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Post-transplantation cyclophosphamide versus conventional graft-versus-host disease prophylaxis in mismatched unrelated donor haematopoietic cell transplantation

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Summary

Post-transplantation cyclophosphamide (PTCy) is an effective strategy to prevent graft-versus-host disease (GVHD) after haploidentical haematopoietic cell transplantation (HCT). We determined the efficacy of PTCy-based GVHD prophylaxis in human leucocyte antigen (HLA)-mismatched unrelated donor (MMUD) HCT. We analysed 113 adult patients with high-risk haematological malignancies who underwent one-antigen MMUD transplantation between 2009 and 2013. Of these, 41 patients received PTCy, tacrolimus and mycophenolate mofetil (MMF) for GVHD prophylaxis; 72 patients received conventional prophylaxis with anti-thymocyte globulin, tacrolimus and methotrexate. Graft source was primarily bone marrow (83% PTCy vs. 63% conventional group). Incidence of grade II–IV (37% vs. 36%, $P = 0.8$) and grade III–IV (17% vs. 12%, $P = 0.5$) acute GVHD was similar at day 100. However, the incidence of grade II–IV acute GVHD by day 30 was significantly lower in the PTCy group (0% vs. 15%, $P = 0.01$). Median time to neutrophil (18 days vs. 12 days, $P < 0.001$) and platelet (25.5 days vs. 18 days, $P = 0.05$) engraftment was prolonged in PTCy group. Rates of graft failure, chronic GVHD, 2-year non-relapse mortality, relapse, progression-free survival or overall survival were similar. Our results demonstrate that PTCy, tacrolimus and MMF for GVHD prophylaxis is safe and produced similar results as conventional prophylaxis in patients with one antigen HLA-MMUD HCT.

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Authorship contributions

R.S.M. contributed to data collection and interpretation of the results, and wrote the manuscript; R.M.S. performed the statistical analysis, interpreted the results and contributed to manuscript writing; J.C. contributed to data collection, reviewed and approved the manuscript; G.R. contributed to data collection, reviewed and approved the manuscript; A.E.H. contributed to patient care, reviewed and approved the manuscript; A.A. contributed to patient care, reviewed and approved the manuscript; M.Q. contributed to patient care, reviewed and approved the manuscript; Q.B. contributed to patient care, reviewed and approved the manuscript; S.A. contributed to patient care, reviewed and approved the manuscript; U.P. contributed to patient care, reviewed and approved the manuscript; C.H. contributed to patient care, reviewed and approved the manuscript; I.K. contributed to patient care, reviewed and approved the manuscript; E.J.S. contributed to patient care, reviewed and approved the manuscript; R.E.C. contributed to study design, reviewed, edited and approved the manuscript; S.O.C. contributed to study design, data collection and interpretation and manuscript writing.

Disclosure of conflicts of interest

The authors do not have any conflicts of interest.

Keywords

HLA-mismatched transplantation; post transplantation cyclophosphamide; MMUD; unrelated donor; GVHD

Despite the availability of more than 10 million potential haematopoietic cell transplantation (HCT) donors in the National Marrow Donor Program registry (NMDP; https://bethematch.org/), the probability of finding a suitable human leucocyte antigen (HLA)-matched donor for HCT varies considerably, from 75% in Caucasians to 16% among other races (Gragert *et al*, 2014). One of the alternative options in such cases is the use of HLA-mismatched unrelated donor (MMUD) HCT, but at the expense of increased risk of graft-versus-host disease (GVHD) and non-relapse mortality (NRM) with reduced progression-free survival (PFS) and overall survival (OS) compared to HLA-matched HCT (Sasazuki *et al*, 1998; Flomenberg *et al*, 2004; Lee *et al*, 2007; Woolfrey *et al*, 2011; Saber *et al*, 2012).

The standard pharmacological GVHD prophylaxis regimen for HLA-matched unrelated (MUD) or related donor (MRD) HCT includes a calcineurin inhibitor (commonly tacrolimus or ciclosporin) and methotrexate (Nash *et al*, 2000; Hiraoka *et al*, 2001; Perkins *et al*, 2010; Saber *et al*, 2012). This is often intensified with *in vivo* T-cell depletion (TCD), generally with antithymocyte globulin (ATG) or alemtuzumab in MMUD HCT (Finke *et al*, 2003; Ayuk *et al*, 2008; Devillier *et al*, 2014; Fuji *et al*, 2015). With this intensive regimen, the incidence of grade II–IV acute GVHD (20–35%), grade III–IV acute GVHD (4–20%) and chronic GVHD (22–67%) in MMUD HCT approaches comparable levels to those seen after MUD HCT (Finke *et al*, 2003; Ayuk *et al*, 2008; Kim *et al*, 2009; Devillier *et al*, 2014; Fuji *et al*, 2015). However, *in vivo* TCD delays T-cell immune reconstitution (Small *et al*, 1997; Duval *et al*, 2002; Bosch *et al*, 2012) and poses heightened risk of bacterial and viral infections, including herpes simplex virus, cytomegalovirus (CMV), Epstein–Barr virus, and infection-related deaths (Bacigalupo *et al*, 2001), as well as fatal post-transplant lymphoproliferative disorder (PTLD) (Small *et al*, 1997; van Esser *et al*, 2001; Finke *et al*, 2009). Alternative improved GVHD prophylaxis regimens are needed. One potential method is the use of high dose post-transplantation cyclophosphamide (PTCy) given on days +3 and +4, which induces transplantation tolerance by inhibiting rapidly proliferating ‘alloreactive’ T-cells (Luznik *et al*, 2012), thereby reducing the risk of GVHD. Several studies reported encouraging outcomes with PTCy in haploidentical HCT in combination with tacrolimus and MMF (O’Donnell *et al*, 2002; Luznik *et al*, 2008), and a number of studies demonstrated its efficacy as the sole GVHD prevention method after myeloablative conditioning in 10/10-MUD and MRD HCT (Luznik *et al*, 2010; Kanakry *et al*, 2014). Its safety and efficacy in MMUD setting is undefined.

