

ORIGINAL ARTICLE

Cyclosporin, methotrexate and prednisolone for graft-versus-host disease prophylaxis in allogeneic peripheral blood progenitor cell transplants

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The utility of GVHD prophylaxis with cyclosporin, MTX and prednisolone (CSA/MTX/Pred) in allogeneic PBPC transplants is not well described although there are published data using this combination after bone marrow transplants. The effectiveness of this regimen on the prevention of GVHD was assessed in 107 consecutive sibling and less-than-ideal donor transplant recipients over a 5-year period and compared to that observed in 65 patients receiving standard CSA and short-course MTX without prednisolone. Oral prednisolone was commenced on day +14 at 0.5 mg/kg per day, increased to 1 mg/kg per day on day +21 to day +34 then gradually tapered and ceased by day +100. The cumulative incidence of acute GVHD (grades II–IV) to day 100 in those receiving prednisolone prophylaxis was lower (52 versus 76%, $P < 0.01$). The onset of symptomatic GVHD requiring systemic treatment was delayed from a median of 41 days post transplant to 92 days. When assessment of the cumulative incidence of symptomatic GVHD continued to day +180 incidence became similar (74 versus 78%), there was no difference between the two groups in rates of relapse, transplant-related mortality, infections or chronic GVHD. We conclude that the addition of prednisolone to CSA/MTX delays the onset of early acute GVHD in PBPC recipients but has no impact on the overall incidence of GVHD.

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Introduction

The severity of GVHD and the effects of prolonged exposure to immunosuppressive therapy impact on transplant-related mortality (TRM) and morbidity. One of the

most common prophylactic combinations used to reduce GVHD is CSA/MTX.^{1,2} With this regimen, grade II–IV acute GVHD still occurs in 30–50% of sibling and up to 80% of unrelated recipients.^{2–4} Several investigators have reported the effects of combining prednisolone with CSA/MTX in reducing GVHD after marrow allografts.^{5–13} The schedule of prophylaxis has varied, with prednisolone commencing at days –7, 0, +14 or +15 and continuing until day +35, +110 or to day +180. A delay in onset of acute GVHD has been described in several series,^{6,8,10} but in only one published report in sibling bone marrow recipients has a significant reduction in the overall incidence of grade II–IV acute GVHD been observed.⁵ In that randomized study, prednisolone was commenced on day +14 at a dose of 0.5 mg/kg per day, increased to 1 mg/kg per day from day +21 to day +34 then gradually tapered and ceased by day +110. There was no significant effect on chronic GVHD, infections or either event-free survival (EFS) or overall survival (OS).

No studies have formally described the impact of prophylactic prednisolone in combination with CSA/MTX for recipients of PBPCs although it has been used in this setting.^{14,15} Nevertheless, as PBPCs are now frequently used as the source of reconstituting stem cells and are associated with an increase in severe acute GVHD and chronic GVHD when compared to bone marrow (with the incidence of chronic GVHD ranging from 46 to 65% at 3 years^{16–18}), the addition of prednisolone to standard GVHD prophylaxis warranted investigation.

In October 2000, we added prednisolone to CSA/MTX as previously described,⁵ for most PBPC recipients with the intent of reducing the incidence of acute GVHD and potentially reducing the incidence of chronic GVHD. This report describes our experience over a 5-year period using this protocol.

Patients and methods

Eligibility

Patients were included for analysis if they had received an unmanipulated filgrastim-mobilized PBPC allograft from either a sibling, other relative or unrelated donor using a conditioning regimen associated with $\geq 90\%$ donor chimerism at 1 month post transplant.^{19,20} Using these criteria

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conditioning with fludarabine (125 mg/m²) and either cyclophosphamide (120 mg/kg) or melphalan 120–140 mg/m² were included. Patients were not eligible for this analysis if they died prior to day +14, received a T cell-depleted transplant or received very low intensity conditioning (fludarabine 125 mg/m²; cyclophosphamide 2 g/m²) the latter known to be associated with mixed rather than complete chimerism early post transplant.

