Cyclosporine, everolimus, and tacrolimus are the cornerstone of immunosuppressive therapy in renal transplantation. These drugs are characterized by narrow therapeutic windows, highly variable pharmacokinetics (PK), and metabolism by CYP3A enzymes. Recently, the decreased activity allele, CYP3A4*22, was described as a potential predictive marker for CYP3A4 activity. This study investigated the effect of CYP3A4*22, CYP3A5*3, and CYP3A combined genotypes on cyclosporine, everolimus, and tacrolimus PK in renal transplant patients. CYP3A4*22 carriers showed a significant lower clearance for cyclosporine (−15%), and a trend was observed for everolimus (−7%) and tacrolimus (−16%). Patients carrying at least one CYP3A5*3 allele had 1.5-fold higher tacrolimus clearance compared with noncarriers; however, CYP3A5*3 appeared to be nonpredictive for everolimus and cyclosporine. CYP3A combined genotype did not significantly improve prediction of clearance compared with CYP3A4*3 or CYP3A4*22 alone. These data suggest that dose individualization of cyclosporine, everolimus, or tacrolimus therapy based on CYP3A4*22 is not indicated.
measured concentrations were above the lower limit of quantification. Baseline characteristics of the included patients are presented in Table 1.

Genotyping
The distributions of all single-nucleotide polymorphisms were in Hardy–Weinberg equilibrium. The distributions of the investigated CYP3A5 and CYP3A4 polymorphisms are listed in Table 2. Allele frequencies found in our data set corresponded with those published previously. To investigate the combined effect of CYP3A4*22 and CYP3A5*3, genotype clusters were made as follows:

- Slow metabolizers (C1): no CYP3A5 activity (CYP3A5*3/*3) and at least one decreased activity allele in CYP3A4 (CYP3A4*22/*22 or CYP3A4*1/*22); intermediate metabolizers group 1 (C2): no CYP3A5 activity (CYP3A5*3/*3) and no decreased activity allele in CYP3A4 (CYP3A4*1/*1); intermediate metabolizers group 2 (C3): carriers of at least one increased activity allele in CYP3A5 (CYP3A5*1/*1 or CYP3A5*1/*3) and at least one decreased activity allele in CYP3A4 (CYP3A4*22/*22 or CYP3A4*1/*22); and extensive metabolizers (C4): carriers of at least one increased activity allele in CYP3A5 (CYP3A5*1/*1 or CYP3A5*1/*3) and no decreased activity allele in CYP3A4 (CYP3A4*1/*1).

Table 1 Baseline characteristics of the patients included in the population PK/PG analyses

|                      | Cyclosporine | Everolimus | Tacrolimus |
|----------------------|--------------|------------|------------|
| Male                 | 187          | 62         | 56         |
| Female               | 111          | 35         | 45         |
| Age (years)          | 51 ± 13      | 51 ± 13    | 50 ± 14    |
| Weight (kg)          | 77 ± 15      | 79 ± 15    | 76 ± 14    |
| Body surface area (m²) | 1.93 ± 0.22  | 1.94 ± 0.22| 1.90 ± 0.22|
| Lean body mass (kg)  | 57 ± 10      | 58 ± 10    | 55 ± 10    |
| Ideal BW (kg)        | 67 ± 9       | 67 ± 8     | 65 ± 9     |
| Height (cm)          | 174 ± 10     | 174 ± 10   | 172 ± 11   |
| Creatinine clearance (ml/min) | 46 ± 30      | 70 ± 25    | 56 ± 35    |

Exposure
- Dose (mg)          | 177 ± 78 (50–500) | 2.49 ± 0.79 (0.75–5.25) | 4.2 ± 1.7 (0.5–12) |
- AUC$_{0–12}$ (µg·h/l) | 5,648 ± 2,574 (702–16,499) | 150 ± 42 (56–336) | 170 ± 81 (49–462) |
- Trough concentration | 219 ± 131 (25–1,209) | 9.3 ± 4.2 (2.6–32) | 10.8 ± 5.5 (3.3–33.6) |

Ethnicity (%)
- Caucasian          | 88           | 86          | 77          |
- Mediterranean      | 3            | 5           | 13          |
- Asian              | 6            | 7           | 9           |
- Black              | 2            | 2           | 1           |
- Other              | 1            |             |             |

Hematocit (l/l) 0.36 ± 0.05 0.38 ± 0.04 0.34 ± 0.04

Underlying disease (n)
- Polycystic kidney disease | 63 | 22 | 16 |
- Glomerulonephritis | 50 | 15 | 7 |
- Diabetes mellitus | 12 | 4 | 22 |
- Hypertension | 50 | 15 | 15 |
- Focal segmental glomerulosclerosis | 13 | 4 | 8 |
- E.c.i.              | 13           | 5           | 5           |
- Interstitial nephritis | 11 | 3 | 3 |
- Urological          | 23           | 10          | 3           |
- Other               | 63           | 19          | 23          |

PK data
- Concentrations (µg/l) | 591 ± 434 (25–2,615) | 15.8 ± 8.1 (2.6–59) | 16.8 ± 10 (3.3–96) |
- Samples per patient | 23 ± 6 (3–37) | 19 ± 8 (7–38) | 9 ± 2 (3–14) |
- Total samples | 6,800 | 1,807 | 921 |

AUC, area under the curve; BW, body weight; E.c.i., e causa ignota (cause unknown); PG, pharmacogenetic; PK, pharmacokinetic.
Table 2 Genotype distribution in study population

| SNP | Frequency and genotype |
|-----|------------------------|
| Cyclosporine (n = 298) | |
| CYPA*4/2 (rs35599367) | 264  *1/*1 32  *1/*2 2  *2/*2 0  NG |
| CYPA*5/3 (rs776746) | 301  *1/*1 48  *1/*3 9  *1/*1 0  NG |
| CYP3A4/CYP3A5 cluster | C1  210  C2  5  C3  52  C4  2  NG |
| Everolimus (n = 97) | |
| CYPA*4/2 (rs35599367) | 87  *1/*1 8  *1/*2 1  *2/*2 1  NG |
| CYPA*5/3 (rs776746) | 90  *1/*3 12  *1/*3 3  *1/*1 1  NG |
| CYP3A4/CYP3A5 cluster | C1  72  C2  0  C3  15  C4  1  NG |
| Tacrolimus (n = 101) | |
| CYPA*4/2 (rs35599367) | 92  *1/*1 7  *1/*2 2  *2/*2 0  NG |
| CYPA*5/3 (rs776746) | 79  *1/*3 18  *1/*3 4  *1/*1 0  NG |
| CYP3A4/CYP3A5 cluster | C1  72  C2  2  C3  20  C4  0  NG |

C1, CYP3A5*1 noncarriers and CYP3A4*22 carriers; C2, CYP3A5*1 noncarriers and CYP3A4*22 noncarriers; C3, CYP3A5*1 carriers and CYP3A4*22 carriers; C4, CYP3A5*1 carriers and CYP3A4*22 noncarriers; NG, not genotyped; SNP, single-nucleotide polymorphism.

