



**HAL**  
open science

## **Personalized Therapy for Mycophenolate: Consensus Report by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology**

Stein Bergan, Mercè Brunet, Dennis A Hesselink, Kamisha L Johnson-Davis, Pawel K Kunicki, Florian Lemaitre, Pierre Marquet, Mariadelfina Molinaro, Ofelia Noceti, Smita Pattanaik, et al.

### ► To cite this version:

Stein Bergan, Mercè Brunet, Dennis A Hesselink, Kamisha L Johnson-Davis, Pawel K Kunicki, et al.. Personalized Therapy for Mycophenolate: Consensus Report by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology. *Therapeutic Drug Monitoring*, 2021, 43 (2), pp.150-200. 10.1097/FTD.0000000000000871 . hal-03216543

**HAL Id: hal-03216543**

**<https://hal.science/hal-03216543>**

Submitted on 23 Jan 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Personalized Therapy for Mycophenolate: Consensus Report by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology

Stein Bergan, PhD,<sup>1</sup> Mercè Brunet, PharmD, PhD,<sup>2</sup> Dennis A. Hesselink, MD, PhD,<sup>3</sup> Kamisha L. Johnson-Davis, PhD,<sup>4</sup> Paweł K. Kunicki, PharmD, PhD,<sup>5</sup> Florian Lemaitre, PharmD, PhD,<sup>6</sup> Pierre Marquet, MD, PhD,<sup>7</sup> Mariadelfina Molinaro, MSciBiol,<sup>8</sup> Ofelia Noceti, PharmD, PhD,<sup>9</sup> Smita Pattanaik, MD,<sup>10</sup> Tomasz Pawinski, PharmD, PhD,<sup>5</sup> Christoph Seger, PD, PhD, FAMH,<sup>11</sup> Maria Shipkova, MD, FEBLM/MB,<sup>12</sup> Jesse J. Swen, PhD,<sup>13</sup> Teun van Gelder, PhD,<sup>13</sup> Raman Venkataramanan, PhD,<sup>14</sup> Eberhard Wieland, MD, FEBLM/MB, EupSpLM,<sup>12</sup> Jean-Baptiste Woillard, PharmD, PhD,<sup>7</sup> Tom C. Zwart, PharmD,<sup>13</sup> Markus J. Barten, MD, PhD,<sup>15</sup> Klemens Budde, MD,<sup>16</sup> Maja-Theresa Dieterlen, RNDr,<sup>17</sup> Laure Elens, PhD,<sup>18</sup> Vincent Haufroid, PhD,<sup>19</sup> Satoshi Masuda, PhD,<sup>20</sup> Olga Millan, PhD,<sup>2</sup> Tomoyuki Mizuno, PhD,<sup>21,22</sup> Dirk J. A. R. Moes, PharmD, PhD,<sup>13</sup> Michael Oellerich, MD,<sup>23</sup> Nicolas Picard, PharmD, PhD,<sup>7</sup> Linda Salzmann, MSc,<sup>24</sup> Burkhard Tönshoff, MD, PhD,<sup>25</sup> Ron H. N. van Schaik, MD,<sup>26</sup> Nils Tore Vethe, PhD,<sup>1</sup> Alexander A. Vinks, PharmD, PhD,<sup>20</sup> Pierre Wallemacq, PhD,<sup>27</sup> Anders Åsberg, PhD,<sup>28</sup> and Loralie J. Langman, PhD<sup>29</sup>

**Abstract:** When mycophenolic acid (MPA) was originally marketed for immunosuppressive therapy, fixed doses were recommended by the manufacturer. Awareness of the potential for a more personalized dosing has led to development of methods to estimate MPA area under the curve based on the measurement of drug

concentrations in only a few samples. This approach is feasible in the clinical routine and has proven successful in terms of correlation with outcome. However, the search for superior correlates has continued, and numerous studies in search of biomarkers that could better predict the perfect dosage for the individual patient have been published. As it was considered timely for an updated and

Received for publication November 3, 2020; accepted January 29, 2021.

From the <sup>1</sup>Department of Pharmacology, Oslo University Hospital and Department of Pharmacy, University of Oslo, Oslo, Norway; <sup>2</sup>Pharmacology and Toxicology Laboratory, Biochemistry and Molecular Genetics Department, Biomedical Diagnostic Center, Hospital Clinic of Barcelona, University of Barcelona, IDIBAPS, CIBERehd, Spain; <sup>3</sup>Department of Internal Medicine, Division of Nephrology and Transplantation, Erasmus MC, University Medical Center Rotterdam, The Netherlands; <sup>4</sup>Department of Pathology, University of Utah Health Sciences Center and ARUP Laboratories, Salt Lake City, Utah; <sup>5</sup>Department of Drug Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Warszawa, Poland; <sup>6</sup>Univ Rennes, CHU Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail)—UMR\_S 1085, Rennes, France; <sup>7</sup>INSERM, Université de Limoges, Department of Pharmacology and Toxicology, CHU de Limoges, U1248 IPPRIT, Limoges, France; <sup>8</sup>Clinical and Experimental Pharmacokinetics Lab, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; <sup>9</sup>National Center for Liver Transplantation and Liver Diseases, Army Forces Hospital, Montevideo, Uruguay; <sup>10</sup>Department of Pharmacology, PGIMER, Chandigarh, India; <sup>11</sup>Risch Laboratory Group, Buchs, SG, Switzerland; <sup>12</sup>Synlab TDM Competence Center, Synlab MVZ Leinfelden-Echterdingen GmbH, Leinfelden-Echterdingen, Germany; <sup>13</sup>Department of Clinical Pharmacy & Toxicology, Leiden University Medical Center, Leiden, The Netherlands; <sup>14</sup>Department of Pharmaceutical Sciences, School of Pharmacy and Department of Pathology, Starzl Transplantation Institute, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania; <sup>15</sup>Department of Cardiac- and Vascular Surgery, University Heart and Vascular Center Hamburg, Hamburg, Germany; <sup>16</sup>Department of Nephrology, Charité Universitätsmedizin Berlin, Berlin, Germany; <sup>17</sup>Department of Cardiac Surgery, Heart Center, HELIOS Clinic, University Hospital Leipzig, Leipzig, Germany; <sup>18</sup>Integrated Pharmacometrics, Pharmacogenomics and Pharmacokinetics (PMGK) Research Group, Louvain Drug Research Institute (LDRI), Université Catholique de Louvain (UCLouvain), Brussels, Belgium; <sup>19</sup>Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Institut de Recherche Expérimentale et Clinique, UCLouvain and Department of Clinical Chemistry, Cliniques Universitaires Saint-Luc, Brussels, Belgium; <sup>20</sup>Department of Pharmacy, International University of Health and Welfare Narita Hospital, Chiba, Japan; <sup>21</sup>Division of Clinical Pharmacology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; <sup>22</sup>Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio; <sup>23</sup>Department of Clinical Pharmacology, University Medical Center Göttingen, Georg-August-University Göttingen, Göttingen, Germany; <sup>24</sup>Risch Laboratory Group, Buchs, SG, Switzerland; <sup>25</sup>Department of Pediatrics I, University Children's Hospital, Heidelberg, Germany; <sup>26</sup>Department of Clinical Chemistry, Erasmus MC University Medical Center, Rotterdam, The Netherlands; <sup>27</sup>Clinical Chemistry Department, Cliniques Universitaires St Luc, Université Catholique de Louvain, LTAP, Brussels, Belgium; <sup>28</sup>Department of Transplantation Medicine, Oslo University Hospital-Rikshospitalet and Department of Pharmacy, University of Oslo, Oslo, Norway; and <sup>29</sup>Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota.

The authors declare no conflict of interest.

Correspondence: Stein Bergan, PhD, Department of Pharmacology, Oslo University Hospital, PO Box 4950 Nydalen, 0424 Oslo, Norway (e-mail: stein.bergan@farmasi.uio.no).

Copyright © 2021 Wolters Kluwer Health, Inc. All rights reserved.

comprehensive presentation of consensus on the status for personalized treatment with MPA, this report was prepared following an initiative from members of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT). Topics included are the criteria for analytics, methods to estimate exposure including pharmacometrics, the potential influence of pharmacogenetics, development of biomarkers, and the practical aspects of implementation of target concentration intervention. For selected topics with sufficient evidence, such as the application of limited sampling strategies for MPA area under the curve, graded recommendations on target ranges are presented. To provide a comprehensive review, this report also includes updates on the status of potential biomarkers including those which may be promising but with a low level of evidence. In view of the fact that there are very few new immunosuppressive drugs under development for the transplant field, it is likely that MPA will continue to be prescribed on a large scale in the upcoming years. Discontinuation of therapy due to adverse effects is relatively common, increasing the risk for late rejections, which may contribute to graft loss. Therefore, the continued search for innovative methods to better personalize MPA dosage is warranted.

**Key Words:** mycophenolate mofetil, mycophenolic acid, limited sampling strategy, Bayesian estimation, personalized, biomarkers, pharmacokinetics, population PK, pharmacogenetics

(*Ther Drug Monit* 2021;43:150–200)

## INTRODUCTION

Mycophenolic acid (MPA), administered as mycophenolate mofetil (MMF) or enteric-coated mycophenolate sodium (EC-MPS), has found its place in combination with tacrolimus as the immunosuppressive drug regimen of first choice for organ transplant recipients.<sup>1</sup> When MMF was approved more than 20 years ago, fixed doses were recommended by the manufacturer. In the following years, there was an increasing awareness of the potential for a more personalized dosing of this drug in all solid organ and stem cell transplantations and even in other indications where MPA has been increasingly used, off-label indications included.<sup>2–4</sup> From a therapeutic drug monitoring (TDM) perspective, the measurement of drug concentrations in plasma or serum is the most obvious method, and this is now the standard practice at many transplant centers.<sup>5,6</sup> The pharmacokinetics (PK) of MPA is complicated because of nonlinear absorption kinetics, enterohepatic circulation, plasma protein binding, and other factors<sup>7</sup> discussed in a following section. This may explain why measurements at single time points have not proven sufficiently informative in prediction of patient outcomes. Therefore, the use of limited sampling strategies (LSSs) has been introduced to estimate MPA area under the curve (AUC), which has proven more successful in terms of correlation with outcome and identification of therapeutic ranges.<sup>8,9</sup> However, the search for superior correlates has continued, and numerous studies in search of biomarkers that could better predict the perfect dosage for the individual patient have been published.

One may question whether these continued investigations are important, considering the relatively low incidence of graft rejection under the immunosuppressive regimens that

are currently applied, especially in low immunological risk patients. First of all, any reduction in rejections is beneficial. With respect to MPA, the explanation for this has 2 aspects. First, the frequency of adverse effects is a problem and will quite often lead to discontinuation of MPA treatment, as illustrated by one study which showed that by the end of the first-year posttransplant half the patients had MPA (MMF) dose reduced or discontinued, mainly because of hematological and other adverse events.<sup>10</sup> If this could be prevented by a more personalized treatment, maybe more patients could be continued on an appropriate MPA dosage. Second, the occurrence of late rejections is still a problem, and a proportion of these will be antibody mediated and possibly driven by the development of donor-specific antibodies (DSA) in the recipient. There are indications that MPA may be a drug that, through its mechanism of action, can provide some protection against the development of DSA, or at least that such rejections are associated with low MPA exposure.<sup>11–13</sup> If so, this would also argue for individual dosing of MPA—keeping in mind that the immunosuppressive treatment will be lifelong.

During the last decade, several reviews have addressed personalized immunosuppression, especially for MPA in which a broad scope of topics have been discussed, such as criteria for analytics, methods to estimate exposure (including pharmacometrics), the potential influence of pharmacogenetics (PG), development of biomarkers, and the practical aspects of implementation of target concentration intervention.<sup>6,9,14–16</sup>

This article has been prepared following an initiative from the Immunosuppressive Drugs Scientific Committee of the International Association of TDM and Clinical Toxicology (IATDMCT) because it was considered time for an updated and comprehensive presentation of consensus on the status of personalized treatment with MPA. The various sections of the article were drafted by members of the Immunosuppressive Drugs Scientific Committee with expertise in each topic supported by invited experts from outside the IATDMCT, combining the expertise within the group with updated nonsystematic literature research. In the following process, the drafts were then reviewed and finalized by all coauthors to ensure consensus. For selected topics, we have included recommendations and applied grading of evidence using criteria as described in Table 1. This article is intended to provide a comprehensive review. Therefore, it includes several biomarkers for which there may not be sufficient evidence to conclude on their usefulness or relevance, but by including these in this article, it will hopefully be useful both as inspiration and support for the implementation of personalized MPA therapy and to suggest directions for further research.

## MYCOPHENOLATE PHARMACOLOGY

### Discovery of MPA

MPA was discovered in 1893 by an Italian physician, Bartolomeo Gosio, as a fermentation product of *Penicillium* species.<sup>17</sup> Initially, MPA was developed as an antibacterial agent, but because of its adverse effects on immune cells, it

**TABLE 1.** Grading of Recommendations and Level of Evidence<sup>568,569</sup>

Category, Grade	Definition
Strength of recommendation	
A	Good evidence to support a recommendation for specific target concentrations or biomarker (BM) monitoring
B	Moderate evidence to support a recommendation for specific target concentrations or BM monitoring
C1	Regardless of poor evidence, recommendation for specific target concentrations or BM monitoring
C2	Poor evidence to support a recommendation for specific target concentrations or BM monitoring
Quality of evidence	
I	Evidence from $\geq 1$ properly randomized, controlled multicentre clinical trial using validated methodology
II	Evidence from $\geq 1$ well-designed cohort or case-controlled non-randomized clinical trial, multiple time series, and standardized methodologies
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports from expert committees

was abandoned. In 1969, it was found that MPA limits the de novo guanosine nucleotide synthesis by inhibiting inosine-5-monophosphate dehydrogenase (IMPDH).<sup>18</sup> MPA seemed to be a 5-fold more potent inhibitor of the type II isoform of IMPDH, which is expressed in activated T and B lymphocytes, than that of the housekeeping type I isoform, which is expressed in most cell types.<sup>19</sup> This “more or less” selective inhibition of lymphocytes made MPA an attractive candidate as an immunosuppressive drug. Anthony Allison, at Syntex, with his wife Elsie Eugui later developed MPA as an immunosuppressive drug.<sup>19</sup> The poor bioavailability of MPA was improved by the synthesis of MMF, a morpholinoethyl ester prodrug.<sup>20</sup> In 1990, Randall E Morris and colleagues published the results of an experimental study performed in collaboration with Allison and Eugui on treatment of heart transplant rejection.<sup>21</sup> This was the starting point of several clinical trials that led to the registration of MMF for the prevention of rejection in kidney transplantation in the mid-1990s.

### Mechanism of Action

The mechanism of action of MPA was elucidated by Allison and Eugui<sup>22</sup> and has been described in detail in several publications.<sup>23–27</sup>

The key effect responsible for the immunosuppressive action of MPA is a potent, noncompetitive, and reversible inhibition of the enzyme IMPDH. This enzyme catalyzes the conversion of IMP to xanthosine-5'-monophosphate (XMP)

in the presence of the cofactor nicotinamide adenine dinucleotide (NAD<sup>+</sup>), and this is the rate-limiting step in the de novo purine synthesis pathway. This results in the depletion of the intracellular pool of guanosine and deoxyguanosine, imbalance between precursors of mRNA, rRNA, and tRNA, nuclear stress, arrest in cell cycle progression at the G0/G1 phase of their cell cycle and thus preventing cell proliferation<sup>24,27</sup> T and B lymphocytes, as well as fibroblasts, are primarily affected because they are strongly dependent on the de novo pathway of purine synthesis in contrast to most other cells that can sustain their purine nucleotide pool through the salvage pathway.

Furthermore, MPA downregulates CD40L [cluster of differentiation (CD); 40; ligand (L)] signaling, a costimulator of antigen-presenting cells in diverse systemic autoimmune diseases. In addition to this major immunosuppressive mechanism of MPA, some further mechanisms may be responsible for additional favorable therapeutic effects. Examples of such mechanisms are alteration of lymphocyte and monocyte recruitment, adhesion, and penetration; apoptosis of activated human T lymphocytes and to lesser degree monocytes; attenuation of cytokine production; antiproliferative effect of MPA on monocytes, fibroblasts, endothelial cells, mesangial cells, and smooth muscle cells; inhibition of mesangial matrix expansion; and alterations in cytoskeletal organization.<sup>22,23</sup> Some of these effects, including reduced expression of important lymphocyte cell surface antigens, may be independent from guanosine depletion.<sup>28,29</sup>

Experimental data suggested that a minor MPA metabolite, acyl-glucuronide MPA (AcMPAG see below), possessed pharmacological and toxicological activity.<sup>30</sup> These observations led to the speculation that the reported activity might contribute to clinical adverse effects. Experience with this metabolite from clinical studies is limited and somewhat controversial. Unfortunately, ex vivo investigation on AcMPAG effects is complicated because of its limited stability in blood samples.<sup>31</sup> Importantly, local concentrations in gut rather than plasma concentrations are more likely to account for gastrointestinal toxicity.<sup>32</sup> Therefore, the current evidence available does not justify the measurement of AcMPAG concentration in routine practice.

### Indications

#### Solid Organ Transplantation

Mycophenolate is marketed under 2 formulations: MMF (CellCept; Roche, Basel, Switzerland) and EC-MPS (Myfortic; Novartis, Basel, Switzerland). MMF and EC-MPS exhibit slight label differences between countries. MMF is indicated together with calcineurin inhibitors (CNIs) with or without glucocorticoids to prevent organ rejection in patients receiving allogeneic renal, cardiac, and liver grafts in adult recipients. In Japan and Australia, the therapeutic indication also covers lung and pancreas rejection prophylaxis. In the pediatric population, MMF is approved for renal transplantation.

The EC-MPS formulation is generally approved for adult renal transplantation. In Canada, it is also approved for adult liver transplant recipients (LTR). However, its safety

and efficacy have not been established in the pediatric population yet. In the United States, the use in renal transplant children is approved for those who are 5 years or older from 6 months posttransplant. Absorption kinetics are different for EC-MPS compared with those for MMF, and important aspects are discussed in the PK section below.

### Off-Label Indications

MPA has several off-label uses in autoimmune diseases such as systemic lupus erythematosus (SLE), lupus nephritis (LN), vasculitis, immunoglobulin (Ig) A nephritis, and others.<sup>33</sup> This is related to (1) its antiproliferative and anti-inflammatory capacity, and as a modulator of fibrosis<sup>34–39</sup>; (2) its role in rescue for patients in whom corticosteroid therapy has failed,<sup>40–44</sup> allowing corticosteroid dose reduction<sup>45</sup>; and (3) its ability to replace azathioprine when azathioprine results in serious adverse events. The potential for improving therapy by personalization of MPA treatment on these indications is discussed in the section on PK monitoring.

### Special Populations

#### Pregnancy

MPA bears a high miscarriage risk and is responsible for congenital malformations of various degrees (orofacial, limb, renal, cardiovascular, and nervous system and fingers) during the first months of pregnancy.<sup>46,47</sup> Teratogenicity is not related to the MMF dose.<sup>48</sup> For these reasons, MPA should be interrupted at least 6 weeks before conception, and fertility preservation should be discussed before starting the treatment.<sup>46,49–52</sup>

#### Male Fertility Preservation

Although the drug label recommends that sexually active men treated with MPA should use reliable contraception, the clinical data do not provide evidence for an increased risk of adverse birth outcomes in children fathered by male transplant patients.<sup>53</sup> It is questionable whether it is wise to switch a stable male transplant patient, wishing to conceive, from MPA to an alternative immunosuppressive drug because this may increase the probability of rejection. It is therefore recommended that patients be informed about the scarcity of evidence for the current warning of potential adverse effects of MMF in men and on the risk of acute rejection in case MMF is discontinued.<sup>46,54</sup>

### Contraindications

Reactions such as IgE-mediated allergy to MMF are not common, and if there is reasonable doubt, one would shift to azathioprine or a mammalian target of rapamycin (mTOR) inhibitor. There is also a single case report where a desensitization protocol was applied.<sup>55</sup>

### Pharmacokinetics

MPA displays nonlinear absorption kinetics, with complex and large intrapharmacokinetic and interpharmacokinetic variability, partly attributed to enterohepatic circulation, plasma protein binding changes, graft function, genetics, and drug–drug interactions (DDIs).<sup>7</sup> The drug is primarily

within the plasma compartment of the blood, with 97%–99% of MPA bound to albumin.

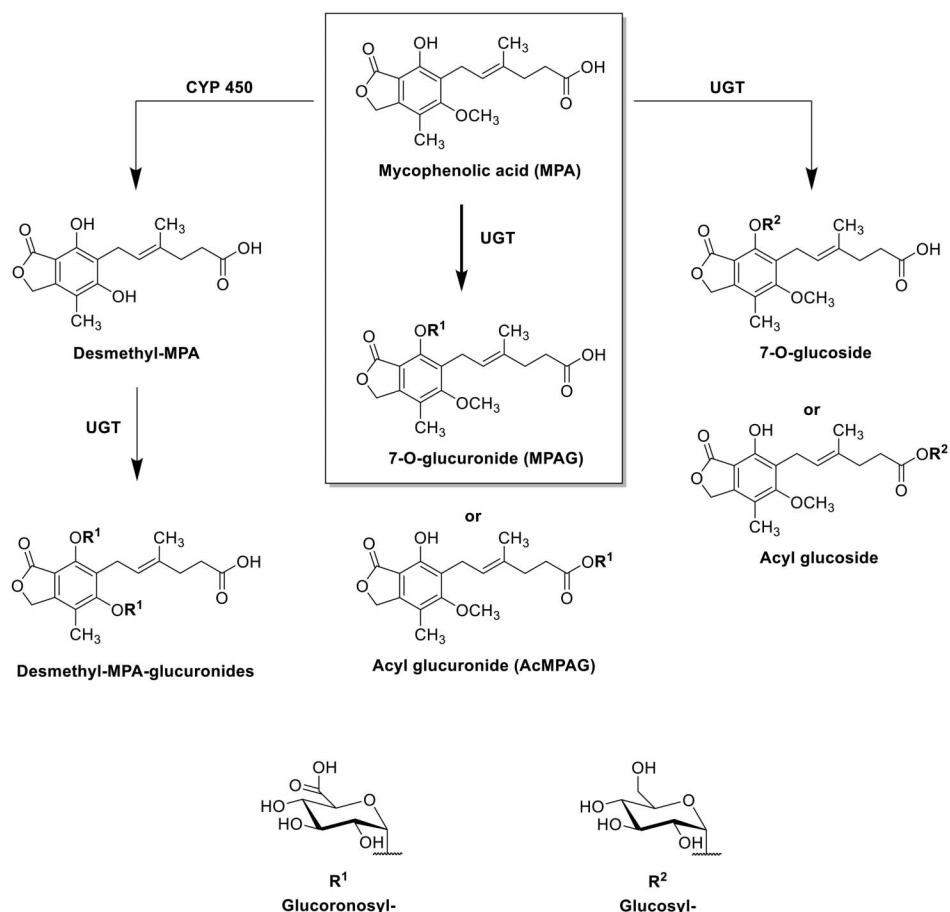
The metabolism of MPA (Fig. 1) is extensive and mostly occurs in the liver, intestine, and kidney through the uridine 5'-diphospho-glucuronosyltransferase (UGT) system. MPA is glucuronidated by several UGTs into the pharmacologically inactive MPA 7-O-glucuronide (MPAG) and the pharmacologically active AcMPAG.<sup>56</sup> Most MPAG undergoes active transport from the hepatocytes into the circulation; among the suggested transporters involved are multidrug resistance-associated protein-3 (MRP3) and multidrug resistance-associated protein-4 (MRP4).<sup>57,58</sup> More than 85% of the metabolites and less than 1% MPA are excreted in the urine.<sup>59</sup> A study in 2019 demonstrated a lower abundance and activity of UGT enzymes in neonatal liver microsomes compared with those in adults.<sup>60</sup> Another study supported the previous study showing a lower level of UGT1A9 and UGT2B7 in infancy increasing to adulthood. In the same study, numerically lower UGT activities were seen in samples from donors older than 65 years, but as only 5 were included these results were statistically inconclusive.<sup>61</sup>

MPAG is excreted from the hepatocyte into the bile by multidrug resistance-associated protein 2 [MRP2, adenosine triphosphate (ATP)-binding cassette subfamily C member 2 (ABCC2)], encoded by the *ABCC2* gene.<sup>62</sup> Glucuronidases in the intestinal flora can convert this metabolite back to MPA, which is subsequently reabsorbed. With MMF, this occasionally results in a second plasma peak of MPA 6–12 hours after oral administration,<sup>63,64</sup> which may contribute 30%–40% of the AUC for MPA. However, the observation of the second peak could also be due to a biphasic absorption.<sup>65</sup> Because MPA is a weak acid, absorption is maximized when pH is low. Because pH variations along the GI tract are described, greater MPA absorption would occur where the pH is lower, as opposed to areas where the pH is higher, and this can account for 2 absorption peaks. With EC-MPS, the absorption is more variable and it is difficult to distinguish between late absorption maximum and a secondary peak due to enterohepatic circulation.<sup>66</sup>

The mean elimination half-life of MPA is 8–16 hours, and final elimination is by active tubular secretion of MPAG in urine. Severe renal impairment has been shown to decrease the binding of MPA to albumin. This can be explained not only by the uremic state itself but also by the reduced elimination of MPAG that then increasingly competes with MPA for albumin binding.<sup>67</sup> This will increase the clearance of MPA and reduce its total concentration while the free concentration will remain the same (free fraction will increase).<sup>68</sup> A sufficiently reduced serum albumin will have the same effect. It has been reported that in the early posttransplant period, in patients with delayed graft function or renal impairment, total MPA exposure was lower.<sup>69–71</sup>

MMF and EC-MPS provide comparable distribution, metabolism, and excretion of MPA. They both exhibit high oral bioavailability, approximately 80%–90%. MPA exposure, based on the AUC, was not significantly lower in elderly than in younger patients receiving the same MMF dose.<sup>72</sup>

After oral administration, MMF can hardly be detected at any time in plasma because it is rapidly de-esterified in the stomach to produce MPA and the inactive mofetil (N-[2-



**FIGURE 1.** Metabolic pathways of MPA.

hydroxyethyl]-morpholine). Absorption takes place partly in the stomach with the remainder in the proximal small intestine. The MPA time–concentration profile after administration of MMF is consistent with rapid absorption of MPA in the early GI tract resulting in MPA reaching a maximum at a time (t<sub>max</sub>) of approximately 0.5–1 hour.<sup>73</sup> Whereas dissolution studies with EC-MPS have shown that because of the enteric coating MPA is maximally released at pH 6.0–6.8, therefore, the drug is being released in the small intestine instead of in the stomach resulting in unpredictable and highly variable t<sub>max</sub> in the range of 1.5–6 hours after administration.<sup>66,74</sup> The maximum concentration (C<sub>max</sub>) was approximately 10%–18% lower during EC-MPS therapy than that during MMF.

The potential variability in PK of MPA with age has only been addressed in a small number of studies. In the study by Tang et al,<sup>72</sup> it was concluded that there were specifically age-related changes for MPA that would influence weight-adjusted dosage, neither in the young nor in the elderly. A review of PK studies in the elderly concluded similarly, although some deviations between results were discussed.<sup>75</sup>

**Effect of Comorbidities on MPA PK**

Under physiologic conditions, the absorption of MPA from MMF and EC-MPS is nearly complete. Gastrointestinal

disturbances may however lead to significantly reduced bioavailability as demonstrated in patients undergoing allogeneic hematopoietic stem cell transplantation with a non-myeloablative preparative regimen.<sup>76</sup> Other factors that may explain the low MPA concentrations observed in patients after hematopoietic stem cell transplantation are the coadministration of cyclosporine (impacting on the enterohepatic recirculation of MPA) and low albumin concentrations (leading to high clearance).

The bioavailability of MPA may also be reduced as a result of changes in the gut microbiota, as demonstrated in a small study where selective bowel decontamination reduced the enterohepatic circulation and hence the bioavailability.<sup>77</sup> In a study of stable renal transplant recipients, it was shown that delayed gastric emptying was associated with a slower absorption of MPA, a longer time to reach peak concentrations, and lower maximum concentrations, but without a significant reduction of the AUC.<sup>78</sup> Similar results have been shown for MMF and EC-MPS in patients with diabetes.<sup>79,80</sup>

How inflammation affects the PK of MPA is unclear, but changes in the expression of UGT in both liver and kidney can be anticipated.

Reduced renal function, whether in native kidneys or a transplanted graft, can affect the MPA PK through several mechanisms as described above, leading to reduced

elimination and higher exposure.<sup>67–71,81</sup> A limited dosage (MMF 1 g daily) is only recommended for patients with chronic renal impairment, GFR <25 mL/min/1.73 m<sup>2</sup>.<sup>82</sup> The effects of variable renal graft function also contribute to explain changes in MPA exposure posttransplant that can be monitored using AUC measurements to guide dosing. There is, however, no general recommendation for dose adjustments in relation to rejection episodes in kidney transplant recipients (KTR).<sup>82</sup> In patients with delayed graft function early after kidney transplantation, lower dose-corrected MPA AUCs have been observed, presumably because of enhanced MPA clearance on account of the elevated MPA-free fraction<sup>83</sup> and in overweight patients also because of higher clearance.<sup>81</sup>

Hypoalbuminemia, probably also high bilirubin, will increase the free fraction of MPA, resulting in reduced exposure because of faster clearance as demonstrated in LTR.<sup>84,85</sup>

## Dosage

The recommended standard oral daily dose for adults is 2 g in KTR, whereas for heart and liver transplants, the oral starting dose is 3 g divided in 2 daily doses.<sup>82</sup> For the EC-MPS formulation, 720 mg is equivalent to MMF 1 g.

The currently recommended dose in pediatric KTR with concomitant cyclosporine A is 1200 mg/m<sup>2</sup> body surface area per day in 2 divided doses; the recommended MMF dose with concomitant tacrolimus or without a concurrent CNI is 900 mg/m<sup>2</sup> per day in 2 divided doses. Data from the Fixed Dose versus Concentration Controlled (FDCC) study suggest that fixed MMF dosing results in MPA underexposure, MPA AUC<sub>0–12</sub> < 30 mg·h/L early posttransplant in approximately 60% of patients.<sup>86</sup> To achieve adequate MPA exposure in most patients, an initial MMF dose of 1800 mg/m<sup>2</sup> per day with concomitant cyclosporine A and 1200 mg/m<sup>2</sup> per day with concomitant tacrolimus for the first 2–4 weeks posttransplant has been suggested.<sup>87</sup>

## Drug–Drug and Food–Drug Interactions

DDIs can occur at several paths of MPA PK. Combination with other drugs or food may lead to PK changes in MPA and metabolites, MPAG, and AcMPAG that may in turn alter the overall exposure to MPA. DDIs can result from decreased absorption from the gut, changes in drug distribution and metabolism, alterations in biliary excretion of MPAG, or reduced hydrolysis of glucuronides in the intestine.<sup>88</sup>

## Interactions at the Absorption Phase

### Proton Pump Inhibitors

Absorption of MPA depends on intragastric pH, with better dissolution of solid dosage form occurring at low pH. Therefore, coadministration of proton pump inhibitors (PPIs) may lead to lower bioavailability, more so for MMF than EC-MPS. Miura et al<sup>89</sup> reported a lower MPA AUC when MMF was coadministered with 30 mg lansoprazole compared with 10 mg rabeprazole or without PPIs. Kiberd, however, did not find PPI-induced differences in MPA AUC in MMF-treated

KTR.<sup>90</sup> A study in healthy volunteers, specifically designed to evaluate the effects of PPIs on MPA PK, showed a 27% drop in MPA AUC if MMF was combined with pantoprazole 40 mg bid, whereas no effect was detected on the PK of EC-MPS.<sup>91</sup> These results were confirmed in a study with omeprazole 20 mg bid.<sup>92</sup> In heart transplant patients, similar findings were obtained.<sup>93–95</sup> Inadequate dissolution of MMF in the stomach at elevated pH is the presumed mechanism of this DDI. The lower MPA exposure may put patients at risk of rejection, and in a retrospective study, PPI use was associated with an increased risk for biopsy-proven acute rejection (BPARG) in Black patients, but not in other patients.<sup>96</sup> Rissling et al described some effects (eg, on C<sub>max</sub> and t<sub>max</sub>) of pantoprazole on EC-MPS and MMF PK in a prospective randomized cross-over trial in renal allograft recipients. These effects, however, were probably not clinically meaningful as MPA AUC and MPAG and IMPDH activity were not affected by the interaction with pantoprazole.<sup>97</sup>

In addition to interaction with MPA absorption, PPIs are substrates for ABCB1 [P-glycoprotein (P-gp)] and inhibit ABCB1-mediated transport, which may lead to a decrease in MPA C<sub>max</sub> and AUC.<sup>98,99</sup>

Antacids containing aluminum hydroxide or magnesium hydroxide significantly reduce MPA absorption because of its chelation by the antacid. The interaction is visible in both the initial and secondary absorption peaks.<sup>100</sup> Antacids administered in the fasting state can decrease the AUC of MPA by 17% and the C<sub>max</sub> by 33%–38%, in comparison with the nonfasting state. In addition, the AUC of MPAG was reduced by 10% and the C<sub>max</sub> by 26%.<sup>100</sup> Still, the authors concluded that the changes in MPA with food and antacid are small in comparison with the interpatient variability and are not likely to have clinically major effects.

### Phosphate Chelators

One study demonstrated that phosphate-binding agents (eg, sevelamer) can interfere with the absorption of MPA by decreasing MPA C<sub>max</sub> and AUC<sub>0–12h</sub> by 36% and 26%, respectively, in adult and pediatric patients. The authors suggested that sevelamer should be given 2 hours after the intake of MMF, alternatively that the MPA levels could be measured and the dose of MMF could be adjusted to compensate for its reduced intake. TDM is recommended when starting or stopping sevelamer.<sup>101</sup>

### Laxatives and Iron Supplements

The concomitant use of MMF and the laxative calcium polycarbophil leads to a decrease in MPA absorption and a reduced AUC and C<sub>max</sub> by more than 50%.<sup>102</sup>

Iron supplements, commonly prescribed to transplant patients to alleviate iron deficient anemia,<sup>103</sup> can reduce the AUC and C<sub>max</sub> of MPA by 90%.<sup>104</sup>

## Interactions at the Metabolism and Transport Phases Including Enterohepatic Circulation

Immunosuppressant drugs, such as cyclosporine A, can cause DDIs when taken in combination with MMF, for both the immediate-release and enteric-coated formulations. In 1997, an article was published that reported higher MPA concentrations in patients treated with tacrolimus, compared

with controls treated with cyclosporine A.<sup>105</sup> Two years later, the Rotterdam transplant group also showed lower MPA concentrations in cyclosporine A-treated patients and suggested that it was not tacrolimus that increased MPA exposure but rather cyclosporine A that reduced MPA concentrations.<sup>106</sup> An experimental study in rats convincingly showed that the cyclosporine A-treated animals had lower MPA concentrations, higher MPAG concentrations, and no second peak in the PK profile.<sup>107</sup> A subsequent study in rats deficient for the transport protein MRP2 confirmed that the effect of cyclosporine A is most likely caused by inhibition of biliary excretion of MPAG into bile, interrupting of the enterohepatic circulation.<sup>62</sup> Clinical observations in KTR in whom cyclosporine A was discontinued confirmed a significant rise in MPA concentrations.<sup>108</sup> In pediatric patients, it was shown that cyclosporine A alters MPA reabsorption through enterohepatic recycling, resulting in an overall decrease in the AUC and C<sub>max</sub> and in increased t<sub>max</sub>.<sup>109,110</sup> Tacrolimus in combination with MMF led to an increase in the tacrolimus AUC by 22%; on the contrary, the MPA PK parameters and exposure were not affected by tacrolimus.<sup>111</sup> Similarly, mTOR inhibitors do not have the same effect as cyclosporine A on MPA exposure.<sup>112,113</sup>

Glucocorticoid drugs are commonly administered with MPA. However, the impacts of glucocorticoid DDIs with MPA have not been clearly elucidated. Glucocorticoids are known to induce the hepatic UGT activity and to decrease the bioavailability of MPA. In transplant recipients, discontinuation of glucocorticoids leads to a modest increase in MPA concentrations.<sup>114</sup>

### NSAIDs

Studies have demonstrated that nonsteroidal anti-inflammatory drugs (NSAIDs) may have an inhibitor effect on the glucuronidation of MPA.<sup>115</sup> A study in patients with childhood-onset SLE on MPA therapy suggested that NSAIDs inhibit the MRP2-mediated MPAG transport: the MPA pharmacokinetic curve in 11 of the 19 patients who received NSAIDs showed no signs of enterohepatic circulation in the later part of their PK profiles, typically after 6 hours, contrary to the patients not treated with NSAIDs.<sup>116</sup>

Broad-spectrum antibiotics may affect the intestinal glucuronidase activity, thus interrupting the enterohepatic circulation, but at another level than the biliary excretion of MPAG. Evidence for this DDI comes from a study in 6 LTR in whom lower MPA concentrations were found while on a selective bowel decontamination regimen (nystatin, tobramycin, and cefuroxime).<sup>77</sup> The median trough concentration of MPA was decreased by 50% when ciprofloxacin or amoxicillin plus clavulanic acid was administered with MPA.<sup>88</sup> Combination therapy of norfloxacin and metronidazole with MMF decreased the MPA AUC by 33%; however, the AUC was not affected when MMF was administered separately from norfloxacin and metronidazole.<sup>88</sup>

Rifampin was shown in one study to reduce the MPA AUC by 17.5%, the C<sub>0</sub> by 48.8%, and to increase MPAG AUC by 34.4%. The authors suggest that their results could be explained by the induction of UGTs and possibly the competitive inhibition of the MRP2 transporter.<sup>117</sup> Similar

effects of rifampicin were described in a case report of a heart and double lung transplant recipient.<sup>118</sup>

Antifungal medications, such as isavuconazole, decrease MPA glucuronidation and thereby increase the exposure to MPA by 35% and reduce C<sub>max</sub> by 11%.<sup>119</sup> By contrast, the AUC and C<sub>max</sub> of MPAG decreased by 24% and 32%, respectively.<sup>119</sup>

### Proton Pump Inhibitors

In addition to interaction with MPA absorption, PPIs are substrates for ABCB1 and inhibit ABCB1-mediated transport, which may lead to a decrease in MPA C<sub>max</sub> and AUC.<sup>98,99</sup>

Cholestyramine can inhibit enterohepatic circulation of MPA and decrease its AUC. At an oral dose of 4 g tid, cholestyramine reduced MPA AUC by 39% in healthy volunteers.<sup>59</sup> As the C<sub>max</sub> was not affected, it seems likely that this was caused by reduced reabsorption rather than by reduced absorption.

### Interactions at the Excretion Phase

The majority of an MPA dose is excreted as MPAG in urine, and the MRP2 transporter protein responsible for the biliary excretion of MPAG plays an important role at the renal tubular level as well. Therefore, the higher MPAG concentrations observed in patients cotreated with cyclosporine A as described above, may partly be due to inhibition of urinary excretion at the level of the renal tubular cells.<sup>120</sup> Both mechanisms have been suggested as potential explanations for the changes in MPAG PK observed in the presence and absence of cyclosporine A.<sup>121</sup>

Drugs that inhibit tubular secretion, such as probenecid, may increase the AUC of MPA by 2-fold and that of MPAG by 3-fold.<sup>122</sup> Salicylate in combination with elevated concentrations of MPAG (>460 mcg/mL) increase the free fraction of MPA.<sup>122</sup>

### Food Interactions

There are limited studies on food–drug interactions with MMF. The consumption of solid food when taking oral doses of MMF can decrease the C<sub>max</sub> by 25%–40%; however, the overall AUC is similar to that in patients on MMF with an overnight fast.<sup>100</sup>

### Galenic Formulations and Generics

MMF and EC-MPS are 2 different drugs, not generics; the molecular weights are 433.49 Da (g/mol) and 320.34 Da (g/mol) for MMF and EC MPS, respectively. Immunosuppressive therapy with MPA started with the registration of CellCept, the innovator MMF product (Roche). The drug has been introduced as follows: 250 mg capsules, 500 mg film-coated tablets, 200 mg/mL powder for oral suspension, and 500 mg powder for intravenous solution.<sup>123</sup> The latter 2 formulations are useful in cases when classic oral administration may be problematic, especially in the early posttransplant period.<sup>124</sup> Original EC-MPS (Myfortic; Novartis) has been introduced with the aim of reducing drug-related gastrointestinal adverse events, as observed in a significant percentage of MMF-treated



patients.<sup>125</sup> MMF and EC-MPS were intended to be different drugs, and as a consequence of the delayed absorption of EC-MPS, the 2 forms of MPA have different PK profiles.<sup>126</sup> The strengths of the second registered EC-MPS have been adjusted to obtain therapeutic equivalence with MMF, and a subsequent meta-analysis of PK data from 3 clinical trials confirmed the AUC equivalence of EC-MPS and MMF for both MPA and metabolite exposure, and for maximum plasma MPA concentrations.<sup>127,128</sup> Because of the different molecular weights [MMF 433.49 Da (g/mol) and EC-MPS 320.34 Da (g/mol)], the 720 mg EC-MPS dose is manufactured as equivalent to 1000 mg of MMF.<sup>125</sup>

The patent for CellCept expired May 3, 2009. Since then, generic products have become an economic alternative for the innovator drug. Several MMF generic products have been registered worldwide, and trade names of the same generic may differ between markets and countries. For EC-MPS, the number of generics is substantially fewer because this drug was introduced later than MMF and it covers a minority of the MPA market. In addition, preparing a bioequivalent enteric-coated formulation is more challenging in comparison with a traditional tablet or a capsule.

