# GENETIC POLYMORPHISMS ON PHARMACOKINETICS AND PHARMACODYNAMICS OF MYCOPHENOLATE MOFETIL IN THAI KIDNEY TRANSPLANT PATIENTS

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A Dissertation Submitted in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy Program in Pharmaceutical Care

Department of Pharmacy Practice

Faculty of Pharmaceutical sciences

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# ภาวะพหุสัณฐานของยีนต่อเภสัชจลนศาสตร์และเภสัชพลศาสตร์ของยาไมโคฟีโนเลตโมฟีติลในผู้ป่วยไทยที่ได้รับการปลูกถ่ายไต

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
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| Thesis Title | GENETIC POLYMORPHISMS ON PHARMACOKINETICS AND PHARMACODYNAMICS OF MYCOPHENOLATE MOFETIL IN THAI KIDNEY TRANSPLANT PATIENTS |
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วนรัตน์ อนุสรณ์เสงี่ยม: ภาวะพหุสัณฐานของยีนต่อเภสัชจลนศาสตร์และเภสัชพลศาสตร์ของยาไมโคฟีโนเลตโมฟีติลในผู้ป่วยไทยที่ได้รับการปลูกถ่ายไต. (GENETIC POLYMORPHISMS ON PHARMACOKINETICS AND PHARMACODYNAMICS OF MYCOPHENOLATE MOFETIL IN THAI KIDNEY TRANSPLANT PATIENT) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร.ดวงจิตต์ พนมวัน ณ อยุธยา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.นพ.เกื้อเกียรติ ประดิษฐ์พรศิลป์, 107 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของภาวะพหุสัณฐานของยีนต่อเภสัชจลนศาสตร์และเภสัชพลศาสตร์ของยาไมโคฟีโนเลตโมฟีติลโดยพิจารณาค่าพารามิเตอร์ทางเภสัชจลนศาสตร์และเภสัชพลศาสตร์ในผู้ป่วยไทยที่ได้รับการปลูกถ่ายไตจำนวน 118 ราย ผู้ป่วยมีอายุเฉลี่ย 45 ปี ส่วนใหญ่ได้รับยาไซโคลสปอรินและทาโครลิมุสร่วมด้วย ขนาดยาเฉลี่ยของไมโคฟีโนเลตโมฟีติลที่ผู้ป่วยได้รับต่อวันเท่ากับ 1211.86±339.10 มิลลิกรัม (พิสัย 500-2000 มิลลิกรัม) ผลการศึกษาพบความถี่ของอัลลีลที่มีภาวะพหุสัณฐานของยีน UGT1A9*1b, UGT1A9-*688T>G*, UGT2B7 *802C>T*, MRP-2 *-24C>T*, IMPDH1 *125G>A*, IMPDH1 *-106G>A* และ IMPDH2 *3757T>C* เท่ากับ 0.49, 0.12, 0.27, 0.23, 0.41, 0.58 และ 0.01 ตามลำดับ ผู้ป่วยจำนวน 78 ราย (ร้อยละ 66.10) มีค่าทำนายพื้นที่ใต้กราฟเวลา 0-12 ชั่วโมงของไมโคฟีโนเลตแอซิด (MPA) อยู่ในช่วงเป้าหมาย (ค่าเฉลี่ย 46.59±8.08 มิลลิกรัม x ชั่วโมง/ลิตร) เมื่อพิจารณาความสัมพันธ์ของภาวะพหุสัณฐานของยีนกับค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ พบว่าผู้ที่มีภาวะพหุสัณฐานของยีน MRP-2 *-24C>T* จะมีค่าพื้นที่ใต้กราฟเวลา 0-12 ชั่วโมงของ MPA น้อยกว่าผู้ที่มียีนปกติอย่างมีนัยสำคัญทางสถิติ (5.044 และ 5.921 มิลลิกรัม x ชั่วโมง/ลิตร/มิลลิกรัม/กิโลกรัม ตามลำดับ, *p*-value = 0.008) ทั้งนี้ยังพบว่าค่าการกำจัดยาในผู้ที่มีภาวะพหุสัณฐานของยีน MRP-2 *-24C>T* จะสูงกว่าค่าการกำจัดยาในผู้ที่มียีนปกติ (0.150 และ 0.120 ลิตร/ชั่วโมง/กิโลกรัม ตามลำดับ, *p*-value = 0.025) แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญของค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ในผู้ที่มีภาวะพหุสัณฐานของยีน UGT1A9*1b, UGT1A9 *-688T>G* และ UGT2B7 *802C>T* ส่วนค่าพารามิเตอร์ทางเภสัชพลศาสตร์นั้นพิจารณาค่า IMPDH activity ซึ่งพบว่า ผู้ที่มีภาวะพหุสัณฐานของยีน IMPDH1 *125G>A* จะมีค่า IMPDH activity ณ เวลาก่อนรับประทานยาและ 2 ชั่วโมงหลังรับประทานยา MMF มื้อเช้าสูงกว่าผู้ที่มียีนปกติอย่างมีนัยสำคัญทางสถิติ ส่วนภาวะพหุสัณฐานของยีน IMPDH1 *-106G>A* และ IMPDH2 *3757T>C* นั้นไม่พบความแตกต่างของค่า IMPDH activity เมื่อเปรียบเทียบกับผู้ที่มียีนปกติ โดยสรุปการศึกษานี้พบผลของภาวะพหุสัณฐานของยีน MRP-2 ต่อเภสัชจลนศาสตร์และผลของภาวะพหุสัณฐานของยีน IMPDH1 ต่อเภสัชพลศาสตร์ของยาไมโคฟีโนเลตโมฟีติลซึ่งอาจเป็นปัจจัยหนึ่งที่ใช้ในการพิจารณาขนาดยาและติดตามผลการรักษาของยานี้ในผู้ป่วยไทยที่ได้รับการปลูกถ่ายไต

ภาควิชา เภสัชกรรมปฏิบัติ ลายมือชื่อนิสิต.....

สาขาวิชา การบริบาลทางเภสัชกรรม ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา 2555 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KEYWORDS : GENETIC POLYMORPHISMS/ PHARMACOKINETICS/ PHARMACODYNAMICS/ MYCOPHENOLATE MOFETIL/ THAI KIDNEY TRANSPLANT PATIENT

WANARAT ANUSORNSANGIAM: GENETIC POLYMORPHISMS ON PHARMACOKINETICS AND PHARMACODYNAMICS OF MYCOPHENOLATE MOFETIL IN THAI KIDNEY TRANSPLANT PATIENTS. ADVISOR: ASSOC.PROF.DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D., CO-ADVISOR: ASSOC.PROF.KEARKIAT PRADITPORNSILPA, M.D., 107 pp.

The objectives of this study were to investigate the influence of genetic polymorphisms on pharmacokinetics and pharmacodynamics of mycophenolate mofetil in 118 Thai kidney transplant patients. Mean age was 45 years old and most patients received concomitant cyclosporine or tacrolimus therapy. Mean daily dose of mycophenolate mofetil was 1211.86±339.10 mg (range 500-2000 mg). Allele frequencies of UGT1A9*1b, UGT1A9 *-688T>G*, UGT2B7 *802C>T*, MRP-2 *-24C>T*, IMPDH1 *125G>A*, IMPDH1 *-106G>A* and IMPDH2 *3757T>C* were 0.49, 0.12, 0.27, 0.23, 0.41, 0.58 and 0.01, respectively. Seventy eight patients (66.10%) had predicted $AUC_{0\text{-}12\ hr}$ of mycophenolic acid (MPA) within target range (mean 46.59±8.08 mg x h/L). When considering the association of gene polymorphisms and pharmacokinetic parameters, patients with MRP-2 *-24C>T* variant had a significantly lower predicted MPA $AUC_{0\text{-}12\ hr}$ than patients with wild-type gene (5.044 and 5.921 mg x h/L/mg/kg, respectively, *p*-value = 0.008). In addition, the oral clearance of MPA in patients with MRP-2 *-24C>T* variant was significantly higher than the oral clearance of MPA in patients with wild-type gene (0.150 and 0.120 L/h/kg, respectively, p-value = 0.025). There were no significantly differences of pharmacokinetic parameters among patients with different polymorphisms of UGT1A9*1b, UGT1A9 *-688T>G* and UGT2B7 *802C>T* SNPs. For pharmacodynamic parameters, IMPDH activity was determined. Patients with IMPDH1 *125G>A* variant had a significantly higher IMPDH activity at predose and 2 hour after morning MMF dose than patients with wild-type gene. No differences of IMPDH activity was found among patients with different polymorphisms of IMPDH1 *-106G>A* and IMPDH2 *3757T>C* SNPs. In summary, the effects of MRP-2 SNP on pharmacokinetics and IMPDH1 SNP on pharmacodynamics of mycophenolate mofetil were documented. These genotypes may be the factor for dose guiding and outcome monitoring in Thai kidney transplant patients.

Department : Pharmacy Practice Student's Signature .....

Field of Study : Pharmaceutical Care Advisor's Signature .....

Academic Year : 2012 Co-advisor's Signature .....

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| | | |
|---|---|---|
| AcMPAG | = | acyl mycophenolic acid glucuronide |
| $AUC_{0\text{-}12\ hr}$ | = | area under the curve 0-12 hours |
| CL/F | = | clearance expressed as a function of bioavailability |
| IMPDH | = | inosine monophosphate dehydrogenase |
| MMF | = | mycophenolate mofetil |
| MPA | = | mycophenolic acid |
| MPAG | = | mycophenolic acid glucuronide |
| MRP-2 | = | multidrug resistance associated protein-2 |
| UGT | = | Uridine diphosphate-glucuronosyl transferase |
| UNL | = | Upper normal limit |

# CHAPTER I

# INTRODUCTION

## 1.1 Rationale and Background

Organ transplantation is the treatment of choice for patients with end-stage organ failure. Long-term use of immunosuppressive drugs can improve graft survival, while adverse drug events will be limited. However, it is well recognized that different transplant recipients respond in different ways to immunosuppressive medication. The genetic variation is a determinant of drug responses. Many non-genetic factors such as organ function and drug interactions are likely to influence the effects of medication. Mycophenolate mofetil (MMF), an ester prodrug of mycophenolic acid (MPA), is an immunosuppressive agent used after solid organ transplantation. In kidney transplant patients, MMF is widely used along with calcineurin inhibitors (cyclosporine and tacrolimus) or the proliferation signal inhibitors (sirolimus and everolimus) and corticosteroid to prevent graft rejection.[1,2]

Following oral administration, MMF is rapidly and totally converted to MPA, which acts by noncompetitive, selective, and reversible inhibition of inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in the *de novo* purine biosynthetic pathway. It inhibits the proliferation of B and T lymphocyte.[2,3] Several uridine diphosphate-glucuronosyltransferases of the family 1 (namely UGT1A8, 1A9, and 1A10) are involved in the glucuronidation of MPA to inactive 7-O-glucuronide MPA (MPAG) and to a lesser extent, acyl glucuronide (AcMPAG) that has pharmacologically active. Of them, UGT1A9 appears to be the most important UGT isozyme, responsible for approximately half of the MPAG formation. MPA metabolites are excreted via the kidney. In addition, MPAG is excreted into the bile via a canalicular transporter, in particular the multidrug resistance-associated protein-2 (MRP-2). In the gut, bacterial

deconjugation transforms MPAG back into MPA, which contributes to the enterohepatic recirculation of MPA.[3,4]

MMF is recommended on a fixed dose (2 g/day). This standard dose regimen has overall proven to be efficient in preventing early acute rejection and improving long-term graft survival. However, it has been shown that there is an intra- and interpatient variability of the pharmacokinetics of MPA. Thus, fixed dose MMF therapy may lead to under- or overimmunosuppression leading to increased risk of acute rejection or drug toxicity, respectively. Factors that affect to MPA pharmacokinetic include ethnicity, time after transplantation, concomitant drugs, serum albumin, renal function, hepatic function, and genetic polymorphisms.[1-5]

A single nucleotide polymorphism (SNP) is a DNA sequence variation of a single nucleotide (adenine [A], thymine [T], cytosine [C], or guanine [G]) that occurs at a frequency of greater than 1% within the general population. SNPs may occur within the coding or noncoding regions of genes, or in the intergenic region between genes. Individual SNPs may cause a change in gene expression or protein function that has a physiological effect on the organism. Identification of the contribution of SNPs in the genes coding for immunosuppressant drug-metabolizing enzymes, transporters and targets to the response of an individual to these drugs has the potential to provide a powerful means of improving the prediction of between-subject variations in the pharmacokinetic or pharmacodynamic response, and thus drug efficacy and side effects. Pharmacogentic research with regard to MPA has focused on the following enzymes: UGT, MRP-2 and the IMPDH type I and II.[6-8]

Considering that UGT isoforms play a significant role in the metabolism of MPA, induction or inhibition of their activity could lead to underexposure to the drug or to the induction of adverse effects. Regarding the pharmacokinetic profile of MPA, the presence of the UGT1A9 *-2152C>T* and *-275T>A* variants of the promoter region showed a lower exposure to MPA.[15] Interestingly, the allele frequencies of UGT1A9 *-2152C>T* and *-275T>A* polymorphisms were not identified in Asian populations.[9,10] UGT1A9*1b was present at high frequency (e.g., 39.0% in Caucasian individuals, 30.1%

in Thai healthy individuals, and 29.6% in Chinese kidney transplant recipients).[11-13] This variant was found in the promoter region at position -118$(T)_{9/10}$ that has been reported *in vivo* to enhance significantly MPA glucuronidation. However, Ramirez et al demonstrated no functional variation in UGT1A9 activity.[11] In clinical study, Zhang et al investigated the effect of UGT polymorphism on the pharmacokinetics of MPA and its metabolites in 98 Chinese kidney transplant recipients. There was a statistically significant increase in MPA $AUC_{6-12}$ in patients with UGT1A9*1b variant ($T_{10}$ = 11.89±8.76, $T_9/T_{10}$ = 11.54±7.62, and $T_9$ = 7.34±4.11 mg x h/mL, *p*-value = 0.041).[13]

Two SNPs of UGT1A9 were identified in Thai healthy volunteers. Allele frequencies of UGT1A9*1b and UGT1A9 *-688T>G* were 0.532 and 0.124. There was no report about the functional polymorphism of UGT1A9 *-688T>G*.[12] Therefore, it was investigated in this study.

As for the UGT2B7 gene, the common UGT2B7 *802C>T* polymorphism was found in 27% of Asians and up to 54% of Caucasian individuals. The impact of this mutation on the glucuronidation activity is still controversial.[9,10] Zhao et al founded the impact of UGT2B7 *802C>T* polymorphism in 89 *de novo* pediatric renal transplant patients. The oral clearance (CL/F) was significantly higher in patients with variant.[14] Other SNP, Djebli et al reported UGT2B7 *-842G>A* polymorphism was associated with higher AcMPAG $AUC_{0-9\ hr}$ in renal transplant patients treated with MMF and sirolimus over three months after transplantation (AA = 4.13, GA = 2.97, and GG = 1.90 mg x h/L, *p*-value = 0.04).[15]

MRP-2 is considered a drug transporter involved in MPAG excretion, both in the liver and the proximal renal tubule, and several SNPs in the MRP-2 gene encoding this transporter have been described.[9,10] The MRP-2 *-24C>T* polymorphism in promoter region was associated with higher MPA trough level in 95 Caucasian kidney transplant patients treated with MMF and tacrolimus because this SNP showed an increasing gene expression and activity. Patients with MRP-2 *-24C>T* variant had significantly more evidences of diarrhea in the first year after transplantation (29.0% of patients with variant and 13.0% of patients with wild-type, *p*-value < 0.049).[16]

Measurement of IMPDH activity is an attempt to evaluate the efficacy of MMF directly. IMPDH is the target enzyme of MPA and has two isoforms, IMPDH1 and IMPDH2 that converts inosine 5'-monophosphate (IMP) into xanthosine 5'-monophosphate (XMP) relying on β-nicotinamide adenine dinucleotide (β-$NAD^+$). MPA has a higher binding affinity for IMPDH2.[17] Maiguma et al founded a wide range of IMPDH activity in 10 health volunteers (5.93-15.28 nmol/h/mg protein). For kidney transplant patients treated with tacrolimus and MMF over 2 months after transplantation, IMPDH activity decreased to 75% and 67% at 1 and 2 hours after MMF dosing, respectively.[18] Glander et al reported a correlation between biopsy-proved acute rejection (BPAR) and interindividual variability of IMPDH activity in 79 patients prior to kidney transplantation. Patients with high pretransplant IMPDH activity and MMF dose reduction were associated with a higher risk of acute rejection (81.81%).[19] Genetic variation in the gene encoding for IMPDH may explain part of the variability in IMPDH activity. The functional IMPDH variants might contribute to the variable response to MPA. Two SNPs of IMPDH1 at intron 7 position (IVS7+125G>A and IVS8-106G>A) have been associated with increased incidence of BPAR in the first year posttransplantation. However, the mechanism of association between IMPDH1 polymorphisms and acute rejection was still to be determined.[20] A polymorphism in intron 7 IMPDH2, *3757T*>C, was associated with three times higher odds of experiencing BPAR at 1 year posttransplantation (odd ratio = 3.39, 95%CI 1.42-8.09, *p*-value = 0.006). Sombogaard et al reported that the IMPDH2 *3757T>C* polymorphism is associated with an increased IMPDH activity in kidney transplant patients. The allele frequency was 6.9%. The area under the time-effect curve for the IMPDH activity over 12 hours was significantly higher in 12 patients with IMPDH2 *3757 C* variant compared with the 68 patients with IMPDH2 *3757 TT* wild-type (336 and 227 μmol/s/mol AMP, respectively, *p*-value = 0.04).[21] However, the study of pharmacogentic polymorphisms on pharmacodynamics is still infancy.

In Thailand, most kidney transplant patients usually maintain MMF dose ranging from 1-1.5 g/day that lower than the recommended fixed dose. Because of no the

adequate therapeutic drug monitoring method or suitable abbreviated MPA AUC estimation, MMF dose is prescribed according to clinical tolerance. Although, there are two studies about MMF pharmacokinetics in Thai kidney transplant recipients. Julasareekul et al conducted the abbreviated MPA AUC equations from 16 patients treated with 1 g/day of MMF, cyclosporine, and prednisolone and Jirasiritham et al determined the optimal sampling time that correlated with the MPA AUC in 46 patients on MMF dose ranging 0.5-2 g/day together with cyclosporine and prednisolone.[22,23] From these results, MPA $AUC_{0-12}$ has shown within 30 to 60 g x h/L; however, it was near the lower limit of the recommend therapeutic interval (MPA $AUC_{0-12}$ = 37.54±0.80 and 34.3 g x h/L, respectively) and the MPA AUC calculation needed several blood samples at long time after morning MMF dose. It is impractical and time consuming for both patient and health care team. Currently, the pharmacogenetics approach is a growing interest and conflict results are still to be considered. Otherwise, multiple genes are involved in regulating drug disposition and the pharmacological effect of the immunosuppressive agents. It would be unrealistic to anticipate that a single gene could regulate multiple and complex drug responses. Multiple gene SNP tests are expected to be more sensitive and promising in predicting efficacy and safety of these drugs. To date, there are no data on the use of these genotypes in guiding mycophenolate dosing. Algorithms or models should be necessarily developed that able to bring together information on the genetic background, pharmacokinetics, pharmacodynamics, and clinical data for predicting drug response in individual patient. In addition, there is no the genetic polymorphism study in Thai kidney transplant patients receiving MMF. Therefore, this study will investigate the genetic polymorphisms on MMF pharmacokinetics and pharmacodynamics that may be helpful in individualized therapeutic dosing, resulting in adequate drug efficacy with minimum adverse effects.

## 1.2 Hypothesis

Genetic polymorphism may influence the pharmacokinetics and pharmacodynamics of MMF. The effects of these polymorphisms could be detected by

differences in the area under the curve 0-12 hr of MPA and IMPDH activity in patients' plasma. The area under the curve 0-12 hr of MPA and IMPDH activity of patients with polymorphic genes would be different from wild-type genotypic patients.

## 1.3 Objectives

1.3.1 To compare pharmacokinetic parameters of MMF in patients with different genotypes of UGT1A9, UGT2B7 and MRP-2.

1.3.2 To compare pharmacodynamic parameters of MMF in patients with different genotypes of IMPDH1 and IMPDH2.

## 1.4 Expected outcomes

The information about the association of genetic polymorphisms with pharmacokinetics and pharmacodynamics of MMF will be used for calculation of appropriate dosage of MMF to achieve optimal immunosuppression and safety personalized therapy.

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# CHAPTER II

# LITERATUERE REVIEWS

## 2.1 Mycophenolate mofetil

Mycophenolate mofetil (MMF) is morpholinoethyl ester of mycophenolic acid (MPA). The drug is available by Roche Pharmaceuticals (trade name CellCept) for oral administration as capsules (250 mg of MMF), tablets (500 mg of MMF), a powder for oral suspension (200 mg/ml when constituted), and MMF hydrochloride 542 mg for intravenous injection (equivalent of MMF 500 mg).[3]

MMF has become one of the cornerstones of immunosuppressive therapy and now is used in the vast majority of maintenance regimens in solid organ transplantation. MPA is a potent, selective, and uncompetitive reversible inhibitor of IMPDH, which is the rate-limiting enzyme in the de novo synthesis of guanosine nucleotides. It is the first of two enzymes responsible for the conversion of inosine monophosphate (IMP) to guanosine monophosphate (GMP), which is then converted guanosine monophosphate, triphosphate, and the deoxyribonucleotide. In the first reaction of guanine nucleotide synthesis, IMPDH catalyzes the nicotinamide dinucleotide (NAD)-dependent conversion of IMP to xanthine monophosphate (XMP). This reaction is irreversible and a committed step for the synthesis of GMP. The enzyme GMP synthetase catalyzes the second reaction the adenosine triphosphate (ATP)-dependent amination of XMP to GMP. Purines can be synthesized by the de novo and the salvage pathways, but IMPDH is only involved in the former pathway (Figure 2.1). De novo purine synthesis is critically important for proliferative responses of human T and B lymphocytes to mitogens, whereas the major salvage pathway is not required for lymphocyte proliferation.[1,17]

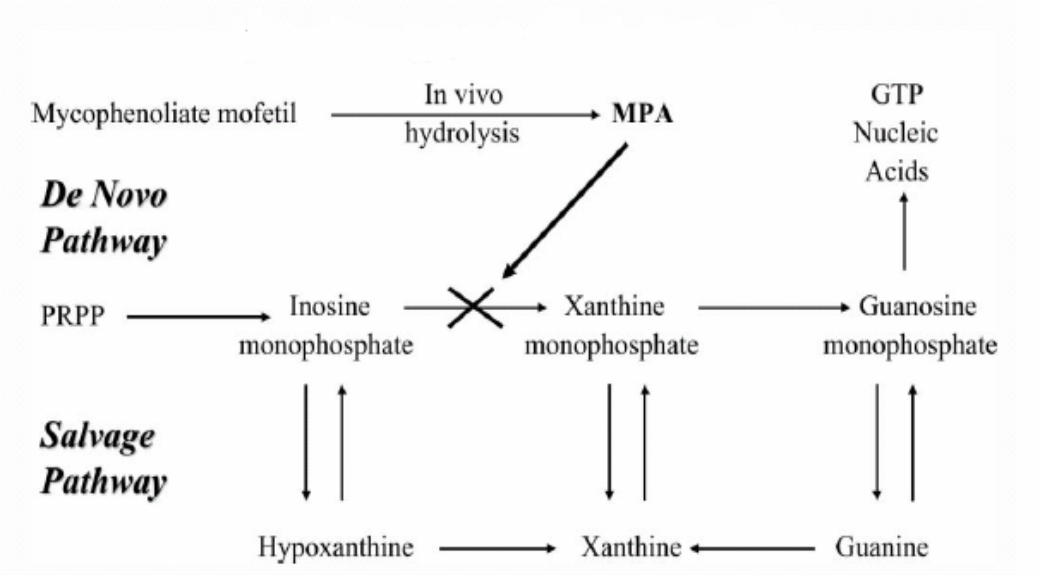


**Figure 2.1** Purine synthesis pathway [17]

Study by Glander et al [19], demonstrated that within the usual target range concentration of MPA, MPA concentrations correlate with the inhibition of IMPDH. Therefore, on average, at peak concentrations, >70% of IMPDH is inhibited whereas at standard trough concentrations, <20% of IMPDH is inhibited.

## 2.2 Pharmacokinetics [1-3,24]

*Absorption*

After oral administration, MMF is rapidly and essentially completely absorbed. MMF is hydrolyzed to MPA by esterase in the stomach, small intestine, and blood. In healthy volunteers, the mean bioavailability of MPA averaged 94% and the time to reach maximal MPA concentration ($t_{max}$) by the oral route is about 1 hour. Food consumption 30 minutes before MMF administration does not affect MPA $AUC_{0\text{-}24hr}$ values. The average $t_{max}$ is delayed slightly, and the $C_{max}$ is lowered by 25%, consistent with delayed gastric emptying in the fed state.

*Distribution*

More than 99% of MPA in blood is retained in plasma. MPA binds extensively to serum albumin in the order of 97-99% in patients with normal liver function. MPA glucuronide (MPAG), the main metabolite of MPA, also displays high serum albumin binding (approximately 82%). The binding of MPA to plasma proteins is influenced by the availability of serum albumin binding sites and competition for these sites by MPAG and urea.

*Metabolism*

Uridine diphosphate glucuronosyltransferases (UGTs) metabolise MPA via glucuronidation in the gastrointestinal tract, liver and kidney. MPAG, the main metabolite, is a phenolic glucuronide, which has no pharmacological activity. MPAG is usually present in plasma at approximately 20- to 100-fold higher concentrations than MPA. At least two other minor metabolites are formed, a 7-O-glucoside and an acyl-glucuronide (AcMPAG). The 7-O-glucoside has no inhibitory effect on IMPDH, while AcMPAG appears to inhibit IMPDH *in vitro* in a concentration-dependent manner and at a pharmacological potency comparable to MPA.

UGT1A9 is believed to be the major isoforms involved in MPA glucuronidation, possibly because of their high hepatic and renal expression. According to in vitro experimentation, UGT1A9 is responsible for 55%, 75% and 50% of MPAG production by the liver, kidney and intestinal mucosa, respectively. MPAG is also formed by UGT1A7, 1A8 and 1A10, which are expressed in the kidney and gastrointestinal tract. UGT2B7 is the only isoform reported to produce AcMPAG. The plasma profile of the MPAG is slightly delayed compared with MPA, which is consistent with MPA being the precursor of MPAG. One hour after both oral and intravenous administration, the concentration of MPAG is higher than that of MPA. The peak level for MPAG is reached 1.25 to 4 hours after dosing. The total plasma AUC of MPAG is five to more than 150 times that of MPA. MPAG is pharmacologically inactive. This glucuronide metabolite is excreted into the bile, a process that is mediated by a canalicular transporter, multidrug resistance-

related protein-2 (MRP-2), and it is converted to MPA via intestinal microflora β-glucuronidase, MPA then is reabsorbed into the systemic circulation. Enterohepatic recirculation of MPA is presumed to account for secondary peak that can occur in the plasma profile of MPA anywhere from 4 to 12 hours after the MMF dose in transplant patients; in healthy subjects it is reached 8 to 12 hours after drug intake. A detectable secondary rise in MPA plasma concentrations was found in 50-60% of kidney allograft recipients.