From October 2000 to 2006 our unit policy was to use prednisolone prophylaxis with CSA/MTX in patients receiving PBPC allografts. The characteristics and transplant outcomes of 107 patients who received this prophylaxis over this period were compared with a group of 65 patients who received CSA/MTX alone, 47 transplanted concurrently between October 2000 and February 2006 and 18 transplanted between August 1997 and September 2000. CSA/MTX alone was given as routine prophylaxis for all patients between 1997 to September 2000 and beyond 2000 in the following selected patients: those undergoing a transplant after conditioning with fludarabine and high-dose cyclophosphamide, the majority as part of an acute myeloid leukaemia study¹⁹ where CSA/MTX was mandated as GVHD prophylaxis ($n=18$), those with invasive fungal infections prior to or during the transplant ($n=9$), in some patients with a very high risk of relapse ($n=13$) in whom an early graft versus leukaemia effect was considered essential and in those with type I diabetes or on other studies that excluded prednisolone prophylaxis ($n=7$). Data were collected at regular intervals over the 5-year period with follow-up being to at least 6 months post transplant.

Donor–recipient matching

Serological and molecular typing for class I and II antigens was performed for all recipient:donor pairs from 2000 onwards. Prior to 2000, class I typing was performed by serology and class II by molecular typing. Donors were defined as sibling (HLA matched at A, B, C and DR) or less-than-ideal donors, encompassing related non-sibling and unrelated donors.

Disease risk at time of transplant

Standard risk disease was defined as acute leukaemia in first remission, chronic myeloid leukaemia in chronic phase and untreated myelodysplasia or myelofibrosis. High-risk disease was defined as acute leukaemia beyond first remission, all lymphoma, myeloma and severe aplastic anaemia.

GVHD prophylaxis

Patients received i.v. CSA at a dose of 3 mg/kg as a 24 h continuous infusion from day –1 except for patients conditioned with fludarabine and melphalan who received 5 mg/kg from days –1 to +1 then 3 mg/kg daily. Once oral medication was tolerated, CSA was continued at 5–6 mg/kg per day with dose adjustments according to toxicity rather than trough levels, although the latter were routinely measured on a weekly basis until day +100. CSA was tapered in the absence of GVHD or relapse from day +100 to day +180. MTX 15 mg/m² i.v. was administered on day +1 and 10 mg/m² per day on days +3, +6 and +11; folic acid rescue was not given routinely. Prednisolone

prophylaxis commenced on day +14 at 0.5 mg/kg per day, increased to 1 mg/kg per day from day +21 to day +34, decreased to 0.5 mg/kg per day from day +35 to day +48, then to 0.25 mg/kg per day from day +49 to day +69 and to 0.12 mg/kg per day from day +70 to day +89 and 0.12 mg/kg on alternate days from day +90 to day +99 and then ceased.⁵

Acute GVHD assessment and treatment

Assessment of acute GVHD was performed according to conventional criteria,²¹ including histological confirmation for gastrointestinal GVHD. Topical therapy or observation was standard management for grade I GVHD. A requirement for additional or new systemic therapy was considered defining for grade II or greater GVHD. This assignment method assisted with delineating between grade I and grade II GVHD as well as defining the failure of prophylactic prednisolone. The day of onset of symptomatic acute GVHD requiring systemic immunosuppressive therapy was recorded. In the prednisolone group, this was identified as the day a patient required an increase in prednisolone or the addition of another systemic agent. In the non-prednisolone group, this was identified as the day a patient required the introduction of prednisolone or another systemic agent to treat acute GVHD.

Acute grade II–IV GVHD was primarily treated with oral prednisolone or i.v. methylprednisolone (MP) and i.v. CSA. MP dose was based on severity of symptoms with the most common initial dose being 2 mg/kg per day. If ineffective, higher doses of up to 1 g per day were given for short periods. Tacrolimus was substituted for CSA in patients with steroid-resistant gut GVHD and anti-thymocyte globulin (ATG) was added if symptoms remained severe. Steroid-resistant skin or liver GVHD was generally managed with the addition of mycophenolate mofetil.

Although traditionally, chronic GVHD has been defined as any GVHD occurring after day +100, it has been recognized that clinical features of acute GVHD may present beyond this time especially in the setting of donor lymphocyte infusions, after immunosuppressive withdrawal or in reduced-intensity conditioning recipients.²² Consequently, for the purposes of this study we prospectively elected to include any first GVHD episode prior to day +180 requiring systemic treatment in the acute GVHD analysis.