Concomitant medication
An overview of concomitant immunosuppressive and nonimmunosuppressive medication with possible interaction of PK in the different groups is presented in Supplementary Table I.

Population PK modeling
The PK data of cyclosporine, everolimus, and tacrolimus were best described by a two-compartmental model with first-order absorption and first-order elimination from the central compartment. The delayed absorption of everolimus and tacrolimus was best described with a lag time, and the delayed absorption of cyclosporine was best described with a transit compartment, using a first-order rate constant describing the transfer from the dose compartment into the transit compartment and subsequently into the central compartment (Figure 1). Random-effect parameters for interindividual variability in clearance (CL) and volume of central compartment (Vc) were identified for all three drugs. Random-effect parameters for interindividual variability in the rate of absorption (K) were identified for cyclosporine and everolimus. For tacrolimus, a random-effect parameter for interindividual variability was identified for bioavailability. Variability between occasions (interoccasion variability) was best described with a random effect on (fixed) bioavailability (F) for cyclosporine, everolimus, and tacrolimus. For everolimus also interoccasion variability on K was identified. The random effects were tested for structural relationship with dose and time to create a model with unbiased and randomly distributed random effects for covariate analysis.

The structural PK model of cyclosporine indicated an apparent clearance (CL/F) of 15.9 l/h, with the bioavailability term fixed to 0.5, an apparent central distribution volume (V/F) of 59.6 l, and an apparent peripheral distribution volume of 348 l. The absorption rate constant was 14.1 h⁻¹. Intercompartmenal clearance was 13.1 l/h. Interoccasion variability was estimated for the fixed bioavailability term and not for clearance because of a better model fit.

The structural PK model of everolimus indicated an apparent clearance (CL/F) of 17.6 l/h, with the bioavailability term fixed to 1, an apparent central distribution volume (Vc/F) of 16.7 l/h, and an apparent peripheral distribution volume of 99.7 l. The absorption rate constant was 2.1 h⁻¹.

The structural PK model of tacrolimus indicated an apparent clearance (CL/F) of 5.7 l/h, with the bioavailability term fixed to 0.5, an apparent central distribution volume (Vc/F) of 59.6 l, and an apparent peripheral distribution volume of 348 l. The absorption rate constant was 42.7 l/h, and lag time was 0.71 h. Interoccasion variability was estimated for the fixed bioavailability term and not for clearance because of a better model fit.

A dose–clearance relationship was observed showing an increase in apparent clearance with increasing dose according to typical value of clearance (TVCL) = [(dose/2.5)⁰.³⁵]. This relationship improved the model fit in terms of objective function. The effect appeared to be caused by strict TDM. Patients with high everolimus blood levels (i.e., with a lower clearance) were titrated to receive lower doses and vice versa to reach the stable target area under the curve (AUC)₁₂−− of 120 µg·h/l. Subsequently, an apparent dose–clearance relationship emerges. Additional tests described by Ahn et al. were performed, and it was confirmed that this effect was caused by strict TDM. Since two different assays were used for the determination of everolimus blood concentrations (liquid chromatography–tandem mass spectrometry and fluorescent polymerization immunoassay (FPIA)), a residual error for each assay was incorporated in the model. The model improved by adding an additive error to the FPIA data. This overestimation of FPIA was expected as investigated previously.

The structural PK model of FPIA was expected as investigated previously.
fixed to 0.23, an apparent central distribution volume \((V_c/F)\) of 20.5 l, and an apparent peripheral distribution volume of which was fixed to 500 l. The absorption rate constant was 0.55 h\(^{-1}\). Intercompartmental clearance was 17.2 l/h, and lag time was 0.809 h. Interoccasion variability was estimated for the fixed bioavailability term. The PK data of cyclosporine showed interindividual variability in \(CL/F\) of 23.5% and interoccasion variability (22.7%). Everolimus data revealed an interindividual variability in \(CL/F\) of 28.8% and interoccasion variability (28.4%). Tacrolimus showed considerably higher interindividual variability in \(CL/F\) of 42.2% and interoccasion variability (35.5%).

### Covariate analysis

**Pharmacogenetics.** In Table 3, the summary of the univariate pharmacogenetic covariate analysis is presented. CYP3A4*22 was significantly associated with cyclosporine \(CL/F\), and patients who carried at least one decreased activity allele in CYP3A4*22 had a 15% lower clearance compared with noncarriers. CYP3A combination showed a significant effect; C1, C2, and C3 showed lower clearance compared with C4 (−16, −2, and −12%, respectively). Everolimus PK did not reveal a significant relation with CYP3A5*3 and CYP3A4*22, nor the CYP3A genotype combination. For tacrolimus, CYP3A5*3 was significantly associated with tacrolimus \(CL/F\). Carriers of at least one CYP3A5*1 allele had 53% higher clearance compared with noncarriers. In contrast, CYP3A4*22 as covariate on \(CL/F\) did not result in a significant objective function drop \((P = 0.218)\). Although not significant, a trend of 16% lower tacrolimus clearance was observed for CYP3A4*22 allele carriers. CYP3A combination showed a significant effect on tacrolimus clearance. C1, C2, and C3 showed lower clearance compared with C4 (−47, −33, and −3%, respectively). Although significant, the genetic covariates explained variability in clearance to a limited degree. In Figure 2, box plots of clearance vs. genotype are presented for cyclosporine, everolimus, and tacrolimus, and the figure also shows the significant variability within the genotype groups.

**Demographics.** The demographic covariates that showed a possible relation with the PK of the drugs in the diagnostic plots were evaluated in the covariate analysis. Univariate analysis \((P < 0.05)\) on cyclosporine showed significant associations for the following demographic covariates: body weight (BW) on \(CL/F\) and \(V_c/F\), prednisolon dose \(\geq 20\) mg on \(K_a\) and \(F\) for cyclosporine, ideal BW on \(V_c/F\) and hematocrit on \(CL/F\) for everolimus. Significant demographic covariates for tacrolimus were prednisolone dose \(\geq 25\) mg on \(F\) and hematocrit on \(CL/F\). The remaining demographic covariates such as ethnicity and other comedication that were evaluated in this study were not significant on \(CL/F\), \(V_c/F\), or \(K_a\).

