OPTIMIZATION OF MYCOPHENOLATE MOFETIL DOSING IN TRANSPLANT PATIENTS

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Mycophenolate Mofetil (MMF) is an immunosuppressive pro-drug of mycophenolic acid (MPA), first approved by the US Food and Drug Administration (FDA) in 1995 for the prevention of rejection post solid organ transplantation (SOT). A narrow therapeutic index, poor predictability of exposure from trough drug level monitoring, large inter transplant as well as inter and intra subject variability and lack of guidelines for optimal dosing are the primary reasons that make it very crucial to apply pharmacokinetic (PK) and pharmacodynamic (PD) concepts to individualize the dosing of MPA in transplant sub-populations. A target range of the active form, MPA measured by the area under the concentration time curve (AUC) in renal transplant recipients of 30-60 mg*h/L has been shown to be associated with better graft and overall survival. This work attempts at optimizing dosing of MMF in pediatric HSCT recipients and in adult lung transplant recipients by using different dosing regimens, therapeutic drug monitoring and modeling approaches. We evaluated the safety and feasibility of a personalized pharmacokinetics-based dosing approach in pediatric HSCT patients using a novel continuous infusion (CI) method of administration of MMF to improve MPA exposure.

We further evaluated the feasibility of a PK and PD focused dosing of MMF in adult lung transplant (LT) recipients. Total plasma concentrations of MPA measured by a UPLC-MS assay were inversely related to the inosine-5′-monophosphate dehydrogenase (IMPDH) activity, with the lowest activity being seen at the maximum MPA concentration. In a pilot study conducted in LT recipients, although statistically not significant, the pre-dose IMPDH activity at the bronchoscopy visit tended to be higher in

patients who rejected compared to those who did not have any rejection episodes. Finally, a robust physiologically based pharmacokinetic (PBPK) model of MPA was built and validated in healthy Caucasian and Chinese volunteers, pediatric patients, kidney transplant recipients and patients with varying degree of renal impairment with an intent to apply this in clinical practice to lung and pediatric HSCT patients. The promising results from this work can serve as the foundation for future studies optimizing the use of MPA in transplant patients.

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ABBREVIATIONS

Ac-MPAG	Acyl mycophenolic acid glucuronide
ACR	Acute cellular rejection
ADAM	Advanced dissolution absorption model
aGVHD	Acute graft-versus-host disease
AMP	Adenosine monophosphate
AR	Acute rejection
ASA	Automated sensitivity analysis
ATG	Antithymocyte globulin
AUC	Area under the concentration-time curve
$AUC_{0-\infty}$	Area under the concentration-time curve between time of drug administration and infinite time
BCS	Biopharmaceutical classification system
BID	Twice daily
BOS	Bronchiolitis obliterans syndrome
BPAR	Biopsy proven acute rejection
CC	Concentration controlled
CI	Continuous infusion

CL	Total systemic clearance
CLint	Intrinsic clearance
Cmax	Maximum concentration
CNI	Calcineurin inhibitor
CsA	Cyclosporine
C_{ss}	Steady state concentration
CV	Coefficient of variance
DDI	Drug-drug-interaction
DLCO	Carbon monoxide diffusing capacity
EC-MPS	Enteric coated mycophenolate sodium
EDTA	Ethylenediaminetetra acetic acid
EHC	Enterohepatic circulation
EMA	European Medicines Agency
ES	Electron spray
FDA	Food and Drug Administration
FEV1	Forced expiratory volume in 1 second
FMW	Fractional difference in molecular mass
FVC	Forced vital capacity
GFR	Glomerular filtration rate
GI	Gastrointestinal
GVHD	Graft-versus-host disease
GVT	Graft-versus-tumor

HLA	Human leukocyte antigen
HPLC	High-performance liquid chromatography
HSCT	Hematopoietic stem cell transplantation
IC_{50}	Half maximal inhibitory concentration
IMP	Inosine monophosphate
IMPDH	Inosine-5′-monophosphate dehydrogenase
IRB	Institutional Review Board
IS	Internal standard
ISEF	Inter-system extrapolation factors
IVIVE	In vitro-in vivo extrapolation
K_a	Absorption rate constant
K_p	Tissue-to-plasma partition coefficient
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LLOD	Lower limit of detection
LLOQ	Lower limit of quantification
LSS	Limited sampling strategy
LT	Lung transplant
LTR	Lung transplant recipient
MAP-BE	Maximum a posteriori Bayesian estimator
MMF	Mycophenolate mofetil
MPA	Mycophenolic acid
MPAG	Mycophenolic acid glucuronide

MRM	Multiple reaction monitoring
MRP	Multidrug resistance protein
NAD+	Nicotinamide adenine dinucleotide
NCA	Non-compartmental analysis
P_{app}	Apparent permeability
PBMC	Peripheral blood mononuclear cell
PBPK	Physiologically based pharmacokinetics
PBS	Phosphate buffered saline
PD	Pharmacodynamic
$P_{eff, man}$	Predicted human permeability
PK	Pharmacokinetic
PO	Oral
QC	Quality control
RAF	Relative activity factor
RMSE	Root-mean-square error
RSE	Relative standard error
SNP	Single nucleotide polymorphism
SOT	Solid organ transplantation
$t_{1/2}$	Half-life
TAC	Tacrolimus
TDM	Therapeutic drug monitoring
T_{max}	Time to Cmax

UGT	Uridine diphosphate glucuronosyltransferases
UPLC-MS/MS	Ultra-performance liquid chromatography coupled with mass spectrometry
UPMC	University of Pittsburgh Medical Center
V_{ss}	Volume of distribution at steady-state
XMP	Xanthosine monophosphate
λ_z	Terminal elimination rate constant

PREFACE

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1.0 INTRODUCTION

1.1 IMMUNOSUPPRESSIVE DRUGS AFTER SOLID ORGAN TRANSPLANTATION

Organ transplantation is the treatment of choice for end-stage organ disease, autoimmune and several genetic disorders. Treatment outcomes in particular acute rejection and infection rates and graft survival of solid organ and stem cell transplantation have dramatically changed over the past decade, primarily due to improvements in immunosuppression and to better medical care post-transplantation. Immunosuppressive drugs form an integral part of the pharmacotherapy. Over the years, the use of induction and maintenance therapy has evolved [1].

Table 1 presents an overview of the currently used immunosuppressive drugs in transplantation[2]. These drugs act on different targets in the immune system and primarily classified as induction therapy and maintenance therapy agents.

1.1.1 Induction regimen

Induction therapy as the name suggests incudes agents used early in transplantation typically intraoperatively or immediately post-transplantation. The goal of induction therapy is to prevent acute rejection by providing a high degree of immunosuppression in the early post transplantation period.

Biological agents are the most normally used for induction therapy. Antithymocyte globulin is the most frequently used induction agent post kidney transplantation. Other induction

therapy agents include basiliximab, daclizumab and alemtuzumab. Induction therapy, by providing a high degree of immunosuppression and thereby delaying the use of calcineurin inhibitors plays an important role in the prevention of CNI-induced nephro-toxicity after transplantation[3] . No standard induction immunosuppressive regimen exists for patients undergoing renal transplantation and the choice of regimen often depends on the preferences of the center and the clinicians.

1.1.2 Maintenance regimen

This is typically initiated post-transplant and often consists of a combination of 2-3 immunosuppressive agents. Calcineurin inhibitors like tacrolimus or cyclosporine form an integral part of maintenance immunosuppression. Tacrolimus and mycophenolate derivatives have largely replaced the use of cyclosporine and azathioprine (AZA), respectively, and are the preferred CNI and antimetabolite agent for maintenance therapy in solid organ and stem cell transplantation. Mycophenolic acid derivatives- the pro-drug mycophenolate mofetil (MMF) and the delayed release enteric coated mycophenolic acid in combination with a CNI have demonstrated lesser risk of early and late allograft rejection, better toxicity profiles and have also prolonged graft survival and hence have become the standard of care in most transplant centers.

Table 1. Overview of immunosuppressive drugs currently used in solid organ transplantation

Brand Name	Drug	Regimen	Comments
Deltasone	Prednisone	Induction/Maintenance	Associated with severe side effects
Imuran	Azathioprine	Maintenance	one-year graft survival of around 70%, higher toxicity
Cellcept/Myfortic	Mycophenolic acid	Maintenance	Similar efficacy as azathioprine, better toxicity profile
Neoral	Cyclosporine	Maintenance	Form backbone of maintenance immunosuppressive regimen Tacrolimus has a lower risk of acute rejection and allograft loss than cyclosporine
Prograf/ Advagraf	Tacrolimus	Maintenance	
Certican	Sirolimus	Maintenance	Place in maintenance immunosuppression still under investigation; often used to limit CNI nephrotoxicity
Rapamune	Everolimus	Maintenance	
	ATG	Induction	Oldest available drug for induction therapy
Mab Campath	Alemtuzumab	Induction	Similar to ATG while less toxic
Simulect	Basiliximab	Induction	Less effective and less toxic than ATG
Nulojix	Belatacept	Maintenance	Promising new agent under investigation

1.2 MYCOPHENOLATE MOFETIL AND ITS USE IN SOLID ORGAN AND STEM CELL TRANSPLANT PATIENTS

Immunosuppressive agents play a critical role in prevention of graft rejection post solid organ as well as GVHD in stem cell transplantation. Calcineurin inhibitors like cyclosporine (CsA) and tacrolimus (TAC) have formed the backbone of a typical immunosuppressive regimen. MMF an anti-proliferative agent was approved by the FDA in 1995 for prophylaxis of organ rejection (accessed from: https://www.gene.com/download/pdf/cellcept_prescribing.pdf). Its approval was based on data from three clinical trials conducted in de-novo kidney transplant recipients at a fixed dose of 1000 mg or 1500 mg administered twice daily for prevention of acute rejection in adult renal transplant recipients. [4-6] It has gained widespread acceptance in all solid organ transplants because of a better toxicity profile and better overall outcomes as compared to azathioprine. [7] The primary mode of action of MPA, the active component of MMF is by inhibition of cell cycle in the S phase by competitive and reversible inhibition of the enzyme IMPDH, which is involved in the conversion of inosine monophosphate (IMP) to xanthosine monophosphate (XMP). Unlike other cell types, T and B Lymphocytes are primarily dependent on this pathway and cannot use the alternative salvage pathway for purine synthesis and proliferation.

1.3 FORMULATIONS OF MYCOPHENOLIC ACID

MMF is a prodrug of the active form MPA and was primarily designed to improve the oral bioavailability of MPA. The morpholinoethyl ester derivative of MPA showed improved solubility at gastric pH < 5 indicating rapid *in-vivo* dissolution in the upper gastrointestinal (GI) tract. [8] Mycophenolate mofetil doses can be converted to equivalent MPA content by multiplying by a factor of 0.739. It is available for oral administration as capsules (250mg), tablets (500mg) and as a powder for suspension (200 mg/mL when constituted). MMF is also available in the intravenous form for patients who are unable to swallow the oral dose, especially during the immediate post-transplant period. It is marketed by Genentech, a member of Roche group.

MPA is also available as a delayed release enteric coated tablet marketed as Myfortic by Novartis Pharmaceuticals. It is available at 180 mg and 360 mg doses. A 720-mg dose of Myfortic (2 x 360 mg) closely approximates the MPA exposure from 1000 mg MMF. [9]

1.4 CLINICAL PHARMACOKINETICS OF MYCOPHENOLIC ACID

Pharmacokinetics of mycophenolic acid has been studied in solid organ transplant patients especially in kidney and liver transplant recipients. Limited data is available on the pharmacokinetics of this drug in other SOT patients and HSCT patients. The pharmacokinetics of this drug shows high inter as well as intra-patient variability. Although several studies report differences in the pharmacokinetics across different transplant patients, MMF is given at a fixed flat dose and is not routinely therapeutically monitored. Different physiological changes occurring as a result of transplantation could alter the exposure of the drug and thereby contribute to the observed variability in MPA pharmacokinetics in these patient populations.

1.4.1 Absorption

Mycophenolic acid is a highly lipophilic drug. Based on biopharmaceutical classification system (BCS) it is a class II drug with high permeability, but low solubility. MMF is rapidly hydrolyzed following oral administration to MPA, reaching undetectable concentrations in plasma within 30 minutes.[10-12] The maximum plasma concentration of MPA is attained within 2 hours after oral dosing. The oral dosage form has a high bioavailability (~96%) leading to a 1:1 dose conversion when switching a patient from an intravenous to oral dosing. Enteric coated mycophenolate sodium (EC-MPS), the delayed release formulation of MPA, attains peak concentrations much later, around ~4 hours. [13] In-vitro dissolution studies of EC-MPS show high solubility at neutral pH as observed in intestine but with limited solubility in the gastric pH. The manufacturer reported bioavailability of EC-MPS to be 72%. Poor post-operative

absorption as seen in certain solid organ transplant recipients can lead to reduced bioavailability of MPA. Any change in the activity or expression of the esterases that are responsible for hydrolysis of MMF can also potentially lead to incomplete absorption of MPA after oral administration of MMF.

1.4.2 Protein Binding and Distribution

Mycophenolic acid has a blood to plasma ratio of 0.59, indicating that it is distributed primarily in the plasma and partitions to a lesser extent in the cell fractions of blood, and supports the rationale of measuring mycophenolic acid levels in plasma. [14, 15] It is more than 95% bound to the plasma protein, albumin. Only the free, unbound form is available for anti-proliferative activity. An *in-vitro* study has shown that an increase in serum albumin from 2 to 4 g/dl serum can decrease the free fraction from approximately 3 to 1.5%[15]. The volume of distribution of MPA at steady state is not significantly different between the intravenous (3.6 L/kg) and oral administration (4L/kg), suggesting that the oral bioavailability of mycophenolic acid is close to 100%. The binding of MPA to albumin depends on the availability of albumin and the competition of MPA with the glucuronide metabolite MPAG, (which is also highly protein bound) for the binding site in albumin. Disease state can have a significant impact on the protein binding and distribution of MPA. Albumin concentrations are low in patients with renal and liver impairment and in the early post-transplant period.

Mycophenolic acid is an intermediate clearance drug with an extraction ratio ranging from 0.3-0.7[16, 17]. For an intermediate clearance drug, factors that may influence its clearance include protein binding, intrinsic enzymatic activity of the liver, and the blood flow to the liver.

Renal impairment seen post-transplant can lead to increase in MPAG levels due to accumulation of MPAG which then leads to displacement of MPA from the binding sites on albumin leading to an increase in the free fraction of MPA and corresponding increase in MPA distribution and clearance. Hepatic impairment can lead to decreased serum albumin concentration and thereby increase the free fraction of MPA resulting in increased clearance. In liver transplant recipients, the concentration of albumin increases with time post transplantation. Pisupati et al. have shown that plasma protein binding of MPA is increased as the concentration of albumin is increased and as the concentration of bilirubin decreased with time post-transplantation[18]. The concentration of MPAG is about 20-100-fold higher than MPA. De Winter et al. conducted a study to evaluate the effect of protein binding on the pharmacokinetics of mycophenolic acid in renal transplant recipients. The results of this study showed that a decrease in plasma albumin concentrations from 0.6 to 0.4 mmol/L, resulted in a decrease in total MPA concentrations, with a negligible effect on free MPA, total MPAG and free MPAG concentrations. The free fraction of both, MPA and MPAG are inversely related to the albumin concentration and were seen to double when albumin concentrations decreased from 0.6 to 0.4 mmol/L. The effect was magnified in patients who were on cyclosporine (MPA: 2.4–5.3%, MPAG: 13.7–26.1%) based immunosuppression therapy as compared to those on tacrolimus (MPA: 2.7–4.1%, MPAG: 14.9–21.3%). The study also showed a decrease in MPA exposure as calculated by the AUC based on total MPA in patients with low albumin concentrations. [19]

1.4.3 Metabolism and Elimination

Mycophenolic acid is primarily metabolized by phase II pathway involving uridine diphosphate glucuronosyltransferases (UGTs) enzymes to MPAG and acyl glucuronide metabolites MPAG and Ac-MPAG metabolites (Figure 1). The plasma concentration of MPAG is about 20-100-fold higher than MPA. MPAG has no activity against IMPDH, but Ac-MPAG has been shown to have some activity against IMPDH, in-vitro.[20, 21] Glucuronidation of MPA primarily occurs in the liver and to certain extent in the kidney and GI tract.[22] UGT1A8 and UGT1A9 and to a minimal extent UGT1A1, 1A7 and 1A10 are involved in the formation of the major metabolite, MPAG. UGT1A8 and 1A10 are primarily responsible for the metabolism of MPA in the GI tract, while UGT1A9 is primarily responsible for hepatic metabolism. Ac-MPAG, the secondary metabolite of MPA is generated by UGT2B7. Due to their high hepatic and renal expression UGT1A9 and UGT2B7 are considered the major isoforms of involved in the metabolism of

MPA[23].

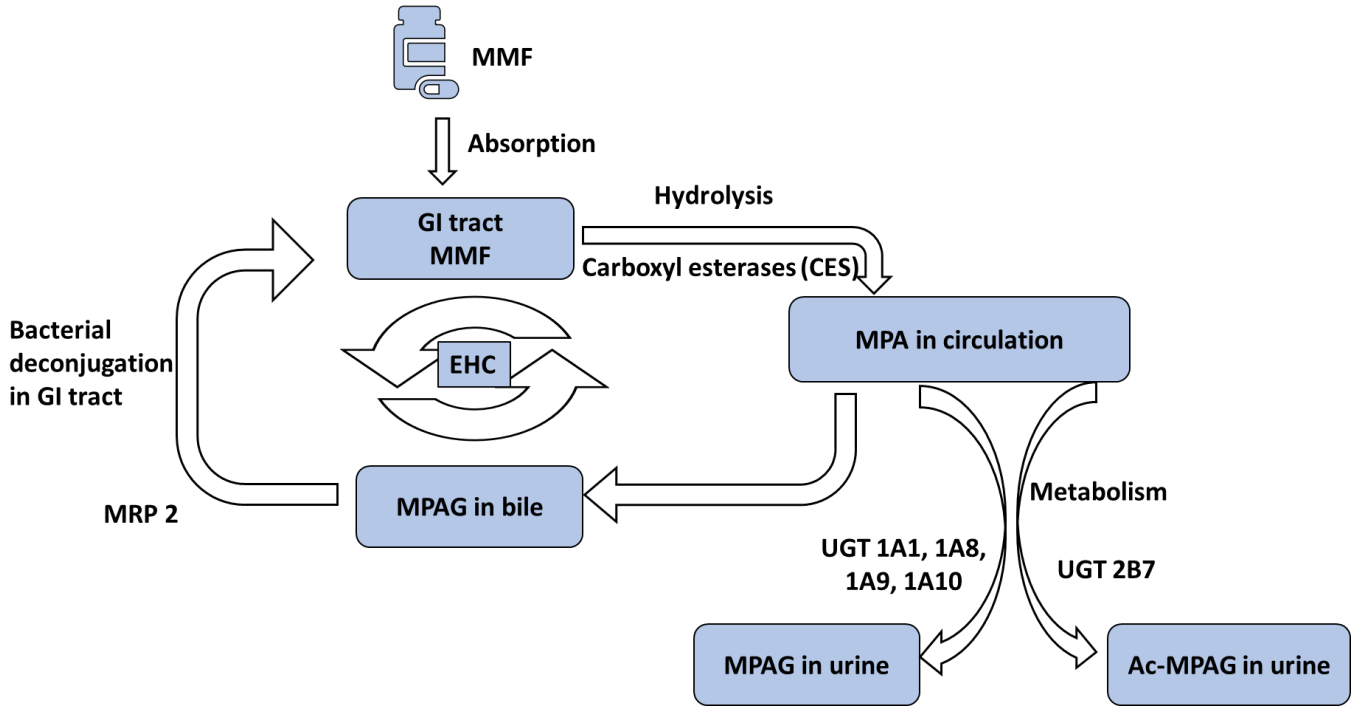


Figure 1. Schematic representation of fate of MMF in human body

EHC: enterohepatic circulation; GI: gastrointestinal; MMF: mycophenolate mofetil; MPA: mycophenolic acid; MPAG:mycophenolic acid glucuronide; Ac-MPAG: Acyl mycophenolic acid glucuronide

Clinical studies conducted with radiolabeled mycophenolate mofetil indicate that 87% of the dose of MPA is excreted in the form MPAG in urine and 6% in faeces as MPA. Excretion of MPAG and Ac-MPAG in the urine is impacted by tubular reabsorption. [10] MPA and Ac-MPAG are detected in small proportion in the urine. The elimination half-life of mycophenolic acid is 16-18 hours in healthy volunteers, and ranges between 8-16 hours in solid organ transplant patients.

The hepatic clearance can be explained by the following equation:

Hepatic Clearance: $Cl_h = Q [(f_u \times Cl_{int})/(Q+ f_u \times Cl_{int})]$

Where,
Cl_h = Hepatic clearance

Q = hepatic blood flow

f_u = fraction of free drug (not bound) and

Cl_{int} = intrinsic capacity of the hepatocytes to metabolize a drug.

For intermediate clearance drugs clearance is determined by the fraction unbound and the intrinsic clearance. Changes in fraction unbound can lead to changes in the total concentration of MPA but does not affect the unbound MPA concentrations.

HSCT recipients show a significant difference in pharmacokinetics of MPA compared to SOT patients. The elimination half-life of MPA is reported as 2-3 hours in adult as well as in pediatric stem cell transplant recipients. Winter et al studied the differences in the clearance of mycophenolic acid across SOT, HSCT patients and patients with autoimmune disease. [24] The MPA clearance was much higher in HSCT patients (45.6 L/h) as compared to other SOT recipients (30.2 L/h) and patients with autoimmune disease (10.7 L/h).

1.4.4 Enterohepatic recycling

Multiple peak phenomenon is seen in the concentration time profiles of several compounds. As discussed in a detailed review by Davies et al[25], this can occur as a consequence of several factors. In case of mycophenolic acid, the secondary peaks occur between 6-8 hours post oral dose. MPA is converted to MPAG in the liver which is secreted into the bile via multi-drug resistance protein (MRP-2) transporter. The MPAG in the gut is then converted to MPA by the

gut bacteria and the reabsorption of MPA results in the secondary peak. The percent contribution of enterohepatic recycling towards the overall MPA exposure is very variable and can range between 10-60% [26-28].

Transplant recipients who are on concomitant cyclosporine dosing have decreased MPA exposure. This occurs as a result of cyclosporine mediated inhibition of biliary secretion of MPAG mediated by MRP2. [29, 30] External factors including but not limited to the type of food taken and the time of food intake can also influence MPA enterohepatic circulation (EHC), further complicating the prediction of MPA pharmacokinetics. Modeling approaches for describing the EHC of MPA have evolved over time. [27, 31-33] Typical empiric compartment models, either one or two compartment models with first order absorption have been used to describe the EHC of MPA. More complex absorption model with inclusion of lag time have been used to describe the EHC. Such models are believed to account for the gall bladder emptying. [34] Other models like the Erlang and transit absorption models are used to describe skewed or delayed absorption profiles. Erlang model successfully elucidates the changes associated with MPA disposition and physiological aspects associated with it. This model includes MPA and its glucuronide metabolite MPAG, and adequately reflects the complex enterohepatic circulation of MPA. [35-37]

1.5 VARIABILITY IN PHARMACOKINETICS OF MYCOPHENOLIC ACID IN TRANSPLANT PATIENTS

Regardless of the dosage form or the type of patient population, large inter- and intra-individual variability in the plasma concentration of mycophenolic acid and its metabolite plasma has been documented in the literature. Several factors have been demonstrated to be significantly associated with the variability in the pharmacokinetics of mycophenolic acid. Physiological factors like albumin concentration, renal and hepatic function as also age, enzyme ontogeny and other factors including drug- drug interactions, contribute to changes in the MPA exposure and clearance. [38] This extreme variability in the exposure calls for routine monitoring of MPA exposure and dose adjustments should be made accordingly to achieve optimal clinical outcomes. Unfortunately, currently there is no personalized dosing approach that is routinely used for MPA in transplant patients.

1.5.1 Age

Clearance of MPA is more rapid in children under the age of 12 years, and therefore higher doses based on body weight basis are required to achieve similar mycophenolic acid exposure compared to that in adults. Population pharmacokinetic studies conducted in pediatric solid organ as well as stem cell transplant have shown age of the patients to have a significant effect on the clearance of MPA.[39, 40]

1.5.2 Liver dysfunction

MPA is primarily metabolized in liver by phase II enzymes. Hepatic impairment is known to alter the pharmacokinetics of MPA. Parker et al.[41] have shown that in patients with hepatic impairment, the serum albumin level is decreased leading to an increase in the unbound or free fraction of MPA, and thereby increasing MPA clearance. In another study conducted in renal transplant patients, mild hepatic impairment seen in the immediate post-transplant phase, resulted in a significantly decreased exposure of total MPA as measured by the area under the concentration time curve (34.3 ± 22.3 mg*h/L vs. 50.2 ± 27.4mg*h/L, p = 0.01 [mean ± SD]) and by trough concentration (1.43 ± 1.07mg/L vs. 2.35 ± 1.62 mg/L, p = 0.03).[42]

1.5.3 Renal dysfunction

Delayed graft function is seen especially in patients receiving cadaveric kidney transplantation.[43-45] A study conducted by Johnson et al in healthy volunteers with varying degree of renal impairment indicated that MPA clearance is unaffected by renal function after a single oral dose of MMF.[46] Most studies conducted in kidney transplant patients however have shown a decrease in MPA exposure in patients with impaired renal function in the immediate post-transplantation period. Changes in pharmacokinetics of MPA post kidney transplantation are primarily time dependent. In studies that have reported a decrease in MPA exposure have proposed that excessive accumulation of MPAG, due to impaired graft function is the reason for decreased MPA exposure. The excess MPAG displaces the albumin bound MPA,

making more MPA available for metabolism by the phase II enzymes. The improvement in renal function in the first months after kidney transplantation and the subsequent increase in the albumin concentration contribute to the progressive increase in MPA dose–corrected exposure seen in the early phase post kidney transplantation. The slow rise in the serum albumin levels after transplantation, leads to reduced free MPA fraction and decreased MPA clearance. Contrasting results were observed in another study in renal transplant recipients with a stable graft function. An inverse relationship was observed between MPA exposure and renal function. The study suggested two plausible but unproven mechanisms via which this increase in exposure is mediated. Firstly, the increased MPAG concentrations in patients because of the impaired renal function can increase MPAG excretion into the bile, leading to increased enterohepatic recycling of MPA and subsequently increased MPA exposure. The other possible reason could be decreased metabolism of MPA by inhibition of UGT probably due to uremic toxins, which can reduce the hepatic clearance of MPA.[47]

1.5.4 Effect of Food and Gastric Emptying

In a study conducted by Bullingham et al in patients with rheumatoid arthritis, food was shown to decrease the maximum concentration (C_{max}) and increase the time required to attain the C_{max} but did not significantly alter the overall exposure of MPA. The time for the occurrence of secondary peak occurring due to enterohepatic recycling was also delayed by 2 hours, suggesting slowed gastric emptying.[48] A clinical trial conducted in kidney transplant patients to study the effect of gastric emptying on the pharmacokinetics of MPA showed similar results.[49] Pharmacokinetic analysis of MPA was performed with simultaneous measurement of gastric

emptying by breath tests using 14C-octanoate/13C-glycine. Delayed gastric emptying was associated with a slower rate of absorption of MPA. The study cautions researchers to account for this effect when developing and validating limited sampling strategies for monitoring MPA exposure in patients with gastric disorders.

1.5.5 Drug-drug interactions

Drug interactions with MPA could result from UGT induction or inhibition or induction or inhibition of MRP2, a transporter involved in the enterohepatic recycling of MPA.Rifampin mediated UGT enzymes induction in the kidney, liver and intestine was first reported in a case study as a possible reason for the decreased MPA exposure when given to patients on MMF [50].

Another study conducted in 8 kidney transplant recipients showed that the MPA 12 hours exposure decreased significantly after rifampin co-administration (17.5% decrease [95% CI, 5.18%-29.9%]; P=.0234). This was primarily mediated by induction of MPA glucuronidation capacity and increased formation clearance of MPA leading to increased exposure to the glucuronide metabolites MPAG and Ac-MPAG [51].

The findings of a retrospective study conducted by Alvarez et al suggested that patients with elevated sirolimus concentrations are at a higher risk for MMF toxicity, while those with lower sirolimus exposure are at a higher risk for [52] rejection suggested a potential DDI between sirolimus and MPA; however the mechanism is still unclear.

Cyclosporine is known to inhibit the EHC of MPAG in the gut leading to decreased MPAG conversion to MPA and thereby decreasing MPA exposure. A 33% increase in clearance

was observed in stem cell transplant patients receiving cyclosporine as compared to those receiving tacrolimus [53].

A study conducted in rheumatoid arthritis patients suggested drug-drug interaction between antacids and MPA; chelation and reduced absorption was suggested as a possible mechanism for the reduced exposure (C_{max} and AUC) of MPA and MPAG due to reduced absorption [48].

Studies conducted with antibacterial agents such as norfloxacin, metronidazole in healthy volunteers suggested a decrease in MPA exposure of 10%, 19% respectively and a 33% reduction for the combination of norfloxacin and metronidazole [54].

The only known report of an increase in MPA exposure is seen in a patient when MMF was given with valproic acid, with inhibition of UGT2B7 as the potential cause of this interaction [55].

Table 2(adapted from Jaklic et al.) [56] summarizes the potential drug-drug-interaction (DDI) affecting MPA pharmacokinetics.

Table 2. Potential DDI affecting MPA pharmacokinetics.

Category	Drug	Effect on MPA Pharmacokinetics	Reference
Immunosuppresant	Cyclosporine	Increase in MPA clearance by 33%	Li et al[53]
Proton Pump Inhibitor	Pantoprazole	Decrease in MPA AUC by 37%	Kofler et al[57]
	Omeprazole	Decrease in MPA AUC by 23%	Kees et al[58]
	Lansoprazole	Decrease in MPA AUC by 25%	Miura et al[59]
Antibiotics	Ciprofloxacin	Decrease in MPA trough concentration by 46%	Goutelle et al[60]
	Amoxicilin/Clavulanic acid	Decrease in MPA trough concentration by 46%	Ratna et al[61]
Anti-epileptic	Valproic acid	Increase in AUC	Annapandian et al[55]

DDI: drug-drug-interaction; MPA: Mycophenolic Acid; AUC: Area under concentration-time curve

1.5.6 Chronopharmacokinetics

There are two publications discussing the chronopharmacokinetics of MPA. A study conducted by Tedesco- Silva H Jr [62] suggested circadian variation in PK as seen by a marked decrease in MPA exposure (C_{max} and AUC) and increase in oral clearance during nighttime as compared to daytime. The effect was greater when MPA was administered as EC-MPS in combination with cyclosporine as compared with everolimus (32% versus 17%). Another study conducted by Satoh et al. [63] reported a significantly higher MPA AUC during the daytime as compared to nighttime (P=0.049). Although chronopharmacologic changes may be clinically less relevant for drugs having a wider therapeutic index, it may be more important for drugs having a narrow therapeutic index.

1.5.7 Genetic Polymorphism

1.5.7.1 UGT polymorphism

Glucuronidation is an essential pathway for metabolism of various endogenous compounds and xenobiotics. The 3 UGT sub families, namely 1A1, 2A and 2B encode for a total of 16 enzymes. 6 of these (UGT 1A1, 1A6, 1A7, 2B4, 2B7 and 2B15) have been characterized for genetic polymorphism.[64] Genetic polymorphisms of UGT enzymes are of toxicological, pharmacological and physiological significance. It is the primary pathway of MPA metabolism. UGTs 1A9, 1A8 and 2B7 are involved in MPA metabolism and this occurs in the liver and to a smaller extent in kidney and intestine [65]. Hepatic UGT1A9 is responsible for >50% of MPA

metabolism to MPAG [22, 66]. UGT1A8 and UGT1A10 are predominantly located in the gastrointestinal tract and have a relatively smaller contribution to disposition of MPA in humans.

The first study exploring the impact of genetic polymorphism of UGTs on MPA exposure was reported in 95 Caucasian kidney transplant recipients, who were on tacrolimus and steroid therapy [64]. The study showed a 50% reduction in the average 12-hour exposure in patients who were either carriers of UGT1A9 – 275T>A or – 2152C>T or both, compared to the non-carriers of this polymorphism. These single nucleotide polymorphisms (SNPs) were associated with a 15-43% reduction in exposure in a long term study spanning over 5 years [67] and in another study a 26% reduction in exposure was observed. [68].

Other UGT1A9 SNPs that are studied include −98T>C (UGT1A9*3), −440C>T and −331T>C. The SNP UGT1A9*3 was associated with higher MPA exposure (AUC or trough concentrations) in patients treated with MMF. The study reported 49% and 54% higher MPA AUC in patients on tacrolimus and cyclosporine, respectively compared to the corresponding non-carriers [69]. Other studies have also reported similar observations [64, 70, 71].

Clinical outcomes like acute rejection were often examined as a secondary outcome in most studies. The authors who studied UGT1A9 – 275T>A or – 2152C>T SNPs also studied UGT2B7 SNPs [64], −125C and −138A, and found that SNPs of 1A9 and 2B7 were significantly associated with development of acute rejection in patients treated with cyclosporine.

1.5.7.2 MRP2 polymorphism

MPA gets converted in the liver to MPAG and undergoes MRP-2 mediated biliary secretion. A study conducted in pediatric kidney transplant recipients showed that patients having MRP2-

24T>C who also had UGT1A9-440C>T or UGT2B7-900A>G showed a 2.2 higher dose-dependent and BSA-normalized MPA-AUC compared with carriers of no or only 1 UGT-SNP. [72] Another study [73] showed that patients with the C24T variant allele in MRP2 who were on MMF and concomitant macrolide treatment showed a significantly reduced exposure as measured by AUC as compared to those who were not on macrolide treatment. However, these studies are confounded by concomitant medication and/or effect of UGT polymorphism. There is no substantial evidence of MRP-2 polymorphism mediated impact on MPA PK.

1.5.7.3 IMPDH polymorphism

MPA acts by inhibiting enzymes IMPDH I and II. MPA PK and PD have shown approximately 10-fold variability among transplant patients. Recently published studies in transplant recipients have hypothesized that inter-individual variability in IMPDH activity may be attributed to SNPs in genes coding for IMPDH I and II.

Reports evaluating the relationship of IMPDH genotype to pharmacokinetic and clinical outcomes are contradictory. A study evaluating 8 SNPs of IMPDH in de novo kidney transplant patients identified the SNP, 3757T>C for IMPDH II with a variant allele (C) to be associated with increased IMPDH activity (p=0.04) in de-novo kidney transplant recipients. [74]

For IMPDH I, 2 SNPs, rs2278293 and rs2278294, are significantly associated the incidence of biopsy-proven acute rejection (BPAR) within a year after kidney transplantation [75, 76].In a larger study [77], rs2278294 SNP was significantly associated with lower risk of rejection and a higher risk of leucopenia during the first year post-transplantation.

1.6 APPROACHES FOR OPTIMIZING MMF DOSING IN TRANSPLANTATION

1.6.1 Fixed dose administration

MMF is often administered at a fixed dose. However, there have been several studies that have explored concentration controlled (CC) MMF dosing based on a target MPA exposure. An extensive study conducted by Wang et al [78] included data from 4 randomized controlled trials that included 1755 kidney transplant patients. The results of this study established no significant differences between concentration controlled and fixed-dose MMF for treatment failure (P=0.52), serum creatinine clearance (weighted mean difference, 2.46; 95% CI, -1.15 to 6.07; P=0.18), total gastrointestinal adverse events (P=0.53), diarrhea (P=0.35), anemia (P=0.12), leukopenia (P=0.25), thrombocytopenia (P=0.41), and malignancy (P=0.23).

A similar study was conducted in 901 kidney transplant recipients where patients were randomized to a concentration-controlled group with a target exposure over a dosing interval as measured by AUC 45µg*hr/ml (n=452) or a fixed-flat dose (n=449) of MMF regimen [79]. The number of patients attaining the target MPA 12-hour AUC values of 30 to 60 mg hr/L was comparable between the two groups. The primary endpoint was treatment failure as measured by a BPAR, graft loss, death within first 12 months post-transplantation. This study did not show a significant difference in the incidence of treatment failure or BPAR (P>0.05) between the concentration-controlled and the fixed-dose groups primarily due to non-adherence to dose adjustments required in the immediate post-transplant phase. This study however did show that there is a significant relationship between immediate post-transplant MPA exposure and BPAR , highlighting the importance of attaining target concentrations early on in these patients.

Another study has explored a weight based dosing of MMF in 53 Asian kidney transplant recipients [80]. This study showed that steady state 12-hr AUC is significantly associated with TBW-adjusted MMF dose. An AUC_{0-12} of 45 μg*hr/ml at steady state could be attained with an MMF dose of 12 mg/kg BID dosing.

The OPTICEPT trial [81] compared clinical outcomes of patients who were either on fixed dose MMF-standard dose calcineurin inhibitor (CNI) (A), concentration controlled MMF-standard dose CNI (B) or concentration controlled MMF- low dose CNI (C). Doses were titrated to maintain MPA concentrations greater than 1.3 μg/ml for cyclosporine treated and 1.9 μg/ml for tacrolimus treated patients. Patients in group C had lower rate of treatment failure. Also, patients that had a higher MPA exposure had significantly less rejection. It can be inferred that concentration controlled MMF can be potentially used in CNI sparing immunosuppressive regimen.

Another trial, CLEAR[82] explored intensified dosing (1.5 g twice daily on days 1 to 5, then 1.0 g twice daily) or standard dosing (1.0 g twice daily) of MMF in combination with tacrolimus and prednisone. Although there were no significant differences in clinical outcomes between the two groups when comparing the overall exposure, AUCs were significantly higher in the 1.5 g twice daily (BID) arm on days 3 and 5, which consequently resulted in a trend for fewer treated acute rejections at 6 months.

Conclusively, these large multi-center clinical trials showed that intensified MMF dosing was well tolerated and resulted in an overall increase in the MPA exposure and reduced acute rejection. The OPTICEPT trial primarily established the non-inferiority of concentration-controlled dosing as compared to fixed dosing of MMF. It also highlighted previously established fact that higher MPA exposure is associated with better clinical outcomes in terms of

acute rejection and overall survival. These studies also emphasized the importance of monitoring AUC rather than trough concentrations owing to the poor correlation between trough concentration and AUC.

1.6.2 Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) refers to the measurement of drug concentration in body fluids at designated intervals to maintain a concentration of a drug in a therapeutic range to optimize drug dosing based on individual exposure [83]. TDM is a common practice for drugs like voriconazole [84], posaconazole, [85] and cyclosporine [86, 87] and tacrolimus [88] which form the backbone of drug treatment in transplantation. TDM is important when there is a correlation between the drug concentration and clinical effect, for drugs having a narrow therapeutic index, for drug showing large inter and inter-patient variability, when there are no good clinical markers of efficacy. For mycophenolate mofetil, trough concentration is a poor representation of overall exposure and is also poorly correlated to the clinical outcomes [89, 90]. Shaw et al [91] evaluated the relationship between MPA PK and clinical outcomes from 11 prospective studies conducted in solid organ transplant recipients. Of these, 8 studies were performed in kidney transplant recipients, and three in heart transplant patients. All these studies have used cyclosporine or tacrolimus as the primary immunosuppressive drug. Overall the following conclusions can be drawn: (i) MPA exposure as measured by the AUC is a good predictor of acute rejection and also some hematologic toxicity; (ii) Targeting a 12 hour AUC of 30-60 μg*hr/ml is associated with better clinical outcomes in terms of graft survival in SOT

recipients, (iii) trough values of MPA is a marginal predictor of rejection events but are highly variable and poorly correlate with MPA AUC.

1.6.3 Pharmacokinetic profiling

Clinical pharmacokinetic profiling refers to quantitation of the rate and extent to which a drug is absorbed distributed and eliminated from patients. Determination of AUC_{0-12} is considered to be the best measure of MPA exposure. This is generally carried out over a dosing interval and is typically less time consuming and relatively easier to perform as compared to pharmacodynamic monitoring. However, such studies are often limited by the volume of sample that can be drawn (especially in special populations like pediatric subjects) and clinical feasibility. Strategies for ideal monitoring of MPA exposure have been largely debated. In a consensus report published by Kuypers et al[92] different characteristics of pharmacokinetic monitoring strategies and the advantages and disadvantages were discussed. The review summarized that single time point measurements although clinically feasible can only be used in the same population that was used to develop the regression equation. It is also limited by inaccuracy of the timing of measurement and may not always have a strong association with the AUC. On the other hand, multiple time point, and Bayesian estimation approaches are better associated with AUC but are more time consuming and require complex mathematical modeling.

Several studies conducted in solid organ and stem cell transplant patients have underlined the importance of AUC monitoring. The target MPA AUC_{0-12} ranges used in these studies have been derived from several studies to be in the range of 30 and 60 μg*hr/ml. The upper limit was determined based on no additional efficacy of higher target AUC. The recommended target MPA

trough concentration of 1.3 µg/ml and 1.9 µg/ml when given with CsA and tacrolimus respectively were derived from the corresponding AUC values, assuming that 80% of patients treated will attain the lower limit of the target AUC exposure.

Two big trials, APOMYGRE and Fixed dose concentration-controlled trials showed the importance of monitoring of MPA AUC. Patients in the APOMYGRE trial achieving a target 12-hour AUC of 40 µg*hr/ml had a significantly lower incidence of biopsy-proven acute rejection (BPAR; 7.7 versus 24.6%; P>0.01). Another study conducted by Kuypers et al [67] in de-novo kidney transplant recipients established similar AUC range. This target level was useful for subjects who had MMF related leukopenia or anemia, however, this AUC had no clinical utility in guiding MMF dosing in patients with gastrointestinal or infectious adverse events. These studies did hint at future strategies for better TDM. It was established through these studies that a Bayesian estimation based on 3 time point within the first 3 hours of MMF dosing can be potentially used to predict the overall exposure. Dosage adjustments helped enable the clinicians to achieve target MPA AUC in 91% by day 14 as compared to 56% in the fixed dosing group.

Table 3 Summary of Limited sampling strategies in transplant patients

Sampling time (hours)	Correlation	Equation used	Method used	Conclusion	Reference
1,3,9	0.82	17.28 + 0.89C1h + 1.76C3h + 6.09C9h	Stepwise multiple regression analysis	71% of estimated AUCs comprised within 85%–115% of actual full AUC	Pawinski et al[93]
1,2,3 and 6	0.89	8.53 + 1.09C1h + 1.07C2h + 1.65C3h + 3.59C6h		83% of estimated AUCs comprised within 85%–115% of actual full AUC	
0,1,3,6	0.84	9.02 + 3.77C0 + 1.33C1 + 1.68C3 + 2.96C6	Previously reported LSS strategies	Superior in predicting $AUC_{0–12}$ in cyclosporine co-treated cohort	Barraclough et al[94]
0,1,2, 4	0.76	6.02 + 5.61C0 + 1.28C1 + 0.9C2 + 2.54C4			
1,2 and 4	0.84	9.328 + 1.311C1 + 1.455C2 + 2.901C4	Multiple regression analysis	Better prediction of MPA AUC in steroid free regimen	Poulin et al[95]
0,1,3 and 6	0.8	12.3 + 4.7 C0+ 1.2 C1+ 2.7 C3+ 1.8 C6	Maximum a posteriori Bayesian estimator (MAP-BE)	Good predictive performance and offers flexibility with respect to sample times	Winter et al[96]
0,2 and 4	0.7	0.26C0 + 2.06C2 + 3.82C4 + 20.38	3 samples at C_0, Multiple regression	Recommended for MPA when restricted to samples collected up to 4 hours	Miura et al [97]
0, 0.5, 2		7.75 + 6.49C0 + 0.76C 0.5h + 2.43C2	Linear regression	(82%) estimated AUC values were within 15% of the value of AUC_{0-12}	Pawinski et al [98]
0,1,2 and 6	0.84	7.99+1.40C2+2.47C4 +9.54C6	Stepwise linear regression analysis	83% of IV and 70% of PO AUC_{0-12} predictions fell within ±20%	Ng et al[99]
1,2,3 and 4	0.81	-	Multiple stepwise linear regression analysis	Appropriate estimation of LSS for MPA in renal transplants treated with EC-MPS	Sommerer et al [100]

AUC: area under concentration-time curve; MPA: mycophenolic acid; IV: intravenous; LSS: limited sampling strategy; EC-MPS: enteric coated mycophenolate sodium.

1.6.4 Limited sampling strategy (LSS)

The main limitation of using AUC based approach is the practical difficulty in having the patient stay in the clinic for over 10 hours. Although the recommended target $AUC_{0\text{-}12hours}$ for MPA is between 30 and 60 µg*hr/ml, most transplant centers find it difficult to implement a 12-hour MPA monitoring primarily due to the technical difficulties and lack of clinical feasibility to conduct multiple repeated sampling.

Prediction of the disposition of a drug from limited blood sampling schedule (often with 3 or fewer concentrations) was proposed as a possible approach by Moore et al [101]. Different single time point, or abbreviated sampling techniques are used for monitoring of MPA exposure in transplant and other patient populations. A summary of the results from major studies is presented in Table 3. Bayesian estimation approaches have also been used to design and validate a limited sampling strategy. TDM of MPA that is based on LSSs is preferred in SOT patients compared with drug dosing that is based on single MPA (trough) concentrations. LSSs provide a good estimation of the AUC within a dosing interval, which is associated with early postoperative efficacy (avoidance of acute rejection) and can thereby improve early graft outcome in terms of acute rejection.

The major advantage of these strategies is the reduced cost; labor and inconvenience on the investigators' end and a shorter hospital stay on the patients' end and faster turnaround time of the results. LSSs are increasingly being used in other clinical setting like bioequivalence studies and PK-PD analysis.

1.6.5 Pharmacodynamic monitoring

Hale et al [102] conducted the first pharmacokinetic-pharmacodynamic (PK-PD) study in transplant recipients. They described a sigmoidal relationship between the MPA exposure and likelihood of rejection and is illustrated in Figure 2. Langman et al [103] reported that the maximum inhibition of IMPDH activity occurred at peak concentrations of MPA and observed a consequent increase in IMPDH activity as the drug was eliminated from the body with very little or no inhibition at 24 hours post-dose. Over the last decade, measurement of IMPDH activity is increasingly being used in an attempt to evaluate the efficacy of MMF directly. IMPDH is an enzyme that is involved in the de-novo synthesis of guanosine nucleotides. It catalyzes the conversion of IMP to XMP in presence of the oxidized form of the coenzyme Nicotinamide adenine dinucleotide (NAD+). Initially radiometric assays were developed for measurement of IMPDH activity. However, these assays were not consistent, and it was difficult to obtain reproducible results using these methods. Several high-performance liquid chromatography (HPLC) method has been reported for measurement of IMPDH activity. IMPDH activity is indirectly determined by measuring XMP *ex-vivo* by incubating peripheral blood mononuclear cell (PBMC) lysates with IMP and NAD+ [104-107]. Studies conducted in renal transplant patients have shown an inverse correlation between MPA concentration and IMPDH activity. In most studies, the maximum inhibition of IMPDH was achieved 1 to 2 hours after dosing. In a number of studies, large inter-patient variability was observed in IMPDH activity, with a relative small intra-patient variability in respectively healthy persons, patients on dialysis and kidney transplant recipients.