Chronic GVHD

Chronic GVHD was graded according to established criteria.²² The incidence and extent of chronic GVHD at 1 and 2 years post transplant were recorded, as were immunosuppressive requirements over that period. Patients were included if they were alive, had reached the 1- and 2-year time point and were in continuous remission from time of transplant to avoid confounding from salvage therapies. Data represent the maximum grade of chronic GVHD between day +180 to 1 year and 1–2 years.

Supportive care

Identical supportive care regimens were given to both groups. Fluconazole was used for fungal prophylaxis from

day 0 until day +100 and thereafter if receiving steroids at a dose of ≥ 10 mg per day; with liposomal amphotericin or voriconazole used in patients with a prior history or current fungal infection. Aciclovir or valaciclovir was used for herpes simplex virus and varicella-zoster prophylaxis and trimethoprim-sulfamethoxazole for pneumocystis jirovecii pneumonia prophylaxis. The early management of CMV infection varied over the study period. Patients between 1997 and 2001 received thrice weekly prophylaxis with 5 mg/kg ganciclovir i.v. or 3 g daily orally from day +21 to day +84.²³ Subsequently, patients were screened for CMV reactivation weekly by PCR from engraftment until day +100 and thereafter if still taking corticosteroids. Patients with a positive PCR test received therapeutic ganciclovir 5 mg/kg twice a day i.v. for 1–3 weeks. Broad-spectrum antibiotics were used for episodes of suspected bacterial sepsis.

Infections

Infections in each group were recorded from day +14 to day +180. Bacterial infections were defined as any culture-positive bloodstream infection (peripheral blood or via a central access device, most commonly Hickman's catheter) or any pneumonia with a positive sputum culture with symptoms requiring i.v. antibiotic therapy. The incidence of the first bacterial infection was recorded.

For the purpose of evaluation of CMV reactivation rates, the occurrence of the first episode of CMV reactivation or invasive CMV infection was recorded. For this particular analysis, patients were excluded if both donor and recipient were CMV seronegative or if they had received prophylactic ganciclovir.

The incidence of new invasive fungal infections was compared between the groups. Only instances of probable or proven invasive fungal infection defined according to published criteria²⁴ were included.

Complications of prophylactic prednisolone

Any modification of prophylactic prednisolone dose for related side effects, notably hyperglycaemia or mood/sleep disturbance was documented. Bone densitometry was performed before transplant and at day +100. Changes were noted as percentage change from baseline at lumbar spine, total hip or femoral neck. Patients were excluded from the analysis of the effects of prednisolone prophylaxis on bone mineral density, if they had received bisphosphonate therapy as part of a trial²⁵ or for myeloma or if no report was available for either assessment.

Additional data

Data were collected concerning the length of stay after transplantation (from day 0 to day of discharge), TRM, relapse and the reasons for and duration of all re-admissions until day +180.

Statistical methods

The primary analysed outcome was the occurrence of grade II–IV acute GVHD as a time to event measure. Patients who died, relapsed, commenced interferon or who had

immunosuppressive therapy withdrawn because of disease progression were included as censored observations on the day of the event. The proportional hazard comparing the two groups was not constant, so the effect of prednisolone on cumulative probability of acute GVHD was assessed using the Wilcoxon (Breslow) test. Data are represented as a Kaplan–Meier failure function (the inverse of the survivor function). The estimated cumulative probability of GVHD at days +50, +100 and +180 are reported with 95% confidence intervals (CI). Cumulative probability of acute GVHD was also analysed by donor source (sibling and less-than-ideal donor) and the predominant conditioning regimens (Cy TBI, Flu Mel and Flu Cy combined) between the two groups.

TRM was any death due to a transplant-related complication, other than relapse of the underlying haematological disease. Analysis times were censored on the day of death from relapse. EFS was defined as survival in complete remission without relapse or death in remission. OS was death from any cause.

The relationships between GVHD prophylaxis and cumulative mortality, EFS or OS were assessed using a Cox proportional hazards model and are represented in terms of a hazard ratio (HR) and 95% CI. An HR >1 indicates a greater probability of death or relapse at any given time in those receiving CSA/MTX/Pred. Data are represented in terms of the Kaplan–Meier failure function for TRM and the survivor function for EFS and OS.

