Drug monitoring for mycophenolic acid in graft-versus-host disease prophylaxis in cord blood transplantation

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Running head: Drug monitoring for mycophenolic acid in CBT

Key words: Drug monitoring, mycophenolic acid, cord blood transplantation

Word count: 3073, Table count: 3, Figure count: 4, Supplemental figure count: 1

What is already known about this subject: Low level of mycophenolic acid after allogeneic hematopoietic stem cell transplantation is associated with high incidence of acute graft-versus-host disease.

What this study adds: Low level of mycophenolic acid after cord blood transplantation is associated with high incidence of acute graft-versus-host disease and human herpesvirus 6 reactivation, whereas high level of mycophenolic acid is associated with high incidence of sepsis before engraftment.

Abstract

Aims: We performed the retrospective analysis to clarify the significance of drug monitoring for mycophenolic acid (MPA), the active form of mycophenolate mofetil (MMF), in prophylaxis for graft-versus-host disease (GVHD) in cord blood transplantation (CBT).

Methods: We retrospectively analyzed the data of 46 patients who underwent first CBT and received GVHD prophylaxis with tacrolimus plus MMF. MPA levels were measured on days 7 and 21, and 24-hour areas under the curve ($AUC_{0\text{-}24}$) were estimated.

Results: The engraftment and 3-year overall survival rates of all patients were 94% and 78%, respectively. The cumulative incidence of sepsis before engraftment was higher in patients with $AUC_{0\text{-}24}$ on day 7 of > 60 μg h/mL than in other patients (33% vs. 6%, $p = 0.02$). The cumulative incidence of grade II–IV acute GVHD was higher in patients with $AUC_{0\text{-}24}$ on day 21 of ≤ 30 μg h/mL than in other patients (80% vs. 50%, $p = 0.04$). The cumulative incidence of human herpesvirus 6 reactivation was higher in patients with $AUC_{0\text{-}24}$ on day 21 of ≤ 48 μg h/mL (median) than in other patients (50% vs. 19%, $p = 0.03$).

Conclusions: Blood level of MPA was associated with the risks of acute GVHD and infection. A prospective trial evaluating the benefit of personalized MMF dosing using MPA levels is needed.

Introduction

Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-SCT). The combination of calcineurin inhibitors (CNIs) with short-course methotrexate (MTX) after allo-SCT has been standard GVHD prophylaxis for more than 20 years.[1, 2] Following the successful use of mycophenolate mofetil (MMF) in the United States and Europe, the use of MMF with CNI in cord blood transplantation (CBT) has recently increased in Japan to promote neutrophil recovery.[3, 4] Wide interpatient variability in blood levels of mycophenolic acid (MPA), the active form of MMF, has been reported even after administering the same dose of MMF.[5, 6] In kidney transplantation, therapeutic monitoring of MPA is reported to be important, with recommended target ranges for areas under the curve (AUCs) of 30–60 μg h/mL. AUCs <30 μg h/mL are associated with transplanted kidney rejection, whereas AUCs >60 μg h/mL are associated with infection and myelosuppression.[6, 7] Similarly, there are some reports on drug monitoring for MPA in bone marrow or peripheral blood stem cell transplantation.[5, 8-11] However, significance of drug monitoring for MPA in CBT was not fully elucidated.[12] In 2015, we reported that a low blood AUC of MPA on day 21 was associated with a high incidence of acute GVHD in the analysis of 24 patients who received CBT.[13] Based on this previous result, we started to increase the dose of MMF in patients with low

MPA AUCs on day 7. In 2018, the number of patients increased sufficiently to perform univariate and multivariate analyses, and the observation times became long enough to evaluate the 3-year overall survival (OS). This retrospective analysis was performed to clarify the association between MPA AUCs and CBT outcomes.