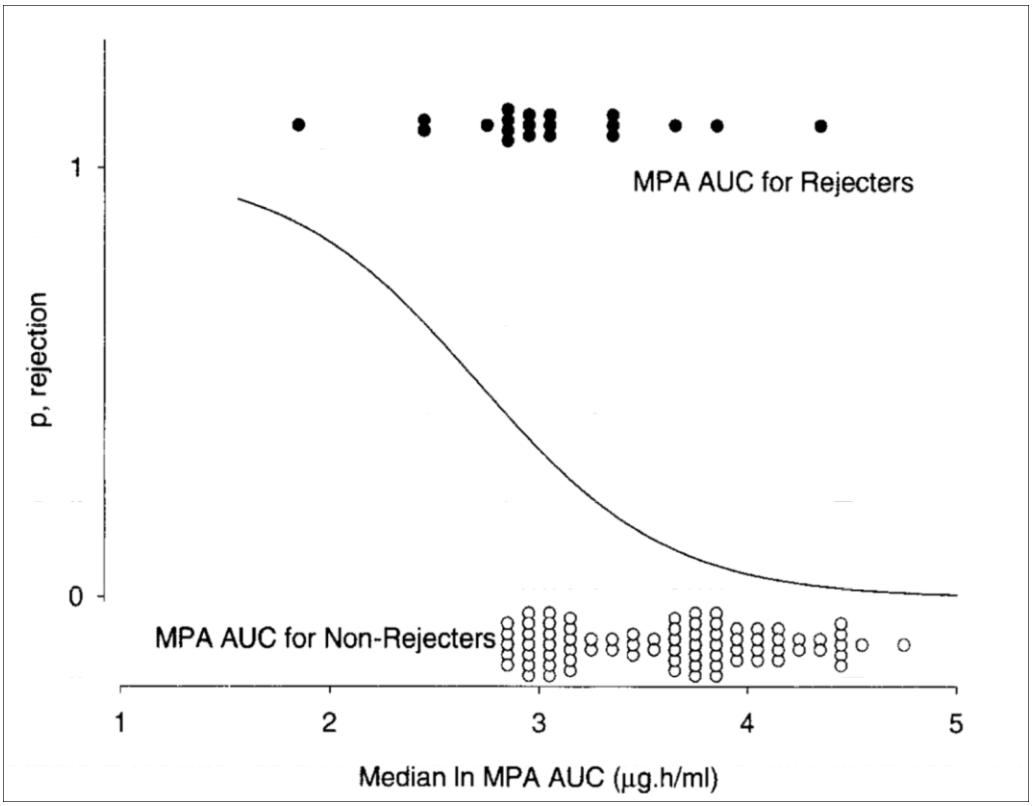


Figure 2. Exposure (AUC)-Response (rejection) relationship of MPA in renal transplant recipients

A study reported by Thi et al. [108] correlated the total MPA and intracellular PBMC MPA concentrations to IMPDH activity. Inhibition of IMPDH activity at 1.5 hours correlated better with total MPA exposure ($p = 0.027$) measured as plasma AUC than with AUC in PBMC ($p = 0.323$) calculated after 3.5 hours. This study suggested that measurement of MPA concentrations in PBMCs does not provide any better correlation with the IMPDH activity than plasma MPA concentrations. There is an inverse relationship between total MPA exposure in plasma and IMPDH activity as shown in Figure 3 [109]. Several other studies have established an integrated population PK-PD model describing an inhibitory E_{max} model in adult and pediatric solid organ transplant recipients [35, 110-112] Budde et al. [112] compared the pharmacokinetics and pharmacodynamics of 2 formulation of MPA: MMF and the extended

release EC-MPS in kidney transplant recipients. They observed a similar degree of inhibition of IMPDH between the 2 formulations. As suggested by Budde et al [109] additional analysis of IMPDH activity can facilitate in better interpretation of the pharmacokinetic data. The estimated half maximal inhibitory concentration (IC_{50}) of 0.97 µg/ml was lower than the observed MPA plasma concentration of 1-5 µg/ml. The integrated PK-PD model in pediatrics estimated an IC_{50} of 1.7 µg/ml [35].

A PK-PD study [113] comparing intensified regimen (days 0 through 14: 2880 mg/day; days 15 through 42: 2160 mg/day; followed by 1440 mg/day) versus standard dosing (1440 mg/day) of EC-MPS suggested that intensified regimen resulted in significantly higher MPA exposure and also lower IMPDH activity on day 3 after transplantation ($P<0.05$). Also, BPAR was lower (2.6%) in the intense regimen group as compared to standard dosing regimen (13.5%). However, the overall safety was comparable between the 2 groups.

MMF dose reductions are known to be associated with inferior graft outcomes. Few studies have investigated the association between transplant naïve IMPDH activity, MMF dose, reduction in dose and the outcomes in transplant recipients. Glander et al. [114] from their study concluded that 62.5% of patients on standard MMF dose (1 g BID.) requiring dose reductions within 3 years post-transplant had significantly lower IMPDH activity. Another trial [115] in renal transplant recipients showed an increasing trend in the baseline IMPDH activity in rejecting as compared to the non-rejecting subjects (p=0.019). The study concluded that quantitation of the recipient's pre-transplant IMPDH activity in PBMC lysate could provide a useful tool for evaluating a recipient's sensitivity to MMF. Monitoring of pre-transplant activity in stem cell transplant recipients revealed contrasting results. A higher IMPDH activity was

associated with a lower rate of grades 2–4 acute and grades 3–4 acute GHVD (P=0.03) and lower day +28 T-cell donor chimerism ≥95. This could be explained by a previous study that showed that higher T cell chimerism was associated with higher probability of acute GVHD. [116]

These studies hint at the potential use of the pre-transplant and continuous IMPDH monitoring in the initial phase post- transplant as criteria to define rejection and infection phenotypes in transplantation.

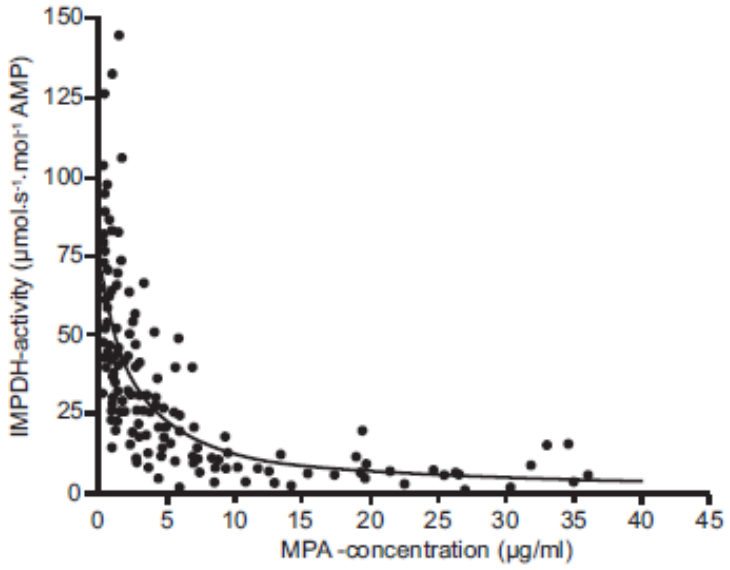


Figure 3 Relationship between total MPA concentration and *ex-vivo* IMPDH activity as fitted using an E_{max} model (r^2=0.68) in 18 stable kidney transplant recipients.

1.6.6 Modeling based MMF optimization

Novel pharmacometric approaches are being used to optimize dosing of drug with narrow therapeutic index. It encompasses the application of mathematical and statistical approaches to characterize the pharmacokinetic and dynamics of the drug. It plays an important role in early stage leading into phase I trials to drug approval and labeling decisions. There has been an increase in the use of modeling and simulation in new drug applications filings submitted to the regulatory agency. Pediatric pharmacometric reviews increased by 5 times from 2000 to 2008. Modeling and simulation will play a significant role in pediatric drug applications with the use rising to a 100% by 2020. [117]

MPA is not just characterized by its narrow therapeutic index, but also has a large inter and intra-patient variability leading to poor dose-exposure response. Most modeling approaches for describing MPA pharmacokinetics have used non-linear mixed effects modeling by describing pharmacokinetic parameters as fixed effects associated with inter and intra individual variability. NONMEM allows use of sparse data across studies conducted with small sample size. Most of population analyses of total and unbound MPA were conducted in adult and pediatric solid organ transplant patients. In most studies a 2- compartment model with and without lag time was used as the structural model. A few advanced mechanistic models have been described to incorporate enterohepatic recycling and exposure associated with it in the model in patients with childhood-onset systemic lupus erythematosus [36], and renal transplant recipients [118].

There were significant differences in pharmacokinetics of MPA across transplant population and across adults and pediatric subjects. For example, clearance was seen to be higher

in adult and pediatric stem cell transplant recipients (24.2L/h -36.9L/h) [53, 119] as compared to solid organ transplant recipients (12.9L/h to 17 L/h). [37, 120]. Table 4 summarizes the median (range) pharmacokinetics of MPA for same dose in adult and pediatric solid organ and HSCT patients. Effect of different covariates like concomitant tacrolimus or cyclosporine administration, MMF dose, albumin concentration, creatinine clearance, age, weight, time pot-transplant, genotype on bioavailability, clearance and volume of distribution were also evaluated.

Table 4. Comparison of Pharmacokinetic parameters of MPA after 1mg BID dose of MMF in adult and pediatric solid organ and HSCT patients.

Pharmacokinetic Parameters	Adult Kidney transplant	Pediatric Kidney transplant	Adult HSCT	Pediatric HSCT
Clearance (L/h) (mean)	32[121]	-	45[24]	42[12]
AUC_{0-12} (μg*hr/mL)	28 (14.4-58.8)[40]	33.5 (13.9-58.4) [40]	19 (8.7-35.2) [122]	12.5 (4.5-49.2) [123]
C_{max} (μg/mL)	8.9 (2.5-24.7)[40]	18.8 (5.3-45.7) [40]	4.8 (2-11.4) [122]	5 (1.8-18) [123]

Bayesian modeling enables estimation of AUC using sparse sampling and D-optimally sampled dataset. By using Bayesian algorithms, flexible and clinically feasible sampling windows can be designed. Bayesian estimation of individual AUC was done based on three blood sample measurements and covariates. The predictive performances of the Bayesian procedure were evaluated in an independent group of patients. Samples drawn at 20 minutes, 1

and 3 hours post dose gave the best estimation of individual AUC with acceptable accuracy (bias 7.7%) and precision (RMSE 12.4%) [124]and in another study with a bias (% RMSE) of -5.7% (20.5%), -8.2% (14.4%), and +0.4% (12.0%) on D7, D30 and more than 3 months post-transplant [125]. Another study has reported sampling times of pre-dose (0 hr), 1 and 4 hours post-dose for estimation of overall AUC [126]. Other studies have used more sampling points for up to 6 [127] and 12 hours [128] for estimation of AUC.

A more clinically practical tool in the form of a web-based Bayesian estimator was developed by researchers in France. The tool individualized MMF dose and estimated pharmacokinetic parameters based on demographic data, co-medication and MPA concentrations sampled at 20min, 1 hour and 3 hours post-dose. A retrospective study evaluating the impact of this tool showed that 72-80% of the AUCs estimated with this tool were within the target 12-hour AUC 0f 30-60µg/mL [129]. Although these methods are accurate (bias <10%), they have inconsistent precision with %RMSE ranging from 4-34% [130]. Nevertheless, these observations do provide substantial evidence that Bayesian dosage prediction can be used clinically for achieving target MPA AUC.

In the past decade, physiologically based pharmacokinetic modeling is increasingly used in the industry for prediction exposure of new drug. It is also used for better understanding the physiological factors that may affect the drug exposure and consequently the drug response. It is a ‘bottom up’ approach as it accounts for a drug’s physico-chemical properties like logP, molecular weight permeability and the pathophysiological factors and trial design into the built model and gives a better control on determining the factors that could affect a drug’s exposure. The classical ‘top down’ approach (Figure 4) is generally based on the observed PK, data, empirical compartmental kinetic data and population PK-PD data. The top down approach is

limited in its capacity to capture the changes in PK due to absorption, EHC and DDIs. Although this approach has been extensively used for describing PK of immunosuppressive drugs like tacrolimus and sirolimus, there is no data on MPA. Recently, Stemkens et al [131], described a PBPK model of MPA using Gastroplus®, a commercially available software. However, this model was not well validated.

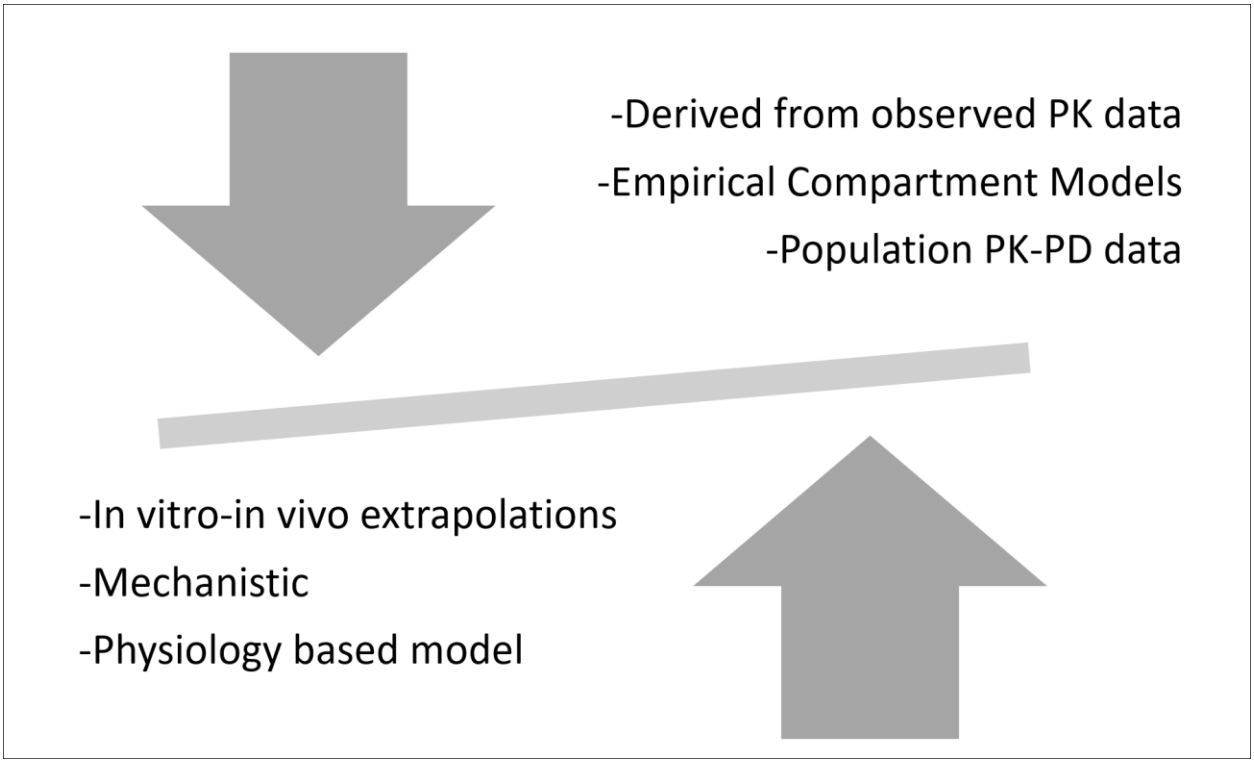


Figure 4 Schematic representation of typical PBPK modeling strategy

1.7 PATIENT POPULATION IN THE CURRENT STUDY

Since its approval for prophylaxis of organ rejection, MMF pharmacokinetics has been extensively studied in kidney and liver transplant recipients. Different techniques to optimize MMF dosing have been studied. The successful prophylactic use of MMF for acute rejection in solid organ transplantation has triggered increasing application of MMF in the prophylaxis and treatment of acute and chronic GVHD in HSCT and in other solid organ settings like lung and heart transplant recipients. Over the last decade MMF has been extensively used off label in HSCT and LT recipients but is not well studied in these populations. MPA monitoring and IMPDH activity measurements have been reported to be beneficial in kidney transplant recipients but it did not increase overall outcomes. This is primarily due to already high graft and patient survival in kidney transplant recipients. However, graft and patient survival is much lower in HSCT and LT recipients. While TAC and CsA monitoring optimizes their overall survival rate, there is scope to improve graft survival with MPA monitoring in lung transplant patients. Through this work we had a unique opportunity to characterize and suggest approaches for optimizing the use of MMF in the less studied HSCT and LT recipients.

1.7.1 Stem cell transplant recipients

Important aspects of the pharmacokinetics of MPA in HSCT patients include lower exposure than other populations with substantial intra and inter-individual variability. Various factors that are unique to stem cell transplantation can lead to differences in pharmacokinetics of MPA in these patients. These patients often suffer from mucosal injury in the GI tract caused by

chemotherapy and/or radiation therapy, presence of GVHD and graft-versus-tumor (GVT) effect and concomitant use of other medications. Also unlike SOT, the immunosuppression is tapered off and eventually discontinued making it very crucial to monitor MPA disposition in the early phase post-transplant when there is a higher likelihood of development of GVHD [132].

The glucuronide metabolite MPAG undergoes enterohepatic recycling and is converted back to MPA by gut bacteria which contributes to 40% of MPA AUC [10]. Enterohepatic recycling is however not observed in stem cell transplant recipients [133, 134]. This may be explained by the mucosal damage associated with chemotherapy and loss of gut bacteria due to the use of antibacterial agents. Another important factor is the relatively lower and highly variable bioavailability seen in HSCT patients reported in a few studies.[135-137]

Third major reason could be the increased MPA clearance. Based on a population pharmacokinetic analysis study in HSCT patients, weight normalized MPA clearance was higher in smaller children and showed a downward trend with increasing body weight [138]. The mechanisms underlying these age-dependent differences are unclear but are probably related to developmental changes in all components of drug disposition.

The relationship between MMF exposures and its efficacy is still unclear, and optimal targets have yet to be defined in HSCT patients, especially in pediatric patients. Current MMF dosing strategies warrant careful evaluation to ensure optimal plasma concentrations, dosing regimens, duration of therapy and upper limit of toxicity for individuals. To elucidate the advantage of MMF use in the HSCT population, multi-center sufficiently powered pharmacokinetic and pharmacodynamics studies focused on personalized dosing are needed to improve clinical outcomes in HSCT patients.

1.7.2 Pediatric patients

MMF had been used off-label in pediatric kidney transplantation before 2001. A study [139] conducted in 1998 showed that MMF in combination with CsA and steroids was well tolerated in children between 4 and12 years for the treatment of acute rejection in kidney transplantation. The US license specifies a much younger age limit of 3 months on the label. In the UK, it is only licensed for kidney transplantation in combination with corticosteroids and cyclosporine in pediatric patients for age 2 years and over.

Many of the studies that reported PK data in children were conducted as a part of adult trials and included only a small number of children. There is a scarcity of exclusive randomized clinical trials in children. The manufacturer recommended pediatric dose is 600 mg/m2. PK profiles of MPA when dosed as 600 mg/m2 twice daily in combination with CsA are comparable with those in adults on 1 g daily dosing [140].

There are no regulatory guidelines for MMF dosing in children and it is often extrapolated from adult data at an initial dosage of around 40 mg/kg/day [141]. Some studies have reported the need for higher doses of up to 50 mg/kg/day [142].

Jacobson et al have studied MMF PK in pediatric HSCT population. Age related changes in clearance and polypharmacy as seen in HSCT patients, and the use of chemotherapy can have a substantial effect on pharmacokinetics in pediatric HCT recipients as compared to other SOT recipients and adult subjects. Very little information is available on the pharmacology of MPA in children following HSCT. In this work, we have characterized the pharmacokinetics of MPA and its metabolite and proposed a novel individualized dosing regimen based on PK.

Owing to higher clearance of MPA, MMF is dosed more frequently at every 8 hours in pediatric patients suggesting a need of a higher and more frequent dosing. Higher MMF doses as used by Jacobson et al. did not increase risk of infection complications and the risk of infection-related mortality as compared to the lower dose. This provides an opportunity to personalize novel MMF dosing strategies that provide a continued higher exposure for GVHD prophylaxis in pediatric HSCT patients.

1.7.3 Lung transplant recipients

In kidney and liver transplant one-year survival rate is over 90% and 5-year survival rate is nearly 80%. In LT recipients the 5-year survival is as low as 50%. Acute cellular rejection (ACR) continues to be a major problem. This highlights the need of better immunosuppression protocols in this patient population. Tacrolimus and cyclosporine are routinely monitored in transplant patients. Although approximately 80 percent of lung transplant recipients in the United States receive MMF as a core constituent of their maintenance immunosuppression, MPA is not routinely monitored. This provides an opportunity for clinical researchers to optimize MMF dosing to improve outcomes in lung transplant recipients. Among the solid organ transplant recipients, lung transplant population is the least studied for monitoring MPA pharmacokinetics. There is a scarcity of PK studies in lung transplant recipients let alone an individualized MMF dosing study based on limited sampling strategy. Also, there have been no reports of IMPDH monitoring in lung transplant recipients. Although immunosuppression regimens have significantly improved in lung transplant recipients, there is no consensus on the optimal dosing

regimen and there are no FDA approved medications to treat rejection making acute and chronic transplant rejection the important obstacles to successful lung transplantation [143]. There are very few studies conducted exclusively in lung transplant patients without cystic fibrosis. All existing studies in LT recipients show that MPA exposure is low in these patients. There are no systematic PK-PD studies conducted to facilitate the understanding of disposition of MPA in this population. Clinical studies should be designed to exclusively focus on identification of MMF dosing strategies to optimize efficacy and minimize rejection in lung allograft recipients. The purpose of a pilot study conducted as a part of this work was to characterize the pharmacokinetic profile and evaluate the pharmacodynamics activity of MPA in adult LT recipients.

1.8 HYPOTHESIS

HSCT and LT recipients are characterized by poor post-transplant outcomes due to higher rates of infection and rejection. These transplant populations provide researchers an opportunity to optimize immunosuppression regimen. The CNI inhibitors, TAC and CsA are monitored routinely and doses are titrated to a target exposure. Although MMF is increasingly being used as a part of maintenance immunosuppression is not monitored and is instead given as a fixed dose. A complete understanding of the pharmacokinetics and pharmacodynamics MMF and its active (MPA) and inactive (MPAG) metabolites in transplant patients will help in optimizing MMF based immunosuppression in transplant patients, especially in the less studied lung transplant and pediatric stem cell transplant recipients. We hypothesize that

1) Fixed-dose flat dose of MMF will lead to suboptimal exposure and a large inter-subject variability in the exposure of MPA in HSCT and LT patients.

2) Pharmacokinetic monitoring and exposure-based individualization in the early post-transplant phase will facilitate a better control on patients' immunosuppression regimen and impact long term outcomes.

3) A PBPK model can form the basis for future modeling-based studies and dosing recommendations in transplant patients.

4) Pharmacokinetic and pharmacodynamic monitoring of MMF in lung transplant recipients could provide a more feasible way to measure mycophenolate activity within lung transplant recipients in order to optimize therapy.

We have evaluated the above hypotheses in pediatric stem cell transplant recipients and lung transplant using novel dosing regimen (Chapter 2.0), PK-PD (Chapter 3.0 and 4.0) and modeling (Chapter 5.0) approaches.

1.9 GAPS IN CURRENT MMF DOSINF AND OPPORTUNITIES FOR IMPROVEMENT

There is extensive data on pharmacokinetics of MPA and MPAG in liver and renal transplant patients. MPA exposure is well correlated with clinical outcomes. Moreover, these solid organ transplant population has more than 80% graft and overall survival in 5 years. Pharmacokinetics of this drug is however not studied extensively in lung and HSCT patients. This provided us a unique opportunity to study the PK of MPA in these populations and improve graft survival in pediatric HSCT and LT patients by personalizing MMF dosing.

Limited sampling strategies for monitoring MPA PK together with PD measurement have been developed, validated and extensively used in kidney and liver transplant recipients but their utility has not yet been established in pediatric HSCT and LT patients owing to low cell count and limitations of PD assay. There is an unmet need of establishing highly sensitive ands robust assays for quantitation of PK and PD activity with a shorter turnaround time and can be potentially used for therapeutic drug PK and PD monitoring of MPA.

PBPK modeling strategies have been established and successfully used for to explain the variability in exposure and bioavailability for other immunosuppressive drugs like sirolimus[144] and provided insight into its disposition and factors contributing to between subject variability. A PBPK model has not been ever developed and validated in healthy or transplant population. This gave us an opportunity to build and validate as PBPK model for MPA and extend its use further to individualize dose adjustments.

1.10 SCOPE AND SPECIFIC AIMS OF THIS WORK

Since the introduction of MMF in 1995 for treatment of rejection in transplantation, significant clinical research has been done to optimize and individualize MMF therapy in transplant patients. All these efforts are targeted to improve graft survival and prevent infection. . In kidney transplant recipients, there is an association between the exposure as measured by the AUC and the rejection free survival outcomes. Graft and patient survival is more than 90% in kidney transplant patients. However, in LT recipients, the national survival rate averages reported in the US by UNOS are at a staggering 51 % at 5 years. Higher incidence of infection is also observed in LT patients. Immunosuppressive drugs play a prominent role in the overall outcomes post LT. The calcineurin inhibitor like CsA and TAC that form the backbone of immunosuppressive therapy are routinely monitored. MMF, which is used in 80% of the transplant centers in the US drugs is not monitored in spite of the high inter and intra-patient variability. It is used off label as a fixed flat dose in LT patients. Although 12-hour AUC of 30-60 µg*hr/ml has been established as a target through various studies in kidney transplant patients, the lack of clinical feasibility of such studies urges for LSS as a viable option for PK monitoring. IMPDH activity, the pharmacological target of MPA is related to the MPA exposure and can be used as a surrogate marker to quantitate MPA based immunosuppression. However, there are very few prospective clinical studies that have explored the PK-PD of MPA in LT recipients. The retrospective study in this work was designed to investigate the association between incidence of infection and rejection during the first-year post lung transplant with trough IMPDH activity. The prospective component of this study was designed as a pilot PK-PD study to characterize the PK of MPA and explore its association with PD response as measured by the *ex-vivo* IMPDH activity.

MPA PK is not well studied in pediatric HSCT patients. There are no guidelines for MMF dosing in pediatric HSCT patients. Also, the kinetics of MPA in these patients is characterized by a short half-life resulting in sub-therapeutic levels within 4 hours of dosing. As HSCT patients are not able to tolerate oral dosing, this necessitates a clinically impractical more frequent IV dosing of MMF. The alternative regimen to maintain optimal levels is a novel 24-hour continuous infusion (CI) of MPA. This CI based dosing strategy has been used for the CNI drugs but has not been tested for MMF. This, to our knowledge is the very first attempt of dosing this drug as a CI to improve outcomes.

The high inter and intra-patient variability in MPA exposure, small therapeutic window and lack of correlation between trough levels of MPA and overall exposure necessitates the need for better dosing strategies for MMF. Therefore, the overall aim of this dissertation was to explore different approaches to individualize the MPA therapy and improve clinical outcomes in MPA treated transplant recipients.

Personalizing drug dosing can be achieved by various approaches. Through this dissertation work we attempted to evaluate different PK, PK-PD and modeling strategies to optimize dosing of MMF in transplant patients. We expect that the observed findings will make for a strong foundation for dosing strategies aiming at individualized MPA therapy in transplant recipients. The objectives for individual chapter are as follows:

1) To evaluate safety and feasibility of AUC-based mycophenolate mofetil targeting for GVHD prophylaxis in pediatric patients undergoing hematopoietic stem cell transplantation. To evaluate the utility of a novel CI dosing regimen of MPA in pediatric HSCT recipients (Chapter 2.0).

2) To explore the pharmacokinetics of MPA in adult lung transplant recipients and explore the PK-PD relationship between MPA exposure and IMPDH activity. (Chapter 3.0)

3) To establish the feasibility of pharmacodynamic monitoring *ex-vivo* in adult lung transplant recipients (Chapter 4.0).

4) To establish and validate a physiologically based pharmacokinetic (PBPK) model of MPA in healthy Caucasian and Chinese adult population and to predict the exposure of MPA in pediatric population (Chapter 5.0).

2.0 PHARMACOKINETICS OF INTRAVENOUS, ORAL AND A NOVEL CONTINUOUS INFUSION OF MYCOPHENOLIC ACID AND ITS GLUCURONIDE METABOLITE IN PEDIATRIC STEM CELL TRANSPLANT RECIPIENTS

2.1 ABSTRACT

Mycophenolate mofetil, an ester prodrug of MPA, is used increasingly for GVHD prophylaxis. Dose-escalation strategies in pediatric hematopoietic stem cell transplantation recipients have failed to achieve target MPA exposure. Here we have described the pharmacokinetics and clinical results of a new MMF dosing approach in pediatric HCT recipients undergoing myeloablative transplantation. We have evaluated the safety and feasibility of a pharmacokinetics-based dosing approach using a novel CI method of administration of MMF in pediatric HSCT recipients. All patients received a myeloablative conditioning with cyclosporine A and MMF for GVHD prophylaxis. Blood samples were collected from 19 patients over a dosing interval. They were initiated on day 0 at a dose of 15 mg/kg every 8 hours. Concentrations of MMF, MPA, MPAG in the plasma were measured using HPLC coupled with mass spectrometry. Non-compartmental pharmacokinetic analysis was performed using WinNonlin. Based on pharmacokinetic data from IV short infusion, MMF was converted to CI to target a C_{ss} of 1.7-3.3 µg/ml corresponding to a total MPA AUC_{0-24} of 40 to 80 µg*hour/mL. The MMF dose was adjusted to maintain a total MPA steady-state concentration (C_{ss}) of 1.7 to 3.3 µg/mL.

During the CI schedule, MPA AUC0-24 was maintained at a mean of 40.1 µg*hour/mL (range, 20.6 to 63.8), and 17 of 19 patients (89%) achieved MPA C_{ss} within target of 1.7 to 3.3 µg/mL. Ninety five percent of patients (18/19) achieved neutrophil engraftment at a median of 13 days (range: 8 to 41) post-transplant and platelet engraftment at 39 days (range: 17 to 298)

days post-transplant. Six of 18 assessable patients (33%) developed stages II to IV acute GVHD and 2 of 15 (13%) developed chronic GVHD. The MMF dose was reduced in 9 patients due to gastrointestinal symptoms (n=6), low blood counts (n=4), and viral infection (n=3). Five patients with acute lymphoblastic leukemia relapsed, of whom 4 have died. Fifteen of 19 patients are alive with a median follow-up of 2.4 years (range, 0.4 to 4.9), with 3-year event-free and overall survival rates of 68% and 79%, respectively.

In this pilot study, we observed no toxic deaths, excellent engraftment, and low rates of grades III to IV acute and chronic GVHD. We observed significantly shorter half-life and higher drug clearance in pediatric HCT recipients compared with stable pediatric renal transplant patients or adult transplant patients. CI regimen deserves further validation in a larger cohort of pediatric patients undergoing myeloablative transplantation.

2.2 INTRODUCTION

Graft-versus-host disease is the major cause of morbidity and mortality after allogeneic HSCT second only to the primary disease that may lead to death in these patients [145]. Different centers implement various GVHD prophylaxis protocols, but they all include administration of a calcineurin inhibitor, either cyclosporine A or tacrolimus, and a second agent, such as methotrexate. The rate of grades II-IV acute GVHD (aGVHD) is high despite the use of methotrexate along with calcineurin inhibitors. Studies have shown that methotrexate post-transplant is often associated with an increase in risk of mucositis, delay in time to myeloid engraftment, and potential pulmonary toxicity [146]. Over recent years, mycophenolate mofetil has increasingly been used off-label in adults and pediatric stem cell transplant patients. Replacement of methotrexate with MMF has been associated with a faster engraftment time and reduced risk of mucositis [147]. Although the incidence of grades II to IV aGVHD appears to be similar, recent data, including a meta-analysis that compared MMF with methotrexate/calcineurin inhibitor, suggested a higher incidence of grades III to IV aGVHD with MMF [148, 149].

Clinical pharmacokinetic studies evaluating the pharmacokinetics of MPA in pediatric HSCT patients [123, 150, 151] are scarce primarily due to concerns over volume of blood that can be drawn for pharmacokinetic analysis [152]. The current dosing strategies in pediatric HSCT patients are based on body weight or body surface area. However, age-dependent pharmacokinetics of MPA has been reported in several studies [39, 40, 123]. The terminal half-

life in adult and pediatric solid organ transplant patients has been reported to be 8 to 12 hours [153]. However, much shorter half-life has been observed in HSCT patients. The observed shorter half-life in HCT patients suggests the need for at least a three or four times daily dosing regimen of MMF in pediatric patients [154]. There are no guidelines for dosing of MMF in pediatric HSCT patients and dosing regimen has primarily been extrapolated from exposure data in solid organ transplant recipients. Exposure response relationships for MPA are much better defined in adult kidney recipients, in whom the established therapeutic exposure is a total MPA $AUC_{0\text{-}12}$ of 30-60 µg*hour/mL or trough concentrations in the range of 1 to 3.5 mg/mL[155]. Pharmacokinetics studies suggest lower MPA exposure in HCT recipients, with some studies showing exposure as low as 30% to 50%, with standard starting MMF doses that are used in kidney transplant recipients. These studies also suggested a higher dose requirement of MMF dose. The unbound MPA AUC in the immediate week post-transplant was seen to be significantly associated with a greater cumulative incidence of grade II-IV GVHD [122, 156, 157]. After intravenous or oral administration of MMF to pediatric HCT patients, the plasma concentration drops to < 1µg/ml within 4 hours post dose. Low MPA trough levels have been associated with higher rates of aGVHD and graft rejection and lower response rates in the treatment of aGVHD [151, 154, 158].

Several dose escalation and frequent dosing strategies have failed to achieve consistent MPA exposure, especially in the early post-transplant period after conditioning therapy [136]. Haentzschel et al.[159] demonstrated feasibility of MMF dosing to a targeted AUC in adult HSCT recipients. However, increasing the dose of MMF has often lead to higher MPA concentrations and corresponding increase in toxicity[150]. Increasing the frequency of dosing, given the short half-life, is also not very practical. An alternative method to achieve target MPA

levels and avoid high drug exposure is to dose MMF as a CI, the way cyclosporine and tacrolimus are administered. Here we have described the pharmacokinetics and clinical outcomes of this novel MMF dosing approach in pediatric HSCT recipients undergoing myeloablative transplantation.

2.3 MATERIALS AND METHODS

2.3.1 Patients

This study was performed in pediatric patients receiving allogeneic stem cell transplant at the Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center between December 2009 and May 2013. The study was approved by the University of Pittsburgh institutional review board (IRB# 09120501) and registered at www.clinicaltrials.gov (NCT01487577). Informed consent was obtained from the guardians, and assent or consent from patients in accordance with the Declaration of Helsinki. Patients meeting the following inclusion criteria were included in the study.

Inclusion Criteria:

1) Age 6 months to 21 years
2) Myeloablative conditioning
3) HLA-identical sibling donor, HLA-A, -B, -C, and -DRB1 allele-matched unrelated volunteer donor, or 4 of 6 HLA-A, -B, and -DRBb1 allele-matched cord blood unit
4) Cord blood units: minimum pre-freezing nucleated cell dose of 3 107 /kg for malignant diseases and 5 107 /kg for nonmalignant diseases.

Exclusion criteria:

1) HIV infection or uncontrolled bacterial, viral, fungal, or other infection
2) Prior HSCT within 12 months

3) Organ dysfunction defined as total serum bilirubin > 2.5 mg/dL or liver enzymes 5 times the upper limit of normal for age, glomerular filtration rate < 70 mL/min/1.73 m2 , left ventricular ejection fraction ≤ 40%, or shortening fraction ≤ 26% and forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and carbon monoxide diffusing capacity (DLCO) ≤ 50% of predicted or if unable to perform pulmonary function tests, O_2 saturation ≤ 92% of room air\
4) Lansky or Karnofsky performance score ≤ 70%.

2.3.2 Study Design

2.3.2.1 Conditioning and GVHD Prophylaxis Regimen

All patients received a myeloablative conditioning regimen consisting cyclophosphamide or busulfan and cyclophosphamide. Patients receiving allogeneic bone marrow or umbilical cord blood transplants received anti-thymocyte globulin (ATG) at a dose of 30 mg/kg/day for 3 days or fludarabine at a dose of 30 mg/m2/day for 5 days. Cyclosporine and MMF were used as GVHD prophylaxis agents. Intravenous cyclosporine was started 2 days prior to transplant as a continuous infusion at a dose of 5 mg/ kg/day and adjusted to maintain whole blood Css of 160 to 250 ng/mL. Patients were switched to oral therapy when they tolerated oral intake and continued upto 100 days. In the absence of aGVHD, the dose of cyclosporine was tapered by approximately 10% per week through day 180.

2.3.2.2 MMF Dosing and Pharmacokinetics

Intravenous MMF was initiated at a dose of 15 mg/kg every 8 hours from day 0 and administered as a constant 2-hour infusion. The first MMF pharmacokinetics study was performed after a minimum of 5 doses to allow achievement of steady state. Based on the total body clearance calculated from the i.v. pharmacokinetics study, MMF was administered as an intravenous continuous infusion over 24 hours using a solution of 10 mg/mL MMF in 5% dextrose in water, at an infusion rate (infusion rate=Css X total body clearance) to achieve a target total MPA $AUC_{0\text{-}24}$ of 40 to 80 µg*hour/mL. Oral dosing of MMF thrice a day was initiated when patients were able to tolerate oral medications. MMF was continued until day 42 and, in the absence of aGVHD, tapered off over 8 weeks. A flowchart of the study design is represented in Figure 5 .

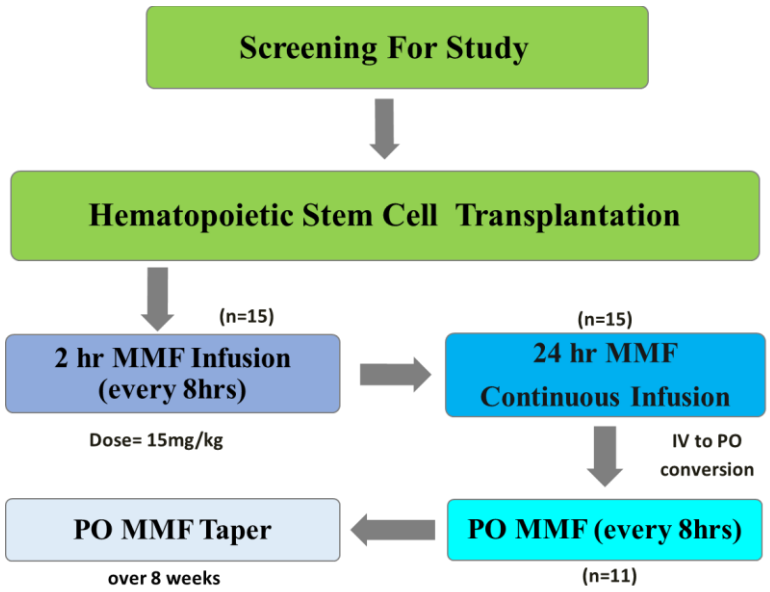


Figure 5. Study Design of Pharmacokinetic study of MMF in pediatric stem cell transplant

2.3.3 Blood Sampling and Assay

Blood samples (2 mL) were drawn in ethylenediaminetetra acetic acid (EDTA)-containing Vacutainer tubes, placed on ice and refrigerated immediately, and centrifuged at 4°C at 3000 rpm for 10 minutes using an Eppendorf Centrifuge 5702R (Hauppauge, NY). Samples were processed within 30 to 60 minutes of blood draw. The plasma concentrations of MMF, total MPA and MPAG were measured using a validated HPLC mass spectrometry method (Waters Corporation, Milford, MA). The coefficient of variation of the assay was less than 10% of all concentrations tested. Serial blood samples were collected within a dosing interval by taking samples just before the dose (0 hour) and at 2, 3, 4, 5, 6, and 8 hours post-dose. While on CI of MMF, total MPA levels were measured thrice a week and on an average MMF dose was adjusted 3 times for patients in order to maintain a total MPA C_{ss} of 1.7 to 3.3 µg/mL. The pharmacokinetics study of oral dosing was performed after a minimum of 5 doses thereby allowing the drug to reach steady state. Serial blood samples were collected over a single dosing interval just before the dose (0 hour) and at 0.5, 1, 2, 3, 6, and 8 hours. While on oral MMF, total trough MPA levels were measured 3 times weekly, with dosing adjustments made to maintain a total MPA trough concentration (C_{trough}) of 1 to 3.5 µg/mL.

2.3.4 Non-Compartmental Pharmacokinetic Analysis

Pharmacokinetic analysis for MMF, MPA and MPAG was done by noncompartmental analysis using WinNonlin software (version 6; Pharsight Corp, Mountain View, CA). The maximum concentration in plasma (C_{max}) and the time to C_{max} (T_{max}) were estimated by visual inspection of

the concentration versus time profiles. The terminal elimination rate constant (λz) was estimated by linear regression of at least the last 3 data points, and half-life ($_{t1/2}$) was calculated by dividing 0.693 by λz. The area under the plasma concentration-time profile from the time of dosing until infinity was calculated by the log-linear trapezoidal method in WinNonlin with extrapolation beyond the last measured concentration, according to: $AUC_{0-\infty} = AUC_{0-8} + C_8/\lambda z$. AUC_{0-8} at steady state is equivalent to $AUC_{0-\infty}$ after a single dose. AUC_{0-24} at steady state was estimated as 3* AUC_{0-8} at steady state Total systemic clearance (CL) was estimated using the equation CL = Dose / $AUC_{0-\infty}$ and the volume of distribution at steady state (Vss) was determined from area under the moment curve using the following equations: $Vss= [(Dose)*(AUMC_{0-\infty})/ (AUC_{0-\infty})^2]$. Results are reported as median and range. We also calculated AUC by using samples collected upto 4 hours.

2.3.5 Safety Assessment

Safety Endpoint and Criteria for MMF Dose Modification Key safety endpoints were day 100 transplant-related mortality, grades III to IV aGVHD, and neutrophil engraftment. Patients were monitored for any grade III or greater adverse events, using Common Terminology Criteria for Adverse Events (version 4.0). Study guidelines for MMF dose reduction or discontinuation were based on BMT Clinical Trials Network trial 0802 (https://web.emmes.com/study/ bmt2/protocol/0802_protocol/0802%20aGVHD_v3.pdf). All patients received antifungal, antiviral, and antibacterial prophylaxis in accordance with standard practice at our institution. Filgrastim was given to all transplant recipients from the day of transplant and discontinued at achievement of myeloid engraftment as per the treating physician.

2.3.6 Clinical Endpoints

Neutrophil engraftment was defined as 3 consecutive days of an absolute neutrophil count > 0.5 x 10^9 /L after transplantation. Platelet engraftment was defined as a platelet count of more than 20,000/mL without transfusion for up to 7 days leading into it. aGVHD was scored based on the modified Keystone criteria and Chronic GVHD diagnosis and grading were based on the 2005 National Institutes of Health Consensus Conference [160, 161]. The major clinical study endpoints were sustained donor engraftment, incidence and severity of GVHD, relapse, and overall survival.

2.3.7 Statistical Analysis

The primary endpoint of the study was to determine the safety and feasibility of personalized AUC-based MMF dosing approach according to the plasma levels. Linear regression was performed top determine the correlation between 4 hour AUC to overall exposure. Secondary endpoints were engraftment as measured by platelet and absolute neutrophil count, the incidence and severity of aGVHD, and the risk associated with persistence and or recurrence of disease. Survival analysis and progression-free survival were estimated from Kaplan-Meier curves using censoring at last follow-up or on death. Categorical variables are represented as frequencies and percentages while continuous variables are summarized as mean± standard deviation and range of values. Owing to the small sample size, nonparametric tests were used, and a multivariate

analysis was not performed. Fisher's exact test was used to compare dichotomous variables, and the Wilcoxon rank sum test was used to compare continuous variables. A statistical comparison of different parameters was made using a paired, 2-tailed Student t-test. $p < 0.05$ was considered statistically significant. All statistical analysis was performed using SPSS.

2.4 RESULTS

2.4.1 Patient and Transplant Characteristics

Patient and transplant characteristics are summarized in Table 5. All assessable patients (N=19) have completed MMF therapy and are included in MMF pharmacokinetic and clinical outcomes analyses with a median follow-up of 2.4 ±1.5 years (range= 0.4 to 4.9). The mean age at transplant was 9.0±6.8 years (range: 0.9 to 21.0). Ten patients were female and 9 were male. Seventeen patients were transplanted for hematologic malignancies and 2 patients for nonmalignant diseases. Eight of 12 cases with acute lymphoblastic leukemia were in second remission, 2 in first remission, 1 in third remission, and 1 primary induction failure; all 3 cases with acute myelogenous leukemia were in first remission. Patients were conditioned with cyclophosphamide-total body irradiation (n =11) or busulfan-cyclophosphamide (n= 8). In addition, of the 12 unrelated donor transplants, 9 patients received equine ATG and 3 received fludarabine.

Table 5. Summary of Patient and transplant characteristics

Number of patients	19
Age, y, mean ± SD (range)	9.0 ± 6.8 (0.9 – 21.0)
Gender (female / male)	10 / 9
Race/ethnicity	
Caucasian	15
Asian Indian	2
Black / Caucasian	1
Black	1
Diagnosis	
ALL	12
AML / Therapy-related MDS	5
Congenital erythropoietic porphyria	1
Severe congenital neutropenia	1
Stem cell source	
Sibling bone marrow	7
Unrelated donor bone marrow	6
Unrelated umbilical cord blood	6
HLA allele match	
Sibling: HLA Identical	7
Unrelated donor: A,B,C,DRb1,DQb1 matched	6
Cord blood: A,B,C,DRb1 matched /mismatched	2 / 4
Conditioning regimen	
Cy + TBI ± Other	11
Bu + Cy ± Other	8
Nucleated cell dose, median, range	
Bone marrow (n=11), x10E08/kg	2.80 (1.76-6.30)
Cord blood, post-thaw (n=4), x10E07/kg	7.09 (4.18-13.10)

2.4.2 Pharmacokinetics of MMF, MPA and MPAG

2.4.2.1 Pharmacokinetics of MMF

It was feasible to prepare MMF solutions to contain adequate MMF for a CI over 24 hours. There were no infusion-related incidents in the patients. The first pharmacokinetics study was performed on intermittent IV, MMF after 3 to 5 doses. Based on the results of the first IV. pharmacokinetics study, MMF was administered as a CI after a median of 6 days (range: 4 to 12) after transplant. Patients were switched from CI to oral MMF after a median of 22 days (range: 7 to 43). The second pharmacokinetics study was performed on conversion to oral dosing after 3 to 5 doses. Patients were on MMF for a median of 94 days (range: 13 to 154), and the drug was weaned off after 47 days (range: 26 to 111).

MMF was quantifiable during the short infusion phase with the maximum levels seen at 2 hours at the end of infusion as seen in Figure 6 . After cessation of infusion the concentrations rapidly declined with most levels being below the limit of quantification for most patients.