The aim of the present retrospective study was to compare the incidence of acute or chronic GVHD in patients who received PTCy in combination with tacrolimus and mycofenolate mofetil (MMF) as a GVHD prophylaxis regimen versus those who received standard GVHD prophylaxis using *in vivo* TCD, tacrolimus and methotrexate after one-antigen HLA-MMUD (9/10 or 7/8 HLA-matched) HCT.

Methods

Study protocol and objectives

A phase-II three arm clinical trial was initiated at the M.D. Anderson Cancer Center in 2009 to assess the safety and efficacy of PTCy after T-cell replete haploidentical, MMUD/MMRD or MUD HCT (protocol 2009-0266, ClinicalTrials.gov Identifier: NCT01010217). The present study is focused on the outcomes of a subset of those patients who underwent one-antigen MMUD HCT. The primary objective of the present study was to compare the incidence of acute or chronic GVHD in these patients to the rates in a separate contemporaneous cohort of patients who received conventional GVHD prophylaxis at our institution.

Patient population

We included all consecutive adult patients with haematological malignancies who received 9/10 HLA-MUD HCT at our institution between 2009 and 2013 after myeloablative or reduced-intensity conditioning regimen (n = 113). Of these, 41 patients received PTCy as a part of GVHD prophylaxis (study group) and 72 patients received conventional GVHD prophylaxis (control group). Participation in the clinical trial was based on preferences of patients and their treating physician and was also contingent on insurance approval. Among the study group, the majority of patients (n = 36/41, 88%) were enrolled in the above mentioned clinical trial (ClinicalTrials.gov Identifier: NCT01010217), whereas five patients (12%) did not qualify for the phase II clinical trial due to insurance reasons, but received the PTCy-based GVHD prophylaxis regimen off-study and were included in the current retrospective analysis. Out of 113 patients, 29 had HLA-DQB1 mismatches. As isolated donor-recipient mismatch at HLA-DQ does not affect survival (Flomenberg *et al*, 2004), we performed a separate analysis of 84 patients who underwent 7/8 HLA-MUD HCT. Out of these, 46 patients received conventional GVHD prophylaxis and 38 patients received PTCy-based prophylaxis. All patients gave signed informed consent according to the Declaration of Helsinki and an Institutional Review Board – approved protocol was obtained for this retrospective study.

Transplantation procedure

Patients in the PTCy group received a conditioning regimen of fludarabine and melphalan with either thiotepa or 200 cGy of total body irradiation (TBI). Melphalan [140 mg/m^2 intravenous (IV) with myeloablative regimen or 100 mg/m^2 with reduced intensity regimen] was given on day −8, followed by fludarabine 40 mg/m^2 IV for 4 days (day −6 to −3). In addition, patients either received thiotepa 5 mg/kg IV on day −7 or TBI 200 cGy on day −1. Reduced doses of melphalan were used for older patients (aged above 55 years) or those with significant comorbidities. Cyclophosphamide 50 mg/kg/day IV was administered on days +3 and +4. Additional GVHD prophylaxis in this arm was provided with tacrolimus and MMF, as previously reported by us (Ciurea *et al*, 2012).

Patients in the conventional GVHD prophylaxis group received various conditioning regimens, such as those based on busulfan/fludarabine (Bu/Flu; 37.5%), fludarabine/melphalan (22.2%), fludarabine/cyclophosphamide (18.1%), and others. Prophylaxis against

GVHD was provided with tacrolimus (dose and schedule as above) and methotrexate 5 mg/m^2 IV on days +1, +3, +6 and +11. Almost all patients received *in vivo* TCD (97.2%) using rabbit ATG (n = 68/72) or alemtuzumab (n = 2/72).

Granulocyte colony-stimulating factor (Filgrastim) 5 μg/kg was administered subcutaneously daily starting day +7 until absolute neutrophil count (ANC) was >1.0 × 10^9/l.

Statistical analysis

Definitions and assessments—High resolution HLA typing was performed for all donor-recipient pairs matching for HLA-A, -B, -C, -DRB1 and –DQB1. Any single antigen or allele mismatch at these loci was defined as "9/10 match," while single antigen or allele mismatch at HLA-A, -B, -C, or -DRB1 was defined as '7/8 match'. The time to neutrophil engraftment was defined as the first of three consecutive days after HCT with an ANC ≥0.5 × 10^9/l, and the time to platelet engraftment as the first of seven consecutive days with a platelet count ≥20 × 10^9/l without platelet transfusion. Primary graft failure was defined as the failure to attain an ANC >0.5 × 10^9/l by day +28 that was maintained for three consecutive measurements, with no evidence of donor-derived cells by bone marrow chimerism studies and no evidence of persistent or relapsing disease. Secondary graft failure was defined as a decline in ANC to <0.5 × 10^9/l for three consecutive days after initial engraftment. Diagnosis and grading of acute and chronic GVHD was defined based on standard criteria (Glucksberg *et al*, 1974; Shulman *et al*, 1980; Przepiorka *et al*, 1995). Chimerism analysis was performed on days 30 and 100 after transplantation and every 3 months thereafter, using a polymerase chain reaction with primer sets flanking microsatellite repeats. Complete donor chimerism was defined as the detection of >95% donor DNA in a sample.