Methods

Data collection

We retrospectively analyzed the data of 46 patients who underwent their first CBT and received GVHD prophylaxis with tacrolimus plus MMF in our institute between October 2011 and July 2016. Tacrolimus was continuously and intravenously administered from day -1. Blood levels of tacrolimus were measured at least three times per week and were maintained at 12–15 ng/mL. After neutrophil engraftment, tacrolimus was orally administered, with trough levels maintained at 5–10 ng/mL. The patients received 10 mg/kg of oral MMF every 8 hours from days -1 to 30 as inpatient treatment. Blood samples were collected immediately before and 1, 2, and 4 hours after the morning administration of MMF on days 7 and 21. Total MPA levels in the plasma were measured using the enzyme multiplied immunoassay technique (EMIT)[14, 15] or the Roche Total Mycophenolic Acid® assay[16] (C_0, C_1, C_2, and C_4, respectively), and C_8 (implying the trough level of the next administration) was assumed to be equal to the C_0 values based on the results of previous

studies.[9, 11, 17, 18] The 8-hour area under the curve ($AUC_{0–8}$) was determined using the linear trapezoidal method, and the 24-hour area under the curve ($AUC_{0–24}$) was calculated as three times the $AUC_{0–8}$. This method is frequently used in the setting of MMF administration three times daily (every 8 hours)[9, 18] because MPA levels peak within 2 hours from administration and decrease linearly after 4 hours.[17, 19] After 2014, the dose of MMF for patients with MPA AUC_{0-24} of <30 μg h/mL on day 7 was increased. The dose of those patients from days 8 to 30 was calculated as follows: [the dose of MMF before day 7] × 40/[the AUC_{0-24} on day 7]. We analyzed the effect of MPA AUC_{0-24} on transplant outcomes. The cut-off values of AUC_{0-24} were set to 30 and 60 μg h/mL, which were the same as the values used for kidney transplantation.[6] We also set the cut-off value as the median. To evaluate human herpesvirus 6 (HHV-6) and cytomegalovirus (CMV) reactivation, peripheral blood was collected at least once weekly until 90 days after transplantation. The HHV-6 DNA copy number was measured using real-time polymerase chain reaction methods, as described previously.[20] The present study was approved by the institutional ethics committee of the Graduate School of Medicine, Kyoto University. All patients provided consent to participate in the present study.

Definition

OS was defined as the time from transplantation to death, and patients who remained alive

at the time of the last follow-up were censored. Complete haematological remission (CR) was defined based on morphology for all the patients, except for those with lymphoma; for lymphoma patients, it was based on positron emission tomography-computed tomographic (PET-CT) imaging.[21, 22] The relapse date of patients who did not achieve CR before and after stem cell transplantation (SCT) was defined as day 1. The patients were divided into two groups according to the conditioning regimen: myeloablative conditioning (MAC) or reduced-intensity conditioning (RIC). MAC and RIC were defined as proposed by Giralt et al.[23] and Bacigalupo et al,[24] respectively. Pre-engraftment immune reaction (PIR) was characterized by the presence of at least three of the following symptoms with no direct consequences of infection or adverse effects of medication six or more days before engraftment, as described previously: a high fever (>38.5°C), skin eruptions, body weight gain greater than 5% of baseline, or peripheral edema.[25, 26] HHV-6 reactivation was defined as plasma HHV-6 DNA level $\geq 10^4$ copies/mL. CMV reactivation was defined as the detection of at least two CMV pp65 antigen-positive cells per 50,000 leucocytes.

Data analysis

Descriptive statistics were used to summarize the variables related to patient demographics and transplantation characteristics. The probability of the OS time was estimated according to the Kaplan–Meier method.[27] To evaluate the influences of confounding factors on OS,

log-rank tests were used for the univariate analysis.[28] The competing risk regression model was used for the multivariate analysis of acute GVHD.[29] The cumulative incidences of engraftment, relapse, and GVHD were calculated, and death without events were considered a competing risk. Landmark analysis was performed to evaluate the effect of MPA level on days 7 and 21, with the landmark day set to days 7 and 21. Mann–Whitney *U* tests were used as non-parametric tests for two groups. Correlation of AUC and single point concentration was assessed by Spearman's correlation coefficient test.[30] The results are expressed as hazard ratios (HRs) and their 95% confidence intervals (CIs). All tests were two sided, and *P*-values <0.05 were considered to indicate statistical significance. All statistical analyses were performed using Stata (version 13.0, Stata Corporation) and EZR (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.3.0).[31]

Results

Patient characteristics

The median observation time of the survivors was 1,352 (range: 103–2,487) days. The median age was 50 (range: 20–66) years. The percentages of patients diagnosed with acute myeloid leukemia, acute lymphoid leukemia, myelodysplastic syndrome, and malignant lymphoma were 43%, 13%, 15%, and 17%, respectively. The percentage of patients with CR was 52%. Fifty-two percent of patients received MAC and 93% received total body irradiation (TBI) (Table 1).

