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ORIGINAL ARTICLE

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Population pharmacokinetics and genetics of oral meltdose tacrolimus (Envarsus) in stable adult liver transplant recipients

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Aims: Meltdose tacrolimus (Envarsus) is marketed as a formulation with a more consistent exposure. Due to the narrow therapeutic window, therapeutic drug monitoring is essential to maintain adequate exposure. The primary objective of this study was to develop a population pharmacokinetic (PK) model of Envarsus among liver transplant patients and select a limited sampling strategy (LSS) for AUC estimation. The secondary objective was to investigate potential covariates including CYP3A/IL genotype suitable for initial dose optimization when converting to Envarsus.

Methods: Adult liver transplant patients were converted from prolonged release tacrolimus (Advagraf) to Envarsus and blood samples were obtained using whole blood and dried blood spot sampling. Subsequently the population PK parameters were estimated using nonlinear-mixed effect modelling. Demographic factors, and recipient and donor CYP3A4, CYP3A5, IL-6, -10 and -18 genotype were tested as potential covariates to explain interindividual variability.

Results: Fifty-five patients were included. A 2-compartment model with delayed absorption was the most suitable to describe population PK parameters. The population PK parameters were as follows: clearance, 3.27 L/h; intercompartmental clearance, 9.6 L/h; volume of distribution of compartments 1 and 2, 95 and 500 L, respectively. No covariates were found to significantly decrease interindividual variability. The best 3-point LSS was t = 0,4,8 with a median bias of 1.8% (−12.5–12.5).

Conclusions: The LSS can be used to adequately predict the AUC. No clinically relevant covariates known to influence the PK of Envarsus, including CYP3A status, were identified and therefore do not seem useful for initial dose optimization.

KEYWORDS
population pharmacokinetics, tacrolimus, therapeutic drug monitoring

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Bart van Hoek and Dirk Jan A.R. Moes Shared senior authorship.

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1 | INTRODUCTION

The introduction of the calcineurin inhibitors cyclosporine and tacrolimus has improved survival of patients after liver transplantation dramatically.[1,2] Tacrolimus inhibits the T-cell mediated immune response by blocking the synthesis of interleukin (IL) and is now the cornerstone in the immunosuppressive regimen post-liver transplantation. Shortly after transplantation, in most centres tacrolimus is combined with induction therapy with the IL-2-receptor antagonist basiliximab and prednisone. In the long term, tacrolimus is frequently combined with mycophenolate mofetil or other immunosuppressants and the dose is reduced to decrease the risk of renal toxicity.

Tacrolimus has a large interindividual variability (IIV) in pharmacokinetics (PK). This variability can be partly explained by genetic differences in metabolizing capacity of enzymes present in the epithelial wall of the gut and in the liver, i.e. cytochrome P450 enzymes CYP3A4 and CYP3A5, impacting first-pass metabolism and thereby bioavailability.[3,4] Drug–drug interactions, food intake and clinical conditions such as diarrhoea also influence the exposure to tacrolimus.[5] Furthermore evidence is accumulating that proinflammatory cytokines are able to down-regulate CYP enzymes.[6,7] IL levels and IL-10, IL-6, IL-18 and tumour necrosis factor-α polymorphisms have been associated with altered tacrolimus PK.[7–9] In contrast to this large PK variability, the therapeutic window of tacrolimus is relatively small; while subtherapeutic exposure increases the risk of graft rejection, supratherapeutic exposure may lead to side effects such as renal toxicity, diabetes, leukopenia and tremors.[10] To achieve adequate exposure early after transplantation, transplant recipients can be genotyped for CYP3A5 polymorphisms in order to adjust the initial starting dose. Further dose optimization is guided by therapeutic drug monitoring (TDM). It is well known that the area-under the concentration–time curve (AUC) is the best link between exposure and effect, superior to trough concentration measurements.[11,12] Nevertheless most centres use trough levels as it is less complicated to perform. A patient-friendly alternative to full AUC measurements, which requires intensive sampling, is a limited-sampling strategy (LSS), based on 3 or 4 time points.[5,13]

Since the introduction of tacrolimus (Prograft) in 1996, various formulations of tacrolimus have been developed, including once-daily regimens (Advagraf, Envarsus, Dailiport), all resulting in different exposure profiles and requiring a formulation-specific LSS. Once-daily meltdose tacrolimus (Envarsus) was developed to increase bioavailability and is being marketed as a formulation with a more consistent exposure due to lower peak-to-trough fluctuations.[14] A first study on the PK of Envarsus among liver and kidney-transplant patients revealed an LSS of t = 0, 4, 8 and 12 hours performed best[15]; however, a more narrow time-frame of sampling would be more feasible in clinical practice and more patient friendly. As this is the only published study so far, the PK properties of this formulation remain relatively unknown. Secondly the impact of genetic polymorphisms is still unclear and could differ from other tacrolimus formulations. Envarsus might have a more prolonged release in the gastrointestinal tract and the expression of metabolizing enzymes decrease towards the more distal parts of the gut.[16]

What is already known about this subject

- Tacrolimus has a small therapeutic window and requires regular therapeutic drug monitoring to individualize the dose. Each formulation requires a specific limited-sampling strategy to minimize blood sampling for AUC measurement.

