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Effect of Early Posttransplantation Tacrolimus Concentration on the Development of Acute Graft-versus-Host Disease after Allogeneic Hematopoietic Stem Cell Transplantation from Unrelated Donors

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Only limited data are available regarding the relationship between blood concentration of tacrolimus and its efficacy in preventing acute graft-versus-host disease (aGVHD). We retrospectively evaluated the effects of the whole blood concentration of tacrolimus, which was measured by an automated microparticle enzyme immunoassay, early after allogeneic hematopoietic stem cell transplantation (HSCT) upon the development of aGVHD. Sixty patients, who underwent allogeneic HSCT from serologically human-leukocyte antigen-matched unrelated donors and received continuous infusion of tacrolimus with short-term methotrexate for GVHD prophylaxis, were included in this study. The target range of the blood concentration of tacrolimus was set at 10 to 20 ng/mL, and the level was maintained within this range in all patients. However, the mean blood concentration of tacrolimus during the third week after HSCT was significantly associated with the grades of aGVHD (17.3 ± 2.1 in patients with grades 0-I vs 15.9 ± 2.8 in II-IV and 14.8 ± 2.1 in III-IV; $P < .05$ and $<.01$, respectively). Multivariate analysis also demonstrated that higher age (≥35) of donor (odds ratio [OR] = 4.28) and lower mean blood concentrations of tacrolimus during the second (OR = 0.75; 95% confidence interval [CI]: 0.58-0.98) and third weeks (OR = 0.76; 95% CI: 0.58-0.98) after HSCT were significant risk factors for grades II-IV aGVHD ($P < .05$). We conclude that the early posttransplantation blood concentration of tacrolimus had a significant impact on the development of moderate-to-severe aGVHD after allogeneic HSCT from an unrelated donor.

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KEY WORDS: Tacrolimus, Graft-versus-host disease, Blood concentration, Allogeneic hematopoietic stem cell transplantation, Unrelated donor

INTRODUCTION

Graft-versus-host disease (GVHD) remains 1 of the major life-threatening complications of allogeneic hematopoietic stem cell transplantation (HSCT), despite the introduction of calcineurin inhibitors such as cyclosporine A (CsA) and tacrolimus. Tacrolimus possesses 100 times greater in vitro inhibitory activity against T cells than CsA, and has been widely used for the prophylaxis of GVHD alone or in combination with methotrexate (MTX) in patients undergoing allogeneic HSCT who are at high risk for developing GVHD [1,2]. There have been 3 randomized trials comparing the efficacy of CsA and tacrolimus in the prophylaxis of GVHD after allogeneic HSCT, all of which indicated that tacrolimus with short-term MTX could prevent the development of acute GVHD (aGVHD) more effectively than CsA with short-term MTX [3-5]. However, the target ranges of the blood concentration of tacrolimus early after transplantation varied significantly among these 3 studies [3-5]. In addition, the descriptions of the actual duration of intravenous administration of tacrolimus, as well as

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the actual blood concentrations of tacrolimus, were insufficient, and either of these parameters could critically affect the drug's efficacy. There have been few studies that evaluated the optimal range of blood concentration of tacrolimus early after allogeneic HSCT for preventing aGVHD. Those studies clearly demonstrated that a higher blood concentration of tacrolimus was associated with its toxicity and transplant-related mortality (TMR), but failed to demonstrate any significant impact of blood concentration of tacrolimus on the incidence of aGVHD [6,7]. Based on these findings, a target range of 10 to 20 ng/mL of tacrolimus was recommended. However, wide target ranges of tacrolimus concentration were set in these studies. In addition, the evaluations of tacrolimus concentrations at a steady state of intravenous infusion were compared rather haphazardly with those of the trough levels of orally administered tacrolimus. We previously showed that the 2 routes of administration had significantly different pharmacokinetic profiles [8]. Therefore, we believe that it is necessary to reevaluate the relationship between the tacrolimus blood concentration and development of GVHD within the steady-state target range between 10 and 20 ng/mL.

Recently, Watanabe et al. [9] reported a significant relationship between the tacrolimus blood concentration and incidence of aGVHD of grades II-IV in pediatric patients. They tried to maintain the blood concentration within 5 to 15 ng/mL, and found that the incidence of aGVHD of grades II-IV was 65.9% in patients with a mean blood concentration ≤7 ng/mL and 34.8% in those with a blood concentration >7 ng/mL. In this study, we retrospectively evaluated the relationship between the blood concentration of tacrolimus within the recommended range and the development of aGVHD within its currently recommended blood concentration in adult patients who underwent allogeneic HSCT from serologically human leukocyte antigen (HLA)-matched unrelated donors.

PATIENTS AND METHODS

Patient and Donor Characteristics

Patients with hematologic disorders who underwent allogeneic HSCT from an unrelated donor at Keio University Hospital were retrospectively evaluated. The inclusion criteria were as follows: (1) T cell-repleted transplantation from an HLA-A, B, and DR serologically matched unrelated donor; (2) use of tacrolimus and short-term MTX for GVHD prophylaxis; (3) administration of tacrolimus intravenously for at least 3 weeks after transplantation without permanent discontinuation; and (4) regular measurement of the whole blood tacrolimus concentration at least 4 times in each week. Among the 64 patients who underwent allogeneic HSCT from an HLA-A, B, and DR serologically matched unrelated donor, 60 patients fulfilled the criteria and were included in the analysis. Four patients were excluded because of early discontinuation of tacrolimus because of multiple organ failure (n = 2), disease relapse (n = 1), and early switch from intravenous to oral (n = 1). The source of stem cells was bone marrow in all patients. The patient characteristics are shown in Table 1. All patients underwent HSCT for the treatment of hematologic malignancies, with the exception of 2 patients who had aplastic anemia. HLA-A and HLA-B antigens were typed using standard serologic or low-resolution techniques. HLA-A, B, and DRB1 alleles were typed using high-resolution DNA techniques, as described previously [10]. Fifty-nine patients received myeloablative conditioning (total body irridiation [TBI]-based, n = 58; busulfan + cyclophophamide, n = 1), and 1 patient received a reduced-intensity regimen (fludarabine + melphalan).

GVHD Prophylaxis

Tacrolimus was administered daily starting on day −1 at a dose of 0.03 mg/kg by continuous intravenous infusion, and the dose was adjusted to maintain the whole-blood tacrolimus concentration between 10 and 20 ng/mL. The dose of tacrolimus had been adjusted by each physician according to the whole-blood tacrolimus concentration and adverse events such as renal dysfunction. The whole-blood

Table 1. Patient and Donor Characteristics

No. of patients	60
Median age	
years (range)	36 (12-56)
Sex	
Male/female	41/19
Diagnosis	
Acute leukemia	31
Chronic myelogenous leukemia	14
Myelodysplastic syndrome	7
Malignant lymphoma	6
Aplastic anemia	2
HLA compatibility	
Serologically match	60
Class I (A and B)	
Allele match	43
Allele mismatch	10
Data not available	7
Class II (DRB1)	
Allele match	45
Allele mismatch	15
Median age of donor	
Years (range)	34 (20-53)
Gender of donor	
Male/female	37/23
Conditioning	
Myeloablative	59
Reduced intensity	1
Methotrexate doses for GVHD prophylaxis	
Four doses (days 1, 3, 6, and 11)	25
Three doses (days 1, 3, and 6)	35

HLA indicates human leukocyte antigen; GVHD, graft-versus-host disease.

concentration of tacrolimus was measured by an automated microparticle enzyme immunoassay. The administration of tacrolimus was switched from intravenous to oral when patients could reliably take oral medication. Basically, MTX at a dose of 15 mg/m^2 was given intravenously on day 1, followed by 10 mg/m^2 on days 3, 6, and 11. MTX on day 11 was omitted if the patients had severe infection, mucositis, and/or liver dysfunction. Twenty-five patients received 4 doses, and 35 received 3 doses of MTX. Among the 35 patients who received 3 doses of MTX, 10 received reduced doses of methotrexate (10 mg/m^2 on day 1, and 7 mg/m^2 on days 3 and 6) according to the local protocol. None of the patients received antithymocyte globulin (ATG) for the prophylaxis of GVHD.

Diagnosis and Grades of aGVHD

The diagnosis of aGVHD was made based on clinical and pathological findings, and graded according to the consensus criteria [11].

Statistical Analysis

The relation between the grades of aGVHD and the concentrations of tacrolimus was evaluated using the Jonckheere-Terpstra test. The difference between the 2 groups was compared using the chi-square test, the Fisher exact test, and Mann-Whitney *U* test as appropriate. Multivariate analysis was performed using multiple logistic regression analysis. *P* values <.05 were considered statistically significant.