### Table 3 Summary of CYP3A4 and CYP3A5 covariate analysis

| Covariate tested | MVOF       | ΔOF        | P value | Var. CL (%) | Expl. Var. (%) | Mean value (%) | 95% CI (%) |
|------------------|------------|------------|---------|-------------|----------------|----------------|------------|
| **Cyclosporine**|            |            |         |             |                |                |            |
| Base model       | −6,750.443|            |         | 23.5        |                |                |            |
| + CYP3A5*3       | −6,751.429| 0.986      | 0.32072 | 23.5        | 0              | 3              | −2 to 8    |
| + CYP3A4*22      | −6,767.684| 17.241     | 0.00003 | 22.7        | 3.4            | −14.5          | −20 to −8  |
| + CYP3A combination | −6,768.565| 18.123     | 0.00041 | 22.7        | 3.4            | C1 −16         | −23 to −9  |
|                  |            |            |         |             |                | C2 −2          | −7 to 3    |
|                  |            |            |         |             |                | C3 −12         | −27 to 4   |
|                  |            |            |         |             |                | C4 0           | −5 to 5    |
| **Everolimus**   |            |            |         | 28.8        |                |                |            |
| Base model       | 5,446.987 |            |         |             |                |                |            |
| + CYP3A5*3       | 5,444.175 | 2.059      | 0.15131 | 28.4        | 1.4            | 12             | −0.3 to 24 |
| + CYP3A4*22      | 5,446.234 | 0.753      | 0.38553 | 28.7        | 0.3            | −7             | −23 to 9   |
| + CYP3A combination | 5,443.734 | 3.253      | 0.19662 | 28.4        | 1.4            | C1 −15         | −29 to −1  |
|                  |            |            |         |             |                | C2 −10         | −19 to −2  |
|                  |            |            |         |             |                | C3 NA          | NA          |
|                  |            |            |         |             |                | C4 0           | −3 to 9    |
| **Tacrolimus**   |            |            |         | 42.2        |                |                |            |
| Base model       | 3,549.937 |            |         |             |                |                |            |
| + CYP3A5*3       | 3,530.215 | 19.722     | 0.00001 | 39.9        | 5.5            | 53             | 25 to 80   |
| + CYP3A4*22      | 3,548.418 | 1.519      | 0.21777 | 41.7        | 1.2            | −16            | −47 to 14  |
| + CYP3A combination | 3,527.993 | 21.717     | 0.00007 | 36.6        | 13.3           | C1 −47         | −69 to −24 |
|                  |            |            |         |             |                | C2 −33         | −46 to −20 |
|                  |            |            |         |             |                | C3 −3          | −48 to 41  |
|                  |            |            |         |             |                | C4 0           | −18 to 18  |

ΔOF, Δ objective function; C1, CYP3A5*1 noncarriers and CYP3A4*22 carriers; C2, CYP3A5*1 noncarriers and CYP3A4*22 noncarriers; C3, CYP3A5*1 carriers and CYP3A4*22 carriers; C4, CYP3A5*1 carriers and CYP3A4*22 noncarriers; Expl. Var. (%), explained variability in percentage of total; MVOF, minimal value of objective function; NA, not applicable; P value, \(\chi^2\) distribution \(P\) value; RSE, relative standard error; Var. CL (%), remaining variability in clearance. Mean value (%) represents the difference in \(CL/F\) compared with the reference group, which is 0.
Figure 2  Box plots representing the average cyclosporine, everolimus, and tacrolimus apparent clearance (l/h) of the different genotype groups with error bars and the number of patients in each group. CYP3A4 (*1/*1 = CYP3A4*22 noncarriers, *1/*22 or *22/*22 = CYP3A4*22 carriers, NG = not genotyped), CYP3A5 (*1/*3 or *1/*1 = CYP3A5*1 carriers, *3/*3 = CYP3A5*1 noncarriers, NG = not genotyped), and CYP3A cluster: (C1: CYP3A5*3/*3 and CYP3A4*22/*22 or CYP3A4*1/*22, C2: CYP3A5*3/*3 and CYP3A4*1/*1, C3: CYP3A5*1/*1 or CYP3A5*1/*3 and CYP3A4*22/*22 or CYP3A4*1/*22, and C4: CYP3A5*1/*1 or CYP3A5*1/*3 and CYP3A4*1/*1, NG = not genotyped). *P < 0.01. Apparent clearance was calculated using the base model.
After the forward inclusion and backward elimination step, the following covariates remained significant (\( P < 0.01 \)):

Cyclosporine: BW on \( CL/F \) and \( V/F \), prednisolon dose \( \geq20mg \) on \( K_e \) and \( F \) (better model fit and objective function drop compared with prednisolon dose on \( CL/F \)), and \( CYP3A4*22 \) on \( CL/F \). Interindividual variability of \( CL/F \) decreased from 23.5 to 22.6%. In Supplementary Table II, all significant covariates improving model fit together with their effects on observed variability are presented for cyclosporine, everolimus, and tacrolimus. Everolimus: ideal BW centered on the population median as exponential function on \( V/F \) improved the model, reducing the random variability between individuals in \( V/F \) by 12%. Hematocrit was lost in the forward elimination step (\( P > 0.01 \)) and was, therefore, not incorporated in the final model. Significant covariates for tacrolimus were found in prednisolone dose \( \geq25 \) on \( F \) (higher objective function drop compared with prednisolon dose on \( CL/F \)), \( CYP3A5*3 \) and hematocrit on tacrolimus \( CL/F \). Incorporation of these covariates decreased the interindividual variability of \( CL/F \) from 42.2 to 39.1%, and the interoccasion variability was reduced from 35.5 to 29.3%.

The population PK parameters obtained with the base and final models are presented in Table 4 (Supplementary Models 1-3). Evaluation of the precision of the PK parameters of all three models was performed with 1,000 bootstrap replications. The percentage of successful runs was 99% for cyclosporine, 82% for everolimus, and 96% for tacrolimus. Moreover, the parameter estimates of the nonsuccessful runs were analyzed and did not deviate from the parameter estimates of the successful runs. The mean values for all fixed-effect parameters were within 15% of those obtained by the final model, indicating good reliability. Since different dosages were used during the study, the performance of the model was evaluated with a prediction-corrected visual predictive check (VPC). Predictive and observed intervals (10%, 90%, and median) are almost identical showing good predictive performance of the final models (Figure 3).

**DISCUSSION**

This is the first comprehensive study investigating the influence of \( CYP3A4*22 \) and \( CYP3A5*3 \) variant alleles and its combined clusters on the PK of the three main kidney transplant immunosuppressive drugs cyclosporine, everolimus, and tacrolimus. This study demonstrates that carriers of the \( CYP3A4*22 \) allele is significantly associated with a decreased cyclosporine clearance. Carriers of the \( CYP3A4*22 \) allele showed 15% lower cyclosporine clearance as compared to noncarriers. Moreover, \( CYP3A \) genotype clusters were significantly associated with cyclosporine and tacrolimus clearance but not with everolimus clearance. Finally, this study also demonstrates that patients carrying at least one \( CYP3A5*1 \) allele have on average 53% higher tacrolimus clearance compared with noncarriers.

