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Non-relapse mortality and mycophenolic acid exposure in nonmyeloablative hematopoietic cell transplantation

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Abstract

We evaluated the pharmacodynamic relationships between mycophenolic acid (MPA), the active metabolite of mycophenolate mofetil (MMF), and outcomes in 308 patients after nonmyeloablative hematopoietic cell transplant. Patients were conditioned with total body irradiation ± fludarabine, received grafts from HLA-matched related (N=132) or unrelated (N=176) donors, and received post-grafting immunosuppression with MMF and a calcineurin inhibitor. Total and unbound MPA pharmacokinetics were determined to day 25; maximum *a posteriori* Bayesian estimators were used to estimate total MPA concentration at steady state (C_{ss}). Rejection occurred in nine patients, eight of whom had a total MPA C_{ss} less than 3 µg/mL. In patients receiving a related donor graft, MPA C_{ss} was not associated with clinical outcomes. In patients receiving an unrelated donor graft, low total MPA C_{ss} was associated with increased grades 3–4 acute graft versus host disease (aGVHD) and increased non-relapse mortality, but not with day 28 T-cell chimerism, disease relapse, cytomegalovirus reactivation, or overall survival. We conclude that higher initial oral MMF doses and subsequent targeting of total MPA C_{ss} to greater than 2.96 µg/mL could lower grades 3–4 aGVHD and non-relapse mortality in patients receiving an unrelated donor graft.

INTRODUCTION

The development of nonmyeloablative allogeneic hematopoietic cell transplantation (HCT) conditioning regimens expanded the availability of this potentially curative procedure to patients who cannot tolerate the toxicity of myeloablative conditioning due to age or comorbidity.^(1, 2) Postgrafting immunosuppression used with these regimens, which aims to facilitate allogeneic engraftment and control graft versus host disease (GVHD), often consists of a calcineurin inhibitor (CNI) and mycophenolate mofetil (MMF).

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During the initial clinical trials of nonmyeloablative HCT, oral MMF was administered every 12 hours (Q12hr), as in solid organ transplant patients. Shortly thereafter, it was recognized that HCT recipients exhibit a shorter half-life of MMF's primary metabolite, mycophenolic acid (MPA),^(3, 4) compared to solid organ transplant recipients.⁽⁵⁾ This finding was particularly important in nonmyeloablative HCT recipients, who rely on the balance between recipient and donor cells to ensure adequate immunosuppression of the recipient, optimal graft-versus-tumor (GVT) effect, and minimal GVHD.

Engraftment was adequately achieved in nonmyeloablative HCT recipients of a human leukocyte antigen (HLA)-matched related donor graft with Q12hr administration of oral MMF. Engraftment proved more challenging, however, for recipients of nonmyeloablative HCT with an HLA-matched unrelated donor graft;⁽³⁾ this led to increasing the daily MMF dose by shortening the oral MMF dosing interval from Q12hr to every 8 hours (Q8hr).⁽⁶⁾ Engraftment rates for patients with HLA-matched unrelated donors were further improved with the use of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell (G-PBMC) grafts instead of bone marrow grafts.⁽³⁾ In patients undergoing nonmyeloablative conditioning with an unrelated donor graft, we have demonstrated that less frequent (Q12hr) MMF dosing and low total MPA area under the plasma concentration-time curve (AUC) are related to a higher risk of graft rejection.^(6, 7) Low total MPA AUC is also related to low (<50%) donor chimerism, but no significant association was observed between total or unbound MPA AUC and acute GVHD (aGVHD) or relapse.⁽⁷⁾

Some HCT centers personalize MMF doses based on MPA pharmacokinetics using either trough concentration,^(8, 9) AUC,⁽¹⁰⁾ or Bayesian estimates of AUC.⁽¹¹⁾ Various investigators have reported a pharmacodynamic association between MPA and clinical outcomes in allogeneic HCT recipients (Supplemental Table 1).^(7-9, 12-14) Many of these studies are, however, from small patient populations (< 75 patients) of adults^(8, 12) or children.^(9, 13) Studies in a homogenous group of HCT recipients with a sufficient number of patients are essential to elucidate any MPA pharmacodynamic associations. In the present study, we retrospectively analyzed data from two cohorts of patients receiving nonmyeloablative HCT from a related or unrelated donor; patients received postgrafting immunosuppression that included either Q12hr (N=167) or Q8hr (N=141) MMF and a CN1. Each cohort was analyzed for possible associations between MPA concentration at steady state (C_{ss}), defined as the AUC divided by the dosing interval, and graft rejection, day 28 T-cell chimerism, grades 2-4 aGVHD, relapse, non-relapse mortality (NRM), cytomegalovirus (CMV) reactivation, and overall survival.

MATERIALS AND METHODS

Patient characteristics and treatment plan

We retrospectively evaluated MPA pharmacodynamics in two separate cohorts of patients who received nonmyeloablative HCT with G-PBMC grafts to treat a variety of hematological malignancies between March 1998 and July 2006 at the Fred Hutchinson Cancer Research Center, Seattle, WA. Given the greater risk of graft rejection among bone marrow recipients compared with G-PBMC recipients receiving Q12hr MMF,⁽³⁾ patients who received bone marrow as the source of stem cells were excluded. Written informed consent was obtained from all patients prior to participation in prospective treatment protocols, which included MPA pharmacokinetic sampling. Fifty-four percent of these ambulatory clinic patients participated in the MPA pharmacokinetic sampling. The Institutional Review Board at the Fred Hutchinson Cancer Research Center approved all study protocols, including this retrospective analysis, and an independent Data Safety Monitoring Board monitored safety in all prospective studies. Patient characteristics are summarized in Table 1.