Endpoints—The primary outcome of interest was incidence of acute or chronic GVHD. We also assessed GVHD occurring within 20 and 30 days of HCT ('early acute' GVHD). Secondary outcomes included rates of graft failure, time to neutrophil and platelet engraftments, attainment of donor chimerism, NRM, PFS and OS, and causes of deaths. NRM was defined as death without evidence of disease persistence or recurrence. PFS was defined as the time from HCT to either death or relapse. OS was defined as the time from HCT to death from any cause.

Statistical procedure—Baseline patient characteristics were compared between the groups using the Wilcoxon rank-sum test for continuous variables and Chi-square test or Fisher's exact test for dichotomous variables. The cumulative incidence of acute GVHD, chronic GVHD, NRM and disease progression were estimated accounting for competing risks. Disease progression or death before GVHD were considered competing risks in the estimation of GVHD incidence. Death with persistent disease or disease progression were competing risks in the estimation of the rate of NRM, and NRM was a competing risk in the estimation of disease progression. Actuarial probabilities of PFS and OS were estimated using the Kaplan–Meier estimator. Cox proportional hazards regression analysis and log rank test were used to compare outcomes between the PTCy and conventional GVHD

prophylaxis groups. The proportionality of the hazards assumption was tested statistically in assessment of the rate of GVHD between the two groups and was found to be met. Predictors of grade II–IV acute GVHD were assessed using Cox proportional hazards analysis. Predictors considered included gender, age, graft source, disease type, disease risk index, conditioning regimens, time between diagnosis and transplant, number of prior chemotherapy regimens, number of prior autologous transplants and year of transplantation. Due to significant differences in the graft source (peripheral blood (PB) or bone marrow (BM)) between the conventional group and the PTCy group, a planned subgroup analysis was performed that was restricted to patients that received only BM grafts. All analyses were performed using STATA 12 [StataCorp LP, College Station, TX, USA and statistical significance was defined at the 0.05 level.

Results

Outcomes of patients with 9/10 HLA-MUD HCT

Patients—A total of 113 consecutive adult patients met the retrospective study inclusion criteria. The PTCy group ($n = 41$) received GVHD prophylaxis with PTCy, tacrolimus and MMF. This group was compared to the conventional GVHD prophylaxis group ($n = 72$) that received *in vivo* TCD (98%) with tacrolimus and methotrexate (94.4%) (Table I). Patient and transplant characteristics were comparable between these groups with the exception of age at transplantation, stem cell source and donor-recipient HLA class mismatch. Patients in the conventional group were marginally older (median age 54 years; range 19–74) than those in the PTCy group (median age 50 years; range 20–64, $P = 0.05$). Also, PB was used more frequently as a graft source in the conventional group (38% vs. 17%, $P = 0.02$). Approximately 88% of patients in the PTCy group had HLA class-I donor-recipient mismatch, compared with about 57% in the control group ($P = 0.001$). Half of the patients in the conventional group and 56% in the PTCy group received myeloablative conditioning regimens. There were no other differences between the groups, including $CD34^+$ and $CD3^+$ cell dose, donor-recipient gender match, donor-recipient CMV serostatus, disease type, disease risk index and prior treatments. The median follow-up in surviving patients was 24 (range 3–49) months in the conventional group and 20 (range 4–43) months in the PTCy group.

Acute and chronic GVHD—The overall cumulative incidences of grade II–IV (37% vs. 36%) or grade III–IV (17% vs. 12%) acute GVHD at day 100 did not differ between the PTCy and the conventional groups, respectively (Fig 1). However, the cumulative incidence of grade II–IV acute GVHD by day 20 was 8% in the conventional arm compared with 0% in the PTCy arm ($P = 0.075$). The corresponding numbers by day 30 were 15% and 0%, respectively, $P = 0.01$. Consistent with these data, the incidence of grade III–IV acute GVHD by day 30 was 8% in the conventional group and 0% in the PTCy group ($P = 0.08$). On the other hand, the cumulative incidence of chronic GVHD was similar between the two groups at 6 months (20% vs. 15%), at 1 year (30% vs. 31%) or 2 years (30% vs. 42%) post-transplant (Table II). Risk factors analysis showed that the use of PTCy was the sole independent predictor of lower risk of grade II–IV acute GVHD by day 30 ($P = 0.01$). None

of the risk factors evaluated, including PTCy use, were shown to predict the rate of grade II–IV acute GVHD within day 100 post-transplant (Table III).

Because of differential use of BM or PB as a graft source between the groups, a subgroup analysis was performed including only those patients who received BM grafts. Again, the rate of grade II–IV acute GVHD by day 30 was significantly lower in the PTCy group; six patients in the conventional group ($n = 45$) experienced GVHD within 30 days of transplantation compared with none in the PTCy group ($n = 34$), $P = 0.03$. Yet again, there were no significant differences in the rate of grade III–IV acute GVHD by day 30, grades II–IV or grade III–IV acute GVHD by day 100, and chronic GVHD at 0.5, 1 and 2 years (Table II).