Cyclosporine, everolimus, and tacrolimus are primarily eliminated by \( CYP3A \) enzymes,\(^ {5,6,31} \) and as shown before in \( in vitro \) and \( in vivo \) studies, \( CYP3A4 \) is involved in their PK.\(^ {5,27,28} \) \( CYP3A4 \) is most likely predominant in cyclosporine and everolimus metabolic clearance, and \( CYP3A5 \) contributes more significantly to tacrolimus metabolic clearance compared with \( CYP3A4.\(^ {5,6} \) In contrast to \( CYP3A5, CYP3A4 \) lacked a reliable genetic marker for prediction of \( CYP3A4 \) expression, which was suitable for dosing adjustments;\(^ {29} \) however, \( CYP3A4*22 \) was recently marked as a potential reliable marker.\(^ {15,16} \) In contrast, as part of our analysis, only a significant influence of \( CYP3A4*22 \) on cyclosporine PK was found, but a trend was also seen in tacrolimus (16% lower clearance (95% confidence interval: \(-47\) to 14%)) and everolimus PK (7% lower clearance (95% confidence interval: \(-23\) to 9%)). This effect is not high enough to justify dose modification based on \( CYP3A4*22 \). In clinical practice, only an effect of at least 20% on clearance will lead to dose adjustments, since these drugs also possess a considerable degree of interindividual variability. Since the clinical studies from which all data were derived were not primarily designed to identify a genotype effect and the fact that we found no clinically relevant genotype effect for \( CYP3A4*22 \), we had to confirm afterwards that our study had enough power. Therefore, we performed a posterior power calculation using the stochastic simulation and estimation tool of the PtsN toolkit to determine the power (95 and 99% confidence) of our study to find a clinically relevant genotype effect (at least 20%) on cyclosporine, everolimus, and tacrolimus PK.\(^ {30,31} \) With the most unfavorable genotype distribution (\( CYP3A4*22 \)) and the least amount of data (tacrolimus), we found a power of 95% (\( \alpha = 0.01 \)) and 91% (\( \alpha = 0.01 \)) in detecting a clinically relevant genotype (at least 20%) effect. It is therefore highly unlikely that our analysis was underpowered and missed a clinically relevant effect of the investigated genotypes due to limited sample size.

In contrast to our findings, the studies of Elens et al.\(^ {15,32} \) and Gijsen et al.\(^ {16} \) showed that \( CYP4A4*22 \) allele carriers required up to 30% lower tacrolimus doses compared with \( CYP3A4*1/*1 \) to reach target trough concentration. However, these exploratory findings have not been confirmed by another research group. Moreover, more recently, Santoro et al.\(^ {11} \) presented a study in 140 renal transplant patients showing that independent effects of \( CYP3A4*22 \) on tacrolimus dose requirements could not be verified. The studies of Elens et al.\(^ {15,32} \) had some limitations such that the data were not corrected for corticosteroid use or hematocrit levels. Corticosteroid and hemocrit levels are known to influence tacrolimus exposure\(^ {32,33} \) and could therefore have influenced their results. The study of Gijsen et al.\(^ {16} \) performed on a small data set had the limitation that they could not correct their results for comedication. Both studies\(^ {15,16} \) only used trough levels in their analysis, which do not give a full insight in PK. The more recent study of Elens et al.\(^ {35} \), in contrast, used an additional 59 whole PK curves to support their conclusion; however, since they were collected only on one occasion, interindividual variability could not be assessed. To investigate whether shrinkage could have been the cause of the lack of significance of the \( CYP3A4*22 \) effect in this study, we also performed the univariate genetic covariate analysis with only the first PK profiles to be able to compare the results in more details with Elens et al. (see Supplementary Table III). The results were the same as with the complete data set, so therefore, the results found in the study of Elens et al.\(^ {25} \) could not be replicated in our study. In another study
### Table 4: Summary of model parameter estimates for cyclosporine, everolimus, and tacrolimus

