UNIVERSITAT DE BARCELONA

Study of the Influence of a Combination of Pharmacogenetic Variables on Tacrolimus Exposure: A Population Pharmacokinetic Approach

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STUDY OF THE INFLUENCE OF A COMBINATION OF PHARMACOGENETIC VARIABLES ON TACROLIMUS EXPOSURE: A POPULATION PHARMACOKINETIC APPROACH.

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STUDY OF THE INFLUENCE OF A COMBINATION OF PHARMACOGENETIC VARIABLES ON TACROLIMUS EXPOSURE: A POPULATION PHARMACOKINETIC APPROACH.

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FRANC ANDREU SOLDUGA

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CERTIFY

That **FRANC ANDREU SOLDUGA,** graduate in Pharmacy by the University of Barcelona, has carried out under our direction, in the Biopharmacy and Pharmacokinetics Unit, Department of Pharmacy and Pharmaceutical Technology and Physical Chemistry at the Faculty of Pharmacy and Food Sciences, University of Barcelona as well as in the Laboratory of Experimental Nephrology University Hospital of Bellvitge, the research work to elaborate his Doctoral Thesis "**STUDY OF THE INFLUENCE OF A COMBINATION OF PHARMACOGENETIC VARIABLES ON TACROLIMUS EXPOSURE: A POPULATION PHARMACOKINETIC APPROACH"** and through this writing signature they authorize its presentation to achieve the Degree of Doctor by the University of Barcelona.

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DEFINITIONS AND ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism and Elimination
AUC	Area Under the Curve
BPAR	Biopsy Proven Acute rejection
C_0	Trough concentration
CL	Clearance
CNI	Calcineurin Inhibitor
CsA	Cyclosporine A
CYP	Cytochrome P450
EC-MPS	Enteric-coated Mycophenolate Sodium
FKBP-12	FK Binding Proteins-12
IIV	Inter-Individual Variability
IOV	Inter-Occasion Variability
K_e	Elimination constant
K_{tr}	Transfer rate constant
L	Liter
LSS	Limited Sampling trategy
MAPB	Maximum A Posteriori Bayesian
MPA	Mycophenolic Acid
MMF	Mycophenolate Mofetil
MT	Mean Transit Time
mTor	Mammalian Target of Rapamycin
npde	Normalized prediction distribution error
Pgp	P-glycoprotein
PK	Pharmacokinetics
POD	post-transplant time
PPAR	Peroxisome Proliferator-activated receptor
PPC	Posterior Predictive Check
PPK	Population Pharmacokinetics
PXR	Pregnane-X-receptor
SNP	Single Nucleotide Polymorphism
rATG	Rabbit Anti-Thymocyte Globulin
Tac	Tacrolimus
TDM	Therapeutic Drug Monitoring
pcVPC	Prediction-corrected Visual Predictive Check

Chapter I

INTRODUCTION

1. RENAL TRANSPLANTATION

Kidney transplantation is the treatment of choice for patients with end-stage chronic kidney disease, with better survival rates and quality of life than patients on long-term dialysis [1]. Renal Transplant is annually increasing reaching in 2015 a total of 2095 renal transplants in Spain according to the Spanish National Transplant Organization.

Advances in immunosuppression have driven kidney transplantation from a scientific curiosity to the optimal treatment for patients with end stage kidney disease. Declining rates of acute rejection have led to improvements in short term kidney transplant survival, culminating in incrementally better long term patient and allograft outcomes.[2] However, long-term results remain suboptimal, immunosuppressant-related nephrotoxicity and chronic allograft rejection are the main causes of allograft loss[3].

1.1 Immunosuppression in renal transplant

Immunosuppression management is not a one-size-fits-all practice. Many factors influence selection of a given regimen, the principal goal being to balance the benefit of rejection prevention against risk of over-immunosuppression. We believe that choice of a regimen should be guided by overall efficacy in addition to immunological and medical risks in individual patients or subpopulations.

Recipients from renal transplant required lifelong administration of immunosuppressive medications to prevent organ rejection. Advances in kidney transplantation have occurred despite relatively few immunosuppression options. Attributing outcome improvement to specific therapies is best appreciated as an evolution through 4 consecutive eras.

Despite excellent short-term outcomes following kidney transplantation, long-term graft function and survival remain suboptimal as half-lives are currently estimated at only ~11 years[1]. Early post-transplant identification of those individuals at highest risk

for developing late graft failure could permit targeted and individualized therapies aimed at improving long-term outcomes.

The immunosuppressive strategies over the past three decades to reduce the incidence of allograft rejection and side-effects of the drugs, and to improve long-term graft and patient survivals. Despite these advances, there is lack of clear evidence of improvement of long-term graft survival because chronic allograft injury continues to cause late renal allografts losses

Thus, the immunosuppressive therapy plays a key role to minimize rejections after solid organ transplantation. The widespread basis for immunosuppression in renal transplant is to use multiple drugs to work on different immunologic targets. The use of a multidrug regimen allows for their different pharmacologic activity at several key steps in the T-cell activation or proliferation to lower dosages of each individual drug, thus producing less drug-related toxicity.[4]

There are three main differentiate clinical immunosuppressive phases during the post-transplant treatment: induction; maintenance and rescue therapy (Table 1).

Table 1. Classification of immunosuppressive agents according to clinical applications.

Induction agents	Maintenance agents	Rescue agents
Polyclonal and monoclonal antibodies: ATG OKT3 Alemtuzumab Rituximab	Calcineurin inhibitors: Cyclosporine Tacrolimus	Mild to moderate cellular rejection: Corticosteroids
Interleukin-2 receptor antagonists: Basiliximab Daclizumab	Anti-metabolites: Azathioprine Mycophenolate mofetil	Moderate to severe cellular rejection: Polyclonal and monoclonal antibodies: ATG OKT3
Methylprednisolone	m-TOR inhibitors: Sirolimus Everolimus	Acute antibody-mediated rejection: Immunoglobulins Rituximab Bortezomib Eculizumab
	Newer agents: Co-stimulation blocker: Belatacept Protein kinase C inhibitor: Sotrastaurin JAK 3 inhibitor: Tofacitinib	

(Kumar A, Shrestha BM. 2016)[5]

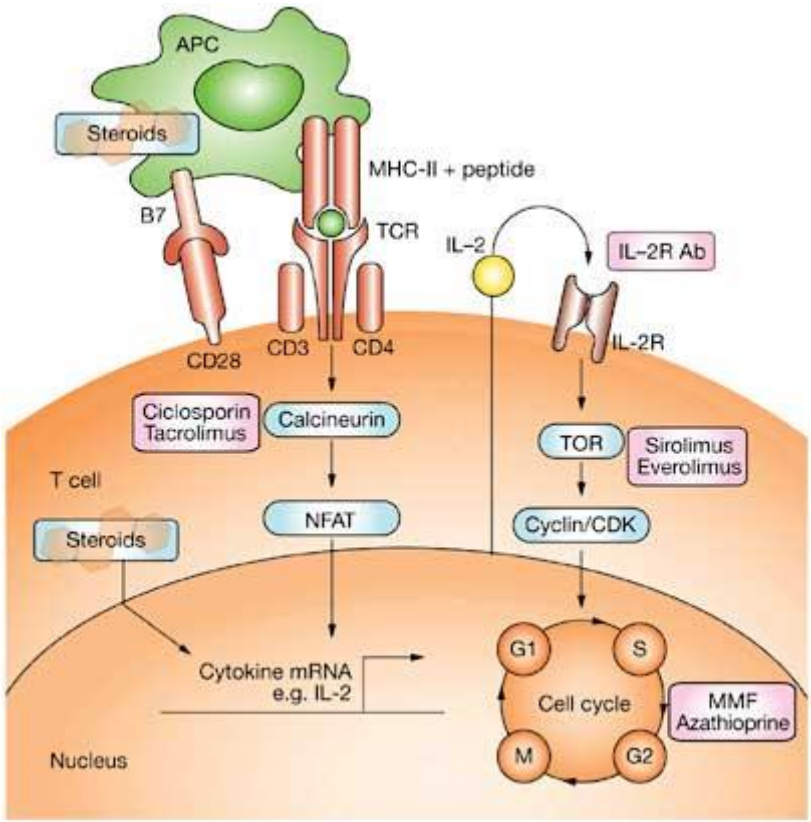
In renal transplant, **induction therapy** consists on to the administration of high doses of immunosuppressants at the time of transplantation, generally prior to organ reperfusion. The goal of induction therapy is to prevent acute rejection during the early posttransplantation period by providing a high degree of immunosuppression at the time of transplantation. Induction therapy is often considered essential to optimize outcomes, particularly in patients at high risk for poor short-term outcomes. All of the induction immunosuppressive agents currently used are biological agents and are either monoclonal (muromonab-CD3, daclizumab, basiliximab, alemtuzumab) or polyclonal (antithymocyte globulin [equine] or antithymocyte globulin [rabbit]) antibodies. No standard induction immunosuppressive regimen exists for patients undergoing renal transplantation. Antithymocyte globulin (rabbit) is the most commonly used agent, whereas basiliximab appears safer. The choice of regimen depends on the preferences of clinicians and institutions[6]. (see table 1)

Rescue agents are used when a transplant rejection is diagnosed. Rejection is identified as acute cellular rejection or acute antibody mediated rejection. Mild cellular rejection used to be treated with corticosteroids, whereas moderate and severe acute cellular rejection is typically treated with rATG. While, the antibody mediated rejection is treated with plasmapheresis, intravenous immunoglobulins and rituximab. Finally, if a refractory antibody mediated rejection is present a proteasome inhibitor (bortezomib) and C5 inhibitor (eculizumab) are used.

Maintenance immunosuppressive therapy is administered to all renal transplant recipients to prevent acute rejection and renal allograft lost. Although an adequate level of immunosuppression is required to dampen the immune response to the allograft, the level of chronic immunosuppression is decreased over time (as the risk of acute rejection decreases) to help lower the overall risk of infection and malignancy. These risks directly correlate with the degree of overall immunosuppression.

The major immunosuppressive agents that are available in various combination regimens[7–9]. The current golden standard of immunosuppressive drug treatment after kidney transplantation, i.e., a combination therapy of tacrolimus and mycophenolate mofetil (MMF), is effective but fraught with side effects[10].

Figure 1. The different Immunosuppressive drug targets on the T-Cell



APC: Antigen Presenting Cells; IL-2: Interleukyne 2; MHC-II: Major Histocompatibility Complex class II; MMF: Mycophenolate Mofetil; NFAT: Nuclear factor of activated T-cells; TCR: T-Cell Receptor; TOR: Target of Rapamycin.
(Jon A Kobashigawa and Jignesh K Patel. 2006)[11]

1.1.1. *Calcineurin Inhibitors (CNIs): Tacrolimus and Cyclosporine*

The CNIs Tacrolimus (Tac) and Cyclosporine (CsA) both binds to different cytoplasmic proteins (cyclophilin and FK binding protein-12 for CsA and Tac, respectively) inhibiting the calcineurin phosphatase. This inhibition prevents the dephosphorilation and translocation of a nuclear factor of activated T-cells (NFAT) involved in the transcription of several cytokine genes that promote T-cell activation and expansion.[8]

CsA[12] is nowadays almost not prescribed and it is not recommended to switch between formulations. Furthermore, CsA is currently being outclassed by Tac due to its better efficacy. In fact many different hospitals centers have established a Tac-based maintenance therapy during the last decade.

Tacrolimus[10] was approved by the regulatory agencies during the mid '90s to prevent to graft rejection in transplant recipients. Over the last decade tacrolimus has become the calcineurin inhibitor of choice for the prevention of rejection in renal transplantation. Tacrolimus exerts its immunosuppressive effects by binding to the intracellular protein FKBP-12 to form an inhibitory complex that blocks the phosphatase activity of calcineurin. A chain of events leads to a complete inhibition of translocation of nuclear factor of activated T-cells, thus preventing cytokine gene transcription and, ultimately, inhibition of T-lymphocyte activation and proliferation. The extensive use of tacrolimus in renal transplantation, coupled with the potential for non-compliance with a standard twice-a-day dosing regimen, has led to the development of a once-daily tacrolimus formulation.

CNIs treatment should be initiated immediately before the chirurgical procedures[13]. The most relevant side effects of CNIs are acute and chronic nephrotoxicity, as well as neurotoxicity, hypertension, hyperlipidemia, and posttransplantation diabetes.

As the use of CNIs have significantly reduced the outcomes in the early stages of renal transplant. Two main studies CAESAR and ELITE-SYMPHONY studied the efficacy and safety of CNIs. CAESAR study [14], a multicentric study using 536 de novo transplant

patients focused in the use of CsA. Three groups were defined: (1) standard-dose cyclosporine, (2) daclizumab induction with low-dose cyclosporine (target CsA C_0 50–100 ng/mL), and (3) daclizumab induction with low-dose cyclosporine, which was withdrawn at 6 months. Concomitantly, all groups received MMF and prednisone. The conclusions of the study were that CsA withdrawal groups presented a higher frequency of BPAR and graft damage, while low-dose CsA was not inferior to standard-dose CsA in terms of BPAR or adverse events.

The ELITE-SYMPHONY study [15,16] was also a multicentric study using 1645 renal transplant patients. This study focused into four different immunosuppressive therapies: (1) standard-dose cyclosporine (target cyclosporine trough, 150–300 ng/mL for 3 months, followed by 100–200 ng/mL), (2) daclizumab induction with low-dose tacrolimus (target tacrolimus trough, 3–7 ng/mL), (3) daclizumab induction with low-dose SRL (target sirolimus trough, 4–8 ng/mL), or (4) daclizumab induction with low-dose CsA (target cyclosporine trough, 50–100 ng/mL). All patients received MMF and Prednisone as concomitant therapy. The study showed that Tac treated group had better renal function and graft survival compared with the other immunosuppressive therapies even after three years of follow-up. Furthermore, SRL treated group presented higher adverse events compared to the other treatment groups. The conclusion of this study group is that using Tac low-dose provided the best efficacy and safety profile in renal transplant. Results of these two previous studies lead to suggest by Improving Global Outcomes (KDIGO) group that tacrolimus should be the first-line CNI used.[13]

1.1.2. *Mycophenolic acid (MPA)*

Mycophenolic acid (MPA) is the principal antiproliferative drug used as coadjuvant to CNI in the maintenance treatment in renal transplantation. The introduction of MPA in the last two decades has manifestly prevailed over the use of azathioprine, especially due to the specificity in the inhibition of the T-cell proliferation.

MPA mechanism of action is by inhibiting inosine monophosphate dehydrogenase, a vital enzyme in the de novo pathway of guanosine nucleotide synthesis. Inhibition of this enzyme prevents the proliferation of most cells that are dependent on the de novo pathway for purine synthesis, including lymphocytes. Specifically, the inhibition of inosine monophospate dehydrogenase, arrests cell cycle in the S phase.[8]

MPA was firstly presented as a prodrug Mycophenolate Mofetil (MMF)[17] and afterwards it was presented in enteric-coated mycophenolate sodium (EC-MPS)[18]. The recommended starting dosage for MMF is one gram given twice daily, and 720 mg given twice daily for the enteric-coated mycophenolate sodium that is the equimolar equivalent of MMF which may lead to the recommended target exposure AUC between 30 and 60 mg·h/L. Principal adverse events related to MPA are urinary tract infection, pain, hypertension and diarrhea.

1.1.3. *mTOR inhibitors*

The mTOR inhibitors sirolimus[19] and everolimus[20] are macrolide antibiotics that inhibit lymphocyte activation and proliferation. Intracellularly, both drugs form a complex with FKBP-12 that binds to and modulates the activity of mTOR, a key regulatory kinase in cytokine-dependent T-cell proliferation[8]. The modulation and inhibition of mTOR stops the cell-division cycle in the G1-to-S phase. Furthermore, the hematopoietic and nonhematopoietic cells-lines are affected by both mTOR inhibitors

The oral dosage of sirolimus[19] for patients with low to moderate immunological risk of rejection is a loading dose of 6 mg and then a 2 mg daily and for patients with high

immunological risk of rejection it is recommended a loading dose of 15 mg and then a 5 mg daily dose. Everolimus[20] dosage is 0.75 mg twice daily without loading dose administered in conjunction with CsA and corticosteroids.

Early posttransplant complications in particular the potential to prolong or increase the occurrence of delayed graft function, as well as poor wound healing, lymphocele formation, pneumonitis, and mucositis, have limited the de novo use of sirolimus[21]. Everolimus presents a similar adverse event profile compared to sirolimus. The principal adverse events recorded for mTor inhibitors (Sirolimus and Everolimus) are peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, increased creatinine, constipation and headache among others

2 TACROLIMUS IN RENAL TRANSPLANT

Tacrolimus displays a considerable interpatient and intrapatient variability associated with its pharmacokinetics (PK) (ADME: Absorption, distribution and elimination (metabolism and excretion) processes), pharmacogenetics, however little is known about the magnitude of the pharmacodynamic (PD) variability of tacrolimus. Multiple factors have been identified as contributors of variability as a consequence of its complex pharmacokinetics.

2.1 Pharmacokinetics

2.1.1 Absorption

Tacrolimus is a lipophilic drug with rapid absorption, however in many cases it has a significant lag time prior to absorption, this causing large variability after its oral administration[22]. Studies in pig mucosa have shown that Tacrolimus is mainly absorbed in the duodenum and jejunum [22]. Overall, peak concentrations are observed between 0.5 and 1 hour post-administration, however, slower absorption processes and secondary peaks can occur due to variability in either the gastric emptying, that strongly impacts the rate and extent of intestinal absorption, or in the dissolution rate at the lumen due to its high lipophylicity. In that sense, it should be noted that tacrolimus belongs to Class II of the biopharmaceutic classification system, showing low solubility and high lipophilicity [23].

2.1.2 First pass effect and bioavailability

Tacrolimus presents a poor and variable bioavailability (Mean value of 25% and ranging from 5% to 95%)[24]. The main contributing factors to this low and variable bioavailability are: i) a low solubility, ii) a pre-systemic metabolism through intestinal and hepatic CYP3A iii) a drug efflux into the lumen by the PgP of which it is substrate iv) CYP3A and PgP genetic polymorphisms.

In effect, the P-glycoprotein (Pgp) present in the gut might lower intracellular concentrations of tacrolimus in the enterocyte by pumping absorbed drug back out into the intestinal lumen[25] resulting in variable pass of tacrolimus into systemic circulation. In fact, the presence of Pgp in the gut wall and the high affinity of some CYP3A substrates to this transporter are postulated to reduce the potential for saturating the enzymes, thus increasing first-pass metabolism for compounds which otherwise would have saturated CYP3A[26]. However, the Pgp effect on tacrolimus first pass is yet controversial as some pre-clinical studies indicated the non-relationship between Pgp and tacrolimus first pass[27,28].

Pre-systemic metabolism through intestinal CYP3A is another of the principal causes of the observed variability in biovailability. A study in renal transplant patients [29] showed that in the absence of ketoconazole, a potent CYP3A inhibitor in the gut and liver, tacrolimus first pass metabolism was about 47% higher than in presence of it. This confirms the contribution of CYP3A to the extensive and variable metabolism of tacrolimus in the gut.

2.1.3 Distribution

Tacrolimus binds extensively to erythrocytes in blood, while in plasma, it is mainly bound to α1-acid glycoprotein and to a lesser extent to albumin as well as to other minor proteins[30–32]. Highly variable plasma protein binding has been reported among hepatic transplant patients with mean unbound fractions of 0.47%, ranging from 0.07 to 0.89%. Distribution to erythrocytes is temperature and concentration-dependent and results in blood to plasma ratios ranging from 15 to 35 among patients[29].This is the reason why whole blood concentrations are used when investigating Tac pharmacokinetics and TDM.

Blood distribution and protein binding of tacrolimus vary significantly over the post-transplantation period, leading to changes in its unbound fraction [32]. In fact, the increase of tacrolimus whole blood concentrations observed in several studies [33] could be attributed to an increase in erythrocyte levels over the post-transplantation period as

the clinical condition of the patients improved. Thus, the erythrocyte fraction remains the main reservoir for tacrolimus in blood.

2.1.4 Elimination

Elimination of tacrolimus occurs mainly by metabolism in the liver. Tacrolimus is considered a restrictive clearance drug. The mean total blood clearance is of 37.5 ml/min, about 3% of the liver blood flow. Renal clearance is less than 1% of the total blood clearance. The elimination half-life is about 12 h (ranging from 4 to 41 h)[34,35].

Metabolism occurs in the liver , through the cytochrome P450, in particular, the CYP3A enzymes subfamily[24] [34] (CYP3A4 and CYP3A5 isoforms). In contrast to cyclosporine, CYP3A5 may play a more dominant role in the metabolism of tacrolimus than CYP3A4. Among all the formed metabolites in renal and liver transplant patients[22,36] (demethyl-, demethylhydroxy-, didemethyl-, didemethylhydroxy- and hydroxy-tacrolimus), demethyl- and demethylhydroxy tacrolimus are the most prevalent representing the 3% and 10 % of the Tacrolimus AUC, respectively [22].

Tacrolimus metabolites are mainly excreted in bile (90 %)[34], while the urinary excretion is only around 2.4%[24]. Metabolites blood concentration increases[37] when a biliary obstruction takes place.

2.2 Pharmacogenetics

During the last years, different SNPs have been found to explain the variability on Tacrolimus pharmacokinetics. The CYP3A4 and CYP3A5 subfamilies are the most attributed to Tacrolimus metabolism. The two CYP3A isoforms contribute to the high variability in the Tacrolimus PK [38]. A variation in a single nucleotide (SNPs) at a specific position in the genome, may lead in the CYP3A genome to different Tacrolimus exposure.

A SNP in the CYP3A5 (rs776746) was strongly associated with CYP3A5 protein expression. Individuals carrying at least one CYP3A5*1 allele (wild-type allele) were found to express large amounts of CYP3A5 protein, whereas individuals homozygous for the CYP3A5*3 allele did not express CYP3A5 protein[39]. In this sense, kidney transplant recipients carrying at least one CYP3A5*1 allele (CYP3A5 expressers) required a higher tacrolimus dose to reach the target whole-blood concentration than CYP3A5 non-expressers (i.e. patients homozygous for the CYP3A5*3 allele) [40–43]. Nevertheless, the CYP3A5*3/*1 SNP alone does not explain the major variability in Tacrolimus exposure.

The CYP3A4*1B SNP (rs2740574) is another polymorphism also related to tacrolimus pharmacokinetics. Although there is a higher dose requirement for patients presenting this variant allele, the CYP3A4*1B SNP has not been a consistent finding and the clinical applicability is questioned [44].

In the recent years, the CYP3A4*22 (rs35599367; C>T) in intron 6 has an allele frequency of around 5 % in Caucasians. The T-variant allele has been linked to reduced CYP3A4 mRNA expression and lower in vitro CYP3A4 enzyme activity[45]. CYP3A4*22 is associated with reduced kidney function in CNIs-treated kidney transplant patients. Subsequently, in 185 cohort of renal patients, Elens et al that patients carrying the T-variant allele presented a lower tacrolimus dose requirement than C-homozygous patients[46]. Therefore, CYP3A4*22 is an important marker for identifying reduced metabolism of CYP3A4 drugs based on inheritable factors.

In vitro studies showed that the catalytic activity towards Tacrolimus is 1.6-fold higher for CYP3A5 than for CYP3A4. Furthermore, in vitro data demonstrated that the importance of CYP3A5*3 allelic status is dependent on the concomitant CYP3A4 activity and that the relative contribution of CYP3A4 or CYP3A5 to Tac metabolism depends on the amount of each counterpart[47,48]. In this sense, Elens et al showed that the CYP3A4*22 SNP is not correlated to the CYP3A5*3 and according to the functional defect associated with *CYP3A* variants, the *CYP3A* genotypes were classified in 3 different clusters[46,49]:

- Extensive metabolizers: patients carrying a CYP3A5*1 allele and with the CYP3A4*22 CC genotype
- Intermediate metabolizers: patients either CYP3A5 expressers carrying a CYP3A4*22 T-variant allele or CYP3A5 non- expressers with the CYP3A4*22 CC genotype
- Poor metabolizers: patients CYP3A5 non-expressers carrying a CYP3A4*22 T-variant allele.

For Tacrolimus exposure, extensive metabolizers (EM) require a higher Tac dose than intermediate metabolizers (IM) who, at their turn, require higher Tac dose than poor metabolizers (PM). The CYP3A4*22 has also been statistically significant when included in new dose-algorithm[50,51]. However, the CYP3A4*22 due its poor prevalence[52], in particular the poor metabolizers phenotypes, not all research groups could have confirmed this finding[53].