As described in Table I, the PTCy group included significantly more patients with HLA class-I mismatches (87.8%) compared with the conventional group (56.9%). Therefore, a separate analysis was performed to determine the effect of GVHD prophylaxis regimens based on HLA class mismatch. In patients with HLA class-I mismatch, PTCy was associated with significantly reduced risk of grade II–IV, but not grade III–IV, acute GVHD by day 30 ($P = 0.01$). However, there were no significant differences in acute grade II–IV GVHD [Hazards Ratio (HR) 1.1, 95% confidence interval (CI) 0.5–2.5, $P = 0.7$] or acute grade III–IV GVHD (HR 1.5, 95% CI 0.4–5.4, $P = 0.5$) by day 100 between PTCy and the conventional groups. It is noteworthy that only five patients in the PTCy group had HLA class-II mismatch. With the confinements of small subgroups of patients, we did not find any difference in the incidence of grade II–IV ($P = 0.4$) or grade III–IV ($P = 0.5$) acute GVHD by day 30, grade II–IV (HR 0.4, 95% CI 0.05–3.1, $P = 0.4$) or grade III–IV (HR 1.4, 95% CI 0.2–13, $P = 0.8$) acute GVHD by day 100, chronic GVHD at 1-year between the PTCy and the conventional prophylaxis group in patients with HLA class-I mismatch (HR 0.8, $P = 0.7$) or HLA class-II mismatch (HR 0.9, $P = 0.9$).

Engraftment—The risk of graft failures did not differ between the PTCy group (primary 2%, secondary 2.4%) and the conventional group (primary 8%, secondary 4%). Two patients in the PTCy group and three patients in the conventional group experienced early death before engraftment could be assessed. The time to neutrophil engraftment was significantly faster in the conventional group (median 12 days; range 8–29 days) compared with the PTCy group (median 18 days; range 13–34 days), $P < 0.001$. Delayed neutrophil engraftment after PTCy was more pronounced in patients who received BM grafts [median 19 days (range 14–34) vs. 12 days (range 9–25), $P < 0.001$]. In patients with PB grafts, the median time to neutrophil engraftment was 14 days (range 13–17) in the PTCy group compared with 12 days (range 8–25) in the conventional group, $P = 0.01$.

Similarly, platelet engraftment was more rapid in the conventional group compared with the PTCy group (median 18 days (range 9–125) vs. 25.5 days (range 11–141), $P = 0.05$). Delayed platelet engraftment with PTCy was observed only in patients who received BM grafts [median 28 days (range 13–141) vs. 19 days (range 12–125), $P = 0.045$], but not in those who received PB grafts (median 13 days in both groups).

Analysis of BM graft recipients showed that 88% (30/34) of the patients in PTCy group and 51% (23/45) in the conventional GVHD prophylaxis group had attained full donor chimerism by day 30. Most patients in both groups achieved complete donor chimerism by 1 year post-transplant – 91% (31/34) and 71% (32/45) in the PTCy and conventional groups, respectively. Higher frequency of mixed chimerism seen in the conventional arm was attributed primarily to the use of busulfan-based conditioning regimens, as we had previously observed (de Lima *et al*, 2004; Alatrash *et al*, 2011).

Other outcomes—Two-year cumulative incidences of NRM (35% vs. 25%), disease progression (20% vs. 31%), PFS (42% vs. 38%) and OS (52% vs. 40%) were similar in the PTCy and the conventional groups, respectively (Fig 2). Likewise, subgroup analysis of BM graft recipients showed comparable outcomes between the groups (Table IV).

Causes of deaths—Disease recurrence or persistence was the leading cause of death in the entire cohort, accounting for about 46% of all deaths. In patients with BM grafts, four deaths occurred due to graft failure or rejection – three in the conventional arm and one in the PTCy arm. Overall, approximately 17% of deaths in the conventional group and 21% in the PTCy group were attributed to infections. Further causes of deaths are summarized in Table V.

Outcomes of patients with 7/8 HLA-MUD HCT

After exclusion of 29 patients with isolated HLA-DQ mismatches, 84 patients were identified as '7/8 HLA-MUD' HCT recipients. Out of these, 38 patients received PTCy-based GVHD prophylaxis while 46 patients received the conventional prophylaxis. No patient in the PTCy group developed acute GVHD by day 30 compared with eight patients in the conventional group ($P = 0.005$) (Table II). Yet again, there were no differences in the incidence of grade II–IV (HR 1, 95% CI 0.5–2.1, $P = 0.9$) or grade III–IV (HR 1.1, 95% CI 0.3–3.3, $P = 0.9$) acute GVHD at day 100, or chronic GVHD at 6 months (HR 0.8, 95% CI 0.2–2.9, $P = 0.7$), 1 year (HR 0.8, 95% CI 0.3–2.2, $P = 0.6$) or 2 years (HR 0.7, 95% CI 0.2–1.9, $P = 0.5$) between the groups. The median time to neutrophil engraftment was 18 days (range 6–34) in the PTCy group and 12 days (range 8–25) in the conventional group, $P = 0.001$. The cumulative incidence of PFS (40% vs. 35%, HR 0.9, 95% CI 0.5–1.5, $P = 0.6$) and OS (51% vs. 41%, HR 0.8, 95% CI 0.5–1.6, $P = 0.6$) was similar between the PTCy group and the conventional GVHD prophylaxis group, respectively. With a median follow-up of 18 months (range 4–43) in the PTCy group and 27 months (range 3–49) in the conventional GVHD prophylaxis group, there were no differences in NRM (HR 1.3, 95% CI 0.6–2.7, $P = 0.5$) or disease progression (HR 0.6, 95% CI 0.3–1.5, $P = 0.3$) between the groups.

Discussion

In this single-institution retrospective analysis, we analysed the outcomes of one antigen HLA-MMUD (9/10-HLA matched) HCT with the use of PTCy as GVHD prophylaxis strategy along with tacrolimus and MMF, as compared to the conventional GVHD

prophylaxis with *in vivo* TCD, tacrolimus and methotrexate. Overall, the rates of acute or chronic GVHD were similar between the groups.