In the European Union and Canada, generic products for narrow therapeutic index drugs (NTIDs) should fulfill more restrictive acceptance criteria for bioequivalence (BE) than “common” generics, that is, for the AUC a 90% confidence interval (CI) for a test to reference ratio within 90.00%–111.11% [European Medicines Agency (EMA)] or 90.00%–112.00% (Canada) instead of 80.00%–125.00% (standard BE and for the United States)<sup>129–131</sup> A discussion keeps smoldering in the transplant community, whether MPA should be classified as an NTID such as CNIs and mTOR inhibitors.<sup>123,132–135</sup> However, the arguments to recognize MPA as an NTID are currently not shared by registration agencies (ie, the EMA maintains classic, wider acceptance criteria for MPA).<sup>123,132,136,137</sup> Irrespective of the classification of MPA as an NTID or not, it is mandatory that the generic products adhere to the criteria for BE. This is not trivial, and in many countries, the authorities have taken measures to secure the high quality of marketed generics, such as strict requirements and repeated inspections of production sites.<sup>128–131,134,138</sup>

We can consider generic formulations of either MMF or EC-MPS as economic alternatives for MPA pharmacotherapy, especially in de novo transplant patients. The patient and family should be informed about the pros and cons of therapy with generics. According to the guideline from the Advisory Committee of the European Society for Organ Transplantation, every conversion between brands needs to be performed under careful TDM and supervision by the physician, whereas the switch between 2 different generics should be managed as the last choice option.<sup>133</sup>

## PHARMACOKINETIC MONITORING

The population PK (POPPK) of MPA is more difficult to describe and requires more complex models than cyclosporine A and tacrolimus, as detailed in the section POPPK modeling of MPA. Moreover, MPA morning trough concentration is not a good surrogate of overall exposure of the drug

in whichever formulation,<sup>139</sup> and AUC estimation has been shown<sup>9,140,141</sup> to be the most effective tool for TDM of MPA, with MMF and EC-MPS.<sup>142</sup> It should also be kept in mind that an additional indication for TDM of MPA, especially in adolescent patients, is the monitoring of drug adherence.<sup>87</sup> As discussed in detail in the section “Measurement of MPA concentration,” the assay for MPA measurement is an important factor when comparing results across studies and for the identification of target trough concentrations or AUCs. In the studies that are discussed in this article, chromatographic assays [detection by ultraviolet (UV) or mass spectrometry (MS)] were typically used.<sup>8,140,143–146</sup> However, other studies used immunoassays alone<sup>142</sup> or according to the local practice, that is, in half the centers,<sup>86</sup> and even without reporting the analytical method.<sup>147</sup> Unless otherwise indicated, the recommendations presented here will refer to concentrations and AUCs obtained by high-performance liquid chromatography (HPLC)-UV or liquid chromatography (LC)-MS.

## Limited Sampling Strategies

A large number of LSSs have been proposed for MPA AUC estimation in KTR on MMF or EC-MPS, relying on multiple linear regression (MLR) (in the form  $AUC = ax \times C1x + by \times C2y \dots + iz \times Cnz$ , where  $C1x, C2y \dots Cnz$  are plasma concentrations measured at times  $x, y \dots z$ , and  $ax, by \dots iz$  the corresponding constants). MLR is a straightforward technique available with every statistics software program, and the resulting equations can be easily implemented in a spreadsheet. Although for MMF most LSSs of 2–3 time points within the first 3 hours postdose were efficient to approximate the full AUC,<sup>9</sup> for EC-MPS it was not straightforward<sup>148</sup>: 3 blood samples were rarely sufficient<sup>142,146</sup> but more often 4 and up to 8 were required,<sup>149,150</sup> over a generally longer period of 4–9 hours after dosing<sup>146,148,150–152</sup> because of the highly variable absorption of the drug and day-to-day fluctuation in enterohepatic cycling of MPA.<sup>9</sup> Importantly, Hougardy et al<sup>143</sup> reported that MPA AUC estimation using a 4-point LSS failed in >30% of patients on EC-MPS, especially when  $C_0$  was the highest concentration measured.

LSSs with maximum a posteriori Bayesian estimation (MAP-BE) have been reported for pediatric and adult patients with different conditions, such as kidney, liver, lung and bone marrow transplantation, or various autoimmune diseases. The publications of such methods have been reviewed<sup>153,154</sup>; all but one pertained to MMF and most used a 3 time point LSS, the most frequent of which was 20 min–1h–3h.<sup>145,155–159</sup> One of the other 3-point LSS was limited to the first 2 hours postdose, in KTR,<sup>160</sup> whereas others included the 4 hours time point.<sup>157,161–163</sup> One MAP-BE in pediatric KTR only required 2 plasma samples,<sup>164</sup> whereas others required 4.<sup>165–168</sup> A MAP-BE was developed for intravenous MMF in hematopoietic stem cell transplant patients,<sup>159</sup> and the bias was –11.7% to 8.7% (with many absolute values <2%) and imprecision 11.2% to 20.5%. The only MAP-BE developed for EC-MPS used 3 (1.5h–2h–4h) or four (1.5h–2h–4h–6h) time points and yielded bias (RMSE) –6.52% (20.8%) and –5.15% (18.3%), respectively,<sup>169</sup> that is, performance comparable with that of MMF MAP-BEs.  $C_0$  was used in less than half of these MAP-BEs because of its very poor

correlation with the AUC,<sup>170</sup> which is probably due to concentration rebounds with variable timing and amplitude. Bayesian estimators are more difficult to develop and use than MLR because they require specialized PK modeling software. A couple of articles compared different MLR and MAP-BE for AUC estimation after oral MMF dosing and showed that they provided highly correlated, although not concordant, AUC estimations in KTR<sup>165</sup> and that the estimation bias with respect to the reference values was very low with both approaches, even sometimes lower with MLR.<sup>33</sup> However, both articles and others concluded that an MAP-BE analysis is preferable because (1) it is flexible with respect to sample timing, as also suggested by equivalent performance of different combinations of sampling time points<sup>156,171</sup>; (2) it is not restricted to a 12-hour dosing interval; (3) it allows visual inspection of the estimated kinetic profile superimposed on experimental data; and (4) it yields a CI for the AUC.<sup>33,165</sup>

There are no MLR, MAP-BE, or other LSSs applicable to all MPA indications, patient populations, or analytical techniques. Different equations, estimators, and LSSs have been proposed for kidney, liver, heart, and lung transplantation, as well as lupus, nephrotic syndrome, and other autoimmune diseases, for adult or pediatric patients, and even for different posttransplant periods<sup>156</sup> and drug combinations.<sup>160</sup> The LSS even differed between reports for the same condition, such as KTR,<sup>156,165</sup> lupus,<sup>158,172</sup> or hematopoietic stem cell transplantation.<sup>167,173</sup> When 2 analytical techniques were used in parallel in different groups of patients, the PK models developed kept the same structure but the estimated parameters were different across conditions, age, and above all analytical techniques.<sup>145</sup> Therefore, it is then recommended to use each LSS and MLR equation or MAP-BE alone for the population<sup>9</sup> and the analytical technique with which they were developed.<sup>145</sup>

Validation on a patient group different from the training set must be made because testing an equation or model in the group of patients used to generate it would be self-fulfilling and likely produce less biased results than in its intended real-world clinical setting. In PK studies with small sample sizes, the jackknife or bootstrap method can also be used to validate an LSS internally, as alternatives to data splitting or external validation. The performance of the estimators can be assessed by comparing the predicted AUC with the “reference AUC” (which is actually another AUC<sub>0–12h</sub> estimate based on all concentration–time points available) and computing the mean prediction error or bias (MPE) and the root mean squared prediction error or precision (RMSE), together with their CIs. The smaller the values of MPE and RMSE, the better the prediction.<sup>174</sup> It is generally accepted that bias >10% and imprecision >25% are unacceptable for routine clinical use. A more clinically orientated method consists of evaluating the proportion of AUCs estimated within a clinically acceptable percent prediction error range (eg, ±20%). It should be noted here that the MPE is the average of individual errors, which means that it provides only limited information on the occurrence of extreme individual errors. Another method consists of expressing the results using the absolute prediction error for a certain percentile of predictions. Calculating the correlation coefficient (r) or coefficient of determination (r<sup>2</sup>) between

predicted and “reference” AUCs is not enough to assess the performance of an estimator.<sup>175</sup> Unfortunately, not all predictors were validated internally or externally, or even evaluated using sound statistics. Therefore, it is strongly advised that LSS-based MLR or MAP-BE should not be used in the absence of convincing performance evaluation and accuracy validation (Tables 2 and 3).

### Summary of Recommendations for LSSs

1. A method for LSSs must be validated in a population separate from the training set before implementation. Validation for each indication and patient category is required.
2. MAP-BE analysis has some preference over MLR due to flexibility in timing of samples and better implementation of covariates.
3. For MMF, 2 or more concentrations are necessary for a reliable LSS, whereas for EC-MPS, 3–4 or more concentration measures are necessary.

### PK Monitoring in Kidney Transplantation

Although approval of MPA implied fixed dosing in adults and weight-adjusted dosing in pediatric patients, it became clear very early that exposure to MPA varied widely between patients. In the late 1990s, a randomized concentration-controlled trial further demonstrated that efficacy was more dependent on MPA AUC<sub>0–12h</sub> than trough plasma concentration while no such relationships were found for toxicity.<sup>140</sup> This was also proof that the MPA AUC was “actionable” and MMF dose adjustment efficient, as the 3 arms of this study had well-separated AUCs. Further proofs came several years later from a retrospective study of approximately 14,000 AUC estimation and dose recommendation requests for 7000 adult KTR posted on a free Web site.<sup>8</sup> It showed, among others, that when dose recommendations were actually applied by the physicians, the subsequent AUC was significantly more often in the recommended AUC range, and the interindividual AUC variability was systematically lower, at all posttransplantation periods. The pending question was then about the efficacy of MPA dose adjustment to improve patient outcome.

### MPA Exposure, Efficacy, and Toxicity—Kidney Transplantation

An article by Metz et al<sup>6</sup> critically reviewed 36 publications dealing with MPA concentration–effect relationships or concentration-controlled dosing (CCD) in KTR. A statistically significant relationship between MPA AUC<sub>0–12h</sub> and acute graft rejection was found in 20 of the 27 patient cohorts (89.1% of the overall population of 3794 patients studied), a trend in an additional 3 cohorts (5.7%), leaving 4 cohorts (5.1%) without such an association. This significant association was true whether patients were on cyclosporine A (12/16 cohorts, 77.8% of the population) or on tacrolimus (7/11 cohorts, 81% of the population). The relationship between MPA AUC<sub>0–12h</sub> and hematological or infectious adverse events was assessed in 22 cohorts (3225 KTR). Only 9 of the 22 cohorts reported a statistically significant association between MPA exposure and toxicities (representing 34% of

**TABLE 2.** Assays Measuring MPA

Method	Manufacturer	Analytical Range (mg/L)	Imprecision	Bias	Specificity (% Cross-Reactivity)
LC-MS/MS	Laboratory developed tests	0.1–50	<5%–10%	Reference method	High if validated appropriately
HPLC-UV, UPLC-UV, and HPLC-Fluorescence	Laboratory developed tests	0.2–50	<5%–10%	Reference method	High if validated appropriately
EMIT	Siemens Healthcare Diagnostics	0.1–15	<5%–10%	~25%	MPAG: ND AcMPAG: 10%–30% MMF: 64%
IMPDH enzyme inhibition assay	Roche Diagnostics	0.4–15	<5%–10%	<10%	MPAG: ND AcMPAG: 6.5%
CEDIA	Thermo Scientific	0.3–10	<5%–10%	~36%	MPAG: ND AcMPAG: 133.3%–177.8%
PETINIA	Siemens Healthcare Diagnostics	0.2–30	<5%–10%	~25%	MPAG: 0.6% AcMPAG: 36.8%–64.5% MMF 28.6%–30.5%

the patients); 2 cohorts reported a trend toward this association, whereas no such association could be found in 11 of the 22 cohorts. When considering the combined CNI therapy, this association was found in 5 of the 6 of the cohorts on tacrolimus (95.8% of the patients), at odds with only 2 of the 11 of the cohorts on cyclosporine A (9.1% of patients). However, in 2 negative cohorts on cyclosporine A, as well as in one positive, a significant association was found between free (unbound) MPA (fMPA) concentrations and these hematological or infectious adverse events.

Rather than studying the exposure–effects relationships following a cross-sectional design, a couple of articles retrospectively investigated the impact of longitudinal exposure to MPA on the time to rejection (or survival without rejection), using joint modeling.<sup>176,177</sup> The first study in 490 KTR found

a significant association between longitudinal exposure to MPA AUC<sub>0–12h</sub> over the first year posttransplantation (described using a polynomial function with a quadratic term) and acute rejection.<sup>176</sup> Interestingly, the MMF dosing strategy (fixed dosing or CCD) was a significant covariate in the model, in addition to patient age. In a further retrospective study in 222 KTR followed-up for 2 years posttransplantation, the same team developed a time-to-event model of immunosuppression efficacy considering longitudinal exposure to MMF and either cyclosporine A or tacrolimus and more potential covariates.<sup>177</sup> They found that the risk of acute rejection, graft loss, or death (combined end point) significantly increased with decreasing MPA AUC and the onset of cytomegalovirus (CMV) infection and disease, whereas it was not associated with longitudinal CNI exposures.

**TABLE 3.** Characteristic Absorbance Maxima and Ion Transitions Used for Detection in Chromatographic Methods\*

	MPA	MPAG	AcMPAG	References
UV detection				
Characteristic absorbance maxima	215 nm, 251 nm, 304 nm	214 nm, 251 nm, 294 nm	215 nm, 251 nm, 306 nm	284
Mass spectrometric detection				
ESI+ [M + H] <sup>+</sup>	m/z 321.1 → <b>207.1</b> ; 303.1; 159.0	—	—	262,331,333,570–573
ESI+ [M + NH <sub>4</sub> ] <sup>+</sup>	m/z 338.2 → <b>207.1</b> 275.2	m/z 514.3 → <b>207.1</b> 321.1 303.0	m/z 514.3 → <b>207.1</b> 321.1 303.0	262,331,570,571,573–576
ESI+ [M+Na] <sup>+</sup>	m/z 343.1 → 229.1 216.0	m/z 519.2 → 343.1	m/z 519.2 → 343.1	259,570
ESI–	m/z 319.0 → <b>191.0</b>	m/z 495.0 → <b>319.0</b> 191.0		577,578

\*Most frequently used ion transitions are given in bold.

Along the years, 4 randomized fixed-dose (FD) versus CCD trials were conducted, all using MMF.<sup>86,144,147,178</sup> However, as underlined by Metz et al,<sup>6</sup> 2 of these used a TDM strategy,<sup>86,147</sup> meaning that a target (AUC or C<sub>0</sub>) range was proposed and dosing adjustment was left to the decision of the physician, whereas the other 2 used a target exposure intervention strategy,<sup>144,178</sup> in the sense that with each AUC estimation a dose recommendation was presented (and most often applied) so as to reach a single predefined AUC<sub>0–12h</sub> target. Of the last 2, APOMYGRE enrolled 137 adult KTR on cyclosporine A and used Bayesian estimation of MPA AUC on days 7, 14, and months 1, 3, and 6 with a target AUC<sub>0–12h</sub> = 40 mg·h/L and a recommended dose to reach it in the CCD arm, as compared with a 2 g/d fixed dose in the comparative arm. It showed a statistically significant and clinically important reduction in a patient's adverse outcome at one year in the CCD arm, mostly due to a highly significant reduction of the incidence of acute rejection.<sup>144</sup> The second study, called OPERA, was conducted in a low-risk population of adult KTR on cyclosporine A with the same CCD strategy and the same tools. It entailed glucocorticoid withdrawal at day 7 in both arms and 2 distinct interventions in the “optimization” arm,<sup>178</sup> that is, a 3 g/d initial MMF dose up to week 2, adjusted thereafter to reach AUC<sub>0–12h</sub> = 40 mg·h/L, as compared to MMF 2 g/d in the control arm. There was no significant difference between the 2 arms, but toxicities associated with MPA and BPAR cases were numerically higher in the “optimization” arm. An explanation for these paradoxical results is that the starting dose was too high for some patients, resulting in drug toxicity, followed by drastic dose decrease or even discontinuation. According to Metz et al,<sup>6</sup> because of the very low incidence of immunological events reported (4% and 2.5% subclinical acute rejection episodes, respectively), this study neither supports nor refutes target AUC<sub>0–12h</sub> intervention. A post hoc analysis of the APOMYGRE and OPERA trials showed that longitudinal exposure to MPA AUC<sub>0–12h</sub> was significantly associated with acute rejection over the first year posttransplantation, with time-dependent thresholds from 35 mg·h/L in the first days to 41 mg·h/L beyond 6 months posttransplantation,<sup>176</sup> which is almost exactly what the original RCCT trial obtained in the intermediate exposure group.

The FDCC trial enrolled 901 adult and pediatric KTR on MMF and cyclosporine A or tacrolimus.<sup>86</sup> In the CCD group, AUC<sub>0–12h</sub> was estimated using multilinear equations, its target range was 30–60 mg·h/L, and no dose recommendation was made. The early dose increments required were generally not applied by clinicians, resulting in similar mean MPA AUCs, proportion of patients within the therapeutic range, and outcomes between the 2 groups. This precludes drawing conclusions about the efficacy of MMF dose adjustment in this study. A post hoc analysis of the data did however confirm a higher risk of rejection in patients with MPA exposure below the target range, which was most pronounced in patients at high immunological risk.<sup>179</sup>

The OPTICEPT study enrolled 720 KTR<sup>147</sup> and was the only trial using MPA C<sub>0</sub> to dose adjust MMF. Two CCD arms, one with standard (A) and one with reduced (B) CNI exposure, were compared with the standard of care of the time

(C), that is, FD MMF and standard CNI exposure. The primary outcome was noninferiority of group A compared with C, based on treatment failure at 12 months (a composite of BPAR, graft loss, loss to follow-up, or withdrawal). In arms A and B, the MPA concentration target was different according to the combined CNI: C<sub>0</sub> ≥ 1.3 mg/L if combined with cyclosporine A and C<sub>0</sub> ≥ 1.9 mg/L if combined with tacrolimus. MMF dose individualization was left to the judgment of the clinician. Again, there was little differentiation among treatment groups in MPA exposure. In patients co-treated with tacrolimus (81.9% of the participants), MPA C<sub>0</sub> was identical at all time points with or without concentration monitoring. Moreover, the noninferiority of group A against the standard of care could be demonstrated, with actually less rejection and treatment failures in group A despite lower CNI exposure. However, the outcomes in groups B and C were identical. Actually, the effectiveness of MPA TDM was not tested in this study because of the lack of differentiation in exposure to MPA between treatment arms.<sup>6</sup>

Metz et al<sup>6</sup> concluded that when critically analyzed, these prospective concentration-controlled trials show that MMF CCD using target exposure intervention leads to effective control of MPA exposure and to improved clinical outcomes. However, a subsequent meta-analysis of the 4 studies, irrespective of their quality, concluded that CCD of MMF cannot be recommended as a routine practice for KTR, but that it may be targeted toward high-risk patients.<sup>180</sup> This highlights the importance of well-designed, well-conducted clinical trials when testing TDM, target concentration intervention, or more largely personalized medicine strategies. Clinical efficacy can only be tested if the procedures are efficient in separating the study arms and providing the clinical intervention intended.<sup>181</sup> The APOMYGRE trial also showed that MMF dose adjustment guided by AUC-MPA, beyond being clinically efficient, was quite affordable if not actually cost-saving: approximately €3757 for each treatment failure avoided, in 2010 euros.<sup>182</sup>

The first pediatric PK/PD study in KTR was published in 2002 by Weber et al,<sup>183</sup> who found in 54 children (aged 2–17 years) that both AUC and predose concentration were associated with the risk of acute rejection. The identified thresholds for AUC (in the initial phase posttransplant) and predose concentration were 33.8 mg·h/L and 1.2 mg/L, respectively. No association was observed between the incidence of adverse events and total MPA exposure, whereas the occurrence of leucopenia and infection was associated with a fMPA AUC<sub>0–12h</sub> > 0.4 mg·h/L. Although most large clinical studies have focused on adult populations, the FDCC trial included 62 pediatric patients.<sup>86</sup> Subgroup analysis indicated that the overall efficacy and tolerability in pediatric patients were comparable with that in adults; however, children younger than 6 years exhibited a higher incidence of adverse events than older children and adults.<sup>184</sup> In the pediatric population, the relationship between MPA concentration and IMPDH enzyme activity has been investigated. Rother et al<sup>185</sup> found no age-related differences in baseline IMPDH activity between healthy children and adults, and comparable inhibition of IMPDH activity by MPA in children (older than 2 years) and adolescents after renal transplantation.

Finally, a study showed that MPA C0 <1.3 mg/L in the long term is associated with the formation of DSA in pediatric KTR, indicating the importance to maintain a minimum concentration of 1.3 mg/L (loosely equivalent to an AUC of 30 mg·h/L) to prevent the formation of DSA.<sup>13</sup>

To the best of our knowledge, no exposure–effect or concentration-controlled study of EC-MPS has been reported in adults or pediatric KTR.

### Summary of Recommended MPA Target Concentration Ranges—Kidney Transplantation

1. In adult KTR treated with MMF in combination with tacrolimus or cyclosporine A, with or without glucocorticoids, a target MPA AUC<sub>0–12h</sub> of 30–60 mg·h/L is recommended (B, II).
2. A target AUC<sub>0–12h</sub> of 30–60 mg·h/L is also recommended in pediatric KTR (B, II).
3. There is no evidence for specific AUC<sub>0–12h</sub> targets beyond the first year after transplantation.
4. No exposure–effect nor concentration-controlled study of EC-MPS has been reported, either in adults or in pediatric KTR.
5. There is no evidence in favor of using MPA C0 to dose adjust MMF or EC-MPS

### PK Monitoring in Liver Transplantation

MMF is indicated in liver transplantation (LT). Combined with low CNI exposure, MPA allows maintaining immunosuppressive treatment efficacy with no increase in acute graft rejection, graft loss, and patient death as compared to high CNI exposure.<sup>186</sup> Hence, the use of MPA offers the possibility of treating LTR de novo without glucocorticoid maintenance treatment,<sup>187</sup> as well as decreasing CNI nephrotoxicity,<sup>188</sup> CNI-induced cardiovascular complications, and diabetes mellitus.<sup>189</sup>

### MPA Exposure, Efficacy, and Toxicity—Liver Transplantation

In LT, MPA exposure increases in a time-dependent manner with sometimes very low exposure during the first 2 postoperative weeks and finally stable concentrations from month 3 onward.<sup>85,190</sup> This low initial exposure might be related not only to low albumin concentrations with high unbound fraction leading to higher MPA clearance but also to biliary drainage and interruption of enterohepatic circulation. MPA presents a large interpatient PK variability and exposure may vary according to which CNI is used.<sup>191</sup> There is a loose correlation between MPA C0 and drug dosage: Hwang et al reported  $r^2 = 0.27$  in 304 LT patients followed in a large monocentric study.<sup>192</sup> Using TDM, it is possible to adjust the drug dose to obtain a defined target, as shown by Kamar et al who obtained more patients in the AUC<sub>0–12h</sub> target of 30–60 mg·h/L during the first postoperative year when MMF dosing was guided by AUC Bayesian estimates obtained using a PK model.<sup>193</sup>

Considering exposure–response relationships in LT, limited data exist. MPA AUC is better correlated to its pharmacodynamic (PD) effect than MPA C0. Actually, Reine et al reported a correlation between AUC<sub>0–4h</sub> and IMPDH

activity in 20 LT patients. The relationship was stronger on days 3–5 ( $r = -0.72$ ) than on week 2–3 ( $r = -0.49$ ).<sup>194</sup> Using serum from patients treated with MPA in a PD functional test, Brunet et al also showed that a C0 of 1 mg/L inhibited cell proliferation.<sup>85</sup> Such a functional approach has been replicated in 27 LT patients, aiming to evaluate the relationship between (total and free) exposure and the inhibition of proliferation of a CEM cell line. Total concentration at 1 hour and free concentration at 1 and 2 hours correlated with the inhibition of proliferation at the same time, whereas AUC<sub>0–12h</sub> correlated with inhibition of proliferation at 2 hours.<sup>195</sup>

Regarding treatment efficacy, MPA C0 has been associated with the onset of acute cellular rejection in a cohort of 210 LT patients (147 adults) treated with MMF. A C0 <1 mg/L was associated with a 2.5 relative risk of rejection. Of note, the cohort was heterogeneous, with adults and children, as well as different associated treatments and periods since MPA introduction.<sup>196</sup> Another observational study, conducted in 56 LT patients, evaluated blinded TDM up to 6 months posttransplantation and the relationship between MPA C0 and efficacy. With a ROC curve analysis, the authors identified a cut-off of 1.73 mg/L associated with acute graft rejection with a 62% prognostic sensitivity and 86% prognostic specificity.<sup>197</sup> In these 2 studies, MPA concentrations were measured using the enzyme-multiplied immunoassay technique (EMIT), which is clearly not the current gold standard.

Finally, even if it was not the purpose of the study, the best evidence in favor of a concentration-controlled strategy came from the study by Saliba et al. In this prospective randomized controlled trial, the authors aimed at evaluating the noninferiority of a glucocorticoid-free treatment (MPA and tacrolimus) with a target MPA AUC<sub>0–12h</sub> between 30 and 60 mg·h/L versus the combination of tacrolimus, glucocorticoids (with a complete discontinuation after 7 months), and a fixed dose of MPA. One hundred eighty LT patients were included in the study. In intention-to-treat, the noninferiority hypothesis was confirmed with 9% of biopsy-proven acute rejections in each arm by 12 months. Safety data showed a higher rate of low hemoglobin as well as low leukocyte and neutrophil counts in the AUC-adjusted arm while diabetes was more frequent in the control arm. No difference was seen on renal function or infections. This study legitimates individually adjusted MPA exposure in patients treated de novo without glucocorticoids.<sup>198</sup> In a 2-stage study, Kim et al retrospectively evaluated a reduced dose of MMF (500 mg bid) in living donor LT patients. They highlighted that low initial exposure (AUC within 15–30 mg·h/L up to day 14) allowed excellent initial (2 weeks) and 1 year efficacy based on protocol biopsies, but in the context of an induction treatment (basiliximab), glucocorticoids, and a relatively high tacrolimus exposure during the first month (C0 = 8–12 ng/mL). Also, there was no comparator in this study.<sup>199</sup>

The relationship between MPA exposure and safety has also been explored in a few studies. In an observational study aiming to establish the exposure levels associated with adverse events in 63 LT patients, treated for a large part with basiliximab, tacrolimus with a C0 target of 5–10 ng/mL and MMF 1 g BID, Hao et al found an association between

adverse events (mainly leucopenia) and  $C_0 > 2$  mg/L,  $C_{max} > 10$  mg/L, and  $AUC_{0-12h} > 40$  mg·h/L.<sup>200</sup> Tredger et al showed that the relative risk of leucopenia, gastrointestinal disturbance, and infection was 3-fold when  $C_0$  was between 3 and 4 mg/L, prompting the authors to propose a  $C_0$  upper limit of 3.5 mg/L.<sup>196</sup> Again, the RCT of Saliba et al suggested that an  $AUC_{0-12h} > 60$  mg·h/L is detrimental.<sup>198</sup> Data on the association between exposure and gastrointestinal adverse events are still conflicting because negative relationships with MPA or metabolite exposure have been reported.<sup>201</sup>

Globally, the exposure–response relationships in adult LT patients are not well documented, and larger observational studies, as well as concentration-controlled versus FD RCTs are needed.

In pediatric LTR, data on the MPA exposure–response relationship are sparse. A large clinical study including 63 children and 147 adults found that MPA  $C_0$  was associated with an increased risk of acute rejection ( $C_0 < 1$  mg/L) and adverse events ( $C_0 = 3-4$  mg/L).<sup>196</sup> Although the number of subjects was limited, all 3 episodes of acute rejection in these pediatric recipients occurred at MPA trough concentration  $< 0.5$  mg/L.<sup>196</sup> Finally, in a small cohort of 15 children (1–15 years), Barau et al found that graft function improved in 13 patients after MMF dose adjustments to target an MPA  $AUC_{0-12h} > 30$  mg·h/L.<sup>202</sup> The observed MPA AUC after dose adjustments ranged from 28.5–68.7 mg·h/L and neither dose reduction nor discontinuation was required because of adverse events at this exposure range.

### Summary of Recommended MPA Target Concentration Ranges—Liver Transplantation

1. In LTR treated de novo with tacrolimus without glucocorticoids, a target MPA  $AUC_{0-12h}$  of 30–60 mg·h/L is recommended (B, II).
2. In living donor LTR treated with basiliximab, tacrolimus, and glucocorticoids, a target MPA  $AUC_{0-12h}$  of 15–30 mg·h/L can be proposed during the first 2 weeks of treatment (C1, II).
3. In patients treated with MMF, an MPA  $C_0$  between 1 and 3.5 mg/L might also be recommended to decrease the risk of rejection and adverse events (C1, II).

### PK Monitoring in Thoracic Transplantation

Approximately 80% of heart and 50% of lung transplant recipients are prescribed MMF as part of a maintenance immunosuppressive regimen, mainly combined with tacrolimus or cyclosporine A or sometimes mTOR inhibitors, with or without concomitant steroids.<sup>203,204</sup> Contrary to MMF, EC-MPS is not approved for thoracic transplantation in Europe or the United States, but it was used at least in a few clinical studies.

### MPA Exposure, Efficacy, and Toxicity—Thoracic Transplantation

In heart transplant recipients, EC-MPS (1080 mg twice daily) and MMF (1500 mg twice daily) resulted in similar MPA exposure.<sup>205</sup> Furthermore, direct comparison in a RCT in 154 de novo heart transplant recipients showed a similar incidence of treatment failure (biopsy-proven or treated acute

rejection, graft loss, or death) at 6 and 12 months posttransplant with EC-MPS and MMF.<sup>206</sup> The overall safety profile was similar for both formulations, but significantly more patients on MMF had dose reductions during the treatment period. An ancillary study in 32 patients showed that steady-state MPA and MPAG  $AUC_{0-12h}$ ,  $C_{max}$ , and concentration minimum ( $C_{min}$ ) were not significantly different between the EC-MPS and MMF groups.<sup>207</sup>

Many other factors contribute to MPA exposure variability in thoracic transplantation. Stable maintenance lung transplant recipients had lower MPA AUC,  $C_{max}$ ,  $C_0$ , and a higher MPAG/MPA metabolic ratio compared with stable heart transplant recipients.<sup>208</sup> These effects on MPA exposure were more pronounced in combination with cyclosporine A than with tacrolimus. The differences may be due to higher albumin and serum creatinine levels as well as a less steroid use in heart transplant recipients. In this study, sex and the presence of cystic fibrosis (CF) had no impact on MPA PK. By contrast, another study showed that stable lung transplant recipients with CF required 30% higher doses of MMF to achieve therapeutic MPA  $C_0$  than patients without CF.<sup>209</sup> Furthermore, a small PK study reported that 5 CF lung transplant recipients had significantly lower  $C_0$ /dose,  $C_{max}$ /dose, and AUC/dose, as well as lower MPAG AUC/dose than 5 patients with no CF.<sup>210</sup> Interestingly, the intra-individual variability across the 3 PK profiles (at least 2 weeks apart) collected from each participant was similarly low in both groups (16.6% and 13.8% for AUC/dose for patients with CF and without CF, respectively). Pancreas insufficiency, GI malabsorption, and lower serum albumin levels in patients with CF are possible explanations for higher MPA apparent clearance (or lower oral bioavailability). Similar to heart transplantation, 50% lower MPA  $C_0$  were observed in lung transplants when MMF was combined with cyclosporine A rather than with tacrolimus.<sup>209</sup> In a case report, a decrease of MPA plasma concentration was detected after plasmapheresis to treat antibody-mediated rejection (ABMR), which is an increasing problem after thoracic transplantation.<sup>211</sup> By contrast, a small study in the early phase after heart transplantation found similarly high exposure (AUC) after intravenous or oral administration of MMF.<sup>212</sup>

MPA PK monitoring is rarely performed in routine after thoracic transplantation.<sup>213,214</sup> The early studies on MPA TDM in thoracic transplantation were systematically summarized in 2 reviews.<sup>215,216</sup> A retrospective study found a significantly lower incidence of acute heart graft rejection for MPA  $C_0 \geq 2$  mg/L as compared to  $< 2$  mg/L, but only in the first year posttransplantation.<sup>217</sup> When analyzed after stratification on CNI blood levels, the difference was only significant in the subgroup with CNI blood concentrations in the target ranges. A prospective, comparative 2-phase study showed that the incidence of heart transplant rejection was significantly lower with a dose-adjusted regimen than a FD MMF regimen, both in combination with tacrolimus.<sup>218</sup> In the first phase where 15 patients were given a fixed MMF dose of 2 g/d, rejection was not seen in the 5 patients with MPA plasma levels  $> 3.0$  mg/L, whereas in the second phase where 30 patients were dose adjusted to reach a target  $C_0$  between

2.5 and 4.5 mg/L, the 3 patients who had a rejection episode had MPA C0 <1.5 mg/L. The incidence of infection was comparable with historical results. Other studies, however, did not find a significant relationship between rejection and MPA C0 or MMF dose.<sup>213,214,219–222</sup> By contrast, some of them reported a significant association between efficacy and MPA AUC<sub>0–12h</sub>, with targets of 36.2 mg·h/L,<sup>213</sup> 40–50 mg·h/L,<sup>219</sup> 50 mg·h/L,<sup>214</sup> or 50–60 mg·h/L<sup>222</sup> to better prevent rejection in thoracic (mostly heart) transplantation. In all these studies, the MMF formulation was used. It has been suggested that similar MPA AUCs would be relevant for EC-MPS, although emphasized that so far this would require that a full AUC must be obtained, including around 8 samples within the dose interval.<sup>214</sup>

Reported results are conflicting with respect to the association between MPA adverse effects and exposure. In heart transplantation, MPA C0/dose and AUC<sub>0–12h</sub>/dose (but not C0 or AUC<sub>0–12h</sub>) were associated with GI symptoms, leucopenia, or anemia in the first 3 months posttransplant.<sup>223</sup> It is known that diarrhea is related to higher doses (ie, higher intestinal epithelium exposure to MPA and metabolites) rather than to systemic exposure to MPA, but it is rather surprising that direct exposure was not linked with hematological adverse effects in this study.<sup>224</sup>

MPA exposure–response relationship data are very limited in pediatric heart and lung transplantation. In a retrospective study including 26 pediatric and young adult heart transplant recipients, MPA C0 <2.5 mg/L were associated with an increased risk of higher grade of rejection, suggesting a target MPA C0 ≥2.5 mg/L to minimize rejection hazard. In addition, pediatric patients on tacrolimus had 40% higher MPA levels than those on cyclosporine A.<sup>225</sup> Discrepant observations were made in pediatric heart transplant recipients, in whom a lower MPA C0 target range of 0.8–2.0 mg/L successfully minimized MPA-related adverse events without any negative impact on graft outcome. In this study, African American pediatric recipients required significantly higher MMF doses (702 ± 235 mg/m<sup>2</sup>) to achieve similar MPA C0 compared with recipients of other ethnicities.<sup>226</sup> Review of 44 pediatric heart transplant patient records in the early 2000s showed that the MMF dose required to achieve the target MPA C0 of >3 mg/L was higher in the immediate posttransplant period and tended to decrease with increasing recipient age.<sup>227</sup> There was no significant association between MPA C0 and efficacy.

### Summary of Recommended MPA Target Concentration Ranges—Thoracic Transplantation

1. In de novo heart transplantation recipients treated with MMF, CNI, and glucocorticoids, MPA AUC<sub>0–12h</sub> > 36 mg·h/L or C0 > 2.0 mg/L is recommended to decrease the risk of acute cellular rejection up to 3–6 months posttransplant (C1 III).
2. In case of gastrointestinal toxicity, a recommendation of dose reduction rather than a target MPA exposure may be made because of the poor concentration–effect relationship (B III).
3. By contrast, in lung transplantation, no evidence-based target can be proposed for MPA C0 or AUC<sub>0–12h</sub> at the

present time to adjust MMF or EC-MPS dose and prevent rejection or avoid adverse events because of the paucity of studies.

4. More evidence is required to establish optimal MPA exposure targets in pediatric thoracic transplant patients.

### PK Monitoring in Stem Cell Transplantation

Prevention of graft-versus-host disease (GVHD) is critically important in hematopoietic stem cell transplantation. In addition to cyclosporine A or tacrolimus, MPA is commonly used for the prevention of GVHD. MPA is also used for the treatment of acute and chronic GVHD that is resistant to steroids. However, it is not approved by the FDA or the EMA in these indications.

Studies have evaluated direct MPA exposure over a dosing interval, using LSSs to estimate MPA exposure, and developed POPPK models to estimate MPA PK parameters and determine patient covariates responsible for the observed variability in exposure. In comparison with data from solid organ transplant patients, the MPA concentrations are low in patients treated for GVHD.<sup>228</sup> It is not entirely clear whether this low exposure is due to limited absorption in gut walls affected by GVHD, to interruption of enterohepatic circulation by broad spectrum antibiotics, or to faster clearance as a result of low protein binding. Some studies have also characterized a PD end point (inhibition of IMPDH activity). These measures have been correlated with clinical outcome such as absence of GVHD, safety (engraftment), and adverse effects.

### MPA Exposure, Efficacy, and Toxicity—Stem Cell Transplantation

Poor correlation has generally been reported between MPA C0 and AUC in various adult transplant patient populations. Interestingly, in 36 patients, a single-point assessment of C2 was shown to be a useful surrogate marker of AUC<sub>0–24h</sub> to predict the incidence of GVHD. This study suggests that individualized MMF dosing in a donor source–dependent fashion may be important for maximizing the benefit of MMF used for prophylaxis of GVHD in allo-hematopoietic stem cell transplant (HSCT).<sup>229</sup> A few studies have focused on developing LSSs to estimate MPA exposure and individualize MPA pharmacotherapy. Blood levels measured at 2, 2.5, 3, 4, and 6 hours after a 2-hour IV infusion of MPA in bone marrow transplantation (BMT) patients provided a good estimate of MPA AUC<sub>0–8h</sub> for Q8-hour dosing.<sup>230</sup> Similarly, a 5 time point LSS consisting of samples immediately before and at 0.25, 1.25, 2, and 4 hours maximum and a posteriori Bayesian estimation after oral MMF administration was shown to predict MPA AUC well in HSCT recipients.<sup>167</sup> A PK study in 34 HSCT recipients on IV MMF resulted in the development of Bayesian estimators based on 3 plasma levels (at 0.33, 2, and 3 hours) that yielded AUC estimates with bias = –12% or –2% and RMSE = 15% or 12%, depending on the PK modeling approach used (individual modeling and nonparametric population modeling, respectively).<sup>159</sup>

Because MPA acts by inhibiting IMPDH activity, based on data from 49 HCT patients, IMPDH activity was modeled using a maximal inhibitory model with an MPA half-maximal

inhibitory concentration (IC<sub>50</sub>) of 3.59 mg/L.<sup>231</sup> In another study, the overall relationship between MPA concentration and IMPDH activity was described by a direct inhibitory maximum effect model with an IC<sub>50</sub> of 3.23 mg/L for total MPA and 57.3 mcg/L for fMPA.<sup>232</sup>

Several studies have demonstrated that MPA plasma exposure is associated with clinical outcomes, with an increasing number of allo-HCT patients needing MPA target concentration intervention.<sup>233</sup> MMF is efficacious in steroid-refractory and steroid-dependent acute or chronic GVHD with a statistically significant correlation between plasma total MPA C<sub>0</sub> in the therapeutic range (1–3.5 mg/L) and clinical response. This study indicated that serum albumin levels should be taken into account when considering MMF dose adjustments.<sup>234</sup> In 83 patients, those with a mean week 1 and 2 total MPA C<sub>0</sub> <0.5 mg/L had an increased day 100 grade III and IV acute GVHD of 26% versus 9% in those with MPA C<sub>0</sub> above 0.5 mg/L ( $P = 0.063$ ). Those patients who received a low total daily MMF dose and had a low mean week 1 and 2 MPA C<sub>0</sub> had a significantly higher (40%) incidence of grade III and IV acute GVHD ( $P = 0.008$ ).<sup>234</sup>

A study by Wakahashi et al found in unrelated allogeneic bone marrow transplantation (allo-BMT) that AUC<sub>0–24h</sub> >30 mg·h/L resulted in the total absence of grade II–IV acute or extensive chronic GVHD and tended to provide a higher overall and disease-free survival, lower relapse rates, and nonrelapse mortality. In the same study, with cord blood transplantation (CBT), the AUC<sub>0–24h</sub> <30 mg·h/L was sufficient to achieve a low incidence of acute or chronic GVHD and high survival.<sup>229</sup>

The relationships between PK or PD markers of MPA and successful GVHD prevention and neutrophil engraftment were evaluated to investigate individualized MPA treatments in HSCT patients. The fMPA AUC<sub>0–24h</sub> was reported to be a better predictor of the prevention of GI GVHD and neutrophil engraftment compared with total MPA in patients receiving CBT. The investigators recommend monitoring of the fMPA AUC<sub>0–24h</sub> with a target range of 405 and 689 mcg·h/L in CBT patients.<sup>235</sup> A prospective study in 56 nonmyeloablative HSCT recipients evaluated plasma concentrations of total MPA, fMPA, and total MPAG and IMPDH activity in peripheral blood mononuclear cells (PBMCs) at 5 time points after the morning dose of oral MMF on day 21. It showed decreasing IMPDH activity with increasing MPA plasma concentration, with maximum inhibition coinciding with maximum MPA concentration in most patients. The relationship between plasma MPA concentration and IMPDH activity was described by a direct inhibitory maximum effect model with an IC<sub>50</sub> of 3.23 mg/L for total MPA and 57.3 ng/mL for fMPA. The day 21 IMPDH area under the effect curve was associated with CMV reactivation, nonrelapse mortality, and overall mortality.<sup>232</sup>

There is a paucity of exposure–response data in pediatric HSCT patients. Because of the lack of exposure–response data in pediatric HSCT patients, adult HSCT targets or targets used in pediatric organ transplantations have been adapted for TDM in pediatric HSCT recipients. McCune et al suggested to adapt adult targets and that an MPA trough  $\geq 1$  mg/L and steady-state average concentration >3 mg/L

(equivalent to AUC<sub>0–12h</sub> > 36 mg·h/L) be regarded as reasonable targets in pediatric HSCT. If fMPA concentrations are available, they suggested an AUC<sub>0–8h</sub> of 200–250 mcg × h/L.<sup>236</sup> In a recent pediatric study including 19 children and young adults (0.9–21 years), Windreich et al investigated an MMF continuous-infusion dosing regimen that was individually adjusted to maintain an MPA steady-state concentration of 1.7–3.3 mg/L.<sup>237</sup> During continuous infusion, the MPA AUC<sub>0–24h</sub> was maintained between 20 and 64 mg·h/L (mean: 40 mg·h/L), and 18 of the 19 patients (95%) achieved hematopoietic donor engraftment. The authors also found that MPA C<sub>ss</sub> in patients with acute GVHD was significantly lower than that in patients without GVHD (1.2 versus 1.8 mg/L).<sup>237</sup> Similarly, Harnicar et al found in their clinical study including children that the higher incidence of grade III to IV acute GVHD was associated with MPA trough concentrations <0.5 mg/L in the first 2 weeks after (dCBT).<sup>238</sup>

### Summary of Recommended MPA Target Concentration Ranges—Stem Cell Transplantation

1. For adult BMT/HSCT patients given MMF, it is suggested that the therapeutic range for total plasma MPA is C<sub>0</sub> 1–3.5 mg/L and AUC<sub>0–24h</sub> (not the AUC<sub>0–12h</sub>) >30 mg·h/L for allo-BMT, whereas AUC<sub>0–24h</sub> <30 mg·h/L would be sufficient in cord blood transplant recipients (B II).
2. For fMPA, an AUC<sub>0–24h</sub> target range of 405–689 μg·h/L has been proposed for cord blood transplant recipients (B II).
3. For pediatric HSCT recipients, based on a single study, a tentative target range of MPA steady-state concentration, C<sub>ss</sub> 1.7–3.3 mg/L (equivalent to AUC<sub>0–8h</sub> 14–26 mg·h/L in a Q8-hour dosing regimen) has been suggested but not validated in larger cohorts (C2 III).
4. Given the lack of MPA POPPK models describing the PK profiles of EC-MPS, future attention should be paid to fill the gap.

