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An Age-Dependent Pharmacokinetic Study of Intravenous and Oral Mycophenolate Mofetil in Combination with Tacrolimus for GVHD Prophylaxis in Pediatric Allogeneic Stem Cell Transplantation Recipients

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Acute graft-versus-host disease (aGVHD) still remains a major limiting factor following allogeneic stem cell transplantation (AlloSCT) in pediatric recipients. Mycophenolate mofetil (MMF), an uncompetitive selective inhibitor of inosine monophosphate dehydrogenase, is a new immunosuppressant agent without major mucosal, hepatic, or renal toxicity compared to other prophylactic aGVHD immunosuppressant drugs. Although there has been an extensive pharmacokinetic (PK) experience with MMF administration following solid organ transplantation in children, there is a paucity of PK data following its use in pediatric AlloSCT recipients. We investigated the safety and PK of MMF as GVHD prophylaxis following intravenous (i.v.) and oral (p.o.) administration (900 mg/m^2 every 6 hours) in conjunction with tacrolimus, after myeloablative (MA) and nonmyeloablative (NMA) conditioning and AlloSCT in 3 distinct age groups of pediatric AlloSCT recipients (0-6 years, 6-12 years, and 12-16 years). Mycophenolic acid (MPA) in plasma samples was measured either by high-performance liquid chromatography (HPLC) or liquid chromatography/mass spectrometry (LC/MS/MS) as we have previously described. Plasma samples were obtained at baseline and at 0.5, 1, 2, 3, 4, and 6 hours after i.v. dosing on days +1, +7, +14, and at 2 time points between day +45 and +100 after p.o. administration post AlloSCT. MPA PK analysis included AUC (0-6 hours), C_{max}, T_{max}, C_{ss}, V_{ss}, C trough (C_0), CL, and $T_{½}$. Thirty-eight patients, with a median age of 8 years (0.33-16 years), 20/18 M:F ratio, 21/17 malignant/nonmalignant disease, 17/21 MA:NMA conditioning, 16 of 22 related/unrelated allografts. Median time to myeloid and platelet engraftment was 18 and 31 days, respectively. Mean donor chimerism on day +60 and +100 was 83% and 90%, respectively. Probability of developing aGVHD grade II-IV and extensive chronic GVHD (cGVHD) was 54% and 34%, respectively. There was significant intra- and interpatient MMF PK variability. There was a significant increase in i.v. MPA area under the curve $(AUC)_{0\text{-}6hour}$ and C_{max} ($P < .0003$) and a significant decrease in CL_{ss} ($P < .002$) and V_{ss} ($P < .001$) on day +14 versus day +7. Children <12 years of age had a significant increase in i.v. MPA T_{max} ($P = .01$), V_{ss} ($P = .028$), and CL_{ss} ($P < .001$) compared to the older age group. There was a trend in increased i.v. MPA CL_{ss} following MA versus NMA conditioning ($P < .054$); i.v. and p.o. MMF administration (900 mg/m^2 every 6 hours) in combination with tacrolimus was well tolerated in pediatric AlloSCT recipients. There was a significant increase in MPA exposure on day +14 versus day +7, suggesting improved enterohepatic recirculation at day +14 post-AlloSCT. Children <12 years of age appear to have a significantly different MPA PK profile compared to older children and adolescents and may require more frequent dosing.

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INTRODUCTION

Allogeneic stem cell transplantation (AlloSCT) remains the only curative option for a number of pediatric malignant and nonmalignant conditions [1]. Acute graft-versus-host disease (aGVHD) is a potentially fatal complication after AlloSCT, contributing to a treatment-related mortality (TRM) of 15% to 30% [2,3]. Current posttransplantation therapies for prophylaxis and treatment of GVHD in pediatric recipients following AlloSCT are only partially effective [4].

Historically, cyclosporine A (CsA) and methotrexate (MTX), with or without prednisone (PDN), have been used for aGVHD prophylaxis after matched related and unrelated AlloSCT, and have been shown to be superior to either drug alone in preventing grade II-IV aGVHD [5,6]. Despite the effectiveness of these regimens, each of these agents are known to be associated with significant organ toxicity [7-9]. Tacrolimus (FK506), another calcineurin inhibitor, is similar to but 50 to 200 times more potent an immunosuppressant than CsA [10,11]. In 3 randomized human trials, FK506 in conjunction with standard low dose MTX was associated with a significantly lower incidence of aGVHD compared with CsA/MTX [12-14].

Mycophenolate mofetil (MMF) is an ester prodrug of the immunosuppressant mycophenolic acid (MPA), which is a potent, reversible, uncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme for de novo purine synthesis during cell division. T and B cell lymphocytes are more dependent on this pathway than other cell types. The rationale for the use of MMF in place of MTX was in part to reduce the risk of mucosal, hematologic, and hepatic side effects often associated with MTX [15-21].

Although MMF is now more commonly utilized in AlloSCT recipients, a scant amount of data is available regarding the pharmacokinetics (PK) in pediatric AlloSCT recipients. Therefore, many centers have extrapolated dosing based on that used in children undergoing kidney transplantation (600 mg/m^2 every 12 hours) or the common adult dose (15 mg/kg every 12 hours). We reported the preliminary results of the safety of MMF and FK506 as GVHD prophylaxis in pediatric AlloSCT recipients [20].

In the current study, we set out to investigate whether MMF and/or its metabolite, MPA, might have a different PK profile between different pediatric age groups and the types of conditioning regimens used (myeloablative [MA] versus nonmyeloablative [NMA]) in pediatric AlloSCT. We report the age dependent PK of intravenous (i.v.) and oral (p.o.) MMF, respectively, in combination with tacrolimus in the early (day +1 to day +14), and late (day +45 to day +100) posttransplant period in pediatric AlloSCT recipients, stratified by conditioning regimens, and the development of aGVHD and chronic GVHD (cGVHD) in this patient population.