Next, we evaluated differences in the incidences of early acute GVHD between the groups. It is known that the occurrence of 'hyperacute' GVHD is associated with lower response rate to GVHD treatment and higher NRM (Saliba *et al*, 2007). 'Hyperacute' GVHD was defined previously as GVHD occurring within 14 days of transplantation, which was evaluated soon after neutrophil engraftment. However, this conventional definition is inapplicable to our cohort of mismatched HCT, the majority of who received BM grafts. In fact, the median time to neutrophil engraftment was 18 days in the PTCy group, and no patient in that group developed grade II–IV acute GVHD by day 20 compared with the conventional group (8%), $P = 0.075$. Additionally, there was statistically significant reduction in the incidence of grade II–IV acute GVHD by day 30 in the PTCy group (0%) compared to 15% in the conventional group, $P = 0.01$.

However, reduced incidence of early acute GVHD did not translate into improvements in incidences of acute GVHD by day 100, chronic GVHD or NRM. As such, the clinical significance of early reduction of GVHD risk is presently uncertain, but may be evident as more patients are enrolled into this trial. Nevertheless, the PTCy-based regimen was at least as effective as the conventional GVHD prophylaxis regimen. Similar results were noted after excluding patients with isolated HLA-DQ mismatched (7/8-HLA matched) unrelated donor HCT; or analysing patients by graft source (BM versus PB) or HLA-class mismatch (class I versus class II).

In contrast to the rates of GVHD observed in haploidentical HCT recipients receiving PTCy (Luznik *et al*, 2008; Bacigalupo *et al*, 2015; Ciurea *et al*, 2015; Sugita *et al*, 2015), we found higher than expected rates of GVHD in our study. The burden of alloreactive T cells is much higher in the major histocompatibility complex (MHC)-mismatched setting and it is pertinent to assume that a greater degree of mismatch at major HLA antigens will result in a more profound T cell proliferation and more efficient clonal deletion by post-transplant cyclophosphamide. Moreover, in the setting of a one antigen MMUD transplant, the higher rates of acute GVHD after PTCy may be related to a higher degree of minor antigen mismatching in addition to less efficient *in vivo* deletion of effector T cells. Indeed, studies of tolerance-induction using MHC-matched skin grafts that differed only in expression of non-MHC, 'minor' histocompatibility antigens followed by cyclophosphamide administration failed to induce complete tolerance (Nirmul *et al*, 1971, 1973).

As noted in prior studies in MUD and MRD HCT (Luznik *et al*, 2010; Kanakry *et al*, 2014), we also observed prolonged time to neutrophil engraftment with the use of PTCy, especially in patients who received BM grafts, where the median time to engraftment was delayed by a week. Nevertheless, almost 90% of BM graft recipients in the PTCy group had achieved complete donor chimerism by day 30, compared with about half of the patients in the conventional GVHD prophylaxis. Although debatable, early achievement of complete donor chimerism, especially in the T-cell compartment, is shown to be associated with better long term disease control in many studies (Molloy *et al*, 1996; Gardiner *et al*, 1997; Bader *et al*, 1998; Shaffer *et al*, 2013). Besides, the overall rates of graft failures were similar between

the PTCy and the conventional GVHD prophylaxis groups. This suggests that the use of PTCy is safe in this setting and is not associated with inferior graft function compared to the conventional GVHD prophylaxis.

With a study cohort including more than 50% of high- or very high-risk haematological malignancies, we observed similar 2-year progression (20% vs. 31%), PFS (42% vs. 38%) and OS (52% vs. 40%) in the PTCy group and the conventional group, respectively. A previous study in MUD and MRD HCT patients receiving Bu/Flu myeloablative conditioning and PTCy experienced higher rates of NRM than what is generally expected with Bu/Flu conditioning (Kanakry *et al*, 2014). The authors speculated that high dose PTCy may add to the toxicity of the conditioning regimen. We used fludarabine and melphalan based conditioning regimen with either thiotepa or TBI 200 cGy in the PTCy group and did not observe any difference in NRM compared to that of the conventional group.

We acknowledge certain limitations in our study. In addition to the inherent flaws of a retrospective analysis, small numbers of patients in different subgroups limited the power of the analysis and our ability to determine the benefits of PTCy in patients with HLA class-I versus class-II mismatches. The impact of different types of mismatches on outcomes after MMUD HCT is well described (Flomenberg *et al*, 2004). Additionally, comparison of data on tempo of immune reconstitution in both the groups is of interest and will be considered in future studies.

In conclusion, our study demonstrates that the use of PTCy, tacrolimus and MMF as GVHD prophylaxis in patients who receive single-antigen MMUD HCT after myeloablative or reduced-intensity conditioning, is safe and at least as effective as the conventional GVHD prophylaxis with *in vivo* TCD, tacrolimus and methotrexate. The PTCy-based regimen results in significantly lower risk of earlier occurrence of acute GVHD, the long-term significance of which is unclear at this time. However, as more patients are being treated it is possible that this early benefit could translate to an improvement in NRM. The use of PTCy does not contribute to additional toxicities; it may be associated with faster and better likelihoods of achieving complete donor chimerism and circumvent the need for *in vivo* TCD. In contrast, engraftment is delayed with PTCy in the recipients of BM grafts. Larger studies are needed to confirm our results.

