the tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide group (p=0·041); 35 (67%) of 52 in the tacrolimus, methotrexate, and bortezomib group (p=0·14); and 28 (57%) of 49 in the tacrolimus, methotrexate, and maraviroc group (p=0·85). Corresponding cumulative incidences of GvHD-free survival are shown in table 2 and figure 2D.

	Controls (n=224)	TAC, MMF, and PTCY (n=92)	p value*	TAC, MTX, and BOR (n=89)	p value*	TAC, MTX, and MVC (n=92)	p value*
Sex							
Female	95 (42%)	30 (33%)	0·13	31 (35%)	0·25	37 (40%)	0·80
Male	129 (58%)	62 (67%)	..	58 (65%)	..	55 (60%)	..
Race							
White	203 (91%)	82 (89%)	0·68	79 (89%)	0·68	81 (88%)	0·54
Black or African American	4 (2%)	3 (3%)	..	3 (3%)	..	1 (1%)	..
Asian	8 (4%)	1 (1%)	..	2 (2%)	..	3 (3%)	..
More than one race	0	1 (1%)	..	1 (1%)	..	0	..
Other or unknown	3 (1%)	4 (4%)	..	4 (4%)	..	6 (6%)	..
Not answered	6 (3%)	1 (1%)	.	0	.	1 (1%)	.
Other characteristics							
Age, years	64 (55–73)	64 (56–72)	0·99	64 (55–73)	0·99	64 (54–73%)	0·98
HCT comorbidity index ≥3	139 (62%)	39 (42%)	0·0018	34 (38%)	0·00021	37 (40%)	0·00049
Donor match							
HLA matched sibling	89 (40%)	29 (32%)	0·20	29 (33%)	0·25	33 (36%)	0·61
HLA matched other relative	3 (1%)	4 (4%)	0·20	1 (1%)	1·00	1 (1%)	1·00
8/8 matched unrelated	119 (53%)	50 (54%)	0·90	53 (60%)	0·32	48 (52%)	0·90
7/8 mismatched unrelated	13 (6%)	9 (10%)	0·23	6 (7%)	0·79	10 (11%)	0·15
Disease Risk Index							
Low	17 (8%)	6 (7%)	..	6 (7%)	..	13 (14%)	..
Intermediate	157 (70%)	67 (73%)	..	65 (73%)	..	59 (64%)	..
High	50 (22%)	17 (18%)	0·54	17 (19%)	0·65	19 (21%)	0·88
Very high	0	2 (2%)	0·084	1 (1%)	0·28	1 (1%)	0·29
Conditioning treatment for transplant							
Fludarabine and busulfan	135 (60%)	42 (46%)	0·018	45 (51%)	0·13	48 (52%)	0·21
Fludarabine and melphalan	65 (29%)	39 (42%)	0·025	35 (39%)	0·082	32 (35%)	0·89
Fludarabine and cyclophosphamide	14 (6%)	1 (1%)	0·076	0	0·013	0	0·013
Fludarabine and total body irradiation	9 (4%)	3 (3%)	1·00	2 (2%)	0·73	3 (3%)	1·00
Fludarabine, cyclophosphamide, and total body irradiation	1 (<1%)	7 (8%)	0·00092	7 (8%)	0·00081	9 (10%)	0·00012
Diagnosis							
Acute myeloid leukaemia	117 (52%)	49 (53%)	0·90	46 (52%)	1·00	49 (53%)	0·90
Acute lymphoblastic leukaemia	14 (6%)	8 (9%)	0·47	12 (13%)	0·043	11 (12%)	0·11
Chronic myelogenous leukaemia	1 (<1%)	3 (3%)	0·076	2 (2%)	0·20	2 (2%)	0·20
Chronic lymphocytic leukaemia	9 (4%)	0	0·063	2 (2%)	0·73	3 (3%)	1·00
Myelodysplastic syndrome	66 (29%)	17 (18%)	0·049	16 (18%)	0·046	15 (16%)	0·0038
Follicular lymphoma	3 (1%)	5 (5%)	0·049	3 (3%)	0·36	6 (7%)	0·020
Diffuse large B-cell lymphoma	9 (4%)	7 (8%)	0·26	4 (4%)	1·00	1 (1%)	0·29
Time to transplantation from diagnosis, months	7·2 (4–18·4)	7·4 (2·4–17·2)	0·85	7·1 (6–20·1)	0·98	6·3 (7·3–19·9)	0·99

Data are number (%) or median (IQR). BOR=bortezomib. HCT=haemopoietic cell transplantation. MMF=mycophenolate mofetil. MTX=methotrexate. MVC=maraviroc. PTCY=post-transplantation cyclophosphamide. TAC=tacrolimus. *Compared with controls.

