

One-year cyclosporine prophylaxis reduces the risk of developing extensive chronic graft-versus-host disease after allogeneic peripheral blood stem cell transplantation

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Background and Objectives. Chronic graft-versus-host disease (GVHD) remains the most common late complication of allogeneic stem cell transplantation, producing significant long-term morbidity and contributing to a substantial risk of late mortality. Chronic GVHD may be more common, more protracted and less responsive to current treatments after peripheral-blood stem cell (PBSC) transplantation than after bone marrow transplantation. The purpose of this retrospective cohort study was to determine whether the hazard of extensive chronic GVHD after allogeneic PBSC transplantation could be decreased by prolonging cyclosporine A (CsA) prophylaxis over 12 months.

Design and Methods. Fifty-seven consecutive patients with hematologic malignancies who had received a PBSC transplant from an HLA-identical sibling were evaluable for chronic GVHD. All patients began CsA tapering at day 50 but 2 different durations of immunosuppression were used: the first 36 patients were allocated to receive a 6-month course with tapering by 5% at weekly intervals (group A), while the following 21 received a 12-month course with tapering by 5% every 2 weeks (group B).

Results. The cumulative incidence of extensive chronic GVHD at 2 years was 69% (95% CI, 53-85%) for group A and 25% (95% CI, 3-47%) for group B with a significantly lower hazard in group B than in group A (HR=0.2; 95% CI, 0.07-0.57; $p=0.0009$). In multivariate analysis, the 12-month CsA tapering schedule was associated with a significantly decreased risk of extensive chronic GVHD (HR=0.2; 95% CI, 0.06-0.66; $p=0.008$). The hazard of transplant-related mortality, relapse and failure to survive in remission was not significantly different among the 2 groups.

Interpretation and Conclusions. One-year CsA prophylaxis seems to be more effective than the standard six-month CsA regimen at preventing extensive chronic GVHD after PBSC transplant from an HLA-identical sibling. Conclusive assessment of the benefits of such prolonged immunosuppression, in terms of better quality of life and minor morbidity, requires both long-term follow-up to evaluate the rates of relapse and secondary tumors and a randomized setting.

Key words: allogeneic peripheral blood stem cell transplantation, chronic graft-versus-host disease, graft-versus-host disease prophylaxis, cyclosporine A.

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A number of studies comparing the outcomes between allogeneic peripheral blood stem cell transplantation (PBSCT) and bone marrow transplantation (BMT) have been carried out, including seven randomized controlled trials,¹⁻⁷ several retrospective cohort studies,⁸⁻¹⁵ one *International Bone Marrow Transplant Registry/European Blood and Marrow Transplantation Group* (IBMTR/EBMT) database review study¹⁶ and one meta-analysis.¹⁷ However, data on the incidence of chronic graft-versus-host disease (GVHD) according to the source of hematopoietic stem cells (BM vs. PB) are controversial. Particularly, the incidence of chronic GVHD after allogeneic PBSCT was not significantly different from that observed after BMT in five out of the seven randomized trials,^{2,3,5-7} whereas the IBMTR/EBMT database review,¹⁶ and meta-analysis,¹⁷ four cohort trials^{8,9,13,14} and two randomized studies,^{1,4} all reported a significantly higher incidence of chronic GVHD following allogeneic PBSCT. The discrepancy in this finding may be accounted for by several factors, such as the limited statistical power of the analyses because of the small number and short follow-up of the evaluable patients, variability of the regimens used for GVHD prophylaxis, variations in cell composition of the granulocyte colony-stimulating factor (G-CSF) mobilized products which may lead to differences in immunologic properties and, finally, *in vivo* immunomodulatory effects of different schedules of recombinant human G-CSF administered for stem cell mobilization.^{6,18}

However, chronic GVHD remains the most common late complication of allogeneic stem cell transplantation, producing significant long-term morbidity and contributing to a substantial risk of late mortality when the extensive form of disease develops. Results of a recent study suggest that chronic GVHD may be more difficult to control when it occurs after PBSCT than when it occurs after BMT.¹⁹ Although a recent randomized clinical trial did not show any significant advantage from an extended regimen of cyclosporine A (CsA) prophylaxis after allogeneic BMT,²⁰ the hypothesis that chronic GVHD might be prevented by prolonging administration of CsA beyond 6 months had been supported by results from single-arm studies.²¹⁻²³ Studies focusing on different durations of immunosuppression for GVHD prophylaxis after PBSCT have not yet been reported. As recently emphasized,²⁴ it may be worthwhile to evaluate different GVHD prophylaxis regimens in order to try to improve prevention of chronic GVHD after PBSCT.