### PK Monitoring in Autoimmune Diseases

It should be noted that the indications discussed under this heading represent off-label use in most countries. In transplant recipients, immunosuppressive treatment often consists of 3 or 4 drugs administered at the same time. In most of the autoimmune indications, MPA is either the only treatment or sometimes it is combined with glucocorticoids. One could therefore argue that in autoimmune diseases efficacy more strongly depends on reaching the therapeutic window of MPA, whereas in transplantation subtherapeutic MPA exposure can be compensated by the concomitant immunosuppressive drugs. This provides a rationale for investigating the concentration–effect relationship in autoimmune diseases.

### Lupus Nephritis

The European League Against Rheumatism–European Renal Association–European Dialysis and Transplant Association (EULAR/ERA–EDTA) published its updated recommendations for the management of LN.<sup>239</sup> For both initial (induction) and long-term (maintenance) treatment of LN, MPA combined with glucocorticoids is recommended as



the first-line treatment. The guideline mentions that the MPA dose may be adjusted according to tolerance and adverse effects, efficacy, and MPA plasma trough levels. No target concentrations were defined. Multiple studies did show a concentration–effect relationship.<sup>240,241</sup> Based on a literature review for LN, a target MPA AUC of 30–45 mg·h/L was proposed. If AUC monitoring is not possible, then the recommendation is to aim above a lower C0 threshold of 3.0 mg/L.<sup>242</sup> In one study, there was a significant correlation between MPA trough concentrations at 12 and 24 hours and MPA AUC<sub>0–12h</sub>, and the combined analysis of effect and toxicity suggested a therapeutic range of 3.5–4.5 mg/L for MPA trough concentration.<sup>243</sup> Although evidence from randomized trials is lacking, we recommend that at least one MPA concentration be measured before the conclusion is drawn that a patient is unresponsive to MPA treatment.<sup>4</sup> The EULAR/ERA–EDTA guideline also mentions the option of combining MPA with a CNI (tacrolimus, cyclosporine A, or the new CNI voclosporin<sup>239</sup>). In contrast to tacrolimus, it is known that cyclosporine A will affect MPA exposure, and therefore, the MPA dose may be different depending on the CNI with which it is co-administered. Whether or not voclosporin will also affect MPA concentrations is unknown.

In a pediatric study in 19 children, an MPA AUC<sub>0–12h</sub> of 30 mg·h/L or higher was associated with improved disease control of childhood-onset SLE.<sup>244</sup> In a pediatric study in 36 children, AUC <44 mg·h/L and AUC/dose <0.06 (h/L) were associated with an increased risk of active disease, suggesting a target AUC<sub>0–12h</sub> > 45 mg·h/L to prevent relapse.<sup>158</sup> In both studies, the exposure–toxicity relationship was not fully characterized, but it is likely that an AUC well above 60 mg·h/L does not provide additional benefit while increasing the risk of adverse drug reactions.<sup>158,244</sup> Finally, a retrospective study analyzing 62 MPA AUC in 27 patients using a logistic regression adjusted for age, sex, LN classification, and time since MMF initiation showed that an MPA AUC >45 mg·h/L was significantly associated with a therapeutic response [odds ratio (OR) 3.6, 95% CI 2.4–9.5, *P* = 0.03].<sup>245</sup>

### Inflammatory Bowel Disease

In the treatment of inflammatory bowel disease, conventional therapy with azathioprine and the CNIs continues to be used, especially in parts of the world where biologics are not covered by insurance.<sup>246</sup> Evidence for the efficacy of MPA for this indication is however limited and published studies are mostly retrospective or uncontrolled.<sup>247</sup>

Colitis as an immune-related adverse event associated with the use of immune checkpoint inhibitors may form a new indication. The first-line treatment is discontinuation of the immune checkpoint inhibitor and high-dose glucocorticoids, and MPA has also been suggested as an alternative.<sup>248,249</sup>

There has not been any investigation of TDM for MPA on these indications.

### Nephrotic Syndrome in Children

A number of studies suggested the need for a higher AUC<sub>0–12h</sub> target (>45 mg·h/L) for the treatment of idiopathic nephrotic syndrome in SLE in children.<sup>250–252</sup> In a prospective multicenter study including 60 pediatric patients with

steroid-sensitive nephrotic syndrome, the incidence of relapse was higher in patients with AUC<sub>0–12h</sub> ≤50 mg·h/L (1.4 relapses per year) than in patients with AUC<sub>0–12h</sub> >50 mg·h/L (0.27 relapses per year).<sup>250</sup> Various adverse effects (mostly minor) were noted in 20 of the 60 patients, but they were not related to MPA exposure.<sup>250</sup> In another study, 168 blood samples from 24 pediatric patients with idiopathic nephrotic syndrome or LN were collected, showing that dose-normalized MPA C0 <2 mg/L per 600 mg/m<sup>2</sup> was associated with a higher risk of proteinuria recurrence<sup>251</sup> and that the erythrocyte count and hemoglobin were negatively correlated with MPA C0.<sup>251</sup> In a retrospective multicenter study including 95 children with steroid-dependent nephrotic syndrome, MPA AUC<sub>0–12h</sub> >45 mg·h/L was significantly associated with a lower relapse rate,<sup>253</sup> whereas no difference in AUC was observed between patients with and without adverse effects.<sup>253</sup>

### Immunoglobulin A Nephropathy and Vasculitis

No targets for MPA TDM have been defined for these indications. Current clinical guidelines on treatment of IgA nephropathy do not recommend MPA as a treatment option.<sup>254</sup> The studies that investigated MPA with or without steroids, compared with either steroid or usual care, were typically small and inconclusive. Rodrigues et al in a review on emerging developments in clinical and translational IgA nephropathy research concluded that the results of clinical trials on the use of MPA for this indication are mixed at best.<sup>255</sup> IgA vasculitis (formerly Henoch–Schönlein Purpura) is believed to be caused by abnormal IgA1 glycosylation. Consensus guidelines suggest as one of the treatment lines for moderate disease (<50% cellular glomerular crescents on renal biopsy plus altered renal function or severe persistent proteinuria, histological class IIIb), the use of glucocorticoids together with an antiproliferative (MMF or azathioprine). MMF belongs to the second-line indication to treat gastrointestinal manifestations of the disease in combination with corticosteroids when the patient develops moderate nephritis.<sup>256</sup>

### Summary of Recommended MPA Target Concentration Ranges—Autoimmune Diseases

1. LN: no target has been definitely recommended, but authors proposed to aim above a lower C0 threshold of 3.0 mg/L, or to an AUC target of 30–45 mg·h/L, before concluding that a patient is unresponsive to MPA treatment (B III).
2. IgA nephropathy: no recommendation to use MPA, no target.
3. Inflammatory bowel disease: limited evidence in favor of MPA efficacy.
4. Nephrotic syndrome in children: AUC<sub>0–12h</sub> > 45 mg·h/L (A II).

### MEASUREMENT OF MPA CONCENTRATIONS

Analytical methods for the determination of MPA plasma concentrations can be divided into 3 main groups: (1) chromatographic methods [HPLC or ultra-high-pressure

liquid chromatography (UHPLC) with either ultraviolet, fluorescence, or MS detection]; (2) immunoassays [EMIT, cloned enzyme donor immunoassay (CEDIA), and particle-enhanced turbidimetric inhibition immunoassay (PETINIA)], and (3) an IMPDH inhibition assay. Methods belonging to the groups (2) and (3) are available for automated general clinical chemistry platforms. Advantages and disadvantages of the different methods vary, and therefore, the choice of which assay to use depends on the laboratories circumstances and requirements. The performance characteristics of the assays are summarized in the subchapters below and in Table 2, as well as in a previously published IATDMCT Consensus Document specifically addressing the requirements for analytical quality in TDM of immunosuppressive drugs.<sup>16</sup>

### Sample Matrix and Stability

In contrast to other immunosuppressive drugs, MPA in blood is distributed extracellularly; therefore, plasma or serum is the appropriate sample matrices for analysis. In an attempt to standardize the collection of immunosuppressive drugs, EDTA plasma has been recommended as the material of choice for MPA in routine TDM services. Heparinized plasma or serum can also be used.<sup>205</sup> To quantify the free drug concentration, deproteinized plasma can be obtained by ultrafiltration (see discussion below).<sup>257</sup> The application of dried blood spots (DBS) and volumetric absorptive micro-sampling (VAMS) tips as an alternative sampling approach in specific clinical situations is gaining interest. Additional sample matrices such as oral fluid, isolated PBMCs, tissue homogenates, or urine find application primarily for research.<sup>258</sup>

In general, the stability of MPA was reported to be up to 8 hours at ambient temperature, 4 days at 2–8°C, and 11 months at –20°C.<sup>16</sup> However, a limited stability of the MPA metabolites (MPAG and AcMPAG), which are also present in the sample, has been demonstrated.<sup>259–262</sup> The *ex vivo* deconjugation of these metabolites may lead to overestimation of MPA concentration, particularly in samples from patients with kidney insufficiency.<sup>263</sup> An overestimation of MPA can also occur in samples containing MMF, for example, those collected during or immediately after an IV application of MMF.<sup>264</sup> Measures to stabilize samples have been reported in the literature.<sup>259–261,265</sup>

Oral fluid was reported to behave similarly to plasma regarding the analyte stability.<sup>266,267</sup> Sampling by the Mitra VAMS tips resulted in stable MPA concentrations for 60 days at 25°C, 30 days at 37°C, 2 days at 50°C, and 50 days at –20°C,<sup>268</sup> whereas with DBS, lower stability was reported.<sup>269</sup> No significant change of MPA and MPAG concentrations was observed after at least 3 repeated freeze–thaw cycles of plasma, oral fluid, or the Mitra VAMS tips.<sup>266–268</sup> However, MPA stability data in oral fluid, with DBS and VAMS, have to be interpreted with caution because samples without additional presence of AcMPAG or MMF were used for the evaluation. Caution should be given to storage of samples for analyses of free drug concentrations because *ex vivo* displacement from the protein-binding site may occur (eg, by free fatty acids in highly lipemic samples,<sup>270</sup> temperature changes, and acidification of the sample *in vitro*

through anaerobic glycolysis) and compromise analytical results.

### Chromatographic Methods

Chromatographic methods have been used for TDM-guided therapy of MPA for more than 20 years, with most of them being laboratory developed tests (LDTs). Chromatographic methods were instrumental for the elucidation of the PK of MPA and the investigation of the PD effects of MPA.<sup>171,228,271–281</sup> According to proficiency testing (PT) information, around 50% of the laboratories are currently using chromatographic procedures for their TDM services, and approximately 60% of those are LC-MS/MS based.

The most important advantages of the chromatographic procedures are that they are specific for the parent compound, possess very broad measuring ranges, and allow simultaneous determination of MPA, MPAG, and AcMPAG. However, the simultaneous analysis is challenged by the very different polarity of the molecules as well as by the large difference between typical therapeutic concentrations observed in patient samples (MPAG  $\approx$  20–100-fold  $>$  MPA  $\approx$  10-fold  $>$  AcMPAG).

In contrast to other immunosuppressive drugs, conventional HPLC methods are still frequently used for MPA determination because they are able to provide adequate measurements of the total drug concentration, which is the main target of the current TDM strategies. Better robustness, lower investment and maintenance costs, and broader availability of the instruments and trained staff are further arguments in support of conventional HPLC. Although the use of fluorescence detection has been reported,<sup>282</sup> most HPLC methods apply ultraviolet detection.<sup>283</sup> The UV absorbance spectra of MPA and its metabolites MPAG and AcMPAG are very characteristic because of each including a different combination of 3 absorbance peaks (Table 3).<sup>284</sup>

When an HPLC system is equipped with a diode-array detector (DAD), chromatographically separated peaks can have simultaneous detection at multiple wavelengths. Therefore, it is possible to gain improved analytical specificity as well as to select the most appropriate absorbance peak(s) for detection and thus to enable a parallel analysis of the 3 analytes and avoid MPAG signal saturation due to its high concentration. Both isocratic- and gradient elution-based procedures have been reported.<sup>283</sup> Regarding liquid chromatography columns, mostly C8- and C18-based materials are used and a shift from conventional (3–5  $\mu$ m) to small (<2  $\mu$ m) particle sizes has allowed faster run times (<5 minutes for MPA plus metabolites) without compromising resolution.<sup>283,285</sup>

There are some advantages of LC-MS/MS, specifically, smaller sample volumes, shorter analytic time, greater specificity, and higher sensitivity.<sup>283,286</sup> The higher sensitivity afforded by LC-MS/MS is particularly beneficial for measurement of free, tissue, or intracellular MPA concentrations and for implementation of microsampling techniques (eg, DBS).<sup>258,283,286</sup> LC-MS/MS does have some drawbacks with relevance for the analysis of MPA and its metabolites,

specifically, matrix effects, isobaric interferences, and in-source fragmentation.

LC-MS/MS with electrospray ionization (ESI) is generally prone to ion suppression, and the typically high MPAG concentrations increase the likelihood of this occurring when measuring MPA. The use of stable isotope-labeled internal standards is an effective way to compensate for matrix effects, and therefore, the use of the commercially available deuterium ( $^2\text{H}$ , D) and carbon-13 ( $^{13}\text{C}$ )-labeled MPA and MPAG analogs is recommended.<sup>16</sup> Furthermore, very high MPAG concentrations may not only contribute to incomplete ionization but also to saturation of the detector, with the consequence of a nonlinear concentration/signal intensity relationship, a phenomenon frequently reported in regard to the analysis of MPAG.<sup>283</sup>

MPAG and AcMPAG are isobaric and their analysis necessitates chromatographic separation. In addition, MPAG and AcMPAG, as well as a commonly used internal standard, the carboxybutoxy ether of MPA, are prone to in-source fragmentation to MPA, which if not appropriately addressed may cause erroneously high MPA concentrations in clinical samples.<sup>283,286</sup> Proper chromatographic separation, monitoring of the transition of the ammonium adduct  $[\text{M} + \text{NH}_4]^+$  for MPA, and choosing more selective MS conditions (eg, reduce acceleration voltages in the ion source) help prevent erroneous results.<sup>283,286</sup>

The scope of LC-MS/MS methods varies considerably.<sup>283</sup> Protein precipitation for sample pretreatment, C18 analytical columns for the chromatographic separation, and positive mode ESI (ESI+) for MS predominate. Various MPA adducts have been reported to be suitable for analysis after ESI+:  $[\text{M} + \text{H}]^+$ ,  $[\text{M} + \text{NH}_4]^+$ , and  $[\text{M} + \text{Na}]^+$  (Table 3). Online sample clean-up performed either with a single analytical column and gradient elution or with 2D chromatography, as well as advanced automation are common trends in routine clinical laboratories.<sup>283,286</sup> UHPLC coupled to triple-quadrupole mass spectrometers is also common and has the advantages of decreased sample volume and analysis time and potentially increased sensitivity.<sup>285</sup>

Discussion about the development, validation, quality assurance, and overall maintenance of analytical procedures for TDM of immunosuppressive drugs is provided in a previously published IATDMCT Consensus Document.<sup>16</sup> An often overlooked, but key issue is that the use of postdose patient samples that contain both the parent drug and its metabolites is necessary for method validation to fully characterize matrix effects, in-source fragmentation, and isobaric interferences.<sup>16</sup> In general, properly designed and maintained chromatographic methods (both conventional HPLC and LC-MS/MS) are in position to easily achieve an analytical precision [coefficient of variation (CV)  $\leq 5\text{--}10\%$ ] and accuracy (analytical bias  $\leq 5\text{--}10\%$ ) and to cover measurement ranges (typically 0.1–50 mg/L for MPA) that fulfill the IATDMCT recommendations.<sup>16</sup>

## Immunoassays

Automated immunoassays were initially used by small clinical laboratories performing routine testing. Advantages included availability and relative ease of use of the apparatus and increased turnaround of results.

## EMIT

The EMIT 2000 MPA Assay has been used in TDM laboratories for more than 20 years and was the first immunoassay introduced for MPA monitoring. It is currently offered by Siemens Healthcare Ltd., and they recommend using the EMIT 2000 MPA Assay on the V-Twin or Viva-E analyzers.<sup>287,288</sup> However, the reagents can be adapted for use on other manufacturers' analytical platforms: a family of Cobas MIRA analyzers,<sup>263,289–299</sup> Hitachi 911,<sup>300</sup> Architect c8000,<sup>301</sup> and Dimension.<sup>301</sup>

The measurement range of the assay stated by the manufacturer is 0.1–15 mg/L. This range is sufficient when monitoring steady-state trough concentrations. However, the Viva-E analyzer studies demonstrated that the linearity was not maintained at the higher concentration range ( $> 10$  mg/L), requiring dilutions of the sample.<sup>288</sup> This emphasizes the necessity for adequate validation studies even of commercial immunoassays on automated platforms. Numerous studies have evaluated the imprecision of the EMIT assay. Overall the intra-assay imprecision ranged from 1.5%–8.1% and interassay imprecision ranged from 1.2%–9.6%.<sup>292–294,301</sup> Analytical specificity is a critical issue for reliability of drug measurements using immunoassay. For EMIT, the overestimation of MPA concentration ranges from 15% to 37.7%. The positive bias is primarily believed to be caused by cross-reactivity with the metabolite AcMPAG, although other factors are also suspected.<sup>263,287–290,292,295–298,301,302</sup>

EMIT may serve as an example of how analytical methods influence TDM. The MPA therapeutic range for trough concentration has been set at 1.0–3.5 mg/L for HPLC methods and at 1.3–4.5 mg/L for EMIT.<sup>295</sup> In addition, POPPK models and Bayesian estimators were even specifically developed for the EMIT technique.<sup>155,169</sup> However, although PK models can be developed for any set of concentrations measured, the use of nonspecific assays makes interpretation of the outcome very difficult.

## PETINIA

About a decade later, in 2011, Siemens introduced the PETINIA MPA assay developed for use on Dimension analyzers. This assay has better reagents stability and a wider calibration range (0.2–30 mg/L). Studies reported intra-assay imprecision from 0.91% to 3.16% and interassay imprecision from 2.8% to 6.0%.<sup>303,304</sup> When compared with reference methods (HPLC or LC-MS/MS), there was a significant positive bias that ranged from 26.3% to 33.5%, depending on the transplant type.<sup>288,304,305</sup> Much like the EMIT assay, it is believed that the high positive bias is due to cross-reactivity with AcMPAG overestimating MPA plasma concentration.<sup>288,303–307</sup>

## CEDIA

Another methodology introduced for MPA monitoring was the CEDIA (from Microgenics Corporation, and later made available from Thermo Scientific). The assay dedicated for automated clinical chemistry analyzers was mainly evaluated on Hitachi 917 instrument and the Indiko analyzer.<sup>308–311</sup>

The linear range was verified to be 0.3–10 mg/L.<sup>310,311</sup> Studies reported intra-assay imprecision ranging from 1.5% to

9.3% and interassay impression ranging from 0.6% to 13.3%.<sup>308–310</sup> The CEDIA MPA assay also demonstrated a significant positive bias of 36.3% over the true MPA concentration on average, depending on the type of transplant. This bias is believed to be due to the cross-reactivity with AcMPAG, a similar issue to that observed for other immunoassays.<sup>308–311</sup>

To the best of our knowledge, no POPPK models or Bayesian estimators have been developed for PETINIA or CEDIA, but Saint-Marcoux et al proposed a procedure to develop Bayesian estimators dedicated to different immunoassays, starting from POPPK models and Bayesian estimators developed with LC-MS/MS and using a simulation approach taking account of the correlation equations between the concentrations measured with each of the immunoassays and LC-MS/MS.<sup>312</sup>

In conclusion, available immunoassays for MPA monitoring (EMIT, PETINIA, and CEDIA) have the advantage of being automated with a relative ease of use of the apparatus and increased turnaround of results. They all, however, suffer from significant overestimation (positive bias) of MPA concentration, which frequently varies with the transplant type. If the laboratorians, pharmacists, and clinicians know and understand the limitations of these methods, they can still be accepted for TDM. According to PT reports, immunoassays are used in approximately 20% of laboratories.

### IMPDH Inhibition Assay for MPA

The Roche Total MPA assay (Roche Diagnostics, Rotkreuz, Switzerland) is based on the drug in vivo mechanism of action. Recombinant IMPDH II combines with inosine monophosphate (IMP) and NAD<sup>+</sup>; the NAD<sup>+</sup> is reduced to form NADH and XMP. The formation of NADH is measured at 340 nm. In the presence of MPA, the activity of IMPDH is inhibited and the formation of NADH is decreased.

As compared to chromatographic methods, the IMPDH inhibition assay has the advantage of being able to be run on automated analytical platforms. Whereas originally the method application was limited to the platforms of the kit manufacturer (COBAS C and Cobas INTEGRA series), a successful open-channel adaptation (ABX Pentra 400 analyzers; Horiba Ltd, Kyoto, Japan) was reported.<sup>313</sup>

The analytical performance of the Roche Total MPA kit was shown to fulfill target acceptance criteria for MPA TDM,<sup>16</sup> except for its lower limit of quantification (LLOQ) of 0.31–0.50 mg/L,<sup>145,313–315</sup> which is higher than that recommended by IATDMCT (0.2 mg/L). Another relative disadvantage of the method is a narrow analytical measurement range with ULOQ of 15 mg/L. This would necessitate sample dilutions if AUC-based TDM strategies are being used. The intra-assay imprecision varied from 0.7% to 5.5% and the inter-assay imprecision from 0.9% to 9.6% throughout the measurement range.<sup>313,314,316</sup>

Because the mechanism of action of the drug is the basis for the assay, it achieved better analytical specificity than immunoassays. In validation studies with LC-MS/MS as the reference, overestimation of MPA concentrations of <5% was demonstrated using samples from kidney, heart, and liver

transplant recipients, as well as from children with idiopathic nephrotic syndrome. This bias was considered of almost no clinical relevance.<sup>145,313,314,316,317</sup>

Method-specific PK models and Bayesian estimators were developed for PK-guided TDM using this IMPDH inhibition-based assay for (adult or pediatric) kidney or lung transplant recipients administered MMF and cyclosporine A, tacrolimus, or sirolimus, at different posttransplant periods.<sup>145</sup>

### Consistency of MPA Results Generated by Different Analytical Methods

MPA is prescribed as a long-term therapy, and the importance of consistent analytical performance of methods and laboratories over long periods is critical. Method inconsistency may have an impact on patient care for several reasons including its effect on clinical decisions regarding alterations in drug dosing and, therefore, also have an impact on long-term patient outcomes. Because the retrospective analysis of the analytical data or the interpretation of pooled data from clinical trials may be used for regulatory purposes or to establish clinical decision points, this may also be impacted by variability in analytical data.

Inconsistency with analytical methods over time is still an issue, not to mention the biases between methods and elevated CVs frequently reaching 10%. A further complicating factor is the low level of method harmonization, particularly with what is perceived as the reference methods, which are almost exclusively LDTs. A patient may be perceived as being above or below the therapeutic target simply because drug concentrations were determined by a different method or laboratory. If the treating clinician is unaware of these methodological differences, it might lead to an inappropriate dosage change and the patient receiving either an insufficient dose and rejecting the organ or receiving a too high dose with the risk of over immunosuppression. The introduction of laboratory- (or method-) specific target ranges as developed with the EMIT assay<sup>295</sup> is a helpful approach to attenuate the impact of between-method differences on patient classification. Still, this approach may pose a hidden danger, particularly when laboratories need to change the methodology at short notice (eg, because of problems with reagent supply) or when transplant physicians have to simultaneously interpret results provided by different laboratories. In addition, because of concentration dependence of the cross-reactivity to AcMPAG in the immunoassays plus a broad interindividual and intraindividual variability of the metabolite concentration,<sup>261</sup> a reliable extrapolation of MPA concentrations measured by immunoassays to respective “chromatographically determined concentrations” is not possible and cannot be recommended. By contrast, because of the very good comparability of MPA concentrations determined with the IMPDH inhibition assay and chromatographic methods, the use of the identical therapeutic targets or target ranges with these techniques seems appropriate.<sup>316</sup>

There has been continuous improvement of analytical performance; however, the methods currently available still have a wide range of performance characteristics, which will

need critical consideration when implementing or changing TDM services for MPA. The current state of assay calibration and PT will be discussed below.

## Method Calibration and Proficiency Testing

### Method Calibration and Measurement Standardization

The applicability and reliability of results generated by laboratories depend on the quality of the data, especially their accuracy. This general remark is valid for any kind of measurement service and is not limited to clinically relevant analytes, such as MPA. Laboratory medicine adopted relatively early the general metrological concept of traceability and established a close relationship with national metrological institutes.<sup>318,319</sup> By founding the Joint Committee for Traceability in Laboratory Medicine (JCTLM), located at the International Bureau of Weights and Measures,<sup>320</sup> chemical and biological entities in laboratory medicine have been raised to the same level of international consistency and used classical SI units for measuring time, weight, and length. Measurement procedure accuracy is achieved through ensuring specificity of the applied methods and is the responsibility of individual laboratory units offering defined measurement services and determined through proper and thorough method validation. The calculation of total error or measurement uncertainty<sup>321–323</sup> can be used to investigate the error components bias and precision, which in combination define the accuracy of a measurement system<sup>324</sup>

Generally, the end user must rely on the quality of the used raw materials, including their thorough characterization and traceability to a higher metrological order. For the quantification of MPA, *in vitro* diagnostic medical device (IVD)-conformité européenne (CE)-certified kits were made available by IVD industry partners (Table 2). MPA measurements generally rely on calibration with pure substance(s), available in high quality from different vendors and including ISO34-certified materials. Whereas in immunoassays or in methods relying on enzymatic reactions solely, single analyte (MPA)-based calibration models in combination with cross-reactivity statements regarding MPA metabolites can be used; chromatographic methods can be designed so that MPA metabolites can be quantified separately. Some laboratories measure both parent drug and its metabolite, MPAG, although the clinical need of such measurement is still undetermined.

If LDTs are produced locally, this responsibility is with the producing laboratory. It must be understood that intended use claims and purity statements on producer certificates must be read with great care to avoid misunderstandings. For example, materials clarified by the US Pharmacopeia standards should not be used for quantitative purposes. Only ISO34 certification ensures complete metrological traceability of a pure compound or solutions made thereof in the sense of a “higher metrological order.”

MPA analysis lacks any kind of high-order measurement procedures or materials provided by metrological institutions, such as the European Commission’s Joint Research Centre<sup>325</sup> or the National Institute of

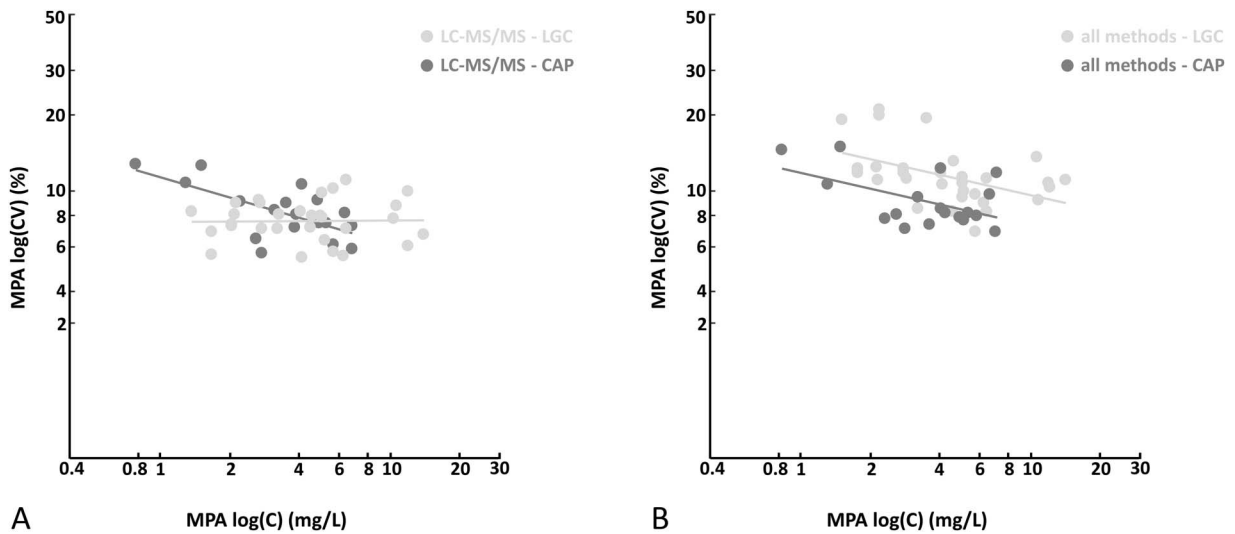
standardization.<sup>326</sup> The JCTLM database<sup>327</sup> shows no entries for candidate reference methods or services. As of now, the only available material with ISO34 certification is the reference material M-106 from Cerilliant (Round Rock, TX). No efforts have been undertaken by the scientific community to establish candidate measurement procedures fit for JCTLM listing. No raw material characterizations meeting ISO34 standards have been published in the JCTLM database after undergoing the standard JCTLM expert team review. The Immunosuppressive Drugs Work Group under the International Federation of Clinical Chemistry and Laboratory Medicine<sup>328</sup> will try to bundle efforts to fulfill the goal of making MPA measurements traceable to SI units by establishing materials and measurement tools of a higher metrological order. However, at the current time, it must be stated that MPA measurement services are not traceable to a higher metrological order.

### Proficiency Testing

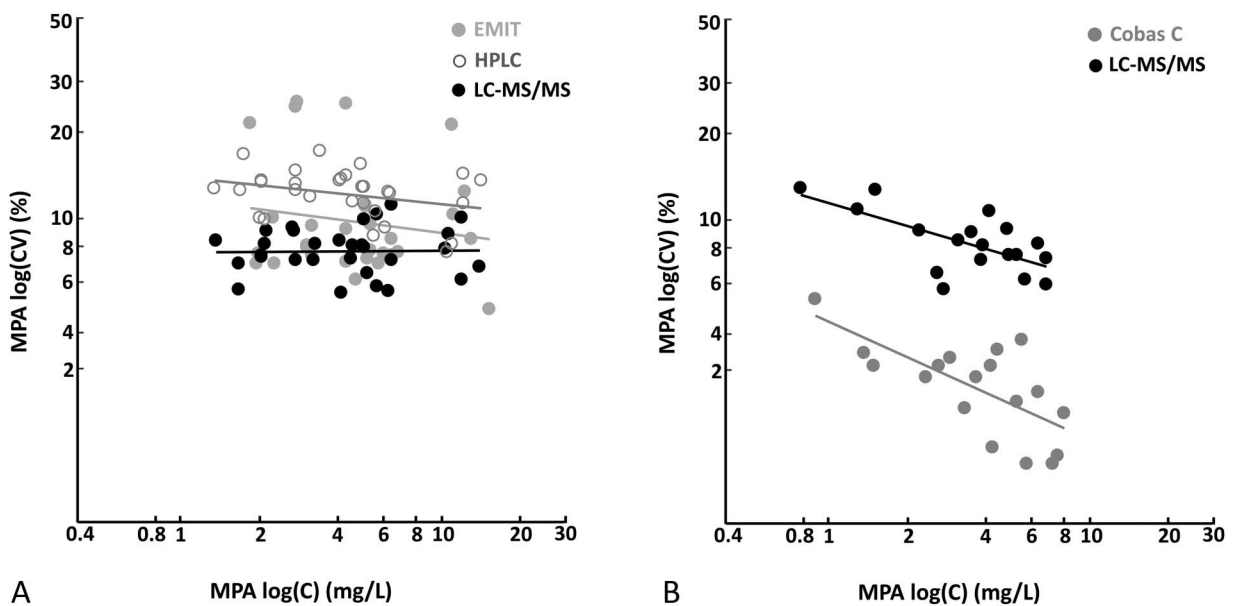
PT for MPA is available from several sources, with LGC Axio PT (as successor to the previous “International Proficiency Testing Scheme”) serving for decades as the largest international cohort with up to 148 participants and the College of American Pathologists (CAP)-based service traditionally more present in the United States. PT cohorts allow an anonymous retrospective analysis of assay performance independent from literature data and diagnostic industry or diagnostic laboratory quality claims. Data analysis of PT challenges (2017–2019) has been performed from CAP and LGC PT data summaries (Figs. 2 and 3). Because of statistical limitations (number of participants), direct CAP and LGC data comparison was only possible for all methods combined (Fig. 2B) and the LC-MS/MS subgroup (Fig. 2A). Overall, MPA measurements show an interlaboratory error in the range of about 5%–20%, with only a limited number of results associated with one of the immunoassays (Fig. 3B) exceeding this number. The overall CV is almost independent from the analyte concentration.

Independent of the scheme, LC-MS/MS subgroup CV is slightly lower than the all methods’ interlaboratory CV and ranges between 5% and 13%. Detailed analysis of the LGC-PT scheme unveils that LC-MS/MS shows better interlaboratory measurement uncertainty compared with HPLC- and EMIT-based measurement services (Fig. 3A). IMPDH-based assay realizations are not sufficiently represented in the LGC cohort to allow subgroup statistics; however, in the CAP scheme a comparison between LC-MS/MS and IMPDH PT results was possible (Fig. 3B). The subgroup measurement uncertainty of laboratories performing the IMPDH assay based on enzyme kinetics (see above) shows uncertainty figures of approximately 2.5-fold lower than the LC-MS/MS-based analysis.

Because the CV value in PT analysis is a combination of systematic and random error components, this difference can be partially attributed to interlaboratory bias contributions from individual calibrator productions in LDT systems that lack traceability to certified reference materials. It is well known from other case studies that this effect is present.<sup>16</sup>



**FIGURE 2.** Comparison of MPA PT data of all CAP (n = 18, spiked samples) and LGC (n = 30, spiked and patient samples) testing rounds from 2017 to 2019. Each data point represents the result of a single PT challenge sample. The participants mean result is plotted against the coefficient of variation of the results. A, The LC-MS/MS subgroups from the 2 schemes are presented (LC-MS/MS subgroup number of participants CAP 20–28, LGC 29–57). B, The corresponding data for all methods are shown (overall number of participants CAP 60–68, LGC 93–148). The overall interlaboratory CV ranges from 5% to 12% in the CAP PT scheme and from 6% to 21% in the LGC PT scheme. For the LC-MS/MS subgroup, the interlaboratory CV ranges from 6% to 13% in the CAP PT scheme and from 5% to 11% in the LGC PT scheme. It is emphasized that the interlaboratory CV in the PT scheme analysis reflects overall interlaboratory measurement uncertainty. It is a combination of unknown systematic (eg, calibration bias related) as well as random (eg, measurement uncertainty) error components.



**FIGURE 3.** MPA PT subgroup data analysis for all CAP (n = 18, spiked samples) and LGC (n = 30, spiked and patient samples) testing rounds from 2017 to 2019. A, All LGC subgroups with a sufficient number of participants to give a subgroup mean and (B) the same data from the CAP scheme. A, In the LGC scheme with an overall number of participants ranging from 93 to 148, LC-MS/MS (29–57 participants), HPLC (18–39 participants), and EMIT (16–27 participants) subgroup data are provided by the PT provider. These 3 subgroups represent 76%–84% of all participants. Interlaboratory subgroup CV ranges from 5% to 11% for LC-MS/MS-, from 8% to 17% for HPLC-, and from 5% to 25% for EMIT-based measurement services. B, In the CAP scheme with an overall number of participants ranging from 60 to 68, LC-MS/MS (20–28 participants) and Cobas C (10–16 participants) subgroup data are provided by the PT provider. These 2 subgroups represent 54%–62% of all participants. Interlaboratory subgroup CV ranges from 6% to 13% for LC-MS/MS- and from 1% to 5% for Cobas C-based measurement services. Because of small numbers of participating laboratories, subgroup analyses do not include CEDIA, HPLC, Siemens Dimension, and Syva EMIT 2000 methods.

However, this does not completely explain the difference between the LC-MS/MS and the Cobas C platforms. It is also likely that intralaboratory random error contributions in LC-MS/MS-based services exceed interlaboratory measurement uncertainty in automated Cobas C installations.

## Alternative TDM Approaches

### Free MPA

The free (unbound) drug is considered the pharmacologically active component, and therefore in general, free drug concentrations are considered more likely to be associated with drug-related effects than the total drug concentrations. For drugs with high protein binding, such as MPA ( $\approx 99\%$  in healthy individuals), small changes in protein binding may result in shifts in total or free drug concentrations that may or may not impact the PD. Consequently, there is an interest in measuring fMPA concentrations to use for TDM.

Because fMPA assays are all LDTs, it should be pointed out that in addition to the usual analytical parameters and issues regarding assay development and validation, sample collection, storage, and pretreatment will all have a significant impact on overall method performance. The matrix for the analysis of fMPA is deproteinized plasma; it is isolated by equilibrium dialysis, ultracentrifugation, or ultrafiltration; the latter being the most frequently used. Many research groups adopted the ultrafiltration procedure originally applied by Nowak and Shaw,<sup>257</sup> but the diversity of conditions reported in the literature is broad, and no data on their comparability are available. Different temperatures used during ultrafiltration ( $37^\circ\text{C}$  or ambient) contribute to differing results.<sup>329,330</sup> It is well known that some microfiltration devices may interfere with the analysis because of drug adsorption on their surface or impurities derived from the filters.<sup>331,332</sup> In addition, different matrices used to prepare assay calibrators may impact result comparability.<sup>329</sup> Currently, no automated ultrafiltration procedure has been published; development of such methods would undoubtedly be important to foster research on the role of fMPA in TDM of MPA.

Some HPLC-UV methods have been developed to determine fMPA, but their LLOQs (5–10 mcg/L) were not compatible with accurate and precise quantitative analysis, particularly of predose fMPA concentrations that are frequently in or below this range.<sup>205,283</sup> The use of fluorescent detection has been proposed, however with a marginal improvement (LLOQ  $\approx 2.5$  mcg/L).<sup>287</sup>

Modifications of the EMIT 2000 MPA Assay<sup>287,329</sup> expected to be compatible with an fMPA analysis on an automated clinical chemistry platform have been reported. Unfortunately, the LLOQ was similar or higher than that of the HPLC procedures and cross-reactivity (discussed above) remained a significant issue.<sup>287</sup>

LC-MS/MS methods have an improved LLOQ of  $\leq 1.0$  mcg/L<sup>283</sup> and clinically acceptable performance (imprecision and bias  $< 10\%$  over the main part of the measurement range). In addition, even with the extra time needed for sample preparation, LC-MS/MS methods can

provide a clinically acceptable turnaround time and the opportunity to measure the free (not protein bound) concentrations fMPA, fMPAG, and fAcMPAG simultaneously.<sup>331</sup> Finally, LC-MS/MS frequently require plasma volumes as low as  $500 \mu\text{L}$ – $200 \mu\text{L}$ ,<sup>267</sup> which is particularly important for pediatric patients.

To provide quality control for the full analytical procedure (sample pretreatment and measurement) and to also outline possible interferences by the MPA metabolites, the use of pooled patient plasma in addition to spiked QC materials is recommended. This also concerns the method development and validation as well.

Despite the theoretical advantage of measuring the biologically active part of MPA, there is not sufficient evidence that monitoring of fMPA concentrations correlates better with clinical outcome than total concentrations and therefore no recommendation for monitoring fMPA in routine services can be given at this time point.

### Intracellular Concentrations

Drug targets for most immunosuppressive drugs are located inside the T cell. Determining concentrations within lymphocytes, or for practical reasons in PBMCs, may have advantages over plasma MPA and may represent a closer reflection of its immunosuppressive activity. MPA also exerts its inhibition of IMPDH activity intracellularly. The amount of drug available inside the cell might, therefore, represent a better surrogate of its immunosuppressive activity.

There are LC-MS/MS methods with sufficient sensitivity for measurement of intracellular MPA concentrations,<sup>333,334</sup> but only few clinical studies have been conducted on this topic. PK-PD analysis in 40 KTR showed minimal if any correlation between PBMCs concentrations and IMPDH activity during the first 10 postoperative days.<sup>335</sup>

A study conducted in KTR reported that patients with rejection ( $n = 15$ ) had lower intracellular trough concentrations of MPA than patients without rejection ( $n = 33$ ), whereas neither plasma trough MPA concentrations, fMPA, nor IMPDH activity was different between the 2 groups. Interestingly, in this study, there was no effect of albumin or MRP2 (ABCC2) phenotype on MPA intracellular concentrations.<sup>336</sup> This study highlights the potential of intralymphocyte MPA monitoring, but there is insufficient evidence to recommend it in routine clinical practice at this time.