Table 1: **Demographics and baseline characteristics**

Toxicities seemed to be similar across the experimental groups. Overall, 238 patients experienced grades 3 and 4 toxicities: 12 (13%) had grade 3 and 67 (73%) had grade 4 events with tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide; ten (11%) had grade 3 and 68 (76%) had grade 4 events with tacrolimus, methotrexate, and bortezomib; and 18 (20%) had grade 3 and 63 (68%) had grade 4 events with tacrolimus, methotrexate, and maraviroc (table 3). The most common toxicities were

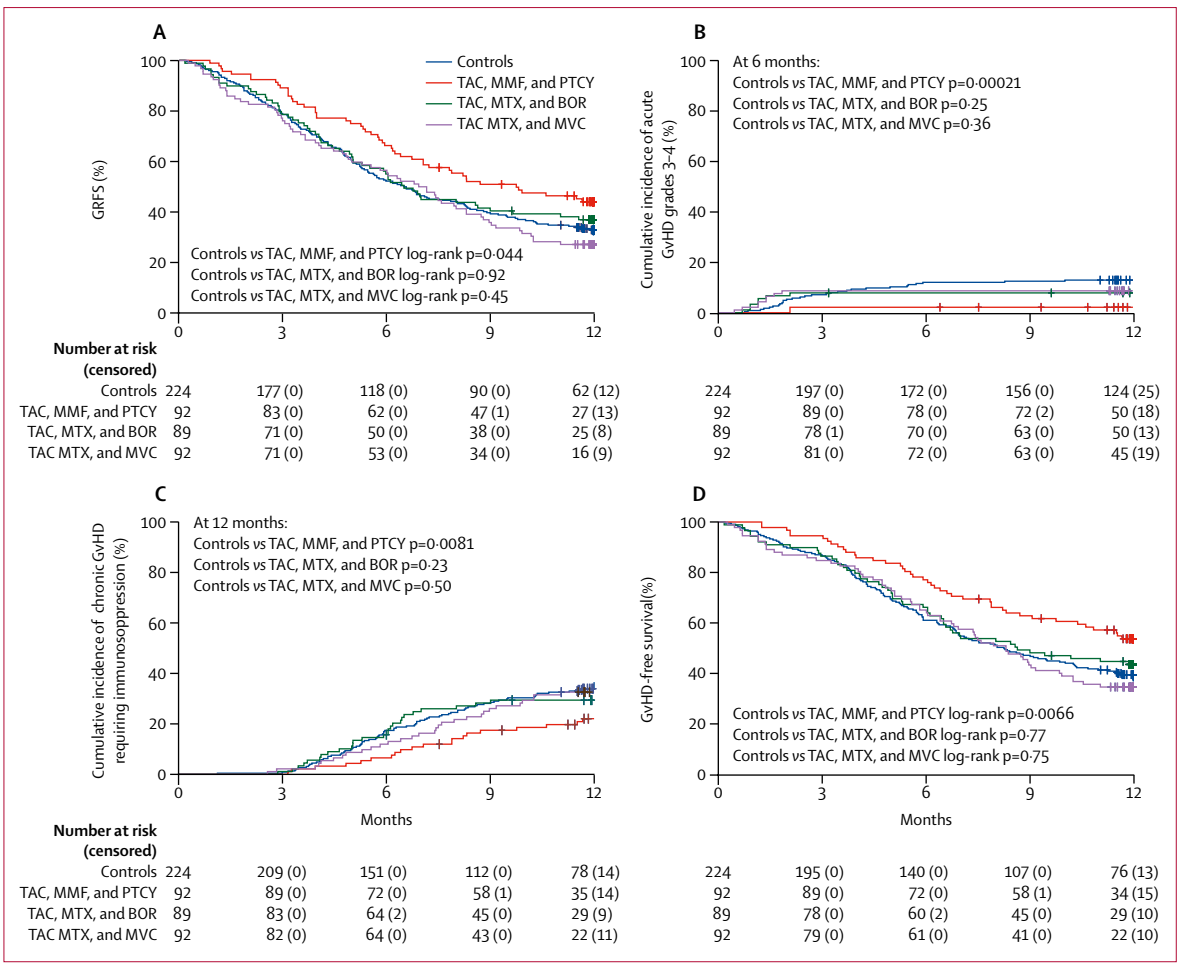

Figure 2: **GvHD outcomes**
BOR=bortezomib. GRFS=graft-versus-host disease-free, relapse-free survival. GvHD=graft-versus-host disease. MMF=mycophenolate mofetil. MTX=methotrexate. MVC=maraviroc. PTCY=post-transplantation cyclophosphamide. TAC=tacrolimus.

haematological and cardiac, which occurred in 77 (84%) and 43 (47%) patients with tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide; 73 (82%) and 44 (49%) with tacrolimus, methotrexate, and bortezomib; and 78 (85%) and 43 (47%) with tacrolimus, methotrexate, and maraviroc. Other toxicities with an incidence of over 30% in all groups included metabolic, gastrointestinal, and pulmonary events (appendix pp 11,12). Causes of death are listed in the appendix (p 8).

Discussion

