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ORIGINAL ARTICLE

Therapy with mycophenolate mofetil for refractory acute and chronic GVHD

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We evaluated the pharmacokinetics and efficacy of oral mycophenolate mofetil (MMF) for treatment of refractory GVHD. In a prospective study of acute GVHD, 9 of 19 patients (47%) had a response and 10 (53%) had no improvement. Survival at 6 and 12 months after the start of MMF was 37 and 16%, respectively. In a retrospective study of acute GVHD, 14 of 29 patients (48%) had a response and 15 (52%) had no improvement. Survival at 6 and 12 months was 55 and 52%, respectively. In a prospective study of chronic GVHD, the cumulative incidence of disease resolution and withdrawal of all systemic immunosuppressive treatment was 9, 17 and 26% at 12, 24 and 36 months, respectively, after starting MMF. Thirteen patients (59%) required additional systemic immunosuppressive treatment for chronic GVHD. Nine of the 42 patients (21%) in the prospective studies discontinued MMF treatment because of toxicity. The area under the curve plasma concentrations of mycophenolic acid seemed to be suboptimal among patients with acute GVHD but not among those with chronic GVHD. MMF can be used effectively for treatment of GVHD.
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Introduction

Acute and chronic GVHD are the most common early and late complications after allogeneic hematopoietic SCT (HSCT) and constitute a major cause of morbidity and non-relapse mortality.[1,2] Depending on the degree of recipient HLA mismatching, the source of the donor graft and other risk factors, 30–80% of graft recipients will develop acute GVHD, usually requiring systemic immunosuppressive treatment.[3–6] Overall response rates in the range of 24–55% have been reported after initial therapy with glucocorticoids or other immunosuppressive medications,[7–12] and many patients require secondary therapy with additional immunosuppressive agents. Overall response rates in the range of 30–75% have been reported for a variety of agents used for the treatment of steroid-resistant or steroid-refractory acute GVHD, although TRM remains high, even when secondary treatment improves GVHD manifestations.[13–21]

Depending on the stem cell source and the time of evaluation, ~30–65% of patients develop chronic GVHD after HSCT, often requiring long-term glucocorticoid treatment.[22,23] In a large retrospective study, the median duration of the treatment for chronic GVHD was 23 months.[24] Both acute and chronic GVHD are associated with an increased risk of TRM, often related to opportunistic infections.[22,25] More effective and less toxic therapies and treatment strategies are needed to improve the management of GVHD and decrease morbidity and non-relapse mortality.

Mycophenolate mofetil (MMF) is the 2-(4-morpholino) ethyl ester of mycophenolic acid (MPA). MMF is rapidly absorbed after oral administration and hydrolyzed to the active metabolite, MPA.[26] MPA selectively and reversibly inhibits inosine monophosphate dehydrogenase, blocking the *de novo* pathway of purine synthesis in T and B lymphocytes.[27–29] Preclinical studies in allogeneic transplant models have shown that MMF is active in preventing graft rejection and GVHD.[30–33] After transplantation of unrelated dog leukocyte antigen-mismatched marrow in dogs, MMF synergizes with CYA to prevent GVHD and improve survival.[34] Phase III clinical studies after kidney transplantation have shown that MMF is effective in the prevention of graft rejection.[35–38] Studies using MMF for the treatment of acute and chronic GVHD after HSCT have reported efficacy in the range of 30–90%.[39–48]

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Two phase II clinical trials and a retrospective review were conducted to evaluate the safety and efficacy of oral MMF for treatment of acute and chronic GVHD. As gastrointestinal toxicity from the conditioning regimen or gut involvement from acute GVHD might affect the absorption of oral MMF, plasma levels of MPA were measured to assess differences between acute and chronic GVHD and to determine the optimal dosing of MMF for the treatment of GVHD.

Patients and methods

Patient enrollment in these two prospective clinical trials began in November 1995 and was completed in December 1997. The Institutional Review Board at the Fred Hutchinson Cancer Research Center approved the trials, and all patients signed consent documents. Patients were approached sequentially for enrollment in each of these trials, provided they met the eligibility criteria summarized below. The original plan was to enroll 20 patients in each study.

For purposes of comparison and validation, results were retrospectively reviewed for all patients who received MMF for secondary therapy of acute GVHD after initial treatment with prednisone after HSCT with myeloablative conditioning regimens between 2000 and 2005. This analysis was also approved by the Fred Hutchinson Cancer Research Center Institutional Review Board.

