



A Limited Sampling Strategy for Therapeutic Drug Monitoring of Mycophenolate Mofetil for Prophylaxis of Acute Graft-Versus-Host Disease in Allogeneic Stem Cell Transplantation

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Abstract

A universally accepted strategy for therapeutic drug monitoring (TDM) of mycophenolate mofetil (MMF) in the prevention of acute graft-versus-host disease (aGVHD) in allogeneic hematopoietic stem cell transplantation (alloHSCT) does not exist. We explored the feasibility of developing a limited sampling strategy (LSS) for TDM of MMF in this setting. Patients undergoing alloHSCT received standard MMF-cyclosporine prophylaxis, with MMF administered twice daily (BD) for matched transplant recipients or thrice daily (TID) in haploidentical transplantation. Intensive blood sampling was carried out on day 7 and area under the concentration–time curve (AUC) of mycophenolic acid (MPA), the active metabolite, was estimated using non-compartmental analysis. The ability of MPA exposure defined by AUC_{0-12} to discriminate between responders (patients who did not develop GVHD) and nonresponders (patients who developed GVHD) was determined by receiver operating characteristic curve analysis. Patients were divided into training and validation sets within BD and TID groups. Mathematical equations were developed from the training set to predict AUC_{0-12} from an abbreviated AUC involving a limited number of sampling points. The equations were validated in the validation set by comparing the MPA AUC_{0-12} predicted from LSS with the observed AUC_{0-12} . It was observed that patients with $AUC_{0-12} \leq 18.99$ mg³h/L had a higher risk of developing aGVHD [odds ratio (OR) = 2.63 (1.17 to 5.87), $P = 0.06$]. The benefit was more in matched transplant recipients [OR = 3.5 (1.30 to 9.49), $P = 0.05$] as compared to haploidentical transplant [OR = 2.8 (0.49 to 15.91), $P = NS$]. Using the mathematical equations, the observed AUC_{0-12} was predicted with 92.31% accuracy in the BD subset and 100% accuracy in the TID subset for a combined accuracy of 94.76%. A set of just three samples that constituted the abbreviated AUC_{1-4} was used to develop the predictive models. The LSS could be employed for the therapeutic monitoring of MMF particularly in patients undergoing matched hematopoietic stem cell transplantation.

Keywords

therapeutic drug monitoring, graft-versus-host disease, hematopoietic stem cell transplantation, mycophenolate mofetil

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Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is performed with increasing frequency in an effort to cure patients with malignant hematological diseases but the therapeutic benefits are limited by the morbidity and mortality associated with graft-versus-host disease (GVHD)¹. Acute GVHD (aGVHD) is defined as any GVHD developing within 100 days of bone marrow transplantation. Current agents for the prevention and treatment of GVHD are partially effective and often result in significant toxicities. Mycophenolate mofetil (MMF), the prodrug of mycophenolic acid (MPA), is a widely used immunosuppressant to prevent graft rejection in solid organ transplantation. Because of its better tolerability, MMF has also been introduced as a replacement for methotrexate in GVHD prophylaxis².

Clinical experience in solid organ allograft recipients has shown that MMF has the ability to decrease the incidence and severity of acute rejection episodes^{3–6}. The safety profile of MMF in this setting is acceptable and does not overlap with the significant toxicities of commonly used immunosuppressant agents. Combination regimens that include MMF have shown that it permits a reduction in dose of cyclosporine, tacrolimus, or corticosteroid without increasing the chances of acute rejections^{7–9}. The systemic exposure of MPA is unpredictable and wide variations in pharmacokinetics have been reported^{2,10–13}. Thus, therapeutic drug monitoring (TDM) of MMF is routinely practiced in solid organ transplants to target a specified area under the concentration–time curve (AUC) based on exposure–effect relationship. TDM using limited sampling strategies (LSSs) has been shown to allow good prediction of the full area-under-the-curve and improve clinical outcomes¹⁴.

Similarly, individualized dosing using TDM approaches could be useful for optimizing MMF treatment in alloHSCT as well¹⁵. However, few such reports are available, and hence, no consensus on TDM strategy exists in this setting¹⁶. There are limited data showing effectiveness of MMF in alloHSCT, much less about the disposition of the drug and its exposure–response relationship in the context of GVHD prophylaxis¹⁷. Several studies in the past have not been successful in developing a strategy for TDM of MMF in alloHSCT^{18,19}.

