Pharmacokinetics and Pharmacodynamics of Once-daily Prolonged-release Tacrolimus in Liver Transplant Recipients

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ABSTRACT

Purpose: Limited published data are available regarding the pharmacokinetic (PK) and pharmacodynamic (PD) variables of prolonged-release tacrolimus (PRT) after liver transplantations. The goal of this study was to compare the PK and PD profiles of PRT in early and stable liver transplant recipients by developing a population PK model of PRT and investigating the profile of calcineurin activity (CNA) in the peripheral blood mononuclear cells.

Methods: A conversion from BID immediate-release tacrolimus (IRT) to once-daily PRT based on a one-to-one daily dose was performed at day 7 (D7) and D90 posttransplantation in groups A (n = 12) and B (n = 12), respectively. Extensive PK samplings, including whole-blood tacrolimus (TAC) concentration, and CNA assessments were performed at D14 and D104 in groups A and B, respectively. TAC concentration–time data (N = 221) were analyzed by using nonlinear mixed effects modeling.

Findings: A 2-compartment model with linear elimination and a delayed first-order absorption characterized by 2 transit compartments best described the PK data. Model-predicted dose-normalized (6.0 mg/d) area under the TAC concentration–time curve over the dosing interval in groups A and B was similar (geometric mean, 235.6 ng/mL · h [95% CI, 139.6–598.7] vs 224.6 ng/mL · h [95% CI, 117.6–421.5], respectively; $P = 0.94$). Area under the CNA versus time curve over the dosing interval did not differ between groups (4897 [3437] and 4079 [1008] pmol/min/10^6 cells; $P = 0.50$). In group A, trough CNA at D14 posttransplantation was statistically higher than that

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measured just before the switch to PRT (ie, D7 posttransplantation) (198 [92] vs 124 [72] pmol/min/10^6cells, n = 8; $P = 0.048$); no statistical difference in TAC concentration was observed ($P = 0.11$). In group B, no statistical difference between D90 and D104 was observed in either trough CNA (149 [78] vs 172 [82] pmol/min/10^6 cells, n = 6; $P = 0.18$) or TAC ($P = 0.17$) concentration. No graft rejection was observed in either of the groups.

Implications: This study suggests that one-to-one dosage conversion to once-daily PRT during the early posttransplantation period could result in significant CNA variations but without causing graft rejection. Further investigations in larger cohorts are warranted to confirm these results. ClinicalTrials.gov identifier: NCT02105155. (*Clin Ther*. 2019;41:882–896) © 2019 Published by Elsevier Inc.

Key Words: calcineurin activity, liver transplantation, pharmacokinetics, prolonged-release tacrolimus.

INTRODUCTION

Tacrolimus (TAC) is a key immunosuppressive agent for the prevention and treatment of allograft rejection in liver transplantation. [1] TAC binds with high affinity to FK-binding protein 12. [2] The drug-receptor complex specifically and competitively binds to and inhibits calcineurin, a calcium- and calmodulin-dependent phosphatase. This process inhibits the translocation of a family of transcription factors (NF-AT), leading to reduced transcriptional activation of cytokine genes such as interleukin (IL)-2 and thereby to a reduction in T-cell proliferation. [3] Because TAC has a narrow therapeutic range and its pharmacokinetics show a significant between-subject variability (BSV), close monitoring of whole-blood trough concentrations is required to avoid underexposure or overexposure. [4] Hence, therapeutic drug monitoring of TAC in liver transplant recipients is the benchmark method in this indication. [1] However, some liver transplant recipients with sufficient exposure to TAC nonetheless experience graft rejection, [5,6] suggesting that whole-blood trough concentration may not be the most appropriate surrogate marker of pharmacodynamic (PD) variables in these patients. Different approaches such as evaluation of TAC intracellular concentration in peripheral blood mononuclear cells (PBMCs) [7] or calcineurin activity (CNA) in PBMCs [6–9] could be helpful to overcome this issue in those patients. However, these approaches are not currently used for the clinical management of liver transplant recipients in daily clinical practice.

Liver transplant recipients are usually treated with BID immediate-release tacrolimus (IRT).* Nonadherence to treatment has been found to be a significant factor associated with graft rejection and graft loss. [10] Once-daily prolonged-release tacrolimus (PRT)† was developed to improve treatment adherence. The Phase III trial conducted in *de novo* liver transplant recipients found that both efficacy and safety profiles were similar between BID IRT and once-daily PRT. [11] The BID dosage of IRT usually shifts to once-daily PRT based on a one-to-one conversion (ie, same daily dose for IRT and PRT). The narrow therapeutic range and the significant BSV in the pharmacokinetics of TAC could result in significant variations in PD parameters in some patients, possibly leading to acute graft rejection within the early posttransplantation period. In this context, exploring both pharmacokinetic (PK) and PD profiles of once-daily PRT at the time of conversion becomes mandatory. However, the PK data of once-daily PRT in liver transplant recipients are sparse. A single-population PK study was conducted to investigate once-daily PRT pharmacokinetics in stable liver transplant recipients, [12] whereas another study using a standard noncompartmental approach characterized its PK profile during the early posttransplantation period. [13] In this context, a population PK study including data from the early and late posttransplantation periods could be interesting to better characterize the PK/PD relation of once-daily PRT in liver transplant recipients. Finally, to the best of our knowledge, the profile of CNA has not been investigated in PBMCs from liver transplant recipients treated with once-daily PRT.

The aim of the present study was to describe the pharmacokinetics of once-daily PRT by using a population approach and to characterize the CNA

* Trademark: Prograf® (Astellas Pharma Canada Inc, Markham, Ontario, Canada).

† Trademark: Advagraf® (Astellas Pharma Canada Inc).

profile in PBMCs in liver transplant recipients treated with once-daily PRT and included in the CONVERSION trial.

PATIENTS AND METHODS

Study Population and Treatment

The CONVERSION trial (ClinicalTrials.gov identifier NCT02105155) is a prospective, randomized, multicenter trial aiming to prove the noninferiority of the early conversion from IRT to PRT versus conversion at 3 months after liver transplantation. Eligible patients (aged >18 years) underwent liver transplantation at day 1 (D1) and started treatment with IRT. A conversion from IRT to PRT was performed at D7 and D90 after transplantation in groups A and B, respectively (Figure 1). The dosage of BID IRT shifted to once-daily PRT based on a one-to-one conversion (ie, same daily dose for IRT and PRT). After conversion, daily dosing was adjusted according to TAC whole-blood trough concentrations with a therapeutic range of 6−10 ng/mL. [1] All patients provided written informed consent. The protocol was approved by the Committee for the Protection of Persons and the French National Agency for Medicines and Health Products Safety.

Two hundred fifty liver transplant recipients were supposed to be included in the CONVERSION trial and 40 of them in the PK/PD study (n = 20 in each group). However, only 90 patients were included in the CONVERSION trial because of numerous simultaneous clinical trials ongoing. Furthermore, many patients refused to participate in the PK/PD study because of the lack of personal gain. In this context, PK and PD data were gathered from 24 patients included in the CONVERSION trial.

