

Mycophenolate mofetil: fully utilizing its benefits for GvHD prophylaxis

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Abstract Mycophenolate mofetil (MMF) has been widely used for the prophylaxis of graft-versus-host disease (GvHD) in hematopoietic stem cell transplantation (HSCT), based on clinical evidence established in organ transplantations. MMF is not a cytotoxic, but rather a cytostatic agent, and there have been several reports of significant advantages in engraftment as well as greatly reduced stomatitis compared to methotrexate (MTX). MMF has been preferred for MTX-free immunosuppression, especially in reduced intensity conditioning, but it is suitable for GvHD prophylaxis for any type of HSCT. Some clinicians doubt its effectiveness, due to the lack of advantage over MTX in acute GvHD prophylaxis, especially in myeloablative conditioning. Pharmacokinetics studies of mycophenolic acid (MPA), the active form of MMF, show large inter- and intra-patient variation, which make interpretations of its clinical usefulness difficult. Nevertheless, several studies, including ours, have demonstrated that relatively higher area under the curve (AUC) of the MPA group leads to significant suppression of acute GvHD in prophylactic use. We propose a model algorithm for optimal dose finding using therapeutic drug monitoring

(TDM) for MPA. Preemptive strategies depending on plasma MPA levels could yield more effective approaches to GvHD prophylaxis, alternative to MTX.

Keywords Mycophenolate mofetil (MMF) · Mycophenolate acid (MPA) · Graft-versus-host disease (GvHD) · Therapeutic drug monitoring (TDM)

Introduction

The prophylaxis for graft-versus-host disease (GvHD) after hematopoietic stem cell transplantation (HSCT) has been developed in the last two decades. Although a combination of calcineurin inhibitor (CI), such as cyclosporin (CsA) or tacrolimus (FK506), plus short-term methotrexate (MTX) has been widely used in clinical practice [1–6], other immunosuppressive drugs, such as steroids, anti-thymocyte globulin (ATG), and campath-1H, are also used as alternative or additional immunosuppressants. Mycophenolate mofetil (MMF) is a type II inosine monophosphate dehydrogenase inhibitor that exerts its immunosuppressive effect by blocking the production of guanosine nucleotide synthesis through the de novo pathway [7, 8]. MMF is widely used for prevention of rejection in organ transplantations. MMF is highly selective, specifically in the suppression of lymphocytes but not myeloid cells; hence, it enables faster engraftment and causes less cytotoxicity, especially stomatitis, compared to MTX. Many clinicians who conduct HSCT favor this drug for GvHD prophylaxis. In addition, MMF has also been utilized as first-line or salvage treatment of acute GvHD, as well as chronic GvHD [9–20]. Despite the increasing infectious complications associated with combined MMF and prednisone regimen, current data have suggested that MMF is an active agent in

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the treatment of acute and chronic GvHD. MMF dosage for GvHD prophylaxis ranges from 1 to 3 g/day according to institutions, because the optimal dose is not provided by therapeutic drug monitoring (TDM). Several studies fail to establish the range of MMF dosage because the concentration of mycophenolic acid (MPA), which is the active and hydrolyzed form of MMF, differs in each individual. Moreover, plasma MPA concentrations after HSCT are apparently lower than after organ transplantation. In this review, the efficacy/limitations in the field of HSCT and analysis of MMF usage based on conditioning, timing, and stem cell source are discussed.

MMF pharmacokinetics and pharmacodynamics in HSCT

Figure 1 shows the pharmacodynamics of MMF. MMF is a morpholinoethyl ester formulation, which enhances the bioavailability of MPA. MPA was first isolated from a *Penicillium* culture, but its efficacy as an antibiotic was limited [21]. MPA was later shown to be a potent inhibitor of nucleic acid synthesis, largely by its ability to inhibit the enzyme IMP dehydrogenase (IMPDH) selectively,

reversibly, and noncompetitively. IMPDH is the rate-limiting enzyme in the de novo synthesis of guanosine monophosphate (GMP) from IMP. Then it was focused for anti-tumor activity [22–26].

The GMP synthesis in lymphocytes is highly dependent on the de novo pathway, while other cells utilize the salvage pathway. Therefore, the blockade of GMP synthesis leads to prevention of T-cell activation, as well as B-cell activation. Thereafter, MMF has been widely used as an immunosuppressive agent.

The bioavailability of MMF after oral administration in healthy individuals was reported to be approximately 94 % [27]. Once orally or intravenously administered, MMF rapidly undergoes de-esterification to form its active compound, MPA [7]. Maximum peak concentrations (C_{max}) of MPA generally occur within 1 or 2 h after MMF administration. MPA is primarily metabolized in the liver by uridine diphosphate glucuronosyl transferases (UGTs) to form the metabolites phenolic MPA-glucuronide (MPAG) and, to a lesser extent, acyl-MPAG (AcMPAG). The latter is pharmacologically active and has been linked to the occurrence of MMF-related adverse effects. The excretion of MPAG is primarily renal. Over 90 % of the administered dose is eventually excreted in the urine,

