



# Clinical aspects of tacrolimus use in paediatric renal transplant recipients

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## Abstract

The calcineurin inhibitor tacrolimus, cornerstone of most immunosuppressive regimens, is a drug with a narrow therapeutic window: underexposure can lead to allograft rejection and overexposure can result in an increased incidence of infections, toxicity and malignancies. Tacrolimus is metabolised in the liver and intestine by the cytochrome P450 3A (CYP3A) isoforms CYP3A4 and CYP3A5. This review focusses on the clinical aspects of tacrolimus pharmacodynamics, such as efficacy and toxicity. Factors affecting tacrolimus pharmacokinetics, including pharmacogenetics and the rationale for routine *CYP3A5*\*1/\*3 genotyping in prospective paediatric renal transplant recipients, are also reviewed. Therapeutic drug monitoring, including pre-dose concentrations and pharmacokinetic profiles with the available “reference values”, are discussed. Factors contributing to high intra-patient variability in tacrolimus exposure and its impact on clinical outcome are also reviewed. Lastly, suggestions for future research and clinical perspectives are discussed.

**Keywords** Tacrolimus · Renal transplantation · Paediatric · Pharmacokinetics · Pharmacogenetics · Intra-patient variability

## Introduction

The calcineurin inhibitor (CNI) tacrolimus (TAC), a macrolide lactone isolated from *Streptomyces tsukubaensis*, is the cornerstone of most immunosuppressive regimens in solid organ transplantation. In paediatric renal transplant recipients TAC has been shown to be more effective than cyclosporine (CsA)-based regimens in preventing acute rejection and improving long-term graft survival [1, 2].

A number of factors playing a role in the pharmacokinetic variability of TAC have been identified. These include patient characteristics, such as age or weight [3, 4], concomitant immunosuppression, such as corticosteroids or other co-medications (e.g. antibiotics) and polymorphism of the genes encoding the enzyme proteins involved in TAC metabolism [4, 5].

The unequivocal benefits of TAC must be counterbalanced against its side effects. Moreover, the multiple drug-to-drug interactions with inducers and inhibitors of the cytochrome

P450 3A (CYP3A) isoforms CYP3A4/A5 increase the risk of TAC under- or overexposure. This review focusses on the clinical aspects of TAC pharmacodynamics, such as efficacy and toxicity, therapeutic drug monitoring (TDM), including pharmacokinetic and pharmacogenetic factors, and the impact of intra-patient variability in TAC pre-dose concentrations  $C_0$  on allograft outcomes.

## Pharmacodynamics of TAC: mechanism of action and toxicity

Tacrolimus exerts its immunosuppressive effects primarily by affecting T-lymphocyte activation through inhibition of the enzyme calcineurin (CN). CN was originally isolated from neuronal tissue and exhibits calcium-binding properties, hence its name. In the cytoplasm calcium binding leads to CN activation and dephosphorylation of the nuclear factor of activated T cells (NFAT). Dephosphorylated NFAT translocates to the nucleus and forms complexes with transcriptional proteins, thereby inducing the transcription of genes required for the proliferation and activation of T-lymphocytes, including interleukin (IL)-2, IL-4 and interferon gamma. TAC forms intracellular complexes with specific immunophilin-binding proteins in the cytoplasm. These complexes bind CN, preventing dephosphorylation and nuclear translocation of

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NFAT, thereby inhibiting T- lymphocyte activation [6]. The mechanism of action of TAC is depicted in Fig. 1.

In addition to T-lymphocytes, CN is expressed in other tissues, including the brain, spleen, thymus, platelets, heart, liver, testes, pancreas and kidney [7, 8]. Numerous actions have been attributed to CN, including a role in programmed death of neuronal cells, cardiac morphogenesis and induction of cardiac hypertrophy, regulation of the Na<sup>+</sup>, K<sup>+</sup>-ATPase in the kidney and neutrophil migration [7, 8]. These diverse actions explain why in addition to immunosuppression, TAC also displays an array of side effects, as listed in Table 1. An important consideration with regard to the use of TAC in adolescents is the fact that in contrast to CsA, TAC is rarely associated with hirsutism, gingivitis and gum hyperplasia, but it can cause alopecia and pruritus in some patients [10].

### Acute and chronic nephrotoxicity

There is evidence to suggest that the use of TAC is associated with a lower risk of nephrotoxicity as compared with CsA. This can be explained by the weaker vasoconstrictive effects and fibrogenic potential of TAC relative to CsA [9] and has been demonstrated in clinical studies [22, 23].