Blockade of MRP2 by an inhibitor, such as cyclosporine, decreases the biliary excretion of MPAG and increases plasma levels of MPAG. This eventually leads to lower plasma levels of MPA because the glucuronide metabolite no longer can be reabsorbed as MPA as a result of disruption of enterohepatic cycling of MPA.

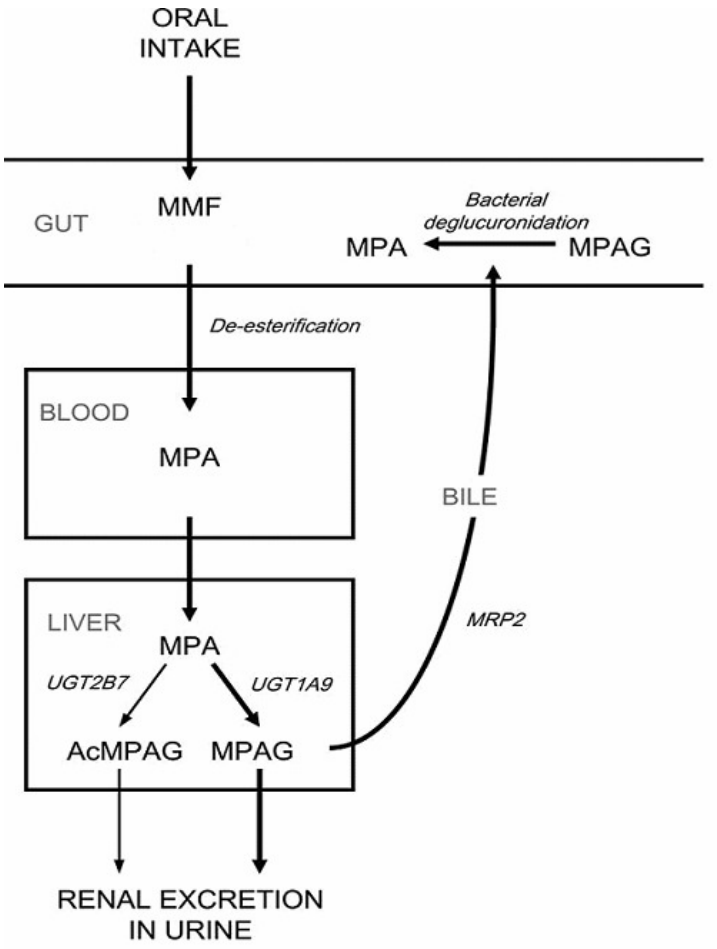


**Figure 2.2** Mycophenolate metabolism pathway[25]

*Elimination*

In healthy subjects about 93% of MPA is excreted in urine, mostly as MPAG (87%), whereas 6% in eliminated in the feces. The mean apparent plasma terminal half life ($t_{1/2}$) of MPA is approximately 17 hours in healthy volunteers. Hemodialysis does not affect the pharmacokinetics of MPA. The drug is undetectable in hemdialysis fluid, although small amounts of MPAG have been detected.

## 2.3 Drug monitoring

The official dose recommendation for MMF in adult kidney transplant recipients is to use 2000 mg/day in combination with a calcineurin inhibitor and corticosteroid. MPA exposure is best reflected by a full MPA $AUC_{0\text{-}12\ hr}$. However, before a full $AUC_{0\text{-}12\ hr}$ can be calculated, at least eight MPA concentration-time samples need to be drawn, covering the total 12-h dosing interval. This is costly, time-consuming, inconvenient for the patient and not feasible in an outpatient clinic setting. MPA predose levels have a weaker correlation with the risk of acute rejection and $AUC_{0\text{-}12\ hr}$, suggesting that predose levels do not always reflect exposure adequately. A suitable alternative may be the estimation of full MPA $AUC_{0\text{-}12\ hr}$ by a limited sampling strategy. Several limited sampling strategies have been proposed for MPA based on multiple regression analyses, and mostly consisted of 3 or 4 samples drawn during the first 2-6 hours of a dosing interval, with correlation coefficients $\leq$ 0.95. The advantage is a reliable estimation of MPA exposure for patients, from no more than a 2- to 6- hour stay. A disadvantage is that MPA plasma samples need to be drawn at exact time-points after MMF dosing. The currently recommended target range of MPA $AUC_{0\text{-}12\ hr}$ is 30 to 60 mg x h/L.[26-28] Three randomized, controlled trials have been shown the benefit of dose adjustment using a limited sampling strategy. The APOMYGRE study compared a fixed-dosage (FD) regimen of MMF 2 g/day with a concentration-controlled (CC) regimen based on MPA AUC calculation using Bayesian estimator that collected blood samples at 20 minutes, 1 and 3 hours after dosing in 137 kidney graft recipients who were receiving cyclosporine

therapy. MMF dose adjustment was calculated to reach target MPA AUC 40 mg x h/L. Patients in the CC group achieved significantly higher MPA AUC within a month after transplantation (median MPA AUC of CC group and FD group were 45.0 and 30.9 mg x h/L, respectively, *p*-value < 0.0001) and had a significantly lower incidence of biopsy-proven acute rejection (BPAR) at month 12 (7.7% in the CC group and 24.6% in the FD group, *p*-value = 0.001). The incidence of treatment failure (including death, graft loss, acute biopsy-rejection, and MMF discontinuation) was significantly lower in the CC group compared to the FD group (29.2% and 47.7%, respectively, *p*-value = 0.03) which interestingly received a higher MMF daily dose in the first 3 months after transplantation.[29] The Fixed Dose-Concentration Controlled (FDCC) study compared a FD regimen of 2 g of MMF with a CC regimen based on abbreviated MPA AUC calculation using Bayesian estimator that collected blood samples at predose, 20, and 120 minutes after dosing (target $AUC_{0\text{-}12\ hr}$ = 30-60 mg x h/L, dose adjustment was made to aim MMF dosing for MPA AUC of 45 mg x h/L) in 901 patients who were treated with cyclosporine or tacrolimus. Early achieving target MPA AUC correlated inversely with the risk for BPAR on day 3 versus BPAR in the first month (*p*-value = 0.009) and versus BPAR in the first year (*p*-value = 0.006); however, a benefit for CC approach could not be demonstrated because there were no difference in the incidence of treatment failure (25.6% in FD group and 25.6% in CC group) and incidence of BPAR (15.5% in FD group and 14.9% in CC group).[30] The Opticept study also compared fixed (2 g) and CC dosing of MMF on the basis of MPA trough concentration (target trough concentration of 1.3 mg/L for the cyclosporine group and 1.9 mg/L for the tacrolimus group) in 720 patients who were on either a standard or a reduced dosage of cyclosporine or tacrolimus. A *post hoc* analysis of 590 patients who were treated with tacrolimus showed that risk for acute rejection was significantly lower (*p*-value < 0.001) in patients who achieved target MPA trough level $\geq$1.6 mg/L at 6 and 12 months after transplantation. In addition, there was a positive correlation between the abbreviated MPA AUC and trough concentration in patient receiving tacrolimus ($r^2$ = 0.6894, *p*-value < 0.001).[31]

A new and promising approach for MMF dose individualization is pharmacodynamic monitoring. Pharmacodynamics directly reflects the drug's biologic effects. The investigation of the mechanism of action of immunosuppressive drugs has provided biomarkers useful for pharmacodynamic assessment. Inhibition of IMPDH activity may, in theory, prove to be a suitable biomarker for pharmacodynamic monitoring of MMF therapy. A relationship between pretransplant IMPDH activity and clinical outcome has been found. Lower pretransplant IMPDH activity was associated with an increased incidence of MMF dose reductions, which is a surrogate end point for adverse events. Furthermore, high pretransplant IMPDH activity was associated with a higher risk of acute rejection. These findings suggest that measurement of IMPDH activity may be suitable to monitor clinical efficacy and toxicity of MMF therapy. A disadvantage of pharmacodynamic monitoring is the technically complex and time-consuming measurement of IMPDH activity, generally consisting of isolation of peripheral blood mononuclear cells (PBMCs) and a high-performance liquid chromatography (HPLC) method.[32]

## 2.4 Genetic polymorphisms and the impact on pharmacokinetics of mycophenolate mofetil

The UGT superfamily, encoded by the *UGT* genes, is responsible for glucuronidation in human. It is comprised of two families (UGT1 and UGT2) and three subfamilies (UGT1A, UGT2A and UGT2B). Glucuronidation results in the formation of hydrophilic, generally less active or inactive glucuronides that are more easily excreted in the urine and bile. Thus these enzymes are of major importance in the detoxification and subsequent elimination of various endogeneous compounds and xenobiotics from the body. SNPs in genes encoding the UGT enzymes may result in altered glucuronidation activity.[9,10]

Interindividual polymorphic regulation of the UGT1A9, UGT2B7 and MRP-2 gene has been reported for MPA metabolism. Table 2.1 summarizes the ethnic frequencies of the SNPs thought to have a clinically relevant effect on MPA transport and metabolism.

**Table 2.1** Frequency of UGT and MRP-2 SNPs [10]

| SNP | rs number | Caucasian frequency | | | Asian frequency | | |
|---|---|---|---|---|---|---|---|
| | | WT | HT | MT | WT | HT | MT |
| **UGT1A9** | | | | | | | |
| *-275T>A* | 6714486 | 0.850 | 0.150 | 0 | 1 | 0 | 0 |
| *-2152C>T* | 17868320 | 0.874 | 0.116 | 0.010 | 1 | 0 | 0 |
| *-440C>T* | 2741045 | 0.583 | 0.383 | 0.033 | 0.978 | 0.022 | 0 |
| *-331T>C* | 2741046 | 0.583 | 0.383 | 0.033 | 0.978 | 0.022 | 0 |
| *98T>C* (*3) | no rs number | 0.969 | 0.031 | 0 | not available | | |
| **UGT2B7** | | | | | | | |
| *802C>T* (*2) | 7439366 | 0.217 | 0.567 | 0.217 | 0.467 | 0.511 | 0.022 |
| *-842G>A* | 7438135 | 0.217 | 0.567 | 0.217 | 0.022 | 0.511 | 0.467 |
| **MRP-2** | | | | | | | |
| *-24C>T* | 717620 | 0.567 | 0.417 | 0.017 | 0.578 | 0.400 | 0.022 |
| *3972C>T* | 740066 | 0.400 | 0.517 | 0.083 | 0.511 | 0.444 | 0.044 |
| *1249G>A* | 2273697 | 0.583 | 0.367 | 0.050 | 0.844 | 0.156 | 0 |

WT = wild-type, HT = heterozygous variant, MT = homozygous variant

UGT1A9 is polymorphically expressed, that identified in the coding and promoter regions of the gene. Two promoter SNPs appear to have the most functional impact and thus have received the most extensive study. The first involves a T to A transition at position -275 and the second involves a C to T transition at position -2152. Heterozygous or homozygous carriers of these SNPs have significantly higher human liver microsomal UGT1A9 protein levels compared with the wild-type promoter (a twofold

increase is seen in those with the *-275T>A* SNP and a 2.3-fold increase is seen in those with both the *-275T>A* and *-2152C>T* SNPs). Additionally, *in vitro* MPA glucuronidation activity is roughly twofold higher in carriers of these SNPs compared with wild-type individuals. It should be noted, however, that because UGT1A9 *-275T>A* and UGT1A9 *-2152C>T* are in strong association with each other, it is difficult to differentiate their respective contributions to UGT1A9 protein levels and glucuronidation activity. The UGT1A9 *-275T>A* and UGT1A9 *-2152C>T* SNPs are found in up to 15% of Caucasian and are absent in Asians.[33] There was a significantly lower MPA $AUC_{0-12}$ in 95 kidney transplant recipients carrying these polymorphisms when compared to the wild-type (31.70±17.6 and 63.60±30.90 mg x h/L, respectively, *p*-value = 0.009). MPA trough level also significantly decreased in patients with variants (1.23±0.25 compared with 2.84±1.64 mg/L in patients with wild-type, *p*-value = 0.04).[34]

Two other *UGT1A9* promoter region SNPs, one involving a C to T transition at position -440 and the second involving a T to C transition at position -331, have been shown to have functional impact. These SNPs are in complete association. Carriers of these SNPs have significantly higher hepatic UGT1A9 protein levels compared with wild-type individuals. A further UGT1A9 SNP with reported functional significance is characterized by nucleotide change from T to C at position 98 of exon 1 (UGT1A9 *98T>C*, otherwise known as UGT1A9*3). This results in a change in amino acid from methionine to threonine at codon 33 of UGT1A9. Lower MPA glucuronidation in the presence of this SNP has been demonstrated. This SNP is only infrequently expressed in Caucasians. There is no data available regarding frequency in other ethnicities. The UGT1A9 *-440C>T* and *-331T>C* had influenced to MPA AUC in 40 Caucasian kidney transplant recipients. MPA $AUC_{0-12}$ was significantly associated with the presence of UGT1A9 *C-440T/T-331C* polymorphisms (TT/CC: 61.50±2.70; TC/CT: 45.40±14.00; CC/TT: 40.80±10.80 mg x h/mL, *p*-value = 0.005).[35]

Regarding UGT2B7 gene, the most common and extensively studied involves a C to T transition at position 802 (UGT2B7 *802C>T*, otherwise termed UGT2B7*2). This

results in a histidine to tyrosine substitution at codon 268. The functional significance of this SNP is controversial. [33,36] An *in vitro* study demonstrated that the *802C>T* variant did not affect the glucuronidation rate of the MPA.[37] In addition, Kagaya et al explored UGT2B7 *C802T* variants did not affect to MPA $AUC_{0-12}$ in 72 Japanese patients on day 28 after kidney transplantation.[38] Zhao et al found the other impact of UGT2B7 *C802T* polymorphism in 89 *de novo* pediatric renal transplant patients. The oral clearance (CL/F) was significantly higher in patients with UGT2B7 *C802T* variant.[14] Other SNPs, van Agteren et al investigated the allele frequency of UGT2B7 *G-840A* polymorphism (44% of patients with heterozygous variant and 28% of patients with homozygous variant). There was no association between UGT2B7 *G-840A* polymorphism and AcMPAG AUC and incidence of adverse effect from MMF (mainly diarrhea and leucopenia).[25]

MRP-2, encoded by the *MRP-2* gene is an ATP-dependant efflux transporter. It is located on the apical plasma membrane of various cell types, including hepatocytes, kidney proximal tubular cells and specialized cells in the intestine and brain. The predominant role of MRP-2 is to export organic anions and xenobiotics out of cells and into the bile, urine and intestinal lumen. MRP-2 is responsible for the biliary excretion of MPAG. SNPs leading to altered MRP-2 activity may influence this process and therefore affect MPA exposure.[33] The most extensively studied is a promoter region SNP that involves a C to T transition at position -24 (MRP-2 *-24C>T*). Data regarding the functional significance of this SNP are conflicting. No difference in MRP-2 mRNA or protein expression was observed in human duodenal or tissue regardless of SNP carrier status. However, a strong trend (*p*-value = 0.055) towards decreased exposure to the anticancer drug irinotecan (which undergoes biliary excretion via MRP-2) has been demonstrated *in vivo* in those carrying this SNP. Other SNPs, Zhang et al reported that patients carrying the heterozygous mutant alleles of MRP-2 *1249G>A* (14.3%) exhibited higher AcMPAG $AUC_{6-12}$ than those with wild-type (GA = 9.60 ± 8.50 and GG = 5.05 ± 3.12 mg x h/mL, *p*-value = 0.016).[13]

## 2.5 Genetic polymorphisms and the impact on pharmacodynamics of mycophenolate mofetil

IMPDH, encoded by the *IMPDH* gene, it is the reversible inhibition of IMPDH in lymphocytes that accounts for the immunosuppressive activity of MPA. The measurement of IMPDH activity might serve as a surrogate marker of MPA-induced immunosuppression. Rother et al showed a large interindividual variability of IMPDH activity in both healthy children and adults (78, [30-184] µmol/s/mol AMP and 83, [26-215] µmol/s/mol AMP).[39] Sanquer et al presented the induction of IMPDH activity occurred in patients treated with MMF more than 18 months after kidney transplantation. Mean IMPDH activity could reach 2-4 times when comparing with mean IMPDH activity of patients with a duration of MMF treatment of 1-3 months.[40] IMPDH1 gene variants have been investigated in 191 kidney transplant recipients treated with MMF and tacrolimus. There were 17 allelic variants with allele frequencies ranging from 0.2-42.7%. In another study, Wang et al identified nine genetic variants in the IMPDH2 gene, with frequencies ranging from 0.5-10.2%. Notably, a novel variant (IMPDH2 *787C>T*) was found that gene encoding could reduce enzymatic activity *in vitro* and a possible clinical correlation with MPA-induced leucopenia in liver transplant patients, but had an allele frequency of only 1.0%.[41] Gensburger et al investigated the associations between the most frequent SNPs in both IMPDH genes and clinical outcomes in 456 kidney transplant patients. For the IMPDH1 rs2278293 and rs2278294 SNPs, the minor allele frequencies were 46% and 36%, respectively. The frequency of variant allele of IMPDH2 *3757T>C* was 9.0%. Only IMPDH1 rs2278294 SNP was associated with a lower risk of BPAR (OR = 0.54, 95%CI [0.34-0.85], *p*-value = 0.0075) and a higher risk of leucopenia (OR = 1.66, 95%CI [1.11-2.48], *p*-value = 0.0139) over the first year posttransplantation. No other IMPDH1 or IMPDH2 polymorphism was significantly associated with any clinical outcome.[42] Ohmann et al explored the association between IMPDH1 *IVS8-106G>A* and IMPDH1 *1572C>T* polymorphisms with gastrointestinal intolerance in pediatric heart recipients ($p$=0.029 and $p$=0.002, respectively). In addition, IMPDH2

*3757T>C* variant was associated with more frequent neutropenia requiring dose-holding ($p$=0.046).[43] Otherwise, the study of Winnicki et al found that no relationship between the IMPDH2 *3757T>C* polymorphism and basal IMPDH activity or enzyme activity during MMF treatment.[44] Therefore, the functional polymorphisms of these SNPs on pharmacodynamics should be ongoing in research.

**Table 2.2** Genotype frequency of IMPDH SNPs [10]

| SNP | rs number | Caucasian frequency | | | Asian frequency | | |
|---|---|---|---|---|---|---|---|
| | | WT | HT | MT | WT | HT | MT |
| **IMPDH1** | | | | | | | |
| *125G>A* | 2278293 | 0.241 | 0.655 | 0.103 | 0.289 | 0.511 | 0.200 |
| *-106G>A* | 2278294 | 0.424 | 0.508 | 0.058 | 0.133 | 0.533 | 0.333 |
| **IMPDH2** | | | | | | | |
| *3757T>C* | 11706052 | 0.792 | 0.208 | 0 | 0.911 | 0.089 | 0 |

WT = wild-type, HT = heterozygous variant, MT = homozygous variant

# CHAPTER III

# RESEARCH METHODOLOGY

## 3.1 Subjects

The study was conducted from April to September 2012 at Post-kidney transplantation Outpatient Clinic at King Chulalongkorn Memorial Hospital, Pramongkutklao Hospital and Police General Hospital.

### Study samples

The subjects of this study were selected from Thai post-kidney transplant patients. The study protocol was reviewed and approved by the institutional review board of the Faculty of Medicine, Chulalongkorn University, Royal Thai Army Medical Department and Police General Hospital. Written inform consent had to be obtained from each individual who was participate in this study after adequate explanation of the aims, methods, objectives and potential hazards of the study and before undertaking and study-related procedures. One hundred and eighteen post-kidney patients fulfilled the following criteria were recruited in this study. The criteria for enrollment included:

*Inclusion criteria*:

1. Currently on mycophenolate mofetil for at least 1 week
2. Age $\geq$ 18 years old
3. Normal liver function (AST and ALT $\leq$ 3 x UNL, serum albumin > 4 g/dL)
4. Agree to participate in the study by signing the inform forms

*Exclusion criteria:*

1. Patients with multiple organ transplantations
2. Patients who are taking concomitant drugs that might have drug interaction such as antacids, cholestyramine, metronidazole, rifampicin

**Sample size determination**

Sample size calculation was based on probability to random patients in each genotype group. Given probability of patients with heterozygous and homozygous variant in the UGT1A9*1b SNP was 0.76 according to data from study of Korprasertthaworn et al[12], sample size was calculated using formula:

$$n = \frac{p(1-p)(Z_{\alpha/2})^2}{E^2} \qquad (\alpha = 0.05 , Z_{\alpha} = 1.96, E \text{ (error)} = 0.1)$$

$$n = \frac{0.76(0.24)(1.96)^2}{(0.1)^2}$$

$$n = 70.07 \sim 71$$

Sample size should be at least 71 cases to include patients with heterozygous and homozygous variant in the UGT1A9*1b SNP enough for comparison.

For UGT1A9 *-688T>G*, probability of patients with heterozygous variant in Thai healthy volunteers was 0.25.[12] Sample size was calculated by the same formula as written above, number of subject should be at least 73 cases.

For UGT2B7 *802C>T*, probability of patients with heterozygous and homozygous variant in Japanese kidney transplant patients was 0.58.[38] Sample size was calculated by the same formula as written above, number of subject should be at least 94 cases.

For MRP-2 *-24C>T*, probability of patients with heterozygous and homozygous variant in Japanese subjects was 0.63.[45] Sample size was calculated by the same formula as written above, number of subject should be at least 90 cases.

For IMPDH1 *125G>A*, probability of patients with heterozygous and homozygous variant in Japanese subjects was 0.67.[46] Sample size was calculated by the same formula as written above, number of subject should be at least 85 cases.

For IMPDH1 *-106G>A*, probability of patients with heterozygous and homozygous variant in Japanese subjects was 0.70.[46] Sample size was calculated by the same formula as written above, number of subject should be at least 81 cases.

For IMPDH2 *3757T>C*, probability of patients with heterozygous variant in Japanese subjects was 0.02.[46] Sample size was calculated by the same formula as written above, number of subject should be at least 8 cases.

In conclusion, sample size in this study should be not less than 94.

## 3.2 Methods

### Study design and procedures

This study was designed as a prospective analytical study. Information was collected from electronic patient database and patient interviewing. Demographic data and laboratory blood test data were recorded in patient medical record form as shown in Appendix A.

Patients used MMF and came to follow up at Post-kidney transplantation Outpatient Clinic at King Chulalongkorn Memorial Hospital, Pramongkutklao Hospital and Police General Hospital were approached to participate in this study by

investigator. After receiving thoroughly explanation about study objectives, methodology and possibilities of injury by blood withdrawing, patients agreed to be in the study signed in the informed consent.

Blood samples of each patient were drawn three times at predose, 30 minutes and 120 minutes after oral morning MMF dose. At each time, 15 mL of whole blood was collected in two tubes (5 mL of ethylenediamine tetraacetic acid (EDTA)-containing tube and 10 mL of lithium heparin-containing tube). The flow chart of study was shown in Figure 3.1.

Blood sample in EDTA-tube was isolated by centrifugation at 3000 g for 10 minutes at 20 $^{o}$C. Plasma was transferred into 1.5 mL of microcentrifuge tube (for MPA concentration analysis) and buffy coat was collected into 1.5 mL of microcentrifuge tube for genomic DNA extraction. All samples were kept at -20 $^{o}$C until analysis.

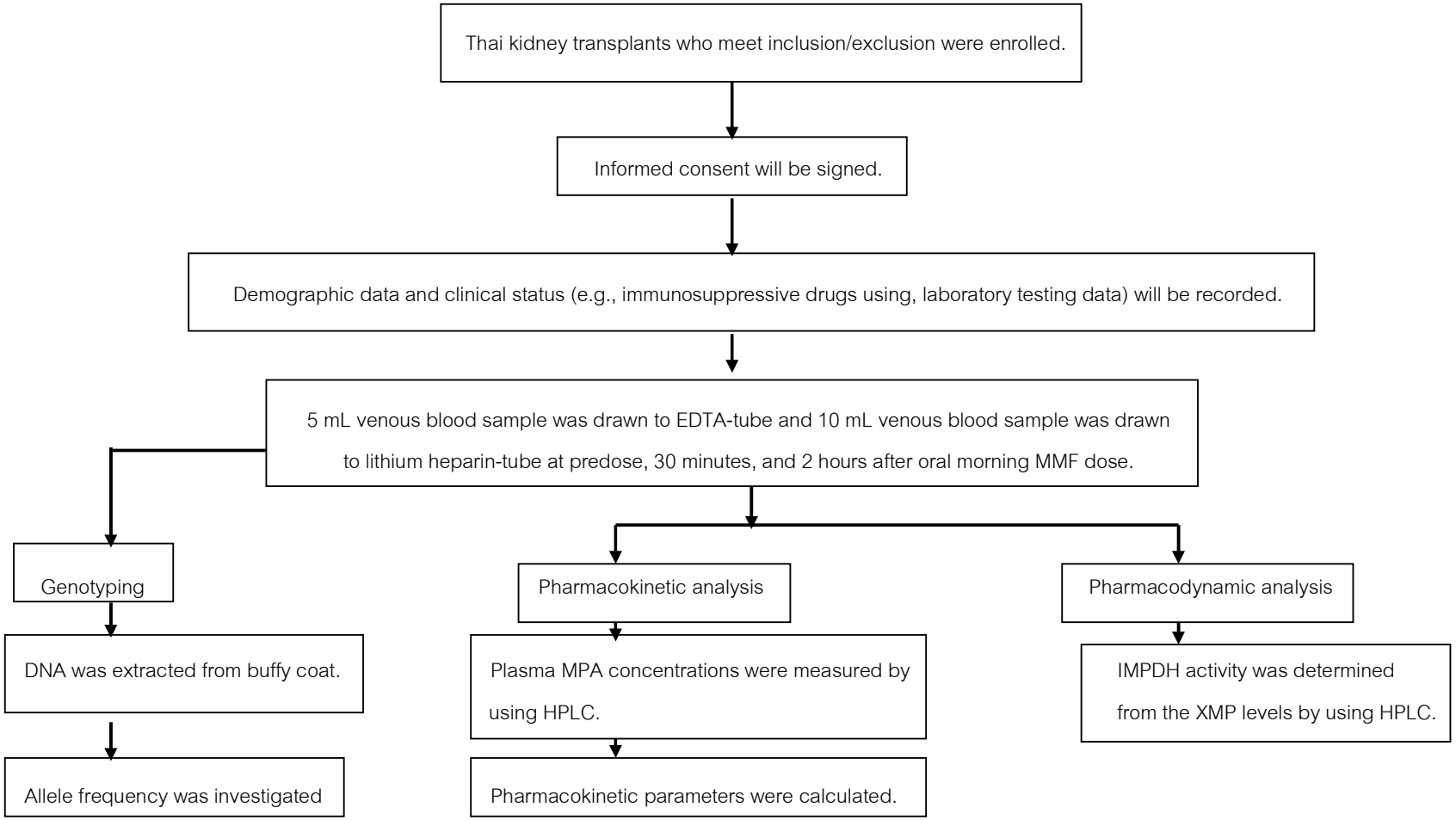


**Figure 3.1** The flow chart of study

Three time points (predose, 30 minutes and 120 minutes after oral morning MMF dose) of blood collection were chosen according to equation that analyzed from the previous study of Kessada Tunwongsa.[47] Full MPA pharmacokinetic data of 20 Thai post-kidney patients taking cyclosporine and mycophenolate mofetil were determined. Serial blood samples were then collected at 0, 0.25, 0.5, 1, 2, 3, 4, 6 and 12 hours. Limited sampling strategy (LSS) was developed and validated using the two-group method. Pharmacokinetic profiles from 11 subjects were randomly assigned as the index group to develop LSS. Multiple regression analysis was performed using SPSS for windows version 17 to generate the LSS. The AUC was the dependent variable and the timed concentrations were the independent variables. Preset criteria for selecting limited sampling equations were a coefficient of determination ($r^2$) $\geq$ 0.8 and a maximum of three concentrations taken at or before 2 hours after drug administration. Stepwise modeling was applied to calculate all possible multiple regression combinations. The profiles from the remaining 8 subjects were then used to validate the developed LSS. Bias was measured by the mean prediction error, whereas precision was measured by the mean absolute error. Common acceptable range of the mean prediction error and the mean absolute error in clinical studies was 15 to 20%.[28]

The $r^2$ value for the three-concentration time points using $C_0$, $C_{0.5}$ and $C_2$ was 0.868. Therefore, this equation was selected. Bias = 1.42% and precision = 9.7%

$$\text{MPA AUC}_{0\text{-}12\,hr} = 17.808 + 5.56*C_0 + 0.548*C_{0.5} + 2.126*C_2$$

## 3.3 Blood sampling and analysis

### 3.3.1 Genotyping analysis

Genomic DNA was extracted from 200 µL of buffy coat sample using a QIAamp DNA blood mini kit (Qiagen, Germany), according to the manufacturer's

protocol. The subjects were genotyped for seven SNPs (Table 3.1) by using a real-time polymerase chain reaction (PCR) method.

