Consideration of the ethnic prevalence of genotypes in the clinical use of tacrolimus

“Polymorphisms in the *CYP3A5* gene explain 40–50% of the variability in tacrolimus dose requirement, at least in Caucasians. The residual variability in tacrolimus clearance is at yet unexplained.”

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Tacrolimus is the mainstay of immunosuppressive therapy after solid organ transplantation for the prevention of acute rejection. Even though the use of tacrolimus-based immunosuppressive therapy has resulted in improved patient and graft survival, there unfortunately are some drawbacks. The associated adverse events, including nephrotoxicity, post-transplantation diabetes mellitus and hypertension remain an important issue [1]. To complicate matters further, the therapeutic range between efficacy and toxicity is narrow and tacrolimus is known to have a high interindividual pharmacokinetic variability [2]. Underexposure to tacrolimus could lead to an increased risk of rejection, whereas overexposure has been associated with an increased risk of toxicity. To individualize tacrolimus therapy, therapeutic drug monitoring (TDM) is routinely performed.

Pharmacogenetic aspects of tacrolimus exposure

The interindividual variability in tacrolimus pharmacokinetics is partly explained by genetic polymorphisms in genes encoding for tacrolimus metabolizing enzymes. *CYP3A5* and to a lesser extent *CYP3A4* are considered the fundamental enzymes involved in the metabolism of tacrolimus [3]. Individuals carrying at least one *CYP3A5**1 allele are referred to as *CYP3A5* expressers, whereas individuals homozygous for the *CYP3A5**3 allele are known as *CYP3A5* nonexpressers.

The *CYP3A5**3 allele causes alternative splicing which results in protein truncation and a severe decrease of functional *CYP3A5* enzyme [4]. It has been consistently demonstrated that *CYP3A5* expressers require at least a 1.5-fold higher tacrolimus dose compared with *CYP3A5* nonexpressers to reach the same exposure [5]. Following standard, bodyweight-based dosing, *CYP3A5* expressers also appear to have a delay in achieving target tacrolimus exposure, in spite of TDM [6]. In one study, *CYP3A5* expressers developed acute rejection earlier compared with nonexpressers [6].

Two randomized-controlled trials (RCTs) in which kidney transplant recipients either received the standard, bodyweight-based tacrolimus dosage or a *CYP3A5* genotype-based tacrolimus dosage (with expressers getting a higher than standard dose and nonexpressers receiving a lower dose) have been conducted. In the first trial, significantly more genotype-dosed patients reached the target range at first steady state compared with bodyweight-based tacrolimus dosing [7]. The second trial, however, found no such advantage of *CYP3A5*-guided tacrolimus dosing [8]. However, neither study demonstrated a decreased risk of acute rejection nor any other clinical benefit, and the conclusions were that optimization of the initial tacrolimus dose using *CYP3A5* pharmacogenetic testing does not improve clinical outcomes when TDM is

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performed [7,8]. It seems that TDM is able to rapidly correct for any tacrolimus exposure outside the target range [9]. Consequently, the time patients may be underexposed typically lasts only a few days, which may not be long enough to cause a clinically relevant increase in the incidence of acute rejection. These findings do not support routinely genotyping kidney transplant recipients for *CYP3A5*.

“Other interethnic differences, including comedication and diet, could turn out to be the missing pieces of the puzzle.”

CYP3A4 genotype has also been associated with altered tacrolimus clearance [10]. The recently discovered *CYP3A4**22 SNP has been associated with lower tacrolimus dose requirements after renal transplantation. After combining both *CYP3A4* and *CYP3A5* genotypes, Elens *et al.* were able to better predict the tacrolimus-metabolizing phenotype of kidney transplant patients [11]. Fairly recently the *CYP3A4**26 SNP was described, which results in exceptionally low tacrolimus dose requirement [12].

Ethnic considerations

In USA in 2015, 55.5% of the transplant recipients were Caucasian, 21.8% African-American (AA) and 15.5% of Hispanic descent [13]. AA kidney recipients are considered a high-risk group, having an increased risk of acute rejection and graft loss compared with non-AA recipients. The reasons for this increased risk are multiple and include differences in immunoreactivity, socioeconomic status, HLA matching, longer time on dialysis and lower rates of living kidney donation. In addition, compared with non-AA transplant recipients, differences exist in the adherence to the immunosuppressive medication and the pharmacokinetics of tacrolimus [14]. AA recipients in general have a higher tacrolimus clearance than Caucasians, and therefore require higher doses to reach the target range [15]. The reasons for these differences are poorly understood.

Part of the differences in tacrolimus pharmacokinetics and transplantation outcomes may relate to differences between populations in the genetics of tacrolimus-metabolizing enzymes. The best-known example is the allelic frequency of the loss-of-function *CYP3A5**3 allele. This SNP is common among Caucasians (90–93%), but is less prevalent among Asians (60–73%) and even more so in AA (32%) [16]. It has been suggested that all AA recipients should therefore receive a higher tacrolimus starting dose (0.3 mg/kg per day) [17].