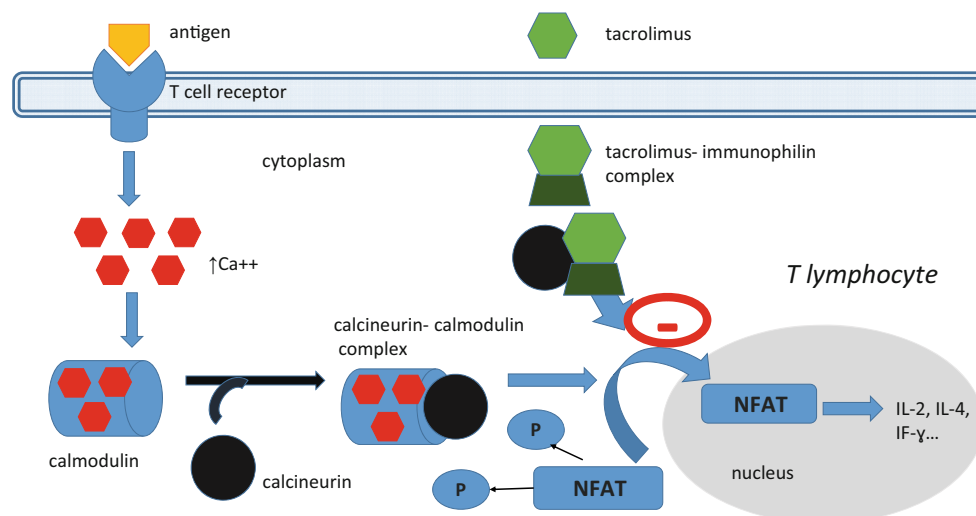
Acute nephrotoxicity usually occurs within several days after the initiation of TAC treatment and may present as oligo- or anuria and a rapid rise in serum creatinine level. The acute nephrotoxic effects are typically reversible upon dose reduction or withdrawal of TAC. There is evidence to suggest that the predisposition to develop acute TAC-induced nephrotoxicity might be associated with the polymorphism of T-lymphocyte regulatory genes [24].

In contrast, chronic TAC-induced nephrotoxicity develops gradually and is clinically manifested as a slow decline in renal function, often with proteinuria and hypertension. While arterial hyalinosis was initially reported as a hallmark of CNI-associated nephropathy [25], more recent data suggest that damage mediated by donor-specific anti-HLA antibodies (chronic antibody-mediated rejection) might also underlie these pathologic changes [26]. Data from adult renal organ transplant recipients suggest an association between a low renal p-glycoprotein expression and the occurrence of histological damage in renal allografts [27, 28].

### Pharmacokinetics of TAC

Tacrolimus is poorly absorbed in the gastrointestinal tract with a low and variable bioavailability in adults (mean 25%; range 4–93%) [29]. It is extensively bound to erythrocytes, with TAC whole blood concentrations exceeding about 15- to 35-fold those measured in plasma. Therefore, TAC whole blood concentrations are most widely used in clinical practice. In turn, > 90% of the TAC present in plasma is bound to proteins, mainly to  $\alpha$ -1 acid glycoprotein and albumin. TAC is metabolised by the liver and intestinal CYP isoenzymes CYP3A4/A5 and is also a substrate to ATP-binding cassette protein B1 (ABCB1). The main route of excretion is the biliary tract and faeces. The elimination half-life of TAC is 12 (range 4–41) h. Liver failure might impact TAC elimination. In contrast, renal function impairment does not have an influence on TAC clearance [30–32].

The major TAC metabolite is the 13-*O* dimethyl metabolite which exhibits a low immunosuppressive activity, but can be



**Fig. 1** Mechanism of action of tacrolimus (TAC). Calcium binding leads to calcineurin (CN) activation and dephosphorylation (P) of the nuclear factor of activated T cells (NFAT). Dephosphorylated NFAT translocates to the nucleus and forms complexes with transcriptional proteins, inducing the transcription of genes required for the activation of T-lymphocytes

(interleukin [IL]-2, IL-4 and interferon gamma [IF- $\gamma$ ]). TAC forms intracellular complexes with immunophilins, and these complexes bind CN, preventing the dephosphorylation and nuclear translocation of NFAT

**Table 1** Summary of the most prevalent side effects of tacrolimus in paediatric solid organ transplant recipients

Side effect	Comments	References
Nephrotoxicity	<ul style="list-style-type: none"> <li>• <b>Acute:</b> oligo/anuria and rapid decline in GFR; reversible upon TAC withdrawal</li> <li>• <b>Chronic:</b> slow decline in GFR, proteinuria and hypertension; histologically hallmarked by interstitial fibrosis and tubular atrophy and hyalinosis</li> </ul>	[9]
Neurotoxicity	<ul style="list-style-type: none"> <li>• <b>Minor symptoms</b> (in up to 60% of patients): insomnia, headache, tremor, photophobia and paresthesia</li> <li>• <b>Serious adverse effects</b> (mostly in children with toxic TAC levels): psychosis, blindness, seizures, mood disorders, aphasia, coma and delirium</li> </ul>	[10, 11]
Cardiovascular	<ul style="list-style-type: none"> <li>• Hypertension</li> <li>• Increased left ventricular wall thickness and myocardial hypertrophy (with TAC concentrations exceeding 15 ng/ml)</li> <li>• Arrhythmia (QT prolongation)</li> </ul>	[12–14]
Gastrointestinal	<ul style="list-style-type: none"> <li>• Diarrhoea, nausea and constipation</li> <li>• Pancreatitis</li> </ul>	[10, 15]
Metabolic disturbances (exacerbated by concomitant corticosteroids)	<ul style="list-style-type: none"> <li>• Impaired glucose intolerance</li> <li>• Post-transplant diabetes: in 7–11% of paediatric renal transplant recipients</li> <li>• Dyslipidemia (less pronounced than with CsA; reported in up to 49% of children)</li> </ul>	[10, 16–18]
Electrolyte disturbances	<ul style="list-style-type: none"> <li>• Renal tubular acidosis type IV with low to normal renin and aldosterone levels and hyperkalaemia</li> <li>• Renal magnesium wasting</li> </ul>	[19, 20]
Infections and malignancy	<ul style="list-style-type: none"> <li>• Routine screening for CMV, EBV and BK infection</li> <li>• Post-transplant lymphoproliferative disorders (especially in children with primary EBV infection post-transplant)</li> </ul>	[10]
Other	<ul style="list-style-type: none"> <li>• Thrombotic thrombocytopenic purpura</li> <li>• Alopecia and pruritus</li> <li>• Idiosyncratic drug reactions</li> <li>• Potential testicular toxicity (possibly lower risk compared with CsA)</li> </ul>	[10, 21]

