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Population pharmacokinetics of unbound mycophenolic acid in adult allogeneic haematopoietic cell transplantation: effect of pharmacogenetic factors

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- There is large variation in the pharmacokinetics of unbound mycophenolic acid (MPA) in adult allogeneic haematopoietic cell transplantation (alloHCT) recipients, and lower exposure is associated with higher rates of acute graft *vs.* host disease (aGVHD).
- Patient-specific clinical factors do not appear to explain the variation between patients.

WHAT THIS STUDY ADDS

- This is the first study to investigate the influence of pharmacogenetic factors on unbound MPA pharmacokinetics in adult alloHCT recipients.
- In addition, the relationship between unbound MPA exposure and aGVHD is reinforced.

AIM

To evaluate pharmacogenetic factors as contributors to the variability of unbound mycophenolic acid (MPA) exposure in adult allogeneic haematopoietic cell transplantation (alloHCT) recipients.

METHODS

A population-based pharmacokinetic (PK) model of unbound MPA was developed using non-linear mixed-effects modelling (NONMEM). Previously collected intensive unbound MPA PK data from 132 adult alloHCT recipients after oral and intravenous dosing of the prodrug mycophenolate mofetil (MMF) were used. In addition to clinical covariates, genetic polymorphisms in UGT1A8, UGT1A9, UGT2B7 and MRP2 were evaluated for their impact on unbound MPA PK.

RESULTS

Unbound MPA concentration–time data were well described by a two compartment model with first order absorption and linear elimination. For the typical patient (52 years of age, creatinine clearance 86 ml min^{-1}), the median estimated values [coefficient of variation, %, (CV)] of systemic clearance, intercompartmental clearance, central and peripheral volumes of MPA were 1610 l h^{-1} (37.4%), 541 l h^{-1} (75.6%), 1230 l (37.5%), and 6140 l (120%), respectively. After oral dosing, bioavailability was low (0.56) and highly variable (CV 46%). No genetic polymorphisms tested significantly explained the variability among individuals. Creatinine clearance was a small but significant predictor of unbound MPA CL. No other clinical covariates impacted unbound MPA PK.

CONCLUSIONS

In adult alloHCT recipients, variability in unbound MPA AUC was large and remained largely unexplained even with the inclusion of pharmacogenetic information. Targeting unbound MPA AUC in a patient will require therapeutic drug monitoring.

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Introduction

Although major advances have been made in allogeneic haematopoietic cell transplantation (alloHCT) with the use of non-myeloablative preparative regimens, high rates of treatment-related mortality (TRM) and acute graft *vs.* host disease (aGVHD) persist. Acute GVHD remains the leading cause of TRM in non-myeloablative alloHCT [1] with the cumulative incidence of aGVHD grades II-IV in non-myeloablative alloHCT estimated to be approximately 67%, and more severe grades III-IV around 30% [2]. Today the mainstay of treatment for the prevention of the development of aGVHD remains prophylaxis with immunosuppressants to inhibit and minimize activity of donor T cells. Mycophenolate, in combination with a calcineurin inhibitor, is now one of the most common immunosuppression regimens used in alloHCT. However, given the development of aGVHD remains a prominent clinical problem despite the inclusion of MMF in immunosuppressive regimens, the need for improved immunosuppressive strategies in alloHCT persists.

MMF is an ester prodrug that is rapidly and extensively (95%) hydrolyzed by esterases found in the blood, gut wall, liver and other tissues to the active moiety, mycophenolic acid (MPA). MPA is extensively bound to serum albumin at (97%) in patients with normal renal and hepatic function, while only the unbound fraction of MPA is pharmacologically active [3]. Unbound MPA pharmacokinetics (PK) displays wide inter- and intra-patient variability in plasma concentrations in both adult and paediatric alloHCT [4–12]. Moreover, in an adult population undergoing alloHCT a relationship between unbound MPA exposure and risk of aGVHD has been demonstrated. Subjects with an unbound MPA AUC(0,12h) of less than 300 ng ml^{-1} h had a higher cumulative incidence of aGVHD grades II-IV compared with those with higher exposure [4]. In a recent population PK analysis of unbound MPA PK in paediatric alloHCT recipients, weight, creatinine clearance and total bilirubin were identified as important clinical covariates impacting unbound MPA clearance [12]. But, even after accounting for these clinical covariates in the final model, the variability in MPA PK remained large. This suggests other clinical or patient-specific factors may be determinants of unbound MPA CL. A potentially important but unexplored source of variation in the PK of unbound MPA in alloHCT recipients include genetic variants of genes involved in MPA metabolism and disposition.

MPA is glucuronidated by several UDP glucuronosyltransferase (UGT) enzymes to the primary inactive metabolite, MPA 7-O-glucuronide (MPAG) [13, 14]. UGT1A9 is considered the main enzyme involved in MPAG formation and is expressed in multiple tissues including the liver, kidney and intestinal mucosa [13]. UGT1A8 and UGT1A10, expressed in the gastrointestinal tract, are also involved in the formation of MPAG [13, 15, 16]. The minor acyl glucuronide metabolite is formed by UGT2B7, located in the liver and kidney, and constitutes approximately 5% of the total MPA metabolic pathway [13]. Following glucuronidation, MPAG is either excreted into the urine via active tubular secretion or carried back into the intestinal lumen via bile through multidrug resistant protein (MRP) transporters, specifically MRP2 [17, 18]. In the intestine, MPAG may be converted back into MPA and reabsorbed into the systemic circulation through enterohepatic recycling [14, 19].

Numerous genetic variants of UGT and MRP2 are known to alter *in vitro* activity or in several clinical studies modify MPA pharmacokinetics. The *UGT1A9*3* exonic variant is associated with decreased *in vitro* catalytic activity towards MPA [16]. In normal volunteers receiving a single dose of MMF, carriers of *UGT1A9*3* had a 30% increase in total MPA area the under concentration–time curve (AUC) [20]. This is in contrast to the high expression *UGT1A9* promoter variants, *-275T>A* and *-2152C>T*, that yield higher rates of MPA glucuronidation [16, 21, 22]. These *in vitro* results have been confirmed in a recent clinical study by Kuypers *et al*. [23] In kidney transplant recipients, individuals carrying at least one *UGT1A9-275T>* or *2152C>T* allele had significantly increased MPA clearance and lower total MPA exposure compared with those lacking a variant allele [23]. MPA glucuronidation is also mediated by the UGT1A8 enzyme which demonstrates the highest enzymatic activity for MPAG production *in vitro* [16]. Several UGT1A8 variants have decreased *in vitro* catalytic activity, specifically *UGT1A8*3* [16, 24]. Although *in vitro* experiments with the *UGT2B7*2* variant have found no change in functional activity, significant increases in both unbound MPA and MPAG exposures were seen in healthy volunteer carriers [20].

More than eight polymorphic variants of MRP2 have been identified to date [25]. However, much of the *in vivo* data regarding the functional significance and clinical importance of these variants is conflicting [25]. In renal transplant recipients, *MRP2-24C>T* and *3972C>T* variant alleles provided protection from low MPA exposure in the presence of mild hepatic dysfunction [26]. In contrast, several other clinical studies in renal transplant recipients have found no influence of MRP2 variants on MPA PK [27, 28]. Conflicting data regarding the significance of MRP2 variants may be dependent on the well described drug–drug interaction between concomitant use of ciclosporin and MPA [29].

To our knowledge, no mycophenolate pharmacogenetic data are available in alloHCT. Drug disposition in alloHCT recipients is complex, and personalized immunosuppression through pharmacogenomics is one potential strategy to reduce the incidence and severity of aGVHD. Given the pharmacogenetic impact on MPA PK in normal volunteers and solid organ transplant recipients, it is reasonable to consider a pharmacogenetic relationship may exist in alloHCT. The primary objective of this study was to develop a population PK model of unbound MPA

in adult alloHCT recipients and to evaluate pharmacogenetic factors as contributors to the variability in PK between patients. As a secondary objective, the relationship between daily unbound MPA AUC and aGVHD was examined.