Patients

The primary therapy for acute GVHD was prednisone or methylprednisolone, initially at 2 mg/kg/day, in addition to continued administration of CYA originally prescribed for prophylaxis. Patients with acute GVHD were eligible for the acute GVHD study (AGVHD Group) if GVHD manifestations worsened after 3 days of primary therapy, showed no improvement after 7 days or persisted for more than 14 days. Patients with skin or gastrointestinal involvement had to have biopsy-proven evidence of GVHD.

Initial treatment for chronic GVHD was prednisone at 1 mg/kg/day, in addition to continued administration of CYA or tacrolimus. Patients with extensive chronic GVHD were eligible for the chronic GVHD study (CGVHD Group) if GVHD manifestations (1) worsened or showed no improvement after at least 2 months of initial treatment, (2) persisted after at least 9 months of treatment in patients with 'high risk' chronic GVHD (progressive onset or plt count $<100\,000/mm^3$ at onset), (3) or for at least 18 months of treatment in patients with 'standard risk' chronic GVHD or (4) if GVHD manifestations recurred during a taper of steroid doses.

Patients were excluded from participation in either study if they were unable to tolerate oral therapy, if the neutrophil count was $<1500/\mu l$ or if they had evidence of recurrent malignancy. Patients with a serum creatinine concentration >2.0 mg per 100 ml were excluded from participation in the acute GVHD study, and those with a creatinine clearance <30 ml/min were excluded from the chronic GVHD study.

Treatment plan and supportive care

All study patients received the oral formulation of MMF at an initial dose of 1 g twice daily for adults and 20 mg/kg per day twice daily for patients who weighed <50 kg. For patients in the AGVHD Group, the dose of MMF could be increased to 1.5 g twice daily if GVHD manifestations had worsened at study day 7 or had not improved by study day 14. The protocol recommended adjustments in the dose of MMF if hematopoietic or gastrointestinal toxicity was suspected. Recommendations were to decrease the MMF dose by 50% if the neutrophil count decreased to $<1000/\mu l$, and to discontinue administration of MMF if the neutrophil count decreased to $<750/\mu l$. Additional recommendations were to decrease the dose of MMF if patients developed gastrointestinal problems not caused by GVHD and to discontinue administration of MMF if problems persisted afterward.

For patients in the AGVHD Group, MMF was administered for 35 days. Treatment with MMF was discontinued if GVHD resolved or there was no response by study day 35. Treatment with MMF could be continued beyond 35 days in patients with a partial response and in those with recurrent GVHD after treatment with MMF was discontinued. Glucocorticoid doses were tapered at the discretion of the attending physician. For patients in the retrospective AGVHD Group, the decision to start MMF therapy, the dosing and route of administration, and the duration of therapy were determined by the attending physician.

For treatment of CGVHD, MMF was administered for 9–12 months. Treatment with MMF was discontinued if GVHD manifestations resolved. If GVHD manifestations improved but persisted after 9–12 months, treatment with MMF was continued for another 9–12 months. If GVHD manifestations showed no improvement or worsened after 2 months, treatment with MMF was discontinued. In the absence of toxicity, administration of CYA or tacrolimus was continued during the initial period of treatment with MMF.

All protocol patients received antifungal prophylaxis with fluconazole. All CMV-seropositive patients had weekly testing for reactivation of CMV, and pre-emptive therapy with ganciclovir was initiated when appropriate. Patients received prophylaxis against Pneumocystis pneumonia with trimethoprim/sulfamethoxazole. Dapsone was given to patients who could not tolerate trimethoprim/sulfamethoxazole.

Pharmacokinetics

Plasma levels of MPA, the active metabolite of MMF, were measured on days 1, 7 and 35 after the start of MMF therapy. Day 1 sampling was started before administration of the first dose of MMF. Specimens were collected immediately before and at 1, 2, 4, 8 and 12 h after ingestion of MMF. Blood was collected in tubes containing EDTA. Blood was centrifuged at 4 °C and the plasma was collected. Plasma was stored at −70 °C and shipped to the University

of Alberta, Canada, for analysis. MPA plasma levels were quantified by HPLC and peak plasma concentration (C_{max}) and area-under-the-curve (AUC) were determined as described earlier.[49]

Evaluation of response

The evaluation and grading of acute and chronic GVHD have been described earlier.[1,2,50] Treatment responses in the AGVHD Group were categorized as a complete response, a partial response or a treatment failure. A complete response was defined as the absence of any symptoms related to GVHD, with no additional agents needed to control the disease. A partial response was defined as the improvement of at least one stage in the severity of acute GVHD in one organ without deterioration in any other organ. Treatment failure was defined as the absence of improvement, deterioration of acute GVHD in any organ by at least one stage, the development of GVHD manifestations in an earlier unaffected organ, or the use of any additional agents to control the disease. Patients were scored for best response at any time after starting treatment with MMF, with follow-up censored at the onset of any subsequent systemic immunosuppressive therapy. This criterion was also used to assess the response to treatment with MMF for patients in the retrospective AGVHD Group.