What this study adds

- This is the first study describing the both the population PK and pharmacogenetics of tacrolimus meltdose in stable liver transplant recipients using nonlinear mixed effects modelling who were converted from Advagraf to tacrolimus meltdose.
- Both 3- and 4- point limited sampling models can be used to accurately predict the AUC_{0-24} in routine clinical care.
- Genetic variability in CYP3A enzymes was of little impact on PK of meltdose tacrolimus in this population.
- The final model will be made publicly available in the commercial model informed precision dosing tool InsightRx.

The primary objective of this study was to develop a population PK model of Envarsus in stable adult liver transplant patients and select an LSS based on maximum accuracy and precision using a maximum a posterori (MAP) AUC estimation. The secondary objective was to explore potential covariates, such as genetic polymorphisms, suitable for initial dose individualization when converting to Envarsus. Additionally, we aimed to implement the model into a point-of-care dosing software (InsightRX Nova), to increase the likelihood that the results of this study are taken up into clinical practice.

2 | METHODS

The study was conducted in compliance with the Declaration of Helsinki and approved by the ethics committee of the Leiden University Medical Center (protocol ID P16.321). The genotyping of donor and recipient was approved separately (protocol ID B19.023). The trial was registered at the Dutch National Trial Registry (www.trialregister.nl), number NTR 6976. All patients gave written informed consent.

2.1 | Study design

This study was an open-label, prospective, PK evaluation study. Stable adult liver transplant patients were eligible in case the following inclusion criteria were met: recipient of a liver transplant at least 6 months prior to entry into the study; age between 18 and 70 years; an Advagraf-based immunosuppressive regimen for at least 3 months with an unchanged dose for at least 2 months prior to enrolment; stable graft function; no infections or other complications at the moment of inclusion into the study. Exclusion criteria included: infections or other complications during inclusion; a direct bilirubin >10 μmol/L or albumin level outside the clinical reference range; allergy or hypersensitivity to tacrolimus; estimated glomerular filtration rate <30 mL/min at time of screening; unstable dosing, and the concomitant use of medications known to affect the PK of tacrolimus at inclusion.

Included patients were converted from prolonged release tacrolimus (Advagraf) after limited sampling AUC measurement (t = 0,2,3 h) to Envarsus using a conversion ratio of 1:0.7.[17] Subsequent doses were adjusted based on patient-specific target whole blood trough concentrations.

2.2 | PK sampling and bioanalytical analysis

For the evaluation of the PK of Envarsus 2 additional AUCs and 1 trough concentration of tacrolimus was measured in addition to routine clinical care. Two weeks after conversion, a full AUC measurement (t = 0,1,2,3,4,6,8,12,24 h) was performed and an abbreviated AUC (t = 0,4,8,12 h) was performed at 3 months after conversion. Samples were measured with a validated LC–MS/MS method[18] using whole blood samples for t = 0,1,2,3,4,6 hours of the full AUC measurement and dried blood spots (DBS) sampling for t = 8,12,24 hours and the abbreviated curve. In case a third AUC was performed for clinical care, this AUC was also included in the modelling process. Figure 1 gives an overview of the inclusion and sampling schedule.

Demographic factors including gender, age, ethnicity, weight, length, primary diagnosis, interacting co-mediation (corticosteroids, azole antifungal agents, rifampicin, dihydropyridins) and basic clinical chemistry (haematocrit, haemoglobin, bilirubin, albumin, liver enzymes and creatinine) were collected. In addition, recipient and donor CYP3A4*22, CYP3A5*3, IL-6, -10 and-18 genotype were determined as variability in these genes have been associated with the PK of tacrolimus.[3,4,7–9]

2.3 | Genotyping assays

DNA was isolated from EDTA blood of liver transplant recipients and spleen or liver tissue from donors. Genotyping of donor and recipient of CYP3A5*3 (rs776746), CYP3A4*22 (rs35599367), IL-18 (rs5744247), IL-6 (rs1800796) and IL-10 (rs1800871) was performed by a TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA), independently and without knowledge of the patient data. These variants are widely recognized.[19,20] Assays were used according to the manufacturer's instructions and performed on 10 ng genomic DNA. Fluorescence detection and genotype calling were performed using the QuantStudio 12 K Flex system (Thermo Fisher Scientific, Bleiswijk, the Netherlands).

To evaluate the effect of the combinations of the CYP3A genotypes of recipients and donors, the following combinations were made for CYP3A4: if recipients and donor are CYP3A4*22 noncarriers: C1; if recipient is CYP3A4*22 noncarrier and donor is CYP3A4*22 carrier: C2; if recipient is CYP3A4*22 carrier and donor is CYP3A4*22 noncarrier: C3; and if both recipient and donor are CYP3A4*22 carriers: C4. For CYP3A5 the coding was as follows; if both recipient and donor are CYP4A5*1 noncarriers: C1; if recipient is CYP4A5*1 noncarrier and donor is CYP4A5*1 carrier: C2; if recipient is CYP4A5*1 carrier and donor is CYP4A5*1 noncarrier: C3; and if both recipient and donor are CYP4A5*1 carriers: C4.[13,21]

2.4 | Model development

One- and 2-compartment models were considered based on a search of the literature and on visual inspection of the data. Various oral absorption models were assessed including linear absorption models with or without lag time, manually added transit-compartments, and the transit-compartment method in which the optimal amount of transit compartments is estimated.[22] Different error models for residual error were assessed including additive, proportional and combined additive and residual models, with or without weighing (conditional weighted residuals). Also, different proportional residual errors for samples measured by means of whole blood samples and DBS was considered.

