Clinical Research

Pharmacokinetic and Pharmacodynamic Analysis of Inosine Monophosphate Dehydrogenase Activity in Hematopoietic Cell Transplantation Recipients Treated with Mycophenolate Mofetil

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A B S T R A C T  
A novel approach to personalizing postgrafting immunosuppression in hematopoietic cell transplantation (HCT) recipients is evaluating inosine monophosphate dehydrogenase (IMPDH) activity as a drug-specific biomarker of mycophenolic acid (MPA)-induced immunosuppression. This prospective study evaluated total MPA, unbound MPA, and total MPA glucuronide plasma concentrations and IMPDH activity in peripheral blood mononuclear cells (PMNCs) at 5 time points after the morning dose of oral mycophenolate mofetil (MMF) on day +21 in 56 nonmyeloablative HCT recipients. Substantial interpatient variability in pharmacokinetics and pharmacodynamics was observed and accurately characterized by the population pharmacokinetic-dynamic model. IMPDH activity decreased with increasing MPA plasma concentration, with maximum inhibition coinciding with maximum MPA concentration in most patients. The overall relationship between MPA concentration and IMPDH activity was described by a direct inhibitory maximum effect model with an IC50 of 3.23 mg/L total MPA and 57.3 ng/mL unbound MPA. The day +21 IMPDH area under the effect curve (AUEC) was associated with cytomegalovirus reactivation, nonrelapse mortality, and overall mortality. In conclusion, a pharmacokinetic-dynamic model was developed that relates plasma MPA concentrations with PMNC IMPDH activity after an MMF dose in HCT recipients. Future studies should validate this model and confirm that day +21 IMPDH AUEC is a predictive biomarker.

INTRODUCTION  
The availability of allogeneic hematopoietic cell transplantation (HCT) has expanded with the development of lower dose nonmyeloablative conditioning regimens, which depend on achieving a delicate balance between recipient and donor cells to obtain immunosuppression of the recipient, optimal antitumor effect, and minimal toxicity [1]. Nonmyeloablative HCT recipients often receive mycophenolate mofetil (MMF) and a calcineurin inhibitor (cyclosporine or tacrolimus) as postgrafting immunosuppression, aiming the pharmacodynamic relationship between MPA and clinical outcomes [7]. Although some HCT centers have proposed personalizing MMF doses based on MPA AUC [8], there is an ongoing debate regarding the benefits of such therapeutic drug monitoring in solid organ transplantation [9].

MPA is a selective, reversible, and noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH) [10]. IMPDH is the rate-limiting enzyme involved in the de novo synthesis of guanosine nucleotides; IMPDH catalyzes the oxidation of inosine 5′-monophosphate to xanthosine 5′-monophosphate (XMP) by a nicotinamide adenine dinucleotide positive-dependent reaction [11]. Characterizing the pharmacodynamic relationship between MPA and IMPDH activity is critical to understanding the potential benefit of alternative MMF dosing strategies in nonmyeloablative HCT recipients. Thus, we sought to characterize the pharmacokinetic-dynamic relationship between this interindividual variability (IIV). MMF is rapidly hydrolyzed to MPA in the gastrointestinal tract. After rapid absorption, MPA undergoes hepatic metabolism by various UDP-glucuronosyltransferase isoenzymes to form MPA glucuronide (total MPAG) [4]. After oral MMF administration, there is considerable between-patient variability in total and unbound MPA area under the concentration-time curves (AUCs) [5,6]. The available pharmacodynamic data in allogeneic HCT recipients suggest a relationship between MPA AUC and clinical outcomes [7]. Although some HCT centers have proposed personalizing MMF doses based on MPA AUC [8], there is an ongoing debate regarding the benefits of such therapeutic drug monitoring in solid organ transplantation [9].

Mycophenolic acid (MPA) undergoes hepatic metabolism by various UDP-glucuronosyltransferase isoenzymes to form MPA glucuronide (total MPAG) [4]. After oral MMF administration, there is considerable between-patient variability in total and unbound MPA area under the concentration-time curves (AUCs) [5,6]. The available pharmacodynamic data in allogeneic HCT recipients suggest a relationship between MPA AUC and clinical outcomes [7]. Although some HCT centers have proposed personalizing MMF doses based on MPA AUC [8], there is an ongoing debate regarding the benefits of such therapeutic drug monitoring in solid organ transplantation [9].