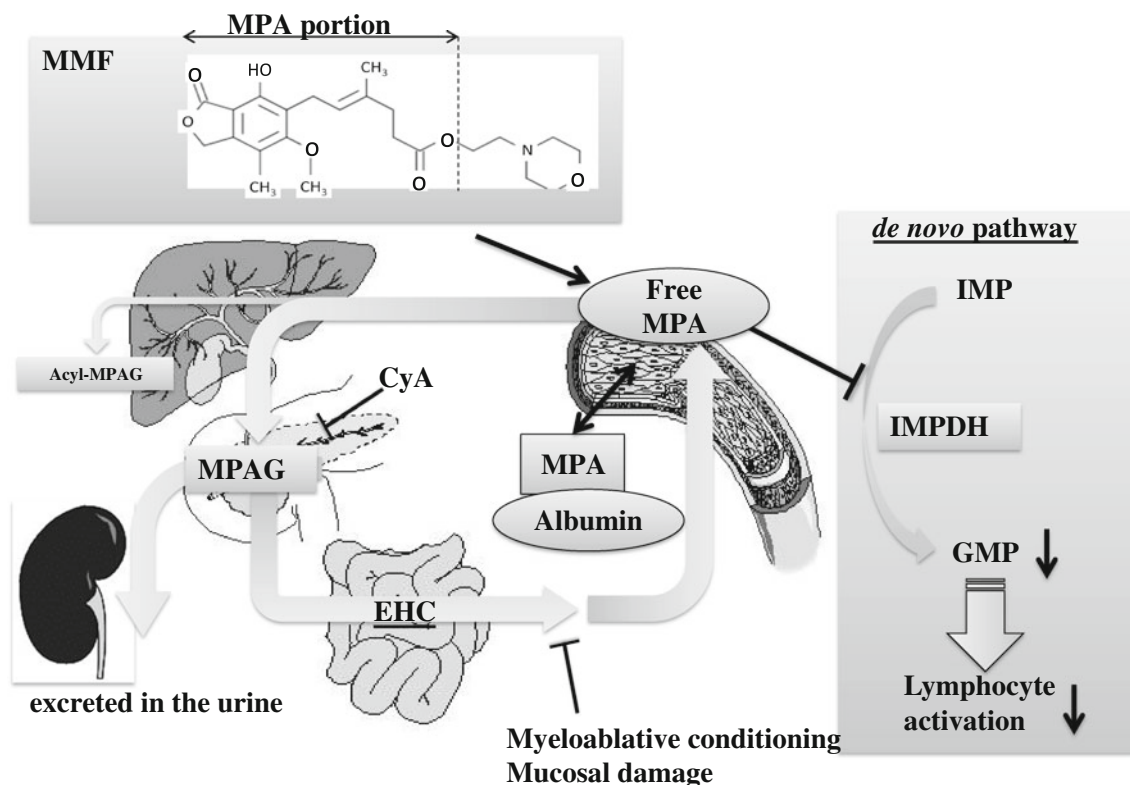


Fig. 1 Summary of pharmacology and pharmacodynamics of MMF. After MMF is administered, MMF is hydrolyzed to MPA, which is the active form, and causes reversible inhibition of IMPDH. MPA is reabsorbed via the enterohepatic circulation. *MMF* mycophenolate mofetil, *MPA* mycophenolic acid, *IMPDH* inosine monophosphate

dehydrogenase, *IMP* inosine monophosphate, *GMP* guanosine monophosphate, *MPAG* MPA glucuronide, *UGTs* uridine diphosphate glucuronosyl transferases, *EHC* enterohepatic circulation, *CsA* cyclosporine

mostly as MPAG. The pharmacokinetics of MMF is complicated by the enterohepatic circulation (EHC) of MPAG, which is excreted into the bile and subsequently hydrolyzed in the intestine and reabsorbed as MPA giving rise to a secondary peak of MPA 6–12 h after MMF administration. However, most studies reported that the second peak of MPA was not found in HSCT, because mucosal damage partly due to conditioning regimen interfered with EHC [28, 29]. Even though EHC is active, detection of the second peak is difficult because of the low plasma MPA levels of HSCT patients 6 h after MMF administration. Furthermore, MPA binds extensively to plasma albumin, and the free MPA fraction is less than 3 %. The free MPA fraction is thought to be responsible for the immunosuppressive effect of MPA. Systemic exposure to MPA when MMF is given in combination with CsA is approximately 30–40 % lower than when given alone or with FK506 or sirolimus. It is because CsA inhibits multidrug resistance associated protein 2 (MRP-2), which has been reported to be responsible for the biliary excretion of MPAG and subsequent MPA EHC [30, 31]. The interference associated with the concomitant use of CsA would be one reason for the inter-patient, as well as intra-patient variations in the plasma MPA levels. While the recommended target range for the MPA area under the curve ($AUC_{0-12\text{ h}}$) in renal transplant recipients is 30–60 mg h/L [32], no standard target range in HSCT has been proposed.

Generally, the plasma MPA levels in HSCT are lower than those in organ transplantation, such as renal transplantation. Considerations on MMF pharmacokinetics for HSCT are listed in Table 1. The major factors are bioavailability and MPA clearance. Indeed, the intestinal mucosal damage due to the myeloablative regimen, including total body irradiation (TBI) and high-dose chemotherapy, and/or the use of broad-spectrum antibiotics deteriorate the

bioavailability after oral administration of MMF. However, because the intravenous formulation of MMF still achieved ten times lower trough blood level of MPA in myeloablative HSCT compared with healthy volunteers [33], the bioavailability of MMF might not be mainly the reason for the lower MPA levels. For MPA clearance, a higher clearance has been reported to be associated with decreased EHC, lower serum albumin levels [34, 35], and in combination with CsA [30, 31]. Pediatric HSCT study using intravenous and oral MMF in combination with FK506 showed that there was a trend in increased MPA clearance following myeloablative conditioning, which caused a more severe mucosal damage and decreased EHC compared to reduced intensity conditioning (RIC) [36]. However, there was no difference in the plasma MPA concentrations in both oral and intravenous regimens. Recently, a pharmacokinetics study has revealed that MPA clearance was increased in HSCT patients compared to renal transplant recipients [37]. Interestingly, the correlation between MPA clearance and CsA trough levels and plasma albumin was significant in a multivariate model. One explanation for the lower MPA levels in HSCT patients compared to those who received organ transplant could be the high MPA clearance as a result of combination with CsA and the high trough and low plasma albumin levels. However, another report on patients treated with RIC did not find a positive correlation between total MPA AUC and serum albumin levels [38]. Therefore, there is still no clear explanation for the lower MPA concentration in HSCT patients.