The Nicotinamide adenine dinucleotide phosphate (NADPH)-CYP oxidoreductase (POR) is another SNP related to Tac exposure. Different polymorphism in the POR gene affects to the CYP-POR complex leading to alterations in the CYP activity[54]. Recently, it has been described that POR*28 (rs1057868; C>T) may affect the dose-requirements. CYP3A5 patients carriers of T-allele for POR*28 required a higher dose than patients CYP3A5 expressers of POR*28 CC homozygous allele[55,56]. On the other side, the CYP3A5 non-expressers are not affected by the POR*28 SNP for Tacrolimus dose-requirement[43].

The Pgp ABCB1 3435T variant allele was associated with 2-fold lower levels of Pgp in the duodenum, and resulted in 50% higher plasma concentration of digoxin. This effect would be explained by less Pgp presence on the apical surface of the membrane would remove less drug from the cells, resulting in increased bioavailability. Anglicheau et al postulated that these SNPs are associated with Tac PK variations in renal transplant recipients[57]. On the other side, the ABCB1 expression in the brush border of proximal tubular epithelial cells and more distally in the renal tubule may contribute to renal elimination, whereas ABCB1 expression at the canalicular surface of hepatocytes controls excretion into bile[58,59].

Finally, other new SNP have been described by their influence on Tac metabolism such as the human pregnane X receptor (PXR; encoded by NR1I2)[60,61], a nuclear transcription factor that regulates the expression of CYP3A and ABCB1; the peroxisome proliferator-activated receptor (PPAR)-a genetic determinant of CYP3A4 activity[62]; or special sub-population such as the CYP3A4*1G SNPs in Chinese transplant patients[63].

2.3 Therapeutic Drug Monitoring of Tacrolimus

An optimal immunosuppressive therapy is essential for the graft survival. The key point of immunosuppressive agents, with narrow therapeutic index, such as Tacrolimus, is to exhibit the desired therapeutic effect with an acceptable tolerability within a narrow range of blood concentrations[64].

As it has been previously described, the high variability in TAC PK leads to an increased risk of therapeutic failure if these agents are used at the same dose in all the renal transplant patients[65]. Therefore, the principal objective for the clinicians is to achieve the optimum equilibrium between therapeutic efficacy and the incidence and severity of adverse events. Understanding and acknowledging all individual factors that influence Tac pharmacokinetics (i.e. patient's age, body weight, pharmacogenomics, concomitant medication, biochemical factors) can help to find the correct Tacrolimus dose for each patient thus reducing the adverse events.

During the last two decades, the high correlation between Tacrolimus blood concentrations and clinical outcomes have supported the use of therapeutic drug monitoring (TDM)[64].

TDM is universally applied to guide tacrolimus dosing. In most transplant centers tacrolimus (predose) concentrations are measured frequently, especially in the early phase after transplantation. Target concentrations have been empirically defined from multiple ranges from 5 to 12 ng/mL[35]. Therefore, the time that patients are exposed to

sub- or supratherapeutic tacrolimus concentrations is therefore likely to be limited. In most cases, TDM helps to correct any (genetically, biochemical or demographic) inter-individual differences in drug exposure.

However, pharmacodynamic variability due to either genetic or other factors should not be dismissed, although, low attention has been focused on it yet. In daily clinical practice, transplant physicians are confronted with patients who experience toxicity despite having tacrolimus concentrations within what is considered the therapeutic window. Likewise, certain patients may reject their grafts at (supra)therapeutic tacrolimus concentrations, while others do not reject at lower exposure. The genetics of the pharmacodynamics of tacrolimus have hitherto been less well-investigated than the genetics of tacrolimus pharmacokinetics.

Multiple studies stress the importance of between- and within-patient variability in Tacrolimus disposition, related to pharmacokinetics PK and pharmacogenetics PG[64,66]. However, the relationship between Tacrolimus whole blood concentrations and efficacy or toxicity has not yet been fully established. In any case, TDM is a key factor to maintain Tacrolimus exposure within therapeutic range, thus avoiding chronic under or over exposure, that is essential for graft survival and limiting adverse events[64].

The best measure of Tacrolimus exposure is the area under the curve (AUC) which correlates the best with all outcomes. However, it requires an intensive sampling so that is very difficult to be implemented in the clinical routine and very difficult to be financially justified[67].

As consequence, two different valid and major strategies for monitoring Tracrolimus exposure have been currently applied in the hospital transplant units: trough concentrations (C_0) monitoring or limited sampling strategy (LSS).

- Different clinical studies have demonstrated a variable but high correlation between Tacrolimus trough concentrations (C_0) and Tacrolimus AUC values. Thus, supporting the use of C_0 as surrogate of Tacrolimus exposure.

Nevertheless, some limitations may apply to this approximation, the high inter-individual and intra-individual variability associated with C_0, as well as, its tendency to increase with post-transplantation time.

- Another strategy consists on the estimation of AUC from a limited sampling strategy (from 2 to 4 blood samples during the first 8 hours post-dose). Several authors have validated their own sampling strategies (cites) based on at least one sample close to the trough concentration (C_0 or C_8), and one sample close the peak concentration (C_1, C_2, C_3).

Due to its practicality and cost-effectiveness, the C_0 strategy is nowadays the most common used in the clinical practice.

However, TDM remains an incomplete tool. It requires achieving the steady state conditions before applying any dose change which may have already passed three days, depending on Tac half-life. Understanding which covariates influence Tac PK would be the first step through dose individualization. In fact, it is important to quantify the influence of each covariate on Tac PK variability. To better adjust and predict Tacrolimus exposure new PK tools, such as population pharmacokinetics (PPK), have been developed to provide information on typical PK parameter values and variability associated with these values within the population. Furthermore, PPK models can offer how specific patient covariates such as age, weight or genotype influence the PK of a drug.

The main alternative is to use a PPK model in a Maximum A Posteriori Bayesian (MAPB) forecasting technique to estimate total drug exposure based on a limited number of drug–concentration measurements generally taken in the first few hours of the dosing interval, for patient convenience. The prerequisite for efficient Bayesian estimation is the availability of an accurate pharmacokinetic model to obtain unbiased and precise estimates of the individual and population parameters.

3 PHARMACOMETRICS

The term Pharmacometrics first appeared in 1982, and since then its importance in making decisions and optimizing in drug development and pharmacotherapy, has been widely recognized. Nowadays pharmacometrics is defined as the science of developing and applying mathematical and statistical methods to characterize, understand and predict drugs' pharmacokinetics and pharmacodynamics, biomarkers and outcomes[68]. In a clinical scenario pharmacometrics can contribute to design safe and effective dosing regimens for use in a patient population. In the last decades, the advent of population pharmacokinetic-pharmacodynamic modeling approaches has represented a major development for this discipline.

3.1 Population Pharmacokinetics

Population pharmacokinetics is defined as the study of variability in drug concentrations between individuals under the same standard dosage regimen[68]. Population pharmacokinetics seeks to identify the measurable pathophysiologic factors that cause changes in the dose-concentration relationship and the extent of these changes so that, if such changes are associated with clinically significant shifts in the therapeutic index, dosage can be appropriately modified.

In classical pharmacokinetic studies a sufficient number of samples must be collected for the PK analysis to be performed on an individual subject basis, whether the aim of the analysis is simply the computation of noncompartmental parameters or it is the estimation of the parameters from a PK model through nonlinear regression or other numerical analyses techniques. This is unfeasible when only sparse data are available such as in the case of traditional therapeutic drug monitoring, with dose assessment following the collection of few concentrations. Development of nonlinear mixed effects models for pharmacokinetic data has supposed to be a significant contribution for pharmacometrics. These models were firstly developed by Sheiner and Beal[69–71] and implemented in NONMEM® and subsequent softwares. This has enhanced the ability to: i) evaluate sparse data, ii) pool rich and sparse data, iii) pool data from different studies,

subject, doses, and other experimental conditions and iv) simulate new circumstances of drug product use.

On the other hand, classical PK analysis require two steps as follows: firstly, the individual PK parameters by non-compartmental or compartmental analysis must be estimated and then, a statistical analysis should be performed to know the mean tendency of PK parameters in the population as well as the associated variability. In a second step, a multivariate analysis is required to find potential correlations between plausible covariates and the calculated PK parameters. Apart from the fact that rich data are required, the principal disadvantages of the two-step analysis are, overestimation of variability associated with the PK parameters and no discrimination between inter- and intra-patient variabilities.

Although these methods have been used for a long-time, they have been replaced by one-step population PK analysis. A population PK analysis allows:

- To estimate the typical value of the PK parameters from the study population
- To quantify the magnitude of the variability related to these PK parameters between the study subjects.
- To identify demographic (body weight, age, sex), pathophysiological (hepatic or renal function), pharmacogenetics (Single Nucleotide Polymorphism), or concomitant drug-related factors that may influence or explain the variability found between the study subjects.
- To quantify the magnitude of the unexplained or error variability in the study population (within individual day-to-day or week-to-week kinetic variability and/or that due to errors in dosage or concentration measurements).

Many mathematical-statistical methods have been developed to build PPK models to be later implemented in the clinical practice for therapeutic drug monitoring. These methods can be classified, from a statistical point of view, based on the distribution of the PK parameters, as parametric or non-parametric. Parametric methods assume a

normal or log-normal distribution of the PK-parameters, whereas non-parametric methods do not make any assumption with regard to the data distribution

NONMEM®[72] is one of the most commonly used NONlinear Mixed Effects Modelling tool in population pharmacokinetic — pharmacodynamic (PK/PD) analysis. The software was developed by the NONMEM® Project Group at the University of California, San Francisco. The fit of drug concentrations (or dependent variable) and independent variable data by models is performed using nonlinear regression methods. Furthermore, mixed effects refer to the model parametrization that combines fixed and random effects. In particular, the fixed effects (THETA) correspond to the PK parameters as well as the regression coefficients of the covariates included in the model to describe part of the unexplained interindividual variability. On the other hand, the random effects correspond to the unexplained interindividual variability (ETA) reflecting the difference between an individual's parameter value and the population value, and also to the variability (EPSILON) reflecting the difference between the observed data for an individual and the model's prediction (also known as residual error)[73,74]. The nonlinear mixed effects modeling allows the simultaneous analysis of the data to determine the fixed (PK parameters) as well as the random effects (interindividual variability and residual error).

3.1.1 Maximum a Posteriori Bayesian Estimation

The population pharmacokinetic analysis, provides prior information about values of typical PK parameters and variability associated with them and about the predictive factors of such variability within the target population. It has shown to be a useful tool to better predict Tacrolimus exposure in each patient when compared to weighted nonlinear least squares regression methods. The process starts by considering the population PK parameters provided by the model as prior information. Then, new data from the patient is considered and the individual a posteriori PK parameters are predicted by using the Maximum A Posteriori Bayesian (MAPB) forecasting technique. This can be feasible from a limited number of drug–concentration measurements as required in the clinical setting. MAPB estimation has been increasingly used in the past

decades for estimating individual pharmacokinetic parameters. MAPB analysis is derived from Bayes theorem, that introduced the concept that prior information can be combined with new observed data[75]. The fitting procedure minimizes the square difference between the measured concentration and its estimate (weighted by the reciprocal of its variance) and at the same time minimizing the squared difference between each population PK parameter and each MPA Bayesian posterior estimate for that patient (weighted by the variance of the model parameter values). Thus, the best overall fit to both types of data will get the MPA Bayesian posterior model for each individual patient. The credibility of the two types of data will determine where the fit will go. A precise analytical assay will pull the fit towards the patient's data. A very uniform population model will pull the individual parameters towards the population prior parameters. Moreover, for individuals with scarce data, the estimates of the individual's parameters will be weighted more by the population parameters than the influence of their data. The individual parameters will shrink then towards the population values, this phenomena called as shrinkage. The extent of shrinkage has consequences on individual predicted parameters and individual predicted concentrations. Then, optimal study designs and analytical assays will be the prerequisites to achieve robust population pharmacokinetic models for unbiased and precise Bayesian estimation when minimum one observation per patient is available.

$$BOF = \sum \left[\frac{(Cobs - Cest)^2}{Var\ Conc\ est} \right] + \sum \left[\frac{(POP\ par - Pipar)^2}{Var\ POP\ par\ est} \right]$$

Despite of this, the major advantage of MAPB estimation with respect to weighted nonlinear regression methods, is the flexibility to calculate individual pharmacokinetic parameters from limited and sparse blood samples. Pre-specified time-points for concentration measurements are not mandatory, this making the logistics easier in the outpatient setting. MAPB estimation is the most common method used in TDM for dose individualization. In addition, MAPB estimation is also commonly used to design optimal (OSS) or limited sampling strategy (LSS). A LSS is the best combination of concentration-time points which will provide the most accurate estimation of one or more individual PK parameters or exposure indices (AUC)[76] when compared to full sampling strategies.

3.2 Tacrolimus Population PK Models

Several Tac population pharmacokinetic models developed using the non-linear mixed effects methods have been reported in adult renal transplant patients (See Table below). Zhao et al [79] have revised and externally evaluated some of them. This allowed to compare models differing in i) analytical methods used for measurement of concentrations used to develop the model, this being a relevant aspect to be taken into account when a published model is going to be applied as support during the TDM ii) number of Patients/samples included iii) identified covariates as predictors of interindividual variability, among other aspects.

Up until now, the majority of them were based on a two-open compartment model with first order absorption. Some models tried to better characterize the delayed absorption process of Tac using transit compartment models (i.e. Erlang distribution model). Most of the models included inter-occasion variability associated with the main PK parameters to better describe PK variability of parameters from one occasion to another. In general, the models achieved to include all well-known clinically plausible covariates such as demographical (age, weight, fat free mass), biochemical (hematocrit[33]), the type of Tac formulation[77], *CYP3A5* [41,42,78] and *CYP3A4*[46,79,80] genotype, which may explain the Tac PK variability. Some of them also considered the inclusion of post-transplant time (POD) although this variable is considered as a surrogate for many time-dependent factors such as albumin, HCT, corticosteroids dose, among other confusing factors. The dose-dependency in clearance was also incorporated in two of the revised models (posar les dues cites) by Zhao et al[79,·] Although the real cause of non-linearity could not be elucidated it was rather related to the POD alterations in absorption due to recovery of gastrointestinal function, the activity of P-glycoprotein, and CYPA3 or concentration dependent–binding to erythrocytes among others.

Analyzing all the information which they provide, the most relevant aspects (model type, population PK parameters and model variability explanation) are highlighted in the table below. It is worth noting that the most influential covariate in all of them when assayed

was the genetic polymorphisms of CYP3A. The prediction-based and simulation-based performances were evaluated for all the models. The population prediction error calculation as accuracy measure allowed to know the credibility of using the population PK parameters as priors and normalized prediction distribution errors (npdes), indicated the feasibility of the model to be used for new scenario simulations.

According to these results, so far, the population PK model that was reported by Storset et al[33] was superior to the others regarding to prediction capability but did not show appropriate capability to be used for simulations. It is worth noting that this model included the most relevant well-known clinically covariates, thus indicating, that dosage of Tac based on hematocrit, CYP3A5 polymorphism and demographic characteristics (fat free mass and/or age) could lead to a better Tacrolimus exposure.

Therefore, further investigation is still required to better explain interindividual variability and to identify the confusing factors leading to the well-known POD influence in Tac PK.

Table 2. Summary of the Most Relevant Published Tacrolimus Population Pharmacokinetic Models.

No. of Patients	Pharmacokinetic model	PK Parameters	Model Variability
Benkali et al[61]			
32 model building	Absorption: Erlang model with 3 transit compartments. Disposition: 2-Compartment model with first-order elimination	k_{tr} = 6.5 h^{-1} CL/F = (θ_1/HCT) L/h θ_1 =863 V1/F = 147 L V2/F= 500L (fixed) Q/F = 60 L/h	IIV k_{tr} = 15 % IIV CL/F = 30 % IIV V1/F=26% IIV Q/F = 63 % IOV k_{tr} =24% IOV CL/F=27% IOV V1/F = 71 % Prop RE = 10 % Add RE = 1.5ng/mL
Benkali et al[81]			
29 model building + 12 external evaluation	Absorption: Erlang model with 3 transit compartments. Disposition: 2-Compartment model with first-order elimination	ktr = 3.3 h^{-1} CL/F = θ_1 $(1 + \theta_2)^{CYP3A5}$ L/h θ_1 = 19 θ_2 = 1.15 V1/F = 486 L K12 = 0.13 h-1 K21 = 0.09 h-1 CYP3A5 = 0 for CYP3A5 non-expresser CYP3A5 = 1 for CYP3A5 expresser	IIV k_{tr} = 52 % IIV CL/F = 35 % IIV V1/F=53% IIV K12 =54% Prop RE=8% Add RE = 0.7ng/mL

Table 2. Summary of the Most Relevant Published Tacrolimus Population Pharmacokinetic Models. (cont.)

No. of Patients	Pharmacokinetic model	PK Parameters	Model Variability
Woillard et al[82]			
73 model building	Absoprtion: Erlang model with 3 transit compartments. Disposition: 2-Compartment model with first-order elimination	$k_{tr} = (\theta_1\ \theta_2^{FORM})$ h-1 θ_1 = 3.34 θ_2 =1.53 CL/F = θ_3 ((HCT/35)$^{\theta 4}$ (θ_5^{CYP3A}5) L/h θ_3 =21.2 θ_4 =–1.14 θ_5 =2.0 V1/F= θ_6 (θ_7 ^{FORM}) L h6 =486 h7 = 0.29 V2/F = 271 L Q/F = 79 L/h FORM = 0 patient received Advagraf®; FORM = 1; patient received Prograf® CYP3A5 = 0 for CYP3A5 non-expresser, CYP3A5 = 1 for CYP3A5 expresser	IIV k_{tr} = 24 % IIV CL/F = 28 % IIV V1/F=31% IIV V2/F = 60 % IIV Q/F=54% IOV k_{tr} =33% IOVCL/F=31% IOV V1/F=75% Prop RE=11.3% Add RE = 0.71ng/mL

Table 2. Summary of the Most Relevant Published Tacrolimus Population Pharmacokinetic Models. (cont.)

No. of Patients	Pharmacokinetic model	PK Parameters	Model Variability
Musuamba et al[83]			
65 model building	Absorption: first order + lag time Disposition 2-Compartment model + first order elimination	k_a = 0.45 h-1 Lag time = 0.1 h CL/F = θ_1 + CYP3A5 + ABCB1 + (HCT/21) θ_2 L/h θ_1 = 16.3 θ_2 =20.6 V1/F = 86.4 L V2/F = 1115 L Q/F = 58.2 L/h Where CYP3A5 = 0 for CYP3A5 non-expresser CYP3A5 = 15.4 for CYP3A5 expresser ABCB1 = 0 for CC-GG-CC non-carriers ABCB1 = 7.6 for CC-GG-CC carriers	IIV k_a =91% IIV Lag time = 61 % IIV CL/F = 32 % IIV V1/F = 55 % IIV V2/F=48% Prop RE = 13% Add RE = 0.88ng/mL

Table 2. Summary of the Most Relevant Published Tacrolimus Population Pharmacokinetic Models. (cont.)

No. of Patients	Pharmacokinetic model	PK Parameters	Model Variability
Zuo et al[79]			
161 (Chinese)	Absorption: first-order absorption Disposition: 1-Compartment model with first order elimination	k_a = 3.09 h^{-1} (fixed) CL/F = θ_1 (HCT/27.9) $^{\theta_2}$ CYP3A L/h θ_1 = 26.6 θ_2 = -0.451 Vd/F = 1090 L - CYP3A = 1.21 for CYP3A5*1/*1 or CYP3A5*1/*3 + CYP3A4*1/*1G or CYP3A4*1G/*1G genotype; - CYP3A = 0.982 for CYP3A5*1/*1, CYP3A5*1/*3 or CYP3A4*1/*1 genotype; - CYP3A = 0.77 for CYP3A5*3/*3, CYP3A4*1/*1G or CYP3A4*1G/*1G genotype; - CYP3A = 0.577 for CYP3A5*3/*3 or CYP3A4*1/*1 genotype	IIV CL/F = 24.2 % IIV V_d/F = 58.5 % Prop RE = 19.8 % Add RRE = 1.47 ng/mL

Table 2. Summary of the Most Relevant Published Tacrolimus Population Pharmacokinetic Models. (cont.)

No. of Patients	Pharmacokinetic model	PK Parameters	Model Variability
Han et al[84]			
102	Absorption: first-order absorption Disposition: 1-Compartment model with first order elimination	k_a = 3.43 h^{-1} Lag time = 0.25 h (fixed) CL/F= θ_1 (1 + θ_2 (POD-9.6)) θ_3 CYP3A5 L/h θ_1 = 21.9 θ_2 = 0.0119 θ_3 = 0.816 Vd/F = 205 L - CYP3A5 = 1 for CYP3A5 non-expresser - CYP3A5 = 0 for CYP3A5 non-expresser	IIV CL/F = 40.9 % IIV k_a =112% IIV Vd/F=59.1% Prop RE = (2 = 3.75)

POD: Post-Operative Days

Table 2. Summary of the Most Relevant Published Tacrolimus Population Pharmacokinetic Models. (cont.)

No. of Patients	Pharmacokinetic model	PK Parameters	Model Variability
Storset et al[33]			
69	Absorption: first-order absorption, with a lag- time. A study-specific absorption rate and lag time improved the data fit of substudy 2 Disposition: 2-Compartment model with and first-order elimination.	$F = [\theta_1 + (1 - \theta_1)/(1+POD/\theta_2)^{-\theta 3})] \cdot [1 + \theta_4/(1 + POD/\theta_5)^{-\theta 6})]$ $\cdot[\theta_7 + (1 - \theta_7)/(1 + AGE/\theta_8)^{\theta 9}] \cdot CYP3A5$ $\theta_1 = 2.04$ ($F_{max,early}$) $\theta_2 = 2.5$ ($F_{early,50}$) $\theta_3 = 9.4$ ($Hill_{Fearly}$) $\theta_4 = 0.28$ ($F_{max,late}$) $\theta_5 = 31$ ($F_{late,50}$) $\theta_6 = 2.5$ ($Hill_{Flate}$) $\theta_7 = 0.43$ ($F_{min,age}$–females) or 0.66 ($F_{min,age}$–males) $\theta_8 = 47$ ($F_{age,50}$) $\theta_9 = -14$ ($Hill_{Fage}$) $k_a = 1.18\ h^{-1}$ k_a, sub-study 2 = $0.38\ h^{-1}$ Lag time = 0.22 h Lag time, substudy 2 = 0.81 h $CL/Fn = \theta_{10} \cdot (FFM/60)^{0.75}$ L/h $\theta_{10} = 20.5$ $V_1/Fn = \theta_{11} \cdot (FFM/60)$ L $\theta_{11} = 107$ L	IIV V_1/Fn=14% IIV V_2/Fn=52% IIV Q/Fn=86% IIV θ_6 ($Hill_{Flate}$) = 113% Corr (CL/Fn, Q/Fn) = 0.74 IOV Fn=16% IOV k_a =60% Prop RE = 16.7 % Study 2 factor = 0.56 Study 3 factor = 0.72

		$V_2/Fn = \theta_{12} \cdot (FFM/60)$ L $\theta_{12} = 424$ $Q/Fn = \theta_{13} \cdot (FFM/60)^{0.75}$ L/h $\theta_{13} = 37.3$ Where CYP3A5 = 0.51 for CYP3A5 expresser CYP3A5 = 1 for CYP3A5 non-expressers. See details on paper for Bioavailability (F) coefficients Tacrolimus whole blood values were standardised to a HCT value of 45 % (see paper for details).	

FFM: Fat free mass, POD: Post-Operative Days

Table 2. Summary of the Most Relevant Published Tacrolimus Population Pharmacokinetic Models. (cont.)