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total and unbound MPA plasma concentrations and ex vivo IMPDH activity in peripheral blood mononuclear cells (PMNCs) in nonmyeloablative HCT recipients receiving MMF as postgrafting immunosuppression.

METHODS

Patient Characteristics

Between November 2008 and February 2012, 105 patients participated in a prospective ancillary biomarker study in nonmyeloablative allogeneic HCT recipients. Study participation influenced neither the conditioning regimen nor postgrafting immunosuppression. Patients (age > 18 years) receiving fludarabine monophosphate (Fludara; Berlex, Montville, NJ) and total body irradiation conditioning, a related or unrelated donor granulocyte colony-stimulating factor–mobilized peripheral blood mononuclear cell (G–PBMC) graft, and postgrafting immunosuppression with a calcineurin inhibitor (cyclosporine or tacrolimus) and MMF were eligible for recruitment in this study. One participant received both G-PBMCs and bone marrow because of inadequate G-PBMC yield from the donor’s apheresis. The choice and kinetics-based dose targeting of the calcineurin inhibitor were determined by the HCT protocol. In addition to cyclosporine or tacrolimus, some participants also received sirolimus as part of their postgrafting immunosuppression.

Exclusion criteria included diagnosis of an immune deficiency disorder or scheduled to receive immunosuppression in addition to fludarabine. Total body irradiation (eg, altemazumab, thymoglobulin) during HCT conditioning to day +28 after graft infusion. This protocol was approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center (FHCRC Protocol 1980, Clinicaltrials.gov NCT00764829). Written informed consent was obtained from all patients before study participation.

The MMF dose and administration frequency were specified by the HCT protocol. MMF doses were based on weight and rounded to the nearest 250-mg dose, as previously described [6]. MMF doses were not adjusted based on MPA plasma concentrations or IMPDH determinations.

This cohort was divided into 2 separate datasets, the development dataset and the validation dataset. The development dataset was used to develop the pharmacokinetic/dynamic model relating plasma MPA concentrations with PMNC IMPDH activity and to validate our previous population pharmacokinetic model [5,6]. The validation dataset was used to validate the pharmacokinetic/dynamic model relating plasma MPA concentrations and PMNC IMPDH. The patient characteristics of the development and validation datasets are shown in Table 1.

Sample Collection

Peripheral blood samples (8 mL drawn into EDTA vacutainers, Supplemental Figure 1) were obtained on day +21. The total MPA, unbound MPA, and total MPAG plasma concentrations and IMPDH activity in PMNCs were quantitated in each sample. All assays were performed on each of the samples. Of the 56 AUCs, 2 were collected on day +19, 17 on day +20, 32 on day +21, 3 on day +22, 1 on day +23, and 1 on day +25. Samples were drawn before and 1, 2, 2.5, and 6 hours after oral MMF for 29 patients (November 2008 to March 2011; development and validation cohorts) and before and 1.25, 2, 3, and 4 hours after oral MMF for 27 patients (April 2011 to February 2012, validation cohort). These patients were primarily treated in the ambulatory clinic, and therefore limited sampling schedules were used to maximize compliance. From November 2008 to March 2011, compliance for pharmacokinetic samples was 63% (41 of 65 patients); this decreased to 58% (6 of 12 participants) when participants were paid per AUC obtained. In April 2011, the sampling schedule was shortened to 4 hours after the oral MMF dose based on a prior analysis [6]. With this change, compliance improved to 76% (26 of 34 participants). In 50 of 56 patients, all 5 samples were collected. Among the remaining participants, 2 had 1 sample collected, 1 had 3 samples collected, 2 had 4 samples collected, and 1 had 6 samples collected. IMPDH activity on day +2 after HCT was planned; this could not be conducted, however, due to myelosuppression resulting from the conditioning regimen.

Table 1 lists the participant characteristics, including biochemistry and concomitant medications associated with MPA pharmacokinetic parameters. Our previous population pharmacokinetic model after intra- venous or oral MMF administration indicated that MPA clearance was significantly increased (by 33.8%) with concomitant cyclosporine and negatively correlated with albumin concentration [6].