PK Data Collection

Extensive PK sampling was performed at D14 posttransplantation (ie, at D7 postconversion) in group A and at D104 posttransplantation (ie, at D14 postconversion) in group B (Figure 1). Blood samples (7 mL) were drawn before next administration (at trough), 0.33, 0.66, 1, 2, 3, 4, 6, 8, and 24 hours after drug intake. Blood samples were also collected right before next drug intake (trough concentration) at D5, D7, D14, D30, D90, and D180 posttransplantation in group A and at D90, D104, and D180 posttransplantation in group B. Whole-blood TAC concentrations were assayed by using an electrochemiluminescence immunoassay (ECLIA) method [14] on a Cobas 8000 (Roche Diagnostics, Meylan, France). The calibration range of the ECLIA method was 1−40 ng/mL with a limit of detection of 0.5 ng/mL. The intermediate precision and accuracy of the ECLIA method were below 8.1% and 5.1%, respectively, at 3 levels of concentrations (2.5, 10.4, and 19.8 ng/mL). The accuracy of our method was

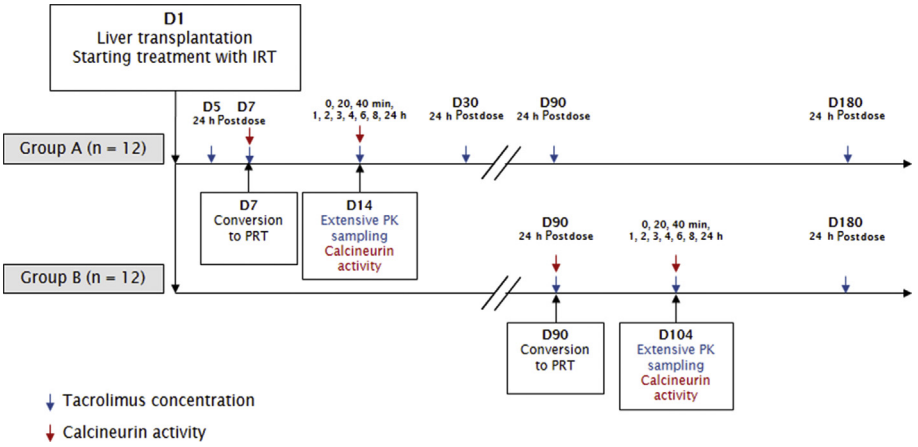

Fig. 1. Design of the pharmacokinetic/pharmacodynamic study in the CONVERSION trial. D = day; IRT = immediate-release tacrolimus; PK = pharmacokinetic; PRT = prolonged-release tacrolimus.

ensured by our participation in the TAC Proficiency Testing Scheme provided by the Cardiac and Vascular Sciences Analytic Unit of St. George's Hospital Medical School (D. Holt PharmD, PhD, London, United Kingdom).

At each follow-up visit, data on body composition and biological parameters were collected: body weight, lean body mass, hematocrit, glomerular filtration rate estimated according to the Cockcroft-Gault formula, alanine aminotransferase, aspartate aminotransferase, albumin, and bilirubin. Lean body mass was estimated according to the formula of McLeay *et al.* [15].

CNA in PBMCs

Trough CNA in PBMCs (just before drug intake) was assayed immediately before the switch to PRT (ie, at D7 and D90 posttransplantation for groups A and B, respectively) (Figure 1). Furthermore, CNA was assayed on the blood samples from extensive PK sampling (D14 for group A and D104 for group B) before next administration (at trough), 0.33, 0.66, 1, 2, 3, 4, 6, 8, and 24 hours after drug intake. For each blood sample, PBMC isolation was performed within 24 hours after blood collection. [16] First, granulocyte depletion was performed to prevent the influence of granulocytes on CNA. [17] For this purpose, the RosettSep kit was used according to the manufacturer's instructions (STEMCELL Technologies, Grenoble, France). Second, PBMCs were isolated by Ficoll density-gradient centrifugation (UNI-SEP Ficoll-tubes; Novamed, Jerusalem, Israel), then washed and counted with Xn-9000 (Sysmex, Villepinte, France). Each sample including 10^6 PBMCs was dried and frozen at −80°C up to analysis. CNA assay was run in duplicate as previously described. [16] Briefly, the PBMC lysates were incubated for 15 minutes at 30°C in analysis buffer including 50 mM Tris-HCl, pH 7.0, 0.1 M ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid, 0.5 mM dithiothreitol, 1 mM $MnCl_2$, 0.3 mg/mL bovine serum albumin, 0.1 mM ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid, 1 mM $CaCl_2$, 0.1 μM calmodulin, and 500 nM okadaic acid. The reaction was initiated by adding a 19 amino acid phosphopeptide (DLDVPIPGRFDRRVSVAAE; Bachem, Voisins-le-Bretonneux, France). Aliquots were sampled at 5 and 10 minutes. The reaction was stopped with 0.5% perchloric acid. Dephosphorylated peptide concentrations were determined by using HPLC coupled with ultraviolet detection. The chromatography system consisted of a Dionex Ultimate 300 equipped with a gradient pump with degas option and gradient mixer, an ultraviolet-visible detector, an autosampler, and a Chromeleon chromatography workstation (Dionex Corporation, Sunnyvale, California). The within-day precision of this method was 13.3%, including all the steps from blood collection to CNA assay. [16] CNA was expressed as picomole of formed dephosphorylated peptide per minute per 10^6 PBMCs (ie, pmol/min/10^6 cells).

Noncompartmental PK Analysis

Whole-blood concentrations of TAC from extensive PK sampling were used to calculate the area under the TAC concentration−time curve over the dosing interval (AUC_{TAC}) by using the trapezoidal rule.

Population PK Analysis

TAC concentration−time data were analyzed according to nonlinear mixed effects modeling by using NONMEM software version 7.4 (ICON Development Solutions, LLC, Ellicott City, Maryland) with Piraña (version 2.9.7) and PsN toolkit (version 4.7.0). Analyses were conducted with a first-order conditional estimation method with interaction. Data processing and plots were performed in R version 3.4.2 software (R Foundation for Statistical Computing, Vienna, Austria). Several structural models were used to fit the concentration−time data. First, 1- and 2-compartment models with first-order absorption and elimination were tested. Because TAC was administered as a prolonged-release formulation, a first-order process with either a lag time or transit compartments with an identical transfer rate constant (k_{tr}) were tested to account for the delay in the absorption phase. The inclusion of BSV and between-occasion variability defined as $OCC_1 \leq$ D28 and $OCC_2 >$ D28 for group A and $OCC_1 \leq$ D105 and $OCC_2 >$ D105 for group B was tested on all PK parameters according to an exponential model:

$$\Theta_i = \Theta_\mu \cdot \exp(\eta_i + \eta_{1i}OCC_1 + \eta_{2i}OCC_2)$$

where Θ_i is the estimate of the parameter for the ith subject, Θ_μ is the population mean estimate of the PK

parameter, η_i is the deviation from the mean for the ith subject with zero mean and variance ω^2, and η_{1i} and η_{2i} are the deviation from the mean for the first (OCC_1) and second (OCC_2) occasion for the ith subject, respectively. Correlation between η of PK parameters was tested by using a ω block structure. The residual unexplained variability was described by using a proportional error model. Model selection was based on the objective function value (OFV = –2 log-likelihood), using the likelihood ratio test to analyze for significant differences in goodness of fit between nested models. A drop of at least 3.84 points (χ [2] test, $\alpha = 5\%$, degree of freedom = 1) between hierarchical models was considered statistically significant. In addition, the plausibility of parameter estimates with their precision (expressed by percent relative SE), η-shrinkage value, and model stability were considered.

