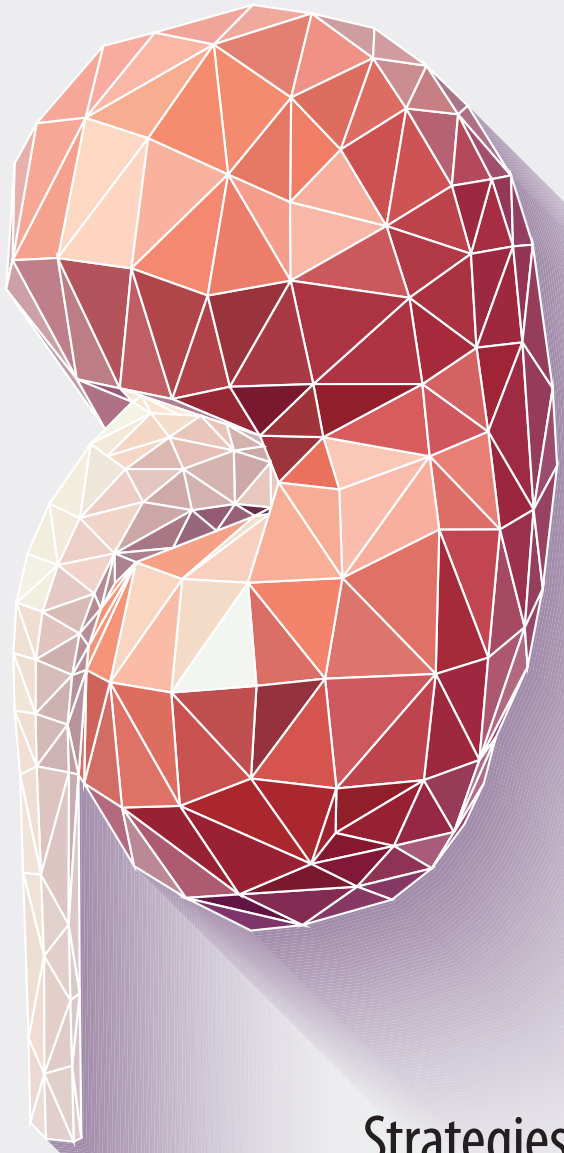

Strategies to optimize tacrolimus treatment and improve long-term clinical outcomes in solid organ transplantation

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Colophon

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STRATEGIES TO OPTIMIZE TACROLIMUS TREATMENT AND IMPROVE LONG-TERM CLINICAL OUTCOMES IN SOLID ORGAN TRANSPLANTATION

Strategieën ter optimalisering van tacrolimus therapie en bevordering van klinische resultaten op lange termijn na een solide orgaantransplantatie

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We can know only that we know nothing. And that is the highest degree of the human wisdom.
Leo N. Tolstoy

Знать мы можем только то, что ничего не знаем. И это высшая степень человеческой премудрости.
Л. Н. Толстой.

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Chapter 1

General Introduction

1. HISTORICAL OVERVIEW

1.1 History of Clinical Transplantation

Organ transplantation is a life-saving treatment for end-stage liver, heart and lung disease. Although end-stage renal disease (ESRD) patients can be treated with dialysis, renal transplantation is the best treatment for the majority of patients with ESRD both in terms of patient survival, quality of life and cost effectiveness. Many of the important developments in the field of organ transplantation have taken place within the past 60 years. The road to successful organ grafting has been long and fraught with problems.

The first experimental transplantation of a kidney between dogs was performed by Emerich Ullmann in Vienna in 1902.[1] A few years later, in 1906, the Frenchman Mathieu Jaboulay connected the renal vessels of a sheep and a pig kidney, respectively, to the brachial vessels of two patients with renal failure.[1,2] Both transplants failed and both patients died. The first use of a human deceased donor kidney for transplantation took place in 1933 when Yu Yu Voronoy, a Ukrainian surgeon, performed the first of a series of six transplantations to treat patients suffering from acute renal failure resulting from mercury poisoning. All the allografts failed, in large part as a consequence of the 6-hours lapse between the donor's death and organ procurement.[2,3] The first "successful" deceased donor kidney transplantation was performed by Richard Lawler and his colleagues, and took place on June 17, 1950 in the United States. A kidney from a woman of approximately the same age, blood group and Rhesus type as the patient was transplanted orthotopically after the removal of her left polycystic kidney.[4] Some 10 months after transplantation, however, the graft was lost as a result of rejection.[5] In 1951, French surgeons Rene Kuss, Charles Dubost and Marceau Servelle performed a series of renal transplantations with kidneys that were recovered from prisoners immediately after their execution by guillotine.[6] Some of these kidney allografts functioned for a limited period of time (days or weeks) but most of the transplanted kidneys were acutely rejected.[1] In 1953, the French surgeon Jean Hamburger performed the first living donor kidney transplantation (from a mother to her son). The kidney functioned well for 3 weeks before being rejected.[6]

Immune responses to alloantigens in the graft, which are proteins that vary from individual to individual, and are thus perceived as foreign by the recipient's immune system, were the major barrier to the success of transplantation. The first modern-day studies in transplantation began at the beginning of the twentieth century, when skin grafting between animals initiated the first series of experiments that eventually led to a scientific understanding of graft rejection. Medawar's work during and after the Second World War studying the rejection of skin grafts had demonstrated the potency of the

immune system.[7] This opened the door to the modern era of human transplantation with the first successful kidney transplant between two identical siblings in 1954 at the Peter Bent Brigham Hospital by Joseph Murray (Nobel laureate in 1990).[1,8] There then followed a series of identical twin transplants. Most of these recipients had a return of normal kidney function and survived for a considerable time.[9,10]

1.2 Pharmacological Immunosuppression

After Medawar's suggestion that rejection was an immunological event, a logical question arose:[7,11] Why not protect the allograft from rejection by suppression of the immune system? The first methods used to achieve immunosuppression included total body irradiation and glucocorticoids.[12] After successes in the field of bone marrow transplantation between siblings, where total body irradiation was used, this was also pursued in renal transplantation but with little success, although three recipients of kidneys from a non-identical twin did achieve relatively long-term function.[13,14] As a result of the profound bone marrow depression, total body irradiation led to patients' deaths from overwhelming infections. Therefore, by the early 1960s it became clear that total body irradiation was not the solution and less toxic methods for immunosuppression were needed.[15] Glucocorticoids alone were then used, also with limited success. The breakthrough came in the late 1950s with the discovery of the immunosuppressive effect of 6-mercaptopurine, which was already in clinical use as an anti-cancer agent.[16,17] 6-mercaptopurine prolonged the survival of kidney allografts in dogs although the survival of the animals remained poor.[18,19] Shortly thereafter (in the early 1960s), 6-mercaptopurine was replaced by its pro-drug azathioprine, a purine analogue which was less toxic. After the first successful series of renal transplantations performed in Denver by Thomas E. Starzl, the combination of azathioprine and prednisone (a synthetic adrenal glucocorticoid) came into widespread use for the next 20 years. Immunosuppression with azathioprine in combination with prednisone was a significant achievement in a time period when dialysis was still in its infancy and renal failure was usually a death sentence, as it enabled transplantation of deceased donor kidneys with a 1-year graft survival of about 60%.[1]

Until the early 1980s, azathioprine in combination with prednisolone was the only immunosuppressive therapy for the prevention of acute rejection after solid organ transplantation. The transplantation field was again revolutionized by the immunosuppressive power of the calcineurin inhibitor (CNI) cyclosporine A (CsA) first described by Borel *et al.*[20] Shortly thereafter, the first clinical study was performed with CsA as a single immunosuppressant, in Cambridge, UK by Calne *et al.*[21] In this early study, it became evident that there was also a downside to CsA, namely its nephrotoxicity. Thomas E. Starzl then decided to decrease the dose of CsA and use it in combination with prednisone in a successful series of 22 kidney recipients.[22] Several trials, comparing the efficacy of CsA

with conventional immunosuppressive treatment consisting of azathioprine and prednisolone, demonstrated that treatment with CsA led to a considerable improvement of transplantation outcomes. CsA-based immunosuppression markedly decreased both the incidence and the severity of acute rejection and increased 1-graft survival to 80%.[23,24] In addition, CsA had advantages over conventional immunosuppression as it decreased the incidence of infections and allowed for reduced doses of prednisolone.[25,26] The impact of CsA on the field of non-renal organ transplantation was even larger and with its introduction heart, lung, and liver transplantation first became realistic therapeutic options.[2] As experience with the new drug increased, it became evident that it did have several important side effects in addition to its nephrotoxicity. These include glucose intolerance, hypertension, gout and hyperlipidemia. This fostered the search for and discovery of other immunosuppressive agents. In the last decade of the twentieth century, such immunosuppressive drugs became available, including tacrolimus, mycophenolate mofetil (MMF), interleukin (IL)-2 receptor antagonists, and the mammalian target of rapamycin inhibitors sirolimus and everolimus, all with different safety and efficacy profiles.

Tacrolimus (Tac) is a CNI that nowadays has replaced CsA as the CNI of first choice for the prevention of graft rejection. Several studies were conducted to compare the efficacy and safety profile of Tac with that of CsA.[27-31] A meta-analysis incorporating 30 trials concluded that Tac is superior to CsA in preventing acute rejection after kidney transplantation. Moreover, Tac-treated recipients had an improved short-term graft survival. However, this comes at the expense of a higher risk to develop post-transplant diabetes mellitus.[32] At present, in most centers, immunosuppressive therapy after renal transplantation consists of the combination of Tac, MMF and glucocorticoids with or without induction therapy with an IL-2 receptor antagonist or a T-lymphocyte depleting agent. With Tac-based immunosuppressive therapy, acute rejection rates during the first post-transplant year have fallen below 20% and 1-year graft survival has risen to above 90%.[33-35]

Despite the considerable improvement of the short-term transplantation outcomes, the availability of modern immunosuppressive drug therapy has not resulted in a comparable improvement in the long-term outcomes. In fact, some studies suggest that long-term graft survival (censored for death of the recipient) may even have decreased in recent years despite an almost halving of the acute rejection incidence during the same time period.[36] Late renal allograft loss is most frequently caused by either the death of a patient with a functioning allograft or due to progressive renal dysfunction. The latter has multiple causes but the nephrotoxicity associated with prolonged treatment with Tac is still considered an important cause of late kidney allograft dysfunction.

2. TACROLIMUS PHARMACODYNAMICS

2.1 Mechanisms of Tacrolimus-mediated immunosuppression

Tacrolimus is a macrolide which was isolated from the fermentation broth of a strain of *Streptomyces*. Like CsA, this agent belongs to the class of CNIs and exerts its immunosuppressive effect mainly by inhibiting the activation of T-lymphocytes, although Tac also has inhibitory effects on B-lymphocytes, natural killer cells and dendritic cells.[37,38] The immunosuppressive properties of Tac result from inhibition of calcineurin, a calcium- and calmodulin-dependent phosphatase. The receptor of Tac is an intracellular immunophilin called FK-binding protein (FKBP)-12.[39] The Tac-FKBP-12 complex inhibits the activity of the enzyme calcineurin, which in turn prevents the dephosphorylation and subsequent activation of the nuclear factor of activated T-cell (NFAT). As a result, by blocking the activation of NFAT, Tac prevents the transcription of several genes, including IL-2 and interferon gamma, that are essential for T-lymphocyte activation and proliferation, and thus for the development of acute rejection.[40,41]

Apart from its effect on the calcineurin-NFAT pathway, Tac also affects the activation of the mitogen-activated protein kinase (MAPK) signaling pathway by inhibiting p38MAPK.[42-44] MAPK signaling is key in T-cell development and activation[45] and acts as an activator of NFAT.[46,47] The phosphorylation status of p38MAPK was recently found to be inversely correlated with Tac whole-blood predose concentrations (C_0) of kidney transplant patients. Furthermore, increased phosphorylated p38MAPK concentrations were associated with a higher T-lymphocyte activation status, which was inhibited by Tac in a dose dependent manner *in vitro*.[48]

2.2 Efficacy

The incidence of acute rejection among kidney transplant recipients has been reduced dramatically since the introduction of CNIs. As a result, 1-year kidney transplant survival has improved considerably. Positive effects of tacrolimus, such as prevention of acute rejection, were found to be related to drug exposure levels.[49-52] In a dose-ranging study conducted in de novo renal transplant recipients (n = 120), patients were randomized to one of three tacrolimus (0.2, 0.3, and 0.4 mg/kg per day) based-regimens designed to achieve a low (5-14 ng/mL), medium (15-25 ng/mL) or high (26-40 ng/mL) C_0. In the group of patients with the highest tacrolimus C_0 range (n = 29), a 62% incidence of toxicity was observed whereas only 10% of the patients developed an acute rejection. In the lowest target C_0 target range group (n = 33), the reverse was observed with 33% of the patients experiencing toxicity but having an acute rejection incidence of 21%. Based on these results, a tacrolimus C_0 range of 5-15 ng/mL was advised to achieve optimal efficacy with minimal toxicity.[53]

2.3 Toxicity

The clinical introduction of Tac has not resulted in the desired improvement of the long-term transplantation outcomes. The disappointing long-term kidney transplant survival can be partly attributed to the considerable toxicity of Tac and other immunosuppressive drugs. Toxicity associated with Tac therapy can occur at drug exposure levels similar to that required for a beneficial effect.[50] Optimal long-term immunosuppression without adverse effects is difficult to achieve and is often complicated by nephrotoxicity, infections, neurotoxicity, cardiovascular disease, and malignancy. The considerable side effects of Tac hamper long-term renal graft and patient survival, and result in additional morbidity. Other studies have reported that higher Tac exposure is associated with an increased risk of nephrotoxicity, neurotoxicity or development of infections.[54-56]

Tac-induced acute and chronic nephrotoxicity are a well-known adverse effects and a serious concern, often leading to irreversible histological renal damage to all renal compartments, including glomeruli, arterioles, and the tubulo-interstitium.[57,58] The acute nephrotoxic effect of Tac manifests clinically as acute oligo-anuria and / or as a rise in serum creatinine. Constriction of the afferent glomerular arteriole and direct tubular toxicity are considered the main mechanisms. The proposed mechanism of chronic Tac-induced nephrotoxicity is chronic ischaemia of the nephron caused by narrowing of the vascular lumen through accumulation of hyaline deposits in the arteriolar wall and prolonged afferent arteriolar vasoconstriction.[57] Although the risk of chronic renal failure in Tac-treated patients is considerable, not all patients develop renal transplant failure because of this phenomenon. Limited evidence suggested that variations in genes involved in the pharmacokinetics (*ABCB1* and *CYP3A5*) and pharmacodynamics (*TGF-β*, *CYP2C8*, *ACE*, *CCR5*) of Tac may influence a patients' risk to develop Tac-induced nephrotoxicity.[59,60]

3. TACROLIMUS PHARMACOKINETICS

Tacrolimus has highly variable and unpredictable pharmacokinetics. In recent years it has become clear that an important part of these between-patient differences in the pharmacokinetics of Tac result from variability in the activity of the Tac metabolizing enzymes (CYP3A4 and CYP3A5) and the drug-transporting protein ABCB1. These metabolizing enzymes and ABCB1 are characterized by considerable variation in their activity and expression, either caused by genetics or by induction and/or inhibition by other substances such as drugs. CYP3A enzymes comprise 30-60% of total CYP content and are responsible for the oxidative metabolism of over 50% of the drugs in use.[61] CYP3A4 and CYP3A5 have largely overlapping substrate specificities. Functional CYP3A4 protein

is expressed in liver, jejunum, colon, and pancreas. Immunohistochemistry analyses show limited expression of this enzyme in the kidney. All individuals synthesize functional CYP3A4 but with up to a 40-fold variation in protein expression and a substantial variability in enzyme activity.[62] Recently, a new mutation, namely *CYP3A4*26*, was found to be associated with CYP3A4 deficiency.[63,64] The expression of CYP3A5 is even more variable, as only some individuals express significantly high levels of functional CYP3A5 protein. CYP3A5 is also expressed in the liver and small intestine but at levels of 10% to 30% of CYP3A4. In the kidney, however, CYP3A5 is the predominant form of CYP3A.[65]

The rate of Tac absorption and its bioavailability vary largely between individuals and is related to first pass metabolism. Its oral bioavailability averages around 25%, but can range from 5% to 90%.[66,67] Peak Tac whole-blood concentrations (C_{max}) are usually achieved within 2 hours, although the time to C_{max} (t_{max}) varies widely.[67] The absorption of Tac occurs mainly in the small intestine and is affected by several factors. Ingestion of food with a moderate fat content may delay the absorption of Tac and reduce its bioavailability. CYP3A5 initially came to light as a contributor to the large inter-individual variation in Tac exposure due to the well-known phenomenon that the Tac dose requirement of patients of African ancestry is about two-fold higher than that of white individuals. Increased first pass metabolism and consequently reduced oral bioavailability, is responsible for this difference rather than a protracted elimination as the half-life of Tac is the same in African and Caucasian patients when the drug is administered intravenously.[68] The poor bioavailability of Tac may also be attributed to the fact that Tac is a substrate of the multidrug-efflux pump ABCB1, previously known as P-glycoprotein (P-gp). ABCB1 is an ATP-driven efflux pump which exports xenobiotics from the cytoplasm or cell membrane to the exterior of the cell, reducing thereby the accumulation of drugs and metabolites within cells. Physiologically, ABCB1 is expressed in the liver (at the canalicular surface of hepatocytes), pancreas, and at the apical surface of mature enterocytes in the small intestine and colon. In the normal human kidney, ABCB1 is expressed in the brush border of proximal tubular epithelial cells, in epithelial cells of Bowman's capsule, glomerular mesangial cells, on the apical membrane of the thick ascending limb of Henle's loop, intracellularly in distal tubules, and on the apical membrane of the collecting duct.[69-71] ABCB1 expression in endothelial cells of renal arteries, arterioles, and glomerular and peritubular capillaries was found to be absent or low.[71,72] In addition, ABCB1 is also expressed in testes, placenta (trophoblasts), on the luminal surface of capillaries in the brain and at the choroid plexus.[73] Finally, ABCB1 is found on various leukocytes, including T and B lymphocytes, and dendritic cells.[74,75] The specific tissue expression of ABCB1 suggests that the protein functions as a protective barrier. Its expression in the intestine is thought to limit the absorption of Tac, whereas its expression in the biliary tract and kidney may facilitate the elimination of Tac and its metabolites.

In blood, Tac binds extensively to erythrocytes, which have a high content of FKBP-12. Whole-blood Tac concentrations are significantly higher (average 15 times, range 4-114 times) than those measured in plasma. Approximately 99% of Tac in plasma is bound to plasma proteins, mainly albumin and α_1-acid glycoprotein. Distribution of Tac between erythrocytes and plasma is dependent on factors such as hematocrit, plasma protein concentrations, and Tac concentration. The volume of distribution of Tac is about 1.4 L/kg.[67] Tac distributes widely into most tissues, including kidney, liver, heart, lungs, spleen, brain, and muscles. This drug crosses the placenta and passes into the breast milk.[76]

Tacrolimus is more rapidly metabolized by CYP3A5 than CYP3A4. The catalytic activity of CYP3A5 (*in vitro*) towards Tac is 1.6-fold higher than that of CYP3A4.[77] More than 15 Tac metabolites are formed through CYP3A-mediated metabolism. 13-*O*-demethyl-Tac is the main breakdown product. This metabolite has an immunosuppressive activity that is one-tenth of that of Tac. The reported elimination half-life of Tac is variable, ranging between 12 and 35 hours.[67] More than 95% of Tac metabolites are eliminated biliary with about 5% being excreted in the urine. Less than 1% of the absorbed amount of Tac is excreted in the urine as unchanged drug.[66,67,78]

In addition to the well-described inter-patient pharmacokinetic variability, the clinical use of Tac is also complicated by considerable intra-patient variability (IPV) in Tac exposure. Several factors can contribute to IPV in Tac exposure, among which medication adherence may be one of the most important determinants. Recipients of a kidney, in comparison to recipients of other organs, demonstrate the highest level of immunosuppressive medication nonadherence,[79] which in turn is related to poorer clinical outcomes.[80] A high Tac IPV may result in a Tac exposure which is outside the therapeutic window. These patients may be at risk for under-exposure and rejection, or Tac toxicity in case of over-exposure.

4. TACROLIMUS PHARMACOGENETICS

It is well recognized that different patients respond differently to the same medication. As described above, the clinical use of Tac is complicated by high inter-patient variability in its pharmacokinetics. In general it has been estimated that genetics can account for 20-95% of variability in drug disposition and effect.[81] Genetic polymorphisms in drug metabolizing enzymes and efflux pumps are potential targets for developing a pharmacogenetic strategy to individualize immunosuppressant therapy. The encoding genes of *CYP3A4*, *CYP3A5*, and *ABCB1* contain numerous single-nucleotide polymorphisms (SNPs) and these polymorphisms have been the subject of a considerable number of studies as they may explain the differences in Tac pharmacokinetics between patients.

4.1 CYP3A

At least 28 SNPs have been identified in the *CYP3A4* gene. The 392A>G SNP (rs2740574), also known as *CYP3A4*1B*, is the most extensively studied *CYP3A4* SNP. The *CYP3A4*1B* polymorphism has been associated with an increased *CYP3A4* transcriptional activity.[82] However, the clinical relevance of this polymorphism is not clarified.[83] The frequency of *CYP3A4*1B* polymorphism depends on ethnicity. It is present in approximately 2-9.6% of Caucasians, 35-67% of Africans, 9.3-11% of Hispanics and 0% of Asians.[84] No reproducible association has been found between the *CYP3A4*1B* variant and the Tac pharmacokinetics. The controversy regarding the functional effect of *CYP3A4*1B*, is most likely explained by the fact that this SNP is in linkage disequilibrium with the *CYP3A5* 6986A>G SNP (see below).[83,85,86]

The polymorphic expression of *CYP3A5* was identified as early as 1990.[87] Its genetic basis was elucidated by Paulussen *et al.* who first identified genetic variants predictive of CYP3A5 expression in liver samples.[88] Kuehl *et al.* described the *CYP3A5*3* variant allele in intron 3 (*CYP3A5* 6986A>G; rs776746), which disrupts the correct splicing of *CYP3A5* transcripts.[89] Individuals who are homozygous for the *CYP3A5**3 allele lack CYP3A5 protein activity, whereas individuals carrying at least one *CYP3A5**1 allele (considered to be the wild-type) were found to express large amounts of CYP3A5 protein. The frequency of these alleles differs between individuals of different ethnicities with approximately 15% of Caucasians expressing functional CYP3A5, whereas as much as 85% of individuals of African descent (sub-Saharan genetic origin) are CYP3A5-expressers. Asian populations fall between these two groups with about 50% carrying the *CYP3A5**1 allele.[89-91]

The identification of SNPs in *CYP3A5* was the first evidence that genetic factors may determine the inter-individual variability in Tac pharmacokinetics. It has been consistently demonstrated that CYP3A5 expressers require approximately two-fold higher Tac doses to reach the same steady-state C_0 than CYP3A5 non-expressers.[57,83,85,92-94] Available data describe a gene-dose effect with lower dose-corrected Tac C_0 in *CYP3A5**1/*1 homozygotes than in *CYP3A5**1/*3 heterozygotes.[95] As a consequence of the higher Tac dose requirement of CYP3A5 expressers, in some investigations there was a delay in achieving the target Tac concentration after standard, bodyweight-based dosing.[95-97] CYP3A5 expressers may therefore be at an increased risk for under-immunosuppression, particularly in the early phase after transplantation, and the development of acute rejection.[92] Several researchers suggested that the Tac starting dose in CYP3A5 expressers should be about two-fold higher than that of CYP3A5 non-expressers.[98] Despite a strong association between the *CYP3A5* 6986A>G SNP and Tac pharmacokinetics, there is no consistent evidence of organ rejection as a consequence of genotype-related under-

immunosuppression.[60] In fact, there's little evidence from randomized-controlled clinical trials that a pharmacogenetic-based approach to Tac treatment will benefit patients.

4.2 ABCB1

Over the last 10 years more than 50 single-nucleotide polymorphisms (SNPs) have been identified in *ABCB1* (previously known as *MDR-1*). The SNPs that have been studied most widely are the C to T transition at position 1236 within exon 12 (rs1128503), the G to T/A transition at position 2677 within exon 21 (rs2032582) and the C to T transition at position 3435 within exon 26 (rs1045642).[99,100] These three SNPs are in strong linkage disequilibrium and their allelic frequency varies between different ethnic groups.[101] Only the 2677G>T/A SNP results in an amino acid substitution (Ala893Ser or Ala893Thr, respectively), whereas *ABCB1* 3435C>T and 1236C>T are synonymous SNPs. The functional impact of these three SNPs is not clear *in vivo* although *in vitro* the ABCB1 3435C>T SNP has been associated with reduced mRNA expression[102] and stability,[103] and more recently, with changes in substrate specificity.[104] With regard to the latter, evidence suggests that the 3435C>T transition affects the timing of co-translational folding and insertion of ABCB1 into the plasma membrane, thereby altering the structure of substrate and inhibitor interaction sites.[104] However, these findings have not been confirmed by other investigators and at present, the consequences of the silent 3435C>T SNP for mRNA expression and stability, as well as protein function, are subject to debate.[105] The functional impact of the 1236C>T and 2677G>T/A, and other *ABCB1* SNPs on ABCB1 expression and function also remains controversial and has been the topic of several review articles.[106,107]

Polymorphisms in *ABCB1* could also contribute to alterations in Tac pharmacokinetics. The *ABCB1* 3435C>T, 1236C>T, and 2677G>T/A SNPs, have been extensively investigated in relation to Tac pharmacokinetics. Taken together, the results of these studies have been negative as they have demonstrated only a limited (if any) effect of *ABCB1* SNPs on Tac disposition.[83,108]

Perhaps even more important than its relation to pharmacokinetics is the influence of genetic variation on Tac pharmacodynamics and consequently transplantation outcome. Several studies investigated the effect of SNPs in *ABCB1* on Tac pharmacodynamics. Similarly, the *ABCB1* SNPs exert no obvious impact on Tac pharmacodynamics, with studies demonstrating conflicting results in regard to the main parameters of acute rejection and nephrotoxicity.[60] However, recently have been found that kidneys from donors with T allele at *ABCB1* rs1045642 are associated with shorter renal allograft survival.[109] It has been hypothesized that a high degree of ABCB1 expression or activity may lead, as a consequence of an increased efflux of Tac out the lymphocytes, to rejection episodes

in transplant recipients. One recent study has shown that recipients *ABCB1* genotype is related to intracellular Tac concentrations in their peripheral blood mononuclear cells.[110] Since increased Tac intracellular concentrations might in turn improved immunosuppressive status and prevention of rejection, *ABCB1* recipient genotyping might be useful to better individualize the Tac therapy after solid organ transplantation.

5. THERAPEUTIC DRUG MONITORING

Optimal immunosuppression is essential to maintain a viable allograft. The well-known large inter- and intra-individual variability of the pharmacokinetics of Tac, as well as its narrow therapeutic window are a challenge to clinicians, who need to select the best treatment and the best dosage for a given patient. Tac is therefore considered to be a critical dose drug. In most transplant centers, monitoring of Tac therapy by use of predose concentrations, a practice known as therapeutic drug monitoring (TDM), is standard of care of (renal)-transplant recipients. In the early years after the clinical introduction of Tac there was debate regarding the appropriate matrix for the determination of Tac concentrations. Because the concentration of Tac is high within the erythrocytes, it proved impractical to measure the drug in plasma or serum, and all our current data on the application of Tac concentrations as a guide to therapy are based on whole blood measurements.[111]

Because Tac dose does not correlate with Tac exposure TDM remains mandatory to optimize clinical outcomes and reduce toxicities. A few multicenter, prospective, randomized-controlled trials have been published to enable the establishment of target Tac whole blood levels in relation to clinical outcome. In renal transplant recipients, Laskow *et al.* found a significant trend for increasing toxicity with increasing maximum Tac C_0 recorded within 7 days after transplantation and for decreasing rates of rejection with increasing minimum Tac C_0.[53] A significant relationship was found between low area under the concentration-time curve (AUC < 200 ng · h/mL) and acute rejection on day 2, but no correlation was demonstrated between rejection and low AUC at 2 weeks or 3 months after kidney transplantation.[52] The $AUC_{0\text{-}12}$ value of 200 ng · h/mL was found to correspond to a Tac C_0 of approximately 10 ng/mL. It was, therefore, suggested that in order to reduce the risk of rejection the minimum Tac C_0 of 10 ng/mL should be achieved by day 2 to 3 post-transplantation.[52] In a prospective 12-momths pharmacokinetic study of Tac in 100 de novo renal allograft recipients these results were confirmed. Kuypers *et al.* demonstrated that on day 7 after transplantation Tac $AUC_{0\text{-}12}$ greater that 150 ng · h/mL was associated with a lower incidence of acute rejection.[56]

The AUC, which is calculated on the basis of a full pharmacokinetic profile, is in general considered as the best marker of drug exposure, but it is difficult to justify for financial and practical reasons. Despite that the correlation between C_0 and $AUC_{0\text{-}12h}$ for Tac is a matter of debate, with generally a better relationship during early phase after transplantation (first month) than later on, most centers still rely on the Tac C_0.[112] Tac 2 hours after dose ingestion (C_2) do not seem to correlate better with AUC than C_0.[111] A more reliable estimation of the total exposure can be obtained by a limited sampling strategy (LSS). This means sampling at limited or optimal sampling times, still allowing for an accurate and precise estimation of the AUC. However, although this LSS adequately predicted Tac exposure, a LSS does not really overcome the logistical and financial disadvantages of a full pharmacokinetic profile. Moreover, it is unlikely to rapidly gain widespread clinical acceptance of LSS because of the lack of any data demonstrating improved clinical outcomes over C_0 monitoring.[113]

Careful interpretation of clinical data allows us to roughly position current effective target Tac C_0 between 5-15 ng/mL, at least in the first year post-transplantation provided that Tac is incorporated in an immunosuppressive regimen with mycophenolate, glucocorticoids, and anti-IL-2 induction. These target ranges are not based on validated guidelines but an observation of what has been evaluated in today's clinical studies.[111,114]

The routine measurement of Tac C_0 has until recently mostly been performed by use of immunoassays, especially the microparticle enzyme immunoassay (MEIA, Abbott Diagnostics, Chicago, IL). In more recent years other immunoassays have been become available, such as the enzyme multiplied immunoassay technique (Dade Behring, now Siemens), the antibody conjugated magnetic immunoassay (ACMIA, Siemens) and the cloned enzyme donor immunoassay (CEDIA, Microgenics). These immunoassays are to some degree biased by cross-reactivity with Tac metabolites, and changes in haematocrit lead to additional bias in the MEIA assay.[115,116] With the recent progress in analytical techniques regarding robustness of measurement, reproducibility, dynamic range (3-4 orders of magnitude) and measurement time (seconds), traditional methods for Tac TDM have been reconsidered. High-performance liquid chromatography (HPLC) based methods have been developed, either with single mass-spectrometric detection (HPLC-MS), or linked to tandem mass spectrometry (HPLC-MS/MS).[111] Some hospital laboratories have already implemented HPLC-MS(/MS) for measurement of samples for patient care, while others still rely on immunoassays.

6. AIMS OF THE THESIS

Although Tac has been in widespread use since the 1990s, there are still a number of unanswered questions related to its clinical use. For example, the optimal starting dose of Tac and the optimal target concentration in both the early and late phase after transplantation, are unknown. At present, the main challenge to transplant physicians is to improve the long-term outcomes of solid organ transplantation while maintaining the good short-term outcomes that have already been achieved. The aim of this dissertation was to try and answer some of these questions and to find strategies to optimize Tac treatment with the ultimate aim to improve the long-term outcomes of solid organ transplantation. More specially, we investigated the following:

1. *Is there a relationship between the currently used, empirically defined, Tac target concentrations and the incidence of acute rejection in kidney transplant recipients?*

TDM of Tac is universally applied. However, contrary to the expectations of most physicians, the concentration-effect relationship for Tac is poorly defined. To justify the widespread implementation of TDM for Tac a concentration-effect relationship should be present. Several studies have attempted to define the optimal Tac concentration range, where both a low incidence of rejection and good tolerability to Tac is achieved. Since the findings of these reports are contradictory, we investigated in a large study population whether the currently applied Tac target C_0 are indeed associated with a lower risk for developing an acute rejection (*Chapter 2.1*).

2. *Does conversion from twice-daily Tac formulation (Prograf®) to a once-daily Tac formulation (Advagraf®) have an effect on p38MAPK phosphorylation in kidney transplant recipients?*

Tac suppresses the phosphorylation of the MAPK pathway by inhibiting p38MAPK. The amount of phosphorylation of this signaling molecule was found to be inversely correlated with Tac whole blood C_0 of kidney transplant recipients. Increased p38MAPK phosphorylation was also associated with a higher T-lymphocyte activation status, which was inhibited by Tac.[42,48] Despite the fact that Tac therapy is routinely monitored by measuring whole blood C_0, some patients who have Tac C_0 that are considered therapeutic, still suffer from acute rejection or toxicities. We speculated that measurement of drug's pharmacodynamics effects may be a better way to perform TDM of Tac. Phospho-specific flow cytometry was used to study the biological effects of conversion from twice-daily to a once-daily Tac formulation on p38MAPK phosphorylation (*Chapter 2.2*). Potentially, pharmacodynamic monitoring of Tac treatment may offer an attractive new method to individualize Tac therapy.

3. *Does the pre-transplant Tac dose requirement predict the post-transplantation dose requirement?*

Tac is a drug with high between-patient variability in its pharmacokinetics. In the early phase after transplantation, following initiation of treatment with a standard Tac dose, many patients have Tac concentrations outside the target range, putting them at risk for rejection or toxicity. Knowledge of the pharmacokinetics of Tac in a patient obtained before transplantation may be helpful in personalizing Tac therapy and achieving therapeutic Tac concentrations early after transplantation. In the study presented in Chapter 3.1 we investigated whether the pre-transplant Tac dose requirement in patients scheduled to undergo living donor kidney transplantation correlates with early post-transplantation dose requirement.

4. *Does pharmacogenetic adaptation of Tac starting dose increase the number of patients having therapeutic Tac exposure early after transplantation and consequently lead to improved clinical outcomes?*

In the area of transplantation it is well known that *CYP3A5* genotype is a strong predictor for the Tac dose needed to reach target concentrations. *CYP3A5* expressers need an approximately 2-fold higher Tac dose than non-expressers, and have lower Tac exposure in the first week after transplantation, which puts them at risk for acute rejection. Our randomized controlled trial was specifically designed to prospectively investigate whether dosing of Tac according to *CYP3A5* genotype leads to earlier achievement of target Tac C_0 and consequently to a better clinical outcome than the standard body-weight based dosing approach (*Chapter 3.2*).

5. *Does high IPV in Tac exposure influence long-term kidney transplant outcomes?*

Tac is known to have a considerable IPV in its pharmacokinetics, which is defined as the fluctuation in Tac concentrations within an individual over a certain period of time during which the Tac dose is unchanged. A high Tac IPV might put patients at risk for episodes of under-immunosuppression, and consequently rejection, as well as toxicity in case of over-exposure. In a large cohort of renal transplant recipients we investigated (during a relatively long follow-up period) whether Tac IPV predicts adverse kidney transplant outcomes (*Chapter 4.2)*.

6. *Does high Tac IPV correlate with progression of graft vascular disease in heart transplant recipients?*

In renal transplant recipients a higher IPV for Tac pharmacokinetics was shown to be associated with impaired long term transplant outcomes. Several other studies have now confirmed our findings in cohorts of kidney transplant patients. There are far less data available on the importance of IPV for long term outcome after other solid organ trans-

plants. In Chapter 4.3 we investigated whether a high IPV in Tac exposure is associated with progression of cardiac allograft vasculopathy as a determinant of long-term survival of heart transplant recipients.

7. *Does conversion from twice-daily Tac formulation (Prograf®) to a once-daily Tac formulation (Advagraf®) lead to a lower intra-patient variability in Tac exposure?*

Non-adherence to the immunosuppressive drug regimen is common and reported to average 23%.[79] Medication non-adherence is considered an important risk factor for poor long-term transplantation outcome. Non-adherence to the immunosuppressive drugs among renal transplant recipients is associated with an increased risk for graft failure.[117] To improve adherence in transplant recipients, a modified-release, oral dosage form of Tac (Advagraf®, Astellas Pharma) has been developed to provide a once-daily dosing alternative. As medication adherence may be an important determinant of IPV in drug exposure, we decided to investigate whether conversion from twice-daily to once-daily Tac formulation leads to a lower IPV in a large cohort of stable renal transplant recipients (*Chapter 4.4*).

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Chapter 2

In search of the therapeutic window of tacrolimus

2.1

Tacrolimus predose concentrations do not predict risk of acute rejection: a pooled analysis from three randomized controlled clinical trials

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¥ This article is dedicated to Henrik Ekberg, who sadly passed away on December 29th, 2012.

ABSTRACT

TDM for tacrolimus (Tac) is universally applied. However, the concentration-effect relationship for Tac is poorly defined. This study investigated whether Tac concentrations are associated with acute rejection in kidney transplant recipients. Data from three large trials were pooled. We used univariate and multivariate analysis to investigate the relationship between BPAR and Tac predose concentration at 5 time points (day 3, 10, and 14, and month 1 and 6 after transplantation). A total of 136/1304 patients experienced BPAR, giving an overall incidence of 10.4%. We did not find any significant correlations between Tac predose concentrations and the incidence of BPAR at the different time points. In the multivariate analysis, only Delayed Graft Function (DGF) and the use of induction therapy were independently correlated with BPAR, with an odds ratio of 2.7 [95% CI: 1.8 - 4.0; $p < 0.001$] for DGF and 0.66 [95% CI: 0.44 - 0.99; $p = 0.049$] for induction therapy. The other variables, including the Tac predose concentrations, were not statistically significantly associated with BPAR. We did not find an association between the Tac predose concentrations measured at 5 time points after kidney transplantation and the incidence of acute rejection occurring thereafter. Based on this study it is not possible to define the optimal target concentrations for Tac.

INTRODUCTION

Tacrolimus (Tac) has almost replaced cyclosporine A (CsA) as the drug of first choice for the prevention of graft rejection after kidney transplantation.[1] Therapeutic Drug Monitoring (TDM) for Tac is universally applied. Requirements for a drug to implement TDM in clinical practice include a high between-patient variability in pharmacokinetics, a relatively low within-patient variability, and a concentration-effect relationship. In order to do TDM, assays to measure drug concentrations also need to be available and ideally, randomized trials should show an improvement in clinical outcome when a drug is dosed based on measured drug concentrations compared to a fixed-dose approach. For Tac several assays are available, but randomized trials showing a benefit of TDM are not available. However, it is not realistic to expect that for Tac such a trial will ever be performed.

Contrary to the belief of many physicians and surgeons, the concentration-effect relationship for Tac is poorly defined. As the most important reason to prescribe Tac to a transplant recipient is the prevention of acute rejection, it is surprising that there are so few data on the concentration-effect relationship of Tac. Based on the current literature there is little support to promote the use of a specific therapeutic window and aim for certain target concentrations.

Several investigators have attempted to identify the optimal Tac concentration range, *i.e.* the one which is associated with the lowest incidence of rejection and with acceptable toxicity, as shown in Table 1. The findings of many of these reports are conflicting and limited by the fact that they were of a retrospective design, included limited numbers of patients, and that the co-immunosuppressive medication used was different from that which is currently considered the gold standard. For the interpretation of the studies that are available an important additional problem is the fact that not all investigators studied Tac concentrations at the same time point after transplantation.

Rodriguez *et al.*[2] recently performed a meta-analysis of 64 studies investigating the correlation between the Tac predose concentration and the incidence of rejection in liver transplant recipients. They concluded that the mean Tac predose concentration during the first month was not correlated with acute rejection. Nevertheless, they suggested that lower Tac predose levels would be more appropriate after liver transplantation to prevent Tac toxicity.

Despite limited evidence for performing TDM for Tac and the exact predose concentrations to aim for, in most transplant centers considerable time and effort is spent on the precise dosing of Tac in order to reach the predefined Tac target concentrations rapidly.

Table 1. Literature

Author, Year	Number of patients	Conclusion
Borobia et al, 2009[15]	57 kidney	The Tac predose concentrations within the first post-operative week are an important predictor of acute rejection
Staatz t al, 2001[16]	29 kidney	Significant relationship between acute rejection and median Tac predose concentrations in the first month.
Bottiger et al, 1999[17]	14 kidney	Concentrations below 10 ng/mL seem to be beneficial with respect to side effects
Kershner et al, 1996[18]	92 kidney	Significant relationship between the Tac concentrations and toxicity
Undre et al, 1999[19]	56 kidney	Mean 12-hour Tac area-under the concentration *vs.* time-curve (AUC_{0-12}) on day 2 after transplantation was significantly lower in 17 patients who experienced acute rejection than in the 39 patients who remained rejection-free
Kershner et al, 1996[18]	721 liver	No relationship between the Tac concentrations and toxicity
Laskow et al, 1996[21]	92 kidney	No significant difference among three different Tac-ranges (5-14 ng/mL, 15-25 ng/mL, and 26-40 ng/mL) with respect to the incidence of rejection
Nashan et al, 2009[21]	60 liver	Tac predose concentrations of 5-8 ng/mL in the first month of transplantation resulted in the same rejection rates as Tac concentrations of 10-15 ng/mL.

Once on target, maintaining patients within the target concentration range also requires careful monitoring.

The aim of the present study therefore was to investigate whether the currently used and empirically-defined Tac target predose concentrations are indeed associated with the risk of developing acute rejection in kidney transplant recipients. We pooled the data of three large randomized-controlled trials (RCTs) and studied the relation between Tac exposure and the incidence of biopsy-proven acute rejection (BPAR).

PATIENTS AND METHODS

Patients and clinical trials

For the present analysis we combined the data of three large, randomized-controlled clinical trials in kidney transplant recipients, the FDCC,[3] Symphony[4] and OptiCept[5] trials. In brief, the main common elements of the three studies were the randomized, open-label, parallel-arm, multicenter design, and the fact that they included a broad spectrum of patients. In general, these patients had a low-to-medium immunological risk and were treated under the respective protocols for at least one year after kidney transplantation. In addition to adults, the FDCC and OptiCept studies enrolled paediatric patients, who were, however, not included in our analysis.

Tac target concentrations

For the present analysis we included only the patients from these three RCTs who received Tac as part of their immunosuppressive regimen from the day of transplanta-

tion and had a minimum of 1 known Tac level. The Tac levels were targeted differently between the studies. For the FDCC study, Tac dosing was according to each center's protocol, and on average was between 10 and 14 ng/mL in the first month, with gradual tapering thereafter. In the Symphony study, Tac levels were targeted at 3-7 ng/mL for the study period. In the OptiCept trial, the Tac predose concentrations were 8-12 ng/mL within the first month, 4-6 or 8-10 ng/mL in the second and third months (depending on the randomization group), and 3-5 or 6-8 ng/mL from the fourth month onwards. Data on Tac dose and predose concentrations, as well as other demographic and clinical characteristics were collected from the databases of the three RCTs and pooled. Tac predose concentrations were studied at day 3 (± 2 days), day 10 (± 2 days), day 14 (± 3 days), month 1 (± 7 days), month 6 (± 4 weeks). We changed the Tac levels that were higher than 30 ng/mL (24 measuring points in total) into missing values, to prevent that non-predose Tac concentrations would be included in the analysis. However, we also performed the analysis with all the Tac levels (including the ones that were higher than 30 ng/mL).

Acute rejection

BPAR was defined as any histologically-confirmed episode for which a Banff score of 1 (mild, grades IA and IIA), 2 (moderate, grades IB and IIB), or 3 (severe, grade III) was recorded. In all three trials, all biopsy samples were assessed by a local pathologist, and rejection was classified according to the revised Banff grading system.[6] For the present analysis, only the first episode of BPAR was investigated. Ongoing or recurrent rejections were not studied.

Statistical analyses

The correlations between Tac concentrations and BPAR were done for BPARs occurring after the time of the Tac concentration measurement, within the remainder of the first post-transplant year tested with the non-parametric Mann Whitney U test at the five different time points. We also did the same analysis for BPARs occurring within the month following the Tac concentration measurement, again for all five time points. We also performed a similar analysis categorizing the patients as high-risk if they had one or more of the following characteristics: delayed graft function (DGF), second or third transplantation, panel reactive antibodies (PRA) of more than 15%, four or more human leukocyte antigen (HLA) mismatches, or were of African descent (black). All other patients were considered as low-risk. We have previously used the same definition for high and low risk.[7] The significance level was stated at 5%. Induction therapy (yes/no)(either ATG of anti ILR monoclonal antibody induction), HLA mismatches (<4 / ≥4), DGF (yes/no), PRA (<15 / ≥15) and number of transplant (first / ≥second transplant) were correlated with the occurrence of BPAR within one month and one year after transplantation by using the Chi Square test. To identify independent risk factors for the development of BPAR, a

binary logistic regression was performed, including all the above mentioned variables, plus median levels of Tac predose concentrations. Statistical analysis was carried out using SPSS version 19 (SPSS / IBM Inc., Chicago, Illinois, USA).

RESULTS

Patient characteristics

In the three clinical trials a total of 1363 renal transplant patients were treated with Tac after transplantation. Of these patients, 1304 met the inclusion criteria and were used for further analysis (Fig. 1). Of these 1304 patients, 358 (27%) participated in the FDCC study, 385 (30%) in the Symphony study, and 561 (43%) in the Opticept study. The patient characteristics are listed in Table 2. A total of 4953 Tac predose concentrations of 1304 patients were available for the analysis (Total predose concentrations of 818 on day 3; 1127 on day 10; 804 on day 14; 1167 on month 1 and 1019 on month 6). The Tac predose concentrations show a substantial range and are depicted in Figure 2A. Twenty-four Tac concentrations were >30 ng/mL (n =13 on day 3; n =4 on day 14, n = 4 on month 1 and n = 3 on month 6). As we were unable to check whether these concentrations were truly predose concentrations or in fact post-dose concentrations, these values were classified as "missing values" and excluded from the primary analysis.