Outcomes of all patients

The cumulative incidence of neutrophil engraftment was 94%. A median of 25 (range: 11–43) days was required for neutrophil engraftment (Figure 1A). Engraftment failure was seen in 3 patients, and they had risks for engraftment failure; one patient had high tumor burden, another patient received weak conditioning (fludarabine 125 mg/m^2 plus melphalan 80 mg/m^2 without TBI) and the other patient transplanted the minimum count of CD34$^+$ cells (0.18×10^5/kg) in this cohort. The cumulative incidences of PIR, grade II–IV acute GVHD, grade III–IV acute GVHD, limited chronic GVHD, and extensive chronic GVHD were 30%, 63%, 11%, 15%, and 13%, respectively. The 3-year OS, cumulative incidence of relapse, and non-relapse mortality rates were 78% (95% CI: 62–87%), 20% (95% CI: 10–33%), and 11%

(95% CI: 4–22%), respectively. After excluding two patients with aplastic anemia, we divided patients with respect to disease status at SCT. The 3-year OS, cumulative incidence of relapse, and non-relapse mortality rates of patients in CR (n = 24) were 82%, 22%, and 13%, (95% CI: 59–93%, 8–41%, and 3–30%), respectively. The 3-year OS, cumulative incidence of relapse, and non-relapse mortality rates of patients in non-CR (n = 20) were 70% (95% CI: 45–85%), 20% (95% CI: 6–40%), and 10% (95% CI: 2–28%), respectively (Figure 1B, C, D). Next, we divided the patients with respect to the number of human leucocyte antigen (HLA) mismatches for graft-versus-host direction. The cumulative incidence of grade II–IV acute GVHD in patients who underwent CBT with more than two HLA antigen mismatches in the HLA-A, B, C, and DRB1 loci (n = 15) was 80% (95% CI: 46–94%), which was significantly higher than that of the other patients (48%, 95% CI: 30–65%, n = 31) (p = 0.02) (Figure 1E). The cumulative incidence of grade II–IV acute GVHD in patients who underwent CBT with more than two HLA allele mismatches in the HLA-A, B, C, and DRB1 loci (n = 24) was 75% (95% CI: 51–88%), which was significantly higher than that of the other patients (41%, 95% CI: 20–61%, n = 22) (p = 0.02) (Figure 1F).

Impact of MPA AUCs on outcomes

None of the cases discontinued MMF administration, except for one case of death before day 30. No patients experienced MMF-related severe toxicities such as diarrhea and myelosuppression. The $AUC_{0\text{-}24}$ of MPA on day 7 (MPA7) was 49.4 ± 24.7 μg h/mL (mean ±

183 standard deviation). There was no association between MPA7 and the incidence of PIR,
184 engraftment rate, engraftment speed or acute GVHD after day 7 (data not shown). In the
185 landmark analysis on day 7, the rate of sepsis between day 7 and engraftment in patients with
186 MPA7 >60 μg h/mL was 33% (95% CI: 9–60%), which was higher than that in the other
187 patients (6%, 95% CI: 1–18%) (p = 0.02) (Figure 2A). The $AUC_{0\text{-}24}$ of MPA on day 21
188 (MPA21) was 47.7 ± 20.3 μg h/mL. After excluding three cases of graft failure, we divided
189 patients with respect to MPA21. In the landmark analysis on day 21, the cumulative incidence
190 of grade II–IV acute GVHD after day 21 in patients with MPA21 ≤30 μg h/mL was 80%
191 (95% CI: 34–96%), which was higher than that in the other patients (50%, 95% CI: 31–67%)
192 (p = 0.04) (Figure 2B). In the landmark analysis on day 21, the cumulative incidence of
193 HHV-6 reactivation after day 21 in patients with MPA21 ≤48 μg h/mL (median) was 50%
194 (95% CI: 28–68%), which was higher than that in the other patients (19%, 95% CI: 6–38%)
195 (p = 0.03) (Figure 2C). Only two patients developed HHV-6 encephalitis, and there was no
196 significant association between the development of HHV-6 encephalitis and the blood level
197 of MPA (data not shown). In the analysis of OS, the cumulative incidence of relapse, chronic
198 GVHD, and reactivation of cytomegalovirus, no significant association was found with the
199 blood level of MPA (data not shown).
200 Next, we surveyed the causes of low MPA levels. The MPA21 of patients with diarrhea on
201 day 21 was significantly lower than that of patients without diarrhea (35.8 vs. 54.5 μg h/mL,