RESULTS

Relationship between the Incidence of Grades of aGVHD and Concentration of Tacrolimus

Among the 60 patients, 13 patients (21.7%) developed grade I aGVHD, 20 (33.3%) developed grade II, 6 (10%) developed grade III, and 5 (8.3%) developed grade IV (Table 2). Among the 31 patients with grades II-IV aGVHD, 27 patients (87.1%) developed aGVHD in the third week or later period after transplantation. The mean blood concentrations of tacrolimus during the first 3 weeks after transplantation ranged between 10.9 and 20.2 ng/mL (median: 16.6 ng/mL). In relation to the grades of aGVHD, there was a significant trend of lower mean concentration of tacrolimus during the first 3 weeks after transplantation ($P < .05$) (Table 2). The comparative analysis of the concentration of tacrolimus in each week during the first 3 weeks after transplantation indicated that the mean concentration of tacrolimus was significantly lower in patients with grades II-IV aGVHD than those with grades 0-I aGVHD in the second and third weeks after transplantation ($P < .05$) (Table 3). The same analysis also indicated that the mean concentration of tacrolimus was significantly lower in patients with grades III-IV aGVHD than those with grades 0-I aGVHD in the third week after transplantation ($P < .01$) (Table 3), but not in the second week. No significant differences were observed in the analysis comparing the concentrations during the first week after transplantation (Table 3).

Table 2. Mean Tacrolimus Concentraion in Relation to Grades of GVHD

Grades of GVHD	N	Mean Concentration (±SD) of Tacrolimus (ng/mL)*
0	16	17.2 ± 1.20†
I	13	16.9 ± 1.38†
II	20	16.1 ± 1.89†
III	6	16.3 ± 2.28†
IV	5	14.1 ± 2.30†

GVHD indicates graft-versus-host disease.
*Mean concentraion during the first 3 weeks after transplantation.
†Significant trend of decrease in relation to grades of GVHD by Jonckheere-Terpstra test ($P < .05$).

Factors Affecting the Development of aGVHD

In addition to the concentration of tacrolimus, the effects of patient age, patient sex, donor age, donor sex, patient–donor sex match, HLA compatibility in class I and II alleles, and the MTX doses on the incidence of grades II-IV aGVHD were evaluated (Table 4). In univariate analysis, older donor age, 3 doses of MTX (vs 4 doses), and blood concentration of tacrolimus were identified as significant factors for developing grades II-IV aGVHD (Table 4). In multivariate analysis, significant factors for developing grades II-IV aGVHD included older donor age and mean tacrolimus blood concentration during the second and third weeks each after transplantation (Table 4). Although lower doses of MTX were not a significant factor by univariate analysis, they showed a strong trend of association with aGVHD ($P = .06$) (Table 4).

Effects of Tacrolimus Concentration on Renal Function

In all patients, the serum creatinine level was measured every day during the first month posttransplantation. Among the 60 patients, the serum creatinine

Table 3. Relationship between Grades of Acute GVHD and Concentration of Tacrolimus at Each Week after Transplantation

Grades of GVHD	N	Mean Concentration (±SD) of Tacrolimus (ng/mL)		
		First Week	Second Week	Third Week
0-I	29	17.3 ± 2.5	16.7 ± 1.9	17.3 ± 2.1
II-IV	31	16.7 ± 3.9*	15.0 ± 3.1†	15.9 ± 2.8†
III-IV	11	15.5 ± 4.8*	15.6 ± 2.7*	14.8 ± 2.1‡

GVHD indicates graft-versus-host disease; SD, standard deviation.
*Not significant compared with grades 0-I.
†Statistically significant compared with grades 0-I ($P < .05$).
‡Statistically significant compared with grades 0-I ($P < .01$).

Table 4. Univariate Analysis for Factors Affecting the Incidence of Acute GVHD (Grades II-IV)

Factors		Univariate Analysis		Multivariate Analysis		
		Incidence of Acute GVHD (%)	*P* Value	Odds Ratio	95% CI	*P* Value
Patient sex	Male versus female	53.7 versus 47.4	.78	—	—	—
Patient age (years)	35 or greater versus <35	56.3 versus 46.4	.61	—	—	—
Donor sex	Male versus female	53.7 versus 47.4	.43	—	—	—
Donor age (years)	35 or greater versus <35	68.0 versus 40.0	.04	4.28	1.15-15.92	.03
Recipient/donor sex	Match versus mimatch	48.0 versus 70.0	.30	—	—	—
HLA class I*	Match versus mimatch	46.5 versus 70.0	.30	—	—	—
HLA class II	Match versus mimatch	51.1 versus 53.3	1	—	—	—
MTX doses	4 doses versus 3 doses	37.1 versus 72.0	.01	3.44	0.97-12.26	.06
Tacrolimus conc. 2nd week	Continuous variable	†	< .05†	0.75‡	0.58-0.98	.03
Tacrolimus conc. 3rd week	Continuous variable	†	< .05†	0.76‡	0.58-0.98	.04

GVHD indicates graft-versus-host disease; CI, confidence interval; HLA, human leukocyte antigen; MTX, methotrexate; conc., concentration.
*Data were missing in 7 patients.
†The results are shown in Table 3.
‡For every increase of 1.0 ng/mL in blood concentration of tacrolimus.

level increased twofold or more during the first month after transplantation as compared with that before transplantation only in 3 patients (5%). There was no significant correlation between the mean blood concentration of tacrolimus during the 3 weeks of the study period and the increase in serum creatinine in all patients ($\rho = 0.11$, $P = .40$). No patients developed posterior reversible encephalopathy syndrome.

DISCUSSION

The prominent features of the present study compared with other studies evaluating the relationship between blood concentration of tacrolimus and aGVHD were: (1) the blood concentrations of tacrolimus were all obtained during its continuous infusion; (2) GVHD prophylaxis was tacrolimus and short-term MTX in all cases; and (3) stem cell donors were all serologically HLA-matched unrelated donors. Furthermore, the target range of blood concentration had been set at 10 to 20 ng/mL, which was strictly monitored, and the mean concentration was successfully maintained within the range in all the patients. In this homogenous population, the blood concentration of tacrolimus had a significant impact on the development of grades II-IV aGVHD. Because a variety of factors reportedly affect the incidence of aGVHD, the variation of those factors should be minimized to evaluate the efficacy of immunosuppressive therapy in preventing aGVHD. Especially in regard to tacrolimus administration, not only its actual blood concentration but also the route of administration (continuous infusion or oral) could have a significant effect on its efficacy. In addition, the steady-state concentrations of intravenous infusion and the trough levels of oral administration were identically evaluated in the previous study [6]. To avoid such potential confounders, this study was designed to evaluate the relationship between the blood concentration of tacrolimus and the development of aGVHD with minimal effects on other factors. This may explain the discrepancy between this study and other studies that did not demonstrate the significance of this relationship [6,7]. On the other hand, a recent report on pediatric patients by Watanabe et al. [9] demonstrated a similarly significant effect of blood concentration of tacrolimus on the incidence of aGVHD; in their study, as in ours, the blood concentrations of tacrolimus were evaluated under continuous infusion (28 days, in their report) [9]. To our knowledge, their report and ours are the only 2 studies demonstrating a clear relationship between the blood concentration of tacrolimus and the development of aGVHD.

However, a number of important differences between the present study and the report of Watanabe et al. [9] should also be discussed. The results of our study indicated that a tacrolimus blood concentration of 15 to 16 ng/mL constituted the dividing line between patients with grades 0-I aGVHD and those with grades II-IV or III-IV aGVHD (Table 3), and thus that 15 to 16 ng/mL might be the recommended concentration for efficacious prophylaxis of aGVHD. However, Watanabe et al. [9] have suggested that this line should be 7 ng/mL. This difference could be explained by the differences in target blood concentration of tacrolimus between the 2 studies (10-20 vs 5-15 ng/mL). In addition, their study included recipients of allogeneic HSCT using various types of stem cells and donors. Because the recipients of stem cells from HLA-identical siblings or cord blood from unrelated donors could be considered at lower risk of developing aGVHD [5,12-15], this could also have partly contributed to the difference in targeted blood concentration of tacrolimus between the 2 studies. It is possible that the recommended targeted blood concentration of tacrolimus could be different among the types of stem cell sources, and this should be examined separately.

Tacrolimus shows its inhibitory activity against activated T cells in vitro in a dose-dependent manner

[16-18]. The results of the present study clinically support this observation. However, it should be considered that tacrolimus also causes side effects, such as nephrotoxicity, when its blood concentration remains within the toxic range (presently considered to be above 20 ng/mL) for a prolonged period [6,7]. Once its toxicity arises, physicians need to discontinue the drug temporarily or decrease the target range. Such an unexpected and unplanned dose adjustment of tacrolimus is likely to increase the risk of developing aGVHD, and this could be 1 of the plausible explanations for the failure of the previous reports to demonstrate the dose-dependent efficacy of tacrolimus, because these reports applied wider and higher (>20 ng/mL) target ranges [6,7]. Therefore, our results suggest that the in vivo efficacy of tacrolimus in preventing aGVHD is dose dependent as long as its concentration is strictly maintained within a less toxic therapeutic range.

In addition to the blood concentration of tacrolimus, we found that the donor age was also a significant factor affecting the development of aGVHD. Older donor age (35 years old or older) increased the incidence of grades II-IV aGVHD with an odds ratio (OR) of 4.28 (95% confidence interval [CI]: 1.15-15.92). There have been a series of reports evaluating the risk factors for aGVHD, and diverse factors have been identified [19-27]. However, the effect of donor age on aGVHD remains controversial. Among those studies, only a few studies identified donor age as a significant factor affecting the incidence of aGVHD; in these studies, older donor age significantly increased the incidence of aGVHD [26,27].