Beginning of utilizing MMF for HSCT

The usefulness of MMF in the field of organ transplantation, especially renal transplantation, by randomized

Table 1 Issues to consider in the use of MMF for acute GvHD prophylaxis and pharmacokinetics of MPA in HSCT

Issues	Comments
Patient/disease-oriented issues	
Conditioning	RIC is more favorable than myeloablative conditioning
Stem cell source	Any type of sources is feasible
Stomatitis	Oral mucosal damage is milder than MTX
Engraftment	Usually faster than MTX
Infectious status	Infections might be increased
Factors affecting pharmacokinetics	
MPA clearance	Might be higher when myeloablative conditioning rather than RIC is used
Serum albumin level	MPA clearance is increased when serum albumin level is decreased
Bioavailability (if oral)	Intravenous formulation is available
Enterohepatic circulation (EHC)	Mucosal damage and antibiotics use reduce EHC
Combination drug for GvHD prophylaxis	CsA interferes with secretion of MPA in bile

MMF mycophenolate mofetil, RIC reduced intensity conditioning, MTX methotrexate, MPA mycophenolic acid, CsA cyclosporine, GvHD graft-versus-host disease

controlled studies had been reported since the mid-1990s [39–41]. In the late 1990s, Storb et al. conducted experimental studies to show the effectiveness of CsA plus MMF in canine HSCT after non-myeloablative conditioning [42, 43]. Subsequently, MMF was introduced for GvHD prophylaxis in human HSCT. The Seattle group showed that the combination of CsA plus MMF was comparable to CsA plus MTX. Thereafter, MMF has been practically employed in the HSCT field, especially for RIC [44, 45], with less clinical trials compared to the application in renal transplantation.

Conventional usage of MMF for acute GvHD prophylaxis

Generally, MMF is initiated at 15–45 mg/kg orally or intravenously twice or thrice daily (the dose is rounded off to the nearest multiple of 250 mg for internal use) from day 0 for 27–40 days, and then it is either stopped or tapered through day 96–180 [29, 38, 46–48]. A report from Fred Hutchinson Cancer Research Center demonstrated that MMF given thrice daily allowed durable engraftment in unrelated HSCT after RIC (fludarabine 90 mg/m² plus TBI 2 Gy) [49]. The infection rate with thrice daily administration was slightly higher than that with twice daily, but treatment-related mortality (TRM) was not increased in thrice daily administration. The probability of acute GvHD was similar in both groups. Another report from the same institute also documented that post-grafting immunosuppression with extended (up to day 180) MMF and shortened (up to day 80) CsA increased the incidence of acute GvHD in unrelated HSCT given RIC [50]. Our small cohort showed that extended MMF administration beyond day 30 is recommended depending on individual risk factors for GvHD, namely (1) HSCT from a mismatched donor, (2) concurrent acute GvHD, (3) eosinophilia ($>0.5 \times 10^9/L$), or (4) fever without infection. The median extended dosing period of MMF was 64.5 days (50–94). The cumulative incidence of grade II–IV acute GvHD was significantly lower (12.5 %) compared to the cessation of MMF administration at day 30 (42.3 %)[51].

The optimal MMF dose is not elucidated so far, but 2–3 g daily of MMF in combination with CI has been mostly used in Western countries, and it might be acceptable to taper according to the patients' status beyond day 30 before CI withdrawal.

Engraftment issue

Primary as well as secondary engraftment failure is one of the most important concerns, especially in HSCT given

RIC or cord blood transplantation (CBT). Previous reports demonstrated that the intensification of the immunosuppressive regimens was the key factor for promotion of engraftment. Conditioning with the use of immunosuppressive agents, such as ATG, campath-1H, fludarabine, cyclophosphamide, and TBI, is an effective way of engraftment [52–56]. As a multicenter experience, fludarabine plus low dose TBI in 38 cases of salvage HSCT for allograft rejection was reported to be well tolerated and resulted in an engraftment rate of 87 % [56]. In this study, MMF was employed for GvHD prophylaxis in combination with CI. Our preliminary experiences also found that the intensification of immunosuppression with MMF in combination with CI enabled the achievement of a successful engraftment as a salvage CBT [57]. Although the mechanism has not been elucidated, several reports have suggested that GvHD prophylaxis regimen containing MMF is associated with faster engraftment [58–61]. One possible explanation is that short-term MTX, but not MMF, often interferes with emerging neutrophil engraftment due to its cytotoxic effect.

Another possibility is that MMF itself might enhance engraftment because patients with mean MPA concentration steady state (C_{ss}) less than 2.5 $\mu\text{g/mL}$ were reported to come across graft rejection [38]. It is well known that treatment with thrice daily MMF significantly increased MPA C_{ss} in plasma [38, 49, 62], despite a negative report [63]. Because free MPA binds reversibly to IMPDH and interrupts de novo purine pathway transiently, high fractionated administration is reasonable to enhance its activity [45]. It is of interest whether the pharmacological increase in C_{ss} by *tid* could contribute to the clinical efficacies to prevent GVHD as well as graft failure.

Trends toward MTX-free immunosuppression

Stomatitis is a frequent complication of the conditioning regimen. Severe oral stomatitis occurs in up to 75 % of cases with myeloablative conditioning [64]. Severe stomatitis often causes problems and increases early mortality rate after HSCT [65]. MTX for GvHD prophylaxis impairs mucosal regeneration after conditioning-related mucosal damage, as well as faster engraftment. In RIC of patients over 55 years old, which is the upper limit for myeloablative conditioning in most facilities, or younger patients with concomitant complications, such as cardiac or infectious diseases, the lesser occurrence of stomatitis and early mortality and faster engraftment by utilizing an alternative immunosuppressant to MTX would be attractive for a safe and secure HSCT.