**Table 3.1** The details of seven SNPs

| SNP | rs number | position | Wild-type | Heterozygous variant | Homozygous variant |
|---|---|---|---|---|---|
| UGT1A9*1b | 3832043 | -118 | $T_{9/9}$ | $T_{9/10}$ | $T_{10/10}$ |
| UGT1A9 | 3806598 | -688 | TT | TG | GG |
| UGT2B7 | 7439366 | 802 | CC | CT | TT |
| MRP-2 | 717620 | -24 | CC | CT | TT |
| IMPDH1 | 2278293 | 125 | GG | GA | AA |
| IMPDH1 | 2278294 | -106 | GG | GA | AA |
| IMPDH2 | 11606052 | 3757 | TT | TC | CC |

Reagents

1. Forward primer (Biosearch Technologies, Canada)
2. Reverse primer (Biosearch Technologies, Canada)
3. FAM-TaqMan BHQplus probe (Biosearch Technologies, Canada)
4. CAL Fluor Orange 560-TaqMan BHQplus probe (Biosearch Technologies, Canada)
5. Type-it Fast SNP probe PCR Master Mix (Qiagen, Germany)
6. Subject DNA (10 ng/μL)

Reaction mix (followed by Table 3.2) was prepared for PCR reaction of each target SNP. Control DNA as positive control and water as negative control were analyzed with patient DNA sample in each SNP. The real-time cycler conditions were 60 $^{o}$C for 1 minute, 95 $^{o}$C for 5 minutes, followed by 45 cycles at 95 $^{o}$C for 15 seconds and

60 °C for 1 minute. The real-time PCR was performed on ABI 7500 Real-Time PCR System (Applied Biosystem, USA), using the manufacturer protocol.

**Table 3.2** Components of reaction mix

| Component | Volume/reaction @ 15 µL |
|---|---|
| 20X Type-it Fast SNP probe PCR Master Mix | 7.5 |
| 20X primer-probe mix | 0.75 |
| Patient DNA (10 ng/µL) | 2 |
| Sterile water | 4.75 |

### 3.3.2 Pharmacokinetic analysis

Mycophenolic acid concentration and its metabolite (mycophenolic acid glucuronide) were performed using HPLC with UV detector method modified from Elbarbry et al [48] and Patel et al [49].

Chemicals

1. Mycophenolic acid (gifted from Roche,Thailand)
2. Mycophenolic acid glucuronide (Toronto Research Chemicals, Canada)
3. Carboxy butoxy ether of mycophenolic acid (as the internal standard of mycophenolic acid) (gifted from Roche, Switzerland)
4. Phenolphthalein β-D- glucuronide (as the internal standard of mycophenolic acid glucuronide) (Sigma)
5. Ortho-phosphoric acid, 85% (Merck, Germany)
6. Acetonitrile HPLC grade (RCI Labscan, Thailand)
7. Methanol HPLC grade (Fisher Scientific, UK)

Instruments

1. High-performance liquid chromatography (HPLC) system (Dionex) consisted of P680 HPLC pump, ASI-100 automated sample injection and UVD 170U detector.

Analytical methods

Samples were prepared using protein precipitation. A 250 µL aliquot of plasma (standard, QC, patient) was spiked with 50 µL of internal standard (carboxy butoxy ether of MPA 60 µg/mL and phenolphthalein β-D- glucuronide 500 µg/mL) and vortexed for 30 seconds. Five hundred µL of 0.1 mol/L cold phosphoric acid in ACN were added and vortexed for 30 seconds. Samples were centrifuged at 16000 rpm for 30 minutes and supernatant was collected in HPLC vial for analysis.

Standard solutions

Standard stock solutions of MPA and MPA glucuronide (MPAG) were prepared by dissolving 5 mg and 10 mg, respectively in 5 mL of methanol. Dilutions of the standard stock solutions for MPA and MPAG were made in methanol range from 0.25 to 60 µg/mL and 5 to 324 µg/mL, respectively for the standard curve and quality control (QC) samples. Two internal standards including carboxy butoxy ether of MPA and phenolphthalein β-D- glucuronide was prepared by dissolving 0.3 mg and 2.5 mg, respectively in 5 mL of methanol. All solutions were stored at -20°C.

Chromatography conditions and equipment

The analytical column was Zorbax Eclipse XDB-C18 (4.6 x 150 mm, particle size 5 µ, Agilent Technologies) protected by a guard column Zorbax Eclipse XDB-C18 (4.6 x 12.5 mm, particle size 5 µ, Agilent Technologies). The chromatographic separation was performed at ambient temperature with gradient elution. The mobile phase components were methanol and 0.15% phosphoric acid. The flow rate remained at 1 mL/min throughout the 16-minute run. For the first 4 minutes of each run, the mobile

phase remained at methanol-0.15% phosphoric acid (45:55% vol/vol), from 4.5-12 minutes, there was a continuous gradient change to methanol-0.15% phosphoric acid (64:36% vol/vol), and from 12.5 to 16 minutes the composition changed back to methanol-0.15% phosphoric acid (45:55% vol/vol). MPA, MPAG and IS were detected at a wavelength of 215 nm. The injection volume was 15 µL.

Method validation of HPLC

Validation of HPLC method including specificity, selectivity, linearity, accuracy and precision were performed (see in Appendix B).

### 3.3.3 Pharmacodynamic analysis

IMPDH activity was expressed as xanthosine monophosphate (XMP) produced per time unit per amount of adenosine monophosphate (AMP). XMP and AMP concentration were performed using HPLC with UV detector method modified from Glander et al. [50]

Chemicals

1. Lymphroprep™ (Axis-Shield PoC, Norway)
2. Phosphate buffered saline tablets, 100 mL (Amresco, USA)
3. EDTA disodium salt, dehydrate (J.T. Baker, USA)
4. Xanthosine 5'-monophosphate disodium salt (XMP) (Santa Cruz Biotechnology, USA)
5. Adenosine 5'-monophosphate monohydrate (AMP) (Sigma)
6. Inosine 5'-monophosphate disodium salt (IMP) (Sigma)
7. β-nicotinamide adenine dinucleotide hydrate (β-$NAD^+$) (Sigma)
8. Bovine albumin fraction V solution (Gibco, USA)
9. Perchloric acid, 70% (Ajax Finechem Pty, Australia)
10. Potassium carbonate (Ajax Finechem Pty, Australia)
11. Potassium chloride (Ajax Finechem Pty, Australia)

12. Sodium dihydrogen phosphate dihydrate (Rankem, India)
13. Potassium dihydrogen orthophosphate (Fisher Scientific, UK)
14. Tetrabutylammonium hydrogen sulfate for ion-pair chromatography (Tokyo-Chemical Industry, Japan)
15. Methanol HPLC grade (Fisher Scientific, UK)

Instruments

1. High-performance liquid chromatography (HPLC) system (Dionex) consisted of P680 HPLC pump, ASI-100 automated sample injection and UVD 170U detector.
2. Thermo-shaker (Biosan)

Analytical methods

*Isolation of human peripheral blood mononuclear cells*

PBMCs were isolated from the 10 mL of whole blood in lithium-heparin containing tubes. Each 5 mL of whole blood was layered on top of 5 mL Lymphoprep™ in sterile 15-mL centrifuge tube. Tubes were then centrifuged at 1200 g for 20 minutes at 20 $^{o}$C. PBMCs were carefully collected from the interphase and washed twice times with 12 mL of phosphate-buffered saline combing with 0.2 mM of EDTA and centrifuged at 800 g for 7 minutes, 4 $^{o}$C. The supernatant was removed and cells adjusted to a density of 10 x $10^{6}$ cells/mL after counting. Cell pellets were lysed with cold sterile water and then stored at -20 $^{o}$C until analysis.

*IMPDH activity assay*

Insoluble fragments of disrupted cells were removed by centrifugation at 5000 g for 1 minute at 4 $^{o}$C after thawing. The incubation buffer consisted of 1 mmol/L IMP, 0.5 mmol/L $\beta$-$NAD^{+}$, 40 mmol/L sodium dihydrogen phosphate dehydrate, and 100 mmol/L potassium chloride with a pH of 7.4. The incubation was initiated by the addition of 100 µL of lysate to 260 µL of incubation buffer in a 1.5-mL microcentrifuge tube and

shaken at 800 rpm Thermo-shaker at 37 °C. After 2.5 hours, the enzymatic reaction was terminated by adding 40 µL of 4 mol/L cold perchloric acid, mixing, and placing the samples on ice for 5 minutes. Precipitated proteins were removed by centrifugation at 5000 g for 5 minutes at 4 °C. The supernatant (340 µL) was transferred to a second 1.5-mL microcentrifuge tube containing 20 µL of 5 mol/L potassium carbonate and then mixed. Samples were stored for 2 hours at -20 °C. After thawing, samples were centrifuged at 5000 g for 5 minutes at 4 °C. The supernatant was collected into HPLC vial for analysis.

Chromatography conditions and equipment

The analytical column was Synergi Fusion-RP 80A (4.6 x 150 mm, particle size 4 µ, Phenomenex) protected by a security guard cartridge Fusion-RP (4 x 3.0 mm, Phenomenex). The chromatographic separation was performed at ambient temperature with isocratic elution. The mobile phase components consisted of a 10:90 (vol/vol) mixture of methanol and buffer containing 50 mmol/L potassium dihydrogen orthophosphate and 7 mmol/L tetrabutylammonium hydrogen sulfate (pH 7.5). XMP and AMP were detected at a wavelength of 254 nm. The injection volume was 40 µL, using a flow rate of 1.2 mL/min and the run time was 15 minutes.

Method validation of HPLC

Validation of HPLC method including specificity, selectivity, linearity, accuracy and precision were performed (see in Appendix B).

## 3.4 Statistical analysis

All data were analyzed using SPSS software for Windows version 17. Distribution of continuous data was evaluated by Kolmogorov-Smirnov test or Shapiro-Wilks test and parametric or nonparametric tests were applied consequently where appropriate. A two-tailed alpha of less than 0.05 was considered statistically significant.

- Demographic data such as gender, donor graft type was shown as descriptive statistics. Continuous variables such as body weight, time after transplantation, serum creatinine were expressed as mean and standard deviation.

- Allele and genotype frequency were shown as percentage and evaluated with the *Chi-square* test.

- Pharmacokinetic parameters and IMPDH activity in different genotypes were compared by analysis of variance (ANOVA) or Kruskal-Wallis test or median test.

## 3.5 Ethical consideration

This study was complied with the standard for gathering subjects' information for confidential in every process since data collection, analysis, conclusion and publication. All data collected from patients were coded in order to protect their confidentially. There had no record any details that led to identify to the subjects. Results from this study may be published in scientific journals or presented at medical meetings but subjects were not been personally identify.

# CHAPTER IV

# RESULTS

## 4.1 Subjects

The study was conducted from April to September 2012 at Post-kidney transplantation Outpatient Clinic at King Chulalongkorn Memorial Hospital, Pramongkutklao Hospital and Police General Hospital. All of the patients gave their consent to participate in this study. One hundred and eighteen Thai kidney transplant patients were genotyped and analyzed pharmacokinetics and pharmacodynamics of MPA.

### Demographic data

Of the 118 patients, age of patients in this study was 45.72±11.96 years old (range 18-69 years old). Most patients were male (86 subjects, 72.90%). The most common causes for kidney transplantation were unknown etiology (56 subjects, 47.50%), chronic glomerulonephritis (24 subjects, 20.30%), hypertension (10 subjects, 8.50%), and IgA nephropathy (10 subjects, 8.50%). Major type of donor graft was cadaveric (67 subjects, 56.80%). Times after posttransplantation ranged from 3 months to 228 months (median 54 months). The number of patients receiving cyclosporine as their immunosuppressive regimen was similar to the number of patients receiving tacrolimus (46.60% and 47.50%, respectively). These data were summarized in Table 4.1. Most patients (48.30%) took 1000 mg MMF daily dose. Mean daily dose of MMF was 1211.86±339.10 mg (range, 500-2000 mg) (Table 4.2).

**Table 4.1** Patient characteristics

| Patient's characteristic | Number of patients (%) |
|---|---|
| Mean age (years old) | 45.72±11.96 |
| Sex | |
| - Male | 86 (72.90) |
| - Female | 32 (29.10) |
| Cause for transplantation | |
| - Unknown | 56 (47.50) |
| - Chronic glomerunephritis | 24 (20.30) |
| - Hypertension | 10 (8.50) |
| - IgA nephropathy | 10 (8.50) |
| - Diabetic nephropathy | 6 (5.10) |
| - Autosomal dominant polycystic kidney disease | 4 (3.40) |
| - Focal segmental glomerulosclerosis | 3 (2.50) |
| - Others | 5 (4.20) |
| Type of donor graft | |
| - Cadaveric | 67 (56.80) |
| - Living | 51 (43.20) |
| Combined immunosuppressant drug | |
| - Cyclosporine | 55 (46.60) |
| - Tacrolimus | 56 (47.50) |
| - Sirolimus | 6 (5.10) |
| - Everolimus | 1 (0.80) |
| Body weight (kg), mean±S.D. | 63.86±13.50 |
| Serum creatinine (mg/dL), mean±S.D. | 1.53±0.66 |
| eGFR (mL/min/1.73m$^2$)*, mean±S.D. | 57.29±21.40 |

* eGFR = estimated glomerular filtration rate was calculated using Modification of Diet in Renal Disease study equation ($175 \times (S_{cr})^{-1.154} \times (Age)^{-0.203} \times (0.742$ if female)).

**Table 4.2** Mycophenolate mofetil daily dose

| MMF daily dose | Number of patients (%) |
|---|---|
| 500 mg | 7 (5.90) |
| 750 mg | 1 (0.80) |
| 1000 mg | 57 (48.30) |
| 1250 mg | 2 (1.70) |
| 1500 mg | 45 (38.10) |
| 1750 mg | 1 (0.80) |
| 2000 mg | 5 (4.20) |

## 4.2 Genotyping study

Seven SNPs including UGT1A9*1b, UGT1A9 *-688T>G*, UGT2B7 *802C>T*, MRP-2 *-24C>T*, IMPDH1 *125G>A*, IMPDH1 *-106G>A* and IMPDH2 *3757T>C* were genotyped in all 118 patients. The allelic frequencies and distribution of genotypes of all genes were in the Hardy-Weinberg equilibrium (Table 4.3). Both allele and genotype frequencies in a population remained constant. They were in equilibrium-from generation to generation unless specific distributing influences were introduced.

**Table 4.3** Allele frequency and genotype frequency of seven SNPs

| Genes | SNP | Allele frequency | | Genotype frequency (number of patient, %) | | | *p*-value* |
|---|---|---|---|---|---|---|---|
| | | Wild-type | Variant | Wild-type | Heterozygous variant | Homozygous variant | |
| UGT1A9*1b | -118($T_{9>10}$) | 0.51 | 0.49 | 33 (27.97) | 55 (46.61) | 30 (25.42) | 0.46 |
| UGT1A9 | -688T>G | 0.88 | 0.12 | 93 (78.81) | 21 (17.80) | 4 (3.39) | 0.06 |
| UGT2B7 | 802C>T | 0.73 | 0.27 | 65 (55.08) | 43 (36.44) | 10 (8.48) | 0.45 |
| MRP-2 | -24C>T | 0.77 | 0.23 | 73 (61.86) | 36 (30.51) | 9 (7.63) | 0.14 |
| IMPDH1 | 125G>A | 0.59 | 0.41 | 41 (34.75) | 58 (49.15) | 19 (16.10) | 0.84 |
| IMPDH1 | -106G>A | 0.42 | 0.58 | 19 (16.10) | 61 (51.69) | 38 (32.21) | 0.50 |
| IMPDH2 | 3757T>C | 0.99 | 0.01 | 115 (97.46) | 3 (2.54) | 0 | 0.89 |

* *Chi*-square test

## 4.3 Pharmacokinetic parameters of mycophenolic acid (MPA)

Range, mean±S.D. and median concentration of MPA and MPAG at predose ($C_0$), 30 minutes ($C_{0.5}$) and 120 minutes ($C_2$) after morning MMF dose of all 118 patients were presented in Table 4.4.

**Table 4.4** Concentrations of MPA and MPAG

| | MPA (µg/mL) | | | MPAG (µg/mL) | | |
|---|---|---|---|---|---|---|
| | $C_0$ | $C_{0.5}$ | $C_2$ | $C_0$ | $C_{0.5}$ | $C_2$ |
| Range | 0.16-15.19 | 0.26-70.94 | 1.13-19.44 | 11.59-142.40 | 8.56-146.26 | 11.78-234.40 |
| Mean±S.D. | 2.59±2.12 | 16.02±13.87 | 6.39±3.71 | 49.29±26.43 | 56.16±29.44 | 80.54±39.91 |
| Median | 2.01 | 12.10 | 5.44 | 44.76 | 50.63 | 76.38 |

MPA $AUC_{0\text{-}12\ hr}$ of each patient was predicted using the limited sampling strategy equation.

$$\text{MPA } AUC_{0\text{-}12\ hr} = 17.808 + 5.56*C_0 + 0.548*C_{0.5} + 2.126*C_2$$

Predicted MPA $AUC_{0\text{-}12\ hr}$ ranged from 22.30 to 149.81 mg x h/L. Most patients (66.10%) had predicted MPA $AUC_{0\text{-}12\ hr}$ within target range (30-60 mg x h/L). Seven patients had predicted MPA $AUC_{0\text{-}12\ hr}$ below 30 mg x h/L and 33 patients had predicted MPA $AUC_{0\text{-}12\ hr}$ above 60 mg x h/L (Table 4.5). When focusing in patients with predicted MPA $AUC_{0\text{-}12\ hr}$ above 60 mg x h/L, MMF daily dose was taken higher than patients with predicted MPA $AUC_{0\text{-}12\ hr}$ within target range and below target range. Estimated GFR of patients with predicted MPA $AUC_{0\text{-}12\ hr}$ above 60 mg x h/L was also significantly lower than patients predicted MPA $AUC_{0\text{-}12\ hr}$ within target range and below target range (*p*-value = 0.013).

**Table 4.5** Predicted MPA $AUC_{0\text{-}12\ hr}$ of all patients

| | MPA $AUC_{0\text{-}12\ hr}$ (mg x h/L) | | | *p*-value* |
|---|---|---|---|---|
| | below 30 | 30-60 | above 60 | |
| Number of patient (%) | 7 (6.93) | 78 (66.10) | 33 (27.97) | |
| Mean±S.D. | 26.78±2.46 | 46.59±8.08 | 79.39±20.15 | |
| Median | 27.12 | 46.97 | 73.25 | 0.000 |
| MMF daily dose (mg) Median | 1000 | 1000 | 1500 | 0.000 |
| Serum creatinine (mg/dL) Median | 1.29 | 1.32 | 1.56 | 0.027 |
| eGFR (mL/min/1.73$m^2$) Median | 62.40 | 59.35 | 48.9 | 0.013 |

* Kruskal-Wallis test

When considering predicted MPA $AUC_{0\text{-}12\ hr}$ in each concomitant immunosuppressant drug, median of MPA $AUC_{0\text{-}12\ hr}$ of patients taking cyclosporine was lower than median of MPA $AUC_{0\text{-}12\ hr}$ of patients taking tacrolimus, sirolimus and everolimus (Table 4.6). No statistically difference of MPA $AUC_{0\text{-}12\ hr}$ and normalized MPA $AUC_{0\text{-}12\ hr}$ was found among different concomitant immunosuppressant drug (*p*-value = 0.084 and 0.521, respectively).

**Table 4.6** Predicted MPA $AUC_{0\text{-}12\ hr}$ in each concomitant immunosuppressant drug

| | Concomitant immunosuppressant drug | | | | |
|---|---|---|---|---|---|
| | Cyclosporine (n = 55) | Tacrolimus (n = 56) | Sirolimus (n = 6) | Everolimus (n = 1) | *p*-value[a] |
| Mean daily dose (mg) | 121.82±45.66 | 4.78±2.67 | 2.67±1.75 | 2.5 | |
| MPA $AUC_{0\text{-}12\ hr}$ (mg x h/L) | | | | | |
| Median | 47.29 | 53.94 | 59.65 | 56.25 | 0.084 |
| Range | 22.30-94.25 | 25.52-149.81 | 37.88-103.46 | - | |
| Normalised MPA $AUC_{0\text{-}12\ hr}$ (mg x h/L)[b] | | | | | |
| Median | 85.99 | 82.68 | 92.89 | 112.51 | 0.521 |
| Range | 39.44-144.07 | 34.03-299.63 | 75.76-206.91 | - | |

[a] Kruskal-Wallis test

[b] Normalised MPA $AUC_{0\text{-}12\ hr}$ was normalized by MMF dose 1000 mg.

## 4.4 Impact of genetic polymorphisms on pharmacokinetic parameters of mycophenolic acid and its metabolite

Four SNPs including UGT1A9*1b, UGT1A9 *-688T>G*, UGT2B7 *802C>T* and MRP-2 *-24C>T* were determined on pharmacokinetics of MPA.

Selected patient's characteristics of each SNP were presented in Table 4.7 and Table 4.8. No statistically differences in patient's demographic data were found among different UGT1A9*1b, UGT1A9 *-688T>G*, UGT2B7 *802C>T* and MRP-2 *-24C>T* genotypes.

Before comparing predicted MPA $AUC_{0\text{-}12\ hr}$ in different genotypes of each SNP, MPA $AUC_{0\text{-}12\ hr}$ was normalised by MMF morning dose and body weight. Clearance of drug (CL/F) was calculated from MPA morning dose divided by predicted MPA $AUC_{0\text{-}12hr}$ and normalised by body weight.

**Table 4.7** Patients characteristic for each UGT1A9*1b and UGT1A9 *-688T*>G genotypes

| Patient's characteristic | UGT1A9*1b | | | | UGT1A9 *-688T>G* | | | |
|---|---|---|---|---|---|---|---|---|
| | *-118($T_{9/9}$)* (n = 33) | *-118($T_{9/10}$)* (n = 55) | *-118($T_{10/10}$)* (n = 30) | *p*-value* | *TT* (n = 93) | *TG* (n = 21) | *GG* (n = 4) | *p*-value* |
| Age (years old), mean±S.D. | 46.03±12.25 | 46.96±12.17 | 43.10±11.18 | 0.360 | 45.26±12.09 | 47.19±11.25 | 48.75±14.59 | 0.703 |
| MMF daily dose (mg), median | 1000 | 1000 | 1000 | 0.440 | 1000 | 1000 | 1250 | 0.914 |
| Serum creatinine (mg/dL), median | 1.55 | 1.34 | 1.44 | 0.099 | 1.36 | 1.55 | 1.49 | 0.188 |
| eGFR (mL/min/1.73m$^2$), median | 50.60 | 61.10 | 53.65 | 0.091 | 57.30 | 50.40 | 55.90 | 0.195 |

* Kruskal-Wallis test

**Table 4.8** Patients characteristic for each UGT2B7 *802C>T* and MRP-2 *-24C>T* genotypes

| Patient's characteristic | UGT2B7 *802C>T* | | | | MRP-2 *-24C>T* | | | |
|---|---|---|---|---|---|---|---|---|
| | *CC* (n = 65) | *CT* (n = 43) | *TT* (n = 10) | *p*-value* | *CC* (n = 73) | *CT* (n = 36) | *TT* (n = 9) | *p*-value* |
| Age (years old), mean±S.D. | 46.38±12.49 | 43.93±11.71 | 49.10±8.80 | 0.378 | 45.67±11.92 | 45.58±12.53 | 46.67±11.96 | 0.970 |
| MMF daily dose (mg), median | 1000 | 1000 | 1500 | 0.478 | 1000 | 1000 | 1500 | 0.133 |
| Serum creatinine (mg/dL), median | 1.34 | 1.42 | 1.48 | 0.375 | 1.40 | 1.36 | 1.47 | 0.714 |
| eGFR (mL/min/1.73m$^2$), median | 58.90 | 56.30 | 53.10 | 0.377 | 55.00 | 61.40 | 43.60 | 0.376 |

* Kruskal-Wallis test

Median of MPA $AUC_{0\text{-}12\ hr}$ and CL/F in different UGT1A9*1b genotypes were shown in Table 4.9. Predicted MPA $AUC_{0\text{-}12\ hr}$ in patients with homozygous variant was higher than predicted MPA $AUC_{0\text{-}12\ hr}$ in patients with wild-type gene and patients with heterozygous variant, however, there was no statistically significance. About CL/F of MPA, patients with homozygous variant had a lower CL/F when comparing with another genotype.

**Table 4.9** Pharmacokinetic parameters in different UGT1A9*1b genotypes

| Pharmacokinetic parameters | *-118*($T_{9/9}$) (n = 33) | *-118*($T_{9/10}$) (n = 55) | *-118*($T_{10/10}$) (n = 30) | *p*-value* |
|---|---|---|---|---|
| Predicted MPA $AUC_{0\text{-}12\ hr}$ (mg x h/L/kg/mg dose) | 5.967 | 5.323 | 5.271 | 0.189 |
| CL/F (L/h/kg) | 0.120 | 0.140 | 0.140 | 0.059 |
| MPAG:MPA ratio at $C_0$ | 19.760 | 18.450 | 22.035 | 0.874 |
| MPAG:MPA ratio at $C_{0.5}$ | 4.930 | 3.440 | 4.565 | 0.370 |
| MPAG:MPA ratio at $C_2$ | 13.210 | 13.940 | 14.110 | 0.887 |

* Kruskal-Wallis test

Among different UGT1A9 *-688T>G* genotypes, median of predicted MPA $AUC_{0\text{-}12\ hr}$ and CL/F were presented in Table 4.10. There were no differences of pharmacokinetic parameters between patients with wild-type and patients with variant genes.