Rates of re-admissions and infections between the two groups were evaluated by the crude cumulative incidence at day +180 and expressed as a relative risk with 95% CI. A two-sample *t*-test was used to compare the mean percentage change in bone mineral density between the two groups. Statistical analyses were performed using GraphPad Prism Version 4.01 (GraphPad Software 2004, San Diego CA, USA) and Stata Version 8.0 (StataCorp 2003, Stata Statistical Software, Release 8.0, Stata Corporation, College Station, TX, USA).

Results

Characteristics of the patients and donors in the two groups are shown in Table 1. Patients were similar for age, sex, disease and risk category at the time of transplant. There was a greater proportion of both reduced-intensity transplants and sibling donors in the CSA/MTX group.

Length of stay and engraftment

Median length of transplant admission was 21 days (range 15–76) in the CSA/MTX/Pred group and 23 days (range 12–52) in patients receiving CSA/MTX. The median time to neutrophil engraftment $\geq 0.5 \times 10^9/l$ was 16 days in both groups and to platelet engraftment $\geq 20 \times 10^9/l$ was 15 days in the CSA/MTX/Pred group and 16 days in the other.

Acute GVHD

The cumulative probability of acute GVHD up to day +180 is shown in Figure 1. Prior to day +100, a striking difference in incidence of GVHD was observed. At day

+50 in the CSA/MTX/Pred group incidence of acute GVHD was 28 (21, 38%) versus 63% (51, 75%) for the CSA/MTX patients, by day +100 for the CSA/MTX/Pred incidence of acute GVHD was 52 (43, 62%) versus 76% (65, 86%) for the CSA/MTX patients. The median day of acute GVHD onset was 92 days for patients receiving prophylactic prednisolone versus 41 days for those receiving CSA/MTX alone.

After day +100 the incidence of new acute GVHD in the CSA/MTX group was minimal, whereas it steadily increased in the CSA/MTX/Pred group. By day +180 the incidence of acute GVHD was similar between the two

groups (74 versus 78%). The characteristics of GVHD occurring from day +100 to day +180 in these patients resembled acute GVHD with the most prominent sites being in the gastrointestinal tract with diarrhoea as the symptom and in the liver.

When the cumulative incidence and risk of acute GVHD were assessed according to donor source (sibling and less-than-ideal donor) and conditioning type (Cy TBI, Flu Mel and Flu Cy combined), the pattern of difference between the two groups was maintained.

No difference in the distribution of severity of acute GVHD was observed between the two groups (Table 2). The number of patients requiring ATG between the two groups was similar, 12 (11%) in those receiving CSA/MTX/Pred and 8 (12%) in the CSA/MTX group.

Cyclosporin levels were assessed in all patients from 2000 onwards and when assessed at day +30 and day +60 levels were similar between those receiving CSA/MTX/Pred and CSA/MTX. At day +30 for the CSA/MTX/Pred group the median CSA level was 295 versus 311 µg/l for the CSA/MTX group. At day +60 for the CSA/MTX/Pred group the median CSA level was 244 versus 256 µg/l for the CSA/MTX patients.

Table 1 Characteristics of patients and donors

	Prophylaxis	
	CSA/MTX/Pred	CSA/MTX
Total no	107	65
Female/male	51/56	30/35
Age at transplant year, median (range)	45 (18–64)	46 (17–60)
<i>Disease, n (%)</i>		
AML	47 (44)	25 (38)
ALL	15 (14)	6 (9)
NHL	17 (16)	8 (12)
MDS/MF	7 (7)	3 (5)
CLL	6 (6)	8 (12)
CML	4 (4)	7 (11)
Myeloma	4 (4)	4 (6)
HL	3 (3)	2 (3)
Other	4 (4)	2 (3)
<i>Conditioning regimen, n (%)</i>		
Cy TBI	43 (40)	17 (26)
Flu Mel	29 (27)	15 (23)
TBI VP16	15 (14)	6 (9)
Bu Cy	9 (8)	4 (6)
Bu Cy VP16	5 (5)	1 (2)
Flu Cy	2 (2)	18 (28)
Other	4 (4)	4 (6)
<i>Disease risk at transplant, n (%)</i>		
Standard	31 (29)	23 (35)
High	76 (71)	42 (65)
<i>Donor, n (%)</i>		
Sibling, fully matched	69 (64)	50 (77)
Unrelated–A, B, C, DR matched	34 (32)	11 (17)
1 antigen mismatch	1 (1)	2 (3)
Related non-sibling–A, B, C, DR matched	3 (3)	2 (3)
Sex mismatched, total no.	41	30
Female donor–male recipient	14/41 (34)	13/30 (43)
Transplanted donor cells	5.1 (1.7–16)	5.6 (1.9–15)
CD34 × 10 ⁶ per kg, median (range)		
CMV + donor and/or recipient	82 (77)	53 (82)