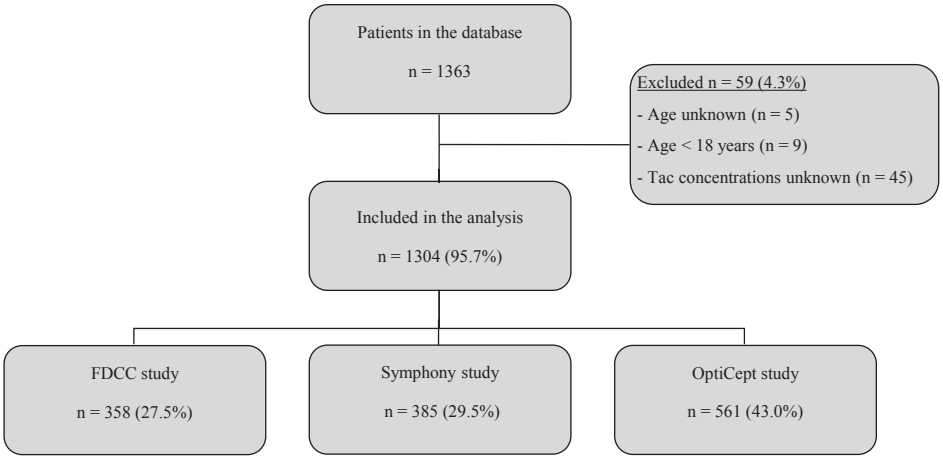


Figure 1. Included patients from the three clinical trials and reasons for exclusion from the study.

Relationship between Tac and BPAR

In this cohort the overall incidence of BPAR was 10.4% (n = 136) within one year after transplantation. The vast majority of BPARs occurred within the first month after transplantation (91/136 = 7%). We univariately tested the relationship between median

Table 2. Patient Characteristics

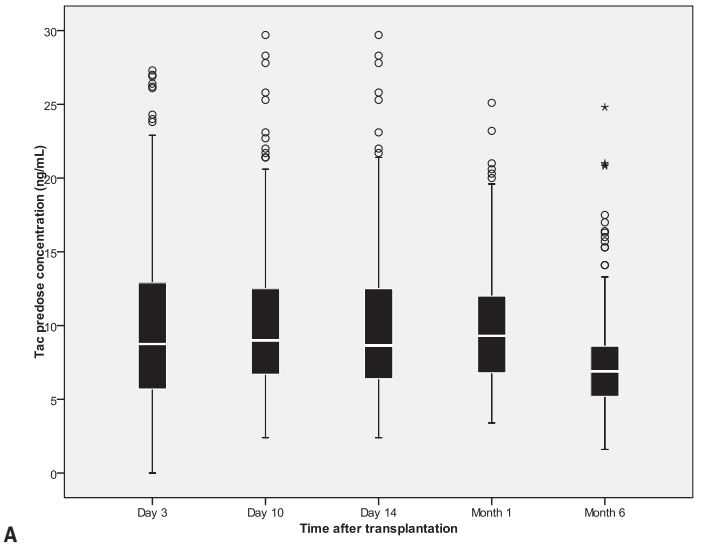
Gender (female / male)	450 (34%) / 854 (66%)
Age (yr; mean (SD)	48 (13.8)
Ethnicity (%):	
- Black	161 (12%)
- Non- Black	1143 (88%)
Transplantation (1st / ≥2)	1219 (94%) / 84 (6%)€
Delayed Graft Function: Yes / No	238 (18%) / 1066 (82%)
Panel reactive antibodies (< 15% / ≥ 15%)	1124 (91.5%) / 105 (8.5%)€
HLA-mismatches (< 4 / ≥4)	709 (54%) / 595 (46%)
Living related / living unrelated / deceased donor	338 (26%) / 183 (14%) / 783 (60%)
Induction therapy: Yes / No	890 (68%) / 414 (32%)

€: For transplantation and PRA there were missing values in 1 and 6 patients, respectively.

Tac predose concentrations and the occurrence of BPAR within the first post-transplant year at 5 different time points, as shown in Table 3A and Figure 2B. We did not find any significant relationship between the Tac concentration and the incidence of BPAR. The results for BPAR within the first month after the Tac measurements did show similar results: again patients that developed a BPAR had Tac predose concentrations that were not different compared to patients without a BPAR, as shown in Table 3B. As for only 61% of the patients a Tac predose concentration was available for day 3 (Table 3A), we have studied the mean Tac predose concentration for each patient, based on samples drawn between day 3 and day 14 and correlated this to BPAR. Again, these Tac concentrations were not significantly different between patients with BPAR and patients without BPAR (10.02 *vs.* 9.97; p = 0.90).

The data were further analyzed by stratification into two groups: patients with a predose concentration < 5 ng/mL *vs.* patients with a predose concentration > 5 ng/mL, and patients with a predose concentration < 10 ng/mL *vs.* patients with a predose concentration > 10 ng/mL. The results are shown in Table 4. There were no statistically significant associations between the Tac predose concentrations and the occurrence of BPAR within one month after the measurement or throughout the rest of the first year after transplantation.

To analyse the risk of BPAR further, we divided the group into high and low immunological risk patients according to the definition described above. The total number of patients defined as being low-risk was 499 (39%) whereas 786 (61%) patients were considered to be high-risk. Nineteen patients were not included in this analysis, because one or more of the variables needed to define their immunological risk were not known. The incidence of BPAR was higher in patients in the high-risk group (100/786 = 12.7%) compared to the low-risk group (36/499 = 7.2%), with an odds ratio of 1.9 for patients



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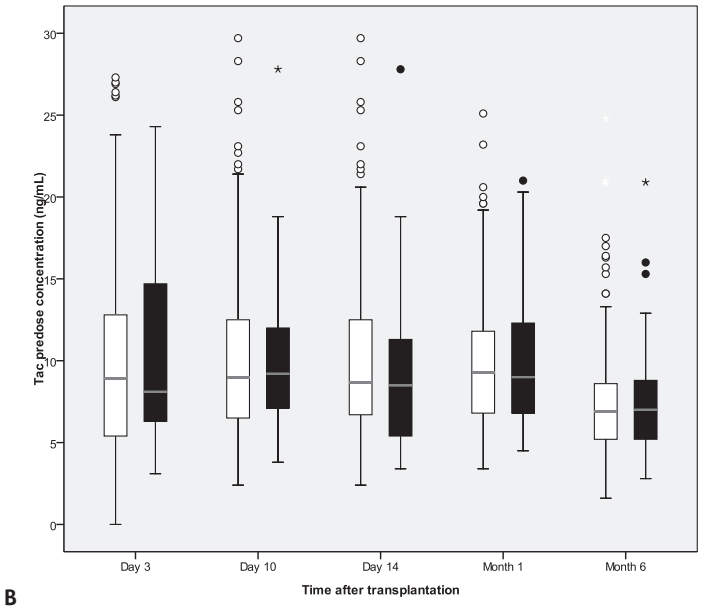

B

Figure 2. A. Boxplots depicting the Tac predose concentrations of all patients at the five different time points after transplantation.
B. Boxplots depicting the Tac predose concentrations of patients experiencing BPAR (black boxes) and patients without BPAR (white boxes) at the five different time points after transplantation.
Bottom, middle, and top lines of each box correspond to the 25th percentile, the 50th percentile (median), and the 75th percentile, respectively. The caps show the 5^{th} and 95^{th} percentiles. The points represent the outliers and the asterisks represent the extreme outliers (more than three times the height of the boxes).

Table 3A. Median Tac predose concentrations and their association with BPAR occurring within the remainder of the first post-transplant year after the Tac concentration measurement.

Post-transplant Time point	Median predose Tac concentration (ng/mL) in patients with BPAR	Median predose Tac concentration (ng/mL) in patients without BPAR
Day 3	Tac: 10.3 [6.5; 17.1; 27.6]Ω n = 135 (61%)¥	Tac: 9.5 [6.0; 14.5; 29.5]Ω n = 1168 (63%)¥
Day 10	Tac: 9.0 [7.0; 11.8; 25.8]Ω n = 92 (85%)¥	Tac: 9.1 [6.6; 12.2; 28.2]Ω n = 1013 (87%)¥
Day 14	Tac: 7.8 [5.6; 10.4; 26.2]Ω n = 65 (72%)¥	Tac: 8.1 [6.2; 11.4; 29.7]Ω n = 722 (62%)¥
Month 1	Tac: 8.7 [5.8; 12.7; 20.2]Ω n = 45 (84%)¥	Tac: 9.7 [7.0; 12.5; 27.6]Ω n = 1050 (90%)¥
Month 6	Tac: 7.5 [6.3; 10.5; 11.0]Ω n = 15 (80%)¥	Tac: 6.8 [5.3; 8.6; 23.6]Ω n = 924 (79%)¥

¥ The percentage of patients of whom the Tac levels were available for analysis at this post-transplant time point. Tac concentrations were related to BPAR occurring after the date of the Tac concentration measurement.
Ω The numbers show the 25th percentile, 75th percentile and the range respectively. For all comparisons no statistically significant differences were found, all p-values were > 0.05.

Table 3B. Median Tac predose concentrations and their association with BPAR occurring within 1 month after the Tac concentration measurement.

Post-transplant Time point	Median predose Tac concentration (ng/mL) in patients with BPAR	Median predose Tac concentration (ng/mL) in patients without BPAR
Day 3	Tac: 11.1 [6.3; 10.5; 11.0]Ω N = 60 (66%)¥	Tac: 9.5 [6.3; 10.5; 11.0]Ω N = 1212 (62%)¥
Day 10	Tac: 9.0 [6.3; 10.5; 11.0]Ω N = 51 (86%)¥	Tac: 9.1 [6.3; 10.5; 11.0]Ω N = 1047 (87%)¥
Day 14	Tac: 8.5 [6.3; 10.5; 11.0]Ω N = 24 (71%)¥	Tac: 8.1 [6.3; 10.5; 11.0]Ω N = 1209 (62%)¥
Month 1	Tac: 8.0 [6.3; 10.5; 11.0]Ω N = 7 (71%)¥	Tac: 9.7 [6.3; 10.5; 11.0]Ω N = 1206 (90%)¥
Month 6	Tac: 7.4 [6.3; 10.5; 11.0]Ω N = 5 (100%)¥	Tac: 6.8 [6.3; 10.5; 11.0]Ω N = 1178 (79%)¥

¥ The percentage of patients of whom the Tac levels were available for analysis at this post-transplant time point. Tac concentrations were related to BPAR occurring after the date of the Tac concentration measurement.
Ω The numbers show the 25th percentile, 75th percentile and the range respectively. For all comparisons no statistically significant differences were found, all p-values were > 0.05.

in the high-risk group *vs.* the low-risk patients [95% CI: 1.3 - 2.8; $p < 0.05$]. First we analysed the Tac concentrations at the different time points for the high-risk group *vs.* the low-risk group. At all the time points the median Tac predose concentrations were not statistically significantly different between the high and low risk groups. We further analysed the Tac concentrations at the different time points within the high and low risk group separately, as shown in Table 5. Again no significantly differences could be found

Table 4. Numbers of patients with Tac concentrations below or above 5 ng/mL (Table 4A) and numbers of patients with Tac concentrations below or above 10 ng/mL (Table 4B) at 5 post-transplant time points, and incidence of BPAR in these patients following that time point.

4A. Tac predose concentrations < / > 5 ng/mL.

Time point	Tac < 5ng/mL	BPAR	Tac > 5ng/mL	BPAR	*P* -value
Day 3	146	10 (6.8%)	671	73 (10.9%)	0.14
Day 10	129	7 (5.7%)	962	71 (7.4%)	0.42
Day 14	92	8 (8.7%)	677	39 (5.8%)	0.27
Month 1	86	2 (2.3%)	1002	36 (3.6%)	0.54
Month 6	185	2 (1.1%)	751	10 (1.3%)	0.79

4B. Tac predose concentrations < / > 10 ng/mL.

Time point	Tac < 10ng/mL	BPAR	Tac > 10ng/mL	BPAR	*P* -value
Day 3	426	40 (9.4%)	391	43 (11%)	0.48
Day 10	619	49 (7.9%)	472	29 (6.7%)	0.26
Day 14	495	32 (6.5%)	274	15 (5.5%)	0.58
Month 1	573	22 (3.8%)	515	16 (3.1)	0.58
Month 6	797	9 (1.1%)	139	3 (2.2%)	0.32

between the patients that developed BPAR and patients without BPAR for the low (Table 5A) as well as for the high risk patients (5B).

We have changed the Tac levels that were higher than 30 ng/mL (24 measuring points in total) into missing values, to prevent that non predose Tac concentrations would be included in the analysis. However, we have also performed the analysis with all the Tac levels (including the ones that were higher than 30 ng/mL), but the results did not change (data not shown).

Explaining BPAR

Next to the Tac predose concentrations, in the univariate analysis, induction therapy, HLA mismatches, DGF, PRA and number of transplants were tested with the occurrence of BPAR within one year after transplantation. Of all 1304 patients 68% used induction therapy, and 9.6% of these patients suffered from a BPAR whereas this percentage was 12.3% in patients who did not use induction therapy after transplantation ($p = 0.13$). We also correlated the incidence of BPAR and the mean Tac concentration of day 3 to day 14 only within patients that did not use induction therapy. The Tac concentration in this group was not statistically different between patients with BPAR and patients without BPAR ($p = 0.53$). To test the influence of HLA mismatching we divided the group into patients that had 0-3 HLA mismatches *vs.* patients that had more than 3 HLA mismatches. There was a significant correlation between the number of HLA mismatches and the oc-

Table 5. Median Tac predose concentrations at different time points after transplantation in patients with BPAR and in patients without BPAR divided into low (5A) and high risk patients (5B).

5A. Low Risk patients.

	LOW-RISK PATIENTS (n = 499) (Total BPAR incidence: 36/499 (7,2%))		
Post-transplant Time point	**Median predose Tac concentration (ng/mL) in patients with BPAR**	**Median predose Tac concentration (ng/mL) in patients without BPAR**	***P*-value**
Day 3	Tac: 10.3 [6.3; 10.5; 11.0]Ω n = 17¥	Tac: 10.1 [6.3; 10.5; 11.0]Ω n = 269¥	0.46
Day 10	Tac: 9.5 [6.3; 10.5; 11.0]Ω n = 20¥	Tac: 9.0 [6.3; 10.5; 11.0]Ω n = 394¥	0.68
Day 14	Tac: 8.9 [6.3; 10.5; 11.0]Ω n = 11¥	Tac: 7.9 [6.3; 10.5; 11.0]Ω n = 302¥	0.73
Day 3- day 14	Tac: 9.2 [6.3; 10.5; 11.0]Ω n = 36¥	Tac: 9.2 [6.3; 10.5; 11.0]Ω n = 454¥	0.63
Month 1	Tac: 10.1 [6.3; 10.5; 11.0]Ω n = 10¥	Tac: 9.3 [6.3; 10.5; 11.0]Ω n = 421¥	0.64

¥: The patients from whom the Tac levels were available for analysis at this post-transplant time point. For month 6 after transplantation the number of patients was too low to perform the analysis and this time point is therefore excluded from the analysis. ΩThe numbers show the 25th percentile, 75th percentile and the range respectively.

5B. High Risk patients.

	HIGH-RISK PATIENTS (n = 786) (Total incidence of BPAR: 100/786 (12,7%))		
Post-transplant Time point	**Median predose Tac concentration (ng/mL) in patients with BPAR**	**Median predose Tac concentration (ng/mL) in patients without BPAR**	***P*-value**
Day 3	Tac: 10.6 n = 66¥	Tac: 9.4 n = 454¥	0.26
Day 10	Tac: 8.7 n = 58¥	Tac: 9.1 n = 600¥	0.98
Day 14	Tac: 7.8 n = 36¥	Tac: 8.1 n = 402¥	0.28
Day 3- day 14	Tac: 9.1 n = 99¥	Tac: 9.3 n = 673¥	0.63
Month 1	Tac: 8.7 n = 28¥	Tac: 9.9 n = 614¥	0.24

¥: The patients from whom the Tac levels were available for analysis at this post-transplant time point. For month 6 after transplantation the number of patients was too low to perform the analysis and this time point is therefore excluded from the analysis. ΩThe numbers show the 25th percentile, 75th percentile and the range respectively.

currence of BPAR. In patients with more than 3 HLA mismatches 12.3% had BPAR *vs.* 8.9% in patients that had 0-3 HLA mismatches (p = 0.046). Also for DGF we found a significant correlation with the occurrence of BPAR (19.7% in patients with DGF *vs.* 8.3 % in those without DGF), p < 0.001). The PRA status was not significantly related to the development of BPAR. PRA was separated into patients that had a PRA < 15% and patients with a PRA

> 15%, in the first group 10.5% developed BPAR and in the last group 8.6% (p = 0.54). We have also studied the development of BPAR within patients that had a first kidney transplantation and compared this to patients that had one or more transplants before. Patients who had been transplanted before had a higher risk of developing BPAR (17.9%) compared with patients who received their first kidney allograft (9.9%; p = 0.021). The variables are listed in Table 6A. Because of the different designs of the studies we have also tested the incidence of BPAR within the different studies (Symphony, Opticept and FDCC). The patients in the Opticept trial suffered significantly less from a BPAR than in the other studies (7.5% vs. 12.2% (Symphony) and 13.1% (FDCC); p = 0.01.

In order to exclude the possibility that some of the other factors associated with the incidence of BPAR have confounded the relationship between Tac concentrations and BPAR we have adjusted for observed confounders and we performed a multivariate analysis which included these variables, as well as the Tac concentrations. Multivariate analysis demonstrated that only DGF and the use of induction therapy were independently correlated to BPAR, with an odds ratio of 2.7 [95%-CI: 1.8 - 4.0; p < 0.001] for DGF, and 0.66 [95%-CI: 0.44 - 0.99; p = 0.049] for the use of induction therapy. The other

Table 6A. Other variables related to BPAR (univariate analysis).

	Patients (%)	**Patients with BPAR (%)**	***P*-value**
DGF	18.3	19.7	< 0.001
No DGF	81.7	8.3	
HLA mismatches > 4	46	12.3	
HLA mismatches < 4	54	8.9	0.046
Number transplantation > 1	6.4	17.9	0.021
Number transplantation = 1	93.6	9.9	
PRA > 15%	13.8	8.6	0.54
PRA < 15%	86.2	10.5	
Induction therapy	68	9.6	0.13
No Induction therapy	32	12.3	

DGF = Delayed graft function; PRA = panel reactive antibody

Table 6B. Multivariate analysis.

	OR (95% CI)	***P*-value**
DGF	2.7 (1.8 – 4.0)	0.0001
Induction	0.66 (0.44 – 0.99)	0.049
Mean Tac concentration day 3 – day 14	0.98 (0.94 – 1.03)	0.48
HLA mismatches < 4	1.47 (1.02 – 2.13)	0.07
Number transplantation > 1	1.71 (0.91 – 3.23)	0.09
PRA > 15%	0.51 (0.17 – 1.53)	0.23

variables, including the Tac predose levels, were not significantly associated with the risk of developing BPAR as shown in Table 6B.

DISCUSSION

We did not find a correlation between the Tac predose concentration measured at 5 time points after transplantation and the occurrence of acute rejection in the period thereafter, within the first post-transplant year. The same was true for BPARs within the first month following the Tac measurement. We investigated a large and heterogeneous study population, and the Tac concentrations measured showed a substantial range, despite rather tight target concentrations defined in the protocols.

The situation for Tac seems to be quite different from mycophenolic acid (MPA). For MPA, a concentration-effect relationship has been shown repetitively[8-9] and for MPA it was also shown that in contrast to patients at low-risk for BPAR for high-risk patients there was a significant difference in the incidence of BPAR depending on the MPA concentrations reached.[7] In the present study, in neither the high-risk nor in the low-risk patients the incidence of acute rejection was dependent on the Tac concentrations. A bit to our surprise the mean Tac concentrations in high risk patients were not different from the Tac concentrations found in the low risk population. We had expected that physicians responsible for dosing Tac would aim for higher Tac concentrations in patients considered to be at presumed higher risk for BPAR, and that they would allow for lower concentrations in patients with a lower risk of rejection. Also in the multivariate analysis the Tac concentrations did not surface as predictor for BPAR.

TDM is generally considered to be required for managing Tac therapy. Often transplant centers have specified the target concentrations for Tac, depending on time post-transplant, on co-medication and presumed risk of rejection. One would think that for a drug so extensively used the evidence for the optimal Tac concentration would be compelling. We show that this is not the case. In the past 15 years we have seen a substantial change in the target Tac concentrations, with targets as high as 20 ng/mL in the early years, and with targets as low as 3-7 ng/mL in the Symphony study. This change in target concentrations was largely reached empirically, and there is only limited evidence for the different targets. This does not imply that TDM for Tac is useless. Without TDM the large between-patient variability in Tac pharmacokinetics would go unnoticed, and extremes in Tac exposure would occur, exposing some patients to toxic levels and others to very low levels. Based on our analysis however it is not possible to conclude that the Tac target concentrations should be above for example 5 or 10 ng/mL. Possibly the

threshold for efficacy is at a concentration that is even lower than the currently applied targets, and it is possible that only when concentrations reach values as low as 1 or 2 ng/mL the incidence of BPAR starts to increase. The same was suggested by Rodriguez[2] who proposed to further lower the Tac concentration in liver transplantation. They even recommended the regulatory authorities and pharmaceutical industry to change the regulatory drug information for lowering the target levels.

This study is a combined analysis of three large clinical trials, and a large number of kidney transplant recipients was included. In spite of the considerable number of patients studied, we could not show an association between the development of acute rejection in 1 month or 1 year after transplantation and the Tac whole blood concentrations. Also adjusting for confounders in a multivariate analysis the results stayed negative. Recently Capron *et al.*[10] also showed that there is no correlation between Tac whole blood concentrations and rejection after liver transplantation. However, they did find a strong correlation between Tac concentrations within peripheral blood mononuclear cells (PBMCs), the site of action of Tac, and the staging of rejection in liver transplant recipients. However, as indicated above, the currently clinically employed assays measure the Tac concentration in whole blood, which is determined to a large extent by the Tac concentration in the erythrocyte fraction. Tac concentrations in PBMCs are not 1:1 correlated with whole blood (or erythrocyte) concentrations, for example due to the presence of drug transporting enzymes in the cell membranes of PBMCs. Therefore Tac concentration within PBMCs might be a better marker of immunosuppressive efficacy than the whole blood predose concentration. Future studies should study the relationship between intracellular Tac concentrations and rejection risk in kidney transplant recipients in more detail.

A limitation to this study is that donor specific anti-HLA antibodies were not routinely measured, and therefore we have no data on correlations between tacrolimus exposure and DSA. Next to this, we had only access to Tac concentrations drawn at predefined time points. These Tac concentrations might not be the last measured concentration prior to diagnosing BPAR and we cannot exclude the possibility that a similar analysis with the last levels drawn would show an association. However, intrapatient variability of Tac is limited and we do not think that we would have achieved another outcome by using the last levels drawn. Another limitation is that the pre-dose concentrations that were investigated in this study do not adequately reflect the exposure to Tac. Kuypers *et al.* in 2004 showed that in contrast to Tac predose concentrations the Tac area under the concentration curve from 0 to 12 hours [AUC(0-12)] was correlated with clinical efficacy, at different time points after transplantation.[11] However, a good correlation between Tac predose concentrations and AUC has been demonstrated. In clinical practice predose concentrations are the preferred method to monitor Tac treatment.[12] In a multivariate

analysis also Australian investigators[13] did not find a correlation between Tac pre-dose concentrations or Tac AUC and incidence of acute rejection, whereas in their study MPA-AUC was correlated to BPAR.

Another explanation might be that other mechanisms, such as innate immunity, which are not calcineurin driven might play a role in the development of acute rejections. These rejections could not be prevent by the use of calcineurin inhibitors, such as Tac and for these type of rejections it is therefore not useful to aim for a specific Tac target. Although T-cells, inhibited by Tac, have a critical role in acute rejection it is known that there is an upregulation of proinflammatory mediators in the allograft before the T cell response, this is due to innate immunity and it is independent of the adaptive immune system.[14]

In this study we have focused only on efficacy, as the incidence of nephrotoxicity was not prospectively collected. Therefore it is not possible form this study to define the upper threshold for Tac treatment.

In conclusion, we did not find an association between the Tac predose concentrations and the incidence of acute rejection after kidney transplantation. Even though it is generally accepted that TDM is essential to maintain the efficacy of Tac, the analysis in this study does not show that TDM, at the used whole blood target ranges, adds to lowering the risk of acute rejection. We do not want to suggest that TDM for Tac can be abolished, but a more critical perception on the relevance of the presumed optimal target concentrations is recommended.

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2.2

Conversion to once-daily tacrolimus results in increased p38mapk activity in t-lymphocytes of kidney transplant recipients

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ABSTRACT

Background: The once-daily formulation of tacrolimus (TAC_{OD}) has been developed to overcome adherence problems. Conversion from the twice-daily TAC (TAC_{BID}) formulation to TAC_{OD} on a 1:1 basis, however, often leads to a decrease of TAC pre-dose concentrations which averages ~15%. Switching between the two TAC formulations may thus influence drug efficacy and necessitates therapeutic drug monitoring. As an additional tool in transplantation diagnostics, phospho-specific flow cytometry was used to study the biological effects of conversion on p38MAPK phosphorylation levels, a kinase involved in T-lymphocyte activation.

Methods: Stable renal transplant recipients (n=12), at least one year after their transplantation, were converted from TAC_{BID} to TAC_{OD} on 1:1 mg for mg base. Co-medication consisted of mycophenolate mofetil (n=10) and prednisolone (n=3). TAC whole-blood pre-dose concentrations were determined by immunoassay before and 3 months after conversion. P38MAPK phosphorylation levels were measured in T-lymphocytes by whole-blood phospho-specific flow cytometry.

Results: Three months after conversion, no significant decreases in TAC pre-dose concentrations (C_0) were found ($p= 0.674$), while p38MAPK phosphorylation increased with 11.4% ($p<0.05$) in CD4+ and with 15.6% ($p<0.05$) in CD8+ T-lymphocytes. The TAC_{BID} C_0 inversely correlated with p38MAPK levels in T-lymphocytes ($r_s= -0.638$, $p <0.05$).

Conclusions: The results demonstrate that p38MAPK phosphorylation levels can be used as a method to determine the biological effects of conversion from TAC_{BID} to TAC_{OD}. This method can be used as a new tool for detailed TAC drug monitoring.

INTRODUCTION

Therapy with the immunosuppressant tacrolimus (TAC) is routinely monitored by measuring whole-blood pre-dose concentrations (C_0). However, lack of efficacy (*i.e.* the occurrence of acute rejection) or toxicity does occur in solid organ transplant recipients who have TAC concentrations that are considered therapeutic. A better way to perform therapeutic drug monitoring (TDM) of TAC may be to measure the drug's pharmacodynamic effects.

TAC inhibits the calcineurin pathway of activated T-lymphocytes resulting in decreased levels of de-phosphorylated Nuclear Factor of Activated T-lymphocytes (NFAT), less production of the cytokine IL-2, and ultimately, inhibition of T-lymphocyte proliferation. Earlier studies demonstrated that the expression of IL-2 in cell samples can be used as a pharmacodynamic tool for TDM of TAC. However, this assay measures the effects of other immunosuppressive drugs as well, and therefore is not specific for TAC. In addition, this assay is time-consuming, costly and may not reflect to TAC toxicity.[1,2] Thus there is an unmet need of better pharmacodynamic assays to monitor TAC treatment, leading to more customized immunosuppressive therapy.[3]

Apart from its effects on the NFAT pathway, TAC also suppress the phosphorylation of the mitogen activated protein kinase (MAPK) pathway.[4] Phosphorylation concentrations of this signaling molecule were recently found to be inversely correlate with TAC whole-blood C_0 of kidney transplant patients. Furthermore, increased phosphorylated p38MAPK concentrations were associated with a higher T-lymphocyte activation status, which was inhibited by TAC in a dose dependent manner *in vitro*.[4,5]

Here, the effect of conversion from the standard, twice-daily TAC formulation (TAC_{BID}) to the once-daily, prolonged-release TAC formulation (TAC_{OD}) on p38MAPK concentrations in kidney transplant recipients is reported. The novel TAC_{OD} formulation was developed to overcome adherence problems. However, whole-blood TAC C_0 may decrease by 9-15% following 1:1 conversion on a mg for mg basis.[6,7] This may lead to sub-therapeutic TAC exposure and may put certain patients at risk for rejection. We speculated that the present assay may be more sensitive than conventional PK monitoring and may discover subtle changes in drug effects.

PATIENTS AND METHODS

Study design and determination of tacrolimus blood concentrations

All twelve patients reported here (for their characteristics see Table 1) participated in a substudy of a larger clinical trial that was reported previously.[7] The aim of the clinical trial was to study the safety of conversion from TAC_{BID} (Prograf®, Astellas Pharma, Leiden, the Netherlands) to TAC_{OD} (Advagraf®, Astellas Pharma, Leiden, the Netherlands) on a 1:1 (mg:mg) basis. The aim of the substudy, which is presented here, was to investigate the effects of this conversion on P38MAPK phosphorylation status. Heparin blood samples for p38MAPK phosphorylation status were collected 1 day before (visit 1) and 3 months after conversion to TAC_{OD} (visit 2). TAC whole-blood C_0 were determined in whole EDTA blood by using the antibody-conjugated magnetic immunoassay (ACMIA) on a Dimension Xpand analyzer (Siemens HealthCare Diagnostics Inc., Newark, DE) according to the manufacturer's instructions. The lower and upper limits of detection were 1.5 ng/mL and 30 ng/mL, respectively. For calculation purposes, TAC C_0 below the detection limit were set at half the detection limit (0.75 ng/mL). Proficiency samples were obtained from the United Kingdom Quality Assessment Scheme (Dr. Holt, St George's Hospital Medical School, London, UK). The laboratory successfully participates in international proficiency testing schemes.

Table 1. Patient characteristics (n = 12)

	Summary measure
Gender (male/female)	2 (16.7%) / 10 (83.6%)
Age (years)	54.7 (19.6 – 69.7)*
Time from transplantation to conversion (years)	4.92 (1 – 12)*
Number of subjects with concomitant MMF therapy	10 (83.3%)
Number of subjects with concomitant steroid therapy	3 (17.6%)
Tac dose (mg/day)	4.3 (1.5 – 10)*
Tac C_0 before conversion (ng/mL)	5.6 (0.75 – 9.8)*

*mean (range)

Whole-blood phospho-specific flowcytometry

P38MAPK phosphorylation was measured according to the manufacturer's instructions and as described previously.[4] In brief, 200 µl of heparinized blood was activated with PMA/Ionomycin (1.6 µM/ 10 µg/mL, Sigma-Aldrich, Steinheim, Germany) and stained with APC-labelled mouse anti-human CD3, Pacific Blue mouse anti-human CD4 and PE-Cy7 mouse anti-human CD8 (BD Biosciences, San Jose, CA) for 30 minutes at 37˚C. Then cells were fixed for 10 minutes with Lyse/fix buffer and treated with permeabilization buffer III (both from BD biosciences) at -20˚C. Samples were stained with the fluorochrome-conjugated mAb PE mouse anti-p38MAPK (clone pT180/pY182, BD Biosciences) for 30

minutes at room temperature and analysed on a FACS Canto II flow cytometer (BD Biosciences). Isotype control IgG1-PE (clone X40, BD Biosciences) and FMO control tubes were included. Interday-variability of the flowcytometer was corrected by using Quantibrite PE beads (BD Biosciences) according to the manufacturer's instructions.

Data analysis and statistics

P38MAPK phosphorylation concentrations were calculated as the Median Fluorescence Intensity (MFI) and normalized using Quantibrite-PE beads. Data and statistical analysis was performed with diva-version 6.0 software (BD Biosciences) and Graph Pad Prism 5.0 (Graph Pad Software Inc., La Jolla, CA) by using paired T test (for p38MAPK phosphorylation after performing log transformation). Spearman tests were used to calculate the correlation between TAC C_0 and p38MAPK phosphorylation. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Conversion from TAC_{BID} to TAC_{OD} resulted in a decrease in C_0 of 4.18% (Figure 1A), which was not statistically significant (p= 0.674). Before conversion the median TAC C_0 was 6.0 ng/ml (range: 0.75 - 9.8 ng/ml); after conversion the median TAC C_0 was 5.4 ng/ml (range: 3.0 – 7.1 ng/ml).

A typical example of induced p38MAPK phosphorylation in CD3+, CD3+CD4+ and CD3+CD8+ T-lymphocytes, respectively is shown in Figure 1B. The MFI levels of p38MAPK increased after stimulation with PMA/Ionomycin.

After conversion to TAC_{OD} p38MAPK phosphorylation concentrations increased significantly, both in CD3+CD4+ (11.4% increase, p =0.034) and CD3+CD8+ T-lymphocytes (15.6% increase, p =0.038, figure 1C).

Next, phosphorylation concentrations were correlated to the TAC C_0. A significant inverse correlation between TAC C_0 and p38MAPK phosphorylation in CD3+ T-lymphocytes was found for TAC_{BID} (r_s = -0.638, p <0.05, Figure 1D), but not for TAC_{OD} (r_s = -0.375, p=0.230, Figure 1D).

DISCUSSION

P38MAPK phosphorylation potential increases significantly after 1:1 conversion from TAC_{BID} to TAC_{OD}, despite an unchanged TAC whole-blood C_0. This observation suggests that measuring p38MAPK phosphorylation is a more sensitive method to measure TAC therapy compared with conventional pharmacokinetic TDM.

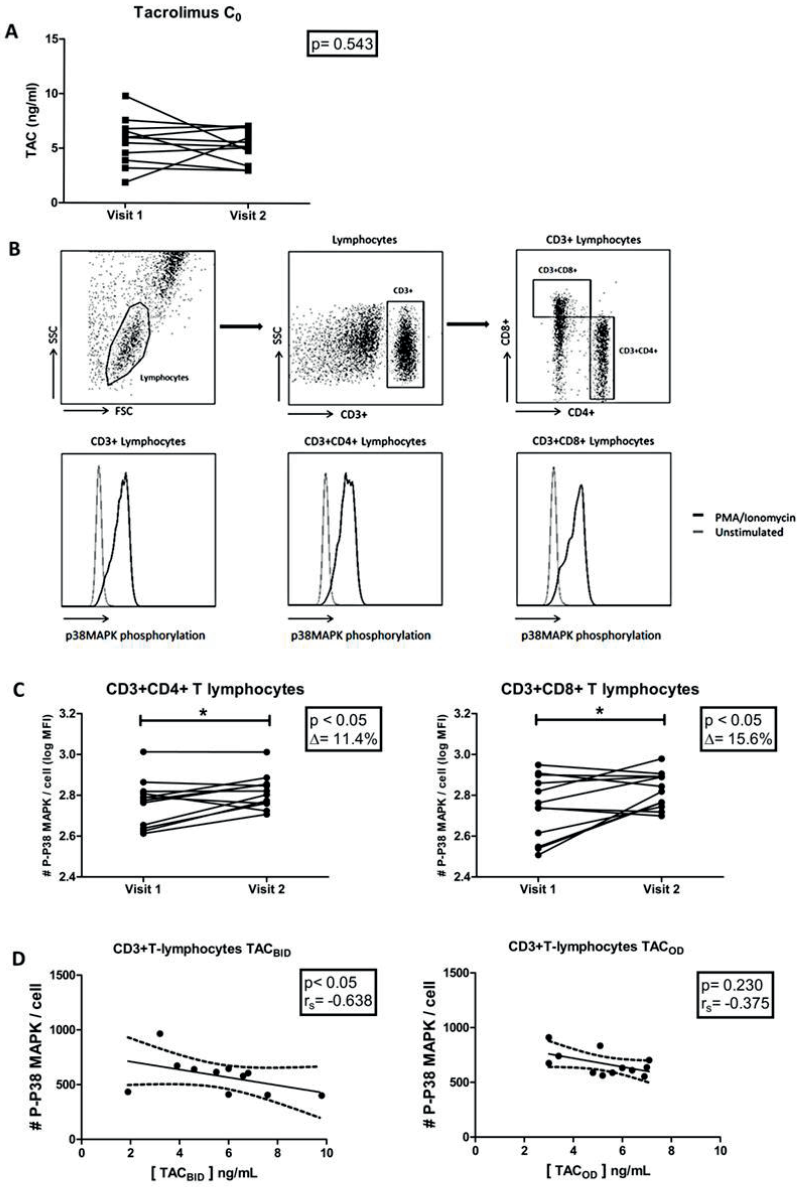

Figure 1. Decreased Tac blood C_0 after conversion from TAC_{BID} to TAC_{OD} lead to increased concentrations of p38 phosphorylation.

A) Tac C_0 in kidney transplant patients before and 3 months after conversion from TAC_{BID} to TAC_{OD} **B)** Dot plots demonstrating the selection of lymphocytes and the CD3+CD4+ and CD3+CD8+ lymphocytes subsets from whole blood samples. The histograms show the p38 phosphorylation for each subset after PMA/Ionomycin stimulation of whole blood for 30 min. **C)** P38 phosphorylation per cell before and 3 months after conversion from TAC_{BID} to TAC_{OD} in CD3+CD4+ (left panel) and CD3+CD8+ (right panel) T-lymphocytes. **D)** Correlation between p38 phosphorylation per cell and TAC_{BID} C_0 (left panel) or TAC_{OD} C_0 (right panel) in CD3+ T-lymphocytes. FSC, forward scatter; SSC, side scatter (n=12).

In this study whole-blood single-cell phospho-specific flowcytometry was used to measure p38MAPK phosphorylation at the single cell level. This technique was earlier used by Nolan et al. who investigated the consequences of growth factor treatment on the profiles of cancer cell signaling networks of T-lymphocytes in tumor immunology.[8] Measuring the p38MAPK phosphorylation is an advantage over the classical pharmacodynamical parameter IL-2 as the phosphorylation concentration in contrast to cytokine concentrations shows the upstream effects in the signaling cascade and consequently may be better linked to clinical outcomes specific for TAC therapy. In addition, phosphorylation of molecules is a rapid process and can be measured with single-cell phospho-specific flow cytometry in whole-blood T-lymphocyte subsets within hours.[4,9]

It has been reported that TAC_{OD} has the same safety and efficacy profile as TAC_{BID}.[10-13] However, these observations were made in a group of patients whereby the clinical outcome for an individual person can be different. Furthermore, the use of TAC_{OD} has been associated with clinical benefit, including improved adherence, a flatter PK profile and better glycemic control.[14] Nonetheless, C_0 may drop considerably after conversion from TAC_{BID} on a 1:1 basis in individual patients and close monitoring of TAC exposure after switching is recommended.[14-16] In the conversion trial, of which the present study was a sub-study, one patient did indeed experience a late acute cellular rejection that was associated with a marked drop in TAC exposure: TAC C_0 decreased from 6,9 ng/ml immediately before conversion to 3,6 ng/ml shortly after conversion.[7]

Increased concentrations of p38MAPK phosphorylation have been associated with more T-lymphocyte activation[17] and consequently may in theory result in a higher risk of acute cellular rejection for kidney transplant patients.[18] Despite this non-significant change in TAC exposure, a significant 11.8% increase in p38MAPK phosphorylation expression was observed demonstrating the high sensitivity of this assay. The present finding suggests that conversion to TAC_{OD} alters exposure to the drug and that this may translate into a change in biologic effect which is not detected by routine C_0 monitoring. Although it could be better to measure the AUC instead of C_0, this will also not show the direct biological effects of a drug.

In addition, these results suggest that both CD4+ and CD8+ T-lymphocytes are less suppressed in their activation potential after conversion. This could indicate that patients have a higher risk for rejection after conversion. In order to test the usefulness of the current phospho-specific flow cytometry assay in the assessment of rejection risk, phosphorylation concentrations of peripheral blood samples from patients with biopsy proven rejection should be compared to samples from patients without rejection.

A limitation of this study is that TAC C_0 was measured with an immunoassay, which not only measures the parent compound but also cross-reacts with several TAC metabolites. Nonetheless, although measurement of TAC concentrations by LC-MS has increased, many transplant centers throughout the world still rely on immunoassays. Second, we tested only a limited number of patients. Therefore, the present findings should be interpreted with caution and be considered as hypothesis-generating.

In the future, it can be interesting to determine the risk of rejection during TAC therapy in kidney transplant patients with the help of phospho-specific flow cytometry. This pharmacodynamical approach could detect the risk of rejection in a more sensitive way than the standard pharmacokinetic method. Furthermore, the assessment of the p38MAPK phosphorylation concentrations is a fast method for therapeutic drug monitoring of TAC and can be used to consider dose adjustment during therapy, although there is no significant difference observed in C_0. This will lead to a more personalized TAC therapy for kidney transplant patients.

Other signaling proteins that are members of the MAPK pathway, such as ERK, can be included in the analysis to find the optimal biological marker for patient monitoring after TAC conversion. Furthermore, it will be essential to start a longitudinal study to investigate the long-term effects of TAC conversion on p38MAPK phosphorylation to assess the phosphorylation concentrations change over time and to define if this change is related to a higher risk for rejection.[12]

CONCLUSION

Conversion from TAC_{BID} to TAC_{OD} is associated with a significant increase in p38MAPK phosphorylation concentrations which was not reflected by TAC whole-blood exposure as determined by C_0. Measurement of p38MAPK phosphorylation status may be a more sensitive way to assess the biological effects of TAC.

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Chapter 3

Predicting tacrolimus dose requirements

3.1

Pre-transplant tacrolimus dose requirements predict post-transplant dose requirements in blood group AB0 incompatible kidney transplant recipients

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ABSTRACT

Background: The aim of this study was to investigate whether pre-transplant tacrolimus (Tac) dose requirements of patients scheduled to undergo living donor kidney transplantation correlate with post-transplantation dose requirements.

Method: The predictive value of Tac dose requirements (defined as the ratio of the Tac predose concentration, C_0, divided by the total daily Tac dose, D) pre-transplantation on this same parameter post-transplantation was assessed retrospectively in a cohort of 57 AB0-incompatible kidney transplant recipients. These patients started immunosuppressive therapy 14 days before transplant surgery. All patients were using a stable dose of glucocorticoids and were at steady-state Tac exposure before transplantation.

Results: Tac dose requirements immediately before transplantation (C_0/D_{before}) explained 63% of the Tac dose requirements on day 3 post-transplantation: $r^2 = 0.633$ (F (1, 44) = 75.97, p <0.01). No other clinical and demographic variables predicted Tac dose requirements early after transplantation.

Conclusion: Steady-state Tac dose requirement before transplantation largely predicted post-transplantation Tac dose requirements in AB0-incompatible kidney transplant recipients. The importance of this finding is that the post-transplantation Tac dose can be individualized based on a patient's pre-transplantation Tac concentration/dose ratio. Pre-transplant Tac phenotyping therefore has the potential to improve transplantation outcomes.

Keywords: Dose requirement, kidney transplantation, tacrolimus.

INTRODUCTION

In many transplant centers, the starting dose of tacrolimus (Tac) is based on a patient's bodyweight. Following the first few dosages, whole-blood Tac concentrations are measured and followed by dose-adjustments in order to reach and maintain the targeted predose concentration (C_0).[1,2] Therapeutic drug monitoring (TDM) is currently the only way to prevent under- or overexposure to Tac. However, this approach is based on `trial and error´ and has no predictive value.[3] In fact, in the early phase after transplantation, many patients have Tac concentrations outside the target range, putting them at risk for rejection or toxicity.[2]

Knowledge of the pharmacokinetics (PK) of Tac in a patient obtained before transplantation may be helpful in personalizing Tac therapy. Several investigators have attempted to predict an individual's response to calcineurin inhibitor (CNI) treatment after transplantation. Lindholm *et al.* evaluated ciclosporin (CsA) PK in patients before kidney transplantation by giving them a single oral and intravenous dose. In this study, pre-transplant bioavailability and clearance of CsA correlated poorly with post-transplant CsA exposure.[4] Likewise, Boots *et al.* observed that there is a poor correlation between Tac exposure after oral administration of a single dose prior to surgery and the required Tac dose after transplantation.[5] Finally, Campbell *et al.* found no difference in the proportion of patients reaching the target Tac C_0 (>10 ng/mL) in the first days post-transplantation when dosing was either based on bodyweight or the pre-transplant 2-hour post-dose (C_2) concentration.[6]

These poor correlations between pre-transplant and post-transplant CNI exposure may be explained by post-operative changes in intestinal motility (*e.g.* ileus), hepatic metabolism, and diet. They may also be explained by the fact that in all above-mentioned studies, PK parameters were assessed after single-dose administration and the patients were therefore not in steady-state. Finally, the use of interacting medication such as glucocorticoids and antibiotics, may have obscured a correlation between pre- and post-transplant PK.[7]

In our transplant program, Tac treatment is not started prior to transplantation except in patients scheduled to receive a blood group AB0-incompatible (AB0i) kidney transplant. In this population, treatment with Tac, mycophenolate mofetil (MMF), and prednisolone is routinely started two weeks before transplant surgery and TDM is performed during this time. These patients are thus in steady-state at the time of surgery and the effects of glucocorticoids on Tac PK may not change much after transplantation.

The primary aim of this study was to investigate whether pre-transplant Tac dose requirements in AB0i kidney transplant recipients predicts post-transplant dose requirements. The secondary aim was to assess the incidence of immunosuppressive drug-related adverse events (AEs) prior to transplantation.

PATIENTS AND METHODS

The AB0i kidney transplant program of the Erasmus MC started in 2006. The clinical results of this program were reported previously.[8] For the present retrospective study, all consecutive patients who received an AB0i kidney transplant up until May 29th, 2013 were included. Data were retrospectively collected. Patients needed to be an adult (≥18 yrs.) and to receive an AB0i kidney. Patients who used Tac interacting drugs at time of initiation of immunosuppressive (14 days before transplantation) therapy were excluded from the study.

Immunosuppressive regimen

Our protocol for AB0i kidney transplantation was published recently.[8] In brief, patients were treated as follows:

Before transplantation: All patients received a single-dose of rituximab (Mabthera®, Roche Pharmaceuticals, Nutley, New Jersey, U.S.A.) intravenously at a dose of 375 mg/m^2 body surface area, four weeks before the scheduled transplant surgery. Two weeks before transplantation, treatment with Tac (Prograf; Astellas Pharma, Leiden, The Netherlands) with a starting dose of 0.1 mg/kg (based on actual body weight) twice daily with a dosing interval of 12 hours, MMF (Cellcept®, Roche Pharmaceuticals; 1000 mg bid), and prednisolone 20 mg once daily were started. Tac was targeted to a C_0 of 10-20 ng/mL. The day before transplantation, after the last immunoabsorption, all patients received 0.5 g/kg human immunoglobulin intravenously (Nanogam®, Sanquin, Amsterdam, the Netherlands).

After transplantation: On days 0, 1, and 2 all patients received prednisolone intravenously at a dose of 50 mg twice daily. Thereafter, patients received 20 mg of prednisolone daily for the first two weeks, and subsequently prednisolone was tapered to 5 mg at month 3. Treatment with Tac was continued aiming for a C_0 of 10-20 ng/mL (weeks 1 and 2) and 5-12 ng/mL, thereafter. MMF was continued at a dose of 1000 mg twice daily for the first two weeks post-transplant.