**Table 4.10** Pharmacokinetic parameters in different UGT1A9 *-688T>G* genotypes

| Pharmacokinetic parameters | *TT* (n = 93) | *TG* (n = 21) | *GG* (n = 4) | *p*-value* |
|---|---|---|---|---|
| Predicted MPA $AUC_{0\text{-}12\ hr}$ (mg x h/L/kg/mg dose) | 5.323 | 6.111 | 4.838 | 0.139 |
| CL/F (L/h/kg) | 0.140 | 0.110 | 0.140 | 0.204 |
| MPAG:MPA ratio at $C_0$ | 19.990 | 17.010 | 36.230 | 0.664 |
| MPAG:MPA ratio at $C_{0.5}$ | 3.900 | 4.790 | 4.190 | 0.643 |
| MPAG:MPA ratio at $C_2$ | 14.110 | 13.940 | 13.210 | 0.789 |

* Kruskal-Wallis test

About UGT2B7 *802C>T*, there were no difference in predicted MPA $AUC_{0\text{-}12\ hr}$ and CL/F between patients with wild-type and patients with homozygous or heterozygous variant genotype (Table 4.11). When considering MPAG:MPA ratio, patients with homozygous variant genotype had higher ratio at all concentrations, especially at $C_{0.5}$, there was a statistically different significance (p-value = 0.041).

**Table 4.11** Pharmacokinetic parameters in different UGT2B7 *802C>T* genotypes

| Pharmacokinetic parameters | *CC* (n = 65) | *CT* (n = 43) | *TT* (n = 10) | *p*-value* |
|---|---|---|---|---|
| Predicted MPA $AUC_{0\text{-}12\ hr}$ (mg x h/L/kg/mg dose) | 5.369 | 5.546 | 5.119 | 0.834 |
| CL/F (L/h/kg) | 0.140 | 0.130 | 0.145 | 0.198 |
| MPAG:MPA ratio at $C_0$ | 18.520 | 20.540 | 26.465 | 0.280 |
| MPAG:MPA ratio at $C_{0.5}$ | 3.330 | 5.470 | 6.015 | 0.041 |
| MPAG:MPA ratio at $C_2$ | 13.730 | 12.990 | 16.400 | 0.645 |

* Kruskal-Wallis test

For MRP-2 *-24C>T*, patients with homozygous variant genotype had a statistically significance lower predicted MPA $AUC_{0\text{-}12\ hr}$ when comparing with patients with wild-type gene and patients with heterozygous variant genotype. In addition, CL/F of patients with homozygous or heterozygous variant genotype trended higher than CL/F of patients with wild-type (Table 4.12).

**Table 4.12** Pharmacokinetic parameters in different MRP-2 *-24C>T* genotypes

| Pharmacokinetic parameters | *CC* (n = 73) | *CT* (n = 36) | *TT* (n = 9) | *p*-value* |
|---|---|---|---|---|
| Predicted MPA $AUC_{0\text{-}12\ hr}$ (mg x h/L/kg/mg dose) | 5.921 | 5.063 | 4.655 | 0.013 |
| CL/F (L/h/kg) | 0.120 | 0.150 | 0.150 | 0.052 |
| MPAG:MPA ratio at $C_0$ | 19.000 | 20.105 | 24.690 | 0.841 |
| MPAG:MPA ratio at $C_{0.5}$ | 4.010 | 4.095 | 2.660 | 0.940 |
| MPAG:MPA ratio at $C_2$ | 14.510 | 12.520 | 13.490 | 0.638 |

* Median test

The pharmacokinetic parameters were compared between patients with wild-type gene and patients with heterozygous or homozygous variant gene of all four SNPs. These data were demonstrated in Table 4.13. About MRP-2 *-24C>T*, there was a statistically difference of predicted MPA $AUC_{0\text{-}12\ hr}$ among patients with wild-type gene and patients with variant gene (*p*-value = 0.008). In addition, CL/F of patients with MRP-2 variant gene was statistically higher than CL/F of patients with wild-type gene (*p*-value = 0.025). When considering the MPAG:MPA ratio at $C_{0.5}$, patients with carriers of UGT2B7 *802C>T* had statistically higher MPAG:MPA ratio than patients with wild-type gene. (*p*-value = 0.012).

**Table 4.13** Pharmacokinetic parameters comparing between patients with wild-type gene and patients with heterozygous or homozygous variant gene

| Pharmacokinetic parameters | UGT1A9*1b | | | UGT 1A9 *-688T>G* | | | UGT2B7 *802C>T* | | | MRP-2 *-24C>T* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *-118(T)*$_{9/9}$ (n = 33) | *-118(T)*$_{9/10}$ and *-118(T)*$_{10/10}$ (n = 85) | *p*-value[a] | *TT* (n = 93) | *TG* and *GG* (n = 25) | *p*-value[a] | *CC* (n = 65) | *CT* and *TT* (n = 53) | *p*-value[a] | *CC* (n = 73) | *CT* and *TT* (n = 45) | *p*-value[b] |
| Predicted MPA $AUC_{0\text{-}12\ hr}$ (mg x h/L/kg/mg dose) | 5.967 | 5.323 | 0.133 | 5.323 | 6.074 | 0.112 | 5.369 | 5.513 | 0.963 | 5.921 | 5.044 | 0.008 |
| CL/F (L/h/kg) | 0.120 | 0.140 | 0.051 | 0.140 | 0.110 | 0.089 | 0.140 | 0.130 | 0.103 | 0.120 | 0.150 | 0.025 |
| MPAG:MPA ratio at $C_0$ | 19.760 | 19.990 | 0.721 | 19.990 | 17.830 | 0.813 | 18.520 | 21.110 | 0.723 | 19.000 | 20.220 | 0.705 |
| MPAG:MPA ratio at $C_{0.5}$ | 4.390 | 3.890 | 0.212 | 3.900 | 4.390 | 0.370 | 3.330 | 5.470 | 0.012 | 4.010 | 3.890 | 1.000 |
| MPAG:MPA ratio at $C_2$ | 13.210 | 13.940 | 0.631 | 13.690 | 15.310 | 0.498 | 13.730 | 14.300 | 0.727 | 14.510 | 12.990 | 0.448 |

[a] Mann-Whitney U test, [b] Median test

## 4.5 Impact of genetic polymorphisms on pharmacodynamic parameters of mycophenolic acid

Three SNPs consisting of IMPDH1 *125G>A,* IMPDH1 *-106G>A and* IMPDH2 *3757T>C* were considered on pharmacodynamics of MPA.

The IMPDH activity was calculated from the measured concentrations of XMP and AMP according to the following equation:

$$\text{IMPDH activity } (\mu mol \times s^{-1} \times mol^{-1} \text{ AMP}) = \frac{\text{produced XMP } (\mu mol/L) \times 10^{6}}{\text{incubation time (s)} \times \text{measured AMP } (\mu mol/L)}$$

IMPDH activity at three time points (predose, 30 minutes and 2 hours after MMF morning dose) of 118 patients was presented in Table 4.14. According to the increase in MPA concentration, the IMPDH activity decreased after 30 minutes and 2 hours after morning MMF dose.

**Table 4.14** IMPDH activity at three time points of 118 patients

| | IMPDH activity (μmol x $s^{-1}$ x $mol^{-1}$ AMP) | | MPA concentration (μg/mL) | |
|---|---|---|---|---|
| | Mean±S.D. (Range) | Median | Mean±S.D. (Range) | Median |
| $T_0$ | 121.23±51.79 (37.34-279.37) | 107.51 | 2.59±2.12 (0.16-15.19) | 2.01 |
| $T_{0.5}$ | 103.96±46.58 (19.96-309.81) | 97.17 | 16.02±13.87 (0.26-70.94) | 12.10 |
| $T_2$ | 106.63±50.38 (22.97-324.97) | 91.88 | 6.39±3.71 (1.13-19.44) | 5.44 |

For IMPDH1 *125G>A*, median of IMPDH activity was compared among the different genotypes (Table 4.15). There were no significantly differences of MPA concentrations at each time point among different genotypes. At $T_0$ and $T_2$, IMPDH activity of patients with homozygous variant was significantly higher than IMPDH activity

of patients with wild-type and patients with heterozygous variant (*p*-value = 0.043 and 0.031, respectively).

**Table 4.15** IMPDH activity in different IMPDH1 *125G>A* genotypes

| | IMPDH activity (μmol x $s^{-1}$ x $mol^{-1}$ AMP) | | | MPA concentration (μg/mL) | | |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_{0.5}$ | $T_2$ | $T_0$ | $T_{0.5}$ | $T_2$ |
| *GG* (n = 41) | 97.70 | 88.58 | 91.94 | 2.16 | 10.64 | 4.99 |
| *GA* (n = 58) | 108.67 | 98.68 | 89.42 | 1.83 | 12.93 | 5.91 |
| *AA* (n = 19) | 129.21 | 110.85 | 113.47 | 2.24 | 16.56 | 5.47 |
| *p*-value* | 0.043 | 0.195 | 0.031 | 0.185 | 0.728 | 0.970 |

* Kruskal-Wallis test

About IMPDH1 *-106G>A*, MPA concentrations at each time point among different genotypes did not differ. In addition, no association between IMPDH activity and SNPs in IMPDH1 *-106G>A* was documented (Table 4.16).

**Table 4.16** IMPDH activity in different IMPDH1 *-106G>A* genotypes

| | IMPDH activity (μmol x $s^{-1}$ x $mol^{-1}$ AMP) | | | MPA concentration (μg/mL) | | |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_{0.5}$ | $T_2$ | $T_0$ | $T_{0.5}$ | $T_2$ |
| *GG* (n = 19) | 97.70 | 85.71 | 88.32 | 1.78 | 9.49 | 4.84 |
| *GA* (n = 61) | 111.00 | 100.15 | 94.10 | 1.99 | 10.67 | 5.93 |
| *AA* (n = 38) | 106.89 | 88.18 | 91.19 | 2.08 | 16.09 | 5.23 |
| *p*-value* | 0.990 | 0.274 | 0.878 | 0.355 | 0.595 | 0.606 |

* Kruskal-Wallis test

Only three patients with heterozygous variant gene of IMPDH2 *3757T>C* was compared with 115 patients with wild-type gene. No differences of IMPDH activity was found among patients with wild-type gene and patients with heterozygous variant gene

(Table 4.17). There was a trend for the higher IMPDH activity at each time point in patients with heterozygous variant gene, but the difference did not reach statistical significance.

**Table 4.17** IMPDH activity in different IMPDH2 *3757T>C* genotypes

| | IMPDH activity (μmol x $s^{-1}$ x $mol^{-1}$ AMP) | | | MPA concentration (μg/mL) | | |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_{0.5}$ | $T_2$ | $T_0$ | $T_{0.5}$ | $T_2$ |
| *TT* (n = 115) | 106.86 | 94.10 | 91.82 | 2.02 | 13.07 | 5.42 |
| *TC* (n = 3) | 111.00 | 108.10 | 109.70 | 1.31 | 3.32 | 7.58 |
| *p*-value* | 0.590 | 0.334 | 0.626 | 0.256 | 0.074 | 0.285 |

* Kruskal-Wallis test

# CHAPTER V

# DISCUSSION

## 5.1 Subjects

One hundred and eighteen patients were included into the study during April to September, 2012. Characteristics of 118 patients were similar to the other studies. Mean age was 45.72 years. The original guideline of MMF dose was fixed at 2 g/day. However, the majority of Thai kidney transplant patients were taking MMF at dose 1 g/day (range 0.5 to 2 g/day). The reasons for taking MMF daily dose below the recommended dose included bone marrow suppression, infection and gastrointestinal intolerance.

## 5.2 Genotyping study

Allele frequencies and genotype frequencies of seven genes including UGT1A9*1b, UGT1A9 *-688T>G*, UGT2B7 *802C>T*, MRP-2 *-24C>T*, IMPDH1 *125G>A*, IMPDH1 *-106G>A*, IMPDH2 *3757T>C* were not significantly different from that reported in the literature for Asian.[12,38,45,46]

## 5.3 Pharmacokinetic parameters of mycophenolic acid

Limited sampling strategy for prediction of MPA $AUC_{0\text{-}12\ hr}$ was developed for using in this study. Most patients (66.10%) had predicted MPA $AUC_{0\text{-}12\ hr}$ within target range (30-60 mg x h/L). Thirty-three patients (27.97%) had predicted MPA $AUC_{0\text{-}12\ hr}$ above 60 mg x h/L. Factors that may affect predicted MPA $AUC_{0\text{-}12\ hr}$ were received MMF daily dose and renal function. Regarding the predicted MPA $AUC_{0\text{-}12\ hr}$ above 60 mg x

h/L, majority of patients had 1500 mg of MMF daily dose and median of eGFR was lower than 60 mL/min/1.73 $m^2$. Decreased renal function leads to reduced renal excretion of MPAG, causing concentrations to become markedly elevated. In patients with eGFR < 25 mL/min/1.73 $m^2$, MPAG accumulates 3- to 6-fold. Accumulated MPAG competes with and displaces MPA from protein binding site thus increasing MPA unbound fraction. In addition, MPAG can be enterohepatically recycled to MPA.[3,4] When considering the predicted MPA $AUC_{0\text{-}12\ hr}$ among different concomitant immunosuppressant drug, patients on cyclosporine had lower exposure to MPA as compared with patients on tacrolimus, sirolimus, and everolimus. Because cyclosporine is an inhibitor of the MRP-2, that inhibiting the active flux of glucuronide metabolite (MPAG) into bile. This explained the reduced of enterohepatic recirculation of MPA in the cyclosporine-treated patients.[51] However, there was no a statistically difference of predicted MPA $AUC_{0\text{-}12\ hr}$ and dose-normalized predicted MPA $AUC_{0\text{-}12\ hr}$ in all concomitant immunosuppressant drugs. Patients on cyclosporine may not require double doses of MMF to achieve the same exposure.

## 5.4 Impact of genetic polymorphisms on pharmacokinetic parameters of MPA and its metabolite

All patients' demographic data among different genotypes of UGT1A9*1b, UGT1A9 *-688T>G*, UGT2B7 *802C>T* and MRP-2 *-24C>T* were not different. Therefore, the differences in pharmacokinetic parameters of MPA were resulted from other factors than demographic data. Differences in genotypes might be one of important factor.

UGT1A9 is the key UGT responsible for glucuronidation of MPA to MPAG in the liver. UGT1A9*1b and UGT1A9 *T-688G* polymorphisms were found in Asian subjects.[12,13] The influence of these polymorphisms on pharmacokinetics of MMF was investigated in this study. Similar to previous study [13], there were no significant differences in pharmacokinetic parameters of MPA among the three UGT1A9*1b

genotype groups. Yamanaka et al found that the insertion of a thymidine in this region (-118) increased 2.6-fold transcription activity. The *-118(T)$_{10/10}$* produced higher *in vitro* glucuronidation rates, suggesting lower MPA exposure.[52] In accordance with *in vitro* data, the predicted MPA $AUC_{0\text{-}12\ hr}$ of patients with *-118(T)$_{10/10}$* carriers in this study seems to be low. For UGT1A9 *T-688G*, it was the first time for studying the functional polymorphism. No significant differences in predicted MPA $AUC_{0\text{-}12\ hr}$ and CL/F among different genotypes were documented. However, in the present study, the frequency of the UGT1A9 *-688GG* genotype was 3.39% (n = 4). The sample size was too small to compare the pharmacokinetic parameters of MPA between UGT1A9 *-688GG* genotype group and UGT1A9 *-688TT* genotype or UGT1A9 *-688TG* genotype groups.

UGT2B7 is involved in another metabolic pathway for conversion of MPA to AcMPAG, a minor metabolite. The measurement of AcMPAG concentrations was not performed in this study. Because of the highly unstable metabolite, to measure AcMPAG concentration reliably, careful sample collection and storage were needed, with acidification of the plasma samples to avoid deglucuronidation of the AcMPAG metabolite. There were no significant differences in predicted MPA $AUC_{0\text{-}12\ hr}$ and CL/F among UGT2B7 *802C>T* genotype groups in the present study. Similar to the results of Bernard et al [37], Kagaya et al [38] and Zhang et al [13], no association with the UGT2B7 *802C>T* and MPA pharmacokinetics was reported. Sawyer et al reported that the plasma concentrations of morphine, the phenotypic probe of UGT2B7, were lower in *TT* patients compared with *CC* and *CT* patients (*p*-value = 0.04), although there was no association with *in vitro* enzyme activities. Sawyer et al proposed in this report that microsomal systems may not reflect *in vivo* activity for UGT2B7, particularly if the polymorphism affected transcriptional activity of UGT2B7.[53] However, UGT2B7 *802C>T* SNPs had the association with other pharmacokinetic parameter. The MPAG:MPA ratio at $C_{0.5}$ in patients with variant genotype was significantly higher than the ratio in patients with wild-type. It can be explained that this metabolic pathway decreased in patients with variant gene, then MPA was more metabolized to MPAG. The same tendency was

found in MPAG:MPA ratio at $C_0$ and MPAG:MPA ratio at $C_2$ in patients with variant genotype but differences did not reach statistical significance.

MPAG is extensively produced in the liver, partly excreted into the bile, and substantially hydrolyzed to MPA in the small intestine, leading to MPA reabsorption, which is estimated to contribute 10-60% of the total MPA exposure. Excretion of MPAG into the bile occurs through membrane drug-efflux transporter (MRP-2).[54] MRP-2 *-24C>T* variant was found to influence MPA pharmacokinetics in this population. Patients with MRP-2 *-24C>T* variant had a significantly lower predicted MPA $AUC_{0\text{-}12\ hr}$ than patients with wild-type gene. It could be described that patients with MRP-2 *-24C>T* variant genotype had a decreased MRP-2 expression and/or activity for exporting MPAG into the bile that affected decrease the enterohepatic recirculation of MPA. Therefore, MPA $AUC_{0\text{-}12\ hr}$ was low in patients with MRP-2 *-24C>T* variant. In addition, clearance of MPA in patient with MRP-2 *-24C>T* variant was significantly higher than patients with wild-type gene. This result was consistent with the decreased enterohepatic recirculation of MPA then MPAG was more excreted. The findings in the present study was similar to The study of Lloberas et al.[55] They determined the relationship between SNPs in MRP-2 gene and MPA pharmacokinetics in 66 kidney transplant patients. At month 3, patients with carriers of the C-24T SNP had a significantly lower MPA $AUC_{0\text{-}12\ hr}$ comparing with patients with wild-type (48.12±4.90 and 68.73±6.78 mg x h/L, *p*-value = 0.023). However, these results in the present study and Lloberas et al differed from the other studies. Naesons et al [16] investigated the impact of MRP-2 polymorphisms on MPA exposure parameters in 95 kidney transplant patients who treated with tacrolimus in combination with 1 (n = 63) or 2 grams (n = 32) MMF divided in two doses. They reported no differences in pharmacokinetic parameters (dose-normalised $AUC_{0\text{-}12\ hr}$ or CL/F) at day 7 after kidney transplantation between non-carriers (n = 54) and carriers (n = 41) of the MRP-2 *-24C>T* SNP. On day 42, 90 and 360 after transplantation, dose-normalised MPA $AUC_{0\text{-}12\ hr}$ were consistently higher in carriers of the MRP-2 *-24C>T* SNP (17.0%, 18.3% and 23.0%, respectively) compared to non-

carrier. These differences reached statistical significance only at six weeks after transplantation (*p*-value = 0.008). The results suggested that the MRP-2 *C-24T* SNP was associated with an increase in expression and activity and enhanced enterohepatic recirculation and a lower oral clearance of MPA. This discrepancy between the findings of the present study and those of Naesons et al can not be explained well because the sample size of two studies were small and the results of Naesons et al may have been biased by the use of the Emit assay for measuring MPA, with cross-reaction between MPA and AcMPAG measurement. In addition, the impact of MRP-2 *-24C>T* genetic polymorphism in other studies were not found the difference in exposure parameters of MPA.[13,55-59]

## 5.5 Impact of genetic polymorphisms on pharmacodynamic parameters of mycophenolic acid

The determination of IMPDH activity was a direct pharmacodynamic parameter of MPA activity. In this study, IMPDH activity was measured in peripheral blood mononuclear cells. Several different assay methodologies have been reported for the measurement of IMPDH activity in different cell types. MPA inhibits both IMPDH1 and IMPDH2 isoforms. However, IMPDH2 was 3.9-fold more sensitive to MPA than the type1 isoform. IMPDH activity assay also measures the activity of both IMPDH1 and IMPDH2.[60]

After oral administration of MMF, IMPDH activity is decreased by 65-100%, persisting for 4-8 hours, and returned to the level of activity before the intake of MMF after 12 hours.[21,44] All studies have found considerable interpatient variability in IMPDH activity, SNPs in the gene encoding for IMPDH could explain part of the variability in IMPDH activity.[61] In this study, genetic polymorphisms influence on MPA pharmacodynamics was observed with more IMPDH activity at predose and 2 hour after morning MMF dose in patients with homozygous variant of IMPDH1 *125G>A* SNP

compared with patients with wild-type and patients with heterozygous variant. It was the first time for comparing the IMPDH activity with IMPDH1 SNPs. Previous studies conducted in kidney transplant patients proved an association of IMPDH1 *125G>A* and IMPDH1 *-106G>A* SNPs with acute rejection and adverse effects.[20,40]

For IMPDH2 *3757T>C* SNP, no difference of IMPDH activity was found in patients with heterozygous variant compared with patients with wild-type. Because of low allele frequency of variant in Asian population, the significant difference did not reach. Sombogaard et al [21] reported that the allele frequency of IMPDH2 *3757T>C* SNP for 80 Caucasian kidney transplant patients was 6.9%. The IMPDH activity 12 hour after MMF intake was significantly higher in the variant carrier group compared with the IMPDH2 3757TT wild-type group (40.8 and 24.5 μmol/s/mol AMP, *p*-value = 0.02).

However, a large cohort study of Shah et al [62] in 1040 kidney transplant patients found that the presence of the A (rs2778293) and G alleles (rs2778294) in the IMPDH1 variants and carriage of the C allele (rs11706052) in the IMPDH2 variant did not increase the risk of rejection or affect graft function by 1 year after transplantation. Furthermore, these polymorphisms did not impact graft or patient survival at 5 years.

# CHAPTER VI

# CONCLUSION

This was the first study which considered the impacts of genetic polymorphisms on pharmacokinetics and pharmacodynamics of mycophenolate mofetil in Thai kidney transplant patients at King chulalongkorn Memorial Hospital, Pramongkutkloa Hospital and Police General Hospital.

Seven SNPs were investigated in 118 subjects. Allele frequencies and genotype frequencies of UGT1A9*1b, UGT1A9 *-688T>G*, UGT2B7 *802C>T*, MRP-2 *-24C>T*, IMPDH1 *125G>A*, IMPDH1 *-106G>A* and IMPDH2 *3757T>C* in this study were similar to other Asian population.

From this study, the presence of *UGT1A9*1b*, *UGT1A9* -688T>G and *UGT2B7* 802C>T SNPs did not cause any significant variation in pharmacokinetic parameters of MPA. Only MRP-2 gene was found the functional characterization on polymorphic variants. In patients with MRP-2 variant, the predicted MPA $AUC_{0\text{-}12\ hr}$ was lower and oral clearance of MPA was higher than those with wild-type.

IMPDH1 *125G>A* SNP might influence MPA pharmacodynamics. Patients with variant gene had higher IMPDH activity than patients with wild-type. Other SNPs, including IMPDH1 *-106G>A* and IMPDH2 *3757T>C*, IMPDH activity did not different between patients with wild-type and variant gene.

Limitations of the present study were the small sample size that inadequate to detect the association of genetic polymorphisms with pharmacokinetic and

pharmacodynamic parameters of MPA. In addition, blood sample occurred only during the absorptive and distributive phase, limiting the ability to measure later time points and include enterohepatic circulation in the overall AUC and IMPDH activity determination.

Further study should be to evaluate the clinical outcomes when using the genetic polymorphisms to guide personalized dosing regimens of MMF and MMF therapy in kidney transplant patients.

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# APPENDICES

# Appendix A

## Patient Medical Record Form

Participant code.....

Date of collection……

Gender [ ] 1. Male [ ] 2. Female

Age……years old Body weight……kg Height…….cm

Date of transplantation……Time after transplantation…..year……mo…...day

Donor graft type [ ] 1. Living [ ] 2. Cadaveric

Medical history for transplantation

[ ] 1. Diabetic nephropathy [ ] 2. Chronic glomerulonephritis

[ ] 3. Hypertensive nephropathy [ ] 4. Obstructive nephropathy

[ ] 5. …….. [ ] 6. …….

Social history alcohol drink……Smoking.....……

**Immunosuppressive drugs using**

| Drug | Dosage regimen | | | | | |
|---|---|---|---|---|---|---|
| | date | date | date | date | date | date |
| Cyclosporine | | | | | | |
| Tacrolimus | | | | | | |
| MMF | | | | | | |
| Sirolimus | | | | | | |
| Steroid ……. | | | | | | |
| | | | | | | |

Concomitant drugs

| Drug | Dosage regimen | | | | | |
|---|---|---|---|---|---|---|
| | date | date | date | date | date | date |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

Therapeutic drug monitoring of immunosuppressive drugs

| Drug | Normal range | date | date | date | date | date |
|---|---|---|---|---|---|---|
| Cyclosporine | $C_0$ | | | | | |
| | $C_2$ | | | | | |
| Tacrolimus | $C_0$ | | | | | |
| Sirolimus | $C_0$ | | | | | |

Laboratory test data

| Labs | Normal range | date | date | date | date |
|---|---|---|---|---|---|
| CBCs | | | | | |
| Hct | | | | | |
| Hgb | | | | | |
| WBC | | | | | |
| - Neutrophil | | | | | |
| - Lymphocyte | | | | | |
| - Monocyte | | | | | |
| - Basophil | | | | | |
| Platelet | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| **Renal function** | | | | | |
| BUN | | | | | |
| SCr | | | | | |
| CrCL | | | | | |
| **Liver function** | | | | | |
| Serum albumin | | | | | |
| AST | | | | | |
| ALT | | | | | |
| Alk Phos | | | | | |
| Total bilirubin | | | | | |
| Direct bilirubin | | | | | |
| **Others** | | | | | |
| Total Cholesterol | | | | | |
| LDL | | | | | |
| TG | | | | | |
| HDL | | | | | |
| | | | | | |

**Blood sampling for MMF pharmacokinetic and pharmacodynamic analysis**

| | **Date** | hr at.... | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MPA level | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| XMP level | | | | | | | | |
| AMP level | | | | | | | | |

# Appendix B

## Validation of HPLC method for pharmacokinetics and pharmacodynamics of MMF

### 1. Validation of HPLC method for MPA and MPAG concentrations

#### 1.1 Specificity and selectivity

Specificity of the method was determined by analyzing six independent sources of drug-free plasma obtained from National Blood Centre, Thai Red Cross Society. No interferences with endogenous substances were observed, in both the chromatogram of drug-free plasma for MMF and in patient samples. The retention time of MPAG, phenolphthalein β-D- glucuronide, MPA and carboxy butoxy ether of MPA were 6.75, 7.35, 11.20, 12.25 minutes, respectively.