No. of Patients	Pharmacokinetic model	PK Parameters	Model Variability
		Asberg et al[85]	
69	Absorption: first-order absorption, with a lag- time Disposition: 2-Compartment model with and first-order elimination.	F = 0.63 (CYP3A5 expressers) F = 1(CYP3A5 non-expressers) k_a = 1.04 h^{-1} Lag time = 1.0 h (first week) Lag time = 0.15 h (week 2–4) Lag time = 0.59 h (after first month) CL/F = θ_1 $(FFM/59)^{0.75}$ L/h (CYP3A5 expressers) θ_1 = 26.7 CL/F = θ_2 $(FFM/59)^{0.75}$ L/h (CYP3A5 non-expressers) θ_2 = 21.2 V1/F = θ_3 (BMI/26) L θ_3 = 177 V2/F = θ_4 (FFM/59) L θ_4 = 3707 Q/F = θ_5 $(FFM/59)^{0.75}$ L/h θ_5 = 19.5	Inter-Quartile Ranges provided: F = 0.12 ka =1.27 Lag time = 1.55 (first week) Lag time = 0.46 (week 2–4) Lag time = 0.57 (after first month) CL/F = 13.2 (CYP3A5 expressers) CL/F = 11.0 (CYP3A5 non-expressers) V1/F = 295 V2/F = 7736 Q/F = 32.3

FFM: Fat free mass, BMI: Body Mass Index

Table 2. Summary of the Most Relevant Published Tacrolimus Population Pharmacokinetic Models. (cont.)

No. of Patients	Pharmacokinetic model	PK Parameters	Model Variability
Bergmann et al[86]			
173	Absorption: first-order absorption, with a lag- time Disposition: 2-Compartment model with and first-order elimination.	k_a = 0.35 h^{-1} Lag time = 0.44 h CL/F = $\theta_1 \cdot \theta_2^{CYP3A5} \cdot (1 + \theta_3 (HCT - 0.33)) \cdot (WT/70)^{0.75} \cdot (1 + \theta_4 (POD - 22.7))$ L/h θ_1= 25.5 θ_2 = 1.60 θ_3 = –1.01 θ_4 = –0.21 V1/F = $\theta_5 (1 + \theta_6 (PredCmax,unbound - 155.5))$ L θ_5 = 113.0 θ_6 = 0.28 V2/F = 1060 L Q/F = 67.9 L/h CYP3A5 = 0 for CYP3A5 non-expresser, otherwise CYP3A5 = 1; POD max at 90 days; PredCmax,unbound is Cmax free prednisolone (nmol/L)	IIV ka = 47.6 % IIV CL/F = 29.5 % IIV V1/F = 46.8 % IIV V2/F = 89.4 % IOV CL/F = 29.9 % IOV V1/F = 126.5 % Corr (V1/F, ka) = 0.677 Corr (V1/F, V2/F) = –0.049 Corr (ka, V2/F) = –0.013 Prop RE = 18.3 %

FFM: Fat free mass; BMI: Body Mass Index; WT: Body Weight; POD: Post-Operative Days;

HYPOTHESIS

The calcineurin inhibitor Tac is used to prevent acute rejection after renal transplant. Unfortunately, the clinical use of Tac is complicated by its considerable toxicity, narrow therapeutic window, and high interindividual pharmacokinetic variability.

Therapeutic drug monitoring is commonly applied to individualize Tac therapy in renal transplant recipients using trough concentrations. When concentrations are out of the target range, the physicians roughly estimate what should be the appropriate change of dose. Despite trough concentrations are the most used exposure parameters, the AUC correlates better with the clinical outcomes. In the clinical setting, an AUC tiered-dosing is not feasible, thus an alternate approach is that based on limited-sampling strategy by means of Bayesian prediction. In this sense, the use of a PPK model can assist for the first dose calculation at the start of treatment but also for dose adaptation based on predefined target by means of MAPB forecasting technique, supporting TDM.

Several Tacrolimus PPK models have been published that includes the CYP3A5 polymorphism to explain part of the interindividual variability. However, recent discovery of new SNPs has led to further investigations on that file aiming to reduce the unexplained interindividual variability in Tacrolimus exposure.

OBJECTIVES

The main objective of the present work was to design a population-based Bayesian prediction tool for initial dose calculation and dose adaptation during the post-transplant period through:

1. Characterizing the Tacrolimus population PK using an intensive sampling and confirming the best limiting sampling strategy to be applied during dose adaptation.
2. To deeply Investigate in tacrolimus pharmacogenetic predictors of interindividual variability
3. Implementing new genetic information as well as other clinical factors to generate a refined population pharmacokinetic model reducing unexplained variability.

LIST OF ORIGINAL ARTICLES

To answer the previous objectives, this thesis was focused on the three original contributions listed below.

1. **Development of a Population PK Model of Tacrolimus for Adaptive Dosage Control in Stable Kidney Transplant Patients.** Franc Andreu, Helena Colom, Josep M Grinyo, Joan Torras, Nuria Lloberas. Therapeutic Drug Monitoring. Volume 37, Number 2, 2015. IF=2.376; Q2

2. **The combination of CYP3A4*22 and CYP3A5*3 SNPs determine tacrolimus dose requirement after kidney transplantation.** Nuria Lloberas, Laure Elens, Ines Llaudó, Ariadna Padullés, Teun van Gelder, Dennis A. Hesselink, Helena Colom, Franc Andreu, Joan Torras, Oriol Bestard, Josep M. Cruzado, Salvador Gil-Vernet, Ron van Schaikh and Josep M. Grinyó. Pharmacogenetics and Genomics. Accepted 07 June 2017. IF=2.184; Q2

3. **A new CYP3A5*3 and CYP3A4*22 cluster influencing tacrolimus target concentrations: A population approach.** Franc Andreu, Helena Colom, Laure Elens, Teun van Gelder, Ronald H. N. van Schaik, Dennis A. Hesselink, Oriol Bestard, Joan Torras, Josep M. Cruzado, Josep M. Grinyó, Nuria Lloberas. Clinical Pharmacokinetics. Online from 03 January 2017. IF=5.216; Q1

Chapter II

DEVELOPMENT OF A POPULATION PK MODEL OF TACROLIMUS FOR ADAPTIVE DOSAGE CONTROL IN STABLE KIDNEY TRANSPLANT PATIENTS

ORIGINAL ARTICLE

Development of a Population PK Model of Tacrolimus for Adaptive Dosage Control in Stable Kidney Transplant Patients

Franc Andreu, PharmD,† Helena Colom, PhD,* Josep M. Grinyó, MD, PhD,† Joan Torras, MD, PhD,† Josep M. Cruzado, MD, PhD,† and Nuria Lloberas, PhD†*

Background: Tacrolimus pharmacokinetics (PK) presents a high variability that hampers its therapeutic use. The aims of this study are to: (1) develop a population pharmacokinetic (PPK) model for tacrolimus and to identify the factors that contribute to the variability of tacrolimus PK in renal transplant patients; and (2) to establish a new Bayesian estimator that can easily and routinely be applied in the hospital. A new PPK model may allow efficacy to be optimized, improve dose regimens, minimize side effects, and decrease the cost of extensive area under the curve (AUC) monitoring.

Methods: PPK analysis of the full PK profiles of 16 patients on 5 occasions was performed with NONMEM 7.2. Biochemical variables (hematocrit, hemoglobin, aspartate aminotransferase, and others) were analyzed.

Results: A 2-open-compartment model with interoccasion variability best described the PK of tacrolimus. Three transit compartments provided the best description of the absorption process. The hematocrit, aspartate aminotransferase, and alanine aminotransferase were not significant in the covariate analysis. External validation with 91 patients proved the good predictability of the model with a bias and precision of 0.37 mcg/L (CI 95%, –0.11 to 1.20 mcg/L) and 0.38 mcg/L (CI 95%, 0.02 to 1.21 mcg/L), respectively. A limited sampling strategy using 1 sampling point at predose (trough concentrations) showed a good performance in AUC_{0-12h} estimation with a correlation between AUC_{full} and AUC_{LSS}, bias and imprecision of $r^2 = 0.75$, 6.78% (range, −16.26% to 30.06%) and 1.42% (lC 95%, 0.14%–3.61%), respectively.

Conclusions: The PPK model developed provides reliable prior information for Bayesian adaptive control of dosage regimens of tacrolimus to achieve the desired AUC goals in stable renal transplant patients.

Key Words: tacrolimus, Pharmacometrics, renal transplant, therapeutic drug monitoring

(*Ther Drug Monit* 2015;37:246–255)

INTRODUCTION

Tacrolimus is a calcineurin inhibitor that forms the cornerstone of provoking immunosuppression to prevent allograft rejection in solid organ and bone marrow transplant patients.[1] It has a narrow therapeutic index and large intra-individual and interindividual variabilities, particularly in the early posttransplant period, which have mostly been attributed to its pharmacokinetics (PK).[1] An erratic absorption process has been reported for tacrolimus because of its lipophylicity.[2] First-pass effect occurs both at the intestinal wall and liver because of the CYP3A isoforms and efflux transport proteins (ABCB1)[3] and cause variable bioavailability ranging from 5% to 93%.[2] Once in the blood stream, tacrolimus is highly bound (up to 99%) both to red blood cells and to α-1 glycoprotein.[4] Therefore, the presence of a low hematocrit and α-1 glycoprotein could explain an increase of tacrolimus clearance related to its hepatic extraction rate values, ranging from 0.566 to 0.598.[5] A large variation in distribution volume values has also been observed (from 0.97 to 104.8 L/kg).[2]

Metabolism by CYP3A4 and CYP3A5 results in 15 different metabolites that are then excreted through the biliary route; less than 1% of the parent compound remains unchanged.[1] Variable and long half-life values have also been previously reported (3.5–50 hours[2]). All this has led to the identification of several factors such as time after transplant, body weight, hematocrit, age, liver function, and polymorphisms[2,6–8] as predictive factors of tacrolimus PKs. As is widely known, therapeutic drug monitoring (TDM) is essential for dose tailoring and the success of renal transplant. Although area under the curve (AUC) has been reported to be the best predictor of efficacy for tacrolimus,[9–11] the good correlation between AUC and trough concentrations (C_{trough})[12,13] has resulted in considering TDM, based on

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C_{trough}, as the exposure measurement to reduce tacrolimus toxicity with optimal immunosuppression.

According to the 2009 European Consensus Conference by the Committee on Tacrolimus Optimization Recommendations,[14] target tacrolimus trough levels should be of 5–15 ng/mL in the early phase after transplantation, 5–10 ng/mL in the long-term treatment, and AUC_{0-12h} between 150 and 200 ng/mL·h. The population pharmacokinetic (PPK) approach is a powerful and helpful tool for TDM. It allows one to identify and include the predictive factors related to organ functions and interactions as prior information for Bayesian adaptive control.[15,16] This can lead to optimized efficacy and improved dose regimens, while minimizing side effects such as nephrotoxicity and neurotoxicity[17,18]; and finally, it can decrease the cost of managing the dosage regimen.

Several studies[19–26] have characterized the PPK of tacrolimus in renal transplant patients. Initially, clearance changes over time were reported on the first models design.[19,20] Later on, study by Benkali et al[21,23] and others[24,26] reported hematocrit as a predictive factor of variability in tacrolimus clearance. The *CYP3A5*3* genotype[22–25] and the *ABCB1* genotype[22] have since been reported to influence tacrolimus clearance.

The aim of this study is to develop a PPK model of tacrolimus using a nonlinear mixed-effect approach. We use data from the Symphony PK Study[27] to identify the factors that contribute to a description of tacrolimus PK variability in renal transplant patients. A PPK model of tacrolimus will also permit useful maximum a posteriori probability Bayesian adaptive control and individualization of its dosage regimens in the clinical care of patients.

METHODS

Patients' Data Collection

The tacrolimus concentration–time data from 16 renal transplant patients of the PK substudy of the Symphony study were used retrospectively.[28] The patients received doses of 1–4 mg of tacrolimus (Prograf) combined with mycophenolate mofetil, daclizumab, and corticosteroids. Details of the criteria for patient selection, treatment allocation, and immunosuppressive comedication have been published previously.[27] Tacrolimus dosages were adjusted to target concentrations between 3 and 7 ng/mL. Blood samples were collected before and 20, 40, and 75 minutes and 2, 3, 4, 6, 8, 10, and 12 hours postadministration on the seventh day and the first, third, sixth, and twelfth month after transplantation. Demographic characteristics, biochemical parameters, and coimmunosuppressive medication (doses and concentrations) were recorded on each occasion. Tacrolimus concentrations were measured using enzyme immunoassay EMIT methods in a Cobas Mira autoanalyzer (Dade Behring, Palo Alto, CA). The interassay imprecision had coefficients of variation of 7.96%, 4.98%, and 5.12% at a level of 5, 11, and 22 ng/mL, respectively, and the lower limit of quantification was 1.22 ng/mL.[29] Retrospectively, 91 tacrolimus C_{trough} at predose (0 hours) were recorded as an external group, from 91 renal transplant patients during the first-year posttransplant treated with the same criteria as the model-building group. These data were used to evaluate the predictive ability of the model. This substudy was conducted in accordance with the Declaration of Helsinki and the guidelines of the International Conference on Harmonization Good Clinical Practice (ICH-GCP) and with the approval of the local ethics committee or institutional review board at each center.[28]

GENOTYPING OF *ABCB1* POLYMORPHISMS

For genotyping, we obtained DNA from the same 16 renal transplant patients. Patients were genotyped for single nucleotide polymorphisms (SNPs) in *ABCB1* gene; exon 12:1236C>T (rs1128503), exon 21:2677 G>T (rs2032582), and the exon 26:3435 C>T (rs1045642). DNA was extracted from a peripheral whole-blood sample using the Wizard Genomic DNA Purification Kit (Promega Corporation, Sydney, Australia) and was stored at −80°C. Genotyping procedures were performed with the MassARRAY SNP genotyping system (Sequenom Inc, San Diego, CA). The method involves multiplex polymerase chain reaction and single base extension assays, designed by the AssayDesigner software (Sequenom Inc), and followed by mass spectrometry analysis with the Bruker Autoflex MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA). Spectral output was analyzed and checked using MassARRAY Typer 3.4 software (Sequenom Inc). The genotyping platform is at the Spanish National Genotyping Center's facilities at Santiago de Compostela University.

PPK Analysis

The PPK analysis was performed using the nonlinear mixed-effects model approach implemented in NONMEM software (version 7.2; ICON Development Solutions, Hanover, MD) using Perl-Speaks-NONMEM (PsN) (version 3.5.3[30]; available at http://psn.sourceforge.net/) and the R package (Xpose 4.2.0). The first-order conditional estimation method with interaction was used throughout all the modeling process.

One-, two-, and three-open-compartment models with linear elimination were fitted to the concentration–time data. The zero-order, first-order with or without lag time, and transit compartment kinetics were tested to establish which best described the absorption process. The transit absorption compartments were assessed by fixing its number from 1 to 10, and the option providing the lowest minimum objective function value (MOFV) was selected. The models were parameterized in terms of absorption rate constant (Ka), distributional clearance (CL_D), apparent volumes of distribution (V), elimination clearance (CL), and mean transit time (MT), when the transit models were implemented. The transfer rate constant (K_tr) was defined from the number of transit compartments and the MT. Interindividual variability (IIV) and interoccasion variability (IOV)[31] were both described using exponential error models, assuming log-normal distributions were tested in all the PK parameters. The covariance between parameters was studied during the modeling process. Additive, proportional, and combined (ie, additive and proportional) error models were tested to establish which best described the residual (RE) variability. To statistically distinguish between nested models, the likelihood ratio test, based

on the reduction of the minimum objective function value (MOFV) was used (ΔMOFV: −2 log likelihood, approximate χ^2 distribution). A significance level of $P < 0.005$ corresponding to ΔMOFV = −7.879 for 1 degree of freedom was considered. For nonhierarchical models, the most parsimonious model with the lowest MOFV according to the Akaike information criterion was chosen.[32] The decrease in MOFV (−2 × log likelihood), parameter precision expressed as relative standard error (%), reductions in IIV associated with parameters, η- and ε-shrinkage values,[33] model completion status, and visual inspection of goodness-of-fit plots were also considered for model selection.

Covariate Analysis

The effect of all clinically meaningful covariates was tested on model parameters using the forward stepwise approach.[34] The covariates evaluated were body weight, body mass index, age, sex, creatinine clearance calculated using the Cockroft-Gault formula as a measure of renal function, plasma albumin concentration, liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total bilirubin (TBIL), hemoglobin, hematocrit, and red blood cells. The influence of continuous covariates on the PK parameters was tested systematically by an established modeling approach according to either a power function (Equation 1), an exponential function or a linear relationship.

$$TVP_j = \theta_1 \left(\frac{COV}{COV_{median}} \right)^{\theta COV}, \quad 1$$

where, θ_1 is the typical value of the jth PK parameter for a patient with the median covariate value in the population (COVmedian), and θCOV is the change in $\ln TVP_j$ per unit change in ln (COV/COVmedian).

The categorical covariates, such as sex or polymorphism, were tested in their respective parameters as indicated in Equation 2, where Z values represent each level of the categorical covariate.

$$\begin{aligned} TVP_j &= \theta_1 \quad \text{for } Z = 0, \\ TVP_j &= \theta_1 \theta_2 \quad \text{for } Z = 1. \end{aligned} \quad 2$$

Covariates were initially explored by univariate analysis and then by forward inclusion and backward elimination procedures. Significance levels of 5% (ΔMOFV = −3.841 units) and 0.1% (ΔMOFV = 10.8 units) were considered during the forward addition and backward elimination steps. Only covariates providing a reduction of IIV associated with parameters of at least 10% were considered clinically relevant and were retained in the model. Model completion status and visual inspection of goodness-of-fit plots were also evaluated for model selection.

Trough Concentration Prediction Ability

The predictive performance of the model developed was assessed in 91 external patients (external evaluation data set). After fixing the parameters at the final model, the maximum a posteriori probability Bayesian estimates of tacrolimus C_{trough} were obtained. The observed concentrations (Obs) of the external data set were compared with the corresponding individual predictions (Ipred) given by the final model. This performance was evaluated in terms of bias [median prediction error (ME), Equation 3] and imprecision [root median squared prediction error (RMSE), Equation 4, also expressed as coefficient of variation] and its corresponding 95% confidence intervals, in accordance with the method proposed by Sheiner and Beal.[35]

$$ME = \text{median}\,(obs - Ipred), \quad 3$$

$$RMSE = \text{median}\left(\sqrt{(Obs - Ipred)^2}\right). \quad 4$$

Internal Evaluation

To evaluate the predictive performance of the final model, a prediction-corrected visual predictive check (predcorrVPC)[36] was also performed based on 1000 simulated replicates of the original data set. Furthermore, the reliability of the results of the analysis was also determined as the inverse of the cumulative density function, normalized prediction distribution error (NPDE),[37] after 1000 simulations of the original data set. The observed and simulated prediction discrepancies should be within the 95% confidence prediction interval (CI) uniformly distributed between 0 and 2. To determine the adequate tacrolimus exposure predicted by the model, a simulation of 50 replicates of the original data set was performed. Subsequently, simulated and observed exposures given by the AUC_{0-12h} values were calculated by the noncompartmental analysis and compared between each other (posterior predictive check).[38]

A nonparametric resampling bootstrap procedure with replacement of 200 replicates was also performed. The median (θ_{iBoot}) and the 95% confidence interval (CI) of each parameter were estimated, and their relative deviation (RD) (Equation 5) from those estimated for the original dataset (θ_i) was evaluated.

$$RD = 100\left(\frac{\theta_i - \theta_{iBoot}}{\theta_i}\right). \quad 5$$

Limited Sampling Strategy

From the model developed, a limited sampling strategy (LSS) was established for clinical use to predict AUC_{0-12h} accurately and precisely using a limited number of samples. The validated final model and all the parameter estimates were used as prior information. Only a combination of 1, 2, or 3 sampling times at predose (C_0) and during the first 4 hours postdose was tested to minimize inconvenience for the patients and due to clinical constraints.[23,25,39] From all the combinations, the best was selected on the basis of its predictive performance with regard to the Bayesian estimation of the AUC of blood concentrations of tacrolimus versus time from 0 to 12 hours after dose (AUC_{0-12h}). The performance of the Bayesian estimation was evaluated through: (1) the

Pearson correlation coefficient test between the AUC predicted by the LSS (AUC_{LSS}) and the AUC predicted by the full sampling (AUC_{full}), and (2) the bias (median percentage prediction error, Equation 6) and imprecision (RMSE, Equation 7) between AUC_{LSS} and AUC_{full} given by the median and the 5th and 95th percentiles.

$$\text{MPPE} = \text{median}\left(100 \times \frac{AUC_{full} - AUC_{LSS}}{AUC_{full}}\right), \quad 6$$

$$\text{RMSE} = \text{median}\left(\sqrt{\left[AUC_{LSS} - AUC_{full}\right]^2}\right). \quad 7$$

Simulations

To establish the optimal dose for target AUC_{0-12h} values of 150–200 ng/mL·h as recommended by European Consensus 2009,[14] an estimation of the doses required to reach this target based on the population clearance (16.5 L/h) was performed. Thus, 500 simulations at initial fixed doses of 2.5, 2.8, 3, and 3.3 mg were performed and the results compared between doses in terms of AUC_{0-12h}.

RESULTS

Baseline Characteristics

Five hundred ninety four tacrolimus concentration–time data values from the 16 renal transplant patients were simultaneously analyzed in the PPK analysis. Demographical and biochemical characteristics are summarized in Table 1. An increase of creatinine clearance from day 7 to 1 year after transplantation was observed. Biochemical variables such as ALT, AST, and TBIL showed stable values except for those of 3 patients who presented moderate hepatic impairment. Hematocrit and albumin levels were highly homogeneous throughout the whole population with median values of 0.37 and 42 g/L, respectively. Concerning C3435T, C1236T, and G2677T MDR1 SNP, 7 of 16 patients presented the CT or GT SNP, 3 of 16 presented the TT SNP, and for 6 patients, no information was available.

TABLE 1. Demographic, Clinical, and Genetic Characteristics of the Studied Population

	Global Median (Min–Max)
Number of patients	16
Weight, kg	68 (35–104)
Age, y	56 (31–72)
Gender (male/female)	10/6
CL_{CR},* mL/min	52.2 (11.6–122.5)
Plasma albumin, g/L	42 (33–47.0)
ALT, U/L	22.4 (6.0–318.0)
AST U/L	18.5 (6.6–104.0)
Serum TBIL, mg/dL	0.47 (0.17–1.22)
Hemoglobin, g/dL	12.3 (7.3–6.5)
Hematocrit	0.37 (0.23–0.49)
Concomitant medication	
MMF doses, mg twice daily	1000
MDR1 polymorphism	
*C3435T (*C/TT)*	7/3
*C1236 (*C/TT)*	7/3
*G2677 (*G/TT)*	7/3

*C3435T (*C/TT) = ABCB1 polymorphism C3435T C carriers versus TT.*
*CL_{CR} = creatinine clearance calculated by Cockroft-Gault.
MMF = mycophenolate mofetil.

During the first 3 months, tacrolimus daily doses ranged from 4.3 to 5.8 mg. Median trough levels achieved at day 7, month 1, and month 3 were 8.1, 7.7, and 7.1 ng/mL, respectively. Only 6 of 594 concentrations (1.0%) were below the limit of quantification. Concentration–time profiles of tacrolimus are presented in Figure 1. Tacrolimus reached its peak concentration around 1 hour after dose, followed by a quick decay phase from 2 to 4 hours after dose, and finally a slower decay phase from 5 to 12 hours after dose. The median AUC_{0-12h} value of tacrolimus calculated by the noncompartmental analysis was 129.3 ng/mL·h. A good correlation ($r = 0.86$, $P < 0.001$) was found between observed C_{trough} (C_0) and tacrolimus exposure values (AUC_{0-12h}) estimated using the noncompartmental approach (Fig. 2).

PPK Analysis

PPK Model

A two-open-compartment model combined with 3 transit absorption compartments and IOV of CL best described the concentration versus time data (MOFV = 1809.65). The inclusion of the IOV associated with CL significantly decreased the MOFV (ΔMOFV = −304.425, $P < 0.001$) and reduced the IIV_{CL} by 9.3%. The 3 transit compartment absorption model best described the absorption process. Residual error was best described by a proportional error model. The different PK parameters estimated from the selected base model are presented in Table 2. The η-shrinkage values were 5.50%, 11.00%, 12.36%, and 13.39% for IIVCL, IIVKa, IIVMT, and IOVCL, respectively. The ε-shrinkage was 6.06%. The Kolmogorov–Smirnov test showed normality for all parameters ($P > 0.05$). Plots of population predicted concentrations and individual predicted concentrations versus observed concentrations (see **Figure, Supplemental Digital Content 1**) did not present any remarkable bias. Conditional weighted residuals were randomly spread around zero, and no trend was detected in the plot.