The conditioning regimen comprised a single fraction of 200 to 300 cGy total body irradiation (TBI) on day 0 with or without fludarabine (30 mg/m²/day intravenously) from day -4 to day -2 (cumulative dose 90 mg/m²).⁽³⁾ In general, the post-grafting CNI was either cyclosporine or tacrolimus given through day +177. MMF was given at two different dose frequencies, either 15 mg/kg Q8hr or Q12hr. Adjusted ideal body weight⁽¹⁵⁾ was used to determine MMF dosing, and all doses were rounded to the nearest 250mg. MMF doses were not adjusted based on MPA plasma concentrations, and patients were asked to take MMF at the same time daily. MMF treatment started on day 0 and, in general, continued until day 27 (related donor) or day 40 (unrelated donor) at which time the MMF dose was reduced by 10% per week in the absence of GVHD. The majority of donor grafts were matched for HLA-A, -B, -C, DRB1 at high resolution DNA typing and DQB1 by intermediate-resolution techniques, with the exception of two related and 34 unrelated donor grafts. Of the 36 patients with mismatched donor grafts, the two related and 22 of the 34 unrelated donor grafts had a 2 allele or antigen mismatch. The median follow-up among patients at the time of last contact was 3.09 years (range, 0.31 – 12.06 years).

Pharmacokinetic analysis

All patients had blood samples scheduled before the morning dose of MMF, and then at 1, 2, 4, 6, and 8 hours following the dose; in patients receiving Q12hr MMF, blood samples were also collected 10 hours after the morning dose. Blood samples were collected in ethylenediaminetetraacetic acid tubes, and total MPA plasma concentrations were quantified by reverse-phase high performance liquid chromatography (HPLC) with UV detection (adapted from Tsina *et al.*).^(7, 16, 17) The dynamic range was 0.2 to 30 µg/mL and the interday coefficient of variation was less than 10%.⁽⁷⁾

Total MPA C_{ss} were evaluated on the following days: one C_{ss} on days 0 through 4, 246 C_{ss} on day 7 (includes days 5 through 9), 25 C_{ss} on days 10 through 18, 236 C_{ss} on day 21 (includes days 19 through 23), and 14 C_{ss} on days 24 through 25. Unbound MPA C_{ss} were collected on the following days: one C_{ss} on days 0 through 4, 178 C_{ss} on day 7 (includes days 5 through 9), 18 C_{ss} on days 10 through 18, 169 C_{ss} on day 21 (includes days 19 through 23), and 10 C_{ss} on days 22 through 25.

Estimation of total MPA AUCs was accomplished using maximum *a posteriori* probability (MAP) Bayesian estimation of pharmacokinetic parameters, incorporating a blend of individualized pharmacokinetic data and a population parameter prior. The MAP Bayesian method used individual patient 0–8 hour (Q8hr administration) or 0–12 hour (Q12hr administration) total MPA concentration-time data, together with a pharmacokinetic model and mean parameter values plus their variance (derived from a population pharmacokinetic analysis of 408 HCT recipients).⁽¹⁵⁾ This integrated model describes the pharmacokinetics of total MPA AUC. The population prior parameters were not changed during this study. The estimated total MPA C_{ss} was calculated by dividing the Bayesian AUC estimate by the dosing interval,⁽⁷⁾ specifically AUC/12 hour in the Q12hr MMF group and AUC/8 hour in the Q8hr MMF group. The C_{ss} term was used to compare the two groups of patients because of the varying administration schedules for oral MMF. The available MPA AUC data from day 0 to day 25 were used to calculate the average total MPA C_{ss} term for each patient.

The correlation between the total MPA trough concentration and MPA C_{ss} was evaluated as well. Because of the high interoccasion (i.e., within patient) variability of MPA absorption rate,⁽¹⁵⁾ the predose MPA sample was not used as the trough concentration. The MPA trough concentration was defined as a concentration-time point collected between 7.5 and 8.5hr post-dose in patients receiving Q8hr MMF and between 9.5 and 10.5hr post-dose in patients receiving Q12hr MMF.

After total MPA concentrations were quantitated, the remaining plasma over one AUC was pooled together to estimate the unbound fraction of MPA. Pooling samples ensured sufficient volume to be above the assay's limit of quantitation. Notably, the unbound fraction of MPA does not change over a total MPA concentration range of 1 to 60 $\mu\text{g/mL}$. (18) The unbound fraction of MPA was separated from the protein-bound MPA through equilibrium dialysis and quantitated as previously described.(7) Specifically, 200 μL of this pooled plasma was placed in a water-tight Teflon dialysis chamber separated by dialysis membranes (Spectrapor 4; 14k molecular weight cutoff; Spectrum Laboratories, Los Angeles, CA), then dialyzed for 2 hours against an equal volume of 10% phosphoric acid buffer (pH 7.2) in a water bath at 37°C. Following this, 120 μL dialysate was mixed with 500 μL acetonitrile; after vortexing and drying, samples were reconstituted in 20 mM phosphate buffer (pH 3.2). The total MPA and the fraction of MPA bound to plasma protein were quantitated, with the unbound drug percentage calculated as follows: unbound MPA = $100 \times (1 - \text{bound MPA})$. The unbound C_{ss} were calculated by multiplying the unbound fraction of MPA by total C_{ss}.(7)

The MPA parameters evaluated for pharmacodynamic relationships were total MPA C_{ss}, unbound MPA C_{ss}, and total MPA trough concentration.

Toxicity

Neutropenia post-HCT was assessed only through day 28, because multiple potential confounding variables (e.g., viral infection or reactivation, corticosteroid therapy) could affect the neutrophil count after day 28. Neutropenia was evaluated by examining daily complete blood counts with differential and assessment of absolute neutrophil count (ANC). CMV reactivation was also evaluated, as it represents a significant consequence of immunosuppressed status; CMV serological status was assessed in each patient and donor prior to HCT. All patients underwent weekly testing to detect the CMV pp65 antigen for the first three months following HCT.

Chimerism and graft rejection

On days 28, 56, and 84 after HCT, all patients' peripheral blood samples were assessed for the percentage of donor CD3⁺ T-cells present. Flow cytometry was used to sort CD3⁺ cells and chimerism was measured using fluorescence *in situ* hybridization and polymerase chain reaction of polymorphic microsatellite regions for sex-mismatched and sex-matched grafts, respectively.(19) If donor CD3⁺ cells were less than or equal to 5% at any of the assessed time points after HCT, then the patient was noted to have graft rejection.

Acute GVHD, chronic GVHD, and disease relapse

Acute GVHD and chronic GVHD (cGVHD) were graded according to established criteria. (20–22) Hematological diseases were classified as low, standard, or high risk of relapse per the Kahl criteria to evaluate relapse rate in a consistent manner.(23) We defined disease relapse or disease progression as disease recurrence following complete remission or progression of persistent disease.