Responses in the CGVHD Group were measured according to two outcomes. Failure was defined as the use of any additional agents to control GVHD within 3 years after starting treatment with MMF, including the resumption of treatment with agents used earlier or the substitution of one calcineurin inhibitor for the other. Success was defined as the discontinuation of all systemic immunosuppressive therapy without recurrent malignancy within 12, 24 and 36 months after starting treatment with MMF. Medications used to treat chronic GVHD were abstracted from records of evaluations in Seattle and from correspondence with the referring physician. In both groups, follow-up for evaluation of GVHD was censored at the onset of recurrent malignancy.

Results

Patient and transplant characteristics

Nineteen patients with acute GVHD and 23 patients with chronic GVHD were enrolled in the two prospective studies, and the retrospective analysis included 29 patients (Table 1). Compared with the prospective AGVHD Group, the patients in the retrospective AGVHD Group were older (median age, 40 years vs 20 years), were more likely to have low-risk hematological malignancies (52 vs 32%), had a higher proportion of unrelated (93 vs 53%) and HLA-mismatched donors (45 vs 37%), and a higher proportion received PBSC grafts (69 vs 21%). Approximately half of the patients in the CGVHD Group had unrelated donors. Most patients in the CGVHD Group had low-risk hematological diseases at the time of the transplant and received BM grafts. Three patients in the CGVHD Group and one in the prospective AGVHD Group had undergone a previous allogeneic HSCT. None of the patients in the retrospective acute GVHD Group had undergone a previous HSCT.

Table 1 Patient and transplant characteristics according to treatment group

Characteristic	*AGVHD*		*CGVHD Group (n = 23)*
	Prospective Group (n = 19)	*Retrospective Group (n = 29)*	
Patient age, median years (range)	20 (4–54)	40 (2–61)	27 (2–57)
Disease risk, n (%)[a]			
Low	6 (32)	15 (52)	15
High	13 (68)	14 (48)	8
Donor type and recipient HLA-matching, n (%)			
Related donor			
HLA-matched	5 (26)	2 (7)	10
HLA-mismatched	4 (21)	0 (0)	3
Unrelated donor			
HLA-matched	7 (37)	14 (48)	4
HLA-mismatched	3 (16)	13 (45)	6
Stem cell source, n (%)[b]			
Marrow	16 (84)	9 (31)	21
PBSC	4 (21)	20 (69)	2
Conditioning regimen, n (%)			
CY/TBI	6 (32)	14 (48)	13
BU/CY	0 (0)	10 (35)	6
BU/TBI	5 (26)	0 (0)	0
Other	8 (42)	5 (17)	4
GVHD prophylaxis, n (%)			
CYA/MTX	15 (78)	23 (80)	16
Tacrolimus/MTX	0 (0)	3 (10)	0
CYA/prednisone	2 (11)	0 (0)	2
Other	2 (11)	3 (10)	5

Abbreviations: AGVHD = acute GVHD; CGVHD = chronic GVHD.
[a]Low-risk disease included CML in chronic phase; acute leukemia in first remission; refractory anemia without excess blasts; and lymphoma in first remission, first untreated relapse or second remission. Three patients with thalassemia, Chediak–Higashi syndrome and paroxysmal nocturnal hemoglobinuria were included in the low-risk category.
[b]One patient in the prospective AGVHD group received PBSC and marrow.