Immunoabsorption: Immunoabsorption was performed using a specific adsorption column for anti-A or anti-B antibodies (Glycorex Transplantation AB, Lund, Sweden) aiming for an anti-donor blood group IgG antibody titer of <1:8 the day before transplantation. The number of immunoabsorptions ranged from 0 to 7 depending on the titer of anti-donor blood group antibodies and the rebound after every session.[8]

Pharmacokinetic and Efficacy endpoints

In all patients, Tac dose and Tac C_0 at days -7 and -1 (before) and days 3, 7, 10, 14, and 30 (after transplantation) were retrospectively collected. Dose-corrected Tac C_0 (hereafter "dose requirement") was calculated by dividing C_0 by the corresponding Tac dose (mg/day).

Immunosuppressive drug-related AEs

Adverse events considered to be related to the use of immunosuppression before transplantation were a) any infection; b) diarrhea or other gastrointestinal side effects not otherwise explained; and c) neurologic events including headache and tremor.

Therapeutic drug monitoring

Pre-transplantation Tac C_0 was measured starting 4-7 days after the initiation of treatment. Tac C_0 was determined after at least 5 unchanged Tac doses (the mean half-life of Tac is 12 hrs),[9] and were therefore considered to represent steady-state concentrations. After transplantation, Tac C_0 was routinely measured three times weekly. The attending physicians could change the daily Tac dose based on the achieved Tac C_0 and/or the clinical situation of the individual patient. No Bayesian prediction tools were used.

Tac C_0 values were determined throughout the study period in whole-blood by use of the ACMIA-Flex assay (Siemens HealthCare Diagnostics, Inc., Newark, DE) on a Dimension XPand (Siemens HealthCare Diagnostics, Inc, Newark, DE). The upper and lower detection limits of this assay were >30 and <1.5 ng/mL, respectively. When a Tac concentration of <1.5 ng/mL was measured, a value of 0.75 ng/mL was imputed (half the lower detection limit); Tac concentrations of >30 ng/mL were set at 30 ng/mL for calculation purposes.

Statistical analysis

Statistical analyses were performed using Statistical Program of Social Sciences version 20 (SPSS Inc., Chicago, Ill., USA). Data distribution was assessed by the Kolmogorov-Smirnov test. As the distribution was mostly skewed, the log-transformed data were used for the statistical analysis. For reasons of clarity, however, the data are presented as median (range) in the results section. Comparison of proportions of patients who were within the target Tac range before (day -1) and after transplantation was undertaken using Chi-squared or Fisher's exact tests, as appropriate. The differences in PK parameters between time points were analyzed with one-way repeated measurements ANOVA analysis. Correlation between pre- and post-transplant Tac dose requirements was analyzed by calculating the bivariate Pearson correlation coefficient *r*. The predictive value of pre-transplant Tac dose requirements was then analyzed by multiple linear regression analysis in which several other clinical variables were included. Statistical significance was defined as a 2-tailed $p \leq 0.05$.

RESULTS

A total of 60 patients received an ABOi renal transplant between March 2006 and May 2013. For the present analysis, 57 patients were included and 3 were excluded. One patient was treated with CsA rather than Tac, and in two patients, no pre-transplantation Tac C_0 were available. The patient characteristics are presented in Table 1. The patient population was predominantly Caucasian (86.0%) and male (61.4%). Their median age at the time of transplantation was 57 years.

Table 1. Patient characteristics.

	Number of subjects (%)
Gender:	
Male / Female	35 (61.4) / 22 (38.6)
Age at the time of transplantation (yr):	57 (19-74)
Ethnicity:	
Caucasian	49 (86.0)
Black	4 (7.0)
Asian	3 (5.3)
Other	1 (1.8)
Primary kidney disease:	
Polycystic kidney disease	13 (22.8)
Glomerulonephritis	11 (19.3)
Hypertensive nephropathy	11 (19.3)
Reflux disease / Chronic pyelonephritis	4 (7.0)
Diabetic nephropathy	3 (5.3)
Other	12 (21.1)
Unknown	3 (5.3)
Number of kidney transplantation:	
1^{st} / 2^{nd} / 3^{rd}	48 (84.2) / 8 (14.0) / 1 (1.8)
Donor type:	
Living-Related / Living-Unrelated	22 (38.6) / 35 (61.4)
Blood group acceptor:	
A / B / AB / 0	9 (15.8) / 9 (15.8) / 1 (1.8) / 38 (66.7)
Blood group donor:	
A / B / AB	33 (57.9) / 14 (24.6) / 10 (17.5)
Renal replacement therapy:	
Hemodialysis	22 (38.6)
Peritoneal dialysis	21 (36.8)
Pre-emptive	14 (24.6)

Tac treatment was started on day -14 ± 2 before transplantation in 54 (94.7%) subjects. In two patients (3.5%) immunosuppressive treatment was also started 14 days before the scheduled transplantation date but due to medical reasons, surgery was postponed, leading to a longer use of immunosuppressive drugs in the period before transplantation (26 and 28 days; see below). In one case, immunosuppressive therapy was started 19 days before transplantation.

A total of 156 C_0 were measured between day -14 and 0, of which 119 were steady-state concentrations. Because most steady-state C_0 were measured approximately one week and one day before transplantation, only days -7 ± 3 and day -1 were analyzed for this study. Steady-state C_0 values were available at these two time points for 39 and 53 patients, respectively.

Table 2 depicts the PK parameters pre- and post-transplantation. Compared with the pre-transplant value on day -1, a significant increase in Tac C_0 and C_0/D at day 3 post-transplantation was observed: 12.4 *vs.* 15.4 ng/mL (24.0% change) and 1.17 *vs.* 1.28 ng/mL per mg/kg per day (9.4% change), respectively (both $p < 0.01$). The Tac daily dose remained stable between day -1 and day 3. There was a significant decrease in the daily dose of Tac, Tac C_0, and C_0/D by day 30 compared with the early post-transplant phase (Table 2). Figure 1 shows the individual changes in Tac C_0/D before transplantation and on the third day after transplantation. Despite the occurrence of a statistically significant increase in the median C_0/D, dose requirements did not change to a clinically relevant degree. In fact, as can be seen from Figure 1, two patients were outliers (C_0/D >1.5 interquartile range (IQR)). When these patients were excluded, the change in the Tac dose requirements was no longer significant.

Table 2. Dose and concentration data pre- (days -7 and -1) and post-transplantation. All values are expressed as median (range).

	Day (-7) n = 39	Day (-1) n = 53	Day (3) n = 50	Day (7) n = 42	Day (10) n = 50	Day (14) n = 49	Day (30) n = 57
Dose (mg/day)	12.0 (2.0-28.0)	12.0 (4.0-20.0)	12.0 (3.0-28.0)	12.0 (0.0-30.0)	12.0 (1.5-34.0)	12.0 (2.0-34.0)	10.0 (2.5-36.0)*
C_0 (ng/mL)	13.6 (0.8-30.0)	12.4 (5.2-30.0)	15.4 (7.1-30.0)**	11.7 (3.9-30.0)***	14.0 (1.7-28.7)	13.9 (4.6-25.5)	11.8 (4.4-29.4)***
C_0/D (ng/mL per mg/day)	1.10 (0.05-3.40)	1.17 (0.34-3.20)	1.28 (0.44-5.85)¥	1.12 (0.37-6.13)¥¥	1.13 (0.28-6.40)	1.08 (0.29-6.45)	1.25 (0.29-4.53)¥

* significant decrease compared with day -1 $p < 0.01$ and day 3 $p < 0.01$
** significant increase compared with day -1 $p < 0.01$
*** significant decrease compared with day 3 $p < 0.01$
¥ significant increase compared with day -1 $p < 0.01$
¥¥ significant decrease compared with day 3 $p < 0.05$

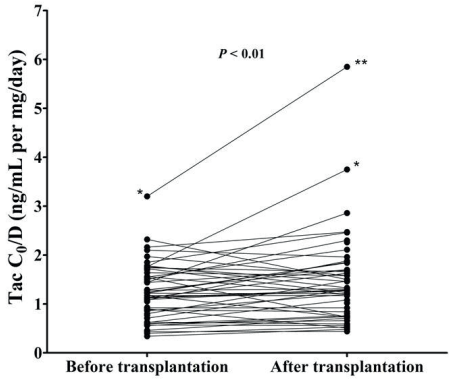

Figure 1. The individual changes of Tac C_0/D before and after transplantation. The single asterisks represent the outliers (more than 1.5 tomes the IQR) and the double asterisks represent the extreme outliers (more than 3 times the IQR).

On the day before transplantation, 30 (52.6%) patients were within the target Tac C_0 range of 10-20 ng/mL. Fifty-three percent (n = 16) of these patients remained so at day 3 after transplantation, whereas 30.0% (n = 9) were above and 3.3% (n = 1) were below the Tac target C_0 range. For 13.3% (n = 4) of patients Tac C_0 values were not available on day 3 after transplantation. Overall, there were 34 (59.6%) patients who were on target at day 3 after transplantation. The proportion of patients on target before transplantation (day -1) was not different from that on day 3 after transplantation (χ^2 = 0.081 (n = 46); p = 0.77; phi = -0.09).

Correlation between pre- and post-transplantation Tac exposure

Tac dose-corrected C_0 on the day before transplantation (C_0/D_{before}) had a significant influence on the Tac C_0/D measured on day 3 after transplantation. The correlation between Tac C_0/D on days -1 and 3 was substantial (r = 0.80; p <0.01). The coefficient of determination (r^2) was 0.633 (F (1, 44) = 75.97, p < 0.01). Therefore, 63.3% of the Tac dose requirements on day 3 after transplantation was explained by C_0/D_{before} (Figure 2). Tac C_0/D on day -1 also correlated with that on day 7 (r^2 = 0.76; p < 0.01). With increasing time after transplantation this correlation became less substantial, although it remained significant: r^2 = 0.45, 0.52, and 0.42 (day -1 *vs.* days 10, 14, and 30 after transplantation, respectively; all p < 0.01). Of the 340 samples analyzed only 5 had a Tac concentration >30.

Age, gender, bodyweight, creatinine clearance, hematocrit and albumin concentration (measured on the day before transplantation) did not correlate with Tac C_0/D post-transplantation.

Previous research suggested that Tac PK is influenced by hematocrit and albumin concentration.[10] Therefore, it was decided to perform multiple regression analysis incorporating hematocrit and albumin concentrations despite the fact that correlation analyses showed

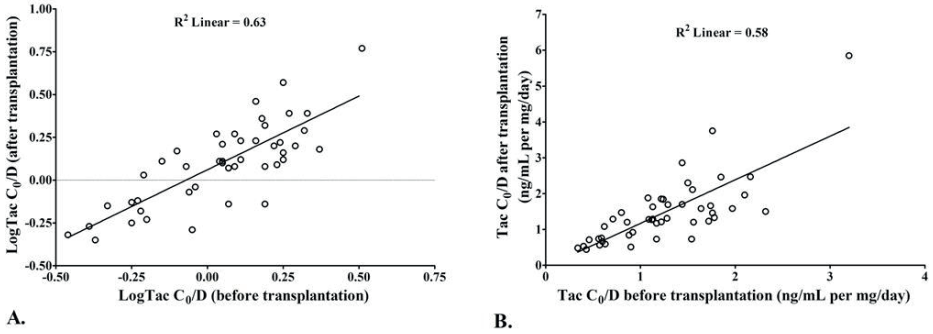

Figure 2. Log-transformed Tac dose-corrected C_0 (C_0/D) on day -1 before transplantation *vs.* log-transformed Tac C_0/D on day 3 after transplantation. The slope of the regression line is not zero (β = 0.70; $p < 0.01$) and therefore, the pre-transplantation Tac C_0/D is useful as a predictor of Tac dose requirements after transplantation (A). Tac C_0/D on day -1 before transplantation *vs.* Tac C_0/D on day 3 post-transplantation: β = 0.90; $p < 0.01$(B).

no significant correlation coefficients for both variables. Sequential multiple regression was performed to determine if addition of these variables improved prediction of C_0/D after transplantation, beyond that afforded by Tac C_0/D_{before}. Hematocrit (mean 0.32 ± 0.05 L/L) and albumin (mean 35 ± 4 g/L) concentrations were found not to predict a significant amount of the variance in Tac dose requirements on day 3 post-transplantation (data not shown).

Immunosuppressive drug-related AEs

During the pre-transplantation treatment phase, gastrointestinal complaints, particularly diarrhea, were the most frequently reported drug-related AE and occurred in 17 (29.8%) patients. One elderly patient had severe diarrhea requiring hospitalization and intravenous rehydration. The incidence of pre-transplant infections was low (n = 2, 3.5%). One week before the scheduled transplantation, one patient suffered from peritoneal dialysis-associated peritonitis. This required temporary discontinuation of immunosuppressive drug treatment (the Tac concentration was >30.0 ng/mL at the time of diagnosis) and antibiotic treatment. The second patient had a respiratory tract infection which necessitated postponement of the transplantation and oral antibiotic therapy. Immunosuppressive therapy before transplantation was associated with neurological AEs in 9 patients (15.8%). Four patients (7.0%) suffered from headache and 7 patients (12.3%) experienced tremor. Tac concentrations exceeded the therapeutic range (>20.0 ng/mL) in 3 of these 9 patients. Three of these patients had Tac concentrations within the therapeutic window and in three patients Tac concentrations were <10 ng/mL.

DISCUSSION

This study demonstrates that Tac dose requirements before transplantation correlate strongly with dose requirements early after transplantation and explain 63% of the variation in dose requirements on post-operative day 3. These data show that therapy with Tac before transplant surgery can provide useful information on what dose of Tac is needed to reach the Tac target concentrations in the immediate post-operative period.

These findings contrast with the observations of the only two published reports that tried to answer the same question. Boots *et al.*, found a poor correlation between pre-transplant Tac exposure (abbreviated AUC) after a single oral dose and Tac doses required to achieve a C_0 of 10 ng/mL after transplantation.[5] Contrary to Boots *et al.,* in connection with the retrospective design of this study, we chose to evaluate Tac exposure using C_0 because of reports demonstrating a significant correlation between $AUC_{0\text{-}24}$ and C_0 and because Tac C_0 is the TDM parameter that is used most frequently in the clinical practice.[1,11] Campbell *et al.,* found no difference in the proportion of patients reaching the Tac target shortly after transplantation between a group of patients who received a standard, bodyweight-based Tac starting dose and a group in which the starting dose was based on a pre-transplant C_2 obtained after a single test dose. The latter group, however, did reach the target Tac concentration significantly faster.[6]

This discrepancy may be attributed to the fact that the patients in previous studies were not in steady-state, whereas in our cohort of AB0i patients, Tac concentrations were determined after at least five stable dosages (which corresponds to five times the half-life of Tac). In addition, the post-operative inducing effect of glucocorticoids on the metabolizing cytochrome P450 enzyme system[7] may not have had an important impact in our study as our patients had been using a stable and pharmacologically relevant prednisolone dose for two weeks prior to transplantation. In the studies by Boots[5] and Campbell[6] that were performed in the pre-transplant setting, no glucocorticoids were co-administered, whereas their patients did receive glucocorticoids after transplant surgery.

Interestingly, although the Tac starting dose is based on bodyweight is routine practice in many transplant centers, including our own, bodyweight was not found to be a covariate predicting Tac dose requirements. This is in line with the observations of Press *et al.* who concluded that there is no rationale for bodyweight-based Tac dosing, and that early under- and overexposure could be related to low and high bodyweight, respectively.[12] Whether or not initiation of Tac treatment one or two weeks prior to transplantation will result in improved outcome compared to starting immunosuppression on the day of surgery has not been investigated in randomized-controlled trials. This is remarkable

as the potential contribution of reaching the target levels as soon as possible, as well as already suppressing the immune system before transplantation, to improved transplantation outcome may be substantial.

Despite the fact that Tac dose requirement is strongly correlated with *CYP3A5* genotype,[13,14] this variable was not studied because recipient DNA was only available for 63% of the (largely Caucasian) study population, thereby precluding a meaningful analysis. Another limitation of the present study is the fact that an immunoassay was used to measure Tac concentrations. This assay is known to cross-react with Tac metabolites and this may have influenced the results.[15,16] However, the same Tac assay was used throughout the study as part of standard clinical care and any analytical bias likely influenced the Tac concentrations before and after surgery would be expected to occur to the same degree. Moreover, a good correlation was observed between pre- and post-transplantation Tac concentrations. It is doubtful that this correlation would have been weaker if more advanced analytical techniques such as mass spectrometry, would have been used.

A strong correlation between pre- and post-transplant Tac dose requirements existed. Therefore it may be argued that by basing the Tac starting dose on pre-transplant, steady-state exposure (obtained after repeated test doses) rather than starting Tac (based on bodyweight) at the time of transplant surgery, one may be able to avert supra-therapeutic Tac concentrations (and possibly toxicity) and prevent under-exposure (and possibly rejection). However, a randomized-controlled clinical trial would be needed to answer this question. Such a strategy of pre-transplant Tac dose requirement phenotyping is a feasible strategy both for patients scheduled to undergo living donor kidney transplantation, as well as for patients that are waitlisted for a deceased donor transplant. In addition, it is a more direct strategy to asses Tac dose requirement as compared with pre-transplant CYP3A phenotyping using a probe drug as suggested by others.[10]

However, the patients described here, were also treated with prednisolone in addition to Tac in the two weeks prior to transplantation. It may well be that when a pre-transplant immunosuppressive regimen without glucocorticoids is prescribed, the correlation of the Tac dose requirements in the pre- and post-transplant (with glucocorticoids) period will be less substantial. There is considerable evidence that glucocorticoids influence Tac PK.[17] Therefore, our results cannot be extrapolated to a setting with a glucocorticoid-free pre-transplant immunosuppressive regimen. Another caveat is the fact that only blood group AB0i kidney transplant recipients were studied here. We feel however, that the results may also apply to blood group AB0 compatible kidney transplant recipients, provided they receive a similar pre-transplantation immunosuppressive regimen.

An alternative strategy could be to start immunosuppression pre-emptively and to perform TDM in the 1-2 weeks before transplant surgery, rather than base the Tac starting dose (started on the day of surgery) on several pre-transplant test doses. This would of course only be feasible in the living kidney donor setting. The present findings suggest that such a strategy may be safe as the number of severe AEs occurring in the pre-transplant phase was low, and gastro-intestinal and neurological toxicity are to be expected when prescribing Tac and MMF. Nonetheless, two patients suffered from serious infections which necessitated temporary discontinuation of immunosuppression and led to postponement of transplant surgery. In addition, one elderly patient required hospitalization because of severe diarrhea. Of the 57 patients in this study, 14 were transplanted pre-emptively. In none of the latter patients, did the initiation of Tac cause severe hyperkaliaemia or a deterioration of renal function necessitating dialysis. Again, a randomized-controlled clinical trial will be necessary to establish whether the possible benefits of such a pre-emptive immunosuppression strategy outweigh the risks.

In conclusion, steady-state, pre-transplant Tac dose requirements strongly correlated with post-transplant dose requirements. Using pre-transplant Tac dose and concentration data, one may be able to predict Tac exposure after transplantation and limit sub -and supratherapeutic exposure. Whether such a strategy will also improve clinical outcomes needs to be addressed in a prospective study.

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3.2

A randomized-controlled trial comparing the efficacy of *CYP3A5* genotype-based with bodyweight-based tacrolimus dosing after living donor kidney transplantation

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ABSTRACT

Patients expressing the cytochrome P450 (CYP) *3A5* gene require a higher tacrolimus dose to achieve therapeutic exposure compared with non-expressers. This randomized-controlled study investigated if adaptation of the tacrolimus starting dose according to *CYP3A5* genotype increases the proportion of kidney transplant recipients being within the target tacrolimus predose concentration range (10-15 ng/mL) at first steady-state. Two hundred forty living-donor, renal transplant recipients were assigned to either receive a standard, bodyweight-based or a *CYP3A5* genotype-based tacrolimus starting dose. At day 3, no difference in the proportion of patients having a tacrolimus exposure within the target range was observed between the standard-dose and genotype-based groups: 37.4% *vs.* 35.6%, respectively; $p = 0.79$. The proportion of patients with a sub-therapeutic (*i.e.* <10 ng/mL) or a supra-therapeutic (*i.e.* >15 ng/mL) Tac C_0 in the two groups was also not significantly different. The incidence of acute rejection was comparable between both groups ($p = 0.82$). Pharmacogenetic adaptation of the tacrolimus starting dose does not increase the number of patients having therapeutic tacrolimus exposure early after transplantation and does not lead to improved clinical outcome in a low immunological risk population.

This study was registered in the Dutch national trial registry (number NTR2226).

INTRODUCTION

Tacrolimus (Tac) is the cornerstone of immunosuppressive therapy after kidney transplantation.[1] The clinical use of Tac is difficult due to its toxicity, narrow therapeutic range, and highly variable pharmacokinetics between individuals.[2] The exposure to Tac correlates with the expression and activity of the Tac-metabolizing enzymes cytochrome P450 (CYP) 3A4 and CYP3A5.[3,4] These enzymes are polymorphically expressed which is in part explained by the presence of single-nucleotide polymorphisms (SNPs) in the *CYP3A4* and *CYP3A5* genes.[5-8]

A SNP at position 6986 of the *CYP3A5* gene (rs776746; 6986A>G) causes a splicing defect resulting in the absence of functional CYP3A5 protein. Patients who are homozygous for the 6986 G allele (designated as *CYP3A5*3*) are therefore expected to lack CYP3A5 activity.[6] Renal transplant recipients (RTRs) carrying one or two *CYP3A5*1* alleles (so called CYP3A5 expressers) require a significantly higher Tac dose compared with CYP3A5 non-expressers.[9,10] In theory, the earlier therapeutic blood concentrations of Tac are attained after transplantation, the more effective the drug is likely to be in preventing acute rejection.[11] The higher dose requirement of patients expressing CYP3A5 may cause a delay in reaching the desired Tac target concentrations. Therefore, these patients are at an increased risk of sub-therapeutic Tac exposure during the first weeks after transplantation, and may be at higher risk to develop early acute rejection.[12]

The KDIGO Transplant Work Group stated that dosing of Tac is important but relatively under-investigated.[11] Despite a statistically significant influence of *CYP3A5* genotype on Tac dose requirement, patients on the waiting list for a kidney transplantation are not routinely genotyped for *CYP3A5* in most transplant centers. Rather, patients receive a standard dose of Tac based on their bodyweight, and with a "trial and error" approach, target concentrations are reached by adjusting the dose based on repetitive concentration measurements, a practice known as therapeutic drug monitoring (TDM). The question is whether the implementation of technological advances such as pre-transplantation genotyping will benefit patient management.[13]

In this randomized-controlled trial, recipients of a living donor kidney transplant were randomized to receive a standard starting dose of Tac based solely on their bodyweight or to receive a starting dose of Tac based on their *CYP3A5* genotype. The goal was to study whether *CYP3A5* genotype-based Tac dosing leads to earlier achievement of Tac target whole-blood exposure and consequently, to a better clinical outcome after kidney transplantation.

PATIENTS AND METHODS

Study design

This was an investigator-initiated, prospective, randomized-controlled, parallel group, open-label, single-centre, clinical trial. Adult patients (≥18 years) who were scheduled to receive a single-organ, blood group AB0-compatible kidney from a living donor at the Erasmus MC, Rotterdam, the Netherlands, were eligible for participation. Patients who received immunosuppressive drug treatment within 28 days prior to transplantation (except glucocorticoids) and/or used any drugs known to interact with Tac at the time of transplantation (see supplementary Table 1 for the list of these drugs) were not included in the study.

The study was approved by the institutional review board of the Erasmus MC (Medical Ethical Review Board number 2010-080) and was registered in the Dutch national trial registry (http://www.trialregister.nl/trialreg/index.asp; number NTR2226, registered 25 Feb. 2010). Written informed consent was obtained from all patients before inclusion and randomization. The study was carried out in compliance with the Good Clinical Practice guidelines.

Intervention and randomization procedure

Patients were enrolled and randomly assigned on a 1:1 basis by one of the coordinating investigators (R.B., N.S., T.v.G., or D.A.H.) to either receive Tac (Prograf®; Astellas Pharma, Leiden, the Netherlands) in a standard, bodyweight-based dose (SDG) of 0.2 mg/kg/day according to the package insert[14] or to receive a dose determined by their *CYP3A5* genotype (GBG): CYP3A5 expressers (*i.e.* carriers of one or two *CYP3A5*1* alleles) received 0.30 mg/kg/day, whereas CYP3A5 non-expressers (*CYP3A5*3* homozygotes) received 0.15 mg/kg/day. The Tac dose was rounded off to the nearest 0.5 mg to enable twice daily oral dosing of an equal dose. The first dose of Tac was administered at 22:00 hr on the night following transplant surgery. During hospitalization, Tac was taken at 10:00 and 22:00 hrs and patients were encouraged to continue doing so after discharge. The randomization was performed by use of sealed, opaque, sequentially-numbered envelopes containing treatment allocation. The random-allocation sequence was generated by an independent statistician by use of a random number generator on a computer. If a patient was assigned to the *CYP3A5* GBG, the individual's *CYP3A5* genotype was revealed to the treating physician by an employee of the clinical chemistry department of the Erasmus MC where the genotyping was performed (see below). The clinical physicians were not aware of the *CYP3A5* genotype of patients in the SDG. Data were collected, monitored and entered by the coordinating investigators, and stored in a hospital-based electronic study database.

Additional (immunosuppressive) treatment

The additional immunosuppressive therapy was identical in both groups and consisted of basiliximab (Simulect®, Novartis Pharma B.V., the Netherlands) in a dose 20 mg administered intravenously on day 0 (immediately before kidney transplant reperfusion) and day 4 after transplantation. Patients also received a starting dose of 1000 mg mycophenolate mofetil (MMF; CellCept®, Roche Pharmaceuticals, the Netherlands) twice daily aiming for plasma mycophenolic acid (MPA) pre-dose concentrations (C_0) between 1.5 and 3.0 mg/L. Prednisolone treatment consisted of an intravenous dose of 50 mg twice daily on days 0-3, followed by 20 mg orally once daily (on days 4-14), after which the dose was tapered to 5 mg at month 3 after transplantation. All patients received trimethoprim/sulfamethoxazole prophylaxis for *pneumocystis jirovecii* pneumonia for at least three months. All patients receiving a kidney from a cytomegalovirus (CMV) positive donor and all patients who were seropositive for CMV received prophylaxis with valganciclovir for a duration of six months.

Endpoints

The primary endpoint was the proportion of patients within the desired Tac whole-blood, C_0 range of 10 – 15 ng/mL at first steady state, *i.e.* on the morning of day 3 after 5 unaltered Tac doses. The Tac C_0 was defined as the whole-blood concentration 12 ± 2 hrs after the previous dose taken the night before at 22:00 hrs. The exact time of Tac intake and C_0 sampling were not recorded. Before C_0 sampling on day 3, no TDM was performed. Hereafter, the clinicians could change the Tac dose based on the measured Tac exposure and/or the clinical situation of the individual patient in both treatment groups according to local protocol. In our transplant unit, the Tac C_0 is routinely measured 3-times weekly during hospitalization and at every outpatient clinic visit. Clinicians were stimulated to use the following formula to calculate the new Tac dose:

New daily Tac dose = (Desired Tac C_0 / Current Tac C_0) x Current Tac dose

The pre-specified Tac C_0 target range was 10.0 – 15.0 ng/mL in week 1-2, 8.0 – 12.0 ng/mL in week 3-4, and 5.0 – 10.0 ng/mL after week 4 post-transplantation.

Secondary pharmacokinetic endpoints included the average Tac C_0 during weeks 1 and 2 after transplantation, the time required to reach the target C_0 range, the number of Tac dose modifications needed to reach the target C_0 range, and the number of markedly subtherapeutic Tac (arbitrarily defined as C_0 <5.0 ng/mL) and markedly supra-therapeutic (defined as C_0 >20.0 ng/mL) C_0 measurements.

Secondary clinical endpoints included the incidence of delayed graft function (DGF; defined as the need for dialysis within the first week after transplantation), the incidence of biopsy-proven acute rejection (BPAR), the incidence of clinically presumed acute rejection, and renal function at month 3 after transplantation. Biopsies were performed only for cause, were reviewed in a blinded fashion by two independent pathologists after the completion of the trial and were graded according to the most recent Banff classification of renal allograft rejection .[15] Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated MDRD study equation.[16] Since the MDRD equation does not estimate GFR sufficiently accurately at values above 60 mL/min we capped the eGFR values at this level. All patients were followed for three months after transplantation or until graft loss occurred (defined as death with a functioning transplant, return to dialysis or re-transplantation).

Safety

The incidence of (serious) adverse events [(S)AE] was registered and these included (the cause of) death, graft loss, cancer, (opportunistic) infections, post-transplant diabetes mellitus (PTDM), neurologic events, and acute Tac-induced nephrotoxicity. PTDM was defined as the use of glucose-lowering medical therapy up until month 3 after transplantation in a patient not needing such treatment before transplantation. Acute Tac nephrotoxicity was defined as any ≥15% increase of serum creatinine with a return to baseline after Tac dose reduction and after exclusion of other causes of renal transplant function deterioration.

Tacrolimus concentration measurement

Tac C_0 was determined in whole-blood in the Nephrology & Transplantation laboratory of the Erasmus MC by use of two different immunoassays, the antibody-conjugated magnetic immunoassay (ACMIA) on a dimension platform (Siemens Healthcare, N.V., the Netherlands) and the enzyme multiplied immunoassay technique (EMIT; Siemens Healthcare N.V., the Netherlands). In the first two years (10/11/2010 to 04/12/2012) of the trial, Tac concentration measurements were measured exclusively by the ACMIA, after which measurements were performed exclusively by use of EMIT. As a result, 76% of the day 3 C_0 measurements were determined by ACMIA and 24% by EMIT. The reason for the forced change in assay was the world-wide shortage of reagents approved by the manufacturer with sufficient patient sample recovery.

Comparison of the ACMIA and EMIT immunoassays demonstrated a high correlation (r = 0.965). The measurements obtained with EMIT were systematically ~15% higher than those obtained with ACMIA. The lower limits of detection were 1.5 ng/mL (ACMIA) and 2.0 ng/mL (EMIT). The upper limit of detection was 30 ng/mL. For calculation purposes, Tac

C_0 below the detection limit were set at half the detection limit (0.75 and 1.0 ng/mL, for ACMIA and EMIT, respectively). Tac C_0 above the detection limit were set at 30 ng/mL. The laboratory participates successfully in the United Kingdom Quality Assessment Scheme (Dr. Holt, St George's Hospital Medical School, London, UK).

DNA extraction and genotyping

DNA was extracted from peripheral blood leukocytes using the Blood DNA kit (Qiagen, Courtaboeuf, France) in accordance with standard protocols. Genotyping of the *CYP3A5*3* allele was performed using TaqMan Assay reagents for allelic discrimination (Applied Biosystems, Courtaboeuf, France) with a 7900 Applied Biosystems thermal cycler as previously described.[9,17] All genotyping was performed *in duplo* according to quality standards of the International Federation of Clinical Chemistry and Laboratory Medicine.

Genotyping was performed during the work-up for transplantation as part of a biobanking programme that has been approved by the institutional review board of the Erasmus MC (Medical Ethical Review Board number 2010-022; approved June 21, 2010). All patients gave written informed consent for participation in this biobanking programme.

Statistical analysis

A study population of 196 patients (98 patients per treatment arm) was considered sufficient to provide a statistical power of 80% to detect a difference between the two groups in the proportion of patients within the target Tac C_0 on day 3 after transplantation, on the basis of a two-sided test and a significance level of 5%, assuming a 40% incidence of the primary end point in the SDG and a 20% increase of this value in the GBG. Taking a 20% drop-out rate into account, a total of 240 patients was included in the study. No interim analysis was planned.

For the analysis of efficacy, the intention-to-treat population was used, which included all randomized patients who were treated with at least one dose of Tac. Categorical variables are reported using frequency tables and percentages, and continuous variables are expressed as medians with ranges. A Chi-square statistic was used to evaluate the null hypothesis of no difference between the SDG and GBG in the proportion of patients within the Tac target C_0 range on day 3 after transplantation. Continuous variables were compared between the groups using the Mann-Whitney *U* test. The time to reach the target Tac C_0 range was estimated with Kaplan-Meier survival analysis, and compared between the groups using the Log Rank test. To estimate the overall effect of *CYP3A5* genotype on the outcome variables Tac daily dose, Tac C_0 and dose-corrected Tac C_0 (a measure for apparent oral clearance and hence, Tac dose requirement), a mixed between-within subjects analysis of variance was used. This analysis was also used to estimate the

overall effect of dosing approach (standard, bodyweight-based dosing and genotype-based dosing) on renal function. All tests were two-tailed and statistical significance was defined as a *p*-value <0.05. Statistical analyses were performed using IBM SPSS version 20 (SPSS Inc., Chicago, IL).

RESULTS

Patients

Between November 10, 2010 (first patient, first visit) and September 30, 2013 (last patient, first visit) 571 patients were screened of whom 254 were eligible for participation (Figure 1). Fourteen patients did not wish to participate. Two-hundred-forty patients were included and randomized. After inclusion, one patient was not transplanted and did not receive Tac. In two cases, an inaccurate Tac starting dose was prescribed. These patients were considered to have protocol violations and were excluded from the analysis, resulting in a total of 237 patients for the intention-to-treat analysis. The characteristics of these patients are described in Table 1. The mean daily starting dose of Tac was 0.20 mg/

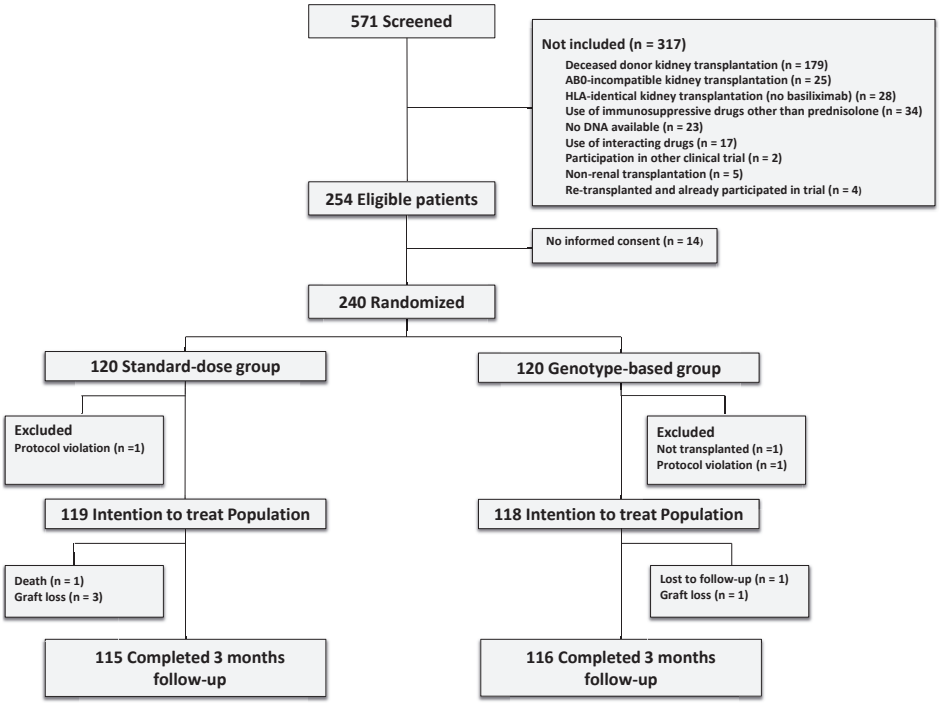


Figure 1. Trial flowchart. All patients who underwent transplantation and received at least one dose of the study drug Tac were included in the intention-to-treat population.

kg in the standard-dose group (SDG) and 0.19 mg/kg in the genotype-based group (GBG). One-hundred-fifteen (96.6%) patients in the SDG and 116 (98.3%) in the GBG group completed the 3-month follow-up period.

The minor allele frequency was 15.2% (Supplementary Table 2). The observed *CYP3A5* genotype distribution was in accordance with the Hardy-Weinberg equilibrium (χ^2 =

Table 1. Baseline characteristics.

	Standard-dose group (n = 119)	**Genotype-based group (n = 118)**	***p***
Gender recipient			
Male / Female	73 (61.3%) / 46 (38.7%)	75 (63.6%) / 43 (36.4%)	0.73
Age of recipient (years)	57 (19 – 79)	55 (19 – 79)	0.55
Ethnicity			0.89
Caucasian	93 (78.2%)	93 (78.8%)	
Asian	13 (10.9%)	10 (8.5%)	
Black	11 (9.2%)	12 (10.2%)	
Other	2 (1.7%)	3 (2.5%)	
Bodyweight (kg)	75.7 (37.6 – 132.0)	81.2 (43.6 – 123.1)	0.21
Length (cm)	173.0 (145.0 – 203.0)	174.0 (151.0 – 196.0)	0.77
BMI (kg/m^2)	25.5 (17.2 – 37.8)	26.2 (18.1 – 42.2)	0.21
Primary kidney disease			
Diabetic nephropathy	21 (17.6%)	23 (19.5%)	
Polycystic kidney disease	17 (14.3%)	18 (15.3%)	
Glomerulonephritis	16 (13.3%)	27 (22.6%)	
Hypertensive nephropathy	16 (13.3%)	13 (11.0%)	
Reflux disease / Chronic pyelonephritis	7 (5.8%)	6 (5.0%)	
Other	6 (5.0%)	4 (3.2%)	
Unknown	36 (30.3%)	27 (22.9%)	
Number of kidney transplantation			0.46
1st	111 (93.3%)	107 (90.7%)	
2nd	7 (5.9%)	9 (7.6%)	
3rd	1 (0.8%)	2 (1.7%)	
RRT* prior to kidney transplantation			0.55
Hemodialysis	46 (38.7%)	44 (37.3%)	
Peritoneal dialysis	20 (16.8%)	24 (20.3%)	
Pre-emptive	53 (44.5%)	49 (41.5%)	
Donor type			
Living-Related / Living-Unrelated	48 (40.3%) / 71 (59.7%)	47 (39.8%) / 71 (60.2%)	0.94
PRA%§ (<15% / ≥15%)	111 (93.3%) / 8 (6.7%)	109 (92.4%) / 8 (6.8%)	0.97
Peak PRA% (<15% / ≥15%)	100 (84.%) / 19 (16.0%)	93 (78.8%) / 24 (20.3%)	0.37

*RRT = Renal replacement therapy; §PRA = Panel reactive antibodies

3.17; p = 0.075) and is comparable to the reported[17] genotype distribution of a largely Caucasian population (Supplementary Table 3).

Efficacy

Primary endpoint

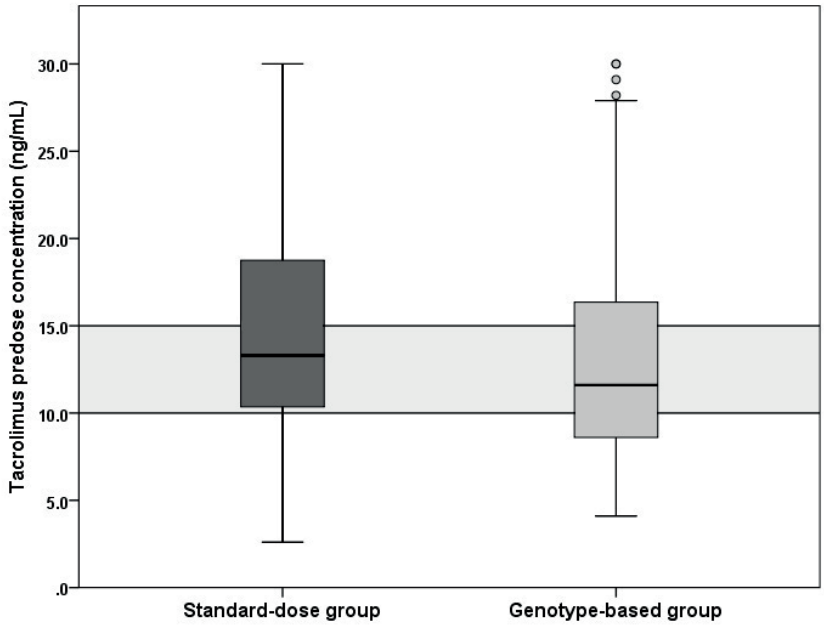
For 203 (85.7%) patients (99 patients in the SDG and 104 patients in the GBG) a Tac C_0 was available on day 3 after transplantation. Most of the missing C_0 at day 3 were samples that had to be collected on a Sunday. Given the predicted 20% drop-out rate, these samples provided sufficient statistical power to study the primary endpoint.

On day 3 after transplantation, the median Tac C_0 was 13.3 ng/mL (2.6 – 30.0 ng/mL) in the SDG *vs.* 11.6 ng/mL (4.1 – 30.0 ng/mL) in the GBG (p = 0.047; Table 2). At this time point, 37 patients (37.4%; 95%-CI 28.5 – 47.0%) in the SDG and 37 patients (35.6%;

Table 2. Tacrolimus dose, pre-dose concentration and dose-corrected pre-dose concentration according to the treatment group.

	Whole group	Standard-dose group	n	Genotype-based group	n
Tacrolimus dose (mg/day)					
Day 3	14.0 (7.0 – 32.0)	15.0 (8.0 – 26.0)	119	13.0 (7.0 – 32.0)	117
Day 7	12.5 (0.0 – 32.0)	12.0 (0.0 – 26.0)	119	13.0 (4.0 – 32.0)	117
Day 10	12.0 (0.0 – 36.0)	11.0 (0.0 – 26.0)	117	12.0 (3.0 – 36.0)	117
Day 14	11.0 (0.0 – 36.0)	10.0 (1.0 – 30.0)	117	12.0 (0.0 – 36.0)	116
Day 30	9.0 (2.0 – 34.0)	8.0 (2.0 – 22.0)	115	10.0 (3.0 – 34.0)	118
Day 60	6.0 (0.0 – 34.0)	6.0 (0.0 – 18.0)	114	6.0 (0.0 – 34.0)	117
Day 90	5.5 (0.0 – 34.0)	5.0 (0.0 – 16.0)	114	6.0 (0.0 – 34.0)	116
Tacrolimus C_0 (ng/mL)					
Day 3	12.2 (2.6 – 30.0)	13.3 (2.6 – 30.0)	99	11.6 (4.1– 30.0)	104
Day 7	12.5 (5.0 – 30.0)	12.7 (5.4 – 30.0)	96	11.9 (5.0 – 30.0)	96
Day 10	11.8 (4.3 – 23.9)	12.1 (5.9 – 22·7)	79	11.5 (4.3 – 23.9)	89
Day 14	11.0 (5.1 – 19.9)	11.1 (5.1 – 19.9)	44	10.6 (5.6 – 18.3)	41
Day 30	9.6 (2.9 – 30.0)	9.3 (2.9 – 30.0)	104	10.3 (3.6 – 23.2)	114
Day 60	8.2 (3.3 – 20.3)	8.6 (3.3 – 20.3)	110	8.1 (3.8 – 19.7)	109
Day 90	7.6 (2.7 – 16.8)	7.5 (2.7 – 16.7)	103	7.9 (3.4 – 16.8)	110
Tacrolimus C_0/dose (ng/mL per mg/kg)					
Day 3	67.8 (13.8 – 195.3)	64·6 (13.8 – 162.0)	97	72.0 (23.4 – 195.3)	104
Day 7	74.9 (20.2 – 355.6)	76.0 (23.5 – 355.6)	93	72.8 (20.2 – 275.6)	96
Day 10	75.6 (22.4 – 678.3)	80.9 (31.6 – 678.3)	77	74.5 (22.4 – 467.0)	88
Day 14	79.6 (10.8 – 531.6)	82.9 (10.8 – 531.6)	41	74.3 (15.8 – 255.9)	41
Day 30	84.6 (16.5 – 647.5)	90.5 (19.1 – 647.5)	101	79.5 (16.5 – 390.6)	113
Day 60	106.7 (14.4 – 439.2)	109.8 (25.7 – 439.2)	106	100.2 (14.4 – 415.7)	106
Day 90	114.2 (19.6 – 775.0)	114.7 (19.6 – 775.0)	100	113.3 (22.6 – 444.0)	109

95%-CI 27.0 – 45.0%) in the GBG were within the target Tac C_0 range (p = 0.79; Figure 2). The proportion of patients with a sub-therapeutic (*i.e.* <10 ng/mL) or a supra-therapeutic (*i.e.* >15 ng/mL) Tac C_0 in the two groups was also not significantly different (Figure 2).



	Standard-dose group	Genotype-based group	*p*
	n = 99	n = 104	
Supra-therapeutic concentration	39 (39.4%)	31 (29.8%)	0.20
Therapeutic concentration	37 (37.4%)	37 (35.6%)	0.79
Sub-therapeutic concentration	23 (23.2%)	36 (34.6%)	0.10

Figure 2. Boxplots depicting the predose Tac concentrations (C_0) on day 3 after transplantation in the standard-dose and genotype-based groups. The boxes depict the median and the 25th and 75th percentiles. The whiskers depict the 5th and 95th percentiles. The dots represent the outliers and the asterisks represent the extreme outliers (more than three times the IQR). The shaded area represents the target Tac C_0 range (10 - 15 ng/mL).

Secondary pharmacokinetic endpoints

Supplementary Figure 1 depicts the average of all Tac C_0 obtained during the first and second week after transplantation. The median of the average of week 1 Tac C_0 in the SDG was 13.1 ng/mL (5.3 – 30.0) *vs.* 12.8 ng/mL (5.2 – 30.0) in the GBG (p = 0.036), whereas the median of the average of week 2 Tac C_0 in the SDG was 12.5 ng/mL (6.7 – 20.3) *vs.* 11.6 ng/mL (5.1 – 22.7) in the GBG (p = 0.010).

There was no significant difference in the median time needed to achieve the target Tac C_0 between the SDG and GBG: 6 days (3 – 17) *vs.* 6 days (3 – 28; $p = 0.72$; Figure 3), nor was there a significant difference in the number of daily dose modifications needed to reach the target Tac C_0: 156 (SDG) *vs.* 129 (GBG); $p = 0.30$ (Supplementary Figure 2).