Statistical analysis

Graphical representation of the pharmacodynamic data are shown by the quartile of the total MPA C_{ss}, with the lower quartile (range: 0.61 to 1.76 $\mu\text{g/mL}$), interquartile range (1.77 to 2.96 $\mu\text{g/mL}$) and upper quartile (range: 2.97 to 4.6 $\mu\text{g/mL}$). Cumulative incidence curves for aGVHD were estimated using methods previously described.(24) Cox regression analysis was used to model the effect of MPA C_{ss} on time-to-event endpoints. Death and relapse were treated as competing risks for analysis of aGVHD and cGVHD. Relapse was treated as

a competing risk for the analysis of nonrelapse mortality (NRM). The effects of MPA C_{ss} on hazard ratios (HRs) were expressed as the interquartile ranges for both related and unrelated donor grafts. Mean MPA C_{ss} were calculated up to day 25 and treated as fixed covariates. Cumulative mean MPA C_{ss} through day 25 was treated as a time-dependent covariate; that is, at each time the covariate represented the mean of all prior concentrations until the onset of GVHD or day 130, whichever occurred first. All reported p-values are two-sided, and those estimated from regression models are derived from the Wald test. No adjustments were made for multiple comparisons.

RESULTS

Pharmacokinetic results

We found considerable inter-individual variation of MPA plasma concentrations in this retrospective analysis of 308 patients from whom 522 MPA AUCs were available. The majority of this data (506 of 522 AUCs, 97%) were collected after oral MMF administration with the remainder (16 of 522, 3%) collected after IV MMF administration. The majority of patients had two MPA AUCs available (67% for total MPA and 43% for unbound MPA); this subgroup is described in detail in Table 2. Of the 522 AUCs, 376 (72%) had both total and unbound MPA AUCs available; the remainder (146, 28%) had only total MPA AUC. Graphical representation of the total MPA C_{ss} pharmacodynamic data are shown by the lower quartile (range: 0.61 to 1.76 µg/mL), interquartile range (1.77 to 2.96 µg/mL) and upper quartile (range: 2.97 to 4.6 µg/mL) (Figure 1 and Figure 2). Since the majority of patients had MPA C_{ss} data available on day 7 and day 21, the data from patients with an MPA C_{ss} on one of those days is described in Table 2. As expected, the total and unbound MPA C_{ss} increased with Q8hr administration compared to Q12hr administration. MMF dosed based on body weight led to considerable interpatient variability, expressed as mean ± standard deviation or fold-range (maximum/minimum). Specifically, with Q12hr administration, there was a 5 to 5.7-fold range for total MPA C_{ss} and 14.7 to 57.9-fold range for unbound MPA C_{ss}. Similarly, total MPA C_{ss} had a 6.3 to 11.4-fold range and unbound MPA C_{ss} had a 6 to 7.7-fold range with Q8hr administration. Recent data demonstrates that limited sampling schedules with Bayesian estimators can accurately estimate C_{ss}.(8, 15) The association of MPA pharmacokinetics with patient characteristics is reported in a separate population pharmacokinetic manuscript.(15) Notably, only the concomitant CNI, dosing weight, and albumin concentration – but not conditioning regimen - were associated with total MPA pharmacokinetics.(15) The effect of the concomitant CNI upon MPA C_{ss} is shown in Supplemental Figure 1, panels A & B.

The association of MPA C_{ss} with the last concentration time point obtained from pharmacokinetic sampling for C_{ss} determination (C_{trough}) was also evaluated. Prior studies have established poor association between these values (R² ranging from 3%(4) to 49%,(4, 7, 14)) which was supported by our own data (Supplemental Figure 1, panels C & D). The total MPA C_{trough} was not associated with clinical outcomes (Supplemental Table 3).

Neutropenia and CMV reactivation

Of the 308 patients, 232 (88 related, 144 unrelated) had an ANC nadir below 500/µL between days –7 and 28. Of those 232, 53 experienced their ANC nadir between days –7 and 7 and 179 between days 7 and 28. Among the 190 patients who were CMV seropositive before HCT, 130 (68%) experienced CMV reactivation. Specifically, 52 of 85 (61%) seropositive related and 78 of 105 (74%) unrelated experienced CMV reactivation. In CMV negative recipients with a CMV positive donor, CMV antigenemia was detected in four of twelve related and one of five unrelated donor graft recipients.

Donor chimerism and graft rejection

Nine of the 308 patients experienced rejection, all of whom received MMF with cyclosporine. A detailed description of the patients who rejected their grafts is reported in Table 3. One rejection occurred in a related donor graft recipient who was conditioned with TBI only; this patient received MMF Q12hr with total MPA C_{ss} of 4.31 μg/mL. The remaining eight patients who rejected their grafts received HLA-matched unrelated grafts after fludarabine/TBI conditioning. Of these eight patients, seven received an HLA-matched graft, six received Q12hr MMF and all had a total MPA C_{ss} < 3 μg/mL. Among the 36 patients who received an HLA-mismatched graft, there was one case of graft rejection. The average total MPA C_{ss} among the 36 patients receiving a mismatched graft was 2.49 μg/mL (range: 1.1–3.97).

Among 308 patients, the majority (234, 76%) had a total MPA C_{ss} less than 3 μg/mL. Eight of 234 patients (3.4%) with a low total C_{ss} (< 3 μg/mL) rejected their G-PBMC grafts, while one of the 74 (1.3%) patients whose C_{ss} was above 3 μg/mL had graft rejection (P=0.36), as described above. Among the 234 patients with total MPA C_{ss} less than 3 μg/mL, 147 (62.8%) were in the Q12hr MMF group and 87 (37.2%) were in the Q8hr MMF group. Of the 141 patients receiving Q8hr MMF, a minority (38%) had an average total MPA C_{ss} greater than 3 μg/mL.

Other studies in the setting of nonmyeloablative HCT have found that low donor T-cell chimerism levels are predictive of graft rejection.(3, 25, 26) Considering this, we evaluated whether total or unbound MPA C_{ss} was associated with the subsequent degree of day 28 donor T-cell chimerism. After adjusting for disease risk, HLA mismatch, mean week 2 CN1 concentration,(27) year of transplant,(27) and female donor to male recipient, there was no statistically significant association between mean total MPA C_{ss} and day 28 donor T-cell chimerism of 50% or lower for patients receiving related (odds ratio (OR) 0.83, 95% CI 0.38–1.82, P=0.63) or unrelated grafts (OR 1.39, 95% CI 0.82–2.37, P=0.23). Among the 32 patients with donor T-cell chimerism of 50% or lower, the average total MPA C_{ss} ranged from 1.1 to 5.3 μg/mL, and average unbound C_{ss} (available in 27 patients) ranged from 9.82 to 66.8 ng/mL. Graphical representation of this data is presented in Figure 3.