Thus, the concept of “MTX-free immunosuppression” has emerged. Sirolimus is the first available inhibitor of the

mammalian target of rapamycin (mTOR) used in HSCT. Since sirolimus poses less nephrotoxicity and neurotoxicity, combination therapy with CI is appealing. There have been many clinical trials of sirolimus and FK506 without MTX for GvHD prophylaxis mainly at Dana-Faber Cancer Institute so far [66–70]. These results indicate that sirolimus may reduce the incidence of acute GvHD, while the severity and incidence of stomatitis and cytomegalovirus (CMV) reactivation are decreased as compared to the MTX-containing regimen [68, 69, 71]. Unfortunately, these results have been discouraged by the increased risk of sinusoidal obstruction syndrome (SOS), especially with busulfan-based conditioning [72]. Moreover, thrombotic microangiopathy (TMA) and renal failure are also documented in GvHD prophylaxis and treatment trials using the sirolimus and CI combination [73–75]. Because MMF has not been shown to increase the risk of SOS or TMA, MTX-free immunosuppression, such as CI plus MMF, might be suitable for GvHD prophylaxis, especially in busulfan-containing regimen. Indeed, MMF has been used as part of the front-line regimen for the prevention of GvHD, especially in the setting of RIC regimen. Similarly to sirolimus, MMF reduces stomatitis [58, 76] and facilitates engraftment [58–61, 76–78].

A recent retrospective study in the comparison of CsA plus MMF and CsA plus MTX after RIC from human leukocyte antigen (HLA)-identical siblings showed a lower tendency of non-relapse mortality (NRM) at day 100 (6 vs. 18 %, $P = 0.04$) [79]. Comparisons of MTX, sirolimus, and MMF for GvHD prophylaxis are summarized in Table 2. Representative reports of comparison for prophylaxis on the use of CI plus MMF versus CI plus MTX are also shown in Table 3.

Why do some clinicians stay away from MMF as conventional prophylaxis?

MMF usage in myeloablative conditioning is controversial. A prospective randomized trial that compared CsA plus

MTX and CsA plus MMF for GvHD prophylaxis in myeloablative HSCT showed significantly less severe stomatitis and more rapid neutrophil engraftment in the MMF arm. The incidence of grade II–IV acute GvHD was similar in the two arms (48 % in the MMF arm and 37 % in the MTX arm) [58] (Table 3). On the other hand, phase I/II study, in which 45 mg/kg/day MMF was given thrice daily for 27 days in combination with CsA, suggested the lack of a significant improvement in the prevention of GvHD compared with historical data for CsA and MTX after myeloablative HSCT from HLA-matched sibling donors [29]. In this study, the incidence of acute GvHD was 62 %. For some physicians, this is the rationale why the combination of CsA plus MMF is considered only when MTX is contraindicated. However, there are not significant differences on the pharmacokinetics of MMF in myeloablative conditioning versus RIC in adult HSCT to date. There is no clear explanation why the benefit of MMF is superior in RIC regimen compared to myeloablative conditioning.

Prophylactic use for related donors (especially from HLA-mismatched donors)

Some studies showed that GvHD prophylaxis by MMF plus CI for either myeloablative or RIC achieved successful engraftment and prevented GvHD similar to MTX plus CI [29, 80, 81] (Table 4). Of note, MMF is also used for HSCT from HLA-mismatched related donors, as well as HLA-matched ones. In Asia, Japanese, and Chinese recipients with no HLA-matched sibling donors received HSCT from two or three loci-mismatched related donor. The usual conditioning regimens, including ATG, and multiple immunosuppressants, such as MMF, were used for GvHD prophylaxis [82, 83]. Moreover, clinical trials from Johns Hopkins University recently evaluated the efficacy of high-dose, post-transplantation cyclophosphamide in addition to FK506 and MMF to prevent GvHD after non-T cell-depleted transplantation from partially HLA-mismatched related donors [84, 85].

Table 2 Comparison of MTX, sirolimus, and MMF

	Stomatitis	Engraftment	SOS/TMA	aGvHD prophylaxis	Infectious complications	Drug interaction
MTX	+++	+	+	+	+	+
Sirolimus	±	++	+++ ^a	++	±	++
MMF	±	++	+	+	++	+

MTX methotrexate, MMF mycophenolate mofetil, SOS sinusoidal occlusive syndrome, TMA thrombotic microangiopathy, aGvHD acute graft-versus-host disease

^a Especially if myeloablative regimens of busulfan and cyclophosphamide were used

Table 3 Representative reports of comparison on calcineurin inhibitor (CI) + MMF versus CI + MTX

References	N	Age (range)	Regimen	Donor	Additional prophylaxis	Neutrophil engraftment (range)	Grade II–IV acute GvHD (95 % CI)	Extensive chronic GvHD (95 % CI)	NRM (95 % CI)
Bornhauser et al. [59]	MMF arm	14	38 (21–63)	Myeloablative	Related	CsA	11 days (9–20)	N.A.	N.A.
	MTX arm	15	38 (20–58)				17 days (10–25)		
Bolwell et al. [58]	MMF arm	21	49 (19–60)	Myeloablative	Related	CsA	11 days (8–24)	48 %	63 %
	MTX arm	19	46 (16–62)				18 days (11–28)	37 %	64 %
Neumann et al. [61]	MMF arm	26	39 (22–57)	Myeloablative	Related	CsA	12 days	38 %	50 %
	MTX arm	67	32 (17–51)				18 days $P < 0.0001$	61 %	45 %
Pinana et al. [79]	MMF arm	52	57 (18–71)	RIC	Related	CsA	15 days (11–27)	38 % (27–54)	6 % (2–17)
	MTX arm	93	54 (23–70)				15 days (10–29)	33 % (25–45)	19 % (12–29)
Perkins et al. [76]	MMF arm	42	49.9 (23–66.2)	Varies (mostly fludarabine/busulfan)	Related/unrelated	FK506	15 days	78 %	38 %
	MTX arm	47	54 (24.9–69.6)				16 days	79 %	45 %
						Platelet recovery	15 days versus 17 days $P < 0.01$		