Diagnostic plots of empirical Bayes estimates of PK parameters in the basic model against all the categorical and continuous covariates indicated no relevant differences between sex or any trends with body weight, body mass index, age, creatinine clearance, plasma albumin concentration, liver enzymes (AST and ALT) (MOFV = 1807.16, RE = 20.7%), TBIL (MOFV = 1805.76, RE = 20.7%), hemoglobin, hematocrit (MOFV = 1806.88, RE = 20.7%), red blood cells (MOFV = 1806.66, RE = 20.7%), or influence of time (MOFV = 2043.33, RE = 24.9%).

From all the covariates, the inclusion of TBIL as a power relationship in tacrolimus clearance significantly reduced the OFV (−3.89, $P < 0.05$), but it was not

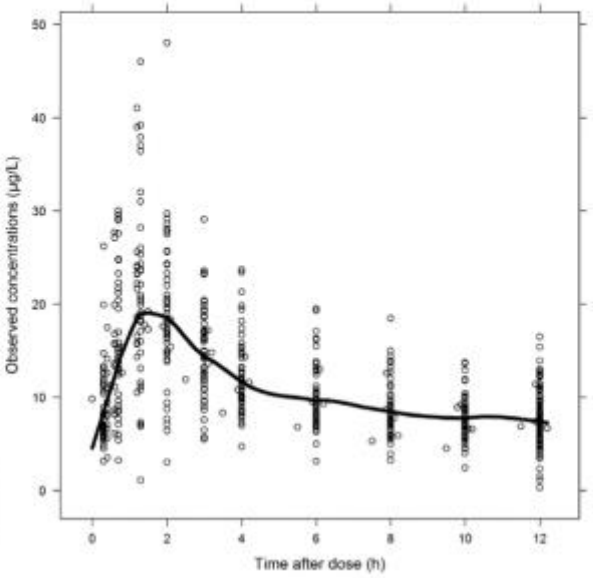

FIGURE 1. Whole-blood concentration–time (postdose) profiles of tacrolimus after an oral administration of 0.5–4 mg tacrolimus twice daily. Open circles: observed data; solid line: locally weighted smoothing of the scatterplot data. High variability was observed in all cases.

considered as clinically relevant because the reduction in IIV associated with clearance was less than 10%.

The percentages of predicted C_{trough} values that fell within the therapeutic interval (3–7 ng/mL) from 1000 simulated data sets like the original after 3 mg fixed doses were compared between the base model and covariate (TBIL) model. The percentage of therapeutic C_{trough} for the TBIL model (31.33% for TBIL under 0.5 mg/dL and 13.81% for TBIL above 0.5 mg/dL) did not improve with respect to the base model (26.28%). Despite the fact that the statistical significance of bilirubin was confirmed, there was no major clinical impact of this covariate, and as a consequence, the base model remained the final model.

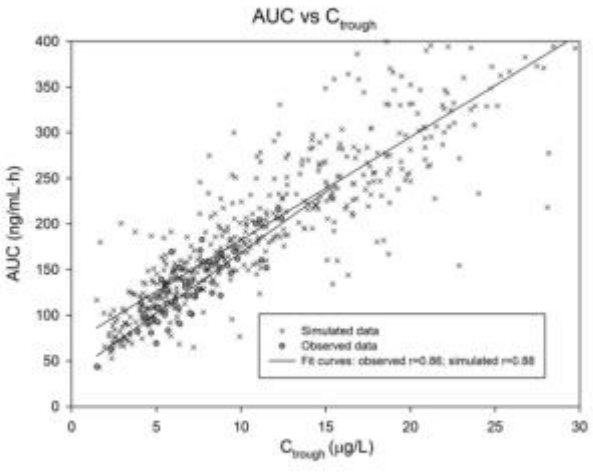

FIGURE 2. Correlation between AUC and C_{trough}. Circles: observed tacrolimus AUC_{0-12h} calculated by noncompartmental analysis versus observed tacrolimus C_{trough}; crosses: simulated tacrolimus AUC_{0-12h} calculated by noncompartmental analysis versus simulated tacrolimus C_{trough}.

TABLE 2. Tacrolimus PPK Estimates for the Final Model

Parameter	Units	Final Model, Estimate (RSE%)	Bootstrap Analysis, Median (95% CI)
Disposition parameters			
CL	L/h	16.50 *(10.9)*	16.47 (13.64–19.75)
V_C	L	9.89 *(21.9)*	10.30 (1.81–17.08)
CL_D	L/h	35.56 *(7.8)*	35.49 (30.09–41.81)
V_P	L	526.03 *(18.0)*	541.08 (382.48–974.67)
Absorption parameters			
Ka	h^{-1}	0.47 *(7.7)*	0.471 (0.402–0.617)
MT	h	0.83 *(10.0)*	0.838 (0.604–1.105)
NN	—	3 [fixed]	3 [fixed]
K_w	h^{-1}	3.61 (—)	3.58 (2.71–4.97)
Interindividual and		interoccasion variabilities	
IOV_{CL}	%	29 *(24)*	28 *(22–36)*
IIV_{CL}	%	39 *(44)*	36 *(16–54)*
IIV_{Ka}	%	35 *(36)*	33 *(15–46)*
IIV_{MT}	%	32 *(47)*	31 *(11–44)*
Residual variability			
Proportional	%	21 *(20)*	21 *(17–26)*

Interindividual, interoccasion, and residual variabilities are expressed as coefficient of variation. All final parameters are shown with the relative standard error indicated by italic numbers in parenthesis.

CL, clearance; CL_D, intercompartmental clearance; K_a, absorption constant; K_w, transfer rate constant; NN, number of transit compartments; V_C, central volume; V_P, peripheral volume.

Trough Concentration Prediction Ability

An acceptable accordance between the observed (Obs) and individual predicted (Ipred) concentrations was found. The median values and the 5th and 95th percentiles of bias and precision were 0.37 mcg/L (−0.11–1.20 mcg/L) and 0.38 mcg/L (0.02–1.21 mcg/L), respectively. The 95% confidence intervals included the zero, suggesting that the model has good predictive power with a coefficient of variation of 6.13% for the predicted value for C_{trough}. The goodness-of-fit of the trough concentration prediction (Fig. 3) revealed a slight underprediction of the results.

Internal Validation

The bootstrap of 200 resamplings (Table 2) confirmed the good accuracy of the PK parameters estimates. All the final model estimates were within the 90% CI of the bootstrap values. The relative deviation of the bootstrap was less than 4.14% for all the parameters. The high correlation ($r = 0.88$, $P < 0.001$) found between predicted AUC_{0-12h} and predicted C_{trough} (Fig. 2) was in agreement with the results obtained from the observed data (observed AUC_{0-12h} and observed C_{trough}), thus confirming the descriptive capacity of the model. The prediction-corrected visual predictive check (Fig. 4) showed that the model describes the mean tendency of the entire data well, although a slight overestimation was found. Overprediction of variability was observed in both confidence intervals of the 5th and 95th percentiles corresponding to the simulated data, mainly at late postdose times. A slight tendency to overpredict was recognized in the mean tendency of the simulated data compared with the observed data set.

NPDE showed normalized prediction discrepancies between the simulated and observed data for the 1000 simulations from the final model displayed as a histogram and dispersion plot with confidence intervals (Fig. 5). The density of predictive model discrepancies (NPDE) followed a theoretical normal distribution, with no extreme values, indicating that the variance of the final model estimations was normal. Discrepancy errors between simulated and observed concentrations had a homogeneous distribution around NPDE = 0, without any specific tendency. A box-plot of observed versus predicted AUC_{0-12h} distributions of the posterior predictive check indicated acceptable predictive capacity (see **Figure, Supplemental Digital Content 2**). The median of simulated AUC_{0-12h} was 141.18 ng/mL·h value: within the 5th and 95th percentiles of the observed AUC_{0-12h} median value, 129.3 (76.8–209.5) ng/mL·h, with no statistical significance in a log-transformed parametric test ($P > 0.05$).

Limited Sampling Strategy

In terms of the AUC_{0-12h} estimation performance, of all the possible LSS combinations, the 2 strategies that provided the best performance in AUC estimation were a two sampling point strategy (at predose and 90 minutes) and a one sampling point strategy at predose. A good correlation was found between the AUC_{Full} and the AUC_{LSS} in both cases ($r^2 = 0.86$ versus $r^2 = 0.75$, respectively). Regarding the bias, the median was 4.04% (range, −13.30% to 16.18%) for the 2 sampling point strategy versus 6.78% (range, −16.26% to 30.06%) for the 1 sampling point strategy. The imprecisions were RMSE = 0.81% (0.15%–2.5%) and RMSE = 1.42% (0.14%–3.61%), respectively. Two individual concentration–time curves for 2 different time periods predicted with the Bayesian forecast from the predose sampling strategy are shown in Figure 6.

Simulations

Table 3 summarizes the exposures given by AUC values after 500 simulations of different dose regimens: 2.5, 2.8, 3.0, and 3.3 mg. Median AUCs increased from 151.4 to 199.9 ng/mL·h with the initial doses targeted between 2.5 and 3.3 mg. Furthermore, all median AUCs were within the 150–200 ng/mL·h target. Of all the initial doses simulated, 3 mg showed the highest AUC_{0-12h} percentages within the theoretical 150–200 ng/mL·h target for patients similar to those used to develop the PPK model.

DISCUSSION

This study provides a clinically applicable PPK model that helps to quantify interpatient and intrapatient variability and to identify the characteristics that may influence the PK of tacrolimus. The full PK profiles of tacrolimus in renal transplant patients were best described by a two-compartment

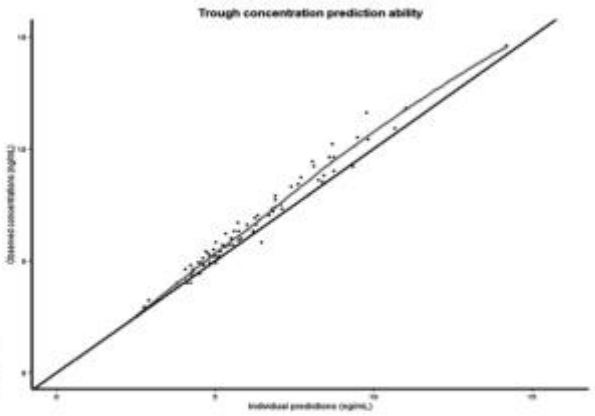

FIGURE 3. Goodness-of-fit plot for the trough concentration prediction ability. Bold line: locally weighted smoothing of the scatterplot data; solid line: identity line.

model with first-order absorption kinetics and lag time given by 3 delayed-transit compartments.

PPK studies including transit models to describe the absorption process are scarce in the literature. This could be due to the lack of sufficient data required for this purpose during the absorption phase. In our study, up to 3 transit compartments were included in a sequential way, coinciding with those estimated by Benkali et al[21] with the Erlang distribution model. The K_{tr} value of our study (K_{tr} = 3.61 per hour and n = 3) was also similar to the value reported by Benkali et al[21] (K_{tr} = 6.5 ± 0.4 per hour). This transit compartment model provided a good description of the absorption process and precision in the parameter estimates (relative standard error < 10%). The observed delay in the absorption process should be expected for a drug with high lipophilicity (log P = 3.3) and poor water solubility (4 mcg/mL)[40] such as tacrolimus, which slows down its absorption in the gut. The population clearance value found in this study (16.5 ± 1.8 L/h) was comparable with previous studies (13.2–40.5 L/h from Antignac et al,[20] 33 ± 11.3 L/h from Staatz et al,[19] 29 ± 0.2 L/h from Musuamba et al,[22] and 28 ± 4 L/h from Benkali et al[21]). This should be expected given that similar populations were included in all these studies. The estimated total distribution volume was 535.92 L, indicative of extensive tissue distribution.

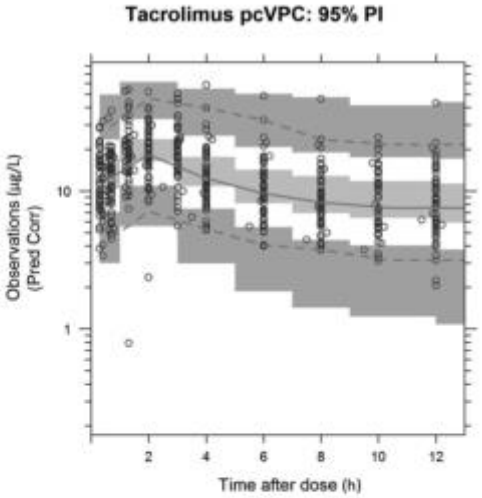

FIGURE 4. Prediction-corrected visual predictive check corresponding to tacrolimus whole-blood concentrations versus time (hours after last dose) profiles. In general, median (solid line), 95th and 5th percentiles (dashed lines) of the observations, as well as the 90% confidence intervals for the median, the 5th and 95th percentiles of the simulated profiles (covered by the light red and blue areas, respectively) are superimposed in each graph.

The low observed IIV associated with CL, Ka, and MT was in accordance with the uniformity of the population in this study. Inclusion of the IOV of the clearance significantly improved the model and reduced the IIV. These results agree with other clinical PPK models reported recently in the literature.[21–25,41] The magnitude of the IOV associated with CL was 29% lower than IIV_{CL} and similar with respect to the IOV_{CL} values reported by Benkali et al[21] (28%), Woillard et al[25] (35%), and Musuamba et al[22] (40%). The inclusion of an IOV_{CL} of such a magnitude benefits dose adjustment based on previous occasions and could lead to improved efficacy and safety.

None of the physiologically plausible covariates led to a significant reduction of variability in clearance and central compartment distribution volume. Previous studies had reported hematocrit,[21] prednisone levels,[41] and the *ABCB1*,[22] the *CYP3A5*3*,[22,24] and the transcriptional control of the human *pregnane*

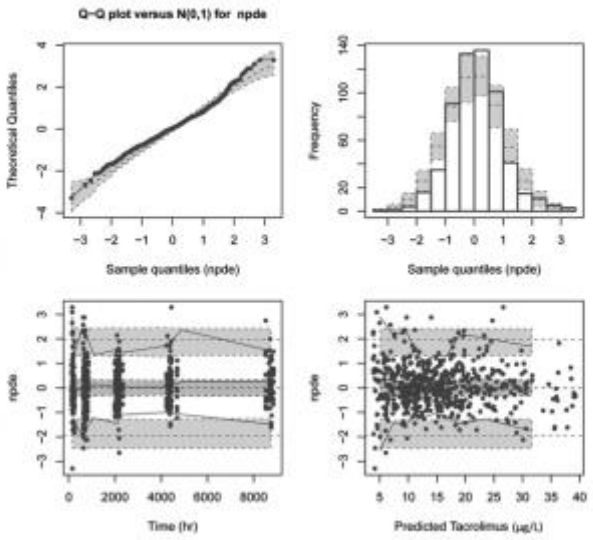

FIGURE 5. NPDE. Upper left panel: Q–Q plot of the matrices based on observation and simulation versus theoretical N (0,2). The line y = x is shown to help evaluate the adequacy between theoretical and both observed and simulated distributions. Upper right panel: distribution of NPDE. Lower left panel: NPDE versus time from the start of the treatment. Lower right panel: NPDE versus predicted tacrolimus concentrations. Dashed lines: 95% prediction interval for a normal distribution. Bold blue points: observed NPDE values. Solid lines: the 5th, 50th, and 95th percentiles of the observed NPDE values. Light blue areas: 90% confidence intervals for the 5th and 95th percentiles of the NPDEs from simulated data. Light red area: 90% confidence interval for the median of the NPDEs from simulated data.

X receptor polymorphisms[21,41] as major predictors of apparent tacrolimus clearance variability. Furthermore, Xue et al[24] linked the body surface area and the red blood count with the apparent peripheral distribution volume. In this study, neither red blood count nor hematocrit reduced the IIV associated with tacrolimus clearance. The median value of hematocrit of our population was 37% (27%–49%), whereas the authors who reported a significant effect of hematocrit on CL reported values of 28.6% (21%–39%).[21] Although comparable ranges were found, in our case, very few patients had low hematocrit values. Given that target patients showed stable hepatic function, a very narrow range of variation was observed in biochemical covariates indicative of hepatic impairment, such as ALT or AST, when tested on CL. Therefore, no statistically significant influence was found. In contrast, in liver transplant patients,[42] both AST and ALT have been reported in a larger range of values and reduced the tacrolimus CL variability.

Bilirubin was the only statistically significant covariate in CL ($\Delta OFV = -3.89$, $P < 0.05$), but it was not clinically significant. However, the reduction of the IIV_{CL} was only

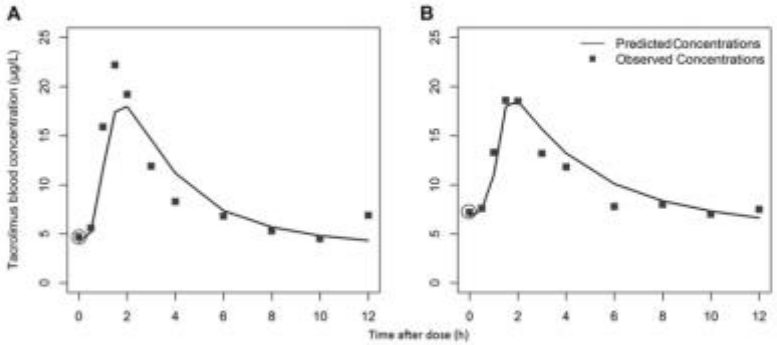

FIGURE 6. Predose LSS. Observed concentrations (square dots) and maximum a posteriori Bayesian predicted concentrations with the predose concentration (solid line) versus time (hours) after last dose for 2 different samples from a patient: (A) at week 1 (B) at month 3. In both cases, the model predicts the observed concentration with only the predose information with optimal accuracy.

TABLE 3. 500 Tacrolimus AUC_{0-12h} Simulations After Different Initial Doses Looking for an AUC Target Between 150 and 200 ng/mL·h

Dose, mg	AUC_{0-12h} Median (SD), ng/mL·h	<150 ng/mL·h, %	Between 150 and 200 ng/mL·h, %	>200 ng/mL·h, %
2.5	151.4 (87.7)	49	22.3	28
2.8	169.6 (98.2)	40	22.9	37
3.0	181.7 (105.3)	35	22.8	42
3.3	199.9 (115.8)	28	22	50

5.12%, and it did not result in much more consistent PK parameters, thus it was not retained in the model. In fact, changes in bilirubin are not only a consequence of hepatic impairment, so it was not considered an adequate predictive covariate of CL to be used for target AUC intervention. Simulations performed after fixed doses of 3 mg with the bilirubin covariate model versus the final selected model without this covariate showed that inclusion of bilirubin had a very low impact on the percentage of the C_{trough} falling within the therapeutic target range. The limited number of patients used in the covariate model building could be responsible for the nonstatistically significant results.

A group of external patients (n = 91) was available for an external validation. The results indicated an acceptable predictive capacity of C_{trough} with a coefficient of variation of 6.13% with better precision than Passey et al.[43] The goodness-of-fit plot showed a slight underprediction with a bias of 0.37 ng/mL that would not result in a major clinical impact. Nevertheless, sparse data from the external validation group could hide some misspecifications of the model. Moreover, results of the internal validation techniques applied (NPDE,[37] visual predictive check,[36] posterior predictive check,[38] and Fig. 2) showed no major discrepancies between observed and simulated data, indicating that the current model adequately described the data for renal transplant patients. The η-shrinkage[33] associated with all the parameters was <20%, this suggests a correct informative distribution of variability, which is important for empirical Bayes estimates. Moreover, the good CL population estimate and the low degree of IIV obtained support the appropriateness of the PPK model devised to predict individual clearance values in new patients, with characteristics similar to our study population, and therefore AUC_{0-12h} related to tacrolimus efficacy.[11,14] Thus, this model could be very helpful for dose tailoring during TDM.

Based on the PPK model, devised simulations of PK profiles after different initial doses allowed us to evaluate the percentage of patients with exposures within an AUC target of 150–200 ng/mL·h. These initial doses are in accordance with those currently used in clinical practice. The 3 mg initial dose included the highest AUC_{0-12h} percentage within the theoretical therapeutic range; although an initial 77% of patients were still outside the target, the dose adjustment during TDM using the model would reduce this percentage.

Moreover, this study supports the development of a Bayesian adaptive control of dosage regimens as a means of estimating the individual PK parameters and the AUC_{0-12h} on the basis of a routinely applicable limited sampling schedule. A two-point strategy (0-hour predose and 90-minute postdose) led to an accurate estimation (bias, 4.04%; range, −13.30% to 16.18%; and imprecision, 0.81%) of the tacrolimus exposure, which is compatible with clinical practice. In the LSS, 1 sampling point at predose also showed an acceptable estimation of the AUC_{0-12h} and better accuracy than Benkali et al,[21] with a narrower bias of 6.78% (range, −16.26% to 30.06%) versus 3% (range, −51% to 110%) and imprecision (1.42% versus 19%). Considering that experience indicates the logistic inconvenience of TDM for some patients, 1 sampling point would be a good LSS to minimize such problems. Thus, Bayesian forecasting from the predose sampling will allow accurate estimation of AUC, considered to be the best predictor of efficacy,[11] and consequently, dose adjustment will not be based on empirical criteria. Application of this predictor tool for target concentration intervention will improve efficacy and safety without any extra cost.

In conclusion, the PPK model devised provides reliable prior information for implementation in Bayesian adaptive control of dosage regimens of tacrolimus to achieve desired AUC values. Futher studies with increased number of patients could allow to successfully include clinically plausible covariates such as hematocrite or polymorphisms to have more accurate adaptive dosage control of tacrolimus. Additionally, studies of different new polymorphisms that may affect tacrolimus exposure should also be considered to be implemented for future PPK models.

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Chapter III

THE COMBINATION OF CYP3A4*22 AND CYP3A5*3 SNPS DETERMINE TACROLIMUS DOSE REQUIREMENT AFTER KIDNEY TRANSPLANTATION

Original article 1

The combination of CYP3A4*22 and CYP3A5*3 single-nucleotide polymorphisms determines tacrolimus dose requirement after kidney transplantation

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Introduction Tacrolimus (Tac) has a narrow therapeutic window and shows large between-patient pharmacokinetic variability. As a result, over-immunosuppression and under-immunosuppression are frequently encountered in daily clinical practice. Unraveling the impact of genetic polymorphisms on Tac pharmacokinetics may help to refine therapy. In this study, the associations of single-nucleotide polymorphisms (SNPs) in drug-metabolizing enzymes (*CYP3A*) with Tac pharmacokinetics were investigated in renal transplant recipients.

Participants and methods In a cohort of 272 kidney transplant recipients, associations between functional genetic variants (*CYP3A4*22* and *CYP3A5*3)* and dose-adjusted predose Tac concentrations (C_0) and daily doses of Tac at days 5–7 and 15 and 1, 3, 6 and 12 months after renal transplantation were evaluated. Patients were genotyped and clustered according to both *CYP3A4*22* and *CYP3A5*3* allelic status: poor (PM) (*CYP3A4*22* carriers with *CYP3A5*3/*3*), intermediate (IM) (*CYP3A4*1/*1* with *CYP3A5*3/*3* or *CYP3A4*22* carriers with *CYP3A5*1* carriers) and extensive *CYP3A*-metabolizers (EM) (*CYP3A4*1/*1* and *CYP3A5*1* carriers).

Results EM had an 88% lower dose-adjusted C_0 compared with IM. PM had a 26% higher dose-adjusted C_0 compared with IM. The percentage of patients with supratherapeutic Tac exposure ($C_0 > 15$ ng/ml) was significantly higher in PM (43.5%) compared with EM (0%) at days 5–7 after transplantation ($P = 0.01$). About 30% of EM had subtherapeutic exposure ($C_0 < 5$ ng/ml) at days 5–7 after transplantation ($P = 0.001$).

Conclusion The combined *CYP3A4* and *CYP3A5* genotype of renal transplant recipients has a major influence on the Tac dose required to reach the target exposure. *Pharmacogenetics and Genomics* 00:000–000

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Introduction

Nowadays, 1-year graft survival of kidney transplants is excellent, mainly as a result of potent, calcineurin inhibitor (CNI)-based, immunosuppressive therapy [1,2]. Tacrolimus (Tac) has now largely replaced ciclosporin as the CNI of choice. Its clinical use is considerably complicated by a high degree of toxicity, including acute and chronic nephrotoxicity. In addition, Tac has a narrow therapeutic window and has a highly variable and unpredictable pharmacokinetic behaviour both between and within individual patients. This wide interpatient variability renders therapeutic drug monitoring (TDM) a necessity. Achievement of therapeutic exposure, assessed by predose concentrations (C_0), is of paramount importance during the early period after transplantation [3]. TDM is thus mandatory and commonly applied in clinical settings for Tac dose adjustment but, similar to any other reactive strategy, TDM is not supportive in the critical first days after therapy initiation. Indeed, the use of predictive factors to move towards a proactive approach by the inclusion of pharmacogenetic markers could aid dose optimization mainly during the early period transplantation when the pharmacokinetic steady state is not yet achieved [4,5].