Methods

Patients

This was a retrospective population pharmacokinetic meta-analysis which utilized unbound MPA PK data obtained in 132 adult alloHCT recipients from three previously published pharmacokinetic or pharmacodynamic studies [4, 30, 31]. All pharmacogenetic data have not yet been previously published. This study was approved by the University of Minnesota Institutional Review Board and Cancer Protocol Review Committee. Patients were eligible to be included in the analysis if they were ≥ 18 years of age, had undergone a related or unrelated non-myeloablative fludarabine-based allogeneic transplant, received both MMF (Cellcept®) and ciclosporin (CSA) for aGVHD immunosuppression, and had both intensive mycophenolate PK data and recipient pretransplant DNA available for analysis. The preparative and immunosuppression regimen can be found in the Appendix S1.

As part of the original study of enrolment, each subject had steady-state intensive mycophenolate pharmacokinetics performed one or two times within 15 days post-transplant. Plasma concentrations of unbound MPA were measured after MMF doses of 1 g every 12 h, 1.5 g every 12 h or 1 g every 8 h either i.v. or oral. Clinical data were collected on each day of PK sampling and included estimates for body size, renal function and hepatic function. Creatinine clearance (CL_{cr}) was estimated by the Cockcroft–Gault equation using ideal body weight. All medications administered within 24 h of pharmacokinetic sampling were recorded. Pre-transplant recipient DNA was used to determine the following UGT and MRP2 single nucleotide polymorphisms using TaqMan probes designed by Applied Biosystems (Foster City, CA): *UGT1A8*2* (rs1042597), *UGT1A8*3* (rs17863762), *UGT1A9* 98T>C (no rs number, primers provided), *UGT1A9 -2152C>T* (rs17868320), *UGT1A9 -275T>A* (rs6714486), *UGT1A10*2* (rs10187694), *UGT2B7* 802C>T (rs7439366), *MRP2-24C>T* (rs717620), *MRP2 3972C>T* (rs3740066), *MRP2 1249G>A* (rs2273697). See Appendix S1 for details of pharmacokinetic sampling, analytic methods and DNA genotyping.

Population PK analysis

A population pharmacokinetic analysis using unbound MPA concentration–time data was carried out using the non-linear mixed-effects modelling program NONMEM (Version VII, Icon Development Solutions, Ellicott City, MD). The first order conditional estimation method with interaction was used throughout the model building and evaluation process to estimate PK parameters and variability. The MMF dose was converted to MPA equivalents according to MPA dose = $F_{MW} \times$ MMF dose, where F_{MW} is the fractional difference in molecular mass between MPA and MMF. For the oral and intravenous formulations of MMF, F_{MW} is equal to 0.739 and 0.682, respectively.

One *vs.* two compartment PK models with first order absorption and linear elimination were compared. The models were parameterized in terms of clearance (CL), central volume (V_c), intercompartmental clearance (Q), peripheral volume (V_p), absorption rate constant (k_a) and bioavailability (*F*), and the analytical solutions implemented in NONMEM (ADVAN1 and ADVAN4) were used in the estimation. To model inter-individual variability (IIV) all PK parameters were assumed to be log-normally distributed. Residual variability was assumed to follow a proportional error model. Additional model parameters such as absorption lag time (ALAG) were implemented in the base model as indicated by the data. Model selection was based on the value of the log-likelihood (NONMEM objective function value, OFV), and visual comparison of standard diagnostic plots. Models were compared statistically with a significance set at $P <$ 0.05 (ΔOFV = −3.84).

Once the structural PK model was established, patient-specific factors (both genetic and non-genetic) were evaluated for their influence on unbound MPA PK parameters. Continuous covariates including body weight, body surface area, albumin, CL_{cr}, CSA trough concentration (obtained on day of PK sampling), and the day of PK sampling relative to stem cell infusion were modelled assuming an exponential relationship. Categorical covariates were modelled proportionally, i.e. the fractional change in clearance when the categorical covariate was true. Categorical covariates evaluated included gender, total bilirubin, concomitant medications and genotype. The final PK covariate model was built following a standard forward addition and backward deletion procedure. Covariates were first added to the model in a stepwise manner in the order of their reduction in the OFV. During forward selection, additions were allowed as long as the decrease in OFV was larger than 2.71, corresponding to a *P* value of <0.1. Once the full model was established, covariates were then eliminated one at a time until the increase in the OFV was less than 6.63 ($P < 0.01$). Details of the covariate model development are available in Appendix S1.

Model evaluation

To evaluate the final model performance, a visual predictive check (VPC) was performed. The final PK model was used to simulate approximately 1000 unbound MPA concentrations at each sampling time point, and the distributions of simulated and observed data were then compared. Patients receiving every 8 h dosing (n = 15)

were not included in the VPC given the small sample size. All concentrations were dose normalized to a MMF 1 g dose. The data were stratified by dose route for presentation.

MPA exposure and clinical outcomes

The relationship between daily unbound MPA exposure (24 h area under the concentration–time curve; AUC(0,24 h)) and clinical study endpoints were investigated. To account for patients receiving both intravenous and oral MMF over the course of therapy, a composite AUC(0,24 h) was calculated weighting the duration of exposure to intravenous dosing and oral dosing. Although data on the day of transition from intravenous to oral MMF were not available, the conversion typically occurred in most subjects within the first week post-transplant. Therefore, all patients were assumed to receive intravenous MMF from day −3 to day 7 relative to transplant and oral MMF from day 8 to day 30. The composite AUC(0,24 h) was then calculated as:

$$\mathrm{AUC}(0{,}24\,\mathrm{h}) = \left(0.32 \times \frac{\text{Daily dose}_{\text{i.v.}}}{\mathrm{CL}}\right) + \left(0.68 \times \frac{\text{Daily dose}_{\text{oral}} \times F}{\mathrm{CL}}\right),$$

where Daily $\text{dose}_{\text{i.v.}}$ and Daily $\text{dose}_{\text{oral}}$ are the MPA equivalent daily intravenous and oral dose a patient received and CL and F are the *post hoc* empirical Bayesian estimates for a patient from the final population PK model.

Statistical methods

Competing-risks survival regression models of neutrophil engraftment and aGVHD (grades II-IV and III-IV) with AUC(0,24 h) as an independent predictor were then fitted in STATA 11 statistical software (StataCorp LP, College Station, TX). Acute GVHD and neutrophil engraftment data were obtained through the University of Minnesota Bone Marrow Transplant Database. GVHD was staged and graded according to the standard University of Minnesota GVHD criteria based on clinical and pathological criteria [32, 33]. Acute GVHD was defined as the day of development of grades II-IV within day 100 of transplantation. The cumulative incidence for aGVHD (grades II-IV and III-IV) was calculated at day 100. Neutrophil engraftment was defined as the first of three consecutive days of an absolute neutrophil count >500 cells μl^{-1}. Patients without neutrophil recovery at day 42 were treated as graft failures. Death was treated as a competing risk for both outcomes. To control for potential confounders, other independent predictors of outcome were tested in the regression models including donor type (related *vs.* unrelated graft), age, weight, gender, and CSA trough concentration. Predictors with P value < 0.1 were included in final the regression models.