MATERIALS AND METHODS

Patients

From January 2004 to May 2008, we investigated the safety profile and age dependent (<6 years, 6-12 years, and 12-16 years) PK of i.v. MMF, in combination with tacrolimus in the early posttransplant period (day +1 to day +14) and p.o. MMF in the late posttransplant period (day +45 to day +100) in 38 pediatric AlloSCT recipients, stratified by conditioning regimen intensity (Table 1). We also evaluated the incidence and severity of aGVHD following MMF/FK506 prophylaxis in pediatric AlloSCT recipients, stratified by allogeneic stem cell source and HLA disparity. Pediatric patients ≤16 years of age with both malignant and nonmalignant conditions, undergoing AlloSCT from both related and unrelated donors using either MA or NMA conditioning regimens were enrolled on a Columbia University Medical Center institutional review board (IRB)-approved protocol. An investigational new drug (IND) exemption for this study was granted by the Federal Food and Drug Administration. All patients and/or parents signed an IRB-approved informed consent and assent (when applicable) prior to study entry. Patients were excluded from the study if they had a known history of hypersensitivity to MMF, MPA, or polysorbate 80 (i.v. formulation), or any ingredient of mycophenolate formulation, had creatinine clearance <40 mL/min/m^2 (determined by Schwartz formula or nuclear glomerular filtration rate), or were pregnant or lactating females. Patients receiving concomitant probenecid, or aluminum and/or magnesium containing antacid (for patients on p.o. MMF only) were ineligible for PK analysis. Only patients scheduled to receive GVHD prophylaxis with tacrolimus and MMF on their AlloSCT protocols were eligible to enter the study. Patients were removed from the study if they reached day +100 posttransplant, developed grade IV toxicity (CTCAE version 3.0) to MMF, had equal to or greater than grade II gastrointestinal GVHD while on p.o. MMF, required to be switched from tacrolimus to another immunosuppressant agent for GVHD prophylaxis or therapy, patient or parent withdrawal from protocol, or death.

GVHD Prophylaxis

FK506 was administered either i.v. at 0.03 mg/kg/day by continuous infusion or p.o. at 0.12 mg/kg/day in 2 or 3 divided doses starting on day −1 or on the first day of conditioning (protocol dependent) as we have previously reported [20]. Doses were adjusted to

Table 1. Patient Characteristics

Number of patients	38
Median age (years, range)	8 (0.33-16)
Sex	
Male	20 (53%)
Female	18 (47%)
Diagnosis/disease status (n = 38)	
Malignant (N = 21)	
ALL (1 CR1, 2 CR2, 1 CR3)	4
AML (6 CR1, 1 CR2, 1 CR3, 2 Rel, 1 PIF)	11
NHL (1 Ref)	1
HD (1 CR2)	1
CML-CP	1
Neuroblastoma (3 PR)	3
Nonmalignant (N = 17)	
SCD	4
Aplastic Anemia	8
WAS	1
Scleroderma	1
HLH	2
MDS	1
Prior transplant history	
Autologous	4
Allogeneic transplant	1
Transplant cell source (allogeneic)/HLA disparity	
Cord blood	21 (55%)
None (6/6)	5
5/6	8
4/6	8
Marrow	6 (16%)
None (6/6)	5
5/6	1
4/6	0
PBSC	11 (29%)
None (6/6)	7
5/6	3
4/6	0
8/10	1
Donor source	
Related	18 (47%)
Unrelated	20 (53%)
Preparative regimen	
Nonmyeloablative	21 (55%)
Myeloablative	17 (45%)

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; CML-CP, chronic myelogenous leukemia in chronic phase; SCD, sickle cell disease; WAS, Wiscott-Aldrich syndrome; HLH, hemophagocytic lymphohistiocytosis; MDS, myelodysplastic syndrome; Rel, relapse; Ref, refractory; PIF, partial induction failure; PBSC, peripheral blood stem cell.

maintain therapeutic trough levels between 10 and 20 ng/mL (Abbott IMX microparticle enzymatic immunoassay [MEIA]), and for tacrolimus-induced toxicity. FK506 was changed to oral dosage when clinically appropriate. MMF was administered daily starting on day +1 at 900 mg/m^2/dose i.v. (over 2 hours) every 6 hours (children <1 year of age or <10 kg received 30 mg/kg i.v. [over 2 hours] every 6 hours). Patients were changed to p.o. MMF when clinically indicated or on/around day +15 at the same dose and interval. During the oral administration, an overnight (8-hour) fasting period was required on days of PK analysis. MMF doses were not modified based on measured MPA concentrations. In those with nonmalignant diseases, MMF was tapered over 4 weeks starting at day +180. In those patients with malignant conditions receiving a related graft, MMF was stopped at day +30 unless signs of equal to or greater than grade II GVHD was present. In those receiving an unrelated graft, MMF was stopped at day +60 unless signs of equal to or greater than grade II GVHD was present. Oral MMF was generously provided by Roche Laboratories, Nutley, NJ. Commercially available i.v. MMF was provided by the NewYork-Presbyterian Hospital pharmacy.

GVHD was graded according to Seattle consensus criteria [22]. The rule of "9" or a burn chart was used to estimate the extent of skin rash. Patients were staged and graded once a week for the occurrence of aGVHD. Once a clinical diagnosis of aGVHD or cGVHD was determined, histological confirmation was obtained if possible.

Sample Collection and Handling

Intravenous MMF PK sample collection

Total MPA calculations were done from the same blood sample. Venous blood samples (2-3 mL) for PK analysis (MPA total) during i.v. MMF administrations were drawn though a central venous line (CVL) and placed in EDTA tubes at time 0 (immediately before dose), and at 0.5, 1, 2, 3, 4, and 6 hours after the administration of the morning IV MMF dose on days +1, +7, and +14.