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As the principal protagonists of CNI pharmacokinetic fate in humans, drug-metabolizing enzymes, efflux pumps and target site genes constitute promising tracks for integrating a pharmacogenetic and personalized dosing strategy into clinical practice. An understanding of the influence of genetic factors on CNI pharmacokinetics (PK) should enable the outlining of the optimal immunosuppressant drug combination and estimating the optimal starting dose for a particular individual. It also has the potential to help in the early identification of patients with an increased risk of drug inefficacy and/or side effects. In particular, Tac and cyclosporine are known to be substrates of the cytochrome P450 (CYP) 3A subfamily [6], which includes three functional members: *CYP3A4*, *CYP3A5* and *CYP3A7*. Both *CYP3A4* and *CYP3A5* are implied in CNI oxidative metabolism, with, for Tac, *CYP3A5* being the more efficient catalyst over *CYP3A4* [7]. The activity of CNI-metabolizing isoenzymes varies markedly between individuals and appears to be explained, in part, by the presence of certain single-nucleotide polymorphisms (SNPs) in the *CYP3A4* and *CYP3A5* genes [8,9].

In-vitro studies showed that the catalytic activity towards Tac is 1.6-fold higher for *CYP3A5* than for *CYP3A4*. Furthermore, in-vitro data showed that the importance of *CYP3A5*3* allelic status is dependent on the concomitant *CYP3A4* activity and that the relative contribution of *CYP3A4* or *CYP3A5* to Tac metabolism depends on the amount of each counterpart [10,11]. Therefore, individual SNP analysis (*CYP3A5*3* and *CYP3A4*22*) influencing Tac PK resulted worth to combine.

The contribution of these two genetic variants to the observed interindividual PK variability has been studied through the years. The *CYP3A5*3* variant is the predominant allele in many populations, and the majority of Caucasians (80%) lack the functional *CYP3A5* protein [12–16]. *CYP3A5* expressers require approximately two-fold higher Tac doses to reach the same blood exposure compared with *CYP3A5* nonexpressers in any population studied [17–27].

Recently, it has been shown that *CYP3A4*22* carriers had higher Tac C_0 during the first week after transplantation and had more supratherapeutic C_0. These effects were independent of the *CYP3A5*3* genotype [7]. However, other authors did not find an association between *CYP3A4*22* and Tac dose-adjusted C_0 [16].

Data suggest that other transporters such as P-glycoprotein, which is encoded by *ABCB1* [6], and multidrug resistance-associated protein 2 encoded by *ABCC2* [28] (and organic anion transporting polypeptide-C (encoded by *SLCO1B1*), may be important in Tac disposition [29–31]. Compared with *CYP3A* SNP, the information on the association between the PK of Tac and SNPs in *ABCC2* and *SLCO1B1* is limited and unexplained pharmacokinetic variability may be explained by these genetic variants. Some studies observed that renal and liver transplant recipients homozygous for the 3435CC *ABCB1* gene required higher Tac doses than carriers of at least one mutated allele 3435T [32,33]. Contradictory data have been published on *ABCC2* variants −24C > T, 1249G > A and 3972C > T [34,35]. Although the influence of three *SLCO1B1* SNP (*SLCO1B1*–11187G > A, *NR1I2* 11156A > C and 11193T > C) on PK parameters and in liver transplant patients has not been observed [17], data on the effect of these SNPs in renal transplant patients are lacking.

The primary objective of the present investigation was to study the association between different SNPs in the recipient's genome and Tac exposure as assessed by Tac C_0 measured at regular time intervals during the first 12 months after kidney transplantation. In addition, the contribution of the different SNPs towards clinical outcomes was explored.

Participants and methods

Patient population

The protocol of the present retrospective and observational study was approved by the Research Ethics Committee of our centre and all patients included provided written informed consent (PR 142/12).

Adult deceased and living donor kidney transplant recipients were recruited between 2005 and 2012 at the Renal Transplant Unit of the Bellvitge University Hospital (Barcelona, Spain). All patients were treated with an immunosuppressive drug regimen consisting of oral Tac (Prograf; Astellas Pharma Europe Ltd., Staines, UK) administered in two equally divided doses in combination with 0.5–1.0 g mycophenolate mofetil (Cellcept; Roche, Basel, Switzerland) twice daily. A Tac oral loading dose of 0.1 mg/kg/day was started on the day of transplantation and subsequently adjusted to achieve the predefined target whole-blood C_0 range of 5–10 ng/ml. The target Tac C_0 was equal for all patients, irrespective of the need for induction therapy. Clinicians adjusted the Tac evening dose to achieve a predefined target concentration range, on the basis of the previous morning C_0 value, and an equally divided daily dose was then recalculated for the following day (new dose = target C_0/measured C_0 × daily dose). No pharmacokinetic modelling program was used to predict dose requirements. Patients using once-daily Tac formulations and patients treated with interacting drugs were excluded [36].

Induction therapy varied depending on the perceived immunological risk and was also used for kidneys from extended criteria donors. Induction therapy (anti-thymocyte globulin (Thymoglobulin; Genzyme Europe, Naarden, the Netherlands), basiliximab (Simulect; Novartis farmaceutica, Camberley, UK) or daclizumab (Zenapaxp; Roche) was used in the case of increased immunological risk. In addition, all patients received intraoperative glucocorticoids according to the local protocol (500 mg intravenous methylprednisolone prereperfusion and 125 mg on day 1), which was followed by 15 mg/day oral prednisone until month 1. Then, prednisone

was tapered progressively over several months to either a daily maintenance dose of 5 mg or complete discontinuation.

Serum creatinine concentrations (μmol/l) and estimated glomerular filtration rates (eGFR; calculated using the Chronic Kidney Disease Epidemiology Collaboration formula), Tac C_0 (ng/ml) and Tac daily doses (mg/day and mg/kg/day) were retrieved from the medical files at the following time points: days 5–7, day 15 ± 2, day 30 ± 2, month 3 ± 7 days, month 6 ± 7 days and month 12 ± 7 days after transplantation (as renal function measure). Dose-adjusted C_0 were calculated by dividing the C_0 by the total daily dose. Demographic characteristics (age, sex, ethnicity and weight) of the patients were recorded and patients were followed for up to 12 months after transplantation.

Clinical outcome variables that were assessed were renal function (eGFR), delayed graft function (DGF) and graft loss. DGF was defined as the need for dialysis therapy within the first postoperative week. Graft loss was defined as return to dialysis or retransplantation (patients with surgical complications and recipients from living donors were excluded from the analysis).

Tacrolimus concentration measurements

Samples were analysed using either the validated enzyme multiplied immunoassay technique with a quantification limit of 2 ng/ml and a linearity over the range of 2 and 30 ng/ml until March 2011. From April 2011, samples were analysed by a validated ultra-performance liquid chromatography–mass spectrometry with a limit of quantification of 0.6 ng/ml and linearity over the range of 0.6 to 44 ng/ml. The correlation of both methods used for Tac analysis (liquid chromatography–mass spectrometry and enzyme multiplied immunoassay technique) was assessed according to Passing and Bablok [37]. There was a good correlation between both techniques, with a slope value of 1.1093 (95% confidence interval: 1.0714–1.1538) and an intersection y-axis value of 0.2073 (95% confidence interval: −0.0385 to −0.3857).

Genotyping

Genomic DNA was extracted from a peripheral whole-blood sample using the Wizard Genomic DNA Purification Kit (Promega Corporation, Sydney, Australia) and was stored at −80°C.

For the genotyping, allelic discrimination reactions were performed using specific TaqMan (Applied Biosystems, Foster City, California, USA) genotyping assays on an ABI PRISM 7500 Fast Real-Time PCR Systems (Applied Biosystems) using 20 ng genomic DNA and according to the manufacturer's instructions. Briefly, the PCR cycle consisted of an initial step of 1 min at 60°C, followed by a denaturation step at 95°C for 30 s and 40 cycles with 95°C for 3 s and 60°C for 30 s. The final post-PCR read was made in 1 min at 60°C. The volume for each reaction was 12 μl, consisting of 5 μl TaqMan GTXpress Master Mix (Biosystems, Foster City, California, USA), 0.125 μl of TaqMan SNP genotyping assay (80 ×) (Applied Biosystems, Foster City, California, USA), containing the primers (4 μmol/l) and the probes (16 μmol/l), and 20 ng genomic DNA. The assays used had already been validated by direct sequencing in the Department of Clinical Chemistry of the Erasmus Medical Center (Rotterdam, the Netherlands), which was in charge of the genetic analyses for the current project.

Recipients were genotyped for *CYP3A4*22* C > T (rs35599367), *CYP3A5*3* G > A(rs776746), *POR*28* C > T (rs1057868), *ABCB1* 3435C > T(rs1045642), *ABCC2* − 24C > T (rs17216163), *ABCC2* 1249G > A(rs2273697), *ABCC2* 4544G > A (rs8187710), *SLCO1B1*1B* 388A > G(rs2306283), *SLCO1B1*5* 521 T > C(rs4149056) and *SLCO1B3* 699 G > A(rs7311358). According to the functional defect associated with *CYP3A* variants [38], we gathered *CYP3A* genotypes and classified patients into three different clusters: poor (PM) (*CYP3A4*22* carriers with *CYP3A5*3/*3*), intermediate (IM) (*CYP3A4*1/*1* with *CYP3A5*3/*3* or *CYP3A4*22* carriers with *CYP3A5*1* carriers) and extensive *CYP3A*-metabolizers (EM) (*CYP3A4*1/*1* and *CYP3A5*1* carriers).

Statistics

Statistical analysis of the primary endpoint included the entire population. Initially, linear models were constructed to explore the effect of categorical variables such as genotype or sex as well as several continuous variables such as haematocrit levels and age, considered fixed factors, on the dose per weight-adjusted C_0 values (expressed as ng/ml/mg/day/kg of body weight). The log-transformed values of the dependent variable (dose-adjusted C_0) were used in all statistical tests. The overall effect of the *CYP3A* genotype and all the continuous covariates on the dependent variable, taking into account interdependent responses from the same patient through the different assayed occasions variability (at 7, 15, 30, 90, 180 and 365 days after transplant), was assessed by the mixed linear models implemented. Differences between patients at baseline were modelled by assuming different random intercepts for each patient. Statistical analysis of secondary objectives (outcome endpoints) included Kruskall–Wallis and U-Mann–Whitney test and χ^2-test for categorical variables and was carried out excluding living donor kidney transplant recipients to compare a homogeneous population. Pearson χ^2-test was also performed to compare the observed genotypic frequencies with Hardy–Weinberg expectations. All analyses were carried out in the R statistical package, version 3.2.2. (R Core Team, Vienna, Austria).

Results

A total of 272 adult recipients of a deceased or a living kidney donor (mean age: 51 ± 15 years) were included in the present study (Table 1). The majority of the patients (87.7%) received additional antibody induction therapy consisting of anti-thymocyte globulin ($n = 88$), basiliximab ($n = 127$) or daclizumab ($n = 14$).

Table 1 Overall characteristics of recipients ($n = 272$)

Recipient characteristics	n (%)
Sex (male/female) ($n = 272$)	179 (65.8)/93 (34.2)
Weight ($n = 271$)	69.59 ± 13.66
Height ($n = 269$)	169.49 ± 61.81
BMI ($n = 268$)	25.16 ± 4.60
Age ($n = 278$)	51 ± 15
Number of transplant ($n = 272$)	
1	217 (79.8)
2	44 (16.2)
≥ 3	11 (4)
Primary kidney disease ($n = 267$)	
Diabetic nephropathy	14 (5.1)
Glomerulonephritis	85 (31.8)
Interstitial nephritis	45 (16.9)
Unknown origin	77 (28.8)
Polycystic kidney disease	33 (12.0)
Hypertensive nephropathy	14 (5.2)
CMV (yes/no) ($n = 266$)	215 (80.8)/51 (19.2)
Total HLA mismatches ($n = 266$)	
0	6 (2.2)
1	7 (2.6)
2	24 (9.0)
3	91 (34.2)
4	86 (32.3)
5	48 (18.0)
6	4 (1.5)
Type of donor ($n = 272$)	
Deceased	232 (85.3)
Living	40 (14.7)
Induction therapy ($n = 261$)	
None	32 (12.3)
ATG	88 (33.7)
Basiliximab	127 (48.7)
Daclizumab	14 (5.4)

The univariate analysis was carried out using log-transformed values of the dependent variable and ANOVA was the statistic used.
ANOVA, analysis of variance; ATG, anti-thymocyte globulin; CMV, cytomegalovirus; HLA, human leukocyte antigen.

Demographic and physiologic data of all included patients are summarized in Table 1. Pharmacokinetic data including Tac C_0 (ng/ml), daily doses (mg/day), daily doses per body weight (mg/kg), dose-adjusted C_0 (ng/ml/mg/day) and dose per weight-adjusted C_0 (ng/ml/mg/kg/day) at six different time points (days 5–7, day 15, months 1, 3, 6 and 12) during the first year after transplantation are presented in Table 2.

Genotyping results/genotype frequencies

Allelic frequencies of the SNPs investigated in the 272 recipients are shown in Table 3. Frequencies observed in the present study were in accordance with reported allele frequencies in a Caucasian population and did not deviate from Hardy–Weinberg distribution. All patients were of Caucasian ethnicity.

The frequencies of the *CYP3A4*1/*1* and **1/*22* genotypes were 242 (91.0%) and 24 (9.0%), respectively. The *CYP3A5*1/*3* and **3/*3* genotypes were observed in 42 (15.8%) and 223 (84.2%) patients, respectively. No *CYP3A5*1/*1* or *CYP3A4*22/*22* homozygous patients were detected. The vast majority [23 out of 24 (95.8%)] of the *CYP3A4*22* carriers were nonexpressers for *CYP3A5* as reported previously [39]. When combining both *CYP3A* allelic statuses and using the previously published *CYP3A* genotype-based classification system [40], 23 Tac recipients were classified as PM, 198 recipients as IM and 41 as EM.

Influence of polymorphisms on Tac exposure and daily dose

Considering the entire follow-up period, *CYP3A4*1/*22* showed higher dose-adjusted C_0 compared with *CYP3A4*1/*1* wild-type patients, with statistically significant differences from days 5–7 to month 6 (Supplementary Fig. S1A, Supplemental digital content 1, *http://links.lww.com/FPC/B245*). *CYP3A4*1/*1* patients presented a 32% decreased Tac dose-adjusted C_0 compared with *CYP3A4*1/*22* patients. In addition, *CYP3A5*3/*3* had significantly higher dose-adjusted C_0 during the entire study period. *CYP3A5*1/*3* patients presented a 51% decreased Tac dose-adjusted C_0 compared with *CYP3A5*3/*3* patients (Supplementary Fig. S1B, Supplemental digital content 2, *http://links.lww.com/FPC/B246*).

The influence of the *CYP3A* combined genotype on Tac dose-adjusted C_0 showed that the differences between the three *CYP3A* genotype clusters were significant during the entire first post-transplantation year (Table 2). EM had 61 and 46% decreased Tac dose-adjusted C_0 compared with PM and IM, respectively (Fig. 1b), and received a higher dose [Supplementary Figs S2A (Supplemental digital content 3, *http://links.lww.com/FPC/B247*) and S2B (Supplemental digital content 4, *http://links.lww.com/FPC/B248*)]. Plots of concentration over time and dose per weight-adjusted C_0 over time by *CYP3A* genotype clusters are shown in Fig. 1a and c, respectively.

None of the *ABCB1 3435C > T*, *ABCC2 − 24C > T*, *ABCC2 1249 G > A*, *ABCC2 4544G > A*, *SLCO1B1 388A > G*, *SLCO1B1 521T > C*, *SLCO1B3 699G > A* alleles tested in this study population correlated with dose-adjusted C_0 (Supplementary Table 1, Supplemental digital content 5, *http://links.lww.com/FPC/B249*).

Overall, mixed-model analysis for repeated measurements for the 12 months of follow-up showed that PM had a statistically significantly lower Tac dose-adjusted C_0 after adjusting for age, sex and haematocrit level compared with IM patients. In contrast, EM had a statistically significantly higher TAC dose-adjusted C_0 after adjusting for age, sex and haematocrit level compared with IM. About 53% of Tac weight-adjusted dose variability was explained within patient and time (data not shown); adding *CYP3A* reduces the variability explained within patient to 45% and finally, after adjusting by age, sex and haematocrit, the % of variability explained by patient effect was 40% (Table 4). However, a population pharmacokinetic model developed with these patients showed that Tac disposition in renal transplant recipients included the *CYP3A5*3* and *CYP3A4*22* genotype, age and haematocrit [41]. EM had 88% a lower Tac dose-adjusted C_0 compared with IM ($P < 0.001$). PM had 26%

Table 2 Tacrolimus C_0, daily dose, daily dose per body weight and dose-adjusted C_0 at days 5–7, day 15, months 1, 3, 6 and 12 after transplantation in the entire study population and considering the *CYP3A* combined genotype

	All recipients studied				Median values			Univariate analysis
	N	Median	SD	95% CI	CYP3A PM recipient	CYP3A EM recipient	CYP3A IM recipient	*P*[a]
C_0 (ng/ml) (days 5–7)	264	8.40	4.97	8.99–10.20	13.6	6.70	8.65	0.000
C_0 (ng/ml) (day 15)	263	8.40	6.45	9.24–10.81	11.25	7.15	8.50	0.000
C_0 (ng/ml) (month 1)	256	8.55	4.44	8.73–9.80	9.75	7.70	8.80	0.085
C_0 (ng/ml) (month 3)	255	7.90	3.30	7.94–8.75	8.15	7.60	7.90	0.574
C_0 (ng/ml) (month 6)	232	7.10	3.51	7.08–7.99	6.60	6.40	7.50	0.115
C_0 (ng/ml) (month 12)	211	6.40	2.96	6.55–7.35	6.20	6.90	6.20	0.230
Dose (mg/day) (day 5–7)	261	8.00	3.35	7.56–8.38	6.00	8.00	7.00	0.000
Dose (mg/day) (day 15)	266	7.00	3.54	6.81–7.66	4.00	9.50	6.00	0.000
Dose (mg/day) (month 1)	263	6.00	3.57	6.24–7.11	4.00	9.50	6.00	0.000
Dose (mg/day) (month 3)	248	4.00	3.29	4.96–5.79	3.00	10.00	4.00	0.000
Dose (mg/day) (month 6)	226	4.00	3.02	4.39–5.19	3.00	8.50	4.00	0.000
Dose (mg/day) (month 12)	200	4.00	2.89	3.96–4.76	2.75	8.00	4.00	0.000
Dose-adjusted C_0 (ng/ml/mg/kg/day) (days 5–7)	258	78.23	85.74	89.66–110.68	117.04	42.79	82.33	0.000
Dose-adjusted C_0 (ng/ml/mg/kg/day) (day 15)	260	91.29	101.63	107.81–132.63	149.88	55.99	92.83	0.000
Dose-adjusted C_0 (ng/ml/mg/kg/day) (month 1)	258	94.96	101.47	111.20–136.08	125.51	58.54	98.56	0.000
Dose-adjusted C_0 (ng/ml/mg/kg/day) (month 3)	245	111.71	112.12	132.24–160.45	182.00	55.70	124.05	0.000
Dose-adjusted C_0 (ng/ml/mg/kg/day) (month 6)	221	118.22	130.51	134.91–169.52	170.80	56.37	130.20	0.000
Dose-adjusted C_0 (ng/ml/mg/kg/day) (month 12)	191	116.85	105.95	137.16–167.40	145.60	78.20	126.73	0.000

CI, confidence interval; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.
[a]Univariate analysis Kruskal–Wallis test.

higher TAC dose-adjusted C_0 compared with IM ($P < 0.001$). Estimated means for dose-adjusted C_0 were 147.7, 111.3 and 56.2 for PM, IM and EM, respectively.

The percent of patients with supratherapeutic C_0 at days 5–7 after transplantation ($C_0 > 15$ ng/ml), considering *CYP3A* genotype clusters status, was significantly higher in PM patients (43.5%) compared with EM (0%) ($P = 0.01$) (Fig. 2). Subtherapeutic values ($C_0 < 5$ ng/ml) were observed in 26.8% of EM at days 5–7 after transplantation, showing a delay in achieving the target Tac exposure in the early period after transplantation ($P = 0.001$, Fig. 2 and Table 5). Although there were difference in the concentrations reached between patients during the first week after transplatation, the majoiry of patients achieved the target within 15 days (Fig. 2).

Influence on clinical outcome

Renal function was not statistically significantly different between *CYP3A5* expressers and nonexpressers. No statistically significant differences were observed in creatinine values, eGFR, the incidence of DGF and acute rejection between the different *CYP3A* genotype groups. Graft loss occurred in 22 patients, 8.8% of EM, 8.7% of IM and 21.1% of PM. These differences were not statistically significantly different between groups [Supplementary Tables 2 (Supplemental digital content 6, *http://links.lww.com/FPC/B250*) and 3 (Supplemental digital content 7, *http://links.lww.com/FPC/B251*)].

Discussion

The aim of the present study was to evaluate the combined influence of *CYP3A4*22* and *CYP3A5*3* SNPs on the PK of Tac throughout the first post-transplant year. We found that Tac PK, as assessed by the dose needed to reach adequate drug exposure, was influenced by *CYP3A4*22* and *CYP3A5*3* SNPS. In addition, we showed the utility of defining genotype clusters according to the *CYP3A* genotype to predict differential dose-adjusted Tac C_0. In particular, patients classified as EM were shown to have a lower mean Tac exposure during the entire study period, which might, over time, lead to subtherapeutic exposure and its consequences such as the development of donor-specific anti-HLA antibodies.

The *CYP3A5*3* allele has been recognized widely as the main genetic factor influencing Tac PK. Patients not expressing *CYP3A5* require two-fold lower Tac doses to reach the same concentrations compared with carriers of at least one *CYP3A5*1* functional allele [42]. This observation has been translated into a promising clinical message as, in a prospective randomized trial, Thervet and colleaguesreported that the proportion of patients reaching the target Tac C_0 (10–15 ng/ml) was significantly higher in the *CYP3A5* genotype adapted-dose group (43.2%) compared with the control group receiving a standard, bodyweight-based dose (29.1%) [43]. In a more recent clinical trial [35], this was, however, not confirmed. In the present study, it is confirmed that patients with the *CYP3A5*3/*3* genotype had significantly higher

Table 3 Recipient allelic frequencies

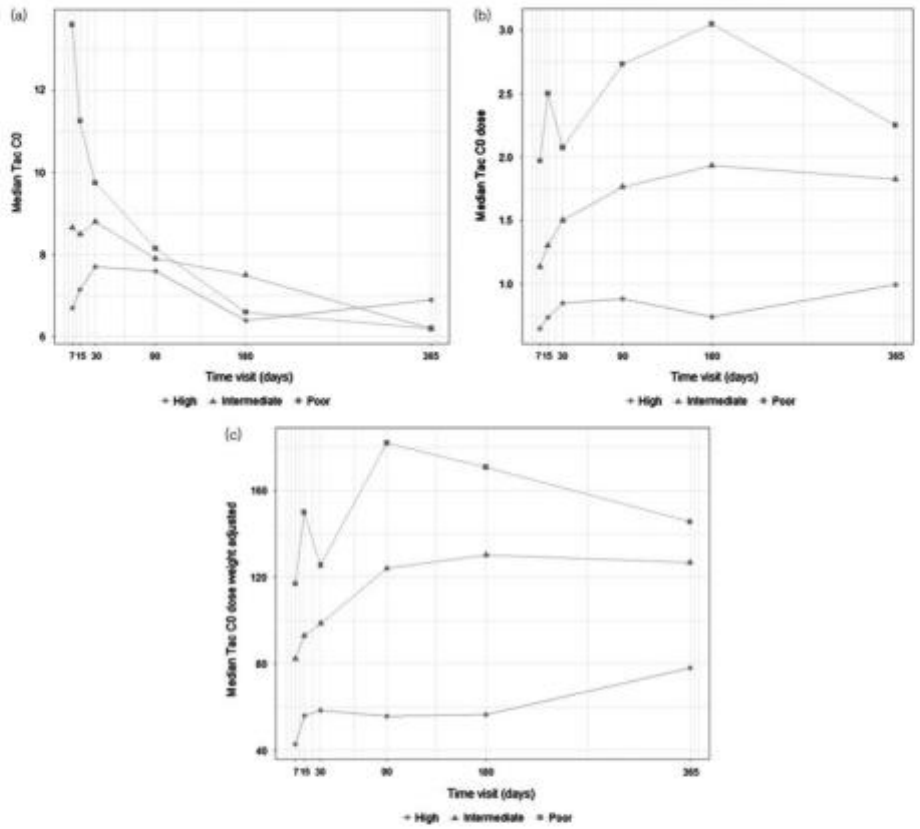
	n (%)
CYP3A4*22	
CYP3A4*1/*1	242 (91.0)
CYP3A4*1/*22	24 (9.0)
CYP3A4*22/*22	0 (0)
CYP3A5*3	
CYP3A5*1/*1	0 (0)
CYP3A5*1/*3	42 (15.8)
CYP3A5*3/*3	223 (84.2)
CYP3A genotype clusters	
CYP3A PM	23 (8.8)
CYP3A IM	198 (75.6)
CYP3A EM	41 (15.6)
POR*28	
POR*1/*1	149 (56.0)
POR*1/*28	96 (36.1)
POR*28/*28	21 (7.9)
ABCB1 3435CT	
CC	84 (31.7)
CT	131 (49.4)
TT	50 (18.9)
ABCC2 24CT	
CC	162 (60.9)
CT	86 (32.2)
TT	18 (6.8)
ABCC2 1249GA	
GG	167 (62.8)
GA	86 (32.3)
AA	13 (4.9)
ABCC 24544GA	
GG	226 (86.3)
GA	36 (13.7)
SLCO1B1 388AGAA	
AG	25 (29.4)
GG	45 (52.9)
SLCO1B1 521TC	15 (17.6)
TT	58 (67.4)
TC	27 (31.4)
CC	1 (1.2)
SLCO1B3 699GA	
GG	6 (2.3)
GA	53 (20.0)
AA	206 (77.7)

dose-adjusted C_0 throughout the first post-transplant year, indicating that, because of a functional defect in *CYP3A5* activity, a lower dose is sufficient to reach the same blood concentrations as *CYP3A5* expressers.