p = 0.006) (Figure 3A). Patients with serum albumin levels <3 g/dL on day 21 tended to have low MPA21 (37.8 vs. 53.8 μg h/mL, p = 0.10) (Figure 3B).

We assessed the efficacy of the dose escalation of MMF. After 2014, the MPA7 of eight patients was <30 μg h/mL and the MMF dose was escalated after day 7. In these patients, the MPA21 of six patients was higher than their MPA7; the MPA 21 of one patient who had moderate diarrhea was less than MPA7 and one patient died before day 21 (Figure 4A). The cumulative incidence of grade II–IV acute GVHD of these eight patients was 43%, which was lower than the incidence of all patients. The MPA7 of 13 patients was 30–60 μg h/mL and the dose of MMF was not escalated after day 7. Among these patients, nine and four patients had MPA21 less and more than their MPA7s, respectively (Figure 4B). The cumulative incidence of grade II–IV acute GVHD of these 13 patients was 78%, which was higher than the incidence of all patients.

Correlation between $AUC_{0\text{-}24}$ and single point concentration

To analyze whether we could reduce the number of blood collections, we investigated the correlation between $AUC_{0–24}$ and single point concentration of MPA. We found that there was stronger correlation between $AUC_{0\text{-}24}$ and C_2 (Correlation coefficient = 0.737, $p = 8.69 \times 10^{-17}$), compared with C_0, C_1 and C_4 (Supplemental Figure 1A-D). However, there was no significant association between C_2 on day 7 and the incidence of sepsis before engraftment,

and between C_2 on day 21 and the incidence of grade II-IV acute GVHD or HHV-6 reactivation (data not shown).

Univariate and multivariate analyses for grade II–IV acute GVHD

After excluding the patients with graft failure and the patients who developed grade II-IV acute GVHD before day 21, we performed univariate and multivariate analyses for grade II–IV acute GVHD after day 21. In the univariate analysis, HLA allele mismatch, non-CR at SCT, and MPA level were associated with a high incidence of grade II–IV acute GVHD (Table 2). Multivariate analysis revealed that non-CR at SCT was associated with a high incidence of grade II–IV acute GVHD (Table 3).

Discussion

In the present study, GVHD prophylaxis with tacrolimus and MMF enabled a high incidence of neutrophil engraftment and prolonged OS compared to those reported in a nationwide survey in Japan.[4, 32] A previous report indicated that 3,000 mg/day of MMF was associated with delayed neutrophil engraftment in CBT,[33] but there was no evidence of an association between the dose of MMF and the rate of neutrophil engraftment. TBI is reportedly necessary for enhanced engraftment in CBT.[34] We administered MMF at 30 mg/kg/day, and 93% of patients received 3–12 Gy of TBI, which may have improved the incidence of neutrophil engraftment. Randomized studies are required to determine the optimal dose of MMF and the best conditioning regimen.

The cumulative incidence of grade II–IV acute GVHD in the present study was higher than that in previous reports.[4, 32] The main reason for this result might be the high percentage of patients who underwent CBT with more than two antigen or allele mismatches in the HLA-A, B, C, and DRB1 loci. In Japan, HLA mismatches are generally evaluated with HLA-A, B, and DRB1 antigens, and fewer than three mismatches were permitted;[35] our institute evaluated HLA mismatches in the same way. Our results suggest that HLA mismatch in CBT should be evaluated with the HLA-A, B, C, and DRB1 alleles to avoid acute GVHD.