Treatment response: AGVHD Groups

In the prospective study, the median onset of acute GVHD was 10 days after HSCT (range 5–30) (Table 2). MMF therapy was started at a median of 42 days after transplantation and 31 days after the diagnosis of acute GVHD. The median duration of MMF administration was 35 days (range 3–416). Seventeen patients received glucocorticoids as initial treatment for acute GVHD and two patients received CYA. Three patients had received one additional systemic immunosuppressive therapy for acute GVHD before starting treatment with MMF. In this group, 6 of 19 patients (31%) had complete resolution of acute GVHD, three (16%) had partial responses and 10 (53%) had no improvement. In three patients, treatment with MMF was stopped after 3 days because of progressive GVHD. One of these patients had no further treatment,

Table 2 Acute GVHD characteristics and treatment responses

Characteristic	*Prospective Group (n = 19)*	*Retrospective Group (n = 29)*
Onset of acute GVHD, median day after HSCT (range)	10 (5–30)	11 (5–34)
Acute GVHD grade at start of MMF therapy, n (%)		
I	0 (0)	3 (10)
II	12 (63)	22 (76)
III	5 (26)	4 (14)
IV	2 (11)	0 (0)
Organ involvement at the start of MMF therapy, n (%)		
Skin	13 (68)	22 (76)
Gastrointestinal	10 (53)	11 (38)
Hepatic	6 (32)	7 (24)
Start of MMF therapy, median day (range)		
From HSCT	42 (27–89)	52 (22–131)
From diagnosis of acute GVHD	31 (14–79)	42 (13–120)
From start of primary therapy	24 (9–68)	35 (4–121)
Clinical response, n (%)		
Complete response	6 (31)	9 (31)
Partial response	3 (16)	5 (17)
Treatment failure	10 (53)	15 (52)
Subsequent chronic GVHD, n (%)		
Yes	13 (93)	22 (88)
No	1 (7)	3 (12)
Not evaluated	5	4

Abbreviation: MMF = mycophenolate mofetil.

and the other two were treated with antithymocyte globulin. All three died within 12 days. At the start of treatment with MMF, 12 patients (63%) had Grade II GVHD, five (26%) had Grade III GVHD and two (11%) had Grade IV GVHD. Eight of the 12 patients (67%) with Grade II GVHD at the start of MMF therapy had a complete or partial response, compared with only one of the seven patients (14%) with Grades III–IV GVHD. All but one of the 14 patients surviving beyond 100 days after HSCT developed chronic GVHD.

In comparing the retrospective AGVHD Group with the prospective AGVHD Group, the main differences were a lower incidence of Grade III–IV GVHD at the onset of MMF therapy (14 vs 37%), and longer median times from HSCT to the onset of treatment with MMF (52 days vs 42 days), from initial diagnosis of GVHD to the onset of treatment with MMF (42 days vs 31 days), and from the onset of steroid treatment to the onset of treatment with MMF (35 days vs 24 days) (Table 2). Patients in the retrospective AGVHD Group had a lower incidence of gastrointestinal (38 vs 53%) and hepatic (24 vs 32%) GVHD. There were no differences in the time of onset of GVHD or the clinical response to MMF. In this group, 9 of 29 patients (31%) had complete response, five (17%) had partial response and 15 (52%) had no improvement. Despite the differences in GVHD severity and interval times at onset of MMF therapy, the response rates in the two AGVHD Groups were virtually identical. Seventy-nine percent of the patients in the retrospective AGVHD Group were initially treated with the oral formulation of MMF.

Table 3 Chronic GVHD characteristics and treatment responses

Characteristic	*CGVHD Group (n = 23)*
Months from transplantation to start of MMF, median (range)	20 (4–91)
No. of prior chronic GVHD therapies, n	
1	13
2	3
None	7
Affected sites at the start of treatment with MMF, n	
Skin	18
Oral	18
Eyes	9
Gastrointestinal tract	4
Lung	4
Liver	4
Vagina	1
No. of sites involved at the start of treatment with MMF, n	
1	4
2	9
3	6
More than 3	4
Immunosuppressive therapy (IS) at study enrollment, n	
CYA or tacrolimus plus glucocorticoids	14
Glucocorticoids alone	4
CYA or tacrolimus alone	2
Other	3
Months of treatment with MMF, median (range)[b]	12 (3–99)
Patients who discontinued all immunosuppressive treatment, n (%)[a]	
12 months	2 (9)
24 months	4 (17)
36 months	6 (26)
Months to discontinuation of immunosuppressive treatment, median (range)	22 (9–35)
Patients requiring additional treatment for GVHD after MMF, *n* (%)[b]	13 (59)

Abbreviations: MMF = mycophenolate mofetil.
[a]Two patients could not be evaluated at 12 months, 9 at 24 months and 9 at 36 months.
[b]One patient was lost to follow-up and could not be evaluated.