GFR, Glomerular filtration rate; TAC, tacrolimus; CsA, cyclosporine A; CMV, cytomegalovirus; EBV, Epstein–Barr virus

quantified in blood [33]. In contrast, the 31-*O*-demethyl metabolite has been shown to be equipotent to TAC and to exhibit a considerable immunosuppressive activity. However, in a clinical setting the concentrations of 31-*O*-demethyl metabolite were too low to allow a pharmacokinetic analysis [33].

### Factors affecting TAC pharmacokinetics

The inter-individual (or between patient) differences in TAC exposure are related to patient characteristics, such as weight and age, laboratory parameters such as haematocrit (Hct), concomitant medications and pharmacogenetic diversity of the *CYP3A4/A5* genes which will be discussed later in this review.

Compared with adults, the impact of weight on TAC pharmacokinetics is much more prominent in studies including paediatric renal transplant recipients [4, 5]. It is known that young children have significantly higher relative TAC dose requirements than adults [3]. The explanation of this phenomenon is not entirely clear. It is assumed that the hepatic *CYP3A4/A5* attains its full adult activity by the age of 1–2 years [34], but the maturation of the intestinal *CYP3A4/A5* and phase II enzymes such as p-glycoprotein remain to be elucidated. Moreover, many factors can be involved in the variability in pharmacokinetics between adults and children, including intestinal uptake, hepatic blood flow and the proportion of liver to body weight, nutrition and underlying disease [10, 30]. In most healthy children weight and age can

be used interchangeably, but this does not always hold true for paediatric patients with chronic kidney disease for whom failure to thrive is common. Naesens et al. reported that children older than 5 years at transplantation had lower weight-normalised TAC dose requirements with increasing age [35]. Knops et al. showed that children exhibit a biphasic course in TAC disposition characterised by a high and stable drug clearance until puberty followed by a significant decline in relative dose requirements thereafter [3]. In contrast, two paediatric pharmacokinetic studies have shown no improvement of an allometric population model after the addition of age as a covariate, leading the respective authors to conclude that adjustments in the TAC dose should be based on the child's weight rather than age [4, 5].

TAC binds to erythrocytes, and the unbound fraction in blood is < 1%. Total TAC concentrations are measured in whole blood, while it is the unbound fraction that exerts pharmacological effects. The increase in total TAC concentration following a rise in Hct does not lead to an increase in the unbound fraction of the drug [36]. Therefore, the changes in TAC concentrations related to changes in Hct level should not lead to a prompt adjustment of dose [37]. Similar considerations apply to TAC dose amendment following rapid changes in plasma proteins.

Factors affecting TAC pharmacokinetics in paediatric renal transplant recipients identified by population modelling are listed in Table 2.

**Table 2** Factors affecting tacrolimus pharmacokinetics in paediatric renal transplant recipients

First author/year of publication	Time since transplantation, days (median; range)	Number of patients	TAC formulation <sup>a</sup>	Weight/age (median; range)	Factors affecting TAC pharmacokinetics
Zhao et al. 2009 [5]	19; 7–54	50	Prograf®	30; 11–62 kg 10; 2–18 years	Weight CYP3A5 genotype Hct
Zhao et al. 2013 [38]	?; 193–4983	22	Advagraf®	45; 17–70 kg 15; 6–23 years	Weight CYP3A5 genotype
Jacobo-Cabral et al. 2015 [39]	244; 50–1230	53	Prograf® ( <i>n</i> = 29) Limustin® ( <i>n</i> = 9) Framebin® ( <i>n</i> = 5) Tenacrine® ( <i>n</i> = 3) Unknown ( <i>n</i> = 7)	48; 11–75 kg 16; 2–19 years	CYP3A5 genotype TAC dose TAC formulation
Prytuła et al. 2016 [4]	486; 342–3720	54	Prograf®	39; 15–86 kg 11; 4–18 years	Weight CYP3A5 genotype γGt
Andrews et al. 2017 [40]	14; 8–42		Prograf® Modigraf®	28; 12–84 kg 9; 2–18 years	Weight eGFR <sup>b</sup> CYP3A5 genotype Deceased donor

*n*, Number of patients; Hct, haematocrit; γGT, gamma glutamyl transpeptidase; eGFR, estimated GFR

<sup>a</sup> Limustin®, Framebin® and Tenacrine® are generic tacrolimus formulations commercialised in Mexico and not used in Europe. Modigraf is a granular formulation of TAC, given twice-daily as a suspension

<sup>b</sup> This is contradictory with the lack of impact of renal function on TAC pharmacokinetics

## Drug–drug interactions

As corticosteroids exhibit CYP3A4-inducing properties, TAC dose requirements may be lower following corticosteroid dose reduction or withdrawal [41]. However, it is possible that this applies only to patients treated with higher doses of corticosteroids as no association has been found between the use of corticosteroids and TAC pharmacokinetics in paediatric pharmacokinetic studies [4, 5].