Results

A summary of the unbound MPA PK data and patient characteristics obtained on the day of PK sampling are provided in Table 1. A total of 132 patients (82 men, 50 women) had both DNA and intensive unbound MPA PK data available, resulting in 80 intravenous and 99 oral evaluable PK

Table 1

Patient characteristics and PK data

	n (%)/median (range)
Number of individual subjects	132
Gender	
Female	50 (38%)
Male	82 (62%)
Age (years)	52 (19–69)
Number of intensive PK profiles	179
i.v. MMF	80
Oral MMF	99
Patient with both i.v. and oral MPA PK profiles	47
Day of PK sample relative to transplant	
i.v.	1 (–2 to 9)
Oral	6 (1 to 15)
MMF dosing regimen	
1 g every 12 h	98
1 g every 8 h	19
1.5 g every 12 h	15
Body weight, actual (kg)	80 (50–149)
Body surface area (m^2)	2.0 (1.5–2.8)
Laboratory chemistries	
Serum creatinine (mg dl^{-1})	0.9 (0.5–2.5)
Creatinine clearance (ml min^{-1})*	86 (30–197)
Serum albumin (g dl^{-1})	2.9 (2–4.3)
Total bilirubin (mg dl^{-1})	0.9 (0.1–20)
Ciclosporin trough concentration (ng ml^{-1})	272 (55–620)
Relevant medications on the day of PK	
Ciclosporin	132 (100%)
Fluoroquinolones	126 (95%)
Fluconazole	95 (72%)
Proton pump inhibitors	76 (58%)
Glucocorticoids	35 (27%)
Ursodiol	25 (19%)
Hormonal contraceptives	16 (12%)
Seizure prophylaxis	3 (2%)
Disease	
Non-Hodgkin's	30 (23%)
Hodgkin's	15 (11%)
Chronic myelogenous leukaemia	19 (14%)
Acute leukaemia	43 (33%)
Myelodysplastic syndrome	19 (14%)
Other	6 (5%)
Stem cell source	
Umbilical cord blood	82 (62%)
Bone marrow	8 (6%)
Peripheral blood stem cells	42 (32%)
Donor type	
Related sibling	43 (33%)
Unrelated donor	89 (67%)

*Creatinine clearance was estimated by the Cockcroft–Gault equation using ideal body weight.

profiles. Forty-seven patients had PK sampling performed following both oral and intravenous MMF. The majority of subjects had adequate kidney and hepatic function as indicated by CL_{cr} and total bilirubin or albumin, respectively. The percentage of individuals classified with mild (>50–80 ml min^{-1}) and moderate (30–50 ml min^{-1}) renal impairment as defined by the FDA regulatory guidelines for PK studies in patients were 33% ($n = 43$) and 5% ($n = 7$), respectively [34]. No patients had severe renal impairment (<30 ml min^{-1}). Ten patients (8%) had a total bilirubin >3 mg dl^{-1}. Concomitant medications considered in the covariate testing are listed in Table 1. All patients were receiving CSA at the time of PK sampling although exposure was highly variable, as represented by CSA trough concentrations. Fifty patients (38%) were taking at least one potential UGT enzyme inducer (corticosteroid, hormonal contraceptive and/or anticonvulsant) at the time of PK sampling.

Genotype frequencies for UGT and MRP2 variants are provided in Table 2. All allele frequencies were found to be in Hardy–Weinberg equilibrium. For *UGT1A9*2* and *UGT1A10*2* no variant alleles were observed (data not shown). In one patient, DNA was limited and genotyping for each variant was not feasible. The *MRP2 1249G>A* genotype was indeterminate in 15 patients. Alleles *UGT1A9-275T>A* and *UGT1A9-2152C>T* were in strong linkage disequilibrium (scale D > 0.99, $P < 0.0001$) as were *UGT1A8*3/UGT1A9-275T>A* (scale D = 0.84, $P < 0.0001$) and *MRP2-24C>T/MRP2 3972C>T* (scale D = 0.96, $P < 0.0001$).

Table 2

Allele frequencies of UGT and MPR2 polymorphisms

Gene	Variant	Genotype	Frequency (%)
UGT1A8	518C>G	C/C	73 (55.3%)
		C/G	52 (39.4%)
		G/G	7 (5.3%)
UGT1A8	830G>A	G/G	125 (94.7%)
		G/A	7 (5.3%)
		A/A	0
UGT1A9	-2152C>T*	C/C	121 (92.4%)
		C/T	9 (6.9%)
		T/T	1 (0.8%)
	-275T>A	T/T	119 (90.2%)
		T/A	12 (9.1%)
		A/A	1 (0.8%)
	98T>C	T/T	128 (97.0%)
		T/C	4 (3.0%)
		C/C	0
UGT2B7	802C>T*	C/C	30 (22.9%)
		C/T	64 (48.8%)
		T/T	37 (28.2%)
MRP2	-24C>T	C/C	97 (72.9%)
		C/T	33 (24.8%)
		T/T	3 (2.3%)
	3972C>T	C/C	69 (52.3%)
		C/T	56 (42.2%)
		T/T	7 (5.3%)
	1249G>A*,†	G/G	75 (64.7%)
		G/A	33 (28.4%)
		A/A	8 (6.9%)

*Genotyping not performed in one patient due to limited DNA. †Genotype indeterminate in 15 patients.

Population PK model building

A total of 1171 quantifiable unbound MPA concentrations were available for population PK modelling and were best described by a two compartment model with first order oral absorption and linear elimination. The range of observed concentrations was 0.6–911 ng ml^{-1}. Eight plasma unbound MPA concentrations were below the level of quantification (1 ng ml^{-1}) and were included in the analysis. Intermediate modelling steps showed that using a two compartment model resulted in a decrease in the OFV by 823 points and a decrease in the unexplained residual variability by 52% compared with a one compartment model. No improvement in the OFV or unexplained residual variability occurred with implementation of a three compartment model.

Further assessment of the two compartment model revealed that fixing bioavailability equal to 1 resulted in an increase in the OFV by 138, and there was a clear worsening in the standard diagnostics plots for the oral data. Therefore, the bioavailability parameter and inter-patient variability in bioavailability were also estimated. Visual inspection of plots of measured concentration and prediction profiles *vs.* time for each patient demonstrated the absorption profiles were adequately described using a first order absorption process. For most patients, there was no delay in the onset of absorption (i.e. lag time), but for a small subset a distinct lag time after oral dosing of approximately 2 h was apparent. Patient characteristics distinguishing this subset of patients could not be identified from the available data. Therefore, a mixture model was implemented that allowed for two sub-populations with different absorption lag time. ALAG was fixed to 0 for the population without a delay in absorption (P1) and ALAG was estimated for the population with a delay in absorption (P2). The mixture model identified eight patients with a delayed absorption (ALAG = 1.96 h) and resulted in a decrease in the OFV of 50 points. Visual inspection of diagnostic plots also showed a distinct improvement in the prediction profiles for all eight patients after inclusion of the mixture model. No other model parameters changed appreciably as a consequence of the inclusion of the mixture model. Allowing for inter-patient variability in lag time did not improve the model and therefore was not included in the final model. Additionally, allowing other absorption parameters (k_a and F) to vary for the two sub-populations by using an extended mixture model did not improve the overall model or predictions. The final two compartment model with first order oral absorption model included the mixture model for absorption lag time.

Covariate analysis

The full covariate model after the stepwise forward addition process identified CL_{cr} (ΔOFV −8.3), albumin (ΔOFV −4.4) and weight (ΔOFV −4.5) to influence unbound MPA CL and weight (ΔOFV −4.9) to influence V_c. All covariates identified were supported by individual Bayesian PK parameter estimates *vs.* covariate plots. In the stepwise backward elimination process, only CL_{cr} was found to remain significant at $P < 0.01$ and remained in the final model. None of the concomitant medications tested had a significant impact on unbound MPA CL. Proton pump inhibitor use did not influence bioavailability. No relationships were identified between unbound MPA PK parameters and genotype for any of the genetic variants tested as based on change in OFV and plots of individual Bayesian PK parameter estimates *vs.* genotype.

Final population PK model

The final unbound MPA population PK model, including parameter estimates and their relative standard errors (RSE), is presented in Table 3. Standard goodness of fit plots of the final model were reasonable (Figure 1). The VPC also demonstrated that the final model performed well in describing the observed data (Figure 2). Large inter-individual variability (IIV) remained in the final population PK model especially for k_a (73%), Q (76%) and V_p (120%).

Table 3

Final population PK model parameter estimates

Population PK parameters	Final model Estimate*	RSE (%)
CL, clearance ($l\ h^{-1}$)	1610	5.8
δ, exponent accounting for CL_{cr} effect on CL (l)	0.207	39.3
$V_{central}$, volume of central compartment	1230	12.5
Q, intercompartmental clearance ($l\ h^{-1}$)	541	13.1
$V_{peripheral}$, volume of peripheral compartment (l)	6140	21.0
k_a, first order rate constant (h^{-1})	0.400	9.3
F, bioavailability	0.560	7.4
P1, proportion of population without delayed absorption	0.91	4.0
P2, proportion of population with delayed absorption	0.09	–
$ALAG_{P1}$, lag time for population without delayed absorption (h)	0, fixed	–
$ALAG_{P2}$, lag time for population with delayed absorption (h)	1.96	1.4
Inter-individual variability (IIV)		
CL, %CV	37.4	23.0
$V_{central}$, %CV	37.5	68.4
Q, %CV	75.6	29.2
$V_{peripheral}$, %CV	120	30.4
k_a, %CV	73.0	24.8
F, %CV	44.7	37.5
Residual intra-individual variability, %CV	42.3	6.9

*Median typical value of the PK parameter in the final model. %CV, coefficient of variation; RSE, relative standard error.