Abbreviations: AML=acute myeloid leukaemia; ALL=acute lymphoblastic leukaemia; CML=chronic myeloid leukaemia; CSA=cyclosporin; HL=Hodgkin lymphoma; MDS=myelodysplasia; MF=myelofibrosis; MTX=methotrexate; NHL=non-Hodgkin lymphoma; Pred=prednisolone.

Cy TBI=cyclophosphamide 120 mg/kg and total body irradiation 12 Gy; Flu Mel=fludarabine 125 mg/m² and melphalan 120 mg/m²; TBI VP16=etoposide 60 mg/kg and fractionated TBI 1320 cGy; Bu Cy=busulphan 16 mg/kg and cyclophosphamide 120 mg/kg; Bu CyVP16=busulphan 16 mg/kg, cyclophosphamide 120 mg/kg and etoposide 500 mg/m²; Flu Cy=fludarabine 125 mg/m² and cyclophosphamide 120 mg/kg.

Chronic GVHD

The patterns of occurrence and severity of chronic GVHD are reported in Table 3. There were 75 (70%) patients in the CSA/MTX/Pred group evaluable at 1 year and 41 (63%) patients in the CSA/MTX group. At 2 years, 48 (45%)

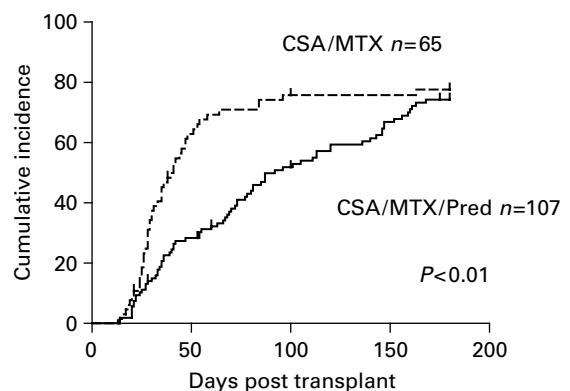


Figure 1 Cumulative incidence of GVHD requiring an increase in or addition of systemic immunosuppression to 180 days post transplant in patients receiving or not receiving prednisolone prophylaxis.

Table 2 GVHD by grade to day +180 between the two groups

Grade, n (%)	Prophylaxis	
	CSA/MTX/Pred (n = 107)	CSA/MTX (n = 65)
0–I	31 (29)	16 (25)
II	52 (49)	36 (55)
III–IV	24 (22)	13 (20)

Abbreviations: CSA=cyclosporin; MTX=methotrexate; Pred=prednisolone.

patients in the CSA/MTX/Pred group were eligible for evaluation in comparison to 31 (48%) patients in the CSA/MTX group. Extensive chronic GVHD at 1 year in the CSA/MTX/Pred group was 72 versus 64% for the CSA/MTX group and at 2 years 66 versus 45% for CSA/MTX patients. The crude incidence of extensive chronic GVHD was high in both groups with a greater amount of extensive chronic in those who had received prior prednisolone prophylaxis. This was likely influenced by an imbalance between the groups with respect to unrelated donors, with 50% of the 2-year survivors in the CSA/MTX/Pred group being recipients of less-than-ideal donor transplants compared to only 16% in the CSA/MTX group.

