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ORIGINAL ARTICLE

Use of mycophenolate mofetil in patients received allogeneic hematopoietic stem cell transplantation in Japan

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Abstract We evaluated the use of mycophenolate mofetil (MMF) after hematopoietic stem cell transplantation (HSCT) in Japan from 1999 to 2008. MMF was administered to 301 patients, including 157 for the prevention of graft-versus-host disease (GVHD), 94 for the treatment of acute GVHD and 50 for the treatment of chronic GVHD. The three most common doses were 500 mg twice daily, 250 mg three times daily and 1,000 mg twice daily, given to 63, 54 and 45 patients, respectively. The incidence of grade II–IV acute GVHD was 30.0% and grade III–IV was 20.0% in the GVHD prevention group. Among treated patients, disappearance or improvement of subjective symptoms occurred in 57.0% of acute GVHD patients and in 52.0% of chronic GVHD patients. With regard to safety, the following major adverse events (grade 3 or more) were recorded: 31 infections, 31 neutropenia, 28 thrombocytopenia, 25 diarrhea and 1 renal disorder. A total of 116 patients developed grade 3 or 4 adverse events, but 79 were successfully treated with supportive treatment. Thus, our findings suggest that MMF is safe and effective for the

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prevention and treatment of GVHD in patients who have received an allogeneic stem cell transplant.

Keywords Mycophenolate mofetil (MMF) · Allogeneic stem cell transplantation · GVHD

1 Introduction

Acute and chronic graft-versus-host disease (GVHD) are important complications following allogeneic hematopoietic stem cell transplantation (HSCT) that can be prevented or treated by immunosuppressive agents such as cyclosporine, tacrolimus, steroids or other therapies [1–3]. Some patients, however, do not respond to these conventional treatments. It is well recognized that mycophenolate mofetil (MMF) is widely used in countries outside Japan, and numerous reports have documented its efficacy for prophylaxis and treatment of GVHD [4–13].

In Japan, MMF is only approved as an immunosuppressant drug for organ transplantation (e.g., renal transplantation) and has not been approved for prophylactic or therapeutic use for GVHD in the field of HSCT. As there have been several reports of experimental MMF use for HSCT in Japan [14, 15], we conducted a nationwide survey to determine the efficacy and safety of MMF in the Japanese population.

2 Patients and methods

2.1 Study design

We retrospectively collected data on MMF use after allogeneic HSCT from related donors. Questionnaires were sent to 228 institutes registered with the Japan Society for Hematopoietic Cell Transplantation (JSHCT). A total of 57 surveys were returned detailing 301 patients undergoing MMF treatment. Data regarding the purpose of treatment, dosage, length of treatment, presence or absence of subjective symptoms of GVHD, GVHD grade and stage (before and after treatment), decrease or increase in concomitant immunosuppressants, effects, adverse events and outcomes were collected. Basic information for each transplantation was extracted from the Transplant Registry Unified Management Program (TRUMP) system, which is a registry used for Japanese patient outcomes [16]. Several demographic data were not available due to the lack of patient entry into the TRUMP system. The effects of MMF with regard to subjective symptoms (none, disappearance, improvement, no change and ingravescence) and the use of steroids (none, withdrawal, dose reduction, no change and dose increase) were assessed by physicians. Adverse events were evaluated by the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE, ver.3). This study was approved by the ethical committees of the Japan Society of Hematopoietic Cell Transplantation and the Nagoya University School of Medicine.

2.2 Statistics

Correlations between the two subgroups were examined using the χ^2 test and Fisher's exact test. P values of less than 0.05 obtained in two-sided tests were considered statistically significant. The data were analyzed with STATA version 10 statistical software (STATA Corp, TX).

3 Results

3.1 Patient background

Patient background data are summarized in Table 1. Patient age ranged from 12 to 70 years (median 41) at the time of transplantation, and there were 173 (57.5%) male and 128 (42.5%) female patients. Among the 301 patients, 97 (32.2%) received a transplant from HLA-matched donor, and 182 (60.5%), from HLA-mismatched donors. Of the HLA-mismatched donors, 66 (36.3%) were 1 locus, 46 (25.3%) were 2 loci and 55 (30.2%) were 3 loci mismatched. There were also 22 patients (7.3%) with missing HLA data. Among the 157 patients who received MMF for GVHD prophylaxis, 119 (75.8%) received a transplant from an HLA-mismatched donor, and among the 50 patients who received MMF as a treatment for chronic GVHD, 17 (34.0%) received a transplant from an HLA-mismatched donor. The graft source was peripheral blood stem cells (PBSCs) in 176 patients, bone marrow (BM) in 101 patients and PBSCs plus BM in 2 patients. The preconditioning regimen was myeloablative in 91 patients and non-myeloablative in 166 patients. Table 1 shows that the primary disease was hematological malignancy in the majority of patients (94.4%) with aplastic anemia or other diseases accounting for the remainder of the patients. Among the patients with the hematological malignancies, 65.9% (162/246, which is clear data of disease status) were in non-complete remission at the time of transplantation.

3.2 MMF administration

The aim of MMF administration was GVHD prevention in 157 patients, acute GVHD treatment in 94 patients and chronic GVHD treatment in 50 patients (Table 1). The daily MMF dosage varied from 250 to 3,000 mg, and the number of doses per day ranged from 1 to 8. The most common dosages and frequencies of MMF administration were

Table 1 Patient characteristics

Variables	Number
Patient number	301
Median age (range)	41 (12–70)
Male/female	173/128
Disease[a]	
Acute myeloid leukemia	78 (46)
Acute lymphoblastic leukemia	66 (44)
Chronic myelogenous leukemia	15 (11)
Myelodysplastic/myeloproliferative syndrome	39 (12)
Malignant lymphoma	75 (41)
Multiple myeloma	11 (8)
Aplastic anemia	3
Other diseases	14 (11)
Purpose of MMF	
GVHD prophylaxis	157
aGVHD treatment	94
cGVHD treatment	50
Graft source[b]	
Bone marrow (BM)	101
Peripheral blood stem cell (PBSC)	176
Both BM and PBSC	2
Donor type[b]	
Matched related	97
Mismatched related	182
1 locus mismatch	66
2 loci mismatch	46
3 loci mismatch	55
Unknown	15

[a] Numbers in parenthesis indicate those of not in complete remission

[b] Twenty-two data were missing for graft source and donor type

500 mg two times per day, 250 mg three times per day and 1,000 mg two times per day given to 63 patients (20.9%), 54 patients (17.9%) and 45 patients (15.0%), respectively. Consequently, 91 patients received 1,000 mg of MMF per day, and 54 patients, 750 or 2,000 mg per day. 59 patients were treated with a daily dose higher than 2,000 mg. There was no consistent pattern between the length and purpose of treatment. MMF administration was discontinued within 30 days in 113 patients (38.4%); however, 19 patients received MMF for more than a year (Fig. 1). Most patients (289 patients, 96.0%) were given MMF concurrently with other immunosuppressants (e.g., cyclosporine, tacrolimus or steroids), and only 12 patients (4%) received MMF alone.