This phase 2 randomised trial tested three approaches for GvHD prophylaxis, comparing each to a non-randomised contemporary cohort using a novel endpoint assessing GvHD relapse and survival. Tacrolimus, mycophenolate mofetil, and post-transplantation cyclophosphamide was the only intervention to have better GRFS than controls. This benefit was mainly driven by lower rates of severe acute GvHD and chronic GvHD requiring immunosuppression, with comparable relapse and survival to controls.

In previous studies, all three treatment agents showed promising activity with respect to prevention of GvHD and its complications. Koreth and colleagues[12] published their findings from a phase 1 study with tacrolimus, methotrexate, and bortezomib for GvHD prophylaxis after reduced-intensity bone marrow transplantation using HLA-mismatched unrelated donors. Bortezomib toxicity was minimal. With a 12-month median follow-up, grade 2–4 acute GvHD occurred in three of 23 patients and chronic GvHD occurred in nine patients. At 1 year, non-relapse mortality was zero, cumulative incidence of relapse or progression was 29%, overall survival was 75%, progression-free survival was 64%, and event-free survival was 59%. In a similar study[13] in 45 patients receiving peripheral blood grafts that were HLA mismatched, the 180-day cumulative incidence of grade 2–4 acute GvHD was 22% and the 1-year cumulative incidence of chronic GvHD was 29%.