Reagents and Chemicals

All nucleotides used as substrates for the enzymatic assay or as chromatographic standards were obtained from Sigma (St. Louis, MO). Nicotinamide adenine dinucleotide was also purchased from Sigma. Acetonitrile, ammonium acetate, methanol, sodium hydroxide, sodium phosphate monobasic, ammonium acetate, ammonium hydroxide, and potassium chloride were all obtained from Thermo Fisher (Waltham, MA). Dulbecco’s PBS was purchased from Invitrogen (Grand Island, NY). Ficoll Hypaque solution (density 1.077 g/mL) was obtained from GE Healthcare (Uppsala, Sweden).

Quantitation of MPA and Total MPAG

Each plasma sample was analyzed for total MPA, unbound MPA, and total MPAG plasma concentrations using reverse phase HPLC with mass
spectrum (MS) detection. For total MPA and total MPAG quantitation, plasma samples (100 μL) with the internal standard (20 μL MPA-d3 and MPA β-D-glucuronide-d3) were combined with 50 μL methanol and 1000 μL acetonitrile, vortexed, and subsequently centrifuged. The supernatant (1 μL) was injected onto a light chromatography (LC/MS running a gradient of 2.0 mM ammonium formate (pH 3.3) and acetonitrile through an Agilent C18 column (2.1 mm × 150 mm × 5 μm; Agilent Technologies, Palo Alto, CA). Monitored ions included m/z 321 for the (M+H)⁺ ion of MPA, m/z 324 for the (M+H)⁺ ion of MPA-d3 (internal standard), m/z 495.2 for the (M+H)⁺ ion of total MPAG, and m/z 498.1 for the (M+H)⁺ ion of total MPAG-d3 (internal standard). The dynamic range was 3 to 15.5 mg/L for MPA and 21.5 to 215 mg/L for total MPAG. The interday coefficient of variation was less than 10.7%.

Unbound MPA plasma concentrations were determined by equilibrium dialysis using Pierce rapid equilibrium dialysis devices (Thermo Fisher). After incubation and processing of plasma samples according to Pierce rapid equilibrium dialysis manufacturer instructions, samples were analyzed using LC/MS as above with slight modifications (i.e., mobile phase was an isocratic mixture of 55% 2.0 mM ammonium formate [pH 3.3] and 45% acetonitrile, with a total run time was 5 minutes). The percentage of unbound (free) MPA was calculated as follows: unbound MPA = 100 × (1-bound MPA).

Isolation of Human PMNCs and IMPDH Activity Assay
PMNCs were isolated within 6 hours of collection by diluting blood in PBS at a 1:1 v:v ratio and subsequently layering on top of Ficol as previously described [12]. In brief, PMNCs were collected, diluted to 10 mL with PBS to wash and centrifuged. The cell counts were quantitated using an HORIBA Diagnostics ABX Micro 60 (Irving, CA), and distilled water was added to the supernatant to adjust the cell concentration to 0.5 × 10⁶ cells/mL. This PMNC count was used to standardize the IMPDH activity measurement. After storage at −80 °C, the IMPDH activity was determined from the conversion of inosine monophosphate to XMP according to procedures adapted from Glander et al. [13] and Daxecker et al. [14]. The incubation reaction mixture included NaH₂PO₄, KCl, inosine monophosphate (8 mL of 6.0 mmol/L) and nicotinamide adenine dinucleotide (8 mL of 4.5 mmol/L made fresh each day). The incubation reaction was started via the addition of the prewarmed cell lysate (standard concentration of 0.5 × 10⁶ cells/mL).

For each incubation, the enzymatic reaction was terminated after 2.5 hours of incubation by the addition of methanol followed by internal standard (8-bromo adenosine 5’-monophosphate), processed, and injected on the LC/MS running a gradient of .1 M ammonium acetate (pH 3.6) to 100% acetonitrile. The mass selective detection was run in the selected ion mode was used. The mass selective detection was run in the selected ion monitoring mode. Monitored ions included m/z 365 for the (M+H)⁺ ion of XMP, m/z 348 for the (M+H)⁺ ion of adenosine monophosphate, and m/z 426 for the (M+H)⁺ ion of 8-bromo adenosine 5’-monophosphate, the internal standard. Typical retention times were 4.3 minutes for XMP, 5.1 minutes for inosine monophosphate, and 7.0 minutes for 8-bromo adenosine 5’-monophosphate. The limit of quantification (signal-to-noise ratio > 60 and coefficient of variation < 2%) was 58 pmol.