IIV and interoccasion variability (IOV) for which occasions 1, 2 and 3 were defined as the occasions of AUC1, AUC2 and AUC3, respectively, were assessed using an exponential model. A covariance matrix with multiple omegas blocks using the same covariance was considered for the interindividual random effects.

FIGURE 1 Study visit and pharmacokinetic sampling schedule. C_{trough}, trough concentration; AUC, area under the curve; LSS, limited sampling schedule

2.5 | Model selection

During model development, candidate models were evaluated for their decrease in objective function value calculated as the −2 log likelihood. A decrease in objective function value of ≥3.84 was considered significant (χ^2, 1 degree of freedom [df], $P < .05$).

In addition, basic goodness-of-fit (GOF) plots, in which the observed concentration is plotted against the individual- and population-predicted concentrations, and the conditional weighted residual errors are plotted against time and against population predicted concentrations, were assessed. Also, parameter precision, shrinkage, IIV and IOV were taken into account during the modelling process.

2.6 | Covariates

After selection of the base model, various covariates were analysed in a stepwise manner (univariate analysis) as well as using automated stepwise covariate modelling (scm). First, bodyweight was a priori included in the model, exponentially using allometric scaling, on clearance (CL) and intercompartmental clearance (Q) with a power exponent of 0.75 and on V1 and V2 with a power exponent of 1.0, based on biological plausibility and extensive previous evidence.[23–25] The effect of body weight was standardized on typical patient of 70 kg. Haematocrit was analysed as covariate on CL and/or V1 based on literature.[26,27]

Recipient and donor CYP3A4*22 and CYP3A5*3 genotype were analysed on CL and F in an univariate analysis. In addition, using automated scm, donor, recipient and combined CYP3A4*22 and CYP3A5*3 genotype, together with IL-6, -10 and -18 genotype were analysed on CL. All covariates were assessed using a forward inclusion criterion of $P < .05$ and backward elimination criterion of $P < .01$. The continuous covariate (haematocrit) was considered (both on CL and on V1) using a linear, hockey-stick, exponential and power condition, and categorical data (pharmacogenetic state) as a linear condition on CL.

2.7 | Model evaluation

The final model was evaluated by means of a prediction-corrected visual predictive check (VPC) based on 500 Monte-Carlo simulations. Binning was adapted manually in such a way that the periods with the densest sampling were in the middle of the bin, since observations were spread around nominal time points.

In addition, the precision of the parameter estimates was further assessed by means of a nonparametric bootstrap with resampling the dataset (n = 1000 times). This way, 1000 new datasets containing different combinations of individuals are generated yielding new parameter estimates and confidence intervals.

2.8 | Limited sampling strategy

Based on the final model, a LSS was developed based in order to predict the first full AUC_{0-24h}. The final model was run as a posthoc method, in which the maximum of evaluations is set to zero (MAXEVAL = 0) and all parameter estimated are fixed. The *true* AUC (AUC_{model}) was calculated as AUC24 = ((DOSE*F)/CL. This AUC was compared to the AUC obtained with different LSS (AUC_{LSS}). One, 2, 3 and 4 time points were taken into account for the construction of an LSS. For practical feasibility, a maximum of 12 hours between drug intake and sampling was allowed.

In addition to the LSS, the correlation between AUC_{model} and trough concentrations was assessed with a Pearson correlation test.

The amount of bias was calculated to compare all LSS with the AUC_{model} and the percentage of patients with an AUC_{LSS} that deviates > 10, 15 and 20% from AUC_{model} was computed. A bias of >20% will probably result in incorrect dosing advice (too high or too low), i.e. outside 80–120% range of a specific preset target AUC.

2.9 | Software

The population PK modelling was carried out using nonlinear mixed-effects modelling (NONMEM v.7.4.1) and PsN (v.4.7.0), Xpose (v 4.7.0).[28–30] Pirana interface was used for run interpretation (v. 2.9.7).[31] The first-order conditional estimation with interaction (FOCE+I) method was for analysis. R statistics (v. 3.4.4) was used for exploratory graphical analysis and for evaluation of the GOF and VPC.[32]

2.10 | Implementation in InsightRX Nova

To provide a certified, robust and ready-to-use tool for application for the model and LSS strategy, the final model was incorporated in the InsightRX Nova software (InsightRX, San Francisco, CA, USA). InsightRX Nova (www.insight-rx.com) is accessible as an online web-application, built around the open-source PKPDsim simulation library for R (pkpdsim.insight-rx.com). Based on TDM and additional clinical patient characteristics, the platform applies MAP Bayesian estimation for derivation of the individual estimates for the population model parameters. The final PK model for Envarsus was implemented in the InsightRX Nova module for tacrolimus dosing in adults. InsightRX Nova adheres to ISO 13485 (Quality Management for Medical Devices) and its quality procedures require the verification of model implementation for numerical accuracy and robustness compared to a gold standard method (NONMEM). Numerical verification was performed for the simulation of tacrolimus concentration data, as well as the calculation of individual estimates and AUCs using the identified LSS strategies and MAP Bayesian estimation.