Oral MMF PK sample collection

Venous blood samples (2-3 mL) for PK analysis (MPA total) were drawn through a CVL and placed in an EDTA tube at time 0 (immediately before dose) and at 0.5, 1, 2, 3, 4, and 6 hours after the morning p.o. MMF dose on 2 different dates at least 1 week apart during PO administration on days +45 to +100.

All samples were placed on ice (4°C) immediately, then plasma was separated and frozen at −80°C until analysis.

MPA Bioanalysis

MPA plasma samples were analyzed using 2 equivalent validated assays: high-performance liquid chromatography (HPLC) [23] and liquid chromatography/mass spectrometry (LC/MS/MS) [24]. In the beginning of the study, HPLC-UV assay was used (Agilent 1100 isocratic system with UV detection [Agilent Technologies, Palo Alto, CA]; Column: Zorbax Eclipse XDB-C18, 4.6 × 150 mm [Agilent Technologies]). Plasma samples were prepared as follows: 0.3 mL of plasma was treated with 1 mL of acetonitrile, vortexed for 0.5 minutes, and centrifuged for 10 minutes at 3000 rpm, 10°C, then 1 mL of supernatant was removed and diluted 1:1 with water, vortexed, and centrifuged again (same conditions). The lower limit of quantification for this method was 0.1 mg/L of

MPA. Three levels of quality controls were utilized: 0.2, 2, and 15 mg/L of MPA, with precision of 0.95 to 5.2% and accuracy of 3.6 to 7.2%.

Approximately halfway through the study, the LC/MS/MS assay was utilized (HPLC: Agilent 1100 system composed of 2 pumps: isocratic and quaternary, degasser, autosampler, and heated column compartment. Column: Zorbax Eclipse XDB-C18, 4.6 × 150 mm [Agilent Technologies]; MS/MS: API 2000 tandem Mass Spectrometer [MDS Sciex, Toronto, Canada] with Valco 10-port switching valve and Analyst® software, version 1.4). Plasma samples for LC/MS/MS assay were prepared as follows: 1 mL of acetonitrile was added to 0.1 mL of plasma, vortexed, and centrifuged as discussed, diluted with water 1:2, then vortexed and centrifuged again. The lower limit of quantification for this method was 0.05 mg/L of MPA. Three levels of quality controls were utilized: 0.5, 5.0, and 15 mg/L of MPA, with precision of 4.1% to 4.5% and accuracy of 0.4% to 5.4%. The powder of MPA generously donated by Roche Laboratories was utilized for preparation of standard and controls.

MPA PK Analysis

For each patient, noncompartmental PK analysis of total MPA concentrations was performed. Plasma concentration-time data were analyzed using standard spreadsheet software (OpenOffice 2.4, OpenOffice.org). The steady-state area under the curve (AUC) 0-6 hours was calculated using the linear trapezoidal method [25]. Steady-state concentration (C_{ss}) was computed by dividing $AUC_{0-\infty}$ by dosing interval (6 hours). Maximum concentration (C_{max}) was determined by visual observation of the highest MPA plasma concentration. T_{max} was the time of C_{max}, and trough concentration (C_0) was the concentration at hour 6 post-MMF dose. MPA clearance was calculated by dividing the total MMF dose by MPA $AUC_{0-\infty}$. Volume of distribution at steady state (V_{ss}) was calculated by multiplying the MPA clearance by mean retention time (MRT) of MPA. Half-life was calculated as a ratio of 0.693 and lambda. Lambda was the slope of a line fitted to the last 3 points (after 2 hours time point) on a semilogarithmic plot of MPA concentration versus time.

Statistics

The continuous variables are summarized as mean and standard deviation, the categoric variables are summarized as percentage. The change in PK measurements at 2 time points were assessed by the signed rank test. The 2-sided *t*-test is used for comparing continuous variables in 2 groups and the analysis of variance (ANOVA) is used for comparing more than 2 groups. Probabilities of neutrophil recovery, platelet recovery, aGVHD and cGVHD, and overall survival (OS) were estimated using the Kaplan-Meier method. Curves were compared by the Log-rank test. Tests with $P < .05$ were considered significant. OS is defined as the time between transplantation and death because of any cause or between transplantation and May 31, 2008. Poor-risk patients were defined as patients with malignant disease with refractory disease and/or complete response (CR) 3 or beyond. The remaining patients were classified as average risk.

The logistic regression was used for the analysis of grade II-IV aGVHD. Variables for the risk of aGVHD included age, sex, conditioning intensity, related versus unrelated donors, HLA 6/6 match versus others, poor risk versus average risk, nonmalignant versus malignant, i.v. MMF aggregate day +7 and day +14 PK (C_{max}, T_{max}, MPA_{trough}, AUC_{0-6}, $T_{½}$, V_{ss}, C_{ss}, Cl_{ss}). Multivariate models were built based on the variables that had a *P*-value of <.2 in the univariate analysis.

RESULTS

Patient Demographics

Thirty-eight AlloSCT patients were studied (Table 1). Twenty patients were male (53%) and 18 patients were female (47%) with a median age of 8 years (0.33-16 years). Twenty-one patients (55%) were transplanted for malignant conditions and 17 patients (45%) were transplanted for nonmalignant conditions. Donor sources were: umbilical cord blood (UCB) (n = 21, 55%), bone marrow (BM) (n = 6, 16%), and peripheral blood stem cells (PBSC) (n = 11, 29%) with 18 patients (47%) undergoing a related SCT and 20 patients (53%) undergoing an unrelated SCT. Four patients had undergone a prior autologous SCT and 1 patient had a prior AlloSCT.