A quality control pooled lysate obtained from the PMNCs of healthy subjects was run in triplicate with every incubation. The mass selective detection was run in the selected ion monitoring mode. Monitored ions included m/z 321 for the (M+H)⁺ ion of MPA, m/z 324 for the (M+H)⁺ ion of MPA-d3 (internal standard), m/z 495.2 for the (M+H)⁺ ion of total MPAG, and m/z 498.1 for the (M+H)⁺ ion of total MPAG-d3 (internal standard). The dynamic range was 3 to 15.5 mg/L for MPA and 21.5 to 215 mg/L for total MPAG. The interday coefficient of variation was less than 10.7%.

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Figure 1. Final pharmacokinetic model characterizing total MPA, unbound MPA, and total MPAG concentrations following oral MMF administration. tlag: lag time; GI, gastrointestinal tract; k10, first-order absorption rate constant; fUB, fraction unbound of MPA; Vc, volume of central compartment of total MPA; Q, intercompartment clearance of total MPA; Vm, volume of peripheral compartment of total MPA; CLMPA, clearance of total MPA; CLMPA/C, clearance of total MPAG; Vm,C, volume of central compartment of total MPAG; kω1, first-order rate constant of EHC.

MPA Population Pharmacokinetic-Dynamic Analysis
Pharmacokinetic (total MPA, unbound MPA, and total MPAG plasma concentrations) and pharmacodynamic (XMP formation in PMNCs to provide IMPDH activity) data were available at each concentration time point. The pharmacokinetic-dynamic models were developed in a sequential manner: the pharmacokinetic model was developed first, then each participant’s pharmacokinetic parameters were fixed for the creation of the pharmacodynamic model.

The initial pharmacokinetic model of Li et al. [6], which was developed in 402 HCT recipients, was modified to include total MPAG (as described by Musumba et al. [15]) and unbound MPA concentrations. Briefly, total MPA pharmacokinetics was described using a 2-compartmental model with first-order elimination and first-order absorption with a lag time (Figure 1). The model was parameterized as clearance (CL), volume of the central compartment (Vc), volume of the peripheral compartment (Vm), and inter-compartment clearance (Q). Total MPAG pharmacokinetics was described using a 1-compartmental model with first-order elimination. It was assumed that MPA is metabolized to total MPAG by a first-order process and that 100% of MPA is converted to total MPAG because urinary MPA data were not available. Collection of urinary excretion data was not possible because participants were treated in the ambulatory clinic. Therefore, total MPAG CL and Vm are apparent clearance and volume of distribution (ie, CL/F and V/F). Enterohepatic recirculation (EHC) of total MPAG was represented as a first-order process between the total MPAG central compartment and the gastrointestinal tract. Unbound MPA concentrations were modeled as [MPAunbound] = [MPAcentral] × fUB with [MPAcentral], [MPAtotal], and fUB representing unbound MPA concentration, total MPA concentration, and the unbound fraction, respectively.

We also sought to validate the covariate effect of the calcineurin inhibitor (cyclosporine or tacrolimus) on MPA clearance [5,6]. MPA clearance was eventually defined by the following equation:

\[
\text{MPA CL} = 24.2 \times \frac{\text{body weight (kg)}}{70} \times \frac{\text{albunin (g/dL)}}{3.4} \times \left(1 + 338 \times \text{CSF}^{-1} \right)
\]

(Eq. 1)

with body weight in kg, albumin in g/dL, and CSF = 1 for cyclosporine and 0 for tacrolimus.

The Bayesian total MPA AUC₂₋₅ of 27 participants who received MMF every 8 hours was calculated. The AUCs of patients receiving concomitant tacrolimus were compared with those of patients taking cyclosporine. In addition, this simulation was executed using population mean parameter estimates to compare total MPA AUC₂₋₅ in patients receiving either concomitant cyclosporine or tacrolimus. Both Bayesian estimations and simulations were performed using NONMEM VII (Icon Development Solutions, LLC, Ellicott City, MD).