Herein, we report the results of a comparison between patients who were allocated to receive a 6-month or 12-month course of CsA prophylaxis after PBSCT from an HLA-identical sibling. The purpose was to assess whether the duration of CsA prophylaxis could affect the development of the extensive chronic GVHD.

Design and Methods

Selection of patients. From January 1998 to November 2001, 57 consecutive patients (median age, 39 years) with hematologic malignancies received glycosylated G-CSF-mobilized PBSC from an HLA-identical sibling and survived relapse-free for at least 100 days after transplant. They were, therefore, evaluable for chronic GVHD. Criteria of selection were also the myeloablative conditioning regimen and CsA-methotrexate (MTX) combination as GVHD prophylaxis. Details on PBSC collection have been described elsewhere.²⁵ Informed consent was obtained from all recipients and donors or their guardians. Patient, donor and graft characteristics are summarized in Table 1. Briefly, 1st complete remission of acute leukemia or 1st chronic phase of chronic myeloid leukemia at time of transplant were classified as low-risk phases. All other disease stages were classified as high-risk phases. Twelve Gy fractionated total body irradiation (TBI) was administered to 4 patients, while the remaining 53 patients were prepared with a chemotherapy-based regimen.

GVHD prophylaxis. Two different durations of GVHD prophylaxis with CsA were used. The first 36 patients (until March 2000) were allocated to receive a 6-month course of CsA (group A), while the following 21 patients received a 12-month course of CsA (group B). The decision to extend the CsA tapering schedule was based on the observation of an increased morbidity due to chronic GVHD in group A compared within 30 concurrent marrow recipients given the same 6-month course of CsA (*data not shown*). The CsA was administered intravenously at the dose of 3 mg/kg of b.w. daily from day -1 and then orally at 12.5 mg/kg of b.w. daily as soon as the oral route was tolerated, monitoring blood CsA levels and maintaining trough levels at 200-400 ng/mL. Factors influencing CsA absorption, such as the CsA formulation, antibiotics and other medications given, were consistent throughout the trial. Starting on day 50, oral CsA was tapered until discontinuation: the schedule was a 5% decrease at weekly intervals in the 6-month group and a 5% decrease every 2 weeks in the 12-month group. Four patients, 2 of group A and 2 of group B, did not receive the planned dose schedule because of CsA toxicity and prematurely interrupted their CsA prophylaxis. In addition to CsA, all 57 patients received a complete (4 doses) short

Table 1. Patient, donor and graft characteristics.

Characteristics	Group A (n=36)	Group B (n=21)	p value
Patient sex, male/female	17/19	14/7	0.18
Donor sex, male/female	14/22	12/9	0.27
Gender combination, female donor-male recipient/other		0.76	
Patient age (years), median (range)	38 (20-59)	41 (7-50)	0.84
Donor age (years), median (range)	37 (18-56)	41 (20-48)	0.76
CMV serology, recipient and donor negatives/other	4/32	1/20	0.64
Diagnosis, ALL-AML/CML/MM	19/9/8	11/5/5	0.99
Disease phase at transplant, low-risk/high-risk	21/15	12/9	1
TBI, no/yes	34/2	19/2	0.62
Total nucleated cell dose ($\times 10^8$ /Kg of the recipient), median (range)	11.7 (4.1-51)	9.7 (5-31)	0.41
Mono-nucleated cell dose ($\times 10^8$ /Kg of the recipient), median (range)	7.5 (4.1-22)	5.9 (2.9-14.7)	0.05
CD3 ⁺ cell dose ($\times 10^8$ /Kg of the recipient), median (range)	3.6 (1.3-7.8)	2.4 (0.8-7.7)	0.01
CD34 ⁺ cell dose ($\times 10^6$ /Kg of the recipient), median (range)	6.15 (3.7-14)	6 (4.2-14)	0.53
Acute GVHD, absent/present	18/18*	13/8*	0.31
Acute GVHD, grade 0-1/grade 2	29/7 [§]	17/4 [§]	0.78

*Hazard ratio for group B vs. group A: 0.63 (95% CI, 0.25-1.5).
[§]Hazard ratio for group B vs. group A: 0.84 (95% CI, 0.25-2.88).

course of MTX, consisting of 15 mg/m² on day 1 and 10 mg/m² on days 3, 6 and 11.