3.3 Adverse events

Adverse events (AEs) associated with MMF administration are listed in Table 2. The major events were neutropenia, infection, thrombocytopenia and myelosuppression. Only

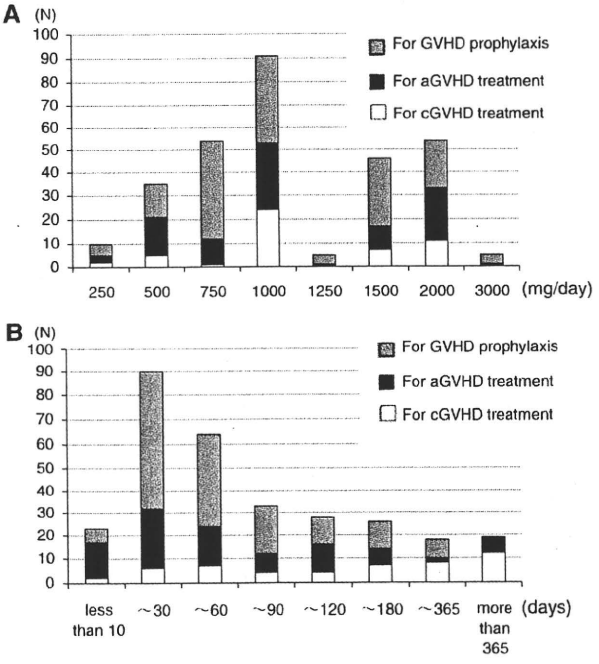

Fig. 1 **a** Initial dose of MMF. MMF was given at a variety of doses ranging from 250 mg per day to 3,000 mg per day. The most common dose was 500 mg twice a day ($N = 67$ among 91 patients taking 1,000 mg per day). **b** Dosing period of MMF. MMF was given for a variety of dosing periods (median 45 days)

three patients (1.7%) developed renal insufficiency with a grade 1, 2 or 4 increase in creatinine. Eighteen patients (6.0%) died from AEs associated with MMF (Table 3). The primary causes of death were infections in 11 patients (including 5 patients with pneumonia, 4 with sepsis and 2 with invasive *Aspergillus* infection), neutropenia in 3 patients, myelosuppression in 2 patients, 1 thrombocytopenia and 1 brain hemorrhage. There were 44 grade 4 AEs: 25 of these patients (56.8%) improved and 15 (34.1%) remained unchanged, but 4 (9.1%) eventually died. The incidence of AEs of grade 3 or higher (except infection) increased in accordance with the daily dosage of MMF (Fig. 2), but most of these AEs improved (Table 4).

3.4 Efficacy of MMF

Among the 157 patients who received MMF for GVHD prophylaxis, the incidences of grade II–IV and grade III–IV acute GVHD were 29.7% (43/145) and 20.0% (29/145), respectively. Limited and extensive chronic GVHD occurred in 21 (18.6%) and 30 (26.6%) patients, respectively ($N = 113$). No significant differences were found in the incidence of grade II–IV acute GVHD between HLA-matched and mismatched transplant patients (9/25 = 36.0 vs. 33/113 = 29.2%, $P = 0.63$), and no significant differences were noted between these two groups with regard to the

Table 2 Adverse events whose relationships to MMF were not necessarily denied

Adverse events: all (grade 3–5)	GVHD prophylaxis ($N = 157$)		aGVHD treatment ($N = 94$)		cGVHD treatment ($N = 50$)		Total ($N = 301$)	
	N	%	*N*	%	*N*	%	*N*	%
Infection	6 (5)	3.8 (3.2)	16 (13)	17.0 (13.8)	9 (8)	18.0 (16.0)	31 (26)	10.3 (8.6)
Diarrhea	6 (5)	3.8 (3.2)	16 (10)	17.0 (10.6)	3 (3)	6.0 (6.0)	25 (18)	8.3 (6.0)
Nausea	7 (2)	4.5 (1.3)	6 (4)	6.4 (4.3)	3 (0)	6.0 (0)	16 (6)	5.3 (2.0)
Vomiting	2 (0)	1.3 (0)	2 (0)	2.1 (0)	1 (0)	2.0 (0)	5 (0)	1.7 (0)
Neutropenia	5 (5)	3.2 (3.2)	21 (20)	22.3 (21.3)	5 (5)	10.0 (10.0)	31 (30)	10.3 (10.0)
Thrombocytopenia	5 (5)	3.2 (3.2)	18 (15)	19.1 (16.0)	5 (5)	10.0 (10.0)	28 (25)	9.3 (8.3)
Myelosuppression	7 (7)	4.5 (4.5)	10 (7)	10.6 (7.4)	4 (4)	8.0 (8.0)	21 (18)	7.0 (6.0)
Gastrointestinal bleeding	3 (2)	1.9 (1.3)	3 (3)	3.2 (3.2)	0 (0)	0 (0)	6 (5)	20.0 (1.7)
Constipation	1 (0)	0.6 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0.3 (0)
Others	5 (3)	3.2 (1.9)	7 (3)	7.4 (3.2)	3 (2)	6.0 (4.0)	15[a] (8[b])	5.0 (2.7)

Numbers in parenthesis indicate those for grade 3 or more toxicity

[a] Others: liver dysfunction (3), creatine kinase elevation (2), hair loss, hemorrhage cystitis

[b] Others: hypocalcemia, brain hemorrhage, septic shock, creatine kinase elevation, abdominal pain, TMA, diabetes mellitus, engraft failure

Table 3 Cause of death potentially associated with MMF

	Number
Infection	11
Pneumonia	5
Bacterial	2
MRSA	1
Fungal	1
CMV	1
Sepsis	4
Invasive *Aspergillus* infection	2
Neutropenia	3
Myelosuppression	2
Thrombocytopenia	1
Brain hemorrhage	1
Total	18

incidence of grade III–IV acute GVHD (6/25 = 24.0 vs. 22/113 = 19.5%, $P = 0.59$). The incidence of chronic GVHD, however, tended to be lower in the HLA-mismatched transplant group (14/23 = 60.9 vs. 35/83 = 42.2%, $P = 0.16$; Fig. 3), although this finding was not statistically significant. The incidences of grade II–IV and III–IV acute GVHD were lower in the subgroup of patients receiving 2,000 mg of MMF daily than in the subgroup receiving 1,000 mg daily (28.6 vs. 37% and 14.3 vs. 28.6% for grade II–IV and III–IV acute GVHD, respectively), although these differences were not statistically significant ($P = 0.51$ and 0.22, respectively). No dose effect was found for chronic GVHD prevention ($P = 0.72$).

Among the 94 patients in the acute GVHD treatment group, subjective symptoms disappeared in 27 (28.7%) and improved in 28 (29.8%). Symptoms remained unchanged in 17 patients (18.1%) and worsened in 22 patients (23.4%). Within this treatment group, 52 patients (55.3%) experienced improvement in their acute GVHD grade. Treatment with combined immunosuppressants was discontinued in 5 patients (5.3%) and reduced in 51 patients (54.3%). Among the 50 patients who received MMF as a treatment for chronic GVHD, the drug was effective against subjective symptoms (i.e., resulted in resolution or improvement) in 52.0% (10.0 and 42.0% experiencing resolution and improvement, respectively). Five patients (10.0%) discontinued combined immunosuppressants, and 29 (58.0%) reduced their dosage. The dosage remained unchanged in 14 patients (28.0%) and increased in only 2 patients (4%) (Fig. 4). In the acute GVHD treatment group, the effectiveness of MMF was higher among patients who had received HLA-matched transplants; however, this difference was not statistically significant for all items evaluated (58–70 vs. 32–69%, $P = 0.18$–0.60). In the chronic GVHD treatment group, the efficacy of MMF against subjective symptoms was higher in the HLA-matched subgroup than in the HLA-mismatched subgroup (17/33 = 51.5 vs. 3/9 = 33.3%, respectively, $P = 0.45$). In contrast, the rate of dosage reduction or discontinuation for combined immunosuppressants was higher in the HLA-mismatched subgroup than in the HLA-matched subgroup (7/9 = 77.8 vs. 21/33 = 63.6%, respectively, $P = 0.69$).