Covariate Analysis

The individual parameter estimates of the base model were used to investigate the correlations with biological and demographic variables. The following covariates were tested for their influence on clearance (CL): age, sex (0 for male and 1 for female), body weight, lean body mass, hematocrit, glomerular filtration rate, aspartate aminotransferase, alanine aminotransferase, albumin, bilirubin, and study group. Because PK data come from a large time period, different values of a covariate for the same patient were included in the data. Continuous covariates were tested according to the linear function:

$$CL = \Theta_{CL} \times (1 + \Theta_{cov} \times (cov - cov_{mean}))$$

where Θ_{CL} is the typical value of CL in the population, cov is the individual covariate value, cov_{mean} is the mean value of a covariate in the studied population, and Θ_{cov} is the fractional change in CL from the mean value of the covariate. Categorical covariates (sex and study group) were tested according to the following equation:

$$CL = \Theta_{CL} \times \Theta_{cov}^{COV}$$

where Θ_{cov} is the estimated influential factor for a covariate, and cov is 1 or 0. In the forward procedure, covariates were tested one-by-one, and a covariate was considered significantly associated with a PK parameter if its inclusion resulted in a drop in OFV of at least 3.84 points (χ^2 test, $\alpha = 5\%$, degree of freedom = 1). In the backward procedure, a full covariate model including the covariates significant in the forward procedure was built. A covariate remained in the final model if its removal resulted in an increase of at least 6.63 points (χ^2 test, $\alpha = 1\%$, degree of freedom = 1) compared with the full covariate model.

Model Evaluation

Diagnostic plots including population predictions (PRED) versus observed concentrations (DV), individual predictions (IPRED) versus DV, conditional weighted residuals versus PRED, and time after dose were generated. Because patients were treated with different doses of TAC, the model validation was performed with a prediction-corrected visual predictive check based on 1000 replicates of the original data set and presented as concentrations versus time after dose and stratified on study group to facilitate interpretation. Finally, 500 bootstrap analyses with resampling using the final model were performed.

Analysis of the Individual PK Parameters

The individual CL values obtained in NONMEM were used to calculate AUC_{TAC} according to the following formula in the model input file:

$$AUC_{ij} = DOSE_i \times F/CL_{ij}$$

where AUC_{ij} is the area under the concentration–time curve over the dosing interval for the ith subject and jth occasion, $DOSE_i$ is the administered dose for the ith subject, CL_{ij} is the individual clearance value for the ith subject and jth occasion, and F is the oral bioavailability of TAC (fixed in the model to 0.23 based on the literature). [18].

Statistical Analysis

The demographic and biological characteristics of the study cohort are presented as median [interquartile range]. PK data are expressed as geometric mean (95% CI), and PD data are expressed as mean (SD). The individual AUC_{TAC} values obtained by using noncompartmental analysis and the population approach were normalized by the median daily dose, which was administered before

extensive PK sampling. Individual AUC_{TAC} values obtained in the noncompartmental analysis were compared with model-predicted AUC_{TAC} values for groups A and B by using nonparametric Wilcoxon paired sample test. AUC_{TAC} data obtained by both noncompartmental analysis and the population approach were compared between groups A and B by using a Wilcoxon unpaired samples test. Because the number of patients per each study group is low, the ratio of the geometric means of $AUC_{TAC\ group\ A}$ over $AUC_{TAC\ group\ B}$, as well as its 90% CI, was calculated in addition to the nonparametric statistical tests to compare AUC_{TAC} between groups A and B.

From data of extensive PK sampling, individual 24-hour area under the CNA versus time curve (AUC_{CNA}) was calculated by using the trapezoidal rule. Only PD data from extensive PK sampling were used to investigate the PK/PD relation. The AUC_{CNA} were compared between groups A and B by using a Wilcoxon unpaired samples test. The relation between AUC_{TAC} and AUC_{CNA} was tested by using Spearman's correlation test. All tests were 2-sided, and they were considered significant when P values were <0.05. Computations were performed by using R software and SAS version 9 (SAS Institute, Inc, Cary, North Carolina).

RESULTS

Patients and TAC Concentrations

The baseline demographic and biological characteristics of 24 patients (n = 12 patients in each group) included in the study are summarized in Table I. Overall, 221 blood samples, including those from therapeutic drug monitoring, were available for the PK analysis. The median number of measurements per individual was 11 (range, 1–13). The sampling time was in the range 0.1–27 hours after drug intake. Four patients who did not have extensive PK sampling (n = 1 in group A and n = 3 in group B) withdrew their informed consent on the day of the analysis because they did not understand that a part of the study included several blood samples drawn throughout the day and that it required them to stay for a longer time in the medical department. For the remaining patients (n = 20), extensive PK sampling was performed at a median of 14 days (range, 13–21 days) and 104 days (range, 95–109 days) after transplantation in groups A and B, respectively. Figure 2 presents TAC concentrations

Table I. Baseline demographic and biological characteristics of groups A and B. Results are presented as median [interquartile] or median (range)

Characteristic	Group A (n = 12)	Group B (n = 12)
Sex (female/male)	3/9	1/11
Age, y	57 [53–60]	59 [54–62]
Body weight, kg	81 [69–86]	74 [67–81]
Lean body mass, kg	15 [13–16]	15 [13–15]
Biological data		
Hematocrit, %	31 [29–33]	38 [34–39]
GFR, mL/min	105 [70–117]	82 [54–91]
AST, UI/L	43 [16–69]	21 [17–25]
ALT, UI/L	85 [26–125]	11 [9–26]
Albumin, g/L	30 [28–34]	40 [36–47]
Bilirubin, µmol/L	24 [17–54]	8.0 [7–11]
Tacrolimus therapy		
Dose, mg/d*	7.0 (2.0–20.0)	5.0 (2.5–12.0)
Trough concentration, ng/mL*	8.5 [5.4–10.2]	5.6 [4.1–6.6]

ALT = alanine aminotransferase; AST = aspartate aminotransferase; GFR = glomerular filtration rate.
* Median value at the time of extensive pharmacokinetic sampling.

versus time after dose at D14 (n = 11) and D104 (n = 9) for groups A and B (data from extensive PK sampling only).

Noncompartmental PK Analysis

AUC_{TAC} values were calculated by using the trapezoidal rule for the 20 patients (n = 11 and n = 9 for groups A and B, respectively) for whom extensive PK data were available. The absolute AUC_{TAC} means obtained by using the noncompartmental analysis were similar between groups A and B (251.3 ng/mL · h [95% CI, 108.5−460.7] and 200.7 ng/mL · h [95% CI, 126.0−302.2], respectively; $P = 0.17$).

At the time of extensive PK sampling, the median dose of PRT was 7.0 mg/d and 5.0 mg/d in groups A and B, respectively; the median dose was 6.0 mg/d regardless of study group. The geometric means of dose-normalized AUC_{TAC} (6.0 mg/d) were similar in groups A and B (234.5 ng/mL · h [95% CI, 130.3−670.6] and 231.0 ng/mL · h [95% CI, 120.2−433.4]). The ratio of the geometric means of $AUC_{TAC\ group\ A}$ over $AUC_{TAC\ group\ B}$ was 1.01 [90% CI, 0.66−1.56] (Table II). The dose-normalized AUC_{TAC} obtained according to noncompartmental analysis was not statistically different between groups A and B ($P = 0.77$).