Diagnosis of GVHD. Acute and chronic GVHD were diagnosed and graded according to the established criteria.^{26,27} Particularly, chronic GVHD was diagnosed according to the grading scheme for chronic GVHD proposed in 1980 and based on data on 20 subjects from Seattle. Chronic GVHD was classified as limited (only localized skin and/or liver involvement) or extensive (generalized skin or limited disease plus involvement of other organs). This system was developed primarily to distinguish patients requiring systemic immunosuppression

and, although different prognostic grading systems predicting survival of patients with chronic GVHD have been reported, it retains its practical relevance. Extensive chronic GVHD was diagnosed in our series on a clinical basis, supported by studies including hematopoietic and chemical parameters, skin and liver biopsies, Schirmer's test and lung function tests.

Treatment of GVHD. The assigned CsA tapering schedule was discontinued at diagnosis of grade > 1 acute and extensive chronic GVHD, for which immunosuppressive therapy including steroids was given. Patients developing grade > 1 acute GVHD received 2 mg/kg of b.w. daily of 6-methyl-prednisolone for 14 days. Steroid treatment was slowly tapered and then discontinued. No patient developed acute GVHD > grade 2. All patients requiring steroids for their grade 2 acute GVHD were still on therapy at day 80. Patients developing extensive chronic GVHD received steroids alone or in combination with CsA or mycophenolate mofetil as 1st line therapy.

Endpoints and statistical methods. Data were collected in an XLS database and imported into an SPSS 7.5 system for statistical analysis, which was based on the principle of the intent to treat with respect to the CsA prophylaxis. The close-out date for analysis was October 20, 2002. The median follow-up for patients who were alive and at risk of extensive chronic GVHD at the time of analysis was 1,255 days for group A (minimum-maximum follow-up, 1033-1356 days) and 518 days for group B (minimum-maximum follow-up, 340-915 days). The endpoints of this retrospective cohort study were to assess whether the incidence of extensive chronic GVHD differed among the 2 groups, with follow-up to either the time of relapse or date of last contact, and to determine the influence of the duration of CsA prophylaxis on transplant-related mortality (TRM), risk of relapse and disease-free survival (DFS). The characteristics of extensive chronic GVHD at the time of diagnosis were also assessed. Patient, donor and graft characteristics were compared using the χ^2 test for categorical and the Mann-Whitney test for continuous variables. Two-sample t-tests were used to compare the doses of nucleated, mono-nucleated, CD3⁺ and CD34⁺ cells administered. Cumulative incidence rates were used to estimate the probabilities of extensive chronic GVHD, TRM and relapse.²⁸ The actuarial probability of DFS was estimated by the method of Kaplan and Meier.²⁹ Cox proportional hazards regression models were used for univariate and multivariate analyses.³⁰ A log rank test was used to compare hazards, and 2-sided *p* values below 0.05 were considered to be statistically significant. In order to evaluate the predictors of extensive chronic GVHD, the following variables were examined in univariate analysis: patients' sex,

donors' sex, gender combination (female donor-male recipient vs. other combinations), patient age (\leq vs. $>$ median and by quartile), donors' age (\leq vs. $>$ median and by quartile), diagnosis (acute leukemia vs. chronic myeloid leukemia vs. multiple myeloma), phase at transplant (low-risk vs. high-risk), duration of CsA prophylaxis (6-month vs. 12-month), and prior acute GVHD (absent vs. present and grade 0-1 vs. grade 2). Finally, since variations in the cell composition of the blood-grafts may affect the occurrence of extensive chronic GVHD,³¹ the cell variables categorized dichotomously (\leq vs. $>$ median) and by quartile were examined in univariate analysis, and the threshold of $8 \times 10^6/\text{Kg}$ CD34⁺ cells was also assessed. Variables found to be significant were tested in multivariate analysis. A significance level of $p=0.05$ was used for the multivariate analysis. Ninety-five per cent confidence intervals (CI) are reported for the main summary statistics.

Results

Patient, donor and graft characteristics. The demographic characteristics of the patients and donors in the 2 groups were similar (Table 1). The number of total nucleated cells and CD34⁺ cells infused was not different between the 2 groups, while the number of CD3⁺ cells given to group B was significantly lower ($p=0.01$) as was the number of the mono-nucleated cells ($p=0.049$) (Table 1).

Limited chronic GVHD. Five patients developed limited chronic GVHD (group A=1; group B=4), at a median follow-up of 273 days (range, 151-336). Taking group A patients as the reference, the hazard of developing limited chronic GVHD was not significantly different for patients in group B (HR=3.8; 95% CI, 0.42-34.41; $p=0.2$).