To assess the efficacy of MMF with regard to total daily dosage, we selected two subgroups: the most frequent dosage (1,000 mg per day) and the maximum dosage (more than 2,000 mg per day). The efficacy rate for every acute GVHD survey item was virtually identical between the 1,000 mg per day ($N = 28$) and 2,000 mg per day ($N = 23$) subgroups

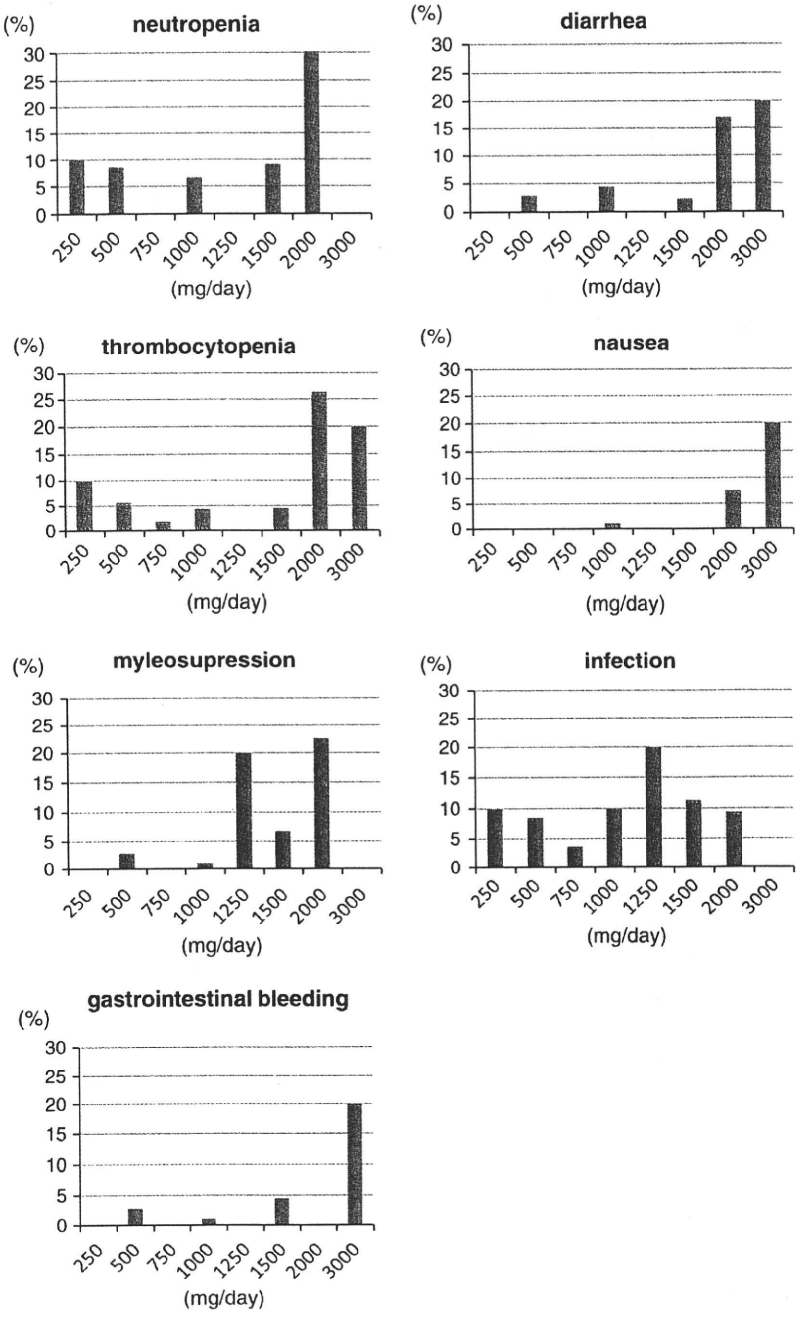

Fig. 2 Frequency of adverse events (grades 3–5) separated by total daily dose. High doses of MMF resulted in higher rates of hematological and gastrointestinal adverse events. Infections developed at all doses of MMF

(47.8–70.8 vs. 33.3–72.7%, respectively, $P = 0.06$-0.97). Among chronic GVHD patients, no difference in dose efficacy was observed between the two dosage subgroups ($N = 24$ in the 1,000 mg per day group and $N = 11$ for patients taking more than 2,000 mg per day, $P = 0.83$–0.91).

3.5 Transplantation outcome

In the GVHD prevention group, engraftment was seen in 122 of 134 evaluable patients (91.0%). Among all 301 patients, 62 (20.7%) relapsed and 169 (56.2%) died after transplantation. The overall survival rate was 41.9% at a median follow-up of 3 years. The main causes of death included disease recurrence in 33 patients (responsible for 19.5% of patient mortality), infection in 26 patients (15.4%), acute GVHD in 26 patients (15.4%) and chronic GVHD in 7 patients (4.1%). Among the 26 deaths due to acute GVHD, 18 patients were in the acute GVHD treatment group. Among the seven patients who died due to chronic GVHD, four were in the chronic GVHD treatment group.

Table 4 Rate of recovery from the adverse events in grades 3–4

	1,000 mg/day ($N = 91$)	More than 2,000 mg/day ($N = 59$)	Total ($N = 301$)
Infection	1/4 (25%)	1/2 (50%)	12/16 (75%)
Diarrhea	3/4 (75%)	7/10 (70%)	10/16 (63%)
Nausea	0/1 (0%)	4/5 (80%)	4/6 (67%)
Neutropenia	6/6 (100%)	12/15 (80%)	24/27 (89%)
Thrombocytopenia	3/4 (75%)	5/14 (36%)	11/24 (46%)
Myelosuppression	1/1 (100%)	8/11 (73%)	12/16 (75%)
Gastrointestinal bleeding	1/1 (100%)	0/1 (0%)	2/5 (40%)

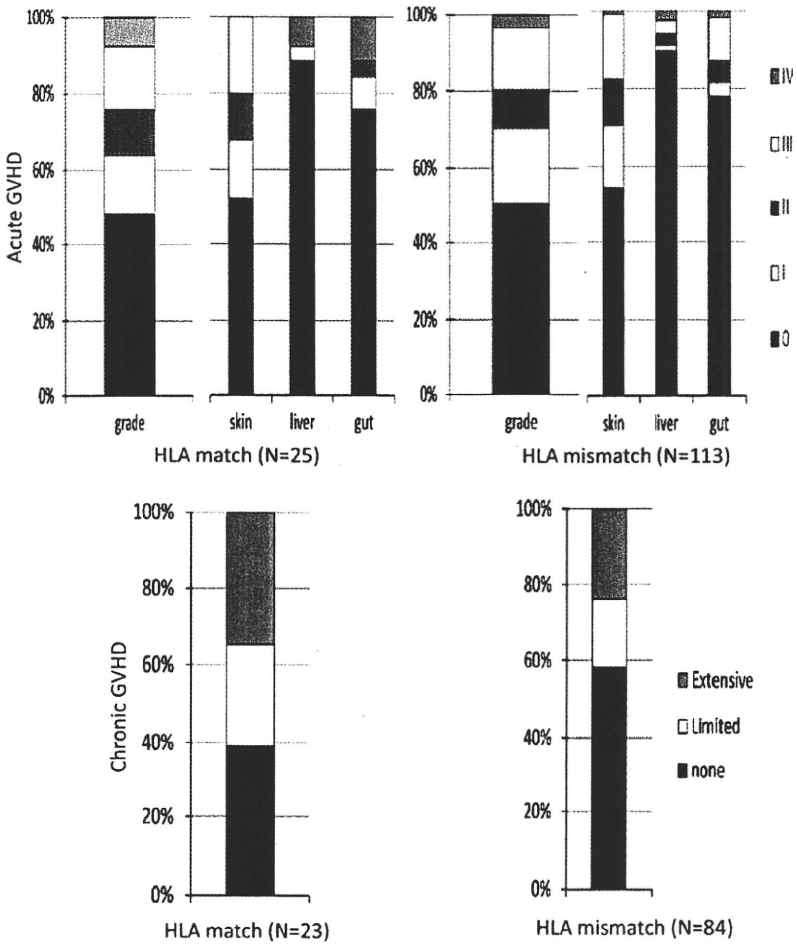

Fig. 3 Incidence of GVHD with prophylactic MMF use. The incidences of grade II–IV acute GVHD were 36.0 and 29.2% in the HLA-matched and -mismatched subgroups, respectively. In contrast, the incidence of chronic GVHD in the HLA-mismatched subgroup was lower (42.2%) than in the HLA-matched subgroup (60.9%)