| PK parameter | Base model | Final model | Expl. Var. (%) | 1,000 bootstrap runs |
|--------------|------------|-------------|----------------|-----------------------|
|              | Mean value | RSE (%)     | Shr. (%)       | Mean value            | RSE (%) | Shr. (%) | Mean value | 95% CI    |
| **Cyclosporine** |            |             |                |                       |         |         |            |           |
| CL/F         | 15.9       | 2           | —              | 15.6                  | 1.8     | —        | 15.6       | 15.1 to 16.1 |
| BW on CL/F   | —          | —           | —              | 0.3                   | 32.7    | —        | 0.3        | 0.09 to 0.48 |
| CYP3A4*22 on CL/F | —      | —           | —              | −0.15                 | 21      | —        | −0.15      | −0.20 to −0.08 |
| F (fixed)    | 0.5        | —           | —              | 0.5                   | —       | —        | 0.5        | —         |
| DDPR ≥20 mg  | —          | —           | —              | −0.12                 | 17.3    | —        | −0.12      | −0.15 to −0.08 |
| Vc/F (l)     | 59.6       | 4           | —              | 56                    | 5       | —        | 55.8       | 50.3 to 61.2 |
| BW on Vc/F   | —          | —           | —              | 0.61                  | 36      | —        | 0.61       | 0.16 to 1.04 |
| Q/F (l/h)    | 13.1       | 5           | —              | 13                    | 7       | —        | 13         | 11.5 to 15.3 |
| Vc/F (l)     | 99.7       | 8           | —              | 90.4                  | 9       | —        | 90.5       | 80.2 to 110.7 |
| K0 (h⁻¹)     | 2.1        | 6           | —              | 2.16                  | 9       | —        | 2.2        | 1.9 to 2.5   |
| DDPR ≥20 mg  | —          | —           | —              | −0.45                 | 11      | —        | 2.5        | −0.53 to −0.34 |
| **Everolimus** |            |             |                |                       |         |         |            |           |
| CL/F         | 16.7       | 4           | —              | 16.7                  | 4       | —        | 16.7       | 15.4 to 17.8 |
| F (fixed)    | 1          | —           | —              | 1                     | —       | —        | 1          | —         |
| Vc/F (l)     | 144        | 5           | —              | 140                   | 5       | —        | 143        | 131 to 156  |
| IBW on Vc/F  | —          | —           | —              | −0.96                 | 28      | —        | −0.95      | −1.55 to −0.39 |
| Q/F (l/h)    | 42.7       | 6           | —              | 43.1                  | 6       | —        | 43.4       | 38.7 to 49.5 |
| Vc/F (l)     | 348        | 22          | —              | 343                   | 20      | —        | 336        | 247 to 585  |
| K0 (h⁻¹)     | 7.3        | 20          | —              | 7                     | 16.3    | —        | 7.1        | 4.7 to 11.1  |
| Dose CL/F (TDM effect) | 0.34 | 31           | —              | 0.34                  | 28      | —        | 0.35       | 0.16 to 0.49 |
| **IIV**      |            |             |                |                       |         |         |            |           |
| IIV CL/F (CV%) | 23.5     | 8           | 10             | 22.6                  | 9.6     | 10       | 22.2       | 18.5 to 26.7 |
| IIV Vc/F (CV%) | 41.5     | 8           | 19             | 42.3                  | 10.4    | 19       | 42.1       | 32.8 to 50.8 |
| IIV K0 (CV%)  | 48.6       | 9           | 21             | 49                    | 10.6    | 23       | 49.1       | 38.5 to 59.0 |
| **IOV**      |            |             |                |                       |         |         |            |           |
| IOV K0 (CV%)  | 127.3      | 11          | 39             | 127.3                 | 11      | 38       | 127.3      | 102.0 to 160.9 |
| IOV F (CV%)   | 26.4       | 6           | 7              | 26.3                  | 5.7     | 6        | 26.2       | 23.3 to 29.5 |
| **Random residual variability** |            |             |                |                       |         |         |            |           |
| σ1 (additive error) | 0.301   | 6           | 10             | 0.297                 | 12.6    | 10       | 0.293      | 0.268 to 0.335 |
| σ1 (proportional error) | 28.8   | 48          | 9              | 28.9                  | 13      | 9        | 28.7       | 21.7 to 34.6 |
| σ2 (proportional error) | 35.1  | 26          | 12             | 30.6                  | 10      | 14       | 30.4       | 25.3 to 37.0 |
| σ3 (additive error) | 115.8  | 16          | 35             | 111                   | 13      | 35       | 108.1      | 84.9 to 136.9 |
| **Random residual variability** |            |             |                |                       |         |         |            |           |
| σ1 (proportional error) | 127.3  | 11          | 39             | 127.3                 | 11      | 38       | 127.3      | 102.0 to 160.9 |
| σ2 (proportional error) | 26.4   | 6           | 7              | 26.3                  | 5.7     | 6        | 26.2       | 23.3 to 29.5 |
| **Random residual variability** |            |             |                |                       |         |         |            |           |
| σ1 (additive error) | 14.5   | 7.7         | 16             | 14.5                  | 7.6     | 15       | 14.3       | 11.8 to 16.7 |
| σ2 (proportional error) | 6.6    | 14.3        | 15             | 6.6                   | 14.1    | 15       | 6.7        | 4.1 to 8.2   |
| σ3 (additive error) | 1.06   | 14          | 15             | 1.06                  | 14      | 15       | 1.08       | 0.8 to 1.44  |

Table 4 Continued on next page
by Elens et al., no significant effect was found for cyclosporine trough concentrations and CYP3A4*22 carrier ship. Our analysis was based on an extensive amount of data consisting of AUCs. Moreover, a wide range of factors possibly influencing PK, including demographic factors and comedication, was also investigated.

The difference in tacrolimus clearance between CYP3A5*1 carriers and noncarriers found in the current analysis was similar to what was published previously. We confirmed with our study that dosing adjustments based on CYP3A5*3 could be indicated to quickly reach target exposure; however, the variability explained by CYP3A5*3 is limited, and the variability within the CYP3A5 genotype groups remains significant, and therefore, close TDM remains essential. The absence of a clinically relevant influence of CYP3A4*22 carrier ship, which makes a further differentiation unnecessary.

Up to now, the only suggested clinically relevant polymorphisms in CYP3A4 enzymes relevant for kidney transplantation are CYP3A5*3 and CYP3A5*6 for tacrolimus, which are primarily found in Africans and have low allelic frequencies in the Caucasian population. CYP3A5*6 was left out of this analysis because of too low allele frequency (<6%). CYP3A4*22 is able to predict CYP3A4 activity; however, the clinical relevancy seems to be limited. The search for a reliable and clinically relevant predictive biomarker for CYP3A4 is still open, although CYP3A4 phenotyping shows more promising results as recently published by de Jonge et al.

The demographic covariates that were identified in this study have been reported in previous studies. The clinical relevancy of the different identified covariates is limited since the explained variability by the individual covariates did not exceed 12%. The effect of prednisolone dose on cyclosporine and tacrolimus bioavailability (high dose, lower bioavailability) can be explained by CYP3A induction in the intestine and has been reported before. The cutoff values were chosen based on literature and highest objective function drop. The PK parameter estimates of the three models were in agreement with those found in previous

### Table 4 Continued

| PK parameter | Base model | Final model | 1,000 bootstrap runs |
|--------------|------------|-------------|---------------------|
| Tacrolimus   |            |             |                     |
| CL/F         | Mean value | RSE (%)     | Shr. (%)            |
|              | 5.7        | 5           | —                   |
| CYP3A5*3 on CL/F | —         | —           | —                   |
| HTC on CL/F + IOV F | —         | —           | —                   |
| F (fixed)   | 0.23       | —           | —                   |
| DDPR ≤25mg (IOV F) | —         | —           | —                   |
| V/F (l)     | 20.5       | 22          | —                   |
| Q/F (l/h)   | 17.2       | 9           | —                   |
| K (h⁻¹)     | 0.55       | 10          | —                   |
| Lagtime     | 0.81       | 7           | —                   |
| IIV         |            |             |                     |
| IIV CL/F (CV%) | 42.2     | 15          | 26                  |
| IIV V/F (CV%) | 124.1     | 13          | 18                  |
| IIV F (CV%)  | 38.1       | 21          | 39                  |
| IOV         |            |             |                     |
| IOV F (CV%)  | 35.5       | 12          | 26                  |
| Random residual variability | | | |
| σ₁ (proportional error) | 17.3 | 5 | 16 |