Incidence of extensive chronic GVHD. Extensive chronic GVHD developed in 24 out of 36 patients in group A and 4 out of 21 patients in group B, for a cumulative incidence at 2 years from transplant of 69% (95% CI, 53-85%) and 25% (95% CI, 3-47%), respectively (Figure 1). The hazard for extensive chronic GVHD in group B was significantly lower than that in group A ($p=0.0009$) (Table 2). Two patients in group A and 2 in group B discontinued CsA at 151-233 and 20-58 days from transplant, respectively, because of toxicity ($n=3$) or refusal ($n=1$). Extensive chronic GVHD developed in 3 out of these 4 patients, 2 of whom died of fatal pulmonary distress and multi-organ failure.

Characteristics of clinical extensive chronic GVHD. The proportions of patients in group A and B with high-risk features of extensive chronic GVHD are shown in Table 3. These features included progressive type of onset progressive, platelet count $< 100 \times 10^9/\text{L}$, treatment with prednisone at

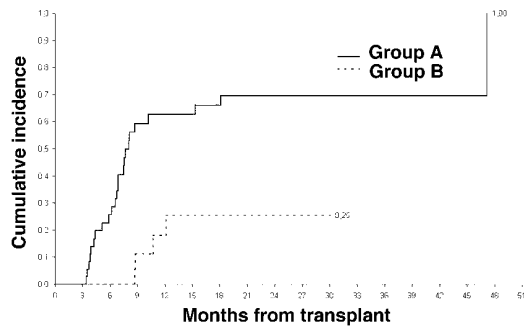


Figure 1. Cumulative incidence of extensive chronic GVHD by CsA prophylaxis (Group A: 6-month course; Group B: 12-month course).

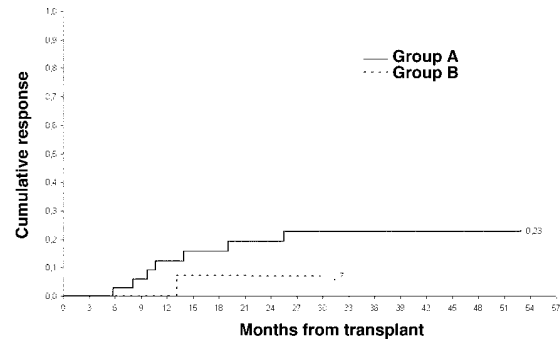


Figure 2. Cumulative incidence of TRM by CsA prophylaxis (Group A: 6-month course; Group B: 12-month course).

Table 2. Influence of duration of CsA prophylaxis on different events: results of univariate analysis.

Duration of CsA prophylaxis	Extensive chronic GVHD		Non-relapse mortality		Relapse		Relapse or death	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
6-mth course	1		1		1		1	
12-mth course	0.2 (0.07-0.57)	0.0009	0.31 (0.04-2.55)	0.25	1.5 (0.5-4.46)	0.46	0.95 (0.38-2.38)	0.92

the time of diagnosis, and extent of skin involvement being $\geq 50\%$ of the body surface area. The small size of group B did not allow any comparison between the cohorts. Skin, mouth, eyes, liver, joints and lungs were the sites most frequently affected by chronic GVHD during the course of the disease; involvement of the vagina was observed in 7 of 15 female patients in group A, while no patient with extensive chronic GVHD in group B was female (Table 3). Bronchiolitis obliterans, keratitis and scleroderma were relatively common complications associated with severe morbidity (Table 3).

Rates of TRM, relapse and DFS. Thirteen of 57 patients (23%) died. For 8 patients the causes of death were transplant-related (group A=7; group B=1) and primarily due to unresponsive chronic GVHD in all of them. The rate of infectious complications was not different between the 2 groups (data not shown). The cumulative incidence of TRM at 2 years was 19% (95% CI, 5-33%) in group A and 7% (95% CI, 1-20%) in group B (Figure 2). The hazard was not significantly different between the 2 groups (Table 2). Relapse, which was the cause of death in 5 patients, occurred in 7 patients in group A and 6 in group B, for a cumulative incidence at

Table 3. Characteristics of extensive chronic GVHD according to the duration of CsA prophylaxis.