4 Discussion

GVHD is one of the leading complications following allogeneic HSCT and is associated with morbidity and mortality. Calcineurin inhibitors and steroids are widely used for GVHD prevention and treatment, but several other immunosuppressive agents have been used for these purposes overseas [17–19]. Since 1997, many promising reports have compared MMF with conventional immunosuppressants [4–13]. In particular, reports focused on GVHD prevention are becoming increasing common due to the use of alternative donor sources [20]. Our current survey demonstrates that the efficacy rate of MMF is approximately 60% for the treatment of acute and chronic GVHD. Furthermore, our results also reveal that MMF is effective for the prevention of GVHD. Especially in HLA-mismatched patients, the frequency of grade III–IV acute GVHD was 20.3%, which was lower than the previous report subjected to HLA-mismatched transplants among Japanese populations [21]. As the efficacy of MMF was

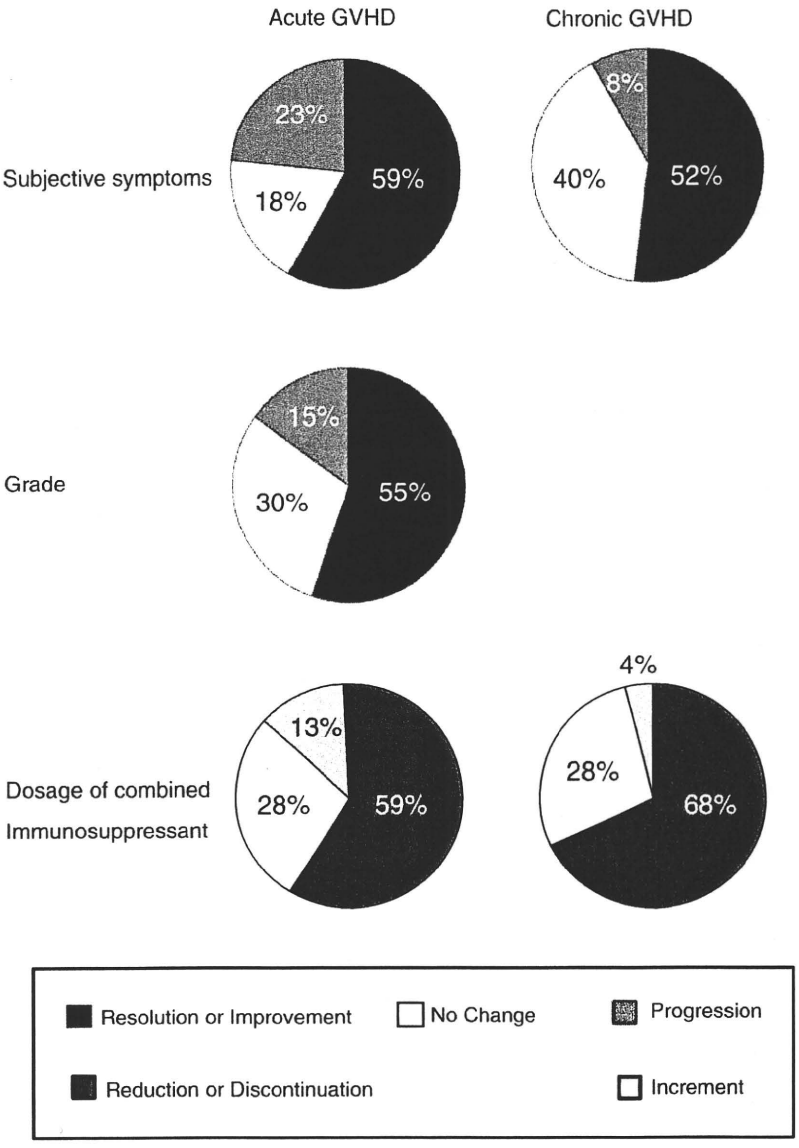

Fig. 4 Response of acute and chronic GVHD during therapeutic MMF use. Subjective symptoms of acute and chronic GVHD resolved in 59 and 52% of the cases, respectively, following the administration of MMF. In addition, 55% of the acute GVHD patients improved the grade of their disease. Finally, 60 and 68% of the acute and chronic GVHD patients, respectively, reduced or discontinued their use of combined immunosuppressant therapy

higher in patients receiving 2,000 mg per day than in those receiving 1,000 mg per day for chronic GVHD prevention, MMF doses of more than 2,000 mg per day are recommended for Japanese patients if the AEs are manageable.

Whether MMF is superior to existing immunosuppressants is a topic of continuing debate. Most previous reports on MMF have been promising, and the response rates for acute and chronic GVHD range from 47 to 71 and 26 to 76.9%, respectively, under various conditions [4, 6, 9–11, 17, 20]. On the other hand, one report suggested that MMF causes no significant improvement in the prevention of GVHD compared to cyclosporine and methotrexate (62 vs. 70%) [12]. Furthermore, another report showed that addition of MMF to an immunosuppressive regimen to control chronic GVHD had no effect (success rate of 15%) [22]. The results in this survey are not statistically different between using MMF and using cyclosporine or tacrolimus as reported in the previous report for the prevention and treatment of GVHD. We would like to emphasize, however, that the patient population in this study consisted mostly of HLA-mismatched donors and non-complete remission recipients (60.5 and 65.9%, respectively; Table 1). Even in this situation, MMF showed comparable efficacy. Therefore, we would like to conclude that MMF has a certain role for immunosuppressants.

Several reports have noted that the incidence of renal damage attributed to MMF (0–12.5%) is lower than that reported for other immunosuppressants like calcineurin inhibitors [4, 5, 11, 12, 23–25]. Our analysis revealed that the incidence of renal insufficiency (serum creatinine > 2 mg/dl)

was 1%. Serum creatinine > 2 mg/dl due to treatment with calcineurin inhibitors can be as high as 50–60 and 56–67% for cyclosporine and tacrolimus, respectively [26, 27]. Thus, MMF will be especially useful for patients with poor renal function.

In conclusion, MMF is tolerable and effective in Japanese patients who have received HSCT. Further studies are warranted to identify suitable candidates and appropriate therapeutic combinations of MMF for the prophylaxis and treatment of GVHD following allogeneic HSCT.

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ORIGINAL ARTICLE

Use of foscarnet for cytomegalovirus infection after allogeneic hematopoietic stem cell transplantation from a related donor

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Abstract Foscarnet is an active agent against cytomegalovirus (CMV) infection after hematopoietic stem cell transplantation (HSCT), as well as ganciclovir. We investigated the usefulness of foscarnet in patients who underwent related allogeneic HSCT. Foscarnet was used in 320 patients with a median age of 45 years (range 15–72). The purpose of administration was CMV disease in 65, preemptive use in 248 and prophylaxis in 7. Totally, 194 patients had a history of prior ganciclovir treatment. The reason for foscarnet use was insufficient therapeutic effect of prior ganciclovir in 99, and adverse event including myelosuppression in 95. The response rate in symptom was 52% for the CMV disease patients. Antigenemia disappeared in 77% of the preemptive treatment and improved in 13% of the patients. No outbreak of CMV disease was recognized. The total effectiveness of therapeutic and preemptive use was significantly higher for patients without prior ganciclovir (91 vs. 76%, $P = 0.001$). Adverse events of grade 3 or higher were recognized in 24%, including electrolyte abnormalities in 11%, neutropenia in 8%, and thrombocytopenia in 8%. Renal damage was only observed in 3% of patients. Foscarnet was concluded to be a safe and effective anti-CMV agent and to be a suitable alternative to ganciclovir.

Keywords Cytomegalovirus infection · Foscarnet · Blood and marrow transplantation · Efficacy · Adverse reaction

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1 Introduction

Cytomegalovirus (CMV) disease is one of the most important infectious complications after allogeneic hematopoietic stem cell transplantation (HSCT), which influences the outcome of the transplantation. The presence of graft-versus-host disease and steroid therapy are associated with the occurrence of CMV infection or reactivation. Ganciclovir is used as a first-line agent for both prophylaxis and the treatment of CMV disease [1–5]. However, approximately one-third of patients receiving ganciclovir develop drug-induced neutropenia or thrombocytopenia [6–9]. Therefore, ganciclovir is unsuitable for use in patients with poor bone marrow function. Another problem is ganciclovir resistant CMV [10–12].