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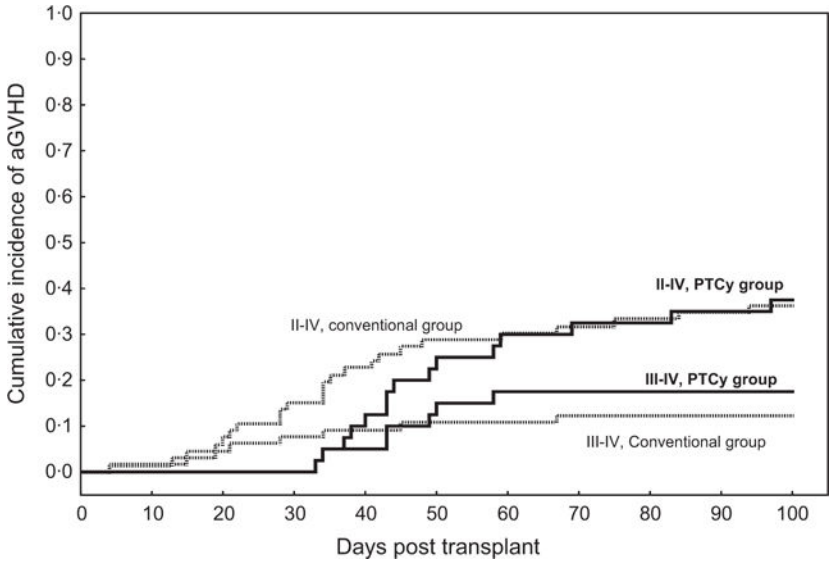

Fig 1.

Cumulative incidence of grade II–IV and III–IV acute graft-versus-host disease (aGVHD) by day 100 in the post-transplant cyclophosphamide (PTCy) group (solid line) compared with the conventional group (dotted line), all patients.

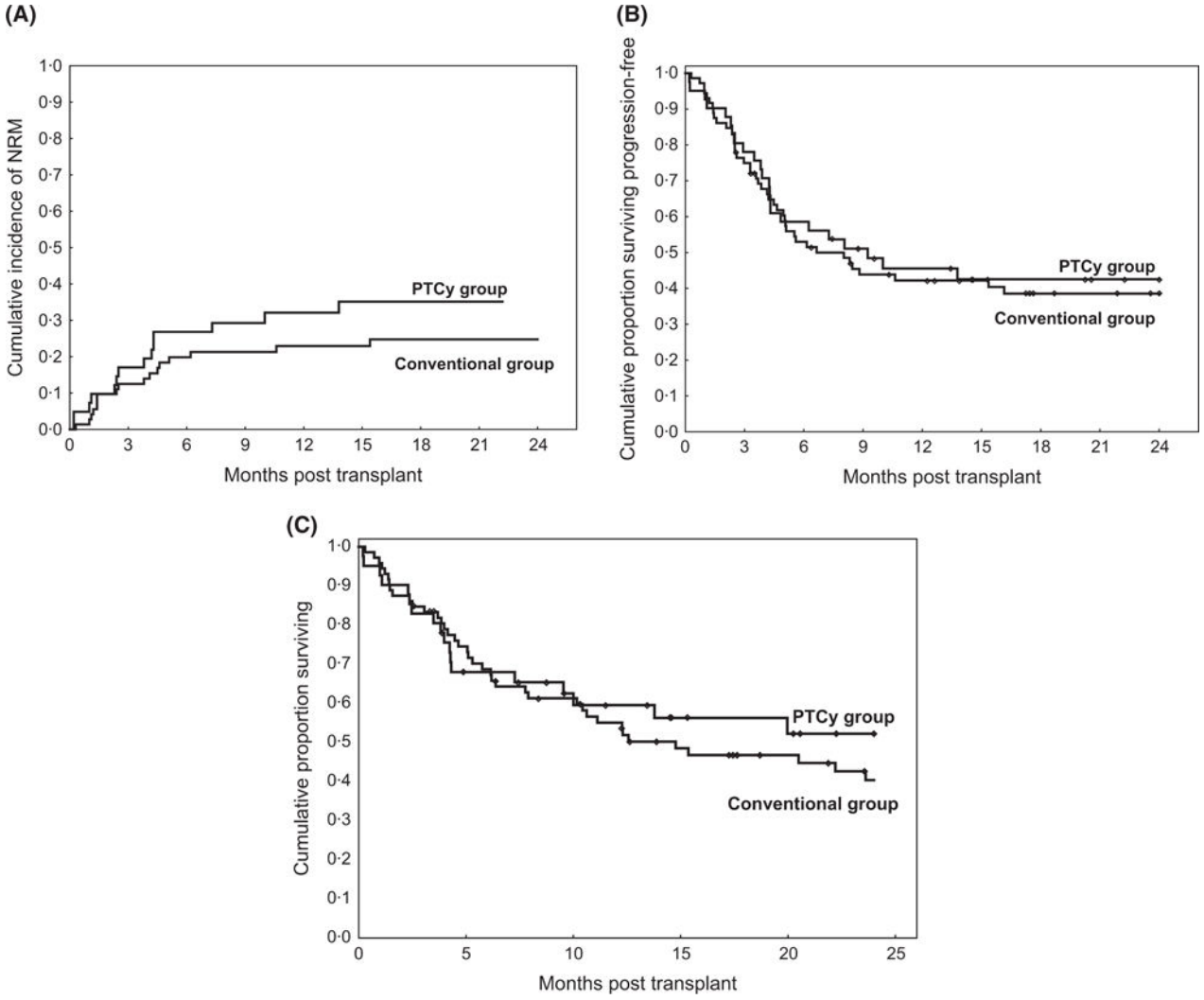

Fig 2.

(A) Cumulative incidence of non-relapse mortality (NRM), (B) progression-free survival and (C) overall survival in the post-transplant cyclophosphamide (PTCy) group compared with the conventional group, all patients.

Table I

Baseline patient characteristics.