The residual unexplained variability also remained high (42%). The final model predicted unbound MPA CL was:

$$CL\ (l\ h^{-1}) = 1610 \times \left(\frac{CL_{Cr}}{86\ ml\ min^{-1}}\right)^{0.207}$$

where 1610 l h^{-1} is the typical value of unbound MPA CL in a patient with the median CL_{cr} value of 86 ml min^{-1} and 0.207 is the exponent accounting for the non-linear effect of CL_{cr} on MPA CL.

Post hoc empirical Bayesian parameter estimates from the final population PK model were used to calculate steady-state unbound MPA 24 h AUC in each patient after a daily MMF dose of 2 g day^{-1} (i.e. 1 g every 12 h) and 3 g day^{-1} (i.e. 1 g every 8 h or 1.5 g every 12 h). The median 24 h AUCs after MMF 2 g day^{-1} were 517 ng ml^{-1} h (IQR, 421–665 ng ml^{-1} h) and 822 ng ml^{-1} h (IQR, 694–1087 ng ml^{-1} h) for oral and intravenous dosing, respectively. MMF 3 g day^{-1} resulted in a median unbound 24 h AUC of 775 ng ml^{-1} h (IQR, 632–997 ng ml^{-1} h) and 1233 ng ml^{-1} h (IQR, 1041–1630 ng ml^{-1} h) for oral and intravenous dosing, respectively. The bioavailability of oral MPA in the typical patient was 0.56 and resulted in lower 24 h AUC after oral dosing compared with intravenous dosing for the same dose strength. Inter-individual variation in bioavailability was high (CV 44.7%) with individual predictions of *F* ranging from 0.17 to 0.95.

Overall the impact of CL_{cr} on unbound MPA CL was small but potentially clinically relevant. For example, a typical patient with a CL_{cr} of 30 ml min^{-1} will have a 33% lower CL than a typical patient with a CL_{cr} of 120 ml min^{-1}. The impact of CL_{cr} on simulated unbound MPA 24 h AUC after oral and intravenous doses of MMF with varying degrees of renal function is shown in Figure 3 (n = 5000 simulated subjects per CL_{cr} group). Due to the large inter-individual variation in CL and bioavailability, even after correcting for CL_{cr} large overlap in 24 h AUC existed between CL_{cr} groups.

Unbound MPA acid exposure and clinical outcomes

Acute graft vs. *host disease* Cumulative incidences of grades II-IV and III-IV acute GVHD at day 100 were 49% (n = 65) and 21% (n = 28), respectively. Mycophenolic acid exposure was defined by a composite of the average daily AUC (AUC(0,24 h)) over the course of therapy. The composite AUC(0,24 h) takes into account differences in exposure after an oral *vs.* intravenous dose, and the amount of time a patient typically received each dose route over the course of therapy. The risk of aGVHD grades II-IV decreased 16% for every 200 ng ml^{-1} h increase in AUC(0,24 h) (P = 0.026). The cumulative incidence of aGVHD grades II-IV by AUC(0,24 h) percentile over the course of the study is shown in Figure 4. The patient at the 25th percentile for AUC(0,24 h) (524 ng ml^{-1} h) had a 37% higher risk of

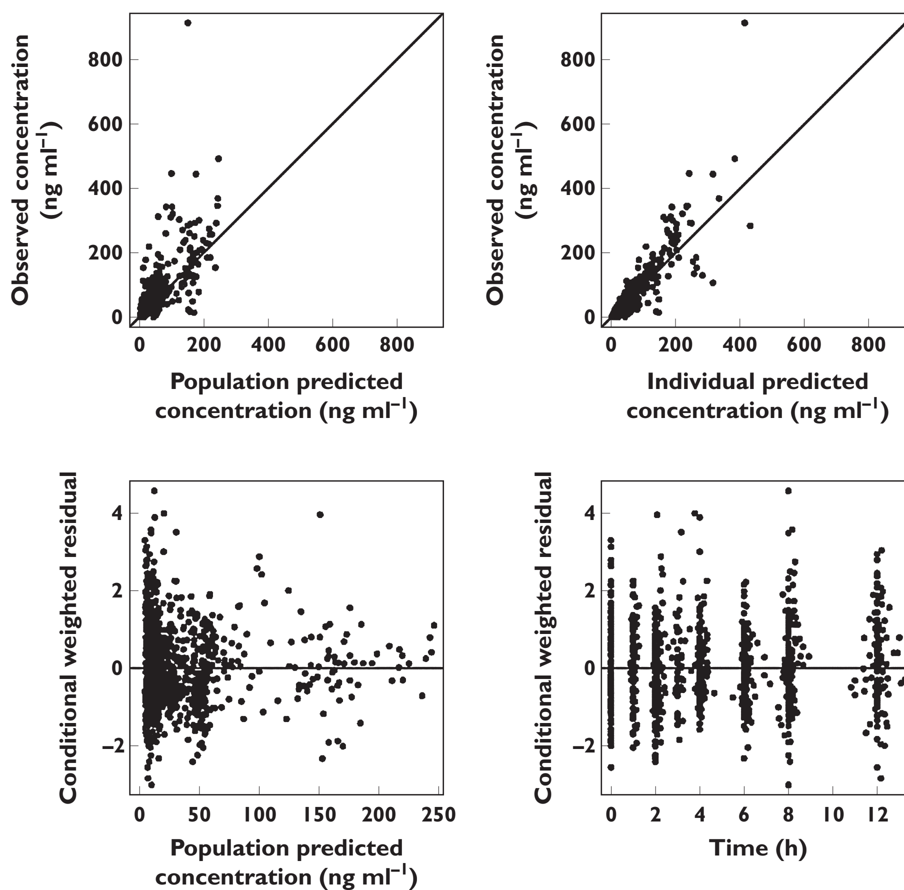

Figure 1

Goodness of fit plots of the final population pharmacokinetic model

aGVHD grades II-IV compared with the patient at the 75th percentile for AUC(0,24 h) (972 ng ml^{-1} h). No other independent predictors of aGVHD grades II-IV, including donor type (related *vs.* unrelated graft), age, weight, gender and ciclosporin trough concentration were identified (all $P >$ 0.3). Unbound MPA AUC(0,24 h) was not predictive of aGVHD grades III-V.

Neutrophil engraftment Primary neutrophil recovery occurred in 126 patients (95%) at a median (range) of 10 days (0–39) following HCT. No relationship was found between unbound MPA AUC24 and neutrophil engraftment. Unrelated cord blood recipient was associated with an increased risk of graft failure ($P < 0.001$).

Discussion

This is the first study to investigate the influence of pharmacogenetic covariates, in addition to clinical factors, as contributors to the variability of unbound MPA exposure in adult alloHCT recipients. We developed a population PK model of unbound MPA using intensive time–concentration data available after both oral and intravenous administration of MMF. The only independent predictor of unbound MPA CL was creatinine clearance. No other clinical covariates, concomitant medications or genetic variants of UGT or MRP2 tested were found to influence unbound MPA CL.