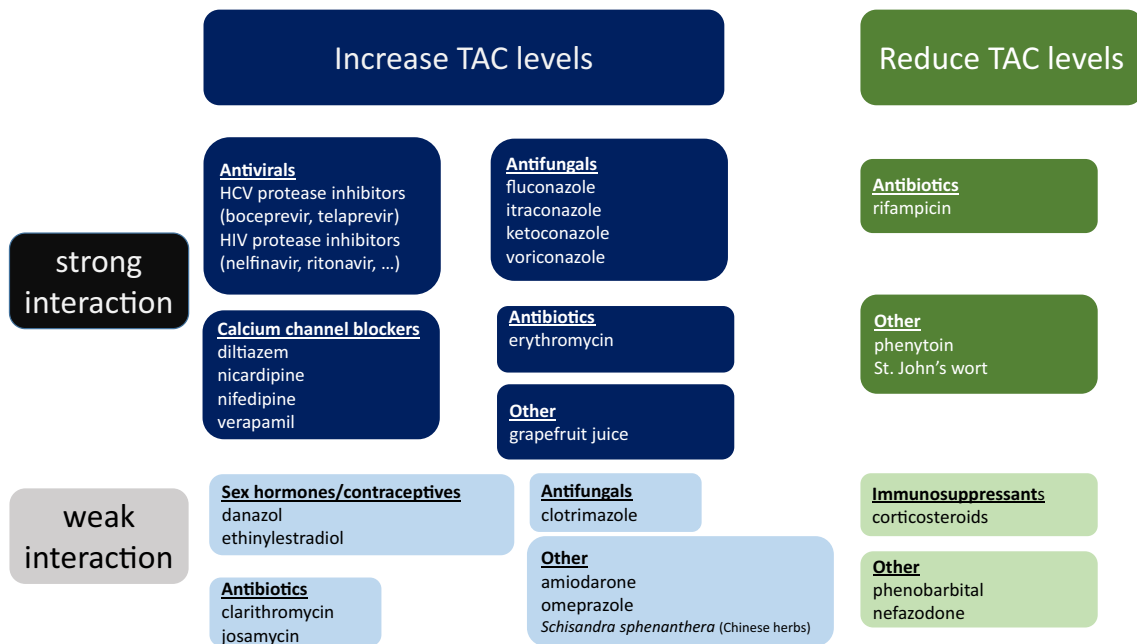
Importantly, in contrast to CsA, TAC has no effect on mycophenolic acid (MPA) exposure. CsA inhibits the multidrug resistance protein 2-mediated transport of 7-*O*-MPA glucuronide into the bile, leading to less MPA exposure. 7-*O*-MPA glucuronide is subject to enzymatic and non-enzymatic hydrolysis in bile and, more importantly, in the intestine, thereby liberating the unconjugated drug MPA, which is then reabsorbed into the systemic circulation. This enterohepatic circulation is responsible for a secondary MPA peak occurring 6–12 h after administration, which can account for up to 10–60% of the total dose-interval MPA-area under the time–concentration curve (AUC) [42]. The consequence of this interaction is that when MPA is used in combination with TAC, its dose should be lower than when it is used in combination with CsA [43]. In the TWIST study, the dose of concomitant mycophenolate mofetil (MMF) in combination with TAC in stable paediatric renal transplant recipients was 600 mg/m<sup>2</sup> [44], while in combination with CsA the MMF dose is usually 1200 mg/m<sup>2</sup>.

Moreover, as TAC is metabolised by CYP3A4/A5, the inducers and inhibitors of these enzymes will have a clinically

significant impact on TAC pharmacokinetics [45]. The clinically relevant drug–drug interactions are depicted in Fig. 2. The use of grapefruit juice or herbal medications by patients receiving TAC should be discouraged. While commencing or discontinuing co-medication(s) that might affect TAC blood levels, a period of meticulous TDM is recommended until the TAC pre-dose concentrations are stable [46]. When using potent CYP3A4/A5 inducers or inhibitors (see also Fig. 2), a preventive TAC dose adjustment of 30% followed by strict TDM may be applied. In case of an acute TAC intoxication, the use of a potent CYP3A4 inducer, such as phenytoin, to accelerate TAC clearance may be considered [47].