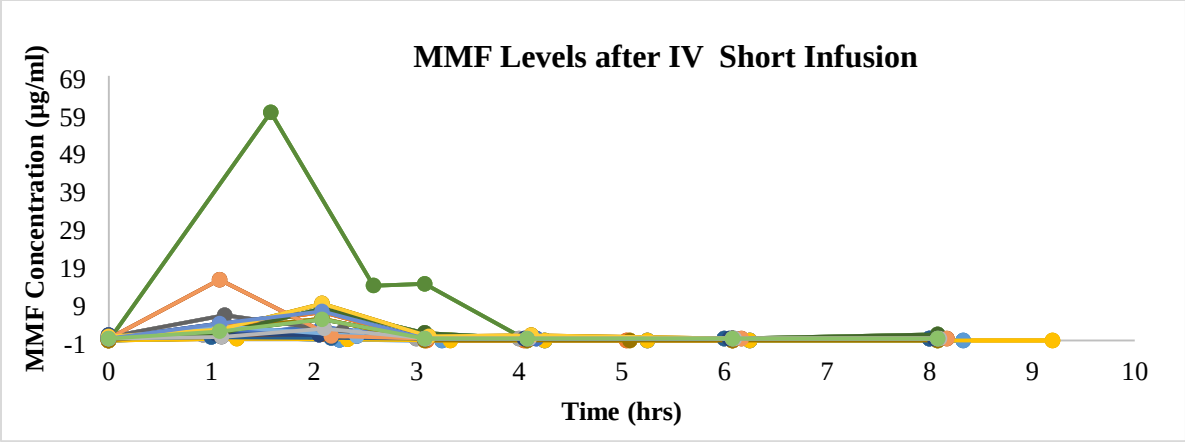


Figure 6. Individual MMF concentration time profiles of 19 HSCT patients after a 2-hour short infusion of MMF given at a dose of 15mg/kg.

2.4.2.2 Pharmacokinetics of MPA

Table 6. Pharmacokinetic parameters of MPA post IV short, IV continuous infusion and Oral dosing (expressed as median (range))

PK parameters (units)	Short Infusion (n = 19)	Continuous Infusion (n = 19)	Oral (n = 14)
Half-life (hours)	1.7 (1.2-2.2)		1.4 (1-2.2)
Volume of distribution (L/kg)	1.6 (1-5.4)		1.3 (.5-4.3)
Clearance (mL/min/kg)	10.2 (6.8-30.8)	15.1 (7.2-73)	10.9 (4.9-26.6)
$AUC_{0\text{-}24}$(µg·hour/mL)	46.5 (16.7-65.9)	40.1 (20.6-63.8)	36.1 (11.9-95.9)
C_{trough} (µg/mL)	0.35 (.1-.7)	—	0.6 (.15-3.8)
C_{avg} (µg/mL)	1.9 (.6-2.7)	1.7 (.9-2.7)	1.5 (.5-4.0)
Mean C_{max}(µg/mL)	5.9 (1.5-9)		5.6 (1.3-12.1)

Short infusion:

There was a considerable inter-patient variation in MPA pharmacokinetics (Figure 7and Table 6). The median values were C_{max} 5.9 µg/mL (range, 1.5 to 9), half-life 1.7 hours (range, 1.2 to 2.2), and AUC_{0-24} 46.5 µg*hour/mL (range: 16.7 to 65.9). There was a 4-fold inter-patient variability in total AUC between patients. The C_{trough} MPA plasma concentrations (median: 0.35 µg/mL; range: 0.1 to 0.7) were poorly correlated with the total AUC_{0-24} (r^2 = 0.37), while the AUC_{0-24} correlated well with the 4-hour abbreviated AUC (r^2 > 0.98) (Figure 8).

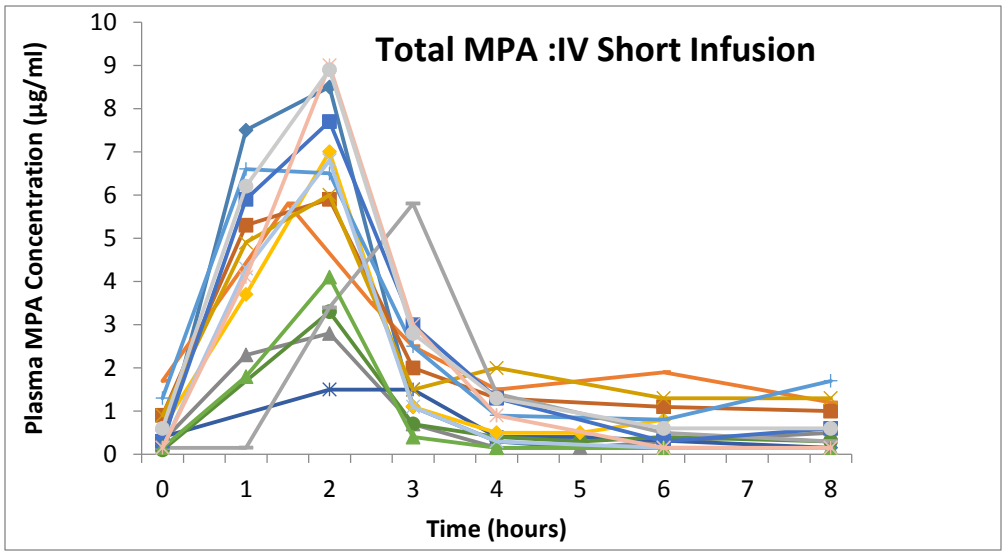


Figure 7. Concentration time profiles of MPA in 19 pediatric HSCT patients on IV short infusion of MMF dosed at 15mg/kg.

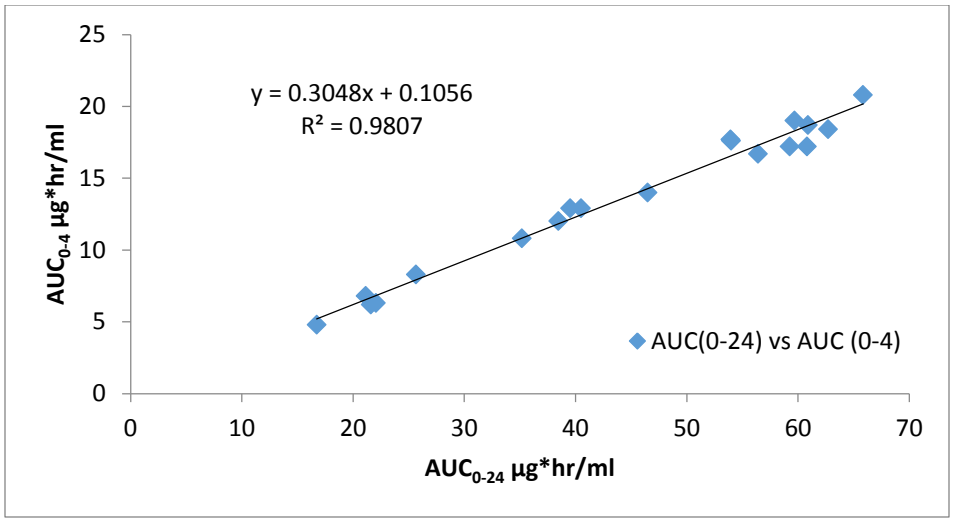


Figure 8. Correlation of estimated AUC_{0-24} (3* AUC_{0-8}) versus AUC_{0-4} of MPA in 19 pediatric HSCT patients on IV short infusion of MMF

Continuous Infusion:

On CI administration of MMF, the median pharmacokinetic values were C_{ss} 1.7 µg/mL (range: 0.9 to 2.7), clearance 15.1 mL/min/kg (range: 7.2 to 73), and AUC_{0-24} 40.1 µg*hour/ mL (range: 20.6 to 63.8). There was a 4-fold variation in the C_{ss} and over 6-fold variation in the total body clearance of MPA. Regression analysis showed no time-dependent change in total body clearance of MPA. With CI administration of MMF, the plasma MPA concentrations were achieved within a 1.7- to 3.3-µg/mL target in 17 of 19 patients (89%) (Figure 9and Table 6). The steady state clearance of individual patient during the IV continuous dosing is depicted in Figure 10 and individual clearance showed no time dependent changes as depicted in Figure 11.

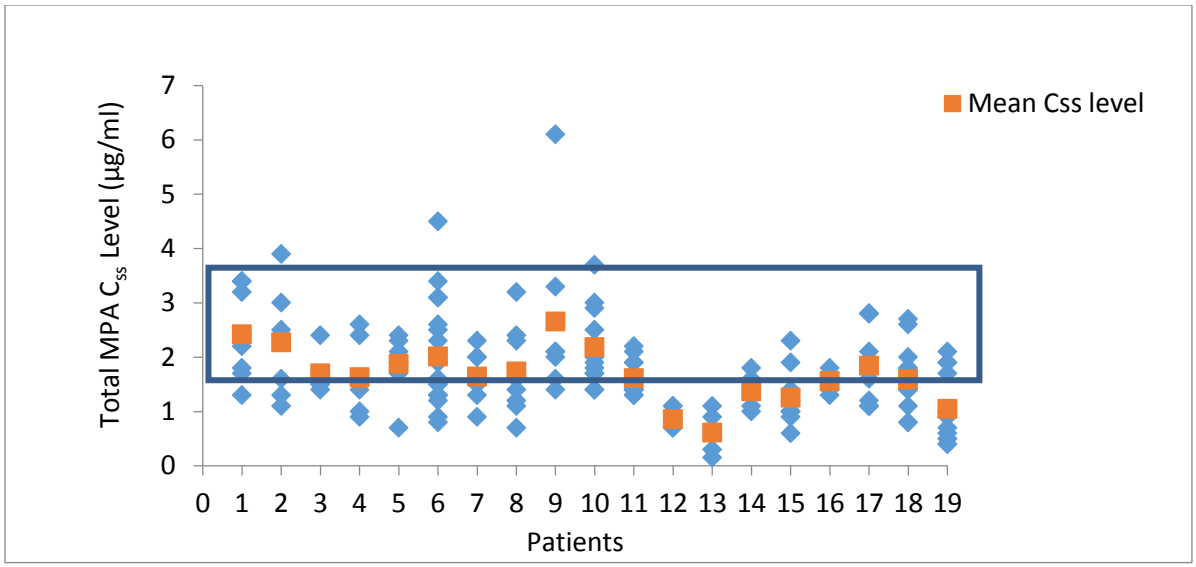


Figure 9. Steady state concentrations in 19 pediatric HSCT patients on continuous IV infusion of MMF

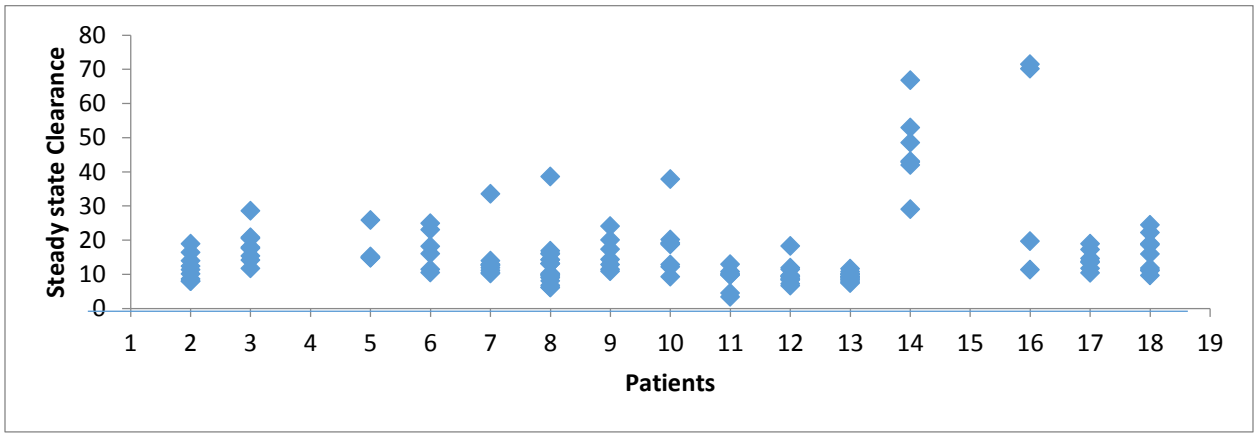


Figure 10. Steady state clearance of 19 HSCT patients on continuous IV infusion of MMF

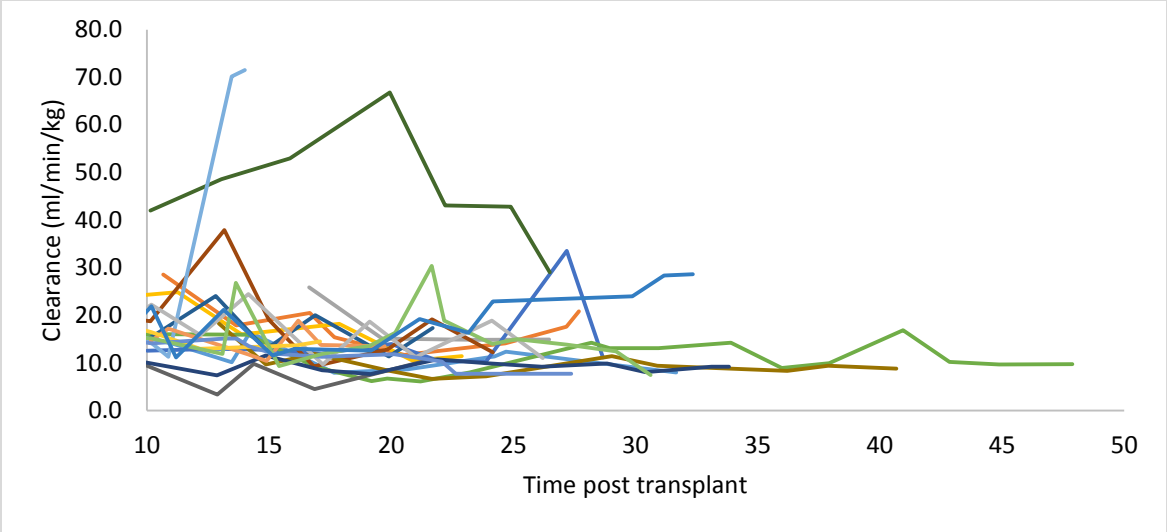


Figure 11. Weight normalized clearance over time in 19 patients receiving 24-hour continuous dosing of MMF

Oral administration:

There was a considerable inter-patient variation in MPA pharmacokinetics (Figure 12and Table 6). The median values were C_{max} 5.6 µg/mL (range, 1.3 to 12.1), half-life 1.4 hours (range: 1 to 2.2), and AUC_{0-24} 36.1 µg*hour/mL (range: 11.9 to 95.9). The C_{trough} MPA plasma concentrations of 0.6 (range: 0.15 to 3.8) were poorly correlated with the total AUC (r2= 0.021); the AUC_{0-8} correlated well with a 4-hour abbreviated AUC ($r^2 > 0.99$) (Figure 13).

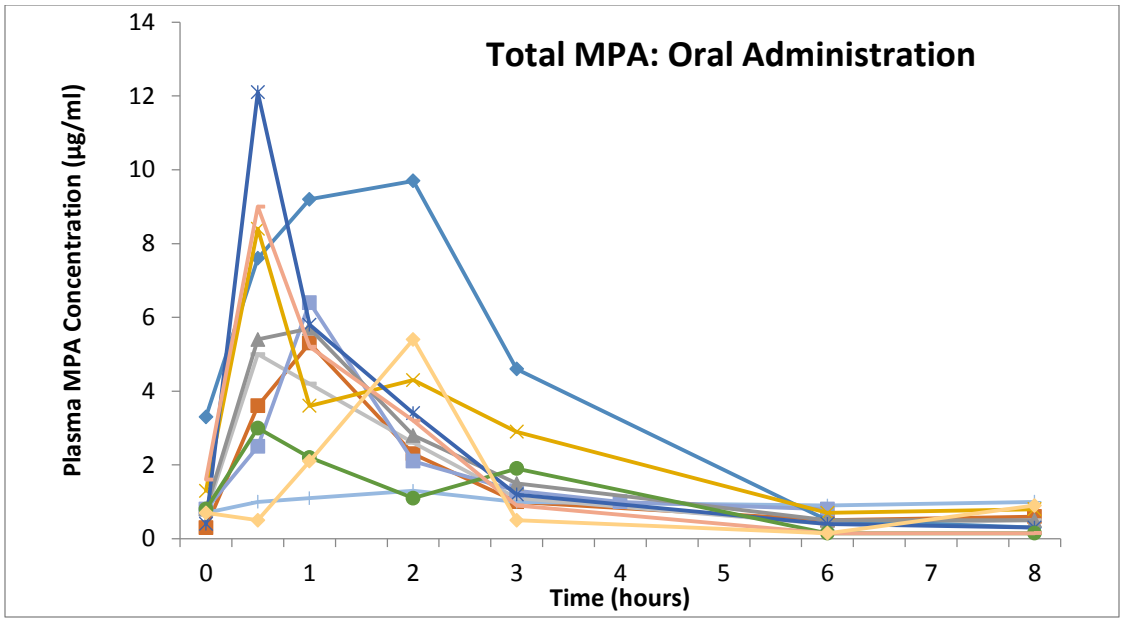


Figure 12. Concentration time profiles of MPA in 14 pediatric HSCT patients after 15mg/kg TID dose of MMF.

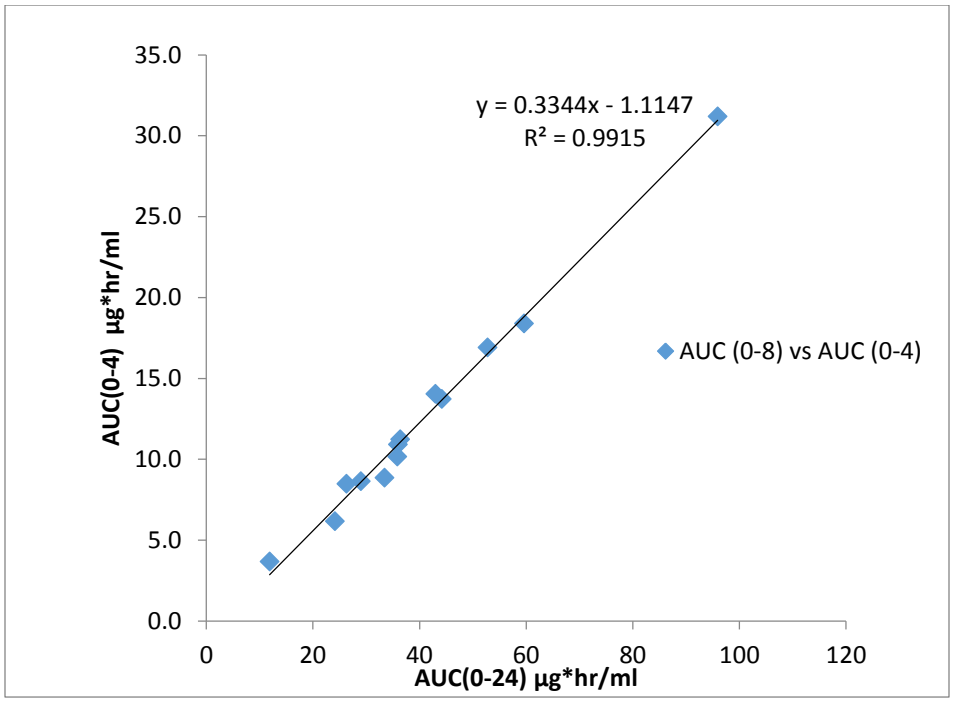


Figure 13. Correlation of estimated AUC_{0-24} hours versus AUC_{0-4} hours of MPA in 14 pediatric HSCT patients on oral MMF dosing.

2.4.2.3 Pharmacokinetics of Mycophenolic acid glucuronide

Plasma concentration versus time plot of MPAG after intravenous administration is shown in Figure 14. The MPAG concentrations were on an average more than 6 times higher than the corresponding MPA concentrations. The derived pharmacokinetic parameters are given in Table 7. The concentrations of MPAG after oral administration were about 12-fold higher as compared to the MPA concentrations after oral dosing (Figure 15). Table 8 shows the MPAG/MPA ratio of the current study in comparison with data from other adult and pediatric solid organ transplant studies. Both adult and pediatric HSCT recipients showed a higher MPAG/MPA ratio for both IV and oral dosing.

Table 7. Pharmacokinetic parameters of MPAG post IV short, IV continuous infusion and Oral dosing in pediatric HSCT patients in the study

	Short Infusion (n = 19)	Continuous Infusion (n = 19)	Oral (n = 14)
AUC_{0-24}(μg·hour/mL)	576.3(213-1896.23)	923.7(36-5376)	1179.2 (458.4-2296.5)
Css (μg/mL)	-	38.49(1.5-224)	-
Mean T_{max} (hours)	2.3 (2-3)	-	2.3(1-8)
Mean C_{max}(μg/mL)	37.1 (14-94.3)	-	67.9(21.3-104.3)

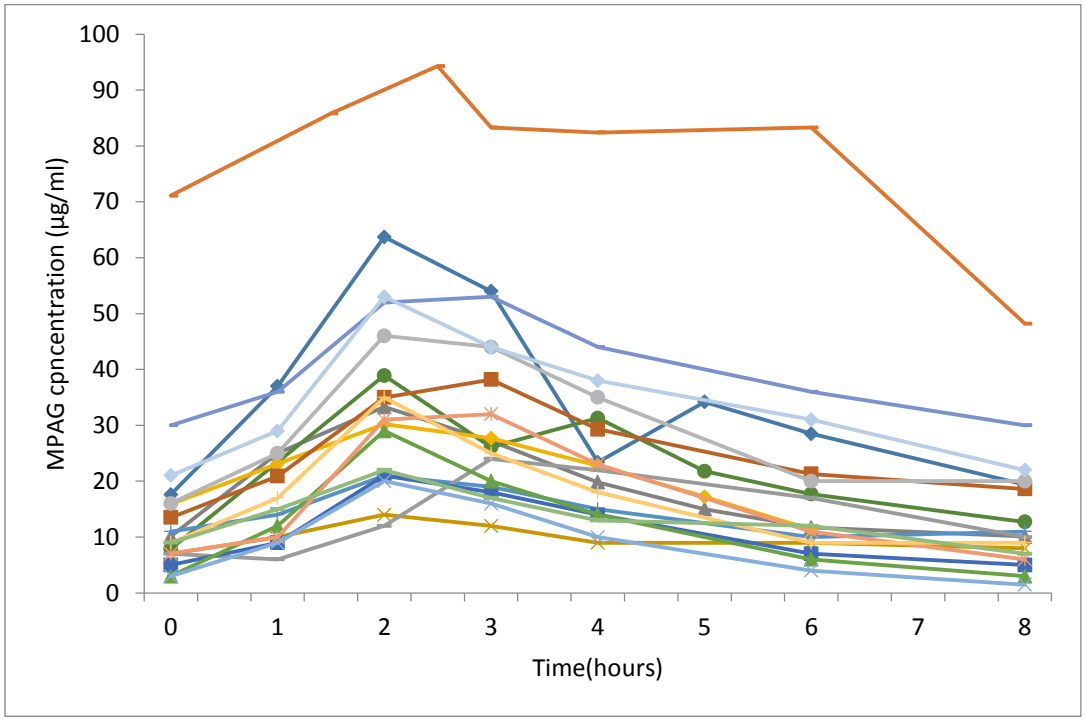


Figure 14. Concentration time profiles of MPAG of 19 pediatric HSCT patients on IV short infusion of MMF

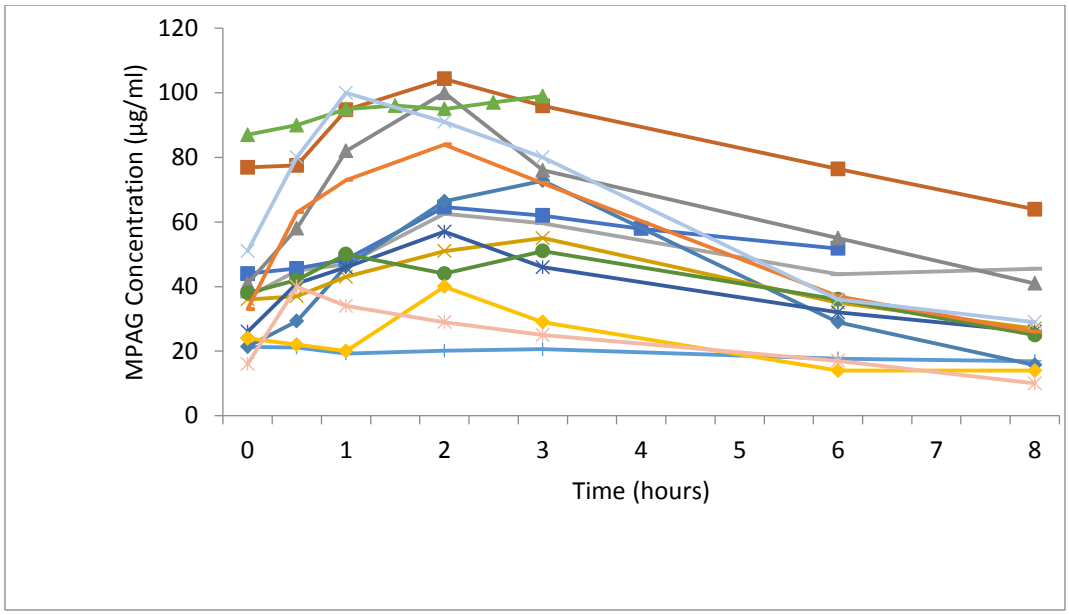


Figure 15. Concentration time profiles of MPAG for 14 pediatric HSCT patients on oral MMF

Table 8. MPAG/MPA AUC ratio of adult and pediatric solid organ and hematopoietic stem cell transplant recipients

Median MPAG/MPA ratio				
Transplant Population	Adult		Pediatrics	
	PO	IV	PO	IV
Post Kidney Tx (>3weeks)	11.7[40]	-	8.9[40]	-
Post Liver Tx (>3weeks) mean	8.0[18]	-	11.6[162]	-
HSCT	29.7 [156]	26.8 [156]	29.4(Current study)	12.1(Current study)

2.4.3 Clinical Outcomes

2.4.3.1 Engraftment

Eighteen of 19 patients (95%) engrafted at a median of 13 days (range: 8 to 41). One patient with severe congenital neutropenia did not achieve donor engraftment and had autologous count recovery by day +43. Patients receiving bone marrow transplants engrafted at 11 days post-transplant (range: 8 to 43), whereas those receiving umbilical cord blood transplants engrafted at 17 days (range: 15 to 41) ($p = 0.01$). All patients achieved platelet engraftment at a median of 39 days post-transplant (range: 17 to 298). Patients receiving bone marrow transplants engrafted at a median of 31 days post-transplant (range: 17 to 298), whereas those receiving umbilical cord blood transplants engrafted at a median of 52 days (range: 28 to 123) ($p = 0.23$). Cord blood

transplant recipients received similar CI doses of MMF (57 ± 25 mg/kg/day) compared with bone marrow transplant patients (44 ± 6 mg/kg/day; $p = 0.19$). Steady-state MPA concentrations were not significantly different between the 2 groups.

2.4.3.2 GVHD

Six of 18 assessable patients (33%) developed grades II to IV aGVHD. Three patients with grade II aGVHD achieved remission with steroid therapy, whereas the other 3 patients with grades III to IV aGVHD required additional agents to go into remission. While on an intermittent IV. schedule, mean MPA levels were 0.5 μg/mL in those with aGVHD versus 0.6 μg/mL in those without aGVHD ($p = 0.4$). Two of 15 assessable patients with a minimum 1-year follow-up developed moderate to severe chronic GVHD. Both patients are in remission; 1 is off treatment and 1 is undergoing systemic immunosuppressive therapy.

2.4.3.3 Overall and Relapse free survival

There were no toxic deaths. The patient with severe congenital neutropenia experienced autologous recovery by 43 days post-transplant. Five patients with acute lymphoblastic leukemia relapsed, of whom 4 have died. Fifteen of 19 patients are alive with a median follow-up of 2.4 years (range: 0.4 to 4.9) and 3-year event-free and overall survival rates of 68% and 79%, respectively (Figure 16). Table 9 shows the overall and event free survival at different stages follow up.

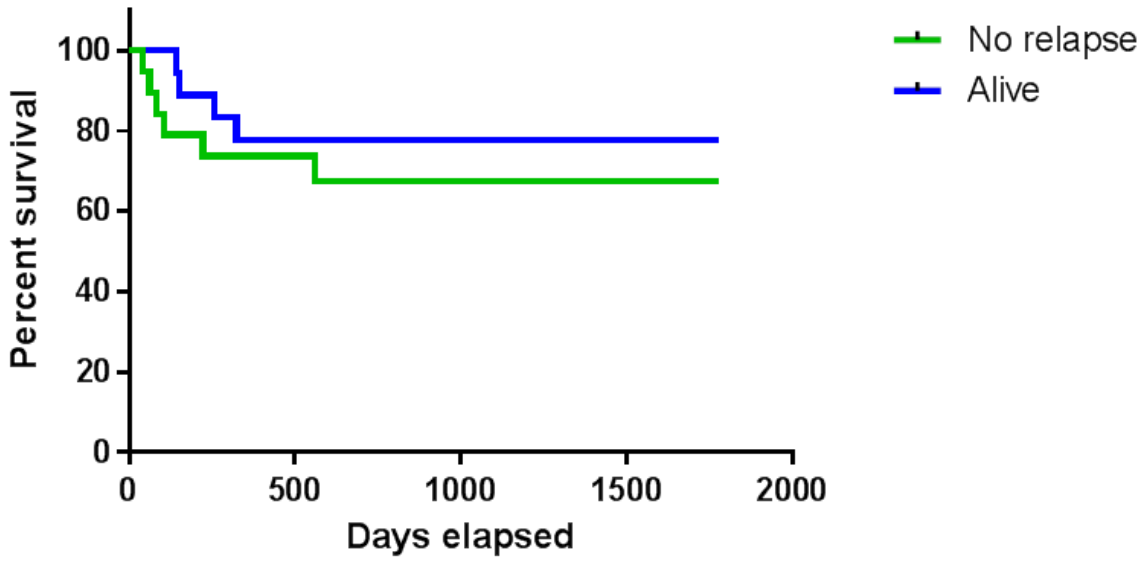


Figure 16. Overall and event-free survival over 5 years post HSCT in 19 pediatric patients

Table 9. Overall and relapse free survival of 19 pediatric HSCT patients at different intervals of follow-up period

Follow-up Period	% Overall Survival	%Event Free survival
Till date	78.9	67.5

2.4.3.4 Correlation of Novel Continuous infusion-based dosing to development of GVHD

Non-parametric analysis was used for statistical analysis of the data owing to the small samples size. A wilcoxon rank sum test was used to measure continuous variables. Patients who developed acute GVHD grade I-IV had steady state levels of less than 1.2 µg/ml whereas patients who had a C_{ss} level of more than 1.8 µg/ml did not develop GVHD (p=0.02).

2.5 DISCUSSION

Pharmacokinetics of MPA and MPAG has been extensively studied in adult HSCT patients on reduced-intensity conditioning; however, the data in myeloablative transplants, especially in the pediatric patients is very limited [133, 163]. Despite the large inter and intra-patient pharmacokinetic variability, MMF continues to be used on a fixed-dose-flat dose schedule [164-166]. A study conducted by Maris et al. [167] has reported that nonmyeloablative conditioning with post grafting cyclosporine and MMF given thrice daily was associated with improved engraftment rate (95%) compared with MMF given twice-daily (85%), as published by the group earlier[168].

The work in this chapter primarily focused on pharmacokinetic guided dosing of MMF to personalize MMF dosing for GVHD prophylaxis in pediatric HSCT patients. To our knowledge this is the first report of pharmacokinetic-guided dosing of MMF based on a target AUC exposure using a 24-hour continuous infusion dosing regimen of MMF in pediatric transplant patients. The main goal of this study was to determine the safety and feasibility of an AUC-based MMF dosing by giving it as a CI over 24 hours. This was feasible in all subjects but did require a dedicated line for IV dosing. All patients started with a 15 mg/kg/dose given thrice a day intravenously. The average daily dose required to maintain patients within the therapeutic range remained close to 45 mg/kg/day during CI and oral dosing. During CI, 17 of 19 patients (89%) achieved MPA C_{ss} within the target of 1.7 to 3.3 μg/mL. In a study conducted by Jacobson et al in 19 pediatric patients receiving stem cell transplant [123], they found a 10-fold variation in

total MPA AUC and a mean AUC_{0-24} of 37.5 μg·hour/mL was observed. Haentzschel et al. [159] have demonstrated feasibility of dosing MMF targeting an exposure as measured by AUC_{0-12} of 35 to 60 μg·hour/mL in 29 adult HCT recipients. Bhatia et al.[150] reported pharmacokinetic data in 38 pediatric HSCT recipients receiving tacrolimus and an initial MMF dose of 900 mg/m^2 every 6 hours intravenously followed by dose adjustments to target total trough concentrations of 1 to 3.5 μg/mL. The total MPA exposure was substantially higher, with an AUC_{0-24} of 157.6 μg·hour/mL compared with 37.5 μg·hour/mL in Jacobson study and 46.5 μg·hour/mL in our study. However, these patients did receive a 2.7-fold higher MMF dose. Dose escalation should be carried out with caution because of concerns about MPA-related hematologic and gastrointestinal toxicities. A comparison of PK parameters in these studies and the current study are shown in Table 10.

In this study, we observed a much higher metabolite to parent exposure ratio (MPAG/MPA) in these subjects consistent with adult HSCT patients. This suggests a higher formation clearance of MPAG in HSCT patients as compared to adult and pediatric SOT patients. Also, the higher MPAG/MPA ratio after oral dosing (29.4) as compared to IV short(12.1) and IV CI (14.8) also suggests the involvement of the gut UGTs in the metabolism of MPA. MPA is highly bound to plasma proteins [15]. This could possibly be explained by saturable protein binding of MPA that may result in greater MPA free fraction and a decrease in total concentration and allowing a greater percentage of the administered drug to be metabolized [169].

Table 10. Comparison of pharmacokinetic parameters across all studies in pediatric HSCT patients on IV and PO dosing of MMF

Study	Dose	Age(years)	N	AUC_{0-8} (µg*hr/ml)	C_{ss} (µg/ml)	C_{max} (µg/ml)
Jacobson et al	15mg/kg q8 iv	0.25-11.7	16	12.6(4.9–49.2)	1.6(0.6–6.1)	5.0(1.8–18.0)
Bhatia et al	900mg/m^2 q6 iv	0.33-16	31	26.82±12.35	4.73±7.22	12.31±7.99
Bhatia et al	900mg/m^2 q6 po	0.33-16	15	26.50±19.03	5.38±3.55	13.15±12.04
Current Study	15mg/kg q8 iv	0.9-21.5	19	14.8(5.6-22)	1.9(0.6-2.7)	5.9(1.5-9)
Current Study	15mg/kg q8 po	0.9-21.5	14	14.0(4.0-32.0)	1.5(0.5-4.0)	5.6(1.3-12.1)
Current Study	24-hour CI	0.9-21.5	19	-	1.7(0.9-2.7)	-

Similar to the results reported by Winter et al. we have also seen a higher MPA clearance in our patient population (10.9 ml/min/kg) as compared to that reported in kidney transplant recipients (7.1 ml/min/kg). A higher MPA clearance can be attributed to low albumin concentrations and concomitant use of cyclosporine. Low albumin concentration leads to lesser

binding of MPA to albumin and availability of more MPA to be eliminated leading to increased clearance and decreased exposure.

Intense blood sampling for pharmacokinetic analysis especially in pediatric patients is inconvenient, and simple methods of quantifying exposure are necessary. Limited sampling strategies have been widely used in kidney transplantation with most of the strategies using a 3 or 4 concentration-time point data over a 12-hour dosing interval after oral administration [93, 98, 170, 171]. There are reports of couple of such studies in HSCT patients [99, 119] . Our results are consistent with previously reported studies showing that trough concentrations are not a good surrogate for MPA overall exposure (R^2= 0.021). Abbreviated AUC as seen in our study, showed a significant correlation between the 4-hour AUC and the 24-hour exposure estimated based on the steady state 8-hour exposure. These results are promising to predict the overall exposure and would allow for a less intensive blood sampling.

The MMF concentrations in our study were below the limit of quantitation for all patients after oral dosing. These observations are in concordance with the data in a study conducted in healthy subjects by Bullingham et al [11]. However, quantifiable MMF concentrations were observed in most patients after an IV short infusion and CI. The plasma concentrations were below quantitation limits in 17 of 19 patients within an hour after stopping the infusion. This suggests that the functional capacity of esterases that are responsible for its metabolism to the active form is intact in these patients. As seen in some other studies MPA exposure is associated with clinical outcomes, and hence optimum exposure in the immediate post-transplant period is very crucial [151, 164-166]. To ensure optimum exposure in the immediate phase post-transplant patients were switched to an individualized IV CI based on pharmacokinetic analysis of the short

infusion regimen. This helped patients not only to achieve but also maintain the desired steady-state levels.

In this study, we have observed low rates of aGVHD (grades II to IV, 33%; grades III to IV, 17%) and chronic GVHD (13%), and all patients with acute and chronic GVHD have responded to therapy. Also, MPA Css was significantly lower, (1.2 versus 1.8 μg/mL) in patients with aGVHD compared with those without aGVHD, respectively. This is consistent with what is known about MPA exposure and outcomes. Harnicar et al. [165]recently reported a higher rate of grades III to IV aGVHD in double cord blood transplant recipients who received doses ≤ 36 mg/kg/day MMF and those with <0.5 μg/mL trough levels of MPA. Adult receiving reduced-intensity double cord blood transplant recipients receiving 3 g/day showed a 49% relative risk reduction in grades II to IV aGVHD rate as compared to the 2g/day dosing [164].

ATG can also play a significant role in GVHD outcomes. Similar to our study, Van Hest et al.[157] used ATG in addition to MMF-cyclosporine A in unrelated donor transplants and observed a low incidence of grades III to IV aGVHD of 7%. In a recent study by Al-Kadhimi et al. [147]conducted in a large cohort of 414 adult patients treated with a uniform MMF-tacrolimus GVHD prevention regimen, found a high rate of grades III to IV aGVHD in sibling donor (22%) and unrelated donor (37%) recipients. Lower incidences of acute and chronic GVHD and non-relapse mortality were reported by studies (including ours) that have used a longer course of MMF with or without taper [172-174].

In our study 95% (18 of 19 patients) achieved donor hematopoietic engraftment. Both neutrophil and platelet count recovery was faster in bone marrow compared with cord blood transplants. Our study is limited by small sample size and heterogeneity in stem cell sources.

Other factors including but not limited to duration of MMF and use of ATG might have affected clinical outcomes in unrelated donor transplants.

Our approach of CI MMF administration may result in more constant IMPDH inhibition and avoid high peak level drug exposure. In renal transplant patients, lower IMPDH baseline activity is associated with lower dosing requirements and presumably lower MPA concentrations [114]. Optimization of MMF based prophylaxis can be further achieved by individualization of MMF dosing based on the IMPDH activity. We have established and validated an assay for *ex-vivo* measurement of IMPDH activity from the conversion of inosine monophosphate to xanthine monophosphate in peripheral blood mononuclear cells. Application of this assay for a PK-PD study has been evaluated in further chapters.

In conclusion, our study shows that individualized MMF dosing by targeting AUC of MPA is feasible, safe, and effective in the early phase after allogeneic HCT. There was a 10-fold variation in the clearance of MPA while patients are on CI. The pharmacokinetics studies showed significantly lower half-life and higher drug clearance in pediatric HCT recipients compared with stable pediatric renal transplant patients. The trough levels poorly correlated with AUC. However, $AUC_{0\text{-}4}$ hour correlated well with $AUC_{0\text{-}24}$. There was no evidence of enterohepatic recirculation of MPA in the study patient population. We observed no toxic deaths, excellent engraftment, and low rates of grades III to IV aGVHD and chronic GVHD. This regimen deserves further validation in a larger cohort of pediatric patients undergoing myeloablative transplantation. In future studies, monitoring IMPDH activity may also facilitate optimizing the dose of MMF.

3.0 DEVELOPMENT AND VALIDATION OF UPLC-MS METHOD FOR QUANTITATION OF MMF, MPA AND MPAG: APPLICATION TO A PROSPECTIVE PK-PD STUDY IN ADULT LUNG TRANSPLANT RECIPIENTS

3.1 ABSTRACT

Mycophenolic acid (MPA) is the active moiety of MMF, a prodrug widely used as part of maintenance immunosuppressive therapy to prevent organ rejection in SOT patients and for GVHD prophylaxis in HSCT patients. Though not routinely monitored like other immunosuppressive drugs, there has been some justification to monitor MPA in transplant patients receiving MMF primarily owing to the large inter and intra-individual variability in PK, dose-related toxicity and the continued risk of acute rejection in organ transplant recipients. A key factor in TDM is the availability of a specific and sensitive assay for measuring all the relevant compounds in a small volume of sample with a shorter turnaround time. In this study, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for simultaneous quantification of MMF, MPA and MPAG in small volume of plasma. Sample preparation comprised of protein precipitation with acetonitrile and subsequent centrifugation, drying and reconstitution of the residue with 50% methanol and 50% water. Deuterium labeled MPA was used as internal standard. The chromatographic separation was achieved on a UPLC HSS T3 column with a gradient flow. LC-MS/MS detection was performed using a triple-stage quadrupole mass spectrometer working in multiple reaction monitoring (MRM) mode with positive ESI. All three analytes were quantified in a single run within 5 min. The assay was linear over a concentration range of 0.01-10 µg/ml for MPA and MMF and from 0.1 -100 µg/ml for MPAG. The lower limits of quantification for MMF and MPA were 0.01 and for MPAG 0.1 µg/ml. Inter and intra-day precisions and accuracies were less within 15%. Sample stability was measured at bench top temperature for up to 24 h, 5 freeze-thaw cycles and at 4 degrees Celsius

in the auto sampler. The described method can be used for quantitation of MMF, MPA and MPAG in plasma samples of small volumes for TDM and pharmacokinetic studies in transplant patients. The applicability of the method was tested in a prospective study conducted in adult lung transplant recipients. The mean AUC of MPA and MPAG was 42.7and 814.1 μg*hr/mL respectively and MPA clearance was 27.1 L/h. The pharmacokinetic parameters observed in this study are comparable to that reported by other groups. Further studies should focus on tailoring MMF dosing strategies that optimize immunosuppressive regimen, improve rejection free survival and minimize toxicity in lung allograft recipients.

3.2 INTRODUCTION

Lung transplant (LT) is the only therapeutic option for patients with end stage lung disease. Although acute clinical outcomes of lung transplant are favorable, long term survival outcomes are often limited by morbidities like Bronchiolitis obliterans syndrome (BOS), especially in the first-year post-transplant.[175] Risk factors for BOS include both acute cellular rejection; antibody mediated rejection and selected infections. [176-181] Immunosuppressive regimens consisting of tacrolimus, MMF, and corticosteroids, are commonly used to prevent rejection episodes after LT. Immune activity and responsiveness to immunosuppressive regimens in LT recipients (LTRs) are heterogeneous which necessitates personalized immunosuppressive treatment approaches. Immunosuppressive regimens consisting of TAC, MMF, and corticosteroids, are commonly used to prevent rejection post LT. In fact, both the immunosuppressive drugs- TAC and MPA, the active metabolite of MMF, demonstrate high inter-patient pharmacokinetic and pharmacodynamic variability. Tacrolimus dosing is individualized in LTRs based on a therapeutic drug monitoring strategy and the history of rejection and infections.[182] Tacrolimus is drug with a narrow therapeutic window and large inter and intra-patient pharmacokinetic variability. Trough blood concentration of tacrolimus is a good surrogate marker of drug exposure as measured by AUC. A therapeutic window has been established for tacrolimus based on the association between probability of acute rejection and adverse effects, with trough blood concentrations. As a result, dosing of tacrolimus is personalized by routine TDM. [183] MMF, the ester form of the active metabolite MPA exhibits high intra and inter-patient pharmacokinetic variability in solid organ transplant recipients.[184]

Studies in renal and heart transplant recipients have demonstrated decreased acute cellular rejection (ACR) episodes when the measured MPA area under the concentration time curve (AUC_{0-12}) falls within the range of 30-60 mg*h/L, when used in combination with cyclosporine and steroids. [185] However, MPA AUC is difficult to measure in clinical practice due to the impracticality of the 12-hour time frame required and the cumbersome process of multiple blood draws. Unfortunately, trough MPA levels do not accurately reflect AUC and cannot be used as a surrogate marker of MPA exposure. For these reasons, MMF is currently dosed on a fixed flat dose strategy, which is suboptimal for LTRs.

Owing to increased sensitivity and quick run time, ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS/MS) based quantification of drug levels in plasma has gained popularity over other conventional methods of drug analysis. However, few methods have been developed to measure MPA and its metabolites simultaneously using UPLC-MS/MShttps://link.springer.com/article/10.1365/s10337-010-1715-6 [186-189] and no studies have been reported to quantify MMF along with MPA and MPAG in the same run. Currently available assays are limited by the volume of sample required for quantitation of all the three analytes MMF, MPA and MPAG.

In the current study, we established a fully validated, sensitive, and accurate UPLC-MS/MS method for the simultaneous quantification of MMF, MPA and MPAG. This method is the first to report a simple two-step sample preparation method to quantify MMF and its metabolites in a plasma. We have used a volume of 50 μL which was diluted 20-fold to quantitate MMF, MPA and MPAG in one single run. This assay was used to quantify plasma samples from lung transplant recipient in this study and is also being used for in other ongoing studies in other solid organ transplant recipients. Furthermore, in this study was aimed to

optimize the sensitivity of the assays so that the required sample volume could be minimized. The added advantage of this assay is the ability to quantitate MMF levels in plasma. Measurement of MMF in plasma can help confirm conversion of MMF to MPA and thereby measure the functional capacity of the esterases involved in hydrolysis of MMF. Incomplete conversion of MMF to MPA can lead to reduced MPA exposure and this is crucial in lung transplant patients that are characterized by lower MPA exposure. The high dilution ratio of 20-fold suggests that the assay can be potentially used to quantitate MMF, MPA and MPAG in much smaller plasma volume. The minimization of the sample volume is very crucial as the assay can be used for conducting pharmacokinetic studies of MPA in pediatric and in other transplant patients, where sample volumes are extremely limited.

3.3 MATERIALS AND METHODS

3.3.1 Materials

UPLC-MS grade methanol, acetonitrile and MS grade water was purchased from Fisher Scientific (Pittsburgh, PA). Formic acid and ammonium acetate was purchased from Sigma-Aldrich (St. Louis, MO). The following chemicals were purchased from Toronto Research Chemicals (TRC): MMF Cat No: M831450, mycophenolic acid (MPA) Cat No: M831500, deuterated mycophenolic acid (MPA d-3) Cat No: M831502 and MPAG Cat No: M831520. Blank plasma was acquired from central reference testing laboratory.