*CYP3A4*22* was originally described by Wang and colleagues, who showed that carriers of the defective allele required lower statin doses to control lipid levels compared with *CYP3A4*1/*1* individuals. They also characterized *in vitro* the functional defect linked to the *CYP3A4*22* intron 6 SNP by showing an allele-specific decrease in *CYP3A4* hepatic mRNA production and a lower microsomal CYP3A4-driven activity. Elens *et al.* [40] reported that carriers of *CYP3A4*22* SNP needed lower Tac doses to reach the target C_0 compared with *CYP3A4*1/*1* patients in de-novo kidney transplant patients. The differences between the different genotype groups were observed at early follow-up time points after transplantation, but not at later time points. This has been confirmed by other investigators who found higher mean Tac C_0 in *CYP3A4*22* carriers at months 3 (13.8 vs. 10.9 ng/ml) and 6 (12.7 vs. 9.4 ng/ml) after transplant [42]. However, some other studies did not find an association between the *CYP3A4*22* allele and Tac C_0 in renal transplant recipients [35,43]. This difference might be because of genetic differences between cohorts and differences in post-transplant immunosuppressive drug protocols. The present study confirmed this influence when considering early time points (i.e. 5–7 days after transplantation) but, in addition, showed that differences were still present at months 3 and 6 after renal transplantation. A post-hoc analysis of Tactique trial showed that the *CYP3A4*22* allele was important to identify patients at risk of supratherapeutic exposure [39]. Three days after the start of Tac administration (i.e. 10 days after surgery), only 11% of the *CYP3A4*22* carriers were within the target range of Tac C_0, whereas among the *CYP3A4*1/*1* carriers, 40% were within the target range. Accordingly, the investigators concluded that carriers of *CYP3A4*22* often reach supratherapeutic concentrations and they assigned an important predictive value to this allele in determining the right Tac starting dose when combined with the *CYP3A5* genotype information.

Some authors have described that the combined genetic score of *CYP3A4* and *CYP3A5* polymorphisms could facilitate rapid dose adjustment of Tac [44,45]. This strategy is also included in a population pharmacokinetic study [41] to aid Tac dose refinement in routine. On combining both *CYP3A* alleles into a metabolism status (PM, IM or EM) and defining three clusters as proposed by Elens *et al.* [38,40], the differences between groups on Tac dose-adjusted concentrations were amplified. As shown in Fig. 1c, almost all differences were statistically significant, also at later time points. These results are in agreement with those of a previously published report [46] where *CYP3A* genotype clusters were studied in a smaller cohort of renal transplant recipients ($n = 49$) who were treated with either Tac or cyclosporine. In this study, in which patients were studied ~8 years after transplantation, the same differences in Tac dose requirement were observed between the three *CYP3A* genotype clusters. Our results indicate that the consideration of the combined *CYP3A4* and *CYP3A5* genotype could help to better predict Tac exposure during the first 6 months after renal transplantation and might aid Tac dose optimization. EM had a 47% decreased Tac dose-adjusted C_0 compared with IM. In contrast, PM had a 36% higher Tac dose-adjusted C_0 compared with IM. These observations indicated that dose adjustment on the basis of the combined *CYP3A4* and *CYP3A5* genotype could increase the number of patients within the therapeutic target, specifically during the first weeks after transplantation. This dosage fine-tuning has the ultimate potential to improve the graft outcome of transplantation by minimizing the drug exposure-related toxicity and subtherapeutic exposure. Supporting this hypothesis,

Fig. 1



Dose-adjusted tacrolimus (Tac) C_0 (ng/ml/kg body weight) according to (a) *CYP3A4*22* or (b) *CYP3A5*1* or (c) *CYP3A* combined genotype over the 12 months after transplantation. Data represent medians.

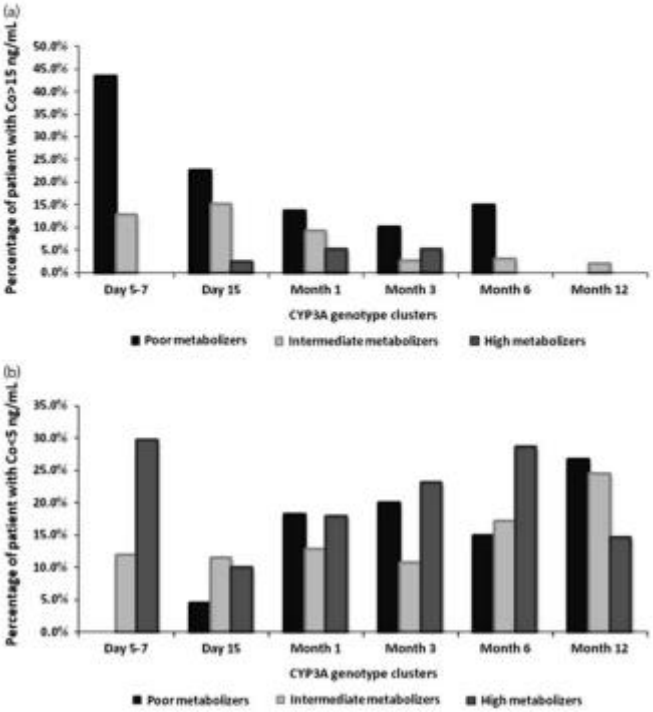
Table 4 Raw and adjusted association of *CYP3A* with Tac C_0 dose and weight adjusted (mixed regression model: $n = 275$ and 1448 observations)

	Standardized β (raw)	*P* value (raw)	Standardized β (adjusted)	*P* value (adjusted)
CYP3A intermediate vs. poor	0.43	0.0052	0.46	0.0011
CYP3A intermediate vs. high	−0.96	<0.0001	−0.90	<0.0001
Time (30 days)	0.17	<0.0001	0.09	0.0001
Sex (male)	–	–	0.16	0.0591
Age 10 years	–	–	0.23	<0.0001
Haematocrit	–	–	0.19	<0.0001

A poor expression of *CYP3A* in relation to an intermediate expression is associated with a higher Tac dose and a higher expression of CYP3 in relation to an intermediate expression is associated with a lower Tac dose both independent of sex, age and haematocrit. Moreover, older patients have a higher haematocrit, which is associated independently with a higher Tac dose.

in the present study, it was observed that the risk of a supratherapeutic C_0 (>15 ng/ml) was significantly higher for PM than for EM (0%) at day 5–7 after transplantation. In addition, a significant delay in reaching the target Tac C_0 was observed in EM. About 29.7% of these patients had subtherapeutic C_0 (<5 ng/ml) levels at day 5–7.

Fig. 2



Distribution of patients on the basis of tacrolimus (Tac) exposure achieved at month 1 after transplantation. (a) percentage of patients with Co>15ng/ml and (b) percentage of patients with C0 < 5 ng/mL

Table 5 Proportion of patients at days 5–7 with tacrolimus concentrations less than 5 or more than 15 ng/ml

	High metabolizers (%)	Intermediate metabolizers (%)	Poor metabolizers (%)	All (%)
C_0 < 5 ng/ml	26.8	10.6	0	12.2
C_0 > 15 ng/ml	0	12.9	43.5	13.8

Associations of genetic variants and normalized Tac dose-adjusted C_0 concentrations.

However, as this is a retrospective analysis, we believe that to validate the recommended starting doses, a prospective randomized-controlled trial is needed to evaluate the risk/benefit ratio of a combined *CYP3A4*/*CYP3A5* genotype-based Tac dosing strategy.

The study has limitations. First, it includes only Caucasian patients and there might be differences in *CYP3A* genotype cluster activity with other cohorts. The second limitation is the relatively low number of PM and IM, which reduces the statistical power and restricts the evaluation of potential confounding factors. However, despite the relatively low number, we could detect a significant influence of this on the Tac concentrations, probably reflecting a very strong impact of the *CYP3A* genotype clusters.

The initial Tac dose on the basis of multiple genotype statuses is being considered in some transplantation centres. However, data reported to date on the possible benefit of such an a priori genotyping strategy are still not very clear. In our study, we did not observe any differences in the clinical outcome. In this sense, the Tactique

trial showed that the reduced time to achieve adequate concentrations in the genotype-based Tac dosing group was not translated into a better clinical response [47]. Nevertheless, as has been discussed by different experts in the field [48], the particular design of the study might not accurately reflect a usual kidney transplant population as they included only patients at low immunological risk for acute rejection and several patients received induction therapy in addition to high mycophenolate mofetil doses. Consequently, the introduction of Tac was delayed until day 7 after transplantation.

In contrast, Tac starting dose on the basis of an individual's *CYP3A5* genotype (GBD) did not increase the proportion of patients in target compared with the standard dose [49]. The proportion of patients with subtherapeutic and supratherapeutic Tac C_0 was comparable between both groups and GBD did not improve the clinical outcome. The main reason for these observations is that the GBD does not explain all variability in Tac PK. Therefore, there is still unexplained PK variability and other genetic variants might explain the residual variability in Tac dose requirement. Interestingly, in the study of Thervet and colleagues, even if the Tac starting dose was defined according to *CYP3A5* expression status, some patients still presented Tac C_0 beyond the therapeutic range after 3 days of Tac therapy. Accordingly, and as explained above, reanalysis of Tactique data to weight the potential additional explicative value of *CYP3A4*22* to elucidate the origin of this remaining PK variability was carried out [39]. As expected, they found that *CYP3A4*22* carriers are more at risk of supratherapeutic concentrations than noncarriers, even if that study had a very particular design not focused on the influence of *CYP3A4*22* carrier status. We believe that future prospective studies with pre-transplantation genotype-based Tac dosing should include not only *CYP3A5*3* but also *CYP3A4*22*. Furthermore, the true clinical benefit remains to be tested in appropriately designed prospective randomized trials to determine the influence of genotype combinations on clinical outcome.

Conclusion

We show that the *CYP3A4*22* and *CYP3A5*3* alleles are all associated independently with Tac exposure during the first year after transplantation. *CYP3A4*1/*22*, *CYP3A5*3/*3* and PM patients had lower dose requirements to achieve the target concentrations. Our study provides arguments for implementation of the combined *CYP3A4* and *CYP3A5* genotype status when deciding on the initial Tac dose.

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Conflicts of interest

D.A. Hesselink has received lecture and consulting fees, as well as grant support from Astellas Pharma. He has also received lecture fees from Chiesi Pharma. For the remaining authors there are no conflicts of interest.

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Chapter IV

A NEW CYP3A5*3 AND CYP3A4*22 CLUSTER INFLUENCING TACROLIMUS TARGET CONCENTRATIONS: A POPULATION APPROACH

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ORIGINAL RESEARCH ARTICLE

A New *CYP3A5**3 and *CYP3A4**22 Cluster Influencing Tacrolimus Target Concentrations: A Population Approach

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Abstract

Background Single nucleotide polymorphisms (SNPs) in the *CYP3A5* and *CYP3A4* genes have been reported to be an important cause of variability in the pharmacokinetics of tacrolimus in renal transplant patients. The aim of this study was to merge all of the new genetic information available with tacrolimus pharmacokinetics to generate a more robust population model with data from renal transplant recipients.

Methods Tacrolimus exposure data from 304 renal transplant recipients were collected throughout the first year after transplantation and were simultaneously analyzed with a population pharmacokinetic approach using NONMEM® version 7.2.

Results The tacrolimus whole-blood concentration versus time data were best described by a two-open-compartment model with inter-occasion variability assigned to plasma clearance. The following factors led to the final model, which significantly decreased the minimum objective function value ($p < 0.001$): a new genotype cluster variable combining the *CYP3A5**3 and *CYP3A4**22 SNPs defined as extensive, intermediate, and poor metabolizers; the standardization of tacrolimus whole blood concentrations to a hematocrit value of 45%; and age included as patients <63 years versus patients ≥63 years. External validation confirmed the prediction ability of the model with median bias and precision values of 1.17 ng/mL (95% confidence interval [CI] −3.68 to 4.50) and 1.64 ng/mL (95% CI 0.11–5.50), respectively. Simulations showed that, for a given age and hematocrit at the same fixed dose, extensive metabolizers required the highest doses followed by intermediate metabolizers and then poor metabolizers.

Conclusions Tacrolimus disposition in renal transplant recipients was described using a new population pharmacokinetic model that included the *CYP3A5**3 and *CYP3A4**22 genotype, age, and hematocrit.

Key Points

This is the first population PK study combining CYP3A5 and CYP3A4 genotype, age, and hematocrit that influence tacrolimus concentrations in renal transplant recipients.

This a externally validated prediction model to propose new clear dosage guidelines for each genotype.

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1 Introduction

Tacrolimus is the most frequently used drug to prevent solid-organ transplant rejection and is mostly combined with anti-proliferative agents and glucocorticoids [1, 2]. Tacrolimus is a narrow therapeutic index drug that requires individual dose titration to achieve a satisfactory balance between maximum efficacy and minimum toxicity [3, 4].

The pharmacokinetic profile of tacrolimus is characterized by a high degree of inter- and intra-individual variability [5]. Although tacrolimus is rapidly absorbed in the gastrointestinal tract, it has a poor bioavailability (17%) [6] that ranges from 5 to 93% [2, 7]. This is mainly attributed to the pre-systemic metabolism of tacrolimus by cytochrome P450 (CYP) 3A and to P-glycoprotein-mediated efflux [8]. Tacrolimus binds extensively to erythrocytes, while in plasma it is mainly bound to α_1-acid glycoprotein and albumin [2, 9, 10]. Multiple factors have been identified as sources of tacrolimus pharmacokinetic variability, including demographic characteristics, food intake, drug–drug interactions, hepatic dysfunction, and hematocrit [9, 11–14]. Two single nucleotide polymorphisms (SNPs) in the *CYP3A4* and *CYP3A5* genes (*CYP3A4**22 and *CYP3A5**3) have also been reported as important causes of inter-individual variability (IIV) [15–19].

The large variability observed in tacrolimus pharmacokinetics makes therapeutic drug monitoring (TDM) essential to optimize tacrolimus dosing. The achievement of therapeutic exposure, routinely assessed using predose concentrations (C_0), is crucial during the early period after transplantation [20]. However, TDM support is less accurate during the first critical days as a consequence of substantial changes in some individual characteristics and is limited by the time delay needed to ensure attainment of pharmacokinetic steady state before performing any dosage adjustment. Pharmacogenetic markers have been shown to assist in improving dose individualization [21, 22]. However, whether a patient has the *CYP3A* genotype is unknown at the time of drug prescription. Ensuring that patients are within the therapeutic tacrolimus concentration range is relevant in the first days post-transplant when the risk of rejection is highest. Thus, the optimization of initial tacrolimus dosing using a pharmacokinetic model including the predictors of pharmacokinetic variability might help to improve the clinical outcome of transplantation.

A previous study by our group [23] showed that clustering patients according to both *CYP3A4**22 and *CYP3A5**3 allelic status defined their metabolic status quite accurately. Extensive metabolizers (EMs) had a lower dose-adjusted tacrolimus C_0 than poor metabolizers (PMs) or intermediate metabolizers (IMs).

Until now, only a few studies on population pharmacokinetic (popPK) models for dose individualization of tacrolimus have been reported. These models included variables such as age, hematocrit [24], the type of tacrolimus formulation [25], and the *CYP3A5* [15, 18, 26] and *CYP3A4* [27–29] genotype. Some of these models included several of these variables but none considered all of them together in the same popPK model. Recently, the importance of *CYP3A5**3 and *CYP3A4**22 as principal SNPs influencing tacrolimus metabolism was confirmed in a large cohort of renal transplant patients [27, 30, 31]. Our group developed a preliminary popPK model of tacrolimus in stable kidney transplant patients. However, the limited sample size did not allow inclusion of genetic information in the final model [32]. The aim of the present study was to include all the newly available genetic information on tacrolimus pharmacokinetics to generate a more robust population model in a larger cohort.

2 Methods

2.1 Patients and Immunosuppressive Therapy

Adult renal transplant recipients treated with an immunosuppressive drug regimen of oral tacrolimus (Prograf®; Astellas Pharma Europe Ltd, Staines, UK) twice daily in combination with 0.5–1.0 g/day of mycophenolate mofetil (MMF) (Cellcept®; Roche, Basel, Switzerland) were included. No Bayesian prediction approach was used for dose tailoring. Demographic and biochemical characteristics were followed and recorded from week 1 to month 12 post-transplantation.

For external evaluation data, immunosuppression consisted of a combination of tacrolimus with MMF or mycophenolate sodium and corticosteroids. For TDM purposes, the tacrolimus C_0 was determined on a daily basis during hospitalization.

2.2 Study Design and Datasets

The discovery cohort consisted of a total of 304 patients. Seven patients in this cohort participated in the pharmacokinetic sub-study of the Symphony study [33] and were intensively sampled. Samples for tacrolimus concentration measurements were collected before and 15 and 30 min and 1, 2, 3, 4, 6, 8, and 12 h after oral dosing on day 7 and months 1, 3, 6, and 12 post-transplantation. The second group consisted of 297 patients who participated in a retrospective pharmacogenetic study carried out between 2005 and 2012 at Bellvitge Hospital (Barcelona, Spain). C_0 was measured on days 7 and 15 and months 1, 3, 6, and 12 after transplantation [23].

External evaluation data were obtained from 59 adult deceased donor renal transplant recipients who were

prospectively recruited between July 2007 and January 2009 at the Cliniques Universitaires St Luc (Brussels, Belgium) and followed during the hospitalization period as previously described [30, 34].

2.3 Tacrolimus Measurement

For the model-building group, until March 2011 samples were analyzed by a validated enzyme-multiplied immunoassay technique with a quantification limit of 2 ng/mL and a linearity over the range of 2–30 ng/mL. From April 2011, a validated ultra-performance liquid chromatography mass spectrometry with a limit of quantification of 0.6 ng/mL and a linearity over the range of 0.6–44 ng/mL was used.

For the validation cohort, tacrolimus C_0 values were measured using a chemiluminescent microparticle immunoassay on the Architect analyzer from Abbott Diagnostics Laboratories (Abbott Park, IL, USA) [30, 34].

2.4 Genotyping

Genomic DNA was extracted from peripheral whole blood using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Sydney, NSW, Australia). For genotyping, an allelic discrimination reaction was performed using specific TaqMan® (Applied Biosystems, Foster City, CA, USA) genotyping assays for each SNP on an ABI PRISM 7500® Fast real-time PCR Systems (Applied Biosystems). All patients were genotyped for *CYP3A5**3 and *CYP3A4**22.

According to the functional defect associated with CYP3A variants [27], we classified patients into three different clusters of CYP3A metabolizers: PMs (*CYP3A4**22 carriers with the *CYP3A5**3/*3 genotype), IMs (*CYP3A4**22 non-carriers with the *CYP3A5**3/*3 genotype or *CYP3A4**22 carriers with the *CYP3A5**1/*1 genotype), and EMs (*CYP3A4**22 non-carriers and *CYP3A5**1 carriers).

2.5 Population Pharmacokinetic Analysis

A popPK analysis was performed with the non-linear mixed-effects model approach using NONMEM® version 7.2 (ICON Development Solutions, Hanover, MD, USA). Perl-Speaks-NONMEM (PsN) version 4.2 [34] (http://psn.sourceforge.net/), R package version 3.1, and Xpose 4.2.0 were used for model evaluation. The first-order conditional estimation method with interaction was used throughout the modeling process.

One-, two-, and three-compartment open models with linear elimination were fitted to the concentration–time data. Zero-order, first-order with or without lag-time, and transit-compartment kinetic profiles were tested to describe the absorption process [35]. IIVs and inter-occasion variabilities (IOVs) [36] defined by fitting exponential error models that assume log-normal distributions were tested. Additive, proportional, and combined error models were tested to describe the residual error (RE) variability. To compare the different nested models statistically, the likelihood ratio test, based on the reduction of the minimum objective function value (MOFV) a significance level of $p < 0.005$ (change in MOFV [ΔMOFV] = –7.879 for 1 degree of freedom) was considered. For non-hierarchical models, the most parsimonious model with the lowest MOFV according to the Akaike information criterion (AIC) was chosen [37]. The decrease in MOFV, parameter precision expressed as percentage relative standard error (RSE%), reductions in IIV associated with parameters, η- and ε-shrinkage values [38], model completion status, and visual inspection of goodness-of-fit plots were also considered for model selection and validation.

2.6 Covariate Analysis

The effect of all covariates was tested on model parameters if the comparison could be considered to be physiologically and clinically meaningful. The covariates evaluated were bodyweight, body mass index, age, sex, hemoglobin, hematocrit and erythrocyte count, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Although the influence of continuous covariates on the pharmacokinetic parameters was tested systematically by an established modeling approach according to linear, exponential, or power-centered relationships, the power function model (Eq. 1) was finally applied:

$$TVP_j = \theta_1 \cdot \left(\frac{COV}{COV_{median}}\right)^{\theta COV} \quad (1)$$

where θ_1 is the typical value of the jth pharmacokinetic parameter (TVP_j) for a patient whose covariate value (COV) is equal to the population median (COV_{median}) and θCOV is the change in $\ln TVP_j$ per unit change in $\ln(COV/COV_{median})$.

The categorical covariates (sex and polymorphisms) were tested on their respective potentially influenced pharmacokinetic parameters by calculating a distinct parameter for each category. Age was tested as a continuous and categorical covariate (Eq. 2). For this purpose, age was sorted into two age groups based on the third quartile of the age distribution of the actual population: patients <63 and ≥63 years old.

$$\begin{aligned} TVP_j &= \theta_1 \quad \text{for} \quad Z = 0 \\ TVP_j &= \theta_1 \cdot \theta_2 \quad \text{for} \quad Z = 1 \end{aligned} \quad (2)$$

Hematocrit was also considered to explain residual variability associated with total blood concentrations, as described by Størset et al. [24] (Eq. 3). A bias between total tacrolimus concentrations and predicted total

concentrations due to hematocrit levels was corrected by linear standardizing of the total blood concentrations with regard to a hematocrit value of 45%.

$$C_{tot} \cong C_b = C_{std} \times R \times \frac{H_{ct}}{45\%} \quad (3)$$

where C_{tot} is tacrolimus total concentration, C_b is tacrolimus bound concentration, C_{std} is the standardized concentration and R is the ratio between the maximum concentration bound to erythrocytes (Cb_{max}) and the unbound concentration leading to half-maximum binding (Cu_{50}) and H_{ct} is hematocrit.