A good TDM strategy should have few but informative set of sampling to make it effective, convenient, and economical. Obtaining multiple samples for TDM to calculate total exposure (AUC) will be expensive, cumbersome, and often not feasible. The present study was aimed at developing a TDM strategy for MMF in alloHSCT using a limited sampling approach.

Materials and Methods

Patients and Settings

A clinical pharmacokinetic (PK) study was performed to assess the feasibility of TDM of MMF in alloHSCT setting.

Forty eight patients aged ≥ 18 years undergoing alloHSCT for hematological malignancies at our center were enrolled into the study from July 2012 to June 2015. MMF was administered at twice daily (BD) or thrice daily (TID) frequency along with cyclosporine (CsA), which is standard institutional practice for the prophylaxis of GVHD. Patients receiving BD regimen were administered 600 mg/m² PO dose of MMF up to a maximum of 1,500 mg per dose and those receiving TID regimen were administered a dose of 15 mg/kg PO up to a maximum of 1,000 mg per dose. Conditioning regimens used were either full intensity such as cyclophosphamide with 12 to 14.4 Gy TBI or reduced intensity such as fludarabine with either melphalan or treosulfan or cyclophosphamide with or without 2 Gy TBI.

Those with matched related or unrelated donors received CsA-MMF prophylaxis from day -1 . All patients who underwent unrelated donor transplants and some patients with 8/10 or 9/10 matched related transplant also received 1 or 2 doses of 2.5 mg/kg of rabbit antithymoglobulin (rATG) with conditioning regimen. Alemtuzumab, wherever indicated for related donor transplantation, was administered at a dose of 10 mg for 3 days with the conditioning regimen. MMF was stopped on day $+30$ if there was no evidence of GVHD. In these patients, MMF was exclusively used at a dose of 600 mg/m² BD to a maximum of 1,500 mg per dose. These patients received CsA at a dose of 1.5 mg/kg intravenous BD from day -1 . CsA tapering was started from day $+60$ to day $+90$ and stopped by day $+150$ to day $+180$ if there was no evidence of GVHD.

Patients who underwent haploidentical transplant received CsA-MMF from day $+5$ till day $+35$. MMF was administered TID in these patients. All these patients also received post-transplant cyclophosphamide (PTCY) at a dose of 50 mg/kg daily on day $+3$ and day $+4$.

PK Sampling

Intensive blood sampling (3 ml each in EDTA tubes) was performed on day 7 of MMF treatment. Samples were collected predose (0 h) and 0.5, 1, 2, 4, 6, 8, and 12 h post dose in BD and 0, 0.5, 1, 2, 4, 6, and 8 h post dose in TID regimen, respectively.

Bioanalysis

Blood samples were centrifuged for 10 min at 3,000 rpm. Plasma was separated and stored at -20°C . Samples were analyzed within 6 months of collection for plasma MPA levels using a validated high-performance liquid chromatography assay.

Estimation of MPA Exposure

AUC was estimated using linear trapezoid rule. The ability of MPA exposure defined by AUC_{0–12} to discriminate between responders and nonresponders was determined by

receiver operating characteristic (ROC) analysis. In the BD regimen, AUC_{0-12} was estimated directly from PK samples collected over the 12-h period. In TID regimen, AUC_{0-12} was calculated from AUC_{0-8} using a multiplication factor of 1.5 as a simple approximation of the measure of exposure over 12 h.

Phase 1 (Feasibility Study, N = 20)

During this phase blood samples were collected at specified time points. Plasma concentration–time curve was plotted for MPA and AUC_{0-12} was estimated for all patients using noncompartmental analysis. A target AUC_{0-12} of 30 mg h/l was set based on monitoring threshold observed in solid organ transplant. A strategy for TDM was deemed necessary if more than 6 out of 20 patients (30%) failed to achieve the target AUC.

Phase 2 (Predictive Models to Calculate AUC_{0-12} from Abbreviated AUCs)

Additional 28 patients (for a total of 48 subjects) were enrolled for development and validation of a predictive model using limited sampling approach. The total pool consisted of 2 subsets of patients; 33 who were administered MMF BD and 15 who received the TID regimen. The least number of samples (LSS) from which AUC_{0-12} could be determined accurately was determined by Pearson's correlation.