Renal toxicity is 1 of the most common adverse effects of tacrolimus. In the present study, in which the target level of tacrolimus was set at 10 to 20 ng/mL, only 5% of the patients experienced renal impairment defined by doubled serum creatinine levels compared with those before transplantation. In addition, there was no significant correlation between the concentration of tacrolimus and an increase in the serum creatinine level. In this study, the dose of tacrolimus was adjusted on a daily basis, not only according to its steady-state blood concentration of tacrolimus but also the serum creatinine level. In addition, efforts, such as hydration and dose adjustment of other nephrotoxic drugs concurrently given, were made to correct renal impairments. Therefore, our experience strongly suggested that tacrolimus could be safely administered at a concentration of 10 to 20 ng/mL if patients were optimally managed.

In conclusion, physicians should recognize the significance of early posttransplantation blood concentration of tacrolimus in preventing aGVHD, and should maintain the concentration between 15 and 20 ng/mL. Further prospective studies are warranted to evaluate the efficacy and safety of this target range of blood tacrolimus concentration in allogeneic HSCT recipients.

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ORIGINAL ARTICLE

M-CSF attenuates severity of chronic GVHD after unrelated BMT

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To study the effects of M-CSF administration on long-term outcomes of unrelated BMT, we retrospectively analyzed data from patients transplanted through the Japan Marrow Donor Program. We obtained data from 54 patients who received M-CSF just after BMT and 500 patients who did not receive M-CSF or G-CSF acted as controls. There were no significant differences between the two cohorts with respect to OS, acute GVHD or relapse. Although the incidence of chronic GVHD was comparable between the two groups, extensive chronic GVHD was observed significantly less often in the M-CSF cohort than in the control group. Multivariate analysis identified M-CSF as a significant factor for attenuating extensive chronic GVHD (relative risk: 0.73; 95% confidence interval: 0.55–0.94; $P = 0.012$). We also found the same results in matched-pair analysis. Our observation suggests the potential for clinical use of M-CSF to dampen severe chronic GVHD.

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Keywords: M-CSF; unrelated BMT; chronic GVHD

Introduction

Chronic GVHD is a major barrier to successful hematopoietic SCT. Because immunosuppressive treatment is necessary for extensive chronic GVHD, it remains a major cause of late death despite its association with lower relapse rates.[1,2] Its pathophysiology is poorly understood, and management strategies beyond systemic corticosteroids have not been established. Neither thalidomide nor prolonged use of calcineurin inhibitors have been successful in preventing chronic GVHD.[3–5]

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M-CSF, a growth factor for cells from the monocyte-macrophage lineage, has been used after chemotherapy and BMT to induce the early recovery of neutrophils and to reduce the incidence of febrile neutropenia.[6,7] A tolerogenic action of M-CSF was reported during the induction of DCs from cord blood.[8,9] Most recently, M-CSF administration before conditioning reduced the severity of GVHD in a mouse model, and it is anticipated that this modality may be adopted as a new strategy for preventing GVHD.[10]

In this study, we retrospectively analyzed data from patients who underwent unrelated BMT through the Japan Marrow Donor Program to study effects of M-CSF administration on long-term outcomes of UR-BMT, and we identified decreased severity of chronic GVHD after M-CSF administration.

Materials and methods

We retrospectively analyzed data from patients who had undergone their first BMT through the Japan Marrow Donor Program between 1993 and 2005 and for whom complete data concerning age, sex, HLA compatibility and M-CSF treatment were available. Acute leukemia, malignant lymphoma and multiple myeloma in the first or second remission, CML in the first or second chronic phase and MDS without leukemic transformation were considered standard-risk diseases, whereas other hematological malignant diseases were considered high-risk diseases. Chronic GVHD was classified as having limited (involving only localized skin and/or liver) or extensive (generalized skin or limited disease plus involvement of other organs) involvement. Diseases that are not malignant were considered benign diseases. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and approval of the study protocol was obtained from the JMDP Institutional Review Board.

Statistical analysis was performed with JMP (version 5.1; SAS Institute Inc., Cary, NC, USA) and R software (www.r-project.org/). OS was analyzed by the Kaplan–Meier method, and the log rank test was used to test the significance of differences. The incidence of GVHD and

Table 1 Patient's characteristics

Factor	*M-CSF (n = 54)*	*No CSF (n = 500)*	P
Gender of patient			
Male	35	297	0.502
Age of patient			
Range	2–59	0–65	
Median	27	31	0.160
Gender of donor			
Male	42	311	0.020
Age of donor			
Median	36	33	0.076
Disease			
Benign	0	19	0.014
Malignancy (standard risk)	35	239	
Malignancy (high risk)	19	242	
ATG			
Yes	1	26	0.220
Number of infused nucleated cells			
Mean ($\times 10^8$ cells/kg)	3.01	2.83	0.521
GVHD prophylaxis			
CYA + MTX	31	314	0.104
Tacrolimus + MTX	22	151	
Others	1	35	
HLA			
Identical	32	320	0.495
Year			
1993–1999	31	179	0.002
2000–2005	23	321	

relapse was estimated by the cumulative incidence method considering death without GVHD and death without relapse as competing risks, respectively, according to EBMT statistical guidelines (http://www.ebmt.org/1WhatisEBMT/Op_Manual/OPMAN_StatGuidelines_oct2003.pdf). The incidence of chronic GVHD was evaluated in patients who survived more than 100 days after transplantation. Factors that significantly affected chronic GVHD were evaluated by the Cox proportional hazards model. For matched-pair analysis, one or two patients were selected from the control cohort by closely matching for recipient age and gender, donor age and gender, disease risk, GVHD prophylaxis, ATG administration, era of transplantation and HLA compatibility.

Results

Fifty-four patients received M-CSF administration within 8 days of undergoing a BMT for a median length of 13 days, and data from 500 patients who received no CSF were used as the control. Forty-five patients started M-CSF injection on the first day after completing BMT. There were no significant differences in the patients' ages or sex, the donors' ages, ATG administration, infused nucleated cell number, GVHD prophylaxis or HLA compatibility (Table 1). The M-CSF cohort (M) group received more transplants from male donors (M, 77.8% vs control, 62.3%; $P = 0.020$) in the nineties (M, 57.4% vs control, 35.8%; $P = 0.002$), and contained more standard-risk diseases than the control cohort (M, 64.8% vs control, 47.8%; $P = 0.014$).

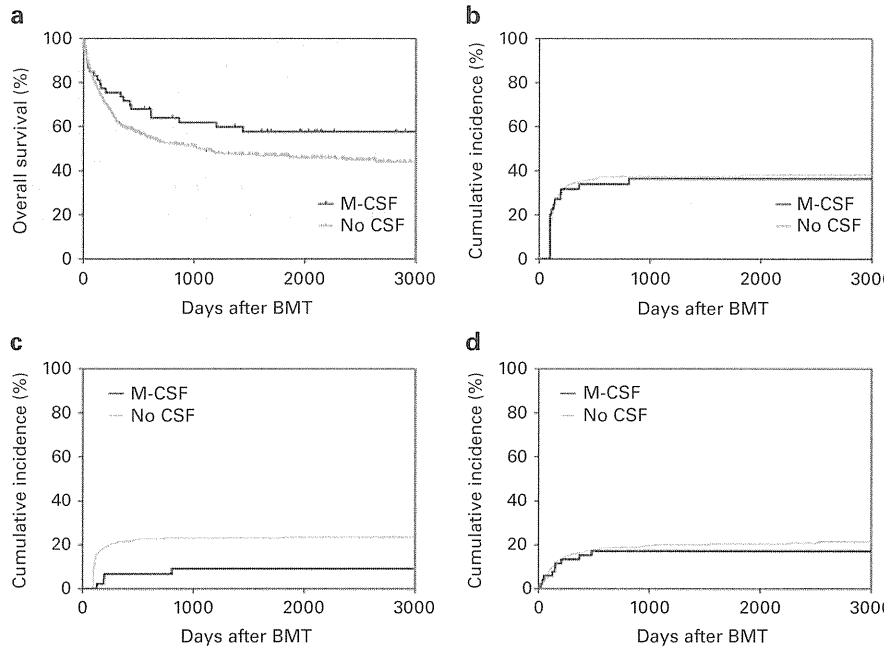

Figure 1 Kaplan–Meier curves showing OS in patients with or without M-CSF administration after BMT (**a**). Cumulative incidence curves showing the probability of chronic GVHD (**b**), extensive chronic GVHD (**c**) or relapse (**d**) in a competing risks setting.

OS of the M cohort group was superior to that of the control cohort (Figure 1a; M, 57.7% vs control, 46.4% at 5 years after transplantation), but the difference was not significant (log rank test, $P=0.084$). There were no significant differences in the incidence or grade of acute GVHD between the two cohorts (Gray test, all acute GVHD, $P=0.316$; grade 2–4, $P=0.691$; grade 3–4, $P=0.855$).