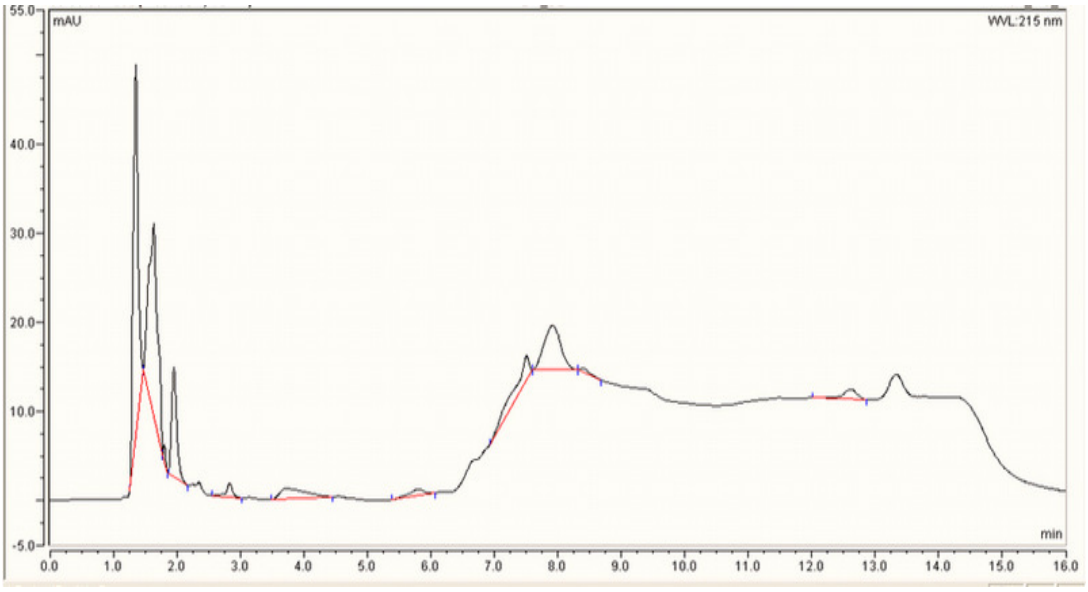


**Figure 1** Chromatogram of drug-free-plasma

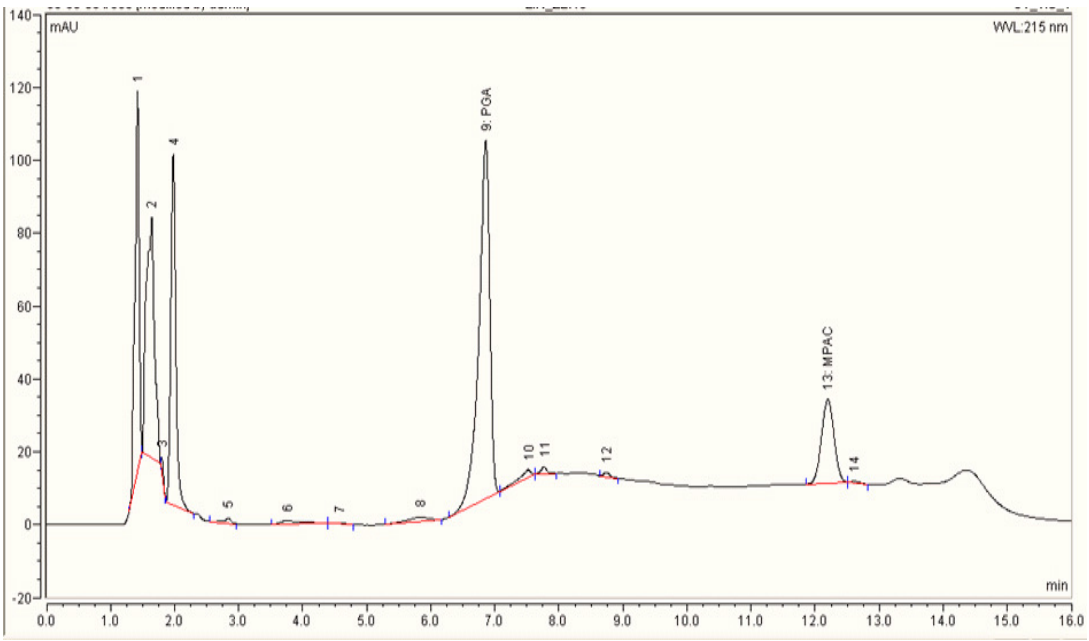


**Figure 2** Chromatogram of extracted blank plasma with internal standard (IS). Retention time of phenolphthalein glucuronic acid (PGA) = 6.75 minutes and carboxy butoxy ether of MPA (MPAC) = 12.25 minutes

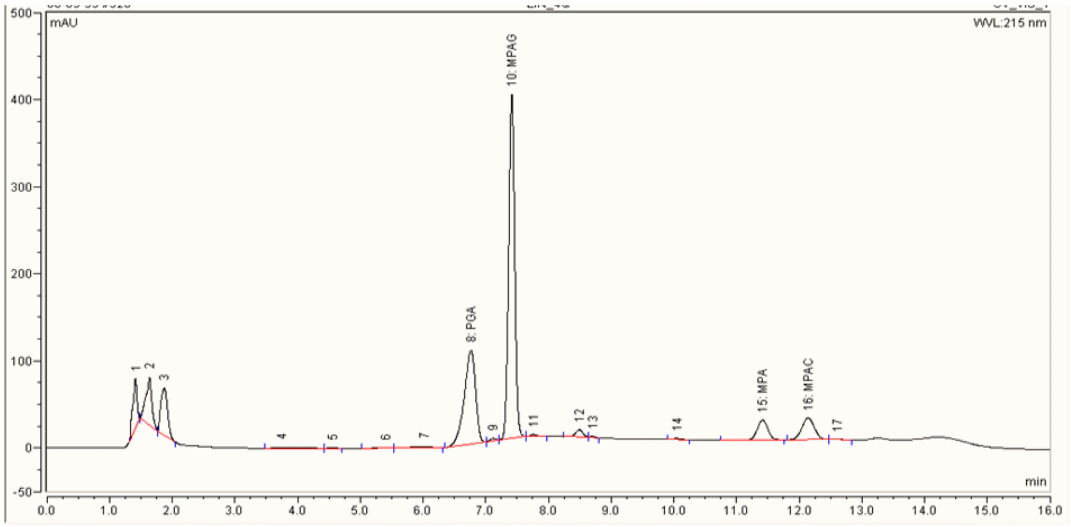


**Figure 3** Chromatogram of drug-spiked-plasma (MPAG 108 µg/mL and MPA 10 µg/mL, respectively)

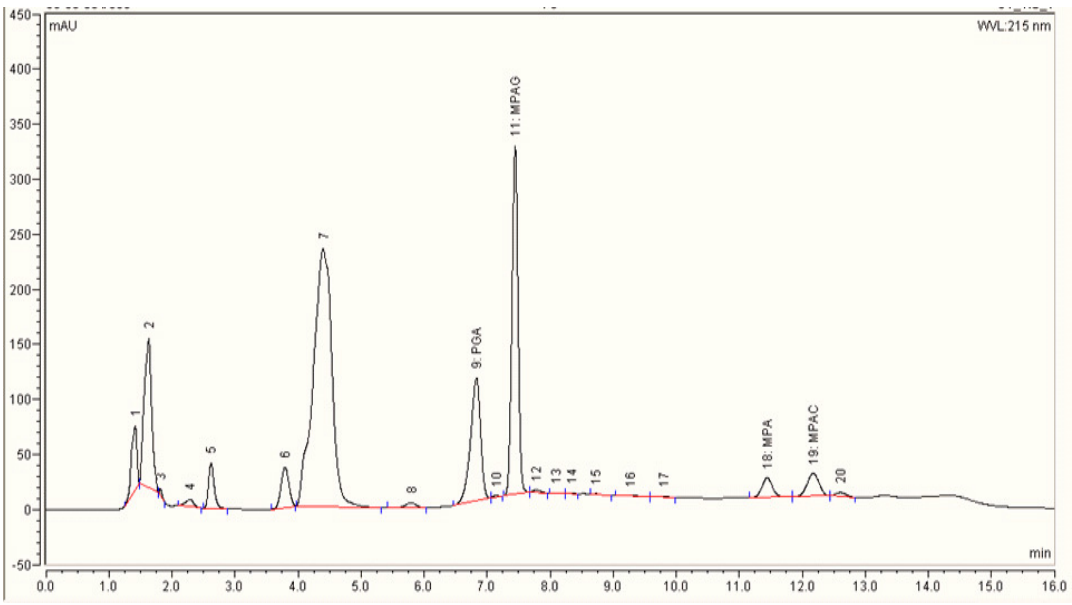


**Figure 4** Chromatogram of kidney transplant patient plasma

1.2 Linearity

Calibration curves were prepared by drug-free plasma spiked with amounts of each analyte (MPA at concentrations 0.25, 1, 5, 15, 30, 60 µg/mL and MPAG at concentrations 5, 10, 54, 108, 216 and 324 µg/mL). The calibration curves were shown in Figure 5 and 6. Peak area ratio of active ingredients to IS versus concentration was plotted. The weighted least squares linear regression equation was calculated using the peak area ratio for every analyte to IS. The coefficient of determination was employed to evaluate the linearity of the calibration curve.

y = Peak area ratio of active ingredients to IS

x = plasma drug concentration

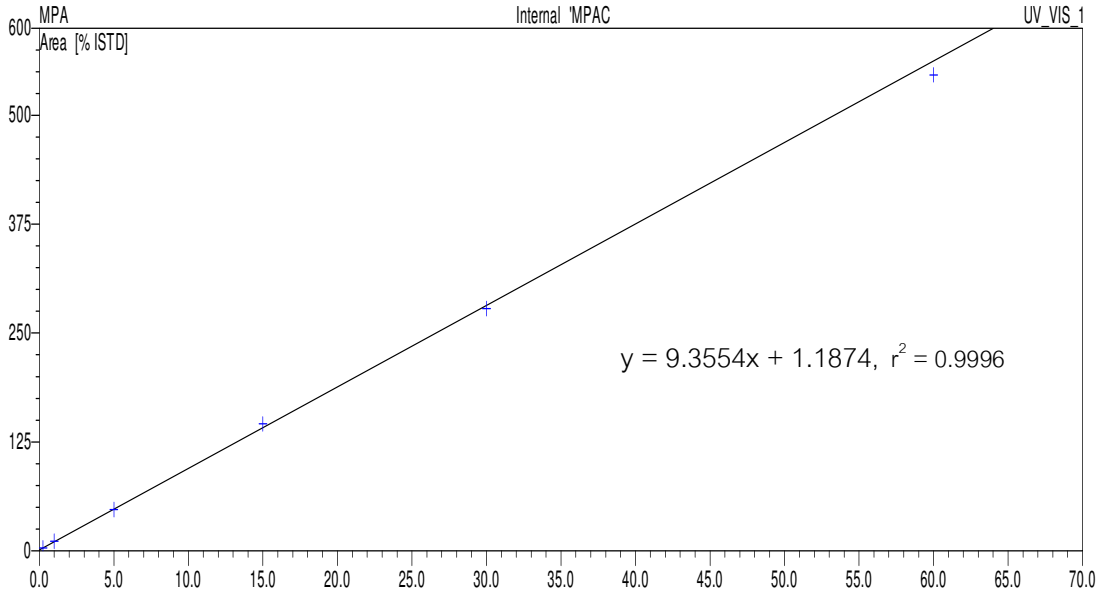


**Figure 5** Calibration curve of mycophenolic acid.

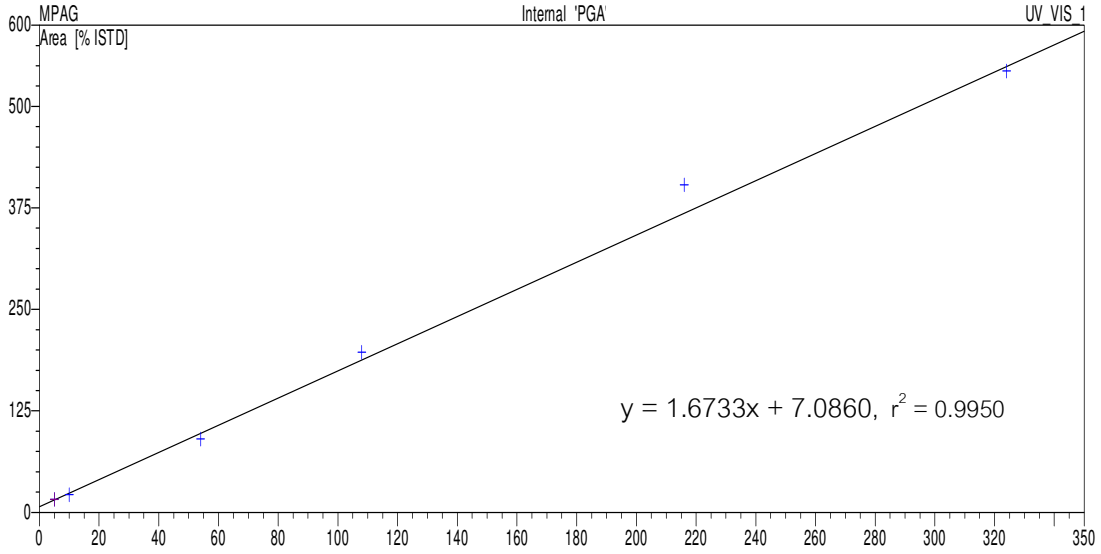


**Figure 6** Calibration curve of mycophenolic acid glucuronide.

1.3 Limit of quantification

Limit of quantification (LOQ) was defined as smallest concentration on the calibration curve which had linear relationship with peak area ratio and had acceptable accuracy and precision within ±20%. The accuracy and precision of intra-day and inter-day of LOQ of MPA and MPAG were shown in Table 1 and Table 2.

1.4 Quality Control (QC)

Accuracy and precision were assessed at the three sets of quality control samples (low, medium and high, 5 determinations at each concentration). For accuracy, the mean value should be within 15% of the actual value was acceptable. The precision measured at each concentration should not exceed 15% of the coefficient of variation (CV). As shown in Table 1 and Table 2, the accuracy and precision of intra-day and inter-day were all in acceptable criteria.

**Table 1** Intra-day and inter-day accuracy and precision of the method for the determination of MPA

| Nominal concentration (µg/mL) | | Intra-day (n = 5) | | | Inter-day (n = 15) | | |
|---|---|---|---|---|---|---|---|
| | | Analyzed conc. (µg/ml) mean±SD | Accuracy (%) | Precision (%CV) | Analyzed conc. (µg/ml) mean±SD | Accuracy (%) | Precision (%CV) |
| LLOQ | 0.25 | 0.24±0.04 | -2.67 | 16.61 | 0.24±0.03 | -4.00 | 12.50 |
| Low QC | 0.75 | 0.76±0.09 | 1.78 | 11.84 | 0.74±0.08 | -1.78 | 10.81 |
| Medium QC | 20 | 18.58±0.56 | -7.10 | 3.01 | 18.86±0.34 | -5.72 | 1.80 |
| High QC | 40 | 37.38±0.71 | -6.55 | 1.90 | 37.87±0.46 | -5.33 | 1.23 |

**Table 2** Intra-day and inter-day accuracy and precision of the method for the determination of MPAG

| Nominal concentration (µg/mL) | | Intra-day (n = 5) | | | Inter-day (n = 15) | | |
|---|---|---|---|---|---|---|---|
| | | Analyzed conc. (µg/ml) mean±SD | Accuracy (%) | Precision (%CV) | Analyzed conc. (µg/ml) mean±SD | Accuracy (%) | Precision (%CV) |
| LLOQ | 5 | 4.52±0.44 | -9.53 | 9.74 | 4.79±0.67 | -4.13 | 13.97 |
| Low QC | 15 | 14.28±0.62 | -4.8 | 4.37 | 12.95±0.71 | -13.67 | 5.52 |
| Medium QC | 81 | 82.54±1.70 | 1.90 | 2.06 | 76.77±1.43 | -5.22 | 1.86 |
| High QC | 162 | 173.73±2.63 | 7.24 | 1.52 | 166.15±2.44 | 2.56 | 1.47 |

1.5 Extraction efficiency

The extraction efficiency from plasma was calculated by comparing peak areas obtained from the plasma samples spiked with MPA and MPAG with those obtained from solution containing the same amount of analytes.

**Table 3** Extraction efficiency of spiked plasma of MPA and MPAG

| MPA (µg/mL) | Efficiency (%) | MPAG (µg/mL) | Efficiency (%) |
|---|---|---|---|
| 0.25 | 89.03 | 5 | 99.04 |
| 0.75 | 96.40 | 15 | 94.40 |
| 20 | 100.14 | 81 | 103.76 |
| 40 | 104.56 | 162 | 104.17 |

## 2. Validation of HPLC method for XMP and AMP concentrations

### 2.1 Specificity and selectivity

Specificity of the method was determined by analyzing incubation buffer solution with IMP and β-$NAD^+$. No interferences with other substances were observed, in both the chromatogram of incubation buffer solution and PBMC lysate of patient samples with incubation buffer solution. The retention time of AMP and XMP were 6.60 and 8.10 minutes, respectively.

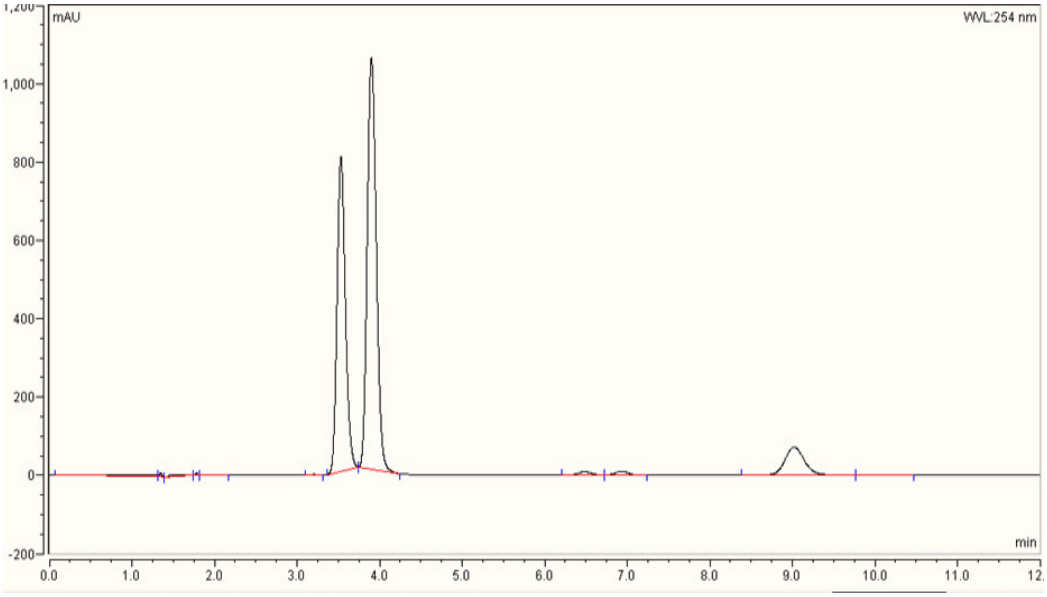


**Figure 7** Chromatogram of incubation buffer solution with IMP and β-$NAD^+$

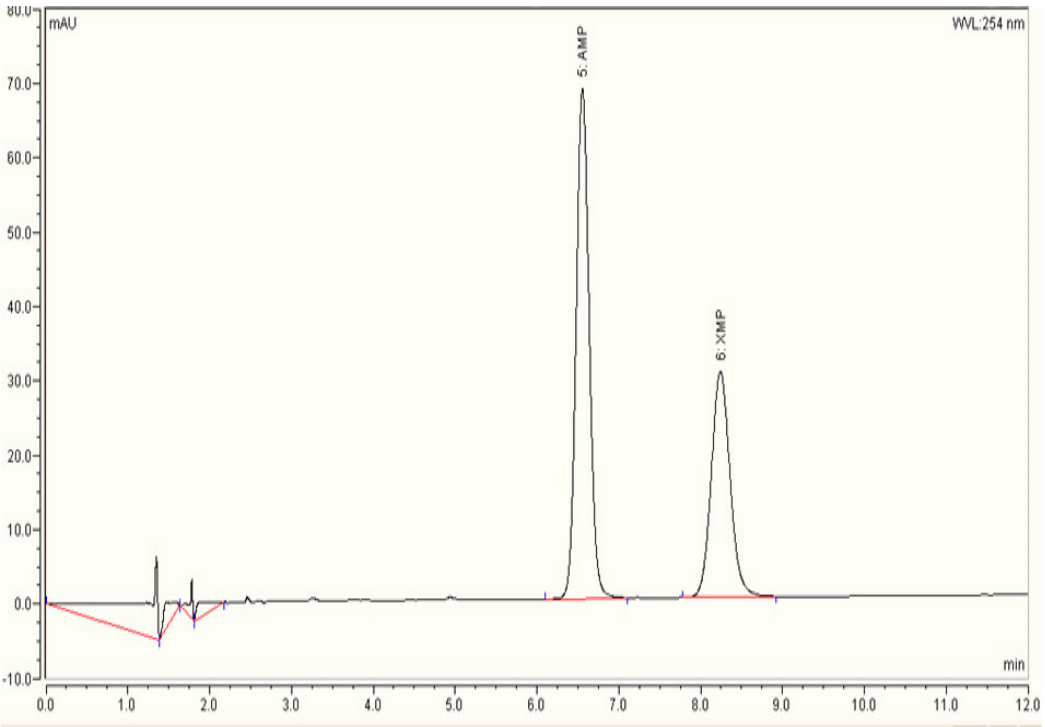


**Figure 8** Chromatogram of incubation buffer solution (without IMP and β-$NAD^+$) and AMP and XMP standard at concentration 150 µmol/L

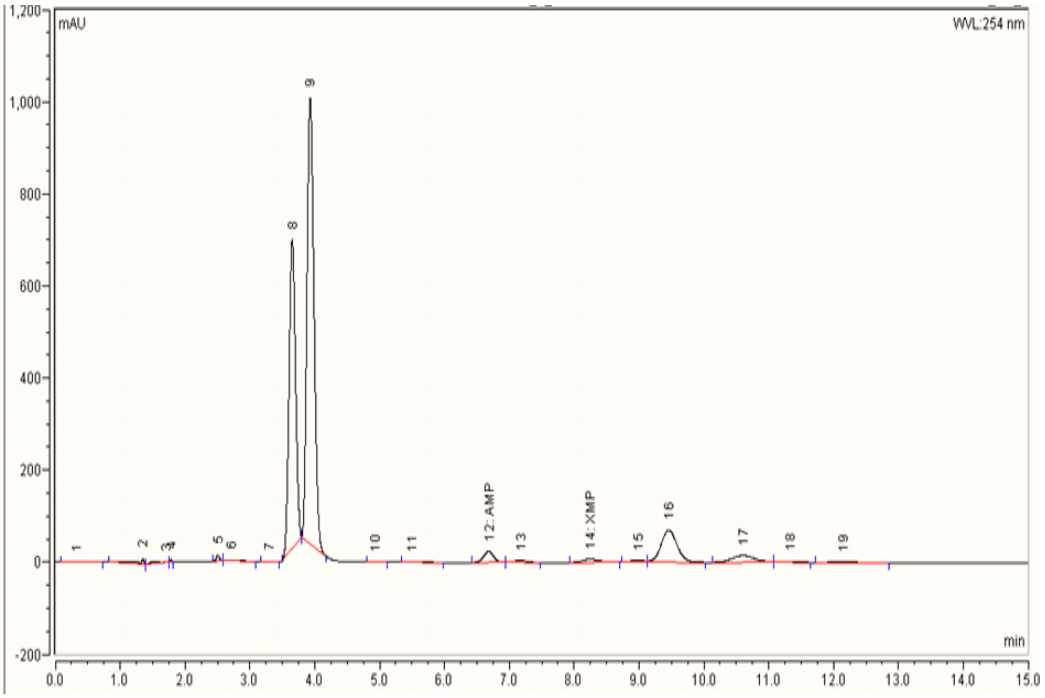


**Figure 9** Chromatogram of PBMC lysate of patient incubated with incubation buffer solution with IMP and β-$NAD^+$

2.2 Linearity

Calibration curves were prepared by external standard solution with amounts of each analyte (XMP and AMP at concentrations 10, 25, 50, 75, 150, 300 µmol/L). The calibration curves were shown in Figure 10 and 11. Peak area of active ingredients versus concentration was plotted. The weighted least squares linear regression equation was calculated using the peak area. The coefficient of determination was employed to evaluate the linearity of the calibration curve.

y = Peak area of active ingredients

x = plasma analyte concentration

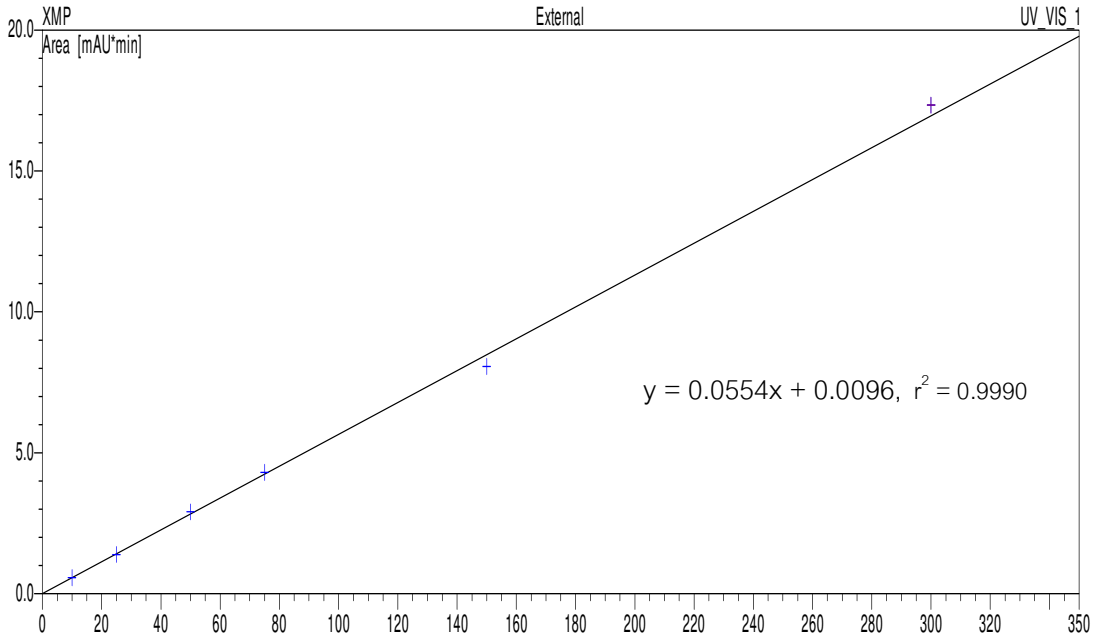


**Figure 10** Calibration curve of XMP

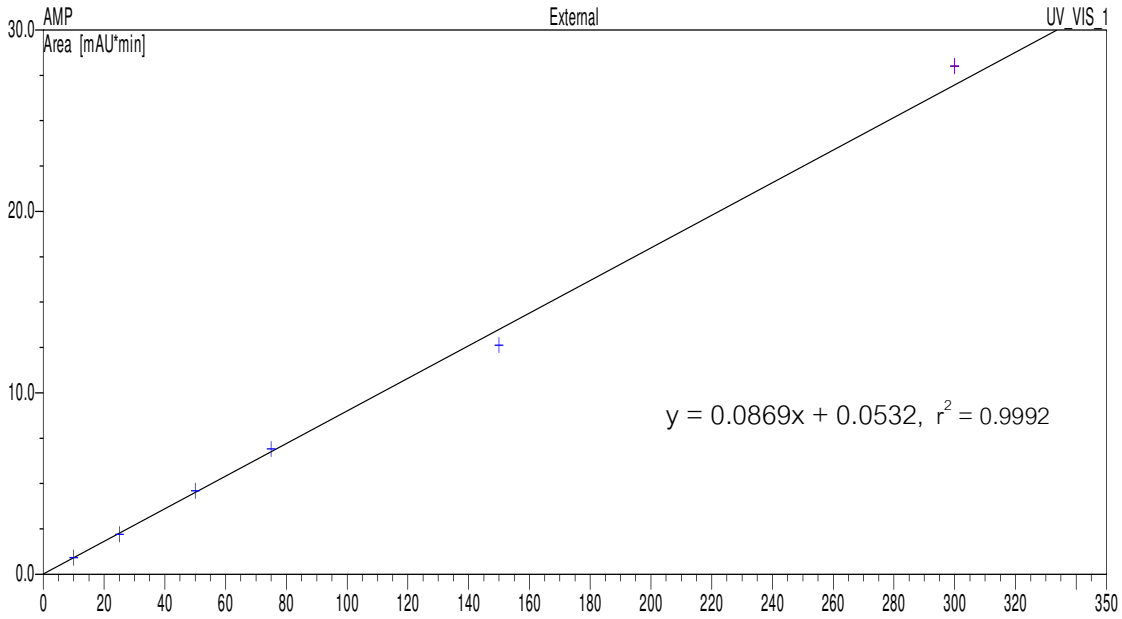


**Figure 11** Calibration curve of AMP

2.3 Limit of quantification

Limit of quantification (LOQ) was defined as smallest concentration on the calibration curve which had linear relationship with peak area and had acceptable accuracy and precision within ±20%. The accuracy and precision of intra-day and inter-day of LOQ of XMP and AMP were shown in Table 4 and Table 5.