3 | RESULTS

3.1 | Demographics

In total, 55 patients were included with a median age of 57 years (range 21–70 years). Median time after transplantation was 67 months (range 6–240 months). Patients were converted from Advagraf (median dose 4 mg, range 1–10 mg) to Envarsus (median dose 2 mg, range 0.75–6 mg). Further baseline characteristics can be found in Table 1.

TABLE 1 Demographic information

Recipient characteristics	
Number of included patients, *n*	55
Median age, y (range)	57 (21–70)
Female, *n* (%)	19 (34.5%)
Weight, kg (range)	81.5 (54–133)
Length, cm (range)	175 (151–189)
Caucasian, *n* (%)	48 (87%)
Indication for transplantation, *n* (%)	
Primary sclerosing cholangitis	12 (21.8%)
Hepatocellular carcinoma	12 (21.8%)
Alcoholic liver disease	7 (12.7%)
Hepatitis C	4 (7.3%)
Polycystic liver disease	4 (7.3%)
Other	16 (29%)
Retransplantation, *n* (%)	5 (9.1%)
Months after transplantation, *n* (range)	67 (6–240)
Exposure	
Dose Advagraf, mg (range)	4 (1–10)
Dose Envarsus, mg (range)	2 (0.75–6)
AUC Advagraf, μg*h/L (range)	166 (38–377)
AUC Envarsus, μg*h/L (range)	144 (25–323)
Concentration time points Envarsus, *n* (range)	15 (8–19)
Comedication, *n* (%)	36 (66%)
Mycophenolate mofetil	27 (49%)
Prednisone	5 (9.1%)
Everolimus	3 (5.5%)
Sirolimus	2 (3.6%)
Azathioprine	2 (3.6%)
Clinical chemistry at AUC Envarsus	
Haemoglobin, mmol/L (range)	8.6 (5.5–10.6)
Haematocrit, L/L (range)	0.41 (0.30–0.51)
Creatinine, μmol/L (range)	96 (48–162)
Albumin, g/L (range)	44 (35–49)
ASAT, U/L (range)	22 (8–86)
ALAT, U/L (range)	23 (7–83)
ALP, U/L (range)	90 (44–438)
GGT, U/L (range)	23 (8–319)

AUC, area under the curve; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transferase.

Of the 55 patients, 53 yielded a total of 748 concentration–time points, which were used for population PK analysis. Two patients were excluded in the PK analysis, because of inconsistencies in the PK data. Figure 2 displays the concentration–time curves of all patients on the full AUC measurement. Pharmacogenetic information of CYP-status was available for 54 patients and from 49 donors. IL-6, -10 and -18 pharmacogenetic information was available for 53 patients and from 48 donors. The frequencies of the pharmacogenetic status of the study population are displayed in Table 2. The frequencies of all genotypes were in HW equilibrium.

3.2 | Population PK model

The PK was best described by a 2-compartment model with delayed absorption described with 1.6 transit compartments with mean transit time of 3.4 hours for absorption. The amount of transit compartments was estimated by the transit-method.[22] The PK parameters along with their % IIV were as follows: CL, 3.27 L/h (34%); Q, 9.6 L/h (24%); volume of distribution of compartment 1, 95 L (141%); volume of distribution of compartment 2, 500 L. Further details are given in Table 3. Bioavailability was fixed to 0.23 and due to poor identification of the second volume of distribution (V2) this parameter was fixed to 500 based on literature.[33,34]

IIV was estimated for the absorption rate constant (Ka), bioavailability (F), CL, volume of distribution of compartment 1 (V1), Q between V1 and volume of distribution 2 (V2). IOV was assessed on

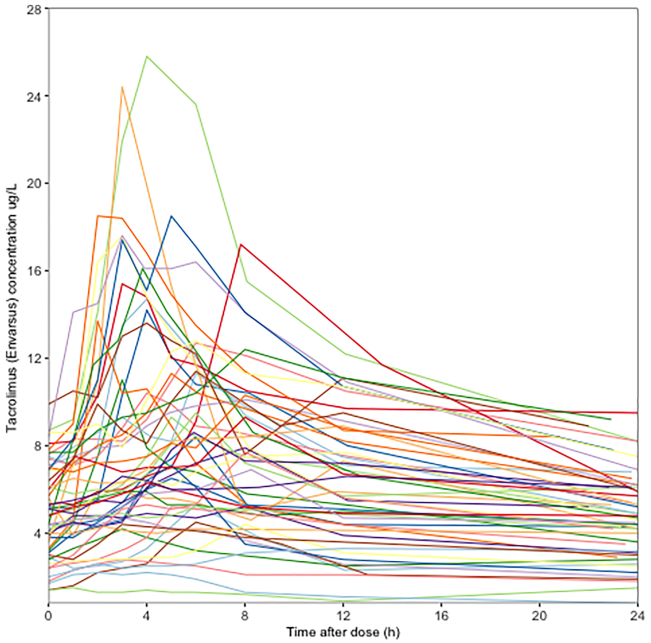

FIGURE 2 Concentration–time curves of all patients at the first area under the curve