Characteristics	Group A (n=24)	Group B (n=4)
Days to extensive chronic GVHD after transplant, median (range)	208 (105-1435)	297 (265-369)
Mode of onset		
progressive	3 (12%)	0
quiescent	11 (46%)	1
de novo	10 (42%)	3
Platelets count $< 100 \times 10^9/L$ at the time of diagnosis	5 (21%)	1
Treatment with prednisone at the time of diagnosis	8 (33%)	1
Extent of skin involvement		
none	4 (17%)	1
$< 50\%$ body surface area	8 (33%)	2
$\geq 50\%$ body surface area	12 (50%)	1
Sites affected at any time		
skin	20 (83%)	3
mouth	13 (13%)	2
eyes	11 (46%)	1
gut	5 (21%)	1
liver	11 (46%)	2
joints	9 (37%)	1
muscles	0	1
vagina	7 (50%)*	0 [§]
esophagus	1 (4%)	0
nails	2 (8%)	0
lungs	8 (33%)	2
serosa	1 (4%)	1
Severe morbidity		
bronchiolitis obliterans	4 (17%)	1
scleroderma	5 (21%)	0
joint contractures	1 (4%)	0
keratitis	5 (21%)	0

*Percentage of female patients with site involvement (7/15).
[§]No female patient in group B.

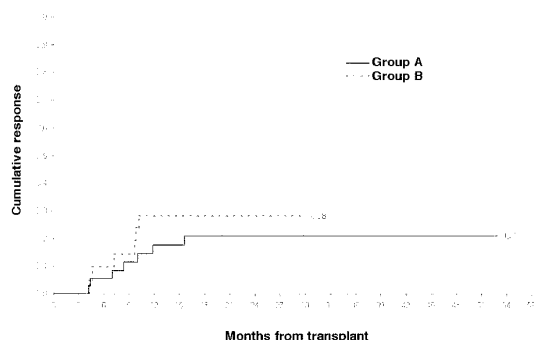


Figure 3. Cumulative incidence of relapse by CsA prophylaxis (Group A: 6-month course; Group B: 12-month course).

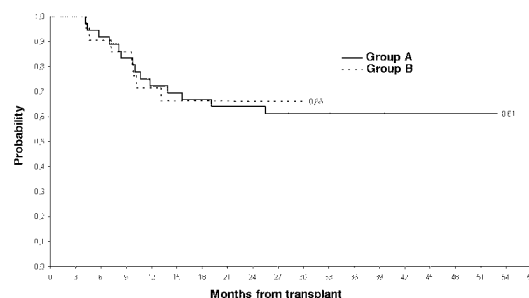


Figure 4. Probability of DFS by CsA prophylaxis (Group A: 6-month course; Group B: 12-month course).

2 years of 21% (95% CI, 7–35%) and 28% (95% CI, 9–47%), respectively (Figure 3). The hazard was not significantly different between the 2 groups (Table 2). The probability of DFS at 2 years was 64% (95% CI, 48–80%) for group A and 66% (95% CI, 46–86%) for group B (Figure 4). The risk of failure to survive without recurrent disease was not significantly different between the 2 groups (Table 2).

Overall Cox regression analysis. In univariate analysis, four factors other than the duration of CsA prophylaxis ($p=0.0009$) were significantly associated with the probability of developing extensive chronic GVHD: donor's sex ($p=0.001$), gender combination ($p=0.02$), disease phase at transplant ($p=0.04$) and CMV serology ($p=0.05$), while a trend towards an association between the CD34⁺ cell dose and extensive chronic GVHD ($p=0.14$) was observed (Table 4). Among the strongest factors known to be associated with the chronic GVHD, neither patient's and donor's age nor prior acute GVHD influenced the likelihood of extensive chronic GVHD (Table 4). In multivariate analysis, after adjustment for the previous five variables and mono-nucleated, CD3⁺ and CD34⁺ cell doses, the female sex of the donor and 6-month CsA tapering schedule were associated with a significantly increased risk of extensive chronic GVHD (Table 5). Finally, a second round of multivariate analysis was carried out testing the acute GVHD together with the other 8 variables; the inclusion of acute GVHD in the model did not change the results significantly (*data not shown*).

Discussion

In this retrospective cohort study comparing a 6-month vs. 12-month course of CsA administration after allogeneic PBSCT we observed a significant advantage for the prolonged CsA prophylaxis in

reducing the incidence of extensive chronic GVHD. The duration of the CsA prophylaxis together with the donor's sex were independent predictors of extensive chronic GVHD.