CI, confidence interval; CL, clearance; CYP, cytochrome P450; DDPR, daily dose prednisolon; Expl. Var. (%), percentage explained of total variability; F, bioavailability; FPIA, fluorescence polarization immunoassay; HTC, hematocrit; IBW, ideal body weight; IIV, interindividual variability; IOV, interoccasion variability; K⁺, absorption rate constant; Lagtime, lagtime of absorption; LCMS, liquid chromatography/tandem mass spectrometry; Q, intercompartmental clearance; Shr. (%), shrinkage (%); TDM, therapeutic drug monitoring; Vc, distribution volume of the central compartment; Vp, distribution volume of the peripheral compartment.
Everolimus. Since everolimus is primarily partitioned into red blood cells and 75% of the plasma fraction is bound to plasma proteins, this relationship can be physiologically explained since length and sex are incorporated in the ideal weight formula.\textsuperscript{2,40} The significant effect of hematocrit on everolimus clearance in the univariate covariate analysis could also be explained by the same mechanism. Ethnicity could not be identified as a covariate on clearance of everolimus or cyclosporine as was found previously by Kovarik et al.\textsuperscript{41} and Hesselink et al.\textsuperscript{36} This difference could be explained by the lack of black patients in our cohort. Although theoretically plausible, we did not find an effect of concomitant medication such as statins, calcium antagonists, sulfamethoxazole/trimethoprim, or proton pump inhibitors on CL/F. This is in accordance with what has previously been described in literature.\textsuperscript{3,41} Comedications known to have a potent effect on the PK of the drugs were avoided for safety reasons.\textsuperscript{42} The remaining variability in clearance between patients of our final model was 22.6% for cyclosporine, 28.8% for everolimus, and 38.9% for tacrolimus. This is only suitable as a predictive marker for tacrolimus clearance, but close TDM remains essential due to the remaining variability between patients with the same genotype. The CYPA4 and CYPA5 combined genotypes do not further improve the predictive performance compared with the predictive performance of the polymorphisms alone. Therefore, the newly discovered CYPA4*22 or CYPA3 combined genotypes are not indicative to be used for dose adjustments in clinical practice to further improve immunosuppressive therapy of cyclosporine, tacrolimus, or everolimus in the investigated patient population.

**Figure 3** Prediction-corrected visual predictive checks with 80% prediction interval of cyclosporine, everolimus, and tacrolimus. The observed concentrations are shown as solid circles. The solid lines with open circles represent the observation intervals. The solid lines represent the prediction interval. The shaded areas around the prediction intervals represent the 95% confidence interval around each of the prediction interval.

**METHODS**

**Cyclosporine**

Clinical data from 298 renal transplant recipients treated with a immunosuppressive regimen cyclosporine (Neoral, Novartis, Basel, Switzerland), prednisolone, and mycophenolate sodium participating in a run in phase of a prospective, open, randomized, multicenter study were studied up to 6 months after transplantation.\textsuperscript{42} Induction therapy consisted of two doses of 20mg basiliximab (Simulect Novartis, Basel, Switzerland) before transplantation and on day 4, rapidly tapered prednisolone dose (50mg twice daily (b.i.d.) intravenously tapered to daily 10mg oral prednisolone). Cyclosporine therapy was started at an oral dose of 4mg/kg b.i.d. and was supported by routine TDM based on AUC\textsubscript{0-12}. TDM was aimed at a target of 5,400 µg/l at the first 6 weeks and 3,250 µg/l thereafter. Cyclosporine concentrations were obtained at steady state at clinical visits, which were scheduled at 1, 5, 12, and 24 weeks after transplantation.

**Everolimus**

Clinical data from 97 stable renal transplant recipients treated with immunosuppressive duotherapy consisting of everolimus (Certican, Novartis) and prednisolone, participating in a prospective, open, randomized, multicenter study were studied up to 6 months after transplantation.
from 6 to 24 months after transplantation. During the first 6 months, patients were treated with an immunosuppressive regimen cyclosporine, prednisolone, and mycophenolate; thereafter, a scheduled biopsy was performed. Patients whose biopsy showed no sign of rejection were included. Subsequently, cyclosporine and mycophenolate were discontinued. Everolimus therapy was started at an oral dose of 3 mg b.i.d. and was supported by routine TDM based on AUC_{0–12 h}. TDM was aimed at a target of 120 μg·h/l. Everolimus concentrations were obtained at steady state at regular clinical visits scheduled at 32, 52, 78, and 104 weeks after transplantation.

**Tacrolimus**

Clinical data from 101 renal transplant patients on an immunosuppressive regimen of tacrolimus (Prograft, Astellas, Leiden, The Netherlands), prednisolone, and mycophenolate mofetil were studied for first two TDM moments after transplantation. Induction therapy consisted of two doses of 20 mg basiliximab (Simulect) before transplantation and, on day 4, rapidly tapered prednisolone dose (50 mg b.i.d. intravenously tapered to daily 10 mg oral prednisolone). Tacrolimus therapy was started at a fixed oral dose of 5 mg b.i.d. and was supported by routine TDM based on AUC_{0–12 h}. TDM was aimed at a target of 160 μg·h/l the first 6 weeks and 120 μg·h/l thereafter. Tacrolimus concentrations were obtained at steady state from 1 to 66 weeks after transplantation with a median of 2 weeks.

The study was approved by the Medical Ethics Committee of Leiden University Medical Center, and patients gave written informed consent.

**Bioanalytics**

TDM was performed on the basis of Bayesian estimation (cyclosporine and tacrolimus) or trapezoidal rule (everolimus) (blood concentration at t = 0, 1, 2, 3, 4, 5, and 6 h (everolimus and tacrolimus) up to 12 h for some patients (cyclosporine) or t = 0, 1, 2, 3, 4 h) in a small number of visits in the everolimus data set) using MW/Pharm 3.5 (Mediware, Groningen, The Netherlands). TDM samples were determined in whole blood by a validated liquid chromatography–mass spectrometric method in two laboratories or by FPIA (Abbott Laboratories, Abbott Park, IL). Tacrolimus blood concentrations were all determined with liquid chromatography–mass spectrometry tandem mass spectrometry, everolimus with liquid chromatography–mass spectrometry tandem mass spectrometry and FPIA, and cyclosporine with FPIA alone. Table 1 shows the samples distribution of the blood concentrations used in this study.

**Genotyping assays**

DNA was isolated from blood from ethylene diamine tetraacetate acid blood collection tubes collected from patients. CYP3A4*22 was determined with TaqMan 7500 (Applied Biosystems, Nieuwerkerk a.d. IJssel, The Netherlands) with predesigned assays, according to the manufacturers’ protocol. CYP3A5*3 was determined with Pyrosequencer 96MA (Isogen, IJsselstein, The Netherlands). Further details with regard to the genotyping protocol are provided in Supplementary Table IV. No inconsistencies were observed. All allele frequencies were in Hardy–Weinberg equilibrium.