Graft-versus-host disease

The median onset of aGVHD was day 35, with 162 patients experiencing aGVHD on or after day 25. Of these, 142 (55 related, 87 unrelated) patients had grades 2–4 aGVHD and 33 (18 related, 15 unrelated) had grades 3–4 aGVHD. One hundred eleven patients with either no or grade 1 aGVHD had a mean day 7 total MPA C_{ss} of 2.64 μg/mL compared with a mean of 2.44 μg/mL among the 190 patients who developed grades 2–4 aGVHD beyond day 7. Of those patients with unbound MPA C_{ss} available, the mean unbound MPA C_{ss} for the 111 patients with either no or grade 1 aGVHD was 24.33 ng/mL, compared with 24.35 ng/mL for the 190 patients who developed grades 2–4 aGVHD. There was no statistically significant association between total MPA C_{ss} and subsequent grades 3–4 GVHD for patients receiving related grafts (HR 1.50, 95% CI 0.62–3.65, P=0.37), as shown in Figure 2a. There was, however, a statistically significant association for those receiving grafts from unrelated donors (HR 0.54, 95% CI 0.29–1.01, P=0.05; Figure 1a). Grades 2–4 aGVHD and cGVHD were not associated with total MPA C_{ss} (Table 4) or unbound MPA C_{ss} (Supplemental Table 2). As reflected in Figure 1a, patients in the upper quartile—specifically, those with a total MPA C_{ss} > 2.96 μg/mL—had the lowest risk of grades 3–4 aGVHD.

Relapse

One hundred thirty-four patients relapsed; 63 had related donors and 71 had unrelated donors. We found no association between mean total MPA C_{ss} and relapse hazard for patients with related (HR 1.07, 95% CI 0.74–1.55, P=0.73) or unrelated donors (HR 1.06, 95% CI 0.76–1.47, P=0.75). There was no association between unbound MPA C_{ss} and relapse for patients with related (HR 1.06, 95% CI 0.77–1.44, P=0.73) or unrelated donors (HR 1.14, 95% CI 0.87–1.50, P=0.34).

Non-relapse and overall mortality

Ninety patients died of NRM. Of these, 35 had a related donor and 55 had an unrelated donor. Among recipients of a related donor graft, we found no association between total MPA C_{ss} and risk of NRM (HR 0.68, 95% CI 0.35–1.34, P=0.27, Figure 2b). For recipients of an unrelated donor graft, there was a lower risk of NRM associated with total MPA C_{ss} (HR 0.58, 95% CI 0.39–0.86, P=0.007). Figure 1b shows that those patients with a total MPA C_{ss} > 2.96 µg/mL had the lowest non-relapse mortality.

We found that there was no statistically significant relationship between total MPA C_{ss} or unbound MPA C_{ss} and overall mortality. Patients receiving a related graft had a hazard of overall mortality of 1.01 (95% CI 0.72–1.43, P=0.96). Those receiving an unrelated graft had a slightly lower hazard of overall mortality; it was not, however, statistically significant (HR 0.78, 95% CI 0.61–1.01, P=0.06).

DISCUSSION

In this analysis, we evaluated MPA pharmacodynamics in 308 consecutive patients who were given nonmyeloablative conditioning before receiving allogeneic grafts to treat hematological malignancies. To our knowledge, this is the largest analysis of MPA pharmacodynamics in HCT patients to date (Supplemental Table 1). Among nonmyeloablative HCT recipients with a related donor graft, MPA C_{ss} was not associated with clinical outcomes (Table 4, Supplemental Table 2). Among patients given nonmyeloablative HCT with an unrelated donor graft, low MPA C_{ss} predicted the severity of aGVHD (Figure 1a), which is consistent with the findings of other investigators.(12, 14) Low total MPA C_{ss} also predicted high non-relapse mortality, potentially due to a higher risk of severe GVHD. Since total MPA C_{ss} was not associated with CMV reactivation or neutropenia (Table 4), oversuppression of the immune system is not apparent. There were, however, few patients who did not experience CMV reactivation, so it may not be possible to observe any adverse effects from elevated MPA C_{ss}.

The initial challenge of reliable engraftment for recipients of a nonmyeloablative HCT from an unrelated donor has been overcome by shortening the MMF dosing interval from Q12hr to Q8hr(6) and using G-PBMC grafts.(3) Low total MPA C_{ss} has previously been related to low (<50%) T-cell donor chimerism measured on days 28, 56, and 84.(7) The optimal day 28 donor T-cell chimerism is 50–90% in nonmyeloablative HCT recipients, with chimerism >40% associated with lower rejection risk.(28) Donor chimerism >50% is associated with higher complete remission rates through the GVT effect, which involves the immunoreactivity of donor cells against recipient cells. Donor chimerism <90% is associated with lower rates of grades 2–4 GVHD.(26, 28) In contrast to our previous study, (7) T-cell chimerism was not associated with total MPA C_{ss} (Figure 3). In this analysis, only day 28 T-cell chimerism was evaluated because all subsequent T-cell chimerism values occur after MMF has typically been discontinued. Thus, the day 28 T-cell chimerism is the only timepoint at which the MMF dose could be personalized to a target MPA C_{ss}. Additional studies regarding the association of MPA C_{ss} with chimerism are needed, as

optimizing day 28 donor T-cell chimerism could lower rates of graft rejection and GVHD while maximizing the GVT effect.(29)