For such cases, foscarnet is an important alternative agent that demonstrates anti-viral activity against all known herpes viruses including CMV [11, 13–15]. In early studies, the dose-limiting toxicities of foscarnet were found to be nephrotoxicity and neurotoxicity, which were seen in up to 50% of patients [16, 17]. Two randomized controlled trials (RCT) comparing the usefulness of preemptive foscarnet versus ganciclovir have been performed for CMV antigenemia [18, 19]. These studies revealed that the effectiveness of foscarnet was equivalent to that of ganciclovir. Adverse reactions and treatment-related mortality of foscarnet were also the same as those of ganciclovir. Renal dysfunction was only noted in 5% of the patients that received foscarnet [19].

The use of foscarnet has also been reported in cord blood transplantation, which is more complicated by viral infection [20]. These studies including the RCT only involved patients who had received foscarnet as an initial therapy. Therefore, we conducted a nationwide study in Japan of the use of foscarnet against CMV infection after related HSCT to investigate the current status, and compared its efficacy and toxicity in patients with and without prior ganciclovir use.

2 Patients and methods

2.1 Study design

This study is a retrospective survey investigating the use of foscarnet after stem cell transplantation. The subjects of this study were patients who received foscarnet after receiving allogeneic transplantation from a related donor in the period from 1998 to 2008. We performed a questionnaire at institutions carrying out allogeneic stem cell transplants in Japan. Data regarding the presence of CMV disease, CMV antigenemia, the reason for foscarnet use, the dose and duration of foscarnet, the effectiveness of therapy, and adverse events were collected. The obtained data were combined with data from the national registry of the Japan Society of Hematopoietic Cell Transplantation, which was collected by the TRUMP system [21]. This study was approved by the Ethical Committees of the Japan Society of Hematopoietic Cell Transplantation and Hyogo College of Medicine.

2.2 CMV antigenemia assay

Cytomegalovirus antigenemia was measured as described previously [22, 23]. Briefly, peripheral white blood cells were attached to slides by cytocentrifugation and stained with HRP-C7 (Teijin, Tokyo, Japan) or C10/C11 (Biotest, Dreieich, Germany) monoclonal antibodies. The number of positive cells was counted per 50,000 attached cells for HRP-C7 and per 150,000 applied cells for C10/C11. The examination was performed in duplicate, and the mean was used for further analyses.

2.3 Definition of CMV disease and infection

CMV diseases were defined as any organ infections by CMV, ideally proven by histopathologic examinations. They include gastroenteritis, pneumonia, retinitis, hepatitis, encephalitis, and cystitis. Patients who presented with interstitial pneumonia accompanied by CMV antigenemia were also diagnosed with CMV disease (pneumonia). For patients who presented with antigenemia and simultaneous diarrhea, gastrointestinal endoscopy and biopsy were recommended, but those who could not receive such diagnostic procedure were regarded as suspicious CMV disease (gastroenteritis). Both CMV antigenemia and CMV disease were regarded as CMV infection.

2.4 Type of therapy

The administration of anti-viral agents for patients without any CMV disease but accompanied by CMV antigenemia with or without febrile complications was defined as preemptive therapy in this study. Therapy of CMV disease was defined as CMV treatment. The use of anti-viral agents for those without antigenemia or CMV disease was regarded as prophylaxis.

2.5 Statistics

Pairwise comparisons were performed using the χ^2 test and Fisher's exact test for categorical variables, and the Mann–Whitney U test for continuous variables. The Kruskal–Wallis test was used to compare multiple groups. P values of <0.05 obtained in 2-sided tests were considered statistically significant. Data were analyzed with the STATA version 11 statistical software (STATA Corp, TX, USA).

3 Results

3.1 Patient characteristics

The background data of 320 patients are shown in Table 1. There were 171 males and 149 females. Their median age was 45 years, and the ages of the patients ranged from 15 to 72 years. The underlying disease of patients was acute myeloid leukemia (AML) in 110, acute lymphoblastic leukemia (ALL) in 59, chronic myelogenous leukemia (CML) in 18, myelodysplastic syndrome (MDS)/myeloproliferative disorder (MPD) in 42, chronic lymphocytic leukemia (CLL) in 2, non-Hodgkin lymphoma (NHL) in 51, Hodgkin lymphoma (HL) in 4, adult T cell lymphoma (ATL) in 16, multiple myeloma (MM) in 10, aplastic anemia (AA) in 6 and 1 each for renal cell carcinoma and virus associated hemophagocytic syndrome. Several demographic data were not available due to the lack of patient entry to the TRUMP system. CMV antibody was positive in both the patient and donor in 189 pairs (59%), in the patient only in 22 cases (7%), and in the donor only in 8 cases (3%), and it was negative in both patient and donor in 4 pairs (1%). Of 289 patients with evaluable data, 113 patients received bone marrow (BM) as a graft, 172 received peripheral blood stem cell (PBSC), and 4 received both BM and PBSC. HLA was matched in 108 of 268 patients but was mismatched in the remaining 160 (155 with serological mismatch and 5 with allele mismatch).

Table 1 Patient characteristics

Variables	Number
Patient number	320
Median age (range)	45 (15–72)
Male/female	171/149
Disease	
Acute myeloid leukemia	110
Acute lymphoblastic leukemia	59
Chronic myelogenous leukemia	18
Myelodysplastic/myeloproliferative syndrome	42
Chronic lymphocytic leukemia	2
Non-Hodgkin lymphoma	51
Hodgkin lymphoma	4
Adult T cell leukemia	16
Multiple myeloma	10
Aplastic anemia	6
Other diseases	2
CMV serology	
Donor +/Patient +	189
Donor +/Patient −	8
Donor −/Patient +	22
Donor −/Patient −	4
Graft source	
Bone marrow (BM)	113
Peripheral blood stem cell (PBSC)	172
Both BM and PBSC	4
Donor type	
Matched related	108
Mismatched related	160

3.2 CMV infection

Foscarnet was administered for CMV disease in 65 patients (20%), including 46 with gastroenteritis, 12 with pneumonia, 2 with retinitis, and one each for hepatitis, encephalitis, and cystitis. Each one other patient developed pneumonia and retinitis accompanied by simultaneous gastroenteritis. On the other hand, 248 (78%) were preemptively treated (only complicated with CMV antigenemia), and 7 (2%) were prophylactically treated. Before foscarnet administration, 194 (61%) patients had received ganciclovir, and one of the patients was treated with cidofovir after ganciclovir use. The reason for changing the anti-viral agent to foscarnet was insufficient therapeutic effect in 99 patients and adverse events due to preceding ganciclovir including myelosuppression in 95 patients. In 126 patients who had not received any anti-viral premedication, foscarnet was used because of poor bone marrow function in 116.

A total of 208 patients (67%) received steroid therapy at the time of foscarnet initiation. The rate of patients under steroid use was 58% for CMV disease, 70% for preemptive foscarnet, and 43% for prophylaxis, but the difference was not significant ($P = 0.08$).