## Pharmacogenetics of TAC

Tacrolimus is metabolised in the liver and intestine by the CYP3A isoforms CYP3A4 and CYP3A5. It is also a substrate for the multidrug efflux transporter glycoprotein that is expressed on various epithelial and endothelial cells and lymphocytes and encoded by the *ABCB1* gene [48]. Polymorphisms of the CYP iso-enzymes 3A4 and 3A5 as well as of the *ABCB1* gene have been extensively studied in adults with regard to TAC pharmacokinetics, and *CYP3A5\*1/\*3* alleles have been shown to be the most significant in terms of TAC dose requirements. Although the CYP3A5 protein in the liver is detectable only in 10–40% of the Caucasian population [49], it may account for up to 50% of total hepatic CYP3A content in some individuals [50]. It has been shown



**Fig. 2** Relevant drug–drug interactions of TAC. *HCV* Hepatitis C virus, *HIV* human immunodeficiency virus. Adapted from the European Medicines Agency website ([www.ema.europa.eu](http://www.ema.europa.eu))

that the incidence of the *CYP3A5\*1* allele (homo- or heterozygote) is around 15–25% in West-European populations [4, 51] and exceeds 50% in African Americans [52, 53].

The *CYP3A5\*1* allele constitutes the wildtype allele, with enzyme activity leading to higher TAC dose requirements, whereas the *CYP3A5\*3* allele results in an absence of protein activity. Carriers of *CYP3A5\*1* are also referred to as *CYP3A5* expressers, whereas *CYP3A5\*3* homozygotes are referred to as *CYP3A5* non-expressers. *CYP3A5* expressers (genotype *CYP3A5\*1/\*1* or *CYP3A5\*1/\*3*) require higher TAC doses to achieve a given TAC concentration. The recommendation for adult solid organ transplant recipients is to increase the standard TAC dose by 1.5- to 2-fold in *CYP3A5* expressers, but not to exceed the daily dose of 0.3 mg/kg, followed by strict TDM [48].

Also in children it has been shown that the *CYP3A5* polymorphism has a significant effect on the pharmacokinetic variability of TAC (see also Tables 2, 3). Children with the *CYP3A5\*1* allele have higher TAC dose requirements than *CYP3A5* non-expressers [54–56]. For children and adolescents with at least one *CYP3A5\*1* allele, a 1.5- to 2-fold increase in dose followed by TDM is recommended, similar to the recommendations for adults [48]. It should be acknowledged that although *CYP3A5* can explain up to 45% of inter-individual TAC pharmacokinetic variability [57], other factors described above can also affect TAC disposition.

Of note, the *CYP3A4\*22* and *POR\*28* (*POR*: P450 oxidoreductase) alleles, two recently identified gene polymorphisms, have been found to impact TAC dose requirements in adults [58, 59] and children [60]. Elens et al. studied the impact of the *CYP3A4\*22* allele (rs35599367, C > T) in a cohort of 185 adult renal transplant recipients, most of whom



were Caucasian. Carriers of the T allele had 33% lower mean TAC dose requirements than carriers of the wildtype C allele. The frequency of the T allele was 3.5% [61]. De Jonge et al. found that the *POR\*28* T allele in *CYP3A5* expressers can lead to TAC underexposure. This effect was alleviated in *CYP3A5* non-expressers [62].

### Rationale for the routine *CYP3A5\*1/\*3* testing

To date, there is no consensus on whether or not solid organ transplant candidates should routinely undergo *CYP3A5* genotyping to guide the TAC dose. The recent guideline on the utility of *CYP3A5* genotyping for TAC dosing in adults provides recommendations for solid organ transplant recipients with a known genotype, but does not advise for or against routine genotyping [48] because to date there is no evidence that routine *CYP3A5* genotyping can positively impact patient outcomes. *CYP3A5* expressers have higher TAC dose requirements and are potentially at risk for underexposure in the early post-transplantation period. A delay in achieving the target TAC concentrations in de novo transplant recipients may lead to acute rejection [63]. However, a Belgian study in adult renal transplant recipients showed that despite de novo renal transplant recipients who were *CYP3A5* expressers needing more time to achieve the target pre-dose TAC concentrations, no association was found between the genetic polymorphism and patient outcomes, such as the incidence of acute rejection or graft dysfunction [58]. A recent Dutch randomised controlled trial investigated if adaptation of the starting TAC dose to the *CYP3A5* genotype as compared with the standard body weight-based dosing would increase the



**Table 3** Factors contributing to the intra- and inter-patient variability in tacrolimus exposure

TAC exposure	factor	effect
<p><b>Inter-patient (between patients) variability</b></p> <p>Variability in TAC concentrations after administration of a given dose between patients</p> 	<p>-weight -age<sup>a</sup> -long-term concomitant medications</p> <p>-Hct</p> <p>-genetic polymorphism <i>CYP3A5</i>*1/*3</p> <p><i>CYP3A4</i>*22</p> <p><i>POR</i>*28</p>	<p>-higher relative exposure with increasing weight and age (lower weight-adjusted dose necessary to achieve target concentration)</p> <p>-higher TAC concentration with increasing Hct</p> <p>-lower TAC concentrations in carriers of the <i>CYP3A5</i>*1 allele</p> <p>-higher TAC concentrations in <i>CYP3A4</i>*22 carriers</p> <p>- lower TAC concentrations in <i>POR</i>*28 carriers and <i>CYP3A5</i> expressers</p>
<p><b>Intra-patient (within patient) variability</b></p> <p>Fluctuating TAC concentrations in a given patient</p> 	<p>-poor therapy adherence -concomitant medications (eg. antibiotics, <i>CYP3A4/A5</i> inducers and inhibitors) -concomitant food ingestion -diarrhea -interchangeable administration of various (generic) TAC formulations</p>	<p>High intra-patient variability in TAC exposure:</p> <p>-increased risk of allograft loss and late acute rejection -increased risk of pathologic changes: fibrosis and tubular atrophy -development of HLA-antibodies</p>