Nevertheless, other endpoints regarding the effectiveness of MMF are associated with total MPA C_{ss} in unrelated donor grafts. Low total MPA C_{ss} is associated with a higher risk of grades 3–4 aGVHD (Figure 1a). We hypothesize that personalizing the postgrafting MMF dose to achieve a target total MPA C_{ss} could lower severe aGVHD and NRM in patients receiving nonmyeloablative conditioning and an unrelated donor graft. Notably, other strategies—such as shortening the MMF dosing interval(6) or prolonged MMF administration (to day 180) and shortened cyclosporine treatment—have not decreased the incidence of GVHD among recipients of unrelated G-PBMC grafts given nonmyeloablative conditioning.(30) To achieve higher MPA C_{ss}, postgrafting MMF doses should be adjusted for the covariates associated with MPA clearance identified in our recent population pharmacokinetic model.(15) In a study of 408 patients, we found that total MPA clearance, adjusted for body weight, is increased in patients receiving cyclosporine as the concomitant CNI and in patients with lower albumin concentrations.(15) Supplemental Figure 1 (A&B) clearly shows that total MPA C_{ss} is lower in those patients who received concomitant cyclosporine compared to those receiving tacrolimus. These data support the recent findings by DeWinter *et al.* that higher MPA clearance in HCT recipients is due to higher predose cyclosporine concentrations and lower albumin concentrations than in renal transplant recipients.(31) All patients who experienced rejection received concomitant cyclosporine (Table 3). Notably, MPA pharmacokinetic parameters are not associated with the graft source.(15) Thus, the apparently different pharmacodynamic associations of total MPA C_{ss} between related and unrelated donors (Table 4) is not due to pharmacokinetic differences.

In addition to characterizing the covariates associated with MPA pharmacokinetics, population pharmacokinetic models can be used in conjunction with limited sampling schedules (LSS) to estimate an individual's MPA C_{ss} without difficult, frequent, and invasive pharmacokinetic sampling. The creation of a population pharmacokinetic model and LSS can greatly facilitate the identification of pharmacodynamic relationships.(32) Our study team has observed an association between reduced total MPA C_{ss} and higher aGVHD and NRM following nonmyeloablative conditioning with an unrelated donor graft. This is consistent with the findings of Jacobson *et al.*, who reported correlations between low total MPA trough concentrations and higher rates of graft failure and between low unbound MPA AUC and more frequent aGVHD in patients receiving different conditioning and grafts than our population.(14) In multi-center studies with an adequately sized HCT patient population receiving a homogenous post-grafting immunosuppressive regimen, this LSS can be used to identify MPA pharmacodynamics associated with clinical outcomes.

Furthermore, a population pharmacokinetic model and LSS would facilitate personalized MMF dosing to a target MPA C_{ss}; these approaches have been used with busulfan(33) and cyclophosphamide(34) in HCT recipients. In the setting of renal transplant, MMF dose personalization using MPA pharmacokinetics suggested lower rejection rates and gastrointestinal toxicity.(35, 36) Two of the three studies used population pharmacokinetic-based LSS to personalize oral MMF doses, reflecting acceptance of these tools in the solid organ transplant community. The target exposures are achieved with higher oral MMF doses and the clinical benefit of higher initial doses of oral MMF in renal transplant patients is being investigated.(36) In nonmyeloablative HCT with an unrelated donor graft, similar prospective studies are needed in which initial oral MMF doses are increased based on covariates associated with total MPA pharmacokinetics. Shortening the administration interval to every 6 hours apparently increased toxicity but did not improve efficacy in a phase I/II study of MMF with cyclosporine as acute GVHD prophylaxis in high-dose conditioning recipients.(4) Therefore, increasing the initial dose while maintaining the

administration interval at 8 hours seems prudent. Notably, initial oral MMF doses would need to be ~33% higher with concomitant cyclosporine to achieve a similar total MPA C_{ss} to that achieved with oral MMF with concomitant tacrolimus, since cyclosporine coadministration increases total MPA clearance by 33.8%.⁽¹⁵⁾ Also, increased total MPA clearance is associated with decreasing albumin concentration; thus, those patients with low albumin would need higher initial oral MMF doses. Further studies, in which oral MMF doses are personalized using a population pharmacokinetic-based LSS, are also needed.

The only pharmacodynamic association between total MPA C_{ss} and outcomes was observed in patients receiving an unrelated donor graft. In patients receiving a related donor graft, future studies should seek to evaluate additional biomarkers, such as inosine monophosphate dehydrogenase (IMPDH) activity. MPA selectively and reversibly inhibits IMPDH activity, which has been associated with rejection in renal transplant patients.⁽³⁷⁾ The feasibility of evaluating IMPDH activity in HCT recipients has been recently established.⁽³⁸⁾ IMPDH activity may be an important biomarker, as it is influenced by MPA concentrations and an individual patient's sensitivity to IMPDH inhibition by MPA.