	Controls (n=224)	TAC, MMF, and PTCY (n=92)	TAC, MTX, and BOR (n=89)	TAC, MTX, and MVC (n=92)
Primary endpoint				
GRFS				
Adjusted 1-year estimate (90% CI)	34% (28–40)	43% (34–54)	35% (27–47)	28% (20–38)
HR (90% CI)	1·00	0·72 (0·54–0·94)	0·98 (0·76–1·27)	1·10 (0·86–1·41)
p value	..	0·044	0·92	0·49
Secondary endpoints				
Acute GvHD grades 2–4				
Cumulative incidence at 180 days (90% CI)	30% (25–36)	27% (20–35)	26% (19–34)	32% (24–40)
HR (90% CI)	1·00	0·85 (0·58–1·24)	0·81 (0·54–1·22)	1·00 (0·69–1·45)
p value	..	0·48	0·41	0·98
Acute GvHD grades 3–4				
Cumulative incidence at 180 days (90% CI)	13% (9–16)	2% (0–5)	8% (4–13)	9% (4–14)
HR (90% CI)	1·00	0·13 (0·03–0·46)	0·53 (0·26–1·08)	0·60 (0·30–1·19)
p value	..	0·0082	0·14	0·23
Chronic GvHD				
Cumulative incidence at 1 year (90% CI)	38% (33–43)	28% (20–36)	39% (30–48)	43% (35–52)
HR (90% CI)	1·00	0·66 (0·45–0·96)	1·07 (0·76–1·51)	1·19 (0·86–1·62)
p value	..	0·069	0·72	0·36
Chronic GvHD requiring immunosuppression				
Cumulative incidence at 1 year (90% CI)	37% (31–42)	22% (15–30)	29% (22–38)	33% (25–41)
HR (90% CI)	1·00	0·59 (0·39–0·89)	0·89 (0·60–1·32)	0·92 (0·64–1·31)
p value	..	0·037	0·63	0·71
Relapse or progression				
Cumulative incidence at 1 year (90% CI)	25% (20–29)	28% (21–37)	24% (17–32)	31% (23–39)
HR (90% CI)	1·00	1·23 (0·83–1·83)	1·01 (0·65–1·57)	1·36 (0·92–2·01)
p value	..	0·37	0·95	0·18
Treatment-related mortality				
Cumulative incidence at 1 year (90% CI)	16% (12–21)	11% (6–17)	17% (11–24)	16% (10–23)
HR (90% CI)	1·00	0·64 (0·36–1·15)	1·09 (0·65–1·83)	0·99 (0·58–1·68)
p value	..	0·21	0·77	0·98
Disease-free survival				
Adjusted 1-year estimate (90% CI)	56% (51–62)	60% (51–68)	58% (49–66)	56% (47–64)
HR (90% CI)	1·00	0·92 (0·66–1·27)	0·98 (0·71–1·37)	1·21 (0·89–1·65)
p value	..	0·68	0·95	0·28
GvHD-free survival				
Adjusted 1-year estimate (90% CI)	37% (31–42)	53% (44–61)	43% (34–52)	34% (26–42)
HR (90% CI)	1·00	0·63 (0·47–0·84)	0·95 (0·72–1·25)	1·05 (0·81–1·36)
p value	..	0·011	0·76	0·75
Overall survival				
Adjusted 1-year estimate (90% CI)	71% (66–76)	71% (63–78)	68% (59–76)	66% (57–74)
HR (90% CI)	1·00	0·98 (0·67–1·44)	1·18 (0·80–169)	1·27 (0·88–1·82)
p value	..	0·94	0·49	0·28

Pointwise probabilities or cumulative incidences are results of the multivariate regression models for each outcome. BOR=bortezomib. GRFS=graft-versus-host disease-free, relapse-free survival. GvHD=graft-versus-host disease. HR=hazard ratio. MMF=mycophenolate mofetil. MTX=methotrexate. MVC=maraviroc. PTCY=post-transplantation cyclophosphamide. TAC=tacrolimus.

Table 2: **Outcomes**

	TAC, MMF, and PTCY (n=92)	TAC, MTX, and BOR (n=89)	TAC, MTX, and MVC (n=92)	Total (n=273)
Blood or lymphatic toxicity	77 (84%)	73 (82%)	78 (85%)	228 (84%)
Cardiac toxicity	43 (47%)	44 (49%)	43 (47%)	130 (48%)
Metabolic toxicity	38 (41%)	40 (45%)	43 (47%)	121 (44%)
Gastrointestinal toxicity	29 (32%)	36 (40%)	46 (50%)	111 (41%)
Intestinal obstruction	2 (2%)	3 (3%)	5 (5%)	10 (4%)
Pulmonary toxicity	31 (34%)	29 (33%)	29 (32%)	89 (33%)
Neurologic toxicity	20 (22%)	33 (37%)	30 (33%)	83 (30%)
Hepatobiliary or pancreas toxicity	18 (20%)	23 (26%)	29 (32%)	70 (26%)
Liver failure	2 (2%)	3 (3%)	4 (4%)	9 (3%)
Dermatologic toxicity	14 (15%)	22 (25%)	27 (29%)	63 (23%)
Musculoskeletal toxicity	17 (18%)	19 (21%)	24 (26%)	60 (22%)
Renal toxicity	10 (11%)	21 (24%)	18 (20%)	49 (18%)
Received dialysis	4 (4%)	5 (6%)	7 (8%)	16 (6%)
Fatigue	14 (15%)	17 (19%)	17 (18%)	48 (18%)
Encephalopathy	6 (7%)	5 (6%)	11 (12%)	22 (8%)
Maximum grade toxicities				
3	12 (13%)	10 (11%)	18 (20%)	40 (15%)
4	67 (73%)	68 (76%)	63 (68%)	198 (73%)
Death	5 (5%)	6 (7%)	9 (10%)	20 (7%)
Infection type				
Bacterial	74 (38)	61 (32)	69 (37)	204 (107)
Viral	59 (32)	51 (20)	31 (22)	141 (74)
Fungal	6 (5)	7 (7)	8 (6)	21 (18)
Protozoal	0 (0)	0 (0)	0 (0)	0 (0)
Other	2 (2)	2 (2)	1 (1)	5 (5)
Infections of unknown cause	17 (16)	21 (15)	11 (8)	49 (39)