TABLE 2 Gene frequencies of study population

Gene	SNP(s)	Nucleotide position and alleles	Genotype	Frequency, *n* (%)
Recipient				
CYP3A4	rs35599367	C > T	C/C	50 (93%)
			C/T	4 (7%)
			T/T	0 (0%)
CYP3A5	rs776746	6986 A > G	G/G	42 (78%)
			G/A or A/A	12 (22%)
IL6	rs1800796	G > C	G/G	48 (90%)
			C/G	3 (6%)
			C/C	2 (4%)
IL10	rs1800871	G > A	G/G	29 (54%)
			A/G	20 (37%)
			A/A	5 (9%)
IL18	rs5744247	G > C	G/G	39 (72%)
			C/G	15 (28%)
Donor				
CYP3A4	rs35599367	C > T	C/C	46 (94%)
			C/T	3 (6%)
			T/T	0 (0%)
CYP3A5	rs776746	6986 A > G	G/G	37 (76%)
			G/A or A/A	12 (24%)
IL6	rs1800796	G > C	G/G	46 (92%)
			C/G	4 (8%)
			C/C	0 (0%)
IL10	rs1800871	G > A	G/G	31 (63%)
			A/G	14 (29%)
			A/A	4 (8%)
IL18	rs5744247	G > C	G/G	37 (76%)
			C/G	12 (24%)
Combination				
CYP3A4			C1	44 (90%)
			C2	3 (6%)
			C3	2 (4%)
			C4	0 (0%)
CYP3A5			C1	27 (55%)
			C2	10 (20%)
			C3	10 (20%)
			C4	2 (4%)

SNP, single nucleotide polymorphism

CL and on F and led to the greatest reduction in OVF for F while GOF plots were similar. An omega block was used for all IOV's to allow for correlation between the IOV of the 3 occasions.

The relationship between CYP and IL-genotype and fixed effects CL, V1 and V2 were first graphically assessed. Secondly, univariate analyses were performed of CYP3A genotype on CL and F. Figures 3 and 4 depict the association between CYP3A4 and CL and between CYP3A5 genotype and CL respectively. Both CYP3A5-genotype of the recipient and haematocrit were significantly correlated to CL in those univariate analyses and reduced unexplained IIV of CL from 34 to 27% when they were both included in the model. Transplant recipients with a functional CYP3A5 enzyme had on average 43% higher CL than nonexpressors. Haematocrit was negatively correlated with CL, i.e. a lower haematocrit led to higher CL. However, in a multivariate analysis using scm, these correlations were no longer significant. Based on this lack of statistical significance and very limited

TABLE 3 Pharmacokinetic estimates from final model

Final model				Bootstrap	
	Mean value	RSE (%)	Shr. (%)	Median value	95% CI
CL/F (L/h)	3.27	8		3.2	2.85–3.89
F (fixed)	0.23			0.23	0.23
Ntrans	1.58	18		1.36	0.36–2.73
MTT (h)	3.39	12		3.39	1.77–5.43
V1/F (L)	94.9	29		90.29	9.03–161.8
Q/F (L/h)	9.62	14		8.29	5.6–14.6
V2/F (L) (fixed)	500			500	500
Ka (h^{-1})	2.97	112		2.66	1.01–3.15
Interindividual variability					
CL/F (CV%)	34	31	27	34	28.5–36.3
V1/F (CV%)	141	17	15	141	122.4–192.8
Q/F (CV%)	24	110	67	23.8	23.4–24.0
Ka (CV%)	174	57	45	174	151.1–180.1
F (CV%)	36	25	25	36.2	30.6–38.2
IOV F (block) (CV%)	19.7	10	28, 40, 98	19.7	13.1–25.8
Random residual variability					
Whole blood (%)	10.5	11	14	10.6	9.2–13.9
DBS (V%)	24.9	9	7	25.2	24.2–29.6

CI, confidence interval; CL, clearance; DBS, dried blood spot; F bioavailability; Ntrans number of transit compartments; MTT mean transit time; V1 distribution volume of central compartment; Q intercompartmental clearance; V2 distribution volume of the peripheral compartment; Ka absorption rate constant; IOV interoccasion variability.