Conditioning

The conditioning regimens were both MA (n = 17, 45%) and NMA (n = 21, 55%). Of the MA regimens, the majority contained busulfan (Bu) 12.8-16 mg/kg (n = 11, 65%); the remainder of the regimens were either total body irradiation (TBI) based (n = 4, 23%) or cyclophosphamide (Cy) 120-200 mg/kg (n = 2, 12%). The NMA regimens all were fludarabine (Flu; 150-180 mg/m^2) based regimens (n = 21, 100%).

Concurrent Medications

None of the patients enrolled in the study received concomitant probenecid, aluminum, and/or magnesium containing antacids, CsA, or cholestyramine. Even though acyclovir may potentially increase serum concentrations of MPA, all patients received acyclovir for HSV prophylaxis from day 0 until engraftment.

Donor Source

Donor sources included 6 6/6 HLA-matched sibling BM, 1 8/10 matched unrelated BM, 7 6/6 HLA-matched sibling PBSC, and 3 5/6 HLA matched PBSC. The majority of patients underwent a UCB-transplant (n = 21, 55%) with 2 6/6 related UCB, 3 6/6 unrelated UCB, 8 5/6 UCB, and 8 4/6 UCB (Table 1).

GVHD

Nineteen of 38 evaluable patients developed grade II-IV aGVHD and 13 developed grade III-IV aGVHD of the skin and intestine. The probability of developing grade II-IV and III-IV aGVHD was 54.4% (CI_{95}: 37.24-68.7) and 41.6% (CI_{95}: 20.3-61.7), respectively (Figure 1). A total of 9 patients developed extensive cGVHD; 5 patients who received a related transplant and 4 patients who received unrelated transplants. The probability of developing cGVHD in all patients was 33.7% (CI_{95}: 15.0-52.3) (Figure 1).

Grade III/IV Toxicity

Grade III toxicity directly, probably and possibly because of MMF included nausea (8%), vomiting (3%), diarrhea (5%), and gastritis (3%). There were no grade III/IV hematologic toxicities definitely, probably or possibly attributed to MMF. Also, no patients in our study developed Grade III/IV nephrotoxicity thought to be attributed to tacrolimus.

Pharmacokinetics

Mean PK parameters following day +1, 7, and 14 i.v. MMF dosing and 2 p.o. MMF dosing between days +45 and +100 for all patients are depicted in Table 2. Data was available in 27, 31, and 32 patients on days +1, +7, +14 following i.v. MMF dosing, respectively. Limited data was available on patients following p.o. MMF dosing between days +45 and +100 because of either death, disease progression, development of severe aGVHD requiring continued i.v. MMF dosing, inability to tolerate p.o. MMF, physician/patient preference, and/or institutional protocol requirements to discontinue MMF at day +30. PK parameters were highly variable with >10-fold interpatient variability in total MPA $AUC_{0\text{-}6}$ as well as total MPA trough concentration. There were no significant differences for most MPA PK parameters on day +1 versus day +7 of dosing, with the exception of total MPA trough concentrations (0.33 versus 0.68 mg/L; $P = .002$). However, there were statistically significant differences in most PK parameters on day +7 versus day +14 of dosing: $AUC_{0\text{-}6}$ and C_{max} were increased significantly on day +14 compared to day +7 (AUC: 33.71 versus 26.82 mgx h/L, $P = .0003$; C_{max}: 16.54 versus 12.31 mg/L, $P = .0003$); whereas clearance and volume of distribution were significantly decreased (CL_{ss}: 1.17 versus 1.40 L/h·kg, $P = .002$; V_{ss}: 3.00 versus 3.35 L/kg, $P = .001$).

We next compared the i.v. (day +1, +7, and +14) aggregate MMF PK between the 3 different age groups (<6 years, 6-12 years, and ≥12 years). There were no significant differences in the following MMF PK parameters between age groups: C_{max}, MPA trough (C_0), C_{ss}, $AUC_{0\text{-}6}$, and $T_{½}$ (Table 3A). Pairwise comparison demonstrated no difference in T_{max} between patients <6 years of age versus 6-12 years and between 6-12 years versus >12 years age groups. However, children <6 years of age had significant higher T_{max} compared to >12 years age group ($P = .001$) (Table 3A).

Similarly, there was no difference in CL_{ss} or V_{ss} between <6 years versus 6-12 years age group; patients in the <6 years group had significant higher CL_{ss} and V_{ss} compared to patients >12 years ($P = .02$ for CL_{ss}; $P = .014$ for V_{ss}) and the 6-12 years age group had significant higher CL_{ss} compared to >12 years group ($P = .001$). When ≤12 years age group was compared to the >12 years group, the results revealed that T_{max}, V_{ss} and CL_{ss} in the ≤12 years group were significantly higher than those in the >12 years group ($P = .01$ for T_{max}; $P = .028$ for V_{ss} and $P = .001$ for CL_{ss}) (Table 3A). There was no significant difference in MMF PK between the 3 different age groups following p.o. MMF administration (Table 3B) (C_{max}: $P = .11$, T_{max}: $P = .25$, MPA: $P = .64$, $AUC_{0\text{-}6}$: $P = .06$, $T_{½}$: $P = .45$, CLA: $P = .42$, Vss: $P = .34$ and C_{ss}: $P = .28$).