3.3.2 Clinical Samples for Analysis

Plasma samples were collected from subjects enrolled in a prospective study conducted in adult lung transplant recipients. This study was approved by the local Institutional Review Board at the University of Pittsburgh. Patients on a fixed stable oral MMF dose were enrolled in the study. Patients were screened, and informed consent was obtained from eligible patients before enrolling them in the study. Samples for pharmacokinetic evaluation were collected prior to the morning dose of a BID regimen. Serial blood samples were collected over the dosing interval at 0.5, 1, 1.5, 2, 4, 6, 9 and 12 hours. Blood samples (4 mL) were obtained from an indwelling arterial line and immediately transferred to sodium EDTA Vacutainer tubes (BD, 39 Franklin Lakes, NJ). Blood samples were centrifuged at 4°C for 10–15 min at 1500 x g. The supernatant plasma was then transferred to cryovials and stored at −80°C until analysis.

3.3.3 Preparation of Calibration Standards and Quality Control Samples

Three separate stock solutions were prepared for MMF, MPA and MPAG in acetonitrile at a concentration of 2 mg/mL. These solutions were spiked into blank plasma to produce standard working concentrations containing MMF and MPA concentrations ranging from 0.01-10µg/ml, while MPAG concentrations ranged from 1-100 µg/ml. Standard working solutions were stored in -80°C freezer until use.

MPA d-3 was used as an internal standard (IS). A stock solution containing the internal standard with a final concentration of 1 mg/mL was prepared in acetonitrile and stored in -80°C until use. A working solution of IS with a concentration of 250ng/ml was prepared fresh in acetonitrile on day of assay. For the internal quality control (QC) samples, a separate stock solution was prepared in methanol at a concentration of 2 mg/ml. QCs were prepared in blank plasma at 4 levels containing MMF and MPA at 0.04 µg/ml (A), 0.4 µg/ml (B), and 4 µg/ml (C) and 8 µg/ml (D) and MPAG at 0.4 µg/ml (A), 4 µg/ml (B), and 40 µg/ml (C) and 80 µg/ml (D) and stored at -80°C until analysis.

Samples were processed by precipitating the protein with acetonitrile as follows-

To 50 µL of plasma sample, 200 µL of acetonitrile containing 250 ng/ml of IS was added to an Eppendorf tube. The sample was vortexed rigorously for 30 seconds and then centrifuged at 15000 rpm for 8 minutes at 4°C. Following centrifugation, 25 µL of supernatant was transferred to another Eppendorf tube and then diluted 20-fold with 475 µL of 50:50 acetonitrile: water. Sample was vortexed for 30 seconds and then 100 µL was transferred to a glass autosampler vial of which 1 µL was injected into the UPLCMS/MS.

3.3.4 UPLC and Mass Spectrometric Conditions

Chromatographic separation and quantification was performed using a Waters Acquity Ultra Performance liquid chromatography (UPLC) H class coupled with a Xevo TQS triple quadrupole mass spectrometer. A Waters Acquity UPLC HSS T3 1.7 um, 2.1*100 mm column (Waters, Milford, MA) was used for the separation of the three analytes and the internal standard. The mobile phase consisted of a gradient of solution A (95% water, 5% ACN with 2mM ammonium acetate and 0.1% formic acid) and solution B (95% ACN, 5% Water with 2mM ammonium acetate and 0.1% formic acid) with an initial composition of 65% A held up until 1 min and changing to 50% at 1.5 min and then transitioning to 100% B held till 4 min and then switching back to 65% A at 4.1 min until the end of run. The flow rate was 0.3 mL/min and the column was maintained at 50°C and an injection volume of 1 µL was used. Analytes were detected via MS/MS with an electrospray ionization source under positive mode. Ultra-high-purity nitrogen was used for source and collision cell gas. The following settings were applied: Capillary voltage 2.5 kV, Cone voltage: 40 V, Desolvation temperature: 500 °C, Cone gas flow 150 l/hr and Desolvation gas flow 850 l/hr. The mass transitions for MMF, MPA, MPA d-3 and MPAG were as follows:

MMF: 433.9>114, MPA: 320.96>206.97, MPA d-3: 323.9>209.9 and MPAG: 518.8> 343.1

The auto sampler tray where the samples were stored during analysis was maintained at 4°C. Sample injection volume was set to 1 µL for all compounds. Calibration standards were injected first in an increasing concentration followed by a set of QCs then the half of study samples, then second set of QCs rest half of the study samples, and lastly the third set of QCs. Between injections, the autosampler needle was washed with methanol/water (1/1, v/v). The

method validation and sample analysis were conducted by constructing calibration curves by plotting the analyte/IS chromatographic peak area ratio against the prepared analyte concentration in each calibration standard. Linear regression was used to fit the calibration curve with a weighting of $1/x^2$. Standards were then back calculated, and QC concentrations were determined by interpolation. QCs failed if they 3/4 were not within ±15% of nominal concentration. Calibration standards that failed to meet this criterion were excluded from regression, and as specified in the FDA bioanalytical guidelines at least ¾ of the calibrators were required to be included in regression. The lower limit of quantification (LLOQ) was defined as the lowest concentration of analyte with acceptable imprecision (≤15%) and mean accuracy (±15% of nominal concentration). At each QC concentration, at least 2 of the 3 replicates of prepared QC samples were required to be within 15%.

3.3.5 Method Validation

Validation was performed by assessment of LLOQ, intra and inter-day accuracy and imprecision, specificity, recovery and matrix effect, stock solution stability, and freeze-thaw stability. Ten plasma calibration standards ranging in concentration from 0.1 – 100 µg/mL for MPAG and 0.01-10 µg/mL for MPA and MMF were prepared and analyzed in duplicate in 5 runs performed on 5 consecutive days. The LLOQ was determined as the minimum concentration that was quantifiable with an accuracy and precision within ±15% of the nominal value. The concentration which yielded a minimum value of a signal-to-noise ratio greater than 3:1 was set as the lower limit of detection (LLOD). Accuracy and precision were determined from replicate samples of spiked QC concentrations. Intra-day validation was performed from 5 replicates of each concentration assayed on the same day within the same analytical batch. Inter-assay

accuracy and precision at each concentration were calculated from a total of 18 replicates assayed over 5 days. Accuracy which is defined as the deviation of the calculated value (E) from that of its true value (T) and is expressed as a percent. It is calculated as relative standard error (RSE%), using the equation RE% = (E-T)/T*100. Precision is calculated as the percent coefficient of variance (%CV) where %CV = standard deviation/mean x 100. An accuracy and precision within ±15% of the nominal value is considered acceptable.

Freeze thaw stability of the analytes was tested with 5 freeze-thaw cycles and room temperature stability was determined over 6 hours. Recovery and matrix effect were determined at all the QC concentrations indicated in Table 12 . For each biological matrix, one QC set (set A, n = 3) was prepared according to normal sample processing method. Another set of QCs (set B, n = 3) was prepared as neat solution in 50% methanol. The third set of QCs (set C, n = 3) was prepared without analyte and spiking the sample into the matrix post-extraction.

3.3.6 Comparison to published methods

Several methods have been developed to quantify MPA and MPAG. This is the first method that has been used to quantitate MMF via UPLC-MS. Therefore, we aimed to expand on these current methods to develop an assay with greater sensitivity. We have used a small amount of sample and diluted it 100-fold. The sample volume was kept at 50 μL to avoid pipetting error. The use of UPLC-MS system facilitated for a shorter run time of 5 minutes. The novel approach of detecting MMF, MPA and MPAG in the same run using a UPLC-MS based separation has been developed for the first time. This method has a potential for analysis of the three analytes,

namely MMF, MPA and MPAG in dried blot spots and for analysis in critically ill patients or pediatric patients where sample volume is a limiting factor.

3.3.7 Pharmacokinetic Analysis

Concentration-time data obtained from 4 lung transplant recipients were used to determine MMF, MPA and MPAG pharmacokinetics. Non-compartmental analysis using WinNonlin® pharmacokinetic software (Standard Edition, Version 6, Apex NC) was used for calculation of pharmacokinetic parameters. The pharmacokinetic parameters determined were: area under the curve for the dosing interval $AUC_{0-\tau}$, and dose normalized $AUC_{0-\tau}$, the maximum concentration obtained during the dosing interval (C_{max}); dose-normalized C_{max}, the time at which C_{max} occurred (T_{max}) and the clearance over the dosing interval.

3.3.8 Statistical Analysis

All statistical analysis for descriptive statistics was performed in Excel (version 14.0, Microsoft Corp., Redmond, WA).

3.4 RESULTS

3.4.1 Mass spectrometric conditions and Standard curve preparation

Table 11 provides the mass spectrometric conditions for each of the compounds.

Table 11. Mass spectrometric conditions for MMF, MPA and MPAG

Analyte	ESI mode	Cone Voltage	Desolvation temperature	MRM transition
MPAG	Positive	40 V	500°C	518.8 → 343.1
MMF	Positive	40V	500°C	433.9→114
MPA	Positive	40V	500°C	320.9→ 206.9

Table 12 provides a summary of the standard and QC concentrations used for the plasma assay. Based on previous knowledge about clinical concentrations of MPA pharmacokinetics [28], calibration curve range was carefully selected to be most appropriate for the biological matrix and analyte to be used.

Table 12. Standard curve and QC concentrations for MMF, MPA and MPAG

Analyte	Matrix	Calibrator Concentration (µg/ml)	QC concentrations (µg/ml)			
			Level 1	Level 2	Level 3	Level 4
MPAG	Plasma	0.1,0.25,0.5,1,2.5,5,10,25,50,100	0.4	4	40	80
MMF	Plasma	0.01,0.025,0.05,0.1,0.25,0.5,1,2.5,5,10	0.04	0.4	4	8
MPA	Plasma	0.01,0.025,0.05,0.1,0.25,0.5,1,2.5,5,10	0.04	0.4	4	8

Figure 17 shows the chromatographic separation of 4 compounds on UPLC -MS and the elution time for each of the compounds is as follows: MPAG (1.26 min), MMF (2.26 min), MPA (3.46 min) and internal standard MPA d-3 (3.46 min).

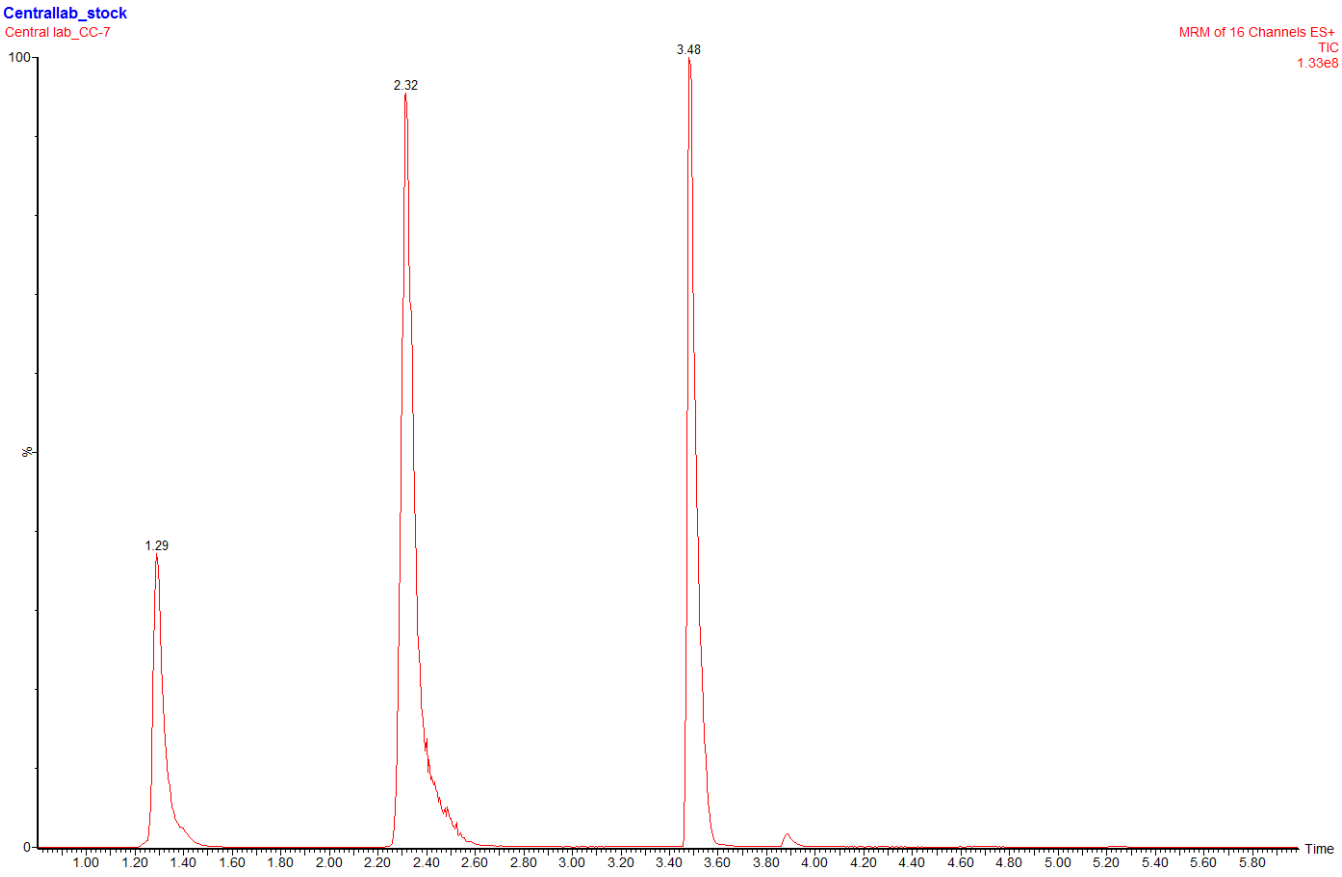


Figure 17. Chromatograms of drug MMF (2.3 min), MPA (3.5 min), MPAG (1.3 min) and deuterated MPA (3.5 min) in a standard solution.

3.4.2 Linearity, Accuracy and Precision

All calibration curves were fitted well by linear regression with a weighting factor of $1/x^2$. In plasma, the standard curve of MMF was linear up to 5 µg/mL. Back-calculation of calibrator and QC concentrations were performed by interpolation and consistently yielded values within 20% of nominal concentration with an r^2 for calibration curves typically being ≥0.99. Intra and inter-day accuracy and precision data at each QC concentration level is summarized in Table 13 and Table 14 respectively. The method was highly accurate and precise. Mean values for intra- and inter-day accuracy (calculated as percent deviation) were within 15%, except for the lowest QC for MPAG which was within 20% while the imprecision in plasma for intra and inter-day assay did not exceed 15%. Figure 18 and Figure 19 shows the calibration curves for the three analytes. The lower limit of quantification in this assay was 0.01 µg/mL for MPA and MMF and 0.1 µg/mL for MPAG.

Table 13. Intra-day accuracy and precision for MMF, MPA and MPAG for n=6 runs on the same day

MMF Concentration (µg/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
0.04	0.0	-0.8	0
0.4	0.4	-7.3	0
4	4.0	-0.8	3.5
8	7.4	-7.3	2.1
MPA Concentration (µg/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
0.04	0.0	0	0
0.4	0.4	-4.2	0
4	4.3	6.7	5.4
8	9.3	15.0	3.7
MPAG Concentration (µg/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
0.4	0.3	-16.7	14.1
4	3.9	-3.3	0.0
40	37.6	-6.0	2.6
80	79.0	-1.3	3.3

Table 14. Inter-day accuracy and precision for MMF, MPA and MPAG for n=20 runs on 4 different occasions

MMF Concentration (µg/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
0.04	0.0	0.0	0.0
0.4	0.4	3.8	8.6
4	3.9	-2.9	4.9
8	7.2	-9.8	4.0
MPA Concentration (µg/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
0.04	0.0	0.0	0.0
0.4	0.4	-1.2	5.5
4	4.2	4.7	8.2
8	8.9	10.6	15.0
MPAG Concentration (µg/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
0.4	0.3	-16.3	19.5
4	3.7	-7.6	5.5
40	39.1	-2.3	4.2
80	82.9	3.7	4.5

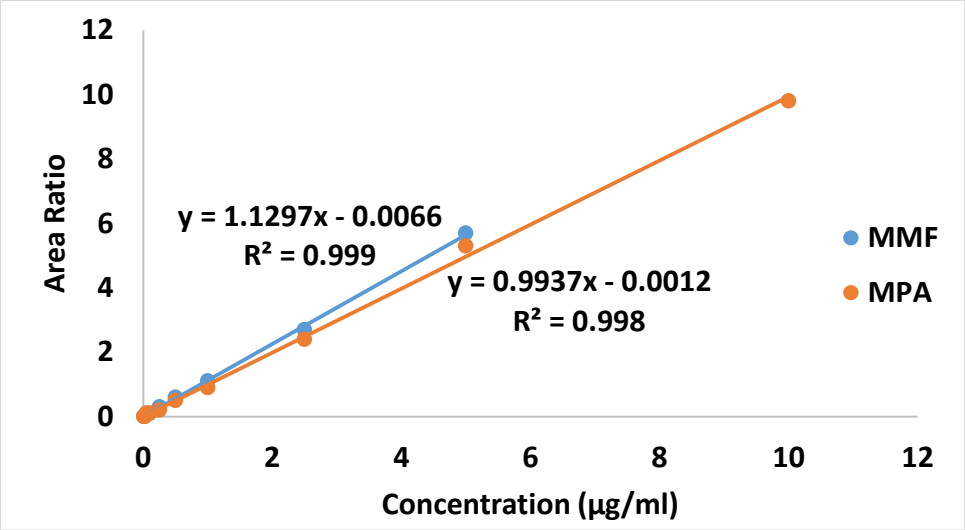


Figure 18. Calibration curve for MMF and MPA in plasma

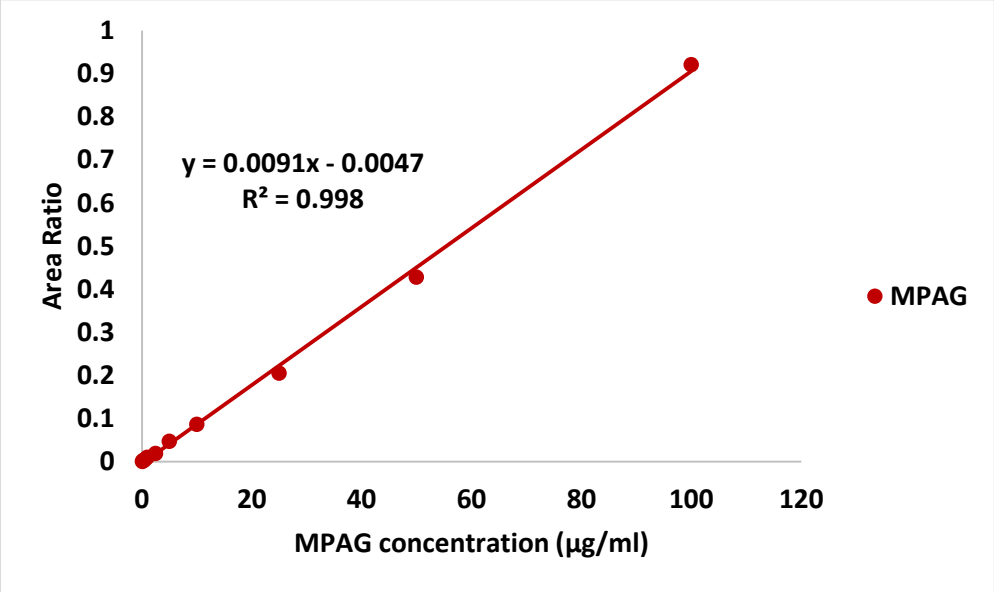


Figure 19. Calibration curve for MPAG in plasma

3.4.3 Recovery and Stability

Analytes at all 4 QC levels pre-extraction were compared to the mean response post extraction. at low, medium and high levels, and IS at the level of use: pre-extraction spiked samples (n=3) are compared with mean response of post-extraction spiked matrix samples (n=3). For MPA, MPAG and MMF the extraction recovery was consistently between 75-100% for MPAG, 75-106.4% for MMF and 75-94.3% for MPA. The matrix effect ranged from 50-88% for MPAG, 75-101.5% for MMF and 95.9-100% for MPA with the lower concentrations having no effect of matrix. The stability data for 5 freeze-thaw cycles, 24 bench-top and 4°C autosampler stability is shown in Table 15.

Table 15. Stability of MMF, MPA and MPAG in human plasma. Reported values are mean % of initial observed

MMF (% stability)			
Concentration (µg/mL)	24-hour at room temperature	5 freeze-thaw cycles	Autosampler at 4°C
0.04	100.0	100.0	100.0
0.4	100.0	118.0	100.0
4	99.3	109.4	102.1
8	100.7	105.7	99.8
MPA (% stability)			
Concentration (µg/mL)	24-hour at room temperature	5 freeze-thaw cycles	Autosampler at 4°C
0.04	100.0	100.0	100.0
0.4	100.0	118.0	104.3
4	109.5	114.8	99.6
8	105.7	126.0	94.5
MPAG (% stability)			
Concentration (µg/mL)	24-hour at room temperature	5 freeze-thaw cycles	Autosampler at 4°C
0.4	100.0	92.9	97.5
4	102.2	99.5	95.7
40	100.9	106.6	106.1
80	100.1	106.1	107.5

3.4.4 Assay Validation in Clinical Use

The developed and validated assay was successfully applied to plasma samples from 4 adult lung transplant recipients receiving MMF as a part of their maintenance immunosuppression. These patients were receiving a fixed dose of 1g BID and at steady state. All patient samples were well above our LLOQ thus confirming the linearity range for this patient population. Figure 20 and Figure 21 show the concentration time profiles for each individual patient. Figure 22 and Figure

23 represent the mean concentration time profiles in these patients for MPA and MPAG respectively.

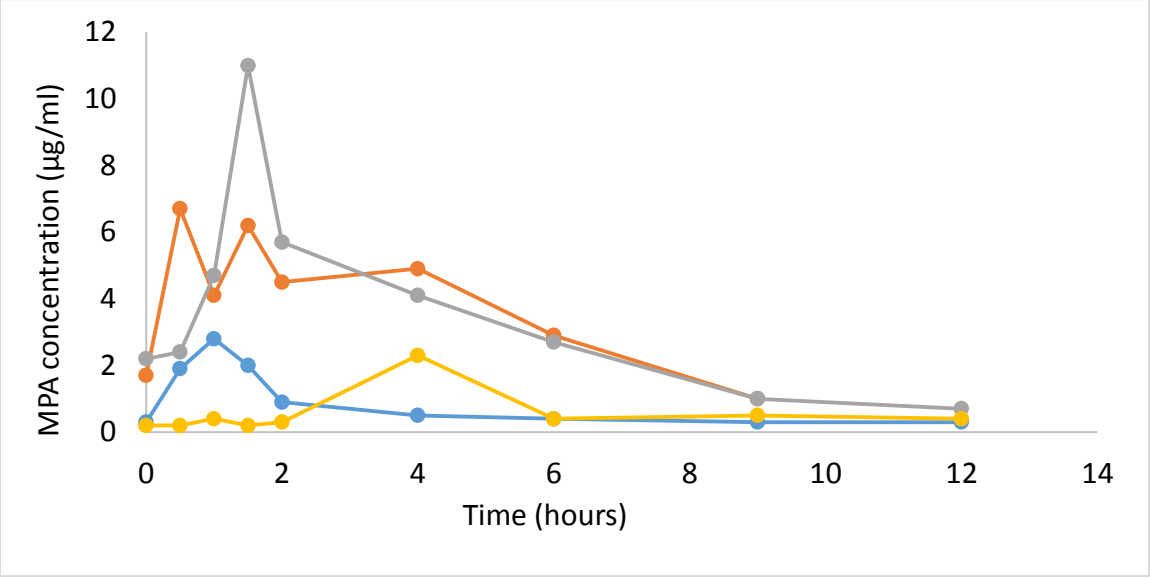


Figure 20. Concentration time profiles of MPA in 4 adult lung transplant recipients.

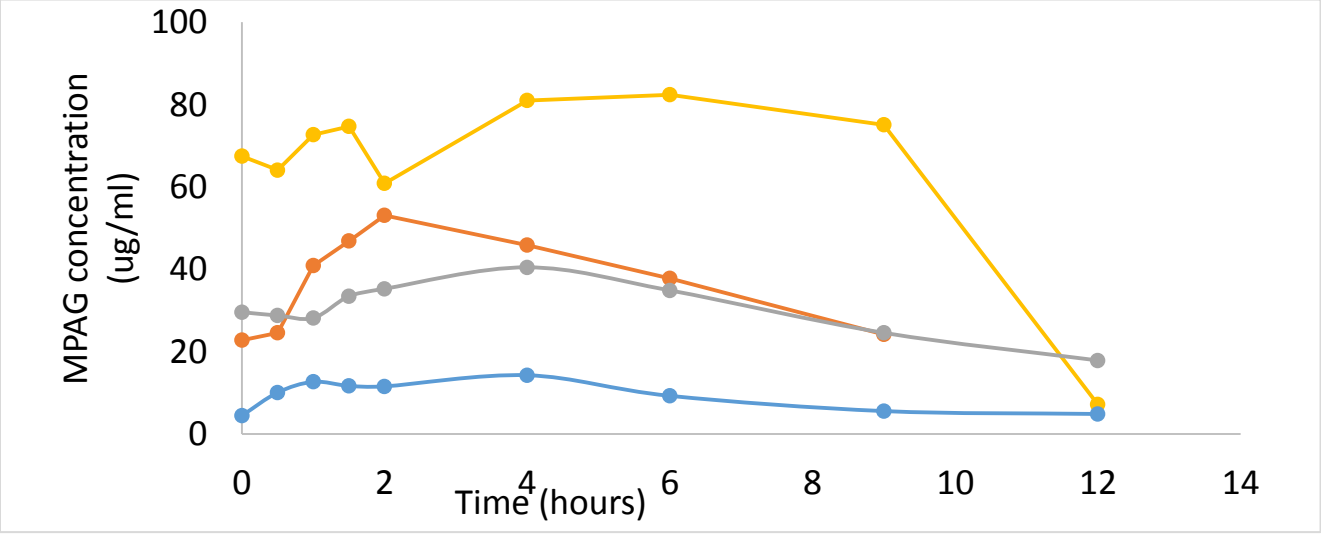


Figure 21. Concentration time profiles of MPAG in 4 adult lung transplant recipients

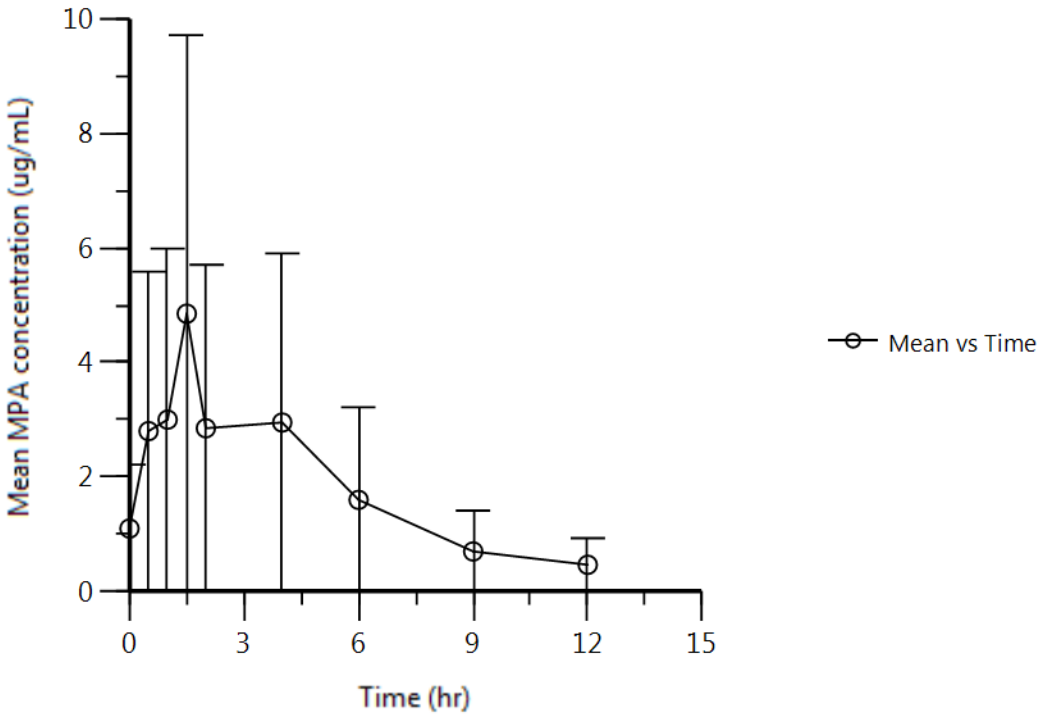


Figure 22. Mean MPA concentration time profile in adult lung transplant recipients

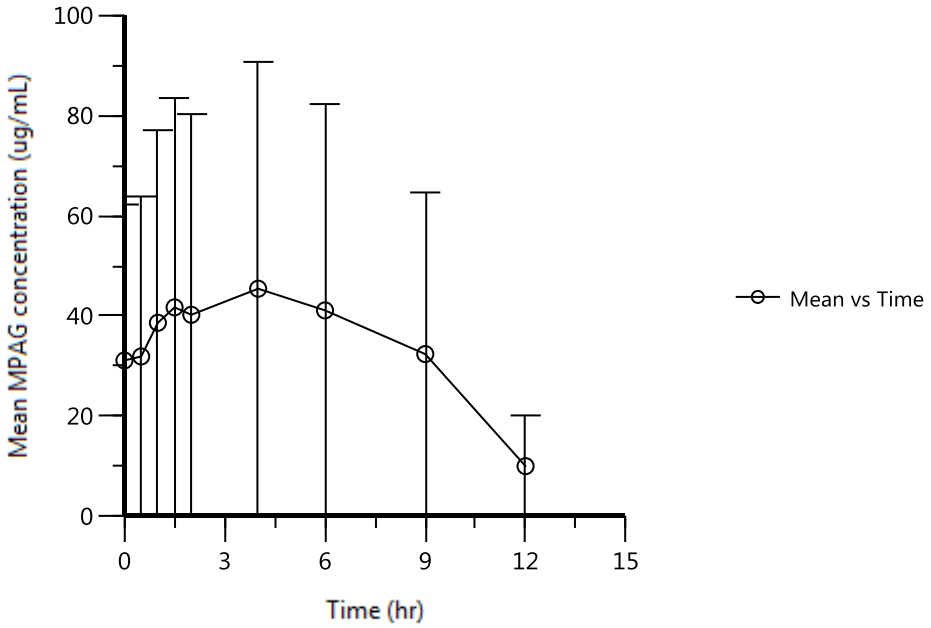


Figure 23. Mean MPAG concentration time profile in adult lung transplant recipients

3.4.5 Pharmacokinetic Analysis of MMF, MPA and MPAG

Table 16 summarizes the pharmacokinetic parameters of MPA and MPAG for the study patients. The lung transplant patients had significantly lower MPA AUC and higher clearance as compared to kidney and liver transplant recipients receiving the same dose. The apparent steady state MPAG/MPA metabolic ratio (12.3) of these patients was comparable to other solid organ transplant patients.[18] suggesting no apparent changes in the formation clearance of MPAG. Overall the pharmacokinetic parameters are comparable to those seen in other lung transplant studies reported by Ensom et al and Ting et al. [190-192].

Table 16. Pharmacokinetic parameters of MPA and MPAG in adult lung transplant recipients

Pharmacokinetic Parameters (Units)	MPA	MPAG
AUC_{0-12} µg*hr/ml	21.3 ± 13.1	407.1 ± 250.2
T_{max}(hours)	1.8 ±1.6	2.7 ± 1.3
Half-life (hours)	4.0 ±1.8	-
Clearance (CL/F) (L/hr)	27.9 ±17.2	
C_{max} (µg/ml)	5.7 ± 3.5	46.8 ± 24.5
MPAG/MPA ratio	12.3	

The AUC_{0-24} correlated well with the abbreviated AUCs calculated over 2-hour ($r^2 > 0.93$) and 4-hour period ($r^2 > 0.98$) as shown in Figure 24.

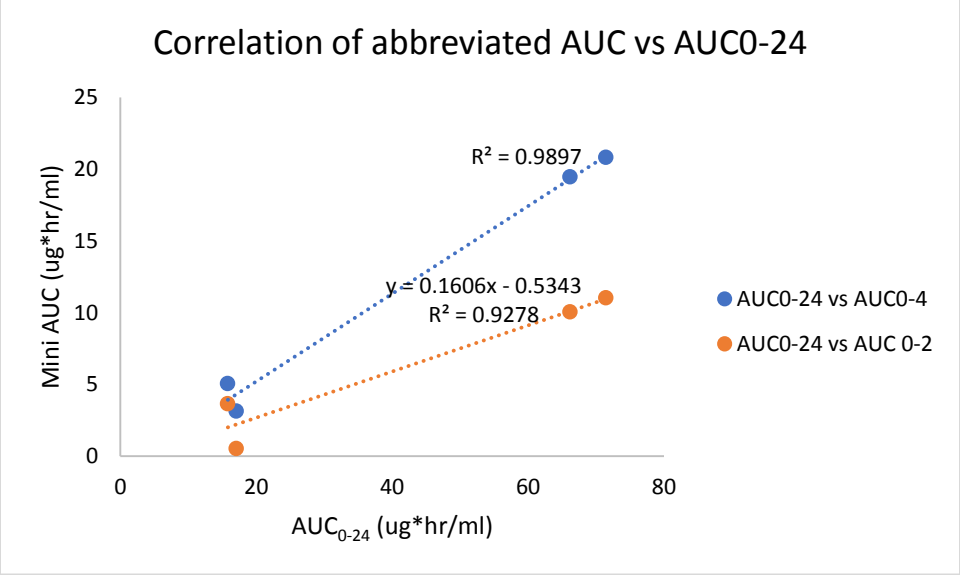


Figure 24. Correlation of mini AUCs with total AUC in 4 adult lung transplant recipients on oral 1 mg BID dose of MMF at steady state.

3.5 DISCUSSION

There are several studies characterizing the inter-patient and intra-patient variability in PK of MPA in other solid organ transplant recipients.[18, 185, 193] To our knowledge, there are only a limited number of studies on the PK of MPA available in the lung transplant patient population. Unlike kidney and liver; lung is not directly involved in the elimination of MPA and hence extrapolations in lung transplant recipients cannot be made based on the pharmacokinetic data in other solid organ transplant recipients. Mycophenolate mofetil exhibits time-dependent changes in drug clearance in kidney and liver transplant recipients.[18, 193] This is primarily due to the changes in protein binding in the immediate post-transplant phase. In kidney transplant patients, for the same MMF dose the total exposure was 30%–50% lower in the first few weeks post-transplantation, as compared to the exposure in the maintenance phase (1-6 months post-transplantation). Renal insufficiency is known to decrease the protein binding of MPA and increase the free fraction. In a study conducted in 33 kidney transplant recipients[194] 13 had poor renal function and had 86% lower AUC and 2-fold higher free fraction and a corresponding higher oral clearance as compared to those having normal renal function on day 4 post-transplant but had comparable exposure on day 28.

While all of the studies in SOT patients measure MPA, none have measured MMF, MPA and MPAG simultaneously. Measurement of MMF can facilitate understanding of functional capacity of estreases that are responsible for conversion of MMF to MPA and measurement of MPAG and the parent to metabolite ratio facilitates understanding of metabolism of MPA. All three compounds and the deuterated internal standard were simultaneously detected, identified, and quantified using a validated UPLC-MS/MS method. All three compounds showed excellent

stability for 24 hours at room temperature. Additionally, we demonstrated that the matrix effect of plasma did not significantly affect the reliability and reproducibility of the assay, for all compounds measured. The high variability in the recovery did not affect quantitation of the clinical samples.

A simple protein precipitation method coupled with a sensitive UPLC-MS/MS method was developed and validated. Advantages of this method include a wide yet clinically relevant concentration detection of MMF, MPA and MPAG, the small sample volume (50µl) and a shorter run time of 5 minutes. Although similar sample volume has been reported in other LC-MS methods, we have diluted the sample 100 times. Validation of this method for a smaller starting sample volume of 10 µl is on-going. Once the method is validated with smaller plasma volume; it can potentially be applied to pharmacokinetic studies of MMF in transplant patients and can thereby facilitate routine therapeutic drug monitoring of MPA and help in optimizing MMF dosing. The utility, sensitivity and suitability of this assay were demonstrated by analysis of samples from a pharmacokinetic study of oral MMF in adult lung transplant recipients. The method was optimized to achieve high sensitivity for the detection of MMF and its metabolites (MPA and MPAG) by 100-fold dilution. The calibration was linear between 0.01-10 µg/ml for MMF and MPA and was linear over the range of 0.1-100 µg/ml for MPAG. To date this is the first report of a highly sensitive UPLC based quantitation method of MMF, MPA and MPAG in lung transplant recipients.

As mentioned by other research groups [195, 196], the chromatographic separation of MPAG and MPA is very crucial as MPAG can undergo in-source fragmentation to MPA, which can potentially co-elute with MPA. A run time of 5 minutes provided adequate time for chromatographic separation of the 2 compounds.

Our study, being one of the only few conducted in lung transplant recipients can help characterize the pharmacokinetic profile and protein binding of MPA in stable lung transplant recipients in the immediate phase and during the maintenance phase post-transplantation. The pharmacokinetic parameters determined from this study are comparable to those reported in other studies[190, 191]. We observed a significant correlation between abbreviated AUC calculated over 2-hour and 4-hour periods with the total steady-state AUC. This study is however limited by the low sample size and therefore, results from our study cannot be generalized to all lung transplant recipients.

The high intra and inter-patient variability warrants for future clinical studies to better understand the PK-PD relationship of MPA and help optimize dosing in this under-studied transplant subpopulation. This variability further emphasized the need for monitoring IMPDH inhibition and using it as a potential marker for rejection and tailoring MMF dosing.

4.0 *EX-VIVO* QUANTITATION OF IMPDH ACTIVITY IN PERIPHERAL BLOOD MONONUCLEAR CELLS: HPLC-MS METHOD ESTABLISHMENT, VALIDATION AND APPLICATIONFOR PHARMACODYNAMIC MONITORING IN ADULT LUNG TRANSPLANT RECIPIENTS

4.1 ABSTRACT

The immunosuppressive response to Mycophenolate Mofetil is based on the degree of inhibition of IMPDH activity. Once in systemic circulation, MMF gets hydrolyzed to the active form mycophenolic acid. Mycophenolic acid is a reversible non-competitive inhibitor of the enzyme IMPDH and is more selective towards T and B lymphocytes. Use of MMF is frequently associated with dose-dependent gastrointestinal or hematological side-effects like diarrhea, gastritis or leukopenia. Consequently, many patients require dose modification or have to discontinue MMF administration, the effect of which is suboptimal immunosuppression and increases the risk of acute rejection (AR). This underlines the importance of measuring the biological response to MMF, and thereby, helps personalize the MMF based immunosuppression. Therefore, measurement of pharmacodynamics effect by quantitating the IMPDH activity may provide direct information on the functional activity of MMF therapy *in-vivo*. Several trials in solid organ transplant recipients have demonstrated large inter-patient variability in baseline IMPDH activity as also the time required for complete recovery of enzyme activity after MMF administration [114, 197, 198].

Limited methods have been used to measure IMPDH activity. A critical requirement of a method is that it can detect IMPDH activity in a patient sample with a low PBMC count. Here we have established a robust LCMS-MS assay for *ex-vivo* quantification of IMPDH in isolated PBMC lysate preparation. The mean intra- and inter-assay accuracies ranged from 90-110%, and intra- and inter-assay imprecision did not exceed 15% for both XMP and adenosine monophosphate (AMP). We determined IMPDH activity in four lung transplant recipients on MMF and

retrospectively evaluated the IMPDH activity in 20 patients within 1-year post lung transplantation. The established assay was able to specifically and reliably measure IMPDH activity *ex-vivo* from patients with low PBMC count. The assay was successfully applied to quantitate and evaluate the pharmacodynamic activity in MMF-treated lung transplant patients. The IMPDH activity was very variable across both the prospective and retrospective studies. In the prospective study in lung transplant recipients, the IMPDH reached a nadir at the maximum concentration of MPA. The single time-point IMPDH activity in the retrospective study did not show any statistically significant association with rejection or infection. However, patients who rejected the graft did show a trend towards higher IMPDH activity. A close and continuous monitoring of the IMPDH could be effectively used as a prognostic marker of rejection.

4.2 INTRODUCTION

Different strategies are used to optimize immunosuppressive drug dosing in transplant patients. Optimization of drug dosing based on exposure is the most common of all the approaches. However, this approach is severely limited by the feasibility of multiple sampling over a dosing interval. Another approach is pharmacodynamic (PD) monitoring, which involves measurement of the biological response of the drug at its site of action. Measurement of PD alone or PK-PD analysis provides a novel method for optimization of drug dosing. This has been reported for CNIs like cyclosporine , tacrolimus [199-201], MMF [103, 202] and azathioprine [203].

MMF, the prodrug of the active form MPA mediates its effect by inhibiting the enzyme IMPDH, which is involved in the de novo biosynthesis of purine nucleotides [204, 205] as represented in Figure 26. Suppression of IMPDH activity in the lymphocytes by MPA inhibits purine synthesis and arrests cell cycle progression in the G1→S phase.[183] Data in renal transplant recipients illustrates that pre-transplant baseline IMPDH activity could predict episodes of ACR and infection post-transplantation.[110, 114, 115, 206] In a renal transplant recipients study conducted by Glander et al. i)IMPDH activity above a cut-off value, ii)MMF dose reduction and iii)age of recipient were shown to be the significant contributors for acute rejection. The study showed that patients with high baseline IMPDH activity and MMF dose reduction had the highest rejection rate (81.8% vs. 36.4%, $p<0.01$). In another study by Vethe et al. the IMPDH enzyme activity strongly correlated with the systemic MPA concentration throughout the dosing interval in all patients receiving MMF [206]. The minimum IMPDH activity with a median reduction of 92 % (range 74–98 %) compared to the baseline levels,

occurred shortly after MMF administration and coincided with the peak MPA which occurred at 1 hour. Chiarelli et al. studied IMPDH activity in healthy subjects and renal transplant recipients on MMF treatment. Sommerer et al.[100] evaluated the association between pharmacokinetic and pharmacodynamic parameters and the risk of adverse events in 66 adult renal transplant patients on EC-MPS. The median MPA concentrations over time were inversely corelated with IMPDH activity over time as seen in Figure 25.

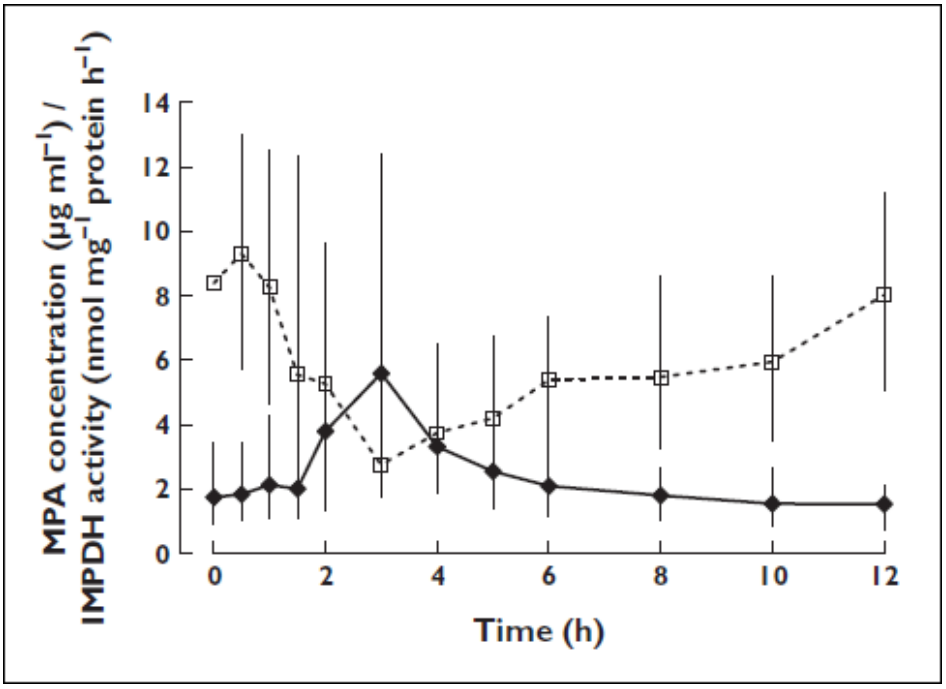


Figure 25. Median MPA concentrations and median IMPDH activities observed over the 12-h sampling period after 1 mg oral dose of EC-MPS in 66 adult renal transplant recipients.

Patients who rejected their graft were seen to have higher baseline IMPDH activity. In a pediatric renal transplant study by Fukuda et al. a simultaneous decrease in IMPDH enzyme

activity was seen with an increase in the MPA plasma concentration, with maximum inhibition coinciding with maximum MPA concentration indicating a direct concentration-response relationship. The EC_{50} value of 1.0 mg/L from the study was well aligned with the clinically established pre-dose (trough) concentration target range of 1.0 to 3.5 mg/L. However, baseline IMPDH activity and the effect of MPA on IMPDH post LT has never been characterized and associated with any clinical outcomes in LTRs. In this study, we have established a method for measuring the IMPDH activity in lung transplant recipients and propose to use this to personalize MMF dosing.

Various assays for measuring the pharmacodynamic effect of MPA by measuring the *ex-vivo* IMPDH activity in mononuclear cells during MPA therapy have been established [104, 106, 207]. In adults on long term MMF administration, IMPDH activity displays wide inter-patient but relatively small intra-patient variability [208]. Recent chromatographic assays have used LCMS- MS for quantification of XMP, the catalytic product of the enzyme, to indirectly measure the inhibition of IMPDH activity [209, 210] (Figure 26). However, these assays are limited by the large number PBMC count (5-10 million cells/ml of lysate) required for quantitation of IMPDH activity. Here we report a highly sensitive robust liquid chromatography-mass spectrometry method based on Bemer et al. with slight modification to measure recipient pre-transplant IMPDH activity *ex-vivo* in isolated peripheral mononuclear cells (PBMCs) lysate based on the quantification of XMP formation normalized to intracellular AMP concentration. The established assay could quantitate IMPDH activity in lysate with PBMC concentration of 1-5 million cells/ml.

This study has retrospectively quantitated IMPDH activity in banked PBMCs from 20 LTRs on MMF maintenance immunosuppression at University of Pittsburgh Medical Center

(UPMC). Thus, this study will address our central hypothesis that IMPDH activity highly influences the incidence of rejection and infections in LTRs. Through this study, we have also established the feasibility of PK-PD analysis by prospectively evaluating relationship between IMPDH activity and MPA exposure in 4 adult lung transplant recipients. IMPDH activity measurement can readily be translated into a routine clinical test in the future and can significantly improve MMF dosing and clinical outcomes in not only LTRs, but also in other solid organ transplant recipients.