The incidence of chronic GVHD was comparable in the two cohorts (Figure 1b; Gray test, $P=0.903$). The incidence of extensive chronic GVHD of the M cohort was significantly lower than in the control (Figure 1c; Gray test, $P=0.021$). The observed occurrence of extensive chronic GVHD was 8.9% in the M cohort and 24.3% in the control. Multivariate analysis revealed that M-CSF administration was a significant factor, as was the age of patients and donors, underlying disease and acute GVHD (Table 2). Although chronic GVHD is associated with a lower relapse rate of the underlying malignant disease, we did not observe any increase of relapse rate in the M cohort (Figure 1d; Gray test, $P=0.533$).

We also performed matched-pair analysis with M-CSF-treated patients and identified a significant decrease in extensive chronic GVHD (Figure 2b; Gray test, $P<0.001$).

Table 2 Multivariate analysis for extended type chronic GVHD using Cox proportional hazard modeling

Factor	*Relative risk (95% CI)*	P
Patient's age	1.01 (1.00–1.02)	0.015
Donor's age	1.02 (1.01–1.04)	0.010
Disease risk		
Standard	1.00	<0.001
High	1.60 (1.16–2.41)	
Benign	0.87 (0.40–1.56)	
Acute GVHD		
No	1.00	0.006
Yes	1.23 (1.06–1.45)	
CSF		
No	1.00	0.012
M-CSF	0.73 (0.55–0.94)	

Abbreviation: CI = confidence interval.
The gender of patients and donors, donor-recipient sex-matching, ATG, administration, GVHD prophylaxis, HLA compatibility and era of transplantation had no significant effect.

The incidence of chronic GVHD was lower in the M cohort without significance (Figure 2a; Gray test, $P=0.066$). There were no significant differences in the incidence or grade of acute GVHD (Gray test, all acute GVHD, $P=0.345$; grade 2–4, $P=0.333$; grade 3–4, $P=0.303$).

Discussion

M-CSF is one of the cytokines that increases after BMT, and is reported to be associated with the development of acute GVHD in children.[11] Therefore, there was a possibility that M-CSF administration might have caused an increase in the severity of acute GVHD, but instead we observed a decrease in the severity of chronic GVHD. In patients with chronic GVHD, both the serum IL-10 level and IL-10 production from mononuclear cells have been shown to decrease after stimulation.[12–14] After stimulation with lipopolysaccharide, M-CSF-induced macrophages produce large amounts of IL-10 instead of IL-12.[15] Recent reports have shown that M-CSF induced DCs with IL-4 in a culture from human cord blood, and that these DCs produced high amounts of IL-10, but not IL-12, a pattern similar to that seen in M-CSF-induced macrophages.[8,9] The low incidence and severity of both acute and chronic GVHD after cord blood transplantation irrespective of HLA disparity, has been well documented.[16–18] M-CSF is elevated during pregnancy and in cord blood.[19,20] Taken together, the above information offers possible explanations for low incidence and severity of GVHD in cord blood transplantation. In this context, our observation suggests that M-CSF administration after UR-BMT might decrease severity of chronic GVHD, through induction of certain types of macrophages and DCs.

Very recently, Hashimoto *et al.*[10] revealed that host macrophages persist in the spleen and lymph nodes after conditioning to reduce allo-reactive donor T cells. Pre-transplant administration of M-CSF limited expansion of donor allo-reactive T cells and improved GVHD outcome in a mouse model. Similarly, M-CSF administration in our analysis might have induced host macrophages to engulf donor T cells.

The present study raises the possibility that M-CSF administration after BMT might be used to control chronic GVHD, although some issues remain to be resolved. First, our study was a retrospective analysis, and a prospective

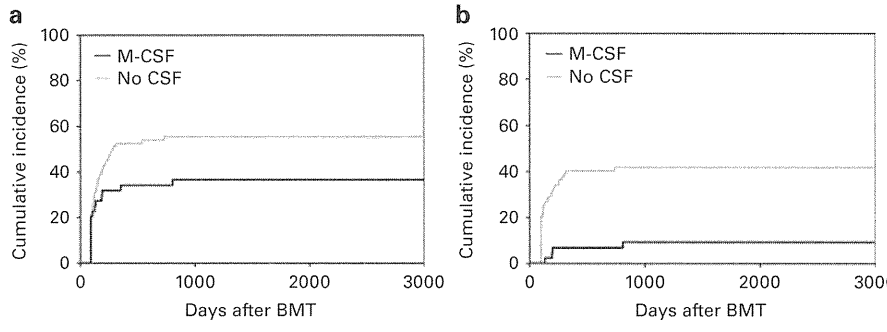

Figure 2 Cumulative incidence curves showing the probability of chronic GVHD (**a**) or extensive chronic GVHD (**b**) in a competing risks setting for matched patients with or without M-CSF.

randomized study is necessary to further evaluate the effects of M-CSF. Second, we used classical diagnostic criteria and severity classifications of chronic GVHD, and did not distinguish between late onset acute GVHD and classic chronic GVHD. Nor did we use the global scoring system that the National Institute of Health consensus development project proposed.[21] Third, most patients started M-CSF injection beginning on the first day after transplantation. Donor T cells are engulfed by splenic macrophages during the first day of transplantation, and before initiation of donor T-cell proliferation.[10] Therefore, there is a possibility that our administration schedule impaired the M-CSF effect on acute and chronic GVHD. The most appropriate administration schedule remains to be clarified.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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ELSEVIER

Immunoglobulin Prophylaxis Against Cytomegalovirus Infection in Patients at High Risk of Infection Following Allogeneic Hematopoietic Cell Transplantation

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ABSTRACT

Reports on the efficacy of intravenous immunoglobulin (IVIG) prophylaxis against cytomegalovirus (CMV) infection after allogeneic hematopoietic cell transplantation (HCT) have often sparked controversy. In addition, we are not aware of any study that has examined whether prophylaxis with IVIG affects the incidence of CMV infection in high-risk patients—those who are elderly or have received human leukocyte antigen (HLA) mismatched HCT. In the present open-label, phase II study, we addressed this question. We enrolled 106 patients in the study. The cumulative incidences of CMV infection at 100 days after HCT were similar in the intervention and the control groups (68% and 64%, $P = .89$; 89% and 87%, $P = .79$, respectively, for patients 55 years or older and those who received HLA-mismatched HCT). In those who received HLA-mismatched HCT, 1-year overall survival after HCT was 46% in the intervention group and 40% in the control group ($P = .31$); for age $\geq$ 55 years, the corresponding values were 46% and 40% ($P = .27$). Our data showed that prophylaxis with regular polyvalent IVIG did not affect the incidence of CMV infections or survival among older patients or those who receive HLA-mismatched HCT.

CYTOMEGALOVIRUS (CMV) infection is still a major complication after allogeneic hematopoietic cell transplantation (HCT). The efficacy of prophylaxis with polyvalent or hyperimmune CMV intravenous immunoglobulin (IVIG) after HCT has been explored since the 1980s. Many clinical trials conducted then and in the early 1990s showed significant prophylactic effects of IVIG against CMV infection and/or CMV pneumonia.[1–9] In addition, earlier meta-analyses (1993 and 1994) also demonstrated the benefits of prophylactic use of IVIG.[10,11] In contrast, the results of a recent placebo-controlled, randomized trial and a meta-analysis found no significant effect on overall or CMV infections.[12,13] However, these previous studies mostly consisted of cases that were human leukocyte antigen (HLA)-matched and underwent myeloablative HCT.

The recent, remarkable development of nonmyeloablative or reduced-intensity conditioning and alternative stem cell sources such as unrelated cord blood and HLA-mismatched donors extended the range of patients eligible for HCT. However, a critical problem is that use of unrelated cord blood or HLA-mismatched donors as sources of stem cells poses a considerable risk of infection after HCT, especially in older patients.[14,15] To our knowledge, there has been no investigation as to whether prophylaxis with IVIG benefits patients who are older or undergo HLA-mismatched HCT, both of whom have a high risk of infection after HCT.

Herein, we conducted an open-label, phase II trial of IVIG prophylaxis after HCT to investigate whether IVIG showed prophylactic effects against CMV infection and

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disease in a population that included patients who were at a high risk of infection.

PATIENTS AND METHODS

Patients' Eligibility, Control Group, and Study Endpoints

This phase II, single-center trial was conducted from July 2006. The protocol was approved by the Institutional Review Boards of our institute. After the concept, procedure, and potential risks of the study were explained to patients who were eligible for the protocol, written informed consent was obtained from all patients. The patients who were scheduled for HCT were eligible for this study irrespective of age, sex, type of graft, conditioning, and graft-versus host disease (GVHD) prophylaxis, HLA, and ABO compatibility. Patients who fulfilled at least one of the following criteria were ineligible for the present study: (1) a history of allergy to immunoglobulin products; (2) a history of severe adverse effects on exposure to the immunoglobulin product; (3) positivity of anti-HIV or hepatitis C virus antibodies, or hepatitis B virus (HBV) antigen; (4) the presence of contraindications to inclusion in this study as determined by the physician in charge. Polyvalent IVIG 5 g per person was given intravenously once per week, from day −7 to day 98 after HCT. A total of 66 consecutive patients who underwent HCT between January 2004 and June 2006 were selected as a historical control group. The primary endpoint was the rate of positive CMV antigenemia in the entire cohort. Secondary endpoints were (1) the peak levels of CMV antigenemia, or the occurrence of CMV disease or CMV pneumonia at 1 year in the entire cohort; (2) the probability of positive CMV antigenemia at day 100 in the subgroup at high risk of infection; and (3) overall survival in the entire cohort or in the subgroup at high risk of infection.