All patients in the prospective AGVHD Group were treated exclusively with the oral formulation.

Treatment response: CGVHD Group

Treatment with MMF was started at a median of 20 months after HSCT (Table 3). MMF was administered for a median of 12 months (range 3–99). Sixteen of the 23 patients (70%) had received at least one systemic immunosuppressive therapy for chronic GVHD in addition to glucocorticoids and the original prophylactic calcineurin inhibitor before starting treatment with MMF. Seven patients had received tacrolimus, six had received azathioprine and four had received thalidomide. When MMF therapy was started, most patients were receiving combination immunosuppressive therapy with a calcineurin inhibitor plus glucocorticoids. Nine patients had chronic

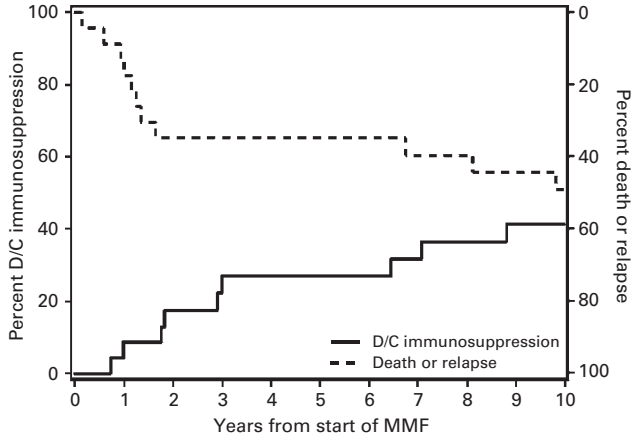

Figure 1 Overall survival and duration of immunosuppression in the CGVHD Group.

GVHD involving two organs and 10 had chronic GVHD affecting three or more organs, most often involving the skin and the oral cavity.

At 12, 24 and 36 months after the start of MMF therapy, two (9%), four (17%) and six (26%) patients, respectively, had discontinued all immunosuppressive therapy after resolution of chronic GVHD at 9–35 (median, 22) months after starting treatment with MMF (Figure 1). Two patients could not be evaluated at 12 months. One had died and the other had recurrent malignancy. At 24 and 36 months, seven additional patients could not be evaluated. One was lost to follow-up, one had recurrent malignancy and five had died. None of the patients who died or had recurrent malignancy had discontinued immunosuppressive treatment after resolution of GVHD. Thirteen of the 22 patients (59%) with follow-up information required additional therapy for chronic GVHD within 3 years after starting treatment with MMF. One patient was lost to follow-up and could not be evaluated.

Toxicity and early discontinuation of MMF

Eight patients in the prospective AGVHD Group discontinued treatment with MMF before day 35 (Table 4). Three had progressive GVHD and discontinued treatment with MMF after 3 days, and one other patient died on day 29. Administration of MMF was discontinued in four patients because of neutropenia ($n=2$), abdominal pain ($n=1$) or pulmonary infiltrate ($n=1$). Doses of MMF were reduced in three other patients because of neutropenia or presumed gastrointestinal toxicity. In the CGVHD Group, 11 patients discontinued administration of MMF prematurely because of gastrointestinal discomfort ($n=4$), neutropenia ($n=1$), lack of efficacy ($n=4$) or recurrent malignancy ($n=2$). Doses of MMF were reduced in one patient because of presumed gastrointestinal toxicity. Altogether, 9 of the 42 patients (21%) in the two prospective studies discontinued MMF treatment because of toxicity.

Pharmacokinetics

Pharmacokinetic testing was completed in 12 patients in the prospective AGVHD Group and 18 in the CGVHD Group. On day 2 (median; range, 1–10) after starting treatment with MMF, the median MPA-AUC was 15.8 (range, 7.6–25.3; mean 17.1 ± 5.5) $\mu g \times h/ml$ in the AGVHD Group, compared with 49.9 (range, 19.2–202.8; mean 62.0 ± 47.9) $\mu g \times h/ml$ on day 2 (median; range, 1–13) in the CGVHD Group (Wilcoxon, $P<0.0001$)). The median C_{max} was 4.8 (range, 1.8–8.6; mean 5.1 ± 2.3) $\mu g/ml$ in the AGVHD Group compared with 12.0 (range, 5.8–24.7; mean 12.8 ± 5.7) in the CGVHD Group (Wilcoxon, $P<0.0001$).