We hypothesized that genetic variants of UGT and MRP2 may contribute to MPA pharmacokinetic variability in alloHCT recipients. However, in the current analysis knowledge of an individual's genetic status proved unlikely to provide a better dosing strategy for MMF as no relationship was found between UGT or MRP2 variants and unbound MPA PK. Drug disposition in alloHCT recipients is complex, and the lack of a genetic effect may be attributed to the impact of co-administered medications on unbound MPA PK through induction or inhibition of UGT and MRP2. AlloHCT recipients often receive 10–20 concomitant medications along with MMF that may mask or confound the impact of any genetic influence on MPA pharmacokinetics. For example, steroids have been shown to induce the expression of several UGT enzymes and may lead to enhanced unbound MPA CL [29]. Fluconazole, a known inhibitor of UGT2B7, was taken by the majority of our

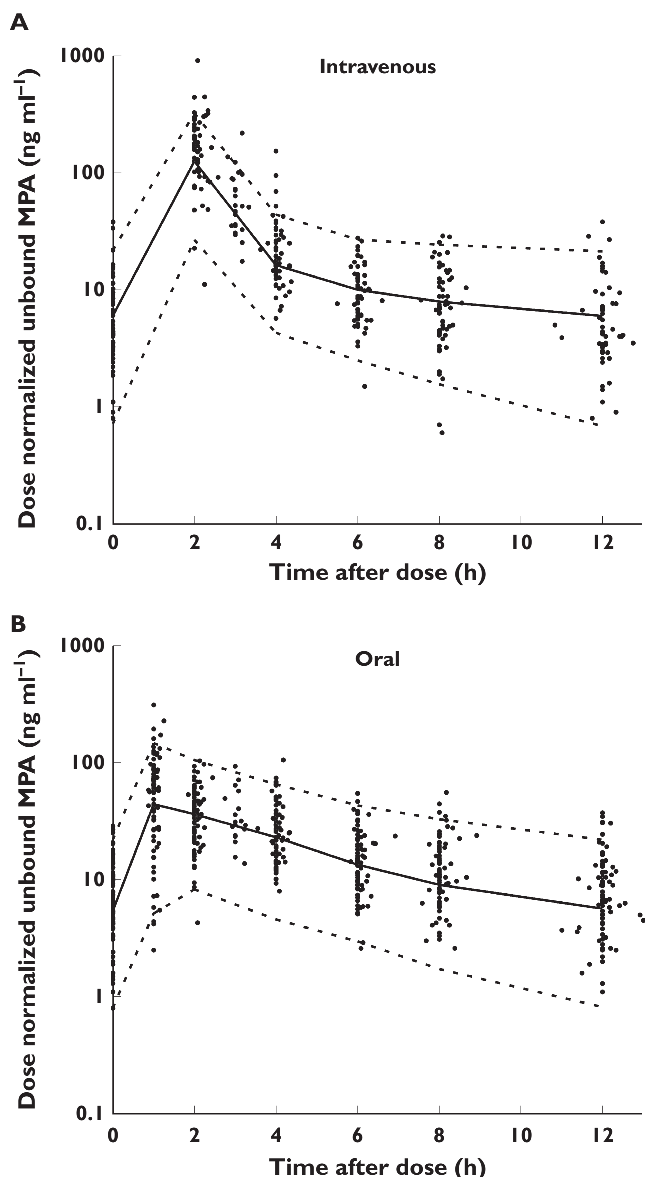

Figure 2

Visual predictive check of the final population pharmacokinetic model. The 5th, 50th and 95th percentiles for 1000 simulated unbound mycophenolic acid concentrations are presented along with the observed data for (A) intravenous and (B) oral route of administration. Concentrations are dose-normalized to a 1g mycophenolate mofetil dose. - - - -, Predicted 5%; ——, Predicted 50%; - - - -, Predicted 95%; ●, Observations

patients and may have masked any potential genotypic effect of UGT2B7 on MPA pharmacokinetics [35]. CSA, an inhibitor of MRP2, leads to decreased biliary excretion of MPAG and enterohepatic recycling of MPA [29]. Kidney transplant recipients receiving CSA have lower MPA concentrations compared with those receiving MMF alone or in combination with tacrolimus or sirolimus [29]. All subjects included in this analysis were receiving CSA at the time of pharmacokinetic sampling. Similarly, quinolones are known substrates for MRP2 and may alter unbound MPA hepatic CL and renal excretion through competition with MPAG or MPA for binding sites [36].

Inter-individual variability in MPA CL remained high (CV 37.4%) in the final PK model even after accounting for creatinine clearance. In addition, large residual variability (CV 42.3%) that represents a combination of within-patient variability, measurement error and model misspecification also remained. We were unable to estimate within-patient variability specifically in our population since the route of administration (i.e. intravenous *vs.* oral) changed within a patient between pharmacokinetic sampling days. Within-patient variability estimates in solid organ transplant patients range between 17–47% [37, 38]. If also present in alloHCT patient, large within-patient variability may make it challenging to detect genetic differences in MPA pharmacokinetics even if present and may even suggest a low genetic component to MPA pharmacokinetic variability [39]. Another possible contributing factor to the lack of a genetic effect seen on unbound MPA pharmacokinetics is the low frequency of several polymorphisms in our population. This may have resulted in inadequate power to detect more subtle genetic effects. Larger and more ethnically diverse populations along with study designs that can capture within-patient variability will be important for any future pharmacogenetic studies of MPA in alloHCT.

CL_{cr} was found to be an independent predictor of unbound MPA CL. While caution should be exercised during covariate selection when a large set of covariates are examined, the strong statistical criteria met for creatinine clearance ($P = 0.004$) combined with its agreement with our previous population PK analysis in paediatric alloHCT recipients and reports in kidney transplantation demonstrating significantly higher unbound MPA concentrations with impaired renal function [12, 40, 41], support retaining it in the final model. On the contrary, serum albumin was identified as a predictor of unbound MPA CL during forward model addition, but it did not maintain statistical significance criteria after backward elimination and therefore was not retained in the final model. This is in agreement with previous studies in kidney transplantation that have shown serum albumin alters total but not unbound MPA CL [38, 42, 43].

Increased risk of leucopenia and infection has been shown in solid organ recipients with an unbound MPA AUC(0,12 h) >400 ng ml^{-1} h [44]. Several case reports have shown neutropenia or engraftment failure in alloHCT patients with both high unbound MPA and severe organ dysfunction, and additional studies suggest that elevated unbound MPA concentrations are associated with adverse effects [45–48]. However, no formal clinical studies have tested this directly, and therefore the therapeutic targets for mycophenolate exposure to minimize toxicity in alloHCT remain unclear. Empiric dose reductions of MMF in patients that develop renal dysfunction should therefore be carefully considered at the present time. Based on the large variability in MPA PK along with the poorly defined