In 5 out of 7 randomized trials the rates of extensive chronic GVHD ranged from 15% up to 71% after PBSCT and from 7% up to 55% after BMT, with a median follow-up ranging from 12 up to 27 months.^{2,4–7} The remaining 2 randomized trials reported probabilities of chronic GVHD at 2 years of 67% and 74% after PBSCT and of 54% and 53% after BMT, respectively, but did not specify the incidence of the extensive disease.^{1,3} The IBMTR/EBMT retrospective analysis showed a 1-year probability of extensive chronic GVHD of 33% after PBSCT and 26% after BMT.¹⁶ Similarly, the reported incidence of extensive chronic GVHD differed very widely between single arm studies, ranging from 17% up to 100%.^{32–34} The lack of statistical power of several studies, the heterogeneity of the regimens used for GVHD prophylaxis and the different G-CSF schedules administered to the donors may account for the variability of the reported data.^{6,18}

Chronic GVHD is usually considered to harbor the beneficial graft-versus-leukemia effect resulting in fewer leukemia relapses. On the other hand, it may be associated with more late deaths and worse long-term quality of life.³⁵ Recently, Lee *et al.*,³⁶ by using 3 different grading systems (limited/extensive, a clinical impression scale and a new severity score), showed that patients identified as having extensive chronic GVHD, severe involvement or high-risk disease were likely to have significantly worse survival than patients without or with less severe chronic GVHD due to greater TRM without the benefit of fewer relapses.

Our study is the first one on the incidence of the extensive chronic GVHD comparing the standard

Table 4. Univariate analysis of risk factors for extensive chronic GVHD.

Factors	No. of patients	Extensive chronic GVHD, HR (95% CI)	p value
Patient sex			
female	26	1	
male	31	0.74 (0.35-1.58)	0.44
Donor sex			
male	26	1	
female	31	3.95 (1.64-9.51)	0.001
Gender combination			
female donor-male recipient	16	1	
other combinations	41	0.39 (0.18-0.86)	0.02
Patient age			
> median of 39 years	28	1	
≤ median of 39 years	29	0.87 (0.41-1.89)	0.75
Patient age			
> 45 years (1 st quartile)	13	1	
> 39 and ≤ 45 years (2 nd quartile)	14	1.16 (0.38-3.47)	0.78
> 31 and ≤ 39 years (3 rd quartile)	14	0.97 (0.34-2.78)	0.96
≤ 31 years (4 th quartile)	16	1.19 (0.41-3.41)	0.74
Donor age			
> median of 37 years	28	1	
≤ median of 37 years	29	0.82 (0.38-1.75)	0.6
Donor age			
> 44 years (1 st quartile)	15	1	
> 37 and ≤ 44 years (2 nd quartile)	13	0.78 (0.27-2.21)	0.65
> 26 and ≤ 37 years (3 rd quartile)	16	0.43 (0.14-1.3)	0.14
≤ 26 years (4 th quartile)	13	1.47 (0.54-3.99)	0.44
Diagnosis			
multiple myeloma	13	1	
chronic myeloid leukemia	14	0.73 (0.24-2.2)	0.58
acute leukemia	30	0.83 (0.33-2.08)	0.7
Disease phase at transplant			
low-risk	33	0.45 (0.2-0.96)	0.04
high-risk	24	1	
CMV serology			
recipient and donor negatives	5	1	
other combinations	52	0.35 (0.12-1.04)	0.05
CsA duration			
6 mo. (group A)	36	1	
12 mo. (group B)	21	0.2 (0.07-0.57)	0.0009
Prior acute GVHD			
present (grade 1-2)	26	1	
absent	31	0.7 (0.33-1.5)	0.37
Prior acute GVHD			
grade 2 (all on steroids at day 80)	11	1	
grade 0-1	46	0.67 (0.28-1.59)	0.36
Total nucleated cell dose			
≤ median of 11.4×10 ⁹ /kg b.w.	26	1	
> median of 11.4×10 ⁹ /kg b.w.	26	1.35 (0.63-2.89)	0.44
Mono-nucleated cell dose			
≤ median of 6.9×10 ⁸ /kg b.w.	28	1	
> median of 6.9×10 ⁸ /kg b.w.	27	1.92 (0.88-4.2)	0.09
CD3 ⁺ cell dose			
≤ median of 3.2×10 ⁸ /kg b.w.	29	1	
> median of 3.2×10 ⁸ /kg b.w.	28	1.3 (0.6-2.76)	0.5
CD3 ⁺ cell dose			
≤ 2.1×10 ⁸ /kg b.w. (1 st quartile)	14	1	
> 2.1 and ≤ 3.2×10 ⁸ /kg b.w. (2 nd quart.)	15	2.41 (0.78-7.41)	0.12
> 3.2 and ≤ 4.2×10 ⁸ /kg b.w. (3 rd quart.)	14	1.65 (0.5-5.44)	0.4
> 4.2×10 ⁸ /kg b.w. (4 th quartile)	14	2.44 (0.79-7.48)	0.11
CD34 ⁺ cell dose			
≤ median of 6×10 ⁶ /kg b.w.	29	1	
> median of 6×10 ⁶ /kg b.w.	28	1.75 (0.82-3.76)	0.14
CD34 ⁺ cell dose			
≤ 8×10 ⁶ /kg b.w.	41	1	
> 8×10 ⁶ /kg b.w.	16	1 (0.42-2.39)	0.98
CD34 ⁺ cell dose			
≤ 5.1×10 ⁶ /kg b.w. (1 st quartile)	14	1	
> 5.1 and ≤ 6×10 ⁶ /kg b.w. (2 nd quartile)	15	0.54 (0.16-1.8)	0.7
> 6 and ≤ 8.9×10 ⁶ /kg b.w. (3 rd quartile)	14	1.54 (0.57-4.14)	0.38
> 8.9×10 ⁶ /kg b.w. (4 th quartile)	14	1.22 (0.44-3.37)	0.32