In the present study, we observed that the blood levels of MPA fluctuated widely among patients after CBT. The kinetics of MMF are influenced by gastrointestinal damage caused by

conditioning, loss of appetite, decreased oral intake, and other drug interactions.[36] Change from oral to intravenous MMF may be one strategy to stabilize blood level;[37] however, intravenous MMF is not available in Japan.

The appropriate level of MPA in CBT is still not confirmed. We suggest that the $AUC_{0\text{-}24}$ on day 7 should be <60 μg h/mL to avoid sepsis before engraftment and that the $AUC_{0–24}$ on day 21 should be >30 μg h/mL to prevent acute GVHD. This range is similar to the adequate $AUC_{0\text{-}24}$ used in kidney transplantation.[6, 7]

CBT is one risk factor of HHV-6 encephalitis,[38] and epidemiologic evidence indicates the association between HHV-6 reactivation and acute GVHD.[39] In the present study, the cumulative incidence of HHV-6 reactivation was higher in patients with $AUC_{0\text{-}24}$ on day 21 of ≤48 μg h/mL (median) than in the other patients. Therefore, maintaining a high level of MPA and suppressing graft-versus-host reaction may prevent HHV-6 reactivation.

Based on these results, we hypothesize that MPA level should be changed before and after engraftment. In kidney transplantation, high MPA level is associated with myelosuppression and infection.[7] Similarly, MPA level in CBT should not be high to prevent bacterial infection before engraftment. After engraftment, alloreactive T cells become activated and excessive activation of alloreactive T cells leads to acute GVHD and delays immune reconstitution. Acute GVHD and delay of immune recovery cause HHV-6 reactivation.[39, 40] Moreover, treatment for GVHD also favors HHV-6 reactivation, as described with other

herpesviruses.[41] Therefore, MPA level should be high to suppress alloreactive T cells and prevent acute GVHD and HHV-6 reactivation. These are hypotheses and should be further explored.

After 2014, we escalated the dose of MMF of patients with low blood levels of MPA on day 7 and succeeded in reducing the incidence of grade II–IV acute GVHD in those patients. However, the levels on day 21 in some patients without dose escalation fell below the levels measured on day 7 and many of them developed grade II–IV acute GVHD. More frequent monitoring of MPA and dose adjustment of MMF is necessary, especially for patients with diarrhea or poor nutrition, whose blood levels of MPA tend to be low.

We focused on AUC and collected blood from patients 4 times per day. To proceed to multicenter study, we analyzed whether we could make the measurement simpler. As a result, there was no significant association between single point concentration and the incidence of acute GVHD or infection. Thus, AUC is a better marker to predict the risk of acute GVHD and infection compared with single point concentration, and 4 times blood collection per day is recommended.

Multivariate analysis revealed that non-CR at SCT was associated with the development of grade II–IV acute GVHD, while a low MPA $AUC_{0\text{-}24}$ on day 21 was not. In some patients with non-CR at SCT, the physicians decided to maintain low levels of MPA, expecting a graft-versus-tumor effect of cord blood. This was the limitation of this small-scale

retrospective study.

The present study also had additional limitations. We measured whole-blood levels of MPA. Levels of unbound form of MPA should be measured to evaluate the direct effect of MMF on lymphocytes.[42] Moreover, we did not survey the single-nucleotide polymorphism (SNP) for the genes coding inosine-5-monophosphate dehydrogenase (IMPDH). IMPDH is an enzyme inhibited by MPA; SNPs in those genes influence the activity of IMPDH and suppress lymphocyte proliferation.[43-45] In addition, our results should be interpreted with caution because of the small sample size and the heterogeneous patient and transplant backgrounds.

In conclusion, blood AUCs of MPA affected the incidence of grade II–IV acute GVHD, sepsis before engraftment, and HHV-6 reactivation in CBT. A multicenter prospective study is required to clarify the appropriate range of MPA AUCs.