TAC tacrolimus, Hct haematocrit, *POR* polymorphism of cytochrome p450 oxidoreductase, *CYP3A* cytochrome P450 3A

<sup>a</sup> in pharmacokinetic paediatric studies age (as opposed to weight) was not a significant covariate in population models [4, 5, 40]

proportion of de novo adult kidney transplant patients who achieved a target TAC  $C_0$  in steady state. The authors of this study found was no difference in the proportion of patients with the sub- or supra-therapeutic TAC  $C_0$ . Moreover, there was no difference in the incidence of acute allograft rejection between the groups [64]. It has to be emphasised that data from adult patients may not be applicable to their paediatric counterparts, especially in children with a low weight. Although currently there is no evidence to support routine *CYP3A5* genotyping in paediatric populations, the personal view of the authors is that this analysis should be performed in resourceful settings as it may be helpful in guiding TAC dose and anticipating potential interactions. Also, the *CYP3A5* polymorphism may be relevant in other aspects of post-transplant care than immunosuppression. Vitamin D is catabolised by *CYP3A4/A5*, which implies that its genetic polymorphism may affect vitamin D concentrations [65].

## TAC dose and TDM

### Recommended starting dose

The recommendations for a patient-tailored TAC starting dose have been based on paediatric population pharmacokinetic models. In de novo renal transplant recipients a starting dose of 0.15 mg/kg twice daily was found to be too high for *CYP3A5* non-expressers and for children weighing > 40 kg and too low for *CYP3A5* expressers and for children with body weight of < 20 kg [5]. The target  $C_0$  was 5–15 ng/ml and the time since transplantation less than 2 months [5]. Recently, Andrews et al. showed a wide range in the starting TAC dose of between 0.27 and 1.33 mg/kg/day in children within 6 weeks after renal transplantation, with a target  $C_0$  of 5–15 ng/ml [40]. These dose recommendations are currently being evaluated in a prospective study.

In a paediatric study including patients beyond the first year from transplantation an additive effect of *CYP3A5*\*1/\*3 polymorphism and weight was reported in TAC dose simulations [4]. A TAC dose of 0.2 mg/kg/day would generate the following values: 5 ng/ml in a child weighing 20 kg and *CYP3A5* expresser; 6 ng/ml in a child weighing 40 kg and *CYP3A5* expresser; 8 ng/ml in a child weighing 20 kg and *CYP3A5* non-expresser; and lastly 10 ng/ml in a child weighing 40 kg and *CYP3A5* non-expresser [4]. These recommendations are similar to those published by Zhao et al. in de novo renal transplant recipients [5].

## Methods of TDM

Therapeutic drug monitoring can be applied in two-ways: (1) a full or abbreviated pharmacokinetic profile where TAC concentrations are measured pre-dose and at given times following ingestion, or (2) pre-dose (trough) concentration  $C_0$ . Obtaining a full 12-h pharmacokinetic profile is cumbersome and often requires a hospital admission. Therefore, abbreviated profiles for estimating TAC exposure have been performed and validated in adults [66] and children, where a profile including concentrations measured pre- and at 60 and 180 min after TAC ingestion showed a good correlation with the 12 h AUC [67]. The abbreviated AUC was predicted using Bayesian estimation. The following formula to calculate the full 12 h AUC was derived:

$$\text{Estimated AUC}_{0-12} = 19.422 + 4.317 \times C_0 + 1.226 \times C_1 + 4.273 \times C_3$$

Although the most accurate way to measure TAC exposure is AUC as opposed to  $C_0$  [68, 69], there is no universal agreement on how often it should be applied. While in some centres AUC (full or abbreviated) is performed routinely at set times following transplantation, others use it on indication only, for example to elucidate the effect of concomitant medications on TAC exposure or in case of unexplained toxicity. In some paediatric renal transplant centers the TAC AUC is not performed routinely.

As performing even an abbreviated pharmacokinetic profile on a regular basis is not feasible, an adequate one-point sampling for estimation of TAC exposure remains essential. In most centres, TAC dose is guided by  $C_0$  obtained from a morning blood sample. To date,  $C_0$  remains an optimal time-point of TAC concentration sampling. TAC pharmacokinetics can be affected by concomitant food ingestion, especially fatty meals. While a standard continental breakfast in most patients does not lead to major changes in TAC exposure [70], it has been shown that the TAC AUC decreases significantly when TAC is taken with a fatty meal [71]. Obtaining a pre-dose TAC concentration from a morning sample in comparison with an

evening sample is more “uniform” because most patients fast overnight and take breakfast only after TAC ingestion.

## TDM: “reference values”

While defining a target TAC  $C_0$ , the analytical method used to measure TAC concentration should be considered. A problem with immunoassays such as the enzyme-linked immunosorbent assay and microparticle enzyme immunoassay is the cross-reactivity of the antibody with TAC metabolites. Nowadays the liquid chromatography–tandem mass spectrometry technique is being increasingly applied, yielding 15–25% lower TAC concentrations in comparison with immunoassays as the cross-reactivity with TAC metabolites is eliminated [72, 73].