3.3 Dosage of foscarnet

The initial dose of foscarnet ranged from 7 mg/kg to 216 mg/kg (median 88 mg/kg, Fig. 1). The dose was

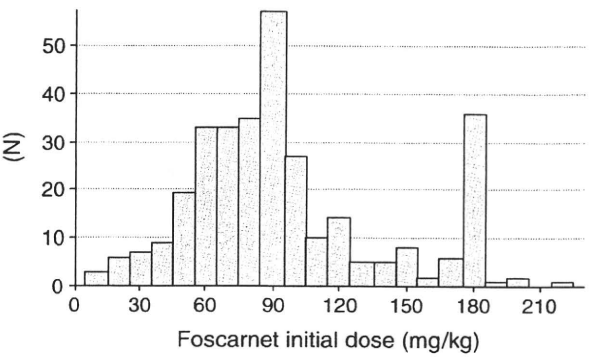

Fig. 1 Initial dose of foscarnet. Foscarnet was given at a variety of doses ranging from 7 to 216 mg/kg (median 88 mg/kg). Two peaks at 90 and 180 mg/kg were seen in the histogram

significantly higher in the patients who had received prior ganciclovir (range 10–216 mg/kg, median 91 mg/kg) than those who had not (range 7–180 mg/kg, median 72 mg/kg) ($P < 0.0001$). The median dose in the preemptive, treatment, and prophylactic groups was 89, 90, and 63 mg/kg, respectively; i.e., it was significantly lower in the prophylactic use group ($P = 0.05$). The initial dose of foscarnet did not have any correlation with creatinine clearance calculated from serum creatinine level and age by the Modification of Diet in Renal Disorder (MDRD) formula ($r = 0.21$, Fig. 2). The duration of foscarnet use ranged from 1 to 163 days (median 20 days) and was significantly shorter for patients who had received prior ganciclovir than those who had not (median 17 vs. 22 days, $P = 0.05$). As there were two peaks at 90 and 180 mg/kg in the dose of foscarnet administered, 5 dose categories (0–39, 40–79, 80–99, 100–159, and 160–220) were defined, and the efficacy and toxicity of foscarnet were estimated according to this categorization.

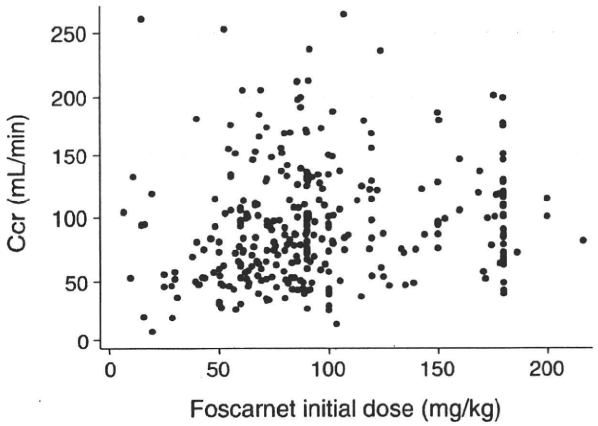

Fig. 2 Relationship between the initial dose of foscarnet and creatinine clearance. Creatinine clearance was calculated from serum creatinine level and age by the Modification of Diet in Renal Disorder (MDRD) formula [Ccr for male = $0.741 \times 175 \times (\text{age})^{(-0.203)} \times (\text{serum creatinine})^{(-1.154)}$, Ccr for female = $0.741 \times 175 \times (\text{age})^{(-0.203)} \times (\text{serum creatinine})^{(-1.154)} \times 0.742$]. No correlation was found ($r = 0.21$)

3.4 Efficacy

Among 65 patients with CMV disease, the symptoms disappeared in 5 (8%) and improved in 28 (44%), no change was seen in 20 (32%), and the symptoms worsened in 10 (16%) (Table 2). One patient was not evaluable with regards to their response, and another patient did not have any symptoms at the initiation of foscarnet because of the effect of prior ganciclovir use. The effectiveness (resolved or improved) was higher in those who did not receive ganciclovir, but the difference was not statistically significant (71 vs. 46%, $P = 0.10$). When the effectiveness in symptom was compared between HLA-matched and -mismatched transplant, the rate was almost comparable ($14/25 = 56\%$ vs. $14/29 = 48\%$, $P = 0.60$). Among 238 evaluable patients who received preemptive CMV therapy, antigenemia was resolved in 183 (77%) and improved in 31 (13%), but was not changed in 17 (7%) and worsened in 7 (3%). No patient developed outbreaks of CMV disease. The effectiveness was higher for those who had not received prior ganciclovir, but the difference was not significant ($93/99 = 93\%$ vs. $121/139 = 87\%$, $P = 0.13$).

Table 2 Response to foscarnet

	Symptoms				Antigenemia			
	Prior GCV		No prior GCV		Prior GCV		No prior GCV	
	N	%	*N*	%	*N*	%	*N*	%
CMV disease								
Disappeared	4	9	1	6	26	65	8	89
Improved/decreased	17	37	11	65	7	18	1	11
No change	18	39	2	12	4	10	0	0
Worsened/increased	7	15	3	18	3	8	0	0
No symptoms/antigenemia	1[a]	–	–	–	7	–	8	–
Unevaluable	1	–	0	–	1	–	0	–
Preemptive								
Disappeared	–	–	105	74	78	80		
Decreased	–	–	17	12	14	14		
No change	–	–	14	10	3	3		
Increased	–	–	5	4	2	2		
No antigenemia	–	–	4[a]	–	–	–		
Unevaluable	–	–	3	–	3	–		

GCV ganciclovir

[a] Symptoms/antigenemia had disappeared after prior GCV

Although the effectiveness in preemptive use was lower in HLA-matched transplant as compared with HLA-mismatched transplant, the difference was not also significant (64/75 = 85% vs. 114/123 = 93%, $P = 0.14$). Among the patients who received prior ganciclovir, the effectiveness was significantly higher in the patients in whom an insufficient effect of ganciclovir was seen compared with those who had suffered an adverse reaction to ganciclovir (64/68 = 94% vs. 57/71 = 80%, $P = 0.02$). The overall effectiveness of treatment and preemptive use was significantly higher in those who had not received prior ganciclovir (91 vs. 76%, $P = 0.001$) because of the low effectiveness in the patients of the CMV disease group who had received prior ganciclovir use. The changing courses of CMV antigenemia are box plotted in Fig. 3a for the patients who received prior ganciclovir and in Fig. 3b for those who did not. After the administration of foscarnet, the CMV antigenemia decreased in both groups ($P < 0.0001$ and $P = 0.01$, respectively).

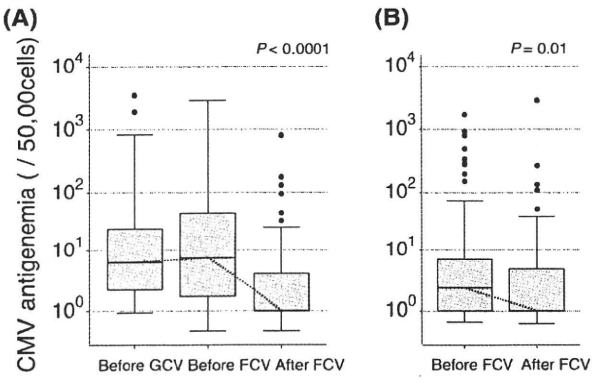

Fig. 3 Change in CMV antigenemia due to foscarnet therapy. The levels of antigenemia before ganciclovir, before foscarnet, and after foscarnet are box plotted. A significant decrease in antigenemia due to foscarnet treatment was observed both (**a**) for patients who had received prior ganciclovir treatment and (**b**) for those who had not

The responses to foscarnet according to the 5 dose categories are summarized in Fig. 4. The symptoms of CMV disease improved in around 50% of patients in every dose category. In the CMV disease patients the response rate of