Population PK Analysis

TAC concentration−time data were described by using a 2-compartment model with linear elimination and a delayed first-order absorption characterized by 2 transit compartments with an identical k_{tr}. Addition of transit compartments to describe the absorption phase resulted in a significant improvement of the model fit: 1 transit compartment dropped OFV by 14 points and 2 transit compartments dropped OFV by 25 points compared with the model without delayed absorption. Further addition of a third transit compartment did not improve the model fit. The PK parameters of the final model were as follows: k_{tr}, CL, volume of distribution of the central compartment (V_c), intercompartmental clearance (Q), and volume of distribution of the peripheral compartment (V_p). The bioavailability (F) of TAC was fixed to the value previously reported in the literature (F = 0.23). [18] Therefore, the PK parameters (CL, V_c, Q, and V_p) were reported as absolute values. BSV was included on k_{tr}, CL, and Q. BSV could not be reliably estimated on V_c and V_p, and inclusion of BSV on F did not improve the model fit; thus, BSV was fixed to zero for these 3 parameters. The addition of covariance between η of the PK parameters did not improve the model fit. Finally, inclusion of between-occasion variability on CL resulted in a drop of 86

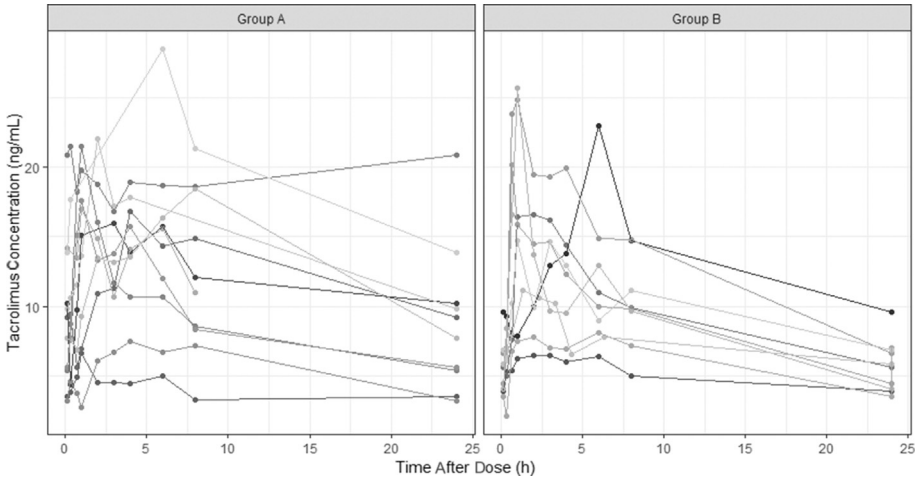

Fig. 2. Individual pharmacokinetic profiles of once-daily prolonged-release tacrolimus from extensive sampling day, corresponding to day 14 for group A (n = 11) and day 104 for group B (n = 9).

Table II. Comparison of area under the tacrolimus concentration−time curve over the dosing interval (AUC_{TAC}) obtained by noncompartmental analysis and a population pharmacokinetic approach

Variable	AUC_{TAC} (ng/mL · h)*		$P^{\dagger}$	$AUC_{TAC\ Group\ A}$/$AUC_{TAC\ Group\ B}$
	Geometric Mean [95% CI]			Ratio of Geometric Means [90% CI]
	Group A (n = 11)	Group B (n = 9)		
Noncompartmental	234.5 [130.3−670.6]	231.0 [120.2−433.4]	0.77	1.01 [0.66−1.56]
Model-predicted	235.6 [139.6−598.7]	224.6 [117.6−421.5]	0.94	1.05 [0.70−1.57]
$P^{\ddagger}$	0.90	0.25	NA	NA

NA = not available.
* Dose-normalized for median daily dose of 6.0 mg.
† Wilcoxon unpaired test comparing noncompartmental and model-predicted AUC between groups A and B.
‡ Wilcoxon paired test comparing noncompartmental and model-predicted AUC for each study group.

points in OFV and decreased the residual variability from 27.8% to 19.8%.

Covariate Analysis

The covariate analysis was performed on CL only as η_{ktr} showed significant deviation from a normal distribution (Shapiro-Wilk test, $P = 0.02$), and η_Q was associated with shrinkage of 35%. The correlation plots between individual CL of OCC_1 and OCC_2 and continuous covariates are presented in Supplemental Figure 1 (in the online version at doi:10.1016/j.clinthera.2019.03.006). Details regarding the lack of influence of sex and study group on CL are presented in Supplemental Figure 2 (in the online version at doi:10.1016/j.clinthera.2019.03.006). In the forward analysis, none of the tested covariates was significantly associated with CL (see Supplemental Table I in the online version at doi:10.1016/j.clinthera.2019.03.006), and thus the final model did not include covariates. The estimates of the final model with corresponding percent relative SE are presented in Table III.

Evaluation of the Final Model

Goodness-of-fit plots depicted in Figure 3 show no major bias of the model based on the IPRED versus DV plot, whereas conditional weighted residuals versus PRED and time after dose were homogeneously distributed around the zero line (although a slight bias at higher PRED values was observed). The prediction-corrected visual predictive check showed that the fifth and 95th percentiles and the median of the simulated data are in good agreement with the fifth and 95th percentiles and the median of the observed concentrations for both groups A and B (Figure 4). Finally, the mean estimates of the PK parameters from 500 bootstrap analyses are in accordance with those estimated by using the original data set (Table III).

Analysis of Individual PK Parameters

Model-predicted absolute AUC_{TAC} at extensive PK sampling (corresponding to OCC_1 for groups A and B) was not statistically different between groups A and B (252.4 ng/mL · h [95% CI, 111.3−510.5] vs 195.2 ng/mL · h [95% CI, 124.9−302.1], respectively; $P = 0.17$, n = 20). Table II presents model-predicted geometric means of AUC_{TAC} values normalized for a median dose of 6.0 mg/d. Dose-normalized AUC_{TAC} were not statistically different between groups A and B (235.6 ng/mL · h [95% CI, 139.6−598.7] and 224.6 ng/mL · h [95% CI, 117.6−421.5] ng/mL · h; $P = 0.94$), and the ratio of the geometric means of $AUC_{TAC\ group\ A}$ over $AUC_{TAC\ group\ B}$ was 1.05 (90% CI, 0.70−1.57). Finally, the comparison of AUC_{TAC} values obtained either by noncompartmental analysis or by population approach showed that both values were similar ($P = 0.90$ and $P = 0.25$ for groups A and B), further validating our PK model.

Table III. Mean pharmacokinetic parameter estimates obtained from the final model and from 500 bootstrap runs with resampling

Parameter	Mean Estimate (%RSE) [Shrinkage]	Bootstrap Mean (95% CI)
k_{tr}, h^{-1}	2.19 (19.7%)	2.14 (1.37–2.92)
CL, L/h	5.09 (8.2%)	5.11 (4.36–5.93)
V_c, L	93.5 (41.0%)	86.9 (54.1–126)
Q, L/h	42.0 (46.2%)	43.1 (20.2–71.9)
V_p, L	135 (21.0%)	142 (88.4–196)
F (fixed)	0.23	0.23
Between-subject variability		
k_{tr} (%CV)	94.5% (22.1%) [13.3%]	80.6% (51.6–111)
CL (%CV)	34.7% (31.5%) [26.1%]	33.8% (13.5–48.4)
Q (%CV)	151% (37.6%) [34.6%]	120% (67–170)
Between-occasion variability *		
CL (%CV)	39.8% (21.3%)	36.2% (22.8–46.6)
Proportional error (%)	19.8% (8.6%) [14.3%]	18.7% (16.0–21.1)

CL = clearance; F = bioavailability; k_{tr} = transfer rate constant between transit compartments; Q = intercompartmental clearance; RSE = relative SE; V_c = volume of distribution of the central compartment; V_p = volume of distribution of the peripheral compartment.
* Occasions (OCC) defined as: OCC1 $\leq$ 28 days and OCC2 > 28 days (group A); OCC1 $\leq$ 105 days and OCC2 > 105 days (group B).