CsA schedule and a more extended tapering of CsA after PBSCT. Prolonged administration of CsA was reported to decrease the risk of chronic GVHD after BMT in three single-arm studies,²¹⁻²³ while a recent randomized trial did not find any significant reduction of the incidence of extensive chronic GVHD in allogeneic bone marrow recipients in whom CsA administration was prolonged for 24 months.²⁰ In the current study, all demographic risk factors for chronic GVHD were equally distributed among the cohorts of patients. Group B received statistically smaller CD3⁺ and mono-nucleated cell loads than did group A. Since T-cells are the mediators of GVHD, this could theoretically have influenced the study results. However, it is our opinion that the higher risk of extensive chronic GVHD among patients in group A was not CD3⁺ cell load-dependent, because no significant difference in the incidence of extensive chronic GVHD was observed when this cell variable was assessed dichotomously and by quartile. Furthermore, Maciej Zaucha *et al.*³¹ recently reported that patients receiving a peripheral blood CD34⁺ cell dose in excess of 8 × 10⁶/Kg from an HLA-identical sibling had a higher risk of developing extensive chronic GVHD, whereas neither CD3⁺ nor CD14⁺ doses were significantly associated with chronic GVHD. In the current study an infused CD34⁺ cell dose above the median of 6 × 10⁶/Kg or above 8 × 10⁶/Kg was not significantly associated with an increased hazard of developing extensive chronic GVHD. As the cell dose is a continuous variable, it is difficult to identify a precise CD34⁺ or CD3⁺ cell dose above which the risk of extensive chronic GVHD is markedly increased. However, we observed a trend towards an association between the CD34⁺ cell dose and extensive disease. Methotrexate has been reported to play a critical role in affecting the development of chronic GVHD after allogeneic PBSCT.^{24,37} However, the higher risk of extensive chronic GVHD among patients of group A was not MTX-regimen dependent, since all patients in each cohort were given a complete MTX course (4 doses). There is a suggestion that the dose of G-CSF might influence the risk of chronic GVHD by inducing qualitative or quantitative changes in cytokines produced by donor T-cells^{6,18,38,39} and also modulating the alloimmune response by cell-mediated suppression of T-cell alloreactivity.^{40,41} Therefore, the results of studies on GVHD prevention by different CsA tapering schedules in recipients of bone marrow are not exhaustive. Both the G-CSF administration and its schedule should be taken in account when the impact of CsA on acute and chronic GVHD following PBSCT is analyzed.

Almost all of our patients with chronic GVHD had extensive disease. This finding is consistent with other reports.^{2,4,13,34,37} Moreover, it is noteworthy that 50% of patients in group B developing chron-

Table 5. Predictors of clinical extensive chronic GVHD.