We next compared the MMF PK following i.v. MMF (days +1, +7, and +14) in pediatric AlloSCT recipients following MA versus NMA conditioning (Table 3C). The intensity of conditioning had no

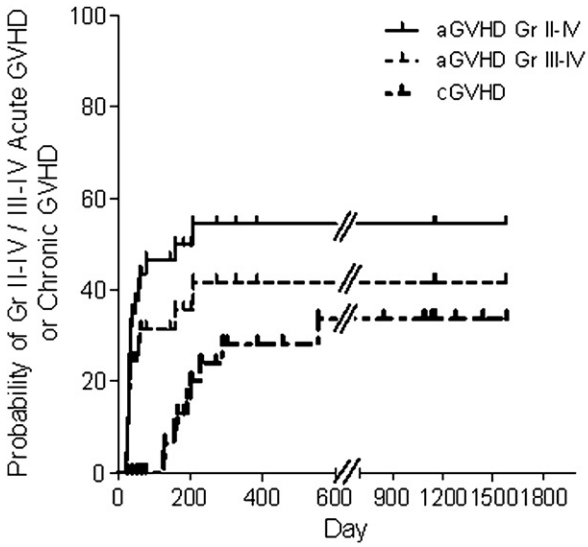

Figure 1. Probability of developing aGVHD or cGVHD. Probability of developing aGVHD grade II-IV (), grade III-IV (), or cGVHD () determined by Kaplan Meier method in patients receiving AlloSCT.

Table 2. Mean PK Parameters (All Patients)

	i.v. MMF Administration			MMF p.o. First[E] Sample	MMF p.o. Second Sample
Mean PK Values	Day +1 (SD) [N = 27]	Day +7 (SD) [N = 31]	Day +14 (SD) [N = 32]	Day +45-100 (SD) [N = 15]	Day +45-100 (SD) [N = 9]
C_{max} (mg/L)	15.4 (19.71)	12.31 (7.99)†	16.54 (13.24)†	13.15 (12.04)	13.20 (8.86)
T_{max} (h)	1.87 (0.47)	1.92 (0.55)	1.75 (0.44)	1.7 (0.98)	1.33 (0.83)
C_0 (mg/L)	0.33 (0.29)*	0.68 (0.56)*	0.72 (0.62)	1.45 (1.46)	1.43 (1.37)
C_{ss} (mg/L)	5.45 (6.34)	4.73 (2.22)	6.46 (4.07)	5.38 (3.55)	6.54 (3.55)
AUC_{0-6} (mg h/L)	32.06 (38.09)	26.82 (12.35)†	33.71 (16.86)†	26.50 (19.03)	26.70 (15.81)
CL_{ss} (L/h·kg)	1.46 (1.04)	1.40 (0.63) *	1.17 (0.63) *	2.21 (1.43)	1.46 (1.01)
V_{ss} (L/kg)	3.04 (1.91)	3.35 (1.58) ‡	3.00 (2.05)‡	9.77 (8.93)	18.33 (35.74)
$T_{1/2}$ (h)	1.02 (0.69)	1.35 (1.01)	2.49 (6.77)	3.17 (2.63)	10.19 (18.06)

SD indicates standard deviation; MMF, mycophenolate mofetil; C_{max}, maximum concentration; T_{max}, time of maximum concentration; C_0, trough concentration; C_{ss}, steady-state concentration; AUC_{0-6}, steady-state area under the curve 0-6 hours; CL_{ss}, steady-state clearance; V_{ss}, volume of distribution; $T_{½}$ half-life.
*$P = .002$.
†$P = .0003$.
‡$P = .001$.

influence on the PK following MA versus NMA conditioning (Table 3C), although there was a very strong trend with increase in CL_{ss} (1.55 versus 1.15) in MA versus NMA conditioning ($P < .054$).

Multivariate Analysis

We determined the effect of age, sex, conditioning intensity, related versus unrelated donor, HLA (6/6 versus other), poor versus average risk, malignant versus nonmalignant, and i.v. MMF PK in aggregate and day +7 and day +14 (C_{max}, T_{max}, $T_{½}$, MPA_{trough}, AUC_{0-6}, C_{ss}, V_{ss}, and CL_{ss}) on the probability of developing grade II-IV aGVHD. Although MA conditioning, HLA <6/6, malignant disease and day +7 C_{max} all were <0.2 in the univariate analysis (Table 4). None of these variables were independently associated with grade II-IV aGVHD in the multivariate analysis.

Survival

The OS for all 38 patients was 61.8% (95% confidence interval [CI]: 48.3-78.4) (Figure 2).

DISCUSSION

In our previous pilot study, we demonstrated in 34 children and adolescents with malignant and nonmalignant disease following 37 AlloSCT, that the prophylactic use of MMF at 15 mg/kg i.v. or p.o. every 12 hours failed to achieve a solid organ transplant target MPA trough level of 1-3.5 mg/L [20]. Eight of those 34 patients received an escalated dose of 900 mg/m^2 i.v. every 6 hours of MMF and obtained an MPA trough level of between 1 and 3.5 mg/L [20]. There was no evidence of increased systemic toxicity following this increase in the dose of MMF [20]. In the current study, this increased dose of MMF (900 mg/m^2 every 6 hours) was given prophylactically in pediatric AlloSCT recipients. Unfortunately, our earlier observations did not hold up in the present analysis in terms of ability to achieve target trough concentrations at the earlier dose as well as the utility of measuring trough concentrations. Recent data in both adults and pediatric AlloSCT recipients suggests the lack of utility of trough concentrations predicting rejection/engraftment and/or risk of GVHD [26,27]. The major known toxicities of MMF, particularly after solid organ transplantation, has included toxicities to the gastrointestinal tract [28]; in particular, nausea and vomiting, and hematopoietic toxicity, especially neutropenia. In the present study, there was a 3% incidence of grade III vomiting and gastritis, a 5% incidence of grade III diarrhea, and an 8% incidence of grade III nausea. This incidence of low-grade gastrointestinal toxicity appears to be similar to previous reports of the use of MMF in adult AlloSCT recipients [16,18,27,29-31] Similarly, there did not appear to be any evidence of any delay in hematopoietic

Table 3A. Mean PK Parameters by Age (Combined i.v. Aggregate)