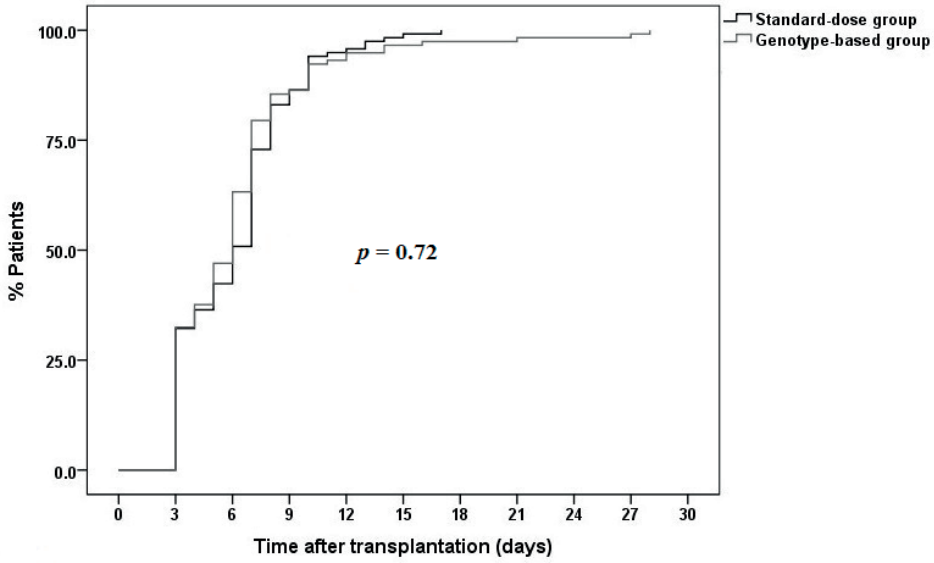


Figure 3. Time to achieve the target Tac C_0 range (10.0 – 15.0 ng/mL).

No significant between-group difference was found in the frequency of being markedly underexposed during the first month after transplantation. During this time period, 12 patients (10.3%; 95%-CI 6.0 – 17.1%) in the SDG and 13 patients (11.1%; 95%-CI 6.6 – 18.1%) in the GBG had one or more Tac C_0 <5.0 ng/mL ($p = 0.83$). Likewise, the number of patients experiencing marked Tac overexposure was comparable between the SDG and GBG: 38 patients (32.5%; 95%-CI 24.7 – 41.4%) in the SDG and 28 (23.9%; 95%-CI 17.1 – 32.4%) patients in the GBG ($p = 0.15$).

Post-hoc pharmacokinetic analyses

To investigate whether *CYP3A5* expressers do require a higher Tac dose to achieve target concentrations compared with *CYP3A5* non-expressers, the whole study population was analysed. On day 3 after transplantation, *CYP3A5* expressers (n = 48) had a 36.3% higher Tac dose requirement (C_0/D) compared with non-expressers (n = 153): 49.1 ng/mL per mg/kg (13.8 – 150.9) *vs.* 77.1 ng/mL per mg/kg (25.8 – 195.3); $p < 0.001$ (Supplementary Table 4). The difference in Tac C_0/D between *CYP3A5* expressers and non-expressers persisted throughout the 3-month follow-up period (Supplementary Table 4 and Supplementary Figure 3). Repeated measurements analysis confirmed that the overall Tac C_0/D

was significantly lower in *CYP3A5* expressers than in non-expressers, indicating a higher dose requirement of the former group (p <0.001).

Tac exposure on day 3 of the patients in the SDG and GBG was analysed according to their *CYP3A5* genotype (Figure 4A). The median Tac C_0 in patients homozygous for *CYP3A5*1* was not statistically significantly different between patients in the SDG and GBG: median 6.8 ng/mL (4.6 – 10.6 ng/mL) *vs.* 9.4 ng/mL (8.1 – 25.7 ng/mL); p = 0.40 (Figure 4A and Supplementary Table 5). Heterozygous *CYP3A5*1* carriers in the SDG had a significantly lower Tac C_0 compared with their counterparts in the GBG: median 10.4 ng/mL (2.6 – 30.0) *vs.* 14.7 ng/mL (6.5 – 30.0); p = 0.032. Among *CYP3A5* non-expressers (those with the *CYP3A5*3/*3* genotype), Tac C_0 was significantly higher in the SDG than in the GBG: 14.5 ng/mL (5.5 – 30.0) *vs.* 11.6 ng/mL (4.1 – 30.0); p < 0.001 (Supplementary Table 5; Figure 4A).

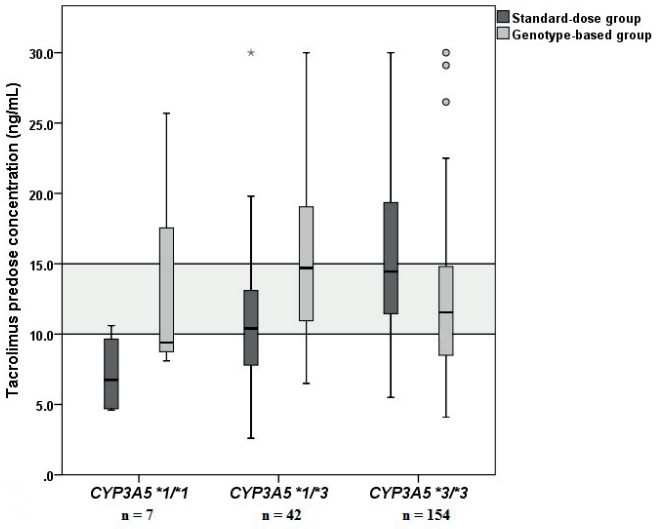
When the proportion of patients within the target Tac C_0 range on day 3 after transplantation was analysed according to *CYP3A5* genotype (expressers and non-expressers), no significant differences were found between the SDG and GBG. Among the *CYP3A5* expressers, 39.1% (95%-CI 22.2 – 59.2%) of the patients in the SDG (n = 23) and 26.9% (95%-CI 13.7 – 46.1%) of the patients in the GBG (n = 26) were within the target Tac C_0 range (p = 0.36). A comparable situation was found among the *CYP3A5* non-expressers: 36.8% (95%-CI 26.9 – 48.1%) of the patients in the SDG (n = 76) and 38.5% (95%-CI 28.4 – 49.6%) of the patients in the GBG (n = 78) were within the target Tac C_0 range (p = 0.84; Figure 4B).

However, on day 3 after transplantation, a trend towards more frequent sub-therapeutic Tac exposure was observed among CYP3A5 expressers in the SDG compared with CYP3A5 expressers in the GBG. The reverse was observed for CYP3A5 non-expressers who were significantly less frequently below the target Tac C_0 in the SDG compared with the GBG (Figure 4B).

Safety

In total, 728 AEs occurred in the SDG and 750 in the GBG (p = 0.56; Tables 3 and 4). Of these, 148 and 167 were judged to be serious (p = 0.40). One patient in the SDG died from bacterial peritonitis. Overall kidney allograft survival (including death with a functioning transplant) was 96.6% in the SDG and 99.2% in the GBG (p = 0.370). Kidney allograft survival censored for death was 97.5% in the SDG and 99.2% in the GBG (p = 0.62). Three patients in the SDG and one in the GBG lost their graft as a result of vascular complications.

A.



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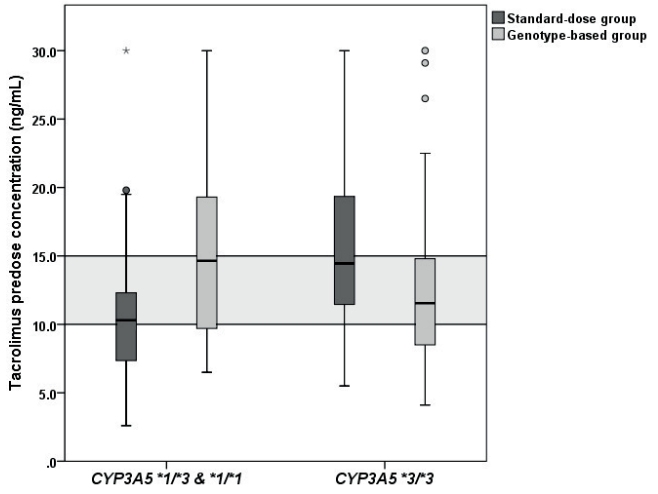

	Standard-dose group	Genotype-based group	*p*
***CYP3A5 *1/*1 & *1/*3* (expressers)**	n = 23	n = 26	
Supra-therapeutic concentration	3 (13.0%)	12 (46.2%)	**0.012**
Therapeutic concentration	9 (39.1%)	7 (26.9%)	0.36
Sub-therapeutic concentration	11 (47.8%)	7 (26.9%)	0.13
***CYP3A5 *3/*3* (non-expressers)**	n = 76	n = 78	
Supra-therapeutic concentration	36 (47.4%)	19 (24.4%)	**0.003**
Therapeutic concentration	28 (36.8%)	30 (38.5%)	0.84
Sub-therapeutic concentration	12 (15.8%)	29 (37.2%)	**0.003**

Figure 4. Boxplots depicting the predose Tac concentrations (C_0) on day 3 after transplantation in the standard-dose and genotype-based groups categorized by *CYP3A5* genotype (4A) and by *CYP3A5* expressers and non-expressers (4B).

The overall incidence of BPAR was 10.5% (n = 25). The BPAR rate was comparable between the SDG and GBG: 10.1% (n = 12) *vs.* 11·0% (n = 13); $p = 0.82$. Also, the severity and sub-type of BPAR was not different between both treatment groups (Table 3). The rate of presumed acute rejection was not significantly different: SDG 4.2% (n = 5) *vs.* GBG 5.1% (n = 6); $p = 1.00$.

The incidence of DGF was comparable between the two groups (Table 4). At month 3 after transplantation, renal function was similar in the SDG and GBG: median eGFR 47 (20 – 60) *vs.* 50 mL/min per 1.73 m^2 (18 – 60), respectively ($p = 0.80$). There was no difference in renal function recovery or the amount of proteinuria between the SDG and GBG (Supplementary Figure 4 and Supplementary Table 6).

There were no significant between-group differences in the incidence of all other AEs, including PTDM ($p = 0.49$), acute Tac-associated nephrotoxicity, and neurotoxicity (Table 4). Tac was tolerated well, and only three patients (all in the GBG) discontinued Tac. Tac was discontinued because of neurotoxicity (n = 2) and thrombotic microangiopathy (n = 1).

Table 3. Incidence of rejection according to the treatment group.

Rejection type	Whole group (n = 237)	Standard-dose group (n = 119)	Genotype-based group (n = 118)
Borderline	5 (2.1%)	3 (2.5%)	2 (1.7%)
Type 1			
1A	0 (0.0%)	0 (0.0%)	0 (0.0%)
1B	1 (0.4%)	1 (0.8%)	0 (0.0%)
Type 2			
2A	9 (3.8%)	4 (3.4%)	5 (4.2%)
2B	7 (3.0%)	3 (2.5%)	4 (3.4%)
Type 3	1 (0.4%)	1 (0.8%)	0 (0.0%)
ABMR*	0 (0.0%)	0 (0.0%)	0 (0.0%)
Mixed ACR[§] and AMBR	7 (3.0%)	3 (2.5%)	4 (3.4%)
Total BPAR[†]	25 (10.5%)	12 (10.1%)	13 (11.0%)

*ABMR = antibody mediated rejection; [§]ACR = acute cellular rejection; [†]Biopsy-proven acute rejection excluding borderline cases

Table 4. Adverse events

Event	Standard-dose group (n = 119)	Genotype-based group (n=118)	*p*
Blood or lymphatic system	**35**	**46**	0.31
Leucopenia	17	19	
Anemia	14	22	
Thrombocytopenia	2	4	
Other	2	1	
Bleeding and thrombotic events	**65**	**57**	0.41
Major bleeding	28	21	
Minor bleeding	28	33	
Thrombosis	9	3	
Cancer	**2**	**3**	0.68
Prostate (Adenocarcinoma)	0	2	
Skin (Basal cell carcinoma)	2	1	
Cardiac	**50**	**52**	1.00
Acute coronary syndrome / myocardial ischemia	3	4	
Cardiac decompensation / volume overload	36	39	
Other	11	9	
Gastro-intestinal	**62**	**49**	0.18
Diarrhea	26	17	
Other gastro-intestinal disorder	36	32	
Infection			
Opportunistic Infection*	**26**	**24**	0.81
BKV	6	8	
CMV	5	3	
EBV	1	1	
HSV	3	3	
VZV	0	1	
Fungal	11	8	
Other infection	**108**	**102**	0.61
Urinary tract infection	77	73	
Upper respiratory tract infection	12	11	
Pneumonia	3	2	
Gastro-intestinal infection	5	7	
Other	11	9	
Locomotor system disorder	**11**	**9**	0.78
Metabolism or nutrition	**137**	**143**	0.94
Post-transplant diabetes mellitus	22	28	
Hypo-/hyperglycemic dysregulation	29	23	
Calcium disorder (hypo-/ hypercalcaemia)	13	19	
Potassium disorder (hypo-/ hyperkalaemia)	19	15	

Table 4. Adverse events (continued)

Event	Standard-dose group (n = 119)	Genotype-based group (n=118)	*p*
Hypophosphataemia	23	23	
Other electrolyte abnormality	7	7	
Liver enzyme abnormality	24	28	
Nervous system	**38**	**45**	0.58
CVA/TIA[§]	0	3	
Tremor	26	26	
Headache	7	8	
Other	5	8	
Skin-related disorders	**8**	**12**	0.54
Surgical or procedural complication	**22**	**31**	0.31
Acute tubular necrosis	8	15	
Delayed graft function	5	6	
Renal infarction	4	2	
Other	5	8	
Tacrolimus-induced nephrotoxicity	**35**	**30**	0.53
Urological complication	**42**	**58**	0.16
Hydronephrosis	21	25	
Urinary leakage	6	8	
Other	15	25	
Wound-related problem	**16**	**11**	0.40
Wound infection	8	5	
Other	8	6	
Other	**49**	**57**	0.59

* BKV = BK virus; CMV = Cytomegalovirus; EBV = Epstein-Barr virus; HSV = Herpes simplex virus; VZV = Varicella zoster virus; [§] CVA = Cerebrovascular accident; TIA = Transient ischemic attack

DISCUSSION

In this study, adaptation of the Tac starting dose based on an individual's *CYP3A5* genotype did not lead to a higher percentage of patients reaching the desired Tac C_0 range on day 3 after kidney transplantation, as compared to a standard, bodyweight-based dosing approach. In addition, *CYP3A5* genotype-based Tac dosing did not result in a lower number of Tac dose modifications, a shorter time to achieve the Tac target C_0, or improved clinical outcome.

This study confirms that CYP3A5 expressers require a significantly higher Tac dose to reach the same target exposure compared with non-expressers.[9,10] Using a standard, bodyweight-based dosing approach, the first median Tac C_0 of CYP3A5 expressers was

lower than that of non-expressers. With *CYP3A5* genotype-based dosing, the proportion of patients with sub-therapeutic Tac exposure tended to decrease among CYP3A5 expressers, whereas it increased significantly among CYP3A5 non-expressers. The reverse was observed with regard to supra-therapeutic Tac exposure (Figure 4B). As a result, the *CYP3A5* genotype-based adjustment of the Tac starting dose did not change the overall proportion of patients within the target Tac C_0 range.

In a randomized-controlled study which included 280 RTRs, Thervet *et al.* demonstrated that *CYP3A5*-based adaptation of the Tac starting dose does increase the proportion of patients on target compared with a standard, bodyweight-based dosing approach. Three days after the start of Tac treatment, significantly more RTRs were within the target Tac C_0 range if Tac was individualized according to *CYP3A5* genotype as compared with standard Tac dosing (43.2% *vs.* 29.1%, respectively; $p = 0.03$).[18] In addition, *CYP3A5*-based Tac dosing was associated with fewer dose modifications and a shorter time to reach the target C_0. However, and in line with the present findings, *CYP3A5* genotype-based Tac dosing did not improve clinical outcomes.[18]

It is unknown why the *CYP3A5*-based Tac dosing approach of Thervet *et al.* was beneficial in terms of early Tac exposure whereas this was not the case in the present study. In both studies, the same Tac starting doses were prescribed and the number of included patients is comparable. However, in contrast to the current study, in which Tac was started directly after transplantation, the initiation of Tac treatment was delayed until day seven after transplantation in the French trial, in which recipients of a deceased donor kidney were also included. Possibly, the between-patient variability in Tac concentrations in the few first days after transplantation was higher than the variability after a week. Early post-operative changes in gastro-intestinal motility and glucocorticoid dose may be responsible for this phenomenon and may have diluted the pharmacogenetic effect.[19,20] Furthermore, during the first week, 82% of the patients in the French study received induction therapy with anti-thymocyte globulin and all patients received a higher than standard dose of MMF.[18] When such a potent immunosuppressive regimen is used in an immunologically low-risk transplant population, a delay in reaching the target Tac exposure may not significantly influence rejection risk.

It may be too early to conclude that the idea of personalising the Tac starting dose on an individual's genotype should be abandoned. *CYP3A5* genotype is currently the strongest known genetic predictor of Tac dose requirement. However, it does not explain all variability.[21,22] In both the present and the French trial,[18] only about 40% of patients in the *CYP3A5* genotype-based dosing group were on target at first steady state. Other genetic variants may explain the residual variability in Tac dose requirement. Recently, several

other such variants including *CYP3A4*22*, *CYP3A4*26*, and *P450 oxidoreductase *28* were found to be associated with Tac dose requirement.[7, 23-25] Also, genetic variants in *CYP3A5* other than *CYP3A5*3*, such as the rare *CYP3A5*6* SNP, which was not determined in this study, may explain some of the inter-individual differences in Tac exposure.[17] Additional variability in Tac exposure may have resulted from variation in the exact timing of Tac intake and C_0 sampling. Although this is a shortcoming of the present study, we feel that this is the way TDM is performed in everyday clinical practice in many transplant centers.

Rather than basing the Tac starting dose on bodyweight and *CYP3A5* genotype, using a dosing algorithm which includes demographic and clinical factors plus multiple genetic variants may optimize early Tac exposure.[26] The concept of using such an algorithm is appealing but these algorithms are complicated (possibly limiting their widespread clinical use). Furthermore, the first validation experiments were not very successful[27] and their performance has not been tested prospectively in clinical trials. An alternative and more simple approach, could be to adjust the starting dose only in selected patients. Post-hoc analyses demonstrated that CYP3A5 expressers tended to be less frequently below the target C_0 range if they received a higher Tac starting dose than standard. If one chooses not to prescribe a reduced dose to CYP3A5 non-expressers, sub-therapeutic Tac exposure in a population as a whole may be reduced. Such a strategy may be especially relevant for patients of African descent who are more often CYP3A5 expressers compared to Caucasians.[28] However, avoiding early sub-therapeutic exposure may come at the expense of more Tac toxicity.[29]

Perhaps the "trial and error" approach we routinely use for Tac dosing is not so bad. Physicians have become highly experienced in TDM and as shown in this and in previous studies, the majority of patients reach the target Tac exposure within 10 days.[30] The outcome of this study is in line with the recommendations of the Royal Dutch Association for the Advancement of Pharmacy which states that although an interaction exists between *CYP3A5* genotype and Tac metabolism, no action is advised because with TDM doses are effectively adjusted.[31] Other guidelines suggest that *CYP3A5* genotyping cannot replace TDM, as other factors (*i.e.* demographic factors and drug-drug interactions) also influence Tac dose requirement.[32]

Our study has weaknesses. First, Tac concentrations were determined with immunoassays which, with the now more widespread availability of more sensitive techniques such as mass-spectrometry (MS), are no longer considered the gold standard. The main reason to use immunological assays was that MS was not used in our centre for the routine determination of Tac at the start of the trial. However, most studies that previously demonstrated an association between *CYP3A5* genotype and Tac pharmacokinetics, were

performed using these same immunoassays and many transplant centres worldwide still rely on them. Second, out of necessity, two types of immunoassays were used to measure Tac C_0. Although this may have added to residual variability in Tac exposure, there was no difference in the primary endpoint between the SDG and the GBG when the analysis was adjusted for assay type (data not shown). Third, the targeted Tac C_0 range may nowadays be considered relatively high.[33] Nonetheless, we feel that it was not excessive[34], the more so given the fact that no induction therapy with T-cell depleting antibodies was prescribed. Furthermore, the optimal Tac exposure early after transplantation remains a matter for debate.[35] Finally, in the French trial,[18] the same Tac C_0 range was aimed for. Because we were aware of this trial, which was ongoing at the time when the present study was planned, and to allow for comparison, it was decided to target the same Tac C_0 range, which was also the standard in our centre at that time. Fourth, and as stated above, one may argue that the *a priori* chances of finding a benefit of a *CYP3A5* genotype-based Tac dosing approach will be highest among *CYP3A5*1* carriers. In Western Europe, the majority of RTRs is of Caucasian descent and if we would have only included patients carrying the minor *CYP3A5*1* allele, this study would have been relevant for about 15% of our transplant population. Fifth and final, although a gene-dose effect has been observed for *CYP3A5* genotype and Tac dose requirement, both *CYP3A5*1* carriers and *CYP3A5*1* homozygotes received the same Tac dose. It was anticipated that the actual number of patients with the *CYP3A5*1/*1* genotype would be limited and therefore it was decided to use a Tac dosing strategy similar to that of the French trial.[18]

In conclusion, basing the Tac starting dose on an individual's *CYP3A5* genotype does not lead to earlier achievement of the target Tac C_0 range or superior clinical outcome as compared with standard, bodyweight-based dosing after kidney transplantation in a low immunological risk population. Therefore, routinely genotyping renal transplant candidates for *CYP3A5* cannot be recommended.

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Supplementary Table 1. Drugs interacting with tacrolimus. Patients using any of these drugs at the time of transplantation were not included in the study.

Drug	Clinical effect
Antibiotics	
Clarithromycin	Increased exposure
Doxycyclin	Increased exposure
Erythromycin	Increased exposure
Rifampin	Reduced exposure
Antiepileptics	
Carbamazepine	Reduced exposure
Phenobarbital	Reduced exposure
Phenytoin	Reduced exposure
Antihypertensive and antiarrhythmic agents	
Amiodarone	Increased exposure
Diltiazem	Increased exposure
Verapamil	Increased exposure
Antimycotic drugs	
Fluconazole	Increased exposure
Itraconazole	Increased exposure
Ketoconazole	Increased exposure
Other	
HIV protease inhibitors	Increased exposure
Theophylline	Increased exposure

Supplementary Table 2. Frequencies of the three *CYP3A5* genotypes in the whole population.

Genotype	Whole population (n = 237)	Standard-dose group (n = 119)	Genotype-based group (n = 118)
*CYP3A5 *1/*1*	9 (3.8%)	4 (3.4%)	5 (4.2%)
*CYP3A5 *1/*3*	54 (22.8%)	27 (22.7%)	27 (22.9%)
*CYP3A5 *3/*3*	174 (73.4%)	88 (73.9%)	86 (72.9%)

Supplementary Table 3. Frequencies of the three *CYP3A5* genotypes in the population according to ethnic group.

Ethnicity	*CYP3A5*1/*1*	*CYP3A5*1/*3*	*CYP3A5*3/*3*
Caucasian (n = 186)	2 (1.1%)	30 (16.1%)	154 (82.8%)
Asian (n = 23)	2 (8.7%)	12 (52.2%)	9 (39.1%)
Black (n = 23)	5 (21.7%)	11 (47.8%)	7 (30.4%)
Other (n = 5)		1 (20.0%)	4 (80.0%)

Supplementary Table 4. Pharmacokinetic data of Tac according to *CYP3A5* genotype (data of the standard-dose and genotype-based dose groups combined).

	***CYP3A5* expressers (*1/*1 & *1/*3)**	**n**	***CYP3A5* non-expressers (*3/*3)**	**n**	***p***
Tacrolimus dose (mg/day)					
Day 3	19.0 (8.0 – 32.0)	62	13.0 (7.0 – 26.0)	174	<0.001
Day 7	18.0 (0.0 – 32.0)	62	12.0 (0.0 – 24.0)	174	<0.001
Day 10	16.0 (1.0 – 36.0)	60	11.0 (0.0 – 22.0)	174	<0.001
Day 14	15.0 (1.0 – 36.0)	60	10.0 (0.0 – 24.0)	173	<0.001
Day 30	12.0 (2.0 – 34.0)	60	8.0 (2.0 – 24.0)	173	0.001
Day 60	12.0 (1.0 – 34.0)	58	6.0 (0.0 – 18.0)	173	<0.001
Day 90	10.0 (0.0 – 34.0)	57	5.0 (0.0 – 18.0)	173	<0.001
Tacrolimus C_0 (ng/mL)					
Day 3	11.2 (2.6 – 30.0)	49	12.8 (4.1 – 30.0)	154	0.205
Day 7	12.2 (7.4 – 22.7)	48	12.5 (5.0 – 30.0)	114	0.829
Day 10	11.7 (5.9 – 18.4)	39	11.8 (4.3 – 23.9)	129	0.615
Day 14	10.0 (5.1 – 15.2)	21	11.4 (5.2 – 19.9)	64	0.040
Day 30	9.4 (2.9 – 15.8)	59	9.6 (3.6 – 30.0)	159	0.373
Day 60	8.6 (3.8 – 20.3)	54	8.0 (3.3 – 14.6)	165	0.116
Day 90	8.0 (3.0 – 12.3)	54	7.6 (2.7 – 16.8)	159	0.609
Tacrolimus C_0/dose (ng/mL per mg/kg)					
Day 3	49.1 (13.8 – 150.9)	48	77.1 (25.8 – 195.3)	153	<0.001
Day 7	50.7 (20.2 – 355.6)	48	82.1 (31.2 – 354.0)	141	<0.001
Day 10	55.4 (22.9 – 678.3)	39	82.7 (22.4 – 467.0)	126	<0.001
Day 14	48.2 (10.8 – 143.2)	21	93.9 (34.6 – 531.6)	61	<0.001
Day 30	56.2 (16.5 – 647.5)	59	93.4 (19.7 – 502.9)	155	<0.001
Day 60	58.5 (14.4 – 185.4)	51	119.0 (21.3 – 439.2)	161	<0.001
Day 90	54.5 (25.8 – 221.9)	52	132.5 (19.6 – 775.0)	157	<0.001

Supplementary Table 5. Pharmacokinetic data of Tac on day 3 after transplantation according to treatment group.

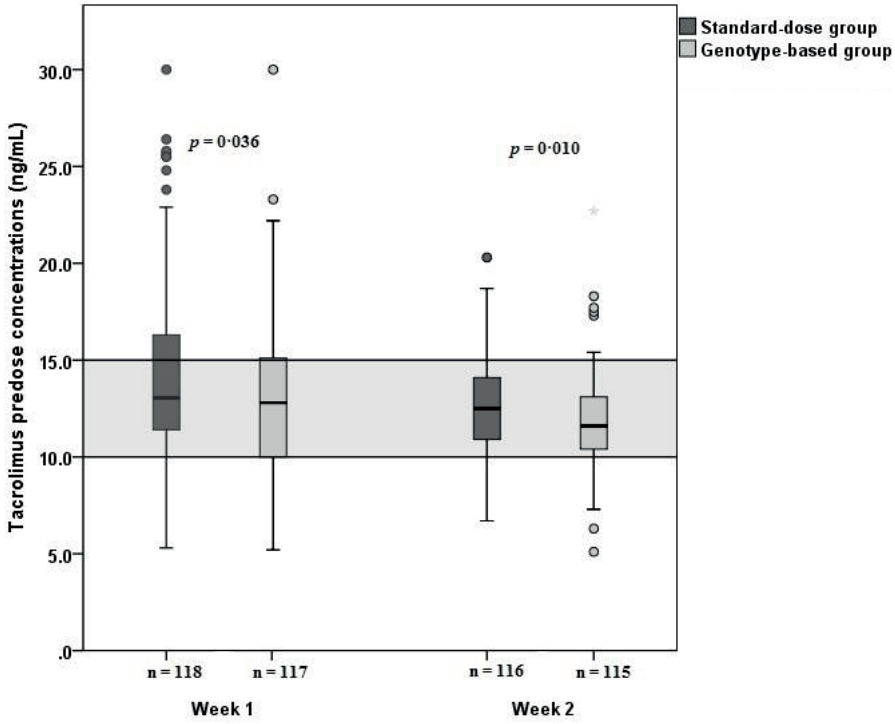
	Standard-dose group	**n**	**Genotype-based group**	**n**	***p***
Tac dose (mg/day)					
All Patients	15.0 (8.0 – 26.0)	119	13.0 (7.0 – 32.0)	117	0.02
*CYP3A5 *3/*3*	15.0 (8.0 – 26.0)	88	12.0 (7.0 – 26.0)	86	< 0.01
*CYP3A5 *1/*3*	14.0 (10.0 – 20.0)	27	24.0 (8.0 – 32.0)	27	< 0.01
*CYP3A5 *1/*1*	14.0 (12.0 – 16.0)	4	20.0 (18.0 – 26.0)	4	0.03
*CYP3A5 *1/*1 & *1/*3*	14.0 (10.0 – 20.0)	31	24.0 (8.0 – 32.0)	31	< 0.01
Tac C_0 at (ng/mL)					
All Patients	13.3 (2.6 – 30.0)	99	11.6 (4.1 – 30.0)	104	0.047
*CYP3A5 *3/*3*	14.5 (5.5 – 30.0)	76	11.6 (4.1 – 30.0)	78	< 0.01

Supplementary Table 5. Pharmacokinetic data of Tac on day 3 after transplantation according to treatment group. (continued)

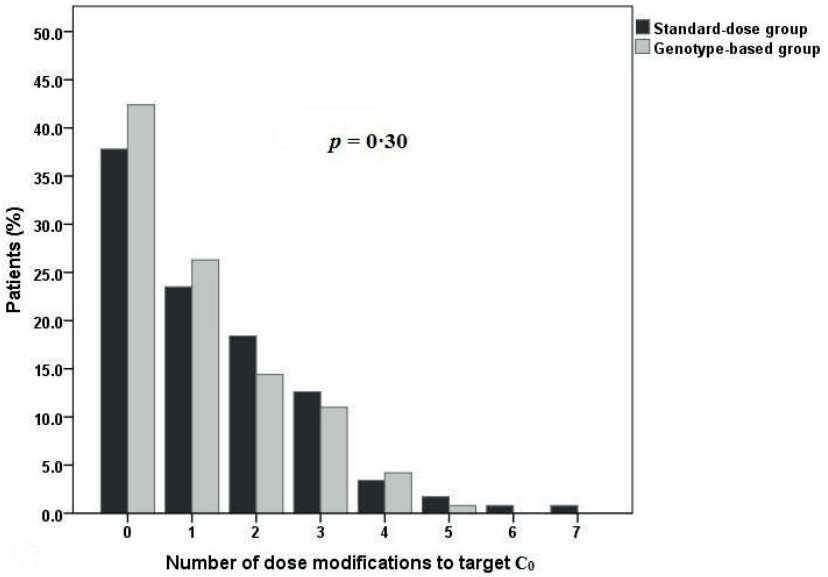
	Standard-dose group	n	Genotype-based group	n	*p*
*CYP3A5 *1/*3*	10.4 (2.6 – 30.0)	19	14.7 (6.5 – 30.0)	23	0.03
*CYP3A5 *1/*1*	6.8 (4.6 – 10.6)	4	9.4 (8.1 – 25.7)	3	0.40
*CYP3A5 *1/*1 & *1/*3*	10.3 (2.6 – 30.0)	23	14.7 (6.5 – 30.0)	26	0.01
Tac C_0/Dose (ng/mL per mg/kg)					
All Patients	64.6 (13.8 – 162.0)	97	72.0 (23.4 – 195.0)	104	0.91
*CYP3A5 *3/*3*	72.3 (26.0 – 162.0)	75	80.1 (25.8 – 195.3)	78	0.97
*CYP3A5 *1/*3*	51.5 (13.8 – 150.9)	18	50.9 (23.4 – 100.8)	23	0.90
*CYP3A5 *1/*1*	34.5 (22.2 – 54.0)	4	30.4 (26.6 – 86.5)	3	0.63
*CYP3A5 *1/*1 & *1/*3*	48.6 (13.8 – 150.9)	22	50.0 (23.4 – 100.8)	26	0.80

Supplementary Table 6. Renal function of the patients (excluding values measured at the time of DGF) according to treatment group.

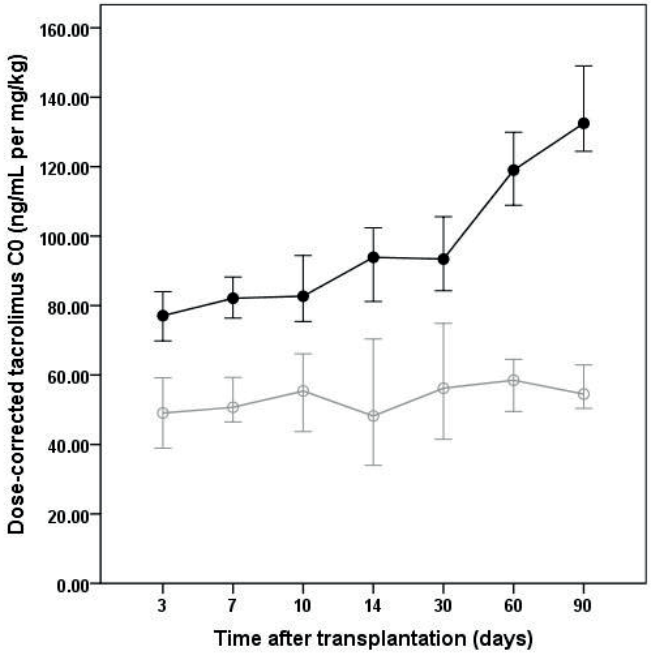
	Standard-dose group	n	Genotype-based group	n
Serum creatinine (µmol/L)				
Day 3	139 (59 - 702)	113	135 (4 - 975)	111
Day 7	132 (66 - 683)	112	137 (64 - 812)	111
Day 10	135 (53 - 501)	98	137 (56 - 458)	93
Day 14	149 (60 - 486)	51	131 (52 - 557)	55
Day 30	121 (58 - 223)	117	127 (52 - 329)	116
Day 60	126 (58 - 313)	116	127 (42 - 363)	116
Day 90	126 (38 - 226)	115	122 (58 - 315)	115
eGFR (mL/min per 1·73 m^2)				
Day 3	44 (7 - 60)	113	44 (5 - 60)	111
Day 7	46 (7 - 60)	112	44 (6 - 60)	111
Day 10	46 (10 - 60)	98	44 (11 - 60)	93
Day 14	40 (10 - 60)	51	48 (10 - 60)	55
Day 30	50 (20 - 60)	117	48 (14 - 60)	116
Day 60	48 (13 - 60)	116	49 (15 - 60)	116
Day 90	47 (20 - 60)	115	50 (18 - 60)	115
Protein/creatinine (mg/mmol)				
Day 3	87.0 (20.3 – 515.4)	76	80.0 (3.1 – 1984.0)	67
Day 7	53.4 (10.3 – 2752.7)	106	58.3 (13.4 – 878.6)	100
Day 10	43.3 (9.2 – 448.5)	57	43.7 (12.7 – 562.5)	67
Day 14	33.4 (7.7 – 1868.0)	46	32.9 (7.1 – 1371.0)	47
Day 30	18.0 (3.3 – 1535.0)	107	24.0 (3.0 – 542.1)	106
Day 60	16.7 (3.8 – 982.3)	105	19.4 (4.9 – 429.6)	105
Day 90	16.4 (3.6 – 1094.0)	107	15.4 (4.7 – 354.3)	104



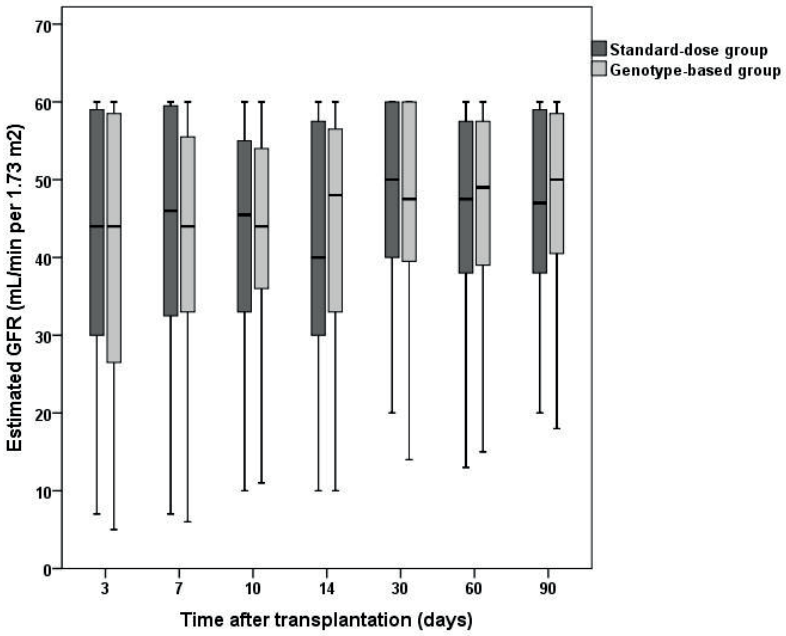
Supplementary Figure 1. Average of all Tac C_0 obtained in the first week (1) after transplantation and the average of Tac C_0 obtained in the second week (2) after transplantation. The boxes depict the median and the 25th and 75th percentile. The whiskers depict the 5th and 95th percentiles. The dots represent the outliers.



Supplementary Figure 2. The number of dose modifications needed to achieve the target Tac C_0 in the standard-dose group and genotype-based group.



Supplementary Figure 3. Tac dose requirement (C_0/D) according to *CYP3A5* genotype. The open circles represent the *CYP3A5* expressers, whereas the closed circles represent the *CYP3A5* non-expressers. Values are depicted as medians. The error bars represent the corresponding 95% confidence intervals.



Supplementary Figure 4. Boxplots describing the distribution of estimated GFR after transplantation (days: 3, 7, 10, 14, 30, 60, and 90). The dark grey boxes represent the standard-dose group (including patients with DGF) whereas the light grey boxes represent the genotype-based group (including patients with DGF). The boxes depict the median and the 25th and 75th percentile. The whiskers depict the 5th and 95th percentiles.

Chapter 4

Intra-patient tacrolimus pharmacokinetic variability

4.1

Intra-patient variability in tacrolimus exposure: causes, consequences for clinical management

Nauras Shuker, Teun van Gelder, Dennis A. Hesselink

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ABSTRACT

Tacrolimus (Tac) is widely used for the prevention of rejection after solid organ transplantation. Finding the optimal balance between effective Tac concentrations and toxicity is a challenge and requires therapeutic drug monitoring. In addition to the well-known inter-patient variability, the clinical use of Tac is also complicated by considerable intra-patient variability (IPV) in Tac exposure. Tac IPV is defined as the amount of fluctuation of whole-blood concentrations over a certain period of time during which the Tac dose remains unchanged. A high IPV in Tac exposure has recently been recognised as a strong risk factor for acute rejection and poor long-term kidney transplantation outcome. In addition to non-adherence, several other factors determine the magnitude of the IPV in Tac exposure. Quantification of IPV is easy and can be easily incorporated into everyday clinical practice as a tool for optimizing transplantation outcomes.

Keywords: Intra-patient variability, kidney, pharmacokinetics, tacrolimus, transplantation

1. INTRODUCTION

Since tacrolimus (Tac) was first introduced into the clinic in the early 1990s, the drug has evolved from an experimental immunosuppressive agent to become the backbone of modern immunosuppressive therapy after renal transplantation.[1] With Tac-based immunosuppression, the incidence of early acute rejection has decreased and 1-year patient and kidney transplant survival have improved considerably. Unfortunately, long-term transplantation outcomes have not improved to a similar degree and many transplanted patients will at some point in their lives suffer from graft failure or premature death.[2]

The causes of long-term kidney allograft loss are multifactorial. About half of successfully transplanted kidneys will fail because of diverse causes such as chronic rejection, late acute rejection, recurrent primary kidney disease, BK virus infection, or nephrotoxicity related to the chronic use of the calcineurin inhibitors (CNIs) Tac and cyclosporine A (CsA).[3] The other half of all graft losses occurs because the recipient dies with a functioning kidney transplant, which is most often caused by cardiovascular disease, malignancy, or infection.[4,5]

The potent immunosuppression provided by Tac and its specific side effects (*e.g.* the induction of diabetes mellitus, hypertension, dyslipidaemia, and nephrotoxicity) undoubtedly contribute to the limited long-term patient and kidney allograft survival. However, attempts to replace Tac with other, equally effective but less toxic immunosuppressive agents, have had limited success. For the next decade or so Tac will remain the first choice immunosuppressive agent, and therefore optimization of Tac-based immunosuppressive therapy is of utmost importance.

Recently, it has become clear that intra-patient variability (IPV) in Tac exposure is a (bio) marker for long-term kidney transplantation outcome.[6] In this review article, the phenomenon of intra-patient variability in Tac pharmacokinetics is explained, the evidence that it predicts long-term transplantation outcome is summarized, and suggestions to use it as a tool for optimizing transplantation results are provided.

2. CLINICAL PHARMACOKINETICS OF TACROLIMUS

Tac pharmacokinetics is characterized by poor bioavailability, which averages 25% (ranging from 5 to 90%).[7] Peak concentrations are usually obtained within 2 hr after oral administration. Tac distributes extensively into tissue and into the cellular fraction of blood. Erythrocytes have a high concentration of the Tac receptor FK-binding protein-12

and this explains why whole-blood Tac concentrations are 15 (range 4 to 114) times higher than those in plasma.[7] More than 90% of the Tac plasma fraction is protein-bound. The drug is mainly excreted in bile (>90%) after biotransformation to at least 15 metabolites by the polymorphically-expressed cytochrome P450 (CYP) 3A4 and 3A5 enzymes. Metabolism largely occurs in the liver but considerable pre-systemic biotransformation takes place in the wall of the intestine. Less than 5% of Tac is eliminated by the kidneys and <1% is eliminated renally as unchanged drug.[7]

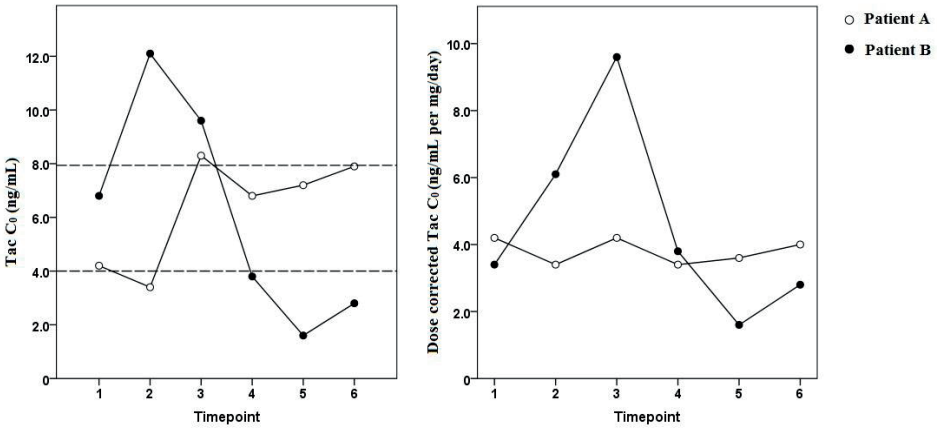
A large variability exists in the pharmacokinetics of Tac between individual patients. Demographic factors and drug-drug interactions are well-known causes of this *inter*-individual variability in Tac disposition.[8] In addition, variation in the genes encoding the metabolizing enzymes CYP3A5 and CYP3A4, and possibly, the drug transporter ABCB1 (previously known as P-glycoprotein; P-gp) and other proteins, explain between-patient differences in Tac pharmacokinetics.[9,10] Another cause of Tac pharmacokinetic variability, which is rarely considered, is circadian rhythm. Several studies have demonstrated that the disposition of Tac is influenced by the time of administration. The Tac C_{max} and AUC after a morning dose is significantly higher than that after an evening dose.[11] Such chronopharmacokinetic changes in Tac exposure may also relate to pharmacodynamic changes, as a lower night-time AUC was found to correspond with the occurrence of acute rejection.[12]

Tac is considered a critical dose drug. A too low exposure to Tac may result in under-immunosuppression and acute rejection, whereas overexposure puts patients at risk for toxicity, which may occur at (whole-blood) concentrations that are considered therapeutic. The high inter-individual variability in its pharmacokinetics, the existence of a concentration-effect relationship, and its narrow therapeutic range have made that therapeutic drug monitoring (TDM) is routinely performed for Tac in most transplant centres throughout the world.[13]

3. INTRA-PATIENT VARIABILITY

In addition to being highly variable *inter*-individually (*i.e.* between patients), Tac pharmacokinetics can also be variable *within* individual patients. This so-called *intra*-patient variability (IPV) in Tac exposure (hereafter referred to as "Tac IPV") is defined as the fluctuation in Tac blood concentrations within an individual over a certain period of time during which the Tac dose is left unchanged.

The large Tac IPV is apparent in everyday clinical practice. Some patients will have a stable Tac exposure that lies within the therapeutic range, whereas others will have highly fluctuating Tac concentrations, often exceeding or falling below the therapeutic range, despite a stable dose and for no apparent reason (Figure 1). It seems reasonable to assume that patients in the latter category are at risk for inferior transplantation outcomes.



	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Mean	SD	CV%	MAD%
Patient A:										
Tac daily dose (mg/day)	1	1	2	2	2	2				
Tac C_0 (ng/mL)	4.2	3.4	8.3	6.8	7.2	7.9				
Tac C_0/dose (ng/mL per mg/day)	4.2	3.4	4.2	3.4	3.6	4.0	**3.8**	**0.38**	**10.0**	**8.8**
Patient B:										
Tac daily dose (mg/day)	2	2	1	1	1	1				
Tac C_0 (ng/mL)	6.8	12.1	9.6	3.8	1.6	2.8				
Tac C_0/dose (ng/mL per mg/day)	3.4	6.1	9.6	3.8	1.6	2.8	**4.6**	**2.88**	**63.4**	**48.4**

Figure 1. Intra-patient variability in Tac exposure (Tac IPV).
An example from clinical practice to illustrate within-patient variability in whole blood Tac C_0. Patient A has a low Tac IPV, whereas patient B has a high Tac IPV. The Tac C_0 of these two patients were measured at regular visits to the outpatient clinic more than one year after transplantation. The target Tac C_0 we aim for one year after transplantation is 4-8 ng/mL (represented by the horizontal lines). The various measures of Tac IPV are given below and were calculated using the formulas from Table 1. The dose-corrected Tac C_0 has a SD of 0.38 and that of patient B of 2.88. Based on the findings of Sapir-Pichhadze *et al.*, the risk that patient B will develop late acute rejection, transplant glomerulopathy, or lose his graft is about twice as high as compared with patient A (hazard ratio 1.84 (95% confidence interval 1.04, 3.25)).[14]

The variance (σ^2) is a commonly used parameter for the quantification of Tac IPV.[14,15] Statistical variance gives a measure of how the data distributes around the mean. Conceptually, it is the expected square difference between an observation and the mean value (denoted as μ). The true variance is not observed, but can be estimated in a data set using the sample variance for an individual patient as described in formula 1 (Table 1).