For a long time the low prevalence of the *CYP3A5**3 allele in AA was considered the cause of the rapid

metabolizer phenotype in this population. However, recent investigations have demonstrated that the two loss-of-function SNPs *CYP3A5**6 and *CYP3A5**7 are quite prevalent among AA individuals with 75% carrying one or more nonfunctional *CYP3A5* alleles (either *3, *6 or *7), and as much as 24% homozygous for all three alleles. This finding implicates that there are substantially more *CYP3A5* nonexpressing AA than previously assumed. The presence of the *6 and *7 SNPs among AA may ‘compensate’ for the lower prevalence of *CYP3A5**3 allele, resulting in a metabolic phenotype similar to that of Caucasians [15,18]. This finding supports the hypothesis that the differences in tacrolimus pharmacokinetics between AA and non-AA recipients might not be genetic.

Residual variability & future perspective

Polymorphisms in the *CYP3A5* gene explain 40–50% of the variability in tacrolimus dose requirement, at least in Caucasians [7,8]. The residual variability in tacrolimus clearance is at yet unexplained. It is likely that other, as yet to be identified genetic variants in enzymes or drug transporter proteins will be discovered in the future but we feel that it is unlikely that the differences between patients of different ethnicity will be completely explained by genetics. Other interethnic differences, including comedication and diet, could turn out to be the missing pieces of the puzzle. When studied under matched conditions, there were no differences in pharmacokinetics and pharmacodynamics of ciclosporin (also a calcineurin inhibitor and *CYP3A* substrate) [19]. This implicates that differences in dietary habits might explain, at least to some extent, the pharmacokinetic differences between Caucasian and AA transplant recipients.

Most of our knowledge on the pharmacogenetics of tacrolimus comes from studies which mostly included non-AA patients. Therefore, this knowledge cannot be directly extrapolated to non-Caucasian patients which make up a relatively large proportion of kidney transplant recipients in USA and UK. Because AA tend to do less-well after transplantation, it is important to improve immunosuppressive drug pharmacotherapy for this group.

Fairly recently, dosing algorithms have been proposed to better individualize the starting dose of tacrolimus [20]. The dosing algorithm developed by Passey *et al.* incorporated a combination of genetic (*CYP3A5* genotype), demographic (age) and clinical (days post-transplant, steroid use and calcium channel blocker use) information [21]. The algorithm was developed in a population that for 20% consisted of AA. It was successfully validated, however it was not able to accurately predict tacrolimus clearance in a prospective

study [22]. The use of dosing algorithms that incorporate more factors than just bodyweight and *CYP3A5* genotype seems a more precise and rational approach. New dosing algorithms need to be developed specifically for AA recipients and should be validated and tested in an independent cohort of AA patients [18]. To justify clinical incorporation of such a population-specific algorithm, a clinical trial should demonstrate the superiority of such an approach over conventional dosing followed by TDM.

An alternative and possibly more simple strategy could be to only adjust the initial tacrolimus dose in a selected group of patients. *Post hoc* analyses of the above-mentioned RCT demonstrated that *CYP3A5* expressers tended to be below the target range less frequently if they received a higher tacrolimus starting dose [8]. AA recipients are more often rapid metabolizers than Caucasians and prescribing these patients a higher starting dose makes sense. It would be interesting to study the utility of comprehensive pharmacogenetic testing in this population. *CYP3A5*1* and *CYP3A5*3* have been the main focus so far, but we now know that this is insufficient in AA transplant recipients. *CYP3A5*6* and *CYP3A5*7* need to be taken into consideration [15]. Given the proactive nature of pharmacogenetic testing, it has the potential to optimize immunosuppressive therapy but it will most likely remain a complementary tool to traditional pharmacokinetic TDM.

A final strategy could be to prescribe a different tacrolimus formulation to AA recipients that has a more favorable pharmacokinetic profile. LCPT (Envirus®; Veloxis Pharmaceuticals, Hørsholm, Denmark) is an extended-release formulation of tacrolimus designed for once-daily administration. The difference between LCPT and other forms of once- and twice-daily tacrolimus products is the proprietary MeltDose® drug delivery technology (Veloxis Pharmaceuticals). MeltDose is designed to improve the bioavailability of drugs with low water solubility (i.e. Biopharmaceutics Classification System Class II compounds). The release of tacrolimus from LCPT tablets and absorption occurs more distally in the small bowel or colon as compared with the conventional twice-daily

formulation [23]. Gut CYP3A activity is largest in the proximal small bowel and progressively decreases downstream in the GI tract [24]. Due to the delayed release effect of LCPT, tacrolimus is mostly released in the distal GI tract, and its absorption is less dependent on CYP3A (and *CYP3A* genotype) which leads to an increased bioavailability, and therefore a decreased dose requirement. It also causes a lower tacrolimus peak concentration, which has been associated with less toxicity [25,26]. The recommended dose of LCPT as described in the package insert is 70% of the standard dose in Caucasian patients and 85% in AA [27]. This suggests that LCPT may improve the pharmacokinetic profile of tacrolimus, particularly in *CYP3A5* expressers. Whether LCPT is a more suitable formulation for rapid tacrolimus metabolizers is currently under investigation [28].

Over the past decade we have learned a great deal about the pharmacokinetics and pharmacogenetics of tacrolimus. This is also the case for ethnic minorities. However, many questions remain unanswered and there is room to improve tacrolimus pharmacotherapy in ethnic minorities. We believe that optimized tacrolimus dosing by use of (ethnicity-specific) dosing algorithms and possibly, the use of delayed-release tacrolimus formulations are rational strategies to achieve this goal. Finally and perhaps most importantly, RCTs should be designed and conducted specifically for patients of non-Caucasian ethnicity.

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