2.4 Quality Control (QC)

Accuracy and precision were assessed at the three sets of quality control samples (low, medium and high, 5 determinations at each concentration). For accuracy, the mean value should be within 15% of the actual value was acceptable. The precision measured at each concentration should not exceed 15% of the coefficient of variation (CV). As shown in Table 4 and Table 5, the accuracy and precision of intra-day and inter-day were all in acceptable criteria.

**Table 4** Intra-day and inter-day accuracy and precision of the method for the determination of XMP

| Nominal concentration (µmol/L) | | Intra-day (n = 5) | | | Inter-day (n = 15) | | |
|---|---|---|---|---|---|---|---|
| | | Analyzed conc. (µg/ml) mean±SD | Accuracy (%) | Precision (%CV) | Analyzed conc. (µg/ml) mean±SD | Accuracy (%) | Precision (%CV) |
| LLOQ | 10 | 9.43±0.12 | -5.71 | 1.32 | 9.98±1.11 | -0.21 | 11.08 |
| Low QC | 30 | 29.59±1.51 | -1.38 | 5.09 | 29.60±2.46 | -1.34 | 8.29 |
| Medium QC | 65 | 64.53±2.52 | -0.72 | 3.91 | 63.63±4.88 | -2.11 | 7.66 |
| High QC | 240 | 234.85±17.48 | -2.15 | 7.44 | 227.64±24.88 | -5.15 | 10.93 |

**Table 5** Intra-day and inter-day accuracy and precision of the method for the determination of AMP

| Nominal concentration (µmol/L) | | Intra-day (n = 5) | | | Inter-day (n = 15) | | |
|---|---|---|---|---|---|---|---|
| | | Analyzed conc. (µg/ml) mean±SD | Accuracy (%) | Precision (%CV) | Analyzed conc. (µg/ml) mean±SD | Accuracy (%) | Precision (%CV) |
| LLOQ | 10 | 9.25±0.50 | -7.47 | 5.36 | 9.64±1.27 | -3.59 | 13.18 |
| Low QC | 30 | 29.37±1.00 | -2.09 | 3.41 | 29.17±2.49 | -2.76 | 8.52 |
| Medium QC | 65 | 64.70±1.69 | -0.46 | 2.61 | 63.81±4.57 | -1.83 | 7.16 |
| High QC | 240 | 233.30±19.94 | -2.79 | 8.55 | 228.41±24.42 | -4.83 | 10.69 |

# Appendix C

Data of individual patient

Table 1. Summary of seven SNPs in each patient

| code | UGT1A9*1b | UGT1A9 | UGT2B7 | MRP-2 | IMPDH1 | IMPDH1 | IMPDH2 |
|---|---|---|---|---|---|---|---|
| | *$T_9$>$T_{10}$* | *T-688G* | *C802T* | *C-24T* | *G125A* | *G-106A* | *T3757C* |
| 1 | $T_{9/10}$ | TT | CC | TT | GG | GG | TT |
| 2 | $T_{9/9}$ | TG | CT | CC | GA | GG | TT |
| 3 | $T_{10/10}$ | TT | CT | CT | GG | GA | TT |
| 4 | $T_{10/10}$ | TT | CC | CC | GG | GA | TT |
| 5 | $T_{9/9}$ | TT | CT | CC | AA | AA | TT |
| 6 | $T_{9/10}$ | TT | CC | CC | AA | AA | TT |
| 7 | $T_{9/9}$ | TG | CC | CC | AA | AA | TT |
| 8 | $T_{10/10}$ | TT | CC | CT | GA | GA | TT |
| 9 | $T_{9/10}$ | TG | CT | CC | GA | AA | TT |
| 10 | $T_{9/10}$ | TG | CT | CT | GG | GA | TT |
| 11 | $T_{10/10}$ | TT | CC | CT | GA | GA | TT |
| 12 | $T_{9/10}$ | TT | CC | CC | GA | GA | TT |
| 13 | $T_{10/10}$ | TT | CC | CC | GA | GA | TT |
| 14 | $T_{9/10}$ | TG | CC | TT | GA | GA | TT |
| 15 | $T_{9/10}$ | TT | CT | CC | AA | AA | TT |
| 16 | $T_{9/10}$ | TG | CC | CT | GG | GA | TT |
| 17 | $T_{9/10}$ | TT | CC | CC | GA | GA | TT |
| 18 | $T_{10/10}$ | TT | CC | CT | GA | GA | TT |
| 19 | $T_{9/9}$ | TT | CC | CT | GA | GA | TT |
| 20 | $T_{10/10}$ | TT | CT | CT | AA | AA | TT |

| code | UGT1A9*1b | UGT1A9 | UGT2B7 | MRP-2 | IMPDH1 | IMPDH1 | IMPDH2 |
|---|---|---|---|---|---|---|---|
| | *$T_9$>$T_{10}$* | *T-688G* | *C802T* | *C-24T* | *G125A* | *G-106A* | *T3757C* |
| 21 | $T_{9/10}$ | TT | CC | CC | GG | GG | TT |
| 22 | $T_{9/10}$ | TG | CC | CT | GA | GA | TT |
| 23 | $T_{9/9}$ | TT | CC | CC | GG | GA | TT |
| 24 | $T_{9/9}$ | TT | CT | CC | GG | GA | TT |
| 25 | $T_{9/9}$ | TG | CC | CT | AA | AA | TT |
| 26 | $T_{10/10}$ | TT | CT | CT | GA | GA | TT |
| 27 | $T_{10/10}$ | TT | CT | CC | GA | AA | TT |
| 28 | $T_{9/10}$ | TG | CC | TT | GA | GA | TT |
| 29 | $T_{9/9}$ | TT | CT | CT | GG | AA | TT |
| 30 | $T_{9/9}$ | TT | CT | CC | GA | GA | TT |
| 31 | $T_{9/10}$ | TT | CC | CC | GA | GA | TT |
| 32 | $T_{9/9}$ | TT | CT | CC | GG | GG | TT |
| 33 | $T_{10/10}$ | TT | CC | CC | AA | AA | TT |
| 34 | $T_{10/10}$ | TT | CT | CC | GA | GA | TT |
| 35 | $T_{10/10}$ | TT | CT | CC | GG | GA | TT |
| 36 | $T_{9/10}$ | TT | CC | CC | GA | GA | TT |
| 37 | $T_{10/10}$ | TT | CC | CC | GA | GA | TT |
| 38 | $T_{10/10}$ | TT | CT | CT | GA | AA | TT |
| 39 | $T_{9/9}$ | TT | TT | TT | AA | AA | TT |
| 40 | $T_{10/10}$ | TT | CC | TT | GG | GA | TT |
| 41 | $T_{10/10}$ | TT | CC | CT | GA | GA | TT |
| 42 | $T_{9/9}$ | TG | CC | CC | GA | GA | TT |
| 43 | $T_{9/10}$ | TG | CC | CC | AA | GA | TT |

| code | UGT1A9*1b | UGT1A9 | UGT2B7 | MRP-2 | IMPDH1 | IMPDH1 | IMPDH2 |
|---|---|---|---|---|---|---|---|
| | *$T_9$>$T_{10}$* | *T-688G* | *C802T* | *C-24T* | *G125A* | *G-106A* | *T3757C* |
| 44 | $T_{9/10}$ | TT | CC | CT | AA | AA | TT |
| 45 | $T_{10/10}$ | TT | CC | CT | GA | GA | TT |
| 46 | $T_{9/10}$ | TT | CT | CC | GG | AA | TT |
| 47 | $T_{10/10}$ | TT | CC | CC | GG | GA | TT |
| 48 | $T_{9/10}$ | TT | CC | TT | GG | GG | TT |
| 49 | $T_{9/9}$ | TT | CC | CC | GA | GA | TT |
| 50 | $T_{9/10}$ | TG | CT | CC | GG | GA | TT |
| 51 | $T_{10/10}$ | TT | CC | CC | GA | GA | TT |
| 52 | $T_{9/10}$ | TT | CT | CT | GA | GA | TT |
| 53 | $T_{9/9}$ | TG | CC | CC | GA | AA | TT |
| 54 | $T_{9/9}$ | TT | TT | CC | GG | GG | TT |
| 55 | $T_{9/10}$ | TG | CC | CC | GG | GA | TT |
| 56 | $T_{9/9}$ | TT | TT | CT | GA | GA | TT |
| 57 | $T_{9/9}$ | TG | TT | CC | GG | GG | TT |
| 58 | $T_{9/10}$ | TT | CC | CC | GA | AA | TT |
| 59 | $T_{10/10}$ | TT | CT | CC | GA | GA | TT |
| 60 | $T_{9/10}$ | TT | CT | CC | GA | GA | TT |
| 61 | $T_{9/10}$ | TT | CC | CC | GA | GG | TT |
| 62 | $T_{9/10}$ | TT | CC | CC | GA | AA | TT |
| 63 | $T_{9/10}$ | TT | CC | CT | GG | GA | TT |
| 64 | $T_{9/9}$ | TG | CC | CC | GG | GA | TT |
| 65 | $T_{9/9}$ | TT | CT | CC | AA | AA | TT |
| 66 | $T_{9/10}$ | TT | CT | CT | GA | GA | TT |

| code | UGT1A9*1b | UGT1A9 | UGT2B7 | MRP-2 | IMPDH1 | IMPDH1 | IMPDH2 |
|---|---|---|---|---|---|---|---|
| | $T_9>T_{10}$ | *T-688G* | *C802T* | *C-24T* | *G125A* | *G-106A* | *T3757C* |
| 67 | $T_{9/10}$ | TT | CC | CT | GA | GA | TT |
| 68 | $T_{9/10}$ | TT | CT | CC | GA | AA | TT |
| 69 | $T_{9/10}$ | TT | CT | CT | GA | AA | TT |
| 70 | $T_{9/10}$ | TT | CT | CT | GA | GA | TC |
| 71 | $T_{10/10}$ | TT | CC | CC | GG | GG | TT |
| 72 | $T_{10/10}$ | TT | CT | CC | GA | AA | TT |
| 73 | $T_{9/10}$ | TT | CC | CC | GA | GA | TT |
| 74 | $T_{10/10}$ | TT | CT | CC | AA | AA | TT |
| 75 | $T_{9/9}$ | TT | CC | CC | AA | GA | TT |
| 76 | $T_{9/9}$ | TT | TT | CT | GG | GG | TT |
| 77 | $T_{9/10}$ | TT | CC | CC | GG | AA | TT |
| 78 | $T_{9/9}$ | TT | TT | CC | GA | GA | TT |
| 79 | $T_{9/9}$ | GG | CC | CC | GA | GA | TT |
| 80 | $T_{10/10}$ | TT | CT | CC | GG | GG | TT |
| 81 | $T_{10/10}$ | TT | CC | CC | AA | AA | TT |
| 82 | $T_{9/10}$ | TT | CT | CC | GG | GA | TT |
| 83 | $T_{9/10}$ | TT | TT | TT | GA | AA | TT |
| 84 | $T_{9/10}$ | TT | CC | CC | GG | GG | TT |
| 85 | $T_{9/9}$ | TG | CC | CC | GA | GA | TT |
| 86 | $T_{9/10}$ | TT | CT | CT | GA | GA | TT |
| 87 | $T_{9/9}$ | TT | CC | CC | GA | GA | TT |
| 88 | $T_{9/9}$ | TT | TT | CT | GG | GG | TT |
| 89 | $T_{9/10}$ | TT | CT | CC | GA | AA | TC |

| code | UGT1A9*1b | UGT1A9 | UGT2B7 | MRP-2 | IMPDH1 | IMPDH1 | IMPDH2 |
|---|---|---|---|---|---|---|---|
| | $T_9>T_{10}$ | *T-688G* | *C802T* | *C-24T* | *G125A* | *G-106A* | *T3757C* |
| 90 | $T_{9/9}$ | GG | CT | CC | GG | GG | TT |
| 91 | $T_{9/10}$ | TT | CT | CT | AA | AA | TT |
| 92 | $T_{9/10}$ | TT | CC | CC | GA | GA | TT |
| 93 | $T_{9/9}$ | TG | CT | CC | GG | AA | TT |
| 94 | $T_{9/10}$ | TT | CT | CT | GA | GA | TC |
| 95 | $T_{9/10}$ | TT | CC | CC | GA | GA | TT |
| 96 | $T_{9/10}$ | TT | CC | CT | GG | AA | TT |
| 97 | $T_{9/10}$ | TT | CT | CT | GG | GG | TT |
| 98 | $T_{9/10}$ | TT | CC | CC | GA | GA | TT |
| 99 | $T_{9/10}$ | TT | TT | CC | GG | GA | TT |
| 100 | $T_{9/10}$ | TT | CT | CC | AA | AA | TT |
| 101 | $T_{10/10}$ | TT | CC | CC | GG | GA | TT |
| 102 | $T_{9/9}$ | GG | TT | CT | AA | AA | TT |
| 103 | $T_{9/10}$ | TT | CC | CC | GA | GA | TT |
| 104 | $T_{10/10}$ | TT | CT | CC | GG | GG | TT |
| 105 | $T_{9/10}$ | TT | CC | CC | GG | GG | TT |
| 106 | $T_{9/10}$ | TG | CC | CC | AA | AA | TT |
| 107 | $T_{9/9}$ | TG | CC | CT | GG | AA | TT |
| 108 | $T_{9/9}$ | TT | CC | CC | GA | AA | TT |
| 109 | $T_{9/10}$ | TT | CC | CC | AA | AA | TT |
| 110 | $T_{9/10}$ | TG | CC | CT | GG | AA | TT |
| 111 | $T_{10/10}$ | TT | CT | TT | GG | GG | TT |
| 112 | $T_{9/9}$ | TT | CC | CC | GA | GA | TT |

| code | UGT1A9*1b | UGT1A9 | UGT2B7 | MRP-2 | IMPDH1 | IMPDH1 | IMPDH2 |
|---|---|---|---|---|---|---|---|
| | *$T_9$>$T_{10}$* | *T-688G* | *C802T* | *C-24T* | *G125A* | *G-106A* | *T3757C* |
| 113 | $T_{10/10}$ | TT | CT | CC | GA | GA | TT |
| 114 | $T_{9/10}$ | TT | CT | CT | GA | AA | TT |
| 115 | $T_{9/10}$ | TT | CC | CT | GG | GA | TT |
| 116 | $T_{9/9}$ | GG | CT | TT | GA | GA | TT |
| 117 | $T_{9/10}$ | TT | CC | CT | GG | GG | TT |
| 118 | $T_{10/10}$ | TT | CC | CC | GA | AA | TT |

Table 2. Data of individual patient

| Code | Sex | Age (years) | Time after transplantation (months) | Graft | Cause for transplantation | Concomitant immunosuppressant drug | | Body weight (kg) | SCr (mg/dL) | eGFR (mL/min/1.73 $m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Drug | Daily dose (mg) | | | |
| 1 | Male | 51 | 89 | Cadaveric | Chronic glomerulonephritis | Tacrolimus | 5 | 77 | 2.31 | 31.9 |
| 2 | Male | 47 | 19 | Cadaveric | ADPKD | Tacrolimus | 9.5 | 73.8 | 2.95 | 24.4 |
| 3 | Male | 69 | 150 | Cadaveric | Unknown | Tacrolimus | 6.5 | 69.5 | 1.36 | 55.2 |
| 4 | Female | 47 | 138 | Living | Unknown | Cyclosporine | 75 | 48.2 | 1.17 | 52.7 |
| 5 | Male | 65 | 64 | Living | Diabetic nephropathy | Tacrolimus | 3.5 | 78 | 1.64 | 45 |
| 6 | Male | 31 | 67 | Cadaveric | FSGS | Cyclosporine | 125 | 78.4 | 1.29 | 69 |
| 7 | Male | 38 | 9 | Cadaveric | Chronic glomerulonephritis | Tacrolimus | 10 | 58 | 2.29 | 34.2 |
| 8 | Female | 37 | 43 | Cadaveric | Unknown | Cyclosporine | 100 | 39.8 | 1.76 | 34.5 |
| 9 | Male | 59 | 126 | Living | Unknown | Cyclosporine | 100 | 87 | 1.28 | 61.1 |
| 10 | Female | 43 | 126 | Living | Unknown | Cyclosporine | 175 | 73 | 3.29 | 16.3 |
| 11 | Male | 34 | 12 | Living | IgA nephropathy | Tacrolimus | 3.5 | 64.5 | 1.31 | 66.6 |
| 12 | Female | 35 | 55 | Living | Chronic glomerulonephritis | Cyclosporine | 150 | 51.5 | 0.93 | 72.9 |
| 13 | Female | 44 | 48 | Living | Unknown | Cyclosporine | 125 | 55.4 | 1.83 | 31.9 |
| 14 | Male | 51 | 37 | Living | Unknown | Cyclosporine | 125 | 58 | 1.76 | 43.6 |
| 15 | Female | 37 | 18 | Living | IgA nephropathy | Cyclosporine | 100 | 45.5 | 1.07 | 61.3 |
| 16 | Male | 30 | 113 | Cadaveric | Unknown | Tacrolimus | 4 | 75.5 | 1.49 | 58.9 |

| Code | Sex | Age (years) | Time after transplantation (months) | Graft | Cause for transplantation | Concomitant immunosuppressant drug | | Body weight (kg) | SCr (mg/dL) | eGFR (mL/min/1.73 $m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Drug | Daily dose (mg) | | | |
| 17 | Female | 67 | 88 | Living | Obstructive nephropathy | Cyclosporine | 50 | 52.9 | 0.83 | 72.9 |
| 18 | Male | 39 | 33 | Cadaveric | Unknown | Cyclosporine | 100 | 52 | 1.22 | 70.3 |
| 19 | Female | 63 | 180 | Cadaveric | Unknown | Cyclosporine | 100 | 71 | 0.78 | 79.3 |
| 20 | Male | 32 | 6 | Living | Chronic glomerulonephritis | Tacrolimus | 10 | 80 | 1.42 | 61.4 |
| 21 | Female | 53 | 64 | Cadaveric | Unknown | Tacrolimus | 5 | 42.8 | 0.76 | 84.6 |
| 22 | Male | 62 | 48 | Living | Unknown | Sirolimus | 1 | 61.6 | 1.01 | 79.6 |
| 23 | Male | 46 | 56 | Living | Unknown | Tacrolimus | 1.5 | 64.5 | 1.06 | 79.9 |
| 24 | Male | 53 | 44 | Cadaveric | Diabetic nephropathy | Tacrolimus | 10.5 | 81.6 | 2.16 | 34.2 |
| 25 | Female | 44 | 15 | Cadaveric | Unknown | Sirolimus | 6 | 59 | 1.64 | 36.2 |
| 26 | Male | 50 | 57 | Living | Hypertension | Tacrolimus | 2 | 79 | 1.44 | 55.2 |
| 27 | Male | 40 | 8 | Living | Others | Tacrolimus | 3.5 | 59.3 | 1.45 | 57.3 |
| 28 | Female | 42 | 23 | Cadaveric | IgA nephropathy | Tacrolimus | 3 | 39.4 | 0.98 | 66.1 |
| 29 | Male | 49 | 34 | Living | Unknown | Tacrolimus | 5 | 61.4 | 1.12 | 74.1 |
| 30 | Male | 36 | 20 | Cadaveric | Unknown | Cyclosporine | 175 | 71.6 | 1.34 | 64.1 |
| 31 | Female | 42 | 114 | Cadaveric | Unknown | Tacrolimus | 2 | 55 | 0.74 | 91.5 |
| 32 | Male | 34 | 66 | Living | Chronic glomerulonephritis | Tacrolimus | 3.5 | 69 | 1.78 | 46.7 |
| 33 | Male | 39 | 25 | Living | Unknown | Cyclosporine | 125 | 60.3 | 1.21 | 71 |
| 34 | Male | 48 | 151 | Living | Unknown | Cyclosporine | 75 | 61.5 | 1.18 | 70 |
| 35 | Female | 46 | 158 | Cadaveric | Unknown | Cyclosporine | 75 | 70 | 1.58 | 37.4 |

| Code | Sex | Age (years) | Time after transplantation (months) | Graft | Cause for transplantation | Concomitant immunosuppressant drug | | Body weight (kg) | SCr (mg/dL) | eGFR (mL/min/1.73 $m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Drug | Daily dose (mg) | | | |
| 36 | Male | 34 | 48 | Living | Unknown | Cyclosporine | 125 | 53 | 1.45 | 59.2 |
| 37 | Male | 18 | 22 | Cadaveric | Unknown | Tacrolimus | 5 | 43 | 2.01 | 46.2 |
| 38 | Male | 61 | 54 | Living | Unknown | Cyclosporine | 100 | 60 | 0.82 | 101.5 |
| 39 | Male | 37 | 43 | Living | Unknown | Cyclosporine | 150 | 60.6 | 2.07 | 38.6 |
| 40 | Female | 36 | 3 | Living | Unknown | Tacrolimus | 7 | 47.2 | 0.67 | 105.9 |
| 41 | Male | 69 | 170 | Living | Unknown | Cyclosporine | 75 | 74 | 4.67 | 13.3 |
| 42 | Male | 20 | 61 | Cadaveric | Unknown | Tacrolimus | 7 | 45 | 1.83 | 50.4 |
| 43 | Male | 41 | 6 | Cadaveric | Unknown | Cyclosporine | 150 | 52 | 1.67 | 48.4 |
| 44 | Male | 57 | 41 | Cadaveric | Unknown | Tacrolimus | 5.5 | 68.6 | 1.3 | 60.5 |
| 45 | Male | 37 | 20 | Cadaveric | IgA nephropathy | Tacrolimus | 5 | 63.9 | 1.56 | 53.5 |
| 46 | Female | 25 | 13 | Cadaveric | Unknown | Tacrolimus | 8 | 46 | 1.07 | 66.4 |
| 47 | Male | 59 | 160 | Living | Unknown | Cyclosporine | 100 | 80 | 1.43 | 53.8 |
| 48 | Female | 68 | 108 | Cadaveric | ADPKD | Cyclosporine | 50 | 54.5 | 1.47 | 37.6 |
| 49 | Male | 57 | 53 | Cadaveric | Unknown | Tacrolimus | 4.5 | 61.1 | 1.31 | 59.9 |
| 50 | Male | 49 | 4 | Living | Unknown | Cyclosporine | 225 | 68.5 | 1.28 | 63.5 |
| 51 | Male | 43 | 110 | Living | Chronic glomerulonephritis | Cyclosporine | 150 | 67.2 | 1.53 | 53.1 |
| 52 | Female | 30 | 63 | Cadaveric | Hypertension | Cyclosporine | 75 | 51.6 | 2.01 | 30.9 |
| 53 | Male | 38 | 9 | Cadaveric | IgA nephropathy | Tacrolimus | 11 | 76 | 1.25 | 68.7 |
| 54 | Female | 51 | 105 | Cadaveric | Lupus nephritis | Tacrolimus | 8 | 89.6 | 1.78 | 31.9 |

| Code | Sex | Age (years) | Time after transplantation (months) | Graft | Cause for transplantation | Concomitant immunosuppressant drug | | Body weight (kg) | SCr (mg/dL) | eGFR (mL/min/1.73 $m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Drug | Daily dose (mg) | | | |
| 55 | Male | 68 | 52 | Cadaveric | Unknown | Cyclosporine | 125 | 62 | 0.8 | 102.2 |
| 56 | Male | 36 | 50 | Living | Unknown | Tacrolimus | 3 | 82.3 | 2.16 | 37 |
| 57 | Male | 54 | 170 | Cadaveric | Unknown | Cyclosporine | 75 | 54.6 | 1.55 | 49.9 |
| 58 | Male | 59 | 26 | Living | Unknown | Tacrolimus | 3 | 62 | 1.64 | 45.9 |
| 59 | Female | 35 | 91 | Cadaveric | Chronic glomerulonephritis | Tacrolimus | 4 | 42.2 | 1.56 | 40.1 |
| 60 | Male | 28 | 117 | Cadaveric | Unknown | Tacrolimus | 2 | 55 | 1.64 | 53.4 |
| 61 | Male | 50 | 183 | Cadaveric | Unknown | Cyclosporine | 75 | 64.9 | 1.24 | 65.6 |
| 62 | Male | 47 | 187 | Cadaveric | Diabetic nephropathy | Cyclosporine | 125 | 57 | 0.92 | 93.7 |
| 63 | Male | 38 | 9 | Cadaveric | Lupus nephritis | Tacrolimus | 5.5 | 70.2 | 1.49 | 56.1 |
| 64 | Male | 55 | 19 | Cadaveric | Unknown | Tacrolimus | 3.5 | 99.4 | 1.47 | 52.9 |
| 65 | Male | 23 | 35 | Living | Unknown | Sirolimus | 3 | 56.5 | 2.84 | 29.5 |
| 66 | Male | 67 | 19 | Cadaveric | Diabetic nephropathy | Tacrolimus | 4 | 60.2 | 0.87 | 93 |
| 67 | Female | 29 | 41 | Cadaveric | Chronic glomerulonephritis | Cyclosporine | 100 | 38 | 1.04 | 66.6 |
| 68 | Male | 54 | 29 | Cadaveric | Unknown | Tacrolimus | 7 | 64 | 1.44 | 54.3 |
| 69 | Male | 40 | 29 | Living | Chronic glomerulonephritis | Tacrolimus | 9 | 79 | 1.35 | 62.2 |
| 70 | Male | 53 | 54 | Living | Unknown | Cyclosporine | 125 | 59 | 1.3 | 61.4 |
| 71 | Female | 55 | 31 | Living | Unknown | Cyclosporine | 150 | 49.5 | 1.16 | 51.6 |
| 72 | Male | 42 | 174 | Cadaveric | IgA nephropathy | Sirolimus | 2 | 67 | 1.8 | 44.2 |
| 73 | Male | 67 | 25 | Cadaveric | Unknown | Sirolimus | 2 | 64.5 | 1.34 | 56.6 |