### Microsampling, including DBS

In theory DBS sampling can be used for TDM of MPA in a clinical setting, especially for remote areas, pediatric setting, or patients who are confined to their homes. DBS or other microsampling techniques have the potential to become wide spread if analytical and clinical performances conform to recommendations for TDM services. In addition to the IATDMCT recommendations on analytics for immunosuppressive drugs mentioned above, a specific guideline on the development and validation of DBS-based methods for TDM was published.<sup>16,337</sup>

**TABLE 4.** Microsampling Approaches Reported for Mycophenolic Acid (MPA) and Its Metabolites

Publication	Compounds	Type of Validation	Volumetric	Correction Factor	Sample Preparation
Koster et al <sup>268</sup>	TAC, EVR, SIR, MPA, TSIR, and CsA	Analytical	Yes (Mitra VAMS)	NA	Multistep extraction with sonification and vortexing
Martial et al <sup>269</sup>	TAC and MPA	Analytical and clinical	No (Whatman 903)	Plasma/DBS = 1.3	One step extraction with vortexing
Zwart et al <sup>338</sup>	TAC, MPA, SIR, EVR, and CsA	Analytical for all and clinical for TAC + MPA	Yes (Hemaxis)	DBS/Plasma = 0.66	One step extraction with vortexing
Iboshi et al <sup>339</sup>	MPA, MPAG, and AcMPAG	Analytical	No	EPC = DBS concentration/1-Hct value	Microwave drying and one step extraction with vortexing
Wilhelm et al <sup>340</sup>	MPA	Analytical	No (Whatman 903)	NA	Two step extraction with vortexing
Arpini et al <sup>341</sup>	MPA	Clinical	No (Whatman 903)	EPC = DBS/[1 - (Hct/100)] Mean or individual Hct	Two step extraction with vortexing
Koster et al <sup>342</sup>	TAC, MPA, SIR, EVR, and CsA	Analytical and clinical only for TAC and CYA	No (Whatman FTA DMPK-C)	NA	One step extraction with vortexing and sonification
Almardini et al <sup>343</sup>	MPA	Only application	Yes, 15 uL plastic (Guthrie cards)	NA	One step extraction with vortexing, dried under 40°C Nitrogen

Publication	Stability Ambient*	Stability Freezer*	Stability Extreme*	Analytical Technique	LLOQ
Koster et al <sup>268</sup>	≥60 d	≥50 d, -20°C	NA	LC-MS/MS	0.100 mg/L
Martial et al <sup>269</sup>	≥240 d	≥240 d 4°C	Unstable at 72°C	LC-MS/MS	0.5 mg/L
Zwart et al <sup>338</sup>	≥180 d	NA	NA	LC-MS/MS	0.2 mg/L
Iboshi et al <sup>339</sup>	MPA: 10 d MPAG: 7 d AcMPAG: 3 d	NA	NA	LC-MS/MS	MPA and MPAG: 0.1 mg/L, AcMPAG: 0.125 mg/L
Wilhelm et al <sup>340</sup>	NA	≥26 d, 4°C	NA	RP-HPLC-DAD	0.74 mg/L
Arpini et al <sup>341</sup>	≥20 d	≥20 d, 4°C	NA	UHPLC-DAD	0.25 mg/L
Koster et al <sup>342</sup>	≥60 d at AT and 37°C	≥60 d, -20°C	14 d, 50°C	LC-MS/MS	0.1 mg/L
Almardini et al <sup>343</sup>	NA	NA	NA	HPLC-UV	0.25 mg/L

AT, ambient temperature; CsA, cyclosporine A; EPC, estimated plasma concentration; EVR, everolimus; Hct, hematocrit; NA, not available; SIR, sirolimus; TAC, tacrolimus; TSIR, tamsirolimus.

\*MPA stability in the presence of MPAG and AcMPAG has not been investigated, except by Iboshi et al.<sup>339</sup>

A limited number of DBS or other microsampling bioanalytical methods for MPA have been described in the literature.<sup>268,269,338–343</sup> The TDM of MPA using DBS/VAMS has been studied in a clinical setting, by applying conventional HPLC equipment or more sophisticated LC-MS/MS methods. Both multi- and single-component assays have been developed. Most methods meet the general criteria of the EMA bioanalytical validation guidelines.<sup>344</sup> Some of the studies also described clinical validations including evaluation of clinical utility.<sup>269,338,342,343</sup> A summary of these assays and types of evaluations are presented in Table 4.

Widespread application in routine practice has not yet been achieved, possibly because of a lack of correlation and a bias compared with venous samples or establishment of appropriate therapeutic ranges. However, the increased need for home sampling methods for monitoring transplant recipients with decreased accessibility to routine health care may increase the acceptability of a slightly higher bias and imprecision compared with conventional plasma methods that use venous blood sampling. Currently, there are

challenges with turnaround time and workload for the assays and also with sample logistics, but this is rapidly improving because of an increasing demand.

One challenge with the microsampling methods described so far is the translation of dried whole blood to plasma concentrations because therapeutic ranges for TDM of MPA are only available for plasma. It has been shown that plasma and DBS/VAMS samples yield different concentrations due to the dilution effect of blood cells, as MPA is present almost exclusively in plasma.<sup>341,345</sup> Theoretically, this effect can be corrected for by using the actual hematocrit in the sample, the mean from recent samples of the patient or a correction factor. The mathematical correction of concentrations measured in whole-blood DBS, or other microsamples, to concentrations equivalent to plasma and serum concentrations could be different depending on the type of sampling technique. More detailed information on the influence of the hematocrit on the results obtained with DBS-based methods and how to deal with this issue can be found in the IATDMCT guideline.<sup>337</sup> Examples of proposed calculations to



estimate the plasma MPA concentrations from the DBS are shown below:

Hematocrit Dependent:

$$\begin{aligned} &\text{Estimated plasma concentration} \\ &= \text{DBS concentration} / [1 - (\text{hematocrit}/100)] \end{aligned}$$

where percent hematocrit is expressed as an integer.

$$\begin{aligned} &\text{Estimated plasma concentration} \\ &= \text{DBS concentration} / (1 - \text{hematocrit}) \end{aligned}$$

where percent hematocrit is expressed as a decimal number.  
Hematocrit Independent:

$$\begin{aligned} &\text{Estimated plasma concentration} \\ &= \text{DBS concentration} \times 1.3 \\ &\text{Estimated plasma concentration} \\ &= \text{DBS concentration} / 0.66 \end{aligned}$$

Only one publication included quantification of MPAG and AcMPAG in addition to MPA; however, the stability of these metabolites seems to be much less than that of MPA, and it was proposed that a microwave treatment could potentially increase the stabilities of MPA, MPAG, and AcMPAG at ambient temperature for 10, 7, and 3 days, respectively.<sup>339</sup>

Because of its minimally invasive handling and the potentially increased stability of the analytes compared with traditional samples, dried blood microsampling can be considered a promising alternative, particularly when venous blood sampling or sample shipment is difficult. More research and experiences with microsampling in TDM of MPA are warranted to establish the utility of this matrix.

### Other Matrices

One of the major advantages gained by the progress in LC-MS/MS methods is sensitivity, thus providing the opportunity to determine the concentration of MPA and its metabolites in alternative sample matrices, such as urine, oral fluid, and tissue samples, for example, from graft biopsies. Some LC-MS/MS procedures to measure urine<sup>259</sup> or tissue<sup>331,346</sup> drug concentrations with satisfactory analytical characteristics have been published.

### Oral Fluid

Assuming that only the nonprotein bound drug (free drug) enters the oral fluid and reflects the pharmacologically active form,<sup>347</sup> combining this with the benefit of being noninvasive makes oral fluid a very attractive matrix. When repeated sampling (eg, to evaluate the AUC) is needed or when sample collection may be difficult (pediatrics), it encourages development of LC-MS/MS methods to measure the concentrations of MPA and its metabolites in oral fluid.<sup>283,286</sup>

LC-MS/MS yields the high analytical sensitivity required to precisely measure the very low drug concentrations in saliva (usual LLOQ <3 mcg/L), but may be challenged by some matrix-specific factors. In particular, the high mucopolysaccharide content may interfere with the pipetting accuracy. Sample pretreatment by sonication and freeze–thaw cycles followed by centrifugation was proposed to facilitate mucopolysaccharides breakdown, but with limited success.<sup>348,349</sup> In addition, sample collection, although convenient for the patient, represents a significant source of variability of the concentration. Insufficient specimen volumes; interference from food particles, substances, and drugs, which can change the pH and the flow of the oral fluid; contamination by blood released from teeth after brushing or flossing; and the microbial flora dependent on dental hygiene can modify the actual concentration or compromise measurement accuracy.<sup>348,350</sup> The wide variety of specimen collection methods (with and without stimulation) and devices may also significantly contribute to result variability.

These issues taken collectively are likely responsible for the very conflicting results generated using oral fluid in the clinical setting. Some studies reported an acceptable correlation of total as well as fMPA concentrations between plasma and oral fluid concentrations,<sup>266,267,351</sup> whereas others found a poor correlation.<sup>350</sup> Therefore, the use of oral fluid for the purpose of MPA TDM cannot be recommended yet, and further studies are needed to identify the most appropriate sampling and sample pretreatment conditions.

### Tissue

An LC-MS/MS method has been developed for the quantification of MPA concentrations in core needle biopsies (weighing as little as 0.1 mg) from KTR taken as part of routine clinical care. The procedure was based on a mechanical tissue homogenization technique instead of enzymatic tissue digestion, to prevent degradation of AcMPAG during sample preparation. It was followed by liquid–liquid extraction to minimize potential matrix interferences.<sup>346</sup> Because of the invasive nature and small sample size, the indications for these types of analytical methods will most likely be restricted to PK or other research settings seeking better understanding of the relationship between plasma and graft concentration and to help predict transplant outcomes.

### Summary of Recommendations for Measurement of MPA Concentrations

1. EDTA plasma, heparinized plasma, and serum are the recommended sample matrices to determine MPA concentrations for TDM services.
2. Samples (EDTA plasma) for analysis of MPA concentrations can be stored up to 8 h at ambient temperature, 4 days at 2–8°C, and 11 months at –20°C. For rare exceptions of this recommendation see section challenges with modeling MPA absorption.
3. Methods of choice for the determination of MPA concentrations for TDM services are those enabling specific analysis of the drug (eg, chromatographic methods that separate MPAG and AcMPAG from MPA, and the IMPDH inhibition assay).

4. If using an immunoassay, information on cross-reactivity with metabolites should be reported with a statement on clinical relevance. Reliable extrapolation/conversion of MPA concentrations measured by immunoassays to respective “chromatographically determined concentrations” is not possible and cannot be recommended. Laboratories should educate the clinicians and pharmacists that values obtained with different methods cannot be used interchangeably.
5. Evidence available suggests that the use of the same target therapeutic ranges with chromatographic methods and the IMPDH inhibition-based assay is possible.
6. Recommended acceptance criteria for analytical performance include:
  - LLOQ  $\leq 0.2$  mg/L,
  - interassay imprecision  $\leq 10\%$ , preferably  $\leq 5\%$ ,
  - analytical bias  $\leq 10\%$ , preferably  $\leq 5\%$ ,
  - Method characteristics, established by comparison with a validated method as described below
    - linear regression slope  $1.0 \pm 0.1$ ,
    - linear regression y-intercept not statistically different from zero, and
    - standard error of the estimate (Syx)  $\leq 10\%$  of the average of the therapeutic concentrations.
7. Because no reference method is available, for method comparison studies the use of a fully validated LC-MS/MS method with calibration traceable to an ISO34-certificated reference material is recommended.
8. Stable isotope-labeled derivatives are preferred as internal standards for LC-MS/MS methods.
9. Laboratories involved in TDM of MPA should participate in an external PT program to allow continuous monitoring of quality. External PT programs that include both spiked samples and pooled patient samples should be preferred.

## PHARMACOGENETICS

PG has emerged rapidly as a tool to attempt to individualize drug treatment by selecting drugs and drug doses based on genetic variation. Several large international consortia including the Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group have systematically reviewed over a 100 gene–drug interactions resulting in more than 50 guidelines providing therapeutic recommendations.<sup>352,353</sup> Concerning immunosuppressive drugs, the available guidelines only provide recommendations concerning the starting dose of tacrolimus in patients who express the cytochrome P450 (CYP) enzyme 3A5.<sup>354</sup> Here, we summarize the available evidence for MPA.

### PG–PK Relationships

MPA undergoes glucuronidation by various members of the UGT family to produce its main metabolite MPAG and several other MPA metabolites, including AcMPAG. The ATP-binding cassette subfamily C member 2 (ABCC2) protein (also referred to as multidrug resistance-associated protein 2; MRP2) is involved in the excretion of MPAG into bile, which then undergoes enterohepatic cycling. Most MPA

is excreted by the kidneys as MPAG. Organic anion transporting polypeptides (OATPs, encoded by the *SLCO* genes), ABCB1 (P-glycoprotein, encoded by the *ABCB1* gene), and CYP2C8, CYP3A4, and CYP3A5 are also involved in MPA PK.<sup>15,355</sup>

Regarding metabolizing enzymes, UGT1A9 accounts for more than 50% of the biotransformation of MPA to MPAG and displays genetic variations.<sup>355</sup> Kuypers et al first demonstrated that *UGT1A9* -2152C>T (rs17868320, c.-2153C>T) and/or -275T>A (rs6714486, c.-276T>A) variant carriers display a significantly reduced MPA exposure compared with noncarriers suggesting a higher activity of these variants toward MPA.<sup>356</sup> Efforts aimed at confirming these findings have yielded conflicting results,<sup>357–367</sup> also in population pharmacokinetic analyses.<sup>368–370</sup>

Another *UGT1A9* variant, *UGT1A9* c.98T>C (or *UGT1A9*\*3a; rs72551330, p.Met33Thr), has been associated with higher MPA exposure in healthy volunteers and KTR in a number of studies,<sup>356,360,361,371</sup> whereas other studies<sup>357,358,364,367</sup> and several population pharmacokinetic analyses<sup>368,369,372</sup> did not detect such an association. Of note, the allelic frequency of *UGT1A9* c.98T>C is relatively low  $<3\%$  (Table 5).<sup>361{Barracough, 2010 #433}</sup> Consequently, only very limited numbers of *UGT1A9* c.98T>C carriers were included in clinical studies that complicates interpretation. Less data are available regarding the association of other *UGT1A9* variants and MPA PK.<sup>15,355,373,374</sup> Genetic variants in *UGT1A7*,<sup>171,375–378</sup> *UGT1A8*,<sup>364,368–370,378–384</sup> *UGT1A10*,<sup>369,383</sup> and *UGT2B7*<sup>171,358,364,367–370,376–380,382–388</sup> have yielded conflicting results.

Among drug transporters, ABCC2 is involved in the biliary excretion of MPAG and displays several genetic variants.<sup>15</sup> The available evidence on associations of *ABCC2* variants with MPA PK is contradictory, with a number of studies supporting<sup>357,366,380,382,389,390</sup> but others opposing<sup>355,358,359,361,363,367,381,387,391</sup> such relationships. Similarly, one population pharmacokinetic study in 65 KTR reported *ABCC2* variants to affect MPA absorption and clearance,<sup>392</sup> whereas others found no associations between *ABCC2* variants and MPA PK.<sup>368–370,377,383,384,393–396</sup> Of note, the interpretation of associative studies on *ABCC2* variants and MPA PK may be complicated in patients receiving concomitant immunosuppressive therapy with cyclosporine A, which is not uncommon in the population receiving MPA. Cyclosporine A exhibits extensive inhibition of *ABCC2*, which likely masks any impact of *ABCC2* variants on MPA PK.<sup>15,374</sup> Aside from *ABCC2*, it has been suggested that ABCB1 is involved in MPA absorption.<sup>397</sup> A study in 338 KTR and 2 smaller studies with 39 and 46 patients with glomerulonephritis found no associations between several *ABCB1* variants [(rs1128503 c.1236C>T, p.Gly412), (rs2032582, c.2677G>T/A, p. Ser893Ala/Thr), and (rs1045642, c.3435C>T, p. Ile1145Ile=)] and MPA PK,<sup>171,376,398</sup> whereas a population pharmacokinetic study in 78 KTR did report the *ABCB1* 3435C>T (rs1045642) variant to affect the clearance of unbound MPA from PBMCs.<sup>396</sup>

Two other drug transporter genes, *SLCO1B1* and *SLCO1B3* encoding OATP1B1 and OATP1B3, respectively, are involved in the hepatic uptake of MPAG, contribute to enterohepatic circulation, and exhibit functional genetic

**TABLE 5.** Most Relevant Pharmacogenetic Markers by Ethnicity: Variant Allele Frequencies of Selected Genes Involved in PK and PD of MPA

Gene	Haplotype	Variant (Other Names)	rs id	Whites	African Ancestry	Asian Ancestry	Admixed Population	References	
<i>UGT1A9</i>	*1C	c.-2153C>T (-2152C>T)	rs17868320	0.075	0.307	0	0.06	579–582 355,361	
		c.-276T>A (-275T>A)	rs6714486	0.015	0.17	0	0.06	355,361	
	*3a	c.98T>C	p.Met33Thr	rs72551330	0.015–0.0158	0.0025	<0.001–0.0022	0.016–0.2	355,357,361,583
<i>UGT2B7</i>	*2	c.802C>T	p.Tyr268His	rs7439366	0.52–0.75	0.0085	0.27–0.4	0.5	355,361,584
		c.211G>T	p.Ala71Ser	rs12233719	NFA	0.008–0.29	0.13	0.0058	584
<i>ABCC2</i>		-24C>T		rs717620	0.232–0.392	0.0145	0.21–0.44	0.18	355,357,380,381
<i>SLCO1B1</i>	*1B	c.388A>G	p.Asn130Asp	rs2306283	0.45	0.267	0.267		355
	*5	c.521T>C	p.Val174Ala	rs4149056	0.0271	0.0084	0.0821	0.17–0.308	355,363,398
<i>SLCO1B3</i>		c.334T>G	p.Ser112Ala	rs4149117	0.982	0.581	0.7–0.921	0.962	355,363,381,398
<i>IMPDH2</i>		c.787C>T (3375C>T)	p.Leu263Phe	rs121434586	<0.01–0.104	<0.01–0.056	<0.01–0.0445		355,585
		c.-95T>G			<0.01	<0.01	<0.01		585
		c.819+10T>C (IVS7+10T>C 3757T>C)		rs11706052	0.106–0.107	0.008–0.0269	0.025–0.062		585

Frequencies are displayed as decimals.

variations.<sup>15</sup> For *SLCO1B1*, 4 studies in solid organ transplant recipients found no associations of any *SLCO1B1* variant or haplotype with MPA PK.<sup>363,366,367,399</sup> One population pharmacokinetic study did report lower MPA clearance in *SLCO1B1* c.388A>G variant (*SLCO1B1*\*1B; rs2306283, p.Asn130Asp) carriers,<sup>377</sup> whereas 2 others reported no effect from any of the *SLCO1B1* variants c.388A>G, *SLCO1B1*\*5 (rs4149056, c.521T>C, p.Val174Ala), *SLCO1B1*\*15 (rs2306283, c.388A.G, p.Asn130Asp/rs4149056, c.521T.C, p.Val174Ala), (rs2291073, c.226+89T>G), (rs2291075, c.597C>T, p.Phe199=), (rs2417955, 1883T>A, intronic), (rs3829306, c.-61-2168C>T), (rs4149026, 10169A>C, intronic), or (rs4149058, c.727+1260A>G) on MPA PK.<sup>383,395</sup> In another study, *SLCO1B1*\*15 (rs2306283/rs4149056) carriers displayed lower MPAG concentrations than noncarriers.<sup>400</sup> Regarding *SLCO1B3*, 2 studies reported reduced MPA exposure in *SLCO1B3* c.334 G (rs4149117) carriers as compared to noncarriers,<sup>363,381</sup> whereas 2 other studies found no associations between *SLCO1B3* variants and MPA PK.<sup>382,390</sup> One population pharmacokinetic study reported an increased distribution volume of MPAG for *SLCO1B3* c.334T>G (rs4149117) carriers, whereas 4 other studies found no effect of *SLCO1B3* on MPA PK.<sup>370,377,383,384,396</sup>

**PG–PD Relationships**

MPA exerts its immunosuppressive effect through inhibition of the IMPDH enzyme, which is involved in the de novo purine synthesis. In particular, the efficacy of MPA

relies on selective targeting of IMPDH2, which is predominantly expressed in activated lymphocytes, over IMPDH1, which is expressed in most cell types.<sup>401</sup> Genetic variation in *IMPDH2* and *IMPDH1* likely results in differential expression of these target enzymes and may explain part of the between-subject variability in an MPA response. Indeed, a large number of genetic variants of *IMPDH2* and *IMPDH1* have been identified.<sup>397,402–405</sup>

Regarding *IMPDH2*, a number of studies have evaluated associations of pharmacogenetic variants with IMPDH2 activity, expression, or PD markers. Most studies focused on the *IMPDH2* 3757T>C (rs11706052) variant, which was associated with increased IMPDH2 activity in 80 KTR receiving MPA therapy,<sup>406</sup> reduced antiproliferative effect of MPA on lymphocytes in 100 healthy volunteers,<sup>407</sup> and increased lymphocyte counts at week 4 and 8 after transplantation in 177 KTR.<sup>408</sup> Regarding other *IMPDH2* variants, in vitro or in silico experiments predicted at least 3 additional variants, *IMPDH2* 787C>T (rs121434586),<sup>409</sup> -95C>T (no rs-number),<sup>410</sup> and 3624A>G(rs4974081)<sup>411–413</sup> to be associated with altered IMPDH2 activity. Regarding *IMPDH1*, to date, to the best of our knowledge, no studies have evaluated associations of the most frequent *IMPDH1* variants [(125G>A, rs2278293, c.579+119G>A) and (rs2278294, c.580-106G>A)] with IMPDH1 activity, expression, or PD markers.<sup>15</sup> One study did find an association between the *IMPDH1* 1079C>T (rs72624960, p.Ser275Leu) variant, only found in Han Chinese, and reduced IMPDH1 activity.<sup>15,405</sup>

The Clinical Pharmacogenetics Implementation Consortium has endorsed *HPRT1*, which encodes hypoxanthine-guanine phosphoribosyl transferase (HGPRT), as actionable pharmacogenomics (provisional level B) for MPA therapy, which indicates that there is a preponderance of weak albeit nonconflicting evidence to support *HPRT1* genotyping as an option to guide MPA therapy.<sup>414</sup> FDA and EMA labels include warnings to avoid MPA therapy in patients with rare hereditary HGPRT deficiencies, including Lesch–Nyhan and Kelley–Seegmiller disease. HGPRT catalyzes an active salvage purine synthesis pathway in non-lymphocyte cell types, allowing for IMPDH-independent guanosine production. Collins et al evaluated the hypothesized association between *HPRT1* gene expression and MPA sensitivity in vivo using a lymphocyte viability assay with material from healthy volunteers, (n = 40) and *HPRT1* mRNA expression was 2.1 times higher in MPA resistant versus MPA sensitive individuals ( $P = 0.049$ ). This observation was confirmed after *HPRT1* siRNA knockout yielding an increase in MPA sensitivity (+12%,  $P = 0.003$ ).<sup>415</sup>

## PG and Clinical Outcomes

Although the available evidence on PG–PK and PG–PD relationships for MPA is limited and in some cases contradictory, various studies have aimed to establish associations between PG and clinical outcomes in patient populations receiving MPA therapy.

## Kidney Transplantation

Regarding metabolizing enzymes, a number of studies have investigated associations of *UGT1A9* variants with clinical outcomes after renal transplantation. Two studies reported an increased risk of BPAR in the first year after transplantation for *UGT1A9* -2152T (rs17868320) and/or -275A (rs6714486) carriers,<sup>361,416</sup> whereas 4 other studies found no relation between *UGT1A9* variants and graft rejection.<sup>356,417–419</sup> Another study did report persistently lower graft function for *UGT1A9* c.98T>C (rs72551330) carriers as compared to noncarriers, but this was not associated with an increased risk of graft rejection or graft loss.<sup>420</sup> Limited evidence is available on variants in other UGTs, with one study reporting associations of *UGT2B7* c.802C>T (rs7439366) with an increased graft rejection risk,<sup>421</sup> whereas others did not observe such relationships for *UGT1A1*\*28 or *UGT2B7* -842G>A/-900G>A (rs7438135).<sup>419,422</sup> Of note, 3 of the abovementioned studies did not specify whether the participants received a fixed or TDM-guided MPA dose.<sup>416,419,420</sup> This is an important shortcoming because any PG effect on MPA PK that may in turn affect long-term clinical outcomes is likely corrected for or overcome by TDM-guided dose adaptation. The other studies reported to have applied fixed MPA dosing strategies. In addition to efficacy-related outcomes, a number of studies have evaluated associations of UGT variants with outcomes related to gastrointestinal and hematological toxicities, which are observed frequently with MPA therapy. Gastrointestinal adverse effects have been suggested to be related to *UGT1A9* -331T>C (rs2741046) and *UGT1A8* 518C>G (*UGT1A8*\*2, rs1042597),<sup>423,424</sup> whereas other studies found no such

associations for other *UGT1A9* variants,<sup>356,417,418,424</sup> *UGT1A1*,<sup>422</sup> *UGT1A8*,<sup>425</sup> or *UGT2B7*.<sup>421,426,427</sup> Hematologic toxicities have been related with several variants, including *UGT1A9* -331T>C (rs2741046), *UGT2B7* 900G>A (rs7438135), and *CYP2C8* -36G>A (rs11572076).<sup>423,428–430</sup> Albeit replicated, the underlying pathophysiological mechanism of the association between *CYP2C8* variants and hematologic toxicity is unclear because *CYP2C8* only has a minor role in MPA metabolism.<sup>418,429</sup> Studies on other *UGT1A9* variants,<sup>356,417,418</sup> *UGT1A8*<sup>425</sup> and *UGT2B7*<sup>421,426</sup> have shown no associations with hematologic toxicity. Also, Oetting et al did not observe any relationship between MPA-related leucopenia and genetic variants in a genome-wide association study in 3213 KTR.<sup>431</sup>

Regarding drug transporters, *ABCC2* variants were not associated with BPAR, gastrointestinal toxicity, or hematological toxicity in a number of studies,<sup>417–419,424,427,430</sup> whereas 2 others did report a relationship between *ABCC2* variants c.-24C>T (rs717620) and 3435C>T (rs1045642) and gastrointestinal toxicity.<sup>357,422</sup> For the OATPs, one study suggested the *SLCO1B1* c.521T>C (*SLCO1B1*\*5, rs4149056) variant to be associated with reduced MPA-related hematological and gastrointestinal toxicity,<sup>417</sup> whereas another reported no such effect.<sup>419</sup> Studies on other *SLCO1B1* variants<sup>430</sup> or *SLCO1B3*<sup>398,399,430</sup> did not report any associations with MPA-related clinical outcomes.

With respect to target enzymes, the *IMPDH2* 3757T>C (rs11706052) variant has been associated with an increased risk of BPAR in a study in 237 KTR (OR = 3.39 95% CI 1.42–8.09,  $P = 0.006$ ),<sup>432</sup> but several efforts at confirming this finding have yielded negative results.<sup>397,408,412,419,433</sup> Regarding toxicity, Pazik et al<sup>408</sup> suggested *IMPDH2* 3757T>C (rs11706052) to be associated with a reduced risk of MPA-related lymphopenia, whereas another study found no such relationship.<sup>430</sup> Another *IMPDH2* variant, -3624A>G(rs4974081), was not associated with MPA-related efficacy or toxicity.<sup>412,430</sup> The *IMPDH2* 787C>T (rs121434586) and *IMPDH2* -95C>T (no rs-number) variants both display a very low minor allele frequency (<1%), which render a clinically relevant contribution to the between-subject variability in MPA response.<sup>401</sup> For *IMPDH1*, 125G>A (rs2278293) and -106G>A (rs2278294), both located in intron 7, 2 studies reported a reduced risk of BPAR after renal transplantation,<sup>397,412</sup> but other studies did not find such a relationship for these variants.<sup>419,433–435</sup> Wang et al reported an increased risk of hematological toxicity for *IMPDH1* -106G>A (rs2278294),<sup>397</sup> whereas another study reported this variant to be protective for hematological toxicity.<sup>430</sup>

## Liver Transplantation

To date, to the best of our knowledge, only one study has evaluated associations between PG and clinical outcomes in LTR receiving MPA therapy.

In a small exploratory study on the *IMPDH2* gene expression level in 16 LTR, Vanozzi et al reported that patients without toxicity (thrombocytopenia, leucopenia, or gastritis) showed lower ( $0.771 \pm 0.300$ ) *IMPDH2* gene expression as compared to patients who did experience

toxicity ( $1.126 \pm 0.656$ ).<sup>436</sup> These authors did not investigate whether the observed variability in *IMPDH2* expression or toxicity was related to specific *IMPDH2* variants.

### Thoracic Transplantation

Limited, and in some cases conflicting, evidence is available for associations between pharmacogenetic variants and clinical outcomes in heart and lung transplant recipients receiving MPA therapy.

Among metabolizing enzymes, 2 *UGT2B7* variants [c.-125T>C (rs7668282) and c.-138G>A (rs73823859)] were associated with increased graft rejection in a study in adult heart (n = 32) and adult lung (n = 36) transplant recipients, whereas *UGT1A7* 622T>C (rs11692021) and 3 variants in the shared *UGT1A* 3'UTR region [1813T>C (rs10929303), 1941G>C (rs1042640), 2042G>C (rs8330)] were associated with reduced anemia ( $P = 0.0256$ ) and reduced leucopenia (0.0237), respectively.<sup>437</sup> Variants in other UGTs, including *UGT1A9*, *UGT1A8*, and *UGT1A1*, were not associated with clinical outcomes in thoracic transplantation.<sup>437,438</sup>

Regarding drug transporters, one *ABCC2* variant [c.-24C>T (rs717620)] was associated with an increased risk of rejection in a study in 290 pediatric heart transplant recipients {5-year freedom of rejection [hazard ratio (HR) = 1.80, 95% CI = 1.01–3.20,  $P = 0.045$ ]},<sup>439</sup> whereas a study in 59 pediatric heart transplant recipients reported an increased risk of gastrointestinal toxicity resulting in drug discontinuation for carriers of this variant.<sup>440</sup> Another study found no such relation for *ABCC2* c.-24C>T (rs717620) in adult heart (n = 32) and lung (n = 36) transplant recipients, but did report a reduced risk of anemia for the *ABCC2* c.1249G>A (rs2273697, p, Val417Ile) ( $P = 0.0108$ ).<sup>437</sup> Other *ABCC2* variants [(c.3563T>A, rs17222723, p.Val1188Glu), (c.4544G>A, rs8187710, p.Cys1283Tyr), and (c.3972C>T, rs3740066, p.Ile1092=)] showed no relations with clinical outcome in thoracic transplant recipients.<sup>437,438</sup> For the OATPs, one study in 275 lung transplant recipients reported associations between *SLCO1B3* c.334T>G (rs4149117) and 699G>A (rs7311358) with decreased 1-year survival [HR = 7.76 (95% CI 1.37–44.04),  $P = 0.021$ ; HR = 7.28 (95% CI 1.27–41.78),  $P = 0.026$ , respectively] and an increased risk for acute rejection [OR = 2.01 (95% CI 1.06–3.81),  $P = 0.031$ ; OR = 2.18 (95% CI 1.13–4.21),  $P = 0.019$ , respectively].<sup>438</sup> In addition, the *SLCO1B3* 699G>A (rs7311358) variant was associated with reduced 3-year survival [HR = 1.97 (95% CI 1.04–3.72),  $P = 0.036$ ], whereas c.334T>G (rs4149117) was not [HR = 1.86 (95% CI 0.99–3.48),  $P = 0.054$ ].<sup>438</sup>

Regarding target enzymes, the *IMPDH1* -106G>A (rs2278294) ( $P = 0.029$ ) and 1572C>T (rs2228075) ( $P = 0.002$ ) variants were associated with increased gastrointestinal intolerance, whereas the *IMPDH2* 3757T>C (rs11706052) variant was associated with increased neutropenia ( $P = 0.046$ ).<sup>440</sup> In a follow-up study in the same population, Ohmann et al combined 5 *IMPDH1* variants [109A>T (rs2288553), 227C>T (rs2288549), 125G>A (rs2278293), -106G>A (rs2278294), and 1572C>T(rs2228075)] in 5 common *IMPDH1* haplotypes, of which the *IMPDH1* “B” haplotype [rs2288553-T, rs2288549-C, rs2278293-T,

rs2278294-T, and rs2228075-T] was associated with increased gastrointestinal intolerance in carriers versus non-carriers (59.1% versus 21.6%,  $P = 0.005$ ).<sup>440</sup> None of the other 4 common *IMPDH1* haplotypes were associated with gastrointestinal intolerance.<sup>440</sup>

### Stem Cell Transplantation

Limited, and in some cases conflicting, evidence is available on the association of pharmacogenetic variants with clinical outcomes in stem cell transplant recipients receiving MPA therapy.

Regarding metabolizing enzymes and drug transporters, variants in *UGT2B7* (-842C>T rs7439366, p.Tyr268His) and *ABCC2* c.-24C>T (rs717620) were evaluated, showing no association with clinical outcomes.<sup>235</sup>

Regarding target enzymes, variants in *IMPDH1* [-106G>A (rs2278294), 125G>A (rs2278293)] have shown conflicting results, with studies reporting protective,<sup>441</sup> hazardous,<sup>442</sup> or no<sup>235</sup> effects on clinical outcomes. For *IMPDH2*, one variant (3757T>C, rs11706052) was evaluated, showing no relationship with clinical outcomes.<sup>235,441</sup>

### Autoimmune Disease

To date, to the best of our knowledge, only one study has evaluated the influence of pharmacogenetic variants in metabolizing enzymes and drug transporters on clinical outcomes in patients with autoimmune diseases treated with MPA.

Yap et al<sup>390</sup> found no association between variants in *UGT1A8* (rs17863762), *UGT1A9* [-275T>A (rs6714486), -2152C>T (rs17868320), and c.98T>C (rs72551330)], *SLCO1B3* [699G>A (rs7311358) and c.334T>G rs4149117], or *ABCC2* [1249G>A (rs2273697), 3972C>T (rs3740066), -24C>T (rs717620), and 3563T>A (rs17222723)] variants and the occurrence of clinical flares or toxicity in Chinese patients with LN (n = 88) receiving MPA therapy.

### MPA Disposition Across Ethnicities

Emerging evidence suggests that there may be differences in the disposition of MPA between patients of distinct ethnicity. In most of the studies, ethnicity was self-reported.

### Whites and Persons of African Origin

Several studies have investigated differences in MPA PK between Whites and people of African origin (PAO). Shaw et al studied MMF and conducted a study on a small population of 33 KTR (including 20 Whites and 13 PAO) and concluded that there was no influence of ethnicity on MPA C<sub>0</sub> and AUC<sub>0–12</sub><sup>443</sup> in the first 3 months. Pescovitz et al studied the effects of ethnicity as well as sex on MPA PK in a slightly larger population (n = 86, 43 Whites and 39 PAO) in a single 12-hour window. The C<sub>min</sub>, C<sub>max</sub>, t<sub>max</sub>, and AUC<sub>0–12</sub> were compared between the ethnic groups as well as sex. They reported that ethnicity, sex, or ethnicity by sex was not significantly associated with MPA or MPAG PK parameters and the systemic exposures were found to be equivalent.<sup>444</sup> In a subsequent study, Burrows et al studied 117 KTR including Whites,

Indo-Asian, and PAO, who were closely monitored for C0 and clinical covariates over a period of 12 months. After performing multivariate analysis, they did not find any influence of ethnicity on C0.<sup>445</sup> In addition to these individual studies, the effect of ethnicity on MPA PK was also investigated in systematic reviews and meta-analyses, including POPPK studies. These studies suggested that although the PAO tend to exhibit a lower MPA exposure in the early posttransplant period, this may be due to the more frequent occurrence of delayed graft function in this subpopulation rather than a true ethnic variability.<sup>446,447</sup> However, a few subsequent independent studies did report an association between ethnicity and pharmacokinetic parameters and determinants: a more rapid MPA clearance and less enterohepatic circulation (23%) in PAO compared with Whites (42%)<sup>448</sup> and significant interaction of ethnicity and sex, as well as ethnicity and formulations of MPA (MMF versus EC-MPS).<sup>449,450</sup> Potential differences in the PD between the 2 ethnicities have also been described. Summarizing the evidence, the effect of ethnicity on MPA disposition is controversial but likely to be small between Whites and PAO.

### Whites and Asians

Several studies investigated the difference between Asians and Whites regarding MPA disposition. The pharmacometric evaluations in Asians were largely performed with data from the Chinese population,<sup>451–454</sup> with a few from Japanese<sup>455,456</sup> and other Asian populations.<sup>457</sup> A direct comparative study of the PK of MPA is not available between Asians and Whites. A comparative analysis of 8 pharmacokinetic studies using MPA in healthy subjects ( $n = 132$ ) did not find any difference between subjects of Chinese and White origin. However, a study conducted in Singapore including Indian, Malay, Chinese, and Eurasians suggests ethnic influence.<sup>457</sup> A systematic review was conducted by Li et al that included 21 pharmacokinetic studies that enrolled participants of all ethnic origins. This study concluded that Asians have a higher dose-normalized  $AUC_{0-12}$  compared with Whites and PAO, indicating a lower MPA dose requirement. As the average body weight of the participants were compiled, it was found that the Asians weighed significantly less compared with Whites and PAO. This study suggested that although body weight may be an important factor contributing to ethnic difference, the role of other variables such as enterohepatic circulation of MPAG, pharmacogenomics, diet, and environmental influences may potentially contribute to ethnic difference.<sup>458</sup> Results from other studies also suggest that a weight-based dosing may minimize the extent of variability in the MPA disposition due to ethnic or sex differences.<sup>457</sup>

### Summary of MPA PG

1. A part of the PK and PD variability of MPA can be explained by pharmacogenetic variation. However, the available data are inconsistent and, in some cases, conflicting. Hence, the effect of genetics on MPA PK seems to be limited. Other variables, including renal function, plasma protein concentration, and the type of concomitant

calcineurin therapy, seem to be more important determinants of MPA PK variability.

- Concerning the effect of genetic variation on the PD between-subject variability, similar conflicting data are reported. The discrepancies relate to the different panels of genetic variants tested, lack of statistical power, and the use of heterogeneous patient populations in terms of immunosuppressive regimens, and co-medication, time after transplantation, and ethnicity.
- Currently, the use of PG to personalize MPA treatment seems to have no added value to TDM, and pre-emptive genotype-based dose adaptation is not recommended.

## PD BIOMARKERS FOR MPA MONITORING

### Drug-Specific PD Biomarkers

A drug-specific biomarker predicts or reflects the molecular response to the drug of interest, exclusively. Such a biomarker will by definition not be influenced by other drugs used concomitantly. Ideally, the biomarker should correlate to the risk of therapeutic failure or adverse reactions, thus providing a basis for drug monitoring and allowing beneficial interventions. The common understanding is that a PD biomarker reflects the direct molecular effect during drug exposure, either in terms of an absolute measure or as a relative response. In addition, the underlying biomarker level may serve as an indirect PD biomarker because it represents the baseline level that the drug is expected to modulate.

In the context of MPA treatment, the drug-specific PD biomarkers are closely linked to the target enzyme IMPDH. The *in vitro* activity of this enzyme in blood cells, and also downstream enzymatic products, has been assessed as both direct response markers and predictive baseline markers for MPA effects. Several studies have demonstrated the potential of this biomarker to guide the initial exposure of MPA and earlier dose adjustments, providing a more accurate assessment of efficacy and toxicity. The IMPDH RNA and protein expressions have also been subject to exploratory biomarker studies. Although these will often be regarded as underlying biomarkers that could predict the sensitivity to MPA or reflect the activation status of immune cells, the IMPDH expression itself may also be influenced by MPA exposure.

### Drug-Specific Biomarkers—IMPDH Activity

#### Background

Monitoring of the IMPDH activity in relevant cells may complement the TDM of MPA. The *in vitro* enzyme activity of IMPDH has been widely studied in blood cells, mainly in isolated PBMCs, from transplant recipients on MPA therapy. One should keep in mind that the measured enzyme activity is an *in vitro* model not identical to the actual *in vivo* activity. Because MPA is an uncompetitive inhibitor of IMPDH (ie, noncompetitive inhibition dependent on preformation of the enzyme–substrate complex),<sup>459,460</sup> its inhibitory effect is maintained under high substrate concentrations. Also, it does not distinguish between the 2 isoenzymes IMPDH types 1 and 2. Although the 2 isoenzymes have indistinguishable catalytic properties, they are not mutually redundant.<sup>461</sup> The type 1

enzyme is constitutively expressed and represents the predominant form in resting cells (including leucocytes and erythrocytes), whereas the type 2 enzyme is upregulated in proliferating cells such as activated lymphocytes.<sup>460,462</sup> Resting cells rely on the salvage pathway for guanine nucleotide biosynthesis, whereas proliferating cells ultimately depend on the de novo synthesis through IMPDH. Thus, MPA exerts selective antiproliferative effects in activated lymphocytes, predominately by inhibiting the type 2 isoenzyme, which is 5-fold more sensitive to MPA compared with type 1.<sup>459</sup> The assayed enzymatic activity that is measured reflects the total IMPDH capacity in cells exposed to MPA.

Methodological principles for the quantification of IMPDH activity in blood samples have evolved from radio-labeled assays<sup>463</sup> into HPLC-UV<sup>464–467</sup> and HPLC-MS/MS methods.<sup>273–275,468</sup> The common steps comprise preparation and lysis of cells, incubation under saturated conditions, and quantification of the produced XMP. Different strategies for normalization of the XMP production in cell lysates have been applied, including normalization to the hemoglobin concentration, cell number, total protein, and adenosine 5'-monophosphate (AMP) content.<sup>271</sup>

Several authors have emphasized that measurement of IMPDH activity integrates the variability of the MPA PK and target enzyme. In the clinical setting, Langman et al showed that the IMPDH activity in whole blood was inversely related to the MPA plasma concentration during the dose interval in KTR.<sup>469</sup> The reversible inhibition of the IMPDH activity during the MPA dose interval was confirmed in whole-blood cells,<sup>465,470</sup> PBMCs,<sup>471</sup> and in CD4<sup>+</sup> cells.<sup>472</sup> Within a standard MPA dose interval at steady state, the maximum reduction of IMPDH activity from the predose level was approximately 90% in isolated whole-blood cells,<sup>470</sup> 80% in PBMCs,<sup>185,471</sup> and 65% in CD4<sup>+</sup> cells.<sup>273</sup> As is apparent from the variability of the results obtained in these studies, the degree of IMPDH inhibition may depend on the selected cell population and on the MPA content retained during the cell preparation. Therefore, direct comparison of results between laboratories would need standardization of assay conditions.