Subsequent to finalization of the population pharmacokinetic model, the relationship between MPA concentrations and IMPDH activity was explored graphically and modeled using an inhibitory maximum effect model:

\[
E = E₀ + \frac{E_{\text{max}} \times C_{\text{MPA}}}{IC_{50} + C_{\text{MPA}}} \quad \text{with } E_0 \text{ as baseline IMPDH activity (minimal inhibition), } E_{\text{max}} \text{ as maximal IMPDH inhibition, } C_{\text{MPA}} \text{ as the MPA plasma concentration, } IC_{50} \text{ as the MPA concentration that causes 50% of maximal IMPDH inhibition, and } r \text{ as the Hill coefficient that governs the slope of MPA concentration versus IMPDH activity.}
\]

Population pharmacokinetic-dynamic analysis was performed using nonlinear mixed effects modeling (NONMEM® VII.1.0). The Monte Carlo Important Sampling expectation maximization method was used throughout the modeling process. Pharmacokinetic models were simultaneously fitted to total MPA, unbound MPA, and total MPAG plasma concentrations. MMF dose and total MPAG concentration were multiplied by 1.08.
donor, 21 (38%) were CMV positive with a CMV-negative donor, 8 (14%) were CMV negative with a CMV-positive donor, and 15 (27%) were CMV negative with a CMV-negative donor. When either the donor or recipient was CMV positive, the CMV antigenemia assay to detect CMV pp65 antigen was performed on a weekly basis for the first 3 months after HCT. Twenty-two participants experienced CMV reactivation post-transplant. Acute GVHD was graded as previously described [17]. Hematological diseases were classified as low, standard, or high risk of relapse per the Kahi criteria to evaluate relapse rate in a consistent manner [18]. We defined disease relapse or disease progression as disease recurrence after complete remission or progression of persistent disease. Clinical endpoints were measured to the time of last clinical follow-up. The median time to last clinical follow-up was 1.4 years (range, 3 to 3.3 years).

Statistical Analysis
IMPDH AUEC was treated as a fixed covariate. Cumulative incidence curves for acute GVHD were estimated using methods previously described [19]. Cox regression analysis was used to model the impact of IMPDH AUEC on time-to-event endpoints. Death and relapse were treated as competing risks for analysis of acute and chronic GVHD. Relapse was treated as a competing risk for the analysis of nonrelapse mortality. The effects of IMPDH AUEC on hazard ratios were expressed as the effect per doubling of IMPDH AUEC. All reported $P$ values are 2-sided, and those estimated from regression models are derived from the Wald test. No adjustments were made for multiple comparisons.

RESULTS
Patient characteristics are summarized in Table 1. The dose, administration route, and administration frequency of MMF were determined by the participant’s HCT transplant protocol.

Population Pharmacokinetic Model
A total of 167 pharmacokinetic samples (total MPA, unbound MPA, and total MPAG) was used for population pharmacokinetic model building. A previously developed MPA pharmacokinetic model was modified to include total MPAG EHC (Figure 1). Total MPAG was assumed to be excreted to the gastrointestinal tract by a continuous first-order process. It was estimated that 29.5% of MPA (ie, EHC $\% = k_{41}/(k_{41} + C_{\text{TOTAL MPAG}/V_{p}})$) underwent EHC. Data fitting was significantly improved when EHC was only integrated with concomitant tacrolimus. The current dataset did not support a more physiologically based EHC model [20]. Of note, the lag time could not be estimated for each participant because his or her food intake was not available. The population mean from the lag time was fixed to a previously estimated value [5]. Pharmacokinetic parameters were well estimated, as shown in Table 2. The visual predictive checks and the time courses of total MPA concentration, unbound MPA concentration, and total MPAG concentration are shown in Supplemental Figure 2.

Covariate analysis identified serum creatinine as a significant covariate of total MPAG CL. Inclusion of serum creatinine in the model resulted in a 24-unit decrease in objective function value and a 9.7% decrease in IV of total MPAG CL. The final pharmacokinetic model included both cyclosporine and serum creatinine as model covariates. Goodness-of-fit plots are presented in Supplemental Figure 3.

Cyclosporine inhibits multidrug resistance-associated protein 2—mediated EHC of total MPAG and thus results in decreased MPA AUC and increased MPA clearance (Supplemental Figure 4). Median total MPA AUC$_{0-8h}$ was increased by 33% in participants receiving concomitant tacrolimus. In addition, simulations using population means demonstrated that the MPA AUC$_{0-12h}$ in participants taking...
Tacrolimus was, on average, 1.38-fold higher than in patients taking cyclosporine. Overall, these results confirmed our previous findings [6] and can be considered as an external validation for Eq. 1 where MPA CL is increased by 33.8% with concomitant cyclosporine.