PRT PD Values

Figure 5 presents the individual CNA profile (log scale) over the dosing interval at D14 for group A (n = 11) and D104 for group B (n = 9). The AUC_{CNA} means were not statistically different between groups A and B (4897 [3437] and 4079 [1008] pmol/min/10^6 cells, respectively; Wilcoxon unpaired *t* test, $P = 0.50$). However, a larger BSV in AUC_{CNA} was observed in group A (70.2% vs 24.7% for groups A and B). No relation was found between AUC_{CNA} and either model-predicted absolute AUC_{TAC} (rho coefficient, $\rho = 0.26$ [95% CI, −0.20 to 0.63]; $P = 0.25$) (Figure 6) or TAC whole-blood trough concentration (rho coefficient, $\rho = 0.20$ [95% CI,−0.27 to 0.59]; $P = 0.39$). The mean trough CNA activity (just before TAC intake) at D14 posttransplantation in group A was statistically higher than that measured just before the switch to PRT (ie, D7 posttransplantation) (198 [92] vs 124 [72] pmol/min/10^6cells, n = 8, respectively; paired *t* test, $P = 0.048$), whereas no statistical difference was observed for TAC whole-blood trough concentration (6.9 [2.3] vs 10.1 [5.4] ng/mL; paired *t* test, $P = 0.11$). Finally, no statistical difference between D90 and D104 was observed for either trough CNA (149 [78] vs 172 [82] pmol/min/10^6cells, n = 6; paired *t* test, $P = 0.18$) or TAC whole-blood trough concentration (8.8 [4.6] vs 5.9 [2.1] ng/mL; paired *t* test, $P = 0.17$). Finally, no graft rejection was observed in either group.

DISCUSSION

PRT is approved by the European Medicines Agency for use in the context of liver transplantations. However, there are limited published data regarding the PK and PD parameters of PRT in this indication. To the best of our knowledge, the present study is the first to assess the PK profile of PRT within the early and late posttransplantation periods by using a population approach. Furthermore, it provides new insights regarding the profile of CNA in PBMCs from liver transplant recipients treated with PRT.

In the population PK analysis, blood concentration–time data of once-daily PRT were described by using a 2-compartment model with

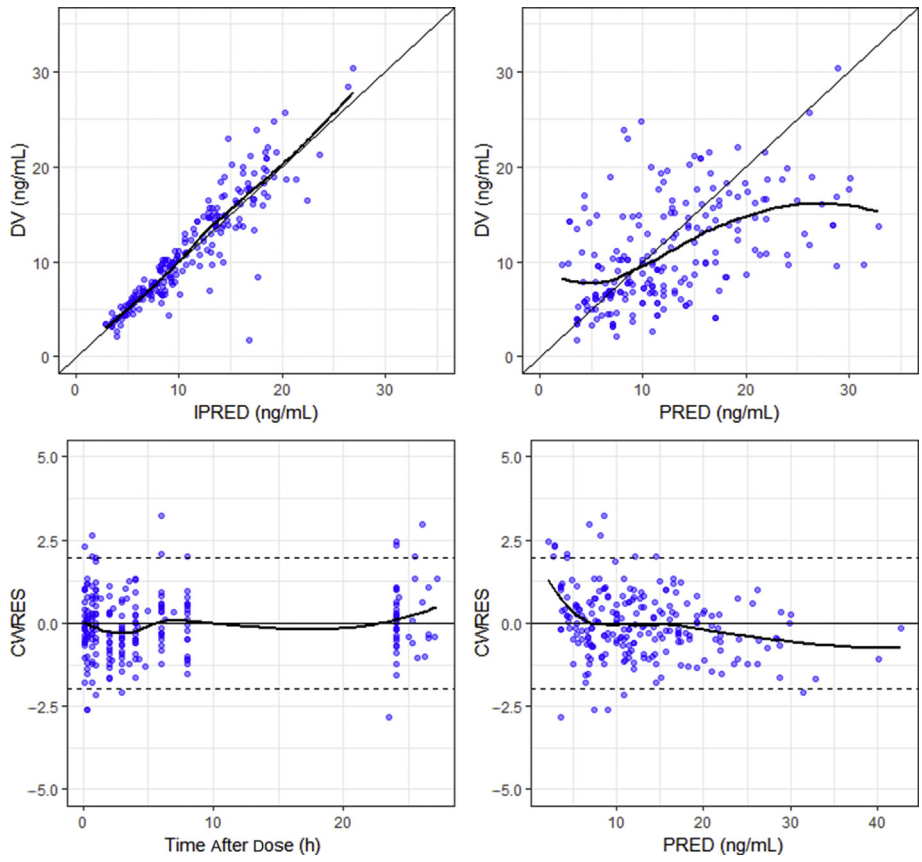

Fig. 3. Goodness-of-fit plots of the final model. CWRES = conditional weighted residuals; DV = observed concentrations; IPRED = individual predictions; PRED = population predictions.

delayed absorption characterized by 2 transit compartments. This model is consistent with a previous population PK study reported by Moes et al [12] in which a 2-compartment model with 3 transit compartments was used to characterize the PK profile of once-daily PRT in 66 stable liver transplant recipients. The mean estimate of CL in our analysis was 5.1 L/h (BSV, 34.7%), which is close to the value reported by Moes et al (4.77 L/h; BSV, 45.4%). The analysis of the demographic and biological covariates on CL did not allow us to identify any significant correlations. This may be due to small sample size and the small dispersion of the covariates in our study. Nevertheless, in stable liver transplant recipients treated with PRT, Moes et al did not report any significant influence of the covariates that we tested on total CL.

It has been reported that the expression of *CYP3A5*1*, both in donor and receiver of a liver transplant, significantly increases CL of TAC in patients treated with PRT. [12] Other studies conducted in kidney transplant recipients treated with PRT also reported the influence of *CYP3A5*1* on CL. [19,20] We could not confirm or contradict these results because pharmacogenetic data were not available in our study. It was decided not to conduct an analysis of *CYP3A5*1* genotype in the CONVERSION study because the frequency of *CYP3A5*1* genotype in the French population is low (13%) [21] and, because the study included a small number of patients, the statistical power would not have been sufficient to draw any firm conclusion. Similarly, using a mixture model in the PK population analysis to identify the subpopulation carrying the