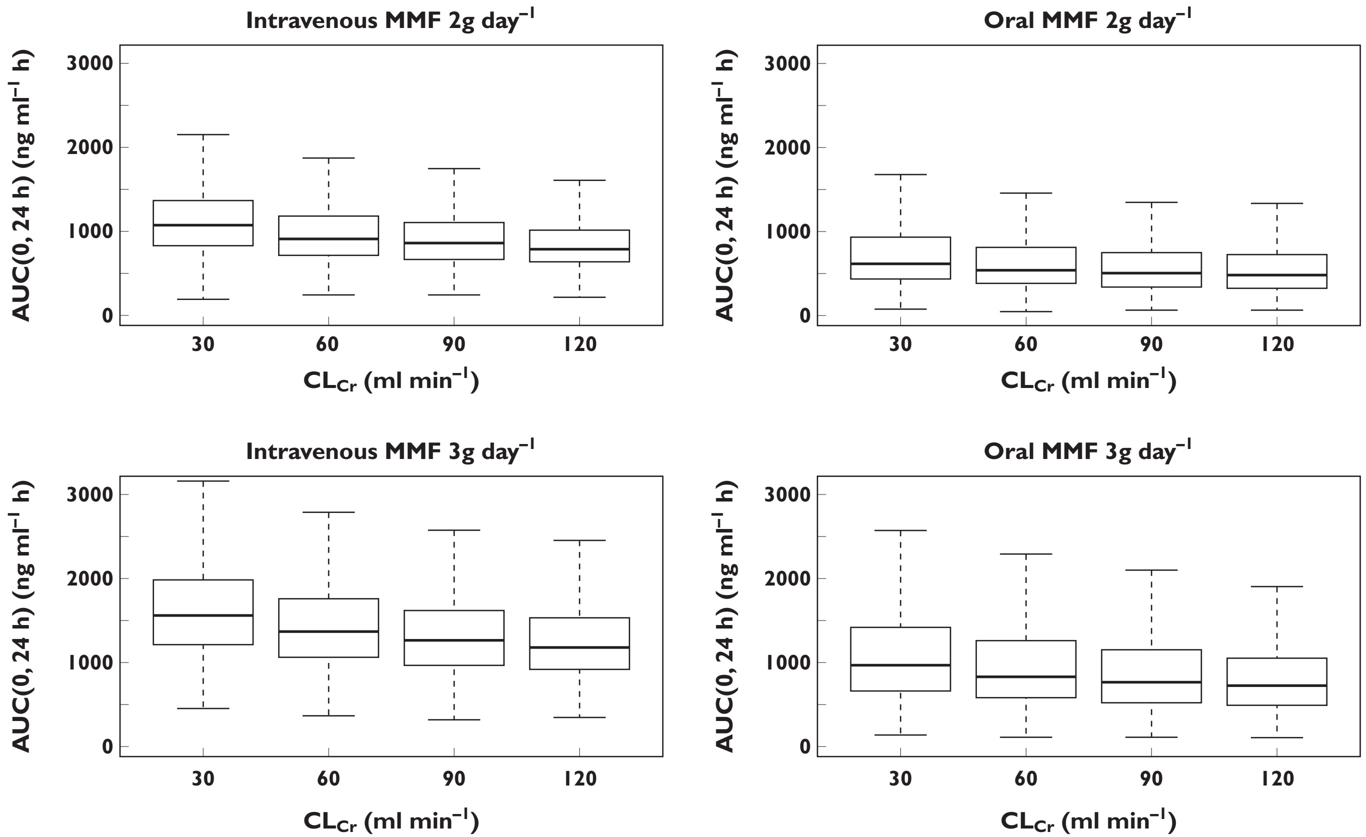

Figure 3

Simulated 24 h AUC (AUC(0,24h)) at various levels of creatinine clearance (CL_{cr}). For each level of CL_{cr}, 5000 subjects were simulated using the final population pharmacokinetic model. Box plots represent the 5th percentile, lower quartile, median, upper quartile, and 95th percentile

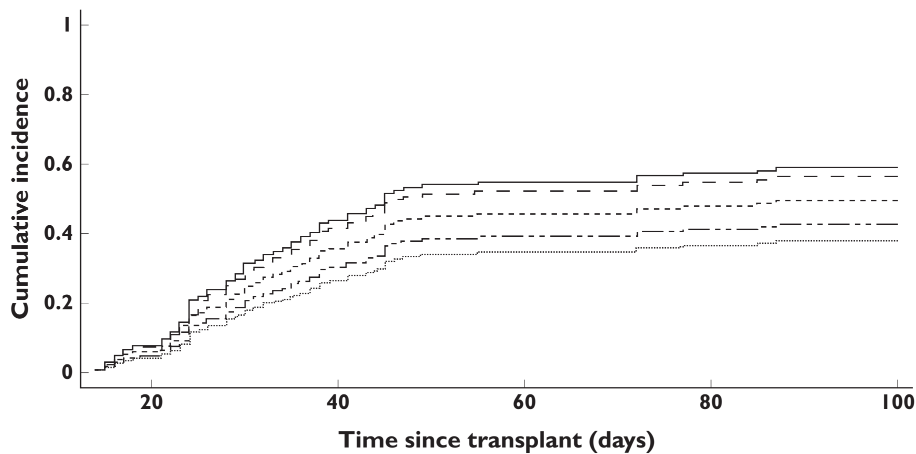

Figure 4

Cumulative incidence of aGVHD grades II-IV by unbound MPA AUC(0,24 h) percentile. AUC(0,24 h) is the average daily AUC over the course of treatment. For each percentile, the corresponding AUC(0,24 h) value in ng ml^{-1} h is shown in parentheses. ——, 10% (= 442 ng ml^{-1} h); - —, 25% (= 524 ng*h ml^{-1}); - - -, 50% (= 739 ng ml^{-1} h); - —, 75% (= 972 ng ml^{-1} h); ········, 90% (= 1147 ng ml^{-1} h)

MPA therapeutic targets, it is recommended that close monitoring of white blood cell counts in conjunction with unbound drug concentrations be used to guide dose modifications of MMF in patients that develop renal impairment. Larger prospective pharmacokinetic–pharmacodynamic studies in alloHCT are urgently needed to define better the relationships between unbound MPA exposure and toxicity.

The bioavailability of MPA following oral administration of MMF was low (56%) and highly variable among alloHCT patients (CV 45%). This is consistent with the median bioavailability of 62% (range, 13–161%) reported in a previous study of 26 adult alloHCT recipients [7]. In contrast, bioavailability in healthy volunteers after oral dosing is high (94%) [49]. The specific mechanism(s) that lead to low and variable bioavailability of MPA in alloHCT patients are still not well understood. Exploratory plots of individual predicted bioavailability and patient-specific clinical covariates did not identify any relationships to explain the variability. Given that the solubility of MMF is markedly decreased at higher pH, elevations in gastric pH with concomitant use of acid lowering medications (antacids, H_2-receptor blockers, PPIs) may limit MPA absorption and bioavailability [3]. Several clinical studies in non-HCT patients have demonstrated a decrease in MMF absorption with comedication of acid lowering drugs, but decreases were modest (lower C_{max} and AUC by 32–57% and 15–27%, respectively) [50–52]. However, our model did not identify any impact of concomitant proton pump inhibitor use on the bioavailability of MPA. It is plausible other factors contribute to lower MPA bioavailability and any additional effect from concomitant PPI therapy may be negligible. Enterohepatic recycling was not observed in our patients based on inspection of the concentration–time profiles for each patient. This is consistent with several other studies that have demonstrated a lack of enterohepatic recycling of MPA in the alloHCT recipient population [4, 53]. In contrast, in kidney transplant recipients enterohepatic recycling may contribute up to 40% of total MPA AUC [54].

There are several implications with significant clinical relevance regarding the low bioavailability of MMF following oral administration. When switching from intravenous to oral MMF, approximately a 75% increase in the dose may be required for the typical patient to maintain similar drug exposure. But given the wide interpatient variability in bioavailability, the specific intravenous to oral dose conversion will vary between individuals, and therefore therapeutic monitoring of unbound MPA drug concentrations would be required to maintain a desired AUC. Currently, the measurement of unbound MPA concentrations is not routinely available at most centres, and the therapeutic monitoring of MMF therapy is performed by measurement of total MPA plasma concentrations (a combined measurement of bound and unbound drug). Several smaller studies in alloHCT suggest the incidence of aGVHD may be lower in patients targeted to achieve either the total MPA AUC between 30–60 μg ml^{-1} h or the total MPA trough between 1 and 3.5 μg ml^{-1} [55, 56]. In contrast, Giaccone *et al*. found no relationship between MPA concentrations and aGVHD but did demonstrate reduced donor T-cell chimerism and higher rates of graft rejection in patients with a total MPA C_{ss} less than 2.5 μg ml^{-1} [10]. Given that prior PK studies have shown a weak correlation between total and unbound MPA concentrations [4] and the association between unbound MPA exposure and development of aGVHD, optimal therapeutic monitoring should be performed by evaluation of pharmacologically active unbound concentrations.

In this study, we used the average daily AUC (AUC(0,24 h)) of unbound MPA during the first 30 days post-transplant as a measure of drug exposure taking into consideration differences in AUC after intravenous and oral dosing during that time period. For every 200 ng ml^{-1} h increase in AUC24, the risk of aGVHD grades II-IV decreased 16%. Our results confirm the association between low unbound MPA AUC and risk of aGVHD in alloHCT recipients [4]. For patients being converted from intravenous to oral MMF early post-transplant, the potential increased risk for development of aGVHD due to lower bioavailability should be considered.

Finally, limitations to our study include use of data from three previously published studies [4, 30, 31], and therefore the pharmacogenetic analysis was retrospective in design. In addition, the dose of MMF and timing of pharmacokinetic analysis was variable between patients. However, the population technique is well-suited to account for underlying variation in individual data. In addition, we did not consider the major metabolite, MPAG, in our pharmacokinetic analysis. Previous pharmacokinetic models in solid-organ transplant recipients have simultaneously modelled MPA and MPAG concentrations [38, 42] and including MPAG concentrations in alloHCT recipients could have improved the predictive ability of our final pharmacokinetic model and ability to detect genetic influences.