Definition of CMV Infection and Disease

CMV antigenemia was defined as detection of the pp65 antigen on peripheral blood leukocytes. The details of the diagnostic method (C7HRP) used are described below. C7HRP testing was performed at least once a week after neutrophil engraftment. CMV disease was defined by established criteria.[16]

Evaluation of CMV Antigenemia (C7HRP)

Peripheral blood treated with EDTA 3 ml, phosphate-buffered saline (PBS) 1.5 mL and 5% dextran solution 0.5 mL were mixed. After standing the specimen for 15 to 20 minutes in a constant-temperature bath at 37°C, the buffy coat was separated and centrifuged at 1200 rpm for 10 minutes, and the supernatant was then extracted. Ice-cold red blood cell-lysis buffer (3 mL) was added to the sediment, the red blood cells were hemolyzed at 4 °C, and the supernatant was extracted again. After 1 mL of the sediment was adjusted to a cell density of approximately 1.5×10^6 cells/mL by dilution with PBS, 100 μL was fixed on a microscope slide by cytospin. The specimen was fixed by immersing in cold acetone for 10 minutes and dried in air at room temperature for 10 minutes. The specimen was submerged in methanol for 100 seconds to block endogenous peroxidase activity, washed with purified water, and reacted with 50 μL of a solution of enzyme-labeled antibody for 60 minutes. After three washes with buffered solution, the specimen was exposed to 100 μL of substrate solution. After a further three washes with buffered solution, counterstaining was performed at room temperature for 30 seconds. The specimen was washed with purified water, and thereafter 50 μL of mounting fluid was added. Finally, it was covered with a coverslip, and the number of CMV-antigen-positive cells was counted by light microscopy.

Management of CMV Infection

Ganciclovir or valganciclovir treatment was started when CMV antigenemia became positive at a level of 0.01 to 0.02% (5 to 10/50,000). In principle ganciclovir (5 mg/kg intravenously twice daily) or valganciclovir (900 mg once a day orally) was given until CMV antigenemia became negative. It was recommended that ganciclovir or valganciclovir treatment be started exclusively in patients who underwent cord blood transplantation or were treated with steroids when CMV antigenemia became positive at any level. The dose of ganciclovir was adjusted appropriately in patients with impaired renal function; ganciclovir was replaced with foscarnet in patients with neutropenia or CMV infection refractory to ganciclovir.

Statistical Analysis

The cumulative incidences of CMV infection and CMV disease were compared between the intervention and control groups with death taken into consideration as a competing risk. The cumulative incidence of CMV disease was calculated using Gray's method, taking death into account. We also evaluated the incidence of grade II to IV acute GVHD, using Gray's method. In this analysis, death not associated with acute GVHD was treated as a competing risk event. Estimated cumulative incidences of CMV infection and survival probability were compared using the log-rank test.

The chi-square test was used to compare categorical variables in the patients' characteristics. Peak levels of CMV antigenemia were compared using the Mann-Whitney U test. Plasma levels of immunoglobulin over time were compared between the two groups using two-way analysis of variance. All P values were two-tailed and considered statistically significant if the values were less than .05. All statistical analyses were performed using PASW Statistics 17.0 (SPSS Inc, Chicago, Ill, USA) or the statistical software environment R, version 2.9.1.

RESULTS

We recruited 109 patients to participate in the study. However, three were not treated according to protocol and were excluded from the analysis. One patient declined consent to participate in the study, one was excluded from enrollment at the physician's discretion, and one patient was found to be positivity for HBV antigen. Patients' characteristics are displayed in Table 1. A total of 106 patients were enrolled in the study from July 2006 to February 2010. In the control group, the number of patients who received reduced-intensity conditioning, T-cell-depleted conditioning, and calcineurin alone as GVHD prophylaxis was significant higher than in the intervention group (Table 1). The intensity of conditioning was categorized as myeloablative or reduced-intensity conditioning according to the American Society for Blood and Marrow Transplantation definition.[17] Median follow-up durations in patients who were alive at the end of the study were 629 days in the intervention group and 1864 days in the control group, respectively.

Table 1. Patient Characteristics

Variables	Immunoglobulin+ (n = 106), n (%)	Immunoglobulin/−ve (n = 66), n (%)	P values
Median age of patients (range)	46 (19–69)	43 (16–69)	
Sex			
Male	62 (58)	34 (52)	.46
Female	44 (42)	32 (48)	
Stem cell source			
BM	55 (52)	42 (64)	.12
PB	27 (25)	17 (26)	
CB	24 (23)	7 (11)	
Conditioning			
Myeloablative	93 (88)	36 (55)	<.001
Reduced intensity	13 (12)	30 (45)	
T-cell depletion	13 (12)	1 (2)	.01
Rituximab treatment in the 2 mo preceding transplantation	2 (2)	1 (2)	.86
HLA-matching			
HLA-matched	60 (57)	44 (67)	.19
HLA-mismatched	46 (43)	22 (33)	
CMV serostatus			
Low	5 (5)	7 (11)	.18
Intermediate	3 (3)	3 (5)	
High	94 (87)	48 (73)	
Disease diagnosis			
AA	6 (6)	0 (0)	.22
AML	34 (32)	32 (48)	
ALL	22 (21)	11 (17)	
CML	5 (5)	2 (3)	
MDS	10 (9)	5 (8)	
MM	0 (0)	1 (2)	
NHL	12 (11)	7 (11)	
ATL	11 (10)	7 (11)	
other	6 (6)	1 (2)	
Acute GVHD prophylaxis			
Calcineurin alone	9 (8)	12 (18)	.01
Calcineurin + MTX	84 (79)	50 (76)	
Calcineurin + MMF	5 (5)	2 (3)	
Other	8 (8)	2 (3)	

The CMV risk was stratified into three groups: low (recipient negative and donor negative); intermediate (recipient negative and donor positive); and high (recipient positive and either donor negative or positive) based on recipient and donor CMV serostatus before hematopoietic cell transplantation. BM, bone marrow; PB, peripheral blood; CB, cord blood; HLA, human leukocyte antigen; CMV, cytomegalovirus; AA, aplastic anemia; AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; ATL, adult T-cell leukemia/lymphoma; GVHD, graft-versus-host disease; MTX, short-term methotrexate; CsA, cyclosporine; MMF, mycophenolate mofetil.

CMV Infection and Disease in the Entire Cohort

The cumulative incidence of CMV infection 100 days after HCT was comparable for the intervention group and control group (66% and 58%, P = .15, respectively; Fig 1). Even in the subset of patients who did not undergo T-cell-depleted conditioning, the cumulative incidence of CMV infection 100 days after HCT was similar for the intervention group and control group (64% and 54%, P = 24, respectively). On comparison of peak CMV antigenemia, these peak levels also did not differ significantly between the intervention group and control group [median (interquartile range): 0.009% (0–0.098) and 0.008% (0–0.12), P = .82, respectively; Fig 2).

One year after HCT, CMV disease had developed in 12 patients in the intervention group (CMV pneumonia: n = 3; CMV gastritis and/or enterocolitis: n = 9) and seven patients (CMV pneumonia: n = 5; CMV gastritis and/or enterocolitis: n = 2) in the control group (13% and 12%, respectively; P = .93). In contrast, prophylactic use of IVIG was weakly associated with a reduced cumulative incidence of CMV pneumonia (3% and 9%, respectively; P = .09).

CMV Infection in the Subgroup at High Risk of Infection

To evaluate prophylactic effects on CMV infection in the population at high risk of infection, we compared the cumulative incidence of CMV infection during the first 100 days after HCT in the intervention and control groups that received HLA-mismatched HCT including cord blood or were ≥55 years old. There was no significant difference between the intervention group and control group in the cumulative incidence of CMV infection 100 days after HCT, looking exclusively at the patients who received HLA-mismatched HCT (68% and 64%, P = .89, respectively) or were older (≥55 years old; 89% and 87%, P = .79, respectively; Fig 3).

Acute GVHD and Overall Survival

There was no significant difference in incidence of grades II to IV acute GVHD, with a rate of 50.2% in the intervention group and 51.9% in the control group (P = .78). In the entire cohort, 1-year overall survival after HCT was 62% in the intervention group, whereas it was 56% in the control group (P = .17). In those who received HLA-mismatched HCT, 1-year overall survival after HCT was 46% in the intervention group and 40% in the control group (P = .31). One-year overall survival after HCT in individuals aged 55 years or more was 46% in the intervention and 40% in the control group (P = .27).