N.A. not applicable, RIC reduced intensity conditioning, CsA cyclosporine, FK506 tacrolimus, MTX methotrexate, MMF mycophenolate mofetil, CI confidence interval, NRM non-relapse mortality, GvHD graft-versus-host disease

Table 4 Representative reports of MMF studies for GvHD prophylaxis in HSCT

References	N	Age (range)	Regimen	Donor	Neutrophil engraftment (range)	Grade II-IV acute GvHD	Extensive chronic GvHD	NRM
CsA-based studies								
Jenke et al. [33]	15	32 (26–57)	Myeloablative	Related: 9 Unrelated: 6	11 days (8–27)	40 %	N.A.	27 %
Niederwieser et al. [77]	52	48 (6–65)	RIC	Unrelated	N.A.	63 %	30 %	29 % at 1 year
Maris et al. [45]	89	53	RIC	Unrelated	15 days (0–55)	52 %	37 % at 1 year	16 % at 1 year
Rodriguez et al. [86]	22	49 (18–66)	RIC	Unrelated	13 days (10–71)	63 %	45 %	32 %
Baron et al. [48]	21	54 (33–66)	RIC	Unrelated	7 days (0–20)	82 %	73 %	11 % at 1 year
Nash et al. [29]	46	49 (18–64)	Myeloablative	Related	15 days (10–20) in PII	62 %	72 %	37 %
Neumann et al. [61]	26	39 (22–57)	Myeloablative	Related	12 days	38 %	50 %	17 %
Giaccone et al. [38]	85	52 (18–70) in twice daily 52 (17–67) in thrice daily	RIC	Unrelated	N.A.	26 % in twice daily 32 % in thrice daily	N.A.	N.A.
Gupta et al. [46]	24	64.5 (60–71)	RIC	Related	13 days (7–27)	45 %	45 %	17 % at 2 years
Maris et al. [49]	103	54 (17–69.6)	RIC	Unrelated	7 days (0–44)	53 %	56 %	19 % at 2 years
Burnstein et al. [90]	110	51 (17–16)	RIC	Cord blood	12 days (0–32)	59 %	23 % at 1 year	26 % at 3 years
Baron et al. [50]	71	56 (17–75)	RIC	Unrelated	N.A.	77 %	45 %	29 % at 1 year
Perez-Simon et al. [87]	44	48 (17–60)	RIC	Unrelated	9 days	53 %	63 %	42 %
Pinana et al. [79]	52	57 (18–71)	RIC	Related	15 days (11–27)	38 %	39 %	25 %
FK506-based studies								
Osunkwo et al. [96]	34	7 (0.5–21)	Myeloablative: 21 RIC: 16	Cord blood: 22 Related: 15	16 days (3–79)	45.4 %	None developed extensive GvHD.	29.4 %
Haentzschel et al. [100]	29	53 (21–69)	Flu 120 mg/m ² + BU 13.8 mg/kg	Related: 7 Unrelated: 22	13 days (10–30)	57 %	19 %	31 %
Sabry et al. [80]	131	54 (20–66)	RIC	Related	10 days (2–27)	19.7 %	76.1 % at 2 year	15.5 % at 7 years
Mizumoto et al. [78]	21	55 (24–66)	RIC	Unrelated	19 days (13–35)	33 %	55 %	19 %
Bhatia et al. [36]	38	8 (0.33–16)	Myeloablative: 17 RIC: 21	Related: 18 Unrelated: 20 (including cord blood)	N.A.	54.4 %	33.7 %	N.A.
Wakahashi et al. [89]	36	43 (33–66) in AUC _{0–24 h} <30 50 (20–66) in AUC _{0–24 h} >30	Myeloablative: 23 RIC: 13	Unrelated: 15 Cord blood: 21	11 days in BMT 20–21 days in CBT	46.7 % in AUC _{0–24 h} <30 15.8 % in AUC _{0–24 h} >30	30.8 % in AUC _{0–24 h} <30 0 % in AUC _{0–24 h} >30	30.6 %

Table 4 continued

References	N	Age (range)	Regimen	Donor	Neutrophil engraftment (range)	Grade II–IV acute GvHD	Extensive chronic GvHD	NRM
Zohren et al. [88]	50	51 (25–67)	RIC	Unrelated	8 days (0–30)	54 %	21 %	26 %
Kanda et al. [91]	27	33 (20–58)	Flu 160 mg/m ² + TBI 1,350 cGy	Dual cord blood	24 days (13–45)	37 %	31 % (95 % CI, 15–49 %)	28 % at 2 years
Uchida et al. [92]	29	62 (52–70)	Varies	Cord blood	19 days (13–32)	63 %	7 % at 2 years	28 % at 1 year

N.A. not applicable, *RIC* reduced intensity conditioning, *CsA* cyclosporine, *FK506* tacrolimus, *MTX* methotrexate, *MMF* mycophenolate mofetil, *CI* confidence interval, *NRM* non-relapse mortality, *GvHD* graft-versus-host disease, *HSCT* hematopoietic stem cell transplantation, *Flu* fludarabine, *TBI* total body irradiation, *BU* busulfan, *AUC* area under the curve

Prophylactic use for unrelated donors (except for umbilical cord blood)

Representative reports of MMF prophylaxis for unrelated donors are listed in Table 4 [38, 45, 47, 49, 77, 78, 86–88]. A large-scale study of eighty-nine patients transplanted under RIC using either oral or intravenous MMF and CsA showed 93 % engraftment. The rates of grade II, III, and IV acute GvHD were 42, 8, and 2 %, respectively [45]. We also showed 15 cases of myeloablative or RIC HSCT from unrelated donors using oral MMF and FK506. All, except one, were engrafted, and grade II–IV acute GvHD occurred in 6 patients (42.8 %) [89]. These results indicate that prophylactic use of MMF plus CI seems not to interfere with primary engraftment and prevent acute GvHD, as well as MTX plus CI, in HSCT from unrelated donors.