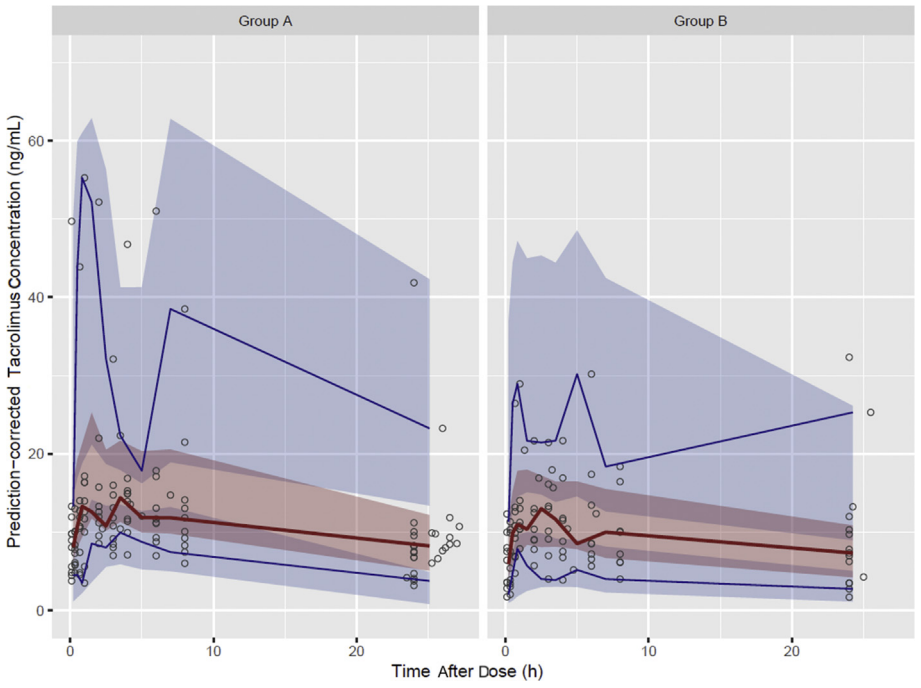

Fig. 4. Prediction-corrected visual predictive check stratified on study group based on 1000 replicates of the original data set using the final model. The blue lines represent the 5th and 95th percentiles of the observed concentrations, the red lines represent the median of the observed concentrations, the blue areas represent the 95% CIs around the 5th and 95th percentiles of the simulated concentrations, the red areas represent the 95% CIs around the median of the simulated concentrations, and the points represent observed concentrations.

*CYP3A5*1* allele would not have been possible. Regarding the genetic polymorphisms of drug transporters such as MDR1, although its influence on TAC PK has been reported, the results remain controversial. [22] In the same way as for the *CYP3A5*1* genotype, our study could not contribute any results regarding the impact of genetic polymorphisms of drug transporters on TAC pharmacokinetics because of the lack of statistical power.

To further evaluate the validity of our model, the individual AUC_{TAC} values obtained by using a population approach were compared with those obtained by using a noncompartmental analysis with data from extensive PK sampling. AUC_{TAC} means of groups A and B obtained by using either a noncompartmental or a population approach were not statistically different ($P = 0.90$ and $P = 0.25$ for groups A and B, respectively). Furthermore, comparison of AUC_{TAC} values obtained by both approaches showed no statistical differences between groups A and B ($P = 0.77$ and $P = 0.94$). Finally, the geometric means of model-predicted dose-normalized AUC_{TAC} in our study (235.6 ng/mL · h [95% CI, 139.6−598.7] and 224.6 ng/mL · h [95% CI, 117.6−421.5] for groups A and B normalized to a median dose of 6.0 mg/d) are close to those previously reported in liver transplant recipients obtained by using a noncompartmental approach. [23] Indeed, in that study, Florman et al reported a mean AUC_{TAC} of 184 (63) ng · h/mL at day 28 posttransplantation in liver transplant recipients treated with PRT (mean dose, 5.2 mg/d). Taken together, these results suggest that the developed model satisfyingly describes the TAC concentration−time data. However, the limitation of

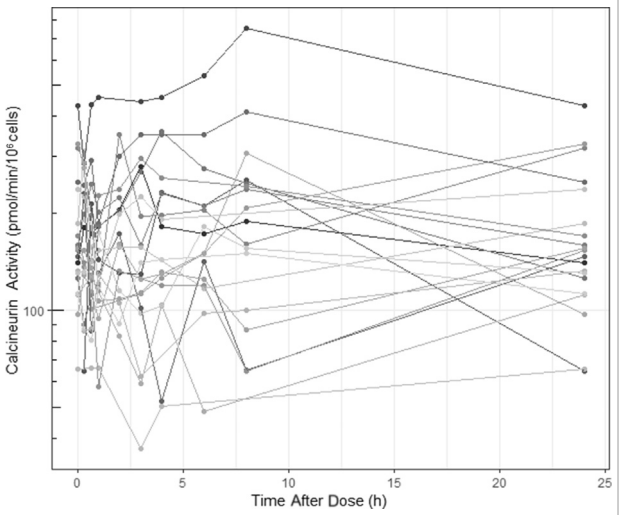

Fig. 5. Individual calcineurin activity profile over the dosing interval at day 14 for group A (n = 11) and day 104 for group B (n = 9).

our PK analysis is the small number of patients. Our results are therefore not conclusive and need to be confirmed in larger cohorts.

Moreover, some individual PK profiles in our study exhibit a second peak of absorption. This finding was previously observed in liver and kidney transplant recipients treated with a different PRT formulation[24,25],‡ and was described by using a double-gamma absorption model. In our analysis, the attempts to describe the second absorption peak did not provide a reliable estimation of the PK parameters, probably due to an insufficient number of samples in the absorption phase or the fact that it was only observed in some patients. In addition, the low number of PK samples in the absorption and distribution phases might be the reason for high BSV on k_{tr} and Q. Although we analyzed the PK data with a model that did not account for the second peak of absorption, the comparison of AUC_{TAC} values obtained with the noncompartmental approach and predicted by using the PK model were in good agreement for both study groups, which shows that our model accurately described the data.

CNA is a surrogate marker of TAC pharmacodynamics. Various PK/PD studies conducted in liver transplant recipients have suggested that assessment of CNA within the early posttransplantation period could be helpful to predict acute graft rejection in patients well exposed to TAC. [6,7] In the present study, no relation was found between AUC_{TAC} and AUC_{CNA} values regardless of the moment of conversion from IRT to PRT, as previously reported. [6–8] Different factors, such as the amount of cytosolic FKBP12 [26] and FKBP13, FKBP51 acting as a reservoir, [2] the genetic polymorphism of the calcineurin catalytic subunit α, [27,28] and the etiology of liver disease before transplant, [29] might significantly influence the CNA in PBMCs regardless of the whole-blood TAC concentration. In addition, Lemaitre et al [7] showed that CNA in PBMCs was not further associated with intracellular concentrations of TAC in liver transplant recipients, which supports our result. The BSV in AUC_{CNA} for group A is in accordance with that reported at D7 and D14 posttransplantation in liver transplant recipients treated with BID IRT. [6–8] However, it was 3-fold higher compared with the BSV in AUC_{CNA} for group B (70.2% vs 24.7%, respectively), whereas AUC_{CNA} means were not statistically different in either group. In addition, absolute AUC_{TAC} values were similar between groups A and B ($P = 0.17$) which altogether suggests that factors other than drug exposure contribute to this variability.

Although patients' characteristics regarding immunophilins (FKBP12, 13, and 51) were probably different between groups, the magnitude of immune response during the early posttransplantation period might also contribute to the large BSV in AUC_{CNA}. In addition, our study shows that the conversion from IRT to PRT in a 1:1 ratio based on total milligram per day dose could also contribute to this variability. Interestingly, trough CNA at D14 in group A was statistically higher than that measured just before the switch to PRT ($P = 0.048$), whereas no difference in TAC whole-blood trough concentration was observed. Furthermore, neither trough CNA nor TAC whole-blood trough concentration at D90 and D104 was different in group B.

Finally, no graft rejection was observed in the present PK/PD study regardless of study group. Although the

‡ Trademark: Envarsus® (Veloxis Pharmaceuticals, Inc, Cary, North Carolina).