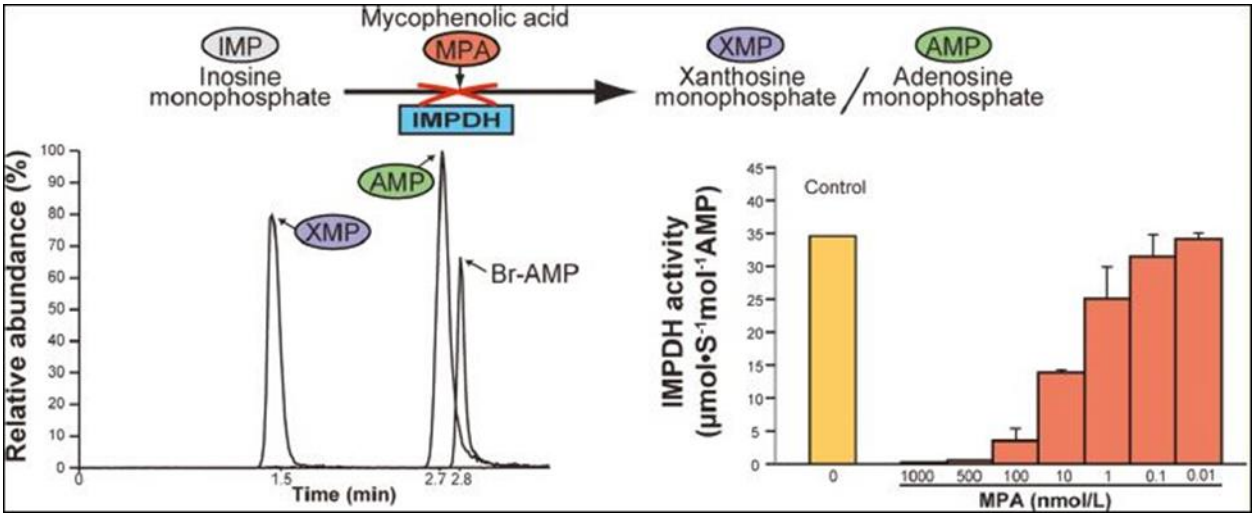


Figure 26. Indirect measurement of MPA based IMPDH activity inhibition by quantification of formed XMP normalized to the intracellular AMP levels.

4.3 METHODS

4.3.1 Chemicals and reagents

IMP, AMP, 8-bromoadenosine 5-monophosphate (Br-AMP), were ordered from Sigma Aldrich. XMP (Toronto Research Chemicals), sodium dihydrogen phosphate, potassium chloride, ammonium acetate, potassium hydroxide(Fisher), Dulbecco's Phosphate Buffered Saline (Invitrogen), Ficoll-Paque solution (GE Healthcare,QC, Canada), SepMate 50 (Stem Cell technologies)

4.3.2 Instruments and Column used

We have used a Hypercarb, 100 mm x 2.1 mm, 5 microns, (Thermo #35005-102130) for the separation of analytes. Chromatographic separation was achieved on an Agilent HPLC/MS system series 1200 with a thermostatically controlled autosampler (Agilent Technologies, Palo Alto, CA) and SCIEX 4000 Q triple quadrupole ion trap mass spectrometer. Thermo Scientific; Sorvall Legend X1R (refrigerated) Centrifuge was used for isolation of PBMCs.

4.3.3 Sample Collection and Handling

4.3.3.1 Prospective Study

This was a prospective PK-PD study in adult lung transplant recipients (IRB Protocol number: PRO15080386) at the UPMC. The Institutional Review Board of the University of Pittsburgh approved the protocol. Patient samples were collected for a prospective PK-PD study over a 12-hour dosing interval post morning oral dose of MMF. The study design is depicted in Figure 27. Upon obtaining informed consent from patient's whole blood was collected in Lithium-heparinized green top tubes.

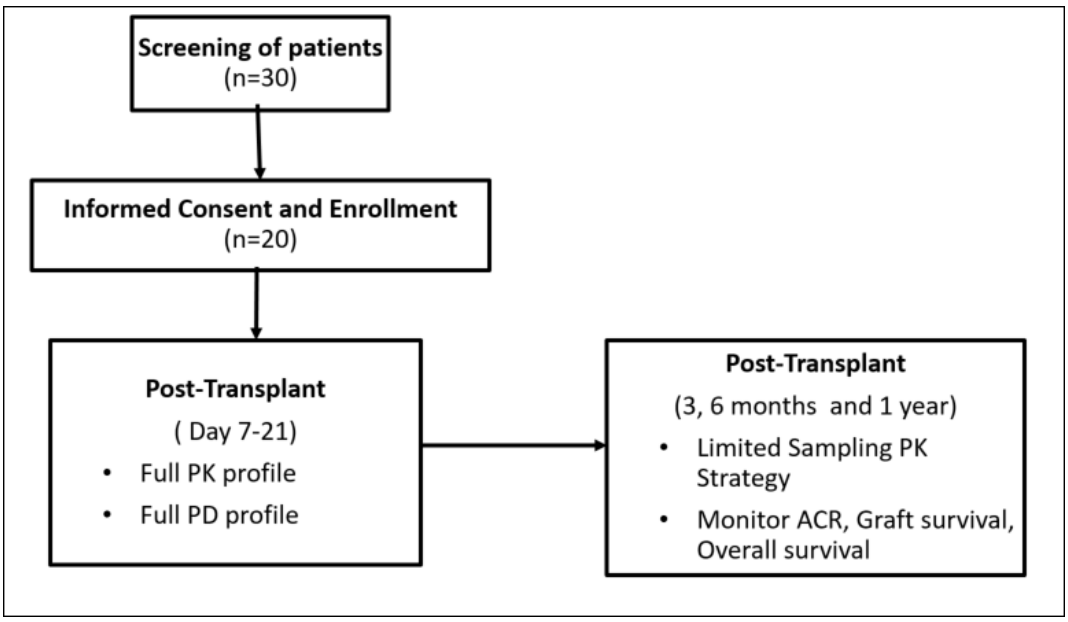


Figure 27. Study design for a prospective PK-PD study in adult lung transplant

For the PD sampling, IMPDH activity measurements were obtained pre-dose and 1, 2, 4, and 6 hours post dose on sampling day by drawing 10 ml blood at each draw. PBMCs were

isolated from lithium heparin-anticoagulated whole blood using falcon tubes with Ficoll-Paque density gradient solution according to the manufacturer's protocol within 6 hours of blood draw.

Inclusion Criteria:

- Patients must be over 18 years of age
- Patients yet to receive a single or double lung transplant.
- Patients must be maintained on a calcineurin inhibitor and mycophenolate mofetil or mycophenolic acid
- Patients must be provided with written informed consent
- Patients must be able to swallow oral medications and must be on same dose of MPA for at least 3 days.

Exclusion Criteria:

- Patients with a known hypersensitivity to MMF.
- Prisoners
- Pregnant women
- Children
- Patients with cystic fibrosis
- Patients with systemic liver disease
- Patients with nasogastric tubes inserted
- Patients with MPA use prior to transplantation
- Concomitantly on interacting medications (e.g. antacid, cholestyramine)
- Hematocrit of less than 8

4.3.3.2 Retrospective Study

This was a retrospective study in adult lung transplant recipients at the UPMC. The Institutional Review Board of the University of Pittsburgh approved the protocol. Patients enrolled in the Breathe-LTE biorepository study were eligible for this study. The study design is depicted in Figure 28. Breathe-LTE is a clinical biorepository at the UPMC with banked blood samples of over 296 lung transplant recipients. PBMC drawn on 20 lung transplant recipients within a year post-transplant were accessed. The banked PBMCs were lysed in a sonicator and the lysate was stored at -80°C until analysis.

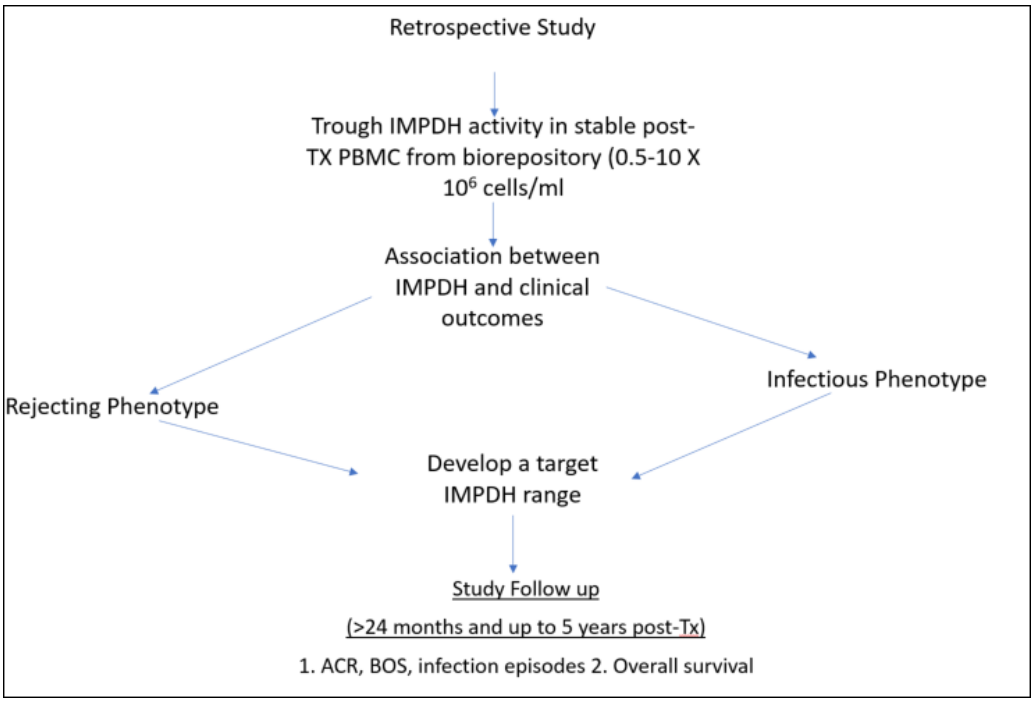


Figure 28. Study design for a retrospective study for pharmacodynamic monitoring in adult lung transplant recipients.

4.3.3.3 PBMC isolation

10 ml blood was diluted with 25 ml (1:2.5) of phosphate buffered saline (PBS). This was layered over 15 ml Ficol-Paque solution in Sep-mate tubes. Sep-mate tubes (50ml) were centrifuged at 1200 x g for 10 minutes at room temperature, with the brake on. Top layer, which contains the enriched MNCs, was poured off into a new Falcon tube not holding the SepMate™ tube in the inverted position for longer than 2 seconds. Cells were then washed with PBS and centrifuged at 300 g for 10 min to yield the pellet. The supernatant PBS was poured off leaving behind about 1.1 ml. The PMNC pellet was re-suspended in the remaining 1.1 mL of PBS, and 1.0 mL of the PBS-cell slurry was transferred to a 2 mL tube. Cells were then manually counted using a hemocytometer and aliquoted and or diluted to yield 5 million cells/ml and were stored at -80°C until analysis.

4.3.4 Assay Method

4.3.4.1 Preparation of stock solution, working solution and incubation buffer, control incubation buffer

Stock solutions were prepared by dissolving XMP (1mg/ml) and Br-AMP (1 mg/ml) in water and AMP (10 mg/ml) in 0.5% NH4OH. All solutions were stored at -80°C. Working solutions were prepared fresh to yield 0.1 mg/ml XMP and 1 mg/ml AMP. Calibration standards were prepared by diluting 50 µL of each working solution with 130 µL of solution consisting of 40 mM sodium dihydrogen phosphate (pH7.4) and 100mM potassium chloride to yield target concentration of AMP ranging from 50-40,000 ng/ml and XMP concentration ranging from 5-4000 ng/ml. The internal standard Br-AMP working solution (10µg/ml) was prepared from stock

solution at the time of assay. 4 quality control samples were prepared for XMP and AMP (XMP: 8,80, 800 and 3500 ng/ml; AMP: 80, 800, 8000 and 35000 ng/ml).

Incubation buffer was prepared to contain of 40 mM sodium dihydrogen phosphate (pH 7.4) and 100 mM potassium chloride containing 1 mM of IMP and 0.25 mM of NAD+. 2 control buffers were prepared with IMP only and NAD+ only respectively.

4.3.4.2 Assay Conditions

IMPDH activity was evaluated in PBMC lysates as described by Bemer et al.[209] with slight modifications. IMPDH activity was calculated based on enzymatic production of XMP and was normalized to the intracellular AMP level. The incubation was initiated at 37°C for 180 min. The reaction was started by adding 50 µL of thawed lysate to 180 mL of incubation mixture consisting of 40 mM sodium dihydrogen phosphate (pH 7.4) and 100 mM potassium chloride containing 1 mM of IMP and 0.25 mM of NAD+.

The incubation reaction was terminated at the end of 3 hours by addition of 1 ml methanol with a repeat pipette. Internal standard (20 µL of 10µg/ml of Br-AMP in deionized water) was then added to each incubation mixture, and then centrifuged at 15000 g for 10 min at 37 °C. The supernatant was then transferred to 12 × 75 disposable culture tubes and evaporated to dryness under a stream of air at 30 °C. The residue was then dissolved in 100µL of deionized water and 5µL was injected on the LC-MS for XMP and 1 µL for AMP quantitation. Prepared samples were stored in the autosampler tray at 5°C. Injection volume was optimized as needed based on changes in instrument response for each compound (magnitude of chromatographic peak areas). Samples were injected in the following order: calibration standards (ascending order of concentrations), analyte-free samples with IS, QC set 1, half of the validation/study samples,

QC set 2, remaining validation/study samples followed by QC set 3. Between injections, the autosampler needle was washed with 60%acetonitrile, 40% water and 0.1% formic acid (v/v). Solvent delay was used to divert the sample to waste for the first 3 minutes and after 7 minutes till the end of the run.

4.3.4.3 Chromatographic conditions

The chromatographic separation was performed on an Agilent HPLC/MS system series 1200 with a thermostatically controlled autosampler (Agilent Technologies, Palo Alto, CA). Thermo Scientific Hypercarb column (2.0mm × 100mm × 5µ, part no. 35005-102130; Thermo Scientific, Bellefonte, PA) was used for separation of the analytes. The mobile phase consisted of A: acetonitrile and B: 0.1 M ammonium acetate in LCMS water pH adjusted to 8.5 with ammonium hydroxide. Elution was achieved using a mobile phase gradient as follows: Starting at 5% acetonitrile for first 0.5 min then increased to 30% at 4 min and was held at 30% till 5 min and then switching back to 5% at 5.1 min. The sample run time was 10 min. The chromatographic gradient used to achieve separation is detailed in Table 17. The injector was maintained at 4 °C. The injection volume was 5 ul for XMP and AMP. The column temperature was set to 30.0 °C and the flow rate was maintained at 0.3 mL/min. The typical retention times were 4.25 min for XMP, 5 min for AMP and 7.0 min for Br-AMP.

Table 17. HPLC mobile phase gradient flow conditions

Time (min)	Solvent A (%)	Solvent B (%)	Flow (mL/min)
0	5	95	0.3
0.5	5	95	0.3
4	30	70	0.3
5	30	70	0.3
5.1	5	95	0.3

4.3.4.4 Mass spectrometric conditions

A SCIEX 4000 Q triple quadrupole ion trap mass spectrometer electron spray (ES) in positive ion mode was used. The temperature of the drying gas (nitrogen) was maintained at 350 °C at a flow of 11 L/min. The MSD was run in the MRM mode. Monitored ions included m/z 365 for the positive ion of XMP, m/z 348 for the positive ion of AMP, and m/z 426 for the positive ion of the internal standard, Br-AMP.

4.3.4.5 Partial Validation

Since this assay was a modification of an existing fully validated bioanalytical assay we have performed a partial validation of the method. Validation of the assay in patient cell lysate preparations was performed. Ten calibration standards ranging in concentration from 5-3500 ng/mL for XMP and 50-35000 ng/ml for AMP were prepared and analyzed in duplicate in 6 analytical runs on 3 consecutive days. Throughout partial method validation and study sample analysis, calibration curves were determined by plotting the analyte to IS chromatographic peak area ratio against the prepared analyte concentration in each calibration standard. Calibration

curves were fit by linear regression for all analytes and a weighting of 1/y2 was applied to all calibration curves. The LLOQ was determined as the minimum value having an accuracy and precision within ±15% of the nominal value. The LLOD was determined by the minimum value with a signal-to-noise ratio of at least greater than 3:1.

Accuracy and precision were determined by analyzing 4 levels of QC samples. AMP and XMP QC samples were prepared to yield A:8 ng/ml XMP and 80 ng/ml AMP, B: 80 ng/ml XMP and 800ng/ml AMP, C: 800ng/ml XMP and 8000 ng/ml AMP and D: 3500 ng/ml XMP and 35000ng/ml AMP. Six samples of each QC were analyzed on 3 days. Accuracy was be expressed as relative standard error (RSE%), was calculated using the equation RSE% = (Calculated value-True value)/True value*100. Precision was calculated as the percent coefficient of variance (%CV) where %CV = standard deviation/mean x 100. A ±15% deviation from the nominal value was considered acceptable. Stability of the analytes was tested for 24 hours at autosampler temperature and compared with freshly prepared calibration standards.

For each cell lysate matrix, one QC set (set A, n = 3) was prepared according to normal sample processing method. Another set of QCs (set B, n = 3) was prepared as neat solution in water. The third set of QCs (set C, n = 3) was prepared without analytes XMP and AMP and spiking the sample into the matrix post-extraction.

4.3.5 Measurement of IMPDH activity

Enzyme activity was expressed as produced XMP (µmol) per time unit (h) per µmol of AMP. As reported by others, here too we have used the concentration of intracellular AMP, rather than cell count or protein concentration was used to normalize results [207, 208]. The normalization of IMPDH activity using AMP better reflects the intracellular level of IMPDH released by disrupted cells and is relatively less affected by extracellular proteins or erythrocyte contamination. The following equation was used (XMP and AMP concentrations are expressed in micromoles per liter and incubation time in seconds):

IMPDH activity (((µmol/second/mol of AMP)) = [produced XMP $_X 10^6$/ (incubation time $_X$ measured AMP)].

Maximum inhibition of IMPDH activity (I_{max}) as described by Sommerer et al.[100] was calculated with the following formula:

$Imax = [1 – (A_{min}/A_{max}) \times 100$,

Where A_{min} = minimum daytime enzyme activity

A_{max} = maximum daytime enzyme activity

4.3.6 Statistical Analysis

Statistical analysis was completed using SPSS software, version 23. In the matrix effects studies, IS-normalized MF values were compared using unpaired t-test (2 tailed). For all statistical tests,

a $p<0.05$ was considered significant. Normality for pharmacodynamic data from the retrospective study was determined using Shapiro Wilk test. T-Test was used for IMPDH activity by those who have rejection within ±3 months window prior to and after IMPDH activity. Univariable logistic regression model for ACR and infection within the ±3-month window with IMPDH as a predictor. Owing to the less enrollment of adult lung transplant patients in the prospective study no statistical analysis was performed.

4.4 RESULTS

4.4.1 Assay Validation

Table 18 shows the optimized mass spectrometric conditions for the assay. Figure 29, Figure 30 and Figure 31 show chromatographic separation for XMP, AMP and the internal standard Br-AMP at their lower limits of quantification.

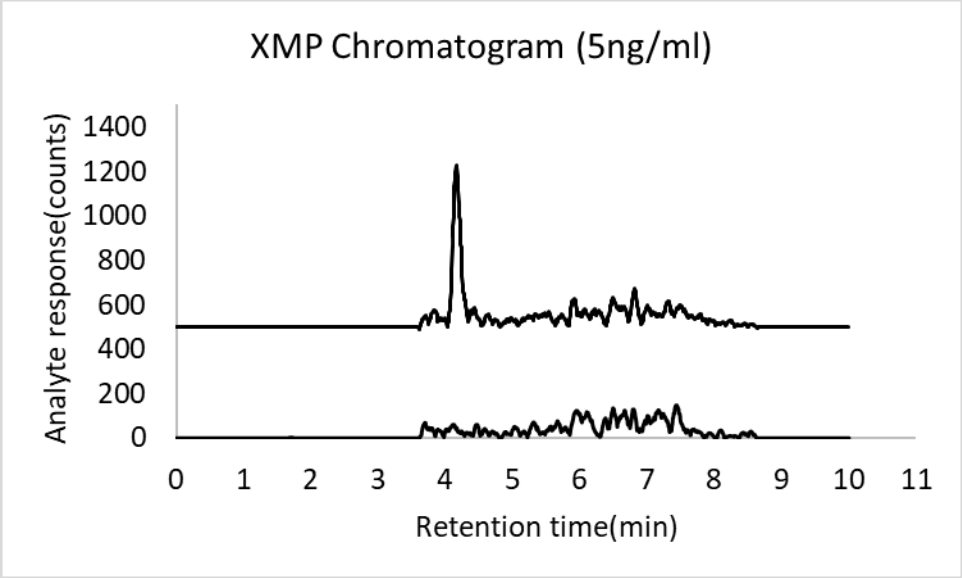


Figure 29. Chromatographic separation of XMP (5ng/ml) against background noise

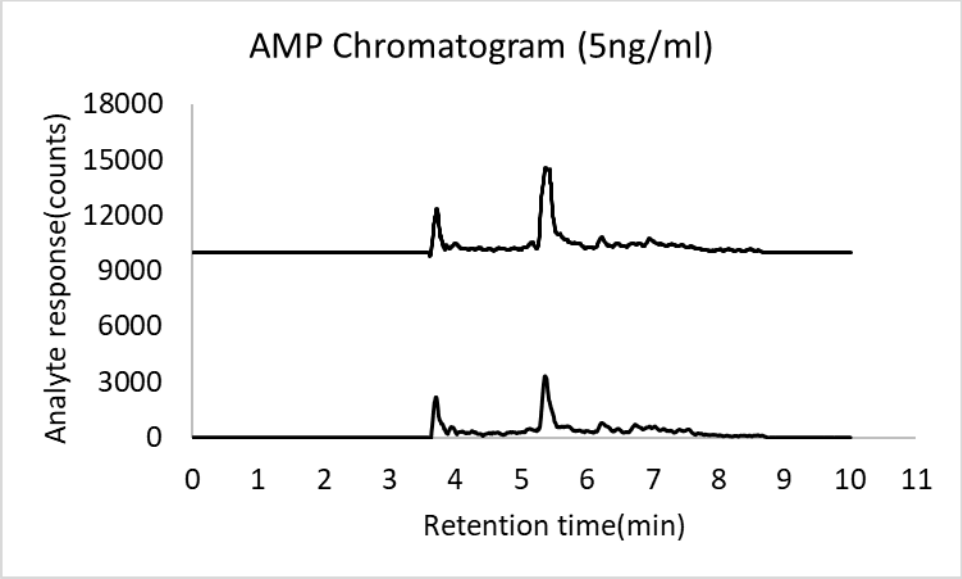


Figure 30. Chromatographic separation of AMP (5 ng/ml) against background noise

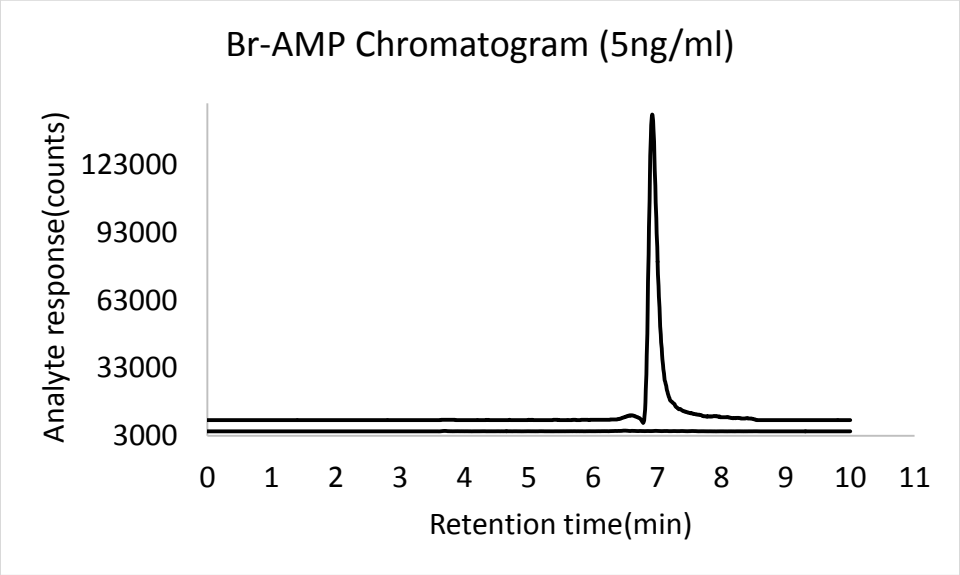


Figure 31. Chromatographic separation of Br-AMP (5ng/ml) against background noise

A partial validation was performed for the IMPDH assay using HPLC-MS. The calibration curves were linear for in the range of 5-4000 ng/ml and 50-40,000 ng/ml for XMP and AMP respectively. Figure 32 and Figure 33 shows the calibration curves for XMP and AMP respectively. The linearity as expressed by the correlation coefficients of the 1/y-weighted

calibration curves were in the range of 0.995 – 0.999 for both the compounds. The lower limit of quantification in this assay was 5 ng/ml and 50 ng/ml for XMP and AMP respectively. The lower limit of detection for both XMP and AMP was 1ng/ml.

Table 18. Mass spectrometric conditions for XMP, AMP and Br-AMP

Analyte	ESI mode	Cone Voltage	Desolvation temperature	MRM transition
XMP	Positive	40 V	500°C	365.5 → 153.3
AMP	Positive	40V	500°C	348.4→136.3
Br-AMP	Positive	40V	500°C	426.1→ 214.4

The intra-day and inter-day precision and accuracy of the assay was less than 14.5% at each of the 4 QC levels. Results of the assay validation for intra and inter-day are presented in Table 19 and Table 20 respectively. The matrix effect at all QC levels in the cell lysate preparation was between 96.9-115% and 103.7-114.5% for XMP and AMP respectively. This demonstrates minimal matrix effects of cell lysate on the reproducibility and reliability of the assay.

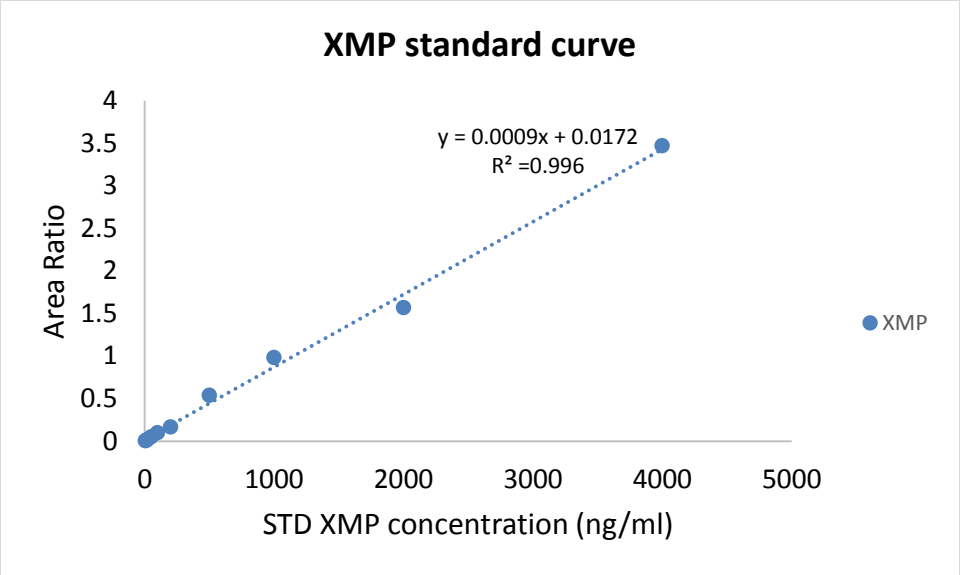


Figure 32. Calibration curve for XMP in phosphate buffer

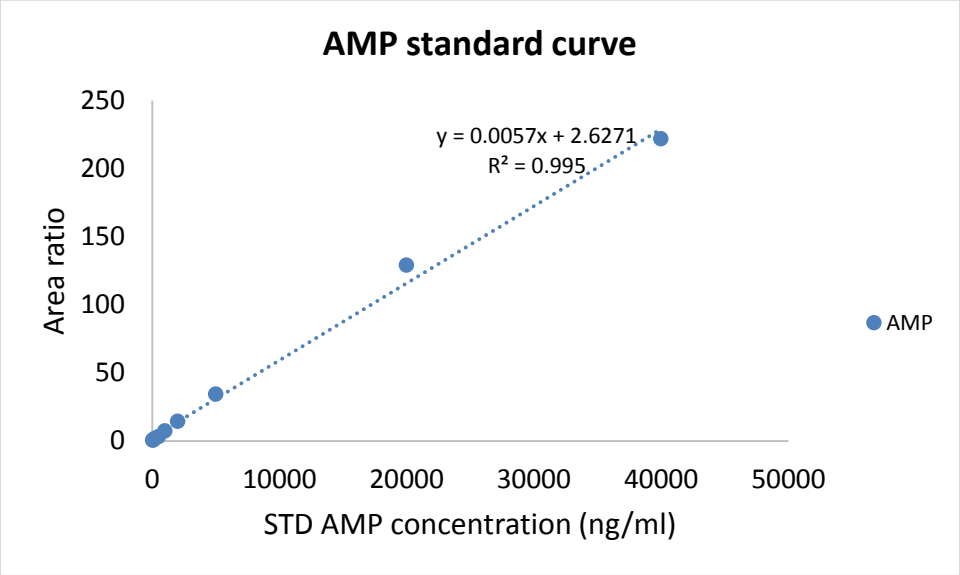


Figure 33. Calibration curve for AMP in phosphate buffer

Table 19. Intra-day accuracy and precision of XMP and AMP

XMP Concentration (ng/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
8	7.7	-3.8	11.3
80	82.5	3.1	12
800	830.5	3.8	1.8
3500	3390	-3.1	7.9
AMP Concentration (ng/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
80	79.7	0	14
800	853.2	-4.2	14.5
8000	815.3	6.7	7.0
35000	30566.7	12.6	14.5

Table 20. Inter-day accuracy and precision of XMP and AMP

XMP Concentration (ng/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
8	7.9	-1.3	2.3
80	86.8	7.9	7.4
800	869.3	8.6	8.8
3500	3710	6.0	13.8
AMP Concentration (ng/mL)	Mean calculated concentration (µg/mL)	Accuracy (% deviation)	Precision (% CV)
80	80.3	0.4	14.4
800	891.8	11.5	11.9
8000	8062.8	0.8	6.5
35000	29767(n=3)	14.8	9.5

4.4.2 Application of Assay to retrospective and prospective studies

The assay was used for quantitation of MPA based IMPDH inhibition in 4 lung transplant recipients from a prospective PK-PD study over a dosing interval and from 20 lung transplant recipients in a retrospective study. All patients received a fixed MMF flat dose of 1 g BID., in combination with a calcineurin inhibitor.

4.4.3 Single time point *ex-vivo* IMPDH activity post-transplant in lung transplant recipients

IMPDH activity was monitored for patients on an existing retrospective biorepository study. PBMCs were isolated from patients at their routine bronchoscopy visits within the first-year post-transplant. Average single time point IMPDH activity monitored in adult lung transplant recipients was 17.8 μmol/second/mol of AMP. IMPDH activity showed high inter-patient variability (~5-fold) from 7 to 33.5 μmol/second/mol of AMP as shown in Figure 34.

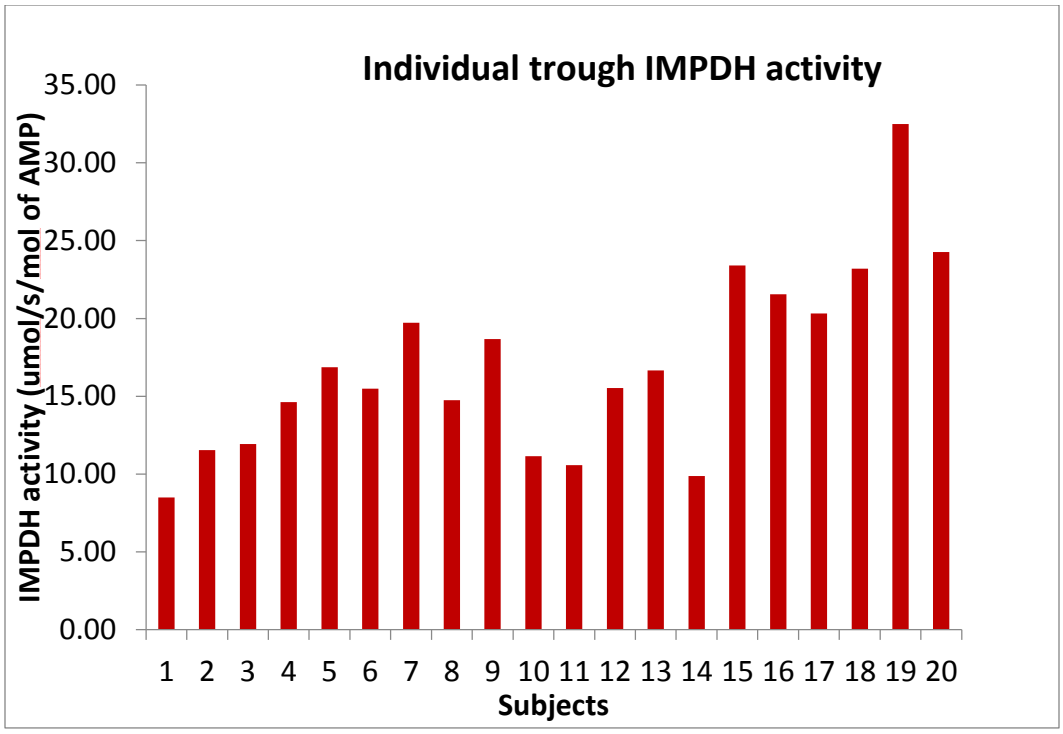


Figure 34. High variability of trough IMPDH activity in 20 adult lung transplant recipients.

. We have used a univariate logistic regression model for ACR and or infection within six months of IMPDH activity measurement with IMPDH as a predictor. The model did not show any statistically significant association between ACR and IMPDH activity and infection and IMPDH activity. Although statistically not significant, patients rejecting the graft had a higher IMPDH activity as seen in Figure 35.

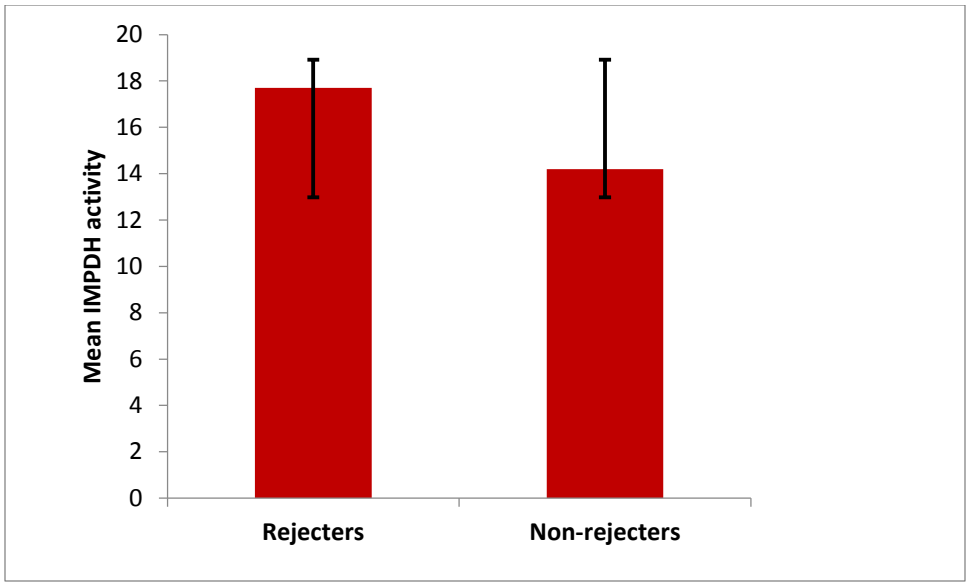


Figure 35. Mean trough IMPDH activity in rejecters and non-rejecters in adult lung transplant recipients.

4.4.4 Pharmacodynamic activity of IMPDH of patients in a prospective study undergoing lung transplantation

IMPDH activity was inversely related to MPA plasma concentration. Figure 36 shows the IMPDH activity of individual patient over a dosing interval. In some patients, the maximum inhibition occurred between 1-2 hours and coincides with the time to peak MPA concentrations as measured in the previous chapter, whereas others exhibited longer inhibition of the activity.Complete inhibition of IMPDH activity was observed only for a short time, followed by a rebound phenomenon after four hours. In the lung transplant patients, mean IMPDH activity was 12.3 μmol/second/mol and revealed large inter-patient variability (range: 1.0-40 μmol/second/mol of AMP). The linear regression for MPA concentration and IMPDH activity is shown in Figure 37. Overall MPA concentrations are not correlated to the IMPDH activity. The mean I_{max} of MPA after oral dose of MMF within the dosing interval was 61.2 %(31.2-93.2%).

This study is the first to evaluate the pharmacokinetics of MPA in relation to inhibition of the pharmacological target enzyme IMPDH in adult lung transplant recipients. This *ex-vivo* measurement of IMPDH activity provided for a good pharmacodynamic marker of MPA-induced immunosuppression at the lymphocyte level. Although preliminary data, this study still does provide the potential utility of combining PK and IMPDH biomarker data to optimize MMF therapy. It also suggests the importance of early monitoring of MPA PK and IMPDH inhibition to improve drug exposure and outcomes.

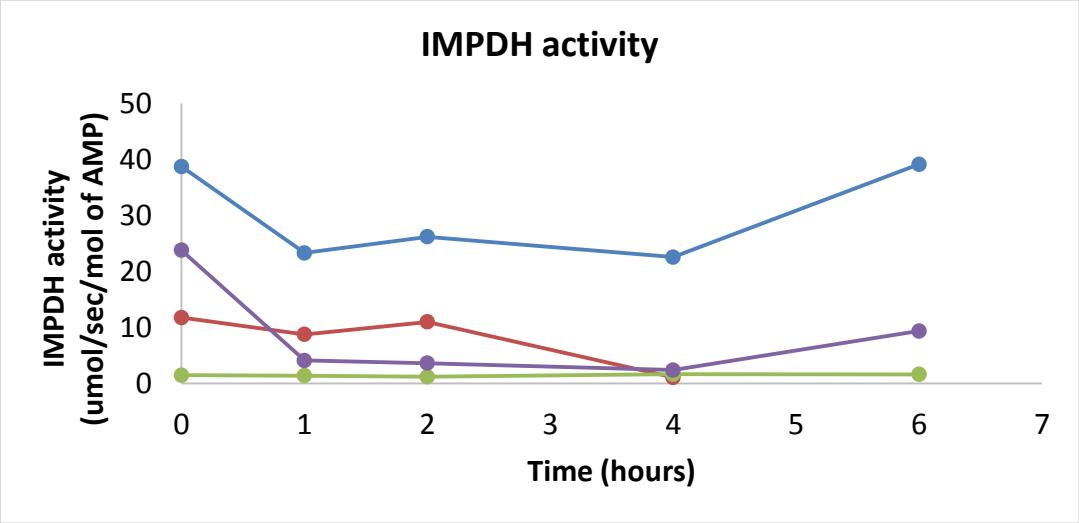


Figure 36. Individual IMPDH activity over time for 4 patients undergoing lung transplantation

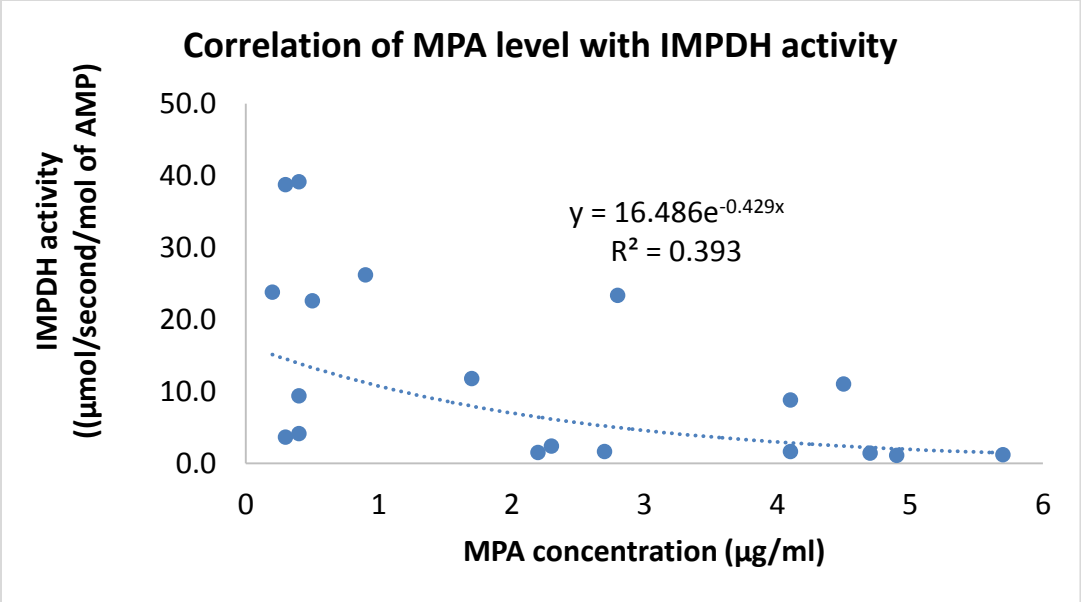


Figure 37. Relationship between post-transplant trough IMPDH activity and corresponding MPA plasma concentration (measured in chapter 3) from prospective study in lung transplant recipients

4.5 DISCUSSION

Therapeutic drug monitoring has become very critical to optimize dosing of immunosuppressants. CNIs that form the backbone of immunosuppressive therapy in solid organ transplant recipients are routinely monitored and the doses are titrated to target trough level. MMF although used extensively in solid organ transplant recipients is however not routinely monitored. Measurement of 12-hour AUC is impractical in standard clinical practice settings. An alternate simpler approach to measure drug exposure is essential to optimize MMF therapy. Pharmacodynamic monitoring of IMPDH activity to determine the efficacy of MMF *ex-vivo* has been a useful approach for optimizing MMF therapy. The pharmacodynamic monitoring is not designed to replace the pharmacokinetic monitoring of MMF, instead to use it along with PK analysis to improve therapeutic monitoring and provide for a more effective way to individualize MMF dosing.

Here we have established and validated a sensitive LCMS assay to explore the IMPDH activity in a retrospective and prospective study in adult lung transplant recipients. In this study, we demonstrated that this HPLC-MS method is useful for simultaneously measurement of XMP and AMP and thereby quantitate the pharmacodynamic activity. The measured IMPDH activity was normalized to the intracellular AMP concentration. This sensitive method required a small number of isolated PBMCs (~1-5 million/ml) for monitoring IMPDH activity in PBMCs. It is convenient for large prospective clinical studies aimed at determining IMPDH activity in any transplantation settings. This chapter provides preliminary data of exploring IMPDH activity quantitation as a promising biomarker for MMF based immunosuppression in solid organ

transplant recipients. Although limited by sample size in both retrospective and prospective studies, these trials do demonstrate the feasibility of PD monitoring.

A fixed dose MMF is used in lung transplant recipients and is similar to the dosing used in renal and heart transplant recipients. However, lung transplant patients have shown a different pharmacokinetic profile as compared to renal transplant recipients. Studies suggest a lower MPA exposure in lung transplant recipients. There is a scarcity of PK studies in lung transplant recipients let alone an individualized MMF dosing study based on PK-PD monitoring. The underlying hypothesis of this clinical trial was that sub-therapeutic levels on MMF in the absence of TDM and concurrent increase in the IMPDH activity could be the cause for high incidence of acute clinical rejection in adult lung transplant recipients. The objective of this study is to describe IMPDH activity in lung transplant recipients maintained on MPA for maintenance immunosuppression and to correlate this activity with clinical phenotypes seen in this population.

This study is the first to document the pharmacodynamic activity of MPA as measured by the inhibition of the pharmacological target enzyme IMPDH in adult lung transplant recipients. Our study is limited, in that we started monitoring IMPDH concentrations at least 6 months post-transplant and thus we have no information on pre-transplant IMPDH activity. Statistical analysis for a power of 80% with an odds ratio for rejection of 3, with an established 2-sided alpha level of 0.05, 70 single time point of IMPDH activity measurements will be required to find significance. Due to small sample size we were not able to establish a threshold for IMPDH activity to facilitate identification of rejecting and infecting phenotypes of IMPDH activity at this point.

Also, for the prospective PK-PD study, owing to the limited enrollment till the time of data analysis we were not able to establish a PK-PD relationship of MMF exposure to the IMPDH inhibition. However, for all patients the maximum concentration of MPA coincided with the maximum inhibition (lowest IMPDH activity).

Single nucleotide polymorphisms (SNPs) in IMPDH-I and -II genes have been independently correlated with acute rejection. IMPDH is subject to genomic variability; and so, the activity is expected to be heterogeneous. Additionally, MPA exposure is influenced by genotype of the MRP2 as represented in Figure 38. The impact of MRP2 SNPs-rs717620, rs3740066 and rs2273697 upon plasma MPA exposure and IMPDH activity will also be evaluated.

	SNP/Genotype	Phenotype	Result
IMPDH	rs2278293	IMPDH I	Higher activity
	rs2278294	IMPDH I	Higher activity
	rs4974081	IMPDH II	Normal activity
	rs11706052	IMPDH II	Normal activity
MRP2	rs717620	Wild type	Normal MPA exposure
	rs2273697	Wild type	Normal MPA exposure
	rs3740066	Heterozygous	Increased MPA exposure

Figure 38. IMPDH and MRP2 genotypes and effect on pharmacokinetics of MPA.

DNA for the IMPDH II SNPs-rs4974081, rs11706052, 787C>T and the IMPDH I SNPs-rs2278293 and rs2278294 will be collected and genotyped using a validated TaqMan® based allelic discrimination assay using the manufacturer protocol. Previous studies have implicated

SNPs associated with IMPDH I with MPA treatment related rejection and will be evaluated as a part of this ongoing trial. Data from this study and future trials with larger number of patients should focus on further investigating whether differences in IMPDH activity can predict efficacy and dose requirements of MPA. Plasma samples from the current study can be used to evaluate the effect of unbound MPA concentration may affect IMPDH activity.

5.0 PREDICTING EXPOSURE OF MYCOPHENOLIC ACID AFTER IV AND ORAL ADMINISTRATION USING A COMBINED *IN VITRO-IN-VIVO* EXTRAPOLATION AND PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING APPROACH

5.1 ABSTRACT

Mycophenolic acid is the active form of the prodrug MMF which was approved in 1995 for prevention of organ rejection in kidney transplant recipients. MPA shows high inter and intra-patient variability across transplant patient populations and yet is not routinely monitored and dosing not individualized. Therapeutic trough monitoring of this drug is not useful owing to the poor correlation between the trough concentration and overall AUC exposure[90, 170, 211] . Over the last decade several pharmacometric approaches have been applied to better capture the inter-subject variability and predict the exposure in solid organ and stem cell transplant populations[35, 212, 213].