**PK modeling**

Nonlinear mixed effect modeling was used to estimate PK parameters from blood concentration–time data. NONMEM (v7.2.1, Icon Development Solutions, Ellicott City, MD) was used for modeling, using PsN toolkit 3.4.2 and Piranã version 2.8.0 (ref. 49) as modeling environment. Results were analyzed using statistical software package R (v2.15.2) and RStudio (v0.97.248; Boston, MA). First-order conditional estimation method with interaction was used throughout the analysis. Model selection was based on statistical significance, goodness of fit, and stability. Throughout the model building process, an altered model was chosen over a precursor model if the difference in the objective functions (~2 log likelihood) was >6.63 (P < 0.01, with 1 degree of freedom, assuming χ^2 distribution).

**Base model**

The model was initially developed strictly PK without covariates. Since only data after oral and not after intravenous administration were available, the absolute oral bioavailability could not be determined. Therefore, the value for bioavailability was fixed. Plots of observed concentration–time data were examined. One- and two-compartmental PK models with first-order elimination were compared to find the best fit of the concentration–time data. The use of transit compartments and a lag time for drug absorption were explored. After building the base model, demographic and genetic covariates were explored.

**Covariate analysis**

Diagnostic plots were constructed of the random effects of clearance, volume, Kᵢ, and F vs. the demographic (age, BW, sex, ethnicity, length, lean BW, ideal BW, body surface area (BSA), BMI (formulas in Supplementary Table V), hematocrit, underlying disease, and comedinations (also weighted residuals vs. comedication plots)) and pharmacogenetic (CYP3A4*22 and CYP3A5*3) characteristics. Polymorphisms were selected based on theoretical relationship and minimal allele frequency (>6%) to assure detection of clinically relevant effect on PK. Based on these diagnostic plots, further testing in the pharmacostatistical model was performed. Subsequently, selected covariate relationships were evaluated by forward inclusion and backward deletion procedure. A covariate effect was only maintained in the model, if the inclusion resulted in a reduction in random variability and improved model fit.

**VPC with prediction correction**

Performance of candidate and final models for cyclosporine, everolimus, and tacrolimus PK models was evaluated using prediction-corrected VPCs, by simulation of 500 simulated data sets. A prediction-corrected VPC differs from a traditional VPC in that both observations and the model predictions are normalized for the typical model prediction in each bin of independent variables.

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**Conflict of Interest.** The authors declared no conflict of interest.
Effect of CYP3A4*22, CYP3A5*3, and CYP3A Genotype

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Cyclosporine, everolimus, and tacrolimus are primarily metabolized by CYP3A enzymes and are characterized by small therapeutic windows and highly variable PK. CYP3A45 genotype has previously been identified as a reliable determinant, and recently, CYP3A4*22 and CYP3A combined genotype (CYP3A44 and CYP3A5) were marked as potential determinants for tacrolimus PK, but their effect on everolimus and cyclosporine are not clarified.

WHAT QUESTION DID THIS STUDY ADDRESS?
Is CYP3A4*22 or CYP3A combined genotype suitable for predicting cyclosporine, everolimus, or tacrolimus dose requirements?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
The effect of CYP3A4*22 on cyclosporine, everolimus, and tacrolimus clearance is less than 20%, and therefore, dosing adjustments based on CYP3A4*22 are not indicated. CYP3A genotype clusters were significantly associated with cyclosporine and tacrolimus clearance; however, they do not further improve predictive performance.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS
These data incontestably demonstrate that CYP3A4*22 polymorphism is not indicated for predicting dose requirements. Moreover, CYP3A combined genotype does not further improve prediction of cyclosporine, everolimus, and tacrolimus dose requirements in renal transplant patients.