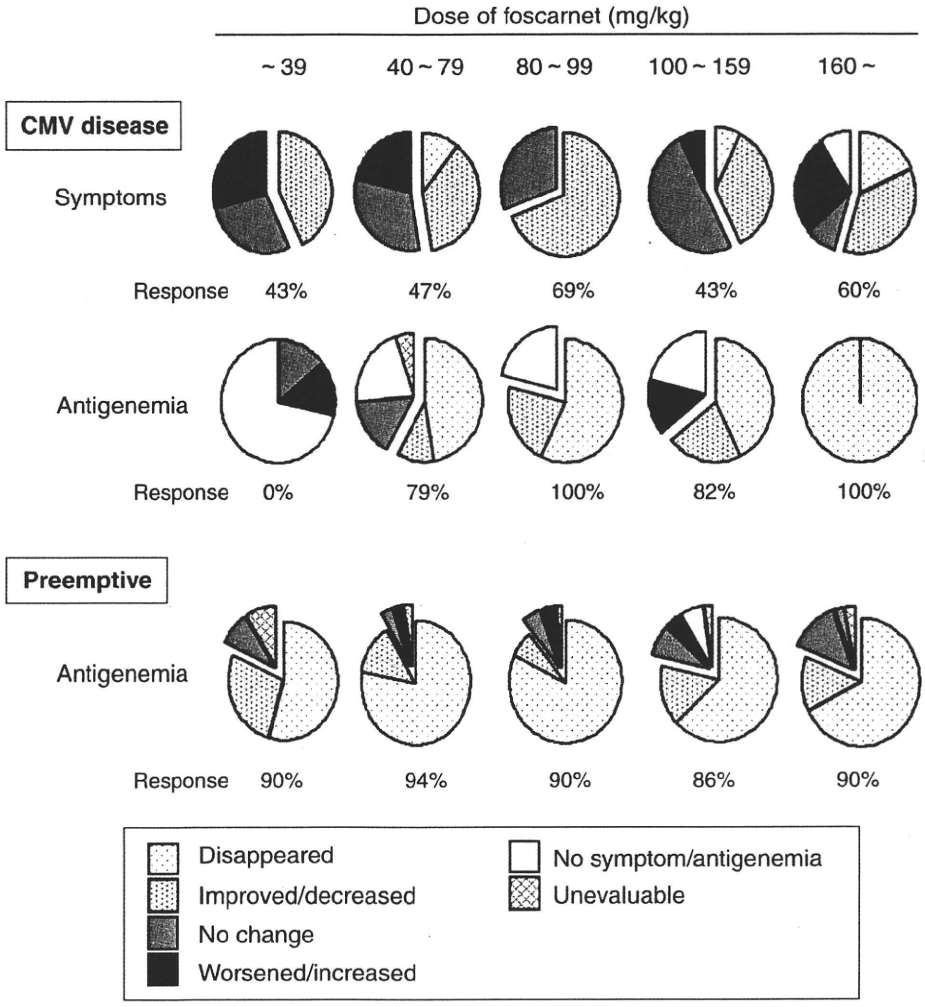

Fig. 4 Response to foscarnet according to 5 dose categories. The number of patients from the CMV disease group was 7 in the <39 mg/kg group, 19 in the 40–79 mg/kg group, 14 in the 80–99 mg/kg group, 14 in the 100–159 mg/kg group, and 11 in the 160 mg/kg or higher group, and those of the preemptive group were 11, 81, 73, 46, and 37, respectively. The response rate was around 50% for symptoms of CMV disease and was generally higher for antigenemia

antigenemia was significantly lower for those received foscarnet <40 mg/kg ($P = 0.01$).

3.5 Survival

The overall survival of all patients who received foscarnet was 34% at a median follow-up of 3 years (Fig. 5a). Patients with CMV disease showed significantly lower survival than those who received preemptive or prophylactic therapy (Fig. 5b, $P = 0.0004$). No significant difference in prognosis was found between the patients with and without preceding other anti-viral agents ($P = 0.21$). A total of 170 patients died, and the main causes of death were disease recurrence in 47, bacterial sepsis in 27, acute/chronic graft-versus-host disease in 25, and fungal infection in 10. The cumulative incidence of transplant-related mortality at 1 year was 30% (95% confidence interval 25–35%). Three patients eventually died of CMV disease, and the cumulative incidence of CMV-associated death at 1 year was 1.0% (95% confidence interval 0.3–2.6%).

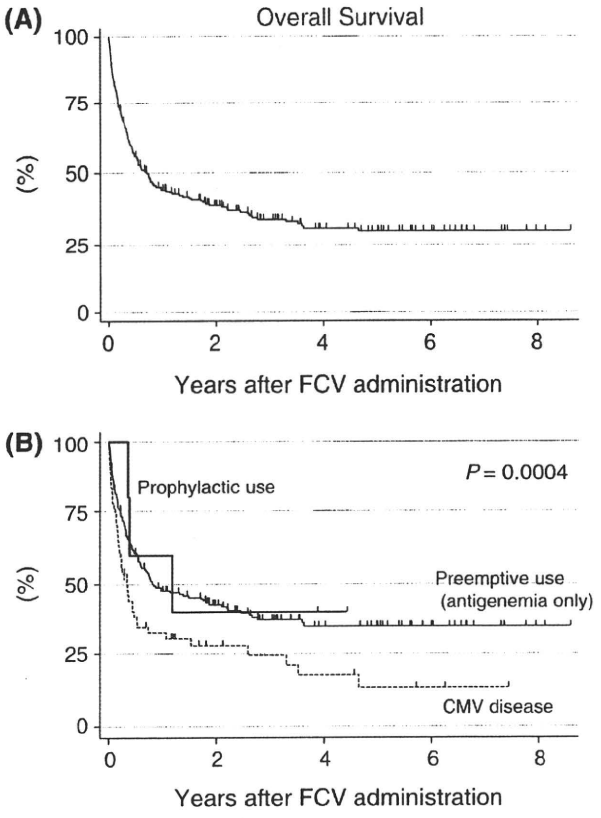

Fig. 5 Overall survival (OS) of patients who received foscarnet therapy. **a** The 3-year OS was 34%. **b** The prognosis of patients with CMV disease was significantly poorer than those of patients who had received preemptive or prophylactic use ($P = 0.0004$)

3.6 Adverse events

Adverse events (irrespective of causal association) of NCI-CTCAE grade 3 or higher are listed in Table 3. The most common adverse event was electrolyte abnormalities, which occurred in 35 patients (11%). The other major toxic events included neutropenia in 27 patients, thrombocytopenia in 26 patients, and bone marrow dysfunction in 11 patients. Renal and hepatic damage developed in 11 and 10 patients, respectively. Adverse events associated with foscarnet included neutropenia in 5 patients; electrolyte abnormalities in 4 patients; thrombocytopenia, renal dysfunction and sensory disturbance in 2 patients each; and bone marrow dysfunction in 1 patient. No patient died of an adverse reaction associated with foscarnet. The total number of patients who developed a grade 3 adverse reaction or higher was 56 (28%) in the patients who received prior ganciclovir and 21 (17%) in those who did not ($P = 0.03$). The rate of adverse events did not differ among the 5 dose categories (Table 4). The duration of foscarnet medication was not different between patients who developed adverse event of grade 3 or more (median 16 days, range 2–121) and those did not (median 20 days,

Table 3 Adverse events during foscarnet treatment

	Prior GCV $N = 198$		No prior GCV $N = 122$		Total $N = 320$	
	N	%	*N*	%	*N*	%
Graft failure	2	1.0	2	1.6	4	1.3
Neutropenia	19	9.6	8	6.6	27	8.4
Grade 3	7	3.5	2	1.6	9	2.8
Grade 4	12	6.1	6	4.9	18	5.6
Thrombocytopenia	19	9.6	7	5.7	26	8.1
Grade 3	6	3.0	0	0.0	6	1.9
Grade 4	13	6.6	7	5.7	20	6.3
BM dysfunction	7	3.5	3	2.5	10	3.1
Grade 3	4	2.0	1	0.8	5	1.6
Grade 4	3	1.5	2	1.6	5	1.6
Renal damage	6	3.0	5	4.1	11	3.4
Grade 3	4	2.0	5	4.1	9	2.8
Grade 4	2	1.0	0	0.0	2	0.6
Electrolyte abnormality	27	13.6	8	6.6	35	10.9
Grade 3	20	10.1	7	5.7	27	8.4
Grade 4	7	3.5	1	0.8	8	2.5
Neurological	3	1.5	1	0.8	4	1.3
Grade 3	3	1.5	1	0.8	4	1.3
Grade 4	0	0.0	0	0.0	0	0.0
Liver damage	9	4.5	1	0.8	10	3.1
Grade 3	7	3.5	0	0.0	7	2.2
Grade 4	2	1.0	1	0.8	3	0.9

BM bone marrow

Table 4 Adverse effects according to foscarnet dose

Dose level (mg/kg)	0–39 $N = 18$ (%)	40–79 $N = 106$ (%)	80–99 $N = 88$ (%)	100–159 $N = 60$ (%)	160– $N = 48$ (%)	Total $N = 320$ (%)
Any grade 3 or higher	33	23	17	25	35	24
Grade 3 or higher, possibly by foscarnet	28	12	13	17	17	15
Grade 3 or higher, definitely by foscarnet	0	2.8	3.4	8.3	6.3	4.4

range 1–322, $P = 0.50$). The difference was not evident for patients with possible and definite association with foscarnet ($P = 0.84$ and $P = 0.22$, respectively). When the adverse events were compared between HLA-matched and -mismatched transplant, the rates were significantly higher in the HLA-matched transplant. Any grade 3 or more toxicity was developed in 36 of 108 HLA-matched and 33 of 160 HLA-mismatched transplant ($P = 0.02$). Of these, 31 and 24, respectively, were possibly due to foscarnet use (29 vs. 15%, $P = 0.009$).