In conclusion, we found that low total MPA C_{ss} was associated with an increased risk of grades 3–4 aGVHD and NRM in recipients of unrelated donor grafts after nonmyeloablative conditioning. These findings were not observed in patients receiving a related donor graft. Studies are needed to identify alternative biomarkers—such as IMPDH activity—in these patients. Future prospective trials should address the clinical benefit of higher initial oral MMF doses – personalized based on covariates – and/or personalizing oral MMF doses to a target MPA C_{ss} above 2.96 µg/mL to lower aGVHD and NRM rates in unrelated donor patients receiving nonmyeloablative conditioning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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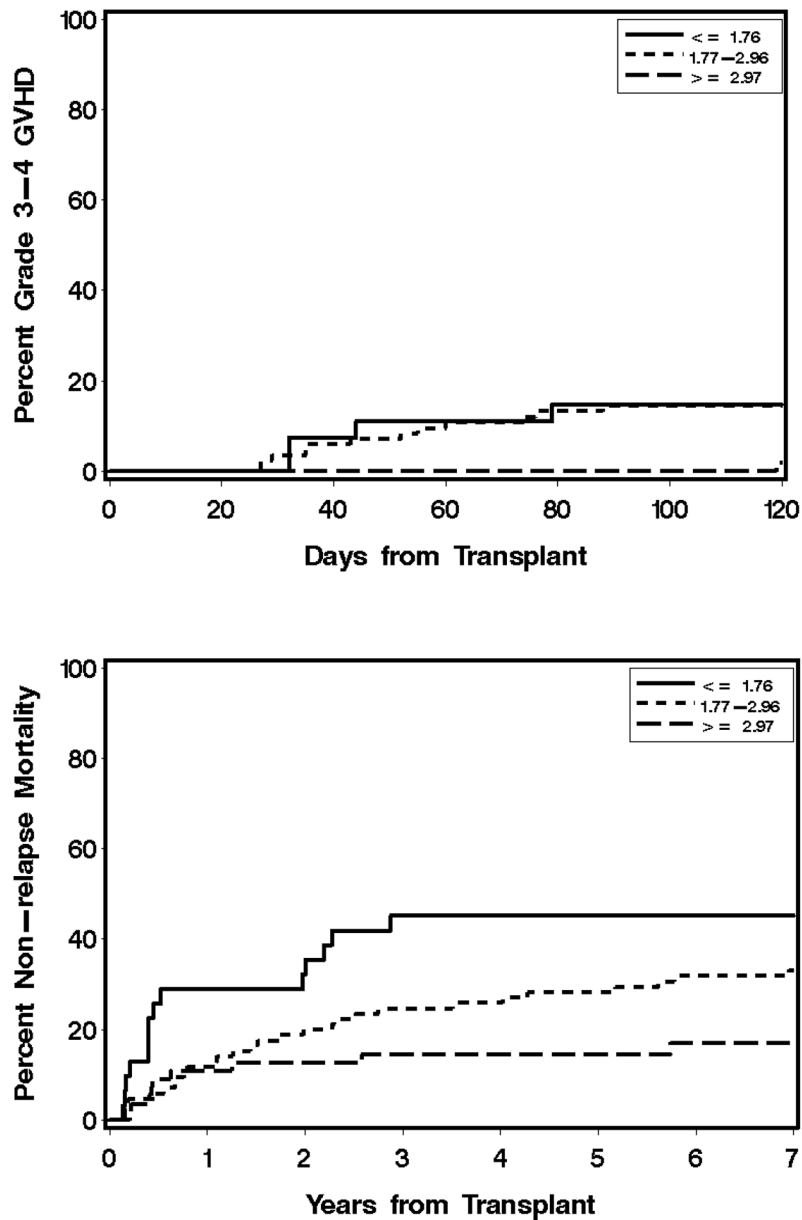


Figure 1. Association of total MPA C_{ss} with grades 3–4 acute GVHD (A) and non-relapse mortality (B) after day 25 in unrelated donor G-PBMC grafts
MPA C_{ss} is the average of all values from day 0 through day 25. Total MPA C_{ss} values are lower quartile (0.61 to 1.76 μg/mL), interquartile range (1.77 to 2.96 μg/mL), and upper quartile (2.97 to 4.6 μg/mL).

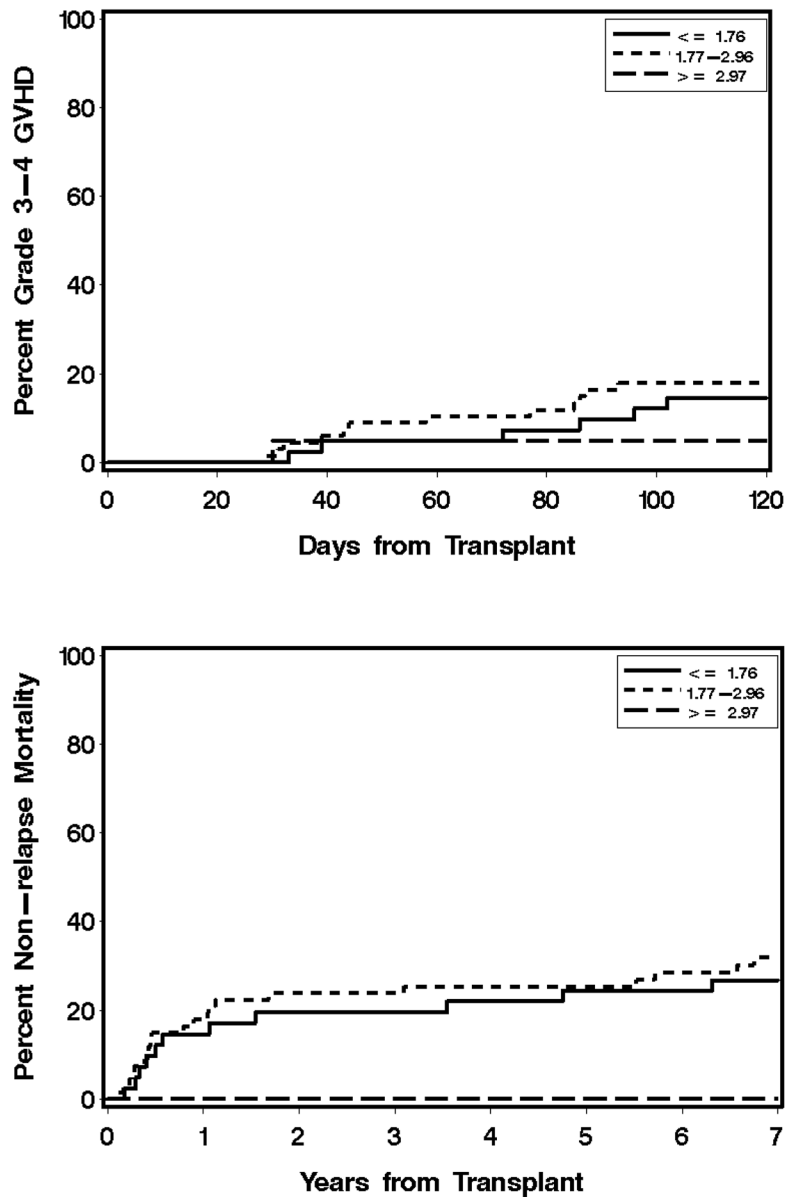


Figure 2. Lack of association of total MPA C_{ss} with grades 3-4 acute GVHD (A) and non-relapse mortality (B) after day 25 in related donor G-PBMC grafts

MPA C_{ss} is the average of all values from days 0 through 25. Total MPA C_{ss} values are lower quartile (0.61 to 1.76 μg/mL), interquartile range (1.77 to 2.96 μg/mL), and upper quartile (2.97 to 4.6 μg/mL).