Variables	Univariate analysis		Multivariate analysis	
	p value		HR (95% CI)	p value
Donor sex	0.001	Male	1	0.02
		Female	4.24 (1.26-14.25)	
Gender combination	0.02	Female donor-male recipient	1	0.27
		Other combinations	0.52 (0.17-1.64)	
Duration of CsA prophylaxis	0.0009	6 mo. course	1	0.008
		12 mo. course	0.2 (0.06-0.66)	
Disease phase at transplant	0.04	High-risk	1	0.11
		Low-risk	0.47 (0.19-1.18)	
CD3+ cell dose	0.5	≤ median of 3.2×10^8 /kg b.w.	1	0.41
		> median of 3.2×10^8 /kg b.w.	1.5 (0.55-4.04)	
CD34+ cell dose	0.14	≤ median of 6×10^6 /kg b.w.	1	0.23
		> median of 6×10^6 /kg b.w.	1.79 (0.68-4.72)	
Mono-nucleated cell dose	0.09	≤ median of 6.9×10^8 /kg b.w.	1	0.35
		> median of 6.9×10^8 /kg b.w.	1.69 (0.56-5.13)	
CMV serology	0.05	Recipient and donor negatives	1	0.09
		Other combinations	0.29 (0.07-1.24)	

ic GVHD had limited disease, 10 times more than in group A. In our model, the female sex of the donor remained as a second significant risk factor for extensive chronic GVHD. Gender mismatch is a known risk factor for acute GVHD in allogeneic BMT⁴² but, so far, this is the first study recognizing gender combination as a possible risk factor for chronic GVHD after allogeneic PBSCT.

The age of the patient and donor, which is one of the strongest predictors of chronic GVHD, did not have a significant impact on the likelihood of extensive chronic GVHD in this study, probably because of the adult age of many of the patients and donors with only a few children included. Acute GVHD is another very strong factor predicting subsequent chronic GVHD. We do not have an explanation of why it was not significant in the present study. It is possible to speculate that the low number of patients with grade 2 acute GVHD and the absence of grade 3-4 acute GVHD played a role.

In the current study, there was no increase in infection-related mortality associated with the longer tapering regimen. On the contrary, the risk of TRM greatly depended on the occurrence of the extensive chronic GVHD, which was the cause of non-relapse mortality in all of the cases. However, the duration of CsA administration did not significantly influence the probability of TRM among the 2 groups. Interestingly, the progressive type of

chronic GVHD, which has been associated with a worse prognosis after BMT,⁴³ was never observed among patients receiving 12-month course of CsA, who never developed signs of extensive chronic GVHD beyond the 6th month after transplant in any case.

Finally, the duration of CsA administration did not affect the probability of relapse and DFS, although our relatively small sample size and short follow-up do not allow any definitive conclusion to be drawn with respect to these outcomes.

In conclusion, the longer tapering regimen seems to be more effective at preventing extensive chronic GVHD after PBSCT from an HLA-identical sibling and there is a suggestion that it may be superior to the standard regimen, considering that no increase in either infection-related mortality or relapse rate was associated with the prolonged prophylaxis. However, any conclusive assessment of the overall benefits of such prolonged immunosuppression, in terms of better quality of life and minor morbidity, requires both a much longer follow-up to evaluate the rates of relapse and secondary tumors and a large randomized, controlled setting.

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Pre-publication Report & Outcomes of Peer Review

Contributions

AM and WA contributed equally to this work and should be considered as the principal authors. API was responsible for transplant co-ordination and data collection. AR, RC and MLM were responsible for the care of patients. AM and LC were responsible for the statistical analyses. MSDP, SF and LDF performed all laboratory investigations. GG was responsible for all the procedures related to the HLA typing and stem cell collection. All authors contributed in revising the manuscript. They are listed according to a criterion of decreasing individual contribution to the work, with the exception of the last author who had a major role as senior author in conceiving the study. We would like to thank Marco Donati for his support in revising the English.

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Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Jordi Sierra, Deputy Editor. The final decision to accept this paper for publication was taken jointly by Professor Sierra and the Editors. Manuscript received November 12, 2002; accepted February 6, 2003.

In the following paragraphs, Prof. Sierra summarizes the peer-review process and its outcomes.

What is already known on this topic

Extensive chronic GVHD is a frequent complication of allogeneic peripheral blood stem cell (PBSC) transplantation. Several single arm studies showed that the frequency of this complication after bone marrow transplants may be reduced by extending the duration of cyclosporine (CSP) prophylaxis; in contrast one randomized trial did not show any benefit from this approach.

What this study adds

This is the first report analyzing the effect of prolonged CSP prophylaxis on the development of extensive chronic GVHD after allogeneic transplantation using PBSC. One year CSP prophylaxis compared to 6 months significantly decreased the incidence of this complication, without having any impact on relapse rate, transplant related mortality or survival.

Caveats

This is a retrospective analysis of two cohorts including a limited number of patients. Only prospective randomized studies will allow firm conclusions to be drawn on this topic.