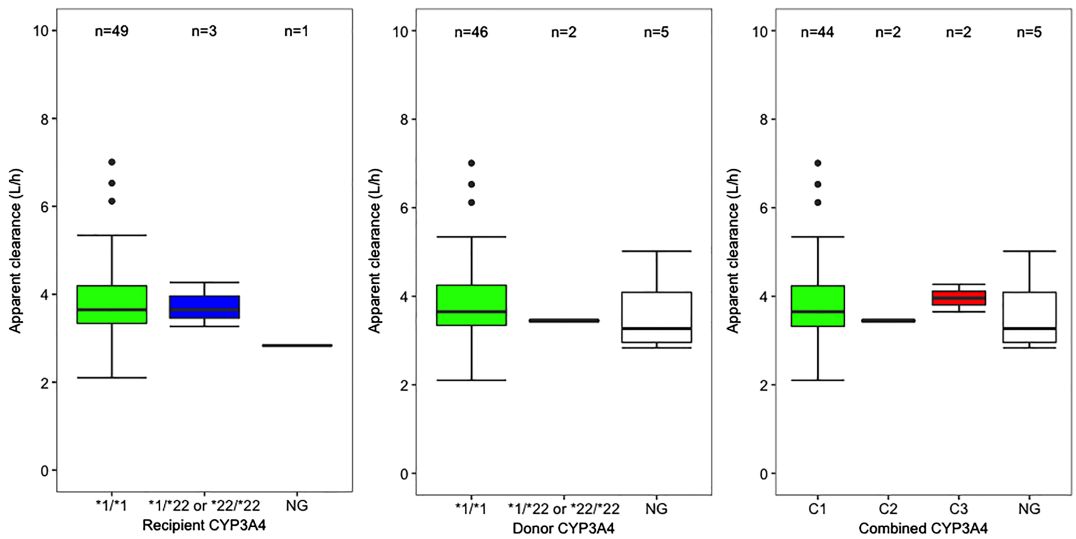

FIGURE 3 Boxplots representing the association between CYP3A4 genotype and apparent clearance (L/h). NG, no genotype

decrease in IIV observed, none of the covariates apart from body weight were included in the final model.

A bootstrap (n = 1000) was performed to assess parameter precision. The median of bootstrap parameter estimates fell within 10% of the estimates obtained from the final model, details are depicted in Table 3.

A prediction-corrected VPC (n = 500) is shown in Figure 5. As can be seen from the VPC, the observed median and 5th and 95th percentiles lie within the predicated area, illustrating good prediction of the model.

In addition, population- and model-predicted *vs*. observed concentrations are shown in Figure S2.

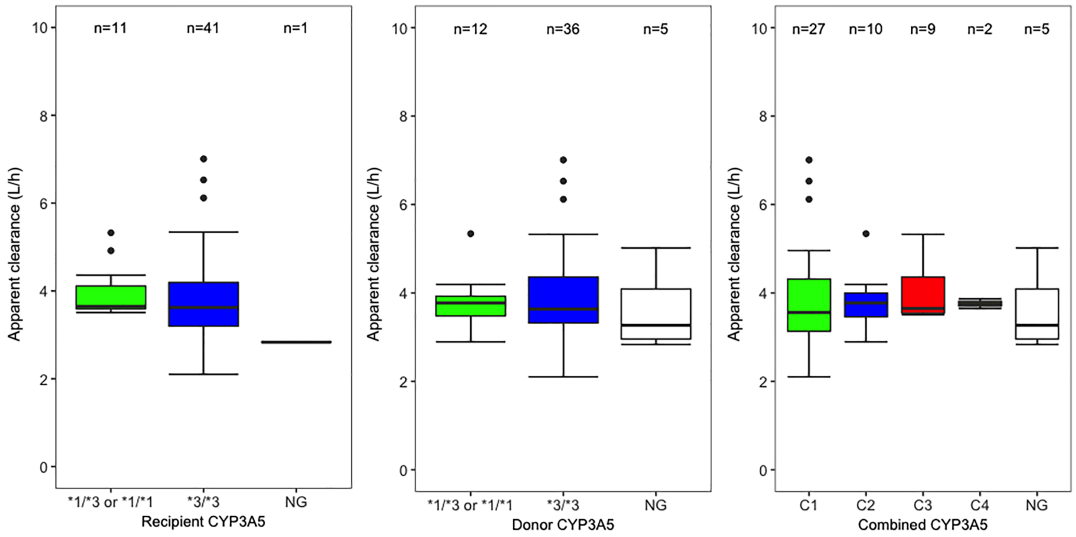

FIGURE 4 Boxplots representing the association between CYP3A5 genotype and apparent clearance (L/h). NG, no genotype

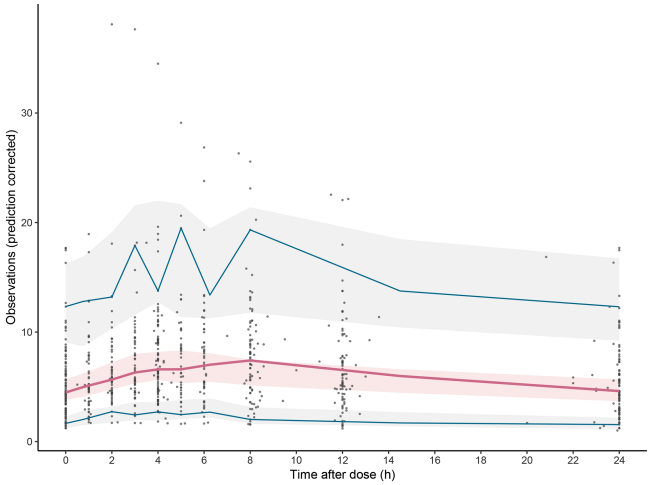

FIGURE 5 Prediction-corrected visual predictive check. Simulated (n = 500) (shaded areas) and observed (circles and lines) tacrolimus concentrations *vs*. time after dose (h). The thick pink line connects the observed median values per bin. The solid blue lines connect the 5th and 95th percentiles of the observations. The blue areas are the 95%confidence interval of the 5th and 95th percentiles. The red area indicates the confidence interval of the median