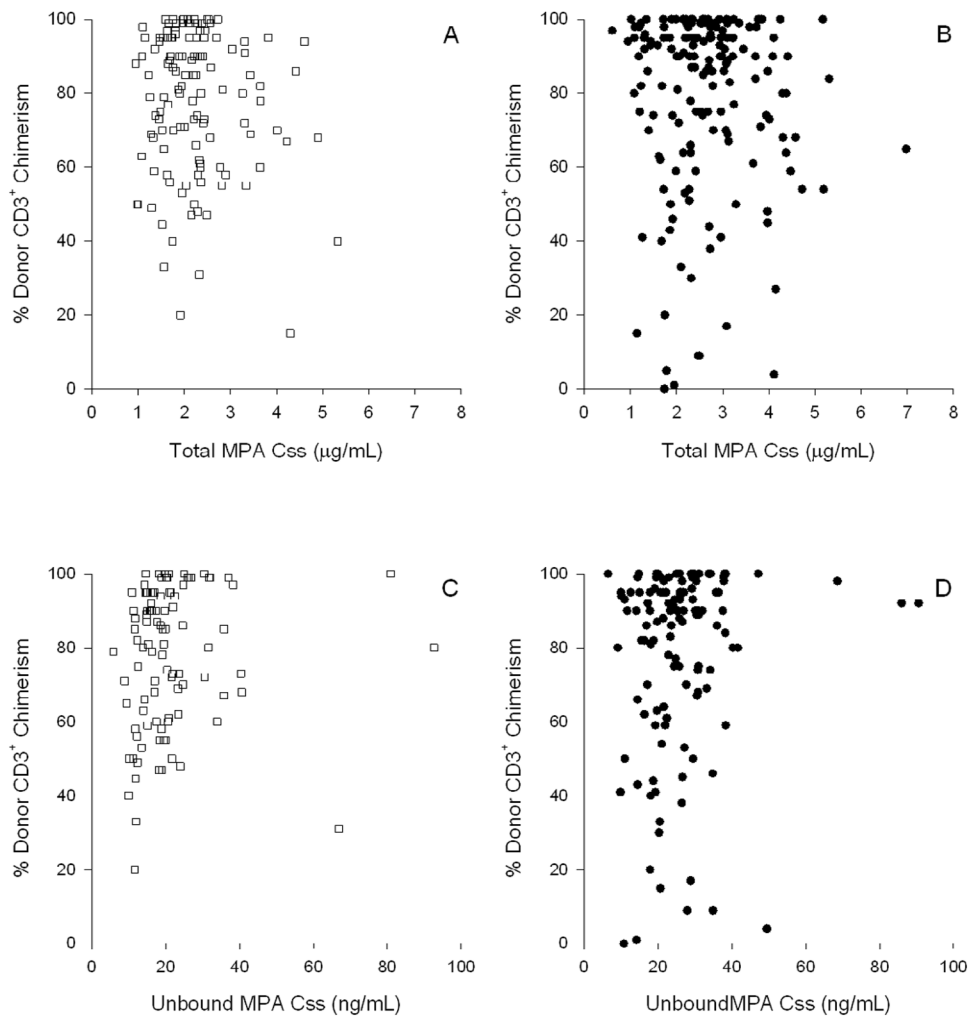


Figure 3. Lack of association between MPA C_{ss}, both total (A, B) and unbound (C, D), with day 28 donor T-cell chimerism in related (A, C) and unrelated (B, D) donor PSBC grafts
MPA C_{ss} is the average of all values from days 0 through 25.

Table 1Patients' Characteristics^a

	Related	Donor Type Unrelated	All patients
Total number	132	176	308
Sex, female/male (% female)	58/74 (44%)	59/117 (34%)	117/191 (38%)
HCT-CI ^b			
0	43 (33%)	44 (25%)	87 (28%)
1–2	35 (26%)	50 (28%)	85 (28%)
3–4	37 (28%)	57 (32%)	94 (30%)
5	15 (11%)	24 (14%)	39 (13%)
Not available	2 (2%)	1 (1%)	3 (1%)
Recipients' ages, yr	54.5 (25.3 – 72.6)	57.8 (9.2 – 74.5)	55.9 (9.2 – 74.5)
Recipients' age < 21 yr	0	5 (2.8%)	5 (1.6%)
CMV seropositive recipients	85 (64%)	105 (60%)	190 (62%)
Kahl Disease risk(23)			
Low	30 (23%)	44 (25%)	74 (24%)
Standard	66 (50%)	80 (45%)	146 (47%)
High	36 (27%)	52 (30%)	88 (29%)
Female donor to male recipient	42 (32%)	40 (23%)	82 (27%)
Donors' age, yr	53 (17 – 76)	33 (18–59)	42 (17–76)
HLA-matched graft	130 (98%)	142 (81%)	272 (88%)
HLA-mismatched graft	2 (2%)	34 (19%)	36 (12%)
Year of transplant			
1998	5 (4%)	0	5 (2%)
1999	21 (16%)	0	21 (7%)
2000	35 (26%)	21 (12%)	56 (18%)
2001	23 (17%)	19 (11%)	42 (14%)
2002	15 (11%)	28 (16%)	43 (14%)
2003	0	25 (14%)	25 (8%)
2004	9 (7%)	37 (21%)	46 (15%)
2005	14 (11%)	27 (15%)	41 (13%)
2006	10 (8%)	19 (11%)	29 (9%)
Conditioning regimen			
2 Gy TBI	30 (23%)	1 (1%)	31 (10%)
2 Gy TBI + auto	20 (15%)	0	20 (7%)
2 Gy TBI + FLU 90mg/m ²	72 (55%)	160 (91%)	232 (75%)
2 Gy TBI + FLU 90mg/m ² + auto	8 (6%)	13 (7%)	21 (7%)
3 Gy TBI + FLU 90mg/m ²	2 (1%)	2 (1%)	4 (1%)
Post-grafting immunosuppression*			
MMF Q8hr	0	141 (80%)	141 (46%)
MMF Q12hr	132 (100%)	35 (20%)	167 (54%)
Cyclosporine+MMF	104 (79%)	147 (84%)	251 (81%)

	Related	Donor Type Unrelated	All patients
Tacrolimus+MMF	28 (21%)	29 (16%)	57 (19%)

^aData shown as median (range) or as number (%); Abbreviations: autologous (auto); cytomegalovirus (CMV), fludarabine monophosphate (FLU), HCT comorbidity index (HCT-CI), human leukocyte antigen (HLA), mycophenolate mofetil (MMF), total body irradiation (TBI).