Table 1. Formulas for the calculation of Tac IPV.

	Parameter	Formula
1	Variance	$\sigma^2 = \sum(X_j - \bar{X})^2/(n-1)$ [1]
2	Coefficient of variation (CV%)	$CV\% = (\sigma/\mu) \times 100$
3	Coefficient of variation (CV%)	$CV\% = \left\{\sqrt{\sum(X_j - \bar{X})^2/(n-1)}/\bar{X}\right\} \times 100$
4	Coefficient of variation (CV%)	$CV\% = \sqrt{e^{\sigma^2_{log}} - 1} \times 100$ [2]
5	Mean absolute deviation (MAD%)	$MAD\% = \frac{1}{n}\sum\frac{abs(X_j - \bar{X})}{\bar{X}} \times 100$ [3]

[1] $\bar{X}$ is the average of all available samples (in the case of Tac IPV, the average of all Tac C_0 measured in time period *j*), X_j is an individual data point (a single Tac C_0 measurement) and n is the number of all available data point (the total number of all available Tac C_0 during time period *j*).

[2] σ^2_{log} is the estimated within-subject variance of the natural log-transformed values.

[3] Abs (...) denotes the absolute value function, so that the quantity $abs(X_j - \bar{X})$ is always a non-negative value. The obtained Tac C_0 has to be corrected to the corresponding daily Tac dose (C_0/D).

The coefficient of variation (CV) is another useful term for the quantification of the IPV.[15-20] The CV is a statistical measure for assessment of the degree of variation, which represents the ratio of the standard deviation (σ) to the μ (formula 2; Table 1). The σ is the square root of the variance (σ^2). In other words, the CV represents the ratio of the square root of the σ^2 to the mean. In a data set, the CV can be estimated using formula 3 (Table 1).

For skewed data, it is often possible to make the distribution of the data normal using a (natural) logarithmic transformation. In this case, an adjusted estimator for CV must be used[16] as described in formula 4 (Table 1). The CV interprets the relative magnitude of the standard deviation. In other words a high/low standard deviation does not automatically mean more/less variable data.

Other investigators have used other statistical measures to calculate Tac IPV, namely the mean absolute deviation (MAD) (formula 5; Table 1).[21] The main difference between MAD and CV is that in the computation of CV, the sum of the squares is taken first and then the square root is taken over the total (= within variance), whereas in the computation of MAD, the square root of each separate term is taken first and then the sum of these

separate terms is taken. The advantage of the MAD is that it less susceptible to outliers, because MAD uses the absolute deviations from the mean, whereas CV uses the squared deviations from the mean.

An important consideration when calculating Tac IPV is the choice of the time period over which Tac concentrations and Tac dosages are collected. In the early phase after transplantation, Tac pharmacokinetics will be subject to variability due to changes in co-medication, including antibiotics and glucocorticoids. Furthermore, during hospitalisation, Tac exposure may change as a result of *e.g.* the inability to ingest the drug or changes in bioavailability.[22] As this inter-occasion variability in Tac exposure may overestimate the true day-to-day variability, in many studies, only Tac concentration measurements obtained during the "stable" phase after transplantation (*i.e.* from 6 months post-transplantation onwards) were included and data from periods of hospitalisation were not considered.

One has to bear the above-mentioned considerations in mind when interpreting the reported magnitude of Tac IPV. A wide range of Tac IPV has been reported in literature with some individuals having a Tac IPV of <5%, and others having a variability of >50% (Figure 2). On average, Tac IPV is between 15 and 30%.[15-21]

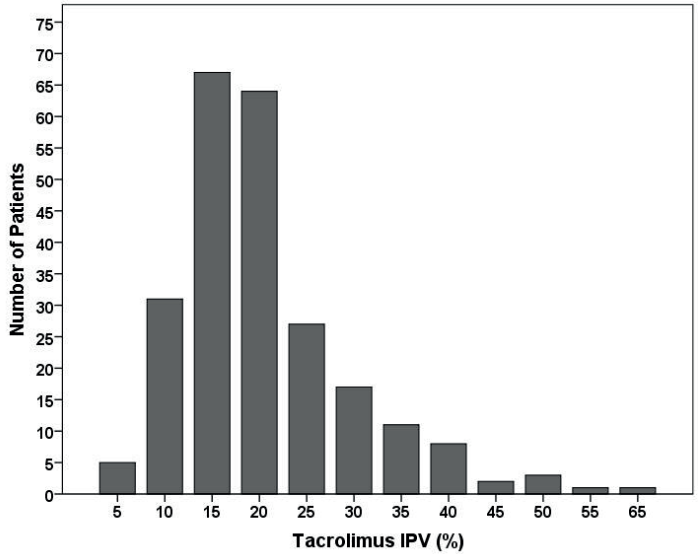


Figure 2. Intra-patient variability in Tac exposure.
Distribution (by 5% intervals) of Tac IPV (*i.e.* MAD, formula 5) in a cohort of 238 stable renal transplant recipients. Adapted from Shuker *et al.*[83] with permission.

4. DETERMINANTS OF INTRA-PATIENT VARIABILITY

Several factors can influence Tac pharmacokinetics and contribute to Tac IPV. In this paragraph these determinants are discussed.

4.1 Type of Tac analytical assay

Worldwide, several different analytical methods to measure Tac are currently in use. Important differences between these techniques may influence Tac IPV. The main disadvantage of immunological methods is cross-reactivity between Tac and its metabolites, which may cause an overestimation of the measured Tac concentration with unacceptable biases. Furthermore, some immunoassays -especially those of the first generation- have insufficient sensitivity to reliably measure Tac in the lower concentration range and suffer from a considerable degree of imprecision (CV% of 18 at lower Tac concentrations (<5.0 ng/mL)).[23-25]

Although better immunoassays are now available, analytical assays based on high-performance liquid chromatography-linked tandem mass spectrometry (HPLC-MS/MS) are more sensitive, selective, and have a higher degree of precision.[26-30] The last decade has seen a gradual shift from immunoassays to HPLC-MS/MS-based Tac concentration measurements and this may add to Tac IPV. However, the latter technique is at the moment not generally available and operational.

4.2 Food

The oral absorption of Tac depends on the fat content of the consumed food and on the time of ingestion. Bekersky *et al.* investigated the influence of a high- *versus* a low-fat meal, relative to the fasting state, on the rate and extent of Tac absorption.[31] The concomitant ingestion of food significantly reduced Tac bioavailability and slowed absorption as compared with the fasted state but did not influence its half-life time.

In other studies, the effect of the timing of a meal on the rate and extent of Tac absorption was investigated. Ingestion of Tac in the fasting state was associated with a significantly higher bioavailability compared with concomitant or delayed (1 – 1½ hr after breakfast) ingestion of Tac.[32,33]

Food that influences the activity of CYP3A and/or ABCB1 can alter the blood concentration of Tac. Grapefruit (*Citrus paradisi*) interacts with a number of drugs, including CNIs.[34,35] Grapefruit juice inhibits the activity of CYP3A and ABCB1, and also down-regulates *CYP3A4* protein expression in the intestinal wall, causing increased Tac exposure.[36]

Egashira *et al.* reported a renal transplant recipient in whom Tac blood concentrations more than doubled after consumption of pomelo (*Citrus maxima* or *Citrus grandis*).[37] Like grapefruit juice, pomelo contains furanocoumarins which are associated with inhibition of CYP3A activity. This observation prompted Egashira and colleagues to investigate the effect of pomelo on the pharmacokinetics of Tac in rats. The clinically-observed interaction between pomelo and Tac was confirmed in this animal experiment.[38] The exposure to Tac was about twice as high in rats pre-treated with 100% pomelo juice compared with animals pre-treated with water. In addition, turmeric (*curcuma longa*) and ginger (*zingiber officinale*) were found to have a comparable effect on Tac pharmacokinetics. Turmeric changes both the function and expression of ABCB1 and CYP3A4, whereas ginger reportedly changes the activity of this same enzyme and efflux transporter.[39,40]

4.3 Diarrhea

Several investigators reported that diarrhoeal illness leads to increased Tac levels.[41-46] Maes *et al.* demonstrated in 26 renal transplant recipients that severe diarrhea (more than 3 loose stools daily) caused a significant (100%) increase in Tac pre-dose concentrations. This rise in Tac concentrations necessitated a Tac dose reduction of 30% to obtain pre-diarrhea Tac exposure.[47] However, less severe diarrhea does not lead to increased Tac exposure.[48] Lemahieu *et al.* demonstrated that the increase in Tac concentrations in patients with diarrhea can be attributed to an increased oral bioavailability. This was the result of reduced intestinal ABCB1 activity which was possibly caused by mucosal injury.[49,50] A shortened intestinal transit time leading to a higher Tac delivery to the ileum, where CYP3A4 expression is lower than in the proximal intestine, could be another explanation for the increased Tac absorption seen during episodes of severe diarrhea.[51]

4.4 Drug-drug interactions

A drug may alter how the body absorbs, distributes, metabolises, or excretes another drug. For Tac, many such drug-drug interactions have been described.[8,52] Most of the described drug interactions with Tac are caused by inhibition or induction of the intestinal and hepatic CYP3A system. Well-known interacting drugs are glucocorticoids, calcium-channel blockers, ritonavir, azole antifungals, rifampin, and several anti-epileptic drugs.[53-55] In addition, non-prescription (over-the-counter) drugs and herbal preparations may also interact with Tac. St John's wort (*Hypericum perforatum*) is one of the most commonly used herbal medicines for the treatment of depression, anxious states, and sleep disorders. Several studies have demonstrated that St John's wort may induce hepatic and intestinal *CYP3A4* and intestinal *ABCB1*, whereas the herb does not appear to influence CYP3A5 expression.[56-58] Mai *et al.* showed that St John's wort decreases the bioavailability of Tac.[59] Treatment with 600 mg/day of St John's wort extract for 14 days reduced the Tac dose-corrected $AUC_{0\text{-}12}$ by 57.8%. To maintain therapeutic Tac concentrations,

dose adjustments from a median of 4.5 mg/day at baseline to 8.0 mg/day under St John's wort treatment were required.[59]

4.5 Genetic factors

The inter-patient variability in Tac exposure is in part explained by genetic differences between individuals. The *CYP3A5*3* allele, which causes a loss a functional CYP3A5 protein, has been repeatedly and consistently associated with Tac exposure.[10,60] Patients who express CYP3A5 (those with the *CYP3A5*1/*3* or *CYP3A5*1/*1* genotype) need an approximately 50% higher dose to reach target concentrations as compared with CYP3A5 non-expressers (those with the *CYP3A5*3/*3* genotype).[10,60,61] Recently, other genetic variants were associated with Tac dose requirement.[62,63]

Whether *CYP3A5* genotype also affects variations in Tac exposure within an individual over time has been the subject of several recent investigations. In a Korean bioequivalence study conducted in healthy volunteers, *CYP3A5* non-expressers had a 52% and 41% higher IPV in Tac C_{max} and $AUC_{0\text{-last}}$ (AUC from time zero to time of the last measurable concentration), respectively as compared with *CYP3A5* expressers.[64] The authors postulated that Tac metabolism in *CYP3A5* non-expressers is fully dependent on the activity of CYP3A4. Because CYP3A4 is very much prone to inhibition/induction, *CYP3A5* non-expressers might have greater Tac pharmacokinetic fluctuations.

Conversely, three studies, conducted in 249, 209, and 118 stable renal transplant recipients, demonstrated no significant association between Tac IPV and *CYP3A5* genotype.[65-67] Although the reasons for these conflicting results are not clear, it may relate to sample size (n = 29 in the study of Yong *et al.*), the populations studied (patients *versus* volunteers), and the fact that in the volunteer study, two single doses of two different Tac formulations were administered in a cross-over design.

4.6 Non-adherence

Adherence to the immunosuppressive drug regimen after renal transplantation is poor and an important risk factor for poor long-term outcomes.[68] Obviously, non-compliance with the prescribed immunosuppressive regimen determines variability in Tac exposure. When patients do not take their medication on certain occasions or "bolus" themselves before visiting the outpatient clinic to make up for missing dosages, Tac concentrations will fluctuate.[15] For an in-depth review of issues pertaining to non-adherence, the reader is referred to the article by Shemesh *et al.* in this edition of *Transplantation Reviews.*

4.7 Generic Tac substitution

Several generic, twice-daily Tac formulations are now available. Substitution of the innovator drug for generic Tac may increase Tac IPV in several ways. First, although manufacturers are required to demonstrate bioequivalence of their generic drug with the innovator drug, there is no requirement to demonstrate bioequivalence with the other generic formulations.[69] Still, the general belief is that all generics can be used interchangeably. In daily practice, prescribing physicians are not always informed when a pharmacist substitutes the brand name drug for a generic preparation. When one generic formulation is substituted for another, it is even less likely that the transplant physician will be notified. Limited data suggest that on average, switching from one formulation to another will result in comparable Tac exposure. However, this may not always be the case on an individual basis and monitoring Tac concentrations after switching is recommended.[70] Perhaps more importantly, patients may inadvertently make medication errors after being switched. There is no requirement for generic formulations to have the same shape and color as the brand formulation. From personal experience we know that some patients do not realize this is the same drug, and take different Tac formulations at the same time causing over-exposure. We have also seen patients that out of confusion decided not to take the drug at all putting them at risk for rejection. Finally, successively prescribing different generic formulations may negatively affect drug adherence, another important contributor to high IPV.[70]

5. INTRA-PATIENT VARIABILITY AND RENAL TRANSPLANTATION OUTCOME

Kahan *et al.* were the first to demonstrate that a high intra-individual variability in CsA exposure increases the risk of developing chronic rejection after kidney transplantation.[71,72] In a cohort of 204 adult renal transplant recipients treated with the oil-based CsA formulation (plus prednisolone), serial pharmacokinetic profiles for CsA were determined. The incidence of chronic rejection over a period of 5 years was 24% among patients with a low variability in CsA exposure *versus* 40% in the highly variable cohort. Healthcare-associated costs were higher among patients with a high intra-individual variability of CsA exposure.[71]

Stoves and Newstead found that among 103 renal transplant recipients, younger age and a highly variable CsA exposure (determined using CsA C_0) while on the micro-emulsion formulation (Neoral®) predisposed these patients to the development of chronic allograft nephropathy.[73] The ROC analysis identified a CV cut-off of 20-24% to predict chronic allograft nephropathy.[73] Other authors have reported worse kidney allograft survival,

poorer renal transplant function, and higher acute rejection rates among patients with a high intra-patient variability of CsA exposure as compared with patients with less variability.[74-76]

The first evidence that a high IPV in the clearance of Tac also negatively affects long-term kidney transplantation outcome, was provided by Borra *et al.*[21] In this study, the Tac IPV of 297 renal transplant recipients treated with Tac and mycophenolate mofetil (MMF) was calculated and based on Tac whole-blood C_0 that were measured at the outpatient clinic between month 6 and 12 after transplantation. Tac IPV was then related to "graft failure", a composite endpoint of graft loss, histologically-proven chronic allograft nephropathy, or doubling of serum creatinine in the period between month 12 after transplantation and last follow-up.[21]

After a mean follow-up of 1849 ± 585 days, 34 of the 297 patients reached the graft failure endpoint. The mean Tac IPV was 17.0%. Based on the mean, the group was then divided into a group with a low Tac IPV (n = 148 with a mean Tac IPV of 9.6%) and a group with a high Tac IPV (n = 149 with a mean Tac IPV of 24.2%). In the group of patients with graft failure, there were significantly more patients with a high IPV (24/34 or 71%) than a low IPV (10/34 or 29%). Among patients who did not reach the primary endpoint, there was no difference in the proportion of patients with a high or low Tac IPV (47.5% *versus* 52.5%, respectively). Tac concentrations were not different between cases and controls and the incidence of acute rejection in the first post-transplant year did not differ between patients with a high or low Tac IPV. Finally, a multivariate Cox regression analysis demonstrated that Tac IPV was a predictor of poor outcome, together with recipient age and the occurrence of an acute rejection episode in the first post-transplant year. The influence of a high Tac IPV on graft survival was comparable to that of acute rejection.[21]

Ro and colleagues reported that a high Tac IPV was associated with more acute rejection after kidney transplantation among 249 adult, Korean patients.[65] Only a few patients lost their grafts and therefore no meaningful analysis of the effect of Tac IPV on long-term transplantation outcome could be performed. Sapir-Pichhadze *et al.* have performed a rigorous statistical analysis of Tac IPV in relation to kidney transplantation outcome.[14] In 356 adult, Canadian renal transplant recipients, a higher Tac IPV was associated with worse long-term outcome (a composite endpoint of late (*i.e.* occurring after year 1) allograft rejection, transplant glomerulopathy, graft loss, or death with a functioning transplant). Furthermore, a dose-response relationship between the height of Tac IPV and the relative hazard of adverse kidney transplant outcomes was observed.[14]

Comparable observations have been made in children. In a group of 144 older (>8 years) pediatric solid organ transplant recipients who had either received a kidney, heart, lung, or liver transplant, a high variability in Tac predose concentrations (assessed by the standard deviation) was an independent risk factor for late allograft rejection and graft loss. A threshold of clinical significance was a standard deviation of the Tac concentration >2. Each 1-point increase above this cut-off was associated with a hazard of 1.58 for graft loss.[77] Hsiau *et al.* reported a higher risk of histologically-confirmed kidney transplant rejection among children with a high Tac IPV as compared to children with a low Tac IPV.[15] ROC analysis demonstrated that a Tac CV% of more than 41% was associated with an increased risk of allograft rejection with an odds ratio of 9.7.[15] In a study which included 69 Dutch children with a kidney transplant, a high Tac IPV (assessed by the CV) was again associated with a higher risk of late rejection but did not significantly correlate with loss of renal function, although this may have been due to the small sample size.[20]

6. INTERVENTIONS AIMED AT REDUCING INTRA-PATIENT VARIABILITY

There is considerable evidence that patients with highly fluctuating Tac concentrations are at increased risk for poor kidney transplantation outcomes and this calls for interventions. Once a patient is identified as having a high Tac IPV (using the proper analytical and statistical methods), physicians should ask about dietary habits, the use of interacting and over-the-counter drugs, possible substitution by generic formulations, and changes in bowel movement. Patients should be instructed to take their Tac in a consistent manner, both regarding to the meal content (if possible) and timing of ingestion relative to consumption of meals. The use of interacting substances should be addressed, and any intestinal illness should be treated.

Improving non-adherence to the immunosuppressive drug regimen may be the most important intervention to reduce Tac IPV and improve long-term transplantation outcomes. Several strategies to do so are available.[78-80] These will not be discussed further here as this topic is covered in-depth by Shemesh *et al.* in this edition of *Transplantation Reviews*.

Finally, switching from the standard, immediate release Tac formulation to a modified-release, once-daily Tac formulation (Tac-OD) should be considered. Advagraf® (Astellas Pharmaceutical Company) has been approved for the prevention of rejection after kidney and liver transplantation.[81] For this formulation, it was demonstrated by Kuypers *et al.* that the use of Tac-OD does lead to better adherence as compared with the standard, twice-daily Tac formulation.[82]

The effect of conversion to Tac-OD on Tac IPV has been the subject of several studies. In a cohort of 129 Taiwanese, stable renal transplant recipients, conversion to Tac-OD led to a significantly lower IPV of Tac C_0 (decreasing from 14.0 to 8.5%).[18] Stifft *et al.* observed a lowering of Tac IPV in $AUC_{0\text{-}24}$ by 3.2% after conversion to Tac-OD in a prospective study including 40 renal transplant patients.[16] However, several other investigators did not find a reduction of Tac IPV after conversion from immediate-release Tac to Tac-OD.[17,19,83] No data regarding Tac IPV of a novel, extended-release OD tacrolimus formulation (Tac-LCPT) is available.[84]

7. CONCLUSIONS

A high Tac IPV is a risk factor for poor long-term outcomes of kidney transplantation. Incorporating algorithms that calculate Tac IPV into electronic patient files may aid clinicians to rapidly recognize patients at risk for inferior transplantation results. Once patients with highly fluctuating Tac concentrations are identified, education with regard to the effects of food and over-the-counter medication on Tac exposure should be attempted. When non-adherence is suspected, interventions aimed at improving drug compliance and possibly, switching to a Tac-OD formulation should be considered. Tac IPV is a cheap and widely available predictor for transplantation outcome that can be easily incorporated into everyday clinical practice. This is an opportunity that the transplant community should not miss.

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4.2

A high intra-patient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation

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ABSTRACT

Tacrolimus is a critical dose drug with a considerable intra-patient variability (IPV) in its pharmacokinetics. We investigated whether a high IPV in tacrolimus exposure is associated with adverse long-term renal transplantation outcomes. Tacrolimus IPV was calculated from pre-dose concentrations measured between 6 and 12 months post-transplantation of 808 renal transplant recipients (RTRs) transplanted between 2000 and 2010. One hundred eighty-eight (23.3%) patients reached the composite end-point consisting of graft loss, late biopsy-proven rejection, transplant glomerulopathy, or doubling of serum creatinine concentration between month 12 and last follow-up. The cumulative incidence of the composite endpoint was significantly higher in patients with high IPV than in patients with low IPV (hazard ratio 1.41, 95%-CI: 1.06 – 1.89; $p = 0.019$). After adjustment for several factors the higher incidence of the composite endpoint for RTRs with a high IPV remained statistically significant (hazard ratio 1.42 (95%-CI: 1.06 – 1.90; $p = 0.019$). Younger recipient age at transplantation, previous transplantation, worse graft function (at month 6 post-transplantation) and low mean tacrolimus concentration at 1-year post-transplantation were additional predictors for worse long-term transplant outcome. A high tacrolimus IPV is an independent risk factor for adverse kidney transplant outcomes that can be used as an easy monitoring tool to help identify high-risk RTRs.

INTRODUCTION

Tacrolimus (Tac) is widely used as part of the immunosuppressive regimen for kidney transplantation. It is a critical-dose drug with a considerable intra-patient variability (IPV) in its pharmacokinetics, which is defined as the fluctuation in Tac concentrations within an individual patient over a certain period of time during which the Tac dose is unchanged.[1,2] A high IPV in Tac exposure may be caused by behavioral factors, interacting co-medication, food,[1-5] and to a lesser extent, genetic factors.[6-8] Whatever the cause, a high Tac IPV may result in a Tac exposure which is outside the therapeutic window. These patients may be at risk for under-exposure and rejection, or Tac toxicity in case of over-exposure.

Late allograft rejection and graft loss remain important problems in the field of solid organ transplantation. The first evidence for the clinical importance of Tac IPV was obtained by Borra *et al.*[9] In this study, it was demonstrated that a high Tac IPV was associated with reduced kidney transplant survival. In a Korean study, it was shown that renal transplant recipients (RTRs) with a high Tac IPV had a significantly higher risk to develop a biopsy-proven acute rejection (BPAR) than patients with a low Tac IPV (hazard ratio, 2.66; 95%-CI: 1.39 – 5.06; $p = 0.003$).[10] Recently, Sapir-Pichhadze *et al.*, in a study which included 356 adult RTRs, observed that a higher Tac IPV was associated with more late allograft rejection, transplant glomerulopathy, graft loss and death with a functioning transplant.[11] In pediatric kidney transplantation, a high Tac IPV has also been associated with increased late rejection and graft loss.[12-14]

A limitation of the above-mentioned studies was their limited sample size and the relatively short follow-up period. The small number of events may have hampered the multivariate analyses of the obtained data. This prompted us to substantially enlarge our original study population[9] and extend the duration of clinical follow-up to evaluate in this extended population whether a high Tac IPV is associated with a composite endpoint consisting of late acute rejection, transplant glomerulopathy, graft loss or doubling of serum creatinine.

SUBJECTS AND METHODS

Patients and setting

This was a retrospective cohort study. The study cohort included RTRs who were transplanted and followed at the Erasmus MC, University Medical Centre Rotterdam, the Netherlands, between January 2000 and December 2010. Adult (age >18 years) RTRs

were included if they were treated with Tac and mycophenolate mofetil (MMF) in the period between 6 and 12 months after kidney transplantation, survived the first post-transplant year and had an estimated glomerular filtration rate (eGFR) of ≥ 25 mL/min at month 12 after transplantation. Patients who were treated between month 6 and 12 with an immunosuppressive regimen which did not consist of Tac plus MMF or who received a multi-organ transplant were not included. Usage of low-dose prednisolone, which is given in our center in the first three post-operative months as a component of the routine immunosuppressive regimen, was not an exclusion condition.

Tac concentrations were determined in whole blood by several kinds of immunoassays. Details on the sensitivity and reproducibility of the EMIT assay in our laboratory have been published previously.[15] Proficiency samples were obtained from the United Kingdom Quality Assessment Scheme (Dr. Holt, St George's Hospital Medical School, London, UK). The laboratory successfully participates in international proficiency testing schemes. The target Tac C_0 between 6 and 12 months post-transplantation was between 4-10 ng/mL.

Endpoints

Because we hypothesized that a high IPV in Tac exposure could result in frequent under-immunosuppression, the outcome of interest was a composite endpoint named "event" which consisted of graft failure [defined as re-transplantation, (re)start of dialysis or an eGFR ≤ 15 mL/min], late BPAR (*i.e.* occurring after month 12), histologically-confirmed transplant glomerulopathy, or doubling of serum creatinine concentration in the period between month 12 after transplantation and last follow-up, taken the serum creatinine concentration at month 12 as a reference. Biopsies were performed for cause only. Patients who died with a functioning graft and who did not have signs of transplant glomerulopathy or acute rejection were considered not to have reached the endpoint and were censored.

Intra-patient variability and outcome variables

The variable of interest was the IPV of Tac. For its calculation, at least 3 pre-dose Tac concentrations (C_0) for an individual patient had to be available. A median of 5 (range: 3 – 11) Tac C_0 measurements were used to calculate Tac IPV. Since RTRs are not on a stable Tac dose in the first phase after transplantation and because they often use interacting drugs [such as for example, antibiotics and (pulse) glucocorticoids] in this period, only data on Tac exposure measured at outpatient clinic visits in the period of 6-12 months post-transplantation were collected. Tac concentration measurements obtained during hospitalization were not considered. As not all patients received a constant drug dose between months 6-12, the obtained C_0 were corrected for the corresponding daily Tac dose (C_0/D). The IPV in Tac exposure (from now on referred to as "Tac IPV") between months 6 and 12 post-transplantation was calculated as:

$$IPV\% = \frac{1}{T}\sum_{t=1}^{T}\frac{abs(X_t - \bar{X})}{\bar{X}} \times 100,$$

where $\bar{X}$ is the mean C_0/D of all available samples in the period of month 6-12 after transplantation; X_t is an individual value of C_0/D measured in the period mentioned; and *T* is the number of all available values for an individual patient.

Statistical analysis

The distribution of baseline characteristics is reported using summary statistics and frequency tables for continuous and categorical variables, respectively. The sample was divided into groups by a dichotomized version of Tac IPV, using the median as threshold. The probability to have reached the composite endpoint as a function of the time since year one after transplantation was calculated using the Kaplan-Meier method, and compared between groups using the log-rank test.

Univariable and multivariable Cox regression analyses were performed to study the association between Tac IPV, other clinical variables and the composite endpoint. The time origin for the survival analysis was one year post-transplantation. Besides Tac IPV (coded as a dichotomous variable), the Cox regression analyses included the following covariates: recipient age at transplantation, recipient gender, recipient ethnicity, primary kidney disease, panel reactive antibody level, donor type (living or deceased donor), transplant number (1 *vs.* > 1), number of HLA-mismatches, transplant year, delayed graft function, eGFR at 6-months post-transplant, acute rejection in the first year, and the mean of the average Tac concentrations measured for an individual patient in the period between 6 and 12 months after transplantation. The covariates in the multivariable Cox regression model were selected from these variables using a backward elimination method with a threshold for removal of $p = 0.20$. We assessed the proportional hazard assumption by testing for an interaction between time and covariates in a multivariable Cox-regression with time-dependent covariates.

To test our hypothesis that high Tac IPV could put the patients who are usually exposed to low Tac concentrations at higher risk to lose their graft than patients who are usually at optimal Tac exposure, effect modification was tested by including the interaction term of IPV and Tac concentration as a covariate in the multivariable Cox regression model. This interaction term was tested in a model that included the main effects of IPV and Tac concentration as covariates (irrespective of the associated p-values), as well as covariates that were selected using backward elimination.

Finally, we considered the possibility of differential effects of Tac IPV in the first 2 years of follow-up (*i.e.* between 12 and 36 months after transplantation) *versus* the remaining follow-up period, by adding a time-dependent covariate to the Cox-regression and testing its significance. This covariate was defined as the Tac IPV (which was measured between 6 and 12 months after transplantation) between 12 and 36 months after transplantation, and as 0 after 36 months.

Patients with missing data for one or more covariates were dropped from the multivariable Cox regression. Statistical analyses were performed using IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL). All tests were two-sided and a p-value < 0.05 was considered statistically significant.

RESULTS

Between January 2000 and December 2010, a total of 1232 adult patients were transplanted and started on Tac/MMF-based immunosuppression. A total of 424 patients were excluded from the present analysis, leaving a final study cohort of 808 patients. The characteristics of these patients are presented in Table 1. The reasons for not including the 424 patients were the following: death within the first year after transplantation (n = 31); graft failure within first year after transplantation (n = 70); GFR below 25 mL/min at month 12 after transplantation (n = 50); multi-organ transplant (n = 4); no treatment with tacrolimus and MMF (n = 179); less than 3 Tac C_0 measurements available (n = 31); insufficient data available (n = 59).

The median follow-up was 1993 days (5.5 years) with a range of 23-5130 days (0.06 -14.1 years) beyond the first year after transplantation. A total of 188 events (23.3%) were documented during 4823 person-years at risk: 68 cases of graft loss, 69 cases of late BPAR, 39 cases of transplant glomerulopathy, and 12 cases of doubled serum creatinine.

At 12 months after transplantation, the median Tac dose was 4.2 mg/day (0.10 – 28.0 mg/day). Among patients who did not reach the composite end point the median Tac dose was 4.2 mg/day (1.0 – 28.0), whereas this was 4.4 mg/day (0.10 – 22.7) among patients who reached the composite end point. The corresponding median Tac C_0 was 7.2 ng/mL (1.8 – 16.5). The median Tac C_0 was 7.4 ng/mL (1.8 – 16.5) and 6.9 ng/mL (2.3 – 15.5) in patients who didn't reach and patients who reached the composite endpoint, respectively.

Table 1. Characteristics of renal transplant recipients.

	Number of patients (n = 808)	Summary measure
Gender recipient		
Male / Female	521 / 287	64.5% / 35.5%
Age of recipient (years)	808	51 (18- 77)
Ethnicity		
Caucasian	618	76.5%
Asian	84	10.4%
Black	61	7.5%
Other	45	5.6%
Primary kidney disease		
Diabetic nephropathy	98	12.1%
Polycystic kidney disease	105	13.0%
Glomerulonephritis	202	25.0%
Hypertensive nephropathy	175	21.7%
Reflux disease / Chronic pyelonephritis pylonephritis	68	8.4%
Other	91	11.3%
Unknown	69	8.5%
Number of kidney transplantation		
1st	662	81.9%
2nd	117	14.5%
≥3rd	29	3.6%
Donor type		
Living / Deceased	519 / 289	64.2% / 35.8%
Delayed graft function		
Yes / No	148 / 658	18.3% / 81.4%
Acute rejection in the first post-transplant year		
Yes / No	165 / 643	20.4% / 79.6%
PRA%[§]	803	0.0 (0.0 – 96.0)
Peak PRA%	804	4.0 (0.0 – 100.0)
HLA mismatches	807	3 (0 – 6)
Transplant year		
2000 - 2005	328	40.6%
2006 - 2010	480	59.4%
Serum creatinine (μmol/L) at 6 months	808	125 (43 – 273)
eGFR* (mL/min/1.73 m^2) at 6 months	808	50 (21 – 90)
Tac C_0** (ng/mL)	808	7.2 (1.8 – 16.5)

[§]PRA = Panel reactive antibodies; *eGFR = estimated glomerular filtration rate; **Mean of the average Tac concentrations measured in the period between 6 and 12 months after transplantation. The summary measure for non-normally distributed variables is the median (range). For binary or categorical variables, the summer measure is the proportion.

The median Tac IPV was 16.2% [range (1.1% – 76.0%); Figure 1]. Dividing patients into two groups based on their variability, using the median as cut-off, resulted in 404 patients in the low-variability group, with a mean variability of 11.0% [median = 11.6% , range (1.1% - 16.1%)] and 404 patients with high variability, with a mean IPV of 25.1% [median = 22.6%, range (16.2% – 76.0%)].

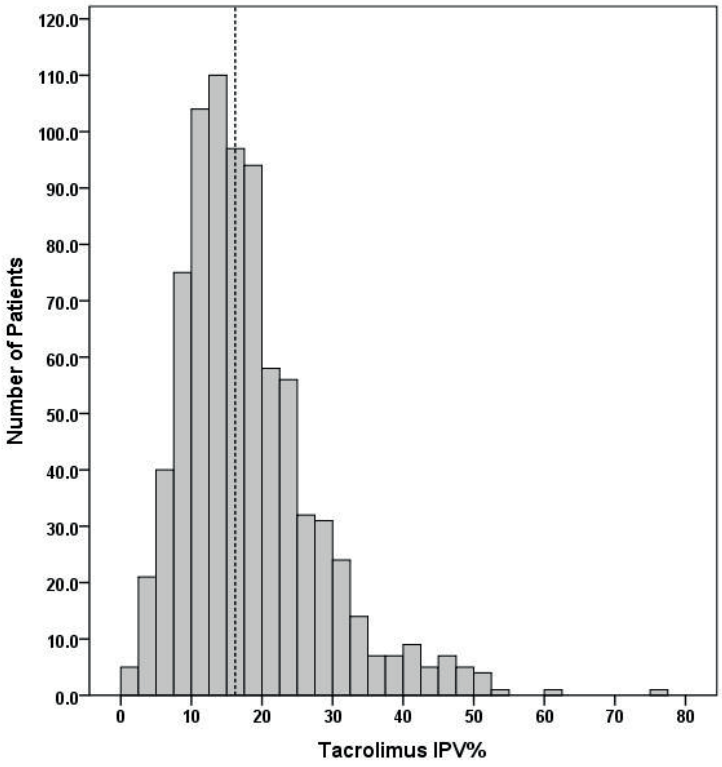

Figure 1. Distribution of Tac IPV in the studied cohort (n = 808). The mean Tac IPV was 18.1% (± 9.7); the median (shown by dotted line) Tac IPV was 16.2% (1.1% - 76.0%).

To visualize the association between Tac IPV and the composite endpoint, a Kaplan-Meier curve was constructed for patients with low and high Tac IPV (Figure 2). Kaplan-Meier analysis demonstrated a cumulative incidence of the composite endpoint of 41.8% by 14-years post-transplant for the composite end-point in patients with low Tac IPV compared with 49.5% in patients with high Tac IPV. As shown in Figure 2, long term transplant outcomes were significantly worse in patients with high Tac IPV (p = 0.018).

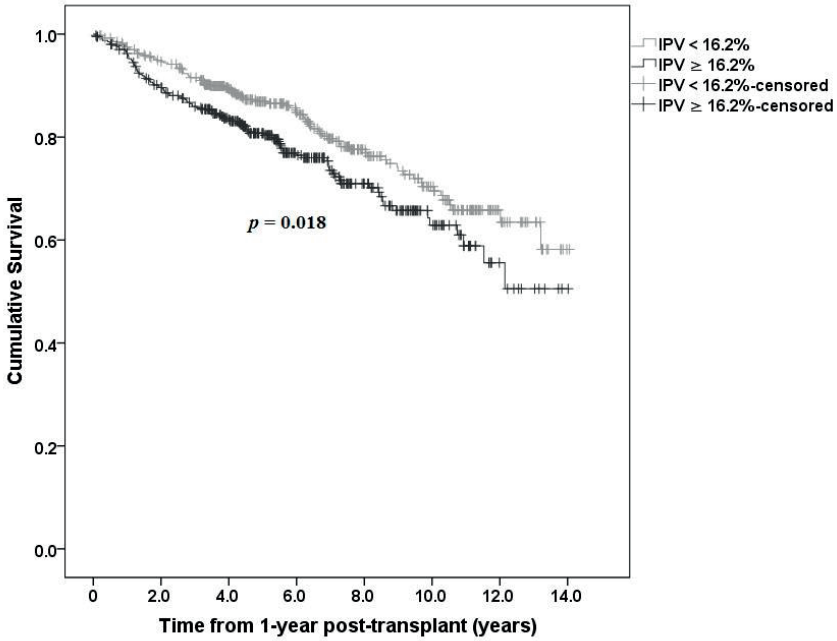

Figure 2. Kaplan-Meier survival curves for patients with low (< 16.2%) and high (≥ 16.2%) Tac IPV. These groups were compared using the log-rank test.

Survival analysis

To determine whether the Tac IPV is a predictor for poor transplant outcome, univariable and multivariable Cox regression analyses were performed. In the univariable analyses, significance was found for six covariates, including Tac IPV (Table 2). Univariable analyses showed a 41.3% (hazard ratio of 1.413, 95%-CI: 1.059 – 1.886; p = 0.019) increase in the risk for the composite end-point for patients with high Tac IPV compared to those with low Tac IPV.

Only four patients (0.5%) were dropped from the multivariable Cox regression because of missing covariate data. The multivariable Cox regression analysis confirmed that high Tac IPV was associated with poor kidney transplant outcome (hazard ratio of 1.42, 95%-CI: 1.059 – 1.903; p = 0.019. Table 3). Also using Tac IPV as a continuous variable the multivariable Cox regression analysis demonstrated a 1.4% increase in the hazard for composite end-points for every one-unit (1%) increase in Tac IPV (hazard ratio of 1.014, 95%-CI: 1.000 – 1.028; p = 0.043). Allowing for differential effects of Tac IPV during the first 2 years of follow-up yielded an estimated hazard ratio of 2.03 during the first 2 years of follow-up and 1.20 during the remaining follow-up period. However the difference between these two hazard ratios was not statistically significant (p = 0.10).

Table 2. Univariable Cox proportional hazards analyses for the influence of clinical variables on the outcome of graft failure censored for death.

	Hazard ratio (95%-CI)	*p*-value
eGFR at 6-months (mL/min)	0.988 (0.979 – 0.998)	0.016
Recipient age at transplantation (year)	0.982 (0.972 – 0.992)	< 0.001
Mean Tac concentration (ng/mL)	0.890 (0.819 – 0.967)	0.006
Transplant number (1st)	1.296 (1.073 – 1.565)	0.007
Tac IPV% (low *vs.* high)	1.413 (1.059 – 1.886)	0.019
Tac IPV% (continuous variable)	1.015 (1.001 – 1.028)	0.030
Acute rejection in the first year	1.425 (1.021 – 1.989)	0.037
Peak PRA(%)	1.005 (1.000 – 1.010)	0.052
PRA(%)	1.005 (0.997 – 1.013)	0.196
Ethnicity		0.452
Caucasian	Reference	
Asian	1.285 (0.826 – 1.999)	0.266
Black	1.327 (0.791 – 2.228)	0.284
Other	0.831 (0.424 – 1.631)	0.591
Primary kidney disease		0.138
Diabetic nephropathy	Reference	
Polycystic kidney disease	0.710 (0.381 – 1.323)	0.281
Glomerulonephritis	0.923 (0.550 – 1.550)	0.762
Hypertensive nephropathy	0.892 (0.519 – 1.535)	0.681
Reflux disease / Chronic pyelonephritis	1.544 (0.861 – 2.767)	0.145
Other	0.799 (0.432 – 1.478)	0.475
Unknown	0.692 (0.331 – 1.445)	0.327
HLA mismatch (none)	1.058 (0.967 – 1.157)	0.217
Transplant year (per year)	1.018 (0.965 – 1.074)	0.518
Recipient gender (male)	0.927 (0.686 – 1.252)	0.620
Delayed graft function (no)	0.923 (0.631 – 1.350)	0.679
Donor type (living)	1.045 (0.778 – 1.404)	0.770

Recipient age at transplantation, eGFR at 6-months post-transplantation, transplant number and the average Tac C_0 measured in the period between 6-12 post-transplantation were also found to be independent predictors for transplant outcome (Table 3). The proportional hazards assumption was not violated, suggesting that the hazard ratios were constant with time.

Based on the mean Tac C_0 at 12-months after transplantation (baseline), the patients were divided into four groups using the quartiles of mean Tac C_0 as cut-off values. The interaction term of Tac C_0 subgroup and Tac IPV was added to the multivariable Cox proportional hazards model to determine the statistical significance of the resulting interaction term.

Table 3. Results of the multivariable Cox regression analysis. Impact of Tac intra-patient variability on the composite endpoint (graft failure, late biopsy-proven acute rejection, transplant glomerulopathy or doubling of serum creatinine concentration) censored for death.

	Hazard ratio (95%-CI)	*p*-value
Recipient age at transplantation (year)	0.980 (0.970 – 0.991)	< 0.001
eGFR at 6-months (mL/min)	0.985 (0.976 – 0.995)	0.002
Tac IPV% (high)	1.420 (1.059 – 1.903)	0.019
Transplant number (>1)	1.505 (1.066 – 2.125)	0.020
Mean Tac concentration (ng/mL)	0.913 (0.839 – 0.994)	0.036
HLA mismatch (none)	1.087 (0.989 – 1.194)	0.084
DGF	0.736 (0.473 – 1.146)	0.175
Donor type (deceased)	0.791 (0.555 – 1.127)	0.194

Dividing patients into groups using the quartiles of the mean Tac C_0 at 12-months post-transplantation as cut-offs resulted in four groups with Tac C_0 as follows: group 1 with Tac $C_0 \leq 6.2$ ng/mL; group 2: 6.2 ng/mL < Tac $C_0 \leq 7.2$ ng/mL; group 3: 7.2 ng/mL < Tac $C_0 \leq 8.2$ ng/mL; and group 4 with Tac $C_0 > 8.2$ ng/mL. There was no significant ($p = 0.59$) modification of the association between Tac IPV and the primary composite end-point by patients in the four Tac C_0 groups. This was also the case when effect modification was tested by including the interaction term of IPV and Tac concentration (coded as a continuous variable) as a covariate in the multivariable Cox regression model ($p = 0.35$).

The estimated hazard ratios as a function of Tac IPV and mean Tac concentrations are shown in Figure 3 (A and B). This figure shows (based on the results of the multivariable model) the influence of Tac IPV and Tac C_0, respectively as continuous variables on the risk of developing the composite endpoint. It is clear that the risk of reaching the composite endpoint (graft failure, late BPAR, transplant glomerulopathy or doubling of serum creatinine concentration) censored for death increases with increasing Tac IPV and decreasing Tac concentrations.

DISCUSSION

This study demonstrates that a high Tac IPV is associated with inferior long-term outcomes after kidney transplantation. Patients with a high Tac IPV had a 1.4 times higher risk of reaching the composite endpoint of graft failure, late BPAR, transplant glomerulopathy, or doubling of serum creatinine concentration. The multivariate analysis showed that the effect of Tac IPV was independent of other known risk factors for poor outcome, such as lower recipient age,[16] number of transplantations and impaired renal allograft function.[17]

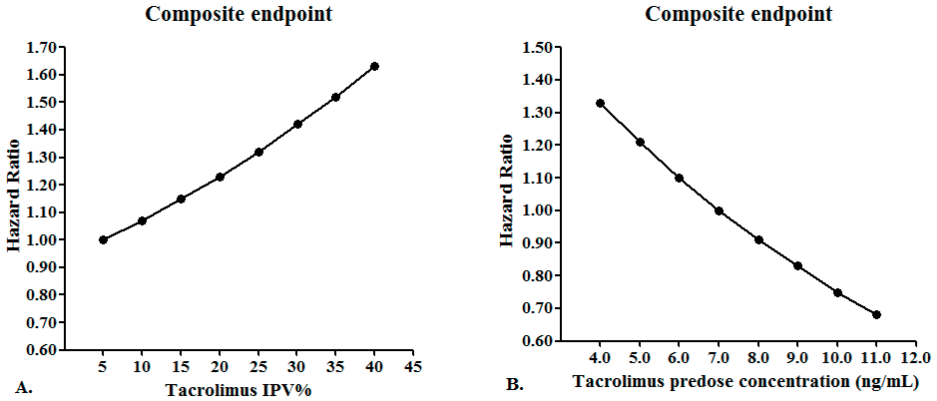

Figure 3. Calculated hazard ratios of the composite endpoint with increasing Tac IPV (A) and decreasing Tac predose concentrations (B). Example: a patient with a high Tac IPV (25%) and low Tac predose concentration (4.0 ng/mL) has a higher risk (1.32 * 1.33 = 1.76) to reach the composite endpoint than a patient with the same Tac IPV but a higher Tac predose concentration (7.0 ng/mL; 1.32 * 1.00 = 1.32).

This study was an extension of the previously published study of Borra *et al.*, and has an almost 3-fold larger study population and a 2-fold longer follow-up period. The present findings are in line with our previous findings, although the association between Tac IPV and long-term graft failure as reported previously[9] was stronger than the association observed here. In the study of Borra *et al.*, patients with a high Tac IPV had a 3-fold higher risk for developing the composite end-point, whereas it was 1.4-fold higher in the present study.

The smaller effect size observed here can be explained by the fact that in the present study, the composite end-point was modified and differed from that used by Borra *et al.* In the latter study, "biopsy-proven chronic allograft nephropathy (CAN)" was included in the composite endpoint in addition to graft loss, and doubling of serum creatinine concentration. CAN may be caused by several clinical entities including among others, calcineurin inhibitor nephrotoxicity, (antibody-mediated) rejection and chronic pyelonephritis.[18] Because the definition of the histopathology of CAN has changed through the years and the histopathologic picture of CAN is not specific, the item "biopsy-proven CAN" was changed into the more specific diagnoses late BPAR and transplant glomerulopathy in the present study. Moreover, a longer follow-up in the present study could be another reason for the smaller effect size we found. This study provides some indication that the effect of Tac IPV on the risk of developing the composite endpoint decreases with time. As has been mentioned previously, patients with high Tac IPV had a 2-fold higher risk than patients with low Tac IPV to develop an event in the first two years of follow-up, whereas this risk was only 1.20-fold higher during the remaining follow-up

period. This finding suggests that the longer follow-up period in the present study may partially explain the smaller effect size we found.