### IMPDH Activity and Its Relation With MPA Exposure and Clinical Outcome

The IMPDH activity in PBMCs shows a high interindividual variability (CV approximately 40%), independent of age, sex, diurnal variation, or dialysis.<sup>185,471</sup>

MPA therapy may lead to induction of the IMPDH activity; in fact, in whole-blood cells and erythrocytes, the measured predose IMPDH activity increases approximately 5- to 20-fold during the first 2 months after transplantation and initiation of MPA therapy.<sup>465,470,473–475</sup> This induction is also associated with increased IMPDH gene expression.<sup>476,477</sup> However, such an induction is not apparent in circulating mononuclear cells. In PBMCs from kidney and heart transplant recipients, the predose IMPDH activity may be restored<sup>478</sup> or partially restored<sup>479</sup> toward pretransplant activity during the first year on MPA therapy. The timing of sample collection has to be considered in relation to possible cut-off values because of the time-dependent alterations in the underlying IMPDH level.

The relationship between IMPDH-based biomarkers and clinical outcome has been explored in transplant recipients. Glander et al first reported that low pretransplant IMPDH activity in PBMCs predicted MMF dose reductions (ie, indirectly associated with adverse reactions) and that high pretransplant IMPDH activity was associated with the risk of acute rejection in KTR.<sup>480</sup> In another study, low IMPDH activity in PBMCs one week after kidney transplantation was also shown to predict MMF dose reductions. Furthermore, measurements of IMPDH activity in PBMCs activated ex vivo did not improve the predictive properties of the biomarker.<sup>478</sup> Also, the longitudinal alteration of predose IMPDH activity in PBMCs has been associated with clinical outcomes. A steeper increasing trend over time for the enzyme activity was observed among long-term KTR experiencing acute rejections.<sup>481</sup> A study in LTR reported associations between high IMPDH inhibition in PBMCs and viral infections and also that low IMPDH inhibition could be associated with biliary complications and reduced retransplantation-free survival.<sup>482</sup> Studies of IMPDH activity have been directed to measurement of IMPDH inhibition in lymphocytes as a metrics of MPA PD. In the studies discussed above, IMPDH activity in such cells within a dose interval was inversely related to MPA concentration. Accordingly, these studies explored the potential for IMPDH inhibition near MPA t<sub>max</sub>, or the area under the IMPDH inhibition curve, as biomarkers of MPA efficacy. In earlier studies investigating the IMPDH activity in red blood cells, unexplained activity increases over time were observed.<sup>470,474,475</sup> Glander et al suggested IMPDH measurement in erythrocytes as a novel and useful strategy for the longitudinal monitoring of MPA treatment.<sup>473</sup> In contrast to the previous hypotheses, this study suggest that in RBCs high IMPDH activity (and IMPDH protein content) may predict side effects of MPA while low activity is associated with the occurrence of rejections. The authors suggested that IMPDH activity in RBCs reflects medium-term MPA exposure through not completely explained pathways could induce IMPDH in the RBCs. They underlined that these associations may not prevail during the first 2 months after initiation of MPA, but these are results that could be relevant for long-term individualization of MPA treatment.

The clinical utility of monitoring IMPDH activity as a PD biomarker of MPA in transplant recipients has previously been discussed by experts of the Biomarker Working Group of the IATDMCT. The quality of evidence and the strength of recommendation to monitor this biomarker were included in the executive summary: Determination of IMPDH activity before transplantation might be useful to KTR at higher risk of acute rejection or MPA-associated side effects (B II); Monitoring IMPDH activity may complement the determination of MPA PK to better guide MPA therapy (B II).<sup>483</sup>

### IMPDH Gene Expression and Its Relation with Clinical Outcome

The IMPDH gene expression (RNA) has been associated with clinical outcome in transplanted patients using MPA. One study based on limited data from KTR reported that the *IMPDH1* gene expression in PBMCs was increased

20-fold during the course of an acute rejection,<sup>477</sup> whereas another study based on more data (44 KTR) reported no such relationship with rejection episodes.<sup>477</sup> An association between low posttransplant IMPDH1 and 2 gene expressions and hematological adverse events during MPA therapy has also been reported.<sup>484</sup> However, the posttransplant *IMPDH1* and 2 gene expressions are apparently influenced by glucocorticoid drugs,<sup>476,485</sup> which make their potential application as posttransplant biomarkers more complex. As a biomarker, pretransplant *IMPDH* gene expression seems more feasible. In KTR, a higher pretransplant *IMPDH2* gene expression in CD4<sup>+</sup> cells was associated with the incidence of acute rejection early after kidney transplantation.<sup>476</sup> Another study indicated a trend where both pretransplant *IMPDH1* and 2 gene expressions in blood samples showed potential to predict acute rejection episodes.<sup>484</sup> In a third study, high and low pretransplant *IMPDH1* gene expressions in PBMCs were significantly related to the risk of acute rejection and hematological complications, respectively, after kidney transplantation.<sup>485</sup>

Feasible laboratory methods have been developed for the determination of IMPDH activity and gene expression. Their relevance as biomarkers depends on the type of sample matrix, timing of sample collection, and principle of normalization. Their reported associations with clinical outcome in transplanted patients on MPA therapy are mainly based on pretransplant measurements in mononuclear blood cells, thus demonstrating potential as biomarkers with predictive properties. The IMPDH enzymatic production rate (activity) has been normalized to cell number and total protein in studies reporting associations between IMPDH activity and outcome. Different reference genes have been used for the normalization of *IMPDH* gene expression in the observational biomarker studies. There is a need for assay harmonization and cross-validation between laboratories for both IMPDH activity and gene expression measurement.

### Summary of Recommendations for IMPDH Activity as MPA-Specific Biomarker

1. Determination of IMPDH activity in PBMCs before transplantation may be useful for predictive risk assessment of acute rejection and MPA-associated side effects in KTR. Methodological conditions and cut-off values should be consolidated before implementation in clinical routine.
2. IMPDH activity has demonstrated potential as a PD biomarker in LTR on MPA. More empirical data are needed in this population of transplanted patients.
3. The increasing trend in IMPDH activity during the first year after transplantation indicates the need to fine-tune specific cut-off values for this PD biomarker.
4. *IMPDH* gene expression before transplantation shows potential for predictive risk assessment of acute rejection and MPA-associated side effects in KTR. Selection of target gene(s), reference genes, sample matrix, and cut-off values should be consolidated before implementation in clinical routine.
5. The potential application of *IMPDH* gene expression as a pretransplant biomarker seems more feasible compared with enzyme activity.

## Drug-Specific Biomarkers—Purine Pool

### Background

Adequate levels of purine nucleotides are necessary to maintain cellular processes. The pool of (deoxy)guanine nucleotides is involved in nucleic acid synthesis (RNA and DNA), signal transduction, energy transfer, and microtubule stabilization. MPA inhibits the de novo (deoxy)guanine nucleotide synthesis, leading to mid-G1 phase arrest as described under section Mechanism of Action. Because there are cross-regulations within and between the de novo and salvage pathway for purine nucleotide synthesis, correlating the plain enzyme activity with the purine pool and cellular effects is not straightforward.<sup>486</sup> Therefore, it will be relevant to investigate the purine nucleotides as PD biomarkers for MPA.

Quantification of purine nucleotides in biological samples may be challenging as they are highly polar compounds with similar chemical characteristics. Ion exchange chromatography with UV detection<sup>479,487,488</sup> and LC-MS/MS methods<sup>489</sup> has equally been applied for the direct measurement of guanine nucleotides. Alternatively, the purine nucleotide pool can be indirectly determined by hydrolysis into purine bases and subsequent quantification with LC-MS/MS.<sup>273</sup>

After induction of IMPDH, the guanosine triphosphate pool in erythrocytes increases during prolonged MPA therapy, as demonstrated in both heart<sup>490</sup> and kidney<sup>487</sup> transplant recipients. With respect to PBMCs, long-term MPA treatment has been reported to cause a reduction of guanosine triphosphate in KTR<sup>488</sup> and a trend toward reduction after heart transplantation.<sup>479</sup> The inhibited de novo synthesis of guanine nucleotides in PBMCs is apparently compensated by long-term upregulation of the purine salvage pathway.<sup>479</sup> The guanine pool in circulating PBMCs showed a decreasing trend over the first week after kidney transplantation and initiation of MPA therapy and appeared to be restored thereafter. A similar trend was observed in KTR not using MPA, indicating that the guanine pool might be influenced also by other factors than MPA. The guanine and adenine pool in PBMCs seem to be highly co-regulated in both resting and circulating ex vivo-activated cells also during clinical exposure to MPA.<sup>478</sup>

The guanine pool in circulating mononuclear cells is rather stable within the MMF dose interval in liver (CD4<sup>+</sup> cells) and kidney (PBMCs) transplant recipients.<sup>273,478</sup> However, a significant decrease 1.5 hours after the MMF dose has been demonstrated when the guanine pool was quantified in ex vivo-activated PBMCs.<sup>478</sup> The activated cells have a considerable need for guanine nucleotides that the salvage pathway cannot fulfill. Therefore, MPA may cause an immediate reduction of guanine nucleotides in immunoreactivated lymphocytes. A study of the molecular PD of MPA in circulating CD4<sup>+</sup> cells early after LT suggested that the guanine to hypoxanthine ratio could be an interesting biomarker. Guanine and hypoxanthine represent the product and substrate of the IMPDH-catalyzed reaction, and this ratio was reduced from day 4 to day 17 posttransplant, whereas the IMPDH activity was stable within the same period.<sup>273</sup> Thus,



the ratio could represent the metabolic consequence of IMPDH inhibition.

### Purine Pool and Its Relation With MPA Exposure and Clinical Outcome

There are limited data on purine nucleotides as PD biomarkers for MPA and the studies have generally included rather small numbers of patients. In the context of MPA therapy, no relevant relationship between purine nucleotide levels and clinical outcome has been reported. Future studies should strive to fill this knowledge gap.

### Summary of Status for Purine Pool as MPA-Specific Biomarker

1. There are limited clinical data on purine nucleotides as PD biomarkers for MPA.
2. The guanine to hypoxanthine ratio in circulating cells might be an interesting biomarker because it represents the metabolic consequence of IMPDH inhibition.

### Drug Nonspecific PD Biomarkers

A drug nonspecific biomarker does not assess the effect of a drug on a molecular target, which directly reflects the drug action, but it monitors the response of an individual to a pharmacological intervention in a broader sense. For an immunosuppressant drug such as MPA, biomarkers that estimate the inhibition of the adaptive immune system, ideally both the cell-mediated and humoral responses would fall into this category. Markers of organ integrity due to successful immunosuppression can also be assessed.

The recognition of non-self plays a pivotal role in T-cell activation and proliferation, followed by the production of antibodies by B cells. In solid organ transplantation particularly, posttransplant (also called *de novo*) donor-specific anti-human leucocyte antigen antibodies (dnDSA) would be favored by under-immunosuppression.<sup>491</sup> T-cell activation relies on antigen presentation to the T-cell receptor and on the proliferating stimulus by mediators such as cytokines. Therefore, monitoring of cytokines inside lymphocytes can also serve as a non-drug-specific biomarker of the immunosuppressive effect. Furthermore, graft damage due to acute and chronic rejection is also a sign of under-immunosuppression.<sup>492</sup> In the case of solid organ transplant the gold standard to assess the type and grade of rejection is a needle biopsy. However, a promising noninvasive biomarker is the measurement of donor-derived cell-free DNA released from the graft into the recipient blood stream in response to an immune-mediated damage.<sup>493</sup>

Apart from biomarkers of cell damage, immune activation biomarkers generally require *ex vivo* cell function assays lasting several days, which complicates monitoring of this type of PD biomarkers.<sup>494</sup>

Because MPA is frequently co-administered with other immunosuppressants such as steroids or CNIs, it could be argued that it will be difficult to filter out the particular effects of MPA. However, the aim of such biomarkers is to assess the overall level of immunosuppression, in line with the common goal of pharmacological intervention that is to prevent immune activation and graft damage.

## Drug Nonspecific Biomarkers: T-Cell Proliferation and Activation

### Background

T-cell proliferation and activation is influenced by MPA in different ways. The major mechanism of action of MPA is the inhibition of the T-cell and B-cell proliferation and the *in vivo* suppression of humoral immune responses through the inhibition of IMPDH.<sup>494</sup> As a result, MPA induce arrest in cell cycle progression at the G0/G1 phase of their cell cycle and thus prevent proliferation.<sup>27</sup> The mechanism is explained by different ways, which have in common to inhibit proper glycosylation and membrane synthesis of T-cell surface markers.<sup>495–497</sup> Inhibition of CD62L expression on T-regulatory lymphocytes (Tregs) was observed in long-term heart transplant recipients treated with an immunosuppressive regimen that included MPA.<sup>498</sup>

### T-Cell Markers for PD Monitoring of MPA and Clinical Outcome after Transplantation

To assess the influence of MPA on T-cell proliferation, 2 well-established proliferation assays can be used: the flow cytometric measurement of the proliferating nuclear cell antigen (PCNA) and the tritium-labeled thymidine ([<sup>3</sup>H]-TdR) incorporation assay. Both methods were used to monitor T-cell proliferation in KTR receiving MPA-containing immunosuppression and detected a decrease in T-cell proliferation after transplantation.<sup>499,500</sup> A decrease of T-cell proliferation is directly associated with a reduced functionality of T cells and displays the immunosuppressive effect after MMF administration. Thus, the monitoring of T-cell proliferation after MMF intake could be useful to quantitate the patient-specific immune function to optimize immunosuppression. PD monitoring of PCNA after MMF intake revealed that the T-cell proliferation decreased in the first hours but returned to baseline levels 4 hours after MMF administration.<sup>501–504</sup> The median MPA half maximal inhibitory concentration (IC<sub>50</sub>) for T-cell proliferation was observed between 1.3 and 1.6 mg/L.<sup>503,504</sup> Also, a dose-dependent inhibition of MPA on both T-cell proliferation and expression of T-cell activation markers was observed in *ex vivo*-stimulated human whole-blood cultures by flow cytometric analysis.<sup>505</sup>

The changes in T-cell activation induced by MPA in solid organ transplant recipients can be measured by flow cytometric analysis of surface activation markers in small amounts of whole blood. For the PD monitoring of MPA, surface antigens such as CD11a (integrin- $\alpha$ L), CD25 [interleukin (IL)-2 receptor], CD71 (transferrin receptor), CD95 (Fas receptor), and CD154 (CD40L) were measured in kidney, heart, and liver transplant recipients (Table 6). All surface markers were upregulated after T-cell activation,<sup>506</sup> and most of the studies used more than one activation marker to demonstrate T-cell activation. A single dose of MMF 1 g was sufficient to inhibit T-cell proliferation in a plasma concentration-proportional manner.<sup>504</sup> A correlation of MPA concentrations was reported with both T-cell activation and T-cell proliferation in KTR.<sup>503</sup> The IC<sub>50</sub> for CD25 and CD71 expression on T cells ranged from 2.1 to 13.1 mg/L and 1.6 to 7.9 mg/L, respectively.<sup>503,504</sup>

Another way to monitor the PD effects of MPA is through immune function scores that combine T-cell proliferation and T-cell functions. For example, a biomarker

**TABLE 6. PD Biomarkers**

Category	PD Biomarker	Assay Principle	Clinical Trials	Used in Clinical Routine	Potential for PD Monitoring of MPA	References
Drug-specific IMPDH activity and gene expression	IMPDH activity	HPLC-UV; LC-MS/MS	No	No	1	Brunet et al <sup>14</sup>
	mRNA expression	RT-PCR	Observational studies, mostly in KTR	No	2	
Drug-specific Purine pool	Purine nucleotides Guanine/hypoxanthine ratio	LC-UV, LC-MS/MS	Limited observational studies	No	2	
Drug nonspecific T-cell proliferation and activation	Proliferation markers: tritium-labeled thymidine ( <sup>3</sup> H]-TdR) PCNA Surface activation markers: CD11a (integrin-αL) CD25 (IL-2 receptor), CD71 (transferrin receptor), CD95 (Fas receptor), CD154 (CD40L) CD62L (L-selectin)	Whole-blood cultures, ex vivo mitogen stimulation Flow cytometry [ <sup>3</sup> H]-TdR	Observational, monocentre, Kidney, heart, and LT	No	2	498,499,501–504,506,507,586
Drug nonspecific B-cell function and activation including DSA	Ex vivo B-cell proliferation B-cell subsets in whole blood Immunoglobulins Donor-specific antibodies CD80/CD86 on CD19 cells	CSFE labeling Flow cytometry ELISA Luminex	Observational studies autoimmune diseases and kidney transplantation	No	2	13,511,513,515
Drug nonspecific Cytokines	IL-1β, IL-2 IL-6, IL-10, IL-17, IL-18, IL-21, and IFN-gamma	Flow cytometry ELISA		No	3	517,518,529
Drug nonspecific Donor-derived cell-free DNA	Donor-derived cell-free DNA (dd-cfDNA)	Digital droplet PCR, NGS	Yes, heart, liver, lung, and kidney transplantation	No	2	539,542,587

1 = high potential, clinical trials; 2 = limited potential, no clinical trials but promising observational studies; 3 = no potential, limited evidence or technically not feasible.

study including 138 renal and 14 combined renal/pancreas transplant recipients used an immune function score combining CD4<sup>+</sup> cell counts, the phagocytic immune cell capacity, reactive oxygen species by neutrophils, and T-cell mitogen-induced proliferative responses.<sup>507</sup> Compared with patients off MPA, those on MPA immunosuppression had a lower immune function score, which could be explained by a reduced lymphocyte mitogen response.<sup>507</sup>

**Summary of Status for T-Cell Proliferation Biomarkers (Drug Nonspecific)**

1. T-cell proliferation and activation are inhibited by MPA in vitro and in ex vivo cell culture experiments by MPA.
2. Immune function scores combining T-cell proliferation and activation biomarkers are specifically reduced in patients under MPA therapy.

3. The use of PD biomarkers based on T-cell proliferation and activation to personalize MPA therapy has not yet been validated clinically.

**Drug Nonspecific Biomarkers—B-Cell Function and Activation Including DSA**

**Background**

In addition to the inhibition of both T-cell and B-cell proliferation and suppression of the humoral immune responses through the inhibition of IMPDH,<sup>508</sup> MPA might attenuate B-cell stimulation by other modes of action. The phosphorylation of signal transducer and activator of transcription 3 (STAT3) has been shown to play a role in memory B-cell formation,<sup>509</sup> and MPA may interfere with this pathway in myeloma cells.<sup>510</sup>

## B-Cell Function and Activation

In patients with lupus erythematosus on MPA treatment, using flow cytometry, Eickenberg et al observed decreased frequencies and numbers of human leucocyte antigen-DR<sup>high</sup> antibody-secreting cells as well as a depletion of antigen-naïve B cells. Consistent with the changes in B-cell subsets in whole blood, lower IgG concentrations were found in patients on MMF than in controls without drug treatment or taking azathioprine.<sup>511</sup>

In vitro proliferation assays with monoclonal antibodies and carboxyfluorescein succinimidyl ester–labeled cells using purified CD27-IgD<sup>+</sup> antigen-naïve and CD27<sup>+</sup> memory B-cell subsets from healthy blood donors showed that MPA abolished B-cell proliferation and differentiation of antibody-secreting cells completely.<sup>511</sup>

Li et al observed in a CD19 B-cell in vitro culture system that MPA was able to induce apoptosis of B cells and to prevent IgM formation in B cells isolated from KTR sensitized against the major histocompatibility complex class I chain-related gene A (MICA) in a dose-dependent manner.<sup>512</sup> Compared with B cells from healthy controls, the effects on B cells from MICA-sensitized transplant patients was significantly more pronounced. Cells were incubated with a B-cell stimulation cocktail including MICA antigen. Apoptosis was assessed by flow cytometry, and antibodies were measured by enzyme-linked immunosorbent assay (ELISA).

In another study with B cells from patients suffering from rheumatoid arthritis, MPA selectively inhibited B-cell activation and potently blocked plasma cell differentiation as assessed by flow cytometry of surface markers and cytokines as well as by measurement of intracellular ATP levels.<sup>27</sup>

Using both a direct in vitro stimulation model and a model incorporating T-cell–dependent human B-lymphocyte activation, Matz et al found that MPA was very effective in inhibiting B-cell proliferation (carboxyfluorescein succinimidyl ester–labeled CD19<sup>+</sup> B cells) and the expression of CD80 and CD86 on CD19<sup>+</sup> B cells at concentrations of 100 ng/mL and 1 mcg/mL, respectively.<sup>513</sup> B cells can act as antigen-presenting cells and express various costimulatory molecules on activation including the CD80/CD86 surface molecules. IgG and IgM formation as measured by ELISA in the cell culture supernatants was also suppressed by MPA in both models, using the direct and indirect stimulation of B cells.

These in vitro and ex vivo models demonstrate the direct inhibitory effects of MPA on B-cell activation, proliferation, and function. The cell isolation and culture models are potentially suitable for a B-cell–specific PD monitoring of MPA. However, most experiments were based on long-term cell culture, which precludes a timely decision of dose adjustments to individualize MPA therapy based on PD monitoring.

## DSA and Its Relation to MPA Exposure

In kidney transplantation, DSA and chronic ABMR are associated with the development of transplant glomerulopathy.<sup>514</sup> A study of 32 pediatric KTR followed up for 8.4 years revealed that MPA trough levels <1.3 mg/L were associated with the formation of DSA.<sup>13</sup> In a study with 617 living

donor KTR, there was however no statistically significant association between the incidence rate of de novo DSA and MPA through concentrations at 1-year posttransplantation.<sup>515</sup> It is therefore unclear whether DSA formation correlates with MPA dosing or drug concentrations. To assess the putative beneficial effect of MPA exposure intensity on DSA formation, the measurement of MPA concentrations and the monitoring of DSA formation in long-term prospective studies with a sufficient number of patients are required.

## Summary of Status for B-Cell Function and DSA as Biomarkers (Drug Nonspecific)

1. Although the effect of MPA on B-cell proliferation and activation as well as antibody formation has been demonstrated in small studies of KTR and patients with autoimmune diseases using ex vivo cell culture experiments, no conclusion can be made regarding the effect of MPA on DSA formation in patients.
2. The use of PD biomarkers based on B-cell function and activation is premature for MPA monitoring.

## Drug Nonspecific Biomarkers—Cytokines

### Background

Cytokines are substances secreted by several types of cells, including lymphocytes, monocytes, granulocytes, and endothelial cells, which mediate immune and inflammatory reactions. Cytokines regulate the complex and dynamic immune response against the implanted graft, and their production and secretion can be modified by immunosuppressive drugs and by the rejection process. This review focuses on current knowledge about the monitoring of changes in the production of some cytokines as a potential tool to predict personal response to MPA and the risk of rejection and infection in transplant recipients.

The few studies evaluating the effect of MPA on cytokine production have mostly been conducted in vitro. They have focused on elucidating the mechanism of action of MPA rather than on assessing cytokines as a potential biomarker for evaluating the response to MPA treatment or as a predictive biomarker of graft outcome in transplant patients treated with this drug. In fact, very few transplant patients receive MPA monotherapy. The most frequent combination in transplant recipients is MPA and a CNI (tacrolimus or cyclosporine A); therefore, the analysis of cytokines as a PD biomarker has been performed within the framework of evaluating possible synergies of action between the 2 drugs rather than as a specific MPA PD biomarker.

Previous studies have reported that MPA is able to promote IL-1b in association with phytohemagglutinin<sup>516</sup> or lipopolysaccharide (LPS),<sup>517</sup> and IL-18 in association with LPS because of its effect on NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3) expression and caspase-1 activation.<sup>518</sup> IL-1b is able to induce the synthesis of chemokines that can modulate macrophage, neutrophil, and T-cell activity.<sup>519</sup> IL-18 is a proinflammatory cytokine and is classified in the IL-1 family, which has important functions on immune regulation, innate immune response, and inflammation.<sup>520</sup> Both cytokines indirectly participate in antiviral responses.

However, this observed effect of MPA on IL-18 production only occurred in the presence of LPS, MPA alone failed to do so. Therefore, patients treated with MPA may not be able to produce IL-18 unless exposed to pathogens. In the presence of opportunistic infections, MPA might contribute to a more efficient host defense against invading germs. In the context of CMV infection, several studies have reported an association between MPA and the risk of CMV infection: specifically, an immunosuppressive regimen containing MPA increases the likelihood of CMV disease.<sup>521,522</sup> Recovery from CMV infection has been shown to be associated with the expansion of natural killer cells and activated viral-specific cytotoxic T lymphocytes<sup>523</sup>; in this regard, MPA may have a specific depressant effect on the proliferation of natural killer cells or CMV-specific cytotoxic T cells. A predominance of both Th1 [T helper cell (Th)] and Th2 cytokines has been reported during CMV+ replication.<sup>524,525</sup> Th1 cytokine production was strongly modified by Tac, whereas CMV-specific Th2 cytokine production was suppressed by antiproliferative drugs, such as MPA.<sup>526</sup>

An important cytokine involved in the earliest phases of acute rejection is IL-17.<sup>527,528</sup> This cytokine has been described in an in vitro study<sup>529</sup> performed in a model of human CD4<sup>+</sup> cell activation; although both drugs, tacrolimus and MPA, decreased Th17-related transcripts, MPA exerted a stronger inhibitory effect on IL-17 production than Tac. In KTR treated with MPA in combination with Tac, those receiving MPA in combination with a minimized dose of tacrolimus tended to have lower circulating IL-17 than patients treated with tacrolimus alone at conventional dose. However, no correlation with MPA exposure was observed. Therefore, the inclusion of MPA in the immunosuppressive regimen could better control Th17 immunity. In the case of CNI minimization, MPA might protect against Th17 over-reactivity.

The only reported effect of MPA on monocyte function is its inhibition of IL-6 and IL-10 production through the phosphorylated Akt (protein kinase B) pathway.<sup>530</sup> However, it seems that the inhibition of Akt phosphorylation is not complete after MPA treatment, and the residual phosphorylation may imply that monocyte functions, such as phagocytosis or differentiation remain intact, suggesting that the innate immune response induced by monocytes after solid organ transplantation may still occur in patients on MPA.

Finally, it is well known that MPA does not, by itself, produce a direct inhibitory effect on the production of IL-2, IL-10, IL-21, and interferon gamma (IFN-g), unlike tacrolimus or cyclosporine A.<sup>531</sup> However, when both types of drugs are combined, some synergistic effect can be observed. The decrease in IL-2 and IFN-g production in patients receiving MPA in addition to CNIs in comparison with those receiving a CNI alone can probably be attributed to the inhibitory effect of MPA on the clonal expansion of activated lymphocytes. The marked decrease in the number of active lymphocytes is probably responsible for the decreased production of these cytokines.<sup>532-534</sup>

## Cytokines and Its Relation With MPA Exposure and Clinical Outcome

There are scarce data on cytokines as PD biomarkers for MPA personal response and clinical outcome. In the context of MPA therapy, no relevant relationship between cytokine production and clinical outcome has been reported.

### Summary of Status for Cytokines as Biomarkers (Drug Nonspecific)

1. Cytokine production does not reflect individual patient susceptibility or response to MPA treatment.
2. In patients receiving combined therapy based on a CNI and MPA, monitoring cytokine production may reflect the synergy of action between the 2 drugs.

### Drug Nonspecific Biomarkers: Donor-Derived Cell-free DNA

The rationale for using donor-derived cell-free DNA (dd-cfDNA) as a biomarker in organ transplantation is based on the fact that organ transplants are also genome transplants.<sup>535</sup> The dd-cfDNA is a marker of graft cell death and is believed to be released into the blood stream as nucleosomes after various damage mechanisms, such as necrosis or in particular apoptosis. The half-life of cfDNA in the circulation is only 30 minutes to 2 hours.<sup>536</sup> This creates the possibility of repeated, noninvasive monitoring for allograft injury through serial measurements. Absolute quantification has been shown to be superior to fractional determination, because dd-cfDNA(%) determinations can be biased by changes that occur in host cfDNA over time.<sup>537,538</sup>

Reviews have concluded that proof of concept has been published for all solid organ transplant types that cfDNA is a promising biomarker for monitoring the health of the graft and that this biomarker could facilitate the detection of under-immunosuppression and find use as a tool for monitoring during immunosuppression minimization.<sup>539,540</sup> So far, several studies have proposed a link between the dd-cfDNA and exposure of one specific immunosuppressant, namely that low tacrolimus exposure was associated with elevated dd-cfDNA levels indicating graft injury possibly related to immune activation.<sup>541-543</sup>

Considering the potential relevance of dd-cfDNA specifically to guide MPA dose adjustments, the underlying hypothesis would be that dd-cfDNA is efficient for the detection of ABMR,<sup>539,544,545</sup> which again is a result of the development of DSA, and that this may be reduced by adequate exposure to MPA.<sup>13</sup> Other studies have concluded that with appropriate methodology, T-cell-mediated rejection can be equally well detected.<sup>540,542,546</sup> However, so far there are no reports that links between MPA exposure and dd-cfDNA have been specifically investigated.

### Summary of Status for Dd-cfDNA as Biomarker (Drug Nonspecific)

1. dd-cfDNA detects under-immunosuppression also in patients treated with MPA who require a higher level of immunosuppression.

2. dd-cfDNA may also prove helpful to guide tapering of immunosuppression. More data are needed to evaluate the utility for MPA therapy specifically.

## POPPK MODELING OF MPA

### Challenges With Modeling MPA Absorption

The PK of MPA is characterized by highly variable absorption profiles and secondary peaks of various intensities, appearing at variable time after dosing. These phenomena lead to a complex absorption profile that cannot be modeled by classical first-order absorption. Therefore, modeling of MPA PK consists in finding models that can account for both the direct absorption of the administered drug and enterohepatic circulation. For this, a sufficient number of samples have to be available to properly describe the 2 processes. Several POPPK models have been developed to specifically characterize MPA enterohepatic circulation. The principle of these models is to use first-order absorption with a lag-time chained to 2 or 3 compartment models.<sup>68,160–162,168,169,232,372,377,394,547–557</sup> More complex models have also been developed, such as the Erlang model that is a simplification of the gamma distribution in which the exponent is an integer that represents the number of virtual transit compartments that the drug has to cross to reach the central compartment,<sup>368,392,558</sup> but they did not always significantly improve the description of MPA profiles.<sup>162</sup> Finally, 1-, 2- or more parallel gamma distributions have been able to describe accurately the multiple MPA absorption peaks seen with MMF in various clinical settings.<sup>157–159,172,173,214,281</sup> To add complexity, EC-MPS exhibits even more intricate MPA absorption profiles (the tlag is much longer and more variable than with MMF),<sup>169,377,392,552</sup> rendering its AUC very difficult to predict accurately using a 3-point LSS in the first 4 hours.<sup>148,169</sup>

Secondary peaks may indeed correspond to enterohepatic recycling of MPAG/MPA when they occur between 4 and 8 hours postdose,<sup>64</sup> but because of their earlier appearance in many patients, they may also correspond to sequential absorptions of the administered dose at various segments of the gastrointestinal tract.<sup>65</sup> It has to be noted that these secondary peaks account for up to 60% (range 10%–60%) of total MPA exposure meaning that modeling the secondary peak accurately will be clinically relevant.<sup>59</sup>

### Modeling MPA and MPAG Conjointly

Efforts have been made to develop POPPK models that describe conjointly the profiles of MPA and its main metabolite MPAG by the inclusion of enterohepatic circulation compartments.<sup>160,171,231,372,553,559–562</sup> Two studies developed a POPPK model for fMPA and MPAG by considering protein binding<sup>68,394</sup> and one encompassed MPA, MPAG, and fMPA.<sup>232</sup>

### Clinically Relevant Covariates

Cyclosporine A comedication decreases the enterohepatic circulation and leads to a decreased MPA AUC in comparison with other comedications, particularly

tacrolimus.<sup>bib68<sup>68,162,169,232,394,549,551,554,562,563</sup></sup>

Interestingly, low plasma albumin concentrations and high MPAG concentrations decreased total MPA exposure by reducing MPA binding to albumin but did not seem to have any effect on unbound MPA concentrations.<sup>68,160,166,168,231,232,377,394,549,551,554,559,560,562</sup>

In some studies, the increase in body weight has been reported as increasing MPA oral clearance (CL), in adults<sup>81,155,372,556</sup> and in children.<sup>563</sup> In lung transplantation, CF clearly affects MPA oral bioavailability<sup>162</sup> or CL,<sup>556</sup> whereas when modeling concerned several types of organ transplants in the same analysis, the type of transplant also affected MPA bioavailability and CL.<sup>162,563</sup> Other covariates were more rarely reported, such as weight on the volume of distribution (Vd) in children<sup>164</sup> or creatinine clearance, albumin, sex on Vd,<sup>550,551</sup> MRP2 variants and EC-MPS or MMF formulations on CL,<sup>392</sup> or UGT1A9 variant on absorption and distribution of MPA from EC-MPS.<sup>377</sup> Finally, in other studies, no covariates were investigated or retained in the models.<sup>156–158,172,312,548,552,553,555</sup> Some of these models were developed in populations in which all patients received cyclosporine A for example, and the influence of the associated CNI could not be tested.

### PK PD Modeling

In patients on the waiting list for LT, Premaud et al showed using a sigmoid inhibitory E(max) model that CD25 and CD71 expression and T-cell proliferation (contrary to IL-2 and TNF- $\alpha$  expression) decreased with increasing MPA concentrations with low estimated IC50 values ( $\leq 2$  mg/L).<sup>504</sup>

Dong et al,<sup>558</sup> in pediatric KTR, used an Emax model for the inhibition of IMPDH by MPA. The final population parameter estimates (and their 95% CIs) were as follows: I0 = 3.45 (2.61, 4.56) nmol h(-1) mg(-1) protein and IC50 = 1.73 (1.16, 3.01) mg L(-1). Emax was fixed to 0.

In HSCT recipients, Li et al<sup>564</sup> also used an Emax model and found for IC50 values of 3.23 mg/L for total MPA and 57.3 ng/mL for fMPA.

Similarly, in HSCT recipients, Yoshimura et al<sup>231</sup> used an Emax model and reported an IC50 = 3.59 mcg/mL for MPA.

### Interface to Estimate AUC Based on LSS and MAP-BE

Few Web services are currently available for the MAP-BE estimation of MPA AUC based on POPPK models. To the best of our knowledge, up to now only 2 companies, InsightRX<sup>565</sup> and MWPharm,<sup>566</sup> and a public university (Limoges University Hospital)<sup>285,567</sup> are providing such platforms.

As an example, ISBA proposes MMF, tacrolimus, and cyclosporine A monitoring by providing individual patient's exposure to the drug (interdose AUC) estimated using MAP-BEs (developed using an Iterative Two-Stage Bayesian method) on the basis of 3 blood samples, generally collected in the first 3 hours after drug intake; the modeled concentration–time curve; and one or a range of recommended dose(s) to reach the therapeutic target. The Web site

has now received about 123,000 requests (March 25, 2020). This Web site is currently proposing several MAP-BEs for MPA, adapted to different patient profiles, immunosuppressive drugs, transplanted organ, or other conditions, drug associations, etc. Some of the POPPK models and MAP-BEs used have been published.<sup>157–159,172,173,214,281</sup>

### Summary of Status for POPPK Modeling of MPA

1. Complex models have been developed to catch the very particular absorption profile and the multiple peaks of MPA.
2. The covariates cyclosporine A, albumin, GFR, and body weight (children) are relevant to explain part of MPA exposure variability.
3. Many LSSs using either MLR or MAP-BE have been reported. MAP-BEs are more accurate, reliable, and less sensitive to sampling time variations than MLR methods, but these methods may not be easy to apply in routine practice. Web interfaces where MAP-BEs are made available can help implement MPA AUC monitoring in routine practice.

### CONCLUSION

There is sufficient evidence to recommend dose adjustments to achieve target MPA concentrations for several indications in solid organ transplantation. As single point measurement (trough level) is a relatively poor predictor of exposure, a LSS combining 3 concentration measurements within the dosing interval is the recommended method for TDM. For some other (off-label) indications and patient populations more research is needed to provide supportive data. Meanwhile, in some fields the extrapolation of recommendations from solid organ transplantation can be applied.

The PG of MPA has been characterized to a large extent, both with respect to genes encoding for proteins involved in the PK of MPA and for PD outcomes. However, at present, there is not sufficient evidence to recommend genotyping transplant recipients (or those on the waiting list) to include this information as a covariate in models for dose adjustment. Furthermore, a range of potential PD biomarkers has been investigated for their potential to correlate with the effect and/or toxicity of MPA. For a few of these biomarkers, for example, IMPDH activity and expression, promising results have been reported. In daily practice, none of these biomarkers has been widely accepted and implemented, partly because of the fact that some of the assays are complicated and labor intensive.

MPA is an established part of the most widely applied immunosuppressive regimens in organ transplantation. In view of the fact that there are very few new immunosuppressive drugs under development for the transplant field, it is likely that MPA will continue to be prescribed on a large scale in the upcoming years. Discontinuation of therapy due to adverse effects is relatively common and late rejections contribute to graft loss. Therefore, the continued search for innovative methods to better personalize MPA dosage is warranted.

### REFERENCES

1. Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2018 annual data report: kidney. *Am J Transpl*. 2020;20(suppl s1):20–130.
2. van Gelder T. Therapeutic drug monitoring for mycophenolic acid is value for (little) money. *Clin Pharmacol Ther*. 2011;90:203–204.
3. Muranushi H, Kanda J, Arai Y, et al. Drug monitoring for mycophenolic acid in graft-vs-host disease prophylaxis in cord blood transplantation. *Br J Clin Pharmacol*. 2020;86:2464–2472.
4. van Gelder T, Berden JH, Berger SP. To TDM or not to TDM in lupus nephritis patients treated with MMF? *Nephrol Dial Transpl*. 2015;30:560–564.
5. Lassailly G, Dumortier J, Saint-Marcoux F, et al. Real life experience of mycophenolate mofetil monotherapy in liver transplant patients. *Clin Res Hepatol Gastroenterol*. 2020;45:101451.
6. Metz DK, Holford N, Kausman JY, et al. Optimizing mycophenolic acid exposure in kidney transplant recipients: time for target concentration intervention. *Transplantation*. 2019;103:2012–2030.
7. de Winter BC, Mathot RA, Sombogaard F, et al. Nonlinear relationship between mycophenolate mofetil dose and mycophenolic acid exposure: implications for therapeutic drug monitoring. *Clin J Am Soc Nephrol*. 2011;6:656–663.
8. Saint-Marcoux F, Vandierdonck S, Premaud A, et al. Large scale analysis of routine dose adjustments of mycophenolate mofetil based on global exposure in renal transplant patients. *Ther Drug Monit*. 2011;33:285–294.
9. Tett SE, Saint-Marcoux F, Staatz CE, et al. Mycophenolate, clinical pharmacokinetics, formulations, and methods for assessing drug exposure. *Transpl Rev (Orlando)*. 2011;25:47–57.
10. Vanhove T, Kuypers D, Claes KJ, et al. Reasons for dose reduction of mycophenolate mofetil during the first year after renal transplantation and its impact on graft outcome. *Transpl Int*. 2013;26:813–821.
11. O'Leary JG, Samaniego M, Barrio MC, et al. The influence of immunosuppressive agents on the risk of de novo donor-specific HLA antibody production in solid organ transplant recipients. *Transplantation*. 2016;100:39–53.
12. Strommen AM, Moss MC, Goebel J, et al. Donor-specific antibodies in a pediatric kidney transplant population-Prevalence and association with antiproliferative drug dosing. *Pediatr Transpl*. 2019;23:e13511.
13. Filler G, Todorova EK, Bax K, et al. Minimum mycophenolic acid levels are associated with donor-specific antibody formation. *Pediatr Transpl*. 2016;20:34–38.
14. Brunet M, Shipkova M, van Gelder T, et al. Barcelona consensus on biomarker-based immunosuppressive drugs management in solid organ transplantation. *Ther Drug Monit*. 2016;38(suppl 1):S1–S20.
15. Picard N, Bergan S, Marquet P, et al. Pharmacogenetic biomarkers predictive of the pharmacokinetics and pharmacodynamics of immunosuppressive drugs. *Ther Drug Monit*. 2016;38(suppl 1):S57–S69.
16. Seger C, Shipkova M, Christians U, et al. Assuring the proper analytical performance of measurement procedures for immunosuppressive drug concentrations in clinical practice: recommendations of the international association of therapeutic drug monitoring and clinical toxicology immunosuppressive drug scientific committee. *Ther Drug Monit*. 2016;38:170–189.
17. Bentley R. Mycophenolic Acid: a one hundred year odyssey from antibiotic to immunosuppressant. *Chem Rev*. 2000;100:3801–3826.
18. Franklin TJ, Cook JM. The inhibition of nucleic acid synthesis by mycophenolic acid. *Biochem J*. 1969;113:515–524.
19. Allison AC, Eugui EM. Mechanisms of action of mycophenolate mofetil in preventing acute and chronic allograft rejection. *Transplantation*. 2005;80(2 suppl):S181–S190.
20. Lee WA, Gu L, Miksztal AR, et al. Bioavailability improvement of mycophenolic acid through amino ester derivatization. *Pharm Res*. 1990;7:161–166.
21. Morris RE, Hoyt EG, Murphy MP, et al. Mycophenolic acid morpholinothylester (RS-61443) is a new immunosuppressant that prevents and halts heart allograft rejection by selective inhibition of T- and B-cell purine synthesis. *Transpl Proc*. 1990;22:1659–1662.
22. Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology*. 2000;47:85–118.