Achieving therapeutic concentrations of TAC can impact patient outcomes. As shown in the SYMPHONY trial, adult de novo renal transplant patients with a target TAC  $C_0$  of 3–7 ng/ml had a higher glomerular filtration rate and a lower incidence of acute rejection 1 year following transplantation than those treated with CsA or sirolimus [74]. However, in the above cited trial no comparison was made between various target TAC  $C_0$  head-to-head and patient outcomes. In adults, a target TAC  $C_0$  between 5 and 10 ng/ml in solid organ transplant recipients has been defined in a regimen including basiliximab, TAC, mycophenolate mofetil (MMF) and corticosteroids [75]. A recent study in adult renal transplant recipients on triple immunosuppression therapy including tacrolimus, MMF and corticosteroids showed that a mean TAC  $C_0$  of < 8 ng/ml was associated with the formation of donor specific antibodies (DSA) and a graded increase in risk with lower mean TAC  $C_0$  [76]. An increased risk of DSA formation might be an important consideration against CNI minimisation in renal transplant recipients.

There are no prospective trials analysing patient outcomes according to the target TAC  $C_0$  in the paediatric literature. In a prospective study on the feasibility of adding basiliximab to the standard immunosuppressive protocol of TAC, azathioprine and corticosteroids, the target TAC  $C_0$  were set at 10–20 ng/ml on days 0–21 and 5–15 ng/ml on days 22–183 [77]. The same target  $C_0$  were applied in the TWIST study that analysed the safety of early corticosteroid withdrawal in paediatric renal transplant recipients on combined therapy with TAC and MMF [44]. In some children TAC is used in combination with a mammalian target of rapamycin inhibitor, everolimus or sirolimus, with the aim to reduce nephrotoxicity; this strategy has proven to be beneficial in terms of cytomegalovirus replication and disease [78]. In this case the target TAC  $C_0$  is reduced.

In particular situations, such as very small kidney recipients or in *CYP3A5* expressers, a three-times daily dosing regimen is sometimes contemplated. The rationale for this approach are the higher peak concentrations required to attain a given AUC

with two-times daily regimens compared with three-times daily regimens. This approach is sometimes applied, but clinical data are very limited [79]. Evidence on the deleterious effect of high peak concentrations on the renal function is also weak. Of note, this approach of dosing three times daily might be challenging in terms of therapy adherence.

Designing a prospective trial with respect to the target TAC  $C_0$  would be challenging keeping in mind the heterogeneity in recipient-, donor- and centre-related characteristics regarding, for example, the underlying cause of end stage renal disease, repeat versus de novo transplantation, donor age and cardiovascular risk factors and concomitant immunosuppression. Moreover, in the era of personalised medicine the “one-dose-fits-all” approach is becoming obsolete. Ideally, the TAC dose and target  $C_0$  and AUC should be customised according to the state of over- and under-immunosuppression, clinically reflected as toxicity and allograft rejection, respectively. In this regard there is a growing interest in clinical application of biomarkers which allow an early and non-invasive detection of allograft injury [80]. An example of such a biomarker is the chemokine C-XC motif ligand 10 (CXCL10). It has been shown that increased urinary concentrations of CXCL10 are associated with acute rejection and BK virus infection in adult and paediatric renal transplant recipients [81].

Similarly to  $C_0$ , the optimal target AUC values in paediatric renal transplant patients have not yet been established. Based on retrospective data and the locally applied protocol, Claeys et al. suggested an AUC of 150 h·ng/ml 1 year after transplantation in paediatric patients with a stable allograft function and around 90 h·ng/ml in the years thereafter [69]. During the first post-transplantation weeks a mean TAC AUC of 190–200 h· $\mu$ g/l have been reported [82, 83]. In patients beyond the first year after transplantation the median AUC of 97 h· $\mu$ g/l corresponded with a TAC  $C_0$  of 4–8  $\mu$ g/l [4].

### Intra-patient variability in TAC concentrations

In contrast to the inter-patient variability, the intra-patient variability (IPV) is defined as fluctuating pre-dose concentrations of a drug in a given period of time. This is also shown in Table 3. The TAC IPV can be quantified by calculating the coefficient of variation, the mean absolute variation or the variance [84].

The coefficient of variation is defined as a ratio of the standard deviation to the mean and can be calculated using the following formula:

$$CV(\%) = (SD \times 100) / X$$

where CV is the coefficient of variation, SD is the standard deviation, and X is the mean value of all analysed concentrations.

In most published studies to date TAC IPV was calculated in stable patients, 6 months from transplantation onwards [84].

There is a wide range of TAC IPV in adult and paediatric populations, from < 5 to > 50%, with average values of 15–30% [84]. Several factors influencing TAC IPV have been identified, including interactions with concomitant medications (see Fig. 2) and food [71], diarrhoea [85] and interchangeable administration of generic TAC formulations [86, 87]. Although conversion from the innovator product Prograf® to generic TAC has been found to be safe and did not lead to a significant TAC IPV in paediatric renal transplant recipients [88], the occurrence of acute allograft rejection shortly following conversion has been reported [88, 89]. Additional studies with extended follow-up are required to define the role of generic TAC in paediatric renal transplantation.