Plasma Concentrations of Immunoglobulin

We compared plasma immunoglobulin concentrations in the intervention and control groups during the 100 days after HCT. The plasma immunoglobulin concentration did not significantly increase in the intervention group as compared to the control group (P = .73; Fig 4).

DISCUSSION

In the present study, prophylaxis with IVIG for CMV infection after HCT did not contribute to a decrease in the incidence of CMV infection and/or disease in both the entire cohort and the subgroups at high risk of infection, although prophylactic IVIG was weakly associated with a decreased incidence of CMV pneumonia. Similar to our

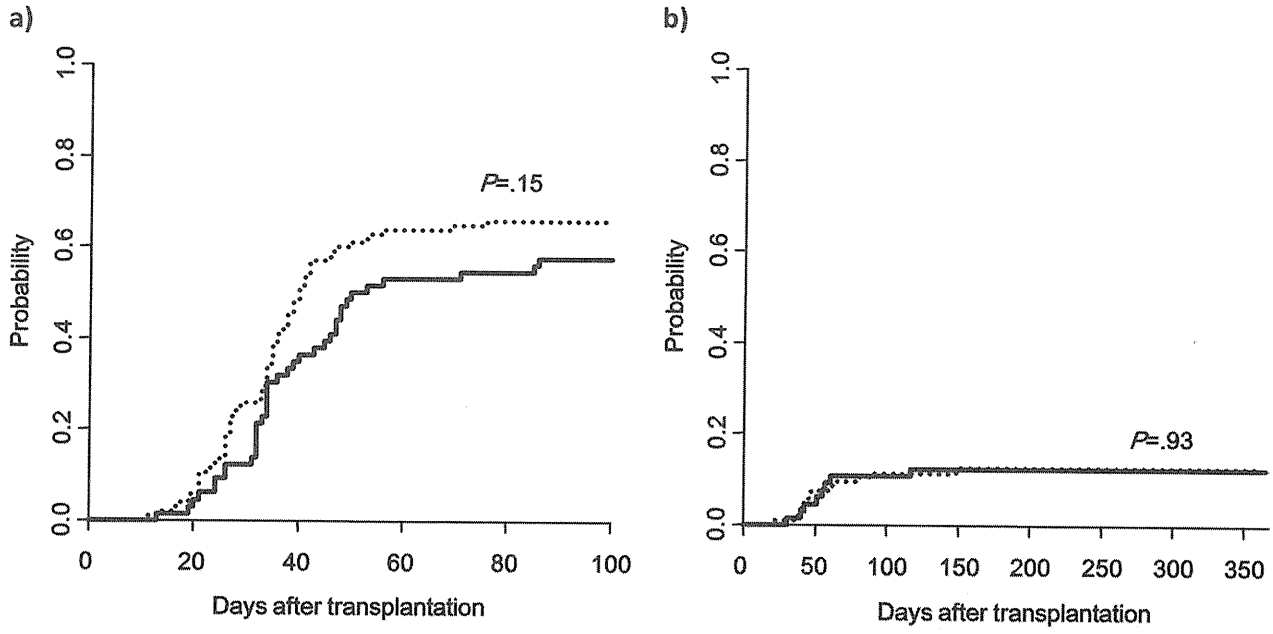

Fig 1. (**a**) The probabilities of detection of cytomegalovirus infection. (**b**) The probabilities of occurrence of cytomegalovirus disease in the groups with and without immunoglobulin prophylaxis. The dashed line indicates the group with immunoglobulin prophylaxis and the solid line indicates the group without immunoglobulin.

results were the findings of recent meta-analysis, which showed that IVIG prophylaxis reduced the rate of interstitial pneumonia but not infection-related outcomes.[13]

Recently, strategies have been developed for prevention and treatment of CMV infection, including antiviral drugs such ganciclovir, valganciclovir, and foscarnet; however, these drug have significant adverse effects, particularly in elderly patients.[18] Therefore, prophylactic use of IVIG appears to be an attractive strategy, particularly in patients of an advanced age and/or with impaired organ function such as renal dysfunction. However, our study could not demonstrate the effectiveness of prophylactic IVIG for CMV infection after HCT in older patients.

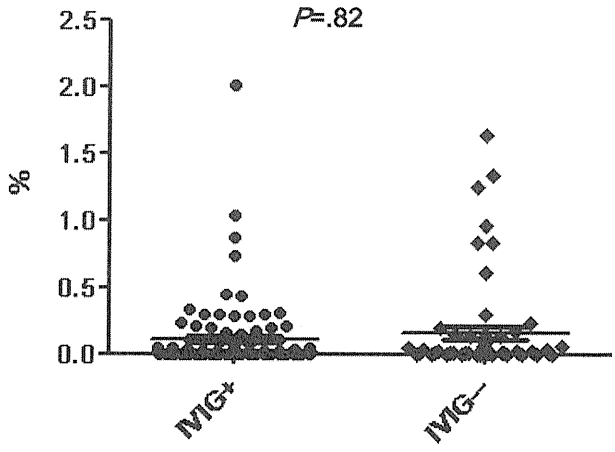

Fig 2. Comparison of peak cytomegalovirus antigenemia (%) in the groups with and without immunoglobulin prophylaxis. IVIG, intravenous immunoglobulin.

According to our data, plasma immunoglobulin levels measured until 100 days post-HCT did not significantly increase in the intervention group compared to those in the control group despite weekly administration of IVIG. We therefore cannot completely rule out the possibility that an insufficient dose of IVIG caused the lack of efficacy. However, the results of a prior placebo-controlled, randomized trial that compared standard-dose and high-dose IVIG did not show effectiveness of high-dose IVIG against viral infections.[12] An important finding was that the half-life of IVIG was fairly short, at about 6 days for both the 250 mg/kg and the 500 mg/kg dosing regimens.[19] We therefore speculate that consumption of immunoglobulin is very rapid after HCT, and it is very hard to sustain effective plasma concentrations of immunoglobulin by regular, prophylactic administration of IVIG.

In the previous literature, risk factors that were identified for CMV infection and/or disease included recipient age, CMV serostatus, herpes simplex virus serostatus, an unrelated donor, reduced-intensity conditioning, and a T-cell-depleted regimen as pretransplant characteristics; GVHD and delayed immune reconstitution were the most important risk factors after transplantation.[18,20–23] HCT from an HLA-mismatched donor and HCT in older patients were significantly related to delayed immune reconstitution and/or a high risk of GVHD.[14,24] In our study, only 9 of 172 patients showed hypoglobulinemia with a plasma immunoglobulin G (IgG) concentration of under 400 mg/dL during follow-up. There-

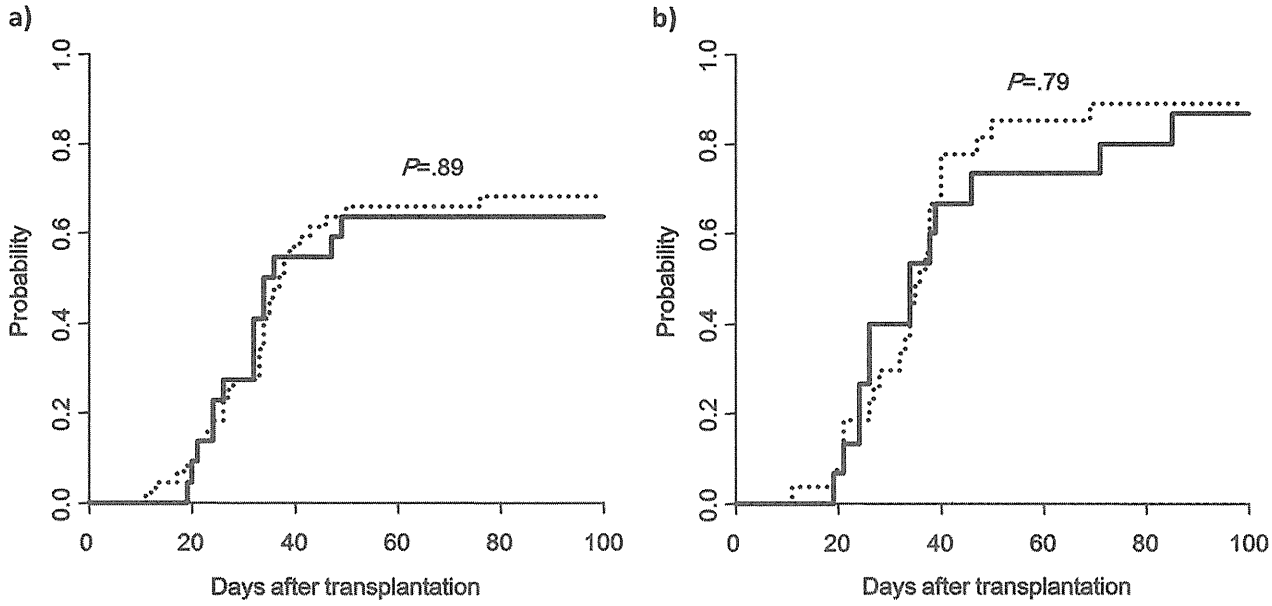

Fig 3. The probabilities of detection of cytomegalovirus infection in the groups with and without immunoglobulin prophylaxis (**a**) in patients who received human leukocyte antigen–mismatched hematopoietic cell transplants and (**b**) in patients ≥ 55 years old. The dashed line indicates the group given immunoglobulin prophylaxis and the solid line indicates the group without immunoglobulin.