We have built and validated a robust mechanistic physiologically based pharmacokinetic model of mycophenolic acid in healthy, Chinese healthy and renally impaired adult volunteers. We have built a full PBPK advanced dissolution absorption model (ADAM) in SimCYP Simulator V15. Physicochemical properties for mycophenolic acid were obtained from literature and a model was build and validated across IV and oral doses of MMF in population cohorts of healthy volunteers, Chinese healthy volunteers and patients with renal impairment. Pharmacokinetic parameters like C_{max}, T_{max}, AUC and Clearance were assessed and compared with the observed data. Parameter estimation using non-linear mixed effects was used to optimize tissue partitioning. Intrinsic clearance for each of the UGT enzymes was extrapolated from recombinant UGT data as reported by Picard et al using rUGT scalar. The simulated clinical trials were carried out in the 100 virtual subjects from population groups of SimCYP's platform: i) healthy volunteers, ii) renal impairment with glomerular filtration rate (GFR) values between

30–60 iii) renal impairment with GFR below 30 and iv) a custom kidney transplant model and v) pediatric population. The model was robust in predicting MPA exposure in Caucasian and Chinese healthy subjects, renally impaired patients and renal transplant recipients. All predicted MPA PK parameters fell well within ±20% range of the corresponding PK parameters calculated from observed studies. Healthy pediatric data was not available to validate the model. The predicted MPS exposure in pediatric population was 5-fold higher than the in-house pediatric HSCT exposure. This predicted difference could be due to physiological changes occurring in HSCT patients. The pediatric model could be used as a template to further build and validate a pediatric HSCT population. Sensitivity analysis revealed that unbound fraction and UGT abundance are the primary drivers of changes in MPA PK and could be responsible for the large inter and intra-patient variability in transplant population. This model predicted exposure can facilitate development of better dosing strategies in adult and pediatric HSCT patients.

5.2 INTRODUCTION

Mycophenolic acid is the active form of the prodrug MMF which was approved in 1995 for prevention of organ rejection in kidney transplant recipients. It is concomitantly given with calcineurin inhibitors like cyclosporine or tacrolimus and corticosteroids. It is given PO as BID or is infused over ≥2 hours, when given intravenously. Two different oral formulations of MPA are available as capsules and tablets as MMF or as MPA in EC-MPS. MMF is an ester derivative of MMF and is readily hydrolyzed by the esterases in the body. Enteric-coated mycophenolate sodium (EC-MPS; myfortic®, Novartis Pharma AG, Basel, Switzerland) is an enteric-coated formulation which was developed to specifically to improve the upper GI tolerability of MPA. Enteric-coated-MPS 720mg is equivalent to MMF 1000 mg and provides similar drug exposure. Mycophenolate Mofetil, the prodrug gets metabolized to MPA by esterases located in the systemic circulation, gut wall, liver and tissues.[26] MPA is undergoes phase II metabolism by UDP glucuronosyl transferases primarily in the liver and kidneys, forming an inactive metabolites MPAG and 7-O-glucoside while a small amount gets metabolized to an active compound acyl-MPA glucuronide. Metabolites are excreted in the urine and bile. MPA is excreted by MRP2 into the bile and MPAG and MPA present in the bile are subjected to MRP2 mediated EHC. EHC shows a high inter-patient variability and is known to contribute 10 to 40% of the AUC of MPA in a dosing interval.[31, 36]

MPA shows high inter and intra-patient variability across different transplant patient populations and yet is not routinely monitored. Trough therapeutic monitoring of this drug is not

recommended owing to the poor correlation between the trough concentration and overall exposure as measured by the AUC [24, 211, 214]. Over the last decade several pharmacometric approaches have been applied to better capture the inter-subject variability and predict the exposure in solid organ and stem cell transplant populations [35, 212, 213].

Several commercially available PBPK platforms like SimCYP (SimCYP Ltd., Sheffield, United Kingdom) and Gastroplus (Simulations Plus, Inc., Lancaster, CA), offer complex clinical trial design options and allow incorporation of multiple routes of drug administration. These software platforms also incorporate population variability, physiologic changes associated with various disease states, and can link exposure in different tissues to pharmacodynamic effects [215]. These softwares facilitate the industry to evaluate the potential clinical impact of physiological and disease specific changes and help design clinical trials. Physiologically based pharmacokinetic (PBPK) modeling is increasingly being used by the industry and the regulatory authorities for *in vitro in-vivo* extrapolations, assessment of risk to health and first in human dosing. [216-218] Inclusion of modeling and simulation approaches in the recent drug interaction regulatory guidance updates from both the European Medicines Agency (EMA) (CHMP, 2012) and the U.S. FDA (CDER, 2012) have demonstrated the acceptance of the use of PBPK modeling to support drug registration by regulatory agencies.

Especially for the purpose of integrating information of different PK properties, physiologically-based pharmacokinetic (PBPK) modeling linked to *in vitro in-vivo* extrapolation (IVIVE) approaches (PBPK– IVIVE) has gained growing interest and application.

In this study, we present a PBPK–IVIVE approach to predict human exposure of active drug after oral prodrug administration. PBPK modeling approach incorporates a drug's physiochemical properties, human physiological variables and population variability estimates to

predict drug exposure. An initial PBPK model describes MPA PK after IV and oral (PO) administrations in healthy Caucasian and Chinese adults and patients with varying degree of renal impairment. The in-built pediatric population in the SimCYP simulator allowed us to predict the exposure of MPA in pediatric patients. However, due to lack of clinical data on healthy pediatric population the model could not be validated. This model can be further used to access the impact of various physiological factors in pediatric transplant patients to improve MPA therapy.

To the best of our knowledge, the use of PBPK modeling in predicting MPA exposure in healthy adult and pediatric and transplantation population has not been explored. The objective of this work was to build and validate a PBPK model for IV and oral MPA in healthy adults and renally impaired and transplant recipients and to explore the factors that could be responsible for the high inter-patient variability associated with this drugs exposure and to determine the dosing required in pediatric HSCT patients based on the simulated data.

5.3 METHODS

A systematic approach was used in developing the PBPK models, starting from screening of drug specific input parameters and clinical pharmacokinetic data from the published literature, to incorporating these data into the software and choosing the right physiological model to the final steps of visual as well as pharmacokinetic evaluation of the model. Physicochemical properties, protein binding, blood to plasma ratio and permeability were obtained from an extensive literature search in Pubmed.[8] The dissolution kinetics data as reported by Lee et al. was digitized and built into the SimCYP simulator. [8]

GetData Graph Digitizer V.2.26 was used to digitize published clinical pharmacokinetic data of mycophenolic acid. A full PBPK model was developed for IV and oral mycophenolic acid formulation using physiochemical properties[219], in-vitro metabolic profiles [23]and published IV mycophenolic acid pharmacokinetic data in healthy subjects. In this stepwise PBPK model, an IV dose was first simulated in healthy volunteers to avoid complexities associated with modeling oral absorption. This approach allowed for optimization of a wide range of drug-dependent parameters that govern the drug disposition, including clearance inputs and the percentage UGT enzyme contributions. When extending the model to oral dosing regimen, parameters from the previous steps were kept, and additional parameters that may potentially influence drug absorption, such as intestinal permeability, were included. Model naïve data for from different pharmacokinetic studies was used to perform inter-study validations. The simulated clinical trials were carried out in the following virtual population groups of SimCYP's platform: i) healthy volunteers, ii) Chinese healthy volunteers iii) renal

impairment with GFR values between 30–60 iv) renal impairment with GFR below 30. All simulations run for 10 clinical trials of 10 subjects in each trial. The overall workflow for the development of model is shown in figure 1. A combined top-down approach using observed pharmacokinetic data and a bottom up using IVIVE approach was used for model building. This was accomplished by first developing and verifying a PBPK model using observed adult clinical data after IV and oral administration followed by simulating mycophenolic acid exposure in Chinese healthy, renally impaired and renal transplant recipients using the PBPK model and comparing the results with observed clinical data. The MMF dose was converted to MPA equivalents according to the equation MPA dose = FMW * MMF dose, where FMW is the fractional difference in molecular mass between MPA and MMF. For the oral and intravenous formulations of MMF, FMW is equal to 0.739.

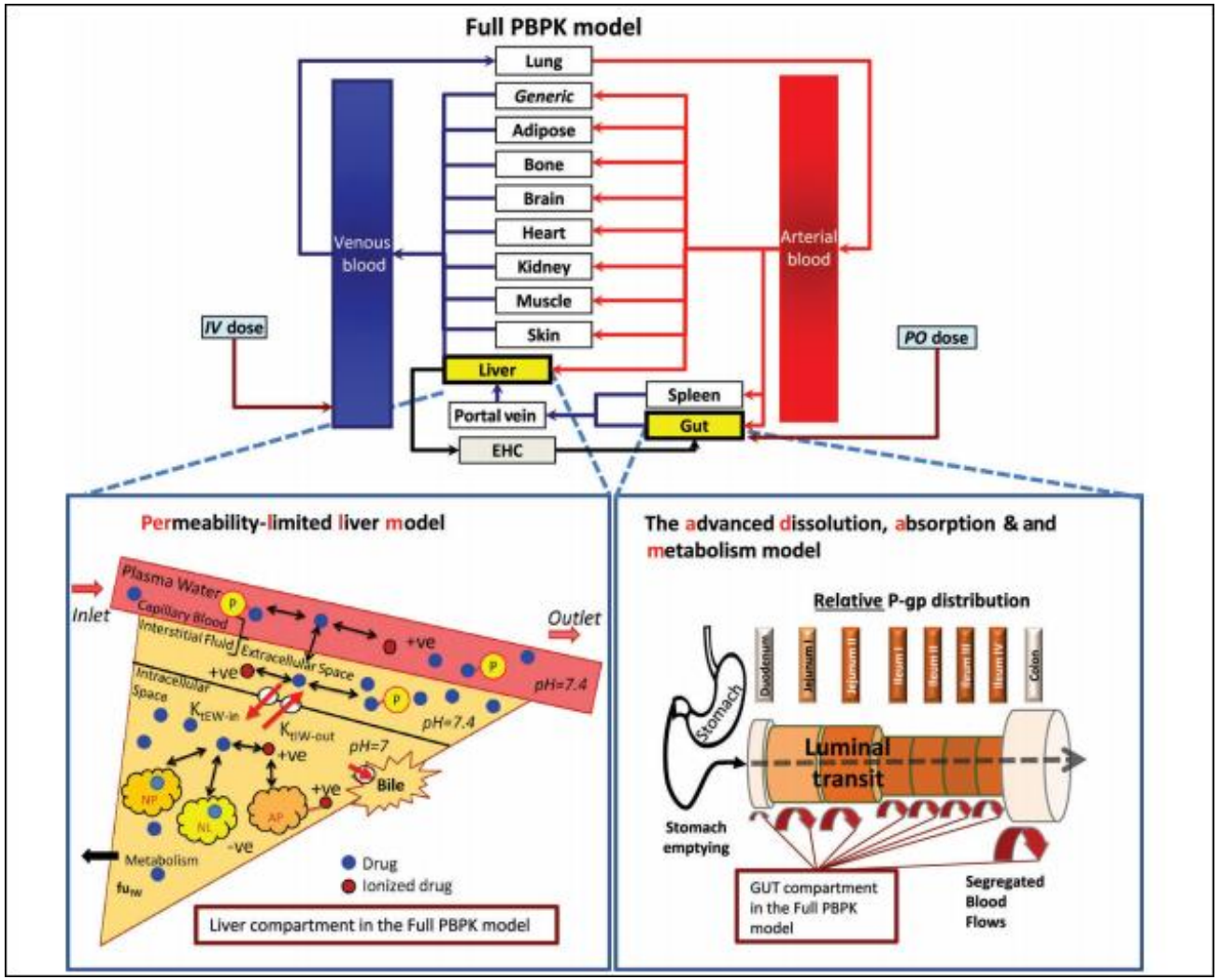


Figure 39. PBPK model used for describing the kinetics of MPA. The absorption of MPA after oral administration was described by the ADAM model. [220]

5.3.1 Absorption

The advanced dissolution, absorption and metabolism (ADAM) model with the Simcyp default values of fasted gastric emptying time (mean 0.4 h with a coefficient of variation [CV] of 38 %) and small intestinal transit time (mean 3.33 h with Weibull distribution, $\alpha = 2.92$ and $\beta = 4.04$) was used to predict drug absorption.[221] The model used is depicted in Figure 39. The predicted human jejunum permeability ($P_{eff,man}$) of mycophenolic acid was 12.3063× 10−4 cm/s, which was obtained using *in vitro* Caco-2 permeability (P_{app}) data calibrated with reference values for propranolol.[222] In addition, the predicted absorbed fraction of mycophenolic acid was 1, which is consistent with mycophenolic acid being a highly permeable drug belonging to BCS class II. The pH-dependent dissolution data of the solid dosage form from Lee et al. was digitized and incorporated into the model. The pH-dependent solubility profile of MPA used in this model is provided in Figure 40.

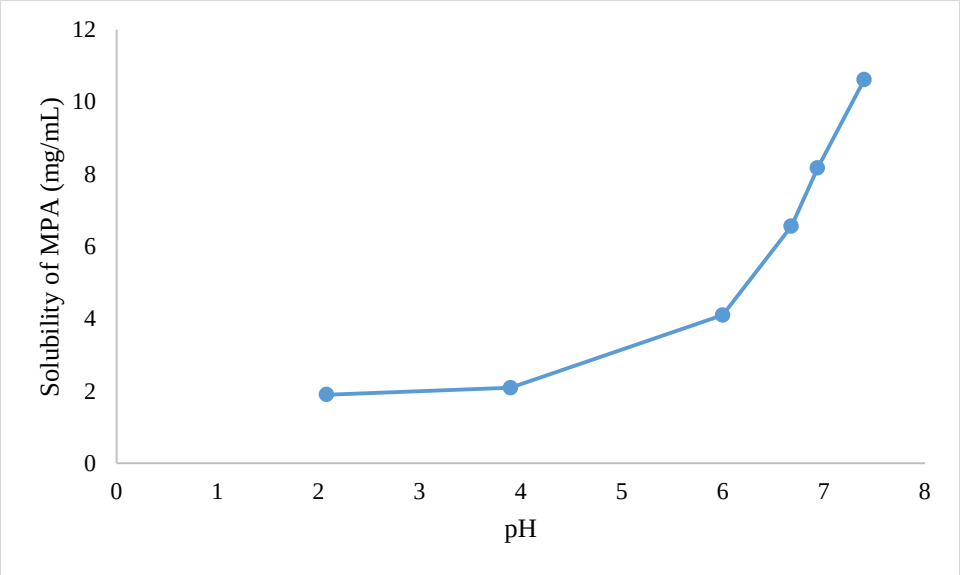


Figure 40. pH-dependent solubility of MPA used in the dissolution profile of the ADAM model.

5.3.2 Distribution

The full PBPK model was used for predicting the volume of distribution. The volume of distribution at steady state (V_{ss}) and the specific tissue-to-plasma partition coefficient (K_p) values were calculated by the Paulin and Theil method with the Berezhkovskiy correction.[223] The model predicted V_{ss} value of 4.34 L/kg by this method was comparable to the reported value of 4 L/kg in the literature.[224] Tissue composition and blood flow rates as reported by SimCYP® for the virtual healthy volunteer population were used for building MPA PBPK model (Figure 41 and Figure 42).

A sequential sensitivity analysis was performed to identify the K_p values that had a significant impact on pharmacokinetic parameters like C_{max}, T_{max} and AUC. Tissue plasma partitioning of MPA in adipose tissue was optimized using non-linear mixed effects modeling using the parameter estimation module in SimCYP simulator.

	Relative Volume of Wet Tissue (%)				AP (mg/g)	Binding Proteins	
	EW	IW	NL	NP		$Kp_{,ALB}$	$Kp_{,LPP}$
Adipose	14.1	3.9	79	0.2	0.4	0.037	0.068
Bone	9.8	34.1	7.4	0.11	0.67	0.1	0.05
Brain	9.2	67.8	5.1	5.65	0.4	0.048	0.041
Gut	26.7	45.1	4.87	1.63	2.84	0.158	0.141
Heart	31.3	44.5	1.15	1.66	3.07	0.157	0.16
Kidney	28.3	50	2.07	1.62	2.48	0.13	0.137
Liver	16.5	58.6	3.48	2.52	5.09	0.086	0.161
Lung	34.8	46.3	0.3	0.9	0.5	0.212	0.168
Muscle	9.1	66.9	2.38	0.72	2.49	0.034	0.059
Pancreas	12	66.4	4.1	0.93	1.67	0.06	0.06

Figure 41. Tissue composition parameters used for building MPA PBPK model in healthy volunteer population.

Screenshot from SimCyp® population-based simulator v15.1 (SimCYP limited, Sheffield, UK). EW: %Extracellular Water; IW : % Intracellular Water; NL : % Neutral Lipids; NP : % Neutral Phospholipids; AP : % Acidic Phospholipids; $Kp_{,Alb}$: tissue-plasma partition coefficient for serum albumin; $Kp_{,LPP}$: tissue-plasma partition coefficient for lipoproteins

Tissue Blood Flow Rates (% cardiac output)

	Male	Female
Adipose	5	8.5
Bone	5	5
Brain	12	12
Stomach and Oesophagus	1	1
Small Intestine	10	11
Villi	6	6
Large Intestine	4	5
Heart	4	5
Kidney	19	17
Liver (Arterial)	6.5	6.5
Liver (Portal)	19	21.5
Lung	100	100
Muscle	17	12
Pancreas	1	1
Skin	5	5
Spleen	2	3

Figure 42. Blood flow rates associated with each of the physiological compartment that were used for building MPA PBPK model in healthy volunteer population.

Screenshot from SimCYP® population-based simulator v15.1 (SimCYP limited, Sheffield, UK).

5.3.3 Elimination

MMF is instantaneously metabolized to the active form MPA by esterases. MPA is extensively metabolized by phase II pathway to its glucuronide metabolites: MPAG and AcMPAG. Intrinsic clearance (CL_{int}) values from in-vitro data in recombinant human UGT enzyme system were used for prediction of drug clearance. The contributions of the individual UGT enzymes that are involved in the metabolism of mycophenolic acid was obtained from the literature. It has been reported that UGT1A9 is the major UGT enzyme, while UGT1A8, UGT1A10, UGT1A1 and UGT2B7 are involved to a minor extent. Here we have used established IVIVE methods using enzyme specific microsomal metabolism data. The inter-system extrapolation factors (ISEF) is used as a scalar to extrapolate recombinant *in-vitro* enzyme activities to *in-vivo* intrinsic clearance by using the following equation:

$$CL_{int} = [\sum_{j=1}^{n}(\sum_{i=1}^{n} \frac{ISEF_{ji} \times Vmax_i(rhCYP_j) \times CYP_j\ abundance}{K_{mi}(rhCYP_j)})] \times \text{MPPGL} \times \text{liver weight}$$

Where ISEF is a scaling factor that corrects for the difference in the activity per unit of enzyme between recombinant systems and liver samples used in experiments whereas, CYPj (UGTs in this case) is the average amount of j^{th} enzyme per mg microsomal protein of human liver and MPPGL is the average amount of microsomal protein per gram of human liver. ISEF is a combination of relative activity factor (RAF) and the abundance of the enzyme and is related to RAF as follows,

$$ISEF = \frac{Vmax\ endogenous\ microsomes\ /\ CYPabundance}{Vmax\ expressed\ enzymes}$$

where RAF can be expressed as, $RAF = \frac{\text{Vmax endogenous microsomes}}{\text{Vmax expressed enzymes}}$

Using the above equations, the RAF for each of the UGTs was calculated using the *in vitro* data from Picard et al and was incorporated into the model (Table 21and Table 22).

Table 21. rUGT scalar calculation from endogenuous microsomal Vmax and enzyme specific Vmax

Enzyme	Abundance (pmol/mg of microsomal protein)			Endogenous Microsomal V_{max}			Enzyme Specific V_{max}	rUGT Scalar		
	MPAG									
	Liver	Kidney	GI	Liver	Kidney	GI		Liver	Kidney	GI
UGT1A1	48	6.1	8.5	5.16	12.94	0.71	0.11	46.91	117.64	6.45
UGT1A7	0	13	7.1	5.16	12.94	0.71	7.14	0.72	1.81	0.10
UGT1A8	0	4.9	5.6	5.16	12.94	0.71	7.27	0.71	1.78	0.10
UGT1A9	31	79	5.9	5.16	12.94	0.71	11.82	0.44	1.09	0.06

Table 22. Physicochemical Properties and In-vitro UGT enzyme kinetics data of Mycophenolic acid

Parameter	Reference Value	Reference
Molecular weight	320.33	Drugbank
LogP	1.6	Lee et al
Compound Type	Monoprotic acid	
pKa	4.8	Lee et al
Blood to plasma ratio	0.6-0.7	Lee et al(optimized)
Fraction unbound in plasma	0.03	
Absorption Model	ADAM	-
Caco 2 permeability (Apical pH 7.4 to Basolateral pH 7.4)	26.33 X 10^{-6} cm/s	
Propranolol permeability	6.582 X 10^{-6} cm/s	
Predicted P_{eff}	12.301 9 10^{-4} cm/s	
Distribution Model	Full PBPK	
Prediction method	Paulin and Theil method	
Elimination	Enzyme Kinetics	Picard et al
UGT1A1 Clint (µL/min/mg protein)	0.26 (Pathway I)	Picard et al
UGT1A7 Clint (µL/min/mg protein)	14.88 (Pathway I)	Picard et al
UGT1A8 Clint (µL/min/mg protein)	5.22 (Pathway I)	Picard et al
UGT1A9 Clint (µL/min/mg protein)	73.86 (Pathway I)	Picard et al
UGT2B7 Clint (µL/min/mg protein)	1.08 (Pathway II)	Picard et al
UGT1A1 Clint (µL/min/mg protein)	0.08 (Pathway II)	Picard et al

5.3.4 Sensitivity Analysis

Sensitivity analysis allows to quantitatively analyze the uncertainty associated with a parameter and its impact on an outcome variable such as clearance or AUC. This uncertainty could be associated with a system parameter (e.g enzyme abundance or blood flow), or an *in vitro* parameter (e.g. intrinsic clearance) that has been determined experimentally. Such knowledge is important for assessing whether certain parameters need to be determined more accurately for the model under study and to get a more comprehensive understanding of the system being modelled.

ASA (Automated Sensitivity analysis) is a user-friendly tool that allows the assessment of changes in an outcome variable (e.g. AUC or CL) as a function of the user-defined input variables (e.g. dose). Here we have performed sensitivity analysis on fraction unbound. MPA, being a highly protein bound drug, its pharmacological activity is largely dependent on the free fraction. It was optimized before incorporating in the model. MPA exposure in terms of AUC and C_{max} was highly sensitive to the fraction unbound. Figure 43 shows the concentration time profiles of MPA for a range of 0.01-0.3 of unbound fraction. MPA is primarily metabolized by UGT1A9, 1A8, 1A2 and 2B7. We predict that changes in UGT abundance can potentially affect the MPA PK. A sesitivity analysis of the effect of abundance of UGTs on the MPA PK was carried out. Figure 44 shows the effect of changes in UGT ezymes' abundance of the concentration time profile of MPA.

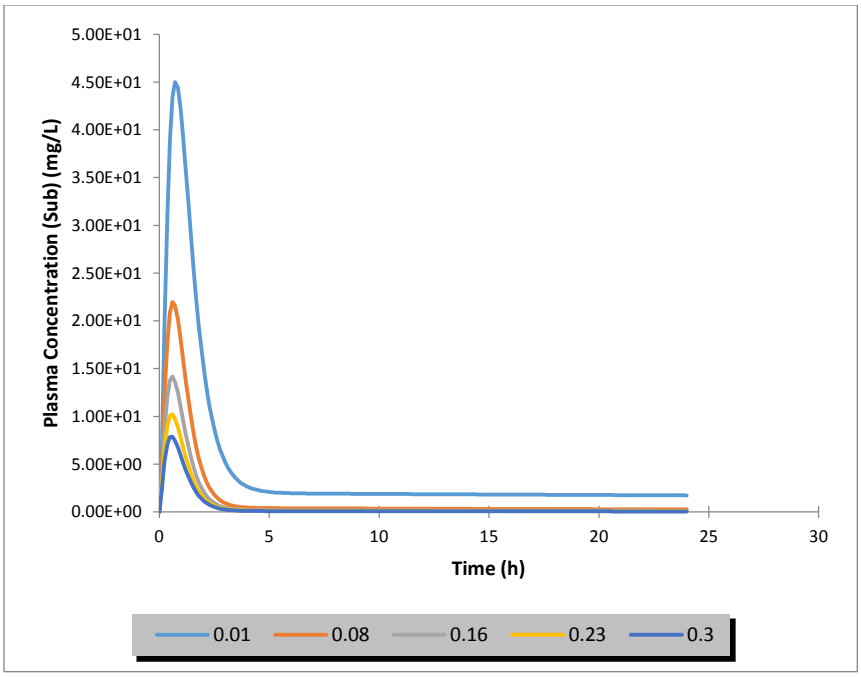


Figure 43. Sensitivity analysis of MPA exposure to unbound fraction of MPA over time

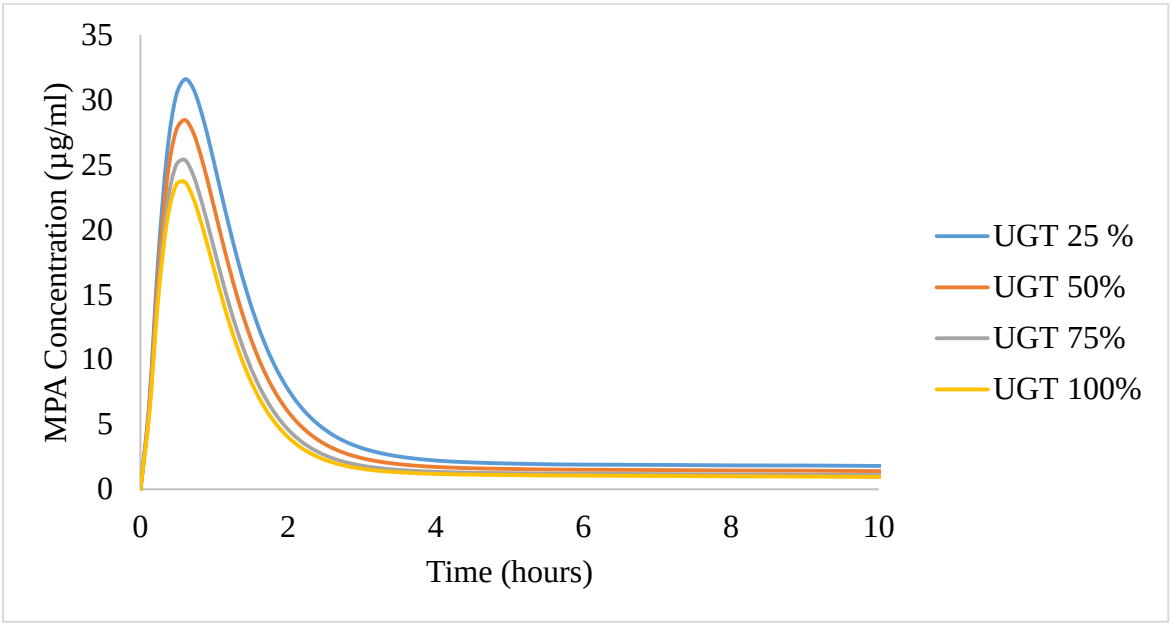


Figure 44. Sensitivity analysis of varying percentages of UGT abundance to predict the MPA exposure

5.3.5 Clinical Pharmacokinetic Data

The PubMed database was screened for single dose pharmacokinetic studies of mycophenolic acid in healthy adults, Chinese healthy volunteers and patients with varying degree of kidney impairment (GFR 30-60, GFR<30) with known age, sex, height and weight data and reported systemic drug concentration–time profiles. Data from a single IV and oral dose of 1500 mg of MMF from Bullingham et al was used to build the model in healthy volunteers.[11] Model naïve data from 4 other studies in adult healthy volunteers [41, 46, 54, 58, 225, 226], 3 Chinese healthy volunteers [32, 227, 228] and 3 in patients with impaired kidney function[46, 229] was used for validation. The pharmacokinetic data were either provided by the author as seen in Bullingham et al. [11] or scanned from the concentration time profiles [46, 54, 58, 226], using the graph digitizer GetData Graph Digitizer V.2.26. However, errors associated with data digitization did add variability to the model-based predictions of concentration time profiles. to the Additionally one publication that reported only the pharmacokinetic parameters, but not the concentration–time profiles [230], after administration of a single oral dose in healthy subjects was used in the comparison of the observed and predicted values of the chosen pharmacokinetic parameters. Table 22 shows the physico-chemical data used to build and validate the model.

5.3.6 Model Evaluation

The developed PBPK model was evaluated using visual predictive checks by overlaying the observed full concentration time profiles on the predicted profiles and a comparison of the observed and predicted values of various pharmacokinetic parameters. For publications where, pharmacokinetic parameters were not available they were calculated by a non-compartmental

analysis (NCA) from each observed profile and compared to its corresponding predicted value in each model (i.e. using the predicted values at the same time points), using Phoenix WinNonLin version 6 software. The area under the drug concentration– time curve from time zero to the time of the last measured concentration (AUC_{last}), C_{max}, T_{max} were compared. There are no established guidelines regarding an acceptable error range that should be used for the evaluation of predicted data by PBPK models. A 2-fold error is commonly used by researchers in this field. [231, 232]Some others have also used a wider (3-fold) range [233] or a stricter (1.5-fold) range. [234] In our study since we are validating a model across different populations we have used a stricter 1.5-fold error as the criterion.

5.4 RESULTS

5.4.1 Healthy adult volunteers

The simulations were performed following multiple single dose administrations of MMF as a 1gm oral dose BID. The selection of the demographics of the virtual population and the dose for the simulation were based on the study details from the clinical study in adults. Ten trials of 10 healthy subjects (i.e., 100 subjects) were run. The observed and predicted pharmacokinetic parameters are shown in Table 23. The predicted concentration time profiles for model building dataset after a 1500 mg single dose of oral MMF are represented in Figure 45.

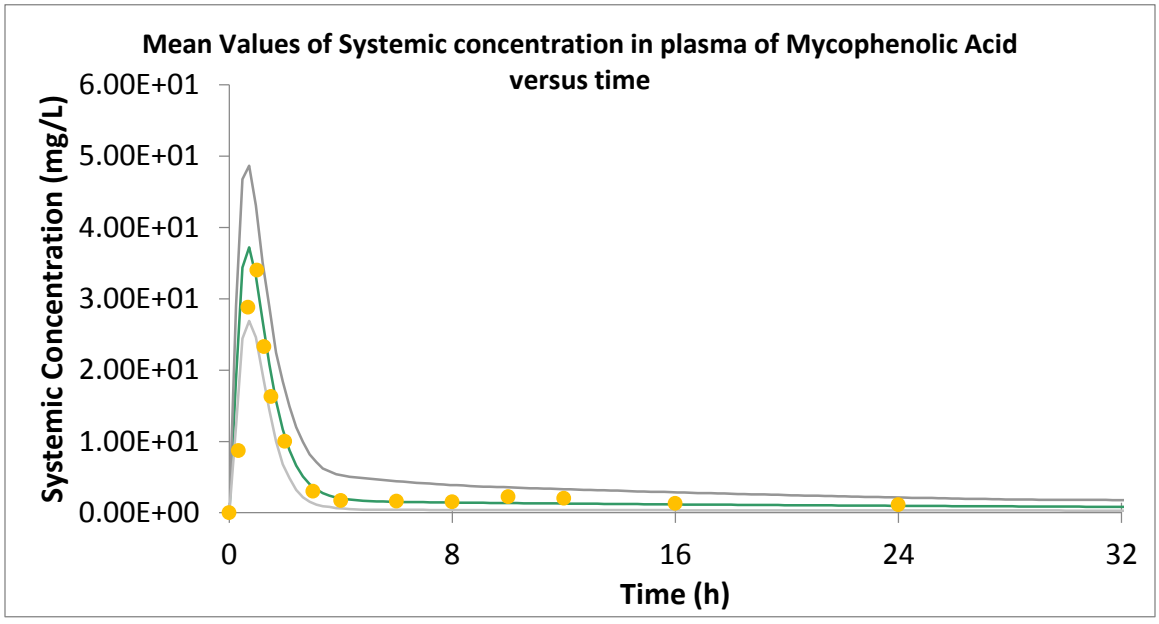
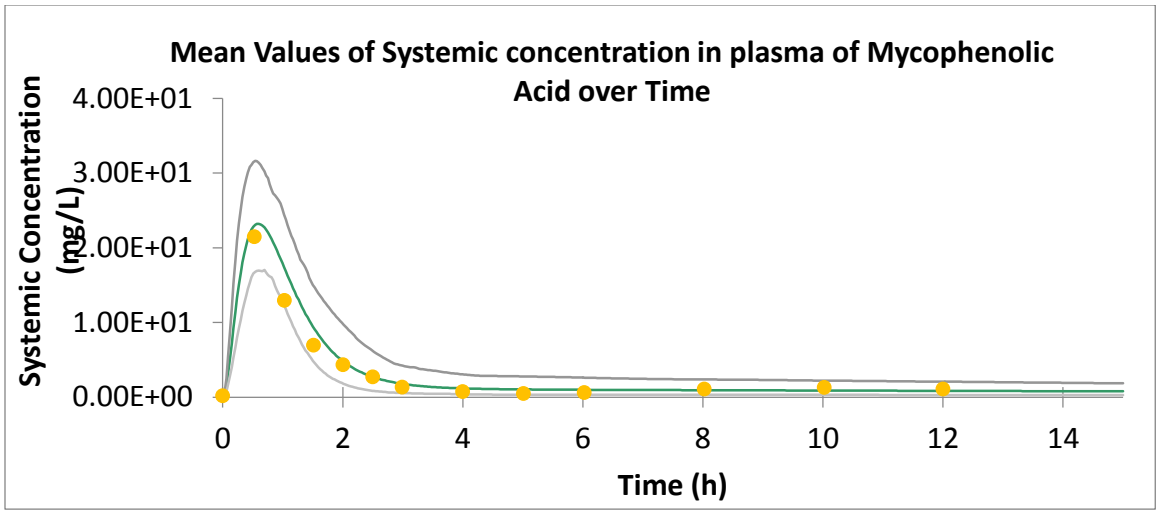
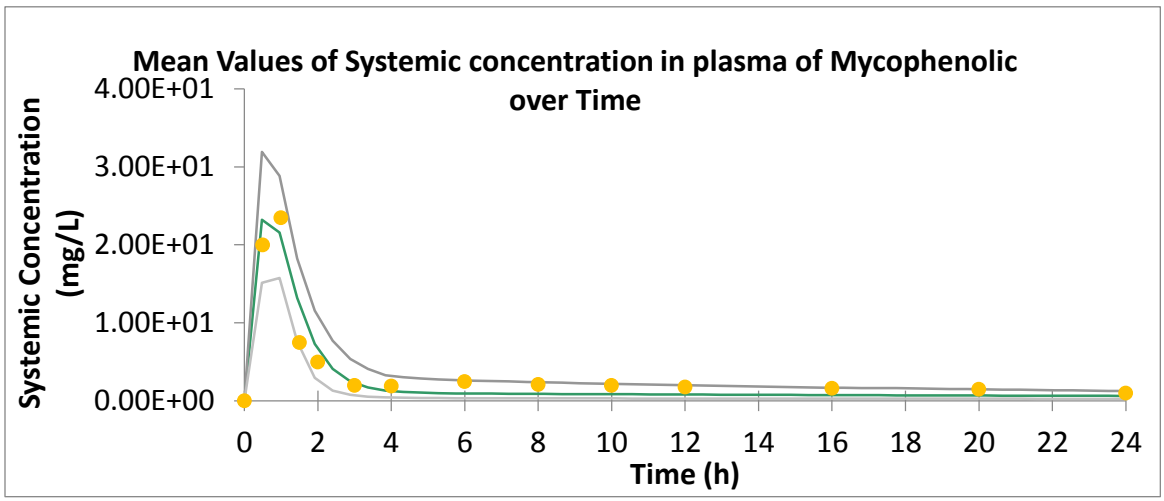


Figure 45. Mean observed (yellow circles) and predicted (green solid line) concentration time profile of MPA for a single 1500 mg dose in healthy volunteers.

Figure 46A, B and C show the predicted concentration time profiles after 1000 mg single oral dose of MMF. The observed concentrations over time are overlaid on the graph. The predicted concentration time profiles for all the model naïve datasets are within 1.5-fold of the observed values. In healthy adults, with the exception of a few concentration observations, the simulated mean profiles with the 5th and 95th percentile curves can capture the observed PK data for all individuals obtained after administration of 1000 mg doses of oral MPA. The calculated PK parameters for virtual population of healthy volunteers, C_{max}, AUC and Clearance, are in good correlation with values from the literature and are presented in Table 23.





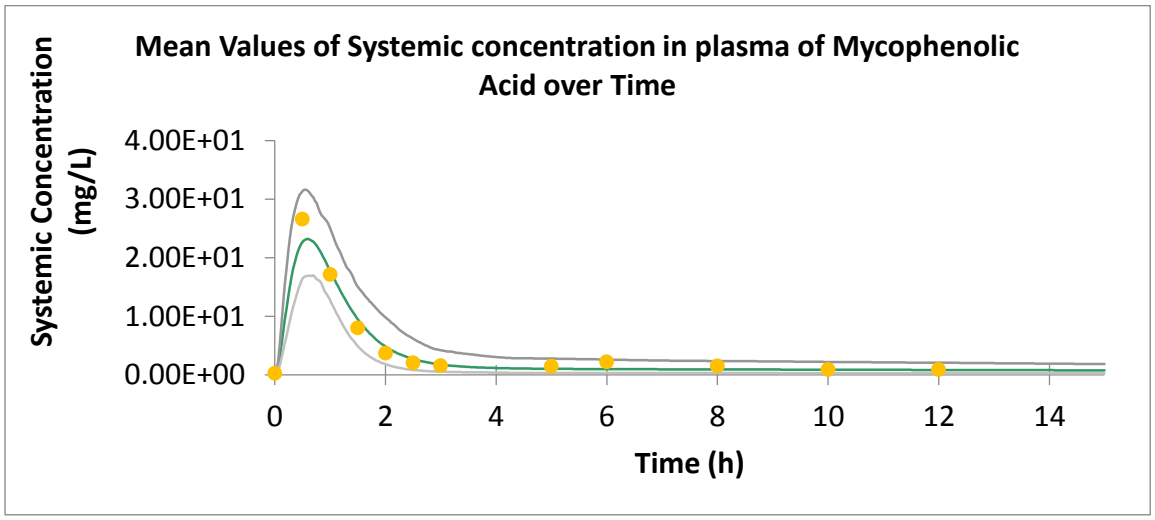


Figure 46. A, B and C: Mean observed (yellow circles) and predicted (green solid line) concentration time profile of MPA for a single 1000 mg dose in healthy Caucasian volunteers.

5.4.2 Healthy Chinese Volunteers

The SimCYP Chinese healthy volunteer population was used to validate the robustness of the model while transitioning between different populations in SimCYP. The predicted concentration time profiles after 1000 mg single oral dose are represented in Figure 47 A, B and C. The calculated PK parameters for virtual population of Chinese healthy volunteers, mainly C_{max}, AUC and Clearance, are within 1.5-fold of the observed values and are presented in Table 23.

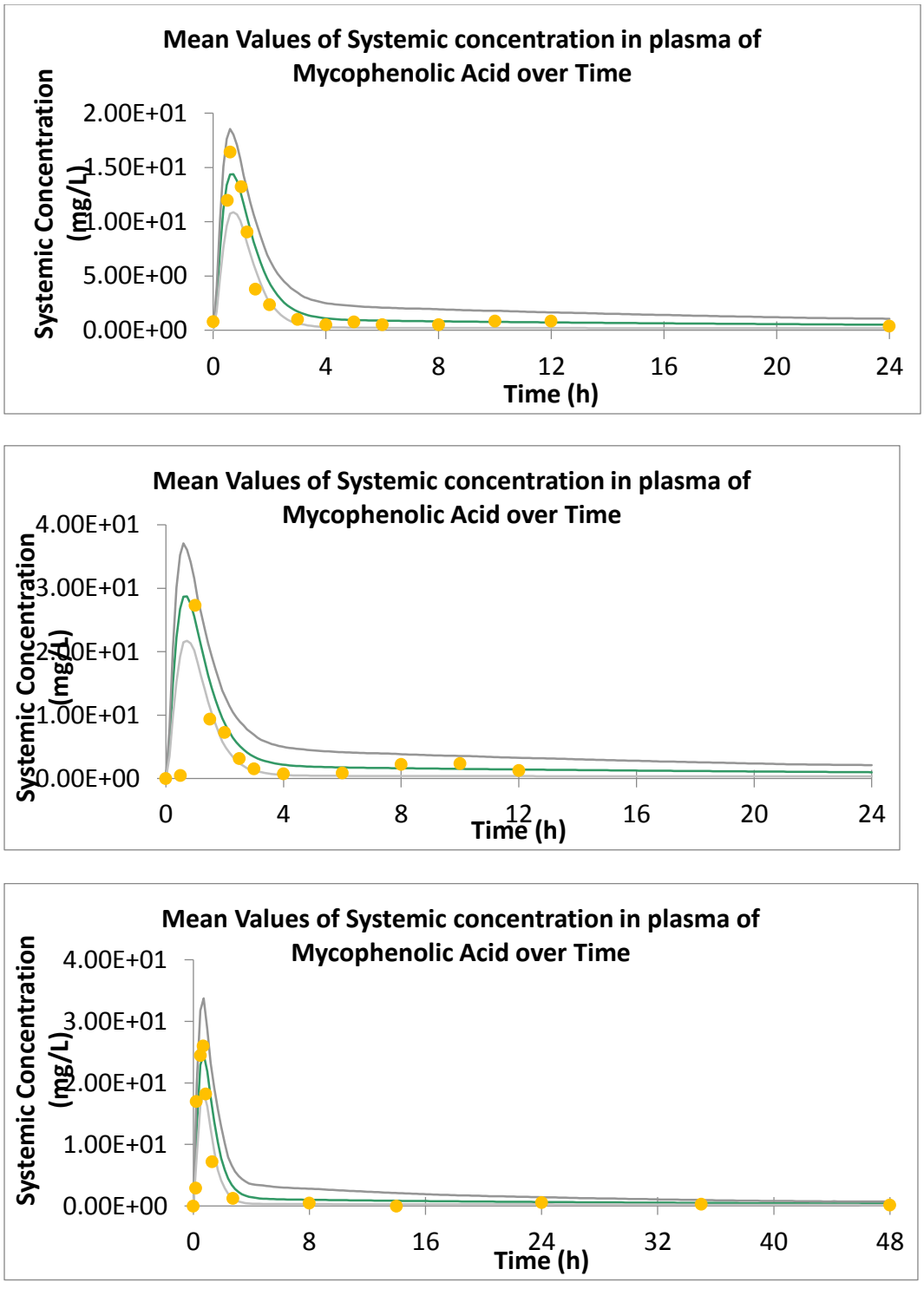


Figure 47. A, B and C: Mean observed (yellow circles) and predicted (green solid line) concentration time profiles of MPA for a single 1000 mg dose in healthy Chinese volunteers from three independent model naïve datasets.

5.4.3 Renally impaired patients

The predicted concentration time profiles for patients with End stage renal disease with varying degrees of GFR are presented in Figure 48A, B and C. Similar to the adult healthy Caucasian and Chinese volunteer predictions, the predicted profiles as also the pharmacokinetic parameters (Table 23) for patients with varying degree of renal impairment were within 1.5-fold of the observed data.