Acknowledgment

The authors thank Emi Furusaka, Tomoko Okuda and Megumi Oka for their expert data management and the hematopoietic stem cell transplantation team members of Kyoto University Hospital for their dedicated care of the patients.

Conflicts of interest statement

The authors declare no conflicts of interest associated with this manuscript.

Funding information

There is no funding associated with this study.

Data availability statement

The data are not publicly available due to privacy or ethical restrictions.

Author contributions

HM, JK, YA and TK designed the research, organized the project, and drafted the manuscript. HM and JK gathered the data and performed the statistical analysis. TS, MH, TY, KY, KM, and ATK contributed to the data analysis and writing of the manuscript. All authors approved the final version of the manuscript for submission for publication.

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Table 1. Patient characteristics

		Number (%) or Median (Range)
Age at SCT (years)		50 (20–66)
Sex	M/F	29 (63) / 17 (37)
Disease	AML/MDS	20 (43) / 17 (37)
	ALL	6 (13)
	NHL	8 (17)
	CML/MPN	1 (2) /1 (2)
	ATL	1 (2)
	AA	2 (4)
PS at SCT	0/1	23 (50) / 14 (30)
	2–3	9 (20)
Serum Albumin at SCT (g/dL)		3.2 (1.9-4.2)
HLA Mismatch	0/1/2	1 (2) / 11 (24) / 10 (22)
(A, B, C, and DRB1 alleles)	3/4/5	11 (24) / 11 (24) / 2 (4)
Disease Status	CR/PR	24 (52) / 2 (4)
at SCT	Others	20 (43)
Days from Diagnosis	< 90	6 (13)
to SCT	90–180	14 (30)
	>180	26 (57)
Conditioning	MAC	24 (52)
	RIC	22 (48)
TBI	0	3 (7)
	3–4	21 (46)
	10–12	22 (48)
Total Nuclear Cell Count ($\times 10^7$/kg)		2.47 (1.94–4.27)
$CD34^+$ Cell Count ($\times 10^5$/kg)		0.57 (0.18–1.91)

Abbreviations: SCT: stem cell transplantation, M: male, F: female, AML: acute myeloid leukemia, MDS: myelodysplastic syndrome, ALL: acute lymphoblastic leukemia, NHL:

484 non-Hodgkin lymphoma, CML: chronic myeloid leukemia, MPN: myeloproliferative
485 neoplasm, ATL: adult T cell leukemia/lymphoma, AA: aplastic anemia, PS: performance
486 status, HLA: human leucocyte antigen, CR: complete remission, PR: partial remission, MAC:
487 myeloablative conditioning, RIC: reduced-intensity conditioning, TBI: total body irradiation.
488

Table 2. Univariate analysis for grade II–IV acute GVHD

Variables	Number	HR	95% CI	*P*
Age at SCT (years)				
<50	20	1		
≥50	20	1.55	0.72–3.35	0.27
PS at SCT				
0–1	34	1		
2–4	6	0.56	0.16–1.90	0.35
HCT-CI at SCT				
0–1	31	1		
>2	9	0.6	0.22–1.63	0.31
Sex mismatch				
Match, M to F	34	1		
F to M	6	0.99	0.61–1.62	0.98
HLA allele mismatch				
0–2	20	1		
3–5	20	2.26	1.05–4.86	**0.04**
Disease status at SCT				
CR	22	1		
Non-CR	18	1.29	1.16–1.45	**<0.001**
Days from diagnosis to SCT				
0–180	16	1		
>180	24	1.01	0.58–1.75	0.98
Conditioning				
MAC	22	1		
RIC	18	0.92	0.41–2.07	0.85
TBI				
No	2	1		
Yes	38	2.41	0.31–18.55	0.40
$AUC_{0\text{-}24}$ of MPA on day 21				
≤30 μg h/mL	10	1		
>30 μg h/mL	30	0.38	0.15–0.91	**0.03**
$CD34^+$ cell count				
$\leq 0.57\times10^5$/kg		1		
$> 0.57\times10^5$/kg		1.27	0.62–2.60	0.51

Abbreviations: GVHD: graft-versus-host disease, HR: hazard ratio, CI: confidence interval, SCT: stem cell transplantation, PS: performance status, HCT-CI: comorbidity index of hematopoietic cell transplantation, M: male, F: female, HLA: human leucocyte antigen, CR: complete remission, MAC: myeloablative conditioning, RIC: reduced-intensity conditioning, TBI: total body irradiation, $AUC_{0\text{-}24}$: 24-hour area under the curve, MPA: mycophenolic acid.