fore, our study results cannot be applied to patients with hypoglobulinemia. Regarding this point, Schmidt-Hieber M et al reported that prophylactic IVIG did not reduce the frequency of episodes of CMV infection in a population with an IgG concentration <400 mg/dL, a natural killer cell count < 100/μL, a $CD4^+$ cell count < 100/μL, or acute or chronic GVHD, all of whom were at high risk of CMV infection.[25]

Our study has several limitations. It was not a randomized trial, and the results might have been affected by an underlying selection bias due to the use of historical controls, although the strategy for prevention of CMV infection did not change significantly in the time between the intervention and control groups, except for the use of prophylactic IVIG. Our study was also limited by the small numbers in the subgroups who were at high risk of infection, the heterogeneity of the disease, the transplant conditioning, GVHD prophylaxis, and the stem cell source. However, we did not observe any trend to indicate a preventive effect of IVIG against CMV infection in our study. Our data demonstrate that prophylaxis with regular polyvalent IVIG did not affect the incidence of CMV infection and survival, even in patients who were at high risk of infection because they received HLA-mismatched HCT or were older.

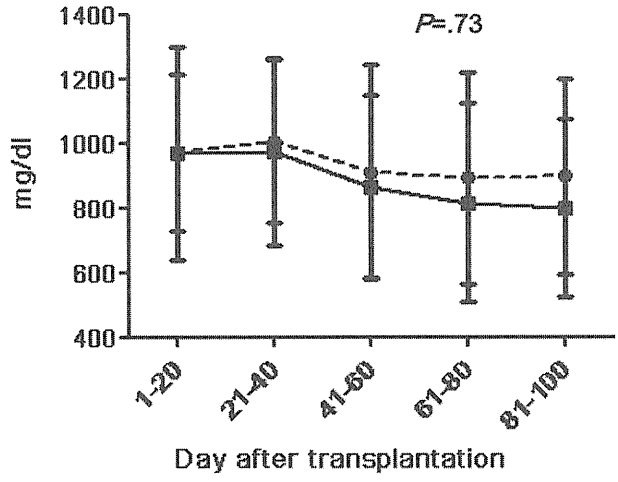

Fig 4. The plasma concentrations of immunoglobulin during the 100 days after transplantation in the groups with and without immunoglobulin prophylaxis. The dashed line indicates the group with immunoglobulin prophylaxis and the solid line indicates the group without immunoglobulin.

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RESEARCH Open Access

Factors that contribute to long-term survival in patients with leukemia not in remission at allogeneic hematopoietic cell transplantation

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Abstract

Background: There has been insufficient examination of the factors affecting long-term survival of more than 5 years in patients with leukemia that is not in remission at transplantation.

Method: We retrospectively analyzed leukemia not in remission at allogeneic hematopoietic cell transplantation (allo-HCT) performed at our institution between January 1999 and July 2009. Forty-two patients with a median age of 39 years received intensified conditioning (n = 9), standard (n = 12) or reduced-intensity conditioning (n = 21) for allo-HCT. Fourteen patients received individual chemotherapy for cytoreduction during the three weeks prior to reduced-intensity conditioning. Diagnoses comprised acute leukemia (n = 29), chronic myeloid leukemia-accelerated phase (n = 2), myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) (n = 10) and plasma cell leukemia (n = 1). In those with acute leukemia, cytogenetic abnormalities were intermediate (44%) or poor (56%). The median number of blast cells in bone marrow (BM) was 26.0% (range; 0.2-100) before the start of chemotherapy for allo-HCT. Six patients had leukemic involvement of the central nervous system. Stem cell sources were related BM (7%), related peripheral blood (31%), unrelated BM (48%) and unrelated cord blood (CB) (14%).

Results: Engraftment was achieved in 33 (79%) of 42 patients. Median time to engraftment was 17 days (range: 9-32). At five years, the cumulative probabilities of acute graft-versus-host disease (GVHD) and chronic GVHD were 63% and 37%, respectively. With a median follow-up of 85 months for surviving patients, the five-year Kaplan-Meier estimates of leukemia-free survival rate and overall survival (OS) were 17% and 19%, respectively. At five years, the cumulative probability of non-relapse mortality was 38%. In the univariable analyses of the influence of pre-transplant variables on OS, poor-risk cytogenetics, number of BM blasts (>26%), MDS overt AML and CB as stem cell source were significantly associated with worse prognosis ($p = .03$, $p = .01$, $p = .02$ and $p < .001$, respectively). In addition, based on a landmark analysis at 6 months post-transplant, the five-year Kaplan-Meier estimates of OS in patients with and without prior history of chronic GVHD were 64% and 17% ($p = .022$), respectively.

Conclusion: Graft-versus-leukemia effects possibly mediated by chronic GVHD may have played a crucial role in long-term survival in, or cure of active leukemia.

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Introduction

Patients with primary refractory or refractory relapsed acute leukemia have an extremely poor prognosis. It has been generally recognized that few cases with primary refractory or refractory relapsed acute leukemia can be cured using conventional chemotherapy alone [1]. While allogeneic hematopoietic cell transplantation (allo-HCT) has the potential to cure even active leukemia, it has not been determined what subgroup can receive a long-term benefit from it.

Several retrospective studies have reported the prognostic factors for allo-HCT in patients not in remission at allo-HCT including untreated first relapse cases [2-8]. However, the factors contributing to long-term survival have not been established because the follow-up periods of these studies were not long enough at less than five years. Importantly, it can be assumed that patients who survive for more than five years without leukemia relapse are most likely cured. Only one large-scale retrospective study has examined long-term outcomes for more than five years following allo-HCT in adult patients with acute leukemia not in remission [9]. This study showed that several pre-transplant variables including complete remission duration, type of donor, disease burden, performance status, age and cytogenetics affected survival. However, whether post-transplant variables such as acute or chronic graft-versus-host disease (GVHD) influenced the post-HCT prognosis was not assessed. To our knowledge, no studies have investigated pre- and/or post-transplant factors which are associated with long-term survival exclusively in adult patients with active leukemia at allo-HCT. Therefore, we comprehensively evaluated the pre- and post-transplant factors which contribute to long-term survival of more than five years in patients with leukemia not in remission at allo-HCT.

Patients and methods

Between January 1999 and July 2009, 42 consecutive patients (24 males and 18 females) with leukemia not in remission, aged 15 to 67 years (median age: 39 years), underwent allo-HCT at our institution. Patients with de novo acute myeloid leukemia (AML; n = 17), acute lymphoblastic leukemia (ALL; n = 12), chronic myeloid leukemia in accelerated phase (CML-AP; n = 2), myelodysplastic syndrome (MDS) overt AML (n = 10) and plasma cell leukemia (n = 1) were included. High-risk AML was defined according to the Eastern Cooperative Oncology Group/Southwest Oncology Group classification as having poor-risk cytogenetics (5/del[5q], 7/del[7q], inv[3q], abn11q, 20q or 21q, del[9q], t[6;9], t[9;22], abn17p, and complex karyotype defined as three or more abnormalities) [10]. High-risk ALL was defined as having poor-risk cytogenetics with either t(4:11), t(9;22), t(8;14), hypodiploidy or near triploidy, or more than five cytogenetic abnormalities [11]. Of study subjects with acute leukemia, cytogenetic abnormalities were intermediate (n = 17, 44%) or poor (n = 22, 56%). Seven patients were primary refractory to induction chemotherapy. The other patients relapsed after conventional chemotherapy (n = 23) or the first or the second HCT (n = 9). The median number of blast cells in bone marrow (BM) was 26.0% (range; 0.2-100) before the start of chemotherapy for allo-HCT. Six patients had leukemic involvement of the central nervous system (CNS). Stem cell sources were related BM (n = 3, 7%), related peripheral blood (PB) (n = 13, 31%), unrelated BM (n = 20, 48%) and unrelated cord blood (CB) (n = 6, 14%). Standard serologic typing was used for human leukocyte antigen (HLA) -A, B and DRB1. Thirty-one pairs were matched for HLA-A, B and DRB1 antigens. Three patients were mismatched for one HLA antigen (two at HLA-A, one at HLA-B), and seven were mismatched for two (two at HLA-A and B, five (all CB) at HLA-B and DRB1). The remaining one patient was mismatched for all three antigens (haploidentical). We classified conditioning regimens into four categories. Standard conditioning (n = 12) comprised a busulfan-based or total body irradiation (TBI)-based (12Gy) regimen. Busulfan was given as a total of 16 mg/kg orally or equivalent dose, 12.8 mg/kg intravenously (i.v.). Intensified conditioning (n = 9) consisted of additional cytoreductive chemotherapy in the three weeks before conditioning, followed by standard conditioning. Of the 21 patients receiving standard or intensified conditioning, 13 patients received the TBI-based regimen. Reduced-intensity conditioning (n = 21) comprised a fludarabine-based (n = 20) and cladribine-based regimen (n = 1). Fludarabine was given as 25-35 mg/m^2 i.v. on five or six consecutive days. Of the 21 patients receiving reduced-intensity conditioning, 14 patients received cytoreductive chemotherapy in the three weeks before conditioning. Prophylaxis for acute GVHD was a calcineurin inhibitor alone (n = 5), calcineurin inhibitor plus short-term methotrexate (n = 32), calcineurin inhibitor plus mycophenolate mofetil (n = 2), or none (n = 3). The calcineurin inhibitor included cyclosporine administered to 33 patients and tacrolimus to six patients.