Age group	n	C_{max} mg/L (SD)	T_{max} h (SD)	C_0 mg/L (SD)	C_{ss} mg/L (SD)	AUC_{0-6} mg h/L (SD)	CL_{ss} L/h·kg (SD)	Vss L/kg (SD)	$T_{1/2}$ h (SD)
<6 yrs	14	13.85 (5.94)	1.89 (0.36)	0.65 (0.56)	5.20 (1.63)	29.46 (9.80)	1.65 (0.59)	3.87 (1.39)	1.20 (0.66)
6-12 years	10	15.93 (10.65)	2.03 (0.11)	0.49 (0.34)	5.90 (3.67)	34.28 (21.19)	1.45 (0.67)	3.22 (1.56)	1.22 (0.51)
≥12-16 years	12	14.12 (7.07)	1.63 (0.44)*	0.67 (0.42)	5.72 (3.00)	28.60 (8.92)	0.88 (0.36)†	2.44 (1.36)‡	2.86 (5.48)

PK indicates pharmacokinetic; i.v., intravenous; C_{max}, maximum concentration; T_{max}, time of maximum concentration; C_0, trough concentration; C_{ss}, steady-state concentration; AUC_{0-6}, steady-state area under the curve 0-6 hours; CL_{ss}, steady-state clearance; V_{ss}, volume of distribution; $T_{½}$, half-life; SD, standard deviation.
*ANOVA *P*-value = .01 (≤12 years versus >12 years).
†ANOVA *P*-value = .001(≤12 years versus >12 years).
‡ANOVA *P*-value = .028 (≤12 years versus >12 years).

Table 3B. Mean PK Parameters by Age (Combined p.o. Aggregate)

Age Group	n	C_{max} mg/L (SD)	T_{max} h (SD)	C_0 mg/L (SD)	C_{ss} mg/L (SD)	AUC_{0-6} mg h/L (SD)	CL_{ss} L/h·kg (SD)	V_{ss} L/kg (SD)	$T_{1/2}$ h (SD)
<6 years	9	14.70 (10.85)	1.36 (0.82)	1.83 (1.48)	5.66 (3.64)	30.18 (19.23)	2.07 (1.05)	8.81 (7.26)	3.32 (2.70)
6-12 years	5	7.03 (5.23)	2.20 (1.15)	1.12 (1.15)	4.49 (2.35)	16.57 (8.01)	2.27 (1.41)	19.09 (25.58)	8.40 (12.46)
≥12-16 years	2	11.85 (11.17)	1.63 (0.53)	1.24 (1.30)	6.07 (0.77)	25.51 (10.26)	0.68 (0)	1.46 (0)	3.36 (3.53)

PK indicates pharmacokinetic; p.o., oral; C_{max}, maximum concentration; T_{max}, time of maximum concentration; C_0, trough concentration; C_{ss}, steady-state concentration; AUC_{0-6}, steady-state area under the curve 0-6 hours; CL_{ss}, steady-state clearance; V_{ss}, volume of distribution; $T_{½}$, half-life; SD, standard deviation.

reconstitution or grade III/IV hematologic toxicity. These safety results compare favorably to adult AlloSCT recipients who tend to receive 25% to 50% of MMF compared to the dose utilized in this prophylactic pediatric AlloSCT trial [15,16,18,27,29-31].

The bioavailability of MMF after p.o. administration in healthy individuals has been reported to be approximately 94% [32]. MMF is rapidly hydrolyzed by esterases to form the active compound MPA [32]. There has been extensive experience in both adults and children receiving solid organ transplants demonstrating that targeting either the total predose MPA trough concentration between 1 and 3.5 mg/L (with concomitant CsA) or 1.9 to 4 mg/L (with concomitant tacrolimus) and/or MPA C_{ss} 2.5 to 5 mg/L has been successfully associated with a decrease in allograft rejection and a reduction in MMF-associated toxicities [33-35].Last, Kagaya et al. [36] demonstrated no PK interactions between MPA and tacrolimus following adult renal solid organ transplantation, suggesting that MMF and tacrolimus can be safely combined.

Therapeutic drug monitoring (TDM) following MMF administration in patients after either solid organ or AlloSCT has been controversial, and is further complicated by the specific manner in which MMF is metabolized [37]. Specifically, MPA, the active metabolite of MMF, is conjugated to the pharmacologically inactive phenolic glucuronide (MPAG), which is excreted primarily by the kidney. The PK of MMF, however, is further complicated by enterohepatic circulation of MPAG, which is excreted into the bile and which is subsequently hydrolyzed in the intestine and reabsorbed as MPA, giving rise to secondary peak of MPA, 6 to 12 hours after MMF administration [38]. Furthermore, MPA binds extensively to plasma albumin and has a free MPA fraction of <3% [38]. Additional confounding factors include significant intra- and interpatient variability following MMF administration and that coadministration of CsA appears to decrease exposure of MPA as well [39-41]. Therefore, a number of variables affect MPA PK, including intra- and interpatient variability, enterohepatic recirculation, renal function, albumin level, and concomitant immunosuppressive therapy.

MMF PK has been extensively studied in adult AlloSCT recipients following both MA and NMA conditioning [27,30,31]. The dosing of MMF in adult AlloSCT recipients has been variable, and has ranged between 12.5 mg/kg every 12 hours to 15 mg/kg every 6 hours [27,30,31]. The total MPA AUC_{0-6} has ranged between 16.4 and 29 mg·h/L [27,30,31]. The MPA C_{max} has ranged between 1.0 and 29 mg/L (Table 5) [27,30,31]. The MPA trough has ranged between 0.01 to 22 mg/L (Table 5) [27,30,31]. Last, the MPA $T_{½}$ has ranged between 0.8 and 7.9 hours (Table 5) [27,30,31]. The influence of MA versus NMA conditioning on MMF PK in adult AlloSCT recipients has not demonstrated any significant differences to date (Table 5) [27,30,31]. In the adult AlloSCT MMF PK studies, few studies have been able to demonstrate achieving an MPA C_{ss} between 2.5 and 5 mg/L, or an MPA trough between 1 and 3.5 mg/L (concomitant CsA) or 1.9 and 4 mg/L (concomitant tacrolimus) as was suggested to be the target ranges following solid organ transplantation [27,30,31,33-35]. Nash et al. [27] demonstrated that total MPA Css > 2.5 mg/L is achievable with either every 8 hours or every 6 hours MMF dosing, however, not with q12 h dosing in adult AlloSCT recipients [27].