**Population Pharmacokinetic-Dynamic Model**

For the pharmacometric analysis, 267 PMNC samples were available for ex vivo IMPDH activity quantitation. Of these, 263 samples had 3 replicates for IMPDH activity and 4 samples had 2 replicates. Within-sample variability was calculated by dividing the lowest XMP formation rate by the highest rate within each sample (ie, minimum/maximum). The within-sample variability ranged from 74% to 100%. Most samples had 2 replicates. Within-sample variability was estimated and fixed to 1.

The concentration-response relationships between MPA concentration and IMPDH activity relationship could be well described by an inhibitory direct effect $E_{\text{max}}$ model (Eq. 2). The final model-based $IC_{50}$ estimate was 3.23 mg/L (relative standard error 10.7%; IV 53.1% CV) for total MPA and 57.3 ng/ml (relative standard error 11.2%; IV 56.3% CV) for unbound MPA. Maximum inhibition could not be estimated from the observed data and was therefore fixed to 1. The Hill coefficient was estimated to be close to 1 and was fixed to 1 in the final parameter estimation. Table 3 summarizes the estimated and fixed population pharmacodynamic parameters.

The final pharmacodynamic model did not include any of the available covariates. Age was negatively correlated with $E_{0}$, with a Pearson correlation of .25 ($P = .06$). Inclusion of age as a model covariate did not result in a significant improvement in model fitting, and age was thus not included in the final model. Notably, the calcineurin inhibitor and graft source (ie, related versus unrelated donor) did not affect the $IC_{50}$ or $E_{0}$ (Supplemental Figure 5).

The final pharmacokinetic-dynamic parameter estimates were updated using a combination of both the model-building and validation datasets (Tables 2 and 3). Both visual predictive check and goodness-of-fit plots demonstrated that the final model precisely described the observed pharmacokinetic and pharmacodynamic data (Supplemental Figures 2 and 3).

**Validation of the Population Pharmacokinetic-Dynamic Model**

The participant characteristics were similar between the model-building and validation datasets (Table 1). Two methods were applied to the validation dataset to externally corroborate the developed population pharmacokinetic-dynamic model. The results from both methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Explanation</th>
<th>Base Model</th>
<th>Final Model</th>
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</thead>
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<tr>
<td>$k_{0}$ (h⁻¹)</td>
<td>First-order rate constant representing both formation and absorption process</td>
<td>.913 (11.7)</td>
<td>.916 (12.4)</td>
</tr>
<tr>
<td>$T_{lag}$ (h)</td>
<td>Lag time of oral absorption</td>
<td>.228 FIXED</td>
<td>.228 (21) FIXED</td>
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<tr>
<td>$CL_{MPA}$ (L/h/70 kg)</td>
<td>Clearance of total MPA</td>
<td>31.4 (5.6)</td>
<td>31.4 (5.7)</td>
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<td>$CL_{TOTAL MPAG}$ (L/h/70 kg)</td>
<td>Clearance of total MPAG</td>
<td>1.29 (7.2)</td>
<td>1.32 (5.9)</td>
</tr>
<tr>
<td>$Q_{mpa}$ (L/h/70 kg)</td>
<td>Intercompartmental clearance of total MPA</td>
<td>11.6 (29.8)</td>
<td>11.5 (27.7)</td>
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<tr>
<td>$k_{1}$ (L/h/70 kg)</td>
<td>First-order rate constant representing total MPAG EHC</td>
<td>.0503 (45.3)</td>
<td>.0558 (44.1)</td>
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<tr>
<td>$V_{c}$ (L/70 kg)</td>
<td>Volume of central compartment of total MPA</td>
<td>25.3 (13.0)</td>
<td>26.5 (12.9)</td>
</tr>
<tr>
<td>$V_{p}$ (L/70 kg)</td>
<td>Volume of peripheral compartment of total MPA</td>
<td>247 (21) FIXED</td>
<td>247 (21) FIXED</td>
</tr>
<tr>
<td>$V_{mpa/fu}$ (L/70 kg)</td>
<td>Volume of central compartment of total MPAG</td>
<td>9.91 (8.9)</td>
<td>9.91 (8.8)</td>
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<tr>
<td>$f_{u}$ (%)</td>
<td>Fraction unbound of MPA</td>
<td>1.76 (2.6)</td>
<td>1.76 (2.7)</td>
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<td>$\beta_{creatinine}$</td>
<td>Power coefficient of creatinine covariate effect on total MPAG clearance</td>
<td>Not estimated (NE)</td>
<td>−.919 (19.5)</td>
</tr>
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</table>

CV indicates coefficient of variation; RSE, relative standard error; $T_{lag}$, lag time.