23. Hackl A, Ehren R, Weber LT. Effect of mycophenolic acid in experimental, nontransplant glomerular diseases: new mechanisms beyond immune cells. *Pediatr Nephrol*. 2017;32:1315–1322.
24. Jurkiewicz A, Lesniewska E, Ciesla M, et al. Inhibition of tRNA gene transcription by the immunosuppressant mycophenolic acid. *Mol Cell Biol*. 2019;40:e00294.
25. Jonsson CA, Carlsten H. Mycophenolic acid inhibits inosine 5'-monophosphate dehydrogenase and suppresses immunoglobulin and cytokine production of B cells. *Int Immunopharmacol*. 2003;3:31–37.
26. He X, Smeets RL, Koenen HJ, et al. Mycophenolic acid-mediated suppression of human CD4+ T cells: more than mere guanine nucleotide deprivation. *Am J Transpl*. 2011;11:439–449.
27. Karnell JL, Karnell FG III, Stephens GL, et al. Mycophenolic acid differentially impacts B cell function depending on the stage of differentiation. *J Immunol*. 2011;187:3603–3612.
28. Gummert JF, Barten MJ, Sherwood SW, et al. Pharmacodynamics of immunosuppression by mycophenolic acid: inhibition of both lymphocyte proliferation and activation correlates with pharmacokinetics. *J Pharmacol Exp Ther*. 1999;291:1100–1112.
29. Gummert JF, Barten MJ, van Gelder T, et al. Pharmacodynamics of mycophenolic acid in heart allograft recipients: correlation of lymphocyte proliferation and activation with pharmacokinetics and graft histology. *Transplantation*. 2000;70:1038–1049.
30. Shipkova M, Wieland E, Schütz E, et al. The acyl glucuronide metabolite of mycophenolic acid inhibits the proliferation of human mononuclear leukocytes. *Transpl Proc*. 2001;33:1080–1081.
31. Patel SR. Bioanalytical challenges and strategies for accurately measuring acyl glucuronide metabolites in biological fluids. *Biomed Chromatogr*. 2020;34:e4640.
32. Treinen-Moslen M, Kanz MF. Intestinal tract injury by drugs: importance of metabolite delivery by yellow bile road. *Pharmacol Ther*. 2006;112:649–667.
33. Abd Rahman AN, Tett SE, Staatz CE. How accurate and precise are limited sampling strategies in estimating exposure to mycophenolic acid in people with autoimmune disease? *Clin Pharmacokinet*. 2014;53:227–245.
34. Bolanos-Meade J, Reshef R, Fraser R, et al. Three prophylaxis regimens (tacrolimus, mycophenolate mofetil, and cyclophosphamide; tacrolimus, methotrexate, and bortezomib; or tacrolimus, methotrexate, and maraviroc) versus tacrolimus and methotrexate for prevention of graft-versus-host disease with haemopoietic cell transplantation with reduced-intensity conditioning: a randomised phase 2 trial with a non-randomised contemporaneous control group (BMT CTN 1203). *Lancet Haematol*. 2019;6:e132–e143.
35. Broen JCA, van Laar JM. Mycophenolate mofetil, azathioprine and tacrolimus: mechanisms in rheumatology. *Nat Rev Rheumatol*. 2020;16:167–178.
36. Cottin V, Brown KK. Interstitial lung disease associated with systemic sclerosis (SSc-ILD). *Respir Res*. 2019;20:13.
37. Crane A, Eltemamy M, Shoskes D. Transplant immunosuppressive drugs in urology. *Transl Androl Urol*. 2019;8:109–117.
38. Gotterer L, Li Y. Maintenance immunosuppression in myasthenia gravis. *J Neurol Sci*. 2016;369:294–302.
39. Morren J, Li Y. Maintenance immunosuppression in myasthenia gravis, an update. *J Neurol Sci*. 2020;410:116648.
40. Mathian A, Miyara M, Cohen-Aubart F, et al. Relapsing polyorchiditis: a 2016 update on clinical features, diagnostic tools, treatment and biological drug use. *Best Pract Res Clin Rheumatol*. 2016;30:316–333.
41. Mieli-Vergani G, Vergani D, Czaja AJ, et al. Autoimmune hepatitis. *Nat Rev Dis Primers*. 2018;4:18017.
42. Bossen L, Gerussi A, Lygoura V, et al. Support of precision medicine through risk-stratification in autoimmune liver diseases—histology, scoring systems, and non-invasive markers. *Autoimmun Rev*. 2018;17:854–865.
43. Santiago P, Schwartz I, Tamariz L, et al. Systematic review with meta-analysis: mycophenolate mofetil as a second-line therapy for autoimmune hepatitis. *Aliment Pharmacol Ther*. 2019;49:830–839.
44. Pape S, Schramm C, Gevers TJ. Clinical management of autoimmune hepatitis. *United Eur Gastroenterol J*. 2019;7:1156–1163.
45. Couderc A, Berard E, Guignon V, et al. Treatments of steroid-dependent nephrotic syndrome in children [in French]. *Arch Pediatr*. 2017;24:1312–1320.
46. Le HL, Francke MI, Andrews LM, et al. Usage of tacrolimus and mycophenolic acid during conception, pregnancy, and lactation, and its implications for therapeutic drug monitoring: a systematic critical review. *Ther Drug Monit*. 2020;42:518–531.
47. Slapak M, Wigmore RA, Demers R, et al. Asanguineous perfusion preservation of canine liver and heart using a simple manuable portable apparatus. *Transpl Proc*. 1969;1:147–152.
48. King RW, Baca MJ, Armenti VT, et al. Pregnancy outcomes related to mycophenolate exposure in female kidney transplant recipients. *Am J Transpl*. 2017;17:151–160.
49. Chandra A, Midtvedt K, Åsberg A, et al. Immunosuppression and reproductive health after kidney transplantation. *Transplantation*. 2019;103:e325–e333.
50. Bitencourt N, Bermas BL. Pharmacological approach to managing childhood-onset systemic lupus erythematosus during conception, pregnancy and breastfeeding. *Paediatr Drugs*. 2018;20:511–521.
51. Colla L, Diena D, Rossetti M, et al. Immunosuppression in pregnant women with renal disease: review of the latest evidence in the biologics era. *J Nephrol*. 2018;31:361–383.
52. Jasiak NM, Park JM. Immunosuppression in solid-organ transplantation: essentials and practical tips. *Crit Care Nurs Q*. 2016;39:227–240.
53. Midtvedt K, Bergan S, Reisaeter AV, et al. Exposure to mycophenolate and fatherhood. *Transplantation*. 2017;101:e214–e217.
54. Kuypers DR, Van Mieghem T, Meijers B, et al. Updated manufacturer and European medicines agency recommendations on the use of mycophenolate acid: balancing the risks for male allograft recipients. *Transplantation*. 2016;100:e50–51.
55. Smith M, Gonzalez-Estrada A, Fernandez J, et al. Desensitization to Mycophenolate Mofetil: a novel 12 step protocol. *Eur Ann Allergy Clin Immunol*. 2016;48:147–148.
56. Shipkova M, Armstrong VW, Weber L, et al. Pharmacokinetics and protein adduct formation of the pharmacologically active acyl glucuronide metabolite of mycophenolic acid in pediatric renal transplant recipients. *Ther Drug Monit*. 2002;24:390–399.
57. Matsunaga N, Wada S, Nakanishi T, et al. Mathematical modeling of the in vitro hepatic disposition of mycophenolic acid and its glucuronide in sandwich-cultured human hepatocytes. *Mol Pharm*. 2014;11:568–579.
58. Berthier J, Benmameri M, Sauvage FL, et al. MRP4 is responsible for the efflux transport of mycophenolic acid beta-d glucuronide (MPAG) from hepatocytes to blood. *Xenobiotica*. 2021;51:105–114.
59. Bullingham RE, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet*. 1998;34:429–455.
60. Bhatt DK, Mehrotra A, Gaedigk A, et al. Age- and genotype-dependent variability in the protein abundance and activity of six major uridine diphosphate-glucuronosyltransferases in human liver. *Clin Pharmacol Ther*. 2019;105:131–141.
61. Badée J, Qiu N, Collier AC, et al. Characterization of the ontogeny of hepatic UDP-glucuronosyltransferase enzymes based on glucuronidation activity measured in human liver microsomes. *J Clin Pharmacol*. 2019;59(suppl 1):S42–s55.
62. Hesselink DA, van Hest RM, Mathot RA, et al. Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2. *Am J Transpl*. 2005;5:987–994.
63. Budde K, Glander P, Kramer BK, et al. Conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium in maintenance renal transplant recipients receiving tacrolimus: clinical, pharmacokinetic, and pharmacodynamic outcomes. *Transplantation*. 2007;83:417–424.
64. Shaw LM, Figurski M, Milone MC, et al. Therapeutic drug monitoring of mycophenolic acid. *Clin J Am Soc Nephrol*. 2007;2:1062–1072.
65. Woillard JB, Debord J, Marquet P. Comment on “population pharmacokinetics of mycophenolic acid: an update”. *Clin Pharmacokinet*. 2018;57:1211–1213.
66. Cattaneo D, Cortinovis M, Baldelli S, et al. Pharmacokinetics of mycophenolate sodium and comparison with the mofetil formulation in stable kidney transplant recipients. *Clin J Am Soc Nephrol*. 2007;2:1147–1155.
67. Meier-Kriesche HU, Shaw LM, Korecka M, et al. Pharmacokinetics of mycophenolic acid in renal insufficiency. *Ther Drug Monit*. 2000;22:27–30.

68. de Winter BC, van Gelder T, Sombogaard F, et al. Pharmacokinetic role of protein binding of mycophenolic acid and its glucuronide metabolite in renal transplant recipients. *J Pharmacokinet Pharmacodyn*. 2009;36:541–564.
69. Zanker B, Schleibner S, Schneeberger H, et al. Mycophenolate mofetil in patients with acute renal failure: evidence of metabolite (MPAG) accumulation and removal by dialysis. *Transpl Int*. 1996;9(suppl 1):S308–S310.
70. Johnson HJ, Swan SK, Heim-Duthoy KL, et al. The pharmacokinetics of a single oral dose of mycophenolate mofetil in patients with varying degrees of renal function. *Clin Pharmacol Ther*. 1998;63:512–518.
71. MacPhee IA, Spreafico S, Bewick M, et al. Pharmacokinetics of mycophenolate mofetil in patients with end-stage renal failure. *Kidney Int*. 2000;57:1164–1168.
72. Tang JT, de Winter BC, Hesselink DA, et al. The pharmacokinetics and pharmacodynamics of mycophenolate mofetil in younger and elderly renal transplant recipients. *Br J Clin Pharmacol*. 2017;83:812–822.
73. Arns W. Noninfectious gastrointestinal (GI) complications of mycophenolic acid therapy: a consequence of local GI toxicity? *Transpl Proc*. 2007;39:88–93.
74. de Jonge H, Naesens M, Kuypers DR. New insights into the pharmacokinetics and pharmacodynamics of the calcineurin inhibitors and mycophenolic acid: possible consequences for therapeutic drug monitoring in solid organ transplantation. *Ther Drug Monit*. 2009;31:416–435.
75. Cossart AR, Cottrell WN, Campbell SB, et al. Characterizing the pharmacokinetics and pharmacodynamics of immunosuppressant medicines and patient outcomes in elderly renal transplant patients. *Transl Androl Urol*. 2019;8(suppl 2):S198–s213.
76. Jacobson P, Green K, Rogosheske J, et al. Highly variable mycophenolate mofetil bioavailability following nonmyeloablative hematopoietic cell transplantation. *J Clin Pharmacol*. 2007;47:6–12.
77. Schmidt LE, Rasmussen A, Norrelykke MR, et al. The effect of selective bowel decontamination on the pharmacokinetics of mycophenolate mofetil in liver transplant recipients. *Liver Transpl*. 2001;7:739–742.
78. Naesens M, Verbeke K, Vanrenterghem Y, et al. Effects of gastric emptying on oral mycophenolic acid pharmacokinetics in stable renal allograft recipients. *Br J Clin Pharmacol*. 2007;63:541–547.
79. Patel CG, Richman K, Yang D, et al. Effect of diabetes mellitus on mycophenolate sodium pharmacokinetics and inosine monophosphate dehydrogenase activity in stable kidney transplant recipients. *Ther Drug Monit*. 2007;29:735–742.
80. Akhlaghi F, Patel CG, Zuniga XP, et al. Pharmacokinetics of mycophenolic acid and metabolites in diabetic kidney transplant recipients. *Ther Drug Monit*. 2006;28:95–101.
81. Kaplan B, Gaston RS, Meier-Kriesche HU, et al. Mycophenolic acid exposure in high- and low-weight renal transplant patients after dosing with mycophenolate mofetil in the Opticcept trial. *Ther Drug Monit*. 2010;32:224–227.
82. EMA. European Medicines Agency: CellCept product information. Available at: [https://www.ema.europa.eu/en/documents/product-information/cellcept-epar-product-information\\_en.pdf](https://www.ema.europa.eu/en/documents/product-information/cellcept-epar-product-information_en.pdf). Published 2015. Accessed November 3, 2020.
83. van Gelder T, Silva HT, de Fijter H, et al. How delayed graft function impacts exposure to mycophenolic acid in patients after renal transplantation. *Ther Drug Monit*. 2011;33:155–164.
84. Jain A, Venkataramanan R, Kwong T, et al. Pharmacokinetics of mycophenolic acid in liver transplant patients after intravenous and oral administration of mycophenolate mofetil. *Liver Transpl*. 2007;13:791–796.
85. Brunet M, Cirera I, Martorell J, et al. Sequential determination of pharmacokinetics and pharmacodynamics of mycophenolic acid in liver transplant patients treated with mycophenolate mofetil. *Transplantation*. 2006;81:541–546.
86. van Gelder T, Silva HT, de Fijter JW, et al. Comparing mycophenolate mofetil regimens for de novo renal transplant recipients: the fixed-dose concentration-controlled trial. *Transplantation*. 2008;86:1043–1051.
87. Tönshoff B, David-Neto E, Ettenger R, et al. Pediatric aspects of therapeutic drug monitoring of mycophenolic acid in renal transplantation. *Transpl Rev (Orlando)*. 2011;25:78–89.
88. Benjanuwattra J, Pruksakorn D, Koonrunsesomboon N. Mycophenolic acid and its pharmacokinetic drug-drug interactions in humans: review of the evidence and clinical implications. *J Clin Pharmacol*. 2020;60:295–311.
89. Miura M, Satoh S, Inoue K, et al. Influence of lansoprazole and rabeprazole on mycophenolic acid pharmacokinetics one year after renal transplantation. *Ther Drug Monit*. 2008;30:46–51.
90. Kiberd BA, Wrobel M, Dandavino R, et al. The role of proton pump inhibitors on early mycophenolic acid exposure in kidney transplantation: evidence from the CLEAR study. *Ther Drug Monit*. 2011;33:120–123.
91. Rupprecht K, Schmidt C, Raspe A, et al. Bioavailability of mycophenolate mofetil and enteric-coated mycophenolate sodium is differentially affected by pantoprazole in healthy volunteers. *J Clin Pharmacol*. 2009;49:1196–1201.
92. Kees MG, Steinke T, Moritz S, et al. Omeprazole impairs the absorption of mycophenolate mofetil but not of enteric-coated mycophenolate sodium in healthy volunteers. *J Clin Pharmacol*. 2012;52:1265–1272.
93. Kofler S, Shvets N, Bigdeli AK, et al. Proton pump inhibitors reduce mycophenolate exposure in heart transplant recipients—a prospective case-controlled study. *Am J Transpl*. 2009;9:1650–1656.
94. Kofler S, Deutsch MA, Bigdeli AK, et al. Proton pump inhibitor co-medication reduces mycophenolate acid drug exposure in heart transplant recipients. *J Heart Lung Transpl*. 2009;28:605–611.
95. Kofler S, Wolf C, Shvets N, et al. The proton pump inhibitor pantoprazole and its interaction with enteric-coated mycophenolate sodium in transplant recipients. *J Heart Lung Transpl*. 2011;30:565–571.
96. Knorr JP, Sjeime M, Braitman LE, et al. Concomitant proton pump inhibitors with mycophenolate mofetil and the risk of rejection in kidney transplant recipients. *Transplantation*. 2014;97:518–524.
97. Rissling O, Glander P, Hambach P, et al. No relevant pharmacokinetic interaction between pantoprazole and mycophenolate in renal transplant patients: a randomized crossover study. *Br J Clin Pharmacol*. 2015;80:1086–1096.
98. Pauli-Magnus C, Rekersbrink S, Klotz U, et al. Interaction of omeprazole, lansoprazole and pantoprazole with P-glycoprotein. *Naunyn Schmiedebergs Arch Pharmacol*. 2001;364:551–557.
99. Blume H, Donath F, Warnke A, et al. Pharmacokinetic drug interaction profiles of proton pump inhibitors. *Drug Saf*. 2006;29:769–784.
100. Bullingham R, Shah J, Goldblum R, et al. Effects of food and antacid on the pharmacokinetics of single doses of mycophenolate mofetil in rheumatoid arthritis patients. *Br J Clin Pharmacol*. 1996;41:513–516.
101. Pieper AK, Buhle F, Bauer S, et al. The effect of sevelamer on the pharmacokinetics of cyclosporin A and mycophenolate mofetil after renal transplantation. *Nephrol Dial Transpl*. 2004;19:2630–2633.
102. Kato R, Ooi K, Ikura-Mori M, et al. Impairment of mycophenolate mofetil absorption by calcium polycarbophil. *J Clin Pharmacol*. 2002;42:1275–1280.
103. Vinke JSJ, Francke MI, Eisenga MF, et al. Iron deficiency after kidney transplantation. *Nephrol Dial Transpl*. 2020; gfaa123. doi: 10.1093/ndt/gfaa123. [epub ahead of print].
104. Morii M, Ueno K, Ogawa A, et al. Impairment of mycophenolate mofetil absorption by iron ion. *Clin Pharmacol Ther*. 2000;68:613–616.
105. Zucker K, Rosen A, Tsaroucha A, et al. Unexpected augmentation of mycophenolic acid pharmacokinetics in renal transplant patients receiving tacrolimus and mycophenolate mofetil in combination therapy, and analogous in vitro findings. *Transpl Immunol*. 1997;5:225–232.
106. Smak Gregoor PJ, van Gelder T, Hesse CJ, et al. Mycophenolic acid plasma concentrations in kidney allograft recipients with or without cyclosporin: a cross-sectional study. *Nephrol Dial Transpl*. 1999;14:706–708.
107. van Gelder T, Klupp J, Barten MJ, et al. Comparison of the effects of tacrolimus and cyclosporine on the pharmacokinetics of mycophenolic acid. *Ther Drug Monit*. 2001;23:119–128.
108. Shipkova M, Armstrong VW, Kuypers D, et al. Effect of cyclosporine withdrawal on mycophenolic acid pharmacokinetics in kidney transplant recipients with deteriorating renal function: preliminary report. *Ther Drug Monit*. 2001;23:717–721.
109. Trkulja V, Lalic Z, Nad-Skegro S, et al. Effect of cyclosporine on steady-state pharmacokinetics of MPA in renal transplant recipients is not affected by the MPA formulation: analysis based on therapeutic drug monitoring data. *Ther Drug Monit*. 2014;36:456–464.



110. Filler G, Lepage N, Delisle B, et al. Effect of cyclosporine on mycophenolic acid area under the concentration-time curve in pediatric kidney transplant recipients. *Ther Drug Monit.* 2001;23:514–519.
111. Kim JH, Han N, Kim MG, et al. Increased exposure of tacrolimus by Co-administered mycophenolate mofetil: population pharmacokinetic analysis in healthy volunteers. *Sci Rep.* 2018;8:1687.
112. Picard N, Premaud A, Rousseau A, et al. A comparison of the effect of ciclosporin and sirolimus on the pharmacokinetics of mycophenolate in renal transplant patients. *Br J Clin Pharmacol.* 2006;62:477–484.
113. Buchler M, Lebranchu Y, Beneton M, et al. Higher exposure to mycophenolic acid with sirolimus than with cyclosporine cotreatment. *Clin Pharmacol Ther.* 2005;78:34–42.
114. Cattaneo D, Perico N, Gaspari F, et al. Glucocorticoids interfere with mycophenolate mofetil bioavailability in kidney transplantation. *Kidney Int.* 2002;62:1060–1067.
115. Vietri M, Pietrabissa A, Mosca F, et al. Mycophenolic acid glucuronidation and its inhibition by non-steroidal anti-inflammatory drugs in human liver and kidney. *Eur J Clin Pharmacol.* 2000;56:659–664.
116. Fukuda T, Brunner HI, Sagcal-Gironella AC, et al. Nonsteroidal anti-inflammatory drugs may reduce enterohepatic recirculation of mycophenolic acid in patients with childhood-onset systemic lupus erythematosus. *Ther Drug Monit.* 2011;33:658–662.
117. Naesens M, Kuypers DR, Streit F, et al. Rifampin induces alterations in mycophenolic acid glucuronidation and elimination: implications for drug exposure in renal allograft recipients. *Clin Pharmacol Ther.* 2006;80:509–521.
118. Kuypers DR, Verleden G, Naesens M, et al. Drug interaction between mycophenolate mofetil and rifampin: possible induction of uridine diphosphate-glucuronosyltransferase. *Clin Pharmacol Ther.* 2005;78:81–88.
119. Groll AH, Desai A, Han D, et al. Pharmacokinetic assessment of drug-drug interactions of isavuconazole with the immunosuppressants cyclosporine, mycophenolic acid, prednisolone, sirolimus, and tacrolimus in healthy adults. *Clin Pharmacol Drug Dev.* 2017;6:76–85.
120. El-Sheikh AA, Koenderink JB, Wouterse AC, et al. Renal glucuronidation and multidrug resistance protein 2-/multidrug resistance protein 4-mediated efflux of mycophenolic acid: interaction with cyclosporine and tacrolimus. *Transl Res.* 2014;164:46–56.
121. Kuypers DR, Ekberg H, Grinyo J, et al. Mycophenolic acid exposure after administration of mycophenolate mofetil in the presence and absence of cyclosporin in renal transplant recipients. *Clin Pharmacokinet.* 2009;48:329–341.
122. *Mycophenolate Mofetil: IBM Micromedex® (Electronic Version).* Greenwood Village, CO: IBM Watson Health. Available at: <https://www.micromedexsolutions.com/>. Accessed October 16, 2020.
123. El Hajj S, Kim M, Phillips K, et al. Generic immunosuppression in transplantation: current evidence and controversial issues. *Expert Rev Clin Immunol.* 2015;11:659–672.
124. Harada N, Yoshizumi T, Yoshiya S, et al. Use of mycophenolate mofetil suspension as part of induction therapy after living-donor liver transplant. *Exp Clin Transpl.* 2020;18:485–490.
125. Kaplan B. Enteric-coated mycophenolate sodium (myfortic(R)): an overview of current and future use in transplantation. *Drugs.* 2006;66:1–8.
126. Salvadori M, Holzer H, de Mattos A, et al. Enteric-coated mycophenolate sodium is therapeutically equivalent to mycophenolate mofetil in de novo renal transplant patients. *Am J Transpl.* 2004;4:231–236.
127. Johnston A, He X, Holt DW. Bioequivalence of enteric-coated mycophenolate sodium and mycophenolate mofetil: a meta-analysis of three studies in stable renal transplant recipients. *Transplantation.* 2006;82:1413–1418.
128. Christians U, Klawitter J, Clavijo CF. Bioequivalence testing of immunosuppressants: concepts and misconceptions. *Kidney Int Suppl.* 2010;S1–S7.
129. Department Of Health And Human Services And Food And Drug Administration. Guidance for industry. Bioavailability and bioequivalence studies for orally administered drug products—general considerations. 2003. Available at: <https://www.govinfo.gov/content/pkg/FR-2003-03-19/pdf/03-6491.pdf>. Accessed October 14, 2020.
130. Health Canada. *Guidance Document—Comparative Bioavailability Standards: Formulations Used for Systemic Effects.* 2018. Available at: <https://www.canada.ca/en/health-canada/services/drugs-health-products/drug-products/applications-submissions/guidance-documents/bioavailability-bioequivalence/comparative-bioavailability-standards-formulations-used-systemic-effects.html>. Accessed October 14, 2020.
131. The European Medicines Agency. *Guideline on the Investigation of Bioequivalence.* 2010. Available at: <https://www.ema.europa.eu/en/investigation-bioequivalence>. Accessed October 14, 2020.
132. van Gelder T. What is the future of generics in transplantation? *Transplantation.* 2015;99:2269–2273.
133. van Gelder T; Substitution EACoG. European Society for Organ Transplantation Advisory Committee recommendations on generic substitution of immunosuppressive drugs. *Transpl Int.* 2011;24:1135–1141.
134. van Gelder T, Gabardi S. Methods, strengths, weaknesses, and limitations of bioequivalence tests with special regard to immunosuppressive drugs. *Transpl Int.* 2013;26:771–777.
135. Helderma JH. Generic substitution for immunosuppressive drugs. *Dial Transplant.* 2011;40:37–40.
136. Yu Y, Teerenstra S, Neef C, et al. Investigation into the interchangeability of generic formulations using immunosuppressants and a broad selection of medicines. *Eur J Clin Pharmacol.* 2015;71:979–990.
137. Reigner B, Grange S, Bentley D, et al. Generics in transplantation medicine: randomized comparison of innovator and substitution products containing mycophenolate mofetil. *Int J Clin Pharmacol Ther.* 2019;57:506–519.
138. Handoo S, Arora V, Khera D, et al. A comprehensive study on regulatory requirements for development and filing of generic drugs globally. *Int J Pharm Investig.* 2012;2:99–105.
139. Budde K, Tedesco-Silva H, Pestana JM, et al. Enteric-coated mycophenolate sodium provides higher mycophenolic acid predose levels compared with mycophenolate mofetil: implications for therapeutic drug monitoring. *Ther Drug Monit.* 2007;29:381–384.
140. van Gelder T, Hilbrands LB, Vanrenterghem Y, et al. A randomized double-blind, multicenter plasma concentration controlled study of the safety and efficacy of oral mycophenolate mofetil for the prevention of acute rejection after kidney transplantation. *Transplantation.* 1999;68:261–266.
141. Knight SR, Morris PJ. Does the evidence support the use of mycophenolate mofetil therapeutic drug monitoring in clinical practice? A systematic review. *Transplantation.* 2008;85:1675–1685.
142. Sommerer C, Muller-Krebs S, Schaefer M, et al. Pharmacokinetic and pharmacodynamic analysis of enteric-coated mycophenolate sodium: limited sampling strategies and clinical outcome in renal transplant patients. *Br J Clin Pharmacol.* 2010;69:346–357.
143. Hougardy JM, Maufort L, Cotton F, et al. Therapeutic drug monitoring of enteric-coated mycophenolate sodium by limited sampling strategies is associated with a high rate of failure. *Clin Kidney J.* 2016;9:319–323.
144. Le Meur Y, Buchler M, Thierry A, et al. Individualized mycophenolate mofetil dosing based on drug exposure significantly improves patient outcomes after renal transplantation. *Am J Transpl.* 2007;7:2496–2503.
145. Marquet P, Saint-Marcoux F, Premaud A, et al. Performance of the new mycophenolate assay based on IMPDH enzymatic activity for pharmacokinetic investigations and setup of Bayesian estimators in different populations of allograft recipients. *Ther Drug Monit.* 2009;31:443–450.
146. Pawinski T, Luszczynska P, Durlik M, et al. Development and validation of limited sampling strategies for the estimation of mycophenolic acid area under the curve in adult kidney and liver transplant recipients receiving concomitant enteric-coated mycophenolate sodium and tacrolimus. *Ther Drug Monit.* 2013;35:760–769.
147. Gaston RS, Kaplan B, Shah T, et al. Fixed- or controlled-dose mycophenolate mofetil with standard- or reduced-dose calcineurin inhibitors: the Optcept trial. *Am J Transpl.* 2009;9:1607–1619.
148. de Winter BC, van Gelder T, Mathot RA, et al. Limited sampling strategies drawn within 3 hours postdose poorly predict mycophenolic acid area-under-the-curve after enteric-coated mycophenolate sodium. *Ther Drug Monit.* 2009;31:585–591.
149. Fleming DH, Mathew BS, Prasanna S, et al. A possible simplification for the estimation of area under the curve (AUC(0)-(1)(2)) of enteric-coated mycophenolate sodium in renal transplant patients receiving tacrolimus. *Ther Drug Monit.* 2011;33:165–170.
150. Jia Y, Peng B, Li L, et al. Estimation of mycophenolic acid area under the curve with limited-sampling strategy in Chinese renal transplant recipients receiving enteric-coated mycophenolate sodium. *Ther Drug Monit.* 2017;39:29–36.

151. Yao X, Huang H, Wei C, et al. Limited sampling strategy for mycophenolic acid in Chinese kidney transplant recipients receiving enteric-coated mycophenolate sodium and tacrolimus during the early post-transplantation phase. *Ther Drug Monit.* 2015;37:516–523.
152. Xu LY, Jiao Z, Liu FY, et al. Pharmacokinetics evaluation of mycophenolic acid and its glucuronide metabolite in Chinese renal transplant recipients receiving enteric-coated mycophenolate sodium and tacrolimus. *Ther Drug Monit.* 2018;40:572–580.
153. Staatz CE, Tett SE. Maximum a posteriori Bayesian estimation of mycophenolic Acid area under the concentration-time curve: is this clinically useful for dosage prediction yet? *Clin Pharmacokinet.* 2011;50:759–772.
154. Dong M, Fukuda T, Vinks AA. Optimization of mycophenolic acid therapy using clinical pharmacometrics. *Drug Metab Pharmacokinet.* 2014;29:4–11.
155. Le Guellec C, Bourgoin H, Buchler M, et al. Population pharmacokinetics and Bayesian estimation of mycophenolic acid concentrations in stable renal transplant patients. *Clin Pharmacokinet.* 2004;43:253–266.
156. Premaud A, Le Meur Y, Debord J, et al. Maximum a posteriori bayesian estimation of mycophenolic acid pharmacokinetics in renal transplant recipients at different postgrafting periods. *Ther Drug Monit.* 2005;27:354–361.
157. Saint-Marcoux F, Guignon V, Decramer S, et al. Development of a Bayesian estimator for the therapeutic drug monitoring of mycophenolate mofetil in children with idiopathic nephrotic syndrome. *Pharmacol Res.* 2011;63:423–431.
158. Woillard JB, Bader-Meunier B, Salomon R, et al. Pharmacokinetics of mycophenolate mofetil in children with lupus and clinical findings in favour of therapeutic drug monitoring. *Br J Clin Pharmacol.* 2014;78:867–876.
159. Labriffe M, Vaidie J, Monchaud C, et al. Population pharmacokinetics and Bayesian estimators for intravenous mycophenolate mofetil in haematopoietic stem cell transplant patients. *Br J Clin Pharmacol.* 2020;86:1550–1559.
160. Musumba FT, Rousseau A, Bosmans JL, et al. Limited sampling models and Bayesian estimation for mycophenolic acid area under the curve prediction in stable renal transplant patients co-medicated with ciclosporin or sirolimus. *Clin Pharmacokinet.* 2009;48:745–758.
161. Zhao W, Elie V, Baudouin V, et al. Population pharmacokinetics and Bayesian estimator of mycophenolic acid in children with idiopathic nephrotic syndrome. *Br J Clin Pharmacol.* 2010;69:358–366.
162. de Winter BC, Monchaud C, Premaud A, et al. Bayesian estimation of mycophenolate mofetil in lung transplantation, using a population pharmacokinetic model developed in kidney and lung transplant recipients. *Clin Pharmacokinet.* 2012;51:29–39.
163. Barau C, Furlan V, Debray D, et al. Population pharmacokinetics of mycophenolic acid and dose optimization with limited sampling strategy in liver transplant children. *Br J Clin Pharmacol.* 2012;74:515–524.
164. Payen S, Zhang D, Maisin A, et al. Population pharmacokinetics of mycophenolic acid in kidney transplant pediatric and adolescent patients. *Ther Drug Monit.* 2005;27:378–388.
165. Hulin A, Blanchet B, Audard V, et al. Comparison of 3 estimation methods of mycophenolic acid AUC based on a limited sampling strategy in renal transplant patients. *Ther Drug Monit.* 2009;31:224–232.
166. de Winter BC, Neumann I, van Hest RM, et al. Limited sampling strategies for therapeutic drug monitoring of mycophenolate mofetil therapy in patients with autoimmune disease. *Ther Drug Monit.* 2009;31:382–390.
167. Li H, Mager DE, Sandmaier BM, et al. Population pharmacokinetics and dose optimization of mycophenolic acid in HCT recipients receiving oral mycophenolate mofetil. *J Clin Pharmacol.* 2013;53:393–402.
168. Langers P, Press RR, Inderson A, et al. Limited sampling model for advanced mycophenolic acid therapeutic drug monitoring after liver transplantation. *Ther Drug Monit.* 2014;36:141–147.
169. Chen B, Shao K, An HM, et al. Population pharmacokinetics and bayesian estimation of mycophenolic acid exposure in Chinese renal allograft recipients after administration of EC-MPS. *J Clin Pharmacol.* 2019;59:578–589.
170. Barraclough KA, Isbel NM, Staatz CE. Evaluation of the mycophenolic acid exposure estimation methods used in the APOMYGERE, FDCC, and Opticcept trials. *Transplantation.* 2010;90:44–51.
171. Sam WJ, Joy MS. Population pharmacokinetics of mycophenolic acid and metabolites in patients with glomerulonephritis. *Ther Drug Monit.* 2010;32:594–605.
172. Zahr N, Amoura Z, Debord J, et al. Pharmacokinetic study of mycophenolate mofetil in patients with systemic lupus erythematosus and design of Bayesian estimator using limited sampling strategies. *Clin Pharmacokinet.* 2008;47:277–284.
173. Saint-Marcoux F, Royer B, Debord J, et al. Pharmacokinetic modelling and development of Bayesian estimators for therapeutic drug monitoring of mycophenolate mofetil in reduced-intensity haematopoietic stem cell transplantation. *Clin Pharmacokinet.* 2009;48:667–675.
174. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. *J Pharmacokinet Biopharm.* 1981;9:503–512.
175. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1:307–310.
176. Daher Abdi Z, Essig M, Rizopoulos D, et al. Impact of longitudinal exposure to mycophenolic acid on acute rejection in renal-transplant recipients using a joint modeling approach. *Pharmacol Res.* 2013;72:52–60.
177. Daher Abdi Z, Premaud A, Essig M, et al. Exposure to mycophenolic acid better predicts immunosuppressive efficacy than exposure to calcineurin inhibitors in renal transplant patients. *Clin Pharmacol Ther.* 2014;96:508–515.
178. Le Meur Y, Thierry A, Glowacki F, et al. Early steroid withdrawal and optimization of mycophenolic acid exposure in kidney transplant recipients receiving mycophenolate mofetil. *Transplantation.* 2011;92:1244–1251.
179. van Gelder T, Tedesco Silva H, de Fijter JW, et al. Renal transplant patients at high risk of acute rejection benefit from adequate exposure to mycophenolic acid. *Transplantation.* 2010;89:595–599.
180. Wang X, Qin X, Wang Y, et al. Controlled-dose versus fixed-dose mycophenolate mofetil for kidney transplant recipients: a systematic review and meta-analysis of randomized controlled trials. *Transplantation.* 2013;96:361–367.
181. Premaud A, Rousseau A, Le Meur Y, et al. Feasibility of, and critical paths for mycophenolate mofetil Bayesian dose adjustment: pharmacological re-appraisal of a concentration-controlled versus fixed-dose trial in renal transplant recipients. *Pharmacol Res.* 2010;61:167–174.
182. Rousseau A, Laroche ML, Venisse N, et al. Cost-effectiveness analysis of individualized mycophenolate mofetil dosing in kidney transplant patients in the APOMYGERE trial. *Transplantation.* 2010;89:1255–1262.
183. Weber LT, Shipkova M, Armstrong VW, et al. The pharmacokinetic-pharmacodynamic relationship for total and free mycophenolic Acid in pediatric renal transplant recipients: a report of the German study group on mycophenolate mofetil therapy. *J Am Soc Nephrol.* 2002;13:759–768.
184. Hocker B, van Gelder T, Martin-Govantes J, et al. Comparison of MMF efficacy and safety in paediatric vs. adult renal transplantation: subgroup analysis of the randomised, multicentre FDCC trial. *Nephrol Dial Transpl.* 2011;26:1073–1079.
185. Rother A, Glander P, Vitt E, et al. Inosine monophosphate dehydrogenase activity in paediatrics: age-related regulation and response to mycophenolic acid. *Eur J Clin Pharmacol.* 2012;68:913–922.
186. Goralczyk AD, Bari N, Abu-Ajjaj W, et al. Calcineurin inhibitor sparing with mycophenolate mofetil in liver transplantation: a systematic review of randomized controlled trials. *Am J Transpl.* 2012;12:2601–2607.
187. Ringe B, Braun F, Schutz E, et al. A novel management strategy of steroid-free immunosuppression after liver transplantation: efficacy and safety of tacrolimus and mycophenolate mofetil. *Transplantation.* 2001;71:508–515.
188. Boudjema K, Camus C, Saliba F, et al. Reduced-dose tacrolimus with mycophenolate mofetil vs. standard-dose tacrolimus in liver transplantation: a randomized study. *Am J Transpl.* 2011;11:965–976.
189. Junge G, Neuhaus R, Schewior L, et al. Withdrawal of steroids: a randomized prospective study of prednisone and tacrolimus versus mycophenolate mofetil and tacrolimus in liver transplant recipients with autoimmune hepatitis. *Transpl Proc.* 2005;37:1695–1696.
190. Benichou AS, Blanchet B, Conti F, et al. Variability in free mycophenolic acid exposure in adult liver transplant recipients during the early posttransplantation period. *J Clin Pharmacol.* 2010;50:1202–1210.