- I) For patients who were dosed BD: Predictive AUC for first 20 patients using concentrations at only 3 time points of all possible combination that is $AUC_{0.5-2}$, AUC_{1-4} , and AUC_{2-6} was calculated and regressed against observed oral AUC_{0-12} and the equation with highest correlation coefficient (r^2) was used to estimate AUC_{0-12} for the remaining 13 patients. To validate the model, the MPA AUC derived from limited sampling model was compared with the MPA observed AUC_{0-12} . The accuracy of the model was determined by its ability to predict AUC falling into “inadequate exposure” and “adequate exposure” as determined by the ROC analysis.
- II) For patients who were dosed TID: Similarly, the first eight patients were employed for development of the predictive model using the same limited sampling time points as above. The predictive model was validated in the remaining patients against the AUC threshold determined in the ROC analysis.

Efficacy Assessment

A pooled analysis of training and validation sets was carried out to determine the efficacy of TDM strategy. Odds ratio with one-sided 90% confidence interval was calculated for the risk of developing aGVHD at exposures below the

threshold AUC_{0-12} determined from ROC analysis. P value less than 0.10 was considered statistically significant.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism, version 6 (GraphPad, San Diego, CA, USA). AUC was also estimated using GraphPad Prism, version 6 software.

Results

Forty-eight patients were enrolled in the study, of which 47 were evaluable for drug exposure. One of the patients who underwent haploidentical transplant was not included in the final analysis since the blood samples could not be analyzed for MPA levels due to hemolysis. Conditioning regimen consisted of fludarabine-based reduced intensity conditioning in 45 cases and myeloablative conditioning with cyclophosphamide plus total body irradiation in two cases. GVHD prophylaxis consisted of CsA and MMF (CSA-MMF) ($N = 25$), CSA-MMF-Alemtuzumab ($N = 2$), CSA-MMF-rATG ($N = 6$), and CSA-MMF-PTCY ($N = 14$). The flow chart of the study is shown in Figure 1. Patient characteristics are summarized in Table 1.

Feasibility Study

The average concentration–time profile of MPA for 20 patients enrolled in the feasibility study is shown in Figure 2. The interpatient coefficient of variation of MPA AUC_{0-12} was found to be 37.18%. The AUC recommended for solid organ transplant is >30 mg*h/L. Fifteen out of 20 patients did not achieve the recommended AUC. Thus, TDM may have a role in optimizing MMF dose in patients undergoing alloHSCT.

Relation Between Acute GVHD and MPA Exposure

The average MPA exposure was 23.15 ± 12.18 mg h/l. ROC analysis showed that an AUC of 18.99 mg*h/L could discriminate between responders (no GVHD) and nonresponders (GVHD) with highest sensitivity and specificity. Patients with $AUC_{0-12} \leq 18.99$ mg*h/L were at higher risk of developing aGVHD compared to those who achieved higher exposure [odds ratio = 2.63 (1.17 to 5.87), $P = 0.06$] (Figure 3). The odds ratio in patients who underwent matched donor transplantation ($N = 33$) was 3.5 (1.30 to 9.49), $P = 0.05$. On the other hand, the odds ratio for haploidentical transplant recipients at the same AUC cut-off was 2.8 (0.490 to 15.91), $P = \text{NS}$.

Development and Validation of LSS

I) *BD dosing.* The minimum number of sampling time points that can accurately estimate the total AUC_{0-12} was modeled