| Code | Sex | Age (years) | Time after transplantation (months) | Graft | Cause for transplantation | Concomitant immunosuppressant drug | | Body weight (kg) | SCr (mg/dL) | eGFR (mL/min/1.73 $m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Drug | Daily dose (mg) | | | |
| 74 | Male | 40 | 108 | Cadaveric | Unknown | Everolimus | 2.5 | 87.5 | 2.47 | 31 |
| 75 | Male | 56 | 137 | Cadaveric | ADPKD | Tacrolimus | 1.5 | 69.8 | 1.92 | 38.7 |
| 76 | Male | 52 | 28 | Cadaveric | ADPKD | Cyclosporine | 150 | 62 | 1.23 | 65.7 |
| 77 | Male | 63 | 108 | Living | Diabetic nephropathy | Cyclosporine | 150 | 103 | 1.34 | 57.2 |
| 78 | Female | 63 | 99 | Living | Unknown | Cyclosporine | 100 | 64 | 0.93 | 64.7 |
| 79 | Male | 28 | 62 | Living | FSGS | Cyclosporine | 125 | 70 | 1.67 | 52.3 |
| 80 | Male | 31 | 49 | Living | Unknown | Cyclosporine | 100 | 46.8 | 1.24 | 72.3 |
| 81 | Male | 50 | 134 | Living | Diabetic nephropathy | Cyclosporine | 100 | 89 | 1.28 | 63.2 |
| 82 | Male | 48 | 3 | Living | Hypertension | Tacrolimus | 10.5 | 58.3 | 1.4 | 57.5 |
| 83 | Male | 52 | 4 | Living | IgA nephropathy | Tacrolimus | 8.5 | 63 | 1.4 | 56.6 |
| 84 | Male | 53 | 159 | Cadaveric | Chronic glomerulonephritis | Tacrolimus | 4 | 63 | 1.3 | 61.4 |
| 85 | Male | 37 | 36 | Cadaveric | Chronic glomerulonephritis | Tacrolimus | 2 | 69.5 | 1.8 | 45.4 |
| 86 | Male | 23 | 7 | Living | Hypertension | Tacrolimus | 2 | 56.5 | 1.1 | 88.2 |
| 87 | Male | 61 | 86 | Living | Chronic glomerulonephritis | Tacrolimus | 3 | 67.5 | 1.5 | 50.6 |
| 88 | Female | 39 | 105 | Living | Chronic glomerulonephritis | Cyclosporine | 75 | 52 | 0.9 | 74.1 |
| 89 | Male | 41 | 104 | Cadaveric | FSGS | Tacrolimus | 6 | 62 | 2.1 | 37.2 |
| 90 | Male | 62 | 228 | Cadaveric | Unknown | Tacrolimus | 2 | 72 | 1.3 | 59.5 |
| 91 | Male | 41 | 166 | Cadaveric | Chronic glomerulonephritis | Cyclosporine | 125 | 62.5 | 1.1 | 78.4 |
| 92 | Male | 55 | 38 | Cadaveric | Unknown | Cyclosporine | 150 | 113 | 2 | 37.1 |

| Code | Sex | Age (years) | Time after transplantation (months) | Graft | Cause for transplantation | Concomitant immunosuppressant drug | | Body weight (kg) | SCr (mg/dL) | eGFR (mL/min/1.73 $m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Drug | Daily dose (mg) | | | |
| 93 | Male | 59 | 159 | Cadaveric | IgA nephropathy | Tacrolimus | 2.5 | 68.5 | 1.4 | 55.1 |
| 94 | Female | 46 | 11 | Cadaveric | Hypertension | Tacrolimus | 3 | 50 | 0.6 | 114.4 |
| 95 | Male | 31 | 23 | Living | Chronic glomerulonephritis | Tacrolimus | 4.5 | 60 | 2.8 | 28.2 |
| 96 | Male | 26 | 88 | Living | Chronic glomerulonephritis | Tacrolimus | 4 | 74 | 1.4 | 65.1 |
| 97 | Male | 36 | 11 | Living | Chronic glomerulonephritis | Tacrolimus | 5.5 | 64 | 1.5 | 56.3 |
| 98 | Female | 62 | 68 | Cadaveric | Chronic glomerulonephritis | Cyclosporine | 75 | 56.5 | 0.7 | 90.1 |
| 99 | Male | 53 | 120 | Living | Hypertension | Cyclosporine | 150 | 72 | 1.4 | 56.3 |
| 100 | Male | 45 | 71 | Living | Chronic glomerulonephritis | Cyclosporine | 150 | 59 | 1.3 | 63.4 |
| 101 | Male | 45 | 88 | Living | Chronic glomerulonephritis | Tacrolimus | 3.5 | 75.5 | 1.2 | 69.6 |
| 102 | Male | 54 | 120 | Cadaveric | Unknown | Cyclosporine | 200 | 60.7 | 2.48 | 29 |
| 103 | Female | 57 | 191 | Cadaveric | Unknown | Cyclosporine | 100 | 52.3 | 2.87 | 18 |
| 104 | Male | 37 | 41 | Cadaveric | IgA nephropathy | Tacrolimus | 1.5 | 67 | 1.95 | 41.3 |
| 105 | Female | 44 | 20 | Cadaveric | Chronic glomerulonephritis | Cyclosporine | 75 | 69.6 | 0.55 | 127.6 |
| 106 | Male | 51 | 34 | Cadaveric | Hypertension | Tacrolimus | 2 | 65 | 2.99 | 23.7 |
| 107 | Male | 48 | 162 | Cadaveric | Unknown | Cyclosporine | 200 | 56.6 | 4.19 | 16.2 |
| 108 | Female | 32 | 20 | Living | Hypertension | Tacrolimus | 4 | 52 | 1.03 | 66 |
| 109 | Male | 42 | 11 | Cadaveric | IgA nephropathy | Tacrolimus | 3 | 54 | 1.65 | 48.9 |
| 110 | Female | 55 | 36 | Cadaveric | Unknown | Sirolimus | 2 | 47 | 0.97 | 63.4 |
| 111 | Female | 32 | 11 | Cadaveric | Chronic glomerulonephritis | Cyclosporine | 250 | 64.5 | 1.71 | 36.8 |

| Code | Sex | Age (years) | Time after transplantation (months) | Graft | Cause for transplantation | Concomitant immunosuppressant drug | | Body weight (kg) | SCr (mg/dL) | eGFR (mL/min/1.73 $m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Drug | Daily dose (mg) | | | |
| 112 | Female | 31 | 8 | Cadaveric | Unknown | Cyclosporine | 225 | 41 | 0.95 | 72.9 |
| 113 | Female | 40 | 61 | Cadaveric | Chronic glomerulonephritis | Cyclosporine | 75 | 82.6 | 1.55 | 39.4 |
| 114 | Male | 42 | 94 | Cadaveric | Obstructive nephropathy | Tacrolimus | 2 | 70 | 2.75 | 27.1 |
| 115 | Male | 53 | 130 | Cadaveric | Hypertension | Cyclosporine | 50 | 52 | 1.45 | 54.1 |
| 116 | Male | 51 | 120 | Cadaveric | Chronic glomerulonephritis | Cyclosporine | 175 | 72.5 | 1.29 | 62.4 |
| 117 | Male | 57 | 85 | Living | Hypertension | Tacrolimus | 2.5 | 92 | 0.74 | 115.9 |
| 118 | Male | 38 | 63 | Cadaveric | Unknown | Cyclosporine | 150 | 52 | 1.13 | 77.2 |

ADPKD = Autosomal dominant polycystic kidney disease

FSGS = Focal segmental glomerulosclerosis

| Code | MMF daily dose (mg) | MPA concentration (µg/mL) | | | MPA $AUC_{0-12}$ (mg*h/L) | Normalized MPA AUC (mg*h/L/1000 mg) | Normalized MPA AUC (mg*h/L/mg dose/kg) | CL/F (L/h/kg) | MPAG concentration (µg/mL) | | | MPAG:MPA ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C_0 | C_0.5 | C_2 | | | | | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 1 | 1500 | 1.42 | 13.59 | 4.38 | 42.46 | 56.62 | 4.36 | 0.17 | 80.44 | 92.05 | 104.89 | 56.65 | 6.77 | 23.95 |
| 2 | 1000 | 2.99 | 2.98 | 2.51 | 41.40 | 82.80 | 6.11 | 0.12 | 86.57 | 82.3 | 81.89 | 28.95 | 27.62 | 32.63 |
| 3 | 1500 | 3.9 | 9.6 | 4.72 | 54.79 | 73.05 | 5.08 | 0.15 | 49.42 | 55.73 | 54.14 | 12.67 | 5.81 | 11.47 |
| 4 | 1000 | 2.69 | 1.95 | 7.97 | 50.78 | 101.55 | 4.89 | 0.15 | 49.82 | 45.04 | 110.97 | 18.52 | 23.10 | 13.92 |
| 5 | 1500 | 5.02 | 3.42 | 8.26 | 65.15 | 86.87 | 6.78 | 0.11 | 69.19 | 54.19 | 59.59 | 13.78 | 15.85 | 7.21 |
| 6 | 1000 | 2.56 | 10.37 | 2.67 | 43.40 | 86.80 | 6.81 | 0.11 | 40.45 | 41.57 | 56.23 | 15.80 | 4.01 | 21.06 |
| 7 | 1000 | 6.18 | 29.35 | 8 | 85.26 | 170.52 | 9.89 | 0.07 | 52.1 | 76 | 98.61 | 8.43 | 2.59 | 12.33 |
| 8 | 1000 | 2.48 | 25.81 | 7.09 | 60.81 | 121.63 | 4.84 | 0.15 | 57.61 | 93.85 | 125.56 | 23.23 | 3.64 | 17.71 |
| 9 | 500 | 0.35 | 0.26 | 1.13 | 22.30 | 89.20 | 7.76 | 0.10 | 16.91 | 15.99 | 17.3 | 48.31 | 61.50 | 15.31 |
| 10 | 1000 | 3.01 | 24.4 | 4.43 | 57.33 | 114.67 | 8.37 | 0.09 | 60.87 | 90.21 | 120.16 | 20.22 | 3.70 | 27.12 |
| 11 | 1500 | 2.84 | 9.69 | 8.37 | 56.70 | 75.60 | 4.88 | 0.15 | 36 | 41.63 | 80.77 | 12.68 | 4.30 | 9.65 |
| 12 | 1000 | 1.3 | 21.7 | 3.75 | 44.90 | 89.80 | 4.62 | 0.16 | 23.99 | 39.85 | 69.89 | 18.45 | 1.84 | 18.64 |
| 13 | 1000 | 0.86 | 4.56 | 5.42 | 36.61 | 73.22 | 4.06 | 0.18 | 66.22 | 76.26 | 118.94 | 77.00 | 16.72 | 21.94 |
| 14 | 1500 | 2 | 11.37 | 8.46 | 53.14 | 70.86 | 4.11 | 0.18 | 66.41 | 67.36 | 114.11 | 33.21 | 5.92 | 13.49 |
| 15 | 1500 | 5.07 | 45.91 | 11.19 | 94.95 | 126.59 | 5.76 | 0.13 | 68.19 | 76.79 | 131.51 | 13.45 | 1.67 | 11.75 |
| 16 | 1500 | 2.31 | 17.85 | 4.19 | 49.34 | 65.79 | 4.97 | 0.15 | 36.58 | 57.44 | 97.42 | 15.84 | 3.22 | 23.25 |
| 17 | 750 | 1.11 | 9.75 | 4.37 | 38.61 | 77.23 | 4.09 | 0.18 | 31.61 | 33.58 | 88.2 | 28.48 | 3.44 | 20.18 |
| 18 | 1500 | 1.8 | 13.21 | 6.62 | 49.13 | 65.51 | 3.41 | 0.22 | 66.51 | 78.46 | 151.53 | 36.95 | 5.94 | 22.89 |

| Code | MMF daily dose (mg) | MPA concentration (µg/mL) | | | MPA $AUC_{0-12}$ (mg*h/L) | Normalized MPA AUC (mg*h/L/1000 mg) | Normalized MPA AUC (mg*h/L/mg dose/kg) | CL/F (L/h/kg) | MPAG concentration (µg/mL) | | | MPAG:MPA ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C_0 | C_0.5 | C_2 | | | | | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 19 | 1000 | 0.16 | 6.83 | 6.95 | 37.22 | 74.43 | 5.28 | 0.14 | 23.93 | 21.8 | 40.45 | 149.56 | 3.19 | 5.82 |
| 20 | 1500 | 6.39 | 31.64 | 9.53 | 90.94 | 121.25 | 9.70 | 0.08 | 48.05 | 77.76 | 81.21 | 7.52 | 2.46 | 8.52 |
| 21 | 1000 | 1.78 | 10.64 | 10.91 | 56.73 | 113.46 | 4.86 | 0.15 | 22.61 | 23.52 | 49.83 | 12.70 | 2.21 | 4.57 |
| 22 | 1000 | 1.39 | 6.46 | 4.14 | 37.88 | 75.76 | 4.67 | 0.16 | 31.2 | 36.53 | 65.34 | 22.45 | 5.65 | 15.78 |
| 23 | 1500 | 4.28 | 2.59 | 7.17 | 58.27 | 77.69 | 5.01 | 0.15 | 38.44 | 26.22 | 43.51 | 8.98 | 10.12 | 6.07 |
| 24 | 1500 | 4.05 | 24.13 | 4.43 | 62.97 | 83.96 | 6.85 | 0.11 | 94.98 | 115.4 | 111.35 | 23.45 | 4.78 | 25.14 |
| 25 | 1500 | 3.15 | 16.56 | 5.87 | 56.88 | 75.84 | 4.47 | 0.17 | 38.67 | 46.14 | 62.99 | 12.28 | 2.79 | 10.73 |
| 26 | 1000 | 1.19 | 17.21 | 3.49 | 41.28 | 82.55 | 6.52 | 0.11 | 33.67 | 40.58 | 49.91 | 28.29 | 2.36 | 14.30 |
| 27 | 1000 | 2.05 | 1.09 | 1.68 | 33.38 | 66.75 | 3.96 | 0.19 | 37.5 | 34.7 | 31.57 | 18.29 | 31.83 | 18.79 |
| 28 | 1000 | 4.09 | 54.67 | 6.66 | 84.67 | 169.33 | 6.67 | 0.11 | 39.11 | 66.54 | 78.49 | 9.56 | 1.22 | 11.79 |
| 29 | 1000 | 1.82 | 1.16 | 9.01 | 47.72 | 95.44 | 5.86 | 0.13 | 24.93 | 22.8 | 31.53 | 13.70 | 19.66 | 3.50 |
| 30 | 1000 | 0.51 | 6.77 | 1.3 | 27.12 | 54.23 | 3.88 | 0.19 | 18.62 | 25.42 | 39.1 | 36.51 | 3.75 | 30.08 |
| 31 | 1000 | 1.51 | 34.86 | 2.75 | 51.15 | 102.31 | 5.63 | 0.13 | 32.67 | 42.42 | 56.28 | 21.64 | 1.22 | 20.47 |
| 32 | 1500 | 3.19 | 22.85 | 5.58 | 59.93 | 79.91 | 5.51 | 0.13 | 34.25 | 41.12 | 80.99 | 10.74 | 1.80 | 14.51 |
| 33 | 1000 | 1.62 | 21.49 | 2.07 | 42.99 | 85.99 | 5.18 | 0.14 | 19.04 | 28.18 | 43.08 | 11.75 | 1.31 | 20.81 |
| 34 | 1500 | 2.34 | 2.03 | 12.76 | 59.06 | 78.74 | 4.84 | 0.15 | 55.06 | 51.26 | 106.29 | 23.53 | 25.25 | 8.33 |
| 35 | 1000 | 0.58 | 4.19 | 11.27 | 47.29 | 94.58 | 6.62 | 0.11 | 23.12 | 27.43 | 72.19 | 39.86 | 6.55 | 6.41 |
| 36 | 1000 | 2.3 | 18.81 | 7.62 | 57.10 | 114.21 | 6.05 | 0.12 | 42.33 | 54.99 | 80.54 | 18.40 | 2.92 | 10.57 |

| Code | MMF daily dose (mg) | MPA concentration (µg/mL) | | | MPA $AUC_{0-12}$ (mg*h/L) | Normalized MPA AUC (mg*h/L/1000 mg) | Normalized MPA AUC (mg*h/L/mg dose/kg) | CL/F (L/h/kg) | MPAG concentration (µg/mL) | | | MPAG:MPA ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C_0 | C_0.5 | C_2 | | | | | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 37 | 1000 | 3.49 | 30.53 | 9.79 | 74.76 | 149.51 | 6.43 | 0.11 | 55.75 | 57.28 | 95.95 | 15.97 | 1.88 | 9.80 |
| 38 | 1000 | 0.84 | 1.83 | 7.29 | 38.98 | 77.96 | 4.68 | 0.16 | 23.03 | 21.86 | 42.79 | 27.42 | 11.95 | 5.87 |
| 39 | 1500 | 2.24 | 28.74 | 7.72 | 62.42 | 83.23 | 5.04 | 0.15 | 67.93 | 72.67 | 101.99 | 30.33 | 2.53 | 13.21 |
| 40 | 1500 | 2.54 | 15.61 | 7.44 | 56.30 | 75.07 | 3.54 | 0.21 | 24.78 | 41.57 | 61.48 | 9.76 | 2.66 | 8.26 |
| 41 | 1000 | 3.77 | 8.94 | 5.93 | 56.28 | 112.55 | 8.33 | 0.09 | 88.51 | 93.09 | 101.95 | 23.48 | 10.41 | 17.19 |
| 42 | 1500 | 7.86 | 33.13 | 9.58 | 100.03 | 133.38 | 6.00 | 0.12 | 93.77 | 122.16 | 141.22 | 11.93 | 3.69 | 14.74 |
| 43 | 1000 | 1.75 | 27.18 | 5.31 | 53.72 | 107.44 | 5.59 | 0.13 | 72.76 | 84.2 | 114.4 | 41.58 | 3.10 | 21.54 |
| 44 | 1000 | 1.88 | 19.73 | 5.47 | 50.70 | 101.40 | 6.96 | 0.11 | 25.38 | 35.32 | 51.27 | 13.50 | 1.79 | 9.37 |
| 45 | 1500 | 1.56 | 29.99 | 8.09 | 60.12 | 80.15 | 5.12 | 0.14 | 23.44 | 31.29 | 57.56 | 15.03 | 1.04 | 7.11 |
| 46 | 1500 | 4.478 | 70.94 | 18.46 | 120.83 | 161.10 | 7.41 | 0.10 | 42.64 | 47.72 | 134.34 | 9.52 | 0.67 | 7.28 |
| 47 | 1000 | 1.36 | 34.21 | 3.92 | 52.45 | 104.90 | 8.39 | 0.09 | 37.25 | 51.15 | 53.83 | 27.39 | 1.50 | 13.73 |
| 48 | 1500 | 3.06 | 9.49 | 15.63 | 73.25 | 97.67 | 5.32 | 0.14 | 109.23 | 106.64 | 123.6 | 35.70 | 11.24 | 7.91 |
| 49 | 1500 | 1.25 | 18.51 | 6.947 | 49.67 | 66.23 | 4.05 | 0.18 | 48.83 | 57.73 | 80.71 | 39.06 | 3.12 | 11.62 |
| 50 | 1500 | 2.9 | 10.67 | 12.57 | 66.50 | 88.67 | 6.07 | 0.12 | 42.17 | 51.16 | 105.89 | 14.54 | 4.79 | 8.42 |
| 51 | 1500 | 2.17 | 34.82 | 5.51 | 60.67 | 80.89 | 5.44 | 0.14 | 69.65 | 73.87 | 106.42 | 32.10 | 2.12 | 19.31 |
| 52 | 1500 | 3.67 | 32.69 | 10.76 | 79.00 | 105.34 | 5.44 | 0.14 | 59.88 | 76.05 | 158.23 | 16.32 | 2.33 | 14.71 |
| 53 | 1500 | 5.14 | 10.09 | 7.36 | 67.56 | 90.08 | 6.85 | 0.11 | 74.28 | 81.11 | 102.17 | 14.45 | 8.04 | 13.88 |
| 54 | 1500 | 3.15 | 13.07 | 4.99 | 53.09 | 70.79 | 6.34 | 0.12 | 72.93 | 101.77 | 102.14 | 23.15 | 7.79 | 20.47 |

| Code | MMF daily dose (mg) | MPA concentration (µg/mL) | | | MPA $AUC_{0-12}$ (mg*h/L) | Normalized MPA AUC (mg*h/L/1000 mg) | Normalized MPA AUC (mg*h/L/mg dose/kg) | CL/F (L/h/kg) | MPAG concentration (µg/mL) | | | MPAG:MPA ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C_0 | C_0.5 | C_2 | | | | | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 55 | 1000 | 2.65 | 3.28 | 11.16 | 58.07 | 116.13 | 7.20 | 0.10 | 45.07 | 39.5 | 73.2 | 17.01 | 12.04 | 6.56 |
| 56 | 1500 | 6.36 | 5.11 | 8.9 | 74.89 | 99.86 | 8.22 | 0.09 | 89.5 | 76.91 | 109.91 | 14.07 | 15.05 | 12.35 |
| 57 | 500 | 1.44 | 7.21 | 2.92 | 35.97 | 143.89 | 7.86 | 0.09 | 35.12 | 30.21 | 52.5 | 24.39 | 4.19 | 17.98 |
| 58 | 1500 | 4.5 | 20.07 | 8.37 | 71.62 | 95.49 | 5.92 | 0.12 | 59.85 | 66.8 | 97.94 | 13.30 | 3.33 | 11.70 |
| 59 | 1000 | 15.19 | 22.91 | 16.46 | 149.81 | 299.63 | 12.64 | 0.06 | 99.19 | 93.86 | 119.93 | 6.53 | 4.10 | 7.29 |
| 60 | 1000 | 1.65 | 1.5 | 2.83 | 33.82 | 67.64 | 3.72 | 0.20 | 21.53 | 17.21 | 19.82 | 13.05 | 11.47 | 7.00 |
| 61 | 1000 | 0.88 | 24.09 | 2.83 | 41.92 | 83.84 | 5.44 | 0.14 | 40.32 | 70.82 | 99.35 | 45.82 | 2.94 | 35.11 |
| 62 | 1000 | 3.3 | 13.94 | 6.74 | 58.12 | 116.25 | 6.63 | 0.11 | 45.49 | 54.39 | 92.27 | 13.78 | 3.90 | 13.69 |
| 63 | 1500 | 0.98 | 11.18 | 5.12 | 40.27 | 53.69 | 3.77 | 0.20 | 89.04 | 90.31 | 169.76 | 90.86 | 8.08 | 33.16 |
| 64 | 1500 | 3.96 | 8.51 | 7.28 | 59.97 | 79.96 | 7.95 | 0.09 | 63.01 | 58.58 | 82.67 | 15.91 | 6.88 | 11.36 |
| 65 | 1000 | 6.81 | 4.22 | 12.52 | 84.60 | 169.20 | 9.56 | 0.08 | 76.41 | 64.95 | 88.23 | 11.22 | 15.39 | 7.05 |
| 66 | 1000 | 0.75 | 2.01 | 10.81 | 46.06 | 92.12 | 5.55 | 0.13 | 11.93 | 12.03 | 44.81 | 15.91 | 5.99 | 4.15 |
| 67 | 1000 | 1.54 | 32 | 3.92 | 52.24 | 104.48 | 3.97 | 0.19 | 28.63 | 69.53 | 89.42 | 18.59 | 2.17 | 22.81 |
| 68 | 1000 | 1.73 | 22.41 | 3.49 | 47.13 | 94.25 | 6.03 | 0.12 | 65.13 | 87.8 | 95.27 | 37.65 | 3.92 | 27.30 |
| 69 | 1500 | 0.95 | 14.02 | 4.98 | 41.36 | 55.15 | 4.36 | 0.17 | 20.05 | 30.59 | 64.7 | 21.11 | 2.18 | 12.99 |
| 70 | 1000 | 0.78 | 7.27 | 4.66 | 36.04 | 72.07 | 4.25 | 0.17 | 45.77 | 44.15 | 59.15 | 58.68 | 6.07 | 12.69 |
| 71 | 1250 | 4.93 | 63.94 | 6.51 | 94.10 | 125.46 | 6.21 | 0.12 | 63.19 | 95.6 | 141.89 | 12.82 | 1.50 | 21.80 |
| 72 | 1000 | 2.92 | 12.65 | 4.84 | 51.27 | 102.53 | 6.87 | 0.11 | 59.99 | 69.24 | 78.84 | 20.54 | 5.47 | 16.29 |

| Code | MMF daily dose (mg) | MPA concentration (µg/mL) | | | MPA $AUC_{0-12}$ (mg*h/L) | Normalized MPA AUC (mg*h/L/1000 mg) | Normalized MPA AUC (mg*h/L/mg dose/kg) | CL/F (L/h/kg) | MPAG concentration (µg/mL) | | | MPAG:MPA ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C_0 | C_0.5 | C_2 | | | | | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 73 | 1500 | 2.31 | 15.71 | 10.9 | 62.43 | 83.25 | 5.37 | 0.14 | 40.02 | 47.32 | 81.9 | 17.32 | 3.01 | 7.51 |
| 74 | 1000 | 3.49 | 15.62 | 4.93 | 56.25 | 112.51 | 9.84 | 0.08 | 72.74 | 75.42 | 93.73 | 20.84 | 4.83 | 19.01 |
| 75 | 500 | 1.01 | 2.83 | 3.04 | 31.44 | 125.75 | 8.78 | 0.08 | 29.07 | 24.66 | 36.15 | 28.78 | 8.71 | 11.89 |
| 76 | 1000 | 1.12 | 2.29 | 19.44 | 66.62 | 133.24 | 8.26 | 0.09 | 31.96 | 31.25 | 47.55 | 28.54 | 13.65 | 2.45 |
| 77 | 1000 | 1.09 | 16.73 | 2.31 | 37.95 | 75.90 | 7.82 | 0.09 | 34.69 | 45.27 | 56.12 | 31.83 | 2.71 | 24.29 |
| 78 | 1000 | 0.25 | 18.56 | 5.27 | 40.57 | 81.15 | 5.19 | 0.14 | 66.24 | 78.71 | 119.5 | 264.96 | 4.24 | 22.68 |
| 79 | 1500 | 0.68 | 18 | 5.96 | 44.12 | 58.83 | 4.12 | 0.18 | 58.31 | 79.01 | 130.54 | 85.75 | 4.39 | 21.90 |
| 80 | 1000 | 2.1 | 34.49 | 4.16 | 57.23 | 114.46 | 5.36 | 0.14 | 55.81 | 93.2 | 97.61 | 26.58 | 2.70 | 23.46 |
| 81 | 1000 | 2.11 | 7.93 | 6.08 | 46.81 | 93.62 | 8.33 | 0.09 | 38.95 | 41.14 | 64.49 | 18.46 | 5.19 | 10.61 |
| 82 | 1500 | 2.59 | 38.86 | 4.14 | 62.31 | 83.07 | 4.84 | 0.15 | 20.22 | 81.12 | 57.73 | 7.81 | 2.09 | 13.94 |
| 83 | 2000 | 3.45 | 47.97 | 4.99 | 73.89 | 73.89 | 4.65 | 0.16 | 52.54 | 58.76 | 74.03 | 15.23 | 1.22 | 14.84 |
| 84 | 1000 | 2.32 | 21.44 | 3.07 | 48.98 | 97.97 | 6.17 | 0.12 | 41.03 | 52.77 | 56.19 | 17.69 | 2.46 | 18.30 |
| 85 | 1750 | 4.81 | 8.06 | 11.23 | 72.84 | 97.12 | 6.75 | 0.11 | 50.82 | 56.44 | 59.12 | 10.57 | 7.00 | 5.26 |
| 86 | 1000 | 1.84 | 1.55 | 1.26 | 31.57 | 63.13 | 3.57 | 0.21 | 26.96 | 27.7 | 11.78 | 14.65 | 17.87 | 9.35 |
| 87 | 1000 | 0.98 | 20.26 | 1.61 | 37.78 | 75.56 | 5.10 | 0.14 | 19.36 | 41.57 | 39.34 | 19.76 | 2.05 | 24.43 |
| 88 | 1000 | 0.82 | 15.13 | 2.35 | 35.65 | 71.31 | 3.71 | 0.20 | 34.34 | 49.85 | 49.05 | 41.88 | 3.29 | 20.87 |
| 89 | 2000 | 2.27 | 1.99 | 13.63 | 60.50 | 60.50 | 3.75 | 0.20 | 56.57 | 49.15 | 66.78 | 24.92 | 24.70 | 4.90 |
| 90 | 1000 | 1.48 | 2.82 | 5.18 | 38.59 | 77.19 | 5.56 | 0.13 | 16.41 | 11.24 | 19.67 | 11.09 | 3.99 | 3.80 |