In summary, following both oral and i.v. administration, plasma concentrations of unbound MPA were well described by a two compartment model with first order absorption and linear elimination. Genetic variation in UGT and MRP2 was not found to influence unbound MPA CL in alloHCT recipients. CL_{cr} was a small but significant predictor of unbound MPA CL, but no other clinical covariates impacted on unbound MPA PK. As previously demonstrated, lower AUC was associated with higher rates of aGVHD. Given the large variation in MPA PK, targeting unbound MPA exposure in a patient will require therapeutic drug monitoring.

Competing Interests

There are no competing interests to declare.

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REFERENCES

1 Soiffer RJ. Hematopoietic Stem Cell Transplantation, 2nd Editon. Totowa, NJ and London: Humana and Springer Distributor, 2008.

2 Majhail NS, Weisdorf DJ, Wagner JE, Defor TE, Brunstein CG, Burns LJ. Comparable results of umbilical cord blood and HLA-matched sibling donor hematopoietic stem cell transplantation after reduced-intensity preparative regimen for advanced Hodgkin lymphoma. Blood 2006; 107: 3804–7.

3 Anonymous. Product Information: CellCept (R) (Mycophenolate Mofetil) Oral and Injectable. South San Francisco, CA: Genetech, 2010.

4 Jacobson P, Rogosheske J, Barker JN, Green K, Ng J, Weisdorf D, Tan Y, Long J, Remmel R, Sawchuk R, McGlave P. Relationship of mycophenolic acid exposure to clinical outcome after hematopoietic cell transplantation. Clin Pharmacol Ther 2005; 78: 486–500.

5 Jenke A, Renner U, Richte M, Freiberg-Richter J, Platzbecker U, Helwig A, Thiede HM, Schafer-Eckart K, Ehninger G, Bornhauser M. Pharmacokinetics of intravenous mycophenolate mofetil after allogeneic blood stem cell transplantation. Clin Transplant 2001; 15: 176–84.

6 Kiehl MG, Shipkova M, Basara N, Blau IW, Schutz E, Armstrong VW, Oellerich M, Fauser AA. Mycophenolate mofetil in stem cell transplant patients in relation to plasma level of active metabolite. Clin Biochem 2000; 33: 203–8.

7 Nash RA, Johnston L, Parker P, McCune JS, Storer B, Slattery JT, Furlong T, Anasetti C, Appelbaum FR, Lloid ME, Deeg HJ, Kiem HP, Martin PJ, Schubert MM, Witherspoon RP, Forman SJ, Blume KG, Storb R. A phase I/II study of mycophenolate mofetil in combination with cyclosporine for prophylaxis of acute graft-versus-host disease after myeloablative conditioning and allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2005; 11: 495–505.

8 Basara N, Blau WI, Kiehl MG, Schmetzer B, Bischoff M, Kirsten D, Gunzelmann S, Fauser AA. Mycophenolate mofetil for the prophylaxis of acute GVHD in HLA-mismatched bone marrow transplant patients. Clin Transplant 2000; 14: 121–6.

9 Bornhauser M, Schuler U, Porksen G, Naumann R, Geissler G, Thiede C, Schwerdtfeger R, Ehninger G, Thiede HM. Mycophenolate mofetil and cyclosporine as graft-versus-host disease prophylaxis after allogeneic blood stem cell transplantation. Transplantation 1999; 67: 499–504.

10 Giaccone L, McCune JS, Maris MB, Gooley TA, Sandmaier BM, Slattery JT, Cole S, Nash RA, Storb RF, Georges GE. Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. Blood 2005; 106: 4381–8.

11 Maris MB, Niederwieser D, Sandmaier BM, Storer B, Stuart M, Maloney D, Petersdorf E, McSweeney P, Pulsipher M, Woolfrey A, Chauncey T, Agura E, Heimfeld S, Slattery J, Hegenbart U, Anasetti C, Blume K, Storb R. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood 2003; 102: 2021–30.

12 Kim H, Long-Boyle J, Rydholm N, Orchard PJ, Tolar J, Smith AR, Jacobson P, Brundage R. Population pharmacokinetics of unbound mycophenolic acid in pediatric and young adult patients undergoing allogeneic hematopoietic cell transplantation. J Clin Pharmacol 2011; Nov 22 [Epub ahead of print].

13 Picard N, Ratanasavanh D, Premaud A, Le Meur Y, Marquet P. Identification of the UDP-glucuronosyltransferase isoforms involved in mycophenolic acid phase II metabolism. Drug Metab Dispos 2005; 33: 139–46.

14 Bullingham RE, Nicholls A, Hale M. Pharmacokinetics of mycophenolate mofetil (RS61443): a short review. Transplant Proc 1996; 28: 925–9.

15 Miles KK, Stern ST, Smith PC, Kessler FK, Ali S, Ritter JK. An investigation of human and rat liver microsomal mycophenolic acid glucuronidation: evidence for a principal role of UGT1A enzymes and species differences in UGT1A specificity. Drug Metab Dispos 2005; 33: 1513–20.

16 Bernard O, Guillemette C. The main role of UGT1A9 in the hepatic metabolism of mycophenolic acid and the effects of naturally occurring variants. Drug Metab Dispos 2004; 32: 775–8.

17 Hesselink DA, van Hest RM, Mathot RA, Bonthuis F, Weimar W, de Bruin RW, van Gelder T. Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2. Am J Transplant 2005; 5: 987–94.

18 Kobayashi M, Saitoh H, Kobayashi M, Tadano K, Takahashi Y, Hirano T. Cyclosporin A, but not tacrolimus, inhibits the biliary excretion of mycophenolic acid glucuronide possibly mediated by multidrug resistance-associated protein 2 in rats. J Pharmacol Exp Ther 2004; 309: 1029–35.

19 van Gelder T, Klupp J, Barten MJ, Christians U, Morris RE. Comparison of the effects of tacrolimus and cyclosporine on the pharmacokinetics of mycophenolic acid. Ther Drug Monit 2001; 23: 119–28.

20 Levesque E, Delage R, Benoit-Biancamano MO, Caron P, Bernard O, Couture F, Guillemette C. The impact of UGT1A8, UGT1A9, and UGT2B7 genetic polymorphisms on the pharmacokinetic profile of mycophenolic acid after a single oral dose in healthy volunteers. Clin Pharmacol Ther 2007; 81: 392–400.

21 Girard H, Court MH, Bernard O, Fortier LC, Villeneuve L, Hao Q, Greenblatt DJ, von Moltke LL, Perussed L, Guillemette C. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. Pharmacogenetics 2004; 14: 501–15.

22 Villeneuve L, Girard H, Fortier LC, Gagne JF, Guillemette C. Novel functional polymorphisms in the UGT1A7 and UGT1A9 glucuronidating enzymes in Caucasian and African-American subjects and their impact on the metabolism of 7-ethyl-10-hydroxycamptothecin and flavopiridol anticancer drugs. J Pharmacol Exp Ther 2003; 307: 117–28.

23 Kuypers DR, Naesens M, Vermeire S, Vanrenterghem Y. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients. Clin Pharmacol Ther 2005; 78: 351–61.

24 Huang YH, Galijatovic A, Nguyen N, Geske D, Beaton D, Green J, Green M, Peters WH, Tukey RH. Identification and functional characterization of UDP-glucuronosyltransferases UGT1A8*1, UGT1A8*2 and UGT1A8*3. Pharmacogenetics 2002; 12: 287–97.

25 Barraclough KA, Lee KJ, Staatz CE. Pharmacogenetic influences on mycophenolate therapy. Pharmacogenomics 2010; 11: 369–90.

26 Naesens M, Kuypers DR, Verbeke K, Vanrenterghem Y. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. Transplantation 2006; 82: 1074–84.

27 Miura M, Kagaya H, Satoh S, Inoue K, Saito M, Habuchi T, Suzuki T. Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients. Ther Drug Monit 2008; 30: 559–64.