4 Discussion

The present study demonstrated that foscarnet is effective for patients with CMV infection who are not suitable for ganciclovir therapy. Sixty percent of the patients had a history of prior ganciclovir, but had demonstrated problems of ineffectiveness and/or adverse reactions. The remaining 40% had poor bone marrow function, and therefore foscarnet had been selected as the up-front use. In both situations, most of the patients were preemptively treated, and prophylactic use was seen in <2% of cases in our series.

The initial dose of foscarnet had two convergent doses, which were 90 and 180 mg/kg. The former corresponds to the maintenance dose, and the latter is the initial dose which was used in most prospective studies [18, 19]. The dose of foscarnet was significantly higher in patients with secondary therapy. This might have resulted from a higher number of more severe patients with CMV infection being present in the secondary therapy group. On the other hand, no dosage differences were found between the various purpose groups (preemptive/prophylactic/treatment). The lack of a correlation between foscarnet dose and creatinine clearance suggested that foscarnet was used irrespective of the renal function of the patient.

The most important adverse reaction of foscarnet was previously described as renal damage including electrolyte abnormalities. In that study, one-third of patients developed renal insufficiency and/or electrolyte disturbance [15]. However, a later study showed that these adverse events occurred less frequently [19]. In our series of patients, electrolyte abnormalities were recognized in 11% of patients, and renal insufficiency was found in no >3% of patients, which was consistent with the findings in the literature [24]. Thus, foscarnet seems to be a safer drug than was initially predicted.

In the preemptive use of foscarnet, >80% of patients showed CMV antigenemia disappearance in both the initial and secondary therapy groups. Foscarnet was highly effective in this setting, but its efficacy was decreased in CMV disease. The efficacy of foscarnet did not correlate with its dose, which was contradictory to a previous dose-finding study [25]. Our findings suggest a need to explore appropriate therapeutic strategies for this agent. Recently, "low-dose" administration of foscarnet at 60 mg/kg/day has been reported to be effective for CMV preemptive treatment [26, 27], which could be an option for future clinical trials. A prospective trial comparing ganciclovir alone and a combination of ganciclovir and foscarnet (half doses of both) was performed for HSCT and organ transplant patients [28]. The efficacy was equivalent for both arms, but adverse events were more frequent in the foscarnet combined arm.

In conclusion, our study shows that foscarnet is a safe and effective agent for treating CMV antigenemia after allogeneic HSCT. It remains to be determined how CMV infections should be treated, as well as how to improve the survival of affected patients.

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Alloantigen expression on non-hematopoietic cells reduces graft-versus-leukemia effects in mice

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Allogeneic hematopoietic stem cell transplantation (HSCT) is used effectively to treat a number of hematological malignancies. Its beneficial effects rely on donor-derived T cell–targeted leukemic cells, the so-called graft-versus-leukemia (GVL) effect. Induction of GVL is usually associated with concomitant development of graft-versus-host disease (GVHD), a major complication of allogeneic HSCT. The T cells that mediate GVL and GVHD are activated by alloantigen presented on host antigen-presenting cells of hematopoietic origin, and it is not well understood how alloantigen expression on non-hematopoietic cells affects GVL activity. Here we show, in mouse models of MHC-matched, minor histocompatibility antigen–mismatched bone marrow transplantation, that alloantigen expression on host epithelium drives donor T cells into apoptosis and dysfunction during GVHD, resulting in a loss of GVL activity. During GVHD, programmed death–1 (PD-1) and PD ligand–1 (PD-L1), molecules implicated in inducing T cell exhaustion, were upregulated on activated T cells and the target tissue, respectively, suggesting that the T cell defects driven by host epithelial alloantigen expression might be mediated by the PD-1/PD-L1 pathway. Consistent with this, blockade of PD-1/PD-L1 interactions partially restored T cell effector functions and improved GVL. These results elucidate a previously unrecognized significance of alloantigen expression on non-hematopoietic cells in GVL and suggest that separation of GVL from GVHD for more effective HSCT may be possible in human patients.

Introduction

Donor immunity in allogeneic hematopoietic stem cell transplantation (HSCT) harnesses beneficial graft-versus-leukemia (GVL) effects; therefore, allogeneic HSCT represents a very potent form of immunotherapy for hematological malignancies (1, 2). Induction of GVL is usually associated with the development of graft-versus-host disease (GVHD), which is a major complication after allogeneic HSCT. T cell depletion of the donor inocula prevents GVHD and leads to a loss of the GVL effect (3–5). Both GVL and GVHD are mediated by donor T cells, which recognize alloantigens presented on host APCs (6, 7). Donor CTLs and inflammatory cytokines are major effectors of GVHD, whereas CTLs are primarily responsible for GVL (8, 9). In patients with advanced-stage leukemia and lymphoma, relapse is still a major cause of mortality after allogeneic HSCT even after the development of severe GVHD. Thus, improvements in our understanding of the pathophysiology of GVHD and GVL are urgently needed to develop more effective therapies for malignant diseases.

Alloantigens are expressed on the three major components in HSCT recipients in the context of GVHD and GVL: hematopoietically derived APCs, GVHD target epithelium, and leukemia cells. Several studies have shown that host APCs are crucial for the induction of both GVHD and GVL (6, 7, 9–11). Alloantigen expression on epithelium is also critical for the induction of GVHD in MHC-matched, minor histocompatibility antigen–mismatched (mHA-mismatched) models of bone marrow transplantation (BMT) (10), but GVHD can occur in the absence of alloantigen expression on epithelium in MHC-mismatched models of BMT (9). However, the effect of alloantigen expression on non-hematopoietic cells such as the epithelium in GVL is not well defined. In this study, we addressed this important issue in mHA-mismatched models of BMT.

Results

Alloantigen expression on host non-hematopoietic cells augments acute GVHD but reduces GVL effects. We generated BM chimeric mice that express alloantigens on APCs, which are essential for the induction of both GVHD and GVL (6, 7, 12). BM chimeras were created by reconstituting lethally irradiated C3H.Sw (C3: H-2^b) mice with 5×10^6 T cell–depleted (TCD) BM cells isolated from C57BL/6 (B6, H-2^b) mice that differ from C3 mice at multiple mHAs ([B6→C3] chimeras). Control chimeras, [B6→B6], were identically created. Four months later, donor repopulation of hematopoiesis was confirmed by flow cytometry as shown previously (6, 9, 12). Thus, [B6→C3] chimeric mice expressed B6-derived mHAs on hematopoietically derived APCs but not on non-hematopoietic target cells. In contrast, [B6→B6] mice expressed B6-derived mHAs on both APCs and target epithelium. These chimeras were used as BMT recipients; they were reirradiated and injected with 5×10^6 TCD BM cells alone or with various doses of $CD8^+$ T cells from C3 donors. After BMT, GVHD mortality was higher in [B6→B6] mice than in [B6→C3] mice (Figure 1A). Clinical GVHD scores (13) in surviving animals were also higher in [B6→B6] mice than in [B6→C3] mice (Figure 1B). Mortality and morbidity from GVHD in [B6→C3] mice were almost equivalent to those in [B6→B6] mice given a 1-log lower T cell dose. This finding confirmed the previous observation of a lack of alloantigen expression on host epithelium significantly reducing GVHD across mHA disparity (10). We

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