Covariates were initially explored by correlation and univariate analysis and then using the forward inclusion ($p < 0.05$) and backward elimination ($p > 0.001$) stepwise procedures [39, 40]. Significance levels of 5% (ΔMOFV = –3.841 units) and 0.1% (ΔMOFV = 10.8 units) were considered during the forward addition and backward elimination steps, respectively. Only covariates providing a reduction of IIV associated with parameters of at least 10% were considered clinically relevant and were retained in the model.

2.7 External Evaluation

The predictive performance of the developed model was assessed in the external evaluation dataset. The bias (median prediction error [MPE]; Eq. 4) and imprecision (root median squared error [RMSE]; Eq. 5) and their corresponding 95% confidence intervals (CIs) were calculated in accordance with Sheiner and Beal [41].

$$\text{MPE} = \text{median}\left(\text{Obs} - I_{pred}\right) \quad (4)$$

$$\text{RMSE} = \sqrt{\text{median}\left(\text{Obs} - I_{pred}\right)^2} \quad (5)$$

where I_{pred} is individual prediction and Obs is observed.

2.8 Internal Evaluation

To evaluate the predictive performance of the final model, a prediction-corrected visual predictive check (predcorVPC) [41] and a posterior predictive check (PPC) [42] were carried out from 1000 simulations of the original dataset. A non-parametric re-sampling bootstrap procedure with replacement of 200 replicates was used to further evaluate precision of model parameters.

2.9 Model-Based Simulations and Dosage Guidelines Definition

The final popPK model was used to define the optimal initial doses for different covariate combinations. Three different scenarios were defined: PM, IM, and EM *CYP3A* metabolizers for patients either <63 or ≥63 years old presenting hematocrit values established at 34%, which corresponded to the overall median population value observed. The age cut-off was based on the age distribution of the actual population.

For each scenario, concentrations that would be achieved after different initial target fixed doses of between 2 and 4 mg every 12 h were stochastically simulated and evaluated 1 month after the start of the treatment. Five hundred simulations were performed in each case. Subsequently, percentages of patients with C_0 values within the therapeutic range after each dose regimen were computed to establish the optimal dose with respect to the target C_0 window (5–10 ng/mL) [3].

3 Results

3.1 Patient Characteristics and Datasets

A total of 1891 tacrolimus whole-blood concentration versus time values were simultaneously analyzed: 329 were obtained from the intensively sampled group ($n = 7$ patients) and 1562 were C_0 values obtained in the second group ('C_0 group'; $n = 297$ patients). In total, 83% of values were C_0 values. Demographic, biochemical, and genetic characteristics of the patients included in the model are summarized in Tables 1 and 2. All patients were Caucasian and genotyped for *CYP3A5**3* and *CYP3A4**22*. No differences in dose-normalized C_0 were found, and nor were there any differences in biochemical and demographic characteristics between the intensive and C_0 groups or among bioanalytical methods. During the study follow-up, the median tacrolimus daily dose was reduced by 2 mg after the first month post-transplant and the median C_0 decreased by approximately 1 ng/mL during this period. A total of three tacrolimus concentrations (0.2%) were below the lower limit of quantification. Both creatinine levels and hematocrit changed significantly from the earlier post-transplant stages to the later stages (paired t test, $p < 0.0001$).

3.2 Population Pharmacokinetic Analysis

3.2.1 Structural Model

The tacrolimus whole-blood concentration versus time data were best described by a two-open-compartment model with first-order absorption kinetics and a lag time. The peripheral distribution volume (V_p) was fixed to 526 L to properly estimate other population parameters.

IOV and IIV were both associated with tacrolimus clearance (CL). The IOV inclusion significantly reduced

Table 1 Overall patient characteristics

Characteristic	Value
Model-building group	
Number of patients	304
Sex (M/F)	200/104
Weight	68 (40–106)
Age	52 (17–83)
Number of transplants	
1	243 (80%)
2	49 (16%)
≥3	12 (4%)
Primary kidney disease	
Diabetic nephropathy	16 (5%)
Glomerulonephritis	97 (32%)
Interstitial nephritis	51 (17%)
Unknown origin	88 (29%)
Polycystic kidney disease	36 (12%)
Hypertensive nephropathy	16 (5%)
CMV (yes/no)	246 (81%)/58 (19%)
Total HLA mismatches	
0	7 (2%)
1	8 (3%)
2	27 (9%)
3	104 (34%)
4	98 (32%)
5	55 (18%)
6	5 (2%)
Polymorphism[a]	
*CYP3A5**1/*3 (n/n)	255/49
*CYP3A4**1/*22 (n/n)	275/29
CLUSTER	
High metabolizer	47 (15%)
Intermediate metabolizer	230 (76%)
Poor metabolizer	27 (9%)
External validation group	
Number of patients	59
Sex (M/F)	21/38
Weight	72 (40–100)
Age	53 (25–76)
Polymorphism[a]	
*CYP3A5**1/*3 (n/n)	41/18
*CYP3A4**1/*22 (n/n)	54/5
CLUSTER	
High metabolizer	36
Intermediate metabolizer	23
Poor metabolizer	0

Data are shown as median (min–max) values for continuous variables and number of cases for discontinuous variables

CMV cytomegalovirus, *F* female, *M* male, *max* maximum, *min* minimum

[a] All polymorphisms respected the Hardy-Weinberg equilibrium. No *CYP3A5**1/*1 or *CYP3A4**22/*22 homozygous patients were detected

the MOFV (ΔMOFV = –368.519, $p < 0.001$) and decreased the IIV associated with CL (IIV_{CL}) by 8.3%. A proportional error model best described the RE distribution. The inclusion of two different REs for each group did not significantly decrease the MOFV (ΔMOFV = –6.785, $p > 0.005$). The pharmacokinetic parameters estimated from the structural model are listed in Table 3.

3.2.2 *Covariate Model*

The most relevant covariate models are summarized in Table 4. When covariates were entered in univariate analysis, neither bodyweight nor other demographical and hepatic variables provided a significant drop in the MOFV ($p > 0.05$).

The inclusion of hematocrit to predict a standardized tacrolimus concentration (Model 2) significantly reduced the MOFV (ΔMOFV = –93.88, $p < 0.001$) compared with the structural model. PredcorVPC using hematocrit as an independent variable showed no systematic prediction error and confirmed the result of better prediction of the model when hematocrit is included when compared to the structural model (data not shown).

The *CYP3A5**1/*3 SNP statistically significantly influenced tacrolimus CL (ΔMOFV = –10.57 for Model 3, $p < 0.05$). In contrast, *CYP3A4**1/*22 was not statistically significant (ΔMOFV = +53.11 for Model 4, $p > 0.05$). A cluster was created as a new covariate in accordance with our previous pharmacogenetic study [23] combining both SNPs. The inclusion of CLUSTER as a covariate (Model 5) significantly decreased the MOFV (ΔMOFV = –108.33, $p < 0.001$) with respect to those models where *CYP3A5**3 and *CYP3A4**22 were included separately. When age was included as categorical covariate, i.e., patients <63 versus ≥63 years old (Model 6), the model fit was also statistically significant when the age effect was targeted at tacrolimus CL (ΔMOFV = –22.27, $p < 0.001$).

The inclusion of the genotype classification CLUSTER in the hematocrit model (Model 7) resulted in a significant drop in MOFV (ΔMOFV = –92.50, $p < 0.001$). The inclusion of CLUSTER, hematocrit, and age together led to the final model, Model 10, which decreased the MOFV the most (ΔMOFV = –27.66, $p < 0.001$). Backward elimination of either CLUSTER or hematocrit out of the final model resulted in a statistically significant increase of ΔMOFV = +107.24 ($p < 0.001$) for Model 8 and ΔMOFV = +98.61 ($p < 0.001$) for Model 9, respectively.

The parameter estimates of the final model are shown in Table 3. The IIV_{CL} was reduced by 27.8% when compared with the structural model. Conditional weighted residuals were randomly spread around zero and no trend was detected (Fig. 1). The bootstrap of 200 re-samplings

Table 2 Demographic and biochemical characteristics and genetic polymorphisms of the model-building and external evaluation patients

Characteristics	Global	Day 7	Day 15	Month 1	Month 3	Month 6	Year 1
Model building							
Number of patients (*n*)	304	281	269	292	268	208	208
Hematocrit (%)	34 (18–59)	30 (21–59)	30 (21–52)	37 (18–53)	39 (23–56)	40 (27–53)	39 (34–42)
Creatinine (μmol/L)	128 (58–953)	209 (71–953)	153 (73–637)	137 (58–765)	126 (68–742)	124 (62–693)	125 (66–678)
ALT (μkat/L)	0.29 (0.07–5.30)	0.28 (0.11–2.61)	0.27 (0.07–1.79)	0.38 (0.16–1.82)	0.30 (0.21–5.30)	0.33 (0.18–1.73)	0.30 (0.27–0.48)
AST (μkat/L)	0.31 (0.10–1.90)	0.45 (0.10–1.90)	0.30 (0.15–1.46)	0.30 (0.13–0.42)	0.35 (0.17–1.73)	0.37 (0.11–0.58)	0.30 (0.20–0.40)
Tacrolimus daily dose (mg)	6.0 (1–20)	7 (1–20)	6 (1–18)	4 (1–18)	4 (1–14)	4 (1–16)	5 (2–6)
Tacrolimus C_0 (ng/mL)	7.7 (1.3–30.0)	8.5 (1.5–25.4)	8.5 (2.0–30.0)	8.3 (2.1–27.2)	7.6 (2.6–22.8)	7.2 (1.3–21.0)	6.4 (2.2–26.5)
External validation (at month 1)							
Number of patients (*n*)	59			59			
Hematocrit (%)	NA	NA	NA	NA	NA	NA	NA
Creatinine (μmol/L)	59.0 (44.2–98.58)	NA	NA	59.0 (44.2–98.58)	NA	NA	NA
Tacrolimus daily dose (mg)	5 (1.5–13)			5 (1.5–13)			
Tacrolimus C_0 (ng/mL)	10.4 (4.8–26.6)			10.4 (4.8–26.6)			

Data are shown as median (min–max) values for continuous variables and number of cases for discontinuous variables

The median tacrolimus C_0 of the validation group was slightly higher than that of the model-building group, probably because patients in the validation group were treated with higher tacrolimus doses than those given to the model-developing group. No statistically significant differences were found in dose-normalized C_0 between the model-building and validation groups

ALT alanine aminotransferase, *AST* aspartate aminotransferase, C_0 predose concentration, *max* maximum, *min* minimum, *NA* data not available

(Table 3) confirmed the good accuracy of the estimates of the pharmacokinetic parameters. All final model estimates were within the 90% CI of the bootstrap values. The relative deviation of the bootstrap median from the population mean was less than 7.5% for all parameters. The predcorVPC (Fig. 2) showed that the model properly described the mean tendency of the entire data. Some misspecifications were detected, such as a slight under-prediction on the 95th percentile for C_0 values. A slight trend to over-prediction of variability was observed for the remaining concentrations associated with the extensive population.

The PPC analysis (Fig. 3) for each cluster level indicated acceptable predictive capability. In all cases, the medians of the simulated C_0 values were close to those of the observed values (8.80, 7.80, and 7.00 vs. 8.51, 7.59, and 6.65 ng/mL for PM, IM, and EM, respectively) when log-transformed values were compared by means of a one-way ANOVA ($p > 0.05$).

3.3 External Evaluation

An external dataset of 407 tacrolimus whole-blood C_0 values from 59 patients was used to determine the prediction ability of the model. The most relevant patient characteristics are depicted in Table 1 and 2. Hematocrit values were not recorded for these patients and they were imputed to 34% based on the overall median population value observed for the developing dataset.

Good correlation between the observed and predicted C_0 values from the external evaluation dataset was found (Fig. 4). The data showed similar C_0 values between observed and individual predicted values. Nevertheless, individual predicted C_0 values were slightly lower than the observed C_0 values. The median values and the 5th and 95th percentiles of bias and imprecision were 1.17 ng/mL (–3.68 to 4.50 ng/mL) and 1.64 ng/mL (0.11–5.50 ng/mL), respectively. Based on a median C_0 of 8.2 ng/mL, this corresponded to a median error of 20.1%.

3.4 Model-Based Simulations and Initial Optimal Dose

The highest percentages of patients with a C_0 within the therapeutic range occurred after 4, 3, and 2 mg every 12 h for EMs, IMs, and PMs, respectively, and hematocrit levels were around 34% in all the cases. Hence, these would be the optimal dose regimens to attain maximum target C_0 coverage, with 44.2, 41.8, and 42.2% of patients, respectively, within the therapeutic C_0 range (5–10 ng/mL). According to our simulations, the dose regimen would be 0.5 mg less for patients ≥63 years than for those <63 years (Fig. 5).

Table 3 Pharmacokinetic parameter estimates for the base and final models

Parameter	Units	Base model [estimate (RSE%)]	Final model [estimate (RSE%)]	Bootstrap analysis (n = 200) [90% CI]
Disposition parameters				
$CL_{\_High}$	L/h	15.6 (6)	20.5 (6%)	18.5–23.7
$CL_{\_Int}$	L/h		12.5 (5%)	11.3–14.4
$CL_{\_Poor}$	L/h		9.1 (7%)	7.6–11.2
$CL_{\_AGE}$	L/h		–0.205 (16%)	–0.262 to –0.142
V_C	L	6.72 (15%)	5.02 (18%)	3.67–20.86
CL_D	L/h	3.7 (22%)	4.2 (50%)	1.2–28.7
V_P	L	526 (fix)	526 (fix)	526 (fix)
Absorption parameters				
k_a	h^{-1}	0.136 (5%)	0.138 (15%)	0.104–0.468
Lag time	h	0.234 (12%)	0.243 (10%)	0.193–0.280
Inter-individual and inter-occasion variabilities				
IOV_{CL}	%	34.7 (7%)	33.3 (9%)	24.4–46.6
IIV_{CL}	%	38.5 (5%)	27.8 (8%)	19.9–35.7
Residual variability				
Proportional	%	6 (11%)	25 (11%)	20–29

The η-shrinkage values were 10% for IIV_{CL} and 24, 22, 26, 32, 30, and 41% (from occasion 1 to 6) for IOV_{CL}

CI confidence interval, CL_{AGE} the change on clearance for patients ≥63 years old, $_{CL\_}$*High* clearance for high metabolizers, *CL_Int* clearance for intermediate metabolizers, *CL_Poor* clearance for low metabolizers, V_C central distribution volume, CL_D intercompartmental clearance, V_p peripheral distribution volume, k_a *absorption rate constant*, IIV_{CL} interindividual variability associated with clearance, IOV_{CL} inter-occasion variability associated with clearance, *RSE*% percentage residual standard error

Table 4 Summary of the covariate stepwise cumulative forward inclusion during model building

Tested model	Reference model	MOFV	ΔMOFV	Covariate relationships	Observations
1		7287.14		Base model: $TVCL = \theta_1$	
2	1	7193.25	–93.88	Base model + HCRT standarized	$p < 0.001$; 4.34% of reduction in IIV associated with CL
3	1	7276.57	–10.57	Base model + *CYP3A5**3	$p < 0.001$; 52.32% of reduction in IIV associated with CL
4	1	7340.25	+53.11	Base model + *CYP3A4**22	$p > 0.05$
5	1	7178.81	–108.33	Base model + CYP CLUSTER	$p < 0.001$; 37.39% of reduction in IIV associated with CL
6	1	7264.86	–22.27	Base model + AGE	$p < 0.001$; 8.70% of reduction in IIV associated with CL
7	5	7086.81	–92.50	Base model + HCRT standarized + CYP CLUSTER	$p < 0.001$; 2.43% of reduction in IIV associated with CL
8	5	7165.88	–12.93	Base model + HCRT standardized + AGE	$p < 0.001$; 26.8% increment of IIV associated with CL
9	5	7157.25	–21.56	Base model + CYP CLUSTER + AGE	$p < 0.001$; 10.97% of reduction in IIV associated with CL
10	7	7058.64	–27.66	Base model + HCRT standardized + CYP CLUSTER + AGE	$p < 0.001$; 15.78% of reduction in IIV associated with CL

ΔMOFV change in minimum objective function value, *CL* clearance, *CYP* cytochrome P450, *HCRT* hematocrit, *IIV* inter-individual variability, *MOFV* minimum objective function value, *TVCL* clearance typical value

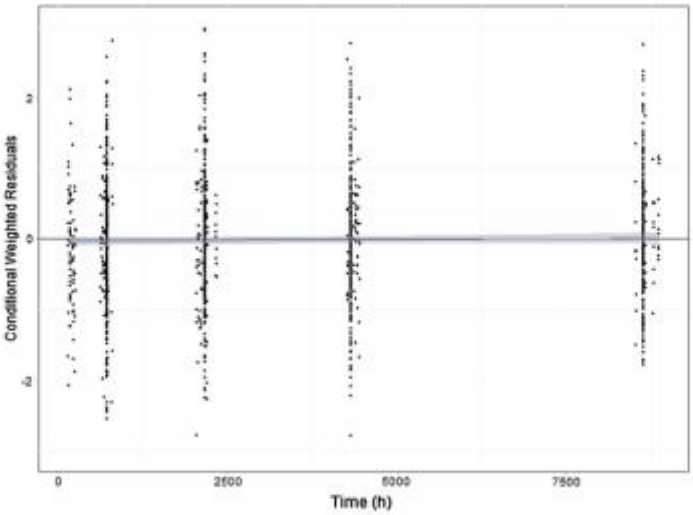

Fig. 1 Conditional Weighted Residuals (CWRES) vs Time plot. *Blue line* represents the local smooth regression (LOESS) curve

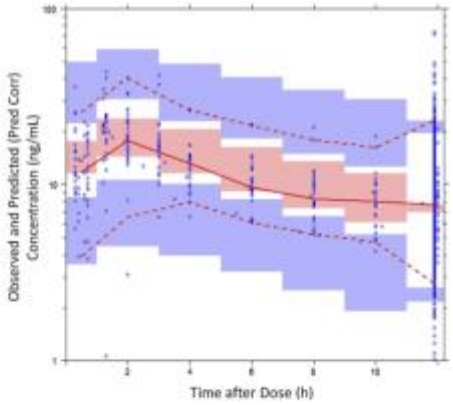

Fig. 2 Prediction Corrected (Pred Corr) Visual Predictive Check (VPC). Prediction-corrected visual predictive check corresponding to tacrolimus whole-blood concentrations versus time (hours after last dose) profiles. In general, median (*solid line*), 95th and 5th percentiles (*dashed lines*) of the observations, as well as the 90% confidence intervals for the median, the 5th and 95th percentiles of the simulated profiles (covered by the light *red* and *blue areas*, respectively) are superimposed

4 Discussion

This is the first popPK study combining *CYP3A5* and *CYP3A4* genotype clustering, age, and hematocrit that demonstrates that these factors influence tacrolimus concentrations in renal transplant patients. A combination of the *CYP3A5**3 and *CYP3A4**22 SNPs, two different patient age categories (<63 and ≥63 years old), and hematocrit were able to predict different tacrolimus elimination rates and whole-blood concentrations.

Our previous study [32], a tacrolimus popPK analysis in stable renal transplant patients using extensive sampling data, provided a clinically applicable model for the tacrolimus starting dose calculation. However, the small sample size and homogeneity of this patient group did not allow us to include genotype or other covariates for initial and Bayesian adaptive control of dose regimens in the model. In addition, previous pharmacogenetic studies [23] identified *CYP3A5**3 and *CYP3A4**22 as the most relevant polymorphisms involved in tacrolimus dose optimization, which led us to develop this new popPK model.

The most relevant finding of the current study is that we were able to appropriately define three different tacrolimus population CL values according to a new combined *CYP3A* genotype categorization previously described by our group [23, 43] as PMs, IMs, and EMs. The three CL values (20.5 ± 1.2, 12.5 ± 0.6, and 9.1 ± 0.6 L/h for EM, IM, and PM patients, respectively) were estimated with good precision (RSE <10%). The EM and IM CL values were in line with those reported in the literature (21.2, 19, 21.2, and 17.9 L/h for CYP3A5 non-expressers and 23.2, 40.8, 26.7, and 21.9 L/h for CYP3A5 expressers, respectively) [22, 44–46]. As expected, the lowest value was observed for PMs, reflecting the effect of *CYP3A4**22 allele carriership. A value of 526 L was considered for V_p according

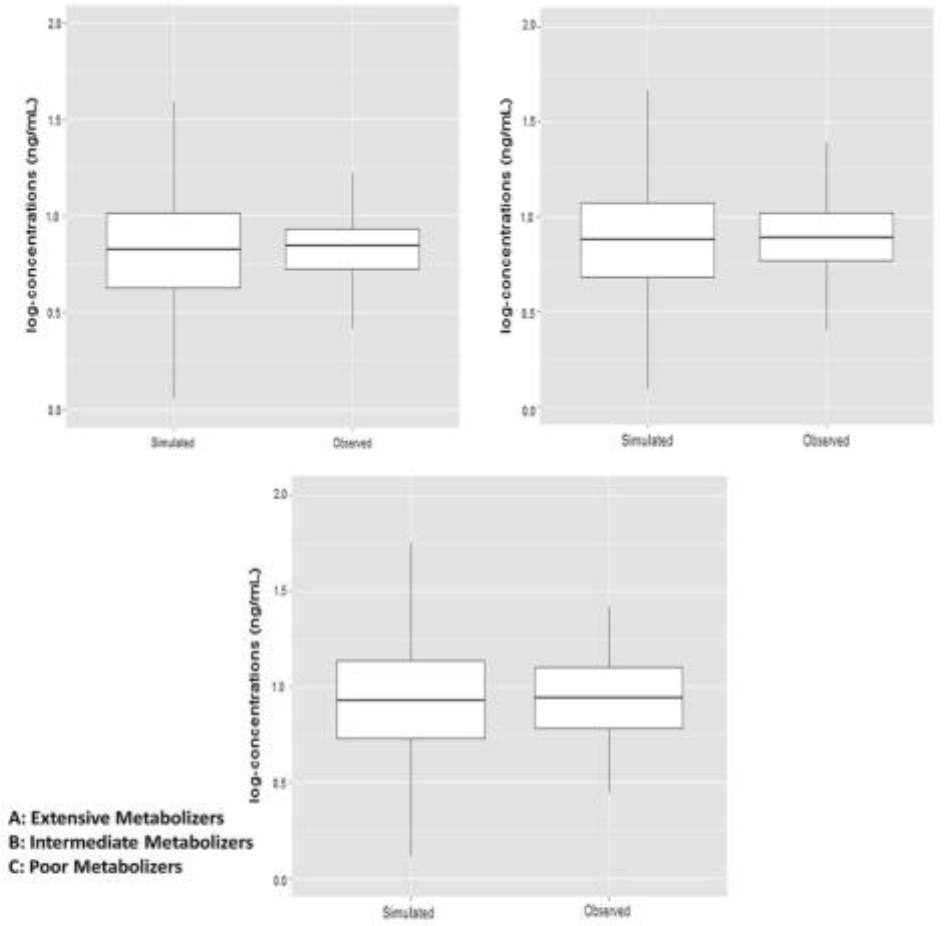

Fig. 3 Boxplots of the distributions of simulated and observed log-transformed C_0 concentrations (PPC) for extensive (*A*), intermediate (*B*) and poor metabolizers (*C*). The *bold horizontal bars* in the middle show the median values, whereas the outer boundaries of the boxes represent the ranges of the 25th and 75th percentiles (interquartile ranges). The whiskers indicate the maximum and the minimum values of C_0. Outliers are not shown in these plots

to our previous popPK study [32] in which an extensive sampling design allowed robust estimation of this parameter. The structural model showed a low (<20%) shrinkage linked to CL that suggests an exact distribution of the variability associated with CL with no major bias to initiate the covariate inclusion. The inclusion of IOV significantly improved the model and was similar to that reported in previous studies (29% [32], 35% [44], and 40% [26]). No statistical significance was found when two different additive components were tested taking into account bio-analytical error.