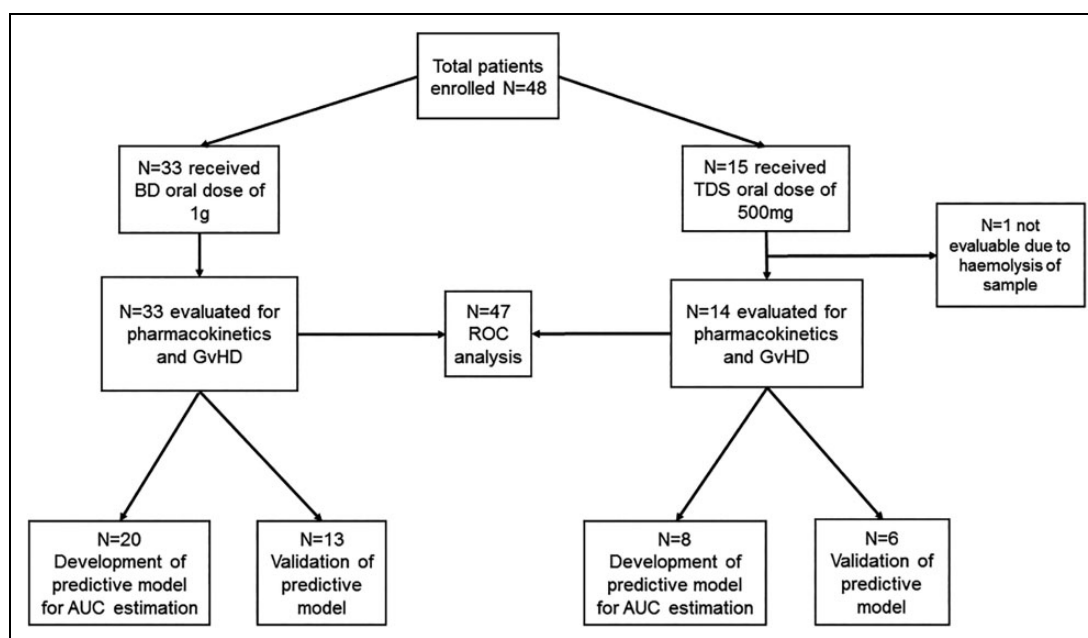


Fig. 1. Study flow chart.

AUC: area under the concentration–time curve; BD: twice daily; GvHD: graft-versus-host disease; TDS: thrice daily; ROC: receiver operating characteristic.

Table 1. Patient Demographics.

Characteristics	Median, range (N = 47—evaluable)
Sex	Male: 35, Female: 12
Age (years)	35, 24–42
Weight (kg)	64, 56–75
Height (cm)	165, 156–170
Diagnosis	AML: 22, ALL: 7, MDS: 5, HL: 3, CML: 6, AA: 1, PMF: 3
Type of transplant	MRT: 29, MUD: 4, HT: 14
ALT (U/l)	33, 29–43
AST (U/l)	17, 14–24
Total bilirubin (mg/dl)	0.75, 0.29–3.0
Serum creatinine (mg/dl)	0.73, 0.28–1.25
Actual dose of MMF (median, IQR)	BD (n = 33) 1,000 (1,000–1,000) TID (n = 14) 500 (500–500)

AA: aplastic anemia; ALL: acute lymphoblastic leukemia; ALT: alanine aminotransferase; AML: acute myeloid leukemia; AST: aspartate aminotransferase; BD: twice daily; CML: chronic myeloid leukemia; HL: Hodgkin lymphoma; HT: haploidentical transplant; IQR: interquartile range; MDS: myelodysplastic syndrome; MMF: mycophenolate mofetil; MRT: matched related transplant; MUD: matched unrelated transplant; PMF: primary myelofibrosis; TID: three times a day.

in 20 patients. Three time points, i.e., AUC_{1-4} had highest correlation with AUC_{0-12} ($r^2 = 0.65$) compared to other limited sample AUCs including $AUC_{0.5-2}$ ($r^2 = 0.38$) and AUC_{2-6} ($r^2 = 0.51$). AUC_{0-12} was predicted from AUC_{1-4} using the equation $AUC_{0-12} = (1.2039 * AUC_{1-4}) + 8.9727$ (Table 2). This model was further validated in 13 patients. Observed and predicted values of MPA were in agreement in

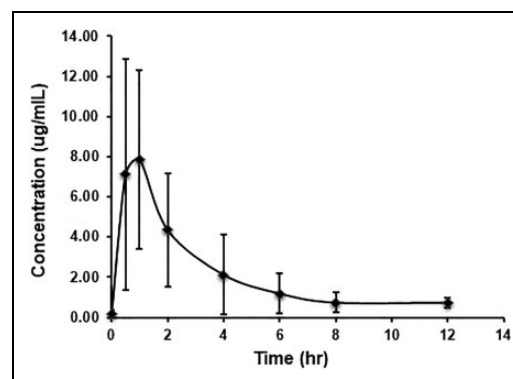


Fig. 2. Concentration–time profile of MPA. Data are shown as mean \pm SD.

MPA: mycophenolic acid.