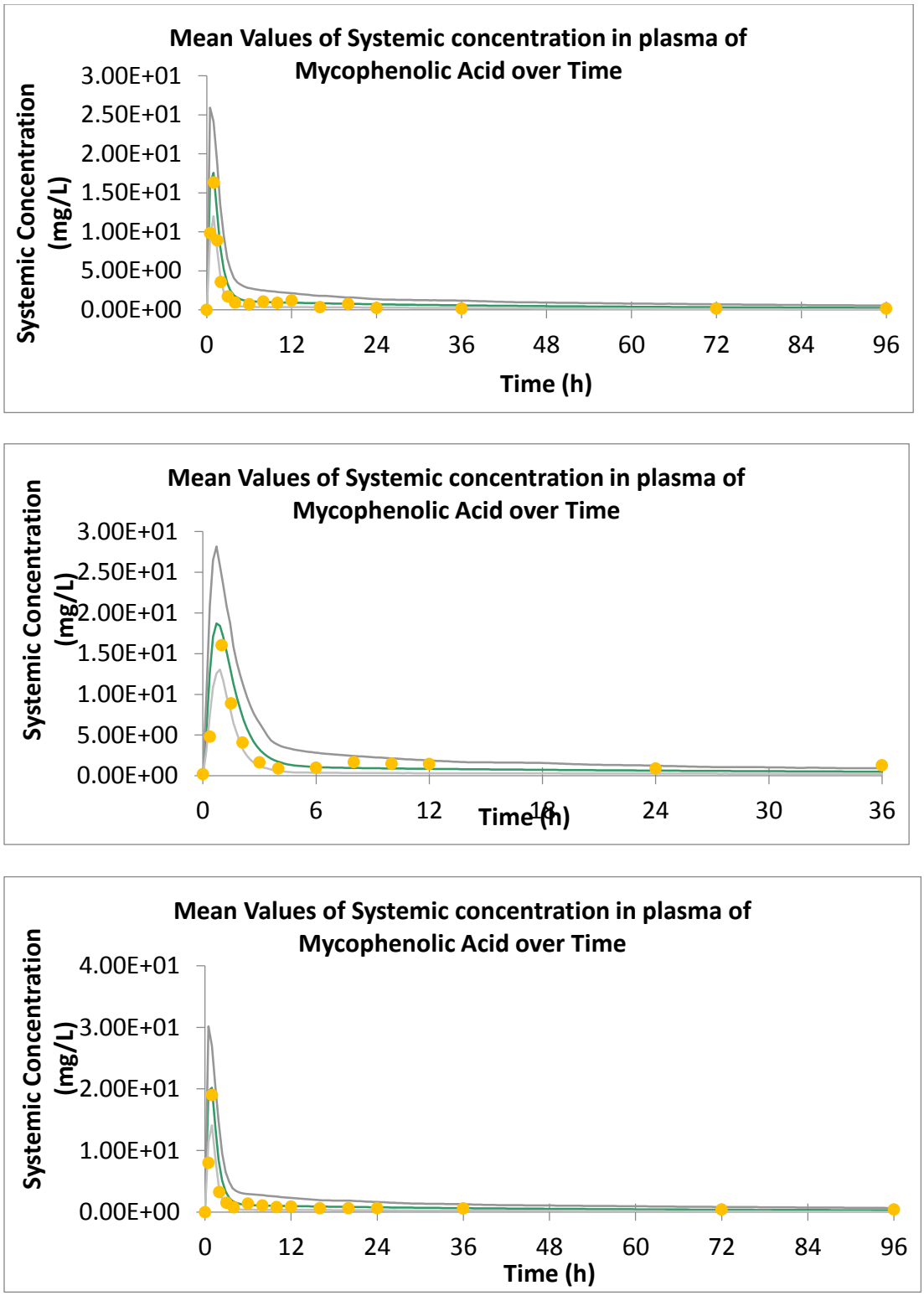


Figure 48. A, B and C: Mean observed (yellow circles) and predicted (green solid line) concentration time profile of MPA for a single 1000 mg dose in patients with varying degree of renal impairment; GRF<30 (A B), GRF:30-60(C)

Table 23. Observed and predicted Pharmacokinetic parameters with fold change for healthy adults, Chinese healthy volunteers and patients with varying degree of renal impairment

Publication	Population	Dose	Route	Model	PK parameters											
					Cmax			Tmax			AUC			Clearance		
					Observed	Predicted	Fold change	Observed	Predicted	Fold change	Observed	Predicted	Fold change	Observed	Predicted	Fold change
Bullingham et al	**Healthy**	1.5 gm	IV	Building												
Bullingham et al	**Healthy**	1.5 gm	Oral	Building	34	37.2	1.09	0.99	0.72	0.73	101	100	0.99	11.58	12.37	1.07
Johnson et al	**Healthy**	1 gm	Oral	Validation	25.3	25.2	1.00	0.75	0.67	0.89	45	-	N/A	N/A	12.37	N/A
Kees et al	**Healthy**	1 gm	Oral	Validation	21.7	22.5	1.04	0.53	0.72	1.36	33.3	39.4	1.18	16.4	13.6	0.83
Rupprecht et al	**Healthy**	1 gm	Oral	Validation	27.9	23.7	0.85	0.53	0.62	1.17	40	39.4	0.99	19.2	16.5	0.86
Parker et al	**Healthy**	1 gm	Oral	Validation	24.3	23.2	0.95	0.5	0.7	1.40	-	-	-	-	-	-
Naderer et al	**Healthy**	1 gm	Oral	Validation	20.4	23.1	1.13	0.5	0.7	1.40	56.2	63.3	1.13	15.52	13.02	0.84
Jiao et al	**Chinese healthy**	1 gm	Oral	Validation	16.4	14.4	0.88	0.6	0.72	1.20	30.9	36.4	1.18	10.2	11.3	1.11
Zhang et al	**Chinese healthy**	1 gm	Oral	Validation	26.4	25.2	0.95	0.8	0.72	0.90	58.3	66.8	1.15	15.8	12.5	0.79
Shen et al	**Chinese healthy**	1 gm	Oral	Validation	35	28.2	0.81	0.81	0.68	0.84	58.34	52.3	0.90	17.1	15.1	0.88
Johnson etal	**GFR<30**	1 gm	Oral	Validation	16.3	17.5	1.07	1	0.96	0.96	78.6	81.2	1.03	-	-	-
Machphee et al	**GFR<30**	1 gm	Oral	Validation	16.01	18.7	1.17	0.66	0.72	1.09	55.7	57.3	1.03	-	-	-
Johnson et al	**GFR 30-60**	1 gm	Oral	Validation	19	20.2	1.06	1	0.96	0.96	75.8	90	1.19	-	-	-

5.4.4 Renal Transplant Population

Virtual renal transplant population was built by modifying existing SimCYP® healthy volunteer population [235]. Since renal transplant recipients have only one functioning kidney, the number of functioning nephrons was halved in the virtual renal transplant population to 0.807 million nephrons per subject from 1.615 million nephrons in healthy adults. However, MPA being primarily a drug metabolized by the liver the changes in stable renal transplant are not expected to cause changes in the MPA PK. In the immediate phase post- transplant, the MPA total concentrations are reduced due to hypoalbuminemia but return to normal after 3 weeks post-transplantation. Figure 49 represents the observed PK profile of MPA 3 weeks post-kidney transplantation[62] overlaid with the model predicted profile. The predicted exposure as measured by $AUC_{0\text{-}12}$ and C_{max} (76.7 µg*hr/ml and 21.4 µg/ml) were within 20% of the observed

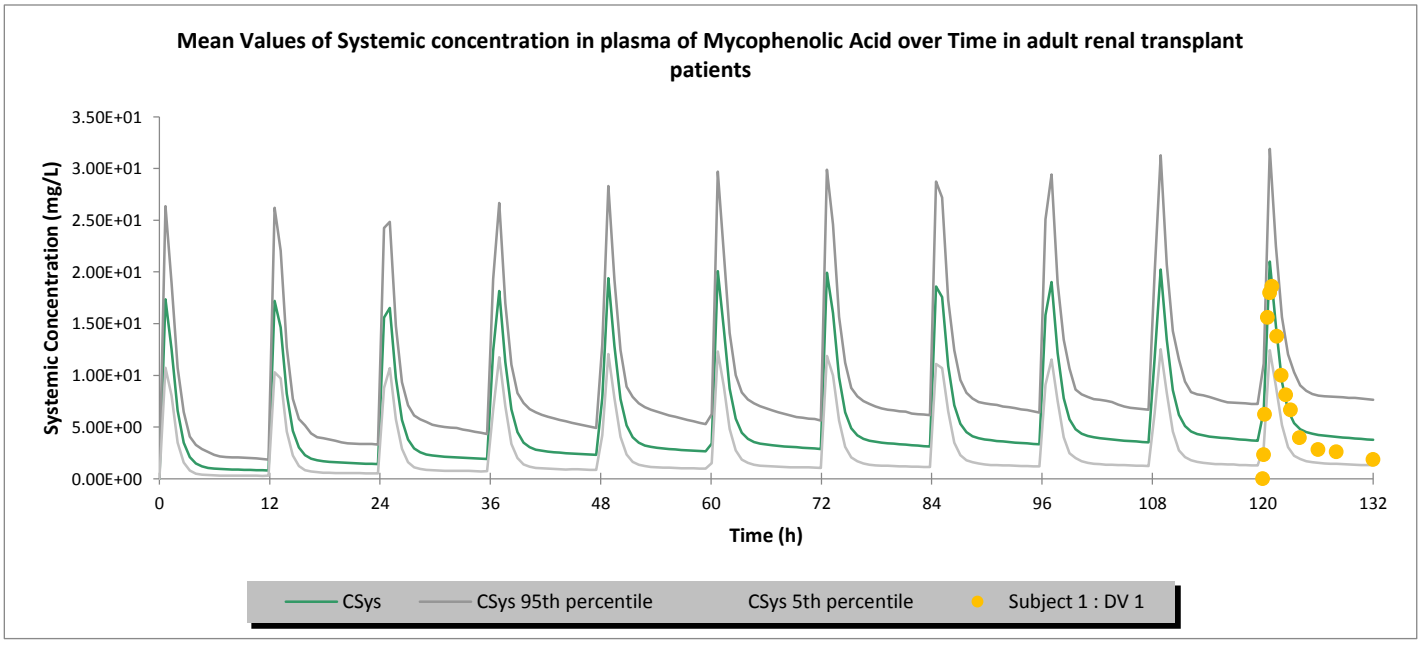


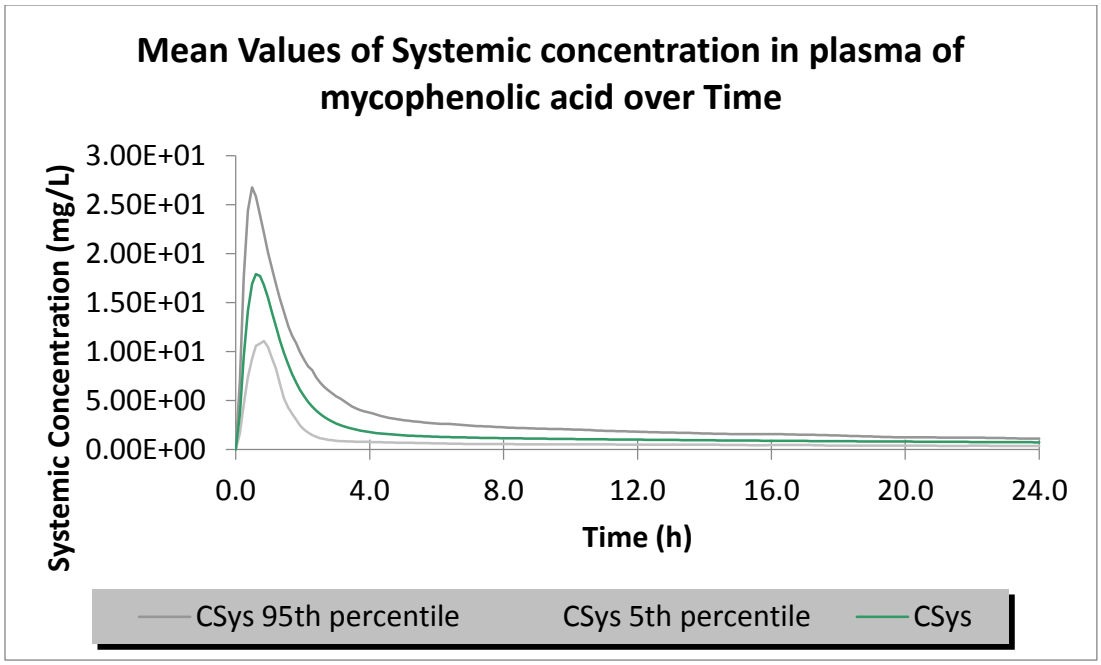
Figure 49. Predicted concentration time profile of MPA (CSys) in stable adult renal transplant recipients at steady state after multiple oral dosing of 1 gm BID.

values (AUC_{0-12}= 61.4 μg*hr/ml and C_{max}= 25.5 μg/ml).

However, the kidney transplant model needs to be refined to incorporate the changes associated with hypoalbuminemia to better predict the MPA PK in the immediate phase post-transplant.

5.4.5 Healthy pediatric population

SimCYP Pediatric version 16 (Release 1) was used as the pediatric PBPK modeling platform with input of drug specific data as used in the MPA PBPK model previously developed for adults. Due to lack of clinical PK data in healthy pediatric and lack of availability of UGT abundance data in pediatric the model could not be validated in this population. The predicted concentration time profiles of MPA for a comparable weight based (15mg/kg) and body surface area-based dosing (600/m^2) are represented in Figure 50A and B respectively.



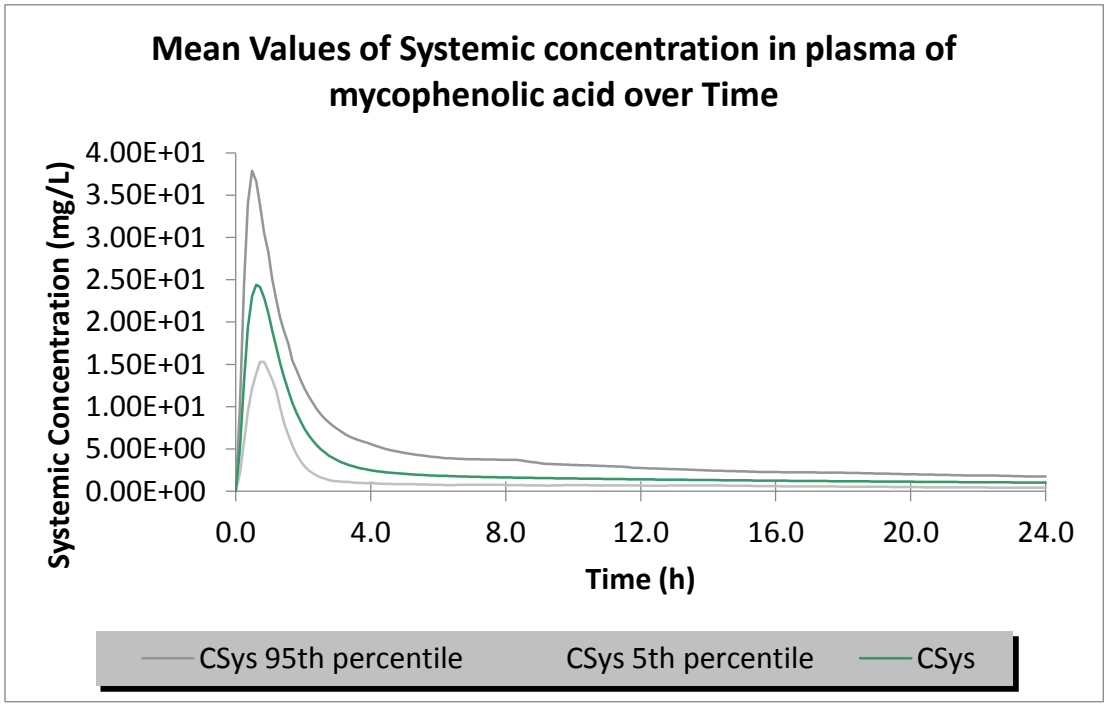


Figure 50 A Predicted plasma concentration time profile of MPA in pediatric population after a single oral dose of 15mg/kg. B Predicted concentration time profile of MPA in pediatric population after a single oral dose of 600 mg/m^2.

We compared the model simulated concentration time profiles and PK parameters from in-house data in pediatric HSCT patients. The model predicted concentration were five times higher than the observed data after a 15mg/kg TID dose of MMF. This suggests a much higher MMF dose requirement. As suggested by the sensitivity analysis, changes in fraction unbound and in the abundancies of UGTs can affect the MPA total concentrations. This hypothesis can be further evaluated on availability of in-vitro data and quantitating unbound concentrations of MPA in future studies. This suggests that protein binding and/or UGT expression could be different in pediatric HSCT patients and hence the healthy pediatric model is not able to capture the PK profiles optimally. This discrepancy in observed and predicted data can also be explained by the wide variability of age in our pediatric HSCT study. The pediatric study was also limited by the wide age range of patients. MPA PK can vary largely based on age of the subjects. PBPK modeling also did predict age related changes in MPA clearance as shown in Figure 51.

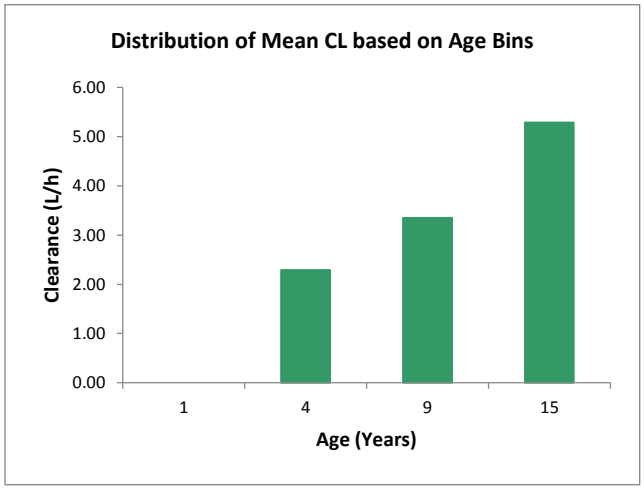


Figure 51. MPA Clearance at different age groups

5.5 DISCUSSION

In this study, we have built and validated full-PBPK models of IV and oral MPA dosing in healthy Caucasian and Chinese and renally impaired patients across clinically relevant doses of MMF. The full-PBPK, ADAM model that we have built incorporates the tissue disposition of MPA and enzymatic metabolism by UGTs. The model is robust across all built-in healthy volunteer populations in SimCYP. Incorporation of the Caco-2 permeability data made the model more mechanistic. Use or permeability-based ADAM model and incorporation of enzyme kinetics data to calculate the RAF values made the model reproducible across populations. This is primarily because rate of absorption (Ka) was predicted based on permeability and dissolution data of MPA unlike the first order model where in the Ka is based on clinical data used to build the model making the model more mechanistic in nature. The model predicted concentration-time profiles are consistent with observed data across 6 independent, model naïve studies. The predicted IV and PO MPA PK parameters are within 1.5-fold range of the corresponding PK parameters calculated from the observed studies. We have also validated the model in a custom built renal transplant population. Plasma concentrations of MPA in patients in special populations like pediatric patients were also predicted for weight based and body surface area-based dosing.

The model was first developed and validated in adult healthy Caucasian patients and was then used to build and validate a model in Chinese healthy volunteer population. The predicted MPA PK did not reveal any differences between the 2 population. Our observations are in line with a population analysis done by Ling et al.[236] to evaluate the ethnic differences in MPA

disposition. The group's population PK model indicated that ethnicity is unlikely to affect MPA PK in healthy subjects and was consistent with NCA results.

Based on predicted concentration time profiles from sensitivity analysis, changes in fraction unbound and UGT abundance can cause significant changes in MPA PK. A better understanding of the protein binding, UGT ontogeny, activity and abundance data in HSCT patients is required to further validate the model in this population. Based on SimCYP simulations, we predict upto a 2-fold increase in AUC for a 25% reduction in abundance of UGTs. Confounding factors in transplantation like organ dysfunction, concomitant medications could be affecting the fraction unbound and could directly influence MPA PK.

Generally, SimCYP® platform represents a "bottom-up" PBPK approach where *in vitro* data are extrapolated to possible *in-vivo* results, in this study, the simulated clinical trials were based on known *in-vivo* parameters. This was to combine clinical data (top-down) to "bottom-up" PBPK models. This study represents a novel approach for the estimation of PK parameters and population variability in MPA across different populations.

PBPK approaches have been previously used to predict exposure of active drug after prodrug administration [237] and to predict exposure in neonates and infants after intravenous administration of oseltamivir [238]. However, predictions in these studies were largely based on extrapolating *in-vivo* animal data and clinical data to predict human exposure and involved extensive model development and refinement. The enterohepatic recycling of MPA is reported to contribute up to 40% to the overall MPA exposure. In our model, ignoring MRP2 based EHC of MPA did not result in a complete misprediction of the PK profile for MPA for all validation datasets. However, on close inspection of the logarithmic concentration time profiles do show that additional *in-vivo* data on the enterohepatic recycling may be required to better understand

the PK profile and tease out the variability associated with clearance in MPA and consequently improve the PBPK model predictions.

We are currently working on building a stem cell transplant population to predict the pharmacokinetics of MPA in these patients and to further facilitate optimization of MMF dosing in HSCT patients. The current model failed to fit the in-house data from the pediatric HSCT patients study. We have used the built-in pediatric population in SimCYP to predict the concentration time profiles in pediatric population. However, due to lack of availability of MPA PK data in healthy pediatric population we were not able to validate the model. The model predicted MPA exposure was five-fold higher as compared to what we observed in our in-house MPA PK data in pediatric HSCT patients. This difference can be attributed to physiological changes, changes in UGT expression and abundancies and/or interaction with other drugs that these patients are being administered concomitantly.

This study will help generate MPA dosing guidance in young patients of varying age and weight. Use of a PBPK approach especially in special populations leverages the limited clinical PK data and can serve as a practical application for the establishment of individual dosing of MMF in transplant patients. This robust ADAM PBPK model of MPA along with future availability of physiological data in transplant patients can be used to build and validated other transplant populations and design dosing guidelines to optimize MMF dosing in transplantation.

6.0 SUMMARY AND FUTURE DIRECTIONS

6.1 SUMMARY

Through this work we have extensively reviewed the literature to present an overview of MPA and its different formulations, the pharmacokinetics, pharmacodynamics and pharmacogenomics. We have also described the effect of physiological factors post-transplant such as albumin levels, renal function and co-medication and other factors that are known to affect the pharmacokinetics in transplant patients. Several pharmacokinetic studies across solid organ and stem cell transplant patients have established MPA AUC from 30-60 mg*h/L as an efficacious therapeutic window. The main objective of this work was to optimize MMF dosing in transplantation using the following approaches:

6.1.1. Pharmacokinetics guided dosing

6.1.2. Pharmacodynamics guided dosing

6.1.3 Model based personalized drug dosing

6.1.1 Pharmacokinetics guided dosing

Pharmacokinetics guided dosing can be attained by a complete PK profiling of the drug and itsd metabolites. This approach although very useful is very tedious and involves the patient having to spend long hours in the hospital. Such studies are often met with poor recruitment and higher number of patients tend to drop out from the study. Limited sampling strategies are a clinically feasible option of PK guided dosing. Better validation of these strategies can lead to

incorporation of PK evaluation as a part of routine TDM. Through the understudied pediatric HSCT and LT patients we have studied this approach of personalized dosing.

6.1.1.1 Optimizing MMF administration in pediatric HSCT patients

MMF dosing in HSCT patients has been primarily extrapolated from data in renal transplant recipients. However, pharmacokinetic studies suggest a lower MPA exposure in HSCT recipients, some as low as 30-50%, with standard starting MMF doses compared to kidney transplant recipients. HSCT patients have a much lower half-life (2-4 hours) as compared to solid organ transplant recipients, which necessitates a dosing regimen of at least three to four times a day which is clinically not practical to achieve in these patients. Low MPA exposure has been associated with higher rates of acute GVHD and graft rejection, and lower response rates in the treatment of acute GVHD. Several strategies like higher MMF dose, increased dosing frequency have been attempted in the HSCT setting to achieve AUC and trough concentration targets similar to those targeted in renal transplants, but the results are not consistent in improving MPA exposure. It is very crucial to maintain optimum MPA exposure in the immediate phase post- HSCT to avoid GVHD. A novel approach to this problem is continuous infusion administration of MMF; a dosing method that has not been previously reported in the literature for this drug. This strategy has been successfully used for CNI immunosuppressive drugs in this population. Continuous infusion allowed for continuous steady state exposure of MPA with minimal fluctuation in MPA levels.

W designed a personalized dosing strategy for pediatric HSCT patients. This involved performing pharmacokinetic analyses of MMF, MPA and MPAG following intermittent IV dosing schedule and then designing a personalized CI, and oral administration schedule for patients. This allowed us to better control factors that contribute to variable exposure of MPA

and helped us to optimize MMF therapy for GVHD prophylaxis. We evaluated the pharmacokinetics of MPA after IV short infusion dose. The personalized CI dosing of MMF was able to achieve a target exposure of AUC_{0-12} of 30-60 μg*hr/mL and MMF oral dose was also individualized before discharge. We have also studied the safety outcomes of this MMF dosing approach in pediatric HSCT recipients undergoing myeloablative transplantation. This is the first report of using a 24-hour MMF IV continuous infusion regimen in transplant patients. The information obtained from this study will be utilized to define future studies to individualize prophylactic and/or therapeutic MMF regimen that can be used to achieve specific target concentrations in the plasma and to optimize efficacy and minimize toxicity.

All patients started with a 15-mg/kg/dose every 8 hour intravenously. The average daily dose required to maintain patients within the desired therapeutic range remained close to 45 mg/kg/day during CI and oral dosing periods. During CI period, 17 of 19 patients (89%) achieved target MPA C_{ss} of 1.7 to 3.3 μg/mL. We observed no delays with neutrophil and platelet engraftment. This regimen had excellent survival outcomes with 3-year event-free and overall survival rates of 68% and 79%, respectively. Although this was feasible in all subjects, they did require a dedicated IV line, and this may put patients at increased risk of central line–associated bloodstream infections. Promising results from the pilot study deserve further validation in a larger cohort of pediatric patients undergoing myeloablative transplantation.

6.1.1.2 MMF PK in adult lung transplant recipients

MMF is increasingly being used in lung transplant recipients, with more than 80% transplant centers across US using MMF as a part of maintenance immunosuppressive regimen. To date, only few studies evaluating MPA PK-IMPDH relationship are available in MPA-treated adult

kidney transplant patients and no data have been reported in lung transplant recipients. The objective of this investigation was to systematically study the PK-PD of MPA in adult lung transplant recipients in early post-transplant period for the first time.

We first established and validated a highly sensitive UPLC-MS assay for quantitation of MMF, MPA and MPAG in plasma. The assay was used to monitor MMF, MPA and MPAG levels in adult lung transplant recipients. This was designed as a pilot study evaluating the PK and PD of MMF, MPA and MPAG in adult LT recipients. MMF PK parameters were determined in 4 adult lung transplant recipients with intense sampling for up to 12 hours within a dosing interval. Pharmacokinetic parameters were comparable to that seen in other published LT studies. AUC_{0-24} did show a good correlation with abbreviated AUCs calculated over 2 and 4-hour time periods. Clearance was comparable to other SOT recipients.

6.1.2 Pharmacodynamic guided dosing

Pharmacodynamic activity can be measured by quantitating the IMPDH activity in PBMC lysate. Currently, PK monitoring is preferred over PD measurement because PD assays are often tedious and time consuming. We have established and validated a rapid, sensitive HPLC-MS/MS assay for XMP and AMP in order to quantify IMPDH activity *ex-vivo* to understand the pharmacodynamic activity of MPA. The utility of IMPDH activity monitoring has been tested in a retrospective and a prospective study in LT recipients. The assay can quantitate IMPDH activity in PBMC lysate with cell concentration as low as 1million/ml making it highly useful in transplant patients with a low PBMC count. The IMPDH activity was highest

pre-dose and dropped with administration of MMF with nadir reaching at the T_{max} of MPA in the prospective LT study.

The retrospective study was designed to investigate the association between incidence of infection and rejection during the first post-transplant year with trough IMPDH activity and genotype in banked PBMC samples in LT recipients surviving at least 1-year post LT. We predicted that LT recipients with low baseline IMPDH activity will have more infections and LT recipients with high baseline IMPDH activity will have more rejection episodes during the first-year post transplantation. The results from the retrospective study wherein IMPDH activity was monitored at a single time point were clinically and statistically inconclusive owing to the low sample size. However, the IMPDH activity tended to be higher in rejecting phenotypes.

6.1.3 Model-based Personalized Drug Dosing

Modeling based approaches entail defining appropriate models that mathematically define the drug exposure, response and effect of physiological and pathological factors that can affect the exposure and in turn clinical outcomes.

We have developed and validated a physiologically based pharmacokinetic model to describe the MPA concentration-time profiles obtained using data from 7 studies in Caucasian healthy volunteers, 3 studies in Chinese healthy volunteers and 3 studies in patients showing varying degree of renal impairment. In this work we have explored the variables that could be responsible for difference in exposure in transplant patients. The data were best described using the ADAM absorption model. The ADAM model[239] enables simulation of the absorption of drugs from solution as well as from solid dosage forms, including modified and controlled release formulations. It also facilitates simulation of metabolic and transporter mediated drug-

drug interactions as well as metabolite formation and relevant interactions in the small intestine. The ADAM model facilitates more mechanistic explanation of gut absorption, transport, metabolism and interactions. We have built a virtual renal transplant population by modifying existing SimCYP® healthy volunteer population[235]. Since renal transplant recipients have only one functioning kidney, the number of functioning nephrons was halved in the virtual renal transplant population to 0.807 million nephrons per subject from 1.615 million nephrons in healthy adults. This custom renal transplant population was used to validate the MPA PBPK model.

We have also simulated concentration time profiles of MPA in pediatric population. The robustness of this model in predicting pediatric MPA PK could not be determined owing to lack of data in healthy pediatric subjects.

Although, SimCYP® platform provides a "bottom-up" PBPK approach where *in vitro* data are extrapolated to possible *in vivo* results, in this study, we have used a middle out approach using *in-vivo* PK data (top-down) as also drug dissolution and permeability data (bottom-up) to simulate concentration time profiles from several clinical trials. This is the only report of a PBPK model for describing PK profile of MPA in healthy volunteers. Our model did not account for the EHC. Although, it did not result in any misprediction of the PK profile for MPA when not incorporating the EHC it does highlight the importance of EHC for improving model performance. The current model shows good predictive performance but does require further validation and refinement using in-vitro data about UGT expression and patient data.

6.2 CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

The discovery and implementation of strategies for monitoring MPA exposure and outcomes has evolved over the past decade and we continue to learn more about safe and effective interventions to optimize MMF-based immunosuppression. This has triggered a shift in dosing strategies as we are seeing a shift from traditional fixed-flat dose regimen to a more individualized approach for dosing MMF.

The success of the novel CI dosing of MMF based on an individualized dosing approach has paved way for future studies in HSCT recipients on MMF dosing. The safety and feasibility of CI dosing that was established through this work can be extended to adult HSCT patients. The design of CI dosing was based on a target $AUC_{0\text{-}12}$ of 30-60 μg*hr/mL. This corresponds to a steady-state concentration of 1-7-3.3μg/mL. This target C_{ss} was used to determine the infusion rate for CI based on individual patient's clearance that was determined from the initial short infusion. This target concentrations and CI strategy of attaining them can be further applied to other SOT and adult HSCT patients. Efforts should be made to further evaluate the clinical feasibility and efficacy of this personalized dosing approach in a larger PK study in both adult and pediatric subjects. IMPDH activity is known to be inhibited during MMF therapy. It will be worthwhile to study the IMPDH activity in patients receiving continuous MMF infusion and determine if improved outcomes are a result of continuous suppression of IMPDH activity.

The most concerning issue in the field of lung transplantation is the lack of improvement in morbidity and mortality. TDM is a valuable clinical tool for guiding dosing of immunosuppressive agents that exhibit a wide pharmacokinetic and pharmacodynamic

variability. However, routine TDM is not practical with MPA therapy. To address this clinical need this is the first attempt of a MPA PK-PD study in LT recipients. Unlike, kidney transplant recipients, LT recipients tend to stay longer in the hospital post-transplantation. This provided us a unique opportunity to conduct a time intensive study when the patients were stable and were at steady-state. We have collected blood samples after the morning MMF dose at 9 time- points over a dosing interval of 12 hours. This facilitated us to study complete PK profile of MPA and MPAG in LT recipients. Abbreviated AUC over 2 and 4-hour periods were able to capture the overall exposure of MPA. This abbreviated AUC can be used as a TDM tool in the maintenance phase post-transplantation. The shorter time (up to 4 hours) required to collect PK samples can thus make TDM a routine procedure when patients come for their bronchoscopy or biopsy visit post-transplantation.

Although the prospective LT study was limited by small number of subjects owing to enrollment issues, the promising results from this prospective study does call for a larger PK-PD study in LT recipients and will facilitate optimization of MMF dosing.

We have also successfully established the feasibility of monitoring IMPDH activity in lung transplant recipients. An HPLC-MS assay for quantitation of IMPDH activity *e-vivo* in lysate with a PBMC concentration as low as 1-5 million/ml was developed and validated. The sensitivity of the assay shall allow for quantitation of IMPDH activity in LT samples with a low PBMC count. This could be further extended to other solid organ transplant recipients. Along with the PK monitoring in the prospective study, we have also determined PD profile up to 6 hours post-dose in LT recipients. We have also retrospectively evaluated single time point IMPDH activity in 20 LT patients within first year post-transplantation. The trial is currently recruiting and will further investigate the association between incidence of infection and

rejection during the first-year post-transplantation with IMPDH activity and genotype in banked PBMC in 70 lung transplant recipients surviving at least 1-year post LT. Proposed sample size for this study was based on association of IMPDH activity with the clinical outcome of rejection or infection tested by logistic regression analysis. Based on a similar study conducted in kidney transplant recipients by Chiarelli et al[115], we assumed an odds ratio for rejection of 3, 80% power and an established 2-sided alpha level of 0.05, seventy single time-point of IMPDH activity measurements will be required to find significance. The study is still ongoing and recruiting additional patients. We predict that LTRs with low IMPDH activity will have more infections and LTRs with high IMPDH activity will have more rejection episodes during the first-year post transplantation.

We do acknowledge that there currently is no established therapeutic range for IMPDH activity supported in the literature. However, we hope that evaluating single time-point activity in 70 patients will help us phenotypically establish IMPDH activity as rejecting or infecting. Based on the results on this study we can further propose a dosing regimen based on the IMPDH activity. We expect that LTRs with lower IMPDH activity will have increased infection episodes and that LTRs with higher IMPDH activity will have increased rejection episodes as seen in other solid organ transplant studies. We also anticipate that IMPDH genotype will predict the respective baseline activity. In the presence of MMF, we further anticipate that MRP2 genotype will influence MPA exposure and thus will influence IMPDH activity in response to MMF. We also anticipate that LTRs receiving IMPDH-adjusted MMF therapy will have improved clinical outcomes. Overall this study on meeting the proposed sample size will address the clinical need of PD monitoring within the field by evaluating the relationship between baseline IMPDH activity and genotype and incidence of rejection and infection events, evaluating the relationship

between MPA exposure and IMPDH activity, and determining the impact of a precision-medicine guided approach to MMF dosing using IMPDH activity after LT. IMPDH activity measurement can readily be translated in to a routine clinical test in the future and can significantly improve MMF dosing and clinical outcomes in not only LTRs, but may also in other SOT patients.

Future studies should be designed to adjust the dose of MMF in LT recipients during the first two weeks to four months after LT to achieve a defined therapeutic range for IMPDH activity and comparing the clinical outcomes of this personalized IMPDH activity based MMF dose-adjusted cohort to a historical standard-of-care group maintained on fixed-dose MMF.

Over the last decade, the need for more robust pharmacometric PK analysis has been recognized and applied. Many pop-PK analyses have been conducted across majority types of transplants and have identified several covariates like age, weight, cyclosporine co-administration as important determinants of variability associated with MPA exposure. Another pharmacometric method is PBPK modeling.

Here, we have attempted to build and validate a PBPK model for MPA to predict the exposure in healthy and renally impaired patients. Based on in-house data, a renal transplant population was built into SimCYP® to predict the exposure of MPA in renal transplant patients. The model was robust and was able to capture MPA PK across healthy Caucasian, healthy Chinese, renally impaired and the custom renal transplant population. Currently there is scarcity of data on UGT abundancies and transporter expression. As MPA is primarily metabolized by UGTs, changes in MPA PK occurring in patients due to changes in UGT expression cannot be captured. Current efforts are focused on fine-tuning this transplant population. We have also simulated the concentration time profiles and predicted the PK parameters in pediatric patients

for a weight based and BSA based dose. Due to absence of healthy pediatric data we were not able to validate the model.

This approach of first developing an adult model by using existing drug and patient data, and scaling down this model to children, helped leverage prior information to mechanistically characterize changes in drug disposition with age. The model can also be used to study the DDI effect of MPA as a victim or perpetrator on other drugs that are metabolized by UGT enzymes. In future, PK data collected from an opportunistic study can be used to evaluate the pediatric population PBPK model. A robust pediatric PBPK model can then be used to perform simulations to identify optimal pediatric dosing and thereby diminish the need for more complex PK trials involving richer sampling.

To conclude, MPA PK and PD demonstrate a high inter and intra-patient variability. TDM approaches based on LSS can be used to tailor MPA exposure. Target IMPDH activity can be used to distinguish phenotypes for rejection and infection in transplant patients and when used along with PK LSS can be used to improve outcomes. PBPK model can be extended to optimize MMF dosing in lung and pediatric HSCT patients. Finally, an integration of all the PK, PD and model-based strategies can be intuitive and can facilitate clinical decision making of MMF dosing in transplantation.

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APPENDIX A [PRO09120501]

A1. ABSTRACT

Graft-versus-host disease (GVHD) remains a major barrier to the success of allogeneic blood and marrow transplant (BMT) therapy. Acute GVHD is seen in 30-80% of patients and, once established, often responds poorly to therapy and is associated with chronic disease and increased risk of death. Although the combination of methotrexate (MTX) and a calcineurin inhibitor has been the "standard of care" for more than a quarter century, there is little consensus on the most effective and least toxic approach to GVHD prevention. MTX use is associated with painful mucositis, delay in engraftment, and potential pulmonary toxicity. For cord blood transplants, commonly, a calcineurin inhibitor is used with corticosteroids and antithymocyte globulin. These transplants are frequently complicated by high rates of infection, hyperglycemia, and hypertension. There is emerging data to support the use of alternative GVHD prophylaxis regimens containing sirolimus, mycophenolate mofetil (MMF), or monoclonal antibodies such as etanercept. In small randomized trials, many of these approaches have been shown to be equivalent or superior, when compared with the standard of cyclosporine A (CsA) and MTX.

Mycophenolate mofetil (MMF), whose metabolite mycophenolic acid (MPA) inhibits proliferation of lymphocytes, is approved for prevention of organ transplant rejection. MMF in combination with CsA is widely used for GVHD prevention in patients receiving reduced-

intensity conditioning BMT. It has also been successfully used in primary and salvage therapy of acute GVHD. In myeloablative transplants, while the GVHD outcomes appear comparable, this regimen appears to have superior toxicity profile in comparison to CsA and MTX, faster hematopoietic engraftment, and reduced severity and duration of mucositis. A number of pharmacokinetics studies including our preliminary data suggest significantly lower MPA exposure in BMT patients with standard starting MMF doses used in kidney transplant recipients. Low total and unbound MPA trough concentrations are associated with higher rates of acute GVHD and graft rejection, and lower response rate in treatment of acute GVHD. MMF dose-escalation has been safely achieved by increasing the dose, shortening dose intervals, or both. However, these attempts to not consistently result in higher MPA trough concentration, especially in the immediate post-conditioning phase. There is also poor correlation between MPA trough concentration and area under the curve concentration (AUC). While most previous studies have used fixed MMF dosing, recently one study in adults has shown feasibility of AUC-based individualized MMF dosing.

This pilot study will use an AUC-based targeting of MMF for GVHD prophylaxis in pediatric patients undergoing allogeneic BMT. We propose a novel continuous infusion (CI) method for MMF administration to achieve total MPA steady state concentration of 1.7-3.3 mcg/mL. Pharmacokinetics studies will be performed on initial intermittent IV dosing, during CI, and following conversion to oral administration of this drug. Total MPA and unbound MPA levels will be measured. This work should lead to a larger-scale multi-center clinical trial to test salient findings emerging from this study in replicate studies of pediatric and adult patients undergoing allo-BMT.

A2. STUDY OBJECTIVE AND SPECIFIC AIMS

Objective: What is the overall purpose of this research study? (Limit response to 1-2 sentences.)

The purpose of this research study is to evaluate safety and feasibility of AUC-based mycophenolate mofetil targeting for GVHD prophylaxis in pediatric patients undergoing myeloablative conditioning.

Specific Aims: List the goals of the proposed study (e.g., describe the relevant hypotheses or the specific problems or issues that will be addressed by the study).

Specific aim 1 is to evaluate safety and feasibility of AUC-based mycophenolate mofetil (MMF) targeting for GVHD prophylaxis in pediatric patients undergoing myeloablative conditioning.

Specific aim 2 is to study MMF pharmacokinetics with continuous infusion and intermittent IV MMF dosing, between IV and oral MMF, and between early and late post-transplant phases.

Specific aim 3 is to describe the relationship between total and unbound MPA levels.

Specific aim 4 is to study the safety and feasibility of continuous-infusion MMF dosing.

Specific aim 5 is to measure salivary MPA and urine MPA and MPAG concentrations, and to correlate these levels with those in the plasma.

Specific aim 6 is to correlate acute and chronic GVHD, engraftment, transplant-related complications, relapse, and survival outcomes with historical controls.

A3. BACKGROUND

MMF for Treatment of Acute and Chronic GVHD

In the BMT setting, MMF has been used for primary and salvage therapy of acute and chronic GVHD. Several published studies have reported treatment responses in the range of 26-81% in acute GVHD and 26-100% in chronic GVHD (see attached file Table 1). MMF has been well-tolerated, and GI complaints, most notably diarrhea, and hematologic toxicities are common adverse effects. Several studies have reported a high incidence of infectious complications, especially in those with refractory acute or chronic GVHD [27-30]. A lower response rate for gut involvement with acute GVHD was noted by Kiehl et al, possibly related to poor absorption following oral administration of MMF and low MPA levels [31].

MMF Pharmacokinetics in HSCT

MMF use in HSCT patients has been primarily extrapolated from data in solid organ transplant recipients [24,35,36]. Standard MMF pediatric dosing for prophylaxis of renal transplant rejection is 600 mg/m2/dose twice daily; maximum dose: 1 g twice daily. MMF dose has been targeted to a total MPA AUC(0-12) of 30-60 mcg*hr/mL or total MPA Ctrough of 1-3.5 mcg/mL [37,38]. Individualization of MMF therapy has been recommended in liver transplant patients [39], and MPA target concentrations (total Ctrough >1 mcg/mL, total Css >3 mcg/ml, and cumulative 24-h unbound AUC >0.600 mcg*h/mL) have recently been proposed in the BMT population [40].

Pharmacokinetics studies suggest lower MPA exposure in BMT recipients, some as low as 30-50%, with standard starting MMF doses used in kidney transplant [24,36,41]. Jacobson and colleagues performed a prospective study in 30 adult patients undergoing nonmyeloablative bone marrow transplant who received MMF 3 g/day, half of whom were dosed at 1.5 g BID and half

of whom were dosed at 1 g TID [41]. A total MPA trough concentration ≥1 mcg/mL was achieved in 20-53% of subjects, while the Css target ≥3 mcg/mL was reached in only 13-27% of subjects. Lower MMF exposure seems to be particularly true during the early post-transplant phase, and these patients have achieved significantly higher steady state levels later in the post-transplant course. Bhatia and colleagues recently reported PK data on a MMF+TAC GVHD prophylaxis regimen in 38 pediatric patients undergoing myeloablative or reduced-intensity transplants [42]. With 900 mg/m2/dose q6h MMF dosing, mean total MPA Ctrough levels were 0.33 mcg/mL on day +1, 0.68 mcg/mL on day +7, and 0.72 mcg/mL on day +14. Furlong and colleagues found the total MPA median AUC and Cmax to be lower in the acute GVHD patients (15.8 mcg*hr/mL and 4.8 mcg/mL, respectively) when compared to those treated for chronic GVHD (49.9 mcg*hr/mL and 12.0 mcg/mL, respectively) ($P<0.0001$) [43]. Jacobson and colleagues studied pharmacokinetics in 19 children undergoing myeloablative BMT with MMF and CsA GVHD prophylaxis and found that even with 15 mg/kg IV q8 hourly dosing, the median total MPA area AUC(0-8) was 12.6 mcg*h/mL and Ctrough 0.27 mcg/mL [44].

A4. SIGNIFICANCE

MMF use in BMT patients has been primarily extrapolated from data in renal transplant recipients. However, pharmacokinetics studies suggest lower MPA exposure in BMT recipients, some as low as 30-50%, with standard starting MMF doses used in kidney transplant. Low MPA exposure has been associated with higher rates of acute GVHD and graft rejection, and lower response rates in the treatment of acute GVHD. Several strategies have been attempted in the BMT setting to achieve AUC and trough concentration targets similar to those targeted in renal transplants, but none have been successful in consistently resulting in higher MPA exposure. A novel approach to this problem is a continuous infusion for MMF administration, a method that has not been previously reported in the literature.

Performing pharmacokinetics analyses on intermittent IV dosing, during continuous infusion, and after conversion to oral administration will allow us to better understand factors correlated with low/high exposure, and its potential causes will help us optimize MMF therapy in our BMT population for GVHD prophylaxis. The information obtained from this study will also be utilized to define future studies to individualize prophylactic and/or therapeutic MMF regimens that can be used to achieve specific target concentrations in the plasma and to optimize efficacy and minimize toxicity.

A5. CONSENT FORM

TITLE: A Pilot Study of Pharmacokinetics-based Mycophenolate Mofetil Dosing for Graft-Versus-Host-Disease Prophylaxis in Pediatric Blood and Marrow Transplantation

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SOURCE OF SUPPORT: Internal (Departmental Funds)

CONSENT FOR A CHILD TO BE A SUBJECT IN A MEDICAL EXPERIMENT AND AUTHORIZATION TO PERMIT THE USE AND DISCLOSURE OF IDENTIFIABLE MEDICAL INFORMATION (PROTECTED HEALTH INFORMATION) FOR RESEARCH PURPOSES

Why Is This Study Being Done?

- Your child's doctor has recommended that your child have a blood and marrow transplant (BMT). This treatment is a way of replacing blood forming cells that were destroyed by treatment of underlying disease. These donor blood forming cells, also called stem cells, are given after chemotherapy with or without radiotherapy to help the bone marrow recover and continue to produce healthy cells. After receiving the donor stem cells, patients undergoing transplant receive medications to suppress their immune system in order to help reduce or

prevent a complication of transplant called graft-versus-host-disease (GVHD). GVHD is a disease that may occur following transplant when the donated cells attack a patient's body's cells because the donated cells see your child's body as "foreign." When GVHD occurs, donor cells can target the skin, liver, and intestines which may cause your child to develop a rash, stomach pains, vomiting, or diarrhea.

- This is a study of giving a medicine called mycophenolate mofetil (MMF) for GVHD prevention. MMF has been approved by the Food and Drug Administration (FDA) for the prevention of organ rejection and has been used in children for over 15 years. Although not FDA-approved for such purposes, it has also been successfully used for treatment and prevention of acute GVHD in adults and children undergoing BMT. MMF belongs to a group of medicines called immunosuppressive agents (drugs used to prevent the rejection of transplanted organs and tissues). It is used to lower the body's immunity in patients who receive transplants.

- In previous clinical trials, MMF has been successfully used for prevention and treatment of GVHD in children. Different patients have been found to have different blood levels of mycophenolic acid (MPA, the active form of MMF) when they are given the same dose of MMF appropriate for their body size. It is NOT the dose of MMF but the amount of MPA in the body that determines the effects and side effects of the drug. Previous clinical trials have shown that low amounts lead to more risk of GVHD. Likewise, high amounts can cause greater side effects of the drug. Other studies have shown that keeping blood MPA levels in a target range leads to better GVHD control. Most studies have given a standard dose of MMF into the vein (IV) based on a patient's size. Recently, it has been shown that MMF dosing can be adjusted to achieve a desired blood level of MPA safely, in individual patients. We would like to test a method of giving MMF by continuous IV infusion over 24 hours to achieve a target-specific amount of MPA in the blood. We will also try to apply the findings from this research to develop a better dosing guideline for MMF, improve the treatment effects of MMF, and minimize side effects.

  The use of MMF as described in this study for control of GVHD is experimental.

  The goals of this study are:

  - To study the handling of MMF by an individual subject's body and use that information to give a MMF dose in a way that is most optimal for its intended use

  - To study the factors (such as age, body weight, blood chemistry, liver function, blood levels of other drugs) that might affect blood MPA levels in different subjects

  We would also like to learn more about differences in drug blood levels between IV versus oral (by mouth) way of giving MMF, and between early and later time periods after BMT. Furthermore, we would like to try measuring MPA levels in the urine and saliva and see how they compare to MPA levels in the blood. Another goal of the study is to learn about the chance of GVHD and other transplant complications with this trial when compared with what is known from previous experience.

- ***What Is The Current Standard Of Treatment For This Disease?***

Participants on this study will receive an allogeneic BMT *(bone marrow or cord blood stem cells from another person).* BMT will be performed for a medical condition for which it is considered to be an accepted, standard treatment. This will be discussed with you, in detail, by your child's doctor. Please read the information about the BMT plan which can be found in Attachment #1, along with the side effects and risks of chemotherapy, GVHD drugs, radiation therapy and BMT which can be found in Attachment #2. As part of BMT preparation, subjects are given medicines to decrease the risk of getting GVHD. The standard treatment for GVHD prevention is to give cyclosporine and methotrexate with or without antithymocyte globulin (ATG) for bone marrow transplants and cyclosporine, prednisone and ATG for cord blood transplants. Subjects receiving umbilical cord blood transplants, mismatched transplants and unrelated marrow receive ATG as part of their GVHD prophylaxis regimen. If you have any specific concerns about any information in the attachments, please discuss with your child's physicians.

What are my child and I being asked to do?

- Your child is being asked to take part in this study which will use a medicine called mycophenolate mofetil (MMF) for GVHD prevention. The effectiveness of MMF depends on blood levels of mycophenolic acid (MPA, the active form of MMF). The purpose of this study is to give MMF to the subject based on the child's blood levels of MPA to make sure that there is the desired (target) amount of the drug in your child's body.
- Your child will receive a standard BMT with one difference. He or she will receive MMF instead of methotrexate if donor bone marrow will be used for transplant, or MMF will be used instead of prednisone if cord blood cells will be used for transplant. This study is testing if methotrexate and prednisone can be safely replaced by MMF, thereby potentially reducing side effects.