End points

The absence of post-transplant remission in some patients biased the calculation of relapse rate, nonrelapse mortality (NRM) and leukemia-free survival (LFS). Therefore, we set five-year overall survival (OS) as the primary end point. OS was defined as time from the date of last transplantation to the date of death or

last follow-up. LFS was defined as time from the date of last transplantation to the date of disease relapse, death during remission or last follow-up. NRM was defined as a death not related to disease. Neutrophil recovery was defined as an absolute neutrophil count of at least 500 cells/mm^3 for three consecutive time points. Platelet recovery was defined as a count of at least 20 000 platelets/mm^3 without transfusion support. Acute GVHD (aGVHD) was defined in accordance with standard criteria [12]. Chronic GVHD (cGVHD) was evaluated in patients surviving for more than 100 days after allo-HCT and was classified into limited or extensive type [13].

Statistical analysis

If the disease for which the patient underwent transplantation was present at the time of death or found at autopsy, we defined disease relapse/progression as the primary cause of death. Unadjusted survival probabilities were estimated using the Kaplan and Meier method and compared using the log-rank tests. Cumulative incidence curves were used in a competing-risks model to calculate the probability of aGVHD, cGVHD and NRM [14]. For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event; for GVHD, death without GVHD and relapse were the competing events; and, for NRM, relapse was the competing event. In order to examine the impact of cGVHD on survival, we performed a landmark analysis, which divided patients according to their prior history of cGVHD at 6 months post-transplant [15]. We excluded from landmark analysis patients who died or relapsed less than 6 months after transplant, and did not use the information on whether or not patients developed cGVHD 6 months after transplant. Multivariable analysis of prognostic factors for the primary outcome could not be conducted due to lack of statistical power. Instead, we performed a landmark analysis, which divided patients according to the significant pre-transplant factors and their prior history of cGVHD at 6 months post-transplant. All P values were 2-tailed and considered statistically significant if the values were less than 0.05. All statistical analyses were performed using the PASW Statistics17.0 (SPSS Inc, Chicago, IL, USA) and the statistical software environment R, version 2.9.1.

Results

The baseline characteristics of the patients are shown in Table 1.

Table 1 Baseline characteristics of study participants

Variable	n (%)	Median (Range)
Male sex	24 (57.1)	
Diagnosis		
de novo AML	17 (40.5)	
ALL	12 (28.6)	
CML-AP	2 (4.8)	
MDS overt AML	10 (23.8)	
PCL	1 (2.4)	
Cytogenetics		
Intermediate	17	
Poor	22	
ECOG PS		
0	2 (4.8)	
1	25 (59.5)	
2	7 (16.7)	
3	8 (19.0)	
Status at allo-HCT		
Primary refractory/Refractory relapse/Untreated MDS overt AML	7/32/3	
No. chemo regimens prior allo-HCT		6 (0-18)
Time from diagnosis to allo-HCT (days)		319 (23-3738)
Marrow blasts at allo-HCT		26.0 (0.2-100)
Conditioning regimen		
Intensified	9 (21.4)	
Standard	12 (28.6)	
Reduced-intensity	7 (16.7)	
Reduced-intensity + cytoreductive chemotherapy	14 (33.3)	
GVHD prophylaxis		
None	3 (7.1)	
Calcineurin inhibitor alone	5 (11.9)	
Calcineurin inhibitor + sMTX	32 (76.2)	
Calcineurin inhibitor + MMF	2 (4.8)	
Donor (HLA-A, B and DRB1 antigens)		
Matched related PB/BM	10/2	
Mismatched related PB/BM	3/1	
Matched unrelated BM	19	
Mismatched unrelated BM	1	
Umbilical cord blood	6	

allo-HCT: allogeneic hematopoietic cell transplantation; HLA: human leukocyte antigen; sMTX: short-term methotrexate; MMF: mycophenolate motefil; BM: bone marrow; PB: peripheral blood.

Engraftment

Neutrophil engraftment was achieved in 33 (79%) of 42 patients. The median time to neutrophil engraftment was 17 days (range, 9-32). In a total of four of 27 evaluable patients, a platelet count > 20 000/μl was not achieved. In the patients that achieved platelet counts of ≥ 20 000/μl, the median time to platelet engraftment was 33 days (range, 13-99). The cumulative probabilities of neutrophil and platelet engraftment were 79% and 55%, respectively.

GVHD

Twenty-four of 42 patients developed aGVHD (eight grade I, nine grade II, five grade III, two grade IV). Twelve of 24 evaluable patients developed cGVHD (one

limited, 11 extensive). At five years, the cumulative probabilities of aGVHD and cGVHD were 63% and 37%, respectively.

NRM

A total of eight patients were alive at the time of this analysis, seven in complete remission (CR). The most common cause of death was disease relapse/progression. Causes of death were disease relapse/progression (n = 27), GVHD (n = 2), sinusoidal obstruction syndrome (SOS) (n = 3), Epstein-Barr virus associated post-transplant lymphoproliferative disorder (n = 1), and adenovirus infection (n = 1). Of six patients with CNS lesion, five died of disease relapse/progression (n = 3), GVHD (n = 1) and SOS (n = 1), and one was alive at last follow-up although another HCT was planned due to BM relapse post-transplant. At five years, the cumulative probability of NRM was 38%. Nine patients died before day 30, and 18 patients died within the first 100 days post-HCT.

LFS and OS

A total of 22 of 33 evaluable patients attained a CR after the allo-HCT. The median follow-up of survivors was 85 months (range, 24-126 months). The five-year Kaplan-Meier estimates of LFS and OS were 17% and 19%, respectively.

Univariable analysis

We analyzed the impact of pre- and post-transplant characteristics on OS after allo-HCT. The factors included age at transplant, sex, primary vs. secondary leukemia, cytogenetics at diagnosis, number of BM blasts, donor type, myeloablative vs. reduced-intensity conditioning, and presence or absence of acute and chronic GVHD. Results of univariable analysis for OS are summarized in Table 2. In the univariable analyses of the impact of pre-transplant variables on OS, poor-risk cytogenetics, number of BM blasts (>26%), MDS overt AML and CB as stem cell source were significantly associated with worse prognosis (p = .03, p = .01, p = .02 and p < .001, respectively). In addition, based on a landmark analysis at 6 months post-transplant, the five-year Kaplan-Meier estimates of OS in patients with and without prior history of cGVHD were 64% and 17% (p = .022) respectively (Figure 1).

Bivariable analysis

We performed the landmark analyses at 6 months post-transplant, which classified patients according to significant pre-transplant factors including poor-risk cytogenetics, number of BM blasts, or secondary leukemia and their prior history of cGVHD at 6 months post-transplant. Results of bivariable analysis for OS are shown in Figure 2, Figure 3 and Figure 4. The groups of patients with intermediate cytogenetics, marrow blast ≤ 26% or primary leukemia, who developed cGVHD less than 6 months after transplant, showed significantly or borderline significantly higher survival rates than those in the other groups (p = .039, p = .147, and p = .060, respectively). The five-year Kaplan-Meier estimates of OS in the patients with intermediate cytogenetics, marrow blast ≤ 26% or primary leukemia in addition to prior history of cGVHD were 75%, 83%, and 64%, respectively.

Table 2 Univariable analysis of impact of pre-transplant variables on overall survival

Variable	Survival (% at 5 y)	Log rank P value
Age at allo-HCT		
< 40	28	0.055
≥ 40	6	
Diagnosis		
MDS overt AML	0	0.015
Others	25	
Cytogenetics		
intermediate	35	0.013
poor	5	
Marrow blasts at allo-HCT		
≤ 26	33	0.013
> 26	5	
Donor source		
Umbilical cord blood	0	<0.001
Others	22	
Conditioning		
Intensified	22	0.087
Standard	42	
Reduced-intensity	0	
Reduced-intensity + cytoreductive chemotherapy	7	

allo-HCT: allogeneic hematopoietic cell transplantation.

Discussion

Our data showed that allo-HCT resulted in long-term disease remission and an eventual cure of active leukemia in a subset of de novo AML or ALL patients with marrow blast ≤ 26% and without poor-risk cytogenetics, possibly by graft-versus-leukemia (GVL) effects mediated through cGVHD.

A retrospective study with a large cohort using data reported to the Center for International Blood and Marrow Transplant Research demonstrated that pre-transplant variables delineated subgroups with different long-