Data are number of patients (%) or number of events (number of patients). BOR=bortezomib. MMF=mycophenolate mofetil. MTX=methotrexate. MVC=maraviroc. PTCY=post-transplantation cyclophosphamide. TAC=tacrolimus.

Table 3: **All toxicities and infections**

The 2-year cumulative incidence of non-relapse mortality was 11% and relapse was 38%. 2-year progression-free survival was 51% and overall survival 64%. This bortezomib-based regimen provided similar outcomes using HLA-mismatched donors to those observed in HLA-matched transplants. Reshef and colleagues[17] hypothesised that CCR5 blockade with maraviroc would inhibit visceral GvHD. In 35 patients, the cumulative incidence of grade 2–4 acute GvHD was 15% on day 100 and 24% on day 180. Acute liver and gut GvHD was not observed before day 100 and remained uncommon before day 180. As hypothesised, serum from patients receiving maraviroc prevented CCR5 internalisation by CCL5 and blocked T-cell chemotaxis in vitro, providing evidence of antichemotactic activity. Finally, post-transplantation cyclophosphamide has been effective at preventing GvHD in both matched and mismatched, as well as in non-myeloablative and myeloablative settings.[20,21,26,27] The mechanism of action suggests an upregulation of regulatory T-cell lymphocytes.[18] Moreover, post-transplantation cyclophosphamide has been associated with low rates of post-transplantation lymphoproliferative disorder,[28] donor-derived leukaemia,[29] and a low immunosuppressive burden after bone marrow transplantation.[30] This approach has been consistently associated with low incidences of grades 3–4 acute GvHD and chronic GvHD. Therefore, these three different approaches were ideal to be compared against the control group as GvHD-preventing regimens. For the control group, the combination of methotrexate and tacrolimus has been used for decades as standard GvHD prophylaxis since it was proven superior to methotrexate and ciclosporine, with incidences of grade 2–4 acute events between 32% and 56% and chronic GvHD between 56% and 76%.[6,7]

Other methotrexate-free regimens have been effective at preventing GvHD. Sirolimus-based combinations have been effective at preventing GvHD in both reduced-intensity and myeloablative settings, but were not more effective than methotrexate-based combinations.[9,31] Also, the use of anti-thymocyte globulin decreased GvHD compared with methotrexate and ciclosporine after myeloablative conditioning regimens.[32] Non-pharmacological strategies such as the use of bone marrow grafts instead of peripheral blood grafts can also decrease the incidence and severity of GvHD.[33] Moreover, the incorporation of anti-thymocyte globulin to post-transplantation cyclophosphamide and bone marrow in a small cohort resulted in a low rate of GvHD.[34] The results of this study suggest that post-transplantation cyclophosphamide could become an effective approach against GvHD.

The selection of the primary endpoint was an issue of intense analysis by the BMT CTN.[22] In GvHD, several competing events can be influenced by a single intervention, making the composite endpoint of great interest. Excessive immunosuppression, for instance, could be effective in preventing GvHD, but could lead to relapse or life-threatening infections, thereby making the intervention ineffective. Conversely, having a patient alive, in remission, with no GvHD and off immunosuppression is the ultimate goal of transplantation. Moreover, these composite endpoints improve efficiency reducing sample size in a clinical trial.[22]

The results related to GvHD also showed interesting patterns. Chronic GvHD requiring immunosuppression in controls was not different than overall chronic GvHD. Patients enrolled in the clinical trial had mandated immunosuppression taper by day 180, whereas in the controls, clinicians followed standard-of-care practices. Controls tended to continue immunosuppression beyond day 180 more frequently and through the development of chronic GvHD, reflecting the higher rate of use of these agents among these patients.