Poor adherence to immunosuppression has been postulated to play a key role in high TAC IPV, especially in adolescents [90]. This can be supported by the fact that adolescents have a poorer long-term graft survival than other age groups and a higher incidence of late acute rejection [91, 92].

High TAC IPV in renal transplant recipients is associated with poor patient outcomes, including inferior allograft survival, higher incidence of acute rejection and an increased risk of histologic lesions, such as moderate to severe fibrosis and tubular atrophy [90, 93–95]. Recently, high TAC IPV has been found to contribute to the development of donor specific HLA-antibodies [96]. The deleterious effect of a high TAC IPV has also been addressed in the paediatric literature. In a retrospective study including 69 paediatric renal transplant patients with a stable renal function, children and adolescents who experienced late acute rejection had a significantly higher variability coefficient than non-rejectors [97].

The potential strategies aimed at reducing of TAC IPV include improving non-adherence [98] and patient education regarding drug and food interactions with TAC. Conversion from the twice-daily to once-daily extended release TAC formulation may also contribute to a better adherence and a lower TAC IPV [99].

### Future research and clinical perspectives

- 1) There is a need for consensus on whether or not paediatric solid organ transplant candidates **should routinely undergo CYP3A5 genotyping** to guide the TAC dose. Selecting the starting dose based on a dosing algorithm that includes CYP3A5 genotype and a number of other variables may prove to be more valuable than genotype only.
- 2) There is a lack of prospective randomised trials comparing different target values for TAC  $C_0$  and AUC on clinical outcomes in paediatric populations. Such studies would be helpful in providing evidence for the **reference values** for TAC  $C_0$  and AUC in paediatric renal transplant recipients.
- 3) Clinical application of customised TAC dose and target  $C_0$ /AUC for each patient; this will require the development and validation of **biomarkers** for a reliable assessment of the state of over- and under-immunosuppression in individual patients



- 4) Attention should be given to the **identification of factors** associated with highly fluctuating TAC concentrations within an individual patient (e.g. poor therapy adherence, concomitant medications, interference with food, diarrhoeal illness, etc.). Interventions to reduce the intra-patient variability (e.g. patient education and switching to once-daily TAC formulation) may improve patient outcome.
- 5) Paediatric studies with sufficiently long follow-up to assess the efficacy and safety of generic TAC formulations will support their implementation.

## Summary

The important points of this review can be summarised as follows:

1. TAC exerts its immunosuppressive effects through binding to calcineurin and inhibition of T-lymphocyte activation. As calcineurin is expressed in various tissues in the human body, TAC also displays a wide array of side effects.
2. TAC has a narrow therapeutic window and therefore TDM is essential. The existing “reference values” for TAC pre-dose concentration and AUC are experience based rather than evidence based.
3. *CYP3A5*\*1/\*3 polymorphism has a significant effect on TAC pharmacokinetics, but there is no consensus whether it should be routinely tested in children prior to renal transplantation. Prospective studies in adults have shown no beneficial effect of *CYP3A5* genotyping on patient outcomes, but there are no paediatric studies.
4. Intra-patient variability in TAC exposure is defined as fluctuating TAC pre-dose concentrations in a given period of time. It is associated with adverse outcomes following renal transplantation (allograft failure, late acute rejection, histologic changes such as fibrosis and tubular atrophy and formation of anti-HLA antibodies).

## Multiple choice questions (answers are provided following the reference list)

1. Which of the following is not a common side effect of tacrolimus
  - a) Hypermagnesaemia
  - b) Hypertension
  - c) Tremor
  - d) Hyperkalaemia
  - e) De novo diabetes mellitus
2. Which of the following statements is true:

- a) TAC has a broad therapeutic window
  - b) Food ingestion does not affect TAC pharmacokinetics
  - c) While commencing treatment with fluconazole the dose of TAC should be increased to avoid underexposure and acute allograft rejection
  - d) Children have higher relative TAC dose requirements than adults
  - e) The difference in relative TAC dose requirements between adults and children can be explained by the maturation of the hepatic *CYP3A4/A5*
3. Which statement regarding TAC pharmacogenetics is true:
    - a) The prevalence of the *CYP3A5*\*1 allele is higher in Western European than in African populations
    - b) *CYP3A5* expressers have lower TAC dose requirements
    - c) It has been shown that the routine pre-transplantation *CYP3A5*\*1/\*3 testing contributes to the lower incidence of acute allograft rejection in the early postoperative period
    - d) It is recommended to increase the standard TAC dose by 1.5- to 2-fold in *CYP3A5* expressers
  4. Which statement is false:
    - a) AUC is a more accurate method of evaluation of TAC exposure than a pre-dose concentration (trough level)
    - b) TAC concentrations should be measured in plasma
    - c) In most centers the target TAC pre-dose concentration 3 months after renal transplantation is set between 5 and 15 ng/ml
    - d) There is a need to develop biomarkers which would allow an early and non-invasive detection of kidney injury
  5. Intra-patient variability in TAC exposure:
    - a) denotes the variability in exposure following administration of a given dose to children with the same weight
    - b) is lowest in adolescents
    - c) is associated with the risk of late acute allograft rejection
    - d) statements *a* and *c* are true
    - e) none of the above statement are true

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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Answers: 1. a; 2. d; 3. d; 4. b; 5. c