Apart from Tac IPV, three other factors proved to be related to long-term kidney transplant failure in multivariate analysis: the recipient's age at transplantation, graft function at six-months after transplantation, the transplant number and the mean of the average Tac concentrations measured between 6 and 12 months after transplantation. An advanced age of the recipient at the time of transplantation was found to be a protective factor. This may be explained by the lower immunological activity of elderly patients .[16-19] It is also not surprising that graft function (eGFR) at baseline predicts the survival time of the graft.[20] Salvadori *et al.* demonstrated in a multivariate analysis, that the effects of several highly relevant parameters from univariable analysis (such as acute rejection and delayed graft function) on 5-year GFR were fully explained by their influence on 1-year GFR. They showed that 1-year GFR was the most relevant predictor for 5-year allograft function.[17] They also demonstrated that immunological risk factor like previous transplantation has an ongoing effect on graft survival beyond year 1.[17] In our study, a low mean of the average Tac C_0 measured in the period between 6 and 12 months after transplantation was found to be another significant predictor for inferior long-term kidney transplantation outcomes. This finding is in line with the results presented by Naesens *et al.*[21] They demonstrated in a multivariate analysis that low mean Tac exposure independently associated with higher increase in biopsy-proven chronicity scores [calculated as the sum of the four basic 'chronic' Banff qualifiers (chronic glomerular damage, interstitial fibrosis, tubular atrophy, and vascular intimal thickening)] between 3 and 12 months after transplantation. Recently, in the DeKAF study, a lower Tac exposure after month 3 was also associated with increased risk of acute rejection.[22] The association between the Tac IPV and poor kidney transplantation outcome was not significantly modified within four patients subgroups based on their mean Tac C_0. Contrary to previous reports[9,23] that suggested that an episode of acute rejection is one of major factors for inferior graft outcome, our multivariate analysis did not confirm that. The reason probably is that the population we studied is a selection that survived at least one year after transplantation with acceptable renal function. Recently, a multivariate analysis performed in 739 living donor recipients found steroid-resistant acute rejection, but not any acute rejection episode, to be significantly associated with death-censored graft loss.[24] Unfortunately, in this retrospective analysis we were unable to distinguish between several types of acute rejection. The major reason for this is that this was a retrospective study and that in the period between year 2000 and year 2010 the Banff classification for kidney transplant rejection was frequently changed.

This multivariable analysis in a large patient population with long follow-up underlines the importance of IPV as a predictor of long-term outcome after kidney transplantation. In our analysis, the median IPV value was used as a cut-off value. It remains unclear if there is a critical threshold for IPV above which the risk of graft loss increases. The cut-off values in the studies by Borra *et al.*[9] and Ro *et al.*[10] (14.9% and 18.0%, respectively) were close to the Tac IPV cut-off value of this study, namely 16.2%.

This study provides good evidence that high Tac IPV increases the risk of poor kidney transplantation outcome. Also the mean Tac concentration at month 12 after transplantation was a significant predictor of long term outcome after kidney transplantation. From Figure 3 (A and B) it can be suggested that in patients with a high Tac IPV (> 16.2%) it is judicious to strive for a Tac C_0 of ≥ 7.0 ng/ml, to reduce the risk of poor kidney transplantation outcomes.

Calculation of Tac IPV is an easy and cheap monitoring tool that may help to identify high-risk patients during routine follow-up visits to the outpatient clinic. Incorporating algorithms that calculate IPV into electronic patient files may assist physicians to recognize these patients. Once a patient is recognized as having a high IPV, physicians need to find out what is the underlying cause, and try to resolve the problem. It is interesting to speculate on the potential causes of Tac IPV.[5] Non-adherence to the therapy is considered an important cause of high variability[25] and has been repeatedly associated with poor transplant outcome.[26] Concomitant diet, over-the-counter medications and repetitive substitution of different (generic) Tac formulations may also contribute to Tac IPV. To avoid a high IPV in Tac exposure, patients should be instructed to take their Tac in a consistent manner, with respect to the meal content and timing of ingestion relative to consumption to meals. Moreover, the use of interacting substances should be addressed and substitution of the innovator drug for generic Tac or one generic formulation for another has to be avoided. Some investigators have reported improved adherence after switching from the twice-daily to the once-daily, modified-release Tac formulation .[27] Others also showed that Tac IPV decreased following a switch to a once-daily formulation.[28,29] This has however, not been a universal finding and at present it is unknown if switching to a once-daily Tac formulation will improve long-term kidney transplantation outcome.[30]

In conclusion, in the largest sample size studied so far, a high Tac IPV was found to be associated with adverse long-term renal transplant outcome. In patients with fluctuating tacrolimus concentrations despite a stable dose, physicians should discuss drug adherence with the patient. To quantify the variability the IPV can be calculated but most likely there is not a critical threshold above which clinical outcome is impaired. In order to

collect more evidence, a prospective evaluation of the use of IPV monitoring to see if it can indeed improve outcomes is needed.

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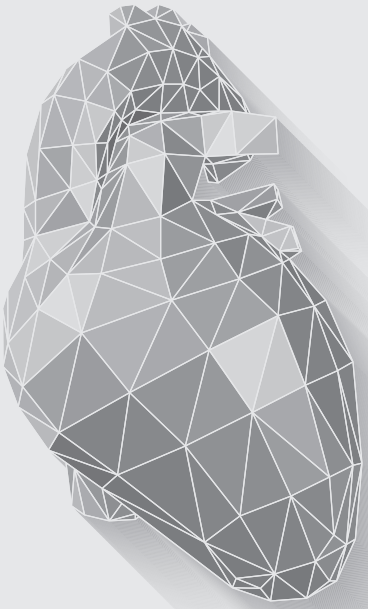

4.3

Is a high intra-patient variability in tacrolimus exposure associated with progression of cardiac allograft vasculopathy after heart transplantation?

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Submitted

ABSTRACT

We hypothesized that a high intra-patient variability (IPV) of tacrolimus (Tac) exposure after heart transplantation may be associated with progression of cardiac allograft vasculopathy (CAV) as a determinant of long-term survival of heart transplant recipients.
Eighty-six heart transplant recipients were included. Patients underwent coronary angiography at year 1 and 4 after transplantation and were divided according to low and high IPV of Tac exposure, with the median variability as cut-off. The primary outcome was the association between Tac IPV and the progression of CAV score between year 1 and 4. Secondary outcome was this association with acute cellular rejection.
There was no significant difference in the proportion of patients with high Tac IPV in the group with progression of CAV (n = 15) as compared with the group without CAV progression (n = 71) (60.0% *versus* 47.9% respectively, $p = 0.57$). There was no significant difference in the proportion of patients with high IPV between the group of 58 patients with one or more acute cellular rejection and the group of 28 patients without rejection (51.7% *versus* 46.4%, respectively, $p = 0.82$). A high IPV in Tac exposure was not associated with progression of CAV nor with acute cellular rejection in heart transplant recipients.

Key Words: Intra-patient variability, tacrolimus, heart transplantation

INTRODUCTION

The immunosuppressive drug tacrolimus (Tac) has a narrow therapeutic range and its clinical use is complicated by a large inter-patient variability in its pharmacokinetics. The determinants for this inter-patient variability have been studied extensively, and include genetic factors, co-medication, and demographic variables.[1-3] Because of this inter-patient variability, the Tac dose is routinely adjusted based on drug concentration measurements in blood in order to reach pre-defined target concentrations.

Tac also displays considerable variability in its pharmacokinetics within a single patient over time, so-called intra-patient variability (IPV). Certain patients who are treated with a stable drug dose will have stable exposure to Tac, but in others Tac concentrations may fluctuate considerably over time despite an unchanged dose. IPV in Tac exposure may be related to non-adherence but factors other than compliance may play a role. Food intake may be a relevant determinant,[4] whereas genetic factors seem less important.[5-7]

Whatever the cause of the IPV in Tac pharmacokinetics may be, patients with a high variability have a Tac exposure more often outside the target range. Such patients may be at risk for under-immunosuppression and rejection, as well as overexposure and toxicity. In a previous study, we demonstrated that a high IPV in the exposure of Tac was associated with poor graft survival after kidney transplantation.[8] Other investigators have reported that kidney transplantation outcomes are also worse in patients who have a high variability in the exposure of cyclosporine.[9,10] Best *et al.*[11] reported that a high IPV in cyclosporine exposure was associated with an increased risk for rejection early after heart-lung transplantation.

The impact of IPV in Tac exposure on graft vascular disease after heart transplantation has not been studied. Cardiac allograft vasculopathy (CAV) is an important problem that limits long-time survival after heart transplantion. CAV is characterized by proliferative thickening of the vascular intima and progressive narrowing of the vascular lumen. This process is related to both immunological and non-immunological mechanisms.[12] These mechanisms include a chronic allo-immune–mediated damage, as well as non-immunological factors such as ischemia-reperfusion damage, donor age, hypertension, hyperlipidemia and CMV-infection. Cellular rejection early and late after heart transplantation is an independent risk factor for the development of CAV.[13,14] A variable exposure to Tac may lead to an increased frequency of cellular rejection and to the development of CAV. The aim of this study was to determine whether a high IPV in Tac exposure is associated with more rapid progression of cardiac allograft vasculopathy in heart transplant patients.

PATIENTS AND METHODS

Study Population

The patients were adults who received a heart transplant at the Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands, between 2000 and 2011. All patients received induction therapy with anti-thymocyte globulin (ATG) followed by a maintenance immunosuppressive regimen consisting initially of a calcineurin-inhibitor (cyclosporine or Tac), prednisolone and mycofenolate mofetil (MMF). The maintenance immunosuppression was thereafter changed according to the tolerance of the drugs and the occurrence of rejection.

Surveillance for rejection was performed by endomyocardial biopsies, weekly for the first 6 weeks, biweekly for the next 3 months, then monthly and finally bimonthly until 1 year after transplantation. A surveillance endomyocardial biopsy was also performed 4 year after transplantation at the time of follow-up coronary angiography (CAG). Additional biopsies were performed when there was clinical suspicion of rejection. Surveillance for CAV was done routinely by performing CAG at 1 and 4 years after transplantation, according to the protocol at our institution. In few patients CAG was performed at a different time due to a clinical indication. As the aim of this study was to investigate the association between IPV in Tac exposure and the progression of CAV, only patients who had survived the first 18 months after transplantation were included. Furthermore, all patients had to use the twice daily oral Tac formulation (Prograf®, Astellas Pharma Inc.) between 6 and 18 months after transplantation.

Study Parameters

The primary outcome of this study was the development of CAV defined as progression of CAV score between the two predefined moments of angiographic follow-up, *i.e.* the 1st and 4th year CAG. The extent of CAV was graded according to the 2010 ISHLT (The International Society for Heart & Lung Transplantation) Cardiac Allograft Vasculopathy nomenclature:[15]

- ***CAV$_0$*** indicates coronary vessels without detectable angiographic lesions.
- ***CAV$_1$*** (mild) indicates angiographic <50% stenosis of the left main coronary artery or <70% stenosis of a primary vessel or any branch stenosis <70% (including diffuse narrowing), in the absence of allograft dysfunction.
- ***CAV$_2$***: (moderate) angiographic <50% left main stenosis, a ≥ 70% stenosis in a single primary vessel or isolated ≥70% stenosis in branches of 2 systems, without allograft dysfunction.

- ***CAV$_3$***: (severe) angiographic ≥ 50% stenosis of the left main coronary artery, or ≥ 70% lesions of two or more primary vessels, or ≥ 70% stenosis in branches of 3 systems, or when CAV_1 or CAV_2 are associated with allograft dysfunction.

Patients in whom the CAV score changed between the 1st and 4th year after transplantation were defined as patients with CAV progression. Patients in whom the CAV score remained constant were defined as patients without CAV progression.

As a secondary outcome we investigated the association between acute cellular rejection and IPV in Tac exposure. Acute cellular rejection was defined as a heart biopsy of at least grade 3A according to the 1990 ISHLT grading system (in the years 2000-2005) or at least grade 2R according to the 2004 ISHLT revised grading system (from 2006 until present).[16] Early acute rejection rates were calculated within the first year after transplantation and late acute rejection was defined as any rejection within the period of 1 year and 4 years after transplantation.

Tac predose concentrations (C_0) were determined throughout the study period in whole-blood by use of immunoassays [the Emit 2000 assay (Syva Company, Dade Behring Inc., Cupertino Calif.) and the ACMIA-Flex assay (Siemens HealthCare Diagnostics, Inc., Newark, DE)] on several analyzers [the IMX (Abbott laboratories, Ill), the Cobas Mira Plus analyzer (Roche Diagnostic Systems, Basel, Switzerland), the V-twin and Dimension XPand (both Siemens HealthCare Diagnostics, Inc, Newark, DE)]. Details on the sensitivity, reproducibility, and sensitivity of the Emit assay in our laboratory were published previously.[17] Proficiency samples were obtained from the International Proficiency Testing Scheme for Immunosuppressive Drugs (Professor Holt, Analytical Services International, St George's University of London). The Tac predose concentration measurements were collected within the period of 18 months post-transplantation.

For the calculation of IPV in Tac exposure, at least 3 C_0 had to be available for an individual patient. Because heart transplant patients are not on a stable Tac dose in the first phase after transplantation and because they often use interacting drugs in this period, only data on Tac exposure measured at outpatient clinic visits in the period of 6-18 months post-transplantation were collected. Tac concentration measurements obtained during hospitalization were not considered. Since not all patients received a constant drug dose between months 6 and 18, the obtained C_0 were corrected for the corresponding daily Tac dose (C_0/D). The IPV in Tac exposure was calculated using the following formula:

$$IPV\ \% = \{[|(X_{mean} - X_1)| + |(X_{mean} - X_2)| \ldots + |(X_{mean} - X_n)|] / n\} / X_{mean} \times 100$$

Where X_{mean} is the mean Tac C_0/D of all the available samples, X_1 is the first available Tac C_0/D sample, X_2 is the second and so on.[8]

Statistical Analysis

The distribution of baseline characteristics is reported using summary statistics and frequency tables for continuous and categorical variables, respectively. Baseline characteristics are compared with the Mann-Withney U test for continuous variables, and the chi-square test for categorical variables. The IPV was correlated with the CAV-grading and also with acute rejection using the chi-square test. Analyses were performed using IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL). All tests were two-sided and a p-value < 0.05 was considered statistically significant.

RESULTS

Between January 2000 and December 2010 a total of 177 patients aged >18 years were transplanted in our center. Of these, 33 died within the first four years after transplantation, 8 did not undergo both angiographic evaluations (at the 1st and 4th year) due to co-morbidities, and 2 patients had severe CAV (CAV score 3) already at the 1st year angiography. These 43 patients were excluded from the analysis. Forty-eight patients used cyclosporine as the primary CNI during the selected time and were therefore excluded from the analysis. As a result a total of 86 patients using Tac and having a angiographic evaluation at the 1st and the 4th post-transplant year was available for the present analysis.

Development of CAV

Of the 86 patients, 15 patients had progression of their CAV score between the 1st and 4th year. Baseline characteristics of the patients are shown in Table 1. There were no significant differences in the age of the recipient, the age of the donor, the ischemia time at transplantation, cholesterol levels, and the presence of hypertension and diabetes mellitus between patients with and those without CAV progression. There was a trend towards more CMV infection in the group of patients with progression of CAV (60.0% *versus* 16.9%; $p = 0.10$). Seventy-one patients had the same CAV score at the 1st year and 4th year CAG, and therefore did not have progression of CAV (Table 2). Systolic left ventricular function was normal in 14 patients with CAV and 70 patients without CAV. One patient in the CAV group and 1 patient in the group without CAV had an impaired systolic left ventricular function (Table 4).

Table 1. Patient characteristics.

	CAV progression (n =15)	No CAV progression (n = 71)	*P*
Gender (% male)	66.7%	63.4%	1.00
Primary heart disease			0.97
Iscemic heart disease	6 (40.0%)	27 (38.0%)	
Dilated cardiomyopathy	6 (40.0%)	32 (45.1%)	
Hypertrophic cardiomyopathy	2 (13.3%)	7 (9.9%)	
Congenital disease	1 (6.7%)	5 (7.0%)	
Recipient age (year)	50 (30 – 61)	49 (24 – 65)	0.72
Donor age (year)	48 (31 – 63)	44 (16 – 64)	0.12
Ischemic time at transplantation (min.)	184 (88 – 260)	188 (88 – 295)	0.60
CMV infection	40.0%	16.9%	0.10
Hypertension	86.7%	70.4%	0.34
Diabetes Mellitus	33.3%	31.0%	1.00
Total Cholesterol (mmol/L)			
at 1 year	4.9 (3.5 – 7.3)	5.0 (3.0 – 8.6)	0.85
at 4 year	4.9 (3.1 – 7.7)	5.2 (2.8 – 9.1)	0.46
Co-medication			
Statin	100.0%	80.3%	0.12
Prednisolone	86.7%	87.3%	1.00
Mycophenolate mofetil	46.7%	53.5%	0.84
Everolimus	26.7%	9.9%	0.10
Sirolimus	0%	1.4%	1.00

* The summary measure for non-normally distributed variables is the median (range). For binary or categorical variables, the summer measure is the proportion.

Table 2. CAV score

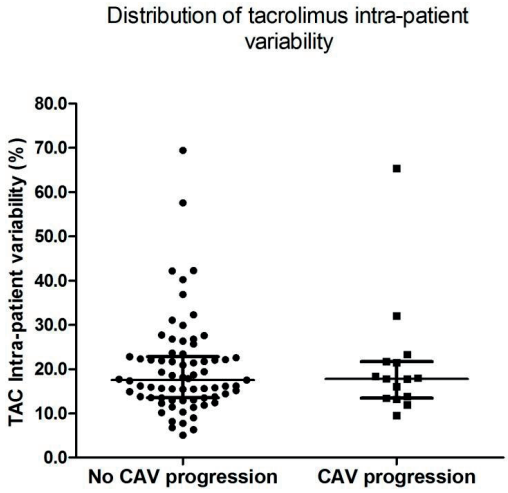
	CAV progression (n = 15)	No CAV progression (n = 71)
1 year CAV score		
CAV_0	12 (80.0%)	68 (95.8%)
CAV_1	3 (20.0%)	2 (2.8%)
CAV_2	0	1 (1.4%)
CAV_3	0	0
4 year CAV score		
CAV_0	0	68 (95.8%)
CAV_1	7 (46.7%)	2 (2.8%)
CAV_2	4 (26.7%)	1 (1.4%)
CAV_3	4 (26.7%)	0

Intra-patient variability and CAV

The patients who developed CAV had an average of 10.8 ± 1.8 Tac concentration measurements to calculate the IPV. This was comparable with the patients without CAV (10.7 ± 2.4 Tac concentration measurements, p = 0.88). The median IPV in Tac pharmacokinetics was 17.7 %, but the variability range was distributed from 5.1% to more than 69.5%. There was no significant difference in the distribution of Tac IPV between patients with progression of CAV score and those without (Figure 1). The median Tac IPV in patients who developed CAV was 17.8% (9.6% -65.4%), whereas this was 17.5% (5.1% - 69.5%) in patients without CAV progression (p = 0.98). Using the 17.7% value as a cut-off, patients were divided into a low and high variability group. The 43 patients in the low IPV group had a median variability of 13.5% (range: 5.1% – 17.7%). The 43 patients in the high IPV group

Table 3. Intra-patient Tac variability in tacrolimus exposure in patients without CAV progression and patients with CAV progression.

Intra-patient variability	No CAV progression (n = 71)	CAV progression (n = 15)	*P*
Low	37 (52.1%)	6 (40.0%)	0.57
High	34 (47.9%)	9 (60.0%)	



	No CAV progression (n = 71)	Progression of CAV (n = 15)
Minimum	5.1%	9.6%
10% percentile	10.2%	11.0%
25% percentile	13.5%	13.4%
Median	17.5%	17.8%
75% percentile	22.8%	21.7%
90% percentile	32.0%	45.4%
Maximum	69.5%	65.4%

Figure 1. Distribution of Tac intra-patient variability among patients with progression of CAV score and those without.

had a median variability of 22.6% (range: 17.7% – 69.5%)%. Progression of CAV score was not correlated with the IPV. There was no significant difference in the proportion of patients with high IPV in the group with development of CAV as compared with the group without CAV (60.0% *versus* 47.9%, p = 0.57) as shown in Table 3.

Intra-patient variability and acute rejection

To test whether the IPV was correlated to the development of acute cellular rejection we divided the patients into those who had one or more rejection episodes in the first year after transplantation and compared them to patients who did not have any rejection in the first year after transplantation. A total of 58 patients experienced acute rejection

Table 4. Rejections

	CAV progression (n=15)	No CAV progression (n = 71)	*P*
Early rejections (< 1 year); n = 115			
Moderate (3A or 2R)	27	85	0.04*
Severe (3B or 3R)	2	1	
Late rejections (> 1 year); n = 13			
Moderate (3A or 2R)	3	9	1.0
Severe (3B or 3R)	0	1	
Number of patients with early rejection			
1	2 (13.3%)	22 (31.0%)	0.06
>1	10 (66.7%)	24 (33.8%)	
Number of patients with late rejection			
1	1(6.7%)	10 (14.1%)	0.07
>1	1 (6.7%)	0	
Left ventricular function			
Normal	14 (93.3%)	70 (98.6%)	0.32
Impaired	1 (6.7%)	1 (1.4%)	

Median number of episodes acute rejection among patients with progression of CAV score was 2.0 (0 – 5), whereas the median number was 1 (0 – 6) in patients without progression of CAV (p = 0.04).

episodes within the first year after transplantation, while 12 patients experienced a late rejection. Twenty-three patients had one rejection episode, whereas 22 had two, and 16 had three or more rejection episodes. Most rejection episodes (n = 108) were treated with a course of intravenous methylprednisolone, 1 episode was treated with (rabbit-ATG), and 17 were treated with an increased oral prednisone dose (Table 4).

Of the patients with a rejection, 48.3% (n = 28) had a low IPV and 51.7% (n = 30) had a high IPV of Tac exposure. When the variability of the patients who did not have a rejection (n = 28) were analyzed, comparable results were found: 53.6% (n = 15) had a low IPV and

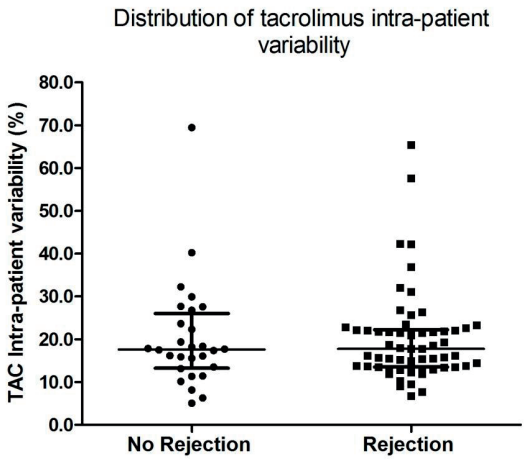
46.4% (n = 13) a high IPV (p = 0.82), as shown in Table 5A. The distribution of the Tac IPV among patients who did develop an acute rejection was also not significantly different to the distribution of Tac IPV in patients who did not have an acute rejection (Figure 2). The median Tac IPV in patients with rejection was 17.8% (6.8% -65.4%), whereas this was 17.6% (5.1% - 69.5%) in patients without rejection (p = 0.96).

Table 5A. Distribution of patients without rejections in the first year and patients with one or more rejections in the first year in the groups with low and high intra-patient variability of tacrolimus.

Intra-patient variability	No rejection (n = 28)	1 or more rejections (n = 58)	*P*
Low	15 (53.6%)	28 (48.3%)	0.82
High	13 (46.4%)	30 (51.7%)	

Table 5B. Distribution of patients without rejections in the first year and patients with three or more rejections in the first year in the groups with low and high intra-patient variability of tacrolimus.

Intra-patient variability	No rejection (n = 28)	3 or more rejections (n = 13)	*P*
Low	15 (53.6%)	5 (38.5%)	0.57
High	13 (46.4%)	8 (61.5%)	



	No Rejection (n = 28)	Rejection (n = 58)
Minimum	5.1%	6.8%
10% percentile	7.9%	11.7%
25% percentile	13.2%	13.5%
Median	17.6%	17.8%
75% percentile	26.0%	22.2%
90% percentile	33.1%	32.5%
Maximum	69.5%	65.4%

Figure 2. Distribution of Tac intra-patient variability among patients who did develop an acute rejection and those who did not.

We hypothesized that a high IPV might lead to multiple rejections. To test this hypothesis we compared the proportion of patients with high intra-patients Tac variability in the group with 3 or more rejections (n = 13 patients) and the group without any rejection (n = 28 patients). The null hypothesis was not rejected, as there were 61.5% (n = 8) and, respectively, 46.4% (n = 13) of patients with a high intra-patient Tac variability in the group with 3 or more rejections as compared with the group without any rejection, respectively (p = 0.57, Table 5B).

DISCUSSION

To the best of our knowledge this study is the first to investigate the IPV in Tac levels in adult heart transplant recipients. We show that the IPV in Tac exposure is not associated with progression of coronary allograft vascular disease within the first 4 years nor with acute rejection after heart transplantation. These data may be in contrast with the significant association between IPV in Tac exposure and kidney allograft survival demonstrated in an earlier study performed by Borra *et al.*[8]

Development of CAV after heart transplantation is a complex process which is immunologically and non-immunologically mediated. The introduction of calcineurin inhibitors has not decreased the development of CAV.[12] Mycophenolate mofetil has been shown to modestly reduce the intima thickness measured by intravascular ultrasound (IVUS) as compared to older immunosuppressive combinations using azathioprine.[12] In a randomized-controlled trial of 600 patients that evaluated everolimus *versus* azathioprine in an immunosuppressive regimen including cyclosporine and corticosteroids, everolimus significantly reduced intima thickness measured by IVUS, although it did not improve graft survival and was associated with dyslipidemia and decreased renal function.[18] It has been shown that CMV infection is an independent risk factor for CAV after transplantation, likely due to alteration of endothelial nitric-oxide synthase pathway.[19] ISHLT guidelines recommend a strict control of cardiovascular risk factors (hypertension, diabetes, hyperlipidemia, smoking and obesity), prophylaxis of CMV infection and the use of statins for the prevention of CAV.[20]

In our study, the group of patients with progression of CAV tended to have more CMV infections compared to the group of patients without progression of CAV, while there was no difference between the groups in terms of other atherosclerotic risk factors, the use of statins or immunosuppressive drugs. The design of the study allowed assessment of progression of CAV between 1 and 4 years post-transplantation excluding donor-related and surgery-related factors. Our hypothesis was that a high IPV in Tac exposure may

influence the development of CAV by lowering the chronic immunosuppressive exposure that allows a good transplant function. However, we found no association between a high intra-patient Tac variability and progression of CAV at 4 years. This suggests that other mechanisms unaffected by the calcineurin inhibitors influence development and progression of CAV.

Earlier studies have shown that there is a cumulative effect of acute rejection on development of CAV and that also late acute rejections increase the risk of CAV.[13,14] In our study there was a trend toward an increased number of patients with more than 1 acute rejections within the first post-transplant year and the progression of CAV after the first year. Contrary to our results, Pollock *et al.*[21] have shown in a pediatric population, that a high variability in Tac levels in 144 solid organ transplantations (including 28 heart transplantations) is an independent risk factor for late graft rejection and graft loss. The difference with our study might be explained by the studied population, as the study of Pollock *et al.* was performed in a population with a median age of 13 year, and by the immunosuppressive treatment used in combination with Tac. Pollock *et al.* mentioned in their study that one of the contributing factors for the high IPV is the documented medication non-adherence. Ensuring adequate adherence in a pediatric population (especially adolescents) is more complicated compared to adults.[22,23]

As opposed to the deleterious effect of high intra-patient Tac variability for the graft survival found in the renal transplant recipients, we found no such effect on progression of CAV in heart transplant recipients. This may be explained by the compensation of the IPV of Tac exposure by a strong immunosuppressive regimen often consisting of three immunosuppressive drugs, including corticosteroids, in our heart transplant recipients. Although the number of patients with at least one rejection was high in both groups, the majority of rejections was moderate and was treated with a course of intravenous methylprednisolone, which may have compensated for the variability of Tac clearance. Furthermore, the large majority of the patients in both groups had preserved graft function, suggesting that rejections were well treated.

This study has several limitations. It is a retrospective study, and the time period that we have chosen for the analysis of Tac pharmacokinetics was between 6 and 18 months after transplantation. The early post-transplant period (< 6 months) is associated with increased Tac exposure variability due to interaction with medications and more frequent hospital admission. A different period of measurement may lead to a different outcome. The number of investigated patients was low, and therefore our study may not have enough statistical power to identify the association between IPV and the progression of vascular disease. The evaluation of CAV was only made by the angiographic assessment,

and did not involve intravascular ultrasound (IVUS) or optical coherence tomography (OCT) for assessment of preclinical stages of coronary disease. Our results are limited to the period of 4 year after transplantation, and therefore cannot be extrapolated to more long-term graft function and survival.

CONCLUSION

A high IPV in Tac exposure was not associated with the development and progression of CAV nor to the development of acute cellular rejection. The use of a combination of two or three immunosuppressive drugs including corticosteroids after heart transplantation may protect against episodes of over –or underexposure to Tac. These findings suggest that the implications of a high IPV of Tac exposure may be organ-specific, and thus less important in heart transplant recipients.

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4.4

Conversion from twice-daily to once-daily tacrolimus does not reduce intra-patient variability in tacrolimus exposure

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ABSTRACT

Background: Intra-patient variability (IPV) in tacrolimus exposure is associated with renal allograft failure. The aim of this study was to investigate whether conversion from the twice-daily tacrolimus formulation (Tac-TD) to a once-daily formulation (Tac-OD) leads to a lower IPV in tacrolimus exposure.

Methods: Two-hundred-forty-seven stable renal transplant recipients were converted from Tac-TD to Tac-OD (Advagraf®) on a 1 mg:1 mg total daily dose basis. After conversion, patients were followed for 12 months and tacrolimus predose whole-blood concentrations (C_0), serum creatinine, estimated glomerular filtration rate (eGFR), and proteinuria were measured. These parameters were compared with those collected at all outpatient visits in the 12-month period (±3 months) before conversion (Tac-TD period). The IPV was calculated based on the dose-adjusted tacrolimus C_0.

Results: The Tac-OD formulation provided an excellent graft survival (100%), a low acute rejection rate (0.8%), and good tolerability. Renal function remained stable: eGFR 48 (16-90) mL/min *vs.* 46 (12-90) mL/min (p=0.15) before and after conversion, respectively. After conversion to Tac-OD, mean C_0 was significantly lower, falling from 5.7 ± 1.5 ng/mL to 5.0 ±1.5 ng/mL, corresponding to a 12% reduction ($p < 0.01$). Both drugs had similar IPV (Tac-TD: 17.3 ±1.6% *vs.* Tac-OD: 16.4 ±1.6%; p =0.31).

Conclusions: Although conversion from Tac-TD to Tac-OD significantly reduces tacrolimus exposure as measured by C_0, and appears safe, it does not reduce IPV in tacrolimus exposure.

Keywords: Intra-patient variability, modified-release, pharmacokinetics, tacrolimus.

INTRODUCTION

Non-adherence to immunosuppressive drug treatment is associated with poor long-term transplantation outcome.[1,2] Modified-release, oral dosage form of tacrolimus (Tac-OD; Advagraf®) provides a once-daily dosing alternative for the immediate-release (twice-daily) Tac formulation (Tac-TD) and significantly improves adherence.[3]

The intra-patient variability (IPV) in a drug's pharmacokinetics is the amount of fluctuation in drug concentrations within an individual over a certain period of time during which the dose is unchanged. A high IPV in the exposure to tacrolimus may put the patient at risk for toxicity (in case of overexposure) or for rejection if concentrations fall below the lower threshold of its narrow therapeutic window.[4] In renal transplant recipients (RTRs) a high IPV in tacrolimus exposure is a risk factor for rejection and long-term treatment failure.[5,6]

Medication adherence may be an important determinant of IPV in drug exposure. Theoretically, the higher adherence to Tac-OD could lower IPV in tacrolimus exposure, and improve transplantation outcomes. Only a few studies, including limited patient numbers, have compared the IPV between Tac-TD and Tac-OD.[7-11] This prompted us to investigate whether conversion from Tac-TD to Tac-OD results in a lower tacrolimus IPV in a larger cohort of stable RTRs.

PATIENTS AND METHODS

Study design

This was a single-center, nonrandomized, study to assess the safety of conversion from Tac-TD to Tac-OD after kidney transplantation. Patients were eligible for enrollment if they met the following inclusion criteria: 1) treatment with a Tac-TD-based immunosuppressive regimen; 2) a need for continued therapy with tacrolimus; 3) age ≥ 18 years; and 4) a follow-up of ≥ 5 months after transplantation.

After inclusion, Tac-TD (Prograf®, Astellas Pharma, Leiden, the Netherlands) was converted (t = 0) to Tac-OD (Advagraf®, Astellas Pharma; only morning dosing) on a 1:1 (milligram:milligram) basis. During both study periods (pre-conversion and post-conversion), tacrolimus doses were adjusted to achieve a predose whole-blood concentrations (C_0) of 4-10 ng/mL. After conversion, patients were followed for 12 months with study assessments and laboratory sampling (tacrolimus C_0 and renal function) at 3-monthly intervals, or whenever deemed necessary by the attending physician. Variables of interest

were tolerability of Tac-OD, renal function, and serious adverse events (SAEs). Clinicians were allowed to monitor tacrolimus exposure and adjust the Tac-OD dose before the first scheduled study visit at month 3.

Within the frame of this study, we studied whether conversion from Tac-TD to Tac-OD influenced Tac IPV. We therefore collected all Tac C_0 measured during visits to the outpatient clinic from the time of conversion up until month 12 (±1 month) *after* conversion (t = 12). To calculate Tac IPV during the use of Tac-TD, we collected the Tac C_0 retrospectively from the year before conversion (t = -12 ±3 months).

As transplant recipients are not on a stable Tac dose in the first phase after transplantation, and because they often underwent interventions that may influence Tac exposure in this period (*e.g.* the use of interacting antibiotics or pulse corticosteroid therapy), only data on Tac exposure from month 5 (and onwards) post-transplantation were considered. Therefore, data on the Tac IPV were derived from patients at least 14 months after transplantation. For the same reason, Tac C_0 that were collected during hospital admission(s) were not included.

Ethics

The study was approved by the institutional review board. The conversion from Tac-TD to Tac-OD was considered to be in the realm of routine clinical care, and therefore, no formal medical ethical approval of this study was required. Nonetheless, all patients were asked if they objected to being switched to Tac-OD, and all gave written consent prior to conversion. For the study of the change in Tac IPV, data were used that were only obtained as part of routine patient care.

Intra-Patient Variability

The variable of interest was the Tac IPV. For its calculation, at least 3 Tac C_0 measurements had to be available for both the Tac-TD and the Tac-OD phase. IPV was calculated as follows:[5]

$$IPV\% = \{[|(X_{mean} - X_1)| + |(X_{mean} - X_2)| \ldots + |(X_{mean} - X_n)|]/n\} / X_{mean} \times 100 \qquad 1.$$

where X_{mean} is the mean Tac C_0 of all available samples, X_1 is the first available Tac concentration measurement, X_2 is the second, and X_n is the n^{th} available Tac C_0. Using this formula the quantity $(X_{mean} - X_n)$ is always expressed as a positive integer (absolute value). Because not all patients received a constant Tac dose throughout the study period, the obtained Tac C_0 were corrected for the corresponding daily Tac dose (C_0/D). The reciprocal of this ratio gives an apparent oral clearance.

Patients were characterised as having a high or low IPV using the median variability of the IPV as the cutoff value.

Tacrolimus C_0 were determined in whole-blood using the ACMIA-Flex immunoassay on a Dimension XPand (both Siemens HealthCare Diagnostics Inc., Newark, USA).

Statistical Analysis

The IPV was calculated using Microsoft Excel 2010. Statistical analyses were performed using Statistical Program of Social Sciences version 20 (SPSS Inc., Chicago, Ill., USA). Data distribution was assessed by visual inspection and the Kolmogorov-Smirnov test. As the distribution was mostly skewed, the log-transformed data were analyzed. Categorical variables are reported using frequency tables and percentages, and continuous variables are expressed as geometric means, unless stated otherwise.

Differences in IPV between the treatment periods were assessed using the paired t test. Differences in median values were tested by the Wilcoxon signed-rank test and distributions were tested by the Mann-Whitney U test. The relationship between various demographic, clinical, and laboratory variables with Tac IPV was assessed using univariate analyses. Variables thet showed a statistically significant relation were then included in a multivariate regression analysis to investigate if they affected the relation between the formulation and IPV.

To quantify the effect of regression to the mean (RTM), the following formula was used:[12, 13]

$$\textit{RTM effect} = \sigma_t(1 - \rho)C(z), \quad -1 \leq \rho \leq 1 \qquad 2.$$

where σ_t is the standard deviation (SD), ρ the correlation between two measures and,

$$C(z) = \varphi(z) / (1 - \Phi(z))$$

where z = (c – population mean) / σ_t if the subjects are selected using a baseline measurement greater than c, and z = (population mean – c) / σ_t if the subjects are selected using a baseline measurement less than c. The terms $\varphi(z)$ and $1-\Phi(z)$ are, respectively, the probability density and the cumulative distribution functions of the standard normal distribution. The calculations were undertaken using the log-transformed data.

Statistical significance was defined as a two-tailed p-value <0.05. The post hoc power calculation was performed using the program 'G*power 3.1.9.2' (Heinrich-Hein-University; Düsseldorf, Germany).

RESULTS

Baseline characteristics and pharmacokinetic outcomes

Two hundred fifty one stable RTRs were enrolled between December 2009 and October 2011 (Table 1 for characteristics). Of these, 247 were converted to Tac-OD. Three patients never took Tac-OD for unknown reasons, and 1 patient declined conversion after signing informed consent. Of these 247 patients, 227 completed the 12-month follow-up (Figure 1). During follow-up, 2 patients died, 5 were lost to follow-up, and in 13 patients treatment with Tac-OD was interrupted (in 8 cases because of side effects; in 4 for unknown reasons). These 12 patients were reconverted to Tac-TD. In 1 patient treatment with Tac-OD was interrupted because of recurrent glomerulonephritis requiring cyclophosphamide and high-dose glucocorticoid treatment.

Most patients (n = 211 or 85%) were converted from Tac-TD to Tac-OD on a 1:1 (milligram:milligram) total daily dose basis. However, in 36 cases, it was decided to change the daily Tac dose at conversion and was therefore increased in 25 and decreased in 11 patients. In 13 patients, the Tac dose was changed at the time of conversion because Tac C_0 was outside the therapeutic range (4-10 ng/mL). In the remainder (n = 23), the clinicians changed the dose because they anticipated a decrease in Tac C_0 or because of Tac toxicity (side effects).

Three months after conversion, 240 patients were still using Tac-OD. Their total daily Tac dose was comparable with that at the time of conversion: 4.0 ±1.8 vs. 4.1 ±1.7 mg/day; P = 0.16. In the first three months after conversion, the daily Tac dose had been left unaltered in 149 but was increased in 51 and decreased in 40 patients based on Tac C_0 measurements.

Tac C_0 decreased from 5.8 ng/mL at baseline to 5.1 ng/mL at month 3, corresponding to a 12% fall (P < 0.01). The dose-adjusted Tac C_0 (C_0/D) also decreased by 14% (P < 0.01; Table 2). The analysis was repeated after exclusion of the 36 patients who were not converted on a 1:1 basis. Again, Tac C_0 decreased significantly, decreasing from 5.7 to 5.0 ng/ml or by 12% (P < 0.01). Tac C_0/D decreased from 1.43 to 1.25 ng/mL per mg/day (P < 0.01).

Clinical outcomes

Ninety-one SAEs occurred in 62 patients (25.1%). Patient survival was 99.2%. One patient died from liver insufficiency caused by hepatitis C virus-related cirrhosis. The second patient died of unknown cause while on holiday. Neither of these deaths was suspected to be related to the use of Tac-OD. One-year graft survival censored for death was 100%.

Table 1. Patient demographics and baseline characteristics.

	Number of subjects (%)
Gender:	
Male / Female	175 (69.7%) / 76 (30.3%)
Age (years):	51 (18–80)*
Ethnicity:	
Caucasian	183 (72.9%)
Black	28 (11.2%)
Asian	24 (9.6%)
Other	16 (6.4%)
Primary kidney disease:	
Hypertensive nephropathy	35 (13.9%)
Polycystic kidney disease	26 (10.4%)
Diabetic nephropathy	25 (10.0%)
Glomerulonephritis	52 (20.7%)
Congenital / Reflux disease	14 (5.6%)
Other	76 (30.3%)
Unknown	23 (9.2%)
Number of kidney transplantation:	
1st / 2nd / 3rd or more	207 (82.5%) / 34 (13.5%) / 10 (4.0%)
Donor type:	
Living / Deceased	175 (69.7%) / 76 (30.3%)
Number of HLA** mismatches:	
0	11 (4.4%)
1	23 (9.2%)
2	54 (21.5%)
≥3	157 (62.5%)
Missing	6 (2.4%)
Time from transplantation to conversion (months):	37 (5-312)*
Immunosuppression at baseline:	
Tac-TD (monotherapy)	29 (11.6%)
Tac-TD and MMF	193 (76.9%)
Tac-TD and prednisone	13 (5.2%)
Tac-TD, MMF and prednisone	8 (3.2%)
Tac-TD and other immunosuppressive drugs	8 (3.2%)

* median (range)
** Human leukocyte antigen

Two patients experienced a biopsy-proven acute rejection. The first patient had a type 1 acute cellular rejection occurring some 3½ years after transplantation and 8 months after conversion to Tac-OD, which was treated with methylprednisolone. The second patient experienced a type 1 acute cellular rejection occurring 3 months after conversion.

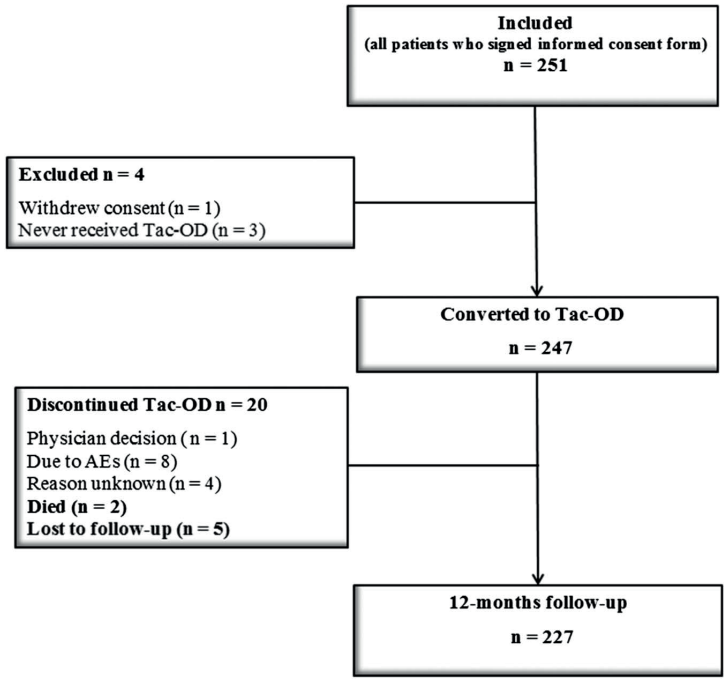

Figure 1. Flow chart.

Table 2. The pharmacokinetic parameters at conversion (t=0) and after conversion to Tac-OD. All values are expressed as geometric mean with standard deviation. The apparent oral clearance is calculated by taking the reciprocal of dose-corrected Tac C_0 (*e.g.,* a Tac C_0/dose of 1.4 ng/ml per mg/day gives an apparent oral clearance of 0.50 L/min).

	t = 0	Month 3	Month 6	Month 9	Month 12
Whole study population (n=247)	n = 247	n = 240	n = 237	n = 234	n = 227
Tac dose (mg/day)	4.0 ±1.8	4.1 ±1.7	4.0 ±1.7	4.0 ±1.7	4.0 ±1.7
Tac C_0 (ng/ml)	5.8 ±1.5	5.1 ±1.5 *	5.1 ±1.4 *	5.2 ±1.4 *	4.9 ±1.5 *
Tac C_0/dose (ng/ml per mg/day)	1.46 ±1.88	1.26 ±1.85 *	1.27 ±1.74 *	1.31 ±1.73 *	1.24 ±1.81 *
Patients available for IPV analysis (n=167)	**t = 0**	**Month 3**	**Month 6**	**Month 9**	**Month 12**
Tac dose (mg/day)	3.8 ±1.7	3.9 ±1.7	3.8 ±1.7	3.9 ±1.7	3.8 ±1.7
Tac C_0 (ng/ml)	5.3 ±1.4	4.8 ±1.5 *	4.9 ±1.4 *	5.0 ±1.3	4.6 ±1.5 *
Tac C_0/dose (ng/ml per mg/day)	1.39 ±1.92	1.25 ±1.80 *	1.27 ±1.70 *	1.33 ±1.72	1.23 ±1.84 *

*significant change ($p < 0.05$) comparing to the measurements at the conversion time point (t = 0).

This rejection may have been triggered by the conversion to Tac-OD as Tac exposure temporarily decreased to a nadir of 3.6 ng/mL.

Three patients developed nonmelanoma skin cancer. Two patients experienced recurrent primary kidney disease (1 case of IgA nephropathy and one of p-ANCA-associated

glomerulonephritis). Twenty-six SAEs were related to hospitalization for an infection. In 56 cases, other reasons necessitated hospitalisation.

Fifty-seven nonsevere adverse events (AEs) occurred in 52 (21.1%) patients. The most frequently reported AEs included skin-related disorders (n = 10, 4.0%), and infections (n = 17, 6.9%), most of which were urinary tract infections (n = 10, 4.0%).

Renal function and proteinuria did not change during follow-up (Table 3). Renal function of the 12 patients who discontinued Tac-OD also remained stable (data not shown). Renal function of the patients (n = 114) who had a decrease in their Tac C_0, also did not change after conversion: [(48 (16-90) mL/min at baseline versus 47 (15-90) mL/min at month 3 after conversion; P = 0.13 versus 47 (12-90) mL/min at month 12 after conversion, P = 0.82]. Apart from a small decrease in HDL-cholesterol, all other clinical and laboratory parameters were unaffected (Table 3).

Table 3. Evolution of clinical and laboratory parameters in the 12 months after conversion from Tac-TD to Tac-OD.