Rates of re-admission and infection

As expected, many patients experienced significant transplant-related morbidity. Re-admission for non-disease-related reasons was common for both groups (Table 4) with prednisolone not increasing the risk of re-admissions (relative risk, RR 1.14 (0.93, 1.40), $P=0.19$), nor did it

impact on the risk of bacterial or fungal infections (Table 5). However, among those receiving prophylactic prednisolone there was some evidence of an increased risk of CMV reactivation with 69% of CSA/MTX/Pred patients having an episode of CMV viraemia compared to 48% of CSA/MTX patients (RR 1.43 (0.97, 2.10), $P=0.05$). As a result, re-admissions for i.v. treatment with ganciclovir for CMV viraemia were more frequent in the CSA/MTX/Pred group. Invasive CMV disease occurred in only one patient from each group affecting the gastrointestinal tract in both cases. Invasive fungal infections were uncommon in both groups. There were no deaths primarily due to CMV disease or fungal infections in either group.

Complications of prophylactic prednisolone

Six patients required a reduction or cessation of prophylactic prednisolone due to hyperglycaemia necessitating the initiation or increase in dose of oral hypoglycaemic therapy or insulin. No patients in the CSA/MTX group required therapy for hyperglycaemia in the absence of prednisolone

Table 3 Chronic GVHD to 2 years post transplant

	Interval post transplant			
	Day +180 to 1 year		1–2 years	
	CSA/MTX/Pred	CSA/MTX	CSA/MTX/Pred	CSA/MTX
Evaluable, <i>n</i>	75	41	48	31
Nil or limited, <i>n</i> (%)	21 (28)	15 (36)	16 (33)	17 (55)
Extensive, <i>n</i> (%)	54 (72)	26 (64)	32 (66)	14 (45)

Abbreviations: CSA = cyclosporin; MTX = methotrexate; Pred = prednisolone.

Table 4 Non-relapse re-admissions for day +180

	CSA/MTX/Pred	CSA/MTX	RR (95% CI)	P-value
Number evaluable for re-admission	103	59		
Re-admitted, <i>n</i> (%)	80 (78)	40 (68)	1.14 (0.93,1.40)	0.19
Median days in hospital for patients re-admitted				
Days (range)	16 (1–123)	12 (1–130)		0.24
<i>Primary reason for re-admission</i>				
GVHD, <i>n</i> (%)	40 (30)	24 (40)		
CMV, viraemia <i>n</i> (%)	40 (30)	9 (15)		
Bacteraemia, <i>n</i> (%)	25 (19)	15 (26)		
Other, <i>n</i> (%)	30 (22)	13 (21)		

Abbreviations: CI = confidence interval; CMV = cytomegalovirus; CSA = cyclosporin; GVHD = graft-versus-host disease; MTX = methotrexate; Pred = prednisolone; RR = relative risk.

Table 5 Infections to day +180

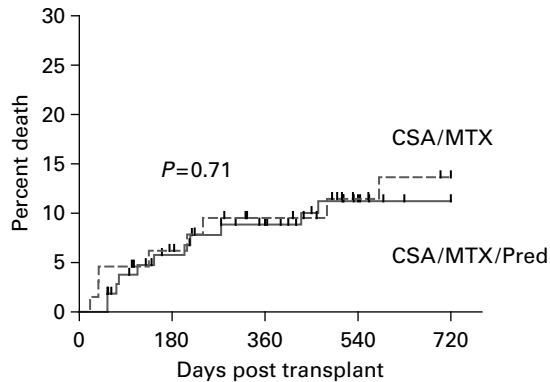
	CSA/MTX/Pred	CSA/MTX	RR (95% CI)	P-value
Type of infection	107	65		
Bacterial, <i>n</i> (%)	36 (34)	17 (26)	1.28 (0.79, 2.09)	0.39
CMV viraemia/other CMV disease, <i>n</i> (%)	50/72 (69)	16/33 (48)	1.43 (0.97, 2.10)	0.05
Invasive fungal, <i>n</i> (%)	5 (5)	4 (6)	0.75 (0.21, 2.73)	0.73

Abbreviations: CI = confidence interval; CMV = cytomegalovirus; CSA = cyclosporin; GVHD = graft-versus-host disease; MTX = methotrexate; Pred = prednisolone; RR = relative risk.

Table 6 Percentage changes in BMD from pre-transplant to day +100

Site	CSA/MTX/Pred			CSA/MTX			P-value
	n	Mean	95% CI	n	Mean	95% CI	
Lumbar spine	64	-5.01	(-5.85, -4.68)	32	-4.53	(-6.07, -2.99)	0.56
Total hip	63	-7.02	(-7.98, -6.06)	29	-6.76	(-8.19, -5.33)	0.77
Femoral neck	63	-7.23	(-8.48, -5.98)	30	-6.36	(-7.94, -4.78)	0.42

Abbreviations: CI = confidence interval; CSA = cyclosporin; MTX = methotrexate; Pred = prednisolone.