Table 4 Reasons for early discontinuation of treatment with MMF

Reason	*Prospective AGVHD Group (n = 19)*	*CGVHD Group (n = 23)*[a]
Death	1	0
Cytopenia	2	1
Gastrointestinal toxicity	1	4
Pulmonary infiltrates	1	0
Uncontrolled GVHD	3	4
Recurrent malignancy	0	2

Abbreviations: AGVHD = acute GVHD; CGVHD = chronic GVHD.
[a]One patient was lost to follow-up.

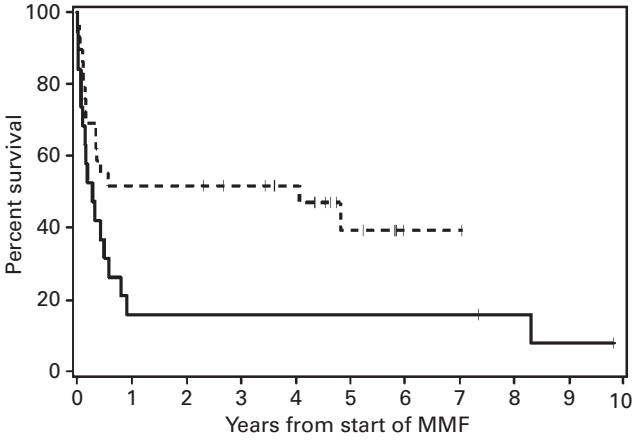

Figure 2 Overall survival in the prospective (solid line) and retrospective (dashed line) AGVHD Groups.

Outcome

In the prospective AGVHD Group, seven (37%) and three (16%) patients were alive at 6 and 12 months, respectively, after starting treatment with MMF. Two patients (11%) died with recurrent malignancy on days 119 and 182, respectively, after starting treatment with MMF. These results contrast with survival rates of 55 and 52% at 6 and 12 months, respectively, in the retrospective AGVHD Group (Figure 2). Two patients (7%) died with recurrent malignancy on days 126 and 158, respectively, after starting treatment with MMF. In the CGVHD Group, 22 patients (96%) were alive 1 year after starting treatment with MMF (Figure 1). Twelve of 23 (52%) patients with follow-up

Table 5 Causes of death

Cause	*Prospective AGVHD Group (n = 19)*	*Retrospective AGVHD Group (n = 29)*	*CGVHD Group (n = 23)*
Respiratory failure (not infection)	1	2	5
Infection	6	7	3
Recurrent malignancy	3	1	0
Cardiopulmonary	2	0	1
GVHD	4	5	1
Multi-organ failure	1	0	1
Secondary malignancy	0	1	0

Abbreviations: AGVHD = acute GVHD; CGVHD = chronic GVHD.

information remain alive at 7.9–10.6 (median, 9.5) years after starting treatment with MMF. One of these patients is still being treated with immunosuppressive medications at the time of the last follow-up. Three patients had recurrent malignancy after treatment with MMF therapy. The causes of death for all prospective study patients are listed in Table 5. In the retrospective group, fungal infection was implicated in six of seven patients who died from infection, compared with three of six patients who died from infection in the prospective group. Overall, the causes of death between the groups were not different.

Discussion

A requirement for additional therapy beyond glucocorticoids to treat acute or chronic GVHD identifies a group of patients with generally poor outcomes. Historically, antithymocyte globulin has been commonly used to treat refractory acute GVHD,[14,20,21,51,52] but a variety of new immunosuppressive agents has been evaluated more recently.[15–19,52,53,54] Depending on the definition of response and time of evaluation, response rates range from 30 to 75%, but survival has been poor, even in studies with high response rates. Survival rates are difficult to evaluate in these studies because many reports did not show the Kaplan–Meier estimates of survival across time. Mortality was commonly associated with uncontrolled GVHD, often complicated by opportunistic infection.

A few reports have described the use of MMF for treatment of refractory acute GVHD in small numbers of patients.[40,41,46–48] Comparisons with our experience are complicated by differences in patient selection, the degree of immunosuppression at the beginning of treatment with MMF, definitions of response and the timing of evaluations. It is unknown whether the patients described in these reports were treated with the parenteral formulation of MMF. In our studies, only the oral formulation of MMF was used. The overall combined response and survival rates among the 42 patients included in these reports were 48 and 38%, respectively. In our prospective study of acute GVHD, the clinical response rate was 47%, but survival at 6 and 12 months after starting treatment with MMF was only 37 and 16%, respectively. Only two patients died from recurrent hematological malignancy. In the retrospective AGVHD Group, the clinical response rate was nearly identical, although the 6- and 12-month survival rates were 55 and 52%, respectively. Two patients died from recurrent hematological malignancy.