191. Chen H, Chen B. Clinical mycophenolic acid monitoring in liver transplant recipients. *World J Gastroenterol*. 2014;20:10715–10728.
192. Hwang S, Lee SG, Ahn CS, et al. A clinical assessment of mycophenolate drug monitoring after liver transplantation. *Clin Transpl*. 2010;24:E35–E42.
193. Kamar N, Marquet P, Gandia P, et al. Mycophenolic acid 12-hour area under the curve in de novo liver transplant patients given mycophenolate mofetil at fixed versus concentration-controlled doses. *Ther Drug Monit*. 2009;31:451–456.
194. Reine PA, Vethe NT, Kongsgaard UE, et al. Mycophenolate pharmacokinetics and inosine monophosphate dehydrogenase activity in liver transplant recipients with an emphasis on therapeutic drug monitoring. *Scand J Clin Lab Invest*. 2013;73:117–124.
195. Minmin S, Zhidong G, Hao C, et al. Correlation between pharmacokinetics and pharmacodynamics of mycophenolic acid in liver transplant patients. *J Clin Pharmacol*. 2010;50:1388–1396.
196. Tredger JM, Brown NW, Adams J, et al. Monitoring mycophenolate in liver transplant recipients: toward a therapeutic range. *Liver Transpl*. 2004;10:492–502.
197. Sarvary E, Nemes B, Varga M, et al. Significance of mycophenolate monitoring in liver transplant recipients: toward the cut-off level. *Transpl Proc*. 2012;44:2157–2161.
198. Saliba F, Rostaing L, Gugenheim J, et al. Corticosteroid-sparing and optimization of mycophenolic acid exposure in liver transplant recipients receiving mycophenolate mofetil and tacrolimus: a randomized, multicenter study. *Transplantation*. 2016;100:1705–1713.
199. Kim H, Yi NJ, Lee J, et al. Safety of reduced dose of mycophenolate mofetil combined with tacrolimus in living-donor liver transplantation. *Clin Mol Hepatol*. 2014;20:291–299.
200. Hao C, Anwei M, Bing C, et al. Monitoring mycophenolic acid pharmacokinetic parameters in liver transplant recipients: prediction of occurrence of leukopenia. *Liver Transpl*. 2008;14:1165–1173.
201. Xia ZW, Jun CY, Hao C, et al. The occurrence of diarrhea not related to the pharmacokinetics of MPA and its metabolites in liver transplant patients. *Eur J Clin Pharmacol*. 2010;66:671–679.
202. Barau C, Barrail-Tran A, Hemerzi B, et al. Optimization of the dosing regimen of mycophenolate mofetil in pediatric liver transplant recipients. *Liver Transpl*. 2011;17:1152–1158.
203. Chambers DC, Cherikh WS, Harhay MO, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: thirty-sixth adult lung and heart-lung transplantation report-2019; focus theme: donor and recipient size match. *J Heart Lung Transpl*. 2019;38:1042–1055.
204. Khush KK, Cherikh WS, Chambers DC, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-sixth adult heart transplantation report—2019; focus theme: donor and recipient size match. *J Heart Lung Transpl*. 2019;38:1056–1066.
205. Kuypers DR, Le Meur Y, Cantarovich M, et al. Consensus report on therapeutic drug monitoring of mycophenolic acid in solid organ transplantation. *Clin J Am Soc Nephrol*. 2010;5:341–358.
206. Kobashigawa JA, Renlund DG, Gerosa G, et al. Similar efficacy and safety of enteric-coated mycophenolate sodium (EC-MPS, myfortic) compared with mycophenolate mofetil (MMF) in de novo heart transplant recipients: results of a 12-month, single-blind, randomized, parallel-group, multicenter study. *J Heart Lung Transpl*. 2006;25:935–941.
207. Hummel M, Yonan N, Ross H, et al. Pharmacokinetics and variability of mycophenolic acid from enteric-coated mycophenolate sodium compared with mycophenolate mofetil in de novo heart transplant recipients. *Clin Transpl*. 2007;21:18–23.
208. Ting LS, Partovi N, Levy RD, et al. Pharmacokinetics of mycophenolic acid and its phenolic-glucuronide and ACYI glucuronide metabolites in stable thoracic transplant recipients. *Ther Drug Monit*. 2008;30:282–291.
209. Gerbase MW, Fathi M, Spiliopoulos A, et al. Pharmacokinetics of mycophenolic acid associated with calcineurin inhibitors: long-term monitoring in stable lung recipients with and without cystic fibrosis. *J Heart Lung Transpl*. 2003;22:587–590.
210. Stuckey L, Clark Ojo T, Park JM, et al. Mycophenolic acid pharmacokinetics in lung transplant recipients with cystic fibrosis. *Ther Drug Monit*. 2014;36:148–151.
211. Kawauchi S, Wada K, Oita A. Changes in blood concentration of mycophenolic acid and FK506 in a heart-transplant patient treated with plasmapheresis. *Int J Clin Pharmacol Ther*. 2019;57:32–36.
212. Armstrong VW, Tenderich G, Shipkova M, et al. Pharmacokinetics and bioavailability of mycophenolic acid after intravenous administration and oral administration of mycophenolate mofetil to heart transplant recipients. *Ther Drug Monit*. 2005;27:315–321.
213. Figurski MJ, Pawinski T, Goldberg LR, et al. Pharmacokinetic monitoring of mycophenolic acid in heart transplant patients: correlation the side-effects and rejections with pharmacokinetic parameters. *Ann Transpl*. 2012;17:68–78.
214. Woillard JB, Saint-Marcoux F, Monchaud C, et al. Mycophenolic mofetil optimized pharmacokinetic modelling, and exposure-effect associations in adult heart transplant recipients. *Pharmacol Res*. 2015;99:308–315.
215. Zuk DM, Pearson GJ. Monitoring of mycophenolate mofetil in orthotopic heart transplant recipients—a systematic review. *Transpl Rev (Orlando)*. 2009;23:171–177.
216. Monchaud C, Marquet P. Pharmacokinetic optimization of immunosuppressive therapy in thoracic transplantation: part II. *Clin Pharmacokinet*. 2009;48:489–516.
217. Yamani MH, Starling RC, Goormastic M, et al. The impact of routine mycophenolate mofetil drug monitoring on the treatment of cardiac allograft rejection. *Transplantation*. 2000;69:2326–2330.
218. Meiser BM, Pfeiffer M, Schmidt D, et al. Combination therapy with tacrolimus and mycophenolate mofetil following cardiac transplantation: importance of mycophenolic acid therapeutic drug monitoring. *J Heart Lung Transpl*. 1999;18:143–149.
219. DeNofrio D, Loh E, Kao A, et al. Mycophenolic acid concentrations are associated with cardiac allograft rejection. *J Heart Lung Transpl*. 2000;19:1071–1076.
220. Cantin B, Giannetti N, Parekh H, et al. Mycophenolic acid concentrations in long-term heart transplant patients: relationship with calcineurin antagonists and acute rejection. *Clin Transpl*. 2002;16:196–201.
221. Groetzner J, Kaczmarek I, Schirmer J, et al. Calcineurin inhibitor withdrawal and conversion to mycophenolate mofetil and steroids in cardiac transplant recipients with chronic renal failure: a word of caution. *Clin Transpl*. 2008;22:587–593.
222. Staats CE, Tett SE. Pharmacology and toxicology of mycophenolate in organ transplant recipients: an update. *Arch Toxicol*. 2014;88:1351–1389.
223. Roan JN, Chou CH, Hsu CH, et al. Dose-normalization for exposure to mycophenolic acid and the early clinical outcome in patients taking tacrolimus after heart transplantation. *Ann Transpl*. 2013;18:43–52.
224. Shaw LM, Korecka M, DeNofrio D, et al. Pharmacokinetic, pharmacodynamic, and outcome investigations as the basis for mycophenolic acid therapeutic drug monitoring in renal and heart transplant patients. *Clin Biochem*. 2001;34:17–22.
225. Gajarski RJ, Crowley DC, Zamberlan MC, et al. Lack of correlation between MMF dose and MPA level in pediatric and young adult cardiac transplant patients: does the MPA level matter? *Am J Transpl*. 2004;4:1495–1500.
226. Siddiqi N, Lamour JM, Hsu DT. The effect of MMF dose and trough levels on adverse effects in pediatric heart transplant recipients. *Pediatr Transpl*. 2015;19:618–622.
227. Dipchand AI, Pietra B, McCrindle BW, et al. Mycophenolic acid levels in pediatric heart transplant recipients receiving mycophenolate mofetil. *J Heart Lung Transpl*. 2001;20:1035–1043.
228. van Hest RM, Doorduyn JK, de Winter BC, et al. Pharmacokinetics of mycophenolate mofetil in hematopoietic stem cell transplant recipients. *Ther Drug Monit*. 2007;29:353–360.
229. Wakahashi K, Yamamori M, Minagawa K, et al. Pharmacokinetics-based optimal dose prediction of donor source-dependent response to mycophenolate mofetil in unrelated hematopoietic cell transplantation. *Int J Hematol*. 2011;94:193–202.
230. Li H, Mager DE, Bemmer MJ, et al. A limited sampling schedule to estimate mycophenolic acid area under the concentration-time curve in hematopoietic cell transplantation recipients. *J Clin Pharmacol*. 2012;52:1654–1664.
231. Yoshimura K, Yano I, Yamamoto T, et al. Population pharmacokinetics and pharmacodynamics of mycophenolic acid using the prospective

- data in patients undergoing hematopoietic stem cell transplantation. *Bone Marrow Transpl.* 2018;53:44–51.
232. Li H, Mager DE, Sandmaier BM, et al. Pharmacokinetic and pharmacodynamic analysis of inosine monophosphate dehydrogenase activity in hematopoietic cell transplantation recipients treated with mycophenolate mofetil. *Biol Blood Marrow Transpl.* 2014;20:1121–1129.
  233. McCune JS, Bemer MJ, Long-Boyle J. Pharmacokinetics, pharmacodynamics, and pharmacogenomics of immunosuppressants in allogeneic hematopoietic cell transplantation: part II. *Clin Pharmacokinet.* 2016;55:551–593.
  234. Hiwarkar P, Shaw BE, Tredger JM, et al. Mycophenolic acid trough level monitoring: relevance in acute and chronic graft versus host disease and its relation with albumin. *Clin Transpl.* 2011;25:222–227.
  235. Yoshimura K, Yano I, Yamamoto T, et al. Pharmacokinetic and pharmacodynamic markers of mycophenolic acid associated with effective prophylaxis for acute graft-versus-host disease and neutrophil engraftment in cord blood transplant patients. *Biol Blood Marrow Transpl.* 2018;24:1441–1448.
  236. McCune JS, Jacobson P, Wiseman A, et al. Optimizing drug therapy in pediatric SCT: focus on pharmacokinetics. *Bone Marrow Transpl.* 2015;50:165–172.
  237. Windreich RM, Goyal RK, Joshi R, et al. A pilot study of continuous infusion of mycophenolate mofetil for prophylaxis of graft-versus-host-disease in pediatric patients. *Biol Blood Marrow Transpl.* 2016;22:682–689.
  238. Harnicar S, Ponce DM, Hilden P, et al. Intensified mycophenolate mofetil dosing and higher mycophenolic acid trough levels reduce severe acute graft-versus-host disease after double-unit cord blood transplantation. *Biol Blood Marrow Transpl.* 2015;21:920–925.
  239. Fanouriakis A, Kostopoulou M, Cheema K, et al. 2019 update of the joint European League against rheumatism and European renal association-European dialysis and transplant association (EULAR/ERA-EDTA) recommendations for the management of lupus nephritis. *Ann Rheum Dis.* 2020;79:713–723.
  240. Djabarouti S, Breilh D, Duffau P, et al. Steady-state mycophenolate mofetil pharmacokinetic parameters enable prediction of systemic lupus erythematosus clinical flares: an observational cohort study. *Arthritis Res Ther.* 2010;12:R217.
  241. Streicher C, Djabarouti S, Xuereb F, et al. Pre-dose plasma concentration monitoring of mycophenolate mofetil in patients with autoimmune diseases. *Br J Clin Pharmacol.* 2014;78:1419–1425.
  242. Luszczynska P, Pawinski T. Therapeutic drug monitoring of mycophenolic acid in lupus nephritis: a review of current literature. *Ther Drug Monit.* 2015;37:711–717.
  243. Neumann I, Fuhrmann H, Fang IF, et al. Association between mycophenolic acid 12-h trough levels and clinical endpoints in patients with autoimmune disease on mycophenolate mofetil. *Nephrol Dial Transpl.* 2008;23:3514–3520.
  244. Sagcal-Gironella AC, Fukuda T, Wiers K, et al. Pharmacokinetics and pharmacodynamics of mycophenolic acid and their relation to response to therapy of childhood-onset systemic lupus erythematosus. *Semin Arthritis Rheum.* 2011;40:307–313.
  245. Godron-Dubrasquet A, Woillard JB, Decramer S, et al. Mycophenolic acid area under the concentration-time curve is associated with therapeutic response in childhood-onset lupus nephritis. *Pediatr Nephrol.* 2021;36:341–347.
  246. Damiao A, de Azevedo MFC, Carlos AS, et al. Conventional therapy for moderate to severe inflammatory bowel disease: a systematic literature review. *World J Gastroenterol.* 2019;25:1142–1157.
  247. Smith MR, Cooper SC. Mycophenolate mofetil therapy in the management of inflammatory bowel disease—a retrospective case series and review. *J Crohns Colitis* 2014;8:890–897.
  248. Spain L, Diem S, Larkin J. Management of toxicities of immune checkpoint inhibitors. *Cancer Treat Rev.* 2016;44:51–60.
  249. Mir R, Shaw HM, Nathan PD. Mycophenolate mofetil alongside high-dose corticosteroids: optimizing the management of combination immune checkpoint inhibitor-induced colitis. *Melanoma Res.* 2019;29:102–106.
  250. Gellermann J, Weber L, Pape L, et al. Mycophenolate mofetil versus cyclosporin A in children with frequently relapsing nephrotic syndrome. *J Am Soc Nephrol.* 2013;24:1689–1697.
  251. Sobiak J, Resztak M, Ostalska-Nowicka D, et al. Monitoring of mycophenolate mofetil metabolites in children with nephrotic syndrome and the proposed novel target values of pharmacokinetic parameters. *Eur J Pharm Sci.* 2015;77:189–196.
  252. Hackl A, Cseppek O, Gessner M, et al. Mycophenolate mofetil therapy in children with idiopathic nephrotic syndrome: does therapeutic drug monitoring make a difference? *Ther Drug Monit.* 2016;38:274–279.
  253. Tellier S, Dallochio A, Guignon V, et al. Mycophenolic acid pharmacokinetics and relapse in children with steroid-dependent idiopathic nephrotic syndrome. *Clin J Am Soc Nephrol.* 2016;11:1777–1782.
  254. Natale P, Palmer SC, Ruospo M, et al. Immunosuppressive agents for treating IgA nephropathy. *Cochrane Database Syst Rev.* 2020;3:CD003965.
  255. Rodrigues JC, Haas M, Reich HN. IgA nephropathy. *Clin J Am Soc Nephrol.* 2017;12:677–686.
  256. Oni L, Sampath S. Childhood IgA vasculitis (Henoch Schonlein purpura)-advances and knowledge gaps. *Front Pediatr.* 2019;7:257.
  257. Nowak I, Shaw LM. Mycophenolic acid binding to human serum albumin: characterization and relation to pharmacodynamics. *Clin Chem.* 1995;41:1011–1017.
  258. Velghe S, Capiou S, Stove CP. Opening the toolbox of alternative sampling strategies in clinical routine: a key-role for (LC-)MS/MS. *Trac-Trends Anal Chem.* 2016;84:61–73.
  259. Klepacki J, Klawitter J, Bendrick-Peart J, et al. A high-throughput U-HPLC-MS/MS assay for the quantification of mycophenolic acid and its major metabolites mycophenolic acid glucuronide and mycophenolic acid acyl-glucuronide in human plasma and urine. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2012;883-884:113–119.
  260. Shipkova M, Armstrong VW, Schneider T, et al. Stability of mycophenolic acid and mycophenolic acid glucuronide in human plasma. *Clin Chem.* 1999;45:127–129.
  261. Shipkova M, Schutz E, Armstrong VW, et al. Determination of the acyl glucuronide metabolite of mycophenolic acid in human plasma by HPLC and Emit. *Clin Chem.* 2000;46:365–372.
  262. Heinig K, Bucheli F, Hartenbach R, et al. Determination of mycophenolic acid and its phenyl glucuronide in human plasma, ultrafiltrate, blood, DBS and dried plasma spots. *Bioanalysis.* 2010;2:1423–1435.
  263. Shipkova M, Schutz E, Armstrong VW, et al. Overestimation of mycophenolic acid by EMIT correlates with MPA metabolite. *Transpl Proc.* 1999;31:1135–1137.
  264. Shipkova M, Armstrong VW, Kiehl MG, et al. Quantification of mycophenolic acid in plasma samples collected during and immediately after intravenous administration of mycophenolate mofetil. *Clin Chem.* 2001;47:1485–1488.
  265. de Loor H, Naesens M, Verbeke K, et al. Stability of mycophenolic acid and glucuronide metabolites in human plasma and the impact of deproteination methodology. *Clin Chim Acta.* 2008;389:87–92.
  266. Wiesen MH, Farowski F, Feldkötter M, et al. Liquid chromatography-tandem mass spectrometry method for the quantification of mycophenolic acid and its phenolic glucuronide in saliva and plasma using a standardized saliva collection device. *J Chromatogr A.* 2012;1241:52–59.
  267. Shen B, Li S, Zhang Y, et al. Determination of total, free and saliva mycophenolic acid with a LC-MS/MS method: application to pharmacokinetic study in healthy volunteers and renal transplant patients. *J Pharm Biomed Anal.* 2009;50:515–521.
  268. Koster RA, Niemeijer P, Veenhof H, et al. A volumetric absorptive microsampling LC-MS/MS method for five immunosuppressants and their hematocrit effects. *Bioanalysis.* 2019;11:495–508.
  269. Martial LC, Hoogtanders KEJ, Schreuder MF, et al. Dried blood spot sampling for tacrolimus and mycophenolic acid in children: analytical and clinical validation. *Ther Drug Monit.* 2017;39:412–421.
  270. Mandla R, Line PD, Midtvedt K, et al. Automated determination of free mycophenolic acid and its glucuronide in plasma from renal allograft recipients. *Ther Drug Monit.* 2003;25:407–414.
  271. Glander P, Sombogaard F, Budde K, et al. Improved assay for the nonradioactive determination of inosine 5'-monophosphate dehydrogenase activity in peripheral blood mononuclear cells. *Ther Drug Monit.* 2009;31:351–359.
  272. Kawanishi M, Yano I, Yoshimura K, et al. Sensitive and validated LC-MS/MS methods to evaluate mycophenolic acid pharmacokinetics and pharmacodynamics in hematopoietic stem cell transplant patients. *Biomed Chromatogr.* 2015;29:1309–1316.

273. Vethe NT, Ali AM, Reine PA, et al. Simultaneous quantification of IMPDH activity and purine bases in lymphocytes using LC-MS/MS: assessment of biomarker responses to mycophenolic acid. *Ther Drug Monit.* 2014;36:108–118.
274. Laverdiere I, Caron P, Couture F, et al. Liquid chromatography-coupled tandem mass spectrometry based assay to evaluate inosine-5'-monophosphate dehydrogenase activity in peripheral blood mononuclear cells from stem cell transplant recipients. *Anal Chem.* 2012;84:216–223.
275. Weissbarth G, Wiesen MHJ, Fietz C, et al. Pharmacodynamic monitoring of mycophenolic acid therapy: improved liquid chromatography-tandem mass spectrometry method for measuring inosin-5'-monophosphate dehydrogenase activity. *Ther Drug Monit.* 2020;42:282–288.
276. Barten MJ, Shipkova M, Bartsch P, et al. Mycophenolic acid interaction with cyclosporine and tacrolimus in vitro and in vivo: evaluation of additive effects on rat blood lymphocyte function. *Ther Drug Monit.* 2005;27:123–131.
277. Oellerich M, Shipkova M, Schutz E, et al. Pharmacokinetic and metabolic investigations of mycophenolic acid in pediatric patients after renal transplantation: implications for therapeutic drug monitoring. German Study Group on Mycophenolate Mofetil Therapy in Pediatric Renal Transplant Recipients. *Ther Drug Monit.* 2000;22:20–26.
278. Kuypers DR, Vanrenterghem Y, Squifflet JP, et al. Twelve-month evaluation of the clinical pharmacokinetics of total and free mycophenolic acid and its glucuronide metabolites in renal allograft recipients on low dose tacrolimus in combination with mycophenolate mofetil. *Ther Drug Monit.* 2003;25:609–622.
279. Glander P, Sommerer C, Arns W, et al. Pharmacokinetics and pharmacodynamics of intensified versus standard dosing of mycophenolate sodium in renal transplant patients. *Clin J Am Soc Nephrol.* 2010;5:503–511.
280. Pisupati J, Jain A, Burckart G, et al. Intraindividual and interindividual variations in the pharmacokinetics of mycophenolic acid in liver transplant patients. *J Clin Pharmacol.* 2005;45:34–41.
281. Premaud A, Debord J, Rousseau A, et al. A double absorption-phase model adequately describes mycophenolic acid plasma profiles in de novo renal transplant recipients given oral mycophenolate mofetil. *Clin Pharmacokinet.* 2005;44:837–847.
282. Shen J, Jiao Z, Yu YQ, et al. Quantification of total and free mycophenolic acid in human plasma by liquid chromatography with fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2005;817:207–213.
283. Syed M, Srinivas NR. A comprehensive review of the published assays for the quantitation of the immunosuppressant drug mycophenolic acid and its glucuronidated metabolites in biological fluids. *Biomed Chromatogr.* 2016;30:721–748.
284. Shipkova M, Armstrong VW, Wieland E, et al. Identification of glucoside and carboxyl-linked glucuronide conjugates of mycophenolic acid in plasma of transplant recipients treated with mycophenolate mofetil. *Br J Pharmacol.* 1999;126:1075–1082.
285. Musuamba FT, Di Fazio V, Vanbinst R, et al. A fast ultra-performance liquid chromatography method for simultaneous quantification of mycophenolic acid and its phenol- and acyl-glucuronides in human plasma. *Ther Drug Monit.* 2009;31:110–115.
286. Shipkova M, Valbuena H. Liquid chromatography tandem mass spectrometry for therapeutic drug monitoring of immunosuppressive drugs: achievements, lessons and open issues. *Trac-Trends Anal Chem.* 2016;84:23–33.
287. Chen B, Gu Z, Chen H, et al. Establishment of high-performance liquid chromatography and enzyme multiplied immunoassay technology methods for determination of free mycophenolic acid and its application in Chinese liver transplant recipients. *Ther Drug Monit.* 2010;32:653–660.
288. Kunicki PK, Pawinski T, Boczek A, et al. A comparison of the immunochemical methods, PETINIA and EMIT, with that of HPLC-UV for the routine monitoring of mycophenolic acid in heart transplant patients. *Ther Drug Monit.* 2015;37:311–318.
289. Beal JL, Jones CE, Taylor PJ, et al. Evaluation of an immunoassay (EMIT) for mycophenolic acid in plasma from renal transplant recipients compared with a high-performance liquid chromatography assay. *Ther Drug Monit.* 1998;20:685–690.
290. Schutz E, Shipkova M, Wieland E, et al. Evaluation of an immunoassay for mycophenolic acid. *Ther Drug Monit.* 2000;22:141–142.
291. Yeung JS, Wang W, Chan L. Determination of mycophenolic acid level: comparison of high-performance liquid chromatography with homogeneous enzyme-immunoassay. *Transpl Proc.* 1999;31:1214–1215.
292. Brunet M, Oppenheimer F, Martorell J, et al. Mycophenolic acid monitoring: evaluation of the EMIT MPA immunoassay in kidney and lung transplantation. *Transpl Proc.* 1999;31:2275–2276.
293. Vogl M, Weigel G, Seebacher G, et al. Evaluation of the EMIT mycophenolic acid assay from dade behring. *Ther Drug Monit.* 1999;21:638–643.
294. Hosotsubo H, Takahara S, Imamura R, et al. Analytic validation of the enzyme multiplied immunoassay technique for the determination of mycophenolic acid in plasma from renal transplant recipients compared with a high-performance liquid chromatographic assay. *Ther Drug Monit.* 2001;23:669–674.
295. Weber LT, Shipkova M, Armstrong VW, et al. Comparison of the Emit immunoassay with HPLC for therapeutic drug monitoring of mycophenolic acid in pediatric renal-transplant recipients on mycophenolate mofetil therapy. *Clin Chem.* 2002;48:517–525.
296. Premaud A, Rousseau A, Le Meur Y, et al. Comparison of liquid chromatography-tandem mass spectrometry with a commercial enzyme-multiplied immunoassay for the determination of plasma MPA in renal transplant recipients and consequences for therapeutic drug monitoring. *Ther Drug Monit.* 2004;26:609–619.
297. Premaud A, Rousseau A, Picard N, et al. Determination of mycophenolic acid plasma levels in renal transplant recipients co-administered sirolimus: comparison of an enzyme multiplied immunoassay technique (EMIT) and liquid chromatography-tandem mass spectrometry. *Ther Drug Monit.* 2006;28:274–277.
298. Irtan S, Azougagh S, Monchaud C, et al. Comparison of high-performance liquid chromatography and enzyme-multiplied immunoassay technique to monitor mycophenolic acid in paediatric renal recipients. *Pediatr Nephrol.* 2008;23:1859–1865.
299. Brown NW, Franklin ME, Einarsdottir EN, et al. An investigation into the bias between liquid chromatography-tandem mass spectrometry and an enzyme multiplied immunoassay technique for the measurement of mycophenolic acid. *Ther Drug Monit.* 2010;32:420–426.
300. Westley IS, Sallustio BC, Morris RG. Validation of a high-performance liquid chromatography method for the measurement of mycophenolic acid and its glucuronide metabolites in plasma. *Clin Biochem.* 2005;38:824–829.
301. Boer K, Brehmer-Streck S, Deufel T, et al. Automated monitoring of C2 and C0 blood levels of mycophenolic acid and cyclosporine on the Abbott Architect c8000. *Clin Biochem.* 2007;40:1163–1167.
302. Schutz E, Shipkova M, Armstrong VW, et al. Therapeutic drug monitoring of mycophenolic acid: comparison of HPLC and immunoassay reveals new MPA metabolites. *Transpl Proc.* 1998;30:1185–1187.
303. Vergara Chozas JM, Saez-Benito Godino A, Zopeque Garcia N, et al. Analytical validation of a homogeneous immunoassay for determination of mycophenolic acid in human plasma. *Transpl Proc.* 2012;44:2669–2672.
304. Ham JY, Jung HY, Choi JY, et al. Usefulness of mycophenolic acid monitoring with PETINIA for prediction of adverse events in kidney transplant recipients. *Scand J Clin Lab Invest.* 2016;76:296–303.
305. Kikuchi M, Tanaka M, Takasaki S, et al. Comparison of PETINIA and LC-MS/MS for determining plasma mycophenolic acid concentrations in Japanese lung transplant recipients. *J Pharm Health Care Sci.* 2018;4:7.
306. Bartoli A, Pitta L, Villani A, et al. Comparison of PETINIA and EMIT immunoassay methods to measure mycophenolic acid concentration in transplanted patients. Abstract P215. 12th International congress of Therapeutic Drug Monitoring and Clinical Toxicology: Stuttgart, Germany October 2–6, 2011. *Ther Drug Monit.* 2011;33:537.
307. Dasgupta A, Tso G, Chow L. Comparison of mycophenolic acid concentrations determined by a new PETINIA assay on the dimension EXL analyzer and a HPLC-UV method. *Clin Biochem.* 2013;46:685–687.
308. Westley IS, Ray JE, Morris RG. CEDIA mycophenolic acid assay compared with HPLC-UV in specimens from transplant recipients. *Ther Drug Monit.* 2006;28:632–636.
309. Shipkova M, Schutz E, Besenthal I, et al. Investigation of the cross-reactivity of mycophenolic acid glucuronide metabolites and of

- mycophenolate mofetil in the Cedia MPA assay. *Ther Drug Monit.* 2010;32:79–85.
310. Dasgupta A, Johnson M. Positive bias in mycophenolic acid concentrations determined by the CEDIA assay compared to HPLC-UV method: is CEDIA assay suitable for therapeutic drug monitoring of mycophenolic acid? *J Clin Lab Anal.* 2013;27:77–80.
311. Jebabli N, Gaies E, Charfi R, et al. Comparative study of two techniques of mycophenolate mofetyl monitoring. *Tunis Med.* 2019;97:1010–1016.
312. Saint-Marcoux F, Debord J, Parant F, et al. Development and evaluation of a simulation procedure to take into account various assays for the Bayesian dose adjustment of tacrolimus. *Ther Drug Monit.* 2011;33:171–177.
313. Parant F, Ranchin B, Gagnieu MC. The Roche Total Mycophenolic Acid(R) assay: an application protocol for the ABX Pentra 400 analyzer and comparison with LC-MS in children with idiopathic nephrotic syndrome. *Pract Lab Med.* 2017;7:19–26.
314. Brandhorst G, Marquet P, Shaw LM, et al. Multicenter evaluation of a new inosine monophosphate dehydrogenase inhibition assay for quantification of total mycophenolic acid in plasma. *Ther Drug Monit.* 2008;30:428–433.
315. Blanchet B, Taieb F, Conti F, et al. Comparison of a new enzymatic assay with a high-performance liquid chromatography/ultraviolet detection method for therapeutic drug monitoring of mycophenolic acid in adult liver transplant recipients. *Liver Transpl.* 2008;14:1745–1751.
316. van Gelder T, Domke I, Engelmayer J, et al. Clinical utility of a new enzymatic assay for determination of mycophenolic acid in comparison with an optimized LC-MS/MS method. *Ther Drug Monit.* 2009;31:218–223.
317. Decavele AS, Favoreel N, Heyden FV, et al. Performance of the Roche total mycophenolic acid(R) assay on the Cobas integra 400(R), Cobas 6000(R) and comparison to LC-MS/MS in liver transplant patients. *Clin Chem Lab Med.* 2011;49:1159–1165.
318. Vesper HW, Myers GL, Miller WG. Current practices and challenges in the standardization and harmonization of clinical laboratory tests. *Am J Clin Nutr.* 2016;104(suppl 3):907S–912S.
319. Siekmann L. Metrological traceability—a concept for standardization in laboratory medicine. *Clin Chem Lab Med.* 2013;51:953–957.
320. Jones GR, Jackson C. The joint committee for traceability in laboratory medicine (JCTLM)—its history and operation. *Clin Chim Acta.* 2016;453:86–94.
321. Westgard JO, Westgard SA. Measuring analytical quality: total analytical error versus measurement uncertainty. *Clin Lab Med.* 2017;37:1–13.
322. Westgard JO. Error methods are more practical, but uncertainty methods may still be preferred. *Clin Chem.* 2018;64:636–638.
323. Theodorsson E. Uncertainty in measurement and total error: tools for coping with diagnostic uncertainty. *Clin Lab Med.* 2017;37:15–34.
324. Menditto A, Patriarca M, Magnusson B. Understanding the meaning of accuracy, trueness and precision. *Accreditation Qual Assur.* 2007;12:45–47.
325. European Commission's Joint Research Centre (JRC). Available at: <https://crm.jrc.ec.europa.eu/>. Accessed October 26, 2020.
326. National Institute of standardization (NIST). Available at: <https://www.nist.gov/srm>. Accessed October 26, 2020.
327. Joint Committee for Traceability in Laboratory Medicine (JCTLM), Bureau International des Poids et Mesures. Available at: <https://www.bipm.org/jctlm/>. Accessed October 26, 2020.
328. Immunosuppressive Drugs (WG-ID), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Available at: <http://www.ifcc.org/ifcc-scientific-division/sd-working-groups/wg-id/>. Accessed October 26, 2020.
329. Rebollo N, Calvo MV, Martin-Suarez A, et al. Modification of the EMIT immunoassay for the measurement of unbound mycophenolic acid in plasma. *Clin Biochem.* 2011;44:260–263.
330. Bittersohl H, Herbinger J, Wen M, et al. Simultaneous determination of protein-unbound cyclosporine A and mycophenolic acid in kidney transplant patients using liquid chromatography-tandem mass spectrometry. *Ther Drug Monit.* 2017;39:211–219.
331. Figurski MJ, Korecka M, Fields L, et al. High-performance liquid chromatography-mass spectroscopy/mass spectroscopy method for simultaneous quantification of total or free fraction of mycophenolic acid and its glucuronide metabolites. *Ther Drug Monit.* 2009;31:717–726.
332. Shipkova M, Niedmann PD, Armstrong VW, et al. Simultaneous determination of mycophenolic acid and its glucuronide in human plasma using a simple high-performance liquid chromatography procedure. *Clin Chem.* 1998;44:1481–1488.
333. Benech H, Hascoet S, Furlan V, et al. Development and validation of an LC/MS/MS assay for mycophenolic acid in human peripheral blood mononuclear cells. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007;853:168–174.
334. Nguyen Thi MT, Capron A, Mourad M, et al. Mycophenolic acid quantification in human peripheral blood mononuclear cells using liquid chromatography-tandem mass spectrometry. *Clin Biochem.* 2013;46:1909–1911.
335. Thi MT, Mourad M, Capron A, et al. Plasma and intracellular pharmacokinetic-pharmacodynamic analysis of mycophenolic acid in de novo kidney transplant patients. *Clin Biochem.* 2015;48:401–405.
336. Md Dom ZI, Coller JK, Carroll RP, et al. Mycophenolic acid concentrations in peripheral blood mononuclear cells are associated with the incidence of rejection in renal transplant recipients. *Br J Clin Pharmacol.* 2018;84:2433–2442.
337. Capiou S, Veenhof H, Koster RA, et al. Official international association for therapeutic drug monitoring and clinical toxicology guideline: development and validation of dried blood spot-based methods for therapeutic drug monitoring. *Ther Drug Monit.* 2019;41:409–430.
338. Zwart TC, Gokoel SRM, van der Boog PJM, et al. Therapeutic drug monitoring of tacrolimus and mycophenolic acid in outpatient renal transplant recipients using a volumetric dried blood spot sampling device. *Br J Clin Pharmacol.* 2018;84:2889–2902.
339. Iboshi H, Yamaguchi H, Suzuki H, et al. Development of a liquid chromatography-tandem mass spectrometric method for quantification of mycophenolic acid and its glucuronides in dried blood spot samples. *Ther Drug Monit.* 2017;39:648–653.
340. Wilhelm AJ, den Burger JC, Chahbouni A, et al. Analysis of mycophenolic acid in dried blood spots using reversed phase high performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2009;877:3916–3919.
341. Arpini J, Antunes MV, Pacheco LS, et al. Clinical evaluation of a dried blood spot method for determination of mycophenolic acid in renal transplant patients. *Clin Biochem.* 2013;46:1905–1908.
342. Koster RA, Veenhof H, Botma R, et al. Dried blood spot validation of five immunosuppressants, without hematocrit correction, on two LC-MS/MS systems. *Bioanalysis.* 2017;9:553–563.
343. Almaradini R, Taybeh EO, Alsous MM, et al. A multiple methods approach to determine adherence with prescribed mycophenolate in children with kidney transplant. *Br J Clin Pharmacol.* 2019;85:1434–1442.
344. European Medicines Agency. *Guideline on Bioanalytical Method Validation.* 2011. Available at: <https://www.ema.europa.eu/en/bioanalytical-method-validation#current-effective-version-section>. Accessed October 18, 2020.
345. Langman LJ, LeGatt DF, Yatscoff RW. Blood distribution of mycophenolic acid. *Ther Drug Monit.* 1994;16:602–607.
346. Md Dom ZI, Noll BD, Coller JK, et al. Validation of an LC-MS/MS method for the quantification of mycophenolic acid in human kidney transplant biopsies. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2014;945–946:171–177.
347. Langman LJ. The use of oral fluid for therapeutic drug management: clinical and forensic toxicology. *Ann N Y Acad Sci.* 2007;1098:145–166.
348. Ghareeb M, Akhlaghi F. Alternative matrices for therapeutic drug monitoring of immunosuppressive agents using LC-MS/MS. *Bioanalysis.* 2015;7:1037–1058.
349. Mendonza AE, Gohh RY, Akhlaghi F. Analysis of mycophenolic acid in saliva using liquid chromatography tandem mass spectrometry. *Ther Drug Monit.* 2006;28:402–406.
350. Brooks E, Tett SE, Isbel NM, et al. Investigation of the association between total and free plasma and saliva mycophenolic acid concentrations following administration of enteric-coated mycophenolate sodium in adult kidney transplant recipients. *Clin Drug Investig.* 2019;39:1175–1184.

351. Ferreira PCL, Thiesen FV, de Araujo TT, et al. Comparison of plasma and oral fluid concentrations of mycophenolic acid and its glucuronide metabolite by LC-MS in kidney transplant patients. *Eur J Clin Pharmacol*. 2019;75:553–559.
352. Swen JJ, Nijenhuis M, de Boer A, et al. Pharmacogenetics: from bench to byte—an update of guidelines. *Clin Pharmacol Ther*. 2011;89:662–673.
353. Relling MV, Klein TE. CPIC: clinical pharmacogenetics implementation Consortium of the pharmacogenomics research network. *Clin Pharmacol Ther*. 2011;89:464–467.
354. Birdwell KA, Decker B, Barbarino JM, et al. Clinical pharmacogenetics implementation Consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing. *Clin Pharmacol Ther*. 2015;98:19–24.
355. Barraclough KA, Lee KJ, Staatz CE. Pharmacogenetic influences on mycophenolate therapy. *Pharmacogenomics*. 2010;11:369–390.
356. Kuypers DR, Naesens M, Vermeire S, et al. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients. *Clin Pharmacol Ther*. 2005;78:351–361.
357. Naesens M, Kuypers DR, Verbeke K, et al. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. *Transplantation*. 2006;82:1074–1084.
358. Baldelli S, Merlini S, Perico N, et al. C-440T/T-331C polymorphisms in the UGT1A9 gene affect the pharmacokinetics of mycophenolic acid in kidney transplantation. *Pharmacogenomics*. 2007;8:1127–1141.
359. Kuypers DR, de Jonge H, Naesens M, et al. Current target ranges of mycophenolic acid exposure and drug-related adverse events: a 5-year, open-label, prospective, clinical follow-up study in renal allograft recipients. *Clin Ther*. 2008;30:673–683.
360. Johnson LA, Oetting WS, Basu S, et al. Pharmacogenetic effect of the UGT polymorphisms on mycophenolate is modified by calcineurin inhibitors. *Eur J Clin Pharmacol*. 2008;64:1047–1056.
361. van Schaik RH, van Agteren M, de Fijter JW, et al. UGT1A9 -275T>A/-2152C>T polymorphisms correlate with low MPA exposure and acute rejection in MMF/tacrolimus-treated kidney transplant patients. *Clin Pharmacol Ther*. 2009;86:319–327.
362. Sanchez-Fructuoso AI, Maestro ML, Calvo N, et al. The prevalence of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T and its influence on mycophenolic acid pharmacokinetics in stable renal transplant patients. *Transpl Proc*. 2009;41:2313–2316.
363. Picard N, Yee SW, Woillard JB, et al. The role of organic anion-transporting polypeptides and their common genetic variants in mycophenolic acid pharmacokinetics. *Clin Pharmacol Ther*. 2010;87:100–108.
364. Fukuda T, Goebel J, Cox S, et al. UGT1A9, UGT2B7, and MRP2 genotypes can predict mycophenolic acid pharmacokinetic variability in pediatric kidney transplant recipients. *Ther Drug Monit*. 2012;34:671–679.
365. Mazidi T, Rouini MR, Ghahremani MH, et al. Impact of UGT1A9 polymorphism on mycophenolic acid pharmacokinetic parameters in stable renal transplant patients. *Iran J Pharm Res*. 2013;12:547–556.
366. Ruiz J, Herrero MJ, Boso V, et al. Impact of single nucleotide polymorphisms (SNPs) on immunosuppressive therapy in lung transplantation. *Int J Mol Sci*. 2015;16:20168–20182.
367. Kiang TKL, Partovi N, Shapiro RJ, et al. Regression and genomic analyses on the association between dose-normalized mycophenolic acid exposure and absolute neutrophil count in steroid-free, de novo kidney transplant recipients. *Clin Drug Investig*. 2018;38:1011–1022.
368. Zhao W, Fakhoury M, Deschenes G, et al. Population pharmacokinetics and pharmacogenetics of mycophenolic acid following administration of mycophenolate mofetil in de novo pediatric renal-transplant patients. *J Clin Pharmacol*. 2010;50:1280–1291.
369. Frymoyer A, Verotta D, Jacobson P, et al. Population pharmacokinetics of unbound mycophenolic acid in adult allogeneic haematopoietic cell transplantation: effect of pharmacogenetic factors. *Br J Clin Pharmacol*. 2013;75:463–475.
370. Resendiz-Galvan JE, Romano-Aguilar M, Medellin-Garibay SE, et al. Population pharmacokinetics of mycophenolic acid in adult kidney transplant patients under prednisone and tacrolimus regimen. *Eur J Pharm Sci*. 2020;150:105370.
371. Levesque E, Delage R, Benoit-Biancamano MO, et al. The impact of UGT1A8, UGT1A9, and UGT2B7 genetic polymorphisms on the pharmacokinetic profile of mycophenolic acid after a single oral dose in healthy volunteers. *Clin Pharmacol Ther*. 2007;81:392–400.
372. Jiao Z, Ding JJ, Shen J, et al. Population pharmacokinetic modelling for enterohepatic circulation of mycophenolic acid in healthy Chinese and the influence of polymorphisms in UGT1A9. *Br J Clin Pharmacol*. 2008;65:893–907.
373. Dupuis R, Yuen A, Innocenti F. The influence of UGT polymorphisms as biomarkers in solid organ transplantation. *Clin Chim Acta*. 2012;413:1318–1325.
374. Lamba V, Sangkuhl K, Sanghavi K, et al. PharmGKB summary: mycophenolic acid pathway. *Pharmacogenet Genomics*. 2014;24:73–79.
375. Inoue K, Miura M, Satoh S, et al. Influence of UGT1A7 and UGT1A9 intronic I399 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *Ther Drug Monit*. 2007;29:299–304.
376. Joy MS, Boyette T, Hu Y, et al. Effects of uridine diphosphate glucuronosyltransferase 2B7 and 1A7 pharmacogenomics and patient clinical parameters on steady-state mycophenolic acid pharmacokinetics in glomerulonephritis. *Eur J Clin Pharmacol*. 2010;66:1119–1130.
377. Han N, Yun HY, Kim IW, et al. Population pharmacogenetic pharmacokinetic modeling for flip-flop phenomenon of enteric-coated mycophenolate sodium in kidney transplant recipients. *Eur J Clin Pharmacol*. 2014;70:1211–1219.
378. Xie XC, Li J, Wang HY, et al. Associations of UDP-glucuronosyltransferases polymorphisms with mycophenolate mofetil pharmacokinetics in Chinese renal transplant patients. *Acta Pharmacol Sin*. 2015;36:644–650.
379. Kagaya H, Inoue K, Miura M, et al. Influence of UGT1A8 and UGT2B7 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *Eur J Clin Pharmacol*. 2007;63:279–288.
380. Zhang WX, Chen B, Jin Z, et al. Influence of uridine diphosphate (UDP)-glucuronosyltransferases and ABCC2 genetic polymorphisms on the pharmacokinetics of mycophenolic acid and its metabolites in Chinese renal transplant recipients. *Xenobiotica*. 2008;38:1422–1436.
381. Geng F, Jiao Z, Dao YJ, et al. The association of the UGT1A8, SLC01B3 and ABCC2/ABCG2 genetic polymorphisms with the pharmacokinetics of mycophenolic acid and its phenolic glucuronide metabolite in Chinese individuals. *Clin Chim Acta*. 2012;413:683–690.
382. Li LQ, Chen DN, Li CJ, et al. Impact of UGT2B7 and ABCC2 genetic polymorphisms on mycophenolic acid metabolism in Chinese renal transplant recipients. *Pharmacogenomics*. 2018;19:1323–1334.
383. Okour M, Jacobson PA, Ahmed MA, et al. Mycophenolic acid and its metabolites in kidney transplant recipients: a semimechanistic enterohepatic circulation model to improve estimating exposure. *J Clin Pharmacol*. 2018;58:628–639.
384. Romano-Aguilar M, Resendiz-Galvan JE, Medellin-Garibay SE, et al. Population pharmacokinetics of mycophenolic acid in Mexican patients with lupus nephritis. *Lupus*. 2020;29:1067–1077.
385. Djebli N, Picard N, Rerolle JP, et al. Influence of the UGT2B7 promoter region and exon 2 polymorphisms and comediations on Acyl-MPAG production in vitro and in adult renal transplant patients. *Pharmacogenet Genomics*. 2007;17:321–330.
386. Guo D, Pang LF, Han Y, et al. Polymorphisms of UGT1A9 and UGT2B7 influence the pharmacokinetics of mycophenolic acid after a single oral dose in healthy Chinese volunteers. *Eur J Clin Pharmacol*. 2013;69:843–849.
387. Genvigir FDV, Nishikawa AM, Felipe CR, et al. Influence of ABCC2, CYP2C8, and CYP2J2 polymorphisms on tacrolimus and mycophenolate sodium-based treatment in Brazilian kidney transplant recipients. *Pharmacotherapy*. 2017;37:535–545.
388. Yu ZC, Zhou PJ, Wang XH, et al. Population pharmacokinetics and Bayesian estimation of mycophenolic acid concentrations in Chinese adult renal transplant recipients. *Acta Pharmacol Sin*. 2017;38:1566–1579.
389. Lloberas N, Torras J, Cruzado JM, et al. Influence of MRP2 on MPA pharmacokinetics in renal transplant recipients—results of the pharmacogenomic substudy within the symphony study. *Nephrol Dial Transpl*. 2011;26:3784–3793.