In the most comparable adult AlloSCT study to our current study, Nash et al. [27], investigated the PK of MMF (450 mg/m^2 [15 mg/kg] administered every 6 hours, every 8 hours, or every 12 hours) in combination with CsA following MA conditioning in adult AlloSCT recipients [27]. Nash et al. [27] demonstrated on day 14 a median MPA AUC_{0-6} of 29 mg·h/L, MPA clearance

Table 3C. Mean PK Parameters (Combined i.v. Aggregate) Stratified by MA versus NMA Conditioning

Conditioning	n	C_{max} mg/L (SD)	T_{max} h (SD)	C_0 mg/L (SD)	C_{ss} mg/L (SD)	AUC_{0-6} mg h/L (SD)	CLss L/h·kg (SD)	V_{ss} L/kg (SD)	$T_{1/2}$ h (SD)
MA	17	15.12 (7.40)	1.83 (0.46)	0.53 (0.37)	5.20 (1.46)	29.77 (8.92)	1.55 (0.68)	3.53 (1.75)	1.20 (0.61)
NMA	19	13.98 (8.07)	1.85 (0.29)	0.69 (0.51)	5.90 (3.49)	31.18 (16.79)	1.15 (0.54)	2.93 (1.25)	2.26 (4.37)

PK indicates pharmacokinetic; MA, myeloablative; NMA, nonmyeloablative; C_{max}, maximum concentration; T_{max}, time of maximum concentration; C_0, trough concentration; C_{ss}, steady-state concentration; AUC_{0-6}, steady-state area under the curve 0-6 hours; CL_{ss}, steady-state clearance; V_{ss}, volume of distribution; $T_{½}$, half-life; SD, standard deviation.

Table 4. Risk of Developing Grade II-IV Acute Graft-versus-Host Disease by Univariate Analysis

Variable	Odds Ratio 95%CI	*P*-Value
Conditioning intensity		
Nonmyeloablative	1.0	
Myeloablative	2.44 (0.7-9.13)	.18
HLA match		
<6/6	1.0	
6/6	0.27 (0.07-1.05)	.059
Disease		
Nonmalignant	1.0	
Malignant	3.7 (0.95-14.09)	.059
Day +7 i.v. MMF PK C_{max}	1.1 (0.96-1.27)	.1722

MMF indicates mycophenolate mofetil; CI, confidence interval.

of 0.54 L/h·kg, MPA C_{max} of 11.4 mg/L, MPA trough of 0.61 mg/L, MPA C_{ss} of 4.84 mg/L and MPA $T_{½}$ of 0.87 hours [27]. In this study there was a suggestion of increased toxicity without improved efficacy in the every 6 hours dosing level. In contrast, we utilized twice the dose of MMF in pediatric AlloSCT recipients following both MA versus NMA conditioning and demonstrated a mean AUC_{0-6} of 29.77 versus 31.18 mg h/L, mean MPA clearance of 1.55 versus 1.15 L/h·kg, mean MPA C_{max} of 15.12 versus 13.98 mg/L, a mean MPA trough of 0.53 versus 0.69 mg/L, mean C_{ss} of 5.2 versus 5.9 mg/L, and a mean $T_{½}$ of 1.2 versus 2.26 hours, respectively (Table 4).

There has been 1 other MMF PK study performed in pediatric AlloSCT recipients following MMF dosing of 15 mg/kg i.v. every 8 hours by Jacobson et al [26]. Jacobson et al. [26] reported MMF PK results in 19 pediatric AlloSCT recipients ≤10 years of age following MA conditioning (n = 15) and in NMA conditioning (n = 4). Steady-state PK sampling was performed at 2 different time points (between day +3 and +7 [week 1], and repeated between day +10 and +14 [week 2]). In comparison to our study, Jacobson et al. [26] reported a median MPA C_{ss} of 1.6 mg/L, total MPA trough concentration of 0.27 mg/L, and median MPA C_{max} of 5.0 mg/L (median of combined values for week 1 and week 2 post-AlloSCT) [26]. Similarly to our study, there was considerable interpatient variability in AUC and MPA trough values. The lower MPA C_{ss}, C_{max}, and MPA trough values reported in Jacobson et al. [26] study are explained by significantly lower doses of MMF (15 mg/kg dose is approximately equivalent to 450 mg/m^2) and longer frequency interval (8 hours versus 6 hours in our study).

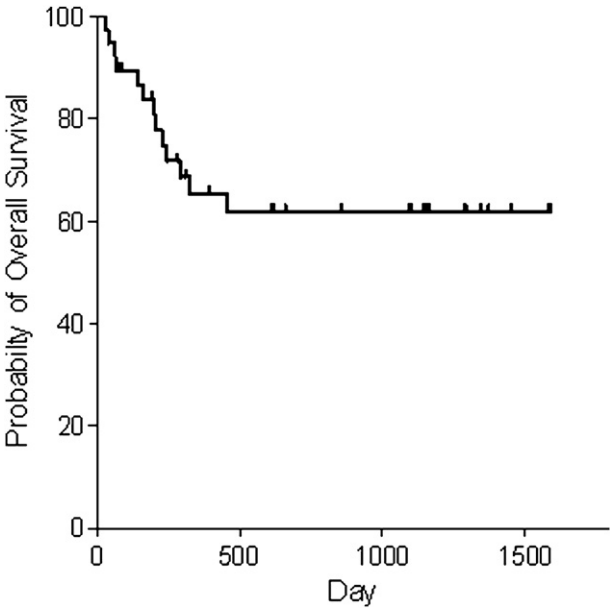

Figure 2. Probability of OS. Probability of OS determined by Kaplan Meier method in all patients following AlloSCT.