* Typical CL values were calculated as described in Eq. 1. Body weight calculations described in Patient Characteristics in Methods.

$\text{Cl}_{\text{TOTAL MPAG}} = \text{Cl}_{\text{pop}} \times \left( \frac{\text{serum creatinine}}{1.12} \right)^{.919}.$

Bioavailability was fixed to 1.

Additive residual error was on a natural logarithmic-scale.
demonstrated that the population pharmacokinetic-dynamic model accurately predicted the observed MPA pharmacokinetics and IMPDH activity in a separate cohort of HCT participants. For the population prediction, the MPE\% for total MPA, unbound MPA, total MPAG, and IMPDH activity were 28.9%, 10.6%, 27.1%, and 1.85%, respectively. For individual Bayesian predictions, MPE\% for total MPA, unbound MPA, total MPAG, and IMPDH activity were 4.85%, 2.0%, .4%, and .97%, respectively. As shown in Supplemental Figure 6, most validation data fall within the 95% confidence interval of the predicted values that were derived from the pharmacokinetic-dynamic parameters estimated from the development dataset.

Pharmacodynamic Relationships

We also sought to evaluate if clinical outcomes could be predicted by the day +21 IMPDH AUEC. The analysis was adjusted for Kahl relapse risk category, female donor to male recipient, and donor type (unrelated versus related). As shown in Table 4, Figure 3, and Supplemental Figure 7, day +21 IMPDH AUEC was associated with CMV reactivation ($P = .003$), nonrelapse mortality ($P = .04$), and overall survival ($P = .03$).

DISCUSSION

The translational relevance of this work is that we created a pharmacokinetic-dynamic model that characterizes the relationship between IMPDH activity with plasma MPA concentrations after oral MMF administration in non-myeloablative HCT patients. Our key results are as follows: (1) weight-based dosing of MMF results in considerable interpatient variability in the inhibition of IMPDH activity, (2) a previous MPA population pharmacokinetic model in HCT recipients has been validated [6], and (3) a population pharmacokinetic-dynamic model of MPA concentrations with IMPDH activity has been developed and validated. No clinical covariates were found for the pharmacodynamic parameters (Supplemental Figure 5), indicating that IMPDH

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Explanation</th>
<th>Estimates* (RSE%) for IMPDH Activity (pmol XMP/10^6 cells/h)</th>
<th>Total MPA (mg/L)</th>
<th>Unbound MPA (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_0$</td>
<td>Baseline IMPDH activity (immediately before day +21 MMF dose)</td>
<td>1370 (5.6)</td>
<td>3.23 (10.7)</td>
<td>57.3 (11.2)</td>
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<td>$IC_{50}$</td>
<td>MPA concentration causing 50% maximal inhibition</td>
<td>27.6 (30.8)</td>
<td>27.3 (31.4)</td>
<td></td>
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<tr>
<td>$IIV_{E_0}$ (CV%)</td>
<td>Interindividual variability of $E_0$</td>
<td>53.1 (34.2)</td>
<td>56.3 (33.4)</td>
<td></td>
</tr>
<tr>
<td>$IIV_{IC_{50}}$ (CV%)</td>
<td>Interindividual variability of $IC_{50}$</td>
<td>.20 (19.3)</td>
<td>.20 (19.3)</td>
<td></td>
</tr>
</tbody>
</table>