The unique design of this study combining a randomised clinical trial with a prospective contemporary registry-based cohort required strategies to minimise

bias. The rationale of using a non-randomised control group was to avoid having a fourth arm in a phase 2 trial, which would be associated with additional challenges and costs, and to improve power and efficiency of the comparisons with the control group for the purpose of identifying promising treatments. However, this trial design introduces potential for bias in the comparison with the control group from several sources. Absence of randomisation is likely to lead to selection bias or confounding. Selection of centres for the control group that were different from those participating in the clinical trial and enrolment of all of their eligible patients is likely to reduce selection bias and generate a real-world comparison, but it also introduces potential confounding between centre effects and treatment effects. The final composition of the control cohort included patients with more comorbidities than those in the trial treatment groups; this difference between the study groups and the controls could represent a tendency to enrol healthier patients in clinical trials. Another challenge was to match the clinical assessments from the trial and from the outcomes database to minimise bias from outcome reporting. We addressed this issue by adding supplemental questions to the CIBMTR forms for the control group. After completion of the trial, all data from the trial and control cohorts were combined and randomly selected for review by an adjudication review committee, which was masked to treatment assignment. The review was done on a subset of all cases following a review plan approved by the data and safety monitoring board. If concordance of the randomly selected 20 patients on the first subset was greater than 95%, review of all cases was not necessary. Despite the non-randomised comparison, the groups were comparable except for a few variables, which were adjusted for in the final models. However, given the non-randomised nature of the treatment versus control comparisons, these should be interpreted with caution. Ultimately, this clinical trial design combining a randomised phase 2 trial with a non-randomised concurrent control proved successful in efficiently identifying promising interventions before phase 3 trial development. However, this study has limitations. Its phase 2 nature and non-randomised control group prevent this from being a definitive trial. Therefore, the BMT CTN is launching a phase 3 trial as a follow on to this study to further explore the post-transplantation cyclophosphamide regimen as a substitute to calcineurin inhibitor-based GvHD prophylaxis.

Contributors

JB-M, RR, RF, MF, AMA, JHA, NLG, SGH, MMH, DAJ, RJJ, BRL, MLM, MM, JP, DLP, DJW, JW, MCP, and JK developed the protocol. JB-M, RR, and JK were study chairs. JB-M, RR, KB, MCP, and JK undertook the study. RF, MF, NLG, BRL, JW, and MCP planned and did the data analysis. MCP was the study officer. JB-M wrote the first draft of the paper. All authors interpreted the results, reviewed the manuscript, actively participated in drafting the final form of the paper, and approved the manuscript.

Declaration of interests

JB-M reports data and safety monitoring board fees from Incyte. RR reports personal fees from Kite, Bristol-Myers Squibb, Incyte, Pfizer, Pharmacyclics, Exelixis, Takeda, and Jazz Pharmaceuticals. KB reports grants from the National Heart, Lung, and Blood Institute (NHLBI). Y-BC reports personal fees from Takeda, Magenta, Incyte, Regimmune, and Kiadis. SAG reports grants from the BMT CTN; grants and personal fees from Celgene, Takeda, Amgen, and Sanofi; personal fees from Jazz, Novartis, Kite, and Bristol-Myers Squibb; and grants from Miltenyi and CSL Behring. SGH reports consulting fees from Incyte. PN reports spousal income from Novartis Pharmaceuticals. DJW reports grants from Incyte. MCP reports personal fees from Pfizer and Medigene. JK reports grants and non-financial support from Prometheus Labs and Millennium; non-financial support from Bristol-Myers Squibb, Novartis, and Miltenyi Biotec; and personal fees from Amgen, Equillium Biotech, and Fortress Biotech. All other authors declared no competing interests.

Data sharing statement

De-identified participant data for BMT CTN 1203 will be deposited in the NHLBI Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC), a publicly available database. Study documents, including the study protocol, informed consent form, data dictionary, and case report forms for data collection, are also available via the repository. Data will become accessible 3 years after the end of clinical activity and 2 years after the primary publication, as anticipated in 2020. Instructions on specimen or data requests and contact information for BioLINCC are also available.

For **BioLINCC** see https://biolincc.nhlbi.nih.gov/home/

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