- It is common to enroll children and adolescents who need BMT in a clinical trial that seeks to improve transplant procedure. Clinical trials include only people who choose to take part. Many different treatment programs are used for BMT in children. You will have a choice between other BMT treatments and this clinical trial.
- Please take your time to make your decision. Discuss it with your friends and family. We encourage you to include your child in the discussion and decision to the extent that he or she is able to understand and take part.

How Many People Will Take Part In This Study?

This study will enroll male and female subjects between the ages of 6 months and 21 years at the Children's Hospital of Pittsburgh. The total number of subjects to be enrolled on this study is expected to be 20.

What Will Happen On This Study That Is Research?

MMF is given every 8 hours to subjects by IV over 2 hours or by mouth. Children who receive MMF as part of standard BMT treatment get periodic blood samples taken for measurement of MPA levels as part of his/her standard medical care. These blood samples are typically taken just before the subject is due to get the next dose and is called a trough level. All study samples will be collected in the Children's Hospital of Pittsburgh, in both the inpatient BMT unit and outpatient BMT clinic.

Your child will receive MMF for GVHD prevention on this study. The part that is research is made up of:

1. Giving MMF in a dose that is individualized to your child based not only on trough levels but by conducting a detailed analysis of how your child's body handles the study drug (called pharmacokinetics). This is done by taking a series of blood samples after a dose and, based on how MMF is cleared from your child's body, calculating the appropriate dose to get the desired target blood level.

Three to seven days after transplant, around a dose of IV MMF, seven blood samples (a half-teaspoon each, or 3 teaspoons total) will be collected at hours 0 (prior to infusion), 2 (at completion of infusion), 3, 4, 5, 6, and 8 from your child's central line. Each blood draw will take up to 5 minutes. Results from these studies will be used to get correct daily dose for your child to be given by continuous IV, as described in the next section.

2. Giving MMF as continuous infusion by IV over 24 hours in order to achieve constant target drug levels during the early part of transplant. During this phase, your child's blood samples will be checked two to three times a week to make adjustments to the MMF dose if necessary. Sometimes, blood samples may be taken twice in a day to check for fluctuations in the MPA drug levels. Each blood draw will take up to 5 minutes.

3. Your child will start taking MMF by mouth instead of IV when he/she is able to tolerate oral medications and is near the time of discharge. Once your child is switched to taking MMF by mouth, a second series of blood samples will be taken. Seven blood samples (a half-teaspoon each, or 3 teaspoons total) will be collected just prior to (0 hr) and at 0.5, 1, 2, 3, 6, and 8 hours after MMF is given by mouth. Results from these studies will be used to find out the correct dose by mouth for your child. Each blood draw will take up to 5 minutes.

4. In older children and adolescents who are able to spit into a sterile container, saliva samples will be collected each time blood is drawn for the pharmacokinetics studies described above in parts (1) and (3). Saliva samples will not be collected from infants, toddlers, and other subjects who are unable to provide samples on their own. MPA levels in saliva will be used to compare to MPA levels in blood. Each collection of saliva will take up to 5 minutes.

5. Also during the two pharmacokinetics studies, urine will be collected from older children and adolescents who are able to provide a voided specimen so that we may measure levels of MPA and one of its metabolites (a chemical that your body converts MPA into). The urine collection will start immediately before the first blood draw and continue for 8 hours, or until after the last blood sample is drawn. Each time your child voids, the urine will be collected in a container and stored in a refrigerator until completion of the collection period. Urine samples will not be collected from infants, toddlers, and other subjects who are unable to provide specimens on their own. Each urine collection will take up to 5 minutes.

6. Whenever possible, the blood samples drawn above will also be used to understand the metabolic effects of MMF in your child's body. This includes measuring levels of inosine

monophosphate dehydrogenase (IMPDH), a chemical in the body that is suppressed, or turned off, by MMF and can potentially serve as an alternative measure of MMF's effectiveness. Results from these tests will not be available to you or your child's doctor and will not be used to make changes to your child's management. These tests may allow us to understand the factors that affect why different subjects handle MMF differently and to explore ways that subjects can get the most benefit from the use of this drug for BMT and minimize its side effects.

In summary, subjects will start MMF therapy with intermittent IV dosing. Based on pharmacokinetics information, it will then be converted to a continuous infusion over 24 hours. Once subjects are able to tolerate oral medications, MMF will be converted to an oral formulation.

How Long Is The Study?

Subjects in this clinical trial are expected to receive the study drug for approximately 3 months. We will continue to review medical information from your child's ongoing care with regards to transplantation, status of the underlying pre-transplant disease, presence of GVHD, other post-transplant outcomes, and results of laboratory tests and other procedures for up to 5 years after the last subject starts the study.

Can my child stop being in the study?

Your child's doctor or the research doctors running the study may decide to take your child off this study under the following circumstances:

- if he/she believes that it is in your child's best interest
- if your child experiences side effects from the study procedure that are considered too severe
- if new information becomes available that shows that another treatment would be better for your child

You can stop your child from participating at any time. However, if you decide to have your child stop participating in the study, we encourage you to talk to the research doctors running the study and your child's regular doctor first.

What are the possible risks, side effects, and discomforts of this research study?

As with any experimental procedure, there may be adverse events or side effects that are currently unknown and not anticipated, and certain of these unknown risks could be permanent, severe or life threatening.

The use of MMF instead of methotrexate or prednisone may cause more complications. The use of MMF as described in this research may cause more side effects than how it is used as standard of care.

It is possible that MMF may be less effective than the current standard treatment.

Risks and side effects related to Mycophenolate Mofetil (MMF) include those which are:

Likely *("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)*	**Less Likely** *("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)*	**Rare, but Serious** *(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)*
• Diarrhea • Stomach pain • Upset stomach • Nausea and vomiting • Difficulty falling asleep or staying asleep	• Pain, especially in the back, muscles, or joints • Constipation	• Swelling of the hands, feet, ankles, or lower legs • Difficulty breathing • Shaking hands that you cannot control • Unusual bruising or bleeding • Headache • Fast heartbeat • Excessive tiredness • Dizziness • Pale skin • Weakness • Blood in stools • Bloody vomit • Loose, floppy muscles • White patches in mouth or throat • Swelling of gums • Vision changes • Rash • Low blood counts • Damage to unborn baby • Limited effectiveness of birth control • Progressive Multifocal Leukoencephalopathy, a rare, life-threatening disease that affects the central nervous system

There is also a risk of breach of confidentiality associated with collection of pharmacokinetic and medical data. However, the investigators will do their best in order to safeguard the confidentiality. Samples will be coded and no subject identifier will be recorded on the sample. Samples will be kept as long as is necessary to complete the study.

For Females:
The study drug can affect an unborn child. Your child should not become pregnant or breast feed a baby while being treated on this study. If your child is sexually active and is at risk of getting pregnant, your child and your child's male partner(s) must use an effective method to avoid pregnancy or your child must not have sex. The study doctor will talk to your child about acceptable methods to avoid pregnancy while he/she is being treated on this study. Your child will have to use the chosen method to avoid pregnancy or abstain (not have sexual intercourse) the whole time he/she is being treated on this study. Your child may need to continue this for a while, even after they finish the cancer treatment, so talk to your child's doctors about the length of time they need to avoid pregnancy or abstain. Natural family planning and the rhythm method will not be permissible means of avoiding pregnancy during study participation. If you/your child have questions about this or want to change your child's method to avoid pregnancy during therapy, please ask your child's doctor. If your child becomes pregnant during the research study, please tell the study doctor and your child's regular doctor immediately.

If your child is nursing a baby, the study drug used in this research could pass into the breast milk. Your child should not nurse a baby for the whole time she is getting the study medicines. Your child may need to continue this for a while, even after she finishes the cancer treatment, so talk to your child's doctors about the length of time she needs to avoid nursing.

For Males:
The study drug can damage sperm. Your child should not father a child while on this study drug as it may indirectly affect an unborn child. If your child is sexually active and is at risk of causing a pregnancy, your child and your child's female partner(s) must use a method to avoid pregnancy that works well or your child must not have sex. The study doctor will talk to your child about the acceptable methods to avoid pregnancy while he is being treated on this study. Your child will have to use the chosen method to avoid pregnancy or abstain (not have sexual intercourse) the whole time he is being treated on this study. Your child may need to continue this for a while, even after he finishes the cancer treatment, so talk to your child's doctors about the length of time your child needs to avoid pregnancy or abstain. Natural family planning and the rhythm method will not be permissible means of avoiding pregnancy during study participation. If you/your child have questions about this or want to change your child's method to avoid pregnancy during therapy, please ask your child's doctor. If your child's partner becomes pregnant during the research study, please tell the study doctor and your child's regular doctor immediately.

Please refer to Attachment #2 for risks and side effects associated with standard transplant procedures.

What are possible benefits from taking part in this study?

We hope that your child will get personal medical benefit from participation in this clinical trial, but we cannot be certain. These potential benefits could include having fewer side effects from GVHD prevention medications and possibly better control of GVHD.

What alternatives are available to my child if I don't give my OK for my child's participation in this study?

Instead of being in this study, your child has these options:

- Current standard therapies, with methotrexate for bone marrow transplants or prednisone for umbilical cord blood transplant, for GVHD prevention
- You may consider some other approach for GVHD prevention than the standard treatment
- You may choose a chemotherapy approach (if BMT is for a type of cancer) instead of transplantation for your child
- You may decide for your child not to receive treatment at this time

Please talk to your child's doctor about these and other options. Participation in this study is entirely up to you. Choosing not to participate in this study will not affect you or your child's present or future relationship with the Children's Hospital of Pittsburgh of UPMC or any hospital or affiliated health care providers or the University of Pittsburgh.

What if there is new information while my child is in this study?

If any information is learned that might affect your willingness to have your child continue participation in this research, you will be informed.

Will an autopsy be requested if my child dies?

Yes. The outcome of this experimental study cannot be predicted, nor can all the side effects be anticipated. If your child dies, information about the cause(s) of death and side effects is very important for future care of patients. Therefore, if your child dies, your permission for an autopsy (post-mortem examination) will be requested. Autopsies for research subjects are offered free of charge to patients who die at Children's Hospital of Pittsburgh (CHP).

May I refuse to give my OK for the use of my child's medical information for the purpose of this research study?

Your OK to use and disclose your child's medical information for the purpose of this research study is completely up to you. However, if you do not provide your OK, your child will not be allowed to participate in this study.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?

Neither you, nor your child's insurance provider, will be charged for the costs of the research procedures performed (MMF pharmacokinetics testing, urine and saliva collection, and other research tests on blood samples) for the purpose of this research study. You or your insurance company will be charged, in the standard manner, for the transplant, hospitalization and regular medical care, including the cost of MMF and the routine clinical monitoring of MMF blood levels.

Will my child be paid for participation in this research study?
You will receive no payment or money for taking part in this study.

Who will pay if my child is injured as a result of taking part in this study?
CHP researchers and their associates who provide services at CHP or UPMC recognize the importance of your child's voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that your child is injured as a result of the research procedures being performed, please contact immediately the Principal Investigator or one of the co-investigators listed on the first page of this form.

There is the possibility with any medical treatment or research that a child may suffer some physical illness or injury. In the unlikely event of an injury or illness resulting from this research, any immediate emergency treatment that may be necessary will be provided without charge at Children's Hospital of Pittsburgh. There is no plan for monetary compensation. You do not, however, waive any legal rights by signing this form.

Who will know about my child's participation in this research study?
Any information about your child obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet or in password-protected computerized records. Your child's identity on these records will be indicated by a case number rather than by his/her name and the information linking these case numbers with your child's identity will be kept separate from the research records.

Will this research study involve the use or disclosure of my child's identifiable medical information?
This research study will result in identifiable information that will be placed into your child's medical records held at CHP. The nature of the identifiable information resulting from your child's participation in this research study that will be recorded in your child's medical record includes results of the testing indicated in this consent form.

Names of participants or material identifying participants (except as described above) will not be released without written permission, unless required by law.

If results of this study are published, your child's identity will remain confidential.

Efforts will be made to keep your child's personal information confidential. We cannot guarantee absolute confidentiality. Your child's personal information may be disclosed if required by law.

It is very unlikely that the research testing might uncover important information about your child's current or future health. If this unlikely event occurs, the researchers may contact your child's doctor about what the research tests might mean. Only the doctor will be notified and the information will not become part of your child's medical record. It will remain confidential. Your child's doctor may discuss this unexpected finding with you. Your child's doctor may

recommend consultation with a genetic counselor or repeat testing in a clinical (not research) laboratory if needed. It is possible that your child's doctor may recommend that no additional action is necessary.

Who will have access to identifiable information related to my child's participation in this research study?

In general, research records are kept confidential. Paper records are stored in locked cabinets, and computerized records are password-protected. There are, however, some disclosures of your child's research-related medical information that may occur.

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your child's identifiable medical information) related to your child's participation in this research study:

- Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your child's identifiable research information (which may include your child's identifiable medical information) for the purposes of monitoring the appropriate conduct of this research study.
- Authorized representatives of the UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your child's identifiable medical information) related to your child's participation in this research study for the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (e.g., laboratory tests, diagnostic procedures) associated with research study participation; (2) addressing correct payment for tests and procedures ordered by the investigators; and/or (3) for internal hospital operations (i.e. quality assurance).
- Authorized representatives of the US Food and Drug Administration (FDA) and the Office for Human Research Protections (OHRP) may review and/or obtain your identifiable health information for the purpose of monitoring the accuracy of research data and to ensure that the research is being conducted according to the FDA regulations. While the FDA has provided its assurance that it will not release your child's identifiable medical information to anyone else, Children's Hospital of Pittsburgh cannot guarantee this.

Research investigators may be required under Pennsylvania law to report any suspicion of child abuse to child protection services. If the investigators learn that your child or someone with whom your child is involved is in serious danger of potential severe harm, they may need to warn those who are in danger and contact other agencies to ensure safety.

For how long will the investigators be permitted to use and disclose identifiable information related to my child's participation in this research study?

The investigators will be permitted to use your child's health information until the study is completed.

The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your child's identifiable medical information)

related to his/her participation in this research study for a minimum of seven years after final reporting or publication of a project.

May I have access to my medical information that results from my child's participation in this research study?
In accordance with the CHP Notices of Privacy Practices document that you have been provided, you are permitted access to information (including information resulting from your child's participation in this research study) contained within your child's medical records filed with his/her health care provider.

Is my child's participation in this research study voluntary?
Your child's participation in this research study, to include the use and disclosure of your child's identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your child's identifiable information for the purposes described above, you will not be allowed, in general, to have your child participate in the research study.) Whether or not you provide your consent for your child's participation in this research study will have no effect on your current or future relationship with the Children's Hospital of Pittsburgh. Whether or not you provide your consent for your child's participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

Your child's physician is involved as an investigator in this research study. As both your child's physician and a research investigator, he/she is interested both in your medical care and the conduct of this research study. Before agreeing to participate in this research study, or at any time during your study participation, you may discuss your child's care with another doctor who is not associated with this research study. Your child is not under any obligation to participate in any research study offered by his/her doctor.

May I withdraw, at a future date, my consent for my child's participation in this research study?
You may withdraw your consent, at any time, for your child's participation in this research study, to include the use and disclosure of your child's identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your child's identifiable medical record information for the purposes described above, your child will also be withdrawn, in general, from further participation in this research study.) Any identifiable research or medical information recorded for, or resulting from, your child's participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for your child's participation in this research study, you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for your child's participation in this research study will have no effect on your or your child's current or future relationship with the Children's Hospital

of Pittsburgh. Your decision to withdraw your consent for your child's participation in this research study will have no effect on your child's current or future medical care at a UPMC hospital or affiliated health care provider or your child's current or future relationship with a health care insurance provider.

****

VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions which I have about my child's rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668).

_____

Participant's (Child's) Printed Name

I understand that, as a minor (age less than 18 years), the above-named child is not permitted to participate in this research study without my consent. Therefore, by signing this form, I give my consent for his/her participation in this research study.

_____ _____

Parent's Name (Print) Relationship to Participant (Child)

_____ _____

Parent's Signature Date

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date

ASSENT (For children who are <u>not</u> capable of understanding the study procedures and their potential discomforts and benefits).

I do not believe the child/my child is capable of giving assent for participation.

_____

Signature of Parents(s)

ASSENT (For children who <u>are</u> capable of understanding the study procedures and their potential discomforts and benefits).

I have explained this research to the child-subject in words and pictures that he/she understands, and I believe he/she understands what this research involves.

_____

Signature of Person Explaining the Research

I believe my child understands what this research involves and that he/she has given his/her assent for participation.

_____

Signature(s) of Parent(s)

VERIFICATION OF EXPLANATION

I certify that I have carefully explained the purpose and nature of this research to (name of child) in age appropriate language. He/she has had an opportunity to discuss it with me in detail. I have answered all his/her questions and he/she provided affirmative agreement (i.e., assent) to participate in this research.

_____ _____

Principal/Co-Investigator Signature Date

CONSENT FOR CONTINUED RESEARCH PARTICIPATION

I understand that I am currently participating in a research study. I further understand that consent for my participation in this research study was initially obtained from one of my parents. I have now reached the age of 18 and I am able to provide direct consent for continued participation in this research study.

The above information has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research registry during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified individual or by the investigator(s) listed on the first page of this consent document at the telephone number(s) given. I understand that I may always request that my questions, concerns or complaints be addressed by a listed investigator. I understand that I may contact the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable. By signing this form I agree to continued participation in this research study.

By signing below, I agree to continue my participation in this research study. A copy of this consent form will be given to me.

_____ _____

Participant's Signature Date

INVESTIGATOR'S CERTIFICATION

I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this study have been explained to the above individual and that any questions about this procedure have been answered.

_____ _____

Investigator's Signature Date

A6. SAMPLE COLLECTION FORM

Sample Bu-Cy-ATG-CSA-MMF form

ABLATIVE PREP ORDERS

Allogeneic BMT using a preparative therapy of busulfan + cyclophosphamide and antithymocyte globulin.

Ht. _____ cm Wt. ____ kg BSA ____ m2

1) Begin hydration on admission (__/__/__) with D5 Sodium Chloride 0.45% + Potassium Chloride 20 mEq/L at _____ ml/hour.Total IV fluids/day = 3000 ml/m2/ day. BMT Team to enter

2) Fosphenytoin _____ mg phenytoin equivalent (PE) (15 mg PE/kg) IV on day –10 (__/__/__) at 4 PM x1, then begin Fosphenytoin _____ mg PE (2.5 mg/kg/dose) every 8 hrs at 4AM on Day -9 (__/__/__) and d/c after last dose 8AM Day -4 __/__/__. Pharmacy staff to enter.

Check Phenytoin level (troughs) on days -8 (__/__/__) and Day -6 (__/__/__)

3) Ondansetron ____ mg IV (0.15mg/kg/dose) 30 min prior to Busulfan on day –9 (__/__/__), then begin ___ mg/day (0.3 mg/kg/day) by continuous infusion at 2 ml/hr and continue daily for EHS - Pharmacy staff to enter.

4) Busulfex (busulfan) ____ mg (1.1 mg/kg/dose < 12kg, 0.8mg/kg dose > 12 kg) IV over 2 hrs every 6 hours x 4 days. Administer on 8-2-8-2 schedule beginning Day –9 __/__/__ at 0800 AM and last dose will be on __/__/__ at 0200 AM (16 doses total) Pharmacy staff to enter. – Doses diluted to final concentration 0.5 mg/ml

5) After the first dose of Busulfan is given at 0800 AM on Day –9 (__/__/__), draw 1-3 ml of blood in a green top tube (Heparin Sodium) at the times given below:

- Draw sample #1 at 1000 AM on Day –9
- Draw sample #2 at 1015 AM on Day –9
- Draw sample #3 at 1030 AM on Day –9
- Draw sample #4 at 1100 PM on Day –9
- Draw sample #5 at 1200 PM on Day –9
- Draw sample #6 at 1300 PM on Day –9
- Draw sample #7 at 1400 PM on Day –9

**Must draw sample from a different catheter
Using a permanent marker, mark each tube with the corresponding sample number and write the exact time on the form provided.

Example: Sample #3 drawn at 1030 would be marked as #3 on the tube and the time "1030" would be written on the label. If the blood is not drawn exactly on the hour or half-hour please write exactly when it is drawn.

After the tube is drawn place it immediately in the ice being stored in the utility room refrigerator

When all the samples are drawn, after 1400 PM, please page Jason at 8288.

6) Cyclophosphamide _____ mg (50 mg/kg/dose) + Mesna ____ mg (15 mg/kg) in D5%W Sodium Chloride 0.45% to final volume ____ ml (200 ml/m2) IV over 1 hour beginning at 10 AM daily on days -5, -4, -3, and -2 (__/__/__, __/__/__, __/__/__). Pharmacy staff to enter.

Cyclophosphamide Monitoring

- I/O's every 4 hours.
- Dip all urine for specific gravity and blood.
- Maintain urine output at least ___ml/hour (2 ml/kg/hour) post-cytoxan with patient urinating every 2-3 hours.
- If urine output < ___ml/hour (2 ml/kg/hour) and any of the following (O>I by 30%; weight decreased; SG ≥ 1.015), then increase IV fluids to ___ ml/hr (by 25%) and reassess.
- If evidence of fluid overload (periorbital or peripheral edema, rales, etc) or I>O by 30% over 8 hours, obtain serum sodium and contact BMT attending to consider Lasix ___ mg (0.5 mg/kg/dose, 40 mg single max dose). BMT team to enter

Do not decrease intravenous hydration without prior approval from BMT Attending.

7) Furosemide ___ mg (0.5 mg/kg/dose, 40 mg single max dose) IV daily at Hours 2 and 6 hrs after each Cytoxan dose daily on days -5, -4, -3, and -2 (__/__/__, __/__/__, __/__/__, and __/__/__). Pharmacy staff to enter.

8) Mesna ____ mg (15 mg/kg) IV over 15 minutes diluted in hydration fluids at Hours 3, 6, 9, and 12 hours after each Cyclophosphamide dose daily on days -5, -4, -3, and -2 (__/__/__, __/__/__, __/__/__, and __/__/__). Pharmacy staff to enter.

9) Methylprednisolone ____mg (0.5mg/kg/dose) IV every 6 hours (2mg/kg/day) with 1st dose 12 hours before Atgam, on day –5 (__/__/__) at 10 PM. Continue every six hours until __/__/__ at 4 am, Day -1. Pharmacy Staff to enter

10) Patient will receive Anti-thymocyte Globulin – Equine (ATGAM®)

Anti-thymocyte Globulin (Equine) (ATGAM®) ____ mg (15 mg/kg/dose; 30 mg/kg/day) IV in Sodium Chloride 0.9% to final concentration 4mg/ml (minimum 120ml) IV over 4 hours every 12 hrs x 6 total doses over 4 hours. Pharmacy staff to enter

Begin at 11 AM on Day –4 (__/__/__) and continue through Day -2 (__/__/__)with last dose __/__/__ 11 PM.

Administer Anti-thymocyte Globulin (ATGAM®) intradermal skin test at 9 AM on Day – 4 (__/__/__) prior to first dose):
0.1 ml. intradermal injection of 1:1000 ATGAM® 0.1 ml Sodium Chloride 0.9% intradermal injection. Pharmacy staff to enter and prepare

DO NOT give diphenhydramine within 72 hours of skin test.
Vital signs every 15 minutes for the first hour and then every hour during infusion.

11) Premedication prior to each Anti-thymocyte Globulin ATGAM® dose every 12 hrs x 6 total doses on Days -4 through Day -2:
a.) Acetaminophen ____ mg (15 mg/kg/dose, max single dose 650mg) PO one hour prior, then every 4 hours as needed for fever >38.5 C. Pharmacy Staff to enter

b.) Diphenhydramine ____ mg (0.5 mg/kg/dose, max single dose 50mg) IV 30 minutes pre-Anti-thymocyte Globulin, then every 6 hours as needed for rash. Pharmacy Staff to enter

12) On day -1 (__/__/__) at 10 AM, decrease hydration fluid rate to ___ ml/hr (85 ml/m2/hr). Total IV fluids should equal 2000 ml/m2/day. BMT team to enter

13) Cyclosporine A ____ mg/day(5 mg/kg/day – dose reduced to 1.65 mg/kg/day due to concurrent voriconazole) IV in D5%W to final volume 48 ml IV at 2 ml/hour beginning on day -2 (__/__/__) at 6 AM, for EHS. Infuse via the same lumen each day. Cyclosporine should always be infused through one dedicated line (Central of Peripheral). Do not switch to another central venous catheter lumen (Broviac, Mediport, PICC or other types of catheter) without prior approval from the medical team. Pharmacy staff to enter.

14) Cyclosporine level at 6 AM Day 0 (__/__/__) and every Monday and Thursday. Draw level via opposite broviac from dose infusion. BMT team to enter.

15) Filgrastim ____ mcg (5 mcg/kg/day; dose rounded to nearest 15 mcg) IV over 1 hour daily. Give the first dose 24 hours after the infusion of the stem cells. Pharmacy staff to enter.

16) MYCOPHENOLATE MOFETIL (15mg/kg/dose) ____ mg IV given as a 2 hour infusion three times a day started Day 0 (__/__/__) at 8 AM

MPA Pharmacokinetic Studies will be performed around the 1600 (4PM) IV dose of Mycophenolate Mofetil on Day +3 (__/__/__).
For Intravenous dose: Blood work will be drawn before the dose and then at 120, 180, 240, 300, 360 and 480 minutes after the start of the infusion. 2 ml of blood will be drawn into green top Vacutainers.

- Draw sample #1 at 1545 AM on Day +3
- Draw sample #2 at 1800 AM on Day +3
- Draw sample #3 at 1900 PM on Day +3
- Draw sample #4 at 2000 PM on Day +3
- Draw sample #5 at 2100 PM on Day +3

- Draw sample #6 at 2200 PM on Day +3
- Draw sample #7 at 2400 PM on Day +3

- All MPA levels will be sent to the lab at the time of blood draw. Do not hold the samples on the unit.
- Cellcept (MMF) should always be infused through one dedicated line (Central of Peripheral). Do not switch to another central venous catheter lumen (Broviac, Mediport, PICC or other types of catheter) without prior approval from the medical team.

[PRO15080386]

APPENDIX B

B1. ABSTRACT

Lung transplantation is a viable treatment option for multiple end-stage pulmonary disorders including cystic fibrosis, COPD, and idiopathic pulmonary fibrosis. Although lung transplantation improves the quality of life and short-term survival of the patient, long-term survival remains poor, with only 55% of patients surviving 5 years post-transplantation. Acute cellular rejection (ACR) is a common complication post-lung transplantation, with more than one-third of patients being treated for acute rejection within one year of transplantation. Furthermore, ACR appears to be a risk factor for the development of bronchiolitis obliterans syndrome (BOS), which is the primary cause of mortality in lung transplant recipients at 5 years. Longer and recurrent episodes of ACR also appear to have a stronger correlation with BOS. ACR is a direct result of inadequate immunosuppression. There are also complications in lung transplant patients that are a result of over-immunosuppression including increased rate of infections and hypogammaglobulinemia. Both of these complications put lung transplant patients at an increased risk of mortality.

Mycophenolate mofetil (MMF), a prodrug of mycophenolic acid (MPA), is commonly used in combination with the calcineurin inhibitors, cyclosporine and tacrolimus, to prevent ACR and to prevent BOS progression. Due to the large variability in the pharmacokinetics of these immunosuppressive drugs, dosing of calcineurin inhibitors (CNIs) are individualized to attain a certain range of trough concentrations, that is known to correlate with drug exposure as measured by the area under the plasma concentration vs time curve (AUC). However, MMF is given at a fixed dose and rotuine monitoring of trough level is not performed as it does not correlate with AUC. Studies in kidney and heart transplant recipients have clearly demonstrated

improved clinical outcomes when the drug exposure as measured AUC (0-12) of MPA falls within the range of 30–60 mg*h/L. We predict that lower MPA exposure (AUC), in the absence of TDM and concurrent increase in the IMPDH activity could be the cause for the high incidence of acute clinical rejection in adult lung transplant recipients. Our long term goal is to optimize MMF dosing based on drug exposure, in order to decrease incidence of ACR in lung transplant patients. Measuring the AUC of MPA is often cumbersome and expensive to perform clinically. Recent studies in kidney transplant and bone marrow patients have focused on the use of limited sampling strategies (LSSs) as an indirect measure of MPA exposure that have the potential to improve and individualize MPA dosing. We hypothesize that LSS can be used as a surrogate marker for drug exposure (AUC) and used for optimizing the dose of MMF.The limited sampling strategy (LSS) and IMPDH measurement could provide a more feasible way to measure mycophenolate activity within lung transplant recipients in order to optimize therapy. The LSS is considered "limited sampling" because it uses less blood draws than the full pharmacokinetic profile. The LSS will use utilize up to 5 blood draws over 4-6 hours whereas the full pharmacokinetic profile utilizes 9 samples over 12 hours. The LSS will be correlated to the full PK profile to provide a more feasible way of measuring mycophenolate within the body.

Mycophenolic acid acts by inhibiting ionosine monophosphate dehydrogenase (IMPDH) in PBMC. The biological response to MPA can therefore be measured by the inhibition of IMPDH activity. We hypothesize that monitoring IMPDH activity can also help in optimizing MMF dosing and minimize ACR burden, BOS progression, hypogammaglobulinemia, and infection episodes in lung transplant patients.

In this pilot study we will identify the optimal sampling time for LSS and evaluate the relationship between MPA exposure and IMPDH activity in lung transplant patients. Subjects will participate in three studies. Study 1 will be performed between days 7-21 post transplantation. Study 2 will be performed around 3 months post transplantation. Study 3 will be performed between months 6-12. Multiple blood samples will be collected from study subjects over a dosing interval and MPA levels will be measured in plasma and IMPDH activity will be measured in PBMC. Results of this pilot study will provide data for a future study of a prospective individualized MMF dosing based on LSS and IMPDH activity in order to minimize ACR.

B2. OBJECTIVE AND SPECIFIC AIMS

Objective: What is the overall purpose of this research study? (Limit response to 1-2 sentences.)
The objective of this study is to establish an optimal limited sampling strategy (LSS) for mycophenolic acid (MPA) in lung transplant patients by characterizing the pharmacokinetics (PK) over a dosing interval and to identify the relationship between MPA exposure and its pharmacological activity (inosine monophosphate dehydrogenase-IMPDH activity) in order to optimize MMF dosing regimen in adult lung transplant recipients. The LSS and IMPDH measurement could provide a more feasible way to measure mycophenolate activity within lung transplant recipients in order to optimize therapy.

Specific Aims: List the goals of the proposed study (e.g., describe the relevant hypotheses or the specific problems or issues that will be addressed by the study).

Aim 1:

To establish a limited sampling strategy to estimate MPA exposure using a minimal number of blood samples collected during a dosing interval in adult lung transplant patients. This will be accomplished by evaluating the pharmacokinetics of MPA over a dosing interval in an adult cohort of lung transplant recipients.

Aim 2:

To determine the activity of MPA by measuring the ex-vivo IMPDH activity in peripheral blood mononuclear cells. IMPDH activity measurements will be obtained pre-transplant (baseline), pre dose, and at 5 additional time points during the full PK profile.

Aim 3:

To characterize the relationship between MPA exposure and IMPDH activity over time in lung transplant patients.

B3. BACKGROUND

Lung Transplantation and Immunosuppression:

Lung transplantation is the treatment of choice for patients with end stage lung diseases including cystic fibrosis. Since the early 1990s, more than 25,000 lung transplants have been performed at centers around the world (1). Maintenance immunosuppressive therapy is administered to all lung transplant recipients to help prevent acute and chronic rejection and the loss of the lung allograft. Calcineurin inhibitors (cyclosporine and tacrolimus) form the backbone of the immunosuppressive regimen. The dose of cyclosporine is targeted to maintain a trough levels of 250 to 300 ng/mL during the initial post-transplant year and at 200 to 300 ng/mL in the later phase. Ninety % of the the lung transplant recipients in the United States are on tacrolimus and are dosed to target a trough level of 5-15ng/ml (2). MPA a selective, reversible, and noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH) that acts by blocking the de novo synthesis of lymphocytes and inhibiting proliferation of activated T and B lymphocytes is also used in most transplant patients. MPA is administered as its prodrug MMF. Approximately 80 percent of lung transplant recipients in the United States receive MMF as a core constituent of their maintenance immunosuppression (2). MMF has replaced azathioprine in cyclosporine or tacrolimus-based anti-rejection regimens in renal and heart transplant recipients. At UPMC Presbyterian Hospital, lung transplant recipients undergo induction immunosuppression with alemtuzumab or basiliximab and are maintained on maintenance immunosuppression thereafter. The preferred maintenance immunosuppression regimen at UPMC consists of tacrolimus, steroids, and mycophenolate mofetil.

MPA pharmacokinetics and Pharmacodynamics in Lung Transplant Patients:

To date only a few studies have characterized the pharmacokinetics of MPA in lung transplant population. Studies conducted in lung and heart transplant recipients have shown significantly lower levels of MPA in lung transplants. However, no prospective study has been conducted to evaluate the impact of baseline IMPDH activity and IMPDH activity during a dosing interval in lung transplant recipients to guide MMF therapy. We predict that baseline IMPDH can serve as a good initial marker to guide the AUC based MMF therapy in adult lung transplant recipients and may serve as an initial marker to guide the exposure and target MPA exposure. (13-14)

Effect of Pharmacogenetics on Mycophenolate Mofetil therapy:

Genes encoding for uridine diphosphate glucuronosyltransferase, organic anion transporting polypeptides, MRP-2 and inosine-5-monophosphate dehydrogenase have shown single nucleotide polymorphisms (SNPs). Several studies conducted in solid organ transplant recipients have demonstrated large inter-individual variability. Such studies have prompted interest in studying the pharmacegentic influence on MMF-based immunosuppression. Prospective studies conducted in solid organ transplant recipients have shown a potential contribution of genetic variants in the UGT1A9, UGT2B7, IMPDH II and MRP2 on pharmacokinetics of MPA (15-19).

PK/PD-Based MMF Targeting in lung transplant patients:

Based on the limited published pharmacokinetic data and paucity of any PD data, this study would examine the feasibility of a limited sampling based PK-PD based MMF targeting in adult lung transplant recipients.

B4. SIGNIFICANCE

Therapeutic drug monitoring has become very crucial to optimal dosing of immunosuppressants. CNIs that form the backbone of immunosuppressive therapy in solid organ transplant recipients are routinely monitored and the doses are titrated to target a trough level. MMF although used extensively in solid organ transplant recipients is however not routinely monitored. This is primarily because MPA trough levels are poorly correlated with the total exposure (AUC). Studies in kidney and heart transplant recipients have demonstrated improved clinical outcomes when the measured AUC (0-12) of MPA falls within the range of 30 – 60 mg*h/L and is given with cyclosporine and steroids. Measurement of 12 hour AUC is impractical in standard clinical practice settings. An alternate simpler approach to measure drug exposure is very essential to optimize MMF therapy.

A fixed dose MMF is used in lung transplant recipients and it is similar to the dosing used in renal and heart transplant recipients. However, lung transplant patients have shown a different pharmacokinetic profile as compared to renal transplant recipients. Studies suggest a lower MPA exposure in lung transplant recipients. A better understanding of the pharmacokinetics of MPA is necessary to optimize MMF therapy.

More recent studies have focused on limited sampling strategies (LSSs) that have the potential to improve MMF monitoring. LSS represents total exposure to MPA by measuring concentrations at fewer time points. There is a scarcity of PK studies in lung transplant recipients let alone an individualized MMF dosing study based on limited sampling strategy. Also there have been no reports of IMPDH monitoring in lung transplant recipients. We predict that sub-therapeutic levels on MMF in the absence of TDM and concurrent increase in the IMPDH activity could be the cause for high incidence of acute clinical rejection in adult lung transplant recipients.

We hypothesize that individualized dosing of MMF based on MPA exposure by LSS and monitoring IMPDH activity will reduce ACR burden, BOS progression, hypogammaglobulinemia, and infection episodes in lung transplant patients. This pilot study will establish the basis for future studied to be performed in lung transplant recipients with PK-PD guided MMF dosing.

B5. CONSENT FORM

TITLE: Limited sampling strategy for predicting exposure and efficacy of mycophenolic acid in adult lung transplant recipients

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Montefiore Hospital, 3459 Fifth Avenue, Pittsburgh, PA 15213

SOURCE OF SUPPORT: Clinical Pharmacokinetics Laboratory Funds, University of Pittsburgh School of Pharmacy

Why is this research being done?

After lung transplant you will receive a drug called mycophenolate mofetil to prevent rejection of the lungs. The primary objective of this study is to understand how mycophenolate mofetil is handled by the body after lung transplantation, and to develop a simple strategy to measure the blood level of mycophenolate mofetil in lung transplant patients that may be more useful in preventing rejection. The secondary objective of this study is to determine the effect of monitoring blood levels of this drug in lung transplant patients. The results of this study may provide valuable information to clinicians on how to more effectively use this drug and minimize rejection of the transplanted lungs.

Who is being asked to take part in this research study?

You are being invited to take part in this research study because you are between the ages of 18 and 65 and will be receiving a lung transplant. You will participate in this study only if you receive mycophenolate mofetil as part of your clinical treatment as a medication to prevent rejection of your transplanted lungs. This study will take place at the University of Pittsburgh Medical Center, Pittsburgh, PA, and will include approximately 20 participants.

How will the study be done?

No medications will be administered for the purposes of this study. You will participate in this study only if you are given mycophenolate mofetil as part of your standard of care, post-transplant course. If you decide to participate in this study, you will undergo a screening procedure and participate in the study at four time points (before your transplant, 7-upto 2 month following transplantation, around 3 months, and around 6 to 12 months following transplantation). The duration of the study will be five minutes before transplant, 12-13 hours at the 7- upto 2 month study period, and not more than 6 hours at the 3 and 6-12 months study period. The entire duration of your participation would be about a year following your transplantation. We will collect information on your medical and surgical history from your hospital, as well as surgical and clinic records during your study participation, and for up to 5 years after transplantation. We will also be testing left over blood samples for certain biochemicals from routine clinical testing samples that were taken from the time of your transplant. Information collected will be confidentially held and used only for the purposes of the research study.

Screening

Screening is required to determine if you are qualified to take part in this clinical study. This will be done following your selection as a recipient for lung transplantation. To determine if you meet the criteria for participation in this study, your doctor will review and collect information about your medical history including but not limited to your age, gender, weight, height, medical history, as well as clinical laboratory test results indicative of your liver and kidney function. If you are a woman of child-bearing age, pregnancy testing is normally performed as part of the standard of care. Once you meet all the study participation criteria, you will be approached by

the investigators to determine your interest in participating in this research study. If you express interest, the study will be explained to you by one of the coordinators and you will be allowed sufficient time to read and understand the consent form and sign the consent. No study procedures will be performed until after you sign the consent.

Pre-transplant
Prior to your transplant, 15 mL (one tablespoon) of blood will be drawn for genetic testing related to the proteins in your body that handle mycophenolate mofetil and mediate its effect. Genetic testing is inherited information (a blueprint) about the structure and functions of cells in the human body that make up the color of our hair and eyes and may influence the way our bodies respond to certain stimuli such as an illness or infections. This activity will take about 5 minutes.

Early Post-transplant study: 7-upto 2 month following transplantation

- Blood samples (4 ml or approximately 1 teaspoon) will be collected before your dose of regularly scheduled mycophenolate mofetil and around 30 minutes, 1 hour, 1.5 hours, 2 hours, 4 hours, 6 hours, 9 hours, and 12 hours after taking the medicine. In addition, there will also be 10 ml (2 teaspoons) of blood drawn at no more than 5 time points during the same time period. A total of 86 ml (6 tablespoons) of blood will be collected over a 12 hour time period.
- This activity will take about 12–13 hours.
- Blood samples taken during this part of the study will be drawn from an existing intravenous line

Post-transplant: 3 months and 6-12 months Post-transplantation
This study will take place around your 3-month and 6-12 months follow-up visits. These visits will occur at the time of the routine bronchoscopy, lavage, and biopsy and will last approximately 4-6 hours. On this day the transplant clinical team will perform routine work-up. You will be asked to hold your morning dose of mycophenolate prior to coming to clinic. Please bring your dose of mycophenolate to clinic with you.

- You will be asked to take your morning dose of mycophenolate in the clinic. Please bring this dose with you to clinic.
- Blood samples (14 ml or 1 tablespoon) will be collected before you take the dose of mycophenolate mofetil and 4 more times after your mycophenolate dose. A total of 70 mL (approximately 5 tablespoons) of blood will be taken
- Blood samples taken during this part of the study will also be drawn from an existing intravenous line

The total volume of blood that will be drawn for the entire study will be 241 mL (approximately 16 tablespoons).

Biological Samples for Future Research
All biological samples already collected during your clinical visits may also be used for future testing related to this study or use of medications in lung transplant patients. The samples that will be collected in this study include blood, plasma, and a type of cell within the blood known as peripheral blood mononuclear cells (PBMCs). The samples will be stored indefinitely in the

Clinical Pharmacokinetics Laboratory at the University of Pittsburgh under the direct supervision of the primary investigator Dr. Raman Venkataramanan. Only members of the research team or laboratory personnel conducting the laboratory tests will have access to the samples. Samples will be labeled with the numerical code assigned to the subject in order to correlate with clinical data obtained during the study and only the study team members will know to whom the sample belongs. You will not be notified about pending results on these tests, as they have no bearing on your medical management. These samples may be shared with other investigators who are interested in transplant, but none of the samples shared will contain your identifiers.

What are the possible risks, side effects, and discomforts of this research study?
There may be certain risks associated with participation in this study. These may include the following:

Blood Draws or Intravenous Line Insertion:
Bruising, bleeding, fainting, swelling, pain and, rarely, infection

Breach of Confidentiality
There is also a remote risk associated with breach of confidentiality related to genetic testing. There is a possibility that, if the results of the research studies involving your biologic materials were to become generally known, this information could impact future insurability, employability, or reproduction plans, or have a negative impact on family relationships, and/or result in stigmatization. To minimize this risk, any information about you obtained from this research will be kept as confidential (private) as possible. All records or samples related to your involvement in this research study will be stored in a secure, double-locked area or password-protected computer database that is accessible only by members of the research team. Your identity on these records and samples will be indicated by your subject identification number rather than by your name, and the information linking your subject identification number with your identity will be kept separate from the research records

What are possible benefits from taking part in this study?
There is no direct benefit from participating in this study. However, the information learned from this study may help others who undergo transplantation in the future.

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?
You will be promptly notified if, during the conduct of this research study, any new information develops which may cause you to change your mind about continuing to participate in this study.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?
Some of the services you will receive during this time are “research only services” that are being done only because you are in the study. These services will be paid for by the study and will not be billed to your health insurance company or you. Examples are certain blood draws, biochemical measurements, and genetic testing.

Some of the procedures that you will undergo during this study are considered to be "routine clinical services" that you would have even if you were not in the study. Examples are the actual lung transplant surgery, other transplant-related tests, use of immunosuppressive medications and routine care medications, hospitalization and all associated costs. These services will be billed to your health insurance company or you, if you do not have health insurance.

Will I be paid if I take part in this research study?
You will not receive any payment for taking part in this clinical research study. Your biological sample or genetic material may lead, in the future, to new inventions or products. If the research investigators are able to develop new products from the use of your biological sample or genetic material, there are currently no plans to share with you any money or other rewards that may result from the development of the new product.

Who will pay if I am injured as a result of taking part in this study?
University of Pittsburgh investigators and their associates who provide services at UPMC recognize the importance of your voluntary participation in their research studies. These individuals and their staff will make every reasonable effort to minimize, control and treat any injuries that may arise as a result of this research. If you believe that you are injured as the result of the research procedures being performed, please contact the principal investigator or one of the investigators listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of UPMC. Your insurance provider may be billed for the costs of this emergency treatment, but none of those costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care. At this time, there is no plan for any additional financial compensation.

Who will know about my participation in this research study?
Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results.

Will this research study involve the use or disclosure of my identifiable medical information?
This research study will involve the recording of past, current and/or future identifiable (pertaining to only you) medical information from your hospital and/or other (e.g., physician office) records. The information that will be recorded will be limited to information concerning your transplant (e.g., diagnostic information, lab results, medications, medical history). This information will be used to determine your eligibility for this study and to follow your response once you are enrolled in the study.

This research study will not result in identifiable information that will be placed into your medical records held at UPMC

Who will have access to identifiable information related to my participation in this research study?

In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information (which may include your identifiable medical information) for the purpose of monitoring the appropriate conduct of this research study.

Authorized representatives from the Food and Drug Administration may review and or obtain your identifiable (which may include your identifiable medical information) related to your participation in this research study for the purposes of monitoring the accuracy and completeness of the research data. While the U.S. Food and Drug Administration understands the importance of maintaining the confidentiality of your identifiable research and medical information, the UPMC and University of Pittsburgh cannot guarantee the confidentiality of this information after it has been obtained by the U.S. Food and Drug Administration.

Authorized representatives of UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of (1) fulfilling orders, made by the investigators, for hospital and health care services (e.g., laboratory tests, diagnostic procedures) associated with research study participation; (2) addressing correct payment for tests and procedures ordered by the investigators; and/or (3) for internal hospital operations (i.e. quality assurance).

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

Records of your participation in this study will be held confidential except as disclosure is required by law or as described in this informed consent document (under "Confidentiality" or "Authorization to Use and Disclose Protected Health Information"). The study doctor, the sponsor or persons working on behalf of the sponsor, and under certain circumstances, the United States Food and Drug Administration (FDA) will be able to inspect and copy confidential study-related records, which identify you by name. Therefore, absolute confidentiality cannot be guaranteed. If the results of this study are published or presented at meetings, you will not be identified.

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?
The investigators may continue to use and disclose, for the purposes described above, identifiable information (which may include your identifiable medical information) related to your participation in this research study for a minimum of seven years after final completion or publication of the project and for as long (indefinite) as it may take to complete this research study.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov as required by US Law. This website will not identify you. At most the website will include a summary of the results. You can search this site at any time.

Is my participation in this research study voluntary?
Your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above, is completely voluntary. (Note, however, that if you do not provide your consent for the use and disclosure of your identifiable information for the purposes described above, you will not be allowed to participate in the research study). Whether or not you provide your consent for participation in this research study will have no effect on your current and future care at a University or Pittsburgh or UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider. If you choose not to participate in this study, it will not impact your care or your chances of receiving a lung transplant.

Your doctor may be an investigator in this research study, and as an investigator, is interested both in your medical care and in the conduct of this research. Before entering this study or at any time during the research, you may discuss your care with another doctor who is in no way associated with this research project. You are not under any obligation to participate in any research study offered by your doctor.

May I withdraw, at a future date, my consent for participation in this research study?
You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable medical record information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study). Any identifiable research or medical information recorded for, or resulting from, your participation in this research study or blood samples collected prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

If I agree to take part in this research study, can I be removed from the study without my consent?

It is possible that you may be removed from the research study by the researchers if you experience unexpected conditions and in the opinion of the investigators that it is in your best interest. If your mycophenolate mofetil is stopped at the recommendation of your physician, you will also be removed from the study. If this happens, your data may still be kept and used depending when your mycophenolate mofetil is discontinued.

VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions which I have about my rights as a research participant will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668). By signing this form I consent to participate in this research study and provide my authorization to share my medical records with the research team. A copy of this consent form will be given to me.

_____ _____

Participant's Signature Date/Time

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date/Time