Variable	Conventional GVHD prophylaxis (*n* = 72)	Post-transplantation cyclophosphamide (*n* = 41)	*P*-value
Age, years; median (range)	54 (19–74)	50 (20–64)	0.05
Diagnosis, *n* (%)			
AML/MDS	30 (42%)	15 (37%)	
ALL	4 (6%)	7 (17%)	
CLL	10 (14%)	3 (7%)	
CML/MPD	8 (11%)	2 (5%)	
Non-Hodgkin lymphoma	14 (19%)	8 (20%)	
Hodgkin lymphoma	4 (6%)	1 (2%)	
Aplastic Anaemia	2 (3%)	4 (10%)	
MM	0 (0%)	1 (2%)	
Lymphoid malignancies	32 (44%)	20 (49%)	0.7
Myeloid malignancies	40 (56%)	21 (51%)	
Disease risk index, *n* (%)			
Very High	9 (13%)	6 (15%)	0.4
High	19 (26%)	13 (32%)	
Intermediate	26 (36%)	7 (17%)	
Low	16 (22%)	11 (27%)	
Missing	2 (3%)	4 (10%)	
Median (range) time to HCT from diagnosis, months	27 (5–319)	15 (3–162)	0.3
Donor/Recipient gender, *n* (%)			
Female/Female	9 (14%)	13 (32%)	0.3
Female/Male	18 (25%)	7 (17%)	
Male/Female	20 (28%)	8 (20%)	
Male/Male	25 (35%)	13 (32%)	
Donor/Recipient CMV, *n* (%)			
Non-reactive/Non-reactive	5 (7%)	2 (5%)	0.5
Reactive/Reactive	26 (36%)	14 (34%)	
Non-reactive/Reactive	36 (50%)	22 (54%)	
Reactive/Non-reactive	5 (7%)	3 (7%)	
Conditioning regimens, *n* (%)			
Myeloablative	36 (50%)	23 (56%)	0.5
Reduced-intensity	36 (50%)	18 (44%)	
HLA mismatches			
HLA class-I mismatch	41 (57%)	36 (88%)	<0.001
HLA class-II mismatch	31 (43%)	5 (12%)	
Graft source, *n* (%)			
Bone marrow	45 (63%)	34 (83%)	0.02
Peripheral blood	27 (38%)	7 (17%)	
Cell dose (bone marrow), median (range)			

Variable	Conventional GVHD prophylaxis (*n* = 72)	Post-transplantation cyclophosphamide (*n* = 41)	*P*-value
$CD34^+$ ($\times 10^6$/kg)	2.4 (0.9–8.5)	2.2 (0.7–7.63)	0.3
$CD3^+$ ($\times 10^5$/kg)	17 (2.6–39)	16 (0.9–47)	0.2
Cell dose (peripheral blood), median (range)			
$CD34^+$ ($\times 10^6$/kg)	6.44 (2.45–16)	13.12 (3.5–41)	0.06
$CD3^+$ ($\times 10^5$/kg)	154 (27–505)	284 (86–381)	0.2
Median (range) number of prior chemotherapies	2 (0–10)	2 (0–7)	0.4
Prior autologous HCT, *n* (%)			
None	65 (90%)	36 (88%)	
One	7 (10%)	4 (10%)	
Two	0 (0%)	1 (2%)	
Year of HCT, *n* (%)			
2009	18 (25%)	1 (2%)	
2010	21 (29%)	9 (22%)	
2011	18 (25%)	13 (32%)	
2012	10 (14%)	14 (34%)	
2013	5 (7%)	4 (10%)	
Median (range) follow-up, in months	24 (3–49)	20 (4–43)	

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; CLL chronic lymphocytic leukaemia; CMV, Cytomegalovirus; GVHD, graft-versus-host disease; HLA, Human Leucocyte Antigen; HCT, haematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; MM, multiple myeloma; MPD, myeloproliferative disorder.

Table II

Acute and chronic GVHD.

	All patients			
	Cumulative incidence (95% CI)			
Variable	**Conventional GVHD prophylaxis (*n* = 65)**	**PTCy (*n* = 40)**	**HR; 95% CI**	***P*-value**
Acute GVHD, day +30				
Grade II–IV	15% (9–27%)	0%	NE	0.01
Grade III–IV	8% (3–18%)	0%	NE	0.08
Acute GVHD, day +100				
Grade II–IV	36% (26–50)	37% (25–56)	0.9 (0.5–1.8)	0.8
Grade III–IV	12% (6–23)	17% (9–34)	1.4 (0.5–3.9)	0.5
Chronic GVHD				
6 months	15% (8–31)	20% (9–44)	1 (0.6–1.7)	0.9
1 year	31% (19–50)	30% (16–57)	0.95 (0.6–1.5)	0.8
2 years	42% (27–68)	30% (16–57)	0.9 (0.5–1.4)	0.6

	Bone marrow grafts only		7/8-HLA matched patients (*n* = 84)	
	PTCy (*n* = 34) vs. Conventional GVHD prophylaxis (*n* = 45)		PTCy (*n* = 38) vs. Conventional GVHD prophylaxis (*n* = 46)*	
Variable	**HR; 95% CI**	***P*-value**	**HR; 95% CI**	***P*-value**
Acute GVHD, day +30				
Grade II–IV	NE	0.03	NE	0.005
Grade III–IV	NE	0.2	–	–
Acute GVHD, day +100				
Grade II–IV	0.9 (0.4–1.8)	0.8	1 (0.5–2.1)	0.9
Grade III–IV	1.9 (0.5–6.7)	0.3	1.1 (0.3–3.3)	0.9
Chronic GVHD				
6 months	1.2 (0.3–4.6)	0.8	0.8 (0.2–2.9)	0.7
1 year	0.8 (0.3–2.6)	0.8	0.8 (0.3–2.2)	0.6
2 years	0.6 (0.2–1.9)	0.4	0.7 (0.2–1.9)	0.5

CI, Confidence Interval; HR, Hazard Ratio; GVHD, graft-versus-host disease; PTCy, Post-transplant cyclophosphamide; HLA, Human Leucocyte Antigen; NE, not evaluable.