12 out of 13 patients at the AUC cutoff of 18.99 mg*h/L giving the model a predictive accuracy of 92.31%.

ii) **TID dosing.** The minimum number of sampling time points that can accurately estimate the total AUC_{0-8} was modeled in eight patients. Once again, the same three time points, i.e., AUC_{1-4} had highest correlation with AUC_{0-8} ($r^2 = 0.69$) compared to other limited sample AUCs including $AUC_{0.5-2}$ ($r^2 = 0.24$) and AUC_{2-6} ($r^2 = 0.08$) (Table 2). AUC_{0-8} was predicted from AUC_{1-4} using the equation $AUC_{0-8} = (1.5164 * AUC_{1-4}) + 2.0295$. The model was further validated in six patients. Observed and predicted values of MPA were in

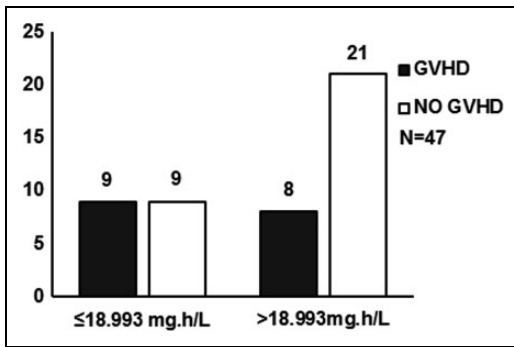


Fig. 3. Relation between GVHD and MPA exposure. MPA exposure is shown as a categorical variable at the AUC_{0-12} cut-off found to have the optimal sensitivity and specificity to discriminate between occurrence and non-occurrence of GVHD. The odds for the occurrence of GVHD at lower exposure was found to be 2.63 (1.17 to 5.87), $P = 0.06$.

AUC: area under the concentration–time curve; GVHD: graft-versus-host disease; MPA: mycophenolic acid.

Table 2. Correlation Between Various Abbreviated AUCs and Total MPA Exposure in Twice-Daily and Thrice-Daily Regimen.

Abbreviated AUCs	Coefficient of determination (r^2) between abbreviated AUCs and AUC_{0-12} (N = 20)	Coefficient of determination (r^2) between abbreviated AUCs and AUC_{0-8} (N = 8)
AUC_{1-4} (sampling time 1, 2, and 4 h)	0.65	0.69
$AUC_{0.5-2}$ (sampling time 0.5, 1, and 2 h)	0.38	0.24
AUC_{2-6} (sampling time 2, 4, and 6 h)	0.51	0.08

AUC: area under the concentration–time curve; MPA: mycophenolic acid.

agreement in all six patients at the AUC cutoff of 18.99 $mg \cdot h/L$ giving the model a predictive accuracy of 100%.

In all, 17 out of 47 patients (36.2%) developed aGVHD, 12 in the BD regimen, and 5 in the TID regimen. The incidence of aGVHD was 36.4% in the BD regimen and 35.7% in the TID regimen ($P = 0.96$). The average AUC_{0-12} was 22.89 ± 10.44 (mean \pm SD) in the BD regimen and 23.76 ± 15.99 (mean \pm SD) in the TID regimen ($P = 0.83$). The predictive ability of combined BD and TID models is shown in Figure 4. The model predicted accurately in 18 out of 19 cases giving it an accuracy of 94.74%.

Discussion

MMF has been successfully introduced into alloHSCT for prophylaxis against acute GVHD. Although not officially licensed^{20,21}, many transplant physicians use it for GVHD prophylaxis in the alloHSCT setting because of its

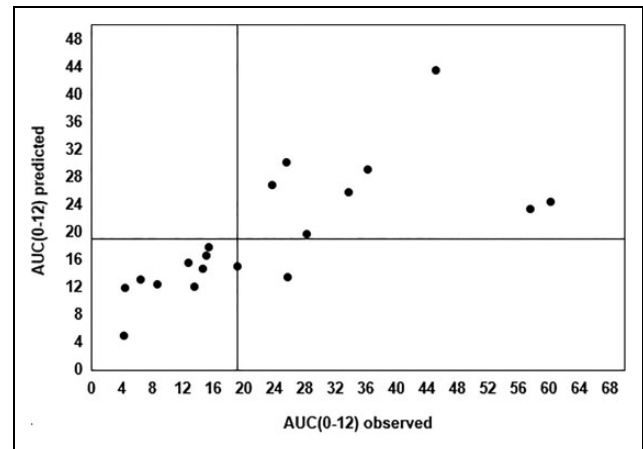


Fig. 4. Scatter plot showing accuracy of prediction of AUC_{0-12} from limited sampling timepoints for combined twice- and thrice-daily regimen of MMF (N = 19). The prediction was 94.74% accurate at the AUC_{0-12} cutoff of 18.99 $mg \cdot h/L$.