Table 2
Comparison of day 7 and day 21 plasma MPA pharmacokinetic data after MMF administration^a

	Day 7			Day 21				
	N	Mean ± SD (range)	Q12hr	N	Mean ± SD (range)	Q8hr		
Total MPA C _{ss} ^b , µg/mL	138 ^b	2.2 ± 0.84 (0.95 – 5.4)	108	2.9 ± 0.94 (1.1 – 6.9)	130	2.1 ± 0.82 (0.94 – 4.7)	106	3.0 ± 0.99 (0.61 – 7.0)
Unbound fraction, %	108	0.94 ± 0.45 (0.43 – 4.3)	70	1.1 ± 0.53 (0.61 – 4.3)	99	0.96 ± 0.42 (0.11 – 3.5)	70	1.1 ± 0.43 (0.59 – 3.0)
Unbound C _{ss} , ng/mL	108	19.6 ± 10.4 (6.43 – 94.3)	70	29.5 ± 12.8 (11.0 – 84.5)	99	20.2 ± 11.4 (1.60 – 92.7)	70	29.8 ± 12.7 (15.0 – 90.6)

^a Abbreviations: concentration at steady state (C_{ss}), mycophenolate mofetil (MMF), mycophenolic acid (MPA), every 8 hours (Q8hr), every 12 hours (Q12hr);

^b C_{ss} was calculated as AUC_{0-τ} divided by τ (with τ as the dosing interval). The C_{ss} term was used because of the varied administration schedules for oral MMF. The majority of participants had two MPA C_{ss} available and are presented in this table. Of the remaining patients (not in this table), 96 had one total MPA C_{ss}, two had three total MPA C_{ss}, 69 patients had no unbound MPA C_{ss}, 104 patients had one unbound C_{ss}, and two patients had three unbound C_{ss} (1%). Total MPA C_{ss} were evaluated on the following days: 1 C_{ss} on days 0 through +4, 246 C_{ss} on day +7 (includes days +5 through +9), 25 C_{ss} on days +10 through +18, 236 C_{ss} on day +21 (includes days +19 through +23), and 14 C_{ss} on days +24 through +25. Unbound MPA C_{ss} were collected on the following days: 1 C_{ss} on days 0 through +4, 178 C_{ss} on day +7 (includes days +5 through +9), 18 C_{ss} on days +10 through +18, 169 C_{ss} on day +21 (includes days +19 through +23), and 10 C_{ss} on days +22 through +25.

Table 3

Detailed description in patients who experienced graft rejection

No.	Donor	Conditioning	Age (yr)	Diagnosis	CD34 dose	CD3 dose	HLA	CD3 ⁺ on day +28	Week2 CSP ^b	MMF ^a		
										MMF frequency	total MPA Css	Unbound MPA Css
1	Sibling	TBI	53.8	MM	10.87	3.29	Identical	15	437	Q12hr	4.30	NA
2	Unrelated	FLU/TBI	60.1	CML	6.28	1.18	A, B, C, DRB1, DQB1	15	443	Q12hr	1.15	20.65
3	Unrelated	FLU/TBI	45.3	AML	0.76	0.31	A, B, C, DRB1, DQB1	9	502	Q8hr	2.47	34.88
4	Unrelated	FLU/TBI	40	MDS	1.48	1.47	A, B, C, DRB1, DQB1	0	309	Q12hr	1.74	10.71
5	Unrelated	FLU/TBI	46.6	NHL	7.77	3.62	A, B, C, DRB1, DQB1	59	576	Q12hr	2.42	19.24
6	Sibling	FLU/TBI	62.8	NHL and MDS	33.75	4.57	Identical	75	584	Q12hr	1.48	12.45
7	Unrelated	FLU/TBI	48.5	CLL	4.90	3.04	A, B, C, DRB1, DQB1	1	339	Q12hr	1.95	14.18
8	Unrelated	FLU/TBI	38.6	CML	6.44	1.61	A, B, C, DRB1, DQB1	40	350	Q12hr	1.68	17.99
9	Unrelated	FLU/TBI	60	NHL	4.08	1.25	A, B, DRB1, DQB1; C antigen mismatch	30	459	Q8hr	2.32	20.28

^a Abbreviations: acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), calcineurin inhibitor (CNI), cyclosporine (CSP), fludarabine monophosphate (FLU), human leukocyte antigen (HLA), myelodysplastic syndrome (MDS), multiple myeloma (MM), mycophenolate mofetil (MMF), mycophenolic acid (MPA), Non-Hodgkin's lymphoma (NHL), total body irradiation (TBI).

^b Rejections only occurred in patients receiving CSP.

Table 4

Effect of increasing mean total MPA C_{ss} as fixed covariate on clinical outcomes occurring after day 25^a

	Donor Type		
	Related (N=132)	Unrelated (N=176)	
	OR/HR (95% CI)	P-value	OR/HR (95% CI) P-value
Day 28 T cell chimerism < 50%	0.83 (0.38–1.82)	0.63	1.39 (0.82–2.37) 0.23
Acute GVHD 2–4	0.86 (0.56–1.32)	0.48	0.79 (0.59–1.07) 0.13
Acute GVHD 3–4	1.50 (0.62–3.65)	0.37	0.54 (0.29–1.01) 0.05
Chronic GVHD	0.68 (0.47–0.99)	0.04	0.97 (0.75–1.27) 0.84
Relapse	1.07 (0.74–1.55)	0.73	1.06 (0.76–1.47) 0.75
Neutrophil nadir	1.53 (0.90–2.61)	0.12	0.87 (0.62–1.24) 0.45
CMV reactivation	1.00 (0.63–1.59)	0.99	0.88 (0.64–1.19) 0.40
Non-relapse mortality	0.68 (0.35–1.34)	0.27	0.58 (0.39–0.86) 0.007
Overall mortality	1.01 (0.72–1.43)	0.96	0.78 (0.61–1.01) 0.06

^aMPA C_{ss} fit as continuous variable (truncated at 97.5th percentile to avoid outliers); HR per unit of MPA C_{ss} (μg/mL), adjusted for Kahl disease risk, antigen/2-allele mismatch, mean CNI concentration during week 2, year of transplant, female donor to male patient, tacrolimus prophylaxis, and fludarbine in conditioning (related donors only). Mean CNI week 2 fit as continuous variable, rescaled as standard deviation units to account for difference between cyclosporine and tacrolimus concentrations.