Table 3. Multivariate analysis for grade II–IV acute GVHD

Variables	Number	HR	95% CI	*P*
HLA allele mismatch				
0–2	20	1		
3–5	20	2.14	0.96–4.79	0.06
Disease status at SCT				
CR	22	1		
Non-CR	18	1.26	1.07–1.48	**0.006**
$AUC_{0\text{-}24}$ of MPA on day 21				
≤30 μg h/mL	10	1		
>30 μg h/mL	30	0.66	0.24–1.78	0.41

Abbreviations: GVHD: graft-versus-host disease, HR: hazard ratio, CI: confidence interval, HLA: human leucocyte antigen, SCT: stem cell transplantation, CR: complete remission, $AUC_{0\text{-}24}$: 24-hour area under the curve, MPA: mycophenolic acid.

Figure legends

Figure 1. Use of mycophenolate mofetil enabled high neutrophil engraftment and feasible overall survival rates.

(A) Cumulative incidence of engraftment. (B) Kaplan–Meier curves of overall survival of patients in CR and non-CR at transplantation. (C) Cumulative incidence of relapse and (D) non-relapse mortality of patients in CR and non-CR. (E) Cumulative incidence of grade II–IV graft-versus-host disease and the number of HLA antigen mismatches (A, B, C, and DRB1). (F) Cumulative incidence of grade II–IV graft-versus-host disease and the number of HLA allele mismatches (A, B, C and DRB1).

Abbreviations: CR: complete remission. HLA: human leukocyte antigen.

Figure 2. $AUC_{0\text{-}24}$ of MPA affected the incidence of sepsis, grade II–IV acute GVHD, and reactivation of human herpesvirus 6.

(A) $AUC_{0\text{-}24}$ on day 7 and cumulative incidence of sepsis before neutrophil engraftment. (B) $AUC_{0\text{-}24}$ on day 21 and cumulative incidence of grade II–IV graft-versus-host disease. (C) $AUC_{0\text{-}24}$ on day 21 and cumulative incidence of reactivation of human herpesvirus 6.

Abbreviations: $AUC_{0\text{-}24}$: 24-hour area under the curve, MPA: mycophenolic acid, GVHD: graft-versus-host disease.

Figure 3. Diarrhea and poor nutrition decreased $AUC_{0\text{-}24}$ of MPA on day 21.

(A) Diarrhea and box plot of $AUC_{0\text{-}24}$ on day 21. (B) Serum albumin levels and box plot of $AUC_{0\text{-}24}$ on day 21. Abbreviations: $AUC_{0\text{-}24}$: 24-hour area under the curve, MPA: mycophenolic acid.

Figure 4. Dose escalation for low $AUC_{0\text{-}24}$ on day 7 increased the $AUC_{0\text{-}24}$ on day 21.

(A) $AUC_{0\text{-}24}$ of MPA on days 7 and 21 among patients with MPA7 ($AUC_{0\text{-}24}$ of MPA on day 7) <30 μg h/mL. The MMF dose of these patients was escalated after day 7. (B) $AUC_{0\text{-}24}$ on days 7 and 21 of patients with MPA7 of 30–60 μg h/mL. The MMF dose of these patients was not escalated after day 7.

Abbreviations: $AUC_{0\text{-}24}$: 24-hour area under the curve, MPA: mycophenolic acid, MMF: mycophenolate mofetil.

Supplemental Figure 1. Correlation between $AUC_{0\text{-}24}$ and single point concentration.

Correlation between $AUC_{0\text{-}24}$ and (A) C_0, (B) C_1, (C) C_2 and (D) C_4.

Abbreviations: $AUC_{0\text{-}24}$: 24-hour area under the curve. *R*: correlation coefficient.