The early onset of acute GVHD in the prospective and retrospective studies of acute GVHD was somewhat unusual and could raise some question about the accuracy of the initial diagnosis, especially because engraftment syndrome can mimic acute GVHD. Engraftment syndrome generally resolves during short-term treatment with high-dose glucocorticoids, whereas GVHD generally resolves more slowly. The persistence of inflammatory manifestations despite initial glucocorticoid treatment suggests that our patients had GVHD rather than engraftment syndrome.

The poor 6- and 12-month survival rates in the prospective acute GVHD study, which was completed in the mid-1990s, prompted a retrospective review of more recent experience with the use of MMF for treatment of steroid-resistant acute GVHD, as MMF is now widely used for secondary treatment of acute GVHD. The reasons for the difference in survival between the two AGVHD Groups are not entirely clear. Although the median age of the prospective AGVHD Group was lower and a higher proportion had HLA-matched related donors, this group also contained higher proportions of patients with high-risk malignancies and Grades III–IV GVHD at the start of MMF therapy. Three patients in the prospective AGVHD Group had progressive GVHD requiring additional immunosuppressive therapy and died within 12 days after starting treatment with MMF. The shorter interval times from HSCT, diagnosis of GVHD and initiation of prednisone treatment to onset of treatment with MMF suggest more aggressive progression of GVHD and greater glucocorticoid resistance in the prospective cohort compared with the retrospective cohort. The similarity of response rates in the two groups, however, argues against this supposition. The favorable survival among patients in the retrospective AGVHD Group may reflect improved management of GVHD, longer experience with the use of MMF and the availability of the i.v. formulation, although most patients in the retrospective group were treated initially with the oral formulation.

One could argue that differences in survival between the two AGVHD Groups might be attributable to advances in transplantation during the years when these groups were treated. Patients in the prospective group had HSCT during 1995–1997, whereas patients in the retrospective group had HSCT during 2000–2005. The latter group might have benefited from improved primary treatment regimens, such as combination therapy with BU and CY. Ten patients (35%) in the retrospective AGVHD Group received this combination of chemotherapy, compared with none in the prospective AGVHD Group. The proportion of patients who received growth factor-mobilized blood cells was higher in the retrospective AGVHD Group than in the prospective AGVHD Group (69 vs 21%). The use of mobilized blood cells likely resulted in earlier neutrophil engraftment and may have influenced immune

reconstitution. Newer antibiotic therapies, particularly for the treatment for fungal infections, were more readily available to patients in the recent cohort. This potential advantage was not validated, however, as the proportions of patients who died from infections were not strikingly different in the two groups.

A variety of agents have been used to treat refractory chronic GVHD,[55–59] and response rates with the use of MMF have been ~40–90%.[42–45] Lopez *et al*.[43] reported on 34 patients who received MMF therapy for treatment of chronic GVHD. Responses were observed in 9 of 10 patients who received MMF for initial treatment of chronic GVHD and in 18 of 24 patients who received MMF as secondary treatment for persistent or progressive chronic GVHD. At a median follow-up of 24 months, 22 of the 30 patients (73%) treated with glucocorticoids at the start of MMF therapy were able to reduce the dose of glucocorticoids, although only one patient was able to discontinue glucocorticoid treatment. The number of patients requiring additional agents to treat chronic GVHD after starting MMF therapy is not reported. Twenty-nine patients (85%) were alive at the time of the analysis. The addition of MMF to a calcineurin inhibitor and glucocorticoids produced 17 complete responses (65%) among 26 pediatric patients with refractory chronic GVHD.[45] Treatment with MMF was started at a median of 14 months after HSCT, and all patients with improvement were able to discontinue treatment with MMF within 3 years. The number of patients who were able to discontinue all immunosuppressive therapy is not reported. Twenty-two patients (85%) were alive at a median of 4.7 years after HSCT.