Furthermore, our study failed to demonstrate any relationship between MMF PK following MA conditioning, versus NMA conditioning, which is consistent with reports in adult AlloSCT recipients [27,30,31]. One of the reasons we did not see a difference in MMF PK based on a regimen intensity is because the majority of our reduced-intensity regimens were rather more of moderate intensity (based on 4 days of Bu plus Flu or high-dose Cy plus Flu, rather then traditional reduced-intensity of 200 cGy TBI/Flu used in the adult studies), which are capable of producing a significant amount of Grade I-II gastrointestinal toxicity, thus, still potentially affecting enterohepatic recycling. This is a potential limitation of our study. Our study did demonstrate a significant increase with time in MMF PK from day 7 to day 14. We demonstrated a significant increase in MPA AUC and MPA C_{max} on day 14 versus day 7, which resulted from a significant decrease in CL_{ss} and V_{ss} on day 7 versus day 14. The detailed mechanism(s) responsible for this time-dependent exposure increase in this patient population is not known. This could be the result of the influence of several factors including an increase in mucosal healing following conditioning that leads to improved drug absorption and to increased enterohepatic recirculation on day 14 versus day 7. Another factor that could contribute to the time-dependent clearance decrease of MPA is the presence of a hypermetabolic/hypercatabolic state in many of these patients, which causes increased drug clearance initially but which normalizes with time [42,43]. Furthermore, there was a significant increase in CL_{ss}, V_{ss}, and T_{max} in children ≤12 years versus the older age group. This preliminary age effect should be confirmed in a larger pediatric cohort.

Another potential limitation of the current study is that we did not monitor prospectively for other drug interactions with MMF besides those mentioned in the Methods section of this article. There may have been patients receiving concurrent metronidazole, fluoroquinolones, proton pump inhibitors, or ganciclovir, all of which can either decrease or increase MPA concentrations.

Table 5. Adult Allogeneic Stem Cell Transplant Mycophenolate Mofetil PK

Author/ Reference	N	Conditioning MA/NMA	MMF Dosing/Interval (mg/kg)	MPA AUC (mg h/L)	C_{ss} (μg/mL)	MPA CL (L/h·kg)	MPA C_{max} (mg/L)	MPA C_0 (mg/L)	MPA $T_{1/2}$ (h)
Jenke et al. [29]	15	MA	12.5-17mg/kg every 12hours	19-25	Not done	N/A	8.6-11.7	0.07-0.34	1.9-2.5
Nash et al. [30]	46	MA	15mg/kg every 6, 8, and 12hours	16-34	1.19-4.84	0.4-1.0	5.9-12.7	0.13-0.64	0.8-1.48
Giaccone et al. [28]	80	NMA	15mg/kg every 8, 12hours	6-45	1.9-3.1	N/A	1.0-29	0.1-22	1.0-7.9

PK indicates pharmacokinetic; MA, myeloablative; NMA, nonmyeloablative; MMF, mycophenolate mofetil; h, hour; MPA, mycophenolic acid; AUC, area under the curve; C_{ss}, steady-state concentration; CL, clearance; N/A, not available; C_{max}, maximum concentration; C_0, trough concentration; $T_{1/2}$, half-life.

The probability of developing grade II-IV aGVHD and cGVHD was 54% (95% CI: 37%-69%) and 34% (95% CI: 15%-52%) in this study, respectively. Although we utilized a higher dose of MMF prophylaxis (900 mg/m^2 every 6 hours), we were not able to demonstrate a decrease in the incidence of grade II-IV aGVHD compared to other pediatric AlloSCT trials [4]. Furthermore, although the numbers were small, i.v. MMF PK in aggregate and on days +7 and +14 has no independent effects on the rate of aGVHD in our multivariate analysis.

In summary, we have demonstrated the safety and PK of both i.v. and p.o. MMF (900 mg/m^2 every 6 hours) following MA and NMA conditioning in pediatric AlloSCT recipients. There was a significant increase in MPA $AUC_{0\text{-}6hr}$ and C_{max} associated with a significant decrease in MPA CL_{ss} and V_{ss} following i.v. MMF on day +14 versus day +7. Furthermore, children <12 years old appear to have a significant increase in CL_{ss}, V_{ss}, and T_{max} compared to 12 to 16 years age groups following i.v. MMF administration. There did not appear to be age differences following p.o. MMF administrations; however, the numbers were too small to draw any firm conclusions. There were no significant differences in i.v. MMF PK following MA versus NMA conditioning. In general, this increased dose of MMF was well tolerated in children. In patients exhibiting signs and symptoms of MMF toxicity (gut or BM) we would recommend obtaining MMF trough concentrations and adjusting doses when elevated MPA levels are present. Monitoring of MMF trough concentrations should be considered in patients at high risk of gut GVHD or when gut absorption is impaired, with consideration of adjustment of dose or frequency of MMF administration when MPA levels are undetectable. Ideally, steady-state levels (aiming for concentrations ≥2.5 μg/mL) [30] should be monitored over trough concentrations as they are more accurate, but the authors recognize that this is not always feasible under nonresearch conditions. Future studies will need to be performed to determine the precise MPA C_0, AUC and/or C_{ss} required to best prevent aGVHD following AlloSCT in pediatric recipients. Last, these results suggest because of a significant interpatient variation, individualized MMF PK measurements may be required in selected pediatric AlloSCT recipients if clinically indicated post transplantation, because the optimal MMF dose and frequency after AlloSCT remains to be elucidated.

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