Prophylactic use for CBT

MMF is usually a feasible option for GvHD prophylaxis in CBT, wherein primary graft failure due to limited stem cell numbers is a concern. Large prospective study consisting of 110 adult umbilical cord blood transplantations demonstrated that the combination of MMF and CsA for GvHD prophylaxis facilitated engraftment (neutrophil engraftment was achieved in 92 % at a median of 12 days) and suppressed grade II to IV acute and chronic GvHD (59 and 29 %, respectively)[90]. In dual CBT, FK506 and MMF (1,000 mg twice daily) were given to 27 patients until at least 60 days after myeloablative conditioning [fludarabine (160 mg/m²) plus TBI 1.350 cGy]. Neutrophil engraftment was achieved in 80 % with a median of 24 days. Grade II to IV acute GvHD occurred in 37 %. Cumulative incidence of total parental nutrition usage was up to 56 %. This reflected the less gastrointestinal mucosal damage caused by MMF than MTX even under myeloablative conditioning [91]. A Japanese group also reported 29 elderly (median age 62) RIC-CBT patients who received FK506 and MMF for acute GvHD prophylaxis. The patients were compared with matched-pair historical controls who received FK506 alone [92]. Primary engraftment until day 60 was significantly higher (90 %) in the FK506 plus MMF group than the control group (69 %). Cumulative incidence of grade II to IV acute GvHD was 63 %. Interestingly, severe pre-engraftment immune reaction (PIR), which was a factor that negatively affected overall survival [93–95], was significantly lower (16 %) in the FK506 plus MMF group than the control group (52 %). Consequently, NRM in the FK506 plus MMF group within 30 days was significantly lower compared to the control group (0 vs. 21 %). Our experiences with 21 adult myeloablative CBT patients, who received FK506 and MMF, also showed

Table 5 Overview of pharmacokinetics studies in HSCT

Reference	N	Age (range)	Regimen	Additional prophylaxis	MMF dosing (mg/kg)	MPA AUC (mg h/L)	C _{ss} (mg/L)	MPA CL (L/h kg)	MPA C _{max} (mg/L)	MPA C _{trough} (mg/L)	MPA T _{1/2} (h)
Kiehl et al. [97]	14	N.A.	Myeloablative	CsA	1–3 g daily	N.A.	N.A.	N.A.	1.64 (median)	0.47 (median)	N.A.
Jenke et al. [33]	15	32 (26–57)	Myeloablative	CsA	12.5–17 mg/kg every 12 h	15.6–59.3	N.A.	0.73–2.73	8.48–38.6	N.A.	1.51–2.45
Maris et al. [45]	19	N.A.	RIC	CsA	15 mg/kg twice daily	20.3 (median)	N.A.	N.A.	5.3 (median)	0.5 (median)	3.0 (median)
Nash et al. [29]	46	49 (18–64)	Myeloablative	CsA	15 mg/kg every 6.8 and 12 h	16.4–34.5	1.19–4.84	0.4–1.0	5.9–12.7	0.13–0.64	0.8–1.48
Giaccone et al. [38]	85	52 (18–70) in twice daily 52 (17–67) in thrice daily	RIC	CsA	15 mg/kg every 8, 12 h	5.8–46.1 in twice daily 8.5–64.8 in thrice daily	1.9 in twice daily 3.8 in thrice daily	N.A.	1.0–29.3	0.8 in twice daily 2.5 in thrice daily	3.4 in twice daily 2.7 in thrice daily
van Hest et al. [28]	15	32 (17–58)	RIC	CsA	750–2,000 mg twice daily	7.6–35	N.A.	N.A.	2.6–23	0–4.0	0.8–5.7
Haentzschel et al. [100]	29	53 (21–69)	Myeloablative	FK506	1,500–2,500 mg (i.v.) twice daily	35.1–43.1	N.A.	N.A.	16–25	N.A.	N.A.
Perez-Simon et al. [87]	8	N.A.	RIC	CsA	1 g every 12 h or 1 g every 8 h	AUC _{0–24 h} : 106.46 (60.2–199.17)	N.A.	N.A.	N.A.	1.11 (0.4–2.6)	N.A.
Royer et al. [99]	15	51 (20–60)	RIC	CsA	750 mg tid if BW <70 kg 1 g tid if BW >70 kg	21.83 (8.96–49.99) at day 7	N.A.	N.A.	N.A.	N.A.	N.A.
Jacobson et al. [63]	30	55 (29–69) in 1 g every 8 h 53 (21–67) in 1.5 g every 12 h	RIC	CsA	1 g every 8 h or 1.5 g every 12 h	AUC _{0–24 h} : 53.59 (22.68–101.99) in 1 g every 8 h 60.9 (35.89–127.24) in 1.5 g every 12 h	2.33 (0.95–4.25) in 1 g every 8 h 2.53 (1.46–5.24) in 1.5 g every 12 h	N.A.	8.09 (1.4–10.8) in 1 g every 8 h 11.74 (7.22–26.5) in 1.5 g every 12 h	0.65 (0.39–8.38) in 1 g every 8 h 0.58 (0.29–4.18) in 1.5 g every 12 h	N.A.
Bhatia et al. [36]	38	8 (0.33–16)	Myeloablative 17 RIC 21	FK506	900 mg/m ² /dose (i.v.) every 6 h	26.82–33.71	4.73–6.46	1.17–1.46	12.31–16.54	0.33–0.72	1.02–2.49
de Winter et al. [37]	38	43 (17–65)	N.A.	CsA	500–2,000 mg daily (median 1,000 mg)	N.A.	N.A.	45.6L/h	N.A.	N.A.	N.A.