*Acute GVHD data missing for one engrafted patient in the conventional GVHD prophylaxis group.

Table III

Univariate analysis of acute and chronic GVHD at day 30 and day 100 (all patients).

		Acute GVHD II–IV, day30			Acute GVHD II–IV, day 100		
	N	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Group							
PTCy group	41	NE		0.01	0.97	0.5–1.8	0.9
Conventional group	72				Ref.		
Gender mismatch							
Female-Male	22	1.7	0.4–6.3	0.45	1.3	0.6–2.6	0.5
Others	84	Ref.			Ref.		
Age, years							
≤40	26	Ref.			Ref.		
41–50	19	1.4	0.2–10.2	0.7	1.3	0.5–3.2	0.5
51–55	17	0.7	0.1–8.1	0.8	0.7	0.2–2.2	0.6
56–60	17	1.5	0.2–10	0.7	1.5	0.6–3.7	0.3
>60	27	1.4	0.2–8.4	0.7	0.7	0.3–1.8	0.5
Graft source							
Bone marrow	74	Ref.			Ref.		
Peripheral blood	32	1.5	0.4–5.4	0.5	0.7	0.3–1.5	0.4
Diagnosis							
Lymphoid malignancies	49	1.8	0.5–6.3	0.4	1.4	0.8–2.6	0.3
Myeloid malignancies	57	Ref.			Ref.		
Disease Risk Index							
Very High	14	Ref.			Ref.		
High	31	0.5	0.1–3.2	0.4	1.3	0.5–3.6	0.6
Intermediate	30	0.7	0.1–4.1	0.7	1.2	0.4–3.3	0.8
Low	27	0.8	0.1–4.4	0.8	0.9	0.3–2.7	0.8
Missing	4	NE			0.7	0.1–6.4	0.7
Conditioning regimen							
Myeloablative	55	Ref.			Ref.		
Reduced-intensity	51	1.6	0.5–5.8	0.4	1.2	0.6–2.1	0.6

		Acute GVHD II–IV, day30			Acute GVHD II–IV, day 100		
	N	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Year of transplantation							
2009	16	Ref.			Ref.		
2010	29	0.3	0.06–2.0	0.2	0.7	0.2–2.0	0.5
2011	30	0.7	0.1–2.9	0.6	1.05	0.4–2.9	0.9
2012	23	NE			0.9	0.3–2.7	0.9
2013	8	0.6	0.1–5.9	0.7	2	0.6–7.1	0.3
2009 vs. >2009		2.6	0.7–10	0.2			

CI, Confidence Interval; HR, Hazard Ratio; NE, Not evaluable; PTCy, Post-transplant cyclophosphamide, GVHD, graft-versus-host disease; Ref., reference.

Table IV

Secondary outcomes.

All patients (*n* = 113)					Subgroup analysis with BM graft (*n* = 79)			
	Cumulative incidence (95% CI)				Cumulative incidence (95% CI)			
Variable	Conventional GVHD prophylaxis (*n* = 72)	PTCy (*n* = 41)	HR; 95% CI	*P*-value	Conventional GVHD prophylaxis (*n* = 45)	PTCy (*n* =34)	HR; 95% CI	*P*-value
NRM, 2 years,	25% (16–38)	35% (23–54)	1.4; 0.7–2.8	0.3			1.6 (0.6–3.8)	0.3
Progression, 2 years	31% (22–44)	20% (22–37)	0.6; 0.3–1.4	0.2			0.6 (0.3–1.5)	0.3
PFS, 2 years	38% (27–50)	42% (27–57)	0.9; 0.5–1.5	0.7	43% (28–58)	56% (37–72)	0.9 (0.5–1.6)	0.8
OS, 2 years	40% (28–52)	52% (35–67)	0.8; 0.5–1.4	0.5	38% (24–52)	44% (27–60)	0.8 (0.4–1.6)	0.5

BM, bone marrow; CI, Confidence Interval; HR, Hazard Ratio; GVHD, graft-versus-host disease; PTCy, Post-transplant cyclophosphamide; NRM, non-relapse mortality; PFS, progression-free survival; OS, overall survival.

Table V

Causes of death, by graft source and GVHD prophylaxis

	Bone marrow graft		Peripheral blood graft	
	Conventional group (*n* = 25)	PTCy group (*n* = 15)	Conventional group (*n* = 17)	PTCy group (*n* = 4)
Disease recurrence/persistence	16 (64%)	5 (33.3%)	7 (41.2%)	–
Acute GVHD	–	3 (20%)	1 (5.9%)	2 (50%)
Chronic GVHD	1 (4%)	–	2 (11.7%)	2 (50%)
Graft failure/rejection	3 (12%)	1 (6.7%)	–	–
Infections	3 (12%)	4 (26.7%)	4 (23.5%)	–
Pneumonia	2 (8%)	–	–	–
Organ failure	–	1 (6.7%)	1 (5.9%)	–
Prior malignancy	–	–	1 (5.9%)	–
Other	–	1 (6.7%)	1 (5.9%)	–

GVHD, graft-versus-host disease; PTCy, Post-transplant cyclophosphamide.