CV indicates coefficient of variation; RSE, relative standard error.  
* Maximum inhibition could not be estimated based on observed data and therefore was fixed to 1. Hill coefficient was estimated close to 1 and was fixed to 1 in the final parameter estimation.  
† The base model is the final model, because none of the evaluated covariates met the criteria for inclusion in the pharmacodynamic model.
The immnosuppressant MMF is an integral component of postgrafting immunosuppression after HCT. HCT recipients receiving MMF dosed by body weight have varying clinical outcomes [21]. We recently constructed MPA population pharmacokinetic models after intravenous MMF [5] or oral MMF [6] administration that revealed considerable IIV in MPA pharmacokinetics. We validated this pharmacokinetic model and refined it by adding concentration-time data of unbound MPA and total MPAG. Oral MMF dosed based on body weight still results in considerable interpatient variability in ex vivo IMPDH activity in PMNCs isolated from participants receiving the same 15-mg/kg dose of oral MMF (Figures 2 and 4). An inhibitory E_max model adequately described the inhibition of IMPDH activity in PMNCs by MPA. The IIV of the pharmacodynamic parameters varied from 27.3% to 56.3% (Table 3). This IIV is greater than the IIV of many pharmacokinetic parameters with the notable exception of the IIV of the volume estimates, which range from 47% to 86.4% (Table 2). The large IIV in the volume estimates could be attributed to the inability to accurately characterize the maximum MPA concentration due to the use of a limited sampling schedule, which is a necessity in this patient population treated in the ambulatory clinic.

We hypothesized that estimating its drug-specific pharmacodynamics (IMPDH activity in PMNCs) could be a predictive measure of an individual’s response to MMF. Determining IMPDH activity may provide a direct

Table 4
Association of Day +21 IMPDH AUEC with HCT Outcomes

<table>
<thead>
<tr>
<th>Events</th>
<th>Odds Ratio/ Hazard Ratio (95% Confidence Interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day +28 T cell chimerism ≥ 95%</td>
<td>.96 (.4-2.6)</td>
<td>.96</td>
</tr>
<tr>
<td>Grades II-IV acute GVHD</td>
<td>.72 (.4-1.3)</td>
<td>.26</td>
</tr>
<tr>
<td>Grades III-IV acute GVHD</td>
<td>.13 (.0-1.0)</td>
<td>.05</td>
</tr>
<tr>
<td>Extensive chronic GVHD</td>
<td>1.38 (.7-2.9)</td>
<td>.38</td>
</tr>
<tr>
<td>Relapse</td>
<td>.95 (.3-3.2)</td>
<td>.93</td>
</tr>
<tr>
<td>CMV reactivation</td>
<td>.29 (.1-1.7)</td>
<td>.003</td>
</tr>
<tr>
<td>Nonrelapse mortality</td>
<td>.23 (.1-1.0)</td>
<td>.04</td>
</tr>
<tr>
<td>Overall mortality</td>
<td>.40 (.2-9)</td>
<td>.03</td>
</tr>
</tbody>
</table>

* Events on or after day +26.
† Day +28 T cell chimerism analyzed as binary endpoint (odds ratio), and all others as time-to-event endpoint (hazard ratio). Odds ratio and hazard ratio are effects per doubling of IMPDH AUEC. All analyses adjusted for Kahl relapse risk category (low, standard, high), donor—recipient gender (female to male, other), and donor (related, unrelated).

Figure 3. Association of IMPDH AUEC (pmol × 10^6 cells) with acute GVHD (A), CMV reactivation (B), nonrelapse mortality (C), and overall mortality (D) in 45 patients receiving an unrelated donor graft. Only events on or after day +26 are included.
variability in IMPDH AUEC (Figure 4B). Although we developed a validated LC/MS method to quantitate IMPDH activity, we could not quantitate IMPDH activity on day +2 because of extremely low white blood cell counts, predominantly due to fludarabine total body irradiation conditioning.

In conclusion, we have shown that adequate number of PMNC can be isolated from HCT recipients on day +21 to quantitate XMP, and thus IMPDH activity, using our highly sensitive assay. We presented an integrated population-based model of total MPA, unbound MPA, and total MPAG plasma concentrations and the associated degree of immunosuppression, as quantified by IMPDH activity in PMNCs. The final model captured the central tendencies and IIV well; there were, however, no clinical covariates associated with the pharmacodynamic parameters. Such a model provides an approach toward individualized oral MMF dosing and a firm rationale for further studies investigating whether dosing MMF on the basis of IMPDH activity can improve clinical outcomes. Subsequent translational studies will be necessary to evaluate whether IMPDH activity after MPA provides a novel biomarker to predict an individual’s sensitivity and response to MMF, with the long-range goal of individualizing postgrafting immunosuppression and/or MMF doses to improve the efficacy and/or decrease the toxicity of non-myeloablative HCT.

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Conflict of interest statement: There are no conflicts of interest to report.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.03.032.

REFERENCES

allogeneic blood stem cell transplantation. Bone Marrow Transplant. 2008;42:113–120.