390. Yap DYH, Tam CH, Yung S, et al. Pharmacokinetics and pharmacogenomics of mycophenolic acid and its clinical correlations in maintenance immunosuppression for lupus nephritis. *Nephrol Dial Transpl.* 2020;35:810–818.
391. Bozina N, Lalic Z, Nad-Skegro S, et al. Steady-state pharmacokinetics of mycophenolic acid in renal transplant patients: exploratory analysis of the effects of cyclosporine, recipients' and donors' ABCC2 gene variants, and their interactions. *Eur J Clin Pharmacol.* 2017;73:1129–1140.
392. Musuamba FT, Mourad M, Haufroid V, et al. A simultaneous d-optimal designed study for population pharmacokinetic analyses of mycophenolic acid and tacrolimus early after renal transplantation. *J Clin Pharmacol.* 2012;52:1833–1843.
393. Colom H, Lloberas N, Andreu F, et al. Pharmacokinetic modeling of enterohepatic circulation of mycophenolic acid in renal transplant recipients. *Kidney Int.* 2014;85:1434–1443.
394. Colom H, Andreu F, van Gelder T, et al. Prediction of free from total mycophenolic acid concentrations in stable renal transplant patients: a population-based approach. *Clin Pharmacokinet.* 2018;57:877–893.
395. Kim JH, Han N, Kim MG, et al. Model based development of tacrolimus dosing algorithm considering CYP3A5 genotypes and mycophenolate mofetil drug interaction in stable kidney transplant recipients. *Sci Rep.* 2019;9:11740.
396. Riglet F, Bertrand J, Barrail-Tran A, et al. Population pharmacokinetic model of plasma and cellular mycophenolic acid in kidney transplant patients from the CIMTRE study. *Drugs R D.* 2020;20:331–342.
397. Wang J, Yang JW, Zeevi A, et al. IMPDH1 gene polymorphisms and association with acute rejection in renal transplant patients. *Clin Pharmacol Ther.* 2008;83:711–717.
398. Bouamar R, Hesselink DA, van Schaik RH, et al. Mycophenolic acid-related diarrhea is not associated with polymorphisms in SLC01B nor with ABCB1 in renal transplant recipients. *Pharmacogenet Genomics.* 2012;22:399–407.
399. Miura M, Satoh S, Inoue K, et al. Influence of SLC01B1, 1B3, 2B1 and ABCC2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *Eur J Clin Pharmacol.* 2007;63:1161–1169.
400. Miura M, Kagaya H, Satoh S, et al. Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients. *Ther Drug Monit.* 2008;30:559–564.
401. Haufroid V, Picard N. Pharmacogenetics biomarkers predictive of drug pharmacodynamics as an additional tool to therapeutic drug monitoring. *Ther Drug Monit.* 2019;41:121–130.
402. Digits JA, Hedstrom L. Species-specific inhibition of inosine 5'-monophosphate dehydrogenase by mycophenolic acid. *Biochemistry.* 1999;38:15388–15397.
403. McPhillips CC, Hyle JW, Reines D. Detection of the mycophenolate-inhibited form of IMP dehydrogenase in vivo. *Proc Natl Acad Sci U S A.* 2004;101:12171–12176.
404. Roberts RL, Geary RB, Barclay ML, et al. IMPDH1 promoter mutations in a patient exhibiting azathioprine resistance. *Pharmacogenomics J.* 2007;7:312–317.
405. Wu TY, Peng Y, Pelleymounter LL, et al. Pharmacogenetics of the mycophenolic acid targets inosine monophosphate dehydrogenases IMPDH1 and IMPDH2: gene sequence variation and functional genomics. *Br J Pharmacol.* 2010;161:1584–1598.
406. Sombogaard F, van Schaik RH, Mathot RA, et al. Interpatient variability in IMPDH activity in MMF-treated renal transplant patients is correlated with IMPDH type II 3757T > C polymorphism. *Pharmacogenet Genomics.* 2009;19:626–634.
407. Winnicki W, Weigel G, Sunder-Plassmann G, et al. An inosine 5'-monophosphate dehydrogenase 2 single-nucleotide polymorphism impairs the effect of mycophenolic acid. *Pharmacogenomics J.* 2010;10:70–76.
408. Pazik J, Oldak M, Podgorska M, et al. Lymphocyte counts in kidney allograft recipients are associated with IMPDH2 3757T>C gene polymorphism. *Transpl Proc.* 2011;43:2943–2945.
409. Wang J, Zeevi A, Webber S, et al. A novel variant L263F in human inosine 5'-monophosphate dehydrogenase 2 is associated with diminished enzyme activity. *Pharmacogenet Genomics.* 2007;17:283–290.
410. Garat A, Cauffiez C, Hamdan-Khalil R, et al. IMPDH2 genetic polymorphism: a promoter single-nucleotide polymorphism disrupts a cyclic adenosine monophosphate responsive element. *Genet Test Mol Biomarkers.* 2009;13:841–847.
411. Genvigir FDV, Cerda A, Hirata TDC, et al. Mycophenolic acid pharmacogenomics in kidney transplantation. *J Translational Genet Genomics.* 2020;4:320–355.
412. Gensburger O, Van Schaik RH, Picard N, et al. Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil. *Pharmacogenet Genomics.* 2010;20:537–543.
413. Mohamed MF, Frye RF, Langae TY. Interpopulation variation frequency of human inosine 5'-monophosphate dehydrogenase type II (IMPDH2) genetic polymorphisms. *Genet Test.* 2008;12:513–516.
414. CPIC Clinical Pharmacogenetics Implementation Consortium. *Genes-Drugs.* Available at: <https://cpicpgx.org/genes-drugs/>. Accessed November 3, 2020.
415. Collins KS, Cheng YH, Ferreira RM, et al. Interindividual variability in lymphocyte stimulation and transcriptomic response predicts mycophenolic acid sensitivity in healthy volunteers. *Clin Transl Sci.* 2020;13:1137–1149.
416. Pazik J, Oldak M, Dabrowski M, et al. Association of UDP-glucuronosyltransferase 1A9 (UGT1A9) gene polymorphism with kidney allograft function. *Ann Transpl.* 2011;16:69–73.
417. Michelon H, Konig J, Durrbach A, et al. SLC01B1 genetic polymorphism influences mycophenolic acid tolerance in renal transplant recipients. *Pharmacogenomics.* 2010;11:1703–1713.
418. Woillard JB, Picard N, Thierry A, et al. Associations between polymorphisms in target, metabolism, or transport proteins of mycophenolate sodium and therapeutic or adverse effects in kidney transplant patients. *Pharmacogenet Genomics.* 2014;24:256–262.
419. Cilio HL, Camargo-Godoy RBO, Souza MF, et al. Polymorphisms in IMPDH2, UGT2B7, and CES2 genes influence the risk of graft rejection in kidney transplant recipients taking mycophenolate mofetil. *Mutat Res Genet Toxicol Environ Mutagen.* 2018;836:97–102.
420. Pazik J, Oldak M, Lewandowski Z, et al. Recipient uridine 5'-diphospho-glucuronosyltransferase UGT1A9 c.98T>C variant determines transplanted kidney filtration rate. *Transpl Proc.* 2014;46:2678–2682.
421. Pazik J, Oldak M, Lewandowski Z, et al. Uridine diphosphate glucuronosyltransferase 2B7 variant p.His268Tyr as a predictor of kidney allograft early acute rejection. *Transpl Proc.* 2013;45:1516–1519.
422. Satoh S, Tada H, Murakami M, et al. Circadian pharmacokinetics of mycophenolic acid and implication of genetic polymorphisms for early clinical events in renal transplant recipients. *Transplantation.* 2006;82:486–493.
423. Prausa SE, Fukuda T, Maseck D, et al. UGT genotype may contribute to adverse events following medication with mycophenolate mofetil in pediatric kidney transplant recipients. *Clin Pharmacol Ther.* 2009;85:495–500.
424. Woillard JB, Rerolle JP, Picard N, et al. Risk of diarrhoea in a long-term cohort of renal transplant patients given mycophenolate mofetil: the significant role of the UGT1A8 2 variant allele. *Br J Clin Pharmacol.* 2010;69:675–683.
425. Betonico GN, Abbud-Filho M, Goloni-Bertollo EM, et al. Influence of UDP-glucuronosyltransferase polymorphisms on mycophenolate mofetil-induced side effects in kidney transplant patients. *Transpl Proc.* 2008;40:708–710.
426. van Agteren M, Armstrong VW, van Schaik RH, et al. AcylIMPAG plasma concentrations and mycophenolic acid-related side effects in patients undergoing renal transplantation are not related to the UGT2B7-840G>A gene polymorphism. *Ther Drug Monit.* 2008;30:439–444.
427. Yang JW, Lee PH, Hutchinson IV, et al. Genetic polymorphisms of MRP2 and UGT2B7 and gastrointestinal symptoms in renal transplant recipients taking mycophenolic acid. *Ther Drug Monit.* 2009;31:542–548.
428. Jacobson PA, Schladt D, Oetting WS, et al. Genetic determinants of mycophenolate-related anemia and leukopenia after transplantation. *Transplantation.* 2011;91:309–316.
429. Bouamar R, E lens L, Shuker N, et al. Mycophenolic acid-related anemia and leukopenia in renal transplant recipients are related to genetic



- polymorphisms in CYP2C8. *Transplantation*. 2012;93:e39–40; author reply e41–32.
430. Varnell CD, Fukuda T, Kirby CL, et al. Mycophenolate mofetil-related leukopenia in children and young adults following kidney transplantation: influence of genes and drugs. *Pediatr Transpl*. 2017;21:10.1111/ptr.13033. doi: 10.1111/ptr.13033.
  431. Oetting WS, Wu B, Schladt DP, et al. Genetic variants associated with immunosuppressant pharmacokinetics and adverse effects in the DeKAF genomics genome-wide association studies. *Transplantation*. 2019;103:1131–1139.
  432. Grinyo J, Vanrenterghem Y, Nashan B, et al. Association of four DNA polymorphisms with acute rejection after kidney transplantation. *Transpl Int*. 2008;21:879–891.
  433. Shah S, Harwood SM, Dohler B, et al. Inosine monophosphate dehydrogenase polymorphisms and renal allograft outcome. *Transplantation*. 2012;94:486–491.
  434. Oetting WS, Schladt DP, Leduc RE, et al. Validation of single nucleotide polymorphisms associated with acute rejection in kidney transplant recipients using a large multi-center cohort. *Transpl Int*. 2011;24:1231–1238.
  435. Kagaya H, Miura M, Saito M, et al. Correlation of IMPDH1 gene polymorphisms with subclinical acute rejection and mycophenolic acid exposure parameters on day 28 after renal transplantation. *Basic Clin Pharmacol Toxicol*. 2010;107:631–636.
  436. Vannozzi F, Filippini F, Di Paolo A, et al. An exploratory study on pharmacogenetics of inosine-monophosphate dehydrogenase II in peripheral mononuclear cells from liver-transplant recipients. *Transpl Proc*. 2004;36:2787–2790.
  437. Ting LS, Benoit-Biancamano MO, Bernard O, et al. Pharmacogenetic impact of UDP-glucuronosyltransferase metabolic pathway and multidrug resistance-associated protein 2 transport pathway on mycophenolic acid in thoracic transplant recipients: an exploratory study. *Pharmacotherapy*. 2010;30:1097–1108.
  438. Tague LK, Byers DE, Hachem R, et al. Impact of SLCO1B3 polymorphisms on clinical outcomes in lung allograft recipients receiving mycophenolic acid. *Pharmacogenomics J*. 2020;20:69–79.
  439. Burckart GJ, Figg WD II, Brooks MM, et al. Multi-institutional study of outcomes after pediatric heart transplantation: candidate gene polymorphism analysis of ABCC2. *J Pediatr Pharmacol Ther*. 2014;19:16–24.
  440. Ohmann EL, Burckart GJ, Brooks MM, et al. Genetic polymorphisms influence mycophenolate mofetil-related adverse events in pediatric heart transplant patients. *J Heart Lung Transpl*. 2010;29:509–516.
  441. McCune JS, Storer B, Thomas S, et al. Inosine monophosphate dehydrogenase pharmacogenetics in hematopoietic cell transplantation patients. *Biol Blood Marrow Transpl*. 2018;24:1802–1807.
  442. Cao W, Xiao H, Lai X, et al. Genetic variations in the mycophenolate mofetil target enzyme are associated with acute GVHD risk after related and unrelated hematopoietic cell transplantation. *Biol Blood Marrow Transpl*. 2012;18:273–279.
  443. Shaw LM, Korecka M, Aradhye S, et al. Mycophenolic acid area under the curve values in African American and Caucasian renal transplant patients are comparable. *J Clin Pharmacol*. 2000;40:624–633.
  444. Pescovitz MD, Guasch A, Gaston R, et al. Equivalent pharmacokinetics of mycophenolate mofetil in African-American and Caucasian male and female stable renal allograft recipients. *Am J Transpl*. 2003;3:1581–1586.
  445. Borrows R, Chusney G, James A, et al. Determinants of mycophenolic acid levels after renal transplantation. *Ther Drug Monit*. 2005;27:442–450.
  446. van Hest RM, Mathot RA, Pescovitz MD, et al. Explaining variability in mycophenolic acid exposure to optimize mycophenolate mofetil dosing: a population pharmacokinetic meta-analysis of mycophenolic acid in renal transplant recipients. *J Am Soc Nephrol*. 2006;17:871–880.
  447. de Winter B, van Gelder T. Why and how to perform therapeutic drug monitoring for mycophenolate mofetil. *Trends Transplant*. 2007:24–34.
  448. Tornatore KM, Sudchada P, Dole K, et al. Mycophenolic acid pharmacokinetics during maintenance immunosuppression in African American and Caucasian renal transplant recipients. *J Clin Pharmacol*. 2011;51:1213–1222.
  449. Tornatore KM, Meaney CJ, Wilding GE, et al. Influence of sex and race on mycophenolic acid pharmacokinetics in stable African American and Caucasian renal transplant recipients. *Clin Pharmacokinet*. 2015;54:423–434.
  450. Nicole LM, Tanguay RM. On the specificity of antisense RNA to arrest in vitro translation of mRNA coding for Drosophila hsp 23. *Biosci Rep*. 1987;7:239–246.
  451. Liang MZ, Lu YP, Nan F, et al. Pharmacokinetics of mycophenolic acid after a single and multiple oral doses of mycophenolate mofetil in Chinese renal transplant recipients. *Transpl Proc*. 2004;36:2065–2067.
  452. Lu XY, Huang HF, Sheng-Tu JZ, et al. Pharmacokinetics of mycophenolic acid in Chinese kidney transplant patients. *J Zhejiang Univ Sci B*. 2005;6:885–891.
  453. Zicheng Y, Peijun Z, Da X, et al. Investigation on pharmacokinetics of mycophenolic acid in Chinese adult renal transplant patients. *Br J Clin Pharmacol*. 2006;62:446–452.
  454. Zhou PJ, Xu D, Yu ZC, et al. Pharmacokinetics of mycophenolic acid and estimation of exposure using multiple linear regression equations in Chinese renal allograft recipients. *Clin Pharmacokinet*. 2007;46:389–401.
  455. Kagaya H, Inoue K, Miura M, et al. Quantification and 24-hour monitoring of mycophenolic acid by high-performance liquid chromatography in Japanese renal transplant recipients. *Yakugaku Zasshi*. 2006;126:1357–1362.
  456. Miura M, Satoh S, Niioka T, et al. Early phase limited sampling strategy characterizing tacrolimus and mycophenolic acid pharmacokinetics adapted to the maintenance phase of renal transplant patients. *Ther Drug Monit*. 2009;31:467–474.
  457. Yau WP, Vathsala A, Lou HX, et al. Is a standard fixed dose of mycophenolate mofetil ideal for all patients? *Nephrol Dial Transpl*. 2007;22:3638–3645.
  458. Li P, Shuker N, Hesselink DA, et al. Do Asian renal transplant patients need another mycophenolate mofetil dose compared with Caucasian or African American patients? *Transpl Int*. 2014;27:994–1004.
  459. Carr SF, Papp E, Wu JC, et al. Characterization of human type I and type II IMP dehydrogenases. *J Biol Chem*. 1993;268:27286–27290.
  460. Zimmermann AG, Spychala J, Mitchell BS. Characterization of the human inosine-5'-monophosphate dehydrogenase type II gene. *J Biol Chem*. 1995;270:6808–6814.
  461. Gu JJ, Stegmann S, Gathy K, et al. Inhibition of T lymphocyte activation in mice heterozygous for loss of the IMPDH II gene. *J Clin Invest*. 2000;106:599–606.
  462. Gu JJ, Spychala J, Mitchell BS. Regulation of the human inosine monophosphate dehydrogenase type I gene. Utilization of alternative promoters. *J Biol Chem*. 1997;272:4458–4466.
  463. Balzarini J, De Clercq E. Assay method for monitoring the inhibitory effects of antimetabolites on the activity of inosinate dehydrogenase in intact human CEM lymphocytes. *Biochem J*. 1992;287:785–790.
  464. Montero C, Duley JA, Fairbanks LD, et al. Demonstration of induction of erythrocyte inosine monophosphate dehydrogenase activity in Ribavirin-treated patients using a high performance liquid chromatography linked method. *Clin Chim Acta*. 1995;238:169–178.
  465. Albrecht W, Storck M, Pfetsch E, et al. Development and application of a high-performance liquid chromatography-based assay for determination of the activity of inosine 5'-monophosphate dehydrogenase in whole blood and isolated mononuclear cells. *Ther Drug Monit*. 2000;22:283–294.
  466. Glander P, Braun KP, Hambach P, et al. Non-radioactive determination of inosine 5'-monophosphate dehydrogenase (IMPDH) in peripheral mononuclear cells. *Clin Biochem*. 2001;34:543–549.
  467. Vethe NT, Bergan S. Determination of inosine monophosphate dehydrogenase activity in human CD4+ cells isolated from whole blood during mycophenolic acid therapy. *Ther Drug Monit*. 2006;28:608–613.
  468. Kalsi K, Marinaki AM, Yacoub MH, et al. HPLC/tandem ion trap mass detector methods for determination of inosine monophosphate dehydrogenase (IMPDH) and thiopurine methyltransferase (TPMT). *Nucleosides Nucleotides Nucleic Acids*. 2006;25:1241–1244.
  469. Langman LJ, LeGatt DF, Halloran PF, et al. Pharmacodynamic assessment of mycophenolic acid-induced immunosuppression in renal transplant recipients. *Transplantation*. 1996;62:666–672.
  470. Vethe NT, Mandla R, Line PD, et al. Inosine monophosphate dehydrogenase activity in renal allograft recipients during mycophenolate treatment. *Scand J Clin Lab Invest*. 2006;66:31–44.

471. Glander P, Hambach P, Braun KP, et al. Effect of mycophenolate mofetil on IMP dehydrogenase after the first dose and after long-term treatment in renal transplant recipients. *Int J Clin Pharmacol Ther.* 2003;41:470–476.
472. Vethe NT, Bremer S, Rootwelt H, et al. Pharmacodynamics of mycophenolic acid in CD4+ cells: a single-dose study of IMPDH and purine nucleotide responses in healthy individuals. *Ther Drug Monit.* 2008;30:647–655.
473. Glander P, Waiser J, Hambach P, et al. Inosine 5'-monophosphate dehydrogenase activity for the longitudinal monitoring of mycophenolic acid treatment in kidney allograft recipients. *Transplantation.* 2020. doi: 10.1097/TP.0000000000003336. [epub ahead of print].
474. Sanquer S, Breil M, Baron C, et al. Induction of inosine monophosphate dehydrogenase activity after long-term treatment with mycophenolate mofetil. *Clin Pharmacol Ther.* 1999;65:640–648.
475. Weigel G, Griesmacher A, Zuckermann AO, et al. Effect of mycophenolate mofetil therapy on inosine monophosphate dehydrogenase induction in red blood cells of heart transplant recipients. *Clin Pharmacol Ther.* 2001;69:137–144.
476. Bremer S, Mandla R, Vethe NT, et al. Expression of IMPDH1 and IMPDH2 after transplantation and initiation of immunosuppression. *Transplantation.* 2008;85:55–61.
477. Sanquer S, Maison P, Tomkiewicz C, et al. Expression of inosine monophosphate dehydrogenase type I and type II after mycophenolate mofetil treatment: a 2-year follow-up in kidney transplantation. *Clin Pharmacol Ther.* 2008;83:328–335.
478. Klaasen RA, Bergan S, Bremer S, et al. Pharmacodynamic assessment of mycophenolic acid in resting and activated target cell population during the first year after renal transplantation. *Br J Clin Pharmacol.* 2020;86:1100–1112.
479. Devyatko E, Zuckermann A, Bohdjalian A, et al. Activation of the purine salvage pathway in mononuclear cells of cardiac recipients treated with mycophenolate mofetil. *Transplantation.* 2006;82:113–118.
480. Glander P, Hambach P, Braun KP, et al. Pre-transplant inosine monophosphate dehydrogenase activity is associated with clinical outcome after renal transplantation. *Am J Transpl.* 2004;4:2045–2051.
481. Chiarelli LR, Molinaro M, Libetta C, et al. Inosine monophosphate dehydrogenase variability in renal transplant patients on long-term mycophenolate mofetil therapy. *Br J Clin Pharmacol.* 2010;69:38–50.
482. Neuberger M, Sommerer C, Bohnisch S, et al. Effect of mycophenolic acid on inosine monophosphate dehydrogenase (IMPDH) activity in liver transplant patients. *Clin Res Hepatol Gastroenterol.* 2020;44:543–550.
483. Budde K, Sommerer C, Rissling O, et al. Target enzyme activity and phosphorylation of pathway molecules as specific biomarkers in transplantation. *Ther Drug Monit.* 2016;38(suppl 1):S43–S49.
484. Sombogaard F, Peeters AM, Baan CC, et al. Inosine monophosphate dehydrogenase messenger RNA expression is correlated to clinical outcomes in mycophenolate mofetil-treated kidney transplant patients, whereas inosine monophosphate dehydrogenase activity is not. *Ther Drug Monit.* 2009;31:549–556.
485. Molinaro M, Chiarelli LR, Biancone L, et al. Monitoring of inosine monophosphate dehydrogenase activity and expression during the early period of mycophenolate mofetil therapy in de novo renal transplant patients. *Drug Metab Pharmacokinet.* 2013;28:109–117.
486. Camici M, Garcia-Gil M, Pesi R, et al. Purine-metabolising enzymes and apoptosis in cancer. *Cancers (Basel).* 2019;11:1354.
487. Goldsmith D, Carrey EA, Edbury S, et al. Mycophenolate mofetil, an inhibitor of inosine monophosphate dehydrogenase, causes a paradoxical elevation of GTP in erythrocytes of renal transplant patients. *Clin Sci (Lond).* 2004;107:63–68.
488. Jagodzinski P, Lizakowski S, Smolenski RT, et al. Mycophenolate mofetil treatment following renal transplantation decreases GTP concentrations in mononuclear leucocytes. *Clin Sci (Lond).* 2004;107:69–74.
489. Xing J, Apedo A, Tymiak A, et al. Liquid chromatographic analysis of nucleosides and their mono-, di- and triphosphates using porous graphitic carbon stationary phase coupled with electrospray mass spectrometry. *Rapid Commun Mass Spectrom.* 2004;18:1599–1606.
490. Weigel G, Griesmacher A, Karimi A, et al. Effect of mycophenolate mofetil therapy on lymphocyte activation in heart transplant recipients. *J Heart Lung Transpl.* 2002;21:1074–1079.
491. Jucaud V, Shaked A, DesMarais M, et al. Prevalence and impact of de novo donor-specific antibodies during a multicenter immunosuppression withdrawal trial in adult liver transplant recipients. *Hepatology.* 2019;69:1273–1286.
492. Kuypers DR. Immunosuppressive drug therapy and subclinical acute renal allograft rejection: impact and effect. *Transplantation.* 2008;85(7 suppl):S25–S30.
493. Oellerich M, Walson PD, Beck J, et al. Graft-derived cell-free DNA as a marker of transplant graft injury. *Ther Drug Monit.* 2016;38(suppl 1):S75–S79.
494. Wieland E, Shipkova M. Lymphocyte surface molecules as immune activation biomarkers. *Clin Biochem.* 2016;49:347–354.
495. Barten MJ, van Gelder T, Gummert JF, et al. Novel assays of multiple lymphocyte functions in whole blood measure: new mechanisms of action of mycophenolate mofetil in vivo. *Transpl Immunol.* 2002;10:1–14.
496. Allison AC, Eugui EM. Purine metabolism and immunosuppressive effects of mycophenolate mofetil (MMF). *Clin Transpl.* 1996;10:77–84.
497. Glomsda BA, Blaheta RA, Hailer NP. Inhibition of monocyte/endothelial cell interactions and monocyte adhesion molecule expression by the immunosuppressant mycophenolate mofetil. *Spinal Cord.* 2003;41:610–619.
498. Klaeske K, Lehmann S, Buttner P, et al. Identification of the immunological profile in rejection-free heart transplantation. *Transpl Immunol.* 2020;59:101259.
499. Stalder M, Birsan T, Holm B, et al. Quantification of immunosuppression by flow cytometry in stable renal transplant recipients. *Ther Drug Monit.* 2003;25:22–27.
500. Daemen BJ, Elsinga PH, Mooibroek J, et al. PET measurements of hyperthermia-induced suppression of protein synthesis in tumors in relation to effects on tumor growth. *J Nucl Med.* 1991;32:1587–1592.
501. Barten MJ, Rahmel A, Garbade J, et al. C0h/C2h monitoring of the pharmacodynamics of cyclosporin plus mycophenolate mofetil in human heart transplant recipients. *Transpl Proc.* 2005;37:1360–1361.
502. Kamar N, Glander P, Nolting J, et al. Effect of mycophenolate mofetil monotherapy on T-cell functions and inosine monophosphate dehydrogenase activity in patients undergoing a kidney transplantation. *Transpl Proc.* 2006;38:2292–2294.
503. Kamar N, Glander P, Nolting J, et al. Pharmacodynamic evaluation of the first dose of mycophenolate mofetil before kidney transplantation. *Clin J Am Soc Nephrol.* 2009;4:936–942.
504. Premaud A, Rousseau A, Johnson G, et al. Inhibition of T-cell activation and proliferation by mycophenolic acid in patients awaiting liver transplantation: PK/PD relationships. *Pharmacol Res.* 2011;63:432–438.
505. Barten MJ, Dhein S, Chang H, et al. Assessment of immunosuppressive drug interactions: inhibition of lymphocyte function in peripheral human blood. *J Immunol Methods.* 2003;283:99–114.
506. Shipkova M, Wieland E. Surface markers of lymphocyte activation and markers of cell proliferation. *Clin Chim Acta.* 2012;413:1338–1349.
507. Hutchinson P, Chadban SJ, Atkins RC, et al. Laboratory assessment of immune function in renal transplant patients. *Nephrol Dial Transpl.* 2003;18:983–989.
508. Hood KA, Zarembski DG. Mycophenolate mofetil: a unique immunosuppressive agent. *Am J Health Syst Pharm.* 1997;54:285–294.
509. Avery DT, Deenick EK, Ma CS, et al. B cell-intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. *J Exp Med.* 2010;207:155–171.
510. Takebe N, Cheng X, Fandy TE, et al. IMP dehydrogenase inhibitor mycophenolate mofetil induces caspase-dependent apoptosis and cell cycle inhibition in multiple myeloma cells. *Mol Cancer Ther.* 2006;5:457–466.
511. Eickenberg S, Mickholz E, Jung E, et al. Mycophenolic acid counteracts B cell proliferation and plasmablast formation in patients with systemic lupus erythematosus. *Arthritis Res Ther.* 2012;14:R110.
512. Li Z, Wen X, Wang Y, et al. Effect of mycophenolic acid and bortezomib on purified human B cells: an in vitro study of long-term functionally stable MICA-sensitized renal recipients. *Exp Clin Transpl.* 2013;11:482–488.
513. Matz M, Lehnert M, Lorkowski C, et al. Effects of sotrastaurin, mycophenolic acid and everolimus on human B-lymphocyte function and activation. *Transpl Int.* 2012;25:1106–1116.

514. Aubert O, Loupy A, Hidalgo L, et al. Antibody-mediated rejection due to preexisting versus de novo donor-specific antibodies in kidney allograft recipients. *J Am Soc Nephrol*. 2017;28:1912–1923.
515. Masaki N, Iwadoh K, Tonsho M, et al. trough level of mycophenolic acid did not affect de novo DSA development in kidney transplantation. *Transpl Proc*. 2019;51:2624–2628.
516. Liu Z, Yuan X, Luo Y, et al. Evaluating the effects of immunosuppressants on human immunity using cytokine profiles of whole blood. *Cytokine*. 2009;45:141–147.
517. Huang XC, He Y, Zhuang J, et al. Mycophenolic acid synergizing with lipopolysaccharide to induce interleukin-1beta release via activation of caspase-1. *Chin Med J (Engl)*. 2018;131:1533–1540.
518. Huang X, Huang Q, He Y, et al. Mycophenolic acid enhanced lipopolysaccharide-induced interleukin-18 release in THP-1 cells via activation of the NLRP3 inflammasome. *Immunopharmacol Immunotoxicol*. 2019;41:521–526.
519. Duitman EH, Orinska Z, Bulfone-Paus S. Mechanisms of cytokine secretion: a portfolio of distinct pathways allows flexibility in cytokine activity. *Eur J Cell Biol*. 2011;90:476–483.
520. Leung BP, Culshaw S, Gracie JA, et al. A role for IL-18 in neutrophil activation. *J Immunol*. 2001;167:2879–2886.
521. ter Meulen CG, Wetzel JF, Hilbrands LB. The influence of mycophenolate mofetil on the incidence and severity of primary cytomegalovirus infections and disease after renal transplantation. *Nephrol Dial Transpl*. 2000;15:711–714.
522. Song AT, Abdala E, Bonazzi PR, et al. Does mycophenolate mofetil increase the risk of cytomegalovirus infection in solid organ transplant recipients?—A mini-review. *Braz J Infect Dis*. 2006;10:132–138.
523. van den Berg AP, van Son WJ, Janssen RA, et al. Recovery from cytomegalovirus infection is associated with activation of peripheral blood lymphocytes. *J Infect Dis*. 1992;166:1228–1235.
524. Sadeghi M, Daniel V, Naujokat C, et al. Dysregulated cytokine responses during cytomegalovirus infection in renal transplant recipients. *Transplantation*. 2008;86:275–285.
525. Essa S, Raghupathy R, Pacsa AS, et al. Th1-type cytokines production is decreased in kidney transplant recipients with active cytomegalovirus infection. *J Med Virol*. 2000;60:223–229.
526. Egli A, Kumar D, Broscheit C, et al. Comparison of the effect of standard and novel immunosuppressive drugs on CMV-specific T-cell cytokine profiling. *Transplantation*. 2013;95:448–455.
527. Antonysamy MA, Fanslow WC, Fu F, et al. Evidence for a role of IL-17 in alloimmunity: a novel IL-17 antagonist promotes heart graft survival. *Transpl Proc*. 1999;31:93.
528. Loong CC, Hsieh HG, Lui WY, et al. Evidence for the early involvement of interleukin 17 in human and experimental renal allograft rejection. *J Pathol*. 2002;197:322–332.
529. Abadja F, Atemkeng S, Alamartine E, et al. Impact of mycophenolic acid and tacrolimus on Th17-related immune response. *Transplantation*. 2011;92:396–403.
530. Kannegieter NM, Hesselink DA, Dieterich M, et al. The effect of tacrolimus and mycophenolic acid on CD14+ monocyte activation and function. *PLoS One*. 2017;12:e0170806.
531. Iwasaki K, Kitahata N, Miwa Y, et al. Suppressive effect of everolimus on IL-2, IL-10, IL-21, and IFNgamma levels: implications for the successful minimization of calcineurin inhibitor use in transplantation. *Ther Drug Monit*. 2019;41:371–375.
532. Millan O, Brunet M, Campistol JM, et al. Pharmacodynamic approach to immunosuppressive therapies using calcineurin inhibitors and mycophenolate mofetil. *Clin Chem*. 2003;49:1891–1899.
533. Grinyo JM, Cruzado JM, Millan O, et al. Low-dose cyclosporine with mycophenolate mofetil induces similar calcineurin activity and cytokine inhibition as does standard-dose cyclosporine in stable renal allografts. *Transplantation*. 2004;78:1400–1403.
534. Barten MJ, Rahmel A, Boci J, et al. Cytokine analysis to predict immunosuppression. *Cytometry A*. 2006;69:155–157.
535. De Vlaminck I, Valantine HA, Snyder TM, et al. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. *Sci Transl Med*. 2014;6:241ra277.
536. Sherwood K, Weimer ET. Characteristics, properties, and potential applications of circulating cell-free dna in clinical diagnostics: a focus on transplantation. *J Immunol Methods*. 2018;463:27–38.
537. Verhoeven J, Boer K, Van Schaik RHN, et al. Liquid biopsies to monitor solid organ transplant function: a review of new biomarkers. *Ther Drug Monit*. 2018;40:515–525.
538. Schutz E, Asendorf T, Beck J, et al. Time-dependent apparent increase in dd-cfDNA percentage in clinically stable patients between one and five years following kidney transplantation. *Clin Chem*. 2020;66:1290–1299.
539. Knight SR, Thorne A, Lo Faro ML. Donor-specific cell-free DNA as a biomarker in solid organ transplantation. A systematic review. *Transplantation*. 2019;103:273–283.
540. Oellerich M, Christenson RH, Beck J, et al. Donor-derived cell-free DNA testing in solid organ transplantation: a value proposition. *J Appl Lab Med*. 2020;5:993–1004.
541. Oellerich M, Schutz E, Kanzow P, et al. Use of graft-derived cell-free DNA as an organ integrity biomarker to reexamine effective tacrolimus trough concentrations after liver transplantation. *Ther Drug Monit*. 2014;36:136–140.
542. Oellerich M, Shipkova M, Asendorf T, et al. Absolute quantification of donor-derived cell-free DNA as a marker of rejection and graft injury in kidney transplantation: results from a prospective observational study. *Am J Transpl*. 2019;19:3087–3099.
543. Kanzow P, Kollmar O, Schutz E, et al. Graft-derived cell-free DNA as an early organ integrity biomarker after transplantation of a marginal HELLIP syndrome donor liver. *Transplantation*. 2014;98:e43–45.
544. Wijnvliet V, Plaeke P, Abrams S, et al. Donor-derived cell-free dna as a biomarker for rejection after kidney transplantation: a systematic review and meta-analysis. *Transpl Int*. 2020;33:1626–1642.
545. Huang E, Sethi S, Peng A, et al. Early clinical experience using donor-derived cell-free DNA to detect rejection in kidney transplant recipients. *Am J Transpl*. 2019;19:1663–1670.
546. Sigdel TK, Archila FA, Constantin T, et al. Optimizing detection of kidney transplant injury by assessment of donor-derived cell-free DNA via massively multiplex PCR. *J Clin Med*. 2019;8:19.
547. Funaki T. Enterohepatic circulation model for population pharmacokinetic analysis. *J Pharm Pharmacol*. 1999;51:1143–1148.
548. Shum B, Duffull SB, Taylor PJ, et al. Population pharmacokinetic analysis of mycophenolic acid in renal transplant recipients following oral administration of mycophenolate mofetil. *Br J Clin Pharmacol*. 2003;56:188–197.
549. Staatz CE, Duffull SB, Kiberd B, et al. Population pharmacokinetics of mycophenolic acid during the first week after renal transplantation. *Eur J Clin Pharmacol*. 2005;61:507–516.
550. van Hest RM, van Gelder T, Vulto AG, et al. Population pharmacokinetics of mycophenolic acid in renal transplant recipients. *Clin Pharmacokinet*. 2005;44:1083–1096.
551. van Hest RM, van Gelder T, Bouw R, et al. Time-dependent clearance of mycophenolic acid in renal transplant recipients. *Br J Clin Pharmacol*. 2007;63:741–752.
552. de Winter BC, van Gelder T, Glander P, et al. Population pharmacokinetics of mycophenolic acid: a comparison between enteric-coated mycophenolate sodium and mycophenolate mofetil in renal transplant recipients. *Clin Pharmacokinet*. 2008;47:827–838.
553. Yau WP, Vathsala A, Lou HX, et al. Mechanism-based enterohepatic circulation model of mycophenolic acid and its glucuronide metabolite: assessment of impact of cyclosporine dose in Asian renal transplant patients. *J Clin Pharmacol*. 2009;49:684–699.
554. de Winter BC, Mathot RA, Sombogaard F, et al. Differences in clearance of mycophenolic acid among renal transplant recipients, hematopoietic stem cell transplant recipients, and patients with autoimmune disease. *Ther Drug Monit*. 2010;32:606–614.
555. Premaud A, Weber LT, Tonshoff B, et al. Population pharmacokinetics of mycophenolic acid in pediatric renal transplant patients using parametric and nonparametric approaches. *Pharmacol Res*. 2011;63:216–224.
556. Wang XX, Feng MR, Nguyen H, et al. Population pharmacokinetics of mycophenolic acid in lung transplant recipients with and without cystic fibrosis. *Eur J Clin Pharmacol*. 2015;71:673–679.
557. Rong Y, Mayo P, Ensom MHH, et al. Population pharmacokinetics of mycophenolic acid Co-administered with tacrolimus in corticosteroid-free adult kidney transplant patients. *Clin Pharmacokinet*. 2019;58:1483–1495.
558. Dong M, Fukuda T, Cox S, et al. Population pharmacokinetic-pharmacodynamic modelling of mycophenolic acid in paediatric renal

- transplant recipients in the early post-transplant period. *Br J Clin Pharmacol*. 2014;78:1102–1112.
559. Cremers S, Schoemaker R, Scholten E, et al. Characterizing the role of enterohepatic recycling in the interactions between mycophenolate mofetil and calcineurin inhibitors in renal transplant patients by pharmacokinetic modelling. *Br J Clin Pharmacol*. 2005;60:249–256.
560. Sam WJ, Akhlaghi F, Rosenbaum SE. Population pharmacokinetics of mycophenolic acid and its 2 glucuronidated metabolites in kidney transplant recipients. *J Clin Pharmacol*. 2009;49:185–195.
561. Wang XX, Liu W, Zheng T, et al. Population pharmacokinetics of mycophenolic acid and its glucuronide metabolite in lung transplant recipients with and without cystic fibrosis. *Xenobiotica*. 2017;47:697–704.
562. Abd Rahman AN, Tett SE, Abdul Gafor HA, et al. Development of improved dosing regimens for mycophenolate mofetil based on population pharmacokinetic analyses in adults with lupus nephritis. *Eur J Drug Metab Pharmacokinet*. 2017;42:993–1004.
563. Zeng L, Blair EY, Nath CE, et al. Population pharmacokinetics of mycophenolic acid in children and young people undergoing blood or marrow and solid organ transplantation. *Br J Clin Pharmacol*. 2010;70:567–579.
564. Li J, Liu Y, Huang J, et al. Evaluation of mycophenolic acid exposure using a limited sampling strategy in renal transplant recipients. *Am J Nephrol*. 2013;37:534–540.
565. Insight-RX. Available at: <https://www.insight-rx.com/>. Accessed October 26, 2020.
566. MwPharm++, MEDIWARE, Inc. Available at: <http://www.mediware.cz/en/home/>. Accessed October 26, 2020.
567. Limoges University Hospital Laboratory of Pharmacology. Available at: <https://pharmaco.chu-limoges.fr/>. Accessed October 26, 2020.
568. *UpToDate Grading Guide*. 2020. Available at: <https://www.uptodate.com/home/grading-guide>. Accessed November 3, 2020.
569. Andrews J, Guyatt G, Oxman AD, et al. GRADE guidelines: 14. Going from evidence to recommendations: the significance and presentation of recommendations. *J Clin Epidemiol*. 2013;66:719–725.
570. Benoit-Biancamano MO, Caron P, Lévesque E, et al. Sensitive high-performance liquid chromatography-tandem mass spectrometry method for quantitative analysis of mycophenolic acid and its glucuronide metabolites in human plasma and urine. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007;858:159–167.
571. Kuhn J, Prante C, Kleesiek K, et al. Measurement of mycophenolic acid and its glucuronide using a novel rapid liquid chromatography-electrospray ionization tandem mass spectrometry assay. *Clin Biochem*. 2009;42:83–90.
572. Delavenne X, Juthier L, Pons B, et al. UPLC MS/MS method for quantification of mycophenolic acid and metabolites in human plasma: application to pharmacokinetic study. *Clin Chim Acta* 2011;412:59–65.
573. Garg U, Munar A, Frazee C. Determination of mycophenolic acid and mycophenolic acid glucuronide using liquid chromatography tandem mass spectrometry (LC/MS/MS). *Curr Protoc Toxicol*. 2018;75:18.21.11–18.21.18.
574. Brandhorst G, Streit F, Goetze S, et al. Quantification by liquid chromatography tandem mass spectrometry of mycophenolic acid and its phenol and acyl glucuronide metabolites. *Clin Chem*. 2006;52:1962–1964.
575. Buchwald A, Winkler K, Epting T. Validation of an LC-MS/MS method to determine five immunosuppressants with deuterated internal standards including MPA. *BMC Clin Pharmacol*. 2012;12:2.
576. Zegarska J, Hryniewiecka E, Zochowska D, et al. Mycophenolic acid metabolites acyl-glucuronide and glucoside affect the occurrence of infectious complications and bone marrow dysfunction in liver transplant recipients. *Ann Transpl*. 2015;20:483–492.
577. Difrancesco R, Frerichs V, Donnelly J, et al. Simultaneous determination of cortisol, dexamethasone, methylprednisolone, prednisone, prednisolone, mycophenolic acid and mycophenolic acid glucuronide in human plasma utilizing liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007;859:42–51.
578. Mohamed MF, Harvey SS, Frye RF. Determination of mycophenolic acid glucuronide in microsomal incubations using high performance liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2008;870:251–254.
579. Whirl-Carrillo M, McDonagh EM, Hebert JM, et al. Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther*. 2012;92:414–417.
580. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*. 2001;29:308–311.
581. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581:434–443.
582. UGT Nomenclature Committee. *UGT Alleles Nomenclature Home Page*. 2005. Available at: <http://www.ugtalleles.ulaval.ca>. Accessed January 14, 2021.
583. Zakerska O, Skrzypczak-Zielinska M, Mikstacki A, et al. Genotype and allele frequencies of polymorphic UGT1A9 in the Polish population. *Eur J Drug Metab Pharmacokinet*. 2013;38:217–221.
584. Stingl JC, Bartels H, Viviani R, et al. Relevance of UDP-glucuronosyltransferase polymorphisms for drug dosing: a quantitative systematic review. *Pharmacol Ther*. 2014;141:92–116.
585. Garat A, Cardenas CL, Lionet A, et al. Inter-ethnic variability of three functional polymorphisms affecting the IMPDH2 gene. *Mol Biol Rep*. 2011;38:5185–5188.
586. Böhler T, Canivet C, Galvani S, et al. Pharmacodynamic monitoring of the conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium in stable kidney-allograft recipients. *Int Immunopharmacol* 2008;8:769–773.
587. Beck J, Bierau S, Balzer S, et al. Digital droplet PCR for rapid quantification of donor DNA in the circulation of transplant recipients as a potential universal biomarker of graft injury. *Clin Chem*. 2013;59:1732–1741.