**Figure 2** Cumulative incidence of TRM in patients receiving or not receiving prednisolone prophylaxis up to 2 years post transplant.

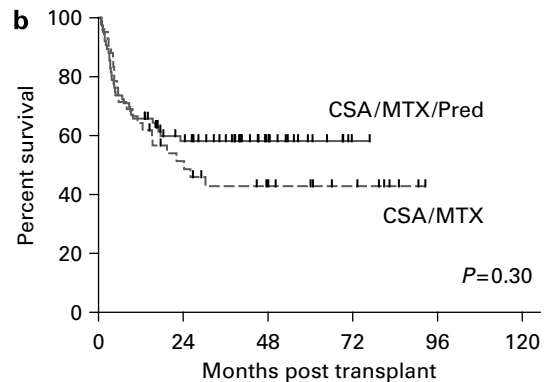
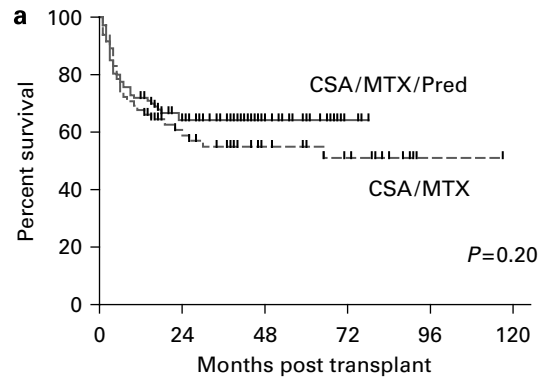
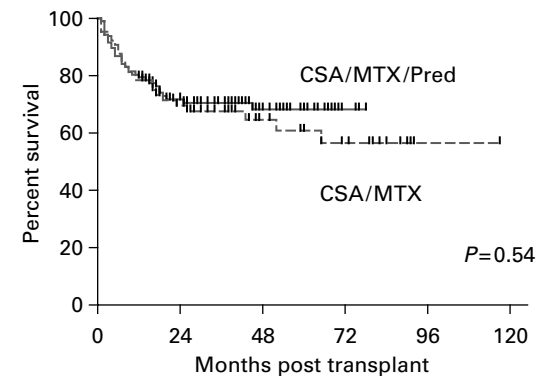
therapy. Five patients receiving CSA/MTX/Pred required a dose reduction of prednisolone for insomnia, agitation or worsening of depression.

Mean percentage changes of lumbar spine, total hip and femoral neck bone mineral density from pre-transplant to day +100 are depicted in Table 6. While overall bone mineral density loss was substantial, consistent with previous reports,²⁵ no difference was observed between the two groups for any site.

Survival and transplant-related mortality

Median follow-up for all surviving patients in remission was between 48 months (range 14–117) in those who received CSA/MTX and 39 months (range 13–78) in the CSA/MTX/Pred patients. When analysed over a 2-year period, TRM was similar in both groups (HR 0.85 (0.33, 2.12), $P=0.71$). The cumulative incidence of TRM for the CSA/MTX/Pred and CSA/MTX groups at day +180 was 5.7 and 6.2%, at 1 year 8.8 and 9.5% and at 2 years 11.2 and 13.6% respectively (Figure 2). Over 2 years, there were seven deaths due to GVHD among those who had received CSA/MTX/Pred, four within the first 180 days. Three deaths from GVHD over 2 years occurred in patients who received CSA/MTX, of which one occurred within the first 180 days.

EFS was not different between the two groups (HR = 0.73 (0.45, 1.18), $P=0.20$; Figure 3a). The estimated probability of EFS for the CSA/MTX/Pred versus CSA/MTX groups at 1 year was 72 (62, 79%) versus 68% (55, 78%) and 64 (54, 73%) versus 58% (45, 69%) at 2 years. In

**Figure 3** (a) EFS in patients receiving or not receiving prednisolone prophylaxis. (b) EFS in patients with high-risk disease receiving or not receiving prednisolone prophylaxis.**Figure 4** OS in patients receiving or not receiving prednisolone prophylaxis.

addition, there was no evidence of a difference in EFS for high-risk disease patients when assessed between the two groups (HR 0.75 (0.43, 1.3), $P=0.30$; Figure 3b). OS between the two groups was also similar (HR = 0.84 (0.48, 1.46), $P=0.54$; Figure 4).