Table 5 continued

Reference	N	Age (range)	Regimen	Additional prophylaxis	MMF dosing (mg/kg)	MPA AUC (mg h/L)	C_{ss} (mg/L)	MPA CL (L/h kg)	MPA C_{max} (mg/L)	MPA C_{trough} (mg/L)	MPA $T_{1/2}$ (h)
Wakahashi et al. [89]	36	43 (33–60) in AUC <30 50 (20–66) in AUC >30	Myeloablative 23 RIC 13	FK506	15–25 mg/kg every 12 h or 1,000 mg every 8 h	AUC _{0–24 h} : 30.4 (median)	N.A.	N.A.	2.5 (median)	N.A.	N.A.

N.A. not applicable, RIC reduced intensity conditioning, CsA cyclosporine, FK506 tacrolimus, MPA mycophenolate mofetil, MMF mycophenolic acid, AUC area under the curve, C_{ss} concentration steady state, C_{max} maximum MPA peak concentrations, MPA CL MPA clearance, C_{trough} concentration at trough, MPA $T_{1/2}$ half-life of MPA

85.7 % engraftment and only 20 % grade II to IV acute GvHD. In pediatric CBT study, the combination of FK506 and MMF was used for GvHD prophylaxis in 22 cases with either myeloablative or RIC regimen [96]. The median time to recovery was 23 days, and grade II to IV acute GvHD occurred in 33.3 % among the evaluated patients.

Collectively in CBT setting, the addition of MMF to CI for the prophylaxis of acute GvHD seems to be feasible in RIC, as well as myeloablative conditioning.

Dose-finding studies and TDM

Many pharmacokinetics studies on MMF had been reported for organ transplantation, especially in renal transplantation. However, limited pharmacokinetics studies for finding the optimal dosage in HSCT have been observed to date. Table 5 shows the summary of pharmacokinetics studies in HSCT. In general, there is a large inter-patient, as well as intra-patient, variation in plasma MPA levels of HSCT patient as seen in organ transplantation. However, the peak of MPA levels in HSCT is significantly lower than those in organ transplantation. Some pharmacokinetics studies on MMF used for acute and chronic GvHD treatment showed that concentration at trough (C_{trough}) of MPA was significantly greater in the treatment responder than the non-responder [97, 98]. However, correlations between the efficacy on the prevention of acute GvHD and the MPA concentration have not been elucidated. Our small retrospective cohort showed that in patients with adjusted MPA AUC_{0–24 h} over 30 mg h/L ($C_{ss} > 1.25$ mg/L), acute GvHD, as well as chronic GvHD, occurred significantly less, especially in HSCT from unrelated bone marrow donors. On the contrary, lower MPA levels were enough to control acute and chronic GvHD in CBT. Moreover, a higher MPA level in CBT posed a tendency of GvHD relapse possibly due to weakened graft-versus-leukemia/lymphoma (GVL) effect of cord blood [89]. This finding is encouraging for prospective dose-finding studies depending on each donor source. Recently, one small prospective study demonstrated that at day 7, patients with AUC_{0–8 h} ≥ 22.5 mg h/L (concentration at steady state (C_{ss}) ≥ 2.8 mg/L) displayed no grade II to IV acute GvHD [99]. As a target range after organ transplantation, it has been suggested to keep C_{ss} MPA between 2.5 and 5 mg/L. In Japan, where HLA homogeneity and less GvHD incidence are more common than in Western countries, lower C_{ss} might be enough to prevent severe acute GvHD. Although MMF of 45 mg/kg/day dose reached a relatively high median C_{ss} MPA, 2.73–3.2 mg/L, it did not significantly reduce the occurrence of acute GvHD compared to historical controls receiving MTX instead of MMF for GvHD prophylaxis [29]. Further pharmacokinetics studies

should be carried out for optimal MMF dose finding, as well as understanding the precise pharmacodynamics of MPA in HSCT and the prevention of GvHD.

Surrogate marker for the prediction of MPA AUC, C_{trough} or C_{max} ?

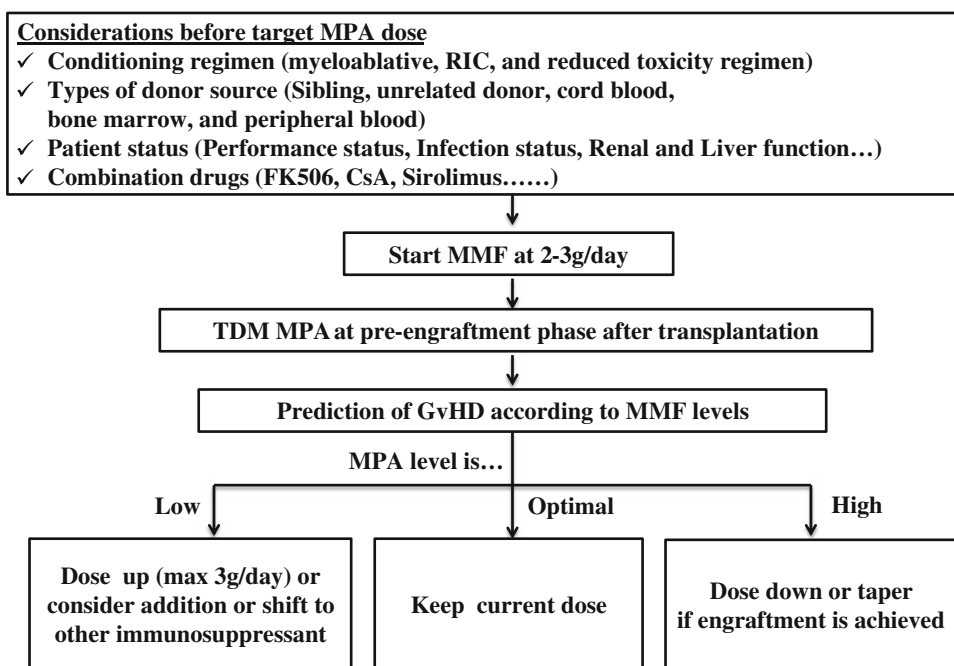
With limited evidences, there is a need to monitor the concentrations of MPA AUC or C_{ss} for evaluating the efficacy in HSCT, as well as in organ transplantation. As a surrogate marker for MPA AUC, the C_{trough} or C_{max} was