	Conversion	Month 12 ± 1	*p*
Plasma creatinine (µmol/L)	132 (55-324)	134 (57-464)	0.38
eGFR (mL/min)	48 (16-90)	46 (12-90)	0.26
Proteinuria (g/L)	0.11 (0.02-3.42)	0.10 (0.01-6.88)	0.80
Protein/Creatinine (mg/mmol)	14.52 (4.00-551.60)	13.51 (3.97-1186.20)	0.57
Hemoglobin (mmol/L)	8.2 (4.9-11.0)	8.3 (5.1-11.4)	0.35
Fasting plasma glucose (mmol/L)	5.6 (3.9-26.7)	5.6 (3.8-22.2)	0.98
Triglycerides mmol/L	1.56 (0.46-6.88)	1.57 (0.45-6.76)	0.45
Total cholesterol (mmol/L)	4.6 (1.8-7.5)	4.5 (2.5-7.5)	0.41
HDL-cholesterol (mmol/L)	1.21 (0.33-4.86)	1.20 (0.63-3.44)	**0.04**
LDL-cholesterol (mmol/L)	2.65 (0.34-5.44)	2.70 (1.20-5.43)	0.59
Systolic blood pressure (mmHg)	135 (80-191)	139 (100-200)	0.65
Diastolic blood pressure (mmHg)	80 (50-110)	80 (58-104)	0.91

eGFR = estimated glomerular filtration rate; LDL = low density lipoprotein;
HDL = high density lipoprotein; Protein/Creatinine = urinary protein/urinary creatinine ratio

Tac-OD was in general tolerated well, and only 8 patients discontinued Tac-OD because of side effects. The AEs that led to discontinuation of Tac-OD were mild and included rash (n = 2), gastrointestinal complaints (n = 2), headache (n = 1), myalgia (n = 1), insomnia (n = 1), and worsening of eczema (n = 2).

Effect of conversion to Tac-OD on Tac IPV

To analyse the influence of Tac formulation on the IPV in Tac apparent oral clearance (hereafter referred to as Tac IPV), stricter inclusion criteria were applied. Eighty patients were excluded, leaving a total of 167 patients for the IPV analyses. In 9 cases, <3 samples were available after conversion because these patients were reconverted to Tac-TD. Seventy-one cases were excluded because the Tac-TD treatment phase was shorter than 9 months.

As observed in the whole group, a significant drop in Tac C_0 (9.5%) and in Tac C_0/D (10%) occurred between conversion and month 3, despite a stable Tac daily dose (Table 2). The mean number of available samples per patient was 6.3 ±2.3 (Tac-TD period) and 5.8 ±2.5 (Tac-OD phase), and was slightly higher before conversion (P = 0.02). In both phases, interpatient variability in IPV was considerable, with some individuals having a variability

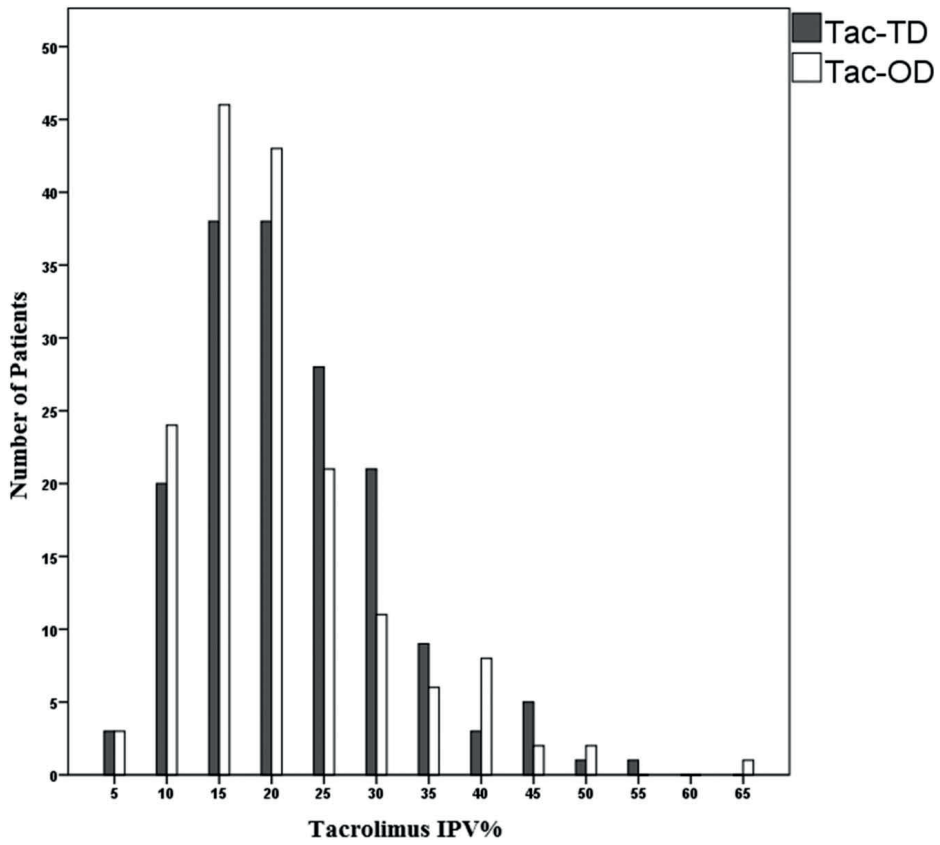

Figure 2. Change of distribution (by 5% intervals) of Tac IPV before and after conversion from Tac-TD to Tac-OD.

of <5% and others >50%. The median IPV for Tac-TD was 17.9% (2.9-51.4%) and for Tac-OD 16.8% (4.2-64.1%), *P* = 0.25. Conversion to Tac-OD did not result in a change in geometric mean Tac IPV: 17.3% ±1.7% *versus* 16.4% ±1.6%, for the Tac-TD and Tac-OD phases, respectively, *P* = 0.31. The conversion also did not alter the distribution of the IPV (*P* = 0.12; Figure 2). When the 71 patients with a Tac-TD phase < 9 months were included, again Tac IVP did not change after conversion to Tac-OD: the median IPV for Tac-TD was 16.9% (1.5%-56.1%) and for Tac-OD 16.5% (4.1%-68.5%), *P* = 0.71.

Patients were classified using the median Tac IPV before conversion (17.9%) as the cut-off. This resulted in 83 patients being classified as having low variability (mean: 11.7% ±1.4%), and 84 as having a high variability (mean: 25.6% ±1.3%). Using this same cut-off, after conversion, the number of patients in the low-variability group rose from 83 to 93 (11.8% ±1.4%), whereas 74 patients ended up in the high-variability group (24.9% ±1.3%). Before conversion, patients with high IPV had a higher number of Tac dose changes in the 12±3 months observation period compared to patients with low IPV [median 0.5 (0-5) *versus* 0.0 (0-3), *P* = 0.02]. After conversion, there were no significant differences in the number of Tac-OD dose adjustments between the two groups: median 1.0 (0-4) *versus* 0.0 (0-3) for the low and high-variability groups, respectively, P = 0.078.

This same analysis restricted to the group of 84 patients with high variability before conversion demonstrated a significant decrease in IPV after conversion: 25.6% ± 1.3% *versus* 17.1% ± 1.6% (P < 0.01, Figure 3B). This effect was even stronger when we included only those patients with an IPV in the upper quartile: IPV decreased from 31.8% ± 1.2% to 18.0% ± 1.6% in the Tac-OD phase (P < 0.01). The reverse was observed for the group of 83 patients who had a low Tac IPV before conversion: The IPV increased from 11.7% ± 1.4% (Tac-TD) to 15.8% ± 1.6% (Tac-OD), P < 0.01 (Figure 3C).

Univariate analyses revealed that age, gender, time after transplantation, baseline creatinine, and haemoglobin concentration had no influence on IPV. Unfortunately, CYP3A5 genotype, which has been correlated with Tac dose requirement,[14] was not available for this cohort. However, there was a direct proportional correlation between the number of samples and IPV, and between the number of dose changes and IPV. In a regression analysis, the effect of switching from Tac-TD to Tac-OD on the IPV remained statistically significant at the same level after adjustment for number of dose changes and the number of samples.

Subsequently, we investigated whether this observation was a treatment effect or whether it was caused by the RTM phenomenon. After conversion, patients with high variability in Tac exposure had a 0.1746 change (decrease) in IPV on the log scale,

corresponding to 8.5% on normal scale. Using equation 2 with σ_t = 0.1263, ρ = 0.05, population mean of 1.2386, c = 1.2538, φ = 0.40, and Φ = 0.47, the estimated RTM effect was 0.1726 (98% of the observed decrease). Hence, it can be concluded that there was no real change in the IPV after conversion.

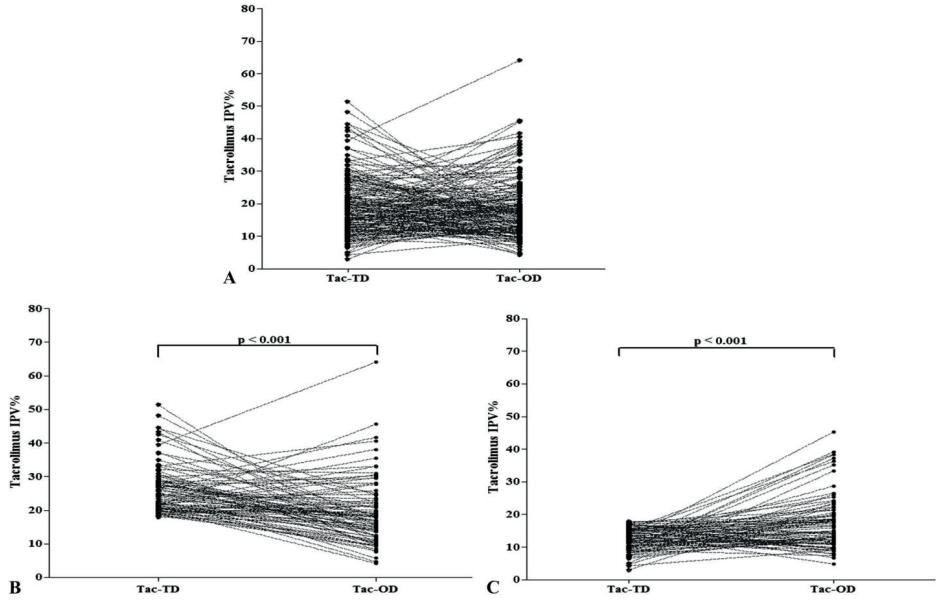

Figure 3. The individual change of Tac IPV before and after conversion from Tac-TD to Tac-OD; A: All patients available for the IPV analysis (n = 167); B: Patients with high IPV before conversion (n = 84) and C: Patients with low IPV before conversion (n = 83).

DISCUSSION

This study demonstrates that converting stable RTRs from Tac-TD to Tac-OD is safe. Graft survival (censored for death) after conversion was 100% and only 2 patients died from causes unrelated to the Tac formulation conversion. Renal function, proteinuria, glucose levels, and blood pressure did not change after conversion. Although a considerable number of SAEs occurred, this is not unusual for RTRs who are immunocompromised and frequently have multiple comorbidities.

Tac-OD was tolerated well, treatment discontinuations due to AEs were infrequent, and drug-related side effects were mild. The nonrandomized design of this study is an obvious limitation, making a formal comparison regarding the safety of both formulations impossible. However, our findings are consistent with safety data of other conversion studies[9,15-17] and the phase II and III trials.[18]

Industry-sponsored trials performed in both stable and *de novo* RTRs showed that conversion from Tac-TD to Tac-OD on a 1:1 (milligram:milligram) daily dose basis yields comparable drug exposure [measured as either the area-under the concentration *vs.* time-curve 0-24 hr. [AUC_{0-24}] or C_0].[19] However, more recent reports demonstrate that conversion to Tac-OD is associated with considerably lower Tac exposure (of up to 15%), necessitating occasional dose changes.[19-21] In line with the latter observations, significantly lower Tac C_0 and Tac C_0/D were observed after conversion in this study. These discrepancies in the changes in Tac exposure after conversion likely relate to differences in inclusion criteria of the various trials, which were stricter in the earlier trials. The present study was a "real world" conversion study, and only a few exclusion criteria applied.

Apart from 1 patient who suffered from acute rejection shortly after conversion, there were no other cases of rejection that contributed to low Tac exposure. It can be argued that the observed lowering of Tac exposure after conversion is not clinically relevant. Neverthless, to prevent the occasional case of marked overexposure or underexposure, at our center, we routinely measure Tac C_0 1-2 weeks after conversion to Tac-OD.

This study shows that conversion from Tac-TD to Tac-OD does not result in a lower Tac IPV, confirming findings of Wehland *et al.*[7] and van Hooff *et al,*[11] which were conducted in smaller patient groups. Other investigators have observed a lowering of Tac IPV after conversion.[8-10] An important limitation of the study of Wu *et al.* is the fact that the number of samples after conversion was larger than before conversion.[9] By mathematical principle, the estimation of IPV (or %CV) will become more precise if the number of samples increases.

In the study of Stifft *et al*, Tac IPV didn't change after conversion when it was calculated using C_0 but decreased when it was calculated using AUC_{0-24}.[10] In the study by Stifft *et al,* the decrease in Tac IPV, calculated using AUC_{0-24}, was 3.2% which, although statistically significantly different, may be considered not to be clinically relevant. In the present study, *we* chose to evaluate Tac exposure using C_0 only because of reports demonstrating a significant correlation between AUC_{0-24} and C_0 for both Tac formulations[8,10,22,23] and because Tac C_0 is the therapeutic drug monitoring parameter that is used most frequently in clinical practice. A change in Tac IPV may thus have remained undetected. The present study did, however, have a power of almost 100% to detect a difference in IPV (data not shown).

In patients with a high Tac variability, conversion resulted in a significantly decreased IPV. This may have been caused by improved adherence as observed by Kuypers *et al.*[3]

We did not measure patient compliance directly. However, because the observed median IPV was not very high before conversion, we feel that the rate of medication adherence in the present, selected population may have been high, with little potential for further improvement by conversion to Tac-OD.

Alternatively, reported changes in IPV after conversion may not have been a true treatment effect but rather the result of RTM. RTM is a statistical phenomenon that can make natural variation in repeated data look like a real change. This occurs when unusually large or small measurements tend to be followed by measurements that are closer to the mean.[12,13] Indeed, about 98% of the decrease in IPV we observed was explained by RTM and therefore not the result of conversion per se. Whether the RTM phenomenon also affected the results of previous studies cannot be determined. However, the chance that this phenomenon occurs increases when a nonrandomized, controlled, or noncrossover design is followed.

In conclusion, conversion from the Tac-TD to the Tac-OD formulation on the same milligram-for-milligram daily dose basis significantly reduces Tac exposure but does not lower Tac IPV. Nonetheless, conversion to Tac-OD seems to be safe.

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Chapter 5

General discussion & summary

GENERAL DISCUSSION

More than sixty years have elapsed since the first successful human kidney transplantation and this procedure has become the treatment of choice for patients with end-stage renal disease. Although the development of powerful and specific immunosuppressive drugs has contributed to the success of this therapy, several challenges remain. Patients still suffer from rejections and kidney allograft loss, while they are also at increased risk of severe infectious complications, cancer and other immunosuppressive drug-related toxicity. In this thesis we tried to find strategies to optimize the immunosuppressive therapy with tacrolimus (TAC) with the primary goal to improve the (long-term) outcomes of solid organ transplantation.

1. Concentration-effect relationship

In Chapter 2 we studied the relationship between the exposure to Tac and the efficacy of this drug. The use of Tac has resulted in a decrease in the incidence of acute rejection and improved early graft survival.[1] The narrow therapeutic range of Tac concentrations, and the high inter-patient pharmacokinetic variability have resulted in the recommendation to perform TDM . Whole blood predose Tac concentrations (C_0) have been found to correlate well with the area under the concentration-curve measurements (AUC), suggesting that C_0 are a good index of overall Tac exposure.[2-5] Despite the fact that Tac has been a widely used immunosuppressive drug for almost 30 years, only a few prospective controlled trails have been conducted to establish the target Tac concentrations in relation to clinical outcome.[6-8] Based on the results of these studies, a Tac C_0 range of 5-15 ng/mL was advised to achieve optimal efficacy with minimal toxicity in the first month post-transplantation. The relationship between Tac exposure and rejection in renal transplantation has been evaluated in several other studies.[4,9-11] The results of these studies are not consistent. Most transplant centers worldwide have nevertheless implemented TDM for Tac, disregarding the fact that only limited evidence is available for performing TDM and that the exact target C_0 range is poorly defined.[12]

An important cause of late loss of kidney allografts is chronic allograft nephropathy. Since episodes of acute rejection, particularly if severe, are considered to be a major risk factor for chronic allograft nephropathy,[13-15] avoiding episodes of acute rejection is an important objective to improve organ survival. This prompted us to investigate whether there is a correlation between the currently used Tac target C_0 and the incidence of acute rejection in a large cohort (n = 1304) of kidney transplant recipients (*Chapter 2.1*). We did not find a correlation between the development of acute rejection in first month or first year after transplantation and the Tac C_0. Over the years the target Tac concentrations substantially changed, with targets as high as 20 ng/mL in the early years, and with targets as low as

3-7 in the Symphony study.[16] We stratified our data into two groups: patients with Tac C_0 < 5.0 ng/mL *vs.* patients with Tac C_0 > 5.0 ng/mL and patients with Tac C_0 < 10.0 ng/mL *vs.* patients with Tac C_0 > 10.0 ng/mL. From this analysis we could not conclude that the target Tac C_0 should be above 5 or 10 ng/mL to avoid renal transplant recipients from an acute rejection. Based on our results one could argue that the threshold for efficacy lies even below the currently used targets. This is in line with the results of a single-center study of 528 prospectively followed kidney transplant recipients, recently reported by Gaynor *et al.*[17] They demonstrated that the hazard rate of developing a first acute rejection during the first 12 months after renal transplantation was significantly higher among patients having a lower Tac C_0 measured prior to the time of rejection occurrence. Gaynor *et al.* reported a 18% lower acute rejection rate for every 1 ng/mL increase in Tac level (p = 0.0003). Use of the cut-off of < 4.0 *vs.* ≥ 4.0 ng/mL for Tac C_0 yielded an even greater association with acute rejection rate, with an estimated hazard ratio of 6.33 (p < 0.0001). Based on these findings Gaynor *et al.* suggested a Tac C_0 cutoff of 4.0 ng/mL as the lower limit of the therapeutic window.[17]

Contrary to our results Israni *et al.* did find an association between Tac C_0 and incidence of acute rejection in the first 6-months post-transplant in a cohort of 1930 patients.[18] Using a multivariate Cox proportional hazards model, they found that each 1 ng/mL decrease in Tac C_0 was associated with a 7.2% increased risk of acute rejection [hazard ratio (HR) = 1.07, 95%-confidence interval (CI) (1.01, 1.14); p = 0.03] in the first 6 months[18] One of the most important discrepancies between our study and Israni *et al.* is that we only had access to Tac concentrations drawn at predefined time points (days 3, 10, 14, and months 1 and 6 after transplantation). These Tac concentrations might not be the last measured concentrations prior to the occurrence of the acute rejection, so we cannot exclude the possibility that a similar analysis with Tac C_0 measured right before diagnosing acute rejection would show an association. In the study of Israni and colleagues clinical data were collected at the time of transplantation and regularly thereafter until allograft failure and maintained in a central database.

Despite the lack of a clear concentration-effect relationship, our study presented in chapter 2 does not led us to propose that TDM of Tac is useless. TDM of Tac remains a major support to patient management, to assess unexpected pharmacogenetic influences on Tac pharmacokinetics. Without Tac TDM the large between-patient variability in Tac pharmacokinetics would be unrecorded putting some patients at risk for overexposure (C_0 > 15 ng/mL) and consequently toxicities and other patients for underexposure (Tac < 4.0 ng/mL) which may lead to higher risk for acute rejection. Moreover, TDM of Tac is useful to assess compliance and in the detection of drug interactions.

1.1. Intracellular tacrolimus concentrations

Due to the fact that the intracellular compartment of lymphocytes is the target site of Tac action, we think that whole blood concentrations can only serve as substitute markers, at least when considering its efficacy. Several small studies have evaluated the potential contribution of measuring Tac intracellular concentrations in peripheral blood mononuclear cells (PBMCs).[19-22] All studies demonstrated a lack of correlation between intracellular Tac concentrations and whole blood concentrations, providing support for the assumption that the whole blood concentration rather serves as a global drug exposure marker, whereas intracellular drug monitoring may more precisely determine whether a patient's immunosuppressant intake is sufficient. Capron *at al.* performed a study that included 96 kidney transplant recipients 6 (5.8%) of whom developed rejection during the first month after kidney transplantation.[20] Rejection tended to be associated with lower Tac concentrations in PBMCs ($p = 0.094$).[20] A later study, also performed by Capron *at al.* demonstrated in liver transplant recipients that clinical rejection established in 12 patients (10.8%) was characterized by a significantly lower mean Tac PBMC concentrations on days 5 and 7 after transplantation ($p < 0.05$).[19] Lemaitre *et al.* explored the pharmacokinetics and pharmacodynamics of Tac in 10 de novo liver transplant recipients by measuring the calcineurin activity as well as whole blood and intracellular Tac concentrations.[22] There was one study participant suffering acute cellular rejection during the study period despite an adequate Tac whole blood concentrations. However, his intracellular Tac concentrations were four-fold lower compared to those of the mean study population.[22] Tac intracellular concentrations displayed a greater variability between patients, and tended to be lower prior to acute rejection. However, despite these encouraging findings, more studies on intracellular Tac concentrations are required and the following questions need to be answered: Can a therapeutic range be defined, and could this kind of monitoring be relevant for both early post-transplant period and long-term examinations? Currently we are working on a prospective study to establish whether measurement of intracellular Tac concentrations in PBMC indeed better correlates with the incidence of acute rejection in the renal transplant recipients (n = 240) during the first year after transplantation.

1.2. Pharmacodynamic markers

Measurement of a drug's pharmacodynamic effect may be a good way to perform TDM of Tac. Several studies have focused on pharmacodynamic markers, which may better reflect efficacy and safety taking into account the inter-patient variability in the immunomodulatory effect of Tac rather than whole blood C_0.[23,24] Diverse methods have been evaluated and validated for the measurement of biomarkers such as lymphocyte proliferation, T-cell surface antigen expression (CD25, CD26, CD71, CD54, CD95, and CD134), and intracellular cytokine synthesis (like IL-2) in stimulated whole blood from treated

transplant recipients.[25] Tac inhibits the calcineurin pathway of activated T-lymphocytes resulting in decreased levels of dephosphorylated NFAT, less production of IL-2, and ultimately, inhibition of T-lymphocyte proliferation. Several studies demonstrated that the protein expression of IL-2 in cell samples can be used as a pharmacodynamic tool for TDM of Tac.[23] However, this assay measures the effects of other immunosuppressive drugs, like steroids, as well, and is therefore not specific for Tac.[24] Recently it was found that the amount of phosphorylation of the p38MAPK, a key factor in T-cell development and activation, is inversely correlated with Tac C_0 of kidney transplant recipients. Furthermore, increased p38MAPK phosphorylation was associated with higher T-lymphocyte activation status, which was inhibited by Tac in a dose dependent way *in vitro*.[26,27]

In a pilot study (*Chapter 2.2*), conducted in 12 stable renal recipients, we evaluated the effect of the conversion from twice-daily to a once-daily Tac formulation on p38MAPK phosphorylation status. All of these patients participated in a non-randomized conversion study, conducted in stable renal transplant recipients (n = 247) to evaluate the safety of the conversion from Tac twice-daily formulation to a once-daily formulation (*Chapter 4.4)*. In this conversion study and in other studies it has been shown that conversion from twice-daily to a once-daily Tac formulation on a 1:1 (milligram : milligram) daily dose basis is associated with significantly lower Tac exposure (of up to 15%).[28-31] Because the incidence of acute rejection after conversion in our study was extremely low (0.8%) we think that observed decrease in Tac exposure after conversion is not clinically relevant. In order to generate additional proof for this statement we decided to investigate the effect of conversion on the pharmacodynamic effect of Tac in this pilot study (*Chapter 2.2*). Despite the fact that in these 12 patients Tac C_0 was not significantly decreased after conversion (6.0% reduction, $p = 0.54$), p38MAPK phosphorylation was significantly increased (11.8% increase; $p = 0.034$), demonstrating the high sensitivity of this assay. Since increased p38MAPK phosphorylation has been associated with more T-lymphocyte activation[32] it may theoretically result in a higher risk of acute cellular rejection for renal transplant recipients.[33] It would be interesting to determine the risk of rejection during Tac therapy in renal transplant recipients with the help of phosphor-specific flow cytometry. If indeed this pharmacodynamic parameter appears to correlate well with the incidence of acute rejection, then in a subsequent clinical trial it could be tested whether this pharmacodynamic approach can be used to decide on dose adjustments during Tac therapy.

2. Individualization of tacrolimus starting dose

Obtaining adequate exposure to Tac as early after transplantation as possible is considered important to ensure maximum efficacy of the drug.[16] With this in mind, it would

make sense to individualize the starting dose of Tac according to a patient's phenotype (*Chapter 3.1*) or genotype (*Chapter 3.2*).

2.1. Phenotyping

In Chapter 3.1 we evaluated whether pre-transplant Tac dose requirement phenotyping is a feasible strategy for patients scheduled for a kidney transplantation to achieve in the early phase after transplantation therapeutic Tac concentrations. The primary goal of the study described in Chapter 3.1 was to investigate whether pre-transplant Tac dose requirements of patients scheduled to undergo living donor kidney transplantation correlate with post-transplant dose requirement. The first attempt to characterize the oral pharmacokinetics of Tac prior to transplantation to assess possible predictors for dose requirements after transplantation, was done by Boots *et al.*[34] They performed a 3-hour AUC prior to transplantation in 71 renal transplant recipients. The Tac doses and the corresponding C_0 (at 0.5, 1, 2, 3, 6, 13 and 26 weeks post-transplantation) were used to calculate the Tac dose necessary to achieve a C_0 of 10 ng/mL. The observed correlation between the 3-hour dose-normalized AUC and the Tac dose required to achieve 10 ng/mL after transplantation was however weak.[34] Subsequently, Campbell *et al.* hypothesized that when the first post-operative Tac dose would be given according to single pre-transplant whole blood concentration 2-hour (C_2) after Tac dosing, the proportion of subjects achieving therapeutic Tac C_0 ($C_0 \geq 10$ ng/mL) within 3 days of kidney transplantation would increase compared with these subjects treated with standard bodyweight-based Tac dose.[35] They demonstrated in a cohort of 90 renal transplant recipients, of whom 84 were included in the intention to treat analysis (control group n = 43; intervention group n = 41), that the proportion of subjects achieving Tac $C_0 \geq 10$ ng/mL within 3 days of kidney transplantation was not significantly different between a group of patients who received a standard, bodyweight-based Tac dose and a group in which post-transplant Tac dose was based on a C_2; 82.9% control *vs.* 93.0% intervention group, $p = 0.19$.[35] The findings demonstrated by Boots *et al.* and Campbell *et al.* are not in line with our results, presented in Chapter 3.1. We evaluated retrospectively the predictive value of Tac dose requirements pre-transplantation on this same parameter post-transplantation in a cohort of 57 ABO-incompotible (ABOi) kidney transplant recipients. This study demonstrated that Tac dose requirements (defined as the ratio of the Tac C_0, divided by the total daily Tac dose, D; C_0/D) before transplantation correlate strongly with dose requirements early after transplantation explaining 63% of the variation in dose requirements on post-transplantation day 3: $r^2 = 0.633$, $p < 0.01$. The choice to perform this study among ABOi kidney transplant recipients, was substantiated by the fact that in this population of kidney transplant recipients the treatment with immunosuppressive therapy (Tac, mycophenolate meofetil, and prednisolone) is routinely started two weeks before transplantation date and TDM is performed during this time. Thus, these

patients are in steady-state at the time of surgery and the effects of glucocorticoids on Tac pharmacokinetic may not change much after transplantation. Whereas patients in previous studies[34,35] were not in steady-state prior transplantation, and pre-transplant Tac pharmacokinetic (based on a single dose) in these patients was correlated and compared with Tac pharmacokinetic and Tac dose requirement determined at least after 3 days post-transplantation (steady-state condition). Another possible explanation for the conflicting results between our study and studies by Boots and Campbell could be the concomitant use of glucocorticoids. Steroids increase the clearance of Tac, possibly due to induction of the cytochrome P450 enzyme system in the liver.[36,37] This inducing effect of glucocorticoids on the metabolizing system may not have had post-operatively an important influence in our study as our patients had been treated with a stable and pharmacologically relevant prednisolone dose for two weeks before transplantation, whereas patients in Boots *et al.* and Campbell *et al.* did receive glucocorticoids only after transplant surgery.

The rationale behind pre-transplantation phenotyping is to shorten the time to reach target Tac exposure, and to avoid substantial over- and under-exposure to this drug. In Chapter 3.1 we have shown a strong correlation between pre- and post-transplant Tac dose requirement. Based on our data it would make sense to base the Tac starting dose on the pre-transplant dose requirement rather than use a standard bodyweight-based starting dose of Tac at the time of transplantation. With this strategy one may be able to prevent under-exposure and rejection, or Tac toxicity in case of over-exposure. However, the possible benefit of such a strategy to start immunosuppressive therapy before transplantation has to be demonstrated in a prospective clinical study.

2.2. Genotyping

Several non-randomized, descriptive studies evaluated the association between the *CYP3A5* 6986A>G variant allele (rs776746, A=*1, G=*3) and Tac pharmacokinetics in transplant recipients. The available literature does provide evidence of a strong association between *CYP3A5* genotype and Tac pharmacokinetics and dose requirement.[38-46] Based on these findings, it was conceived that pre-transplantation genotyping for *CYP3A5* and using this pharmacogenetic information to choose the starting dose of Tac, would be a promising option to personalize Tac therapy and this idea justified a prospective, randomized-controlled clinical trial. The first prospective randomized-controlled study (Tactique study) which investigated whether a *CYP3A5* genotype-based Tac dosing strategy is beneficial was conducted by Thervet *et al.*[47] They demonstrated in 280 renal transplant recipients that *CYP3A5*-based adaptation of the Tac starting dose increases the proportion of patients reaching the target Tac C_0 as compared with a standard, bodyweight-based dosing approach. Furthermore, by a *CYP3A5* genotype-based

approach, the time to reach the Tac target exposure range was shortened (8 *versus* 25 days after the start of Tac in the genotype-based and standard-dose group, respectively). Nonetheless, a *CYP3A5* genotype-based Tac dosing approach was not associated with a reduction of the incidence of acute rejection nor an improvement of any other clinical outcome.[47] This may have resulted from the fact that in the study of Thervet *et al.* Tac was started as late as day 7 after transplantation. In the first post-transplant week, most patients received powerful immunosuppression with antithymocyte globulin and high-dose mycophenolate which is not the standard in any transplant centers. In a recently published study this French investigation group have also demonstrated that the adaptation of initial Tac dose according to *CYP3A5* genotype does not lead to better long-term transplantation outcomes of the 236 kidney transplant recipients who participated in the Tactique study.[48]

Our randomized-controlled trial (*Chapter 3.2*) was specifically designed to prospectively investigate the added value of pre-transplant genotyping for *CYP3A5* in kidney transplantation. This study -conducted among 240 *de novo* renal transplant recipients- shows that a *CYP3A5*-based adaptation of Tac starting dose does not lead to a higher proportion of patients reaching the target Tac C_0 range on day 3 after kidney transplantation as compared to a standard, bodyweight-based dosing approach. In addition, *CYP3A5*-based dosing of Tac was not associated with a shorter time to achieve the Tac target C_0 nor with improved clinical outcome. These findings demonstrate that a *CYP3A5*-genotype based approach to Tac treatment is not beneficial for kidney transplant recipients receiving standard, Tac-based, combination immunosuppressive therapy from the day of transplantation and in whom the Tac dose is adjusted by routine Tac blood concentration monitoring (TDM). This is in line with the available guidelines for pharmacogenetics and Tac dosing which suggest that *CYP3A5* genotyping cannot replace TDM, as other demographic and clinical factors also have impact on Tac dose requirement.[49,50]

These two randomized-controlled trials should lead to the conclusion that the idea of personalizing the Tac dose on an individual's genotype should be abandoned. Up until now, *CYP3A5* is the strongest known genetic predictor of Tac dose requirement, however it does not explain all variability.[51,52] In our study and in the study of Thervet, around 40% of patients in the *CYP3A5* genotype-based dosing group were within the target Tac C_0 range at first steady state. Other genetic variants contribute to the residual variability in Tac dose requirement. Recently, several genetic variants have been found to be associated with Tac dose requirement. The *CYP3A4*22* SNP (rs35599367; C>T) in intron 6 has an allele frequency of about 5% in Caucasians. The T-variant allele has been associated with reduced *CYP3A4* mRNA expression and lower in vitro CYP3A4 enzyme activity.[53] Elens *et al.* have demonstrated that de novo kidney transplant recipients who carry 1 or 2

T alleles require significantly lower Tac doses to reach the target C_0 than wild-type CC patients. This effect was independent of *CYP3A5* genotype.[54] These findings were confirmed by the same researchers in an independent cohort of kidney transplant recipients and a cohort of pediatric heart transplant recipients.[55,56] Pallet *et al.* confirmed these results in a cohort of 186 kidney transplant recipients, in whom the *CYP3A4*22* allelic variant was associated with a slower Tac metabolism and higher systemic exposure in the early post-transplant period. The patients carrying this gene variant may require 30% lower Tac doses than patients with the *CYP3A4*1/*1* genotype.[57] Therefore, multiple genotype-based dosage adjustment taking into account *CYP3A5* and *CYP3A4* allelic variants might optimize initial Tac doses more accurately than if based on *CYP3A5* alone.

Besides the polymorphisms in the genes expressing the Tac-metabolizing enzymes CYP3A4 and CYP3A5, several other polymorphisms have been shown to also affect Tac pharmacokinetics. Nicotinamide adenine dinucleotide phosphate (NADPH)-CYP oxidoreductase (POR) is a protein that functions as an electron donor for CYP mono-oxygenase enzymes (including CYP3A) and is therefore essential for CYP-mediated drug oxidation.[58] Actually, polymorphisms in the P450 oxidoreductase (POR) genes have been demonstrated to modulate the activity of CYP3A enzymes. Several studies provide evidence that the *POR*28* SNP (rs1057868; C>T) is associated with additional increases (25%) in Tac dose requirements in patients carrying a *CYP3A5*1* allele. Moreover, patients expressing CYP3A5 and carrying the *POR*28 T*-variant allele also required a significantly longer time to reach the target Tac C_0 and were more often underexposed in the early phase after transplantation.[59,60]

Polymorphisms in the gene expressing the drug transporter ABCB1 could also contribute to alterations in Tac pharmacokinetics. Due to the importance of ABCB1 in the absorption of Tac several SNPs in the *ABCB1* gene have been extensively investigated in relation to Tac pharmacokinetics. The *ABCB1 3435C>T* (rs1045642), *1236C>T* (rs1128503), and *2677G>T/A* (rs2032582) SNPs, which are in linkage disequilibrium, have received the most attention in this respect. Taken together, the results of these studies have been disappointing as they have demonstrated only a limited (if any) effect of *ABCB1* SNPs on Tac disposition.[44,61]

Taken together, several SNPs in genes other than *CYP3A5* have been associated with Tac dose requirement. Their impact, however, appears to be smaller than that of *CYP3A5*3*. If these additional genetic variants do indeed explain residual variability in Tac dose requirement, development of an accurate polygenic algorithm could be a good approach to help physicians to decide on an individual's starting dose.[62] By combining genetic information with demographic and clinical variables, which are found to be associated

with Tac dose requirements such as age, weight, days after transplantation, albumin, hemoglobin, the use of calcium channel blockers and corticosteroid use, predictive algorithms may be developed that will allow for more reliable dosing, thereby limiting early over- and under-exposure.[62,63]

3. Tacrolimus intra-patient variability

Strategies targeting adequate exposure to Tac as early after transplantation as possible are being pursued in the effort to improve short-term outcomes. However, adequate exposure to immunosuppressive drugs in maintenance treatment is crucial for long term graft survival. In *Chapter 4* of this thesis we have studied a recently identified (bio)marker for long-term kidney transplantation outcome, namely the intra-patient variability (IPV) in Tac exposure. Borra *et al.* was the first to provide evidence that high IPV in Tac exposure is associated with poor graft survival after kidney transplantation.[64] Subsequently, several investigators have confirmed the finding of Borra *et al.* both in adults[65-67] and in pediatric[68-70] renal graft recipients. All these studies were limited by a relatively small number of events and short follow-up period. This prompted as to enlarge our original study population (Borra *et al.*) and extend the duration of the follow-up to evaluate the impact of high IPV in Tac exposure on a composite endpoint consisting of late cute rejection, transplant glomerulopathy, graft loss (censored for death) or doubling of serum creatinine (*Chapter 4.2*). Using multivariate analysis we have now confirmed in a cohort of 808 renal transplant recipients that IPV in Tac exposure is an independent risk factor for inferior long-term outcomes after kidney transplantation. Patents with high IPV had a 1.4 times higher risk of reaching the composite endpoint (hazard ratio 1.42, 95%-CI: 1.06 – 1.90; p = 0.019). Moreover, we have shown that the risk of developing the composite endpoint increases with increasing Tac IPV and decreasing Tac C_0 (*Chapter 4.2; Figure 3*). Hence, it has been suggested that in patients with high IPV (>16.2%) it is judicious to strive for higher Tac C_0 (> 7.0 ng/mL), to reduce the risk of poor kidney transplantation outcomes. Incorporating algorithms that calculate IPV in Tac exposure into electronic patient records may assist physicians to recognize patients with high intra-patient Tac variability. Based on the available data, a prospective evaluation of the use of Tac IPV monitoring to investigate whether it can indeed improve outcomes could be suggested.

The impact of IPV in Tac exposure on graft survival after heart transplantation had not been studied before. Cardiac allograft vasculopathy (CAV) is a problem that limits long-time survival after heart transplantation. We decided to evaluate whether a high IPV in Tac exposure is associated with more rapid progression of CAV in heart transplant recipients (*Chapter 4.3*). In contrast to the renal transplant recipients, we failed to find a relationship between the IPV in Tac exposure and the risk on CAV. These findings suggest that the implication of a high IPV of Tac exposure might be organ-specific, and thus

less important in heart transplant recipients. The discrepancy between renal and heart transplant recipients might be explained by the fact that Tac C_0 (measured between 6 -12 months after transplantation) in heart transplant recipients are substantially higher than in renal transplant recipients (11.0 ng/mL *vs.* 6.9 ng/mL, respectively). Due to the higher exposure fluctuations in exposure downwards may not reach levels associated with under-immunosuppression. Although the main immunosuppressive regimen in both populations consists of Tac, mycophenolate mofetil and prednisolone, the heart transplant recipients use the prednisolone for a longer period of time and they have more outpatient visits in the first year post-transplantation than renal transplant recipients. Therefore, a progression in the disease and eventually non-compliance to the drug therapy might be noticed earlier. These differences may have contributed to the contrasting outcomes of the two studies. It is however, also possible that we have missed a positive correlation, as the population in the study with heart transplant recipients was quite smaller and the number of events was considerable lower than in the study with renal transplant recipients. This study in heart transplant patients may not have had sufficient statistical power to identify the association between the IPV in Tac exposure and the progression of CAV. Consequential studies with a higher number of investigated patients (higher number of events) and longer follow-up period, which would allow to conduct multivariable Cox-regression analysis, should be performed to clarify whether progression of CAV is associated with the IPV in Tac exposure among heart transplant recipients.

Several factors can influence Tac pharmacokinetics and contribute to Tac IPV.[71] Obviously, IPV in Tac exposure may be related to non-adherence to the immunosuppressive drug regimen and non-adherence was found to be an important risk factor for poor long term outcomes.[72] A meta-analysis showed that non-adherent renal graft recipients had a seven-fold increase in the risk for graft failure.[72] Across a variety of therapeutic classes it has been proved that less frequent dosing regimens resulted in better medication adherence. To improve adherence in transplant recipients, a prolonged-release oral dosage form of Tac (Advagraf®, Astellas Pharma) has been developed to enable once daily dosing alternative with a similar safety profile as the widely used immediate-release (twice daily) formulation (Prograf®, Astellas Pharma). In a recently published study of Kuypers *et al.* it was shown that a once daily regimen of Tac was significantly superior to a twice daily regimen. This study demonstrated that 81.5% of the renal transplant patients randomized into the once daily group remained persistent with treatment during the follow up period of 6 months, whereas this percentage was about 72% in the twice daily group. Among patients who remained persistent with the regimen, 88.2% of the once daily group and 78.8% of twice daily group (p = 0.0009) took the prescribed number of daily doses.[73] Theoretically, the improved adherence to Advagraf® could contribute to

a reduced intra-patient Tac exposure variability. Since there is a significant association between the IPV in Tac exposure and graft survival, we investigated (*Chapter 4.4*) whether Advagraf® exhibits a lower IPV in Tac exposure than Prograf®. In this non-randomized conversion study, conducted in a cohort of 247 stable renal transplant recipients it was shown that conversion from Tac twice-daily to Tac once-daily formulation on the same milligram-for-milligram daily dose basis does not result in a lower Tac IPV (calculated using C_0). This finding is in line with findings provided by Wehland *et al.*[74] and Hooff *et al.*[75] which were also conducted in stable renal transplant recipients but in a smaller patient groups. However, other studies (also in smaller populations) have demonstrated a reduction of Tac IPV after conversion from twice-daily to a once-daily formulation.[76-78] Alloway *et al.* demonstrated in a cohort of 67 stable kidney transplant recipients that IPV in Tac exposure (calculated using AUC_{0-24}) significantly decreased after conversion to a once-daily Tac formulation in African-American patients (n = 12).[76] However, no significant decrease in Tac IPV was observed among Caucasians in this study. Owing to different dissolution properties, Advagraf® is typically released and absorbed further along the gastrointestinal tract, while Prograf® is characterized by an absorption more proximally, primarily in the proximal small intestine. It has been reported that the expression of CYP3A enzymes reduces progressively along the length of the gastrointestinal tract.[79] Consequently, the CYP3A status (activated or inhibited) may have less impact on the intra-patient Tac exposure variability by use of Advagraf® compared with Prograf®. Given that about 85% of Africans are *CYP3A5* expressers, this information could explain the findings of Alloway *et al.* namely, the four-fold lower Tac IPV in African-American patients compared with Caucasians after conversion from twice-daily to once-daily Tac formulation.[76] Another factor contributing to lower variability in black patients may be the fact that CYP3A5 is more stably expressed, whereas CYP3A4 expression is more variable, and more influenced by environmental factors. The vast majority of our study population was Caucasian (73%) about 15% of whom were *CYP3A5* expressers.[80] Conversion from Prograf® to Advagraf® in a cohort of 129 Taiwanese stable renal graft recipients also led to a significant decrease in mean IPV of Tac C_0: 14.0% ± 7.5% before conversion *vs.* 8.5% ± 5.0% ($p < 0.05$).[78]

In the study of Stifft *et al.* the IPV in Tac exposure did not change after conversion from twice-daily to once-daily Tac formulation when it was calculated using C_0. However, they did find a significant change in Tac IPV after conversion when it was calculated using AUC_{0-24}.[77] The IPV in Tac AUC_{0-24} decreased with 3.2% after conversion, which although statistically significantly different, may be considered not to be clinically relevant. In our study, we chose to evaluate the IPV in Tac exposure using C_0 based on the reports demonstrating a significant correlation between AUC_{0-24} and C_0 for both Tac formulations. Moreover, C_0 is the TDM parameter that is used most frequently in clinical practice. An

important weakness of our study is that we did not measure patient compliance directly. However, because the observed median IPV in Tac C_0 was not very high before conversion (17.9%), we feel that the rate of medication adherence in the selected population may have been high, with a little potential for further improvement by conversion to Tac once-daily formulation.

4. Conclusions of this thesis

In this thesis several studies are reported with the aim to improve the use of Tac after solid organ transplantation. A striking first finding was the lack of an association between the Tac C_0 and the incidence of acute rejection after kidney transplantation. This observation in our view does not imply that TDM of Tac can be abolished. We have shown that monitoring of pharmacodynamic markers such us monitoring of p38MAPK phosphorylation in T-lymphocytes by whole blood phospho-specific flow cytometry could be another alternative for TDM of Tac.

In this thesis we have shown that steady-state, pre-transplant Tac dose requirements strongly correlate with post-transplant dose requirements. Using information on pre-transplant Tac dose requirement (Tac dose and concentration data), may be able to predict Tac exposure after transplantation and limit under- and over-suppression.

Adapting the starting Tac dose according to a patient's *CYP3A5* genotype does not lead to earlier achievement of the target Tac C_0 range or superior clinical outcome as compared with standard, bodyweight-based dosing approach. Therefore, routinely genotyping renal transplant recipients for *CYP3A5* cannot be recommended.

A high IPV in Tac exposure is associated with poor long-term kidney transplant outcome in kidney transplant recipients. However, a high IPV in Tac exposure was not found to be associated with the progression of CAV in heart transplant recipients.

Conversion from immediate-release (twice-daily) Tac formulation (Prograf®) to a modified-release (once-daily) Tac formulation (Advagraf®) on the same milligram-for-milligram daily dose basis significantly reduces Tac exposure but does not lower IPV in Tac exposure.

ALGEMENE DISCUSSIE

Zestig jaar geleden werd de eerste succesvolle niertransplantatie bij de mens uitgevoerd. Inmiddels is niertransplantatie de voorkeurs behandeling voor patiënten met eindstadium nierfalen. De ontwikkelingen op het gebied van immunosuppressieve geneesmiddelen hebben de overleving na niertransplantatie sterk verbeterd, maar er zijn nog diverse uitdagingen. Nog steeds krijgen veel patiënten een afstoting en velen van hen krijgen een ernstige infectie, maligniteit of een andere bijwerking van de immunosuppressieve therapie. Het onderzoek zoals beschreven in dit proefschrift had als doel om de behandeling met tacrolimus (Tac) verder te optimaliseren, waarbij de nadruk lag op het verminderen van de risico's in de eerste fase na transplantatie en het verbeteren van de lange termijn uitkomsten.