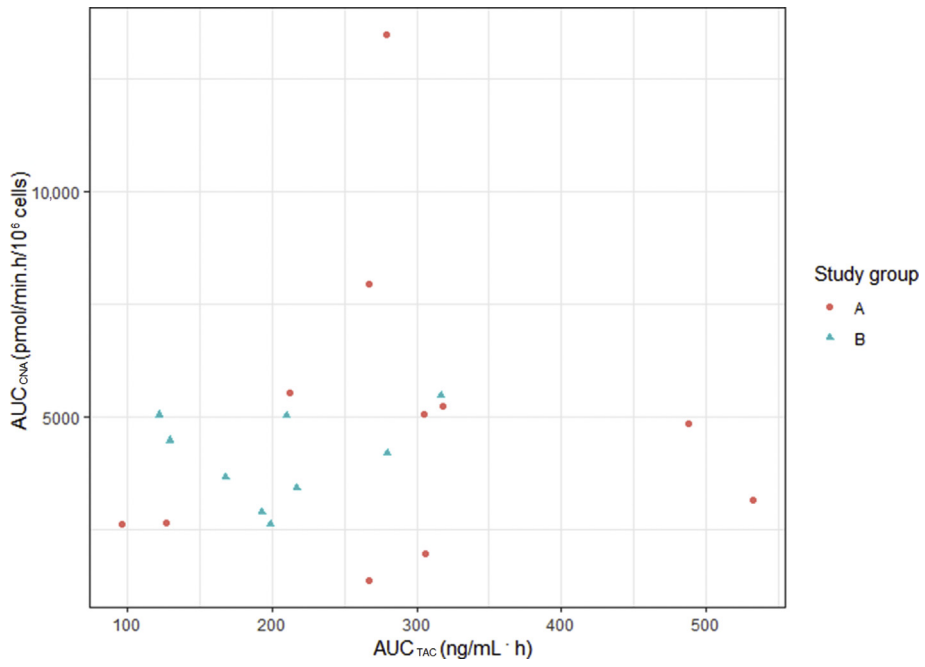

Fig. 6. Relationship between area under the tacrolimus concentration—time curve over the dosing interval (AUC_{TAC}) and the 24-hour area under the calcineurin activity curve (AUC_{CNA}) in liver transplant recipients treated with once-daily prolonged-release tacrolimus. Calcineurin activity (CNA) is expressed for 10^6 cells.

number of patients was limited, these results suggest that the conversion from IRT to PRT during the early posttransplantation period could modify the PD profile of calcineurin without causing graft rejection. Further investigations with a larger cohort of patients should be conducted to confirm this result.

CONCLUSIONS

We developed a population PK model for PRT to evaluate the PK/PD relationship for TAC in early and stable liver transplant recipients. The results suggest that one-to-one dosage conversion from BID IRT to once-daily PRT during the early posttransplantation period could modify CNA in PBMCs, which might not be related to the TAC PK profile. The advantage of our study is the PK and PD comparison between early and stable transplant recipients. Using both a noncompartmental analysis and a population approach, we showed that the mean AUC_{TAC} values between groups A and B were not statistically significantly different. Therefore, the model we developed can be used to predict TAC whole-blood concentrations in liver transplant recipients under the same conditions and dosing regimen as specified in our study. However, because the sample size in the present study is low, our results should first be confirmed in larger cohorts.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article. Sponsors were not involved in Methodology, interpretation of the results and discussion.

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Drs. Conti, Calmus, and Blanchet were responsible for conceptualization; Drs. Allard, Puszkiel, Conti, Noé, Kamar, Thomas-Schoemann, Vidal, Calmus, and Blanchet were responsible for investigations; Drs. Allard, Puszkiel, Chevillard, Blanchet, and White-Koning performed data analysis; Dr. Simon was responsible of methodolgogy and project management; and Drs. Puszkiel, Chevillard, Calmus, and Blanchet were responsible for writing the original draft of the manuscript. All authors reviewed and edited the manuscript.

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SUPPLEMENTARY TABLE S1

Results of the covariate analysis using the base model (forward step). Continuous covariates were tested according to the linear function.

Parameter	Mean estimate (%RSE)											
	Base model	BW on CL [a]	AGE on CL	LBM on CL	HT on CL	GFR on CL [a]	AST on CL	ALT on CL	BIL on CL [a]	ALB on CL [a]	GRP on CL	SEX on CL
OFV	704.622	704.512	704.442	704.618	704.539	704.625	704.612	704.060	704.622	704.622	704.181	704.614
ΔOFV [b]		-0.110	-0.180	-0.004	-0.083	0.003	-0.010	-0.562	0.020	0.004	-0.441	-0.008
k_{tr} (h^{-1})	2.19 (20%)	2.19	2.19 (27%)	2.19 (29%)	2.19 (35%)	2.19	2.19 (37%)	2.18 (24%)	2.19	2.19	2.18 (33%)	2.19 (28%)
CL (L/h)	5.09 (8%)	5.10	5.09 (11%)	5.09 (11%)	5.07 (15%)	5.09	5.10 (13%)	5.14 (12%)	5.09	5.09	5.41 (11%)	5.07 (15%)
V_c (L)	93.5 (41%)	93.7	93.8 (38%)	93.6 (52%)	93.4 (19%)	93.5	93.4 (21%)	92.8 (41%)	93.5	93.5	92.7 (61%)	93.4 (61%)
Q (L/h)	42.0 (46%)	41.9	41.9 (25%)	42.0 (29%)	42.3 (55%)	42	42.1 (47%)	42.2 (64%)	42.0	42.0	42.4 (54%)	42.1 (72%)
V_p (L)	135 (21%)	135	135 (18%)	135 (36%)	135 (21%)	135	135 (22%)	136 (15%)	135	135	136 (45%)	135 (46%)
F (fixed)	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Covariate effect		0.0023	0.00612 (274%)	0.00363 (300%)	0.00607 (221%)	0.000001	0.000268 (1530%)	0.000755 (50%)	0	0.000001	0.881 (23%)	1.02 (25%)
Between-subject variability												
k_{tr} (CV%)	94.5% (22%)	94.6%	94.6% (21%)	94.5% (21%)	94.4% (26%)	94.5%	94.5% (27%)	94.4% (22%)	94.5%	94.5%	94.4%	94.5% (22%)
CL (CV%)	34.7% (31%)	34.1%	33.9% (42%)	34.7% (34%)	36.2% (58%)	34.7%	35.1% (59%)	35.4% (32%)	34.7%	34.7%	35.1% (30%)	34.9% (33%)
Q (CV%)	151% (38%)	150%	150% (31%)	151% (28%)	151% (47%)	151%	151% (46%)	150% (44%)	151%	151%	152% (33%)	151% (36%)
Between-occasion variability [c]												
CL (CV%)	39.8% (21%)	40.1%	40.1% (24%)	39.9% (23%)	38.9% (45%)	39.8%	39.6% (42%)	38.2% (24%)	39.8%	39.8%	39.2% (23%)	39.8% (36%)
Proportional error (%)	19.8% (9%)	19.7%	19.7% (9%)	19.7% (9%)	19.7% (7%)	19.7%	19.7% (10%)	19.7% (9%)	19.7%	19.7%	19.7% (10%)	19.7% (9%)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; BIL, bilirubin; BW, body weight; CI, confidence interval; CL, clearance; CV, coefficient of variation; GFR, glomerular filtration rate; F, bioavailability; HT, hematocrit; LBM, lean body mass; ktr, transfer rate constant; OFV, objective function value; Q, inter-compartmental clearance; RSE, relative standard error; Vc, volume of distribution of the central compartment; Vp, volume of distribution of the peripheral compartment