AUC: area under the concentration–time curve; MMF: mycophenolate mofetil.

synergistic action with CsA^{22,23}. However, its reportedly high interindividual variability in pharmacokinetics leads to toxicity or lack of therapeutic effect at standard doses^{24–26}. This makes MMF an ideal candidate for TDM-based dosing.

A high variability in MPA pharmacokinetics was observed in our patients as well. Our study shows that an AUC_{0-12} of ~ 19 $mg \cdot h/L$ is required for adequate GVHD prophylaxis, whereas the reported threshold in solid organ transplant is 30 $mg \cdot h/L$ ²⁷. In general, we observed lower exposures in our patients compared to solid transplant setting possibly due to concomitant administration with CSA. CSA reduces the enterohepatic recirculation of MPA resulting in lower total exposure of MPA^{28,29}. Even in recipients of kidney transplant, MPA exposure was shown to be significantly lower in patients who received concomitant CSA as compared to those who received tacrolimus. Calcineurin inhibitors influenced the urinary excretion of MPA in kidney transplant recipients by interfering with its renal tubular secretion³⁰. Previously, the relationship between AUC_{0-12} of unbound MPA of less than 0.3 $mg \cdot h/L$ was shown to be associated with significantly higher incidence of aGVHD following alloHSCT³¹. Considering MPA is approximately 98% protein bound, this translates to a total MPA AUC_{0-12} of approximately 15 $mg \cdot h/L$. The corresponding threshold in solid organ transplantation for the prevention of organ rejection is 30 $mg \cdot h/L$. A similar study in lung and heart transplant also reported lower MMF exposure when administered concomitantly with CSA as compared to tacrolimus³².

A few studies have demonstrated the utility of TDM of MMF in alloHSCT. Windreich and colleagues demonstrated the utility of LSS for the TDM of MMF in pediatric patients undergoing HSCT. In their study, they set a lower threshold

of 40 mg h/l as the target AUC_{0-24} considering the fact that MPA exposure in HSCT is 30% to 50% of that achieved for similar doses in kidney transplantation. Using this strategy they achieved excellent engraftment and low rates of acute GVHD³³. In a retrospective study of 36 patients who underwent unrelated alloHSCT, AUC_{0-24} correlated with GVHD incidence and overall survival²⁹. Jacobson P et al demonstrated the utility of monitoring exposure (AUC_{0-6} or AUC_{0-12}) of unbound MPA for effective prophylaxis of aGVHD in alloHSCT³¹. In spite of these efforts, there is no clear consensus on the preferred MPA PK monitoring parameters and target range in alloHSCT recipients³⁴. Our study has demonstrated that an MPA AUC of ~ 19 mg h/l is required for adequate aGVHD prophylaxis, and that the AUC can be estimated with reasonable accuracy from a LSS involving only three time points. Interestingly, patients who underwent matched donor transplantation had a higher odds ratio as compared to haploidentical transplant recipients who in addition to CsA-MMF also received PTCY. Thus, the failure of prophylaxis was higher in matched transplant recipients if the patients did not achieve the desired exposure, but it did not seem to matter so much in the PTCY cohort with only two out of seven patients developing aGVHD at less than optimal exposure. PTCY might offset the effects of low MPA exposure making MPA levels irrelevant. Therefore, haploidentical transplant recipients are unlikely to benefit from therapeutic monitoring of MMF. We could not make any conclusions in patients receiving alemtuzumab or rATG due to a small number of patients subjected to this regimen.

Limited sampling strategies are widely used in kidney transplantation. Most of the TDM strategies for MMF in renal transplantation have been described in combination with tacrolimus and very few with CsA. Most sampling strategies in kidney transplantation use 3 or 4 time points over a 12-h dosing interval after oral administration^{35,36}. In fact, sampling up to 3 to 6 h post dose has been shown to be reasonably accurate for the prediction of total AUC^{37,38}. Brooks et al and Willis et al examined the predictive performance previously published LSS studies in prospective cohorts of patients^{37,39}. A wide range of agreement was found when predicted AUCs were compared with full AUCs (range: $r^2 = 0.16$ to 0.91). In the study by Brooks et al, only 2 out of 19 models had correlation of determination greater than 0.75. However, these equations also used six to eight sampling time points over 8 h. Only two out of seven models having three sampling time points had r^2 greater than 0.65. In general, predictive models with more number of samples fared better. The r^2 in our study ranged from 0.65 (BD regimen) to 0.69 (TID regimen). However, the accuracy of our models to predict the total AUC with respect to threshold AUC was 92.31% and 100%, respectively. Thus, the correlation of determination should be judged in the context of sensitivity and specificity of the model. Also, fewer sampling time points make the TDM strategy financially viable and acceptable to physicians and patients alike.