1. Concentratie-effect relatie

In Hoofdstuk 2 is de relatie bestudeerd tussen de expositie aan Tac, uitgedrukt als de (volbloed) concentratie en de effectiviteit van het geneesmiddel. Het gebruik van Tac heeft geleid tot een belangrijke reductie van de incidentie van acute afstoting en het geneesmiddel heeft de korte termijn resultaten duidelijk verbeterd.[1] Tac is een zogenaamd "critical dose drug", wat wil zeggen dat de effectieve (werkzame) concentraties dicht liggen bij de toxische concentraties. Deze nauwe therapeutische index, alsmede de grote inter-individuele variabiliteit van Tac concentraties bij een vergelijkbare dosering, heeft geleid tot het advies om dit geneesmiddel te doseren op geleide van bloedconcentraties. Dit principe staat bekend als "Therapeutic Drug Monitoring (TDM)". Volbloed dalspiegels van Tac (C_0) correleren vrij goed met de expositie gedurende een doseringsinterval -gemeten als "area under the concentration-versus-time curve" (AUC)-, hetgeen suggereert dat deze C_0 een goede maat is voor TDM van Tac.[2-5] Jammer genoeg zijn er maar een paar prospectieve, gecontroleerde studies verricht om de streefconcentratie van Tac vast te stellen.[6-8] De resultaten van deze studies ondersteunen de momenteel geadviseerde streef Tac C_0 van 5-15 ng/mL in de eerste maand na transplantatie. Diverse andere onderzoekers hebben getracht om de optimale Tac C_0 range te definiëren, waarbij een goede verdraagbaarheid is gekoppeld aan een laag risico op afstoting.[4,9-11] De resultaten van deze studies zijn niet eenduidig en worden vaak gehinderd door de retrospectieve studieopzet. De meeste transplantatiecentra in de wereld hebben TDM voor Tac in hun programma's geïmplementeerd, ofschoon het bewijs dat dat nuttig is beperkt is en de streefconcentraties slecht gedefinieerd zijn.[12]

Een belangrijke oorzaak van laat verlies van transplantaten is het proces dat van oudsher bekend staat als "chronic allograft nephropathy". Omdat het optreden van acute rejectie, en vooral van ernstige acute rejectie, gezien wordt als een belangrijke risicofactor voor

het krijgen van chronic allograft nephropathy,[13-15] is het vermijden van dergelijke afstotingen een belangrijk middel om de lange termijn transplantaatoverleving te verbeteren. Wij onderzochten daarom het verband tussen de hoogte van de Tac C_0 en de incidentie van acute rejectie in een groot cohort (n = 1304) niertransplantaat ontvangers (*Hoofdstuk 2.1*). Deze patiënten werden met de huidige standaard immunosuppressieve "cocktail" behandeld en de gerealiseerde Tac expositie was variabel en lag tussen de 2,5 en 29 ng/mL. Ondanks het feit dat een zeer groot aantal patiënten onderzocht werd in deze analyse en er betrouwbare data uit grote klinische studies beschikbaar waren, konden we geen correlatie vinden tussen het optreden van acute rejectie in de eerste maand of in het eerste jaar na niertransplantatie en de hoogte van de Tac C_0. In de afgelopen 20 jaar is de streefwaarde van de Tac concentratie substantieel veranderd, met streefwaarden van 20 ng/mL en hoger in de eerste jaren na introductie van Tac en streefwaarden tussen 3 en 7 ng/mL in de Symphony studie.[16] We hebben de beschikbare data onderverdeeld in twee groepen: patiënten met een Tac C_0 < 5,0 ng/mL *vs.* patiënten met Tac C_0 > 5,0 ng/mL en de andere groep omvatte patiënten met een Tac C_0 < 10,0 ng/mL *vs.* patiënten met Tac C_0 > 10,0 ng/mL. Uit deze aanvullende analyse kunnen we niet concluderen dat de streefwaarde van de Tac C_0 al dan niet boven 5 of 10 ng/mL moet zijn om acute rejectie na niertransplantatie te vermijden. Wij kunnen derhalve geen streefwaarde voor de Tac C_0 definiëren. Gebaseerd op onze studieresultaten zou zelfs gesteld kunnen worden dat de drempel voor effectiviteit lager ligt dan de momenteel gangbare streefwaarde van 5 tot 15 ng/mL. Onze conclusies zijn in overeenstemming met de resultaten van een recentelijk gepubliceerde studie, uitgevoerd door Gaynor *et al.*[17] Zij rapporteerden op basis van een prospectief vervolg van 528 niertransplantatie patiënten, dat het risico op het ontwikkelen van een acute rejectie gedurende de eerste 12 maanden na transplantatie significant hoger was in patiënten met lagere Tac C_0, gemeten voorafgaand aan het ontstaan van acute rejectie. Deze studie heeft gedemonstreerd dat elke 1 ng/mL stijging in de Tac C_0 het risico op een acute rejectie met 18% deed dalen (p =0,0003). Door gebruik te maken van een grenswaarde voor Tac C_0 (< 4,0 *vs.* ≥ 4,0 ng/mL) werd een sterkere associatie met de incidentie van acute rejectie gevonden (hazard ratio (HR) = 6,33, p < 0,0001). Op basis van deze bevindingen suggereren Gaynor *et al.* dat de onderste limiet van de Tac therapeutische range C_0 4,0 ng/mL moet zijn.[17]

Israni *et al.* vonden ook in een cohort van 1930 patiënten een verband tussen de Tac C_0 en de incidentie van acute rejectie in de eerste 6-maanden na transplantatie.[18] In een multivariaat analyse vonden zij dat elke 1 ng/mL afname van de Tac C_0 in die periode het risico op afstoting met 7,2% deed stijgen [hazard ratio (HR) = 1,07, 95%-betrouwbaarheidsinterval (BI) (1,01, 1,14); p = 0,03].[18] Een belangrijk verschil tussen onze studie en die van Israni *et al.* is dat wij alleen toegang hadden tot de Tac concentraties die afgenomen waren op een aantal vooraf gedefinieerde tijdspunten (dag 3, 10, 14, en maand 1 en 6 na

transplantatie). Deze Tac concentraties zijn veelal niet de laatst gemeten concentraties voorafgaande aan een afstoting. Daarom kunnen we de mogelijkheid niet uitsluiten dat een vergelijkbare analyse van onze data, met de Tac C_0 gemeten net voor een afstoting, wel een significante relatie zou laten zien.

Onze studieresultaten moeten niet leiden tot de conclusie dat TDM voor Tac zinloos is. Aangezien Tac een geneesmiddel is met een grote inter-individuele farmacokinetische variabiliteit, blijft TDM voor dit geneesmiddel nodig. Na een standaarddosering Tac zullen verschillende individuen dus grote variaties in Tac expositie kunnen vertonen. Indien TDM niet wordt uitgevoerd, wordt deze grote inter-individuele variabiliteit niet gedetecteerd met als consequentie dat sommige patiënten bloot worden gesteld aan een te hoge Tac concentratie (C_0 > 15,0 ng/mL) wat op zijn beurt de kans op toxiciteit verhoogt, terwijl andere patiënten juist een hogere kans zouden kunnen hebben op acute rejectie als gevolg van een te lage blootstelling (C_0 < 4,0 ng/mL). Bovendien is TDM van belang bij het beoordelen van therapietrouw en het detecteren van geneesmiddelinteracties.

1.1. Intracellulaire tacrolimus concentraties

Tacrolimus remt de vermeerdering van lymfocyten en dit is het belangrijkste immunosuppressieve werkingsmechanisme van dit geneesmiddel. Het lijkt daarom logisch om de concentratie van Tac te bepalen in deze cellen en niet in het volbloed dat voor een groot gedeelte uit plasma, rode bloedcellen en bloedplaatjes bestaat. Een aantal kleine studies heeft de mogelijke meerwaarde van het meten van intracellulaire Tac concentraties in mononucleaire cellen uit het perifere bloed ("peripheral blood mononuclear cells"; PBMCs) onderzocht.[19-22] Al deze studies toonden een slechte correlatie tussen de intracellulaire Tac concentratie en de volbloed concentratie, wat de veronderstelling ondersteunt dat de intracellulaire Tac concentratie een betere afspiegeling is van de mate waarin het immuunsysteem wordt onderdrukt. Capron e*t al.* voerden een studie uit bij 96 niertransplantaat ontvangers waarvan er 6 een afstoting ontwikkelden in de eerste maand na transplantatie.[20] Deze afstotingen waren geassocieerd met een lagere Tac concentratie in de PBMCs (p = 0.094).[20] In een vervolgonderzoek van Capron *et al.* werd bij levertransplantaat ontvangers aangetoond dat de 12 patiënten met een afstoting een significant lagere concentratie Tac in PBMCs hadden op dag 5 en dag 7 na transplantatie in vergelijking met de patiënten die geen rejectie kregen (p < 0.05).[19] Lemaitre *et al.* bestudeerden de farmacokinetiek en farmacodynamiek van Tac in 10 levertransplaat ontvangers door naast de volbloed en de intracellulaire Tac concentraties ook de calcineurine activiteit te meten.[22] In dit onderzoek maakte één patiënt een acute cellulaire rejectie door ondanks een adequate Tac volbloed concentratie. Echter, de intracellulaire Tac concentraties van deze patiënt waren viervoudig lager dan die van de andere patienten.[22] De intracellulaire Tac concentraties kenden ook een grotere variabiliteit tussen

patiënten en leken lager voorafgaande aan het diagnosticeren van een acute rejectie. Ondanks deze bemoedigende bevindingen is het bewijs dat het meten van de intracellulaire Tac concentraties nuttig is niet geleverd en is meer onderzoek nodig. Bovendien moet nog een therapeutisch venster voor deze intracellulaire concentraties worden gedefinieerd, zowel voor de vroege fase na transplantatie als voor de onderhoudsbehandeling. Onze onderzoeksgroep werkt tegenwoordig aan een prospectieve studie om in een studiepopulatie van 240 niertransplantatie patiënten te bepalen of intracellulaire Tac concentraties daadwerkelijk beter correleren met de incidentie van acute rejectie in het eerste jaar na transplantatie dan met de Tac concentratie in de volbloed.

1.2. Farmacodynamische markers

Het bepalen van het farmacodynamisch effect van een geneesmiddel is op theoretische gronden een veelbelovende manier om TDM uit te voeren. In diverse studies is gezocht naar farmacodynamische parameters die de inter-individuele variatie in de immunosuppressieve werking van Tac beter weergeven dan de volbloed C_0.[23-24] Diverse methoden zijn ontwikkeld en gevalideerd voor het meten van biomarkers zoals; lymfocyten proliferatie, T-cel oppervlakte antigeen expressie (CD25, CD26, CD71, CD54, CD95 en CD134) en intracellulaire cytokine synthese (zoals IL-2) in gestimuleerd bloed van transplantatie patiënten.[25] Zoals hierboven beschreven remt Tac de productie van IL-2, hetgeen resulteert in een vermindering van de proliferatie van T-lymfocyten. Verschillende studies hebben aangetoond dat de IL-2 eiwitexpressie gebruikt kan worden als een methode voor farmacodynamische TDM voor Tac.[23] Echter, deze assay meet ook de effecten van andere immunosuppressieve geneesmiddelen zoals prednison en is dus niet specifiek voor Tac.[24] Recent werd bij niertransplantaat ontvangers vastgesteld dat de mate van fosforylering van het "mitogen activated protein kinase" (p38MAPK), een belangrijke stap in de T-cel ontwikkeling en activatie, omgekeerd evenredig is gerelateerd aan de Tac C_0. Ook bleek een hoge p38MAPK fosforylering geassocieerd te zijn met meer T-lymfocyt activatie.[26,27] Hoofdstuk 2.2 beschrijft een pilot farmacodynamische studie. In een studiepopulatie van 12 stabiele niertransplantaat ontvangers hebben we het effect van het omzetten van deze patiënten van tweemaal daags naar eenmaal daags Tac formulering op de p38MAPK fosforylering bestudeerd. Deze 12 patiënten hebben eerder deel genomen aan een niet-gerandomiseerde conversie studie ter evaluatie van de veiligheid van de omzetting van stabiele niertransplantaat ontvangers van tweemaal daags Tac formulering naar een eenmaal daags formulering. In onze conversie studie bevestigden wij de eerder gedane observatie dat volgend op een 1:1 omzetting van tweemaal daags naar eenmaal daags Tac formulering, de Tac expositie significant daalt (tot 15% lager).[28-31] Dat deze verandering klinisch niet relevant is blijkt uit de extreem lage incidentie van rejectie rondom dergelijke omzettingen. In onze studie maakten 2 van de 247 patiënten (0.8%) rondom de omzetting een acute rejectie door en in één van die gevallen leek deze

direct gerelateerd te zijn aan een te lage Tac expositie. Om meer steun te krijgen voor de veronderstelling dat de 15% daling in Tac volbloed concentraties niet klinisch relevant is, besloten wij om deze farmacodynamische studie uit te voeren. Ofschoon de Tac C_0 in deze 12 patiënten niet significant veranderde (p = 0.54), nam de p38MAPK fosforylering significant toe (11.8% toename; p = 0.034). Deze bevinding suggereert dat TDM van de farmacodynamiek van Tac gevoeliger is dan de klassieke farmacokinetische TDM middels volbloed concentraties. Aangezien een toegenomen p38MAPK fosforylering gekoppeld is aan meer activatie van T-lymfocyten[32] zou dit dus theoretisch kunnen leiden tot rejectie.[33] Het zou interessant zijn om bij patiënten met een niertransplantaat het risico op afstoting te vervolgen middels fosfo-specifieke flowcytometrie van p38MAPK. Als deze farmacodynamische parameter inderdaad sterk geassocieerd blijkt te zijn met rejectie, dan zou in een prospectieve studie bestudeerd kunnen worden of het uitvoeren van TDM op basis van deze parameter de klinische uitkomst verbetert.

2. Individualiseren van tacrolimus startdosering

Zoals uitgelegd in paragraaf 1 is de optimale C_0 range voor Tac niet onomstotelijk vastgesteld. Veel transplantatiecentra houden echter de waardes aan zoals beschreven in de bijsluiter door de producent van Tac (7 - 20 ng/mL). Daarnaast zijn er centra die een lagere Tac concentratie (5 - 15 ng/mL) nastreven welke vergelijkbaar is met de concentraties die in de Symphony studie zijn aanbevolen.[16] Een andere vraag is hoe deze streefconcentratie zo snel mogelijk is te bereiken. Het snel bereiken van een adequate Tac concentratie wordt gezien als een belangrijke factor in de preventie van rejectie.[16] Het zou daarom goed zijn om bij initiatie van Tac behandeling te onderzoeken in hoeverre het fenotype (Hoofdstuk *3.1*) of genotype (Hoofdstuk *3.2*) van de patiënt kan helpen in het individualiseren van de startdosering.

2.1. Fenotyperen

In Hoofdstuk 3.1 is onderzocht of de dosisbehoefte voorafgaand aan transplantatie, voorspelt hoeveel Tac een patiënt nodig heeft na transplantatie. Een eerste studie met dezelfde vraagstelling werd uitgevoerd door Boots *et al.*[34] Bij 71 niertransplantatie patiënten werd een 3-uurs AUC voorafgaand aan de niertransplantatie gemeten na een eenmalige proefdosis Tac. De Tac dosis en de corresponderende C_0 (op 0,5; 1; 2; 3; 6; 13 en 26 weken na transplantatie) werden gebruikt om de Tac dosis te berekenen die nodig zou zijn om een C_0 van 10 ng/mL te bereiken. In dit onderzoek bleek de correlatie tussen de Tac dosisbehoefte voor en na transplantatie zwak.[34] Campbell *et al.* onderzochten of het kiezen van een startdosering op basis van de volbloed concentratie 2 uur na Tac inname, de zogenaamde C_2, na een eenmalige testdosis voorafgaand aan transplantatie resulteerde in een hoger percentage patiënten in het therapeutisch raam na transplantatie ($C_0 \geq 10$ ng/mL).[35] De controlegroep kreeg een Tac dosis gebaseerd op lichaamsge-

wicht. In een groep van 84 patiënten (controle groep n = 43; interventie groep n = 41) konden zij geen voordeel aantonen van het gebruik van informatie verkregen tijdens de testdosis. De proportie patiënten met een Tac C_0 ≥ 10 ng/mL binnen de eerste drie dagen na niertransplantatie was niet significant hoger in de interventie groep (82,9% in controle groep *vs.* 93,0% in interventie groep, p = 0,19).[35] De belangrijkste tekortkoming van beide studies is het feit dat de farmacokinetische gegevens van Tac voorafgaand aan transplantatie werden bepaald na een eenmalige Tac toediening en dat deze na transplantatie gecorreleerd werden aan Tac farmacokinetiek in "steady-state" toestand.

In een groep van 57 AB0-incompatible (AB0i) niertransplantaat ontvangers hebben we retrospectief onderzocht of de Tac dosisbehoefte (uitgedrukt als de ratio van de Tac C_0 gedeeld door de Tac dosering; C_0/D) vóór transplantatie de dosisbehoefte na transplantatie kan voorspellen. In ons onderzoek vonden wij dat de Tac dosisbehoefte vóór transplantatie sterk correleerde met de dosisbehoefte na transplantatie. Maar liefst 63% van de variabiliteit in dosisbehoefte na transplantatie kon worden verklaard met de farmacokinetische gegevens verkregen voorafgaand aan transplantatie: r^2 = 0,633; p < 0,01. Deze studie was juist in deze groep AB0i niertransplantatie ontvangers uitgevoerd omdat bij hen de combinatie Tac, mycofenolaat mofetil en prednisolon al twee weken voorafgaand aan de transplantatie was gestart. Daarom waren de patiënten in ons onderzoek al in "steady-state" ten tijde van de operatie. Bovendien gebruikten de patiënten een stabiele dosis prednisolon. Glucocorticoïden verhogen de klaring van Tac, mogelijk door inductie van het cytochroom P450 enzym systeem in de lever.[36,37] In onze studie was gebruik van prednisolon dus geen storende variabele. In de eerder genoemde studies[34,35] waren patiënten echter niet in stabiele toestand, werd vaak het advies gebaseerd op de effecten van een enkele dosering en was er pretransplantatie meestal geen co-medicatie met prednisolon.

Aangezien er een sterke correlatie bestaat tussen de Tac dosisbehoefte vóór en na transplantatie, zouden patiënten die voor transplantatie al starten met Tac theoretisch gezien sneller een therapeutische concentratie bereiken dan patiënten die volgens het huidige protocol (start immunosuppressiva op de dag van transplantatie) worden behandeld. Echter, het mogelijke voordeel van het pre-emptief starten met immunosuppressiva therapie vlak voor transplantatie zou nader onderzocht moeten worden in een prospectief gerandomiseerde studieopzet.

2.2. Genotyperen

Een belangrijke voorspellende factor voor de Tac dosisbehoefte van een individuele patiënt is het cytochroom *P450 (CYP) 3A5* genotype. Het CYP3A5 enzym speelt een belangrijke rol in het metabolisme van Tac en sinds 1990 is bekend dat dit enzym polymorf

tot expressie komt. Verschillende studies hebben de associatie tussen aanwezigheid van het *CYP3A5* 6986A>G variant allel (rs776746, A=*1, G=*3) en de Tac farmacokinetiek in transplantatie patiënten onderzocht. Uit deze onderzoeken werd geconstateerd dat *CYP3A5* non-expressers (*CYP3A5*3/*3*) een hogere dosis-gecorrigeerde dalspiegel en een hogere piekspiegel hebben (C_0/D en C_{max}), een hogere dosis-gecorrigeerde AUC en een kortere tijd tot het bereiken van de streefconcentratie. De onderhoudsdosering is bij *CYP3A5* non-expressers lager dan bij *CYP3A5* expressers (*CYP3A5*1/*3* en *CYP3A5*1/*1*).[38-46] Vanwege het sterke verband tussen het *CYP3A5* genotype en de Tac dosisbehoefte is de gedachte ontstaan dat door genotyperen voor *CYP3A5*, voorafgaande aan de transplantatie, voor elk individu een passende startdosering kan worden gekozen. Dit zou wellicht tot het sneller bereiken van de streefconcentratie kunnen leiden en misschien ook tot betere uitkomsten van niertransplantatie. De eerste prospectieve gerandomiseerd-gecontroleerde studie die onderzocht of een op *CYP3A5* genotype-gebaseerde startdosering Tac inderdaad voordelen heeft, werd uitgevoerd in Frankrijk door Thervet *et al.*[47] Zij toonden aan in hun onderzoek, waar 280 niertransplantatie patiënten aan meededen, dat een op *CYP3A5*-gebaseerde aanpassing van de Tac startdosering leidt tot een toename van het percentage patiënten dat de C_0 streefwaarde bereikt. Bovendien was, dankzij de *CYP3A5* genotype gebaseerde behandeling, de tijd tot het bereiken van de streefwaarde korter dan in de groep die de standaard startdosering kreeg (8 *vs.* 25 dagen na start van de behandeling). Echter waren de incidentie van acute afstoting of andere klinische uitkomsten vergelijkbaar in beide groepen.[47] Dit werd mogelijk verklaard door het feit dat in de studie van Thervet *et al.* pas 7 dagen na de transplantatie gestart werd met Tac. In de eerste week na transplantatie werden de patiënten namelijk behandeld met krachtige immunosuppressie, zoals anti-thymocyten globuline en hoge doses mycofenolzuur, hetgeen in de meeste Nederlandse transplantatie centra geen standaardbehandeling is. In een recente studie heeft deze Franse onderzoeksgroep aangetoond dat de aanpassing van de Tac startdosering op basis van het *CYP3A5* individu genotype ook niet tot betere lange termijn transplantatie uitkomsten heeft geleid.[48]

Onze gerandomiseerde studie (Hoofdstuk *3.2*) was ook specifiek ontworpen om de meerwaarde van het *CYP3A5* genotyperen voorafgaand aan niertransplantatie te onderzoeken. Wij vonden dat een op *CYP3A5*-gebaseerde startdosering Tac niet leidt tot verhoging van het percentage patiënten dat de streefwaarde voor Tac C_0 op dag 3 na transplantatie bereikt. Evenmin was er een kortere tijd tot het bereiken van die streefwaarden. De klinische uitkomsten waren niet beter dan het gangbare beleid van een alleen op lichaamsgewicht gebaseerde dosis. Beschikbare richtlijnen erkenden tot nu toe wel dat het *CYP3A5* genotype een duidelijke invloed heeft op de Tac dosisbehoefte maar genotyperen werd niet geadviseerd, omdat voor Tac intensieve TDM wordt uitgevoerd en

eventuele verschillen in expositie zodoende snel kunnen worden gecorrigeerd. Op basis van onze resultaten is er geen reden die richtlijn te herzien.[49,50]

Ons eigen en het Franse onderzoek zijn niet het definitieve bewijs dat er geen toekomst is voor het genotyperen van niertransplantatie patiënten om de Tac dosis te individualiseren. Hoewel *CYP3A5* de sterkste genetische voorspeller is voor de dosisbehoefte is er nog veel resterende (niet door genetisch polymorfisme verklaarde) variabiliteit.[51,52] Deze zogenaamde residuele variabiliteit bedroeg in onze studie en in die van Thervet, ongeveer 60%. Overige genetische variaties hebben waarschijnlijk ook bijgedragen aan de resterende variatie in dosisbehoefte. Het *CYP3A4*22* polymorfisme (rs3559936; C>T) in intron 6 heeft een allel frequentie van ongeveer 5% in Kaukasiërs. Het T-variant allel leidt tot verminderde *CYP3A4* mRNA expressie en tot lagere *in vitro* CYP3A4 enzym activiteit.[53] Elens *et al.* toonden aan dat patiënten die drager zijn van 1 of 2 T allelen significant minder Tac nodig hebben om de C_0 te bereiken dan wild-type (CC) patiënten. Dit effect was onafhankelijk van het *CYP3A5* genotype.[54] Dezelfde onderzoekers bevestigden deze bevinding in een tweede groep niertransplantaat ontvangers en ook in een groep kinderen met een harttransplantatie.[55,56] Pallet *et al.* toonden ook aan dat het *CYP3A4*22* polymorfisme is geassocieerd met de Tac dosisbehoefte van 186 niertransplantatie patiënten. Dragers van het *CYP3A4*22* lijken een 30% lagere Tac dosis nodig te hebben dan patiënten met het *CYP3A4*1/*1* genotype.[57] Dat betekent dat een dosisadvies gebaseerd op *CYP3A5* en *CYP3A4*22* genotype wellicht accurater is dan wanneer dit uitsluitend op het *CYP3A5* genotype wordt gebaseerd.

Naast de polymorfismen in de genen die coderen voor de metaboliserende enzymen CYP3A4 en CYP3A5, is voor een aantal andere polymorfismen ook aangetoond dat zij de Tac farmacokinetiek beïnvloeden. Nicotinamide adenine dinucleotide phosphate (NADPH)-CYP oxidoreductase (POR) is een eiwit dat als elektronen-donor fungeert voor CYP mono-oxygenase enzymen (waaronder CYP3A). POR is daarom van groot belang voor de CYP-gemedieerde geneesmiddel-oxidatie.[58] Polymorfismen in het *POR* gen moduleren de activiteit van CYP3A enzymen. Verschillende studies hebben aangetoond dat het *POR*28* polymorfisme (rs1057868; C>T) leidt tot een toename van de Tac dosisbehoefte (25%) in dragers van het *CYP3A5*1* allel (zogenaamde CYP3A5 expressers). Patiënten die CYP3A5 tot expressie brengen en tegelijkertijd het *POR*28* T-variant allel dragen hebben significant meer tijd nodig om de streefwaarde van Tac C_0 te bereiken en bij hen komen vaker sub-therapeutische Tac concentraties voor in de eerste weken na transplantatie.[59,60]

Polymorfismen in het gen dat codeert voor het transporteiwit ABCB1 zouden ook kunnen bijdragen aan variabiliteit in de Tac farmacokinetiek. De effecten van dit polymorfisme

zijn met name op de absorptie van Tac uit de darm onderzocht. De *ABCB1* 3435C>T (rs1045642), 1236C>T (rs1128503), en 2677G>T/A (rs2032582) polymorfismen hebben hierbij de meeste aandacht gekregen. Echter, in grote lijnen gaven dergelijke studies een negatief resultaat en werd geen grote bijdrage gevonden van deze drie polymorfismen op de Tac kinetiek.[44,61] Er lijkt momenteel dan ook geen plaats te zijn voor het genotyperen van patiënten voor *ABCB1*.

Samenvattend, naast de polymorfismen in de genen die coderen voor het CYP3A5 enzym werden verschillende andere polymorfismen in diverse genen geassocieerd met Tac farmacokinetiek. Op zijn best hebben deze genetische variaties slechts een gering effect op de Tac farmacokinetiek en vele malen kleiner dan dat van *CYP3A5*3*. Ondanks het feit dat de individuele bijdrage van deze polymorfismen gering is, zouden ze theoretisch wel in een doseringsalgoritme kunnen worden geïncorporeerd om zo de beste startdosering te berekenen.[62] De voorspellende waarde van een dergelijk algoritme wordt wellicht groter indien genetische factoren worden gecombineerd met klinische en demografische variabelen, zoals leeftijd, plasma albumine en hemoglobine concentratie, lichaamsgewicht en co-medicatie (zoals b.v. calciumantagonisten of glucocorticoïden).[62,63]

3. Intra-patiënt variabiliteit

Het bereiken van adequate Tac concentraties in de vroege post-operatieve periode is vooral bedoeld om de korte termijn resultaten te verbeteren. Echter is het ook voor de onderhoudsbehandeling van belang dat de concentraties van de immunosuppressieve geneesmiddelen zo veel mogelijk binnen de streefwaarden blijven. In *Hoofdstuk 4* wordt de betekenis van een recent geïdentificeerde parameter voor lange termijn uitkomsten beschreven, namelijk de intra-patiënt variabiliteit van de Tac expositie, hierna te noemen "IPV". Grote schommelingen van de Tac concentraties binnen een individuele patiënt zorgen voor een toegenomen risico op periodes met onder- en over-immunosuppressie. Als gevolg daarvan kan de patiënt afstotingen respectievelijk toxiciteit ervaren. Borra *et al.* hebben aangetoond dat een hoge IPV in de Tac expositie geassocieerd is met slechtere transplantaatoverleving na niertransplantatie.[64] Sinds de publicatie van deze studie hebben verschillende andere onderzoekers de relevantie van de IPV als voorspeller voor de lange termijn uitkomst bevestigd, zowel in volwassenen[65-67] als in kinderen met een niertransplantaat.[68-70] In de meeste van deze onderzoeken waren de aantallen patiënten en events beperkt en de follow-up kort. Om die reden besloten wij onze oorspronkelijke studie-populatie zoals beschreven door Borra *et al.* uit te breiden en hen langer te volgen. (*Hoofdstuk 4.2*). Met een multivariate analyse bevestigen we in een cohort van 808 niertransplantatie patiënten dat IPV in Tac expositie een onafhankelijke risicofactor is voor een slechte transplantaat uitkomst. Patiënten met een hoge IPV hadden een 1,4 keer hogere kans op het bereiken van het samengestelde primaire eindpunt (transplan-

taatverlies, late acute rejectie, transplantaat glomerulopathie en verdubbeling van het serum creatinine): hazard ratio 1,41, 95%-CI: 1,06 – 1,89; p = 0,019). Mogelijk kan bij patiënten met een hoge IPV (>16.2%) een hogere Tac C_0 (> 7.0 ng/mL) bescherming bieden tegen rejectie. Artsen zouden bij de behandeling van hun patiënten niet alleen aandacht voor de absolute blootstelling aan Tac moeten hebben, maar ook oog moeten hebben voor de schommelingen in de Tac concentraties. Deze IPV zou relatief eenvoudig in het elektronisch patiëntendossier kunnen worden gegenereerd.

De betekenis van de IPV voor de prognose na harttransplantatie was nog niet eerder bestudeerd. Chronische allograft vasculopathie ("CAV") na harttransplantatie is een aandoening die vergelijkbaar is met chronische afstoting na niertransplantatie. Wij bestudeerden daarom of de IPV in Tac expositie ook geassocieerd is met snellere progressie van CAV na harttransplantatie (*Hoofdstuk 4.3*). In tegenstelling tot de studie na niertransplantatie vonden wij bij de harttransplantatie patiënten geen relatie tussen de IPV en (de progressie van) CAV, wat suggereert dat de impact van IPV mogelijk orgaanspecifiek is. Mogelijk kan dit verschil in uitkomst worden verklaard doordat de Tac C_0 (gemeten tussen 6 -12 maanden na transplantatie) na harttransplantatie aanzienlijk hoger was dan bij niertransplantatie ontvangers (11,0 ng/mL *vs.* 6,9 ng/mL, respectievelijk). De schommelingen in de concentraties naar beneden zouden daardoor wellicht niet hebben geleid tot sub-therapeutische expositie, terwijl hogere spiegels die bij niertransplantatie leiden tot nefrotoxiciteit bij een harttransplantatie patiënt niet leiden tot schade aan het getransplanteerde hart. Een ander verschil tussen de twee onderzochte populaties is dat na harttransplantatie de behandeling met prednison langer wordt voortgezet en ook worden patiënten na harttransplantatie vaker op de polikliniek terug gezien. Door de meer frequente controles zou de ziekte progressie en eventuele therapieontrouw de behandelaars mogelijk ook eerder zijn opgevallen. Het is echter ook mogelijk dat we een positief verband hebben gemist doordat de populatie harttransplantatie patiënten te klein was en dit onderzoek te weinig statistisch onderscheidend vermogen had. Wij zouden dan ook willen adviseren om een soortgelijke studie te herhalen in een groter cohort.

Verschillende factoren kunnen de Tac farmacokinetiek beïnvloeden en bijdragen aan de IPV.[71] Uiteraard kan de IPV groter zijn naarmate de patiënt minder therapietrouw is. Van therapieontrouw weten we dat het de kans op nierfalen na transplantatie zevenvoudig vergroot.[72] Het minder vaak per dag doseren van een geneesmiddel lijkt de therapietrouw te bevorderen, zoals aangetoond voor diverse klassen van medicamenten. Voor transplantatie patiënten is een Tac formulering met vertraagde afgifte ontwikkeld (Advagraf®, Astellas Pharma). In een Belgisch onderzoek bleek het eenmaal daagse Tac regime duidelijk superieur aan het twee maal daagse regime, aangezien 81,5% van de eenmaal-

daagse groep therapietrouw bleef, tegen 72% van de twee maal daagse groep.[73] De betere therapietrouw aan Advagraf® zou ook een gunstig effect op de IPV kunnen hebben. In Hoofdstuk 4.4 beschrijven we een studie waarin we onderzochten of gebruik van Advagraf® inderdaad tot een lagere IPV leidt dan Prograft®. In deze niet-gerandomiseerde conversie-studie, uitgevoerd bij 247 stabiele niertransplantatie patiënten, vonden wij geen afname van de IPV (gebruik makend van herhaalde C_0 metingen). Ook Wehland *et al.*[74] en van Hooff *et al.*[75] vonden geen afname van de IPV na conversie van Tac-Prograft® naar de eenmaal-daagse formulering. Echter, een aantal andere studies vond wel degelijk een verschil in IPV.[76-78] Alloway *et al.* toonden aan dat in 67 niertransplantaat ontvangers de IPV in Tac expositie (gebruikmakend van $AUC_{0\text{-}24}$) wel significant daalde na omzetting naar de eenmaal-daagse formulering.[76] Dit werd enkel geobserveerd in de Afro-Amerikaanse patiënten (n = 12) en dus niet in de Kaukasische patiënten. Vanwege de vertraagde afgifte wordt Tac uit Advagraf® meer distaal in het darmkanaal vrijgegeven dan Prograft®. Omdat de expressie van CYP3A enzymen naar distaal in het darmkanaal afneemt,[79] zouden wisselingen in CYP3A activiteit door bijvoorbeeld dieet invloeden wel eens minder van invloed kunnen zijn op de IPV van Advagraf®. Omdat 85% van de Afro-Amerikaanse patiënten *CYP3A5* expresser is, zou dat de resultaten van Alloway *et al.* in de deze subgroep kunnen verklaren.[76] Immers is CYP3A5 activiteit minder onderhevig aan invloeden van omgevingsfactoren, terwijl *CYP3A4* expressie meer variabel is. In ons onderzoek was de meerderheid van de patiënten Kaukasisch (73%) en slechts 15% van hen was *CYP3A5* expresser.[80] Omzetting van Prograft® naar Advagraf® in 129 Taiwanese nierontvangers gaf ook een significante verlaging van de IPV van Tac C_0: 14,0% ± 7,5% voor conversie *vs.* 8,5% ± 5,0% ($p < 0,05$) erna.[78]

In een mooi opgezet onderzoek toonden Stifft *et al.* aan dat de IPV van Tac expositie niet verandert door omzetting naar eenmaal-daags, indien berekend op basis van de C_0. Echter bleek de IPV berekend op basis van een $AUC_{0\text{-}24}$ wel significant te dalen.[77] De IPV nam af met 3,2%, hetgeen weliswaar statistisch significant was, maar niet klinisch relevant. In ons eigen onderzoek werd alleen de C_0 gemeten en we kunnen slechts speculeren over de mogelijke uitkomst indien we $AUC_{0\text{-}24}$ hadden gemeten. C_0 is in de dagelijkse praktijk de meest gebruikte parameter aan de hand waarvan de Tac dosering wordt aangepast. Verder werd in ons onderzoek de therapietrouw niet rechtstreeks gemeten. Omdat de mediane IPV in de Tac C_0 niet erg hoog was voorafgaand aan de omzetting (17,9%), zou het kunnen zijn dat wij een groep hebben bestudeerd die al therapietrouw was, waardoor er nauwelijks meer ruimte was voor verbetering na introductie van de eenmaal daagse formulering.

4. Conclusies van de dissertatie

In dit proefschrift beschrijven wij een aantal studies die bijdragen aan het optimaliseren van het voorschrijven van Tac aan patiënten die een orgaantransplantatie hebben ondergaan. Een eerste opvallende bevinding van ons onderzoek was het ontbreken van een associatie tussen de hoogte van de Tac C_0 en het optreden van acute rejectie na niertransplantatie. Deze observatie impliceert ons inziens niet dat TDM voor Tac kan worden afgeschaft. Met TDM sporen we patiënten op die (ver) buiten het therapeutische venster liggen, en in die patiënten is het aanpassen van de dosis wel bijdragend. In een pilot studie hebben we aangetoond dat juist een farmacodynamische parameter, zoals p38MAPK fosforylering in T-lymfocyten middels flowcytometrie, het biologisch effect van Tac beter reflecteert en daarmee ook tot betere associaties met optreden van rejectie leidt. Mogelijk kan een dergelijke bio-marker in de toekomst de voorschrijver helpen om de juiste dosering te kiezen.

Het nadeel van de momenteel toegepaste strategie van TDM voor Tac is dat dit een 'trial and error' benadering is, die geen voorspellende waarde heeft. Bij veel patiënten duurt het lang voordat de Tac streefconcentratie na transplantatie wordt bereikt en zolang de expositie te laag is loopt de patiënt een risico op afstoting. In dit proefschrift tonen wij aan dat de Tac dosisbehoefte voor transplantatie goed voorspelt welke dosis een patiënt na niertransplantatie nodig heeft. Informatie over de farmacokinetiek van Tac, verkregen voorgaand aan de transplantatie, zou derhalve kunnen worden gebruikt om een dosering te kiezen waarmee de patiënt aansluitend aan de transplantatie behandeld kan worden. Zo kan onder- en overbehandeling worden tegengegaan.

In een gerandomiseerde klinische studie hebben wij aangetoond dat *CYP3A5* genotype gebaseerde aanpassing van de Tac startdosering niet leidt tot het eerder bereiken van de beoogde Tac C_0 ten opzichte van het gangbare beleid van een op lichaamsgewicht-gebaseerde Tac startdosering. Ook de klinische uitkomsten waren vergelijkbaar in beide groepen. Het implementeren van *CYP3A5* genotypering voorafgaand aan niertransplantatie wordt daarom niet aanbevolen.

In de studie met het tot dusverre grootste aantal patiënten stelden we vast dat een hoge intra-patiënt variabiliteit in Tac expositie een belangrijke en onafhankelijke risicofactor is voor een slechte transplantaat uitkomst na niertransplantatie. We bevelen artsen dan ook aan om de intra-patiënt variabiliteit in de dagelijkse zorg te meten en te gebruiken. De IPV in Tac expositie bleek echter geen vergelijkbaar effect te hebben op de prognose van harttransplantatie patiënten.

Omdat therapietrouw een mogelijke determinant is van de IPV, onderzochten we of het omzetten van de tweemaal-daagse Tac formulering naar een eenmaal-daagse formulering de IPV verlaagt. In onze studie zagen we weliswaar dat volgend op een 1:1 omzetting de Tac concentraties daalden (reductie van 15%), maar dat de IPV in Tac expositie onveranderd bleef. Verlaging van de IPV is derhalve geen argument om patiënten te converteren naar het duurdere eenmaal-daagse Tac preparaat.

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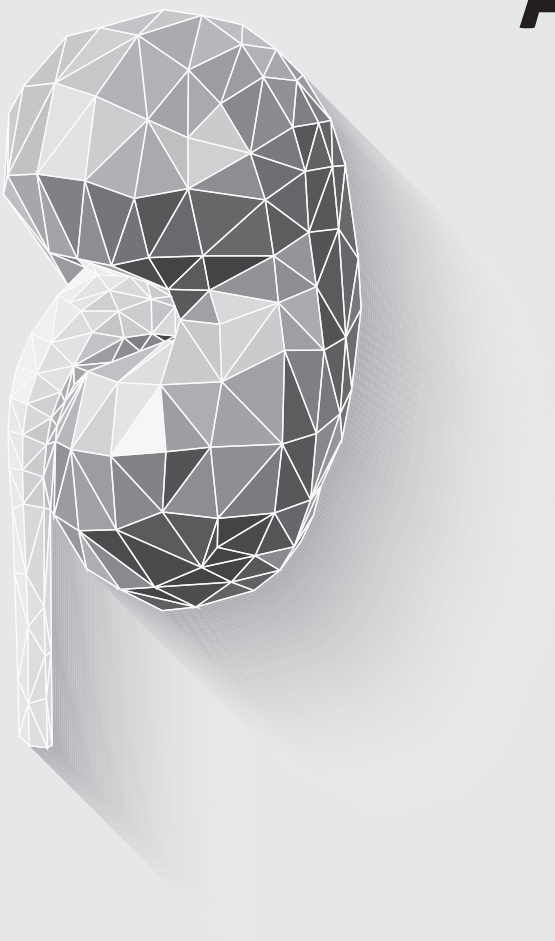
Appendix



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Nauras, Rotterdam Mei 2016

Наурас, Роттердам Май 2016

نورس، روتردام ایار 2016

PhD PORTFOLIO

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Promotor(s):
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	Year
1. 1. PhD training	
General courses	
CPO mini course	2011
Courses Molecular Biology	2011
BROK course	2013
NIH course Principles of Clinical Pharmacology	2013
Biomedical English Writing & Communication	2013
Introductory course on Statistics & Survival Analysis	2013
Survival Analysis (Erasmus Winter Programme)	2015
Seminars & Workshops	
Presentation training skills	2011
Systematic literature search in Pubmed	2012
Endnote course	2012
Solid Phase Extraction Seminar (Waters®)	2013
Workshop on Photoshop and Illustrator	2014
Integrity in Science	2015
Department Journal Club	2010 - 2015
Clinical Pharmacology meeting	2011 - 2014
Presentations (oral)	
Pharma meeting, Rotterdam, the Netherlands	2012
Annual Congress Dutch Transplantation Society, Leiden, the Netherlands	2014
ESOT International Transplant Congress, Brussels, Belgium	2015
International Congress on Therapeutic Drug Monitoring & Clinical Toxicology, Rotterdam, The Netherlands	2015
Presentations (poster)	
International Congress on Therapeutic Drug Monitoring & Clinical Toxicology, Salt Lake City, USA	2013
Annual Congress Dutch Transplantation Society, Leiden, the Netherlands	2014
Annual Congress Dutch Transplantation Society, Leiden, the Netherlands	2014
ESOT International Transplant Congress, Brussels, Belgium	2015
International Congress on Therapeutic Drug Monitoring & Clinical Toxicology, Rotterdam, The Netherlands	2015
International Conferences	
Siemens European Symposium on Clinical and Translational Immunosuppression, Barcelona, Spain	2011
The Transplantation Society - Transplantomics & Biomarkers in Transplantation, La Jolla, USA	2012
International Congress on Therapeutic Drug Monitoring & Clinical Toxicology, Salt Lake City, USA	2013
ESOT International Transplant Congress, Brussels, Belgium	2015
International Congress on Therapeutic Drug Monitoring & Clinical Toxicology, Rotterdam, The Netherlands	2015

Annual Congress Dutch Transplantation Society, the Netherlands	2011 - 2014
Clinical Review Symposium, Utrecht, The Netherlands	2011 - 2014
Other	
Molmed day, Erasmus MC	2011, 2012
PhD day	2012
1. 2. Teaching Activities	
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Supervising	
Ling Huang (Research Master student at Erasmus University Rotterdam)	2013
Femke de Man (Master's student in medicine)	2013

LIST OF PUBLICATIONS

Presented in this thesis

- Shuker N, Bouamar R, van Schaik R.H.N, *et al.* A randomized-controlled trial comparing the efficacy of *CYP3A5* genotype-based with bodyweight-based tacrolimus dosing after living donor kidney transplantation. *Am J Transplant.* 2015 Dec 29.
- Shuker N, de Man F.M, de Weerd A.E, *et al.* Pre-transplant tacrolimus dose requirements predict early post-transplant dose requirements in blood-group AB0 incompatible kidney transplant recipients. *Ther Drug Monit.*2016 Apr;38(2):217-22.
- Kannegieter N.M, Shuker N, Vafadari R, *et al.* Conversion to Once-daily Tacrolimus Results in increased p38MAPK Activity in T-lymphocytes of Kidney Transplant Recipients. *Ther Drug Monit.* 2016 Apr;38(2):280-4.
- Shuker N, van Gelder T, Hesselink D.A. Intra-patient variability in tacrolimus exposure: causes, consequences for clinical management. *Transplant Rev.* 2015 Apr;29(2):78-84.
- Shuker N, Cadogan M, van Gelder T, *et al.* Conversion from twice-daily to once-daily tacrolimus does not reduce intra-patient variability in tacrolimus exposure. *Ther Drug Monit.* 2015 Apr;37(2):262-9.
- Bouamar R, Shuker N, Hesselink D.A, *et al.* Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomized Controlled clinical trials. *Am J Transplant.* 2013 May;13(5):1253-61.
- Shuker N, Shuker L, van Rosmalen J, *et al.* High intra-patient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation. *Transpl Int. 2016 in Press.*
- Shuker N, Bouamar R, Hesselink D.A, *et al.* Is high intra-patient variability in tacrolimus exposure associated with progression of cardiac allograft vasculopathy after heart transplantation. *Submitted*

Other Publications

- Li P, Shuker N, Hesselink D.A, *et al.* Do Asian renal transplant patients need another mycophenolate mofetil dose compared with Caucasian or African American patients? *Transplant Int.* 2014 Oct;27(10):994-1004.
- Elens L, Bouamar R, Shuker N, *et al.* Clinical implementation of pharmacogenetic in kidney transplantation: calcineurin inhibitors in the starting blocks. *Br J Clin Pharmacol.* 2014 Apr;77(4):715-28.

- Bouamar R, Elens L, Shuker N, *et al.* Micophenolic acid-related anemia and leucopenia in renal transplant recipients are related to genetic polymorphisms in CYP2C8. *Transplantation.* 2012 May 27;93(10).
- Shuker N, Bouamar R, Weimar W, *et al.* ATP-binding cassette transporters as pharmacogenetic biomarkers for kidney transplantation. *Clin Chem Acta.* 2012 Sep 8;413(17-18):1326-37.

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Nauras Shuker was born on September 10th, 1981 in Kharkov, Ukraine. In the year 1998 she graduated from secondary school in Moscow and moved to the Netherlands. After accomplishing a pre-university education at Melanchthon College in Rotterdam in 2002, she started her study Pharmacy at the University of Utrecht. In August 2010 she obtained her master's degree. Subsequently, Nauras started her professional career at the Department of Internal Medicine, division of Nephrology and Transplantations, as a clinical researcher. In 2011 she was happy to receive a grant from the Dutch Kidney Foundation, and got the opportunity to start her PhD project under supervision of Prof. dr. T. van Gelder, Prof. dr. W. Weimar, dr. D.A. Hesselink and dr. B.C.P. Koch. The project was performed in collaboration with the Department of Internal Medicine and the Department of Hospital Pharmacy of Erasmus Medical Center in Rotterdam and was funded by project grants from the Netherlands Organization for Scientific Research (NOW) and the Dutch Kidney Foundation.
In September 2015 she took a position as a pharmacist at the pharmacy of the Erasmus MC Oncology Institute.