Discussion

This study demonstrates that the use of prophylactic prednisolone delays the onset of acute GVHD in PBPC recipients. In addition, it is associated with reductions in the incidence of acute GVHD requiring systemic therapy to

day + 100 in both sibling and unrelated recipients. Despite this effect, the overall acute GVHD incidence to day + 100 was 52% for patients receiving prophylactic prednisolone. Although this is consistent with other reports of acute GVHD in PBPC recipients,^{4,16} we had anticipated the addition of prednisolone would have had a greater effect on reducing GVHD. Ruutu *et al.*⁵ reported an incidence of grade II–IV GVHD of 13% for bone marrow recipients receiving prophylactic prednisolone up to day +110. Despite using a similar schedule this could not be replicated in our cohort of PBPC recipients where we observed an overall acute GVHD incidence of 52% to day + 100. This was non-randomized therapy with the majority of data collected retrospectively and certain imbalances existed between the two groups analysed, in particular the proportion of unrelated donor transplants and the imbalance between conditioning regimens. These factors limit the scope of the analysis. To minimize these sources of bias, a subset analysis including donor source and conditioning type was assessed with the patterns of difference in cumulative incidence of acute GVHD between the two groups being maintained.

The most likely explanation for different outcomes between this study and Ruutu *et al.*⁵ is that the effects of adding prednisolone differ between recipients of BM grafts and recipients of PBPC grafts. However, other points of difference between the studies in terms of design could also contribute to the variations in observed outcome. Our analysis included recipients of both unrelated and sibling transplants, whereas in the previous study only sibling transplants were included. In addition, our management of acute GVHD differed, in that we did not treat grade I GVHD nor did we use MP at a dose of 10 mg/kg per day in patients with acute GVHD. The dose and tapering schedule for CSA was also different; we used a higher oral dose and tapered CSA in the absence of GVHD from day + 100 to day + 180 in comparison to continuing it until 1 year. These differences may explain the higher incidence of acute GVHD that we experienced.

Despite these methodological differences, it appears that the addition of prednisolone to CSA/MTX does not have the same benefit in PBPC transplants as observed for bone marrow transplants. By day + 180, acute GVHD incidence was similar between the two groups, demonstrating that prophylactic prednisolone most likely delayed acute GVHD onset rather than preventing its occurrence. Similarly, we could find no evidence that the addition of prednisolone prophylaxis impacted on the rates of severe or fatal GVHD.

Several previous randomized studies in sibling bone marrow transplants,^{6–10} although varying in duration of prednisolone prophylaxis and in some, comparing CSA/MTX/Pred to CSA/Pred have generally found no reduction in acute GVHD incidence. In the unrelated donor bone marrow transplant setting, grade II–IV GVHD incidence did not significantly differ with the addition of prednisolone to CSA/MTX.²⁶

Our study confirms previous observations that the addition of prophylactic prednisolone has no significant effect on chronic GVHD onset.^{5–9,12,13} Extensive chronic GVHD remained a substantial toxicity for both groups.

Consistent with other reports,^{16–18} the use of PBPCs as the stem source is likely to have contributed significantly to high rate of chronic GVHD observed in our study.

We found that the use of prednisolone prophylaxis was generally well tolerated although 10% of patients did require dose reduction or cessation due to hyperglycaemia and neuropsychiatric problems. Use of prednisolone did not contribute to an increased risk of bacterial or fungal infections or a greater loss of bone mineral density. There was some evidence of more CMV reactivation in those receiving prednisolone prophylaxis although we did not analyse whether this was independent of other factors such as donor:recipient CMV serostatus and related versus unrelated donor source. There was no evidence of an effect on survival, with EFS, OS and TRM being similar between the two groups.

In conclusion, this study demonstrated that prednisolone prophylaxis slows the rate of onset of acute GVHD in PBPC recipients but is unable to reduce GVHD incidence or diminish its severity. More effective prophylactic regimens are required.

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