In this study, most patients had received a variety of agents in addition to a calcineurin inhibitor and glucocorticoids for the management of chronic GVHD before beginning treatment with MMF. In addition, the median time from HSCT to the beginning of treatment with MMF was nearly 20 months, suggesting that this group of patients may have had more resistant chronic GVHD. Despite these unfavorable risk factors, the 96% 1-year survival of the CGVHD Group compares favorably with the results of other studies reporting the use of MMF for first- or second-line treatment of chronic GVHD. The 5-year survival rate was 74%, which is similar to patients with newly diagnosed 'standard risk' chronic GVHD.[60] The rate of discontinuation of all immunosuppressive treatment at 1, 2 and 3 years after the start of MMF in the CGVHD Group compares favorably with historical results. Even more impressive is the observation that the 26% 3-year cumulative incidence of discontinued immunosuppressive treatment after resolution of chronic GVHD was comparable to historical results in patients with newly diagnosed chronic GVHD.[24]

The ability to administer treatment over a prolonged period of time and the availability of an i.v. and oral formulation are advantages for the use of MMF, allowing for multi-agent therapy, particularly for chronic GVHD. Although treatment with MMF was scheduled to stop after 35 days in the acute GVHD study, six patients continued treatment for more than 35 days. In the CGVHD Group, the median duration of MMF therapy was 11.6 months. Consistent with earlier reports, most patients showed good tolerance to MMF therapy, although four patients in the AGVHD Group and five in the CGVHD Group discontinued treatment with MMF therapy prematurely because of suspected toxicity generally related to gastrointestinal complaints and neutropenia. The rate of discontinuation reflects, in part, the fact that safety concerns were of prime importance in these early studies investigating the use of MMF for treatment of GVHD. As their experience was limited, the investigators had a low threshold in deciding to discontinue administration of MMF. As the use of MMF has expanded, for both treatment and prophylaxis, dose adjustments are more likely the first step to managing potential toxicities before discontinuing administration of MMF. A direct causal link between MMF treatment and the toxicities observed in these studies could not always be made. In some cases, adverse effects had multiple causes, particularly when administration of MMF was discontinued because of cytopenia or gastrointestinal complaints, which occur frequently after HSCT even in the absence of treatment with MMF.

At the time these studies were conducted, only the oral formulation of MMF was available. Pharmacokinetic analysis indicates that the AUC and C_{max} of MPA were lower in patients in the prospective AGVHD Group compared with the CGVHD Group, possibly because of the to reduced bioavailability or interference with the enterohepatic recirculation. Ten of 19 patients in the AGVHD Group had evidence of gastrointestinal GVHD when MMF therapy was started. Low levels of MPA and decreased bioavailability after administration of oral MMF have been noted previously in acute GVHD prevention studies.[61,62] In a study evaluating MPA levels after the administration of oral MMF for initial treatment of acute GVHD, Kiehl *et al*.[63] reported lower MPA levels among patients with gastrointestinal GVHD compared with those with skin GVHD. This difference was not noted in our small sampling of patients who had pharmacokinetic sampling performed. They also observed that trough plasma MPA concentrations were higher among patients with improvement in GVHD manifestations than among those who had no improvement.

The standard dose of MMF at our center for treatment of acute GVHD is 30 mg/kg per day in divided doses, or ~1 g twice daily. In renal transplant studies, the AUC of MPA after oral administration predicts allograft rejection among patients receiving CYA.[64,65] In these pharmacokinetic studies, an increased MMF dose correlated with an increased AUC of MPA. van Gelder *et al*.[64] showed that after kidney transplantation, the day 7 mean MPA-AUC levels of 17, 27 and 43.9 $\mu g \times h/ml$ resulted in biopsy-proven acute rejection rates of 28, 15 and 12%, respectively. Similarly, Hale *et al*.[65] found that an MPA-AUC of 15, 25 and 40 $\mu g \times h/ml$ yielded efficacy rates of 50, 75 and 90% after kidney transplantation. The median MPA-AUC within 10 days after the start of MMF in our prospective AGVHD study was only 15.8 $\mu g \times h/ml$. The MPA-AUC among patients with chronic GVHD was consistent with results from solid organ transplant recipients.

Oral MMF can be used successfully for treatment of refractory acute and chronic GVHD. The long-term survival rate in the prospective AGVHD Group was low,

although more recent experience using MMF as secondary treatment is more encouraging. The difference should be interpreted with caution, as the improved outcome in the more recent cohort of patients is based on a retrospective analysis. The challenge remains to control the response of donor cells against recipient alloantigens, while at the same time allowing protective immune responses against pathogens. Results with the use of MMF for treatment of steroid-refractory chronic GVHD have encouraged the development of phase III trials to establish the benefits and risks of MMF or MPA for initial treatment of chronic GVHD.

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