1. Kuypers, D.R., de Jonge, H., Naesens, M., Lerut, E., Verbeke, K. & Vanrenterghem, Y. CYP3A5 and CYP3A4 but not MDRI single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. Clin. Pharmacol. Ther. 82, 711–725 (2007).
2. Kovarik, J.M. et al. Everolimus Phase 2 Study Group. Longitudinal assessment of everolimus in de novo renal transplant recipients over the first post-transplant year: pharmacokinetics, exposure-response relationships, and influence on cyclosporine. Clin. Pharmacol. Ther. 89, 45–56 (2001).
3. Morse, G.D., Holdsworth, M.T., Verdu, R.C., Gerbasi, J. & Walsh, J.J. Pharmacokinetics and clinical tolerance of intravenous and oral cyclosporine in the immediate postoperative period. Clin. Pharmacol. Ther. 44, 654–664 (1988).
4. Kirchner, G.I., Meier-Wiedenbach, I. & Manns, M.P. Clinical pharmacokinetics of everolimus. Clin. Pharmacokinet. 43, 83–95 (2004).
5. Jacobsen, W., Serkova, N., Hausen, B., Morris, R.E., Benet, L.Z. & Christians, U. Comparison of the in vitro metabolism of the macrolide immunosuppressants sirolimus and RAD. Transplant. Proc. 33, 514–515 (2001).
6. Dai, Y. et al. Effect of CYP3A45 polymorphism on tacrolimus metabolic clearance in vitro. Drug Metab. Dispos. 34, 836–847 (2006).
7. Lown, K.S. et al. Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. Clin. Pharmacol. Ther. 62, 248–260 (1997).
8. Christians, U., Jacobsen, W., Benet, L.Z. & Lampen, A. Mechanisms of clinically relevant drug interactions associated with tacrolimus. Clin. Pharmacokinet. 41, 813–851 (2002).
9. Knops, N., Lerutchenko, E., van den Heuvel, B. & Kuypers, D. From gut to kidney: transporting and metabolizing calcineurin-inhibitors in solid organ transplantation. Int. J. Pharm. 452, 14–35 (2013).
10. Press, R.R. et al. Explaining variability in tacrolimus pharmacokinetics to optimize early exposure in adult transplant recipients. Drug. Ther. Monitor. 31, 167–197 (2009).
11. Santoro, A.B., Struchiner, C.J., Felipe, C.R., Tedesco-Silva, H., Medina-Pestana J.O. & Suarez-Kurtz G. CYP3A5 genotype, but not CYP3A4*1b, CYP3A4*22, or hemocrit, predicts tacrolimus dose requirements in Brazilian renal transplant patients. Clin. Pharmacol. Ther. 94, 201–202 (2013).
12. Zhao, W. et al. Population pharmacokinetics and pharmacogenetics of tacrolimus in de novo pediatric kidney transplant recipients. Clin. Pharmacol. Ther. 86, 609–618 (2009).
13. Thetwe, E. et al. Optimization of initial tacrolimus dose using pharmacogenetic testing. Clin. Pharmacol. Ther. 87, 721–728 (2010).
14. de Jonge, H., de Loor, H., Verbeke, K., Vanrenterghem, Y. & Kuypers, D.R. In vivo CYP3A4 activity, CYP3A4 genotype, and hematocrit predict tacrolimus dose requirements and clearance in renal transplant patients. Clin. Pharmacol. Ther. 92, 366–375 (2012).
15. Elens, L. et al. A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. Clin. Chem. 57, 1574–1583 (2011).
16. Gijsen, V.M. et al. CYP3A4*22 and CYP3A combined genotypes both correlate with tacrolimus disposition in pediatric heart transplant recipients. Pharmacogenomics 14, 1027–1036 (2013).
17. Elens, L., Bouamar, R., Hesselin, D.A., Hautriod, V., van Gelder, T. & van Schaik, R.H. The new CYP3A4 intron 6 C>T polymorphism (CYP3A4*22) is associated with an increased risk of delayed graft function and worse renal function in cyclosporine treated kidney transplant patients. Pharmacogenet. Genomics 22, 373–380 (2012).
18. Fanta, S. et al. Pharmacogenetics of cyclosporine in children suggests an age-dependent influence of ABCB1 polymorphisms. Pharmacogenet. Genomics 18, 77–80 (2008).
19. Moes, D.J., Press, R.R., de Fijter, J.W. & Kuypers, D.R. CYP3A5 variant allele frequencies in Dutch Caucasians. J. Clin. Pharmacol. 48, 325–329 (2008).
20. Yates, C.R. et al. The effect of CYP3A4 and MDRI polymorphic expression on cyclosporine oral disposition in renal transplant patients. J. Clin. Pharmacol. 43, 555–564 (2003).
21. van Schaik, R.H., van der Heiden, L.P., van den Aker, J.N. & Lindemans, J. CYP3A4 variant allele frequencies in Dutch Caucasians. Clin. Chem. 48, 1688–1671 (2002).
22. Kuypers, D.R., Claes, K., Evenepoel, P., Maes, B. & Vanrenterghem, Y. Long-term pharmacokinetic study of the novel combination of tacrolimus and sirolimus in de novo renal allograft recipients. Ther. Drug Monit. 23, 447–451 (2001).
23. Ato, J.E., Birnbaum, A.K. & Burger, R.C. Inherent correlation between dose and clearance in therapeutic drug monitoring settings: possible misinterpretation in population pharmacokinetic analyses. J. Pharmacokinet. Pharmacodyn. 32, 703–718 (2005).
24. Moes, D.J., Press, R.R., de Fijter, J.W., Guchelaar, H.J. Population pharmacokinetics and pharmacogenetics of everolimus in renal transplant patients. Clin. Pharmacokin. 51, 467–480 (2012).
25. Yates, C.R. et al. Differential influence of two cyclosporine formulations on everolimus pharmacokinetics: a clinically relevant pharmacokinetic interaction. J. Clin. Pharmacol. 49, 141–175 (2009).
26. Bergstrand, M., Hocker, A.C., Wallin, J.E. & Karlsson, M.O. Prediction correct visual predictive checks. <http://www.go-acop.org/acop2009/posters {ACOP .}> = 2009.
27. Murray, B., Hawes, L., Lee, R.A., Watson, R. & Roderer, M.W. Genes and beans: pharmacogenomics of renal transplant. Pharmacogenomics 14, 783–798 (2013).
28. Kovarik, J.M., Kalbag, J., Figueiredo, J., Roully, M., Frazier, O.L. & Rordorf, C. Differential influence of two cyclosporine formulations on everolimus pharmacokinetics: a clinically relevant pharmacokinetic interaction. J. Clin. Pharmacol. 42, 95–99 (2002).
29. Lemeshow, S., Moes, B.D., Verbeke, K. & Vanrenterghem, Y. CYP3A4 and P-glycoprotein activity in healthy controls and transplant patients on cyclosporin vs. tacrolimus vs. sirolimus. Am. J. Transplant. 4, 1514–1522 (2004).
30. Staatz, C.E., Goodman, L.K. & Tett, S.E. Effect of CYP3A4 and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: part I. Clin. Pharmacokinet. 49, 141–175 (2010).
31. Kang, D., Schwartz, J.B. & Verotta, D. Sample size computations for PK/PD population models. J. Pharmacokin. Pharmacodyn. 32, 685–701 (2005).
32. Bertrand, J., Comets, E., Lafont, C.M., Chenel, M. & Mentre, F. Pharmacogenetics and population pharmacokinetics: impact of the design on three tests using the SAEM algorithm. J. Pharmacokin. Pharmacodyn. 36, 317–339 (2009).
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32. Elens, L. et al. Impact of CYP3A4*22 allele on tacrolimus pharmacokinetics in early period after renal transplantation: toward updated genotype-based dosage guidelines. Ther. Drug Monit. 35, 608–616 (2013).
33. van Duijn, E.M., Boots, J.M., Christiaans, M.H., Stolke, L.M., Undre, N.A., van Hooff, J.P. Increase in tacrolimus trough levels after steroid withdrawal. Transpl. Int. 16, 721–725 (2003).
34. Kniepeiss, D. et al. The role of CYP3A5 genotypes in dose requirements of tacrolimus and everolimus after heart transplantation. Clin. Transplant. 25, 146–150 (2011).
35. Picard, N. et al. The role of CYP3A5 genotypes in dose requirements of tacrolimus and everolimus after heart transplantation. Clin. Transplant. 25, 146–150 (2011).
36. Press, R.R. et al. Explaining variability in ciclosporin exposure in adult kidney transplant recipients. Eur. J. Clin. Pharmacol. 66, 579–590 (2010).
37. Antignac, M., Barou, B., Fainiotti, R., Lechat, P. & Uren, S. Population pharmacokinetics and bioavailability of tacrolimus in kidney transplant patients. Br. J. Clin. Pharmacol. 64, 750–757 (2007).
38. Benkali, K. et al. Population pharmacokinetics and Bayesian estimation of tacrolimus exposure in renal transplant recipients on a new once-daily formulation. Clin. Pharmacokinet. 49, 683–692 (2010).
39. Botza, P.L. Pharmacokinetic-Pharmacodynamic Modeling and Simulation. (Springer Science and Business Media, New York, 2008).
40. Kovarik, J.M., Hsu, C.H., McMahon, L., Berthier, S. & Rordorf, C. Population pharmacokinetics of everolimus in de novo renal transplant patients: impact of ethnicity and comedication. Clin. Pharmacol. Ther. 70, 247–254 (2001).
41. Koster, R.J., van Benten, M., Beijnen, J.H., Schellens, J.H. & Huijten, A.D. Pirfla and PCluster: a modeling environment and cluster infrastructure for NONMEM. Comput. Methods Programs Biomed. 101, 72–79 (2011).
42. Karlsson, M.O. & Holford, N. A tutorial on visual predictive checks. PAGE. Abstracts of the Annual Meeting of the Population Approach Group in Europe. ISSN 1871–6032.