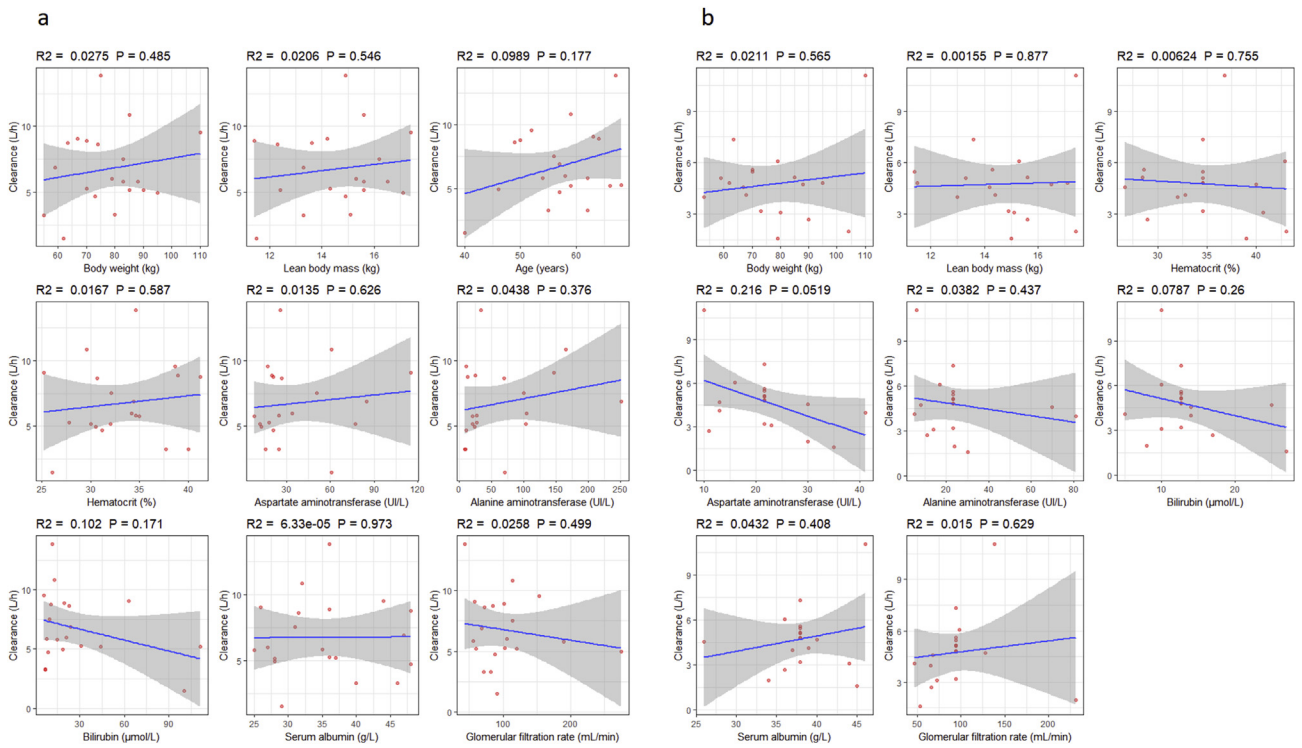
[a] model convergence was not obtained

[b] ΔOFV with the base model

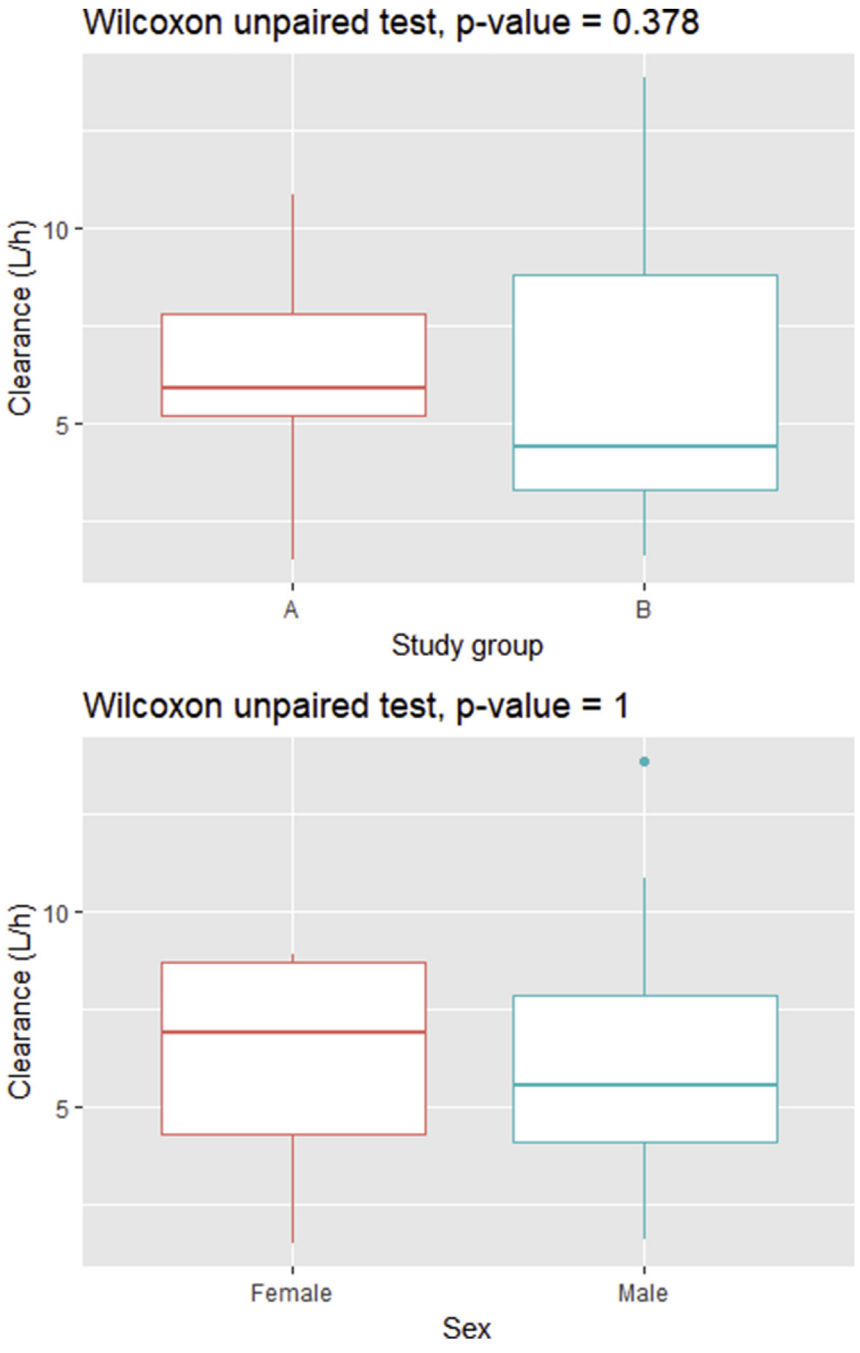
[c] occasions (OCC) defined as: OCC1 <= 28 days and OCC2 > 28 days (group A); OCC1 <= 105 days and OCC2 > 105 days (group B)

APPENDIX A. SUPPLEMENTARY DATA

The following are the supplementary data to this article:



Supplementary Fig. S1. (a) Correlation plots between individual absolute clearance (CL) obtained from the population approach and continuous covariates at the first pharmacokinetic occasion ($OCC_1 \leq 28$ days for group A and $OCC_1 \leq 105$ days for group B); (b) Correlation plots between individual absolute clearance (CL) obtained from the population approach and continuous covariates at the second pharmacokinetic occasion ($OCC_2 > 28$ days for group A and $OCC_2 > 105$ days for group B).



Supplementary Fig. S2. Box-plots for individual absolute clearance (CL) obtained from the population approach and categorical covariates.