The novel *CYP3A* cluster as predictor of tacrolimus CL contributed highly to a better description of the IIV, with a reduction of a 37.4% with respect to the structural model. In recent years, the *CYP3A5* genotype has often been proved to be a strong factor influencing of tacrolimus CL [26, 47, 48]. *CYP3A4**22, first described by Elens et al. [49], was recently also shown by Moes et al. [48] to

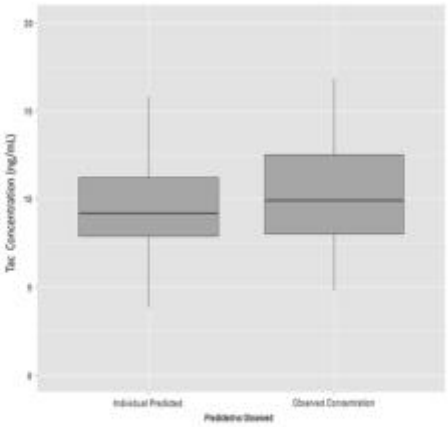

Fig. 4 Boxplot of the distributions of predicted and observed C_0 concentrations from the external evaluation dataset. The *bold horizontal bars* in the middle show the median values, whereas the outer boundaries of the boxes represent the ranges of the 25th and 75th percentiles (interquartile ranges). The whiskers indicate the maximum and the minimum values of C_0. Outliers are not shown in these plots

independently influence tacrolimus CL_o as confirmed in our previous pharmacogenetic study [23], despite the fact that they could not demonstrate the *CYP3A4**22 effect, probably due to the relatively small sample size of their tacrolimus-treated subgroup. Thus, merging both *CYP3A* polymorphisms in a new cluster, defining three subpopulations, improved characterization of the tacrolimus elimination process.

To improve model predictability, patients were also categorized according to age (<63 or ≥63 years old). The inclusion of age as a factor in the tacrolimus CL values resulted in a statistically significant drop in the IIV. Our model is the first to include age as a consideration for tacrolimus dosing. This is in accordance with findings from Jacobson et al. [50], in which calcineurin inhibitor trough concentrations were more than 50% higher in older than in young adults. In contrast, Åsberg et al. [22] tested the inclusion of age, assuming a linear decline in CL in patients older than 50 years, but the covariate was not retained in their final model.

The inclusion of hematocrit refined our model fit by linearly standardizing whole-blood concentrations to a hematocrit of 45%. This strategy, first reported by Størset et al. [24], was applied to our data and allowed prediction of whole-blood concentrations closer to the observed data by removing the variability in blood concentrations due to the extent of hematocrit binding. This resulted in an appropriate model for dose adjustment based on whole-blood concentrations that overcomes the inconvenience associated with monitoring free drug concentrations, which can be highly time consuming and incur larger costs. However, albumin could not be tested to evaluate its influence on tacrolimus distribution.

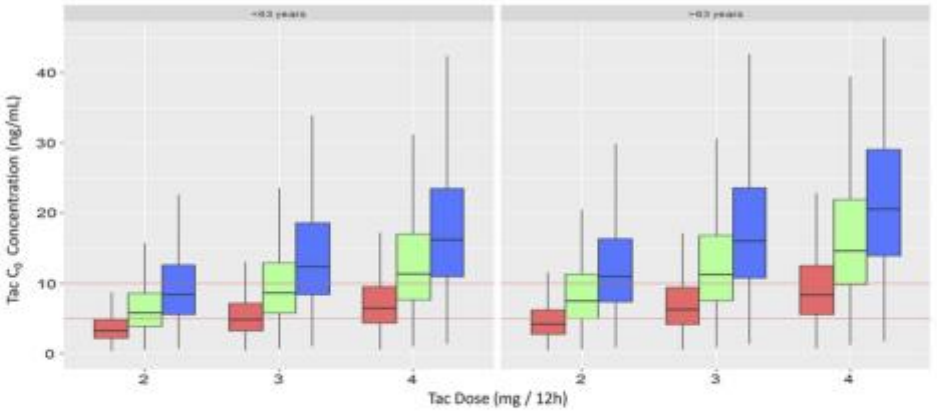

Fig. 5 Boxplots of the distributions of simulated C_0 concentrations for extensive (*red*), intermediate (*green*) and poor metabolizers (blue) after 2, 3 and 4 mg/12h fixed dose either for patients <63 years old (*left*) or 63 years old. The *bold horizontal bars* in the middle show the median values, whereas the outer boundaries of the *boxes* represent the ranges of the 25th and 75th percentiles (interquartile ranges). The whiskers indicate the maximum and the minimum values of C_0. Outliers are not shown in these plots. *Red lines* indicate the therapeutic interval of 5-10 ng/mL

Until now, there have been no popPK models in the literature using the combination of the cluster of *CYP3A5**3 and *CYP3A4**22 polymorphisms, age, and hematocrit to describe tacrolimus pharmacokinetics. According to our results, and assuming hematocrit values around 34%, the EMs showed CL values of around 2.25-fold higher than PMs, while the IMs had CL values around 1.37-fold higher than PMs.

The internal validation techniques (visual predictive check and PPC) confirmed the predictive capability of the model, although a slightly over-predicted variability was found for non-C_0 values. This was also confirmed by the external validation, which showed acceptable bias (1.17 ng/mL) and imprecision (1.64 ng/mL) values for predose tacrolimus concentrations when compared with other previously reported externally validated models. In the current study, a median error of 20.1% for future predictions was found, which is similar to that described by Åsberg et al. [22]. These results confirmed the power of this model for predicting individual tacrolimus CL values using C_0 values with regard to the individual levels of hematocrit, and the age and *CYP3A5**3 and *CYP3A4**22 SNPs of each patient.

Although the relationship between CYP genotype, dose requirement, and its clinical value remains to be proven [51], Størset et al. [52] successfully used a computerized dose individualization to improve target tacrolimus concentrations after renal transplant without including *CYP3A* genotyping [52]. However, Shuker et al. [54] were not able to increase the number of patients in the tacrolimus therapeutic range early after transplantation. In this study, a popPK model with Bayesian prediction was not applied for the dose prediction, suggesting that the inclusion of the *CYP* genotype in a popPK model might improve tacrolimus dose optimization [53]. Furthermore, high tacrolimus IIV is a risk factor for adverse events [54, 55], which a popPK model with Bayesian prediction could take into account.

In our study, 60% of EMs, IMs, and PMs were within the target minimum concentration. Simulations showed that the *CYP3A* EMs cluster may require approximately 2-fold higher doses than PMs. Moreover, IMs may require approximately 1.5-fold higher doses than PMs. These dose recommendations confirm the results from our previous study [23] in which PMs showed a higher percentage of patients with supra-therapeutic C_0 values (C_0 >15 ng/mL) than did EMs. Our results simulated that, in order to minimize the percentage of patients falling outside the therapeutic range, different tacrolimus initial doses of 4, 3, and 2 mg every 12 h for EMs, IMs, and PMs, respectively, should be considered for those with a hematocrit level of around 34% in all cases.

5 Conclusions

In summary, we have developed and validated a new popPK model. This model includes individual patient characteristics such as *CYP3A5**3 and *CYP3A4**22 genotype, age, and hematocrit to best describe tacrolimus disposition in renal transplant patients. Our popPK model accurately estimates the mean and individual tacrolimus CL values using Bayesian forecasting and can be used as a reliable tool for starting dose calculation and posterior dose adjustment. Considering the importance of optimizing the percentage of patients within the C_0 target directly from the start of the treatment, we have used this fully validated prediction model to propose new clear dosage guidelines for each genotype cluster. Testing the effect of *ABCB1* diplotype in *CYP3A4**22 carriers, as reported by Vanhove et al. [56], in a more refined popPK model with an increased cohort size including full/partial extensive samplings before day 7 post-transplant might be of interest. A future prospective clinical trial using our popPK model is required to validate the contribution of *CYP3A5**3 in combination with *CYP3A4**22 before initiation of and during tacrolimus therapy.

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Compliance with Ethical Standards

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Conflict of Interest Franc Andreu, Helena Colom, Laure Elens, Teun van Gelder, Ronald H. N. van Schaik, Dennis A. Hesselink, Oriol Bestard, Joan Torras, Josep M. Cruzado, Josep M. Grinyó and Nuria Lloberas declare that they have no conflicts of interest that might be relevant to the contents of this manuscript.

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Chapter V

GENERAL DISCUSSION

Nowadays, Tacrolimus, a calcineurin inhibitor is the cornerstone of most immunosuppressive protocols for renal transplant. Tac is usually combined with proliferation inhibitors such as mycophenolate. Although some efforts to find good biomarkers for individualizing immunosuppressive therapy have been done[87], physicians still rely on pharmacokinetic exposure measures, usually based on therapeutic drug monitoring to individualize doses based on predefined target values.

As it has been reported there is a good correlation between tacrolimus exposure and the clinical outcomes and this has supported the use of therapeutic drug monitoring[64]. However, Tac has low therapeutic index and large inter and intraindividual PK variabilities. After many investigations, much is yet to know about dosages at the early stages after transplantation and changes of them with time. Its high PK variability leads to an increased risk of therapeutic failure[88], particularly at the early stages of transplant. On the other hand, from our research we learned that tacrolimus trough concentrations correlated with total exposure is yet controversial being most desirable to implement an AUC tiered-dosing. This is not feasible in the clinical setting; thus, an alternate approach is that based on limited-sampling strategy by means of Bayesian prediction. In this sense, the use of a PPK model can assist for the first dose calculation at the start of treatment but also for dose adaptation based on predefined target by means of MAPB forecasting technique, supporting TDM

Several PPK models have been reported describing the Tac PK and quantifying its variability. In addition, the most relevant predictive factors of the PK variability including demographic, clinical and genetic variables have been identified. However further investigations, particularly on the influence of genetic polymorphisms are still required. At the time the aims of the current work were planned, none of the existing models had been externally evaluated, however a recent publication of Zhao et al. has shown that among all the existing models only one can be recommended for prediction. Models included the CYP3A5 polymorphism as the most influential covariate followed by haematocrit and post-organ transplantation. Previous evidence of new a CYP3A4 SNPs which is not correlated with the CYP3A5 led to further investigations to identify possible

genetic combinations as better predictors of interindividual variability to be considered for initial dose calculations and later for dose adaptations.

To fulfill these purposes, the first step of our work was to characterize the PK behavior of Tac by developing a population PK model from data from an intensive sampling design and in turn allowed to establish a limited sampling strategy. It is worth noting that the intensive sampling also enabled a more physiological description of the delayed absorption process reported for Tac in transplant patients[24]. Of note, Tac shows a rapid absorption due to its high lipophilicity (log P= 3.3)[88], but delayed absorption can occur due to reduced gastrointestinal motility after transplantation. Some models have also been focused to obtain the most effective simplified blood sampling strategy to better predict Tac exposure. Nevertheless, no studies got in the clinical and efficient application of their respective models through the external validation, although recently Zhao et al. [89] have addressed this issue.

The requirement of confirming the best sampling strategy and necessity of aims of the current thesis are focused on this line, our first study[90] provided a clinically applicable PPK model that helped, to quantify inter- and intra-patient variability and to identify the characteristics that may influence the PK of tacrolimus. Full PK Tac profiles in renal transplant patients were best described by a two-compartment model with first-order absorption kinetics and lag time given by three delayed-transit compartments. PPK studies including transit models to describe the absorption process are scarce in the literature. Probably, due to the lack of sufficient data required for modelling the Tac absorption phase. In our first model[90], up to three transit compartments were included in a sequential way, coinciding with those estimated by Benkhali et al[61] with the Erlang distribution model. The K_{tr} value of our study (K_{tr}=3.61 h^{-1} and n=3) was also similar to the value reported by Benkhali et al[61] (K_{tr}=6.5±0.4 h^{-1}). This transit-compartment model provided a good description of the absorption process and adequate precision in the parameters estimates (RSE<10%). The population CL value found in this first study (16.5±1.8 L/h) was comparable with previous studies (13.2-40.5 L/h[91], 33±11.3 L/h[92], 29±0.2 L/h[78] and 28±4 L/h[61]). This should be expected given that similar populations

were included in all these studies. The estimated total distribution volume was 535.92 L, also in line with other studies was indicative of extensive tissue distribution.

The low observed IIV associated with CL, Ka and MT was in accordance with the uniformity of the population in the present study. Inclusion of the IOV_{CL} significantly improved the model and reduced the IIV. These results agree with other clinical population PK models reported recently in the literature[61,63,78,82,93,94]. The magnitude of the IOV associated with CL was 29% lower than IIV_{CL} and similar with respect to the IOV_{CL} values reported (28%[61], 35%[82] and 40%[78]). The estimated value of IOV_{CL} warns about the inconvenience of the dose adjustment based on observations from more than one previous occasion. Although the structural model showed a low shrinkage linked to CL, the small sample size and the homogeneity of the patient group did not allow us to include genotype or other demographical or biochemical covariates into the model.

To determine which was the best sampling strategy to assess exposure during TDM, a LSS was applied for this first model. Thus, one sampling point at predose showed an acceptable estimation of the AUC_{0-12h} and better accuracy than Benkali et al[61] with a narrower bias of 6.78% (from -16.26% to 30.06%) *vs* 3% (from -51% to 110%) and imprecision (1.42% *vs* 19%). Then, one sampling time at C_0 would be a good LSS and this can make the logistics easier in the outpatient setting.

At the same time, further investigations culminated in new promising CYP3A SNPs, such as CYP3A1*B and CYP3A4*22 that demonstrated to influence TAC exposure variability. Despite our attempts in that sense, the few number of patients included in our first Population PK model development, prevented the inclusion of the most relevant genetics variables as predictors of variability in the model. This was the start point to propose a new pharmacogenetic study to identify the most relevant findings postulated in the literature related to Tac exposure.

The primary objective of the second paper[95] was to evaluate the combined influence of *CYP3A4*22* and *CYP3A5*3* SNPs on the PK of Tac during the first post-transplant year. We demonstrated that Tac PK, assessed by the dose needed to reach adequate drug

exposure, was influenced by both *CYP3A4*22* and *CYP3A5*3* SNPS. In addition, we demonstrated the utility of defining genotype clusters according to *CYP3A* genotype[49] to predict differential dose-adjusted Tac C_0.

*CYP3A5*3* allelic status remained the most significant parameter explaining the observed differences, in accordance with the greater involvement of that isoenzyme in the oxidative metabolism of Tac [96]. Furthermore, *in vitro* data demonstrated that the importance of *CYP3A5*3* allelic status is dependent on the concomitant CYP3A4 activity and that the relative contribution of CYP3A4 or CYP3A5 to Tac metabolism depends on the amount of each counterpart[47,48].

This study confirmed as well the importance of recently reported CYP3A4*22 SNP[46,97] which patients required lower Tac doses to reach the target C_0 when compared with *CYP3A4*1/*1* patients in *de novo* kidney transplant patients. The CYP3A4*22 effect was observed in our study at early time-points (*i.e.* 5-7 days after transplantation) but additionally, showed that differences were still present at months 3 and 6 after renal transplantation. This was in accordance with the retrospective analysis of Tactique trial which showed that the *CYP3A4*22* allele was important to identify patients at risk of supra-therapeutic exposure[98].

In addition, we demonstrated the utility of defining three genotype clusters (Poor, Intermediate and Extensive metabolizers by Elens et al.[46]) according to *CYP3A* genotype to predict differential dose-adjusted Tac C_0. In fact, the CYP3A genotype classification according to both allelic status (i.e., *CYP3A4*22* and *CYP3A5*3* SNP) increased the strength of this association. Patients classified as extensive metabolizers showed a lower mean Tac exposure during the entire study period, which might over time lead to sub-therapeutic exposure. It could have consequences in the development of donor-specific anti-HLA antibodies.

Our results indicated that the consideration of the combined *CYP3A4* and *CYP3A5* genotype could help to better predict Tac exposure during the first 6 months of renal transplant and might assist Tac dose optimization. Extensive metabolizers had a 47%

decreased Tac dose-adjusted C_0 when compared to intermediate metabolizers. In contrast, poor metabolizers had a 36% higher Tac dose-adjusted C_0 when compared to intermediate. These observations indicated that dose adjustment based on *CYP3A* combined polymorphisms could increase the number of patients within the therapeutic target concentrations, specifically during the first months after transplantation. This dosage fine-tuning has the ultimate potential to improve the graft outcome of transplantation by minimize the drug exposure-related toxicity and sub-therapeutic exposure. Supporting this hypothesis, in this second study it was observed that the risk of a supra-therapeutic C_0 (>15 ng/mL) was significantly higher for poor metabolizers (45.3%) than for extensive metabolizers (0%) at day 5-7 after transplantation. In addition, a significant delay in reaching the target Tac C_0 was observed in extensive metabolizers. About 29.7% of these patients had infra-therapeutic C_0 (<5 ng/mL) levels at day 5-7.

In summary, this second study showed that the *CYP3A4*22 and CYP3A5*3* alleles are all independently associated with Tac exposure during the first year after transplantation. Poor metabolizers patients related to the cluster of *CYP3A4*1/*22* and *CYP3A5*3/*3,* had lower dose requirements to achieve the target concentrations. It provides proofs for implementation of the combined *CYP3A*4 and 5 genotype status when deciding on the initial Tac dose.

This information provided by the second study with a bigger cohort of renal transplants patients, lead to joint these new data with those from intensive sampling patients of the first cohort. Combining data from both studies allowed to refine the previous model taking it as starting point.

In the third study[99] three different Tac subpopulation CLs according to the new combined *CYP3A* genotype cluster[49,95] (poor, intermediate and extensive metabolizers) were estimated as previously mentioned. The three values of CL (20.5±1.2, 12.5±0.6 and 9.1±0.6 L/h for extensive, intermediate and poor metabolizer patients, respectively) were estimated with good precision (RSE<10%). The extensive and intermediate CL values were in line with those reported in the literature (21.2, 19, 21.2 and 17.9 L/h for

CYP3A5 non-expressers; and 23.2, 40.8, 26.7 and 21.9 L/h for CYP3A5 expressers for [82,84,85,93], respectively). As expected, the lowest value was observed for poor metabolizers, reflecting the effect of *CYP3A4*22* allele carriership. A value of 526L was considered for peripheral distribution volume according to the first study[90] where the extensive sampling design allowed a robust estimation of this parameter. The base model showed, like our first model, a low (<20%) shrinkage linked to CL that suggests an exact distribution of the variability associated with CL with no major bias to initiate the covariate inclusion. The inclusion of IOV significantly improved the model and was similar to that reported in our previous model as well as in other studies [29% [90], 35% [82] and 40% [78]]. No statistical significance was found when two different additive components were tested accounting for the bioanalytical error.

The novel *CYP3A* cluster as predictor of Tac CL highly contributed to a better description of the IIV with a reduction of a 37.4% with respect to the base model. As mentioned in the second study, the *CYP3A5* genotype has been commonly proved as a strong factor influencing Tac CL[61,78,100] and the *CYP3A4*22* as described by Elens et al. [101] also influences Tac CL despite the fact that other study from Moes et al could not demonstrate the *CYP3A4*22* effect probably due to their relatively small sample size of Tac-treated subgroup[100]. Thus, merging both *CYP3A* polymorphisms in a new cluster, defining three subpopulations, improved and confirmed the characterization of the Tac elimination process.

To improve model predictability, patients were also categorized per age (<63 or ≥63 year old). The inclusion of age in the Tac CL resulted in a statistically significant drop of the IIV. Our model is the first including age to be considered for Tac dosing. This result was in accordance with findings from Jacobson et al[102] which CNI troughs concentrations were more than 50% higher in older than young adults. In contrast, Åsberg et al [85] tested the inclusion of the age assuming a linear decline of CL in patients older than 50 years, but the covariate was not retained in their final model.

Hematocrit refined our model fit by linearly standardizing whole-blood concentrations to a hematocrit of 45%. This strategy, firstly reported by Størset et al. [33], was applied to

our data allowing to better predict whole-blood concentrations closer to the observed data by removing the variability in whole-blood concentrations due to the extent of hematocrit binding. This resulted in an appropriate model for dose adjustment based on whole-blood concentrations that overcomes the inconveniences associated with free concentrations monitoring which can be highly time consuming and occasioning larger costs. However, similarly to other models reported the body weight did not resulted statistically significant.

Until now, there are no PPK models in the literature using the combination of the cluster of *CYP3A5*3* and *CYP3A4*22* polymorphisms, age and hematocrit to describe Tac PKs. According to the results obtained in this refined PPK model and assuming hematocrit values around the 34%, the extensive metabolizers showed CL values around 2.25-fold higher than poor metabolizers, meanwhile the intermediates had CLs around 1.37–fold higher compared to poor metabolizers.

The internal validation techniques used (VPC, PPC and NPDE) confirmed the predictive capability of both models, although a slightly over-predicted variability was found for non-predose concentrations in the second model. The predictive capability was also confirmed by respective external validations in both models using different C_0 Tac concentrations. When compared to other previously reported externally validated models, both models indicated an acceptable bias and imprecision of 0.37 ng/mL and 0.38; and bias 1.17 ng/mL and imprecision 1.64 values for the first and second model, respectively. The median errors of 6.1% and 20.1% for future predictions were found similar to described by Åsberg et al [85]. These results confirmed the power of both models for predicting individual Tac CL values using pre-dose concentrations using for the second model the individual levels of hematocrit, the age and the *CYP3A5*3* and *CYP3A4*22* SNPs of each patient.

Størset et al successfully used a computerized dose individualization improving target Tac concentrations after renal transplant without including *CYP3A* genotyping[103]. However, Shuker et al[104] was not able to increase the number of patients in Tac therapeutic range early after transplantation. In this study, a PPK model with Bayesian

prediction was not applied for the dose prediction suggesting that the inclusion of *CYP* genotype in a PPK model might improve Tac dose optimization [105]. A very limited number of PPK studies[99,106] have included a CYP genotype combination in their models and their relationship between CYP genotype, dose requirement and its clinical value still remains to be proven[107]. More precisely, the improvement obtained in PK levels considering the CYP genotype might not have a relevant impact on its long-term clinical endpoints which might become controversial[104]. On the other hand, a high Tac IIV has been recently proven as a risk factor for adverse events [108,109]. This might be a key point that using a well

In this third study, 60% of extensive, intermediates and poor metabolizers were within the target minimum concentration in accordance with the percentages out of the therapeutic interval obtained second study obtained. Simulations showed that *CYP3A* extensive cluster may require about 2-fold higher doses compared to poor metabolizers. Moreover, intermediates may require about 1.5-fold higher doses compared to poor metabolizers. These dose recommendations confirm the results from the second study[95] of this thesis describing that poor metabolizers showed a higher percentage of patients with supra-therapeutic $C_{0,}$ (C_0>15 ng/mL) compared with extensive metabolizers. Our results simulated that, to minimize the percentage of patients falling outside the therapeutic range, different Tac initial doses of 4, 3 and 2 mg every 12 hours for extensive, intermediate and poor metabolizers, respectively should be considered for a hematocrit levels around the 34% in all the cases.

This final refined model including all explained genetic, demographic and biochemicals covariates could be a useful tool to demonstrate a better dose optimization using MAPB analysis compared to the actual empirical dosage. Next step would be to perform an external evaluation to prove that the model can predict adequately the AUC as a better surrogate marker of efficacy. A clinical trial is ongoing to prove if the Tac dose adjustment by PPK modelization is better than the actual clinical routine of tac TDM.

Chapter VI

CONCLUSIONS

1. A Tacrolimus population PK model was designed to characterize accurately the population absorption phase as well as quantify the inter and intra-individual variability.

2. The first population PK model led to obtain an optimal sampling strategy using only trough concentrations for dose tailoring through Bayesian prediction.

3. The *CYP3A4*22 and CYP3A5*3* alleles are all independently associated with Tac exposure during the first year after transplantation. Proofs that a combined *CYP3A*4 and 5 genotype cluster is of relevant importance when deciding on the initial Tac dose.

4. Poor metabolizers patients related to the cluster of *CYP3A4*1/*22* and *CYP3A5*3/*3,* had lower dose requirements to achieve the target concentrations. The risk of a supra-therapeutic C_0 (>15 ng/mL) was significantly higher for poor metabolizers (45.3%) than for extensive metabolizers (0%) at day 5-7 after transplantation.

5. Extensive metabolizers patients related to the cluster of *CYP3A4*1/*1* and *CYP3A5*1/*3*, had higher dose requirements to achieve the target concentrations. A 29.7% of extensive metabolizers patients had a risk of infra-therapeutic C_0 (<5 ng/mL) levels at day 5-7 after transplantation.

6. A new refined PPK model was developed using the combination of the cluster of *CYP3A5*3* and *CYP3A4*22* polymorphisms, age and hematocrit to describe Tacrolimus pharmacokinetics. Three different Tac subpopulation CLs according to the new combined *CYP3A* genotype cluster (poor, intermediate and extensive metabolizers) were identified.

7. The *CYP3A* extensive metabolizers patients may require about 2-fold higher doses compared to poor metabolizers. Moreover, intermediate metabolizers may require about 1.5-fold higher doses compared to poor metabolizers.

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