28 Zhang WX, Chen B, Jin Z, Yu Z, Wang X, Chen H, Mao A, Cai W. Influence of uridine diphosphate (UDP)-glucuronosyltransferases and ABCC2 genetic polymorphisms on the pharmacokinetics of mycophenolic acid and its metabolites in Chinese renal transplant recipients. Xenobiotica 2008; 38: 1422–36.

29 Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of mycophenolate in solid organ transplant recipients. Clin Pharmacokinet 2007; 46: 13–58.

30 Jacobson P, El-Massah SF, Rogosheske J, Kerr A, Long-Boyle J, Defor T, Jennissen C, Brunstein C, Wagner J, Tomblyn M, Weisdorf D. Comparison of two mycophenolate mofetil dosing regimens after hematopoietic cell transplantation. Bone Marrow Transplant 2009; 44: 113–20.

31 Jacobson P, Green K, Rogosheske J, Brunstein C, Ebeling B, DeFor T, McGlave P, Weisdorf D. Highly variable mycophenolate mofetil bioavailability following nonmyeloablative hematopoietic cell transplantation. J Clin Pharmacol 2007; 47: 6–12.

32 Weisdorf DJ, Snover DC, Haake R, Miller WJ, McGlave PB, Blazar B, Ramsay NK, Kersey JH, Filipovich A. Acute upper gastrointestinal graft-versus-host disease: clinical significance and response to immunosuppressive therapy. Blood 1990; 76: 624–9.

33 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED. 1994 consensus conference on acute GVHD grading. Bone Marrow Transplant 1995; 15: 825–8.

34 Anonymous. Guidance for Industry: Pharmacokinetics in Patients with Impaired Renal Function-Study Design, Data Analysis, and Impact on Dosing and Labeling. Rockville, MD: Center for Drug Evaluation and Research, 1998.

35 Uchaipichat V, Winner LK, Mackenzie PI, Elliot DJ, Williams JA, Miners JO. Quantitative prediction of *in vivo* inhibitory interactions involving glucuronidated drugs from *in vitro* data: the effect of fluconazole on zidovudine glucuronidation. Br J Clin Pharmacol 2006; 61: 427–39.

36 Naderer OJ, Dupuis RE, Heinzen EL, Wiwattanawongsa K, Johnson MW, Smith PC. The influence of norfloxacin and metronidazole on the disposition of mycophenolate mofetil. J Clin Pharmacol 2005; 45: 219–26.

37 Guillet BA, Simon NS, Purgus R, Botta C, Morange S, Berland Y, Pisano PS. Population pharmacokinetics analysis of mycophenolic acid in adult kidney transplant patients with chronic graft dysfunction. Ther Drug Monit 2010; 32: 427–32.

38 van Hest RM, van Gelder T, Vulto AG, Shaw LM, Mathot RA. Pharmacokinetic modelling of the plasma protein binding of mycophenolic acid in renal transplant recipients. Clin Pharmacokinet 2009; 48: 463–76.

39 Ozdemir V, Kalow W, Tang BK, Paterson AD, Walker SE, Endrenyi L, Kashuba AD. Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. Pharmacogenetics 2000; 10: 373–88.

40 Shaw LM, Mick R, Nowak I, Korecka M, Brayman KL. Pharmacokinetics of mycophenolic acid in renal transplant patients with delayed graft function. J Clin Pharmacol 1998; 38: 268–75.

41 Kaplan B, Meier-Kriesche HU, Friedman G, Mulgaonkar S, Gruber S, Korecka M, Brayman KL, Shaw LM. The effect of renal insufficiency on mycophenolic acid protein binding. J Clin Pharmacol 1999; 39: 715–20.

42 de Winter BC, van Gelder T, Sombogaard F, Shaw LM, van Hest RM, Mathot RA. Pharmacokinetic role of protein binding of mycophenolic acid and its glucuronide metabolite in renal transplant recipients. J Pharmacokinet Pharmacodyn 2009; 36: 541–64.

43 van Hest RM, Mathot RA, Pescovitz MD, Gordon R, Mamelok RD, van Gelder T. Explaining variability in mycophenolic acid exposure to optimize mycophenolate mofetil dosing: a population pharmacokinetic meta-analysis of mycophenolic acid in renal transplant recipients. J Am Soc Nephrol 2006; 17: 871–80.

44 Weber LT, Shipkova M, Armstrong VW, Wagner N, Schutz E, Mehls O, Zimmerhackl LB, Oellerich M, Tonshoff B. The pharmacokinetic-pharmacodynamic relationship for total and free mycophenolic acid in pediatric renal transplant recipients: a report of the german study group on mycophenolate mofetil therapy. J Am Soc Nephrol 2002; 13: 759–68.

45 Jacobson P, Long J, Rogosheske J, Brunstein C, Weisdorf D. High unbound mycophenolic acid concentrations in a hematopoietic cell transplantation patient with sepsis and renal and hepatic dysfunction. Biol Blood Marrow Transplant 2005; 11: 977–8.

46 Mudge DW, Atcheson BA, Taylor PJ, Pillans PI, Johnson DW. Severe toxicity associated with a markedly elevated mycophenolic acid free fraction in a renal transplant recipient. Ther Drug Monit 2004; 26: 453–5.

47 Kaplan B, Gruber SA, Nallamathou R, Katz SM, Shaw LM. Decreased protein binding of mycophenolic acid associated with leukopenia in a pancreas transplant recipient with renal failure. Transplantation 1998; 65: 1127–9.

48 Jacobson PA, Rydhom N, Huang J, Baker KS, Verneris MR. High-unbound mycophenolic acid concentrations in an infant on peritoneal dialysis following hematopoietic cell transplant. Bone Marrow Transplant 2007; 40: 911–2.

49 Bullingham R, Monroe S, Nicholls A, Hale M. Pharmacokinetics and bioavailability of mycophenolate mofetil in healthy subjects after single-dose oral and intravenous administration. J Clin Pharmacol 1996; 36: 315–24.

50 Rupprecht K, Schmidt C, Raspe A, Schweda F, Shipkova M, Fischer W, Bucher M, Kees F, Faerber L. Bioavailability of mycophenolate mofetil and enteric-coated mycophenolate sodium is differentially affected by pantoprazole in healthy volunteers. J Clin Pharmacol 2009; 49: 1196–201.

51 Bullingham R, Shah J, Goldblum R, Schiff M. Effects of food and antacid on the pharmacokinetics of single doses of mycophenolate mofetil in rheumatoid arthritis patients. Br J Clin Pharmacol 1996; 41: 513–6.

52 Miura M, Satoh S, Inoue K, Kagaya H, Saito M, Suzuki T, Habuchi T. Influence of lansoprazole and rabeprazole on mycophenolic acid pharmacokinetics one year after renal transplantation. Ther Drug Monit 2008; 30: 46–51.

53 van Hest RM, Doorduijn JK, de Winter BC, Cornelissen JJ, Vulto AG, Oellerich M, Lowenberg B, Mathot RA, Armstrong VW, van Gelder T. Pharmacokinetics of mycophenolate mofetil in hematopoietic stem cell transplant recipients. Ther Drug Monit 2007; 29: 353–60.

54 Bullingham RE, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. Clin Pharmacokinet 1998; 34: 429–55.

55 Osunkwo I, Bessmertny O, Harrison L, Cheung YK, Van de Ven C, del Toro G, Garvin J, George D, Bradley MB, Wolownik K, Wischhover C, Levy J, Skerrett D, Cairo MS. A pilot study of tacrolimus and mycophenolate mofetil graft-versus-host disease prophylaxis in childhood and adolescent allogeneic stem cell transplant recipients. Biol Blood Marrow Transplant 2004; 10: 246–58.

56 Haentzschel I, Freiberg-Richter J, Platzbecker U, Kiani A, Schetelig J, Illmer T, Ehninger G, Schleyer E, Bornhauser M. Targeting mycophenolate mofetil for graft-versus-host disease prophylaxis after allogeneic blood stem cell transplantation. Bone Marrow Transplant 2008; 42: 113–20.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1
Detailed methodology