| Code | MMF daily dose (mg) | MPA concentration (µg/mL) | | | MPA $AUC_{0-12}$ (mg*h/L) | Normalized MPA AUC (mg*h/L/1000 mg) | Normalized MPA AUC (mg*h/L/mg dose/kg) | CL/F (L/h/kg) | MPAG concentration (µg/mL) | | | MPAG:MPA ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C_0 | C_0.5 | C_2 | | | | | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 91 | 1500 | 1.19 | 0.84 | 2.21 | 29.58 | 39.44 | 2.47 | 0.30 | 27.2 | 20.4 | 22.41 | 22.86 | 24.29 | 10.14 |
| 92 | 2000 | 1.84 | 15.81 | 2.59 | 42.21 | 42.21 | 4.77 | 0.15 | 114.17 | 139.15 | 135.1 | 62.05 | 8.80 | 52.16 |
| 93 | 1000 | 1.36 | 4.08 | 3.45 | 34.94 | 69.88 | 4.79 | 0.15 | 33.85 | 26.67 | 62.32 | 24.89 | 6.54 | 18.06 |
| 94 | 1000 | 1.31 | 3.32 | 7.58 | 43.03 | 86.05 | 4.30 | 0.17 | 17.48 | 18.87 | 23.05 | 13.34 | 5.68 | 3.04 |
| 95 | 1500 | 8.39 | 40.13 | 6.9 | 101.12 | 134.82 | 8.09 | 0.09 | 69.06 | 77.99 | 113.08 | 8.23 | 1.94 | 16.39 |
| 96 | 2000 | 1.59 | 32.68 | 11.13 | 68.22 | 68.22 | 5.05 | 0.15 | 109.3 | 127.22 | 234.4 | 68.74 | 3.89 | 21.06 |
| 97 | 1500 | 3.89 | 5.07 | 4.84 | 52.50 | 70.01 | 4.48 | 0.16 | 44.45 | 48.22 | 38.81 | 11.43 | 9.51 | 8.02 |
| 98 | 1250 | 0.98 | 9.38 | 11.23 | 52.27 | 69.70 | 3.94 | 0.19 | 23.16 | 29.42 | 74.27 | 23.63 | 3.14 | 6.61 |
| 99 | 2000 | 3.1 | 2.97 | 3.33 | 43.75 | 43.75 | 3.15 | 0.23 | 52.24 | 45.4 | 55.28 | 16.85 | 15.29 | 16.60 |
| 100 | 1500 | 1.67 | 21.76 | 4.08 | 47.69 | 63.59 | 3.75 | 0.20 | 69.04 | 73.04 | 106.64 | 41.34 | 3.36 | 26.14 |
| 101 | 1500 | 2.09 | 2.4 | 4.6 | 40.52 | 54.03 | 4.08 | 0.18 | 39.71 | 33.48 | 42.81 | 19.00 | 13.95 | 9.31 |
| 102 | 1500 | 1.3 | 3.79 | 4.57 | 36.83 | 49.10 | 2.98 | 0.25 | 71.02 | 49.77 | 74.03 | 54.63 | 13.13 | 16.20 |
| 103 | 1000 | 3.97 | 10.61 | 9.02 | 64.87 | 129.74 | 6.79 | 0.11 | 142.4 | 126.08 | 177.63 | 35.87 | 11.88 | 19.69 |
| 104 | 500 | 0.24 | 7.69 | 1.29 | 26.10 | 104.40 | 6.99 | 0.11 | 24 | 26.58 | 51.13 | 100.00 | 3.46 | 39.64 |
| 105 | 1000 | 0.49 | 2.1 | 2.87 | 27.78 | 55.57 | 3.87 | 0.19 | 22.69 | 21.06 | 26.3 | 46.31 | 10.03 | 9.16 |
| 106 | 1500 | 0.3 | 1.26 | 2.52 | 25.52 | 34.03 | 2.21 | 0.33 | 48.57 | 46.1 | 45.9 | 161.90 | 36.59 | 18.21 |
| 107 | 1000 | 2.02 | 28.92 | 3.68 | 52.71 | 105.42 | 5.97 | 0.12 | 89.27 | 101.65 | 144.61 | 44.19 | 3.51 | 39.30 |
| 108 | 500 | 1.9 | 22.58 | 2.78 | 46.66 | 186.62 | 9.70 | 0.08 | 12.44 | 18.58 | 28.93 | 6.55 | 0.82 | 10.41 |

| Code | MMF daily dose (mg) | MPA concentration (µg/mL) | | | MPA $AUC_{0-12}$ (mg*h/L) | Normalized MPA AUC (mg*h/L/1000 mg) | Normalized MPA AUC (mg*h/L/mg dose/kg) | CL/F (L/h/kg) | MPAG concentration (µg/mL) | | | MPAG:MPA ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C_0 | C_0.5 | C_2 | | | | | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 109 | 1500 | 7.89 | 35.05 | 8.41 | 98.76 | 131.68 | 7.11 | 0.10 | 55.23 | 58.97 | 82.42 | 7.00 | 1.68 | 9.80 |
| 110 | 1000 | 8.6 | 22.52 | 11.99 | 103.46 | 206.91 | 9.72 | 0.08 | 48.33 | 41.71 | 70.44 | 5.62 | 1.85 | 5.87 |
| 111 | 1500 | 2.16 | 7.53 | 8.13 | 51.23 | 68.30 | 4.41 | 0.17 | 53.32 | 50.11 | 116.82 | 24.69 | 6.65 | 14.37 |
| 112 | 1000 | 1.47 | 1.12 | 5.9 | 39.14 | 78.28 | 3.21 | 0.23 | 12.01 | 8.56 | 16.8 | 8.17 | 7.64 | 2.85 |
| 113 | 1000 | 1.82 | 6.824 | 4.27 | 40.74 | 81.49 | 6.73 | 0.11 | 139.74 | 146.26 | 187.65 | 76.78 | 21.43 | 43.95 |
| 114 | 1500 | 1.87 | 38.56 | 3.62 | 57.03 | 76.04 | 5.32 | 0.14 | 55.39 | 77.94 | 98.38 | 29.62 | 2.02 | 27.18 |
| 115 | 500 | 1.99 | 4.89 | 2.1 | 36.02 | 144.07 | 7.49 | 0.10 | 15.13 | 15.87 | 39.32 | 7.60 | 3.25 | 18.72 |
| 116 | 500 | 0.65 | 8.522 | 1.39 | 29.05 | 116.19 | 8.42 | 0.09 | 11.59 | 16.69 | 29.12 | 17.83 | 1.96 | 20.95 |
| 117 | 1500 | 1.68 | 2.27 | 11.17 | 52.14 | 69.52 | 6.40 | 0.12 | 33.58 | 26.68 | 56.46 | 19.99 | 11.75 | 5.05 |
| 118 | 1000 | 0.73 | 11.56 | 4.88 | 38.58 | 77.15 | 4.01 | 0.18 | 27.44 | 29.95 | 66.5 | 37.59 | 2.59 | 13.63 |

| Code | Produced XMP (µmol/L) | | | Measured AMP (µmol/L) | | | IMPDH activity (µmol x $s^{-1}$ x $mol^{-1}$ AMP) | | |
|---|---|---|---|---|---|---|---|---|---|
| | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 1 | 67.28 | 75.21 | 69.98 | 28.68 | 61.11 | 61.41 | 260.65 | 136.75 | 126.62 |
| 2 | 27.94 | 41.52 | 40.16 | 20.43 | 28.14 | 25.17 | 151.96 | 163.94 | 177.28 |
| 3 | 103.44 | 82.56 | 81.63 | 41.14 | 29.61 | 37.53 | 279.37 | 309.81 | 241.67 |
| 4 | 60.02 | 75.35 | 84.11 | 28.1 | 34.34 | 28.84 | 237.33 | 243.80 | 324.05 |
| 5 | 92.86 | 89.73 | 90.31 | 39.32 | 35.55 | 51.99 | 262.41 | 280.45 | 193.01 |
| 6 | 66.61 | 68.94 | 65.28 | 40.41 | 56.21 | 68.25 | 183.15 | 136.27 | 106.28 |
| 7 | 90.03 | 63.93 | 93.3 | 39.42 | 31.71 | 31.9 | 253.76 | 224.01 | 324.97 |
| 8 | 38.59 | 22.98 | 37.68 | 49.52 | 49.72 | 65.07 | 86.59 | 51.35 | 64.34 |
| 9 | 81.92 | 81.76 | 64.23 | 97.82 | 142.28 | 114.39 | 93.05 | 63.85 | 62.39 |
| 10 | 117.46 | 101.61 | 109.73 | 96.96 | 85.11 | 74.12 | 134.60 | 132.65 | 164.49 |
| 11 | 81.6 | 59.19 | 71.53 | 57.17 | 52.63 | 58.07 | 158.59 | 124.96 | 136.87 |
| 12 | 81.91 | 60.31 | 40.37 | 76.19 | 78.25 | 38.46 | 119.45 | 85.64 | 116.63 |
| 13 | 104.89 | 79.93 | 89 | 83.22 | 62.37 | 61.16 | 140.04 | 142.39 | 161.69 |
| 14 | 100.62 | 77.52 | 72.64 | 87.67 | 76.24 | 73.13 | 127.52 | 112.98 | 110.37 |
| 15 | 94.85 | 67.72 | 86.28 | 120.87 | 102.3 | 115.47 | 87.19 | 73.55 | 83.02 |
| 16 | 60.79 | 62.95 | 80.52 | 71.24 | 74.33 | 91.81 | 94.81 | 94.10 | 97.45 |
| 17 | 106.51 | 87.07 | 92.74 | 123.71 | 126.54 | 127.79 | 95.66 | 76.45 | 80.64 |
| 18 | 106.34 | 54.85 | 56.36 | 123.98 | 67.98 | 73.36 | 95.30 | 89.65 | 85.36 |
| 19 | 72.46 | 68.52 | 73.04 | 126.63 | 111.02 | 97.2 | 63.58 | 68.58 | 83.49 |

| Code | Produced XMP (µmol/L) | | | Measured AMP (µmol/L) | | | IMPDH activity (µmol x $s^{-1}$ x $mol^{-1}$ AMP) | | |
|---|---|---|---|---|---|---|---|---|---|
| | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 20 | 89.07 | 85.95 | 84.59 | 58.97 | 85.59 | 68.77 | 167.83 | 111.58 | 136.67 |
| 21 | 66.91 | 68.8 | 58.83 | 79.9 | 91.03 | 89.8 | 93.05 | 83.98 | 72.79 |
| 22 | 60.44 | 150.36 | 63.75 | 77.62 | 85.17 | 93.26 | 86.52 | 196.16 | 75.95 |
| 23 | 87.6 | 91.95 | 89.29 | 100.65 | 110.12 | 105.43 | 96.70 | 92.78 | 94.10 |
| 24 | 94.22 | 67.48 | 77.62 | 77.42 | 63.13 | 62.71 | 135.22 | 118.77 | 137.53 |
| 25 | 97.64 | 83.46 | 110.5 | 79.25 | 77.65 | 69.92 | 136.89 | 119.42 | 175.60 |
| 26 | 60.55 | 55.82 | 75.95 | 93.59 | 92.65 | 97.22 | 71.89 | 66.94 | 86.80 |
| 27 | 96.02 | 110.78 | 88.3 | 142.91 | 125.39 | 142.94 | 74.65 | 98.16 | 68.64 |
| 28 | 86.67 | 50.85 | 87.3 | 104.82 | 95.57 | 102.24 | 91.87 | 59.12 | 94.87 |
| 29 | 83.23 | 82.97 | 86.03 | 103.01 | 104.89 | 82.07 | 89.78 | 87.89 | 116.47 |
| 30 | 66.91 | 62.91 | 54.85 | 59.24 | 69.23 | 62.54 | 125.50 | 100.97 | 97.45 |
| 31 | 72.24 | 66.19 | 52.63 | 81.03 | 98.34 | 108.81 | 99.06 | 74.79 | 53.74 |
| 32 | 71.1 | 61.88 | 87.32 | 80.86 | 105.55 | 118.45 | 97.70 | 65.14 | 81.91 |
| 33 | 83.36 | 66.46 | 83.26 | 90.68 | 83.47 | 76.31 | 102.14 | 88.47 | 121.23 |
| 34 | 65.04 | 53.51 | 41.59 | 66.19 | 58.29 | 51.73 | 109.18 | 102.00 | 89.33 |
| 35 | 63.7 | 61.2 | 41.6 | 105.14 | 113.28 | 83.41 | 67.32 | 60.03 | 55.42 |
| 36 | 87.11 | 60.01 | 50.34 | 83.39 | 67.07 | 60.45 | 116.07 | 99.42 | 92.53 |
| 37 | 78.28 | 62.89 | 80.88 | 64.4 | 65.39 | 81.13 | 135.06 | 106.86 | 110.77 |
| 38 | 57.85 | 62.55 | 60.59 | 72.67 | 87.85 | 114.97 | 88.45 | 79.11 | 58.56 |

| Code | Produced XMP (µmol/L) | | | Measured AMP (µmol/L) | | | IMPDH activity (µmol x $s^{-1}$ x $mol^{-1}$ AMP) | | |
|---|---|---|---|---|---|---|---|---|---|
| | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 39 | 66.34 | 49.85 | 55.39 | 54.14 | 53.2 | 45.32 | 136.15 | 104.11 | 135.80 |
| 40 | 63.09 | 57.78 | 46.9 | 62.68 | 61.24 | 46.55 | 111.84 | 104.83 | 111.95 |
| 41 | 86.72 | 95.48 | 83.69 | 62.6 | 83.96 | 73.48 | 153.92 | 126.36 | 126.55 |
| 42 | 64.95 | 23.71 | 62.74 | 91.22 | 30.75 | 79.24 | 79.11 | 85.67 | 87.97 |
| 43 | 57.54 | 35.91 | 55.69 | 49.48 | 39.84 | 48.27 | 129.21 | 100.15 | 128.19 |
| 44 | 51.25 | 58.59 | 77.44 | 36.86 | 55.89 | 78.27 | 154.49 | 116.48 | 109.93 |
| 45 | 42.69 | 32.93 | 62.75 | 34.37 | 37.43 | 48.31 | 138.01 | 97.75 | 144.32 |
| 46 | 57.12 | 37.05 | 39.87 | 62.46 | 87.71 | 31.16 | 101.61 | 46.93 | 142.17 |
| 47 | 83.29 | 67.42 | 30.82 | 82.61 | 81.21 | 51.44 | 112.03 | 92.24 | 66.57 |
| 48 | 31.81 | 38.12 | 39.02 | 65.76 | 93.43 | 66.83 | 53.75 | 45.33 | 64.87 |
| 49 | 46.87 | 63.18 | 80.04 | 36.55 | 56.92 | 57.41 | 142.48 | 123.33 | 154.91 |
| 50 | 62.49 | 45.57 | 61.26 | 82.27 | 89.08 | 93.25 | 84.40 | 56.84 | 72.99 |
| 51 | 46.92 | 35.51 | 46.66 | 35.64 | 38.58 | 43.58 | 146.28 | 102.27 | 118.96 |
| 52 | 64.75 | 54.95 | 73.45 | 59.67 | 49.31 | 60.41 | 120.57 | 123.82 | 135.10 |
| 53 | 41.49 | 21.7 | 15.52 | 46.22 | 60.52 | 52.42 | 99.74 | 39.84 | 32.90 |
| 54 | 113.1 | 71.91 | 22.04 | 64.72 | 82.14 | 28.24 | 194.17 | 97.27 | 86.72 |
| 55 | 26.52 | 39.16 | 16.15 | 78.91 | 68.93 | 57.17 | 37.34 | 63.12 | 31.39 |
| 56 | 48.49 | 40.15 | 48.99 | 60.18 | 48.52 | 88.84 | 89.53 | 91.94 | 61.27 |
| 57 | 22.2 | 24.86 | 20.78 | 32 | 34.24 | 28.22 | 77.08 | 80.67 | 81.82 |

| Code | Produced XMP (µmol/L) | | | Measured AMP (µmol/L) | | | IMPDH activity (µmol x $s^{-1}$ x $mol^{-1}$ AMP) | | |
|---|---|---|---|---|---|---|---|---|---|
| | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 58 | 110.88 | 73.05 | 75.09 | 106.1 | 104.87 | 107.2 | 116.12 | 77.40 | 77.83 |
| 59 | 115.91 | 93.57 | 77.24 | 81.78 | 69.1 | 62.66 | 157.48 | 150.46 | 136.96 |
| 60 | 94.37 | 60.83 | 53.65 | 74.68 | 69.63 | 68.71 | 140.41 | 97.07 | 86.76 |
| 61 | 48.83 | 25.69 | 40.93 | 89.67 | 143.01 | 73.54 | 60.51 | 19.96 | 61.84 |
| 62 | 73.01 | 53.74 | 72.86 | 113.4 | 90.96 | 117.41 | 71.54 | 65.65 | 68.95 |
| 63 | 39.06 | 72.22 | 29.47 | 41.45 | 38.43 | 42.28 | 104.70 | 208.81 | 77.45 |
| 64 | 68.63 | 92.75 | 32.53 | 50.54 | 65.78 | 16.98 | 150.88 | 156.67 | 212.86 |
| 65 | 65.39 | 73.03 | 36.23 | 59.02 | 58.15 | 59.85 | 123.10 | 139.54 | 67.26 |
| 66 | 79.92 | 70.3 | 12.27 | 42.28 | 48.99 | 24.35 | 210.03 | 159.44 | 55.99 |
| 67 | 63.58 | 46.05 | 10.02 | 65.31 | 47.25 | 48.46 | 108.17 | 108.29 | 22.97 |
| 68 | 62.21 | 49.42 | 52.68 | 45.16 | 45.32 | 57.37 | 153.06 | 121.16 | 102.03 |
| 69 | 92.45 | 72.28 | 63.79 | 71.2 | 71.82 | 81.98 | 144.27 | 111.82 | 86.46 |
| 70 | 44.5 | 46.77 | 42.1 | 63.28 | 52.38 | 42.64 | 78.14 | 99.21 | 109.70 |
| 71 | 68.26 | 49.8 | 55.8 | 72.55 | 82.4 | 80.19 | 104.54 | 67.15 | 77.32 |
| 72 | 73.13 | 76.82 | 86.98 | 92.98 | 105.17 | 88.19 | 87.39 | 81.16 | 109.59 |
| 73 | 114.84 | 92.92 | 55.97 | 63.59 | 66.44 | 42.49 | 200.66 | 155.40 | 146.36 |
| 74 | 32.49 | 40.71 | 33.35 | 35.09 | 68.39 | 42.7 | 102.88 | 66.14 | 86.78 |
| 75 | 27.84 | 21.68 | 32.15 | 43.38 | 41.15 | 39.74 | 71.31 | 58.54 | 89.89 |
| 76 | 92.87 | 77.55 | 72.25 | 90.05 | 100.99 | 97.89 | 114.59 | 85.32 | 82.01 |

| Code | Produced XMP (µmol/L) | | | Measured AMP (µmol/L) | | | IMPDH activity (µmol x $s^{-1}$ x $mol^{-1}$ AMP) | | |
|---|---|---|---|---|---|---|---|---|---|
| | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 77 | 52.27 | 49.82 | 60.38 | 67.3 | 78.04 | 83.58 | 86.30 | 70.93 | 80.27 |
| 78 | 81.42 | 54.29 | 63.09 | 43.93 | 39.89 | 29.59 | 205.93 | 151.22 | 236.90 |
| 79 | 78.97 | 73.33 | 76.47 | 99.25 | 107.29 | 92.69 | 88.41 | 75.94 | 91.67 |
| 80 | 62.35 | 58.38 | 71.54 | 64.83 | 73.23 | 71.33 | 106.86 | 88.58 | 111.44 |
| 81 | 82.85 | 45.38 | 41.3 | 83.01 | 73.98 | 62.79 | 110.90 | 68.16 | 73.08 |
| 82 | 68.68 | 66.33 | 61.63 | 73.08 | 85.91 | 71.8 | 104.42 | 85.79 | 95.37 |
| 83 | 71.49 | 37.59 | 108.78 | 159.29 | 48.03 | 133.45 | 49.87 | 86.96 | 90.57 |
| 84 | 56.05 | 40.92 | 52.93 | 77.81 | 60.9 | 58.06 | 80.04 | 74.66 | 101.29 |
| 85 | 95.41 | 91.86 | 82.09 | 93.96 | 80.91 | 82.41 | 112.83 | 126.15 | 110.68 |
| 86 | 97.76 | 100.03 | 118.79 | 118.31 | 149.97 | 183.46 | 91.81 | 74.11 | 71.94 |
| 87 | 56.85 | 41.85 | 73.39 | 85.28 | 107.29 | 151.22 | 74.07 | 43.34 | 53.92 |
| 88 | 103.87 | 67.05 | 75.18 | 43.86 | 54.07 | 51.62 | 263.14 | 137.78 | 161.82 |
| 89 | 112.44 | 112.05 | 103.06 | 112.3 | 115.17 | 103.32 | 111.25 | 108.10 | 110.83 |
| 90 | 77.1 | 91.97 | 93.97 | 87.81 | 86.17 | 98.89 | 97.56 | 118.59 | 105.58 |
| 91 | 52.15 | 80.52 | 94.03 | 62.86 | 80.71 | 92.23 | 92.18 | 110.85 | 113.28 |
| 92 | 110.58 | 88.32 | 99.31 | 78.85 | 95.53 | 85.94 | 155.82 | 102.73 | 128.40 |
| 93 | 25.8 | 26.04 | 16.49 | 43.3 | 44.41 | 31.61 | 66.20 | 65.15 | 57.96 |
| 94 | 105.56 | 23.9 | 24.16 | 105.67 | 21.33 | 30.21 | 111.00 | 124.50 | 88.86 |
| 95 | 98.89 | 97.67 | 61.91 | 40.24 | 71.78 | 76.84 | 273.06 | 151.19 | 89.52 |

| Code | Produced XMP (µmol/L) | | | Measured AMP (µmol/L) | | | IMPDH activity (µmol x $s^{-1}$ x $mol^{-1}$ AMP) | | |
|---|---|---|---|---|---|---|---|---|---|
| | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 96 | 116.63 | 72.16 | 81.28 | 79.43 | 81.88 | 98.36 | 163.15 | 97.92 | 91.82 |
| 97 | 146.23 | 102.02 | 92.7 | 84.33 | 72.9 | 72.7 | 192.67 | 155.49 | 141.68 |
| 98 | 41.37 | 41.09 | 47.17 | 65.73 | 66.55 | 74.03 | 69.93 | 68.60 | 70.80 |
| 99 | 73.11 | 48.03 | 61.68 | 87.38 | 62.51 | 76.28 | 92.97 | 85.37 | 89.84 |
| 100 | 103.08 | 59.98 | 79.86 | 89.03 | 81.89 | 102.26 | 128.65 | 81.38 | 86.77 |
| 101 | 72.54 | 46.67 | 64.38 | 110.55 | 73.69 | 98.72 | 72.91 | 70.37 | 72.46 |
| 102 | 90.08 | 69.11 | 73.95 | 81.53 | 78.26 | 72.41 | 122.76 | 98.12 | 113.47 |
| 103 | 70.97 | 48.85 | 103.82 | 60.84 | 45.29 | 105.53 | 129.61 | 119.84 | 109.31 |
| 104 | 84.9 | 87.01 | 66.63 | 96.97 | 108.99 | 83.82 | 97.28 | 88.70 | 88.32 |
| 105 | 129.49 | 127.21 | 91.02 | 171.45 | 164.91 | 110 | 83.92 | 85.71 | 91.94 |
| 106 | 142.82 | 137.25 | 156.11 | 59.49 | 66.06 | 73.98 | 266.75 | 230.85 | 234.46 |
| 107 | 143.29 | 73.07 | 103.15 | 117.13 | 82.92 | 113.84 | 135.93 | 97.91 | 100.68 |
| 108 | 50.02 | 48.14 | 62.77 | 96.39 | 120.73 | 94.57 | 57.66 | 44.30 | 73.75 |
| 109 | 128.05 | 119.41 | 89.15 | 85.28 | 84.65 | 41.94 | 166.84 | 156.74 | 236.18 |
| 110 | 46.3 | 39.79 | 8.43 | 60.49 | 58.32 | 17.94 | 85.05 | 75.81 | 52.21 |
| 111 | 115.03 | 50.14 | 91.04 | 74.25 | 54.98 | 72.64 | 172.14 | 101.33 | 139.26 |
| 112 | 90.55 | 71.25 | 44.48 | 71.34 | 59.08 | 55.4 | 141.03 | 134.00 | 89.21 |
| 113 | 51.08 | 117.88 | 104.34 | 61.97 | 111.85 | 107.56 | 91.59 | 117.10 | 107.78 |
| 114 | 128.32 | 105.06 | 120.74 | 177.34 | 185.18 | 194.82 | 80.40 | 63.04 | 68.86 |

| Code | Produced XMP (µmol/L) | | | Measured AMP (µmol/L) | | | IMPDH activity (µmol x $s^{-1}$ x $mol^{-1}$ AMP) | | |
|---|---|---|---|---|---|---|---|---|---|
| | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 | C_0 | C_0.5 | C_2 |
| 115 | 90.74 | 30.85 | 48.46 | 135.14 | 38.2 | 57.05 | 74.61 | 89.73 | 94.38 |
| 116 | 39.6 | 35.48 | 40.73 | 57.96 | 66.09 | 49.62 | 75.91 | 59.65 | 91.20 |
| 117 | 114.85 | 10.17 | 42.52 | 151.05 | 17 | 70.77 | 84.48 | 66.47 | 66.76 |
| 118 | 68.63 | 51.83 | 88.62 | 116.52 | 90.87 | 117.82 | 65.44 | 63.38 | 83.57 |

# VITA

Miss Wanarat Anusornsangiam was born on the third of January in 1977 at Roi-Et Hospital, Roi-Et. She graduated Bachelor degree in Pharmaceutical Sciences (first class honors) from Faculty of Pharmaceutical Sciences, Khon Kaen University in 1998 and Master degree in Clinical Pharmacy from Faculty of Pharmacy, Mahidol University in 2002. Her current position is a lecturer at Faculty of Pharmacy, Mahasarakham University.