Previous studies in alloHSCT failed to show good correlation between total observed AUC and AUC predicted from limited time points used in kidney transplant setting. Again, we believe this could be due to concomitant administration of CSA in alloHSCT, which interferes with the enterohepatic recirculation and renal tubular secretion of MPA. Thus, there is a need to develop and utilize limited sampling approaches specific to alloHSCT. The three-point sampling identified by us is unique in that sense and hence generalizable to patients undergoing alloHSCT.

TDM strategies have to justify the costs involved. The three-point sampling can be done for a total cost of less than 25 USD. While we did not carry out a detailed pharmacoeconomic analysis, it is certainly going to be cost effective considering the fact that more than one-third of our patients had exposure below the desired cutoff.

The study had a few limitations. We did not measure free MPA and MPAG (7-O-MPA-glucuronide) levels. Free MPA levels are known to correlate with the toxicity of MMF^{14,40-42}. However, in the setting of alloHSCT with multiple coadministered drugs, assessment of toxicity and attributing its causality to a given drug would have been difficult. Moreover, this study was designed to develop a strategy for TDM of MMF with respect to GVHD outcomes. Therefore, we did not attempt to measure free MPA levels. Also, measurement of free levels is more expensive and technically challenging and hence it is unlikely to translate into routine practice. MPAG, on the other hand, is pharmacologically inactive. However, estimating its levels may have shed light on the reasons for low MPA AUC observed in our study because CsA is known to inhibit MRP2-mediated biliary secretion of MPAG and corresponding enterohepatic recycling of MPA⁴³⁻⁴⁵. Secondly, the patient population was highly heterogeneous in terms of donor sources, conditioning regimens, and GVHD prophylaxis regimens including the frequency of administration of MMF. Establishing the relationship between MMF exposure and the risk of aGVHD is very difficult in a small yet heterogeneous sample. Also, we had patients who were treated with different MMF regimens, which necessitated a conversion factor of 1.5 to be used on AUC_{0-8} to obtain AUC_{0-12} . This conversion gives the average exposure of MPA over a 12-h duration. The rationale for the conversion factor is provided in supplemental file S1.

Undoubtedly, the sample size in the training and validation set was relatively small. In bone marrow transplantation, sample size is often limited by feasibility, especially in single-center studies. We did not have a formal sample size calculation, and the sample size was dictated by what was feasible in a reasonable time span. Even in kidney transplant, studies evaluating limited sampling strategies for TDM of MMF have typically had small sample size in the range of 20 to 40 patients^{36,37,46}. While TDM was found to benefit the matched transplant subset (MRT and MUD), the overall odds ratio for the pooled analysis was somewhat lower, albeit statistically significant, attributable largely to the

haploidentical subset who do not seem to derive benefit from TDM. Thus, future studies to validate these findings should focus only on matched transplant recipients.

To conclude, we have demonstrated that MPA exposure determines the outcome of GVHD prophylaxis, particularly in patients undergoing matched transplantation. The total exposure can be predicted from limited number of samples with acceptable accuracy. The findings are applicable to patients undergoing alloHSCT receiving CsA and MMF for aGVHD prophylaxis, but not for other combinations of MMF. The proposed strategy facilitates dosage adaptation in clinical practice as it provides a simple and effective method to monitor MPA even in resource-constrained settings.

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Ethical Approval

Ethical approval to report this study was obtained from Institutional Ethics Committee-III (IEC-III Approval number 65) of Tata Memorial Centre, Navi Mumbai, India.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Institutional Ethics Committee-III's (Approval number 65) approved protocols, Good Clinical Practice guidelines and the Declaration of Helsinki.

Statement of Informed Consent

Written informed consent was obtained from all the patients for their anonymized information to be published in this article.


Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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