Moreover, the model-predicted AUC (AUC_{model}) calculated as described in section 2.18 correlated with an additionally calculated trapezoidal AUC (AUC_{trap}) with a Pearson correlation coefficient (R^2) of 0.95 indicating good predictive power (shown in Figure S1). For this analysis, only full AUCs (occasion 1) were considered.

3.3 | Limited sampling strategy

Limited sampling strategies (LSS) were systematically assessed on the final model by means of *posthoc* analysis within NONMEM. The results of the different LSS are shown in Table S1 and Figure 6.

Linear regression of the full AUC_{model} on trough concentration (C0) gave the following regression formula: 8.836 + C_{trough} × 28.256. Correlating the AUC calculated with this formula to the full AUC_{model} gave R^2 of 0.89, about 15% of the patients had >20% bias and in >25% of patients the bias was >15%. Using only C0 in a LSS resulted in >20% bias in 12% of patients and in >15% bias in 23%.

The best 4-point LSS were t = 0,3,6,8 hours and t = 0,3,6,12 hours, which had a median bias of 0.6% (range −8.9 – 7.2) and 1.4% (range −7.5 – 6.2) as compared to the full AUC_{model} respectively. The best 3-point LSS was t = 0,4,8 hours with a median bias of 1.8% (range −12.5 – 12.5). All 3 LSS resulted in a predicted AUC that had >20% bias in <10% of the patients with the full AUC. Median absolute percentage prediction error and median percentage prediction error were <5%.

3.4 | Implementation in InsightRX Nova

The final PK model was successfully implemented in InsightRX Nova and numerically verified against a gold standard. Supplementary file S2 shows the verification of the model in InsightRX Nova.

4 | DISCUSSION

This is the first study describing the population PK of tacrolimus melt-dose in liver transplant recipients using nonlinear mixed-effects modelling yielding primary PK parameters including clearance and volume of distribution and assessing genetic variability as a possible explanation for PK variability. Both whole blood samples obtained by venous sampling as well as DBS finger-prick samples were used to develop the model. A 2-compartment model with delayed absorption using transit compartments and linear elimination best described the PK of meltdose tacrolimus (Envarsus). The variability in elimination of

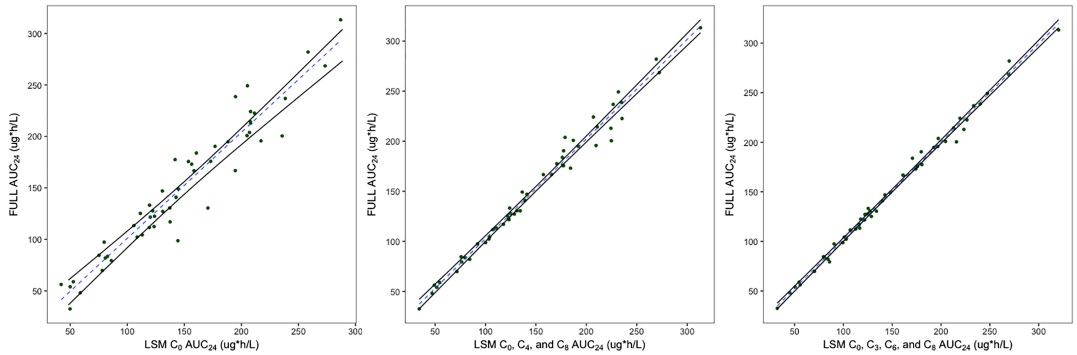

FIGURE 6 Regression plots of AUC_{model}*vs*. 3 limited sampling strategies. First panel: AUC_{model}*vs*. limited sampling model (LSS) of C0; second panel: AUC_{model}*vs*. limited sampling model (LSS) of C0, C4 and C8 hours and third panel: AUC_{model}*vs*. LSS of C0, C3, C6 and C8 hours. AUC, area under the curve

volume of distribution was not impacted by haematocrit, haemoglobin, nor by CYP3A4 and CY3A5 or IL-6, −10 or −18 genotype.

The mean apparent clearance and volume of distribution of compartment 1 and 2 were 3.27 L/h and 94.9 L and 500 L (fixed), respectively. We found a lower clearance as compared to the study by Hénin *et al*. among kidney transplant recipients treated with meltdose tacrolimus,[34] where 20 L/h was found. This difference is probably caused by the differences in bioavailability, which is assumed to be 1 for their population and was fixed to 0.23 in the current study, so CL/F was similar (14.2 L/h when back-calculated).