Table 6 Adverse effects of MMF

Hematological toxicities
Leukocytopenia
Anemia
Thrombocytopenia
Gastrointestinal toxicities
Nausea
Vomiting
Abdominal pain
Diarrhea
Infectious toxicities
Viral infections
CMV
EBV
HSV
Fungal infections
Aspergillosis
Candidiasis
Others

MMF mycophenolate mofetil, CMV cytomegalovirus, EBV Epstein–Barr virus, HSV herpes simplex virus

Fig. 2 New proposal algorithm for optimizing MMF dose. First, start MMF at 2–3 g/day. Second, monitor MPA concentration at pre-engraftment phase after transplantation. Third, adjust the MMF dosage according to the estimation of GvHD risk or switch to/add another immunosuppressant, such as steroids, if MPA concentration is too low



often monitored. Haetzshel et al. [100] reported that a significant correlation was observed between C_{max} and AUC for MPA in 28 patients evaluable at all points. Our data also showed that the concentration at 2 h (C_{2h}) after MMF administration was well correlated with AUC of MPA [89]. These results were encouraging for the utilization of C_{max} as surrogate marker of the AUC of MPA.

Toxicities and adverse effects

The adverse effects of MMF are listed in Table 6. The most common toxicities are gastrointestinal toxicity, opportunistic infections, and pancytopenia. Most of the physicians’ concern is the gastrointestinal toxicity, which is usually manifested as diarrhea. MMF can produce colitis with focal ulcerations, marked apoptosis, and intense acute and chronic inflammation [101]. Histological features of MMF-related colitis are remarkably similar to the ones associated with intestinal GvHD. We are able to distinguish MMF-related colitis from intestinal GvHD only when there is improvement or resolution of symptoms on the withdrawal or reduction of MMF. This may cause some problems when we estimate the efficacy of MMF for salvage therapy of acute interstitial GvHD. However, few previous reports on prophylactic use of MMF discontinuation due to diarrhea until day 30 after HSCT have been published. There are insufficient data on whether MMF can also produce lesions in the upper gut or not.

Infectious complications, including CMV reactivation, are also common and can be serious adverse effects. In HSCT patients within day 100 after the transplantation,

CMV infection, as well as antigenemia, are most likely to happen because of insufficient immune recovery after conditioning regimen and probably with acute GvHD. Prospective trial of 85 patients for HCST with RIC demonstrated that an elevated unbound C_{ss} was significantly associated with CMV reactivation [38]. A retrospective study of 21 consecutive patients with acute and extensive chronic GvHD showed the occurrence of 22 opportunistic or serious viral or bacterial infections in 10 patients [12]. Because MMF also prevents immune recovery, preemptive therapy should be required for CMV reactivation by monitoring CMV antigenemia, as well as empiric therapy for bacterial and fungal infections. Leukocytopenia, anemia, and thrombocytopenia are alarming when we worry about primary and secondary engraftment after HSCT.

A relationship between high MPA AUC values and drug-related adverse effects has been reported in some studies [102–105]. In a prospective, randomized, double-blind, multicenter, controlled study in 150 renal transplant patients, a dose-dependent increase in adverse effects was reported in the first 6 months post-transplant [106]. According to these data, it appears that an MPA AUC_{0–12 h} above 60 mg h/L may increase the risk of toxicity, although such high plasma MPA levels could be hardly achieved in HSCT as described above.

Conclusions and future directions

The relationship between MMF pharmacokinetics/pharmacodynamics and its effectiveness in HSCT is still obscure. However, MMF has been widely used for GvHD prophylaxis, as well as treatment of HSCT, in Europe and the USA.

One of the most important aspects of the pharmacokinetics of MMF is the wide intra-patient and inter-patient variations in the plasma MPA levels even under the same daily dose. On the other hand, similar to organ transplantation where higher MPA is correlated with lower rejection rate, higher MPA would correlate with the suppression of immune reactions, such as acute GvHD in HSCT. Then, we proposed a model of algorithm for the optimal dose finding using TDM of MMF (Fig. 2). For GvHD prophylaxis, MMF should be started at 2–3 g/day. In the earlier days after HSCT, such as at the pre-engraftment phase, the plasma MPA levels should be monitored (MPA AUC is preferred, but MPA $C_{2 h}$ might be an alternative). If the MPA level is low, based on the prediction of upcoming GvHD as determined by individual risk factors such as conditioning, donor type and combination immunosuppressant, MMF dosage should be increased up to the maximum (3 g/day), or other immunosuppressants, such as steroids if the maximum dose had been administered,

should be added/shifted to. For example, MPA AUC_{0–24 h} at day 9 or 16 should be >30 mg h/L for Japanese ordinary unrelated BMT. On the other hand, if MPA levels are high enough to prevent acute GvHD, MMF should be tapered as soon as the engraftment is achieved. In our study, we could predict that MPA AUC_{0–24 h} <30 mg h/L at day 9 or 16 would be usually enough for single unit Japanese CBT. The risk for relapse is higher at higher MPA levels. Thus, the MMF dose must be kept at the minimal requirement.

In conclusion, MMF is a safe and effective prophylaxis for the prevention of acute GvHD, as well as its treatment. MMF has been frequently used in RIC regimen and CBT. In a myeloablative setting, MMF has not been used by some clinicians due to limited clinical studies. To elucidate the advantage of the prophylactic use of MMF depending on the donor sources in the myeloablative regimen as well as RIC, larger prospective studies accompanying TDM are needed.

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