The clearance found in our model was comparable to Advagraf PK (4.8 L/h)[13] in the same population. Our volume of distribution of the first compartment (94.9 L) was also comparable to data on Advagraf (87.3 L) but we fixed the volume of the second volume of distribution to 500 L, while for Advagraf this was 142 L.[13] Hénin *et al*. developed a 1-compartment model for Envarsus in which the volume of distribution was 451 L, which is more comparable to our total volume of distribution of compartments 1 and 2.[34]

Other literature on the PK of meltdose tacrolimus did not report any primary parameters.[14,15,35]

The absorption of tacrolimus was challenging to describe given the major IIV of this process, as can be seen in differences in Cmax and Tmax (Figure 2), and also IIV was observed (data not shown). Our absorption model used the Savic-code to estimate the amount of absorption compartments (n = 1.7).[22] Woillard *et al*. described the absorption of meltdose tacrolimus in both liver and kidney transplant patients and designed the absorption in 2 phases using a sum of 2 gamma distributions.[15] Hénin *et al*. used a 3-phase absorption profile distinguishing between fast and slow absorption.[34] Advagraf PK among liver transplant patients was described by 3 absorption transit compartments.[13] Those examples illustrate the complex absorption profile of tacrolimus controlled release formulations. The observed variability in absorption is expected to be even higher under less controlled situations, i.e. in clinical practice, given the known extrinsic factors impacting first-pass effect and exposure such as interacting drugs or diarrhoea.

In addition, we observed good agreement between simulated (AUC_{model}) and observed (AUC_{trap}) AUCs (Figure S1), confirming that the model is good for its purpose.

No covariates were identified to significantly decrease IIV of apparent clearance or volume of distribution. Based on our results, for meltdose tacrolimus, the effect of genotype on Envarsus PK seems at least less pronounced than with other tacrolimus formulations, and perhaps even absent; however, this should be confirmed in a larger study. This is in contrast to earlier findings of tacrolimus PK for Advagraf and Prograft formulations, where higher clearance was observed in patients that were (engrafted with a liver from) CYP3A5*1 carriers.[13,34,36,37] The sample size of nondominant CYP3A4 and CYP3A5 genotypes was relatively low in the current study, which may have hampered adequate estimation of the impact of genetic variability. Another factor that may contribute to the lack of effect of genetic variability is the longer residual time of meltdose tacrolimus in the gastrointestinal tract. CYP3A4 expression is less abundant after the duodenum while CYP4A5 expression remains equal in the gastrointestinal tract.[38] The overall effect may be higher absorption and less influence of genetic variability in metabolizing capacity.

Ideally, the results of the developed model are compared using an external validation cohort. Although no validation cohort was available in the current study, the model is considered fit-for-purpose given the results of the VPC, the bootstrap and the agreement between AUC_{model} and AUC_{trap}.

The results of our study with meltdose tacrolimus confirm the need for AUC-based TDM, instead of trough concentration monitoring, as one cannot rely on trough concentrations to predict the AUC. To simplify AUC-based TDM, MAP LSS can be helpful. Various LSS were evaluated based on statistical measures such as the absolute and relative percentage prediction errors and based on clinical interpretation (% bias), as the correlation between the predicted and true AUC is not informative enough on the variability and precision.[13,39,40] Various limited-sampling models were able to predict the AUC_{0-24h} in this population, with t = 0,4,8 hours and t = 0,3,6,8 hours being the best 3- and 4-point models fitting our data

respectively. The 4-point LSS resulted in statistically (absolute) better prediction of the AUC but is slightly more inconvenient for the patient due to an extra timepoint. The total time window is 8 hours for both LSS. The best LSS for Woillard *et al*. was t = 0,8,12 hours.[15] This regimen was slightly inferior in our model and has a slightly larger time window between the first and last sample. A time window of 8 or 12 hours needs DBS sampling to make it clinically feasible.

Finally, we demonstrated how the meltdose tacrolimus PK model could be implemented in a validated point-of-care precision dosing platform. This tool facilitates the application of this model and LSS in clinical practice, especially when integrated with electronic medical records.

5 | CONCLUSION

The PK of Envarsus in stable adult liver transplant patients was adequately described by a 2-compartment model with delayed absorption described with transit compartments. Variability in CYP3A4 and CYP3A5 status was of much less impact on CL for Envarsus and could not reduce IIV of CL in a clinically significant way, opposed to what is known for other tacrolimus-formulations.[34,36,37] A 3-point LSS predicted the AUC with maximal 12.5% bias. A 4-point LSS led to even lower bias. This LSS can be used in routine clinical care to adequately predict AUC and facilitate dose-individualization with a reduced burden for both patients and the clinic.

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COMPETING INTERESTS

L.M., M.B., D.M., J.S., B.R. and B.H. have no conflicts of interest that are relevant to the content of this manuscript.

CONTRIBUTORS

M.B. was responsible for conducting the study and collecting the data. L.M. and D.M. were responsible for analysing the data, modelling the PK. L.M. and M.B. were responsible for writing the manuscript. J.S. was responsible for conducting the pharmacogenetics screening. All other authors have critically reviewed the manuscript and provided additional comments. All authors have approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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